Complete removal of biomass from oilseed radish as a cover crop decreased nitrous oxide emissions

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Abstract

One of the many benefits of utilizing cover crops in crop rotations is their carbon sequestrating effect. However, frost-sensitive cover crops could emit high levels of the potent greenhouse gas nitrous oxide. To avoid a system where the effect of carbon sequestration is simultaneously mitigated through nitrous oxide emissions, it is important to identify which methods that are able to decrease nitrous oxide emissions. A field trial was performed with 4 treatments of the frost sensitive cover crop oilseed radish; (1) untreated, (2) cut and removed, (3) uprooted and removed and (4) addition of a high C: N ratio material for immobilisation of nitrogen. The hypothesis was that all treatments 2-4 would decrease nitrouse oxide emissions from oilseed radish, but that treatment 3 would have the largest effect. The mean cumulative emissions over the whole measuring period of 78 days were 774.3, 459.2, 271.2 and 651.7 g N_2O-N ha^{-1}, for treatments 1, 2, 3 and 4, respectively. Only treatment 3 was significantly different from the other treatments and proved to have potential in decreasing N_2O emissions from oilseed radish. However, the results need to be confirmed through further studies, as well as the treatments economic and practical feasability. The results for treatments 2 and 4 were not as expected, but raised questions and impulses for further research.

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1. Introduction

A large proportion of the greenhouse gasses emitted globally originates from agriculture, forestry and other Land use; 13% of CO_2 emissions, 44% of CH_4 emissions and 82% of N_2O emissions (Jia et al., 2019). On the other hand, soil carbon (C) sequestration in the green sector might be one of the main solutions for mitigating climate change over the next ten to twenty years, (Minasny *et al.*, 2017). FAO (2021) emphasizes the use of cover crops as a main part of conservation agriculture for C sequestration and a recent global meta analysis by Mcclelland, Paustian and Schipanski (2020) estimated that the cover crop contribution of stabilised carbon to soil C stocks could be said to be on average 1.11 Mg C/ha, which equals an increase of 12% relative to a no cover crop control. By simultaneously sequestering C and enhancing soil health (Jian, Du and Stewart, 2020), cover crops could be an approach in mitigating climate change, without compromising future food safety.

Cover crops that are grown between main crops retain nutrients in their biomass, thereby limiting nutrient losses (Norberg and Aronsson, 2020). Cover crops add organic material to the soil as aboveground biomass but also in the form of roots and root exudates; this enhances soil microbial activity, soil structure and water retention ability of the soil (*Jordbruksverket*, 2012). Cover crops are also used for their weed suppressing ability (Jian, Du and Stewart, 2020) . Due to soils in temperate climates having a net percolation through the soil profile during the autumn and winter periods, cover crops are often used as nitrate (NO_3^-) sink (Hu, Sørensen and Olesen, 2018); cover crops have been shown to decrease N leaching by 50-70 % contrasted to bare fallow (Basche et al., 2014; Valkama et al., 2015).

1.1 Cover crops and N_2O

Besides leakage of NO_3^- , N may be lost from soils as gas from mainly nitrification or denitrification (Butterbach-Bahl *et al.*, 2013). Agricultural soils are the largest anthropogenic source of N_2O emissions (EEA, 2022). N_2O, whose potential to aggravate global warming is 265 times higher than that of CO_2 (IPCC, 2019) is

emitted during crop residue decomposition (Abalos, 2021). Some authors have found high emissions of N_2O from the decomposition of cover crops
(Dörsch, 2000; Li *et al.*, 2015). These emissions can in some cases cancel out the C sequestering effect of cover crops (Lugato, Leip and Jones, 2018; Xia et al., 2018).The effect of cover crop decomposition on N_2O emissions is dependent on the managment practices of cover crops, and at what point in the growing season these are carried out. For example untreated cover crops that are left to grow in the field during the winter period can decrease soil mineral N availability in the soil, which can decrease winter N_2O emissions (Wagner-Riddle and Thurtell, 1998; Foltz et al 2021). However, frost sensitive cover crops that are left in the field during the winter periods, and therefore die and wither during frost, have been noted to give rise to substantial amounts of N_2O emissions when decomposed (Li et al, 2015; Olofsson and Ernfors, 2022).Whether plants are killed off by active termination or by frost damage, the resulting addition of fresh plant material during late autumn or winter, a time when soils are influenced by high moisture content and freeze-thaw cycles, could potentially increase N_2O emissions from soils (Risk, Snider and Wagner-Riddle, 2013). Due to the emissions of N_2O being regulated by an intricate composition of processes, the effect of cover crop presence can be difficult to forecast (Abalos et al., 2022a). However, the main N_2O producing process during winter in temperate climates can most often be assumed to be denitrification (Groffman et al., 2009).

1.2 Freeze-thaw cycles and crop characteristics

Conditions facilitating denitrification are especially likely to arise during the freezing and thawing of soils in cold climates in winter and spring (Risk, Snider and Wagner-Riddle, 2013). Non-growing season freeze- thaw cycles have been shown to make up a large part of the annual N_2O emissions (Wagner-Riddle and Thurtell, 1998; Risk, Snider and Wagner-Riddle, 2013).

N_2O production during freeze-thaw cycles is believed to be induced by increased biological activity and physical and chemical changes taking place in the soil (Risk, Snider and Wagner-Riddle, 2013). When infiltration is hindered in frozen subsoils, the thawed surface layer of the soil consequently becomes more wet, which gives rise to denitrification facilitating conditions (Dörsch, 2000). The amount of N20 emissions produced during denitrification is strongly influenced by the water content of the soil, often expressed as the water filled pore space (WFPS), since this regulates the availability of oxygen for denitrifiers (Butterbach, 2013). With some variation depending on soil type, N20 emissions reach an optimum at a wfps of 70-

80% (Davidsson et al, 2000). At even higher wfsp values, the denitrification is completed with N2 as the main product (Butterbach, 2013).

Denitrifier access to C and nitrogen (N) is another key factor affecting the extent of N_2O emissions during FTC (freeze-thaw cycles). At thaw, dead microbes, fine roots and dissolved components from soil aggregates also become available for microbial decomposition (Risk, Snider and Wagner-Riddle, 2013). If living plants are present in the field, these will also die during frost and add new organic material. The amounts of C and N released are regulated by the type of plant material that is decomposed. A common measure to predict N_2O emissions from plant material is to study its C:N ratio (Chen et al., 1995). Plant materials with a C:N ratio below 20-30 are expected to induce net N-mineralisation, and thus a net increase of available N, while a C:N ratio higher than 30 are expected to induce net N-immobilization (Robertson and Groffman, 2006). When microbes decompose C rich materials they scavenge the surrounding soil for N, which limits substrate availability for nitrification and denitrification (Robertson and Groffman, 2006) Therefore, the C:N ratio provides information on the availability of ammonium and nitrate to microbes (Robertson and Groffman, 2006), thus, predicting N_2O emissions. Recent research has suggested that plant materials with a low C:N ratio are associated with higher N_2O emissions during FTC (Abalos, Rittl, et al., 2022b).

Newly dissolved organic C at FTC can give rise to increased N_2O fluxes by directly feeding heterotrophic denitrifiers. (Mørkved et al., 2006; Mitchell et al., 2013). Labile C can also increase N_2O fluxes in an indirect way; by stimulating soil respiration, anaerobic conditions are induced, which in turn is a precursor to further N_2O emissions (Mørkved et al., 2006). Only a fraction of the plant material C is easily available for microbial decomposition. Depending on plant origin, residues consist of varying proportions of lignin, cellulose, hemicellulose and soluble C , with the latter being the directly consumable fraction (Kriaučiunie *et al.,* 2012). There is a relationship between size of the labile C fraction and plant material senescence. Abalos et al., (2022b) and Lashermes *et al.*(2022) found that a low physiological maturity was related to high concentrations of water soluble C, which in turn was related to high levels of N_2O emissions. Frost sensitive cover crops generally have a low C/N ratio and low physiological maturity and thus run the risk of emitting high levels of nitrous oxide when they freeze and die.

