Emissions of nitrous oxide associated with frost-killed cover crops

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Abstract

Agriculture is responsible for approximately 80% of anthropogenic emissions of the potent and long-lived greenhouse gas nitrous oxide (N_2O). N_2O emissions from agricultural soils are characterized by high temporal and spatial variability and often peak in short bursts related to events such as precipitation, fertilization and soil thawing. Frost-sensitive cover crops (CCs) could potentially enhance N_2O emissions during winter. This thesis work investigated the thaw-related N_2O fluxes induced by three different cover crops (oilseed radish, (*Raphanus sativus*), phacelia (*Phacelia tanacetifolia*) and oats (*Avena* sativa)) by field measurements during a six-week winter period when the CCs were successively terminated by low temperatures. It was hypothesized that the magnitude of the emissions would be related to the content of nitrogen (N) and soluble carbon (C) components in the CC aboveground biomass, as these are substrates for denitrifying soil bacteria. Results showed that oilseed radish plots had the highest cumulative emissions of N_2O during the study period, at 3.3 kg ha^{-1}, whereas phacelia and oat plots each emitted 1.4 kg ha^{-1}, all significantly higher compared to control plots without CCs. The content of N and soluble C components in aboveground biomass could not fully explain the emissions of N_2O. However, it is possible that belowground biomass was important, or that other qualities of the biomass were influential in governing thaw-related N_2O flux.

Keywords: Nitrous oxide, cover crops, greenhouse gases, freeze-thaw cycles

Table of contents

List of tables

List of figures

Abbreviations

AFOLU	Agriculture, forestry and other land use
C	Carbon
CH_4	Methane
CO_2	Carbon dioxide
CO_2-eq.	Carbon dioxide equivalents
FTC	Freeze-thaw cycles
GHG	Greenhouse gas
GWP100	Global warming potential on a 100-year timescale
IPCC	Intergovernmental Panel on Climate Change
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
NO	Nitric oxide
NO_3^-	Nitrate
N_2O	Nitrous oxide
SE	Standard error
WFPS	Water-filled pore space

1. Introduction

The climate is changing and the agricultural sector is one of the important drivers. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), the three most important greenhouse gases (GHG), are together responsible for 85% of the radiative forcing caused by anthropogenic emissions (IPCC, 2013), but the latter two are more important in an agricultural context. More specifically, Agriculture, Forestry and Other Land use (AFOLU) make up 13% of CO_2 emissions, 44% of CH_4 emissions, and 82% of N_2O emissions (Jia *et al.*, 2019). Carbon sequestration is suggested as a method for mitigating the climate impact of agriculture, and growing cover crops (CCs) is one promising tool for accomplishing this. However, when CCs die and add plant material to the soil, there is a risk of increased emissions of other GHG, especially those of N_2O.

N_2O is a long-lived and potent GHG with a global warming potential on a 100-year timescale (GWP100) corresponding to 298 CO_2-equivalents (CO_2-eq.) (Myhre *et al.*, 2013). N_2O is currently also the most important substance emitted that causes stratospheric ozone depletion (Ravishankara *et al.*, 2009). The atmospheric concentration of N_2O has risen by 20% since pre-industrial times and continues to increase (Ciais *et al.*, 2013). Agriculture is almost solely responsible for the 82% of anthropogenic emissions caused by AFOLU (Jia *et al.*, 2019), and it is likely that these emissions will increase in the future due to a predicted increase in global demand for food (Ciais *et al.*, 2013). Nitrogen (N) losses in the form of N_2O from agricultural land are commonly calculated as 1% of applied N (Venterea *et al.*, 2012) but, in reality, several interacting factors govern N_2O emissions and understanding how these are affected by management practices offers great mitigation potential.

1.1. N_2O production and regulation

Several processes produce N_2O (Butterbach-Bahl *et al.*, 2013), but nitrification and denitrification are the two most important ones, together responsible for more than 80% of the total global N_2O emissions (Fowler *et al.*, 2015). Both processes can occur in terrestrial and aquatic systems, but it is well-established that soils are the

dominant source of atmospheric N_2O (Butterbach-Bahl *et al.*, 2013). In *nitrification,* ammonium (NH_4^+) is oxidized to nitrate (NO_3^-) and in the process, N_2O and nitric oxide (NO) are produced as by-products (Gregorich *et al.*, 2015). NO_3^- formed in nitrification can then be reduced to N_2 in *denitrification*, where N_2O and NO are produced as intermediates. In wet climates, such as in Sweden, denitrification is the main process responsible for N_2O emissions (Henriksson *et al.*, 2015)

There are three fundamental factors that are required for denitrification to occur (Phillips, 2008). Firstly, denitrification requires oxygen-limited conditions. Soil water content, which strongly influences the possibility of oxygen to diffuse through soil, is therefore an important factor controlling denitrification rates (Firestone and Davidson, 1989). Castellano *et al.* (2010) found that N_2O production from denitrification is optimal at a rather wide range of soil water-filled pore space (WFPS) of 63-98%, depending on landscape position and soil properties. Secondly, denitrification requires electron donors in the form of organic C, and thirdly, electron acceptors in the form of NO_3^- (Phillips, 2008). The three requirements are in turn influenced by factors such as soil properties, climate and management practices, making N_2O emissions complex and difficult to predict (Henriksson *et al.*, 2015)

1.2. Freeze-thaw cycles

Emissions of N_2O are characterised by a large spatial and temporal variability. They peak in short 'bursts' and these are often related to events such as fertilization, precipitation, or soil thawing (Henriksson *et al.*, 2015). A significant part of annual emissions occur during freeze-thaw cycles (FTC) in winter and spring, and a Canadian five-year study of N_2O emissions concluded that non-growing season emissions comprised 30-90% of the annual emissions, and that these were tightly linked to soil thawing (Wagner-Riddle *et al.*, 2007). Why emissions peak when soils thaw is not entirely clear, although several mechanisms have been suggested. A review by Risk *et al*. (2013) summarizes that earlier studies often argue that thawing simply releases previously produced N_2O trapped under snow and frozen soil, but that more recent studies point to the importance of *de novo* production as well.

