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ORIGINAL ARTICLE

Nitrification inhibitors reduce N_2O emissions induced by application of biogas digestate to oilseed rape

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Abstract Winter oilseed rape (WOSR) is the major oil crop cultivated in Europe and the most important feedstock for biodiesel. Up to 90% of the greenhouse gas (GHG) emissions from biodiesel production can occur during oilseed rape cultivation. Therefore, mitigation strategies are required and need to focus on direct nitrous oxide (N_2O) emission as one of the largest GHG contributors in biodiesel production. Earlier studies show that nitrification inhibitors (NIs) can reduce N_2O emissions derived from N-fertilization. Since information on the effect of biogas digestates with or without NIs on N_2O emissions from WOSR fields is scarce, the aim of this study was to evaluate their effects on N_2O emissions, mineral N dynamics, and oil yield in WOSR production fertilized with digestate. The study was conducted at five sites across Germany over three years resulting in 15 full site-years data sets. Across all sites and years, N_2O emission from WOSR fertilized with biogas digestate (180 kg NH_4^+-N $ha^{-1}yr^{-1}$) ranged between 0.2 and 3.5 kg N_2O–N ha^{-1} yr^{-1}. Due to the reduction of the

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nitrate concentrations following digestate application, application of NI significantly reduced annual N_2O emission by 36%. Our results demonstrate that NI can be an effective measure for reducing N_2O emissions from digestate application, but its effectiveness depends on soil and weather conditions, and ultimately on the site-specific potential for N_2O production and release. There was no effect of NI application on grain and oil yield.

Keywords GHG reduction · N-fertilization · Winter oilseed rape · Nitrification inhibition · Yield-related emission · Biodiesel

Introduction

During the last decade, demand of biofuel in Europe has been growing with rapeseed oil as it is the most important feedstock (Hamelinck et al. 2012; Aldhaidhawi et al. 2017). Oilseed rape (*Brassica napus* L.) production in the European Union increased by 38% between 2000 and 2014 (FAOSTAT 2017). However, there has been a long controversial discussion whether rapeseed cultivation for biofuel production is environmentally sound. The potent greenhouse gas nitrous oxide (N_2O) is emitted during the cultivation of feedstock in the field, accounting for 75 to 90% of the total GHG emissions in biodiesel production (66.7–119.5 g CO_2 MJ_{fuel}^{-1}; Hoefnagels et al. 2010). Nitrous oxide contributes to both the greenhouse effect (e.g., 100-year Global Warming Potential of 298; Myhre et al. 2013) and to stratospheric ozone depletion (Crutzen 1981; Ravishankara et al. 2009). More than half of the entire anthropogenic N_2O emission is originated from agricultural soils (IPCC 2006). Nitrification and biological denitrification are main sources for N_2O production in soils (Bremner 1997). Apart from these two processes, the contribution of further microbial and chemical N transformations to the total N_2O release from soils, such as i.e. nitrifier-denitrification or chemo-denitrification, is currently discussed (Shaw et al. 2006; Butterbach-Bahl et al. 2013). Since all processes of N_2O production in soils rely on mineral N as a substrate, N-fertilization has frequently shown to enhance N_2O emissions from agricultural soils (Stehfest and Bouwman 2006; Jungkunst et al. 2006; Kaiser and Ruser 2000).

In the European Union, primary energy production from biogas production has increased from 2.5 billion m^3 methane equivalent in 2000 to 18 billion m^3 in 2015, representing half of the global biogas production (Scarlat et al. 2018). The residual digestates after anaerobic digestion of the biogas feedstocks are valuable fertilizers which gain importance with increasing biogas production. To close the nutrient cycle within renewable energy production, digestate from biogas plants are promoted as a substitute for mineral N-fertilizer. Anaerobic digestion changes the chemical composition of the biogas substrate, resulting in higher NH_4^+ contents, higher pH values and lower carbon contents (Möller and Müller 2012; Wolf et al. 2014). The application of organic N-fertilizers such as digestates on soils might result in small-scale anaerobic zones with increased oxygen consumption due to the input of easily available C-sources favoring N_2O release from denitrification (Flessa and Beese 1995) and thus increasing N_2O emissions when compared to mineral N-fertilizer application (Jones et al. 2007).

Nitrification is one of the main sources of N_2O formation in soils and it provides NO_3^- which serves as an initial substrate for N_2O production via denitrification. Nitrification inhibitors (NIs) inhibit the enzyme ammonia monooxygenase (AMO) which catalyzes the first step of the nitrification process carried out by microorganisms (oxidation of NH_4^+ to hydroxylamine), thus stabilizing NH_4^+. It has often been reported that application of organic fertilizers with NIs reduces NO_3^- leaching, increases N-use efficiency and enhances yields (i.e. Di and Cameron 2007; Fangueiro et al. 2009). A meta-analysis from Abalos et al. (2014) showed an increase in productivity of approximately 5% when N-fertilizers were applied with an NI. However, this study included only one experiment with an organic fertilizer (cattle slurry) other than urine.

Due to the reduction of substrate availability for microbial N_2O production, NIs are a promising tool for N_2O mitigation. Akiyama et al. (2010) and Ruser and Schulz (2015) reported a N_2O mitigation potential

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of approximately 35% by using NIs. The exploitation of this mitigation potential depends widely on site-specific conditions such as weather, soil properties and management practices (Volpi et al. 2017). To date, only few studies investigated the effect of NIs on N_2O emissions from soils fertilized with biogas digestates (e.g. Severin et al. 2016; Wolf et al. 2014). Under laboratory conditions (50 days), N_2O emission from a digestate treatment with 3,4-dimethylpyrazole phosphate (DMPP) was 70% lower than from a treatment without NI (Severin et al. 2016). Wolf et al. (2014) reported a similar reduction of the N_2O emission between 37 and 62% in the first weeks following digestate application under field conditions. However, despite this high short-term mitigation, the annual N_2O emission did not differ significantly between the treatments of Wolf et al. (2014).

Due to the paucity of information on the impacts of biogas digestates in WOSR production at the present, the aims of this study were to quantify the effects of a nitrification inhibitor added to digestate on N_2O emission, mineral N dynamics, and WOSR oil yield. We further aimed to quantify whether it would be possible to reach the CO_2 reduction goals of the European Directive on the promotion of the use of energy from renewable sources (EU-RED-II, Annex V: EC 2018) with biogas digestates as N-fertilizers in WOSR.

Material and methods

Study sites, experimental design and management

This 3 y study encompassed five experimental sites geographically located in areas representative of WOSR production across Germany (Table 1). A companion report by Ruser et al. (2017) provided further details. Three experimental sites where established in northern Germany (Hohenschulen, Dedelow and Berge), where is the main WOSR cultivation area. One site was located in central Germany (Merbitz) and one site in southern Germany (Ihinger Hof). Soil and environmental characteristics of each study sites are shown in Table 1.

A randomized split-plot design with four replicate blocks was established at each site. Crop rotation was winter oilseed rape (var. 'Visby')—winter wheat (*Triticum aestivum* L., var. 'Julius')—winter barley (*Hordeum vulgare* L., var. 'Tenor' in Berge, var. 'Meridian' in Hohenschulen and var. 'Souleyka' at all other sites) with the crops as main plot factor. The main plots were managed according to best management practices in Germany (e.g. the compliance of environmental standards such as the German Fertilizer Ordinance (DüV 2006) or the cross compliances of the common agricultural policy of the European Union (Regulation 73/2009). In each main plot of the WOSR seven treatments were established. Here, we report the results from the treatments biogas digestate without NI and biogas digestate with NI. Oilseed rape was the only crop within the crop rotation that received biogas digestates. Plot size varied slightly across study sites due to differences in the dimensions of the farming equipment; the minimum size was 3 m × 9 m (27 m^2).

Before seeding of the WOSR, soil was conventionally plowed with a moldboard plow (0–0.3 m). Oilseed rape was sown between end of August and early September at all sites and in all years (40 to 45 seeds m^{-2}, at inter-row spacing of 0.36 m). As a common practice for WOSR production in Germany, N-fertilization was split into two doses. The first dose was applied at the beginning of the growing season in spring and the second approximately four weeks later. For each N-fertilization 90 kg NH_4^+–N ha^{-1} was applied as liquid-based digestate, resulting in a total of 180 kg NH_4^+–N ha^{-1} yr^{-1}. Characteristics of the digestates are shown in Table S1. At the study site Dedelow the whole N amount was applied with only one single application in 2014.

