Received: 15 March 2021 | Revised: 26 May 2021 | Accepted: 24 June 2021

DOI: 10.1111/gcbb.12879

GCB-BIOENERGY BIOPRODUCTS FOR A SUSTAINABLE BIOECONOMY Open Access

WILEY

ORIGINAL RESEARCH

N_2 and N_2O mitigation potential of replacing maize with the perennial biomass crop *Silphium perfoliatum*—An incubation study

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Funding information
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.

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 N_2O 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 ^{15}N gas flux method was used to quantify N source-specific N_2 and N_2O fluxes. Cumulated N_2O emissions and N_2+N_2O 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 $N_2O/(N_2+N_2O)$ ratios (N_2Oi). N_2O emissions originating from sources other than the ^{15}N-labelled NO_3 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 ^{15}N pool indicate that under the experimental conditions with high moisture and NO_3^- 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

^{15}N 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 (CO_2) and nitrous oxide (N_2O) 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 CO_2 and especially N_2O 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 N_2O losses from denitrification especially in poorly drained and fine-textured soils (Rochette, 2008). However, reduced oxygen (O_2) availability due to reduced gas diffusivity has also been reported to favour N_2O reduction to dinitrogen (N_2) and thus lower the $N_2O/(N_2+N_2O)$ product ratio (N_2Oi) of denitrification (Müller & Clough, 2014), decreasing the share of N_2O 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, N_2Oi is shifted towards more N_2O 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 N_2O 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 (Neugschwandtner et al., 2014; Pugesgaard et al., 2015).

Few studies to date have directly compared the differences in N_2 and N_2O 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 N_2 and N_2O 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 preferable 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 (^{15}NGF) to quantify N_2 fluxes and source-specific N_2O fluxes from soil under two different cropping systems in controlled laboratory conditions.

It was expected that soil derived N_2 and N_2O 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 N_2 (lower N_2Oi) because of a longer residence time due to reduced gas diffusivity and greater availability of labile C; (1.b) the N_2Oi from cup plant soil is lower because of the impact of conditions favouring N_2O reduction, that is, reduced gas diffusivity and more labile C have a greater impact than lower pH due to less intensive management; (1.c) N_2O 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 N_2+N_2O 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 N_2O emissions due to incorporation of litter and denitrification activities in the earthworm gut.

TABLE 1 Soil characteristics at the maize and cup plant sampling site in September 2019; mean ± SD ($n \geq 3$)

	Maize	Cup plant
Bulk density (g cm^{-3})	1.40 ± 0.04	1.42 ± 0.03
Soil texture (mass %)		
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

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}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% O, 2.7% N, 77% He, 350 ppm CO_2 and 250 ppb N_2O, and the flow rate was set to 11 ml min^{-1}. The low background N_2 in the gas mixture improved the sensitivity of the isotope ratio mass spectrometry (IRMS) measurements (Lewicka-Szczebak et al., 2017), while CO_2 and N_2O 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.

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 KNO_3 to reach a target level (83 mg kg^{-1}) equivalent to 230 kg NO_3-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 ^{15}N KNO_3 fertilizer was applied dissolved in 316 ml irrigation solution per incubation vessel with 0.01 M $CaCl_2$ (equivalent to 19.4 l m^{-2} precipitation). The stabilizing effect of Ca^{2+} 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 ($N_{min} = NO_3^-$-N+NH_4^+-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. N_{min} 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 NO_3^--N and NH_4^+-N were analysed colorimetrically using a continuous flow analyser (SA 5000, Skalar Analytical B.V.). Then ^{15}N enrichment of extractable NO_3 ($^{15}aNO_3$)$^-$ was determined by analysing the N_{min} extraction solution using chemical conversion of NO_3^- to N_2O 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 NO_3^- and NH_4^+ 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

TABLE 2 Overview of the 14 treatments ($n = 4$). The Litter treatment consisted of 6 g (DM) crop-specific litter and the Worm+Litter treatment consisted of 6 g litter and two individuals of the earthworm species Lumbricus terrestris

	WFPS level	Control[a]	Bare Soil	Litter	Worm+Litter
Maize	High	x	x	x	x
	Low	n.e.[b]	x	x	x
Cup plant	High	x	x	x	x
	Low	n.e.[b]	x	x	x

[a]The control was not treated to defaunation by heating.

