N_2O emissions from a loamy soil cropped with winter wheat as affected by N-fertilizer amount and nitrification inhibitor

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Abstract

Nitrogen (N) fertilization leads to the release of reactive N species, which can be detrimental to the environment. Nitrification inhibitors (NIs) are substances capable of retarding the oxidation of ammonium to nitrate, which can increase N use efficiency of applied N fertilizer and decrease N losses such as the release of the greenhouse gas nitrous oxide (N_2O). Adaption of N fertilizer amount to plant demand might also decrease N surpluses and thus lower N_2O emissions. We investigated the effects of N fertilizer amount (0, 120, 180, and 240 kg N ha^{-1} a^{-1}) and the use of the NI 3,4-dimethylpyrazol phosphate, DMPP, on annual N_2O emission from a soil cropped with winter wheat in a 2 year field experiment. N_2O fluxes were affected by N level and by use of DMPP with higher fluxes under high N amounts and treatments without NI. Application of DMPP led to a reduction of annual emissions by 45%. Interestingly, also winter emissions (8–12 months after N fertilization) were decreased by DMPP. In this period, a complete degradation of DMPP was assumed. The reason for this effect remains unclear. Wheat yield and quality were unaffected by DMPP, whereas grain yield was increased with N fertilizer amount in the first year. Nevertheless, response curves of grain yield-related N_2O emissions over all data showed lower optimal N fertilizer doses when DMPP was used. Application of DMPP at suboptimal N rates could help to achieve a better profitability with simultaneous reduction of the product scaled emission.

Introduction

Nitrogen (N) fertilization is essential to ensure high cereal yields and grain protein contents, as an increasing demand for cereals of over 9 billion people in 2050 is expected (Ladha et al. 2005). Wheat (*Triticum aestivum* L.) ranks among the most cultivated cereals, with a worldwide harvested area of 2.2 x 108 ha in 2013 (FAO 2015). Depending on climatic conditions, fertilizer-N amount and soil type, the N use efficiency (NUE, defined here as the difference between N-grain amount of a fertilized and an unfertilized treatment divided by N fertilizer amount) of mineral N fertilizers can be as low as 19-25% (Riley et al. 2011; Thomason et al. 2000). N fertilizer not transferred into the harvested crop remains in the field, contributing to N surpluses (the difference between fertilizer N applied and N removed from the field) which are susceptible to be lost into the environment (Cassman et al. 2002). Management measures which can improve the NUE of fertilizer-N and so reduce N surpluses after harvest, allow for the reduction of the total N fertilizer amount (Pasda et al. 2001).

One important path of N loss into the environment is the release of the climate relevant trace gas nitrous oxide (N_2O) from agricultural soils. With 4.1 Tg N_2O-N ha^{-1} a^{-1} agricultural soils account for 66% of the anthropogenic N_2O emissions (Davidson and Kanter 2014). Nitrous oxide accounts for 6.4% of the total global radiative forcing (Butler and Montzka 2015) and it is further involved in the depletion of stratospheric ozone (Crutzen 1970). It is generally agreed that the main microbial pathways contributing to N_2O production in agricultural soils are nitrification and denitrification (Braker and Conrad 2011), whereas other microbial pathways, as i.e. nitrifier denitrification, might also release considerable amounts of N_2O (Wrage-Mönnig et al. 2018). Since all these processes rely on mineral N as substrate for N_2O production, N_2O emission increases with the amount of N fertilizer applied (Stehfest and Bouwman 2006; Lebender et al. 2014).

High N_2O emissions have frequently been observed outside the cropping season, most often in conjunction with tillage (Lebender et al. 2014) or freeze/thaw cycles in middle and high latitudes (Wertz et al. 2016; Wagner-Riddle et al. 2017). High soil moisture favouring anaerobic conditions, easily available C and N compounds released after disruption of aggregates and lysis of microorganisms, and damaging of N_2O reductase during frost have been discussed as possible reasons for the high N_2O fluxes during thaw (Risk et al. 2013; Wertz et al. 2016). Emissions outside the cropping season account for 50% of the annual N_2O emission at German study sites (Kaiser and Ruser 2000) and can offset management induced N_2O reduction of the preceding cropping period on an annual basis (Ruser et al. 2001). Therefore, a reliable assessment of management strategies on the N_2O reduction potential requires the determination of annual N_2O fluxes in agricultural systems (Flessa et al. 1995).

A major part of the N uptake by plants can occur either as ammonium (NH_4^+) or nitrate (NO_3^-). This can impact plant physiology and soil pH, where NH_4^+ uptake is energetically more effective (Hawkesford et al. 2012). Oxidation of NH_4^+ to NO_3^- through nitrifiers occurs rapidly in agricultural soils, bearing the risk of NO_3^- leaching mostly in sandy soils (Singh and Verma, 2007). An efficient tool to stabilize NH_4^+ after fertilization, is the use of nitrification inhibitors (NIs). NIs are compounds able to retard the oxidation of NH_4^+ through the inhibition of the enzyme ammonia monooxygenase (AMO) (Subbarao et al. 2006). This reduces directly N_2O production during nitrification process, indirectly during denitrification as a result of a lower substrate availability and it also reduces NO_3^- leaching by prolonging the adsorption time of NH_4^+ in the top soil (Akiyama et al. 2010; Ruser and Schulz 2015). In contrast, NIs may enhance NH_3 emissions from surface applied organic fertilizers (Kim et al. 2012).

A common NI used under European practice conditions is 3,4-dimethyl pyrazolphosphate (DMPP) which can be applied with several mineral and organic N fertilizers. The mode of action of DMPP is still unclear. Chaves et al. (2005) suggested DMPP might inhibit nitrification by indiscriminately binding to membrane-bound proteins, as earlier work with Nitrapyrin, another heterocyclic N compound, reacted in this manner (Vannelli and Hooper, 1992). Florio et al. (2014) speculated DMPP could inhibit the transcription of bacterial and archaeal genes and serve as readily available C and N substrate for other microbial groups. Ruser and Schulz (2015) mentioned that DMPP is supposed to act as a metal chelator of copper, which is a cofactor of the AMO enzyme.

Although several studies have proven a high N_2O reduction potential of DMPP during the vegetation period in different production systems, like cereal (Linzmeier et al. 2001; Weiske et al. 2001; De Antoni Migliorati et al. 2014; Huérfano et al. 2015), grassland (Menéndez et al. 2006) or vegetable cultivation (Pfab et al. 2012), there is only little information on the effect of DMPP on annual N_2O emissions. Akiyama et al. (2010) calculated a 50% decrease in the relative N_2O emission by the application of DMPP. However, these findings were based on solely 12 field experiments, with a duration between 3 and 32 weeks, without sites with distinct frost periods and thus with only mild freeze thaw cycles.

The effect of DMPP on N_2O emissions after harvest is not clear. When compared to a conventionally fertilized treatment in a field experiment with vegetables, Pfab et al. (2012) measured a significant reduction of 45% in the N_2O emission after DMPP application in July. Due to high soil temperatures in the period July until September, it was assumed that DMPP was more or less completely degraded. Unexpectedly, the reduction in the N_2O fluxes lasted more than 7 months. Similarly, Pfab et al. (2012) measured lower soil respiration rates in the DMPP treatment during winter, indicating a negative effect of DMPP on heterotrophic and potentially denitrifying microorganisms. Under sub-tropical conditions, Scheer et al. (2017) found that DMPP used in a vegetable production system was able to reduce N_2O emissions during the vegetation period. However, this mitigation was offset by post-harvest emissions, fully compensating for the emissions during the vegetation period, thus leading to no NI effect on an annual basis. The authors of the study attribute these high emissions to higher soil mineral nitrogen (N_{min}) contents after harvest as a result of the use of NIs.

The frequently shown increased NUE with NI application gives the opportunity to reduce the amount of N fertilizer. Alonso-Ayuso et al. (2016) achieved the same yield and N uptake for maize with 170 kg N ha^{-1} without NI and 130 kg N ha^{-1} with DMPP. The reduction of N fertilizer would also decrease indirect N_2O emissions such as emissions during production and distribution of N fertilizer, nitrate loss or ammonia volatilization. For a reliable estimation of the effect of NIs on yield and N_2O emissions, Rose et al. (2018) claimed the need of field studies with at least one suboptimal N rate considerably below the recommended N amount. In their meta-analysis, they found only 10 datasets fulfilling this requirement. Within this dataset, there was no study with DMPP application in wheat. Rose et al. (2018) also pointed out the need to evaluate the economic viability of reduced N fertilization with NIs, since the use of NIs is afflicted with additional costs. The aim of this work was to test the following three main hypotheses: (1) increasing N fertilizer application increases N surpluses and annual N_2O emission; (2) the application of DMPP reduces the N_2O emission of a soil cropped with winter wheat in the cropping period as well as in the period after harvest; and (3) a reduction of the N fertilizer dose in combination with DMPP application is environmentally sound due to the reduction of N_2O emissions and economically viable.

Material & Methods

Field experiment

Field experiments were carried out at the experimental farm of the University of Hohenheim "Heidfeld-hof", South of Stuttgart, Southern Germany (48° 42' 59" N; 9° 11' 42" E), 400 m above sea level. The mean annual temperature during the last ten years was 10.2°C, the mean annual precipitation was 628 mm (2007 – 2016). Soil type is a Haplic Luvisol derived from periglacial loess ("Filder Plateau").

C_{org} of the topsoil (0-0.3 m) was 1.19%, $N_t$was 0.121%, and soil texture of the stone free soil was silt dominated (2% sand, 72% silt, and 26% clay). The initial pH (0.01 M $CaCl_2$) was 6.7.

The previous crop of the site was winter wheat and basic fertilization took place in 27 October 2010 with phosphorus, sulphur and potassium (23-23-43 kg ha^{-1}, respectively). Since availability of seeds was limited, two different winter wheat varieties were sown in the two experiment years: Toras (1st year, sowing date 13 October 2010) and Schamane (2nd year, sowing date 6 October 2011). Despite of the fact that both varieties have very similar characteristics (i.e. ear emergence, maturity) and belong to the same wheat yield and quality group (Federal Plant Variety Office, 2013), this might bias the year effect in statistical analyses. Nitrogen fertilization took place on 4 April 2011 and 29 March 2012 as either ammonium sulphate nitrate (ASN, –NI treatments) or ENTEC26® (ASN plus DMPP, +NI treatments), except for the unfertilized treatment (N0). Splitting of N fertilization in this region was shown not to lead to differences in grain yield or quality (Schulz et al. 2015). Therefore, fertilizer was applied in one dose at growth stage 27-32 (Zadoks et al. 1974) which should also improve comparability of the +NI and –NI treatments.

The 28 plots (3m x 5m) used for this investigation were selected from a fully randomized block experiment with four replicates. In order to avoid possible DMPP or N surplus effects on the second year, the same experimental design was carried out at an adjacent site in the second year (Figure S. 1). Each plot was divided into a sampling and a harvest plot (1.5m x 5m each). Gas and soil samples were collected in the sampling plot. The harvest plot was subjected to harvesting for plant analysis (C and N). Fertilizer amount N2 (1st year 175, 2nd year 180 kg N ha^{-1}) was calculated according to the German Fertilizer Ordinance (DüV 2006) ("best management practice"), in treatment N1, fertilizer amount was 30% reduced (both years 120 kg N ha^{-1}) whereas in treatment N3 it was 30% increased (1st year 230, 2nd year 240 kg ha^{-1}). Wheat was harvested with a plot harvester on 11 August 2011 and on 2 August 2012. For a more detailed information of the field management see Table S. 1 (Supplement material).

Short term experiment on mini plots

Several trace gas studies at our investigation site showed temporal C limitation for N_2O release (Pfab 2012, Seiz et al. 2019). Low N_2O fluxes following N fertilization in our study seemed to confirm such a limitation (Figure 1). Based on these results we initiated a two week short field experiment in the same field, adjacent to the main field experiment (Figure S. 1). We used a three factorial split-plot design (n=3), with factor soil moisture (dry and wet) as main plots and N amount (0, 75 and 150 kg N ha^{-1} applied as NH_4NO_3) and C application (0 and 360 kg C ha^{-1} as glucose) as subplots (1m x 1m). The short term experiment took place after the wheat harvest of the second experimental year (19–30 August 2013) after removing straw residues. One week before gas sampling a shallow soil tillage operation was done. Wet plots were watered with 61 mm at the beginning and in the middle of the experiment in order to achieve 80% WFPS. Dry plots were not watered and covered with a white plastic cover in case of rain.

Water, C and N were applied to the plots two times (19 and 26 August 2013) and gas and soil samples were taken one, two and four days after treatment application.

