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1 **Artificial Microbiome-Selection to Engineer Microbiomes That Confer Salt-Tolerance to Plants**
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11
12 **Abstract:** We develop a method to artificially select for rhizosphere microbiomes that confer salt-tolerance
13 to the model grass *Brachypodium distachyon*. We differentially propagate microbiomes within the back-
14 ground of a non-evolving, highly-inbred plant population, and therefore only microbiomes evolve in our
15 experiment, but not the plants. To optimize methods, we conceptualize artificial microbiome-selection as a
16 special case of *indirect selection*: We do not measure microbiome properties directly, but we use host per-
17 formance (e.g., biomass; seed set) as an indicator to infer association with rhizosphere microbiomes that
18 confer salt-tolerance to a plant. We previously called this indirect-selection scheme *host-mediated indirect*
19 *selection on microbiomes* (Mueller & Sachs 2015). Our methods aim to maximize evolutionary changes
20 due to differential microbiome-propagation, while minimizing some (but not all) ecological processes af-
21 fecting microbiome composition. Specifically, our methods aim to maximize microbiome perpetuation be-
22 tween selection-cycles and maximize response to artificial microbiome-selection by (a) controlling micro-
23 biome assembly when inoculating seeds at the beginning of each selection cycle; (b) using low-carbon soil
24 to enhance host-control mediated by carbon secretions of plants during initial microbiome assembly and
25 subsequent microbiome persistence; (c) fractionating microbiomes before transfer between plants to per-
26 petuate and select only on bacterial and viral (but not fungal) microbiome components; and (d) ramping of
27 salt-stress between selection-cycles to minimize the chance of over-stressing plants. Our selection protocol
28 generates microbiomes that enhance plant fitness after only 1-3 rounds of artificial selection on rhizosphere
29 microbiomes. Relative to fallow-soil control treatments, artificially-selected microbiomes increase plant
30 fitness by 75% under sodium-sulfate stress, and by 38% under aluminum-sulfate stress. Relative to null
31 control treatments, artificially-selected microbiomes increase plant fitness by 13% under sodium-sulfate
32 stress, and by 12% under aluminum-sulfate stress. When testing microbiomes after nine rounds of differ-
33 ential microbiome propagation, the effect of bacterial microbiomes selected to confer tolerance to sodium-
34 sulfate stress appears specific (these microbiomes do not confer tolerance to aluminum-sulfate stress), but
35 the effect of microbiomes selected to confer tolerance to aluminum-sulfate stress appears non-specific (se-
36 lected microbiomes ameliorate both sodium- and aluminum-sulfate stresses). Complementary metagenomic
37 analyses of the artificially selected microbiomes will help elucidate metabolic properties of microbiomes
38 that confer specific versus non-specific salt-tolerance to plants.
39
40 **INTRODUCTION**
41 A challenge in plant-microbiome research is engineering of microbiomes with specific and lasting benefi-
42 cial effects on plants. These difficulties of microbiome engineering derive from several interrelated factors,
43 including transitions in microbiome function during plant ontogeny, and the sheer complexity of microbi-
44 ome communities, such as the hyperdiverse rhizosphere or phyllosphere microbiomes containing countless
45 fungal, bacterial, and viral components (Bulgarelli *et al* 2013; Pfeiffer *et al* 2013; Roossinck 2015). Even
46 when beneficial microbiomes can be assembled experimentally to generate specific microbiome functions
47 that benefit a plant, microbiomes are often ecologically unstable and undergo turnover (i.e., microbiome
48 communities change over time), for example when new microbes immigrate into microbiomes, when ben-
49 eficial microbes are lost from microbiomes, or when beneficial microbes evolve under microbe-microbe
50 competition new properties that are detrimental to a host plant.

One strategy to engineer sustainable beneficial microbiome-function uses repeated cycles of differential microbiome-propagation to perpetuate between hosts only those microbiomes that have the most desired fitness effects on a host (Figure 1). Such differential propagation of microbiomes between hosts can therefore artificially selects for microbiome components that best mediate, for example, 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 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 build on the original methods of Swenson *et al* (2000) by modifying experimental steps that are critical to improve (i) microbiome perpetuation and (ii) response to artificial microbiome-selection. These experimental improvements include controlling microbiome assembly when inoculating seeds; low-carbon soil to enhance host-control exerted by seedlings during initial microbiome assembly and early plant growth; harvesting and perpetuating 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.

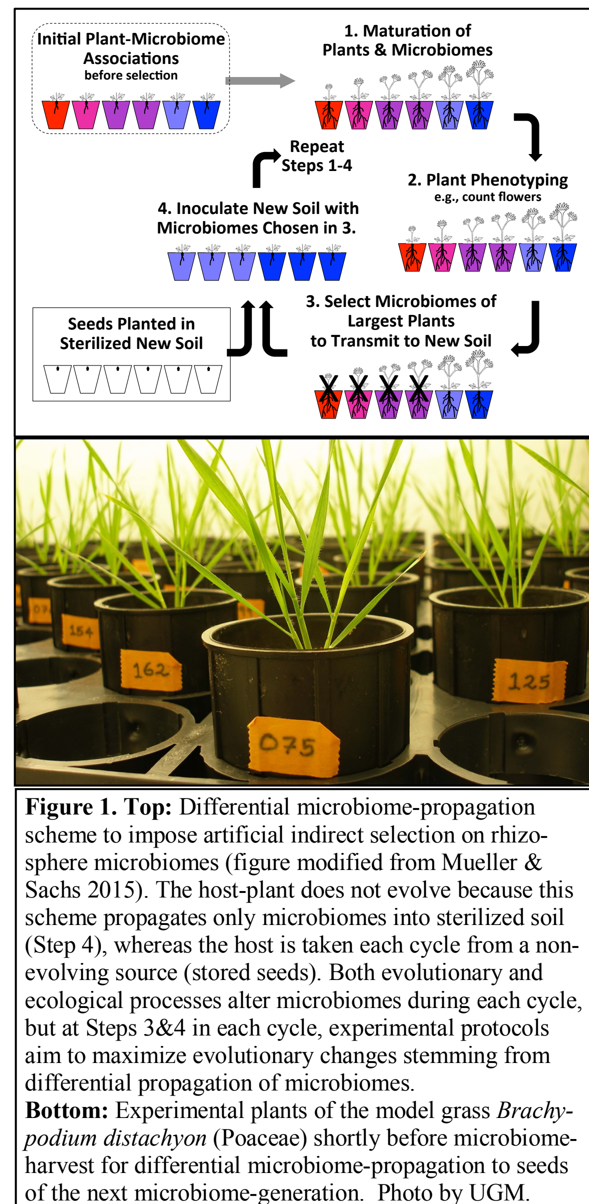

Figure 1. Top: Differential microbiome-propagation scheme to impose artificial indirect selection on rhizosphere microbiomes (figure modified from Mueller & Sachs 2015). The host-plant does not evolve because this scheme propagates only microbiomes into sterilized soil (Step 4), whereas the host is taken 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 microbiomes are 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 host phenotype that is dependent on microbiome properties. The efficacy of indirect selection depends on correlations between microbiome properties and host trait, and indirect microbiome-selection should therefore be more efficient

102 if such correlations can be maximized experimentally, for example by controlling ecological priority-effects
103 during initial microbiome assembly, or by increasing host-control over microbiome assembly and microbi-
104 ome persistence. Second, because a typical host experienced a long history of evolution to monitor and
105 manipulate its microbiomes (a process called *host control*; Sachs *et al* 2004, 2010; Kiers *et al* 2007; Berg
106 & Smalla 2009; Bright & Bulgheresi 2010; Hillman & Goodrich-Blair 2016; Lareen *et al* 2016; Foster *et*
107 *al* 2017), indirect microbiome-selection uses the host as a kind of "thermostat" to help gauge and adjust the
108 "temperature" of its microbiomes, then propagate desired microbiomes between hosts (Mueller & Sachs
109 2015; Figure 1). Based on theory (Foster & Wenseleers 2006; Williams & Lenton 2007; Fitzpatrick 2014;
110 Mueller & Sachs 2015), such *host-mediated indirect* selection on microbiomes can be easier than direct
111 selection on microbiomes, particularly with hosts that exert strong host-control over assembly and stability
112 of their microbiomes (Scheuring & Yu 2012; Coyte *et al* 2015; Mueller & Sachs 2015). Third, as in Swen-
113 son *et al* (2000), we used an inbred strain of a plant-host (here, *B. distachyon* genotype Bd3-1) to ensure
114 constancy of the host-genetic environment within and between selection-cycles, such that microbiomes
115 evolve in a specific plant-genotype background; we have previously called this method *one-sided selection*
116 (Mueller & Sachs 2015) because only the microbiomes change between propagation cycles, whereas the
117 host is taken each cycle from stored, inbred stock (Figure 1). The host therefore cannot evolve and micro-
118 biome-selection proceeds within the background of a genetically constant and homogenous host.
119
120 ***Definition of Microbiome Engineering & Microbiome Selection:*** Microbiome engineering by means of
121 differential microbiome propagation (Figure 1) alters microbiomes through both ecological and evolution-
122 ary processes. Ecological processes include changes in community diversity, relative species abundances,
123 or structure of microbe-microbe or microbe-plant interaction networks. Evolutionary processes include ex-
124 tinction of specific microbiome members; allele frequency changes, mutation, or gene transfer between
125 microbes; and differential persistence of microbiome components when differentially propagating micro-
126 biomes at each selection cycle. These processes can be interdependent (e.g., in the case of eco-evolutionary
127 feedbacks; Strauss 2014; Theis *et al* 2016; De Meester *et al* 2019), and some processes can be called either
128 ecological or evolutionary (e.g., loss of a microbe from a microbiome community can be viewed as evolu-
129 tionary extinction or as a result of ecological competition), but for the design of an efficient microbiome-
130 selection protocol, it is useful to think about ecological processes separately from evolutionary processes.
131 Microbiome-selection protocols aim to maximize evolutionary changes stemming from differential micro-
132 biomes propagation (Steps 3&4 in Figure 1), for example by optimizing microbiome inheritance during
133 microbiome transfers between hosts, or by optimizing microbiome re-assembly after such transfers (e.g.,
134 by facilitating ecological priority effects at initial host inoculation). Although both evolutionary and eco-
135 logical processes alter microbiomes during each propagation cycle (Figure 1), as a shorthand, we refer here
136 to the combined evolutionary and ecological changes resulting from host-mediated indirect artificial selec-
137 tion on microbiomes as 'microbiome response' due to 'microbiome selection'.
138

139 METHODS

140 We describe all experimental steps and analyses in great detail in the Supplemental Information, and sum-
141 marize here the basic experimental approach:
142 ***Maximizing microbiome perpetuation*:** To select for microbiomes that confer salt-tolerance to plants, we
143 used a differential host-microbiome co-propagation scheme as described in Swenson *et al* (2000), Mueller
144 *et al* (2005), and Mueller & Sachs (2015). Because both evolutionary and ecological processes alter micro-
145 biomes during and between selection-cycles (i.e., microbiome-generations), we designed a protocol that
146 improved on these earlier selection-schemes by (i) maximizing evolutionary microbiome changes stem-
147 ming from differential propagation of whole microbiomes occurring at Step 3 in Figure 1, while (ii) mini-
148 mizing some, but not all, ecological microbiome changes that can occur at any of the steps in the selection
149 cycle (e.g., we tried to minimize uncontrolled microbe-community turnover). In essence, our protocol
150 aimed to maximize microbiome-perpetuation (i.e., maximize microbiome-inheritance of key microbes) and
151 thus enhance efficacy of artificial indirect selection on microbiomes. To increase microbiome-inheritance,

