Plant Soil
https://doi.org/10.1007/s11104-026-09004-6

RESEARCH ARTICLE

Interactions between plant growth and soil moisture determine the N_2O source strength of farmed landscape depressions

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Received: 10 February 2026 / Accepted: 13 August 2026

Abstract
Background Topographic depressions within agricultural fields contribute disproportionately to regional nitrous oxide (N_2O) emissions. These low-lying areas accumulate nutrients and fine particles through water inflow and erosion, creating conditions conducive to elevated N_2O emissions. Because depressions remain part of fields, understanding how crop presence and management influence their N_2O source strength is essential.
Methods A greenhouse mesocosm experiment was conducted using soil collected from an agricultural depression under either drained or partially waterlogged conditions (water table maintained 10 cm below the soil surface). Wheat was sown at three dates (57, 43, and 29 days before waterlogging) to represent different vegetative stages and capacities for N uptake. Dissolved organic carbon (DOC), dissolved nitrogen (N), root growth, and N_2O emissions were continuously monitored. A ^{15}N-labelled fertilizer was applied during the waterlogging period to trace fertilizer-derived N in emitted N_2O, soil, and plant biomass.
Results Early-sown wheat was more strongly impaired by waterlogging than later-sown treatments but nevertheless significantly reduced N_2O emissions compared with the unplanted control, irrespective of water regime. Early-sown plants also acted as stronger N sink, indicating that greater plant N acquisition

Responsible Editor: Yu Jiang.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-026-09004-6.

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Published online: 20 August 2026

contributed to reduced N availability for N_2O production. In contrast, trends in DOC and cumulative N_2O emissions across seeding dates were less consistent, particularly under waterlogged conditions.

Conclusions Plant N uptake emerged as the primary mechanism reducing N_2O emissions under the tested conditions. Although a general trend of lower N_2O emissions with increasing plant age and N uptake was observed, the relationship was not strictly linear, due to plant age-specific interactions with waterlogging. Nevertheless, the results suggest that establishing crops in depression areas can mitigate N_2O emissions by strengthening plant competition for available N, while the magnitude of this mitigation depends on both seeding date and waterlogging conditions.

Keywords Denitrification · Root growth · Winter wheat · Waterlogging

Introduction

Among the greenhouse gases (GHG) emitted during crop production, nitrous oxide (N_2O) plays a predominant role, due to its 273 times higher global warming potential than carbon dioxide (CO_2) over a 100-year timescale and its effective radiative forcing of 0.21 ± 0.03 W m^{-2} (Forster et al. 2021; IPCC 2023). Agricultural activities and direct and indirect emissions during crop production account for 52% of the global anthropogenic N_2O emissions (Tian et al. 2020). In countries with more intensive farming, such as Denmark, the agricultural sector contributes up to 82% of national N_2O emissions (Albrektsen et al. 2021). In soils, N_2O is produced mainly during microbial processes of the nitrogen (N) cycle, particularly nitrification and denitrification (Butterbach-Bahl et al. 2013). Nitrification is an aerobic microbial process that converts ammonium (NH_4^+) to nitrate (NO_3^-) through the intermediate nitrite (NO_2^-) (Butterbach-Bahl et al. 2013). Denitrification is the reduction of NO_3^- and NO_2^- to dinitrogen (N_2) under oxygen-limited conditions. It is strongly controlled by NO_3^- availability, and labile carbon (C) serving as an energy source, while soil moisture plays a crucial role by controlling soil aeration (Butterbach-Bahl et al. 2011). Still, denitrification remains inherently unpredictable, mainly due to the high spatial and temporal dynamics of abiotic and biotic processes within a heterogeneous soil matrix (Groffman et al. 2009).

The topography of eastern Denmark's arable land is characterized by widespread poorly drained depressions typical of glacial landscapes. These small, low-lying depression areas, ranging from 7 to 80 m in diameter (Elberling et al. 2023), create favorable conditions for denitrification due to their landscape position and associated hydrological, physical, and biotic soil properties. Consequently, these depression areas have been identified as N_2O emissions hotspots, releasing more than 80 times higher N_2O emissions than surrounding upland soils and contributing up to 30% of total regional N_2O emissions (Elberling et al. 2023). The low-lying position of these depressions results in a significant accumulation of precipitation, surface runoff, drainage water, and in some cases, is temporarily affected by groundwater, leading to frequent waterlogging, especially in the period between late fall and spring. Given that N_2O production via denitrification occurs when the water-filled pore space (WFPS) ranges from 30 to 90% WFPS and peaks at 65% WFPS (Davidson 1991), such concentrated soil moisture conditions partly explain why these depressions act as N_2O emission hotspots.

Water flow into depressions is associated with the mass transport of both solid and dissolved compounds from upslope positions. Over time, this erosion process leads to an increased accumulation of silt and clay fractions in depression areas compared to upslope positions. In addition to the deposition of fine particles, erosion also contributes to the accumulation of substantial amounts of soil organic matter (SOM) in depressions (Ashiq et al. 2021; Suriyavirun et al. 2019). The accumulation of fine particles and SOM alters soil physical properties, increasing water retention and consequently extending periods of low soil gas diffusivity (Ashiq et al. 2021; Gaultier et al. 2006). In addition to altering physical soil properties, the increased SOM content in depression soils provides a large pool of readily mineralizable material. This leads to an elevated supply of labile C and

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mineral N (SMN, including NO_3^- and NH_4^+), of which labile C fuels microbial processes such as nitrification and denitrification (Stuchiner et al. 2024). Furthermore, studies have consistently shown that depression and upslope soils harbor distinct microbial communities, including different N cycling and denitrifying communities (Krichels et al. 2025; Liu et al. 2025; Zhou et al. 2022). Under transient soil moisture conditions, the specific composition of N_2O-producing and N_2O-reducing microbes contributes to the contrasting N_2O source strengths of landscape depressions compared to surrounding upslope areas (Krichels et al. 2025; Liu et al. 2025).

To achieve a process-based understanding of N_2O emission from landscape depressions, it is essential to consider factors beyond soil properties alone. Most depressions are situated within cultivated fields, where the vegetation (crop) is not specifically adapted to frequent waterlogging, and where N inputs are substantial. These inputs arise both from direct fertilization and from N transport via runoff and leaching from upslope areas, further fueling nitrification and denitrification rates and consequently N_2O emissions (Vaidya et al. 2023). Plants have been shown to influence soil denitrification in various ways, either by stimulating or suppressing this process (Engelaar et al. 2000; Malique et al. 2019; Senbayram et al. 2020). Plant growth regulates the distribution of SMN. The N uptake rate depends on plant development, physiological status, root traits, and environmental stress (Engels and Marschner 1995). For example, high plant N uptake rates during the early active growing period can reduce N_2O emissions from soils following high N input, as plants act as a strong NO_3^- sink, thereby reducing the availability of N for denitrification (Abalos et al. 2018; Inselsbacher et al. 2010; Legay et al. 2020). Despite competing for NO_3^-, plants also contribute to soil C via labile C compounds from root exudation, decaying roots, and overall rhizodeposition (Mueller et al. 2024; Rummel et al. 2021), thereby potentially fueling denitrification (Dalal and Allen 2008). Similar to plant N uptake, the composition and quantity of root exudates vary with plant age, species, and environmental conditions (Badri and Vivanco 2009).

Managing excessive soil water in Denmark's rolling landscapes is challenging (Nielsen 2010; Skriver and Hedegård 1973). Although man-made near-surface drainage exist in more than half of the agricultural area, many systems are old and poorly maintained or function intermittently, leading to persistent or episodic waterlogging in depressional areas (Motarjemi et al. 2021). Therefore, large-scale intensification of drainage is technically challenging and constrained by environmental considerations, while tillage-based solutions are increasingly incompatible with reduced-tillage and no-till practices adopted to improve soil structure, reduce erosion, and enhance soil carbon sequestration. Land leveling is similarly constrained by cost, landscape heterogeneity, and environmental regulations. These hydrological conditions in depressions create soil environments conducive to denitrification, while also influencing crop performance and survival. Consequently, the risk of denitrification-derived N_2O emissions increases, particularly when crop failure occurs or the crop stand is poor, yet depressions still receive fertilizer. Therefore, improving the mechanistic understanding of plant-soil interactions under waterlogging remains highly relevant. In the long term, identifying key regulatory management factors, such as seeding and fertilization timing, and their interactions at the process level, could support the development of management strategies to reduce N_2O emissions not only from Danish crop production but in general agricultural landscapes characterized by periodic waterlogging.