[b]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_2O) 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 unsteady 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 | ^{15}N labelling and $^{15}N_2$ and $^{15}N_2O$ isotope analysis

To elucidate the N_2 and N_2O emission and related processes based on stable isotope ratios, each column was amended with ^{15}N-labelled NO_3^- with the aim of reaching a ^{15}N enrichment of 60 at% in the soil after tracer addition.

Gas samples for ^{15}N 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-Szczebak 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_2O was reduced in a Cu oven to N_2. Nitrogen isotope mass ratios ^{29}R ($^{29}N_2/^{28}N_2$) and ^{30}R ($^{30}N_2/^{28}N_2$) from N_2, N_2+N_2O and N_2O 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_2O 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_2O 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_2O fluxes and the ^{15}N 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 (Hauck & Bouldin, 1961) caused by high enrichment of NO_3^-. The N_2 and N_2O fluxes were calculated using the formulas given by Spott et al. (2006) and Russow et al. (1996). The apN_2, apN_2+N_2O, apN_2O and fractions of gas species originating from the labelled pool (fp) were quantified for N_2 (fp_N_2), N_2+N_2O (fp_N_2+N_2O) and N_2O (fp_N_2O; see Supporting Information). Based on the initial and final NO_3 content and its ^{15}N enrichment ($^{15}aNO_3$), both net nitrification and, through pool dilution, gross nitrification were calculated (Deppe et al., 2017; Hart et al., 1994). Furthermore, N_2O emissions that did not originate from the labelled pool (fn_N_2O, see Supporting Information) divided by gross nitrification gave an estimate of N_2O formation from nitrification, that is, N_2O yield of nitrification.

Cumulated fluxes were calculated by linear interpolation using fp_N_2+N_2O, fp_N_2 and fp_N_2O 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_2O originating from the labelled pool at total N_2O (N_2Ot) in the sample ($Fp\_N_2O = fp\_N_2O/N_2Ot$) and the product ratio of denitrification [$N_2Oi = fp\_N_2O/(fp\_N_2+N_2O)$] 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 quasibinominal

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 µm diameter, Figure S1). Soil-gas diffusivity (D_p/D_0) at low WFPS was 0.055 ± 0.018 and 0.070 ± 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 ± 0.005 compared to maize soil with 0.019 ± 0.008: The gas diffusivity in cup plant soil was 52.6 ± 73.4% and 20.5 ± 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_{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_{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 organic carbon (SOC):N ratio and N_{min} in in 0–10 and 10–20 cm at the end of incubation; ±SD; $n \geq 4$

			NO_3^--N (mg N kg^{-1})		NH_4^+-N (mg N kg^{-1})	
Soil	**WFPS**	**SOC:N**	**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

TABLE 4 Mean cumulated CO_2 and N_2O flux ± standard deviation per treatment and WFPS level ($n \geq 3$)

	CO_2 flux (mg C m^{-2} h^{-1})		N_2O flux (µg N m^{-2} h^{-1})	
	WFPS high	**WFPS low**	**WFPS high**	**WFPS low**
Maize				
Bare Soil	31.5 ± 5.1	31.6 ± 4.0	1808.2 ± 592.0	49.0 ± 68.7
Litter	77.4 ± 4.7	84.8 ± 3.1	1504.9 ± 801.6	92.4 ± 85.8
Worm+Litter	—	99.7 ± 1.0	—	136.0 ± 38.3
Cup plant				
Bare Soil	43.3 ± 8.8	38.2 ± 6.8	1184.8 ± 818.6	5.6 ± 2.1
Litter	103.8 ± 8.2	90.7 ± 7.0	264.9 ± 125.6	26.6 ± 20.3