152 we added protocol steps of established techniques, most importantly (i) facilitation of ecological priority-
153 effects during initial microbiome assembly (Fierer *et al* 2012; Scheuring & Yu 2012), thus increasing mi-
154 crobiome-inheritance by controlling in each selection-cycle the initial recruitment of symbiotic bacteria
155 into rhizosphere microbiomes of seedlings; and (ii) low-carbon soil to enhance carbon-dependent host-
156 control of microbiome-assembly and microbiome-persistence (Bais *et al* 2006; Bulgarelli *et al* 2013;
157 Mueller & Sachs 2015; Coyte *et al* 2015; Tkacz & Poole 2015). Theory predicts that any experimental steps
158 increasing fidelity of microbiome-perpetuation from mother-microbiome to offspring-microbiome should
159 increase the efficacy of host-mediated microbiome selection (Mueller & Sachs 2015; Zeng *et al* 2017).
160 ***Maximizing microbiome heritability*:** In each microbiome-propagation cycle (microbiome generation), we
161 inoculated surface-sterilized seeds taken from non-evolving stock (inbred strain Bd3-1 of the grass *Brachy-*
162 *podium distachyon*; Vogel *et al* 2006; Garvin *et al* 2008; Vogel & Bragg 2009; Brkljacic *et al* 2011), using
163 rhizosphere bacteria harvested from roots of those plants within each selection line that exhibited the great-
164 est above-ground biomass (Figure 1). Microbiome-selection within the genetic background of an invariant
165 (i.e., highly inbred) plant genotype increases *microbiome heritability*, defined here as the proportion of
166 overall variation in plant phenotype that can be attributed to differences in microbiomes between plants.
167 By keeping plant genotype invariant, microbiome heritability increases because a greater proportion of the
168 overall plant-phenotypic variation in a selection line can be attributed to differences in microbiome-associ-
169 ation. This increases an experimenter's ability to identify association with a beneficial microbiome (Swen-
170 son *et al* 2000). In essence, microbiome selection within the background of a single, homogenous plant-
171 genotype increases reliability of the host-phenotype as indicator of microbiome effects, and thus should
172 increase the efficiency of indirect selection on microbiomes.
173 ***Harvesting rhizosphere microbiomes & selection scheme*:** Each selection line consisted of a population of
174 eight replicate plants, and each selection treatment had five replicate selection lines (i.e., 40 plants total per
175 treatment). To phenotype plants on the day of microbiome harvesting, we judged above-ground growth
176 visually by placing all eight plants of the same selection line in ascending order next to each other (Figure
177 S3), then choosing the two largest plants for microbiome harvest. For all plants, we cut plants at soil level,
178 then stored the above-ground portion in an envelope for drying and subsequent weighing. For each plant
179 chosen for microbiome harvest, we extracted the entire root system from the soil, then harvested rhizosphere
180 microbiomes immediately to minimize microbiome changes in the absence of plant-control in the rhizo-
181 sphere. Entire root structures could be extracted whole because of a granular soil texture (Profile Porous
182 Ceramic soil), with some loss of fine roots. Because we were interested in harvesting microbiomes that
183 were in close physical association with roots, we discarded any soil adhering loosely to roots, leaving a root
184 system with few firmly attached soil particles. We combined the root systems from the two best-growing
185 plants of the same selection line, and harvested their mixed rhizophere microbiomes by immersing and
186 gently shaking the roots in the same salt-nutrient buffer that we used also to hydrate soils (details in Sup-
187 plemental Information). Combining root systems from the two best-growing plants generated a so-called
188 *mixed microbiome* harvested from two 'mother rhizospheres', which we then transferred within the same
189 selection line to all eight 'offspring plants' (i.e., germinating seeds) of the next microbiome generation
190 (Figure 1).
191 ***Microbiome fractionation with size-selecting filters before microbiome propagation*:** To simplify meta-
192 genomic analyses from propagated microbiomes, we used size-selecting filters (Bakken & Olsen 1987;
193 Mueller & Sachs 2015) to filter microbiomes harvested from rhizospheres of 'mother-plants', thus captur-
194 ing only bacteria (and possibly also viruses) for microbiome propagation to the next microbiome-genera-
195 tion, but eliminating from propagation between microbiome-generations any larger-celled soil-organisms
196 with filters (i.e., we excluded filamentous fungi, protozoa, algae, mites, nematodes, etc.). This fractionation
197 step distinguishes our methods from those of Swenson *et al* (2000) and Panke-Buisse *et al* (2015), both of
198 which used 'whole-community' propagation to transfer between generations all organism living in soil,
199 including the fungi, protozoa, algae, and multicellular organisms that were excluded from propagation
200 through size-selecting filtering in our experiment.
201 ***Salt-stress treatments and experimental contrasts*:** Using different selection-lines, we selected for benefi-
202 cial microbiomes conferring salt-tolerance to either sodium-sulfate [Na_2SO_4] or to aluminum-sulfate

203 [$Al_2(SO_4)_3$]. Such an experimental contrast involving two main treatments (i.e., the two salt stresses) ena-
204 bles an experimenter to compare evolving microbiomes using metagenomic time-series analyses, as well
205 as to identify candidate microbes (indicator taxa) and microbial consortia that differ between sodium- and
206 aluminum treatments and that may therefore confer specific salt-tolerance to plants.
207 ***Control treatments*:** To evaluate the effects of selection treatments, we included two non-selection control
208 treatments. In *Fallow-Soil Microbiome-Propagation Control*, we harvested microbiomes from fallow soil
209 (no plant growing in a pot; microbiomes are harvested from root-free soil, collected at the same depth of
210 roots in pots with plants), then propagated the harvested microbiomes to sterile fallow soil of the next
211 microbiome-generation. In *Null Control*, we did not inoculate germinating seeds with any microbiomes,
212 but microbes could enter soil from air (i.e., microbial "rain"), as was the case also for all other treatments.
213 ***Number of selection cycles (microbiome generations)*:** Our complete experiment involved one baseline
214 Generation (Generation 0, Table S1a) to establish initial microbiomes in replicate pots; 8 rounds of micro-
215 biome-selection (i.e., differential microbiome-propagation) (Generations 1-8, Table S1b); and one final,
216 ninth round (Generation 9, Table S1c) to evaluate the effects of the engineered microbiomes on flower-
217 production and seed-set, for a total of 10 microbiome-generations.
218 ***Ramping of salt stress*:** We increased salt stresses gradually during the selection experiment, by (i) increas-
219 ing between generations the molarity of the water used to hydrate dry soil before soil-sterilization and
220 planting (Table S2); and (ii) increasing correspondingly between generations also the molarity of the water
221 used to hydrate pots at regular intervals (Table S3). Over the 10 generations, sodium-sulfate molarity in the
222 sodium-stress treatments increased from 20 millimolar (mM) to 60 mM, and aluminum-sulfate molarity in
223 the aluminum-stress treatments increased from 0.02 mM to 1.5 mM (Tables S2 & S3). The salt stresses of
224 the baseline generation were chosen because, in pilot experiments, these stresses caused minimal delays in
225 germination and growth compared to unstressed control plants. The logic of ramping and adjusting salt-
226 stresses stepwise between selection cycles are explained further in the Supplemental Information.
227
228

229 RESULTS

230 Artificially-Selected Microbiomes Confer Increased Salt-Tolerance to Plants

231 Figure 2a-d shows the changes in relative plant-fitness (above-ground dry biomass) during 8 rounds of
232 differential microbiome propagation. Relative to Fallow-Soil Control and Null Control treatments, selected
233 microbiomes confer increased salt-tolerance to plants after only 1-3 selection-cycles, for both microbiomes
234 selected to confer tolerance to sodium-stress (Figure 2a&b) or to aluminum-stress (Figure 2c&d). Relative
235 to Fallow-Soil Controls, artificially selected microbiomes increase plant fitness by 75% under sodium-sul-
236 fate stress ($p<0.001$), and by 38% under aluminum-sulfate stress ($p<0.001$). Relative to Null Control plants,
237 artificially selected microbiomes increase plant fitness by 13% under sodium-sulfate stress, and by 12%
238 under aluminum-sulfate stress. Although repeated rounds of differential microbiome-propagation improved
239 plant fitness between successive microbiome-generations (particularly in the Null Controls; Figure 2b&d),
240 interactions between treatment and generation were not statistically significant (see Supplemental Infor-
241 mation). This implies that fitness-enhancing effects of microbiomes from selection-lines therefore were
242 realized after one or a few rounds of differential microbiome-propagation (e.g., Figure 2b&d), and there
243 was insufficient statistical support that, under the gradually increasing salt stress, any additional rounds
244 further resulted in greater plant biomass of selection-lines relative to control-lines. However, because plants
245 were exposed to greater salt stresses in later selection cycles (see ramping of salt stress, Table S2), the
246 selected microbiomes must have had correspondingly greater beneficial effects on the stressed plants. Se-
247 lected microbiomes of later generations therefore helped plants tolerate greater salt stresses to allow plants
248 grow the same biomass as plants in somewhat earlier generations experiencing somewhat lesser salt stress.
249
250 The phenotypic effect on plants due to the evolving microbiomes fluctuated during the eight rounds of
251 differential microbiome propagation (Figures 2a-d; Table S1). Such fluctuations can occur in typical artifi-
252 cial selection experiments (e.g., chapters 10, 12 & 18 in Garland & Rose 2009), but fluctuations may be

more pronounced when artificially selecting on microbiomes (Blouin *et al* 2015) because additional factors can contribute to between-generation fluctuations. Specifically, across the eight selection cycles in our experiment, the observed fluctuations could have been due to (i) uncontrolled humidity changes and correlated humidity-dependent water-needs of plants (humidity was not controlled in our growth chamber), consequently changing the effective salt-stresses; (ii) the strong ramping of salt-stress 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 consequently random microbe-microbe interactions; or (iv) other such uncontrolled 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 generation and when humidity varied most (see Supplemental Information), whereas fluctuations were less pronounced during the last three generations 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.

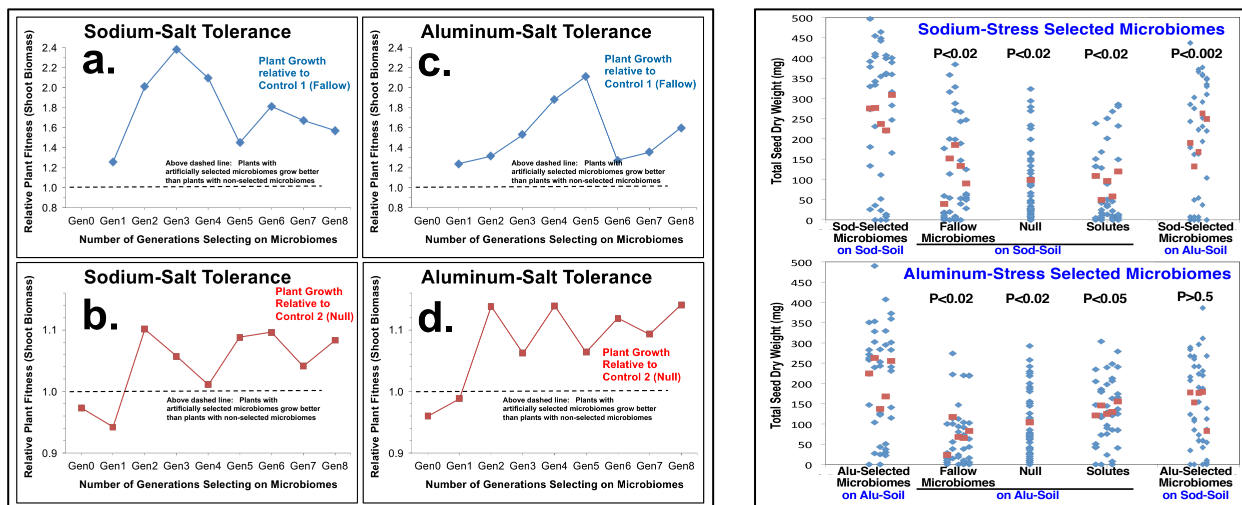

Figure 2. Host-mediated artificial selection on microbiomes to confer salt-tolerance to the grass *Brachypodium distachyon*. **Left:** Microbiomes were propagated for 8 selection cycles (generations = Gen), using the microbiome propagation scheme in Figure 1. Two salt stresses (sodium-sulfate vs. aluminum-sulfate) are contrasted, and plant fitness of selection-lines is shown relative to two non-selected control treatments. In *Fallow-Soil Microbiome-Propagation Control*, microbiomes were harvested from fallow soil (pot with no plant), then propagated to sterile fallow soil in pots 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 5 selection lines each, with 8 plants per line. **Right:** At the end of the experiment after a 9th selection cycle (Generation 9), plants were grown to seed for 68 days. Total seed weight is plotted in blue for individual plants (plants of same selection line are vertically above each other), the averages for each of the 5 selection lines are plotted in red. Two additional controls were added in Generation 9: *Solute-Control*, filtering out bacterial & fungal components, to test only solutes & viral microbiome components that are propagated; and *2x2 Cross-Fostering* to test for specificity of evolved microbiome function. All controls are significantly different from selection treatments, 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 microbiomes selected to confer tolerance to aluminum-sulfate appears non-specific (these microbiomes confer tolerance to both sodium- and aluminum-sulfate stress; $p>0.5$), but the effect of bacterial microbiomes selected to confer tolerance to sodium-sulfate appears specific (these microbiomes do not confer salt-tolerance to aluminum-sulfate stress; $p<0.002$).

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 Control* (*Solute Control*) and *Cross-Fostering*, to help elucidate some of the mechanisms underlying the salt-tolerance-conferring effects of selected microbiomes on seed production. In the Solute 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 bacterial microbiomes in the selection-lines. Plants receiving these bacterial-free filtered solutes (i) had significantly poorer seed-production compared to plants that received these same solutes together with the live bacterial microbiomes ($p<0.02$ for sodium-stress treatment; $p<0.05$ for aluminum-stress treatment; Tables S3 & S4); and (ii) had 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 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 sodium-stress (SOD) and aluminum-stress (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 microbiomes selected to confer tolerance to aluminum-sulfate appears non-specific (these aluminum-selected microbiomes confer tolerance to both sodium- and aluminum-sulfate stress; $p>0.5$), but the effect of bacterial microbiomes selected to confer tolerance to sodium-sulfate appears specific (these sodium-selected microbiomes do not confer salt-tolerance to aluminum-sulfate stress; $p<0.002$) (Figure 2 right, rightmost-graphs). The underlying microbial and metabolic mechanisms conferring specific and non-specific salt-tolerance are unknown, but metagenomic comparisons of the sodium-selected versus aluminum-selected microbiomes should help generate hypotheses that can be tested in future studies.

