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# Evaluating nitrous oxide emissions in low input systems using different cover crop strategies over the winter period

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#### ABSTRACT

The integration of cover crops (CCs) in low-input systems is a widely adopted practice to re-capture surplus nitrogen (N) and avoid excessive losses to the environment by leaching or N2O emissions. Closing the N cycle within an agricultural system is therefore economically beneficial and lowers the negative impact of inorganic N on soil and water bodies. However, it is debated if pollution swapping occurs to some extent and if  $N_2O$  emissions increases as a result of decreased N leaching. An experiment was conducted to systematically evaluate grass vs. non-grass CCs, frost tolerant vs. non-frost tolerant CCs as well as high residual N vs. low residual N from the precrop, in a low input system which receives no additional fertilizer. Furthermore, the extent to which N2O losses occur from different seeded CC species and mixtures (Sinapis alba/Vicia sativa, Brassica rapa/Vicia villosa and Lolium perenne/Trifolium repens) was investigated over two experimental years on a sandy soil located in northern Germany. The annual N2O emissions were investigated on a weekly basis using the static closed chamber method. The non-grass CCs had the highest annual N<sub>2</sub>O emissions (2.5 kg N<sub>2</sub>O-N ha<sup>-1</sup>) compared to grass (1.9 kg N<sub>2</sub>O-N ha<sup>-1</sup>). The frost-killed CC led to higher N<sub>2</sub>O emissions (3.3 kg N<sub>2</sub>O-N ha<sup>-1</sup>), especially after the first year when high residual N was still present due to the pre-crop. This indicates that the type of CC used, frost tolerance as well as residual N from the pre-crop will affect N2O emissions. The low N2O emissions observed in the current study indicate that pollution swapping as a result of avoided N leached due to the use of CCs, as shown in a previous paper from the same experiment, is not occurring under these low-input systems. Furthermore, grass used as CC has low N2O emissions and a high N uptake and therefore the potential to close the N cycle and improve on-farm N budgets.

# 1. Introduction

Nitrous oxide ( $N_2O$ ) emissions are considered the foremost important greenhouse gas (GHG) emitted from the agricultural sector at field level. It has a 121-year atmospheric life span and a global warming potential (GWP) of 273 when compared to  $CO_2$  over a 100-year period (IPCC, 2021). Surplus nitrogen (N) in intensive cropping systems are of major concern as it contributes towards  $N_2O$  emissions and N-leaching, thereby negatively affecting climate and environmental health parameters. To reduce these negative impacts, the broader agricultural sector calls for mitigation strategies to reduce  $N_2O$  emissions that can be

implemented by farmers. These strategies should however be economically and practically viable on farm-level to promote implementation across various soil, climate and farming system conditions. One such strategy includes the incorporation of cover crops (CCs) in arable systems to capture surplus soil N during the winter season and improving the total N-balance of the farming system (Möller et al., 2008; Pandey et al., 2018). Nitrogen is released during the subsequent spring season when the CC residues decompose, which is available for uptake by the succeeding crop. Additional benefits of CCs include improved weed- and erosion control, nutrient retention, positive effects on soil structure and an increase in herbage yield through N transfer to the subsequent crop.

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The amount of accumulated N made available by CCs is subjected to the soil N-status, the temperature after seeding CCs and soil moisture which allows favorable plant growth conditions of CCs before winter (Böldt et al., 2021; Reheul et al., 2017).

Due to heterotrophs living off soil denitrifiers, N2O-fluxes can be increased by additional plant residual biomass with a low C:N ratio. If easily decomposable organic matter coincides with a high soil N-status, N<sub>2</sub>O emissions can be heavily increased (Hassan et al., 2022). Accordingly, previous studies indicate that CCs have little effect on N2O emissions annually (Basche et al., 2014; Sanz-Cobena et al., 2014). However, CCs may have a significant effect on N2O emissions on a shorter timescale during the winter period in the northern parts of Europe, where the net effect depends on the crop C:N ratio, management after termination as well as prevailing weather conditions (Essich et al., 2020; Frimpong and Baggs, 2010). Cover crops may enhance N2O emissions after termination or die-off due to frost when the plant material returns to the soil (Taghizadeh-Toosi et al., 2022). Indeed, incorporating CC residues into the soil and increased precipitation can result in short-term increases in N<sub>2</sub>O emissions (especially leguminous CCs) (Mitchell et al., 2013). Farmers often include frost-sensitive CCs to minimize the use of herbicides or intensive soil tillage actions when terminating CCs (Storr et al., 2021) but this approach may result in elevated N2O emissions and greater risk for nitrate (NO3) leaching (Gollner et al., 2020). It is therefore important to carefully consider CC species and agronomic management options when aiming to minimize N2O emissions from agricultural soils.

Four classes of CC species are available to farmers and consist of legumes, nonlegume broadleaf, grasses and brassicas. Legume and nonlegume species are most commonly planted by farmers as both types utilize available soil N leading to improved N use efficiency (Thorup-Kristensen et al., 2003). However, nonlegume CCs are more effective in reducing soil NO<sub>3</sub>-N content and therefore lowers the risk of N leaching in systems with a positive N field-balance. Nonlegume CCs can therefore decrease soil N<sub>2</sub>O emissions by decreasing the soil NO<sub>3</sub> pool, which is the principle substrate for the denitrification process (Baggs Karlen and Huggins, 2014; Liebig et al., 2015). In contrary, legume species may increase N<sub>2</sub>O emissions markedly when easily decomposable matter with a low C:N ratio is mineralized (Flessa et al., 2002). Thus, enhanced N<sub>2</sub>O emissions from leguminous CCs have been observed; however, legume species used as CC deliver more N for succeeding crops (Abdalla et al., 2019).

