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Root trait responses to drought are more heterogeneous than leaf trait responses

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Abstract

1. Drought can strongly modify plant diversity and ecosystem processes. As droughts are expected to intensify in the future, it is important to better understand plant responses to this global driver. Root traits are an overlooked but powerful predictor of plant responses to drought because they are in direct contact with the soil environment and are responsible for taking up nutrients and water.

2. Here, we determine which root traits are sensitive to drought and the magnitude of that response. We also tested whether root trait relationships with shoot biomass are affected by drought and to what extent all these responses depend on plant species identity. To do so, we conducted a glasshouse experiment with 24 plant species grown in pots (10 replicates per species), which included grasses, forbs and legumes. All replicates were well watered during the first month and then half of them were kept under drought (30% water holding capacity [WHC]), with the other half serving as control (70% WHC). After 2 months of treatment, leaf and root traits were measured.

3. Leaf traits had a strong and more uniform response to drought compared to root traits. Root trait responses were variable and differed among plant species. Overall, grasses and several forbs had increased root diameter with drought while forbs had decreased specific root surface area (SRSA) and specific root length (SRL). Increase of root diameter and reduction of root elongation or sacrificing fine roots are different strategies that may promote nutrient and water acquisition, depending on plant species identity.

4. Our results identify changes in root morphological traits as mechanisms to likely tolerate drought and highlight that, although such drought responses are species-specific, they are phylogenetically clustered.

KEYWORDS
global change, grassland ecosystem, plant traits, root diameter, root tissue density, specific root length, specific root surface area, WinRhizo

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1 | INTRODUCTION

Plant traits provide a means of explaining the role of plant diversity in ecosystem functioning. Given this explanatory power, there has been a lot of interest in measuring the response of plant traits to climatic factors. This research, though, has been biased towards leaf traits, neglecting the study of root traits. This bias is unfortunate because root traits are important drivers of many ecosystem processes such as carbon and nutrient cycling, as well as the formation and structural stability of soil (Bardgett, Mommer, & De Vries, 2014). This lack of research on root traits hampers our ability to predict how plant diversity and ecosystem functioning will respond to climate change drivers, like drought episodes, which are predicted to intensify in the future due to warmer temperatures and potential decline in seasonal rainfall (Bodner & Robles, 2017).

Data available on root trait responses to drought are limited to a small number of plant species. As a result, conclusions about plant strategies in terms of root trait responses seem idiosyncratic or at best premature. For example, some studies report plant species producing thinner roots with high specific root length (SRL) and specific root surface area (SRSA) in response to drought, a strategy interpreted as an improvement of water acquisition with low investment (Comas, Becker, Cruz, Byrne, & Dierig, 2013; Debinski, Wickham, Kindscher, Caruthers, & Germino, 2010). Other studies, by contrast, report that plant species produce thicker roots with a low SRL and SRSA, which has been shown to diminish the risk of hydraulic rupture (Zimmermann, 1983; Zufferey, Cochard, Ameglio, Spring, & Viret, 2011). Thicker roots have been associated with high nutrient and water acquisition through mycotrophy (Brundrett, 2002; Comas et al., 2012), and with osmoregulation due to the storage of non-structural carbohydrates (NSC; Chaves, 1991; Galvez, Landhäusser, & Tyree, 2011; Yang, Zhang, Li, Xu, & Wang, 2016). Recent meta-analyses found that drought decreases root length, and increases root diameter (Zhou et al., 2018).

We expect that these contrasting patterns in root trait responses to drought to be (at least partly) explained by phylogeny. This is because root traits are related to distinct resource acquisition strategies that have evolved across and within angiosperm clades. For example, many grass species exhibit traits associated with a fast growth acquisitive strategy, such as high SRL, SRSA and low diameter (Comas et al., 2013; Padilla et al., 2013; Ravenek et al., 2016; but see de Vries, Brown, & Stevens, 2016), and thus closely related species are more likely to share root trait values and, as a consequence, a similar response to drought. In addition, different clades of plants have evolved a variety of mechanisms to cope with drought. These can be categorized into dehydration avoidance, dehydration escape, dehydration tolerance, dormancy and desiccation tolerance (Volaire, 2018). Dehydration avoidance relies on mechanisms that maintain the plant's water status by decreasing water loss through reduced stomatal conductance and restricted shoot growth; maintaining water uptake through physiological, biotic or root morphological adjustments (Brunner, Herzog, Dawes, Arend, & Sperisen, 2015) appears to be one of the first strategies plants use to face drought (Volaire, 2018). With this strategy, potential shifts

in root traits such as SRL, SRSA, root tissue density (RTD), root diameter (RAD), or root:shoot ratio become relevant, as roots are the first organ in contact with the soil and thus the first that face water scarcity.

We also expect that drought will alter the relationship among root traits and with shoot traits as part of the strategies to cope with a lack of water. Such relationships are expected based on theories like the root economic spectrum (RES) which posit that root traits that are positively associated with water and nutrient uptake capacity, like high SRL, should correlate negatively with RTD and diameter (Reich, 2014). However, evidence for these trends is still weak and inconsistencies with the expected RES trends are common (Valverde-Barrantes & Blackwood, 2016). Similarly, evidence suggests that root traits, such as SRL and RTD, are independent of each other and from the leaf economic spectrum (Kramer-Walter et al., 2016).

