



# Waterlogging effects on $\text{N}_2\text{O}$ and $\text{N}_2$ emissions from a Stagnosol cultivated with *Silphium perfoliatum* and silage maize

Björn Kemmann<sup>1</sup> · Thorsten Ruf<sup>2</sup> · Amanda Matson<sup>1</sup> · Reinhard Well<sup>1</sup>

Received: 9 July 2022 / Revised: 30 September 2022 / Accepted: 4 October 2022 / Published online: 23 November 2022  
© The Author(s) 2022, corrected publication 2025

## Abstract

European policy recommends that biomass production occur on marginal land, such as poorly draining Stagnosols. Compared to annual cropping, perennial crops may better mitigate  $\text{N}_2\text{O}$  emissions at such sites, through more complete denitrification. To test that hypothesis, we compared  $\text{N}_2$  and  $\text{N}_2\text{O}$  fluxes from the soils of a perennial crop (cup plant, *Silphium perfoliatum* L.) and an annual crop (silage maize, *Zea mays* L.). Intact soil columns (35 cm height, 14.4 cm diameter) were incubated for 37 days. The soils were fertilized with 60 or 120 kg N ha<sup>-1</sup> and exposed to successive phases of waterlogging: free drainage, waterlogging of 1/3-, and waterlogging of 2/3- of the column. Source-specific  $\text{N}_2\text{O}$  and  $\text{N}_2$  fluxes were measured using the <sup>15</sup>N gas flux method. Denitrification was higher in cup plant than maize soil and total N losses from denitrification were dominated by emissions from the third phase. Cup plant soil emitted  $33.6 \pm 78.1$  mg N m<sup>-2</sup> and  $95.8 \pm 64.4$  mg N m<sup>-2</sup> more  $\text{N}_2\text{O}$  than maize soil in the low and high N treatments, respectively. The product ratio of denitrification ( $\text{N}_2\text{O}_i = \text{N}_2\text{O} / (\text{N}_2 + \text{N}_2\text{O})$ ) increased with waterlogging in maize soil, while remaining stable in cup plant soil. Emissions from the top 10 cm dominated the  $\text{N}_2\text{O}_i$  rather than  $\text{N}_2$  fluxes from the saturated soil. This study did not show  $\text{N}_2\text{O}$  mitigation in cup plant soil, instead highlighting the complexity of plant-soil effects on denitrification. We clearly showed that the application of a general  $\text{N}_2\text{O}_i$  for agricultural soils across annual and perennial cropping is not recommended.

**Keywords** Cup plant · Denitrification · Di-nitrogen · Maize · Nitrous oxide · <sup>15</sup>N gas flux method

## Introduction

Biomass cultivation for producing bioenergy gets increasingly criticized due to broad environmental concerns ranging from soil degradation to negative impact on biodiversity (Herbes et al. 2014), and due to its competition with food production for arable land (Haberl et al. 2012). To avoid increased carbon dioxide (CO<sub>2</sub>) emissions through indirect land-use change, it has been strongly recommended that biomass production be restricted to sites less suitable for food production (Directive (EU) 2018/2001, 2018).

Marginal conditions for food production can be found at sites that are difficult to cultivate due to harsh environmental conditions (Blanco-Canqui 2016; Shortall 2013). The

hydromorphic, fine textured soils (Stagnosols, IUSS 2015)) in the cold, winter-wet climate of low mountain ranges are one example of marginally productive land suitable for biomass production in central Europe. However, under these conditions, the most common biomass crop in Europe, silage maize (*Zea mays* L.), does not reach its full yield potential due to the slow soil warming and challenging crop management at low mountain range sites. Perennial crops may overcome some of the drawbacks of annual cropping at these sites. According to Grunwald et al. (2020), cup plant (*Silphium perfoliatum* L.) is particularly suitable for low mountain ranges, since these sites meet the high water demand of cup plant and in turn benefit from the soil preservation of the perennial crop. Due to the perenniality of the roots and the long growing season, cup plant is reported to mitigate N leaching and erosion compared to annual crops (Grunwald et al. 2020). However, as a green harvested crop, cup plant has a relatively high nitrogen (N) demand compared with other perennial crops, e.g., miscanthus (Ruf and Emmerling 2021), leading to concerns about N fertilizer addition and related losses.

✉ Björn Kemmann  
bkemmann@hotmail.com

<sup>1</sup> Thünen Institute of Climate-Smart Agriculture, Brunswick, Germany

<sup>2</sup> Department of Soil Science, University of Trier, Trier, Germany

Field-derived greenhouse gas (GHG) emissions during crop production are a critical consideration when assessing the sustainability of bioenergy from biomass (Directive (EU) 2018/2001, 2018; Jin et al. 2019). While the use of marginal land may reduce carbon- (C) related emissions from indirect land-use change, these potential gains must be weighed against increases in other GHGs, particularly nitrous oxide ( $\text{N}_2\text{O}$ ) at the hydromorphic sites.  $\text{N}_2\text{O}$  causes ozone depletion in the stratosphere, has a global warming potential 273 times greater than  $\text{CO}_2$  over a one-hundred-year time horizon (GWP100, Forster et al. (2021)), and is mainly produced by nitrification and denitrification, two microbial processes of the N cycle in soils. In agricultural soils, denitrification is usually the main source of emitted  $\text{N}_2\text{O}$  (Butterbach-Bahl et al. 2013; Li et al. 2016; Ostrom et al. 2010). Denitrification is the microbial reduction of nitrate ( $\text{NO}_3^-$ ) to  $\text{N}_2$ , where  $\text{N}_2\text{O}$  is an intermediate product. The key consideration, therefore, is not only whether denitrification occurs, but the product ratio ( $\text{N}_2\text{O}_i = \text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$ ). While any denitrification activity represents  $\text{NO}_3^-$  removal from the soil–plant system, complete denitrification to  $\text{N}_2$  does not pose a risk through GHGs. Therefore, both total  $\text{N}_2 + \text{N}_2\text{O}$  emissions and the  $\text{N}_2\text{O}_i$  are necessary to understand denitrification and its role as GHG source (Ciarlo et al. 2007; Jahangir et al. 2012; Scheer et al. 2020). On a global scale, total denitrification ( $\text{N}_2 + \text{N}_2\text{O}$  emissions) is currently estimated based on land-use-specific  $\text{N}_2\text{O}_i$  (e.g., Scheer et al. (2020) and Schlesinger (2009)). However, cropping system-specific  $\text{N}_2\text{O}_i$  under field conditions are widely unknown.

Organic C sources serve as electron donors for denitrification processes, while N oxides such as  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and  $\text{N}_2\text{O}$  serve as electron acceptors. Hence, soil aeration, organic C, and  $\text{NO}_3^-$  availability are all key factors controlling denitrification. Land use and management has an impact on each of these: soil aeration (Soane et al. 2012), C availability (Palm et al. 2014), and N mineralization and N availability (Balesdent et al. 2000). Thus, it can be assumed that soil-derived N emissions from a system managed for annual cropping may differ substantially from those of a perennial cropping system. However, in order to predict management and cropping system effects and their potential to cause or mitigate denitrification, it is important to understand the mechanisms involved.

Soil aeration is controlled through bulk density and soil moisture. No-till systems generally have higher bulk densities than conventionally tilled systems (Palm et al. 2014). Soils that do not undergo frequent rearrangement of aggregates through tillage have fewer large pores ( $> 1000 \mu\text{m}$ ) than tilled soils (Kay and VandenBygaart 2002). Smaller pores are saturated faster and drain slower than more transmissive pores of less compacted, tilled soil (Weninger et al. 2019). Soil under perennial crops, representing a no-till system, should then presumably have lower soil gas diffusivity

than annual crops in a conventionally tilled system (Ball et al. 1999; Rochette et al. 2008), and possibly be more prone to complete denitrification, especially in fine textured soil (Chapuis-Lardy et al. 2007). Generally, denitrification emissions increase with increasing water-filled pore space (WFPS) up to an optimum of around 80% WFPS for  $\text{N}_2\text{O}$  (Butterbach-Bahl et al. 2013), with  $\text{N}_2$  emissions increasing through to complete soil saturation. Thus, above the ‘tipping point’ of soil moisture for  $\text{N}_2\text{O}$  emission, the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  might exceed  $\text{N}_2\text{O}$  formation thus leading to lower  $\text{N}_2\text{O}$  fluxes. This tipping point can be reached through severe anoxic conditions in saturated soil or the prolonged residence time of  $\text{N}_2\text{O}$  due to low soil gas diffusivity. Denitrification is known to be potentially very high in the saturated zone of hydromorphic soils (Well et al. 2005), and could be particularly relevant in the afore-mentioned, periodically waterlogged Stagnosols of low mountain ranges. With such soil conditions, the potentially lower aeration under cup plant could increase denitrification, but also increase the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$ , while in maize soil, the higher pore volume may result in more aeration, limiting  $\text{N}_2\text{O}$  reduction.

Th C availability for denitrifiers can be an important factor in determining the  $\text{N}_2\text{O}_i$ . Since the final reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  is energy-intensive (Saggar et al. 2013), abundant available C as the energy source for denitrification can decrease the  $\text{N}_2\text{O}_i$  by enabling the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  (Firestone 1982; Firestone and Davidson 1989). However, there is no one management system (i.e., till vs. no-till) that clearly favors C availability. Tillage-induced mineralization (Balesdent et al. 2000) and incorporation of organic matter (Van Den Bossche et al. 2009) induces the release of C and  $\text{NO}_3^-$  and enhances  $\text{O}_2$  consumption. Perennial no-till systems are reported to facilitate soil organic C (SOC) accumulation due to increased root biomass (Don et al. 2012; Gauder et al. 2016; Monti and Zatta 2009) and canopy litter (Amougou et al. 2012; Schoo, et al. 2017a, b). The net effect of perennial no-till vs annually tilled soils on  $\text{N}_2\text{O}$  and  $\text{N}_2$  fluxes is thus not clear and both maize and cup plant have the potential to enhance denitrification through C availability.

As a key substrate for denitrification,  $\text{NO}_3^-$  availability has a clear impact on  $\text{N}_2\text{O}$  and  $\text{N}_2$  emissions and the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  in arable soil (Firestone et al. 1979; Senbayram et al. 2012). Numerous field experiments have compared the  $\text{N}_2\text{O}$  emissions from annual and perennial biomass crops (Drewer et al. 2012; Gauder et al. 2012; Walter et al. 2015), and consistently show that perennial systems have lower  $\text{N}_2\text{O}$  emissions. However, in these field studies, the perennial crops received less N. Therefore, the effect of the different cropping systems on other soil properties affecting denitrification (i.e., bulk density, C availability) is unclear. In studies where perennial biomass crops received comparable amounts of N fertilizer to annual crops, perennial crops

exhibited equal or higher  $\text{N}_2\text{O}$  emissions than the annual crops (Ferchaud et al. 2020; Jørgensen et al. 1997). Given the higher N demand of cup plant as compared to other perennial crops (Ruf and Emmerling 2021), cup plant relative to maize may provide a considerable risk for high denitrification losses. To assess this risk, more information is needed on the interactions between N availability and other soil properties related to the cropping system.

In the last decades, numerous denitrification studies have been conducted under fully saturated or constantly submerged soil conditions, as these conditions are assumed to cause substantial gaseous N losses to the environment (Friedl et al. 2016; Reddy et al. 1978; Well et al. 2005). In the field, however, soil water conditions are more dynamic. Varying stages of soil saturation are likely to be more common than full waterlogging, particularly on slow draining Stagnosols. Information on denitrification with changing soil saturation at different soil depths, which would be typical for Stagnosols and more representative of field conditions than static waterlogging, is lacking. To assess the effects of cup plant and maize cultivation on  $\text{N}_2$  and  $\text{N}_2\text{O}$  emissions, an incubation experiment using the  $^{15}\text{N}$  gas flux method was conducted, focusing on the potential for complete denitrification during periods of waterlogging. Field conditions occurring between fall and spring were mimicked as closely as possible using a dynamic soil water regime ranging from moist soil up to waterlogging 10 cm below the soil surface. The main question of the present study was to test if the cultivation of cup plant on Stagnosols results in soil conditions that mitigate  $\text{N}_2\text{O}$  emissions due to more complete reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$  compared to maize cultivation. To answer this question, we had three specific objectives: (1) to describe the impact of waterlogging in different soil depths on denitrification and the emission of  $\text{N}_2\text{O}$  and  $\text{N}_2$ ; (2) to test, using intact cores, the effect of N availability on the formation and reduction of  $\text{N}_2\text{O}$ , given the different soil properties that develop under cup plant and maize cultivation; and finally (3) to compare denitrification, particularly the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$ , from maize and cup plant soil.

## Materials and methods

### Soil selection, sampling of soil cores, and their preparation

Intact soil cores were taken from a maize (*Zea mays*) and an adjacent cup plant (*Silphium perfoliatum*) field in Gronig (49.520° N, 7.073° E, 365 m.a.s.l., mean annual temperature 9 °C, mean annual precipitation 1031 mm), western Germany. The extraction sites were in close vicinity of each other (< 80 m). The cup plant stand was established in 2017, while the maize field followed maize in rotation

with a rye winter cover crop (Table S.1). The soil monoliths extracted either from the maize or the cup plant field are referred as maize or cup plant soil. The soil at both sites is a Hypereutric Stagnic Cambisol (Loamic, Aric, Humic) (IUSS 2015). The fine textured soil (silt loam) is characterized by temporal water logging due to a reduced total pore volume and a higher bulk density below ploughing depth of 25 cm (Table S.2). Plant row spacing was 75 cm for both crops. To minimize the number of factors affecting N cycling (e.g., plant uptake, rhizosphere effects), living plants were excluded from the experiment by taking the columns from the inter-row area representative for > 50% of both fields.

In September 2021, an area of 7.5 m<sup>2</sup> was marked out in the adjacent fields, and was irrigated by drip irrigation (approximately 20–25 mm) to minimize structural damage while pushing cylinders in the soil. Following irrigation, intact, 35-cm-high soil columns were collected in Plexiglas cylinders/inner diameter = 14.4 cm, height = 40 cm height). Cylinders were used as liners in a steel auger that was pushed into the soil with an electric motor hammered auger. Columns were carefully extracted by digging. Parallel to the column extraction, bulk density measurements were conducted with 100 cm<sup>3</sup> steel cylinders at the following depths: 5–10 cm, 15–20 cm, and 30–35 cm (each depth  $n = 4$ ).

After transport to the incubation facility, each column was irrigated with 4 L solution of 0.01 M  $\text{CaCl}_2$ , equivalent to 245.6 mm, for 13 days to remove mineral  $\text{NO}_3^-$  from the soil, create comparable conditions in cup plant and maize soil, and allow homogenous  $^{15}\text{N}$  labeling. The amount of irrigated water is comparable to the precipitation over the winter at the extraction sites. To exclude the effects from growing plants during the subsequent incubation, emerging weeds were carefully removed and few individual, sprouting cup plants were killed selectively by brushing the emerging leaves with glyphosate (24 g glyphosate/l, Roundup Powerflex, Bayer AG, Leverkusen, Germany) to avoid soil disturbance by removing the plants due to their relatively deep rooting. In total, six cup plant soil cores were treated with the herbicide; three were used to determine initial N content, leaving only three for flux measurements (highest share of treated cores in one treatment was 22% in the 60 kg N treatment in the first phase).