In this study, we aimed to elucidate the potential key drivers and their interactions influencing N_2O emissions under controlled conditions mimicking soil and moisture levels typical of these depression sites. We focused particularly on plant-soil interactions and plant age, as determined by seeding date. The developmental stage entering winter determines the plant's habitus in spring, when waterlogging typically occurs and the first fertilization is applied. Hence, seeding date strongly affects the depletion of available NO_3^- via plant N uptake and the supply of labile C to the soil at this early stage. However, waterlogging may severely impede these processes, potentially leading to varying risks of elevated N_2O emissions. To understand these interactions and the resulting influence on N_2O emissions, we carried out a greenhouse mesocosm experiment, using soils from a topographic depression site and wheat sown at different dates, resulting in varying plant stages of vegetative development. The plants were grown under either drained or partially waterlogged conditions, with the water table kept constant at 10 cm below the soil surface. An additional ^{15}N fertilizer application was made seven days after the onset of the waterlogging period. We hypothesized that:

(1) growing wheat reduces soil N_2O emissions by lowering soil NO_3^- availability through plant N uptake, and this reduction is stronger in early-sown wheat plants, which, being at more advanced vegetative growth stages, exhibit higher N uptake compared to intermediate- and late-sown plants;
(2) early-sown wheat is more susceptible to waterlogging because a larger proportion of its root system is exposed to waterlogged soil, and the higher O_2 demand of its more developed roots impairs N uptake, thereby elevating N_2O emissions compared to intermediate- and late-sown plants;
(3) under waterlogged conditions, the effect of plant age on soil N_2O emissions is more pronounced than under drained conditions, due to age-related plant-soil interactions, particularly the allocation of fresh, readily available plant-derived C substrates.

Materials and methods

Experimental setup

In this experiment, we studied the effect of two soil moisture levels on N_2O emissions and the fate of N in a simulated depression soil profile cultivated with winter wheat (*Triticum aestivum* L.) with plant age. The experimental treatments consisted of two distinct soil water regimes: freely drained soil and waterlogged conditions up to 10 cm below the soil surface. The second factor tested in this experiment was the influence of the plant age of winter wheat and thus the development of the root system, together with the plant N uptake, on the fate of the soil N pool. Therefore, three different plant ages of winter wheat were induced by different sowing dates: early, intermediate, and late sowing (Fig. 1). Mesocosms without wheat plants were used as controls. The fully factorial design was replicated four times, which resulted in a total of 32 mesocosms. Here, the term 'waterlogging'

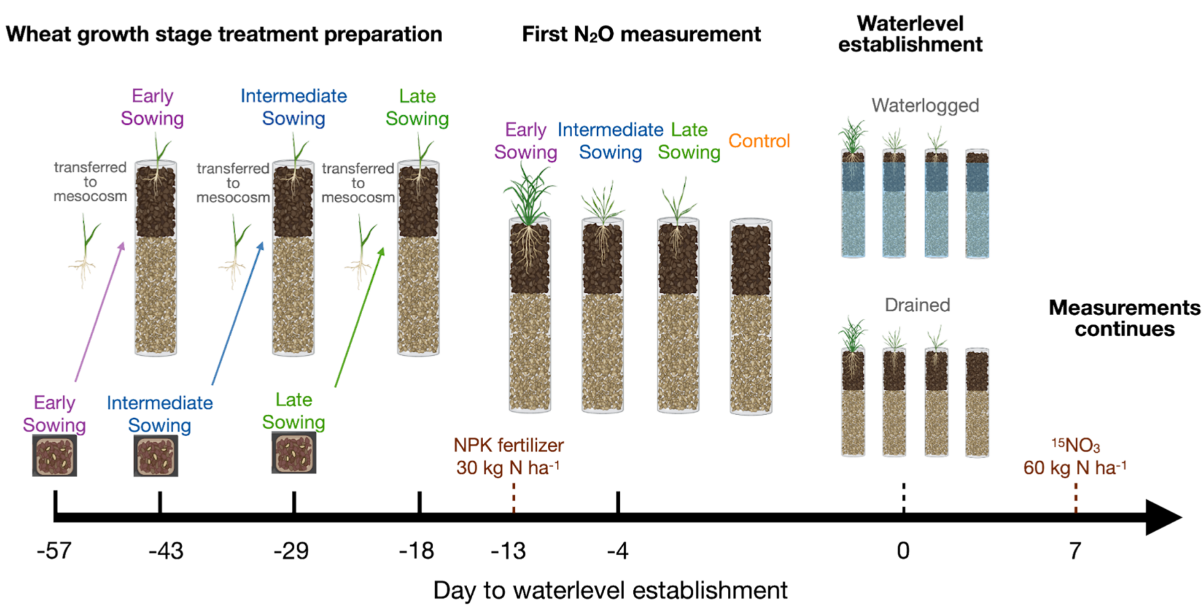

Fig. 1 Schematic overview of the experiment. Wheat seeds were sown on day − 57, − 43, and − 29 for early, intermediate, and late-sown treatments, respectively. Seedlings were transferred to mesocosm 14 days (early and intermediate-sown) and 11 days (late-sown) after sowing. The two fertilizers were applied on day − 13 and day 7. The first N_2O measurement was conducted on day − 4. The water level was established on day 0. Additional measurements (soil solution, root images) were taken after day 0 onward and continued throughout the experiment

refers to soil saturation, even when no standing water is visible on the soil surface.

Topsoil material (Ap horizon, 0–10 cm) for the experiment was collected from a glacial depression at a long-term trace gas monitoring site in Sorø (55.49076 N, 11.59585 E), Denmark. The soil is classified as a Luvisol according to the WRB (FAO, 2014). Cover crops were seeded in late summer of the previous growing season, before the soil sampling. The subsoil material (coarse sand) for the mesocosm setup was sampled at the experimental farm of the University of Copenhagen at Taastrup. The soil type was an Arenosol according to the WRB (FAO 2014). Both soils were air-dried and sieved through a 2 mm mesh. Visible roots and stones were picked out by hand. Further soil properties are presented in Table 1.

The mesocosms were constructed from transparent acrylic glass cylinders with an inner diameter of 11 cm and a height of 75 cm (Figure S1). The bottoms were sealed by gluing acrylic glass plates onto the cylinder ends. The lower section (30–75 cm) of each mesocosm was packed with the sandy subsoil (Table 1), while the upper 30 cm was packed with the topsoil collected from the depression site. During packing, a spiked plate was used to compact the soil column to a bulk density of 1.5 g cm^{-3}, comparable to field conditions.

To control water levels, wicks were installed through drilled holes prior to filling the mesocosms with soil (Figure S1). For the drained treatments, a single hole (7 mm drill bit) was drilled at the bottom of each mesocosm (70 cm depth) and fitted with a wick that extended upward to the top, with its upper end placed in a water reservoir. This setup allowed a continuous water supply from above, while excess water drained through the lower end of the wick, exiting the hole, thereby maintaining constant soil moisture. For the waterlogged treatments, two holes were drilled at 10 cm and 70 cm depths (Figure S1). A wick was installed, extending from the lower hole (70 cm) to the top of the mesocosm, with both ends of the wick hanging out of the mesocosm as the drained treatments. A second, shorter piece of wick was placed in the hole at 10 cm. After filling the soils, the mesocosms were pre-wetted using this wick system, and the soil water level was maintained until the establishment of the partial waterlogging. Partial waterlogging (10–75 cm) was established on day 0 by cutting the wick at 70 cm and sealing the hole with duct tape, while the 10 cm wick remained active. Here partially waterlogged conditions were mimicked, as previous field study showed that N_2O emissions are often higher when depressions are not fully waterlogged (Elberling et al. 2023). The water table was kept constant to reduce hydrological variability and focus on the effects of plant development on N_2O emission. A 24-day waterlogging period was applied to represent mild field waterlogging conditions.

For the planted treatments, winter wheat was germinated on a peat substrate, and four seedlings were transferred to each mesocosm approximately two weeks after seeding. The three sowing dates relative to the waterlogging establishment (day 0) were: early sowing (57 days before establishment of water levels, − 57), intermediate sowing (day − 43), and late sowing (day − 29). The first N_2O measurements were conducted on day − 4, corresponding to 53 days, 39 days, and 25 days after seeding, for the early-, intermediate-, and late-sown plants, respectively (Fig. 1). To facilitate wheat growth, an NPK fertilizer (14–3–23) was applied to all mesocosms at a rate of 25 mg $mesocosm^{-1}$, equivalent to 30 kg N ha^{-1}, on day − 13 (first fertilization). Additionally, soils were labeled with $Ca(^{15}NO_3)_2$ (5 at% ^{15}N) at a rate of 50 mg $mesocosm^{-1}$, equivalent to 60 kg N ha^{-1}, on day 7 (second fertilization). Both fertilizers were dissolved in deionized water and applied to the soil surface via pipette (10 mL per mesocosm).

