

Drought increases soil nitrous oxide emissions and nitrogen availability under plant cover in arable soils

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Abstract

Background and aims

Climate change induces risks for summer droughts, which increases the need to better understand how different plant communities respond to drought and how they affect soil processes leading to nitrous oxide (N₂O) emissions. We investigated how drought and plant cover regulate nitrogen (N) cycling, substrate availability, and N₂O emissions under controlled conditions.

Methods

A greenhouse pot experiment was conducted with oat (*Avena sativa*) grown alone or undersown with Italian ryegrass (*Lolium multiflorum*) or alfalfa (*Medicago sativa*), and with bare soil, under moist (WFPS 70%) and dry (WFPS 40%) conditions. N₂O emissions, plant N uptake and soil N pools were measured after a 39-day growing period. A complementary incubation study assessed gross nitrification and N immobilization rates.

Results

Bare soil showed higher N₂O emissions and mineral N accumulation than vegetated treatments, whereas plant cover enhanced N uptake and decreased residual soil N. Drought decreased N₂O emissions from bare soil by 79%, but increased emissions to 2.3-3.9-fold in vegetated soils. Higher N₂O emissions under drought coincided with increased soil NO₃⁻ contents, while plant N uptake and gross nitrification rates remained unchanged.

Conclusion

Plant cover enhanced N retention and reduced N₂O emissions compared with bare soil, demonstrating its importance for N₂O mitigation. Contrasting drought responses between bare and vegetated soils suggest that plant-mediated changes in N availability regulate N₂O production under water-limited conditions. Managing soils to maintain good plant growth may therefore help mitigate N₂O emissions during drought and upon rewetting.

INTRODUCTION

Nitrous oxide (N₂O) is a potent greenhouse gas (GHG), having a global warming potential 273 times higher than carbon dioxide (CO₂) during 100 years (Forster et al. 2021). Agriculture is considered one of the biggest sources of anthropogenic N₂O emissions due to nitrogen (N) fertilizer use and manure

management (Abdalla et al. 2019; Butterbach-Bahl et al. 2013; Reay et al. 2012; Syakila and Kroeze 2011). Even though agricultural N₂O emissions have decreased in Finland, agriculture contributes about 60% of the total N₂O emissions (Antman et al. 2015). In soils, N₂O is produced by multiple microbiological processes, mainly denitrification and nitrification, that are controlled by soil water, oxygen and substrate availability, and plant rhizospheric processes (Butterbach-Bahl et al. 2013; Dong et al. 2025). In a previous greenhouse experiment, we found that species interactions and plant growth stages strongly impact N₂O emissions, with cover crops intercropped with barley mitigating soil N₂O emissions during early growth of the plants (Bore et al. 2024). Plants can influence soil N₂O dynamics through several interacting mechanisms. Plants compete with rhizosphere microbes for available N in soil, reducing substrate availability for microbial N₂O formation (Kuzyakov and Xu 2013; Moreau et al. 2015). In addition, plant water uptake can decrease soil water content and increase air-filled pore space, suppressing creation for anaerobic microsites where denitrification occurs (Rohe et al. 2021; Rummel et al. 2021). The resulting increase in air-filled porosity may also enhance N₂O diffusivity, facilitating its escape from soil and potentially increasing emissions (Lussich et al. 2024). Conversely, plants stimulate microbial activity through root growth and root exudates, supplying labile carbon (C) to the rhizosphere (Guyonnet et al. 2017; Henry et al. 2008; Wang et al. 2021). As a result, an enhanced microbial respiration can deplete oxygen in localized hotspots, promoting the formation of anaerobic microsites and denitrification, even under relatively dry conditions (Lussich et al. 2024).

In Finland and across northern Europe, global climate change predictions indicate an increasing risk of spring and summer droughts, as higher temperatures enhance evapotranspiration, and lead to earlier snowmelt and earlier soil drying in spring (Ruosteenoja et al. 2018; Ruosteenoja and Jylhä 2021). In dry conditions, N may accumulate in soils as plants are unable to access and take up N, when drought stress restricts transpiration, and hence photosynthesis and growth (Farooq et al. 2009; Zeid and Shedeed 2006). Besides reduced N uptake by plants, NH₄⁺ and NO₃⁻ accumulate in soil during drought due to strongly decreased hydraulic conductivity and constrained movement of nutrients in dry conditions, limiting the access of microbes to NH₄⁺ and NO₃⁻ (Parker and Schimel 2011; Schaeffer et al. 2017). Such accumulation of mineral N in soil during drought increases the risk of large N₂O emission pulses upon rewetting of soil (Harris et al. 2021).

Although several studies have investigated the effects of drought on soil N cycling and plant growth (Deng et al. 2021; Farooq et al. 2009; Gloser et al. 2020; Hartmann et al. 2013; Homyak et al. 2017) the interactive effects on plants and N₂O emissions have received less attention. Also, the impact of undersown cover crops and plant cover on N₂O emissions under drought remain underexplored.

We conducted a pot experiment in controlled conditions to evaluate the direct and interactive effects of plant cover and drought on soil N₂O emissions. Two cover crop species – Italian ryegrass (*Lolium multiflorum*) and alfalfa (*Medicago sativa*) – sown under oat (*Avena sativa*), oat sown alone and bare soil controls were set up. We quantified their impact on N cycling processes, N substrate availability and N₂O emissions in controlled moist and dry soils. We hypothesized that: 1) Overall, plant N uptake reduces soil

mineral N availability, resulting in lower N₂O emissions in vegetated treatments compared to bare soil; 2) Drought limits plant growth and N uptake, increasing soil mineral N availability and potentially enhancing N₂O production; and 3) drought-induced suppression of microbial activity (as indicated by reduced soil respiration, gross nitrification, and immobilization rates) is the dominant control on N₂O emissions, overriding increased mineral N availability, resulting in lower N₂O emissions across all treatments.

MATERIALS AND METHODS

- Soil material and preparation

Clay soil was collected in spring 2021 and 2023 at the TWINWIN biodiversity experiment (Cappelli et al. 2024) at Viikki Research Farm, University of Helsinki, Finland (60° 13' 29" N 25° 01' 13" E) from a depth of 0–30 cm in a field where the soil is tentatively classified as Luvic Stagnosol (IUSS Working Group WRB 2022). The soil contained 44% clay, 36% silt and 20% sand, and total C and N of 3.6% and 0.30%, respectively. The initial NH₄⁺-N and NO₃⁻-N contents were 5.04 and 5.11 mg kg⁻¹ dry soil, respectively. The soil was air-dried, milled, and sieved through a 2 mm mesh, and visible plant material and stones were removed by hand prior to use in the pot experiment.

- Experimental design and setup

The pot experiment was conducted in a greenhouse at the Viikki Campus, University of Helsinki, Finland. The soil was mixed with deionized water to obtain water-filled pore space (WFPS) of either 70% (moist) or 40% (dry). Moistened soil was packed into polyvinyl chloride pots (diameter 7 cm, height 30 cm) to a height of 27 cm and to a dry bulk density of 1.10 g cm⁻³ to reflect the average bulk density at the field site. To make sure the bulk density would be the same throughout the whole pot, the soil was compressed into the pot in six 4.5 cm layers using a Universal Materials Testing Machine (LR10K Plus, LLOYD Instruments/Ametek, US).

Pots were divided into two plant cover treatments: vegetated and bare soil treatments. Each vegetated treatment was replicated 6 times at two different moisture conditions: moist (70% WFPS) and dry (40% WFPS), with the dry condition used to simulate drought. Bare soils were replicated 3 times at the given moisture conditions, resulting in a total of 42 pots (Fig. 1). The vegetated pots were fertilized with nutrient amounts corresponding to N 100 kg ha⁻¹, P 12 kg ha⁻¹, K 50 kg ha⁻¹, Mg 40 kg ha⁻¹, Mn 10 kg ha⁻¹ and B 0.2 kg ha⁻¹. The nutrients were mixed into the top 4.5 cm soil layer of the vegetated pots as an aqueous solution, and correspondingly less deionized water was added into this layer so as not to exceed the aimed final WFPS of 70%. Bare soil pots were prepared similarly by adding only deionized water (no fertilizer) to obtain the WFPS of either 70% or 40%.

