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Plant and Soil

Controlled natural selection of soil microbiome through plant-soil feedback confers resistance to a foliar pathogen

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Full Title:	Controlled natural selection of soil microbiome through plant-soil feedback confers resistance to a foliar pathogen
Article Type:	Research Article
Keywords:	controlled natural selection; plant-microbiome interactions; plant immunity; Pseudomonas syringae DC3000; salicylic acid; soil suppressiveness
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Title:

Controlled natural selection of soil microbiome through plant-soil feedback confers resistance to a foliar pathogen

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Abstract

Background and aims

The rhizosphere microbiome has been shown to contribute to nutrient acquisition, protection against biotic and abiotic stresses and, ultimately, changes in the development and physiology of plants. Here, using a controlled natural selection approach, we followed the microbial dynamics in the soil of *Arabidopsis thaliana* infected with the foliar pathogen *Pseudomonas syringae* DC3000 (*Pst*).

Methods

Plants were iteratively cultivated on a pasteurized soil inoculated with the soil microbial community of the previous iteration isolated from the rhizosphere of plants infected with *Pst* (pst-line) or not (mock-line). Modification of soil microbial communities was assessed through an amplicon-based metagenomic analysis targeting bacterial and fungal diversity. Plant fitness and transcript abundance of stress hormone related genes were also analysed.

Results

At the tenth and eleventh iterations, we observed a reduction in disease severity of 81% and 85% in pst-lines as compared to mock-lines. These changes were associated with (i) a diminution of transcript abundance of salicylic acid related genes whereas jasmonic acid, ethylene or abscisic acid related genes were not modified and (ii) a shift in soil bacterial, but not in fungal, composition.

Conclusions

Our study suggests that these changes in soil bacterial composition are mediated by plant-soil feedback in response to *Pst* and resulted in an activation of SA-related immune response in the plant. This supports the concept of applying plant-soil feedbacks to enhance soil suppressiveness against foliar pathogens.

Keywords: controlled natural selection; plant-microbiome interactions; plant immunity; *Pseudomonas syringae* DC3000; salicylic acid; soil suppressiveness

Introduction

Plant growth and development is strongly influenced by its environment. Among biotic factors, plant pathogens constitute a major threat to agriculture and food security around the world. The outcome of plant-pathogen interaction is governed by complex mechanisms resulting from long-term evolutionary processes but also from short-term responses not directly related to plant defence mechanisms and relying on the interaction with other

organisms or changes in the environment (Mitter et al. 2013; Pieterse et al. 2016). Plants shapes their rhizosphere through exuded carbon and other metabolites (Bais et al. 2006; Doornbos et al. 2012). Upon pathogen attack, changes in root exudates composition, often termed as "cry for help", help to recruit beneficial organisms such as plant growth promoting microbes (PGPM) enabling the assemblage of a protective rhizosphere microbiome (Rolfe et al. 2019) which can, in return, influence plant resistance to pathogen infection. Several studies already showed that recruitment of soil microorganisms can lead to soil suppressiveness; *i.e.* to a low level of disease development even though a virulent pathogen and susceptible host are present (Weller et al. 2002). Soil suppressiveness has been demonstrated for many soilborne disease like take-all of wheat, *Fusarium* wilts, potato scab or apple replant disease. Soil suppressiveness, relies on the selection of beneficial organisms by the plants after repeated ulterior infections with the pathogen (Weller et al. 2002; Berendsen et al. 2012). However, the concept of soil suppressiveness is not restricted to soilborne pathogen and can be applied to other diseases even those caused by aboveground pathogens or pests (Pineda et al. 2017, 2020).

For soilborne pathogens, the soil microbial community might influence disease severity through direct impact on pathogenic microorganisms (i.e. through competition or production of antimicrobial compounds) thus influencing the disease incidence and severity (Berendsen et al. 2012). On the contrary, the impact of soil microbial community on aboveground disease severity is mediated by systemic changes in plant development and defence responses, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR). In general, SAR is induced during a compatible host-pathogen interaction whereas ISR is elicited by PGPM. SAR and ISR acts antagonistically and relies on the activation of complex salicylic acid (SA) and jasmonic acid-ethylene (JA-ET) signalling pathways respectively, leading to localized and systemic defences (Pieterse et al. 2009). Activation of SAR or priming of ISR confers to the plant a broad-spectrum tolerance or resistance to ongoing and subsequent infections by pathogens and pests.

Thus, the soil microbiome has emerged as a key component of plant immunity, which has led to plant immunity no longer being seen as an innate genetic feature (Pieterse et al. 2016; Pineda et al. 2017). However, most of the studies about plant immunity to aboveground pathogens, conferred by beneficial microorganisms, focused on the impact of individual strains. Indeed, several soil microbial strains conferring a resistance in *Arabidopsis thaliana* to *Pseudomonas syringae* DC3000 (*Pst*) have already been identified (Mitter et al. 2013) and it was already shown that upon challenge by *Pst*, *A. thaliana* is able to recruit specific beneficial microbial strains to counteract

subsequent infections (Rudrappa et al. 2008; Yuan et al. 2018). Improvement of our knowledge, enhanced by the rapid development of inexpensive high-throughput DNA-sequencing, push towards integrating the role of soil microbiome in the understanding of plant immunity (Lemanceau et al. 2017).

In this work, through natural selection in a controlled environment (Conner 2003) we investigated the contribution of soil microbiome to *A.thaliana* adaptation to the foliar pathogen *Pst*. To do so, plants were divided in two lines, depending on whether they were (i) inoculated with *Pst* or (ii) a saline buffer. Then, a new batch of plants were iteratively cultivated on a pasteurized soil inoculated with the soil microbial community of the previous iteration (whether inoculated with *Pst* or not) during eleven iterations. This allowed us to mimic the dynamics of a soil microbial community exposed to successive monocultures infected or not with *Pst*. The inoculation of the foliar pathogen, in one line and not the other, aimed at testing if the pathogen can induce a modification of soil microbial community mediated by the plant. Modification of soil microbial communities by the plant in response to *Pst* was assessed through an amplicon-based metagenomic analysis targeting bacterial and fungal diversity. Plant fitness and transcript abundance of stress hormone related genes were also analysed to finely describe plant response to (i) *Pst* and (ii) changes in soil microbial composition.

Materials and Methods

Experimental set-up

Arabidopsis thaliana Col-0 seeds were surface-sterilized and stratified at 4°C for 2 days before sowing. Plants were cultivated in a growth chamber (Snijders, Netherlands) for 5 weeks at 22°C with daily cycles of 10 h light (100-130 µmol m^{-2} s^{-1}) and 14 h dark and 70% relative humidity. Initially (iteration 0), plants were cultivated in pots filled with a mixture of vermicompost, pasteurized natural sandy soil and pasteurized TS3 Aquasave substrate (pH=6.0, Recipe-No.: 316; Klasman-Deilmann, Lithuania) in 1:2:7 (v:v:v) ratio. Only vermicompost, originating from vegetable residues, was not pasteurized to provide a primary source of rich, diverse and abundant microbial community (Pathma and Sakthivel 2013). For the natural soil (cambisol with moor from CEREEP station, Saint-Pierre-Lès-Nemours, France), only the first 20 cm were sampled, excluding plant material before air-drying and sieving at 2 mm. Substrates were pasteurized for 72 h at 80 °C in a dry oven.

