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Research

Mimicking a rainfall gradient to test the role of soil microbiota for mediating plant responses to drier conditions

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Oikos
127: 1776–1786, 2018
doi: 10.1111/oik.05443

Subject Editor: Eric Seabloom
Editor-in-Chief: Dries Bonte
Accepted 19 June 2018

Plant interactions with soil microbiota are important drivers of biodiversity and ecosystem function, but climate change can modify these interactions by directly altering the soil community, which can affect the direction and magnitude of such interactions. We manipulated water quantity and soil microbiota of two populations of three plant species that differ in their interactions with soil microbiota and assessed germination and biomass production under conditions that mimicked a rainfall gradient in southeastern Spain. We assessed the importance of soil microbiota from home and away (drier) sites for alleviating or exacerbating the effects of drier conditions. Our results suggest that home soil microbiota enhanced germination of the legume *Trifolium stellatum*. Conversely, we found that the grass, *Lagurus ovatus*, and the forb, *Sisymbrium erysimoides*, produced more biomass under moderate drying with soil microbiota from a drier site than with home soil microbiota, suggesting that dry-adapted soil microbiota alleviated the negative effects of drier conditions for these species. This maintenance of productivity with dry-adapted soil microbiota under drier conditions was found despite simultaneous reductions in leaf dry matter content and root-to-shoot ratio that would typically be less optimal traits changes under reduced water availability. Severe water limitation resulted in decreased plant biomass regardless of the plant species and soil inoculum, indicating a threshold effect whereby severe water limitation on growth supersedes the beneficial effects of soil microbiota. Overall, our results show that species identity, the severity of water limitation, and soil microbiota interact to determine the response of plants to drier conditions.

Keywords: climate change, facilitation, mutualism, plant–climate interactions, species distributions, semiarid

Introduction

The effect of climate change on the distribution and range shifts of plants depends on interactions among plants, between plants and abiotic factors, and between plants and other trophic levels (Liancourt et al. 2013, Alexander et al. 2015,

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Tomiolo et al. 2015, O'Brien et al. 2017a). Interactions between plants and soil microbiota can exacerbate or alleviate the effects of warmer or drier conditions on plants (Valladares et al. 2014, Kaisermann et al. 2017, Lozano et al. 2017), but climatic conditions may alter the strength and magnitude of interactions between plants and soil microbiota which may contribute to the ability of plants to persist, and potentially adapt, under novel climates (Ruiz-Lozano et al. 1995, Singh et al. 2010, Tomiolo et al. 2015). Therefore, assessing plant–soil–climate interactions is important for understanding and predicting plant responses to novel climatic conditions and the factors that mediate those responses.

Soil microbial communities are important drivers of productivity, diversity and invasion of plant communities (Reinhart and Callaway 2006, Schnitzer et al. 2011, van der Putten et al. 2013, Wagg et al. 2014, Lozano et al. 2017). Because climate directly affects soil microbial diversity and function (Singh et al. 2010, Sheik et al. 2011, Maestre et al. 2015, Sayer et al. 2017, Delgado-Baquerizo et al. 2018), climate change may also alter the direction of plant–soil feedbacks that maintain plant community function. However, the effect of climate on plant–soil interactions is likely specific to plant species or functional groups. If plant nutrient and water uptake is highly dependent on soil microbial communities that have co-evolved with the plant species or population under a specific microclimate, then climatic alterations to the soil microbial community may negatively affect plant establishment, growth and survival (Pregitzer et al. 2010, Rodríguez-Echeverría 2010, Arguello et al. 2016). This dependency may be particularly important for plant species establishing mutualisms with soil mycorrhizal fungi or nitrogen-fixing bacteria (Rodríguez-Echeverría et al. 2012, Bennet et al. 2017). In contrast, species that are less specific in their associations with soil microbial communities for resource availability and uptake may benefit from novel soil microbial communities (Bever et al. 1997, Kaisermann et al. 2017) and take advantage of soil microbiota better adapted at resource acquisition under the new climatic conditions (Lau and Lennon 2012, Gehring et al. 2017). Therefore, soil microbial communities can either benefit or limit plant performance in new environments and climates, but the direction of this effect depends on plant species identity and functional traits that determine their resource-uptake and growth strategies (Lau and Lennon 2012, Schweitzer et al. 2014, Tomiolo et al. 2015).

Shifts in plant species distribution under climate change depends on the cumulative effects on the populations that comprise that species (Valladares et al. 2014), especially because populations vary in their phenotypes across the distribution range of the species (Volis et al. 1998, Mägi et al. 2011). Furthermore, just as biotic associations are important mediators for matching species to environments across climatic gradients (Maestre et al. 2015, Tomiolo et al. 2015), the fitness of populations across the distribution range of a species is likely mediated by interactions with soil microbes (van der Putten 2012). Therefore, soil microbiota likely influences the response of populations to climatic variables such as water and temperature (Valladares et al. 2014, Gehring et al. 2017).

In addition, functional traits may be good metrics to gauge population responses to climatic variables (O'Brien et al. 2017b, Griffin-Nolan et al. 2018). For example, leaf dry matter content (LDMC), which is associated with photosynthetic capacity, relative growth rate and leaf longevity (Cornelissen et al. 2003), is regulated by construction costs related to plant water status. Alternatively, the functional equilibrium theory of biomass allocation (Poorter et al. 2012) predicts that plants will allocate relatively more biomass to roots if the limiting factor for growth is belowground (e.g. nutrients, water). Therefore, LDMC and root-to-shoot ratio would increase across populations as water availability decreases (Cornelissen et al. 2003, Poorter et al. 2012).