All mesocosms were wrapped with lightproof plastic (Groflective, Germany), which was black on the inside and white on the outside, to darken the soil and limit soil heating. Greenhouse conditions were maintained at 20˚C during the day and 15˚C at night under a 16/8 h light–dark cycle. It is acknowledged

Table 1 Initial sieved soil properties of the topsoil and subsoil used before filling them into the mesocosms

	Total C (g kg^{-1})	Total N (g kg^{-1})	NO_3^- (mg N kg^{-1})	NH_4^+ (mg N kg^{-1})	pH_{water}	Clay (%)	Silt (%)	Sand (%)
Topsoil	27.7	2.6	83.1	5.4	6.75	6.2	25.6	68.2
Subsoil	1.8	0.3	28.3	13.5	7.11	5.1	4.1	89.9

that the greenhouse temperature and light regime were more representative of spring and summer conditions. The exact timeline is shown in Fig. 1.

N_2O flux measurement

The soil N_2O flux was measured using the static chamber method on day −4, 2, 7, 8, 9, 10, 13, 14, 16, 17, 20, and 24 (Anthony et al. 1995). A transparent gas-tight lid was placed on each mesocosm (height: 29 cm), with wheat plants carefully rolled inside. Tubes connected the lid with an Off-Axis ICOS laser (GLA151-N2OM1, ABB, Quebec City, Canada), which recorded a mean N_2O concentration in ppm every 10 s. During N_2O measurements, the wheat plants were carefully rolled inside the lid. The chamber closure time was 5 min. For the $^{15}N_2O$ measurement, two gas samples were collected for each mesocosm unit; the first one was taken when the lid was placed on the mesocosm tube (t0), and the second one was taken after 5 min (t5). Gas samples were analyzed with an isotopic N_2O analyzer (LGR 914–0027, ABB, Quebec City, Canada).

Root monitoring

Root growth was monitored by taking digital images from the outside of the mesocosm tubes after removing the lightproof foil. To ensure reproducibility, a box with the camera (Sony DSC-QX10 4.45–44.5 mm) was mounted onto the mesocosm. Three images were taken to ensure complete coverage of one entire circumference of the mesocosm surface across its full depth (0–30, 30–60, and 60–75 cm). The images were always taken from the same side of the mesocosm. Root images were captured four times (days −6, 8, 14, 20) during the experiment. The root images were analyzed with Rootpainter, which was trained to segment roots from each image (Smith et al. 2022). Root segmentation for all images was performed using a model trained on a subset of randomly selected images. Planar root length (pRL [cm]), which represents root length from a 2D sample origin, was extracted from the segmented root pictures (Wacker et al. 2025).

Soil solution monitoring and final sampling of soils and plants

For soil solution sampling, an additional hole was drilled (1.5 mm drill bit) at 15 cm depth to install small porous soil solution samplers (Rhizon MOM sampler, 5 cm long, 0.15 µm pore size, Rhizosphere Research Products B.V, Netherlands). Soil solutions were sampled on day 7, 10, 13, 14, 16, 17, 20, and 24, using a 50-mL syringe and the above-described Rhizon samplers. Samples were filtered with 0.45 µm syringe filters (LLG Labware syringe filters) and then stored at −20 °C until analysis. Soil solution dissolved organic carbon (DOC) was measured with a DOC analyzer (Multi N/C 2100s, Analytik Jena, Germany), and SMN concentrations were measured colorimetrically with a continuous flow analyzer (AA3 CFA, Seal Analytical, USA).

At the end of the experiment (day 24), soil samples were collected from four depths (0–10, 10–30, 30–50, and 50–75 cm). Eight grams of fresh soil were extracted with 1 M KCl (1:5, w:v). The suspension was shaken for one hour at room temperature, filtered through 0.45 µm syringe filters (LLG Labware syringe filters), and stored at −20 °C until analysis. SMN concentrations in the soil KCl extracts were measured colorimetrically with a continuous flow analyzer. Total aboveground biomass was harvested at the end of the experiment by cutting the wheat plants 1 cm above the soil surface, and then oven-dried at 60 ˚C for 48 h. Dried plant material as well as soil samples were finely ground by ball-milling. Plant and soil total C, N, and $\delta^{15}N$ values were determined by an elemental analyzer (Flash 2000 Organic Elemental Analyzer, coupled to a Delta V Advantage isotope ratio mass spectrometer, Thermo Fisher Scientific, Germany).

Calculations and statistical analyses

All calculations and statistical analyses were performed in R using RStudio (R Core Team 2023). Unless stated otherwise, results are presented as means ± 1 standard error.

The N_2O fluxes were calculated as the concentration increase during the five-minute chamber closure time, based on the high-frequency concentration measurements using the R package 'gasflux' (Fuß et al. 2020). The $^{15}N_2O$ flux was determined from the increase in $^{15}N_2O$ concentration between the two manual gas samples taken at time points t_0 and t_5. Cumulative N_2O and $^{15}N_2O$ emissions were calculated by applying the trapezoidal rule after linear interpolation between measurement dates (Dorich et al. 2020).

A simple ^{15}N balance was established based on the recoveries in aboveground biomass N, soil total N, and N_2O emissions. The percentage of N derived from fertilizer (Ndff) in each target pool (aboveground biomass N, soil total N, and N_2O emissions) was calculated as follows (Jamali et al. 2016):

$$N_{\mathrm{dff}}(\%) = \mathrm{atom\%}\,{}^{15}\mathrm{N}_{\mathrm{target\ pool}} \mathrm{atom\%}\,{}^{15}\mathrm{N}_{\mathrm{fertilizer}} \times 100 \quad (1)$$

where atom%$^{15}N_{target\ pool}$ is the ^{15}N enrichment in the aboveground biomass, soil total N, and N_2O–N pool, and atom%$^{15}N_{fertilizer}$ is the enrichment of the applied fertilizer solution. Natural abundance correction was performed using measured reference soil and plant material (internal laboratory standards). A correction for the background of ^{15}N in N_2O emission could not be applied as no unlabeled treatment was established, but the overestimation was considered to be negligible in this study.

The ^{15}N recovery ($^{15}N_{recovery}$ in %) in a given pool was then calculated as follows:

$$^{15}\mathrm{N\ recovery} = N_{\mathrm{dff}}(\%) \times N_{\mathrm{target\ pool}}(\%) \times \mathrm{dry\ mass}_{\mathrm{target\ pool}} \mathrm{rate\ of}\ {}^{15}\mathrm{N\ fertilizer\ applied} \quad (2)$$

where $N_{target\ pool}$ is the N concentration in % of the respective pool (biomass, soil, or N_2O), and dry $mass_{target\ pool}$ refers to the total dry mass in grams of aboveground biomass, soil, or total N emitted as N_2O.

Spearman's rank correlation coefficient (r) was used to assess relationships between cumulative N_2O emissions, pRL, aboveground biomass N, aboveground biomass $\delta^{15}N$, and soil NO_3^- content at harvest (0–10 cm and 10–30 cm). Similarly, Spearman's r was applied to evaluate correlations between daily N_2O flux, days after wheat sowing, and soil solution properties (DOC, TN, NO_3^-–N, and NH_4^+–N). Treatment effects on cumulative N_2O emissions were tested using a linear model after log10-transformation of the response variable. Treatment effects on aboveground biomass N, soil C, soil N, and pH at harvest were also assessed using linear models. Post-hoc tests and pairwise comparisons were conducted using the R package 'emmeans'. Treatment effects on soil NO_3^- and NH_4^+ at harvest were analyzed with the Kruskal–Wallis test, followed by Dunn's post-hoc test.

To analyze the influence of plant age (days after sowing), SMN, and DOC on daily N_2O flux, a generalized additive model was fitted using the R package 'mgcv' (Wood 2011; Wood and Augustin 2002). A square root transformation of daily N_2O fluxes was used due to lower generalized cross-validation (GCV) scores than no transformation or a log-transformation. Generalized additive model selections were based on Akaike's information criterion (AIC).

