bioRxiv preprint doi: https://doi.org/10.1101/081521; this version posted October 17, 2016. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Artificial Microbiome-Selection to Engineer Microbiomes That Confer Salt-Tolerance to Plants

Ulrich G Mueller[1,*], Thomas E Juenger[1], Melissa R Kardish[1,3], Alexis L Carlson[1], Kathleen Burns[1], Chad C Smith[1], David L De Marais[1,2]

[1] Department of Integrative Biology, University of Texas at Austin, 1 University Station #C0990, Austin TX 78712, USA
[2] Department of Organismic & Evolutionary Biology, Arnold Arboretum, Harvard University, 1300 Centre Street, Boston MA 02131, USA
[3] Center for Population Biology, University of California, Davis, CA, USA

* Corresponding author: Ulrich G. Mueller, umueller@austin.utexas.edu

Abstract: We use a differential microbiome-propagation method to artificially select for rhizosphere microbiomes that confer salt-tolerance to the model grass *Brachypodium distachyon*. To optimize methods, we conceptualize microbiome-selection within a host-focused quantitative-genetic framework (Mueller & Sachs 2015), rather than within a multi-level selection framework ("artificial ecosystem selection" *sensu* Swenson *et al* 2000). Artificial microbiome-selection represents a special case of *indirect selection* that quantifies host phenotype (but does not measure microbiome properties directly) and uses host phenotype to infer presence of microbiomes that interact beneficially with a host; we have called this indirect-selection scheme previously *host-mediated indirect selection on microbiomes*. We improve on the methods of Swenson *et al* by modifying several protocol steps to maximize evolutionary changes due to differential microbiome-propagation, while minimizing some (but not all) ecological processes affecting microbiome composition. Specifically, our methods aim to improve microbiome perpetuation and response to artificial microbiome-selection by (a) controlling microbiome assembly when inoculating seeds; (b) low-carbon soil to enhance host-control during initial microbiome assembly and subsequent microbiome persistence; (c) microbiome-fractionation to propagate and select only on bacterial and viral (but not fungal) microbiome components; and (d) ramping of salt-stress between selection-cycles to minimize the chance of either under-stressing or over-stressing plants. Depending on salt-stress and control treatments, our protocol generates microbiomes that enhance plant fitness after only 1-3 rounds of host-mediated indirect selection on rhizosphere microbiomes. When testing microbiomes after nine rounds of differential microbiome propagation, the effect of bacterial microbiomes selected to confer tolerance to sodium-sulfate stress appears specific (these microbiomes do not confer such tolerance under aluminum-sulfate stress), but the effect of microbiomes conferring tolerance to aluminum-sulfate stress appears non-specific (selected microbiomes ameliorate both sodium- and aluminum-sulfate stresses). Ongoing metagenomic analyses of the artificially selected microbiomes will help elucidate metabolic properties of microbiomes that confer specific versus non-specific salt-tolerance to plants.

INTRODUCTION

A challenge in plant-microbiome research is engineering of microbiomes with specific, sustained beneficial effects on plants. These difficulties of microbiome engineering derive from several interacting factors, including environmental influences that modulate microbiome effects on a plant-host; transitions in microbiome function during plant ontogeny; and the sheer complexity of microbiome communities impacting plant fitness, such as the hyperdiverse rhizosphere or phyllosphere microbiomes containing countless fungal, bacterial, and viral components (Bulgarelli *et al* 2013; Pfeiffer *et al* 2013; Roossinck 2015). Even when beneficial microbiomes can be assembled experimentally to generate specific microbiome functions that benefit a plant, microbiomes are often ecologically unstable and undergo turnover (i.e., microbiome communities change over time), for example when new microbes can immigrate into microbiomes, or beneficial microbes can be lost from microbiomes under microbe-microbe competition.

One strategy to engineer sustainable beneficial microbiome-function uses repeated cycles of differential microbiome-propagation to perpetuate between hosts only those microbiomes that appear to have the most desired fitness effects on a host (Figure 1), and thus artificially select for (i.e., differentially propagate between hosts) microbiome components that best mediate stresses that impact host fitness (Swenson *et al* 2000; Williams & Lenton 2007; Mueller & Sachs 2015). Only two published studies have used this approach so far (Swenson *et al* 2000; Panke-Buisse *et al* 2015); both studies tried to select on rhizosphere microbiomes of the plant *Arabidopsis thaliana*, and both studies needed more than 10 selection-cycles to generate an overall weak and highly variable phenotypic response to microbiome-selection (e.g., increase in above-ground biomass of plants by an average of about 10%; Swenson *et al* 2000). We here improve this differential microbiome-propagation method to artificially select for rhizosphere microbiomes that confer salt-tolerance to the model grass *Brachypodium distachyon* (Figures 1 & 2). Specifically, we built on the original methods of Swenson *et al* (2000) by modifying experimental steps that seemed critical to improve microbiome perpetuation and response to artificial microbiome-selection (e.g., controlled microbiome assembly when inoculating seeds; low-carbon soil to enhance host-control by germinating seedlings during microbiome assembly and subsequent plant growth; harvesting and perpetuation of microbiomes that are in close physical contact with plants; short-cycling of microbiome-generations to select for microbiomes that benefit seedling growth; microbiome-fractionation to eliminate possible transfer of pathogenic fungi; ramping of salt-stress between selection-cycles to minimize the chance of either under-stressing or over-stressing plants).

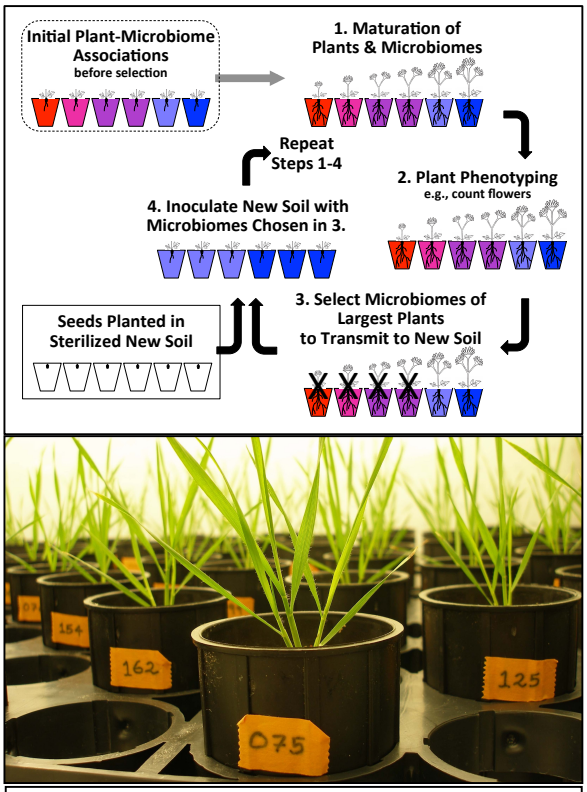

Figure 1. Top: Differential microbiome-propagation scheme to impose artificial indirect selection on rhizosphere microbiomes (figure modified from Mueller & Sachs 2015). The host-plant does not evolve because this scheme propagates only microbiomes into sterilized soil (Step 4), whereas the host is taken each cycle from a non-evolving source (stored seeds). Both evolutionary and ecological processes alter microbiomes during each cycle, but at Steps 3&4 in each cycle, experimental protocols aim to maximize evolutionary changes stemming from differential propagation of microbiomes.
Bottom: Experimental plants of the model grass *Brachypodium distachyon* (Poaceae) shortly before microbiome-harvest for differential microbiome-propagation to seeds of the next microbiome-generation. Photo by UGM.

***The Logic of Host-Mediated Indirect Artificial Selection on Microbiomes*:** To optimize microbiome-selection experiments, we found it useful to conceptualize the selection process within a host-focused quantitative-genetic framework (Mueller & Sachs 2015), rather than within a multi-level selection framework preferred by Swenson *et al* (2000, "artificial ecosystem selection"). Both frameworks capture the same processes, but a host-focused quantitative-genetic framework is more useful to identify factors that can be manipulated to increase efficacy of microbiome-selection (Mueller & Sachs 2015). First, because microbiome-selection aims to shape a fitness component of the host-plant (e.g., growth rate, stress tolerance, disease resistance), and because it is typically easier to measure plant phenotypes rather than measure microbiome properties, *selection is indirect* (microbiomes are not measured directly, but evaluated indirectly by measuring host performance). Indirect selection is an established breeding technique that is often used when the indirectly-selected trait is difficult or costly to measure (Falconer & Mackay 1996), as is the case also for microbiome properties, compared to the ease of measuring a microbiome-dependent host phenotype. The efficacy of indirect selection depends on correlations between microbiome properties and host trait, and indirect microbiome-selection should therefore be more efficient if such correlations can be maximized

experimentally, for example by controlling ecological priority-effects during initial microbiome assembly, or by increasing host-control over microbiome assembly and microbiome persistence. Second, because a typical host experienced a long history of evolution to monitor and manipulate its microbiomes (a process called *host control*; Sachs *et al* 2004, 2010; Kiers *et al* 2007; Berg & Smalla 2009; Bright & Bulgheresi 2010; Hillman & Goodrich-Blair 2016; Lareen *et al* 2016), indirect microbiome-selection uses the host as a kind of "thermostat" to help gauge and adjust the "temperature" of its microbiomes, then propagate desired microbiomes between hosts (Mueller & Sachs 2015; Figure 1). Based on theory (Foster & Wenseleers 2006; Williams & Lenton 2007; Fitzpatrick 2014; Mueller & Sachs 2015), such *host-mediated indirect* selection on microbiomes can be easier than direct selection on microbiomes, particularly with hosts that exert strong host-control over assembly and stability of their microbiomes (Scheuring & Yu 2012; Coyte *et al* 2015; Mueller & Sachs 2015). Third, as in Swenson *et al* (2000), we used an inbred strain of a plant-host (here, *B. distachyon* genotype Bd3-1) to ensure constancy of the host-genetic environment within and between selection-cycles, such that microbiomes evolve in a specific plant-genotype background; we have previously called this method *one-sided selection* (Mueller & Sachs 2015) because only the microbiomes change between propagation cycles, whereas the host is taken each cycle from stored, inbred stock and therefore cannot evolve (Figure 1).

Definition of Microbiome Engineering & Microbiome Selection: Microbiome engineering via differential microbiome propagation (Figure 1) alters microbiomes through both ecological and evolutionary processes. Ecological processes include changes in community diversity, relative species abundances, or structure of plant-microbe and microbe-microbe interaction networks. Evolutionary processes include extinction of specific microbiome members; allele frequency changes, mutation, or gene transfer between microbes; and differential persistence of microbiome components when differentially propagating microbiomes at each selection cycle. Effective microbiome-selection protocols aim to maximize evolutionary changes stemming from such differential microbiomes propagation (Steps 3&4 in Figure 1), for example by optimizing microbiome inheritance in between-host microbiome transfers, or by optimizing microbiome re-assembly after such transfers (e.g., by facilitating ecological priority effects at initial host inoculation). Although both evolutionary and ecological processes alter microbiomes during each propagation cycle, as a shorthand, we will refer here to the combined evolutionary and ecological changes resulting from *host-mediated indirect artificial selection on microbiomes* as 'microbiome response' due to 'microbiome selection'.

METHODS

To select indirectly on microbiomes and evolve beneficial microbiomes that confer salt-tolerance to plants, we used a differential host-microbiome co-propagation scheme as described in Swenson *et al* (2000), Mueller *et al* (2005), and Mueller & Sachs (2015) (detailed methods in Supplemental Information below). Because both evolutionary and ecological processes alter microbiomes during and between selection-cycles (i.e., microbiome-generations), we designed a protocol that improved on earlier selection-schemes by (a) maximizing evolutionary microbiome-changes stemming from differential propagation of microbiomes, while (b) minimizing some, but not all, ecological microbiome-changes. In essence, our protocol aimed to maximize microbiome-perpetuation (i.e., microbiome-inheritance of core microbes) and thus enhance efficacy of artificial indirect selection on microbiomes. To increase microbiome-inheritance, we added several protocol steps of established techniques, most importantly (a) facilitation of ecological priority-effects during initial microbiome assembly (Fierer *et al* 2012; Scheuring & Yu 2012), thus increasing microbiome-inheritance by controlling in each selection-cycle the initial recruitment of symbiotic bacteria into rhizosphere microbiomes of seedlings; and (b) low-carbon soil to enhance carbon-dependent host-control of microbiome-assembly and microbiome-persistence (Bais *et al* 2006; Bulgarelli *et al* 2013; Mueller & Sachs 2015; Coyte *et al* 2015; Tkacz & Poole 2015). Using different selection-lines, we selected for beneficial microbiomes conferring salt-tolerance to either sodium-sulfate [Na_2SO_4] or to aluminum-sulfate [$Al_2(SO_4)_3$] [This experimental contrast enables us, in analyses not reported here, to compare evolving microbiomes using metagenomic time-series analyses, as well as identify candidate taxa and microbial consortia that differ between sodium- and aluminum treatments and may therefore confer specific salt-tolerance

to plants.] In each microbiome-propagation cycle (microbiome generation), we inoculated surface-sterilized seeds taken from non-evolving stock (inbred strain Bd3-1 of the grass *Brachypodium distachyon*; Vogel *et al* 2006; Garvin *et al* 2008; Vogel & Bragg 2009; Brkljacic *et al* 2011) with rhizosphere bacteria harvested from roots of those plants within each selection-line that exhibited the greatest above-ground biomass (Figure 1). Because the plant-host could not evolve between selection-cycles, whereas microbiomes could change under differential microbiome-propagation, we therefore used a so-called *one-sided selection* scheme (Mueller & Sachs 2015). In our quantitative-genetic framework, microbiome-selection within the genetic background of the single plant genotype Bd3-1 increases so-called 'microbiome heritability' (defined here as the proportion of overall variation in plant phenotype that can be attributed to differences in microbiomes between plants), because a greater proportion of the plant-phenotypic variation can be attributed to differences in microbiome-association, therefore increasing an experimenter's ability to identify association with a beneficial microbiome. In essence, microbiome selection within the background of a single plant-genotype increases reliability of host-phenotype as indicator of microbiome effects, and thus should increase the efficiency of indirect selection on microbiomes (this experimental trick was already used by Swenson *et al* 2000). To simplify metagenomic analyses and eliminate fungal pathogens from propagated microbiomes, we used size-selecting filters (Bakken & Olsen 1987; Mueller & Sachs 2015) to filter microbiomes harvested from rhizospheres of 'mother-plants', thus capturing only bacteria (and possibly also viruses) for microbiome propagation to the next microbiome-generation, but eliminating from propagation between microbiome-generations any larger-celled soil-organisms with filters (i.e., we excluded fungi, protozoa, algae, mites, nematodes, etc.). This fractionation step distinguishes our methods from those of Swenson *et al* (2000) and Panke-Buisse *et al* (2015), both of which used 'whole-community' propagation to transfer between generations all organism living in soil, including the fungi, protozoa, algae, mites, and nematodes that were excluded from microbiome-propagation through size-selecting filtering in our experiment. To evaluate the effects of selection treatments, we included two non-selection control treatments. In *Fallow-Soil Microbiome-Propagation Control*, we harvested microbiomes from fallow soil (no plant growing in soil), then propagated the harvested microbiomes to sterile fallow soil of the next microbiome-generation. In *Null Control*, we did not inoculate germinating seeds with any microbiomes, but microbes could rain into soil from air, as was the case also for all other treatments. Our complete experiment involved one baseline Generation (Generation 0, Table S1a) to establish initial microbiomes in replicate pots; 8 rounds of microbiome-selection (i.e., differential microbiome-propagation) (Generations 1-8, Table S1b); and one final, ninth round (Generation 9, Table S1c) to evaluate the effects of the engineered microbiomes on flower-production and seed-set, for a total of 10 microbiome-generations (Figure 2). We describe all experimental steps and analyses in greater detail in the below Supplemental Information.

RESULTS

Artificially-Selected Microbiomes Confer Increased Salt-Tolerance to Plants: Figure 2a-d shows the changes in relative plant-fitness (above-ground dry biomass) during 8 rounds of differential microbiome propagation. Relative to Fallow-Soil-Control and Null-Control treatments, selected microbiomes confer increased salt-tolerance to plants after only 1-3 selection-cycles, for both microbiomes selected to confer tolerance to sodium-stress (Figure 2a&b) or to aluminum-stress (Figure 2c&d). Relative to Fallow-Soil-Controls, artificially-selected microbiomes increase plant fitness by 75% under sodium-sulfate stress ($p<0.001$), and by 38% under aluminum-sulfate stress ($p<0.001$). Relative to Null-Control plants, artificially-selected microbiomes increase plant fitness by 13% under sodium-sulfate stress, and by 12% under aluminum-sulfate stress, but neither of these fitness-enhancing effects was statistically significant. Although repeated rounds of differential microbiome-propagation seemed to improve plant fitness between successive microbiome-generations (particularly in the Null-Controls; Figure 2b&d), interactions between treatment and generation were not statistically significant (see Supplemental Information). Any fitness-enhancing effects of microbiomes from selection-lines therefore seemed to be realized already after one or a few rounds of differential microbiome-propagation (e.g., Figure 2b&d), and there was insufficient statistical support that any additional rounds under gradually increasing salt stress further increased the fitness-enhancing effects of microbiomes in the selection-lines relative to control-lines.