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 selecting 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 generations (Swenson *et al* 2000). To address these problems, we adopted 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) facilitate ecological priority-effects during initial microbiome assembly (Fierer *et al* 2012; Scheuring & Yu 2012), thus increasing microbiome-inheritance by steering the initial recruitment of symbiotic bacteria into rhizosphere microbiomes of seedlings; (b) propagate microbiomes harvested from within the sphere of host-control (i.e., microbiomes in close physical proximity to roots) (Yan *et al* 2017; Shi *et al* 2016), whereas Swenson *et al* (2000) harvested also microbes from outside the sphere of host control; (c) enhance carbon-dependent host-control of microbiome-assembly and host-control of 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); and (d) ramping of salt-stress between selection-cycles to minimize the chance of either under-stressing or over-stressing plant. Without additional experiments, it is not possible to say which of these experimental steps was most important to increase efficacy and response to microbiome-selection. The observation that it is

possible to artificially select for microbiomes that confer drought tolerance to wheat grown in high-carbon potting soil (Metro-Mix 900, Jochum 2019) suggests that the low-carbon soil thought to be important in our experiment may not be essential for plant-mediated microbiome selection.

Our conceptual approach of *host-mediated indirect selection on microbiomes* (Mueller & Sachs 2015), combined with our experimental approach aiming to capitalize on evolved mechanisms of *host-control* (Sachs *et al* 2004; Mueller & Sachs 2015; Foster *et al* 2017), 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" selection 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 capture some aspects of host-microbiome population-biology (van Opstal & Bordenstein 2015; Theis *et al* 2016; Garcia & Kao-Kniffin 2018), we found that a multi-level selection view provides few useful insights for the design of microbiome-selection experiments. In contrast, basic principles of quantitative-genetics, microbial-ecology, and host-microbiome evolution (Coyte *et al* 2015; Mueller & Sachs 2015; Tkacz & Poole 2015; Rillig *et al* 2016; Wright *et al* 2019) and particularly the host-centric concept of *host control* (Sachs *et al* 2004; Foster *et al* 2017) allowed us to identify and adjust critical experimental steps and parameters in our differential microbiome-propagation scheme.

Compared to two other experiments of host-mediated 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, for example, 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 conducted our experiment in a more stable greenhouse environment; and (c) we selected for microbiomes conferring specific benefits (salt-tolerance), rather than the non-specific, general-purpose beneficial microbiomes selected by 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) growth improvement 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 very controlled greenhouse conditions that are very different from outdoor conditions, this seems a remarkable enhancement of plant productivity compared to traditional plant breeding.

An interesting result is that microbiomes selected to benefit growth of plants during the early vegetative phase (biomass of 4-week-old plants, well before flowering; Figure 2 left) generated microbiomes with benefits that translated also into enhanced plant fitness during the reproductive phase by increasing seed set of 10-week-old plants (Figure 2 right). Rhizosphere microbiomes of grasses can change significantly during plant ontogeny (Edwards *et al* 2018), and therefore microbiomes selected to serve one function such as early growth may not necessarily optimize other functions such as seed set. Our observation that microbiome selection to promote early growth (Figure 2 left) also promotes increased seed set (Figure 2 right) therefore implies that (a) some of the same bacteria benefitting plants during the early vegetative phase under the tested salt stresses also benefit plants during reproductive phase in *B. distachyon*, despite possible changes in overall microbiome communities during plant ontogeny; (b) seed set is intrinsically tied to optimal early growth in *B. distachyon*, possibly by accelerating the timing of flowering; and (c) microbiome-selection experiments aiming to increase seed productivity do not necessarily have to select on seed set as

391 a measured phenotype, but can shorten each selection cycle by selecting on other plant phenotypes, such as
392 early vegetative growth.
393
394 Because our experiment was the first systematic attempt to improve the methods of Swenson *et al* (2000),
395 we predict that it should be possible to further optimize protocols of differential microbiome propagation.
396 Microbiome-selection therefore could emerge as a novel tool to engineer and elucidate microbiome-func-
397 tions in controlled laboratory environments, and possibly also in those natural environments that allow
398 control of key parameters affecting microbiome harvest, microbiome transfer, and microbiome inheritance.
399 Such optimization of microbiome-selection should ideally be informed by metagenomic analyses of exper-
400 imental contrasts (e.g., comparison of microbiomes selected to confer either sodium-stress versus alumi-
401 num-stress tolerance to plants) and by time-series analyses across microbiome-propagation cycles, to iden-
402 tify candidate microbes and microbial consortia important in mediating stresses.
403

404 **FUTURE RESEARCH TO IMPROVE METHODS OF MICROBIOME-SELECTION**

405 To expand on our methods of artificial microbiome selection, we outline a series of additional experiments
406 that should generate insights into key parameters that determine efficacy of microbiome selection. Arias-
407 Sánchez *et al* (2019) and Xie *et al* (2019) recently summarized criteria for microbiome-selection experi-
408 ments that are not host-mediated (e.g., selection on CO_2 emission by a complex microbiome, in absence of
409 a plant host), Lawson *et al* (2019) summarized protocols for engineering any kind of microbiome (e.g., by
410 using bottom-up and top-down design criteria), and we focus here on methods of host-mediated microbiome
411 selection that aim to improve performance of a plant host. Because host-mediated microbiome selection
412 leverages traits that evolved to assemble and control microbiomes (so-called host control; Mueller & Sachs
413 2015, Foster *et al* 2017), the first four experiments explore whether factors promoting strong microbiome-
414 control by a plant host could improve efficacy of microbiome selection:

415 *(1) Artificial mirobiome-selection on endophytic vs. rhizosphere microbiomes*: Microbiomes internal to a
416 host (e.g., endophytic microbes of plants) require some form of host infection, and therefore could be
417 under greater host control than external microbiomes, such as rhizoplane or rhizosphere microbiomes.
418 Consequently, under stresses that are mediated by host-controlled microbes, it may be easier to obtain
419 a response to microbiome-selection when targeting selection on endosphere microbiomes versus exter-
420 nal microbiomes. This prediction can be tested in an experiment that compares, in separate selection
421 lines in the same experiment, the responses to microbiome selection when harvesting and propagating
422 only endophytic microbiomes versus only rhizosphere microbiomes. This prediction may not hold for
423 stresses that require stress-mediation by microbes in the external microbiome compartment of roots
424 (e.g., microbes that detoxify toxins, such as aluminum, before they enter the root and then affect the
425 plant negatively; for example microbes that chelate toxins external to the plant in the rhizosphere, Ma
426 *et al* 2001; Aggarwal *et al* 2015); however, this prediction about a key role of host control for the
427 efficacy of microbiome selection should hold for many other stresses that are mediated by microbes
428 that a plant permits to enter into the endophytic compartment.

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

436 *(3) Varying host control by varying carbon-content in soil*: A third approach to test host control is to com-
437 pare efficacy of microbiome selection in low- versus high-carbon soil. Microbial growth in some soils
438 is limited by carbon, and many plants therefore regulate their soil-microbiomes by carbon secretions
439 (Zahar Haichar *et al* 2016; Sasse *et al* 2018). We therefore hypothesized that a low-carbon soil (like
440 the carbon-free PPC soil in our experiment) may facilitate host control and consequently also microbi-
441 ome-selection. This hypothesis remains to be tested, for example in a microbiome-selection experiment
442 contrasting response to selection between soils with different carbon content. The observation that it is

443 possible to artificially select for microbiomes that confer drought tolerance to wheat grown in high-
444 carbon potting soil (Jochum 2019) suggests that low-carbon soil may not be essential for plant-mediated
445 microbiome selection, but low-carbon soil could be a facilitating condition.
446 *(4) Varying resource-limited host control by varying seed size*: A fourth approach to test host control could
447 be to compare the efficacy of microbiome selection between plant species with large seeds versus small
448 seeds (e.g., *Brachypodium* versus *Arabidopsis*), or between seedlings of the same plant species grown
449 from small versus large seeds. A germinating seed has to allocate resources to above-ground growth to
450 fix carbon and to below-ground growth to access nutrients and water, and seedlings growing from re-
451 source-rich large seeds therefore may be better able to allocate additional resources to manipulate mi-
452 crobiomes effectively, for example by root secretions. If such resource-allocation constraints exist for
453 young seedlings, this could explain why our microbiome-selection experiment with *B. distachyon* ap-
454 pears to have generated stronger and faster response to microbiome selection compared to other such
455 experiments with *Arabidopsis thaliana* (Swenson *et al* 2000; Panke-Buisse *et al* 2015). The best tests
456 of seed-resource dependent efficacy of microbiome selection will not be comparisons between species,
457 however, but within-species comparisons of the efficacy of microbiome selection within host popula-
458 tions grown from resource-rich large seeds versus resource-poor small seeds of the same plant species.
459 *(5) Propagation of fractionated vs. whole microbiomes*: Experimental microbiome-propagation between
460 host generations can be complete [all soil-community members are propagated between hosts, as in
461 Swenson *et al* (2000) and Panke-Buisse *et al* (2015)] or microbiomes can be fractionated by excluding
462 specific microbial components, as in our protocol where we propagated only organisms of bacterial or
463 smaller sizes. We used fractionated microbiome-propagation because (a) we were more interested in
464 elucidating contributions to host fitness of the understudied bacterial components than the fungal com-
465 ponents (e.g., mycorrhizal fungi), and (b) fractionation simplifies analyses of the microbiome-responses
466 to selection (e.g., bacterial microbiome components, but not necessarily fungal components, need to be
467 analysed with metagenomic techniques). However, because fungal components and possible synergis-
468 tic fungal-bacterial interactions cannot be selected on when using our fractionated microbiome-propa-
469 gation scheme, we hypothesized previously (Mueller & Sachs 2015) that selection on fractionated mi-
470 crobiomes may show attenuated selection responses compared to selection on whole microbiomes.
471 This can be tested in an experiment comparing the response to microbiome selection when propagating
472 fractionated versus whole microbiomes, for example by using different size-selecting filters.
473 *(6) Propagation of mixed vs. un-mixed microbiomes*. When propagating microbiomes to new hosts, it is
474 possible to propagate mixed microbiomes harvested from different hosts, or only un-mixed microbi-
475 omes. Mixed vs. un-mixed propagation schemes therefore represent two principal methods of microbi-
476 ome selection (Swenson *et al* 2000; Williams & Lenton 2007; Mueller & Sachs 2015; Rillig *et al* 2016).
477 We hypothesized previously (Mueller & Sachs 2015) that mixed propagation may generate a faster
478 response to microbiome-selection, but the respective advantages of mixed versus un-mixed propagation
479 have yet to be tested. Mixed propagation may be superior to un-mixed propagation, for example if
480 mixing generates novel combinations of microbes with novel beneficial effects on a host (Mueller &
481 Sachs 2015), or may merge previously separate networks of microbes into a superior compound net-
482 work (so-called community-network coalescence; Rillig *et al* 2016), or generate novel competitive in-
483 teractions between microbes that increase microbiome stability (Coyte *et al* 2015).
484
485
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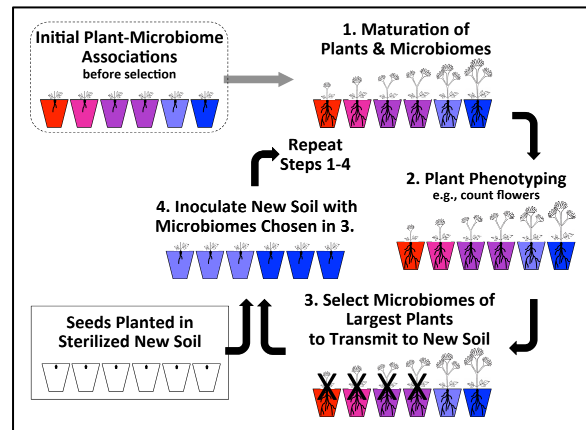
620

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 microbiome 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). We chose to conduct the experiment with *B. distachyon* because it is a model for biofuel and cereal crops, including research on salt stresses and water-use efficiency (De Marais & Juenger 2011; De Marais et al 2016).



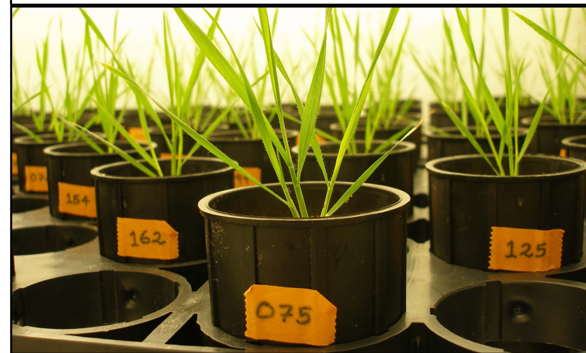


Figure S1. Top: Differential microbiome-propagation scheme to impose artificial indirect selection on rhizosphere microbiomes (figure modified from Mueller & Sachs 2015). The host-plant does not evolve because this scheme propagates only microbiomes into sterilized soil (Step 4), whereas the host is 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.