We used two populations of three plant species from three different functional groups (a legume, a grass and a forb) to assess the population level variation in their responses to climate change (water availability) and the interactive effects of climate and soil microbiota for plant establishment and productivity. Populations came from two sites (a wet and intermediate site) and soil from three sites (wet, intermediate, and dry sites) across a rainfall gradient from ~700 to ~250 mm of rain per year. We simulated three scenarios (Fig. 1): 1) plant populations growing with soil microbiota from their home site under drier conditions (home inoculum and drier climate); 2) plant populations growing with soil microbiota from a more arid site and under drier conditions (away inoculum and drier climate); and 3) plant populations growing with soil microbiota from their home site and home rainfall conditions (i.e. a control of home inoculum and home rainfall). We monitored germination, survival, biomass production, and plant traits to assess the importance of soil inoculum for plant performance under drier conditions. We hypothesized that: 1) drier conditions will have negative effects on germination, survival and biomass, and the magnitude of these negative effects will be altered by the origin of soil inoculum; 2) the effects of home and away inocula on plant performance will vanish as water limitation increases (a threshold effect); and 3) the importance of soil inocula for drought resistance will vary with the plant species; whereby the legume, which relies on multiple soil mutualists, will benefit most from co-evolved home soil microbiota under drier conditions, plant species that rely less on mutualistic associations with specific microbes (i.e. the grass and forb) will benefit most from dry-adapted soil microbiota (away biota).

Methods

Experimental design

The experimental design was based on the natural rainfall gradient that decreases west to east across Andalucía, Spain with all sites having similar annual temperature and soil characteristics (e.g. soil pH around 7.8). The highest rainfall site

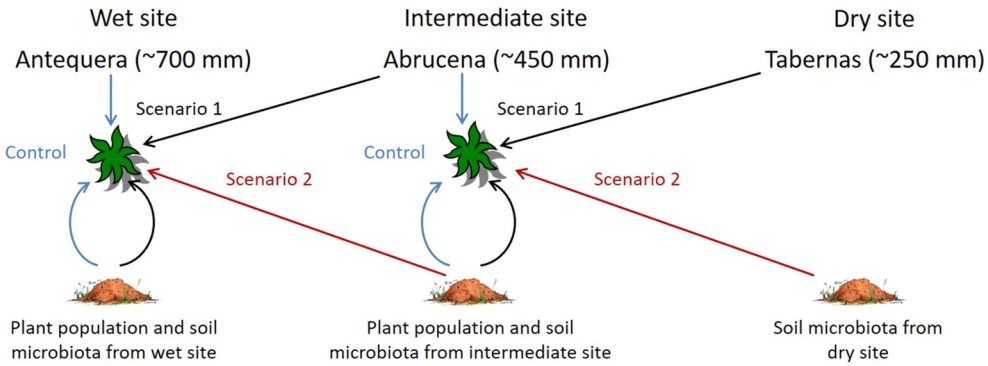

Figure 1. Experimental design. We used three treatments to simulate possible scenarios: 1) plants growing in a drying climate and interacting with home soil microbiota (black lines; drier:home treatment), 2) plants growing in a drying climate and interacting with soil microbiota from a drier site (red lines; drier:away treatment) and 3) plants growing in their home climate with home soil microbiota (blue lines; control). We used three functional groups (legume, grass and forb), represented by *Trifolium stellatum*, *Lagurus ovatus* and *Sisymbrium erysimoides*, respectively. Seeds were collected both in wet and intermediate sites along a natural rainfall gradient while soil to be used for inoculation was collected in the three sites. Seeds were sown into pots inoculated with soil microbiota either from the same population (home inocula) or collected from the next drier site (away inocula). Drier conditions were simulated by watering with the equivalent to the next drier site for each plant population. Using multiple populations of species allowed us to test the effect of shifting climates for populations across a range of the distributions of species. We predicted that species identity would alter the role of home and away soil microbiota under drying conditions and that the importance of microbiota would decrease with decreasing soil water availability.

in the west was near Antequera (~700 mm per year and a mean temperature ~16°C), the intermediate rainfall site was near Abrucena (450 mm per year and a mean temperature ~15°C) and the lowest rainfall site in the east was near Tabernas (250 mm per year and mean temperature ~17°C). Therefore, rainfall decreases by approximately 50% with each site from west to east. Almost all precipitation for the year falls between September and April. Soil was collected under *Retama spaherocarpa* (*Retama*) to use as inoculum from the three sites (see details of soil collection below), and seeds of the three species were collected from the wet (Antequera) and intermediate (Abrucena) sites, which are separated by about 160 km.

Both populations of the species received soil inoculum consisting of either their home soil or soil from the next drier site (away). In addition, each population received water in proportion to the rainfall at their home site or a 50% reduction relative to their home site. Therefore, there were three levels of watering, 100% rainfall of the wet site (150 ml per watering), 50% rainfall of the wet site, which is equivalent to the intermediate site (75 ml per watering) and 25% of the wet site, which is equivalent to the dry site (37.5 ml per watering). The wet site population received soil inoculum from the wet site (home soil inoculum) and from the intermediate site (a soil inoculum from a drier site), and the intermediate site population received soil inoculum from the intermediate site (home soil inoculum) and from the dry site (a soil inoculum from a drier site; see Fig. 1 for a diagram of the experimental design). Combined, the study was a partial factorial design whereby each population received two watering treatments (equivalent to 100% and 50% of the home precipitation; i.e. control and drier) and two soil inocula treatments (from home and the drier site; i.e. home and away) to make three treatments for each population: 1) the 'control' with 100% home watering and home soil, 2) the 'drier:home' with 50% reduced watering and home soil, and 3) the 'drier:away' with 50% reduced watering and away soil (from next drier site).