For the natural selection in a controlled environment experiment (Fig. 1), we established two "lines" (later denoted as pst-line and mock-line) of soil/plants microcosms (n=8), with one plant in each. After 4 weeks of growth, three leaves of each plant were inoculated with *Pst* or a saline buffer (10 mM $MgCl_2$) for pst- and mock-line respectively. One week later, the disease severity on pst-lines was evaluated by quantification of *Pst* DC3000 in leaf tissues (as colony forming units, CFU). Since it was not technically possible to separate the soil from plant roots, the entire soil (including plants roots) from the different microcosm respective to their line, were retrieved and pooled. Pooling of the soil was done since it does not generates consistent inference bias (Allen et al. 2021) and eases preparation of new microcosms. After pooling, soil was mixed to be used as "inoculum" to inseminate the substrate for the next iteration. The cultivation substrate consisted of inoculum (pooled total soils from the previous iteration), pasteurized natural soil and pasteurized substrate TS3 in a ratio 1:2:7 (v:v:v). On these, *Arabidopsis* seeds were sown; this was performed for 11 iterations. Note that vermicompost, except for the first iteration, was not used for the rest of the controlled natural selection experiment as its physical and chemicals properties is difficult to standardize and characterize, which can lead to the introduction of non-controlled bias between two iterations. During iterations 1-6, plants were watered with demineralized water with no fertilizers; starting the 7th iteration plants were first watered with distilled water for 3 weeks and then with ¼ Steiner solution (Steiner 1961), due to the reinforcement of deficiency symptoms (symptoms that gradually got worse during the first generations). Substrate water content was kept at 60-80% of total water capacity (w:w). From the fourth to the last iteration, individual pictures of the plants were taken at 7 d.p.i. (days post inoculation with *Pst*). Total leaf area and rosette diameter was measured using ImageJ software (1.52v) and PHENOPSIS 3.0 ImageJ macro (Rymaszewski et al. 2018).

Inoculation with pathogens and evaluation of disease severity

The inoculation with *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) was performed according to literature (Katagiri et al. 2002) with the following modifications: bacteria were cultivated overnight on LB medium plates containing 50 $\mu g.\mu l^{-1}$ rifampicin. Bacteria were washed out from the plate and resuspended in 10 mM $MgCl_2$ up to OD_{600}=0.0005. Four-week-old plants were syringe-infiltrated with the suspension: three leaves at the similar developmental stage (middle age leaves: 8th-9th-10th leaves) were infiltrated according to (Berens et al. 2019). At 7 d.p.i., three discs (6 mm in diameter) were sampled on three leaves from each plant and pooled for evaluation of disease severity. The discs were homogenized in 10 mM $MgCl_2$, in a 2mL Eppendorf tube, with 1 g of 1.3 mm

150 silica beads using a FastPrep-24 instrument (MP Biomedicals, USA). The resulting homogenate was subjected to
151 serial 10x dilutions and pipetted onto LB plates. Colonies were counted after 1–2 days of incubation at 28 °C.
152
153 *Plant gene expression*
154 Gene expression was monitored in plants of iterations 10 and 11, for both lines, 7 days post inoculation. Expression
155 of defence-related genes was performed as in (Leontovyčová et al. 2019). Briefly, leaf discs (approximately 100
156 µg fresh weight) were immediately frozen in liquid nitrogen. The tissue was homogenized similarly as for CFU
157 quantification. Total RNA was isolated using Spectrum Plant Total RNA kit (Sigma-Aldrich, USA) and treated
158 with a DNase DNA-free kit (Ambion, USA). Subsequently, 1 µg of RNA was converted into cDNA with M-MLV
159 RNase H^- Point Mutant reverse transcriptase (Promega Corp., USA) and an anchored oligo dT21 primer
160 (Metabion, Germany). An equivalent of 6.25 ng of RNA was loaded into a 10-µl reaction with a SYBR Green
161 qPCR Kit (ThermoFisher, USA). Gene expression was quantified by qPCR using a LightCycler 480 SYBR Green
162 I Master kit and LightCycler 480 (Roche, Switzerland). The PCR conditions were 95 °C for 10 min followed by
163 45 cycles of 95 °C for 10 s, 55 °C for 20 s, and 72 °C for 20 s. Melting curve analysis was then conducted. Relative
164 gene expression was evaluated using ΔΔCt, where the genes of interest were normalized to the housekeeping gene
165 *AtTIP41* and relative to the mock-line. Gene of interest are associated with stress-hormone signalling pathways:
166 *PR1*, *PR2*, *PR5* and *ICS1* for SA-signalling pathway (Qi et al. 2018) ; *PDF1.2*, *AOS*, *LOX2* for JA pathway (Leon-
167 Reyes et al. 2010); *ACS2*, *ERF1* for ethylene pathway (Wang et al. 2002); *ABI1* and *LEA4-1* for ABA pathway
168 (Dalal et al. 2009; Cao et al. 2017). A list of the primers is available in Supplementary Table S1.
169
170 *Soil DNA extraction and sequencing*
171 DNA was extracted from 500 mg of -20°C frozen soils following the (Griffiths et al. 2000) protocol with some
172 modifications according to (Nicolaisen et al. 2008) and (Monard et al. 2013). Lysing Matrix E tubes (MP
173 Biomedicals, California, USA) were used for the cell lysis and agitated at 30 $m.s^{-1}$ for 3 min in a bead beater.
174 Glycogen (0.1 mg) was added to precipitate nucleic acids for 2 h at 4 °C that were subsequently pelleted by
175 centrifugation at 18 000 **g** for 30 min at 4 °C. Nucleic acids were resuspended in 50 µl of DNase–RNase-free
176 water.
177
178 Bacteria and fungi libraries were constructed according to the Illumina protocol "16S metagenomic sequencing
179 library preparation" using a two steps polymerase chain reaction (PCR) approach. The following primer sets were

used for bacteria and fungi: 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') (Klindworth et al. 2013), ITS1f (5'-CTTGGTCATTTAGAGGAAGTAA-3') (Gardes and Bruns 1993) and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White et al. 1990), respectively. Each primer set contains a supplementary sequence, the overhang adapter: forward overhang (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse overhang (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'). PCRs were conducted in 25 µl containing each bacterial or fungal primer (0.2µM), 12.5µl 2X TransTaq® Hifi PCR SuperMix II (TransGen Biotech, Beijin, China), 2 µl of tenfold diluted DNA and ultrapure water to reach the final volume. The amplification conditions were as followed: for bacteria, 3 min at 95°C, 25 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C and a final 5 min extension step at 72°C; for fungi, 4 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C and a final 10 min extension step at 72°C. Two independent PCR replicates were performed for each sample, the PCR products were pooled and purified using the Agencourt® AMPure® XP beads system. The second PCR reaction attached specific indexes (i5, i7) to identify each sample and the Illumina sequencing adapters (P5, P7) using the Nextera XT index kit, 1X KAPA2G Robust mastermix (Kapa Biosystems, Wilmington, Massachusetts, USA) and the SmartChip technology (Wafergen, Fremont, USA). The amplification conditions consisted in 3 min at 95°C, followed by 8 cycles of 30 s at 95°C, 30 s at 55°C and 30 s at 72°C, and a final 5 min extension step at 72°C. After the polymerase chain reactions, pooled amplicons were collected from the chip(s) via centrifugation using single-use components supplied in the SmartChip TE Collection Kit and were purified with the Agencourt® AMPure® XP beads system.

