



## Timing of grass renewal regulates nitrous oxide emissions from a drained boreal peatland

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### ABSTRACT

Drained peatlands in northern latitudes have been used for forage production for decades. They are recognized sources of nitrous oxide (N<sub>2</sub>O) emissions. In boreal regions, grassland renewal is mandatory to maintain high forage productivity and quality, yet renewal practices involving ploughing can significantly increase N<sub>2</sub>O emissions. Here, we assessed the effect of plough timing (autumn ploughing with spring reseeded (AP) vs summer ploughing with immediate reseeded (SP)) on N<sub>2</sub>O emissions from a drained peatland in Eastern Finland. We conducted a three-year field experiment measuring N<sub>2</sub>O fluxes using manual chambers during snow-free seasons and snow gradient method during the snow-covered periods. Results showed that AP (88 kg N<sub>2</sub>O-N ha<sup>-1</sup>) emitted 2.6 times more N<sub>2</sub>O than SP (34.5 kg N<sub>2</sub>O-N ha<sup>-1</sup>) over the three years and exhibited greater interannual variability in its N<sub>2</sub>O emissions. Ploughing in the autumn led to sustained higher N<sub>2</sub>O emissions for longer periods and affected the following non-growing season and annual emissions considerably. Similarly, three-year yield-scaled N<sub>2</sub>O emissions were 2.2 times more in AP (3.7 kg N Mg<sup>-1</sup>) than in SP (1.7 kg N Mg<sup>-1</sup>). We found that grassland renewal increased the yield compared to the yields prior to renewal. These findings suggest that summer ploughing with prompt reseeded is a more sustainable practice for grassland renewal on drained peatlands, offering reduced N<sub>2</sub>O emissions without compromising productivity.

### 1. Introduction

Grasslands, both natural and managed, play a crucial role in global ecosystems, covering approximately 40 % of the Earth's land surface and serving as significant carbon sinks (Bai and Cotrufo, 2022; Bardgett et al., 2021). These ecosystems are vital for biodiversity conservation, soil health, environmental sustainability, and climate regulation. In some regions, including boreal areas, drained peatlands are used for intensive short term grassland production. These areas are essential for agricultural production, however, they present significant opportunities and challenges in the context of climate change mitigation and adaptation (Bai and Cotrufo, 2022; Olesen and Bindi, 2002).

Peatlands, both pristine and drained, represent critical ecosystems that store approximately one-third of the global soil carbon (C) pool and significant amounts of nitrogen (N) (Hugelius et al., 2020). The drainage of peatlands for agriculture, particularly for grassland establishment, has emerged as a major concern, inducing profound changes in soil

hydrology and biogeochemistry (Bhattarai et al., 2018; Lin et al., 2022; Maljanen et al., 2010). One of the most significant consequences of peatland drainage and subsequent grassland management is the increased nitrous oxide (N<sub>2</sub>O) emissions. N<sub>2</sub>O is a potent greenhouse gas and a significant contributor to stratospheric ozone depletion (Ravishankara et al., 2009; Sommerfeld et al., 1993). In boreal regions, grassland management on drained peatlands impacts significantly on N<sub>2</sub>O emissions (Gerin et al., 2023; Regina et al., 2004). For instance, in Finland, organic soils drained for agriculture cover less than 10 % of total arable land, while contributing 25 % of the national anthropogenic N<sub>2</sub>O emissions and 43 % of emissions from agricultural soils (Official Statistics of Finland, 2024).

The production of N<sub>2</sub>O in soil mainly originates from two microbial pathways: aerobic nitrification, which is the oxidation of ammonium (NH<sub>4</sub><sup>+</sup>) to nitrite (NO<sub>2</sub>) and nitrate (NO<sub>3</sub>), and anaerobic denitrification, which is the reduction of NO<sub>3</sub> and NO<sub>2</sub> to nitric oxide (NO), N<sub>2</sub>O and dinitrogen (N<sub>2</sub>) (e.g. Firestone and Davidson, 1989). N<sub>2</sub>O emissions from

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grasslands on drained peatlands are governed by a complex interplay of biogeochemical processes, soil properties, and environmental conditions: (1) Water table depth controls the balance between aerobic and anaerobic processes in peat soils, influencing  $\text{N}_2\text{O}$  production (Offermanns et al., 2023). (2) Soil temperature affects microbial activity and biogeochemical process rates. In the boreal region, frequent freeze–thaw cycles are expected due to fluctuating air temperatures, especially in spring, and these cycles are known to contribute significantly to  $\text{N}_2\text{O}$  emissions. (Pihlatie et al., 2010; Wagner-Riddle et al., 2017). (3) Mineral N availability directly corresponds to variations in  $\text{N}_2\text{O}$  emissions in grassland ecosystems (Shurpali et al., 2016; Wang et al., 2024). (4) Grass species composition and growth dynamics influence  $\text{N}_2\text{O}$  emissions through effects on soil structure, organic matter input, and nutrient uptake (Abalos et al., 2014; Maljanen et al., 2007; Silvan et al., 2005).

Management practices in boreal grasslands on drained peatlands significantly influence the factors mentioned above and, consequently,  $\text{N}_2\text{O}$  emissions (Wang et al., 2024). Grassland renewal is a common practice to maintain productivity and forage quality. In north-western Europe, it is typically carried out every five to ten years (Buchen et al., 2017; Ammann et al., 2020). On organic soils this is most often done by ploughing, which involves radical soil disturbance (MacDonald et al., 2011; Olesen and Bindi, 2002; Reinsch et al., 2018). This process is necessary to incorporate organic matter, control weeds, and prepare the seedbed for reseeding. However, the timing of ploughing is a critical yet understudied factor in  $\text{N}_2\text{O}$  emissions from these managed grasslands (Reinsch et al., 2018; Velthof et al., 2010). The traditional autumn ploughing has been favored in boreal grasslands for its practical advantages, such as better distribution of farm labor over the year and potentially improved soil structure for spring sowing (Ammann et al., 2020; Velthof et al., 2010). However, this practice can increase  $\text{N}_2\text{O}$  emissions during the non-growing season (NGS) due to microbial decomposition of exposed organic matter when nutrient uptake by plants is minimal (Offermanns et al., 2023; Velthof et al., 2010). Additionally, this practice is counterintuitive to the Finland Climate Action that urges the farmers to not leave any agricultural land without a proper vegetation cover at any time of the year (Ministry of Agriculture and Forestry, 2023). An alternative approach, summer renewal, involves ploughing followed by prompt reseeding in the middle of the growing season (GS), after the first grass cut (Ringselle et al., 2019; Velthof et al., 2010). This method may offer several advantages in the context of boreal grasslands: (1) quick establishment of new grass cover following ploughing may limit  $\text{N}_2\text{O}$  emissions by allowing rapid plant utilization of released nutrients (Merbold et al., 2014); (2) ploughing during the warmest and driest part of the GS may limit microbial activity and associated  $\text{N}_2\text{O}$  production in these typically wet organic soils (Buchen et al., 2017; Clagnan et al., 2020); (3) drier soil conditions during summer ploughing can reduce soil compaction compared to autumn ploughing, potentially improving soil structure and grass growth (Calvelo-Pereira et al., 2022). Despite these potential benefits, the impact of summer renewal on  $\text{N}_2\text{O}$  emissions from boreal grasslands on drained peatlands has not been thoroughly investigated.

To assess the impact of grass renewal timing on  $\text{N}_2\text{O}$  emissions, we conducted a three-year field experiment on a perennial grassland on a drained peatland in Eastern Finland. We measured  $\text{N}_2\text{O}$  emissions from two treatments, autumn ploughing (AP) and summer ploughing (SP) using closed-static chamber for snow-free period and snow gradient method for snow-covered period. We aimed to address the following research questions: (1) How does the timing of grass renewal (AP vs. SP) affect  $\text{N}_2\text{O}$  emissions over a three-year period? (2) What effect do different grass renewal timings have on soil variables that regulate  $\text{N}_2\text{O}$  emissions? (3) Could summer ploughing be an effective alternative to traditional autumn ploughing for mitigating  $\text{N}_2\text{O}$  emissions from drained peatlands? We hypothesized that SP could reduce  $\text{N}_2\text{O}$  emissions due to earlier establishment of new vegetation after ploughing, that outcompetes microbial competition for nutrient uptake and  $\text{N}_2\text{O}$

production. By evaluating the effect of grassland renewal timing on  $\text{N}_2\text{O}$  emissions, our findings will contribute towards the optimization of sustainable agricultural practices on drained peatlands and the associated  $\text{N}_2\text{O}$  production and emissions.

## 2. Materials and methods

### 2.1. Study site

The study site is a 6.97 ha peatland in Maaninka, eastern Finland (63.1380° N, 27.2462° E). The averaged (1991–2020) mean annual air temperature and mean annual precipitation of the region are 3.8 °C and 612 mm respectively (Jokinen et al., 2021). The snow cover typically lasts from late November until the end of April.

The field was drained in 2013. From 2013–2020, the field was used as a vegetative buffer zone area for nearby waterways, with 1–2 grass harvests per year and no fertilizer or herbicide application. The average peat depth at the experimental area is 70 cm, while it ranges from 25 cm to 100 cm in the field (Fig. 1). The key soil physiochemical properties (mean  $\pm$  standard deviation) are pH ( $\text{H}_2\text{O}$ )  $5.3 \pm 0.1$ , electrical conductivity  $1.1 \pm 0.2 \text{ mS m}^{-1}$ , soil organic carbon  $26.99 \pm 4.11 \text{ g kg}^{-1}$ , total nitrogen  $1.94 \pm 0.27 \text{ g N kg}^{-1}$ , C:N ratio  $13.9 \pm 0.59$ , and total phosphorus  $3.5 \pm 0.9 \text{ g N kg}^{-1}$ .

