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REGULAR ARTICLE

Grassland species root response to drought: consequences for soil carbon and nitrogen availability

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Abstract
Background and Aims Root traits are increasingly used to predict how plants modify soil processes. Here, we assessed how drought-induced changes in root systems of four common grassland species affected C and N availability in soil. We hypothesized that drought would promote resource-conservative root traits such as high root tissue density (RTD) and low specific root length (SRL), and that these changes would result in higher soil N availability through decreased root N uptake, but lower C availability through reduced root exudation.
Methods We subjected individual plants to drought under controlled conditions, and compared the response of their root biomass, root traits, and soil C and N availability, to control individuals.
Results Drought affected most root traits through reducing root biomass. Only SRL and RTD displayed plasticity; drought reduced SRL, and increased RTD in small plants but decreased RTD in larger plants. Reduced root biomass and a shift towards more resource-conservative root traits increased soil inorganic N availability but did not directly affect soil C availability.
Conclusions These findings identify mechanisms through which drought-induced changes in root systems affect soil C and N availability, and contribute to our understanding of how root traits modify soil processes in a changing world.

Keywords Aboveground-belowground linkages · Plant functional traits · Plasticity · Soil microbial properties · Soil processes · Climate change

Introduction

Ecologists are increasingly using plant traits for explaining and predicting ecosystem functioning. These approaches generally use the leaf economics spectrum (Wright et al. 2004), where exploitative leaf traits that maximise photosynthesis, such as high specific leaf area and leaf nitrogen content, maximize plant growth and nutrient uptake, and accelerate rates of soil nutrient and carbon cycling (e.g. Fortunel et al. 2009; Garnier et al. 2004; Grassein et al. 2015; Laughlin 2011; Orwin et al. 2010). Recently, ecologists have shifted their focus from aboveground plant functional traits to belowground traits for explaining soil and ecosystem processes (Bardgett et al. 2014). In contrast to leaves, roots are in contact with the soil and modify the soil environment directly by penetrating the soil, taking up water and

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nutrients, releasing root exudates, and through the process of root turnover (Bardgett et al. 2014). Therefore, root functional traits might be better predictors of soil and ecosystem processes than leaf traits. Indeed, in recent work, root traits have been shown to explain a range of ecosystem properties and processes better than leaf traits. For example, root traits have been found to explain soil microbial community composition better than leaf traits in field and pot experiments (Legay et al. 2014; Orwin et al. 2010), as well as the availability of inorganic N and rates of denitrification and nitrification (Cantarel et al. 2015; Moreau et al. 2015; Orwin et al. 2010), and plant performance at a population level (Schroeder-Georgi et al. 2016).

As a consequence of this growing body of evidence that links root traits to measures of ecosystem functioning, it has been proposed that root traits are central to ecosystem response to climate change (Bardgett et al. 2014). Root system properties have been shown to respond to climate change (Beidler et al. 2015; Nie et al. 2013), and these changes in root traits might have cascading effects on soil properties and ecosystem functioning. Particularly drought, which is expected to increase in some regions with global climate change, can have strong impacts on soil functioning by killing soil microbes and animals and causing a flush in C and N mineralization upon rewetting (as reviewed by Borken and Matzner 2009). Drought also strongly impacts on plant communities and can cause changes in aboveground and belowground species abundances through impacting on individual plant growth. Moreover, drought can alter root system architecture, and induce phenotypic plasticity in root traits. Thus, because drought simultaneously affects root systems and soil nutrient availability, there is the potential of these plant and soil responses to interact: roots can directly affect soil properties via uptake of water and nutrients, but roots can also indirectly affect soil nutrient and C availability via root exudation and turnover. In addition, root traits can display plasticity to changes in soil nutrient concentrations (e.g. Hodge 2004; Lambers et al. 2003).

Plants can cope with drought through drought avoidance and drought tolerance strategies, which both depend on a combination of different functional traits. Drought avoidance strategies include high water use efficiency and low stomatal conductance through dense leaves, and investing in high root to shoot ratio; drought tolerance involves increased osmoprotectants and accumulation of carbohydrates in plant tissues (Brunner et al. 2015; Kooyers 2015). These strategies are likely related to plant functional traits associated with the trade-off between fast growth or acquisitive-resource-use strategies and slow growth or conservative-resource-use strategies: slow growth confers stress resistance by reducing C demand for growth, thereby allowing for greater investment in defence traits (Chapin et al. 1993). In support of this hypothesis, drought tolerance through foliage senescence has been linked to resource-conservative growth strategies (Perez-Ramos et al. 2013), and traits like high root tissue density (RTD) and root dry matter content (RDMC) have been linked to drought resistance (Fort et al. 2013; Ryser 1996; Tjoelker et al. 2005; Wahl and Ryser 2000). However, in contrast with this hypothesis, thinner roots and the ability to elongate roots into deeper soil layers—both root traits linked to resource-acquisitive strategies—have been linked to drought avoidance and maintained growth under drought conditions (Comas et al. 2013; Padilla et al. 2013; Perez-Ramos et al. 2013; Zwicke et al. 2015).

In addition to overall growth responses of plants to drought, plants can display considerable phenotypic plasticity under drought by allocating more C to their roots (Poorter et al. 2012), but also by adjusting root morphological and chemical traits. For example, Padilla et al. (2013) found that grassland species that increased their specific root length (SRL) also increased their aboveground growth under pulsed water supply, which supports the above findings that resource-acquisitive plants are best at drought-avoidance strategies. In contrast, and in line with the drought-tolerance strategy, fine roots can be the first to be sacrificed under drought, thus reducing SRL (Brunner et al. 2015). Drought can also increase nonstructural carbohydrates in roots (Brunner et al. 2015; Zwicke et al. 2015), thereby increasing RDMC (Zwicke et al. 2015) and potentially RTD.

These drought-induced changes in plant growth and root traits likely have consequences for soil N and C availability. Reduced plant growth under drought conditions implies lower plant uptake and use of water and N. In addition, if drought promotes acquisitive traits such as high SRL, this might result in higher uptake of soil water and N when drought conditions are relieved. In contrast, if drought increases resource-conservative traits such as high RTD, plant uptake of water and N might be reduced upon rewetting (De Vries and Bardgett 2016; Grassein et al. 2015). In addition, it has been suggested that roots with resource-conservative traits would have lower rates of root exudation than those with resource-exploitative traits (Roumet et al. 2008). Root traits might indirectly affect C and N availability through affecting microbial activity or community composition; for example, resource-conservative root traits might select for microbial communities with a higher C/N ratio through low-N litter and root exudates (Drake et al. 2013), or associations with mycorrhizal fungi (Bardgett et al. 2014). If drought causes root senescence, then mineralization of dead root tissue after rewetting has the potential to either decrease or increase concentrations of dissolved organic C (DOC), dissolved organic N (DON) and inorganic N in soil (Freschet et al. 2012; Freschet et al. 2013). While it is well known that drought followed by rewetting causes a flush in soil C and N mineralization, it is not known whether concurrent root responses to drought mitigate or exacerbate this flush.