Trailing-hose application method was used at all sites to apply the digestate between the plant rows. The width of the surface applied digestate was 0.12 m, corresponding to 1/3 of the whole area. In contrast, an injection technique was used for the first digestate application in 2013 at the site Berge.

To avoid sulfur deficiency, 90 kg S ha^{-1} were applied to all plots as kieserite ($MgSO_4$) every spring. After harvest, soil was plowed at each site.

Piadin® (SKW, Piesteritz, Germany), which is a pyrazole derivate, was used as nitrification inhibitor. According to the producer's recommendation we applied 5 L Piadin® solution ha^{-1}. It was added to the digestates directly before application. The two active compounds in Piadin® (1H-1,2,4-triazole and 3-methylpyrazole) have been shown to inhibit

Table 1 Meteorological, soil chemical and physical characteristics of the study sites

Study site		Berge	Dedelow	Ihinger hof	Hohenschulen	Merbitz
Coordinates		N 52° 61′ 67 ″	N 53° 36′ 57″	N 48° 73′ 76 ″	N 54° 31′ 34 ″	N 51° 61 ′ 62 ″
		E 12° 78′ 33 ″	E 13° 82′ 71 ″	E 8° 92′ 36 ″	E 9° 99′ 34 ″	E 11° 91 ′ 12 ″
MAP	[mm yr^{-1}]	503	485	688	732	520
2013/14/15		615/482/570	446/561/414	923/763/544	462/409/562	700/456/429
MAT	[°C]	8.7	8.4	8.3	8.9	9.0
2013/14/15		9.4/13.0/10.6	8.7/9.9/9.7	8.6/10.4/10.1	8.1/9.6/8.8	9.1/10.7/10.4
Clay	[%]	5.7	10.0	3.2	10.5	15.8
Silt	[%]	19.9	30.9	78.2	29.4	67.8
Sand	[%]	74.4	59.1	18.6	60.1	16.4
Soil texture[¥]		Sandy loam	Sandy loam	Silty loam	Sandy loam	Silty loam
pH	0.01 M	6.5	7.4	6.8	5.9	6.6
C_{org}	[%]	1.15	0.75	1.68	1.87	1.18
N_t	[%]	0.09	0.10	0.20	0.12	0.11
Soil type[§]		Luvisol	Luvisol	Haplic Luvisol	Haplic Luvisol/ Anthrosol	Haplic Chernosem

MAP: Long-term mean annual precipitation and annual precipitation in the single experimental years. MAT: Long-term mean annual air temperature (2 m) and annual mean air temperature in the single experimental years. [§]IUSS Working Group WRB (2015). [¥]Measured in the topsoil (0–0.30 m)

nitrification efficiently (Aulakh et al. 2001; Barneze et al. 2014; Wu et al. 2017).

Flux measurement and calculations of flux rates and greenhouse gas (GHG) emission

Between 2013 and 2015, N_2O flux rates were measured using the closed chamber method (Mosier and Hutchinson 1981). Briefly, chamber frame bases (0.71 m × 0.27 m) were installed between the plant rows. We used the CO_2 fluxes as indicator for soil respiration. Although the chamber frame bases did not include growing WOSR plants, we cannot exclude root respiration from WOSR also contributing to CO_2 fluxes through diffusion into the chamber headspace from beneath the frames. This bias was not quantified for our chamber setting. Fluxes were measured at least once a week and supplemented with additional, event-oriented measurements after events which were frequently shown to increase N_2O fluxes (i.e. N-fertilization, tillage, heavy rain, and frost-thaw cycling). During gas sampling, dark closed chamber covers were placed airtight on the chamber frame bases. Four gas samples were taken periodically (every 15–20 min) from the chamber headspace and transferred into pre-evacuated glass vials. The N_2O and CO_2 concentrations in the gas samples were analysed in the laboratories of the participating research groups by various gas chromatographs equipped with ^{63}NI electron capture. Analytical laboratory inter-comparability was verified by conducting blind inter-comparison measurements between the laboratories involved in the study at the beginning of the experiment. Each laboratory achieved a coefficient of variance below 2% on ten repeated measurements of an ambient N_2O standard gas (Ruser et al. 2017). The GC instrumentation of the laboratories is given in Table S5.

Flux rates were calculated using the R (R Core Team 2017) package gasfluxes. Measured fluxes were subjected to a rigorous quality check using CO_2 accumulation above the freezing point since a large number of personnel was involved in the comprehensive gas sampling (e.g., about 60,000 gas samples were taken in the whole study during the three experimental years) and missing or discarded fluxes were filled by multiple imputation (Honaker et al. 2011).

Cumulative annual N_2O emissions were calculated for the periods between 1 January and 31 December. This calendar year cycle was chosen since it covered

all soil management and N-fertilization measures of the WOSR as well as it captures the time when increased soil mineral N contents were expected. Furthermore, cumulative N_2O emission during the fertilization period was calculated for each year and each site separately. The fertilization periods started with the date of the first digestate application and ended four weeks after the second N application (Table S2).

For the calculation of the cumulative N_2O emission, we linearly interpolated between two consecutive sampling dates. We also calculated the oil yield-related N_2O emission by dividing the annual N_2O-N emission by the oil yield.

We compared the GHG emissions including measured or calculated N_2O emissions from soils fertilized with biogas digestate without NI against the default reduction value claimed by the European Directive on the promotion of the use of energy from renewable sources (EU-RED-II Annex V: EC 2018). Frequently GHG emissions from agricultural products are calculated with IPCC emissions factors. The IPCC emission factor (IPCC-EF) for calculating direct N_2O emissions based on the N-input is globally applicable and assumes a linear relationship between N-input and N_2O emissions. Therefore, the uncertainty of so calculated N_2O emissions is high; the (IPCC-EF for direct N_2O emissions) ranges between 0.1% and 1.8%. (IPCC 2019) The Global Nitrous Oxide Calculator (GNOC, see https://gnoc.jrc.ec.europa.eu/) takes the non-linearity of N_2O flux response with varying N-fertilizer amount into account and allows for the calculation of site- und crop-specific N_2O emissions with a lower uncertainty when compared to the IPCC-EF. Therefore, GNOC was used in this study for comparison with the measured N_2O emissions at the experimental sites. The calculated direct N_2O emissions were compared with measured data during the period from 2013 to 2015 at the various experimental sites.

Default input data from the latest report of the Joint Research Centre (JRC) of the European Commission were used for all activity data not measured in the study, such as diesel supply and consumption, pesticides, non-N-fertilizer, and others (Edwards et al. 2019). Moreover, default data of the EU-RED-II Annex V (EC 2018) were used for processing of rapeseed to biodiesel as well as for distribution and storage. Based on those default data for processing and distribution the required GHG savings from cultivation were calculated for 50%, 60%, and 65% total GHG savings, when digestate was used as N-fertilizer. The GHG savings were calculated based on default GHG emissions of fossil-based diesel. The revised EU-RED-II requires 50% GHG-savings for biofuel processing facilities installed before 2015, 60% GHG reduction for biofuels from facilities installed between 2015 and 2020, and 65% for biofuels from facilities installed from 2021 onwards.

We considered the following two scenarios:

- Taking 50% of total–N in digestate into account to estimate N_2O emission with the GNOC approach as recommended by JRC (Edwards et al. 2019).
- Taking the average ammonium–N content (57%) of the digestates used in the field trials into account to estimate N_2O emission by GNOC.

Environmental, soil, and plant analyzes

Weather stations were installed at each study site directly next to the experimental plots. We detected air temperature at 2 and 0.05 m height and daily precipitation. Data loggers (LogTag, TRIX-8, CIK solutions, Karlsruhe, Germany) were used to determine soil temperature in 0.05, 0.1 and 0.2 m soil depth. Soil samples were taken concurrently with each gas sampling from 0 to 0.3 m depth. We used soil augers with an inner diameter of 0.02 m. According to the share of surface area covered by digestate, two soil samples were taken aside and one in the middle of the digestate bands in each plot. We mixed the three samples of each plot to one homogenized sample. The four samples from the replicate plots were then pooled resulting in one composite sample per treatment and sampling date.