Determination of N_2O flux rates and further measurements in the field

Gas fluxes were measured at least weekly between 15 March 2011 and 28 February 2013. In periods where high flux rates were potentially expected (e.g. after N fertilization, tillage, rewetting of dry soil in summer or during freeze/thaw cycles in winter) additional measurements were conducted, in order to obtain a higher resolution of the trace gas fluxes and to avoid under- or overestimates of the cumulative N_2O emissions. Gas fluxes were determined using the closed-chamber method (Hutchinson and Mosier 1981). Circular PVC bases with an inner diameter of 0.3 m and a height of 0.15 m were installed permanently on the field at a depth of 0.08 m in the middle of each sampling plot. During the vegetation period additional PVC extensions of 0.3 or 0.6 m of height were used (Flessa et al. 1995). For gas sampling dark PVC chambers with a height of 0.16 m were airtightly placed on the circular base. Four gas samples were taken periodically with evacuated vials (volume=22.4 ml) during the enclosure of 45 minutes. N_2O and CO_2 concentrations of the samples were measured with a gas chromatograph (5890 series II, Hewlett Packard) equipped with a ^{63}Ni electron capture detector (ECD) and an autosampler (HS40, Perkin Elmer). Gas flux rates were calculated using the linear slope of the trace gas concentrations in the chambers atmosphere over time as described by Ruser et al (1998). Since we used dark chambers CO_2 fluxes were not calculated as long as the base rings were covered with wheat plants. Outside the cropping season CO_2 fluxes from bare soil were used as an indicator for soil respiration and carbon availability.

Simultaneously to each trace gas measurement we determined soil temperature in 0.1 m depth. Air temperature in 2 m height and daily precipitation were recorded approximately 300 m away from our experiment. These data were provided by the agricultural research station (Landwirtschaftliches Technologiezentrum Augustenberg 2015).

Laboratory analysis

Simultaneously to each gas sampling, soil samples were taken from a mixed sample of eight soil cores (0 – 0.3 m deep and 14 mm diameter). We pooled and homogenized the soil samples over the four replicated plots. Samples were kept cold in the field and were frozen until further analysis. Soil moisture was calculated gravimetrically after drying an aliquot of the soil samples for 24 h at 105°C. Water filled pore space (WFPS) was calculated assuming a bulk density of 1.25 Mg m^{-3} for the plough horizon (0 – 0.3 m) as follows:

$$WFPS = \frac{gravimetric\ water\ content \times soil\ bulk\ density}{total\ soil\ porosity}$$

Where the soil porosity is calculated as:

$$Soil\ porosity = \frac{((1 - soil\ bulk\ density))}{2.65}$$

and where 2.65 Mg m^{-3} (density of quartz) is the assumed particle density of the soil. For determination of the N_{min} concentrations, soil samples were thawed and 30 g of fresh soil were immediately extracted with 60 ml of a 0.5 M K_2SO_4 solution. NO_3^- and NH_4^+ concentrations were measured with a flow injection analyser (3 QuAAtro.AQ2.AACE, SEAL Analytical, UK).

Yield and plant analysis

Aliquots of the straw and grains were dried for 48 h at 60°C and ground. C- and N- analyses were performed with an elemental analyser (vario MAX CN, Elementar Analysensysteme, Hanau). NUE (also found in literature as apparent recovery efficiency of grain) was calculated as the difference of the N amount in grain of a fertilized treatment and the unfertilized control divided by the rate of N fertilizer applied.

Cumulative emissions, statistical analysis, and further calculations

Cumulative N_2O emissions were calculated stepwise, i.e. assuming a constant flux rate until next sampling date. Cumulative emissions were calculated for different time periods ("seasons"), defined as "vegetation period" which included all N fertilizer applications (15 March – 11 August 2011 and 6 March – 9 August 2012), "tillage" (12 August – 11 November 2011 and 10 August – 29 November 2012) and "winter" (16 November 2011 – 7 March 2012 and 30 November 2012 – 21 March 2013).

A complex treatment structure (augmented factorial) was chosen for the statistical analysis based on Piepho et al. (2006). The Proc Mixed procedure in SAS (9.4 TS1M0 SAS Institute Inc., Cary, NC, USA.) was used in order to investigate the effects of N fertilization, N fertilizer level, use of nitrification inhibitor and year on harvest indicators, cumulative N_2O emission and N_2O flux rates. N_2O flux rate and cumulative emission models included the fixed effect season. Variables were log-transformed in order to meet the model assumption of normality of residuals and variance homogeneity, when necessary. N_2O flux rates were transformed using a Box-Cox transformation SAS macro (Piepho 2017).

A repeated measures analysis of variance with a spatial power covariance matrix was used in order to compensate for serial autocorrelation with different sampling frequency. Selection of standardized regression parameters was done by checking the significance ($p < 0.05$) of the variable and the models' Akaike Information Criterion (AIC) for response variables soil temperature, soil NH_4^+ and NO_3^- amount, WFPS and the difference between the actual WFPS and the WFPS at the previous sampling (ΔWFPS).

A stepwise multiple regression model of N_2O fluxes was conducted additionally on the same response variables using the statistical program R (R Core Team 2016) and the stepAIC function in R package MASS (Venables and Ripley, 2002). Linear regression of individual variables were conducted in order to investigate how certain variables affect N_2O fluxes. When necessary, N_2O fluxes were log-transformed in order to fulfil normality of residuals and variance homogeneity. The R package relaimpo was used in order to calculate relative importance of selected variables (Grömping 2006).

Statistical analyses of the short experiment were performed with the statistical program R (R Core Team 2016). Effect of the factors soil moisture, N amount and C application on N_2O and CO_2 flux rates of the short experiment were assessed with a repeated measures model using the R package nlme (Pinheiro et al. 2017) with block, date and treatments as fixed effects, date as repeated term, with the interaction block and moisture plots as subject. Fluxes were log-transformed in order to achieve normal distribution of residuals. Least-square means and letter display for pairwise comparisons were performed using the R packages lsmeans (Lenth 2016) and multcomp (Hothorn et al. 2008).

For an assessment of the response of yield, gross margin (defined as revenue minus fertilizer cost), as well as grain yield and grain N related N_2O emissions to N amount, a quadratic fit was performed with the statistical program R using NI as a dummy variable in order to assess the effect of DMPP on response curves. NI variables included N0 in both levels. Optimal N amounts were calculated as the maximal point of the quadratic function. For calculation of gross margin, revenues for three quality classes (staggered according to protein content) were calculated using 2017 mean prices from the Mannheim commodity exchange (less than 11.5% protein: 161 Mg^{-1}; between 11.5 and 13.5% protein: 167 Mg^{-1}; over 13.5% protein 169 Mg^{-1}) and September 2017 prices for ASN (0.885 kg N^{-1}) and ENTEC26® (1 kg $N^{-1)}$. For calculation of standard errors of optimal N values the function deltamethod from the R package msm (Jackson 2011) was used.

N balance was calculated as the difference between N applied and N removed with wheat grain. All graphs were done with the graphical R package ggplot2 (Wickham 2009).

Weather conditions

The mean air temperature during the vegetation period was 14.9°C and 13.9°C in the 1st and 2nd experimental year, respectively. Only during February 2012 a long frost period took place. Precipitation was in part low, especially during the first year. Compared to a 4-year mean (2007 – 2010), cumulative precipitation in the first 13 weeks after N application in the first experimental year was 47% lower. In order to avoid water shortage in the 2nd year, we decided to irrigate the experiment when the weekly amount of precipitation was 20% lower than the 10-year mean precipitation. This was the case only once (29 May 2012, Figure 1f).

Warm and humid conditions were observed in the period between autumn 2011 and January 2012, followed by frost temperatures without snow cover (Figures 1d and 1f). We observed yellowing leaf tips and a slightly silage odour at the beginning of the second vegetation period. These symptoms are typical for insufficient winter hardening and frost injuries (Klein 2006; Peltonen-Sainio et al. 2011). As snow cover in February 2012 varied among districts and regions, winter wheat yields in the region of Stuttgart varied also between 6.3 and 7.3 Mg ha^{-1} (Regional Database Germany 2017). Our results (Table 2) are in concordance with the grain yield of the regions most affected by the low winter temperatures. During spring and summer 2012 high precipitation and warm weather conditions promoted leaf rust in all treatments. This might have had negative effects on grain yield of the 2nd experimental year.

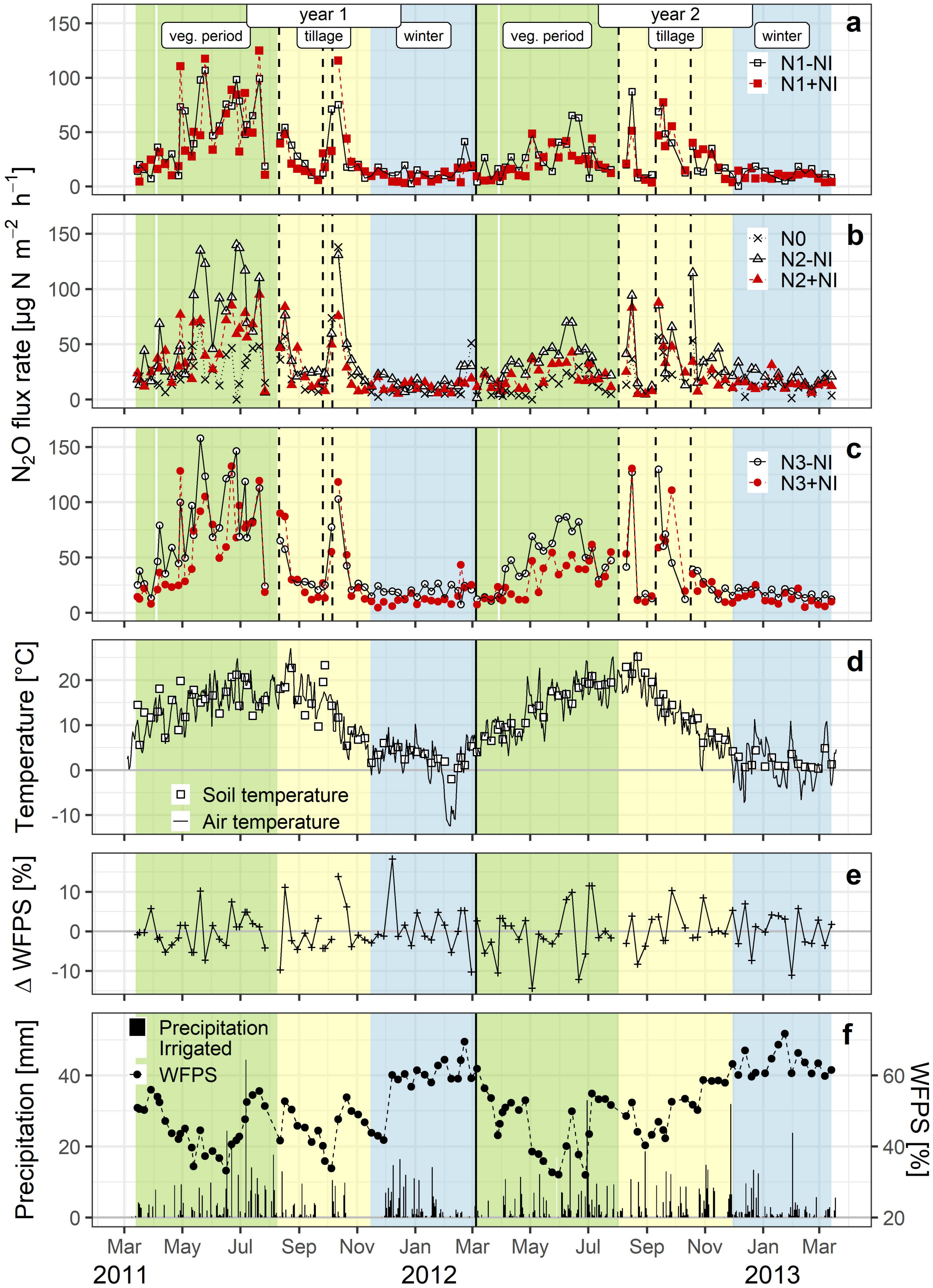

Figure 1: **a–c** Mean N_2O flux rates (n = 4) during the experimental period as affected by N-fertilizer rate (N0, N1, N2, N3) and use of nitrification inhibitor DMPP (- NI, + NI). Solid white and broken black lines indicate fertilization and tillage events, respectively. **d** Mean daily air temperature (2 m height) and soil temperature in 0.1 m depth during gas sampling (mean over all treatments); **e** mean difference between the actual WFPS and the WFPS at the previous sampling (ΔWFPS); **f** daily precipitation, irrigation and mean water-filled pore space (mean over all treatments) in the experimental period. Note only one irrigation event (29 May 2012).

Results and Discussion

Temporal N_2O flux pattern

A high spatial and temporal variability of N_2O fluxes was measured, with fluxes ranging between 0 and 158 μ N_2O-N m^{-2} h^{-1} (Figure 1a-c). Despite high soil mineral N contents (Figure 2), N_2O fluxes most often remained low after N applications (Figure 1a-c). Generally, high N_2O pulses during the vegetation period occurred after rewetting of dry soil, which was indicated by the highest changes in soil moisture (ΔWFPS, Figure 1e). During the first distinct N_2O pulse (20 May 2011), the flux rate in treatment N3-NI was 5 times higher than on 14 April 2011 in the same treatment. Soil moisture and soil temperature and NO_3^- content did not differ on these two sampling dates. In contrast ΔWFPS was 10% on 20 May and -5% on 14 April 2011. Low N_2O fluxes on 14 April and high N_2O fluxes on 20 May hint on the importance of strong changes in soil moisture for N_2O release.

