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Post-drought organic carbon mineralization leads to high productivity and nutrient uptake efficiency of perennial grassland after rewetting

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ABSTRACT

Grasslands often recover well from drought, with some even surpassing non-drought-stressed controls in productivity long after drought release. However, the mechanisms responsible for such post-drought productivity outperformance remain unclear. In this study we examine how rewetting after drought influences important short- and longer-term soil microbial processes (i.e. nitrogen mineralization, potential enzyme activities) and consequent plant nutrient availability and uptake. For this, a field experiment was set up where an established perennial ryegrass sward under different N-fertilization levels was subjected to either a 2-month experimental summer drought followed by rewetting or to rainfed control conditions.

Rewetting after drought led to an immediate pulse in gross N-mineralization and NH_4-consumption rates. Both rates increased by >230% and >430% in formerly drought-stressed subplots compared to controls in plots not N-fertilized and N-fertilized during drought, respectively. Importantly, gross N mineralization rates correlated significantly with extractable soil organic carbon contents at the end of drought. Concurrently, drought and rewetting significantly increased NO_3–N, P, K, S, Fe, Zn, and Mn availability during the 1st but not the 2nd month after rewetting, except for K. Aboveground productivity of perennial ryegrass responded positively to NO_3–N availabilities during the 1st month after rewetting, leading to productivity outperformance of formerly drought-stressed plots compared to controls. These results suggest that short-term productivity outperformance of perennial grasslands in the 1st month after rewetting is driven by an increase in NO_3–N availability caused by a rewetting-induced pulse in N-mineralization of organic substrates accumulated during drought. Although effects of drought and rewetting on nutrient availability were only observed in the 1st month after rewetting, grassland productivity outperformance persisted in the 2nd month after rewetting. This indicates that soil drought legacy increased plant nutrient uptake efficiency, explaining longer-term outperformance effects when effects of drought and rewetting on nutrient availability were no longer apparent.

1. Introduction

Grasslands have a high capacity to recover from drought and even outperform non-drought control swards in terms of productivity once drought stress is released (Hofer et al., 2016; Matos et al., 2020; Wilcox et al., 2020). Such post-drought outperformance effects have been reported for different grassland ecosystems (Matos et al., 2020; Zhou et al., 2022), with previously drought-stressed grasslands showing increased productivity even during the following seasons (Hahn et al., 2021; Ru et al., 2023). In a recent study we showed that post-drought outperformance of productivity in perennial ryegrass is mainly driven by drought- and rewetting-induced soil legacy effects, which increase plant performance after drought release (Schärer et al., 2023). The mechanisms behind such soil drought legacies and their role in short-term and longer-term post-drought productivity outperformance has, however, not yet been resolved.

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Drought and rewetting can severely affect a range of processes in the soil. As soils dry, the water and osmotic potential in the soil matrix decline while soil air content increases. This can impair the mobilization, transport, and thus the availability of water and water-soluble nutrients (Schimel, 2018). Under severe drought, these changes can ultimately restrict plant growth, reduce microbial activity, induce microbial dormancy and mortality, and alter both plant and microbial community composition (Manzoni et al., 2012; Canarini et al., 2017).

As soils are rewetted, soil conditions undergo a radical transition with rapid increases in soil matric and osmotic potentials. This often leads to a pulse in soil microbial activity upon rewetting with a subsequent increase in nutrient availability also known as the 'Birch effect' (Birch, 1958a,b; Borken and Matzner, 2009; Jarvis et al., 2007). Several non-exclusive processes can explain this effect. Reduced microbial activity during drought can lead to an accumulation of labile soil organic matter which may serve as substrate for mineralization once drought is released (Borken and Matzner, 2009). Rewetting can induce the microbial release of osmoregulatory solutes previously taken up during drought; under decreasing water potentials, microorganisms accumulate amino acids, amino acid derivates, carbohydrates, and inorganic ions such as K+ to maintain cell turgor (Killham and Firestone, 1984; Schimel, 2018; Wood, 2015). As soil water potentials increase following rewetting, the release of these substrates can lead to increased mineralization and nutrient availability (Halverson et al., 2000; Kieft et al., 1987; Schimel et al., 2007). When microbes are not able to adjust to the rapidly changing osmotic potential, their cells can burst. The cell content which then is released into the soil matrix becomes available for subsequent mineralization and uptake (Sparling et al., 1985; Sparling and Ross, 1988). However, since the survival of microorganisms during drought heavily depends on resource availability (Schimel, 2018), it is likely that the amount of the released osmotic solutes, as well as the magnitude of a mineralization pulse upon rewetting depend on nutrient availability.

Besides biotic processes, drying and rewetting of soil can also lead to a physical breakdown of soil aggregates which releases previously inaccessible inorganic nutrients and organic matter that serve as potential substrates once drought stress is released (Adu and Oades, 1978; Denef et al., 2001; Fierer and Schimel, 2003; Haygarth et al., 1998). All these processes may lead to higher post-drought plant nutrient availability and improve plant nutrition and thus recovery. However, the rewetting-induced effects on post-drought nutrient availability and mineralization are only short-lived (Borken and Matzner, 2009; Cabrera, 1993; Franzluebbers et al., 1996; Song et al., 2017), leaving the main processes driving longer-term post-drought productivity outperformance unknown.

In the present study, we investigate rewetting-effects on microbial activity, study the implication of these effects for plant nutrient supply, and identify the role of rewetting-induced effects on mineralization and plant nutrient supply in explaining short- and longer-term productivity outperformance in perennial grasslands. For this, we set up a field experiment where we exposed an established *L. perenne* L. sward under different N-fertilization regimes to a 2-month experimental summer drought followed by rewetting in 2019 and repeated the experiment in the following year. Specifically, we tested how drought and rewetting affects (1) plant aboveground productivity recovery, (2) plant nutrient availability and uptake, (3) extractable soil organic carbon contents, and (4) microbial short- and longer-term gross N mineralization rates, NH_4 consumption rates, and potential activities of extracellular enzymes involved in the soil organic matter N, P and C cycling. Further, (5) we tested whether (1–4) are affected by different N-fertilization levels during drought and at the beginning of recovery.

2. Material and methods

2.1. Site conditions and experimental setup

The field trial was set up in 2018 nearby the Agroscope institute Zurich-Reckenholz, Switzerland (47°26′16″ N, 8°31′39″ E, 487 m a.s.l.) as a pure perennial ryegrass (*L. perenne* L., cultivar "Allodia") sward. The moderately deep clay soil at the site consisted of 21 % clay, 39 % silt, 38 % sand, and 2.2 % humus, and contained 59 mg kg^{-1} water-extractable phosphorus, 21 mg kg^{-1} water-extractable potassium, and 142 mg kg^{-1} water-extractable magnesium at a pH of 6.6. Mean annual temperature and precipitation in the reference period 1991–2020 at the MeteoSwiss weather station situated nearby was 9.9 °C and 1018 mm, respectively.

We established the pure perennial ryegrass sward in 2018 with a sowing density of 0.25 g m^{-2} and a seed row distance of 13.5 cm. We chose perennial ryegrass as a model species as it is a well-studied and the most widely used species in temperate forage grasslands (Lee et al., 2010; Turner et al., 2012). We carried out the experiment with three experimental factors: experimental summer drought, N-fertilization during drought, and N-fertilization at the beginning of recovery, where each factor consisted of two levels (drought and N-fertilization treatments are described in detail below). The experimental layout followed a split-plot design with four replicates, arranged on the site in four blocks. In each block, the two-by-two combinations of N treatments were randomly allocated to four main plots. In each main plot, three subplots (3 × 5 m) were established, one for the rainfed control, one for the drought treatment in 2019 and one for the drought treatment in 2020. Plots were cut six times per growing season and fertilized with 240 kg N ha^{-1} ammonium nitrate per year to account for the estimated annual N removal via hervest and to prevent non-drought-induced N limitation. The total annual productivity of rainfed control subplots was 1392 g m^{-2} in 2019 and 777 g m^{-2} in 2020, which was a slightly drier year than 2019 (Fig. S1).

