Effects of cover crops on nitrous oxide (N_2O) emissions in cereal cropping

Khadija Aziz

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Swedish University of Agricultural Sciences, SLU
Faculty of Natural Resources and Agricultural Sciences
Department of Ecology
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Supervisor:	**Rong Lang, SLU, Department of Ecology**
Co-supervisor:	Peter Dörsch, Norwegian University of Life Sciences, Department of Environmental Science and Natural Resource Management
Examiner:	Thomas Kätterer, SLU, Department of Ecology

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Swedish University of Agricultural Sciences
Faculty of Natural Resources and Agricultural Sciences (NJ)
Department of Ecology

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Abstract

More than 60% of anthropogenic nitrous oxide (N_2O) emissions are attributed to agricultural activities. N_2O production in soils highly depends on the N availability along with other factors such as soil moisture content, which provide suitable conditions for nitrification and denitrification. Cover crops (CCs) are used in agriculture to take up the excess N from the field to reduce nitrate leaching and assimilate carbon, which has been reported to result in increased soil organic carbon stocks. Despite these promising benefits, high N_2O emissions are recorded from agricultural soils containing CCs, particularly during winter and early spring. The freeze and thaw cycles in these off-seasons may not only cause the release of trapped N_2O, but also stimulate its *de novo* formation. Hence, due to high off-season emissions, the benefit of carbon capture by the CCs can be offset. The aims of this thesis were to investigate the effect of different cover crop types (legumes, non-legumes, brassicas, and herb mixture) on N_2O emissions, and to assess potential trade-offs between carbon accretion by CCs and N_2O emissions. N_2O fluxes of approximately one year were analyzed to assess seasonal and treatment effects of CCs. The results showed that the effect of having CCs in the cereal cropping system was species dependent. Ryegrass (Italian and perennial) and a herb mixture suppressed N_2O emissions compared with the control, particularly during winter, while oilseed radish increased N_2O emissions. Legumes (winter and summer vetch, Phacelia) grew poorly and had no effect. Nitrous oxide emissions showed strong seasonal patterns with off-season emissions accounting for more than 80% of the annual emission. Among the different cover crop species, the highest cumulative N_2O emissions were recorded from oilseed radish (10.5–14.2 kg N_2O-N ha^{-1} y^{-1}), which is frost-killed and has a low C/N ratio. Perennial ryegrass (2.6 kg N_2O-N ha^{-1} y^{-1}) and the herb mixture (3.54 kg N_2O-N ha^{-1} y^{-1}) had the lowest emissions. Assuming an extra C sequestration by CCs of 320 kg C ha^{-1} yr^{-1}, increase in N_2O emissions by CCs must be kept below 2.5 kg N_2O-N ha^{-1} y^{-1} to avoid offsetting the expected C gain. Oilseed radish increased N_2O emissions by more than 2.5 kg N_2O-N ha^{-1} y^{-1}, mainly because of large winter emissions, and can therefore not be recommended for enhancing soil C sequestration under Nordic conditions. All other CCs had no effect or reduced N_2O emissions, supporting the idea that cover crops can be used to combat GHG emissions by “carbon farming”.

Keywords: cover crops, nitrous oxide, C sequestration, greenhouse gases, off-season emissions, freeze-thaw cycles

Table of contents

List of tables

List of figures

Abbreviations

C	Carbon
CCs	Cover crops
FFR	Field flux robot
GHG	Greenhouse gas
GM	Geometric mean
GWP	Global warming potential
N	Nitrogen
NMBU	Norwegian University of Life Sciences
SLU	Swedish University of Agricultural Sciences
SNK	Student Newman Keuls Test
SOC	Soil organic carbon
SOM	Soil organic matter
WFPS	Water filled pore spaces

1. Introduction

Agricultural soils are responsible for more than 60% of the anthropogenic N_2O emissions globally (IPCC, 2007). The estimated total emission of N_2O from Norwegian agriculture is 6.1 Gg N_2O-N yr^{-1}. About 66% of this (3.8 Gg N_2O-N yr^{-1}) is estimated to be directly emitted from soils, attributed to organic and inorganic fertilizers, crop residues and cultivation of organic soils. The remainder is attributed to indirect sources such as leached nitrate (NO_3^-), volatilized ammonia (NH_3) and animal grazing on cultivated or uncultivated land (Tesfai 2016).

Cover crops assimilate carbon (C) in the above and belowground biomass and are known to increase soil organic carbon (SOC) stocks over time, which makes them a promising tool to improve soil quality, sequester C and help reduce greenhouse gas (GHG) emissions from arable soils (Kaye and Quemada 2017). Based on these advantages, cover crops have been proposed to be included in Norwegian cereal production. However, due to incorporation of N-fixing legume cover crops and given the cold climate in Norway, with short growing seasons and long winter period, use of CCs may be a potential source of increased nitrous oxide, particularly in off-season (Sturite et al. 2021). Hence, it is important to quantify the N_2O emissions from these crops to evaluate if they truly are a viable option for mitigating net GHG emissions.

The present study was conducted within the framework of the Norwegian-funded project "CAPTURE -- Fangvekster som klimatiltak i norsk kornproduksjon". The major aim of the project is to evaluate the effect of cover crops on direct N_2O emissions under Norwegian conditions, especially during winter and early spring, and to assess potential trade-offs between carbon accretion by CCs and N_2O emissions.

1.1. Background

1.1.1 Cover crops

Cover crops (CCs) are crops planted below or after harvesting the main/cash crop (Abdalla et al. 2019). Cover crops were originally used to avoid soil erosion and nitrogen (N) leaching from bare arable soils (Battany and Grismer 2000). Plant species used as cover crops range from annual to perennial and biennial plants, which are planted as single species or in mixtures (Abdalla et al. 2019). Cover crops are expected to produce high biomass to ensure maximum soil coverage, have a balanced C/N ratio so that they do not reduce nitrogen (N) for the main crop and be resistant to rapid decomposition (Kocira et al. 2020).

Cover crops can be assigned to five classes: grasses, brassicas, legumes, non-leguminous herbs, and mixtures. Grasses such as ryegrass are commonly used as CCs to decrease nutrient leaching and increase soil organic matter content (Wang et al. 2021). They are known for their rapid growth and good establishment and are, depending on the species used, winter hardy, i.e., can tolerate frost and cold winters (Islam et al. 2021). They can be perennial, or biennial such as Italian ryegrass, which requires two years to complete its life cycle. They have a low N content (C/N ratio > 20) and require fertilizer for optimal growth when the soil concentrations of residual N after the main crop are low (Sainju et al. 2005).

Brassicas such as radishes and turnips act as scavengers of N due to their deep and extended root system. They take up N from both upper and deeper soil layers. However, they are unable to retain N for a long period as their residues decompose rapidly. They are not very winter hardy and can be damaged by a light frost or killed by a hard freeze (Gruver et al. 2014).

Unlike grasses and brassicas, legumes are not dependent on mineralization of soil organic matter to cover their N demand, as they can fix N_2 from the atmosphere via rhizobial symbiosis. Due to high N concentrations (i.e., C/N < 20), legumes are used in cropping systems without additional sources of N fertilizers (Sainju et al. 2005; Constantin et al. 2011) as they readily release plant available N when mulched or incorporated into the soil.

Mixtures of different cover crop species, e.g., bicultures of legume and non-legumes can be beneficial as they are high-yielding and supply carbon for soil C sequestration while the legumes provide adequate amounts of N to the soil (Sainju et al. 2005; Abdalla et al. 2019). In addition to nutrient cycling, cover crops also improve soil quality by increasing the water holding capacity, stabilizing soil aggregates, and enhancing soil porosity. Other ecosystem services of cover crops in agroecosystems include enhancing biodiversity, reducing erosion, and improving water quality (Daryanto et al. 2018).

1.1.2 Cover crops and soil organic carbon

Soils constitute the largest terrestrial pool of C, storing about 2500 Pg of C up to one meter depth (Batjes 1996). About 12% of this carbon is found in agricultural soils, making them an important sink and/or source for global CO_2 (Schlesinger 1997). Carbon is stored in soil as soil organic matter (SOM), which is a heterogenous mixture of partially decomposed and/or stabilized organic material derived from above and belowground plant litter (including rhizodeposition), as well as microbial and faunal necromass (Totsche et al. 2010). The SOC fraction (elemental C) constitutes approximately 58% of the total SOM (Stockmann et al. 2013); other components include nitrogen, phosphorous and sulphur. Carbon entering the soil serves as a substrate for microbial growth, which in turn stimulates the decomposition of SOM. Microbial decomposition and root/rhizosphere respiration result into CO_2 production thus reversing CO_2 assimilation by photosynthesis (Luyssaert et al. 2007).

Globally, SOC stocks are known to increase with decreasing mean annual temperature (Tarnocai et al. 2009). Therefore, boreal ecosystems and arctic regions store a larger portion (about 1672 Gt) of the global soil C than temperate and tropical regions (Post et al. 1982). Current accelerated rates of global warming directly influence the drivers of terrestrial carbon fluxes, by altering net primary productivity, and stimulating microbial activity. Changes in these drivers significantly affect the amount of CO_2 released back to the atmosphere (Stockmann et al. 2013). As the quantity and quality of organic matter returned to soils is shaped by plants, the ability of agricultural soils to sequester carbon is high if they are managed properly. Use of cover crops promotes carbon sequestration, as they add organic matter to the soil via above and belowground biomass and stabilize the soil structure (Nair et al. 2015). Several studies reported a positive effect of cover crops on SOC stocks in agricultural systems (Wang et al. 2010; Venkatesh et al. 2013). Poeplau et al. (2015) investigated the effect of perennial ryegrass on SOC stock changes in three long-term (16-24 years) field experiments in Sweden. They reported an increase in SOC stocks by using perennial ryegrass as a cover crop with a mean annual sequestration rate of 0.32 ± 0.08 Mg C ha^{-1} yr^{-1}.

1.1.3 Formation and consumption of nitrous oxide in soils

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential (GWP) 298 times higher than carbon dioxide (CO_2) in a 100-year timeframe (Myhre et al. 2013). The main processes leading to N_2O formation and emission in soils are the microbial N transformations nitrification, denitrification and nitrifier denitrification (Abdalla et al. 2011; Fowler et al. 2015) (Figure 1).

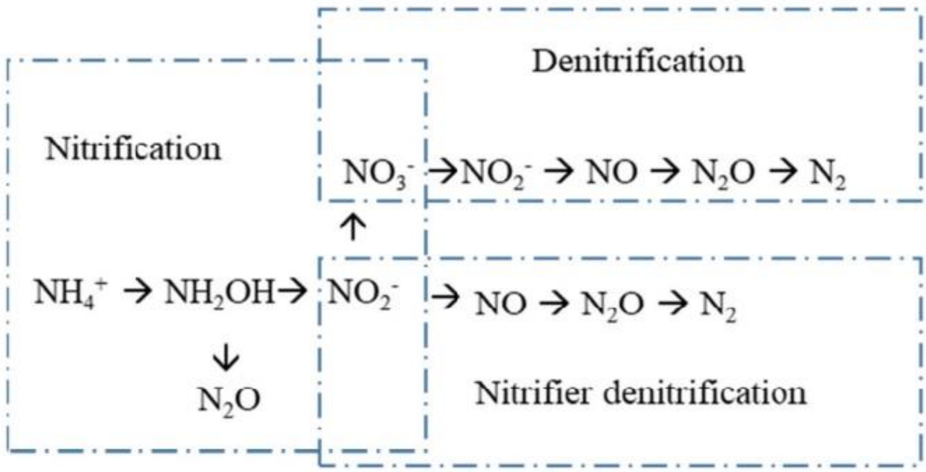

Figure 1. Major N_2O formation processes (adapted from Wang et al. 2021).

Nitrification is an aerobic process in which ammonia (NH_3) is oxidized to nitrate (NO_3^-) via nitrite (NO_2^-) with N_2O produced as a by-product. Ammonia is firstly converted into nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB) and archaea (AOA) in a two-step process, producing hydroxylamine as an intermediate. Nitrous oxide is either produced oxidatively from hydroxylamine (AOB and AOA), or by enzymatic reduction of nitrite (NO_2^-), a process equivalent to that of denitrification. Nitrite is then oxidized further to nitrate (NO_3^-) by nitrite oxidizing bacteria (NOB). Little is known about the N_2O yield of the latter process. In denitrification, NO_3^- or NO_2^- are used as electron acceptors during anoxic respiration reducing them to gaseous N (NO, N_2O, N_2). Nitric oxide (NO) and N_2O are stoichiometric intermediates, which can escape to the atmosphere (Smith 2010; Wang et al. 2021). The ability to denitrify is widespread among phylogenetically unrelated taxa of heterotrophic microorganisms (bacteria and fungi) (Jones and Hallin 2019).