TABLE 5 Coefficient with effect size, lower and upper 95% confidence intervals (CI), and p values of linear regression models for CO_2 and N_2O flux and the residual standard error

	$CO2_i = \sum \beta_j x_{ij} + \varepsilon_i, \varepsilon_i \sim N(0, \sigma^2)$			$Log10\ (N_2O)_i = \sum \beta_j x_{ij} + \varepsilon_i, \varepsilon_i \sim N(0, \sigma^2)$		
	β	**95% CI**	p **value**	β	**95% CI**	p **value**
Intercept	31.6	(38.04, 25.10)		1.40	(1.84, 0.97)	
Cup plant soil	6.6	(15.79, −2.51)	0.15	−0.67	(−0.06, −1.23)	0.03
high WFPS	−0.1	(9.04, −9.26)	0.98	1.83	(2.45, 1.22)	<0.01
Litter	53.2	(62.40, 44.10)	<0.01	0.43	(1.05, −0.18)	0.16
Soil:WFPS	−5.2	(18.12, −7.75)	0.42	0.22	(1.09, −0.65)	0.61
Soil:litter	−0.7	(12.22, −13.66)	0.91	0.17	(1.04, −0.70)	0.69
WFPS:litter	−7.3	(5.64, −20.24)	0.26	−0.56	(0.34, −1.40)	0.22
Soil:WFPS:litter	15.3	(33.55, 3.04)	0.10	−0.48	(0.75, −1.71)	0.43
σ	6.27			0.422		

However, soil ($p < 0.001$) and WFPS ($p < 0.3$) had interacting ($p < 0.01$) effects on CO_2 emissions. Treatments with litter on the soil surface had significantly higher CO_2 emissions than Bare Soil. Therefore, cumulated CO_2 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 CO_2 flux of 0.14 ± 0.01 mg C g^{-1} litter m^{-2} h^{-1} and 0.16 ± 0.03 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 CO_2 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 | N_2O emissions

The comparison of mean cumulated N_2O fluxes in cup plant between the non-defaunated Control and the Bare Soil revealed no significant difference.

While N_2O fluxes in maize decreased initially and were approaching a constant level at the end of observation period, N_2O 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 N_2O 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 N_2O 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 N_2O emissions could not be quantified.

3.4 | N_2 and N_2O emissions from the ^{15}N-labelled pool

All columns at the high WFPS level exhibited detectable pool-derived N_2, N_2+N_2O and N_2O fluxes (fp_N_2, fp_N_2+N_2O and fp_N_2O 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_N_2, fp_N_2+N_2O, fp_N_2O or fn_N2O fluxes.

Similar to the total N_2O (N_2Ot) flux from the GC measurements, fp_N_2O decreased or remained at the same level during the observation period in both soils (Figure 1). The fp_N_2 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_N_2 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_N_2O fluxes and consequently also to the fp_N_2+N_2O fluxes (Table 6). In each of the maize treatments, fn_N_2O fluxes were higher than in treatments with cup plant soil ($p < 0.0001$). However, the contribution of fn_N_2O to total emission in the high emitting treatments (high WFPS) was very low (<7%).

Nevertheless, N_2 and N_2O fluxes differed significantly between the two WFPS levels in both soils, except for the Litter treatment of cup plant soil.

N_2O 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 ($R = 0.59$, $p < 0.05$).