2.2. Experimental drought and rewetting treatment

To induce experimental summer drought in the growing seasons 2019 and 2020, we installed rain-out shelters excluding 100 % precipitation from the beginning of June 2019 to mid-August 2019 (9.5 weeks in total) and from the beginning of June 2020 to mid-August 2020 (10 weeks in total). The rain-out shelters consisted of a tunnel-shaped steel frame (3 × 5.5 m with a height of 140 cm, Hortuna AG, Winikon, Switzerland) covered with a 200 μm thin, UV permeable plastic foil ('Gewächshausfolie Lumisol clear AF' from Hortuna AG, Winikon, Switzerland). To allow air circulation, the shelters were open at both ends and had a ventilation opening of 35 cm height along the entire length of both sides as well as on the top. For more information on the shelter design see Hofer et al. (2016). During experimental summer drought in season 2019 and 2020, the shelters excluded 229 mm and 227 mm of precipitation, respectively, which equals 13 % and 27 % of the annual precipitation in 2019 and 2020, respectively. This led to an average of 0.57 MPa and 0.78 MPa lower soil water potential in drought-stressed compared to rainfed control field subplots during the 2nd month of experimental drought in 2019 and 2020, respectively (Fig. S1). To end the drought period, the field was naturally rewetted by rain events starting on August 8 with 31 mm over 6 days in 2019, and by rain events starting on August 13, which precipitated 21 mm of rain within 6 days in 2020. In 2020, we supplemented the precipitation event by artificial irrigation of all previously droughted and rainfed control subplots with 30 mm on August 18 to ensure successful rewetting.

2.3. N-fertilization treatment

To assess how belowground nutrient dynamics and plant productivity during drought recovery responded to different levels of N supply at the beginning of recovery as well as to legacy effects of different N-levels during drought, we induced two distinct factorial N-fertilization treatments: N-fertilization during drought ('-Ndr' or '+Ndr') and N-fertilization at the beginning of recovery ('-Nrec' and '+Nrec'). This resulted in the following N-fertilization treatment combinations: plots receiving no N-fertilizer during drought and at the beginning of recovery (-Ndr/-Nrec), plots receiving two times 47.5 kg N ha^{-1} during drought (once at the beginning of drought and once after 1 month drought) but no N-fertilizer at the beginning of recovery (+Ndr/-Nrec), plots receiving no N-fertilizer during drought and 50 kg N ha^{-1} at the beginning of recovery (-Ndr/+Nrec), and plots receiving two times 35 kg N ha^{-1} during drought and 50 kg N ha^{-1} at the beginning of recovery (+Ndr/+Nrec). N-fertilization treatments were equally applied to drought and rainfed control subplots. A detailed overview of applied fertilizer amounts can be found in Table S1.

2.4. Plant measurements

To determine aboveground productivity, grass was cut to 7 cm height in a central strip of 1.5 × 5 m per subplot (3 × 5 m) using an experimental plot harvester (Hege 212; Wintersteiger AG, Ried im Innkreis, Austria). The fresh weight of the harvested biomass was directly measured at the site with an integrated balance inside the plot harvester. Dry weight – fresh weight ratios were determined by collecting one biomass subsample of each harvested subplot and drying it at 60 °C for 48 h. Aboveground productivity was then determined as the dry weight of the total harvested biomass inside the central subplot strip.

To assess legacy effects of drought and rewetting on plant nutrient uptake, the harvested aboveground biomass was analyzed for leaf nitrogen (N), phosphorus (P) and potassium (K) contents. For this, the previously dried subsamples collected during harvests were ground with a cutting mill (Mühle SM1, Retsch, Germany) until passing through a 0.75 mm sieve. Leaf N concentrations were determined by complete combustion and gas chromatography using an elemental analyzer (Vario MAX CN Elemental Analyzer, Elementar Analysensysteme, Germany). Leaf P and K contents were measured using an inductively coupled plasma optical emission spectrometer (ICP-OES; Arcos FHS 16, Spectro, Germany). Leaf nutrient contents were corrected for residual moisture content of the samples to correspond to 100 % dry matter. Aboveground plant nutrient uptake was calculated as the product of leaf nutrient content and aboveground productivity.

2.5. Soil measurements

2.5.1. Plant nutrient availability

To assess plant nutrient availability after rewetting, two cation and two anion PRS® probes per subplot were installed vertically at 2–8 cm soil depth and for two consecutive periods of 14 days per recovery regrowth (for exact installation and retrieval dates see Table S2). PRS® consist of a 10 cm^2 ion-exchange membrane fixed in a PE-frame. We installed PRS® probes inside a central subplot strip with a minimum distance of 75 cm from each subplot side to minimize potential effects of lateral water flow into the experimental drought subplots. Since plant roots partially compete with PRS® for nutrient uptake, PRS® were installed in plant exclusion cylinders to avoid potential confounding effects on nutrient availability after drought (Hofer et al., 2017; Huang and Schoenau, 2011). For this, we installed one cylinder (Ø 25 cm, 20 cm height) out of rigid polyvinyl chloride (PVC–U) in each subplot center on July 24 and 28 in 2019 and 2020, respectively, i.e. two weeks before drought release, and removed aboveground plant material and major root material in the upper 5 cm soil layer. After each 14d-installation period, PRS® were retrieved, carefully brushed and rinsed with DI water to ensure full removal of soil residues. Probes of the same recovery regrowth period (1st month or 2nd month after drought and rewetting) and subplot were pooled and stored in clean zip-lock bags at 4 °C until shipping to Western AG Innovations Inc. for analysis.

2.5.2. Determination of extractable soil organic carbon pool

To assess the effects of drought and rewetting on soil organic carbon pools, we measured extractable organic carbon (EOC). For this, seven soil samples (2.8 × 8 cm up to a soil depth of 10 cm) were taken randomly over a central subplot strip using a 28 mm-diameter soil corer ('Pyrkhauer', Goecke GmbH & Co. KG, Schwelm, Germany). Samples were taken at the end of experimental drought in 2019 (July 29) as well as 6 weeks (September 16) and 13 weeks (November 7) after rewetting. Samples of each subplot were pooled, sieved to 2 mm, and fine roots were removed manually. Then, one aliquot (4 g) per sample was extracted with 30 ml 1 M KCl and analyzed for EOC using a TOC/TN Analyzer (TOC-V CPH E200V/TNM-122V; Shimadzu, Austria). To calculate EOC on a soil dry weight basis, dry weight - fresh weight ratios were determined by weighing 5 g of fresh soil and subsequently drying it at 60 °C for 48h.

2.5.3. Gross N mineralization and NH_4 consumption

We measured gross N mineralization rates and NH_4 consumption to assess rewetting effects on gross nitrogen turnover using soil aliquots of the same soil samples used for determination of EOC. Due to methodological reasons, the soil needed to be rewetted for measuring gross mineralization and NH_4 immobilization. Therefore, rates measured at the end of the experimental drought likely represent the immediate response of the microbial community to rewetting (Maxwell et al., 2022). Microbial gross N mineralization and NH_4 consumption rates were determined via the ^{15}N-isotope pool dilution method described by Kirkham and Bartholomew (1954). For this, we added 0.5 ml $^{15}NH_4SO_4$ (10 at%) tracer solution to duplicated soil aliquots of 4 g. Concentrations of tracer solutions were calculated according to previously photometrically determined NH_4 pool sizes and ranged between 0.125 and 2 mM. After ^{15}N-label addition, samples were incubated at 25 °C and one aliquot was extracted after 4 and 12h with 20 ml 1 M KCl each. The extracts were stored at −20 °C until further analysis. Gross N mineralization and NH_4 consumption rates were measured by microdiffusion of NH_3 using acid traps. For this, 100 mg MgO was hydrolyzed in 10 ml extract and placed together with the acid traps on an orbital shaker for two days. After drying the acid traps for at least 24 h, total N contents and atom-percent excess of ^{15}N was measured by EA-IRMS (EA 1110, CE Instruments, Italy) coupled to a Finnigan MAT Delta Plus IRMS via ConFlo III interface (Thermo Fisher Scientific, MA, USA). Gross N mineralization rates and NH_4 consumption rates were calculated following Kirkham and Bartholomew (1954).