FTC can also affect fluxes of CH_4 although this is less studied than for N_2O and large uncertainties concerning the flux response at FTC remain. However, in comparison to N_2O, the thaw-related response of CH_4 is generally smaller and of less importance in terms of the climate impact (Kim *et al.*, 2012)

1.3. Cover crops and N_2O

Cover crops (CCs) have received increased attention due to their potential to mitigate climate change by sequestering C in agricultural soils (Chahal *et al.*, 2020). There is, however, no consensus in the literature regarding the effect that CCs have on N_2O emissions (Basche *et al.*, 2014), and few studies have been conducted on this topic. Mørkved *et al.* (2006) argue that frost-sensitive cover crops could stimulate N_2O emissions as their decayed plant material becomes available to soil microorganisms when spring meltwater infiltrates the soil. Li *et al.* (2015) argue similarly, that frost-killed CCs may contribute significantly to C and N substrate availability for denitrifying bacteria with their decomposing above- and belowground biomass. However, research conducted on cover crops and N_2O emissions often neglect winter emissions (Singh and Kumar, 2021), lack comparisons of several different cover crops (Foltz *et al.*, 2021) or risk missing emission peaks due to sparse measurements (Liebig *et al.*, 2010; Petersen *et al.*, 2011). There is therefore a major knowledge gap concerning winter emissions of N_2O induced by CCs, especially with regard to varying winter-hardiness.

Both the quantity and the quality of the frost-killed CC plant material could influence denitrification. Essich *et al.* (2020) concluded that the carbon to nitrogen ratio (C/N) of crop residues was a major determinant of N_2O emissions after harvest, where crop residues with lower C/N ratios induced higher N_2O emissions compared to crop residues with high C/N ratios. The same conclusion was drawn by Huang *et al.* (2004) in a study of N_2O emissions after incorporation of plant residues with different C/N ratios. In soils with high concentrations of NO_3^-, it is possible that denitrification instead is limited by the supply of labile C (Mitchell *et al.*, 2013). Large fractions of labile C in frost-killed decomposing CC biomass could therefore potentially also enhance N_2O emissions. Senbayram *et al*. (2012) found that application of organic fertilizers containing high amounts of labile C induced higher denitrification rates compared to application of organic fertilizers containing more recalcitrant C.

1.4. Aim and hypothesis

The aim of this thesis was to contribute to agricultural GHG mitigation by identifying CCs that generate high N_2O emissions during winter. The aim was addressed by testing the following hypothesis:

- The magnitude of N_2O emissions induced by frost-killed CCs will be related to the total N and soluble C contents of the CC biomass.

2. Methodology

2.1. Experimental setup

The field work for this thesis was conducted at the SITES Lönnstorp Research Station located in Scania, Southern Sweden. The soil type at the studied field was a loam with 22% clay and 3% organic material (Hansson *et al.*, 2021). Measurements and sampling were performed in plots sown with cover crops for the research project "Effective weed control and increased carbon sequestration through strip-till establishment of field crops in withered cover crops" (referred to as the strip-till project from here on) (Figure 1). In the strip-till project, five frost-sensitive CCs were sown on August 23rd, 2020, in pure stands or mixtures, in a randomized block design with four blocks. Each treatment was represented by one plot measuring 6x15 m within each block. Of the five CCs in the strip-till project, four were sown in pure stands but one (buckwheat) had already been frost-killed by December 2020 (approximately a month before the start of this project) and were therefore not included in this study. The three CCs included were oilseed radish (*Raphanus sativus*; OSR), phacelia (*Phacelia tanacetifolia*; Ph) and oats (*Avena sativa*; O). Ploughed plots with bare soil (no sown CC) were used as control. In total, 16 plots were included in this project, four for each treatment, including controls.

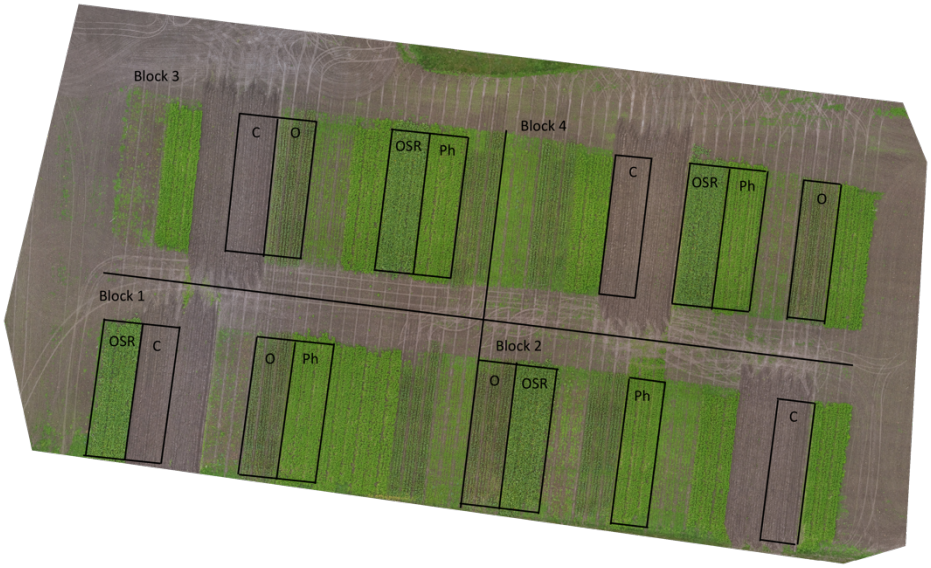

Figure 1. Overview of the experimental setup of the strip-till project at the SITES Lönnstorp Research Station. The black lines and boxes show the four blocks and the 16 plots included in this study. Photo by Ryan Davidson, adaptation by Felicia Olofsson.

