Plant Soil (2021) 459:217–233
https://doi.org/10.1007/s11104-020-04750-7

REGULAR ARTICLE

Nitrate uptake and carbon exudation – do plant roots stimulate or inhibit denitrification?

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Received: 12 May 2020 / Revised: 25 September 2020 / Accepted: 20 October 2020 / Published online: 6 November 2020

Abstract
Background and aims Plant growth affects soil moisture, mineral N and organic C availability in soil, all of which influence denitrification. With increasing plant growth, root exudation may stimulate denitrification, while N uptake restricts nitrate availability.
Methods We conducted a double labeling pot experiment with either maize (*Zea mays* L.) or cup plant (*Silphium perfoliatum* L.) of the same age but differing in size of their shoot and root systems. The ^{15}N gas flux method was applied to directly quantify N_2O and N_2 fluxes in situ. To link denitrification with available C in the rhizosphere, $^{13}CO_2$ pulse labeling was used to trace C translocation from shoots to roots and its release by roots into the soil.
Results Plant water and N uptake were the main factors controlling daily $N_2O + N_2$ fluxes, cumulative N emissions, and N_2O production pathways. Accordingly, pool-derived $N_2O + N_2$ emissions were 30–40 times higher in the treatment with highest soil NO_3^- content and highest soil moisture. CO_2 efflux from soil was positively correlated with root dry matter, but we could not detect any relationship between root-derived C and $N_2O + N_2$ emissions.
Conclusions Root-derived C may stimulate denitrification under small plants, while N and water uptake become the controlling factors with increasing plant and root growth.

Keywords Nitrous oxide · Dinitrogen · Nitrogen mineralization · Rhizodeposition · ^{15}N ^{13}C labeling · Carbon cycling

Responsible Editor: Jorge Durán.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-020-04750-7.

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Introduction

Soil conditions for denitrification have frequently been studied with the main prerequisites being availability of nitrate (NO_3^-) and easily decomposable organic substances, and oxygen deficiency (Burford and Bremner 1975; Firestone et al. 1979). Growing plants modify all these parameters, particularly the availability of the main substrates (NO_3^- and C_{org}) and soil moisture, and

may thus play an important role in regulating denitrification in situ (von Rheinbaben and Trolldenier 1984).

Plant N uptake largely controls concentration and distribution of mineral N in soils. Amounts and rates of plant N uptake depend on plant species, age, physiological status, root size, and nutritional status. N uptake rates of maize and cereals remain low during the first two months of growth, then increase linearly with increasing biomass reaching a maximum around the time of flowering (Novák and Vidovič 2003, Malhi et al. 2011).

Plant roots contribute to organic C input to the soil through rhizodeposition and decaying roots and root hairs. Thus, total and available concentration of C_{org} is higher in the rhizosphere compared to bulk soil (Cheng et al. 1993). The amount of rhizodeposited C and its quality depend on plant species, age, and development (Gransee and Wittenmayer 2000; Vancura 1964; Vancura and Hovadik 1965), and plant nutrient status (Carvalhais et al. 2011). In general, younger plants translocate a higher share of assimilated C belowground than mature plants (Kuzyakov and Domanski 2000; Nguyen 2003), and perennial plants translocate a higher share of assimilated C belowground than annual plants (Husáková et al. 2018; Pausch and Kuzyakov 2018).

C and N availability are closely interrelated in the rhizosphere: Under low mineral N concentrations, root morphology is altered, and exudation related to root mass is increased (Paterson and Sim 1999). In addition, the composition of maize root exudates is altered under N deficiency (Carvalhais et al. 2011). On the other side, N fertilization decreases the portion of below-ground translocated C (Kuzyakov and Domanski 2000).

Several studies have tried to disentangle the effects of N and C availability on denitrification with contradictive results. Higher denitrification rates were measured from planted compared to bare soil (Senbayram et al. 2020; Vinther 1984). Some studies showed a strong influence of roots (Philippot et al. 2009), increasing denitrification rates with increasing root biomass (Klemedtsson et al. 1987), and higher potential denitrification activity in rhizosphere soil compared to bulk soil (Hamonts et al. 2013; Malique et al. 2019). Higher denitrification rates in planted soils have been associated with higher C_{org} availability in the rhizosphere (Bakken 1988; Philippot et al. 2009). In addition, denitrification rates correlated with soil NO_3^- content (Philippot et al. 2009; von Rheinbaben and Trolldenier 1984). In contrast, other studies found no differences between planted and unplanted soil (Haider et al. 1985). Denitrification was increased only with poorly growing plants (von Rheinbaben and Trolldenier 1984) or when root biomass started to decrease (Haider et al. 1987), and NO_3^- availability did not affect denitrification (Haider et al. 1987; Hamonts et al. 2013). The majority of these studies measured potential denitrification applying the acetylene inhibition method (Yoshinari and Knowles 1976), which is considered outdated due to a number of drawbacks such as inhibiting nitrification (Groffman et al. 2006).

Accordingly, it is still unclear whether growing plants stimulate denitrification through root exudation or restrict it through NO_3^- uptake. Reliable measurements of N_2 fluxes and $N_2O/(N_2O + N_2)$ ratios in the presence of plants are scarce. Direct measurement of N_2 fluxes is only possible in either artificial N_2-free atmosphere (Scholefield et al. 1997, Senbayram et al. 2020) or by applying highly enriched ^{15}N labeled NO_3^- (Hauck and Melsted 1956). The latter is used in the ^{15}N gas flux technique which enables direct quantification of N_2O and N_2 produced from the labelled NO_3^- pool and estimation of processes contributing to N_2O and N_2 formation including denitrification, co-denitrification, or nitrification and nitrifier denitrification (Buchen et al. 2016, Laughlin and Stevens 2002).

This study aimed to directly quantify N_2O and N_2 fluxes from soil with plants of the same age but different size of shoot and root systems and to relate denitrification to C availability from root exudation. As plant water uptake may also affect denitrification (von Rheinbaben and Trolldenier 1984), we aimed to keep soil moisture constant by continuous irrigation. We hypothesized that (I) plant N uptake governs NO_3^- availability for denitrification. When plant N uptake is low due to smaller root system or root senescence, N_2O and N_2 emissions are increased. (II) Denitrification is stimulated by higher C_{org} availability from root exudation or decaying roots increasing total gaseous N emissions and decreasing their $N_2O/(N_2O + N_2)$ ratios.

Materials and methods

Experimental concept

The experiment consisted of a pre-cultivation phase followed by the experimental phase. A schematic overview of both phases is presented in Fig. 1. In the pre-

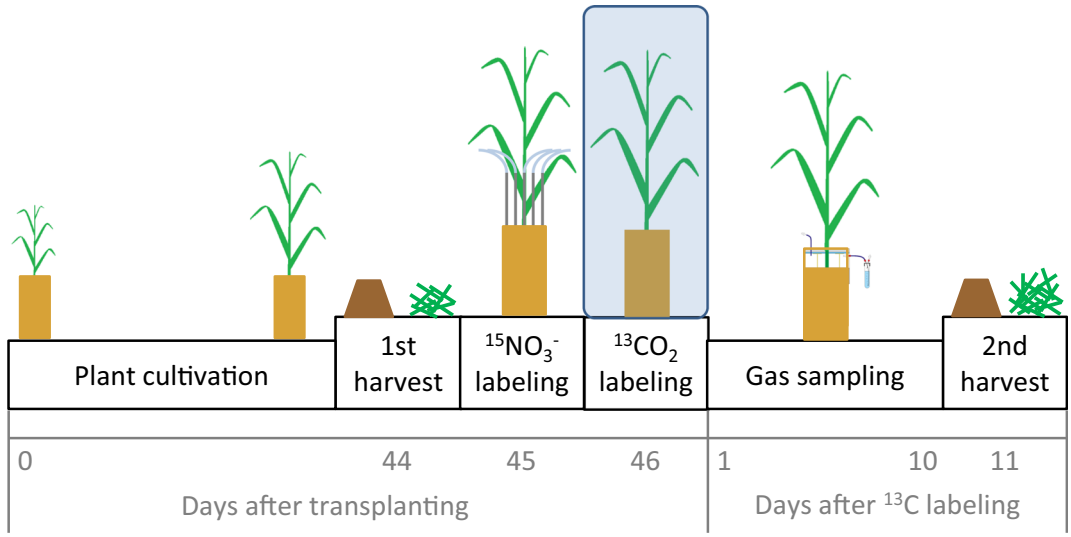

Fig. 1 Timeline of the experiment: pre-cultivation phase from day 0–46 and experimental phase from day 47–57 after transplanting

cultivation phase, plants were raised under controlled conditions. Maize plants (*Zea mays* L. cv. Ronaldinio) were grown under different N fertilization to obtain plants with different root and shoot biomass. As a second species, cup plant (*Silphium perfoliatum* L.) was included, a bioenergy plant that can produce similar aboveground biomass as maize (Gansberger et al. 2015) but has a higher root:shoot ratio. As cup plant is a perennial plant, it likely transfers more C belowground and exudes more organic substances than maize (Husáková et al. 2018; Pausch and Kuzyakov 2018). In all treatments, the N supply was scheduled to assure that at the end of the pre-cultivation phase, the soils were equally depleted in plant available N. With respect to background N supply, this permitted nearly equal starting conditions for the subsequent stable isotope labeling experiment.