N_2O emissions can be described as arising in ”hot moments” and ”hot spots” over the season, which refers to how a large part of the total N_2O from denitrification can be derived from activity in small areas during short timeperiods (Jacinthe et al., 1998; McClain et al., 2003). Fysical factors of a location in the soil affect the diffusion of oxygen and the effectiveness of denitrification reactant transportation, as well as how long they will be present in the spot (Groffman et al., 2009).Combined with the distribution of organic matter pactches in the profile, this controls the intensity of denitification hotspots (Groffman *et al.*, 2009). Hotmoments are induced by events that cause several denitrification reactants to coincide, such as the drying-rewetting and freezing and thawing of soil (Groffman et al., 2009). The annual emissions of N_2O from agricultural ecosystems in temperate climates is to a large extent dominated by the ”hot moments” of emissions that arise in relation to FTC (Chen et al., 1995; Teepe, Brumme and Beese, 2000).

The C and N composition of plant material varies depending on if it has above or below ground origin. Generally, roots have a higher C/N ratio than shoots and a higher concentration of the recalcitrant C fraction lignin (Rasse, Rumpel and Dignac, 2005). Therefore, roots tend to have a slower decompositon, and mineralisation, than shoots (Rasse, Rumpel and Dignac, 2005). However, root biomass is more exposed to denitrification due to already being present in the soil where decomposition can be more rapid due to more variation in moist conditions and less N limitation Chaves et al., 2021; Chen et al.,2014) All in all, the importance of roots for the promotion of N_2O from denitrification is difficult to establish.

1.3 Oilseed radish

Oilseed radish (Raphanus sativus var. oleiformis) (OSR) is a commonly used cover crop in cold climates such as Sweden and Finland, where the time period between harvest of the main crop (July-September) and the first frost during autumn (September-December) is relatively short, which makes it important with a fast growing cover crop such as OSR (Norberg and Aronsson, 2020). The tap root of OSR loosens up the soil which opens up the soil structure for the following crop (Jordbruksverket, 2012) and has a root depth that reaches far down in the soil profile (Norberg and Aronsson, 2020; Thorup-Kristensen, 2001). A study by Sapkota et al. (2012) found that OSR could reach a rooting depth of 210 cm during the autumn period, compared to ryegrass that reached a depth of 99 cm. In addition to solving some of the effects of soil compaction n (Williams and Weil, 2004) the tap root enables the OSR to efficiently retrieve N from the deeper soil layers, thereby counteracting N leakage (Norberg and Aronsson, 2020). this too is of great importance in a humid region as Sweden, where the growing season of the main crop is relatively short; the combination of soil without vegetation from approximately October to April and a net percolation through the soil profile increases the risk of N-leaching (Norberg and Aronsson, 2020). Furthermore, OSR can have a nematode sanitizing effect (Schmidt, Finckh and Hallmann, 2017). Even though the growing season is shorter for cover crops compared to main crops, and characterized by low temperatures, OSR can contribute a substantial amount of C to the soil (Mutegi et al., 2011). The C input occurs both during the growing period, as root exudates and the death of fine roots, and after the growing period as crop residues.

However, since oilseed radish is a frost sensitive cover crop, there is a risk that it releases the N too early in the season, in winter or even late autumn, and that way offsets the C effect through nitrate leakage or FTC induced N_2O emissions. Some studies have been able to see elevated N_2O emissions when oilseed radish is terminated by frost damage (Li *et a*l 2015;Olofsson and Ernfors, 2022; Dörsch 2000) while others have seen low emissions with no obvious difference to control treatment (Taghizadeh-Toosi et al., 2022). The emissions from OSR are in many cases higher than those from other cover crop species (Thomas et al 2017, Ernfors 2021, Dörsch 2000). Other brassicas such as mustard (*Sinapis arvensis*) have also been noted to have higher emissions than other cover crops (Lashermes 2021; Janz et al., 2022).

Olofsson and Ernfors (2021) measured field emissions of nitrous oxide from oilseed radish and two other frost sensitive cover crops over a 43 day period in winter. Compared to ploughed control plots without cover crops, all cover crop species emitted significantly higher levels of N_2O compared to the control; 1.8, 0.7 and 0.6 kg N_2O -N ha^{-1}, for OSR, phacelia (*Phacelia tanacetifolia*) and oats (*Avena sativa*), respectively. OSR increased N_2O emissions significantly more than the emissions from oats and phacelia; relative to the control treatment OSR increased emissions more than twice as much as phacelia. Since OSR and phacelia had similar amounts of aboveground biomass, the authors concluded that other factors, such as root biomass, could be a regulating factor for N_2O emissions associated with frost-killed cover crops. There is thus a risk concerning OSR that it will emit high levels of N_2O and since it is a species with several valuble characteristics as a cover crop, further research is needed on how the high N_2O emissions of OSR could be mitigated.

1.4 Potential measures against elevated N_2O emissions of OSR

By applying suitable management practices in the field on OSR, the FTC induced N_2O emissions might be reduced. There are several possible measures that could be examined to decrease N_2O emissions from OSR cover crops; two of these would be to (1) remove cover crop biomass or to (2) promote immobilisation of N, in order to decrease the availability of substrates for denitrification.

If the crop biomass is removed before the first frost, nitrification will be deminished due to reduced availability of C and N during FTC. Removal of cover crop biomass has therefore been suggested as a method to decrease N_2O emissions. For OSR this can be done either by grazing or harvesting. Harvesting of OSR by machine cutting has been examined by Li et al (2015), but harvesting of the whole plant has not yet been studied. Removing all of the OSR plant material except fine roots is currently not an established method in practice, but would be technically possible and could be a useful method if it decreases N_2O emissions substantially.

If soil denitrification is N limited, adding an N-immobilizing material with a high C:N ratio could be an effective measure in decreasing N_2O emissions by limiting the substrate availability for nitrification and denitrification (Robertson and Groffman, 2006) In a laboratory experiment, (Chaves et al., 2005) co-incorporated straw, immature compost and sawdust with crop residues of celery, and found that cumulative N_2O emissions were reduced by more than 50%. Rothardt et al. (2021)used organic amendments with high C:N ratios and found reductions in nitrous oxide emissions during autumn and winter by up to 45 %. However, N_2O reduction by immobilisation seems not to have been studied for cover crops.

1.5 Aim and hypothesis

The aim of this thesis was to identify management methods for decreasing N_2O emissions from the frost sensitive cover crop OSR.

The aim was addressed by testing the following hypothesis:

Hypothesis 1: The magnitude of N_2O emitted from OSR will be related to the total amount of aboveground biomass and coarse roots left in the collars.

Hypothesis 2: Adding a high C:N ratio material on the soil surface will decrease N_2O emissions.