N_2O emissions from soils are regulated by several environmental and management factors. Dominant factors are C and N availability, soil moisture, temperature, pH and mineral N content as affected by fertilizer application (Signor et al. 2013). Increased soil available carbon boosts soil microbial activity and consumes oxygen as most microbes are carbon heterotroph and prefer oxygen as electron acceptor for energy metabolism and growth (Steinbach et al. 2006). Input of easily available C can thus create anoxic microzones in the soil, which supports denitrification (Schlüter et al. 2019). The product ratio of denitrification, i.e., the N_2/N_2O ratio is an important controller for N_2O emissions from denitrification and depends among others on the carbon to nitrate ratio (Stein et al. 2003) and the soil pH (Bakken et al. 2012). Incubation with different concentrations of glucose in sandy and silty loam soils indicated that higher SOC content increased microbial activity and N_2O consumption (Weier et al. 1993). However, studies by Köster et al. (2015) and Saggar et al. (2013) showed that this effect is only valid for soils with low NO_3^- concentrations.

As mineral N species (NO_3^- and NH_4^+) are direct substrates for nitrification and denitrification, type and amount of mineral N in the soil and soil aeration affect whether N_2O is mainly produced from nitrification or denitrification. Therefore, most studies find a positive relationship between mineral N input and N_2O emissions. However, the rate of N_2O emission varies for different climates and soil types (Farquharson, 2016). As NO_3^- produced from nitrification is used by denitrifiers to produce N_2O or N_2, the concentration of NO_3^- in soils plays an important role to decide whether NO_3^- will be reduced to N_2O or N_2 (Scholefield et al. 1997; Bol et al. 2003). Weier et al. (1993) found that higher NO_3^- soil concentrations resulted in lower N_2/N_2O ratio as it inhibited the conversion of N_2O to N_2.

Soil moisture content plays a significant role in regulating N_2O emissions, as it controls whether oxic or anoxic conditions will prevail in soils (Wang et al. 2021). When the water filled pore space (WFPS) is greater than 60%, the amount of available oxygen (O_2) decreases in the soil pores creating anaerobic microsites that allow for denitrification and hence N_2O production (Ciarlo et al. 2007; Friedl et al. 2016). Nitrification, on the other hand, is the main source of N_2O emissions with WFPS between 35% – 60% (Bateman et al. 2005). It is important to note that oxic and anoxic processes proceed simultaneously in the soil matrix of drained upland soils, which makes it difficult to attribute N_2O emissions unequivocally to one or the other process without using stable isotope tracing.

Soil temperature directly influences the microbial growth and activity (Lesschen et al. 2011). The optimal temperature range for nitrification is between 20 and 35℃ (Parton et al. 2001), but this can vary according to climatic regions (Lai et al. 2018). Generally, N_2O fluxes are low at lower temperature because of less soil microbial activity. However, freeze and thaw cycles due to temperature variation often result in high N_2O emissions. This is due to episodic release of trapped N_2O in the soil when the ice melts and formation of *de novo* N_2O when anaerobic conditions prevail in the soil due to thawing favouring denitrification (Dörsch et al. 2004).

N_2O emissions are also influenced by agronomic management such as type and amount of fertilizer and time of application. Fertilizers in any form (either synthetic or organic) serve as an extra source of N in the form of ammonium (NH_4^+), nitrate (NO_3^-), or organic N, hence the amount of fertilization has a strong impact on N_2O emissions (Zimmermann et al. 2018). The heterogenous nature of the above-mentioned proximal factors (such soil C and N content, water content and temperature) that are regulated by distal factors such as management, soil type and climate, make N_2O emissions spatially and temporally variable (Sirivedin and Gray, 2006; Tesfai 2016).

Apart from being sources of N_2O, soils also serve as N_2O sink under certain conditions. Consumption of N_2O is indicated by negative fluxes. The main process behind consumption of N_2O is denitrification, in which N_2O is reduced to N_2 when mineral N content is low and soil moisture content ranges between medium to high (Chapuis-Lardy et al. 2007).

1.1.4 N_2O emissions from agricultural soils and CCs

Agricultural soils are a significant source of N_2O. Cover crops have an ambiguous effect on soil N cycling as they either take up N from the soil and alter N availability for the main crop or provide N in the system for example by fixing atmospheric N_2 in case of legumes. Cover crops with low C/N ratios decompose rapidly and provide labile C and N to the microbes which under certain conditions (as mentioned above) can stimulate N_2O production (Kaspar, and Singer 2011).

Various studies report strong seasonal patterns of N_2O emissions from agricultural soils. During the growing season, increased N availability due to fertilizer application and mineralization of SOM is the main driver for N_2O production. Cover crops can affect N availability by taking up extra N, thus reducing emissions. On the other hand, organic N stored in crop residues or cover crops can be released during freezing and thawing cycles in winter and spring, fueling extensive winter emissions. Off-season (non-growing season) emissions are known to comprise 30-90% of the total annual emissions in temperate regions (Wagner-Riddle et al. 2008; Olofsson and Ernfors 2022). Even though such high emissions are produced during the off-season, only few studies have reported on the effect of different cover crops on winter N_2O emissions.

1.2. Research Questions

The aim of this study was to assess the effect of cover cropping on soil N_2O emissions under Norwegian conditions with emphasis on off-season emissions, i.e., emissions outside the main cropping season (winter and early spring). The results were evaluated with respect to expected increases in soil C sequestration by cover crops to identify potential trade-offs between enhanced carbon sequestration and GHG emissions. It was hypothesized that cover crops decrease N_2O emissions during the growing season but increase emissions in winter. Specifically, the following research questions are addressed:

1. How do different cover crops affect N_2O emissions in the growing and off-season?
   1.1. What is the overall effect of cover crop on annual N_2O emissions?
   1.2. What is the seasonal effect of the cover crops?
2. How does the measured surplus N_2O emissions from cover crops balance with the expected increase in carbon sequestration?

2. Material and Methods

2.1. Study site

The study site is located in Southern Norway, Ås (59°39′47″N, 10°45′42″E) on the campus of the Norwegian University of Life Sciences (NMBU). The soil type is classified as an Umbric Epistagnic Retisol (IUSS, 2015) and is tile drained. The soil texture can be classified as a silty light clay with 23% clay, 55% silt and 22% sand. Basic soil properties are presented in Table 1. The 30-year normal (1997-2000) average annual temperature and precipitation is 5.7 ℃ and 795 mm (Wolff et al. 2018). Between April 2021 – April 2022, the temperature varied from -16.3℃ (in December) to 29.2℃ (in July). October was the wettest month with a total precipitation of 157 mm. The maximum snow depth of 19 cm was measured in January 2022 (Norwegian Meteorological Institute, Oslo). Based on field observations, the off-season (winter) was snow poor.

Table 1. Soil parameters of experimental site.

Soil property	mean ± standard deviation
Total C	2.45 ± 0.24%
Total N	0.25 ± 0.03 %
Loss on ignition OM	6.13 ± 0.43% w/w
pH	6.10

2.2. Experimental design

A field site with 36 experimental plots (each 40m^2) was established in spring 2020 (Figure 2) with barley (*Hordeum vulgare*) as the main crop. In total 12 treatments, consisting of five different cover crops species (grasses, legumes, brassicas, non-leguminous herbs, a herb mixture) and two controls (main crop) were planted in the experimental plots during spring and summer 2021 in a complete randomized design (Table 2). The 12 treatments with three replicates were randomly allocated to 36 plots. Fertilizer YaraMila 22-3-10 NPK was applied at two rates referred to as N1 (120 kg N ha^{-1}) and N2 (25 kg N ha^{-1} after the harvest of the main crop). Only a few treatments i.e., treatments 2,4,6,10 received N2.

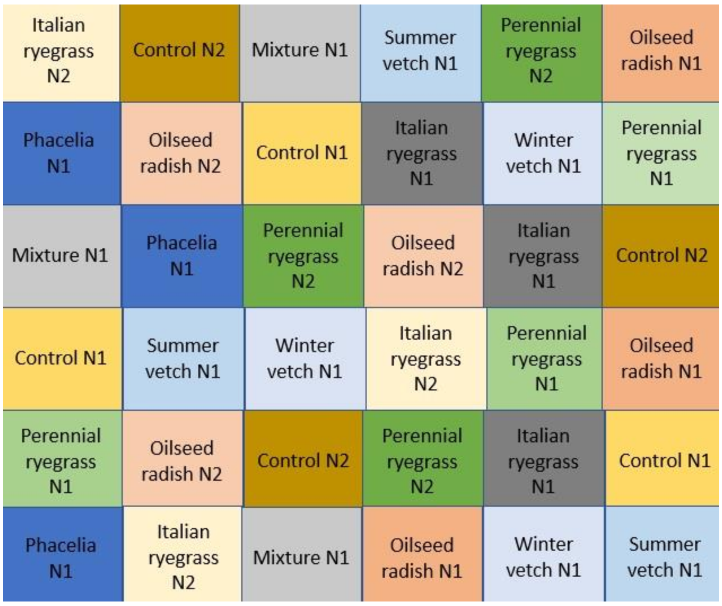

Figure 2. Layout of the experimental plots.

Table 2. List of treatment types with sowing time and fertilization strategy

Treatment	Treatment type	Sowing time	Fertilisation strategy
1	Control (Barley)	Spring 2021	N1
2	Control (Barley)	Spring 2021	N1 + N2
3	Perennial ryegrass	Spring 2021	N1
4	Perennial ryegrass	Spring 2021	N1 + N2
5	Italian ryegrass	Spring 2021	N1
6	Italian ryegrass	Spring 2021	N1 + N2
7	Summer vetch	Summer 2021	N1
8	Winter vetch	Summer 2021	N1
9	Oilseed radish	Summer 2021	N1
10	Oilseed radish	Summer 2021	N1 + N2
11	Phacelia	Summer 2021	N1
12	Mixture*	Spring 2021	N1

*Mixture (30% Perennial ryegrass (*Lolium perenne, L.*), 8% Italian ryegrass (*Lolium multiflorum, L.*), 13% Timothy (*Phleum prantense, L.*), 20% Meadow fescue (*Festuca pratensis, L.*), 5+5+5% White (*Trifolium repens, L.*), red (*Trifolium pratense, L.*) and crimson (*Trifolium incarnatum, L.*) clover, 5% Birdsfoot trefoil(*Lotus corniculatus, L.*), 5% Camelina (Camelina sativa, L.), 3% Herbs

(Common chicory (*Cichorium intybus, L.*), Salad burnet (*Sanguisorba minor, L.*), Caraway (*Carum carvi, L.)),* 1 % Phacelia (*Phacelia tanacetifolia, L.*)).

Different cover crops were selected for measuring their biomass yield and effect on N_2O emissions under Norwegian conditions. Known properties of the selected cover crops are given in Table 3.

Table 3. Cover crops used in the current study.