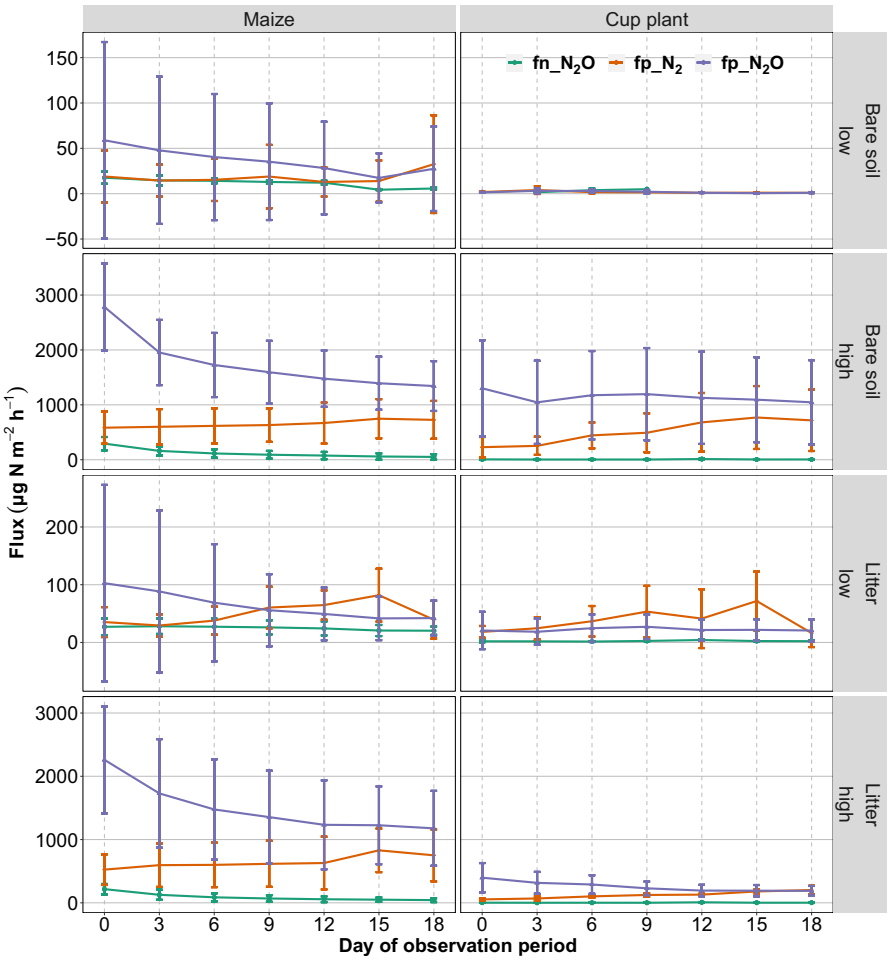

FIGURE 1 Mean not pool-derived N_2O (fn_N_2O), pool-derived N_2 (fp_N2) and N_2O (fp_N_2O) flux from maize and cup plant soil per treatment and WFPS level over time. All data are based on mass spectrometry measurements. Error bars depict ±1 SD

TABLE 6 Mean cumulated fluxes (μg N m^{-2} h^{-1}) and standard deviation ($n = 4$) from ^{15}N data

	High WFPS		Low WFPS	
	Maize	**Cup plant**	**Maize**	**Cup plant**
Bare Soil				
fp_N_2	653.3 ± 331.3	521.1 ± 361.6	16.3 ± 25.2	1.8 ± 0.9
fp_N_2O	1701.2 ± 550.8	1135.1 ± 790.7	35.4 ± 61.7	1.8 ± 1.0
fp_N_2+N_2O	2354.4 ± 798.5	1656.4 ± 1097.7	51.7 ± 86.9	3.6 ± 1.7
fn_N_2O	112.3 ± 68.3	5.7 ± 3.0	13.0 ± 2.3	3.2 ± 1.5
Litter				
fp_N_2	651.3 ± 345.4	122.0 ± 17.5	52.0 ± 25.5	41.5 ± 31.8
fp_N_2O	1456.3 ± 729.6	252.1±120.0	62.8 ± 80.5	22.4 ± 20.4
fp_N_2+N_2O	2107.6 ± 1041.2	374.0 ± 103.1	114.5 ± 92.9	64.4 ± 43.2
fn_N_2O	86.3 ± 55.1	2.6 ± 1.1	25.1 ± 11.8	2.7 ± 0.4
Worm+Litter				
fp_N_2	—	—	132.2 ± 73.1	—
fp_N_2O	—	—	94.4 ± 30.9	—
fp_N_2+N_2O	—	—	226.6 ± 86.8	—
fn_N_2O	—	—	30.5 ± 8.6	—