The multifunctional benefits of CCs have accelerated the integration of CCs in arable systems in the last decade, especially in low-input systems. This is also in line with the European Nitrogen Assessment (ENA), which identifies challenges and threats associated with N pollution (Sutton et al., 2017), while creating better farmer awareness on CCs. As a result, the arable land covered with CCs is rapidly expanding in northwest Europe, which is triggered by the green transition of the common agricultural policy (Destatis, 2021; Kathage et al., 2022; Smit et al., 2019). In Germany, the recent fertilization regulations, with its updated fertilizer planning and maximum allowed N application rates, acts as an additional incentive for planting CCs as they can efficiently close the N cycle (Bodirsky et al., 2012) and minimize losses.

Pollution swapping is a term used to define the increase in one pollutant as a result of a measure implemented to combat a different pollutant (Stevens and Quinton, 2009). For example, cover crops are used to reduce N losses in the form of leaching over the winter period, especially on sandy soils. However, whether this reduction in N-leaching lead to the increase of  $N_2O$  emissions warrants further research. On average, the effect of CCs on  $N_2O$  is not clear, as certain studies indicate an increase in  $N_2O$  emissions through CCs while other studies show no consistent effect (Cavigelli et al., 2012; Jarecki et al., 2009; Smith et al., 2011). Moreover, CCs are increasingly promoted as a GHG mitigation strategy and therefore the understanding of the relationship between CCs (as well as different CCs used) and  $N_2O$  emissions is of importance (Eagle and Olander, 2012). It is therefore necessary to evaluate to which

extent (if so) pollution swapping is occurring in terms of  $N_2O$  emissions when N-leaching losses are reduced.

In the current study, we aimed to assess the use of grass vs. non-grass CCs, frost tolerant CCs as well as high and low residual N from pre-crop on the N-cycling and GHG emissions during winter in northern Germany. We hypothesized that grass used as CC and frost-tolerant species are more effective in reducing  $N_2O$  emissions compared to non-grass and frost-killed CCs by decreasing the  $NO_3$  pool in the soil. Furthermore, we evaluate to which extent pollution swapping is occurring i.e. if high  $N_2O\text{-}N$  losses occur due to the cultivation of CCs in order to reduce N-leaching over the winter period on sandy soils.

#### 2. Materials and methods

#### 2.1. Experimental site description

A two-year field experiment was conducted at an experimental field in Bovenau' (54.32 N, 9.80 E), located in the 'Vorgeest' of the federal state Schleswig-Holstein, Germany. Historically, the site was managed as arable land with a three-year conventional crop rotation (winter rape (*Brassica napus*) – winter wheat (*Triticum aestivum*) – winter barley (*Hordeum vulgare*)). The crop rotation was shifted towards a more diversified arable system and in 2012 a non-N-fertilized ryegrass/red clover mixture was established for two years and mulched three times annually so the effects of N loss and recovered N could be linked to the cover crops. The soil type at the experimental site is classified as a Cambisol (IUSS Working Group, 2022). Soil properties were 5.6% clay, 15.9% silt and 78.5% sand with a 1.5% Corg in the topsoil (0–30 cm). The soil pH was 5.5 and the soil bulk density was 1.53 g cm<sup>3</sup>.

Meteorological data were obtained from a weather station located 0.25 km from the experimental site (Germany's National Meteorological Service – DWD-station 06105 "Ostenfeld"). The area has a humid-temperate climate with a mean annual temperature of 8.8 °C and mean annual rainfall of 826 mm. The weather conditions during the two experimental years (2015/2016 and 2016/2017) are presented in Fig. 1. Winter was defined as months December-February, spring as March-May, summer as June-August, autumn as September-November.

#### 2.2. Experimental layout and treatments

A new crop rotation was established in late summer of 2014 with ryegrass/red clover (Lolium perenne/Trifolium pratense) - summer wheat (Triticum aestivum) – winter triticale (Triticosecale cv. Securo) – fieldpea (Pisum sativum cv. Alvesta) - oats (Avena sativa cv. Max). A CC field experiment was established in May 2015 within the aforementioned crop rotation. The field experiments were carried out over two experimental years and onwards referred to as 2015 (August 2015 to July 2016) and 2016 (August 2016 to July 2017), respectively. The CC treatments were established after cereal (CE) and a field pea (PE) in order to create different N residual levels to test the effect of the previous crop on CC biomass production, N retention and N2O emissions. Winter triticale was sown at a rate of 95 kg ha<sup>-1</sup>. The PE was sown at a rate of 260 kg ha<sup>-1</sup>. The experiment was laid out as a split plot design (block, previous crop, and cover crop) with three different CCs and two controls as treatments, replicated four times. Plots size were 4.5  $\times$  8.5 m. Three different cover crop treatments and two different controls were implemented, as described below. A timeline of the experimental sowing, biomass sampling, and harvesting is presented in Fig. 2.

The different CCs used in the experiment consisted out of three different CC species with different winter hardiness. A frost-killed white mustard (WM; *Sinapis alba* cv. Litember), a frost-tolerant turnip rape (TR; *Brassica rapa L.* var. silvestris cv. Jupiter) and a winter-hardy perennial ryegrass (RG; *Lolium perenne* cv. Kubus). The seeding rates for WM and RG were 20 kg ha $^{-1}$  and 12 kg ha $^{-1}$  for TR. Plots under green fallow (GF; natural vegetation, volunteer pre-crop) and bare fallow (BF) served as the controls.