The amplified products of bacteria and fungi were quantified by qPCR (light cycler 480, Roche, Meylan, France) with, per well, 2.67 µl of PCR product, 4 µl of premix containing 2X of KAPA SYBR FAST qPCR Master Mix (KAPA library quantification kit for illumina, Kapa Biosystems, Wilmington, USA) and the Illumina primers P5 (5'-AATGATACGGCGACCACCGA-3') and P7 (5'-CAAGCAGAAGACGGCATACGA-3'). The qPCR program consisted in 3 min at 95°C, followed by 45 cycles of 30 s at 95°C, 45 s at 60°C, 20 s at 72°C and a final melting curve step of 0.05 s at 95°C, 1 min at 65°C and an increase of 0.06°C/s from 65°C to 97°C. Then, the amplified products were combined in a unique pool in equimolar ratio and sequenced using 2x250 bp paired-end Illumina MiSeq with 15% PhiX at the 'Human and Environmental Genomic' platform (Rennes, France).

Amplicon-based metagenomic analysis

The sequence reads obtained after sequencing were analysed using Find Rapidly OTUs with Galaxy Solution (FROGS) pipeline at the Genotoul platform following the standard operation procedure. Unless specified otherwise, data were processed using default parameters. Briefly, reads were pre-processed to filter out sequences without primers, that are too small or of poor quality (ambiguous bases) and to trim the primers. Sequences were clustered using the Swarm algorithm (Mahé et al. 2014). Chimera sequences were detected and then removed. Clustered OTU were filtered to remove OTU that are present in at least two samples and containing at least 0.00005% of all sequences. Taxonomy was assigned to OTU using SILVA (release 132) and UNITE (release 7.1) as reference database for 341F/785R and ITS1F/ITS2 reads respectively.

Statistical and microbial community analyses

All statistical analyses were performed using the R software (R Core Team 2020). Bacterial and fungal communities diversity analyses were performed with the R packages Phyloseq (McMurdie and Holmes 2013). Data were rarefied to 9220 and 14753 sequences/samples for bacteria (341F/785R) and fungi (ITS1F/ITS2) reads, respectively. Correspondence analysis was performed with the *ade4* package (Thioulouse et al. 2018). To identify the OTUs the most associated with pst- and mock- lines, the 200 OTUs with the highest absolute contribution to the first axis were isolated. A specific subset of OTUs associated to the pst-line and the mock-line was obtained by filtering on the data of the eleventh iteration. For the pst-line, only the OTUs detected in all three replicates of the pst-line and not detected in at least one replicate of the mock-line were kept. For the mock-line, only the OTUs detected in all three replicates of the mock-line and not detected in at least one replicate of the pst-line were kept. This provided a specific set of 78 and 34 OTUs for pst- and mock-line respectively.

Results

Plant resistance to Pst but not growth increased between pst- and mock-line

To determine the influence of the iterative inoculation of plants with a leaf pathogen on the associated rhizosphere microbial communities, we performed a natural selection in a controlled environment experiment (Fig. 1). One week after infiltration with *Pst*, inoculated leaves showed strong symptoms of infection (necrotic lesions surrounded by diffuse chlorosis) (Fig. 2a). On the contrary, for plants infiltrated with saline buffer, no leaf damage nor necrosis was observed (Fig. 2a). Plant growth (Fig. 2b), monitored through the projected leaf area (PLA) showed a progressive decrease starting from the fourth iteration up to the eighth. At the ninth iteration, PLA increased to values close to that of the fourth iteration and remained steady, up to the last iteration. For the fourth

and seventh iteration, we observed a significant difference in PLA between the plants of the pst- and the mock-line (p-value < 0.05, Wilcoxon test). No significant differences (p-value > 0.05, Wilcoxon test) were observed for the other iterations. At the tenth and eleventh iterations, PLA of plants in the mock-line, inoculated with *Pst* did not differ from plants in the pst-line (Fig. 1b). For both 10th and 11th iterations in which additional plant of the mock line were used to compare plant susceptibility to the pathogen in both pst- and mock-lines, the number of bacteria was significantly lower (p-value < 0.05, Wilcoxon test) in plants from the pst-line versus those of the mock-line: -81% (Fig. 2c) and -85% (Supplementary Fig. S1) for iteration 10 and 11, respectively.

Gene expression

Among all tested genes, we detected a significant (p-value < 0.05, Wilcoxon test) differences between lines only in expression of genes associated with the SA-pathway (*PR1*, *PR2* and *PR5*) and SA biosynthesis gene *ICS1* (Fig. 3 for i10 and Supplementary Fig. S2 for i11). The SA-related response was stronger in the mock line than in the pst-line in two subsequent iterations. For the other genes, no significant differences (p-value > 0.05, Wilcoxon test) in transcripts levels was detected between mock- and pst-lines (Fig. 3 for i10 and Supplementary Fig. S2 for i11).

Rhizosphere microbiome community dynamics

Soil bacterial and fungal diversity, assessed through the phylogenetic analysis of 16S rRNA and ITS gene amplicons, was strongly impacted at first by the experimental setup (Supplementary Fig. S3). For bacterial diversity, species richness almost doubled the first iteration, going from 365 to 645 and 698 for mock and pst-line respectively. Species richness then stabilized around 840 for the following iterations (three times more as compared to initial soil). A similar pattern was observed for fungal diversity, with an increased species richness from 28 to 110 for both mock and pst-line in the first iteration. Species richness strongly varied the next iterations, before decreasing from the sixth iteration. For the last three iterations, species richness was around 40 (1.5 times more as compared to initial soil). For bacterial communities, Shannon H-index, followed a similar pattern than species richness with an initial increase from 5 to 5.6 and remained stable for the following iterations. For fungal communities, Shannon H-index remained stable the first four iterations (around 2.4) before decreasing and reaching 1.3 the last iterations.