2.5.4. Microbial enzyme activities

Potential activities of extracellular enzymes related to the C, N and P cycle were measured on fresh soil aliquots using an adapted fluorometric approach (Kaiser et al., 2010). For this, 1 g of the previously sieved soil sample was suspended in 100 ml sodium acetate buffer (50 mM, pH 6.5) and ultrasonicated at low energy. After that, 200 μl soil suspension was pipetted into black microtiter plates together with 50 μl substrate in five analytical replicates. Detailed information on the enzyme functions and on the specific substrates used to determine potential enzyme activities can be found in Table S3. Measurements assessing leucine-aminopeptidase activity were calibrated using amino-methylcoumarin (AMC) as standard, for the other enzymes methylumbelliferyl (MUF) was used as calibration standard. Potential activities were measured fluorometrically at an excitation wavelength of 365 nm and an emission wavelength of 450 nm (Tecan Infinite M Nano plate reader, Werfen, Austria) every 40 min after incubation of the plates at 20 °C in the dark and four times in total. Potential activity was calculated as the increase in fluorescence over time.

2.6. Statistical analyses

The effects of drought and rewetting on aboveground productivity, plant nutrient availability, gross mineralization and NH_4 consumption rates, potential extracellular enzyme activities, and plant N, P, and K uptake were analyzed using linear mixed-effects models for each variable, sampling event (time after drought and rewetting), and year separately with the nlme package (Pinheiro et al. 2020) using the restricted maximum likelihood (REML) approach. Drought treatment, N fertilization during drought, and N fertilization at the beginning of recovery were defined as fixed factors. 'Block' and 'main plot' were defined as random factors and considered for spatial correlation in the split-plot design. The final model included all main effects, two-way, and three-way interactions. To achieve homoscedasticity and normal distribution of residual variance, variables were natural log-transformed prior to analysis. Marginal and conditional R^2 of models were calculated following Nakagawa and Schielzeth (2013) using the MuMIn package (Bartoń, 2022). All data processing, statistical analyses and graphical visualisations were performed using the statistical software R, version 4.0.2 (R Core Team, 2020). Because the effects of drought and rewetting were almost equal in both experimental years and more variables were assessed in 2019 compared to 2020, the 2019 results are presented in the main text while results from 2020 are presented in the Supplement for reasons of readability. However, where responses diverged between years, this is clearly stated in the main text.

3. Results

Rewetting after severe drought significantly increased aboveground productivity of perennial ryegrass in the recovery regrowths 1 and 2 months after rewetting (both $P < 0.001$) (Fig. 1, Table S4). One month after rewetting, this effect was significantly more pronounced when plots were N-fertilized during drought (drought x Ndr, $P < 0.001$). Specifically, drought and subsequent rewetting increased aboveground productivity by on average 15 g m^{-2} (45 %) and 40 g m^{-2} (97 %) in plots not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr), respectively. In the 2nd month after rewetting, previous drought increased aboveground productivity by on average 22 g m^{-2} (32 %) over all N-fertilization treatments. N-fertilization during drought (+Ndr) further significantly increased plant aboveground productivity 1 month after rewetting by 55 g m^{-2} (83 %, $P < 0.001$) and by 15 g m^{-2} (22 %, $P < 0.001$) in the 2nd recovery regrowth 2 months after rewetting. However, this effect was less pronounced 2 months after rewetting if plots were N-fertilized at the beginning of recovery ($P < 0.05$). Concurrently, aboveground productivity was also significantly increased by N-fertilization at the beginning of recovery (+Nrec), resulting in 79 g m^{-2} (108 %) and 26 g m^{-2} (53 %) higher productivity in +Nrec compared to -Nrec in the 1st ($P < 0.001$) and in the 2nd month after rewetting ($P < 0.001$), respectively.

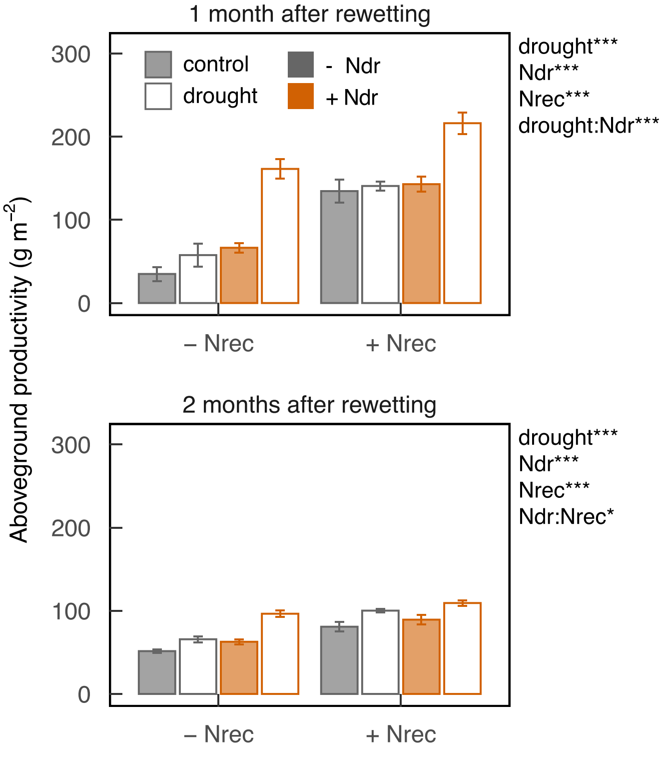

Fig. 1. Aboveground productivity after rewetting (means ± SE, n = 4) of rainfed control (filled bars) and formerly drought-stressed and rewetted subplots (empty bars) at the end of the 1st and the 2nd recovery regrowth 1 and 2 months after rewetting, respectively, in 2019. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. For better readability, only statistically significant treatment interactions are displayed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). For more statistical information see Table S4. Results for 2020 can be found in Fig. S2 and Table S4.

Rewetting after severe drought significantly increased plant N, P and K uptake in both recovery months (all $P < 0.001$, Fig. 2, Table S5) and had a more pronounced effect on plant nutrient uptake under + Ndr compared to -Ndr during the 1st month after rewetting (all $P < 0.001$). In the 1st month after rewetting, drought increased plant N uptake by 0.4 g N m^{-2} (30 %) and 2.7 g N m^{-2} (140 %), plant P uptake by 0.04 g P m^{-2} (22 %) and 0.33 g P m^{-2} (101 %), and plant K uptake by 0.7 g K m^{-2} (49 %) and 3.1 g K m^{-2} (150 %) in -Ndr and +Ndr plots, respectively. In the 2nd month after rewetting, drought increased N uptake by on average 0.9 g N m^{-2} (26 %), plant P uptake by on average 0.09 g P m^{-2} (29 %), and plant K uptake by on average 0.9 g K m^{-2} (43 %) across all N-fertilization treatments. Ndr significantly increased plant N, P and K uptake during the 1st month after rewetting by 1.5 g N m^{-2} (87 %), 0.19 g P m^{-2} (68 %), and 0.7 g K m^{-2} (90 %), respectively (all $P < 0.001$). During the 2nd recovery regrowth only plant N uptake was still significantly increased by Ndr, resulting in 0.4 g N m^{-2} (13 %, $P < 0.01$) higher plant N uptake in +Ndr compared to -Ndr. Nrec significantly increased plant nutrient uptake during both months after rewetting (all $P < 0.001$). Specifically, Nrec increased plant N, P, and K uptake by 1.9 g N m^{-2} (193 %), 0.28 g P m^{-2} (163 %), and 2.6 g K m^{-2} (256 %) during the 1st month after rewetting, respectively, and by 0.6 g N m^{-2} (39 %), 0.11 g P m^{-2} (46 %), and 0.9 g K m^{-2} (55 %) in the 2nd month after rewetting, respectively.