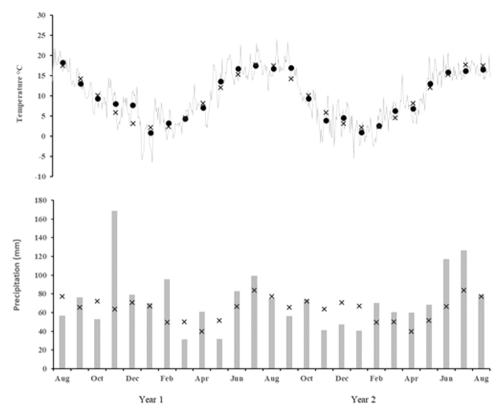


Fig. 1. Daily mean air temperature (°C) (monthly average temperature indicated with black dots) and cumulative monthly precipitation at the experimental site during the two experimental years (August 2015 to August 2017). Long-term (1991–2020) averages for temperature and precipitation indicated with x.

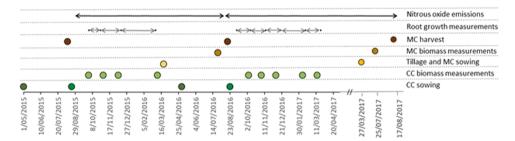


Fig. 2. Timeline of selected field operations with the main crop (MC)/pre-crop (either a field pea or a cereal) and cover crops (CC) either ryegrass undersown in May or white mustard, and turnip rape sown after harvest of the main crop (MC).

In late May, the RG treatment was undersown into the main crop. The other CCs (WM and TR) were sown after harvest of the main crop in late August. In March, all CC were incorporated into the soil by mulching, roto-tilling and ploughing to a depth of 25 cm for seedbed preparation of the subsequent crop. Based on the six-year crop rotation, the succeeding crops following the CC were PE after CE and oats after PE. A pneumatic drill was used to sow all cover and main crops at a row distance of 12.5 cm.

A previous study was done by Böldt et al. (2021) using different cover crops to evaluate the effect on N losses in the form of N leached over the winter period in northern Germany. The aforementioned study used the same treatments and was carried out under the same conditions as the current study. The current study is therefore an extension of the aforementioned study where the focus of the current study was on investigating  $N_2O$  emissions as a possible pathway for N loss. Further information pertaining all treatments and N leaching results can be found in the published version by Böldt et al. (2021).

# 2.3. N<sub>2</sub>O measurements

Nitrous oxide flux measurements were conducted using the static chamber method (Hutchinson and Mosier, 1981). Basal rings made from polyvinyl chloride (PVC) (60 cm diameter and 20 cm height) were installed to a soil depth of 10 cm in each plot. These collars were only removed briefly during harvesting and tillage operations. The PVC chambers (60 cm diameter and 35 cm height) were deployed onto the basal rings during measurement and secured with a butyl rubber band to avoid any gas leakages.

Four gas samples were collected weekly between 10:00 and 11:00 am. The first gas measurement was taken immediately after the deployment of the chambers onto the basal rings, and subsequently after 20, 40, and 60 min. A fan within each chamber allowed for homogenized air conditioning before sampling. Samples were obtained using a 30 mL syringe through a septum cap located at the top of the chamber and were immediately transferred into 12 mL pre-evacuated exetainers (Labco, High Wycombe, UK).

Gas samples were analyzed for the presence of N2O using a gas

chromatograph (SCION 456-GC, Bruker, Leiderdorp, Netherlands) equipped with a  $^{63}$ Ni electron-capture-detector using He as carrier gas. Samples were injected using an autosampler (model 271 LH, Gilson Inc., Middleton, WI, USA). Data were processed using the software Compass CDS (Version 3.0.1). The N<sub>2</sub>O flux was calculated based on the increased chamber headspace concentration within 60 min of chamber deployment. The change of gas concentration in the chamber headspace during measurement was calculated through means of linear regression.

#### 2.4. Plant and soil analysis

The belowground biomass (BGB) were measured during the CC season using the ingrowth core method with three cores per plot. For this measurement, mesh bags (synthetic fiber net, mesh size 1 mm, diameter 4 cm and length 60 cm) were filled with pre-sieved (≤1 mm) and rootfree topsoil from the same experimental site and placed into cores. These cores were installed into the soil at an angle of 45° relative to the soil surface and at a vertical depth of 30 cm (Steingrobe et al., 2000). The ingrowth cores were installed in November and remained in the soil until the end of March. After sampling the cores, the roots were washed over a 0.63 mm sieve and manually separated from other soil constituents. The belowground net primary production was provided by the cumulative root growth in the bags over the entire period. The root material was dried at 58  $^{\circ}$ C, milled in a ball-mill and the C- and N content (belowground N) were determined through the DUMAS combustion method in a C/N-Analyzer (Vario Max CN, Elementar Analysensysteme, Hanau, Germany).

The aboveground biomass (AGB) of the CCs was sampled throughout the CC growth period using quadrants (0.25  $\rm m^2)$  by cutting the plant material to ground level. Above- and belowground samples from each plot were dried (48 h at 40  $^{\circ}$ C) and milled (Cyclotec mill, Foss, Hillerød, Denmark) to a particle size of 1 mm to use for further analyses. Subsequently all samples were analyzed for C, N and ash contents as explained above.

The volumetric water content as a fraction of the total pore space was determined and used to calculate the water filled pore space (WFPS). A particle density of  $2.65~{\rm g~cm^{-3}}$  was assumed.

$$WFPS(\%) = \frac{SWC}{1 - \left(\frac{BD}{PD}\right)} \quad x100$$

where WFPS is the water filled pore space (%), SWC is the volumetric soil water content (vol%), BD is the soil bulk density (1.53 g cm $^{-3}$ ) and PD refers to the particle density.  $N_{min}$  content was defined as the sum of the  $NO_3^-$ -N and  $NH_4^+$ -N contents.  $N_{min}$  (kg ha $^{-1}$ ) was calculated using the appropriate bulk densities for the corresponding soil depths.

