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Research

Post-drought compensatory growth in perennial grasslands is determined by legacy effects of the soil and not by plants

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Summary

- Grasslands recovering from drought have repeatedly been shown to outperform non-drought-stressed grasslands in biomass production. The mechanisms that lead to the unexpectedly high biomass production in grasslands recovering from drought are, however, not understood.
- To disentangle plant-intrinsic and plant-extrinsic (soil) drought legacy effects on grassland recovery from drought, we designed a factorial field experiment where *Lolium perenne* plants that were exposed to either a 2-month drought or to well-watered control conditions were transplanted into control and drought-stressed soil and rewetted thereafter.
- Drought and rewetting (DRW) resulted in negative drought legacy effects of formerly drought-stressed plants (DRW_p) compared with control plants (Ctr_p) when decoupled from soil-mediated DRW effects, with DRW_p showing less aboveground productivity (−13%), restricted N nutrition, and higher $\delta^{13}C$ compared with Ctr_p. However, plants grown on formerly drought-stressed soil (DRW_s) showed enhanced aboveground productivity (+82%), improved N nutrition, and higher $\delta^{13}C$ values relative to plants grown on control soil (Ctr_s), irrespective of the plants' pretreatment.
- Our study shows that the higher post-drought productivity of perennial grasslands recovering from drought relative to non-drought-stressed controls is induced by soil-mediated DRW legacy effects which improve plant N nutrition and photosynthetic capacity and that these effects countervail negative plant-intrinsic drought legacy effects.

Introduction

Drought stress is an abiotic disturbance, which has major impacts on plants and ecosystems. While most studies are addressing immediate drought effects (i.e. drought resistance), mechanisms explaining how and why plants recover from drought (i.e. the capacity of a plant or ecosystem to return to an undisturbed condition (Hodgson *et al.*, 2015)) are rarely addressed (Ingrisch & Bahn, 2018; Gessler *et al.*, 2020; Zhou *et al.*, 2022).

Grasslands are ecosystems that have a particularly high capacity to recover from drought (Hofer *et al.*, 2016; Kreyling *et al.*, 2017; Karlowsky *et al.*, 2018; Wilcox *et al.*, 2020). In contrast to trees in which severe hydraulic stress often leads to irreparable loss of conductivity (Tyree & Sperry, 1989; Arend *et al.*, 2016), grasses are able to abandon their aboveground tissues and endure belowground by relying on dehydration-tolerant meristematic root and shoot tissue (Volaire *et al.*, 2014, 2020; Hasibeder *et al.*, 2015). Grasses therefore do not depend on a tall and hydraulically intact structural aboveground biomass to recover from drought. They can quickly restore their canopy by the production of new tissue even when all aboveground biomass was lost during drought (Hofer *et al.*, 2016; Kreyling *et al.*, 2017; Karlowsky *et al.*, 2018; Mackie *et al.*, 2018; Stuart-Haëntjens *et al.*, 2018; Hahn *et al.*, 2021). There is accumulating evidence that the biomass production of formerly drought-stressed grasslands can even outperform nonstressed controls once drought stress is released (Hofer *et al.*, 2016; Kreyling *et al.*, 2017; Ingrisch *et al.*, 2018, 2023; Volaire *et al.*, 2020; Hahn *et al.*, 2021; Ru *et al.*, 2023).

Post-drought compensatory growth patterns (i.e. increased aboveground productivity of formerly drought-stressed grasslands compared with nonstressed controls after rewetting) have been reported for a range of different grassland ecosystems (Matos *et al.*, 2020; Zhou *et al.*, 2022). While an increase in post-drought plant growth *prima facie* suggests that there are no negative drought legacy effects on the plant level, this in turn conflicts with the fact that the maintenance of intact meristematic tissue during drought as well as the re-establishment of abandoned tissue after drought require a substantial investment of resources (Volaire *et al.*, 2018, 2020; Guo *et al.*, 2020). Hence, it is likely that negative drought legacy effects are countervailed by plant-intrinsic or plant-extrinsic drought- and rewetting-induced processes, which promote plant growth and lead to net growth increase once drought stress is released.

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Several intrinsic or extrinsic mechanisms have been proposed as explanations for post-drought compensatory regrowth patterns in grasslands: Intrinsic processes (1) could be related to grasses filling up reserve pools during drought before photosynthesis ceases (by preferably investing energy into storage rather than growth), enabling fast regrowth when drought stress is released (Muller *et al.*, 2011; Hasibeder *et al.*, 2015; Hofer *et al.*, 2017a; Karlowsky *et al.*, 2018; Guo *et al.*, 2020). It has also been suggested that grasses could follow an internally controlled seasonal growth cycle as shown in Starr *et al.* (2001), Rosa *et al.* (2015), and Möhl *et al.* (2022), which is interrupted by drought and completed by compensatory physiological activity and increased growth after rewetting (Arend *et al.*, 2016; Schwieger *et al.*, 2022). Extrinsic processes (2) have been related to soil rewetting, which causes a pulse in soil nutrient availability facilitating plant growth after drought release (Jarvis *et al.*, 2007; Borken & Matzner, 2009; Hofer *et al.*, 2016; Ingrisch *et al.*, 2018; Sundert *et al.*, 2020) and/or the accumulation of fertilizers, in particular nitrogen, in managed grasslands during a drought (Hahn *et al.*, 2021). The role, amplitude, and longevity of such intrinsic and extrinsic drought legacy effects in contributing to post-drought plant recovery and compensatory growth has, however, not yet been specifically examined, leaving the physiological and biogeochemical mechanisms that drive post-drought recovery of grasslands poorly understood.

In this study, we designed an experiment where we treated a perennial ryegrass (*Lolium perenne*) grassland with a severe summer drought and transplanted drought-stressed and control plants (Ctr_p) into control and drought-stressed soil that we rewetted thereafter. This design enabled us to (1) disentangle intrinsic processes in the plant from extrinsic processes in the soil as drivers of post-drought recovery and compensatory growth in grasslands, (2) identify the biochemical and physiological mechanisms that lead to net-positive or net-negative intrinsic or extrinsic drought legacy effects on plant productivity, and (3) test whether N-fertilization during drought affects intrinsic and/or extrinsic drought legacy effects on post-drought grassland recovery.