### Incubation set up and experimental design

The Plexiglas cylinders (volume = 6515 cm<sup>3</sup>) served as incubation vessel, as previously described in Kemmann et al. (2021). Briefly, a needle plate as irrigation lid on the top and a base plate at the bottom of the Plexiglas cylinder were sealed by flat rubber sealings airtight. The porous base plate contained a polyamide membrane with a 0.45 µm pore size and bubble point of 100 kPa (ecoTech, Bonn, Germany) and was connected to leachate collection bottles held at defined

negative pressure to allow controlled drainage. In addition to the connections for the irrigation and fertilizer solution, the lid contained an inlet and outlet for the synthetic gas flow. The artificial atmosphere containing 20% O<sub>2</sub>, 2.7% N<sub>2</sub>, 77% He, 350 ppm CO<sub>2</sub>, and 250 ppb N<sub>2</sub>O flowed through the 815 cm<sup>3</sup> headspace and had a mean flow rate of 11 ml min<sup>-1</sup>. The reduced N<sub>2</sub> concentration was used to improve the sensitivity of the isotope ratio mass spectrometry (IRMS) measurements (Lewicka-Szczebak et al. 2017), whereas CO<sub>2</sub> and N<sub>2</sub>O were added to establish atmospheric conditions of these gases. Gas chromatography (GC) measurements, flow measurements, valve control, irrigation, and temperature were controlled by the fully automated system (Hantschel et al. 1994; Kemmann et al. 2021) (see supplement). The experiment was conducted constantly at 10 °C. Temperature and soil moisture regimes were set to mimic field conditions occurring from mid-October to mid-March.

Treatments were two N levels, 60 kg NO<sub>3</sub>-N ha<sup>-1</sup> (low N) and 120 kg NO<sub>3</sub>-N ha<sup>-1</sup> (high N), and soils from the two cropping systems: cup plant soil and maize soil; in total four treatment combinations with a minimum of five replicates (Fig. 1).

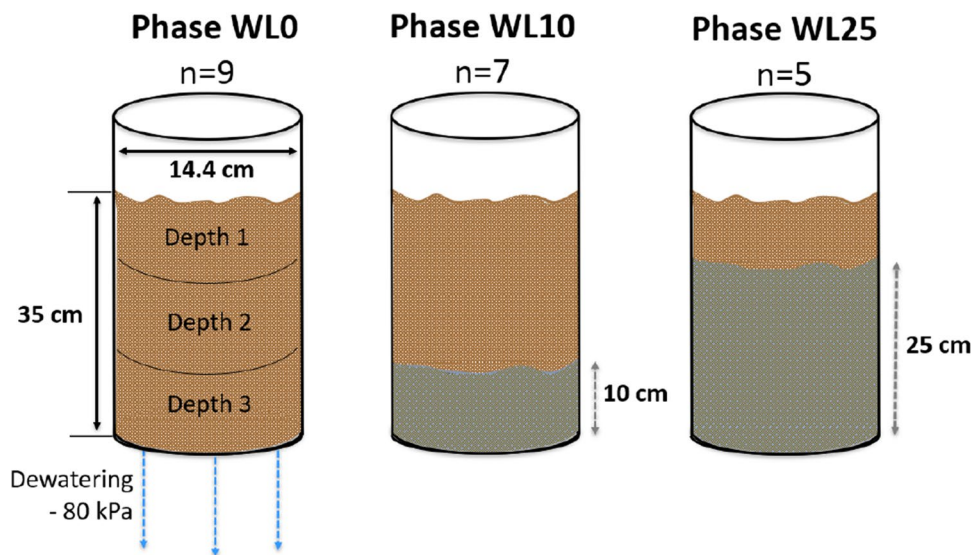
The dynamic experiment (Fig. 1) consisted of three phases with different water levels: drainage with pressure head of -80 kPa for 239 h (phase WL0), waterlogging in the lower 10 cm (25–35 cm) for 336.5 h (phase WL10), and waterlogging in the lower 25 cm (10–35 cm) for 321.5 h (phase WL25). The waterlogging was established by slowly pumping water (approximately 50 to 100 ml for 10 cm of waterlogging) through the base plate into the soil in order to avoid entrapped air bubbles in the waterlogged soil. Detailed information about the experimental setup can be found in the supplement (Fig. S.7). The number of days in phase WL10 and WL25 include the time for adjusting the water

level (around 48–72 h). As soil columns designated to be used for soil sampling were included in the incubation, the start of the dynamic experiment consisted of a total of 36 soil columns (Fig. 1). Additional eight soil columns were destructively sampled after label application but prior the start of the experiment (time 0 sample).

### Soil analysis

Two days after finishing <sup>15</sup>N labeling (time 0, t<sub>0</sub>) and after each phase, two mesocosms per treatment combination were sampled destructively. At t<sub>0</sub>, during phase transition, or at the end of the experiment, columns were sampled destructively in three soil depths, where each depth represented 1/3 of the soil column (0–11.7, 11.7–23.3, 23.3–35 cm). For simplicity, throughout the rest of the paper, these depths are referred to as depth 1 (0–12 cm), depth 2 (12–23 cm), and depth 3 (23–35 cm). All analyses were conducted in each depth of the sampled column. Soil moisture was determined immediately after dividing columns into the three soil layers. Gravimetric water content was determined by drying the soil for 48 h at 105 °C. Free water in the column at the time of destructive sampling could not be quantified, due to loss during the sampling process. Therefore, water content in the waterlogged soil layers could not be precisely determined. One subsample of fresh soil was stored at -20 °C and thawed over night at 4 °C for determination of soil mineral N content ( $N_{\min} = \text{NO}_3^- \text{-N} + \text{NH}_4^+ \text{-N}$ ). For the  $N_{\min}$  extraction, 400 g of homogenized fresh soil was filled into a 1 L PE bottle. The extractions were carried out by shaking the sample with 2 M KCl at a ratio 1:1.5 (w/v) for 1 h in an overhead shaker. The extraction solution was filtered (MN 614¼ filters, Macherey & Nagel,

**Fig. 1** Schematic representation of the mesocosms with the soil columns with sampling depths and number of replicates in the three consecutive phases with increasing waterlogging level (WL0, WL10, and WL25)



Düren, Germany) and stored afterwards at  $-20\text{ }^{\circ}\text{C}$  until analysis.  $\text{NO}_3^-$  and  $\text{NH}_4^+$ -N concentrations were analyzed colorimetrically with a continuous flow analyzer (SA 5000, Skalar Analytical B.V., Netherlands). The large soil sample size was used to account for soil heterogeneity, since soil cores were not disturbed and mixed prior to incubation.  $^{15}\text{N}$  enrichment of extractable  $\text{NO}_3^-$  ( $^{15}\text{aNO}_3$ ) and  $\text{NH}_4^+$  ( $^{15}\text{aNH}_4$ ) was quantified by analyzing the same extract by chemical conversion of  $\text{NO}_3^-$  to  $\text{N}_2\text{O}$  and  $\text{NH}_4^+$  to  $\text{N}_2$ , respectively, and online analysis by mass spectrometry (Dyckmans et al. 2021).

Another fresh soil subsample for determination of the water extractable organic C (WEOC) was stored at  $4\text{ }^{\circ}\text{C}$  overnight after destructive sampling. WEOC was extracted by gently shaking 5 g (dry mass basis) of fresh soil for one minute in 20 ml in pure water. Afterwards, solution was centrifuged for 10 min at 12,000 g and the supernatant was filtered through a  $45\text{ }\mu\text{m}$  syringe filter. For analysis of the WEOC concentration in the extractant, WEOC was oxidized and C emissions quantified by NDIR detection (Dimatoc 2000, Dimatec, Essen, Germany). WEOC analysis was only conducted on the remaining 20 columns after phase WL25. Soil acidity was measured with pH-meter (FE20, Mettler Toledo, Urdorf, Switzerland) after shaking 5 g (dry mass basis) of fresh soil in 20 ml in  $0.01\text{ M CaCl}_2$  for 1 h. Soil for the determination of total C and N was air dried and ground before analyzing in an elemental analyzer (TruMac CN analyzer, Leco, St. Joseph, USA). Soil organic C (SOC) was determined indirectly by dry combustion as the difference of total C and total inorganic C. Soil pH, SOC, and total N were only analyzed on three columns per treatment combination ( $n = 3$ ).

## Gas analysis

Gas samples from all mesocosms connected to the automated incubation system were analyzed online with a gas chromatograph (GC, Shimadzu GC-2014, Shimadzu, Duisburg, Germany) equipped with a flame ionization detector (FID), electron capture detector (ECD), and a thermal conductivity detector (TCD).

Each column was measured in intervals of 4.5, 3.5, and 2.5 h in phase WL0, WL10, and WL25, respectively. In each measuring sequence, three measurements of empty vessels with a representative headspace exchange rate as mesocosms were integrated for monitoring background  $\text{N}_2\text{O}$  and  $\text{CO}_2$  concentrations in the gas mixture. Furthermore, for calibration six standards (0.33, 0.55, 2.01, 6.94, 40.4, 130 ppm  $\text{N}_2\text{O}$ ) were measured regularly. Repeated measurements of these standards determined an analytical precision of the GC of consistently  $< 2\%$  CV.

## $^{15}\text{N}$ labeling and isotope analysis of $\text{N}_2$ and $\text{N}_2\text{O}$

For  $^{15}\text{N}$  labeling the soil columns were amended with a  $\text{K}^{15}\text{NO}_3$  solution containing  $0.01\text{ M CaCl}_2$  to achieve a target enrichment of 60 atom%  $^{15}\text{N}$ . Since the  $^{15}\text{N}$  gas flux method ( $^{15}\text{NGF}$ ) assumes homogeneous distribution of  $^{15}\text{N}$ ,  $\text{Br}^-$  percolation pre-tests were conducted to identify the best practicable irrigation scheme to reach homogeneously labeling. Based on these pre-tests, the  $^{15}\text{N}$  tracer solution was applied by replacing soil water 1.33 times with an alternating regime of two irrigation and flooding cycles. The irrigation was conducted by applying 1 L of fertilizer solution through the overhead irrigation plate. The flooding was conducted from the bottom by slowly pumping fertilizer solution through the filter membrane in the base plate into the mesocosm (Fig. S.7).

For  $^{15}\text{N}$  gas analysis, 12 ml Exetainers (Labco Ltd., Lam-peter, UK) were connected to the gas flow at the outlet of each mesocosm, guaranteeing a flushing of at least 1200 times the Exetainer's volume in 24 h. Samples from each mesocosm plus two blanks (background concentration of the gas mixture) were analyzed at  $t_0$  and then in a three day intervals. Therefore, each phase had four sampling dates. Sampling dates were selected to cover each phase equally during the time period when soil moisture conditions were stable. Therefore, the time for establishing the water level of each phase was excluded, because individual columns behaved differently in establishing the water level and thus did not provide comparable conditions.

Dissolved  $\text{N}_2\text{O}$  and  $\text{N}_2$  in the soil water were analyzed at the end of incubation to estimate accumulation of denitrification products in the water-saturated layers. Soil water samples were drawn from the baseplate with a syringe and immediately transferred into a 100 ml serum bottle containing a gas mixture of 3% atmospheric air in helium with slight overpressure of 40 hPa. Serum bottles with the samples were shaken for 1 h intensively on a horizontal lab shaker at constant  $10\text{ }^{\circ}\text{C}$  to equilibrate dissolved gases with the headspace. After shaking, two aliquots of 12.5 ml of headspace air were transferred into evacuated Exetainers for gas analysis by GC and IRMS.

IRMS analysis of gas samples was conducted as described in Lewicka-Szczebak et al. (2013) with a modified Gas-Bench II (Thermo Scientific, Bremen, Germany) with an automated sampling and online sample preparation (PAL Systems, Zwingen, Switzerland). Before samples were analyzed in the triple collector IRMS (MAT 253, Thermo Scientific, Bremen, Germany),  $\text{N}_2\text{O}$  is reduced in Cu oven to  $\text{N}_2$ . Therefore, the isotopocule mass ratios  $^{29}\text{R}$  ( $^{29}\text{N}_2/^{28}\text{N}_2$ ) and  $^{30}\text{R}$  ( $^{30}\text{N}_2/^{28}\text{N}_2$ ) from  $\text{N}_2$ ,  $\text{N}_2 + \text{N}_2\text{O}$ , and  $\text{N}_2\text{O}$  were measured. Through these measurements, the fraction originating of the labeled pool (fp) of  $\text{N}_2$  (fp\_ $\text{N}_2$ ),  $\text{N}_2 + \text{N}_2\text{O}$  (fp\_ $\text{N}_2 + \text{N}_2\text{O}$ ), and  $\text{N}_2\text{O}$  (fp\_ $\text{N}_2\text{O}$ ) were quantified. The analytical precision

of the IRMS measurements were  $< 7\%$  and  $< 0.01\%$  CV for  $^{30}\text{R}$  and  $^{29}\text{R}$ , respectively, which corresponds to a standard deviation of  $< 10^{-6}$  for both ratios.

## Calculations and statistics

The bulk densities (BD) measured in the field were systematically higher than the mean column average BDs, presumably due to slight loosening during sampling of the soil cores or due to more pronounced soil swelling following (drip) irrigation. Therefore, column average BD was used for calculations of the  $^{15}\text{N}$  labeling. However, the BD trends observed in the field were also observed in the extracted soil columns.

For flux calculations, the mass concentrations ( $C$ ) were calculated according the ideal gas law from the mole fraction ( $n$ ) of  $\text{CO}_2$ ,  $\text{N}_2\text{O}$ , and  $\text{N}_2$  provided from the GC and IRMS measurement:

$$C = n * \frac{M * 273.15K}{22.4136 \frac{\text{L}}{\text{mol}} * (273.15K + T)} \quad (1)$$

where  $M$  is the molar mass and  $T$  the temperature ( $^{\circ}\text{C}$ ). Mass flow ( $f$ ,  $\text{mg C}$  or  $\mu\text{g N m}^{-2} \text{h}^{-1}$ ), therefore, was calculated by  $C$  and the flow rate ( $Q$ ) provided from the flow meter:

$$f = \frac{C * Q}{A} \quad (2)$$

where  $A$  denotes the soil surface of the column in  $\text{m}^2$  and  $Q$  is the flow rate in  $\text{ml min}^{-1}$ .

Cumulated fluxes were obtained by integrating the emissions according the trapezoidal law from each phase separately or from the whole observation period after linear interpolation. Treatments were evaluated based on their cumulated emissions.

