N2 and N2O mitigation potential of replacing maize with the perennial biomass crop Silphium perfoliatum—An incubation study

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Abstract
Sustainability of biogas production is strongly dependent on soil-borne greenhouse gas (GHG) emissions during feedstock cultivation. Maize (Zea mays) is the most common feedstock for biogas production in Europe. Since it is an annual crop requiring high fertilizer input, maize cropping can cause high GHG emissions on sites that, due to their hydrology, have high N2O emission potential. On such sites, cultivation of cup plant (Silphium perfoliatum) as a perennial crop could be a more environmentally friendly alternative offering versatile ecosystem services. To evaluate the possible benefits of perennial cup plant cropping on GHG emissions and nitrogen losses, an incubation study was conducted with intact soil cores from a maize field and a cup plant field. The 15N gas flux method was used to quantify N source-specific N2 and N2O fluxes. Cumulated N2O emissions and N2+N2O emissions did not differ significantly between maize and cup plant soils, but tended to be higher in maize soil. Soils from both systems exhibited relatively high and similar N2O/(N2+N2O) ratios (N2Oi). N2O emissions originating from sources other than the 15N-labelled NO3 pool were low, but were the only fluxes exhibiting a significant difference between the maize and cup plant soils. Missing differences in fluxes derived from the 15N pool indicate that under the experimental conditions with high moisture and NO3 level, and without plants, the cropping system had little effect on N fluxes related to denitrification. Lower soil pH and higher bulk density in the cup plant soil are likely to have reduced the mitigation potential of perennial biomass cropping.

KEYWORDS
15N gas flux method, biomass cropping, cup plant, emissions, incubation, maize, nitrogen, nitrous oxide

1 | INTRODUCTION

Methane produced by biomass fermentation is a valuable renewable decentralized energy source that, in contrast to wind and solar energy production, is capable of providing base load power. In Germany, the acreage of maize as a feedstock for biogas plants has increased substantially since 2000 (EUROSTAT, 2020). This increase has led to concerns...
Regarding the environmental constraints related to this renewable energy source, namely resultant soil compaction, reduced biodiversity and loss of soil organic matter (Jacobs et al., 2017; Ruf & Emmerling, 2018; Schorpp & Schrader, 2016).

Perennial biomass crops offer potential for sustainable intensification of biomass production due to the versatility of ecosystem services (Emmerling, 2014; Gardiner et al., 2010; Yang et al., 2018). Several new perennial crops have been suggested as alternatives to maize (Z. mays; Franzaring et al., 2015; Schmidt et al., 2018), one of which is the yellow-flowering cup plant (S. perfoliatum), which is currently grown on approximately 3000 ha in Germany (FNR, 2020). Cup plant stands are used for up to 15 years and receive no tillage during that time (Gansberger et al., 2015). Cup plant is exposed to the same potential of soil compaction due to multiple passing during the application of biogas digestate and harvest operations as maize, since the same heavy equipment is used in both crops. In contrast to annual maize soil compaction in cup plant is not mitigated by frequent tillage. Hence, less disturbed soils tend to have a higher bulk density, higher surface organic matter content and a more structured pore size distribution compared with conventionally tilled soils (Mangalassery et al., 2013; Palm et al., 2014). Furthermore, belowground biomass, including roots and soil biota, is increased under perennial biomass cropping due to less disturbance (Don et al., 2012; Emmerling, 2014; Schorpp & Schrader, 2016).

The greenhouse gas (GHG) balance of biogas production is strongly affected by carbon dioxide (CO₂) and nitrous oxide (N₂O) emissions during feedstock cultivation (Crutzen et al., 2016), thus GHG emissions need to be considered when evaluating the sustainability of a cropping system (European Parliament, Council of the European Union, 2018). Emissions of CO₂ and especially N₂O from biomass production are variable since local climate and soil properties, the cultivated crop and management intensity have an impact on field emissions (Don et al., 2012; Peyrard et al., 2017).

Nitrous oxide in soil is produced by microbial processes, predominantly nitrification and denitrification. Soil structure and aeration are key drivers controlling microbial activity (Ball, 2013). No-till soils that tend to have a higher bulk density reach higher water-filled pore space (WFPS) values than tilled soils due to reduced total porosity (Palm et al., 2014). Consequently, episodic anaerobic conditions are more frequent in no-till systems favouring denitrification. This increases the potential for N₂O losses from denitrification especially in poorly drained and fine-textured soils (Rochette, 2008). However, reduced oxygen (O₂) availability due to reduced gas diffusivity has also been reported to favour N₂O reduction to dinitrogen (N₂) and thus lower the N₂O/(N₂+N₂O) product ratio (N₂Oi) of denitrification (Müller & Clough, 2014), decreasing the share of N₂O from denitrification.

Soil pH is also a controlling factor in multiple soil microbial processes and nitrogen (N) turnover (Kunhikrishnan et al., 2016). Nitrification activity is reduced with decreasing pH, but the net effect of pH on potential denitrification is still uncertain (Parkin et al., 1985; Qu et al., 2014; Šimek & Cooper, 2002). However, N₂Oi is shifted towards more N₂O at low pH (Šimek & Cooper, 2002). Soil pH in perennial land use systems is lower than in crops with a high return margin due to less frequent liming (Goulding, 2016). Thus, differences in nitrification and denitrification can be expected between fields used for annual versus perennial crop cultivation.

Carbon (C) and N substrate availability and their interaction with soil biota need to be considered for the assessment of soil-borne GHG emissions of cup plant cropping in comparison with silage maize. In contrast to maize, cup plant produces more litter during its late reproductive growth due to the shedding of senescent leaves (Gansberger et al., 2015). Litter serves as an important labile C source for microbial processes, that is, N turnover and denitrification. Carbon availability to soil microbiota is further increased through litter incorporation by earthworms, which are more abundant in perennial systems (Emmerling, 2014). Furthermore, earthworms are known to be able to contribute substantially to soil-borne N₂O emissions due to denitrification in the earthworm gut (Giannopoulos et al., 2011; Lubbers et al., 2013; Schorpp et al., 2016). Compared with conventional cropping, perennial cropping systems under no-till management allocate more C and potentially mineralizable N to soil organic matter (Gauder et al., 2016) through more belowground biomass and litter input (Don et al., 2012; Luo et al., 2010) and less mineralization of organic matter due to the absence of annual soil disturbance (Neugswandtner et al., 2014; Pugesgaard et al., 2015). Few studies to date have directly compared the differences in N₂ and N₂O formation in soils of annual and perennial biomass cropping systems. Emissions of GHG from annual and perennial biomass cropping systems have only been studied in the field or with incubated disturbed soil and under different N rates. These studies have focused on maize, miscanthus (M. giganteus and M. lutarioriparius) and short-rotation coppices (Gauder et al., 2012; Mi et al., 2018). However, they did not investigate source-specific N₂ and N₂O fluxes: a requirement for fully understanding the processes involved. Source and emission partitioning is very challenging and causes large uncertainties in field studies (Zaman et al., 2021), making it preferrable for such studies to be conducted in the laboratory. Therefore, in this study, a microcosm experiment was conducted with undisturbed soil cores using the ¹⁵^N gas flux method (¹⁵NGF) to quantify N₂ fluxes and source-specific N₂O fluxes from soil under two different cropping systems in controlled laboratory conditions.
It was expected that soil derived \(\text{N}_2\) and \(\text{N}_2\text{O}\) emissions from the perennial cup plant cropping system would differ from the emissions of the annual maize system due to the impact of tillage on soil structure, fertilizer application and liming intensity. Thus, the specific hypotheses for this study were: (1.a) the undisturbed cup plant soil emits a higher fraction of total denitrification products as \(\text{N}_2\) (lower \(\text{N}_2\text{O}\)) because of a longer residence time due to reduced gas diffusivity and greater availability of labile C; (1.b) the \(\text{N}_2\text{O}\) from cup plant soil is lower because of the impact of conditions favouring \(\text{N}_2\text{O}\) reduction, that is, reduced gas diffusivity and more labile C have a greater impact than lower pH due to less intensive management; (1.c) \(\text{N}_2\text{O}\) emissions from sources other than denitrification are higher in maize because conditions for nitrification are more favourable due to better aeration/diffusivity, a narrower C:N ratio and higher pH; (2.a) there is only a small litter effect on \(\text{N}_2\) + \(\text{N}_2\text{O}\) emissions without earthworms due to the supply of labile C near the soil surface only; and (2.b) a treatment with litter and earthworms strongly enhances \(\text{N}_2\text{O}\) emissions due to incorporation of litter and denitrification activities in the earthworm gut.