The phenotypic effect on plants due to the evolving microbiomes fluctuated during the 8 rounds of differential microbiome propagation (Figure 2a-d; Table S1); such fluctuations can occur during experimental evolution when selecting on genomic variation (e.g., chapters 10, 12 & 18 in Garland & Rose 2009), but fluctuations may be more pronounced when selecting on microbiomes (Swenson *et al* 2000; Blouin *et al* 2015) because additional factors can contribute to between-generation fluctuations. Specifically, in our experiment, the observed fluctuations could have been due to (i) uncontrolled changes in humidity modulating water-needs of plants and consequently changing the effective salt-stress; (ii) our salt-stress ramping during the first five selection-cycles, possibly resulting in excessively stressed plants in Generations 4 & 5 (see discussion in Supplemental Information; Tables S2 & S3); (iii) random microbiome changes (microbiome drift) and ecological microbe-microbe interactions; or (iv) unknown factors. The fluctuations in plant fitness are most prominent during the first five selection-cycles (Figure 2a-d) when we increased salt-stress 2-fold to 5-fold between each selection-cycle and when humidity varied most (see Supplemental Information), whereas fluctuations were less pronounced during the last three cycles when we changed salt-stress only minimally and humidity was relatively stable (Tables S2 & S3). These observations seem consistent with known responses of *B. distachyon* to environmental stresses (De Marais & Juenger 2011) predicting that artificial selection on microbiomes conferring salt-tolerance to plants should be most efficient under experimental conditions that rigorously control soil moisture, salt-stress, humidity, and plant transpiration.

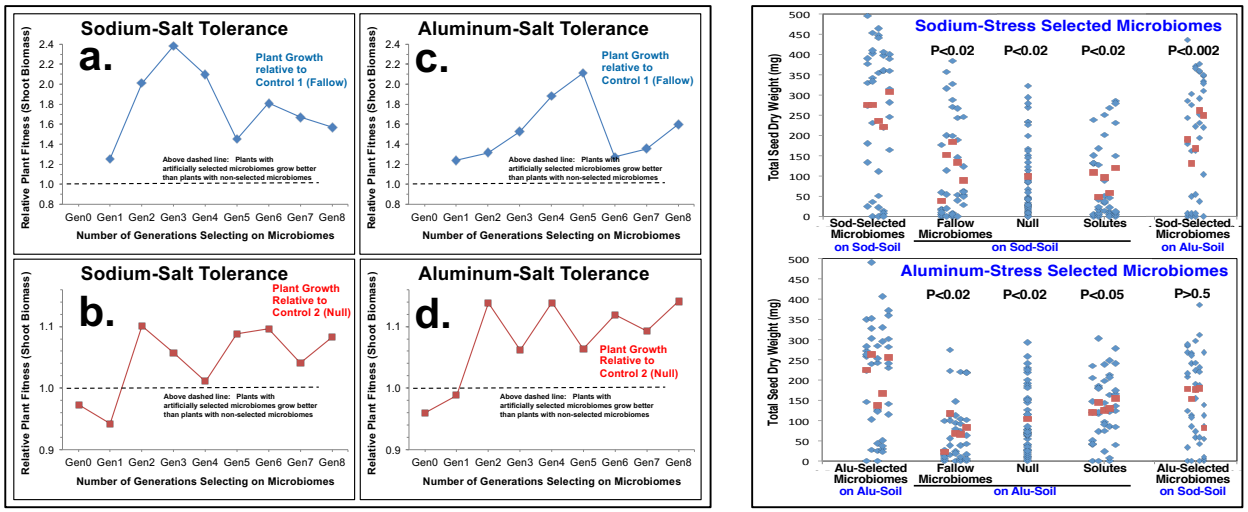

Figure 2. Host-mediated artificial selection on microbiomes conferring salt-tolerance to the grass *Brachypodium distachyon*. **Left:** Microbiomes were propagated for 8 cycles (generations = Gen), using the microbiome propagation scheme in Figure 1. Two salt stresses (sodium-sulfate vs. aluminum-sulfate), and 2 Control treatments are contrasted. Plant fitness of selection-lines is shown relative to non-selected controls. In *Fallow-Soil Microbiome-Propagation Control*, microbiomes were harvested from fallow soil (pot with no plant), then propagated to sterile fallow soil in pot of next microbiome-generation. In *Null Control*, plants did not receive a microbiome inoculum, but microbes could "rain in" from air as in all treatments. Selection and fallow-soil treatments had each 5 selection lines, with 8 plants/line. **Right:** At the end of the experiment after a 9th selection-cycle (Generation 9), plants are grown to seed (68 days). Total seed weight is plotted in blue for individual plants (plants of same selection line are vertically above each other); the average is plotted in red for each line. Two additional controls were added in Generation 9: *Solute-Control* (filtering out bacterial & fungal components, to test only solutes & viral components that are propagated); and *2x2 Cross-Fostering* to test for specificity of evolved microbiome function. All controls are significantly different from Selected Microbiomes, except for Aluminum-Selected Microbiomes tested under Sodium-Stress (p-values are shown above each Control, and are corrected using the false discovery rate for post-hoc comparisons; see Supplemental Information, Tables S3 & S4). **Conclusion:** Host-mediated artificial selection on microbiomes can generate bacterial microbiomes conferring salt-tolerance to plants; this effect is due to bacterial microbiomes and not due to solutes (plant secretions, viruses, etc.) in soil. The effect of bacterial microbiomes conferring tolerance to sodium-sulfate appears specific (these microbiomes do not confer such tolerance under aluminum-sulfate stress, $p<0.002$), but the effect of microbiomes conferring tolerance to aluminum-sulfate appears non-specific ($p>0.5$).

Effect of Artificially Selected Microbiomes on Seed Production: In the last microbiome-generation after a ninth microbiome-selection cycle (Generation 9), we grew plants for 68 days to quantify the effect of our artificially-selected microbiomes on seed production. We also added two control treatments, Solute-Transfer and Cross-Fostering, to help elucidate some of the mechanisms underlying the salt-tolerance-conferring effects of microbiomes on seed production. In the Solute-Transfer Control treatments, we eliminated with 0.2µm filters live cells from the harvested microbiomes in the selection lines, to test the growth-enhancing effects of root secretions and viruses that may be co-harvested and co-transferred with microbiomes in the selection-lines. Plants receiving these bacterial-free filtered solutes had (i) significantly poorer seed-production compared to plants that received these same solutes together with the live artificially selected bacterial microbiomes ($p<0.02$ for sodium-stress treatment; $p<0.05$ for aluminum-stress treatment; Tables S3 & S4); and (ii) seed production comparable to plants from Null-Control treatments ($p>0.7$ for sodium-stress treatment; $p>0.25$ for aluminum-stress treatment; Tables S3 & S4) (Figure 2 right). These findings indicate that any plant secretions or viruses co-harvested with bacterial microbiomes did not account for the fitness-enhancing effects of the selected microbiomes conferring salt-tolerance to plants, and that any co-harvested solutes (e.g., root secretions) and viruses appear to affect plant growth like Null-Control treatments.

Specificity Test by Crossing Evolved SOD- and ALU-Microbiomes with SOD- and ALU-Stress: In the *Cross-Fostering* Control of the last microbiome-generation (Table S1c), we crossed harvested microbiomes from the SOD and ALU selection-lines with the two types of salt-stress in soil, to test specificity of the salt-ameliorating effects of the microbiomes. The effect of selected bacterial microbiomes conferring tolerance to SOD-stress appears specific (these microbiomes do not confer such tolerance under aluminum-sulfate stress, $p<0.005$), but the effect of microbiomes conferring tolerance to ALU-stress appears non-specific ($p>0.5$; the selected microbiomes ameliorate both SOD- and ALU-stress) (Figure 2 right). The underlying microbial and metabolic mechanisms conferring specific and non-specific salt-tolerance are unknown, but metagenomic comparisons the sodium- versus aluminum-selected microbiomes should help generate hypotheses that can be tested in follow-up studies.

Flowering Phenology and Harvest Index in Generation 9: Results will be added in a forthcoming version of this manuscript.

Metagenomics of Artificially-Selected and Cross-Fostered Microbiomes: Results will be added in a forthcoming version of this manuscript.

DISCUSSION

Our study aimed to improve the differential microbiome-propagation scheme that was originally developed by Swenson *et al* (2000), then test the utility of our improved methods by artificially and indirectly select on microbiomes to confer salt-stress tolerance to plants. Swenson's original whole-soil community propagation scheme failed to generate consistent and sustained benefits for plant growth; specifically, Swenson's growth enhancement due to the putatively selected communities was overall minor when averaged across all propagation-cycles (an average of about 10% growth enhancement), and highly variable between successive cycles (Swenson *et al* 2000). To address these problems, we applied in our experiment ideas from quantitative-genetics, microbial-ecology, and host-microbiome evolution to optimize experimental steps in our microbiome-propagation scheme, with the aim to improve perpetuation of beneficial microbiomes and improve microbiome assembly at the seedling stage. Specifically, our methods aimed to (a) enhance carbon-dependent host-control of microbiome-assembly and microbiome-persistence by using low-carbon soil (Bais *et al* 2006; Bulgarelli *et al* 2013; Mueller & Sachs 2015; Coyte *et al* 2015; Tkacz & Poole 2015); (b) facilitate ecological priority-effects during initial microbiome assembly (Fierer *et al* 2012; Scheuring & Yu 2012), thus increasing microbiome-inheritance by biasing the initial recruitment of symbiotic bacteria into rhizosphere microbiomes of seedlings; (c) propagate microbiomes harvested from within the sphere of host-control (i.e., microbiomes in close physical proximity to roots) (Yan *et al* 2016; Shi *et al* 2016); and (d) ramping of salt-stress between selection-cycles to minimize the chance of either under-stressing or over-

stressing plant. Without further testing, it is not possible to say which of these experimental steps was most important to increase efficacy and response to microbiome-selection.

Our conceptual approach of *host-mediated indirect selection on microbiomes* (Mueller & Sachs 2015), combined with our experimental approach capitalizing on evolved mechanisms of *host-control* (Sachs *et al* 2004; Mueller & Sachs 2015), differs from other approaches that view microbiome-selection primarily as a multi-level selection process ("artificial ecosystem selection" sensu Swenson *et al* 2000; "meta-organism" or "hologenome" selection sensu Voss *et al* 2015, Rosenberg & Zilber-Rosenberg 2016, Theis *et al* 2016; "multi-layered" selection sensu Shapira 2016). While these multi-level views appear to capture some aspects of host-microbiome population-biology (van Opstal & Bordenstein 2015; Theis *et al* 2016), we found that a multi-level selection view provides few useful insights for design of microbiome-selection experiments. In contrast, basic principles of quantitative-genetics, microbial-ecology, and host-microbiome evolution (particularly the host-centric concept of *host control*; Sachs *et al* 2004; Coyte *et al* 2015; Mueller & Sachs 2015; Tkacz & Poole 2015) allowed us to identify and adjust critical experimental steps and parameters in our differential microbiome-propagation scheme.

Compared to two other tests of microbiome-selection (Swenson *et al* 2000; Panke-Buisse *et al* 2015), our selection scheme appears to generate more pronounced and more stable effects on plant phenotype as a result of host-mediated microbiome-selection. Except for the initial two selection-cycles (Figure 2a-d), our selected microbiomes consistently outperformed in subsequent selection-cycles the non-selected microbiomes of the control conditions (in contrast, Swenson *et al*'s experiments sometimes resulted in selected microbiomes that were outperformed by control microbiomes). Our methods may have generated more stable microbiome-effects because (a) only bacteria, but no fungi were propagated between generations (Swenson *et al* suspected fungal diseases as cause of complete devastation of plant populations in several of their selection cycles); (b) we may have worked in a more stable greenhouse environment; and (c) we selected for microbiomes conferring specific salt-tolerance rather than the possibly more non-specific, general-purpose beneficial microbiomes selected in Swenson *et al* and Panke-Buisse *et al*. After only 1-3 selection cycles, our selected microbiomes consistently outperformed the control microbiomes, with averages of 75% (SOD) and 38% (ALU) growth improvement relative to Fallow-Soil Controls, and 13% (SOD) and 12% (ALU) relative to Null Controls (Figure 2a-d). Most importantly, when quantifying plant fitness by total seed production in the final Generation 9, our selected microbiomes outperformed Fallow-Soil Controls, Null-Controls, and Solute-Controls by 120-205% (SOD) and 55-195% (ALU) (Figure 2 right). Although we achieved these results under controlled and thus not generalizable greenhouse conditions, this seems a remarkable improvement of plant productivity compared to traditional plant breeding.

Because our experiment was the first systematic attempt to improve the methods of Swenson *et al* (2000), we predict that it should be possible to further optimize protocols of differential microbiome propagation. Consequently, microbiome-selection could emerge as a novel tool to engineer and elucidate microbiome-functions in controlled laboratory environments, and possibly also in those natural environments that allow control of key parameters affecting microbiome harvest, microbiome transfer, and microbiome inheritance. Such optimization of microbiome-selection should ideally be informed by metagenomic analyses of experimental contrasts (e.g., comparison of microbiomes selected to confer either sodium- versus aluminum-tolerance to plants) and by time-series analyses across microbiome-propagation cycles, to identify candidate microbes and microbial consortia important in mediating stresses (see our forthcoming metagenomics analyses of the microbiomes propagated across 10 microbiome-generations in our experiment).

FUTURE RESEARCH DIRECTIONS

We propose five kinds of experimental variants to expand on our methods of host-mediated indirect selection on microbiomes. Because host-mediated microbiome selection leverages host-control traits that evolved to assemble and manipulate microbiomes (Mueller & Sachs 2015), the first three experiments explore whether conditions promoting strong host-control can improve efficacy of microbiome-selection:

(1) *Artificial selection on endophytic vs. rhizosphere microbiomes*: Microbiomes internal to a host (e.g., endophytic microbes of plants) require some form of host-infection, and therefore could be under greater host-control than external microbiomes (e.g., rhizoplane or rhizosphere microbiomes). Consequently, it should be easier to obtain a response to microbiome-selection when targeting selection on the microbiomes in the endosphere versus external compartment. This prediction can be tested in an experiment that compares, in separate lines of the same experiment, the responses to microbiome selection when harvesting and propagating only endophytic microbiomes vs. rhizosphere microbiomes.

(2) *Microbiome-selection in two genetic backgrounds differing in host-control*: A second approach to test for the role of host-control is to compare microbiome-selection in two different host-genotypes, such as two inbred strains of the same plant species. For example, different host-genotypes may recruit into symbiosis different kinds of microbes. Such differences in host-controlled microbiome assembly could result in differences in microbiome-selection, and a microbiome artificially-selected within one host-genotype to improve one particular host trait may produce a different phenotypic effect when tested in a different host-genotype.

(3) *Varying host-control by varying carbon-content in soil*: Microbial growth in some soils is limited by carbon, and many plants therefore can regulate their soil-microbiomes by carbon secretions (REF). A plant-host with strong carbon-based control over microbiome-assembly and –persistence can therefore be tested in different soils with different carbon content, and a low-carbon soil (like the PPC soil used in our experiment) therefore may facilitate microbiome-selection, while a high-carbon soil may preclude easy microbiome-selection.

(4) *Propagation of fractionated vs. whole microbiomes*: Experimental microbiome-propagation between host generations can be complete (all soil-community members are propagated between hosts; as in Swenson *et al* 2000 and Panke-Buisse *et al* 2015) or microbiomes can be fractionated by excluding specific microbial components, as in our protocol where we propagated only organisms of bacterial size or smaller. We used fractionated microbiome-propagation because (a) we were more interested in elucidating contributions to host fitness of the understudied bacterial components than the fungal components (e.g., mycorrhizal fungi), and (b) fractionation simplifies analyses of the microbiome-responses to selection (e.g., bacterial microbiome components, but not necessarily fungal components, need to be analyzed by with metagenomic techniques). However, because fungal components and possible synergistic fungal-bacterial interactions are eliminated by fractionated microbiome-propagation, we hypothesized previously (Mueller & Sachs 2015) that selection on afractionated microbiomes may slow or reduce selection-response compared to selection on whole-microbiomes. This can be tested in an experiment comparing the response to microbiome-selection when propagating fractionated versus whole microbiomes (e.g., using different size-selecting filters).

(5) *Propagation of mixed vs. un-mixed microbiomes*. When propagating microbiomes to new hosts, it is possible to propagate mixed microbiomes harvested from different hosts, or only un-mixed microbiomes. Mixed vs. un-mixed propagation schemes therefore represent two principal methods of microbiome selection (Swenson *et al* 2000; Williams & Lenton 2007; Mueller & Sachs 2015). We hypothesized previously that mixed propagation could generate a faster response to microbiome-selection, but the respective advantages of mixed versus un-mixed propagation have yet to be tested. Mixed propagation may be superior to un-mixed propagation, for example if mixing generates novel combinations of microbes with novel beneficial effects on a host (Mueller & Sachs 2015), or generate novel competitive microbe-microbe interactions that increase microbiome stability (Coyte *et al* 2015)

ACKNOWLEDGEMENTS

We thank Shane Merrell for help with greenhouse maintenance; John Willis for suggesting the fallow-soil control treatment; Hannah Marti for suggesting the thermostat metaphor; Joey Knelman, John Vogel, Hannah Marti, Rong Ma, Emma Dietrich, and Andy Fang for comments on the manuscript; and the Stengl Lost Pines Endowment of the University of Texas at Austin, the CNS Undergraduate Research Fellowship Program, and the National Science Foundation (awards 0919519 & 1354666 to UGM) for funding.

394 **REFERENCES**

395 Aggarwal A, Ezaki B, Munjal A, Tripathi BN. 2015. Physiology and biochemistry of aluminum toxicity
396 and tolerance in crops. Pp 35-57 in *Stress Responses in Plants* (Tripathi BN & Müller M, eds), Springer
397 International Publishing.

