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Soil moisture incidentally selects for microbes that facilitate locally adaptive plant response

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While a plant's microbiome can facilitate adaptive phenotypes, the plant's role in selecting for these microbes is unclear. Do plants actively recruit microbes beneficial to their current environment, or are beneficial microbes only an incidental by-product of microbial adaptation? We addressed these questions through a multigeneration greenhouse experiment, selecting for either dry- or wet-adapted soil microbial communities, either with or without plants. After three plant generations, we conducted a full reciprocal transplant of each soil community onto wet- and dry-treated plants. We found that plants generally benefited from soil microbes, and this benefit was greater whenever their current watering conditions matched the microbes' historical watering conditions. Principally, the plant's presence was not necessary in the historical treatments for this environmental matching benefit to emerge. Moreover, we found microbes from droughted soils could better tolerate drought stress. Taken together, these results suggest that the moisture environment selects for microbes that benefit plants under those specific moisture conditions, and that these beneficial properties arise as a by-product of microbial adaptation to the watering environment and not as a co-adapting plant–microbe system. This work highlights that understanding the selective agents on these plant-associated microbes will lead to a better understanding of plant adaptation.

1. Introduction

The degree to which a plant's phenotype is adapted to its environment is often considered a product of the plant's evolutionary history and genetics [1,2]. However, a plant's phenotype is plastic, in part due to the composition and function of its associated microorganisms [3,4]. Plants are colonized by diverse communities of microorganisms that can influence plant phenotype through their various activities including but not limited to, nutrient cycling, decomposition, hormone production and pathogen activity [5–7]. While these plant–microbe interactions are key in determining the fitness of both the plant and microbial partners, the evolutionary history and selective agents behind many of the microbial traits that influence plant phenotype are unclear. Identifying the selective pressures on plants' associated microbes, and how this selection influences their impacts on host adaptation will provide insight to these microbes' operation and evolution, as well as their contribution to plant phenotype. To this end, herein we investigated the selective agents on plant-associated microbes that determine these microbes' impact on plant adaptation to different soil moisture environments, specifically focusing on the separate contribution of the host plant and the abiotic environment in selecting for microbes that mediate locally adaptive plant phenotypes.

Recent work has highlighted that plant hosts are frequently colonized by microbial communities that mediate locally adaptive plant phenotypes [8]. For example, prior work has found that plants in a drought experiment had the highest fitness when provided with microbes collected from plants whose

wet/dry-watering environment matched the plants' current watering environment [9–12], and similar results have been found in linking microbes to plant adaptation to serpentine soils and across temperature and salinity gradients [13–15]. These patterns may indicate that plants and microbes adapt to environmental stress as a coevolving system, which implies that the plant acts as a significant agent in selecting for microbes that facilitate its locally adaptive phenotype.

Indeed, through their root morphology, chemistry, root exudation patterns, immune signalling etc. plant roots select upon their root-associated microbes, provisioning important resources and habitats for many soil microbes and influencing which microbes can colonize the plant root [16–20]. Prior work has hypothesized that plants may have evolved mechanisms to preferentially recruit microbes that provide an adaptive value to the plant; namely, that plants use a 'cry for help' in response to various environments, wherein plants exposed to stress adaptively alter their root exudates and chemistries to recruit microbes beneficial to the current environment [21–23]. Similarly, the microbial dependence on the plant for resources may select from microbes with cooperative traits that support locally adaptive plant phenotypes that maintain plant health and thus resources for the microbes [24]. The mutual dependence of plants and microbes could potentially align plant and microbial fitness in the face of environmental stress. Therefore, there may be selection for microbes that facilitate locally adaptive plant phenotypes, because the microbial traits that increase plant fitness will also consequently benefit microbial fitness.

However, the plant is not the sole selective agent on their associated microbes. Many root-associated microbes are not fully obligate on the plant and can also be free-living in the soil [25]. Consequently, these microbes must navigate multiple dimensions of selection besides the plant—especially environmental stress. Any microbial traits that benefit the plant in a given environment may thus alternatively be the result of microbial adaptation to that environment, with any benefit to the plant only an incidental by-product. For example, traits that help microbes persist in the face of environmental stress will directly benefit their own fitness under similar environmental conditions. However, by maintaining important microbial function under environmental stress, these microbial traits may also end up benefiting plants. Importantly, these beneficial traits would be an incidental by-product of selection on these microbes from the local environment.

To understand the origin of microbial interactions beneficial to the plants in their local environment, we compared the contribution of the host plant and the abiotic environment in selecting for microbes that facilitate locally adaptive benefits to the plant. In addressing these questions, we used a long-term greenhouse experiment, exposing soil microbial communities to various selective watering environments (wet versus dry) for multiple plant generations. Following these selective treatments, we used a full reciprocal cross and inoculated these microbial communities back onto plants under the two original watering treatments, allowing us to evaluate these microbes' contribution to plant adaptation to the historical watering treatments. To evaluate the importance of plant selection on microbes, we included two plant conditioning species. Additionally, we critically included a 'no plant' control treatment to determine if plants and microbes need to adapt to drought stress *together*. By comparing the fitness of plants inoculated with microbes from 'plant' and 'no plant' treatments, we can identify the importance of the plant versus the environment in mediating selection on the microbes, and the subsequent contribution to plant phenotypes adaptive to moisture environment.

2. Material and methods

(a) Greenhouse experiment

(i) Selection phase (generations 1–3)

We established a long-term greenhouse selection experiment to investigate selective agents on plant-associated microbes and their impact on plant adaptation. For three plant generations, we exposed soil microbes to two selective moisture treatments, wet or dry. We refer to this treatment as the historic moisture selection treatment. We crossed this moisture treatment with three plant treatments: (i) *Brassica rapa* (standard stock lines, Wisconsin Fast Plants Program, University of Wisconsin); (ii) *Arabidopsis thaliana* (Col-0 lines, Arabidopsis Biological Resource Center, Columbus, OH, USA), and (iii) no plants present. We refer to this treatment as the historical plant selection treatment. Altering the presence of plants in this experiment altered the presence of plant selection on the microbes, and including the two separate plant species allowed us to evaluate the importance of species-specific specialization of soil microbes to the selective plant.