Vegetated pots were sown with either oats alone, or oat with Italian ryegrass (*Lolium multiflorum*) or oat with alfalfa (*Medicago sativa*). Oat seeds were disinfected with Baytan I seed dressing fungicide (triadimenol; Bayer Crop Science) before pre-germination. All seeds were pre-germinated at room

temperature (20°C) for three days before sowing. Due to slower growth, alfalfa was sown one week before oat and Italian ryegrass. Four pre-germinated seeds were sown into each pot. After sowing, the pots were placed in a completely randomized order on two tables. Soil moisture conditions were maintained by weighing the pots every 2–3 days and adding water if needed (Fig. S1). One week after the sowing of oats and Italian ryegrass, plants were thinned to two plants per pot (i.e. each pot contained either two oats, or one oat with Italian ryegrass or alfalfa). Top and bottom ends of the pots were then sealed with plastic caps. The top caps had two holes that allowed plants to grow through the cap, and two holes with 3-way valves for gas collection as described in Bore et al. (2024). To ensure equally good start of plant growth in both moisture treatments, half of the pots were randomly selected for drought treatment after the 3rd leaves of oats had emerged on day 14. The pots in the drought treatment were allowed to dry out to 40% WFPS and then maintained at 40% WFPS for the rest of the 35-day experiment (from sowing of oats). During the experiment, the temperature at the greenhouse was 20°C during daytime (6 am to 8 pm) and 15°C during nighttime (8 pm to 6 am). Mean daytime photosynthetically active radiation (PAR) ranged from 8 to 417 $\mu\text{mol m}^{-2} \text{s}^{-1}$ among experiment days, with an overall mean of 92 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Due to low natural sunlight during the experiment, high pressure sodium lights (400 W) were used.

- Soil N₂O and CO₂ flux measurements

All pots were watered for the last time 35 days after the start of the experiment, followed by soil N₂O and CO₂ flux measurements during the days 36–39. The edges of the caps were sealed with aluminum foil tape, and the openings for plants were sealed using silicone to make the pots airtight. At this time, the oats were still in a vegetative growth stage. On the next day, the headspace of all pots was scrubbed for three minutes by bubbling the headspace air through flasks of 2 M NaOH solution and water by a peristaltic pump to remove CO₂ before circulating it back to the headspace. N₂O and CO₂ gas samples were taken through 3-way valves at 6, 12, 24, 48 and 72 hours after the first scrubbing. To prevent anaerobic conditions, the 3-way valves were left open for 1 hour after each sampling at 6 and 12 hours, and thereafter for 12 hours after sampling at 24 and 48 hours. The headspaces were scrubbed each time before closing the 3-way valves for gas accumulation. At each gas sampling, 10 ml samples of headspace air were taken into 3 ml He-flushed and pre-evacuated vials (Exetainer, Labco Ltd., Lampeter, UK) for N₂O and CO₂ concentration analysis. The samples were analyzed for N₂O and CO₂ with a gas chromatograph (Agilent Technologies 7890B GC custom, Santa Clara, CA, United States) equipped with ECD, FID and TCD detectors (Yli-Halla et al. 2017).

- Plant and soil sampling

On day 39, after the last gas samples were taken, the pots were sampled destructively (Fig. 2). Aboveground plant biomass was harvested at the soil surface and pots were cut open. The soil inside the pot was divided into top and bottom layers, bigger roots were removed by hand, and soil was sieved to get out smaller roots. After removing the roots, the soil was homogenized, and soil samples were taken for mineral N analysis as well as for extractable N (EN) and microbial biomass nitrogen (MBN)

analysis by chloroform-fumigation extraction (CFE). Mineral N soil samples were stored at -20°C prior to mineral N extraction and analysis, whereas fresh soils were processed for CFE immediately after sampling. Rest of the soil was stored at -20°C.

- Plant and soil analyses

Individual plants and washed root samples were dried at 60°C for 48 h before weighing. Dried plant and root samples were ball-milled and subsamples of about 2 mg were weighed into tin capsules and analyzed with Delta plus XP isotope ratio mass spectrometry (IRMS) with a conflo III interface (Thermo Scientific) for total C and N. Dry-matter and moisture contents of the fresh soil was determined by drying about 6 g of soil in 105°C for 24 h. Mineral N from frozen soil samples (20 g) was extracted by 50 ml of 2 M KCl. The samples were shaken for 2 h by a horizontal reciprocal shaker (180 rpm/min) and centrifuged at $2825 \times g$ for 10 min, after which the extracts were filtered with ashless Whatman Grade 589/3 blue ribbon filter papers (Whatman, Maidstone, UK) and stored frozen at -20°C until the analysis. Mineral N (NH_4^+ -N and NO_3^- -N) concentrations of extracts were analyzed with a Gallery Plus Discrete Analyzer (Thermo Scientific™, USA). Soil MBN and EN contents were determined with the CFE method (Vance et al. 1987) using a modified extraction concentration of 0.05 M K_2SO_4 (Černý et al. 2003; Makarov et al. 2013). For soils subjected to the drought treatment, two replicate subsamples of 7 g of soil were weighed, and 1 ml of Milli-Q water (resistivity < 18.2 MΩ cm) was added. For moist treatments, two 8-g subsamples were weighed. One subsample was immediately extracted with 40 ml of 0.05 M K_2SO_4 , whereas the other was fumigated with chloroform for 24 hours in a sealed vacuum desiccator prior to extraction. Both extractions were shaken on a horizontal reciprocal shaker at 200 rpm for 30 minutes, followed by filtering through Whatman ashless No. 42 filter paper (Whatman, Maidstone, UK) and subsequently through 0.45 µm syringe filter (VWR; 25mm, PES, non-sterile, USA). The filtered extracts were analyzed for dissolved total N using a TOC-L CSH analyzer (Shimadzu Corporation, Kyoto, Japan). The unfumigated sample represented EN, while MBN was calculated as the difference in dissolved total N between the fumigated and unfumigated samples, applying a correction factor for extraction efficiency ($k_{\text{EN}} = 0.54$) (Brookes et al. 1985; Joergensen and Mueller 1996).

- Plant and soil N calculations per pot

Nitrogen accumulation in shoot biomass was calculated for each individual plant by multiplying the shoot dry biomass by its N content. The total aboveground N accumulation per pot was obtained by summing the N mass of the two individual plants. Similarly, root N accumulation was calculated by multiplying the total root dry biomass by root N content. As roots were not separated by an individual plant, root N accumulation was determined for each individual pot only.

Soil mineral N, EN and MBN were analyzed separately for both top and bottom layers. Pot level mineral N content of individual pots was then calculated as mass-weighted averages by dividing the sum of N amounts (mg) in both layers by the total dry soil mass of the two layers. Final values are expressed as N mg kg⁻¹ dry soil.

- Flux calculation

Gas fluxes (F) of N_2O ($N \mu g kg^{-1} h^{-1}$) and CO_2 ($C mg kg^{-1} h^{-1}$) were calculated from the gas concentration change in the headspace during a 5-hour closure (Eq. 1). N_2O fluxes were calculated from the difference between the N_2O concentration in ambient air (measured outside the pots as scrubbing was not expected to affect N_2O concentrations) and that in the headspace at the end of chamber closure. For CO_2 fluxes scrubbing was assumed to take out all CO_2 in the headspace. Thus, CO_2 fluxes were calculated from the difference between the CO_2 concentration 0 and that in the headspace at the end of chamber closure.

$$F = \frac{dC}{dt} \times \frac{V}{m_{soil}} \times \frac{M}{V_m} \times \frac{T_0}{T} \# (1)$$

where $\frac{dC}{dt}$ is the slope of the linear regression of concentration against time ($m^3 m^{-3} h^{-1}$) during each closure, V is the headspace volume (m^3), m_{soil} is the dry soil mass (kg), M is the molar mass of N_2 ($28.01 g mol^{-1}$) or C ($12.01 g mol^{-1}$), V_m is the molar volume of an ideal gas at standard conditions ($0.0224 m^3 mol^{-1}$), and T and T_0 are the air temperature during sampling (K) and the standard temperature ($273.15 K$), respectively.

To estimate the cumulative N_2O and CO_2 fluxes over the 72-h gas sampling period, flux measurements were linearly interpolated between observation times to calculate the hourly fluxes. Because the first measurement was taken at 6 h, the fluxes for hours 1–5 were obtained by linear extrapolation based on the slope between the first two measurements (6 and 12 h). The resulting hourly flux series (1–72 h) was then used to calculate cumulative fluxes as the time-integrated sum of hourly fluxes.