2. Methodology

2.1 Experimental setup

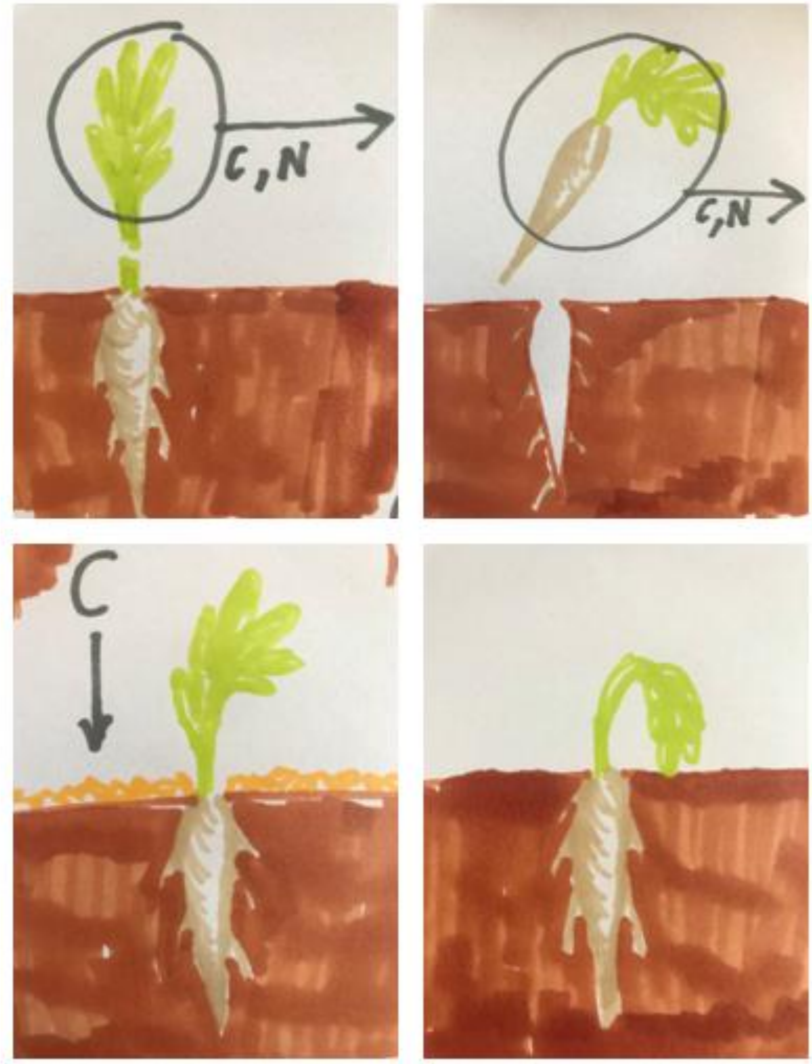
The field experiment was performed at the SITES Lönnstorp research station located in Scania in southern Sweden, on a sandy loam (Hansson et al 2022) Based on soil samples, the soil pH at the experimental site was 7.4 and the C/N ratio was 10.4. Gas measurements were carried out in field plots of oilseed radish (OSR) sown on 23rd of August 2021 for an ongoing project, hereafter referred to as the "strip-till project". The strip-till project examines the effect on C sequestration and weed control when field crops are strip sown in withering frost sensitive cover crops, as part of a conservation agriculture practice (Hansson et al, 2022; FAO, 2019).

For the gas measurements, stainless steel collars (0.564 x 0.564 m) were installed into the soil on the 14th of December, to a depth of 0.2 m. The top of the collar was comprised by a metal furrow located at the soil surface in which chambers for gas measurements could be placed. The collars were left in the ground during the whole measuring period. The experimental setup involved 4 treatments (including control) in three replicate blocks in plots of OSR. The collars were placed at least 60 cm from the field plot edge and with a distance of 20 cm between the collars. Each block had 8 collars, 2 for each treatment. The treatment for each frame was The treatments where randomly distributed within the 4 frames in every row in each plot. To prevent the damaging of the cover crop, aboveground biomass belonging to plants with roots inside the frame were also moved inside the frame. Similarly, aboveground biomass belonging to plants outside the frame was removed from the frame.

The trial involved the following 4 treatments of OSR

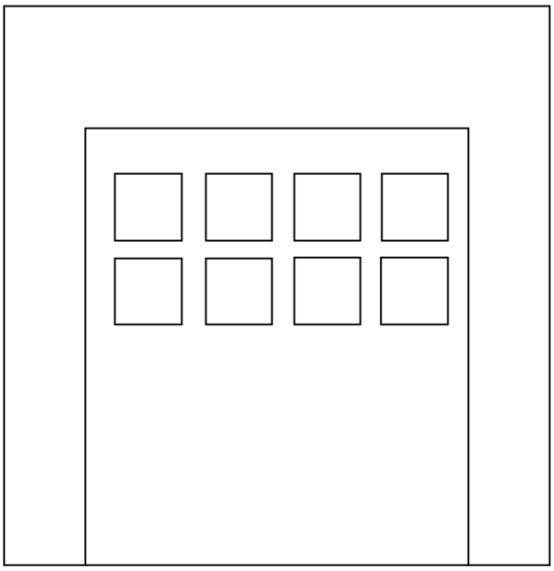
1. Harvest on 15 December by cutting the plant material at a height of 2-3 cm, thereby leaving the root and stubble in the soil (CUT).
2. Harvest on 15 December by pulling the plants up with the roots, removing both all aboveground biomass and coarse roots (UR).

3. Application of 159 g of fine wood chips (1-4 mm) on 17 december, corresponding to 5 tonnes per ha in dry weight (IMM).
4. Control with untreated OSR (UO).



Figur 1 From top left corner to lower right corner, treatments CUT, UR, IMM and UO.

Figur 2. Overviwe of the experimental setup of the strip-till project at the SITES Lönnstorp Research Station. The black boxes show where the oilseed radish was located, in three blocks. Photo by Ryan Davidson adapted by Emma Lövgren.



Figur 3. Overview of the location of the collars within the plots. The inner larger box with black lines represent plot edges and the eight small boxes shows where the steel collars were installed.

Figur 4. Collars in block 1 on the second measurement day on 30 December with the four different treatments before weed removal.

2.2 Gas measurements

Between 20 December and 3 March, 13 gas measurements were carried out once a week. Two measurements were however divided onto two dates; On the second measurement, gas samples from block one were collected on 20 December and samples from block 2 and 3 on 21 December, and on the fifth measurement date samples from block one and two were collected on 18 January and samples from block three on 19 January.Measurements on the 20 and 21 December have been counted as if they were measured on 21 December and measurements on 18 and 19 January have been counted as if they were measured on 19 January.

Another exception was the last two measurements, that were made over the course of 24 hours on 9 March, as compared to the normal sampling duration of about 7 hours during daytime. Two rounds of gas measurements were done at each block, with a lower measuring frequency during daytime when measurements were normally performed.

Figur 5. The state of the OSR on 6 December 2021.

For gas measurements, non-steady state chambers (Livingston and Hutchinson, 1995) were used. The chambers used had two different heights, 100 cm and 62 cm, and these were distributed during sampling so that for the two collars belonging to the same treatment within the block, one of them had a 100 cm chamber and one of them a 62 cm chamber. On a few occasions, when a chamber failed, a treatment could have only large chambers or only small chambers on the two collars (2022-03-09, round two of diurnal measurements: treatment UO had only 62 cm chambers when block 1 was measured. UR had only 62 cm chambers when block 2 and block 3 were measured.

Figur 6. Gas measurement chambers of two different heights, 100 and 62 cm.

Before measuring, the metal collars were filled with water up to 1 cm from the collar edge to ensure that the space between the collar and chamber-bottom was sealed. If there was ice in the collars, this was removed before the collars were filled with water. Before each measurement at a new collar, the chamber was ventilated by being swayed three times back and forth with the silicone stopper removed. After that, the chamber was placed on the collar, carefully, to prevent air being pressed into the frames and generating a pressure chock. 2 samples were taken from each of the 8 chambers via a pump. The first sample (t1) was taken 1 minute after the chamber was placed on the collar and the second (t60) 59 minutes after the first. The pump circulated the air from the chamber to a 6 ml glass vials (Exetainer ®, Labco, UK) and back again. In total, every block took 96 minutes to measure. Gas samples were collected in the same order every measurement day, starting with block 1 and ending with block 3. On most measurement days, samples were collected from block 1 between 9–11, block 2 at 11-13 and block 3 from 13-15. Finally, by analyzing gas samples on a gas chromatograph (HP7890A, Agilent, Wilmington, USA) gas fluxes could be calculated from the change in N_2O concentration over time. CO_2 and CH_4 fluxes were also analyzed on the gas chromatograph. CH_4 fluxes are not presented here and CO_2 is used as a proxy for microbial activity. CO_2 could not be used as a complete measure of soil respiration since the total flux of CO_2 is much larger than the flux of N_2O to which the measuring time is adapted. Therefore, the chamber will eventually become saturated with CO_2, which causes the increase in CO_2 concentration to decline with time.