In the first generation, soil microbial communities were established in small 'cone-tainer' pots and filled with approximately 120 ml of a locally produced 'root-wash' soil mix: equal parts of calcined clay, torpedo sand and field soil (University of Illinois Plant Care Facility, https://pcf.aces.illinois.edu/soil-mixes/). To provide a microbial community that could be the target of selection by the various selective treatments, soil was amended 5% by volume with a live soil inoculum field-collected from a decades-old tallgrass prairie restoration site (40.128577, −88.140734). There were 30 replicate pots in each treatment, for a total of 180 pots (30 replicates × 2 watering treatments × 3 plant treatments). At the start of each of the first three generations, we planted five seeds of the designated species in each pot. All pots were watered to saturation daily for the first week after sowing to facilitate successful germination, after which we thinned pots down to three plants and the moisture treatments were imposed: in the wet treatment, pots were watered to saturation every 2 days; in the dry treatments, we weighed pots and watered to 14% gravimetric water content every 2 days. We chose this water content target for the dry treatment because a pilot study demonstrated this water content represented a significant stress compared with the well-watered control, while still allowing for plant survival. These watering treatments thus allow for differing selective environments, which can be used to evaluate plant adaptation to each moisture environment. Plants were grown in the greenhouse on a 26°C/24°C day/night schedule, supplemented with 14 h of daily light. We continued these treatments for a total of three plant generations, with each generation lasting approximately 50 days. Between each generation, plant shoots were pruned, and new seeds of the appropriate species were sown into pots to continue the plant selection treatments.

(ii) Test phase (generation 4)

After three generations of selection on the soil microbial communities, we examined how the resulting microbial communities mediated the plant's response to the selective moisture environments. To this end, we performed a full reciprocal transplant on the soil from all pots, using soils from each pot as inoculum for a new generation of plants. To control for potential nutritional/chemical changes in soils due to the historic selective treatments, the soils from each pot were divided into two, with one portion being autoclaved. Both live and sterile (autoclaved) components

were used as separate sources of inoculum into freshly sterilized soil in this final generation. Comparing subsequent plant growth between paired plants inoculated with the live and those inoculated with sterile soils would allow us to compare the microbial impact of these historic selection treatments apart from the nutritional/chemical changes to the soil. This approach is similar in design to plant-soil feedback experiments [26]. We freeze-dried 5 g soil inoculum from each of these pots to be used for nutrient analysis. Additionally, for future microbial analyses, we stored 1 g of soil from each mesocosm in a 10 ml solution of 70% phosphate-buffered saline (PBS) and 30% glycerol and stored at −80°C.

Each soil inoculum was inoculated into two pots, with each assigned to either a wet or a dry treatment, which we refer to as the contemporary treatment. This made a total of 720 pots: 180 historical pots × 2 microbe treatments (live versus sterile) × 2 contemporary watering treatments (wet versus dry). The inoculum composed 8% by volume of the total soil with the remainder being a sterilized root-wash mix. We seeded all pots with *B. rapa* seeds (standard stock lines, Wisconsin Fast Plants Program, University of Wisconsin) and thinned to one seedling after germination. Pots were placed in a framed tray and blocked such that two replicates of each treatment combination existed in each frame; each treatment's location was randomized within each block. Identical to the previous watering treatment; pots were watered to saturation daily for the first week after planting to facilitate successful germination, after which moisture treatments were imposed, as described above.

In this fourth generation, we assessed the degree to which the historic selective watering and plant regimes on the soil microbial communities impacted plant adaptation to their contemporary watering environment, using multiple plant traits as our proxies for fitness, including biomass and height. Seven weeks after the initiation of this final generation, we harvested both above- and below-ground biomass of the *Brassica* plants over 2 days. After harvest, we measured the height of each plant to the nearest quarter centimetre, and gently washed root tissues to remove soil particles. All tissues were oven dried at 75°C for 72 h and then weighed.

(b) Assessing traits of soil inoculum

(i) Characterizing soil nitrogen

While we were primarily interested in the impact of the historic selective treatments on the microbial inoculum, these treatments may have also altered the nutrition/chemistry of these soils. We tested the impact of these treatments specifically on soil nitrogen, as nitrogen seems a likely candidate for a nutrient that could be altered by these treatments while also impacting plant fitness. To this end, we conducted a KCl-extraction on a random subset of 15 soil inoculum samples within each treatment that had been collected from the end of the selection phase and freeze-dried (see above), followed by a colorimetric analysis for NH_4^+ using a modified Berthelot-salicylate method [27].

(ii) Profiling microbial community respiration over a moisture gradient

Either through ecological filtering or the evolution of the microbial community, we hypothesized that the historic watering treatment may have altered the optimal moisture range in which these microbial communities were active, becoming more adapted to their historic watering conditions. Microbial activity may be important in influencing plant tolerance to specific moisture environments [28]. Consequently, we used microbial respiration as a proxy for generalized microbial activity, and we measured the microbial respiration of a random subset of inocula (see below) across a range of soil moisture conditions. This measure is somewhat akin to the microbial communities' 'moisture niche', as previously described by others [29].