### 2.2. Study design and experimental setup

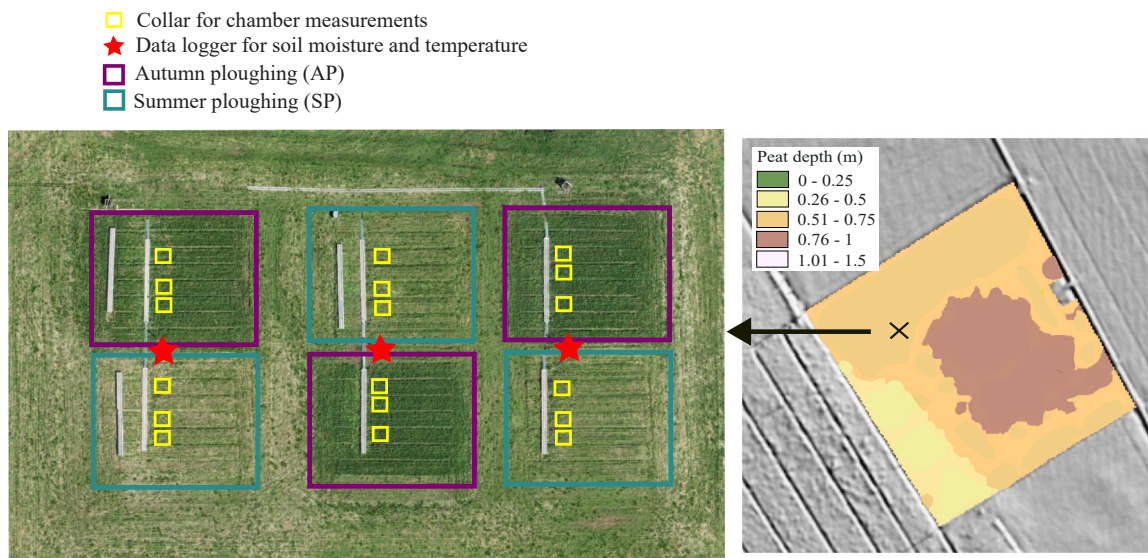
We conducted the  $\text{N}_2\text{O}$  flux measurements for three years, starting from May 2021 to May 2024. The measurement years are hereafter referred to as first year (1.5.2021–30.4.2022), second year (1.5.2022–30.4.2023) and third year (1.5.2023–30.4.2024). In May 2021, the experimental setup was established within the main field, which had two treatments: autumn ploughing (AP) and summer ploughing (SP). Each treatment was applied to three separate blocks (biological replicates) and within each block, there were three adjacent experimental plots (technical replicates) totaling 18 plots, 9 plots per treatment (each plot  $1.5 \times 10 \text{ m}$ ) (Fig. 1). During 2021, all plots received two rounds of fertilization and harvest. On 24th of August, AP plots were treated with the herbicide Roundup Flex (glyphosate) at a rate of  $6 \text{ l ha}^{-1}$ , applied in  $200 \text{ l ha}^{-1}$  of water. AP plots were ploughed in September, while SP plots were left with grass cover over winter. In May 2022, AP plots were sowed with a mixture of timothy (*Phleum pratense*, cv. “Uula”) and meadow fescue (*Festuca pratensis*, cv. “Inkeri”), using a cover crop of wheat (*Triticum aestivum* cv. “Anniina”) and oat (*Avena sativa* cv. “Taika”). The SP plots were ploughed on 7th of July, one week after the first grass harvest that occurred in June 2022, followed by a rapid seedbed preparation and sowing on 8th of July. Due to excessive rainfall during the rest of the growing season after establishment, the plots were unable to be harvested again in the fall, leading to overwintering of both grass and cover crop. In 2023, all plots had the same management with two rounds of fertilization and harvest (Table 1).

### 2.3. $\text{N}_2\text{O}$ measurements and flux calculation

We used both closed-static chamber for snow-free seasons and snow gradient methods for snow-covered period to measure  $\text{N}_2\text{O}$  fluxes during the study period. Measurements were made between 7:00 and 17:00, weekly from May to September representing the GS, and biweekly from October to April considered here as the NGS. Additional measurements were conducted during the weeks with management practices, when possible.

#### 2.3.1. $\text{N}_2\text{O}$ flux measurements during snow-free periods

To facilitate the gas flux measurements, collars with water grooves ( $60 \text{ cm} \times 60 \text{ cm} \times 15 \text{ cm}$ ) were installed at the end of each plot when the plots were established (hereafter permanent collars) and removed



**Fig. 1.** Map of the experimental site showing spatial distribution of peat depth and experimental plot layout at the study site. Color gradient indicates peat depth (m) variation across the experimental field and the placement for plot trial inside the field is marked with X. Violet color represents AP treatment and blue color SP, yellow squares the placement of permanent collars and stars the location of loggers with sensors for soil volumetric water content and soil temperature measurements at 5 cm depth.

**Table 1**

Agricultural management practices applied to experimental plots under autumn ploughing (AP) and summer ploughing (SP) treatments during the three-year study (2021–2024). Herbicide application for weed control was conducted exclusively on AP plots on August 24, 2021. # Sowing was done together with the fertilization.

Year	Treatment	Fertilization 1 (kg N ha <sup>-1</sup> )	Harvest 1	Fertilization 2 (kg N ha <sup>-1</sup> )	Harvest 2	Ploughing
1 (May 2021–April 2022)	AP	75 (19th May)	17th June (grass)	70 (17th June)	27th July (grass)	6th September
	SP	75 (19th May)	17th June (grass)	70 (17th June)	27th July (grass)	No ploughing
2 (May 2022–April 2023)	AP	106# (31st May)	17th August (cover crop as whole grain)			No ploughing
	SP	89.7 (24th May)	27th June (grass)	69# (8th July)		7th July
3 (May 2023–April 2024)	AP	120 (22nd May)	27th June (grass)	70.4 (28th June)	10th August (grass)	No ploughing
	SP	120 (22nd May)	27th June (grass)	70.4 (28th June)	10th August (grass)	No ploughing

only to facilitate ploughing and sowing. After each management event, the collars were placed back in the exact same location. During snow-free seasons, N<sub>2</sub>O fluxes were measured using closed-static chamber method. The concentrations of N<sub>2</sub>O in the chamber headspace were analyzed with gas chromatography in year 2021 (hereafter, N<sub>2</sub>O-GC) and with a portable trace gas analyzer (LI-7820 LI-COR Biosciences; Lincoln, NE, USA) designed for N<sub>2</sub>O concentration measurement in years 2022 and 2023 (hereafter, N<sub>2</sub>O-LI). During the flux measurements, permanent collars were covered with opaque chambers for 20 min for N<sub>2</sub>O-GC and 2–4 min for N<sub>2</sub>O-LI. The first two measurements were conducted with the opaque chamber placed directly into the soil (similar as N<sub>2</sub>O-GC) with the method described by Maljanen et al. (2006). Different chamber sizes were used to minimize disturbance to the vegetation, and extensions were applied when plant height exceeded the chamber height.

For N<sub>2</sub>O-GC, gas samples were taken from the chamber headspace with 60 ml syringes at 5, 10, 15, and 20 min after the enclosure and were stored in pre-evacuated vials (Labco® UK). Gas samples were analyzed with a gas chromatograph (Agilent 7890 Agilent Technologies, Inc., Wilmington, DE, USA) equipped with flame ionizer and electron capture detectors, and a nickel catalyst for converting CO<sub>2</sub> to CH<sub>4</sub>. The system featured a 2 ml sample loop and a backflush system to separate water from the sample and flush the precolumn between runs. The precolumn and analytical columns were 1.8 and 3.0 m long steel columns packed with 80/100 mesh Hayesep Q (Supelco Inc., Bellefonte, PA, USA). Nitrogen served as the carrier gas. A standard gas mixture of known N<sub>2</sub>O

concentration was used for calibration with seven concentration points. The detection limit for N<sub>2</sub>O was 1.2 µg N m<sup>-2</sup> h<sup>-1</sup>. Samples were fed into the gas chromatograph by an autosampler (222 XL Liquid handler, Gilson Medical Electronics, France) and analyzed within one month of sampling.

For N<sub>2</sub>O-LI, a certain gas volume from the chamber headspace was pumped continuously for measurement duration (i.e., 2–4 min) into the LI-7820 gas analyzer and pumped back to the chamber headspace after the concentration's measurement which is based on optical feedback-cavity enhanced spectroscopy. Total of 120–240 data points (60 data points/min) representing N<sub>2</sub>O concentrations were collected during the 2–4 min of measurement. The system was equipped with a thermometer to monitor chamber temperature and an electric fan to ensure air mixing. Measurement was recorded every second and typically lasted 2–4 min depending on the chamber size and extra collars.

### 2.3.2. N<sub>2</sub>O flux measurement during snow cover periods

During snow-cover seasons, when the snow depth exceeded 10 cm, N<sub>2</sub>O fluxes were measured using the snow gradient method (Sommerfeld et al., 1993). Gas samples were collected at 10 cm intervals from the snow surface to the soil surface using syringes connected to a metal probe (diameter 3 mm, length 100 cm) that was pushed through the snowpack. The gas samples were stored and analyzed with a previously described gas chromatograph. Snow samples were concurrently collected using a PVC cylinder (diameter 10.2 cm) for porosity measurements. Snow porosity was calculated using the density of pure ice

(0.9168 g cm<sup>-3</sup>).

### 2.3.3. N<sub>2</sub>O flux and cumulative emission calculations

N<sub>2</sub>O fluxes in both N<sub>2</sub>O-GC and N<sub>2</sub>O-LI techniques were calculated from the linear regression of gas concentration over time (i.e., slope) using Eq. 1:

$$F = \frac{p_0 \cdot k \cdot V \cdot M}{R \cdot T \cdot A}, \quad (1)$$

where F is the gas flux (g m<sup>-2</sup> h<sup>-1</sup>), p<sub>0</sub> is 101.3 kPa, k is the slope of the flux (ppm h<sup>-1</sup>), V is the volume of the chamber headspace (m<sup>3</sup>), M is the molar mass (g mol<sup>-1</sup>) of the respective gas, R is the ideal gas constant (8.314 J k<sup>-1</sup> mol<sup>-1</sup>), T is the temperature (K) inside the chamber, A is the collar area (m<sup>2</sup>).

For N<sub>2</sub>O-GC we used R<sup>2</sup> > 0.8 for simultaneously measured CO<sub>2</sub> as a quality criterion for selecting good N<sub>2</sub>O data points. All individual gas flux measurements meeting the criteria were included in the data analysis and those that did not meet the criteria were excluded. For N<sub>2</sub>O-LI, data trimming for anomalies caused by chamber placement and removal was manually performed and the fluxes were accepted based on visual inspection (three measurements were rejected due to obvious measurement error). During the three years of experiment, we observed 29 instances of non-linearity between N<sub>2</sub>O concentrations and measurement time (Table S1 and Fig. S1). To avoid under- or overestimation in flux calculation at those time events, we applied a non-linear function, Hutchinson and Mosier exponential model, available in R package called “goFlux” (Rheault et al., 2024) to calculate the N<sub>2</sub>O fluxes.

The N<sub>2</sub>O fluxes from the snowpack were calculated based on the N<sub>2</sub>O concentration gradient between the snow surface and the soil surface, and diffusion rate of N<sub>2</sub>O through the snowpack, according to Fick’s law of diffusion (Eq. 2):

$$J_{\text{gas}} = D_{\text{gas}} \left( \frac{d_{\text{gas}}}{dz} \right) f, \quad (2)$$

where J<sub>gas</sub> is the diffusive flux for gas (g cm<sup>-2</sup> s<sup>-1</sup>), D<sub>gas</sub> is the diffusion coefficient of respective gas in the air (N<sub>2</sub>O 0.139 cm<sup>2</sup> s<sup>-1</sup>), d<sub>gas</sub>/dz is the vertical concentration gradient of respective gas in the profile (N<sub>2</sub>O g cm<sup>-3</sup> cm<sup>-1</sup>), and f is the air-filled porosity of the snow (cm<sup>3</sup> cm<sup>-3</sup>) (Maljanen et al., 2003; Sommerfeld et al., 1993). Intermediate samples taken every 10 cm were used to assess the reliability of the concentration profiles, not for flux calculation. Simultaneously measured CO<sub>2</sub> was used to select good data points, and when CO<sub>2</sub> flux was negative, the measurements were rejected (11 measurements in total).