In their meta-analysis study, Kim et al. (2012) calculated an increase of N_2O fluxes by almost 5 times in cropland after rewetting. As the content of available organic matter in soils may increase with soil drying, organic substrates for soil microorganisms may be highly available when soil is rewetted (Zsolnay 1996). Consequently, the following increased C turnover creates anoxic microsites, which promotes denitrification and so, N_2O release.

In the vegetation period of the first year, rewetting events occurred more often than in the second experimental year (Figure 1e). As a result, the share of the emission in this period to the total annual N_2O emission was significantly higher in the first year (61% in the 1st year versus 46% in the 2nd year) (Table 3).

Regardless of N fertilization, soil tillage after harvest steadily increased N_2O fluxes in all treatments (Table 1). Tillage events were shown to increase C turnover inside the aggregates (Six et al. 1999), to increase potential nitrification and denitrification (Staley et al. 1990) and to enhance C and N availability from crop residues for soil microbes (Granli and Bøckman 1994) thus stimulating denitrification. As a result N_2O emissions were often shown to be increased after tillage (i.e. Mutegi et al. 2010). Mutegi et al. (2010) attributed increased N_2O emissions after soil disturbance under wet, yet warm autumn conditions, to an increased soil organic matter and crop residue turnover and N mineralisation, which lead to an elevated O_2 consumption rate, stimulating N_2O production through denitrification. This might also have been the case in our study, since straw was incorporated after harvest at a period when mean daily air temperature was the highest, which we registered in both years (average air temperature 27°C and 26.3°C, first and second year respectively).

Additionally to N and soil moisture limitation and since high fluxes most often occurred under conditions of increased C availability (rewetting and tillage), we thus also hypothesized a temporary carbon limitation at our study site. This was confirmed by our short-term field experiment studying the effect of C, N and WFPS availability on the same field (Table 4). We found a significant difference between the trace gas fluxes in treatment N75 and N150, when C was added. Although N application and soil moisture had a significant effect, the highest stimulating effect on N_2O fluxes was the addition of carbon on N_2O fluxes when N was added. Although we could not steadily raise WFPS in the wet treatment (mean WFPS dry 51.3%, wet 53.7%) – probably due to both a rapid horizontal and lateral water movement – we found a significant effect of watering on N_2O fluxes.

In unsaturated soils, denitrification is often limited by soil C availability (Robertson and Groffman 2015). Soil C can promote denitrification both directly, by providing electrons to heterotrophs, including denitrifiers, and indirectly, by reducing O_2 availability (and so decreasing soil redox potential) through C mineralization (Robertson and Groffman 2015). In a series of soil incubation experiments, Gilliam et al. (2008) investigated the effect of C, N and WFPS on N_2O emission. In their experiment,

Table 1: Type 3 tests of fixed effects and back transformed least square means of significant interactions. Selection of standardized regression parameters was done for the response variables soil temperature, soil NH_4^+ and NO_3^- amount, WFPS and the difference between the actual WFPS and the WFPS at the previous sampling (ΔWFPS). β= Standardized coefficient

Effect	Num DF	Den DF	F Value	p value	β
block × year	6	626	0.47	0.8300	
year	1	711	0	0.9460	
year×season	4	469	29.84	<0.0001	
year×season×date	6	269	32.24	<.0001	
fertilization	1	625	62.47	<0.0001	
fertilization×N fertilizer level	2	1281	37.26	<0.0001	
fertilization×NI	1	1280	72.94	<.0001	
fertilization×N fertilizer level×NI	2	1281	3.03	0.0488	
fertilization×year×season	5	387	7.98	<0.0001	
WFPS	1	1943	57.1	<0.0001	-0.061
ΔWFPS	1	2412	40.97	<0.0001	0.032

Least square means of interactions[1]
[μ N_2O-N m^{-2} h^{-1}]

fertilization	N fertilizer level	Use of NI	
No	N0	N0	12.5f
Yes	N1	-NI	18.2cd
Yes	N2	-NI	24.1b
Yes	N3	-NI	29.2a
Yes	N1	+NI	14.9e
Yes	N2	+NI	16.6de
Yes	N3	+NI	20.2c

	Fertilization[2]		
Year-season	N0	fertilized	p value
1-vegetation	14.3ab	31.5a	<0.001
1-tillage	20.6a	21.7bc	0.786
1-winter	9.5c	14.1d	<0.001
2-vegetation	6.0d	18.6c	<0.001
2-tillage	17.1ab	23.7b	0.052
2-winter	11.3bc	14.7d	0.044

[1] Least square mean N_2O fluxes not sharing any letter are significantly different for fertilization, use of NI, N level or season within fertilization treatments ($p < 0.05$).
[2] p values of difference between fertilization treatments within year and season.

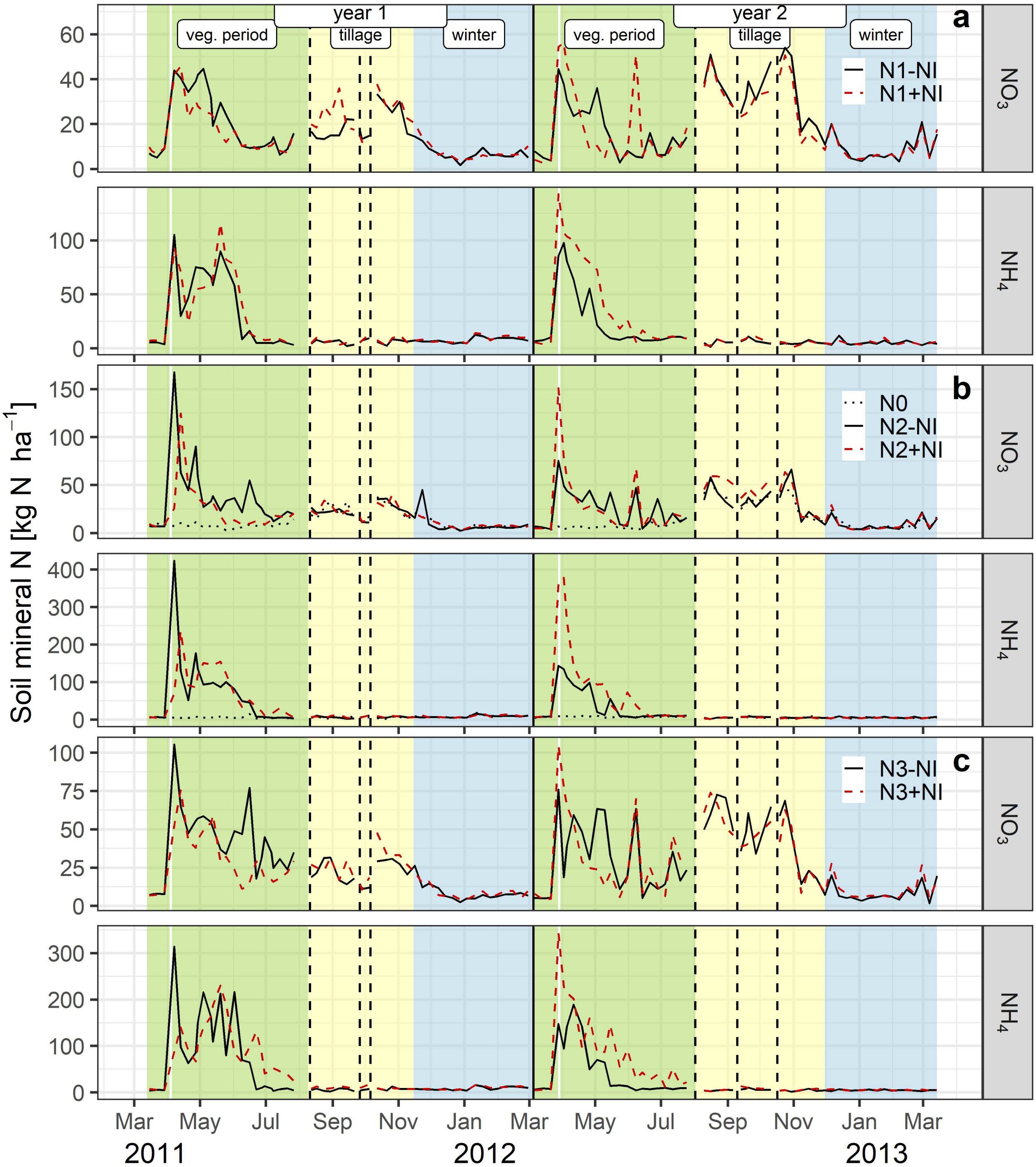

Figure 2: **a–c** NO_3^- and NH_4^+ contents (0–0.3 m depth) during the experimental period as affected by N-fertilizer dose (N0, N1, N2, N3) and use of nitrification inhibitor DMPP (- NI, + NI). Solid white and broken black lines show fertilization and tillage events, respectively. Note different y axis scaling.

C application alone was the main driver for N_2O emissions in a two factorial experiment (C × N) and in a three factorial experiment (C × N × WFPS), C and WFPS mainly regulated N_2O fluxes. Ju et al. (2011) reported that nitrification may become the main N_2O source in a winter wheat/summer maize rotation when C availability was limiting. In their study an ammonium application increased emissions significantly, when compared to nitrate, and NO_3^- was able to enhance N_2O emissions only if C was added. They attributed these results to a low readily oxidable C combined with a well-aerated loamy soil, thus favouring conditions for nitrification.

A distinct frost/thaw cycle occurred only once (February 2012, Figure 1d). We did not measure increased N_2O fluxes during the frost and the following thawing phase (Figure 1a-c). In contrast, high N_2O flux rates were measured at the same study site and in the same winter period on an adjacent plot experiment with a vegetable crop rotation (data not shown). The absence of distinct N_2O pulses during thawing may again be due to substrate limitation for N_2O production in our wheat experiment.

Table 2: Least square means of N_2O fluxes as affected by N (NH_4NO_3) and C (glucose) application and soil moisture (M) for the short field experiment

Treatment			N_2O flux
N	C	M	
kg ha^{-1}	kg C ha^{-1}		μ N_2O-N m^{-2} h^{-1}
0	0	Dry	90
0	360	Dry	144
75	0	Dry	174
75	360	Dry	1225
150	0	Dry	271
150	360	Dry	2252
0	0	Wet	157
0	360	Wet	108
75	0	Wet	537
75	360	Wet	3034
150	0	Wet	773
150	360	Wet	6417
Significant effects ($p < 0.05$)			C×N, M×N, M, C, N

A stepwise multiple regression calculated over the whole dataset showed that the variables soil temperature, WFPS, soil NO_3^- and ΔWFPS were able to explain 15% of the variability of N_2O fluxes, whereas NH_4^+ did not enter the regression model (Table S.5). Except for WFPS, all variables entered into the model with positive estimates. Although N_2O fluxes can increase with increasing soil moisture, the opposite effect was observed for the whole annual data set as covariate in the repeated measures analysis and as a regression variable in the multiple regression analysis (Table 1 and Table S.5). This result reflects the relationship between WFPS and emissions driven by seasonal events, with higher emissions during the comparatively dry vegetation and tillage period and lower emissions in the C limited humid winter, without freeze-thaw events.

Effect of fertilization and fertilizer amount on N_2O emission and yield

N fertilization significantly increased N_2O emissions (Tables 1 and 3). In both experimental years, the unfertilized control treatment showed the lowest N_2O emission (2.1 and 1.3 kg N_2O-N ha^{-1} a^{-1}). Annual N_2O emissions from the fertilized treatments ranged between 1.7 and 4.0 kg N_2O-N ha^{-1} a^{-1} and increased with the amount of N fertilizer (Table 3).

Annual N_2O emissions from our experiment were in the same order of magnitude as N_2O emissions reported for different winter cereal fields in Germany (Kaiser and Ruser 2000; Jungkunst et al. 2006). N fertilization was frequently shown to enhance N_2O emissions from arable soils since it provides the substrates for microbial N_2O production in soil (i.e. Stehfest and Bouwman 2006). Although

Table 3: Least squares means of cumulative N_2O emission per season and year as affected by significant effects [N fertilizer level and use of nitrification inhibitor (DMPP)].