Materials and Methods

Field experiment

Setup of the drought experiment in the field As a first step, we set up a field trial where an established grassland was subjected to severe summer drought. The experiment was situated nearby the Agroscope Institute Zurich-Reckenholz, Switzerland (47°26′16″N, 8°31′39″E, 487 m above sea level). The soil at the site was a moderate-profound clay soil with a top-soil layer consisting of 21% clay, 39% silt, and 38% sand. The soil had a pH of 6.6 and contained 2.2% humus, 59 mg kg^{-1} phosphorus, 21 mg kg^{-1} potassium, and 142 mg kg^{-1} magnesium. Mean annual temperature and precipitation of the region in the reference period 1991–2020 were 9.9°C and 1018 mm, respectively.

The field trial was set up in 2018 as a pure perennial ryegrass (*Lolium perenne* L., cultivar 'Allodia') sward to exclude any effects of drought-induced shifts in species abundance. The sward had a sowing density of 0.25 g m^{-2} and a seed row distance of 13.5 cm. Perennial ryegrass was chosen as a model species for the experiment since it is a well-studied and the most important species in temperate forage grasslands (Morvan-Bertrand *et al.*, 2001; Amiard *et al.*, 2004; Lee *et al.*, 2010; Turner *et al.*, 2012; Guo *et al.*, 2020). The field experiment was arranged in a complete block design with two factorial treatments (experimental summer drought and N-fertilization during drought). The field was split up into four replicates which were divided into four randomized blocks each, of which two blocks (per replicate) were exposed to the same N-fertilization treatment. Each block consisted of two plots (3 × 5 m each): one well-watered control plot and one plot experiencing experimental summer drought in the 2020 growing season. Plots were cut six times during the 2019 growing season and four times until the end of the experimental drought in mid-August 2020. Total annual yield was 1384 g dry matter (DM) m^{-2} in 2019 and 647 g DM m^{-2} until the 4th cut in mid-August 2020 in nonstressed control plots. Before the 2020 growing season, all plots grew under rainfed control conditions and were fertilized with 240 kg N ha^{-1} ammonium nitrate per year.

Drought and N-fertilization treatments before transplantation To induce drought in the field, we set up a 2-month rain exclusion experiment (1st factor with two levels: control, drought) in summer 2020. Rain-out shelters excluding 100% precipitation were installed from the beginning of June 2020 to mid-August 2020 (10 wk in total). These 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 and translucent plastic foil ('Gewächshausfolie Lumisol clear AF' from Hortuna AG). For more technical information on the shelter design, see Hofer *et al.* (2016). During the entire drought period, the shelters excluded 227 mm rain which equals 27% of the annual precipitation in 2020 and 43% of the precipitation between January 2020 and monolith excavation on 8 August 2020 (Supporting Information Fig. S1a). This led to a 1.1 MPa lower soil water potential in drought-stressed compared with control field plots at the end of experimental drought (Fig. S1b) and resulted in a total reduction in aboveground productivity of 76% and 53% for N-fertilized and non-N-fertilized stress plots compared with controls, respectively, during the 2-month drought period including two cuts (Fig. S1c).

To assess whether different N availabilities during drought affected plant-intrinsic and plant-extrinsic drought legacy effects on plant recovery after rewetting, we induced a N-fertilizer treatment during experimental drought. Half of the control and drought-stressed plots were N-fertilized with 35 or 47.5 kg N ha^{-1} at the start (3 June) and in the middle of the experimental drought period (7 July, after 5 weeks of drought), while the other half of the plots remained unfertilized. The distinct amount of N-fertilizer applied originated from two N-fertilization treatments which were pooled for this experiment (for detailed information on N-fertilization treatments, see Table S1). Until 1 month before drought, all plots received 90–115 kg N ha^{-1} in three N-fertilization doses. N-fertilizer was

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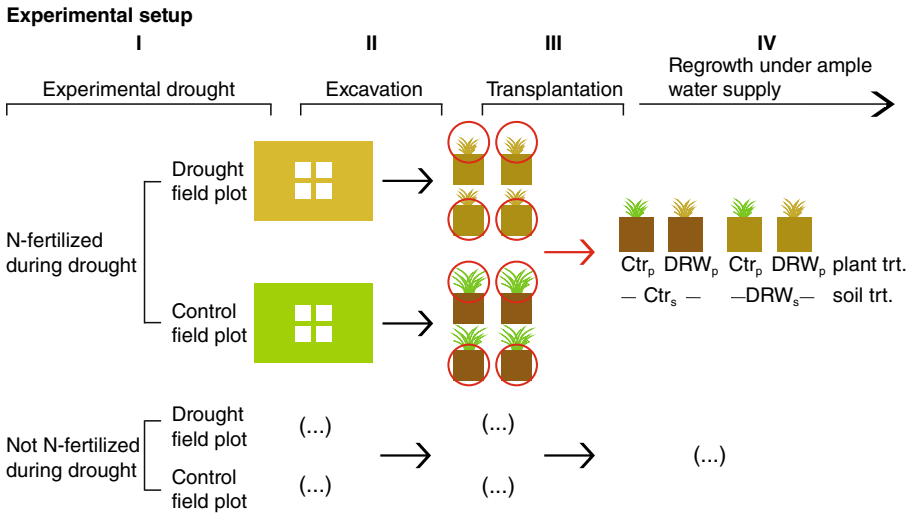

Fig. 1 Conceptual figure showing the setup of the transplantation experiment. Field plots were either N-fertilized or not N-fertilized during experimental drought. As indicated by '(…)', experimental drought, excavation, transplantation, and regrowth under ample water supply were equally performed in plots not N-fertilized as in N-fertilized plots. Red circles indicate the monolith part used for transplantation (plants or soil). Transplantation led to the following soil and plant combinations: control plants (Ctr_p) and formerly drought-stressed plants (DRW_p) either grown on formerly drought-stressed soil (DRW_s) or on control soil (Ctr_s).

applied in the form of ammonium nitrate (27% N). Since the drought and N-fertilization treatments were applied before transplantation (i.e. before the start of the pot experiment), they are further referred to as 'prior treatments'.