2.3 Soil water content and soil temperature

Soil volumetric water content (VWC) was measured using a TDR soil moisture meter (Fieldscout TDR 300, Specmeters, Aurora, USA) at a depth of 0–12 cm. Soil temperature was measured using a hand held probe thermometer at 5 cm depth. The measurements were done 10–30 cm from the frames, at places where the ground had not been disturbed. Soil water content and soil temperature was recorded on each measuring day, except for when the ground was frozen.

2.4 Biomass sampling

Plant samples were collected for dry weight, total C and N contents and C fractions, for above and belowground biomass separately. The samples were taken from the biomass harvested in treatments 1 and 2 (with weeds included in the total biomass). In treatment 1, the aboveground biomass was harvested, except for 2-3 cm of stubble. In treatment 2 almost all biomass was harvested except for fine roots. To mimic machine harvest, plants in treatment 1 were cut on a height of 2-3 cm. Below ground biomass was rinsed with water before drying to avoid soil contamination. The samples were dried in 70℃ over night on the day of harvest, then dried again in 70℃ to constant weight. The dried samples were weighed to calculate the removal of biomass per m^2 for treatments 1 and 2. After that, samples were milled in a knife mill, and subsamples of 6 (±0,2) mg were weighed into tin capsules and analysed using an elemental analyzer (Flash 2000, Thermo Scientific, Bremen, Germany). The remains of the samples after C and N samples had been taken were homogenized, and 2 representative samples for above and belowground biomass were analysed for lignin, cellulose, hemicellulose and soluble components, through the van Soest method (Goering and Van Soest, 1970; AFNOR, 2013).

For treatments 1 and 2, the OSR was harvested on the 15th of December. To avoid disturbing the soil before measuring gas, the weeds were harvested after the measurement on the 30th of december. It is therefore worth noting that during the two first measuring days, some weeds were still present in the field.

2.5 Soil sampling and analyses

Soil samples were collected on the 10th of February for determination of bulk density, total N and C and pH. 3 samples from each block were collected at least 50 cm from plot borders. The samples were collected directly in 400 cm3 (ø 7.1 cm, height 10 cm) stainless steel cylinders, which made it possible to retrieve undisturbed soil samples from the top 10 cm of the soil. The soil samples were stored in room temperature until 8 June when they were dried in 105 ℃ to constant weight and weighed afterwards to determine bulk density. Samples were then pooled to attain a representative subsample for the C and N analysis. The sample was milled in a ball mill, and from the pulverized sample 3 subsamples of 5 (±0.29)g were analyzed using the same elemental analyser as for the plant samples

2.6 Meterological data

To calculate gas flux, hourly means of temperature and atmospheric pressure (LantMet, accessed May 2022) were selected for the times of measurement for the location at Lönnstorp. Daily means of precipitation, air temperature and soil temperature were also retrieved to compare with data of soil water content and soil temperature that were collected during gas measurements.

2.7 Calculations and statistics

Emissions of N_2O and CO_2 were calculated for the whole period (20 December to 3 March), period 1 (20 December to 19 January) and period 2 (19 January to 3 March). This was done by interpolating linearly between the times of measurement, for each treatment in each block (the mean value of the two collars with the same treatment was used in the calculations). For soil temperature at 5 cm depth and WFPS, which were measured in connection to each gas measurement, the mean values were weighted according to the lengths of the periods in between. On the dates when soil temperature and WFPS values were missing, since the soil was frozen, it was assumed that soil temperature was zero and WFPS remained unchanged since the previous date. For the biomass calculations, the weeds were included in the total aboveground biomass, since their root biomass was very small in relation to the aboveground biomass, especially in comparison to the OSR.

Differences between treatments were analysed using a univariate general linear model, with Tukey post-hoc tests and a significance level of $p < 0.05$. Stepwise linear regression was used to find correlations between the mean N_2O emission values, for the whole period, period 1 and period 2, and four other variables: soil temperature at 5 cm depth, WFPS, CO_2 flux (as an indicator of heterotrophic microbial activity) and time of day for the gas measurements. Ln transformations of the N_2O data were used when needed, to obtain normality and homoscedasticity of the residuals. The Brench-Pagan test was used to check for heteroscedasticity. All statistical analyses were carried out using SPSS software (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp).

3. Results

3.1 N_2O

The mean cumulative emissions of N_2O-N were, from highest to lowest, 774.3 g ha^{-1} 79 d^{-1} (SE 229.9), 651.7 g ha^{-1} 79 d^{-1} (SE 211.0), 459.2 g ha^{-1} 79 d^{-1} (SE 94.3), and 271.2 g ha^{-1} 79 d^{-1} (SE 57.6) for untreated OSR (UO), immobilisation (IMM), cut (CUT) and uprooted (UR), respectively (Table 1). Mean cumulative N_2O-N emissions from UR plots were lower than at all other plots: UO ($p=0.002$), IMM ($p=0.006$), CUT ($p=0.042$). Emissions of N_2O were higher in the beginning of the study period and lower towards the end, with all treatments reaching an emission peak before 19 January. (Figure 6). Since the N_2O emission pattern between 21 December to 19 january was high emissions for all treatments, while between 24 January to 9 April the emissions were lower, the data was divided into two periods accordingly. Mean values for all treatments during period 1 and 2 are presented in Table 2 and Figure 9 and 10. During period 1, the emissions from UR was smaller than from UO ($p=0.008$) and IMM ($p<0.028$). Likewise, CUT emissions were smaller than those from UO ($p=0.010$) and IMM ($p=0.037$). In period 2 there was no significant difference between any of the treatments. There was however a tendecy to higher emissions from the CUT treatment compared to the UR treatment ($p=0.083$).

3.2 Crop and soil variables

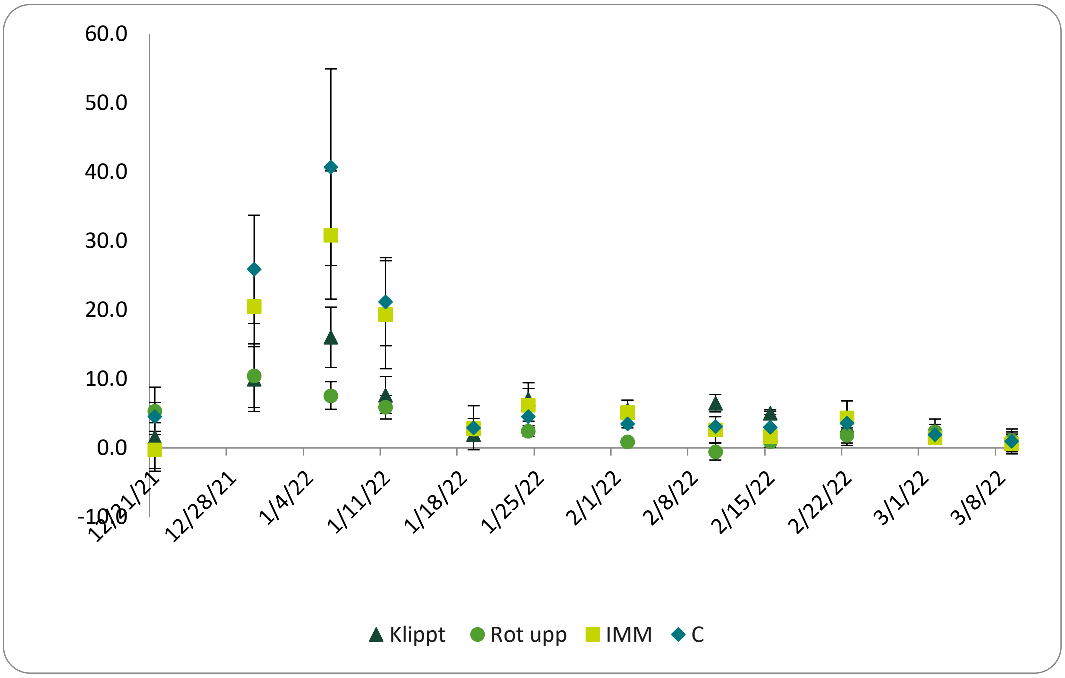
As for N_2O, CO_2 emissions were generally higher in the beginning of the study period, even though the decrease was less dramatic in period 2 (Figure 7). The mean cumulative emissions of CO_2-C, largest to smallest, were 716.4 g ha^{-1} (SE 53.6), 696.4 g ha^{-1} (SE 51.3), 343.4 g ha^{-1} (SE 18.1) and 332.6 g ha^{-1} (SE 38.0) for IMM, UO , CUT and UR, respectively. UR had lower emissions than UO ($p=0.005$) and IMM ($p=0.004$), as did CUT for both UO ($p=0.006$) and IMM ($p=0.005$).