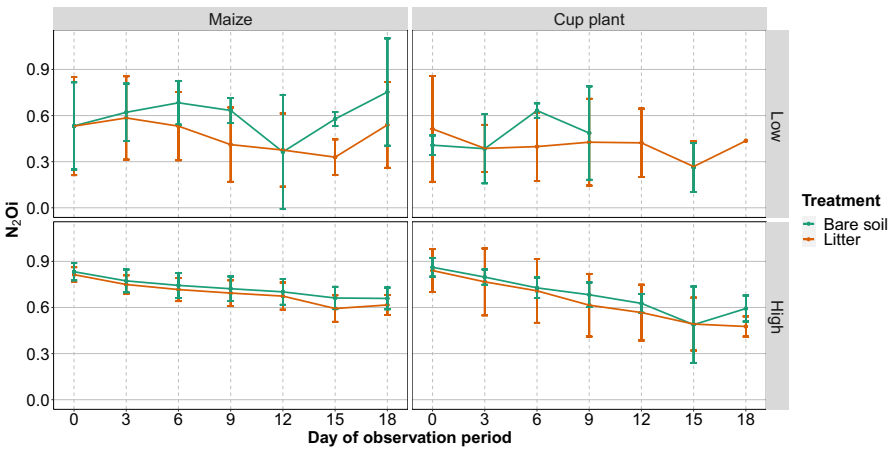

FIGURE 2 Denitrification product ratio (N_2Oi) by treatment over time by standard deviation. Facet by soil and WFPS level. Missing error bars or values indicate weak ^{15}N signal in the sample

The mean contribution of N_2O derived from the ^{15}N-labelled pool to the total N_2O flux (Fp_N_2O) from both soils was higher ($p < 0.0001$) at high WFPS level (mean Fp_N2O = 0.96±0.04) than in the low WFPS treatments for both soils (mean Fp_N_2O = 0.56 ± 0.25; Table S5), but no significant differences were observable between maize and cup plant soil. A tendency for increasing Fp_N_2O 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_2O and fp_N_2+N_2O (N_2Oi), although fp_N_2 increased more than fp_N_2O due to the addition of litter and/or earthworms (Figure 1). Therefore, slightly lower N_2Oi values were observed in the Litter and Worm+Litter treatments compared with the Bare Soil. At low WFPS the N_2Oi was significantly lower than in high WFPS treatments, thus WFPS levels were the only influential effect on N_2Oi ($p < 0.01$). Over the observation period, N_2Oi decreased in maize treatments at high WFPS by 0.01029 day^{-1} and in cup plant treatments by 0.02057 day^{-1} (Figure 2). N_2Oi was more variable at low WFPS, but there were also decreasing tendencies over time. Overall, the decrease in N_2Oi was more pronounced in cup plant soil. This coincided with a greater decrease in N_2O fluxes and a higher increase in N_2 fluxes in cup plant soil during the observation period.

The ^{15}N enrichments of the active nitrate N pool producing N_2 and N_2O (apN_2 and apN_2O 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

