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RESEARCH ARTICLE

Heathland management affects soil response to drought

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Abstract

1. Drought can affect ecosystem functioning by altering plant–soil interactions, posing a significant threat to vulnerable ecosystems like heathlands. In heathlands, ongoing nitrogen deposition increases the dominance of fast-growing grasses over the slow-growing shrub *Calluna vulgaris*. These changes above-ground can influence soil dynamics and heathlands' responses to drought. Here, we assessed whether the legacy effects of drought on heathland soils depended on mowing times as a management practice commonly used to regenerate *Calluna* and decrease the abundance of fast-growing grasses.
2. Using a long-term field experiment, we investigated if soil response to drought differed under *Calluna* and grasses, as well as under *Calluna* plants of different growth stages through different mowing times. We hypothesized that drought would decrease soil C and nutrient pools underneath grasses and younger *Calluna* plants through its impact on soil microbial communities.
3. Our results show that long-term drought decreased soil C but only underneath grasses and old *Calluna*, while under young *Calluna*, soil C increased under drought when compared to control conditions. Bacterial and fungal community composition differed between drought and control and were affected by the growth stage of *Calluna* but not by plant growth strategies. Furthermore, drought and *Calluna* growth stage-induced changes in bacterial communities directly affected total soil C and indirectly by reducing microbial C, which was positively related to total soil C.
4. *Synthesis and applications*. Understanding ecosystem response to drought is crucial for maintaining biodiversity and mitigating climate change feedbacks. Heathlands are threatened by high nitrogen deposition, a lack of management and an increasing frequency of drought. Our results emphasize the importance of frequent mowing (decadal) as a management practice that promotes a younger and more *Calluna*-dominated plant community for reducing soil C losses under future climate change-induced drought conditions. The active management of heathlands is essential not only for keeping above-ground vegetation dynamics, but also for maintaining below-ground soil nutrient and carbon pools.

KEYWORDS
drought, heathland, legacy effects, management, soil carbon, soil microbial community

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1 | INTRODUCTION

One of the consequences of climate change is the increase in intensity and frequency of extreme weather events, such as drought (Samaniego et al., 2018), which is threatening many different ecosystems. Drought can have a strong impact on ecosystem functioning through its effects on plants and soils by decreasing primary productivity (Xu et al., 2019), reducing nitrogen fixation (Zheng et al., 2020) and decreasing soil respiration, potentially impacting carbon (C) and nitrogen (N) storage (Anderegg et al., 2015). Quantifying how ecosystems respond to drought, and, in particular, how soils respond to drought, can therefore shed light on potential mechanisms feeding back to climate change itself. Furthermore, as drought can threaten the persistence of certain plant species (Zhang et al., 2019), quantifying its effects becomes even more important for already vulnerable ecosystems, such as heathlands (De Graaf et al., 2009).

Heathlands are human-shaped, intensively managed ecosystems that have developed in extremely nutrient-poor soils and that, like many other ecosystems, have been under threat by climate change and nitrogen deposition (Fagúndez, 2013). Heathlands provide many ecosystem services and are considered important habitats for conservation by the Natura 2000 (Schellenberg & Bergmeier, 2021). These ecosystems are dominated by ericaceous shrubs, such as *Calluna vulgaris* (hereafter *Calluna*), a perennial plant with a lifespan of approximately 30 years. Traditionally, heathlands have been managed to promote *Calluna* regrowth by using different forms of grazing, mowing or fire (Fagúndez, 2013). However, due to land abandonment and high levels of nitrogen deposition, the dominance of more nutrient-demanding grasses has increased over *Calluna* (Aerts, 1989). In fact, models predict that if heathlands are not mowed at short-term intervals, the combination of high levels of N deposition with drought may lead to the exclusion of *Calluna* in favour of grasses (Britton et al., 2001). Moreover, Meyer-Grünefeldt et al. (2015) have shown that these combined effects of N and drought on *Calluna* may also depend on growth stage: seedlings were highly susceptible to drought in a glasshouse experiment when fertilized with N, but the building stage of *Calluna* (ca. 10 years old) was not affected by drought in the field with high N addition. These findings suggest that heathland management and global change together can affect vegetation dynamics, not only by changing competitive interactions between different plant species, but also by differentially impacting the growth stages of the dominant species.

Drought can also negatively affect heathland functioning through changes in plant productivity and soil processes (Reinsch et al., 2017), but these effects may differ depending on the growth strategy of the plant species. Fast-growing resource-acquisitive plant species tend to exhibit lower drought resistance but potentially higher resilience when compared to slow-growing resource-conservative species (Pérez-Ramos et al., 2013). In heathlands, the dominant plant species have very contrasting growth strategies: *Calluna* is a slow-growing perennial shrub, while *Molinia caerulea* and *Deschampsia flexuosa* are fast-growing resource-acquisitive grasses that prefer higher nutrient availability (Fagúndez & Pontevedra-Pombal, 2022). In response to drought, *Calluna* plants have either increased their total biomass (Andresen et al., 2010) or remained neutral in short-term (Haugum et al., 2021) and long-term studies (Kongstad et al., 2012). Contrary to expectations, the total biomass of *D. flexuosa* was unaffected by drought (Andresen et al., 2010). Below-ground, the effects of drought might manifest themselves more clearly: reduced root growth with increased root nitrogen concentration have been shown for *Calluna*, and increased root phosphorus uptake for *D. flexuosa*, under drought conditions (Arndal et al., 2013). In the long term, however, it is unclear if these growth-related differences also influence how soil nutrient availability and carbon stocks respond to drought.

Distinct plant growth strategies can also affect how different soil microbial communities respond to drought. Drought can directly affect soil microbial communities by causing osmotic stress and reduced nutrient diffusion (Hartman & Tringe, 2019), which can lead to a general decrease in microbial activity (Abbasi et al., 2020). However, drought can impact soil fungal and bacterial communities differently, with the first having found to be more stable in their network interactions than the latter (de Vries et al., 2018). Drought can, in the short term, increase fungal dominance in heathland soils (Jensen et al., 2003) and, in the long term, increase microbial resilience to drought (de Nijs et al., 2019), but these results have not taken into account the potential role that associating with different plant species may have in shaping these microbial communities. Below-ground, resource-acquisitive plant species are more often associated with bacterial-dominated soil communities, while resource-conservative species have been more associated with fungal-dominated soil communities (de Vries et al., 2012). These differences in rhizosphere communities driven by resource-acquisition strategies have also been shown to affect soil nutrients and carbon cycling under drought conditions for different grassland species (de Vries et al., 2016). However, it remains unknown whether these drought effects can be generalized across ecosystems, and if they persist even after drought has ceased. Here, we aimed to understand if the legacy effects of drought in heathland soils are mediated by different plant growth strategies.