Cover crop species	Characteristics	References
Perennial ryegrass (*Lolium perenne*)	Perennial, persistent, rapid growth & establishment, shallow fibrous roots, frost tolerant, medium winter hardiness, C/N ratio > 20, mean root depth 1 m.	https://keys.lucidcentral.org/keys/v3/pastures/Html/Perennial_ryegrass.htm#Plant%20description https://www.generalseedcompany.ca/products/forage/perennial-ryegrass
Italian ryegrass (*Lolium multiflorum*)	Biennial, rapid growth & establishment, shallow fibrous adventitious roots, frost tolerant, medium winter hardiness C/N ratio > 20, shallow fibrous roots.	https://smallgrains.wsu.edu/weed-resources/common-weed-list/italian-ryegrass/
Summer vetch (*Vicia sativa*)	Legume, annual, frost tolerant, C/N ratio < 20, shallow but strong root system.	https://www.cotswoldseeds.com/species/69/vetch
Winter vetch (*Vicia villosa*)	Hairy vetch, Legume, annual, frost tolerant, C/N ratio < 20, shallow, taproot can grow upto 2 -3 feet, most roots in 20 cm.	http://www.omafra.gov.on.ca/english/crops/facts/cover_crops01/hairyvetch.htm#family
Oilseed radish (*Raphanus sativus*)	Brassicas, poor winter hardiness, C/N ratio < 20, root depth upto 1 feet.	https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/arpmcpg11828.pdf
Phacelia (*Phacelia tanacetifolia*)	Dense shallow roots at 3-4 cm of topsoil, fast establishment, medium winter hardy.	https://www.cotswoldseeds.com/species/44/phacelia
Herb Mixture "Grønn bro"	To be undersown cereals, recommended for "regenerative soil cultivation", partly overwintering	https://www.norgesfor.no/produkt/strand-nr-52/

2.3. Management

In mid-April 2021, before sowing crops, the field that has been ploughed during autumn was harrowed and fertilized (N1), where all plots received 120 kg N ha^{-1} (Table 4). Barley was sown in late April, followed by sowing of some of the cover crops (Italian ryegrass, Perennial ryegrass, and herb mixture) in their respective plots. Permanent frames for manual GHG measurements were installed at the end of April. At the end of May, loggers for measuring soil temperature and moisture were installed. In early June, the plots were sprayed against weeds and irrigated once due to warm and dry weather. In late July, three weeks before the harvest, the rest of the cover crops (summer vetch, winter vetch, oilseed radish and Phacelia) were sown in the remaining plots. Barley was harvested in mid-August and the straw was removed. One day after the harvest, selected treatments received an additional fertilization (N2) of 25 kg N ha^{-1}. In early September the field was irrigated. For aboveground biomass measurements, cover crops were harvested in early November.

Table 4. Experimental field management.

Date	Management
2021-04-19	N1 application - 120 kg N ha^{-1}
2021-04-26	Sowed barley
2021-04-27	Sowed ryegrasses and mixture
2021-05-07	First weekly manual flux measurement
2021-05-28	Installation of data loggers
2021-06-07	Herbicides Basagran + MCPA
2021-06-08	Irrigation ~25 mm
2021-07-26	Sowed raddish, vetchs and Phacelia
2021-08-18	Harvested barley
2021-08-19	N2 application - 25 kg N ha^{-1}
2021-08-20	Started measurements by FFR
2021-09-02	25 mm irrigation
2021-11-03	Biomass cuts

2.4. GHG flux measurements

The N_2O emissions were measured by two different methods. Manual chambers were used to measure N_2O fluxes weekly from all plots during the growing season until barley was harvested (2021/05/07 – 2021/08/18). After the harvest, between

2021/09/19 – 2021/10/12, the field flux robot (FFR) was used. Due to technical issues with the FFR, a few measurements in early November were taken manually, whereas between 2021/11/15 - 2022/04/30, all the measurements were taken with the robot. GHG flux measurements throughout the study period were carried out by the CAPTURE project group members.

2.4.1. Manual chambers

A static chamber method (Rochette and Bertrand 2008) was used to measure N_2O fluxes by manually placing aluminium chambers (0.51x0.51x0.20m) on frames permanently installed in the experimental plots (Figure 3). The aluminium chambers are equipped with a 3-way sampling port and a 3 mm diameter pressure equilibrium tube. To measure N_2O flux, chambers were placed manually on each frame and air samples were taken every 15 minutes during a total deployment time of 45 minutes. Air samples (15 mL) were drawn from the chambers using 0.02 L polypropylene syringes. These samples were then transferred to 12.5 mL glass vials. Gas chromatography was used to analyse concentrations of N_2O and CO_2 from air samples. More details about the method can be found in Russenes et al. (2019).

Figure 3. Manual chambers used for measuring N_2O fluxes.

2.4.2. Field flux robot

The field flux robot (FFR) was used to measure N_2O fluxes outside the growing season. FFR is a mobile autonomous robot that uses an automatic fast box technique (Hensen et al. 2006, Cowan et al. 2014) for monitoring N_2O emissions at high spatial and temporal resolution. The FFR has two movable collarless chambers that are deployed automatically by pressing them onto the soil. To achieve air-tight closure, the chambers are lined at the bottom with a ring of closed-pore foam rubber with a flexible gas tight membrane attached to their inner side, which is compressed when the chamber is pressed onto the soil. Chamber air is pumped alternatively from the left and the right chamber to a tuneable diode N_2O/CO laser (DLT-100, Los Gatos Research, California, USA) and CO_2/H_2O infrared gas analyser (LI-840A, LI-COR Biosciences, Nebraska, USA). The robot was purposefully programmed to deploy the chambers randomly along a stretch of 1.5 m within each experimental plots to capture small-scale spatial variability (Figure 4).

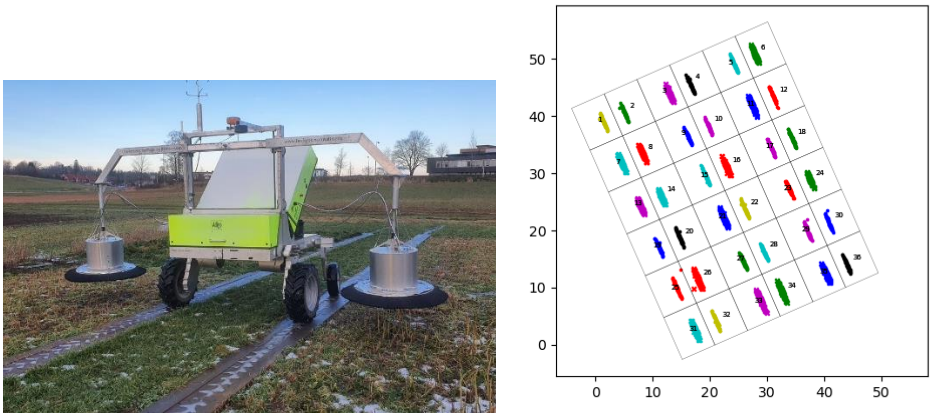

Figure 4. Field flux robot (left) and GPS located field sampling area (right).

The deployment time of chambers was 3 minutes during which the sampled air circulated between the two chambers and the analysers switching every 20 seconds between the chambers. These measured N_2O concentrations were post-processed using a Python script developed by Capture group project members (Molstad, 2015). More details about the FFR can be found in Byers et al. (2021).

2.4.3 N_2O flux calculations

Based on linear slope estimates, the change in N_2O concentrations over time was used to calculate N_2O flux rates according to equation 1:

$$F(N_2O) = \frac{d[N_2O]}{dt} \times \frac{Vc\ X\ Mn}{A\ X\ Vm} \qquad \text{(Equation 1)}$$

Where $F(N_2O)$ is the emission flux measured in µg N_2O-N m^{-2} h^{-1}, $d[N_2O]/dt$ is rate of change in N_2O concentration over time (ppmv h^{-1}), Vc is the chamber volume (L), Mn is the molecular mass of N in N_2O (g mol^{-1}), A is the chamber area (m^2), and Vm is the molecular volume (L mol^{-1}) at the chamber temperature (Tan et al. 2009). Ideal gas law was used to calculate Vm:

$$Vm = \frac{R \times (T_c + 273.15)}{P} \qquad \text{(Equation 2)}$$

Where R is the ideal gas constant, Tc is the temperature in °C, P is the pressure. To get Tc from the manual measurements (2.4.1), the temperature inside the aluminium chambers was measured after every deployment. For FFR (2.4.2), as the chamber deployment time was short, air temperature taken from downscaled meteorological data (Norway M. 2020-2021) was assumed to be equal to the chamber temperature.

Fluxes were calculated automatically from linear slopes over a movable time window of 100s. The time window was guided by the best linearity of CO_2 accumulation.

The estimated fluxes were checked visually and erroneous strong negative fluxes excluded before cumulating N_2O emission rates to seasonal sums (kg N_2O-N ha^{-1} $period^{-1}$) by linear interpolation (i.e., by trapezoidal integration). For most of the sampling dates during off-season, fluxes were measured twice a day. Averaged daily N_2O fluxes were used for calculating cumulative N_2O fluxes to avoid bias by high midday fluxes (especially during the diurnal freezing and thawing periods). Cumulative fluxes were calculated for different time periods (Table 5).

Table 5. Time periods selected for calculating cumulative N_2O fluxes.

Period	Days	Date
Complete period	356	2021/05/07- 2022/04/30
Growing season	158	2021/05/07- 2021/10/17
Off-season	198	2021/10/17 - 2022/04/30
1. Off-season I (Winter)	126	2021/10/17 - 2022/02/15
2. Off-season II (Late winter & spring thaw)	72	2022/02/15 - 2022/04/30

2.5. Soil variables

Two data loggers (Decagon Em50) were installed in two control plots (south and north in the experimental field) to continuously measure soil temperature (℃) and volumetric water content (m^3 m^{-3}). Each logger was connected to 5 combined time-domain reflectometry (TDR) thermistor probes which were installed at 5 cm depth.

2.6. Biomass sampling

During the harvest (2021/8/18), biomass samples of barley were taken from all plots (1.5 m * 6 m = 9 m^2) by Wintersteiger combine harvester. These samples were dried at 60℃ for 2 days to calculate the barley grain yield in kg ha^{-1} and barley nitrogen (N) yield in kg N ha^{-1}. The aboveground biomass samples of cover crops were taken in frames (0.5 m *0.5 m = 25 m^2) in November (2021/11/2). These samples were then sorted (separating weeds and stubble) and dried at 60℃ to calculate the dry matter yields (DMY) in kg ha^{-1} and aboveground standing C and N in kg ha^{-1}.

2.7. Statistical analysis

A linear mixed effect model was devised by Rong Lang (SLU) in SAS to analyse the daily average N_2O fluxes. Daily averaged N_2O fluxes were log-transformed to test treatment and seasonal effects. Log transformation was needed for parametric analysis as the data were not normally distributed and highly skewed. Treatment, season, and their interaction were set as fixed effects. To account for the temporal autocorrelation in the repeated measurements, plot number was set as repeated subject, and first-order antedependence ANTE(1) was chosen as covariance structure after assessing the model residuals. Least-square means of N_2O fluxes from cover crop treatments were compared specifically for the control N1 treatment. Being a better representative of the conventional agricultural practice only the control N1 treatment was selected for this comparison. Since effects of second fertilizer application (N2) on N_2O emissions was not analysed in this study, so control N2 was not used. Similarly, seasonal N_2O fluxes of cover crop treatments were compared specifically to fluxes of control N1 in the growing season and off-season, respectively.

For cumulative N_2O fluxes, one-way ANOVA was conducted in R to analyse the treatment effects for the different periods given in Table 5. Barlett test and Shapiro test were used to check the ANOVA assumptions of equal variances and normal distribution. In cases where variance was unequal, Welch's ANOVA was used and Games Howell pairwise comparison was performed when the treatment effect was significant in Welch ANOVA. Repeated measures two-way ANOVA was conducted to analyse the treatment and seasonal effects on N_2O emissions when different time periods were compared with each other i.e., off-season with growing season, and off-season I with off-season II. Student Newman Keuls (SNK) test and Kruskal-Wallis test were used to compare the differences in means of treatments, seasons, and their interaction.

3. Results

3.1. Soil temperature and moisture

Figure 5 shows the average hourly soil temperature measured in 5 cm depth for the study period (May 2021 – April 2022). The highest average temperature was recorded to be 30.6 ℃ in August whereas the lowest was -7.5 ℃ in February. The first soil frost was observed on October 17, when the soil temperature dropped below 0℃. This was also set as a cut off for the growing season. Snow cover was minor and unstable throughout winter 2021/22. Diurnal freeze-thaw cycles were observed in the winter period (Off-season I) and more pronounced during the spring thaw period (Off-season II).