For annual emission calculations, the gaps in the data were filled by using time weighted averages, by multiplying the average daily flux of two consecutive measurements by the number of days between the measurements. Measured and interpolated daily values were then summed up together to get annual cumulative N<sub>2</sub>O sum. Cumulative emissions were first calculated separately to every measuring point and then averaged by treatment. Cumulative emissions were also calculated separately for each GS and NGS.

## 2.4. Supporting measurements

### 2.4.1. Environmental conditions

In snow-free periods, soil temperature (TM-80N, Tenmars electronics co., Ltd, Taiwan) and soil volumetric water content (HH2 Moisture Meter, Delta-T Devices Ltd, UK) were manually measured at 5 cm depth near every collar simultaneously with N<sub>2</sub>O flux measurements. Similarly, the water table level was manually measured from monitoring wells near each collar. In addition, data loggers were installed on 11.11.2021 to continuously measure soil volumetric water content and temperature (ZL6 Data Logger METER Group, equipped with TEROS 12 sensors installed at 5 cm depth, recording data with 30-minute intervals). Water table level was followed in 30-minute intervals by using

U20L-04 HOBO Water Level Data Loggers (Onset Computer Corporation), and the results were calculated using the HOBOWare Pro Software (Onset Computer Corporation).

During snow-cover periods, snow depth was measured weekly from six independent sampling locations in the middle of the intensive site, situated near the continuous data loggers (Fig. 1). From the same locations, frost depth was measured using 1 m long tubes buried vertically in soil, which contained methylene blue solution that turns transparent when frozen. Air temperature and precipitation data were obtained from the weather station operated by Finnish Meteorological Institute (FMI) located in Maaninka, Kuopio, approximately 3 km from the study site.

### 2.4.2. Soil ammonium and nitrate concentration

Topsoil samples (0–10 cm) were collected from each measurement plot using a stainless-steel corer (length 25 cm, diameter 2.4 cm) four times during the 2021 GS and five times during the 2022 and 2023 GS. For each sampling event, four samples were taken from different locations within each plot and combined to form one composite sample per plot. The soil samples were sieved (4 mm) and extracted on the following day. The concentrations of mineral N were measured from soil-KCl extracts (v:v, 1:3). We extracted mineral N from 30 ml of soil using 100 ml of 1 M KCl. The extract was obtained by shaking the soil slurry for 1 h at 175 rpm and filtering through filter paper (Whatman ashless Ø 185 mm filters). The concentrations of NH<sub>4</sub><sup>+</sup> in soil extracts were analyzed spectrophotometrically at 650 nm with the colorimetric method as described in Fawcett and Scott (1960). Similarly, NO<sub>3</sub><sup>-</sup> concentration was determined from soil extracts spectrophotometrically at 544 nm using the colorimetric method (Miranda et al., 2001). The spectrophotometric determination of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> was done with FLUOstar Omega microplate reader (BMG Labtech). Soil gravimetric water content (GWC) was determined by drying a subsample at 65 °C for approximately 24 h. In addition, electrical conductivity and pH were measured from samples mixed with distilled H<sub>2</sub>O using EC (HQ40d, Hach Co., CO, USA) and pH meters (UB-10, Denver Instrument, CO, USA).

### 2.4.3. N<sub>2</sub>O concentrations in soil profile

Soil profile N<sub>2</sub>O concentrations were determined using PVC soil gas collectors (diameter 9 mm, length 2 m) (Kammann et al., 2001). The collectors were installed at depths of 5, 20 and 40 cm in the soil and sampling tube (length 60 cm, of which approximately 20–50 cm remained above the soil surface depending on installation depth) was brought to soil surface for sampling. Gas samples were collected with 60 ml syringes equipped with three-way-valve monthly during GS and twice a month during NGS. The samples were stored in vials and analyzed with previously described gas chromatograph until April of 2023. From May 2023 forward the samples were analyzed with a different gas chromatograph (Agilent 8890 Agilent Technologies, Inc., Wilmington, DE, USA). The gas samples were analyzed within two months of sampling.

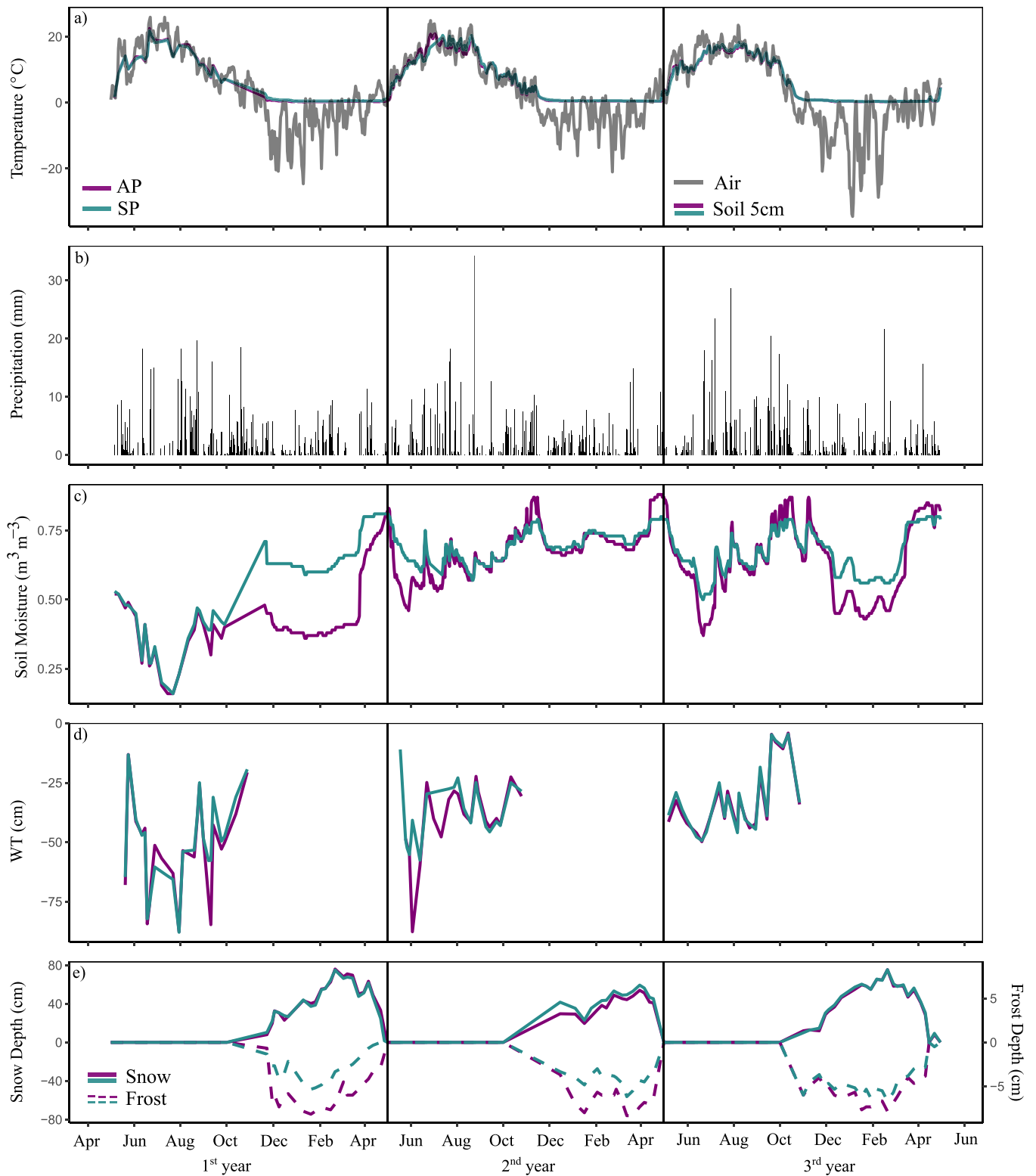
### 2.4.4. Vegetation characteristics

Canopy height was measured manually every time when N<sub>2</sub>O fluxes were measured. Once the cover crops exceeded the height of the grass, the average of oat and wheat height was used as the canopy height. Biomass inside the collars was manually cut to a height of 7 cm at the same time as the rest of the field was harvested. Cereals were harvested as whole grain. This procedure was followed for all harvests, except for the second harvest of SP in the second GS. The harvested biomass was then dried in the oven at 60 °C for 48 h for determination of dry matter content. To estimate the biomass of cereals inside the collars for SP in the second GS, we used slope and intercept obtained by regressing biomass from ten fixed locations from the entire field against the biomass within the collars from first year and first harvest in the second year. The biomass from the ten fixed locations was obtained by harvesting biomass manually using a 0.1 m<sup>2</sup> metallic collar. Biomass carbon and nitrogen

contents were analyzed using in-house method JOK3016 (LECO). Leaf area index (LAI) was measured using a plant canopy analyzer (model: LAI-2000, LiCor) with a 180° view cap from plots.

2.5. Statistical analysis

Statistical analysis and graphical presentations were made using R statistical software (R version 4.4.2). For normally distributed data, we used two-way analysis of variance (ANOVA) to test the statistical



**Fig. 2.** Environmental and soil conditions during the three-year study period: a) daily mean air (black) temperature and soil temperature at 5 cm depth (AP violet, SP blue) (°C), b) daily precipitation (mm), c) volumetric soil moisture at 5 cm depth ( $m^3 m^{-3}$ ), d) water table (WT) depth (cm) below soil surface and e) snow height (cm, solid line) with depth of frost penetration (cm, dashed line). In all plots color indicates experimental treatments, violet, AP and blue, SP.

significance of measured variables (Table S4) between the treatments and measurement time with Tukey's test as a post hoc test to assess significant differences between the interactions (treatments x time points). For non-normally distributed data we used non-parametric Kruskal-Wallis test to test the significant difference between the treatments and Dunn's test as a post hoc test to test the significant difference between the measurement time points of the variables. Before statistical test, data normality was assessed using histograms and QQ-plots and

data was transformed to achieve normal distribution when needed and possible.