## 2 | MATERIALS AND METHODS

### 2.1 | Soil selection, sampling of soil cores

Undisturbed soil cores were taken in fall 2019 from the inter-row area of one maize (\(Z.\ mays\)) field and one cup plant (\(S.\ perfoliatum\)) field in Gronig (49.31°N, 7.07°E) in western Germany. The soils are referred to below as maize soil and cup plant soil. Both sampling sites were close to one other (-80 m apart). The cup plant at the sampling site was established in 2016, while the maize followed winter barley with a subsequent winter cover crop mixture. Management history of the fields is provided in Table S2. The soil type is a Hypereutric Stagnic Cambisol (IUSS Working Group WRB, 2015) and texture in the upper 20 cm is silty loam (Table 1). The sites are showing stagnic soil properties, characterized by temporal waterlogging and prone to soil compaction, and due to the slope (7° south-east) of the fields, also prone to erosion. Undisturbed soil monoliths 20 cm high were taken using a motor-hammered auger in Plexiglas cylinders with an inner diameter of 14.4 cm and height of 30 cm.

To enable homogeneous isotopic labelling and create two distinguishable WFPS levels, the sampled cores were air dried by leaving the open cores in the greenhouse for 18 days. The cores were subsequently defaunated at 60°C for 24 h to eliminate earthworms in the columns. Pre-tests showed that defaunation at 60°C disturbed soil structure less than freezing. Corresponding to the lower WFPS treatment of 67.5%, the soil had to be dried to at least 16.6% gravimetric water content (w/w) for homogeneous labelling. Only limited precision of established WFPS levels could be achieved due to heterogeneous bulk densities (Table S1). To determine the effect of the defaunation procedure on GHG emission, four replicates of each soil were not defaunated. Prior to the start of the incubation experiment, the soil cores were pre-incubated for 6 days at 15°C without moistening the soil. Rewetting was not possible for pre-incubation because irrigation at the start of the incubation experiment was required to apply \(^{15}\text{N}\) nitrate homogeneously.

### 2.2 | Incubation setup and treatments

The incubation vessels consisted of a Plexiglass cylinder with an inner diameter of 14.4 cm holding the soil core, an irrigation lid on top and a base plate. Flat rubber seals were used to make the vessels airtight. The lid contained an inlet and an outlet to channel a synthetic gas mixture through the 1670 ml headspace of the column. The gas mixture contained 20% \(\text{O}_2\), 2.7% \(\text{N}_2\), 77% \(\text{He}\), 350 ppm \(\text{CO}_2\) and 250 ppb \(\text{N}_2\text{O}\), and the flow rate was set to 11 ml min\(^{-1}\). The low background \(\text{N}_2\) in the gas mixture improved the sensitivity of the isotope ratio mass spectrometry (IRMS) measurements (Lewicka-Szczebak et al., 2017), while \(\text{CO}_2\) and \(\text{N}_2\text{O}\) were added to maintain approximately atmospheric levels. Flow rate was measured automatically bi-hourly by a flow metre. Besides flow and GC measurements, the automated system also controlled the irrigation and temperature (Hantschel et al., 1994). The experiment was performed under constant moisture (67.5% and 82.5% WFPS) and temperature (15°C) conditions.

### Table 1 | Soil characteristics at the maize and cup plant sampling site in September 2019; mean ± SD (n ≥ 3)

| Soil texture (mass %) | Maize | Cup plant |
|----------------------|-------|-----------|
| Sand                 | 22.79 ± 0.34 | 17.71 ± 0.43 |
| 2000–630 µm          | 2.72 ± 0.24  | 2.58 ± 0.37  |
| 630–200 µm           | 6.14 ± 0.17  | 5.95 ± 0.12  |
| 200–63 µm            | 13.92 ± 0.17 | 9.18 ± 0.46  |
| Silt                 | 56.49 ± 0.56 | 59.07 ± 1.41 |
| 63–20 µm             | 20.71 ± 0.12 | 19.68 ± 0.18 |
| 20–6.3 µm            | 20.69 ± 0.47 | 23.16 ± 0.18 |
| 6.3–2 µm             | 15.08 ± 0.35 | 16.23 ± 1.21 |
| Clay <2 µm           | 22.79 ± 0.34 | 17.71 ± 0.43 |
| NO\(_3\)-N (mg N kg\(^{-1}\)) | 33.46 ± 6.13 | 2.38 ± 1.01 |
| NH\(_4\)-N (mg N kg\(^{-1}\)) | 12.89 ± 1.02 | 5.43 ± 3.75 |
| SOC (g C kg\(^{-1}\)) | 17.44 ± 2.18 | 16.77 ± 1.34 |
| pH                   | 5.62 ± 0.09  | 5.04 ± 0.10  |
The incubation was conducted in the absence of living plants to exclude rhizosphere effects as far as possible. The inter-row area covered >50% of the acreage in both row crops. The conditions in the mesocosms were intended to mimic the situation early in the vegetation period or in the advanced reproductive growth stages in autumn after harvest, when plant–soil interactions are less pronounced. Soil conditions comparable with those in this experiment, especially WFPS levels, occur between October and April at the sampling sites.

Differences in soil nitrate content between the two soils were removed by adding KNO₃ to reach a target level (83 mg kg⁻¹) equivalent to 230 kg NO₃-N ha⁻¹. All columns were fertilized to the same N level. Nitrate addition was calculated and applied per surface area to account for the differing bulk densities in the two soils. The optimal irrigation scheme had been tested in Br⁻ percolation pre-tests to achieve homogeneous labelling as far as possible and create distinguishable WFPS levels. According to the pre-tests, a minimum of 315 ml was required to achieve these aims. The stabilizing effect of Ca²⁺ was used to prevent excessive particle dispersion (Klute & Dirksen, 1986). Additional water for establishing the different WFPS levels was added in a second irrigation event.