### 2.5. Statistical analysis

The statistical software R 4.2 (2022) was used to evaluate the data. The data evaluation started by defining an appropriate statistical linear model. Data distribution was assumed to be normal and heteroscedastic with regard to the different soil cultivation and fertilization treatments. These assumptions were based on a visual graphical residual analysis (Kozak and Piepho, 2018). The statistical model included Experimental year \* pre-crop \* CC treatment as well as their interaction term as fixed factors. Significance of factors was declared at p < 0.05. Based on this model an analysis of variance (ANOVA) was conducted to test the hypothesis of the experiment. Furthermore, multiple contrast tests (e.g., see Bretz et al., 2011) were implemented in order to compare the several levels of the tested treatments. Furthermore, linear regression analyses were used to predict the relationship between accumulated N2O-N losses (kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup>) in relation to increased levels of N in the roots (kg N ha<sup>-1</sup> year<sup>-1</sup>) as well as the nitrate (NO<sub>3</sub>-N) (kg N ha<sup>-1</sup> year<sup>-1</sup>) levels in the soil in early spring and winter.

#### 3. Results

#### 3.1. Daily N2O fluxes

The daily N2O fluxes over the two experimental years (August 2015 to August 2017) for the various CCs treatments with either a field pea or a cereal as a pre-crop are shown in Fig. 3. We observed N<sub>2</sub>O emissions to be episodic with small fluxes throughout the measurement period (0.006 kg N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup> on average), except in winter in the first year, where higher fluxes were observed (0.01 kg  $\rm N_2O\text{-}N~ha^{-1}~day^{-1}$  on average). The WFPS was also highest during the winter months in year one, likely as a result of the higher precipitation associated during these months in northern Germany. Daily N2O emission spikes were generally lower in the second year compared to the first year and were associated with higher N<sub>min</sub> values in the first year. The daily N<sub>2</sub>O-N fluxes ranged up to  $\sim 0.8 \text{ kg N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$  in the BF treatment. High daily  $N_2\text{O}$ fluxes were observed in the GF and BF treatments, which occurred during January and February 2016 and reached peak fluxes of around 0.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> day<sup>-1</sup>, respectively. From our results, we observed that a field pea as pre-crop resulted in higher daily N<sub>2</sub>O emissions compared to a cereal as pre-crop during both years of the experiment.

#### 3.2. Accumulated N2O fluxes

The mean accumulated N2O emissions of the various CCs are shown in Fig. 4 and averaged 1.7  $\pm$  0.8 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup> over the twoyear trial period. Accumulated N2O emissions ranged from 1.1 to 3.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup> during the first year of study and in the second from 0.8 to 1.9 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup>. The highest accumulated N<sub>2</sub>O emissions (3.3 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup>) were observed in year one in both the WM and TR treatments whereas the BF treatment resulted in the lowest accumulated  $\rm N_2O$  emissions (0.8 kg  $\rm N_2O\text{-}N~ha^{-1}~year^{-1})~during$ the second year. Pre-crop had an effect on the accumulated N2O emissions. A significant difference was found between the WM treatment compared to the other treatments in the first year of study with a cereal crop as pre-crop. The RG treatment had higher accumulated N2O emissions compared to the WM, GF and BF treatments with a field pea as pre-crop in the second year, but RG only differed from BF when cereal served as pre-crop. The BF treatment differed from all other treatments in the second year when a field pea served as pre-crop. Pre-crop had a significant effect in year one between treatments TR, WM and GF. Accumulated emissions were reduced from the first to second year of study over all treatments. Emissions were reduced as much as 2.1 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup> in the WM treatment from the first to second year. Significant differences were also observed between years for the TR and GF treatments when the field pea served as pre-crop. The only difference observed between years, when cereal served as the pre-crop, was observed in the WM treatment. The total amount of accumulated N2O emissions over all treatments were reduced from 14.4 to 7.3 kg N2O-N ha<sup>-1</sup> year<sup>-1</sup> from year one to year two when a field pea served as precrop and reduced from 6.5 to 5.6 kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup> when a cereal crop served as pre-crop.

# 3.3. Nitrogen uptake above and belowground

The highest aboveground N uptake was observed in the TR treatment (118.2 kg ha $^{-1}$ ) and the lowest value (2.3 kg ha $^{-1}$ ) was observed in the BF treatment, which also differed significantly from all other treatments in both experimental years (Table 1). Both the TR and WM treatment, compared to the other treatments, had a higher N uptake aboveground in year one when a cereal was used as pre-crop. However, the aboveground N uptake for these two treatments (TR and WM) were significantly reduced in the second year of study compared to the first year. Within the first experimental year, pre-crop significantly had an effect on the aboveground N-uptake in the RG and GF treatments. The BF treatment differed from all other treatments in both years. The results