We used the MicroResp system [30], to measure the CO_2 respiration of these microbial communities collected from the end of the selection phase. This system is relatively high-throughput, allowing us to measure microbial respiration across a variety of samples and soil moisture contents. Briefly, we created soil microcosms by filling each well in a deep-well plate with 0.75 g of sand amended with 1.5% R2B medium (Research Products International, Mt. Prospect, IL) by weight to provide resources for microbial growth. We autoclaved and oven-dried these deep-well plates, and then added sterile water to wells across a 12-step soil water gradient, ranging from 23% to 10% gravimetric soil water content. After adding sterile water, we allowed these soil microcosms to equilibrate for 24 h, after which we inoculated each well with 15 µl of inoculum from a soil sample that had been stored at −80°C in PBS and glycerol (see previous description). We included control wells that were not inoculated with any microbial inoculum, but rather only with sterile water. We additionally prepared 96-well plates filled with a cresol red CO_2 trap gel. These CO_2 traps have a colorimetric dye that changes colour according to the concentration of CO_2. Following their inoculation, these deep-well plates were clamped to the plates with the CO_2 traps using a rubber gasket, sealing each soil microcosm with a single aligned CO_2 trap well. Consequently, increased soil respiration in each soil microcosm resulted in an increased colorimetric change in the corresponding well. We read these plates' absorbance at 570 nm on a plate reader, for an initial reading. We then incubated these deep-well plates in the dark for 6 h at 25°C, following which we measured the colorimetric change of the 96-well CO_2 traps by again measuring their absorbance.

From the original 180 pots from the initial selection phase, we characterized the respiration of 60 samples, randomly choosing 10 inocula from each of the six historical watering and plant treatment combinations. Samples were randomly designated a location on a 96-well plate, and run over the course of two trials, with four plate systems in each trial.

(c) Data analysis

(i) Greenhouse biomass analysis

We used plant above- and below-ground biomass, and height as our measures of plant adaptation to the contemporary watering treatments. While these measures assume that increasing biomass or height for a given watering condition represented increased plant adaptation to that environment, prior work has shown a strong correlation between *B. rapa* reproductive fitness and above-ground biomass (see electronic supplementary material data within [29]). While below-ground biomass or height are not frequently used as proxies for plant fitness, it can be beneficial to use multiple traits related to plant health to fully encapsulate plant fitness [31,32]. These measures may be particularly useful in evaluating plant health in relation to droughted conditions, wherein plants may differentially allocate their resource allocations to above- and below-ground tissues.

To examine the impact of our various treatments on plant adaptation, we constructed mixed effects models for each of these plant traits. In these models, we included as fixed effects: the contemporary watering treatment (wet or dry), the microbial inoculum's historic watering treatment (wet or dry), the microbial inoculum's historic plant treatment (*A. thaliana* (='other' plant), *B. rapa* (='same' plant), or no plant), as well as the status of the microbial inoculum (live or sterile). To control for variation, we additionally included the greenhouse block, and the original source pot as a random effects. We square-root transformed above-ground biomass and height, and log-transformed below-ground biomass to meet assumptions of normality (see electronic

supplementary material, figure S1 for residual plots in checking model assumptions). We constructed models and evaluated the fixed effects by using the lme4 and lmerTest packages [33,34] in the R statistical environment. We used Tukey honestly significant difference (HSD) *post hoc* comparisons to evaluate differences between groups.

In order to assess the impact of microbes on plant adaptation, we first focused on the terms in these models representing the interaction between the microbe's historical selective treatments and the status of the microbial inoculum term (live versus sterile). These interaction terms would indicate if live microbial inoculum was needed for any apparent plant adaptation to the selective treatments. As parsing the results of multiple plant traits can be difficult, we additionally used a separate MANOVA that combined the three plant traits (above- and below-ground biomass, and plant height) to evaluate these treatments' impact on this suite of plant traits.

We further investigated the sign and direction of the microbial fitness impact on plants with a derived variable that quantified the difference between inoculated and sterile treatments. Every original pot from the selection phase had generated a live and sterile pair in the final testing phase. We calculated a 'microbial effect' for each plant trait by dividing each live inoculum plant trait value by that of the corresponding paired sterile plant trait value [26]. If the microbial effect is greater than 1, then the live microbial inoculum increased the plant trait, and if the microbial effect is less than 1, then the live microbial inoculum decreased the plant trait.

We used these microbial effects to create mixed-effects models similar to those described above. We included as fixed effects: the contemporary watering environment (wet or dry), historical watering treatment (wet or dry), the historical plant treatment (*Brassica* or *Arabidopsis* or no plant), and the interaction of these terms. To control for variation, we included block and the source pot as a random effects (see electronic supplementary material, figure S1 for residual plots in checking model assumptions). We again additionally built a MANOVA that combined the three microbial effects on the plant traits. Data were log-transformed to generate log-response ratios. In all these models, we were specifically interested in the interactions involving the historical water and historical plant treatments, as our primary question was focused on the impact of plant selection on microbially mediated plant adaptation. For example, in the contemporary dry treatment, based on prior work, we expected that microbes from historical dry treatments and with plants would facilitate higher plant fitness than the historical wet treatments [12]. However, will this benefit depend on the historic presence of the plant?

(ii) Microbial community respiration analysis

Following the methods outlined by the MicroResp manual, we transformed each well reading into a respiration rate. Using this respiration data, we characterized the range of respiration for soils from the two watering treatments across the water content gradient. Specifically, using a maximum-likelihood method with the bblme package [35], we generated models for respiration by fitting data to a nonlinear function that was used by others [29] to similarly describe microbes' functional moisture profile,

$$R = R_{\max}\left(\exp\left[-\left|\frac{W - W_{\text{opt}}}{\sigma}\right|^{\tau}\right]\right).$$

The response variable in this model, R, is respiration, and is modelled as a function of: $R_{\max}$, maximum respiration; W, soil water content; W_{opt}, soil water content corresponding to the maximum respiration (i.e. the optimum); σ, the rate at which respiration declined after maximum respiration; and τ, a shape parameter. We compared models between the two watering treatments by examining the estimates for these parameters. We specifically focused on the water content for maximum respiration (W_{opt}, optimal water content), as changes in this parameter might indicate a shift in the adaptation of these soil communities to specific water contents. We compared the breadth (defined as b) of the estimated respiration curves between historic watering treatments, calculated using various parameters from the model

$$b = \sigma(-\log_{10}x)^{1/\tau},$$

where x defines the range of water content that is some proportion of $R_{\max}$ [29]. By assigning x to 0.5, this breadth parameter can be qualitatively described as the range of water content where respiration is at least 50% of the maximum respiration.