In this study, we measured leaf and root trait responses to drought of 24 plant species that include grasses, forbs and legumes. With this information, we addressed four questions: (a) which root morphological trait and carbon allocation adjustments occur across plant species in response to drought, and how do they compare to leaf trait adjustments; (b) to what extent are these traits phylogenetically clustered; (c) how does drought modify the relationship between root and leaf traits; (d) how do these changes in the root–leaf relationship impact on shoot biomass, and to what extent is variation in these explained by phylogeny.

2 | MATERIALS AND METHODS

2.1 | Species selection

We selected 24 plant species belonging to three different plant functional groups: eight grasses (*Arrhenatherum elatius, Festuca brevipila, Holcus lanatus, Poa angustifolia, Anthoxanthum odoratum, Lolium perenne, Festuca rubra, Dactylis glomerata*), 13 forbs (*Achillea millefolium, Armeria maritima* ssp.*elongata, Artemisia campestris, Berteroa incana, Daucus carota, Galium verum, Hieracium pilosella, Hypericum perforatum, Plantago lanceolata, Potentilla argentea, Ranunculus acris, Rumex thyrsiflorus, Silene vulgaris*) and three legumes (*Trifolium repens, Vicia cracca, Medicago lupulina*). All these species are common and frequently co-occur in Central European grasslands. Plant species will be referred to by their generic name from here on (except for the two *Festuca* species to which we refer as *F. brevipila* and *F. rubra*). Seeds of these plant species were obtained from commercial suppliers in the region (Rieger-Hofmann GmbH, Blaufelden).

2.2 | Experimental design

In September 2016, we collected sandy loam soil (% N 0.07, % C 0.77, pH 6.66) from Dedelow, Brandenburg, Germany (53°37′N, 13°77′W) where our plant species naturally grow. Soil was sieved

(4 mm mesh size) and homogenized to use as substrate. We established the experiment in a controlled glasshouse growth chamber with a daylight period set at 12 H, 50 klx, and a temperature regime at 22/18°C day/night with relative humidity of ~40%. Prior to germination, we surface-sterilized seeds with 4% sodium hypochlorite for 5 min and 75% ethanol for 2 min and then thoroughly rinsed with sterile water. Then, seeds were germinated in trays with sterile sand and transplanted into deep pots (11-cm diameter, 30-cm height) 5 days after germination. Pots were filled with 3 L (i.e. 2,500 g) of soil and one individual seedling per plant species was planted into the centre of each pot (for a total of 10 replicate pots per plant species).

All plants were well-watered during the first month of growth. Then, half of the pots (i.e. five replicates of each plant species) were kept under drought treatment by maintaining ~30% water holding capacity (WHC) while the other half were maintained as control at ~70% WHC for 2 months. The pot weight increase due to plant biomass growth (i.e. ~3 g dry weight at the end of the experiment) had a minor effect on the WHC calculations. Pots were weighed every 2 days and their moisture content adjusted gravimetrically to keep them at their respective WHC during 2 months. This experimental design included 24 plant species × two water treatments × five replicates = 240 pots. Seedlings that died during the first 2 weeks were replaced. All pots were randomly distributed in the chamber and their position shifted twice to homogenize environmental conditions during the experiment.

At harvest, we clipped the plant above-ground material while the below-ground compartment, consisting of both soil and roots of each pot, was divided into three sections every 10 cm of depth (upper, middle and bottom sections). The soil and roots from these sections were dried at 27°C in a forced-air drying oven for 3 weeks to stabilize samples (no molds or similar organisms were visible in these soils after drying and storage). After that, we first collected roots by hand. Then, following Kuzyakov, Biriukova, Turyabahika, and Stahr (2001), we spread the soil on a filter paper to capture small and fine roots (<1 mm) by electrostatic. This was done by manually collecting root pieces attracted to an electrostatically charged polyethylene plate (we charged the plate by means of intensive rubbing for 4–5 s on a stretched wool fabric) in order to ensure the capture of all fine roots. This method allows for collection of fine root pieces and a fast storage of root samples until further processing. This latter point was important because given the large amount of root samples it helped avoid risk of root degradation.

2.3 | Measurements

2.3.1 | Root morphological traits

We measured the traits of fine roots (i.e. <2 mm in diameter which included mostly first to third order roots). Specifically, we measured: length, surface area, volume and root average diameter (RAD). These traits were measured on a rehydrated root sample from the middle section of the below-ground compartment (i.e. the section in between 10- and 20-cm depth) using the WinRhizo^{TM} scanner-based system (v.2007; Regent Instruments Inc.). This rehydration method provides accurate and unbiased root trait values as shown in previous studies that report high linear correlations (Pearson's $r = 0.93$) between root traits measured on fresh and rehydrated material for these same species (Bergmann, Ryo, Prati, Hempel, & Rillig, 2017). Further, we took root samples with no signs of fragmentation. For the analysis described below, we used data collected from roots found in the middle section of the pot. We did this because (a) it contained the largest amount of root biomass, (b) there were no differences in root traits with respect to the other two section (Table S1) and (c) it facilitated comparison among species.