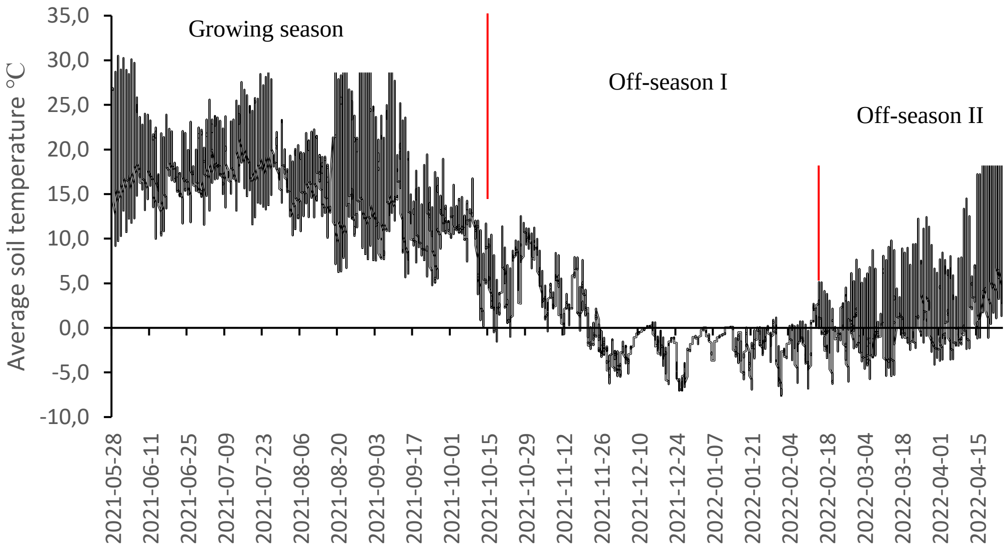

Figure 5. Average soil temperature measured at 5 cm depth in the experimental field.

Soil moisture content measured as volumetric water content m^3 m^{-3} at 5 cm depth is shown in Figure 6. It can be seen that soil moisture content varied throughout the study period. The highest and lowest average soil moisture content was 0.46 and

0.11 m^3 m^{-3}, respectively, both occurring in March 2022. Low soil moistures coincided with periods of soil freezing, and reflect the fact that TDR does not detect frozen water.

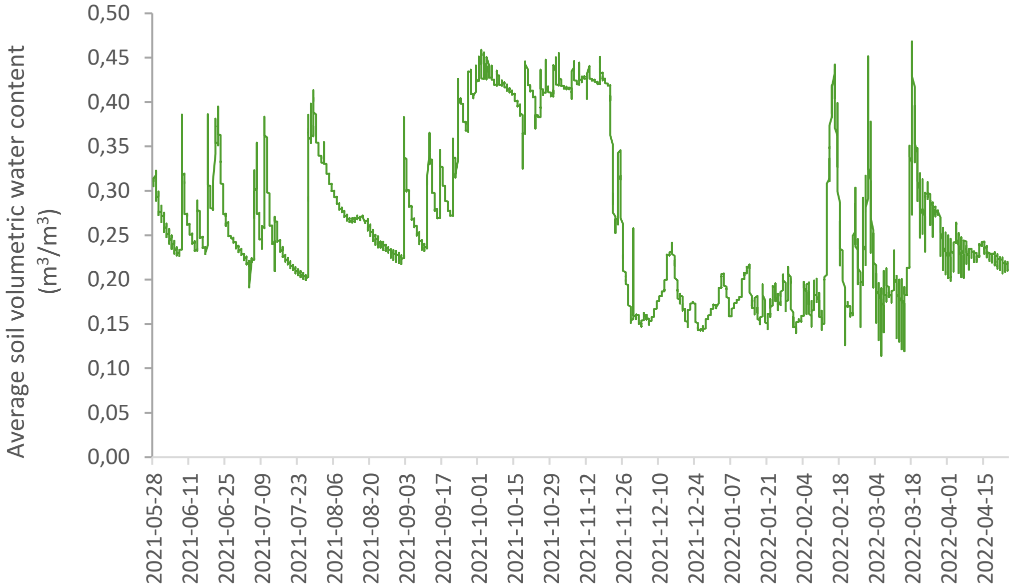

Figure 6. Average soil moisture content measured in 5 cm depth.

3.2. N_2O flux dynamics

3.2.1. Descriptive statistics

Descriptive statistics of daily averaged N_2O fluxes (µg m^{-2} h^{-1}) from cover crop treatments for the entire study period are shown in Table 6. The highest mean N_2O flux was measured in N-fertilized oilseed radish (N2), 200.9 µg m^{-2} h^{-1}, whereas the lowest in perennial ryegrass, 30.8 µg m^{-2} h^{-1}. Standard deviation reflected the high temporal variability of the fluxes. Skewness and kurtosis showed that the data were not normally distributed and needed to be transformed prior to further statistical analysis.

Table 6. Descriptive statistics of N_2O fluxes (µg N_2O-N m^{-2} h^{-1}) from CC treatments.

Treatments	Mean	Standard Error	Standard Deviation	Kurtosis	Skewness	Range	Minimum	Maximum
Control	109.0	18.6	258.2	33.7	5.2	2358.8	-63.0	2295.9
Control N2	161.7	28.2	391.1	21.1	4.4	2572.5	-27.5	2545.1
Perennial ryegrass	30.8	4.0	56.2	16.3	3.6	429.1	-28.6	400.5
Perennial ryegrass N2	114.1	62.2	864.4	168.4	12.7	11688.9	-30.1	11658.8
Italian ryegrass	66.7	10.7	148.8	33.6	5.1	1319.6	-20.7	1298.9
Italian ryegrass N2	67.7	9.1	127.4	17.6	3.7	1019.5	-30.6	988.9
Summer vetch	116.5	25.5	355.5	56.7	7.0	3397.2	-17.9	3379.3
Winter vetch	124.1	23.6	328.0	56.6	6.7	3411.5	-20.3	3391.2
Oilseed radish	178.3	38.6	534.5	104.5	9.3	6690.0	-186.7	6503.3
Oilseed radish N2	200.9	61.0	856.2	125.0	10.4	10881.7	-17.2	10864.5
Phacelia	66.0	8.5	119.0	10.3	3.0	784.3	-26.9	757.4
Herb mixture	37.0	5.4	74.6	36.3	5.2	733.8	-28.6	705.2

3.2.2. Dynamics of N_2O fluxes

A temporal pattern in N_2O emissions was observed in all treatments with pronounced N_2O emission peaks (µg m^{-2} h^{-1}). The first peak occurred after spring fertilization in all treatments while the second fertilizer application in fall (25 kg N ha^{-1}) did not result in any appreciable peak.

Several episodic emission peaks were observed in off-season; both in early winter (off-season I) and late winter during spring thaw (off-season II). Figure 7 gives an example for the above mentioned temporal pattern.

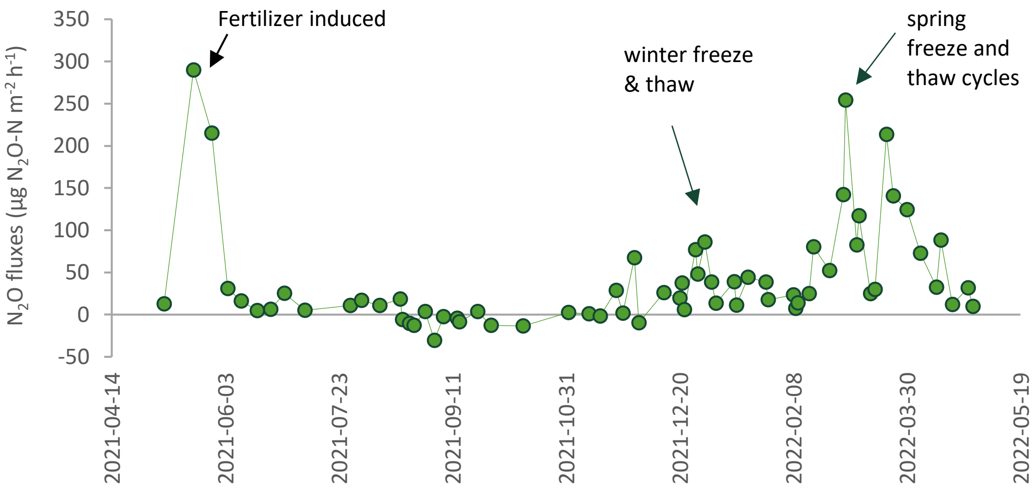

Figure 7. Dynamics of N_2O fluxes from treatment 6 - Italian ryegrass N2 (Plot 1).

In some plots with oilseed radish (Figure 8), emissions peaks were noted after the first few frosts in early winter.

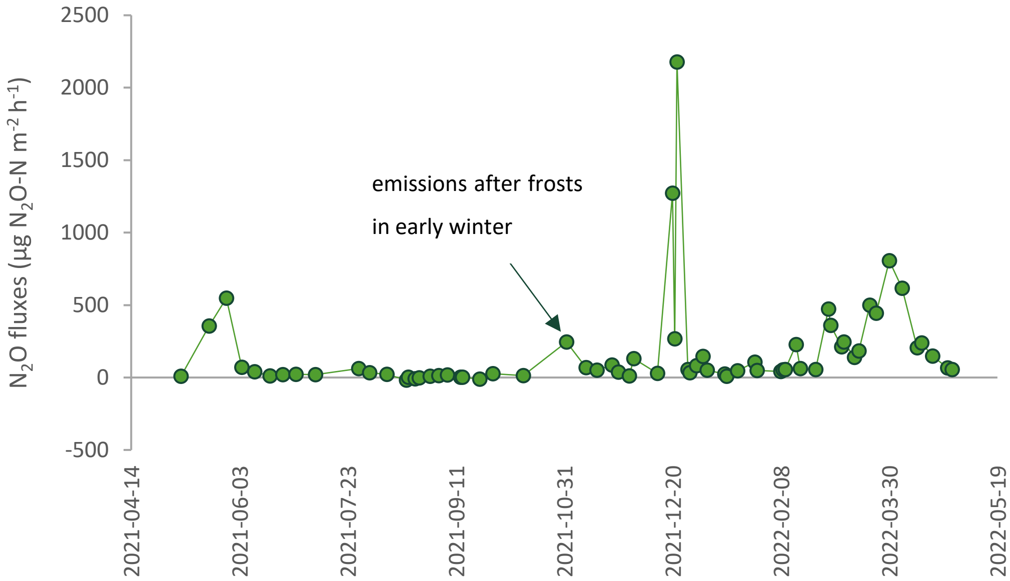

Figure 8. N_2O fluxes from treatment 9 – oilseed radish N1 (Plot 34).

3.2.3. Effects of cover crops on N_2O fluxes

The results from the linear mixed model showed that the effect of treatment, season and their interaction (treatment*season) on daily N_2O fluxes was significant ($p < 0.0001$).

When compared with Control N1, cover crop treatment 3 (Perennial ryegrass), 4 (perennial ryegrass N2), 5 (Italian ryegrass), 7 (summer vetch), 11 (Phacelia) and 12 (mixture) emitted significantly less N_2O, whereas treatment 9 (oilseed radish N1) and 8 (winter vetch) emitted more N_2O, which was not significantly different from control N1 (Figure 9). Hence, the majority of CC treatments had a positive effect in terms of reducing N_2O emissions as compared with a control without CCs.

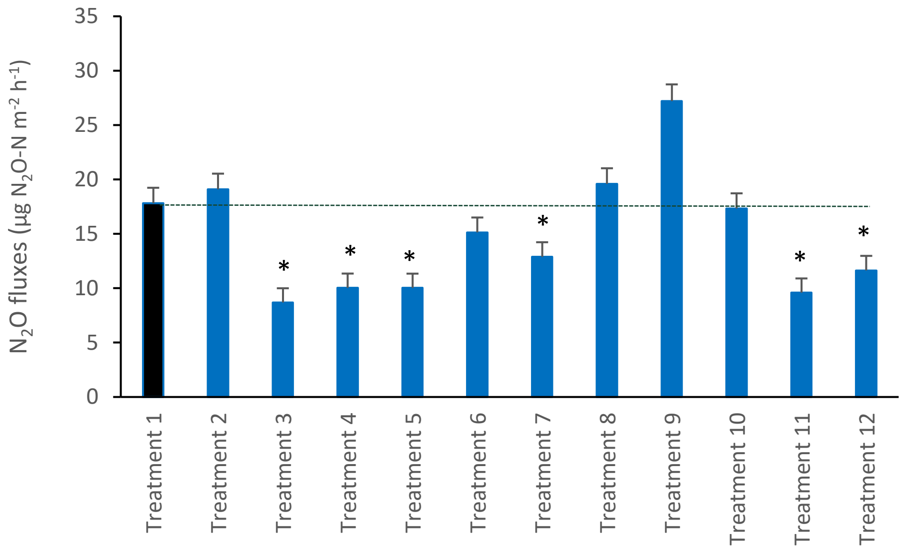

Figure 9. Treatment effects: mean N_2O fluxes as compared to control N1 (indicates statistical significance where p <0.05).*

Off-season daily N_2O fluxes were significantly higher than the growing season fluxes ($p < 0.0001$). Significant interactions as compared to growing season*control N1 at $p<0.05$ are given in Figure 10. Off-season*treatment 9 & 10 (light blue), emitted significantly higher N_2O emissions as compared to the control N1 whereas other treatments given in Figure 10 emitted less N_2O.