398 Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere inter-
399 actions with plants and other organisms. *Annu Rev Plant Biol* 57: 233-66.
400 DOI: 10.1146/annurev.arplant.57.032905.105159

401 Bakken LR, Olsen RA. 1987. The relationship between cell size and viability of soil bacteria. *Microb Ecol*
402 13: 103-114. DOI: 10.1007/BF02011247

403 Blouin M, Karimi B, Mathieu J, Lerch TZ. 2015. Levels and limits in artificial selection of communities.
404 *Ecol Lett* 18: 1040–1048. DOI: 10.1111/ele.12486

405 Brkljacic J, Grotewold E, Scholl R, Mockler T, Garvin DF, Vain P, Brutnell T, Sibout R, Bevan M, Budak
406 H, Caicedo AL, Gao C, Gu Y, Hazen SP, Holt BF, Hong S-Y, Jordan M, Manzaneda AJ, Mitchell-Olds
407 T, Mochida K, Mur LAJ, Park C-M, Sedbrook J, Watt M, Zheng SJ, Vogel JP. 2011. *Brachypodium* as
408 a model for the grasses: today and the future. *Plant Physiol* 157: 3–13.
409 DOI: 10.1104/pp.111.179531

410 Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. 2013. Structure and functions
411 of the bacterial microbiota of plants. *Annu Rev Plant Biol* 64: 807-838. DOI: 10.1146/annurev-arplant-
412 050312-120106

413 Coyte KZ, Schluter J, Foster KR. 2015. The ecology of the microbiome: Networks, competition, and sta-
414 bility. *Science* 350: 663-666. DOI: 10.1126/science.aad2602

415 Delhaize E, Ryan PR. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiology* 107: 315-321.

416 Des Marais DL, Juenger TE. 2011. *Brachypodium* and the abiotic environment. Pages 291-311 in *Genet-*
417 *ics and Genomics of* Brachypodium (Vogel JP, ed). Springer International Publishing.
418 DOI: 10.1007/7397_2015_13

419 Des Marais DL, Razzaque S, Hernandez KM, Garvin DF, Juenger TE. 2016. Quantitative trait loci asso-
420 ciated with natural diversity in water-use efficiency and response to soil drying in *Brachypodium dis-*
421 *tachyon*. *Plant Science* 251: 2-11. DOI: 10.1016/j.plantsci.2016.03.010

422 Dodd IC, Pérez-Alfocea F. 2012. Microbial amelioration of crop salinity stress. *J Exp Bot* 63: 3415-3428.
423 DOI: 10.1093/jxb/ers033.

424 Fierer N, Ferrenberg S, Flores GE, González A, Kueneman J, Legg T, Lynch RC, McDonald D, Mihaljevic
425 JR, O'Neill SP, Rhodes ME, Song SJ, Walters WA. 2012. From animalcules to an ecosystem: Applica-
426 tion of ecological concepts to the human microbiome. *Annu Rev Ecol Evol Syst* 43: 137-155. DOI:
427 10.1146/annurev-ecolsys-110411-160307

428 Garland T, Rose MR. 2009. *Experimental Evolution*. University of California Press.

429 Garvin DF, Gu YQ, Hasterok R, Hazen SP, Jenkins G, Mockler TC, Mur LAJ, Vogel JP. 2008. Develop-
430 ment of genetic and genomic research resources for *Brachypodium distachyon*, a new model system for
431 grass crop research. *Crop Science* 48: S69–S84. DOI: 10.2135/cropsci2007.06.0332tpg

432 ISO/FDIS 10390 (2005) Soil quality – Determination of pH. International Organization for Standardization,
433 2005. www.iso.org/iso/catalogue_detail.htm?csnumber=40879
434 www.ecn.nl/docs/society/horizontal/pH_standard_for_validation.pdf

435 Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environ-
436 ments. *Proc Natl Acad Sci USA* 109: 14058–14062. DOI: 10.1073/pnas.1202319109

437 Lodeyro AF, Carrillo N. 2015. Salt stress in higher plants: mechanisms of toxicity and defensive responses.
438 Pp 1-33 in *Stress Responses in Plants* (Tripathi BN & Müller M, eds), Springer International Publishing.

439 Luef B, Frischkorn KR, Wrighton KC, Holman HY, Birarda G, Thomas BC, Singh A, Williams KH,
440 Siegerist CE, Tringe SG, Downing KH, Comolli LR, Banfield JF. 2015. Diverse uncultivated ultra-small
441 bacterial cells in groundwater. *Nat Commun* 6: 6372. DOI: 10.1038/ncomms7372

442 Mueller, U.G. Gerardo NM, Aanen DK, Six DL, Schultz TR. 2005. The evolution of agriculture in insects.
443 *Annu Rev Ecol Evol Syst* 36: 563–595. DOI: 10.1146/annurev.ecolsys.36.102003.152626

444 Mueller UG, Sachs JL. 2015. Engineering microbiomes to improve plant and animal health. *Trends Micro-*
445 *biol* 23: 606-617. DOI: 10.1016/j.tim.2015.07.009

446 Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. 2015. Selection on soil microbiomes
447 reveals reproducible impacts on plant function. *ISME J* 9: 980–989. DOI: 10.1038/ismej.2014.196

448 Pfeiffer JA, Sporb A, Koren O, Jinb Z, Tringe S, Dangl JL, Buckler ES, Ley RE. 2013. Diversity and
449 heritability of the maize rhizosphere microbiome under field conditions. *Proc Natl Acad Sci USA* 110:
450 6548–6553.

451 Priest HD, Fox SE, Rowley ER, Murray JR, Michael TP, Mockler TC. 2014. Analysis of global gene ex-
452 pression in *Brachypodium distachyon* reveals extensive network plasticity in response to abiotic stress.
453 *PLoS One* 9: e87499. DOI: 10.1371/journal.pone.0087499

454 Roossinck MJ. 2015. Plants, viruses and the environment: Ecology and mutualism. *Virology* 479-480: 271-
455 277. DOI: 10.1016/j.virol.2015.03.041

456 Scheuring I, Yu DW. 2012. How to assemble a beneficial microbiome in three easy steps. *Ecol Lett* 15:
457 1300–1307. DOI: 10.1111/j.1461-0248.2012.01853.x

458 Shi S, Nuccio EE, Shi ZJ, He Z, Zhou J, Firestone MK. 2016. The interconnected rhizosphere: High net-
459 work complexity dominates rhizosphere assemblages. Ecol Lett 19: 926-936. DOI: 10.1111/ele.12630.

460 Swenson W, Wilson DS, Elias R. 2000. Artificial ecosystem selection. *Proc Natl Acad Sci USA* 97: 9110–
461 9114. DOI: 10.1073/pnas.150237597

462 Tkacz A, Poole P. 2015. Role of root microbiota in plant productivity. *J Exp Bot* 66: 2167–2175. DOI:
463 10.1093/jxb/erv157

464 Vogel JP, Garvin DF, Leong O, Hayden DM 2006. *Agrobacterium*-mediated transformation and inbred line
465 development in the model grass *Brachypodium distachyon*. *Plant Cell, Tissue & Organ Culture* 84: 199-
466 211. DOI: 10.1007/s11240-005-9023-9

467 Vogel J, Bragg J. 2009. *Brachypodium distachyon*, a new model for the Triticeae. In *Genetics and Genomics*
468 *of the Triticeae*, pp. 427–449. Edited by GJ Muehlbauer & C Feuillet. New York: Springer.

469 Yan Y, Kuramae EE, de Hollander M, Klinkhamer PGL, van Veen JA. 2016. Functional traits dominate
470 the diversity-related selection of bacterial communities in the rhizosphere. *ISME J* in press. DOI:
471 10.1038/ismej.2016.108

472

SUPPLEMENTAL INFORMATION: METHODS & RESULTS

Protocol Outline: We used a differential host-microbiome co-propagation scheme as described in Swenson *et al* (2000) and in Mueller *et al* (2005) (Figure S1), but we added to this scheme protocol steps of established techniques, including (a) microbiome-fractionation using size-selecting filters (Bakken & Olsen 1987; Mueller & Sachs 2015); (b) ramping of stress in successive selection-cycles (Garland & Rose 2009); (c) facilitation of priority-effects during microbiome assembly (Fierer *et al* 2012; Scheuring & Yu 2012) by capping pots for the first 4 days of the germination stage (i.e., we used a so-called *semi-open system*; Mueller & Sachs 2015), thus controlling in each selection-cycle the initial recruitment of symbiotic bacteria into rhizosphere microbiomes of seedlings; and (d) low-carbon soil to enhance carbon-dependent host-control of microbiome-assembly and -persistence (Bais *et al* 2006; Bulgarelli *et al* 2013; Mueller & Sachs 2015; Coyte *et al* 2015). In each microbiome-propagation cycle ('Microbiome Generation' = Gen), we inoculated surface-sterilized seeds taken from non-evolving stock (inbred strain Bd3-1 of the grass *Brachypodium distachyon*, derived via single-seed-descent inbreeding from the source accession; Vogel *et al* 2006; Garvin *et al* 2008; Vogel & Bragg 2009; Brkljacic *et al* 2011) with rhizosphere bacteria harvested from roots of those plants of each selection-line that exhibited the greatest above-ground biomass (Figure S1). Because the plant-host could not evolve between selection-cycles, whereas microbiomes could potentially evolve due to differential microbiome propagation, our selection-scheme was *one-sided selection* (Mueller & Sachs 2015). Both evolutionary and ecological processes alter microbiomes during and between selection-cycles, but our protocol aimed to maximize evolutionary changes stemming from differential microbiome-propagation (Figure S1). To focus indirect selection only on bacterial communities, we filtered the microbiomes harvested from rhizospheres of 'mother-plants', perpetuating only bacteria (and likely also viruses) to the next generation, but eliminating from propagation between microbiome-generations any larger-celled soil-organisms with filters (i.e., we excluded fungi, protozoa, algae, mites, nematodes, etc.). This fractionation step distinguishes our methods from those of Swenson *et al* (2000) and from the replication of that study by Panke-Buisse *et al* (2015), both of which used differential 'whole-community' propagation to transfer between generations all organism living in soil, including the larger-celled fungi, protozoa, algae, mites, and nematodes that were excluded through size-selecting filtering in our experiment. Our complete experiment involved one baseline Generation (Generation 0, Table S1a) to establish initial microbiomes in replicate pots; 8 rounds of microbiome-selection (i.e., differential microbiome-propagation) (Generations 1-8, Table S1b); and one final ninth round of selection (Generation 9, Table S1c) to evaluate the effects of the engineered (i.e., evolved) microbiomes on flower-production and seed-set, for a total of 10 Generations. Our entire selection experiment lasted 300 days from 3. January -29. October 2015 (Table S2).

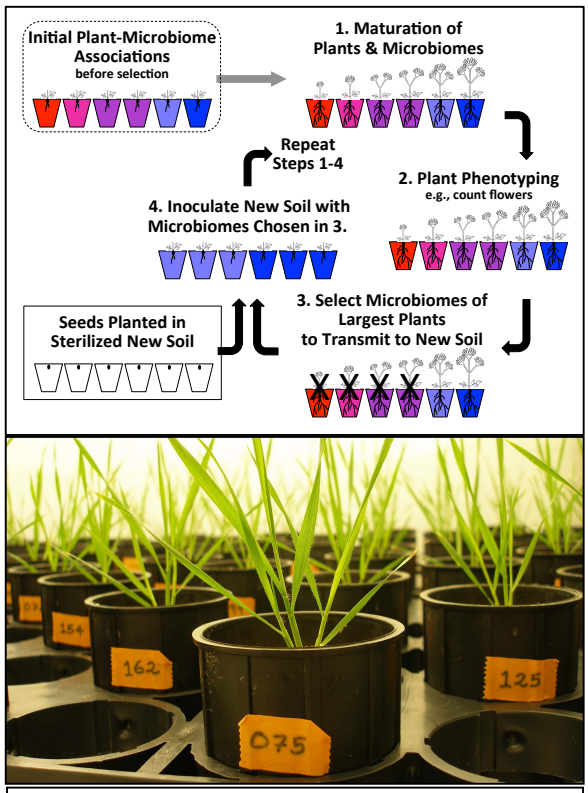

Figure S1. Top: Differential microbiome-propagation scheme to impose artificial indirect selection on rhizosphere microbiomes (figure modified from Mueller & Sachs 2015). The host-plant does not evolve because this scheme propagates only microbiomes into sterilized soil (Step 4), whereas the host is taken each cycle from a non-evolving source (stored seeds). Both evolutionary and ecological processes alter microbiomes during each cycle, but at Steps 3&4 in each cycle, experimental protocols aim to maximize evolutionary changes stemming from differential propagation of microbiomes. **Bottom:** *Brachypodium distachyon* Bd3-1 plants in our growth chamber shortly before a microbiome-harvest for differential microbiome-propagation to soils/seeds of the next microbiome-generation. Photo by UGM.

Logic of Salt-Stress Ramping: We used ramping of salt-stress (Mueller & Sachs 2015) to ensure that plants were neither under-stressed nor excessively over-stressed during any selection-cycle of our microbiome-selection experiment, and thus facilitate that microbiomes can gradually improve under differential microbiome-propagation to confer increasingly greater salt-tolerance to plants under increasingly greater salt-stress. The experimental rationale of stress-ramping is as follows: if salt-stress is too weak, all plants grow well, any salt-stress-mediating microbiomes will make little or no difference to plants, and no microbiome-mediated variation in plant-phenotype may emerge that could be used as direct target for indirect selection on microbiomes; in contrast, if salt-stress is excessive, all plants suffer severely, and any observed variation in plant-phenotype may be due to microbiome-unrelated effects emerging under excessive stress, such that possible beneficial effects of salt-stress-mediating microbiomes are dwarfed and masked by the excessive stress. Stress-ramping is therefore an experimental trick that permits an experimenter to continuously adjust stress during a selection experiment, particularly in experimental evolution where the evolving effect sizes cannot be known *a priori* (i.e., in our experiment, it was not possible to predict *a priori* the approximate effect sizes due to beneficial microbiomes that emerge as a result of multiple rounds of differential microbiome-propagation).

Table S2 shows the ramped salt-concentrations for the two salt treatments of soils in our experiment, Na_2SO_4 (sodium-sulfate, henceforth *SOD-soil treatment*) and $Al_2(SO_4)_3$ (aluminum-sulfate, *ALU-soil treatment*). We chose the particular two salt stresses because sodium-cations are a problem in saline and sodic soils (e.g., Lodeyro & Carrillo 2015), and aluminum-cations are a problem because aluminum inhibits, at even minimum concentrations, plant growth in low-pH soils (Delhazie *et al* 1995; Aggarwal *et al* 2015). Our maximum sodium-salt stress of 75 mMolar salt-concentration sodium-sulfate of water used to hydrate soil and water plants during the experiment is not quite comparable to the salt stress of 500 mMolar sodium-chloride used by Priest *et al* (2014) because (a) the two experiments used different kinds of salts and (b) Priest *et al* spiked salt stress after initial growth of plants in the absence of salt-stress, whereas plants in our experiments were salt-stressed from seed and stress continuing throughout the entire selection cycle.

Table S2. Salt concentrations (Millimolar = mMolar) of salt-nutrient solutions used to hydrate soil for each selection cycle (= Microbiome-Generation = Gen); the recipes to mix these solutions; and growth parameters for each Generation. In the short-cycled Generations 0-8, time was too short for plants to flower, and we quantified plant-performance by visually estimating above-ground biomass (see *Phenotyping of Plants*). In Generation 9, plants were grown for 68 days to produce seeds, and we quantified plant-performance as total seed weight per plant.

	Microbiome-Generation (Selection Cycle)									
	Gen 0	**Gen 1**	**Gen 2**	**Gen 3**	**Gen 4**	**Gen 5**	**Gen 6**	**Gen 7**	**Gen 8**	**Gen 9**
Sodium-Sulfate Concentration	**20 mMolar**	**30 mMolar**	**50 mMolar**	**60 mMolar**	**70 mMolar**	**75 mMolar**	**60 mMolar**	**60 mMolar**	**60 mMolar**	**60 mMolar**
1-molar sodium-sulfate	240 mL	360 mL	600 mL	720 mL	840 mL	900 mL	720 mL	720 mL	720 mL	1200 mL
Dyna-Gro fertilizer	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	400 mL
e-pure water	12 L	12 L	12 L	12 L	12 L	12 L	12 L	12 L	12 L	20 L
number of pots (plants)	100	100	100	100	100	100	100	100	100	200
Aluminum-Sulfate Concentration	**0.02 mMolar**	**0.04 mMolar**	**0.08 mMolar**	**0.20 mMolar**	**1.0 mMolar**	**3.0 mMolar**	**1.0 mMolar**	**1.0 mMolar**	**1.5 mMolar**	**1.5 mMolar**
1-molar aluminum-sulfate	240 µL	480 µL	960 µL	2.4 mL	12 mL	36 mL	12 mL	12 mL	18 mL	30 mL
Dyna-Gro fertilizer	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	240 mL	400 mL
e-pure water	12 L	12 L	12 L	12 L	12 L	12 L	12 L	12 L	12 L	20 L
number of pots (plants)	100	100	100	100	100	100	100	100	100	200
Start date (= microbiome transfer/inoculation date)	03. Jan 2015	25. Jan 2015	14. Feb 2015	07. Mar 2015	31. Mar 2015	25. Apr 2015	27. May 2015	22. Jun 2015	20. Jul 2015	20. Aug 2015
Number of days plants allowed to grow until microbiome harvest & transfer to next generation	22	20	21	24	25	32	26	28	31	68

Number of leaves of well-growing plants at day of microbiome harvest & transfer	9-11	9-11	9-11	10-13	8-10	11-13	11-14	17-22	25-30	plants allowed to grow to seed
Number of plants	200	200	200	200	200	200	200	200	200	400
Weight bin of seeds used for planting	5.8-5.9 mg	5.7-5.8 mg	5.5-5.6 mg	5.4-5.5 mg	5.3-5.4 mg	5.2-5.3 mg	5.1 mg	5.0 mg	4.9 mg	4.6-4.8 mg

A second pre-planned feature of our experimental design was to use 'short-cycling' in the initial selection-cycles (cycling at about 20-day intervals; plants grew to about the 9-13 leaf stage, but did not have sufficient time to flower), and then to increase lengths of selection-cycles gradually as plants became more stressed under the ramped salt-concentrations and plants needed more time to grow to the 9-13 leaf stage when plants have root-systems large enough for microbiome harvest. Although we planned lengthening the duration of selection-cycles during our multi-generation experiment, we did not pre-plan at the beginning of our experiment the exact length of each selection-cycle, because the exact transfer dates were dependent also on time-constraints of the main experimenter (UGM) handling the microbiome-transfers. Because we increased salt-stress during the 10-Generation experiment (Table S2), plant growth was slower in later generations (see growth trajectories of plants recorded in Table S1a-c).