For further analysis, the samples were sieved (< 2 mm) and stored frozen. To determine the mineral N content, 80 g of fresh soil were extracted with 200 ml of a 1.25 10^{-2} M $CaCl_2$ solution. Photometric analysis was used to measure the concentrations of NO_3^- and NH_4^+ in the extracts. Since these measurements were also done separately at the several research laboratories across Germany and thus with different analytical systems, we conducted an inter-laboratory comparison with all laboratories involved in the study. Detection limits were calculated based on DIN 32,645

(2008) and shown together with the manufacturer and model information of analyzers in Table S4.

Soil moisture was determined gravimetrically by drying soil at 105 °C for 24 h. Bulk density of the topsoil was determined using stainless steel cylinders (100 ml) before and after each tillage operation. Water-filled pore space (WFPS) in the A_p horizon (0–0.3 m depth) was calculated as follows:

$$\text{WFPS} = \text{gravimetric soil moisture} \times \text{soil bulk density} \times \text{total porosity}^{-1} \quad (1)$$

with soil porosity calculated as

$$\text{Soil porosity} = 1 - \text{soil bulk density} \times 2.65^{-1} \quad (2)$$

where 2.65 Mg m^{-3} (particle density of quartz) was the assumed particle density of the soil.

Plant biomass yield was measured on 1 m^2 cuttings. Moisture content of the plant samples was determined by drying for three days at 60 °C (straw and pods separately). Composite subsamples of the crop straw and grain were analyzed for C and N using an elemental analyzer (vario Max CN, Elementar Analysensysteme, Hanau, Germany). Oil content of the WOSR seeds was measured using near-infrared spectroscopy (NIRSystem 5000, Foss, Hamburg, Germany).

In 2017 the German Fertilizer Ordinance, which regulates the maximum amount of N-fertilizer, was revised. In contrast to the preceding ordinance (DüV 2006), the maximum rate of N applied with digestate in arable land was reduced from 170 kg N ha^{-1} based on NH_4-N to 170 kg N ha^{-1} based on total N (DüV 2017).

Meteorological conditions

The annual precipitation varied between 409 mm (Hohenschulen in 2014) and 923 mm (Ihinger Hof in 2013) (Table 1). Except for Hohenschulen, the long-term mean annual precipitation at each study site lay within the range of the annual precipitation in the three single experimental years, indicating that our measurements were conducted in a period with site typical precipitation characteristics at Dedelow, Ihinger Hof, Merbitz, and Berge. In contrast, precipitation during the whole study period in Hohenschulen was 35% lower than the long-term mean hinting on a potential water deficit for that region. At Ihinger Hof, Merbitz and Berge, precipitation in the first experimental year (2013) was 34%, 35%, and 22% higher when compared to the long-term mean. Particularly spring and summer 2015 was very dry at almost every study site.

Mean annual air temperature ranged between 8.1 °C (Hohenschulen in 2013) and 13.0 °C (Berge in 2014). Except for the study site Hohenschulen, annual temperature was predominately higher than the long-term mean at all remaining study sites and in all three experimental years.

Statistical methods

A mixed model approach using SAS PROC MIXED for the comparison of cumulative N_2O emissions, oil yield and oil yield-related N_2O emissions was used. The model can be described as follows:

$$y_{hijkl} = \mu + a_h + l_j + \tau_i + (al)_{hj} + (a\tau)_{hi} + (l\tau)_{ji} + (al\tau)_{hij} + b_{hjkl} + e_{hijkl},$$

where a_h, l_j and τ_i are the fixed main effects for the hth year, jth site and ith level of nitrification inhibitor (NI). $(al)_{hj}$, $(a\tau)_{hi}$, $(l\tau)_{ji}$, and $(al\tau)_{hij}$ are the fixed interaction effects of corresponding main effects. b_{hjkl} is random block effects. e_{hijkl} is the plot error effect with a site-by-year-specific variance. As data for N_2O emissions were repeatedly taken, block effects and error effects were allowed to have a first order autocorrelation variance–covariance structure if this decrease the AIC (Wolfinger 1993). The assumptions of normally distributed residuals and a homogeneous variance (beside the heterogeneity accounted for) were checked graphically. After finding significant effects via F tests in the second stage of the analysis, least square means for corresponding effects were calculated and compared with a Tukey-test at $\alpha = 0.05$. Additionally, least square means of cumulative N_2O emission and their standard errors were calculated for each site-by-year-by-NI treatment combination using equation 3.

These means were additionally compared using Fishers LSD test to show single site-by-year results.

Furthermore, for each site, a multiple regression analysis for N_2O flux rates was performed in order to explore the main processes of N_2O release. To include block and treatment effects within multiple regression approach, dummy variables were created and included per default within the model. The best model per site was selected via adjusted R^2 after fitting all possible models. The following explaining variables were used: air temperature (2 m), soil extractable NO_3^- and NH_4^+ concentrations, water-filled pore space (WFPS) and CO_2 flux rates. From this approach, the correlation was determined as the square root of the partial R^2 value. Additionally, selected variables were tested via F-test and the result and their slope estimates were presented. Analyses were performed in SAS 9.4 (SAS Institute, Cary NC, USA). Plots and graphics were created with SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany).

Results

N_2O flux rates

Nitrous oxide fluxes showed a high spatial and temporal variability (Fig. 1). Increased fluxes were often measured after digestate application in conjunction with precipitation events. With 458 µg N_2O-N m^{-2} h^{-1} the highest flux rate during the entire experimental period was determined in the treatment without NI at the study site Merbitz on 8th May 2013. This peak occurred after the second fertilizer application one day after a heavy rainfall event (42 mm d^{-1}). Similarly, high N_2O flux rates after N-fertilization were also measured 2013 in Berge, 2015 in Dedelow, and 2014 and 2015 in Hohenschulen.

In cases where the application of digestates induced a considerable increase of the N_2O fluxes (i.e. when flux rates after N-fertilization exceeded 50 µg N_2O-N m^{-2} h^{-1}), the flux rates were higher in the treatment