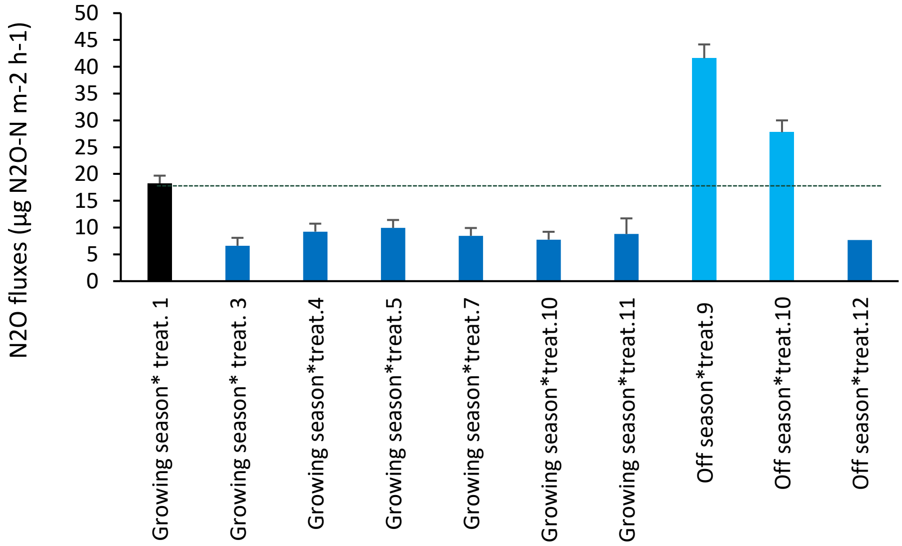

*Figure 10. Interaction effects: mean N_2O fluxes for season*treatment. Treatments with light blue bars emitted more N_2O as compared to Control N1* season.*

3.3. Cumulative N_2O fluxes

Table 7 shows the mean cumulative emission for different periods. Emissions for the entire observation period ranged from 2.65 to 14.20 kg N_2O-N ha^{-1}. These large range was mainly caused by differences between treatments in off-season emission. Overall, treatments with perennial ryegrass and herb mixture had the lowest cumulative N_2O emissions, whereas the highest emissions were found in oilseed radish treatments.

Table 7. Cumulative N_2O fluxes from cover crops with standard deviation.

Treatment	Name	Cumulative N_2O emissions (kg N_2O-N ha^{-1} $period^{-1}$)			100 * (off-season/complete period)
		Complete period	Growing season	Off-season	
1	Control	8.75 ± 2.04	2.38 ± 0.48	6.36 ± 2.17	72.6
2	Control (N2)	13.80 ± 6.58	3.42 ± 1.84	10.3 ± 4.95	74.6
3	Perennial ryegrass	2.65 ± 0.89	1.31 ± 0.45	1.34 ± 0.77	50.6
4	Perennial ryegrass (N2)	7.31 ± 8.45	1.71 ± 0.84	5.6 ± 8.02	76.6
5	Italian ryegrass (N1)	5.31 ± 1.24	2.07 ± 0.62	3.24 ± 0.67	61
6	Italian ryegrass (N2)	5.60± 2.41	2.27 ± 0.917	3.34 ± 1.53	60
7	Summer vetch	9.02 ± 3.31	1.98 ± 0.50	7.04 ±3.43	78
8	Winter vetch	10.70 ± 5.14	4.44 ± 4.04	6.26 ±1.22	58.5
9	Oilseed radish	10.50 ± 3.19	2.03 ± 0.516	8.49 ±3.05	80.8
10	Oilseed radish (N2)	14.20 ± 15.2	1.48 ± 0.45	12.70 ±15.1	89.4
11	Phacelia	5.51 ± 0.65	1.66 ± 0.12	3.85 ±0.9	69.8
12	Mixture	3.54 ± 0.52	2.24 ± 0.48	1.30 ±0.12	36.7

3.3.1. Overall impact of CCs

Results from Welch's ANOVA for the complete period (356 days) showed that the mean cumulative N_2O fluxes of all treatments were significantly different ($p=0.0239$). The order of treatments according to mean cumulative N_2O emission was: Perennial ryegrass < Herb mixture < Italian ryegrass < Phacelia < Italian ryegrass (N2) < Perennial ryegrass (N2) < Control < Summer vetch < Oilseed radish < Winter vetch < Control (N2) < Oilseed radish (N2) (Figure 11). The pairwise Games Howell test showed that no significant differences were found between different treatments (as $p > 0.05$) due to the high variability within the data.

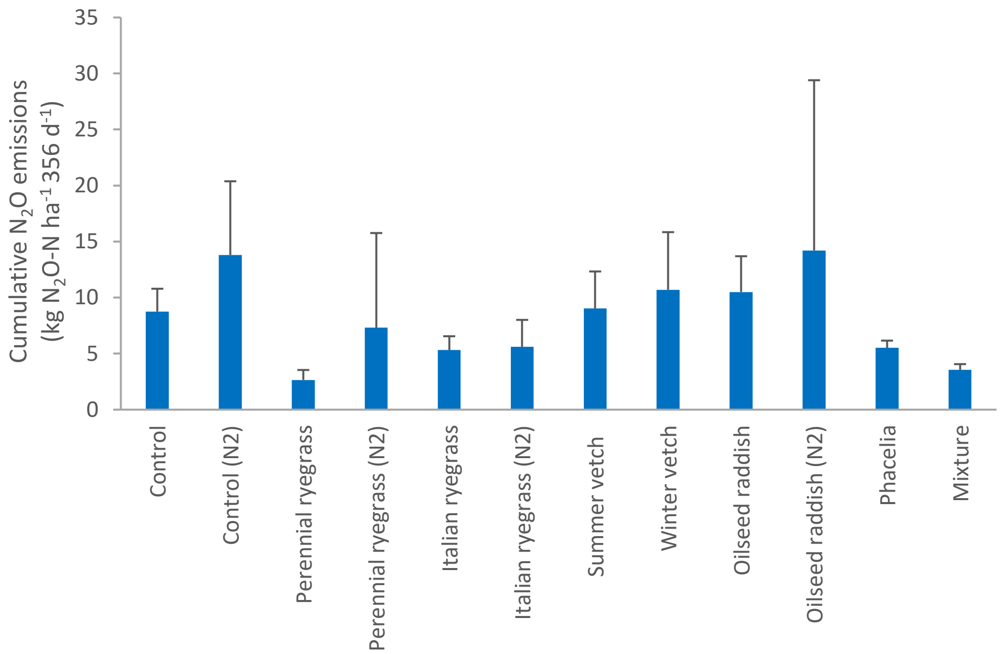

Figure 11. Cumulative N_2O emissions from CC treatments for complete period (356 days). Error bars represent standard deviation.

3.3.2. Seasonal impacts of CCs on N_2O fluxes

Although large variability was observed within treatments, strong seasonal patterns in cumulative N_2O fluxes were monitored, i.e., extremely higher fluxes in the off-season (198 days) as compared to the growing season (158 days) (Table 7, Figure 12). Statistically significant differences were found in the seasonal means (growing and off-season) by the SNK test, indicating that the seasonal effect was more prominent than the treatment effect. The off-season N_2O emissions accounted for 36% to 89% of the total N_2O emissions measured for the complete period of 356 days.

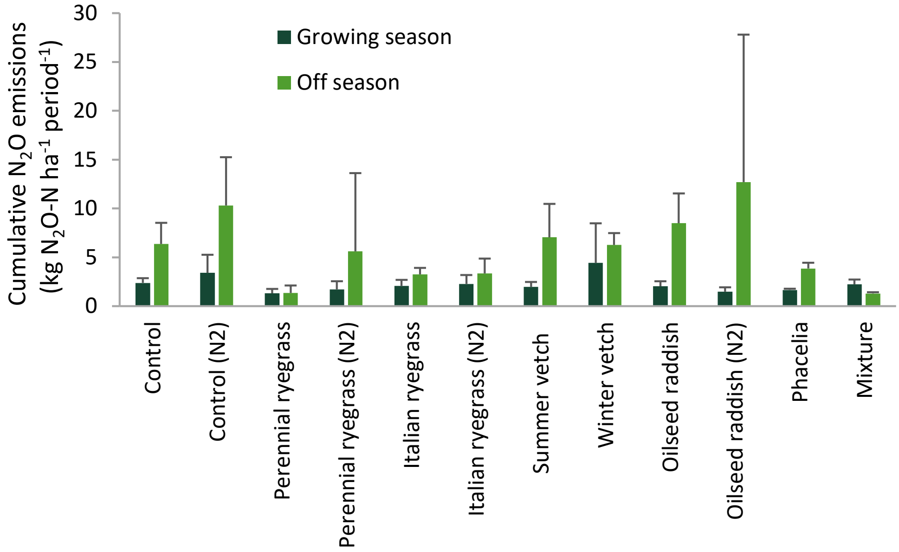

Figure 12. Cumulative N_2O emissions during growing season (158 days) and off-season (198 days) from CCs, SD repesented by the error bars.

3.3.2.1. Growing season

For the complete growing season (158) days, no significant differences in cumulative N_2O emissions were found between the treatments at $p > 0.05$. The lowest cumulative mean 1.31 kg N_2O-N ha^{-1} 158 d^{-1} was measured for perennial ryegrass, whereas the highest measured emission was from winter vetch i.e. 4.44 kg N_2O-N ha^{-1} 158 d^{-1} (Figure 13).

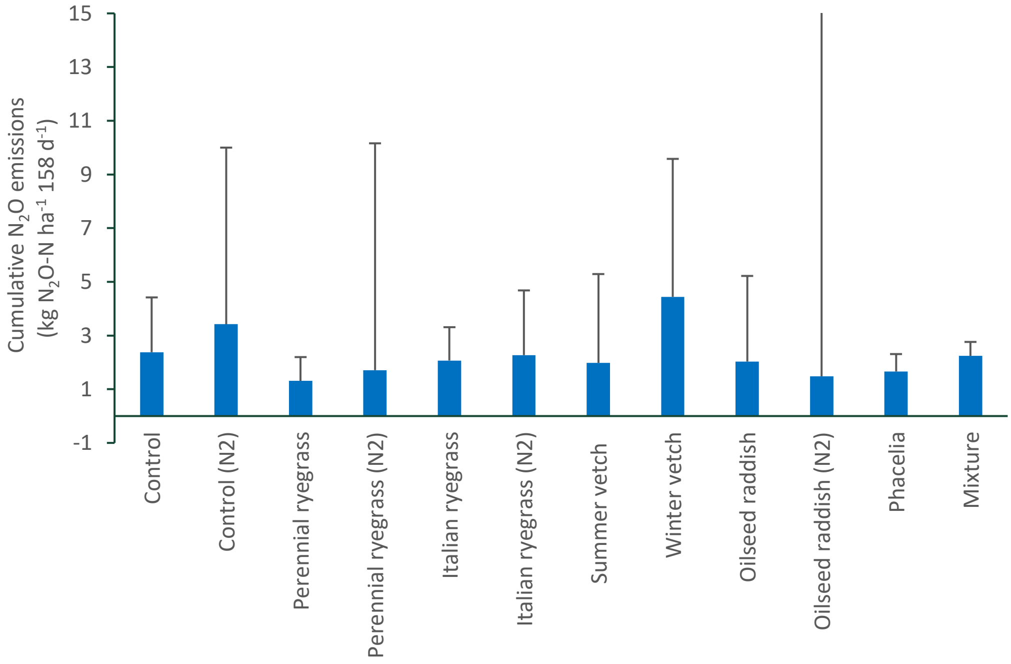

Figure 13. Cumulative growing season (158 days) N_2O emissions. SD repesented by the error bars.