Plant N availability measured in the field To assess plant N availability in the different treatments, we installed two cation and two anion PRS® probes per field plot at 5 cm soil depth and for two consecutive periods of 14 d per regrowth (for exact installation and retrieval dates see Table S2). PRS® probes were installed in a central plot strip (1.5 × 5 m) and with a minimum distance of 75 cm from each plot side to minimize the effects of lateral water flow into the experimental drought plot. PRS® consist of a 10 cm^2 ion-exchange membrane fixed in a PE frame. Since plant roots partially compete with PRS® for nutrient uptake, we installed PRS® in plant exclusion cylinders to avoid potential confounding effects on N availability after drought (Huang & Schoenau, 2011). For this, one cylinder (Ø 25 cm, 20 cm height) out of rigid polyvinyl chloride (PVC-U) was installed in the center of each field plot on 28 July, that is 2 wk before drought release. Right after installation, aboveground plant material and major root material in the upper 5 cm soil layer were removed. After each installation period, PRS® were retrieved, carefully brushed, and rinsed with DI water to ensure full removal of soil residues. Probes of the same regrowth and field plot were pooled and stored in clean zip-lock bags at 4°C until shipping to Western AG Innovations Inc. for analysis.

Main transplantation experiment

Setup of the transplantation experiment Just before drought release in August 2020 and one day after field swards were cut at 7 cm height using an experimental plot harvester (Hege 212; Wintersteiger AG, Ried im Innkreis, Austria), we excavated four monoliths (20 × 20 × 20 cm each) per plot from 16 control and 16 drought plots at the field site in total. Monoliths were excavated from a central plot strip (1.5 × 5 m) and with a minimum distance of 75 cm from each plot side to exclude mitigating effects of potential lateral water flow on the drought treatment. Each monolith contained two 20 cm long rows of grass plants. From the 16 control and 16 drought field plots, one half was N-fertilized while the other half was not N-fertilized during drought, resulting in a replication of eight plots per factorial combination of prior drought and prior N-fertilization treatments. After excavation, the monoliths were placed separately in closed plastic bags for transportation to the laboratory to prevent excessive evaporative water loss. From the four collected monoliths per field plot, two were used only for soil and two only for plant excavation (Fig. 1). To excavate the perennial ryegrass plants from dry soil, we carefully loosened the soil by hand to avoid any rewetting effects before transplantation and then rinsed remaining soil particles off. To excavate plants from well-watered control soil (Ctr_s), we removed the soil from roots by washing them as well as possible while keeping the structure of the grass rows intact. To account for potentially differing root damage on formerly drought-stressed plants (DRW_p) and Ctr_p during the removal of soil from the roots, roots were cut and standardized to 4 cm length. For soil excavation, all plant material was removed and discarded, and the soil was homogenized using a 2 mm sieve. The sieved soil was transferred into pots (16 × 16 × 16 cm), and the excavated *L. perenne* plant rows were planted diagonally in the pot while maintaining the original row distance from the field. This resulted in a three-factorial transplantation experiment with eight treatments combining two levels of prior plant treatment (formerly control or drought-stressed plants) with two levels of prior soil treatment (formerly control or drought-stressed soils) and two levels of prior N-fertilization treatment (N-fertilized during drought or not N-fertilized during drought) with eight replicates (Fig. 1). After finishing the transplantation, perennial ryegrass plants in all treatments were re-trimmed to 7 cm leaf length to remove any aboveground biomass produced between the last field harvest and transplantation. After that, soils were rewetted with 3 × 100 ml tap water per pot in an interval of 30 min between each rewetting dose to minimize throughflow. Finally, the pots were put in the glasshouse and kept at day and night temperatures of 18°C and 10°C, respectively, during the 1st recovery regrowth, and at 12°C and

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6°C, respectively, during the 2nd recovery regrowth after drought, following the seasonal climate trends the plants would have experienced in the field. Pots were kept in a well-watered condition by irrigating them every 2–3 d with 3 × 100 ml tap water.

Plant physiological response variables To assess the impact of plant-intrinsic and plant-extrinsic (soil) drought legacy effects on aboveground productivity, the plants of each pot were cut manually at 7 cm height 1 and 2 months after rewetting. Aboveground productivity was determined after drying the collected leaves at 80°C for 48 h and weighing. Aboveground productivity is reported as g pot^{-1}.

To assess legacy effects of prior treatments on plant mineral nutrition, we measured leaf N content. For this, dried leaf material was ground into a fine powder with a horizontal ball mill (MM 400; Retsch, Haan, Germany). Then, 0.5–1 mg of the ground leaf material was transferred into 8 × 5 mm tin capsules (OEA Labs Ltd, Cornwall, UK) and loaded to a Flash 2000 elemental analyzer coupled to a DELTA Plus XP continuous-flow IRMS via a ConFlo IV interface (Thermo Fisher Scientific, Bremen, Germany). Leaf N content was determined by a thermal conductivity detector (TDC) coupled to the Flash 2000 elemental analyser. Leaf N content was divided by specific leaf area (Methods S1; Fig. S2) to obtain leaf N contents per area. Aboveground plant N uptake was calculated as the product of leaf N content and aboveground productivity.

Leaf bulk $\delta^{13}C$ values were determined as a time-integrating indicator for foliar gas exchange. $\delta^{13}C$ values are affected by stomatal conductance and carbon assimilation rates and are therefore a well-suited indicator for the intrinsic water use efficiency (iWUE), which is the ratio of net C assimilation over stomatal conductance (Farquhar *et al.*, 1982, 1989). To measure $\delta^{13}C$ values, the same dried and ground leaf bulk material was used for leaf N content analysis. Also, leaf bulk $\delta^{13}C$ was analyzed simultaneously with and on the same equipment as leaf N. $\delta^{13}C$ values were standardized relative to the Vienna Pee Dee Belemnite (VPDB) with an analytical precision of ±0.17‰. Isotopic measurements were conducted in the Stable Isotope Ecology Laboratory at the Department of Environmental Sciences of the University of Basel, Switzerland.

Statistical analyses

To statistically test the effect of drought and rewetting (DRW) on aboveground productivity, plant N availability rates, aboveground plant N uptake, leaf N contents, and leaf bulk $\delta^{13}C$, we applied a linear mixed model for each variable separately using the maximum likelihood (ML) approach with the NLME package (Pinheiro *et al.*, 2020). Prior soil treatment, prior plant treatment (except for plant N availability), prior N-fertilization treatment, and regrowth were defined as main factors for the fixed model term. To account for repeated measurements and the nested study design, replicate/block/pot were defined as random factors and considered for temporal and spatial correlation. Model simplification was performed by comparing complex and simplified models using the Akaike Information Criterion corrected for small sample sizes (AIC_c) calculated following Burnham & Anderson (2002). Based on these AIC_c values, the model containing all four main factors including their two-way interactions was chosen for all tested variables. To achieve homoscedasticity and normal distribution of residual variance, plant N availability data were natural log-transformed before analysis. Marginal and conditional R^2 of the models were extracted using the MUMIN package (Bartoń, 2022). All data processing, statistical analyses, and graphical visualizations were performed using the statistical software R v.4.0.2 (R Core Team, 2020).