When comparing bacterial (Fig. 4a) and fungal (Fig. 4b) communities between two successive iterations within the same line (*i.e.* legacy effect), we observe that for both soil lines, shift in beta-diversity was the highest between the initial soil and the first iteration (i0-i1), with values around 0.63 and 0.72 for bacterial and fungal communities respectively. Between successive iteration beta-diversity then decreased in both lines through the experiment and remained low from the sixth to the last iteration for both lines (mock and pst) and bacterial and fungal communities. When compared, the dissimilarity in bacterial composition between the mock and pst-line for the same iteration (*i.e.* inter treatment dissimilarity), increased between the first and fourth iteration and then remained stable at high values till the end of the iteration process (Fig. 4c). Dissimilarity between bacterial communities of the same line (*i.e.* intra treatment dissimilarity) also increased during the first three iterations and then decreased and remained low for both mock- and pst-line (Fig. 4c). From the third and up to the last iteration, inter-treatment (mock *vs.* pst) dissimilarity was higher than the intra-treatment (mock or pst) dissimilarity. For fungal communities (Fig. 4d), an increase in both intra and inter-treatment dissimilarity was also observed in the first iterations. Intra and inter dissimilarity then decreased from i3 up to i11, with only a brief increase at i10 for the pst-line community (thus increasing the inter-treatment dissimilarity). As fungal communities did not appear to be impacted by *Pst,* we only consider bacterial communities in further analyses.

Bacterial sub-community differences between pst- and mock- lines

Correspondence analysis (CA, Fig. 5a) confirmed the strong influence of the experimental setup during the first iterations, as already shown by between successive iteration beta-diversity (Fig. 4a). In the CA map, samples are distributed according to their iterations, with samples from initial soil (i0) and first iteration (i1) contributing the most to both the first and second axis. Besides, the bacterial communities of i0 and i1 samples show a great divergence with those of following iterations (i2 to i11). The last iterations (i5 to i11) are joined on the CA map and contribute poorly to the first and second axis, showing a relative stabilization of microbial community structure (Fig. 4a and 4c). To have a better view of what happens for the last iterations, CA was performed using only the samples corresponding to the last four iterations (i8 to i11) (Fig. 5b). It shows a strong impact of the *Pst* inoculation on microbiome composition as samples are distributed along the first axis according to the line (pst or mock), with samples of the mock-line being mainly on the left and pst-line on the right. Interestingly, the second axis separates the samples according to their iterations, with samples of pst-line and mock-line for a given iteration nearly mirroring according to the second axis.

Following CA, we selected the OTUs that contribute the most, positively, or negatively, to the first axis. We identified (Fig. 6a) a specific set of 78 OTUs whose abundance increased through the iterative process in the pst-line but not in the mock line. These OTUs which represented less than 1 % of the relative abundance during the first two iterations represented up to 11% in the last iteration. These OTUs were mostly related to the phyla Proteobacteria (4 %), Planctomycetes (3 %) and Bacteroides (1.8 %) (Fig. 6a) with families such as *Tepidisphaeraceae*, *Xanthobacteraceae* and *Caedibacteraceae* being the most abundant in pst-line but not in mock-line (Supplementary Table S2 and S3). In the mock-line, the abundance of these OTUs did not change and has remained broadly close to 1% during the whole experiment. In soils of the mock-line, we also identified a specific set of 34 OTUs (Fig. 6b) which abundance increased during the iterative process. These OTUs represented less than 2% of the relative abundance during the first two iterations and up to 4.4 % in the last iteration. These OTUs are mostly related to the phyla Proteobacteria (2.1 %) and Bacteroides (1.1 %). A decrease in the abundance of these OTUs was observed in soils iteratively exposed to *Pst*. They represented up to 2.5 % of the total abundance in the first iterations and dropped close to 0.5% in the last iterations.

Discussion

Susceptibility to Pst decreased in the line iteratively exposed to Pst

Iterative exposure of soil to plants inoculated with *Pst* did not significantly impact plant growth and development as there were no differences between the mock- and pst-lines during the iterative process (Fig. 2b). However, from the 4th to the 8th iteration, plant growth decreased. This might be due to an uncontrolled environmental factor. This phenomenon might have been accentuated by the small pot size, chosen to maximize the volume occupied by the roots to increase the rhizosphere (and so the interactions with soil microorganisms). Fertilization of the plants with a Steiner solution, started at the seventh iteration, might have allowed them to overcome this effect in the last iterations (i9 to i11). Although no significant differences were observed between mock- and pst-lines regarding plant growth (except for iteration 4 and 7), plant susceptibility to *Pst* was strongly modulated. Plants grown on soils of iteration 10, *i.e.* soil microbiome that has been ten times consecutively exposed to plants exposed to *Pst*, appeared far less susceptible to the disease. This was shown by a decrease of 81 and 85 % in *Pst* density in pst-line as compared with mock-line, at iterations 10 and 11 respectively. Interestingly, the increased resistance in pst-line observed at the tenth and eleventh iterations was coupled with the lower transcription of *PR1*. *PR1* is the canonical SA-responsive gene, used as an indicator of the triggering of SA signalling (Janda and Ruelland

2015). During *Pst* infection, SA signalling (i.e. *PR1* expression) turns on rapidly (16-24 hpi) peaking around 48 hpi and gradually decreasing afterwards (De Vos et al. 2005; Yang et al. 2015). The increased resistance at 7 dpi in pst-line, correlated with lower SA signalling at this very moment, might indicate that defence mechanisms were activated in pst-line allowing the plant to overcome the infection faster. This activation could be linked to the presence of PGPM or molecules inducing SA related defence mechanisms (Pieterse et al. 2009). For example, *Pst* infection resistance in *A. thaliana* plants induced by the PGPM *Bacillus cereus* AR156 and *Burkholderia phytofirmans* PsJN (Bp) is associated to higher levels of transcripts of SA related genes in the first stage of the infection but lower in the late stage of the infection as compared to non-elicited plants (Niu et al. 2011; Su et al. 2017). On conditioned soils (*i.e.* by growing in the same soil, multiple generations of *A. thaliana* inoculated aboveground with *Pst*), a similar decrease of endogenous SA level was observed (Yuan et al. 2018). Conversely, we did not detect any significant differences in transcript abundance of JA-responsive (*PFD1.2*) or biosynthetic genes (*AOS*, *LOX2*), ET- (*ACS2*, *ERF1*) or ABA-associated (*ABI1*, *LEA4-1*) genes between pst- and mock-lines, consistently with the antagonistic role of those hormones (Robert-Seilaniantz et al. 2011). Even if we did not see any significant differences for other hormones than SA, involvement of other hormones like JA or ET cannot be totally excluded. There is more and more evidence of a strong interplay of plant hormones, even between antagonistic ones, to finely tune plant response to pathogens, with often a time-lagged regulation of hormones levels (Liu et al. 2016; Klessig et al. 2018). This interplay between plant hormones plays an important role in plant-microbiome interactions as several soil organisms can interact with plant hormone signalling, modulating *in fine* hormone levels and thus plant response to pathogens (Hase et al. 2003; Dodd et al. 2010; Puga-Freitas and Blouin 2015; Trivedi et al. 2021).