Results

Aboveground and belowground biomass

Aboveground biomass significantly increased with plant age, and waterlogging reduced biomass in the intermediate- and early-sown treatments but not in the late-sown treatments (Table 2). The highest aboveground biomass N was observed in the drained, early-sown treatment (0.4 ± 0.05 g N $mesocosm^{-1}$, Table 2). Although waterlogging alone had only a minor, non-significant negative effect on aboveground biomass N content ($p = 0.10$), the largest decrease occurred in the early-sown wheat (Table 2). In contrast, waterlogging increased aboveground biomass N contents in late-sown wheat compared with drained late-sown wheat.

The pRL increased significantly with plant age ($p < 0.001$, Table 2) and over the course of the experiment (Fig. 2). Certain treatments, such as intermediate-sown wheat, exhibited a more pronounced reduction in root growth rates two weeks after waterlogging onset (Table 2, Fig. 2). However, the overall effect of waterlogging on pRL was not statistically significant ($p = 0.3$). The pRL was significantly positively correlated with aboveground biomass and N content ($r = 0.66$, $p < 0.001$, Table 3), and significantly negatively correlated with aboveground ^{15}N content ($r = -0.73$, $p < 0.001$, Table 3).

Table 2 Aboveground biomass, aboveground biomass N content, N concentration, ^{15}N content, and planar root length (pRL) of wheat at the end of the experiment, presented as mean ± 1 standard error ($n = 4$)

Sowing	Water level	Biomass (g mesocosm^{-1})	Biomass N (g mesocosm^{-1})	Biomass N (%)	Biomass ^{15}N (mg mesocosm^{-1})	pRL (cm)
late	drained	3.0 ± 0.4 c	0.07 ± 0.01 b	2.2 ± 0.2 a	1.1 ± 0.3 bc	238.0 ± 69.4 c
	waterlogged	3.9 ± 0.3 c	0.1 ± 0.01 b	2.7 ± 0.2 a	1.8 ± 0.1 c	248.5 ± 72.1 bc
intermediate	drained	7.4 ± 1.0 bc	0.2 ± 0.04 b	2.0 ± 0.3 a	2.3 ± 0.5 abc	803.4 ± 210.0 a
	waterlogged	6.1 ± 1.6 c	0.2 ± 0.04 b	2.4 ± 0.1 a	2.1 ± 0.5 abc	584.5 ± 132.4 ab
early	drained	16.9 ± 1.1 a	0.4 ± 0.05 a	2.4 ± 0.3 a	4.3 ± 0.4 a	814.1 ± 72.7 a
	waterlogged	12.4 ± 1.7 ab	0.2 ± 0.04 b	1.8 ± 0.2 a	2.3 ± 0.3 ab	732.8 ± 117.7 ab

Lowercase letters indicate a significant difference across treatments ($p < 0.05$)

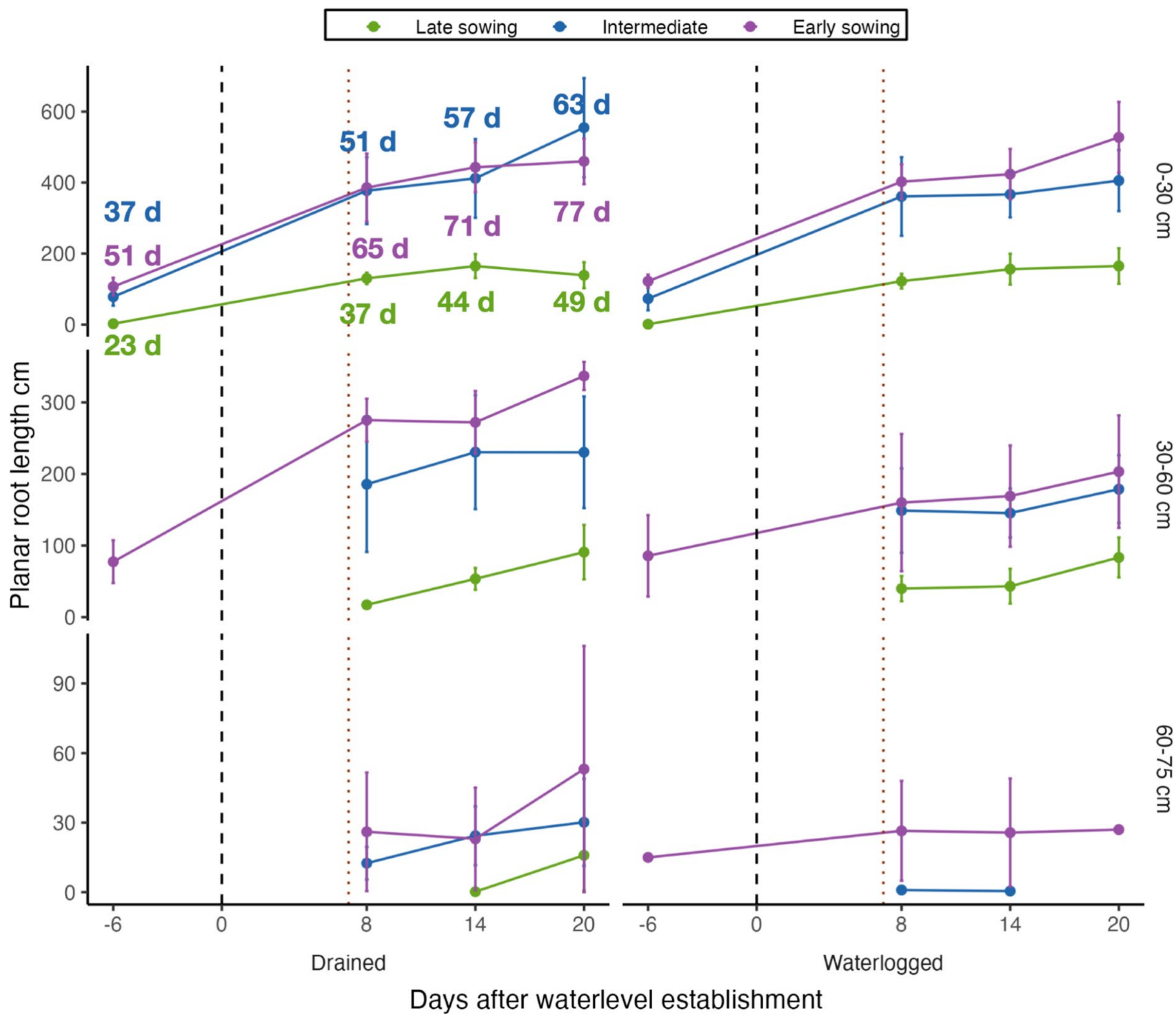

Fig. 2 Planar root length of three depth increments (0–30, 30–60, and 60–75 cm), the number beside the data points in the top left facet indicates days after seeding, mean ± 1 standard error (n = 4). The vertical black dashed lines indicate water level establishment on day 0; the vertical red dotted lines indicate 5% enriched $^{15}NO_3^-$ application at a rate of 60 kg N ha^{-1} on day 7. Note the Y-axis scale decreases with soil depth

Table 3 Correlation coefficients for the relationship between cumulative N_2O emissions, planar root length (pRL), aboveground biomass N content, aboveground biomass ^{15}N content, and soil NO_3^- (0–30 cm) at the time of destructive sampling at the end of the experiment

	cumulative N_2O	pRL	biomass N content	biomass ^{15}N content	NO_3^- 0–30 cm
cumulative N_2O	1	−0.30	−0.52**	0.28	0.46*
pRL		1	0.66***	−0.73***	−0.45*
biomass N content			1	−0.63**	−0.51*
biomass ^{15}N content				1	0.47*
NO_3^- 0–30 cm					1

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Soil solution dynamics and final soil properties

At the first soil solution sampling date (day 7, Fig. 3), DOC concentrations were highest in the early-sown (173 ± 135 mg C L^{-1}) and intermediate-sown treatments (250 ± 156 mg C L^{-1}), compared to the low concentrations observed in the control (62 ± 35 mg C L^{-1}) and late-sown treatments (61 ± 29 mg C L^{-1}) under drained conditions. After these initial high values, DOC concentrations in the drained treatments declined and stabilized at low levels similar to those of the control and late-sown treatments (Fig. 3A). In the waterlogged treatments, DOC concentrations showed more variability but remained within the range observed under drained conditions (Fig. 3A). Across both soil water conditions, the only consistent trend was that DOC concentrations were higher in the intermediate and early-sown treatments compared to the late-sown and control treatments, even with a slight increase towards the end of the experiment (Fig. 3A).