We inoculated seeds with rhizosphere bacteria harvested from roots of those plants of the previous selection cycle that exhibited the greatest above-ground biomass (Figure S1). Because the plant-host could not evolve between selection-cycles (seeds were taken from non-evolving stock), 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 on bacterial communities, we filtered the microbiomes harvested from rhizospheres, perpetuating only bacteria (and possibly 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 a 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

671 9, Table S1c) to evaluate the effects of the engineered (i.e., evolved) microbiomes on flower-production
672 and seed-set, for a total of 10 Generations. Our entire selection experiment lasted 300 days from 3. January
673 -29. October 2015 (Table S2).

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

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

699 **Table S2. Salt concentrations (Millimolar = mMolar) of salt-nutrient solutions used to hydrate soil** for each
700 selection cycle (= Microbiome-Generation = Gen); the recipes to mix these solutions; and growth parameters for each
701 Generation. In the short-cycled Generations 0-8, time was too short for plants to flower, and we quantified plant-
702 performance by visually estimating above-ground biomass (see *Phenotyping of Plants*). In Generation 9, plants were
703 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 to grow sufficiently large root systems for microbiome harvest, but plants 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. 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.

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 well, 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 (see last row in Table S2). 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, 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 μmol/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

744 & Sons, Tangent, Oregon, USA) filled with autoclaved PPC soil (Profile Porous Ceramic soil, Greens-
745 Grade™ Emerald, Natural Color; PROFILE Products LLC, Buffalo Grove, IL, USA). To permit autoclav-
746 ing of soil in the Deepots prior to planting, we pressed heat-tolerant fiberglass-fill into the bottom of each
747 pot to plug bottom-drainage holes, then compacted dry PPC soil into each pot until the soil level reached
748 15mm below the pot margin. Each plug consisted of a fiberglass square (9.5cm x 9.5cm) cut from an insu-
749 lation-sheet (R-13 EcoTouch Insulation Roll; 38cm width; GreenGuard-certified, formaldehyde-free), then
750 pressed firmly into the bottom of a pot. After compacting soil in all pots used for a given selection-cycle
751 (200 pots in selection-cycles Generation 0-8; 400 pots in the final Generation 9) we carefully equalized soil
752 levels between all pots.

753 According to the manufacturer's website (www.profileevs.com/products/soil-amendments/profile-porous-
754 ceramic-ppc), PPC soil is a calcined, non-swelling illite, non-crystalline opal mineral; it has 74% pore
755 space, with 39% capillary (water) pores and 35% non-capillary (air) pores; pH = 5.5; cation-exchange-
756 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
757 5% of the remainder combining all other chemicals (e.g., CaO, MgO, K_2O, Na_2O, TiO_2). We chose PPC
758 soil for three reasons: First, PPC has a very homogeneous consistency because of its uniform particle size;
759 soil-quantity and soil-quality are therefore easy to standardize between pots. Second, whole root systems
760 can be easily extracted from hydrated soil with little rupture of roots. Third, because the manufacturer
761 exposes PPC soil to high temperature (heated in a rotary kiln at 1200 degrees Fahrenheit, then de-dusted),
762 the soil contains minimum carbon, and we believed that such low- or no-carbon soil could facilitate a plant's
763 ability for carbon-mediated host-control (via carbon secretions by roots; see above *Protocol Outline*) (Bais
764 *et al* 2006; Bulgarelli *et al* 2013; Mueller & Sachs 2015; Coyte *et al* 2015) of microbiome-assembly and
765 microbiome-stability.

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

791 The salt-concentration of the baseline Generation 0 (Table S2) was determined in a salt-gradient pilot ex-
792 periment as that salt-concentration that caused a minimal, but just noticeable, delay in germination and a

793 minimal growth-rate reduction. Because aluminum-sulfate delays germination and attenuates growth at far
794 lower concentrations than sodium-sulfate, concentrations in the ALU-treatment (Table S2) were lower by
795 several orders of magnitude than the concentrations of sodium-sulfate in the SOD-treatment. For ramping
796 of salt-stress, pre-planned step-increments in salt-concentration between selection-cycles were likewise in-
797 formed by our pilot experiments, which suggested increments for aluminum-sulfate concentrations of about
798 two- to five-fold for the first few microbiome-generations, and less than two-fold increments for sodium-
799 sulfate concentrations, with gradual decrease in step-increments in later microbiome-generations so as not
800 to over-stress plants (Table S2). Because we had to prepare hydrated soil for the next selection-cycle about
801 1-2 weeks before the end of a given cycle, we had to decide salt-stress increments for the next selection-
802 cycle well in advance, using information from relative growth of younger plants in the sodium-sulfate and
803 the aluminum-sulfate treatments. Decisions on salt-increments between Generations therefore typically in-
804 volved some informed guessing, to adjust salt concentrations for the next cycle such that plants in either
805 treatment were projected to germinate and grow at about the same rate (i.e., we aimed for plants in either
806 salt treatment to grow to comparable sizes in the same time during a selection cycle). With such projected
807 equal growth between sodium- and aluminum-treatments, microbiomes could be harvested at the end of a
808 selection cycle from plants of comparable sizes (typically 9-15 leaves at the time of microbiome harvesting)
809 regardless of whether a plant was stressed with aluminum-sulfate or sodium-sulfate (i.e., sodium-treated
810 plants were not behind in growth compared to aluminum-treated plants, or vice versa). A second pre-
811 planned feature of our salt-ramping design was to increase salt-stress in successive selection-cycles as long
812 as differences in effect-sizes seemed to increase between salt- and control-treatments, but to reduce the salt-
813 stress if differences in effect-sizes diminished or disappeared, possibly because of over-stressing the plants
814 (see above *Logic of Salt-Stress Ramping*). This seemed to happen in Generations 4 & 5 (see Figure 1 in
815 main text), and salt-stress was therefore reduced somewhat in the subsequent four Generations 6-9 (Table
816 S2).

817 For hydration of 100 pots, we mixed, in a large carboy, 12 liter double-distilled e-pure water at a 50:1-ratio
818 with 240ml Dyna-Gro 9-7-5 (Nutrient Solutions, Richmond, CA; www.dyna-gro.com/795.htm), plus an
819 aliquot of 1-Molar salt solution (Table S2 lists salt-aliquots in recipes for salt-nutrient mixes) to generate
820 the specific salt-stress planned for a particular selection-cycle. [To prepare 1-Molar ALU-salt stock, we
821 dissolved 307.94g anhydrous aluminum-sulfate in 900ml e-pure water in a 1-liter bottle; to prepare 1-Molar
822 SOD-salt stock, we dissolved 289.98g decahydrous sodium-sulfate in 900ml e-pure water in a 1-liter bottle;
823 then filter-sterilized each salt solution to prepare sterile stock.] We used different carboys to prepare salt-
824 nutrient mixes for the different salt treatments (SOD, ALU). The nutrient concentration in each mix (Table
825 S2) was sufficient such that plants did not need additional fertilization during each selection-cycle of 20-
826 30 days during Generations 0-8 when we quantified plant fitness as above-ground biomass production, and
827 plants even had sufficient nutrients to flower and grow seed during the 68 days of the last Generation 9
828 when we quantified plant fitness as seed production. For both salt treatments, fertilizer-salt solutions had a
829 pH = 3.75 before addition to soil, but because of the buffering capacity of PPC soil (natural pH = 5.5, see
830 above), the hydrated soil had a pH of about 5.0-5.5 after autoclaving soils, using the pH-measurement
831 procedure in ISO/FDIS 10390 (2005). After hydration of all pots, we immediately autoclaved all pots (to
832 minimize the time that any live microbes in the soil could consume any of the nutrients), and we autoclaved
833 in separate 1-liter flasks at the same time 800ml of each of the unused salt-nutrient solutions; these auto-
834 claved salt-nutrient solutions were used later during planting, and as buffer (at half-concentration) to sus-
835 pend microbiomes harvested from rhizospheres for microbiome-transfers (see *Planting & Microbiome-*
836 *Harvest* below).

837 **Autoclaving of Soil:** After hydration of soil by carefully pouring exactly 94ml of fertilizer-salt solution
838 into a pot, we leveled and smoothed the soil-surface in a pot with the bottom of a glass (same size as interior
839 diameter of a pot); taped to each pot a label of autoclavable label-tape (Fisherbrand^{TM}) with a pre-written
840 pot-number (#001-100 for pots of SOD-treatment; #101-200 for pots of ALU-treatment) to the top side of
841 each pot (Figure S1); then used pre-cut pieces of aluminum foil to cap the top and wrap the bottom of each

842 pot to prevent possible microbial contamination during seed-
843 stratification (see below *Planting & Stratification*). Wrapped
844 pots were arranged vertically in large autoclave trays (67 pots
845 per tray, 3 trays total), the trays were covered with sheets of
846 aluminum foil, then all pots in these 3 trays were sterilized
847 simultaneously in a large autoclave. Hydration, labeling and
848 capping of a set of 200 pots needed typically 5-6 hours. The
849 subsequent autoclaving procedure lasted about 10 hours over-
850 night, starting in the evening with a first cycle of 35 minutes
851 autoclaving (121C° temperature, 20 atm pressure) with a
852 slow-exhaust phase lasting 90 minutes; followed by overnight
853 exposure to high temperature in the unpressurized autoclave;
854 followed in the morning by a second cycle of 35 minutes au-
855 toclaving with a 90-minute slow-exhaust phase. This strin-
856 gent autoclaving regime was sufficient to sterilize PPC soil,
857 because plating on PDA-medium of about 0.5g soil (n=2 SOD
858 pots, n=2 ALU pots) taken with a sterile spatula from the in-
859 terior of such autoclaved pots produced no visible microbial
860 growth within a month of incubation at room temperature. Af-
861 ter cooling of autoclaved pots in the foil-covered trays at room
862 temperature for at least 16 hours, we planted seeds into the
863 sterilized soil (one seed per pot; see below *Planting*).

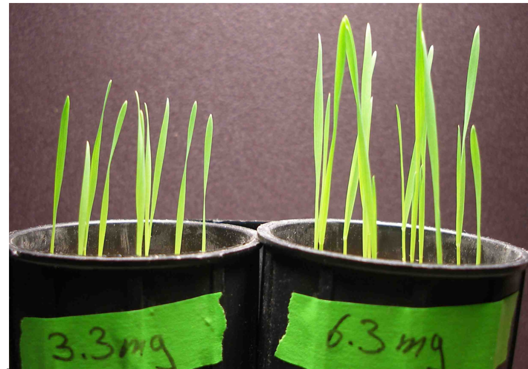


Figure S2. Pilot experiment illustrating growth variation of *B. distachyon* Bd3-1 plants growing under identical conditions from seeds weighing either 3.3mg or 6.3mg. The seed-weight range tested here includes about 90% of the 6000 seeds that we harvested for our microbiome-selection experiment. We 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.

864 **Seeds Preparation & Binning of Seeds by Weight:** To have enough seeds for our 10-generation selection
865 experiment, we first grew *Brachypodium distachyon* Bd3-1 plants under standardized light conditions (14h
866 light, 10h dark) and room temperature in well-fertilized and well-watered greenhouse soil, harvested about
867 6000 seeds from these plants, then dried and stored seeds at room temperature (see above *Preparation of*
868 *Brachypodium distachyon Seeds*). For our experiment, we used only long-awn seeds; that is, we discarded
869 any short-awn seeds positioned peripherally in inflorescences (spikelet), and we discarded also any mis-
870 shapen or discolored seeds. We used only long-awn seeds because these kind of seeds grow in more stand-
871 ardized central positions in a spikelet, because we could grasp an awn with a forceps during weighing and
872 planting without risk of injuring a seed, and because we could plant seeds vertically into soil with only the
873 awn protruding above the soil to reveal the exact location of a seed during later microbiome inoculation
874 (see below *Seed Inoculation*). To weigh each seed accurately, we first removed any attached glumes to
875 weigh only the seed with its awn. One experimenter pre-weighed each seed to bin seeds by weight to the
876 nearest 0.1mg, then a second experimenter re-weighed all seeds in bins 4.5mg – 6.0mg again (i.e., each
877 seed was weighed twice). To help reduce within-treatment variation in plant-phenotype (specifically here,
878 reduce seed-weight-dependent maternal effects on plant-phenotypes, as illustrated in Figure S2), we used
879 seeds of only a narrow weight-window for each microbiome-selection cycle. We used seeds of 5.9&5.8mg
880 weight for the initial baseline Generation 0, then we used up seeds of bins of gradually decreasing seed-
881 weight (5.9&5.8mg, 5.8&5.7mg, 5.6&5.5mg, …), as shown in Table S2 for each microbiome-generation.