3. Results

(a) Plant traits

Under both contemporary wet and dry conditions, both the MANOVA and each individual plant trait (above- and below-ground biomass, and height) were significantly and similarly impacted by the main effects of the soil microbial status (live versus sterile) and the historical watering condition, but not the historical plant treatment (table 1). Overall, plants were larger (both higher biomass and were taller) when paired with live microbes (figure 1; above-ground $p < 0.001$, below-ground $p < 0.001$, height $p < 0.001$, MANOVA $p < 0.001$). Plants also tended to be larger when grown under soils that had experienced historically dry conditions (figure 1; above-ground $p < 0.001$, below-ground $p < 0.001$, height $p < 0.001$, MANOVA $p < 0.001$), a fact that may be partially due to a more substantial draw-down of nutrients under historic wet conditions during the selection phase of the experiment (electronic supplementary material, figure S4).

While there were several significant interactions in these models, multiple interactions across the various treatments made their effects difficult to parse. Therefore, we constructed additional mixed-effects models that were the same structure as the previous models, but split the data into the contemporary wet and contemporary dry treatments (electronic supplementary material, table S1 and figures S2 and S3 for residual plots in checking model assumptions). As we are primarily interested in adaptation, examining models specific to each of these contemporary watering environments will allow us to evaluate specifically adaptation to either the wet or the dry environment. Within these smaller models, we found the same two statistically significant two-way interactions across all plant traits and both contemporary watering treatments: historical watering by historical plant, and historical watering by microbial condition (see electronic supplementary material, table S1 for details). These interactions arose because plant trait differences under historically wet and historically dry soils depended both on the historical plant and on the soil microbial status (figure 1). For example, for above-ground biomass, the magnitude and direction of the historically wet versus dry treatments' impact on biomass changed across the microbial treatments. Plants grown in contemporary wet conditions largely had higher above-ground biomass when paired with live microbes from historically dry soils. Plants grown in contemporary dry conditions had significantly higher above-ground biomass when paired with live microbes from historically dry soils, but there was no

Table 1. Mixed model ANOVAs for the effects of the contemporary watering treatment (wet versus dry), historic plant conditioning treatment (*A. thaliana* versus *B. rapa* versus no plant), historic watering treatment (wet versus dry), the microbial status (live versus sterile), and their interactions on multiple plant traits, including above- and below-ground biomass, as well as height. We additionally show a MANOVA that combines all three plant traits together. We display both *F* and *p*-values for each model term. Terms with an associated *p*-value less than 0.05 are in italics. + $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

model term	d.f.	above-ground biomass	below-ground biomass	plant height	MANOVA
contemporary water	1	*107.11****	0.97	*550.92****	*292.19****
historic water	1	*36.16****	*31.32****	*16.77****	*13.04****
historic plant	2	2.78^{+}	*2.74**	1.26	1.98^{+}
microbe	1	*67.72****	*39.64****	*39.01****	*24.16****
contemporary water × historic water	1	0.19	1.68	1.44	1.50
contemporary water × historic plant	2	1.28	0.14	2.34^{+}	*2.87***
historic water × historic plant	2	*10.04****	1.25	1.32	*5.27****
contemporary water × microbe	1	1.93	0.93	*8.62***	*9.39****
historic water × microbe	1	2.69	*4.22**	0.23	2.43
historic plant × microbe	2	*3.49**	1.33	0.11	1.91^{+}
contemporary water × historic water × historic plant	2	*3.53**	1.58	*4.90***	*3.04***
contemporary water × historic water × microbe	1	*26.03****	*28.60****	*32.36****	*14.65****
contemporary water × historic plant × microbe	2	0.12	0.02	0.24	0.53
historic water × historic plant × microbe	2	0.51	1.22	1.47	1.40
contemporary water × historic plant × historic water × microbe	2	0.41	1.65	0.73	1.25

difference in plant biomass under different historical watering conditions with sterilized microbial inoculum. Similar patterns were observed in the other plant trait measures (figure 1). These results suggest that microbes play a positive role in plant response to dry conditions.

The microbially mediated effect was similar across all plant traits and the MANOVA, with these traits being significantly influenced by the interaction between the historic watering treatment and contemporary watering treatment, and no other factors (table 2; above-ground $p < 0.001$, below-ground $p < 0.001$, height $p < 0.001$, MANOVA $p < 0.001$). As above, these interactions can be difficult to parse, and we therefore constructed two additional mixed-effects models that were the same structure as the previous models, but split the data into the contemporary wet and contemporary dry treatments (see electronic supplementary material, figures S2 and S3 for residual plots in checking model assumptions). In these additional models, across all plant traits and the MANOVA, in both contemporary watering environment, the historic watering treatment was highly significant, while all other factors had no significant impact on the microbial impact (see electronic supplementary material, table S2 for all *p*-values). In general, we found that soil microbes largely were neutral or had a positive benefit to the plants (figure 2; most values are at 1 or higher). Plants received the largest benefit to biomass and height from microbes when paired with microbes whose historical watering environment matched the plant's contemporary watering environment. For example, under contemporary wet conditions plants received more beneficial microbial effects with the wet microbes than the dry microbes. (Figure 2*a–c*; above-ground $p < 0.001$, below-ground $p < 0.001$, height $p < 0.001$, MANOVA $p < 0.001$). Under contemporary dry conditions, plants received more beneficial microbial effects with the dry microbes than the wet microbes (figure 2*d*,*e*; above-ground $p < 0.001$, below-ground $p < 0.001$, height $p < 0.001$, MANOVA $p < 0.001$). Importantly, we highlight that as there was no significant interaction in these models between the historic plant and historic watering environment, the impact of the historic selective watering treatment in mediating locally adaptive plant phenotypes was not dependent on interactions with the plant.