Rewetting after severe drought significantly increased NO_3–N, Fe ($P < 0.001$), S, Zn, Mn ($P < 0.01$), and K ($P < 0.05$) availability during the 1st month after rewetting, but had no significant effect on NH_4–N and P availability in 2019 (Fig. 3, Table S6). In detail, drought and rewetting increased NO_3–N availability by on average 99 μg 10 cm^{-2} 28 d^{-1} (67 %), K availability by 9 μg 10 cm^{-2} 28 d^{-1} (48 %), S availability by 8 μg 10 cm^{-2} 28 d^{-1} (33 %), Fe availability by 17 μg 10 cm^{-2} 28 d^{-1} (126 %), Zn availability by 0.26 μg 10 cm^{-2} 28 d^{-1} (59 %), and Mn availability by 1.8 μg 10 cm^{-2} 28 d^{-1} (77 %). During the 2nd month after rewetting, only K availability was still significantly increased by previous drought, showing an on average 4 μg 10 cm^{-2} 28 d^{-1} (48 %, $P < 0.05$) higher availability in formerly drought-stressed subplots compared to controls, with a more pronounced effect in +Ndr compared to -Ndr ($P < 0.05$). Ndr marginally increased S, Fe, and Mn availability during the 1st month after rewetting ($P < 0.1$) but did not affect the availability of the other nutrients measured during the same period and on nutrient availability in the 2nd month after rewetting. Nrec marginally increased NO_3–N, Fe, and Zn availability during the 1st month after drought ($P < 0.1$) and had no further effect on nutrient availability during the whole recovery period after rewetting.

Effects of drought and rewetting under different N-fertilization regimes on the hitherto reported values assessed in 2019 were similar in 2020 (Figs. S2–10). However, in contrast to 2019, previous drought significantly increased P availability by 1.7 μg 10 cm^{-2} 28 d^{-1} (104 %) ($P < 0.01$, Fig. S5, Table S6), but did not significantly affect S, Fe, and Zn

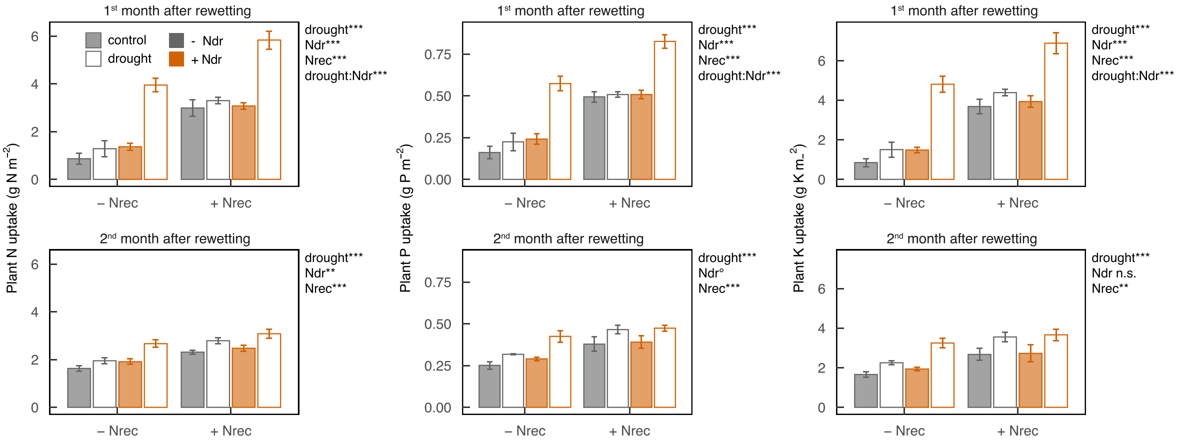

Fig. 2. Plant N, P and K uptake (means ± SE, n = 4, in g N per m subplot area) in rainfed control (filled bars) and formerly drought-stressed and rewetted subplots (empty bars) during the 1st and 2nd month after rewetting in 2019. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. For better readability, only statistically significant treatment interactions are displayed (°$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). For more statistical information see Table S5. Results for 2020 can be found in Fig. S3 and Table S5.

availability (Figs. S6–7, Table S6) during the 1st month after rewetting in 2020. The different effects of drought and rewetting on these variables were mainly driven by distinct responses to Nrec across years. While -Nrec showed similar drought and rewetting effects in 2020 as in 2019 across all N-fertilization treatments, +Nrec resulted in either no, or opposite responses to previous drought in 2019 compared to 2020.

Extractable organic carbon (EOC) contents at the end of experimental drought were significantly increased in drought-stressed subplots compared to rainfed control subplots ($P < 0.001$) (Fig. 4, Table S7) and had a more pronounced effect in +Ndr compared to -Ndr ($P < 0.05$). In detail, drought increased EOC contents by 21 μg C g^{-1} soil dry weight (DW) (44 %) and 57 μg C g^{-1} DW (80 %) in -Ndr compared to + Ndr, respectively. +Ndr significantly increased EOC contents during drought by on average 26 μg C g^{-1} DW (36 %). Neither drought nor the N-fertilization treatments affected EOC contents measured 1 and 2 months after rewetting significantly. However, drought effects on EOC differed among -Nrec and +Nrec 1 month after rewetting, with drought positively affecting EOC in +Nrec (significant drought:Nrec interaction, $P < 0.01$) and showing inconsistent effects on EOC in -Nrec (significant drought:Ndr:Nrec interaction, $P < 0.05$).

Drought and rewetting significantly increased gross N mineralization rates and NH_4-immobilization rates measured 4–12 h after soil sampled at the end of experimental drought was rewetted in the lab (both $P < 0.001$) (Fig. 5, Table S8). This effect was almost doubled in +Ndr compared to -Ndr ($P < 0.5$), resulting in a rewetting-induced increase in gross N mineralization of on average 1.1 μg N g^{-1} d^{-1} (235 %) in -Ndr and 1.9 μg N g^{-1} d^{-1} (435 %) in +Ndr, and in an increase in NH_4 consumption of on average 1.5 μg N g^{-1} d^{-1} (299 %) and 2.3 μg N g^{-1} d^{-1} (476 %) in -Ndr and +Ndr, respectively. However, the positive effect of Ndr on the rewetting-induced increase of gross N mineralization and NH_4 consumption was less pronounced in +Nrec compared to -Nrec (significant drought:Ndr:Nrec interaction, $P < 0.05$). One and two months after rewetting, neither drought nor N-fertilization significantly affected gross N mineralization or NH_4 consumption. However, 2 months after rewetting, significantly distinct interaction effects of Ndr and drought on gross N mineralization and NH_4 consumption in -Nrec compared to + Nrec were observed.