Natural selection by the Pst-infected plants of a soil microbiome alleviating Pst associated symptoms

Since new seeds were sown at the beginning of each iteration, and a fresh *Pst* inoculum was used for each infection, the effects observed on plant susceptibility could not be attributed to plant adaptation to the pathogen neither to the pathogen adaptation, as already observed in other studies (Luna et al. 2012; Meaden and Koskella 2017). Modulation of plant susceptibility to *Pst* on the final iterations (i10 and i11) can therefore only be due to changes in soil biological, physical or chemical properties as a small proportion of soil was transferred between iterations (10 % of final volume). However, changes in soil physical and chemical properties were limited as soil was destructurated by breaking aggregates between two successive iterations and plants were fertilized using a nutritive solution after the seventh iteration. Soil biological properties, especially bacterial dynamics, thus

appeared as a key element in the alleviation of *Pst* susceptibility in our experiment. Bacterial and fungal communities tended to stabilize along the iterative process (Fig. 4a) as the legacy effect increases (*i.e.* for a same line, divergence between two successive iterations tended to decrease). Aboveground inoculation with *Pst* impacted bacterial communities (Fig. 4c) but not fungal communities (Fig. 4d). Enrichment of rhizospheric microorganisms following infections has been extensively reported in suppressive soils (Raaijmakers and Mazzola 2016; Cordovez et al. 2019). On these soils, suppression of a specific subset of disease caused by soilborne phytopathogenic fungi, oomycetes, bacteria or nematodes is generally observed. It has been strongly suggested that this protection is mediated by changes in the soil microbiome composition (Sanguin et al. 2009; Mendes et al. 2011; Raaijmakers and Mazzola 2016). In our study, the iterative cultivation of plants on a pasteurized soil inoculated with the soil microbial community of the previous iteration (whether inoculated with *Pst* or not) acted as a driving force to shape soil microbiome composition. This resulted in the selection by the plant, likely through its root exudates (Bais et al. 2006; Rudrappa et al. 2008; Yuan et al. 2018), of bacterial families such as *Burkholderiaceae*, *Caulobacteraceae*, *Sphingobacteriaceae* and *Sphingomonadaceae* (Supplementary Table S2), that helped the plants to overcome the infection as already shown in other pathosystems (Chapelle et al. 2016). After ten iterations, this microbial community is stable and can be therefore transferred to a new soil. For soilborne pathogens, it have been demonstrated that specific suppressiveness of soils can be transferred to conducive ones by mixing small amounts (1 to 10 % w/w) of the suppressive soil into the conducive soil (Mendes et al. 2011; Raaijmakers and Mazzola 2016). A similar mixing ratio seems to be also valid for diseases aboveground pathogens as shown in our study comforting that the concept of soil suppressiveness can be extended to aboveground pathogen or pests. However, the soil community trait selected by the plants differs according to the type of pathogen (soilborne or foliar). For soilborne pathogens, modifications induced by soil microbiome likely lead to changes in physicochemical soil properties and activation of specific antagonistic traits that restrict pathogen infection such as production of antimicrobial compounds or siderophores (Raaijmakers et al. 2009; Schlatter et al. 2017). For foliar pathogens, as in our study, such direct impact of soil microbiome on the pathogen seems unlikely. Here, the mechanisms involved is therefore systemic and indirect, and relies extensively in the modulation by soil microbiome of the plant immunity related to SA homeostasis as demonstrated in the current and previous studies (Yuan et al. 2018).

To conclude, active shaping of soil microbiome by plant-soil feedbacks during pathogen infection allows to recruit a stable microbiome leading to a reduction in the susceptibility to the disease. This indirect mechanism could rely

upon a systemic response induced by soil organisms and is mediated in the plant through SA signalling. The use of similar controlled natural selection approaches could lead to the development of new agricultural strategies as the complex microbiomes obtained can be introduced to other soils via soil transplantation. Such approach needs to be associated to breeding strategies that optimize interactions with beneficial microbes, as plant interaction with soil organisms is a major driver of terrestrial ecosystems functioning.

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References

Allen WJ, Sapsford SJ, Dickie IA (2021) Soil sample pooling generates no consistent inference bias: a meta-analysis of 71 plant–soil feedback experiments. New Phytologist 231:1308–1315. https://doi.org/10.1111/nph.17455

Bais HP, Weir TL, Perry LG, et al (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annual review of plant biology 57:233–66. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends in Plant Science 17:478–486. https://doi.org/10.1016/j.tplants.2012.04.001

Berens ML, Wolinska KW, Spaepen S, et al (2019) Balancing trade-offs between biotic and abiotic stress responses through leaf age-dependent variation in stress hormone cross-talk. Proceedings of the National Academy of Sciences of the United States of America 116:2364–2373. https://doi.org/10.1073/pnas.1817233116

Cao M-J, Zhang Y-L, Liu X, et al (2017) Combining chemical and genetic approaches to increase drought resistance in plants. Nature Communications 8:1183. https://doi.org/10.1038/s41467-017-01239-3

Chapelle E, Mendes R, Bakker PAHM, Raaijmakers JM (2016) Fungal invasion of the rhizosphere microbiome. The ISME Journal 10:265–268. https://doi.org/10.1038/ismej.2015.82

Conner JK (2003) Artificial Selection: A Powerful Tool for Ecologists. Ecology 84:1650–1660

Cordovez V, Dini-Andreote F, Carrión VJ, Raaijmakers JM (2019) Ecology and evolution of plant microbiomes. Annual Review of Microbiology 73:69–88. https://doi.org/10.1146/annurev-micro-090817-062524

Dalal M, Tayal D, Chinnusamy V, Bansal KC (2009) Abiotic stress and ABA-inducible Group 4 LEA from Brassica napus plays a key role in salt and drought tolerance. Journal of Biotechnology 139:137–145. https://doi.org/10.1016/j.jbiotec.2008.09.014

De Vos M, Van Oosten VR, Van Poecke RMP, et al (2005) Signal Signature and Transcriptome Changes of Arabidopsis During Pathogen and Insect Attack. Molecular Plant-Microbe Interactions® 18:923–937. https://doi.org/10.1094/MPMI-18-0923

Dodd ICC, Zinovkina NYY, Safronova VII, Belimov AAA (2010) Rhizobacterial mediation of plant hormone status. Annals of Applied Biology 157:361–379. https://doi.org/10.1111/j.1744-7348.2010.00439.x

Doornbos R, van Loon L, Bakker P (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agronomy for Sustainable Development 32:227–243. https://doi.org/10.1007/s13593-011-0028-y

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Molecular Ecology 2:113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Griffiths RI, Whiteley AS, O'Donnell AG, Bailey MJ (2000) Rapid method for coextraction of DNA and RNA from natural environments for analysis of ribosomal DNA- and rRNA-based microbial community composition. Applied and Environmental Microbiology 66:5488–5491. https://doi.org/10.1128/AEM.66.12.5488-5491.2000