(b) Soil data: microbial moisture niche and nitrogen

Historic watering treatments produced different moisture niches for soil microbial communities (electronic supplementary material, table S3 and figure 3). While our models predicted similar respiration for the two historic watering treatments towards the wet end of the water gradient, they significantly diverged towards the dry end of the gradient (figure 3*a*). Specifically, models predicted significantly higher respiration at lower water contents for soils from the historic dry treatments compared with soils from the historic wet treatments. The maximum respiration for soils from the historic dry treatments occurred at a significantly lower water content than soils from the historically wet treatments (figure 3*b*), and the niche breadth of soils from the historic dry treatments was significantly wider than that of the soils from the historic wet treatments (figure 3*c*).

We additionally found significant differences in the nitrogen content between treatments from soils sampled at the end of the 3-generation conditioning phase (electronic supplementary material, figure S4). Namely, there was higher

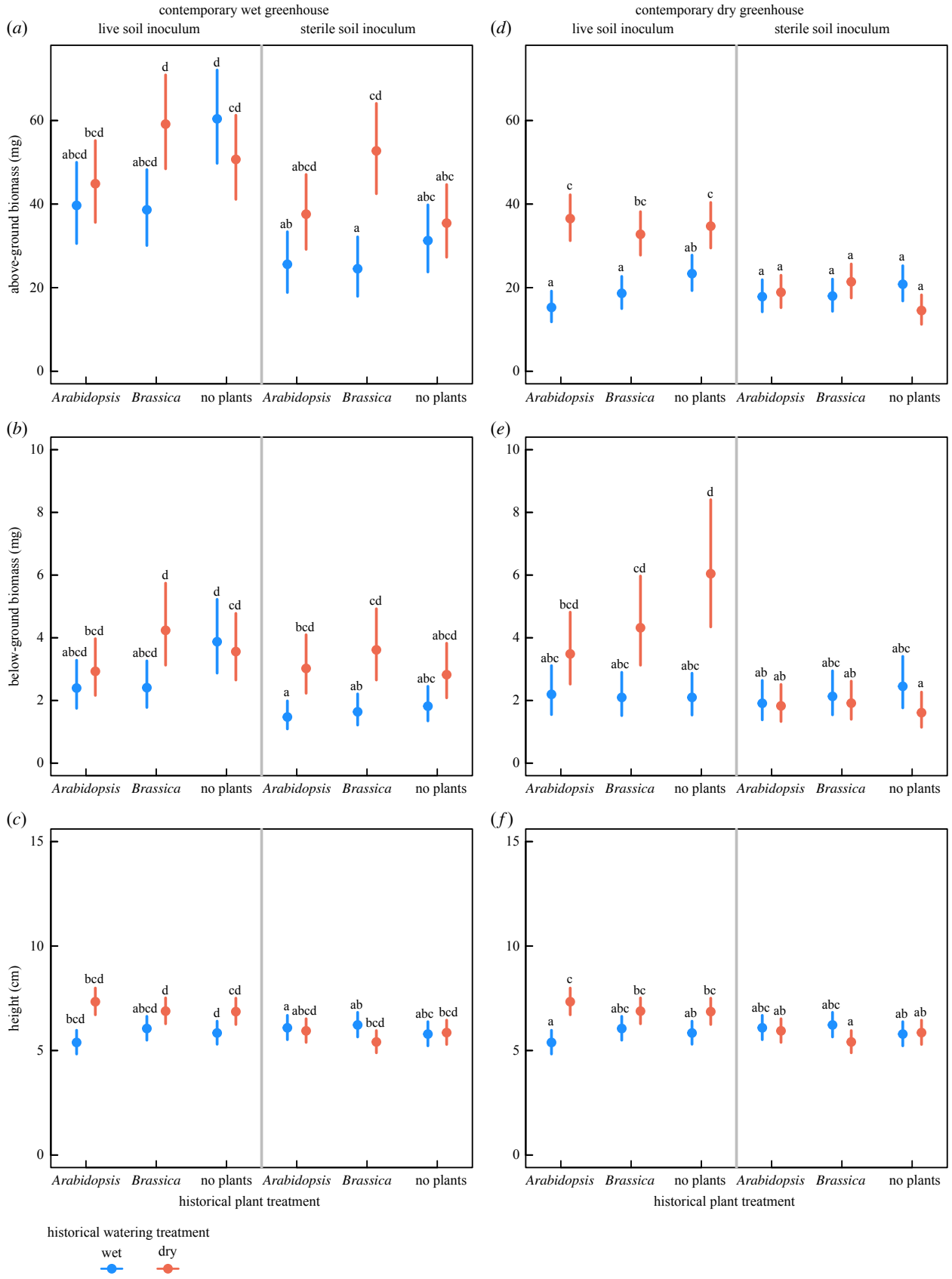

Figure 1. Various plant traits from *B. rapa* plants in our greenhouse experiment, including above-ground biomass (*a*) and (*d*), below-ground biomass (*b*) and (*e*), and height (c) and (*f*). Data are grouped by the present greenhouse watering treatment (wet, panels (*a*), (*b*) and (*c*), or dry, panels (*d*), (*e*) and (*f*)), the microbial inoculum's historic watering treatment (wet or dry, coded as blue or orange), the microbial inoculum's historic plant treatment (*A. thaliana* or *B. rapa* or no plant), as well as status of the microbial inoculum (live or sterile). Data were square-root transformed in the analysis, but were back-transformed here for interpretation. We present here estimated marginal means, with bars representing 95% confidence intervals generated from the standard error. Lettering represents *post hoc* Tukey HSD, with groups that differ in their lettering being significantly different from one another. As we are primarily interested in adaptation to each contemporary water environment, comparisons across the contemporary watering treatments may not be informative. We therefore based *post hoc* comparisons off the reduced models (seen electronic supplementary material, tables S1 and S2) representing each contemporary watering environment.