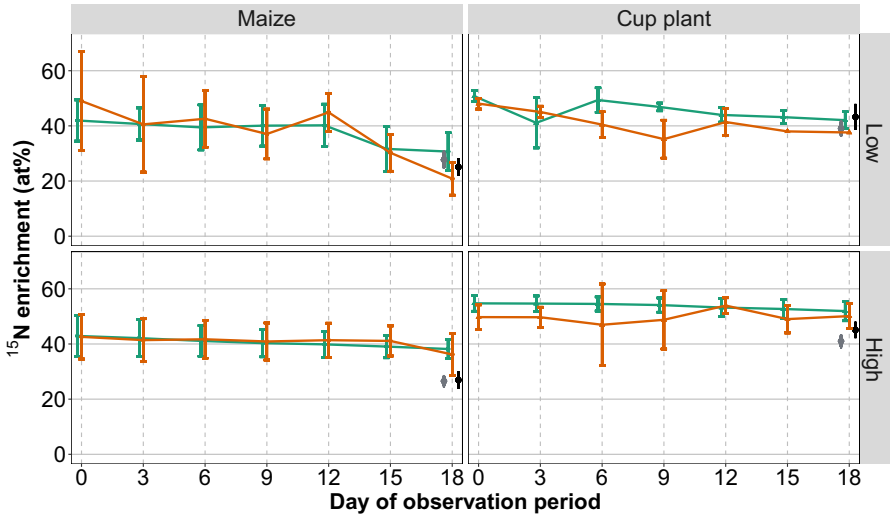

FIGURE 3 ^{15}N enrichment of the NO_3 pool producing N_2 (apN_2, orange) and N_2O (apN_2O, green) during the observation period averaged over soil and WFPS level ($n = 8$). Error bars depict ±1 SD. Points with standard deviation depict $^{15}aNO_3$ in 0–10 cm (grey) and 10–20 cm (black)

both soils (Figure 3). There were significant differences in apN_2O values between the soils at high WFPS ($p < 0.001$) and at low WFPS ($p < 0.001$), while differences in apN_2 were only significant at high WFPS ($p < 0.05$). In contrast, the ^{15}N enrichment of the extracted NO_3^- ($^{15}aNO_3$) 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, $^{15}aNO_3$ 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 $^{15}aNO_3$ between the WFPS levels were less pronounced than in ap values. Mean apN_2 and apN_2O values were higher ($p < 0.01$) than final $^{15}aNO_3$ in the extractant of the high WFPS treatments.

4 | DISCUSSION

4.1 | Comparison with field and laboratory studies

Relatively high N_2O emissions can be expected from fine-textured gleysols with high N_{min} content in which high moisture conditions are frequent and soil aeration is thus reduced (Rochette, 2008). Consequently, the present study's incubation with undisturbed gleyic soil cores under moist to wet conditions exhibited relatively high N_2O emissions, which is in agreement with other observations (Gauder et al., 2012). However, the soil moisture and relatively high NO_3^- content in the columns in the present study presumably led to a substantially higher N_2Oi compared with the observation from maize soil made by Buchen et al. (2016).

For example, the cumulated N_2O 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 N_2O to N_2+N_2O coming from denitrification (N_2Oi) was more than 10 times higher (0.58 ± 0.17) than the N_2Oi 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 CO_2, N_2 and N_2O emissions and N cycling

4.2.1 | Treatment effects

Incorporation of labile C sources such as litter is known to increase CO_2 and N_2O emissions substantially (Köbke et al., 2018; Senbayram et al., 2012), as observed in the CO_2 results of both maize and cup plant soils: CO_2 fluxes increased in the order Worm+Litter > Litter > Bare Soil. No similar pattern was observed with N_2 and N_2O fluxes. Dry litter material with a high C:N ratio remaining on the soil surface is reported to cause fewer N_2O 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 N_2O 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 N_2 and N_2O emissions.

4.2.2 | Interaction of soil structure and water content on CO_2, N_2O and N_2 emissions and the N_2Oi

Soil structure is an important factor for GHG emissions through its influence on gas diffusion (Petersen et al., 2008; Schlüter 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 ($p = 0.051$), 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 ($p < 0.01$) 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; Schlüter et al., 2018; van der Weerden et al., 2012).