Hase S, Van Pelt JA, Van Loon LC, Pieterse CMJ (2003) Colonization of Arabidopsis roots by Pseudomonas fluorescens primes the plant to produce higher levels of ethylene upon pathogen infection. Physiological and Molecular Plant Pathology 62:219–226. https://doi.org/10.1016/S0885-5765(03)00059-6

Janda M, Ruelland E (2015) Magical mystery tour: Salicylic acid signalling. Environmental and Experimental Botany 114:117–128. https://doi.org/10.1016/j.envexpbot.2014.07.003

Katagiri F, Thilmony R, He SY (2002) The Arabidopsis Thaliana-Pseudomonas Syringae Interaction. The Arabidopsis Book 1:e0039. https://doi.org/10.1199/tab.0039

Klessig DF, Choi HW, Dempsey DA (2018) Systemic Acquired Resistance and Salicylic Acid: Past, Present, and Future. Molecular Plant-Microbe Interactions® 31:871–888. https://doi.org/10.1094/MPMI-03-18-0067-CR

Klindworth A, Pruesse E, Schweer T, et al (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research 41:1–11. https://doi.org/10.1093/nar/gks808

Lemanceau P, Blouin M, Muller D, Moënne-Loccoz Y (2017) Let the Core Microbiota Be Functional. Trends in Plant Science xx: https://doi.org/10.1016/j.tplants.2017.04.008

Leon-Reyes A, Van der Does D, De Lange ES, et al (2010) Salicylate-mediated suppression of jasmonate-responsive gene expression in Arabidopsis is targeted downstream of the jasmonate biosynthesis pathway. Planta 232:1423–1432. https://doi.org/10.1007/s00425-010-1265-z

Leontovyčová H, Kalachova T, Trdá L, et al (2019) Actin depolymerization is able to increase plant resistance against pathogens via activation of salicylic acid signalling pathway. Scientific Reports 9:1–10. https://doi.org/10.1038/s41598-019-46465-5

Liu L, Sonbol F-M, Huot B, et al (2016) Salicylic acid receptors activate jasmonic acid signalling through a non-canonical pathway to promote effector-triggered immunity. Nature Communications 7:13099. https://doi.org/10.1038/ncomms13099

Luna E, Bruce TJA, Roberts MR, et al (2012) Next-Generation Systemic Acquired Resistance. Plant Physiology 158:844–853. https://doi.org/10.1104/pp.111.187468

Mahé F, Rognes T, Quince C, et al (2014) Swarm: Robust and fast clustering method for amplicon-based studies. PeerJ 2014:1–13. https://doi.org/10.7717/peerj.593

McMurdie PJ, Holmes S (2013) Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8:. https://doi.org/10.1371/journal.pone.0061217

Meaden S, Koskella B (2017) Adaptation of the pathogen, Pseudomonas syringae, during experimental evolution on a native vs. alternative host plant. Molecular Ecology 26:1790–1801. https://doi.org/10.1111/mec.14060

Mendes R, Kruijt M, de Bruijn I, et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science (New York, NY) 332:1097–100. https://doi.org/10.1126/science.1203980

Mitter B, Brader G, Afzal M, et al (2013) Advances in Elucidating Beneficial Interactions Between Plants, Soil, and Bacteria. In: Sparks DLBT-A in A (ed). Academic Press, pp 381–445

Monard C, Gantner S, Stenlid J (2013) Utilizing ITS1 and ITS2 to study environmental fungal diversity using pyrosequencing. FEMS Microbiology Ecology 84:165–175. https://doi.org/10.1111/1574-6941.12046

Nicolaisen MH, Bælum J, Jacobsen CS, Sørensen J (2008) Transcription dynamics of the functional tfdA gene during MCPA herbicide degradation by Cupriavidus necator AEO106 (pRO101) in agricultural soil. Environmental Microbiology 10:571–579. https://doi.org/10.1111/j.1462-2920.2007.01476.x

Niu D-D, Liu H-X, Jiang C-H, et al (2011) The Plant Growth–Promoting Rhizobacterium Bacillus cereus AR156 Induces Systemic Resistance in Arabidopsis thaliana by Simultaneously Activating Salicylate- and Jasmonate/Ethylene-Dependent Signaling Pathways. Molecular Plant-Microbe Interactions® 24:533–542. https://doi.org/10.1094/MPMI-09-10-0213

Pathma J, Sakthivel N (2013) Molecular and functional characterization of bacteria isolated from straw and goat manure based vermicompost. Applied Soil Ecology 70:33–47. https://doi.org/10.1016/j.apsoil.2013.03.011

Pieterse CMJ, de Jonge R, Berendsen RL (2016) The Soil-Borne Supremacy. Trends in Plant Science 21:171–173. https://doi.org/10.1016/j.tplants.2016.01.018

Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nature Chemical Biology 5:308–316. https://doi.org/10.1038/nchembio.164

Pineda A, Kaplan I, Bezemer TM (2017) Steering Soil Microbiomes to Suppress Aboveground Insect Pests. Trends in Plant Science 22:770–778. https://doi.org/10.1016/j.tplants.2017.07.002

Pineda A, Kaplan I, Hannula SE, et al (2020) Conditioning the soil microbiome through plant–soil feedbacks suppresses an aboveground insect pest. New Phytologist 226:595–608. https://doi.org/10.1111/nph.16385

Puga-Freitas R, Blouin M (2015) A review of the effects of soil organisms on plant hormone signalling pathways. Environmental and Experimental Botany 114:. https://doi.org/10.1016/j.envexpbot.2014.07.006

Qi G, Chen J, Chang M, et al (2018) Pandemonium Breaks Out: Disruption of Salicylic Acid-Mediated Defense by Plant Pathogens. Molecular Plant 11:1427–1439. https://doi.org/10.1016/j.molp.2018.10.002

R Core Team (2020) R: A Language and Environment for Statistical Computing. https://www.r-project.org/, Vienna, Austria

Raaijmakers J, Paulitz T, Steinberg C, et al (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil 321:341–361. https://doi.org/10.1007/s11104-008-9568-6

Raaijmakers JM, Mazzola M (2016) Soil immune responses. Science 352:1392 LP – 1393. https://doi.org/10.1126/science.aaf3252

Robert-Seilaniantz A, Grant M, Jones JDG (2011) Hormone crosstalk in plant disease and defense: more than just JASMONATE-SALICYLATE antagonism. Annual Review of Phytopathology 49:317–343. https://doi.org/10.1146/annurev-phyto-073009-114447

Rolfe SA, Griffiths J, Ton J (2019) Crying out for help with root exudates: adaptive mechanisms by which stressed plants assemble health-promoting soil microbiomes. Current Opinion in Microbiology 49:73–82. https://doi.org/10.1016/j.mib.2019.10.003