The  $^{15}\text{N}$  enrichment of  $\text{N}_2$  and  $\text{N}_2\text{O}$  and of the active  $\text{NO}_3^-$ -pool undergoing denitrification ( $\text{ap}$ :  $\text{apN}_2$ ,  $\text{apN}_2 + \text{N}_2\text{O}$ , and  $\text{apN}_2\text{O}$ ) were calculated based on the assumption of the non-random distribution of isotopocules in the gas samples (Hauck and Bouldin 1961). Calculations of  $\text{N}_2$ ,  $\text{N}_2 + \text{N}_2\text{O}$ , and  $\text{N}_2\text{O}$  fluxes were conducted according to Spott et al. (2006) and Russow et al. (1996).  $\text{N}_2\text{O}$  flux from other sources than the labeled pool ( $\text{fn}_{\text{N}_2\text{O}}$ ) were calculated as  $(1 - \text{Fp}_{\text{N}_2\text{O}}) * \text{N}_2\text{O}_t$  (where  $\text{Fp}_{\text{N}_2\text{O}}$  is the ratio of pool derived  $\text{N}_2\text{O}$  at total  $\text{N}_2\text{O}$  in the sample (background corrected), lower case  $t$  = total, see Kemmann et al. (2021)). Detailed information about the calculation of the IRMS data can be found in the supplement.

Cumulated fluxes of pool derived  $\text{N}_2$ ,  $\text{N}_2 + \text{N}_2\text{O}$ , and  $\text{N}_2\text{O}$  as well as  $\text{N}_2\text{O}$  from other sources than the labeled pool were calculated with the formulas 1 and 2 using  $\text{fp}_{\text{N}_2}$ ,  $\text{fp}_{\text{N}_2 + \text{N}_2\text{O}}$ ,  $\text{fp}_{\text{N}_2\text{O}}$ , and  $\text{fn}_{\text{N}_2\text{O}}$ . Where concentrations in the samples were below detection limit of the IRMS, half of

the detection limit was imputed for calculation of cumulated fluxes. The product ratio of denitrification ( $\text{N}_2\text{O}_i$ :  $\text{fp}_{\text{N}_2\text{O}} / \text{fp}_{\text{N}_2 + \text{N}_2\text{O}}$ ) and  $\text{Fp}_{\text{N}_2\text{O}}$  were calculated for each sample and corresponding cumulated fluxes. When the imputation of half detection limit had a significant effect on the cumulated emissions, these fluxes were omitted for further calculations of the two ratios. This was the case when the cumulated flux was not 5 times greater than the theoretical cumulated flux of half the detection limit. The IRMS data set with four sampling dates per phases overestimates cumulated  $\text{N}_2\text{O}$  emissions from the GC measurements with a higher temporal resolution by  $3.2 \pm 10.6\%$ . Therefore, calculation and comparisons were all based on the IRMS dataset, unless otherwise stated.

Dissolved gas concentrations were calculated from head-space concentrations according the Henry's law using the  $\text{fp}_{\text{N}_2}$  and the  $\text{N}_2\text{O}$  concentrations from the GC (further information in the supplement). The molar solubilities were obtained according to Wilhelm et al. (1977).

The observation period of phase WL0 started two days after the  $t_0$  sampling and 4 days after finishing fertilizer application because soil moisture conditions did not reach stable conditions before that date.

Calculations and statistical analysis were conducted using the version 4.1.1 of the statistic software R (R core team, 2020). Normal distributed explanatory variables were analyzed using an analysis of variance (ANOVA). Cumulated fluxes were analyzed using a linear mixed effect models with soil core as random effect. When assumption of normality were violated (e.g.,  $\text{N}_2\text{O}_i$ ,  $\text{Fp}_{\text{N}_2\text{O}}$ ), generalized linear mixed models were fitted with quasibinomial distribution family using a logit link function with the R package nlme (Pinheiro et al. 2020) or the glmmTMB package (Magnusson et al. 2017) with a beta distribution and logit link function when random effects were implemented. To handle the high variability of N emissions, flux data was log10 transformed. Data transformation for  $\text{CO}_2$  data was in most cases not necessary. Statistic tests in each phase were conducted with all replicates in each phase,; however, the fluxes were only plotted with those columns that lasted for the whole incubation time. All values, if not mentioned differently, are the arithmetic means  $\pm 1$  standard deviation.

## Results

### Physical soil properties and water content

Soil properties varied substantially between the soils and within soils. The bulk density (BD) was  $1.44 \pm 0.03 \text{ g cm}^{-3}$  and  $1.42 \pm 0.02 \text{ g cm}^{-3}$  in cup plant and maize soil (Fig. S.1), respectively, and thus significantly ( $p < 0.01$ ) higher in cup plant soil. Depth-specific bulk densities

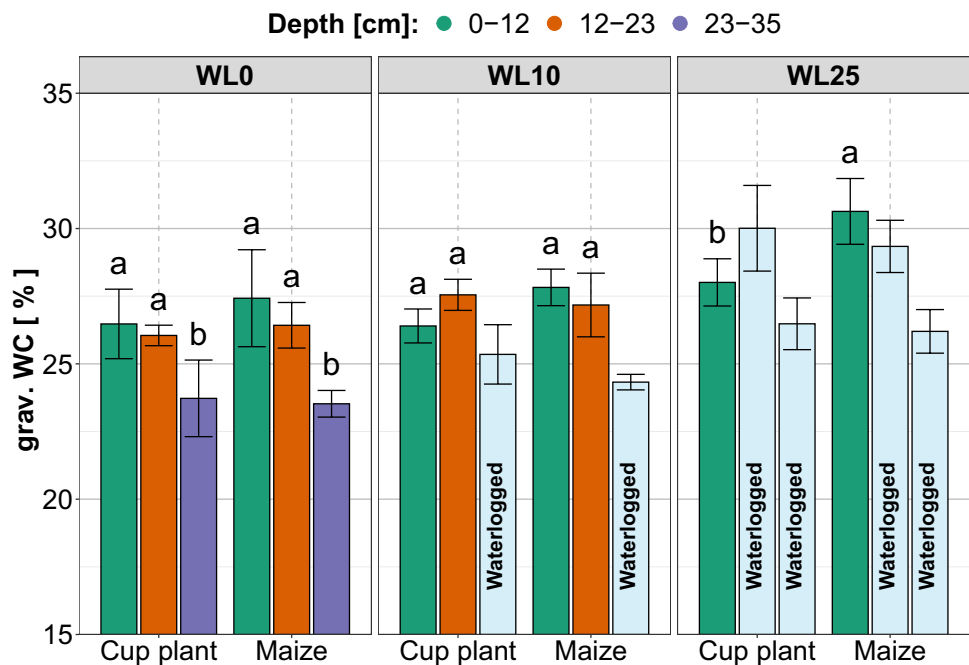
(BD) in the mesocosm could not be determined accurately. Density measurements in the field indicated following trends in BD and total pore volume (PV): maize 5–10 cm ( $BD = 1.43 \text{ g cm}^{-3}$ ,  $PV = 46.0\%$ ) < 15–20 cm ( $BD = 1.44 \text{ g cm}^{-3}$ ,  $PV = 41.0\%$ ) < 30–35 cm ( $BD = 1.69 \text{ g cm}^{-3}$ ,  $PV = 36.2\%$ ); and cup plant 5–10 cm ( $BD = 1.49 \text{ g cm}^{-3}$ ,  $PV = 43.9\%$ ) > 15–20 cm ( $BD = 1.43 \text{ g cm}^{-3}$ ,  $PV = 46.2\%$ ) < 30–35 cm ( $BD = 1.64 \text{ g cm}^{-3}$ ,  $PV = 38.0\%$ ). While the analysis of variance did not show significant differences in WC between soils in the WL0 and WL10 phases, the WC in WL25 supported the BD field measurements, showing significantly higher WC in depth 1 in maize than cup plant soil but not in depth 2 and 3 (Fig. 2). In phase WL25, the WC in depth 1 increased from phase WL10 by 2.8% (w/w,  $p = 0.001$ ) in maize soil and by 1.6% (w/w,  $p = 0.21$ ) in cup plant soil. Soil WC for each phase is shown in Fig. 2. From t0 to the end of the drainage phase (WL0), the WC in maize soil decreased significantly ( $p = 0.01$ ), while no change ( $p = 0.70$ ) in WC could be observed in cup plant soil. There were no

significant differences in phase WL0 and WL10, although depth 2 in phase WL10 appeared to be higher than in the first phase. In phase WL10 and WL25, water saturation was visually present in the waterlogged depths.

### Chemical soil properties and C availability

Soil pH was significantly lower ( $p < 0.001$ ) in cup plant ( $4.9 \pm 0.1$ ) compared to maize soil ( $5.2 \pm 0.1$ , Table 1). Total N decreased with soil depth. This decrease was more pronounced in cup plant soil than maize soil, while the decrease in SOC was comparable between the soils (Table 1). Due to a higher ( $p = 0.002$ ) total N content in maize compared to cup plant soil, the C:N ratio also differed ( $p < 0.001$ ) between the soils, especially in depth 3 where the C:N ratio was lower in maize soil than cup plant soil. Total C and N contents in depth 3 were about 50% lower in both soils than in the soil above (Table 1). Water extractable organic C (WEOC) did not differ between the soils ( $p = 0.77$ ). However, as with

**Fig. 2** Gravimetric water content of the three soil depths in phase WL0 ( $n = 4$ ), WL10 ( $n = 4$ ), and WL25 ( $n = 10$ ). Letters indicate significant differences within each phase. Error bars show  $\pm 1$  standard deviation



**Table 1** Soil organic C (SOC), total N content, water extractable organic C (WEOC), C:N ratio ( $n = 3$ ) and pH in depth 1 (0–12 cm), 2 (12–23 cm), and 3 (23–35 cm) from cup plant and maize soil ( $n = 6$ )

| Depth | Soil      | SOC (g C/kg)  | N (g N/kg)   | WEOC (mg C/kg) | C:N          | pH           |
|-------|-----------|---------------|--------------|----------------|--------------|--------------|
| 1     | Cup plant | 16.4 ± 0.4 ab | 1.9 ± 0.1 ab | 2.4 ± 1.7 a    | 8.6 ± 0.2 a  | 4.9 ± 0.1 cd |
|       | Maize     | 16.7 ± 0.8 a  | 2.1 ± 0.1 a  | 2.2 ± 0.9 a    | 8.0 ± 0.4 ab | 5.1 ± 0.1 b  |
| 2     | Cup plant | 14.7 ± 1.3 c  | 1.8 ± 0.2 b  | 0.6 ± 0.7 b    | 8.3 ± 0.2 ab | 4.8 ± 0.1 d  |
|       | Maize     | 14.9 ± 0.8 bc | 1.9 ± 0.1 ab | 0.8 ± 0.3 b    | 7.9 ± 0.8 ab | 5.3 ± 0.1 a  |
| 3     | Cup plant | 7.5 ± 0.9 d   | 1.0 ± 0.1 c  | 0.5 ± 1.2 b    | 7.3 ± 0.4 b  | 5.0 ± 0.0 c  |
|       | Maize     | 7.8 ± 1.0 d   | 1.3 ± 0.1 c  | 0.3 ± 1.0 b    | 6.1 ± 1.0 c  | 5.3 ± 0.0 a  |

SOC, WEOC decreased significantly ( $p < 0.03$ ) with depth (Table 1).

### Soil mineral N

The target mineral N ( $N_{\min} = \text{NO}_3\text{-N} + \text{NH}_4\text{-N}$ ) contents for the high and low N treatments were 24 and 12 mg N kg<sup>-1</sup>, respectively. With 26.2 mg N kg<sup>-1</sup> and 14.5 mg N kg<sup>-1</sup> ( $n = 2$ ), the maize  $N_{\min}$  contents in phase WL0 were slightly above target. In contrast,  $N_{\min}$  contents in phase WL0 in cup plant soil were with 26.0 mg N kg<sup>-1</sup> and 8.9 mg N kg<sup>-1</sup> ( $n = 2$ ), both above and below the initial targets. Coinciding with the decreasing gravimetric water content,  $\text{NO}_3^-$  decreased with soil depth, resulting in lower  $\text{NO}_3^-$  content in the denser depth 3. However, the <sup>15</sup>N enrichment of the  $\text{NO}_3^-$  (<sup>15</sup>aNO<sub>3</sub>) in the lower depths (2 and 3) did not differ from depth 1, indicating that fertilizer solution replaced soil water sufficiently in the lower soil. In depth 2 and 3 of cup plant soil,  $\text{NO}_3^-$  content, especially in the high N treatment, started to decrease during WL10 and WL25 (Table 2). In maize soil, the  $\text{NO}_3^-$  content increased in depth 1 and 2 in the absence of waterlogging, while  $\text{NO}_3^-$  content in depth 3 remained relatively stable (Table 2). Ammonium ( $\text{NH}_4^+$ ) content did not differ ( $p = 0.96$ ) between

the N treatments.  $\text{NH}_4^+$  contents were affected by the soil ( $p < 0.001$ ) and were non-significantly lower in maize soil treatments ( $1.2 \pm 1.4$  mg  $\text{NH}_4\text{-N}$  kg<sup>-1</sup>) than in cup plant soil ( $2.4 \pm 1.3$  mg  $\text{NH}_4\text{-N}$  kg<sup>-1</sup>). In cup plant soil,  $\text{NH}_4^+$  remained at a constant level (2.1 and 2.7 mg  $\text{NH}_4\text{-N}$  kg<sup>-1</sup> in phase WL0 and WL25, respectively), whereas  $\text{NH}_4^+$  content in maize soil decreased (2.1 and 1.0 mg  $\text{NH}_4\text{-N}$  kg<sup>-1</sup> in phase WL0 and WL25, respectively) throughout the observation period (Table S.3).

In cup plant, soil  $\text{NO}_3^-$  content was negatively correlated ( $r = -0.53$ ,  $p < 0.001$ ) with the  $\text{NH}_4^+$  content. The <sup>15</sup>aNH<sub>4</sub><sup>+</sup> in cup plant soil was with  $17.6 \pm 6.5$  atom% significantly higher ( $p < 0.001$ ) than in maize soil with  $0.6 \pm 0.4$  atom% (Fig. S.2).