Two stainless-steel collars were installed in each included plot on the 8th of January and left in the ground for the entire study period. The collars, 32 in total, were inserted to a depth of 20 cm so that the bottom of the channels at the top of the collars as far as possible were level with the soil surface. Due to uneven soil surfaces, this varied to some extent. The collars were placed in spots with vegetation representative for the plot and not close to plot edges or in tractor tracks. To avoid damaging the plants, all biomass belonging to plants growing within the collars was carefully placed on the inside of the collar while biomass from plants growing on the outside of the collar was moved to the outside. Figures 2-5 display four installed collars, one for each treatment, on the day of installation.

Figure 2. Newly installed collar on the 8th of January in a plot with oilseed radish (OSR).

Figure 3. Newly installed collar on the 8th of January in a plot with phacelia (Ph).

Figure 4. Newly installed collar on the 8th of January in a plot with oats (O).

Figure 5. Newly installed collar on the 8th of January in a control (C) plot.

2.2. Gas measurements

Gas flux was measured on 13 occasions, from January 9th to February 21st, 2021. Measurements were performed approximately twice a week, but dates were continually adjusted depending on weather conditions. For example, no measurements were conducted under a cold period of approximately two weeks when the ground was frozen and N_2O emissions presumably low, whereas three measurements in four days were conducted after this cold period in an attempt to catch a potential emission peak at soil thawing.

Non-steady state (closed) multi-component gas chambers were used (Livingston and Hutchinson, 1995). The chambers were sealed with water poured into the collar channels into which the chambers thereafter were placed. If there was ice in the channels, this was removed, if possible, otherwise new water was poured on top of the ice. Before placing the chambers in the water-filled channels, each chamber was ventilated by removing the silicone stopper and swinging the chamber from side to side three times. The chambers were then carefully placed in the collar channel to avoid pressure changes that could force gases from the soil. Samples were collected from each chamber using a pump that circulated the air through 6 ml glass vials (Exetainer ®, Labco, UK) for 1 minute. Two samples from each chamber were collected, the first sample (T1) 1 minute after the chamber was placed in the collar channel, and the second sample (T60) 60 minutes after the first. Measurements were performed in two rounds of approximately two hours each. The 16 plots of block 1 and 2 were sampled first and the 16 plots of block 3 and 4 thereafter. This means that at almost all occasions, measurements in block 1 and 2 were performed just before noon whereas measurements in block 3 and 4 were performed after noon. Finally, samples were analysed on a gas chromatograph (HP7890A, Agilent, Wilmington, USA) to determine concentrations of primarily N_2O, but also CO_2 and CH_4.

The volume of the measurement system, i.e. the air enclosed within the chambers once placed in the collars, is needed to calculate the gas flux. Since the depth from the collar top to the soil surface varied between collars, the volume had to be determined for each individual measurement system. To achieve this, nine measurements of the depth from the collar top to the soil surface were conducted in each collar to calculate a mean depth that in turn was used in system volume calculations. These measurements were conducted on the 16th of March. Similarly, if chamber height was affected by ice in the collar channels, this was adjusted for in the system volume calculations.

2.3. Soil water content and soil temperature

Soil volumetric water content of the top 12 cm of soil was measured using a TDR soil moisture meter (Fieldscout TDR 300, Specmeters, Aurora, USA) and soil temperature was measured using a hand-held probe thermometer. Both measurements were made, within a distance of 10-30 cm from each collar, at every gas measurement date, unless the soil was frozen. Temperature measurements were not conducted at the first measurement date due to faulty equipment.

2.4. Biomass sampling

Samples for determining dry-weight biomass, C/N ratio and fractions of C compounds in the plant tissues were collected on January 13th, 2021. All sample areas were chosen to be representative of the vegetation in the plot and were located at least 50 cm from plot borders. Two samples were collected, one to determine dry-weight biomass and one to analyse C/N ratio and C compound fractions.

To determine biomass dry-weight, all cover crop plant material from 1 m^2, both living plant tissues and plant residues at the soil surface, was collected from each cover crop plot in each block (16 samples in total). The samples were washed by hand to remove soil, dried at 70°C for two days and weighed.

A second biomass sample was taken from each of the 16 plots for analysis of the C/N ratio and fractions of C compounds in the cover crop plant tissues. These samples were taken from 1 m^2 of each OSR plot, whereas a larger sample area of 1,75 m^2 was needed from the O and Ph plots to ensure sufficiently large biomass samples for the analyses. To avoid soil contamination, biomass was cut five cm above the ground in the sample areas. Plant residues on the soil surface were not collected. The biomass samples were dried at 70°C for two days. After drying, two samples (the O samples from block 3 and 4) did not reach the minimum weight of approximately 55 g needed for both analyses and had to be complemented with a few grams of plant material from the samples analysed for dry-weight biomass. A representative subsample of a few grams was collected for analysis of total N and total C, whereas the main part of each sample was analysed for C compound fractions by the van Soest method (Goering and Van Soest, 1970; AFNOR, 2013). Each subsample was cut into pieces of about 1-2 cm and a 3-4 g subsample of these plant clippings was ground to a fine powder in a knife mill. 5.0 (±0.5) mg of the powder was weighed into a tin capsule and analysed for total N and total C content on an elemental analyser (Flash 2000, Thermo Scientific, Bremen, Germany).

2.5. Soil sampling

Two different soil samples were collected from each plot on the 26th of January, one to determine bulk density and one to analyse soil mineral N concentration. Sample areas were located at least 50 cm from plot borders.

For bulk density, stainless steel cylinders with a volume of 400 cm^3 were used to collect undisturbed soil samples from the top 10 cm of soil. The samples were dried at 105° overnight and then weighed to determine bulk density. For soil mineral N concentration, soil samples were collected from the top 10 cm of the soil, immediately frozen and later analysed for NO_3^- and NH_4^+ content (ADAS method 53; Eurofins Food and Agri Sweden AB, Kristianstad, Sweden).