Rudrappa T, Czymmek KJ, Paré PW, Bais HP (2008) Root-Secreted Malic Acid Recruits Beneficial Soil Bacteria. Plant Physiology 148:1547 LP – 1556. https://doi.org/10.1104/pp.108.127613

Rymaszewski W, Dauzat M, Bédiée A, et al (2018) Measurement of Arabidopsis thaliana Plant Traits Using the PHENOPSIS Phenotyping Platform. Bio-protocol 8:e2739. https://doi.org/10.21769/BioProtoc.2739

Sanguin H, Sarniguet A, Gazengel K, et al (2009) Rhizosphere bacterial communities associated with disease suppressiveness stages of take-all decline in wheat monoculture. New Phytologist 184:694–707. https://doi.org/10.1111/j.1469-8137.2009.03010.x

Schlatter D, Kinkel L, Thomashow L, et al (2017) Disease Suppressive Soils: New Insights from the Soil Microbiome. Phytopathology® 107:1284–1297. https://doi.org/10.1094/PHYTO-03-17-0111-RVW

Steiner AA (1961) A universal method for preparing nutrient solutions of a certain desired composition. Plant and Soil 15:134–154. https://doi.org/10.1007/BF01347224

Su F, Villaume S, Rabenoelina F, et al (2017) Different Arabidopsis thaliana photosynthetic and defense responses to hemibiotrophic pathogen induced by local or distal inoculation of Burkholderia phytofirmans. Photosynthesis Research 134:201–214. https://doi.org/10.1007/s11120-017-0435-2

Thioulouse J, Dufour AB, Jombart T, et al (2018) Multivariate analysis of ecological data with ade4

Trivedi P, Mattupalli C, Eversole K, Leach JE (2021) Enabling sustainable agriculture through understanding and enhancement of microbiomes. New Phytologist n/a: https://doi.org/10.1111/nph.17319

Wang KL-C, Li H, Ecker JR (2002) Ethylene Biosynthesis and Signaling Networks . The Plant Cell 14:S131–S151. https://doi.org/10.1105/tpc.001768

Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual review of phytopathology 40:309–48. https://doi.org/10.1146/annurev.phyto.40.030402.110010

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJBT-PCRP (eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Yang L, Li B, Zheng X, et al (2015) Salicylic acid biosynthesis is enhanced and contributes to increased biotrophic pathogen resistance in Arabidopsis hybrids. Nature Communications 6:7309. https://doi.org/10.1038/ncomms8309

Yuan J, Zhao J, Wen T, et al (2018) Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome 6:156. https://doi.org/10.1186/s40168-018-0537-x

Statements & Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

TK, LB, ER and RPF conceived and designed the study. TK and BJ performed the experiment. CM performed the soil DNA extraction and sequencing. RPF analysed the data. TK, MB, SJ, ER and RPF interpreted the data. TK, ER and RPF wrote the first manuscript and all authors contributed to the revision of the manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure legends

Fig. 1 Experimental set-up for the iterative exposure of soil microbiome to *A. thaliana* inoculated with the foliar pathogen *Pst*. Before starting the iterative process (iteration 0), plants were sown and grown for four weeks on pasteurized substrate mixed with vermicompost (providing the initial microbial inoculant). The iterative process was started (i1) by dividing the first batch of plants (i0) in two lines : i/ pst-line, infiltrated with a *Pst* strain, and ii/ mock-line, infiltrated with saline buffer (plain and empty red arrows respectively). This will allow to assess if the plant could recruit different microbial communities when exposed or not to a pathogen. Seven days after inoculation, disease susceptibility was assessed on the *Pst*-inoculated line. Then, for each pot of both lines, a small fraction of soil was collected and pooled with samples from the same line; an aliquot fraction of pooled soil was kept for further amplicon-based metagenomic analysis. Thus, pooled soil sample from the parental generation was used to inoculate a soil microbial community within the pasteurized substratum of the offspring one at a 1:9 (v:v) ratio to mimic the dynamics of a soil microbial community exposed to successive monocultures. *A. thaliana* seeds were sown on this inseminated soil to pursue the iterative process for eleven iterations. Each iteration is denoted i_n, with n, the iteration number ranging from 1 to 11 and consisting in the three following steps: i/ sowing of *A.thaliana* on soil inoculated with the microbial community of the previous iteration. After four weeks, ii/ leaves infiltration with *Pst* strain or a saline buffer (for pst- and mock-line respectively). Finally, iii/ soil collection, respective to its line, to prepare a new batch of soil and pursue the iterative process. For the tenth and eleventh iteration, five additional experimental set-ups were prepared for mock lines to assess disease susceptibility while continuing the iteration process. Green and blue colours are associated with pst- and mock-line respectively. Red colour is associated with the pathogen *Pst* and grey colour with initial soil before starting the iterative process. Soil microbial composition has been characterized for samples indicated with asterix

Fig. 2 Leaf traits of *Arabidopsis thaliana* along the iterative process. (a) Representative images of plants from the tenth iteration, 7 days post inoculation (d.p.i.): mock ($MgCl_2$) treated (left panel) and *Pst* inoculated (right panel). Red triangles point out mock or *Pst* infiltrated leaves (empty or plain triangle respectively). White scale bar represents 10 mm. (b) Evolution of rosette area. Boxplots show the total projected leaf area at 7 d.p.i for mock (blue) or pst (green) lines from iteration 4 to 11 (points indicates values for biological replicates, n=8). (c) Boxplot comparing pathogen resistance of plants from mock- (n=5) or pst-line (n=8) at the tenth iteration (i10; points indicates values for biological replicates, see also i11 in Supplementary Fig. S1). For each plant, three leaves were

infiltrated with *Pst* before CFU quantification at 7 d.p.i. Asterix indicates significant differences and "ns" indicates non-significant differences (**p-value < 0.01, *p-value < 0.05, ns p-value > 0.05, Wilcoxon test)

Fig. 3 Impact of the soil microbiome on hormone related gene expression of *Arabidopsis thaliana* from mock- or pst-line upon *Pst* infection. (a-e) SA-related, (f-h) JA-related, (i-j) ABA related and (k-l) ET related gene expression at 7 d.p.i. Data are those from iteration 10. Boxplots show Log2 transformed differential expression levels (ΔΔCt) of the genes of interest normalized to the housekeeping gene *AtTIP41* and relative to infected plants from the mock line (points indicates values for biological replicates, n=5 and n=8 for mock-line and pst-line respectively) at the tenth iteration. Symbols indicates significant differences (*p-value < 0.05, (.) p-value < 0.1), and "ns" indicates non-significant (p-value > 0.1) differences (Wilcoxon test)

Fig. 4 Legacy and *Pst* effect on bacterial and fungal community structure along the iterative process. Bray-Curtis dissimilarity (Beta-diversity) between successive iterations (i^{th} and $(i+1)^{th}$) in pst- and mock-lines for bacterial (a) and fungal (b) community. Intra and inter group Bray-Curtis dissimilarity (Beta-diversity) for bacterial (c) and fungal (d) community. Green and blue solid lines represent the median (n=3) and intervals represent the minimum and maximum values for pst and mock treatment, respectively. Grey solid line represents the median (n=3) and intervals represent the minimum and maximum value of beta-diversity between pst and mock treatment

Fig. 5 Community structure for bacteria along the experiment. (a) Correspondence analysis on bacterial diversity of i0 to i11. First and second axis account for 25 and 13 % of total variance respectively. (b) Correspondence analysis on bacterial diversity of iterations i8 to i11. First and second axis account for 21 and 16 % of total variance respectively. OTUs with the highest absolute contribution to the first axis are highlighted in blue and green, defining the soil bacterial sub-community of mock- and pst-lines respectively. Other OTU are shown in light grey. Symbols indicates the iteration: triangle for i8, inverted triangles for i9, squares for i10 and diamonds for i11. Mock- and pst-line barycenters are in blue and green respectively.