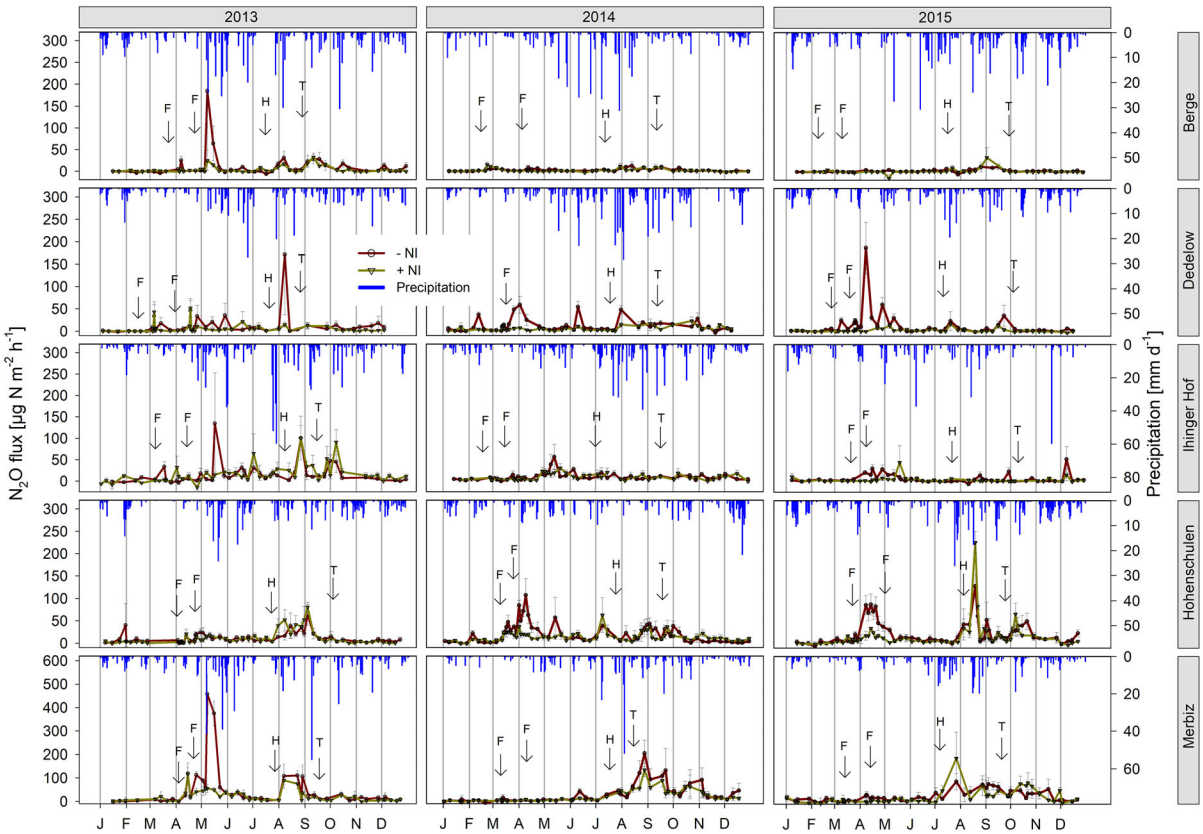

Fig. 1 Temporal pattern of the mean N_2O flux rate (n = 4) of the treatment with (+ NI) and without (–NI) nitrification inhibitor and daily rainfall as affected by study site and experimental year. Note: different y-axis scaling. Abbreviations: F = fertilization; T = tillage; H = harvest

Table 2 Site-specific multiple regression analysis for N2O fluxes. The best model was selected via adjusted R2. Partial R2 values and results from estimating and testing slope effects are presented for variables included in the best model selected.

Study site	Estimated slope effects	R^2						F-value	*p*-value
		CO_2	NO_3^-	NH_4	T	WFPS	$\sum$		
Berge	0.00077	0.06					0.06	32.1	< .0001
			0.00					2.3	0.1283
Dedelow	0.00138	0.14					0.18	104.0	< .0001
	0.00109		0.01					11.1	0.0009
	0.00258				0.00			2.68	0.1020
	0.00311					0.02		15.4	< .0001
Ihinger Hof	0.00107	0.20					0.23	148.2	< .0001
	0.00115		0.00					2.8	0.0949
	−0.00138			0.00				1.9	0.1685
	0.00326				0.01			4.7	0.0313
	0.00159					0.01		8.0	0.0048
Hohenschulen	0.00187	0.29					0.34	238.2	< .0001
	0.00354		0.04					31.2	< .0001
	0.00359				0.01			13.2	0.0003
	0.00334					0.00		2.35	0.1261
Merbitz	0.00163	0.17					0.26	209.1	< .0001
	0.00252		0.03					40.3	< .0001
	0.01646				0.04			48.8	< .0001
	0.00793					0.02		26.4	< .0001

CO_2 = CO_2 flux rates; NO_3 = NO_3^-–N concentration in the top soil (0–0.3 m); NH_4 = NH_4^+–N

without NI than in the treatment with NI (Fig. 1). On 8th May 2013, the mean N_2O flux rate from the treatment with NI in Merbitz was by factor 8 lower (55 µg N_2O-N m^{-2} h^{-1}) when compared to the treatment without NI application.

Approximately two weeks after the second digestate application the N_2O fluxes decreased and they were on background level four weeks after N-fertilization. Except for Berge and Ihinger Hof (both in 2014 and 2015) N_2O flux rates increased after harvest and less frequently after soil tillage. Winter fluxes were generally low at all sites and in all years (Fig. 1).

For all experimental sites, N_2O fluxes were significantly correlated ($p < 0.001$, Table 2) with CO_2 fluxes. Except for the sandy site Berge, we also found a positive correlation between the N_2O fluxes and soil moisture. Additionally, N_2O fluxes in Dedelow, Hohenschulen and Merbitz were also positive correlated with soil nitrate contents (Table 2).

Annual N_2O emission and N_2O emission during the fertilization period

Annual N_2O emission varied between 0.2 kg N_2O-N ha^{-1} yr^{-1} and 3.5 kg N_2O-N ha^{-1} yr^{-1}. The study site Merbitz revealed the highest annual N_2O emissions in every experimental year and in both treatments (-NI and + NI). Except for the treatment without NI in 2013 (measured in Hohenschulen), Berge showed the lowest annual N_2O emissions.

When compared to the treatment without NI, the application of the NI significantly reduced the annual N_2O emission over the whole data set by 36% (all sites and years, $p = 0.0027$, Table 3). With regard to the single years and study sites, the mean annual N_2O emission in the treatment with NI was only significantly ($p < 0.05$, Table 4) lower for 3 of the 15 site-years: at Dedelow in 2014 (50% less than -NI) and in 2015 (73% less than -NI), and at Merbitz in 2013 (49% less than -NI). Cumulative N_2O emission during the

Table 3 ANOVA table of annual N_2O emissions as affected by NI, year, experimental site and their interactions. Furthermore, least square means and their standard errors for the two NI levels are presented

Effect	Num DF	Den DF	F value	*p* value
NI	1	28.2	10.84	0.0027
Year	2	21.8	1.78	0.1926
Site	4	17.8	26.31	< 0.0001
NI × Year	2	20.7	0.53	0.5971
Year × Site	8	20.5	3.42	0.0116
NI × Site	4	18.1	1.75	0.1821
NI × Site × Year	8	19.5	1.36	0.2750
Least square means [kg N_2O-N ha^{-1} yr^{-1}]				
–NI		+ NI		**0.0027**
Mean	SE	Mean	SE	
1.38	0.09	0.99	0.09	

NI = nitrification inhibitor, Num *DF* = number of degrees of freedom, Den *DF* = denominator degrees of freedom, *SE* = standard error

fertilization period varied between 0.01 and 1.82 kg N_2O-N ha^{-1} $period^{-1}$ (Table 4). During the fertilization period, the mean N_2O emission in the treatment with NI tended to be lower in 12 out of 15 observations with 6 observations being statistically significant. The N_2O mitigation in this period varied between 65% (Hohenschulen in 2015) and 97% (Dedelow in 2015) (Table S6).

Outside the fertilization periods, the mean N_2O emission varied between 0.16 and 1.63 kg N_2O-N ha^{-1} (Table 4). The emission in this period accounted for between 48 and 99% of the annual N_2O emissions (Table S6). Except for Dedelow in 2014, NI application did not affect N_2O emissions outside the fertilization periods. The higher N_2O emissions in the + NI treatment outside the fertilization period in Dedelow 2014 were mainly the result of higher N_2O fluxes following heavy rainfall events at the begin of June and August.

Mineral N in the topsoil

Digestate application often increased NO_3^- concentrations in the topsoil (Fig. 2). With 19.4 mg NO_3^-–N kg^{-1} (corresponding to approximately 75 kg N ha^{-1}) highest concentration after digestate application was measured mid-March 2015 at the study site Ihinger Hof. Following digestate applications NO_3^- concentrations decreased with increasing N demand of the growing WOSR. Nitrate concentrations increased again either shortly before or directly after harvest of the WOSR. At all study sites and in nearly every experimental year highest NO_3^--concentrations of up to 31.5 mg NO_3^-–N kg^{-1} (Dedelow September 2014) were measured in this post-harvest period.

When compared to digestate application without NI, usage of the NI significantly decreased the NO_3^--concentrations in the topsoil during the fertilization period (Fig. 3). The median NO_3^--concentration in this period was 32% lower in the + NI treatment. This effect of the NI on lowering NO_3^--concentration in the fertilization period was highly significant with a high coefficient of determination (R^2 = 0.79).

The NH_4^+ concentrations in the plow layer increased after digestate application at all sites (Table S2) whereas their contribution to total mineral N outside the fertilization period was negligible (< 5 kg NH_4^+-N ha^{-1}, data not shown). We did not find any correlation between the NH_4^+-N concentrations and the N_2O flux rates, neither for the whole data set nor for a single site or year.