To account for all necessary measurements, each treatment was replicated 19 times (Table 1). At the end of the pre-cultivation phase, the first set of replicates (1–6) was harvested to determine shoot and root biomass, N and C content, and ^{15}N and ^{13}C background concentrations. The second set of replicates (7–12) was labeled with $^{15}NO_3^-$ and $^{13}CO_2$, and gases evolving from soil were measured for the following 10 days. At the end of the experiment, replicates 7–12 were harvested. Replicate 13 was used to determine ^{13}C uptake during $^{13}CO_2$ pulse labeling and replicates 14–19 were used to determine ^{13}C background values in soil-emitted CO_2.

Pre-experimental plant cultivation

The soil for the experiment was collected from a long-term experimental field site of the Höhere Landbauschule Rotthalmünster, Germany (latitude N48°21′, longitude E13°11′, elevation 360 m above sea level) in summer 2016. It was sieved to 10 mm, air dried, and stored at 4 °C until setup of the experiment. The soil was classified as a Haplic Luvisol with a silty loam texture (19% clay, 71% silt, 10% sand). Soil

Table 1 Overview of replicates in the experiment

Replicate	Labeling	Sampling of plants and soil	Details
1–6	-	1st harvest (44 days)	Determination of dry matter, C and N background values
7–12	$^{15}NO_3^-$ and $^{13}CO_2$	Final harvest (57 days)	Measurement of N_2O, N_2, and CO_2, determination of dry matter, total C and N, ^{13}C and ^{15}N content
13	$^{13}CO_2$	Plants sampled directly after $^{13}CO_2$ labeling	Determination of total $^{13}CO_2$ uptake during labeling
14–19	Treated similar to 7–12, but not labeled	No sampling of plants and soil	Determination of ^{13}C background in CO_2

properties were: total C 1.23%, total N 0.14%, C:N 8.76, pH ($CaCl_2$) 6.74.

Seven kg dry soil was mixed with fertilizers (0.14 g P kg^{-1} as $Ca(H_2PO_4)_2$, 0.2 g K kg^{-1} as K_2SO_4 and 0.04 g Mg kg^{-1} as $MgSO_4$ * 7 H_2O including 0.135 g S kg^{-1}) and filled in pots of 15 cm diameter and 35 cm height to a bulk density of 1.3 g cm^{-3}. Soil moisture was adjusted to 60% water holding capacity (WHC, 21.9% gravimetric water content) and watered regularly. TDR soil moisture sensors (Decagon Devices, Pullman, USA) were used to monitor soil water content during plant growth.

Maize seeds (*Zea mays* L. cv. Ronaldinio) were germinated on wet paper for 4 days. Cup plant (*Silphium perfoliatum* L.) had been pre-cultivated for 2 years in 5 cm-pots and had 2–4 leaves. Per pot, either one germinated maize seed or one cup plant seedling was transplanted. Plants were cultivated in a climate chamber (Weiss, Loughborough, UK) with a diurnal cycle of 16 h day (light intensity 300 µmol m^{-2} s^{-1}, air temperature 25 °C, relative humidity 50%) and 8 h night (18 °C, 60%). Daytime included 4 h sunrise and 4 h sunset when light intensity, temperature, and relative humidity were gradually adjusted. All pots were fully randomized weekly to avoid microclimatic effects.

Maize N fertilization differed between treatments to achieve plants of different size: Maize S (no N fertilization, small plants), Maize M (0.05 g N kg^{-1} (0.35 g N pot^{-1}, as NH_4NO_3 split in 7 doses), medium sized plants), and Maize L (0.086 g N kg^{-1} (0.6 g N pot^{-1}, as NH_4NO_3 split in 7 doses), large plants). Cup plants were fertilized like Maize M (0.05 g N kg^{-1} (0.35 g N pot^{-1}, as NH_4NO_3 split in 7 doses)).

Experimental $^{15}NO_3^-$ and $^{13}CO_2$ pulse labeling

Replicates 7–12 of each treatment received 0.1 g N kg^{-1} ^{15}N-labeled $Ca(NO_3)_2$ (~ 60 at% $^{15}N_2$, Campro Scientific GmbH, Berlin, Germany) 45 days after transplanting. The tracer was dissolved in H_2O_{dest} and applied by injection with stainless steel needles as described by Buchen et al. (2016). Briefly, ^{15}N fertilizer solution was injected via 12 needles to 6 depths (2.5, 7.5, 12.5, 17.5, 22.5 and 27.5 cm) aiming for optimal three-dimensional homogenous label distribution (Wu et al. 2011). Per injection point, 10 ml tracer solution were injected via a peristaltic pump (Ismatec, Wertheim, Germany) to simultaneously increase soil water content to 75% water-filled pore space (WFPS, equivalent to 80% WHC).

After injection of ^{15}N tracer solution, all pots were closed with acrylic glass lids with a hole for the plant shoot, leaving a small headspace (2–3 cm) between soil surface and lid. Pots were then sealed with silicone paste (Tacosil 171, Thauer & Co. KG, Dresden, Germany). Plants were labeled with ^{13}C in four separate chambers made from translucent greenhouse film (one for each treatment Maize S, Maize M, Maize L, and Cup plant). In each chamber, ^{15}N labeled replicates 7–12 and the non-labeled replicate 13 were labeled with ^{13}C. To enrich the chamber atmosphere with $^{13}CO_2$, 60 ml of 5 *M* H_2SO_4 were added to 5 g $Na_2{}^{13}CO_3$ (99 at%) dissolved in H_2O_{dest} in each chamber. For internal chamber ventilation, two fans were installed. The plants were pulse labeled in the $^{13}CO_2$ enriched atmosphere for 5 h. Before opening each chamber, an air sample was analyzed for CO_2 concentration to ensure that maximum amounts of CO_2 had been taken up by plants. Chambers were opened and CO_2 evolving from the soil was trapped. Replicate 13 was harvested directly after chamber opening to estimate the amount of ^{13}C assimilated during labeling.

To determine natural abundance background of ^{13}C in CO_2, replicates 14–19 were used. 0.1 g N kg^{-1} was injected using $Ca(NO_3)_2$*6 H_2O dissolved in H_2O_{bidest}, and pots were sealed using the same methods as described above.

Irrigation

After pots had been sealed with silicon paste, plants were irrigated by injecting water through a valve on the bottom of the pots. To irrigate pots without applying too much pressure, peristaltic pumps (Watson-Marlow, Zollikon, Switzerland) with a pumping rate of 1.5 ml min^{-1} were used. Target soil moisture was 75% water-filled pore space (WFPS, equivalent to 80% WHC) during the gas sampling phase. To estimate irrigation demand, TDR soil moisture sensors (Decagon Devices, Pullman, USA) were used to monitor soil water content during the experiment in one replicate for each treatment. In addition, all pots were weighed one, three, and five days after ^{13}C labeling to compare whether irrigation demand differed between pots. As pot weights were comparable within treatments, sensor data were used to compare soil moisture.

Gas sampling

One additional pot was filled with dry quartz sand, sealed with silicone paste as described before, and used as a reference to determine background gas concentrations. To flush the headspace of all pots with CO_2-free air, pressurized air was first run through a glass column filled with soda lime (pellets made of NaOH and $Ca(OH)_2$ mixture) to remove CO_2. For trapping CO_2 emitted from soils, the outlet tubes of the pots' headspaces were connected to glass tubes containing 15 ml of 1 *M* NaOH solution. Starting one day after labeling, NaOH solution was changed in intervals of 6, 12, or 24 hours. To determine ^{13}C background in CO_2, replicates 14–19 were treated similarly: the headspaces were flushed for 6 hours and CO_2 was trapped in glass tubes containing 15 ml of 1 *M* NaOH solution. To estimate the total CO_2 efflux, the C concentration of the NaOH solutions was determined with a TIC-analyzer (multi N/C 2100S, Analytik Jena, Jena, Germany). For ^{13}C measurements, CO_2 trapped in NaOH was precipitated as $SrCO_3$ with an excess of 1 *M* $SrCl_2$ solution. The precipitants were centrifuged, washed with deionized water until the pH was neutral, the precipitate was frozen, and then freeze-dried with a rotation vacuum concentrator (RVC 2–25 CDplus, Martin Christ, Osterode am Harz, Germany) and a cooling trap (CT 02–50, Martin Christ, Osterode am Harz, Germany), both connected to a vacuum pump.