While NO_3^- concentrations reached up to 261 mg N L^{-1}, NH_4^+ concentrations remained consistently below 4 mg N L^{-1} (Fig. 3B, C). NO_3^- in soil solution decreased within 7 days after the second fertilizer amendment (day 7) in the drained treatments, and within 9 days in waterlogged treatments (Fig. 3 B, C). Under drained conditions, soil solution NO_3^- concentrations followed the trend: early < late < intermediate < control. In contrast, under waterlogged treatments, early- and intermediate-sown treatments showed similar concentrations, with controls showing the highest concentrations. Plant age was weakly but significantly positively correlated with DOC concentration (r = 0.16, Table S2), while it was significantly negatively correlated with both TN and NO_3^- (r = −0.31 and r = −0.68, respectively, Table S2).

The following patterns were observed for the KCl extractable SMN in 0–30 cm depth at the end of the experiment: the unplanted control generally had lower NH_4^+ (2.3 ± 0.2 mg N kg^{-1}) but higher NO_3^- contents (91.3 ± 25.2 mg N kg^{-1}, Fig. 4) than the treatments with plants (4.4 ± 0.2 and 35.6 ± 4.2 mg N kg^{-1} for NH_4^+ and NO_3^-, respectively). Hence, NO_3^- content in 0–30 cm at the end of the experiment was negatively correlated with plant N uptake, which was increasing with plant age (aboveground biomass N content, r = −0.51, $p < 0.05$, Table 3). However, this trend in NO_3^- content in 0–30 cm was not consistently observed across the seeding date treatments. Intermediate-sown treatments had the highest NO_3^- contents across both soil moisture levels, followed by late- and early-sown treatments (46.2 ± 10.3, 34.4 ± 5.5, and 26.1 ± 2.1 mg N kg^{-1}, respectively, Fig. 4). The unplanted control had higher NO_3^- in 30–75 cm depths among all treatments (Fig. 4A). Late-sown treatments had the highest NO_3^- in 30–50 cm depth compared to intermediate-sown and early-sown treatments. No other statistically significant relationships were observed for either soil layer (0–10 cm and 10–30 cm; Table S1). Soil organic C in 0–10 cm was, on average, significantly higher in planted treatments than in the unplanted control ($p < 0.001$), while differences among the wheat treatments were smaller (Table S1). Further detailed soil information (total C, total N, $\delta^{15}N$ of total N) can be found in the supplement in Table S1.

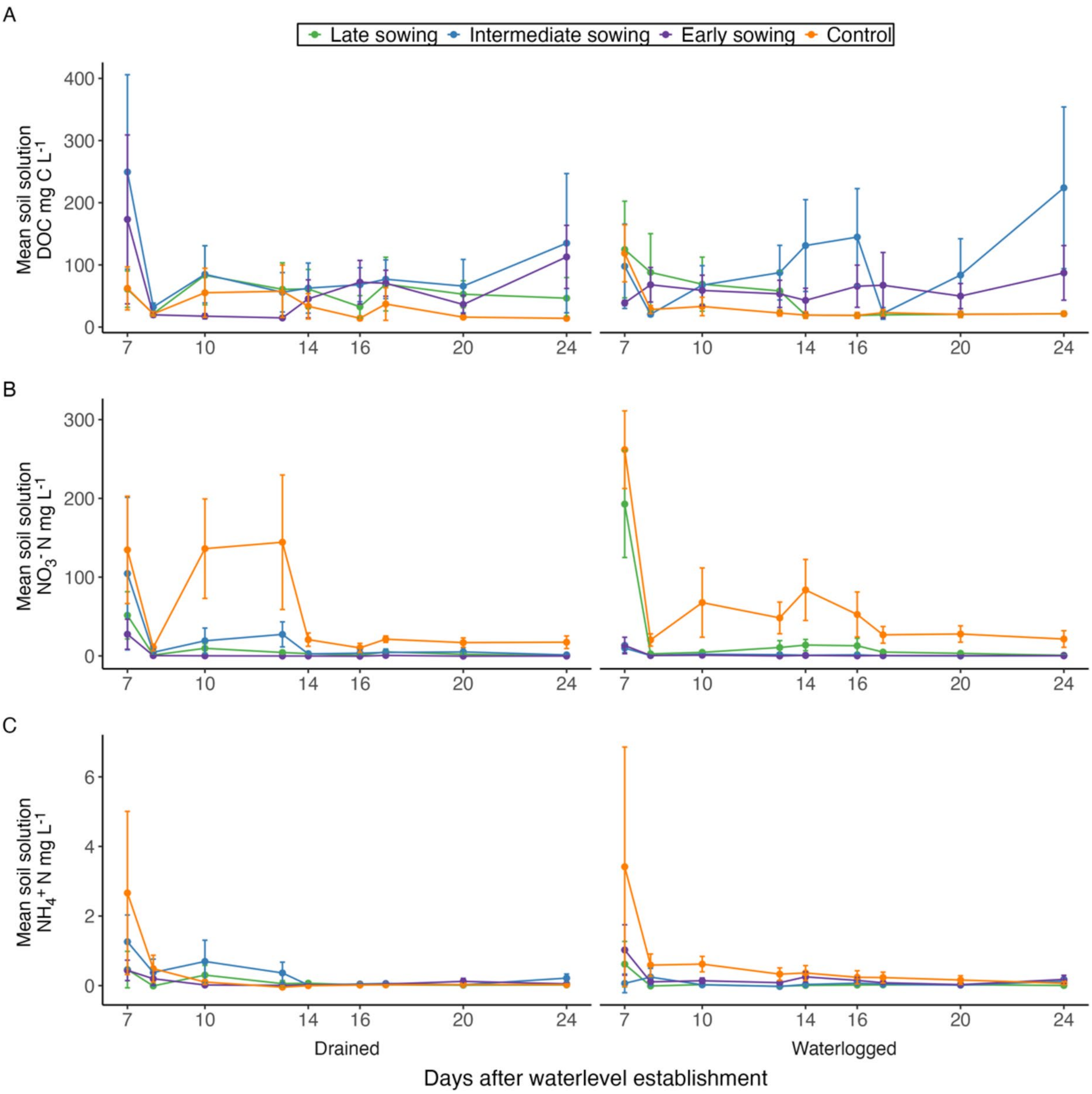

Fig. 3 Mean concentrations of (**A**) dissolved organic carbon (DOC; mg C L^{-1}), (**B**) NO_3^- (mg N L^{-1}), (**C**) NH_4^+ (mg N L^{-1}) in soil solution, shown as mean ± 1 standard error (n = 4). The first fertilization was applied on day − 13 at a rate equivalent to 30 kg N ha^{-1}, and the second on day 7 at 60 kg N ha^{-1}

N_2O flux and ^{15}N recovery

N_2O flux during the experiment

High N_2O fluxes were observed from all mesocosms immediately following the application of the $^{15}NO_3^-$ solution (day 7; Fig. 1) and over the subsequent three days (Fig. 5A). The highest N_2O fluxes occurred in intermediate-sown wheat treatments under waterlogged conditions (0.85 ± 0.46 mg N_2O–N m^{-2} h^{-1}), followed by the drained control treatments (0.35 ± 0.25 mg N_2O–N m^{-2} h^{-1}) on these days (Fig. 5A).

Treatments with early-sown wheat had the lowest cumulative N_2O emissions among all treatments, ranging from 0.73 to 2.62 mg N_2O–N m^{-2}