2.6. Meteorological data

Meteorological data were obtained from a weather station at the SITES Lönnstorp Research Station (LantMet, no date). An hourly mean of temperature and atmospheric pressure was selected based on the time of measurements and used to calculate gas flux. Data on daily means of precipitation and air temperature were used for comparison with the measurements of soil water content and soil temperature, respectively.

2.7. Calculations and statistics

Cumulative emissions for N_2O, CO_2 and CH_4 were calculated by linear interpolation of emission values between sampling dates, for each of the 16 plots. Mean cumulative emissions of N_2O and CH_4 for each treatment were also converted into CO_2-eq. using GWP100 from Myhre et al. (2013) to compare the climate impact of the respective gas. Cumulative emissions of CO_2 were not used in the same way as N_2O and CH_4 because the chosen measurement method was adjusted for gases with lower flux rates and the results for CO_2 should therefore only be considered as approximate. Instead, it was used as a proxy for soil respiration, indicating relative decomposition rates in the CC plots. Weighted means were calculated for soil temperature and WFPS. Since soil temperature and WFPS lacked data for several of the measurement days, these were gapfilled where appropriate (one data point for WFPS and two data points for soil temperature) before weighting and calculating means. Data from another, temporally close (1-2 days) measurement day was used to gapfill.

The mean cumulative emission values of N_2O and CH_4 and the mean values for the *soil variables* (soil temperature, WFPS, CO_2 flux, and soil NO_3^-) and the *crop variables* (dry-weight biomass, C/N ratio, N in biomass per m^2, soluble components, and soluble components in biomass per m^2) were compared between treatments using a univariate general linear model and post hoc Tukey test in the statistical program SPSS. All data was checked for normality and homoscedasticity of the residuals. Since controls lacked data for some variables (i.e. those related to biomass), they were excluded from the analyses of crop and soil variables.

3. Results

3.1. Crop variables

Mean values for all crop variables are presented in Table 1. O plots produced less biomass in comparison with OSR ($p=0.002$ and Ph ($p=0.003$) plots while there was no significant difference in biomass between OSR and Ph ($p=0.964$). O biomass had higher C/N ratios in comparison with OSR ($p=0.001$) and Ph ($p=0.001$) while there was no significant difference between OSR and Ph ($p=1.000$). O had less N in biomass per m^2 in comparison with both OSR ($p<0.001$) and Ph ($p=0.001$) while there was no significant difference between OSR and Ph ($p=0.770$). OSR biomass had higher shares of soluble components compared to Ph ($p=0.008$) and O ($p<0.001$) biomass (Figure 6) In comparison to O, Ph biomass had higher fractions of soluble components ($p=0.011$). O had less soluble components in biomass per m^2 compared to OSR ($p<0.001$) and Ph ($p=0.001$). There was a tendency of more soluble components in biomass for OSR in comparison with Ph ($p=0.059$).

3.2. Soil variables

Mean values for all soil variables are presented in Table 1. OSR plots had higher mean soil temperature in comparison with Ph ($p=0.046$) and O ($p=0.008$) plots while there was no significant difference in the comparison of Ph and O ($p=0.321$) (Figure 8, graph C). There were no significant differences in mean WFPS (Figure 8, graph D), soil NO_3-N or cumulative emissions of CO_2-C between the CC treatments ($p=0.782$, $p=0.336$ and $p=0.103$, respectively).

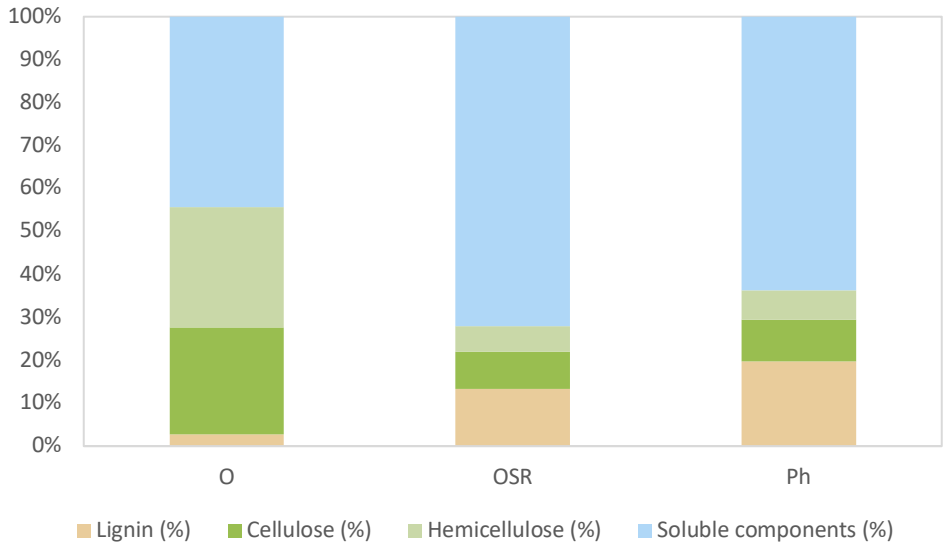

Figure 6. Fractions of carbon compounds in ash-free cover crop biomass. OSR=oilseed radish, Ph=phacelia, and O=oats.

Table 1. Mean values (and standard errors) of the crop variables dry-weight biomass, C/N ratio, N in biomass, soluble components, and soluble components in biomass, as well as the soil variables soil temperature, water-filled pore space (WFPS), NO_3-N, and CO_2-C flux, for all treatments. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different.