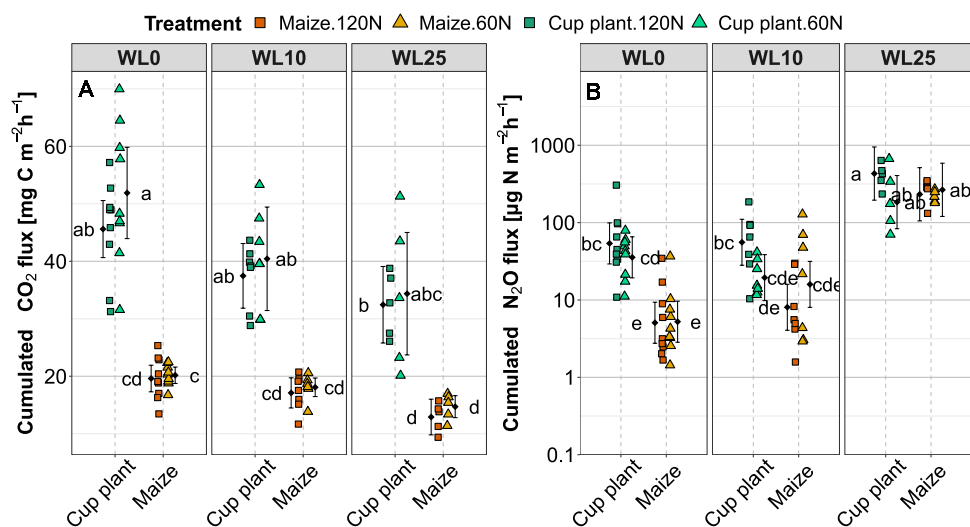
### CO<sub>2</sub> fluxes

Cumulated CO<sub>2</sub> emissions were significantly ( $p < 0.001$ ) higher from cup plant soil ( $41.8 \pm 11.2$  mg C m<sup>-2</sup> h<sup>-1</sup>) than from maize soil ( $17.7 \pm 3.6$  mg C m<sup>-2</sup> h<sup>-1</sup>) (Table S.5). Furthermore, there was a significant interaction of soil x phase ( $p < 0.001$ ) on the CO<sub>2</sub> emissions. The N level had no effect on the CO<sub>2</sub> emissions ( $p = 0.13$ ) (Fig. 3). CO<sub>2</sub> fluxes were the highest at the beginning of phase WL0, especially from

**Table 2** Nitrate ( $\text{NO}_3^-$ ) content at the end of the three phases of increasing waterlogging (WL; 0, 10 and 25 cm of soil saturation) at soil depth 1 (0–12 cm), 2 (12–23 cm) and 3 (23–35) and the high N (120 kg N ha<sup>-1</sup>) and low N (60 kg N ha<sup>-1</sup>) treatment. Values shown are mean  $\pm$  1 standard deviation (WL0:  $n = 2$ , WL10:  $n = 2$ , and WL25:  $n = 5$ )

| Depth | Soil      | WL0<br>mg $\text{NO}_3\text{-N}$ kg <sup>-1</sup> |                | WL10<br>mg $\text{NO}_3\text{-N}$ kg <sup>-1</sup> |                 | WL25<br>mg $\text{NO}_3\text{-N}$ kg <sup>-1</sup> |                   |
|-------|-----------|---------------------------------------------------|----------------|----------------------------------------------------|-----------------|----------------------------------------------------|-------------------|
|       |           | High N                                            | Low N          | High N                                             | Low N           | High N                                             | Low N             |
| 1     | Cup plant | 33.1 $\pm$ 4.3                                    | 12.7 $\pm$ 3.1 | 36.3 $\pm$ 0.2                                     | 9.7 $\pm$ 6.4   | 34.7 $\pm$ 5.0 b                                   | 16.1 $\pm$ 6.2 bc |
|       | Maize     | 31.3 $\pm$ 2.6                                    | 17.4 $\pm$ 1.3 | 39.7 $\pm$ 0.8                                     | 11.7 $\pm$ 14.3 | 46.7 $\pm$ 5.3 a                                   | 27.4 $\pm$ 1.9 a  |
| 2     | Cup plant | 24.6 $\pm$ 7.8                                    | 4.5 $\pm$ 1.2  | 21.9 $\pm$ 0.2                                     | 13.6 $\pm$ 17.8 | 16.6 $\pm$ 7.7 c                                   | 7.0 $\pm$ 4.8 d   |
|       | Maize     | 23.1 $\pm$ 7.8                                    | 14.0 $\pm$ 0.3 | 31.1 $\pm$ 0.7                                     | 18.0 $\pm$ 2.2  | 31.0 $\pm$ 3.0 b                                   | 16.4 $\pm$ 0.9 b  |
| 3     | Cup plant | 14.8 $\pm$ 9.2                                    | 2.0 $\pm$ 1.5  | 9.9 $\pm$ 4.6                                      | 3.9 $\pm$ 4.7   | 5.6 $\pm$ 2.9 d                                    | 1.8 $\pm$ 1.7d    |
|       | Maize     | 14.1 $\pm$ 8.7                                    | 9.4 $\pm$ 0.0  | 19.1 $\pm$ 0.2                                     | 10.3 $\pm$ 1.1  | 16.4 $\pm$ 1.3 c                                   | 8.5 $\pm$ 0.5 cd  |

**Fig. 3** Cumulated CO<sub>2</sub> fluxes (A) and cumulated total N<sub>2</sub>O fluxes (B) from a 37-day incubation of intact soil cores. Soils (from cup plant and maize cropping systems) were fertilized with 60 kg or 120 kg N ha<sup>-1</sup>. Black diamonds and error bars show estimated marginal means and 95% confidence interval ( $n \geq 5$ )



cup plant soil. Flux rates showed a decreasing trend with each phase. In both soils,  $\text{CO}_2$  dropped markedly with the establishment of phase WL25 but increased again afterwards. Cup plant soil exhibited higher variability in  $\text{CO}_2$  fluxes than maize soil (Fig. 3).

### **$\text{N}_2\text{O}$ fluxes dynamics from the GC dataset**

Similar to the  $\text{CO}_2$  emissions, there was a significant effect of the soil  $\times$  phase ( $p < 0.001$ ) interaction, and additionally, an interaction of soil  $\times$  N level ( $p = 0.03$ ), whereas the main effect of N level was not significant ( $p = 0.31$ ). However, corresponding to the significant main effect of soil ( $p < 0.001$ ),  $\text{N}_2\text{O}$  emissions from cup plant soil were always equal or higher than from maize soil (Fig. 3). In cup plant soil, the N level had a significant effect ( $p = 0.04$ ) on the  $\text{N}_2\text{O}$  emissions while  $\text{N}_2\text{O}$  emissions maize soil were not affected by the N level ( $p = 0.44$ ).

At the beginning of phase WL0,  $\text{N}_2\text{O}$  were  $109.6 \pm 97.7 \mu\text{g N m}^{-2} \text{h}^{-1}$  and  $15.1 \pm 15.0 \mu\text{g N m}^{-2} \text{h}^{-1}$  from cup plant and maize soil, respectively, and declined slowly toward the end of phase WL0. Pairwise comparison of the cumulated  $\text{N}_2\text{O}$  emissions in phase WL0 showed that emissions from cup plant soil at both N levels were significantly ( $p < 0.001$ ) higher than those from maize soil at both N levels (Fig. 3). During the establishment of WL10, fluxes from cup plant soil dropped and increased again after 2 days reaching a level comparable to the end of phase WL0 (Fig. 3). Emissions from maize soil did not show a similar dynamic during establishment of phase WL10. In phase WL10, the only significant difference observed was that emissions from high-N maize soil were lower than the high-N cup plant soil ( $p = 0.01$ ). In contrast to WL10, only  $\text{N}_2\text{O}$  from maize dropped due to the increase of the water level in WL25, whereas fluxes from cup plant soil were apparently not affected (Fig. 4). After raising the water level in WL25, fluxes from both soils increased substantially. The

$\text{N}_2\text{O}$  from cup plant soil peaked between day 31–33 with a maximum of  $1302.4 \mu\text{g N m}^{-2} \text{h}^{-1}$  and  $1194.1 \mu\text{g N m}^{-2} \text{h}^{-1}$  for the high and low N treatment, respectively. Fluxes from maize soil were still increasing at the end of incubation and thus ended with maximum flux rates of  $809.4 \mu\text{g N m}^{-2} \text{h}^{-1}$  and  $686.2 \mu\text{g N m}^{-2} \text{h}^{-1}$  in the high and low N treatment, respectively.

### **$\text{N}_2$ and $\text{N}_2\text{O}$ fluxes from the $^{15}\text{N}$ labeled pool**

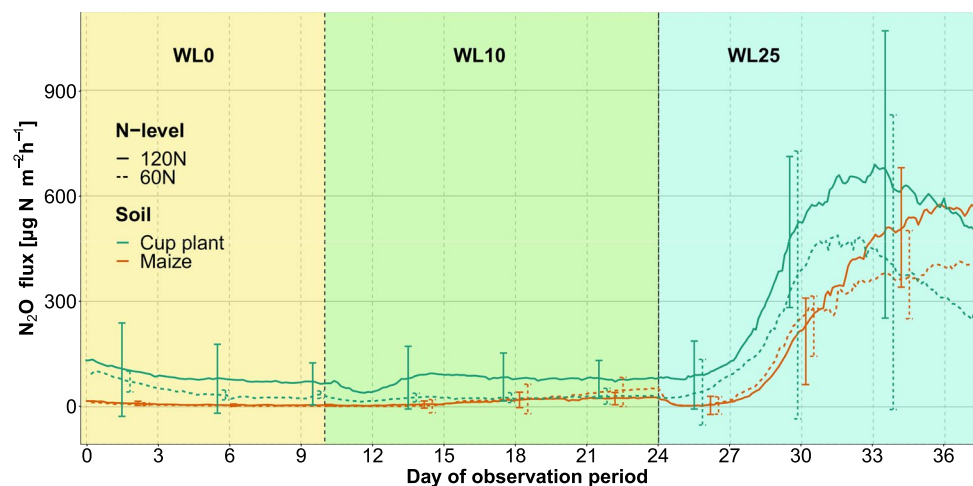
The pool-derived  $\text{N}_2\text{O}$  fluxes ( $\text{fp\_N}_2\text{O}$ ) closely followed the total  $\text{N}_2\text{O}$  fluxes ( $\text{N}_2\text{O}_t$ ) (Fig. 4; Fig. S.6) as indicated by the fraction of pool-derived fluxes in the total fluxes ( $\text{fp\_N}_2\text{O}/\text{N}_2\text{O}_t = \text{Fp\_N}_2\text{O}$ ).  $\text{Fp\_N}_2\text{O}$  was relatively high ( $> 0.84$ ) throughout the observation period and did not differ ( $p = 0.38$ ) between cup plant soil ( $0.93 \pm 0.08$ ) and maize soil ( $0.90 \pm 0.12$ ). The N level had a significant effect on the  $\text{Fp\_N}_2\text{O}$  ( $p = 0.02$ ), but  $\text{Fp\_N}_2\text{O}$  did not differ between phases ( $p = 0.66$ , Fig. S.5).

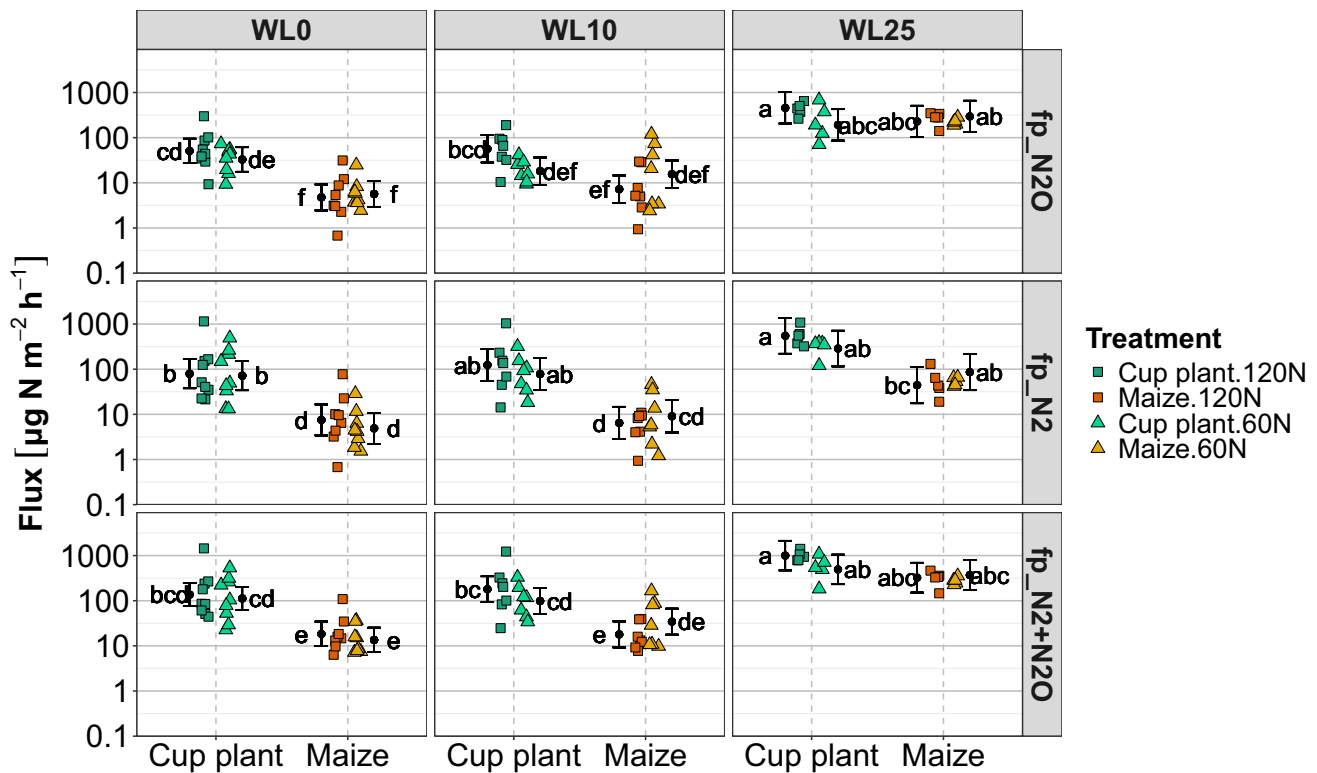
The soil  $\times$  phase interaction ( $p < 0.001$ ) and the soil  $\times$  N level interaction ( $p = 0.02$ ) had a significant effect on the cumulated  $\text{fp\_N}_2\text{O}$  emissions. While  $\text{fp\_N}_2\text{O}$  emissions from cup plant soil was always equal or higher than from maize soil (soil effect  $p < 0.001$ , Fig. 5). As shown with the  $\text{N}_2\text{O}_t$ , the N level only had an effect on the  $\text{fp\_N}_2\text{O}$  in cup plant soil ( $p = 0.04$ ), not in maize ( $p = 0.3$ ). Aside from the high N cup plant treatment, the cumulated  $\text{fp\_N}_2\text{O}$  emissions in phase WL0 and WL10 were relatively low (3 times the detection limit, Table S.6).

The  $\text{fp\_N}_2$  emissions were only affected by soil ( $p < 0.001$ ) and phase ( $p < 0.001$ ). There were no significant interactions. The coefficients of variation of  $\text{fp\_N}_2$  ( $94.9 \pm 50.7\%$ ) and  $\text{fp\_N}_2\text{O}$  ( $75.5 \pm 36.8\%$ ) were comparable ( $p = 0.12$ ).