Here, our aim was to quantify root response to drought of four common British grassland species of contrasting ecological strategies, and to assess whether changes in root biomass and traits had cascading effects on C and N availability in soil. We hypothesized that 1) resource-acquisitive root traits such as high SRL would be reduced by drought, while resource-conservative traits such as high RTD would be promoted; 2) the reduction in root biomass and resource-acquisitive traits as a result of drought would result in higher soil N availability through decreased root N uptake; and 3) the reduction in root biomass and resource - acquisitive traits would result in lower soil C availability through reducing the microbial C/N ratio and lower rates of root exudation. We also hypothesized that drought would least affect aboveground biomass and root traits of the more slow-growing species compared to fast-growing species We tested these hypotheses by growing individual plants in pots under controlled conditions, subjecting them to drought, and comparing the response of their aboveground biomass, root biomass, root traits, and soil C and N availability, to control individuals over time.

Materials and methods

Experimental set up

We chose four common temperate grassland species based on previous knowledge of their root traits and growth strategies, namely *Anthoxanthum odoratum*, *Dactylis glomerata*, *Leontodon hispidus*, and *Rumex acetosa*. These four species represented two grasses and two herbs of differing growth strategies, with, of the grasses, *A. odoratum* being slower growing than *D. glomerata*, and, of the herbs, *L. hispidus* being slower growing than *R. acetosa* (Suppl. Table 1). We selected these four species, based on their widely differing root traits, from a species pool of 24 grassland species of which we previously measured root and leaf traits (Suppl. Table 1). The two grasses were characterized by high SRL, but *D. glomerata* had higher RTD and RDMC, and lower root nitrogen content (RNC) than *A. odoratum*. The two herbs had low SRL, but *R. acetosa* had considerably higher RTD and RDMC, and lower RNC, than *L. hispidus*.

Soil (silt loam of the Brickfield 2 association, %N 0.19, %C 2.35, pH 4.75, as in De Vries and Bardgett (2016)) was collected from the Lancaster University Field station (54 ° 1′ N, 2 ° 46’W a.s.l), sieved (4 mm mesh size), homogenized, and stored at 4 °C until use. Individual plants were germinated in plug trays (in the same soil used for the main experiment) and transferred into pots after four weeks. Pots (1 L, 13 cm diameter, 10 cm height) were filled with 750 g of sieved fresh soil with one individual plant planted into the centre of each for three replicate pots. Pots were arranged in a fully randomized block design, with one replicate for each treatment in each block, in the glasshouse (16 h/8 h and 20 °C/16 °C day/night). Pots were weighed daily and their moisture content was adjusted gravimetrically with an accuracy of ±0.5 g. All pots were kept at 60 % water holding capacity (WHC) for four weeks before the drought event (plants were 10 weeks old when subjected to drought). During the two-week drought period, which started when the plants were 10 weeks old and ended when they were 12 weeks old, half of the pots were droughted to 30 % WHC and then kept at this value for two weeks. Although the different species might have reached this moisture level after slightly different time periods, keeping the soil at the same moisture level rather than applying equal water stress to the plants more realistically mimics cessation of rainfall under field conditions. At the end of the two week drought, the droughted pots were carefully rewetted to 60 % WHC, ensuring no water was leached from the pots. The control pots were kept at 60 % WHC throughout the experiment.

On five sampling dates, 24 pots (four species x drought vs. control x three replicates (blocks)) were destructively harvested, resulting in a total number of 120 pots. Sampling dates were before the drought (plant age of 10 weeks), at the end of the drought (12 weeks), two weeks (14 weeks), six weeks (18 weeks) and ten weeks (22 weeks) after ending the drought. Aboveground plant parts were cut to the base, dried at 60 °C for 48 h, weighed, and ground for further analysis. Roots were carefully separated from soil, washed, and stored in 15 % ethanol until further analysis. Soil was sieved (4 mm mesh) and kept at 4 °C until further analysis.

Soil analyses

Water-extractable inorganic N (NO_3^- and NH_4^+), dissolved organic N (DON), and DOC concentrations were analysed after shaking 5 g of soil in 35 ml of Milli-Q water 10 min. DOC extracts were filtered (0.45 μm) and total and inorganic C were determined using a Shimadzu 5000 A TOC analyser, after which DOC was then calculated by subtracting the amount of inorganic C from the total C in the extracts. Total N was determined by oxidation with potassium persulfate (Ross 1992), and measurement of the NO_3^- and NH_4^+ was determined using a Bran + Luebbe Autoanalyser 3. DON was calculated by subtracting the amount of inorganic N in un-oxidised extracts from the total N in oxidized extracts.

Microbial biomass C and N were measured using the chloroform fumigation extraction method as described by Brookes et al. (1985). Briefly, 5 g of fumigated and un-fumigated soil samples were extracted in 25 ml of 0.5 M K_2SO_4 by shaking for 30 min. Dissolved organic carbon (DOC) and total N were measured by a Bran + Luebbe AutoAnalyser 3, and microbial C and N was calculated as the difference between fumigated and un-fumigated samples, using a k_{EN} of 0.54 and a k_{EC} of 0.35. All measures done on fresh soil were calculated per gram dry soil, as determined gravimetrically after 24 h oven-drying at 105 °C.

Plant analyses

Analysis of structural root traits (total root length, average root diameter and root volume) was carried out using the WinRhizo® root analysis software (Regent Instruments Inc., Canada) and an Epson flatbed scanner, using the batch analysis feature. Whole root systems were scanned; while fine roots (<2 mm) arguably respond stronger to environmental change than coarse roots (>2 mm), only a small fraction of grassland species roots are coarse (Suppl. Figure 1), and the ratio between fine and coarse root length was not affected by drought (Suppl. Figure 1). After analysis, roots were blotted dry, weighed, dried at 60 °C for 24 h, re-weighed, and analysed for root N content (RNC) and root carbon content on an Elementar Vario EL elemental analyzer (Hanau, Germany). SRL was calculated by dividing the dry biomass by the total root length (cm g^{-1}). RTD was calculated by dividing the weight of the dry biomass by the root volume (g cm^{-3}). Dry matter content of aboveground tissues and roots (RDMC) was calculated as dry weight divided by fresh weight.

Calculations and statistical analyses

All variables were checked for normality and homogeneity of variances, and log transformed if necessary. To assess the effects of species, time, and drought on soil properties, plant aboveground and root biomass, root traits, and soil properties, we used three-way analysis of variance (ANOVA) with replicate (block) as random effect, using the aov function in R. In addition, we calculated the effect of drought relative to control treatments for all these properties as follows:

$$\text{Relative drought effect (RDE)} = \frac{\text{Drought-Control}}{\text{Control}}$$

Here, the value for drought is the individual observation, and the value for control is the mean of the three control replicates of the same treatment. When the 95 % confidence interval of this RDE did not overlap with zero, the difference between drought and control was statistically significant. We also analysed global treatment effects on the RDE using two-way ANOVA with factors species and time. To assess whether changes in root traits in response to drought could be attributed to true plasticity or to differences in ontogeny as a result of drought, we fitted linear mixed effects models for each trait, with root biomass and drought as explanatory variables, and block as a random term, using the package nlme in R. We also analysed changes in suites of root traits by PCA (vegan package in R).