Crop and soil variables	**C**	**OSR**	**Ph**	**O**
Dry-weight biomass (g m^{-2})	n/a	127 (11)^{a}	124 (8)^{a}	62 (9)^{b}
C/N ratio	n/a	7 (0.5)^{a}	7 (0.2)^{a}	14 (1.0)^{b}
N in biomass (g m^{-2})	n/a	6.6 (0.6)^{a}	6.2 (0.7)^{a}	1.9 (0.4)^{b}
Fraction of soluble components (%)	n/a	59.1 (0.6)^{a}	47.4 (1.3)^{b}	36.7 (2.1)^{c}
Soluble components in biomass (g m^{-2})	n/a	75 (6)^{a}	59 (4)^{a}	23 (2)^{b}
Soil temperature (°C)	0.43 (0.04)	0.49 (0.02)^{a}	0.44 (0.03)^{b}	0.42 (0.01)^{b}
WFPS (%)	62 (2)	72 (3)^{a}	69 (2)^{a}	69 (2)^{a}
NO_3-N (mg kg^{-1} dry matter)	5.1 (0.4)	7.8 (1.2)^{a}	8.4 (1.9)^{a}	5.4 (0.4)^{a}
CO_2-C (kg ha^{-1})	127 (7)	916 (94)^{a}	828 (48)^{a}	689 (33)^{a}

3.3. N_2O

Emissions of N_2O were low in the beginning of the study period but increased gradually and reached the highest levels in the end of the study period (Figure 12, A). The mean cumulative emissions of N_2O-N (Figure 7) were, from highest to lowest, 3335 g ha^{-1} $43d^{-1}$ (standard error (SE) 292), 1448 g ha^{-1} $43d^{-1}$ (SE 227), 1422 g ha^{-1} $43d^{-1}$ (SE 113), and 489 g ha^{-1} $43d^{-1}$ (SE 115) for OSR, O, Ph and C, respectively. Mean cumulative N_2O-N emissions from OSR plots were higher compared to plots with O ($p<0.001$) and Ph ($p<0.001$). Mean cumulative N_2O-N emissions from C plots were lower compared to all CC treatments ($p<0.001$ in each comparison). When converted into CO_2-eq., the mean cumulative emissions of N_2O-N corresponded to 146, 424, 432, and 994 kg CO_2-C ha^{-1} for C, Ph, O, and OSR, respectively.

3.4. CH_4

CH_4 flux varied between low emissions or slight uptake during the study period (Figure 8, graph B). The mean cumulative emissions of CH_4-C were 76 g ha^{-1} (SE 24), 46 g ha^{-1} (SE 22), and 24 g ha^{-1} (SE 10) for C, OSR, and Ph, respectively, whereas O had a mean cumulative uptake of 9 g CH_4-C ha^{-1} (SE 41). There were no differences in mean emissions/uptake in comparisons of the different treatments ($p=0.220$). When converted into CO_2-eq., the mean cumulative emissions/uptake of CH_4 corresponded to emissions of 2.6, 1.6, and 0.8 kg CO_2-C h^{-1} for C, OSR, and Ph, respectively, and an uptake of 0.3 kg CO_2-C h^{-1} for O.

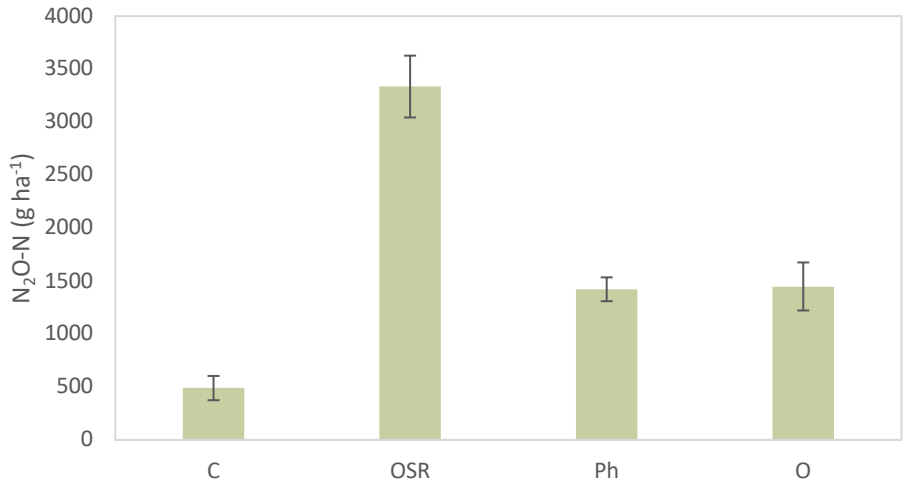

Figure 7. Mean cumulative emissions of N_2O-N for each treatment during the full study period. Error bars represent standard error. OSR=oilseed radish, Ph= phacelia, O=oats, and C=control.