### 3. Results

#### 3.1. Environmental and soil conditions

The mean annual air temperature and mean annual precipitation was

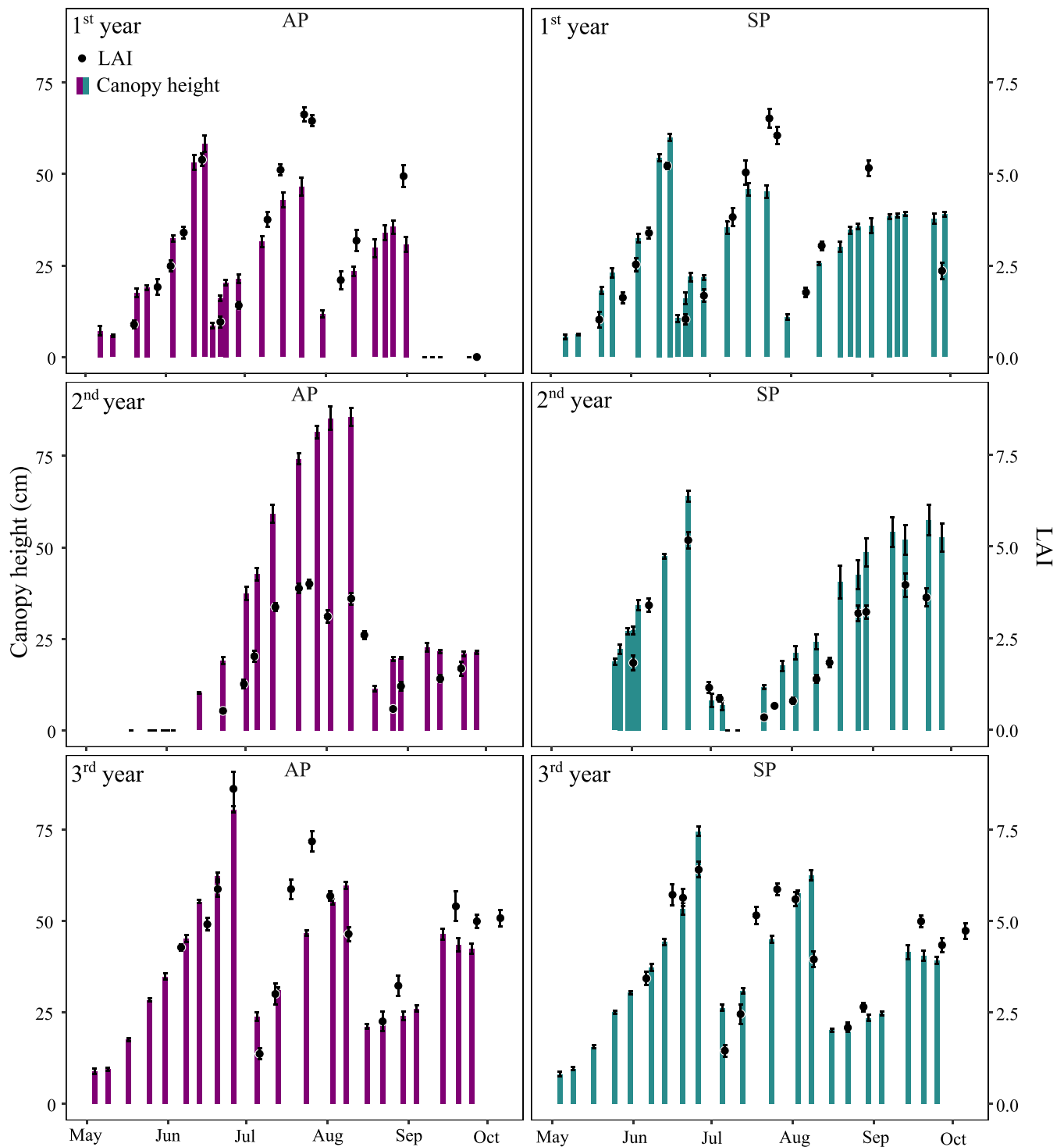


Fig. 3. Temporal dynamics of vegetation development showing leaf area index (LAI, circles) and canopy height (cm, bars) between treatments AP (left panel) and SP (right panel). Data shown for grass in the first and third years, and combined grass-cover crop measurements in the second year. Values are mean and error bars represent standard deviation (n = 9).

4.4 °C and 622 mm for the first year, 4.6 °C and 578 mm for the second year, and 3.2 °C and 661 mm for the third year, with no significant difference among the years (Fig. 2a, b). The GS lasted 160, 167, and 162 days in the first, second, and third year, respectively. The soil temperature at 5 cm depth varied significantly among all years ( $p < 0.05$ ) and the mean soil temperature was 2.2, 6.4 and 6.3 °C in the first, second, and third year, respectively, with a maximum of 22.5 °C on 21st of June in the first year and minimum of 0.2 °C on 10th of January in the first year (Fig. 2a). There was a significant difference ( $p < 0.05$ ) in the soil temperature between the treatments in the first year, however, not in the second and third year. Mean soil moisture at 5 cm depth was 0.54, 0.69 and 0.65  $\text{m}^3 \text{m}^{-3}$  in the first, second, and third year, respectively, and varied from 0.16 (on 15th of July in the first year) to 0.88  $\text{m}^3 \text{m}^{-3}$  (on 23rd of April in the second year) (Fig. 2c). Soil moisture differed significantly between all years ( $p < 0.05$ ). In the first and third year, soil moisture was significantly higher in SP than in AP ( $p < 0.05$ ), while in the second year no significant difference between treatments was observed. On average, water table level was -52.0, -34.7 and -32.2 cm below the soil surface in the first, second, and third year, respectively, and ranged from 0 (indicating water saturation) to -100 cm during the three-year study (Fig. 2d). The water table level was significantly ( $p < 0.05$ ) lower in the first year compared to second and third year, however, there was no difference between the treatments in any of the years. There was no significant difference in snow depth between the years and treatments. Maximum snow depth was 83 cm in the first, 64 cm in the second and 80 cm in the third year (Fig. 2e). Permanent snow cover lasted 160, 160, and 159 days in first, second, and third year, respectively. Frost depth was significantly ( $p < 0.05$ ) greater in AP compared to SP in first and second year, however, there was no difference between the treatments in the third year (Fig. 2e). In general, the maximum frost depth was greater in AP than in SP in all years; 11, 9 and 9.5 cm in AP and 7, 7 and 8.5 cm in SP in the first, second and third year, respectively.

### 3.2. Vegetation dynamics and biomass yield

Vegetation development followed a seasonal trend with harvests and renewal practices (Fig. 3). In the first year, both treatments showed similar vegetation characteristics until AP was ploughed, with maximum LAI of 7.5 and 7.7 for AP and SP, respectively, with corresponding maximum canopy heights of 72 cm and 64 cm. In the second year, LAI did not vary significantly between the treatments (maximum of 4.6 for AP and 6.3 for SP), however, AP had significantly ( $p < 0.05$ ) higher canopy height (95 cm) compared to that in SP (80 cm). In the third year, maximum LAI was 8.6 for AP and 6.4 for SP, with corresponding canopy heights of 85 cm and 78 cm, with no significant differences between the treatments.

Total biomass yield showed significant treatment effects in second

and third year, with AP producing higher yields compared to SP ( $p < 0.05$ ; Tables 1 and 2). Both treatments increased yield following renewal compared to pre-renewal (year 1 vs. 3,  $p < 0.05$ ). Biomass C% proportion remained relatively consistent, with an average of 43 % C across treatments and years (Table 2). However, nitrogen content showed treatment differences in the second year, with AP having lower values than SP ( $p < 0.05$ ).

### 3.3. N<sub>2</sub>O emission dynamics

#### 3.3.1. Temporal flux patterns

N<sub>2</sub>O emissions showed different temporal patterns in response to plough timing over the three years (Fig. 4). The daily averaged fluxes showed both positive (emission) and negative (uptake) N<sub>2</sub>O rates ( $\text{mg m}^{-2} \text{d}^{-1}$ ) and ranged from 0.47 to 120.30 for the first, 0.03–472.13 for the second and -0.18–19.15 for the third year (Fig. 4).

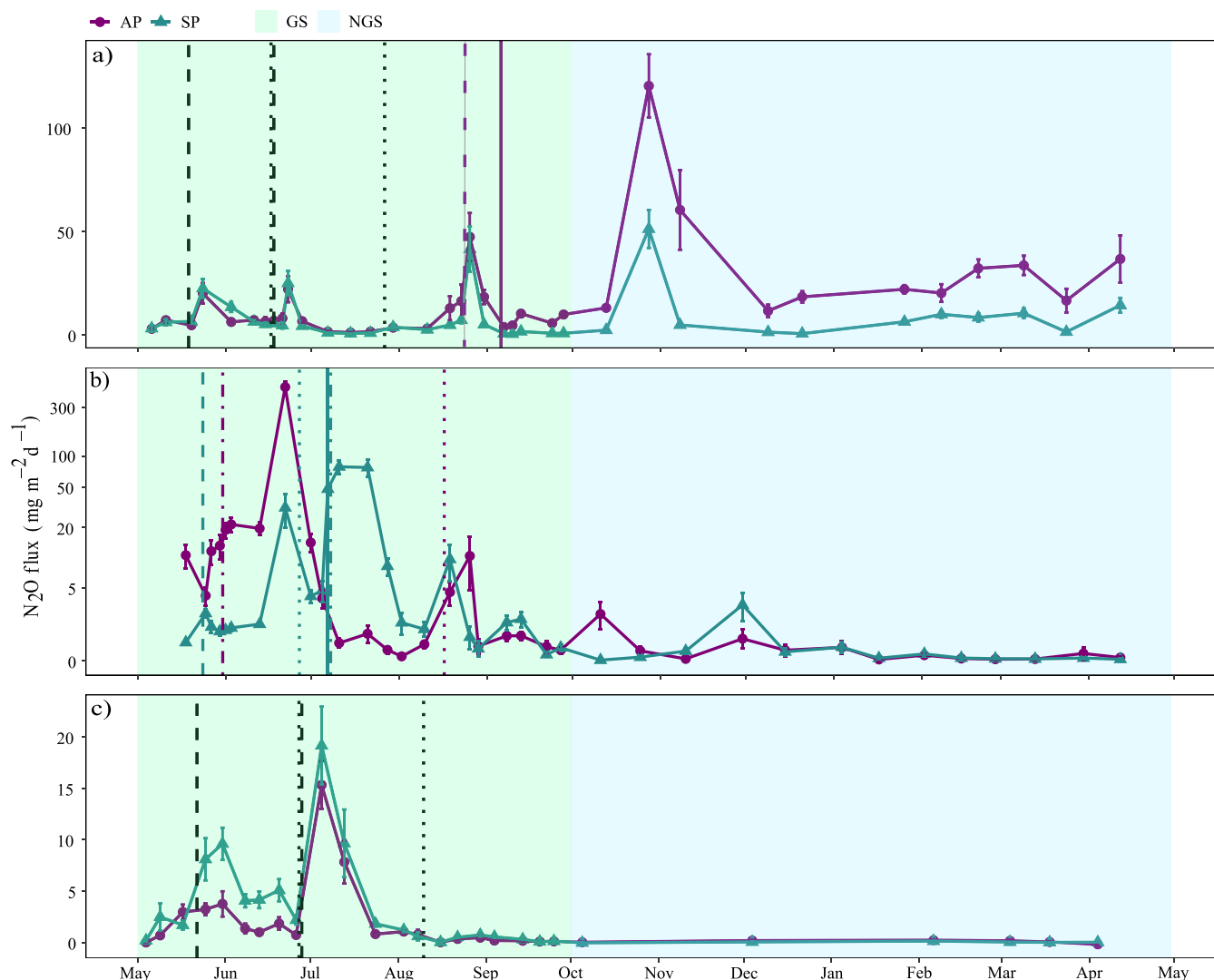
During the first year, AP and SP showed similar N<sub>2</sub>O emission patterns regardless of the implied management practices and the seasons (Fig. 4a). Both fertilization events marginally increased N<sub>2</sub>O emissions with rather similar rates in AP and SP. Similarly, both harvesting events also increased N<sub>2</sub>O emissions in AP and SP; however, the increment was higher after the second harvest in both the treatments. The N<sub>2</sub>O emissions increased sharply in both AP and SP, after the commencement of NGS. After ploughing (early September, Table 1), AP did not show any immediate response to N<sub>2</sub>O emissions. However, AP showed considerably higher N<sub>2</sub>O emissions than non-ploughed SP throughout the NGS. There was a significant ( $p < 0.05$ ) difference in the fluxes between the treatments in the first year, with annual mean of  $17.3 \pm 22.2 \text{ mg m}^{-2} \text{d}^{-1}$  for AP and  $7.9 \pm 11.1 \text{ mg m}^{-2} \text{d}^{-1}$  for SP (Fig. 4a).