Drought and rewetting reduced potential activities of microbial extracellular enzymes related to the N- and C-cycle one month after rewetting. Specifically, drought and rewetting reduced potential leucine-aminopeptidase and exochitinase activity by on average 11 nmol h^{-1} g^{-1} (−6 %) ($P < 0.1$) and 30 nmol h^{-1} g^{-1} (−22 %) ($P < 0.01$), respectively, while β-glucosidase (C cycle) and phosphatase (P cycle) activities were not significantly affected by drought one month after rewetting (Fig. 6, Table S9). However, 2 months after rewetting, β-glucosidase and phosphatase activities were significantly increased by previous drought, showing on average 22 nmol h^{-1} g^{-1} (20 %) increased β-glucosidase and 62 nmol h^{-1} g^{-1} (17 %) increased phosphatase activities in formerly drought-stressed subplots compared to controls. Ndr marginally increased leucine-aminopeptidase activity 1 month after rewetting by on average 14 nmol h^{-1} g^{-1} (8 %) ($P < 0.1$) and significantly increased positive effects of previous drought on β-glucosidase activity 2 months after rewetting ($P < 0.05$). Apart from that, enzyme activities were not significantly affected by any of the N-fertilization treatments.

Aboveground productivity of perennial ryegrass increased linearly with increasing NO_3–N availability in the 1st month after rewetting including both years of measurement ($P < 0.001$, $R^2c = 0.68$, Fig. 7).

Gross N mineralization rates measured 4–12 h after soil sampled at the end of experimental drought was rewetted in the lab responded linearly to EOC contents at drought end ($P < 0.001$, $R^2c = 0.43$, Fig. 8).

4. Discussion

This study assessed important belowground microbial and nutrient cycling processes with the goal to explain post-drought productivity outperformance in perennial grasslands. Our experiment revealed that grasses formerly exposed to a 2-month experimental summer drought were N- but not P- or K-limited. The grassland profited from a rewetting-induced short-term pulse in NO_3–N availability, resulting in productivity outperformance once drought stress was released. These findings were consistent over two years. We observed an immediate, but short-term pulse in gross N-mineralization and NH_4 consumption when drought-treated soil was rewetted, suggesting that the observed pulse in NO_3–N availability was likely driven by increased mineralization and nitrification once drought stress was released. The positive relationship between gross N mineralization rates and extractable soil organic carbon (EOC) contents at drought end suggests that the post-drought increase in N availability is driven by increased mineralization of organic substrates accumulated during drought. Positive N-fertilization effects as well as a significant interaction with drought stress on EOC during drought imply that the drought-induced increase in EOC and thus the post-drought pulse in NO_3–N availability is at least partially driven by increased root exudation. However, concurrently increased availability of nutrients not directly dependent on mineralization processes (K, Fe, Zn, and Mn) also imply that other processes next to the accumulation of organic

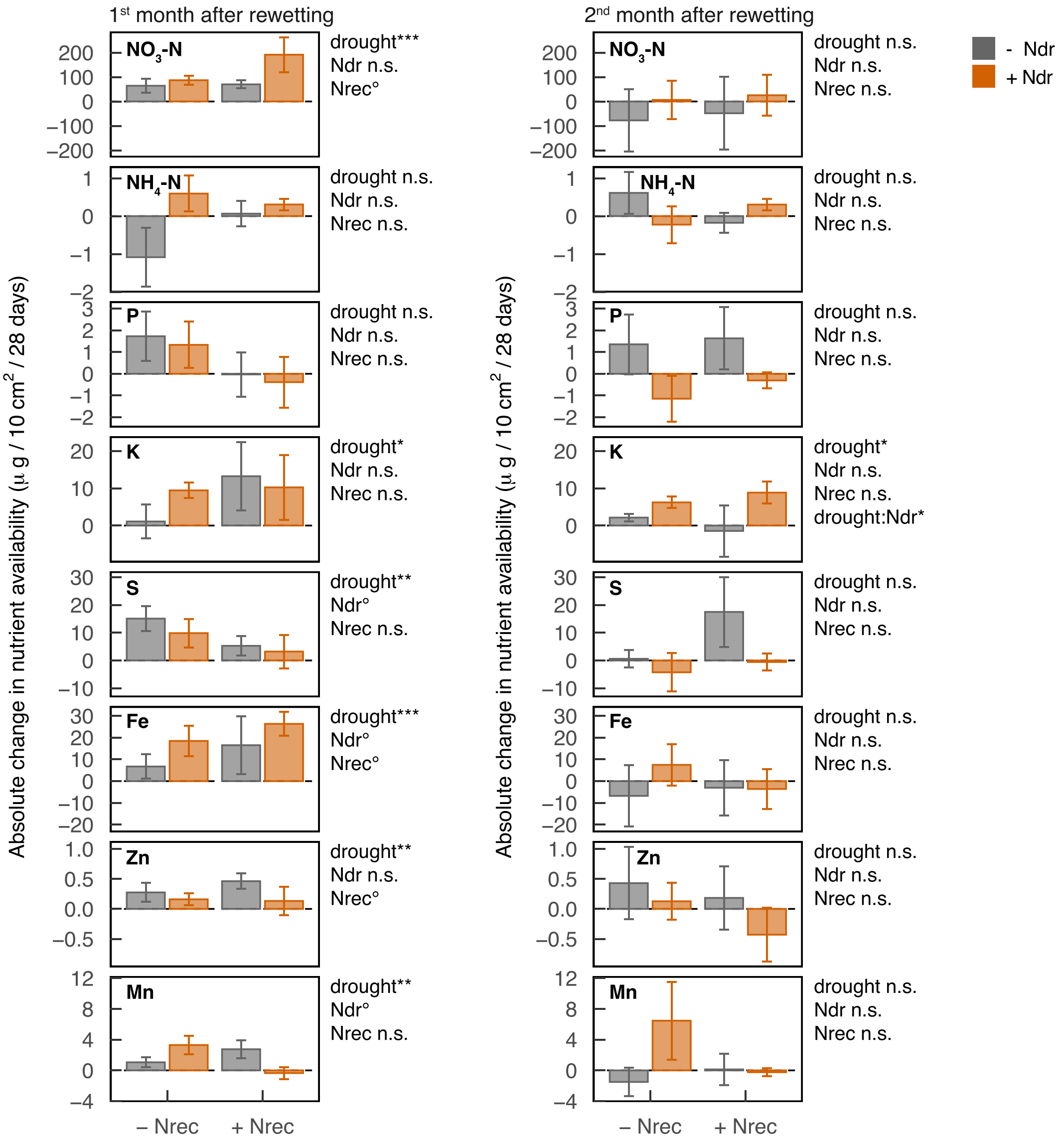

Fig. 3. Absolute changes in nutrient availabilities due to drought and rewetting (means ± SE, n = 4) in formerly drought-stressed subplots compared to controls during the 1st and the 2nd month after rewetting in 2019. Nutrient availabilities were measured with plant root simulator (PRS®) probes. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. Statistical analysis was performed on the raw data (Figs. S4–7). For better readability, only statistically significant treatment interactions are displayed (°$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). For more statistical information see Table S6. Results for 2020 can be found in Figs. S4–7 and Table S6.

substrates during drought increased short-term post-drought nutrient availability.

Yet, the initial rewetting-induced pulse in N mineralization and NH$_4$ consumption was only short-lived, as no or negative drought-legacy effects were observed on gross N mineralization, NH$_4$ consumption, and potential enzyme activities around 1 month after rewetting. Also, drought and rewetting had no legacy effects on nutrient availabilities in the 2nd month after rewetting, except for persistently increased K availabilities in formerly drought-stressed subplots compared to controls. Nevertheless, plant N and P uptake was still higher in previously drought-stressed plants compared to controls. This indicates that previously drought-stressed grasses took up nutrients more efficiently, which improved their nutrition and explains longer-term productivity outperformance.

