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# Frost killed cover crops induced high emissions of nitrous oxide

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# HIGHLIGHTS

# GRAPHICAL ABSTRACT

- High N<sub>2</sub>O emissions from frost-killed cover crops
- More than twice as high  $N_2O$  emissions from oilseed radish than from phacelia or oats
- Similar quantity and quality of aboveground biomass in oilseed radish and phacelia

Mean cumulative emissions of  $N_2O$ -N over 43 days from cover crops dying, and subsequently starting to decay, due to frost. Error bars represent standard errors.



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#### ABSTRACT

Establishing a cover crop after harvest of a main crop in late summer or early autumn can have several advantages, including weed control, decreased nitrate leaching and an increased potential for carbon sequestration. However, the addition of fresh plant material to the soil in late autumn or winter, either by active termination of the cover crop or by frost damage, could be a risk factor for nitrous oxide emissions, due to the simultaneous occurrence of wet soil conditions and freeze-thaw cycles. We measured field emissions of nitrous oxide from three cover crops – oil-seed radish, (*Raphanus sativus* var. *oleiformis*), phacelia (*Phacelia tanacetifolia*) and oats (*Avena sativa*) – over a 43-day period in winter. All three cover crops were sensitive to frost and died, wilted and started to decompose during this period. The cover crops increased nitrous oxide emissions, relative to controls that were ploughed in autumn, by 1.8, 0.7 and 0.6 kg N<sub>2</sub>O-N ha<sup>-1</sup>, for oilseed radish, phacelia and oats, respectively. We conclude that the choice of cover crop species and management options for cover crops need to be further researched to minimise their contribution to nitrous oxide emissions from agriculture.

#### 1. Introduction

The climate is changing and the agricultural sector is one of the important drivers and regulators, through emissions of carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O), but also uptake of CO<sub>2</sub> (IPCC, 2013). Agriculture, Forestry and Other Land use (AFOLU) make up 13% of CO<sub>2</sub> emissions, 44% of CH<sub>4</sub> emissions and 82% of N<sub>2</sub>O emissions, globally (Jia et al., 2019). Carbon sequestration has been suggested as a method for mitigating the climate impact of agriculture, and growing cover crops (CCs) is one promising tool for accomplishing this (Poeplau and Don, 2015). In some studies, CCs have also decreased N<sub>2</sub>O emissions during the winter season, particularly for over-wintering CCs (Wagner-Riddle and Thurtell, 1998; Foltz et al., 2021), adding to their climate change mitigation benefits. In other studies, emissions have been high (Dörsch, 2000; Li et al., 2015), suggesting N<sub>2</sub>O emissions induced by CCs could offset a significant part of their carbon sequestration benefits. There is as yet no general consensus in the literature regarding the effect that CCs have on N<sub>2</sub>O

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emissions (Basche et al., 2014; Hansen et al., 2019; Guenet et al., 2021) and relatively few studies have been conducted specifically on this topic.

A significant part of annual emissions of N2O can occur during freezethaw cycles in winter and spring (Risk et al., 2013). A Canadian five-year study of N2O 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). Substantial emissions of N<sub>2</sub>O can also occur when fresh, green plant material is added to the soil (Velthof et al., 2002; Lashermes et al., 2022), which happens when CCs are damaged or killed by frost during winter. Mørkved et al. (2006) argue that frost-sensitive cover crops could stimulate N<sub>2</sub>O 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. Dörsch (2000) measured emission peaks of (1) 0.16 kg  $N_2$ O-N ha<sup>-1</sup> d<sup>-1</sup> from autumn-sown, frost-killed oilseed radish in connection to soil thawing, (2) 0.48 kg  $N_2$ O-N ha<sup>-1</sup> d<sup>-1</sup> from frost-killed leguminous cover crops, and (3) 0.65 kg  $N_2$ O-N ha<sup>-1</sup> d<sup>-1</sup> from a thawing mulch of autumn-sown mustard, indicating frost-kill of cover crops as a high-risk situation with regard to N<sub>2</sub>O emissions.

CCs can be terminated, in late autumn or in spring, using herbicide or soil incorporation (Thorup-Kristensen and Dresbøll, 2010), but if they are sensitive to frost they can instead be allowed to die from low temperatures. The use of frost sensitive CCs has been suggested as a way of avoiding the need for herbicide or intensive cultivation to terminate a cover crop (Storr et al., 2021). Similarly, frost sensitive companion crops have been proposed for weed control and increased nitrogen use efficiency in winter rapeseed, without the need for herbicide to terminate the companion crop (Verret et al., 2017). The risk for N<sub>2</sub>O emissions may be particularly high with such practices, but emissions are likely to vary depending on cover crop species or species mixture.