In cup plant soil,  $\text{fp\_N}_2$  decreased during phase WL0 from  $357.8 \pm 515.6 \mu\text{g N m}^{-2} \text{h}^{-1}$  to  $91.51 \pm 148.1 \mu\text{g N m}^{-2} \text{h}^{-1}$ . During phase WL0 and WL10,  $\text{fp\_N}_2$  from cup plant soil

**Fig. 4**  $\text{N}_2\text{O}$  flux over the time course (37 days) of an incubation of intact soil cores from a maize and cup plant cropping system. Soils were fertilized with 60 kg or 120 kg N  $\text{ha}^{-1}$  and underwent three phases of increasing waterlogging (WL; 0, 10, and 25 cm of soil saturation). Error bars show  $\pm 1$  standard deviation ( $n = 5$ )





**Fig. 5** Cumulated emissions of pool-derived gaseous N fluxes ( $fp_{N_2O}$ ,  $fp_{N_2}$ , and  $fp_{N_2+N_2O}$ ) from intact soil cores incubated for 37 days. Soils were fertilized with 60 kg or 120 kg N  $ha^{-1}$  and under-

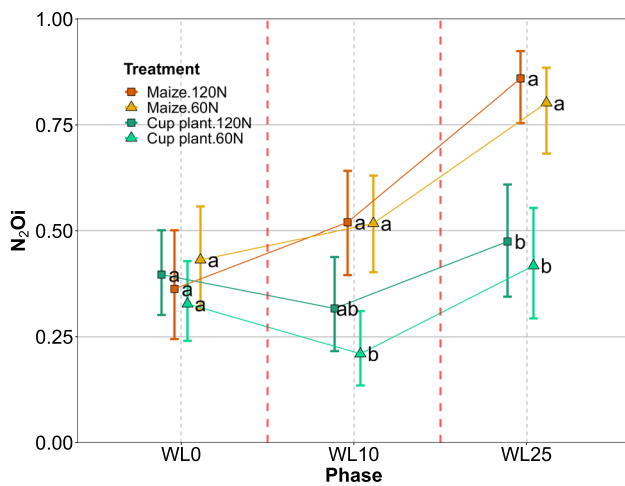
went three phases of increasing waterlogging (WL; 0, 10 and 25 cm of soil saturation). Black dots and error bars show estimated marginal means ( $n \geq 5$ ) and 95% confidence interval

ranged between 100 and 270  $\mu\text{g N m}^{-2} \text{h}^{-1}$  (Table S.6) and increased significantly ( $p < 0.02$ ) by 100–200% in phase WL25 compared to phase WL10. In phase WL10, the greatest increase of  $fp_{N_2}$  was observed in the cup plant soil, at the high N level. In maize soil,  $fp_{N_2}$  started phase WL0 at  $17.2 \pm 17.5 \mu\text{g N m}^{-2} \text{h}^{-1}$  and remained constant during phase WL0 and WL10. In phase WL25,  $fp_{N_2}$  in maize increased significantly ( $p < 0.001$ ) by 600% and 1700% in the low and high N treatments, respectively (Table S.6). Pairwise comparison within the phases revealed that  $fp_{N_2}$  in phase WL0 and WL10 from maize soil were, with  $< 20 \mu\text{g N m}^{-2} \text{h}^{-1}$ , significantly ( $p < 0.003$ ) lower than those from cup plant soil (Fig. 5). In phase WL25, cup plant and maize soil only differed in  $fp_{N_2}$  emissions at the high N level ( $p = 0.001$ ), although cumulated fluxes from maize soil were nearly one magnitude lower (Table S.6). Compared to the  $fp_{N_2O}$  dynamic,  $fp_{N_2}$  in cup plant soil apparently reacted more to the establishment of phase WL10 than  $fp_{N_2O}$  (Fig. S.8). Furthermore, in phase WL25,  $fp_{N_2}$  was constantly increasing toward the end of incubation, unlike  $fp_{N_2O}$ . However, in maize soil, the increase of  $fp_{N_2}$  by establishing WL25 was not as pronounced as it was for  $fp_{N_2O}$  (Fig. S.8).

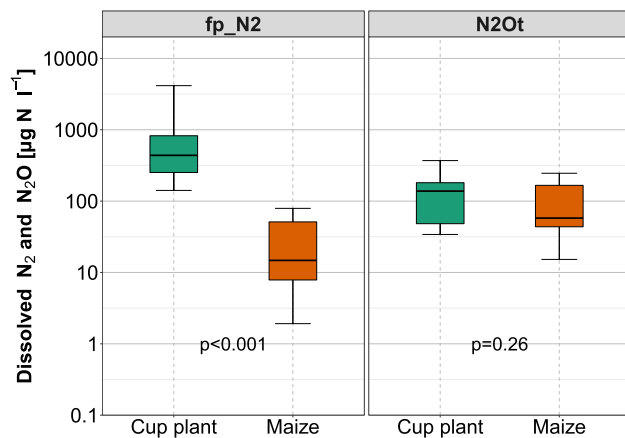
The soil  $\times$  phase interaction ( $p = 0.001$ ) had a significant effect on the cumulated  $fp_{N_2+N_2O}$ . Testing  $fp_{N_2+N_2O}$  showed that cup plant soil exhibited a higher denitrification rate in phase WL0 and WL10 (both  $p < 0.001$ ) and marginally higher in phase WL25 ( $p = 0.055$ ) than maize soil (Fig. 5).

### The $N_2O_i$ in the head space

In phase WL0, the product ratio of denitrification in the head space gas ( $N_2O_i$ :  $fp_{N_2O}/(fp_{N_2+N_2O})$ ) did not differ between cup plant and maize soil (Fig. 6). In phase WL10, the  $N_2O_i$  in maize ( $p > 0.14$ ) and cup plant ( $p > 0.11$ ) was not significantly different than in phase WL0 (Fig. 6). In phase WL25, the  $N_2O_i$  in maize soil was  $102.7 \pm 68.1\%$  higher ( $p < 0.002$ ) than in phase WL0 (Fig. 6), corresponding to the substantial increase of  $fp_{N_2O}$  emissions in relation to  $fp_{N_2}$  in phase WL25 (Table S.6). In contrast, increasing waterlogging in the cup plant soil caused simultaneous increases in both  $fp_{N_2O}$  and  $fp_{N_2}$  (Fig. S.6), resulting in no change in  $N_2O_i$  ( $p > 0.41$ ). The N level had no effect ( $p = 0.40$ ) on the  $N_2O_i$  in either soil.



**Fig. 6** The mean product ratio ( $N_2O_i = fp\_N_2O / (fp\_N_2 + N_2O)$ ) and 95% confidence interval ( $n \geq 5$ ) of denitrification in intact soil cores incubated for 37 days. Soils were fertilized with 60 kg or 120 kg N  $ha^{-1}$  and underwent three phases of increasing waterlogging (WL; 0, 10, and 25 cm of soil saturation). Letters show significant differences between the treatments within one phase



**Fig. 7** Dissolved gas concentrations of pool derived  $N_2$  ( $fp\_N_2$ ) and total  $N_2O$  ( $N_2Ot$ ) in the soil solution of cup plant and maize soil at the end of a 37-day incubation of intact soil cores from a cup plant and maize cropping system ( $n = 5$ ). For simplification, box plots were averaged over the 120 and 60 kg N  $ha^{-1}$  treatment

**Table 3** Total emitted N, expressed in mg N  $m^{-2}$  37.4 days $^{-1}$  (897 h) from incubating intact soil cores from a cup plant and maize cropping system, where fluxes were measured using  $^{15}N$  tracing (added N pool) ( $n = 5$ ). Two N levels were established (120 kg N  $ha^{-1}$  and

## Dissolved $N_2$ and $N_2O$ from the $^{15}N$ labeled pool

Measured concentration of dissolved  $fp\_N_2$  ranged from 3.7 to 7649.3  $\mu g N L^{-1}$  and the concentration of  $N_2Ot$  ranged from 15.51 to 376.0  $\mu g N L^{-1}$ . The  $N_2Ot$  and  $fp\_N_2$  dissolved gas concentrations showed the same treatment effects as the  $N_2Ot$  and  $fp\_N_2$  emissions (Table S.9). As with the cumulated emissions in phase WL25, dissolved  $N_2Ot$  did not differ between the soils ( $p = 0.26$ , Fig. 7) but they were slightly affected by N level ( $p = 0.04$ ). N level had no effect on dissolved  $fp\_N_2$  ( $p = 0.68$ ), but dissolved  $N_2$  concentrations were higher ( $p < 0.001$ , Fig. 7) in cup plant soil than in maize soil.

## Total N emissions to the head space

Total emitted N ( $fp\_N_2 + N_2Ot$ ) was clearly dominated by the emissions in phase WL25, which masked the differences of the previous phases since only 34.5% ( $154.3 \pm 215.6$  mg N  $m^{-2}$  24 days $^{-1}$ ) in cup plant and 10.4% ( $11.8 \pm 9.4$  mg N  $m^{-2}$  24 days $^{-1}$ ) of total emissions in maize soil were emitted in the first two phases (Table S.7). Furthermore, total emitted N was not affected by the N level ( $p = 0.13$ ). Cup plant soil emitted significantly more N (equivalent to  $4.2 \pm 3.0$  kg N  $ha^{-1}$  37.4 days $^{-1}$ ,  $p < 0.001$ ) than maize soil (equivalent to  $1.1 \pm 0.3$  kg N  $ha^{-1}$  37.4 days $^{-1}$ ). In contrast to the individual  $N_2O$  emissions in the single phases and N levels where soil had a significant effect ( $p < 0.001$ ) on  $fp\_N_2O$  and  $N_2Ot$ , total cumulated  $N_2O$  emissions over the entire observation period were only slightly higher in cup plant than in maize soil ( $fp\_N_2O$   $p = 0.04$ ;  $N_2Ot$   $p = 0.03$ , see Table 3).

## Discussion

### Denitrification in partially waterlogged soils

The aim of our experimental setup was to mimic field conditions at marginally productive, wet to waterlogged sites, only excluding the effect of active plants. Under the tested

60 kg N  $ha^{-1}$ ). Emissions from the labelled pool ( $fp\_N_2O$ ,  $fp\_N_2$  and  $fp\_N_2 + N_2O$ ), from other sources including nitrification ( $fn\_N_2O$ ) and total  $N_2O$  ( $N_2Ot$ ; all sources) are shown

| Soil      | N level | $N_2Ot$             | $fp\_N_2O$          | $fp\_N_2$           | $fp\_N_2 + N_2O$    | $fn\_N_2O$      | $N_2Ot + fp\_N_2$   |
|-----------|---------|---------------------|---------------------|---------------------|---------------------|-----------------|---------------------|
| Cup plant | 120 N   | $193.0 \pm 57.1$ a  | $190.1 \pm 55.1$ a  | $350.1 \pm 349.3$ a | $529.5 \pm 384.3$ a | $2.9 \pm 2.1$ a | $543.1 \pm 387.2$ a |
|           | 60 N    | $118.3 \pm 75.5$ ab | $111.7 \pm 73.9$ ab | $181.1 \pm 92.6$ a  | $289.1 \pm 108.4$ a | $6.7 \pm 2.3$ a | $299.4 \pm 108.1$ a |
| Maize     | 120 N   | $97.2 \pm 29.7$ ab  | $95.6 \pm 28.8$ ab  | $24.2 \pm 13.6$ b   | $116.6 \pm 39.1$ b  | $1.6 \pm 1.1$ a | $121.5 \pm 40.5$ b  |
|           | 60 N    | $84.7 \pm 19.8$ b   | $80.6 \pm 18.8$ b   | $20.2 \pm 4.1$ b    | $102.0 \pm 22.2$ b  | $4.1 \pm 1.1$ a | $104.8 \pm 22.7$ b  |

conditions, high  $N_2$  and  $N_2O$  emissions could be expected (Bronson and Fillery 1998; Friedl et al. 2016; Well et al. 2003), due to extremely reduced aeration in saturated soil favoring denitrification. However, although theory states that high denitrification is likely, there are few studies reporting direct measurements of total denitrification ( $N_2$  and  $N_2O$ ), which can be compared with the presented results. Anderson et al. (2014) addressed annual denitrification losses from landscapes that are prone to temporal waterlogging, comparable to the conditions mimicked in this study, by a mass balance approach. They reported landscape averaged annual denitrification losses around  $45 \text{ kg N ha}^{-1} \text{ a}^{-1}$ . Wendland et al. (2009) reported in a study that focused on N removal from groundwater that  $> 50 \text{ kg N ha}^{-1} \text{ a}^{-1}$  denitrification losses can be expected from periodically waterlogged Stagnosols of temperate climates. Interestingly, directly upscaling the results of this study results in  $49 \text{ kg N ha}^{-1} \text{ a}^{-1}$ , matching very closely with the values reported by Anderson et al. (2014) and Wendland et al. (2009). However, upscaling our results (or other short-term incubations) to a per-year basis almost certainly results in an overestimation, since we had waterlogged soils for over 2/3 of the incubation period, whereas field sites would only be expected to be partially waterlogged for perhaps 3–4 months of the year and the soil mineral N contents during these 3–4 months are usually lower than in our study. A more accurate upscaling of our results (assuming that our conditions are representative for 2 months in a year: early fall where high soil mineral N contents coincides with high precipitation; and late spring where fertilizer is applied on wet soils) would contribute less than  $10 \text{ kg N}$  to total annual denitrification losses. Additionally, the relatively high product ratio of denitrification in this study ( $N_2O_i > 0.3$ , Fig. 6) contradicts reports from other denitrification incubations (albeit with re-packed soils, not comparable conditions or measured in micro lysimeters), under high soil moisture, where  $N_2$  was by far the predominant product of denitrification (Bronson and Fillery 1998; Friedl et al. 2016; Weier et al. 1993). Possible explanations for these differences are discussed in the next section.

The  $N_2O$  flux rates from cup plant ( $0.6\text{--}1302.4 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ ) and maize soil ( $0.0\text{--}809.4 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ ) in the present study were in the range of the  $N_2O$  fluxes from the same cup plant ( $-19.7$  to  $371.1 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ ) and maize ( $-25.2$  to  $1955.6 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ ) field, observed in a 2-year field experiment at the extraction sites (Kemmann 2022). The fact that the observed emissions were not significantly different from reported field emissions suggests that we were able to achieve our goal of field-like conditions with incubation of intact soil cores. Furthermore, emissions in the current study were much lower (measured  $N_2O$  fluxes were  $5\times$  and  $\times$  lower in cup plant and maize soil, respectively) than a previous incubation study that also used intact cores

from the same extraction sites (Kemmann et al. 2021). The observed low emissions in the current study could be explained by the absence of rapid changes in soil moisture or N supply throughout the observation period, which are known to boost mineralization and microbial activity (Appel 1998; Kuzakov et al. 2000). Rapid changes, also known as hot moments, can disproportionately contribute to cumulative emissions (Groffman et al. 2009). Such rapid changes did occur in the previous study, through a dry pre-incubation and higher  $NO_3^-$  addition (Kemmann et al. 2021).