The treatments were without earthworms and litter (Bare Soil), litter without earthworms (Litter), litter with earthworms (Worm+Litter) and the non-defaunated soil cores (Control). The control treatment was setup only in combination with the high WFPS level. All other treatments were fully crossed with the two WFPS levels (67.5% and 82.5%) and the two soils. Therefore, the experiment consisted of a total of 14 treatments and 56 columns (n = 4; Table 2).

The initial litter on the surface of the columns was carefully removed before incubation. For treatments with litter amendment (Worm+Litter, Litter, Table 2), dried and chopped (2 cm) plant material from the sampling sites was added to the soil surface. Maize litter, which did not include cobs and stems, had a C:N ratio of 31 ± 1.6 (± here and hereafter is always standard deviation) and cup plant litter a C:N ratio of 50 ± 0.2. Two individuals of the species Lumbricus terrestris L. were introduced into columns of the Worm+Litter treatment. This species is commonly used as a model organism for deep-burrowing anectic earthworms. The amount of 6 g (DM) maize or cup plant material per column was applied to satisfy the food demand of the earthworms in both treatments containing litter. The earthworms were added immediately before irrigation. Additionally, the litter was moistened with 5 ml pure water to create a moist environment for the earthworms before irrigation started. In all cup plant treatments, the earthworms had to be replaced due to high mortality in the first few days. This was done without removing the burrowed dead earthworms. Measurements from these columns were discarded from the data analysis.

### 2.3 Soil and litter analysis after incubation

At the end of incubation, each column was sampled destructively. The soil columns were divided into 0–10 cm and 10–20 cm layers. To analyse soil mineral N (Nmin = NO₃⁻-N + NH₄⁺-N) content, 400 g of homogenized fresh soil was filled into a 1 l PE bottle. The large samples were used to reduce bias due to soil heterogeneity. Soil was stored at −20°C and thawed at 4°C overnight before extraction. Nmin was extracted with 2 M KCl solution (extraction ratio: 1:1.25 w/v) and shaking for 60 min using an overhead shaker. Subsequently, extraction solution was filtered (MN 614¼ filters, Macherey & Nagel) and stored at −20°C until analysis. Concentrations of NO₃⁻-N and NH₄⁺-N were analysed colorimetrically using a continuous flow analyser (SA 5000, Skalar Analytical B.V.). Then ¹⁵N enrichment of extractable NO₃⁻(¹⁵aNO₃⁻) was determined by analysing the Nmin extraction solution using chemical conversion of NO₃⁻ to N₂O and online analysis by mass spectrometer (GAM 200, InProcess Instruments, Bremen, Germany) coupled to a membrane inlet (Eschenbach et al., 2017, 2018). The 1:1.25 extraction ratio was compared with the standard ratio of 1:4 (n = 24). There was no significant difference in mean values and variance of NO₃⁻ and NH₄⁺ concentration between the extraction ratios. Soil water content was determined separately during destructive sampling of the soil cores directly after incubation. WFPS was calculated from bulk density, gravimetric water content, and an

| WFPS level | Control | Bare Soil | Litter | Worm+Litter |
|------------|---------|-----------|--------|-------------|
| Maize      |         |           |        |             |
| High       | x       | x         | x      | x           |
| Low        | n.e.    | x         | x      | x           |
| Cup plant  |         |           |        |             |
| High       | x       | x         | x      | x           |
| Low        | n.e.    | x         | x      | x           |

*The control was not treated to defaunation by heating.

*Not established.
assumed particle density of 2.65 g cm$^{-3}$. Soil pH was determined with a pH metre (FE20, Mettler Toledo) after shaking for 1 h in 0.01 M CaCl$_2$ (1:4 w/v). Total C and N content in soil were determined after drying at 40°C for 2 days with an elemental analyser (TruMac CN analyser, Leco).

Soil organic C (SOC) was determined indirectly by dry combustion as the difference of total C and total inorganic C.

Soil texture analysis were conducted by wet sieving (2000–63 µm) and the Köhn-pipette technique (<63 µm).

### 2.4 Gas analysis

Soil mesocosms were integrated into an automated incubation system (Hantschel et al., 1994; Säurich et al., 2019). Every incubation vessel was measured within a period of 6 h. Blanks for measuring background concentrations of the gas mixture and five standards for calibrations were regularly integrated into the measurement sequence. Gas samples and standards were analysed online with a Shimadzu GC-2014 (Shimadzu) equipped with a flame ionization detector (FID), electron capture detector (ECD) and thermal conductivity detector (TCD). The analytical precision was determined by repeated measurements of standards (0.33, 0.55, 2.01, 6.94, 40.4, 130 ppm N$_2$O) and was consistently <2% CV. The first days of incubation were not considered for flux calculations because irrigation after the dry pre-incubation and technical issues with the valve system led to unstable conditions. Therefore, only data after day 9 of the experiment, when stable conditions were reached, were evaluated. The period after day 9 is referred to below as the observation period.

### 2.5 15N labelling and 15N$_2$ and 15N$_2$O isotope analysis

To elucidate the N$_2$ and N$_2$O emission and related processes based on stable isotope ratios, each column was amended with 15N-labelled NO$_3^-$ with the aim of reaching a 15N enrichment of 60 at% in the soil after tracer addition.

Gas samples for 15N isotope analysis were taken by connecting two 12 ml Exetainers with rubber septa (Labco Ltd.) to the outlet flow of the columns. The Exetainers were flushed 1200 times (24 h) before being disconnected from the gas flow. After day 9 of the incubation experiment, samples from each column plus one blank were collected every 3 days.

Gas samples were analysed as described in Lewicka-Szczechak et al. (2013). Samples were processed by a modified GasBench II (Thermo Scientific) with online preparation and automated sampling (PAL Systems). Isotope mass ratios were determined with a connected triple collector IRMS (MAT 253, Thermo Scientific). During the isotope analysis in this setup, N$_2$O was reduced in a Cu oven to N$_2$. Nitrogen isotope mass ratios $^{29}$R ($^{29}$N/$^{28}$N$_2$) and $^{30}$R ($^{30}$N/$^{28}$N$_2$) from N$_2$, N$_2$+N$_2$O and N$_2$O were measured. The IRMS had an analytical precision of <7% CV for $^{30}$R and of <0.01% CV for $^{29}$R, which is equivalent to a standard deviation of <1e-6 for both ratios.

### 2.6 Calculations and statistics

CO$_2$ and N$_2$O fluxes were calculated from GC and airflow data as mass flow per area and time. Cumulated fluxes were calculated as the integral of the time series from day 9 to day 27 after linear interpolation. Further analyses were all conducted with cumulated CO$_2$ and N$_2$O fluxes.

Soil-gas diffusivity was calculated with a structure-dependent water-induced linear reduction model (SWLR) as described by Moldrup et al. (2013) based on bulk density, WFPS and a porous media complexity factor.