As mentioned in section 2.3, some of the cover crops were not sown before harvest. Only perennial (N1+N2; n=6), Italian ryegrass (N1+N2; n=6) and the herb mixture (n=3) were sown in spring and the rest after 82 days. This allowed us to lump all plots without CCs for the first 82 days and compare those which had CCs on them, thereby increasing the statistical power. The results (Figure 14) showed that controls or plots without CCs had the highest cumulated emissions in this period i.e. 2.07 kg N_2O-N ha^{-1} 82 d^{-1} whereas perennial ryegrass had the lowest 1.40 kg N_2O-N ha^{-1} 82 d^{-1}.

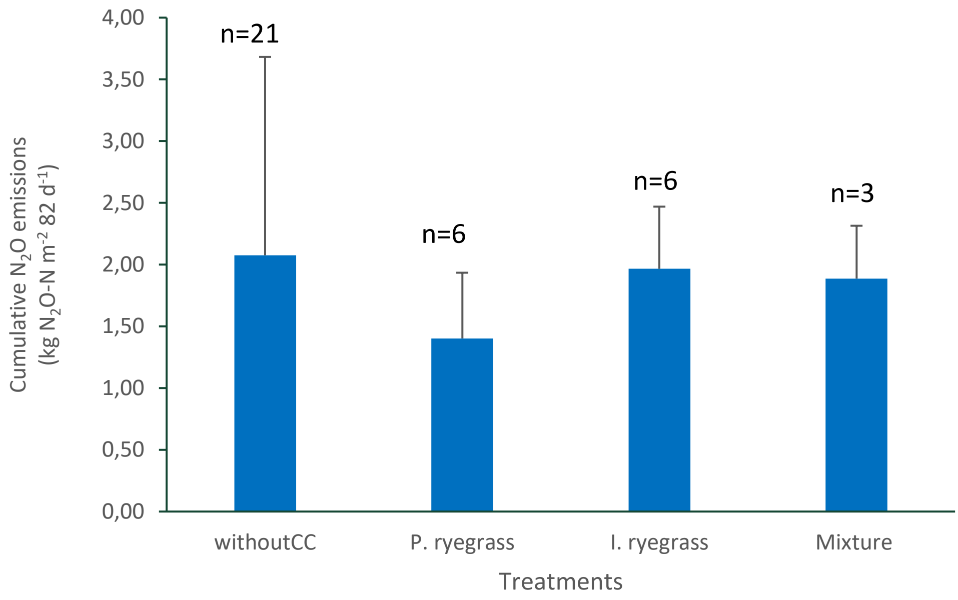

Figure 14. Cumulative N_2O emissions for 82 days. SD repesented by the error bars.

3.3.2.2. Off-season

For the complete off-season (198) days (Figure 15), significant differences were found in mean cumulative N_2O emissions between the treatments at $p < 0.05$. The lowest cumulative mean, 1.3 kg N_2O-N ha^{-1} 198 d^{-1}, was measured for the herb mixture, followed by perennial ryegrass 1.34 kg N_2O-N ha^{-1}198 d^{-1}. Oilseed radish (N2) had the highest mean cumulative flux 12.7 kg N_2O-N ha^{-1}198 d^{-1}. Off-season emissions followed the order: mixture < perennial ryegrass < Italian ryegrass < Italian ryegrass (N2) < Phacelia < perennial ryegrass (N2) < winter vetch < control < summer vetch < oilseed radish < control (N2) < oilseed radish (N2).

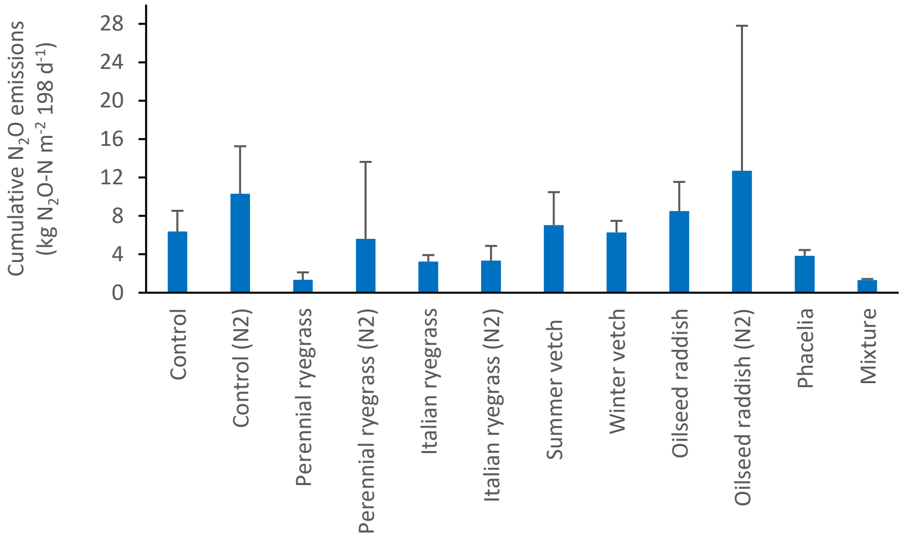

Figure 15. Cumulative off-season (198 days) N_2O emissions. SD repesented by the error bars.

Table 8 and Figure 16 show the cumulative N_2O fluxes for off-season divided into two periods i.e., off-season I (126 days) and off-season II (72 days). Statistically significant differences were found between the seasonal (off-season I and off-season II) means indicating that seasonal effect is more prominent than the treatment effect.

In off-season I, no significant differences were observed between treatments ($p > 0.05$). The higher mean fluxes were measured from oilseed radish (3.4 kg N_2O-N ha^{-1} 126 d^{-1}), oilseed radish N2 (1.81 kg N_2O-N ha^{-1} 126 d^{-1}) and control N2 (1.79 kg N_2O-N ha^{-1} 126 d^{-1}) whereas perennial ryegrass (0.334 kg N_2O-N ha^{-1} 126 d^{-1}) and mixture (0.332 kg N_2O-N ha^{-1} 126 d^{-1}) had the lowest mean cumulative fluxes.

Significant differences were found between treatments in off-season II (15 Feb – April) at $p = 0.0003$. The post hoc test identified significant differences between treatment 3 (perennial ryegrass) and 9 (oilseed radish) with a p-value of 0.014 and between treatment 9 (oilseed radish) and 12 (mixture) with a p-value of 0.026. Overall extremely high cumulative emissions were measured for this period. The highest emission was measured from oilseed radish (N2) (10.9 kg N_2O-N ha^{-1} 72 d^{-1}) whereas the lowest was measured for mixture (0.97 kg N_2O-N ha^{-1} 72 d^{-1}). Emissions in off-season II (72 days) accounted for approximately 60 to 92% of the complete off-season (198 days) emissions. This highlights that late winter and spring thaw contribute most to non- growing season emissions.

Table 8. Off-season cumulative N_2O emissions.

Treatment	Name	Cumulative N_2O emissions (kg N_2O-N ha^{-1} $period^{-1}$)			100* (off-season II/off-season complete)
		Off-season (complete)	Off-season I	Off-season II	
1	Control	6.36 ± 2.17	0.606 ± 0.09	5.75 ±2.25	90.4
2	Control (N2)	10.3 ± 4.95	1.79 ±1.62	8.54 ±3.77	82.9
3	Perennial ryegrass	1.34 ± 0.77	0.334 ±0.17	1 ±0.6	74.6
4	Perennial ryegrass (N2)	5.6 ± 8.02	1.22 ±1.58	4.39 ±6.44	78.4
5	Italian ryegrass	3.24 ± 0.67	0.56 ±0.41	2.68 ±0.418	82.7
6	Italian ryegrass (N2)	3.34 ± 1.53	0.802 ±0.29	2.54 ±1.55	76.0
7	Summer vetch	7.04 ± 3.43	0.551 ±0.30	6.49 ±3.57	92.2
8	Winter vetch	6.26 ± 1.22	0.969 ±0.20	5.29 ±1.24	84.5
9	Oilseed radish	8.49 ± 3.05	3.4 ±2.49	5.1 ± 0.60	60.1
10	Oilseed radish (N2)	12.7 ± 15.1	1.81 ±1.98	10.9 ±13.1	85.8
11	Phacelia	3.85 ± 0.59	0.421 ±0.10	3.43 ±0.50	89.1
12	Mixture	1.3 ± 0.12	0.332 ±0.07	0.971 ±0.19	74.7

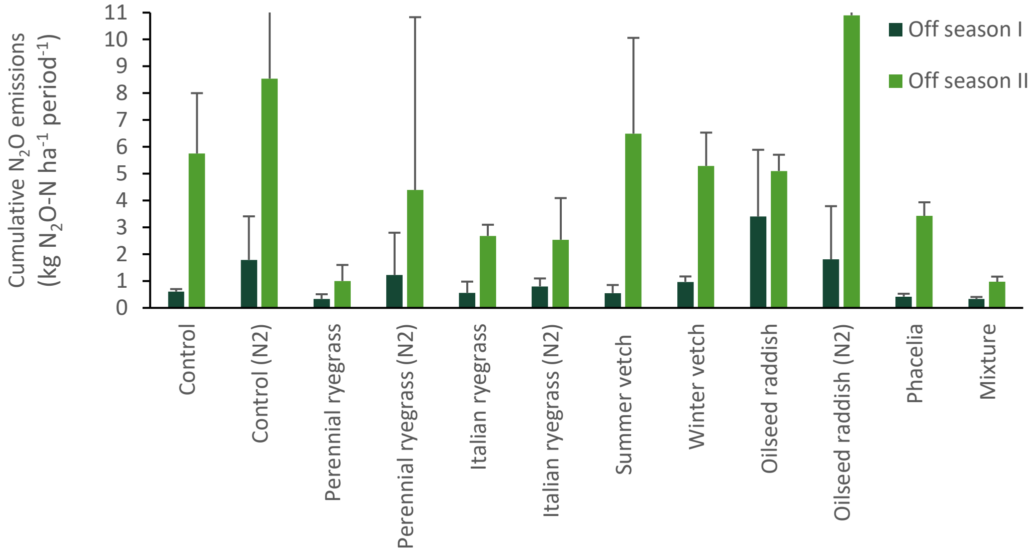

Figure 16. Cumulative N_2O emissions from off-season I (126) and off-season II (72).

3.4. N_2O Flux variability

High spatial and temporal variability was observed within and between the treatments. One example of variability measured in different plots of the oilseed radish N2 treatment is given in Figure 17, where the highest flux in plot 8 was measured to be 496.8 µg m^{-2} h^{-1} whereas the highest flux in plot 16 was 10,864 µg m^{-2} h^{-1}. This resulted in large standard deviations of treatment means, even when cumulating fluxes.

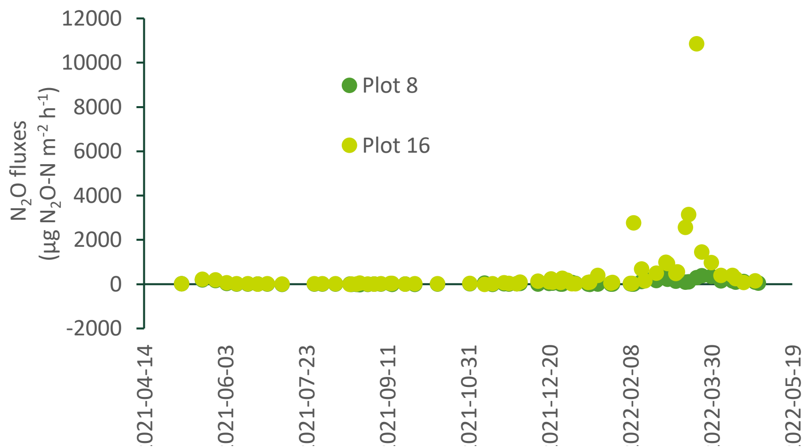

Figure 17. Spatially variable N_2O fluxes from different plots of treatment 10 – oilseed radish N2.

3.5. Biomass yields

Measurement of aboveground biomass in CC treatments in late autumn (kg dry matter ha^{-1}) showed that Phacelia, summer vetch and winter vetch did not establish well, whereas all other CCs showed reasonable aboveground yields (Figure 18).