### ***$N_2O$ and $N_2$ emissions with consecutively increasing water level***

Denitrification products and their ratio measured in head-space gas concentrations are generally interpreted as being representative of denitrification from the entire soil. However, incubating intact soil cores under different waterlogging levels does provide very different conditions for denitrification in the individual soil depths. Therefore, our first objective was to analyze and discuss  $N_2O$  and  $N_2$  emissions for each phase individually.

### **Drainage phase (WL0)**

Prolonged phases of high  $N_2$  and  $N_2O$  emissions can be observed if soil structure (i.e., due to compacted or no-till managed soil) results in persistent anaerobic conditions in the soil due to a high water content through reduced drainage (Harrison-Kirk et al. 2015; Rochette 2008). In this study, the WL0 phase was designed to specifically observe whether differences in physical soil properties between the maize and cup plant soil affected drainage and related emissions. Although the bulk densities and pore structure of the individual soil depths could not be determined, particularly the drainage phase but also the two waterlogging phases gave an approximation of differences in soil moisture (Fig. 2,  $t_0$  moisture not shown) that would be controlled by soil structure. Using those differences as proxies, we were able to show, based on changes in gravimetric water content, that the annually tilled maize soil drained faster than the denser cup plant soil. The faster drainage was presumably due to the 3.8 vol.% more total pore space in 0–20 cm previously observed by Kemmann et al. (2021) in the same soil, and may explain the consistently low  $fp\_N_2 + N_2O$  emissions from maize soil ( $< 35 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$ ), in contrast to cup plant soil, where slower drainage may have caused the gradual decrease in  $fp\_N_2 + N_2O$  emissions from  $475 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$  at the start of WL0, to  $130 \text{ } \mu\text{g N m}^{-2} \text{ h}^{-1}$  at the end of WL0. Although the difference in BD between the two soils was seemingly marginal (cup plant was  $0.02 \text{ g cm}^{-3}$  higher than maize;  $p = 0.01$ ), a strong negative effect of compaction  $> 1.3 \text{ g cm}^{-3}$  on soil aeration has been reported

for fine textured soils with a moisture tension  $< 100$  kPa (Stepniewski 1981). Therefore, the effect of reduced drainage on gaseous N emission might be particularly relevant for the tested soils ( $BD > 1.4 \text{ g cm}^{-3}$ ), explaining the higher  $fp\_N_2O$  and  $fp\_N_2$  emissions in cup plant compared with maize soil in phase WL0. Additionally, the soil gas diffusivity ( $D_p/D_0$ ) as calculated by Moldrup et al. (2013) could be estimated from a previous study (Kemmann et al. 2021). In 0–20 cm, under comparable conditions (moisture and texture) as in phase WL0,  $D_p/D_0$  was 0.009 and 0.019 for cup plant and maize, respectively. Therefore, at any later point than phase WL0, increased soil moisture would have caused a lower  $D_p/D_0$  that was far below the critical anaerobicity value of  $< 0.02$  (Stepniewski 1981). Given the lower soil aeration of the cup plant compared to the maize soil, it can be concluded that the tested soil, from a relatively young cup plant stand (no tillage in the last 5 years), is already more prone to denitrification than the annually tilled soil. However, the role of bioturbation, bio-pores, and other biotic aspects affecting soil aeration might become increasingly important with stand age (Blanco-Canqui 2010; Bonin et al. 2012).

### Phase of 10 cm waterlogging (WL10)

The WL10 phase tested the potential for significant denitrification losses in the lower soil depths of cup plant and maize soil. The saturated zone of hydromorphic soils provides conditions for intense denitrification (Well et al. 2005). However, while the potential for microbial denitrification is present in subsoils (Barrett et al. 2016), denitrification rates often decrease exponentially with soil depth (Jahangir et al. 2012; Luo et al. 1998). In the present study, although there was potential for denitrification in the subsoil, as seen by the slightly increased  $fp\_N_2$  emissions in cup plant soil in WL10 compared to WL0 (Fig. 5), water saturation in the lower 10 cm of soil had only an insignificant effect on cumulated  $fp\_N_2$  and  $fp\_N_2O$  emissions to the headspace (Fig. 5; Fig. S.6). This may be reflecting changes in C availability. Given anoxic conditions and the presence of  $NO_3^-$ , denitrifier abundance and activity, particularly in subsoils, is often constrained by C availability (Barrett et al. 2016; Dhondt et al. 2004; Jahangir et al. 2012). In the present study, N was added to all 3 depths of both tested soils, but there were significantly lower C concentrations (ca. 50%) measured in depth 3 compared to the soil above. Furthermore, root biomass under perennial crops is often higher than annual crops (Don et al. 2012; Gauder et al. 2016; Monti and Zatta 2009). Along with increased root biomass under perennial crops, Jesus et al. (2016) and Liang et al. (2012) reported a higher microbial biomass in soil of perennial biomass systems compared to an annual maize soil. The higher  $CO_2$  emissions from cup plant soil in this study (Fig. 3) suggest

that a higher microbial biomass and/or root biomass was present. Not only the presence of roots, but also the quality of the C source (Barrett et al. 2016; Morley and Baggs 2010) and likely the distribution, will have a significant effect on denitrification (Loecke and Robertson 2009). Schoo et al. (2017a, b) observed a higher fine roots fraction at total root biomass in maize than cup plant, while cup plant forms thick roots resulting in a patchy distribution of root litter. Patches of organic matter provide conditions of high microbial activity where  $O_2$  consumption exceeds  $O_2$  supply by diffusion and thus favors denitrification (Kravchenko et al. 2017; Loecke and Robertson 2009). Hence, C availability and distribution is a plausible explanation for the difference between the two soils and the lack of any changes in  $fp\_N_2$  or  $fp\_N_2O$  emissions in maize soil (Fig. 5; Fig. S.6) during phase WL10, despite the presence of strong reducing conditions in 25–35 cm. We note that three methodological points need to be considered with respect to C availability. First, we did measure soil C, and saw no significant difference between the soils (Table 1). The standard procedures for soil C measurement that we used (SOC, WEOC), however, did not account for large root fragments (visible roots are excluded when subsampling soil for analysis). Second, the simulated rain prior to the experiment, with which  $NO_3^-$  was washed out, could have aggravated existing C limitation due to the removal of soluble organic C (Kindler et al. 2011). Third, since the different phases were established sequentially and not in parallel, substrates for denitrification (labile C and  $NO_3^-$ ) might have been depleted to some extent before the start of phase WL10, especially in denitrifying microsites. Increasing substrate limitations might thus have balanced increasing oxygen limitation.

### Phase of 25 cm waterlogging (WL25)

The WL25 phase highlighted the potential for significant denitrification losses in the upper soil depths of cup plant and maize soil. The uppermost layer of soil is known to have the highest microbial activity and denitrifying potential because of high substrate availabilities (Luo et al. 1998; Parkin 1987; Staley et al. 1990). Jahangir et al. (2012) observed significantly higher denitrification rates from 0 to 10 cm of soil than from soil layers below. Dobbie and Smith (2006) also observed exponentially increased  $N_2O$  emissions caused by increased WFPS in the uppermost soil due to a shallow ground water table in the field. In this study, near-surface waterlogging resulted in more pronounced increases in the fluxes of  $fp\_N_2O$  than  $fp\_N_2$  from both soils and both N levels; these were, on average, between  $5\times$  and  $23\times$  higher compared to phase WL10 (Fig. 5).

The stronger increase of  $fp\_N_2O$  compared to  $fp\_N_2$  with the establishment of phase WL25 was not in line with observations from previous denitrification studies, where

the predominant product of denitrification was  $N_2$  at high soil moistures (Bronson and Fillery 1998; Friedl et al. 2016; Rudaz et al. 1999; Weier et al. 1993). This difference may be reflecting the specific soil depth where denitrification occurred. In contrast to the experimental design of Bronson and Fillery (1998), while the gravimetric water content in the top 10 cm increased during WL25 (2.8% ( $p=0.001$ ) in maize and by 1.6% ( $p=0.06$ ) in cup plant soil; Fig. 2), the top 10 cm of soil was not itself waterlogged. Therefore, due to capillary rise in the top 10 cm of soil, soil moisture conditions may have been more favorable for  $N_2O$  emissions (i.e., < 80% WFPS; Butterbach-Bahl et al. (2013)), than for  $N_2$  emissions. The capillary rise, which is closely associated with soil structure, was apparently more pronounced in maize than cup plant soil. Since no similar observation of N emissions from the boundary layer between depths 2 and 3 were made in phase WL10, it is likely that the  $fp\_N_2O$  emissions from depth 1 dominated the  $N_2Ot$  emissions in phase WL25.

The increase in  $fp\_N_2$  due to the establishment of phase WL25 was comparable or less pronounced than the increase in  $fp\_N_2O$  ( $5\times$  in cup plant soil and  $11\times$  in maize soil higher than in phase WL10). In saturated soil, a time lag between the actual denitrification ( $NO_3^-$  consumption) and the emission of  $N_2$  at the soil surface can be expected, due to impeded gas diffusion (Friedl et al. 2016). However, in this study,  $fp\_N_2$  and  $fp\_N_2O$  emissions in both soils started to increase simultaneously around day 27, which does not indicate a significant time lag between the emission of  $fp\_N_2$  and  $fp\_N_2O$  (Fig. S.6). This suggests that both species were produced in close temporal and spatial proximity to each other. Given the assumption that the  $N_2O$  was produced under the less reducing conditions in depth 1, it is likely that, especially at the beginning of phase WL25 (day 27–33), the top 10 cm was also the origin of the majority of  $fp\_N_2$ . Further indications for this assumption are given by the fact that the active pool producing  $N_2$  ( $apN_2$ ) was more comparable to the labeled  $NO_3^-$  ( $^{15}aNO_3$ ) of depth 1 than that of the soil below. For example, in the high N treatment for cup plant,  $apN_2$  was 47 atom% and  $^{15}aNO_3$  of depth 1 was 48 atom%, whereas  $^{15}aNO_3$  was 49 and 55 atom% in depths 2 and 3, respectively (Table S.4). Therefore, the contribution from  $N_2$  produced and/or  $N_2O$  reduced in the saturated zone to the head space emissions was unexpectedly low.

### Nitrate consumption in saturated soil

This experiment was conducted using two N levels (60 kg  $NO_3-N\ ha^{-1}$  and 120 kg  $NO_3-N\ ha^{-1}$ ), in order to explore the effect of a differing  $NO_3^-$  concentration on denitrification. Both the stimulation of  $N_2O$  formation through increased substrate availability and the inhibition of  $N_2O$  reduction are possible with higher N (Senbayram et al. 2012; Weier et al.

1993). Interestingly, there was only a slight increase in  $N_2O$  emissions (both  $N_2Ot$  and  $fp\_N_2O$ ) at the higher N level in cup plant soil ( $p<0.05$ ), while no effect was observed in maize soil ( $p>0.3$ ). The N rate had no significant effect on the  $N_2Oi$  ( $p=0.40$ , Fig. 6) in either soil, suggesting that no substantial inhibition of  $N_2O$  reduction occurred. Overall, these observations support other studies that have shown that mineral N content is not always the most important explanatory variable for  $N_2O$  emissions (Rochette et al. 2008) or the  $N_2Oi$  (Kemmann et al. 2021).

While  $NO_3^-$  availability did not clearly affect measured gas emissions in the headspace, there was evidence that it affected  $NO_3^-$  consumption in the saturated soil. Mineral N was highly variable between columns in each of the four treatments (Table 2). However, based on the average change in  $NO_3^-$  content in high N cup plant soil, there was more  $NO_3^-$  consumption in the saturated soil depths (loss of  $24.7\pm 20.9\ kg\ N\ ha^{-1}$  from the beginning of WL10 to the end of WL25), than in the other three treatments (in maize a net  $NO_3^-$  production of  $0.6\pm 22.0\ kg\ N\ ha^{-1}$  occurred). As increased  $NO_3^-$  consumption was not reflected in the  $N_2+N_2O$  surface emissions, we explore here other potential explanations of that  $NO_3^-$  loss, focusing on the high N cup plant treatment.

Separated by depth,  $NO_3^-$  losses were observed both in depth 2 (loss of  $9.0\pm 12.9\ kg\ N\ ha^{-1}$  during phase WL25) and in depth 3 (loss of  $15.4\pm 16.2\ kg\ N\ ha^{-1}$  across phase WL10 and WL25, Table 2). Our measured surface fluxes only explained a  $NO_3^-N$  loss of  $5.4\pm 3.9\ kg\ N\ ha^{-1}$  (Table 3). However, with an expected lag time between denitrification and emission (Friedl et al. 2016), it is possible that our incubation period may have been too short to see activity from the saturated layers, due to the extremely low gas diffusivity in water and resulting accumulation of produced  $N_2$  (Well et al. 2001). This is indicated both by the increase in  $fp\_N_2$  fluxes that we observed near the end of our incubation (Fig. S.6), and by the dissolved gas concentrations at the end of WL25 (Fig. 7), which showed that some of the denitrification products had built up in the saturated layers (Well and Myrold 1999). Our estimated upscaling of the dissolved gases (Table S.9) accounted for another  $1.2\pm 1.8\ kg\ N\ ha^{-1}$  of  $NO_3^-$  loss. This increases the directly measured denitrification losses to  $6.6\pm 5.8\ kg\ N\ ha^{-1}$ , which represents just under 27% of the observed  $24.7\ kg\ N\ ha^{-1}$  loss. However, measurement of dissolved gases shows only those gaseous denitrification products that are trapped in the liquid phase, and excludes the gases accumulated in encapsulated gas bubbles. Bubbles typically occur during the rise of the water table in the soil (Fayer and Hillel 1986). Therefore, a possible source of ‘missed’ denitrification activity may have been entrapped  $N_2O$  and  $N_2$ . It is possible for a significant portion of produced  $N_2$  to accumulate in gas bubbles that are either entrapped during saturation (Well and Myrold 1999)

or newly formed when  $N_2$  production by denitrification is in excess of gas solubility (Blicher-Mathiesen et al. 1998) due to the low solubility of  $N_2$  (Wilhelm et al. 1977). Assuming a WFPS between 90 and 100% in the waterlogged soil layers, dissolved  $N_2$  may only reflect part of the total accumulated  $N_2$  in the saturated layers (between 15 and 100%; see calculations in Table S.10). Thus,  $N_2$  trapped in bubbles could theoretically account for up to  $7.1 \text{ kg N ha}^{-1}$  of additional denitrification that occurred, but was not captured through headspace or dissolved gas measurements. Measured (dissolved gas + headspace flux,  $6.6 \text{ kg N ha}^{-1}$ ) and estimated (trapped  $N_2$  in bubbles,  $7.1 \text{ kg N ha}^{-1}$ ) denitrification losses account for up to 55% ( $13.7$  of  $24.7 \text{ kg N ha}^{-1}$ ) of total  $NO_3^-$  loss observed in the saturated soil in the high N cup plant soil, which was in the range of N losses explained by denitrification reported by Matheson et al. (2002) in a mass balance study from submerged riparian soil. Future studies should target methods to directly measure entrapped  $N_2$  and  $N_2O$ , as it has the potential to cause significant underestimations of denitrification activity in saturated soil.