Results

Drying and rewetting of ryegrass (further referred to as 'prior plant DRW treatment') significantly reduced aboveground productivity in all prior soil and N-fertilization treatments and in both recovery regrowths, resulting in an on average 0.21 g pot^{-1} (13%) lower aboveground productivity in DRW_p compared with Ctr_p ($P < 0.01$; Fig. 2; Table 1). Drying and rewetting of soil (further referred to as 'prior soil DRW treatment') significantly increased grass aboveground productivity in all prior plant and N-fertilization treatments and in both recovery regrowths ($P < 0.001$) with a greater effect in priorly N-fertilized compared with non-N-fertilized plants (significant prior soil treatment – prior N-fertilization interaction, $P < 0.001$). Specifically, prior soil DRW treatment increased aboveground productivity in the 1st recovery regrowth by on average 0.33 g pot^{-1} (18%) and 0.78 g pot^{-1} (51%) for plants priorly non-N-fertilized and N-fertilized, respectively. In the 2nd recovery regrowth, the effect of prior soil treatment on aboveground productivity was greater compared with the 1st recovery regrowth with DRW_s showing on average 0.34 g pot^{-1} (66%) and 1.29 g pot^{-1} (130%) higher aboveground productivity compared with Ctr_s in the non-N-fertilized and N-fertilized treatment, respectively (significant regrowth – prior soil treatment interaction $P < 0.05$). N-fertilization during drought affected aboveground productivity of all treatments positively ($P < 0.001$) and increased aboveground productivity by on average 76% and 26% in DRW_s and Ctr_s, respectively. Interestingly, the effect of prior N-fertilization on the recovery of aboveground productivity was slightly but not significantly higher during the 2nd compared with the 1st recovery regrowth. Overall, aboveground productivity of the 1st recovery regrowth after DRW was higher than mean aboveground productivity of the 2nd recovery regrowth after DRW ($P < 0.01$).

Plant N availability in formerly drought-stressed field soil (DRW_s) was significantly higher than plant N availability in control field soil (Ctr_s) in both N-fertilization treatments and during both recovery regrowths ($P < 0.01$; Fig. 3; Table 2). Specifically, prior soil DRW treatment increased plant N availability by 106 μg N/10 cm^2/28 d (85%) and 276 μg N/10 cm^2/28 d (169%) in non-N-fertilized and N-fertilized field plots, respectively, during the 1st recovery regrowth. The DRW-induced pulse in N availability persisted during the 2nd recovery regrowth, though at a much lower level of 53 μg N/10 cm^2/28 d (28%) and 34 μg N/10 cm^2/28 d (12%) higher N availability in DRW_s compared with Ctr_s in non-N-fertilized and N-fertilized field

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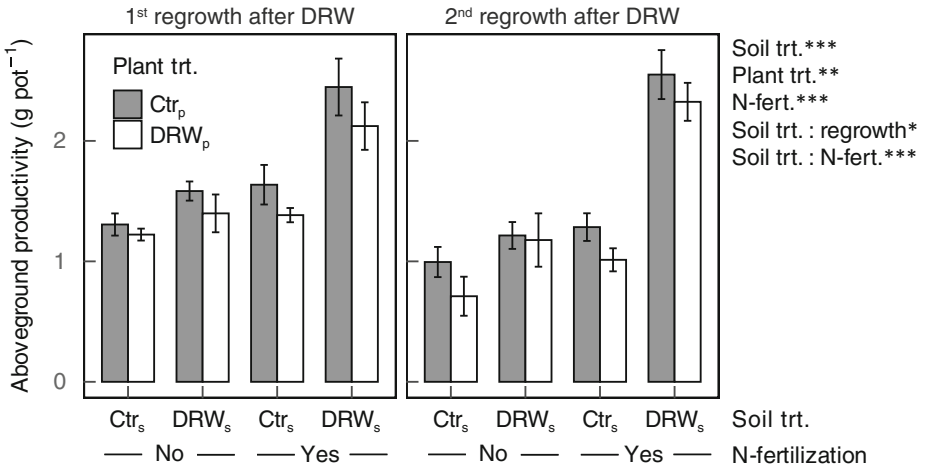

Fig. 2 Aboveground productivity of perennial ryegrass in g dry matter (DM) per pot (mean ± SE, $n = 8$) of the 1st and 2nd recovery regrowth under adequate water availability after prior drought and rewetting (DRW) of soil and plants either non-N-fertilized or N-fertilized during drought. Bar filling indicates prior plant treatment with filled bars representing control plants (Ctr_p) and empty bars representing formerly drought-stressed and rewetted plants (DRW_p). Prior soil treatment with plants grown in control soil (Ctr_s) and in previously dried and rewetted soil (DRW_s), as well as the prior N-fertilization treatment, are indicated along the *x*-axis. Asterisks indicate statistically significant treatment or treatment interaction effects from a linear mixed effects model which included both recovery regrowths (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Due to better readability, only statistically significant treatment interactions are displayed. For more statistical information, see Table 1.

plots, respectively (significant prior soil treatment – regrowth interaction $P < 0.001$). Prior N-fertilization slightly but not significantly increased N availability of Ctr_s and DRW_s, showing the most pronounced increase in DRW_s during the 1st recovery regrowth.

Prior plant DRW treatment consistently reduced aboveground plant N uptake during recovery in all prior soil and N-fertilization treatments by on average 6 mg N pot^{-1} (17%; $P < 0.01$; Fig. 4; Table 1). Prior soil DRW treatment significantly increased aboveground plant N uptake in all prior plant and N-fertilization treatments and in both recovery regrowths ($P < 0.001$) with a greater effect on aboveground plant N uptake in priorly N-fertilized compared with non-N-fertilized plants (significant prior soil treatment – prior N-fertilization treatment interaction $P < 0.001$). Specifically, prior soil DRW treatment increased plant N uptake by 14 mg N pot^{-1} (42%) and 47 mg N pot^{-1} (111%) in non-N-fertilized and N-fertilized plants in the 1st recovery regrowth, respectively, and by 5 mg N pot^{-1} (31%) and 43 mg N pot^{-1} (161%) in non-N-fertilized and N-fertilized plants in the 2nd recovery regrowth, respectively. Prior N-fertilization increased overall plant N uptake of all treatments and regrowths ($P < 0.001$). Aboveground plant N uptake was significantly lower in the 1st compared with the 2nd recovery regrowth ($P < 0.001$).