Preparation of *Brachypodium distachyon* Seeds: Prior to the start of the microbiome-selection experiment, we harvested about 6000 seeds from 36 plants (*B. distachyon* strain Bd3-1; Garvin *et al* 2008; Vogel & Bragg 2009) grown simultaneously at room temperature under constant light-cycle (14h light, 10h dark) in well-homogenized, well-watered and well-fertilized greenhouse potting soil. Seeds were air-dried at room temperature for 4 months, mixed, then weighed individually to the nearest 0.1mg to generate cohorts of seeds of equal weight (binned to within 0.1mg). To reduce within-generation phenotypic variation due to differences in seed-weight-dependent maternal effects, we used seeds of only one or two adjacent weight-bins for each generation. We used seeds of 5.9&5.8mg weight for the initial baseline Generation 0, then we used up seeds of bins of gradually decreasing seed-weight (5.9&5.8mg, 5.8&5.7mg, 5.6&5.5mg, …), as shown in Table S2 for each microbiome-generation. All microbiome selection-cycles used seeds from this stored (non-evolving) seed-stock of Bd3-1 plants grown under identical conditions prior to the selection experiment, and microbiomes were therefore selected under a so-called *one-sided selection* scheme in the single plant-genotype background Bd3-1 (only microbiomes can change between selection-cycles, but the plant-host cannot evolve; Mueller & Sachs 2015).

Growth Chamber: For the multi-generation selection experiment, we grew plants under constant temperature (24°C) and constant light-cycle (20h light 4AM-midnight, 4h dark) in a walk-in growth chamber (model MTPS72; Conviron, Winnipeg, Canada) at the Welch Greenhouse Facility of the University of Texas at Austin. The chamber was not humidity-controlled, and chamber humidity therefore varied with outdoor humidity/rainfall and with any heating (in winter) affecting humidity of the air circulating in the Greenhouse Facility. Because of unusual rainfall in spring 2015, humidity was highest in the growth chamber during Generations 4 & 5 (Table S1 & S2), and lowest during selection-cycles 0-2 and 7-9. Unfortunately, we did not monitor exact humidity with a hygrometer in the chamber, but we recorded in a journal any days of high humidity. We grew plants on two shelves (each 120cm x 100cm) in the Conviron chamber, under fluorescent lights (Sylvania T8 fluorescent tubes spaced at 10cm, plus a center row of T2 fluorescent spiral-bulbs) generating a light-intensity of 192 $\mu mol/m^2/s$ at soil level. Except for preparation of pots and planting of seeds, we performed all experimental steps for artificial microbiome-selection in this chamber, including microbiome-harvesting from rhizospheres, microbiome-fractionation (filtering), and microbiome-transfers to surface-sterilized seeds planted in sterile soil (details below).

Soil & Pot Preparation: We grew plants from surface-sterilized seeds, each planted individually in the center of its own D50-Deepot (5cm pot diameter, 17.8cm depth, total volume 262ml; model D16H; Stewe & Sons, Tangent, Oregon, USA) filled with autoclaved PPC soil (Profile Porous Ceramic soil, Greens-Grade™ Emerald, Natural Color; PROFILE Products LLC, Buffalo Grove, IL, USA). To permit autoclaving of soil in the Deepots prior to planting, we pressed heat-tolerant fiberglass-fill into the bottom of each

pot to plug bottom-drainage holes, then compacted dry PPC soil into each pot until the soil level reached 15mm below the pot margin. Each plug consisted of a fiberglass square (9.5cm x 9.5cm) cut from an insulation-sheet (R-13 EcoTouch Insulation Roll; 38cm width; GreenGuard-certified, formaldehyde-free), then pressed firmly into the bottom of a pot. After compacting soil in all pots used for a given selection-cycle (200 pots in selection-cycles Generation 0-8; 400 pots in the final Generation 9) we carefully equalized soil levels between all pots.

According to the manufacturer's website (www.profileevs.com/products/soil-amendments/profile-porous-ceramic-ppc), PPC soil is a calcined, non-swelling illite, non-crystalline opal mineral; it has 74% pore space, with 39% capillary (water) pores and 35% non-capillary (air) pores; pH = 5.5; cation-exchange-capacity of 33.6 mEq/100g; and a chemical composition of 74% SiO_2, 11% Al_2O_3, 5% Fe_2O_3, and less than 5% of the remainder combining all other chemicals (e.g., CaO, MgO, K_2O, Na_2O, TiO_2). We chose PPC soil for three reasons: First, PPC has a very homogeneous consistency because of its uniform particle size; soil-quantity and soil-quality are therefore easy to standardize between pots. Second, whole root systems can be easily extracted from hydrated soil with little rupture of roots. Third, because the manufacturer exposes PPC soil to high temperature (heated in a rotary kiln at 1200 degrees Fahrenheit, then de-dusted), the soil contains minimum carbon, and we believed that such low- or no-carbon soil could facilitate a plant's ability for carbon-mediated host-control (via carbon secretions by roots; see above *Protocol Outline*) (Bais *et al* 2006; Bulgarelli *et al* 2013; Mueller & Sachs 2015; Coyte *et al* 2015) of microbiome-assembly and microbiome-stability.

Soil Hydration & Salt-Stress Treatments: After compacting soil into each pot with a wooden dowel and equalizing soil levels between all pots used in a selection-cycle, we hydrated each pot with 94ml of a fertilizer-salt solution (Table S2). The fertilizer concentrations in this solution was identical in each selection-cycle (i.e., we added the same amount of fertilizer to soil of each microbiome generation), but we increased salt-concentrations gradually between successive selection-cycles in order to ramp salt-stress, as shown in Table S2 for the two salt-stress treatments, Na_2S0_4 (decahydrate sodium-sulfate, MW=322.2g; *SOD-soil*) and $Al_2(S0_4)_3$ (anhydrous aluminum-sulfate, MW=342.15; *ALU-soil*). We chose the particular two salt stresses because sodium is a problem in saline soils (e.g., Lodeyro & Carrillo 2015), and aluminum is a problem because aluminum inhibits, at even minimum concentrations, plant growth in low-pH soils (Delhazie et al 1995; Aggarwal et al 2015). Because of this pH-dependent growth-attenuating effect of aluminum in soil, we suspected that it may be easier to select for a microbiome conferring tolerance to aluminum salt, for example by selecting for a microbiome that increases soil pH (i.e., artificial microbiome-selection would select against acidifying bacteria in microbiomes). We therefore were able to formulate this *a priori* hypothesis on a possible pH-based mechanistic basis of a microbiome-conferred tolerance to aluminum-salt. In contrast, we did not formulate a similarly specific mechanistic hypothesis for why a microbiome could confer tolerance to sodium-salt, although a number of hypotheses have been suggested in the literature, such as changes in phytohormone concentrations influencing plant physiology, or indirect physiological effects on transpiration rates (Dodd & Pérez-Alfocea 2012). We included this second selection-regime selecting for microbiomes conferring sodium-tolerance because a dual experimental design of two soil-treatments (aluminum- and sodium-salt stress) offered two advantages: (i) we could contrast evolving microbiomes between aluminum- versus sodium-treatments to identify candidate bacterial taxa or candidate consortia that may be important in mediating microbiome-conferred salt-tolerance to plants; and (ii) we could cross selection history with selection stress in the last Generation 9 to test for possible specificities of evolved microbiomes, as explained further in *Crossing Evolved SOD- and ALU-Microbiomes with SOD- and ALU-Stress*.

The salt-concentration of the baseline Generation 0 (Table S2) was determined in a salt-gradient pilot experiment as that salt-concentration that caused a minimal, but just noticeable, delay in germination and a minimal growth-rate reduction. Because aluminum-sulfate delays germination and attenuates growth at far lower concentrations than sodium-sulfate, concentrations in the ALU-treatment (Table S2) were lower by several orders of magnitude than the concentrations of sodium-sulfate in the SOD-treatment. For ramping

of salt-stress, pre-planned step-increments in salt-concentration between selection-cycles were likewise informed by our pilot experiments, which suggested increments for aluminum-sulfate concentrations of about two- to five-fold for the first few microbiome-generations, and less than two-fold increments for sodium-sulfate concentrations, with gradual decrease in step-increments in later microbiome-generations so as not to over-stress plants (Table S2). Because we had to prepare hydrated soil for the next selection-cycle about 1-2 weeks before the end of a given cycle, we had to decide salt-stress increments for the next selection-cycle about well in advance, using information from relative growth of younger plants in the sodium-sulfate or aluminum-sulfate treatments. Decisions on salt-increments between Generations therefore typically involved some informed guessing, to adjust salt concentrations for the next cycle such that plants in either treatment were projected to germinate and grow at about the same rate (i.e., we aimed for plants in either treatment to grow to comparable sizes at about the same time during a cycle). With such projected equal growth between sodium- and aluminum-treatments, microbiomes could be harvested at the end of a selection-cycle from plants of comparable sizes (typically 9-15 leaves at microbiome-harvesting) regardless of whether a plant was stressed with aluminum-sulfate or sodium-sulfate (i.e., sodium-treated plants were not far behind in growth compared to aluminum-treated plants, or vice versa). A second pre-planned feature of our salt-ramping design was to increase salt-stress in successive selection-cycles as long as differences in effect-sizes seemed to increase between salt- and control-treatments, but to reduce the salt-stress if differences in effect-sizes diminished or disappeared, possibly because of over-stressing the plants (see above logic underlying *Stress Ramping*). This seemed to happen in Generations 4 & 5 (see Figure 1 in main text), and salt-stress was therefore reduced somewhat in the subsequent four Generations 6-9 (Table S2).

For hydration of 100 pots, we mixed, in a large carboy, 12 liter double-distilled e-pure water at a 50:1-ratio with 240ml Dyna-Gro 9-7-5 (Nutrient Solutions, Richmond, CA; www.dyna-gro.com/795.htm), plus an aliquot of 1-Molar salt solution (Table S2 lists salt-aliquots in recipes for salt-nutrient mixes) to generate the specific salt-stress planned for a particular selection-cycle. [To prepare 1-Molar ALU-salt stock, we dissolved 307.94g anhydrous aluminum-sulfate in 900ml e-pure water in a 1L bottle; to prepare 1-Molar SOD-salt stock, we dissolved 289.98g decahydrous sodium-sulfate in 900ml e-pure water in a 1L bottle; then filter-sterilized each salt solution to prepare sterile stock.] We used different carboys to prepare salt-nutrient mixes for the different salt treatments (SOD, ALU). The nutrient concentration in each mix (Table S2) was sufficient such that plants did not need additional fertilization during each selection-cycle of 20-30 days during Generations 0-8 when we quantified plant fitness as above-ground biomass production, and plants even had sufficient nutrients to flower and grow seed during the 68 days of the last Generation 9 when we quantified plant-fitness as seed production. For both salt treatments, fertilizer-salt solutions had a pH = 3.75 before addition to soil, but because of the buffering capacity of PPC soil (natural pH = 5.5, see above), the hydrated soil had a pH of about 5.0-5.5 after autoclaving soils, using the pH-measurement procedure in ISO/FDIS 10390 (2005). After hydration of all pots, we immediately autoclaved all pots (to minimize the time that any live microbes in the soil could consume any of the nutrients), and we autoclaved in separate 1L flasks at the same time 800ml of each of the unused salt-nutrient solutions; these autoclaved salt-nutrient solutions were used later during planting, and as buffer (at half-concentration) to suspend microbiomes harvested from rhizospheres for microbiome-transfers (see *Planting* and *Microbiome-Harvest* below).

Autoclaving of Soil: After hydration of soil by carefully pouring exactly 94ml of fertilizer-salt solution into a pot, we leveled and smoothed the soil-surface in a pot with the bottom of a glass (same size as interior diameter of a pot); taped to each pot a label of autoclavable label-tape (Fisherbrand™) with a pre-written pot-number (#001-100 for pots of SOD-treatment; #101-200 for pots of ALU-treatment) to the top side of each pot (Figure S1); then used pre-cut pieces of aluminum foil to cap the top and wrap the bottom of each pot to prevent possible microbial contamination during seed-stratification (see below *Planting & Stratification*). Aluminum-wrapped pots were arranged vertically in large autoclave trays (67 pots per tray, 3 trays total), the trays were covered with sheets of aluminum foil, then all pots in these 3 trays were sterilized simultaneously in a large autoclave. Hydration, labeling and capping of a set of 200 pots using the above methods needed typically 5-6 hours. The subsequent autoclaving procedure lasted about 10 hours overnight,

starting in the evening with a first cycle of 35min autoclaving (121C° temperature, 20atm pressure) with a slow-exhaust phase lasting 90min; followed by overnight exposure to high temperature in the unpressurized autoclave; followed in the morning by a second cycle of 35min autoclaving with a 90min slow-exhaust phase. This stringent autoclaving regime was sufficient to sterilize PPC soil, because plating on PDA-medium of about 0.5g soil (n=2 SOD pots, n=2 ALU pots) taken with a sterile spatula from the interior of such autoclaved pots produced no visible microbial growth within a month of incubation at room temperature. After cooling of autoclaved pots in the foil-covered trays at room temperature for at least 16 hours, we planted seeds into the sterilized soil (see below *Planting*).

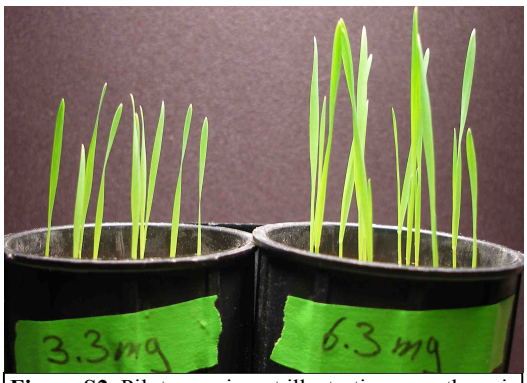

Figure S2. Pilot experiment illustrating growth variation of *B. distachyon* Bd3-1 plants growing under identical conditions from seeds weighing either 3.3mg or 6.3mg. The seed-weight range tested here includes about 90% of the 6000 seeds that we harvested for our microbiome-selection experiment. We used seeds of a narrow weight-window of only 0.1mg or 0.2mg for each microbiome-selection cycle (see Table S2), to help reduce within-generation and within-treatment variation in plant-phenotype (specifically here, reduce seed-weight-dependent maternal effects on phenotypes), Photo by UGM.

Seeds Preparation & Binning of Seeds by Weight: To have enough seeds for our 10-generation selection experiment, we first grew *Brachypodium distachyon* Bd3-1 plants under standardized light conditions (14h light, 10h dark) and room temperature in well-fertilized and well-watered greenhouse soil, harvested about 6000 seeds from these plants, then dried and stored seeds at room temperature (see above *Preparation of Brachypodium distachyon Seeds*). For our experiment, we used only long-awn seeds; that is, we discarded any short-awn seeds positioned peripherally in inflorescences (spikelet), and we discarded also any misshapen or discolored seeds. We used only long-awn seeds because these kind of seeds grow in more standardized central positions in a spikelet, because we could grasp an awn with a forceps during weighing and planting without risk of injuring a seed, and because we could plant seeds vertically into soil with only the awn protruding above the soil to reveal the exact location of a seed during later microbiome inoculation (see below *Seed Inoculation*). To weigh each seed accurately, we first removed any attached glumes to weigh only the seed with its awn. One experimenter pre-weighed each seed to bin seeds by weight to the nearest 0.1mg, then a second experimenter re-weighed all seeds in bins 4.5mg – 6.0mg again (i.e., each seed was weighed twice). To help reduce within-treatment variation in plant-phenotype (specifically here, reduce seed-weight-dependent maternal effects on plant-phenotypes, Figure S2), we used seeds of only a narrow weight-window for each microbiome-selection cycle. We used seeds of 5.9&5.8mg weight for the initial baseline Generation 0, then we used up seeds of bins of gradually decreasing seed-weight (5.9&5.8mg, 5.8&5.7mg, 5.6&5.5mg, …), as shown in Table S2 for each microbiome-generation.