Study species

We used three annual species differing in functional group. *Trifolium stellatum* is a legume that forms symbiosis with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi. *Lagurus ovatus* is a grass that can associate with arbuscular mycorrhizal fungi but not with nitrogen-fixing bacteria, and *Sisymbrium erysimoides* is a crucifer forb with overall low affinity for soil mutualists (Gerdemann 1968). All three species are annuals, although *Sisymbrium* can persist for more than one growing season under some conditions. Natural populations of the focal species co-occur along the rainfall gradient and beyond (~250 to ~1500 mm of rain per year), although *T. stellatum* is present at low abundance at the dry site. For each species, seeds were collected from two populations (one from the wet site and the other from the intermediate site) in May–June 2015. Seeds were cleaned, surface sterilized by stirring in 70% ethanol solution for 1 min followed by 2 min in 5% NaOCl solution and then rinsed five times in deionized water. Seeds were placed in sterile Eppendorf tubes until sowing. Because germination success varied among species, the number of seeds per Eppendorf tube was adjusted to ensure a similar number of viable plants after germination. *Lagurus* and *Sisymbrium* had five seeds each, and *Trifolium* had 20 seeds. One Eppendorf tube contained seeds of only one species to be sown as a monoculture in one pot (totaling 48 pots per species).

Soil inoculum

We choose to collect soil inoculum from under *Retama* instead of in open areas because the legume and grass are almost exclusively present under *Retama* as rainfall decreases while the forb can be found both under and outside *Retama* canopies (Pugnaire and Lázaro 2000, Pugnaire et al. 2004, Armas et al. 2011). Therefore, inocula from under *Retama* more accurately mimics the microbiota associated with these three species under dry conditions while also standardizing it for all three species. Furthermore, by sampling soil inocula from beneath *Retama* shrubs we minimized differences across the gradient in soil nutrients, organic matter and soil texture, which are relatively similar under *Retama*, and in turn promoting homogeneity of the soil microbial community across the gradient.

At each site, we collected soil under eight different *Retama* shrubs. Samples were kept separate allowing for multiple unique soil inoculum replicates of each site (8 soils × 3 sites = 24 soils). Soils were collected five days before the start of the experiment (24 June 2016, at the beginning of the dry season) from the surface (0–10 cm; the normal rooting and resource uptake zone for this system and were most of the soil microbiota thrives) using trowels sterilized with ethanol between samples. Soil was placed in sterile bags after collection for the five days prior to inoculum preparation.

Inoculum was made by mixing 300 g of soil in 1.2 l of sterile deionized water (ca 1:2; v:v). The solution was filtered through a 0.5-mm sieve to remove soil particles while allowing fungal spores, hyphae, soil bacteria and micro-fauna to pass (van de Voorde et al. 2012). We used soil suspensions instead of sieved soil to minimize differences in nutrient and organic matter content between inocula (Rodríguez-Echeverría et al. 2013). We used the total soil suspension to assess the effect of the soil community and not separate groups of the soil microbiota. Each inoculum was poured into three (dry site), six (wet site) or nine (intermediate site) replicate pots (1.5 l pots, 15 cm high and filled with 1 l of substrate). The soil inoculum from the dry site was used as the drier:away treatment for seed populations from the intermediate site. Therefore, each dry site inoculum (n = 8) was used in three pots, one per plant species (3 species × 1 treatment × 8 dry site soil inoculum replicates = 24 pots). The intermediate site soil inoculum was used in three treatments: 1) the control for the intermediate site seed populations, 2) the drier:home for intermediate site seed populations and 3) the drier:away for the wet site seed populations. Therefore, each inoculum was used in nine pots, one per plant species per watering treatment (3 species × 3 treatments × 8 intermediate site soil inoculum replicates = 72 pots). The wet site soil inoculum was used in the control and the drier:home treatment of the wet site seed populations for a total of six pots, one per species per watering treatment (3 species × 2 treatments × 8 wet site soil inoculum replicates = 48 pots). There was a total of 144 pots filled with a mixture of 25% standard topsoil rich in nutrients (MO = 50%, pH = 7.5; enriched with N:P:K 5-6-5), 25% of field soil (Lozano et al. 2017) and 50% sand. This mixture was filtered through a 5-mm sieve to remove large particles and sterilized by autoclaving three times to a temperature of 120°C for 1 h each time. Major nutrient contents of the soil substrate (SE; n = 16) were 1.92 mg of N (0.19), 0.25 mg of P (0.02) and 2.51 mg of K (0.19). This soil substrate controlled the nutrient conditions while providing similar pH and structural conditions to that of field soil across all sites.

The pots were placed in a climate chamber at the Estación Experimental de Zonas Áridas in Almería, Spain. The chamber was set to 15°C for 12 h of night time and 18°C for 12 h of day time (average photosynthetically active radiation = 300 μmol m^{-2} s^{-1}) to simulate average ex situ germination and growing conditions of the field sites. Approximately 150 ml of soil inoculum was applied to every pot to allow the solution to reach all depths of the pot (ca 25% volume density). Three days after the solution was applied seeds were sown into every pot and hand watering began at the 3 treatment levels (150, 75 and 37.5 ml of distilled water) every three to four days.

Germination and harvest

Germinated seeds (i.e. radicle emergence) were recorded on days 14 and 28 in every pot, and the highest number from these two censuses was used as total germination per pot. If final abundance was higher than the maximum germination, then that was used as total germination, but this occurred for only four pots, which each had an additional one plant. No seeds germinated in 13 pots, and an additional one pot had only one seedling that subsequently died, reducing the total replicates to 130 pots at the final harvest (11 of the forb, two of the legume and one of the grass pots were, thus, excluded). Plants grew for approximately 120 days to reach full maturity and bloom (although not all the plants matured at exactly the same time, none of the plants were senescing at harvest). After this period, the number of plants was counted, and plants were harvested. The largest healthy and fully developed leaf from each pot was removed, fully hydrated in a falcon tube with moist cotton, weighed at full moisture, dried for 72 h at 60°C and re-weighed to calculate leaf dry matter content (the ratio of leaf dry mass to saturated mass). Roots of all plants were cleaned of soil, and plants were dried for at least 72 h at 60°C. Once dry, roots and shoots were weighed separately. Because the roots of plants were intertwined, plants could not be separated for measurement of individual level biomass.