We performed Structural Equation Modelling (SEM) to test how changes in root biomass and root traits affected soil microbial biomass and soil C and N availability. SEM is a robust statistical method to test how experimental data fit a hypothesised causal structure that is well suited for investigating interactions between multiple traits and ecosystem functioning based on prior knowledge (Garcia-Palacios et al. 2013; Grace 2006). We constructed an a priori model based on our hypotheses and theoretical knowledge of plant-microbe controls on C and N availability in soil (Fig. 1). Data were log-transformed where necessary, and rescaled to correct for large differences in variances. We fitted our a priori model to the rescaled data using the lavaan package in R. First, we fitted models using PC axes 1 and 2 scores of all root traits. Then, we fitted a model including the root traits that showed the strongest relationships with root biomass and inorganic N as demonstrated by correlation analysis. Finally, we fitted models to all traits separately. We used model modification indices and stepwise removal of non-significant relationships, and tested the effect of these removals on AIC and model fit using a likelihood ratio test, as in De Vries & Bardgett (2016). After obtaining the final, most parsimonious models, we re-analysed the fit of those models by using a multi-group approach with drought and control as groups, allowing all parameters to be freely estimated in each group. This approach gives information on differences in out hypothesised relationships between drought and control treatments. We evaluated the fit of our final models using a minimum set of parameters, including χ^2, root mean square error of approximation (RMSEA), and comparative fit index (CFI). Adequate model fits are indicated by a non- significant χ^2-test ($P < 0.05$), high probability of a low RMSEA value ($P > 0.05$) (Grace 2006; Pugesek et al. 2003), and high CFI (> 0.95) (Byrne 1994).

While our hypothesized causal structure for the SEMs was that drought-induced changes in root traits would affect soil concentrations of DOC and inorganic N, we acknowledge the possibility that drought-induced changes in soil nutrients could affect root traits. Therefore, we also examined SEMs in which individual root traits responded to changes in soil inorganic N.

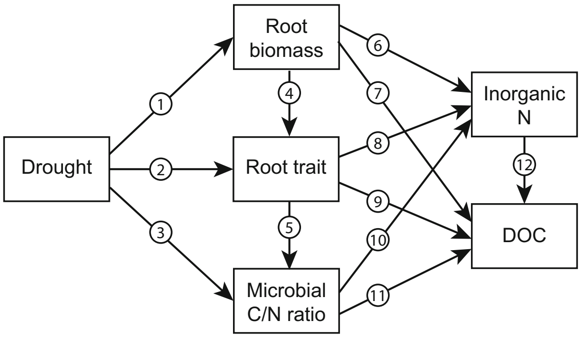


Fig. 1 Our a priori Structural Equation Model (SEM). Drought can affect root biomass, root traits, and microbial C/N ratio directly, through cell death and root shedding (*arrows 1–3*). Root biomass affects root traits through ontogenetic relationships (*arrow 4*), and root traits can affect microbial C/N ratio through root chemical quality, root exudation, and root turnover, and through direct associations with arbuscular mycorrhizal fungi (*arrow 5*). Root biomass can affect soil inorganic N and dissolved organic carbon (DOC) availability by taking up nutrients and water, and through exuding C compounds as root exudates (*arrow 6* and *7*). Root traits can affect inorganic N and DOC concentrations by enhancing or decreasing root N uptake and root exudation (*arrow 8* and *9*). Microbial C/N ratio can affect inorganic N by mineralization and immobilization processes, and DOC by decomposing soil organic matter and by using root exudates as a substrate (*arrow 10* and *11*)

Results

Plant biomass and root trait changes over time in control treatments

In the control pots, both aboveground biomass and root biomass increased with plant age. *A. odoratum* had the highest aboveground biomass at the final sampling, while *R. acetosa* had the highest root biomass; *R. acetosa* had the greatest root mass fraction. *D. glomerata* had the lowest root biomass (Suppl. Fig. 2, Table 1). When the plants had reached an age of 22 weeks, the two herbs each had a greater root mass fraction than the two grasses (Suppl. Fig. 2).

Root traits of control plants were also strongly affected by plant age and species identity. RNC and SRL decreased with plant age, while RTD increased (Suppl. Fig. 3, Table 1). RDMC varied over time, and root diameter was not affected by plant age. *L. hispidus* had the highest RNC, and *A. odoratum* the lowest, although these differences had disappeared at the final sampling date (Suppl. Fig. 3). SRL was the trait that differed most strongly between species, with *A. odoratum* having the highest values and *R. acetosa* the lowest. In line with these differences in SRL, *R. acetosa* had the highest RTD and RDMC, while *D. glomerata* had the lowest RTD. *L. hispidus* had the highest root diameter overall, but at the final sampling, the grasses had a significantly smaller root diameter than the herbs (Suppl. Fig. 3).

PCA showed that root traits of all four species followed a trajectory from high SRL and RNC in young plants, to high RTD in older plants from positive to negative values along PC1 (Fig. 2, Harvest $F_{3,62} = 63.2$, $P < 0.001$). In addition, plant species were separated on both axes (PC1 and PC2 scores Species $F_{3,62} = 156$, $P < 0.001$ and $F_{3,62} = 130$, $P < 0.001$, respectively), but this species effect depended on sampling date along PC2 (PC2 scores Species × Harvest interaction $F_{9,62} = 4.26$, $P = 0.0002$).

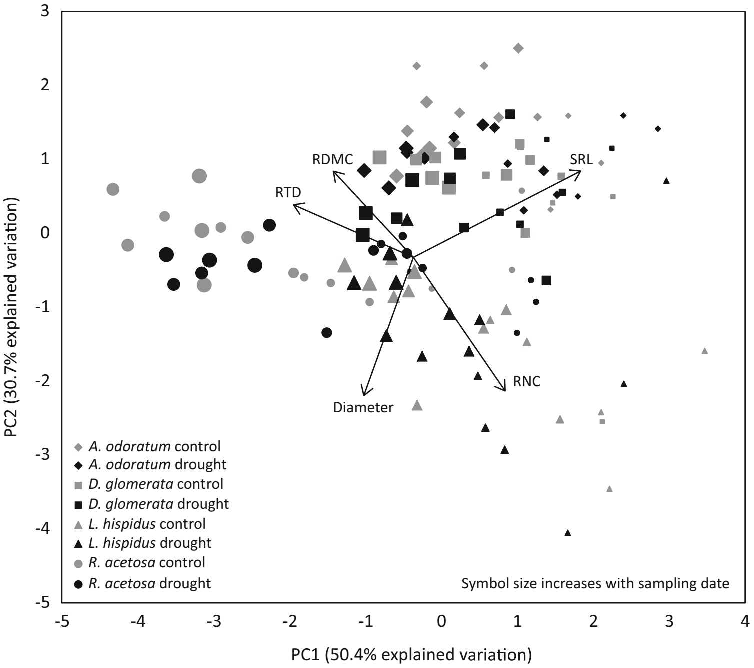


Fig. 2 PCA biplot for the five root traits analysed. Symbols represent individual observations. Different symbols indicate different species. *Black* symbols are drought treatments, *grey* symbols are control treatments, and symbol size increases with sampling date. Abbreviations: SRL, specific root length; RDMC, root dry matter content; RNC, root N content; RTD, root tissue density

Drought effects on plant biomass

There was no overall effect of drought on aboveground biomass (Table 1). However, when calculating the RDE, aboveground biomass of *A. odoratum* and *L. hispidus* was reduced at the end of the two-week drought, while aboveground biomass of *R. acetosa* was not immediately reduced, but two weeks after the drought had ended. Aboveground biomass of *L. hispidus* suffered most under drought; it was reduced by 31 % compared to control plants (RDE of −0.31, Fig. 3). Drought did not affect aboveground biomass of *D. glomerata*.