- Incubation experiment

To identify the effects of soil moisture and rewetting on N_2O emissions, gross nitrification and immobilization rates, in the bare soil and oat treatments, we conducted a follow-up incubation experiment. Gross nitrification and immobilization rates were determined using the ^{15}N isotope pool dilution method following Davidson et al. (1991). Frozen soils from all replicate pots from the pot experiment were combined to produce one homogenized sample per soil and drought combination. From each homogenized sample, fresh soil was weighed into small plastic bags to represent 10 g dry soil, based on a separate gravimetric water content determination. Soil N was enriched with either 4 atom% excess labelled $NH_4^{15}NO_3$ or $^{15}NH_4NO_3$ ($0.02 M$) with a volume of 0.2 ml ($10 \mu g N g^{-1}$ soil d.w.). The soil moisture content was then set back to the original WFPS (either 70% or 40%) by adding milliQ-water. Rewetting treatment was established by increasing WFPS from 40% to 70%. After adding the solution, the soil was mixed cautiously, and the bags were closed in 1 L glass jars. The jars were flushed with ambient air before being closed and pressurized with 90 ml of additional ambient air. Each treatment was replicated 5 times for the 48-h incubation and an additional 5 times for initial mineral N analyses. The jars were incubated for 48 h at a constant room temperature of $20^\circ C$.

Headspace gas samples (10 ml) were collected into 3 ml He-flushed and pre-evacuated vials (Exetainer, Labco Ltd., Lampeter, UK) at 0, 12, 24, 48 h for N₂O concentration analysis. N₂O concentrations were determined using a gas chromatograph described above. Headspace N₂O concentrations (ppm) were converted to N₂O–N concentrations (µg l⁻¹) using the molar mass of N in N₂O (28.0134 g mol⁻¹) and the molar volume of an ideal gas at incubation temperature (24.05 l mol⁻¹). The total mass of N₂O–N in each bottle was calculated by multiplying the concentration by the headspace volume. Headspace volume was adjusted after each sampling to account for removal of a 10 ml gas sample. N₂O production rates were calculated from the increase in N₂O–N mass between successive sampling times and expressed per unit dry soil mass and time (µg N₂O–N kg⁻¹ dry soil h⁻¹). Cumulative N₂O–N emissions over the 48-h incubation period were calculated by integrating interval-specific fluxes over time. Fluxes for the 0–12, 12–24, and 24–48 h intervals were multiplied by the duration of the corresponding interval (12, 12, and 24 h, respectively), and the products were summed to obtain cumulative emissions (µg N₂O–N kg⁻¹ dry soil).

The initial mineral N content and the content at the end of the incubation were determined with 2 M KCl-extraction as described above but using an extraction ratio of 1:4. Filtrates were stored frozen at -20°C before the analysis of NH₄⁺ and NO₃⁻ concentrations and the enrichments of ¹⁵N in the extracted NH₄⁺ and NO₃⁻. Mineral N contents were measured with a discrete analyzer (Gallery Plus Discrete Analyzer, Thermo Scientific™, USA). The components of ¹⁵N enrichments of extractable NH₄⁺ and NO₃⁻ were concentrated on acidified filter paper (Sørensen and Jensen 1991) and the ¹⁵N contents of filter papers were analyzed with elemental analyzer (CE 1110, Thermo Electron, Milan, Italy) coupled in a continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (IRMS; Thermo Scientific, Bremen, Germany).

Gross nitrification rates (*m*) and gross immobilization rates (*i*) were calculated using the ¹⁵N-isotope pool dilution principle and equations (2) and (3) from Kirkham and Bartholomew (1954):

$$m = \frac{(M_0 - M_{48})}{t} \times \frac{\log(H_0 M_{48} / H_{48} M_0)}{\log(M_0 / M_{48})} \quad \# (2)$$

$$i = \frac{(M_0 - M_{48})}{t} \times \frac{\log(H_0 / H_{48})}{\log(M_0 / M_{48})} \quad \# (3)$$

where *m* is the gross nitrification rate (mg kg⁻¹ h⁻¹) derived from the NH₄¹⁵NO₃ labelled treatment and *i* is the gross immobilization rate (mg kg⁻¹ h⁻¹) derived from the ¹⁵NH₄NO₃ labelled treatment. *M*₀ and *M*₄₈ represent the mass of the ¹⁴⁺¹⁵N pool per unit mass of soil at time 0 and 48 h, respectively. *H*₀ and *H*₄₈ are the corresponding masses of excess ¹⁵N at time 0 and 48 h; and *t* is the incubation time (h).

The calculations were done using the atom percent excess values that were obtained by subtracting the natural abundance, measured from unlabeled soil (0.418 at% ¹⁵NO₃ and 0.523 at% ¹⁵NH₄), from the ¹⁵N

atom percent values of labelled samples.

Statistical analysis

All statistical analyses were performed with the R statistical software version 4.4.1 (R Core Team 2024). For the pot experiment, we tested the effects of plant cover, drought and their interaction on soil N cycling and N₂O emissions using factorial two-way analysis of variance (ANOVA). The experimental design included four plant cover treatments: oat (Oat), oat-alfalfa mixture (Oat + AA), oat-Italian ryegrass mixture (Oat + IR) and bare soil (Bare), under two moisture conditions (moist and dry). For each response variable (N₂O-N, CO₂-C, NH₄⁺-N, NO₃⁻-N, EN, MBN, plant shoot and root biomass, shoot and root N uptake and shoot and root N concentration), we fitted linear models including the plant cover treatment, soil moisture treatment, and their interaction as fixed effects. The interaction term was included to test whether the effect of drought differed among plant cover treatments. Data from the incubation experiment (cumulative N₂O emissions, gross nitrification, and gross immobilization rates) were analyzed similarly using factorial two-way ANOVA. The model included plant cover (bare soil and soil previously cultivated with oats), soil moisture (dry, moist and rewetted) and their interaction as fixed effects.

Model assumptions were evaluated using graphical and statistical diagnostics. Residuals versus fitted values were visually inspected to assess homoscedasticity and independence, and Q-Q plots and histograms were examined to evaluate normality. Normality was further tested with the Shapiro-Wilk test, and the homogeneity of variances across plant cover and moisture treatments was assessed with Levene's test. When diagnostic plots or tests indicated violations of normality or heteroscedasticity, the response variable was log-transformed (N₂O-N, CO₂-C, NO₃⁻-N, EN and MBN) and the model was refitted.

Analysis of variance was performed using the `anova()` function from the `stats` package in R (R Core Team 2024), and the effects of factors were evaluated using sequential (Type I) sums of squares. Because replication differed among vegetated and bare soil treatments, model terms were entered in an order reflecting the experimental design (plant cover, moisture, and their interaction). When significant main or interaction effects were detected, post-hoc pairwise comparisons of estimated marginal means were performed with the *emmeans* package (Lenth 2025). Compact letter displays using `multcomp` package (Hothorn et al. 2008) were used to denote plant cover treatments that differed significantly ($p < 0.05$). Microsoft Copilot was used to assist in writing R codes (Microsoft 2026).

RESULTS

- Pot experiment
- Cumulative N₂O-N

Cumulative N₂O-N emissions were significantly affected by plant cover treatment (log-transformed $F_{(3,34)} = 102.8$, $p < 0.001$), soil moisture condition (log-transformed $F_{(1,34)} = 31.51$, $p < 0.001$), and their

interaction (log-transformed $F_{(3,34)} = 19.36$, $p < 0.001$; Fig. 3). Bare moist soil had the largest emissions, whereas drought reduced the emissions of bare soil from 8.50 to 1.72 $\mu\text{g N}_2\text{O-N kg}^{-1}$ ($p < 0.001$). Presence of plants drastically decreased N_2O emissions by 82–99% from both moist and dry soil compared with bare soil. Cumulative N_2O emissions were smallest under oats in moist soil (0.081 $\mu\text{g N}_2\text{O-N kg}^{-1}$) and largest under oat-alfalfa mixture in dry soil (0.351 $\mu\text{g N}_2\text{O-N kg}^{-1}$). Plants decreased the emissions much more under moist conditions than under drought across all vegetated treatments ($p < 0.02$), with Oat alone showing the strongest response (3.9-fold higher N_2O emissions under dry than moist conditions), followed by the plant mixtures Oat + IR (2.9-fold higher) and Oat + AA (2.3-fold higher).