4.1. *Rewetting-induced pulse in NO$_3$–N availability explains short-term post-drought productivity outperformance*

Drought and rewetting resulted in productivity outperformance of formerly drought-stressed grasses compared to controls during the 2-month post-drought period assessed, regardless of whether plots were N-fertilized during drought or not. The magnitude of the outperformance effect (45–97 %) was in line with observations of previous studies reporting 30–90 % increased aboveground productivity of formerly drought-stressed perennial grasslands compared to controls (Hahn et al., 2021; Hofer et al., 2016, 2017). Prior N-fertilization during drought significantly increased post-drought productivity outperformance, which suggests that processes leading to higher post-drought productivity of formerly drought-stressed swards are related to plant N nutrition. These effects were consistent among the two study years (Fig. S2, Table S4). However, the productivity outperformance after rewetting did not compensate for productivity losses

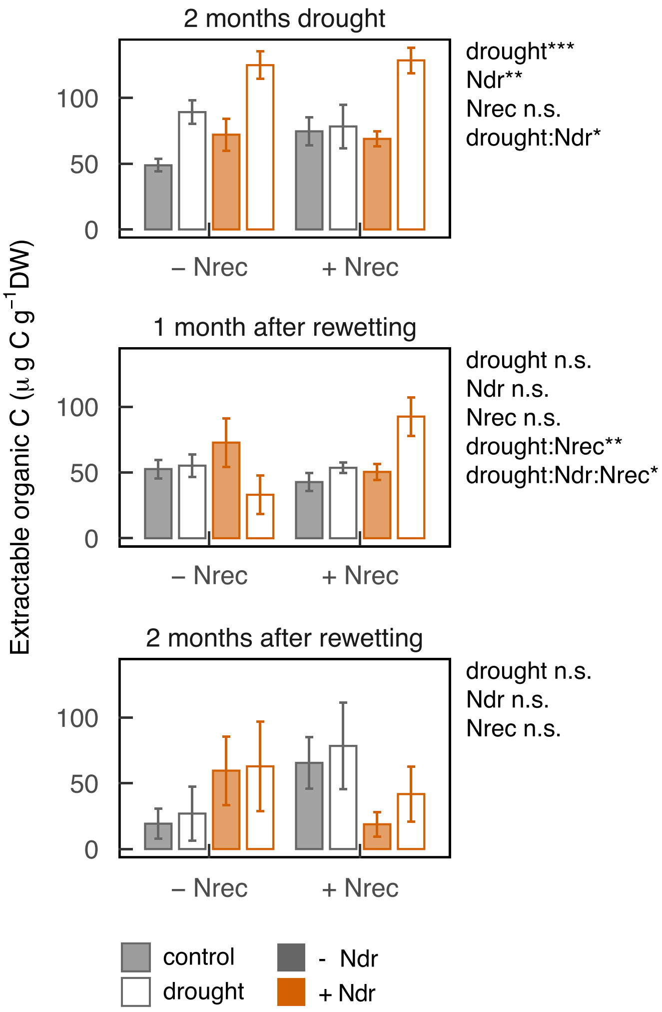

Fig. 4. Extractable organic carbon contents in the soil (means ± SE, n = 4) on a soil dry weight (DW) basis in rainfed control subplots (filled bars) and drought-stressed subplots (empty bars) at the end of experimental drought, and 1 and 2 months after rewetting in 2019. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. For better readability, only statistically significant treatment interactions are displayed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). For more statistical information see Table S7.

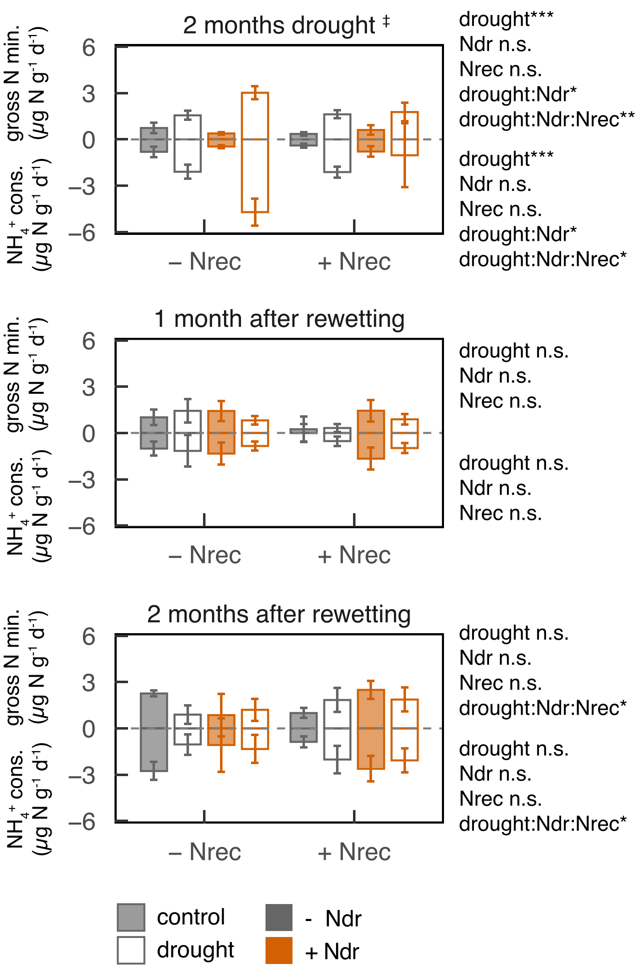

Fig. 5. Short-term to longer-term rewetting effects on gross N mineralization rates and NH_4 consumption rates (means ± SE, n = 4) in rainfed control subplots (filled bars) and drought-stressed subplots (empty bars) measured 4–12 h after soil sampled at the end of experimental drought was rewetted in the lab (‡), as well as 1 month and 2 months after rewetting in 2019. Gross N mineralization and NH_4 consumption rates are displayed on the same y-axis. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. For better readability, only statistically significant treatment interactions are displayed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). For more statistical information see Table S8.

during drought (Fig. S11, Table S4).

Concurrently, we observed a significant rewetting-induced pulse in NO_3–N, K, S, Fe, Zn, and Mn availability during the 1st month after rewetting in 2019, of which effects on NO_3–N, K, and Mn availability were consistent in 2020 irrespective of the N-fertilization treatment (Figs. S4–5 & S7, Table S6). Also, we observed a tendency towards a similar effect on P availability in 2019, which was significant in 2020 (Fig. S5, Table S6). Positive drought and rewetting effects on nutrient availability have been reported before (e.g. in Hofer et al. (2017); Bünemann et al. (2013); Sundert et al. (2020); Johnston et al. (2014); Haynes and Swift (1991)). However, since the mobilization pathways of the abovementioned nutrients are very diverse, a rewetting-induced increase in their availability suggests the involvement of different biotic and abiotic processes which are discussed below.

Plants in our experiment profited from the rewetting-induced nutrient pulse. Bailey et al. (2000) proposed critical leaf nutrient contents under which a nutrient would limit the productivity of field-grown perennial ryegrass, which are 28, 2.5 and 20 mg g^{-1} N, P and K, respectively. Based on these values, the leaf N, P and K contents in our experiment (Figs. S8–10) suggest that control and drought subplots of all N-fertilization treatments were N limited during drought and the 1st month after rewetting but not P- or K-limited. Hence, plants likely profited from the increased post-drought NO_3–N availability. A significant positive relationship of aboveground productivity with NO_3–N availability during the 1st month after rewetting (Fig. 7) corroborates this finding and suggests that increased post-drought NO_3–N availability explains the productivity outperformance of formerly drought-stressed grasses observed in the 1st month after rewetting.