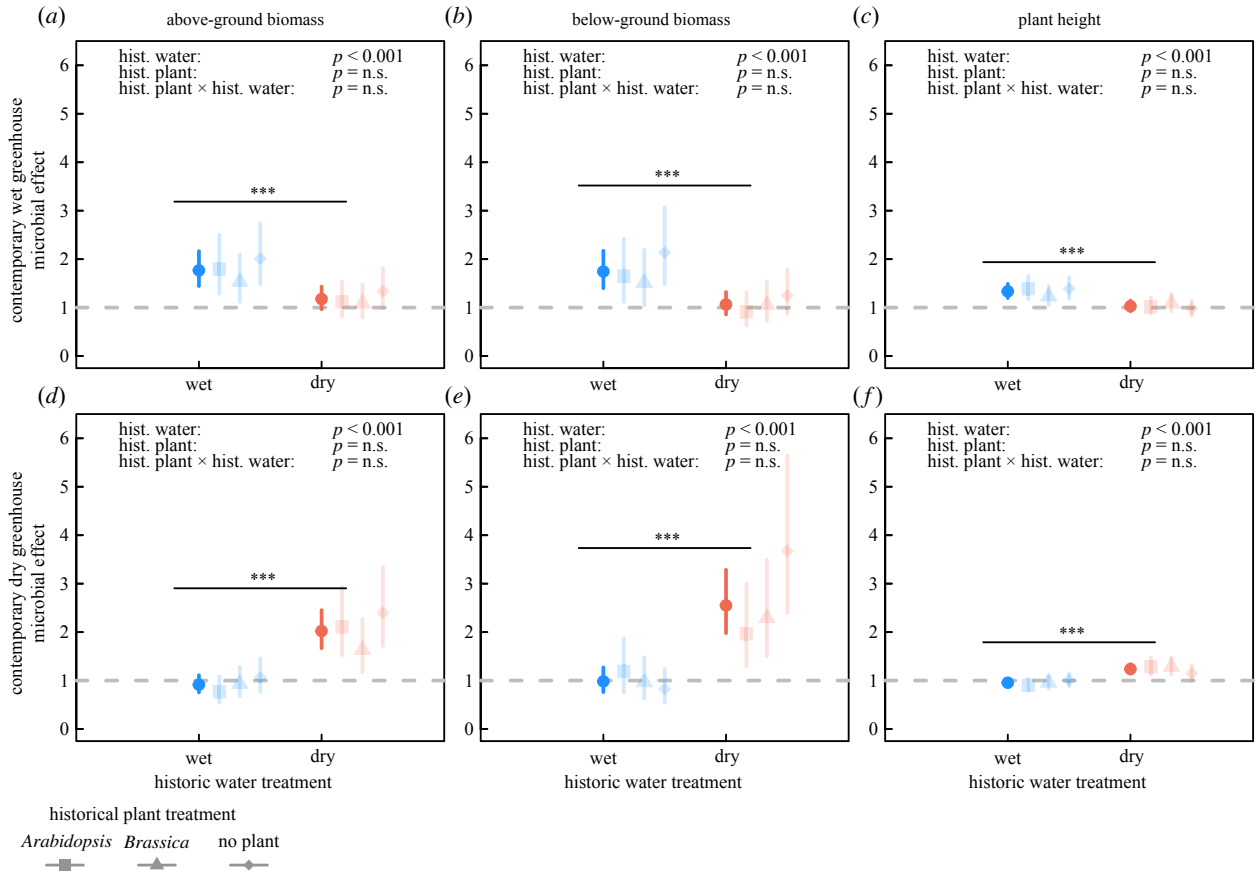

Figure 2. Impact of microbes on *B. rapa* various traits in our greenhouse experiment, including above-ground biomass (*a*) and (*d*), below-ground biomass (*b*) and (*e*), and height (*c*) and (*f*). Microbial impact is calculated as the log-response ratio of the plant trait from plants provided with live inoculum versus the plant biomass from plants provided with sterile inoculum. The main effects of the historic watering environment on each trait are presented, though we display their interactions with the historic plant treatments with transparent lines. Data are grouped by the present greenhouse watering treatment (wet, panels (*a*), (*b*) and (*c*), or dry, panels (*d*), (*e*) and (*f*)), the microbial inoculum's historic watering treatment (wet or dry, coded as blue or orange), and the microbial inoculum's historic plant treatment (*A. thaliana* or *B. rapa* or no plant). The dashed grey line at 1 is provided as reference for the impact of microbe. We present here estimated marginal means, with bars representing 95% confidence intervals generated from the standard error. The statistical significance of each comparison is indicated using the symbology as follows: n.s. $p > 0.10$; + $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table 2. Mixed model ANOVAs for the effects of the contemporary water treatment (wet versus dry), historic plant conditioning treatment (*A. thaliana* versus *B. rapa* versus no plant), the historic watering treatment (wet versus dry), their interactions on the microbial effect on multiple plant traits, including above- and below-ground biomass, as well as height. We additionally show a MANOVA that combines all three plant traits together. Note, that this these models contrast from the previous models as there is no 'Microbe' term as the response variable here was the effects of the microbes on biomass (the ratio of plant biomass provided with live soils versus plant biomass provided with sterile soils). This table includes the results from the separate models for plants under the contemporary wet treatment and those under the contemporary dry treatment. We display both *F* and *p*-values for each model term. Terms with an associated *p*-value less than 0.05 are in italics. + $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

model term	d.f.	above-ground biomass	below-ground biomass	plant height	MANOVA
contemporary water	1	0.38	1.73	2.19	2.40^{+}
historic water	1	*4.27**	*3.90**	0.01	0.11
historic plant	2	2.62^{+}	1.44	0.04	0.82
contemporary water × historic water	1	*43.26****	*39.31****	*38.54****	*15.08****
contemporary water × historic plant	2	0.07	0.15	0.16	0.54
historic water × historic plant	2	0.22	1.71	1.90	1.14
contemporary water × historic water × historic plant	2	0.81	1.65	0.72	0.94

nitrogen in soils from the historically dry treatments than the historically wet treatments. To determine if these differences in nitrogen content were driving plant health, we correlated nitrogen content with the residuals from the models described above (table 1) but found no significant effect (electronic supplementary material, figure S5).