In the second year, AP and SP showed varying patterns of N<sub>2</sub>O emissions, especially in the GS, when management practices were implied at different time points and treatments had diverse vegetation species (Fig. 3 and Fig. 4b). The second year started with higher N<sub>2</sub>O emissions from the autumn ploughed AP than non-ploughed SP indicating a strong legacy of ploughing on N<sub>2</sub>O emissions. The N<sub>2</sub>O emissions from AP were further increased soon after fertilization and seeding, reaching a maximum flux of  $472 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$ , the highest rate among all measurement years. SP, which was fertilized a few days earlier than AP (Table 1), also showed increased N<sub>2</sub>O emissions after fertilization, however with a longer delay and a lower peak ( $78 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$ ) than AP. The N<sub>2</sub>O emissions from both treatments decreased to a same level at the start of July, just prior to the renewal of SP. After ploughing, reseeding and fertilization of SP in July, the N<sub>2</sub>O emissions increased sharply and displayed a consistently higher flux rates for approximately one month, whereas emissions from AP were decreased further. The N<sub>2</sub>O emissions increased similarly between AP and SP during early autumn and both treatments showed a peak in emissions after AP harvest. Thereafter, interestingly, N<sub>2</sub>O emissions from SP

**Table 2**

Dry matter yield, nitrous oxide emissions (annual and yield-scaled annual emissions) and carbon (C%) and nitrogen (N%) proportion of the harvested biomass. The dry matter yield ( $\text{kg dw ha}^{-1}$ ) was estimated using the biomass harvested from the measurement collars. The total N<sub>2</sub>O emissions ( $\text{N}_2\text{O-N kg ha}^{-1}$ ) represents the annual estimates. The yield-scaled N<sub>2</sub>O emissions ( $\text{kg N Mg}^{-1}$ ) represent the amount of N<sub>2</sub>O emitted per unit of biomass harvested. Carbon content (%) is expressed as the mean across all harvests conducted that year. Different lowercase letters indicate significant difference ( $p < 0.05$ ) between the treatments inside each year and different uppercase letters indicate significant differences between the years inside the treatments. \* Indicates that AP had one end-of-season harvest (cover crops), while SP had one mid-summer grass harvest. #SP second harvest value represents estimated average biomass amount at the end of GS from the whole field.

	Year	Harvest 1 kg dw ha <sup>-1</sup>	Harvest 2 kg dw ha <sup>-1</sup>	Total kg dw ha <sup>-1</sup>	Annual N <sub>2</sub> O-N kg ha <sup>-1</sup>	Yield-scaled annual N <sub>2</sub> O emissions (kg N Mg <sup>-1</sup> )	C%	N%
AP	1	3130	2750	5880 <sup>a,A</sup>	50.7 <sup>a,A</sup>	8.6 <sup>a,A</sup>	43.1 <sup>a,A</sup>	2.5 <sup>a,A</sup>
	2*	7600		7600 <sup>a,B</sup>	35.0 <sup>a,B</sup>	4.6 <sup>a,B</sup>	44.1 <sup>a,B</sup>	1.3 <sup>a,B</sup>
	3	6790	3830	10 600 <sup>a,C</sup>	2.3 <sup>a,C</sup>	0.2 <sup>a,C</sup>	43.4 <sup>a,A</sup>	2.0 <sup>a,C</sup>
	Total				88.0 <sup>a</sup>	3.7 <sup>a</sup>		
SP	1	3190	2870	6070 <sup>a,A</sup>	17.0 <sup>b,A</sup>	2.8 <sup>b,A</sup>	43.3 <sup>a,A</sup>	2.5 <sup>a,A</sup>
	2*	4740	(3870)#	4740 <sup>b,B</sup>	13.8 <sup>b,A</sup>	2.9 <sup>b,A</sup>	43.6 <sup>a,A</sup>	2.6 <sup>b,A</sup>
	3	5340	3960	9300 <sup>b,C</sup>	3.7 <sup>a,B</sup>	0.4 <sup>b,B</sup>	43.6 <sup>a,A</sup>	2.1 <sup>a,B</sup>
	Total				34.5 <sup>b</sup>	1.7 <sup>b</sup>		



**Fig. 4.** Daily  $\text{N}_2\text{O}$  fluxes ( $\text{mg m}^{-2} \text{d}^{-1}$ ) from autumn ploughing (AP, violet) and summer ploughing (SP, blue) treatments during the growing (GS, green shade) and non-growing (NGS, blue shade) season of the a) first year, b) second year, and c) third year. Various management practices are indicated by different vertical lines: fertilization (dashed), ploughing (solid), herbicide application (widely spaced dashed), harvest (dotted), and seeding (dash-dot). All management practices for the second year were employed at different time points for AP and SP, which are indicated by the respective treatment colors. All management practices for the first and third year were applied simultaneously for both treatments, and are shown in a single black color, except for ploughing and herbicide application in the first year, which were only done for AP and is therefore indicated by its own color. Values represent means  $\pm$  standard error of mean ( $n = 9$ ). Note: Scale of  $\text{N}_2\text{O}$  flux rates (y-axis) is different for different years.

decreased sharply, whereas those from AP increased temporarily before both treatments showed similarly low and sustained  $\text{N}_2\text{O}$  fluxes throughout the NGS (Fig. 4b). Although the annual mean  $\text{N}_2\text{O}$  emissions in the second year were higher in AP ( $17.69 \pm 79.33 \text{ mg m}^{-2} \text{d}^{-1}$ ) compared to SP ( $8.03 \pm 19.53 \text{ mg m}^{-2} \text{d}^{-1}$ ), there was no significant difference between the treatments.

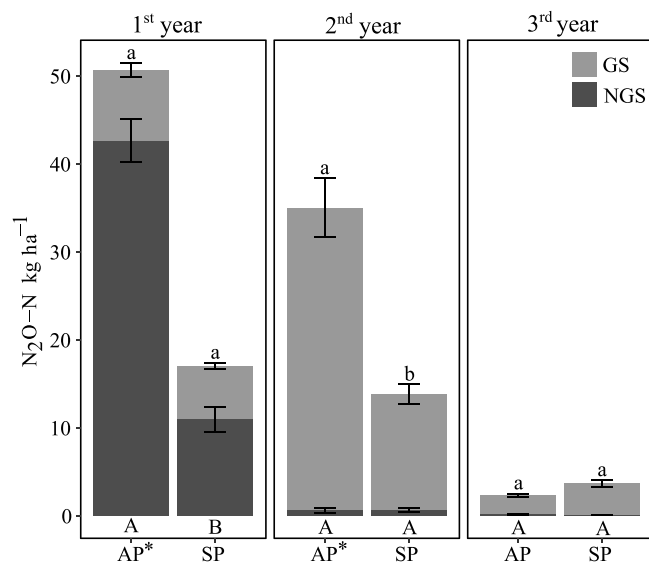
By the third year, after the grass had been renewed in both treatments, the  $\text{N}_2\text{O}$  emission patterns remained rather similar in both the treatments, except after first fertilization, which showed a contrasting trend between the treatments (Fig. 4c). The  $\text{N}_2\text{O}$  emissions increased after the first fertilization in SP, however remained unchanged in AP. Nevertheless,  $\text{N}_2\text{O}$  emissions showed a similar pattern with the highest emission rates in the third year, in both AP ( $15.3 \text{ mg m}^{-2} \text{d}^{-1}$ ) and SP ( $19.2 \text{ mg m}^{-2} \text{d}^{-1}$ ) after the first harvest and second fertilization. After that, the  $\text{N}_2\text{O}$  emissions declined sharply in both AP and SP and showed a sustained lower emissions rate for rest of the year without any effects from second harvest. The annual mean  $\text{N}_2\text{O}$  emissions were similar in AP ( $1.59 \pm 3.23 \text{ mg m}^{-2} \text{d}^{-1}$ ) and SP ( $2.68 \pm 4.40 \text{ mg m}^{-2} \text{d}^{-1}$ ), even

though SP had significantly ( $p < 0.05$ ) higher flux rates during the GS.

### 3.3.2. Seasonal mean, annual cumulative and yield-scaled $\text{N}_2\text{O}$ emissions

The mean  $\text{N}_2\text{O}$  emissions rates of GS and NGS varied between the measurement years for AP and SP. The mean  $\text{N}_2\text{O}$  emissions rates during the first, second and third year GS were  $9.54 \pm 9.78$ ,  $27.85 \pm 99.48$ , and  $2.03 \pm 3.55 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$  for AP and  $6.98 \pm 9.5$ ,  $12.32 \pm 23.52$ , and  $3.43 \pm 4.74 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$  for SP, respectively. Similarly, the mean  $\text{N}_2\text{O}$  emissions rates during the first, second and third year NGS were  $35.0 \pm 31.56$ ,  $0.48 \pm 0.67$ , and  $0.07 \pm 0.15 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$  for AP and  $10.11 \pm 14.33$ ,  $0.45 \pm 0.86$ , and  $0.03 \pm 0.06 \text{ mg N}_2\text{O m}^{-2} \text{d}^{-1}$  for SP, respectively.