882 **Planting & Stratification:** For planting of seeds in sterile soil, we first surface-sterilized Bd3-1 seeds in a
883 laminar flow-hood by gently shaking the seeds for 8 minutes in 10% bleach [Chlorox®; 4ml bleach added
884 to 36ml autoclaved e-pure water in a 50ml Falcon tube; plus 4µl Tween80-surfactant (Sigma-Aldrich, Saint
885 Louis, MO, USA) to promote wetting of seeds], then rinsing the seeds three times to wash off bleach (suc-
886 cession of three 1-minute gentle shaking, each in 40ml e-pure autoclaved water in a 50-ml Falcon tube). In
887 pilot tests, such surface-sterilized seeds placed on PDA-medium did not lead to bacterial or fungal growth.
888 After rinsing, we blotted seeds on autoclaved filter paper, then air-dried the seeds in an open Petri dish in
889 the flow-hood while preparing the flow-hood for planting inside the hood. To plant one seed into the center
890 of a pot, we removed the aluminum-foil lid from a pot inside the flow-hood, pushed a narrow hole into the
891 center of the soil with a flame-sterilized fine-tipped forceps (#5 forceps), then inserted a seed into that hole

892 such that the seed was positioned vertically in the soil and only the awn was protruding above the soil (i.e.,
893 the pointed tip of a seed was just below the soil surface). Because seeds used for a selection cycle had been
894 binned to within 0.1mg weight (i.e., all seeds were of same size for each selection cycle), seeds were there-
895 fore planted at the same depth (to within about 0.5-1.0 mm identical depth), and any differences in initial
896 germination rate (i.e., appearance of the shoot at soil surface) was unlikely due to differences in planting
897 depth between seeds. To solidify the soil around each seed, we applied 4ml autoclaved salt-nutrient solution
898 (same concentration that was used to hydrate soil in a given selection-cycle; Table S2) with a 5ml pipette
899 to flush soil into the hole and completely cover each seed (excepting the awn protruding vertically above
900 the soil surface). We covered each pot with a translucent, ethanol-sterilized lid (inverted Mini Clear Plastic
901 Bowl 40ct; Party City, Rockaway, NJ, USA). The lids prevented entry of airborne microbes into each pot,
902 but did not seal pots completely and permitted some gas exchange. Each lid measured 5.7cm diameter x
903 3.8cm height, and fit snugly only each pot such that a series of 50 capped pots could be kept in a rack (D50T
904 rack, see above) without the lids interfering with each other. We placed each rack of 50 capped pots into its
905 own ethanol-sterilized plastic tub (Jumbo Box; Container Store, Coppell, TX), covered the box with a boxes
906 plastic lid, then sealed the spaces at the side of each lid by wrapping lid&tub with a 2-meter-long strip of
907 10-cm-wide Parafilm to prevent entry of contaminants during subsequent cold-storage for stratification of
908 seeds. We moved each tub into cold-storage immediately after completing the planting of 50 pots. For
909 stratification, we stored the tubs with planted seeds in a 5°C cold-room for about 5 days (range 4-10 days,
910 the duration differing slightly between selection-cycles because of scheduling-constraints affecting plant-
911 ing). Planting of a set of 200 seeds (4 racks of 50 pots each) using the above methods needed typically 4.5-
912 5.5 hours.

913 **Preparations for Microbiome-Harvesting:** To prepare salt-nutrient buffer-solution for microbiome har-
914 vesting, we used the autoclaved salt-nutrient solution that we had prepared for hydration of soil for a par-
915 ticular selection-cycle (Table S2), then diluted the solution to half-concentration by addition of an equal
916 volume of autoclaved e-pure water. We decided to use for microbiome-harvesting the salt-nutrient solution
917 at half-concentration, because we were concerned that the full-concentration may have too high osmolarity
918 compared to the osmolarity that may exist in the soil after weeks of root- and microbiome-growth in the
919 soil; this dilution precaution may not have been necessary, and it may be possible to propagate microbiomes
920 even when using full-concentration of salt-nutrient solution. Aliquots of 45ml of the sterile, half-concen-
921 tration salt-nutrient buffer were added to 50ml Falcon tubes in a laminar-flow hood, and these tubes were
922 then pre-labeled with relevant information (SOD vs ALU treatment; Generation #; date of microbiome-
923 harvest) to save time on the actual day of microbiome-harvest. To sterilize microfilters needed for fraction-
924 ation of harvested microbiomes (2µm Whatman™ filters; model Puradisc 25 GD2 Syringe Filter, 25mm
925 diameter; Whatman PLC, United Kingdom), we wrapped filters individually in aluminum-foil, then auto-
926 claved these in a 15min-exposure fast-exhaust cycle. On the evening before the day of microbiome-harvest,
927 we set up a custom-made flow-hood on a bench in our Conviron growth-chamber, sterilized the inside of
928 the hood by spraying liberally with 100% ethanol, then allowed the flow of clean air to purify the inside of
929 the hood overnight. Our custom-made hood was constructed of a large plastic tub placed on its side, with
930 the lid cut half so that a lid-portion affixed to the tub could shield the inside of the hood from above (like a
931 sash on a regular flow-hood), whereas the bottom half was kept open to permit access to the inside of the
932 hood. To generate a flow of clean air through the hood, we cut a large hole into the top of the hood (i.e.,
933 one of the original sides of the tub now resting on its side) to fit into that hole the top portion of an air
934 purifier (model HPA104 Honeywell HEPA Allergen Remover, with HEPA filter of 0.3 microns; Honeywell
935 International Inc., Morris Plains, NJ, USA). We operated the purifier at medium flow-setting, which gen-
936 erated an even flow through the hood and minimized any air-vortices that could draw impure air into the
937 hood at high flow-setting. In a pilot test, Petri-plates with PDA-medium, exposed overnight to the flow
938 inside our hood, revealed no visible growth within seven days of incubation of these plates at room tem-
939 perature. Early on a day of a between-generation microbiome-transfer, we moved the tubs with racks of
940 planted, cold-stratified seeds from the cold-room into our growth-chamber, to have sufficient time for com-
941 pletion of all microbiome-harvests and -transfers (the total time needed on the day of microbiome harvest

942 for completion of harvests/transfers of all lines was 8-10 hours, plus an additional 2 hours for distribution
943 of pots in pre-determined randomized arrangements across 8 racks used to support pots in the growth-
944 chamber). We began microbiome-harvests and -transfers immediately after moving pots into the growth
945 chamber, so transferred microbiomes would interact with seeds at the very early stages of germination.

946 **Phenotyping of Plants; Quantification of Above-Ground Biomass:** To select the two best-growing
947 plants from a particular selection line on the day of microbiome-harvest and -transfer, we moved all eight
948 pots from a selection line into a separate, ethanol-sterilized rack, recorded the number of leaves of each
949 plant, and arranged plants visually by apparent above-ground biomass into a size-ranked series (Figure S3).
950 We chose visual sizing rather than weighing for phenotyping of plants, because visual evaluation of all
951 eight plants in a selection-line needed only about 5-10 minutes (including recording the number of leaves
952 for all eight plants), and because microbiomes could be harvested immediately after visually identifying a
953 particular plant for microbiome harvest without first having to cut and weigh above-ground biomass of all
954 plants in a selection line. We harvested rhizosphere microbiomes from only those two plants within a se-
955 lection line that we visually judged to have grown the largest and second-largest above-ground biomass
956 (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 *Phenotyping of Plants; Quantification of Above-Ground Biomass*. Photo by UGM.

957 To test the accuracy of our visual rankings, we later compared these rankings with dry above-ground (shoot)
958 biomass. To weigh shoot-biomass, we cut each plant at soil-level at the time of microbiome harvesting,
959 stored above-ground biomass for drying in an individual paper envelope (Coin Envelope 8cm x 14cm),
960 dried these envelopes/plants for at least two weeks at 60°C in a drying oven, then weighed dry biomass for
961 each plant to the nearest 0.1mg. Although we judged above-ground plant-biomass visually on the day of
962 microbiome harvesting, of the 80 lines judged during our entire experiment (5 SOD-lines + 5 ALU-lines
963 judged each Generation, times 8 Generations; Table S1), we picked for microbiome harvest the combination
964 of largest (#1) and second-largest (#2) plant in 56.25% of the cases; the largest (#1) and third-largest (#3)
965 plant in 27.50%; the largest (#1) and fourth-largest (#4) plant in 6.25%; the second-largest (#2) and third-
966 largest (#3) in 5.00%; the second-largest (#2) and fourth-largest (#4) in 5.00%; and never any lower-ranked
967 combination. In cases when we did not identify visually the combination of #1 and #2 plants as later deter-
968 mined by dry weight, the slightly lighter #3 or #4 plants were typically within 0.2-4mg (0.5-9% of total
969 dry-weight) of the two best-growing plants in the same selection-line (Table S1). Moreover, because har-
970 vested microbiomes of the two chosen plants were mixed for propagation to the next microbiome-genera-
971 tion (see below *Microbiome Mixing*), we harvested in 100% of the cases the microbiomes from either the
972 best-growing or second-best growing plant into the mixed microbiome that we then propagated to the next
973 microbiome generation (i.e., a microbiome of one of the two best-growing plants was always included in
974 the propagated microbiome mix). In sum, therefore, our method to visually judge plant-size was both time-
975 efficient (about 5-10 minutes needed to visually size all plants in a selection-line and record number of
976 leaves for each plant), ant our method was also accurate to identify those plants that had grown biomass
977 well above-average within any given selection-line (i.e., our methods were accurate to visually identify
978 plants that were likely associated with microbiomes that conferred salt-tolerance to plants).

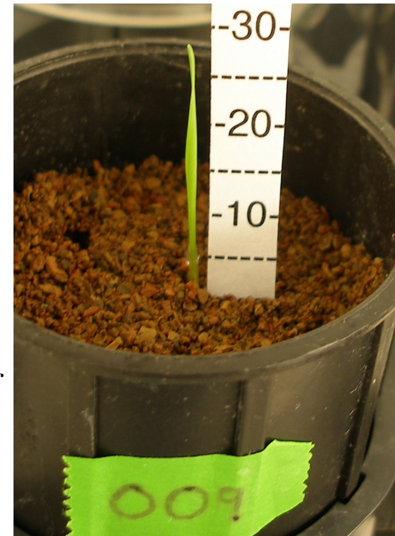


Figure S4. Measuring length of the first leaf on Day 3, using an ethanol-sterilized dry paper-strip with a millimeter-scale. Photo by UGM.

In some cases, on the day of microbiome-harvest, more than two plants of the same selection line appeared to have 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) after Day 5, recording the number of leaves grown by a plant every other day up to a time when plants had grown about 10 leaves; and (iii) once plants had grown about 10 leaves, visual ranking of relative plant size (visual appearance of overall biomass) on a 10-point scale from 1-9, using the protocol below. ***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 when the first shoots became visible after 55-70 hours (Table S1). To quantify this early growth each selection-cycle, 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 millimeter with an ethanol-sterilized ruler (millimeter scale 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 the translucent 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, thus facilitating priority effects in microbiome recruitment into the initial microbiome assembled by a plant. ***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). We counted the number of leaves regularly after Day 6 (Table S1), typically every other day. 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 plants of average size to be scored as 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 high repeatability of this 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 day of microbiome-harvesting.

1030 **Microbiome-Harvesting from a Rhizosphere & Microbiome Mixing:** We performed all steps of micro-
1031 biome harvest and microbiome transfer in a clean-air flow-hood (see above) set up on a bench inside our
1032 growth-chamber (i.e., we did not have to move microbiomes/pots of selection lines outside the growth
1033 chamber), and we sterilized hands and work-surfaces regularly with 100% ethanol to prevent contamination
1034 and cross-contamination of samples. After choosing the two plants with the greatest above-ground biomass
1035 (see above *Phenotyping of Plants*), we cut each plant at soil level with ethanol-sterilized scissors, stored the
1036 above-ground portion in an envelope for drying, and harvested rhizosphere microbiomes immediately to
1037 minimize microbiome changes in the absence of plant-control in the rhizosphere. To extract the root-system
1038 from a pot (Deepot) with minimal contamination, we held the shoot-stub at the soil surface with ethanol-
1039 sterilized forceps, tilted the pot such that PPC-soil would gradually loosen and fall out when squeezing the
1040 plastic pot, until the root-structure could be extracted as a whole by gentle pulling at the main root with the
1041 forceps. In most cases, the entire root structure could be extracted whole, with some loss of fine roots
1042 embedded in spilled soil. Because we were interested in harvesting microbiomes that were in close physical
1043 association with a plant (i.e., we were interested in rhizoplane bacteria, plus any endophytic bacteria if they
1044 were released during root processing as a result of any root damage), we discarded any soil adhering loosely
1045 to the roots. We dislodged loosely adhering soil by knocking the root-system gently against the wall of an
1046 autoclaved aluminum-pan (e.g., Hefty EZ Foil Roaster Pan; 32cm length x 26cm width, vertical depth
1047 11cm) such that any dislodged soil would fall into the pan without the roots contacting any discarded soil.
1048 We then cut off the top 2 cm of the root-system (i.e., roots close to the soil surface), then transferred the
1049 remaining root-system into a 50 ml Falcon tube filled with 45 ml of salt-nutrient buffer (the same buffer
1050 used also to hydrate soils of the subsequent microbiome-generation, but diluted to half-concentration to
1051 suspend harvested microbiomes; see above *Preparations for Microbiome-Harvesting*). We repeated this
1052 process with the second plant chosen for microbiome-harvest from the same selection line, and added this
1053 second root-system to the same Falcon tube as the first root-system. Combining both root-systems for mi-
1054 crobiome-harvesting generated a so-called mixed-microbiome collected from two 'mother rhizospheres'
1055 (see *Mixed Microbiome Propagation*; and Box 3 in Mueller & Sachs 2015), which we then transferred
1056 within the same selection line to all eight 'offspring plants' of the next microbiome-generation.