The characterization of the N$_2$ and N$_2$O fluxes and the 15N enrichment of the NO$_3^-$ pool undergoing denitrification (ap) was based on the assumptions of the non-random distribution of isotopocules in the gas samples (Hauk & Bouldin, 1961) caused by high enrichment of NO$_3^-$ in the labelled pool. The N$_2$ and N$_2$O fluxes were calculated using the formulas given by Spott et al. (2006) and Russow et al. (1996). The apN$_2$, apN$_2$+N$_2$O, apN$_2$O and fractions of gas species originating from the labelled pool (fp) were quantified for N$_2$ (fp$_{N_2}$), N$_2$+N$_2$O (fp$_{N_2+N_2O}$) and N$_2$O (fp$_{N_2O}$; see Supporting Information). Based on the initial and final NO$_3$ content and its 15N enrichment (15aNO$_3$), both net nitrification and, through pool dilution, gross nitrification were calculated (Deppe et al., 2017; Hart et al., 2014). Furthermore, N$_2$O emissions that did not originate from the labelled pool (fn$_{N_2O}$, see Supporting Information) divided by gross nitrification gave an estimate of N$_2$O formation from nitrification, that is, N$_2$O yield of nitrification.

Cumulated fluxes were calculated by linear interpolation using fp$_{N_2}$+N$_2$O, fp$_{N_2}$ and fp$_{N_2}$O as well as fn$_{N_2O}$. If measurements were below the IRMS detection limit, concentrations were imputed as half-detection limit. This imputation had only a negligible impact on the resulting cumulated fluxes since the time series also contained fluxes that were higher by several orders of magnitude. Furthermore, the fraction of N$_2$O originating from the labelled pool at total N$_2$O (N$_2$O$_t$) in the sample (fp$_{N_2O}$ = fp$_{N_2O}$/N$_2$O$_t$) and the product ratio of denitrification [N$_2$Oi = fp$_{N_2O}$/fp$_{N_2+N_2O}$] were calculated from each sample as well as from cumulated fluxes.

R version 3.6.2 (R Core Team, 2019) was used for all statistical analyses. Cumulated emissions of the cropping systems and treatments were analysed by comparing the means with analysis of variance (ANOVA). When assumptions of normality were violated, that is, ratios (N2Oi, Fp$_{N2O}$), generalized linear models were fitted using a quasibinomial
distribution family and logit link function. In the case of time series, generalized least squares models were fitted with the R package nlme (Pinheiro et al., 2020) to account for autocorrelation. All gaseous N fluxes were log10-transformed to remove variance heterogeneity, whereas transformation of CO$_2$ data was not necessary. The Control treatment (not defaunated) was omitted from further analyses after comparison with the Bare Soil treatment. Moreover, only Worm+Litter treatments where both earthworms had survived and no earthworms had been replaced were taken into account. Hence, only three of four Worm+Litter maize columns at low WFPS provided valid results, which appeared not to be sufficiently robust for conclusions to be drawn on the effect of the Worm+Litter treatments. Hence, for most tests the Worm+Litter treatments were not considered.

3 | RESULTS

3.1 | Soil parameters

There was no significant difference in mean WFPS between the soils at the low level (Table S1), however, mean WFPS in cup plant soil at the high WFPS level was higher than in maize soil ($p < 0.01$). At the high WFPS level, the differing WFPS corresponded to the difference in bulk density affecting the pore size distribution of both cropping systems, causing 3.8 vol.% more pore space in maize soil ($<50 \mu$m diameter, Figure S1). Soil-gas diffusivity ($D_p/D_a$) at low WFPS was $0.055 \pm 0.018$ and $0.070 \pm 0.033$ in cup plant and maize soil respectively. Whereas at high WFPS it was significantly ($p < 0.001$) reduced in cup plant soil with $0.009 \pm 0.005$ compared to maize soil with $0.019 \pm 0.008$: The gas diffusivity in cup plant soil was $52.6 \pm 73.4\%$ and $20.5 \pm 57.6\%$ lower than in maize soil at the high and low WFPS level respectively. The soil had therefore a significant ($p = 0.001$) effect on gas diffusivity.

Similar to the physical properties, soil chemical parameters also varied substantially within and between the cropping systems. The pH in maize soil was higher than in the cup plant soil ($p < 0.001$, Table 1) but was constant over soil depth in both soils. In contrast, SOC, total N as well as the C:N ratio did not differ between the soils. N$_{\text{min}}$ content increased during the incubation. This increase was greater in low WFPS treatments. At low WFPS, NO$_3^-$ N content increased from the initial 83.7 mg N kg$^{-1}$ DM on average by 44.6% (+37.39 mg N kg$^{-1}$ DM) in maize soil and by 11.2% (+9.33 mg N kg$^{-1}$ DM) in cup plant soil. The increase at high WFPS was less pronounced or non-existent, that is, 22.3% (+18.7 mg N kg$^{-1}$ DM) and −3.1% (−2.6 mg N kg$^{-1}$ DM) in maize and cup plant soil respectively. Nitrate content in both soils was higher in the upper soil ($p < 0.01$), except in cup plant columns with a low WFPS level. Total NH$_4^+$-N content in maize decreased from the initial content at the start of incubation (11.55 ± 1.02 mg N kg$^{-1}$ DM) to the end of incubation (3.90 ± 3.15 mg N kg$^{-1}$ DM). Whereas in cup plant, total NH$_4^+$-N content only tended to increase between the start (4.80 ± 3.31 mg N kg$^{-1}$ DM) and end of incubation (7.08 ± 5.07 mg N kg$^{-1}$ DM). Furthermore, the variability in NH$_4^+$ content was higher in the lower soil layer (10–20 cm) across the WFPS levels, apart from maize columns with low WFPS (Table 3). Except in the treatments in which the earthworms died, the addition of worms and/or litter had no effect on N$_{\text{min}}$ content.

3.2 | CO$_2$ fluxes

The non-defaunated Control had mean cumulated CO$_2$ fluxes of 19.5 ± 2.8 and 31.2 ± 5.3 mg C m$^{-2}$ h$^{-1}$ in maize and cup plant soil, respectively, which was significantly lower than the fluxes of the Bare Soil treatments with 31.5 ± 5.1 and 43.3 ± 8.8 mg C m$^{-2}$ h$^{-1}$ respectively (Table 4). Hence, the heat treatment increased the mean cumulated CO$_2$ flux by 38.1 ± 18.5% and 27.9 ± 23.7% in maize and cup plant relative to the non-defaunated Control.

In the Worm+Litter treatment in maize and the Litter treatments in both soils, there was a trend of continuously decreasing CO$_2$ fluxes, while fluxes in the Bare Soil treatments decreased only slightly (Figure S4). The decrease in CO$_2$ fluxes in maize was more pronounced and steadier than in cup plant soil. Furthermore, WFPS had no effect on CO$_2$ flux.

The post hoc test showed no difference ($p = 0.07$) between CO$_2$ fluxes of the Bare Soil treatments of both soils.