Table 1 ANOVA table for treatment (Species, Sampling date, Drought, and all possible interactions) effects on biomass and traits. For each treatment, F-values with degrees of freedom for the factor and the residuals are given, and *P*-values to three digits

		Aboveground biomass	Root biomass	Root mass fraction	RNC	SRL	RDMC	RTD	Diameter
Species	$F_{3,62}$	26.1	80.6	69.0	27.2	198.8	34.1	148.0	128.5
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.0001	<0.001
Sampling	$F_{3,62}$	54.5	164.0	24.0	22.7	38.5	2.10	168.0	6.17
	P	<0.001	<0.001	<0.001	<0.001	<0.001	0.109	<0.001	0.001
Drought	$F_{1,62}$	0.88	7.46	2.35	4.86	7.33	2.99	5.46	3.18
	P	0.352	0.008	0.130	0.031	0.009	0.089	0.023	0.079
Species × Sampling	$F_{9,62}$	1.45	4.09	4.83	1.08	2.53	1.33	4.11	2.59
	P	0.188	<0.001	<0.001	0.393	0.015	0.241	<0.001	0.013
Species × Drought	$F_{3,62}$	0.310	2.88	1.55	1.51	5.80	2.59	4.65	2.76
	P	0.818	0.043	0.210	0.222	0.002	0.061	0.0054	0.050
Sampling × Drought	$F_{3,62}$	1.57	3.34	0.41	2.68	4.06	0.512	1.32	1.33
	P	0.205	0.025	0.746	0.055	0.011	0.675	0.277	0.274
Species × Sampling × Drought	$F_{9,62}$	0.649	0.82	0.62	0.65	1.86	0.937	0.69	1.55
	P	0.750	0.596	0.774	0.750	0.075	0.500	0.713	0.150

RNC root nitrogen content, *SRL* specific root length, *RDMC* root dry matter content, *RTD* root tissue density

In contrast to aboveground biomass, root biomass was significantly affected by the two-week drought, and this effect depended on both sampling time and species identity (Drought × Species and Drought × Harvest interaction effects, Table 1). Drought reduced the root biomass of *A. odoratum* most strongly at the end of the two-week drought period; at the second harvest *A. odoratum* had a RDE of −0.46, meaning that biomass of drought plants was 46 % lower than biomass of control plants (Fig. 3). In addition, and similar to aboveground biomass, root biomass of *L. hispidus* was reduced at the end of the drought, while root biomass of *R. acetosa* was only significantly reduced one week after ending the drought (Fig. 3).

Drought effects on root traits

Overall, drought increased RNC, and the extent to which RNC was affected did not differ between species (Species × Drought interaction, Table 1). However, the RDE on RNC did differ between species and sampling times. RNC of *D. glomerata* was strongly increased at the end of the drought, but this trait also had higher values in *R. acetosa* at the end of the drought and two weeks after ending the drought (Fig. 3). In contrast, RNC in *A. odoratum* was reduced compared to control treatments after 10 weeks of recovery.

Drought also affected SRL, but this effect varied across species and sampling times (Table 1). RDE calculations showed that SRL was significantly reduced in *A. odoratum* at the end of the drought, and in *L. hispidus* after two weeks of recovery (Fig. 3). In contrast, SRL was strongly increased in *R. acetosa* two weeks after ending the drought; droughted plants had an SRL that was 70 % higher than that of control plants (RDE = 0.70, Fig. 3). SRL of *D. glomerata* was not affected by drought at any sampling time.

RDMC varied across sampling times and species, and as a result there was no significant overall drought effect on this trait (Table 1). However, after calculating the RDE, RDMC was strongly reduced in *A. odoratum* at the end of the drought; values of this trait were 60 % lower in droughted plants than in control plants (Fig. 3). In addition, RDMC was reduced in *L. hispidus* at the end of the drought, and in *R. acetosa* two weeks after ending the drought (Fig. 3). In contrast to RDMC, RTD was affected by drought, and this drought effect varied across species and sampling times (Table 1). RDE's showed that this trait was mostly lower in drought plants than in control plants. However, drought plants only had significantly lower RTD than control plants for *A. odoratum* at the end of the drought, and for *R. acetosa* two weeks after the drought (Fig. 3).

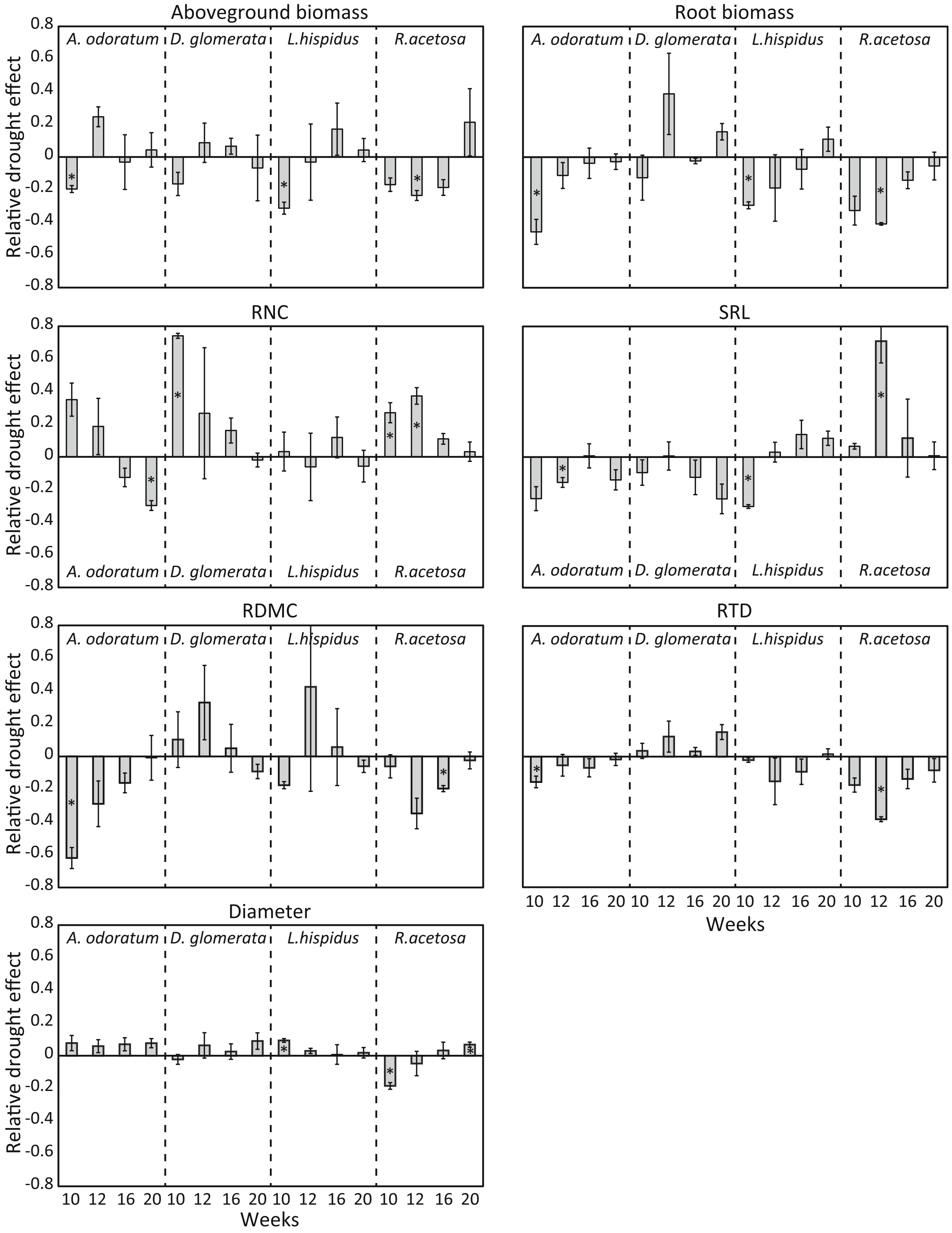
Finally, root diameter was weakly affected by drought in interaction with species identity (Table 1). For most species and most samplings, the RDE was positive, meaning root diameter was higher in droughted plants compared to control plants (Fig. 3). However, this increase in root diameter as a result of drought was only significantly different from zero for *L. hispidus* at the end of the drought. In contrast, root diameter of *R. acetosa* was significantly reduced at the end of the drought (Fig. 3).