^{13}C enrichment in precipitated $SrCO_3$ was analyzed: Natural abundance samples were measured on an elemental analyzer NA 11,100 (CE Instruments, Milano, Italy) linked to a Delta Plus gas-isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a ConFlo III interface (Finnigan MAT, Bremen, Germany). For enriched samples, depending on capacity, one of the following combinations was used: (i) elemental analyzer Flash 2000 (Thermo Fisher Scientific, Cambridge, UK) linked to a Delta V Advantage gas-isotope ratio mass spectrometer (Thermo Electron, Bremen, Germany) via a ConFlo III interface (Thermo Electron, Bremen, Germany), or (ii) elemental analyzer NA1108 (Fisons-Instruments, Milan, Italy) linked to a Delta C gas-isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a ConFlo III interface (Thermo Electron Cooperation, Bremen, Germany).

For N_2O and N_2 sampling, the airflow through the pots' headspace was interrupted to accumulate gases in the headspace. After 1 h, duplicate samples were taken using a syringe and filled in pre-evacuated 12-ml Exetainer® glass vials (Labco, High Wycombe, UK). Samples were analyzed for N_2O concentration using a gas chromatograph (GC 7890A, Agilent, Santa Clara, USA). The analytical precision of the GC was determined by repeated measurements of standard gases (300 ppb N_2O) and was consistently < 3%. The second duplicate was analyzed for *m/z* 28 ($^{14}N^{14}N$), 29 ($^{14}N^{15}N$) and 30 ($^{15}N^{15}N$) of N_2 using a modified GasBench II preparation system coupled to an isotope ratio mass spectrometer (MAT 253, Thermo Fisher Scientific, Bremen, Germany) according to Lewicka-Szczebak et al. (2013). This system allows a simultaneous determination of mass ratios ^{29}R (29/28) and ^{30}R (30/28) of three separated gas species (N_2, $N_2 + N_2O$, and N_2O), all measured as N_2 gas after N_2O reduction in a Cu oven. Typical repeatability of ^{29}R and ^{30}R (1 σ of 3 replicate measurements) was 5×10^{-7} for both values. For each of the analyzed gas species, the fraction originating from the ^{15}N-labeled pool with respect to total N in the gas sample (*Fp*) as well as the ^{15}N enrichment of the active ^{15}N-labeled N pool (a_p) producing N_2O (a_{p_N2O}) or $N_2 + N_2O$ (a_{p_N2+N2O}) were calculated after Spott et al. (2006) as described in Lewicka-Szczebak et al. (2017).

Harvest and analyses of plant and soil material

Before labeling (44 days after transplanting), replicates 1–6 were harvested. Eleven days after ^{13}C labeling (57 days after transplanting), all labeled plants (replicates 7–12) were harvested. At both harvests, plants were separated into shoots and roots including maize crown roots. As all pots were densely rooted, a separation of rhizosphere and bulk soil was not possible. Roots were shaken gently to separate them from soil and washed. From replicates 7–12, a subsample of root washing water was analyzed for water-extractable organic C (WEOC) and the amount of ^{13}C lost during root washing was determined. To estimate their amount in soil, fine roots were picked by hand from a subsample of soil (~ 400 g soil) for a defined time. All plant material and a soil subsample were dried at 60 °C, milled in a ball mill and analyzed for total C, ^{13}C, total N, and ^{15}N content using an elemental analyzer coupled to a gas-isotope ratio mass spectrometer as described earlier. For determination of water-extractable organic C (WEOC) content, a subsample of fresh soil was analyzed according to Chantigny et al. (2007). Briefly, fresh soil was

homogenized with deionized water (1:2 w/v). Samples were centrifuged and filtered with 0.45 µm polyether sulfone filters (Labsolute, Renningen, Germany), split in two subsamples and stored at -20 °C. The extracts were analyzed for total C, organic C, and total N content using a multi N/C® Analyzer (Analytik Jena, Jena, Germany).

For determination of soil mineral N content, a subsample of 50 g was frozen at -20 °C. Frozen samples were extracted with a 2 *M* KCl solution (1:5 w/v) for 60 min on an overhead shaker (85 rpm). The extracts were filtered with 615 ¼ filter paper (Macherey – Nagel GmbH & Co. KG, Düren, Germany), split in two subsamples, and stored at -20 °C. The extracts were analyzed colorimetrically for the concentrations of NO_3^- and NH_4^+ using a San^{++} continuous flow Analyzer (Skalar Analytical B.V., Breda, The Netherlands). ^{15}N concentration in NH_4^+ and NO_3^- was analyzed using an automated sample preparation unit for inorganic nitrogen coupled to a membrane inlet quadrupole mass spectrometer (QMS, GAM 200, InProcess, Bremen, Germany) as described in detail by Eschenbach et al. (2017, 2018). In parallel subsamples, soil water content was determined by oven drying at 105 °C.

Calculations and statistics

Plant N uptake was calculated by multiplying dry mass with the respective tissue N content (root, shoot).

Cumulative CO_2 emissions were calculated from CO_2 trapped in NaOH, CO_2 fluxes were calculated by dividing cumulative CO_2 through the respective trapping time (6, 12, or 24 h). ^{13}C recovery in CO_2 ($^{13}C_{recovery;\ CO2}$, mg) was calculated as the excess (above background) ^{13}C concentration multiplied with the total CO_2 trapped (CO_2, mg CO_2-C):

$$^{13}C_{recovery;CO_2} = (^{13}C_{CO_2} - {}^{13}C_{NA;CO_2}) * CO_2 \quad (1)$$

where $^{13}C_{CO2}$ is the ^{13}C enrichment of CO_2 (at%) trapped after labeling and $^{13}C_{NA;\ CO2}$ is the natural ^{13}C background (at%) from unlabeled plants (replicates 14–19). ^{13}C recovery in soil ($^{13}C_{recovery;\ soil}$, mg) was calculated as follows:

$$^{13}C_{recovery;soil} = (^{13}C_{soil} - {}^{13}C_{NA;soil}) * C_{soil} * mass_{soil} \quad (2)$$

where $^{13}C_{soil}$ is the enrichment of ^{13}C (at%) of the soil C pool after labeling, $^{13}C_{NA}$; soil is the natural abundance of ^{13}C in soil before labeling (at%), C_{soil} is the total content of C in soil (mg g^{-1}), and $mass_{soil}$ is the mass of soil per pot (g). *Relative* $^{13}C_{recovery}$ (% of recovered ^{13}C) of a particular pool (CO_2, soil) was calculated by dividing the amount of ^{13}C recovered in that pool ($^{13}C_{recovery;\ pool}$) by the sum of the amount of ^{13}C recovered in all pools (CO_2, shoot, root, soil, root washing water).

$$Relative\,^{13}C_{recovery} = \frac{^{13}C_{recovery;pool}}{\sum {}^{13}C_{recovery;pool}} * 100 \quad (3)$$

Total N_2O fluxes (f_{tot}, µg N kg^{-1} h^{-1}) were calculated from GC measurements:

$$f_{tot} = \frac{(C_H - C_B)}{t} * \frac{V}{m} \quad (4)$$

where C_H is the mass concentration in the headspace and C_B is the background concentration in the reference pot (µg N m^{-3}) corrected by the chamber temperature according to the ideal gas law, t is the accumulation time (h), V is the volume of the headspace (m^3), and m is the dry mass of soil per pot (kg).