### Table 3

| Soil        | WFPS | SOC:N | NO$_3^-$N (mg N kg$^{-1}$) | NH$_4^+$N (mg N kg$^{-1}$) |
|-------------|------|-------|---------------------------|---------------------------|
|             |      |       | 0–10 cm                  | 10–20 cm                  | 0–10 cm                  | 10–20 cm                  |
| Maize       | High | 8.8 ± 0.2 | 119.4 ± 13.0           | 85.5 ± 14.4              | 2.9 ± 0.9               | 9.3 ± 7.8               |
|             | Low  |        | 151.6 ± 28.4           | 90.7 ± 9.0               | 2.8 ± 0.8               | 1.8 ± 0.3               |
| Cup plant   | High | 8.9 ± 0.1 | 86.5 ± 16.6            | 75.6 ± 12.6              | 3.4 ± 2.7               | 12.1 ± 9.9              |
|             | Low  |        | 93.9 ± 6.4             | 92.1 ± 5.9               | 3.9 ± 1.9               | 8.93 ± 7.3              |
However, soil ($p < 0.001$) and WFPS ($p < 0.3$) had interacting ($p < 0.01$) effects on CO2 emissions. Treatments with litter on the soil surface had significantly higher CO2 emissions than Bare Soil. Therefore, cumulated CO2 fluxes in the Litter treatments were 157.4% (+49.6 ± 6.8 mg C m$^{-2}$ h$^{-1}$) and 138.9% (+56.6 ± 12.6 mg C m$^{-2}$ h$^{-1}$) higher for maize and cup plant soils, respectively, compared with the Bare Soil treatments. This represents a litter-induced CO2 flux of 0.14 ± 0.01 mg C g litter m$^{-2}$ h$^{-1}$ for maize and cup plant litter respectively. The addition of earthworms plus litter in maize at low WFPS increased the cumulated CO2 flux by 216.8% (+68.2 ± 4.1 mg C m$^{-2}$ h$^{-1}$) compared with Bare Soil, and was therefore comparable to the litter-induced effects of treatments in maize and cup plant.

### 3.3 | N2O emissions

The comparison of mean cumulated N2O fluxes in cup plant between the non-defaunated Control and the Bare Soil revealed no significant difference.

While N2O fluxes in maize decreased initially and were approaching a constant level at the end of observation period, N2O fluxes in cup plant were relatively stable from the beginning (Figure S6). This was more apparent in the high WFPS maize treatments, but was also observed in the Litter and Bare Soil treatments at low WFPS. The Worm+Litter treatment in maize with low WFPS ($n = 3$) showed fluctuating fluxes, with a small increase during the first day of the observation period (Figure S6). The variance within the treatments was relatively constant throughout the observation period except in the maize soil Litter treatment, where at low WFPS, the variance declined over time.

Cumulated N2O fluxes were 15 and 53 times higher at high WFPS than at low WFPS in maize and cup plant soils respectively (Table 4). Bare Soil treatments tended to have the lowest emissions at low WFPS, while Bare Soil treatments at high WFPS tended to have higher emissions than the Litter treatments.

No significant effect ($p < 0.2$) on the cumulated N2O flux due to the addition of litter (and earthworms) could be observed within one soil WFPS levels compared to the bare soil (litter effect, Table 5). However, for the Worm+Litter treatment in maize at low WFPS, cumulated fluxes had a tendency ($p < 0.5$) to be higher (+177.6%, +87.0 ± 78.7 µg N m$^{-2}$ h$^{-1}$) than in Bare Soil. In the Litter treatment in maize at low WFPS, cumulated fluxes had a tendency to be higher (+88.6%, +43.4 ± 109.9 µg N m$^{-2}$ h$^{-1}$) than.
those in Bare Soil. Conversely, mean cumulated flux in the Litter treatment at high WFPS in maize was lower (−20.2%, −304.2 ± 996.5 µg N m⁻² h⁻¹). In cup plant soil at low WFPS, mean cumulated flux in the Litter treatment was higher (+372.5%, +21.0 ± 20.5 µg N m⁻² h⁻¹) than the Bare Soil treatment, whereas at high WFPS, mean cumulated flux in the Litter treatment was lower (−347.3%, −919.9 ± 828.2 µg N m⁻² h⁻¹) than in Bare Soil. These tendencies were inconsistent and insignificant (p > 0.4), therefore litter-induced N₂O emissions could not be quantified.

3.4 N₂ and N₂O emissions from the ¹⁵N-labelled pool

All columns at the high WFPS level exhibited detectable pool-derived N₂, N₂+N₂O and N₂O fluxes (fp_N₂, fp_N₂+N₂O and fp_N₂O respectively), except for one maize Bare Soil column and one cup plant Bare Soil column at low WFPS, where fluxes were sometimes below detection.

Defaunation had no consistent effect on fp_N₂, fp_N₂+N₂O, fp_N₂O or fn_N2O fluxes.

Similar to the total N₂O (N₂Ot) flux from the GC measurements, fp_N₂O decreased or remained at the same level during the observation period in both soils (Figure 1). The fp_N₂ fluxes remained at the same level in the low WFPS treatments or increased in the high WFPS treatments during the observation period in both soils, with this increase being more pronounced in cup plant soil than in maize soil (Figure 1).

Mean cumulated fp_N₂ flux was on average more than twice as high in maize soil than in cup plant soil (Table 6), but the associated standard deviation was substantially higher and the difference was therefore not significant. The same applied to fp_N₂O fluxes and consequently also to the fp_N₂+N₂O fluxes (Table 6). In each of the maize treatments, fn_N₂O fluxes were higher than in treatments with cup plant soil (p < 0.0001). However, the contribution of fn_N₂O to total emission in the high emitting treatments (high WFPS) was very low (<7%).

Nevertheless, N₂ and N₂O fluxes differed significantly between the two WFPS levels in both soils, except for the Litter treatment of cup plant soil.

N₂O fluxes from non-labelled N sources were consistently lower in the Litter treatments regardless of WFPS level, except in maize at a low WFPS. Furthermore, fn_N2O correlated positively with mineralized N at high WFPS in both soils (R = 0.59, p < 0.05).

![Figure 1](image_url)
The mean contribution of N\textsubscript{2}O derived from the 15N-labelled pool to the total N\textsubscript{2}O flux (Fp\_N\textsubscript{2}O) from both soils was higher (p < 0.0001) at high WFPS level (mean Fp\_N\textsubscript{2}O = 0.96 ± 0.04) than in the low WFPS treatments for both soils (mean Fp\_N\textsubscript{2}O = 0.56 ± 0.25; Table S5), but no significant differences were observable between maize and cup plant soil. A tendency for increasing Fp\_N\textsubscript{2}O was only observed when litter and earthworms were added, however, it was not significant (p > 0.5).

Furthermore, soil and treatment had no significant effect on the ratio between fp\_N\textsubscript{2}O and fp\_N\textsubscript{2}O+N\textsubscript{2}O (N\textsubscript{2}Oi), although fp\_N\textsubscript{2} increased more than fp\_N\textsubscript{2}O due to the addition of litter and/or earthworms (Figure 1). Therefore, slightly lower N\textsubscript{2}Oi values were observed in the Litter and Worm+Litter treatments compared with the Bare Soil. At low WFPS the N\textsubscript{2}Oi was significantly lower than in high WFPS treatments, thus WFPS levels were the only influential effect on N\textsubscript{2}Oi (p < 0.01). Over the observation period, N\textsubscript{2}Oi decreased in maize treatments at high WFPS by 0.01029 day\textsuperscript{-1} and in cup plant treatments by 0.02057 day\textsuperscript{-1} (Figure 2). N\textsubscript{2}Oi was more variable at low WFPS, but there were also decreasing tendencies over time. Overall, the decrease in N\textsubscript{2}Oi was more pronounced in cup plant soil. This coincided with a greater decrease in N\textsubscript{2}O fluxes and a higher increase in N\textsubscript{2} fluxes in cup plant soil during the observation period.