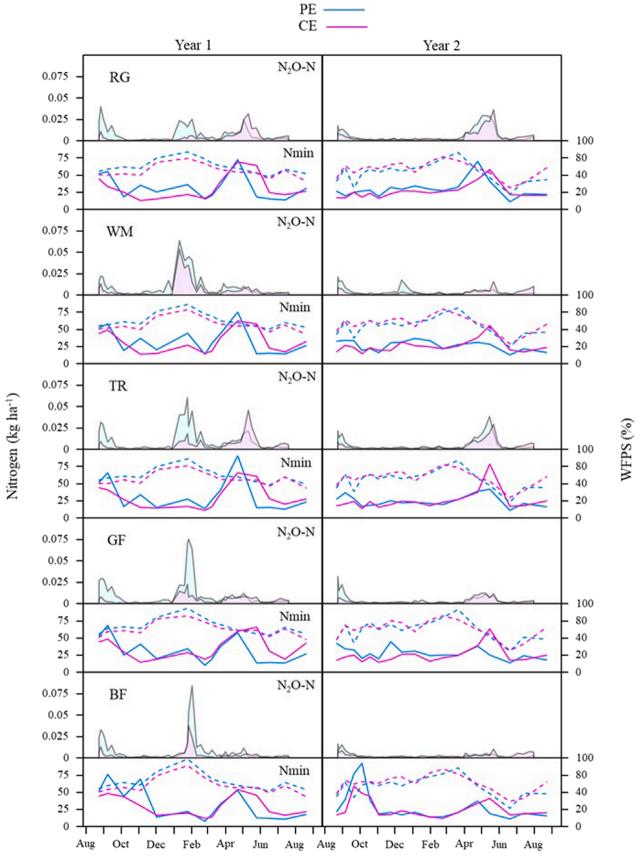


Fig. 3. Daily  $N_2O$ -N fluxes,  $N_{min}$  ( $NH_4^+$ -N and  $NO_3$ -N) and WFPS (%) of the various catch crops over the two experimental years. A field pea (PE) or a cereal (CE) served as pre-crop. RG = ryegrass, WM = white mustard, TR = turnip rape, GF = green fallow, BF = bare fallow.  $N_{min}$  is presented by the solid line and the WFPS (%) is presented by the dashed line.

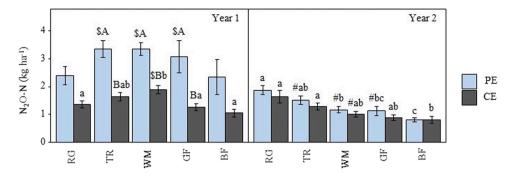


Fig. 4. Accumulated  $N_2O$  emissions (kg  $N_2O$ -N ha<sup>-1</sup> year<sup>-1</sup>) of the various CCs over the two experimental years. A field pea (PE) or a cereal (CE) served as pre-crop. RG = ryegrass, WM = white mustard, TR = turnip rape, GF = green fallow, BF = bare fallow. Small letters indicate significant differences between treatments, capital letters between pre-crop and \$ and # between years. Standard errors are shown.

**Table 1**Above-, belowground and total nitrogen uptake (kg ha<sup>-1</sup>) of the various CCs over the two experimental years. A field pea (PE) or a cereal (CE) served as pre-crop. RG = ryegrass, WM = white mustard, TR = turnip rape, GF = green fallow, BF = bare fallow. Small letters indicate significant differences between treatments, capital letters between pre-crop and \$ and # between years. Standard errors are shown in brackets.

Variable	Year	Pre-Crop	RG		TR		WM		GF		BF	
Naboveground	1	PE	89.0 <sup>Aa</sup>	(11.76)	118.2ª	(13.09)	113.1 <sup>a</sup>	(9.40)	97.5 <sup>Aa</sup>	(14.75)	3.6 <sup>b</sup>	(1.52)
(kg ha-1)		CE	$43.0^{Ba}$	(3.28)	74.2 <sup>\$b</sup>	(4.68)	91.2 <sup>\$b</sup>	(5.40)	$37.8^{Ba}$	(7.38)	2.3 <sup>c</sup>	(0.56)
	2	PE	100.7 <sup>a</sup>	(8.64)	66.7 <sup>a</sup>	(15.12)	74.2 <sup>a</sup>	(15.62)	84.3 <sup>a</sup>	(16.33)	$2.9^{\mathrm{b}}$	(0.46)
		CE	$71.7^{a}$	(16.88)	32.9 <sup>#a</sup>	(4.95)	35.7 <sup>#a</sup>	(8.40)	$32.0^{a}$	(7.12)	$3.6^{\mathrm{b}}$	(0.26)
$N_{belowground}$	1	PE	30.2 <sup>\$a</sup>	(2.92)	17.8 <sup>\$ab</sup>	(1.50)	$12.0^{\rm b}$	(1.85)	13.6 <sup>ab</sup>	(3.44)		
(kg ha-1)		CE	43.6 <sup>a</sup>	(3.57)	31.0 <sup>ac</sup>	(2.67)	17.3 <sup>bc</sup>	(1.94)	$21.3^{$bc}$	(3.44)		
	2	PE	$50.9^{\#a}$	(7.84)	37.7 <sup>#ab</sup>	(2.67)	$21.8^{b}$	(1.59)	31.5 <sup>ab</sup>	(4.04)		
		CE	$41.3^{a}$	(7.57)	44.7 <sup>a</sup>	(2.62)	31.7 <sup>a</sup>	(1.81)	45.3 <sup>#a</sup>	(5.55)		
$N_{total}$	1	PE	119.0 <sup>a</sup>	(12.50)	90.7 <sup>a</sup>	(21.20)	83.4 <sup>a</sup>	(19.00)	111.0 <sup>a</sup>	(16.70)	$3.6^{b}$	(1.52)
(kg ha <sup>-1</sup> )		CE	86.6 <sup>a</sup>	(5.10)	70.1 <sup>a</sup>	(15.40)	$72.3^{a}$	(15.90)	59.1 <sup>a</sup>	(9.60)	$2.3^{\rm b}$	(0.56)
	2	PE	152.0 <sup>a</sup>	(13.60)	91.9 <sup>a</sup>	(91.90)	88.7 <sup>a</sup>	(18.60)	$116.0^{a}$	(16.40)	$2.9^{\mathrm{b}}$	(0.46)
		CE	113.0 <sup>a</sup>	(12.70)	62.7 <sup>a</sup>	(62.20)	56.8 <sup>a</sup>	(12.30)	77.3 <sup>a</sup>	(7.70)	$3.6^{\mathrm{b}}$	(0.26)

also indicate that RG is the only treatment where a high aboveground N uptake was observed in both experimental years.