There was a main effect of drought on suites of root traits along PC2 (PC2 scores $F_{1,62} = 15.4$, $P < 0.001$; Fig. 2), but along PC1, the effect of drought on root traits depended on species (PC1 scores Species × Drought interaction $F_{3,62} = 3.8$, $P = 0.001$; Fig. 2).

To test whether these changes in root traits as a result of drought were due to either phenotypic plasticity or root senescence and not just the consequence of changes in root biomass, we fitted models including the interaction term between drought and root biomass. For the drought-induced changes in root traits to be true plasticity, the effect of drought would have to explain variation over and above the effect of root biomass, i.e. drought would have to alter the relationship between biomass and traits. All root traits, including those that were not calculated from biomass, were strongly related to root biomass, consistent with their change with plant age (Suppl. Fig. 4). However, only for SRL and RTD the relationship of these traits with root biomass was affected by drought and thus these traits displayed true plasticity in response to drought. For both traits, there was an interaction between the effect of drought and biomass, indicating that the slope of their relationship with biomass depended on whether a drought was imposed (Suppl. Fig. 4). Our data suggest that SRL was reduced by drought in small or young plants, while RTD was reduced by drought in larger or older plants (Suppl. Fig. 4).

Drought effects on soil properties

Soil concentrations of inorganic N, DON, and DOC all showed very strong temporal patterns, with inorganic N generally being reduced towards the end of the experiment, while DON and DOC peaked halfway (Fig. 4, Table 2). There was no overall effect of drought on concentrations of DON and DOC. In contrast, DIN


Aboveground biomass
Root biomass
RNC
SRL
RDMC
RTD
Diameter
Relative drought effect
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
A. odoratum
D. glomerata
L.hispidus
R.acetosa
*
10 12 16 20 10 12 16 20 10 12 16 20 10 12 16 20
Weeks

◀ **Fig. 3** Relative drought effect on aboveground and root biomass and root traits, for the four species and the four sampling dates after imposing drought. Week 10 is at the end of the two week drought. Bars indicate mean ± 1SE ($n = 3$). Positive values indicate an increase as a result of drought, negative values a decrease, and bars marked with an asterisk indicate that the 95 % confidence interval does not overlap with zero (i.e. a significant difference between drought and control). Abbreviations: SRL, specific root length; RDMC, root dry matter content; RNC, root N content; RTD, root tissue density

concentrations increased sharply in response to drought, and then dropped again. In addition, the temporal dynamics of inorganic N depended on species identity (Species × Harvest interaction, Table 2), with *L. hispidus* having the highest concentrations at the start of the experiment (Fig. 4).

Microbial biomass N mirrored the dynamics observed for inorganic N; it decreased sharply at the end of the drought, and increased towards the end of the experiment. Similarly, microbial biomass C decreased as a result of drought. However, in contrast to microbial biomass N, microbial biomass C decreased towards the end of the experiment, resulting in a decreasing microbial C/N ratio (Fig. 5). The temporal dynamics and drought response of these microbial C and N pools did not depend on species identity (Table 2).

Interactions between root traits and soil properties

Our final overall SEM including PC axis 1 (PC1) scores, which represented a gradient from high RTD and RDMC to high SRL and RNC (Fig.4), fit the data very well (Fig. 6). PC axis 2 (PC2) scores, which represented a gradient from high diameter and RNC to high RDMC and SRL, were removed from this model following