Soil nutrients

A 5-g subset from the top 0 to 5 cm of soil without roots from all pots was collected and air-dried at the end of the experiment. A systematic sample of the treatments (eight with intermediate site and four with dry and wet site inocula) were sent to the Ionomic Lab at the Centro de Edafología y Biología Aplicada del Segura, Murcia, Spain, for the analysis

of soil nutrients. Briefly, the analysis assessed total C and N content using a C/N analyzer, and micronutrients were determined after acid digestion by inductively coupled plasma (ICP) emission spectrometry. Phosphate (PO_4^{3-}), nitrate (NO_3^-), and sulphate (SO_4^{2-}) concentrations in water extracts (1:10 soil:water) were analyzed by HPLC. Soil nutrients were not significantly different between treatments (which was expected because the substrate was standardized), which indicates the inoculum wash did not affect the nutrient levels among treatments (see ANOVA in the Supplementary material Appendix 1 Table A1).

Statistical analysis

Germination and final abundance were analyzed as a function of population (a factor with two levels; wet and intermediate sites), functional group (a factor with 3 levels; legume, grass and forb), watering (a factor with two levels; control and reduced), soil inoculum × watering (a factor with three levels; control, drier:home and drier:away) and all possible two-way interactions. This model structure allowed a contrast which first compares control versus reduced watering effects followed by the three level factor for our three treatments (control:home, drier:home and drier:away) that tested the additional effect of home and away soil inocula in drier conditions relative to control conditions with home soil inoculum. We used a generalized linear-mixed model with a quasi-Poisson distribution – which allowed for the estimation of the dispersion parameter – and an identity link function. We weighted the response by the total number of seeds planted in each pot (5 for the grass and forb and 20 for the legume) to account for abundance differences. A random term for *Retama* plant (i.e. *Retama* soil inoculum) nested in site (a factor with 24 levels) was used to account for the eight different *Retama* plants used for soil collection at each site (see ANOVA of Wald statistics in the Supplementary material Appendix 1 Table A2).

Total biomass per pot was analyzed with a Gaussian distribution and the same fixed and random terms as germination (see ANOVA of Wald statistics in the Supplementary material Appendix 1 Table A3). However, we used an additional covariate for plant abundance in the pot to account for differences in plant numbers between treatments and populations. Biomass was log-transformed to meet assumptions of normality. Leaf dry matter content and root to shoot ratio were analyzed using the same model as biomass (see ANOVA of Wald statistics in Supplementary material Appendix 1 Table A4). Root to shoot ratio was log transformed to meet assumptions of linearity. All linear mixed effects models were performed with the asreml-R package (ASReml 3, VSN International, Hemel Hemsted, UK), using R 3.3.2 (<www.r-project.org>). Results shown in the figures and text are model means with 95% confidence intervals (CIs). If the 95% CIs of treatments do not cross other treatment means, then estimates are significantly different at $p < 0.05$, and no further post-hoc tests or pairwise testing is required to prove significance (Hector 2015).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.hh73sc6> (O'Brien et al. 2018).

Results

Because of high survival in all treatments of species and populations, germination and final abundance showed nearly identical patterns (Fig. 2, Supplementary material Appendix 1 Fig. A1), and therefore, we present only the results of germination. At the treatment level, averaged across all populations and species, the control and drier:home treatments had identical germination quantities (3.6 seeds, 95% CI: 3.0–4.2) while the drier:away treatment had significantly lower germination (2.9 seeds, 95% CI: 2.4–3.5). At the level of functional group, the grass and forb showed no significant differences between treatments, but the legume had significantly lower germination in the drier:away treatment (3.9 seeds, 95% CI: 3.2–4.6) than the control (5.0 seeds, 95% CI: 4.3–5.8) and drier:home (5.5 seeds, 95% CI: 4.7–6.3) treatments. Differences between populations were tested by comparing the drier:away treatment for the wet site populations and the control treatment for the intermediate site populations because these treatments had identical soil inoculum and watering quantity (i.e. a standardized environment). However, germination in both treatments was statistically indistinguishable across species, with an average of 3.4 seeds (95% CI: 2.6–4.2) for the drier:away treatment in the wet site and 3.3 seeds (95% CI: 2.5–4.1) for the control treatment in the intermediate site.

Forb and grass species from the wet site had statistically indistinguishable biomass between the control and drier:away treatments, while these species populations had significantly lower biomass than the control in the drier:home treatment (Fig. 3a, b), indicating a negative effect of the home inoculum under drier conditions. However, the biomass of wet site population of the legume was unaffected by drier conditions regardless of soil inoculum origin. The dry treatments had a greater effect on the plant populations from intermediate site than on plant populatins from the wet site (Fig. 3a, c), and this response was similar for the plants from the intermediate site populations inoculated with either home or away soil inocula (Fig. 3d). A threshold effect of drying was apparent whereby the most water-limited treatment caused a decrease about two times greater in biomass (difference between drier and control for plants from the intermediate site = –3.3 g, 95% CI: –4.3 to –2.5; Fig. 3d) than the moderate water-limited treatment (difference between drier and control for plants from the wet site = –1.4 g, 95% CI: –2.6 to –0.3; Fig. 3b). Biomass production was not different between populations for any of the species.