We calculated the ^{15}N enrichment of the active NO_3^- pool undergoing denitrification (ap_N_2O, $ap\_N_2O + N_2$) from the non-random distribution of N_2 and/or N_2O isotopologues using calculations by Spott et al. (2006) as described by Buchen et al. (2016) and Lewicka-Szczebak et al. (2017):

$$a_p = \frac{^{30}X_m - a_{bgd} * a_m}{a_m - a_{bgd}} \quad (5)$$

where a_p is the ^{15}N abundance of the ^{15}N labeled NO_3^- pool undergoing denitrification, a_{bgd} is the measured ^{15}N abundance of atmospheric background N_2, a_m is the measured ^{15}N abundance of N_2 or N_2O

$$a_m = \frac{^{29}R + 2 * {}^{30}R}{2(1 + {}^{29}R + {}^{30}R)} \quad (6)$$

and $^{30}x_m$ is the measured fraction of m/z 30 in N_2 and converted N_2O:

$$^{30}x_m = \frac{^{30}R}{1 + {}^{29}R + {}^{30}R} \quad (7)$$

The fraction of N derived from the active NO_3^- pool (*Fp*) was calculated using Eq. (8) if ^{30}R was significant and otherwise Eq. (9) was used. In the latter case, ap_N_2O was assumed to be identical with ap_N_2 and $ap\_N_2O + N_2$ and was thus used when calculating

Fp_N_2 and $Fp\_N_2O + N_2$ from Eq. 9. If ap_N_2O of a sampling date was not available, the mean value from the other replicates from the same sampling date was used as best estimate. *Fp* calculated from Eq. (9) with a given ^{29}R is relatively insensitive to changes in *ap* between 0.4 and 0.6 since the nominator yields values between 0.48 and 0.5. Hence, uncertainty in the estimation of *ap* within that range causes minor uncertainty in calculated *Fp* (Well and Myrold 1999). Because *ap* values in our study were typically between 0.4 and 0.6, we assume that uncertainty in *Fp* calculation from the missing of individual *ap* values was small.

$$Fp = \frac{a_m - a_{bgd}}{a_p - a_{bgd}} \tag{8}$$

$$Fp = ({}^{29}R_{sa} - {}^{29}R_{bgd})/(2a_p(1 - a_p)) \tag{9}$$

where lower case *sa* and *bgd* denote sample and background (ambient air), respectively.

Fp values were multiplied with respective total sample N concentration (N_2O, N_2) to obtain pool-derived gas concentrations (in ppm). Then, pool-derived fluxes (*fp*) were calculated from concentrations similar to Eq. (4). The same calculations were used for N_2O, N_2, and $N_2O + N_2$, resulting in respective values for fractions of pool-derived N and for the respective ^{15}N abundances of the active N pools (ap_N_2O, ap_N_2, $ap\_N_2O + N_2$). Non-pool derived N_2O fluxes were calculated by subtracting pool-derived N_2O fluxes from total N_2O fluxes.

The ratio of denitrification end products was calculated from pool-derived N_2O (fp_N_2O) and $N_2O + N_2$ ($fp\_N_2O + N_2$):

$$Product\ ratio = \frac{fp\_N_2O}{fp\_N_2O + N_2} \tag{10}$$

Cumulative N_2O, N_2, and $N_2O + N_2$ emissions were calculated by linear interpolation of fluxes. The % of $N_2 + N_2O$ emitted with respect to added N was estimated by dividing cumulative pool-derived $N_2O + N_2$ emission by the amount of N added with $^{15}NO_3^-$ labeling.

All statistical analyses were performed using the statistical software R version 3.6.0 (R Core Team 2019). Means and standard deviations were calculated over all replicates. For harvest data, cumulative CO_2, and ^{13}C recovery a one-way ANOVA was calculated followed by Tukey's HSD post-hoc test at $p \leq 0.05$ to separate treatment effects. As cumulative N emissions were not normally distributed, the Kruskal-Wallis rank sum test was used followed by LSD post-hoc test at $p \leq 0.05$ to separate treatment effects. To compare soil and plant samples between harvests, and to test whether soil $^{15}a\_NO_3^-$ contents at final harvest and ap_N_2O or $ap\_N_2O + N_2$ at last sampling date differed, a t-test was used at $p \leq 0.05$. Simple linear regression models were tested to analyze the effects of soil and plant parameters on CO_2 and N fluxes and cumulative emissions.

Results

Plant growth after labeling

Shoot dry matter increased significantly in all treatments between the first and the second harvest, but differences between treatments did not change (Table 2, results of 1st harvest are displayed in supplementary table S1). Root dry matter significantly decreased in Maize S and M until the end of the experiment. Increases in root dry matter in Maize L and Cup plant were not significant. Root:shoot ratio decreased in all treatments but remained twice as high in cup plant compared to maize, which is typical for perennial plants compared to annual plants (Husáková et al. 2018). Nitrogen content increased in previously unfertilized Maize S plants and was similar in all maize treatments at the final harvest. Nitrogen content in cup plant shoots and roots was significantly greater than in all maize treatments and total N uptake corresponded with N fertilization Maize L > Maize M = Cup plant > Maize S. Soil NO_3^- content analyzed at the end of experiment was on average still twice as high in Maize S compared to all other treatments.

Total N_2O and pool-derived $N_2O + N_2$ fluxes and cumulative emissions

Total and pool-derived N fluxes were highest in Maize S but followed a similar pattern in all treatments (Fig. 2a + c). Total N_2O fluxes strongly increased in Maize S reaching highest values on day 3 (11.3 μg N_2O-N kg^{-1} h^{-1}, Fig. 2a) and a second smaller peak on day 6 (5.9 μg N_2O-N kg^{-1} h^{-1}). Pool-derived $N_2O + N_2$ fluxes followed a similar general pattern as total N_2O fluxes (Fig. 2c) and peaks were detected at similar times as N_2O peaks, in Maize S on day 3 (48 μg N_2-N kg^{-1} h^{-1}) and larger

Table 2 Harvest data of final harvest at the end of the experiment (replicates 7–12, 57 days after transplanting/ 11 days after $^{13}CO_2$ labeling)

	Maize S			Maize M			Maize L			Cup Plant		
Shoot dry matter (g pot^{-1})	63.5 ± 2.7	c	***	89.8 ± 4.0	b	***	115.3 ± 10.8	a	***	34.9 ± 5.4	d	***
Root dry matter (g pot^{-1})	10.1 ± 1.6	c	***	14.5 ± 0.8	b	***	21.5 ± 4.2	a		19.7 ± 1.8	a	
Root : Shoot ratio	0.16 ± 0.02	b	***	0.16 ± 0.01	b	***	0.19 ± 0.02	b	***	0.58 ± 0.09	a	
Shoot N content (%)	1.19 ± 0.04	b	***	1.14 ± 0.08	b	***	1.05 ± 0.14	b		2.47 ± 0.28	a	
Root N content (%)	1.06 ± 0.10	b	***	0.97 ± 0.08	b		0.95 ± 0.08	b		1.59 0.19	a	
NO_3^- content (mg kg^{-1})	3.39 ± 2.01	a		1.54 ± 1.02	ab		1.04 ± 0.43	b		1.61 ± 0.83	ab	
NH_4^+ content (mg kg^{-1})	1.46 ± 0.19	ab	***	1.94 ± 0.36	a	***	1.64 ± 0.25	ab	***	1.41 ± 0.39	b	***
WEOC content (mg kg^{-1})	6.65 ± 0.95	a	***	7.35 ± 0.66	a	***	7.56 ± 2.69	a	***	8.10 ± 1.24	a	***
N uptake (shoot + root) (g pot^{-1})	0.86 ± 0.35	c		1.16 ± 0.087	b		1.41 ± 0.054	a		1.17 ± 0.044	b	

Shoot dry matter includes cob dry matter

Different letters in one row indicate a significant difference ($p < 0.05$) between treatments

***indicates a significant difference ($p < 0.05$) to first harvest

peaks on day 6.5 (67 μg N_2-N kg^{-1} h^{-1}) and day 9.5 (61 μg N_2-N kg^{-1} h^{-1}). Total N_2O and pool-derived $N_2O + N_2$ fluxes in all other treatments followed a similar pattern but on a lower scale. The product ratio (N_2O/($N_2O + N_2$), Fig. 2d) of pool-derived fluxes followed a similar pattern in all treatments. The product ratio decreased for the first days after onset of incubation reaching values between 0.2 and 0.5 as N_2 became the dominant end product of denitrification. It shortly increased and peaked on day 3.5, then decreased again until day 6 to values between 0 and 0.2. After day 6.5, the product ratio ranged between 0 and 0.5 until the end.

Total and pool-derived cumulative N emissions were 20–43 times higher in Maize S compared to all other treatments (Table 3). No significant differences were detected between the other treatments. Similarly, recovery of added NO_3^- in $N_2O + N_2$ was highest in Maize S and not significantly different in the other treatments. The mean $N_2O/(N_2O + N_2)$ ratio ranged from 0.14 to 0.16 in maize treatments and was 0.24 in cup plant.