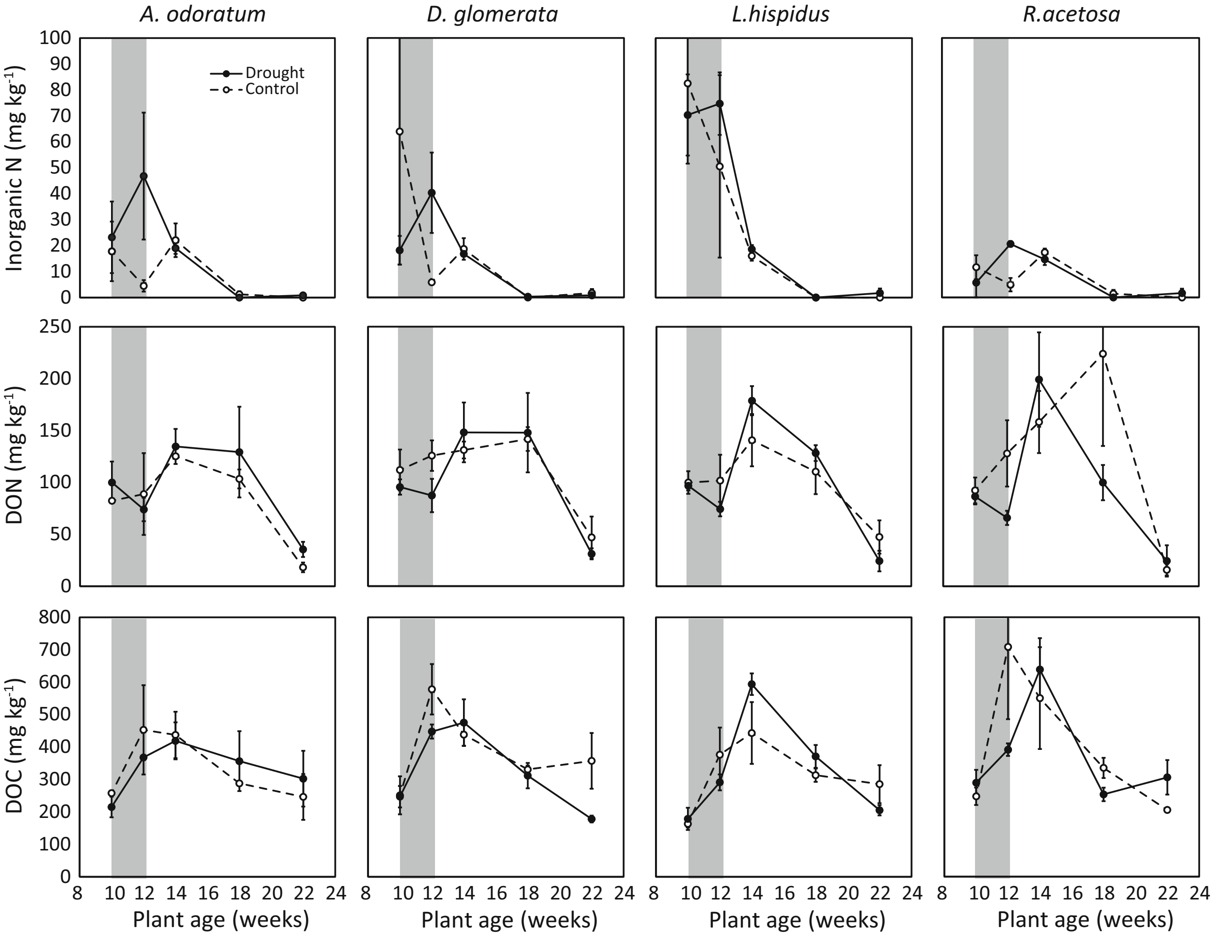


Fig. 4 Soil inorganic N, DON, and DOC availability over time in drought and control treatments for all four species. The shaded area indicates the two-week drought. Symbols and bars represent mean ± 1SE ($n = 3$)

Table 2 ANOVA table for treatment effects (Species, Sampling date, Drought, and all possible interactions) on soil and microbial properties. For each treatment, F-values with degrees of freedom for the factor and the residuals are given, and *P*-values to three digits

		Inorganic N	DON	DOC	Microbial biomass N	Microbial biomass C	Microbial biomass C/N
Species	$F_{3,62}$	1.59	1.42	0.89	0.99	1.18	0.39
	P	0.201	0.244	0.450	0.402	0.323	0.768
Sampling	$F_{3,62}$	174.1	34.6	25.1	41.7	89.9	85.2
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Drought	$F_{1,62}$	12.3	0.72	0.86	6.24	2.91	10.6
	P	0.0008	0.401	0.359	0.015	0.093	0.002
Species × Sampling	$F_{9,62}$	2.76	0.64	1.25	1.30	0.74	0.89
	P	0.009	0.758	0.283	0.255	0.668	0.543
Species × Drought	$F_{3,62}$	0.09	1.06	0.91	0.46	1.79	0.04
	P	0.961	0.38	0.441	0.710	0.158	0.99
Sampling × Drought	$F_{3,62}$	11.6	2.04	2.02	13.2	5.17	11.1
	P	<0.001	0.117	0.120	<0.001	0.003	<0.001
Species × Sampling × Drought	$F_{9,62}$	0.93	1.13	1.42	1.14	0.89	0.36
	P	0.505	0.357	0.200	0.346	0.54	0.95

DON dissolved organic nitrogen, *DOC* dissolved organic carbon

model selection. In our overall model, root biomass strongly reduced inorganic N. PC1 scores decreased with greater root biomass, and decreased PC1 scores subsequently increased inorganic N availability (negative relationship between PC1 scores and soil inorganic N). In addition, a wider microbial C/N ratio was linked to higher concentrations of inorganic N and DOC; additionally, DOC concentrations were positively affected by inorganic N concentrations. Root biomass and microbial C/N ratio were strongly correlated. To test whether this causal structure was affected by drought, we re-analysed our final model using a multi-group approach. We found that few relationships differed between drought and control treatments (Fig. 6). However, in the drought treatment, the relationship between microbial C/N ratio and inorganic N and DOC availability was disrupted, as was the relationship between PC1 scores and inorganic N (Fig. 6). Importantly, the overall strong correlation between root biomass and microbial C/N ratio was primarily caused by the drought treatment.

All our SEMs for individual root traits included similar relationships as our model including PC1 scores, and were in line with the general shift from high RTD and RDMC to high SRL and RNC along PC1 (Suppl. Fig. 5). Specifically, high SRL reduced inorganic N concentrations, while RNC and RTD increased these (Suppl. Fig. 5). However, in the models including RDMC and RTD there was a link between root traits and microbial C/N ratio; a higher RDMC and RTD resulted in a wider microbial C/N ratio. Overall, the strength and direction of relationships between root biomass, root traits, and inorganic N did not differ much between drought and control treatments (Suppl. Figs. 6, and 7).

In our SEMs in which changes in inorganic N affects root traits, we found that root biomass controlled soil inorganic N availability, which then caused a feedback to root traits (Suppl. Fig. 6). Specifically, high inorganic N concentrations were linked to greater RNC, RDMC, and RTD, but to lower SRL. Model AIC for these models were consistently worse than for our models in which root traits controlled inorganic N availability (Suppl. Fig. 8).

Discussion

Our primary aim in this study was to quantify how drought responses of the root systems of four common grassland species affect soil C and N availability. We hypothesized that drought would, across all species, induce a shift towards more conservative root traits, i.e. lower SRL and RNC, and higher RDMC and RTD, and that this would result in higher soil N