- Mineral N contents

By the end of the experiment the initial $\text{NH}_4^+\text{-N}$ content of 5.04 mg kg^{-1} had decreased by 60–64% in the bare soil treatments, whereas in the presence of plants the $\text{NH}_4^+\text{-N}$ content had decreased by 92–95% from the initial content of 22.4 $\text{mg NH}_4^+\text{-N kg dry soil}^{-1}$ after fertilization to 1.40 $\text{mg NH}_4^+\text{-N}$. Consequently, $\text{NH}_4^+\text{-N}$ content was 3–45% lower in vegetated than in bare soil treatments ($F_{(3,34)} = 3.61$, $p = 0.023$) across both moisture conditions (Fig. 4a). Drought alone did not significantly affect $\text{NH}_4^+\text{-N}$ content across plant cover treatments.

Soil $\text{NO}_3^-\text{-N}$ content ranged from 0.143 to 40.1 $\text{mg NO}_3^-\text{-N kg}^{-1}$ across all treatments (Fig. 4b). Initially, soil $\text{NO}_3^-\text{-N}$ contents were 22.5 mg kg^{-1} in the fertilized vegetated treatments and 5.11 mg kg^{-1} in unfertilized bare soil. By the end of the experiment, presence of plants had decreased $\text{NO}_3^-\text{-N}$ content by 96–100%, whereas bare soil showed a 6–7-fold increase compared with the initial contents. Accordingly, $\text{NO}_3^-\text{-N}$ content was significantly smaller in the vegetated treatments compared to the bare soil treatments (log-transformed $F_{(3,34)} = 63.60$, $p < 0.001$) in both dry and moist conditions. Under drought, $\text{NO}_3^-\text{-N}$ content was significantly larger across all plant cover treatments compared to moist conditions (log-transformed $F_{(1,34)} = 38.16$, $p < 0.001$), with no significant plant cover $\times$ moisture interaction (log-transformed $F_{(3,34)} = 1.75$, $p = 0.176$).

- Plant shoot and root N uptake

Under moist conditions, plant shoot N uptake was on average about nine times higher than root N uptake (Fig. 5). Drought further increased the shoot N : root N ratio, indicating a shift in N allocation toward aboveground biomass under water stress. The strongest response occurred in the oat-Italian ryegrass mixture, where the ratio increased from 9 to 14, whereas the oat-alfalfa mixture showed only a modest increase from 11 to 12.

Total shoot N uptake differed significantly between the plant cover treatments ($F_{(2,30)} = 4.45$, $p = 0.02$) with largest values observed in the oat-alfalfa mixture, and smallest in oat under both moisture conditions. The total shoot N uptake in the oat-Italian ryegrass mixture did not differ from that of either

oat or oat-alfalfa mixture (Fig. 5a). Oat shoot N uptake was consistently higher when oat plants were grown in a mixture compared with oat plants alone. In the oat-alfalfa mixture, oat shoots contained on average 55 mg N per plant under both moisture conditions, compared with 28 and 30 mg N per plant in the oat plants grown alone in moist and dry soil, respectively. A similar pattern was observed in the oat-Italian ryegrass mixture where oat shoot N uptake was 41 and 46 mg N per plant in moist and dry soil, respectively. Drought did not have a significant effect on shoot N uptake ($F_{(1,30)} = 1.93$, $p = 0.18$).

Root N uptake (Fig. 5b) in different plant cover treatments did not differ significantly within a moisture level ($F_{(2,30)} = 0.75$, $p = 0.480$), but it was significantly decreased by drought ($F_{(1,30)} = 7.37$, $p = 0.011$). In oats grown alone, drought decreased root N uptake by 15%, whereas the decrease was 4% in the oat-alfalfa mixture and 28% in the oat-Italian ryegrass mixture.

- Incubation experiment
- Gross nitrification rate

Plant cover $\times$ moisture interaction had a significant effect on soil gross nitrification rates ($F_{(1,2)} = 11.40$, $p < 0.001$) indicating that the effect of soil moisture on gross nitrification depends on plant cover (Fig. 6). In bare soil, gross nitrification was highest under dry conditions ($0.418 \pm 0.035 \text{ mg kg}^{-1} \text{ h}^{-1}$), 90% lower under moist conditions ($0.041 \pm 0.045 \text{ mg kg}^{-1} \text{ h}^{-1}$; $p < 0.001$), and 54% lower under rewetted conditions ($0.191 \pm 0.039 \text{ mg kg}^{-1} \text{ h}^{-1}$; $p < 0.001$) compared with dry soil. In contrast, soil moisture treatment did not significantly affect gross nitrification in the soil previously cultivated with oats. Consequently, gross nitrification was significantly greater in bare than oat soil under dry conditions ($p < 0.001$), whereas no differences were observed under moist or rewetted conditions ($p > 0.49$).

DISCUSSION

- Drought had contrasting effects on N_2O emissions in bare and vegetated soil

Our results showed that drought had contrasting effects on soil N_2O emissions depending on the presence of plants. Plants can therefore alter soil's response to drought, with direct implications for nutrient cycling and greenhouse gas fluxes. Overall, vegetated soils emitted less N_2O than bare soils, indicating that growing plants help to mitigate soil N_2O emissions. As expected, the presence of growing plants with high plant N uptake significantly reduced mineral N contents of soil leading to lower N supply to N_2O production. This was supported by Rummel et al. (2021), who found that plant N uptake was one of the main factors controlling N_2O emissions.

Contrary to our hypothesis that drought decreases N_2O emissions across all treatments due to suppression of microbial activity, we found that drought increased the emissions from vegetated soils, even if it decreased them from bare soil. The lower N_2O emission from dry bare soil aligns with previous meta-analyses from field studies by Homyak et al. (2017) and Li et al. (2020), where the lower N_2O

emissions during dry conditions were explained by reduced microbial activity and decreased denitrification due to limited soil moisture. In our study, the bare soil pots under drought, unlike the corresponding vegetated pots, experienced dry conditions throughout the 39-day experiment (in comparison to 25 days for vegetated soil). This could have altered the microbial activity differently compared with the vegetated treatments. Substantially higher gross nitrification rates and final NO_3^- content, decreased CO_2 emissions (Fig. S2) and lower microbial biomass N (Fig. S3) in bare soil under drought give support to altered microbial activity, which is also consistent with drought-induced reduction in overall microbial activity found by Manzoni and colleagues (2012).

The presence of plants can modify N_2O dynamics by root O_2 consumption and stimulation of microbial activity through C input from root exudates, which can lead to the formation of anaerobic microsites (Malique et al. 2019). In soils with high NO_3^- content, this can further increase denitrification and N_2O production. However, in soils with limited NO_3^- availability, the N uptake by plants may reduce denitrification rates as plants and microbes compete for the same mineral N (Smith and Tiedje 1979). Thus, plants may enhance or inhibit denitrification in soil (Rummel et al. 2021).

In the vegetated treatments, soil mineral N was dominated by NH_4^+ , whereas in bare soil NO_3^- was the predominant form. Both Homyak et al. (2017) and de Nica et al. (2020) found that soil NH_4^+ contents typically increased under reduced rainfall, whereas NO_3^- contents do not change. In contrast, our results showed that NO_3^- content was higher under drought than in moist conditions, while NH_4^+ content showed little or no response to drought across plant cover treatments, suggesting that drought primarily affected NO_3^- dynamics rather than NH_4^+ availability. Under drought, plant NO_3^- uptake is known to decrease (Gloser et al. 2020), which leads to increased NO_3^- content in soil. This could lead to a risk of higher N_2O emissions. Contrary to our expectations, plant N uptake was not affected by drought. This could be due to majority of N uptake occurring in the early growing stages of the plants (Plett et al. 2020; Zhu et al. 2025), before the actual drought conditions in soil started. Higher NO_3^- contents in vegetated treatments under drought may reflect altered microbial N transformations, such as nitrification and denitrification (Parker and Schimel 2011; Sullivan et al. 2012). Nitrification is thought to dominate N_2O production when the soil WFPS is below 60%, while denitrification dominates above WFPS 60% (Linn and Doran 1984; Wang et al. 2023). This division of N_2O forming processes and soil moisture condition is, however, shown to be arbitrary because denitrification can occur also in aerobic microsites within well aerated soils. For instance, Harris et al. (2021) found that the proportion of N_2O formed by denitrification increased under drought and even 80 to 90% of N_2O was produced by denitrification at such low WFPS as 20–30%. This is further supported by Friedl et al. (2021), who found that in clay and loam soils denitrification is the main N_2O production pathway even under oxic conditions ($\text{WFPS} \leq 60\%$). In this study, soil cultivated with oats showed no drought-induced increase in gross nitrification, suggesting that higher NO_3^- content under drought was driven by reduced microbial NO_3^- consumption rather than enhanced nitrification. Many previous studies have shown that soil microbial biomass increases under

drought (e.g. Homyak et al. 2017; Parker and Schimel 2011; Schaeffer et al. 2017; Schimel 2018; Séneca et al. 2020). Our results on short term drought showed a slightly lower microbial biomass N under drought, also in the presence of plants. In principle, this could lead to reduced microbial immobilization of mineral N, potentially contributing to higher soil NO_3^- contents and enhanced N_2O emissions in planted treatments as suggested in Wang and Song (2024). However, the results of our incubation experiment did not indicate significant changes in microbial N immobilization by drought (Fig S4). Instead, soils that were vegetated prior to the incubation increased the immobilization rate in all moisture contents.