The 15N enrichments of the active nitrate N pool producing N\textsubscript{2} and N\textsubscript{2}O (apN\textsubscript{2} and apN\textsubscript{2}O respectively) at the beginning of the observation period tended to be higher in cup plant soil than in maize soil (Figure 3). Moreover, ap values tended to be higher in the high WFPS treatments. A slight linear decline in ap values could be observed at high WFPS, whereas ap values fluctuated more at low WFPS in

|               | High WFPS | Low WFPS |               | High WFPS | Low WFPS |
|---------------|-----------|----------|---------------|-----------|----------|
|               | Maize     | Cup plant| Maize         | Cup plant |
| Bare Soil     |           |          |               |           |          |
| fp\_N\textsubscript{2} | 653.3 ± 331.3 | 521.1 ± 361.6 | 16.3 ± 25.2 | 1.8 ± 0.9 |
| fp\_N\textsubscript{2}O | 1701.2 ± 550.8 | 1135.1 ± 790.7 | 35.4 ± 61.7 | 1.8 ± 1.0 |
| fp\_N\textsubscript{2}+N\textsubscript{2}O | 2354.4 ± 798.5 | 1656.4 ± 1097.7 | 51.7 ± 86.9 | 3.6 ± 1.7 |
| fn\_N\textsubscript{2}O | 112.3 ± 68.3 | 5.7 ± 3.0 | 13.0 ± 2.3 | 3.2 ± 1.5 |
| Litter        |           |          |               |           |          |
| fp\_N\textsubscript{2} | 651.3 ± 345.4 | 122.0 ± 17.5 | 52.0 ± 25.5 | 41.5 ± 31.8 |
| fp\_N\textsubscript{2}O | 1456.3 ± 729.6 | 252.1 ± 120.0 | 62.8 ± 80.5 | 22.4 ± 20.4 |
| fp\_N\textsubscript{2}+N\textsubscript{2}O | 2107.6 ± 1041.2 | 374.0 ± 103.1 | 114.5 ± 92.9 | 64.4 ± 43.2 |
| fn\_N\textsubscript{2}O | 86.3 ± 55.1 | 2.6 ± 1.1 | 25.1 ± 11.8 | 2.7 ± 0.4 |
| Worm+Litter   |           |          |               |           |          |
| fp\_N\textsubscript{2} | —         | —        | 132.2 ± 73.1  | —         |
| fp\_N\textsubscript{2}O | —         | —        | 94.4 ± 30.9  | —         |
| fp\_N\textsubscript{2}+N\textsubscript{2}O | —         | —        | 226.6 ± 86.8 | —         |
| fn\_N\textsubscript{2}O | —         | —        | 30.5 ± 8.6  | —         |
both soils (Figure 3). There were significant differences in apN2O values between the soils at high WFPS (p < 0.001) and at low WFPS (p < 0.001), while differences in apN2 were only significant at high WFPS (p < 0.05). In contrast, the 15N enrichment of the extracted NO3− (15aNO3) clearly differed between the soils, regardless of WFPS level. Maize soil had a mean nitrate pool enrichment of 37.2 ± 2.2 at%, which was lower than the 42.1 ± 2.6 at% in the cup plant soil. In maize soil, 15aNO3 did not differ (p < 0.2) with depth. However, enrichment in the lower soil layer in cup plant columns was higher (p < 0.001) than in 0–10 cm soil depth, regardless of WFPS level. Differences in 15aNO3 between the WFPS levels were less pronounced than in ap values. Mean apN2 and apN2O values were higher (p < 0.01) than final 15aNO3 in the extractant of the high WFPS treatments.

4 | DISCUSSION

4.1 | Comparison with field and laboratory studies

Relatively high N2O emissions can be expected from fine-textured gleysols with high Nmin content in which high moisture conditions are frequent and soil aeration is thus reduced (Rochette, 2008). Consequently, the present study’s incubation with undisturbed gleysic soil cores under moist to wet conditions exhibited relatively high N2O emissions, which is in agreement with other observations (Gauder et al., 2012). However, the soil moisture and relatively high NO3− content in the columns in the present study presumably led to a substantially higher N2Oi compared with the observation from maize soil made by Buchen et al. (2016).

For example, the cumulated N2O fluxes from maize soil at high WFPS (~1800 µg N m−2 h−1) in this study were slightly lower than the cumulated fluxes (~2900 µg N m−2 h−1) observed by Rummel et al. (2020) in a soil incubation study of disturbed soil with a comparable texture, N rate and incorporated maize material. Gauder et al. (2012) observed mean cumulated fluxes of 41 µg N m−2 h−1 over 1 year in maize fertilized with 240 kg N ha−1, which is more in the range of the low WFPS treatments (49–136 µg m−2 h−1) in the present study. Furthermore, the emitted ratio of N2O to N2+N2O coming from denitrification (N2Oi) was more than 10 times higher (0.58 ± 0.17) than the N2Oi reported by Buchen et al. (2016; 0.02 ± 0.01) under field conditions on a histic gleysol with high organic matter content.

4.2 | Factors controlling CO2, N2 and N2O emissions and N cycling

4.2.1 | Treatment effects

Incorporation of labile C sources such as litter is known to increase CO2 and N2O emissions substantially (Köbke et al., 2018; Senbayram et al., 2012), as observed in the CO2 results of both maize and cup plant soils: CO2 fluxes increased in the order Worm+Litter > Litter > Bare Soil. No similar pattern was observed with N2 and N2O fluxes. Dry litter material with a high C:N ratio remaining on the soil surface is reported to cause fewer N2O emissions than incorporated litter (De Ruijter et al., 2010; Giannopoulos et al., 2011; Huang et al., 2004). This is consistent with the observation here, where no significant litter-induced N2O emissions were observed after surficial addition of plant material with a C:N ratio above 30.

The >50% mortality of the earthworms was substantially higher than that reported in other incubation studies (Giannopoulos et al., 2011; Schorpp et al., 2016). Apparently, commercially grown earthworms, which are used to optimized substrate, struggle with the harsh soil conditions in sampled soil cores (Lowe & Butt, 2007). The Worm+Litter
treatments were omitted from further analyses and discussion. Thus, earthworm effect could not be evaluated and litter addition had only a negligible effect on N₂ and N₂O emissions.