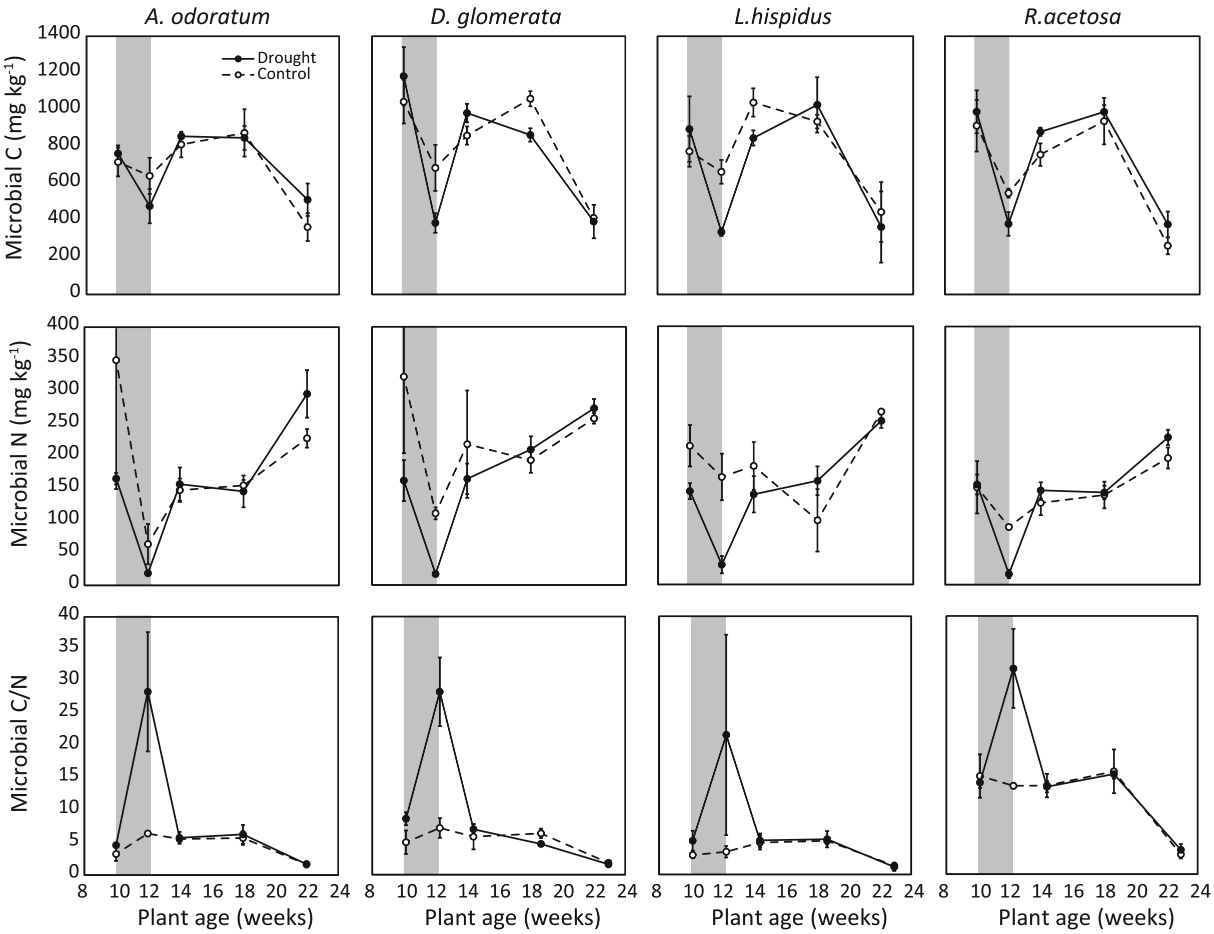


Fig. 5 Microbial C, N, and C/N ratio over time in drought and control treatments for all four species. The shaded area indicates the two-week drought. Symbols and bars represent mean ± 1SE ($n = 3$)

availability through decreased root N uptake. Our results support our hypotheses: we found that drought strongly affected plant growth and root systems, and that these changes in root systems had cascading effects on soil DOC and inorganic N concentrations. Specifically, and as we hypothesized, reduced biomass and a shift towards more resource-conservative root traits was linked to higher levels of soil inorganic N, indicating a reduced capacity for root N uptake.

We found all root traits to strongly change over time and as plants grew bigger: RNC and SRL decreased with plant growth and root biomass while RTD increased, indicating a shift from investing in thin, long roots for maximum N uptake and growth, to investing in coarser, thicker roots for maximum longevity. These findings broadly follow field-based observations by Craine et al. (2003), although these authors found no effect of increasing root biomass on SRL, but a decrease in root diameter. We found that drought reduced root biomass in most species, and because root traits were strongly linked to root biomass, drought also affected root traits. The only root traits that changed independent of the drought effect on root biomass were SRL and RTD: drought tended to reduce SRL, while RTD tended decrease in larger plants. This relatively minor display of root plasticity in response to drought might be because of the inescapable nature of our simulated drought, and because roots had become pot-bound in the final sampling. Under more realistic conditions, plants might be able to adjust their C balance and modify their root traits, for example by increasing their root biomass and SRL in search for water in deeper soil layers (Comas et al. 2013; Padilla et al. 2013).

These drought-induced changes in root systems had clear effects on soil inorganic N and DOC concentrations. Our SEMs revealed that root biomass was the

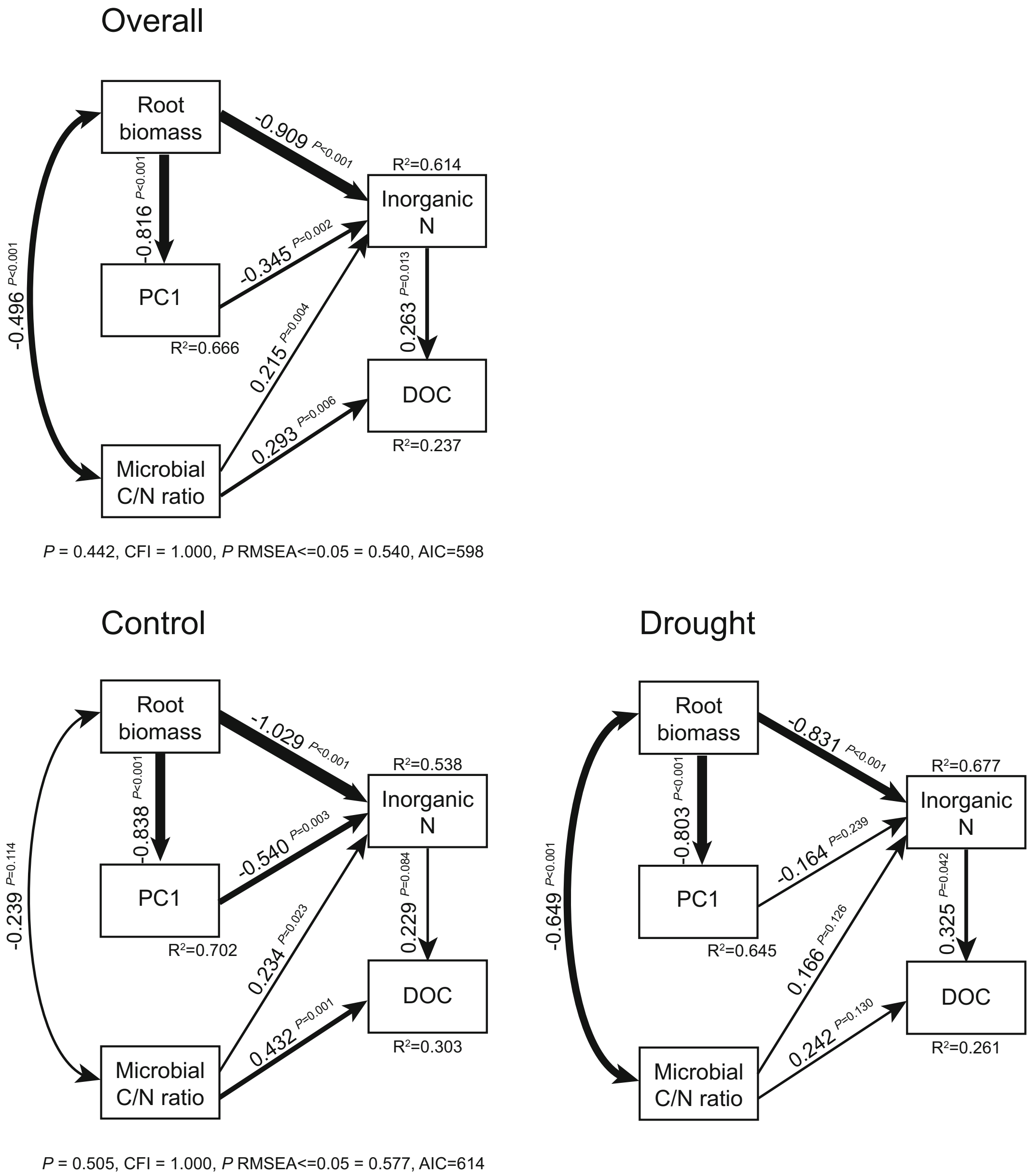


Fig. 6 Final overall SEM indicating causal relationships between PC axis 1 scores for root traits, microbial properties, and soil C and N availability (top), and final SEM using multi-group modelling to examine the effect of drought on these causal relationships. Both models fit the data well; model indicators of fit are given under each model. Arrow size represents standardized effect sizes, values alongside arrows indicate standardized effect size and significance, and values underneath boxes indicate variation explained by the model