Aside from denitrification, other processes that consume  $NO_3^-$  include dissimilatory nitrate reduction to  $NH_4^+$  (DNRA) and immobilization. At the end of the incubation, the much higher recovery of  $^{15}N$  in the cup plant soil extractable  $NH_4^+$  (Fig. S.2) shows that much more of the  $^{15}N$ -labeled  $NO_3^-$  was transferred to the  $NH_4^+$ -pool in cup plant soil than in maize soil. In the saturated soil layers, the high  $^{15}aNH_4$  could be due to DNRA (Rütting et al. 2011). DNRA transforms  $NO_3^-$  into the more biologically available  $NH_4^+$  (Burgin and Hamilton 2007), and has been shown to account for 50% of  $NO_3^-$  loss in unplanted riparian soil (Matheson et al. 2002). The retention of N in the form of less mobile  $NH_4^+$  in the soil via DNRA can substantially differ between soils under different plant species (Shi et al. 2020). Since  $NH_4^+$  formed through DNRA can be preferably immobilized in organic forms (Burgin and Hamilton 2007), DNRA occurring in the cup plant soils of this study could explain why the total extractable  $NH_4^+$  content did not increase significantly, but the pool was enriched by 18 atom%  $^{15}N$ . Microbial immobilization and re-mineralization (Azam et al. 1988) might additionally explain up to 25% of  $NO_3^-$  loss under waterlogged conditions (Matheson et al. 2002). Therefore, in the saturated layers of cup plant soil,  $NO_3^-$  loss was likely caused by a combination of denitrification, DNRA, and immobilization. In comparison, these processes appeared to be less active in maize soil, where net  $NO_3^-$  production was observed (Table 2).

### **$N_2O$ emissions and its reduction to $N_2$**

In view of the higher  $N_2O$  emissions from cup plant soil than maize soil, the main hypothesis of the presented study was not confirmed. Cup plant soil did not emit less  $N_2O$

than the reference maize soil due to an increased reduction to  $N_2$  with increasing waterlogging. Cup plant soil had, in general, higher  $fp\_N_2 + N_2O$  fluxes than maize soil ( $p < 0.02$ , Table 3), and at no point during the experiment did this enhanced  $N_2O$  reduction balance the higher gross  $N_2O$  formation (Fig. 6). Denitrification from soil depth 1 was disproportionally contributing to emissions of  $N_2O$  and to a smaller extent also  $N_2$ , so that other aspects, such as prolonged residence time and strong reducing conditions in the saturated soil below the 10 cm of soil depth, were not decisive for total N emissions at the soil surface.

Although not associated with less  $N_2O$  emissions, the conditions in the soil cultivated with cup plant were more favorable for the reduction of  $N_2O$  to  $N_2$ , which was evident from the surface emissions (Fig. 6), but also from dissolved gas analysis (Fig. 7). As previously discussed, this was likely due to C availability and soil structure, pointing out another substantial difference between the soils. The lower  $N_2O$  reduction in maize soil cannot simply be explained by an inhibitory effect of  $O_2$  on  $N_2O$  reductase (Morley et al. 2008) in the better aerated maize soil, since the  $N_2O_i$  measured in the dissolved gas in the saturated soil was also significantly higher than in cup plant soil (Table S.9). An inhibition of the expression of  $N_2O$  reductase due to a low soil pH (Raut et al. 2012) in maize can also be excluded, because the pH was significantly higher than in cup plant soil. However, the slightly lower pH might have had an effect on the Cu mobility in cup plant soil. Shen et al. (2020) reported an increased  $N_2O$  reduction with increased Cu availability. Along with the different N cycling (e.g., occurrence of DNRA) in cup plant soil, limited  $N_2O$  reduction in maize soil could be due to land use and management related effects on the composition of the microbial community (Cavigelli and Robertson 2000; Domeignoz-Horta et al. 2015; Hargreaves and Hofmockel 2014; Jangid et al. 2008; Maul et al. 2019). In comparison with annual systems, perennial cropping systems have a higher abundance of  $N_2O$  reducers of the clade II, but not of the more common clade I (Domeignoz-Horta et al. 2015). The recent development of new primers for the clades I (Zhang et al. 2021) and II (Chee-Sanford et al. 2020) of *nosZ* gene might further elucidate the diversity of the denitrifier community under different cropping systems.

### **Conclusion**

In the absence of living plants, the soil from the perennial cup plant field generally exhibited higher denitrification rates than the soil from maize cropping, thus providing an increased risk of N loss via denitrification. With increasing waterlogging, the reduction of  $N_2O$  to  $N_2$ , under the tested conditions, did not increase as much as total denitrification in either soil. The cultivation of the perennial biomass crop

did not result in soil properties that caused less N<sub>2</sub>O emissions than maize soil. Therefore, the potential to mitigate N<sub>2</sub>O emissions through more complete reduction by changing from an annual silage maize cropping to perennial cup plant cropping remains hypothetical.

In the direct comparison of maize and cup plant soil, maize soil exhibited a significantly lower reduction of N<sub>2</sub>O to N<sub>2</sub> under waterlogged conditions than cup plant soil. Given that the parent material was the same in the two cropping systems, this suggests that each cropping systems had a strong influence on the mechanism controlling denitrification in that soil. This mechanism is likely related to differences in C availability, soil structure, and the composition of the microbial community, but we are unable to identify a clear explanation for the inhibited N<sub>2</sub>O reduction in the maize soil. Nonetheless, this study clearly demonstrated that even under the same soil and climatic conditions, annual and perennial land use systems can strongly influence the product ratio of denitrification. Conclusively, our results therefore stressed that denitrification losses and its implication for the cropping system's N use efficiency in biomass cultivation should not simply be estimated based on constant product ratios of denitrification across annual and perennial cropping systems.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00374-022-01673-6>.

**Acknowledgements** We would like to thank Andreas Kirch for the support in the field and Stefan Burkart for the assistance with the mesocosm facility. Furthermore, we would like to thank the technicians from the isotope and the soil laboratory at the Thünen-Institute for Climate-Smart Agriculture for analyzing all the samples: Martina Heuer, Jennifer Giere and Ute Rieß, Ute Tambor, Andrea Niemeyer, and Claudia Wiese.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This study was funded by the Fachagentur Nachwachsende Rohstoffe e.V. (FNR, Agency for Renewable Resources, Germany) through the project BESTLAND (FKZ: 22403618 and 22404217): Greenhouse gas mitigation potential, soil and biodiversity conservation of perennial biomass cropping on temporal waterlogged (pseudogleyic) sites.

**Data availability** The datasets generated during and/or analyzed during the current study are available in the OpenAgrar repository, <https://doi.org/10.3220/DATA20220922104041> or is available from the corresponding author.

## Declarations

**Conflict of interest** The authors declare no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated

otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Amougou N, Bertrand I, Cadoux S, Recous S (2012) *Miscanthus* × *giganteus* leaf senescence, decomposition and C and N inputs to soil. *GCB Bioen* 4:698–707
- Anderson TR, Goodale CL, Groffman PM, Walter MT (2014) Assessing denitrification from seasonally saturated soils in an agricultural landscape: a farm-scale mass-balance approach. *Agric Ecosyst Environ* 189:60–69
- Appel T (1998) Non-biomass soil organic N—the substrate for N mineralization flushes following soil drying–rewetting and for organic N rendered CaCl<sub>2</sub>-extractable upon soil drying. *Soil Biol Biochem* 30:1445–1456
- Azam F, Mahmood T, Malik K (1988) Immobilization–remineralization of NO<sub>3</sub>-N and total N balance during the decomposition of glucose, sucrose and cellulose in soil incubated at different moisture regimes. *Plant Soil* 107:159–163
- Balesdent J, Chenu C, Balabane M (2000) Relationship of soil organic matter dynamics to physical protection and tillage. *Soil till Res* 53:215–230
- Ball BC, Scott A, Parker JP (1999) Field N<sub>2</sub>O, CO<sub>2</sub> and CH<sub>4</sub> fluxes in relation to tillage, compaction and soil quality in Scotland. *Soil till Res* 53:29–39
- Barrett M, Khalil M, Jahangir MMR, Lee C, Cárdenas LM, Collins G, Richards KG, O'Flaherty V (2016) Carbon amendment and soil depth affect the distribution and abundance of denitrifiers in agricultural soils. *Environ Sci Pollut Res* 23:7899–7910
- Blanco-Canqui H (2010) Energy crops and their implications on soil and environment. *Agron J* 102:403–419
- Blanco-Canqui H (2016) Growing dedicated energy crops on marginal lands and ecosystem services. *Soil Sci Soc Am J* 80:845–858
- Blicher-Mathiesen G, McCarty G, Nielsen L (1998) Denitrification and degassing in groundwater estimated from dissolved dinitrogen and argon. *J Hydrol* 208:16–24
- Bonin C, Lal R, Schmitz M, Wullschlegel S (2012) Soil physical and hydrological properties under three biofuel crops in Ohio. *Acta Agriculturae Scandinavica, Section B - Soil Plant Sci* 62:595–603
- Bronson K, Fillery I (1998) Fate of nitrogen-15-labelled urea applied to wheat on a waterlogged texture-contrast soil. *Nutr Cycl Agroecosyst* 51:175–183
- Burgin AJ, Hamilton SK (2007) Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. *Front Ecol Environ* 5:89–96
- Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philos Trans R Soc B* 368:20130122
- Cavigelli MA, Robertson GP (2000) The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology* 81:1402–1414
- Chapuis-Lardy L, Wrage N, Metay A, Chotte JL, Bernoux M (2007) Soils, a sink for N<sub>2</sub>O? A review. *Glob Chang Biol* 13:1–17
- Chee-Sanford JC, Connor L, Krichels A, Yang WH, Sanford RA (2020) Hierarchical detection of diverse Clade II (atypical) *nosZ* genes using new primer sets for classical-and multiplex PCR array applications. *J Microbiol Meth* 172:105908

- Ciarlo E, Conti M, Bartoloni N, Rubio G (2007) The effect of moisture on nitrous oxide emissions from soil and the  $N_2O/(N_2O+N_2)$  ratio under laboratory conditions. *Biol Fertil Soils* 43:675–681
- Dhondt K, Boeckx P, Hofman G, Van Cleemput O (2004) Temporal and spatial patterns of denitrification enzyme activity and nitrous oxide fluxes in three adjacent vegetated riparian buffer zones. *Biol Fertil Soils* 40:243–251
- Directive (EU) 2018/2001 of the European Parliament and of the council of 11 December 2018 on the promotion of the use of energy from renewable sources (2018)
- Dobbie K, Smith K (2006) The effect of water table depth on emissions of  $N_2O$  from a grassland soil. *Soil Use Manag* 22:22–28
- Domeignoz-Horta L, Spor A, Bru D, Bizouard F, Leonard J, Philippot L (2015) The diversity of the  $N_2O$  reducers matters for the  $N_2O$ :  $N_2$  denitrification end-product ratio across an annual and a perennial cropping system. *Front Microbiol* 6:971
- Don A, Osborne B, Hastings A, Skiba U, Carter MS, Drewer J, Flessa H, Freibauer A, Hyvönen N, Jones MB (2012) Land-use change to bioenergy production in Europe: implications for the greenhouse gas balance and soil carbon. *GCB Bioen* 4:372–391
- Drewer J, Finch JW, Lloyd CR, Baggs EM, Skiba U (2012) How do soil emissions of  $N_2O$ ,  $CH_4$  and  $CO_2$  from perennial bioenergy crops differ from arable annual crops? *GCB Bioen* 4:408–419
- Dyckmans J, Eschenbach W, Langel R, Szewc L, Well R (2021) Nitrogen isotope analysis of aqueous ammonium and nitrate by membrane inlet isotope ratio mass spectrometry (MIRMS) at natural abundance levels. *Rapid Commun Mass Spectrom* 35:e9077
- Fayer M, Hillel D (1986) Air encapsulation: II. Profile water storage and shallow water table fluctuations. *Soil Sci Soc Am J* 50:572–577
- Ferchaud F, Peyrard C, Léonard J, Gréhan E, Mary B (2020) Large variations in  $N_2O$  fluxes from bioenergy crops according to management practices and crop type. *Atmosphere* 11:675–699
- Firestone M (1982) Biological denitrification. In: Stevenson FJ (ed) *Nitrogen in agricultural soils*. American Society of Agronomy, Madison, WI, pp 289–326
- Firestone M, Smith M, Firestone R, Tiedje J (1979) The influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Sci Soc Am J* 43:1140–1144
- Firestone M, Davidson E (1989) Microbiological basis of  $NO$  and  $N_2O$  production and consumption in soil. In: Andreae MO, Schimel DS (eds) *Exchange of the trace gases between terrestrial ecosystems and the atmosphere*. Report for the Dahlem Workshop on Exchange of Gases Between Terrestrial Ecosystems and the Atmosphere. Wiley, New York, pp 7–22
- Forster P, Storelvmo T, Armour K, Collins W., Dufresne JL, Frame D, Lunt DJ, Mauritsen T, Palmer MD, Watanabe M, Wild M, Zhang H (2021) The Earth's energy budget, climate feedbacks, and climate sensitivity. In: Masson-Delmotte V, Zhai P, Pirani A, Connors S.L, Péan C, Berger S, Caud N, Chen Y, Goldfarb L, Gomis M.I, Huang M, Leitzell K, Lonnoy E, Matthews JBR, Maycock TK, Waterfield T, Yelekçi O, Yu R, Zhou B (eds), *Climate change 2021: the physical science basis*. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, pp. 923–1054
- Friedl J, Scheer C, Rowlings DW, McIntosh HV, Strazzabosco A, Warner DI, Grace PR (2016) Denitrification losses from an intensively managed sub-tropical pasture—Impact of soil moisture on the partitioning of  $N_2$  and  $N_2O$  emissions. *Soil Biol Biochem* 92:58–66
- Gauder M, Butterbach-Bahl K, Graeff-Hönniger S, Claupein W, Wiegel R (2012) Soil-derived trace gas fluxes from different energy crops—results from a field experiment in southwest Germany. *GCB Bioen* 4:289–301
- Gauder M, Billen N, Zikeli S, Laub M, Graeff-Hönniger S, Claupein W (2016) Soil carbon stocks in different bioenergy cropping systems including subsoil. *Soil till Res* 155:308–317
- Groffman PM, Butterbach-Bahl K, Fulweiler RW, Gold AJ, Morse JL, Stander EK, Tague C, Tonitto C, Vidon P (2009) Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. *Biogeochemistry* 93:49–77
- Grunwald D, Panten K, Schwarz A, Bischoff WA, Schittenhelm S (2020) Comparison of maize, permanent cup plant and a perennial grass mixture with regard to soil and water protection. *GCB Bioen* 12:694–705
- Haberl H, Sprinz D, Bonazountas M, Cocco P, Desaubies Y, Henze M, Hertel O, Johnson RK, Kastrup U, Laconte P (2012) Correcting a fundamental error in greenhouse gas accounting related to bioenergy. *Energy Policy* 45:18–23
- Hantschel RE, Flessa H, Beese F (1994) An automated microcosm system for studying soil ecological processes. *Soil Sci Soc Am J* 58:401–404
- Hargreaves SK, Hofmockel KS (2014) Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. *Biogeochemistry* 117:67–79
- Harrison-Kirk T, Thomas SM, Clough TJ, Beare MH, van der Weerden TJ, Meenken ED (2015) Compaction influences  $N_2O$  and  $N_2$  emissions from  $^{15}N$ -labeled synthetic urine in wet soils during successive saturation/drainage cycles. *Soil Biol Biochem* 88:178–188
- Hauck R, Bouldin D (1961) Distribution of isotopic nitrogen in nitrogen gas during denitrification. *Nature* 191:871–872
- Herbes C, Jirka E, Braun JP, Pukall K (2014) Der gesellschaftliche Diskurs um den, Maisdeckel “vor und nach der Novelle des Erneuerbare-Energien-Gesetzes (EEG) 2012, The social discourse on the “Maize Cap “ before and after the 2012 amendment of the German Renewable Energies Act (EEG). *GAIA-Ecol Perspect Sci Soc* 23:100–108
- IUSS WG (2015) World reference base for soil resources 2014 (update 2015). International soil classification system for naming soils and creating legends for soil maps, World Soil Resource Reports 106
- Jahangir MM, Khalil MI, Johnston P, Cardenas L, Hatch D, Butler M, Barrett M, O'flaherty V, Richards KG (2012) Denitrification potential in subsoils: a mechanism to reduce nitrate leaching to groundwater. *Agricult Ecosyst Environ* 147:13–23
- Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH, Jenkins MB, Endale DM, Coleman DC, Whitman WB (2008) Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biol Biochem* 40:2843–2853
- Jesus EDC, Liang C, Quensen JF, Susilawati E, Jackson RD, Balser TC, Tiedje JM (2016) Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. *GCB Bioen* 8:481–494
- Jin VL, Schmer MR, Stewart CE, Mitchell RB, Williams CO, Wienhold BJ, Varvel GE, Follett RF, Kimble J, Vogel KP (2019) Management controls the net greenhouse gas outcomes of growing bioenergy feedstocks on marginally productive croplands. *Sci Advances* 5:eaav9318
- Jørgensen RN, Jørgensen BJ, Nielsen NE, Maag M, Lind A-M (1997)  $N_2O$  emission from energy crop fields of *Miscanthus* “Giganteus” and winter rye. *Atmos Environ* 31:2899–2904
- Kay B, VandenBygaart A (2002) Conservation tillage and depth stratification of porosity and soil organic matter. *Soil till Res* 66:107–118
- Kemmann B, Wöhl L, Fuß R, Schrader S, Well R, Ruf T (2021)  $N_2$  and  $N_2O$  mitigation potential of replacing maize with the