Following digestate application the NH_4^+-N/NO_3^--N ratio in the treatment + NI at the study sites Dedelow, Ihinger Hof and Hohenschulen was higher than in the treatment without NI, this was also measured at the site Berge in the third year (Figure S1). For the study site Merbitz (silty loam texture), this effect was not observed.

Effect of NI on grain yield, oil yield and oil yield-related N_2O emission

The WOSR grain yield was not affected by NI application, over the whole experimental period and all sites it varied between 2.8 Mg ha^{-1} yr^{-1} and 5.7 Mg ha^{-1} yr^{-1} (Table S3). The corresponding mean WOSR oil yield was 2.0 Mg ha^{-1} yr^{-1}, it ranged between 1.2 and 2.7 Mg ha^{-1} yr^{-1} (Table 5). Oil yield was not affected by NI application, neither in one single experimental year nor at any of the study sites.

Oil yield-related emission varied over the experimental sites and years between 0.1 and 1.9 kg N_2O-N

Table 4 N_2O emissions on an annual base, during the fertilization period, and outside the fertilization period as affected by site, year and nitrification inhibitor (+ NI; –NI). Means not sharing any letter are significantly different within one site, year, and period by Fishers LSD-test at the 5% level of significance (bold marked)

Study site	Year	N_2O emission					
		Annual [kg N_2O-N ha^{-1}]		Fertilization period† [kg N_2O-N ha^{-1}]		Outside fertilization period [kg N_2O-N ha^{-1}]	
		–NI	+ NI	–NI	+ NI	–NI	+ NI
Berge	2013	1.08[a]	0.40[a]	0.42	0.08[a]	0.66[a]	0.33[a]
	2014	0.27[a]	0.22[a]	0.07[a]	0.05[a]	0.20[a]	0.16[a]
	2015	0.19[a]	0.24[a]	0.01[a]	0.01[a]	0.18[a]	0.23[a]
	2013–2015	0.51	0.29	0.17	0.05	0.35	0.24
Dedelow	2013	1.13[a]	0.47[a]	0.22[a]	0.09[a]	0.91[a]	0.38[a]
	2014	**1.20[a]**	**0.64[b]**	**0.15[a]**	**0.03[b]**	**1.05[a]**	**0.60[b]**
	2015	**1.10[a]**	**0.25[b]**	**0.44[a]**	**0.01[b]**	0.68[a]	0.24[a]
	2013–2015	1.14	0.45	0.27	0.04	0.87	0.41
Ihinger Hof	2013	1.40[a]	1.72[a]	0.06[a]	0.10[a]	1.34[a]	1.63[a]
	2014	0.94[a]	1.15[a]	0.06[a]	0.07[a]	0.88[a]	1.09[a]
	2015	0.54[a]	0.35[a]	**0.19[a]**	**0.04[b]**	0.35[a]	0.32[a]
	2013–2015	0.96	1.08	0.11	0.07	0.86	1.01
Hohenschulen	2013	0.98[a]	0.96[a]	0.12[a]	0.08[a]	0.86[a]	0.87[a]
	2014	1.54[a]	1.21[a]	**0.47[a]**	**0.20[b]**	1.07[a]	1.01[a]
	2015	1.88[a]	1.28[a]	**0.49[a]**	**0.15[b]**	1.39[a]	1.13[a]
	2013–2015	1.47	1.15	0.36	0.14	1.10	1.00
Merbitz	2013	**3.53[a]**	**1.78[b]**	**1.82[a]**	**0.51[b]**	1.71[a]	1.27[a]
	2014	2.64[a]	1.90[a]	0.05[a]	0.04[a]	2.60[a]	1.87[a]
	2015	2.23[a]	2.23[a]	0.17[a]	0.03[a]	2.06[a]	2.20[a]
	2013–2015	2.80	1.97	0.68	0.19	2.12	1.78

† Fertilization period started with the day of the first digestate application and ended 4 weeks after the second digestate application (Table S2)

Mg^{-1} oil ha^{-1} (Table 5). Oil yields were not affected by NI application. NI application reduced oil yield-related N_2O emissions in only 3 of the 15 tested site-years (Dedelow in 2014 and 2015 and Merbitz in 2013).

GHG emissions from digestate application

Calculated direct N_2O emissions with GNOC were generally higher than the measured N_2O emissions at the experimental sites (Table 6). The only exception was the study site Merbitz with lower GHG emissions in the GNOC calculation.

Newly established facilities for biodiesel production have to prove that 65% GHG reduction is achieved when replacing fossil fuel by biodiesel. This target could be achieved at all experimental sites except for Merbitz, if measured direct N_2O emissions and calculated indirect N_2O emissions were used. When N_2O emissions were calculated with GNOC taking 50% of the total N in digestate into account as recommended by JRC, all experimental sites would meet the 65% GHG reduction target.

Using the average NH_4^+-N content of the applied digestate (57%) instead of 50% of the total N for the GNOC calculation did not change the overall picture. A GHG reduction of 65% could be achieved at all

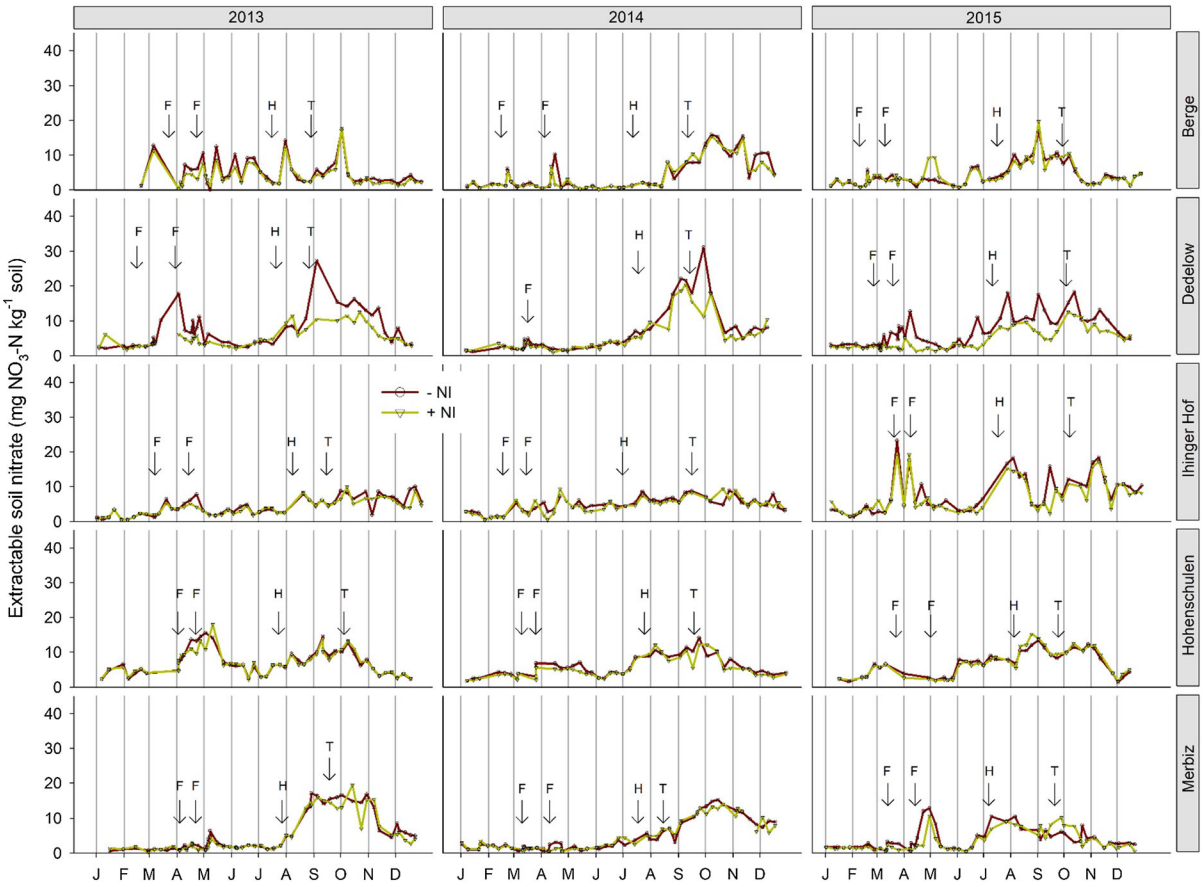

Fig. 2 Temporal pattern of the extractable soil NO_3^- concentrations (0–0.3 m depth) in the treatment with (+ NI) and without (–NI) nitrification inhibitor as affected by study site and experimental year. Abbreviations: F = fertilization; T = tillage; H = harvest