In this study, we sampled soils 2 years after the end of a long-term drought experiment in a Central European Atlantic heathland to determine whether drought had a legacy effect on soil nutrients, soil carbon pools and soil microbial communities and whether these effects depended on the mowing times used as a management practice for this ecosystem. More specifically, we investigated if these drought legacy effects on rhizosphere soils would differ between soil collected underneath plant taxa with contrasting growth strategies and from underneath *Calluna* plants at different growth stages as a result of different mowing times. When comparing the two different plant taxa, *Calluna* and fast-growing grasses, we hypothesized a stronger legacy effect of drought on soil underneath fast-growing grasses, leading to a different community composition for fungi and bacteria than under control, and a general decrease

in plant-available nutrients and carbon in comparison to soils underneath slow-growing *Calluna*, where we expected less impact of drought legacy. Similarly, when comparing different growth stages of *Calluna*, we hypothesized a stronger legacy effect of drought on soils underneath younger *Calluna* than underneath older *Calluna*. Consequently, we hypothesized that total soil C and N decreased in response to drought underneath fast-growing grasses and young *Calluna* plants.

2 | MATERIALS AND METHODS

2.1 | Experimental site description

We sampled soil from a long-term drought experiment that was originally set up in 1998 in Oldebroek, The Netherlands (52°24′ N, 05°55′ E) in a managed heathland. Permission to access this field site was granted by the Royal Netherlands Army (Koninklijke Landmacht). The site has an acidic, nutrient-poor sandy haplic podzol soil with an annual average rainfall of 1072 mm and a growing season average rainfall of 566 mm (Kopittke et al., 2014). The experimental design included three control and three drought plots of 5 × 4 m each (see Figure S1). The drought treatment was imposed during the growing season (usually for 2–3 months between April and September, Kopittke et al., 2014) from 1999 until 2018 by an automatic retractable transparent and waterproof cover that prevented rain from reaching the ground in each drought plot (Beier et al., 2004). As a result of the drought treatment, growing season precipitation was reduced by ca. 33% and soil moisture was reduced by ca. 60% (de Nijs et al., 2019). Each 20-m^2 plot was subdivided into three parts, of which one was mowed in 2009, one in 2013 and one was not mowed. These three different mowing times have led to three different growth stages of *Calluna*: young (2013), intermediate (2009) and old (not mowed since approximately 1984, Figure S1).

2.2 | Soil sampling

Soil was collected in June 2020 from underneath three individuals of *Calluna* from each of the three parts of the plot (not mowed, mowed in 2009 and mowed in 2013) and from underneath three grass plants of *Deschampsia flexuosa* or *Molinia caerulea* only from the 2013 mowed part of the plots, all randomly selected (Figure S1). The vegetation in the 2013 mowed part of the plots was largely dominated by *Calluna* (cover ranging from 43.5% in control to 25.6% in drought plots at the end of the drought experiment in 2018), while grasses had an average cover of 3% in drought and control plots (Table S2). Individual soil cores of 1.5 cm diameter and 15 cm depth were taken from underneath the plants until a total amount of at least 90 g of fresh soil was reached (ca. 4–7 cores). This resulted in a total of 69 samples (3 *Calluna* samples × 3 growth stages (1984—old, 2009—intermediate and 2013—young) × 6 plots (3 drought and 3 control) = 54 *Calluna* samples and 3 grass samples × 5 plots (2 control and 3 drought) = 15 samples of grasses), as unfortunately in one of the control plots there were no individuals of the two grass species in the 2013 mowed part of the plot (Table S2).

2.3 | Soil nutrient analysis

Fresh soil samples were sieved at 2 mm, homogenized and kept at 4°C for 36 h prior to extractions. To measure dissolved organic carbon and nitrogen (DOC and DON), we conducted water extractions using a 1:2.5 weight ratio with fresh soil samples, which were shaken at 160 rpm for 2 h. On these unfiltered water extracts, we measured pH with a pH meter (Consort C831). These samples were then filtered using a Whatman 45 mm filter, and total nitrogen, ammonia and nitrate were measured on an autoanalyser (San ++, Skalar, Breda, The Netherlands), while DON was measured on the Vario TOC cube analyser (Elemental GmbH, Langenselbold, Germany) after acidification of samples with HCl. The values for total C were assumed to be appropriate indicators of the DOC since the samples were acidified prior to measurements and no inorganic C was measured. For plant-available nitrogen (PAN), we conducted a 1 M KCl extraction using a 1:5 soil-to-solution ratio, where samples were shaken for 1 h at 160 rpm, centrifuged at 2000 rpm for 10 min and filtered using a Whatman 45 mm filter. Extracts were diluted 10 times and analysed on the autoanalyser for nitrate and ammonia (PAN) and for orthophosphate (plant-available phosphorus—PAP).

To quantify total soil C and N, air-dried soil samples were analysed with the Vario EL cube CN elemental analyzer (Elementar GmbH, Langenselbold, Germany). Total soil P (TP) was determined by extraction with sulphuric acid from samples previously burned for 24 h at 500°C, a step that also allowed for the determination of soil organic matter content (SOM). Briefly, for measuring TP, sulphuric acid was added to the burned soil samples, which were then shaken for 16 h at 200 rpm, centrifuged for 10 min at 2000 rpm and filtered using 0.2 µm Whatman membrane filters. TP in the filtered extracts was determined colorimetrically following Murphy and Riley (1962) and using the Cecil visible spectrometer CE1010 (Cecil Instruments Limited, Cambridge, UK). Soil microbial biomass nitrogen and carbon were determined by fumigation extraction as described by Vance et al. (1987) and Brookes et al. (1985), with a k_{ec} factor of 0.54 and 0.45 respectively. A total number of 11 samples were excluded from the final data analysis due to mishandling of samples in the laboratory or values being below the detection limit of equipment (for details and final sample numbers, see Table S2).