1057 **Microbiome-Fractionation with Microfilters:** To dislodge microbes from roots and from soil-particles
1058 adhering to roots, we turned a closed Falcon tube upside-down 50 times, then permitted the solids to settle
1059 in the bottom of the tube for 1 minute. A 1cm-deep sediment of PPC-soil particles typically accumulated
1060 in the bottom cone of a Falcon tube, with the roots settling on top of this sediment, and small particles and
1061 colloids remaining suspended in the salt-nutrient buffer. We aspirated 20 ml of this suspension with a sterile
1062 20 ml syringe (external syringe diameter fitting into a 50 ml Falcon tube), then attached to the syringe's
1063 Luer-lock a 2 μm Whatman microfilter (model Puradisc 25 GD2 Syringe Filter, 25 mm diameter; Whatman
1064 PLC, United Kingdom), then filtered the aspirated suspension into an empty sterile 50 ml Falcon tube.
1065 Making sure that the exterior of the syringe did not become contaminated during this first filtering, we
1066 repeating this step with the same syringe to filter another 15-20 ml of the suspension, then mixed the com-
1067 bined filtrates by inverting the Falcon tube several times. The total volume of 35-40 ml filtrate was suffi-
1068 cient to inoculate 8 'offspring plants' each with 4 ml filtrate (total of 8 x 4 ml = 32 ml needed). In pilot
1069 tests, plating on PDA-medium 10 μL of this filtrate (2μm filter) yielded thousands of bacterial colony-
1070 forming-units (CFUs) but no fungal CFUs within 24 hours growth; whereas plating on PDA-medium 50
1071 μL of this same filtrate that had been filtered a second time with a 0.2μm filter (VWR Sterile Syringe Filter,
1072 0.2μm polyethersulfone membrane, 25mm diameter; Catalog #28145-501; retains even the small-sized *Bre-*
1073 *vundimonus diminuta*) did not yield any visible microbial growth on PDA plates kept for 7 days at room
1074 temperature. These results justified addition of a third control-treatment in Generation 9 (0.2μm filtration
1075 of suspension) to test growth-promoting effects of chemicals and viruses co-harvested with the harvested
1076 microbiomes (see below *Crossing Evolved SOD- and ALU-Microbiomes with SOD- and ALU-Stress*). Alt-
1077 hough a 0.2 μm filter may not eliminate ultra-small bacteria (e.g., Luef *et al* 2015; we did not use filters of
1078 smaller pore size because it was too difficult to press liquid through such filters with a hand-held syringe),
1079 our control comparison between 2.0 μm-filtered and 0.2 μm-filtered bacterial microbiomes can still test

1080 whether the bulk of the bacterial microbiome (in size range 0.2-2.0 µm) or alternatively any smaller-sized
1081 organisms (viruses, ultra-small bacteria) plus solutes in the soil are responsible for conferring salt-tolerance
1082 to plants in our experiment.

1083 **Inoculation of Seeds; Transfer of Microbiomes to Plants of the Next Microbiome-Generation:** During
1084 planting, the 200 pots of each microbiome-generation had been ordered numerically in the 4 racks used for
1085 stratification in the cold-room, so it was easy to locate in these racks a pot with a particular number that had
1086 been assigned to a specific selection-line and needed to be inoculated with a microbiome. To inoculate a
1087 seed planted in a particular pot, we moved the pot into our clean-hood in our growth chamber, opened the
1088 pot's translucent cap inside the hood (using one hand to hold the pot while opening the cap with thumb and
1089 index finger of that same hand), then used a 5 ml pipetter to transfer 4 ml of the microbiome-filtrate to the
1090 center soil in a pot where a seed had been planted before vernalization/stratification. We spread the 4 ml
1091 filtrate across an area with a radius of about 5mm around a seed, applying some of the filtrate directly onto
1092 the seed (the exact location of the seed was indicated by its awn protruding above the soil; see *Planting*
1093 above), and we spread some of the filtrate also in a circular motion onto the surrounding soil within 5 mm
1094 distance of a seed. To keep the filtrate well-mixed during the time needed to inoculate all 8 'offspring soils'
1095 of the same selection-line, we repeatedly mixed the filtrate in the Falcon tube with the pipette-tip before
1096 aspirating a 4 ml-aliquot to inoculate the next pot. We then taped a small tag of labeling-tape to the lid of
1097 each pot that had received an inoculum (as a check to verify later that all pots had received an inoculate, no
1098 pot was accidentally skipped, or any pot was accidentally inoculated twice), then we returned the pot to its
1099 appropriate position in one of the four racks. After inoculation of all 200 plants within a Generation, all
1100 pots were distributed among the 8 racks used to support plants in the growth chamber (see below *Random-*
1101 *ization of Pot-Positions in Racks*).

1102 Each pot was capped for first 4 days to promote priority effects during microbiome establishment (i.e.,
1103 capping prevented immigration of extrinsic microbes into the soils/microbiomes for the first 4 days; see
1104 above *Planting*), but all caps were removed on Day4 because the tallest plants (35-40mm tall on Day4)
1105 were close to reaching the cap-ceiling. We monitored growth during first 5 days (see above *Phenotyping*)
1106 by recording length of the first leaf on Days 2-5, and recording day of appearance of the second leaf (typi-
1107 cally on Days 6 or 7; Table S1). Seeds that did not germinate or that germinated very late (i.e., no above-
1108 ground growth visible by Day 4) were extracted from pots with forceps and inspected. Most of these seeds
1109 had failed to grow both a rootlet and shoot by Day4, but some seeds had grown a rootlet but no shoot. In a
1110 typical microbiome-generation, about 88-100% of the plants showed a visible shoot within the first 3 days
1111 (Table S1). Germination rates were therefore good overall, and most lines had the planned 8 replicates
1112 (sometimes 7 replicates, rarely 6 replicates, if some seeds failed to germinate; see Table S1). Germination-
1113 rates were often minimally higher in the Null-Control treatments compared to other treatments of the same
1114 soil-stress (slightly fewer non-germinating seeds in Null-Controls), and, across all plants, germination-rates
1115 were minimally higher in ALU-soil than in SOD-soil (see Tables S1a-c); we did not analyze these trends
1116 for statistical significance because differences were minimal, but we simply note here these general patterns
1117 became apparent only when pooling information across all 10 selection cycles.

1118 **Randomization of Pot-Positions in Racks in Growth-Chamber:** Deepots were supported in D50T racks
1119 (Stewe & Sons, Tangent, Oregon, USA). Each rack can hold a total of 50 pots (5 rows of 10 pots each), but
1120 to prevent contact of leaves from different plants and to reduce accidental between-pot transfer of microbes
1121 during watering (see below *Watering*), we used only 25 rack-positions (25 pots per rack, total of 8 racks,
1122 for a total number of 200 pots per selection cycle). Pots within a selection line were first assigned by block-
1123 ing to particular rack (e.g., of the 8 replicates within a selection line, one replicate was assigned to each of
1124 the 8 racks). Within each rack, however, we randomly assigned pot positions, using the *Random Sequence*
1125 *Generator* option at Random.Org (www.random.org/sequences/). For Generations 0-8 (growth cycles 0-8),
1126 Tables S1a&b lists pot positions (#1-#25) from different treatments within each rack (Rack #1-8), corre-
1127 sponding 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	

1128 For the final Generation 9 when we added two more control-treatments (details below), we randomized 400
1129 pot-positions by first assigning a pot to one of 8 racks, then randomizing position within each of the 8 racks
1130 (50 pots/rack; the position-numbering of pots shown for Generation 9 in Table S1c for each rack is num-
1131 bered consecutively, starting in left top corner, without leaving empty spacer-slots between pots).

1132 The 8 racks were positioned in two groups of 4 racks each on two comparable shelves at either side of the
1133 growth chamber. Within each selection cycle, we rotated these 8 racks in clockwise rotation each day (mov-
1134 ing one rack from right shelf to left shelf, and one rack from left to right shelf), and at the same time we
1135 also turned each rack (such that the rack-side facing the chamber wall one day faced the chamber center the
1136 next day). This rotation-turning scheme aimed to minimize possible environmental influences dependent
1137 on location of a rack on the two shelves, and to reduce any minimal differences in light-level, air-circulation,
1138 or any such uncontrolled environmental factors that may exist between different positions on the two
1139 shelves in our growth chamber. Despite our effort to minimize rack effects through daily rack-rotation and
1140 rack-turning, as well as randomization of processing order (e.g., watering, phenotyping, microbiome-har-
1141 vesting), we had occasionally racks of poorer or better plant growth (e.g., Rack 7 of Generation 9 had lower
1142 average seed production compared to other racks, because many plants in that rack did not flower, or flow-
1143 ered late). We do not know the exact causes for occasional small rack-effects, because we believe we treated
1144 plants across all racks equally.

1145 **Starter Inoculum for Microbiomes at Beginning of the Experiment for Baseline Generation 0:** We
1146 used a single inoculum-batch to inoculate all replicate pots of the initial baseline Generation 0. To prepare
1147 that inoculum, we filtered bacterial communities from a mix of roots and adhering soil taken from three
1148 principal sources: (a) root-systems with adhering soil of three local grass species (*Bromus* sp., *Andropogon*
1149 sp., *Eragrostis* sp.) collected into individual plastic bags on 3.Jan.2015 (about 90 minutes before microbi-
1150 ome harvesting) at restored native habitat at Brackenridge Field Lab of the University of Texas at Austin
1151 (www.bfl.utexas.edu/); (b) root-systems with adhering soil of 40 16-day-old *B.distachyon* Bd3-1 plants
1152 grown in PPC-soil Deepots as part of a pilot experiment quantifying the effect of salt in soil on the growth
1153 rate of *B. distachyon* (see below *Salt Treatments*); and (c) old root-systems with adhering soil of 15 Bd3-1
1154 plants grown in PPC-soil Deepots, but that had been stored in the soil/Deepots in a cold-room (6°C) for 7
1155 months after completion of a previous low-nutrient microbiome-selection experiment. We combined roots
1156 and rhizosphere soils from these three sources in order to capture a diversity of microbes into our starter
1157 inoculum, and we included Bd3-1 rhizospheres in order to capture specific microbial taxa that may be
1158 readily recruited by *B. distachyon* into its rhizosphere microbiomes. We suspended this mix of roots and
1159 rhizosphere soil in 200 ml e-pure water, blended the mix for 30 seconds in an autoclaved Waring blender
1160 to generate a liquid slurry, allowed the solids to settle in the blender for 1 minute, then decanted the super-
1161 natant into a separate autoclaved beaker. Adding each time 200 ml e-pure water, we repeated this blend-
1162 ing/decanting with the remaining slurry three more times to collect a total of about 600 ml supernatant.
1163 Using vacuum filtration, we pre-filtered this supernatant in a Buchner funnel through filter paper (Ahlstrom
1164 filter paper S02-007-42), eliminating larger particles suspended in the supernatant. To harvest only bacterial
1165 microbiome components (and viruses) from this pre-filtrate, we filtered the supernatant a second time in a
1166 laminar-flow hood, using a 60 ml syringe fitted with a 2 μm Whatman™ microfilter (Puradisc 25 GD2
1167 Syringe Filter, 25 mm diameter; Whatman PLC, United Kingdom) to generate the bacterial mix for inocu-
1168 lation of replicate pots of our initial baseline Generation 0. Because the Puradisc filters became clogged
1169 after filtration of about 70-100 ml supernatant, we used 8 Puradisc filters to process about 600 ml of filtrate.