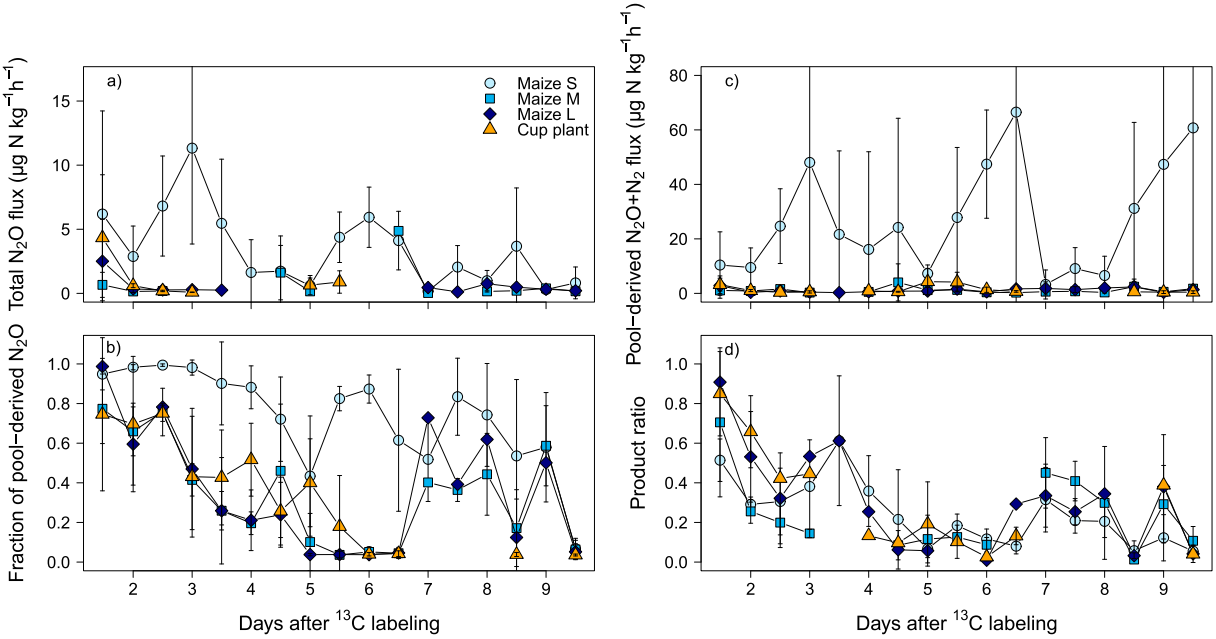

Fig. 2 Total N_2O fluxes (**a**), fraction of pool-derived N_2O (Fp_N_2O) (**b**), pool-derived $N_2O + N_2$ fluxes (**c**), and product ratio $N_2O/(N_2O + N_2)$ of pool-derived fluxes (**d**). Means and standard deviation for n = 1–6

Table 3 Total cumulative N_2O, pool-derived cumulative N_2 and $N_2O + N_2$ emissions, and product ratio of pool-derived fluxes

	Total cumulative N_2O		Pool-derived cumulative N_2		Pool-derived cumulative $N_2O + N_2$		$N_2O/(N_2O + N_2)$ ratio of cumulative pool-derived emissions		^{15}N recovered in $N_2O + N_2$	
	($\mu g\ N\ kg^{-1}$)		($\mu g\ N\ kg^{-1}$)		($\mu g\ N\ kg^{-1}$)				% of added ^{15}N	
Maize S	643.3 ± 310.1	a	4219.0 ± 3963.6	a	4830.4 ± 4235.1	a	0.16 ± 0.06	n.s.	0.690 ± 0.605	a
Maize M	25.3 ± 40.0	b	132.9 ± 110.9	b	159.2 ± 132.9	b	0.15 ± 0.07	n.s.	0.023 ± 0.019	b
Maize L	15.4 ± 25.4	b	97.7 ± 96.4	b	120.7 ± 114.8	b	0.14 ± 0.14	n.s.	0.017 ± 0.016	b
Cup Plant	31.6 ± 39.5	b	105.5 ± 101.0	b	128.7 ± 122.0	b	0.24 ± 0.21	n.s.	0.018 ± 0.017	b

Means and standard deviation for n = 6. Different letters in one column indicate a significant difference, n.s. indicates no significant difference ($p < 0.05$) between treatments

^{15}N enrichment of N pools and pool-derived fraction of N_2O

Treatments did not exhibit continuous patterns of ap and Fp values throughout the experiment. The fraction of N_2O derived from the active labeled NO_3^- pool (Fp_N_2O) decreased during the experiment showing that the contribution of N_2O from sources other than the labeled NO_3^- pool increased with time (Fig. 2b). Fp_N_2O was close to 1.0 in Maize S for three days after labeling, then decreased to 0.4 on day 5, and ranged between 0.5 and 0.8 until the end of the experiment. For the other treatments, Fp_N_2O continuously decreased until day 5/6. After day 6.5, Fp_N_2O increased in Maize M and L, fluctuating between 0.1 and 0.6. At the last day, Fp_N_2O was < 0.07 in all treatments.

The time course of ^{15}N enrichment of the active NO_3^- pool producing N_2O and N_2 (ap_N_2O, $ap\_N_2O + N_2$) was different in Maize S than in the other treatments. During the first days after labeling, ^{15}N enrichment of the active NO_3^- pool producing N_2O and N_2 (ap_N_2O, $ap\_N_2O + N_2$) was close to 60 at% in all treatments (Fig. 3). In Maize S, ap_N_2O and $ap\_N_2O + N_2$ were higher than 50 at% during the whole experiment and only decreased on the last day. In all other treatments, ap_N_2O and $ap\_N_2O + N_2$ continuously decreased until day 6.5. On day 7, ap-values were higher than 50 at% and decreased again until the end of the experiment. ^{15}N enrichment of the total soil NO_3^- pool ($^{15}a\_NO_3^-$) was measured at final harvest and was mostly significantly lower ($p < 0.05$) than ^{15}N enrichment of the active NO_3^- pool producing N_2O and N_2 (ap_N_2O and $ap\_N_2O + N_2$) from the last gas measurement (Fig. 3, Supplementary table S2).

Soil moisture and its effect on N fluxes

Data from soil moisture sensors showed that soil moisture content was higher in Maize S than all other treatments for the first days after labeling (Fig. 4). However, it was lower than the targeted value of 75%WFPS. As all plants respired large amounts of water, it took a few days to adjust irrigation amounts to plant water demand, and soil moisture could not be kept constant throughout the experiment.

In Maize S and Cup plant, soil moisture increased with irrigation three days after labeling reaching values around 70%WFPS. In Maize M and L, soil moisture increased five days after labeling reaching values around 55%WFPS. In Cup plant, soil moisture stayed on a similar level around 70%WFPS with fluctuations due to water uptake and irrigation. Although soil moisture was in a similar range in Maize S and Cup plant from day 4 to 6 and in all maize treatments after day 7, total N_2O and pool-derived $N_2O + N_2$ fluxes were always highest in Maize S. Thus, we did not find significant relationships between N fluxes and soil moisture during the experiment indicating that soil moisture was not the only factor controlling gaseous N losses (Table 5, supplementary table S3, supplementary figure S1). However, the $N_2O/(N_2O + N_2)$ ratio of pool-derived fluxes decreased with increasing soil moisture (%WFPS, adj. R^2=0.14, $p < 0.05$) indicating that increasing soil moisture stimulated N_2O reduction.