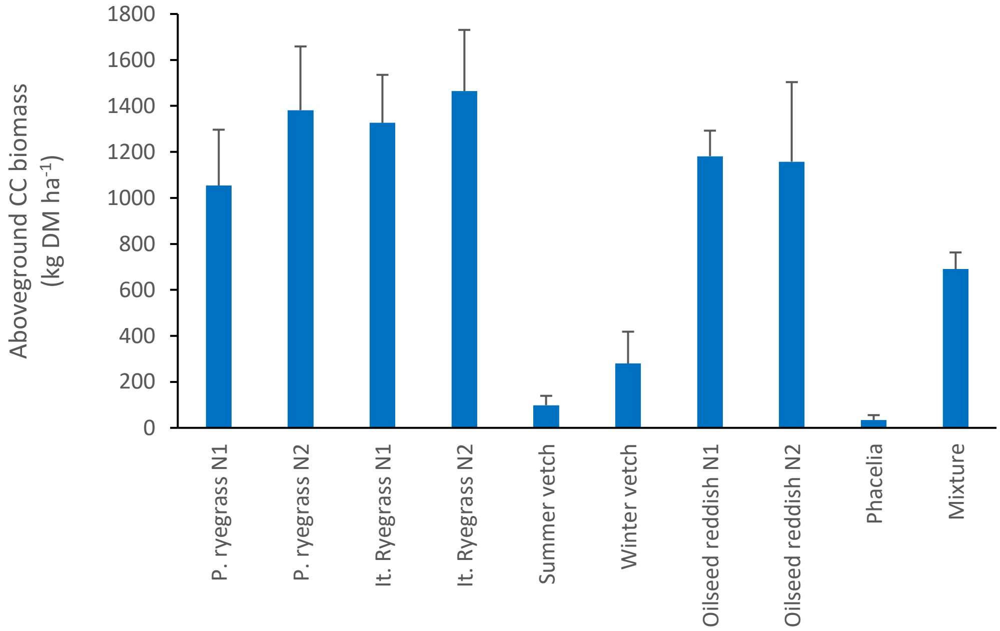

Figure 18. Standing aboveground biomass of cover crops.

Figure 19 shows the C/N ratios of the aboveground biomass. As expected the legumes (summer vetch, winter vetch, Phacelia) and the oilseed radish had lower C/N ratios as compared to Italian ryegrass, perennial ryegrass and mixture.

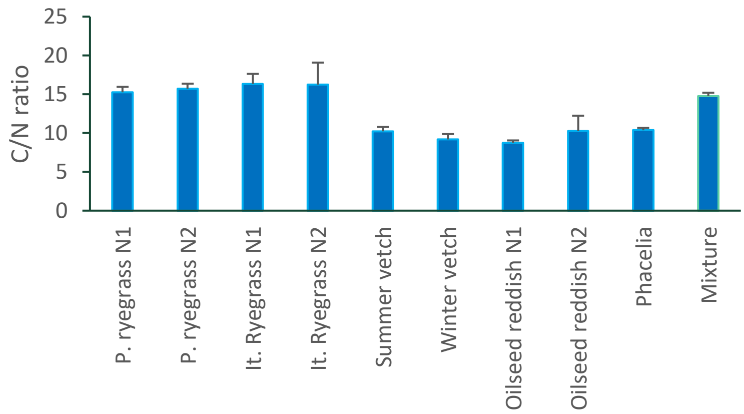

Figure 19. Cover crop C/N ratios.

Figure 20 shows the grain yield of barley. There were no significant differences with treatments, suggesting that CCs grown in summer did not impose a yield penalty on the main crop.

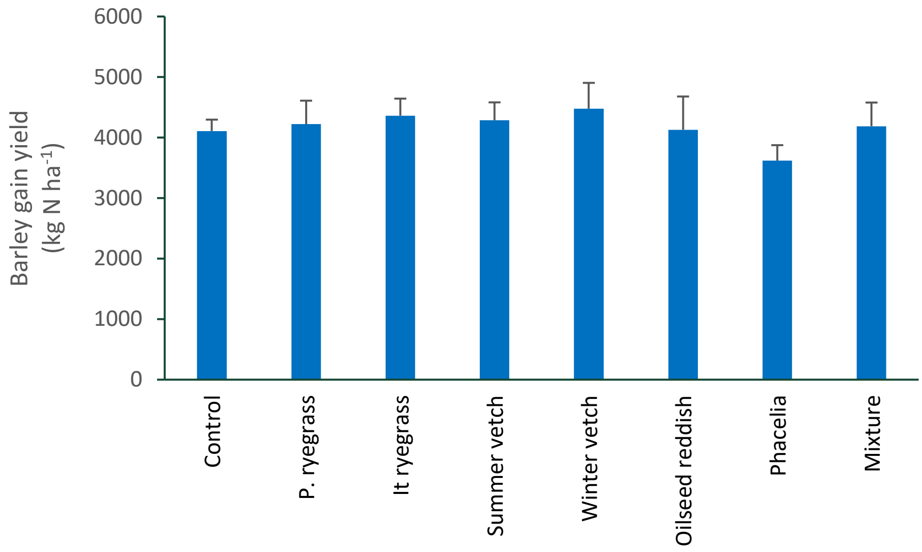

Figure 20. Barley grain yields.

3.6. Potential offset of C sequestration's climate effect by N_2O emissions

According to Poeplau et al. (2015), who studied long-term field trials with cover crops in Sweden, the mean C sequestration rate attributed to the use of perennial ryegrass as cover crop was 0.32 ± 0.28 Mg C ha^{-1} y^{-1}. The same mean C sequestration rate of 0.32 ± 0.28 Mg C ha^{-1} y^{-1} by use of CCs was reported by Poeplau and Don (2015) in a global meta-analysis, where they compiled data from 37 different sites to estimate the global potential of CCs for C sequestration. As the dataset in their meta-analysis was comprised of studies where winter CCs were not harvested, CCs were the only additional carbon inputs and included 27 different cover crop species (including legumes and non-legumes), this value was selected to evaluate the GHG trade-offs for this thesis. To evaluate the amount of additional N_2O emissions admissible before cancelling out the climate effect of this C sequestration, both C sequestration and N_2O emissions were converted to CO_2 equivalents (taking account for their respective GWPs) and the amount of N_2O equivalent to 320 kg C sequestration ha^{-1} y^{-1} was calculated:

Estimated C sequestration = 320 kg C ha^{-1} y^{-1}
Converting sequestered C into CO_2 taken up by the soil = 320 * 44/12 =1173 kg CO_2 ha^{-1} y^{-1}
Calculating equivalent N_2O emissions = 1173 / 298 (GWP of N_2O) = 3.93 kg N_2O ha^{-1} y^{-1}
Converting to kg N_2O-N ha^{-1} y^{-1} = 3.93 * 28/44 =2.50 kg N_2O-N ha^{-1} y^{-1}

This back-of-the-envelope calculation suggests that an extra emission of 2.5 kg N_2O-N ha^{-1} y^{-1} in any CC treatment would cancel out the expected cooling effect of enhanced C sequestration. Keeping this assumption in mind, calculations were done to see whether the change in N_2O emissions in CC treatments relative to the controls are high enough to cancel out the cooling effect by the expected C gain (Table 9). For this, cumulative N_2O emissions of the controls were subtracted from the CC treatments. Only the oilseed radish-treatment 10 exceeded the 2.5 kg extra N_2O-N and thus would offset the expected carbon gain, whereas all other CC treatments had a positive effect, i.e. did not offset the carbon gain, but much to the contrary, would support a GHG saving effect by reduced N_2O emissions.

Table 9. Calculations for assessing GHG tradeoffs.

Treat. No.	Name	Cumulative N_2O 356 days	Cumulative N_2O (CC - Control 1)	Cumulative N_2O (CC – Control N2)	Cumulative N_2O (CC - average controls)
1	Control	8.75			
2	Control (N2)	13.8			
3	Perennial ryegrass	2.6	-6.1	-11.1	-8.6
4	Perennial ryegrass (N2)	7.3	-1.4	-6.5	-4
5	Italian ryegrass	5.3	-3.4	-8.5	-6
6	Italian ryegrass (N2)	5.6	-3.3	-8.2	-5.7
7	Summer vetch	9.02	0.3	-4.8	-2.2
8	Winter vetch	10.7	1.9	-3.1	-0.6
9	Oilseed radish	10.5	1.7	-3.3	-0.8
10	Oilseed radish (N2)	14.2	5.4	0.4	2.9
11	Phacelia	5.5	-3.2	-8.3	-5.8
12	Mixture	3.5	-5.2	-10.3	-7.7

4. Discussion

From the results it can be implied that both the environmental factors and the attributes of the cover crops influence N_2O emissions. Generally, addition of cover crops in the cropping system was beneficial, as it did not affect the grain yield of the main crop and the daily N_2O fluxes for most of the CCs treatments were lower as compared to the control (without cover crops).

4.1. Seasonal effects

The seasonal effect was very prominent in both daily and cumulative N_2O emissions. Off-season cumulated N_2O emissions accounted for more than 50% of the total N_2O emissions (except for the mixture). The magnitude of these emissions was also higher when compared with the growing season emissions, i.e., cumulative emissions for growing season ranged between 1.41 – 4.4 kg N_2O-N ha^{-1} $period^{-1}$ whereas for the off-season they ranged between 1.34 – 12.7 kg N_2O-N ha^{-1} $period^{-1}$. These results are supported by multiple studies that also find higher off-season or non-growing season N_2O emissions as compared to the growing season emissions (Li et al. 2015; Thomas et al. 2017; Ejack & Whalen 2021; Ekwunife et al. 2022). The magnitude of off-season emissions highlights the significance and variability of winter N_2O emissions.

Emissions peaks were observed in two distinct off-season periods i.e. winter or freezing (off-season I) and spring thaw (off-season II). Although the microbial activity has been found to be reduced during the winter or frozen soil conditions, it is not completely inhibited which may lead to substantial formation and emissions of N_2O from the frozen soil (Ekwunife et al. 2022). When the soil is frozen, after initial microbial cell lysis, soil microorganisms become gradually acclimatized to the sub-zero soil temperatures (Maljanen et al. 2007; Smith et al. 2010). The microbial activity is further supported to some extent by the relatively warmer soil that is present under the snow cover (Ekwunife et al. 2022). These activities include decomposition of OM, and mineralization of organic N (Maljanen et al. 2007). N_2O produced under such circumstances remains trapped in the soil and eventually increase its concentration (Byers et al. 2021). Some of this trapped N_2O escapes the soil surface and is released into the atmosphere through cracks, when soil temperature increases slightly, leading to mild thawing of the soil (Teepe et al. 2001). According to Smith et al. (2010), denitrification is the main process causing N_2O production during this time because of their lower susceptibility to the colder temperatures as compared to nitrifiers.

In late winter or early spring, increasing soil temperature (especially during the day), leads to melting of ice and/or the creation of cracks in the soil surface layer, which causes release of the trapped gases. During spring thaw, when the soil temperature increases and ice melts, favourable conditions for nitrification and/or denitrification result into production of *de novo* N_2O. The magnitude of these biologically driven N_2O fluxes could be up to five times greater than the concentration of trapped N_2O (Risk et al. 2014). Denitrification is the principal source of N_2O formation during spring thaw (Van Groenigan et al. 2005). Increased soil temperature, leads to melting of ice/snow, which in turn increases the soil moisture content and enhances anerobic conditions (Pelster et al. 2013; Chen et al. 2020). Anaerobic conditions are also facilitated by the partly frozen subsoil, which reduces the soil drainage and increases soil saturation (Nyborg et al. 1997). Substrates required by denitrifiers are provided by the crop residual C and N and also from freeze and thaw stress inducing microbial death (Teepe et al. 2001; Chen et al. 2020). When the subsoil thaws it enhances soil drainage conditions (in the top soil) and creates aerobic conditions in the top soil, favouring N_2O production by nitrification (Ekwunife et al. 2022). A substantial amount of N_2O is produced and released in spring due to the diurnal freezing and thawing cycles.

In the growing season, fertilizer induced N_2O emission peaks were observed after the first fertilization. As N_2O production mainly depends on the availability of N in the soil (Akiyama et al. 2000), fertilizer application that adds additional N into the soil is a major driver of emissions during the growing season (Chen et al. 2008; Signor et al. 2013).