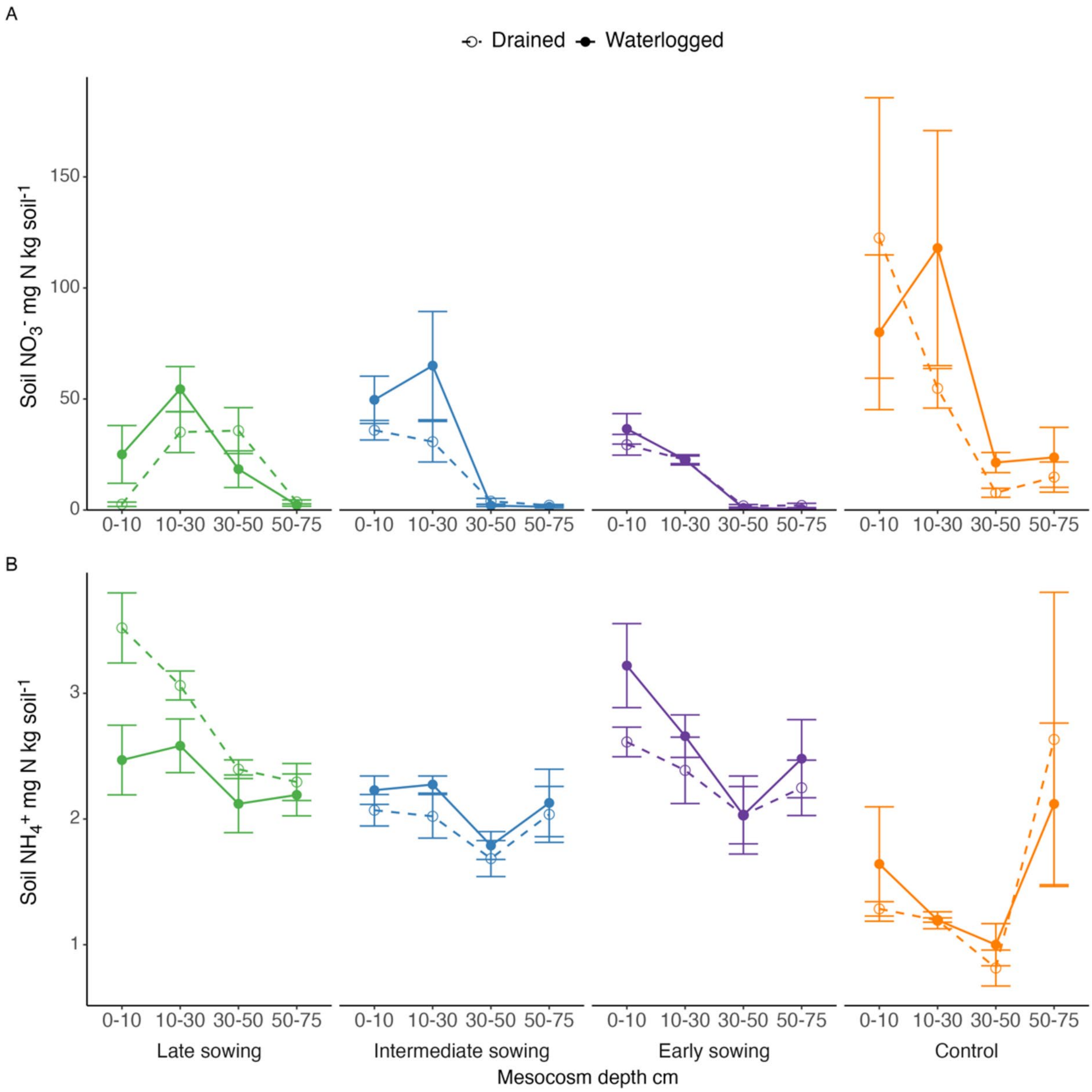

Fig. 4 **A** Soil NO_3^- (mg N kg soil^{-1}) and (**B**) NH_4^+ (mg N kg soil^{-1}) at destructive sampling at the end of the experimental period, measured in four depth increments of the mesocosm. Values represent mean ± 1 standard error ($n=4$)

across the two soil moisture regimes (Fig. 5B). In contrast, the intermediate-sown, waterlogged treatment exhibited the highest cumulative N_2O emissions among all planted treatments, while the control treatments showed the overall highest cumulative N_2O emissions, ranging from 62.6 to 72.1 mg N_2O–N m^{-2} (Fig. 5B). Cumulative N_2O emissions from early-sown treatments were significantly lower than those from the unplanted control ($p=0.0015$).

Plant age had a significant effect on cumulative N_2O emissions ($p<0.001$, Fig. 5B), but emissions did not decrease linearly with plant age, following the order: intermediate->late->early-sown wheat. Across treatments, N_2O emissions tended to be higher

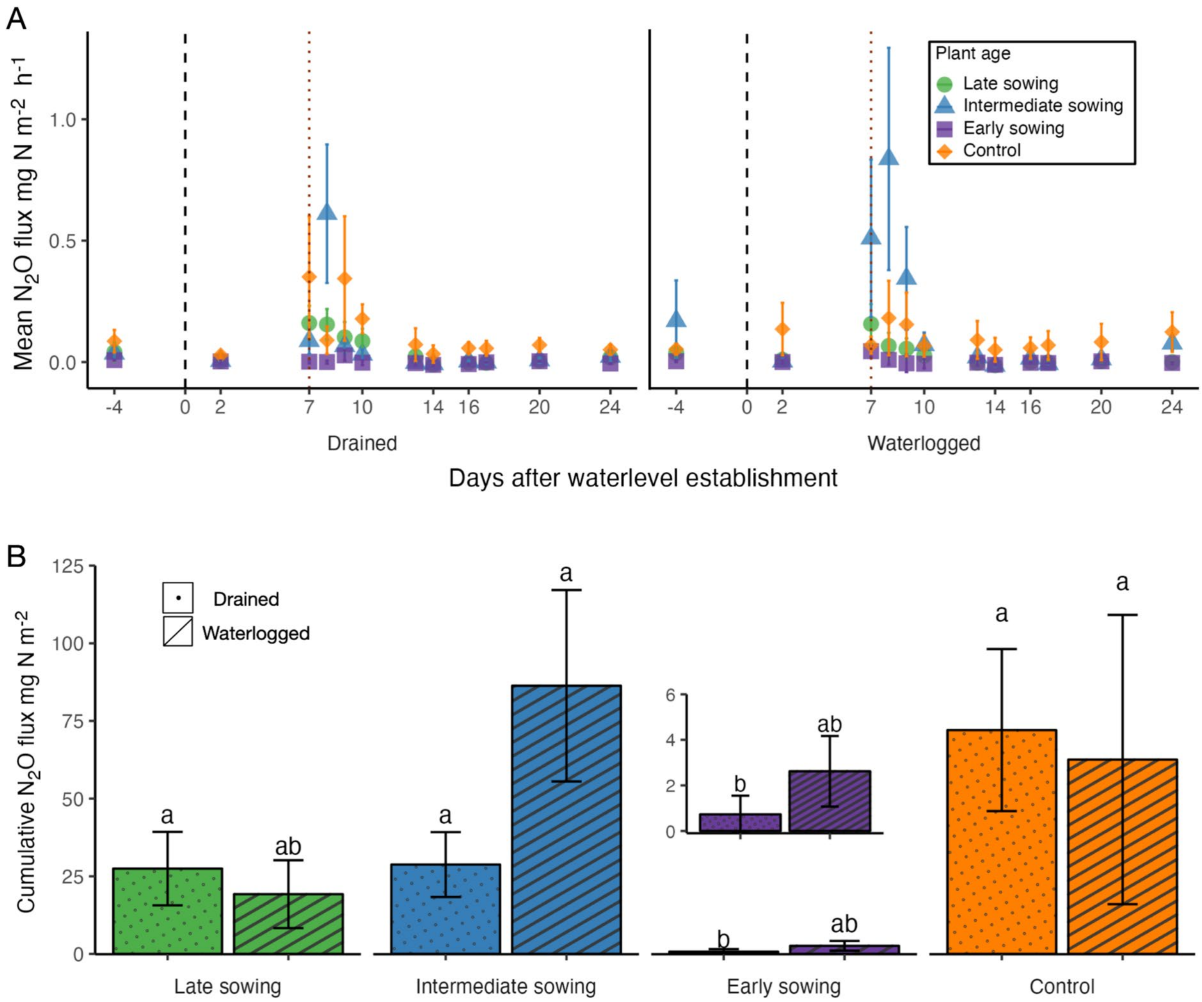

Fig. 5 **A** N_2O fluxes over the course of the experiment (mg N_2O–N m^{-2} h^{-1}). (**B**) Cumulative N_2O emissions (mg N_2O–N m^{-2}), shown as mean ± 1 standard error ($n = 4$). Vertical black dashed lines indicate the establishment of water level treatment on day 0, and vertical red dotted lines indicate the application of 5% enriched $^{15}NO_3^-$ at a rate of 60 kg N ha^{-1} on day 7

under waterlogged than under drained conditions, although this trend was not statistically significant ($p = 0.11$). Notably, waterlogging had a pronounced effect on intermediate-sown mesocosms, which resulted in a threefold increase in cumulative N_2O emissions compared to the corresponding drained treatment.

The generalized additive model explained 58.4% of the deviance in daily N_2O flux (Table S3, Figure S2). In both intermediate and late-sown treatments, regardless of the soil moisture regime, interactions between days after seeding and soil solution DOC significantly affected daily N_2O flux ($p = 0.02$ for waterlogged late-sown treatment; $p < 0.001$ for the other three treatments, Table S3). Soil solution SMN significantly influenced daily N_2O flux in the drained intermediate-sown treatment ($p = 0.014$, Table S3).