2.4 | Soil DNA extraction, amplification, amplicon sequencing and data processing

We extracted microbial DNA from ~0.50 g of subsampled soil with the DNeasy® PowerSoil® Kit (QIAGEN GmbH, Hilden, Germany)

following the manufacturer's protocol. We amplified the bacterial 16S ribosomal DNA region with primer pair 515F–806R and the fungal internal transcribed spacer 1 region (ITS1) with the primer pair ITS1-F–ITS2 and sent PCR products to be sequenced, more details on DNA amplification, PCR conditions and library constructions can be found in Supporting Information. Samples were sequenced using the Illumina MiSeq sequencer at Earlham Institute (Norwich, UK). Unfortunately, one soil sample from underneath grasses in a control plot showed no amplification for the ITS region despite many attempts and had to be discarded. QIIME2 (version 2020.8; Bolyen et al., 2019) was used for quality control, feature tabulation and taxonomic classification of both 16S and ITS reads, and more details on the processing of sequencing data can be found in the Supporting Information.

2.5 | Statistical analysis

All statistical analyses were done using R version 4.1.2 (R Core Team, 2021). To quantify the legacy effect of drought underneath the different plant growth strategies, underneath *Calluna* at different growth stages and their interactions on the different soil properties, we used linear mixed-effect models with the experimental plot as a random factor in the model. For the comparisons between plant taxa of different growth strategies, only samples taken from the 2013 mowed part of the plots for *Calluna* and grasses were used. All models were fit with maximum likelihood using the function 'lme' from package 'nlme' (Pinheiro et al., 2022). When a factor or an interaction was considered significant, we ran a post-hoc analysis with the function 'emmeans' from package 'emmeans' (Lenth, 2022). We analysed the effect of drought, plant taxon and *Calluna* growth stage on the composition and structure of bacterial and fungal communities with a permutational analysis of variance with the function 'adonis' in the package 'vegan' (Oksanen et al., 2020), using 'strata' for nesting samples within plots and Bray–Curtis as a measure of dissimilarity, followed by a post-hoc test using the function pairwiseAdonis (Martinez Arbizu, 2020). These results were visualized with nonmetric multidimensional scaling (NMDS) analysis, with the function 'metaMDS' in vegan.

2.6 | Structural equation modelling

To gain a better mechanistic understanding of the effects of drought on soil C underneath *Calluna* at different growth stages, we constructed a structural equation model (SEM). We used the function 'psem' in package piecewiseSEM (Lefcheck, 2016) to test our hypothesized relationships between drought, *Calluna* growth stages, plant-available N, microbial community composition, microbial C and soil C (see Figure S2 for our priori model). We used NMDS axis one scores (see above) for bacterial and fungal communities. We used Fischer's C score to evaluate whether the data fit these models (where $p > 0.05$ indicates a good fit), and we used a stepwise removal of non-significant relationships and compared models based on Akaike information criterion values (Lefcheck, 2016).

3 | RESULTS

3.1 | Drought decreases total soil C and N under fast-growing grasses

The drought legacy effect on soil depended on plant growth strategy for microbial biomass N, SOM and total soil C and N (Table 1). Soil microbial biomass N was significantly lower under drought only underneath fast-growing grasses, and it showed no change underneath *Calluna* (Figure 1; Table 1, Taxon × Drought interaction). Total soil C, total soil N and SOM were lower for the drought legacy plots than for control plots only underneath fast-growing grasses and were not affected by drought legacy underneath *Calluna* (Figure 1; Table 1, Taxon × Drought interaction). Soil C:N ratio was affected only by plant growth strategy, with lower values found underneath grasses under drought, but post-hoc analysis showed no significant differences between treatments (Figure S3; Table 1). Microbial biomass C was significantly lower in plots with drought legacy than in control plots underneath both plant taxa (Figure 1; Table 1, post-hoc test $p = 0.01$). We also found that PAN was generally higher under fast-growing grasses than under *Calluna* in both control and drought and, for both taxa, that there was a general decrease in PAN under drought. For all the other measured variables (microbial C:N ratio, DON, PAP, DOC, total soil P and soil pH), we found no significant legacy effect of drought or of the distinct taxa (Table 1; Figure S3).

3.2 | Drought legacy effects on soil are stronger underneath old *Calluna* plants

We found significant interactive effects of drought legacy and growth stage of *Calluna* on SOM, DON, total soil C and soil C:N (Table 2). In general, drought reduced all these properties in soils underneath old *Calluna* but not under young- and intermediate-stage *Calluna* plants, with the exception of soil C:N (Figure 2; Table 2). Soil underneath intermediate-stage *Calluna* plants under drought legacy exhibited the highest soil C:N ratios compared to the other growth stages and drought treatments (Figure 2). PAN decreased with decreasing growth stage of *Calluna*, being lowest underneath young *Calluna* plants. We also found a tendency for an effect of drought treatment on PAN, decreasing it further underneath young *Calluna*, but without any difference in soils underneath old and intermediate *Calluna* plants in comparison to the control conditions (Figure S4). There was no significant legacy effect of drought on any of the other variables (Table 2; Figure S4).

TABLE 1 ANOVA table from linear mixed-effect model results for the effects of drought and plant taxa of different growth strategies on soil parameters, statistically significant values where $p \leq 0.05$ are highlighted in bold.

	Taxon				Drought treatment				Taxon × Drought			
Soil properties	**numDF**	**denDF**	***F***	***p***	**numDF**	**denDF**	***F***	***p***	**numDF**	**denDF**	***F***	***p***
Microbial C:N ratio	1	26	0.04	0.85	1	26	0.02	0.89	1	26	0.37	0.55
Microbial biomass C	1	27	0.14	0.71	1	27	**6.53**	**0.02**	1	27	2.72	0.11
Microbial biomass N	1	26	0.09	0.76	1	26	**5.37**	**0.03**	1	26	**5.32**	**0.03**
Plant-available N	1	27	**11.69**	**0.002**	1	27	6.50	6.50	1	27	1.43	0.24
Dissolved organic N	1	27	2.24	0.15	1	27	0.34	0.56	1	27	2.48	0.13
Plant-available P	1	27	0.05	0.82	1	27	0.02	0.89	1	27	0.01	0.90
Dissolved organic C	1	27	0.08	0.78	1	27	1.59	0.22	1	27	1.82	0.19
Soil organic matter	1	27	0.10	0.75	1	27	3.25	0.08	1	27	**6.55**	**0.02**
Total soil N	1	27	0.01	0.94	1	27	3.07	0.09	1	27	**8.02**	**0.01**
Total soil P	1	27	0.85	0.36	1	27	0.25	0.62	1	27	1.35	0.25
Total soil C	1	27	0.07	0.80	1	27	3.02	0.09	1	27	**6.15**	**0.02**
Soil C:N ratio	1	27	**3.95**	**0.05**	1	27	1.99	0.16	1	27	1.10	0.30
Soil pH	1	27	0.94	0.34	1	27	3.48	0.07	1	27	0.22	0.64

