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RESEARCH ARTICLE

Root traits of grasslands rapidly respond to climate change, while community biomass mainly depends on functional composition

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Abstract

1. Current challenges of functional responses in plant communities to climate change call for multi-factorial experiments. Moreover, studies on climate change should focus on below-ground responses since absorptive roots largely control soil C allocation and resource acquisition. Thus, we aimed to understand biomass allocation and traits of absorptive roots in young mesocosm grasslands subjected to simultaneous manipulation of three components of climate change.
2. We tested grassland biomass and root traits under climate change while manipulating functional composition. Using 64 mesocosms with designed grasslands within four chambers of a controlled-environment facility ('ecotron'), we simulated two contrasting IPCC climate change scenarios for elevated [CO₂] and temperature ('eCO₂' and 'eT'). We applied normal vs. reduced precipitation of early summer in Central Europe. We also tested the effect of functional composition by varying the proportion of grasses and forbs in the communities. Specifically, we quantified above- and below-ground biomass, root diameter (RD), root tissue density (RTD), specific root length (SRL), and root length density (RLD).
3. Functional composition played a significant role in biomass allocation of the grasslands, with grass-dominated communities producing more below-ground biomass than forb-dominated ones, and the opposite pattern registered above-ground. Below-ground biomass did not respond to climate change factors, whereas root trait values responded significantly during early establishment of the grasslands. A higher RD indicated a more conservative strategy under reduced precipitation, while eT and eCO₂ led to higher RTD. We detected interactive effects between climate change and functional composition on root traits. Moreover, root biomass primarily occupied the upper soil layer, while a warm and CO₂-rich environment promoted root allocation to the lower soil layer. Grass-dominated communities quickly colonized all available soil volume, while forb-dominated ones accumulated more root biomass in the upper soil layer.

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4. In the mesocosm grasslands, root trait variation rather than root biomass reflected below-ground adjustments to climate change. Furthermore, functional composition and the associated trait diversity modulated biomass allocation. Thus, establishing plant communities that are more resilient to climate change must consider the functional and taxonomic composition of the seed mixtures designed to restore urban grasslands.

KEYWORDS

absorptive root traits, biomass allocation, climate scenario, fine roots, plant functional types, urban grasslands

1 | INTRODUCTION

Anthropogenic increase in $[\text{CO}_2]$ leads to higher global temperature, changes in precipitation, and more frequent and intense climatic extremes (IPCC, 2021), with adverse effects on biodiversity and ecosystem services (MAE, 2005). Yet, while climate change manifests through multiple environmental factors, most studies used single-factor experiments or simplistic combinations of climate-change components as affecting grassland plant communities, disregarding offsetting and enhancing effects among them (Luo et al., 2008; Mikkelsen et al., 2007), but see, for example, Pilon et al. (2013) for the impacts of increased temperatures, reduced precipitation and elevated CO_2 on the root system of grasslands. Still, while the functional composition of plant communities modifies the responses to climate change either via 'insurance effects' of increased functional redundancy (Gonzalez & Loreau, 2009) or due to the buffering effects of more complementary species (Chen et al., 2022; Isbell et al., 2015; Wagg et al., 2017), abiotic and biotic interactions may result in unexpected outcomes that are poorly understood.

Moreover, most climate-change studies focus on the above-ground component of ecosystems, while the below-ground responses might be at least as important, since roots largely control potential responses to climate change (Bardgett et al., 2014; Freschet, Roumet, et al., 2021). In particular, absorptive roots, that is with dominantly resource acquisition function and a relatively small diameter ($\leq 2\text{mm}$ in diameter; Freschet, Pagès, et al., 2021), can inform about below-ground C allocation (Bardgett et al., 2014; Freschet & Roumet, 2017), and strategies for water and nutrient acquisition (Eapen et al., 2005; Freschet, Pagès, et al., 2021). Thus, focusing on different aspects of the below-ground compartment (biomass allocation, functional traits, community composition, etc.) and their responses to interacting climate-change components is needed to provide more information for the predictive modelling of grassland responses to climate change (Mikkelsen et al., 2007).

Climate-change components affect root C allocation and absorptive root traits, thus moderating ecosystem functions (Freschet, Roumet, et al., 2021). Absorptive root productivity and biomass tend to increase under elevated $[\text{CO}_2]$ (' eCO_2 ') across ecosystems (Nie et al., 2013), as well as under higher temperatures (' eT '), which stimulate absorptive root production to enhance soil exploration

and water acquisition (Wang et al., 2021). Similarly, plants exposed to reduced precipitation tend to increase water-absorbing surfaces (i.e. fine roots; Zhang et al., 2019). Furthermore, while eCO_2 leads to changes in root diameter (RD), root length and rooting depth (Nie et al., 2013), eT has shown equivocal effects on root traits (Wang et al., 2021). Likewise, root traits respond to reduced precipitation by adopting resource-conservative strategies like increased RD, higher root tissue density (RTD) and reduced specific root length (SRL; Bardgett et al., 2014; Zhou et al., 2018). Furthermore, eCO_2 increases water use efficiency (WUE) in plants and offsets the effects of a reduced soil water status under moderate drought or eT on root production and traits (Arndal et al., 2018). Thus, combinations of climate-change components allow the detection of realistic non-additive below-ground responses (Mueller et al., 2018).

Plant communities react differently to changes in environmental conditions, including modulating biomass allocation and root traits in response to climate changes. For example, species richness and functional diversity positively correlate to absorptive root biomass, especially in grasslands (Mommer et al., 2010), as a result of increased complementarity in resource use (Isbell et al., 2015) or species asynchronous performances (Haughey et al., 2018; Valencia et al., 2020). Grasses generally produce thinner roots but have more root biomass (Roumet et al., 2008), accumulating in the topsoil (Fargione & Tilman, 2005). Grasses may be more impacted by decreased precipitation than forbs, which have thicker roots in deeper soil layers (Berendse, 1982; Mommer et al., 2010; Roumet et al., 2008). Moreover, the diversity of plant functional types will foster diversity of root traits, for example, forbs have lower SRL and root length density (RLD) than grasses (Bakker et al., 2019), leading to higher resistance and resilience to environmental stress (Bakker et al., 2019; Barkaoui et al., 2016; Wang et al., 2020).

As the vertical distribution of roots determines water and nutrient acquisition, root distribution may vary with community composition and respond differently to climate-change components. In grasslands, the majority of root biomass occurs in the upper 30 cm of soil (Jackson et al., 1996; Mommer et al., 2010), while vertical root distribution varies among species, plant age and density, soil characteristics, and climate (Freschet, Pagès, et al., 2021). Vertical root biomass distribution and length density show how plants exploit the soil profile (Freschet, Pagès, et al., 2021) and explain their resource acquisition strategies and potential resource

partitioning (Mueller et al., 2013). These characteristics also reveal community effects on soil stability (Gould et al., 2016), water infiltration and percolation (Fischer et al., 2015, 2019). Therefore, to refine predictions about the consequences of climate change on ecosystem functioning, investigations should consider whether community responses occur only in the upper soil layer or across the soil profile.