The CO_2 emissions during period 1 and 2 showed the same patterns of significant differences as the emissions for the whole study period (Table 2). During period 1, the significance values were as follows: UO-CUT ($p<0.002$), UO-UR ($p<0.004$) IMM-CUT ($p<0.001$), IMM-UR ($p<0.002$) and in period 2: UO-CUT ($p<0.005$), UO-UR ($p<0.014$), IMM-CUT ($p<0.004$), IMM-UR ($p<0.009$).

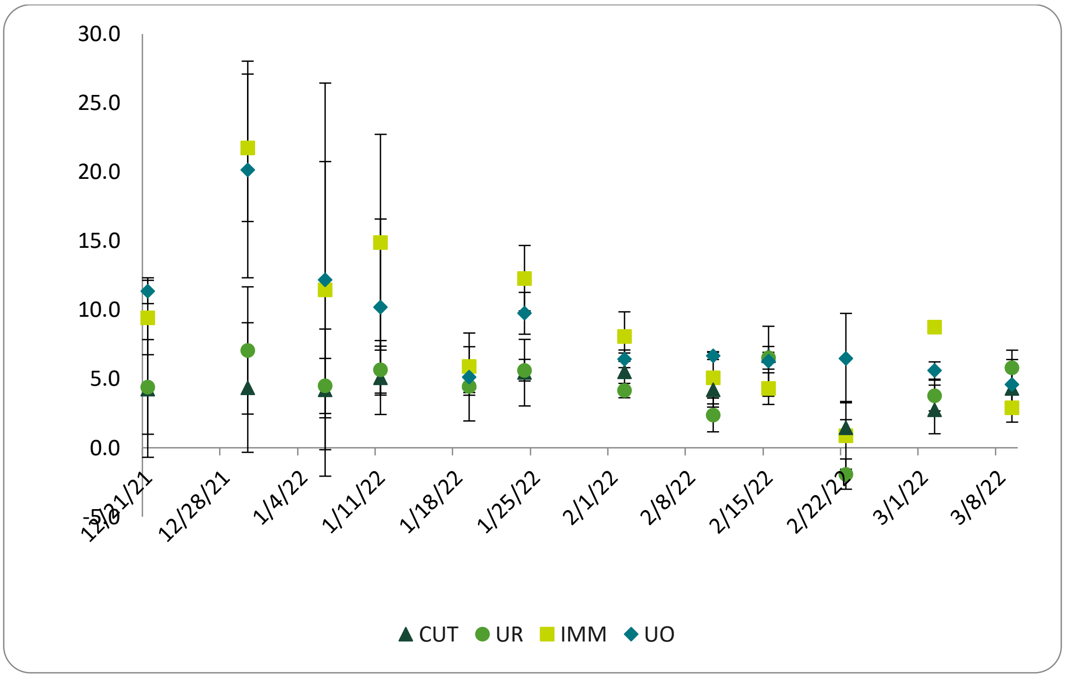
Aboveground biomass made up 87 % of the total biomass and contained 91 % of the total biomass N (Table 1). The C:N ratio was 11.1 for aboveground biomass and 18.0 for belowground biomass. Soil temperature at 5 cm depth, air temperature at 20 cm height, rainfall and WFPS are presented in figures 8 to 11. According to the stepwise linear regression, CO_2 explained 38 % of the variation in N_2O in period 1 ($R^2=0.38$). For period 2, time of the day explained 33% of the variation ($R^2=0.33$). For both periods CO_2 explained 46% of the variation and time of day explained another 7% ($R^2=0.46$ and 0.53, respectively). No other variables contributed.

Table 1. Mean cumulative emissions of N_2O-N and CO_2-C for each treatment during the full study period and mean values of dry weight biomass and N in biomass for all treatments, for above and belowgound biomass. Numbers within brackets show standard error.

	CUT	UR	IMM	UO
N_2O-N (kg ha^{-1})	459.2 (943)	271.2 (576)	651.7 (211)	774.3 (222.9)
CO_2-C (kg ha^{-1})	343.4 (181)	332.6 (38.0)	716.4 (53.6)	696.4 (51.3)
Biomass aboveground (dry weight) (g m^{-2})	27 (7.70)	0	202 (12.66)	202 (12.66)
Biomass belowground (dry weight) (g m^{-2})	30.99 (2.83)	0	30.99 (2.83)	30.99 (2.83)
N in aboveground biomass (g m^{-2})	0.88	0	6.59	6.59
N in belowground biomass (g m^{-2})	0.67	0	0.67	0.67



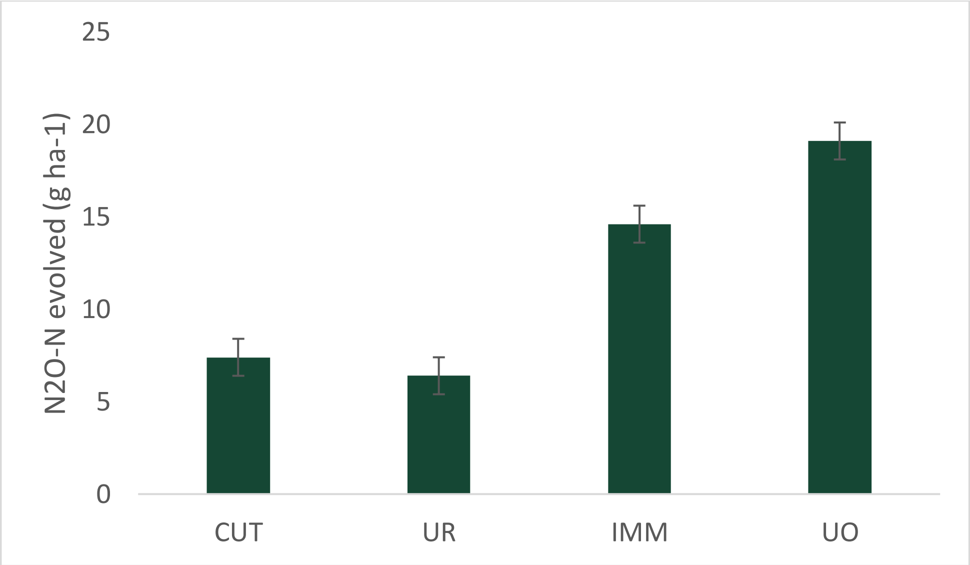
Figur 7. Emissions of N_2O-N (g ha^{-1} d^{-1}) over the whole measurement period. Error bars represent standard error.