Planting & Stratification: For planting of seeds in sterile soil, we first surface-sterilized Bd3-1 seeds in a laminar flow-hood by gently shaking the seeds for 8 minutes in 10% bleach [Chlorox®; 4ml bleach added to 36ml autoclaved e-pure water in a 50ml Falcon tube; plus 4µl Tween80-surfactant (Sigma-Aldrich, St. Louis, MO, USA) to promote wetting of seeds], then rinsing three times to wash off bleach (succession of three 1-minute gentle shaking, each in 40ml e-pure autoclaved water in 50-ml Falcon tubes). In pilot tests, such surface-sterilized seeds placed on PDA-medium did not lead to bacterial or fungal growth. After rinsing, we blotted seeds on autoclaved filter paper, then air-dried the seeds in an open Petri dish in the flow-hood while preparing the flow-hood for planting inside the hood. To plant one seed into the center of a pot, we removed the aluminum-foil lid from a pot inside the flow-hood, pushed a narrow hole into the center of the soil with a flame-sterilized fine-tipped forceps (#5 forceps), then inserted a seed into that hole such that the seed was positioned vertically in the soil and only the awn was protruding above the soil (i.e., the pointed tip of a seed was just below the soil surface). Because seeds used for a selection-cycle had been binned to within 0.1mg weight (i.e., all seeds were of same size), seeds were therefore planted at the same depth (to within about 0.5-1.0 mm identical depth), and any differences in initial germination rate (i.e., appearance of the shoot at soil surface) was unlikely due to differences in planting depth between seeds. To solidify the

745 soil around each seed, we applied 4ml autoclaved salt-nutrient solution (same concentration that was used
746 to hydrate soil in a given selection-cycle; Table S2) with a 5ml pipette to flush soil into the hole and com-
747 pletely cover each seed (excepting the awn protruding vertically above the soil surface). We covered each
748 pot with a translucent, ethanol-sterilized lid (inverted Mini Clear Plastic Bowl 40ct; Party City, Rockaway,
749 NJ, USA). The lids prevented entry of airborne microbes into each pot, but did not seal pots completely and
750 permitted some gas exchange. Each lid measured 5.7cm diameter x 3.8cm height, and fit snugly only each
751 pot such that a series of 50 capped pots could be kept in a rack (D50T rack, see above) without the lids
752 interfering with each other. We placed each rack of 50 capped pots into its own ethanol-sterilized plastic
753 tub (Jumbo Box; Container Store, Coppell, TX), then sealed the cover of each tub with a 2-meter-long strip
754 of 10-cm-wide Parafilm to prevent entry of contaminants during subsequent cold-storage for stratification
755 of seeds. We moved each tub into cold-storage immediately after completing the planting of 50 pots. For
756 stratification, we stored the tubs with planted seeds in a 5°C cold-room for about 5 days (range 4-10 days,
757 the duration differing slightly between selection-cycles because of scheduling-constraints affecting plant-
758 ing). Planting of a set of 200 seeds (4 racks of 50 pots each) using the above methods needed typically 4.5-
759 5.5 hours.

760 **Preparations for Microbiome-Harvesting:** To prepare salt-nutrient buffer-solution for microbiome har-
761 vesting, we used the autoclaved salt-nutrient solution that we had prepared for hydration of soil for a par-
762 ticular selection-cycle (Table S2), then diluted the solution to half-concentration by addition of an equal
763 volume of autoclaved e-pure water. We decided to use for microbiome-harvesting the salt-nutrient solution
764 at half-concentration, because we were concerned that the full-concentration may have too high osmolarity
765 compared to the osmolarity that may exist in the soil after weeks of root- and microbiome-growth in the
766 soil; this dilution precaution may not have been necessary, and it may be possible to propagate microbiomes
767 even when using full-concentration of salt-nutrient solution. Aliquots of 45ml of the sterile, half-concen-
768 tration salt-nutrient buffer were added to 50ml Falcon tubes in a laminar-flow hood, and these tubes were
769 then pre-labeled with relevant information (SOD vs ALU treatment; Generation #; date of microbiome-
770 harvest) to save time on the actual day of microbiome-harvest. To sterilize microfilters needed for fraction-
771 ation of harvested microbiomes (2µm Whatman™ filters; model Puradisc 25 GD2 Syringe Filter, 25mm
772 diameter; Whatman PLC, United Kingdom), we wrapped filters individually in aluminum-foil, then auto-
773 claved these in a 15min-exposure fast-exhaust cycle. On the evening before the day of microbiome-har-
774 vesting, we set up a custom-made flow-hood on a bench in our Conviron growth-chamber, sterilized the
775 inside of the hood by spraying liberally with 100% ethanol, then allowed the flow of clean air purify the
776 hood overnight. Our custom-made hood was constructed of a large plastic tub placed on its side, with the
777 lid cut half so that a lid-portion affixed to the tub could shield the inside of the hood from above (like a sash
778 on a regular flow-hood), whereas the bottom half was kept open to permit access to the inside of the hood.
779 To generate a flow of clean air through the hood, we cut a large hole into the top of the hood (i.e., one of
780 the original sides of the tub now resting on its side) to fit into that hole the top portion of an air purifier
781 (model HPA104 Honeywell HEPA Allergen Remover, with HEPA filter of 0.3 microns; Honeywell Inter-
782 national Inc., Morris Plains, NJ, USA). We operated the purifier at medium flow-setting, which generated
783 an even flow through the hood and minimized any air-vortices that could draw impure air into the hood at
784 high flow-setting. In a pilot test, Petri-plates with PDA-medium, exposed overnight to the flow inside our
785 hood, revealed no visible growth within seven days of incubation of these plates at room temperature. Early
786 on a day of a between-generation microbiome-transfer, we moved the tubs with racks of planted, cold-
787 stratified seeds from the cold-room into our growth-chamber, to have sufficient time for completion of all
788 microbiome-harvests and -transfers (the total time needed on the day of microbiome-harvest for completion
789 of harvests/transfers of all lines was 8-10 hours, plus an additional 2 hours for distribution of pots in pre-
790 determined randomized arrangements across 8 racks used to support pots in the growth-chamber). We be-
791 gan microbiome-harvests and –transfers immediately after moving pots into the growth chamber, so trans-
792 ferred microbiomes would interact with seeds at the very early stages of germination.

793 **Phenotyping of Plants; Quantification of Above-Ground Biomass:** To select the two best-growing
794 plants from a particular selection-line on the day of microbiome-harvest and -transfer, we moved all eight

795 pots from a selection-line into a separate, ethanol-sterilized rack, recorded the number of leaves of each
796 plant, and arranged plants visually by apparent above-ground biomass into a size-ranked series (Figure S3).
797 We chose visual sizing rather than weighing for phenotypinc, because visual evaluation of all eight plants
798 in a selection-line needed only about 5-10min (including recording number of leaves for all eight plants),
799 and because microbiomes could be harvested immediately after visually identifying a particular plant for
800 microbiome-harvest without first having to cut and weigh above-ground biomass of all plants in a selection
801 line. We harvested rhizosphere microbiomes from only those two plants within a selection-line that we
802 visually judged to have grown, by the day of microbiome-harvest, the greatest and second-greatest above-
803 ground biomass (Figure S3).

Figure S3. Plants of the same selection-line ranked by visually estimating above-ground biomass. The 8 plants were grown in 8 different racks (one plant per rack) in randomized positions in each rack, and the 8 plants were moved to a separate ethanol-sterilized rack for visual comparison immediately before microbiome harvesting from the two largest plants. See text for further details on *Quantification of Above-Ground Biomass*. Photo by UGM.

804 To test the accuracy of our visual rankings, we later compared these rankings with dry above-ground (shoot)
805 biomass. To weigh shoot-biomass, we cut each plant at soil-level at the time of microbiome-harvesting,
806 stored above-ground biomass for drying in an individual paper envelope (Coin Envelope 8cm x 14cm),
807 dried these envelopes/plants for at least two weeks at 60°C in a drying oven, then weighed dry biomass for
808 each plant to the nearest 0.1mg. Although we judged above-ground plant-biomass visually on the day of
809 microbiome-harvesting, of the 80 lines judged during our entire experiment (5 SOD-lines + 5 ALU-lines
810 judged each Generation, times 8 Generations; Table S1), we picked for microbiome-harvest the combina-
811 tion of largest (#1) and second-largest (#2) plant in 56.25% of the cases; the largest (#1) and third-largest
812 (#3) plant in 27.50%; the largest (#1) and fourth-largest (#4) plant in 6.25%; the second-largest (#2) and
813 third-largest (#3) in 5.00%; the second-largest (#2) and fourth-largest (#4) in 5.00%; and never any lower-
814 ranked combination. In cases when we did not identify visually the combination of #1 and #2 plants as later
815 determined by dry weight, the slightly lighter #3 or #4 plants were typically within 0.2-4mg (0.5-9% of
816 total dry-weight) of the two best-growing plants in the same selection-line (Table S1). Moreover, because
817 harvested microbiomes of the two chosen plants were mixed for propagation to the next microbiome-gen-
818 eration (see below *Microbiome Mixing*), we included in 100% of the cases microbiomes from either the
819 best-growing or second-best growing plant into the mixed microbiome that we then propagated to the next
820 microbiome-generation (i.e., a microbiome of one of the two best-growing plants was always included in
821 the propagated microbiome mix). Lastly, of the 160 plants chosen for microbiome-propagation during the
822 entire experiment, in 90% of the cases did we propagate in a mix the microbiome from the best-growing
823 plant within a selection line (i.e., in only 10% of the cases did we not include the microbiome from the best-
824 growing plant). In sum, our method to visually judge plant-size was both time-efficient (about 4 minutes
825 needed to visually size all plants in a selection-line), but our method was also accurate to identify those
826 plants that had grown biomass well above-average within any given selection-line (i.e., our methods were
827 accurate at visually identifying plants that were likely associated with microbiomes that conferred salt-
828 tolerance to plants).

829 In some cases, on the day of microbiome-harvest, more than two plants of the same line appeared to have
830 the largest above-ground biomass. To decide between those plants for microbiome-harvest, we considered

as a second criterion also the growth trajectory recorded from the day of germination to the day of microbiome-harvest, choosing then the plant with the best growth-trajectory. We quantified growth trajectory of plants during each generation with three methods: (i) measuring the length of the first leaf on Days 2-5; (ii) counting of number of leaves grown by a plant; and (iii) visual ranking of relative plant size (visual appearance of overall biomass) on a 10-point scale from 1-9. ***Length of first leaf*:** After moving pots from the cold-room into the growth-chamber, the fastest-growing shoots became visible, as they pushed through the soil, after about 44 hours in the early, low-salt Generations, but growth rate was somewhat slower in the later, high-salt Generations (Table S1). To quantify this early growth, we estimated length of the first leaf during Days 2 & 3 visually without lifting the translucent lids from pots, but measured leaf-length on Days 4 & 5 to the nearest mm with an ethanol-sterilized ruler (mm gradation printed on paper strip; Figure S4) held next to the growing leaf, using a different sterile paper-ruler for each plant so as not to transfer microbes between pots. In blind, repeat evaluations (Table S1), the visual sizing on Days 2 & 3 is accurate to about ±0.5mm for leaves less than 15mm tall, and accurate to about ±2mm for plants larger than 25mm. Despite the somewhat lower accuracy of the visual leaf-length estimation compared to the precise measurement with a ruler, we chose to visually size plants on Days 2 &3 because that method allowed us to leave the pots covered with lids, thus preventing any influx of microbes when lifting a lid; plants therefore interacted only with the experimentally-transferred microbiomes for a total of 4 days without any influx of additional microbes. ***Counting leaf number*:** The fastest-growing plants showed growth of a second leaf typically late on Day 5 (in the early low-salt Generations) or on Day 6 (in the later high-salt Generations) (e.g., compare records for Days 5 & 6 in Generations 2 & 5 in Table S1). We counted the number of leaves regularly after Day 6 (see Table S1). Because different plants with the same number of leaves differed in size of their youngest leaves (e.g., on the same day, some plants showed beginning growth of leaf #3, other plants extensive growth of leaf #3), we also recorded during these counts the relative size of each plant's youngest leaf on a 5-point scale [see Table S1b&c: double-minus "–" (= well-below average for plants with that particular leaf number); single-minus "-" (= below average); average; single-plus "+" (= above average); double-plus "++" (= well above average)]. ***Above-ground biomass estimated on a 10-point scale ranging from 0-9*:** This third method gave the most precise estimate of above-ground biomass once plant had grown more than 10 leaves, and we used this method therefore every generation to obtain a relative measure of above-ground biomass a few days before the microbiome-harvesting. An experimenter first looked over all plants to gain an impression of the largest plants, of the average-sized plants, and of the smallest plants, then subdivided the entire range on a subjective 0-9 point-scale, with average-sized plants to be centered around a value of 4.5 on the 0-9 point-scale. Evaluating all plants rack-by rack, the experimenter scored and recorded sizes of all 200 plants in a Generation, then blindly re-scored all plants again rack-by-rack, then calculated an average between the two scores for each plant. Comparison of the first and second size-values for each plant (see Table S1b&c) showed that about 70% of the blind re-scoring were identical to the first score; and in most of the remaining 30% cases, the scores of the same plant differed by only a 1-point-value, and only in very exceptional cases (<2%) the scores differed by a 2-point value. Because of this repeatability of our blind scoring method, we used this method every generation to obtain estimates of the relative above-ground biomass of each plant 1-3 days before each microbiome-harvesting.

Figure S4. Measuring length of the first leaf on Day 3, using an ethanol-sterilized dry paper-strip with a millimeter-scale. Photo by UGM.

Microbiome-Harvesting from a Rhizosphere & Microbiome Mixing: We performed all steps of microbiome-harvest and -transfer in a clean-air flow-hood (see above) set up on a bench inside our growth-chamber (i.e., we did not have to move microbiomes/pots of selection-lines outside the growth chamber),

880 and we sterilized hands and work-surfaces regularly with 100% ethanol to prevent contamination of sam-
881 ples. After choosing the two plants with the greatest above-ground biomass (see above *Phenotyping of*
882 *Plants*), we cut each plant at soil level with ethanol-sterilized scissors, stored the above-ground portion in
883 an envelope for drying, and harvested rhizosphere microbiomes immediately to minimize microbiome
884 changes in the absence of plant-control in the rhizosphere. To extract the root-system from a pot (Deepot)
885 with minimal contamination, we held the shoot-stub at the soil surface with an ethanol-sterlized forceps,
886 tilted the pot such that PPC-soil would gradually loosen and fall out when squeezing the plastic pot, until
887 the root-structure could be extracted as a whole by gentle pulling at the main root with the forceps. In most
888 cases, the entire root structure could be extracted whole, with some loss of fine roots embedded in spilled
889 soil. Because we were interested in harvesting microbiomes that were in close physical association with a
890 plant (i.e., we were interested in rhizoplane bacteria, plus any endophytic bacteria if they were released
891 during root processing as a result of any root damage), we discarded any soil adhering loosely to the roots.
892 We dislodged loosely adhering soil by knocking the root-system gently against the wall of an autoclaved
893 aluminum-pan (e.g., Hefty EZ Foil Roaster Pan; 32cm length x 26cm width, vertical depth 11cm) such that
894 any dislodged soil would fall into the pan without the roots contacting any discarded soil. We then cut off
895 the top 2cm of the root-system (i.e., roots close to the soil surface), then transferred the remaining root-
896 system into a 50ml Falcon tube filled with 45ml of salt-nutrient buffer (the same buffer used also to hydrate
897 soils of the subsequent microbiome-generation, but diluted to half-concentration to suspend harvested mi-
898 crobiomes; see above *Preparations for Microbiome-Harvesting*). We repeated this process with the second
899 plant chosen for microbiome-harvest from the same selection line, and added this second root-system to the
900 same Falcon tube as the first root-system. Combining both root-systems for microbiome-harvesting gener-
901 ated a so-called mixed-microbiome collected from two 'mother rhizospheres' (see *Mixed Microbiome*
902 *Propagation*; and Box 3 in Mueller & Sachs 2015), which we then transferred within the same selection-
903 line to all eight 'offspring plants' of the next microbiome-generation.

904 **Microbiome-Fractionation with Microfilters:** To dislodge microbes from roots and from soil-particles
905 adhering to roots, we turned a closed Falcon tube upside-down 50 times, then permitted the solids to settle
906 in the tube for 1 minute. A 1cm-deep sediment of PPC-soil particles typically accumulated in the bottom
907 cone of a Falcon tube, with the roots settling on top of this sediment, and small particles and colloids re-
908 maining suspended in the salt-nutrient buffer. We aspirated 20ml of this suspension with a sterile 20ml
909 syringe (external syringe diameter fitting into a 50ml Falcon tube), then attached to the syringe's Luer-lock
910 a 2µm Whatman microfilter (model Puradisc 25 GD2 Syringe Filter, 25mm diameter; Whatman PLC,
911 United Kingdom), then filtered the aspirated suspension into an empty sterile 50ml Falcon tube. Making
912 sure that the exterior of the syringe did not become contaminated during this first filtering, we repeating
913 this step with the same syringe to filter another 15-20ml of the suspension, then mixed the combined filtrates
914 by inverting the Falcon tube several times. The total volume of 35-40ml filtrate was sufficient to inoculate
915 8 'offspring plants' each with 4ml filtrate (total of 8x4ml = 32 ml needed). In pilot tests, plating on PDA-
916 medium 10 µL of this filtrate (2µm filter) yielded thousands of bacterial colony-forming-units (CFUs) but
917 no fungal CFUs within 24 hours growth; whereas plating on PDA-medium 50 µL of this same filtrate that
918 had been filtered a second time with a 0.2µm filter (VWR Sterile Syringe Filter, 0.2µm polyethersulfone
919 membrane, 25mm diameter; Catalog #28145-501; retains the small-sized *Brevundimonus diminuta*) did not
920 yield any visible growth on PDA plates kept for 7 days at room temperature. These results justified addition
921 of a third control-treatment in Generation 9 (0.2µm filtration of suspension) to test growth-promoting ef-
922 fects of chemicals and viruses co-harvested with the harvested microbiomes (see below *Crossing Evolved*
923 *SOD- and ALU-Microbiomes with SOD- and ALU-Stress*). Although a 0.2 µm filter may not eliminate ultra-
924 small bacteria (e.g., Luef *et al* 2015; we did not use filters of smaller pore size because it was too difficult
925 to press liquid through such filters with a hand-held syringe), a comparison between 2.0µm-filtered and
926 0.2µm-filtered bacterial microbiomes can still test whether the bulk of the bacterial microbiome (in size
927 range 0.2-2.0µm) or any smaller-sized organisms (viruses, ultrasmall bacteria) plus solutes in the soil is
928 responsible for conferring salt-tolerance to plants in our experiment.