We selected grasslands as model to test the effects of different components of climate change on below-ground biomass allocation and traits. We focused on restored grasslands mainly designed for urban environments, usually species-poor, intensively managed and established under challenging environmental conditions. Despite increasing efforts to detect root responses to climate change in grasslands (Nie et al., 2013; Wang et al., 2020, 2021), experiments simulating the simultaneous impacts of multiple climate change components are scarce or produce contradicting results (see, e.g. Arndal et al., 2018; Mueller et al., 2018; Pilon et al., 2013). Moreover, the role of community composition on absorptive root responses is less known, and community biomass and functional traits are rarely combined. Hence, we addressed climate-change effects, represented by eCO_2 and eT , and interacting with reduced precipitation on young grasslands designed for urban settings. We specifically studied allocation of community biomass and traits of absorptive roots, utilizing a mesocosms approach under two simulated climate-change scenarios for $[CO_2]$, temperature and precipitation in early summer. We also manipulated the proportion of grasses vs. forbs to test how the functional composition of grasslands modulates their response to climate change. We expected increased WUE resulting from eCO_2 to counterbalance the drying effect of warming and reduced precipitation on the communities such that below-ground biomass would not be negatively affected by warmer temperatures under RCP8.5 scenario interacting with reduced precipitation. Reduced precipitation should instead have adverse effects on communities when interacting with RCP2.6. More specifically, we asked:

- (i) Does climate change, grassland functional composition and their interaction affect biomass allocation to above- and below-ground compartments in mesocosm grasslands?
- (ii) What is the effect of climate change, grassland functional composition and their interaction on the root traits yielded by mesocosm grasslands?
- (iii) Do the below-ground responses of mesocosm grasslands to climate change vary with soil depth?

2 | MATERIALS AND METHODS

2.1 | Experimental design

In a mesocosm experiment in a controlled-environment facility ('ecotron', sensu Roy et al., 2021), we simulated the early establishment (i.e. the first 3 months after sowing) of model grasslands. The

communities were designed to improve the biodiversity of urban road verges in Central Europe by sowing mixtures of target species onto bare soil (Rojas-Botero, Teixeira, & Kollmann, 2023; Figure 1). We manipulated community functional composition by controlling the proportion of 'forbs' vs. 'grasses'. We simulated eCO_2 and eT to represent climate-change scenarios (RCP scenarios) expected by IPCC (2021) for the end of the century in Central Europe and specifically adapted to South Germany. Additionally, we manipulated two precipitation conditions, that is normal vs. reduced precipitation of early summer in Central Europe.

2.2 | Grassland communities

We designed four grassland communities with different proportions of grasses and forbs ('forb proportion'). The reference mixture contained only grasses (i.e. F0; five species). We also designed communities by mixing the five species of grasses (F0) with 26 forb species in two different proportions (50% and 75% forbs, i.e. F50 and F75, respectively, thus totalling 31 species each). An only-forb community containing the 26 forb species was also tested (100% forbs, F100). We used native forb and grass species suitable for urban grasslands, produced by a certified supplier of regional seeds (Table S1). The mixtures were assembled by weight and adjusted to each tested forb proportion, whereby each species of the respective functional type grass or forb had the same proportion (Table S1). The final mixture seed density per mesocosm was $4400 \text{ seeds m}^{-2}$. The seed mass sown per mesocosm was F0=0.86 g, F50=1.43 g, F75=1.74 g and F100=2.01 g.

2.3 | Ecotron experiment

We ran the experiment for 10 weeks in four walk-in chambers (Figures S1–S3) located at the TUMmesa ecotron (described in Roy et al., 2021); more information is provided as Supporting Information. After 27 days of establishment of the grasslands, we simulated environmental conditions of two climate-change scenarios (RCP2.6 and 8.5; in two chambers per scenario), reflecting environmental parameters of early summer (May–July) in an urban setting (Munich, Germany). RCP2.6 was the control, with $[CO_2]$ and temperature at current values, while RCP8.5 represented worst-case climate change, with nearly doubled $[CO_2]$ and $+3^\circ C$ air temperature (see Supporting Information, Figure S4 for additional information on climate change simulation). Precipitation was manually controlled in each community. The mean precipitation recorded in Munich from May to July 2000–2019 was distributed over the experimental period. Reduced precipitation represented a 50% reduction in water input, mimicking a water-scarce early-growing season. Indeed, we simulated only precipitation variations in early summer conditions (May–July) to test the effects of a particularly dry phase of the growing season, whereas mean annual precipitation values are not expected to change much for Central Europe (IPCC, 2021).

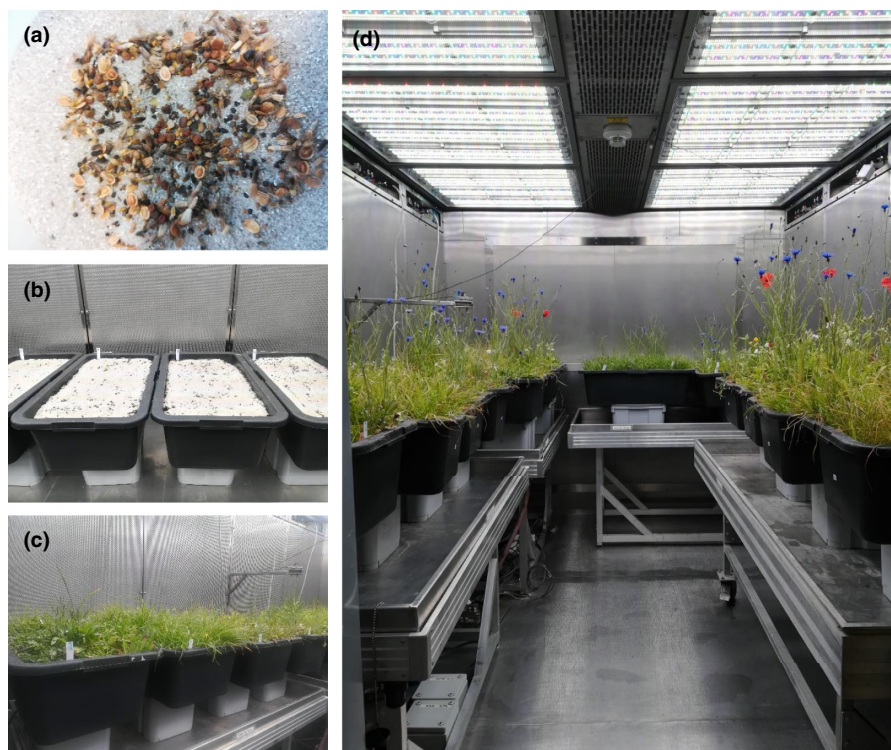


FIGURE 1 Experimental setup for testing the effects of climate change and functional composition on mesocosm grasslands in four chambers of a controlled-environment facility ('ecotron'). Selected regional seed mixtures of urban grasslands (a) were sown onto bare substrate (b) and allowed to develop under different climate-change scenarios and manipulated precipitation (c) until the 67th day, when above- and below-ground biomass was harvested (d). The last picture gives an overview of one chamber used within the ecotron.