Overall the N uptake belowground increased over all treatments (except RG with a cereal as pre-crop) from the first to second year of the experiment and regardless of pre-crop. The highest belowground N uptake were observed in the RG treatment (50.9 kg ha<sup>-1</sup>) and the lowest value (12 kg ha<sup>-1</sup>) was observed in the WM treatment (Table 1). Significant differences were found between the RG and WM treatments except in the second year when cereal was used as a pre-crop. Moreover, no differences between the treatments were found in the second year of the experiment when a cereal was used as pre-crop. Significant increases in N uptake belowground was observed between experimental years in the RG and TR when a field pea served as pre-crop. The same was

observed for the GF treatment but with a cereal as pre-crop.

The relationship between accumulated  $N_2O$ -N losses (kg  $N_2O$ -N  $ha^{-1}$ ) in relation to  $N_{belowground}$  (kg N  $ha^{-1}$ ) as well as the nitrate (kg N  $ha^{-1}$ ) levels in the soil in late autumn and early spring are shown in Fig. 5

The accumulated  $N_2O$ -N losses (kg  $N_2O$ -N ha $^{-1}$  year $^{-1}$ ) decrease as a result of increasing N found belowground (kg N ha $^{-1}$  year $^{-1}$ ). The RG treatment indicated the most N stored belowground (Table 1) compared to the other treatments. Treatments such as TR and WM, especially within the first year, had less N stored belowground (kg N ha $^{-1}$  year $^{-1}$ ) and led to the most  $N_2O$ -N losses (kg  $N_2O$ -N ha $^{-1}$  year $^{-1}$ ). The nitrate concentration (kg N ha $^{-1}$  year $^{-1}$ ) had a weak relationship with  $N_2O$ -N losses (kg  $N_2O$ -N ha $^{-1}$  year $^{-1}$ ) in early spring. However, a field pea (PE)

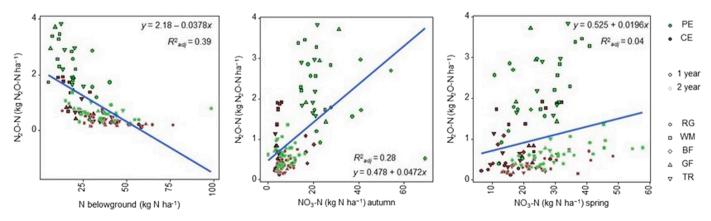


Fig. 5. The linear relationships between accumulated  $N_2O$ -N losses (kg  $N_2O$ -N ha<sup>-1</sup>) in relation to increased levels of N in the roots (kg N ha<sup>-1</sup>) as well as the nitrate (NO<sub>3</sub>-N) (kg N ha<sup>-1</sup>) levels in the soil in late autumn (November) and early spring (March).

used as pre-crop resulted in higher accumulated N<sub>2</sub>O-N losses (kg N<sub>2</sub>O-N ha<sup>-1</sup> year<sup>-1</sup>) within the first year during early spring. A stronger relationship was observed between accumulated N2O-N losses (kg N2O-N ha<sup>-1</sup> year<sup>-1</sup>) and the nitrate concentration (kg N ha<sup>-1</sup> year<sup>-1</sup>) in late autumn. The field pea (PE) used as pre-crop led to higher accumulated  $N_2O$ -N losses (kg  $N_2O$ -N ha<sup>-1</sup> year<sup>-1</sup>), especially in the first year. Less nitrate (kg N ha<sup>-1</sup> year<sup>-1</sup>) was found in the soil during autumn compared to early spring. Generally the roots of the CCs take up the N which leads to low N losses in the form of leaching (Böldt et al., 2021). Furthermore, Fig. 5 depicts this relationship and highlights that when N is captured belowground through the CCs, losses of N through other pathways are generally considered to be low. Furthermore, Fig. 5 indicates the ability of CCs to capture N belowground from autumn to spring and consequently shows the relatively low effect on N2O emissions over the winter period. From the previous study done by Böldt et al. (2021), which indicated low leaching values, and from the current study further showing low N2O emissions, we observed that N2O emissions as a result of pollution swapping due to low N leaching losses is not occurring.

#### 4. Discussion

#### 4.1. Environmental factors on measured N2O fluxes

Environmental variables such as soil moisture and temperature are key drivers influencing N<sub>2</sub>O diffusion between the soil and atmosphere (Signor and Cerri, 2013). A look into the near future could also mean that raising temperatures together with more frequent events of unstable weather patterns could further enhance the release of N2O emissions under certain favorable conditions. During the first year of the study, we observed an increase in temperature from January (-9.5 °C) up to 11.2°C in February. This increase in temperature, together with the high soil moisture, led to good soil conditions for N to be mineralized. Furthermore, these conditions affect the relative rates of nitrification and denitrification (Dobbie and Smith, 2001; Firestone and Davidson, 1989) by influencing the microbial activity (Oertel et al., 2016; Signor and Cerri, 2013). These processes of nitrification and denitrification are therefore accelerated (Akiyama et al., 2000; McAuliffe et al., 2020) and, as a result, increased N2O fluxes were observed in the current study. This pattern of N2O fluxes indicates the high sensitivity of N2O emissions to short-term fluctuations according to weather as well as the physical soil conditions, i.e. WFPS. It is therefore important to highlight that more frequent weather events would imply that mitigation options need to be investigated and applied so that agriculture could find solutions to adapt to such future events and conditions.

The highest daily N2O fluxes in the current study were observed when WFPS was close to or higher than 60% in year one (Fig. 3). Gao et al. (2014) also reported the highest production of N<sub>2</sub>O occurs around 60% WFPS. The probability of high N<sub>2</sub>O peaks is further enhanced with the incorporation of decaying herbage and under the lack of aeration in the soil due to high water content or low porosity (Li et al., 2016). Therefore, the elevated N2O fluxes observed in the current study were coinciding with water saturated soils and further enhanced at high temperatures. In the second year, the WFPS reached almost the same values as in the first year but the N2O fluxes were noticeably lower and seem to have been driven by high soil mineral N, rather than the WFPS. It has been reported that the management of CCs effects N cycling bacteria communities in the soil (Cazzaniga et al., 2023; Romdhane et al., 2019). Furthermore, soil properties impacts denitrifier abundance and bacterial communities (Jha et al., 2017) linked to denitrification (Bowen et al., 2020). It is possible that DNA extraction analysis to determine dentrifiers from soil could provide additional information about the denitrification potential of the experimental site. This, however, points to a limitation in the study which could have given insight on how soil-denitrifying bacteria are influenced by field management as well as the CC strategy.