Both the quantity and the quality of cover crop plant material could influence  $N_2O$  emissions. 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 and that 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  $N_2O$  emissions originating from heterotrophic denitrification are instead limited by the supply of labile C (Huang et al., 2004; Mitchell et al., 2013). Lashermes et al. (2022) found the labile C content of crop residues to be the best predictor of  $N_2O$  emissions, at a water filled pore space (WFPS) of 60%. Both a low C/N ratio and a high content of labile C in decomposing CC biomass could thus potentially contribute to high  $N_2O$  emissions.

Since research conducted on cover crops and N<sub>2</sub>O emissions have often either neglected winter emissions (Singh and Kumar, 2021), lacked comparisons of several different cover crops (Foltz et al., 2021), or risked missing emission peaks due to sparse measurements (Liebig et al., 2010; Petersen et al., 2011), there is still a major knowledge gap concerning winter emissions of N<sub>2</sub>O induced by CCs, particularly with regard to differences between CC species. In order to identify differences in N<sub>2</sub>O emissions between frost sensitive non-legume cover crop species and assess the magnitude of these emissions, we measured N<sub>2</sub>O fluxes from plots with oilseed radish (*Raphanus sativus* var. *oleiformis*), phacelia (*Phacelia tanacetifolia*) and oats (*Avena sativa*) from January 9th to February 21st, which was the period when these cover crops died from frost, wilted and started to decompose, during the 2020–2021 winter season. Plots without a cover crop, which were instead ploughed in the autumn, were used as controls.

# 2. Materials and methods

#### 2.1. Experimental setup

The field work 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" (Hansson et al., 2021). Frost-sensitive CCs were sown on August 23rd, 2020, after harvest of field cress (*Lepidium campestre*), ploughing and harrowing. The  $6 \times 15$  m plots were arranged in a randomized block design with four blocks. Two stainless-steel collars ( $0.56 \times 0.56$  m) were installed in each of the 16 plots on the 8th of January and left in the ground for the entire study period. The collars were inserted to a depth of 0.2 m and each had a channel at the top that was approximately level with the soil surface.

#### 2.2. Gas measurements

Gas fluxes were measured on 13 occasions, from January 9th to February 21st, 2021. Since only a limited number of measurement days were permitted due to time and budget restraints, these were allocated to periods of thawing whereas no measurements were performed during periods when the ground was completely frozen. Days with frozen soil, defined as days when the air temperature did not rise above 0 °C, were assumed to have zero emissions, in order to obtain a conservative estimate of total emissions. Emissions of N2O were measured using manual non-steady state vented polypropylene chambers of 0.60 m height, 0.57  $\times$  0.57 m in area, on the previously mentioned stainless steel collars. The connection between chamber and collar was sealed by filling the channel at the top of the collar with water. Samples were collected using a pump that circulated air through a 6 ml glass vial (Exetainer ®, Labco, UK) for 1 min. Two samples from each chamber were collected, 1 and 61 min after the chamber was closed (t1 and t61). Measurements in block 1 and 2 were generally performed before noon and measurements in block 3 and 4 were performed after noon. Samples were analysed for N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> on a gas chromatograph (HP7890A, Agilent, Wilmington, USA). CH<sub>4</sub> results are not presented here.

#### 2.3. Soil water content and soil temperature

Soil volumetric water content in the top 0.12 m 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, at approximately 0.05 m depth. Both measurements were made within a distance of 0.1–0.3 m 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. Meteorological data were obtained from a weather station at the SITES Lönnstorp Research Station, approximately 450 m from the experimental field (LantMet, accessed March 2021).

#### 2.4. Biomass and soil sampling

Cover crop samples for determining biomass dry-weight, C/N ratio and biochemical composition were collected on January 13th, 2021. All sample areas were located at least 0.5 m from plot borders. Two samples were collected from each plot, one to determine biomass dry-weight and one for analysis of total C content, total N content and biochemical composition. For the 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. The samples were washed by hand to remove soil, dried at 70 °C for two days and weighed.