In addition to the treatment-specific generalized additive model results, correlation analyses provided a broader overview across all treatments. Daily N_2O fluxes were positively correlated with soil solution DOC, TN and NO_3^- (r = 0.12, 0.21 & 0.36, $p < 0.05, < 0.001$, and < 0.001, respectively; Table S2), and negatively correlated with plant age, measured as days after sowing (r = −0.36, $p < 0.001$, Table S2), reflecting the general decline in fluxes throughout the experiment. At the end of the experiment, cumulative N_2O emissions were significantly negatively correlated with aboveground biomass N content (r = −0.52, $p < 0.01$, Table 3), and significantly positively correlated with soil NO_3^- content at the time of destructive sampling (r = 0.42, $p < 0.05$, Table 3).

The recovery of ^{15}N in the measured N pools

The recovery of ^{15}N in soil total N, aboveground biomass N, and cumulative N_2O pools is summarized in Table 4. On average, 22.2 ± 1.49% of the applied ^{15}N was recovered from the soil N pools (including SMN and total N) of planted mesocosms. In comparison, in the unplanted control mesocosms, 38.3 ± 14.5% was recovered in the soil total N. Across treatments, ^{15}N recovery in soil was higher in waterlogged mesocosms (28.9 ± 5%) than in drained mesocosms (23.6 ± 5.8%).

The ^{15}N recovery in aboveground biomass N was higher in drained treatments (56.7 ± 12.5%) than in waterlogged treatments (46.2 ± 7.7%). Sowing date had a pronounced and consistent effect on ^{15}N recovery in aboveground biomass N ($p = 0.006$, Table 4): Early-sown treatments had the highest ^{15}N recovery (89.8 ± 12.8%), followed by intermediate and late-sown treatments (69.2 ± 10.2% and 46.9 ± 5.98%, respectively). Waterlogging alone had only a marginal effect on aboveground biomass ^{15}N recovery ($p = 0.16$), but its interactions with sowing date significantly influenced the outcome ($p = 0.01$). Differences in aboveground biomass ^{15}N recovery were more pronounced under drained than under waterlogged conditions (Table 4). However, there is some uncertainty in these estimates, evidenced by > 100% ^{15}N recovery in aboveground biomass in the early-sown drained treatments. Only a small fraction of the applied ^{15}N (< 0.2%) was recovered from N_2O emissions across all treatments, resulting in minimal and non-significant differences between treatments. However, higher ^{15}N recoveries in emitted N_2O tended to occur under waterlogging and in treatments with less or no (control) plant N uptake. Among the main factors, plant age had a stronger effect on ^{15}N recovery of emitted N_2O than soil moisture ($p = 0.11$ vs. $p = 0.27$).

Discussion

Plant response to waterlogged soil conditions

Under field conditions, winter wheat is normally sown in mid-September in Denmark (Olesen et al. 2012). When prolonged winter waterlogging occurs, the plants are still in their early growing stage. In the early- and intermediate-sown wheat, waterlogging treatments reduced aboveground biomass by 22% and pRL by 14% (Table 2). This aligns with previous reports of > 20% reductions in winter wheat grown under waterlogging in both field and controlled experiments (Dickin et al. 2009; Herzog et al. 2016; Khabaz-Saberi et al. 2006; Steffens et al. 2005). In contrast, waterlogging increased aboveground biomass and pRL in late-sown wheat. The higher aboveground biomass and pRL in waterlogged compared to drained late-sown wheat indicates that these plants were not water stressed under this treatment.

Table 4 ^{15}N recovery (%) from cumulative $^{15}N_2O$, soil total N, aboveground biomass N, and the sum of the three N pools, mean ± standard error ($n = 4$)

Sowing	Water level	Cumulative $^{15}N_2O$	Soil total ^{15}N	Aboveground biomass ^{15}N	Sum
late	drained	0.13 ± 0.08	17.6 ± 2.9	36.0 ± 9.0 b	53.7 ± 11.6
	waterlogged	0.09 ± 0.05	28.8 ± 4.8	57.8 ± 2.4 b	86.7 ± 4.5
intermediate	drained	0.09 ± 0.08	18.8 ± 2.4	73.5 ± 17.7 ab	92.4 ± 18.2
	waterlogged	0.17 ± 0.07	21.6 ± 1.8	65.0 ± 12.5 b	86.7 ± 11.5
early	drained	0.02 ± 0.01	21.9 ± 3.4	117.5 ± 13.7 a	139.4 ± 15.3*
	waterlogged	0.01 ± 0	24.6 ± 4.7	62.1 ± 8.0 b	86.7 ± 5.1
control	drained	0.02 ± 0.01	36.3 ± 24.4	/	36.3 ± 24.4
	waterlogged	0.21 ± 0.18	40.4 ± 19.7	/	40.6 ± 19.8

Lowercase letters indicate a significant difference between treatments ($p < 0.05$)

*Likely reflects subsampling variability in heterogeneous plant material, amplified by the large biomass and low ^{15}N enrichment

In our study, the waterlogging treatment was maintained with the water table positioned 10 cm below the soil surface, thereby leaving the upper 10 cm aerated. This, in turn, may explain why waterlogging did not cause effects on plant growth as severe as those reported for completely waterlogged conditions. The comparable pRL in the upper 30 cm under drained and waterlogged conditions further indicates less severe waterlogging effects (Fig. 2). Consequently, the reduction in above- and particularly belowground growth was less pronounced than previously reported by Dickin et al. (2009). This can be attributed to the fact that the wheat in our experiment was grown under drained conditions for a longer period (at least 29 days before the establishment of waterlogging, Fig. 2) than under waterlogged conditions (24 days). The period of partial waterlogged conditions (24 days, 10 cm below the soil surface) may have been insufficient to reduce or kill off the already established root biomass in the waterlogged soil (Dickin et al. 2009). Moreover, increased nodal root growth might have compensated for reductions in seminal root growth caused by waterlogging, explaining the minor differences in pRL between drained and waterlogged treatments.

Dynamics of N and C in the soil and soil solution

As hypothesized, the concentrations of N in soil and soil solution were closely linked to plant development. Soil solution NO_3^- concentrations were moderately negatively correlated with both plant age ($r = -0.68$, Table S2) and pRL ($r = -0.45$, Table 3), indicating that N sink strength increased with plant age. This was reflected not only in soil solution but also in final SMN contents at the end of the experiment, particularly NO_3^-. In addition to fertilizer N inputs, mineralization of organic material in SOM-rich depression soils likely contributed substantially to the availability of SMN (Shi et al. 2024). Comparing SMN contents of the unplanted control and planted treatments revealed that plant uptake was the dominant factor in depleting (reducing) SMN, regardless of soil moisture conditions (Fig. 3). Even in the SMN-rich soil used, wheat plants effectively reduced SMN concentrations through uptake, underscoring the greater susceptibility of unplanted soils to N losses via leaching and denitrification. The lower subsoil NO_3^- content in both the early- and intermediate-sown wheat treatments, compared to the late-sown treatment, indicates plant-mediated reduction in the risk of N losses from the rooting zone through subsoil denitrification or leaching. Similar patterns have also been observed under field conditions, where early-sown winter wheat effectively reduced soil NO_3^- concentrations during the early growth stage (Rasmussen and Thorup-Kristensen 2016).

In contrast to NO_3^-, DOC concentrations in the soil solution showed less clear trends (Fig. 3). DOC was only weakly correlated with plant age ($r = 0.16$; Table S2). Nonetheless, the intermediate-sown treatments almost consistently exhibited the highest DOC concentrations, followed by the early-sown and then the late-sown wheat. This nonlinear pattern with plant age likely reflects a combined effect of plant development and waterlogging-induced stress, as both the quantity and quality of root exudates change with plant growth (Chaparro et al. 2013; Santangeli et al. 2024) and biochemical responses of roots to waterlogging (Daniel and Hartman 2024). Another explanation for the lack of clear DOC trends across sowing dates is dilution. Soil solution was sampled at 15 cm depth, whereas the water table in waterlogged treatments was at 10 cm depth. Consequently, root exudates might have been more diluted in waterlogged compared to drained treatments, potentially masking any increases in exudation or plant-age-dependent effects caused by waterlogging stress (e.g., Henry et al. 2007). Dilution might also partially explain why DOC concentrations in drained treatments were consistently higher than in waterlogged treatments.

Although the effect of waterlogging on plant-age-related DOC dynamics is difficult to interpret, the observed increase in DOC concentrations from late- to early- and intermediate-sown treatments, with smaller differences between the early- and intermediate-sown wheat treatments, suggests that plant age-dependent effects of direct contribution to labile C pools exist. However, these effects are considered nonlinear and not sufficiently captured by our data. Nonetheless, the combined results from soil solution NO_3^- and DOC measurements, together with above- and belowground plant growth and N uptake, indicate that early-sown plants are more negatively affected by waterlogging than late-sown plants.