2.3.2 | Biomass traits

We measured root and shoot mass after drying samples at 70°C for 48 hr.

2.3.3 | Above-ground traits

We also measured specific leaf area (SLA) and leaf dry matter content (LDMC) following standard protocols (Cornelissen et al., 2003).

2.3.4 | Chemical and physical traits

We measured leaf and root C and N contents with an Elemental Analyzer (EuroEA, HekaTech). Soil temperature (Hobo 1-800, Onset Computers) at depth of 15 cm was monitored continuously during the experiment in additional control pots under drought and non-drought conditions.

2.4 | Statistical analyses

2.4.1 | Phylogenetic relationships

We used a phylogenetic tree by subsetting our plant species from the Daphne phylogeny (a phylogeny that encompasses all species in the trait databases BIOLFLOR, PLANTATT, and BioBase 2003 and that includes a large European flora; Durka & Michalski, 2012; Figure S1). Then, we calculated a phylogenetic distance matrix of the plant species by using the function 'cophenetic' from the R package STATS version 3.5.3. We then subjected the distance matrix to a principal coordinate analysis by using the function 'cmdscale' from the same package. By doing so, we summarize the amount of variance explained by phylogenetic distance into a few independent principal axes (Diniz-Filho, de Sant'Ana, & Bini, 1998; Legendre & Legendre, 1998). The first four principal coordinate analysis axes, which represented ~80% of phylogenetic variation

(i.e. 47.15%, 12.81%, 8.66% and 6.42% respectively), were extracted and used as the phylogenetic covariate in further analysis (referred as 'phylogeny' from now on).

2.4.2 | Redundancy analysis and linear models

We performed a redundancy analysis (RDA) to assess the response of root and shoot traits to drought and whether such response was species specific. To account for phylogenetic relatedness, we included the first four principal coordinate analysis axes of the phylogenetic distance matrix as covariate (i.e. in the RDA the traits are the standardized response variables, drought treatment and species identity are explanatory variables, while the phylogeny is the covariate, see Supporting Information S1).

For the RDAs, we used data from all replicates (i.e. the plant traits values and the eigenvectors of the first four principal coordinate analyses axes from the phylogenetic distance matrix). RDAs (one for leaf and other for root traits) and significance testing were performed using the function 'rda' and 'ANOVA.cca', respectively, both from the package VEGAN (Oksanen et al. 2019). SRL showed multicollinearity (i.e. vif > 10; Borcard, Gillet, & Legendre, 2018), and thus it was dropped from the final RDA and instead we used SRSA, a similar functional trait.

Additionally, we performed linear models using generalized least squares (GLS) to determine which plant traits significantly responded to drought treatment. Drought was included as a fixed factor while phylogeny and soil temperature were included as covariates. Leaf (shoot mass, SLA, LDMC, leaf C and N) and root traits (RAD [mm], RTD [root dry weight per volume mg/cm^3], SRL [cm/mg], SRSA [cm^2/mg], root to shoot ratio, total root mass, root C and N) were transformed when necessary to meet normality assumptions. In addition, for each trait (except SLA, leaf N and SRL), we accounted for heterogeneity in the water treatment by using the 'varIdent' function from the NLME package. SLA and LDMC were not calculated for *Trifolium* as leaves were shrivelled when collected, which would cause biases in leaf area and LDMC measurements (Pérez-Harguindeguy et al., 2013). For the correlations among traits, we used Model-II regression (by using the function 'lmodel2' from the same name package; Pierre, 2018) as no trait can be considered an independent variable (i.e. all traits are response variables subject to measurement error). Finally, we also calculated Pearson's correlations between phylogeny, root and leaf traits relative index of interaction (RII; explained below). All analyses were conducted using R version 3.5.3 (R Core Team, 2019). Results shown throughout the text and figures are mean values ± 1 *SE*.

2.4.3 | Relative index of interaction

We determined the direction and magnitude of the effect of drought on each plant trait by using the relative interaction index (RII; Armas, Ordiales, & Pugnaire, 2004). This index provides a means to determine whether trait values are higher or lower under drought relative to non-drought (control) conditions. It is calculated as a ratio as follows:

$$RII_{trait} = (Y_{drought} - Y_{non-drought})/(Y_{drought} + Y_{non-drought}), \quad (1)$$

where $Y_{drought}$ is the growth of the trait when the plant grew under drought (30% WHC) and $Y_{non-drought}$ is the growth of the trait when the plant grew under non-drought conditions (70% WHC). This index was calculated for each plant species. RII has positive values when trait values are greater under drought than in non-drought and negative values when the opposite is true. In addition, we performed a priori contrast tests to assess whether RII values were significantly different from zero (indicating neutral or no significant effect).

3 | RESULTS

3.1 | Root trait responses to drought are more heterogeneous than leaf trait responses

Leaf and root trait responses were affected by drought and plant species identity (Table 1). Overall, leaf trait responses to drought were homogenous across all plant species (Figures 1a and 2). Plant species exhibited decreased shoot mass, SLA, and leaf N under drought relative to the control treatment while LDMC increased in response to drought (Figure 2). This can be further visualized in the ordination along the RDA axes, where most plant species grown under drought are left-clustered compared to the ones grown under control (Figure 1a; Tables 1 and 2).