3.3 | Soil microbial community composition is affected by drought legacy and by the growth stage of *Calluna*, but not by plant growth strategy

Drought had a significant legacy effect on the composition of bacterial and fungal communities in soil underneath *Calluna* and grasses (Figure 3) and underneath *Calluna* at different growth stages (Figure 4; Table 3). Unexpectedly, we did not find any effect of plant growth strategy, alone or in interaction with drought legacy, on the composition of soil microbial communities (Figure 3a,b). Bacterial communities underneath *Calluna* and grasses had *Proteobacteria* and *Acidobacteria* as the most abundant groups under control, and both decreased in relative abundance in response to drought legacy (Figure S7); fungal communities underneath both plant taxa in control treatments were more dominated by Archeorhizomycetes, but under drought legacy, Leotiomycetes and Mortierellomycetes increased their relative abundance (Figure S5).

We found a significant effect of *Calluna* growth stage on both bacterial and fungal community composition, but only a significant interaction effect of growth stage and drought on bacterial community composition (Figure 4; Table 3). However, post-hoc comparisons showed significant differences in bacterial communities in response to drought only for intermediate-stage *Calluna* (adjusted $p = 0.015$, Table S3), and a marginally significant difference for young *Calluna* (adjusted $p = 0.06$, Table S3). In general, bacterial communities underneath *Calluna* at all growth stages had Acidobacteria and Proteobacteria as the most relative abundant phyla in all treatments (Figure S8). Young- and intermediate-stage *Calluna* plants under drought had a higher relative abundance of Bacteroidetes, which were present in similar abundance in both treatments in old *Calluna* plants (Figure S8). For fungal communities, there were similar responses to drought legacy across all growth stages, with increases in relative abundance for Leotimycetes and Mortierellomycetes and decreases in Archeorhizomycetes (Figure S6).

3.4 | Drought legacy and growth stage of *Calluna* indirectly drive changes in total soil C

Our SEMs showed that both drought and *Calluna* growth stages indirectly affected soil C through their effects on the bacterial community and microbial biomass C (Figure 5; Fischer's $C = 15.94$, $p = 0.45$ and $df = 16$). The best model explained 39% of the variance in soil C, and showed that *Calluna* growth stage affected plant-available N and soil fungal communities, but here there was no direct effect of these variables on total soil C nor indirectly via microbial C. The SEM also included a direct effect of drought on both fungal and bacterial communities, but only bacterial community was associated with changes in microbial C that led to changes in soil C. Furthermore, bacterial community composition directly and strongly affected total soil C.

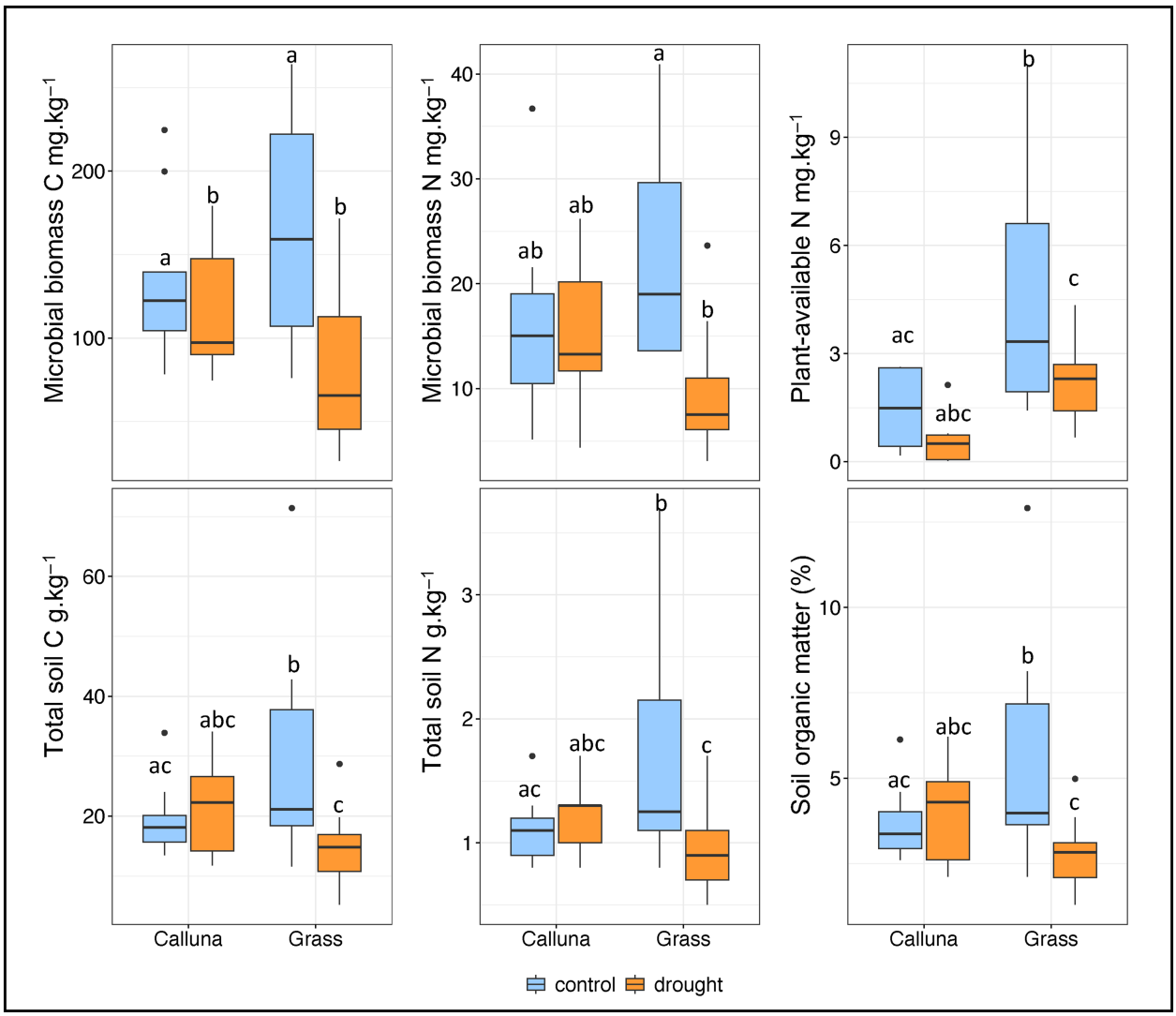

FIGURE 1 The legacy effects of drought on different soil carbon and nitrogen pools in samples collected underneath plant taxa with different growth strategies. Calluna represents *Calluna vulgaris*, and grass represents samples collected underneath *Molinia caerulea* and *Deschampsia flexuosa*. Boxplots show the median, 1st and 3rd quartiles, along with outliers (data outside the whiskers) indicated by individual dots. The *y*-axis scales are different for each individual soil parameter shown in this figure. Different lower-case letters above whiskers represent significant treatment differences ($p \leq 0.05$).