- perennial biomass crop *Silphium perfoliatum*—an incubation study. *GCB Bioen* 13:1649–1665
- Kemmann B (2022) Assessment of field-derived greenhouse gas mitigation potential in biomass production by replacing maize with cup plant (*Silphium perfoliatum*) in low mountain ranges. Dissertation, University of Kiel
- Kindler R, Siemens J, Kaiser K, Walmsley DC, Bernhofer C, Buchmann N, Cellier P, Eugster W, Gleixner G, Grunwald T (2011) Dissolved carbon leaching from soil is a crucial component of the net ecosystem carbon balance. *Glob Change Biol* 17:1167–1185
- Kravchenko A, Toosi E, Guber A, Ostrom N, Yu J, Azeem K, Rivers M, Robertson G (2017) Hotspots of soil  $N_2O$  emission enhanced through water absorption by plant residue. *Nature Geosci* 10:496–500
- Kuzyakov Y, Friedel J, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biol Biochem* 32:1485–1498
- Lewicka-Szczepak D, Well R, Giesemann A, Rohe L, Wolf U (2013) An enhanced technique for automated determination of  $^{15}N$  signatures of  $N_2$ , ( $N_2+N_2O$ ) and  $N_2O$  in gas samples. *Rapid Commun Mass Spectrom* 27:1548–1558
- Lewicka-Szczepak D, Augustin J, Giesemann A, Well R (2017) Quantifying  $N_2O$  reduction to  $N_2$  based on  $N_2O$  isotopocules—validation with independent methods (helium incubation and  $^{15}N$  gas flux method). *Biogeosciences* 14:711–732
- Li X, Sørensen P, Olesen JE, Petersen SO (2016) Evidence for denitrification as main source of  $N_2O$  emission from residue-amended soil. *Soil Biol Biochem* 92:153–160
- Liang C, Jesus EDC, Duncan DS, Jackson RD, Tiedje JM, Balser TC (2012) Soil microbial communities under model biofuel cropping systems in southern Wisconsin, USA: impact of crop species and soil properties. *Appl Soil Ecol* 54:24–31
- Loecke TD, Robertson GP (2009) Soil resource heterogeneity in terms of litter aggregation promotes nitrous oxide fluxes and slows decomposition. *Soil Biol Biochem* 41:228–235
- Luo J, Tillman R, White R, Ball P (1998) Variation in denitrification activity with soil depth under pasture. *Soil Biol Biochem* 30:897–903
- Magnusson A, Skaug H, Nielsen A, Berg C, Kristensen K, Maechler M, van Benthem K, Bolker B, Brooks M, Brooks MM. (2017) Package ‘glmmTMB’. R Package Version 0.2. 0
- Matheson F, Nguyen M, Cooper A, Burt T, Bull D (2002) Fate of  $^{15}N$ -nitrate in unplanted, planted and harvested riparian wetland soil microcosms. *Ecol Eng* 19:249–264
- Maul JE, Cavigelli MA, Vinyard B, Buyer JS (2019) Cropping system history and crop rotation phase drive the abundance of soil denitrification genes *nirK*, *nirS* and *nosZ* in conventional and organic grain agroecosystems. *Agric Ecosyst Environ* 273:95–106
- Moldrup P, Chamindu Deepagoda TTK, Hamamoto S, Komatsu T, Kawamoto K, Rolston DE, de Jonge LW (2013) Structure-dependent water-induced linear reduction model for predicting gas diffusivity and tortuosity in repacked and intact soil. *Vadose Zone J* 12:0026
- Monti A, Zatta A (2009) Root distribution and soil moisture retrieval in perennial and annual energy crops in Northern Italy. *Agric Ecosyst Environ* 132:252–259
- Morley N, Baggs EM (2010) Carbon and oxygen controls on  $N_2O$  and  $N_2$  production during nitrate reduction. *Soil Biol Biochem* 42:1864–1871
- Morley N, Baggs E, Dörsch P, Bakken L (2008) Production of  $NO$ ,  $N_2O$  and  $N_2$  by extracted soil bacteria, regulation by  $NO_2^-$  and  $O_2$  concentrations. *FEMS Microbiol Ecol* 65:102–112
- Ostrom NE, Sutka R, Ostrom PH, Grandy AS, Huizinga KM, Gandhi H, von Fischer JC, Robertson GP (2010) Isotopologue data reveal bacterial denitrification as the primary source of  $N_2O$  during a high flux event following cultivation of a native temperate grassland. *Soil Biol Biochem* 42:499–506
- Palm C, Blanco-Canqui H, DeClerck F, Gatere L, Grace P (2014) Conservation agriculture and ecosystem services: an overview. *Agric Ecosyst Environ* 187:87–105
- Parkin TB (1987) Soil microsites as a source of denitrification variability. *Soil Sci Soc Am J* 51:1194–1199
- Pinheiro J, Bates D, DebRoy S, Sarkar D (2020) R Core Team. 2020. nlme: linear and nonlinear mixed effects models. R package version 3.1–147. URL: <https://CRAN.R-project.org/package=nlme>.
- Raut N, Dörsch P, Sitaula BK, Bakken LR (2012) Soil acidification by intensified crop production in South Asia results in higher  $N_2O/(N_2+N_2O)$  product ratios of denitrification. *Soil Biol Biochem* 55:104–112
- Reddy K, Patrick W Jr, Phillips R (1978) The role of nitrate diffusion in determining the order and rate of denitrification in flooded soil: I. Experimental results. *Soil Sci Soc Am J* 42:268–272
- Rochette P (2008) No-till only increases  $N_2O$  emissions in poorly-aerated soils. *Soil Till Res* 101:97–100
- Rochette P, Angers DA, Chantigny MH, Bertrand N (2008) Nitrous oxide emissions respond differently to no-till in a loam and a heavy clay soil. *Soil Sci Soc Am J* 72:1363–1369
- Rudaz A, Wälti E, Kyburz G, Lehmann P, Fuhrer J (1999) Temporal variation in  $N_2O$  and  $N_2$  fluxes from a permanent pasture in Switzerland in relation to management, soil water content and soil temperature. *Agric Ecosyst Environ* 73:83–91
- Ruf T, Emmerling C (2021) Different life-form strategies of perennial energy crops and related nutrient exports require a differentiating view specifically concerning a sustainable cultivation on marginal land. *GCB Bioen* 13:893–904
- Russow R, Stevens R, Laughlin R (1996) Accuracy and precision for measurements of the mass ratio 30/28 in dinitrogen from air samples and its application to the investigation of N losses from soil by denitrification. *Isotopes Environ Health Stud* 32:289–297
- Rütting T, Boeckx P, Müller C, Klemetsson L (2011) Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* 8:1779–1791
- Saggar S, Jha N, Deslippe J, Bolan N, Luo J, Giltrap D, Kim D-G, Zaman M, Tillman R (2013) Denitrification and  $N_2O$ :  $N_2$  production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts. *Sci Tot Environ* 465:173–195
- Scheer C, Fuchs K, Pelster DE, Butterbach-Bahl K (2020) Estimating global terrestrial denitrification from measured  $N_2O:(N_2O+N_2)$  product ratios. *Curr Opin in Environ Sustain* 47:72–80
- Schlesinger WH (2009) On the fate of anthropogenic nitrogen. *Proc Natl Academy Sci* 106:203–208
- Schoo B, Kage H, Schittenhelm S (2017) Radiation use efficiency, chemical composition, and methane yield of biogas crops under rainfed and irrigated conditions. *Eur J Agron* 87:8–18
- Schoo B, Schroetter S, Kage H, Schittenhelm S (2017) Root traits of cup plant, maize and lucerne grass grown under different soil and soil moisture conditions. *J Agron Crop Sci* 203:345–359
- Senbayram M, Chen R, Budai A, Bakken L, Dittert K (2012)  $N_2O$  emission and the  $N_2O/(N_2O+N_2)$  product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. *Agric Ecosyst Environ* 147:4–12
- Shen W, Xue H, Gao N, Shiratori Y, Kamiya T, Fujiwara T, Isobe K, Senoo K (2020) Effects of copper on nitrous oxide ( $N_2O$ ) reduction in denitrifiers and  $N_2O$  emissions from agricultural soils. *Biol Fertil Soils* 56:39–51
- Shi X, Wang J, Müller C, Hu H-W, He J-Z, Wang J, Huang Z (2020) Dissimilatory nitrate reduction to ammonium dominates soil

- nitrate retention capacity in subtropical forests. *Biol Fertil Soils* 56:785–797
- Shortall O (2013) “Marginal land” for energy crops: exploring definitions and embedded assumptions. *Energy Policy* 62:19–27
- Soane BD, Ball BC, Arvidsson J, Basch G, Moreno F, Roger-Estrade J (2012) No-till in northern, western and south-western Europe: a review of problems and opportunities for crop production and the environment. *Soil till Res* 118:66–87
- Spott O, Russow R, Apelt B, Stange CF (2006) A  $^{15}\text{N}$ -aided artificial atmosphere gas flow technique for online determination of soil  $\text{N}_2$  release using the zeolite K strolith SX6 . *Rapid Commun Mass Spectrom* 20:3267–3274
- Staley T, Boyer D, Caskey W (1990) Soil denitrification and nitrification potentials during the growing season relative to tillage. *Soil Sci Soc Am J* 54:1602–1608
- Stepniewski W (1981) Oxygen diffusion and strength as related to soil compaction. II. Oxygen diffusion coefficient. *Polish J Soil Sci* 14:3–13
- Van Den Bossche A, De Bolle S, De Neve S, Hofman G (2009) Effect of tillage intensity on N mineralization of different crop residues in a temperate climate. *Soil till Res* 103:316–324
- Walter K, Don A, Flessa H (2015) Net  $\text{N}_2\text{O}$  and  $\text{CH}_4$  soil fluxes of annual and perennial bioenergy crops in two central German regions. *Biomass Bioen* 81:556–567
- Weier K, Doran J, Power J, Walters D (1993) Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Sci Soc Am J* 57:66–72
- Well R, Myrold DD (1999) Laboratory evaluation of a new method for in situ measurement of denitrification in water-saturated soils. *Soil Biol Biochem* 31:1109–1119
- Well R, Augustin J, Davis J, Griffith S, Meyer K, Myrold D (2001) Production and transport of denitrification gases in shallow ground water. *Nutr Cycl Agroecosyst* 60:65–75
- Well R, Augustin J, Meyer K, Myrold D (2003) Comparison of field and laboratory measurement of denitrification and  $\text{N}_2\text{O}$  production in the saturated zone of hydromorphic soils. *Soil Biol Biochem* 35:783–799
- Well R, H per H, Mehranfar O, Meyer K (2005) Denitrification in the saturated zone of hydromorphic soils—laboratory measurement, regulating factors and stochastic modeling. *Soil Biol Biochem* 37:1822–1836
- Wendland F, Behrendt H, G mann H, Hirt U, Kreins P, Kuhn U, Kunkel R, Tetzlaff B (2009) Determination of nitrogen reduction levels necessary to reach groundwater quality targets in large river basins: the Weser basin case study, Germany. *Nutr Cycl Agroecosyst* 85:63–78
- Weninger T, Kreiselmeier J, Chandrasekhar P, Julich S, Feger K-H, Schw rzel K, Bodner G, Schwen A (2019) Effects of tillage intensity on pore system and physical quality of silt-textured soils detected by multiple methods. *Soil Res* 57:703–711
- Wilhelm E, Battino R, Wilcock RJ (1977) Low-pressure solubility of gases in liquid water. *Chem Reviews* 77:219–262
- Zhang B, Penton CR, Yu Z, Xue C, Chen Q, Chen Z, Yan C, Zhang Q, Zhao M, Quensen JF (2021) A new primer set for Clade I nosZ that recovers genes from a broader range of taxa. *Biol Fertil Soils* 57:523–531

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.