In the case of root traits, while phylogeny on its own accounted for 24% of the variation (almost double compared to what phylogeny explains in leaf traits), drought treatment and species identity combined accounted for 48% of the remaining variation (Table S2). SRSA along with root C, N, RTD and shoot mass were the most phylogenetically structured traits (Figure S2).

Drought strongly influenced root traits (drought main effect: $F_{1,188} = 2.99$, $p = 0.008$) but this influence was species specific, as indicated by the interaction term of drought and species identity in the RDA analysis ($F_{23,188} = 1.57$, $p = 0.01$). That is, root

TABLE 1 Results from redundancy analyses on leaf and root traits response to drought accounting for phylogenetic relatedness. Pseudo-F values and p-values in parentheses. Number of permutations: 999

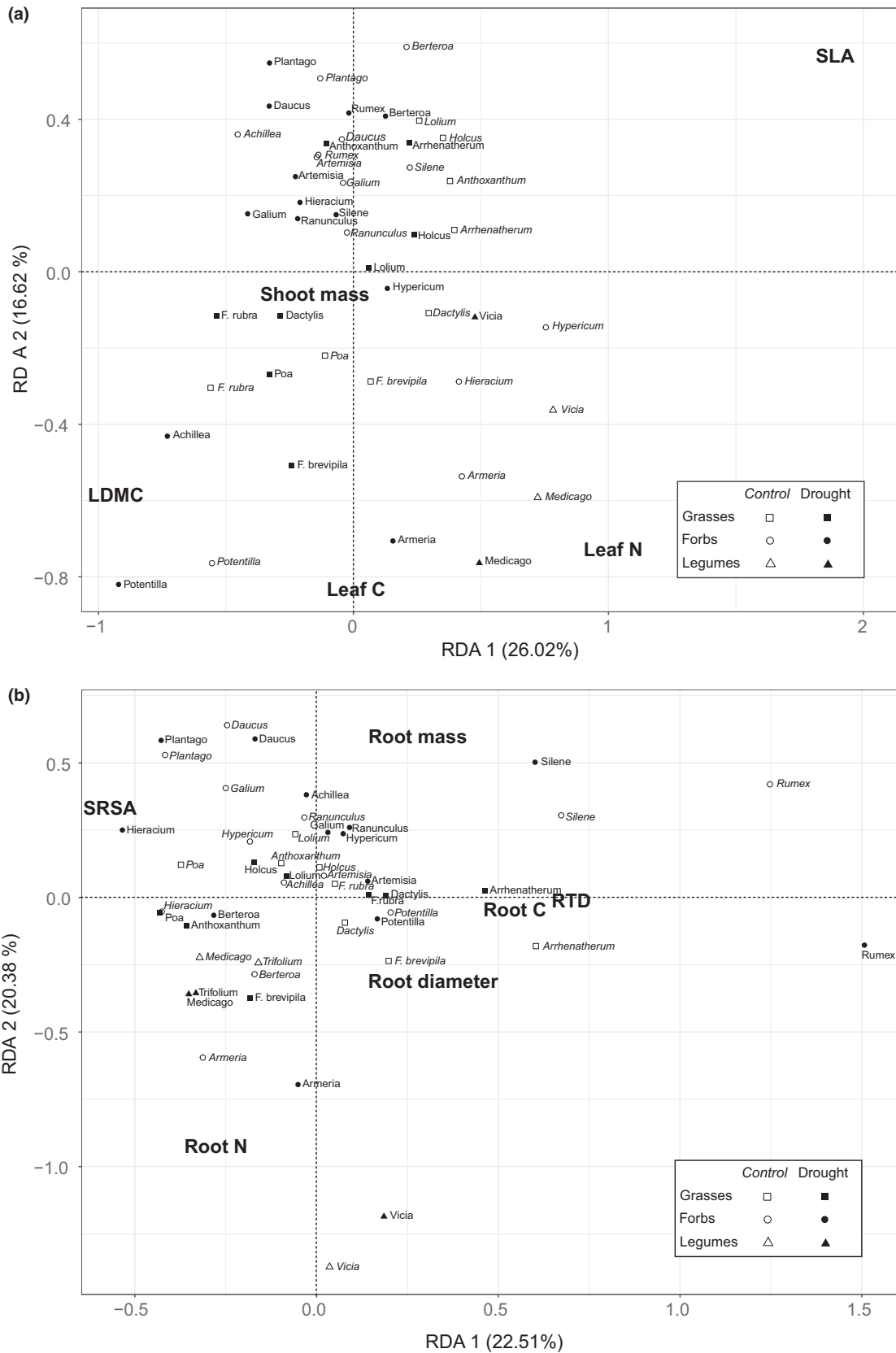| Factors	df	Leaf traits	Root traits
Drought (D)	1	25.33 (<0.01)	2.99 (<0.01)
Plant species (Ps)	22	12.57 (<0.01)	12.76 (<0.01)
D × Ps	23	1.25 (0.07)	1.57 (<0.01)
R^2 adjust		0.47	0.41