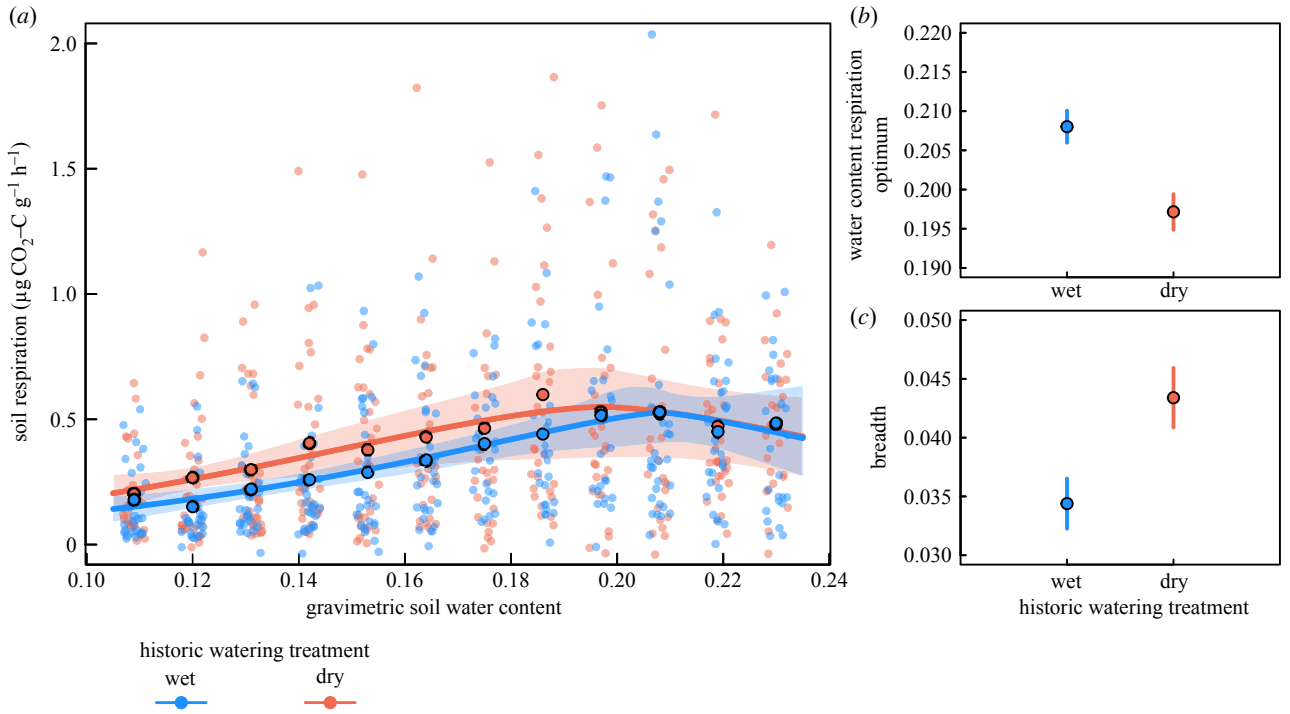

Figure 3. Models for microbial respiration across a soil moisture gradient. Separate models were constructed for the two historical soil treatments, with the soil microbes from the historic dry treatments denoted in orange and the soil microbes from the historic wet treatments denoted in blue. (*a*) The curves for the maximum-likelihood models fit to the data using a biologically relevant model described in Lennon *et al.* [29]. Confidence intervals over the prediction interval were estimated via bootstrapping. Mean respirations of each treatment group at each water content level are displayed with solid points, while the actual respiration data are displayed with semi-transparent points. Data were jittered along the *x*-axis for ease of viewing. (*b*) Estimates for the niche water optimum (based on the gravimetric soil water content) for the two historic watering treatments. These values were parameters estimated as a part of the models. (*c*) Estimates for the niche breadth for the two historic watering treatments. These values were estimated based on various parameters extracted from the models, as described by Lennon *et al.* [29], and can described as the range of water content where respiration rate is 50% of the maximum respiration.

4. Discussion

We generally found that soil microbes maximally increased plant growth (i.e. microbial effect) when plants were paired with soil microbes whose historical moisture environment matched the plant's contemporary moisture environment. For example, under contemporary wet conditions, plants received maximal benefit from microbes when paired with microbes from the historical wet treatments, while under contemporary dry conditions, plants received maximal benefit to biomass from microbes when paired with microbes from the historical dry treatments. Consistent with prior work [8,12,13,15,36,37], these results suggest the historical moisture environments selected for microbes that mediate plant local adaptation to the selective moisture environment. Importantly, we observed this same pattern of microbial fitness benefits across all historical selective plant treatments, including those that did not include a historical association with a plant. This suggests that wet/dry microbial fitness benefits to plants emerge as an incidental by-product of selection on these microbes from the moisture environment and not as part of a co-adapting plant–microbe system.

Why should selection for (e.g.) dry-adapted microbes result in fitness benefits for plants under dry conditions? One possible explanation is that the moisture environment may select for microbes with traits that both benefit themselves and also incidentally benefit plants as a by-product. For example, dry soil conditions may select for increased microbial biofilm production, because microbial biofilms can prevent microbial desiccation [29]. While these biofilms are a direct microbial response to drought, microbial biofilms can also support plant drought tolerance if the biofilms develop as a rhizosheath around plant roots, protecting the roots from desiccation [38]. Thus, while the production of these biofilms may be a selfish trait from the perspective of the microbes, they incidentally positively impact the fitness of the plant (i.e. by-products/incidental cooperation, see [39]).