We used 64 plastic containers ('mesocosms', $70 \cdot 40 \cdot 23 \text{ cm}^3$ [W · D · H] and 40L volume) with drainage holes to establish the mesocosm grasslands. For the lowest 10 cm, we used a mixture of gardening substrate (20% C organic, pH 6.8) and washed sand (30:70). For the upper 10 cm, we used substrate commercialized for landscaping and establishing urban lawns (mean pH 8.1 by the end of the experiment). In all, 16 mesocosms were placed on four tables (i.e. four per table) inside each of the four chambers at the TUMmesa ecotron (Figure S5). Four mesocosms (i.e. one per table) were randomly assigned to one 'forb proportion' mixture (and consecutively for the other mixtures). At the same time, the precipitation treatments were randomized and applied on two tables in each chamber. Thus, each mesocosm contained a combination of forb proportion, precipitation and RCP scenario, that is four replicates for each treatment combination. After sowing all prepared seed mixtures in one single event and thereby avoiding priority effects, we allowed the experimental communities to germinate and develop under similar conditions, well-watered, with temperature, $[\text{CO}_2]$, and light period similar to current values in May, to promote homogeneity in species composition and plant density of the grassland communities. By doing so, we increased the reliability of the compositions tested. We manually removed typical opportunistic colonizers of urban substrates emerging from the seed bank of the substrate for 4 weeks after sowing the seed mixtures to avoid their dominance in the mesocosms (e.g. *Setaria pumila*, *Agrostis stolonifera*, *Atriplex* sp., *Plantago major* and

Polygonum aviculare). We applied the RCP scenarios and precipitation treatments from the 28th day after sowing until the 67th day.

2.4 | Above- and below-ground biomass sampling and root trait measurement

After 67 days, we harvested the above-ground biomass 2 cm above soil level and dried it for 72 h at 70°C . Immediately after harvest, we collected two soil cores per mesocosm using an 8-cm diameter soil corer, $\geq 10 \text{ cm}$ from the mesocosm sides and $\geq 30 \text{ cm}$ from each other. A total of 15-cm depth was sampled in each soil core. At collection, there was no evidence of root accumulation at the bottom of the mesocosm since the grasslands were still young. We collected 128 soil cores and kept them frozen at -25°C before processing. In the laboratory, the samples were defrosted. Then, each core was divided into two layers, that is 0–6 cm ('upper') and 6–15 cm ('lower layer'). We divided the cores into these two portions, because 0–6 cm reflect more accurately the most common soil depth encountered in urban roadsides, where these same seed mixtures are being tested (see Dietzel et al., 2023). A 6–15 cm depth is a less common soil depth in the urban settings where these grasslands are implemented. Each of the resulting 256 sub-cores was processed separately. We cleaned the roots thoroughly with tap water using 250 μm and 2-mm metal sieves.

After cleaning, the samples were stored at 5°C in a solution of 50:50 distilled water:ethanol 70%.

Since we studied young communities, and the samples were primarily fine roots, we assumed that the root system mainly consisted of absorptive roots (cf. Freschet & Roumet, 2017; McCormack et al., 2015). We measured traits of these roots from a representative subsample of each sub-core. Washed roots were gently pressed between two sheets of blotting paper to remove excess water and then weighed to determine the fresh biomass per subsample. Then, we soaked root subsamples in a 1 g·L⁻¹ toluidine blue solution for 20 min to increase contrast and obtain high-quality images. After staining, roots were rinsed and immediately scanned. For scanning, we spread them on a transparent acrylic tray filled with water and set a 16-bit grey-scale of 1200 dpi resolution, which was necessary due to the small size of the roots. We used an Epson V700 Photo scanner to produce digital images of all the samples. The following root traits were calculated (Freschet, Pagès, et al., 2021): (i) RTD (g·cm⁻³) as the ratio between root dry biomass and root volume; (ii) SRL (m·g⁻¹) as the ratio between root length and root dry mass and (iii) RLD (cm·cm⁻³) as the root dry mass per unit of soil volume (g·m⁻³) multiplied by the SRL. The calculations were conducted separately for the upper and lower soil layers and aggregated across the entire soil profile for overall responses.

After scanning, each root subsample and the corresponding primary sample were oven-dried at 60°C for 48 h and weighed with a high-precision scale to determine dry biomass. All digital images were analysed using WinRHIZO software (Pro STD4800, Regent Instruments Inc.). Total above- and below-ground biomass was expressed relative to the sampled area. In the case of total below-ground biomass, we pooled the biomass values from the two cores of each mesocosm and the two soil depths. Root biomass was kept separated according to the two soil depths to test for depth effects. The root:shoot ratio was calculated as the ratio between total below- and above-ground biomass.

2.5 | Statistical analyses

Our initial models for overall responses of biomass (above- and below-ground, root: shoot ratio) and root traits (RD, RTD, SRL and RLD) tested the main effects of RCP scenario, precipitation, and forb proportion, and potential interactions between RCP scenario and precipitation, forb proportion and RCP scenario, and forb proportion and precipitation. Initial statistical models considered up to two-way interactions among explanatory variables. To avoid pseudo-replication, below-ground biomass and trait data from the two mesocosm cores were pooled for all further analyses.

To account for the effect of soil layer on root biomass allocation and root traits (RD, RTD, SRL and RLD), we determined the direction and magnitude of the effect of depth on each trait using the relative interaction index (RII; Armas et al., 2004). This index provided a means to determine whether root trait values were higher or lower in the upper soil layer compared to the assessed lower soil layer; it was calculated as follows:

$$RII_{\text{trait}} = (Y_{\text{top layer}} - Y_{\text{bottom layer}}) / (Y_{\text{top layer}} + Y_{\text{bottom layer}}), \quad (1)$$

where $Y_{\text{top layer}}$ was the trait value at the upper soil layer and $Y_{\text{bottom layer}}$ the trait value at the lower soil layer. The index was calculated for each mesocosm. The RII had positive values when root traits were larger in the upper soil than in the lower soil layer, and negative values when the opposite was true. Utilizing t-tests, we assessed whether RII values were significantly different from zero (indicating a significant effect of soil depth). We also assessed the effect of RCP scenario, precipitation and forb proportion on the RII obtained.

Considering the nestedness of the mesocosms in ecotrons, we first formulated linear mixed models (LMM) using each chamber-ID as random intercept, resulting in singularity cases due to zero variance among the random factors. Therefore, we modelled all responses of interest with linear models (LM) and used beta regression, which is suitable for proportion data, to analyse the proportion of root biomass in the upper soil layer. We considered chamber-ID as a fixed factor to control for its impact, that is four levels for that factor, which is considered unsuitable for random intercepts (Bolker et al., 2009). Yet, the perfect linearity between chamber-ID and RCP scenario, including chamber as a fixed effect, resulted in the redundancy of model estimates. Therefore, we dropped 'chamber-ID' factor from all models.