Prior plant DRW treatment consistently reduced leaf N content per unit leaf area by on average 0.01 mg N cm^{-2} (10%; $P < 0.001$; Fig. 4; Table 1). Prior soil DRW treatment significantly increased leaf N content per unit leaf area in all prior plant and N-fertilization treatments and over both regrowths ($P < 0.001$) with a greater effect in priorly N-fertilized compared with non-N-fertilized plants (significant prior soil treatment – prior N-fertilization treatment $P < 0.05$). In detail, prior soil DRW treatment increased leaf N content per unit leaf area in the 1st recovery regrowth by on average 0.2 mg N cm^{-2} (15%) and 0.4 mg N cm^{-2} (34%) for plants priorly non-N-fertilized and N-fertilized, respectively. In the 2nd recovery regrowth, the effect of prior soil treatment was much smaller than in the 1st recovery regrowth (significant prior soil treatment – regrowth interaction $P < 0.05$) and persisted only in N-fertilized treatments where it significantly increased leaf N content by on average 0.2 mg N cm^{-2} (16%). Prior N-fertilization increased leaf N content per unit leaf area over both recovery regrowths ($P < 0.01$) and leaf N contents per unit leaf area were significantly higher in the 1st than in the 2nd recovery regrowth ($P < 0.001$).

Prior plant DRW treatment significantly reduced leaf bulk $\delta^{13}C$ values by *c.* 0.5‰ in DRW_p compared with Ctr_p in both recovery regrowths ($P < 0.001$; Fig. 4; Table 1). Prior soil DRW treatment significantly increased $\delta^{13}C$ values in all prior plant and N-fertilization treatments ($P < 0.001$) and had a greater effect on $\delta^{13}C$ in the 1st than in the 2nd recovery regrowth (significant prior soil treatment – regrowth interaction $P < 0.001$). In the 1st recovery regrowth, DRW_s showed on average 0.9‰ higher $\delta^{13}C$ values than Ctr_s, whereas in the 2nd recovery regrowth $\delta^{13}C$ of DRW_s were on average 0.2‰ higher than Ctr_s. Prior N-fertilization significantly increased $\delta^{13}C$ values in the overall recovery period ($P < 0.05$) but had a less pronounced effect compared with prior soil or plant treatment. Leaf $\delta^{13}C$ values were significantly lower during the 2nd compared with the 1st recovery regrowth ($P < 0.001$).

Discussion

The aim of this study was to determine and disentangle the contribution of plant-intrinsic and plant-extrinsic (i.e. soil-mediated) drought legacy effects on the recovery and post-drought

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Table 1 Summary of the linear mixed effects models for the effects of number of recovery regrowth, prior drought and rewetting treatment of soil (soil trt.) and plants (plant trt.) as well as N-fertilization during drought on aboveground plant productivity, plant N uptake, leaf N contents per area, and leaf bulk $\delta^{13}C$ of perennial ryegrass during recovery under adequate water availability.

Variable	Factor	numDF	denDF	*F*-value	*P*-value	R^2_m	R^2_c
Aboveground productivity	Regrowth	1	56	10.08	0.002**	0.66	0.73
	Soil trt.	1	43	128.00	< 0.001***		
	Plant trt.	1	43	11.21	0.002**		
	N-fertilization	1	14	42.53	< 0.001***		
	Regrowth : Soil trt.	1	56	5.23	0.026*		
	Regrowth : N-fertilization	1	56	2.37	0.130		
	Regrowth : Plant trt.	1	56	0.00	0.944		
	Soil trt. : N-fertilization	1	43	40.37	< 0.001***		
	Soil trt. : Plant trt.	1	43	0.01	0.912		
	Plant trt. : N-fertilization	1	43	0.83	0.368		
Plant N uptake	Regrowth	1	53	95.15	< 0.001***	0.77	0.86
	Soil trt.	1	41	192.03	< 0.001***		
	Plant trt.	1	41	7.72	0.008**		
	N-fertilization	1	11	50.32	< 0.001***		
	Regrowth : Soil trt.	1	53	0.00	0.992		
	Regrowth : N-fertilization	1	53	1.55	0.218		
	Regrowth : Plant trt.	1	53	1.83	0.182		
	Soil trt. : N-fertilization	1	41	48.99	< 0.001***		
	Soil trt. : Plant trt.	1	41	0.79	0.380		
	Plant trt. : N-fertilization	1	41	0.00	0.994		
Leaf N content per area	Regrowth	1	48	200.96	< 0.001***	0.61	0.83
	Soil trt.	1	41	29.14	< 0.001***		
	Plant trt.	1	41	15.67	< 0.001***		
	N-fertilization	1	11	11.68	0.006**		
	Regrowth : Soil trt.	1	48	4.68	0.036*		
	Regrowth : N-fertilization	1	48	1.81	0.185		
	Regrowth : Plant trt.	1	48	2.61	0.113		
	Soil trt. : N-fertilization	1	41	4.47	0.041*		
	Soil trt. : Plant trt.	1	41	0.75	0.391		
	Plant trt. : N-fertilization	1	41	2.83	0.100		
Leaf bulk $\delta^{13}C$	Regrowth	1	50	100.83	< 0.001***	0.41	0.87
	Soil trt.	1	41	28.25	< 0.001***		
	Plant trt.	1	41	20.27	< 0.001***		
	N-fertilization	1	11	5.32	0.042*		
	Regrowth : Soil trt.	1	50	19.72	< 0.001***		
	Regrowth : N-fertilization	1	50	0.23	0.631		
	Regrowth : Plant trt.	1	50	0.21	0.648		
	Soil trt. : N-fertilization	1	41	0.12	0.735		
	Soil trt. : Plant trt.	1	41	0.07	0.795		
	Plant trt. : N-fertilization	1	41	0.17	0.685		

Asterisks indicate statistically significant treatment or treatment interaction effects (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

compensatory growth of a *L. perenne* grassland after drought and rewetting (DRW). Our experiment revealed that formerly drought-stressed plants (DRW_p) have lower aboveground productivity, lower nitrogen (N) uptake, and lower $\delta^{13}C$ values compared with Ctr_p. This suggests that DRW impaired plant recovery when decoupled from any extrinsic, soil-mediated DRW effects. Instead, plants grown on formerly drought-stressed soil (DRW_s) had a higher aboveground productivity, higher N uptake, and reduced $\delta^{13}C$ values compared with plants grown on Ctr_s, independently of the prior plant treatment. These effects were consistent for two recovery regrowths and irrespective of N-fertilizer applications during drought. In the soil, we observed a pulse in plant N availability after DRW in DRW_s compared with Ctr_s. In summary, our experiment shows that during regrowth under adequate water availability, a previous drought resulted in plant-intrinsic constraints such as reduced plant N uptake, lower plant physiological activity, and reduced plant growth. However, these constraints were more than compensated by extrinsic, soil-mediated drought legacy effects. Increased plant N availability after DRW improved plant N uptake and enhanced plant physiological activity which ultimately resulted in compensatory growth of formerly drought-stressed plants outperforming nonstressed controls once drought stress was released.