Even if we do not eliminate ultra-small bacteria (e.g., Luef *et al* 2015), we can still test whether the bulk bacterial microbiome, with bacterial sizes such as *Bosea* (Fig.6), can be engineered by microbiome selection (see also Fig.1 right, where our filtering was adequate to identify the bacterial microbiome fraction responsible for conferring salt-tolerance to plants).

Inoculation of Seeds; Transfer of Microbiomes to Plants of the Next Microbiome-Generation: During planting, the 200 pots of each microbiome-generation had been ordered numerically in the 4 racks used for stratification in the cold-room, so it was easy to locate in these racks a pot with a particular number that had been assigned to a specific selection-line and needed to be inoculated with a microbiome. To inoculate a seed planted in a particular pot, we moved the pot into our clean-hood, opened the lid inside the hood (using one hand to hold the pot while opening the lid with thumb and index finger of that same hand), then used a 5ml pipetter to transfer 4ml of the microbiome-filtrate the center soil in a pot. We spread the 4ml filtrate across an area with a radius of about 5mm around a seed, applying some of the filtrate directly onto the seed (the exact location of the seed was indicated by its awn protruding above the soil; see *Planting* above), and we spread some of the filtrate also in a circular motion onto the surrounding soil within 5mm distance of a seed. To keep the filtrate well-mixed during the time needed to inoculate all 8 'offspring soils' of the same selection-line, we repeatedly mixed the filtrate in the Falcon tube with the pipette-tip before aspirating a 4ml-aliquot to inoculate the next pot. We taped a small tag of labeling-tape to the lid of each pot that had received an inoculum (as a check to verify later that all pots had received an inoculate, no pot was accidentally skipped, or any pot was accidentally inoculated twice), then we returned the pot to its appropriate position in one of the four racks (all pots were later distributed among 8 racks used to support plants in the growth chamber; see below *Randomization of Pot-Positions in Racks*).

Each pot was capped for first 4 days to promote priority effects during microbiome establishment (i.e., capping prevented immigration of extrinsic microbes into the soils/microbiomes for the first 4 days; see above *Planting*), but all caps were removed on Day4 because the tallest plants (35-40mm tall on Day4) were close to reaching the cap-ceiling. We monitored growth during first 5 days (see above *Phenotyping*) by recording length of the first leaf on Days 2-5, and recording day of appearance of the second leaf (typically on Days 6 or 7; Table S1). Seeds that did not germinate or that germinated very late (i.e., no above-ground growth visible by Day 4) were extracted from pots with a forceps and inspected. Most of these seeds had failed to grow both a rootlet and shoot by Day4, but some seeds had grown a rootlet but no shoot. In a typical microbiome-generation but depending on microbiome-treatment, about 88-100% of the plants showed a visible shoot within the first 3 days (Table S1). Germination rates were therefore good overall, and most lines had the planned 8 replicates (sometimes 7 replicates, rarely 6 replicates, if some seeds failed to germinate; see Table S1). Germination-rates were often minimally higher in the Null-Control treatments compared to other treatments of the same soil-stress (slightly fewer non-germinating seeds in Null-Controls), and, across all plants, germination-rates were minimally higher in ALU-soil than in SOD-soil (see Tables S1a-c); we did not analyze these trends for statistical significance because differences are minimal, but we simply note here these general patterns emerging only when pooling information across all 10 Microbiome-Generations.

Randomization of Pot-Positions in Racks in Growth-Chamber: Deepots were supported in D50T racks (Stewe & Sons, Tangent, Oregon, USA). Each rack can hold a total of 50 pots (5 rows of 10 pots each), but to prevent contact of leaves from different plants and to reduce accidental between-pot transfer of microbes during watering (see below *Watering*), we used only 25 rack-positions (25 pots per rack, total of 8 racks, for a total number of 200 pots per selection-cycle). Pots within a selection line were first assigned by blocking to particular rack (e.g., of the 8 replicates within a selection-line, one each was assigned to each of the 8 racks). Within each rack, however, we randomly assigned pot positions, using the *Random Sequence Generator* option at Random.Org (www.random.org/sequences/). For Generations 0-8 (growth cycles 0-8), Tables S1a&b lists pot positions (#1-#25) from different treatments within each rack (Rack #1-8), corresponding to the following pot arrangement:

#1		#2		#3		#4		#5	
	#6		#7		#8		#9		#10
#11		#12		#13		#14		#15	
	#16		#17		#18		#19		#20
#21		#22		#23		#24		#25	

For the final Generation 9 when we added two more control-treatments (details below), we randomized 400 pot-positions by first assigning a pot to one of 8 racks, then randomizing position within each of the 8 racks (50 pots/rack; the position-numbering of pots shown for Generation 9 in Table S1c for each rack is numbered consecutively, starting in left top corner, without leaving empty spacer-slots between pots). The 8 racks were positioned in two groups of 4 racks each on two comparable shelves at either side of the growth chamber. Within each selection cycle, we rotated these 8 racks in clockwise rotation each day (moving one rack from right shelf to left shelf, and one rack from left to right shelf), and at the same time we also turned each rack (such that the rack-side facing the chamber wall one day faced the chamber center the next day). This rotation-turning scheme aimed to minimize any environmental influences dependent on location of a rack on the two shelves, and to reduce any minimal differences in light-level, air-circulation, or any such uncontrolled environmental factors that may exist between different positions on the two shelves.

Despite our effort to minimize rack effects through daily rack-rotation and rack-turning, as well as randomization of processing order (e.g., during watering, phenotyping, microbiome-harvesting), we had occasionally racks of poorer or better plant growth (for example, compared to other racks of Generation 9, Rack 7 of Generation 9 had unusually low average seed production, because many plants in that rack did not flower, or flowered late). We do not know the exact causes for these occasional small rack-effects, because we believe we treated plants across all racks equally (e.g., our watering-protocol required that we check all racks after watering, and we do not believe we missed watering any rack, but we cannot rule out this possibility with absolute certainty).

Starter Inoculum for Microbiomes at Beginning of the Experiment: We used a single inoculum-batch to inoculate all replicate pots of the initial baseline Generation 0. To prepare that inoculum, we filtered bacterial communities from a mix of roots and adhering soil taken from three principal sources: (a) root-systems with adhering soil of three local grass species (*Bromus* sp., *Andropogon* sp., *Eragrostis* sp.) collected into individual plastic bags on 3.Jan.2015 (about 90 minutes before microbiome harvesting) at restored native habitat at Brackenridge Field Lab of the University of Texas at Austin (www.bfl.utexas.edu/); (b) root-systems with adhering soil of 40 16-day-old *B.distachyon* Bd3-1 plants grown in PPC-soil Deepots as part of a pilot experiment quantifying the effect of salt-additions to soil on the growth-rate of *B. distachyon* (see below *Salt Treatments*); and (c) old root-systems with adhering soil of 15 Bd3-1 plants grown in PPC-soil Deepots, but that had been stored in the soil/Deepots in a cold-room (6°C) for 7 months after completion of a previous low-nutrient microbiome-selection experiment. We combined roots and rhizosphere soils from these three sources in order to capture a diversity of microbes into our starter inoculum, and we included Bd3-1 rhizospheres in order to capture specific microbial taxa that may be readily recruited by *B.distachyon* into its rhizosphere microbiomes. We suspended this mix of roots and rhizosphere soil in 200ml e-pure water, blended the mix for 30 seconds in an autoclaved Waring blender to generate a liquid slurry, allowed the solids to settle in the blender for 1 minute, then decanted the supernatant into a separate autoclaved beaker. Adding each time 200ml e-pure water, we repeated this blending/decanting with the remaining slurry three more times to collect a total of about 600ml supernatant. Using vacuum filtration, we pre-filtered this supernatant in a Buchner funnel through filter paper (Ahlstrom filter paper S02-007-42), eliminating larger particles suspended in the supernatant. To harvest only bacterial microbiome components (and viruses) from this pre-filtrate, we filtered the supernatant a second time in a laminar-flow hood, using a 60ml syringe fitted with a 2µm Whatman™ microfilter (Puradisc 25 GD2 Syringe Filter, 25mm diameter; Whatman PLC, United Kingdom) to generate the bacterial mix for inoculation of replicate

1019 pots of our initial baseline Generation 0. Because the Puradisc filters became clogged after filtration of
1020 about 70-100ml supernatant, we used 8 Puradisc filter to process about 600ml of filtrate. We reserved 500ml
1021 of this filtrate for inoculation of 160 randomly-assigned pots in a Bacterial-Inoculate treatment (80 Bacte-
1022 rial-Inoculate with SOD soil, 80 Bacterial-Inoculate with ALU soil), and filtered the remaining 100ml with
1023 0.2µm filters (VWR Sterile Syringe Filter, 0.2µm polyethersulfone membrane, 25mm diameter; Catalog
1024 #28145-501) for inoculation of 40 pots in Null-Control treatments (20 Null-Control with SOD soil, 20 Null-
1025 Control with ALU soil). The Null-Control treatments tested, after elimination of bacteria, the effect of any
1026 chemicals and viruses that may have been co-harvested from rhizosphere roots and soils. Seeds in the Bac-
1027 terial-Inoculum and the Null-Control treatments were inoculated following the procedure described above
1028 (see *Inoculation of Seeds*), except that each seed of Generation 0 received 2ml inoculate, whereas each seed
1029 of subsequent microbiome-generations received 4ml inoculate transferred between generations. During in-
1030 oculation of seeds, we mixed the stock filtrates regularly to prevent bacterial sedimentation and to insure
1031 standardized inoculation of all replicates in a treatment. We needed about 3 hours to complete the entire
1032 process from root collection to conclusion of all filtration steps, and another 2 hours to apply inoculate-
1033 aliquots of the filtrates to each of the assigned pots. We then moved all pots immediately into our growth
1034 chamber, and set out all 200 pots of Generation 0 into randomized positions in 8 racks (see above *Random-*
1035 *ization of Pot Positions*; Table S1).

1036 To test for live bacteria in our 2µm-filtrate used as the Starter Inoculum, we plated on PDA-medium (2
1037 replicate plates) 10 µL each of the 2µm filtrate and maintained plates at room temperature; the plates
1038 showed thousands of bacterial colony-forming-units (CFUs) within 24 hours, but no fungal growth within
1039 7 days. To test for absence of live bacteria in our 0.2µm-filtrate, we plated on PDA-medium (3 replicate
1040 plates) 50 µL each of the 0.2µm-filtrate; these platings did not yield any visible growth on the PDA plates
1041 kept for 7 days at room temperature. These results indicate a great abundance of live bacteria (and appar-
1042 ently no live fungi) in our initial inoculum, and justified addition of a third control-treatment in Generation
1043 9 (0.2µm filtration of suspension to test growth-promoting effects of chemicals and viruses co-propagated
1044 with the harvested bacterial microbiomes; see below).

1045 **Selection of Microbiomes from Generation 0 to Inoculate Plants from Generation 1:** At the start of
1046 our experiment, we did not assign microbiomes (i.e., pot numbers) from Generation 0 to specific selection
1047 lines, to permit selecting the best-growing plants from Generation 0 to contribute microbiomes to the se-
1048 lection-lines starting with Generation 1. We chose this particular assignment rule because random assign-
1049 ment to selection lines would result in some cases for a poorly-growing plant to contribute microbiomes to
1050 Generation 1, and we wanted to increase the chance of obtaining a response to microbiome-selection in the
1051 fewest rounds of selection. To select plants for harvesting and propagation of rhizosphere microbiomes, we
1052 ranked, separately for plants in the SOD and ALU treatments, the plants in the Bacterial-Inoculate treat-
1053 ments of Generation 0 by relative size, then picked the 10 best-growing plants of each salt-treatment to
1054 contribute microbiomes to the selection-lines that we started with Microbiome-Generation 1 (Table S1).
1055 On Day 22 of Generation 0 (day of microbiome-harvest and –transfer; Table S1), we first ranked plants by
1056 relative size-scores (i.e., average size-score from three scores received by a plant on Days 18, 19, 20; see
1057 protocol *Above-Ground Biomass Estimated on a 10-Point Scale*), then used number of leaves recorded on
1058 Day 21 as a second criterion to differentiate between plants of equal average size-score. Among the 10 best-
1059 growing plants within each of the SOD and ALU salt-treatments, we paired plants randomly to generate 5
1060 combinations (2 plants each) for mixing of harvested microbiomes within each pair (harvested root-systems
1061 were combined from the two plants to harvest a mixed microbiome from both plants, as described above
1062 for *Microbiome Mixing*). Within each of the SOD and ALU treatments, the 5 mixed microbiomes from
1063 Generation 0 were assigned randomly to 5 SOD and 5 ALU selection-lines (each with 8 'offspring-micro-
1064 biome' replicates per line) starting with Generation 1. Microbiomes were harvested and processed from
1065 chosen rhizospheres as described above. At the end of Generation 0, like at the end of each other Generation,
1066 we cut all plants at soil level to preserve above-ground growth for later weighing of dry biomass for each
1067 plant (Table S1; see also *Phenotyping*).

Salt- and Control-Treatments in Generation 0-9; Sample Sizes Per Treatment: Starting with Generation 1 and continuing until the last Generation 9, we included the two aforementioned salt-treatments (SOD and ALU soil) with *5 SOD Microbiome-Selection* Lines (8 replicates each, for a total of 40 replicates) and with 5 *ALU Microbiome-Selection* Lines (8 replicates each, for a total of 40 replicates). Also starting with Generation 1 and continuing until the last Generation 9, we included two control treatments for each of the SOD and ALU treatments, *Null-Control* (on SOD- and on ALU-soils) and *Fallow-Soil Microbiome Propagation* (on SOD- and on ALU-soils). ***Control 1, Fallow-Soil Microbiome Propagation***: For this control, we harvested microbiomes from fallow soil (from pots without a plant), then propagated the harvested microbiome to sterile fallow soil to perpetuate 'Fallow-Soil Microbiomes' in the absence of plant influences (e.g., absence of plant secretions into the soil). Fallow-soil pots were treated throughout each selection-cycle exactly like pots with plants; for example, these fallow-soil pots received the same amount of water whenever all other pots were watered. Each Fallow-Soil-Line had only one replicate pot, so a microbiome harvested from fallow-soil was propagated to a single pot of the next selection-cycle to continue a particular selection-line; the same harvested microbiome was also transferred to pots with plants of the next cycle to assay the effect of a harvested fallow-soil-microbiome on plant growth (but these microbiomes were not propagated to subsequent Generations). We chose a control of fallow-soil microbiome-propagation because this treatment resembles the kind of microbiome conditions that many plants encounter in horticulture and agriculture (soils are left fallow for some time before planting). Changes in fallow-soil microbiomes between Generations reflect ecological changes over time, as well as any microbial immigration from external sources (e.g., airborne microbes raining into the soil; perhaps also accidental cross-contamination between soils from different pots). We initially allocated 8 test-replicate plants/line to test the effect of each harvested fallow-soil microbiomes on plant growth (total of 5x8=40 replicates for SOD, 5x8=40 replicates for ALU), but we reduced the number of these test-replicates/line in later Generations (first to 6 test-replicates/line in Generation 4; then 4 test-replicates/line in Generation 5-9), because it became clear during the first few Generations that plants receiving fallow-soil-microbiomes grew poorly, far inferior to plants in the corresponding selection-lines (i.e., we could differentiate averages between fallow-soil and microbiome-selection lines even with the smaller number of test-replicates in the fallow-soil controls). **Control 2, *Null-Control***: For this control, plants received no experimental microbial inoculation; instead, plants receive an aliquot of the same sterile salt-nutrient buffer that we used to harvest microbiomes. Null-Control-plants grow initially under sterile conditions (before caps are lifted on Day4), but airborne microbes can "rain in" from the air after Day4. In pilot experiments, Null-Control-plants invariably grew better during the first 10-20 days than any plant inoculated with microbiomes (see Table S1a-c), possibly because Null-Control-plants do not need to expend resources to mediate interactions with microbes, or because Null-Control-plants do not have to compete with microbes for access to nutrients. Despite the microbially unusual soils of Null-Control-plants, we included this control treatment because it was easy to set up replicates (no microbiomes needed to be harvested to inoculate Null-control soils), because Null-Control-conditions were easy to standardize within Generations, and because null-control-conditions may even be reasonably standardized between Generations if microbial immigration (i.e., rain of airborne microbes) into Null-Control-soils can be assumed to be reasonably constant over time. We initially allocated 10 replicates of SOD pots and 10 replicates of ALU pots to Null-Controls, but we increased the number of replicates in later Generations for the Null-Control treatments (first we increased to 20 replicates in Generation 4, then to 30 replicates in subsequent Generations) in order to improve the estimates (reduce confidence intervals) of the average growth of plants in Null-Control treatments. Sample sizes for all treatments are summarized for all Generations in Table S1a-c.