We conducted likelihood ratio tests to identify the most parsimonious models until at least the main factors were considered as explanatory variables in the models. The final model structures are provided in the corresponding result tables of the supplement (Table S2). Significance levels were set in all cases at $p=0.05$, and the fit of the models was checked based on the behaviour of residuals with the DHARMa package (Hartig, 2022). In cases where heterogeneity of residuals was detected, response variables were log-transformed to improve model fit (root: shoot, RTD). Tests of pairwise comparisons and interactions were performed based on estimated marginal means with emmeans (Lenth, 2022). We conducted all analyses in R, version 4.1.2 (R Core Team, 2021).

3 | RESULTS

3.1 | Biomass allocation in response to climate change and functional composition

Biomass allocation to the above-ground compartment of the mesocosm grasslands was affected by climate change, that is it was larger under RCP8.5 and lower under reduced precipitation (Figure 2a,b). In contrast, climate change did not affect below-ground biomass (Figure 2d,e), while forb proportion, depicting 'grasslands' functional composition, was a strong driver of above- and below-ground biomass (Figure 2c,f). Grass-only communities produced more below-ground biomass than all other compositions and grasslands with an even forb:grass proportion had greater below-ground biomass than forb-only grasslands (Table S3). Although not statistically significant,

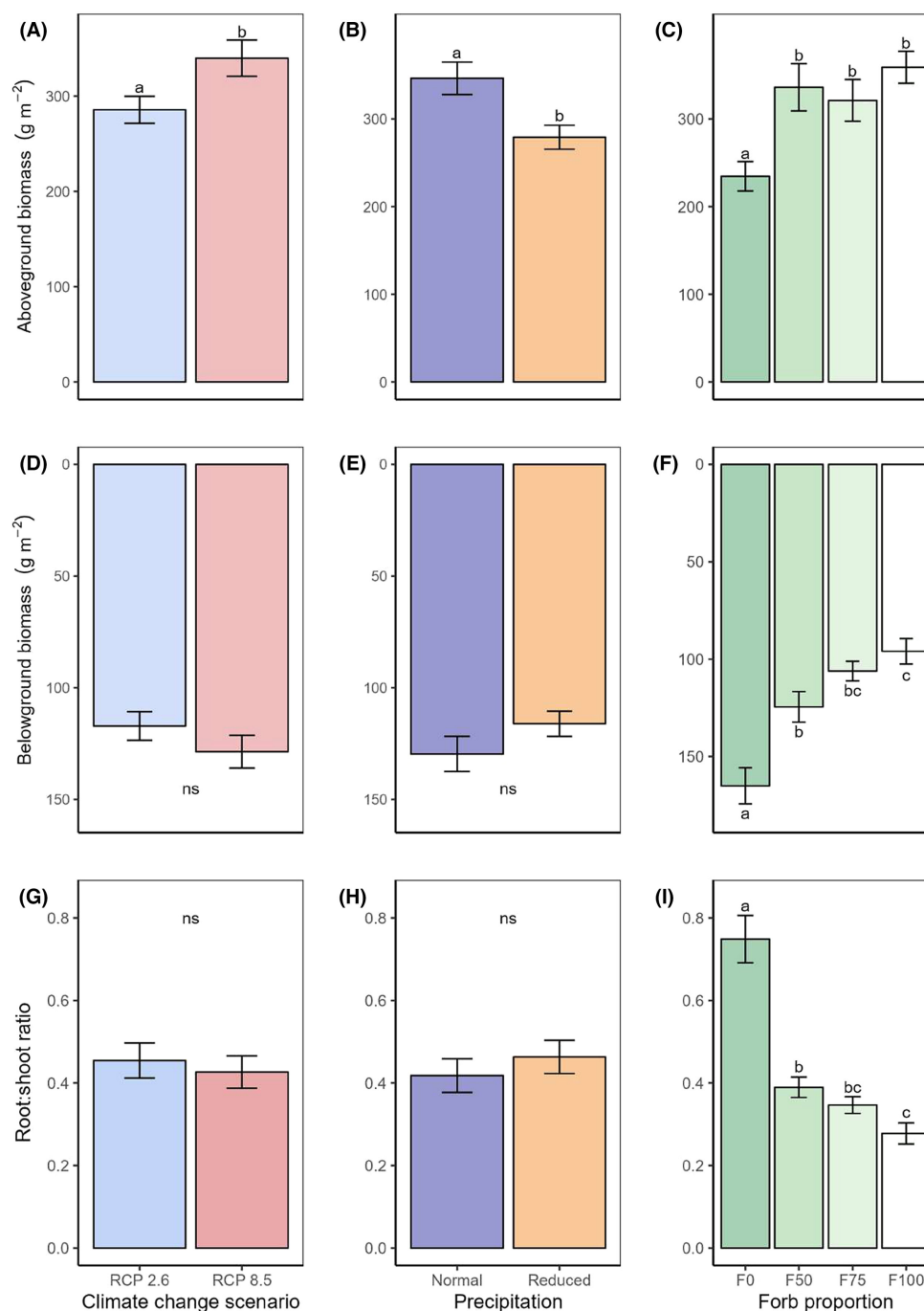


FIGURE 2 Biomass allocation of mesocosm grasslands responded to community composition rather than to simulated climate change in an ecotron. Shown are the above-ground biomass allocation (a–c), below-ground biomass allocation (d–f) and root:shoot ratio (g–i) of the grasslands as a response to climate change scenarios, precipitation and forb proportions (means \pm 1 SE; different letters depict significant differences; ns, not significant).

below-ground biomass tended to increase under RCP8.5 (i.e. elevated CO₂ and temperature), and to decrease under reduced precipitation, matching above-ground patterns. Above- and below-ground biomass showed an opposite response to forb proportion, whereby grass-only communities produced less above-ground biomass than the other communities, and the opposite occurred below-ground. Moreover, under RCP8.5, root allocation tended to be lower than

above-ground biomass, while under reduced precipitation, the communities allocated more biomass below-ground (Figure 2f,g). Only forb proportion controlled root:shoot ratio, with a larger ratio found in grass-only communities compared to all other functional compositions (Figure 2f; Tables S4 and S5). The interaction between climate change and functional composition did not affect biomass allocation in the mesocosms.

3.2 | Root trait responses to climate change and functional composition

RD did not respond to RCP scenario, but its values were larger under reduced precipitation, as well as in communities with higher proportions of forbs (Figure 3a–c). RTD was larger under RCP8.5, and lower

under reduced precipitation, while there was no clear response to forb proportion (Figure 3d–f). SRL was not responsive to RCP scenario or precipitation, but its value decreased with increasing forb proportions in the community (Figure 3g–i). RLD was lower under RCP8.5 and reduced precipitation as well as with increasing forb proportion in the community (Figures 3j–l); see Table S6 for complete model outputs.