#### 4.2. The effect of pre-crop on annual N<sub>2</sub>O emissions

The associated daily N2O flux spikes were less intense in the second experimental year of the current study compared to the first experimental year. The sequence of pre-crops had an effect on N losses, however, the CCs used in the study can act as a mitigating strategy towards the reduction of N<sub>2</sub>O emissions and N leaching in organic systems. Legumes are often used in low-input systems to fixate N. Furthermore, it has been shown that legumes have low N<sub>2</sub>O emissions (Schmeer et al., 2014). Therefore, legumes are often used as mitigation strategy to lower N losses to the environment. However, our field experiment indicates differences in pre-crop effects between experimental years caused by various factors. One of these factors could be the incorporation from plant residues which are associated with enhanced N2O emissions. The incorporation of residues by tillage increases soil respiration and N2O fluxes as a result of microbial stimulation (Krauss et al., 2017). It is evident from the current study that differences were found between pre-crop used and that a field pea (PE) used as pre-crop led to higher N<sub>2</sub>O emissions when compared to a cereal (CE) used as pre-crop. The same was observed in Benoit et al. (2015), which found the highest N<sub>2</sub>O emissions to crops after legumes. Moreover, Rochette and Janzen (2005) mentioned that one should expect higher N<sub>2</sub>O emissions from legume residues than from cereals as legumes have easily degradable N and C. Pugesgaard et al. (2017) supports this finding and concluded that legume rich CCs are an important source of N2O emissions. We also observed this in our linear regressions (Fig. 5) which indicated higher N2O losses for the PE compared to the CE, especially in the first year. This is particularly true after spring. The N<sub>2</sub>O losses are most likely the result of decomposing PE residues rather than the incorporation of the CCs. The CCs are successfully removing excess N from the soil when the two experimental years are being compared.

The synchronization between N mineralization following CC termination and the N demand of the subsequent crop are factors to consider, apart from the critical N uptake of the CC. In the current study, a high residual mineralizable N was measured after PE which confirms a high N surplus and therefore a high mineralization rate after the harvest. Therefore, the risk of N leaching and N lost as N<sub>2</sub>O were increased. The biomass N and the C:N ratio of the CC residue, time of CC termination and incorporation as well as prevailing weather conditions will affect the potential transfer of N to the subsequent crop. From our results, the field pea used as pre-crop increased the biomass of the CC, due to increased N<sub>min</sub> after harvest. However, in the second year, the CCs were much more effective in utilizing N compared to the bare fallow treatment. This makes farm management difficult to synchronize N uptake from plants with the N release from crop residues because of a great diversity of N mineralization patterns. This will affect the farmers' decision in terms of planting, harvesting and choosing which CCs to use. Furthermore, changing weather patterns will also contribute to management difficulties.

Background emissions of  $N_2O$  deriving from the mineralization of soil organic matter may exceed the  $N_2O$  emissions as a result of N input in the same year (Bouwman et al., 2002). This could help explain why we observed higher  $N_2O$  emissions in the first year compared to the second year in our study. It may occur that soil N and organic C influence  $N_2O$  emissions from organically managed soils comparatively to the N input (Skinner et al., 2014).

#### 4.3. Does the cover crop reduce the likelihood for $N_2O$ -losses?

A consensus in literature does not yet exist regarding the effect of CCs on  $N_2O$  emissions. A meta-analysis conducted on  $N_2O$  fluxes from agricultural soils under organic and non-organic management reported a mean average value of  $3.22\pm0.85\ kg\ N_2O\text{-N}\ ha^{-1}\ year^{-1}$  in organic arable systems (Skinner et al., 2014). The mean value over treatments from the current study (1.69  $\pm$  0.80 kg  $N_2O\text{-N}\ ha^{-1}\ year^{-1})$  was almost half of that, which indicates that the values obtained from our study are

at the lower end of emission values reported in organically managed, low-input systems.

We observed an increase in N2O peaks which occurred during winter in year one and in summer during year two. However, it is known that fluxes of N2O are closely related to environmental conditions as well as soil characteristics (Davidson and Swank, 1986). The risk of high daily N2O fluxes are enhanced under drying/wetting, compact and/or wet soil, mechanical mixing of crop residues into the soil and freezing/thawing cycles (Hansen et al., 2019). The peaks in N2O fluxes observed were likely due to higher rainfall and freeze/thaw cycles during winter (Fig. 1) in the first year and highly mineralizable N in the soil paired with optimal soil conditions during the summer of the second year. Frost-killed CCs are commonly used on farms as management strategy instead of tillage, to incorporate crop residues into the soil. In this case, tillage can be reduced as well as the associated N2O emissions. However, in areas that receive high rainfall during winter, especially on sandy soils, the risk of N-leaching is increased (Böldt et al., 2021; Smit et al., 2021). Furthermore, frost-killed CCs also have the potential to increase N<sub>2</sub>O emissions as easily available N and degradable C are released from cells blasted by frost. However, the frost-killed CC (WM) used in our study did not lead to higher N<sub>2</sub>O emissions compared to the other treatments, indicating a different pathway of N loss and possibly pollution swapping.