The concurrent increase in N_2O emissions despite low nitrification rates indicates that N_2O production under drought in vegetated soil was unlikely to be directly linked to increased nitrification. Instead, elevated NO_3^- availability together with the supply of labile C may have favored N_2O production processes such as denitrification or nitrifier-denitrification (Rummel et al. 2021). These observations align with previous studies showing that nitrifiers and denitrifiers are not entirely inhibited by drought; rather, both groups can remain active under dry soil conditions (Krichels et al. 2023; Séneca et al. 2020; Sullivan et al. 2012). Although total denitrification generally declines with decreasing soil moisture (Ciarlo et al. 2007; Friedl et al. 2021; Groffman and Tiedje 1988), dry conditions often increase the proportion of denitrified N release as N_2O relative to N_2 (Ciarlo et al. 2007; Friedl et al. 2021; Saggar et al. 2013). Consequently, a higher $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ production ratio may occur even when overall denitrification rates are decreased. In addition, elevated NO_3^- concentrations have been shown to increase the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio of denitrification (Saggar et al. 2013). Together, these mechanisms could explain the higher N_2O emissions observed in the vegetated treatments under drought than under moist conditions.

- Plant species effects on N dynamics

Because root traits and exudates differ among plant species, the effects of drought on denitrification and other soil microbial processes are likely plant species-specific (Hou et al. 2025; Williams et al. 2022; Williams and Vries 2020). However, plant species had only minor effects on N_2O emissions compared to the dominant influence of presence of any plant species and drought. In our earlier study, we found that Italian ryegrass grown alone led to significantly higher N_2O emissions during early growing season compared to the emissions from plant mixtures of barley and Italian ryegrass (Bore et al. 2024). In our experiment under moist conditions, the mixture of oat and alfalfa showed slightly higher N_2O emissions as compared to oat only, whereas the emissions under oat and Italian ryegrass mixture did not differ from either treatment. This indicates that N_2 fixing plants may stimulate soil N_2O formation in the rhizosphere as shown in Dong et al. (2025). These differences, however, disappeared under drought, indicating that drought overrides the effects of different plant species on the N_2O emissions.

Lack of plant species effects on soil N pools and N_2O emissions could be due to the relatively short experimental duration (39 days) at early growth stages of plants. For instance, the root hairs on alfalfa

radicles become infected with nitrogen fixing bacteria (*Rhizobium meliloti*) typically within 4 weeks after germination and activation of N_2 fixation typically takes several weeks (Mueller and Teuber 2007). This is also the time needed for nodulation and nitrogenase activity to begin, while N_2 fixation rates typically increase gradually and remain relatively low during early growth. The interaction of species could have also reduced the plant-species specific effects on soil N pools and N_2O emissions.

In the mixtures with N_2 fixing plant species, the root interaction between plants species enhances N_2 fixation due to plants competing for soil mineral N, as well as biomass production and N accumulation in all plants (Zhao et al. 2020). The high N recovery in below- and above-ground biomass of oat–alfalfa mixture, however, indicates that alfalfa enhanced N retention in plant biomass and altered N partitioning within the plant–soil system (Fig. S5). While the total shoot biomass was highest in oat alone (Fig. S6a), the individual oat plants attained greater biomass when grown in mixtures, indicating improved N availability by alfalfa and/or reduced competition under mixed planting. In our study, the stronger N uptake by oat intercropped with alfalfa likely limited any accumulation of legume-derived N in soil, resulting in an enhanced N capture. In addition, the N contents (Fig. S7) in both shoots and roots were consistently higher under dry conditions, indicating that drought reduced plant biomass formation more than N uptake, which led to a relative increase in plant N contents, rather than greater total N accumulation.

The N pools and plant N uptake in the oat–Italian ryegrass mixture differed minimally from those in oats grown alone. This likely reflects functional similarity between the two grass species. In our previous study (Bore et al. 2024), however, we found that Italian ryegrass alone released significantly more N_2O emissions than when sown together with barley, highlighting the importance of plant species and diversity effects on N_2O emissions.

- Implications for managing N_2O emissions in agroecosystems under drought

Our results indicate that plant cover can influence how drought affects soil N cycling and subsequent N_2O emissions. Drought increased NO_3^- accumulation in soil, increasing the risk of N_2O pulses following rewetting. However, the magnitude of this response depended strongly on previous plant cover. Soils previously cultivated with oat maintained substantially lower N_2O emissions after rewetting than bare soils (Fig. S8). This suggests that plant N uptake may reduce the accumulation of mineral N available for N_2O production following rainfall. As early-summer droughts are projected to become more frequent under climate change, these findings highlight the importance of maintaining active plant N uptake during periods prone to drought, thereby limiting NO_3^- accumulation and subsequent N_2O emissions upon rewetting.

Moreover, as plant–soil feedbacks may differ under longer-term or repeated drought events, the responses we observed during early plant growth stages may not reflect those in mature plant communities with fully developed root systems. Field-based and long-term studies are therefore needed

to assess whether the effects of plant cover on N cycling and N₂O emissions under drought persists under more complex and variable environmental conditions.

CONCLUSIONS

This experiment provides new insights into how plant–soil interactions regulate N₂O emissions under drought conditions. The 25-day drought during early growth stages of plants revealed that the presence of a plant, irrespective of plant species, mitigated soil N₂O emissions compared to bare soils.

However, when the plant growth and soil N uptake was impeded by drought, soil N₂O emissions were higher than those in the corresponding vegetated soil under moist conditions. Despite this increase, N₂O emissions remained substantially lower than those from drought-affected bare soil. The accumulation of NO₃[−] content in bare soil during drought likely creates conditions for high N₂O emissions upon rewetting, as observed in moist and rewetted bare soil. These findings highlight the critical role of growing plants in limiting drought-induced N accumulation and mitigating post-drought N₂O pulses. Our results also suggest that early-season drought can impair plant establishment, increasing the risk for N₂O emissions. Ensuring successful crop establishment and minimizing drought stress appear thus key strategies for reducing agricultural N₂O emissions.

Declarations

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Pauliina Turunen: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – original draft, Writing – review & editing, Visualization. **Xuhui Luo:** Investigation, Writing – review & editing. **Ezekiel K. Bore:** Conceptualization, Methodology, Writing – review & editing. **Anne Viinikainen:** Investigation, Formal analysis. **Asko Simojoki:** Conceptualization, Writing – review & editing, Supervision. **Markku Koskinen:** Conceptualization, Writing – review & editing, Supervision. **Per Lennart Ambus:**

Methodology, Writing – review & editing. **Kristiina Karhu**: Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Mari Pihlatie**: Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Data availability

The dataset generated during and/or analyzed during the current study are available in Zenodo (<https://doi.org/10.5281/zenodo.20305928>)