Year	Season (days)	Fertilizer level				Nitrification inhibitor		
		N0	N1	N2	N3	N0	-NI	+NI
		g N_2O-N ha^{-1}						
1	Vegetation (149)	892c	1684b	1834ab	2262a	892c	2140a	1708b
1	Tillage (96)	820a	721a	849a	892a	820a	877a	762a
1	Winter (113)	283b	303b	375ab	414a	283b	440a	297b
1	Annual (358)	2058c	2768b	3137b	3690a	2058c	3546a	2851b
2	Vegetation (149)	430c	860b	963b	1472a	430c	1277a	894b
2	Tillage (119)	571c	740bc	861ab	1060a	571b	921a	837a
2	Winter (112)	270b	260b	395a	382a	270b	414a	279b
2	Annual (380)	1322c	1912b	2353b	3015a	1322c	2725a	2128b
1 and 2	Annual	1690d	2340c	2745b	3352a	1690c	3136a	2489b

Least squares means not sharing any letters are significantly different (between fertilizer levels or use of DMPP; $p < 0.05$).

fertilizer amount significantly affected N_2O fluxes (Table 1), NO_3^- was able to account only for 2% of the variance in the multiple regression model (Table S.4). One possible reason for the marginal importance of soil mineral N as a driver for N_2O fluxes, might be the upper mentioned temporary C limitation for denitrifiers, impeding N_2O production for large periods although sufficient NO_3^- was available. Another explanation might be the highly dynamic nature of the soil NO_3^- pool: i.e. Burton et al. (2008) found no direct correlation between N_2O fluxes and the actual soil NO_3^- concentration, but rather an integrated NO_3^- measure, which should reflect the accumulated effects of NO_3^- on soil microbiology. Except for the tillage phase of the first year, where N_2O fluxes showed a high spatial heterogeneity, fertilization-related differences on a cumulative basis occurred in every single phase, with higher N_2O losses at higher N fertilizer doses (Table 3).

In addition to the relationship between the N_2O fluxes and the main drivers we also found a negative linear correlation between the cumulative N_2O emissions after harvest in the second experimental year and the C/N ratio of the chopped and incorporated straw ($p < 0.001, R^2 = 0.37$). This was in agreement with Kaiser et al. (1998), who also reported a decrease of the N_2O emission in winter with increasing dry matter to N ratio of incorporated wheat, barley and oilseed rape residues. Increasing C/N-ratios might have led to an immobilization of mineral N in autumn and thus reduced substrate availability for N_2O production in soil over winter. The low straw C/N ratio of the 2nd year might have been a consequence of leaf rust, since environmental conditions and field management were optimal for its development (humid conditions during summer and repeated cultivation of winter wheat on the same field). As leaf rust can have an effect not only on yield, but also on N grain content (Bancal et al. 2008; Devadas et al. 2014), N translocation from straw to grains in the second year was probably disrupted (Table 2), leaving up to 87 kg N ha^{-1} as straw-N in the field (Table S.3). This effect is reflected in the higher soil NO_3^- content during the tillage period in the 2nd year (Figure 2a-c). The positive correlation between NO_3^- content in the topsoil and the N_2O emission over the entire experimental dataset (Table S.5) explains the high contribution of the N_2O emission in this tillage period to the total annual emission (Table S.4).

Table 4: Least square means for significant ($p < 0.05$) effects of several agronomic variables as affected by year, N fertilizer level and use of a nitrification inhibitor

Significant single effects	Fertilization		Nitrification inhibitor			Fertilizer level				Year	
	N0	fert	N0	-NI	+NI	N0	N1	N2	N3	1	2
Grains per ear, n	27.1^{b}	32.9^{a}									
Straw C/N, ratio						112^{a}	86^{b}	71^{c}	62^{d}	121^{a}	69^{b}
Straw-N, kg N ha^{-1}						13.1^{d}	25.2^{c}	31.1^{b}	39.6a		
N_2O/grain-N, g N_2O-N kg^{-1} grain-N	27.5^{a}	21.2^{b}	27.5^{a}	23.5^{b}	18.9^{c}						
N_2O/grain yield, g N_2O-N kg^{-1} grain			0.47^{ab}	0.5^{a}	0.4^{b}	0.47^{ab}	0.4^{b}	0.43^{b}	0.52^{a}	0.5^{a}	0.4^{b}

Significant interactions	Year				Year							
	1	1	2	2	1	1	1	1	2	2	2	2
	Fertilization				Fertilizer level							
	N0	fert	N0	fert	N0	N1	N2	N3	N0	N1	N2	N3
Ears, n m^{-2}	315^{c}	395^{b}	309^{c}	514^{a}								
Straw yield, Mg ha^{-1}	2.9^{c}	3.9^{b}	3.8^{b}	5.7^{a}								
Grain yield, Mg ha^{-1}					4.1^{d}	5.9^{c}	6.6^{ab}	7^{a}	3.3^{d}	6.2^{bc}	6.2^{bc}	6^{c}
TGW, g					43.6^{bc}	46^{ab}	47.1^{a}	46.7^{a}	42.5^{c}	41.7^{c}	39.4^{d}	37.4^{d}
Straw-N, kg N ha^{-1}	9.2^{c}	17.7^{b}	18.7^{b}	55.8^{a}								
Grain-N, kg N ha^{-1}					69.6^{e}	120.7^{cd}	148.3^{b}	166.7^{a}	55.2^{e}	110.1^{d}	121.5^{c}	127.5^{c}
NUE, %						34.8^{bc}	39.6^{ab}	38.2^{b}		45.7^{a}	36.8^{b}	30.1^{c}
N balance, kg N ha^{-1}					-69.6^{e}	-0.7^{d}	26.7^{c}	63.3^{b}	-55.2^{e}	9.9^{d}	58.5^{b}	112.5^{a}

TGW = Thousand grain weight, *NUE* = Nitrogen use efficiency, also defined as apparent recovery efficiency of grain.
Mean values (or medians, for backtransformed data) not sharing any letter are significantly different for each N level or use of NI with $p < 0.05$.

The mean amount of N released as N_2O by all fertilized treatments in the period after harvest (tillage and winter) of the 1st and 2nd year were 1.2 and 1.3 kg N ha-1, respectively and contributed to 39% and 54% of the annual N_2O emission. In a study by Röver et al. (1998), only a small difference of the N_2O emissions from a soil cropped with winter wheat between the fertilized and unfertilized treatments was measured. Most of the emissions were registered during winter driven by freeze-thaw events, with a high share of the winter emissions to annual losses. In our experiment, the period after harvest was also responsible for a high share of the annual N_2O losses (Table S.4), nevertheless due to repeated soil tillage and not freeze-thaw events.

N_2O fluxes increased with increasing N fertilizer rates (Table 1). Cumulative emissions of the periods following harvest were also significantly higher at elevated N fertilizer rates (Table 3). Increasing N fertilizer amount increases the substrate availability for nitrification and denitrification and thereby N_2O emissions (Granli and Bøckman 1994; Shcherbak et al. 2014). Although several studies have found a nonlinear relationship between these two variables, with an exponential increase of N_2O fluxes above maximum crop N uptake (i.e. Shcherbak et al. 2014), a linear response has often been observed for N application rates similar or below crop demand (Liu et al. 2012; Lebender et al. 2014). Nevertheless, N_2O emissions from soils cropped with cereals appear to be lower than those of other arable crops (Dobbie et al. 1999). Kaiser and Ruser (2000) found a better correlation between N balance and annual N_2O emission than between N fertilizer amount and N_2O emission, because N balance considers both plant N uptake and N mineralization. In our experiment, the increasing N_2O emission with increasing N fertilizer level in the periods after harvest underlines this effect; high N balances due to high N fertilizer level and the above mentioned lower N translocation into the grains in the 2nd year (Table 2, straw and grain-N) lead to high N_2O losses (Table 3).

Effect of nitrification inhibitor on N_2O emission and yield indicators

N_2O fluxes were significantly reduced by DMPP (Table 1), leading to a reduction of annual emissions of 45% (both years, all N fertilizer levels, unfertilized control subtracted, Table 3). The reduction was significant during the vegetation period and, despite high variation of weekly fluxes, also significant for the cumulated emissions during the winter season (Table 3).

As expected, soil NH_4^+ was repeatedly higher in the +NI treatments, especially in the N2 and N3 fertilizer levels during the vegetation period (Figure 2b-c). A tendency of increased soil NH_4^+ amount with the use of DMPP was observed, even more clearly in the 2nd year. The period until NH_4^+ reached a pre-fertilization level was longer with DMPP and with higher N application rate (1st year=−NI: 11 weeks; +NI: 12 to 16 weeks. 2nd year=−NI: 7 to 10 weeks; +NI: 10 to 17 weeks). During soil tillage events, similar NO_3^- amounts were found throughout all treatments with higher values in the second year. A decrease in NO_3^- in the sampled soil layer was measured with the beginning of the winter period. At harvest, soil NH_4^+ was low in all treatments. After harvest, soil NO_3^- was similar across treatments.

An effect on nitrification related variables (higher soil NH_4^+ , lower soil NO_3^- and lower N_2O fluxes) by the use of DMPP as a nitrification inhibitor with NH_4^+ and NH_4^+ releasing fertilizers has already been confirmed across climates, soil types and soil characteristics (Akiyama et al. 2010; Ruser and Schulz 2015). Nevertheless, most of these results derive from measurements just from the vegetation period and often from sites without high emission events after harvest (i.e. tillage, freeze-thaw cycles). Compared to other field studies with DMPP in winter wheat our results fit in the range of emission reduction. Weiske et al. (2001) reported a reduction of 49% during the cropping season for measurements in South Germany. Similar N_2O reduction potentials were found also under contrasting climatic conditions, i.e. under oceanic climate (39-83% reduction in wheat; Huérfano et al. 2015), under semi-arid Mediterranean conditions (53-72% reduction in barley; Abalos et al.

2017), and under sub-tropical climate conditions (71-81% vegetable production and maize-wheat; Scheer et al. 2014 and De Antoni Migliorati et al. 2014).

Pfab et al. (2012) reported an annual N_2O reduction between 40 and 45% after N-fertilization together with DMPP from vegetable production at the same study site as our experiment. Although this vegetable system received higher amounts of N-fertilization and differed markedly in management, N_2O reduction was in the same range.

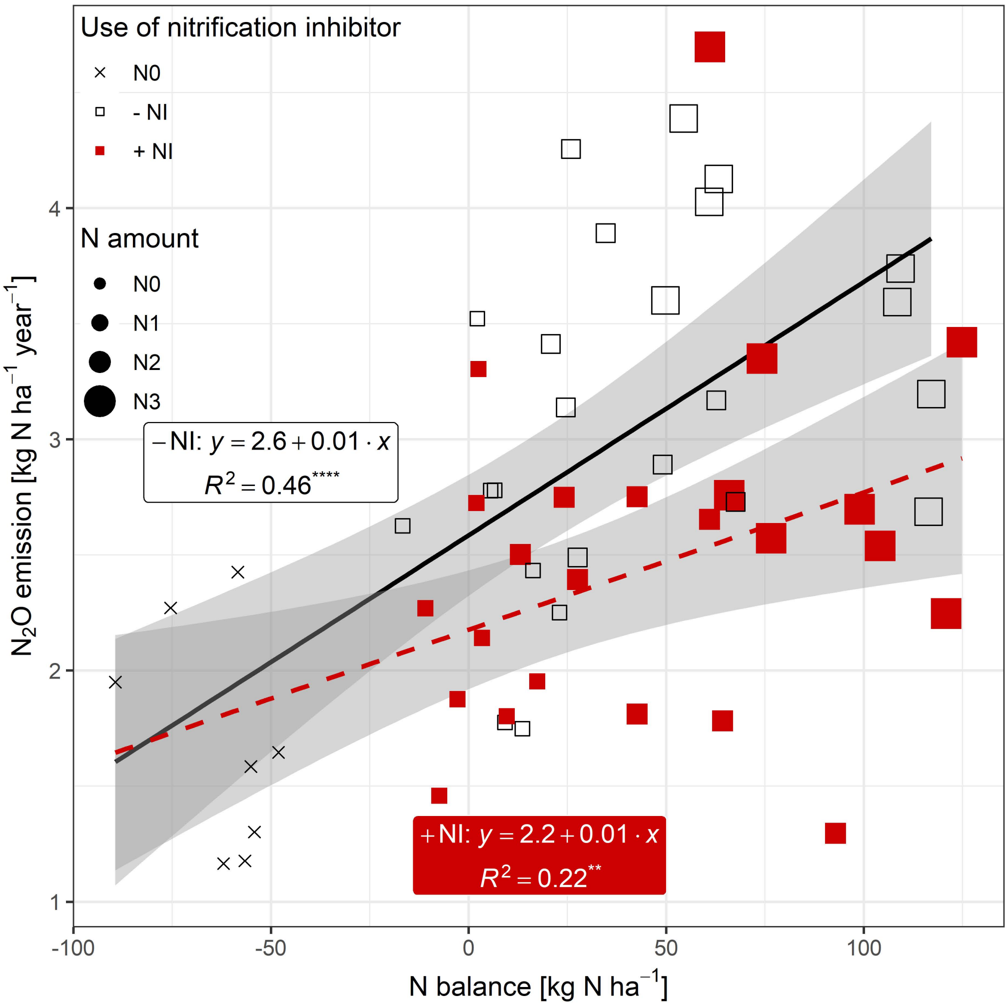

Figure 3: Relationship between N balance and annual N_2O emission, as affected by application of nitrification inhibitor DMPP (- NI, + NI) and N fertilization rate (N0, N1, N2, N3). Solid and broken lines stand for linear regression (± standard error in grey) of treatments with and without nitrification inhibitor, respectively. Equations, R^2 value and significance of regressions are indicated in a white (- NI) and black (+ NI) box ($***p \leq 0.0001; **p \leq 0.01$).