A drought-induce increase in root biomass, as previously observed by (Arndal et al., 2013, 2018; Hofer et al., 2017), could have also promoted nutrient uptake and, consequently, post-drought productivity of formerly drought stressed grasses. However, drought effects on root growth and morphology in grasslands vary widely (Byrne et al., 2013; Denton et al., 2017; Hahn et al., 2021; Vries et al., 2016). In a parallel study, we were able to separate plant from soil effects and could demonstrate clearly, that processes in the soil determine post-drought

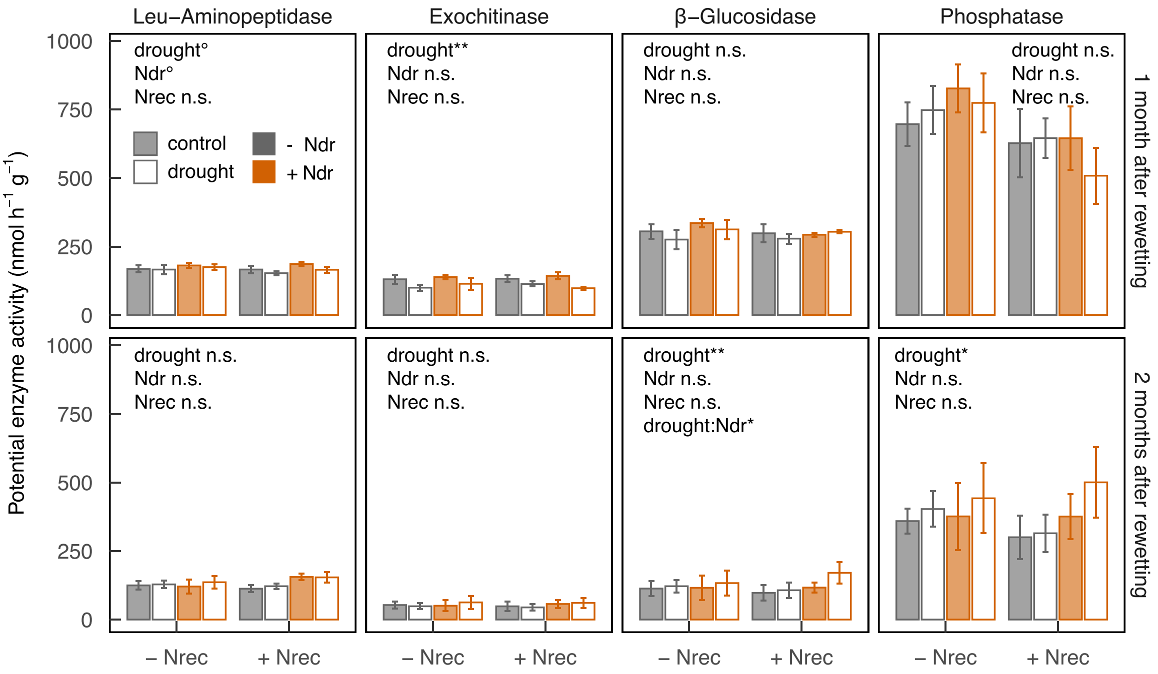

Fig. 6. Potential extracellular microbial enzyme activity rates (means ± SE, n = 4) in rainfed control (filled bars) and formerly drought-stressed and rewetted subplots (empty bars) 1 month and 2 months after rewetting in 2019. Plots priorly not N-fertilized during drought (-Ndr) and N-fertilized during drought (+Ndr) are indicated in gray and orange, respectively. N-fertilization at the beginning of recovery is indicated along the x-axis with plots not N-fertilized (-Nrec) and plots N-fertilized (+Nrec) upon rewetting. For better readability, only statistically significant treatment interactions are displayed (°$P < 0.1$, *$P < 0.05$, **$P < 0.01$). For more statistical information see Table S9.

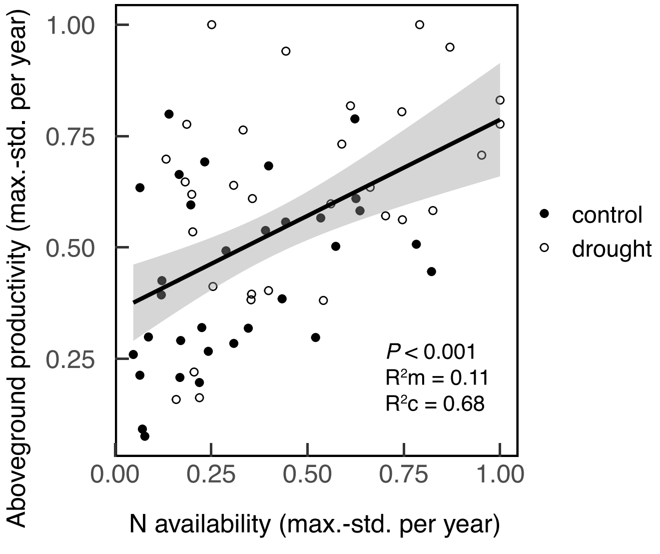

Fig. 7. Relationship of aboveground productivity 1 month after rewetting and NO_3–N availability in the 1st month after rewetting measured in 2019 and 2020 and maximum-standardized over the years. Statistical information represents the summary of a mixed model regression analysis.

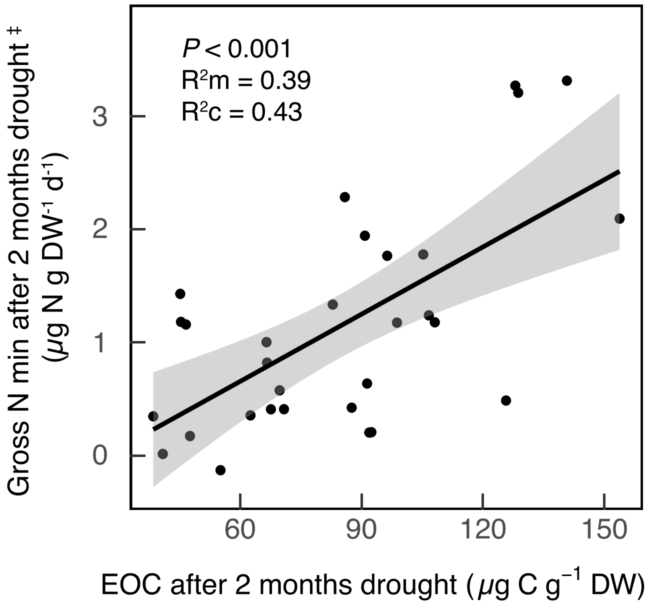

Fig. 8. Relationship between gross N mineralization measured 4–12 h after soil sampled at the end of experimental drought was rewetted in the lab (‡) and extractable organic carbon (EOC) contents at drought end in 2019. Statistical information represents the summary of a mixed model regression analysis.

productivity of formerly drought stressed grasses (Schärer et al., 2023). In fact, drought and rewetting caused negative plant intrinsic drought legacy effects in perennial ryegrass which diminished positive soil drought legacy effects on productivity outperformance irrespective of fertilization. We therefore suggest that drought effects on root growth or morphology may have played only a minor and/or counteracting role in the observed post-drought outperformance in nutrient uptake and productivity.

4.2. *Accumulated organic substrates during drought led to a short-term pulse in N-mineralization and NO_3–N availability after rewetting*

Rewetting after a 2-month severe summer drought led to an immediate pulse in gross N mineralization within the first 12 h after the rewetting event. This is in line with studies reporting short-term increases in mineralization and microbial activity in response to drought and rewetting (Birch, 1958a,b, 1964; Borken and Matzner, 2009). However, the increase in gross N mineralization did not result in increased post-drought NH_4–N availability. Instead, a pulse in NO_3–N availability during the 1st recovery regrowth was observed, likely because the pulse in gross N mineralization was accompanied by a concurrent increase in NH_4 consumption rates in formerly drought-stressed subplots due to enhanced nitrification (Braun et al., 2018). Importantly, the magnitude of the pulse in gross N-mineralization observed immediately after rewetting correlated positively with soil EOC contents at the end of drought. This implies that the pulse in N mineralization observed immediately after rewetting was mainly fueled by organic substrates accumulated during drought, either through the mineralization of labile substrates, or by stimulation of soil organic matter mineralization via a priming effect (defined as the increase in decomposition after labile organic matter input (Bingeman et al., 1953; Fontaine et al., 2003)).