Plant functional traits showed contrasting responses to soil inoculum under dry conditions. The forb showed similar LDMC under drier and control conditions, but the grass had significantly lower LDMC under dry conditions than in

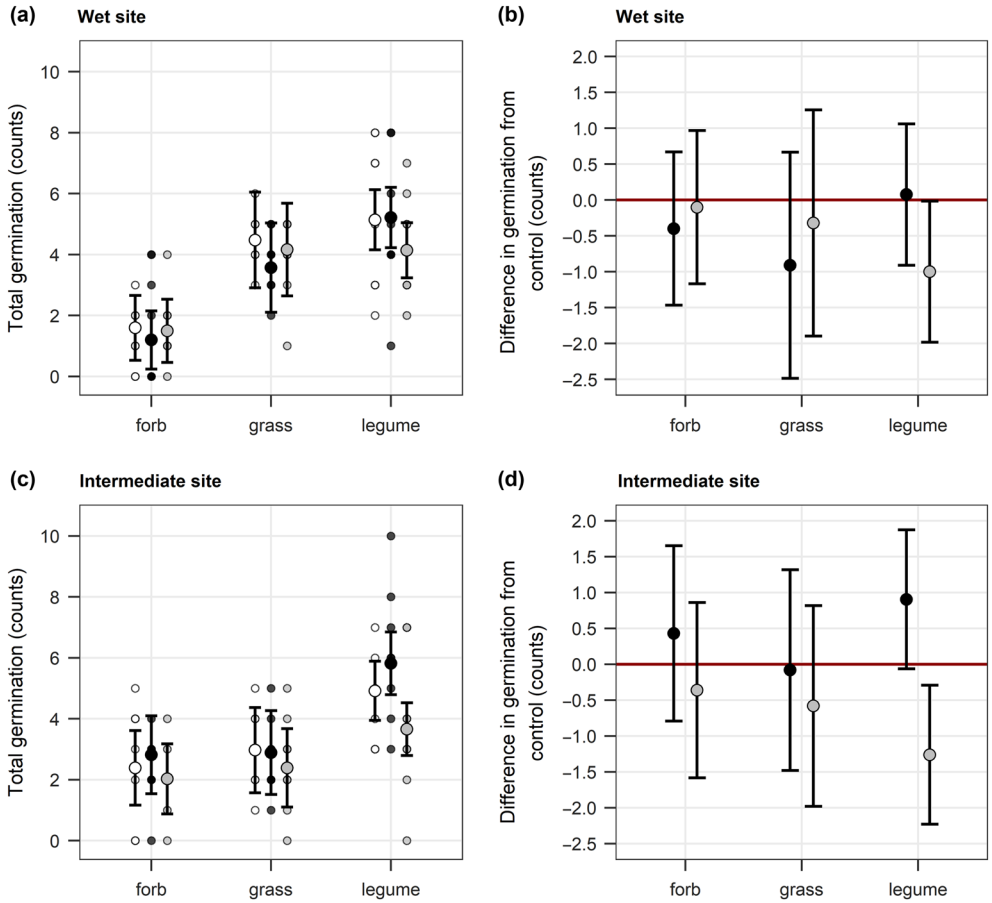

Figure 2. Germination for each treatment of each population of each species. Germination (95% CI) for the (a) wet site populations of each species in the control (○), drier:home (●) and drier:away (●) treatments. (b) Differences between the control and the home and away inoculum in germination for the wet site populations. (c) Intermediate site populations of each species in the control, drier:home and drier:away treatments. (d) Differences between the control and the home and away inoculum in germination for the intermediate site populations. Away soil inocula came from the next drier site in the gradient – i.e. the intermediate site for the wet site populations and the dry site for the intermediate site populations.

the control, regardless of soil inoculum origin (Fig. 4a, b). The pattern was reversed for root-to-shoot ratios whereby the home soil inoculum promoted the maintenance of the root-to-shoot ratios of the grass while the forb had significantly lower root-to-shoot ratio under dry conditions than in the control, regardless of soil inoculum origin (Fig. 5a, b). In contrast, the legume had similar LDMC and root-to-shoot ratios regardless of water or soil inoculum treatment. The intermediate site population of the legume had a significantly higher LDMC than the wet site population (Fig. 4c, 5c), but there were no differences in functional traits between the populations of the grass and forb.

Discussion

Our manipulations of water and soil microbiota for two populations of three plants support our predictions that soil microbiota can alter plant responses to drier conditions, but plant species identity mediates the direction and magnitude of soil microbiota effects. Germination of the legume benefited from home soil under drier conditions whereas the grass and forb showed no response to inoculum, but the biomass of the grass and forb increased when inoculated with soil microbiota from drier sites under drier conditions. However, the benefits of soil microbiota from dry sites on plant biomass were not observed under the most severe water-limited conditions, which supports our hypothesis that increasing aridity will constrain the benefits of soil microbiota. Combined, these results suggest that plant functional group and soil microbiota interact to determine plant responses to reduced water availability associated with climate change, although severe water limitation could undermine these interactions (O'Brien et al. 2017c).

Germination

In contrast to our hypothesis, decreasing soil water availability did not show strong effects on germination. Semiarid systems maintain a soil seed bank that germinates after autumn rainfall (Pugnaire and Lázaro 2000), but a minimum amount of rain is needed to stimulate germination (Noy-Meir 1973,