In the field experiment of Pfab et al. (2012) the N_2O reducing effect of DMPP lasted nearly the whole winter period, for more than four months after N fertilization, despite high temperatures after application. Since DMPP must have been completely degraded in this period, Pfab et al. (2012) hypothesized that DMPP could have an effect on denitrifiers, because soil respiration was also decreased, indicating an inhibiting effect of DMPP on the activity of the heterotrophic microflora and thus potentially also on denitrification. A reduction of soil respiration was also reported for a DMPP application to winter wheat (Weiske et al. 2001) and for two incubation experiments (Maienza et al. 2014; Florio et al. 2016). The recommended dose of DMPP did neither effect non-target organisms

(Tindaon et al. 2012) nor the activity of enzymes involved in denitrification (Müller et al. 2002), but reduced the copy numbers of the soil bacterial 16S rRNA (Florio et al. 2016), and therefore the effect of DMPP on CO_2 release remains unclear.

In our experiment, soil CO_2 release after harvest did not differ among fertilized treatments (Figure S. 3). Nevertheless, respiration of growing volunteer wheat weeks after harvesting and later of the sown wheat plants, made the differentiation between soil and plant respiration not possible. Since the conditions for a complete mineralization of DMPP were sufficient (long time after application, high temperatures, high oxygen availability through repeated soil tillage), a long term change of microbial community could be a reason for the reduction of N_2O emissions during winter. Such a shift of the microbial community due to the application of DMPP was reported in incubation experiments: while Maienza et al. (2014) found decreased relative abundance of phospholipid fatty acids (PLFA) indicative of fungi and gram-negative bacteria and increased gram-positive bacteria. Florio et al. (2016) using community-level physiological profiling (CLPP) found a "shift in the pattern of C sources used by the heterotrophic microbial community".

Yield indicators in our experiment were not affected by the use of DMPP while yield related N_2O emissions were reduced (Table 2). In contrast, Pasda et al. (2001) reported higher grain yields, lower crude protein contents and a lower thousand grain weight in the DMPP treatment when compared to a treatment without DMPP from a large field experiment with three annual data sets from nine study sites in Germany and France. Overall DMPP application resulted in a lower N removal. However, in the study of Pasda et al. (2001) ASN was applied in two doses, so that splitting could have had an effect on yield indicators. Another reason for the discrepancy between our results and the data from Pasda et al. (2001) could be the low precipitation during the vegetation period and the loamy soil texture of our site. In our experiment NO_3^- in the treatment without DMPP was not leached into deeper soil layers (data not shown) and it was therefore still available for the growing crop. Our results were in full agreement with field studies from Northern Spain (Huérfano et al. 2015) and Northern Greece (Polychronaki et al. 2012), where grain yield, yield related indicators and grain quality of winter wheat were not affected by DMPP. In the study by Polychronaki et al. (2012) other factors influenced grain yield, such as N amount at seeding and experimental year. Huerfano et al. (2015) observed a decrease of grain protein when ENTEC26® was applied in one dose, nevertheless there was no difference between split ASN and ENTEC26® treatments. The authors' hypothesis for DMPP's lack of effectiveness was that cereal crops using modern cultivars under new management systems report only modest reductions or no changes in grain weight even at increased N doses (Hay and Walker, 1989, as cited by Lloveras et al. 2001).

It has been shown that the surplus mineral N from fertilization was correlated with the annual N_2O release from soils under different agricultural crops (Kaiser and Ruser 2000; van Groenigen et al. 2004; Ruser et al. 2008). In our experiment we found a positive relationship between N balance and the annual N_2O emission (Figure 3). One of the reasons for N surpluses in this experiment were e.g. the high N amount in the straw remaining in the field during the 2nd year. The slope of this relationship was significantly decreased by the use of DMPP (Table S.6) by 0.5% kg N_2O -N kg surplus-N^{-1} year^{-1}.The relationship between fertilizer N dose and yield for both years followed a quadratic function with an optimal N dose of 192±24 and 224±36 kg ha^{-1} for fertilizer with and without NI, respectively (Fig. 4a). An economic assessment gave a similar picture with an optimal amount dose of 159±17 and 195±32 kg N ha^{-1} and respective maximal revenue of 883±29 and 909±23 ha^{-1} for ENTEC and ASN, respectively (Fig. 4b). Use of DMPP had no significant effect on yield and revenue curves. Nevertheless, quadratic regressions of grain yield and grain-N related emissions on N amount were significantly affected by DMPP. When ASN was used, 77 kg N ha^{-1} were needed to reach the minimal emission of 0.44 kg N_2O-N Mg^{-1}. Adding DMPP allowed to increase N amount by 54% yet still reducing minimal yield-related emissions by 20% (Fig. 4c). Annual N_2O losses per grain-N showed a similar pattern, however the use of DMPP led to a small reduction of N amount (9%) reducing minimal grain-N related emissions by 22% (Fig. 4d).

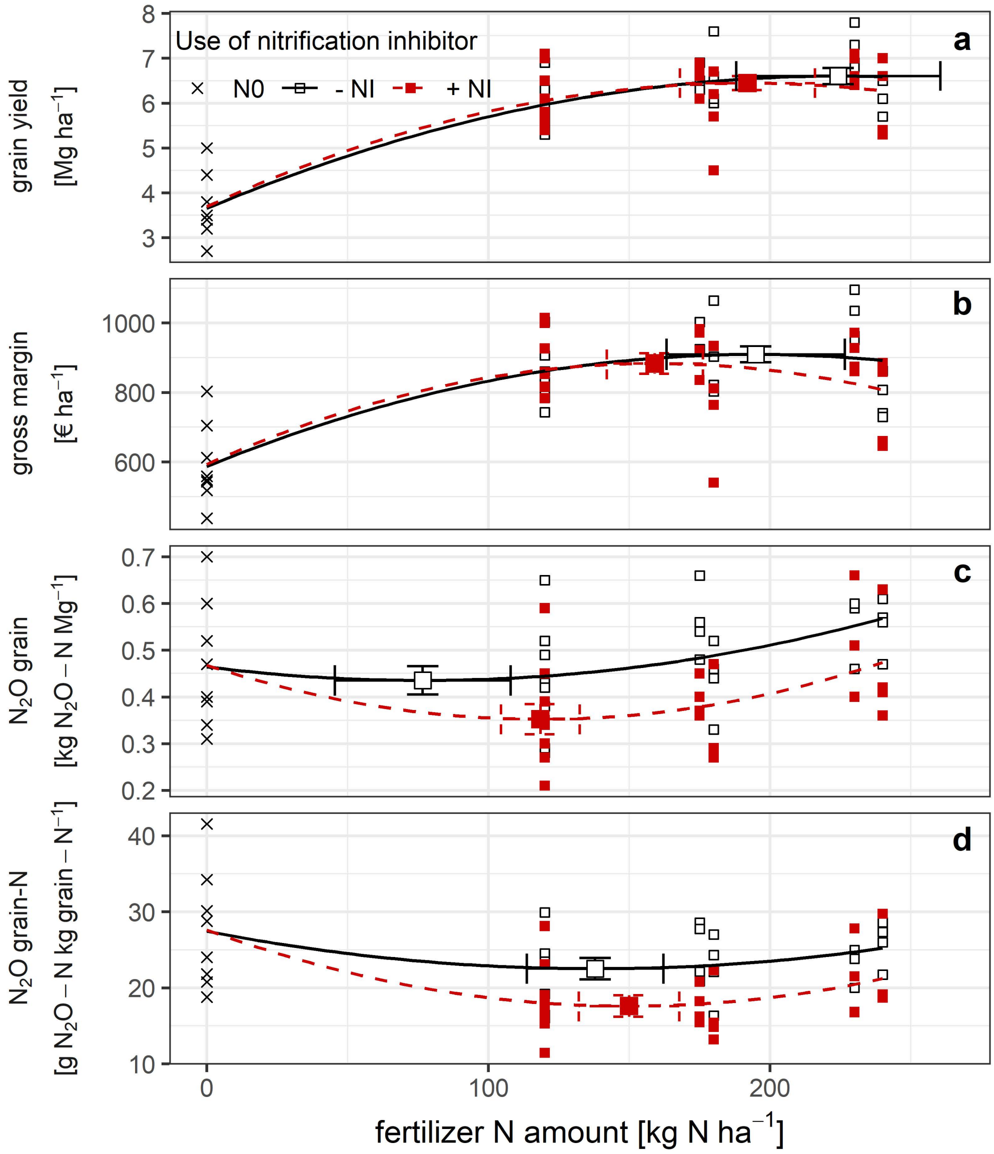

Figure 4: Relationship between N amount and **a** grain yield, **b** gross margin (calculated as revenue minus fertilizer cost), **c** N_2O per grain and **d** N_2O per grain-N, as affected by use of nitrification inhibitor DMPP (- NI, + NI). Optimal N amounts for all variables were calculated as the vertex of the quadratic function ($\pm$ standard error).

Lebender et al. (2014) reported yield scaled N_2O emissions for winter wheat on different German sites. The lowest emissions were obtained at 127 and 150 kg ha^{-1}, at a grain yield of 8.1 and 8.9 Mg ha^{-1}, respectively. Reported economic optima were 240 and 233 kg N ha^{-1} at a grain yield of 9.5 and 9.9 Mg ha^{-1}, respectively. Our results differ with lower optima for yield scaled emissions and revenue, although many factors could have an influence on these results, such as less precipitation between March and July ($\approx$100 mm) at our sites, lesser number of N rates, different cultivars and soil types, as well as lower prices for wheat grain and N fertilizer. Nevertheless, the response curves show a similar pattern, with lowest yield scaled emissions at N rates which are suboptimal from a yield and economical point of view.

For different crops Rose et al. (2018) reported a shift of the optimum N rate in yield response curves towards lower N fertilizer amounts for enhanced fertilizers when compared to conventional fertilizers. The extent of this shift for our data set was -32 kg N ha^{-1} a^{-1} ($p = 0.84$). Since we investigated only four N fertilizer rates, the yield and revenue response curves did not show the same pattern as described by Rose et al. (2018). In the meta-analysis on the effect of inhibitors on crop productivity and NUE by Abalos et al. (2014), they found higher crop yields by the use of inhibitors on medium and coarse textured soils. Nitrate leaching, as one of the main paths of N loss in agricultural systems, was probably not of importance in our experiment, due to the silt dominance in our study site and the deep rooting system of wheat plants (Kirby 2002). Thus, the use of DMPP did not bring a benefit for the wheat plants in the investigated N doses. But, as pointed out by Rose et al. (2018), optimal N amount can vary greatly depending on many factors – and so the N dose at which wheat plants can produce more grain – and enhanced N availability of +NI treatments was not enough to bring a profit after all.

Conclusion

N surpluses and cumulative N_2O emissions increased with increasing rates of N fertilizer application, confirming the first hypothesis of our study. Our second hypothesis could be confirmed, as we measured a significant reduction of N_2O emissions by the application of DMPP not only during the vegetation period (-35% 1st year; -45% 2nd year; all N levels, excluding emissions from N0 treatment) but also during winter (-91% 1st year; -94% 2nd year; all N levels, excluding emissions from N0 treatment). Annual N_2O emissions were reduced when DMPP was used. As this reduction took place almost a year after the application of the NI – thereby after the mineralisation of DMPP was presumably undergone – DMPP did most likely change soil microbial community, as observed in two soil incubation experiments (Maienza et al. 2014; Florio et al. 2016). The use of DMPP also significantly reduced the relationship between N surplus and annual N_2O emission.

We partially confirmed our third hypothesis of a mitigation of N_2O emissions by a reduction of the N fertilizer amount, without a decrease in yield. A significant yield decrease of 10.6% was measured during the first year, when 30% less N amount then recommended was applied. Fertilizer amount had no effect on grain yield the second year, nevertheless a harsh winter without snow cover and leaf rust might have negatively influenced N grain filling. The use of different wheat varieties might have biased the year effect, though. An economic assessment shows that the use of DMPP together with different N doses did not influence grain yield, gross margin and neither its optimal N amount. Nevertheless, when relating N_2O losses to grain yield or grain-N (giving an insight on grain protein), we found a significant effect of DMPP on these variables. Response curves of grain yield-related N_2O emissions showed that, when DMPP is used as a fertilizer additive, N_2O losses per grain yield can be minimized, even if N amount increases by 54%. These results reveal that the use of DMPP at suboptimal N rates, can help to close the gap between profitability and product scaled emissions.