In this experiment, differences in fluxes and cumulated emissions of N_2 and N_2O 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 N_2O increases, and therefore N_2O is more likely to be reduced to N_2 (Schlüter 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 N_2Oi was expected in the high WFPS treatments. Interestingly, a higher N_2Oi (+0.23 ± 0.17 and +0.19 ± 0.24 higher N_2Oi in maize and

TABLE 7 Coefficient with effect size, lower and upper 95% confidence intervals (CI), and *p* values of linear regression models for ^{15}N pool-derived N fluxes (fp_N_2, fp_N_2+N_2O, fp_N_2O) and non-pool-derived N_2O (fn_N_2O) and the residual standard error (σ)

	Log10 (flux)$_i$ = $\sum \beta x_{ij} + \varepsilon_i$, $\varepsilon_i \sim N(0, \sigma^2)$											
	fp_N_2+N_2O			**fp_N_2O**			**fp_N_2**			**fn_N_2O**		
	β	**95% CI**	***p* value**	***β***	**95% CI**	***p* value**	***β***	**95% CI**	***p* value**	***β***	**95% CI**	***p* value**
Intercept	1.22	(0.79, 1.65)		0.96	(1.48, 0.44)		0.85	(1.22, 0.47)		1.11	(0.84–0.14)	
Cup plant soil	−0.70	(−0.09, −1.31)	0.03	−0.75	(−0.02, −1.48)	0.05	−0.64	(−0.11, −1.16)	0.02	−0.63	(−0.20, −1.06)	0.01
High WFPS	2.13	(2.74, 1.52)	<0.01	2.25	(2.98, 1.52)	<0.01	1.92	(2.44, 1.39)	<0.01	0.86	(1.22, 0.50)	<0.01
Litter	0.72	(1.33, 0.11)	0.02	0.59	(1.32, 0.14)	0.11	0.82	(1.34, 0.29)	<0.01	0.25	(0.61, −0.11)	0.16
Soil:WFPS	0.34	(1.20, −0.53)	0.43	0.29	(1.32, −0.74)	0.57	0.42	(1.16, −0.33)	0.26	−0.62	(−0.08, −1.16)	0.03
Soil:litter	0.48	(1.34, −0.38)	0.27	0.41	(1.44, −0.62)	0.42	0.48	(1.23, −0.27)	0.20	−0.30	(0.24, −0.84)	0.26
WFPS:litter	−0.78	(0.08, −1.65)	0.08	−0.67	(0.36, −1.70)	0.19	−0.81	(−0.07, 1.56)	0.03	−0.35	(0.13, −0.84)	0.15
Soil:WFPS:litter	−0.85	(0.38, −2.07)	0.17	−0.73	(0.73, −2.19)	0.31	−0.95	(0.11, −2.00)	0.08	−0.07	(0.79, −0.64)	0.83
σ	0.42			0.50			0.36			0.23		

cup plant soils respectively) at high WFPS was observed (Table S5). This suggests that in both systems, gross N_2O production exceeds consumption at high WFPS, probably due to high NO_3^- 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 N_2O formation during denitrification (Butterbach-Bahl et al., 2013), the N_2Oi results implied that soil moisture in the high WFPS treatments was, in fact, not above optimum conditions for N_2O formation.

The expected impact of the perennial system on macroscale 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 O_2 and N_2O). Indications of spatial heterogeneity of N_2 and N_2O production can be obtained by comparing the ^{15}N enrichment of NO_3^- in bulk soil ($^{15}aNO_3$) with the ^{15}N enrichment of the NO_3^- pools undergoing denitrification (apN_2, apN_2O; 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 $^{15}aNO_3$ are distinguishable, that is, due to missing dilution by nitrification in the absence of O_2 in denitrifying hotspots. Comparing apN_2O and apN_2 in relation to $^{15}aNO_3$ revealed differences between the two soils and their hotspots of N_2 and N_2O formation. While in maize soil the apN_2O and apN_2 did not differ significantly from one other (Figure 3), in cup plant soil at high WFPS apN_2O was significantly higher than apN_2, indicating distinguishable N_2O and N_2-producing microsites. In cup plant soil, the lower apN_2 suggests that these microsites had a lower $^{15}NO_3^-$ availability than N_2O-forming microsites, indicating a spatial separation of these hotspots. Higher apN_2O than apN_2 may indicate that in cup plant soil, relatively isolated hotspots existed where O_2 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 apN_2 and apN_2O in cup plant soil clearly indicated more heterogeneity in N_2 and N_2O production than in maize soil.