CO_2 and $^{13}CO_2$ efflux

The time course of cumulative CO_2 efflux and ^{13}C enrichment in CO_2 was similar in all treatments (Fig. 5a + b). CO_2 efflux was similar in Maize M and Maize L where it increased almost linearly during the

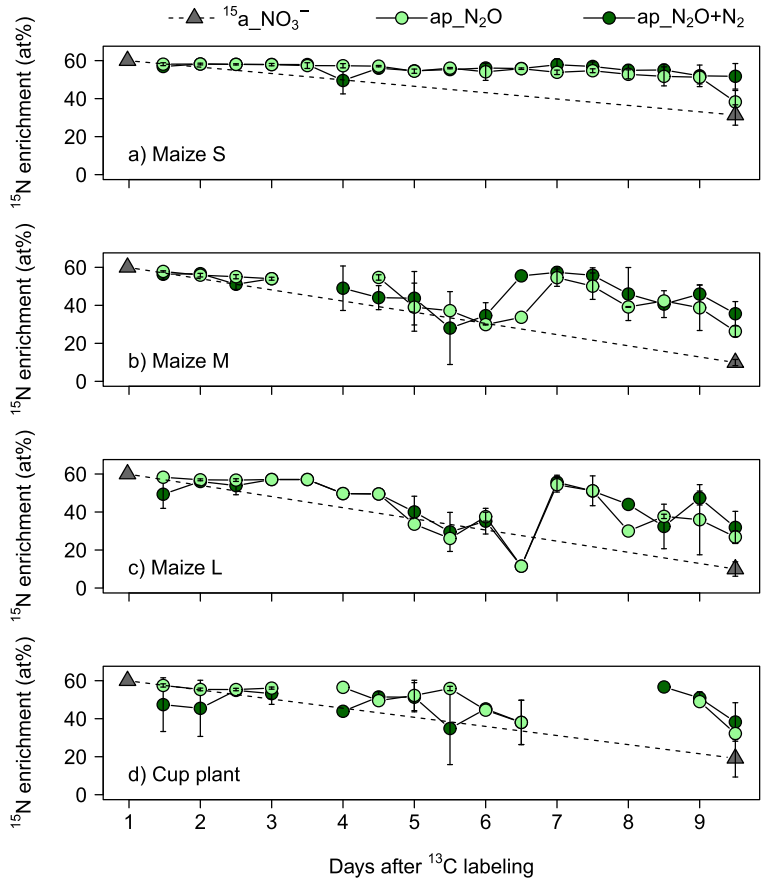

Fig. 3 ^{15}N enrichment of the active N pool undergoing denitrification (ap_N_2O, ap_N_2O+N_2) and ^{15}N enrichment of total soil NO_3^- pool ($^{15}a\_NO_3^-$). Means and standard deviation for n =6. When not visible, error bars are smaller than the symbols

whole experiment, and total cumulative CO_2 was only slightly higher in Maize L than in Maize M (Table 4). Lowest cumulative CO_2 efflux was measured under Maize S plants where efflux decreased considerably after 1.5 days. Cumulative CO_2 efflux under cup plants was significantly lower than from Maize L and not statistically different from the other two maize treatments (Table 4). Overall, cumulative CO_2 efflux was positively correlated with root dry matter at final harvest (adj. R^2=0.36, $p < 0.01$, Table 5).

^{13}C enrichment in CO_2 strongly decreased in all treatments two days after labeling until the end of CO_2 sampling (Fig. 5b). Highest ^{13}C enrichment of soil emitted CO_2 was measured under Cup plant and lowest in Maize L. It strongly decreased two days after labeling until the end of CO_2 sampling. No statistically significant differences ($p < 0.05$) were found in relative ^{13}C recovery in CO_2, soil, or soil + CO_2 (Table 4), but overall, mean relative ^{13}C recovery in soil increased with root dry matter, indicating that root-derived C recovered in soil increased with root biomass.

Interactions between N emissions, soil NO_3^- content, and C_{org} availability

Simple linear regression models were tested to identify effects of plant growth, N uptake, and C_{org} availability on cumulative N_2O and N_2 emissions (Table 5, supplementary table S3). Total cumulative N_2O and pool-derived cumulative $N_2O + N_2$ emissions were significantly ($p < 0.01$) negatively correlated with root dry matter (adj. R^2=0.41 and adj. $R^2 = 0.32$, respectively) and plant N uptake (adj. R^2=0.49 and adj. $R^2 = 0.33$, respectively) indicating that gaseous N losses were highest under plants with small root system and low N uptake. In addition, total cumulative N_2O emissions and soil NO_3^- content at final harvest were positively correlated (adj. R^2=0.10, $p < 0.05$). As cumulative CO_2 emissions were positively correlated with root dry matter and cumulative N emissions negatively, total cumulative N_2O and pool-derived cumulative $N_2O + N_2$ emissions were negatively correlated with cumulative CO_2 efflux (adj. R^2=0.36, $p < 0.01$). No correlations were found

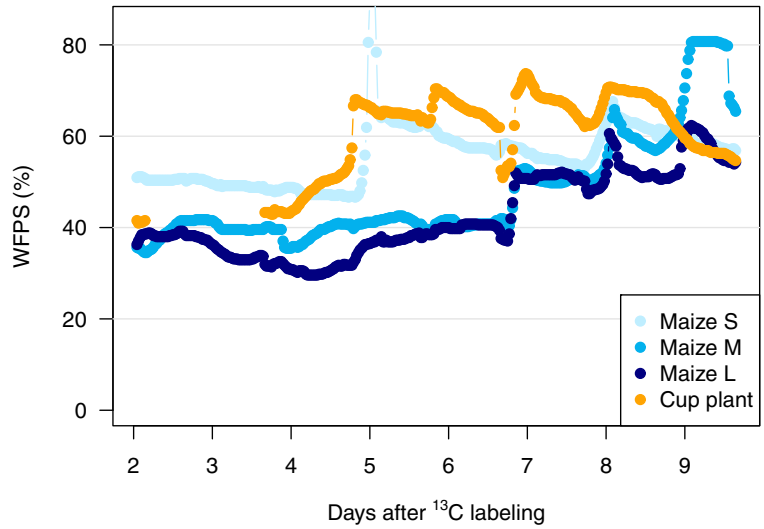

Fig. 4 Soil moisture as % water filled pore space measured with soil moisture sensors (n = 1)

between total or pool-derived cumulative N emissions or $N_2O/(N_2O + N_2)$ ratio and ^{13}C recovery in soil and/or CO_2. However, we identified a weak but significant positive relationship between $N_2O/(N_2O + N_2)$ of pool-derived fluxes and CO_2 efflux (adj. R^2=0.11, $p < 0.01$).

Discussion

Effect of soil moisture and plant N uptake on N_2O and N_2 emissions

Cumulative N emissions were 20–40 times higher in Maize S compared to all other treatments. Plant transpiration strongly affected soil moisture which was highest in Maize S during the first 4 days after ^{13}C labeling. Soil moisture is an important control of denitrification as it regulates O_2 concentration and diffusion in soil (Schlüter et al. 2018, Rohe et al. 2020). Plant roots constantly alter soil moisture and its distribution in soil by root water uptake. Accordingly, previous studies reported that plant growth controlled soil moisture and denitrification rates (Bakken 1988; von Rheinbaben and Trolldenier 1984). In our study, when soil moisture increased with irrigation, N_2O and, especially, N_2 fluxes increased shortly thereafter. Furthermore, the $N_2O/(N_2O + N_2)$ ratio of pool-derived fluxes decreased with increasing soil moisture which is consistent with N_2 being the dominant end product of denitrification under high WFPS (Davidson 1991). Although soil moisture was highest in Cup plant from day 4 to 8, and similar in Maize treatments from day 6 to 9, N fluxes were highest in Maize S throughout the experiment. Thus, differences in soil moisture alone cannot explain the vast differences in N fluxes between Maize S and the other treatments in our study.

Maize S plants were characterized by lowest root dry matter of all treatments and lower shoot dry matter compared to the other maize treatments. Furthermore, soil NO_3^- content at final harvest was more than twice as high in Maize S compared to all other treatments. The relationship between soil NO_3^- content at the end of the experiment and cumulative N_2O emissions was weak (0.10, $p < 0.05$). However, N uptake was negatively correlated with total cumulative N_2O emissions and pool-derived cumulative $N_2O + N_2$ emissions (adj. R^2=0.49, $p < 0.001$ and adj. $R^2 = 0.33$, $p < 0.01$, respectively) indicating that NO_3^- availability played an important role in regulating denitrification. Our results show clearly that an increase in soil moisture led to increasing $N_2O + N_2$ fluxes, but N fluxes remained on a low level when NO_3^- availability was low due to rapid plant N uptake. Only when both N and water uptake were low, high NO_3^- availability and high soil moisture led to strongly increased N losses.