The annual cumulative  $\text{N}_2\text{O}$  emission (GS+NGS) was significantly ( $p < 0.05$ ) higher in AP than SP, however only in the first ( $50.7$  vs  $17.0 \text{ kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$ ) and second ( $35.0$  vs  $13.8 \text{ kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$ ) year (Fig. 5 and Table 2). By the third year, the annual cumulative emissions decreased in both AP ( $2.3 \text{ kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$ ) and SP ( $3.7 \text{ kg N}_2\text{O-N ha}^{-1} \text{yr}^{-1}$ ), however the decrease was statistically significant



**Fig. 5.** Cumulative N<sub>2</sub>O emissions (kg N<sub>2</sub>O-N ha<sup>-1</sup>) for autumn ploughing (AP) and summer ploughing (SP) treatments during growing seasons (GS) and non-growing seasons (NGS) across the three-year period. Different lowercase letters indicate significant difference ( $p < 0.05$ ) in GS between the treatments inside each year and different uppercase letters indicate significant differences in NGS between the treatments inside each year. Significant differences ( $p < 0.05$ ) in annual emissions between the treatments are indicated with asterisk. Values represent means  $\pm$  standard error of mean ( $n = 9$ ).

between all years ( $p < 0.05$ , first vs. second vs. third) in AP, whereas it was significantly ( $p < 0.05$ ) lower only in the third year compared to first and second year in SP. The yield-scaled N<sub>2</sub>O emissions were significantly ( $p < 0.05$ ) higher in AP compared to SP in the first and second year whereas it was significantly ( $p < 0.05$ ) higher in SP than AP in the third year (Table 2). The total yield-scaled emissions from the three-year study were significantly ( $p < 0.05$ ) lower in SP (1.7 kg N Mg<sup>-1</sup>) compared to AP (3.7 kg N Mg<sup>-1</sup>).

The seasonal total N<sub>2</sub>O emissions (i.e., totals for the GS and NGS) varied considerably within the treatments and between the measurement years, especially first and second year (Fig. 5). In the first year of AP, NGS produced significantly ( $p < 0.001$ ) more (42.62 kg N<sub>2</sub>O-N ha<sup>-1</sup>, 84 % of the first year cumulative) N<sub>2</sub>O than GS. However, this pattern was reversed in the second year of AP where GS produced significantly ( $p < 0.001$ ) more (34.42 kg N<sub>2</sub>O-N ha<sup>-1</sup>, 98 % of the second year cumulative) N<sub>2</sub>O than NGS. The seasonal total N<sub>2</sub>O emissions from SP were significantly different only in the second year, where GS produced significantly ( $p < 0.001$ ) more (13.16 kg N<sub>2</sub>O-N ha<sup>-1</sup>, 95 % of the second year cumulative) N<sub>2</sub>O than NGS. When compared between the measurement years, the total emissions from second year GS were significantly ( $p < 0.001$ ) higher compared to total GS emissions from first and third year for both AP and SP. Similarly, the total emission from first year NGS were significantly ( $p < 0.001$ ) higher compared to total NGS emissions from second and third year for both treatments. All three years cumulative sum shows a treatment effect (Table 2), with AP (88.0  $\pm$  10.7 kg N<sub>2</sub>O-N ha<sup>-1</sup>) emitting nearly threefold more N<sub>2</sub>O than SP (34.5  $\pm$  1.3 kg N<sub>2</sub>O-N ha<sup>-1</sup>).

### 3.4. Soil profile N<sub>2</sub>O concentrations and nitrogen dynamics

The soil profile N<sub>2</sub>O concentrations in AP ranged from 0.05 to 701 ppm, 0.01–504 ppm and 0.01–34.8 ppm, and in SP from 0.01 to 680 ppm, 0.01–587 ppm and 0.01–70 ppm, in first, second and third year, respectively (Table S2). In AP, maximum N<sub>2</sub>O concentrations reached 657, 582, and 701 ppm at depths of 5, 20, and 40 cm, respectively. SP showed consistently lower concentrations, with corresponding

values being 587, 426, and 680 ppm, respectively (Table S2).

Soil NH<sub>4</sub><sup>+</sup> concentrations ranged from 0.16 to 0.98 mg N g<sup>-1</sup> across the three years in both treatments, with similar ranges observed within each year (Table S3). Both treatments showed lower NH<sub>4</sub><sup>+</sup> concentrations in the second year compared to the first and third years. Highest NH<sub>4</sub><sup>+</sup> concentrations were observed in May of the first year and in June of the third year in both treatments, though these differences were not significant.

Similarly, soil NO<sub>3</sub><sup>-</sup> concentrations showed no significant differences between the treatments, ranging from 0.02 to 0.25 mg N g<sup>-1</sup> across the three years in both treatments (Table S3). Highest NO<sub>3</sub><sup>-</sup> concentrations were observed in AP during June of both first and second years, while SP showed peaks only in June of the first year, though these differences were insignificant.

Other soil properties were stable between treatments, with mean pH of 5.3 and organic matter content of 64.1 %, showing no significant ( $p > 0.05$ ) treatment differences.

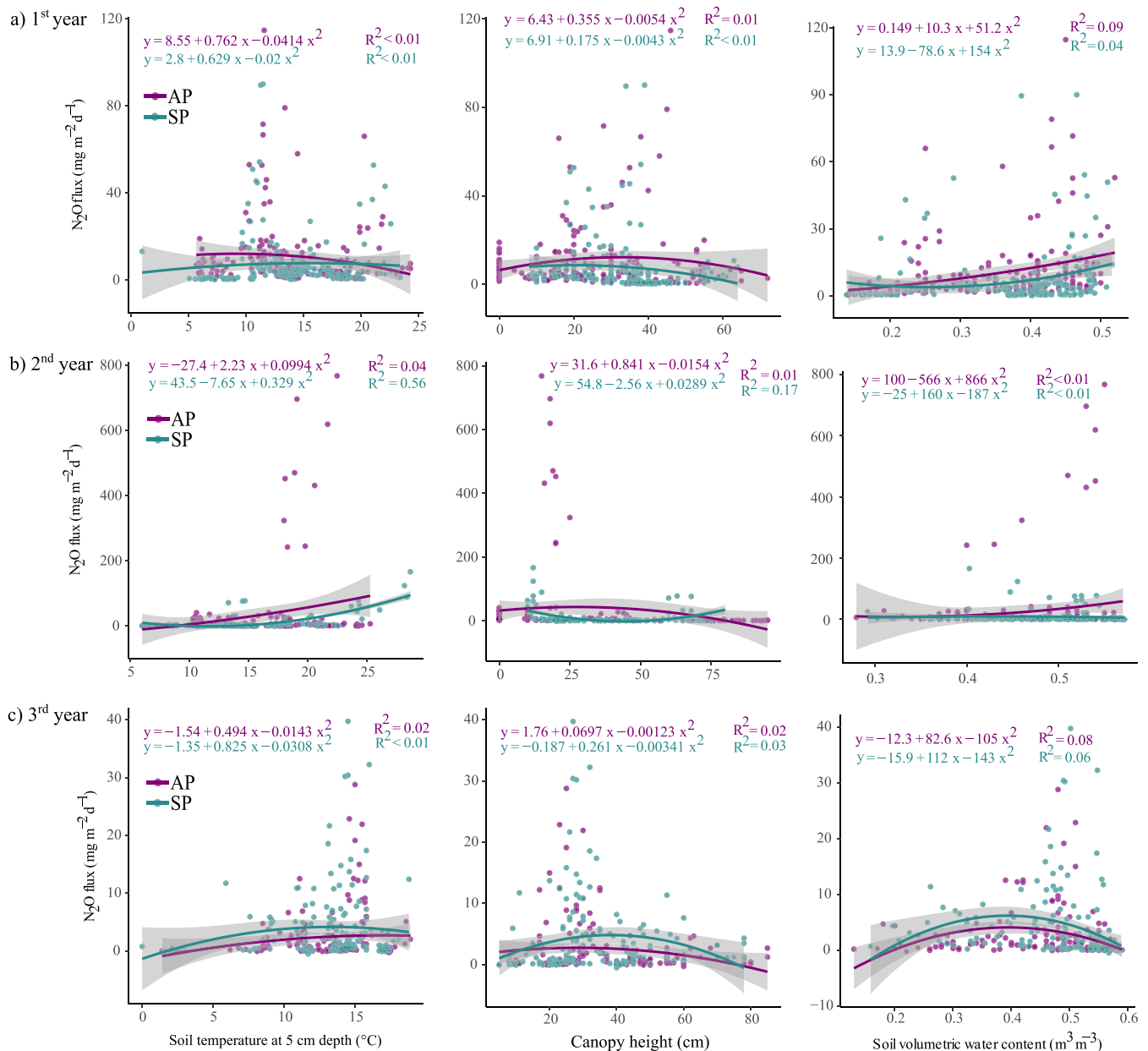
### 3.5. Relationship between N<sub>2</sub>O emissions and environmental conditions

Soil temperature showed a contrasting relationship with the N<sub>2</sub>O emissions between the GS and NGS, with rather consistent trend between the years of each season (Figs. 6, 7). In the GS of first and second year (Fig. 6), we found higher N<sub>2</sub>O emissions with higher soil temperatures in both treatments. Interestingly, the first-year soil temperature exhibited a dual high N<sub>2</sub>O emission peak, first between 10–14 °C and second between 20–25 °C in both the treatments. Contrastingly, the NGS showed high N<sub>2</sub>O emissions mostly at lower temperatures ( $\sim 0$  °C) in all years in both the treatments (Fig. 7).

Soil moisture also showed a contrasting relationship with N<sub>2</sub>O emissions between the GS and NGS with inconsistent trend between the treatments and over the years. Treatment AP showed higher N<sub>2</sub>O emissions with higher soil moisture in GS in all years, whereas treatment SP exhibited a similar relationship only in the first and second year GS. Interestingly, soil moisture in the first year also showed a dual high N<sub>2</sub>O emissions peak similar to soil temperature, first between 0.20–0.30 m<sup>3</sup>m<sup>-3</sup> and second between 0.40–0.50 m<sup>3</sup>m<sup>-3</sup> in both the treatments. Contrastingly, in the NGS treatment AP showed higher N<sub>2</sub>O emissions with lower moisture ( $< 0.4$ – $0.5$  m<sup>3</sup>m<sup>-3</sup>) during the first and second year whereas SP showed higher N<sub>2</sub>O emissions with higher soil moisture in the first year and lack any typical trend in the second year. In the third year NGS, both treatments showed an almost identical relationship with the soil moisture. The AP and SP showed both emissions and uptake of N<sub>2</sub>O closer to 0.6 m<sup>3</sup>m<sup>-3</sup> soil moisture.

The relationship of N<sub>2</sub>O with canopy height in the GS varied between the years. The first year showed an increasing N<sub>2</sub>O emissions with progressively increasing canopy height in both the treatments, however up to only a certain height ( $\sim 45$  cm). In contrast, second and third year showed high N<sub>2</sub>O emissions only during early phase of vegetation growth when plant heights were  $< 25$  cm in both the treatments.