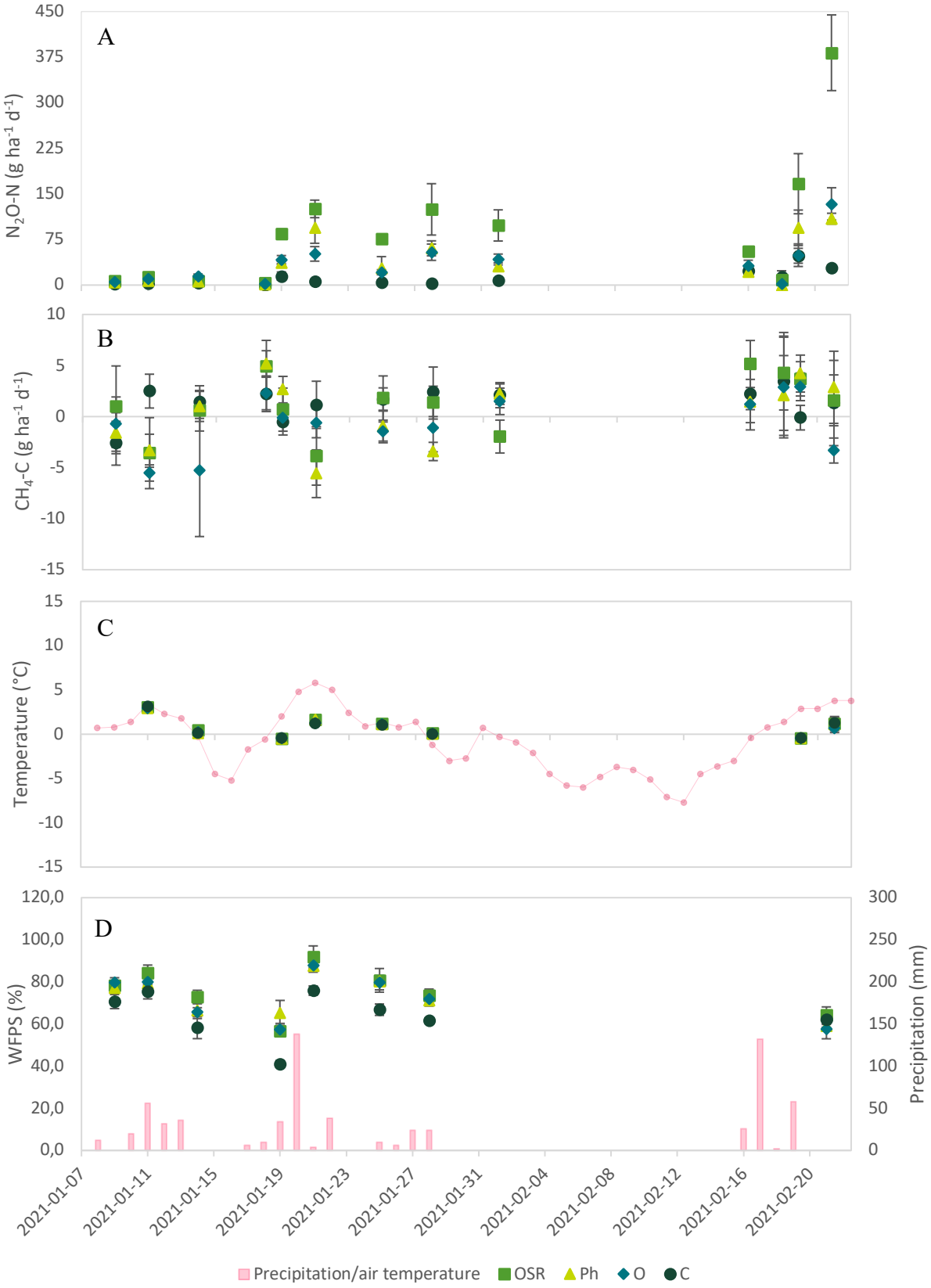

Figure 8. A: Emissions of N_2O-N (g h^{-} d^{-1}). B: Emissions of CH_4-C (g ha^{-1} d^{-1}). C: The dotted line represents air temperature (°C) and the differently shaped and colored treatment markers represent soil temperature (°C). D: Water-filled pore space (WFPS) (%,) displayed as differently shaped and colored treatment markers (left y axis) and precipitation (mm) displayed as bars (right y axis). All error bars represent standard error. OSR=oilseed radish, Ph= phacelia, O=oats, and C=control.

4. Discussion

4.1. Emissions of N_2O and CH_4

The primary goal of this study was to quantify and compare emissions of N_2O induced by three frost-sensitive CCs during the part of winter when they were successively terminated by frost. The results show that OSR induced higher emissions compared to Ph and O and, furthermore, that these emissions were very high for such a limited time. Mean accumulated emissions of N_2O from OSR plots during the six-week study period were 3.3 kg ha^{-1} $43d^{-1}$. A publication by the Swedish advisory organization Hushållningssällskapet summarizes studies that have measured N_2O emissions from agricultural land in Northern Europe and concludes that *annual* emissions normally range between 0-10 kg N_2O-N ha^{-1}, although estimations are difficult due to the high variability (Henriksson *et al.*, 2015). Worth noting is that most of the included studies reported mean annual emissions that were much lower than 10 kg ha^{-1} and yr^{-1}.

The highest peak in emissions recorded in this thesis work was the mean OSR flux on the last measurement day, which amounted to 0.38 kg N2O-N ha-1 d-1. In comparison, Dörsch (2000) measured peaks of 0.16 kg N2O-N ha-1 d-1 from fields with autumn-sown, frost-killed OSR in connection to soil thawing in Southern Germany. The winter before, frost-killed leguminous CCs and a field mulched with an autumn-sown mustard CC induced thaw-related emission peaks of 0.48 and 0.65 kg N2O-N ha-1 d-1, respectively (Dörsch, 2000). Hence, the emission peaks recorded in this thesis work are within the range of what others have measured. Whether or not the peak observed on the last day of measurements had already reached its maximum, or if it would continue to rise or decrease quickly or gradually, is impossible to say. A review by Kim et al. (2012) summarized studies showing N_2O flux responses from a few days up to a month after thawing. N_2O emissions have also been shown to correlate to the intensity of freezing (Risk *et al.*, 2013). The longer and colder the period before thawing, the higher the thaw-related N_2O flux tend to be. This is in agreement with the results in the present study. A short cold period that briefly froze the uppermost layers of the soil did not result in as high emissions as did the longer cold

period of approximately two weeks (Figure 8, graphs A and C). Since this two-week cold period was one of the major ones during that winter, the thaw-related N_2O flux was likely one of the main peaks of the season.

Due to the longer period of cold weather during the two first weeks of February, measurement dates were separated into two groups, one group before (9 occasions) and one group after (4 occasions) said two-week period. Measurements were mainly concentrated to the periods just after thawing, based on the assumption that this correlated with emission peaks. Emissions were assumed to be lower during cold periods because the low temperatures would decrease N_2O production, and the frozen soil would limit the possibility of produced N_2O to diffuse into the atmosphere. The cold period was nonetheless included in calculations of accumulated flux during the full study period by assuming that a mean of the measured flux from the closest measurement occasion before (1st of Feb) and after (16th of Feb) the cold period would be representative for the days of the cold period. Thus, gas flux during this period may be either over- or underestimated. Combined field and laboratory trials conducted in Germany showed that N_2O can be both produced and emitted from frozen soil at rates higher than those during the cropping season, but lower than those during thaw events (Röver *et al.*, 1998). To assume zero emissions during the two-week cold period would therefore likely have underestimated the N_2O flux. Additionally, measured N_2O flux on 1st and 16th of February were neither two of the lowest nor highest measured rates in comparison with the other measurement occasions and were therefore considered suitable for making the estimation. Excluding the two-week cold period rendered similar relative results in accumulated N_2O emissions where OSR induced the highest flux while Ph and O induced less than OSR but more than C (results not presented).