4.2. Treatment effects

Although high variability in N_2O emissions was observed within different CC treatments, the treatment effects were found to be significant in the daily N_2O fluxes. For cumulative N_2O emissions, during the growing season, most of the CCs reduced the nitrous oxide emissions. This is illustrated in Figure 14 where cumulated N_2O emissions from perennial ryegrass, Italian ryegrass and mixture were less than those in the controls during the peak fertilizer-induced N_2O emissions. This reflects the importance of having cover crops in the cropping system, in terms of reducing N_2O emissions during the growing season. CCs take up extra N / NO_3^{-1} from the soil and hence reduce soil N_2O production by limiting N availability (Liebig et al. 2015; Kim et al. 2015).

As compared to the growing season, higher N_2O emissions were emitted from all treatments in the off-season. Oilseed radish had the highest cumulative fluxes i.e., between 8.49 – 12.7 kg N_2O-N ha^{-1} $period^{-1}$ in the off-season (198 days) accounting for 89% of the total N_2O emissions (356 days). The results from aboveground biomass sample analysis showed that oilseed radish developed quite well (1100 kg DM ha^{-1}) in the experimental plots and had a low C/N ratio <10. Moreover, frost-killed raddish plants were also observed in the field during winter. Due to its deep, extended taproot, oilseed radish scavenges N easily from the soil profile and has a low C/N ratio (Gruver et al. 2014). However, it is highly susceptible to decompose due to its low winter hardiness. Olofsson and Ernfors (2022) measured N_2O emissions from experimental plots having oilseed radish, Phacelia and oats as CCs over a period of 43 days during winter in Southern Sweden. Along with N_2O emissions, they also analyzed the biochemical composition of the aboveground biomass of the respective CC species. They found that the soluble C content in oilseed radish was higher as compared to the other CCs, thus, releasing provide more labile C into the soil upon decomposition. Hence, in winter, oilseed radish decomposition provides both labile C and N in the soil which could fuel the N_2O emissions under anaerobic conditions (Thorup-Kristensen, 2006; Li et al. 2015; Petersen et al., 2011). Perennial ryegrass and the species mixture, on the other hand had the lowest emissions even in off-season. Mixtures, containing both legume and non-legume CCs, have the ability to fix atmospheric N_2 and are useful for recycling of soil residual nitrate, as they do not decompose easily (Tonitto et al. 2006). Perennial ryegrass is very frost tolerant, and efficiently reduces N leaching by taking up soil N (Thomsen, 2005).

Li et al. (2015) also reported highest cumulative N_2O emissions from fodder radish treatments (1158 g N_2O-N ha^{-1}) and lowest from perennial ryegrass (29 g N_2O-N ha^{-1}) in winter from an organic cropping system in Denmark. Thomas et al. (2017) reported high NO_3^- concentrations in soils with oilseed radish whereas the concentration of NO_3^- was lower in soils with perennial ryegrass. High availability of NO_3^- in soil can lead to increased N_2O emissions under anaerobic conditions as microbes use NO_3^- as an electron acceptor (Cho et al. 1997; Gillam et al., 2008). Although belowground biomass was not investigated in this thesis, high N_2O emissions from soils planted with oilseed radish have been reported to be influenced by its root biomass (Li et al. 2015; Olofsson and Ernfors 2022). Hence, in addition to the decomposition of aboveground biomass, the nutrient rich root biomass that is already present in the soil can be an additional source of substrates for microbes for N_2O production. The importance of root biomass was highlighted by Li et al.

(2015), where the harvested oilseed radish plots emitted more N_2O emissions as compared to the unharvested plots. These emissions were attributed to the large amount of root biomass of oilseed radish present near the soil surface. Being less winter hardy, the roots of oilseed radish might be more susceptible to degradation and provide mineral N during the freeze and thaw cycles for N_2O production. Olofsson and Ernfors (2022) reported that among oilseed radish, Phacelia and oats, oilseed radish had the highest fraction of soluble C compounds in plant tissues.

4.3. CC carbon sequestration & N_2O emissions

An increase in soil C sequestration by the use of CCs is reported in multiple studies (Eagle et al. 2012; Lal 2015; Kaye and Quemada 2017). According to Poeplau and Don (2015), the estimated amount of mean C sequestration by use of cover crops was 0.32 ± 0.28 Mg C ha^{-1} y^{-1}. The corresponding equivalent climate impact of this C sequestration rate is 2.50 kg N_2O-N ha^{-1}. Thus, the oilseed radish treatment in our study negated the benefit of carbon sequestration, as the N_2O emissions in this treatment exceeded from those in the control more than 2.50 kg N_2O-N ha^{-1}. Nitrous oxide emissions in the other cover crops treatments did not offset the carbon gain or added additional climate change mitigation effect compared with the control. Hence, choosing the right type of CC for the system is important as the trade-off between C sequestration and N_2O emissions depends on the type of cover crop species and the climatic conditions. Additional N_2O emissions from CCs such as oilseed radish can reduce or adversely influence the GHG mitigation potential of CC achieved by C sequestration. As the majority of these emissions were emitted in off-season, winter emissions from CCs should be given special attention and measured robustly to account for GHG trade-offs and sustainable agricultural practices.

4.4. Limitations and outlook

Large spatial and temporal variability was observed in the N_2O fluxes, which makes it difficult to understand the dynamics of N_2O, especially through the statistical analysis. Having more replicates per treatment and data for more than one year may provide a better understanding about the sources of this high variability. Data of another year (or even more years) can also help determining the inter annual variation of N_2O fluxes due to changes in temperature and precipitation, especially during winter.

Although the effect of fertilizer additions was not analyzed in this study, there was a trend of higher N_2O emissions from N2 treatments. Therefore, it would be interesting to analyze this effect in the future studies.

Due to high variability in daily fluxes, as illustrated in Figure 17, cumulative N_2O emissions, especially those for the winter period, may result in overestimation due to data aggregation. As the freeze and thaw cycles result in extremely high but short episodic N_2O fluxes, the chances of overestimation by accumulating data are higher in winter then underestimation. Therefore, the results from this study may not be comparable with other studies as the magnitude of N_2O emissions may have been too high. Hence, simple accumulation and/or use of daily mean N_2O fluxes might not be the best way to deal with N_2O data especially for Norwegian winters, where effects of freeze thaw cycles are quite pronounced. One method to deal with such skewed data in order to obtain more accurate flux estimates could be to use geometric means (GM) when scaling flux data for a year. Figure 21 shows a comparison between N_2O emissions calculated using accumulation of arithmetic mean values and geometric means. It can be seen that estimates given by the geometric means are lower as they avoid overestimation caused by aggregating data.

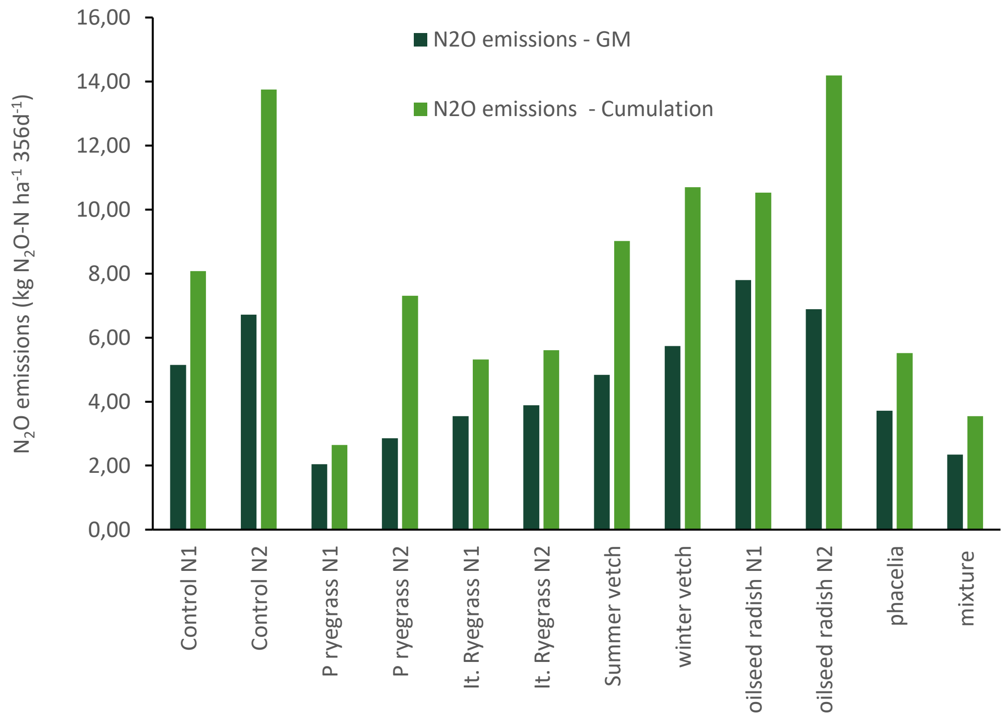

Figure 21. Comparison of N_2O emissions calculated for 356 days by accumulation of arithmetic daily means and using geometric means.

It is difficult to estimate the systematic difference between N_2O measurements taken by manual chambers and FFR. Both methods use closed chambers. The FFR was used more frequently on the field as compared to the manual chambers. Even with its short deployment time (3 mins) there is a chance that it measures extremely high fluxes due to its ability to measure at a frequency of 1Hz. Hence difference between the equipment could lead to additional uncertainty which was not easy to address within the framework of this thesis.

5. Conclusion

The following conclusions are drawn from this study:

1. Overall, CCs are a positive addition in cereal cropping as they significantly reduce N_2O emissions compared to the controls without CCs.
2. No negative effect of CCs was found on barley grain yield, which is important from a sustainable agricultural point of view.
3. N_2O emissions are highly variable, which makes it difficult to understand their dynamics.
4. Off-season emissions were significantly higher than those during the growing season and should be given special attention in the future studies.
5. Accumulation of daily arithmetic mean N_2O fluxes during winter may lead to overestimation of the N_2O emissions.

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Popular science summary

Agricultural soils contribute to a significant amount of anthropogenic nitrous oxide (N_2O) emissions. These emissions are regulated by factors such as C and N availability, soil moisture, and mineral N content affected by fertilizer application. Various studies report strong seasonal patterns of N_2O emissions i.e., high N_2O emissions during the off-season (winter and early spring). Cover crops (CCs) are crops mainly used to reduce N leaching and avoid soil erosion from bare arable soils. They are also known to increase soil organic carbon (SOC) stocks overtime as they assimilate carbon. Due to these advantages, CCs have been proposed to be included in Norwegian cereal production. However, organic N stored in cover crops can be released during freeze and thaw cycles in winter and spring, which fuels extensive winter emissions. Even though high emissions (30-90%) are produced during the off-season, only a few studies report winter N_2O emissions from soils containing cover crops. The aim of this thesis was to evaluate the effect of different cover crop types on N_2O emissions (especially during the off-season) in a Norwegian field cropped with spring barley. Furthermore, the potential trade-offs between carbon assimilation by CCs and N_2O emissions were also assessed in this study. N_2O emissions from 12 treatments, consisting of five different cover crops species (grasses, legumes, brassicas, non-leguminous herbs, a herb mixture) and two controls were measured in an experimental field between May 2021- April 2022. These fluxes were then analysed to assess the seasonal treatment effects of CCs. The results showed that N_2O emissions differed between treatments. Ryegrass (Italian and perennial) and the herb mixture reduced N_2O emissions, particularly during winter, whereas oilseed radish increased N_2O emissions. High N_2O emissions from the oilseed radish plots can be attributed to its lower winter hardiness, which makes this cover crop species susceptible to decompose easily in winter. Due to its low C/N ratio, the biomass of oilseed radish provides easily accessible C and N that are required for N_2O formation. The seasonal patterns of N_2O emissions were very prominent with off-season N_2O emissions accounting for more than 80% of the total measured N_2O emissions. During the off-season, trapped N_2O is released from the soil surface through cracks. Along with this, formation of *de novo* N_2O is favoured by freeze and thaw cycles during winter and spring. C sequestration rate by CCs was assumed to be 320 kg C ha^{-1} yr^{-1} in this study. The corresponding equivalent climate impact of this sequestration rate was calculated

as 2.50 kg N_2O-N ha^{-1}. Among all CCs, only the oilseed radish treatments offset the expected C gain as the N_2O emissions from these treatments were higher than 2.50 kg N_2O-N ha^{-1} y^{-1}. Hence, choosing the right type of cover crops is very important to avoid high N_2O emissions under Nordic climatic conditions. As the major part of the N_2O was released outside the growing season of the main crop, this study emphasizes the need for measurements during several winter emissions.

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