The relationship between snow depth and N<sub>2</sub>O emissions varied between the treatments in all NGS. In the first year NGS, AP showed broad distribution of higher N<sub>2</sub>O emissions with snow depth ranging from 0 cm (with highest rates) to 40 cm whereas SP showed rather linear relationship with snow depth, however with comparatively smaller N<sub>2</sub>O emissions. In the second year both AP and SP showed higher N<sub>2</sub>O emissions with no snow cover (0 cm) with some exception of higher N<sub>2</sub>O emissions between 30–40 cm snow depth. In the third year, both treatments showed an almost identical relationship with snow depth. The AP and SP both showed higher N<sub>2</sub>O emissions with higher snow depth with few occasions of N<sub>2</sub>O uptake at smaller snow depth ( $\sim 0$  cm).



**Fig. 6.** The relationships between N<sub>2</sub>O emissions and soil and plant variables during growing season of all three years. Left, middle and right panel represents the relationship between N<sub>2</sub>O fluxes (mg m<sup>-2</sup> d<sup>-1</sup>) and soil temperature at 5 cm depth (°C), canopy height (cm) and soil volumetric water content (m<sup>3</sup> m<sup>-3</sup>), respectively, for the first (a), second (b) and third (c) year. The color in each plot represents treatments, violet for AP and blue for SP.

## 4. Discussion

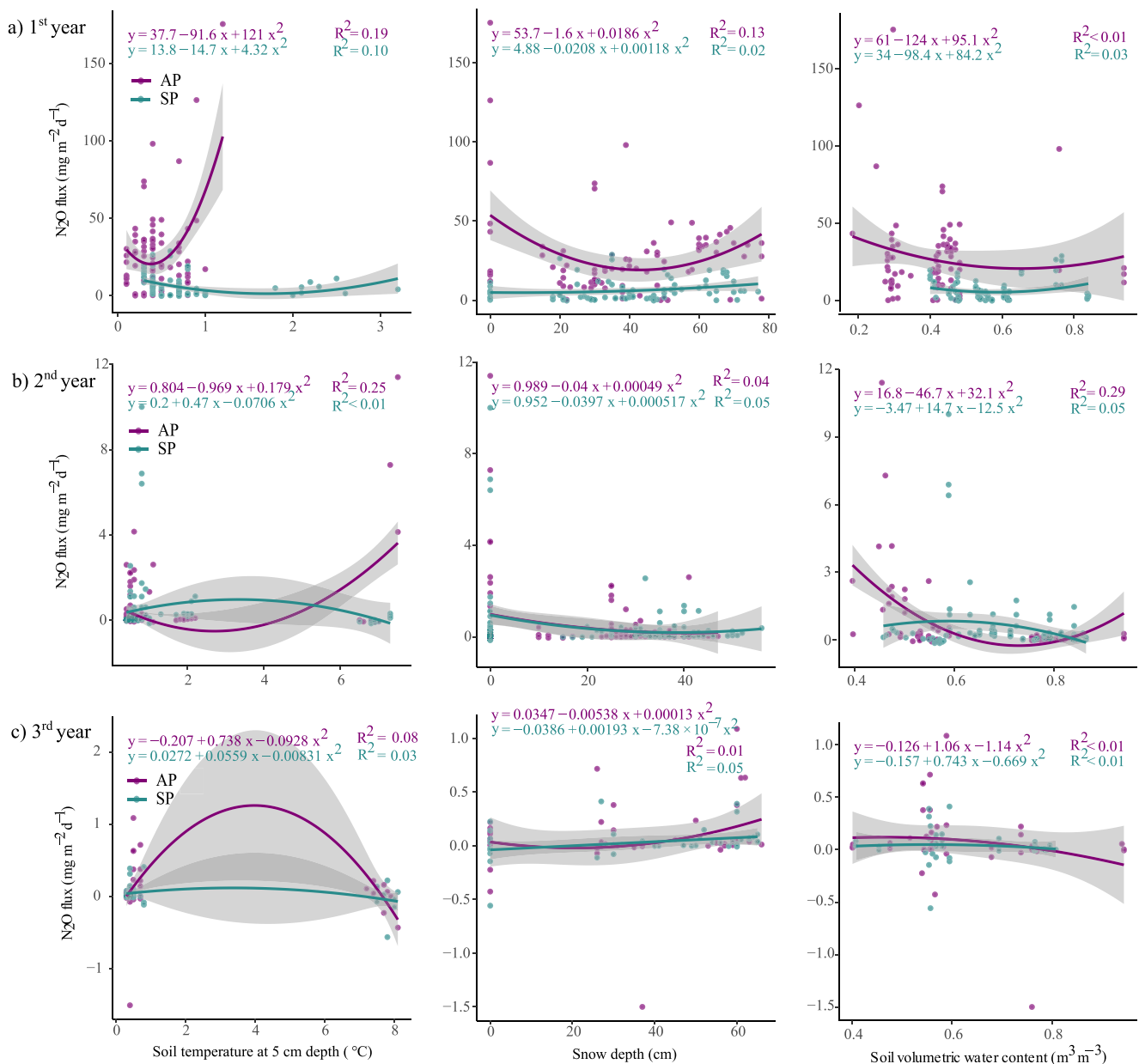
### 4.1. Grassland renewal in the autumn leads to higher N<sub>2</sub>O emissions than in the summer

The three-year field experiment suggests that the traditional autumn ploughing with spring reseeding results in higher N<sub>2</sub>O emissions than summer ploughing and reseeding from a grassland on drained organic soil. The measured N<sub>2</sub>O emissions from SP were within the reported range of emissions from the first year after grassland renewal (0 – 29.1 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, Buchen et al., 2017). However, the emissions from AP in our study (50.7 kg N ha<sup>-1</sup> yr<sup>-1</sup>) were one of the highest recorded from the boreal region for agricultural organic soils (e.g. Maljanen et al., 2010; Gerin et al., 2023; Honkanen et al., 2024), although even higher annual emissions (144.5 kg N ha<sup>-1</sup> yr<sup>-1</sup>) have been reported in temperate climate (Offermanns et al., 2023). The high

flux rates from AP after ploughing are in line with those measured after grassland renewal on organic soil (12.58 mg N m<sup>-2</sup> h<sup>-1</sup> Offermanns et al., 2023). The average N<sub>2</sub>O emissions from the third year after grassland renewal in our study were lower than the reported average for N<sub>2</sub>O emissions from peat soils with perennial grass swards in Nordic countries (9.5 ± 10.2 kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>, Maljanen et al., 2010). The highest increase in the cumulative emissions were seen in AP after ploughing and again after reseeding and fertilization, and in SP after ploughing and reseeding, which suggests that the management practices had a strong effect on the rates and annual estimates of N<sub>2</sub>O.

### 4.2. Stronger and longer legacy of autumn than summer ploughing on N<sub>2</sub>O emissions

We found that ploughing in the autumn led to a longer period of increased N<sub>2</sub>O emissions and consequently increased during the



**Fig. 7.** The relationships between N<sub>2</sub>O emissions and soil variables during non-growing season of all three years. Left, middle and right panel represents the relationship between N<sub>2</sub>O fluxes (mg m<sup>-2</sup> d<sup>-1</sup>) and soil temperature at 5 cm depth (°C), snow depth (cm) and soil volumetric water content (m<sup>3</sup> m<sup>-3</sup>), respectively, for the first (a), second (b) and third (c) year. The color in each plot represents treatments, violet for AP and blue for SP.

following NGS and second year annual N<sub>2</sub>O emissions (Figs. 4, 5). This effect could likely be associated with the plough induced positive effects on soil physiochemical properties supporting the activities of N<sub>2</sub>O producers during the following seasons. Ploughing has been found to increase N<sub>2</sub>O emissions from grasslands (Buchen et al., 2017; Offermanns et al., 2023; Velthof et al., 2010) and it is mostly explained by plough induced positive effects on soil porosity (aeration), temperature and mineralization of peat and plant residues (Ammann et al., 2020; Nykänen et al., 1995), all supporting towards increasing mineral N availability and N<sub>2</sub>O producer's activities. Glyphosate application prior to ploughing is a common agricultural practice which has been reported to increase greenhouse gas emissions during the weeks after application (e.g. Gerin et al., 2023). In this study, we observed a similar peak in emissions in both treatments after herbicide (glyphosate) application solely on AP plots, which suggest that the peak was rather due to increased precipitation at similar time (Fig. 2b) than from the glyphosate.

The unploughed SP during the first NGS exhibited a similar trend as ploughed AP (Fig. 4) suggesting that underlying N<sub>2</sub>O production mechanisms of both treatments were similar during the period, and this is also supported by comparable soil profile N<sub>2</sub>O concentrations (Table S2). Nevertheless, SP showed smaller N<sub>2</sub>O fluxes than AP during the first NGS, suggesting a partial reduction of N<sub>2</sub>O to N<sub>2</sub> in SP treatment. SP had low NO<sub>3</sub> concentrations (Table S3) and supplied labile carbon from vegetation (Fig. 3), which provided easily available energy for denitrifiers and could therefore have promoted the reduction of N<sub>2</sub>O (Baggs and Philippot, 2010; Bore et al., 2024). Additionally, SP had higher soil moisture and lower depth of frost penetration (Fig. 2), which may have further enhanced complete denitrification (reducing N<sub>2</sub>O to N<sub>2</sub>) and/or stimulated the reduction of already formed N<sub>2</sub>O, thereby decreasing net surface N<sub>2</sub>O emissions.

The autumn ploughing legacy continued beyond NGS and affected N<sub>2</sub>O emissions of the following GS. Higher N<sub>2</sub>O emissions from AP compared to SP in the beginning of the second GS (Fig. 4b) suggest that

plough induced changes to soil were still favorable for N<sub>2</sub>O production. The N<sub>2</sub>O emissions were enhanced even further after reseeded and fertilization leading to highest rates of N<sub>2</sub>O emissions from our study (Fig. 4b), indicating a strong positive effect of seeding and fertilization on N<sub>2</sub>O emissions. Supporting this, we found increased soil profile N<sub>2</sub>O concentrations in AP compared to SP throughout the second GS with rather high concentrations even after 27 days of fertilization (Table S2). Increased amount of labile C from germinating seeds and together with fertilization has been shown to promote N<sub>2</sub>O emissions from organic soils (Bhattarai et al., 2019; Bore et al., 2024). The treatment SP also showed an increased N<sub>2</sub>O emissions after ploughing, reseeded and fertilization, however, the size of the N<sub>2</sub>O peak was remarkably lower spread over a shorter period of peak emissions. Moreover, SP had remarkably lower soil profile N<sub>2</sub>O concentrations compared to AP (Table S2) despite receiving higher amount of fertilizer and higher moisture content than AP (Table 1). In the second GS, the higher soil moisture (Fig. 2c) and N via fertilization (Table 1) should have promoted higher N<sub>2</sub>O emissions in SP, however, AP exhibited remarkably higher N<sub>2</sub>O emissions than SP suggesting that the legacy of ploughing in the autumn left the soil in optimal conditions for N<sub>2</sub>O production until the GS of the second year. In addition, plant type may have influenced the flux rates during the second GS, as annual crops have been reported to produce higher N<sub>2</sub>O emissions than perennial species in some studies (Maljanen et al., 2004), although in this study the effect of plant type could not be assessed separately.