Emissions or uptake of CH_4 had, in comparison to N_2O, low impact in terms of global warming potential. Three treatments (C, OSR and Ph) induced emissions of CH_4 over the 43-day study period, but all of these amounted to less than 2% of the corresponding N_2O emissions when converted to CO_2-eq. Similarly, the uptake of CH_4 observed for O was very low (0.06%) compared to the emitted N_2O from O plots expressed in CO2-eq. This is in line with results from a review by Kim *et al.* (2012), who concluded that the magnitude of the response to soil thawing normally is much smaller for CH_4 compared to other gases.

4.2. Crop variables influencing N_2O emissions

N_2O emissions from thawing soils has been shown to derive mainly from denitrification (Wagner-Riddle *et al.*, 2008) and CCs have the potential to enhance this through several mechanisms. The decomposing CC biomass can provide significant portions of substrate to denitrifying soil bacteria (Li *et al.*, 2015). Furthermore, the decomposing CC biomass could stimulate microbial activity that depletes soil oxygen and increases the anaerobic volume of the soil (Mørkved *et al.*, 2006). Indeed, results in a study of N-dynamics under frost-sensitive CCs showed that mineral N increased from December to mid-February, during the same time as the frost-sensitive CCs were successively terminated by cold temperatures (Storr *et al.*, 2020).

The results from this study enable us, firstly, to single out O as qualitatively different in comparison to OSR and Ph. O had less biomass that also contained less N and less soluble components per m^2. Decomposition of O biomass would therefore provide less NO_3^- and labile C, the substrates needed for denitrification (Phillips, 2008). Secondly, OSR had a higher fraction of soluble C components in plant tissues compared to both Ph and O. When combined with biomass production, OSR and Ph had more soluble components per m^2 compared to O and should therefore provide more soluble C to denitrifying bacteria. The ability of the CCs to provide N and soluble C as substrate to denitrifying bacteria through their decomposing plant tissues has been argued to be potentially important for winter emissions of N_2O (Li *et al.*, 2015), and was hypothesized to govern the magnitude of N_2O emissions. Had the hypothesis been correct, OSR and Ph would have induced the highest N_2O emissions and O the least. However, our results show that OSR induced the highest N_2O emissions (approx. 3.3 kg N_2O-N ha^{-1}) whereas Ph and O induced lower and similar N_2O emissions (approx. 1.4 kg N_2O-N ha^{-1} each). The hypothesis can therefore not be confirmed in full, and it is possible that other factors also contribute. This analysis assume that all soluble components are equally available to the soil denitrifying bacteria. However, research on N_2O emissions as affected by different carbon substrates showed that a high fraction of soluble compounds not necessarily induces high N_2O emissions (Senbayram *et al.*, 2012). Biogas residues, with a high proportion of *recalcitrant* soluble components, induced lower emissions compared to other substrates. It is possible that the availability of the soluble components differed somewhat between the different CC species. In comparison to OSR, Ph had a slightly higher fraction of lignin (Figure 6) that potentially could "protect" the more labile compounds in the plant tissues from degradation. Thus, less soluble components would be available to the denitrifying soil bacteria. Liu *et al.* (2014) associated greater "woodiness" of CCs with less frost-induced phosphorus leaching.

The analysis also assumes that similar proportions of produced biomass is made available to soil decomposers from each CC, which was probably not the case. Although all three CCs were frost-killed during the study period, they differed somewhat in their frost-hardiness and consequently, the time of biomass degradation and soil contact of the aboveground biomass. By visually observing the CCs being gradually frost-killed during the study period, Ph was estimated to be the least frost-tolerant and also having the most soil contact with its decomposing aboveground biomass (results not presented). This is in line with a study on eight CC species (including OSR and Ph) in central and southwest Sweden where Ph was observed to be the least frost-tolerant (Liu *et al.*, 2014). Consequently, the relatively high N_2O flux of OSR cannot be explained by it being frost-killed first and its biomass therefore supplying proportionally more substrate to soil denitrifying bacteria in comparison with Ph and O during the six-week study period. On the other hand, there is a possibility that the relatively low N_2O flux of Ph could be explained by its low frost-tolerance, if this induced emissions *before* the study period started. The CCs had been affected by cold weather before the start of the study period, and Ph had been more affected in comparison to OSR and O.

The amount and quality of belowground biomass of the CCs was not investigated in this project but could have influenced N_2O by the same mechanisms as aboveground biomass. Within the previously mentioned strip-till project, 5-10 plants from each plot were harvested on October 19th, 2020 to investigate the relationship of above- and belowground biomass. This showed that Ph allocated proportionally less biomass belowground compared to OSR and O (5% of total biomass compared to 15% and 19%, respectively) (Thomas Prade, personal communication). Li *et al.* (2015) measured N_2O emissions and attributed the comparatively high emissions induced by fodder radish (also *Raphanus* sativus) to it having a relatively large amount of root biomass close to the surface where it easily became available to denitrifying bacteria when decomposed. In contrast to aboveground biomass, root biomass has the "advantage" of already being present in the soil where denitrification occur. A study by Li *et al.* (2016) showed that fodder radish induced higher winter emissions of N_2O after autumn harvest of the aboveground biomass compared to leaving the biomass untouched over winter, which further indicates the importance of root biomass. On the other hand, root biomass of rapeseed residues has been shown to decompose more slowly in comparison to other plant tissues (Trinsoutrot *et al.*, 2000), which would delay flux responses in comparison. However, it is possible that the root biomass could offer part of the explanation to why OSR induced higher N_2O emissions compared to the qualitatively similar Ph, and why Ph did not induce higher N_2O emissions compared to O which in terms of both quantity and quality of aboveground biomass should induce least N_2O emissions.