4 | DISCUSSION

Here, we sought to determine whether the legacy effect of 18 years of drought on heathland soils depends on plant growth strategy and on the management of this ecosystem. In line with our hypothesis, we found that 18 years of drought affected total soil C, microbial C and N and plant-available N, and that this was modified by plant taxa with contrasting growth strategies. Specifically, drought reduced soil C underneath grasses and old *Calluna* plants, suggesting that grass encroachment as a result of N deposition and abandoned management reduces the capacity of heathlands to store C under future climates, and highlights the importance of frequent (decadal) mowing to retain this capacity. Moreover, our results suggest that this loss of soil C is mediated by changes in soil bacterial community composition, shedding light on how management practices impact microbial control on the maintenance of C stored in heathland soils.

4.1 | The effects of plant growth strategy on the response of soil C and nutrient pools to drought

In line with our expectations, we found that soil microbial C and N and SOM underneath fast-growing grasses were more vulnerable to the legacy effects of drought than in soils underneath *Calluna*. One possible reason for these differences may lie in the fact that fast-growing grasses may be decreasing their organic inputs to soils through reduced growth and root exudation under drought more strongly than the slow-growing *Calluna*, as it has recently been shown that root exudation can vary with plant growth strategy

TABLE 2 ANOVA table from linear mixed-effect model results for the effects of drought and *Calluna* growth stage on different soil parameters, where statistically significant values ($p \leq 0.05$) are highlighted in bold.

	Calluna growth stage				Drought treatment				Growth stages × Drought			
Soil properties	**numDF**	**denDF**	***F***	***p***	**numDF**	**denDF**	***F***	***p***	**numDF**	**denDF**	***F***	***p***
Microbial C:N ratio	2	44	0.24	0.78	1	44	0.99	0.32	2	44	0.72	0.49
Microbial biomass C	2	46	0.26	0.77	1	46	0.003	0.95	2	46	2.34	0.10
Microbial biomass N	2	44	0.51	0.60	1	44	0.09	0.76	2	44	0.55	0.58
Plant-available N	2	46	**7.89**	**0.001**	1	46	3.33	0.07	2	46	1.64	0.20
Dissolved organic N	2	46	0.60	0.55	1	46	0.15	0.69	2	46	**4.11**	**0.02**
Plant-available P	2	42	0.49	0.61	1	42	2.27	0.13	2	42	1.79	0.17
Dissolved organic C	2	46	2.40	0.10	1	46	0.82	0.37	2	46	0.51	0.60
Soil organic matter	2	45	2.95	0.06	1	45	0.01	0.93	2	45	**3.03**	**0.05**
Total soil N	2	46	2.98	0.06	1	46	0.03	0.86	2	46	1.80	0.17
Total soil P	2	46	0.57	0.56	1	46	1.80	0.18	2	46	1.16	0.32
Total soil C	2	46	2.19	0.12	1	46	0.22	0.64	2	46	**3.13**	**0.05**
Soil C:N ratio	2	46	1.19	0.31	1	46	0.79	0.37	**2**	**46**	**8.17**	**0.001**
Soil pH	2	46	0.14	0.87	1	46	0.22	0.64	2	46	0.59	0.55

(Williams et al., 2022). Furthermore, these decreases in root exudation as a response to drought have been proposed as one mechanism by which plant species can affect ecosystem functioning (Williams & de Vries, 2020), as they can potentially trigger more soil respiration, decreasing the amount of total soil C (de Vries et al., 2019). While we here can only speculate about potential mechanism, our study's observed differences in soil responses to drought, especially related to changes in total soil C mediated by plant growth strategy, highlights how mowing at short time intervals can be important not only as a management practice aimed at reducing the encroachment of grasses in heathlands but also for reducing potential carbon losses from soil.

Surprisingly, we found that the contrasting plant growth strategies of *Calluna* and grasses did not result in changes in soil microbial community composition, with only drought legacy having a strong significant effect on bacterial and fungal communities underneath the different plant growth strategies. This result goes against most studies to date that have shown strong differences in rhizosphere fungal and bacterial communities driven by distinct plant functional traits and growth strategies across many different ecosystems (Dassen et al., 2017; de Vries et al., 2012; Han et al., 2023). We offer three possible reasons on why microbial communities did not differ between plant growth strategies here. First, an in vitro study found that heathland grass species were colonized by strains of fungi belonging to the Helotiales order (Zijlstra et al., 2005), which encompasses ericoid mycorrhizae species that are commonly present in the roots of *Calluna* (Johansson, 2001). This suggests that these plant species may share similar symbionts. In fact, in soils underneath both plant taxa, one of the most relative abundant groups of fungi is the class Leotiomycetes (of which Helotiales is part), and, for both taxa, we found that this class increased in relative abundance in response to drought legacy (Figure S9). Second, and a possible limitation of this present study, is that soil samples were collected underneath two different grass species, which may be causing additional variation in microbial community composition. Third and final, despite these species clearly growing in separate tussocks, we cannot rule out that the root systems of grasses and *Calluna* might have been intertwined and could be sharing rhizosphere microbial communities. Nevertheless, our results indicate the potential for drought effects to overrule any differences that could otherwise be driven by the growth strategies, further stressing how strong and likely homogenizing the legacy effects of this unique 18-year drought can be for soil microbial communities in heathlands.