The second biomass sample, for analysis of total C and N content and biochemical composition, was collected from  $1 \text{ m}^2$  in the oilseed radish plots, whereas a larger sample area of  $1.75 \text{ m}^2$  was needed in the oats and phacelia plots, to ensure sufficiently large biomass samples for the analyses. To avoid soil contamination, this biomass was cut 0.05 m above the ground and plant residues on the soil surface were not collected. The biomass samples were dried at 70 °C for two days. After drying, two samples

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(the oat samples from block 3 and 4) did not reach the minimum weight of approximately 55 g needed for analyses and had to be complemented with a few grams of plant material that was added from the samples analysed for biomass dry-weight.

For analysis of total N and C, a representative subsample of a few grams was ground to a fine powder in a knife mill and 5.0 ( $\pm 0.5$ ) mg of the powder was weighed into a tin capsule and analysed using an elemental analyser (Flash 2000, Thermo Scientific, Bremen, Germany). The remaining part of each sample was extracted and analysed according to the van Soest proximate analysis method (Goering and Van Soest, 1970; AFNOR, 2013), to estimate its biochemical composition. The material was extracted in a neutral detergent solution, separating neutral detergent soluble compounds (NDS) from a remaining fraction of "neutral detergent fibre" (NDF). NDS is an estimate of the cell content fraction and will be referred to in the following as "soluble compounds". NDF is an estimate of the cell wall fraction. NDF was extracted in an acid detergent solution, separating acid detergent soluble compounds (ADS) from "acid detergent fibre" (ADF). ADS and ADF are estimates of the hemicellulose fraction and the combined cellulose and lignin fractions, respectively. ADF was treated with 72% sulfuric acid, leaving behind the "acid detergent lignin" (ADL) fraction, which is an estimate of the lignin fraction. Ash content was determined by dry combustion and the biochemical fractions were calculated as percentages of the total dry matter.

Soil samples were collected from each plot on the 26th of January, one to determine bulk density and one for analysis of soil mineral N concentrations. Samples were collected at least 0.5 m from plot borders. For bulk density, stainless steel cylinders with a volume of 400 cm<sup>3</sup> were used to collect undisturbed soil samples from the top 0.1 m of soil. The samples were dried at 105° overnight and weighed. For soil mineral N concentrations, soil samples were collected from the top 0.1 m 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).

#### 2.5. Calculations and statistics

Cumulative emissions of  $N_2O$  and  $CO_2$  were calculated by linear interpolation of emission values between sampling dates, including the dates with assumed zero emissions due to completely frozen soil, for each of the 16 plots. The duration of gas accumulation in the chambers, in relation to the size of the chambers, was chosen to fit the generally low flux rates of  $N_2O$ , which meant that the  $CO_2$  flux values were most likely underestimated due to saturation in the headspace, according to previous linearity tests on the same chambers (data not shown). However, the  $CO_2$ flux values were used as a proxy for soil respiration, indicating relative respiration rates in the cover crop plots. For soil temperature and water filled pore space (WFPS), the mean values for the whole measurement period were weighted based on the different lengths of the periods between measurements. For the dates when measurements could not be made due to frozen soil, soil temperature was assumed to be 0 °C and WFPS was interpolated between the closest measurements, since it could be assumed not to change in frozen soil. For January 9th, when the thermometer was faulty, soil temperatures were assumed to be the same as for January 11th, which was the measurement closest in time.

The mean cumulative emission values of N<sub>2</sub>O and CO<sub>2</sub>, the weighted mean values of soil temperature and WFPS, the soil mineral N values and the crop variables (biomass dry-weight, C/N ratio, N in biomass per m<sup>2</sup>, soluble compounds and soluble compounds in biomass per m<sup>2</sup>) were analysed using a univariate general linear model and post hoc Tukey test in SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp). A significance level of p < 0.05 was used. Controls were excluded from the analyses of crop and soil variables since no cover crop biomass was present and soil conditions could have been strongly affected by the lack of cover crop biomass – and our focus was on explaining differences between cover crops.

#### 3. Results

#### 3.1. Crop and soil variables

The oat plots produced less aboveground biomass (p = 0.002 and p = 0.002), with a higher C/N ratio (p < 0.001 and p < 0.001) and less N per m<sup>2</sup> (p < 0.001 and p = 0.001) in comparison with oilseed radish and phacelia, respectively (Table 1). Oilseed radish had higher shares of soluble compounds (NDS; Goering and Van Soest, 1970) in its aboveground biomass compared to phacelia and oats (p < 0.001 and p < 0.001) (Table 1). In comparison to oats, phacelia aboveground biomass had a higher share of soluble compounds (p = 0.002). The amount of soluble compounds in aboveground biomass on an area basis ( $g m^{-2}$ ) was lower for oats than for both oilseed radish and phacelia (p < 0.001 and p < 0.001, respectively) but did not differ between the latter two (Table 1). The non-soluble (cell wall) fraction of the oat biomass was dominated by hemicellulose and cellulose, with very low lignin contents, while for oilseed radish and phacelia approximately half of the non-soluble fraction was lignin (data not shown).