1170 We reserved 500 ml of this filtrate for inoculation of 160 randomly-assigned pots in a Bacterial-Inoculate
1171 treatment (80 Bacterial-Inoculate with SOD soil, 80 Bacterial-Inoculate with ALU soil), and filtered the
1172 remaining 100 ml with 0.2 µm filters (VWR Sterile Syringe Filter, 0.2µm polyethersulfone membrane, 25
1173 mm diameter; Catalog #28145-501) for inoculation of 40 pots in Null-Control treatments (20 Null-Control
1174 with SOD soil, 20 Null-Control with ALU soil). The Null-Control treatments controlled for, after elimina-
1175 tion of bacteria, the effect of any chemicals and viruses that may have been co-harvested from rhizosphere
1176 roots and soils. Seeds in the Bacterial-Inoculum and the Null-Control treatments were inoculated following
1177 the procedure described above (see *Inoculation of Seeds*), except that each seed of Generation 0 received 2
1178 ml inoculate, whereas each seed of subsequent Generations 1-9 received 4 ml inoculate transferred between
1179 generations. During inoculation of seeds, we mixed the stock filtrates regularly to prevent bacterial sedi-
1180 mentation and to insure standardized inoculation of all replicates in a treatment. We needed about 3 hours
1181 to complete the entire process from root collection to conclusion of all filtration steps, and another 2 hours
1182 to apply inoculate-aliquots of the filtrates to each of the assigned pots. We then moved all pots immediately
1183 into our growth chamber, and set out all 200 pots of Generation 0 into randomized positions in 8 racks (see
1184 above *Randomization of Pot Positions*; Table S1).

1185 To test for live bacteria in our 2 µm filtrate used as the Starter Inoculum, we plated on PDA-medium (2
1186 replicate plates) 10 µL each of the 2 µm filtrate and maintained plates at room temperature; the plates
1187 showed thousands of bacterial colony-forming-units (CFUs) within 24 hours, but no fungal growth within
1188 7 days. To test for absence of live bacteria in our 0.2 µm filtrate, we plated on PDA-medium (3 replicate
1189 plates) 50 µL each of the 0.2 µm-filtrate; these platings did not yield any visible growth on the PDA plates
1190 kept for 7 days at room temperature. These results indicate (i) a great abundance of live bacteria (and ap-
1191 parently no live fungi) in our initial inoculum, and (ii) elimination by the 0.2 µm filters of live bacteria that
1192 would be apparent when plating out such filtrate on PDA plates. The latter justified our use of a third
1193 control-treatment in Generation 9 (0.2 µm filtration of suspension to test growth-promoting effects of chem-
1194 icals and viruses co-propagated with the harvested bacterial microbiomes; see below).

1195 **Selection of Microbiomes from Generation 0 to Inoculate Plants from Generation 1:** At the start of
1196 our experiment, we did not assign microbiomes (i.e., pot numbers) from Generation 0 to specific selection
1197 lines, to permit selecting the best-growing plants from Generation 0 to contribute microbiomes to the se-
1198 lection-lines starting with Generation 1. We chose this particular assignment rule because random assign-
1199 ment to selection lines would result in some cases for a poorly-growing plant to contribute microbiomes to
1200 Generation 1, and we wanted to increase the chance of obtaining a response to microbiome-selection in the
1201 fewest rounds of selection. To select plants for harvesting and propagation of rhizosphere microbiomes, we
1202 ranked, separately for plants in the SOD and ALU treatments, the plants in the Bacterial-Inoculate treat-
1203 ments of Generation 0 by relative size, then picked the 10 best-growing plants of each salt-treatment to
1204 contribute microbiomes to the selection-lines that we started with Microbiome-Generation 1 (Table S1).
1205 On Day 22 of Generation 0 (day of microbiome harvest and microbiome transfer; Table S1), we first ranked
1206 plants by relative size-scores (i.e., average size-score averaged across three scores received by a plant on
1207 Days 18, 19, 20; see protocol *Above-Ground Biomass Estimated on a 10-Point Scale*), then used number
1208 of leaves recorded on Day 21 as a second criterion to differentiate between plants of equal average size-
1209 score. Among the 10 best-growing plants within each of the SOD and ALU salt-treatments, we paired plants
1210 randomly to generate 5 combinations (2 plants each) for mixing of harvested microbiomes within each pair
1211 (i.e., harvested root-systems were combined from the two plants to harvest a mixed microbiome from both
1212 plants, as described above for *Microbiome Mixing*). Within each of the SOD and ALU treatments, the 5
1213 mixed microbiomes from Generation 0 were assigned randomly to 5 SOD and 5 ALU selection lines (each
1214 with 8 'offspring microbiome' replicates per line) that started with Generation 1. Microbiomes were har-
1215 vested and processed from chosen rhizospheres as described above. At the end of Generation 0, as well as
1216 at the end of each subsequent Generation, we cut all plants at soil level to preserve above-ground growth
1217 for later weighing of dry biomass for each plant (Table S1; see also above *Phenotyping*).

1218 **Salt- and Control-Treatments in Generation 0-9; Sample Sizes Per Treatment:** Starting with Genera-
1219 tion 1 and continuing until the last Generation 9, we included the two aforementioned salt-treatments (SOD
1220 and ALU soil) with *5 SOD Microbiome-Selection* Lines (8 replicates each, for a total of 40 replicates) and
1221 with 5 *ALU Microbiome-Selection* Lines (8 replicates each, for a total of 40 replicates). Also starting with
1222 Generation 1 and continuing until the last Generation 9, we included two control treatments for each of the
1223 SOD and ALU treatments, *Null-Control* (on SOD- and on ALU-soils) and *Fallow-Soil Microbiome Prop-*
1224 *agation* (on SOD- and on ALU-soils). ***Control 1, Fallow-Soil Microbiome Propagation***: For this control,
1225 we harvested microbiomes from fallow soil (from pots without a plant), then propagated the harvested
1226 microbiome to sterile fallow soil to perpetuate 'Fallow-Soil Microbiomes' in the absence of plant influences
1227 (e.g., absence of plant secretions into the soil). Fallow-soil pots were treated throughout each selection-
1228 cycle exactly like pots with plants; for example, these fallow-soil pots received the same amount of water
1229 whenever all other pots were watered. Each Fallow-Soil-Line had only one replicate pot, so a microbiome
1230 harvested from fallow-soil was propagated to a single pot of the next selection-cycle to continue a particular
1231 Fallow-Soil-Line; a portion of the same microbiome harvested from fallow-soil was also transferred to pots
1232 with plants of the next cycle to assay the effect of a harvested fallow-soil-microbiome on plant growth (but
1233 those microbiomes were later not propagated to subsequent Generations; i.e., these inoculations of control
1234 plants aimed at assaying the effect of un-selected fallow-soil-microbiomes on plant growth under the in-
1235 creasing salt stress that we increased stepwise between Generations; see above *Logic of Salt-Stress Ramp-*
1236 *ing*). We chose a control of fallow-soil microbiome-propagation because this treatment resembles the kind
1237 of microbiome conditions that many plants encounter in horticulture and agriculture (soils are left fallow
1238 for some time before planting). Changes in fallow-soil microbiomes between Generations reflect ecological
1239 changes as microbe communities change over time, as well as any microbial immigration from external
1240 sources (e.g., airborne microbes raining into the soil; perhaps also unintended accidental cross-contamina-
1241 tion between soils from different pots). We initially allocated 8 control-replicate test-plants per Fallow-
1242 Soil-Line to test the effect of each harvested fallow-soil microbiomes on plant growth (total of 5x8=40
1243 replicates for SOD, 5x8=40 replicates for ALU), but we reduced the number of control-replicate test-plants
1244 for each of the 5 Fallow-Soil-Control replicates per line in later Generations (first reducing the number to
1245 6 control-replicate test-plants per line in Generation 4; then reducing the number to 4 control-replicate test-
1246 plants per line in Generation 5-9), because it became clear during the first few Generations that plants
1247 receiving fallow-soil-microbiomes grew poorly under the salt stresses, far inferior to plants in the corre-
1248 sponding selection-lines where plants received artificially selected microbiomes (i.e., we could differentiate
1249 averages between fallow-soil and microbiome-selection lines even with the smaller number of control-
1250 replicate test-plants in the fallow-soil controls). **Control 2, *Null-Control***: For this control, plants received
1251 no experimental microbial inoculation; instead, these control plants received on the day of microbiome
1252 transfer an aliquot of the same sterile salt-nutrient buffer that we used to harvest microbiomes and then
1253 transfer to seeds of the next Generation. Because our pots were capped for the first 4 days of seed germi-
1254 nation, Null-Control-plants grow initially under sterile conditions (before caps are lifted on Day4), but air-
1255 borne microbes can enter the sterile soil and rhizospheres of Null plants from the air after Day4 once caps
1256 are lifted from pots. In pilot experiments, Null-Control plants invariably grew better during the first 10-20
1257 days than any plant inoculated with microbiomes (see Table S1a-c), possibly because Null-Control plants
1258 do not need to expend resources to mediate interactions with microbes, or because Null-Control plants do
1259 not have to compete with microbes for nutrients in the soil. Despite the microbially unusual soils of Null-
1260 Control plants, we included this control treatment because it was easy to set up (no microbiomes needed to
1261 be harvested to inoculate Null-Control soils), because Null-Control conditions were easy to standardize
1262 within Generations, and because Null-control Conditions may even be standardized between Generations
1263 if microbial immigration (i.e., rain of airborne microbes) into Null-Control soils can be assumed to be rel-
1264 atively constant over time. We initially allocated 10 replicates of SOD pots and 10 replicates of ALU pots
1265 to Null-Controls, but we increased the number of replicates in later Generations for the Null-Control treat-
1266 ments (first we increased to 20 replicates in Generation 4, then to 30 replicates in subsequent Generations)
1267 in order to improve the estimates (reduce confidence intervals) of the average growth of plants in Null-
1268 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. We found in pilot experiments that 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, thus leaching nutrients and salts. Keeping pot weights well below 260g at all times therefore prevented leaching of nutrients and salt. To prevent cross-contamination (microbe-exchange) between pots, we did not use 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 of plant growth (we removed caps during the afternoon of Day 4), 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 Day 5 of each selection-cycle, and thereafter approximately every 2 days (sometimes also at 1-day or 3-day intervals, depending on humidity in the growth chamber and on experimenter time-constraints), but we did not pre-plan to follow a rigorous 2-day watering schedule (see Table S3). We typically watered 15-25 ml per pot 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, 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 0.5 milliliter 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 of the carboy, mounted the ethanol-sterilized Seripetter Dispenser onto the carboy, 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). Table S3 summarizes the exact watering schedules, volumes of water added, and salt concentrations of the water added.

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 well 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, because in salt-free soils virtually all plants would have flowered.

1317 **Table S3. Ramping Salt-Stress Between and Within Microbiome-Generations.** Soil in each pot was initially hy-
1318 drated with 102 ml salt-solution [94 ml added to soil prior to autoclaving; 4 ml during planting; and 4 ml during
1319 microbiome inoculation in Generations 1-9 (2 ml in Generation 0); see *Planting* and *Inoculation of Seeds*]. In the
1320 baseline Generation 0, plants were watered only with unsalted water, but starting with Generation 1, we increased
1321 salt-stress *within* each Generation by watering with salted water. Because we capped pots for the first 4 days to control
1322 initial microbiome assembly, we started watering each Generation on Days 5 or 6. Pots of Generations 0-3 were
1323 watered more because of low humidity (because of heating of Greenhouse Facility in winter) and pots of Generations
1324 4&5 were watered less because of high humidity (unusual rainfall in spring, increasing general humidity; see *Growth*
1325 *Chamber*). Pots were watered more in the second half of Generation 9 because plants grew large and transpired more
1326 water. Plants of Generations 0-8 were short-cycled to grow only for 20-30 days before microbiome transfer (to about
1327 10-15 leaves for the largest plants); plants in Generation 9 were grown for 68 days to permit ripening of seeds.
1328 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**

Analysis of Relative Plant Performance: Because successive generations were not grown under precisely identical conditions (e.g., we had to increase the duration of selection-cycles in later generations because plant-growth decelerated slightly under the increasing salt-stress; we had to adjust watering schedules because of uncontrolled humidity in our growth chamber), 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 during 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 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; Solute-Control 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; and (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). 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 *Solute Control*, 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 Control* 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

1361 specificity of the salt-tolerance-conferring effects of the microbiomes. This Cross-Fostering treatment al-
1362 lowed us to address the question whether the salt-tolerance-conferring effects of the *SOD-selected* micro-
1363 biomes confer these effects only under SOD-stress, or also in ALU-stress; and vice versa the additional
1364 question whether the salt-tolerance-conferring effects of the *ALU-selected* microbiomes confer these effects
1365 only under SOD-stress, or also in ALU-stress. This basic cross-fostering design was inspired by the exper-
1366 imental methods developed by Lau & Lennon (2012), except that, in contrast to Lau & Lennon (2012), our
1367 plant-populations did not evolve, and that we artificially selected on microbiomes (whereas in Lau & Len-
1368 non plant populations evolved under artificial selection and microbiomes were allowed to change ecologi-
1369 cally, without artificial microbiome selection).