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Figures

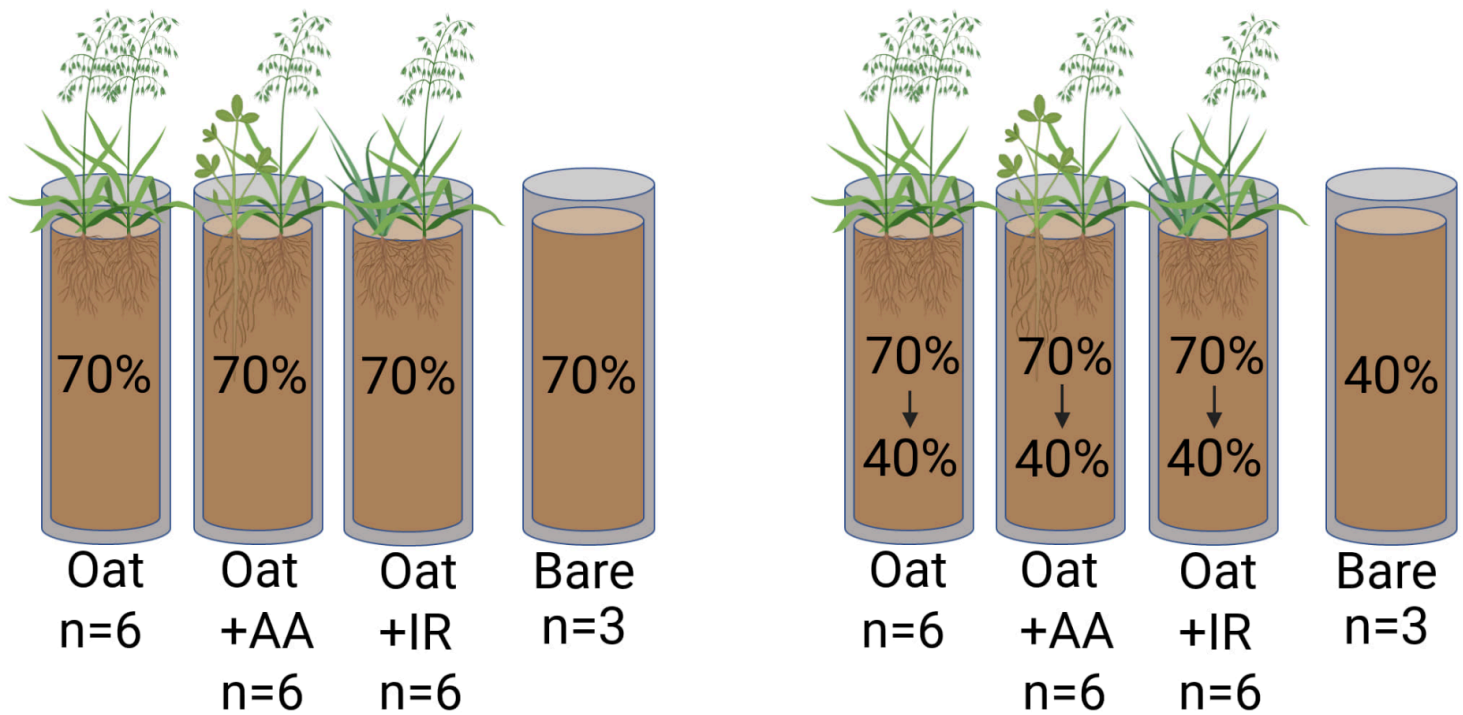


Figure 1

A schematic illustration of the experimental study design. Plant cover treatments included oat alone (Oat), oat with alfalfa (Oat + AA), oat with Italian ryegrass (Oat + IR), and bare soil. The vegetated treatments were kept at 70% WFPS for two weeks after which half of the pots were assigned for drought treatment (40% WFPS). Bare soil treatments were kept constantly either at 70% or 40% WFPS. Created in BioRender. Turunen P (2026) <https://BioRender.com/93iko36>

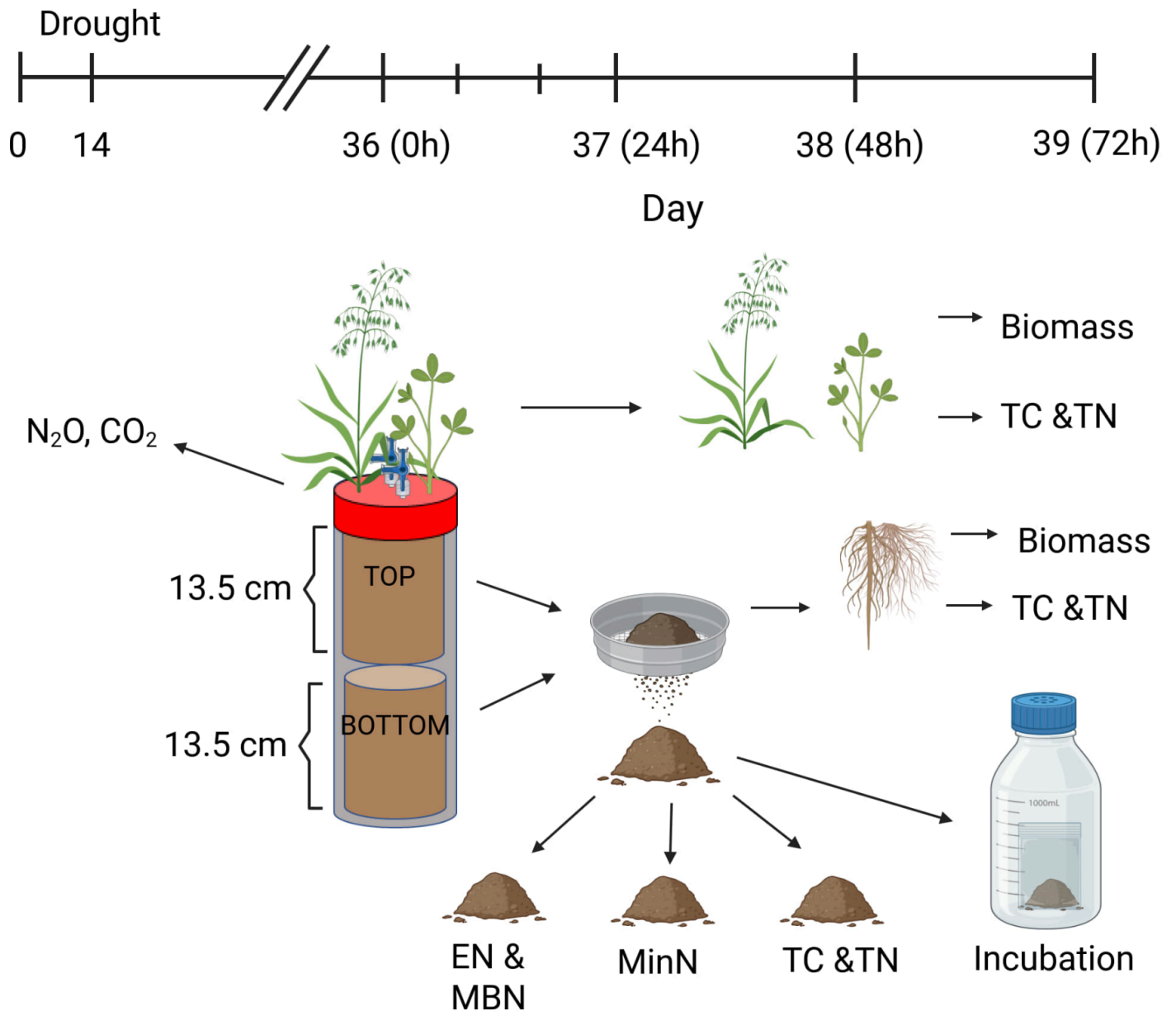


Figure 2

Soil and plant sampling and analyses at the end of the experiment. Drought treatment started on day 14. Gas samples of N_2O and CO_2 were taken at times 6, 12, 24, 48 and 72 h after the first scrubbing (0 h). Plants and roots were sampled for biomass as well as total C and N (TC and TN). Soil samples were taken from top (13.5 cm) and bottom (13.5 cm) layers of the soil column for extractable N (EN), microbial biomass N (MBN), mineral N (MinN), TC and TN, and for incubation experiment. Created in BioRender: Turunen P (2026) <https://BioRender.com/es501yn>