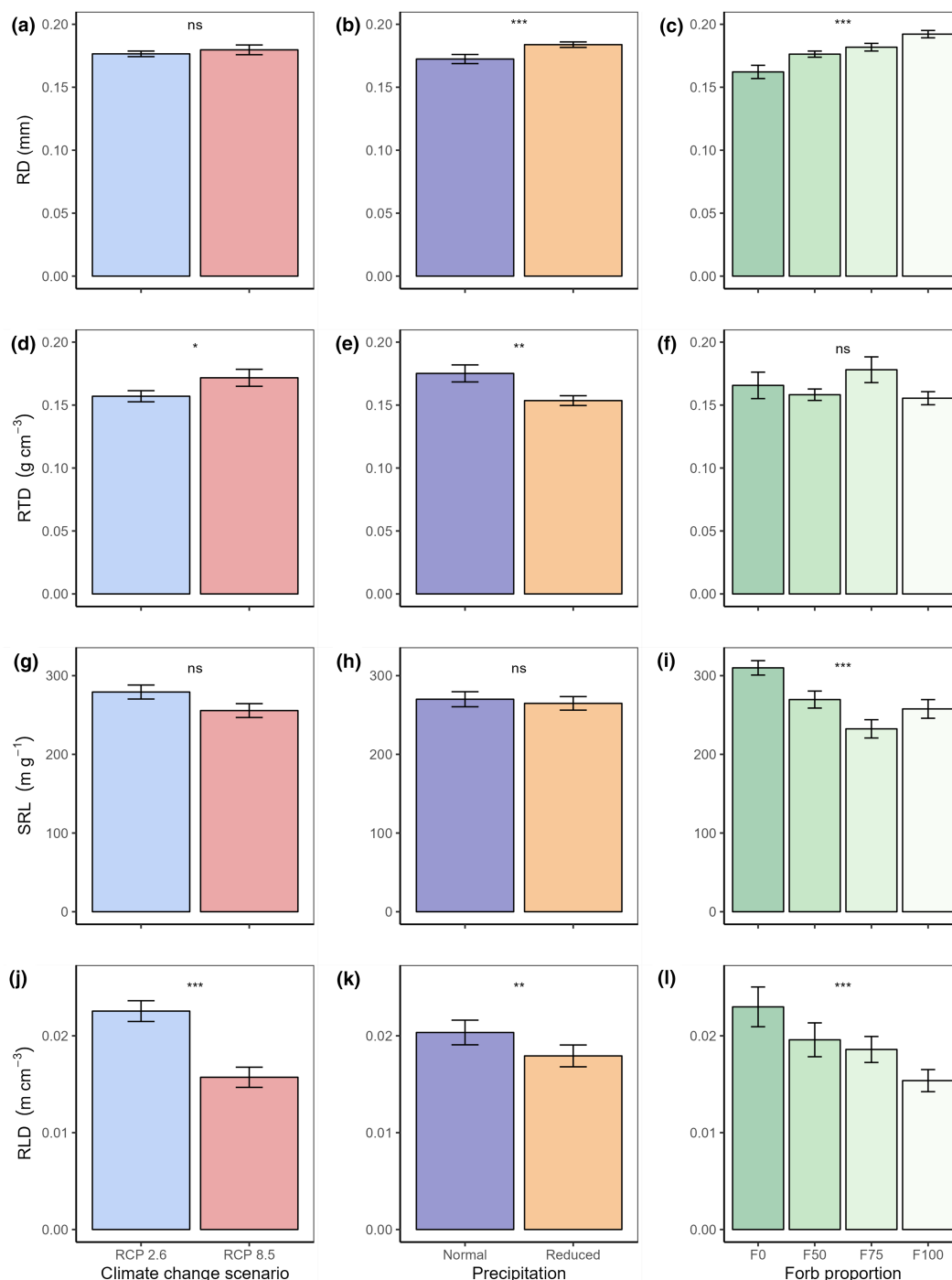


FIGURE 3 Absorptive root traits of mesocosm grasslands respond to climate change and functional composition in an ecotone. Main effects of climate change scenario (a, d, g, j), precipitation (b, e, h, k) and forb proportion (c, f, i, l) on root traits of experimental grasslands (RD, root diameter; RLD, root length density; RTD, root tissue density; SRL, specific root length; means \pm 1 SE; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant).

3.3 | Functional composition modulates the response of root traits to climate change

There were interactive effects of forb proportion with either RCP scenario or precipitation on the root traits of the mesocosm grasslands (Figure 4). Although not strong in all detected interactions, forb-only communities changed the direction of the effect in comparison to the other functional compositions (Table S6). While RD was generally larger under reduced precipitation in forb-only communities, RD did not change in response to reduced precipitation. RTD was generally positively affected by RCP8.5, but in forb-only communities, RTD was lower under RCP8.5. Furthermore, SRL tended to decrease under RCP8.5 in most communities, but in forb-only SRL was higher under RCP8.5 than under RCP2.6. Finally, reduced precipitation negatively affected RLD, whereas in forb-only communities, RLD increased under reduced precipitation.

3.4 | Below-ground responses to climate change and functional composition depend on soil depth

RCP8.5 led to less root biomass allocation in the top soil layer, while precipitation had no effect (Figure 5a,b). Furthermore, forb-only communities had a larger proportion in the upper soil layer (0–6 cm) than grass-only communities (Figure 5c; Table S7).

Differential responses of root traits were found according to soil layers. Values of RD were generally larger at the lower soil layer but did not change in response to RCP scenario. Under normal precipitation, RD values were significantly larger than under reduced precipitation in the lower soil layer (Figure 6a,b). In grass-only (F0) and F50 communities, RD was larger in the lower soil layer, whereas in forb-dominated communities (F75 and F100), RD did not change with depth (Figure 6c). Overall, RTD had larger values in the upper soil layer, while the magnitude of such a pattern was significantly larger under RCP2.6 than RCP8.5 (Figure 6d–f). Under climate-change-related conditions, SRL was not affected by soil depth, while F50 and F100 communities had larger SRL either in the upper or lower soil layers, respectively (Figures 6g–i). Finally, RLD was always larger in the upper soil layer but did not vary according to RCP scenario, precipitation or forb proportion (Figures 6j–l).

4 | DISCUSSION

4.1 | Climate change and functional composition affect biomass allocation above-ground but not below-ground

Climate change only affected biomass allocation above-ground in the mesocosm grasslands. In contrast, biomass tended to increase below-ground under RCP8.5 and to decrease under reduced