4.2 | The effects of *Calluna* growth stage on the response of soil C and nutrient pools to drought

Drought legacy reduced DON and soil C pools more strongly underneath older *Calluna* plants than underneath intermediate and young stages. Under ambient conditions, Kopittke et al. (2013) found no differences in soil C stocks between *Calluna* plants of

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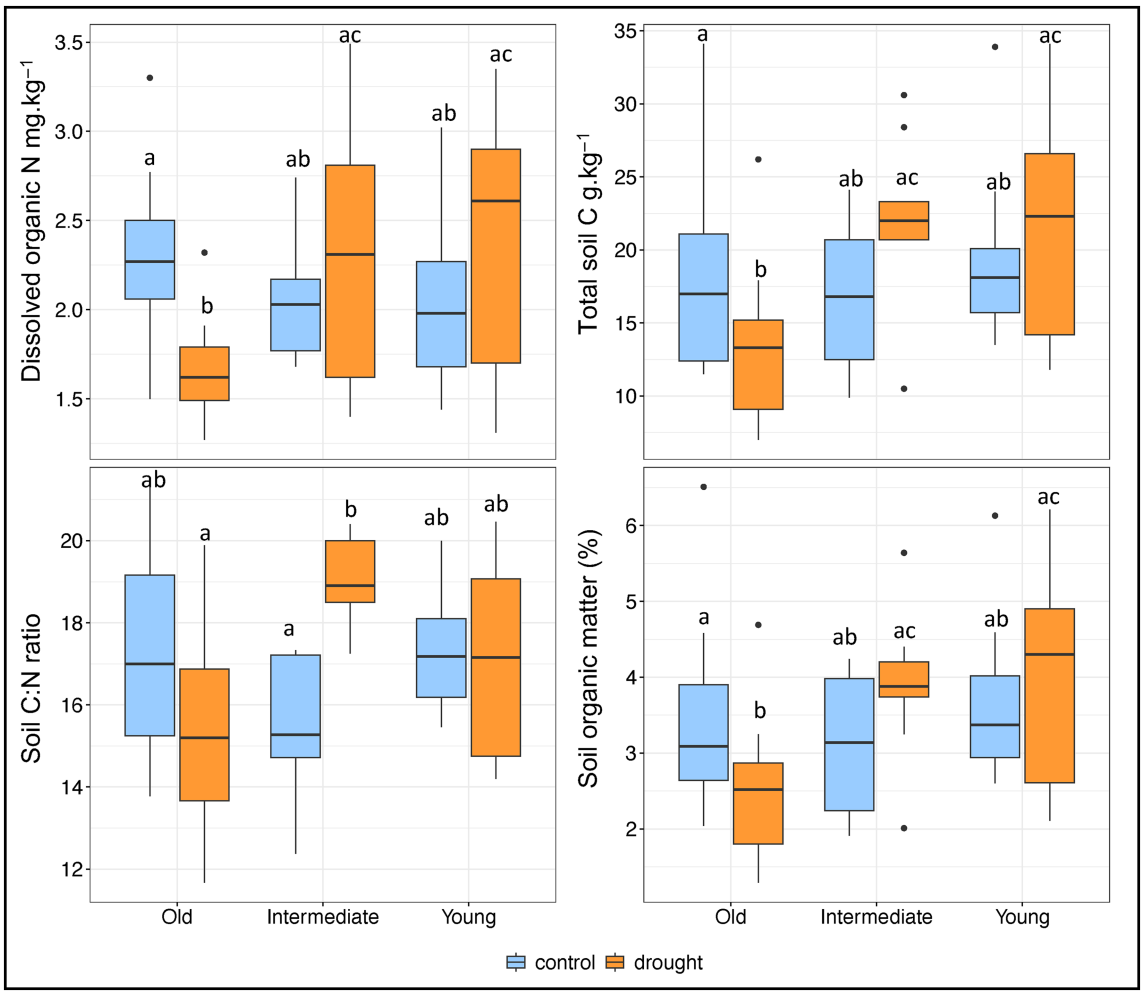

FIGURE 2 The legacy effects of drought on soil C and N parameters underneath *Calluna* at different growth stages. Boxplots represent the median, 1st and 3rd quartiles, along with outliers showed by individual dots (data outside the whiskers) indicated by individual dots. The *y*-axis scales are different for each individual soil parameter shown in this figure. Different lower-case letters above whiskers represent significant treatment differences ($p \leq 0.05$).

different growth stages, which is in line with our 'control' results. Our results show that young- and intermediate-stage *Calluna* plants might not be so strongly affected by drought, and can continuously supply C to soils, which is in contrast to recent findings that seedlings from *Calluna* were more susceptible to drought in terms of growth in comparison to intermediate-stage *Calluna* plants (ca. 10 years old; Meyer-Grünefeldt et al., 2016). As *Calluna* plants age and come close to the end of their 30-year life cycle, they tend to significantly reduce their photosynthetic rates, which can potentially reduce their carbon inputs to soil, and eventually decrease net ecosystem exchange (Li et al., 2023). This reduced carbon allocation to soils might also influence the microbial community underneath these plants.

We found stronger differences in bacterial and fungal community composition in response to drought underneath young and intermediate *Calluna* than underneath old *Calluna* plants (Table S3). Previous research in heathlands has shown that drought can negatively affect soil fungal diversity underneath *Calluna* plants in the short term (Toberman et al., 2008), and can shift both bacterial and fungal community composition in the long term (Seaton et al., 2022). To our knowledge, no previous study has investigated how the growth stage of *Calluna* plants can mediate these responses to drought, but our findings are in line with findings from other systems that rhizosphere microbiome recruitment depends on the plant's developmental stage (Chaparro et al., 2014). *Calluna* has a lifespan of approximately 30 years, and in its last stages, there is very low biomass allocation below-ground (Schellenberg & Bergmeier, 2021). The smaller changes in microbial communities underneath old *Calluna* plants in response to drought found in our study therefore suggest that these plants at this older stage, with potentially lower rhizodeposition, might have limited influence on the composition of soil microbial communities on their rhizosphere. The young- and intermediate-stage *Calluna* plants, on the other hand, are at their maximum size and vitality, which could result in altered root exudation in response to drought, mediating the assembly of different microbial communities.