Unlike AP, SP showed immediate (not sustained) peak of N<sub>2</sub>O emissions after ploughing, reseeded and fertilization (Fig. 4). Such increase in N<sub>2</sub>O emissions in SP could be explained by the availability of mineral N via fertilization and its efficient utilization by N<sub>2</sub>O producers on vegetation free soil. Fertilization following ploughing and in the absence of vegetation, a situation like SP in our study, is known to induce N<sub>2</sub>O emission peaks (Wang et al., 2024). The NGS (second year) and the GS (third year) following the SP establishment did not show any considerable higher N<sub>2</sub>O emissions and did not affect seasonal and annual (third year) N<sub>2</sub>O estimates. However, SP showed higher N<sub>2</sub>O emissions at the beginning of the third GS (Fig. 4c), likely due to increased C and N availability from decomposed cover crop residues, which stimulated the activities of N<sub>2</sub>O producers (MacDonald et al., 2011; Regina et al., 2004).

#### 4.3. Varying effect of soil variables and plant height on N<sub>2</sub>O emissions

We found that during GS, higher soil moisture and temperature were associated with elevated N<sub>2</sub>O emissions in both treatments (Fig. 6), suggesting that they are key soil variables controlling N<sub>2</sub>O dynamics. Typically, higher temperatures and moisture conditions are known to increase microbial activities and anoxia, respectively that can lead to increased denitrification and N<sub>2</sub>O production (Elder and Lal, 2008; Maljanen et al., 2003). On the contrary, we found a contrasting relationship of N<sub>2</sub>O fluxes with soil moisture and temperature in the NGS. Higher N<sub>2</sub>O emissions closer to 0 °C in all years in both treatments and in both moisture regimes independent of treatments, (e.g. high N<sub>2</sub>O fluxes at lower moisture in AP in the first and second year and high N<sub>2</sub>O fluxes at higher moisture in SP only in the first year NGS, Fig. 7) suggest a considerable effect of ploughing on N<sub>2</sub>O emission dynamics. Our result of higher N<sub>2</sub>O emissions at temperatures near 0 °C during NGS agrees with many previous findings (Honkanen et al., 2024; Maljanen et al., 2003; Regina et al., 2004;) and could be a result of fluctuating freeze-thaw conditions in the soil surface that may have resulted release of nutrients from plant and microbial (dead) residues, providing substrates for N<sub>2</sub>O source processes (Regina et al., 2004; Koponen and Martikainen, 2004). After ploughing, AP exhibited lower soil moisture leading to more aerobic conditions which could have stimulated aerobic N<sub>2</sub>O source process (i.e., nitrification) inducing higher N<sub>2</sub>O emissions. Additionally, a lack of nutrient competitor, i.e., vegetation after ploughing could have further supported the activities of N<sub>2</sub>O producing

microbes by increasing mineral N availability leading to higher N<sub>2</sub>O emissions. On the contrary, un-ploughed and vegetated SP had undisturbed conditions which could have retained higher soil moisture generating more anoxic conditions in the first NGS. Additionally, presence of vegetation in SP could have depleted mineral N and supplied substantial labile C – a typical condition for N<sub>2</sub>O reduction to N<sub>2</sub> (Baggs et al., 2010) leading to lower N<sub>2</sub>O emissions. Lower magnitude of N<sub>2</sub>O emissions with more instances of N<sub>2</sub>O uptake rates in both treatments in the third year (Fig. 7) could have resulted to lower surface N<sub>2</sub>O emissions (Fig. 4) and annual estimates (Fig. 5).

A wider distribution of high N<sub>2</sub>O emissions with wider range of snow depth in the first NGS in the ploughed AP than un-ploughed and vegetated SP suggests that snow depth had smaller contribution than ploughing itself (Honkanen et al., 2024; Elder et al., 2008) to N<sub>2</sub>O production. We found that during the second year, smaller canopy height, representing the early phase of vegetation growth, increased N<sub>2</sub>O emissions considerably. This could likely be associated with the availability of fresh labile carbon from germinating seeds to early developing root systems serving as energy source of N<sub>2</sub>O producers (Bore et al., 2024; Bhattarai et al., 2019). Additionally, smaller N<sub>2</sub>O emission with higher canopy heights in both treatments in all years suggest that vegetation is an important driver for lowering N<sub>2</sub>O emissions from a drained peatland likely by outcompeting N<sub>2</sub>O producers for mineral N (e.g., NO<sub>3</sub>).

The inter- and intra-annual variation in relationships between N<sub>2</sub>O emissions and soil and environmental variables suggests that the management practices overrode the effect of individual factors on N<sub>2</sub>O emissions (Maljanen et al., 2010). In addition, the exceptionally high emission peaks for boreal organic soil measured in our study during the second GS were probably result of several optimal conditions for N<sub>2</sub>O production, including enhanced microbial activity together with soil conditions that did not limit the diffusion and release of N<sub>2</sub>O to the atmosphere, as Offermanns et al., (2023) have demonstrated also. However, what drives high N<sub>2</sub>O emissions in both high and low moisture regimes in NGS, and both N<sub>2</sub>O emissions and uptake at equal strength at a constant moisture level when grassland is fully established, will require further investigation to better understand the effect of grass renewal on N<sub>2</sub>O emissions.

#### 4.4. Challenges and opportunities of grass renewal timing on a drained peatland

It has been reported that N<sub>2</sub>O emissions from grass renewal years can be remarkably higher compared to grass years (Ammann et al., 2020; Elder and Lal, 2008; Honkanen et al., 2024; MacDonald et al., 2011; Offermanns et al., 2023; Regina et al., 2004; Velthof et al., 2010). Nevertheless, grass renewal is mandatory every few years to maintain reasonable yields and its quality. Therefore, an urgent need for management practices that can lower the N<sub>2</sub>O emissions from grassland renewal is evident. Our study shows that yield-scaled N<sub>2</sub>O emissions were remarkably lower from summer ploughing (SP) during the renewal years, although autumn ploughing (AP) produced higher yields compared to SP. The traditional ploughing i.e., AP, produced higher yields in the second year, however, we could not estimate the yields from SP due to excessive soil moisture conditions that led to suboptimal working environment for the farm machinery. Thus, a correct yield-scale estimation of SP did not happen. This could be one of the major challenges to implementing SP as a management practice despite its positive results. Due to intrinsic moisture holding nature of peat, longer periods of high-water table can exist due to even small changes in hydrogeology (e.g., via precipitation) obstructing field operations in the spring. Additionally, high workload due to short growing seasons compels farmers to complete as many tasks as possible in the autumn posing a high risk of leaving active photosynthetic period unutilized in the GS. In Finnish boreal conditions, the ploughing and reseeded are often possible only after the end of May or beginning of June, whereas suitable

conditions for photosynthesis usually start a few days after snow melt (beginning of May) on drained peat soils. Although the trade-off between agricultural productivity and N<sub>2</sub>O mitigation has primarily been a concern in crop cultivation (Kim et al., 2023), it is equally important to consider this trade-off in forage production, particularly in northern latitudes where ruminant-based systems are the main source of dietary protein. Therefore, proper planning of the field activities and its timely and successful execution and local weather could stand as challenges and seem reasonably important factors to consider when implementing summer renewal.

#### 4.5. Limitations and future research directions

Our study showing significant variability in annual estimates owing to differences in the timing of ploughing for grassland renewal in the first and second year and no difference at all in the third-year highlights that there is a need for multiyear studies covering multiple grass rotations on drained agricultural peat soils covering wider geographical distribution. Different field properties combined with varying climatic conditions and peatland's hydrogeological status can influence the implementation of certain management practices affecting N<sub>2</sub>O emissions and annual estimates. We acknowledge that with weekly or even biweekly chamber measurements, there is a high risk of missing short-term emission peaks and thus underestimating the emissions (Barton et al., 2015). In addition, the use of gap-filling methods to compensate for these missing data points may also result in overestimation, depending on the assumptions and temporal variability of emissions. Therefore, more frequent, or even continuous measurements e.g., with automated chamber system or eddy covariance method would be recommended to capture highly heterogeneous N<sub>2</sub>O production dynamics for more accurate emission estimation. In addition, we did not determine N (e.g., NO<sub>3</sub>) losses through leaching, which has been identified as potential risk related to renewing grasslands (Buchen et al., 2017; Velthof et al., 2010). Leaching of NO<sub>3</sub> could affect the N<sub>2</sub>O emission dynamics beyond the actual field e.g., ditches surrounding peatlands (Hyvönen et al., 2013). Therefore, future studies should consider this aspect of grassland renewal to estimate N<sub>2</sub>O emissions accurately. To fully quantify the greenhouse gas mitigation potential of summer renewal practice, the emissions of carbon dioxide and methane need to be addressed as well, as carbon dioxide can account for the majority of the total greenhouse gas balance (Gerin et al., 2023; Offermanns et al., 2023) and methane emissions from inundated drained organic soils could be high (Maljanen et al., 2010).

## 5. Conclusions

This study suggests that switching the timing of ploughing from autumn to summer could be a feasible practice to mitigate N<sub>2</sub>O emissions from grass cultivation on boreal organic soils. Ploughing in the autumn did not induce immediate N<sub>2</sub>O emission peak like summer ploughing. However, autumn ploughing and subsequent reseeding and fertilization in the spring resulted in a longer period of increased emissions and consequently higher annual emissions. In contrast, summer ploughing with rapid establishment of new vegetation after ploughing remarkably reduced the N<sub>2</sub>O emissions from grassland renewal. Soil temperature and moisture were two important variables that affected N<sub>2</sub>O flux dynamics year around, whereas canopy height affected the emissions during GS. Yield-scaled N<sub>2</sub>O emissions were significantly lower in SP compared to AP, suggesting SP could be an effective mitigation strategy for N<sub>2</sub>O emissions. Given the high global warming potential of N<sub>2</sub>O and the relatively large contribution of drained organic soils to agricultural greenhouse gas emissions, our findings offer relevant insights into climate change mitigation. Future research with more frequent measurements over multiyear in varying climatic conditions and peatlands across wider geography are needed to further assess and confirm the role of summer ploughing as an effective management

practices for N<sub>2</sub>O emissions reduction from grasslands on drained peatland.

## CRedit authorship contribution statement

**Semberg Sanni Liisa Emilia:** Writing – review & editing, Writing – original draft, Visualization, Investigation. **Virkajarvi Perttu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Manninen Petra:** Writing – review & editing, Investigation. **Hem Raj Bhattarai:** Writing – review & editing. **Shurpali Narasinha:** Writing – review & editing, Supervision, Conceptualization. **Li Yuan:** Writing – review & editing.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Perttu Virkajarvi reports financial support was provided by Finnish Ministry of Agriculture and Forestry. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.agee.2025.110155.

## Data availability

Data will be made available on request.

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