All treatments in the current study showed an increase in N2O fluxes in February 2016 when temperatures rose from below zero to about 11 °C. In the first year of our study, we observed that the frost-killed CC (WM) resulted in higher N2O emissions compared to the winter hardy CC (RG). However, during the second year, the opposite occurred and the frost-killed CC (WM) led to less N2O emissions compared to the winter hardy CC (RG) treatment. Frost which occurred in January in the experimental year 2016 of the current study, most likely killed off the white mustard CC. This could have led to higher levels of N in the soil as white mustard (specifically the leaves) produce high N litter materials that can undergo fast degradation (Gentsch et al., 2022). Consequently, N2O fluxes can increase during thawing of the soil as a result of this easily degradable C and N in the plant material. Furthermore, roots and stubble are left when CCs die off during frost which suggests that N and C in roots of frost-sensitive CCs can be an important driver of N2O emissions after thawing (Hansen et al., 2019). The same was observed in Westphal et al. (2018) when high daily N<sub>2</sub>O fluxes were observed during the winter/spring thaw cycle.

The frost sensitive WM CC had the highest  $N_2O$  emissions with the cereal used as pre-crop. Fluxes were low until the onset of frost which occurred in January in the first year of the study (Fig. 3). The same observation was made in Hansen et al. (2019) which reported elevated levels of  $N_2O$  emissions after frost. However, even though the WM CC resulted in the highest  $N_2O$  emissions in the first year in our experiment, the value is still considered to be low when compared to values found in literature. For this reason, the benefits of using a frost sensitive CC, at least in our study, outweighs the negative associated environmental effects.

In the current study, the  $N_2O$  emission measurements were carried out on a weekly basis. We have to acknowledge the fact that some values for freezing and thawing events, which might have occurred outside of the measurement period, could have been missed as a result of weekly measurements. This could have led to the underestimation of the total  $N_2O$  emissions. On the other hand, the use of linear interpolation of  $N_2O$  fluxes could also overestimate the  $N_2O$  emissions if such peak events would have been recorded. The usage of Automated chambers, with a higher temporal resolution of measurements, could have been used to improve the significance of results. However, the static closed chamber method used in the current study, is a well-known method accepted and adopted in  $N_2O$  measurement trials.

#### 4.4. The role of cover crops on the N-budget in arable systems

Nitrogen inputs often exceed the N outputs on farming systems causing an imbalance of N which could reach more than the quality standard of 11.3 mg NO<sub>3</sub>-N L<sup>-1</sup> and pose a threat to groundwater bodies. Cover crops have been proven to increase soil health, crop performance and reduce N leaching through the uptake of N that would otherwise be lost (Scavo et al., 2022). From the current study, we observed the N<sub>2</sub>O losses from CCs to be at the lower spectrum of values reported elsewhere. The RG CC used in our study showed comparable N2O emissions to the other CC although more N was accumulated in the above- and belowground biomass. Furthermore, this aboveground N could be removed through harvesting or be grazed by animals, further reducing excess N on soils and closing the N cycle. In general, the belowground N uptake increased from year one to year two which indicates less N losses to the environment. The same was observed in a study by Wang et al. (2021) which showed that the presence of ryegrass used as CC decreased the nitrate content in soil as well as led to low N2O emissions.

Böldt et al. (2021) demonstrated the ability of different CCs to take up residual N as well as reduce N leaching when compared to the control, a bare fallow over autumn/winter. In the same study, the frost-killed CC showed higher N leaching compared to the other CCs. This in turn could explain our results from the current study which showed that the frost-killed CC had low  $N_2O$  emissions, compared to the other treatments. A bigger proportion of N is therefore lost in the form of leaching rather than being emitted as  $N_2O$  and the possibility of pollutant swapping is occurring to some extent. However, according to Böldt et al. (2021), Smit et al. (2021) and Vogeler et al. (2022), using CCs over the winter period resulted in low N leaching. From the current study, low  $N_2O$  emissions were also observed. For this reason, we cannot agree that pollutant swapping is occurring to a large extent within our low-input system.

Furthermore, the RG used as CC had a much higher N uptake compared to the green and bare fallow treatments. Meisinger et al. (1991) also concluded that grass species are much more efficient in reducing N leaching. A higher N content in belowground biomass was observed for our RG treatment compared to the other treatments. This is in line with higher amounts of N allocated to roots from grass species compared to non-grass species (Pietola and Alakukku, 2005; Redin et al., 2018). This indicates that incorporating grass CCs have the ability to capture N and use it more efficiently. This in turn could lead to more effective N cycling and overall decrease the N surplus thereby containing N within the cycle and adding to a better N budget on-farm in low-input systems.

# 5. Conclusion

Our results indicate that incorporating CCs, at least during one year, had higher emissions compared to the bare fallow treatment. However, the  $N_2 O$  emissions observed are still considered to be low. The frost-killed CC did not result in higher  $N_2 O$  emissions compared to the other CCs used in this study. However, mineralization of residues from frost-killed CC can still lead to other pathways of N losses and therefore the possibility of nutrient swapping still exists. Even though the RG CC treatment lead to slightly higher  $N_2 O$  emissions compared to the control, it is still considered to be low and therefore the added benefits of including grass as CC in field rotations outweighs the negative associated environmental effects. Furthermore, the grass CC had a higher uptake of N indicating additional benefits in terms of closing the N gap and improving on-farm N budgets in low-input systems.

# CRediT authorship contribution statement

**Reinsch Thorsten:** Conceptualization, Visualization, Writing – review & editing. **Kluß Christof:** Data curation, Formal analysis, Writing – review & editing. **Taube Friedhelm:** Supervision, Writing – review &

editing. **Loges Ralf:** Conceptualization, Methodology, Project administration. **Smit Hendrik Petrus Jordaan:** Visualization, Writing – original draft, Writing – review & editing. **Böldt Matthias Johannes:** Formal analysis, Investigation, 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.

#### Data Availability

Data will be made available on request.

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