Effect of nitrate availability and soil moisture on pools and processes contributing to N_2O formation

Different NO_3^- pools and N turnover processes contributed to N_2O formation throughout the experiment. The ^{15}N enrichment of the total soil NO_3^- pool ($^{15}a\_NO_3^-$) decreased from 60 at% after labeling to 10–30 at% until the end of the experiment as unlabeled organic N was

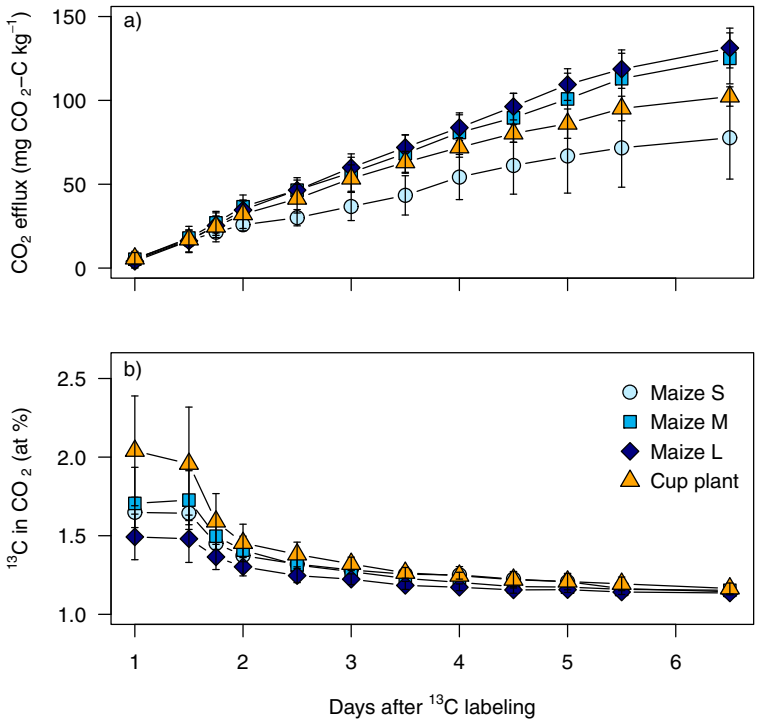

Fig. 5 Cumulative CO_2 efflux from soil (**a**) and ^{13}C enrichment of CO_2 flux from soil (**b**). Means and standard deviation for n = 6. When not visible, error bars are smaller than the symbols

mineralized and diluted the labeled NO_3^- pool (Buchen et al. 2016; Deppe et al. 2017). Thus, denitrification of unlabeled NO_3^-, as well as nitrification, nitrifier denitrification, and coupled nitrification-denitrification may have contributed to N_2O formation (van Groenigen et al. 2015; Wrage-Mönnig et al. 2018; Wrage et al. 2001).

In Maize S, $^{15}a\_NO_3^-$ at final harvest was significantly higher than in all other treatments, indicating that nitrification was less relevant in this treatment. Accordingly, the fraction of N_2O derived from the active labeled NO_3^- pool (Fp_N_2O) was > 0.5 throughout the experiment indicating that most N_2O was lost through denitrification from labeled NO_3^-. The ^{15}N enrichment of the active NO_3^- pool producing N_2O and $N_2O + N_2$ (ap_N_2O and $ap\_N_2O + N_2$) stayed close to its initial value of 60 at% highlighting that N_2O and N_2 were mainly lost from anoxic microsites where labeled NO_3^- had not been diluted by nitrification (Buchen et al. 2016).

In the other treatments, ap_N_2O, ap_N_2, and Fp_N_2O did not exhibit continuous trends. While values first decreased due to dilution with NO_3^- from nitrification, values increased after day 6.5 (Fig. 3), presumably due to the slight increase in WFPS after irrigation on day 6.5 (Fig. 4). Increasing soil moisture increased denitrification rates in anoxic hotspots, which corresponds with increasing N_2O and especially N_2 fluxes in Maize M and L. At the same time, it restricted nitrification and thus decreased the share of nitrification-dependent processes

Table 4 Cumulative CO2 emissions, relative 13C recovery in soil and CO2

	Cumulative CO_2		relative ^{13}C recovery in CO_2		relative ^{13}C recovery in soil		relative ^{13}C recovery in soil + CO_2	
	(mg C kg^{-1})		(% of recovered ^{13}C)		(% of recovered ^{13}C)		(% of recovered ^{13}C)	
Maize S	77.7 ± 24.6	c	4.24 ± 1.16	n.s.	0.57 ± 3.11	n.s.	4.81 ± 3.72	n.s.
Maize M	125.0 ± 15.2	ab	5.83 ± 3.34	n.s.	1.11 ± 5.71	n.s.	6.94 ± 3.73	n.s.
Maize L	131.2 ± 11.9	a	3.73 ± 1.38	n.s.	3.37 ± 1.62	n.s.	7.10 ± 1.62	n.s.
Cup Plant	102.3 ± 5.8	bc	4.57 ± 1.83	n.s.	2.13 ± 1.37	n.s.	6.70 ± 1.87	n.s.

Mean and standard deviation for n = 6. Different letters in one column indicate a significant difference, n.s. indicates no significant difference ($p < 0.05$) between treatments

Table 5 Results of simple linear regressions between parameters

response	predictor	adjusted R^2	*p*-value	n
total N_2O flux	CO_2 flux	-0.0136	0.8788	74
pool-derived $N_2O + N_2$ flux	CO_2 flux	0.0157	0.1458	74
mean total N_2O flux	% water-filled pore space	-0.0252	0.9179	35
mean pool-derived $N_2O + N_2$ flux	% water-filled pore space	-0.0158	0.5111	35
$N_2O/(N_2O + N_2)$ ratio (pool-derived)	**% water-filled pore space**	**0.1365**	**0.01402**	**35**
Cumulative CO_2	**DM Root 2nd harvest**	**0.3574**	**0.00121**	**24**
Total cumulative N_2O	**DM Root 2nd harvest**	**0.4090**	**0.00046**	**24**
Pool-derived cumulative $N_2O + N_2$	**DM Root 2nd harvest**	**0.3210**	**0.00231**	**24**
Total cumulative N_2O	**Plant N uptake**	**0.4894**	**8.5×10^{-05}**	**24**
Pool-derived cumulative $N_2O + N_2$	**Plant N uptake**	**0.3300**	**0.00197**	**24**
Total cumulative N_2O	**Soil NO_3^- content 2nd harvest**	**0.0983**	**0.0477**	**24**
Pool-derived cumulative $N_2O + N_2$	Soil NO_3^- content 2nd harvest	0.0131	0.2656	24
Total cumulative N_2O	**cumulative CO_2**	**0.3573**	**0.00121**	**24**
Pool-derived cumulative $N_2O + N_2$	**cumulative CO_2**	**0.3641**	**0.00107**	**24**
Total cumulative N_2O	recovered ^{13}C in soil + CO_2	-0.0068	0.3683	24
Pool-derived cumulative $N_2O + N_2$	recovered ^{13}C in soil + CO_2	0.0051	0.302	24
$N_2O/(N_2O + N_2)$ ratio (pool-derived)	recovered ^{13}C in soil + CO_2	-0.0389	0.7138	24

Significant regressions ($p \leq 0.05$) are marked in bold

contributing to N_2O formation (reflected in increasing Fp_N_2O). Simultaneously increasing ap_N_2O and ap_$N_2O + N_2$ in Maize M and L on Day 7 indicate that ^{15}N enrichment of the active labeled NO_3^- pool was still close to 60 at% in microsites where denitrification took place. The rise of actual ap-values back towards initial values is in line with a change in the anaerobic volume where denitrification occurs (Bergstermann et al. 2011). A recent study conducted with the same soil but without plants showed that O_2 concentration in repacked soil cores was highly variable and average O_2 saturation decreased with increasing soil moisture while the anaerobic soil volume fraction increased with increasing soil moisture and soil depth (Rohe et al. 2020). After day 6.5, $N_2O + N_2$ were predominately lost from domains that had been continuously anoxic or were most distant from oxic domains and thus were less diluted with unlabeled NO_3^-. We anticipate that in these microsites O_2 concentrations had been low enough to prevent nitrification during the first days so that the labeled pool was not diluted by unlabeled NO_3^- from nitrification.

While fungal co-denitrification has been reported as the dominant N_2O source in a planted soil with high NO_3^- content (Laughlin and Stevens 2002), our data provide no indications for co-denitrification, because ap_N_2O and ap_$N_2O + N_2$ were always higher than ^{15}a_NO_3^-, but co-denitrification would lead to ap lower than ^{15}a_NO_3^- due to hybrid formation of N_2O or N_2 (Spott and Stange 2007).

Thus, in our study, N_2O and N_2 fluxes mainly derived from denitrification of labeled $^{15}NO_3^-$ in anoxic microsites, while nitrification simultaneously occurred in more oxic parts of the soil, potentially contributing to formation of unlabeled N_2O.

Effect of root-derived C on N emissions

One of the core hypotheses of this study was that availability of root-derived C is a key driver of denitrification in planted soils. It was based on a number of studies reporting higher denitrification activity in rhizosphere compared to bulk soil which was explained by higher soil C (Hamonts et al. 2013; Klemedtsson et al. 1987; Malique et al. 2019 ; Smith and Tiedje 1979). Detectable rhizodeposition and C flow into belowground respiration result from C translocation from shoots to roots (Remus and Augustin 2016). Thus, we used $^{13}CO_2$ pulse labeling to trace C translocation from shoots to roots, its release by roots into the soil, and $^{13}CO_2$ emitted from soil.