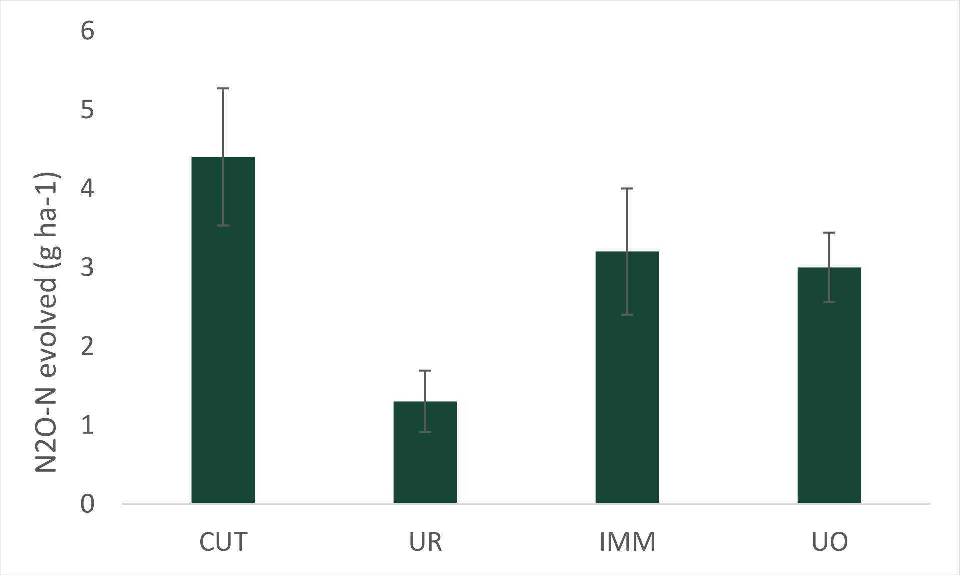
Figur 8. Emissions of CO_2-C (g ha^{-1} d^{-1}) over the whole measurement period. Error bars represent standard error.

Table 2. Mean cumulative emissions of N_2O-N and CO_2-C for each treatment during period 1 (2021-12-21 - 2022-01-19) and period 2 (2022-01-24 – 2022-03-09). Numbers within brackets represent standard error. Values that have different letters in superscript were significantly different in the statistical analysis.

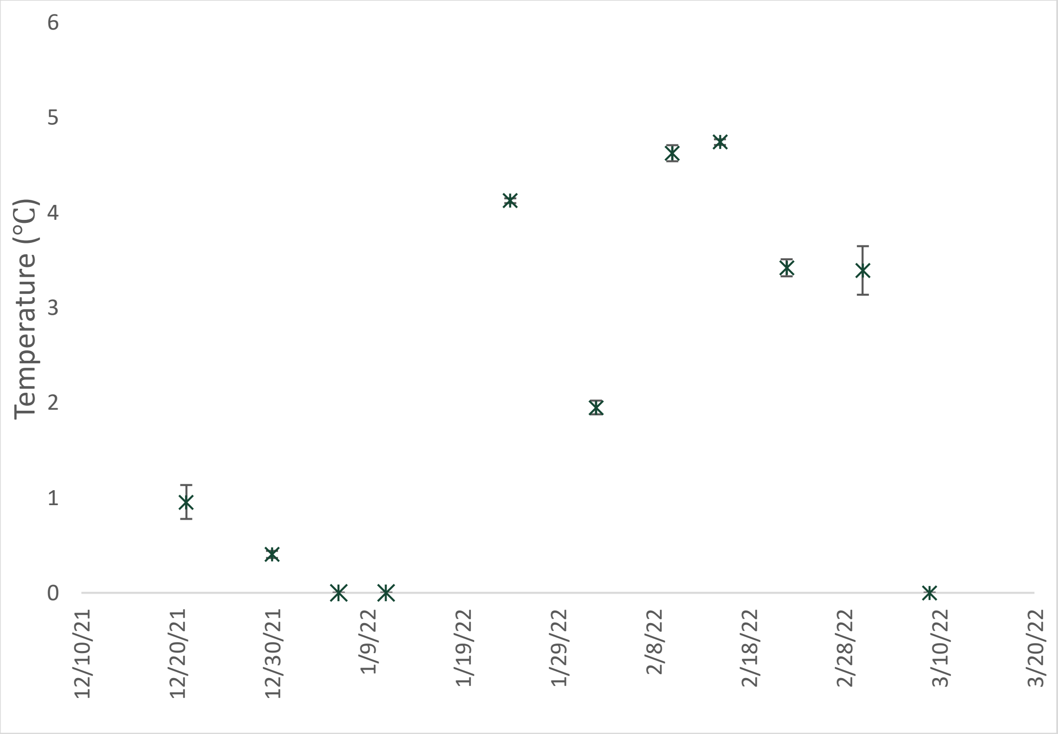
	N_2O		CO_2	
Treatment	Period 1	Period 2	Period 1	Period 2
CUT	7.4 (2.69)^{a}	4.4 (0.87)^{a}	4.6 (0.17)^{a}	4.3 (0.67)^{a}
UR	6.4 (1.27)^{a}	1.3 (0.39)^{a}	5.2 (0.52)^{a}	3.8 (1.08)^{a}
IMM	14.6 (5.84)^{b}	3.2 (0.80)^{a}	12.7 (2.69)^{b}	6.0 (1.47)^{b}
UO	19.1 (7.03)^{b}	3.0 (0.44)^{a}	11.8 (2.42)^{b}	6.5 (0.60)^{b}



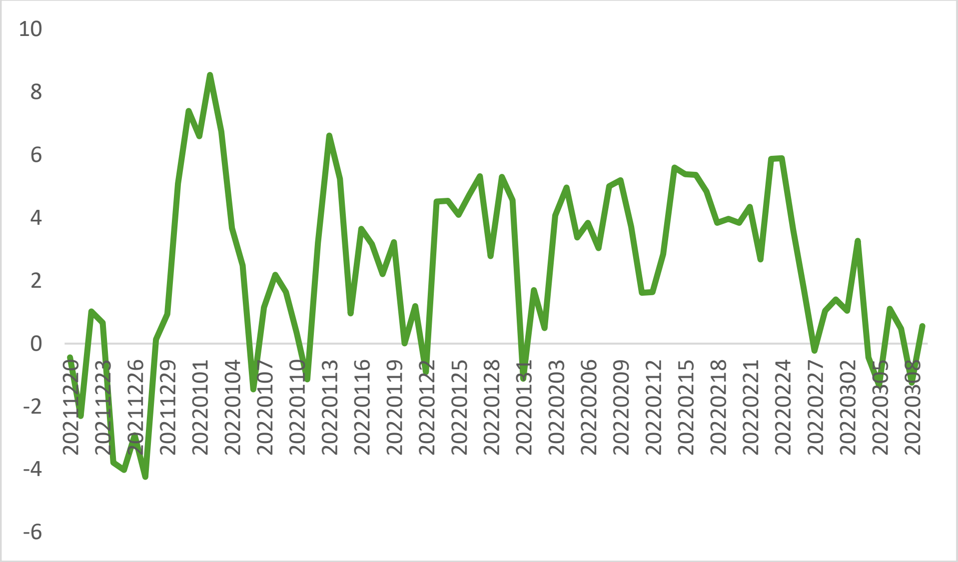
Figur 9. Mean cumulative emissions of N_2O-N during period 1 (2021-12-21 - 2022-01-19).