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References

Abalos D, Jeffery S, Sanz-Cobena A, Guardia G, Vallejo A (2014) Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agric Ecosyst Environ 189:136–144. https://doi.org/10.1016/j.agee.2014.03.036

Abalos D, Sanz-Cobena A, Andreu G, Vallejo A (2017) Rainfall amount and distribution regulate DMPP effects on nitrous oxide emissions under semiarid Mediterranean conditions. Agric Ecosyst Environ 238:36–45. https://doi.org/10.1016/j.agee.2016.02.003

Akiyama H, Yan X, Yagi K (2010) Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: metaanalysis. Glob Chang Biol 16:1837–1846. https://doi.org/10.1111/j.1365-2486.2009.02031.x

Alonso-Ayuso M, Gabriel JL, Quemada M (2016) Nitrogen use efficiency and residual effect of fertilizers with nitrification inhibitors. Eur J Agron 80:1–8. https://doi.org/10.1016/j.eja.2016.06.008

Bancal MO, Roche R, Bancal P (2008) Late foliar diseases in wheat crops decrease nitrogen yield through N uptake rather than through variations in N remobilization. Ann Bot 102:579–590. https://doi.org/10.1093/aob/mcn124

Braker G, Conrad R (2011) Diversity, structure, and size of N2O-producing microbial communities in soils—what matters for their functioning? Adv Appl Microbiol 75:33–70. https://doi.org/10.1016/B978-0-12-387046-9.00002-5

Butler JH, Montzka SA (2015) The NOAA Annual Greenhouse Gas Index (AGGI). http://www.esrl.noaa.gov/gmd/aggi/aggi.html. Accessed 15 Jul 2015

Cassman KG, Dobermann A, Walters DT (2002) Agroecosystems, nitrogen-use efficiency, and nitrogen management. AMBIO A J Hum Environ 31:132–140. https://doi.org/10.1579/0044-7447-31.2.132

Chaves B, Opoku A, Neve S, Boeckx P, Cleemput OV, Hofman G (2005) Influence of DCD and DMPP on soil N dynamics after incorporation of vegetable crop residues. Biol Fertil Soils 43:62–68. https://doi.org/10.1007/s00374-005-0061-6

Crutzen P (1970) The influence of nitrogen oxides on the atmospheric ozone content. Q J R Meteorol Soc 96:320–325. https://doi.org/10.1002/qj.49709640815

Davidson EA, Kanter D (2014) Inventories and scenarios of nitrous oxide emissions. Environ Res Lett 9:105012. https://doi.org/10.1088/1748-9326/9/10/105012

De Antoni Migliorati M, Scheer C, Grace PR, Rowlings DW, Bell M, McGree J (2014) Influence of different nitrogen rates and DMPP nitrification inhibitor on annual N_2O emissions from a subtropical wheat–maize cropping system. Agric Ecosyst Environ 186:33–43. https://doi.org/10.1016/j.agee.2014.01.016

Devadas R, Simpfendorfer S, Backhouse D, Lamb DW (2014) Effect of stripe rust on the yield response of wheat to nitrogen. Crop J 2:201–206. https://doi.org/10.1016/j.cj.2014.05.002

Dobbie KE, McTaggart IP, Smith KA (1999) Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. J Geophys Res 104:26891. https://doi.org/10.1029/1999JD900378

DüV (2006) Verordnung über die Anwendung von Düngemitteln, Bodenhilfsstoffen, Kultursubstraten und Pflanzenhilfsmitteln nach den Grundsätzen der guten fachlichen Praxis beim Düngen (Düngeverordnung – DüV, German legislation on fertilization). Bundesgesetzblatt Jahrgang 2006 Teil I Nr. 2, 20. Bonn, Germany

FAO (2015) World fertilizer trends and outlook to 2018. FAO, Rome

Federal Plant Variety Office (2013) Beschreibende Sortenliste - Getreide, Mais, öl- und Faserpflanzen, Leguminosen, Rüben. Zwischenfrüchte, Hannover

Flessa H, Dörsch P, Beese F (1995) Seasonal variation of N_2O and CH_4 fluxes in differently managed arable soils in southern Germany. J Geophys Res 100:23115–23123. https://doi.org/10.1029/95JD02270

Florio A, Clark IM, Hirsch PR, Jhurreea D, Benedetti A (2014) Effects of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on abundance and activity of ammonia oxidizers in soil. Biol Fertil Soils 50:795–807. https://doi.org/10.1007/s00374-014-0897-8

Florio A, Maienza A, Dell'Abate MT, Stazi SR, Benedetti A (2016) Changes in the activity and abundance of the soil microbial community in response to the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). J Soils Sediments 16:2687–2697. https://doi.org/10.1007/s11368-016-1471-9

Gillam K, Zebarth B, Burton D (2008) Nitrous oxide emissions from denitrification and the partitioning of gaseous losses as affected by nitrate and carbon addition and soil aeration. Can J Soil Sci 88:133–143. https://doi.org/10.4141/CJSS06005

Granli T, Bøckman O (1994) Nitrous oxide from agriculture. Nor J Agric Sci 12:125

Grömping U (2006) Relative importance for linear regression in R: the package relaimpo. J Stat Softw 17:1–27. https://doi.org/10.18637/jss.v017.i01

Hawkesford M, Horst W, Kichey T, Lambers H, Schjoerring J, Møller IS, White P (2012) Functions of macronutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Elsevier, London, pp 135–189

Hay RK, Walker AJ (1989) An introduction to the physiology of the crop yield. Longman Sci. and Tech., New York

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical J 50:346–363

Huérfano X, Fuertes-Mendizábal T, Duñabeitia MK, González-Murua C, Estavillo JM, Menéndez S (2015) Splitting the application of 3,4-dimethylpyrazole phosphate (DMPP): influence on greenhouse gases emissions and wheat yield and quality under humid Mediterranean conditions. Eur J Agron 64:47–57. https://doi.org/10.1016/j.eja.2014.11.008

Hutchinson G, Mosier A (1981) Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci Soc Am J 45:311–316. https://doi.org/10.2136/sssaj1981.03615995004500020017x

Jackson CH (2011) Multi-state models for panel data: the MSM package for R. J Stat Softw 38:1–29. https://doi.org/10.18637/jss.v038.i08

Ju X, Lu X, Gao Z, Chen X, Su F, Kogge M, Römheld V, Christie P, Zhang F (2011) Processes and factors controlling N_2O production in an intensively managed low carbon calcareous soil under sub-humid monsoon conditions. Environ Pollut 159:1007–1016. https://doi.org/10.1016/j.envpol.2010.10.040

Jungkunst HF, Freibauer A, Neufeldt H, Bareth G (2006) Nitrous oxide emissions from agricultural land use in Germany—a synthesis of available annual field data. J Plant Nutr Soil Sci 169:341–351. https://doi.org/10.1002/jpln.200521954

Kaiser E-A, Ruser R (2000) Nitrous oxide emissions from arable soils in Germany—an evaluation of six long-term field experiments. J Plant Nutr Soil Sci 163:249–259. https://doi.org/10.1002/1522-2624(200006)163:3%3c249:AIDJPLN249%3e3.0.CO;2-Z

Kaiser EA, Kohrs K, Kücke M, Schnug E, Heinemeyer O, Munch JC (1998) Nitrous oxide release from arable soil: importance of N-fertilization, crops and temporal variation. Soil Biol Biochem 30:1553–1563. https://doi.org/10.1016/S0038-0717(98)00036-4

Kim D-G, Saggar S, Roudier P (2012a) The effect of nitrification inhibitors on soil ammonia emissions in nitrogen managed soils: a meta-analysis. Nutr Cycl Agroecosystems 93:51–64. https://doi.org/10.1007/s10705-012-9498-9

Kim D-G, Vargas R, Bond-Lamberty B, Turetsky MR (2012b) Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research. Biogeosciences 9:2459–2483. https://doi.org/10.5194/bg-9-2459-2012

Kirby EJM (2002) Botany of the wheat plant. In: Curtis BC, Rajaram S, Gómez Macpherson H (eds) Bread wheat. FAO, Rome. http://www.fao.org/3/y4011e/y4011e05.htm

Klein RN (2006) Freeze injury to Nebraska Wheat. In: University of Nebraska Lincoln Ext. http://extensionpubs.unl.edu/publication/9000016363742/freeze-injury-tonebraska-wheat/. Accessed 21 Aug 2017

Ladha JK, Pathak H, Krupnik TJ, Six J, van Kessel C (2005) Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. Adv Agron 87:85–156. https://doi.org/10.1016/S0065-2113(05)87003-8

Landwirtschaftliches Technologiezentrum Augustenberg (2015) Agrarmeteorologie Baden-Württemberg. https://www.wetter-bw.de

Lebender U, Senbayram M, Lammel J, Kuhlmann H (2014) Impact of mineral N fertilizer application rates on N_2O emissions from arable soils under winter wheat. Nutr Cycl Agroecosystems 100:111–120. https://doi.org/10.1007/s10705-014-9630-0

Lenth RV (2016) Least-squares means: the R package lsmeans. J Stat Softw 69:1–33. https://doi.org/10.18637/jss.v069.i01

Linzmeier W, Gutser R, Schmidhalter U (2001) Nitrous oxide emission from soil and from a nitrogen-15-labelled fertilizer with the new nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). Biol Fertil Soils 34:103–108. https://doi.org/10.1007/s003740100383

Liu C, Wang K, Zheng X (2012) Responses of N_2O and CH_4 fluxes to fertilizer nitrogen addition rates in an irrigated wheat-maize cropping system in northern China. Biogeosciences 9:839–850. https://doi.org/10.5194/bg-9-839-2012

Lloveras J, Lopez A, Ferran J, Espachs S, Solsona J (2001) Bread-making wheat and soil nitrate as affected by nitrogen fertilization in irrigated mediterranean conditions. Agron J 93:1183–1190. https://doi.org/10.2134/agronj2001.1183

Maienza A, Bååth E, Stazi SR, Benedetti A, Grego S, Dell'Abate MT (2014) Microbial dynamics after adding bovine manure effluent together with a nitrification inhibitor (3,4 DMPP) in a microcosm experiment. Biol Fertil Soils 50:869–877. https://doi.org/10.1007/s00374-014-0907-x

Menéndez S, Merino P, Pinto M, González-Murua C, Estavillo JM (2006) 3,4-Dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands. J Environ Qual 35:973–981. https://doi.org/10.2134/jeq2005.0320

Müller C, Stevens RJ, Laughlin RJ, Azam F, Ottow JCG (2002) The nitrification inhibitor DMPP had no effect on denitrifying enzyme activity. Soil Biol Biochem 34:1825–1827. https://doi.org/10.1016/S0038-0717(02)00165-7

Mutegi JK, Munkholm LJ, Petersen BM, Hansen EM, Petersen SO (2010) Nitrous oxide emissions and controls as influenced by tillage and crop residue management strategy. Soil Biol Biochem 42:1701–1711. https://doi.org/10.1016/j.soilbio.2010.06.004

Pasda G, Hähndel R, Zerulla W (2001) Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops. Biol Fertil Soils 34:85–97. https://doi.org/10.1007/s003740100381

Peltonen-Sainio P, Hakala K, Jauhiainen L (2011) Climate-induced overwintering challenges for wheat and rye in northern agriculture. Acta Agric Scand Sect B Plant Soil Sci 61:75–83. https://doi.org/10.1080/09064710903535977

Pfab H (2011) Nitrous oxide emission and mitigation strategies: measurements on an intensively fertilized vegetable cropped loamy soil. Dissertation. University of Hohenheim

Pfab H, Palmer I, Buegger F, Fiedler S, Müller T, Ruser R (2012) Influence of a nitrification inhibitor and of placed N-fertilization on N_2O fluxes from a vegetable cropped loamy soil. Agric Ecosyst Environ 150:91–101. https://doi.org/10.1016/j.agee.2012.01.001

Piepho HP (2017) Box-Cox transformation SAS macro. https://biostatistik.uni-hohenheim.de/fileadmin/einrichtungen/biostatistik/Tools_und_Macros/SAS-Macros/boxcox_macro.sas. Accessed 28 Sep 2018

Piepho HP, Williams ER, Fleck M (2006) A note on the analysis of designed experiments with complex treatment structure. HortScience 41:446–452. https://doi.org/10.21273/HORTSCI.41.2.446

Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2017) nlme: Linear and nonlinear mixed effects models. R package version 3.1-139, https://CRAN.R-project.org/package=nlme

Polychronaki E, Douma C, Giourga C, Loumou A (2012) Assessing nitrogen fertilization strategies in winter wheat and cotton crops in northern Greece. Pedosphere 22:689–697. https://doi.org/10.1016/S1002-0160(12)60054-X

R Core Team (2016) R: a language and environment for statistical computing Regional Database Germany (2017)

Hektarerträge ausgewählter landwirtschaftlicher Feldfrüchte–Jahressumme–regionale Ebenen (Code 115-46-4-B). https://www.regionalstatistik.de/genesis/online/data. Accessed 9 Mar 2017

Riley H, Hoel BO, Kristoffersen AØ (2011) Economic and environmental optimization of nitrogen fertilizer recommendations for cereals in Norway. Acta Agric Scand Sect B Soil Plant Sci 62:1–14. https://doi.org/10.1080/09064710.2011.629620

Risk N, Snider D, Wagner-Riddle C (2013) Mechanisms leading to enhanced soil nitrous oxide fluxes induced by freeze–thaw cycles. Can J Soil Sci 93:401–414. https://doi.org/10.4141/cjss2012-071