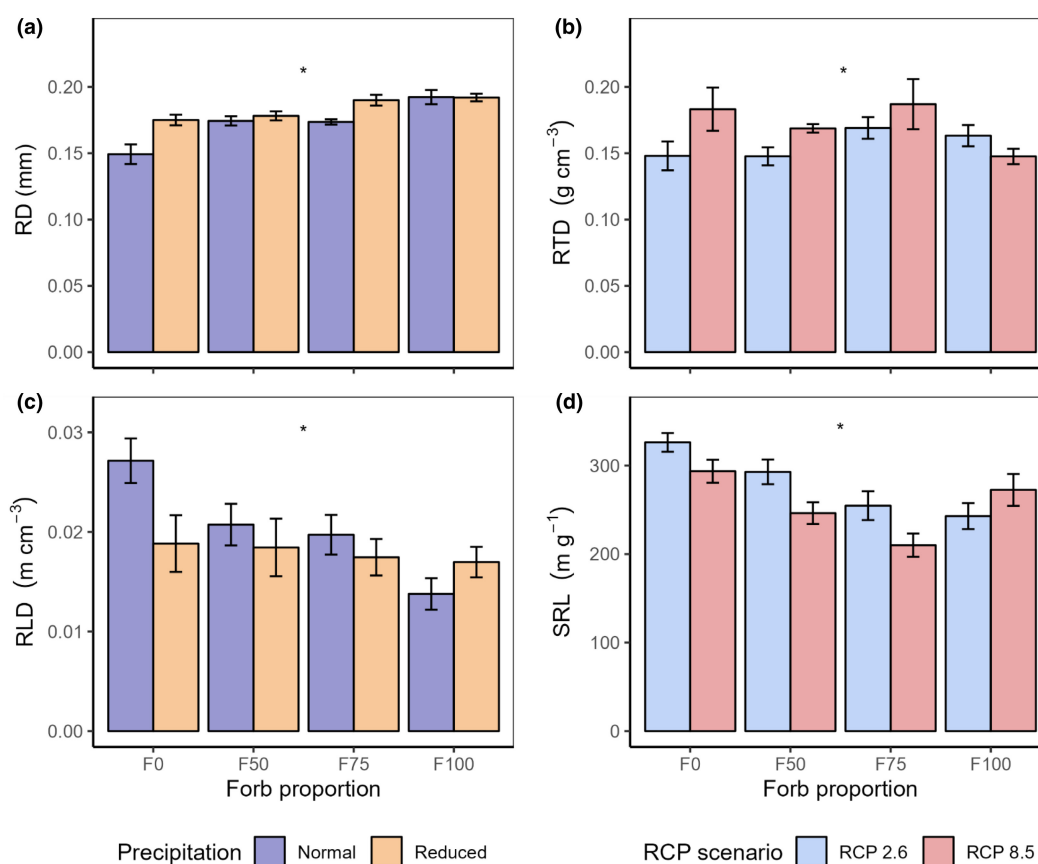


FIGURE 4 Interactive effects of forb proportion and precipitation on (a) root diameter (RD) and (c) root length density (RLD), and forb proportion and climate change scenario on (b) root tissue density (RTD) and (d) specific root length (SRL). Error bars depict means ± 1 SE (*, $p < 0.05$).

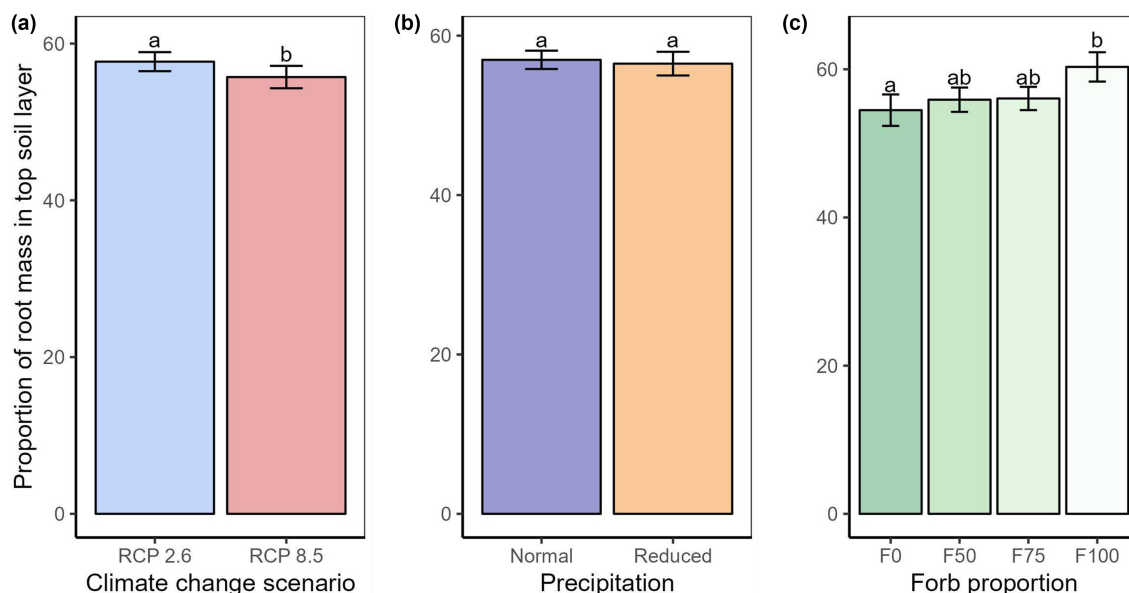


FIGURE 5 A large share of below-ground biomass accumulated in the upper soil layer in mesocosm grasslands. The proportion (expressed in %) of root biomass in the grasslands' upper soil layer (0–6 cm) is shown in response to (a) climate change scenario, (b) precipitation and (c) forb proportion. Proportions were modelled using beta regression (means \pm SE; different letters depict significant differences).

precipitation. Our results confirm previous findings showing that a combination of eCO_2 and eT did not affect root biomass and other below-ground responses (Mueller et al., 2018). However, increased below-ground biomass may still be observed under climate change (especially eCO_2 ; Arndal et al., 2018), and the lack of consensus in the responses to eCO_2 and eT suggests that factors other than climate might play a role in the allocation of below-ground resources, for example, grassland age, nutrient status and soil texture (Mueller et al., 2018). We also found that the root:shoot ratio tended to decrease under RCP8.5, contradicting previous findings (Arndal et al., 2018; Nie et al., 2013), and resulted from the disproportionate increase in above-ground biomass under eCO_2 and eT compared to below-ground biomass as well as the fact that root:shoot ratio mainly responded to functional composition. Similarly, decreased precipitation tended to reduce root biomass, although the response was weak, likely due to the low intensity and duration of the imposed water stress (Zhou et al., 2018). In general, drought reduces root biomass of grassland species through hydraulic failure and adverse effects on overall plant photosynthesis (Arndal et al., 2018; Lozano et al., 2020; Wang et al., 2020), and delayed carbon allocation to the roots under water stress (Hasibeder et al., 2015). Even though reduced precipitation negatively affected above- and below-ground biomass in the mesocosm grasslands, the decrease in above-ground allocation under reduced precipitation was greater than below-ground. Thus, the root:shoot ratio tended to increase under water stress, though only slightly changing.

We showed that functional composition (i.e. forb proportion) predicted below- and above-ground biomass allocation, being the most important root:shoot ratio driver. While communities containing more species (Bakker et al., 2021), or certain functional

compositions (Bessler et al., 2009; Ravenek et al., 2014) produce more biomass, our species-poor grass-only community produced most root biomass. This can be explained by the large productivity of grasses together with the variability of root traits within this functional type (Bakker et al., 2019), which promoted a fast use of resources in the available soil profile and increased biomass production and water uptake even under water stress (Bakker et al., 2021; Fischer et al., 2019). In contrast, forb-dominated communities, containing legumes that enhance grassland performance (Bakker et al., 2019; Marquard et al., 2009), produced less root biomass. Indeed, a negative effect of legumes on root biomass production was previously described (Bessler et al., 2009) and might explain the contrasting response in forb-dominated vs. grass-only communities.