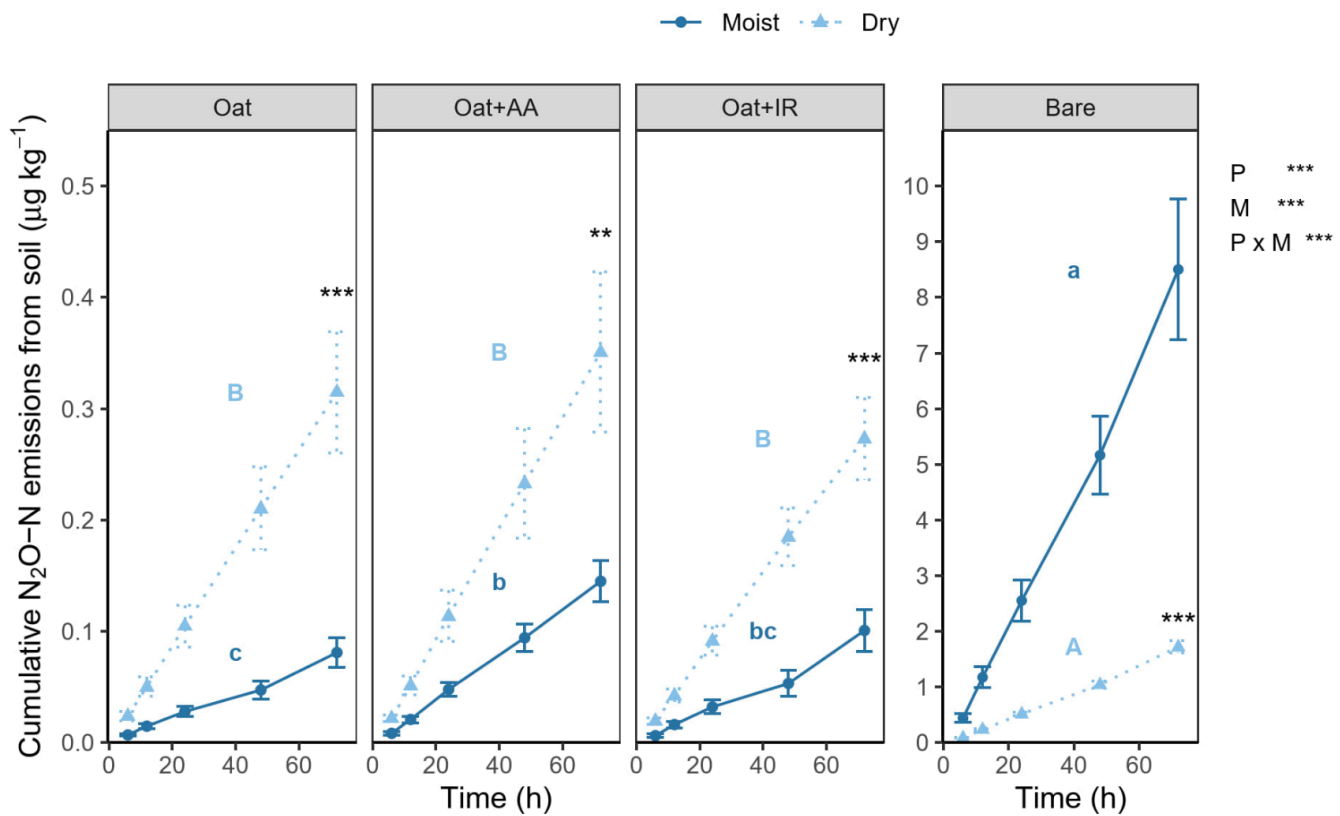


Figure 3

Mean cumulative soil N_2O emissions ($\pm$ SE) during a 72-h period in soil under oats (Oat, $n=6$), soil under oat-alfalfa mixture (Oat+AA, $n=6$), soil under oat-Italian ryegrass mixture (Oat+IR, $n=6$) and in bare soil (Bare, $n=3$). Solid dark blue lines indicate moist (70% WFPS) and dotted light blue lines indicate dry (40% WFPS) conditions. Two-way ANOVA reveal significant effects of plant cover treatment (P), soil moisture (M), and their interaction (P $\times$ M) and the asterisks within panels indicate significant effects of drought within each plant cover treatment (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant). Lowercase and uppercase letters indicate significant differences among plant cover treatments within moist and dry conditions, respectively ($p < 0.05$). Note the difference in y-axis scale between the vegetated and bare soil treatments

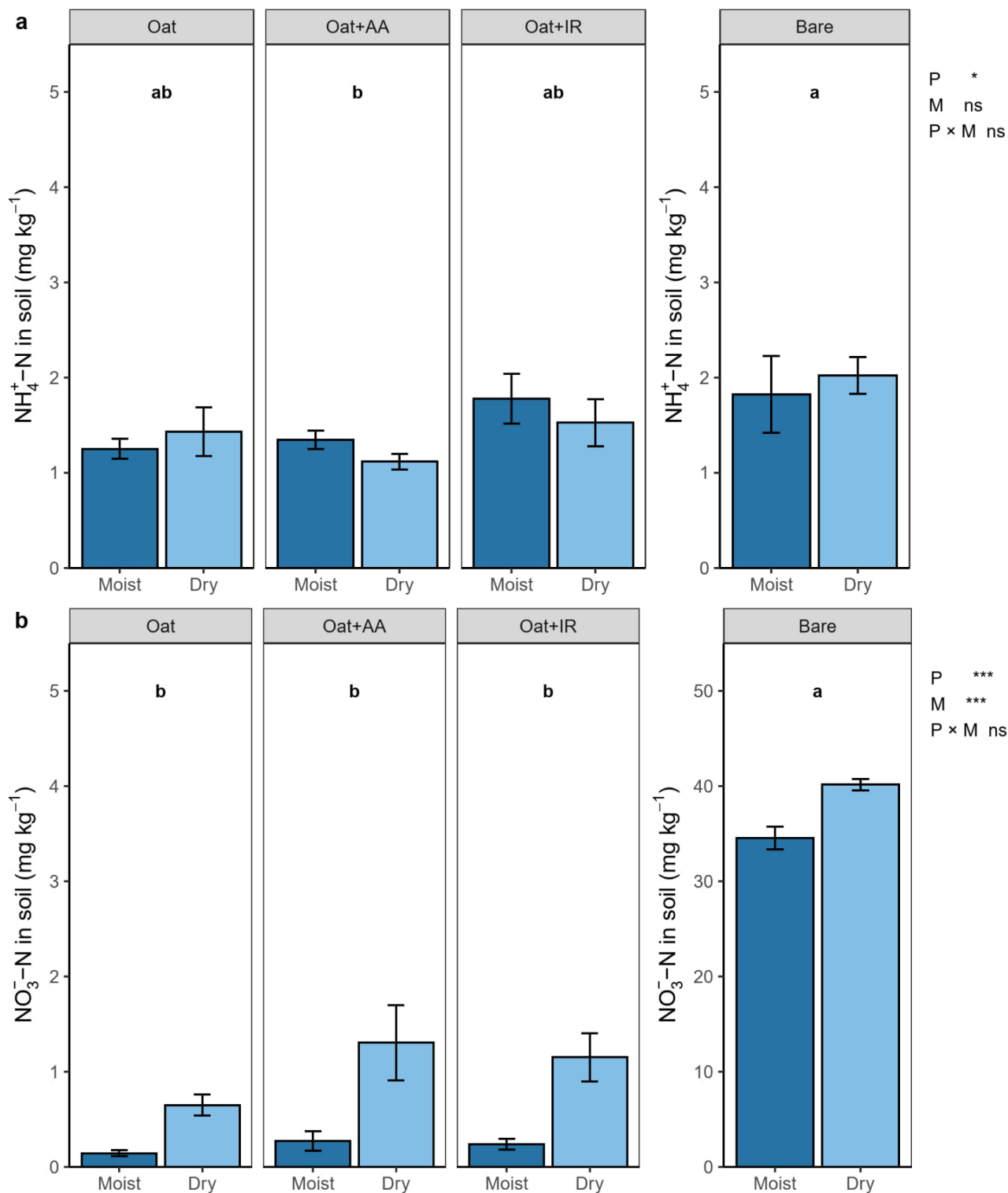


Figure 4

Soil $\text{NH}_4^+\text{-N}$ (**a**) and $\text{NO}_3^-\text{-N}$ (**b**) contents under oats (Oat, n=6), oat-alfalfa mixture (Oat+AA, n=6), oat-Italian ryegrass mixture (Oat+IR, n=6) and in bare soil (Bare, n=3) at the end of experiment. Bars represent treatment means $\pm$ standard errors. Two-way ANOVA shows significant main effects of plant cover treatment (P) on both $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, whereas soil moisture treatment (M) only show

significant effect on NO_3^- -N. Plant cover $\times$ soil moisture interaction ($P \times M$) did not have a significant effect on either NH_4^+ -N or NO_3^- -N (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns = not significant). Lower case letters denote significant differences between plant cover treatments based on the estimated marginal means (Tukey adjustment $p < 0.05$). Note the different NO_3^- -N axis scales in vegetated and bare soil treatments (b)