Robertson GP, Groffman PM (2015) Nitrogen transformation. In: Paul EA (ed) Soil microbiology, ecology, and biochemistry, 4th edn. Elsevier, London, pp 421–446

Rose TJ, Wood RH, Rose MT, Van Zwieten L (2018) A reevaluation of the agronomic effectiveness of the nitrification inhibitors DCD and DMPP and the urease inhibitor NBPT. Agric Ecosyst Environ 252:69–73. https://doi.org/10.1016/j.agee.2017.10.008

Röver M, Heinemeyer O, Kaiser EA (1998) Microbial induced nitrous oxide emissions from an arable soil during winter. Soil Biol Biochem 30:1859–1865. https://doi.org/10.1016/S0038-0717(98)00080-7

Ruser R, Schulz R (2015) The effect of nitrification inhibitors on the nitrous oxide (N_2O) release from agricultural soils—a review. J Plant Nutr Soil Sci 178:171–188. https://doi.org/10.1002/jpln.201400251

Ruser R, Flessa H, Schilling R, Steindl H, Beese F (1998) Soil compaction and fertilization effects on nitrous oxide and methane fluxes in potato fields. Soil Sci Soc Am J 62:1587–1595. https://doi.org/10.2136/sssaj1998.03615995006200060016x

Ruser R, Flessa H, Schilling R, Beese F, Munch JCC (2001) Effect of crop-specific field management and N fertilization on N_2O emissions from a fine-loamy soil. Nutr Cycl Agroecosyst 59:177–191. https://doi.org/10.1023/A:1017512205888

Ruser R, Sehy U, Weber A, Gutser R, Munch JC (2008) Main driving variables and effect of soil management on climate or ecosystem-relevant trace gas fluxes from fields of the FAM. In: Schröder P, Pfadenhauer J, Munch J (eds) Perspectives for agroecosystem management—balancing environmental and socio-economic demands. Elsevier, London, pp 79–120

Scheer C, Rowlings DW, Firrel M, Deuter P, Morris S, Grace PR (2014) Impact of nitrification inhibitor (DMPP) on soil nitrous oxide emissions from an intensive broccoli production system in sub-tropical Australia. Soil Biol Biochem 77:243–251. https://doi.org/10.1016/j.soilbio.2014.07.006

Scheer C, Rowlings D, Firrell M, Deuter P, Morris S, Riches D, Porter I, Grace P (2017) Nitrification inhibitors can increase post-harvest nitrous oxide emissions in an intensive vegetable production system. Sci Rep 7:1–9. https://doi.org/10.1038/srep43677

Schulz R, Makary T, Hubert S, Hartung K, Gruber S, Donath S, Döhler J, Weiß K, Ehrhart E, Claupein W, Piepho H-PH-P, Pekrun C, Müller T (2015) Is it necessary to split nitrogen fertilization for winter wheat? On-farm research on Luvisols in south-west Germany. J Agric Sci 153:575–587. https://doi.org/10.1017/S0021859614000288

Seiz P, Guzman-Bustamante I, Schulz R, Müller T, Ruser R (2019) Effect of crop residue removal and straw addition on nitrous oxide emissions from a horticulturally used soil in South Germany. Submitted to Soil Sci Soc Am J

Shcherbak I, Millar N, Robertson GP (2014) Global metaanalysis of the nonlinear response of soil nitrous oxide (N_2O) emissions to fertilizer nitrogen. Proc Natl Acad Sci USA 111: 9199–9204. https://doi.org/10.1073/pnas.1322434111

Singh SN, Verma A (2007) The potential of nitrification inhibitors to manage the pollution effect of nitrogen fertilizers in agricultural and other soils: a review. Environ Pract 9:266–279. https://doi.org/10.1017/S1466046607070482

Six J, Elliott ET, Paustian K (1999) Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci Soc Am J 63:1350. https://doi.org/10.2136/sssaj1999.6351350x

Staley TE, Boyer DG, Caskey WH (1990) Soil denitrification and nitrification potentials during the growing season relative to tillage. Soil Sci Soc Am J 54:1602. https://doi.org/10.2136/sssaj1990.03615995005400060015x

Stehfest E, Bouwman LF (2006) N_2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutr Cycl Agroecosyst 74:207–228. https://doi.org/10.1007/s10705-006-9000-7

Subbarao G, Ito O, Sahrawat K, Berry WL, Nakahara K, Ishikawa T, Watanabe T, Suenaga K, Rondon M, Rao IM (2006) Scope and strategies for regulation of nitrification in agricultural systems—challenges and opportunities. CRC Crit Rev Plant Sci 25:303–335. https://doi.org/10.1080/07352680600794232

Thomason WE, Raun WR, Johnson GV (2000) Winter wheat fertilizer nitrogen use efficiency in grain and forage production systems. J Plant Nutr 23:1505–1516. https://doi.org/10.1080/01904160009382118

Tindaon F, Benckiser G, Ottow JCG (2012) Evaluation of ecological doses of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (DMPP) and 4-chloromethylpyrazole (ClMP) in comparison to dicyandiamide (DCD) in their effects on dehydrogenase and dimethyl sulfoxide reductase activity in soil. Biol Fertil Soils 48:1–8. https://doi.org/10.1007/s00374-011-0655-0

van Groenigen JW, Kasper GJ, Velthof GL, van den Pol-van Dasselaar A, Kuikman PJ (2004) Nitrous oxide emissions from silage maize fields under different mineral nitrogen fertilizer and slurry applications. Plant Soil 263:101–111. https://doi.org/10.1023/B:PLSO.0000047729.43185.46

Vannelli T, Hooper AB (1992) Oxidation of nitrapyrin to 6-chloropicolinic acid by the ammonia-oxidizing bacterium Nitrosomonas europaea. Appl Environ Microbiol 58:2321–2325

Venables WN, Ripley BD (2002) Modern applied statistics with S, 4th edn. Springer, New York

Wagner-Riddle C, Congreves KA, Abalos D, Berg AA, Brown SE, Ambadan JT, Gao X, Tenuta M (2017) Globally important nitrous oxide emissions from croplands induced by freeze–thaw cycles. Nat Geosci 10:279–283. https://doi.org/10.1038/ngeo2907

Weiske A, Benckiser G, Herbert T, Ottow JCG (2001) Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol Fertil Soils 34:109–117. https://doi.org/10.1007/s003740100386

Wertz S, Goyer C, Zebarth BJ, Tatti E, Burton DL, Chantigny MH, Filion M (2016) The amplitude of soil freeze-thaw cycles influences temporal dynamics of N_2O emissions and denitrifier transcriptional activity and community composition. Biol Fertil Soils 52(8):1149–1162. https://doi.org/10.1007/s00374-016-1146-0

Wickham H (2009) ggplot2. Springer, New York

Wrage-Mönnig N, Horn MA, Well R, Müller C, Velthof G, Oenema O (2018) The role of nitrifier denitrification in the production of nitrous oxide revisited. Soil Biol Biochem 123:A3–A16. https://doi.org/10.1016/j.soilbio.2018.03.020

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. Weed Res 14:415–421. https://doi.org/10.1111/j.1365-3180.1974.tb01084.x

Zsolnay A (1996) Dissolved humus in soil waters. In: Piccolo A (ed) Humic substances in terrestrial ecosystems. Elsevier Science, Amsterdam, pp 171–223

Supplementary Material

Table S1: Overview of the field management

Date	Field management
12 October 2010	Ploughing 30 cm deep
13 October 2010	Sowing 8 cm deep variety Toras
27 October 2010	P-K-S fertilization (23-43-23 kg ha^{-1} respectively)
15 March 2011	Begin of experiment
30 March 2011	Herbicide application
4 April 2011	Nitrogen fertilization
11 April 2011	Herbicide and plant growth regulator application
11 April 2011	Harvest and stubble cultivation 10 cm deep
26 September 2011	Cultivator 15 cm deep
6 October 2011	Sowing 8 cm deep variety Schamane
4 March 2012	Change of experimental site
29 March 2012	Nitrogen fertilization
10 April 2012	Herbicide application
8 May 2012	Herbicide application
29 March 2012	Irrigation (17 mm)
2 August 2012	Harvest and stubble cultivation 10 cm deep
10 September 2012	Cultivator 15 cm deep
17 October 2012	Cultivator 15 cm deep
	Sowing 8 cm deep

Table S2: Covariance parameter estimates from repeated measures ANOVA of N_2O fluxes.

Covariance Parameter	Subject	Group year - season	Estimate
Variance	plot	1 - vegetation	0.1212
SP(POW)	plot		$-2.73 * 10^-10$
Variance	plot	1 - tillage	0.08288
SP(POW)	plot		0.8083
Variance	plot	1 - winter	0.03874
SP(POW)	plot		0.5868
Variance	plot	2 - vegetation	0.05533
SP(POW)	plot		0.000469
Variance	plot	2 - tillage	0.08911
SP(POW)	plot		0.7404
Variance	plot	2 - winter	0.04578
SP(POW)	plot		0.6969

Table S3: Mean values of several agronomic variables as affected by year, N fertilizer level and use of a nitrification inhibitor.

Year	1							2						
Nitrification inhibitor		– NI			+ NI				– NI			+ NI		
N fertilizer level	N0	N1	N2	N3	N1	N2	N3	N0	N1	N2	N3	N1	N2	N3
ears [n m^{-2}]	317	395	401	420	356	386	443	311	513	577	499	508	495	512
Straw yield [Mg ha^{-1}]	2.9	3.6	3.9	4.2	3.6	4	4.1	3.8	5.6	6	5.9	5.8	5.3	5.7
Grain yield [Mg ha^{-1}]	4.1	5.8	6.6	7.2	5.9	6.6	6.8	3.3	5.9	6.6	6	6.5	5.8	6.1
TGW [g]	43.6	45.8	47.5	46.6	46.3	46.7	46.8	42.5	40	38.7	37.1	43.3	40	37.7
Grains per ear [n]	29.2	32.7	35	37.2	36.4	37.9	33.5	25.1	29.2	30.1	32.5	29.5	29.1	31.6
Straw C/N [ratio]	138	112	97	89	125	90	89	87	49	45	30	60	50	39
Straw-N [kg N ha^{-1}]	9.4	14.8	18.2	21.4	13.1	20.1	20.7	18.9	51.7	57.7	86.9	42.3	47.1	67.3
Grain-N [kg N ha^{-1}]	70	121	149	173	121	148	161	55	104	128	127	116	115	128
NUE [%]		34.7	39.7	40.8	34.8	39.5	35.5		41	40.6	30	50.4	33.1	30.2
N balance [kg N ha^{-1}]	-69.6	-0.6	26.5	57.1	-0.8	26.9	69.5	-55.2	15.5	51.8	112.8	4.2	65.2	112.3
Grain protein [%]	9.7	11.8	12.9	13.7	11.7	12.8	13.5	9.6	10.2	11.1	12.1	10.2	11.4	12
N_2O/grain-N [g N_2O-N kg^{-1} grain-N]	30.6	24.5	24.8	23.4	21.7	17.7	20.7	24.4	19.8	22.4	25.9	15.5	16.4	21.6
N_2O/grain yield [g N_2O-N kg^{-1} grain]	0.52	0.51	0.56	0.56	0.45	0.4	0.49	0.41	0.35	0.43	0.55	0.28	0.33	0.46

Table S4: Mean cumulative N_2O emissions for seasons and year as affected by N fertilizer level and use of nitrification inhibitor (DMPP). Share of season for the annual emission is given in percentage.

	Nitrification inhibitor		– NI			+ NI		
	N fertilizer level	N0	N1	N2	N3	N1	N2	N3
Year	Season (days)	g N_2O-N ha^{-1} (% of annual emission)						
1	vegetation (149)	905 (45)	1812 (61)	2210 (60)	2555 (64)	1622 (62)	1554 (60)	2039 (62)
1	tillage (96)	866 (41)	737 (26)	1013 (28)	961 (23)	734 (28)	727 (28)	951 (28)
1	winter (113)	287 (14)	377 (13)	451 (13)	520 (13)	253 (10)	318 (12)	354 (10)
1	annual (358)	2058 (100)	2927 (100)	3675 (100)	4036 (100)	2609 (100)	2599 (100)	3344 (100)
2	vegetation (149)	436 (34)	966 (48)	1223 (44)	1796 (55)	785 (45)	761 (43)	1226 (45)
2	tillage (119)	605 (44)	768 (37)	1089 (38)	1055 (31)	769 (43)	765 (39)	1170 (42)
2	winter (112)	282 (21)	318 (15)	508 (18)	453 (14)	218 (12)	359 (18)	330 (12)
2	annual (380)	1322 (100)	2052 (100)	2820 (100)	3304 (100)	1772 (100)	1886 (100)	2725 (100)

Table S5: Summary table of stepwise multiple regression analysis. Selection of regression parameters was done for the response variables soil temperature, soil NH_4^+ and NO_3^- amount, WFPS and the difference between the actual WFPS and the WFPS at the previous sampling (ΔWFPS). Relative importance was added for selected variables.