4.2 | Below-ground changes resulted from modified root traits and functional composition

In contrast to biomass, root traits showed rapid responses to RCP8.5, as RTD was larger in grasslands developing under this climate-change scenario. This suggests an investment in high-quality roots (i.e. with higher carbon content) associated with a potential conservative resource-use strategy (Freschet et al., 2017), by investing a higher amount of C for long-lasting tissue (Nie et al., 2013), instead of increasing SRL or RLD to enhance soil exploration (Ma, 2021; Mueller et al., 2018). Water reduction also produced significant responses in roots traits, that is increasing RD, and decreasing RTD and RLD, as shown by Lozano et al. (2020) and Zhou et al. (2018). Notably, high RD impedes hydraulic damage of roots by anatomical adjustments, including long-lived fine roots (Lozano et al., 2020; Weemstra et al., 2016).

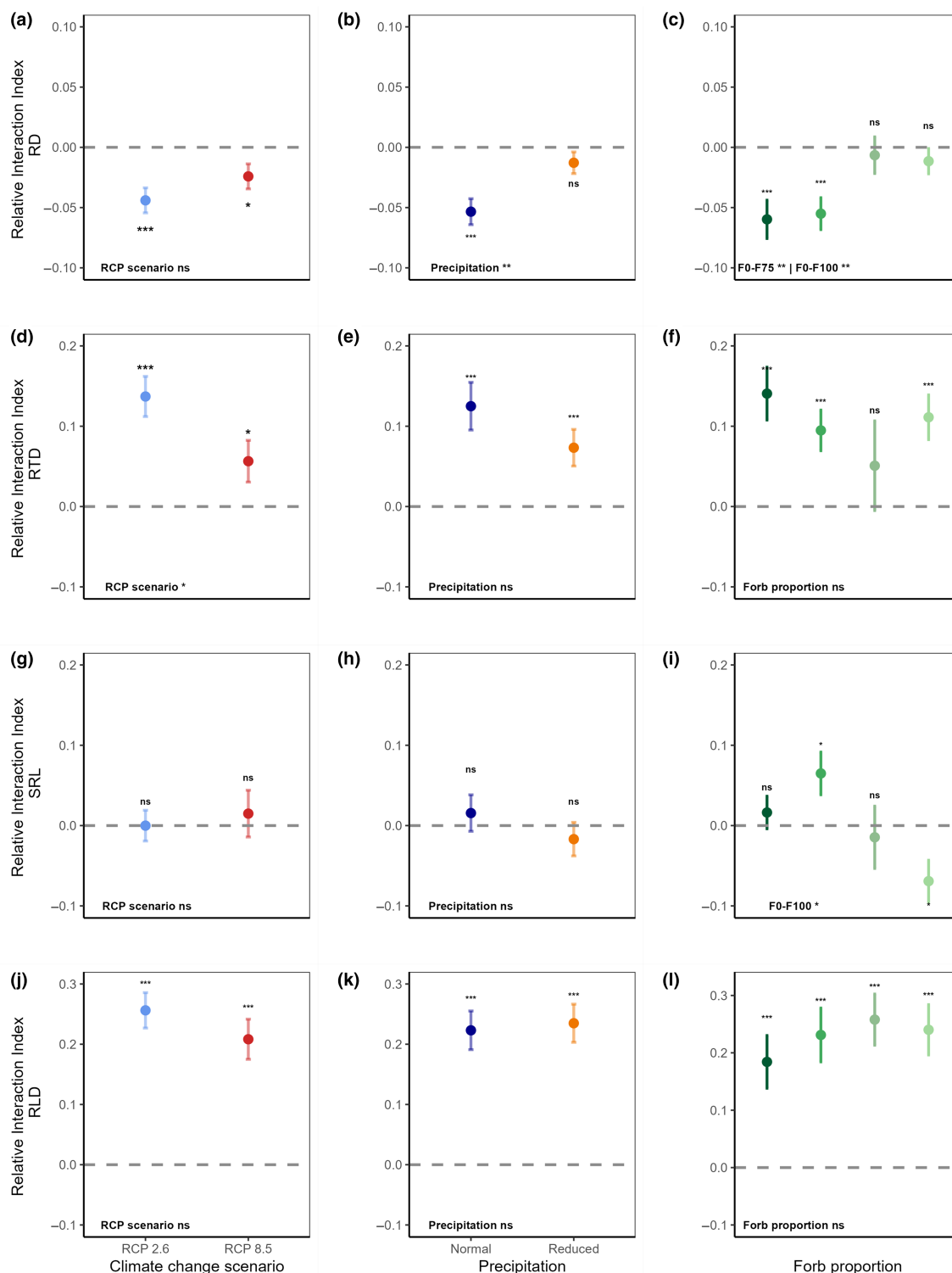


FIGURE 6 Variation with soil depth of absorptive root traits assessed for two soil layers (0–6 cm, upper soil layer; 6–15 cm, lower soil layer) in mesocosm grasslands in response to climate-change scenario, precipitation and forb proportion. Assessed traits were root diameter (RD; a–c), root tissue density (RTD; d–f), specific root length (SRL; g–i) and root length density (RLD; j–l). The relative interaction index (RII) compares trait values at the upper vs. lower soil layer. Positive values indicate higher trait values in the upper than in the lower soil layer, while negative ones show the opposite. RII above or below zero (grey dashed line) differs significantly over soil layers (means ± 1 SE; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant; one-sample t -tests). Effects of climate change, precipitation and forb proportion are presented at the bottom left of each panel (pairwise t -test for climate change scenario and precipitation, and ANOVA for forb proportion). Note the difference in y -scales.

Root traits were also controlled by grassland functional composition. Traits such as low RD, and high SRL and RLD depict strategies for soil exploration in grasses (Comas et al., 2013; Ravenek et al., 2014; but see de Vries et al., 2016), whereby a high RLD underscores their competitiveness for nutrients and water (Ravenek et al., 2014). Interestingly, climate-change effects interacted with functional composition to alter root traits. Forb-only communities were shifting the responses of root traits to either RCP scenario or precipitation, suggesting that grasses largely determined the community response of root traits to climate change. In mixtures containing grasses and forbs, root production of certain grass species may have been favoured, increasing their root:shoot ratio without affecting the growth of other species (Mommer et al., 2010). Thus, by becoming more abundant below-ground, grass roots drive the community traits under climate change to values closer to those of grass-dominated communities.

4.3 | Soil-depth effects on root biomass and traits in response to climate change and functional composition

The mesocosm grasslands accumulated the largest share of the biomass in the upper soil layer. Even though we included various species within each functional type, thus reflecting diversity in rooting depth and morphology, the communities densely occupied the upper soil layer as typical for near-natural and experimental grasslands (Jackson et al., 1996; Ma, 2021; Mommer et al., 2010). An increased share of root biomass in the lower soil layer under RCP8.5 suggests greater soil exploration for water and nutrients in deeper soil layers under eCO₂ and eT to sustain increased above-ground biomass (Arndal et al., 2018; Iversen, 2010; Nie et al., 2013). Conversely, reduced precipitation did not change the share of root biomass in the upper soil layer.