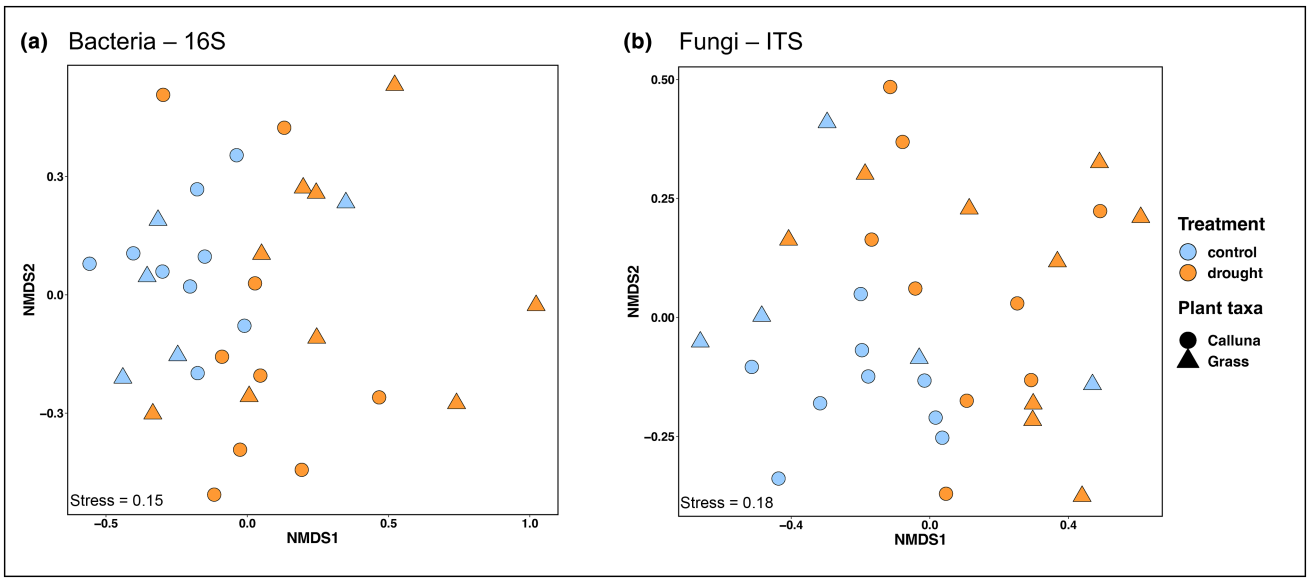

FIGURE 3 Nonmetric multidimensional scaling ordination showing Bray–Curtis dissimilarity-based composition of soil bacterial (a) and fungal communities (b) as affected by drought and plant taxa with contrasting growth strategies (*Calluna* and Grass). Each point on the figure represents a microbial community from one soil sample. ITS, internal transcribed spacer; NMDS, nonmetric multidimensional scaling.

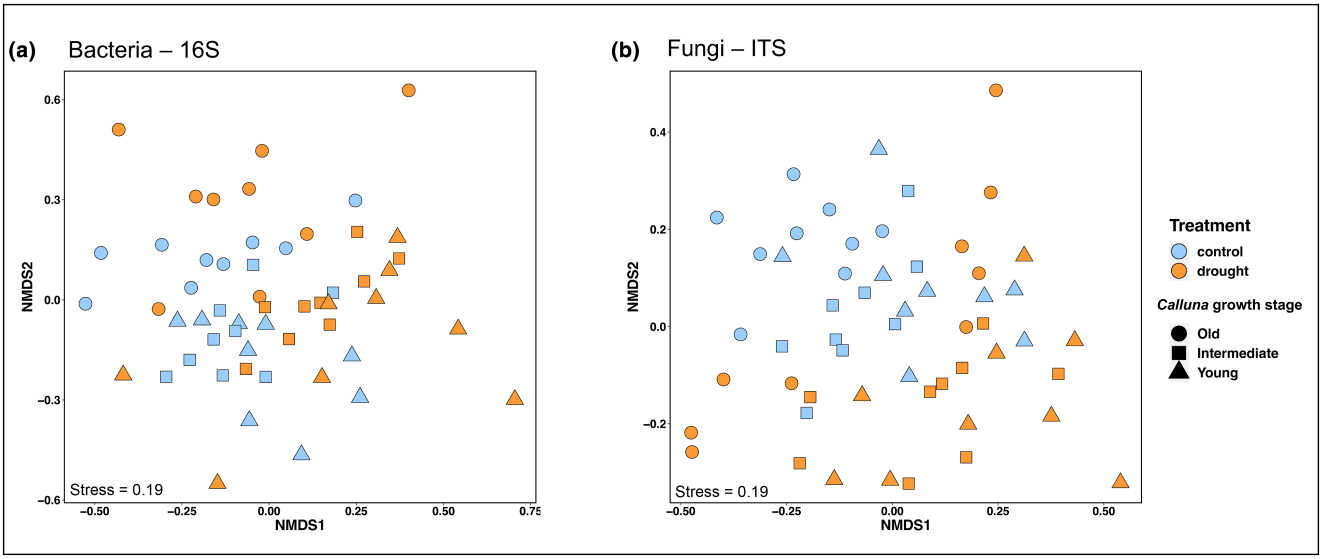

FIGURE 4 Nonmetric multidimensional scaling ordination showing Bray–Curtis dissimilarity-based composition of soil bacterial (a) and fungal communities (b) as affected by drought and *Calluna* growth stages. Each point on the figure represents a microbial community from one soil sample. ITS, internal transcribed spacer; NMDS, nonmetric multidimensional scaling.

4.3 | Drought effects on soil C were mediated by bacterial communities

Our fitted SEM helped us to gain a better understanding of the potential mechanisms governing the observed changes in total soil C underneath different *Calluna* stages in response to drought. The model showed an important role of bacterial community composition in governing total soil C, both directly and indirectly through changes in soil microbial biomass C. In contrast, fungal communities were not directly related to the observed changes in soil C. Soil bacterial networks have been shown to be not only less stable under drought than fungal networks, but also to have stronger links to CO_2 fluxes after drought recovery (de Vries et al., 2018), further suggesting that drought-induced changes in bacterial communities have repercussions for soil C pools. Drought may select for opportunistic bacteria that respond quickly to changes in moisture and resource availability, resulting in a faster decomposition of below-ground C inputs and reducing total soil C, as has been shown in our experiment (de Nijs et al., 2019). Our observed changes in bacterial community composition are almost equally determined by drought and the growth stage of *Calluna*, confirming that our findings for lower soil C underneath old *Calluna* plants in response to drought are mediated by bacterial communities.

TABLE 3 Permutational analysis of variance table for the effects of drought legacy, plant taxon and *Calluna* growth stage on the composition of bacterial and fungal communities. Statistically significant results ($p \leq 0.05$) are highlighted in bold.