The mean WFPS, per treatment, varied between 41 and 92% during the measurement period, and the soil temperature at 0.05 m depth was never above +3.3 °C, for any of the plots (Fig. 1). There was a thin layer of snow, 0.01–0.02 m, in the plots during parts of the period, but the soil was never completely covered. Over the whole measurement period, there were no significant differences in mean soil temperature at 0.05 m, mean WFPS or cumulative emissions of CO<sub>2</sub>-C between the cover crop

#### Table 1

Dry-weight, C/N ratio, total N content and soluble compounds (NDS; Goering and Van Soest, 1970) in cover crop aboveground biomass, as well as soil temperature, soil water-filled pore space (WFPS), soil NO<sub>3</sub>-N and NH<sub>4</sub>-N contents, and a soil respiration proxy (CO<sub>2</sub>-C flux measured under suboptimal measurement conditions), for all treatments. The letters in superscript indicate significant differences between treatments. If two treatments share the same letter, they are not significantly different. Standard errors are presented in parenthesis.

Cover crop and soil variables	Control with no cover crop, autumn ploughed	Oilseed radish	Phacelia	Oats
Above-ground cover crop biomass				
Total dry-weight (g m <sup>-2</sup> )	n/a	127 (11) <sup>a</sup>	124 (8) <sup>a</sup>	62 (9) <sup>b</sup>
C/N	n/a	7.3 (0.5) <sup>a</sup>	7.3 (0.2) <sup>a</sup>	13.9 (1.0) <sup>b</sup>
$N_{tot} (g m^{-2})$	n/a	6.6 (0.6) <sup>a</sup>	$6.2(0.7)^{a}$	$1.9(0.4)^{b}$
Soluble compounds (g $m^{-2}$ )	n/a	75 (6) <sup>a</sup>	59 (4) <sup>a</sup>	23 (2) <sup>b</sup>
Fraction of soluble compounds (%)	n/a	59 (1) <sup>a</sup>	47 (1) <sup>b</sup>	37 (2) <sup>c</sup>
Soil variables				
Temperature, 0.05 m (°C)	0.4 (0.0)	$0.5 (0.0)^{a}$	$0.5(0.0)^{a}$	$0.4 (0.0)^{a}$
WFPS (%)	62 (2)	$72(3)^{a}$	69 (2) <sup>a</sup>	70 (2) <sup>a</sup>
NO <sub>3</sub> -N (mg kg <sup><math>-1</math></sup> dry matter) <sup>1</sup>	5.1 (0.4)	7.8 (1.2) <sup>a</sup>	8.4 (1.9) <sup>a</sup>	5.4 (0.4) <sup>a</sup>
$NH_4-N$ (mg kg <sup>-1</sup> dry matter) <sup>1</sup>	0.6 (0.3)	0.9 (0.5) <sup>a</sup>	1.6 (1.0) <sup>a</sup>	0.1 (0.1) <sup>a</sup>
Proxy for respiration (kg $CO_2$ -C ha <sup>-1</sup> 43d <sup>-1</sup> )	87 (8)	714 (77) <sup>a</sup>	677 (42) <sup>a</sup>	538 (22) <sup>a</sup>

<sup>1</sup> Sampled 2021-01-26.



**Fig. 1.** A: Emissions of N<sub>2</sub>O-N (g ha<sup>-1</sup> d<sup>-1</sup>). B: The dotted line represents air temperature (°C) and the treatment markers represent soil temperature at 0.05 m (°C). C: Water-filled pore space (WFPS; %; left y axis) and precipitation (mm) displayed as bars (right y axis). All error bars represent standard error.

treatments (p = 0.192, p = 0.684 and p = 0.095, respectively). For the soil samples, collected on 2021-01-26, soil NO<sub>3</sub>-N and total soil Nmin (NO<sub>3</sub>-N + NH<sub>4</sub>-N) did not differ between cover crop treatments (p = 0.281 and p = 0.192, respectively) (Table 1).