Plants show negative intrinsic drought legacy effects

Prior plant DRW treatment resulted in impaired recovery in terms of productivity with DRW_p yielding less aboveground DM compared with Ctr_p in both recovery regrowths. Hence, prior plant DRW treatment led to net-negative plant-intrinsic drought

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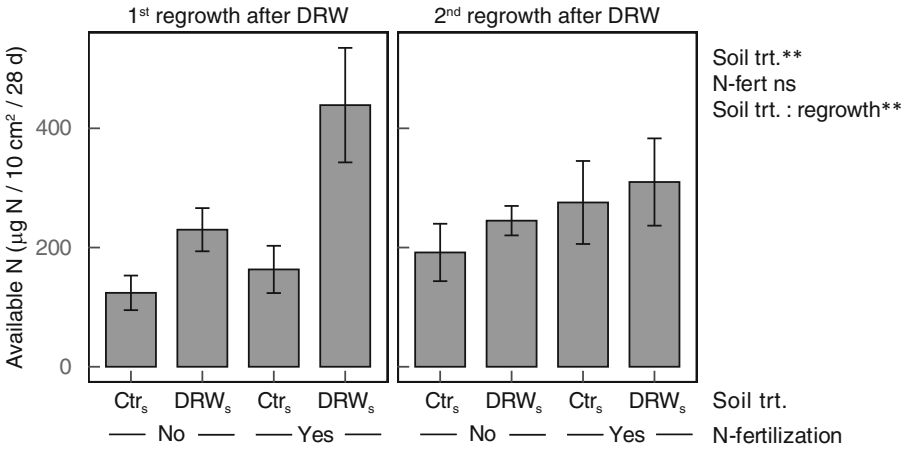

Fig. 3 Plant N availability (mean ± SE, $n = 4$) accumulated over 28 d during the 1st and 2nd recovery regrowth after drought and rewetting (DRW) in the field experiment. Prior soil treatment with plants grown in control soil (Ctr_s) and in previously dried and rewetted soil (DRW_s) as well as the prior N-fertilization treatment are indicated along the *x*-axis. Asterisks represent the level of statistical significance of a treatment or treatment interaction from a linear mixed effects model, which included both recovery regrowths (**, $P < 0.01$). ns, not statistically significant. For more statistical information, see Table 2.

Table 2 Summary of the linear mixed effects model for the effects of number of recovery regrowth, prior drought and rewetting treatment of soil (soil trt.) as well as N-fertilization during drought on post-drought plant N availability measured in the field.

Variable	Factor	numDF	denDF	*F*-value	*P*-value	R^2_m	R^2_c
Available N	Regrowth	1	13	3.36	0.090°	0.44	0.89
	Soil trt.	1	6	17.13	0.006**		
	N-fertilization	1	3	1.54	0.302		
	Regrowth : Soil trt.	1	13	10.14	0.007**		
	Regrowth : N-fertilization	1	13	0.92	0.356		
	Soil trt. : N-fertilization	1	6	0.08	0.789		

Asterisks indicate statistically significant treatment or treatment interaction effects (°, $P < 0.1$; **, $P < 0.01$).

legacy effects once drought stress was released. In regard to recent studies suggesting that formerly drought-stressed grasses could benefit of greater investment into reserve pools during drought for post-drought regrowth (Muller *et al.*, 2011; Hofer *et al.*, 2017a; Volaire *et al.*, 2020), our results suggest that ryegrass did either not increasingly invest in building up energy reserves during drought, or the acquired reserves could not fully compensate for the amount of tissue lost during drought. Also, observing negative DRW legacy effects on ryegrass when decoupled from any soil effects speaks against the theory that compensatory growth after DRW results from an internally controlled seasonal life cycle interrupted by drought.

Our results suggest that severe drought resulted in physiological constraints, which could not fully recover until 2 months after rewetting. Decreased leaf bulk $\delta^{13}C$ values in DRW_p indicate lower iWUE and point toward lower photosynthetic capacity relative to stomatal conductance of DRW_p compared with Ctr_p (Farquhar *et al.*, 1982, 1989). The additional comparison of $\delta^{13}C$ with leaf $\delta^{18}O$ values not affected by prior plant treatment (Fig. S3; Table S3) suggests that changes in $\delta^{13}C$ are driven by a reduced photosynthetic capacity rather than stomatal conductance (Scheidegger *et al.*, 2000). A reduced photosynthetic capacity can be explained by a lower N content per leaf area (Field & Mooney, 1986; Reich *et al.*, 1998; Wright *et al.*, 2001; Lambers *et al.*, 2008), which is the result of reduced plant N uptake in DRW_p compared with Ctr_p plants (Congreves *et al.*, 2021). Lower photosynthetic capacity and thus reduced production of assimilates then translated into the reduced aboveground productivity observed in DRW_p compared with Ctr_p.

The increase in aboveground productivity, N uptake, leaf N, and $\delta^{13}C$ of N-fertilized DRW_p to non-N-fertilized Ctr_p levels confirms our interpretation that differences in post-drought physiological performance and productivity between Ctr_p and DRW_p are related to N nutrition. Restricted access to soil N could be the consequence of either impaired root regrowth or restricted root N uptake capacity after DRW. Studies examining post-drought root growth dynamics in grasslands without separating soil and plant DRW effects report increased but delayed post-drought root production, indicating that increased root regrowth was mainly supported by newly assimilated carbon (Newton *et al.*, 1996; Wedderburn *et al.*, 2010). Hence, it is likely that the effect of impaired plant N access on photosynthesis restricted root regrowth and, in turn, further limited N access of DRW_p. This underlines the importance of plant N availability for grasslands when recovering from drought.