Another possible explanation is that soil microbes locally adapted to the contemporary watering environment may maximize plant fitness by preventing the dormancy of beneficial microbes. This maintains crucial microbial functions that are necessary for plant health. Indeed, in our study (as well as many others) plants were healthier when inoculated with live soil compared with sterile soil, highlighting the general benefit to the plant of the soil microbial community. These broadly beneficial microbes may be involved in nutrient cycling, decomposition, pathogen suppression etc. [40,41]; some of these broadly beneficial traits probably evolved for the microbial benefit, and not the plants', with any benefit incidental to the plant. Each of these beneficial microbial taxa probably has a range of environments within which they are physiologically active [42]; if the current environment is outside a microbe's range, that taxa probably becomes dormant in the community or becomes locally extinct [43]. The historic watering treatments may select for these beneficial microbes locally adapted to those conditions, causing these microbes to be available to aid the plant; for example, under contemporary dry conditions, microbes with dry-adapted traits may be available to benefit and influence the plant, while wet-adapted microbes are dormant.

This second explanation receives some support from our observed microbial respiration data, because the optimal respiration for the microbial communities from the historical dry treatment was at a lower water content than the wet treatments. Moreover, these dry communities had higher respiration relative to the wet communities under lower soil moisture conditions, and they had a wider respiration breadth. To the extent that soil respiration is an indicator of overall microbial activity, these differences in respiration may indicate that the historic dry treatments led to the adaptation of these microbial communities to lower soil water contents; thus, these dry-adapted microbes may have been better able to continue to provide beneficial functions to plants under low water conditions. We do note, however, that these respiration measures may also be limited because (i) these differences in respiration were quite small relative to the overall variance of respiration rate, and (ii) total respiration broadly profiles microbial activity across this water gradient, and we do not know which particular microbial taxa were the main responders in respiration, nor do we know how they influence plant health. Isolating specific microbes from these soils and assessing their functional capacity under a range of moisture conditions could follow up and test these hypotheses.

While thus far we have used the term 'selection' to refer to potential changes in soil microbes by the environment, it is unclear if these results are the product of natural selection (e.g. microbial evolution), ecological selection (i.e. filtering), or even shifts in microbial dormancy of the community. In the context of soil microbial communities, with short generation times, rapid mutation rates and frequently dormant microbes, distinguishing between these is not possible without molecular tools. Indeed, we unfortunately conducted no sequencing in this study. Sequencing could potentially identify microbial mechanisms influencing the plant adaptation observed herein. We suggest that future work addressing similar questions could specifically use shotgun metagenomic-based approaches to identify specific microbial genes under selection or could use culture-based approaches to track the evolution of model microbes.

This experiment was designed to investigate the impact of selection on the microbes, and that selection's subsequent impact on the plant. Consequently, we generated a microbial effects index that compared fitness measures between the live and sterile treatments. However, a caveat to our study is that even within the sterile treatments, there were significant differences between the historical watering treatments. For example, this was particularly notable in the contemporary wet greenhouse treatments, inoculated with sterile soils selected upon by *Brassica* under the historically dry conditions, which had substantially higher plant biomass than any other sterile inoculum. Differences in plant performance within the sterile soil treatments were potentially related to the changes in soil nutrition/chemistry during the selection phase. For example, we note that soils from historically wet conditions had lower nitrogen than soils from historically dry conditions in the two plant-containing treatments (electronic supplementary material, figure S4). This is likely to reflect greater nitrogen uptake by well-watered plants during the selection stage, and this might lead to soil fertility differences that influenced plant growth during the final stage of our experiment. We did not find that soil nitrogen correlated with plant biomass (electronic supplementary material, figure S5), but it is hard to rule out all possible soil chemical/nutritional changes due to these historic treatments without an extensive profile of soil properties. We note that the high fitness of the droughted *Brassica* soil compared with the 'no plant' treatments suggests that *Brassica* is somehow promoting future plant growth through an abiotic means, though the mechanism is unclear. Because our microbial effects index was generated from paired samples, we argue that pre-existing nutritional/chemical changes from historical treatments should be carried over to both live and sterile inocula. Thus, nutritional/chemical changes to the soil should be controlled for in the microbial effects index, and any remaining differences between treatments can be attributed to changes in the soil microbes. We found a clearer signal of historical–contemporary environmental matching in the microbial effects analysis (figure 2), suggesting that microbes are largely responsible for wet–dry fitness benefits.

5. Conclusion

Our work suggests that the soil moisture environment, independent of the plant, selects for soil microbial communities that maximize plant biomass when under the same moisture environment. These results, as well as that of others, emphasize the significant impact of microbes on their host's fitness and suggest that these plant–microbe interactions may frequently play an adaptive role for the host, and may be influential in shaping each partner's evolution [44–46]. Importantly, this work may indicate that, as least in the timeframe of this study, the plant may be relatively unimportant in guiding the functional evolution of some of its microbial partners, with any beneficial effects of the microbes to the plant only an incidental by-product of the selective abiotic environment. We do emphasize, however, that many microbes coevolve with their partners, including rhizobia and mycorrhizal fungi [47,48], neither of which is associated with the plant species we used. Such microbes, however, are a part of some of the most intricate plant–microbe symbioses, and they may not be representative of the plant interactions with the diffuse soil microbial community.

As many microbial processes benefit their host plants, it may be intuitive to use an adaptationist paradigm to explain the emergence of these beneficial microbes, with these beneficial microbes being selected with the express purpose of aiding the plant. However, our results highlight that some of these locally adaptive and beneficial microbes may arise independently of the plant, with any benefit to the plant only as a by-product of environmental selection.

Host–microbe interactions appear ubiquitous, with all eukaryotic organisms apparently colonized by large communities of microorganisms [49,50]. Given their frequent significant impact on host adaptation, we need a strong understanding of the selective agents on these host-associated microbes, and their impact on host adaptation. Future studies can follow our work herein by explicitly examining the microbial traits that underpin host fitness and phenotype, and investigating the selective drivers of these traits.

Data accessibility. The data supporting this contribution is available on Zenodo and can be accessed using the following link: https://doi.org/10.5281/zenodo.7798871 [51].

Additional information is provided in electronic supplementary material [52].

Authors' contributions. K.D.R.: conceptualization, formal analysis, investigation, methodology, writing—original draft, writing—review and editing; A.C.Y.: conceptualization, resources, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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