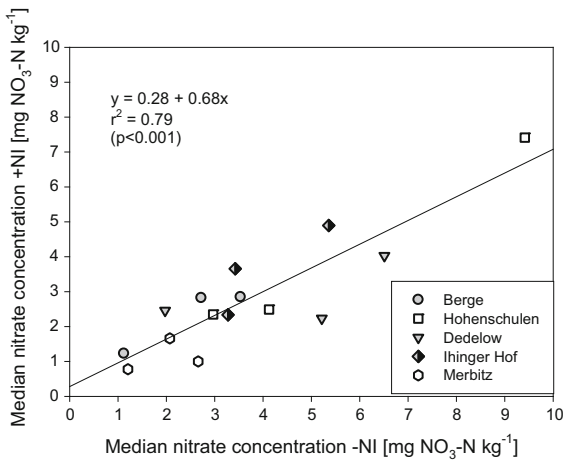

Fig. 3 Effect of nitrification inhibitor on the median NO_3^- concentration (0–0.3 m depth) during the single fertilization periods. Each point represents the median NO_3^- concentration of between 6 and 12 sampling dates during the fertilization periods

experimental sites, although Hohenschulen just met the requirement.

Discussion

N_2O flux rates as affected by environmental conditions and soil drivers

Nitrous oxide release at our five study sites showed a high temporal variability with increased flux rates after N-fertilization in conjunction with rainfall. Several studies in arable fields reported similar N_2O flux patterns and mainly explained the increased flux rates with rainfall enhancing denitrification (Ambus and Christensen 1994; Flessa et al. 1995; Kaiser et al. 1996). After fertilization, ammonium in liquid manure stripes is rapidly oxidized to nitrate (Delin and

Table 5 Oil yield and oil yield-related N_2O emission as affected by site and year and nitrification inhibitor (+ NI; –NI). Means not sharing any letter are significantly different within one site and year by Fishers LSD-test at the 5% level of significance (bold marked)

Study site	Year	Oil yield [Mg ha^{-1} yr^{-1}]		Oil yield-related N_2O emission [kg N_2O-N Mg^{-1} oil ha^{-1}]	
		–NI	+ NI	–NI	+ NI
Berge	2013	1.33[a]	1.21[a]	0.80[a]	0.34[a]
	2014	1.81[a]	1.79[a]	0.15[a]	0.12[a]
	2015	1.48[a]	1.40[a]	0.13[a]	0.18[a]
	2013–2015	1.54	1.47	0.36	0.21
Dedelow	2013	2.68[a]	2.60[a]	0.41[a]	0.18[a]
	2014	2.64[a]	2.58[a]	**0.46[a]**	**0.25[b]**
	2015	2.05[a]	2.11[a]	**0.54[a]**	**0.12[b]**
	2013–2015	2.46	2.43	0.47	0.18
Ihinger Hof	2013	1.89[a]	1.82[a]	0.77[a]	0.95[a]
	2014	1.79[a]	1.64[a]	0.54[a]	0.72[a]
	2015	1.86[a]	1.84[a]	0.29[a]	0.19[a]
	2013–2015	1.85	1.76	0.53	0.62
Hohenschulen	2013	2.10[a]	2.08[a]	0.47[a]	0.46[a]
	2014	2.47[a]	2.47[a]	0.63[a]	0.50[a]
	2015	2.09[a]	2.02[a]	0.72[a]	0.63[a]
	2013–2015	2.22	2.19	0.60	0.53
Merbitz	2013	1.87[a]	1.84[a]	**1.89[a]**	**0.96[b]**
	2014	2.10[a]	2.07[a]	1.24[a]	0.91[a]
	2015	1.66[a]	1.63[a]	1.38[a]	1.34[a]
	2013–2015	1.88	1.85	1.50	1.07

Strömberg 2011), thus increasing substrate availability for denitrification. Oxygen (O_2) diffusion in soil water is approximately 10^4 times lower than in soil air (Heincke and Kaupenjohann 1999). Consequently, high soil moisture reduced O_2 diffusion from the atmosphere into and within the moist soil after rainfall. Additionally, high microbial O_2 demand during C turnover of easily available C from cattle slurry further stimulated formation of anaerobic soil conditions and thus promoted denitrification (Flessa and Beese 1995, 2000). As shown for cattle slurry, oxygen depletion in the upper soil centimeters during intense nitrification of the NH_4^+ can further enhance the development of anaerobic conditions after surface application (Van Nguyen et al. 2017), this might also hold true for the application of digestates.

Besides high N_2O flux rates measured after digestate applications, we also found increased N_2O release after harvest of WOSR which, depending on study site and year, lasted up to six weeks. These high fluxes coincided with increased NO_3^- contents of the topsoil post-harvest. Consistently, Ruser et al. (2017) reported enhanced post-harvest N_2O fluxes from WOSR plots in the same field experiment from treatments fertilized with mineral N. We found a significant correlation between N_2O and CO_2 fluxes, which was also confirmed by Ruser et al. (2017). This indicates a positive relationship between C-heterotrophic microbial activity, including activity of the denitrifying community, and N_2O release. Our results are in good agreement with Walter et al. (2015) who also explained the higher post-harvest N_2O emissions from WOSR fields in comparison to winter wheat fields with a higher NO_3^- availability as substrate for denitrification under WOSR.

The output of the explorative multiple linear regression analysis indicated that (i) CO_2 flux was the main explanatory variable in the regression model at all study sites and that (ii) further significant parameters such as soil moisture and NO_3^- concentration at some sites also hint on denitrification as the main N_2O source in our experiment. Michaelis–Menten kinetic studies for denitrification in agricultural soils derived K_M values in the range between 4

Table 6 Study site specific mean WOSR yield, mean measured direct N_2O emissions in the -NI treatments, indirect N_2O emissions calculated with GNOC, direct N_2O emission calculated with GNOC, total GHG emissions either based on measured or calculated direct N_2O emissions, default values (EU-RED-II, annex V: EC 2018) for maximum GHG emissions for 50%, 60%, or 65% CO_2 savings. Calculations according to the JRC (2019) recommendations assuming NH_4^+–N accounting for 50% of the total digestate N or based on measured NH_4^+–N accounting for 57% of the total N

Study site	Berge	Dedelow	Ihinger Hof	Hohenschulen	Merbitz
Yield [Mg ha^{-1} yr^{-1}]	3.19	5.23	3.77	4.43	3.92
Direct emissions, measured values [kg N_2O–N ha^{-1}]	0.51	1.14	0.96	1.47	2.80
Direct emissions, measured values [g CO_{2eq} MJ^{-1}]	3.04	4.15	4.85	6.32	13.60
50% NH_4^+-N according to JRC (2019)					
Σ Indirect emissions according to GNOC [kg N_2O–N ha^{-1}]		0.26	0.26	0.73	0.76	0.26
Σ Direct emissions according to GNOC [kg N_2O-N ha^{-1}]		1.72	2.76	1.69	2.90	1.70
Total from N_2O based on GNOC [g CO_{2eq} MJ^{-1}]		6.86	6.37	7.07	9.11	5.51
Total CO_{2eq} based on GNOC and JRC defaults [g CO_{2eq} MJ^{-1}]		11.99	11.50	12.24	14.24	10.64
Total CO_{2eq} based on measured direct N_2O emissions [g $CO_{2eq}MJ^{-1}$]		4.56	5.07	8.52	9.58	14.83
57% NH_4^+-N based on measured NH_4^+ concentrations					
Σ Indirect emissions according to GNOC [kg N_2O–N ha^{-1}]		0.29	0.29	0.80	0.80	0.29
Σ Direct emissions according to GNOC [kg N_2O-N ha^{-1}]		1.91	3.06	1.85	3.25	1.86
Total from N_2O based on GNOC [g CO_{2eq} MJ^{-1}]		7.63	7.06	7.75	10.17	6.05
Total CO_{2eq} based on GNOC and JRC defaults [g CO_{2eq} MJ^{-1}]		12.76	12.19	12.88	15.30	11.18
Total CO_{2eq} based on measured direct N_2O emissions [g CO_{2eq} MJ^{-1}]		4.77	5.20	8.90	9.91	15.01
Maximum GHG for 50% savings [g CO_{2eq} MJ^{-1}]				29.5		
Maximum GHG for 60% savings [g CO_{2eq} MJ^{-1}]				19.9		
Maximum GHG for 65% savings [g CO_{2eq} MJ^{-1}]				15.2		

and 13 mg NO_3^--N kg^{-1} soil (Klemedtsson et al. 1977; Limmer and Steel 1982; Mosier et al. 1983). In our experiment, NO_3^- concentrations measured during periods with enhanced N_2O fluxes were well within this K_M range indicating that NO_3^- as substrate for denitrification was never limiting at all study sites.