FIGURE 1 Redundancy analysis (RDA) for plant species subjected to drought and non-drought conditions and (a) leaf traits (shoot mass, specific leaf area (SLA), leaf dry matter content (LDMC), leaf C and N) and (b) root traits (root diameter, root tissue density (RTD), root biomass, specific root surface area (SRSA), root C and N). RDA results are summarized in a bi-dimensional plot and include phylogeny as a covariate in the model. Plant species under drought (in black) and control (in white) conditions; phylogenetically related species have the same symbol shape
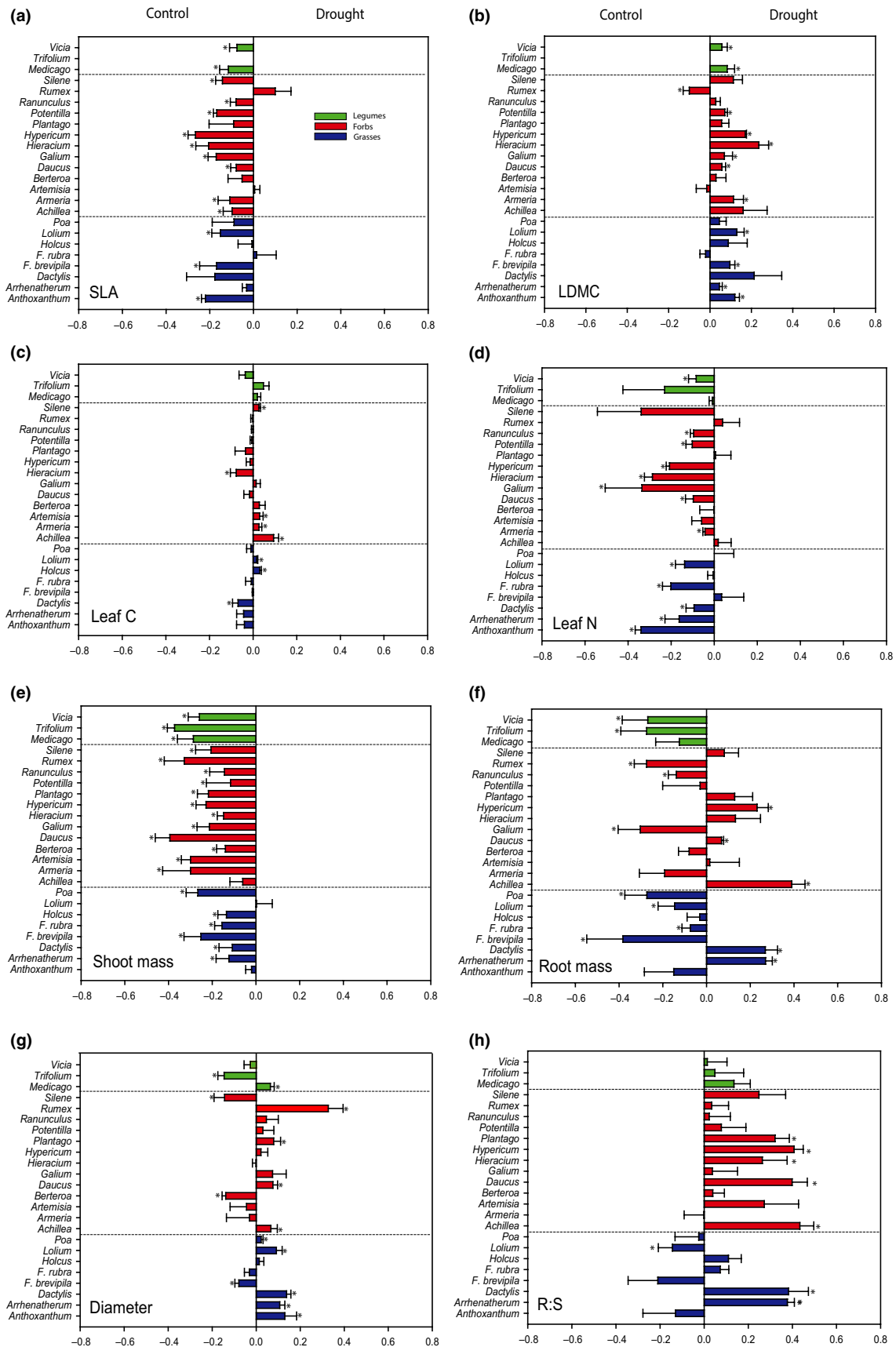
FIGURE 2 Magnitude of drought effects on (a–e) leaf and (f–m) root traits for each plant species. RII compares the trait value in the drought versus non-drought (control) conditions. Positive values indicate higher trait value in drought than in control treatment and negative values indicate the opposite. RII values different significantly from zero are indicated by asterisk (*$p < 0.05$)
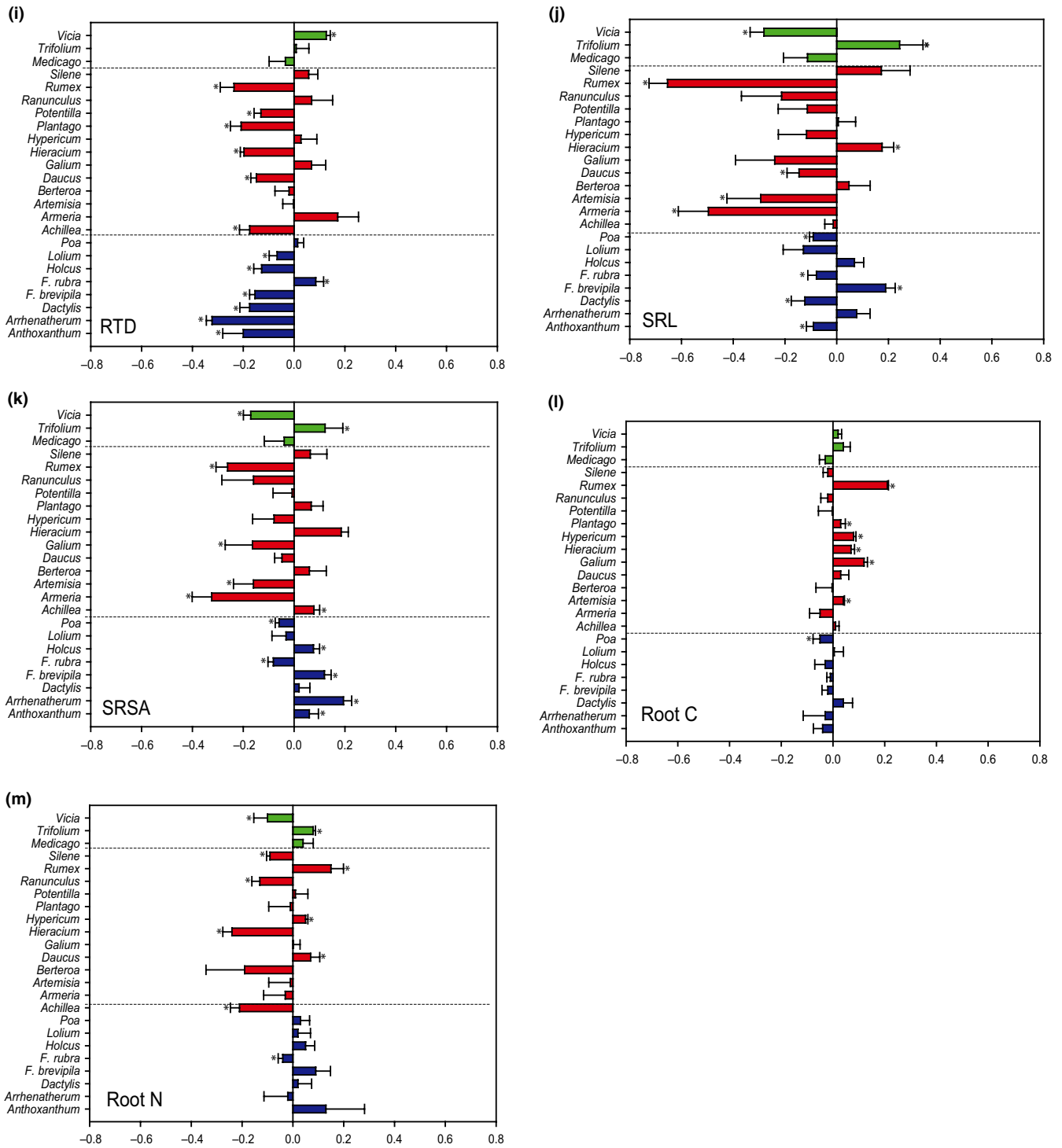
FIGURE 2 (Continued)