Drought-induced increases in EOC have been reported before (Canarini et al., 2017; Deng et al., 2021; Fuchslueger et al., 2014) and were mainly attributed to a lower drought-sensitivity of microbial enzymatic activity compared to microbial uptake (Canarini et al., 2017; Fuchslueger et al., 2014; Steinweg et al., 2013), or to reduced leaching of dissolved organic carbon pools into deeper soil layers (Deng et al., 2021). Just recently, however, studies revealed increased amounts of recently assimilated C in soil organic C pools during drought (Fuchslueger et al., 2014; Karlowsky et al., 2018; Ulrich et al., 2022) as well as in the respiration pulse observed upon rewetting (Ingrisch et al., 2018) and suggest that increased plant rhizodeposition under drought including root exudation might play an important role in the response of organic C pools and thus microbial activity upon rewetting (Grayston et al., 1997; Gunina and Kuzyakov, 2015; Ingrisch et al., 2018; Karlowsky et al., 2018).

Also, N-fertilization was reported to increase root exudation and EOC contents (Bowen, 1969; Embacher et al., 2008; Sun et al., 2015; Zhu et al., 2016). Since both drought and N fertilization increased EOC (along with aboveground productivity, Fig. S11) at the end of drought in our study, and N fertilization during drought amplified the positive effects of drought on EOC, we hypothesize that the accumulated EOC during drought at least partially results from persistent or increased root exudation. Here we provide empirical evidence that the rewetting-induced pulse in NO_3–N availability during the 1st recovery regrowth is related to a drought-induced accumulation of organic substrates. Concurrently increased availability of other inorganic nutrients which can originate from soil organic matter (S, and P in 2020) (Paul and Clark, 1989) in formerly drought-stressed subplots compared to controls further indicate a possible priming effect caused by the accumulation of extractable organic matter (Gunina and Kuzyakov, 2015). However, direct quantifications are necessary to confirm this.

One month after rewetting, neither drought nor rewetting still had a detectable effect on gross N mineralization or NH_4 consumption. Potential extracellular enzyme activities related to the C, N, and P cycle were either not (β-glucosidase & phosphatase) or negatively (leucine-aminopeptidase & exochitinase) affected by drought and rewetting one month after rewetting. Furthermore, the effects of drought and rewetting on NO_3–N and S (and P availability in 2020, Fig. S5) were no longer apparent in the 2nd month after rewetting. This supports previous findings that rewetting-induced increases in microbial activity are short-lived (Borken and Matzner, 2009; Cabrera, 1993; Franzluebbers et al., 1996; Song et al., 2017). Moreover, our results suggest that the immediate post-drought productivity outperformance of previously drought-stressed plants could be explained by the accumulation of organic substrates during drought which fueled N-mineralization and thus N availability upon rewetting.

4.3. *Post-drought pulse in the availability of non-mineralized nutrients suggests the involvement of further abiotic and biotic drought- and rewetting-induced processes*

We observed increased availability of mineral nutrients which do not depend directly on microbial mineralization processes, such as K (in both recovery regrowths), Fe, Mn and Zn (in the 1st month after rewetting) (Paul and Clark, 1989). Drought- and rewetting-induced increases in K availability have been reported before (e.g. Sundert et al. (2020); Venterink et al. (2002); Zhang et al. (2022)) and were mainly attributed to a rewetting-induced microbial release of K during osmoregulation or cell lysis, or to a rewetting-induced increase in K-solubilization by K-solubilizing microbes (Kieft et al., 1987; Sundert et al., 2020; Unger et al., 2010). Also increased Fe, Mn and Zn availabilities following drought and rewetting were reported before and related to increased oxidation during drought (Nelson, 1977; Bartlett and James, 1980; Haynes and Swift, 1991). However, recently Sardans et al. (2023) suggested that also drought-induced increases in plant exudation (Karlowsky et al., 2018; Ulrich et al., 2022) could lead to increased mobilization and availability of K and Fe in the soil. A significant correlation between Fe availability during the 1st recovery regrowth and EOC levels at drought end (Table S10) could point towards such a chelation-driven Fe-mobilization. For K, however, such a relation was not found (Table S10). Hence, the processes responsible for the observed increase in K and metal availability cannot be conclusively determined. Also, Fe and Zn availabilities showed diverging responses to drought and rewetting in 2020 compared to 2019 (Figs. S6–7, Table S6), indicating that processes determining post-drought metal availability might depend on factors we did not assess.

4.4. *Drought and rewetting improved longer-term plant nutrient uptake efficiency which affected belowground nutrient turnover*

During the 2nd month after rewetting, effects of drought and subsequent rewetting on nutrient availabilities were no longer observable except for a persistently increased K availability. Nonetheless, formerly drought-stressed grasses still showed higher aboveground productivity compared to rainfed controls. Despite similar N and P availabilities in controls and formerly drought-stressed subplots during the 2nd month after rewetting, formerly drought-stressed plants showed increased N and P uptake, suggesting that these plants were able to take up N and P more efficiently compared to controls (Moll et al., 1982). Plant nutrient uptake efficiency can be influenced by root architecture and anatomy (Griffiths and York, 2020) and is positively related to prior plant nutritional status (Schärer et al., 2023). While effects of drought on root growth seem to be diverse (Arndal et al., 2013, 2018; Byrne et al., 2013; Denton et al., 2017; Hahn et al., 2021; Hofer et al., 2017; Vries et al., 2016), rewetting drought-stressed grasses increased root growth in previous studies (Fuchslueger et al., 2016; Newton et al., 1996; Wedderburn et al., 2010). We therefore suggest that the post-drought pulse in nutrient availability increased not only aboveground but also belowground plant productivity, thereby increasing the efficiency for plant nutrient uptake. This likely explained longer-term productivity outperformance even after drought- and rewetting-induced legacy effects on nutrient availability had ceased.

Further, we observed small but significant positive longer-term drought-legacy effects on potential β-glucosidase and phosphatase activities and a tendency towards increased leucine-aminopeptidase rates 2 months after rewetting. During the 2nd month after rewetting, previously drought-stressed subplots showed increased plant N and P uptake compared to controls, despite similar N and P availabilities across treatments. This could have enforced plant-microbial competition for nutrients, potentially driving increased microbial N and P acquisition activities in the drought-stressed subplots. At the same time net N mineralization rates were still significantly increased in formerly drought-stressed subplots compared to rainfed controls (Fig. S12), while gross N mineralization rates were unaffected two months after rewetting, suggesting that microbial N immobilization decreased. Nevertheless, positive drought-legacy effects on the potential activities of enzymes related to the C, N, and P cycle could indicate that drought and subsequent rewetting resulted in persisting effects on belowground nutrient cycling.

In summary, we observed that grasses exposed to a 2-month experimental summer drought and subsequent rewetting profited from an increased NO_3–N availability during the 1st month after rewetting, leading to post-drought productivity outperformance of formerly drought-stressed grasses compared to rainfed controls. The increase in NO_3–N availability resulted from an immediate, rewetting-induced pulse in N-mineralization of organic substrates accumulated during drought, which likely partially originate from plant rhizodeposition. The post-drought pulse in nutrient availability persistently increased nutrient uptake efficiency of formerly drought-stressed grasses compared to controls, which explained longer-term productivity outperformance of perennial ryegrass even when the rewetting induced pulse in nutrient availability had already ceased. Our findings imply that drought- and rewetting-induced processes can lead to persisting effects on plant performance and productivity that might affect future responses to recurrent drought events.

CRediT authorship contribution statement

Marie-Louise Schärer: Writing – original draft, Visualization, Project administration, Investigation, Formal analysis, Conceptualization. **Lucia Fuchslueger:** Writing – review & editing, Validation. **Alberto Canarini:** Writing – review & editing, Validation. **Andreas Richter:** Writing – review & editing, Resources. **Andreas Lüscher:** Writing – review & editing, Supervision, Resources, Conceptualization. **Ansgar Kahmen:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109744.

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