Roots tend to grow toward the water supply (Cassab et al., 2013; Eapen et al., 2005), which came exclusively from above in our experiment. Thus, rainfall is captured by a dense root system in the upper soil layer. Furthermore, the functional composition also controlled the proportion of root biomass allocation across the soil layers. The largest share of biomass in the upper layer occurred in forb-only compared to grass-only communities. Whereas this seems counter-intuitive, all mixtures containing forbs consisted of 19% annuals and 19% legumes, some primarily shallow rooters (cf. Fry et al., 2018), thus explaining the preferential top-layer allocation of roots in the forb-only grasslands at the time of harvest. Moreover, because our experiment represented first-year grassland communities and the soil volume was not yet fully exploited by the roots, we cannot rule out that biennial and perennial forbs would develop deeper roots over time and change root allocation across the soil profile, with a rather increasingly exploitative strategy (e.g. higher root mass and length per unit soil volume, higher root to shoot ratio) allowing for optimized use of limited resources in the overall shallow soil profile of the mesocosms (Fry et al., 2021).

RD values were larger in the lower soil layer, especially for grass-dominated communities. Fine roots of grasses were more abundant at the upper soil layer, causing more water scarcity in lower soil layers and fostering larger RD values. A higher value of SRL in the upper soil layer also indicated that grass communities preferentially exhibited an acquisitive resource strategy in the upper soil. In contrast, forb grasslands depicted a more acquisitive strategy in the lower soil layer, where some species were more likely to develop at harvest time. A larger RLD in the upper soil layer underlines that resource uptake by the plant community mainly occurred in the soil subsurface (Mommer et al., 2010). While grasslands grown in shallow mesocosms usually have larger RLD (Fry et al., 2021), due to reduced availability of soil volume and thus of resources that will be rapidly depleted, we posit that urban grasslands also have high RLD because the lack of space forces roots to become increasingly exploitative, especially in periods of water scarcity.

5 | STUDY LIMITATIONS

We conducted a mesocosm experiment in a strictly controlled environment ('ecotron') to simulate urban grasslands implemented in road verges and exposed to climate change. While we acknowledge some of the responses may not be generalized as long-lasting responses (de Boeck et al., 2015), our study combined three components of climate change and manipulated the functional composition of the grasslands, which is still rare. Despite the direction of changes in biomass and root:shoot ratio may shift over time (Arndal et al., 2018), given that the root system was in the first year of development, nutrients were not depleted, and space not fully occupied. Still, our findings can help to improve the understanding and modelling of ecosystem processes and their response to climate change (Bardgett et al., 2014).

Moreover, plants grown in pots under eT might be exposed to slightly higher temperatures below-ground (Poorter et al., 2012), which could hamper comparisons with natural or semi-natural grasslands. Nonetheless, a warm and shallow soil compartment is typical for roadsides with soil sealing and high exposure to urban heat. Thus, our results are realistic to the conditions experienced by urban grasslands.

6 | CONCLUSIONS

This study contributes to understanding grassland responses to multi-factorial climate change and its interaction with grassland functional composition. Above-ground responses to climate change occur early in developing grasslands, while changes in below-ground biomass are less evident. Root traits respond rapidly to climate change and are thus sensitive predictors of below-ground responses of young grasslands. A warmer and CO₂-richer climate favours conservative root strategies, that is long-lasting tissues and deeper soil exploration, while water stress promotes thicker roots to

avoid vessel damage. Importantly, functional composition controls biomass allocation, determines root traits and modulates trait responses to climate change.

Thus, taxonomic and functional community composition should be considered when conducting climate-change experiments. This is also true for adapting urban grasslands to climate change. We highlight the importance of root traits in ecosystem functioning, particularly in urban ecosystems, where the effects of climate change are enhanced. Understanding grassland responses to climate change is crucial for predicting their functioning and the delivery of ecosystem services and improving the design of seed mixtures and grassland management in a changing world.

AUTHOR CONTRIBUTIONS

Sandra Rojas-Botero, Leonardo H. Teixeira, Johannes Kollmann and Soizig Le Stradic conceived the ideas and designed the study; Sandra Rojas-Botero, Leonardo H. Teixeira and Soizig Le Stradic conducted the experiment; Sandra Rojas-Botero, Paula Prucker, Veronika Kloska, Leonardo H. Teixeira and Soizig Le Stradic collected the data; Sandra Rojas-Botero and Paula Prucker analysed the data; Sandra Rojas-Botero led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available from the mediaTUM Repository <https://doi.org/10.14459/2023MP1706017> (Rojas-Botero, Teixeira, Prucker, et al., 2023).

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SUPPORTING INFORMATION

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

Appendix A. Description of the ecotron facility 'TUMmesa'.

Figure S1. View of the ecotron facility ('TUMmesa') of the Technical University of Munich, campus Weihenstephan (Freising, DE).

Figure S2. Characteristics of the ecotron facility ('TUMmesa') used for the mesocosm experiment on functional grassland responses to climate change.

Figure S3. Walk-in chambers of the ecotron facility 'TUMmesa' used for the experiment with grasslands with four functional compositions, subjected to two climate scenarios.

Appendix B. Extended methods.

Figure S4. Climatic conditions during the mesocosm experiment on grassland performance in four chambers ('C') within the ecotron TUMmesa.

Figure S5. Experimental design deployed in the walk-in ecotron TUMmesa to test the effect of climate change (RCP 2.6 vs. 8.5), precipitation (normal vs. reduced), and four functional compositions (forb proportion F0, F50, F75, F100) on biomass allocation and root traits in mesocosm grasslands.

Table S1. Species used in the mesocosm grasslands designed for temperate urban environments of Central Europe to test the effects of climate-change scenario, precipitation, and functional composition ('forb proportion') on biomass allocation and root traits.

Appendix C. Extended results.

Table S2. List of models considered in the study after model simplification.

Table S3. Pairwise comparison of the effects of functional composition ('forb proportion') on above- and below-ground production of the mesocosm grasslands.

Table S4. ANOVA tables displaying the effect of climate change scenario (RCP scenario), precipitation, and functional composition (forb proportion) on root:shoot ratio of mesocosm grasslands.

Table S5. Pairwise comparison of the effects of functional

composition ('forb proportion') on the root:shoot ratio of mesocosm grasslands.

Table S6. Summary tables of the best models describing the response of morphological root traits, across the soil profile in mesocosm grasslands ($n=64$) to climate change scenario (RCP; two levels: 2.6 and 8.5), precipitation (Precip; two levels: normal and reduced), forb proportion (F; four levels: F0, F50, F75, F100).

Table S7. Summary table of the best model describing the response of proportion of root biomass in the upper soil layer (i.e. 0–6 cm) to climate change scenario (RCP scenario), precipitation, and functional composition (forb proportion) in mesocosm grasslands.

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