Drought and rewetting increased plant N availability

We observed a significant pulse in plant N availability in the field after DRW irrespective of the N-fertilization treatment, although N availability was higher in plots N-fertilized than in non-N-fertilized plots. This is in line with previous findings reporting higher mineral soil N and plant N availability after DRW (Borken & Matzner, 2009; Niboyet *et al.*, 2017; Hofer *et al.*, 2017b;

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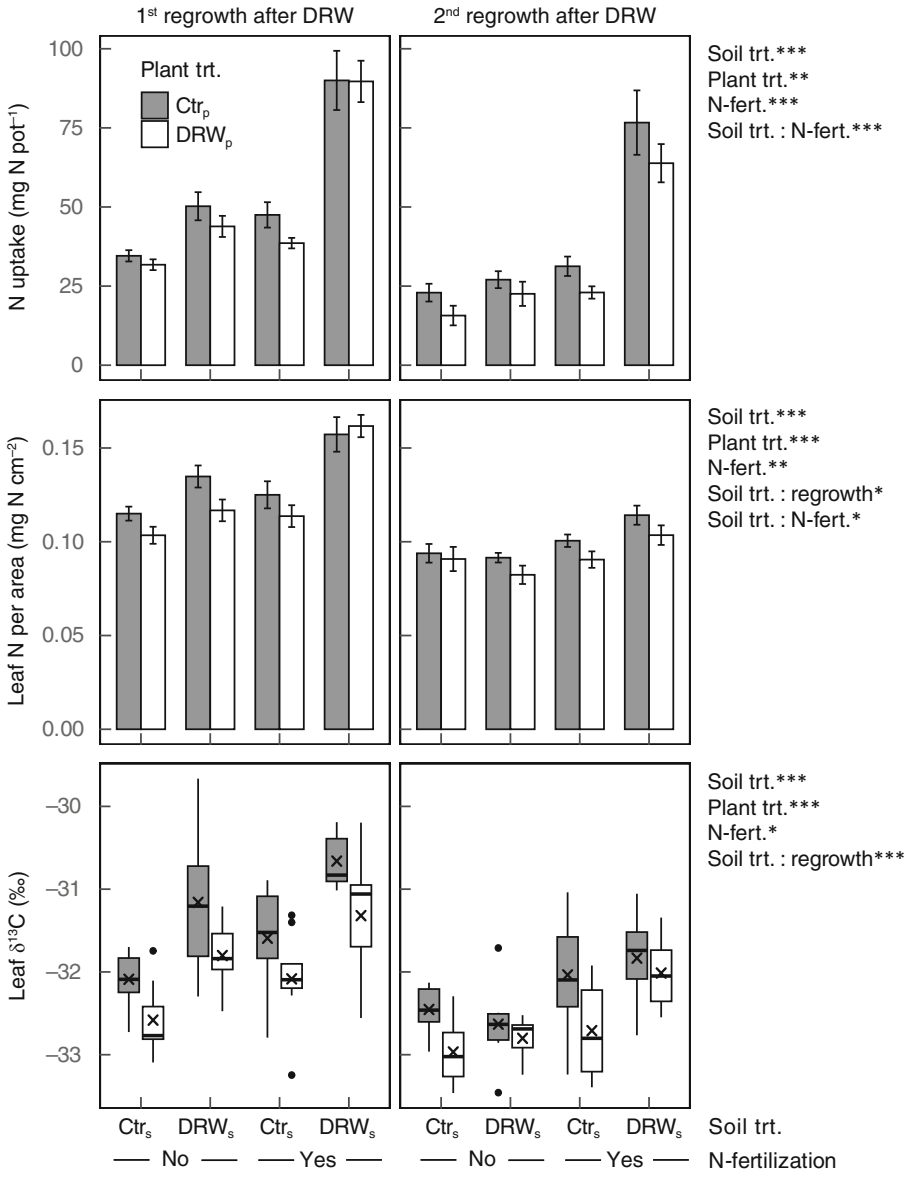

Fig. 4 Aboveground plant N uptake, leaf N content per area (means ± SE, $n = 8$), and leaf bulk $\delta^{13}C$ values ($n = 8$) of perennial ryegrass N-fertilized and non-N-fertilized during drought and measured in the 1st and the 2nd recovery regrowth after drought and rewetting (DRW). Boxes in the $\delta^{13}C$ boxplot indicate the interquartile range (IQR), the horizontal lines inside the boxes represent group medians, crosses inside the boxes indicate group means, whiskers represent ±1.5 IQR, and the black dots beyond the whiskers represent outliers. Bar/box filling indicates prior plant treatment with filled bars/boxes representing control plants (Ctr_p), and empty bars representing formerly drought-stressed and rewetted plants (DRW_p). Prior soil treatment with plants grown in control soil (Ctr_s) and in previously dried and rewetted soil (DRW_s), as well as the prior N-fertilization treatment, are indicated along the *x*-axis. Asterisks indicate statistically significant treatment or treatment interaction effects from linear mixed effects models, which included both recovery regrowths (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Due to better readability, only statistically significant treatment interactions are displayed. For more statistical information, see Table 1.

Mackie *et al.*, 2018). Soil nutrient pulses induced by DRW can be caused by a range of non-exclusive processes. These are the accumulation of nutrients not taken up during drought that become available during the process of rewetting (e.g. Homyak *et al.*, 2017), mineralization of previously unavailable organic substrates either accessible through physical breakdown of soil aggregates or released by microbial cell lysis or osmoregulation during DRW (e.g. Birch, 1958, 1960; Sparling & Ross, 1988; Wu & Brookes, 2005) and/or the release of plant-available inorganic nutrients through physical breakdown of soil aggregates, microbial cell lysis, or microbial osmoregulation during the process of DRW (e.g. Haygarth *et al.*, 1998; Unger *et al.*, 2010; Bünemann *et al.*, 2013; Schimel, 2018; Sundert *et al.*, 2020). These processes all potentially lead to higher post-drought nutrient availability (Jarvis *et al.*, 2007; Borken & Matzner, 2009; Dodd *et al.*, 2015). More enriched leaf $\delta^{15}N$ values in leaves of DRWs ryegrass compared with Ctr_s ryegrass, which would point to increased N-mineralization in DRWs soils (Kahmen *et al.*, 2008) could, however, not been observed (Fig. S4; Table S3). This suggests that the pulse in N availability in the field originates from other above-mentioned processes than increased N-mineralization.