#### $3.2. N_2O$

Emissions of N<sub>2</sub>O were low in the beginning of the study period but increased gradually and reached their highest levels at the end of the period (Fig. 1). The start and end dates of the study were predetermined and all samples were sent for analysis after the last sampling date, which is why the measurements were not continued. The mean cumulative emissions of N<sub>2</sub>O-N, over the 43-day period, were 2.1 (standard error (SE) 0.17), 0.9 (SE 0.12), 1.0 (SE 0.11) and 0.3 (SE 0.06) kg ha<sup>-1</sup> for oilseed radish, oats, phacelia and control, respectively. Mean cumulative N<sub>2</sub>O-N emissions were higher for oilseed radish compared to oats (p < 0.001) and phacelia (p < 0.001) and lower from control plots compared to all cover crop treatments (p < 0.001, p = 0.008 and p = 0.025 for oilseed radish, phacelia, and oats, respectively).

#### 4. Discussion

#### 4.1. Emissions of N<sub>2</sub>O

The primary goal of this study was to quantify and compare emissions of N<sub>2</sub>O induced by three frost-sensitive CCs during the part of winter when they died, wilted and started to decompose. The results show that oilseed radish induced higher emissions compared to phacelia and oats and, furthermore, these emissions were very high for such a limited time. For comparison, annual C sequestration by cover crops was estimated at an average of 0.56 Mg ha<sup>-1</sup> yr<sup>-1</sup> by Jian et al. (2020) and the N<sub>2</sub>O emissions at our oilseed radish plots, after subtracting control plot emissions, correspond to an emission of approximately 0.22 Mg CO<sub>2</sub>-C ha<sup>-1</sup> over only 43 days (GWP<sub>100</sub>; Forster et al., 2021). The highest emission peak recorded was the oilseed radish flux on the last measurement day, which amounted to 0.38 kg N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup>. This was an order of magnitude higher than the maximum emission peak for fodder radish of 0.03 kg N<sub>2</sub>O-N ha<sup>-1</sup> d<sup>-1</sup> in the study by Li et al. (2015), but similar to some of the winter emission peaks measured by Dörsch (2000).

Due to limitations in time, our measurements were performed during a restricted period. When the first measurement was conducted on the 9th of January, CCs had been slightly affected by a cold period at the end of December, when temperature dropped below 0 °C for three days (LantMet, no date). The week leading up to the first measurement had also been cold, with temperatures dropping just below 0 °C (LantMet, no date). It is thus possible that some freeze-thaw induced emissions occurred before measurements started, and the high N<sub>2</sub>O emissions on the last measurement day suggest that emissions may have continued. Assuming zero emissions when the soil was completely frozen may have led to underestimations of total N2O fluxes, but seemed justified since WFPS values were high and the blocking of soil pores by ice was illustrated by the pooling of water on the soil surface on days of snow melt. WFPS was lower in the control plots, however, which could have underestimated fluxes from these plots, exaggerating the difference between controls and CC treatments. Including control plots that were not ploughed would have provided a closer comparison to the cover crop plots, which would have facilitated the interpretation of the results, but such plots were not available. The use of linear interpolation of N<sub>2</sub>O flux values between measurement days is always problematic, since N<sub>2</sub>O emissions are typically highly variable, but interpolating between measurements made in daytime during periods of diurnal freeze-thaw cycles may also cause systematic overestimations. Automatic chambers, that sample at all hours, might be especially suitable for these conditions, but were not available for this study.

#### 4.2. Crop variables influencing N<sub>2</sub>O emissions

 $N_2O$  emissions from thawing soils have been shown to derive mainly from denitrification (Wagner-Riddle et al., 2008) and cover crops have the potential to enhance denitrification through several mechanisms. The decomposing cover crop biomass can provide significant amounts of C and N substrates to denitrifiers, which has been argued to be potentially important for winter emissions of  $N_2O$  (Li et al., 2015). Furthermore, the decomposing cover crop biomass could stimulate the general heterotrophic microbial activity, depleting soil oxygen and increasing the anaerobic volume of the soil (Mørkved et al., 2006).

The crop variables measured enable us, firstly, to single out oats as quantitatively and qualitatively different in comparison to oilseed radish and phacelia. Oats had less aboveground biomass, which also contained less N and less soluble compounds per  $m^2$ . Decomposition of the oat biomass would therefore have provided less of the NO<sub>3</sub><sup>-</sup> and labile C needed for denitrification (Phillips, 2008). Secondly, oilseed radish had a higher fraction of soluble C compounds in its plant tissues compared to phacelia, but the total amount of soluble compounds per  $m^2$ , in the aboveground biomass, was not significantly different. The relatively low emissions from oats could thus be due to a lower input of substrate, while the lower emissions from phacelia could not be explained by differences in substrate inputs.