trait responses were more heterogeneous depending on species identity. The species-specific nature of these drought responses is also evident from the clustering patterns of species along RDA axes (Figure 1b). For example, species such as the forbs *Armeria*, *Artemisia*, *Galium*, *Hypericum* and *Rumex*, clustered right along RDA 1 under drought compared to the ones grown under non-drought (Figure 1b), indicating a decrease in SRSA and an increase in root diameter or root C under drought for these species (Figures 1b and 2).

The heterogeneous nature of root trait responses to drought is further evident when inspecting the variation in individual traits (Figure 2). Overall, the value for SRSA was lower in drought compared to control conditions for several species (*F. rubra*, *Poa*, *Armeria*, *Artemisia*, *Galium*, *Rumex* and *Vicia*) while the opposite was true for species such as *Anthoxanthum*, *Arrhenatherum*, *F. brevipila*, *Holcus*, *Achillea* and *Trifolium* (Figure 2). Root diameter was greater under drought compared to control in most species (Table 2; Figure 2).

TABLE 2 Results from general linear models on leaf and root traits response to drought. Leaf dry matter content (LDMC), specific leaf area (SLA), root average diameter (RAD), Root tissue density (RTD), specific root length (SRL), specific root surface area (SRSA). Drought was considered as a fixed factor while phylogeny and soil temperature were used as covariate. F-values, and p-values in parentheses. Heteroscedasticity was corrected for all traits (except SLA, leaf N and SRL). Variables were log-transformed except root mass and SRL that were sqrt-transformed and SRSA that was not transformed. All significant values (<0.07) in bold