Meanwhile, the pulse in N availability observed in the field after DRW was greater when plots were N-fertilized during drought, which is in line with findings of Hofer *et al.* (2017b). Bulk leaf $\delta^{15}N$ values of N-fertilized swards closer to 0‰ (Fig. S4; which corresponds to the average $\delta^{15}N$ value of synthetic N-fertilizers (Bateman & Kelly, 2007)) suggest that the greater pulse in N availability observed in N-fertilized compared with non-N-fertilized DRWs swards was mainly a result of accumulated fertilizer-N solubilized during the process of rewetting. This implies that the increase in aboveground productivity, plant N uptake, leaf N, and leaf $\delta^{13}C$ observed in N-fertilized compared with non-N-fertilized treatments was an effect of increased N availability at recovery start.

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Higher plant N availability after drought mitigates plant-intrinsic legacy effects and leads to post-drought compensatory growth

Although we observed negative plant-intrinsic drought legacy effects on plant productivity, plants grown on formerly drought-stressed soil showed post-drought compensatory growth in both recovery periods, in both N-fertilization treatments, and irrespective of the prior plant treatment (DRW_p or Ctr_p). The amplitude of this effect is comparable to observations of DRW effects on grassland productivity in previous studies under field conditions, which report a 30–90% increased aboveground productivity of formerly drought-stressed plots compared with control plots with and without N-fertilization during drought (Hofer *et al.*, 2016, 2017b; Hahn *et al.*, 2021).

Our experiment shows that compensatory growth concurred with increased plant N availability, greater plant N uptake, and higher leaf N contents per area observed in DRW_s than in Ctr_s in both N-fertilization treatments. This concurrence is corroborated by a significant correlation of aboveground productivity with leaf N in the transplantation experiment (Fig. S5) as well as a significant relationship of aboveground productivity of field swards with available N measured in the field (Fig. S6). Increased plant N uptake and leaf N contents in DRW_s compared with Ctr_s suggest a better photosynthetic performance of DRW_s compared with Ctr_s through improved N nutrition as indicated by higher leaf $\delta^{13}C$ of DRW_s relative to Ctr_s. The latter is supported by a strong correlation of leaf $\delta^{13}C$ values with leaf N content per area (Fig. S7), implying that photosynthesis was increased by improved N nutrition in DRW_s compared with Ctr_s. Our findings are in accordance with previous studies that explain post-drought compensatory growth and increased photosynthetic capacity of formerly drought-stressed grasses with higher nutrient availability (Albert *et al.*, 2011; Hofer *et al.*, 2016; Ingrisch *et al.*, 2018; Karlowsky *et al.*, 2018; Sundert *et al.*, 2020; Ru *et al.*, 2023). We therefore suggest that increased N availability after DRW improved N nutrition and photosynthetic capacity of formerly stressed perennial ryegrass, which mitigated negative plant-intrinsic drought legacy effects and led to a net post-drought compensatory growth of DRW_s compared with Ctr_s. Since most grass species respond positively to higher nutrient availability in terms of growth, it is probable that the post-drought mechanisms affecting aboveground productivity of *L. perenne* observed in this study also account for other grass species. Post-drought compensatory effects have been found in grasses of different life forms and origins (Albert *et al.*, 2011; Volaire *et al.*, 2020; Wilcox *et al.*, 2020; Hahn *et al.*, 2021; Ru *et al.*, 2023). However, it remains unclear whether plant-intrinsic and plant-extrinsic effects would be similar after a drought of higher intensity and/or duration. Increasing drought intensity and duration have been both related positively and negatively to the post-drought release of soil N (Borken & Matzner, 2009; Guo *et al.*, 2014; Zhou *et al.*, 2022) and have been shown to reduce plant reserves required to re-establish once drought stress is released (McDowell *et al.*, 2008; Yang *et al.*, 2022).

In summary, we observed no evidence of plant-intrinsic DRW legacy effects contributing to post-drought compensatory growth. In contrast, negative plant-intrinsic drought legacy effects such as restricted N nutrition, lower photosynthetic capacity, and lower aboveground productivity of formerly stressed perennial ryegrass compared with controls were observed when disentangled from any soil-mediated drought legacy effects. Plant-intrinsic drought legacy effects were, however, more than compensated when perennial ryegrass grew on formerly stressed soil. There, grasses benefitted from increased plant N availability in the soil, leading to improved N uptake, higher photosynthetic capacity, and higher aboveground productivity of formerly drought-stressed grasses compared with grasses grown on Ctr_s, irrespective of the prior plant treatment. Hence, we conclude that the effect of post-drought compensatory growth observed in perennial ryegrass is driven by soil-mediated drought legacy effects leading to increased soil nitrogen availability, which allowed to compensate for negative plant-intrinsic drought legacy effects.

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Competing interests

None declared.

Author contributions

M-LS, AL and AK designed the experiment. M-LS performed the experiment and collected and analyzed the data. M-LS wrote the manuscript with the contribution of AL and AK

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Data availability

The data that support the findings of this study are openly available on figshare at doi: 10.6084/m9.figshare.23584764.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Precipitation, soil water potential, and aboveground productivity data measured in the field.

Fig. S2 Specific leaf area (SLA) of the 1st and the 2nd recovery regrowth after drought and rewetting.

Fig. S3 Leaf bulk $\delta^{18}O$ values of the 1st and 2nd regrowth after drought and rewetting.

Fig. S4 Leaf bulk $\delta^{15}N$ values of the 1st and 2nd regrowth after drought and rewetting.

Fig. S5 Relationship between leaf N content per area and aboveground productivity.

Fig. S6 Relationship between plant-available N and aboveground productivity, both measured in the field.

Fig. S7 Relationship between leaf N content per area and leaf bulk $\delta^{13}C$.

Methods S1 Methodological information on data shown exclusively in the Supporting Information.

Table S1 Field harvests and N-fertilization doses applied before transplantation.

Table S2 Installation, replacement and deinstallation dates of PRS® membranes at the field site.

Table S3 Summary of the linear mixed effects models for the effects of number of recovery regrowth, prior drought and rewetting treatment of soil (soil trt.) and plants (plant trt.) as well as N-fertilization during drought on specific leaf area, leaf bulk $\delta^{18}O$ and leaf bulk $\delta^{15}N$.

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