strongest driver of soil inorganic N concentrations: increasing root biomass was linked to lower soil inorganic N, presumably through greater plant N uptake (Craine et al. 2003). Importantly, and although minor compared to the effect of root biomass, root traits explained additional variation in soil inorganic N concentrations. Specifically, and as we hypothesized, higher SRL was associated with reduced inorganic N, while higher RTD was associated with higher inorganic N concentrations, confirming that plants that invest in short-lived, thin and long roots have the highest N uptake rates (De Vries and Bardgett 2016). In contrast with our expectation, higher RNC was associated with higher soil inorganic N concentrations. We propose two possible mechanisms for this observation. First, it is possible that root turnover, through decomposition of dying roots, resulted in the mobilisation of inorganic N; in this case, roots high in N would release most N in the soil (Fornara et al. 2009; Silver and Miya 2001). Alternatively, it is possible that RNC responded to soil N availability, rather than controlling it; high soil N availability would then result in high RNC (Craine et al. 2003). However, our alternative SEMs in which soil inorganic N affected root traits did not fit the data as well as our hypothesised SEMs. Moreover, these models resulted in counter-intuitive relationships where high inorganic N resulted in high RDMC and RTD (Suppl. Fig. 8). Third, roots with high RNC might be releasing high-N root exudates, or exudates that are high in labile C or with low pH that mobilise N (Bardgett et al. 2014). Importantly, these findings do not only highlight the overarching importance of plant roots in controlling soil N availability, they also suggest that drought impairs the capacity of plant roots to take up N, through reducing root biomass, but also through reducing SRL and increasing RTD.

In contrast to many recent studies that found strong links between root traits and soil microbial properties, we only found the root traits RDMC and RTD affected microbial C/N ratio. The microbial C/N ratio is a coarse measure of characterizing microbial communities that informs on C vs. N limitation of soil microbes, with high C/N ratios indicating N limitation (Kaye and Hart 1997) and/or a high abundance of fungi relative to bacteria (Bloem et al. 1997; Van Veen and Paul 1979). Our observation that the microbial C/N ratio increased with greater RDMC and RTD is consistent with previous observations. It supports the notion that fungi are able to degrade more recalcitrant organic material (Hunt et al. 1987; Newman 1985), and with decomposition of these more recalcitrant roots being limited by N (Kaye and Hart 1997). Also, it supports the hypothesis that high C/N ratio is a trait that confers microbial resistance to desiccation (De Vries and Shade 2013). However, it is surprising that we found no link between RNC and microbial C/N ratio, since litter chemistry has been found to be a more important control on root decomposition rates than structural traits (Birouste et al. 2012). We found that an increased microbial C/N ratio resulted in higher inorganic N and DOC availability, indicating a reduced microbial capacity to immobilize N (De Vries and Bardgett 2016), and a greater microbial capability to decompose organic matter. We found no direct effect of root traits on DOC concentrations in soil, indicating that here, changes in root traits only affected DOC concentrations through affecting microbial activity. Despite the absence of a direct causal relationship, root biomass and microbial C/N ratio responded in tandem to drought: root biomass was reduced at the end of the drought, while microbial C/N ratio was increased, potentially supporting observations that the proportion of fungi relative to bacteria increases under drought (Bapiri et al. 2010; De Vries et al. 2012; Gordon et al. 2008).

We hypothesized that the species with the slower growth rates, i.e. *A. odoratum* and *L. hispidus*, would be least affected in their biomass by drought. Alternatively, resource-conservative root traits would confer drought resistance. Despite the inescapability of our simulated drought, the effects of drought on aboveground and root biomass were moderate. Drought reduced both aboveground and root biomass, but root biomass was more affected than aboveground biomass. Yet, in contrast to previous work, the root mass fraction of our four species was not affected by drought, indicating that there was no whole-plant plasticity in response to drought (Poorter et al. 2012). In stark contrast with our hypotheses, we found that drought most reduced both aboveground and root biomass of *A. odoratum* and *L. hispidus* – the species that we hypothesized would be least affected by drought because of their slow growth rates. Interestingly, and in contrast with earlier field-based observations (Mamolos et al. 2001), in a glasshouse-based study similar in scale and duration to ours, Padilla et al. (2013) found no effect of pulsed water supply on both biomass and root traits of *A. odoratum*. However, these authors only sampled at one time point, and might therefore have missed a dynamic change in root traits. In addition, we hypothesized that *R. acetosa* would be resistant to drought because of its high RTD and low SRL. However, and in contrast with findings from field studies on *R. obtusifolius* (Gilgen et al. 2010) and *R. acetosella* (Mamolos et al. 2001), drought consistently reduced both aboveground and root biomass of *R. acetosa* over the entire duration of our experiment. An explanation for these discrepancies might be that in the field, *Rumex* species take advantage of their deep taproot system to maintain water uptake and photosynthesis during drought (Gilgen et al. 2010), whereas in our relatively small pots, *R. acetosa* was not able to fulfill its ecological niche. Another explanation for the strong drought effect on *R. acetosa* might be that this was the only non-mycorrhizal species we included. Although beyond the scope of our experiment, it is well known that associations with mycorrhizal fungi can increase a plant's resistance to drought (Ruiz-Lozano et al. 2012). Finally, our finding that *D. glomerata* was hardly affected by drought does correspond to findings from pot and field-based studies (Lelievre et al. 2011; Poirier et al. 2012; Staniak and Kocon 2015; Zwicke et al. 2015).

In conclusion, our results give insight in how root system responses to drought can modify soil DOC and inorganic N availability: reduced root biomass and a shift towards more resource-conservative traits resulted in higher soil inorganic N concentrations, likely through reduced plant uptake of inorganic N, but did not directly affect soil C availability. This reduced plant N uptake coincided with a flush of N availability as result of reduced microbial biomass under drought. In addition, drought, and to a lesser extent increased RTD, selected for microbial communities with a lower capacity to immobilize N, again contributing to high N availability, and a higher capacity to decompose C, leading to greater soil C availability. Together, these changes in root systems and microbial properties can contribute to greater N loss from soil after drought. In conclusion, our results identify the mechanisms through which drought-induced changes in root systems and microbial communities can affect soil C and N availability. This knowledge contributes to our understanding of how root traits can modify soil and ecosystem processes in a changing world.

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Author contributions F.T.d.V. conceived the experiment, analysed and interpreted data, and wrote the manuscript. C.B. contributed to conceiving the experiment, set up the experiment, performed lab work, and contributed to writing the manuscript. C.S. contributed to setting up and running the experiment, and to writing the manuscript.

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