Factors	df	Leaf traits					Root traits						
		Shoot mass	LDMC	SLA	Leaf C	Leaf N	RAD	RTD	Root mass	SRL	SRSA	Root N	Root C
Drought	1	**29.1 (0.01)**	**19.6 (0.01)**	**10.2 (0.01)**	0.1 (0.76)	**6.5 (0.01)**	3.2 (0.07)	3.1 (0.07)	0.00 (0.99)	0.25 (0.61)	0.5 (0.48)	0.01 (0.97)	2.2 (0.14)
Phylogeny	1	**24.4 (0.01)**	**88.3 (0.01)**	0.03 (0.95)	0.7 (0.40)	**176.0 (0.01)**	**71.0 (0.01)**	**108.2 (0.01)**	**74.9 (0.01)**	**225.5 (0.01)**	**290.4 (0.01)**	**80.1 (0.01)**	**5.6 (0.01)**
Soil temperature	1	1.1 (0.29)	2.6 (0.11)	1.1 (0.30)	0.0 (0.86)	0.3 (0.54)	0.03 (0.95)	0.00 (0.99)	0.72 (0.39)	0.1 (0.73)	0.14 (0.70)	0.4 (0.53)	0.2 (0.66)

Only *Trifolium*, *Silene*, *Berteroa* and *F. brevipila* showed a different pattern.

Root mass and root N clustered on opposite ends along the RDA (Figure 1b); illustrating opposite response patterns to drought. Plant species such as *Arrhenatherum*, *Dactylis* and *Achillea* under drought, were clustered together (upward) compared to the ones grown under control conditions, indicating an increase in root biomass with drought (Figure 1b), while the opposite pattern was found for *F. rubra*, *Lolium*, *Poa*, *Galium*, *Ranunculus* and *Rumex* (Figures 1b and 2). Following that pattern, *Arrhenatherum* and *Achillea* had decreased root N with drought (Figure 2). Our results show that even within plant functional groups species identity plays a key role in responses to drought.

3.2 | Root trait–shoot biomass relationships were stronger under drought conditions

Under non-drought (control) conditions, the five plant species with a higher shoot mass (i.e. *Armeria*, *Artemisia*, *Daucus*, *Rumex* and *Trifolium*) showed no consistent patterns across their root traits (Figure S3a). *Rumex* showed a high RTD and root mass and a low SRL and root C; *Daucus* showed a high SRSA and SRL values, while *Armeria* showed high RAD and root N values. By contrast, under drought conditions (Figure S3b) the five plant species with higher shoot mass (i.e. *Anthoxanthum*, *Dactylis*, *Lolium*, *Achillea* and *Potentilla*) showed similar mean values in the seven root traits (except *Anthoxanthum*, which had higher SRSA and SRL values in comparison to the other plant species). Overall, when considering the 24 species, we observed lower variability under drought than under control conditions for most of the traits, based on the standard deviation of each trait (Figure S4; Table S3). In addition, we found a higher correlation between root traits and shoot biomass under drought than under control conditions (Figure S5). For instance, R^2 increased from 0.04 in the control to 0.12 under drought (RAD and shoot mass, Figure S5a), a similar pattern was found for RTD, SRSA and SRL (Figure S5b–d). In summary, most of the root traits correlated with shoot mass under drought but not under control conditions (Table S4).

4 | DISCUSSION

4.1 | Phylogeny explains more variation in root traits than leaf traits

For the 24 plant species growing under control conditions, we found that root traits are more phylogenetically clustered than leaf traits. This result is congruent with recent comparative studies assessing the role of evolutionary history in root trait variation using a larger set of plant species and field data at global scale. For example, Ma et al. (2018) found that root traits are strongly influenced by phylogeny; and Valverde-Barrantes, Freschet, Roumet, and Blackwood (2017) found them to be more phylogenetically structured than leaf

traits. Although our results align with the idea that root diameter and root N are phylogenetically structured traits, we found that SRSA, a root trait not considered by Valverde-Barrantes et al. (2017), was one of the most phylogenetic structured traits. Thus, our result supports the view that selective pressures that took place early on in plant evolution have a strong influence on the expression of root traits today.

4.2 | Root trait responses to drought are more heterogeneous than leaf trait responses

Leaf and root traits differed in their response to drought. Leaf traits had a stronger response to drought than root traits and the direction of the response was consistent across plant species (i.e. independent of the plant species identity after taking into account variation by phylogeny). For example, all plants species under drought had reduced leaf area (which is correlated with decreased photosynthetic activity) and increased leaf thickness (which would be indicative of more rigid cell walls (Markesteijn, Poorter, Paz, Sack, & Bongers, 2011). These responses enable leaves to maintain turgor and minimize cell damage under drought, and this has been related to an increase in drought survival (Bongers, Olmo, Lopez-Iglesias, Anten, & Villar, 2017). The decrease in SLA, which is strongly correlated with LDMC, is associated with drought tolerance (Markesteijn et al., 2011). The stronger impact of drought on leaves than on root traits may be linked to differences in the physiology of root and leaves. Roots are the organs responsible for taking up water and are the first responders to many kinds of stress (Brunner et al., 2015; Weemstra et al., 2016). Thus, due to their ability to grow towards wetter patches in the soil (hydrotropism; Eapen, Barroso, Ponce, Campos, & Cassab, 2005), roots can minimize the effect of water scarcity. For leaves, higher water stress is expected because of the extremely high loss of water during transpiration as stomata are opened to absorb CO_2 (Beerling & Franks, 2010), and due to the reduced amount of water that reaches them (as roots require water for their own metabolism (Bais, Loyola-Vargas, Flores, & Vivanco, 2001) and as the pressure in the xylem becomes excessive under drought, breaking the water column (cavitation; Zimmermann, 1983; Zufferey et al., 2011)).