Watering During Each Selection Cycle: We watered pots such that the total weight (pot plus hydrated soil) stayed between 200-250g and did not exceed 260g, because a pot would be over-hydrated if the total weight reached 260-270g or more, which would result in dripping of excess water from the bottom of the pot. Keeping pot weights well below 260g therefore prevented leaching of nutrients and salt. To prevent cross-contamination (microbe-exchange) between pots, we did not use the time-efficient technique of bottom-hydration by immersing racks in a waterbath, but we watered pots individually, only from above, and

always with autoclaved water that we dispensed with a Seripetter Dispenser (adjustable to dispense volumes of 2.5-25ml; BrandTech Scientific Inc; Essex, CT, USA) mounted on a 6-liter carboy. Because we kept pots capped during the first 4 days (we removed caps during the afternoon of Day4), soils remained well-hydrated during germination (little water evaporated from soil, humidity inside the cap was near 100%). We watered for the first time on Day5 of each selection-cycle; and thereafter approximately every 2 days (sometimes also at 1-day or 3-day interval, depending on humidity in the growth chamber and on time-constraints), but we did not plan to follow a rigorous 2-day watering schedule (see Table S3). We typically watered 15-25 ml depending on water loss, which depended on humidity in the growth chamber and on the size of plants (humidity was greatest during Generations 4&5 because of unusually high rainfall in spring 2015). To determine the volume to be watered on a given day, we selected six pots haphazardly from the 4 racks in the two front rows, and weighed these on a scale (sterilizing the surface of the scale with 100% ethanol before placing a pot onto the scale). The difference between the average weight of these six pots and 255mg was the maximum quantity of water to be added to each pot. The amount to be watered could be varied to the nearest 0.5ml with the carboy-mounted Seripetter Dispenser. To prepare carboys, we filled each with 6 liter of e-pure water, and autoclaved the water to ensure sterile watering. Immediately before watering, we quickly opened a carboy to add a specific volume of 1-Molar salt solution to generate a desired salt-concentration in the water (details in Table S3), mixed the contents by vigorous shaking in the carboy, mounted the ethanol-sterilized dispenser, flushed the dispenser five times to eliminate any ethanol in the dispenser, then began the watering. During the days when the Seripetter Dispenser was not used, we mounted it on a 1-Liter bottle with 100% ethanol, and kept the entire dispenser filled with ethanol to prevent growth of microbial biofilms inside the dispenser. We used different carboys dedicated to watering of SOD-salt and ALU-salt, to minimize cross-contamination of salts between treatments. In each round of watering, we first watered all pots of the SOD-treatment, then rinsed the dispenser with 100% ethanol, then watering all pots of the ALU-treatment. To minimize the chance of accidentally adding the wrong salt-water to a pot (e.g., accidentally watering ALU-soil with SOD-water, or vice versa), we labeled pots of the different salt treatments with different colors (white-label for pots ##001-100 to indicate SOD-treatment, and green-label for pots ##101-200 to indicate ALU-treatment). The exact watering schedules, volumes of water added, and respective salt-concentrations are summarized in Table S3:

Table S3. Ramping Salt-Stress Between and Within Microbiome-Generations. Soil in each pot was hydrated with 102ml salt-solution [94ml added to soil prior to autoclaving; 4ml during planting; and 4ml during microbiome inoculation in Generations 1-9 (2ml in Generation 0); see *Planting* and *Inoculation of Seeds*]. In the baseline Generation 0, plants were watered only with unsalted water, but starting with Generation 1, we increased salt-stress *within* each Generation by watering with salted water. Because we capped pots for the first 4 days to control initial microbiome-assembly, we started watering each Generation on Days 5 or 6. Pots of Generations 0-3 were watered more because of low humidity (because of heating of Greenhouse Facility in winter) and pots of Generations 4&5 were watered less because of high humidity (unusual rainfall in spring, increasing general humidity; see *Growth Chamber*). Pots were watered more in the second half of Generation 9 because plants grew large and transpired more water. Plants of Generations 0-8 were short-cycled to grow only for 20-30 days before microbiome-transfer (to about 10-15 leaves for the largest plants); plants in Generation 9 were grown for 68 days to permit ripening of seeds.
SOD = sodium-sulfate; ALU = aluminum-sulfate.

	Microbiome-Generation (Selection-Cycle)																			
	Gen 0		**Gen 1**		**Gen 2**		**Gen 3**		**Gen 4**		**Gen 5**		**Gen 6**		**Gen 7**		**Gen 8**		**Gen 9**	
Initial Soil Hydration (see also Table S2)	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**	**SOD**	**ALU**
	100 ml 20 mM	100 ml 0.02 mM	102 ml 30 mM	102 ml 0.04 mM	102 ml 50 mM	102 ml 0.08 mM	102 ml 60 mM	102 ml 0.2 mM	102 ml 70 mM	102 ml 1.0 mM	102 ml 75 mM	102 ml 3.0 mM	102 ml 60 mM	102 ml 1.0 mM	102 ml 60 mM	102 ml 1.0 mM	102 ml 60 mM	102 ml 1.5 mM	102 ml 60 mM	102 ml 1.5 mM
Watering Schedule																				
Day 5	15ml 0 mM	15ml 0 mM	15ml 0 mM	15ml 0 mM	15ml 15 mM	15ml 0.16 mM	15ml 15 mM	15ml 0.20 mM	10ml 15 mM	10ml 3.0 mM	-	-	-	-	15ml 15 mM	15ml 2.0 mM	20ml 15 mM	20ml 2.0 mM	20ml 15 mM	20ml 2.0 mM
Day 6	23ml 0 mM	23ml 0 mM	20ml 0 mM	20ml 0 mM	25ml 15 mM	25ml 0.16 mM	-	-	-	-	5ml 15 mM	5ml 5.0 mM	15ml 15 mM	15ml 4.0 mM	-	-	-	-	-	-
Day 7	-	-	-	-	-	-	25ml 15 mM	25ml 0.60 mM	25ml 15 mM	25ml 3.0 mM	-	-	-	-	15ml 15 mM	15ml 2.0 mM	25ml 15 mM	25ml 2.0 mM	-	-
Day 8	17ml 0 mM	17ml 0 mM	20ml 15 mM	20ml 0.02 mM	25ml 15 mM	25ml 0.16 mM	-	-	-	-	-	-	-	-	-	-	-	-	20ml 15 mM	20ml 2.0 mM
Day 9	-	-	-	-	-	-	25ml 15 mM	25ml 0.90 mM	-	-	10ml 15 mM	10ml 10.0 mM	25ml 15 mM	25ml 4.0 mM	15ml 15 mM	15ml 2.0 mM	25ml 15 mM	25ml 2.0 mM	-	-

Day 10	25ml 0 mM	25ml 0 mM	20ml 0 mM	20ml 0 mM	25ml 15 mM	25ml 0.24 mM	-	-	-	-	-	-	-	-	-	-	-	-	15ml 15 mM	15ml 2.0 mM
Day 11	-	-	-	-	-	-	25ml 15 mM	25ml 0.90 mM	25ml 15 mM	25ml 5.0 mM	-	-	15ml 15 mM	15ml 4.0 mM	-	-	15ml 15 mM	15ml 2.0 mM	-	-
Day 12	25ml 0 mM	25ml 0 mM	25ml 15mM	25ml 0.02 mM	25ml 15 mM	25ml 0.32 mM	-	-	-	-	-	-	-	-	15ml 15 mM	15ml 2.0 mM	-	-	-	-
Day 13	-	-	-	-	25ml 15 mM	25ml 0.32 mM	25ml 15 mM	25ml 1.20 mM	-	-	20ml 15 mM	20ml 15.0 mM	25ml 15 mM	25ml 5.0 mM	-	-	15ml 15 mM	15ml 2.0 mM	20ml 15 mM	20ml 2.0 mM
Day 14	25ml 0 mM	25ml 0 mM	25ml 15mM	25ml 0.04 mM	-	-	-	-	-	-	-	-	-	-	20ml 15 mM	20ml 2.0 mM	-	-	15ml 15 mM	15ml 2.0 mM
Day 15	-	-	-	-	25ml 15 mM	25ml 0.40 mM	25ml 15 mM	25ml 1.50 mM	10ml 15 mM	10ml 5.0 mM	-	-	-	-	-	-	15ml 15 mM	15ml 2.0 mM	-	-
Day 16	25ml 0 mM	25ml 0 mM	25ml 15mM	25ml 0.08 mM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Day 17	-	-	-	-	25ml 15 mM	25ml 0.60 mM	-	-	-	-	20ml 15 mM	20ml 15.0 mM	15ml 15 mM	15ml 5.0 mM	20ml 15 mM	20ml 2.0 mM	15ml 15 mM	15ml 2.0 mM	18ml 15 mM	18ml 2.0 mM
Day 18	25ml 0 mM	25ml 0 mM	25ml 15 mM	25ml 0.08 mM	-	-	25ml 15 mM	25ml 1.80 mM	15ml 15 mM	15ml 5.0 mM	-	-	-	-	-	-	-	-	-	-
Day 19	-	-	-	-	25ml 15 mM	25ml 0.90 mM	-	-	-	-	-	-	-	-	20ml 15 mM	20ml 2.0 mM	-	-	-	-
Day 20	25ml 0 mM	25ml 0 mM	**Transfer to Gen2**	**Transfer to Gen2**	-	-	25ml 15 mM	25ml 1.80 mM	-	-	-	-	25ml 15 mM	25ml 3.0 mM	-	-	20ml 15 mM	20ml 2.0 mM	18ml 15 mM	18ml 2.0 mM
Day 21	-	-			**Transfer to Gen3**	**Transfer to Gen3**	-	-	20ml 15 mM	20ml 5.0 mM	25ml 15 mM	25ml 15.0 mM	-	-	15ml 15 mM	15ml 2.0 mM	-	-	-	-
Day 22	**Transfer to Gen1**	**Transfer to Gen1**					10ml 15 mM	10ml 1.80 mM	-	-	-	-	-	-	-	-	-	-	-	-
Day 23							**Transfer to Gen4**	**Transfer to Gen4**	-	-	-	-	-	-	20ml 15 mM	20ml 2.0 mM	25ml 15 mM	25ml 2.0 mM	20ml 15 mM	20ml 2.0 mM
Day 24									10ml 15 mM	10ml 5.0 mM	-	-	20ml 15 mM	20ml 3.0 mM	-	-	-	-	-	-
Day 25									**Transfer to Gen5**	**Transfer to Gen5**	20ml 15 mM	20ml 15.0 mM	-	-	20ml 15 mM	20ml 2.0 mM	-	-	-	-
Day 26											-	-	**Transfer to Gen7**	**Transfer to Gen7**	-	-	25ml 15 mM	25ml 2.0 mM	18ml 15 mM	18ml 2.0 mM
Day 27											-	-			20ml 15 mM	20ml 2.0 mM	-	-	-	-
Day 28											-	-			**Transfer to Gen8**	**Transfer to Gen8**	20ml 15 mM	20ml 2.0 mM	20ml 15 mM	20ml 2.0 mM
Day 29											20ml 15 mM	20ml 15.0 mM					-	-	-	-
Day 30											-	-					10ml 15 mM	10ml 2.0 mM	-	-
Day 31											10ml 15 mM	10ml 15.0 mM					**Transfer to Gen9**	**Transfer to Gen9**	20ml 15 mM	20ml 2.0 mM
Day 32											**Transfer to Gen6**	**Transfer to Gen6**							20ml 15 mM	20ml 2.0 mM
Day 33																			-	-
Day 34																			20ml 15 mM	20ml 2.0 mM
Day 35																			-	-
Day 36																			20ml 15 mM	20ml 2.0 mM
Day 37																			-	-
Day 38																			20ml 15 mM	20ml 2.0 mM
Day 39																			20ml 15 mM	20ml 2.0 mM
Day 40																			-	-
Day 41																			20ml 15 mM	20ml 2.0 mM
Day 42																			-	-
Day 43																			20ml 15 mM	20ml 2.0 mM
Day 44																			-	-
Day 45																			-	-
Day 46																			20ml 15 mM	20ml 2.0 mM
Day 47																			20ml 15 mM	20ml 2.0 mM
Day 48																			20ml 15 mM	20ml 2.0 mM
Day 49																			-	-
Day 50																			20ml 15 mM	20ml 2.0 mM
Day 51																			-	-
Day 52																			20ml 15 mM	20ml 2.0 mM
Day 53																			-	-
Day 54																			-	-
Day 55																			20ml 15 mM	20ml 2.0 mM
Day 56																			20ml 15 mM	20ml 2.0 mM
Day 57																			20ml 15 mM	20ml 2.0 mM
Day 58																			20ml 15 mM	20ml 2.0 mM
Day 59																			20ml 15 mM	20ml 2.0 mM
Day 60																			-	-
Day 61																			20ml 15 mM	20ml 2.0 mM
Day 62																			-	-
Day 63																			20ml 15 mM	20ml 2.0 mM
Day 64																			-	-
Day 65																			20ml 15 mM	20ml 2.0 mM
Day 66																			-	-
Day 67																			20ml 15 mM	20ml 2.0 mM
Day 68																			15ml 15 mM	15ml 2.0 mM
Day 69																			15ml 15 mM	15ml 2.0 mM
Day 70																			**End**	**End**

Flowering: Because we short-cycled plants in Generations 0-8 and harvested microbiomes when plants were relatively young (20-30 days old; the largest plants had typically 10-15 leaves), only few plants bolted

and developed flowers during the short-cycled Generations 0-8; all these cases of flowering were in Generations 1 & 8 (Table S1a&b), whereas no plants flowered in Generation 0 and in Generations 2-7. The long light-phase (20h light, 4h dark) stimulated flowering uniformly in each Generation, but our short-cycling scheme aimed to harvest microbiomes before plants began to flower. Because of scheduling-constraints, Generation 8 was grown for slightly longer (31 days) than earlier Generations, which could explain the flowering in some of these plants, but it is unclear why some plants began to flower in the far shorter Generation 1 (20 days). Plants in Generation 9 were grown for 68 days to permit seeds to ripen, and most of these plants flowered (Table S1c). The fact that not all plants flowered in Generation 9, and the observation that onset of flowering was delayed in the control-treatments (Table S1c), indicate that plants were indeed stressed by the salts (in salt-free soils, virtually all plants would have flowered).

Analysis of Relative Plant Performance: Because successive generations were not grown under precisely identical conditions (e.g., we had to adjust watering schedules because of uncontrolled humidity in our growth chamber; we had to increase the duration of selection-cycles in later generations because plant-growth decelerated slightly under the increasing salt-stress), we plot in Figure 1 plant-performance as relative above-ground biomass (rather than absolute biomass), relativizing the observed dry-biomass of a specific plant by average biomass across all plants in that plant's salt treatment (i.e., biomass of a SOD-plant is relativized with respect to average biomass across all SOD-plants; likewise for ALU plants). The overall average across all plants within a salt-treatment in a given Generation is therefore 1, and plants (and selection-lines) performing poorer than the average have scores <1, whereas plants or lines performing above the average have scores >1 (see Figure 1). To calculate relative plant performance, we used the dry-biomass grown by a plant by the last day of its selection-cycle, as summarized in Figure 1 and in Tables S1a-c. Records of absolute above-ground dry-biomass for all plants of all Generations are also given in Tables S1a-c. In Generation 9, we allowed plants to grow for 68 days to permit ripening of seeds; plant performance and metagenomic information of plants in Generation 9 are therefore not quite comparable to previous Generations, but treatments within Generation 9 can be compared.

Crossing Evolved SOD- and ALU-Microbiomes with SOD- and ALU-Stress in Generation 9: At the end of the experiment in Microbiome-Generation 9, we modified protocols in three important ways: (a) we grew plants for 68 days to permit flowering and ripening of seeds, because seed production seemed a more informative estimator of plant fitness than the proxy of above-ground biomass used in earlier Generations; (b) we doubled the total number of pots to 400 (i.e., 400 plants) to permit addition of two control treatments (in addition to Fallow-Soil and Null-Control treatments already used in earlier Generations); and (c) we added these two control treatments to understand the mechanistic basis of the salt-tolerance-conferring effects of microbiomes in the SOD and ALU selection-lines. The first additional control was *Solutes*, where we filtered out all live cells from the harvested microbiomes in the selection lines (using a 0.2µm filter; see above *Microbiome-Fractionation with Microfilters*), to test the growth-enhancing effect of plant-secreted solutes and viruses that may be co-harvested and co-transferred with microbiomes in the selection-lines. The second control was 2x2 Cross-Fostering where we crossed harvested microbiomes from the SOD and ALU selection-lines with the two types of salt-stress in soil (i.e., microbiomes harvested from SOD-selection-lines were tested in both SOD-soil and in ALU-soil; microbiomes harvested from ALU-selection-lines were tested in both SOD-soil and in ALU-soil) to test specificity of the salt-tolerance-conferring effects of the microbiomes (do the salt-tolerance-conferring effects of the *SOD-selected* microbiomes confer these effects only in SOD-soil, or also in ALU-soil? and vice versa, do the salt-tolerance-conferring effects of the *ALU-selected* microbiomes confer these effects only in SOD-soil, or also in ALU-soil?). This basic cross-fostering design was inspired by the experimental methods developed by Lau & Lennon (2012), except that, in contrast to Lau & Lennon (2012), our plant-populations did not evolve, and that we artificially selected on microbiomes (whereas in Lau & Lennon plant populations evolved under artificial selection and microbiomes were allowed to change ecologically, without artificial microbiome-selection, in parallel to the artificially-selected evolutionary changes in the plant populations).