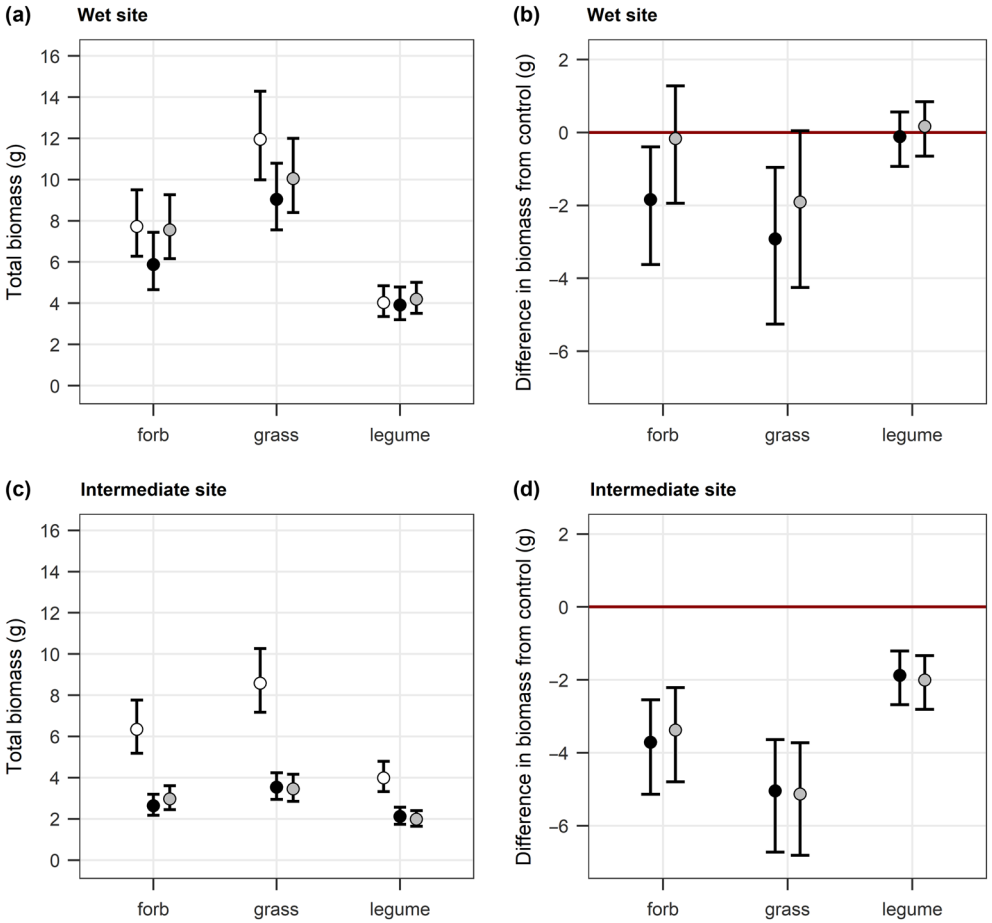

Figure 3. Total biomass for each treatment of each population of each species. Total biomass (95% CI) for the (a) wet site populations of each species in the control (○), drier:home (●) and drier:away (●) treatments, (b) differences between the control and the home and away inoculum in biomass production for the wet site populations, (c) intermediateI site populations of each species in the control, drier:home and drier:away treatments, (d) differences between the control and the home and away inoculum in biomass production for the intermediate site populations. Away soil inocula came from the next drier site in the gradient.

Chesson et al. 2004, Jiménez et al. 2011, 2016). In the absence of temperature limitation, mass germination in deserts occurs only after a threshold amount of precipitation (effective rain) with smaller amounts resulting in only scattered germination (Kigel 1995). Overall, this effective rain is considered to be ca 10–20 mm in deserts with winter rains, while this threshold could be even 10 times higher in deserts with summer rains (Gutterman 1993, Kigel 1995). Our design ensured that the minimum amount of moisture was achieved to initiate germination and may account for the nonsignificant differences between control and drier treatments.

Home soil microbiota promoted germination of the legume irrespective of the watering treatment, which supports our argument that the legume would be more dependent on co-evolved soil microbiota than the grass and forb. These results support previous research that found soil and rhizospheric rhizobial bacteria might enhance germination through the production of enzymes and hormones (Kremer 1993, Requena et al. 1997, Shweta et al. 2008, Miransari and Smith 2009, Delshadi et al. 2017a, b, Lozano et al. 2017) and that inoculum paired with plants from the same location further promotes these positive effects on germination (Requena et al. 1997). The significantly lower germination with away soil microbiota in drier conditions suggests that soil microbiota that promote germination of the legume were absent or had lower activity in the away soil relative to the home soil microbiota. The response of the legume germination to away soil was consistent for both the wet and intermediate site (i.e. the soil inoculum from the intermediate site did not promote germination of the wet site), which suggests populations of the legume had distinct relationships with their local soil microbiota. Due to the closer dependency on soil microbiota it is possible that positive local feedbacks are established in whichthe legume may promote soil microbiota that assist germination through breaking the seed coat and allowing imbibition (Baskin and Baskin 2000), but the ecological and evolutionary advantages of this strategy are unclear and require more experimental support.

Biomass

Biomass results support all our main hypotheses. First, under moderate water limitation, the grass and forb were able to sustain biomass production with soil microbiota from drier