4.2.3 | Availability of labile C and N

Labile C sources and NO_3^- 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 O_2 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 NO_3^- availability and a more pronounced ^{15}N 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 NO_3^- immobilization and therefore a possible limitation of NO_3^- availability for denitrification.

The more intensive N mineralization at high WFPS was positively correlated with the flux of N_2O from non-labelled sources (fn_N_2O), which is a potential risk for nitrification N losses of maize soil. However, in maize soil at high WFPS, the contribution of fn_N_2O to the total N_2O flux (N_2Ot) was relatively low (<7%). The share was much higher at low WFPS (up to 46%), but absolute emissions from these treatments were negligible. The N_2O yield from nitrification in maize soil at high WFPS was around 0.2 ± 0.1% and at low WFPS the N_2O yield from nitrification was 0.04 ± 0.03%, showing that the observed N_2O 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 N_2O in these tested soils.

4.2.4 | Contrasting effect of pH on N_2Oi

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 N_2O/N_2+N_2O ratio (N_2Oi) is negatively correlated with soil pH due to post-transcriptional inhibition of the N_2O reductase and increased O_2 consumption due to increased microbial activity, which shifts the product stoichiometry towards more N_2O 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 N_2Oi 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 N_2Oi values similar to maize soil, even though their soil structure and reduced gas diffusivity (Schlüter et al., 2019), lower NO_3^- and labile C content would favour lower N_2Oi (Senbayram et al., 2012).

4.2.5 | Revisiting the hypotheses

Since N_2O and N_2 emissions and N_2Oi were not statistically different between maize and cup plant soils, but maize soil exhibited consistently higher tendencies of N_2 and N_2O emissions, the main working hypothesis that N_2O and N_2 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 N_2 emissions from denitrification, and thus hypothesis 1a could not be accepted. However, an apparent separation of N_2 and N_2O-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 N_2Oi, that is, predominant emissions of N_2 from denitrification.

A potential N_2O mitigation due to a reduced N_2Oi caused by conditions favouring N_2O 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 N_2O reduction to N_2 by reduced gas diffusivity due to soil structure (affecting N_2O and O_2) and the hotspot-forming patchy distribution of organic matter (locally increased O_2 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 NO_3^- content) on N_2O 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 NO_3^- availability favouring N_2O formation. This could result in an elevated N_2Oi in maize soil due to the preferred reduction of NO_3^- over N_2O (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_N_2O fluxes, total soil-borne N_2O and N_2 emissions and the N_2Oi 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 N_2O 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 N_2 and N_2O emissions and N cycling

This study excluded field processes such as NO_3^- uptake by plants, O_2 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 N_2O mitigation potential of perennial cropping is strongly influenced by the management and management history.

5 | CONCLUSION

Although we expected lower N_2O and higher N_2 emissions from the perennial system, there were no significant differences in N_2 and N_2O emissions or the product ratio of denitrification (N_2Oi) 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_2O 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_2O) 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_2O reduction by preventing excessive acidification and frequently high NO_3^- availability to reduce N_2O emissions from cup plant cropping. Moreover, conditions which favour denitrification should generally be reduced since cup plant soil did not exhibit a lower N_2Oi in order to optimize cup plant cropping as a climate-friendly alternative to maize through N_2O 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.

ACKNOWLEDGEMENTS

The authors thank Frank Hegewald, Ute Tambor and Martina Heuer for their technical support in the field and in the laboratory, the farmers and Balazs Grosz. Open Access funding enabled and organized by Projekt DEAL.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Open agrar at https://doi.org/10.3220/DATA20210630134418, reference number: https://doi.org/10.3220/DATA20210630134418.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: 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 Bioenergy*, 13, 1649–1665. https://doi.org/10.1111/gcbb.12879