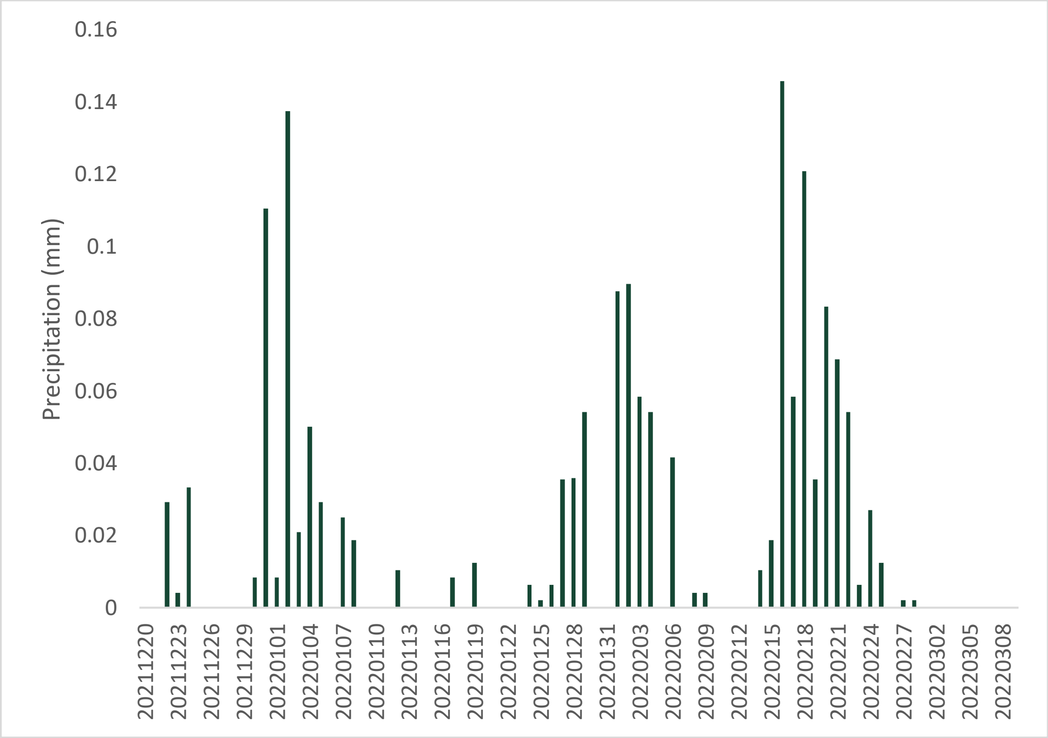
Figur 10. Mean cumulative emissions of N_2O*-N during period 2 (2022-01-24 – 2022-03-09).*



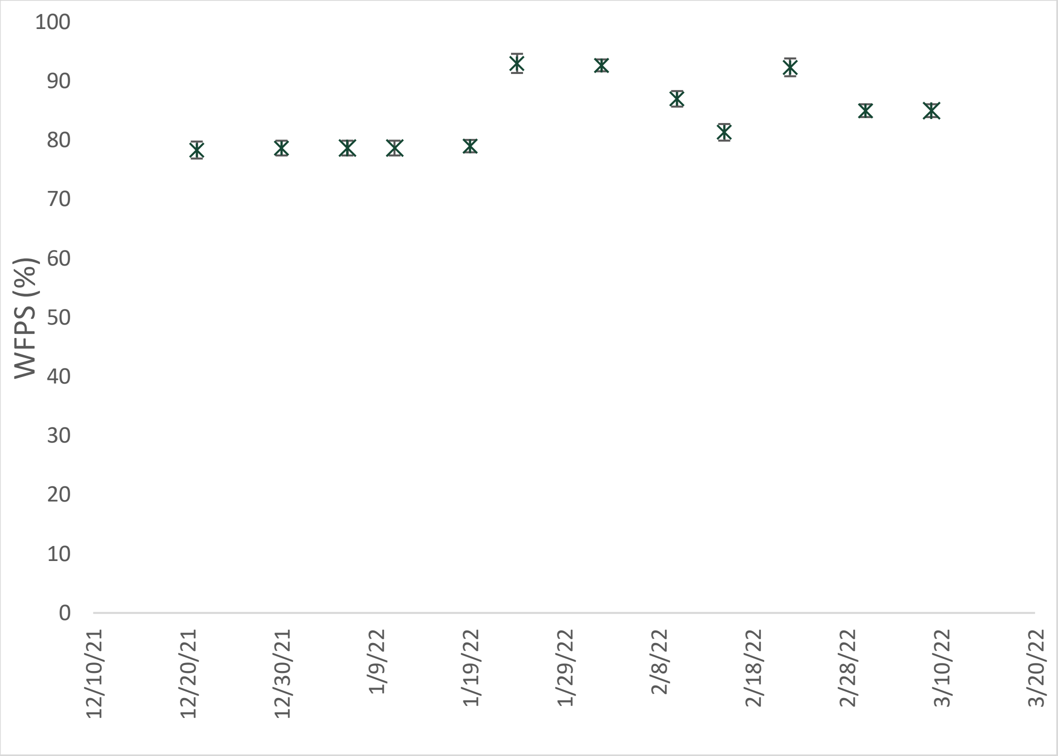
*Figur 11. Soil temperature at 5 cm depth over the whole measurement period. The * symbol indicates that soil temperature could not be measured since the soil was frozen and soil temperature was then assumed to be 0 °C.*



Figur 12. Air temperature (°C), measured at 20 cm from the ground (LantMet, accessed May 2022).



Figur 13. Rainfall during the measurement period (LantMet, accessed May 2022).



*Figur 14. Water filled pore space (WFPS) over the measurement period. The * symbol indicates that the soil was frozen and it was assumed that the WFPS had not changed since the previous measurement date.*

4. Discussion

The measured N_2O values did to some extent confirm hypothesis 1, considering that they correlated with how much biomass that was present in the collars. However, since UO was the only treatment that was significantly lower than the other treatments, removing all of the OSR biomass seems to be a better solution than the cutting of aboveground biomass for N_2O emission reduction from the frost sensitive cover crop OSR. Hypothesis 2, that an immobilising material can decrease N_2O emissions of OSR, was not supported by the experimental results.
The N_2O values measured for UO in this study were 0.69 kg N_2O-N ha^{-1} (over 78 days), which was lower than those measured on OSR during winter by Olofsson and Ernfors (2022) (2.1 kg N_2O-N ha^{-1} over 43 days) and Dörsch (2000) (2.36-4.79 kg N_2O-N ha^{-1} over 212 days), but in the same order of magnitude as values measured by Li (2015) (0.878 kg N_2O-N ha^{-1} over 174 days).

4.1 The effect of biomass removal on N_2O emissions

4.1.1 Period 1

Generally, emission levels were higher during period 1, compared to period 2. During this period, the most intense decomposition took place, after the freezing of OSR in december when temperatures went below 0 ºC (Fig.9). The high emissions are likely explained by C and N that was consequently released. At the same time, WFPS levels were probably conducive to nitrous oxide emissions (74–84 %) (Fig.11) (Butterbach-Bahl *et al.*, 2013). High initial N_2O peaks (”hot moments”) after addition of fresh plant material with a low C:N ratio have also been seen in other studies (Pfab et al., 2011; Seiz et al., 2019; Schmatz et al., 2020). It seems possible that the contact surface between the newly dead plant material and the soil surface meets the criteria for possible ”hotspots”, which could also be the case for on-plant surfaces, since weather conditions were wet.

Treatments IMM and UO, that had no biomass removed, emittied signficantly higher levels of N_2O during period 1 compared to CUT and UR (Table 2) for which the biomass was relatively small (CUT) or only constituted by remaining fine roots (UR). IMM and C did not differ significantly from each other, nor did CUT and UR. These were expected results, indicating that N_2O emissions in period 1 were mainly related to the decomposition of organic material, which was supported by the results of the stepwise lineral regression; 38% of the variation in N_2O emissions could be explained by the variation in CO_2.

4.1.2 Period 2

The lower emissions of period 2 were probaly primarily linked to the fact that the organic material was largely decomposed and presumably most of the easily available C and N had been consumed. Even though temperatues were below zero during period 2, the FTC were less intense, which might also have contributed to lower emissions (Libby et al., 2020).Furthermore, high rainfall caused WFPS to reach levels over 90% at several occasions; this might have lowered the proportion of N_2O out of the total denitrification products (Risk, Snider and Wagner-Riddle, 2013).

During period 2, there were no significant differences between any of the treatments. It was surprising that IMM and UO did not emit higher levels of N_2O since no biomass had been removed from these treatments and since all biomass was frost killed during period 1 and at the end of period 2 it was very withered. Similar results were seen in a study by Li et al (2015) who compared N_2O emissions from untreated OSR with OSR that had been harvested with a grass cutter during autumn. No significant difference was seen between treatments in winter and Li proposed that the unexpectedly high emissions from the cut treatment might be connected to disturbance at harvest. This explanation cannot be extrapolated to our results, since the UR treatment was more disturbed and did not emit higher levels of N_2O than CUT. Moreover, the CUT treatment in this experiment was harvested manually and exposed to what should be negligible levels of disturbance. Furthermore, Li had a higher amount of biomass retained in the cut treatment after harvest compared to the biomass retained in this trial, which might explain the relatively high emissions from their cut treatment. Altogether, the fact that there was no significant difference in emissions from the cut and uncut treatments of this trial in period 2 should have another explanation.