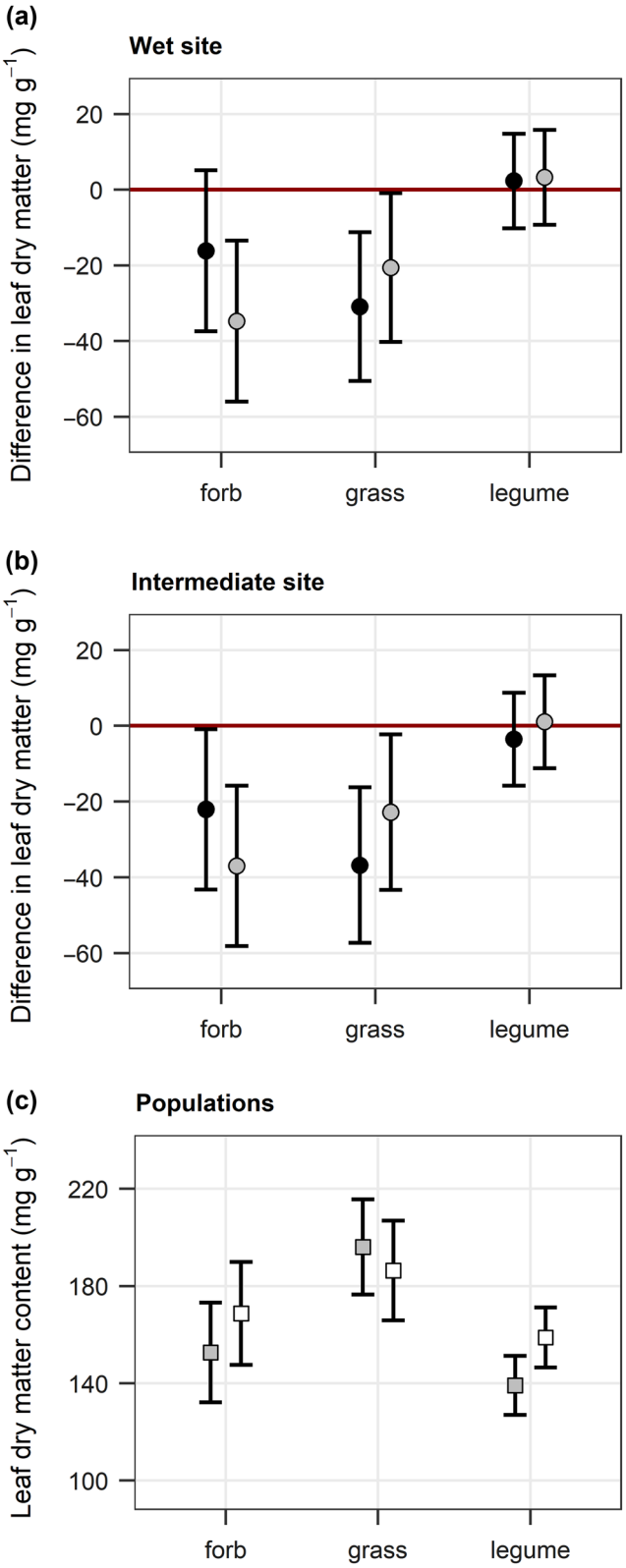

Figure 4. Leaf dry matter content changes for each species and treatment. Difference in leaf dry matter content (95% CI) between the control and drier:home (●) and drier:away (●) treatments for the (a) wet site populations of each species and the (b) intermediate site populations of each species. (c) Baseline leaf dry matter content (mg g^{-1}) of the wet (■) and intermediate (□) site populations of each species.

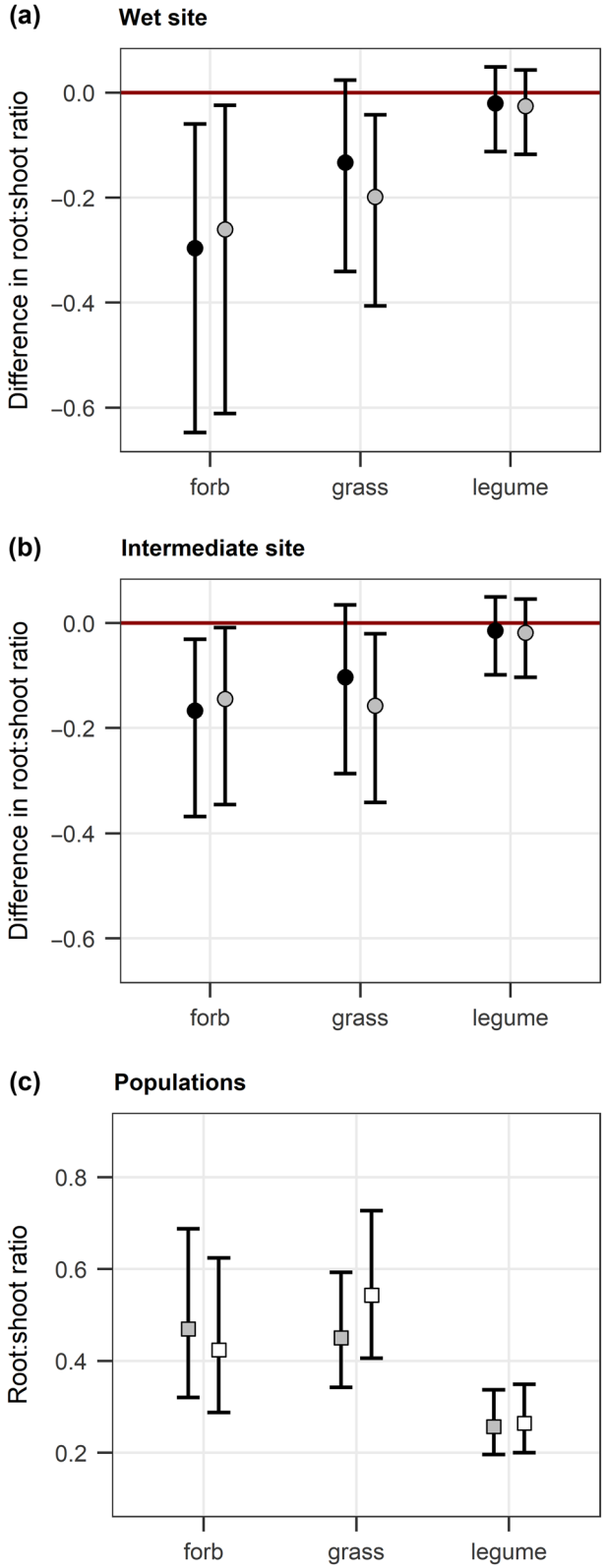

Figure 5. Root to shoot ratio changes for each species and treatment. Difference in root to shoot ratio (95% CI) between the control and drier:home (●) and drier:away (●) treatments for the (a) wet site populations of each species and the (b) intermediate site populations of each species. (c) Baseline root to shoot ratio of the wet (■) and intermediate (□) site populations of each species.

sites but not with home soil microbiota, supporting the argument that soil microbiota mediate plant responses to changing climatic conditions. The soil microbiota from drier sites may be better adapted for nutrient cycling with increasing water limitation (Compant et al. 2010, Vurukonda et al. 2016a, b), which alleviated the effect of drying soils on plants and in turn promoted plant growth (Lau and Lennon 2012). An alternative hypothesis could be that negative effects of pathogens in home soil may have been enhanced by drier conditions thereby causing a decrease in growth, while away soil inoculum may have offered populations a release from those pathogens (Van Grunsven et al. 2007). However, there was no evidence of pathogens in our experiment, and in semiarid environments that are resource-limited, pathogen accumulation and activity may be less common than in more productive sites (Tomiolo et al. 2015). Our experimental design does not allow us to rule out that the soil microbiota from the drier sites might also benefit plants under wet conditions. However, a positive effect of away soil in wet conditions would not invalidate the fact that soil inocula from drier sites improves biomass production under the drier conditions forecasted for the future.