In a lab study with 20 different soils, Gödde and Conrad (2000) reported a significant relationship between the N_2O production rate and the CO_2 release rate, whereas N_2O production during nitrification was not correlated with the CO_2 flux. A low degree of the coefficient of determination (R^2 = 0.06) between N_2O and CO_2 flux rates and no further correlating soil driver (e.g., NO_3^- or soil moisture) implies different main sources such as nitrification at this site. Note that the presented multiple linear regression analysis was performed for each site separately and thus the influence of site-specific variables was not considered. Further, static parameters such as clay contents or soil classification do not change over short periods. Although they might be helpful for the estimation of a site-specific potential for N_2O release, they are unhelpful to analyze the high temporal dynamics of N_2O flux rates which are mainly driven by events such as N-fertilization or precipitation occurrence.

N_2O emission after digestate application as affected by the study site

Over all sites and experimental years, cumulative N_2O emission ranged between 0.2 and 3.5 kg N_2O-N ha^{-1} yr^{-1}. Walter et al. (2015) summarized the results from N_2O measurements in WOSR fields in Germany, Spain, France and UK. The emissions reported by Walter et al. (2015) were higher than the emissions measured in our experiment. For mineral N-fertilizer amounts between 172 and 195 kg N ha^{-1} yr^{-1} (n = 6) the annual N_2O emissions varied between 1.71 and 5.69 kg N_2O-N ha^{-1} yr^{-1} with a mean

emission of 3.42 kg N_2O-N ha^{-1} yr^{-1}. This mean emission value was close to our highest annual N_2O emission (3.5 kg N_2O-N ha^{-1} yr^{-1} in Merbitz in 2013). In agreement with our lower emissions, measurements of Ruser et al. (2017) in the same experiment in treatments receiving different amounts of mineral N-fertilizer also showed lower emissions when compared to the whole data set of Walter et al. (2015).

The mean annual N_2O emission in the treatments without NI decreased in the following order: Merbitz > Hohenschulen > Dedelow > Ihinger Hof > Berge. Except for the site Ihinger Hof, annual N_2O emissions decreased from loamy to sandy textures of the soils. Sandy soil texture and low C_{org} contents (Berge and Dedelow) result in a low water holding capacity and thus in good soil aeration limiting denitrification (Bouwman et al. 2002). Pelster et al. (2012), Stehfest and Bouwman (2006) and Leip et al. (2011) reported similar results for N_2O emissions from soils with varying soil texture. The Dedelow and Hohenschulen sites had more or less a similar soil texture. The twofold higher C_{org} content in the top soil of Hohenschulen compared to the topsoil in Dedelow might be one reason for the higher N_2O emission in Hohenschulen. Further, the lower pH values at Hohenschulen (pH: 5.9) might also be a reason for the higher N_2O emission when compared to the site Dedelow (pH: 7.4). As shown by Russenes et al. (2016) even small differences in soil pH affect N_2O emission in periods with high denitrification losses with an increasing share of N_2O to the total product ratio of denitrification with decreasing soil pH. When compared to Hohenschulen, the higher pH and the lower C_{org} content at the site Ihinger Hof might also have been a reason for the lower N_2O emission at Ihinger Hof.

In keeping with Ruser et al. (2017), highest annual N_2O emission in both treatments within every site was measured in the experimental year with the highest precipitation. This would as well confirm our assumption of denitrification as the major N_2O source.

Between 48.4% and 98.8% of the annual N_2O emission occurred outside the fertilization period. A major part of these emissions stemmed from the post-harvest period, indicating the importance of this period for annual N_2O budgets from WOSR fields. As already pointed out by Ruser et al. (2017) frost periods during our experiment were short and the air temperature only slightly below 0 °C. Therefore, frost-thaw cycles did not significantly contribute to the annual N_2O emissions.

Effect of NI on N_2O emissions after digestate application

Over the whole data set, annual N_2O emission from WOSR fields fertilized with biogas digestate was reduced by 36% when a NI was applied ($p = 0.0027$). For single sites and years, the reduction was very efficient (> 45%) and thus statistically significantly in 3 data sets with comparably high annual emissions and with low emissions outside the fertilization period. When compared to the -NI treatment, this decrease in N_2O release is in good agreement with reduction potentials reported by Akiyama et al. (2010) and by Ruser and Schulz (2015) who both calculated a N_2O mitigation of approximately 35% from publications that had tested a range of available NIs.

Nitrification inhibitors inhibit the ammonia monooxygenase (AMO) as the first step during nitrification, and therefore, directly decrease the release of N_2O from nitrification. Due to lower NO_3^- concentration as substrate for denitrifiers, NIs can also reduce N_2O emissions from denitrification (Ruser and Schulz 2015). Additionally, the N_2/N_2O ratio increases with decreasing NO_3^- concentration as a result of a competitive effect of NO_3^- and N_2O as a terminal electron acceptor during denitrification (Blackmer and Bremner 1978; Yamulki et al. 1995). This effect would further decrease N_2O release from denitrification due to lower NO_3^- concentrations.

The lower NO_3^- concentrations in the + NI treatment during the fertilization period clearly show the effect of reduced NO_3^- availability after NI application. This effect was even observed on an annual base, although the differences in annual median NO_3^- concentrations between + NI and –NI treatment were only low. The inhibitory effect of NI on NH_4^+ oxidation after digestate application was also confirmed by higher NH_4^+-N/NO_3^--N ratios at most study sites (Figure S1). This ratio did not differ between + NI and –NI at Merbitz, the study site with the highest clay content within our experiment. Since the NO_3^- concentrations during the fertilization period at Merbitz were higher in the treatment without NI, we infer that the NH_4^+-N/NO_3^--N ratio did not

respond to NI application as a result of low NH_4^+ extraction recoveries for soils with considerably high clay contents when 0.01 M $CaCl_2$ solution is used as extraction solution (Li et al. 2012).

Except for the site Ihinger Hof, we found significant lower cumulative N_2O emission during the fertilizer period for study sites where we also found a positive correlation between N_2O flux rates and NO_3^- concentration in the topsoil. For the Berge site, this also shows that despite a lower NO_3^- availability in the + NI treatment, the potential to decrease N_2O emissions was not realized because good aeration as a result of the low water-holding capacity limited denitrification. Similar results with almost no effect of NI after slurry application under dry conditions and distinct reduction of the N_2O emission at higher soil moisture conditions were also reported by Lin and Hernandez-Ramirez (2020).

Comparing single years and sites, mean cumulative N_2O emission in the -NI treatment during the fertilization period was significantly higher than in the + NI treatment in 6 of the 15 data sets. While not statistically significant, the mean N_2O emission from the -NI treatment in the fertilization period was higher than in + NI in further 6 data sets. Although the mixed model revealed a significant N_2O mitigation for NI application on an annual basis, only 3 annual data sets showed significances in single years and at single sites. Besides the well-known high spatial variability of N_2O fluxes (i.e., Hénault et al. 2012; Röver et al. 1999), this discrepancy could also be explained in part by the high post-harvest N_2O emissions masking the beneficial NI effect during the fertilization period. Indeed, all sites with a significant N_2O reduction during the fertilization period, but without significant annual effects, showed between two- and threefold higher N_2O emissions outside the fertilization period. This result clearly demonstrates the need of further investigations with the aim of reducing post-harvest N_2O emissions from WOSR.