4.2.2 | Interaction of soil structure and water content on CO₂, N₂O and N₂ emissions and the N₂Oi

Soil structure is an important factor for GHG emissions through its influence on gas diffusion (Petersen et al., 2008; Schlueter et al., 2018; van der Weerden et al., 2012). As reviewed in Bronick and Lal (2005), it is often assumed that one benefit of perennial cropping will be improved soil structure (i.e. aggregation, porosity and aeration), through less frequent disturbance. However, less frequent disturbance in perennial systems can also lead to higher bulk density, due to the absence of frequent loosening of compacted layers by tillage (Palm et al., 2014; Skaalsveen et al., 2019). In this experiment, bulk density did not differ significantly between sites (ρ = 0.051), but was slightly lower in maize soil (1.40 ± 0.04 g cm⁻³) than in cup plant soil (1.42 ± 0.03 g cm⁻³). However, the bulk density in conventionally tilled fields changes over time as visualized by Ellert and Bettany (1995); these soil cores were taken in autumn, as late as possible after tillage, which may explain the minimal difference. Furthermore, physical soil properties such as bulk density varied substantially within replicate soil columns of both soils (Table 1; Figure S1), which reflects spatial heterogeneity at the sampling sites (Ball et al., 2000; Dekker et al., 1999).

Although the difference in bulk density between the two soils was small, it caused significantly higher WFPS (ρ < 0.01) in cup plant soil (Table S1). WFPS, which depends on porosity and pore size distribution, is an important factor for microbial and physical processes such as denitrification and gas diffusion (Petersen et al., 2008; Schlueter et al., 2018; van der Weerden et al., 2012).

In this experiment, differences in fluxes and cumulated emissions of N₂ and N₂O occurred only between different WFPS levels (Table 7). Oxygen availability decreases with increasing WFPS level due to reduced gas diffusivity in the water-filled pore system, resulting in a non-linear denitrification response (Weier et al., 1993). Moreover, with decreased diffusivity, the residence time of N₂O increases, and therefore N₂O is more likely to be reduced to N₂ (Schlueter et al., 2019). Hence, according to the slightly higher bulk density and lower pore volume in cup plant soil, which is mainly caused by a lower fraction of pores with >0.2 µm diameter (Figure S1), a lower N₂Oi was expected in the high WFPS treatments. Interestingly, a higher N₂Oi (+0.23 ± 0.17 and +0.19 ± 0.24 higher N₂Oi in maize and...
cup plant soils respectively) at high WFPS was observed (Table S5). This suggests that in both systems, gross N₂O production exceeds consumption at high WFPS, probably due to high NO₃⁻ availability (Yin et al., 2020). Although WFPS was 79.5 ± 3.2% and 84 ± 3.3% in cup plant and maize soils, respectively, which is at the upper limit of the optimum range (70%–80% WFPS) for most soils for N₂O formation during denitrification (Butterbach-Bahl et al., 2013), the N₂Oi results implied that soil moisture in the high WFPS treatments was, in fact, not above optimum conditions for N₂O formation.

The expected impact of the perennial system on macro-scale pore structure is also closely related to the distribution of organic matter and is thus important for microenvironments in which the majority of biological processes in the soil are concentrated (Schlüter et al., 2018), that is, hotspots of denitrification around incorporated organic matter (Kravchenko et al., 2017; Parkin, 1987; Schlüter et al., 2019). The absence of tillage leads to a reduced gas diffusivity (Figure S2) and a patchy distribution of organic substrates: organic litter (dead roots) and input by bioturbation (Braakhekke et al., 2013; Christensen, 2001). This supports the assumption that the absence of frequent tillage (soil mixing) leads to a more spatially heterogeneous distribution in denitrification activity due to the patchy distribution of substrate and its interaction with soil structure (longer diffusion path of O₂ and N₂O). Indications of spatial heterogeneity of N₂ and N₂O production can be obtained by comparing the ¹⁵N enrichment of NO₃⁻ in bulk soil (¹⁵aNO₃) with the ¹⁵N enrichment of the NO₃⁻ pools undergoing denitrification (apN₂, apN₂O; Buchen et al., 2016; Deppe et al., 2017; Lewicka-Szczebak et al., 2013; Zaman et al., 2021). Spatially distinct distribution of denitrification is indicated when ap values and ¹⁵aNO₃ are distinguishable, that is, due to missing dilution by nitrification in the absence of O₂ in denitrifying hotspots. Comparing apN₂O and apN₂ in relation to ¹⁵NO₃ revealed differences between the two soils and their hotspots of N₂ and N₂O formation. While in maize soil the apN₂O and apN₂ did not differ significantly from one other (Figure 3), in cup plant soil at high WFPS apN₂O was significantly higher than apN₂, indicating distinguishable N₂O and N₂-producing microsites. In cup plant soil, the lower apN₂ suggests that these microsites had a lower ¹⁵NO₃⁻ availability than N₂O-forming microsites, indicating a spatial separation of these hotspots. Higher apN₂O than apN₂ may indicate that in cup plant soil, relatively isolated hotspots existed where O₂ became limiting due to increased microbial respiration during mineralization/nitrification (Zhu et al., 2015), providing additional unlabelled reduced N and more complete denitrification. While there may be other possible explanations, larger differences between apN₂ and apN₂O in cup plant soil clearly indicated more heterogeneity in N₂ and N₂O production than in maize soil.

### 4.2.3 Availability of labile C and N

Labile C sources and NO₃⁻ availability are also known to be important factors in controlling denitrification (Weier et al., 1993). In bulk soil, total content and distribution of these two substrates for denitrification can vary substantially between perennial or no-till systems and intensively managed annual cropping systems (Neugschwandtner et al., 2014; Palm et al., 2014; Yuan et al., 2018). Untilled soil from perennial systems commonly has a gradient in the content of these substrates that decreases with soil depth.

Apparent mineralization and nitrification rates were higher in maize soil while total amount of SOC and N did not differ between the soils. This is consistent with the fact that potentially mineralizable organic matter in no-till systems is protected from decomposition within aggregates (Six et al., 2002) while it is easier accessible in maize soil. READILY decomposable organic matter and better O₂ availability due to the higher soil-gas diffusivity in maize soil resulted therefore in more than 150% higher nitrification rates (gross and net, Table S4) than in cup plant soil. However, the gaseous N loss from maize was comparable with the emissions from the cup plant soil. Therefore, the balance of net nitrification and gaseous N losses in maize soil was positive, resulting in increased NO₃⁻ availability and a more pronounced ¹⁵N pool dilution in maize soil. In contrast, the balance of net nitrification and gaseous N losses in cup plant soil was balanced or even negative (i.e. −0.44 g N m⁻² in Litter at high WFPS, Table S4), indicating an apparent NO₃⁻ immobilization and therefore a possible limitation of NO₃⁻ availability for denitrification.

The more intensive N mineralization at high WFPS was positively correlated with the flux of N₂O from non-labelled sources (fn_N₂O), which is a potential risk for nitrification N losses of maize soil. However, in maize soil at high WFPS, the contribution of fn_N₂O to the total N₂O flux (N₂Ot) was relatively low (<7%). The share was much higher at low WFPS (up to 46%), but absolute emissions from these treatments were negligible. The N₂O yield from nitrification in maize soil at high WFPS was around 0.2 ± 0.1% and at low WFPS the N₂O yield from nitrification was 0.04 ± 0.03%, showing that the observed N₂O yields were in middle of the range of 0.01%–1.8% reported in the literature (Deppe et al., 2017; Nadeem et al., 2020). This indicates that nitrification was only a minor source of N₂O in these tested soils.