4.3. Soil variables influencing N_2O emissions

Next to availability of the substrates labile C and NO_3^-, oxygen-limited conditions is the third requirement for denitrification, tightly linked to soil water content. Mean values of measured WFPS were all within the range, albeit in the lower parts of the range, of optimum conditions for denitrification (65-85%) (Henriksson *et al.*, 2015) for the three CC treatments while C plots had slightly lower WFPS. However, Bateman and Baggs (2005) showed that denitrification bore sole responsibility for N_2O emissions already at 70% WFPS. Furthermore, there are two reason why WFPS might have been underestimated in this study. Firstly, at two measurement occasions (19th of Jan and 21st of Feb) following soil thaw, the uppermost layers of soil were thawed while the soil beneath was still frozen. It was possible to insert the soil moisture meter into the soil but the lower parts (approximately 1-2 cm) of the sensors were inserted into harder, frozen soil. The absence of liquid water surrounding the lower parts of the sensor might have led to a measured value that underestimated the WFPS in the thawed, upper layers of the soil. For example, the field conditions the 21st of February were extremely wet, but the measurements indicated a WFPS of approximately 60%, which was lower than several other measurement days with field conditions that appeared less wet. Secondly, soil water content measurements were not performed in the frames within which gas measurements were made and conditions were slightly different within the frames compared to the outside. Especially in the end of the study period, when soils were thawing after the longer cold period, water in the field tended to run off into depressions such as ditches at field borders or tractor tracks. The frames, inserted 20 cm into the ground, past the thawed layers, prevented water from escaping to lower ground. Consequently, the frames were partly water-filled while plot conditions on the outside of the frames appeared less wet. The slightly different conditions within the frames could have led to an over- or underestimation of the emitted N_2O in comparison to the rest of the field. Values of WFPS from soil surrounding the installed frames might therefore have underestimated soil water content within the frames. The wetter conditions within the frames likely led to higher rates of denitrification (Bateman and Baggs, 2005), but the product ratio of N_2O (N_2O/N_2O+N_2) likely decreased with increasing WFPS (Weier *et al.*, 1993).

Soil temperature influence N_2O emissions by increasing denitrification rates when temperature rises (Sommerfeld *et al.*, 1993). CCs could indirectly influence soil temperature through shading or insulating the soil with their canopy covers. The results from this study show that mean soil temperature was higher in OSR plots compared to the two other CC treatments, similarly to N_2O emissions. It is however unlikely that this is a driving factor of differences in emissions due to the size of the actual temperature difference. OSR plots had a mean temperature 0.05°C and 0.07°C higher compared to Ph and O, respectively. In other words, it is likely too

small to have had any major effects on denitrification rates. An explanation of the temperature difference could be that OSR appeared to have the most upright-standing biomass of the three CC treatments and therefore also the thickest boundary layer that could insulate the soil against cooling air temperatures.

While WFPS was likely underestimated, the calculated mean soil temperature was likely overestimated since measurements were impossible when soil was frozen. Mean soil temperature for the entire period was calculated on the available data from measurements in unfrozen soil. Consequently, estimated means for both temperature and WFPS are likely not entirely correct, but are still useful to compare the different treatments. Finally, the lack of significant differences in terms of soil NO_3-N and CO_2-C flux also suggests similar access to N substrates and rate of decomposing activity in the different treatment plots.

4.4. Measurement methodology

Non-steady state chambers has been the most common method for assessing N_2O emissions for decades, largely because it is relatively inexpensive and easy to adopt to a range of different field situations (Rochette and Bertrand, 2008). However, correct design and deployment is necessary to obtain valid results. The use of chambers is an invasive method that can affect factors such as soil temperature, soil water content and turbulence regime, which in turn affects N_2O production and transport (Rochette, 2011). The chambers used in this study were, for example, insulated and white (reflective) to avoid temperature changes, vented to avoid pressure changes, and provided with a fan for mixing of the headspace air. The chamber design fulfills the requirements stated by Rochette (2011). There were primarily two weaknesses in the deployment of the chambers in this study. Firstly, the chambers were installed the day before the first measurement. This may have damaged roots and therefore influenced root activity and gas flux (Rochette, 2011). Ideally, the chambers would have been installed at least a few days before the first measurement to allow any effect on root activity to stabilize to pre-insertion levels. Secondly, the cold weather at some measurements occasions caused the water in the collar channels to freeze with which made it more difficult to ensure a tight water-seal between the chamber and the collar. Finally, it could also be argued that the sampling strategy of the measurements were “chasing peaks” and that this, combined with linear interpolation, risks an overestimation of the N_2O flux (Dorich *et al.*, 2020). However, Barton *et al.* (2015) summarizes studies that demonstrate that it is appropriate, in short-term studies, to use a high measurement frequency when flux likely is higher and less frequent measurements in the intervening periods. Furthermore, the observed N_2O flux values during the study period were

both high and low. Consequently, they were not all observed during emission peaks. Other measurement approaches, such as micrometeorological approaches, are less intrusive but more costly (Rochette, 2011) and removes the possibility for replicated and blocked field experiments (Chadwick *et al.*, 2014), which would have made them unsuitable in this study.

5. Conclusion

The high emissions of N_2O observed in this study suggest that frost-killed CCs might have a substantial influence on annual flux budgets. Furthermore, there was a relatively large difference in emissions induced by the different CCs, indicating mitigation potential. More knowledge about this is important to enable the choice of suitable winter CCs that do not risk off-setting a potential positive climate impact of C sequestration. OSR, the CC that produced the most N and soluble C per m^2 of biomass, induced the highest emissions of N_2O as hypothesized, but the proportions of these fractions in the aboveground biomass failed to explain the relatively low N_2O emissions induced by the qualitatively similar Ph. It is therefore possible that other factors, such as root biomass, were involved in governing N_2O emissions associated with frost-killed CCs. More research is needed to validate the results in this study and investigate a wider range of CCs. Knowledge is also needed to understand what qualities or characteristics of the CCs that influence N_2O emissions, and suitable management practices that could mitigate the effects.

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