Second, under severe water limitation, biomass production decreased significantly relative to controls, and differences between home and away soil microbiota were statistically indistinguishable, supporting the hypothesis that a threshold exists on the ability of soil communities to buffer the negative effects of increasing aridity (Ogle and Reynolds 2004, Schwinning and Sala 2004, Miranda et al. 2009a). The large reduction in biomass in all species in the lowest watering treatment supports the argument for this threshold effect (Tielbörger and Kadmon 2000, O'Brien et al. 2017c), which is likely due to a water limitation on cell expansion and division (Körner 2015) that cannot be overcome by the presence of soil microbiota from drier climates. We suggest the existence of a climatic threshold that, when crossed, could mean local plant extinctions, loss of front-edge populations and potentially decreased genetic variation within species (Kaisermann et al. 2017). Although our experiment used mean-based drying scenarios (i.e. reduced average rainfall amount), we expect seasonal changes in rainfall quantity, frequency and timing to also interact with soil microbiota and alter plant responses under a changing climate (Miranda et al. 2009b, 2011, Mitchell et al. 2016, Wagg et al. 2017).

Finally, the differential effect of home and away microbiota on biomass for the grass and forb was contrasted by statistically indistinguishable biomass production between home and away soil microbiota for the legume, which highlights the role of plant functional group in determining the importance of soil microbiota under a changing climate. Although the mechanisms explaining these species differences cannot be directly elucidated from our experiment, it is possible that the dual symbiosis established by the legume promotes tolerance to nutrient and water deficit during soil drying (Aguilera et al. 2016, Vurukonda et al. 2016a). Further experiments eliminating soil microbes are needed to differentiate between the effect of plant adaptation and soil microbial interactions in coping with drier conditions.

Traits

The response of traits to soil inoculum origin and drier conditions seemed to contrast the results of biomass for the grass and forb. Although total biomass benefited from away soil inoculum under drier conditions, the effect on LDMC and root-to-shoot ratio followed the opposite pattern. In other words, the grass and forb had lower LDMC and root-to-shoot ratios under drier conditions with away soil inoculum, but it was expected that these traits would be higher in drier conditions (Schenk and Jackson 2002, Poorter et al. 2012, Pérez-Harguindeguy et al. 2013). This suggests that dry-adapted microbial species may be buffering plants from water and nutrient limitations (Vurukonda et al. 2016a), and thereby reducing the demand for increasing leaf construction costs (LDMC) or belowground biomass allocation (root-to-shoot ratio). Recent work has highlighted the importance of identifying appropriate response traits to climatic variables such as precipitation (O'Brien et al. 2017b, Griffin-Nolan et al. 2018). Our results suggest that leaf and allocation traits measured here are less useful for assessing trait shifts in this semiarid system than physiological traits such as nonstructural carbohydrate concentrations or hydraulic conductance. More research examining physiological traits may offer better insights into the role of functional traits for mediating plant responses to changing precipitation patterns.

Population differences

Germination responses paralleled biomass responses in our plant populations, suggesting a lack of plasticity among populations (Valladares et al. 2014) which could point that genetic variation within these plant species is not a strong determinant of their responses to drought. The high interannual variability of precipitation in Mediterranean climates may reduce local adaptation of ecotypes across the selected precipitation gradient. Populations at the edge of the climate envelope of the species may be more resistant to shifts but may also be living at physiological thresholds that once crossed lead to mortality and population loss. The significantly higher leaf dry matter content of the intermediate site population supports the argument that leading edge populations may be better adapted to drier conditions due to those populations experiencing more frequent and severe droughts than populations more centrally located along the species distribution (Valladares et al. 2014). However, additional research is required that would subject all populations to extreme water limitation as populations may vary in their gene expression during severe water limitation and in turn plasticity in their response.

Conclusions

The effect of soil microbiota on plant responses to drier conditions depended on plant functional group and the severity of water limitation. Our results indicate that soil microbiota from drier environments promoted plant growth of the forb and the grass under moderate drying, suggesting a faster adaptation of soil microbiota to drier conditions; by

contrast, the legume was more dependent on co-evolved soil microbiota. Therefore, legumes may be more sensitive and less buffered by soil microbiota to drying conditions than grasses and forbs in these semiarid systems. However, severe water shortage strongly limited growth of all plant species, suggesting a threshold of plant growth to drier conditions and a limitation of soil microbiota to alleviate those negative impacts. Therefore, even though dry-adapted soil microbiota may delay negative effects of drying on plants, the increased temperatures and decreased rainfall predicted by climate change scenarios might decouple plant–soil interactions that promote plant germination and productivity in semiarid systems, leading to changes in community composition and ecosystem function.

Acknowledgements – Funding – MOB was supported by a Swiss National Science Foundation Advanced Post.Doc Mobility Fellowship (P300PA_167758). CA was supported by the Spanish Government under a Ramón y Cajal contract (RYC-2012-12277). FIP and CA acknowledge funding from MINECO (CGL2014-59010-R and CGL2017-84515-R). SRE was supported by the Portuguese Science Foundation - FCT (IF/00462/2013).
Author contributions – MOB designed the experiment, analyzed the data and wrote the manuscript. CA and SRE provided input on the design and contributed to writing and revisions. JAM contributed revisions and preparation of soil analysis. FMU, ALE and DJA carried out the experiment and provided logistical support. FIP contributed to revisions and provided logistical support.

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Supplementary material (available online as Appendix oik-05443 at <www.oikosjournal.org/appendix/oik-05443>). Appendix 1.