Phenotyping of Plants and Microbiome-Harvesting in the Last Generation 9: In contrast to Generations 0-8 when we used early growth (above-ground biomass during first 3-4 weeks) of non-flowering plants as host-phenotype to select indirectly on microbiomes, in the last Generation 9, we allowed plants to mature for 68 days (10 weeks), such that plants could flower and seeds could ripen. Because of the longer growth, some plants started to senesce towards the end of Generation 9 and some individual flower-stalks of a plant started to dry (no plant had dried completely by the end of Generation 9); comparisons of metagenomic information from Generation 9 versus earlier Generations therefore need to be interpreted with caution (i.e., metagenomic information from Generation 9 is best compared between treatments within that Generation). Despite these limits of metagenomic comparisons pertaining to Generation 9, we decided to grow plants to seed in this last Generation because we were interested in understanding how treatment differences in above-ground biomass apparent at Days 20-30 (when we harvested microbiomes in Generations 0-8) would translate into treatment differences in flowering and seed-set if plants were allowed to grow longer. Apart from the longer duration of Generation 9, a second difference is likely also the gradually increasing salt-concentration in soils of Generation 9 that were watered with weakly salted water for 68 days (see *Watering*), in contrast to watering with weakly salted water for only 20-30 days in earlier Generations. At the end of Generation 9, all plants were cut at soil level, above-ground biomass was preserved for each plant in individual envelopes (for drying and later weighing of seeds and overall biomass; see above), and each root systems was extracted from its pot and placed into an autoclaved aluminum-tub for further processing. Harvested root-systems of plants from Generations 0-8 were comparatively small (filling about 30-60% of the soil-volume in each pot), but root-systems at the end of Generation 9 were large and permeated the entire soil-volume in each pot. We shook-off most of the adhering soil from each root system of Generation 9, cut off and discarded the top-most 2cm portion with sterile scissors, then cut the remaining root-system lengthwise (top to bottom) to preserve half of the root-system in 100% ethanol (for metagenomic iTAG screens), whereas we flash-froze (in liquid nitrogen) the other half of the root-system for possible later transcriptomics analyses. For some of the best-growing plants in the SOD- and ALU-selection-lines, we also preserved a representative portion of the root-system in sterile 20% glycerol (for storage at -80°C for possible later isolation of microbes). Processing all root-systems (nearly 400 plants) took considerable time over three successive days (Days 68-70 of Generation 9). Although we processed plants from 3 racks on Day 68 (Racks #3, #8, #7), from 3 racks on Day 69 (Racks #6, #2, #4), and from 2 racks on Day 70 (Racks #1, #5), we summarize all weight-data of these plants in Table S1c in columns labeled Day 68.

Flowering Phenology and Harvest Index in Generation 9: Methods and results will be added in a forthcoming version of this manuscript.

Statistical Analyses: We performed all analyses in R v3.3.1. Differences in plant biomass among treatments in Generations 1-8 of the selection experiment were assessed by fitting the data to a generalized linear mixed model with a gamma error distribution. Line was entered as a random effect; generation, treatment, and their interaction were entered as fixed effects. In Generation 9, the effect of selection history on plants grown in different salt environments was determined by fitting the data to a linear mixed model with a Gaussian error distribution. Line was entered as a random effect; selection history (sodium- or aluminum-sulfate), salt exposure in Generation 9 (sodium- or aluminum-sulfate), and their interaction were entered as fixed effects. We also compared total seed weight per plant among microbiome-selection treatment (plant present), Null-Control, and Fallow-Soil Control (no plant present) in Generation 9. Kruskal-Wallis tests were used because the data did not meet the assumptions of the linear model.

Statistical significance of fixed effects in the GLMMs were assessed with likelihood ratio tests and Tukey tests employed for post-host comparisons of treatment means. Mann-Whitney U-tests were used for non-parametric post-hoc comparisons between treatment means, correcting p-values using the false discovery rate. Selection of the appropriate error distribution for the GLMMs was evaluated by visual inspection of Q-Q plots, and homoscedasticity was assessed using plots of the residuals of the model against the fitted values. All hypothesis tests were two-tailed with alpha=0.05.

MOLECULAR METHODS

DNA extraction: Methods will be added in a forthcoming version of this manuscript.

PCR amplification: The V4-V5 region (approx. 412bp) of the 16S rRNA gene was amplified for all samples using the primer pair 515F-Y/926R (Parada *et al* 2015). Amplification was performed according to the Joint Genome Institute's iTag Sample Amplification QC Protocol: (http://1ofdmq2n8tc36m6i46scovo2e.wpengine.netdna-cdn.com/wp-content/uploads/2016/04/iTag-Sample-Amplification-QC-v1.3.pdf).

16S iTag Screening: Methods and results will be added in a forthcoming version of this manuscript.

RESULTS

Effects of differential microbiome propagation under sodium-sulfate (SOD) stress: We found a significant main effect of treatment on plant biomass over 8 generations of microbiome selection under sodium-sulfate stress (LRT: Treatment, Chisq=27.8, p<0.001, Generation, Chisq=381.8, p<0.001; Treatment x Generation, Chisq=15.2, p=0.37; Figure 2 left). Plant biomass was 75% higher in the plant-present microbiome selection lines (beta=0.57 ± 0.06, z=10.0, p<0.001) than in the fallow-soil control lines, and 66% higher than in the null-control line (beta=0.50 ± 0.07, z=7.4, p<0.001). There was no significant difference in biomass between plant-present microbiome selection and the null-control treatment (beta=0.07 ± 0.06, z=1.1, p=0.29). The lack of a significant interaction between treatment and generation indicates that gains in plant biomass were realized quickly after the experiment was initiated, and that the advantage of the plant-present and null-control treatments over the fallow-soil treatment were maintained as the concentration of sodium-sulfate was ramped up over the course of the experiment.

Generation 9, SOD-treatments: We measured total seed weight in the final Generation 9 of the experiment and found significant difference among treatments (Kruskal-Wallis Chisq=10.6, p=0.01; Figure 2 right). Total seed weight in the plant-present microbiome selection lines were 168% greater compared to the null-control line, 120% greater than the fallow-soil-control lines, and 205% greater than plants grown in soil that was inoculated with filtrate (0.2µm filter) from the soil of plant-present microbiome selection lines (Figure 2 right; Table S3).

Table S3. Mann-Whitney pairwise comparisons of total seed weight in the sodium-sulfate (SOD) treatments. Values represent the test statistics (p-value) for each treatment comparison. Significant comparisons are indicated in bold. Np = Fallow soil microbiome propagation control, Null=Null control line, Pp=Plant-present selection line, PpFilt=Plant-present selection line filtrate.

	Np	Null	Pp
Null	100 (0.50)		
Pp	**0 (0.02)**	**20 (0.02)**	
PpFilt	20 (0.50)	90 (0.71)	**20 (0.02)**

Effects of microbiome propagation under aluminum-sulfate (ALU) stress: Unlike the sodium-sulfate experiment, we found a significant interaction between treatment and generation under aluminum-sulfate stress (LRT: Treatment, X1=25.7, p<0.001; Generation, Chisq=753.7, p<0.001, Treatment x Generation, X1=26.6, p=0.02). The interaction was due to a drop in plant biomass in the fallow-soil treatment in Generations 4 and 5 (Figure 2). To calculate a conservative estimate of the effect size of our treatments on plant biomass, we re-ran the analysis excluding generations four and five, which eliminated the significant interaction between treatment and generation (LRT: Treatment, Chisq=17.8, p<0.001; Generation, X1=614.5,

p<0.001, Treatment x Generation, Chisq=7.67, p=0.66). In the reduced dataset, we found that plant biomass in plant-present microbiome selection lines were 38% larger than fallow-soil lines (beta=0.32 ± 0.04, z=8.9, p<0.001) but not significantly different from the null-control line (beta=0.09 ± 0.4, z=2.3, p=0.06). Null-control line plants were 26% larger than fallow-soil plants (beta=0.23 ± 0.04, z=5.1, p<0.001).

Generation 9, ALU-treatments: As in the sodium sulfate experiment, total seed weight in the final Generation 9 was significantly different among treatments (Kruskal-Wallis: Chisq=9, p=0.02; Figure 2 right). Total seeds weight in the plant-present microbiome selection lines were 194% greater than in the fallow-soil lines, 101% greater than the null-control line, and 55.4% greater than in the filtrate lines (Table S4). Plants with filtrate-inoculated soil produced total seed weights that were 89.2% greater than plants grown in the fallow-soil control (Figure 2 right; Table S4).

Table S4. Mann-Whitney pairwise comparisons of total seed weight in the aluminum-sulfate (ALU) treatments. Values represent the test statistics (p-value) for each treatment comparison. Significant comparisons are indicated in bold. Np = Fallow soil microbiome propagation control, Null=Null control line, Pp=Plant-present selection line, PpFilt=Plant-present selection line filtrate.

	Np	Null	Pp
Null	80 (0.55)		
Pp	**0 (0.02)**	**30 (0.03)**	
PpFilt	**0 (0.02)**	70 (0.29)	**20 (0.05)**

Interactions between selection history and salt stress on plant fitness: We examined whether indirect host-mediated selection produced microbiomes that were adapted to specific types of salt stress or produced a generalized improvement in plant fitness across among different types of salt stress by growing plants with microbiomes from selection lines under both sodium- and aluminum-sulfate stress. There was a significant interaction between selection history and the type of salt stress to which plants were exposed in the last generation on seed mass (Analysis of deviance: Selection history, $F_{1,8}$=<0.01, p=0.99; Salt exposure, $F_{1,141}$=5.82, p=0.017; Selection history x Salt exposure, $F_{1,141}$=6.42, p=0.012; Figure 2 right), indicating that performance in sodium-sulfate or aluminum-sulfate depended upon which salt the microbiome was selected on during the course of the experiment.

We conducted post-hoc comparisons of the treatment means and found that plants grown with microbiomes selected in sodium sulfate had total seed weights that were 70.1% greater when exposed to sodium-sulfate stress compared to exposure of aluminum-sulfate stress in Generation 9 (beta=108 ± 31.0, z=3.5, p=0.002). In contrast, plants grown in microbiomes selected in aluminum-sulfate did not differ in similar total seed weight, regardless of whether they were exposed to sodium- or aluminum-sulfate in Generation 9 (beta=4.2 ± 31.8, z=0.13, p=0.99). The effect of exposure to different kinds of salt stress on plant fitness thus depends upon the selection history of the soil microbiome.

Unlike total seed weight, there was no interaction between selection history and the type of salt stress on total plant biomass, however there was a trend toward plants growing larger in aluminum-sulfate compared to sodium-sulfate irrespective of the selection history (Analysis of deviance: Selection history, F=0.14, p=0.72; Salt exposure, F=3.71, p=0.056; Selection history x Salt exposure, F=1.38, p=0.24).

References

Aggarwal A, Ezaki B, Munjal A, Tripathi BN. 2015. Physiology and biochemistry of aluminum toxicity and tolerance in crops. Pp 35-57 in *Stress Responses in Plants* (Tripathi BN & Müller M, eds), Springer International Publishing.

1341 Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. 2006. The role of root exudates in rhizosphere inter-
1342 actions with plants and other organisms. *Annu Rev Plant Biol* 57: 233-66.
1343 DOI: 10.1146/annurev.arplant.57.032905.105159

1344 Bakken LR, Olsen RA. 1987. The relationship between cell size and viability of soil bacteria. *Microb Ecol*
1345 13: 103-114. DOI: 10.1007/BF02011247

1346 Brkljacic J, Grotewold E, Scholl R, Mockler T, Garvin DF, Vain P, Brutnell T, Sibout R, Bevan M, Budak
1347 H, Caicedo A, Gao C, Gu Y, Hazen S, Holt BF, Hong S, Jordan M, Manzaneda AJ, Mitchell-Olds T,
1348 Mochida K, Mur LAJ, Park C-M, Sedbrook J, Watt M, Zheng S, Vogel JP. 2011. *Brachypodium* as a
1349 model for the grasses: today and the future. *Plant Physiol* 157: 3–13. DOI: 10.1104/pp.111.179531

1350 Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. 2013. Structure and functions
1351 of the bacterial microbiota of plants. *Annu Rev Plant Biol* 64: 807-838. DOI: 10.1146/annurev-arplant-
1352 050312-120106

1353 Coyte KZ, Schluter J, Foster KR. 2015. The ecology of the microbiome: Networks, competition, and sta-
1354 bility. *Science* 350: 663-666. DOI: 10.1126/science.aad2602

1355 Delhaize E, Ryan PR. 1995. Aluminum toxicity and tolerance in plants. *Plant Physiology* 107: 315-321.

1356 Des Marais DL, Juenger TE. 2011. *Brachypodium* and the abiotic environment. Pages 291-311 in *Genet-*
1357 *ics and Genomics of* Brachypodium (Vogel JP, ed). Springer International Publishing.
1358 DOI: 10.1007/7397_2015_13

1359 Des Marais DL, Razzaque S, Hernandez KM, Garvin DF, Juenger TE. 2016. Quantitative trait loci asso-
1360 ciated with natural diversity in water-use efficiency and response to soil drying in *Brachypodium dis-*
1361 *tachyon*. *Plant Science* 251: 2-11. DOI: 10.1016/j.plantsci.2016.03.010

1362 Dodd IC1, Pérez-Alfocea F. 2012. Microbial amelioration of crop salinity stress. *J Exp Bot* 63: 3415-3428.
1363 DOI: 10.1093/jxb/ers033.

1364 Fierer N, Ferrenberg S, Flores GE, González A, Kueneman J, Legg T, Lynch RC, McDonald D, Mihaljevic
1365 JR, O'Neill SP, Rhodes ME, Song SJ, Walters WA. 2012. From animalcules to an ecosystem: Applica-
1366 tion of ecological concepts to the human microbiome. *Annu Rev Ecol Evol Syst* 43: 137-155. DOI:
1367 10.1146/annurev-ecolsys-110411–160307

1368 Garland T, Rose MR. 2009. *Experimental Evolution*. University of California Press.

1369 Garvin DF, Gu YQ, Hasterok R, Hazen SP, Jenkins G, Mockler TC, Mur LAJ, Vogel JP. 2008. Develop-
1370 ment of genetic and genomic research resources for Brachypodium distachyon, a new model system for
1371 grass crop research. *Crop Science* 48: S69–S84. DOI: 10.2135/cropsci2007.06.0332tpg

1372 ISO/FDIS 10390 (2005) Soil quality – Determination of pH. International Organization for Standardization,
1373 2005. www.iso.org/iso/catalogue_detail.htm?csnumber=40879
1374 www.ecn.nl/docs/society/horizontal/pH_standard_for_validation.pdf

1375 Lau JA, Lennon JT. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environ-
1376 ments. *Proc Natl Acad Sci USA* 109: 14058–14062. DOI: 10.1073/pnas.1202319109

1377 Lodeyro AF, Carrillo N. 2015. Salt stress in higher plants: mechanisms of toxicity and defensive responses.
1378 Pp 1-33 in *Stress Responses in Plants* (Tripathi BN & Müller M, eds), Springer International Publishing.

1379 Luef B, Frischkorn KR, Wrighton KC, Holman HY, Birarda G, Thomas BC, Singh A, Williams KH,
1380 Siegerist CE, Tringe SG, Downing KH, Comolli LR, Banfield JF. 2015. Diverse uncultivated ultra-small
1381 bacterial cells in groundwater. *Nat Commun* 6: 6372. DOI: 10.1038/ncomms7372

1382 Mueller, U.G. Gerardo NM, Aanen DK, Six DL, Schultz TR. 2005. The evolution of agriculture in insects.
1383 *Annu Rev Ecol Evol Syst* 36: 563–595. DOI: 10.1146/annurev.ecolsys.36.102003.152626

1384 Mueller UG, Sachs JL. 2015. Engineering microbiomes to improve plant and animal health. *Trends Micro-*
1385 *biol* 23: 606–617. DOI: 10.1016/j.tim.2015.07.009

1386 Panke-Buisse K, Poole AC, Goodrich JK, Ley RE, Kao-Kniffin J. 2015. Selection on soil microbiomes
1387 reveals reproducible impacts on plant function. *ISME J* 9: 980–989. DOI: 10.1038/ismej.2014.196

1388 Parada A, Needham DM, Fuhrman JA. 2015. Every base matters: assessing small subunit rRNA primers
1389 for marine microbiomes with mock communities, time-series and global field samples. *Environ Micro-*
1390 *biol* 18: 1403–1414. DOI: 10.1111/1462-2920.13023.

1391 Priest HD, Fox SE, Rowley ER, Murray JR, Michael TP, Mockler TC. 2014. Analysis of global gene ex-
1392 pression in *Brachypodium distachyon* reveals extensive network plasticity in response to abiotic stress.
1393 *PLoS One* 9: e87499. DOI: 10.1371/journal.pone.0087499

1394 Scheuring I, Yu DW. 2012. How to assemble a beneficial microbiome in three easy steps. *Ecol Lett* 15:
1395 1300–1307. DOI: 10.1111/j.1461-0248.2012.01853.x

1396 Swenson W, Wilson DS, Elias R. 2000. Artificial ecosystem selection. *Proc Natl Acad Sci USA* 97: 9110–
1397 9114. DOI: 10.1073/pnas.150237597

1398 Vogel JP, Garvin DF, Leong O, Hayden DM 2006. Agrobacterium-mediated transformation and inbred line
1399 development in the model grass *Brachypodium distachyon*. *Plant Cell, Tissue & Organ Culture* 84: 199-
1400 211. DOI: 10.1007/s11240-005-9023-9

1401 Vogel J, Bragg J. 2009. *Brachypodium distachyon*, a new model for the Triticeae. In *Genetics and Genomics*
1402 *of the Triticeae*, pp. 427–449. Edited by GJ Muehlbauer & C Feuillet. New York: Springer.

1403