Call:
lm(formula = $(\text{n2oflux} + 3)^{0.0915}$ soil temperature + WFPS +ΔWFPS + NO3, data = n2o)

Residuals :

Min	1Q	Median	3Q	Max
-0.54547	-0.06835	0.00837	0.07341	0.36587

Coefficients:	Estimate	Std. Error	t value	$Pr(>\|t\|)$	Relative importance (%)
(Intercept)	1.38	$1.91 * 10^{-02}$	$7.24 * 10^{+01}$	$< 2 * 10^{-16}$ ***	
soil temperature	$3.61 * 10^{-03}$	$4.11 * 10^{-04}$	8.78	$< 2 * 10^{-16}$ ***	6.7
WFPS	$-2.12 * 10^{-03}$	$3.06 * 10^{-04}$	-6.93	$5.04 * 10^{-12}$ ***	4.6
ΔWFPS	$2.80 * 10^{-03}$	$3.50 * 10^{-04}$	7.99	$1.91 * 10^{-15}$ ***	1.4
NO3	$5.84 * 10^{-04}$	$9.98 * 10^{-05}$	5.85	$5.36 * 10^{-09}$ ***	2.0

—

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.1131 on 3185 degrees of freedom
(2 observations deleted due to missingness)
Multiple R-squared: 0.147, Adjusted R-squared: 0.1459
F-statistic: 137.2 on 4 and 3185 DF, p-value: $< 2.2 * 10^{-16}$

Table S6: Summary table of linear regression analysis of the effect of N balance and use of DMPP on annual N_2O emission for both years.

Call:
lm(formula = annualN_2O Ntype + Nbalance:Ntype)

Residuals :

Min	1Q	Median	3Q	Max
-1.4328	-0.5610	-0.0576	0.4735	2.1582

Coefficients:	Estimate	Std. Error	t value	$Pr(>\|t\|)$
(Intercept)	2.584617	0.126709	20.398	$< 2 * 10^{-16}$ ***
NtypeENTEC	-0.408458	0.179592	-2.274	0.02653 ***
NtypeASN:Nbalance	0.010961	0.002136	5.13	$3.27 * 10^{-06}$ ***
NtypeENTEC:Nbalance	0.005941	0.002036	2.918	0.00495 **

—

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Residual standard error: 0.6856 on 60 degrees of freedom
Multiple R-squared: 0.4165, Adjusted R-squared: 0.3873
F-statistic: 14.28 on 3 and 60 DF, p-value: $3.95 * 10^{-07}$

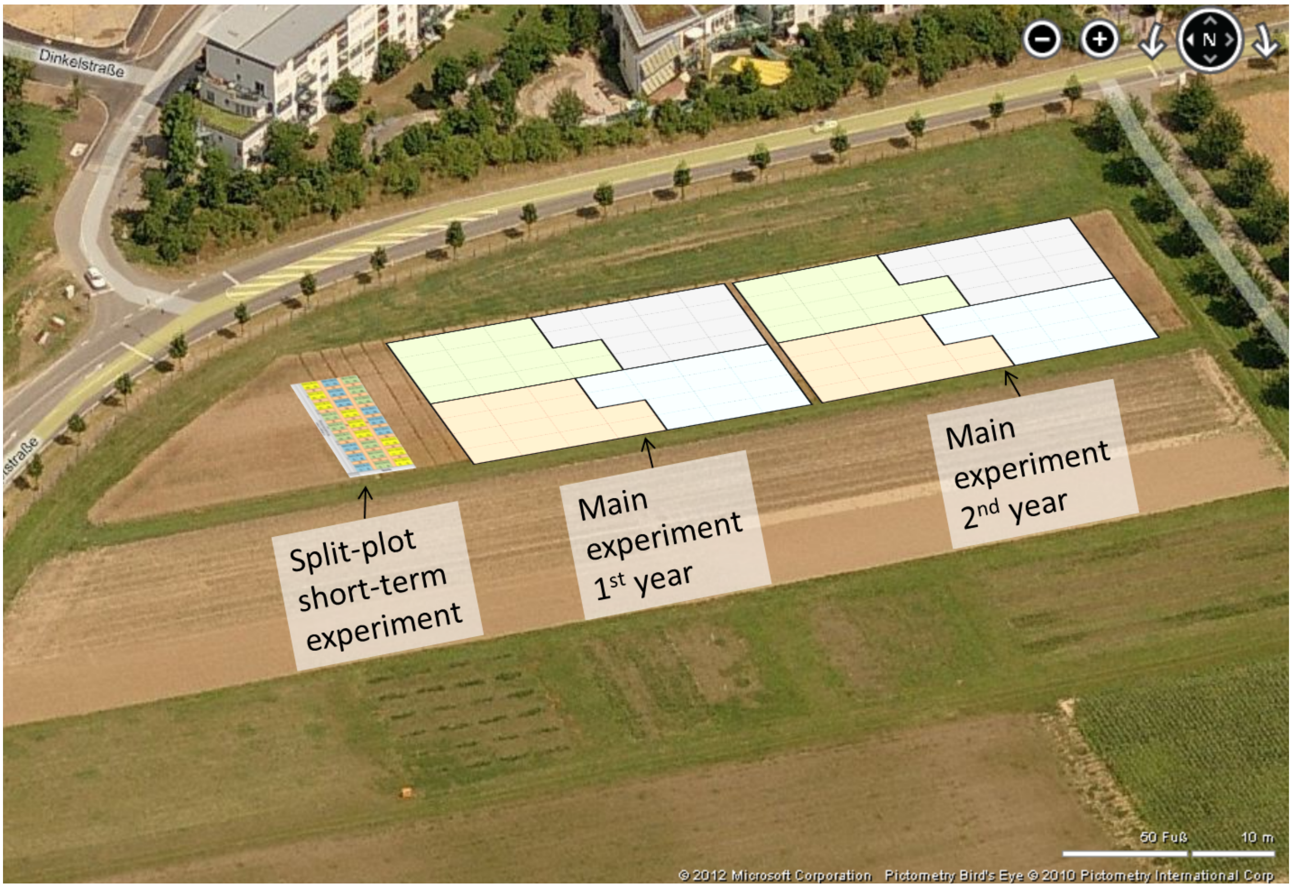

Figure S1: Aerial photography of the experimental field depicting the position of the main field experiment in both years and the short-term experiment.

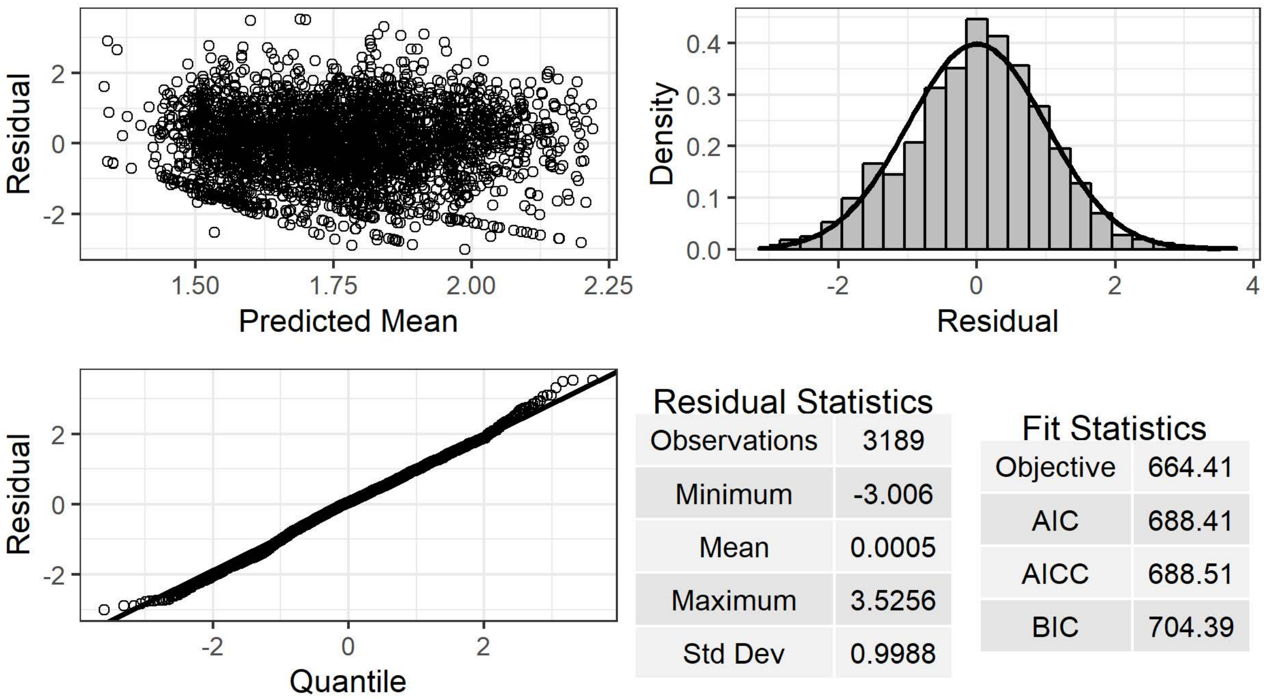

Figure S2: Studentized residuals of repeated measures ANOVA of N_2O fluxes.

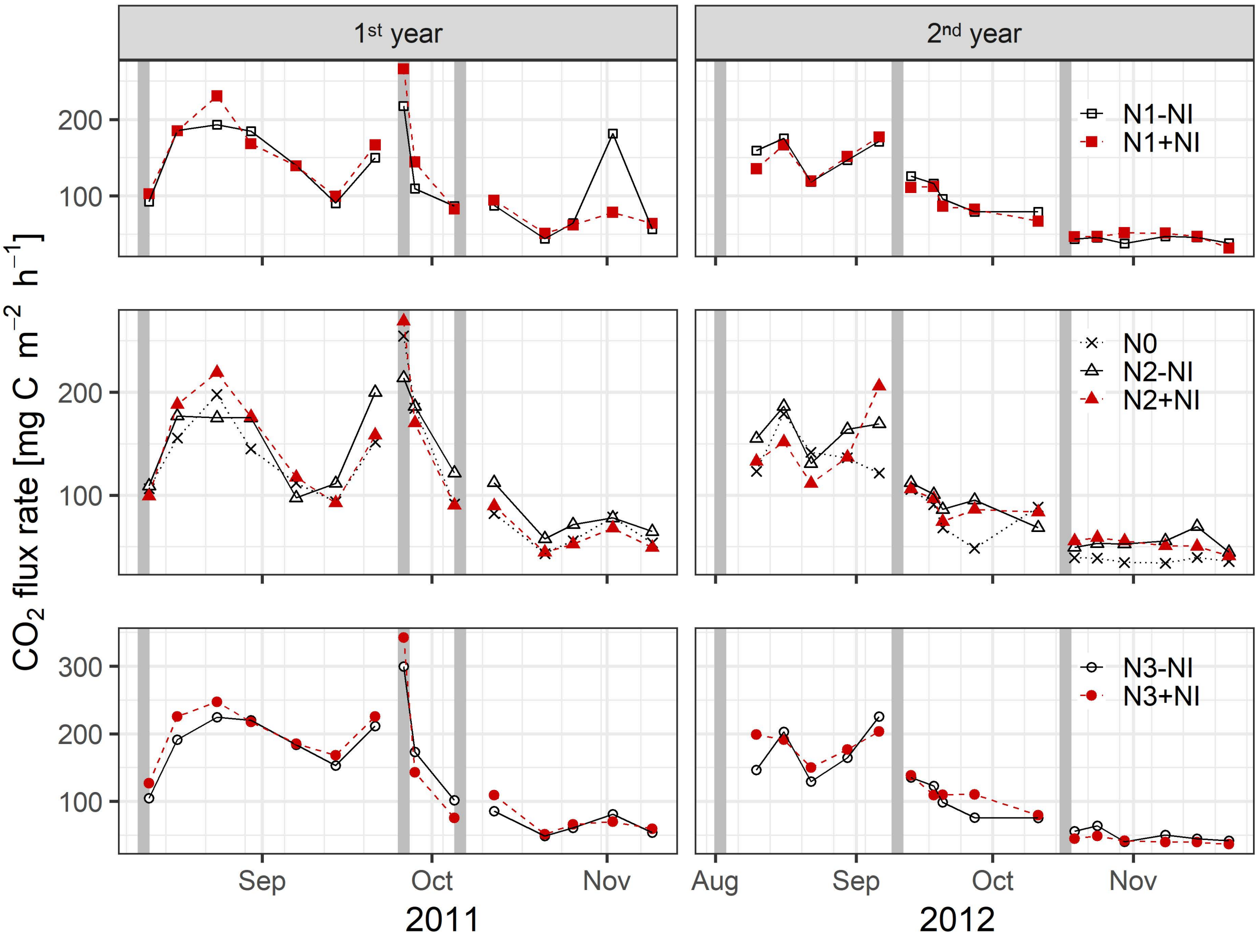

Figure S3: Mean CO_2 flux rates (n = 4) during the "tillage" periods as affected by N-fertilizer rate (N0, N1, N2, N3) and use of nitrification inhibitor DMPP (-NI, +NI). Solid grey lines indicate tillage events.