### 4.2.4 Contrasting effect of pH on N₂Oi

Another observed difference between maize and cup plant soil was soil pH. The different pH values correlated with the management intensities of the two cropping systems. Maize columns had a significantly higher pH (5.6 ± 0.1) than cup
plant columns (5.0 ± 0.1). Nitrification is heavily controlled by soil acidity (Norton & Ouyang, 2019). In contrast, potential denitrification is less clearly affected by soil pH (Liu et al., 2010; Qu et al., 2014). However, the N₂O/N₂+N₂O ratio (N₂Oi) is negatively correlated with soil pH due to post-transcriptional inhibition of the N₂O reductase and increased O₂ consumption due to increased microbial activity, which shifts the product stoichiometry towards more N₂O at higher pH (Liu et al., 2010; Nadeem et al., 2020; Senbayram et al., 2019). The pH range measured here was comparable with that in soils studied by Russenes et al. (2016), who observed a negative correlation between N₂Oi and increasing soil pH. No such correlation was found in this study. However, the difference in pH between the soils could have contributed to the fact that cup plant soil columns exhibited N₂Oi values similar to maize soil, even though their soil structure and reduced gas diffusivity (Schlüter et al., 2019), lower NO₃⁻ and labile C content would favour lower N₂Oi (Senbayram et al., 2012).

4.2.5 | Revisiting the hypotheses

Since N₂O and N₂ emissions and N₂Oi were not statistically different between maize and cup plant soils, but maize soil exhibited consistently higher tendencies of N₂ and N₂O emissions, the main working hypothesis that N₂O and N₂ emissions differ between the two cropping systems could not be proven conclusively. Furthermore, the absence of frequent physical soil disturbance and soil mixing in the perennial system did not result in predominant N₂ emissions from denitrification, and thus hypothesis 1a could not be accepted. However, an apparent separation of N₂ and N₂O-producing hotspots was evident in the cup plant soil, indicating a heterogeneous and patchy distribution of organic matter. This heterogeneous distribution of organic matter is most likely creating isolated hotspots of microbial activity, and thus causing favourable conditions for complete denitrification (Kinoshita et al., 2017; Sarker et al., 2018; Schlüter et al., 2019). However, this feature of the cup plant soil did not coincide with significantly lower N₂Oi, that is, predominant emissions of N₂ from denitrification.

A potential N₂O mitigation due to a reduced N₂Oi caused by conditions favouring N₂O reduction could not be observed and thus hypothesis 1b was not supported. The lower pH in the perennial cropping system potentially counteracted the effect favouring N₂O reduction to N₂ by reduced gas diffusivity due to soil structure (affecting N₂O and O₂) and the hotspot-forming patchy distribution of organic matter (locally increased O₂ consumption). Other counteracting factors besides soil pH are possible, that is, altered denitrifying community in the soil of these two very distinct managed cropping systems (Ai et al., 2017; Ouyang et al., 2018). Moreover, high variability, especially in N emissions and factors controlling denitrification, that is, substrate availability, bulk density and pH, could interact and thus interfere with the expected differences between the soils. Therefore, the negative effects of less intensive management observed in this experiment (low pH, relatively high NO₃⁻ content) for N₂O emissions appeared to outweigh the potential benefits of cup plant cropping on more complete denitrification.

However, better aeration and less protected organic matter were associated with higher N mineralization/nitrification in maize soil, resulting in a significantly higher NO₃⁻ availability favouring N₂O formation. This could result in an elevated N₂Oi in maize soil due to the preferred reduction of NO₃⁻ over N₂O (Senbayram et al., 2012; Weier et al., 1993). Although, substrate availability was higher in maize soil and N mineralization was more intensive and thus coincided with higher fn_N₂O fluxes, total soil-borne N₂O and N₂ emissions and the N₂Oi were not significantly different from cup plant soil. This indicates that the tested maize soil with higher substrate availability and better aeration is more prone to emit N₂O from sources other than denitrification, thus supporting hypothesis 1c. Overall this suggests that the effect of cup plant cropping on the soil did not provide significant potential for mitigation of GHG emissions from the field.

4.3 | Importance of crop management on N₂ and N₂O emissions and N cycling

This study excluded field processes such as NO₃⁻ uptake by plants, O₂ consumption by root respiration or supply of labile C by root exudation and root litter. These interact at field scale with parameters quantified here at laboratory scale, and would presumably result in significant differences between these two cropping systems. Field flux studies are needed to verify the transferability of results from this incubation experiment to field scale.

The three factors of substrate availability, soil structure/compaction and soil pH can be controlled by agronomic management such as tillage, crop rotation, fertilization and liming (Booth et al., 2005; Goulding, 2016; Habteselassie et al., 2006; Rochette, 2008). The use of cover crops, frequent liming, tillage and the application of organic and synthetic fertilizer are common and best agricultural practice in silage maize production. This management practice was manifested in the high N cycling activity and soil pH in this experiment. However, cup plant management is considered to be less intensive, mainly because only one annual fertilizer application, usually biogas digestate, is common. At the study sites, this less intensive management resulted in a lower pH, wider soil C:N ratio and reduced gas diffusivity, and also caused comparable GHG emissions to those of the maize system. Hence, the N₂O mitigation potential of perennial cropping is strongly influenced by the management and management history.
5 | CONCLUSION

Although we expected lower N\textsubscript{2}O and higher N\textsubscript{2} emissions from the perennial system, there were no significant differences in N\textsubscript{2} and N\textsubscript{2}O emissions or the product ratio of denitrification (N\textsubscript{2}O/N\textsubscript{2}) between undisturbed soil cores from the cup plant field or the reference (maize). Thus, the soil under the perennial biomass crop did not offer potential to mitigate N\textsubscript{2}O emissions under the tested conditions.

Soil sampled from a maize-cropping system provided more substrate for denitrification and had more active N cycling, whereas soil originating from a perennial cup plant cropping system was more susceptible to detrimental denitrification losses (N\textsubscript{2}O) because of slight acidification and reduced gas diffusivity that led to more anaerobic conditions. Observed differences between the two cropping systems were related to soil properties, that is, gas diffusivity, pH and N turnover, which could not be controlled in this experiment and were a result of management history. Therefore, measures should be taken to promote N\textsubscript{2}O reduction by preventing excessive acidification and frequently high NO\textsubscript{3}\textsuperscript{–} availability to reduce N\textsubscript{2}O emissions from cup plant cropping. Moreover, conditions which favour denitrification should generally be reduced since cup plant soil did not exhibit a lower N\textsubscript{2}O in order to optimize cup plant cropping as a climate-friendly alternative to maize through N\textsubscript{2}O mitigation. This could be achieved through an optimized liming and fertilization strategy and the prevention of soil compaction due to field operations. Furthermore, to verify the potential of perennial cup plant cropping to mitigate GHG, a complete life cycle assessment is necessary, accounting for all input and output variables in this production system.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open agrar at https://doi.org/10.3220/DATA2 02106301344418, reference number: https://doi.org/10.3220/ DATA202106301344418.

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