Thus, our results not only show that root trait responses depended on plant species identity, but also that leaf and root trait responses to drought are uncoupled.

4.3 | Root diameter as a strategy to tolerate drought

Our results showed that most species increased root diameter as a response to drought. Thicker roots with lower tissue density may support faster nutrient acquisition (Wahl & Ryser, 2000; Withington, Reich, Oleksyn, & Eissenstat, 2006) because of their greater dependence on mycorrhizal fungi (Brundrett, 2002; Kong et al., 2017; Weemstra et al., 2016). Likewise, thicker roots may increase the reserve of NSC (Guo, Mitchell, & Hendricks, 2004), which

can be utilized to maintain osmoregulation and osmoprotection (Chaves, 1991). Although there is evidence that under drought conditions thick roots increased NSC accumulation (Yang et al., 2016), whether the increase in root diameter of fine roots is directly linked with an accumulation of NSC remains to be tested. Thus, the apparent disadvantage of building an expensive root system with thicker roots under water-limited conditions, may be further compensated by long-lived roots (Kong et al., 2017; Weemstra et al., 2016). Our results support recent studies (Zhou et al., 2018; Zhou, Wang, Bai, Zhang, & Zhang, 2019) showing that plants under drought increase root diameter.

4.4 | Specific root surface area as a strategy to tolerate drought

Additionally, our results showed that several plant species (most forbs and some grasses) had reduced SRSA and SRL (i.e. the amount of fine roots) as a response to drought. In fact, we found that the length of roots whose diameter was lower than 0.1 mm was smaller under drought than under control conditions (Figure S6). This response can be interpreted as a drought coping mechanism when taking into consideration water flow dynamics. On the one hand, as soon as water availability decreases, the turgor potential of the plant cells diminishes; as a consequence, many turgor-driven processes, such as root elongation, slow down (Bardi & Malusà, 2012). On the other hand, sacrificing fine roots may prevent the propagation of embolism in the plant, which diminishes the risks of hydraulic rupture under drought (Zufferey et al., 2011). The reduction in fine absorptive roots also could explain the decrease in leaf N under drought conditions. This aligns with He and Dijkstra (2014), who demonstrate that drought has a stronger negative effect of plant N uptake, which can be related with reduced microbial activity, net N mineralization (Borken & Matzner, 2009) and nutrient diffusivity (Rouphael, Cardearelli, Schwarz, Franken, & Colla, 2012) under drought. By contrast, several grasses and other plant species had increased SRL and SRSA, which is a strategy mainly linked to fast-growing species that allows them to quickly uptake water and nutrients under drought conditions (Comas et al., 2013; Fort, Jouany, & Cruz, 2013; Tjoelker, Craine, Wedin, Reich, & Tilman, 2005).

4.5 | Root carbon allocation as a strategy to tolerate drought

Our results showed that some plant species had increased root biomass under drought, likely promoting water and nutrient acquisition (Burri, Sturm, Prechsl, Knohl, & Buchmann, 2014; Palta & Gregory, 1997); this response is sometimes linked to an increasing requirement for osmotically active C compounds under drought (Chaves, Maroco, & Pereira, 2003). Likewise, the decline of root respiration with drought (Huang & Fu, 2000; Thorne & Frank, 2009) may contribute to the increased root biomass. However, most species

showed a reduction in root biomass as a response to drought. This reduction may be because plants were dying from hydraulic failure and/or because they allocated more to photosynthetic or reproductive tissues. For example, we observed an early flowering under drought (e.g. *Trifolium* had 2 ± 1.2 flowers under drought versus 0 flowers under non-drought, after 1 week with drought) which is a well-known drought escape mechanism in plants (Shavrukov et al., 2017). Likewise, drought may reduce the amount and speed of carbon allocation to root biomass by about 50% (Hasibeder, Fuchslueger, Richter, & Bahn, 2015).

5 | CONCLUSIONS

Most species had increased root diameter with drought, while others had increased or decreased SRSA, SRL (root fineness) and root biomass depending on the plant species identity. Our results show the individual strategies of 24 different plant species for tolerating drought. Further research is needed in order to know how these root traits responses to drought change under field conditions where different biotic and abiotic factors are involved. As global climate models predict an increase in short term but extreme 'pulse droughts' and in long-term but subtle 'press-droughts' (Hoover & Rogers, 2016), root trait adjustments in response to drought may be potentially affected. Our results support the inclusion of root traits and their role in ecosystem functioning in future models in order to better project responses of terrestrial ecosystems to global change, specifically to drought.

AUTHORS' CONTRIBUTIONS
Y.M.L., C.A.A.-T. and M.C.R. conceived the ideas and designed methodology; Y.M.L., I.C.F. and C.A.A.-T. established and maintained the experiment in the greenhouse; Y.M.L. and C.A.A.-T. analyzed the data; Y.M.L. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY STATEMENT
Data are available in the Dryad Digital Repository https://doi.org/10.5061/dryad.4f4qrfj91 (Lozano, Aguilar-Trigueros, Flaig, & Rillig, 2020).

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SUPPORTING INFORMATION

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