For the stepwise linear regression that was carried out for period 2, time of the day was the only significant explanatory factor for the variation in N_2O emissions. This in turn could either be related to the temperature or to the block factor, since measurements in the blocks were carried out at different times over the day. During period 2, the soil surface normally froze over night and thawed at day. Another trial that was made in the same field in March 2022, to investigate the possibility of diurnal fluctuations in N_2O emissions, could identify a similar pattern of time of day dependecy, with higher emissions in the morning when the soil had just started to thaw (unpublished data). Unfourtunately, only blockwise soil temperature measurements were carried out, outside the frames, as to not disturb the soil within the frames. Possibly, the amount of biomass left within the frames in UO and IMM could have had an insulating effect, which made the FTC less intense, leading to lower N_2O emissions than expected.

4.1.3 Total emissions

Over the whole measuring period, the UR treatment was significantly lower than IMM and UO, but there were no other significant differences between treatments. CUT was only significantly lower during period 1, not for the sum of the periods. If the measuring period would have been longer, the dynamics of period 2, with high emissions from the cut treatment, could have dominated the total emissions. Also, if the removal of biomass in CUT did indeed lead to more intense FTC and therefore larger N_2O emissions from the small amount of biomass left, long periods of freeze- thaw cycling during the winter could possibly result in higher total N_2O emissions after cutting, defeating the purpose of the treatment.

4.2 The effect on of adding an immobilising material on N_2O-emissions

IMM did not significantly lower N_2O emissions compared to control. This could be a result of not incorproràting the wood chips into the soil, which can be of some importance (Chaves, 2005). Another explanation might be that not enough material was added, the soil surface was only partially covered, or that the particles were too large. The idea of adding an immobilising material was for it to decrease the amount of available N for denitrifiers, providing that the soil N is of belowground origin or N that has percolated down into the soil profile from lysating plants aboveground. Emissions could also come directly from the aboveground biomass that is being decomposed at the soil surface. However, considering that high emission peaks were registered for IMM and C in the beginning of period 1, when OSR plants were still upright standing and no considerable amount of withered biomass was present at the soil surface, the N_2O emissions could have to a large extent originated from the aerial parts of OSR. Denitrification on aerial parts of the plant should be possible; the plant is close to the soil surface and soil containing denitrifiers are likely to have splashed onto stem and leaves. Due to moist weather conditions and decomposition, the plant surfaces were usually wet, presumably providing anaerobic conditions.

5. Conclusions

UR proved to have potential in decreasing N_2O emissions from OSR, but the results need to be confirmed through further studies. More broadly, research is also needed to determine the economic and practical feasability of the UR treatment. The results for treatments 2 and 4 were not as expected, but raised questions and impulses for further research, eg. the question of whether the cutting of biomass made FTC more intense, thereby increasing N_2O emissions. Further research should also be undertaken with other types of immobilsing materials and other quantities of material, possibly combined with incorporation.

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Popular science summary

Cover crops that are grown between main crops can be utilised as a way of capturing carbon dioxide (CO2) from the atmosphere by stabilising it as carbon (C) in the soil. Since growing cover crops can also have many soil health enhancing effects, they are an important part of mitigating climate change, without compromomising future food safety. But, it has been seen that cover crops, and especially frost sensitive cover crops, can emit high levels of nitrous oxide (N2O). N2O is a very potent greenhouse gas, which means that these emissions could potentially cancel out the desirable C capturing effect from cover crops. This raises the question: why do frost sensitive cover crops give rise to N2O emissions?

There are many processes that produce N2O in soils, but the main N2O producing process during winter in temperate climates can often be assumed to be denitrification. There are three main parameters that need to coincide for denitrification to occur, that is 1. Microbially available NO3- 2. Microbially available C, and 3. Oxygen limited conditions. Cover crops contain both NO3- and C, so when frost sensitive cover crops are left in the field over winter, and therefore die and wither during frost, they release NO3- and C, which becomes available for microbes to decompose. This in itself doesn't create nitrous oxide, but, if conditions are also oxygen limited, which it often is during winter when the soils and plants freeze and thaw, repeatedly, conditions become favourable for denitrification, and nitrous oxide production.

One of these frost sensitive species is oilseed radish (Raphanus sativus var. oleiformis) (OSR). It has been seen to emit high levels of N2O winter emissions, emissions that are in many cases higher than those from other cover crop species. At the same time, it is a species with several valuble characteristics as a cover crop. Therefore, further research is needed on how the high N2O emissions from OSR could be mitigated.

The aim of this thesis was to identify management methods for decreasing N2O emissions from the frost sensitive cover crop OSR. This was done by performing a field trial with four different treatments of OSR, including control.
If the crop biomass is removed before the first frost, a large part of the C and N needed for the denitrification is not present when the soil starts to freeze and thaw,

which should decrease N2O emissions. Therefore, two treatments of OSR were 1. Harvest by cutting the plant material at a height of 2-3 cm, which meant that root and stubble were left in the soil, and 2. Harvest by pulling the plants up with the roots, removing both aboveground biomass and coarse roots.
If soil denitrification is nitrogen limited, adding a C rich material, with a high level of C in relation to N, could decrease the N2O emissions. This is due to the fact that when microbes decompose C rich materials they scavenge the surrounding soil for N, which limits substrate availability for nitrification and denitrification. Therefore, the third treatment of OSR was 3. Application of of fine wood chips. Finally, the fourth treatment was 4. Control with untreated OSR, left to wither in the field.

The field experiment was performed at the SITES Lönnstorp research station. 13 gas measurements were carried out about once a week between the 20 December and 3 March in field plots of OSR. Finally, the samples from the gas measurements were analysed on a gas chromatograph, so that gas fluxes could be calculated from the change in N2O concentration over time.

774.3 g ha-1 was the highest cumlative emission and it came from untreated OSR. Emissions from the uprooted plots were significantly lower than all other plots. During the first half of the study period, emissions from uprooted and cut were significantly smaller than those from the immobilisation treatment and untreated oilseed radish. These were expected results, indicating that N2O emissions in period 1 were mainly related to the decomposition of organic material. During the second half of the study period, there were no significant differences between any of the treatments. It was surprising that the immobilisation and untreated did not emit higher levels of N2O since no biomass had been removed from these treatments. One possible explanation for this is that the biomass that was left in the immobilisation and control plots could have had an insulating effect, while the removal of biomass in the cut treatment led to more intense freezing and thawing, and therefore larger N2O emissions from the small amount of biomass left. Since uprooted was the only treatment that was significantly lower than the other treatments over the whole study period, removing all of the OSR biomass seems to be a better solution than the cutting of aboveground biomass.

The immobilisation did not significantly lower nitrous oxide emissions compared to control. This could be a result of that wood chips were not incorporated into the soil, that not enough material was added or that the particles were too large. Another possible explanation is that the N2O emissions might not even be of soil origin. High emissions peaks from immobilisation and untreated OSR were registred already in the beginning of the study period; a time when the plants were still pretty upright standing and no considerable amount of withered biomass was present at the soil surface. This could imply that emissions have possibly originated from the aerial parts of the plant.

These results need to be confirmed through further studies. More broadly, research is also needed to determine the economic and practical feasibility of the uprooted treatment. The results for the cut and immobilisation treatments were not as expected, but it raised questions and impulses for further research, for example the question of whether the cutting of biomass made the freezing and thawing of the soil more intense. Further reaserach should also be done with other types of immobilising materials and other quantities of material, possibly combined with incorporation.

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