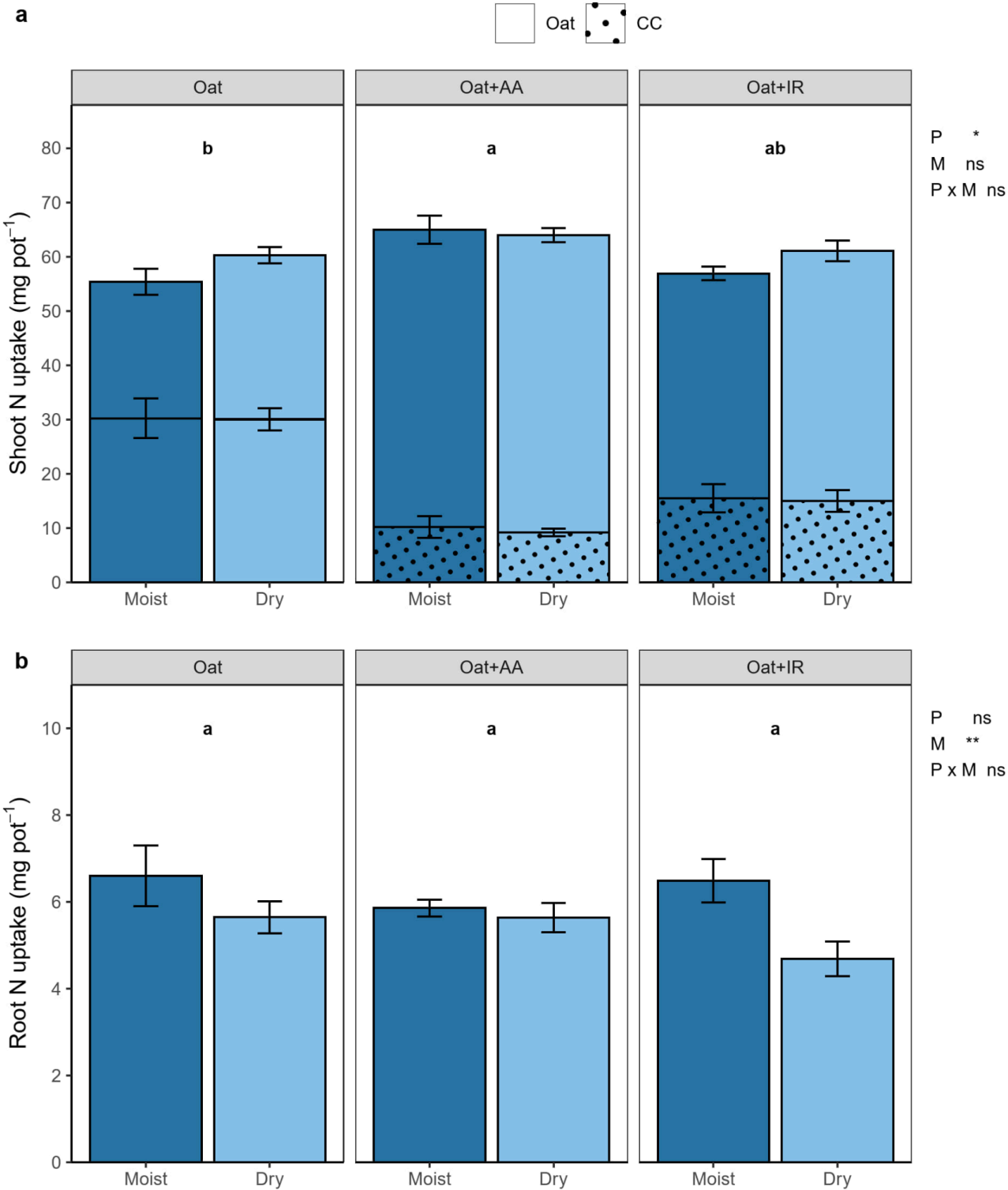


Figure 5

Nitrogen uptake (mean $\pm$ SE) by shoots (a) and roots (b). The stacked bars in panel a, show the separate N uptakes of both plants in the pot. The effects of moisture and plant cover treatments were statistically tested only for the combined total N uptake by both plants in the pot. Two-way ANOVA revealed significant main effects of plant cover treatments (P) on shoot N uptake, and soil moisture treatment (M) on root N uptake. Plant cover $\times$ soil moisture interaction (P $\times$ M) did neither affect shoot nor root N uptake (*p < 0.05; **p < 0.01; ***p < 0.001; ns = not significant). Different letters denote significant differences among vegetated treatments based on the estimated marginal means (Tukey adjustment, p < 0.05)

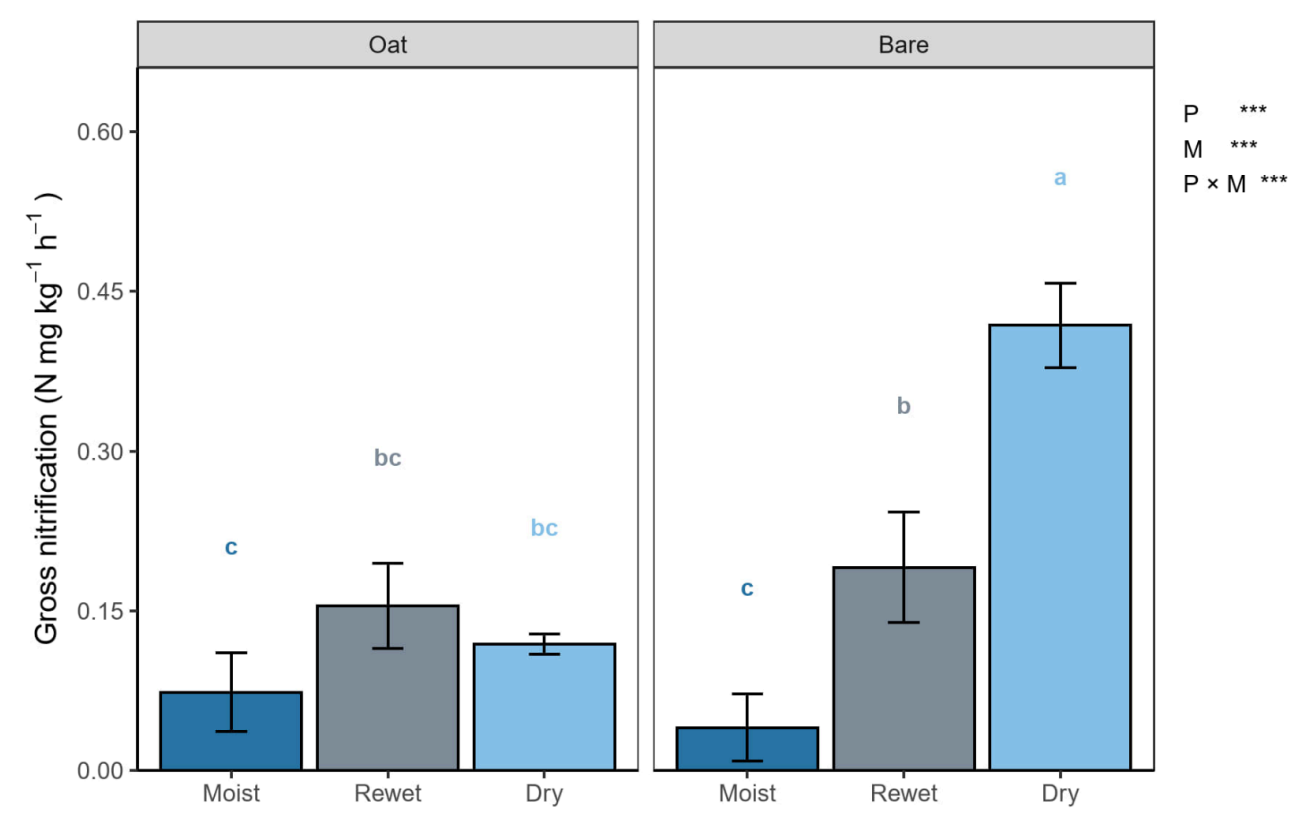


Figure 6

Mean ($\pm$ SE) gross nitrification rates (mg N kg⁻¹ h⁻¹) in soil previously cultivated with oats (n=5) and in bare (n=5) soil. Two-way ANOVA revealed that plant cover $\times$ soil moisture interaction (P $\times$ M) had a significant effect; gross nitrification being affected by moisture in bare soil but not in soil under oats. Lowercase letters indicate significant differences among treatments (p < 0.05)

Supplementary Files

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