1370 **Phenotyping of Plants and Microbiome-Harvesting in the Last Generation 9:** In contrast to Genera-
1371 tions 0-8 when we used early growth of plants (above-ground biomass during first 3-4 weeks) as host-
1372 phenotype to select indirectly on microbiomes, in the last Generation 9, we allowed plants to mature for 68
1373 days (10 weeks), such that plants could flower and seeds could ripen. Because of the longer growth, some
1374 plants started to senesce towards the end of Generation 9 and some individual flower-stalks of some plants
1375 started to dry (no plant dried completely by the end of Generation 9); comparisons of metagenomic infor-
1376 mation from Generation 9 versus earlier Generations therefore need to be interpreted with caution (i.e.,
1377 metagenomic information from Generation 9 is best compared between treatments within that Generation).
1378 Despite these limits of metagenomic comparisons pertaining to Generation 9, we decided to grow plants to
1379 seed in this last Generation because we were interested in understanding how treatment differences in
1380 above-ground biomass apparent at Days 20-30 (when we harvested microbiomes in Generations 0-8) would
1381 translate into treatment differences in flowering and seed-set if plants were allowed to grow longer. Apart
1382 from the longer duration of Generation 9 to permit flowering, a second important difference is likely the
1383 gradually increasing salt-concentration in soils of Generation 9 that were watered 34 times with salted water
1384 over 68 days (Table S3), in contrast to watering with salted water fewer times over the shorter 20-30 days
1385 in Generations 0-8 (9-12 waterings, depending on the Generation; see Table S3). At the end of Generation
1386 9, all plants were cut at soil level, above-ground biomass was preserved for each plant in individual enve-
1387 lopes (for drying and later weighing of seeds and overall biomass; see above), and each root systems was
1388 extracted from its pot and placed into an autoclaved aluminum-tub for further processing. Harvested root-
1389 systems of plants from Generations 0-8 were comparatively small (filling about 30-60% of the soil-volume
1390 in each pot), but root-systems at the end of Generation 9 were large and extended through the entire soil-
1391 volume in each pot. We shook-off most of the adhering soil from each root system of Generation 9, cut off
1392 and discarded the top-most 2cm portion with sterile scissors, then cut the remaining root-system lengthwise
1393 (top to bottom) to preserve half of the root-system in 100% ethanol (for metagenomic screens of bacterial
1394 communities), whereas we flash-froze (in liquid nitrogen) the other half of the root-system for possible later
1395 transcriptomics analyses. For some of the best-growing plants in the SOD- and ALU-selection-lines, we
1396 also preserved a representative portion of the root-system in sterile 20% glycerol (for storage at -80°C for
1397 possible later isolation of microbes). Processing all root-systems (nearly 400 plants) took considerable time
1398 over three successive days (Days 68-70 of Generation 9). Although we processed plants from 3 racks on
1399 Day 68 (Racks #3, #8, #7), 3 racks on Day 69 (Racks #6, #2, #4), and 2 racks on Day 70 (Racks #1, #5),
1400 we summarize all weight-data of these plants in Table S1c in columns labeled Day 68.

1401 **Statistical Analyses: *Plant Biomass, Generations 1-8*:** We performed all analyses in R v3.3.1. We as-
1402 sessed differences in above-ground plant biomass (dry weight) among treatments of Generations 1-8 by
1403 fitting the data to a generalized linear mixed model with a gamma error distribution. Line was entered as a
1404 random effect; generation, treatment, and their interaction were entered as fixed effects. Statistical signifi-
1405 cance of fixed effects in the GLMMs were assessed with likelihood ratio tests and Tukey tests employed
1406 for post-host comparisons of treatment means. Selection of the appropriate error distribution for the
1407 GLMMs was evaluated by visual inspection of Q-Q plots, and homoscedasticity was assessed using plots
1408 of the residuals of the model against the fitted values. Because plants were short-cycled in Generations 1-8
1409 (i.e., grown long enough so plants produce typically 9-15 leaves, too short to bolt and produce flowers; see

1410 Table S2), plants did not produce any seeds, and therefore only above-ground plant biomass (dry weight)
1411 could be compared between treatments of Generations 1-8.

1412 **Statistical Analyses: *Total Seed Weight, Generation 9*:** Because plants were grown long enough to flower
1413 in Generation 9, we compared total seed weight per plant among microbiome-selection treatments (plant
1414 present; microbiomes are harvested from plants for transfer to seeds), Fallow-Soil Control (no plant present;
1415 microbiomes are harvested from fallow soil for transfer to seeds), and Null-Control (no initial microbiome
1416 inoculation, microbes establish in microbiomes when microbes "rain in" from the air). Because plants were
1417 strongly salt-stressed in Generation 9 and many plants therefore did not flower or only produced very few
1418 seeds, the distribution of data was not normal (Figure S5 top-left). We therefore attempted several data-
1419 transformations to approximate normality, including *square-root(seed weight)* transformation (Figure S5
1420 top-right), *log(seed weight)* transformation [excluding the plants that generated zero seeds because log(0)
1421 is undefined; Figure S5 bottom-left], and *log(seed weight +1)* transformation (making it possible to retain
1422 the plants that produced zero seeds, because seed-weight values of all plants was increased by 1mg; Figure
1423 S5 bottom-right). None of these transformations generated a distribution that approximated normality (Fig-
1424 ures S5b-d). We therefore used Kruskal-Wallis tests for non-parametric evaluation of treatment differences;
1425 and we used Mann-Whitney U-tests for non-parametric post-hoc comparisons between treatment means,
1426 correcting p-values using the false discovery rate. All tests were two-tailed with alpha=0.05.

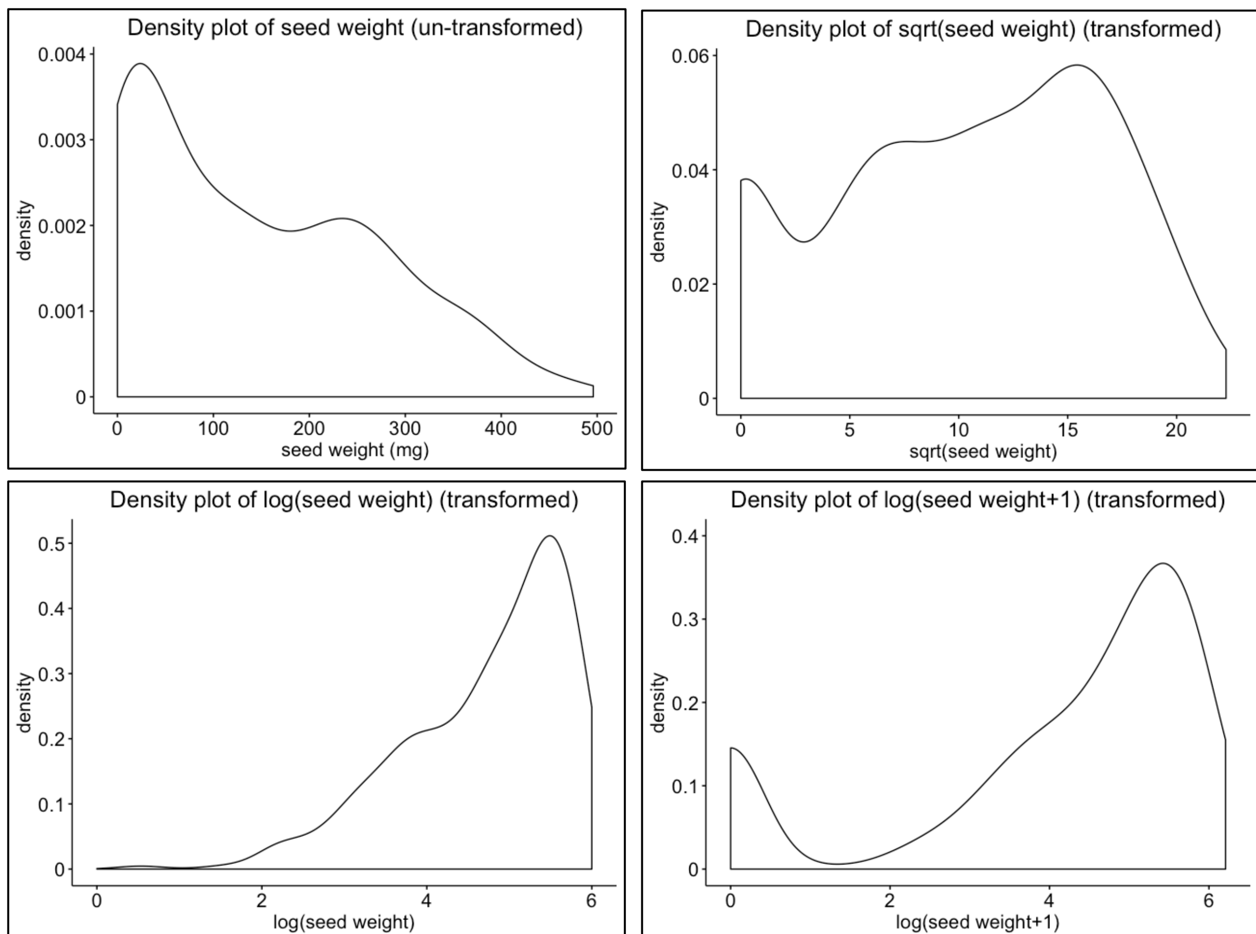


Figure S5. Top-left: untransformed seed-weight data in milligram (mg), indicating a skewed distribution, with many plants producing no or few seeds because of the extreme salt stress during Generation 9. **Top-right:** square-root transformed seed-weight data. **Bottom-left:** log-transformed seed-weight data, excluding seed-weights of zero because log(0) is not defined. **Bottom-right:** log-transformed seed-weight+1 data. None of the three transformations generated a distribution that approximated normality, and we therefore used non-parametric tests to evaluate differences in seed production between treatments.

RESULTS

Generations 1-8: 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 the fallow-soil and the null-control treatments (beta=0.07 ± 0.06, z=1.1, p=0.29). The lack of a significant interaction between treatment and generation (Chisq=15.2, p=0.37) indicates that gains in plant biomass were realized quickly in the first few selection cycles, and that the advantage of the plant-present treatment over the fallow-soil treatment was 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 in parentheses) for each comparison. Significant comparisons are indicated in bold. Np = Fallow soil microbiome-propagation control, Null=Null control line, Pp=Plant-present microbiome-selection line, PpFilt=Plant-present microbiome-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)**

Generations 1-8: 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, Chisq=25.7, p<0.001; Generation, Chisq=753.7, p<0.001; Treatment x Generation, Chisq=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 4 and 5, which eliminated the significant interaction between treatment and generation (LRT: Treatment, Chisq=17.8, p<0.001; Generation, Chisq=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 in 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 plants generated 26% greater biomass 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 in 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).

1468 **Table S4.** Mann-Whitney pairwise comparisons of total seed weight in the aluminum-sulfate (ALU) treat-
1469 ments. Values represent the test statistics (p-value in parentheses) for each comparison. Significant com-
1470 parisons are indicated in bold. Np = Fallow soil microbiome-propagation control, Null=Null control line,
1471 Pp=Plant-present microbiome-selection line, PpFilt=Plant-present microbiome-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)**

1472

1473 **Interactions between selection history and salt stress on plant fitness:** By growing plants with microbi-
1474 omes from selection lines under both sodium- and aluminum-sulfate stress (*Cross-Fostering Control*), we
1475 examined whether microbiome selection produced microbiomes that conferred a salt-specific effect on
1476 plants (e.g., whether microbiomes selected to confer tolerance to SOD conferred such tolerance only under
1477 SOD stress but not to ALU stress), or alternatively whether selected microbiomes produced a generalized
1478 improvement in plant fitness for both SOD and ALU stress. There was a significant interaction between
1479 selection history and the type of salt stress to which plants were exposed in the last generation on seed mass
1480 (Analysis of deviance: Selection history, $F_{1,8}$=<0.01, p=0.99; Salt exposure, $F_{1,141}$=5.82, p=0.017; Selection
1481 history x Salt exposure, $F_{1,141}$=6.42, p=0.012; Figure 2 right), indicating that performance under SOD-stress
1482 or ALU-stress in Generation 9 depended upon which salt the microbiome was selected on during Genera-
1483 tions 0-8.

1484 We conducted post-hoc comparisons of the treatment means and found that plants grown with microbiomes
1485 selected in sodium sulfate had total seed weights that were 70.1% greater when exposed to sodium-sulfate
1486 stress compared to exposure of aluminum-sulfate stress in Generation 9 (beta=108 ± 31.0, z=3.5, p=0.002).
1487 In contrast, plants grown in microbiomes selected in aluminum-sulfate did not differ in similar total seed
1488 weight, regardless of whether they were exposed to sodium- or aluminum-sulfate in Generation 9 (beta=4.2
1489 ± 31.8, z=0.13, p=0.99). The effect of exposure to different kinds of salt stress on plant fitness thus depends
1490 upon the selection history of the soil microbiome.

1491 Unlike total seed weight, there was no interaction between selection history and the type of salt stress on
1492 total plant biomass, however there was a trend toward plants growing larger under ALU-stress compared
1493 to SOD-stress irrespective of the selection history (Analysis of deviance: Selection history, F=0.14, p=0.72;
1494 Salt exposure, F=3.71, p=0.056; Selection history x Salt exposure, F=1.38, p=0.24).
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1496

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