Interrelated effects of plant age and waterlogging on N_2O emission

In landscape depressions within arable land, hydrological conditions, nutrient accumulation, and their direct effects on N cycling and microbial community composition have been reported to determine N_2O source strength (Elberling et al. 2023; Krichels et al. 2025; Liu et al. 2025). However, the role of plants in studies focusing on landscape depressions as N_2O emission hotspots is often neglected (Krichels et al. 2025; Liu et al. 2025). Here, we included plants of different ages, achieved through different seeding dates, as an experimental factor to mimic field conditions in which different waterlogging occurs at different times during the growing season. In the present mesocosm study, where other environmental conditions were kept constant, we aimed to elucidate how plant age and partial waterlogging jointly regulate N_2O emission from depression soil.

The presence of plants reduced available NO_3^- and was generally associated with a tendency toward lower N_2O emissions compared to the unplanted control (Fig. 5B). The near absence of N_2O peaks after fertilization in the early-sown waterlogged treatments (Fig. 5A) supports the first hypothesis: increased plant N uptake reduces the risk of high N_2O emissions during subsequent waterlogging periods, likely due to reduced NO_3^- availability. Overall, the low NO_3^- content in planted treatments and even the late-sown, waterlogged treatment underscores the role of plant-driven competition for N with denitrifiers, thereby limiting reactive N and reducing the risk of N_2O emissions (Rummel et al. 2021). This implies that delayed or missing crop growth in waterlogged or flooded depressions in fields may lead to an even larger source of N_2O, as NO_3^- transported from surrounding areas cannot be taken up by plants.

Partial waterlogging at 10 cm below the soil surface only slightly increased N_2O emissions (+32.2 ± 15.0%; $p = 0.11$) compared to findings by Kemmann et al. (2023) under similar hydrological conditions in intact soil. In that study, enhanced N_2O emissions were partly attributed to increased moisture in the uppermost 10 cm of soil, with generally high microbial activity and denitrification potential (Jahangir et al. 2012), due to capillary rise. Such a response might be absent in this study, since sieved and repacked soil was used (Lewis and Sjöstrom 2010). Therefore, waterlogging under field conditions may exert a stronger effect on N_2O emissions than observed in this experiment. Besides the isolated effect of partial waterlogging, strong plant-mediated effects on soil moisture and related N_2O emission are unlikely, as emissions between drained and waterlogged unplanted soil were not statistically different ($p = 0.73$).

Early-sown wheat was more impaired by waterlogging than late-sown wheat. However, this did not translate into consistently elevated N_2O emissions from the apparently more stressed wheat following the second fertilizer addition to waterlogged soil (Fig. 1). Therefore, the second hypothesis, that early-sown wheat would be more affected by waterlogging, leading to higher N_2O emissions from fertilizer applied to partially waterlogged soil, is not fully supported. Among the planted treatments experiencing waterlogging-induced stress, only the intermediate-sown treatments exhibited N_2O emissions comparable to or higher than the unplanted control (Fig. 5). This indicates complex interactions between direct plant-related factors (e.g., N sink strength, root-derived labile C) and indirect microbial-mediated rhizosphere effects that vary with plant age and waterlogging.

While plant age-specific plant-soil interactions and their effects on N_2O emission remain complex, there was a clear trend of reduced N_2O emissions with increasing N immobilization in aboveground biomass. Although age-specific effects were evident under waterlogging, the patterns were less pronounced than under drained conditions. Therefore, the results do not support the third hypothesis that plant age effects on N_2O emissions would be more pronounced under waterlogged than drained conditions. One explanation is that the observed results reflect soil conditions integrated over both the non-waterlogged (day − 57 to 0) and waterlogged (day 0 to 24) periods, which might have reduced contrasts among treatments differing in waterlogging exposure relative to total plant growth. For instance, NO_3^- pools might have already been depleted, or at least reduced, particularly in treatments with early-sown plants (Fig. 4), before waterlogging was even established.

Transferability to field conditions

In the field, depression soils are periodically waterlogged or experience rapid fluctuations in soil water content and surface ponding, resulting in a heterogeneous hydrological regime that often enhances overall denitrification-driven N_2O losses (Groffman et al. 2009; Kemmann et al. 2023; Tomasek et al. 2019). In our mesocosm setup, hydrological conditions were intentionally simplified to isolate the interactive effects of plant age and partial waterlogging on N_2O emissions. However, the use of constant water level and disturbed rather than intact soil likely led to an underestimation of waterlogging effects on N_2O emissions. The observed N_2O emissions are most likely applicable to periodically waterlogged agricultural depression soils with relatively fine-textured, nutrient-rich soils. Nevertheless, soil texture, soil C content, nutrient availability and wheat cultivar traits may further modulate the magnitude of the observed effects on N_2O emissions.

Furthermore, both the duration of waterlogging and the water table height strongly shape crop responses under field conditions. When temperatures range between 4–12 °C, winter wheat can recover from prolonged waterlogging (lasting 42 to 80 days), with yields reaching 82–96% of controls (Belford et al. 1985; Herzog et al. 2016). This resilience is particularly relevant in depression areas, where wheat often experiences late-winter to early-spring waterlogging. If winter wheat is able to restore growth and yield after waterlogging, it can still take up N remaining in the soil from earlier waterlogged periods, thereby helping to reduce subsequent N losses (Fageria and Baligar 2005). Shorter periods of waterlogging during later stages, such as flowering and grain filling, have been shown to reduce grain yield by 50–70%, thereby reducing the amount of N exported at harvest (Luxmoore et al. 1973; Ploschuk et al. 2018).

Conclusion

Overall, waterlogged soil emitted more N_2O than drained soils, however, the magnitude of emissions strongly depended on plant age and plant growth stage. The intermediate-sown wheat treatment produced higher N_2O emissions compared to soils with either early- or late-sown wheat. This response coincided with the greatest reduction in total root length and the highest soil DOC and NO_3^- concentrations, suggesting that reduced plant N uptake, altered root activity and increased availability of C and N substrates together enhanced N_2O emissions. These findings indicated that the N_2O source strength of depressions is strongly regulated by plant-mediated controls on soil N and C availability, which determine whether these landscape features function as strong N_2O hotspots. In frequently waterlogged depression soils, limited crop performance and N uptake may substantially increase N_2O emissions, whereas successful plant establishment can partially mitigate N_2O emissions even under waterlogged conditions. More broadly, our results identify plant age as a critical regulator of plant–soil interactions and associated N_2O emissions in agricultural depressions, highlighting the importance of incorporating crop phenology into assessments of N_2O source strength in heterogeneous, waterlogged field environments. These insights complement field-based observations and provide a process-based framework for interpreting the occurrence and intensity of N_2O hotspots in agricultural systems.

Acknowledgements This study was funded by the Independent Research Fund Denmark 'Limiting N_2O emission from hot spots in Danish agricultural soils—linking crop roots and nitrate dynamics to develop new strategies to mitigate trace gases' (funding number: 0217-00322B). P.A. has also been supported by the Pioneer Center for Landscape Research in Sustainable Agricultural Futures (Land-CRAFT), funded by the Danish National Research Foundation grant number P2. We are grateful to Pauline Rummel for her insightful comments and suggestions that helped improve this manuscript.

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Björn Kemmann: Writing – review & editing, Software, Formal analysis.

Per Ambus: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Bo Elberling: Writing – review & editing, Supervision, Funding acquisition.

Michael Dannenmann: Methodology, Writing – review & editing.

Kristian Thorup-Kristensen: Writing – review & editing, Supervision, Funding acquisition.

Daniel M.N. Poultney: Writing – review & editing, Supervision.

Carsten W. Mueller: Conceptualization, Methodology, Writing – review & editing, Validation, Supervision, Funding acquisition.

Funding Open access funding provided by Copenhagen University. This study was funded by the Independent Research Fund Denmark 'Limiting N_2O emission from hot spots in Danish agricultural soils—linking crop roots and nitrate dynamics to develop new strategies to mitigate trace gasses' (funding number: 0217-00322B). P.A. has also been supported by the Pioneer Center for Landscape Research in Sustainable Agricultural Futures (Land-CRAFT) funded by the Danish National Research Foundation grant number P2.

Data availability Data is available upon request.

Declarations

Competing interests The authors declare that they have no conflict of interest.

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