Dedelow 2014 was an exceptional data set because in contrast to all other study sites and also to Dedelow in 2013 and 2015, it showed an effect of the NI also outside the fertilization period. In 2014 digestate application was applied in Dedelow with only one single dose and the fertilization period (defined as period between day of first digestate application and four weeks after last application) was therefore very short (28 days). At the beginning of June, the N_2O flux rates following rainfall were higher in the –NI treatment than in the + NI treatment indicating that the NI was still active approximately ten weeks after application. This flux event occurred outside the short fertilization period and was one reason for the higher N_2O emission in the -NI treatment outside the fertilization period. The second reason was an emission event after harvest with higher N_2O fluxes from the -NI treatment. The reason for these higher fluxes remains unclear because one could expect that the NI was completely degraded after more than 18 weeks in soil. However, some indication for a longer activity has been shown by Pfab et al. (2012) who reported an inhibitory effect of 3,4 dimethylpyrazol phosphate (a similar compound as 3-methylpyrazol in Piadin®) 15 weeks after application to cauliflower fields.

Application of NIs with organic fertilizers can decrease NO_3^- leaching and lower N_2O and NO emissions from soils, but it also bears the risk of increasing NH_3 losses (Qiao et al. 2015). For a full environmental assessment of NIs and their potential trade-offs, future studies must therefore also consider NH_3 losses.

NI effects on grain yield, oil yield and oil yield-related N_2O emission

The yields measured at the study sites were in the same range as yields published from the official German WOSR yield statistics; 4.0, 4.5, and 3.9 Mg ha^{-1} yr^{-1} in 2013, 2014, and 2015, respectively (German Federal Statistical Office 2017) indicating that the selection of our study sites fulfilled the claim of representability for WOSR production in Germany. Application of the NI neither showed any effect on grain yield nor on oil yield. Consistent with our study, Wolf et al. (2014) also found no effect of NI on the yield of maize fertilized with biogas digestate. Generally, NIs were shown to be efficient in increasing crop yield, N uptake or N use efficiency when applied on sandy soils or in systems with high precipitation or irrigation (Pasda et al. 2001). Wolf et al. (2014) measured at a study site with a high sand content (62%) where an increase in N uptake or maize yield could have been expected. However, they also reported the need for irrigation due to water shortage, and hence, it can be presumed that most of NO_3^- was not leached and thus it was still available for plant

uptake even in the treatment without NI. Further, N-fertilization and oil yield were shown to be negatively correlated. Hegewald et al. (2016) reported only minor increases in oil yield (0.04 Mg ha^{-1} yr^{-1}) when N-fertilization was increased from 120 to 180 kg N ha^{-1} yr^{-1}. As pointed by Rathke et al. (2006), N-fertilization increases the crude protein content of rapeseeds at the expense of oil concentration. Since we fertilized with 180 kg N ha^{-1} yr^{-1} in our study, we considerably exceeded the N requirements for maximum oil yield, and therefore, an effect of NI on oil yield could not be expected.

For their data set with mineral N-fertilization in WOSR, Walter et al. (2015) reported oil yield-related N_2O emissions ranging between 0.09 and 53.3 kg N_2O-N Mg^{-1} oil ha^{-1} yr^{-1} with a median emission of 2.7 kg N_2O-N Mg^{-1} oil ha^{-1} yr^{-1}. The upper boundary of their data (53.3 kg N_2O-N Mg^{-1} oil ha^{-1} yr^{-1}) was clearly marked as an outlier which was the result of complete yield loss. Similar to the area-related N_2O emissions, oil yield-related N_2O emissions in our study were also lower because N_2O emissions were lower and oil yields were similar to the yields reported by Walter et al. (2015). The application of NI in our study reduced oil yield-related N_2O emissions in the same data sets as in the area-related annual N_2O emission data. This was the result of lower N_2O emissions in the –NI treatments whereas oil yields did not differ.

GHG emission from digestate application

The digestate related N_2O emission factor was 0.45 (N_2O emission from fertilized treatment corrected for N_2O emission from an unfertilized control and related to total N from the digestates. Mean over all digestate treatments without NI; range: 0.01—1.23; data not shown). It was lower than the emission factor calculated for mineral N-fertilization at the same study site (0.6; Ruser et al. 2017). The corresponding emission was distinctively lower when compared to the emission modelled with the GNOC tool (except for study site Merbitz). For treatments fertilized with mineral N in the same experiment, Ruser et al. (2017) also reported higher N_2O emissions calculated with GNOC when compared to the measured data.

As mentioned above, fertilization regulation in Germany has changed after the completion of our study, and digestates now belong to the category of "organic fertilizers", resulting in a mandatory cap of 170 kg total N $ha^{-1}yr^{-1}$. If it would be possible to fertilize digestate rates as in our study, 65% savings (relative GHG compared to fossil fuels, here biodiesel from rapeseed) would be achieved at every site (assuming 50% NH_4^+-N of total N). The contrasting GHG emission resulting from data calculated with GNOC or with IPCC Tier 1 emission factors and measured data (Table 6) clearly shows the necessity of more reliable regional-specific estimation of direct N_2O emissions.

Styles et al. (2015) also reported the avoidance of fossil resource depletion from a broad consortium of biofuel crops, including WOSR. However, it is difficult to compare their GHG data directly with ours because they used the IPCC Tier 1 emission factor to calculate N_2O emissions and considered indirect land use effects. Further studies either with digestates of another origin (biowaste and sewage sludge), different crop fertilized with digestate or with different production target (vehicle fuel) showed that substitution of mineral N-fertilizer through digestates result in a distinct reduction of the GHG emissions (Junker et al. 2015), but other reports also stressed the ample uncertainty of total GHG emissions due to the high share of soil borne N_2O emissions and their challenging estimation (Havukainen et al. 2018; Börjesson et al. 2015; Smeets et al. 2009). Moreover, GHG calculations for biofuels are based on annual N_2O emissions and they do not account for future N_2O emissions due to the mineralization of organic N of the applied digestates.

Conclusion

Nitrification inhibitors can as effectively mitigate direct N_2O emissions from biogas digestates as from synthetic ammonium-based fertilizers. However, our results show that the mitigation potential strongly depends on site characteristics: reduction of annual N_2O emissions is strongest if N_2O emission potential of the site is high and if a large proportion of emissions occurs during the fertilization period. The fact that 80% of our analyzed site-years did not show a significant effect of NI application on annual N_2O emission hampers general recommendations. The NIs may efficiently reduce direct N_2O emissions if

specifically applied to high emitting sites and high emitting crops (e.g., vegetables), but we found no evidence of a general reduction of annual N_2O emission. We did not determine NI effects on nitrate leaching and indirect N_2O emission. In particular, at sandy sites and for crops with high risk of nitrate leaching, NI effects might become more important on indirect N_2O emission than direct N_2O emission.

There was no positive effect of NI application on WOSR yield. This result was probably influenced by the generally high fertilization rate. The effects might be different in ground water protection areas where farmers have to reduce nitrogen application rates to avoid nitrate leaching. Overall, our results indicate that application of organic fertilizers such as digestate can help to save greenhouse gas emissions from industrial fabrication of synthetic N-fertilizers based primarily on fossil fuels. We found no evidence that N_2O emission from N application is higher for digestate than synthetic nitrogen fertilizer. The value of NIs with respect to mitigation of direct N_2O emission is probably restricted to sites and crops with high N_2O emission.

Future studies on the effects of NIs applied to WOSR fields fertilized with digestates should also focus on determining the magnitudes and trade-offs of indirect N_2O emissions associated with i) a potential increase in NH_3 volatilization caused by using NIs as well as ii) a decreased risk for NO_3^- leaching due to reduced soil NO_3^- concentrations over the fertilization period as also caused by using NIs. The latter seems particularly important for a proper evaluation of GHG release from sandy soils that showed a low potential for direct N_2O emissions. Further investigation should also consider the development of mitigation strategies for high N_2O emissions that can happen following WOSR harvest. This is crucially needed as such postharvest N_2O emissions can negate and even reverse the significant mitigating effect of NIs that occurred following digestate application over the early vegetative period of WOSR.

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