We found a positive correlation between root dry matter at final harvest and cumulative CO_2 efflux

(adj. R^2=0.36, $p < 0.01$) and, on average, relative ^{13}C recovery in soil increased with increasing root dry matter indicating that root exudation increased with root dry matter. However, we could not detect any relationship between total or pool-derived N fluxes and total CO_2 efflux or root-derived C and cumulative N emissions or the ratio of gaseous products.

Most previous studies investigating plant effects on denitrification did not measure N_2O and N_2 emissions under growing plants, but either potential denitrification (Klemedtsson et al. 1987; Malique et al. 2019; Smith and Tiedje 1979) or denitrification capacity (Hamonts et al. 2013) from soil samples taken from rhizosphere and/or bulk soil. In those studies, conducted under anoxic conditions with unlimited NO_3^- supply, higher C availability in rhizosphere soil samples led to higher emissions of N_2O and N_2. However, when separation of bulk soil and rhizosphere was not well-defined due to densely rooted soil in pots, no differences in potential denitrification were found (Malique et al. 2019). In the few studies with growing plants, higher denitrification rates were measured during the first weeks after emergence (Senbayram et al. 2020), with poorly growing plants (von Rheinbaben and Trolldenier 1984), or when root biomass was decreasing (Haider et al. 1987). No stimulation of denitrification was found when actively growing maize plants were compared to unplanted soil (Haider et al. 1985). Accordingly, root-derived C may stimulate denitrification when soil NO_3^- is not limited. We were not able to estimate the effect of C availability on denitrification as NO_3^- limitation and O_2 inhibition were the factors controlling denitrification in our study.

Plant root effects on denitrification

The activity of denitrifying microorganisms in soil is primarily controlled by availability of O_2, NO_3^-, and C_{org} (proximal factors, Groffman et al. 1988). Plant roots affect these through rhizodeposition, root respiration, N and water uptake (distal factors, Groffman et al. 1988). Figure 6 shows how proximal and distal factors change during plant and root growth and how these

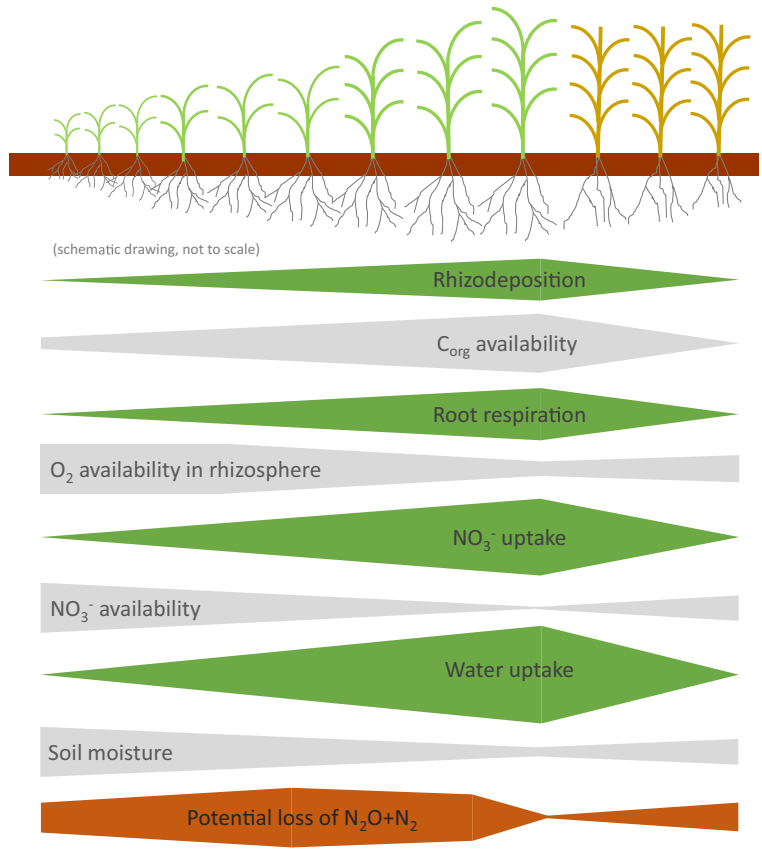

Fig. 6 Conceptual drawing of plant root effects (distal factors, green/grey) on drivers of denitrification (proximal factors, light grey) and potential $N_2O + N_2$ losses (orange/dark grey). Based on two assumptions: (i) NO_3^--based fertilizer is only added before plant growth and (ii) root water uptake is the main regulator of soil moisture

affect denitrification in planted soil. The presented conceptual drawing is based on two assumptions: (i) NO_3^--based fertilizer is only added before plant growth and (ii) root water uptake is the main regulator of soil moisture.

In most agricultural soils, available C_{org} is low. With increasing root growth, rhizodeposition and root turnover increase C_{org} availability. At the same time, root respiration and microbial activity increase, decreasing O_2 concentrations in the rhizosphere. This offers favorable conditions for denitrifying microorganisms as long as sufficient NO_3^- is available (Klemedtsson et al., 1987; Senbayram et al., 2020; Stefanson, 1972). As N uptake increases with plant and root growth, NO_3^- becomes limited for denitrifiers (Haider et al., 1985). Furthermore, increasing water uptake decreases soil moisture and restricts formation of anoxic microsites for denitrification (Bakken 1988, von Rheinbaben and Trolldenier, 1984). Accordingly, our study showed that with increasing plant and root growth, plant water and N uptake became the most important controls of denitrification. Similarly, soil moisture can vary strongly under field conditions depending on precipitation and plant water uptake. When NO_3^- is available (i.e. after fertilization), increasing soil moisture after rainfall can lead to strongly increased $N_2O + N_2$ emissions (Buchen et al. 2017, Ruser et al. 2017).

Overall, plants continuously change boundary conditions and substrate availability for denitrification in soil, and it requires high technical input and equipment to keep experimental conditions stable and controlled. However, as plants do control these conditions so strongly, it is very exciting and very important to further investigate these processes to understand and predict N cycling, denitrification, and gaseous N losses on the field scale. Further research is thus indispensable.

Conclusions

We aimed to investigate how plants control the main substrates for denitrification (NO_3^- and C_{org}) through N uptake and root exudation. To our knowledge, this is the first study combining in situ measurements of $N_2O + N_2$ fluxes with estimations of root-derived C availability.

Plant water uptake was a main factor controlling soil moisture and, thus, daily $N_2O + N_2$ fluxes, cumulative N emissions, and N_2O production pathways. However, N fluxes remained on a low level when NO_3^- availability was low due to rapid plant N uptake. Only when both N and water uptake were low, high NO_3^- availability and high soil moisture led to strongly increased N losses. Our study provides evidence that most N losses originated from denitrification in small anoxic hotspots where NO_3^- was not diluted by nitrification. Simultaneously occurring nitrification in oxic parts of the soil potentially contributed to formation of unlabeled N_2O.

Total CO_2 efflux was positively correlated with root dry matter, but there was no indication of any relationship between recovered ^{13}C from root exudation and cumulative N emissions. We anticipate that higher C_{org} availability in pots with large root systems did not lead to higher denitrification rates, as NO_3^- was limiting denitrification due to plant N uptake. Overall, we conclude that root-derived C stimulates denitrification only when soil NO_3^- is not limited and low O_2 concentrations enable denitrification.

Acknowledgements The authors thank Simone Urstadt, Finn Malinowski, Bridith Angulo Schipper, Ilse Thaufelder, and Jakob Streuber for help during experimental and laboratory work. We are thankful to Tomor Krasniqi for weighing endless numbers of C:N samples and to the Centre for Stable Isotope Research and Analysis of the University of Göttingen for analysis of C and N isotopes of soil and plant samples. Further, we thank Dr. Daniel Ziehe and Kerstin Gilke for GC analyses, Dr. Anette Giesemann and Martina Heuer for IRMS analyses of gas samples, and Dr. Wolfram Eschenbach for analysis of ^{15}N in NO_3^- and NH_4^+. We thank Prof. Dr. Jürgen Böttcher for soil classification and Prof. Kenneth Albrecht and Dr. Pedro Gerstberger for supplying cup plant seedlings. We thank Dr. Bernd Steingrobe for his feedback on the manuscript. Furthermore, we acknowledge two anonymous reviewers for their advice to improve the quality of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) through the research unit DFG-FOR 2337 (DI 546/4 − 1, We 1904/10 − 2): Denitrification in Agricultural Soils: Integrated Control and Modelling at Various Scales (DASIM).

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