	16S—bacterial community				ITS—fungal community			
Source of variation	**df**	**Sums of square**	**Pseudo-*F***	***p***	**df**	**Sums of square**	**Pseudo-*F***	***p***
(a) Plant taxon								
Taxon	1	0.21	1.21	0.19	1	0.37	1.35	0.164
Drought treatment	1	**0.6**	**3.47**	**0.001**	**1**	**0.68**	**2.49**	**0.008**
Taxon × Drought	1	0.14	0.85	0.64	1	0.25	0.93	0.48
Residuals	28	4.86	0.83		28	7.71	0.95	
Total	31	5.82			31	9.03		
(b) *Calluna* growth stage								
Growth stage	2	**0.58**	**1.88**	**0.002**	2	**0.82**	**1.71**	**0.04**
Drought treatment	1	**0.56**	**3.68**	**0.001**	1	**1.49**	**6.23**	**0.001**
Growth × Drought	2	**0.41**	**1.33**	**0.05**	2	0.47	0.98	0.456
Residuals	48	7.39	0.82		48	11.5	0.8	
Total	53	8.95			53	14.29		

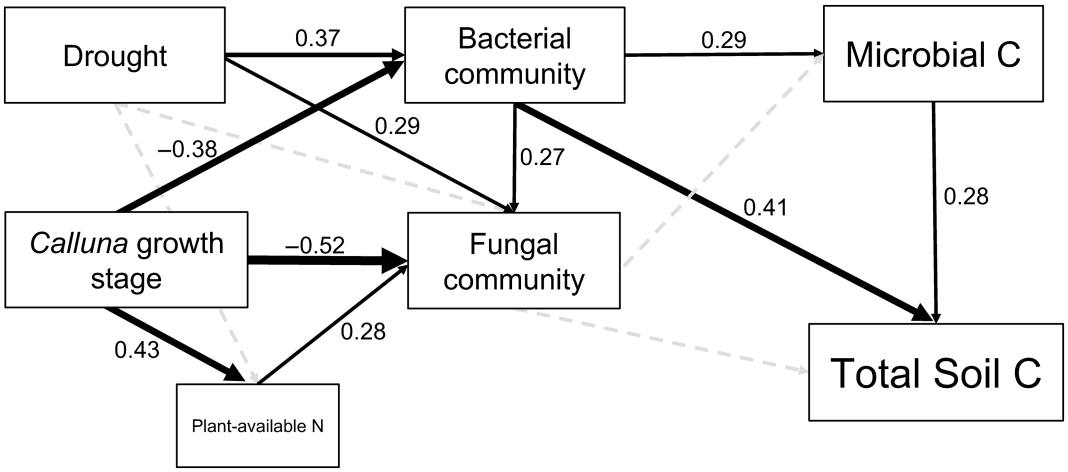

FIGURE 5 Structural equation model on the effects of drought and *Calluna* growth stages on total soil carbon (C). Different arrows represent different relationships between variables, where dashed arrows are non-significant relationships and filled arrows are significant ones for which the standardized coefficients are presented, and their strength is represented by the weight of the arrow. Bacterial and fungal communities refer to the microbial community composition represented by the first axis of the nonmetric multidimensional scaling ordination based on Bray–Curtis dissimilarities for each microbial group.

5 | CONCLUSION

As heathlands are among one of the most important endangered landscapes of Europe, threatened by high levels of N deposition and abandoned management (Fagúndez, 2013), our results here support the recent calls for a need to actively manage these ecosystems (Schellenberg & Bergmeier, 2021) and reinforce the importance of regular mowing (decadal) as a management practice to sustain not only above-ground vegetation dynamics, but also below-ground soil nutrient and carbon pools in the face of climate change. Our findings reinforce what has already been shown for ambient conditions: *Calluna* has greater CO_2 sequestration potential than encroaching grasses in heathlands (Quin et al., 2015). Moreover, our results suggest that N deposition, which has been suggested as the leading cause for the encroachment of grasses into heathlands (Aerts, 1989; Fagúndez, 2013), and has been found to increase *Calluna*'s susceptibility to drought (Meyer-Grünefeldt et al., 2015), can also be a threat for soil C storage in heathlands under future climate scenarios, where extreme events like drought are increasing in frequency (Samaniego et al., 2018). By looking at both the soil responses underneath grasses and *Calluna* and those of *Calluna* at different growth stages to the legacy effects of drought, we provide evidence of the importance of regular (decadal) mowing as a management practice to not only promote a young and more *Calluna*-dominated vegetation in heathlands, but also for optimizing ecosystem C storage under the increasing occurrence of drought.

AUTHOR CONTRIBUTIONS

Mariana Gliesch, Albert Tietema and Franciska T. de Vries conceived the ideas and designed the methodology. Mariana Gliesch, Leonardo Hijonosa, Celia Martin and Yahan Hu collected the data. Mariana Gliesch led the data analysis in collaboration with Evelien Jongepier. Mariana Gliesch led the writing of the manuscript together with Franciska T. de Vries. All authors contributed to the draft and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are publicly available from the FigShare repository on https://doi.org/10.21942/uva.24146508.v3 (Gliesch Silva & De Vries, 2023). The raw DNA sequences have been deposited in NCBI under project number PRJNA746101.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Experimental design of the study site in Oldebroek, the Netherlands.

Figure S2. Hypothesized structural equation model explaining the expected relationships between drought, *Calluna* growth stages and Total Soil C.

Figure S3. The legacy effects of drought on different soil carbon and nutrient pools in samples collected underneath different plant taxa.

Figure S4. The legacy effects of drought on soil carbon, nutrient pools and pH underneath *Calluna* of different growth stages.

Figure S5. The legacy effects of drought on the relative abundance of different fungal classes underneath *Calluna* and grasses.

Figure S6. The legacy effects of drought on the relative abundance of different fungal classes underneath *Calluna* at different growth stages.

Figure S7. The legacy effects of drought on the relative abundance of different bacteria phyla underneath *Calluna* and grasses.

Figure S8. The legacy effects of drought on the relative abundance of different bacteria phyla underneath *Calluna* at different growth stages.

Figure S9. Relative abundance of Leotimycetes fungi in soils underneath *Calluna* and grasses in response to drought legacy.

Table S1. Relative cover (%) of *Calluna* and grasses in the experimental plots.

Table S2. Total number of samples.

Table S3. Post-hoc comparisons for differences in how bacterial community composition based on 16S sequencing responded to drought underneath *Calluna* plants of different growth stages.

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