However, substrate inputs may not have been proportional to cover crop biomass – although all three cover crops were frost-killed during the study period, their dynamics of biomass degradation and degree of soil contact varied. When visually observing the cover crops being gradually frost-killed, we noted that phacelia seemed to die first and to have the most contact between its decomposing aboveground biomass and the soil. Consequently, the relatively low N<sub>2</sub>O flux induced by phacelia, despite biomass qualities similar to those of the oilseed radish, could not be explained by it being frost-killed last and its biomass therefore supplying relatively less substrate to soil denitrifying bacteria in comparison with oilseed radish and oats. The lack of significant differences in terms of soil NO<sub>3</sub>-N and CO<sub>2</sub>-

C flux also suggests similar access to N and C substrates in the different treatment plots.

The amounts and qualities of belowground biomass of the cover crops could have influenced N<sub>2</sub>O emissions via the same mechanisms as for the aboveground biomass. Li et al. (2015) measured N<sub>2</sub>O emissions in the field 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 became easily 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 occurs. Root biomass was not measured within our study, but 5-10 plants from each plot were harvested from the same field on October 19th, 2020 for another study, to determine the ratio between aboveground and belowground biomass. This sampling showed that phacelia allocated proportionally less biomass belowground, compared to oilseed radish and oats (Personal communication: Thomas Prade, Swedish University of Agricultural Sciences). It is possible that root biomass explains, at least partly, why oilseed radish induced much higher N<sub>2</sub>O emissions compared to the qualitatively and quantitatively similar phacelia, and why phacelia did not induce higher N<sub>2</sub>O emissions compared to oats, which, based on both quantity and quality of aboveground biomass, could have been expected to induce the lowest N2O emissions.

#### 4.3. Soil variables influencing N<sub>2</sub>O emissions

Oxygen-limitation in the soil is a requirement for heterotrophic denitrification, and closely linked to soil water content. Mean values of measured WFPS were close to optimum conditions for denitrification (Davidson et al., 2000; Butterbach-Bahl et al., 2013) during the study, with slightly lower values for the control plots. There were no differences between the three cover crops with regard to WFPS. WFPS could have been underestimated, since the soil moisture and bulk density values used were measured outside the collars, to avoid disturbance. Especially at the end of the study period, when soils were thawing, water in the field tended to run off into depressions such as tractor tracks. The collars prevented water from escaping laterally and conditions within the collars appeared wetter than outside. A higher water content within the frames may have led to either overestimations or underestimations of emitted N2O in comparison to the rest of the field, by either higher denitrification rates or lower  $N_2O/(N_2 + N_2O)$  ratios (Davidson et al., 2000; Chen et al., 2013). Soil temperature influences N<sub>2</sub>O emissions by increasing denitrification rates when temperature rises (Sommerfeld et al., 1993) and cover crops could influence soil temperature by providing a shading and/or insulating canopy cover. In this study, however, there were no significant differences in soil temperature between cover crop treatments. We suggest further research into: (1) the influence of physical and chemical characteristics of cover crops on N<sub>2</sub>O emissions, (2) mitigation strategies based on active termination of freeze-sensitive cover crops, instead of relying on frost kill, and (3) mitigation strategies based on the removal of residues - e.g. for biogas production or fodder, with subsequent returning of digestate or manure to the field.

## 5. Conclusions

The high emissions of  $N_2O$  observed in this study suggest that frostkilled cover crops may have a substantial influence on annual flux budgets. Furthermore, there was a relatively large difference in emissions induced by the different cover crops, indicating mitigation potential. More knowledge about this is important, to enable the choice of suitable winter cover crops that do not risk offsetting a potential positive climate impact of C sequestration. Oilseed radish increased  $N_2O$  emissions more than twice as much as phacelia, relative to the control treatment, despite similar amounts and qualities of aboveground biomass. This indicates that other factors, such as root biomass, are involved in governing  $N_2O$  emissions associated with frost-killed CCs. More research is needed to validate the results of this study and investigate a wider range of cover crops. Identifying the mechanisms behind the high emissions would help explaining the variability among cover crop species and extrapolating the results to species that have not been studied closely.

#### CRediT authorship contribution statement

**Felicia Olofsson:** Investigation, Data curation, Formal analysis, Writing – original draft, Project administration. **Maria Ernfors:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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