Fig. 6 Main changes in the bacterial community in response to *Pst* inoculation. Relative abundance and phylum associated with (a) *Pst* treatment (78 OTUs) and (b) mock treatment (34 OTUs). Bar height represents the relative abundance (% of total abundance) of each OTU. Colour indicates taxonomic classification of OTUs at the phylum level

Fig.1

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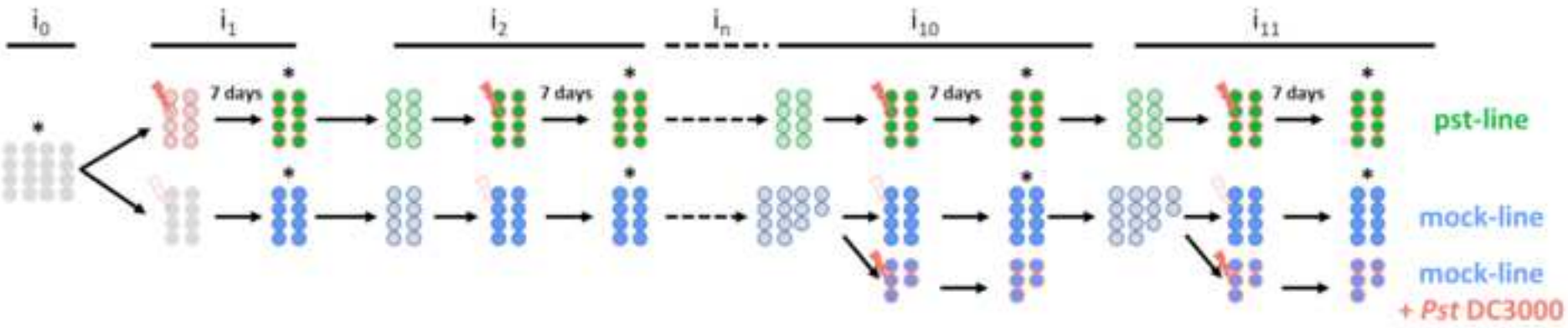

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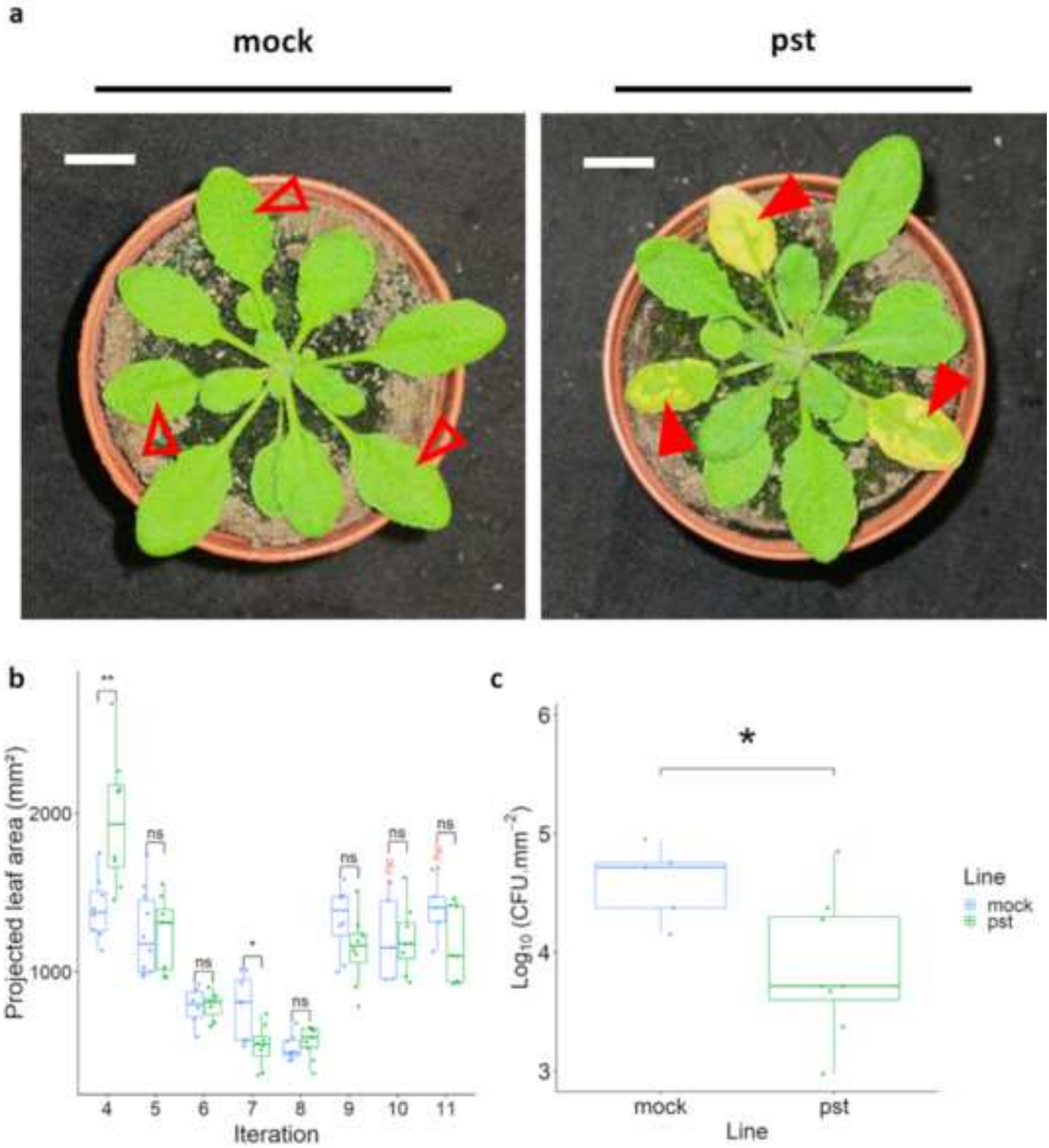

Fig.3

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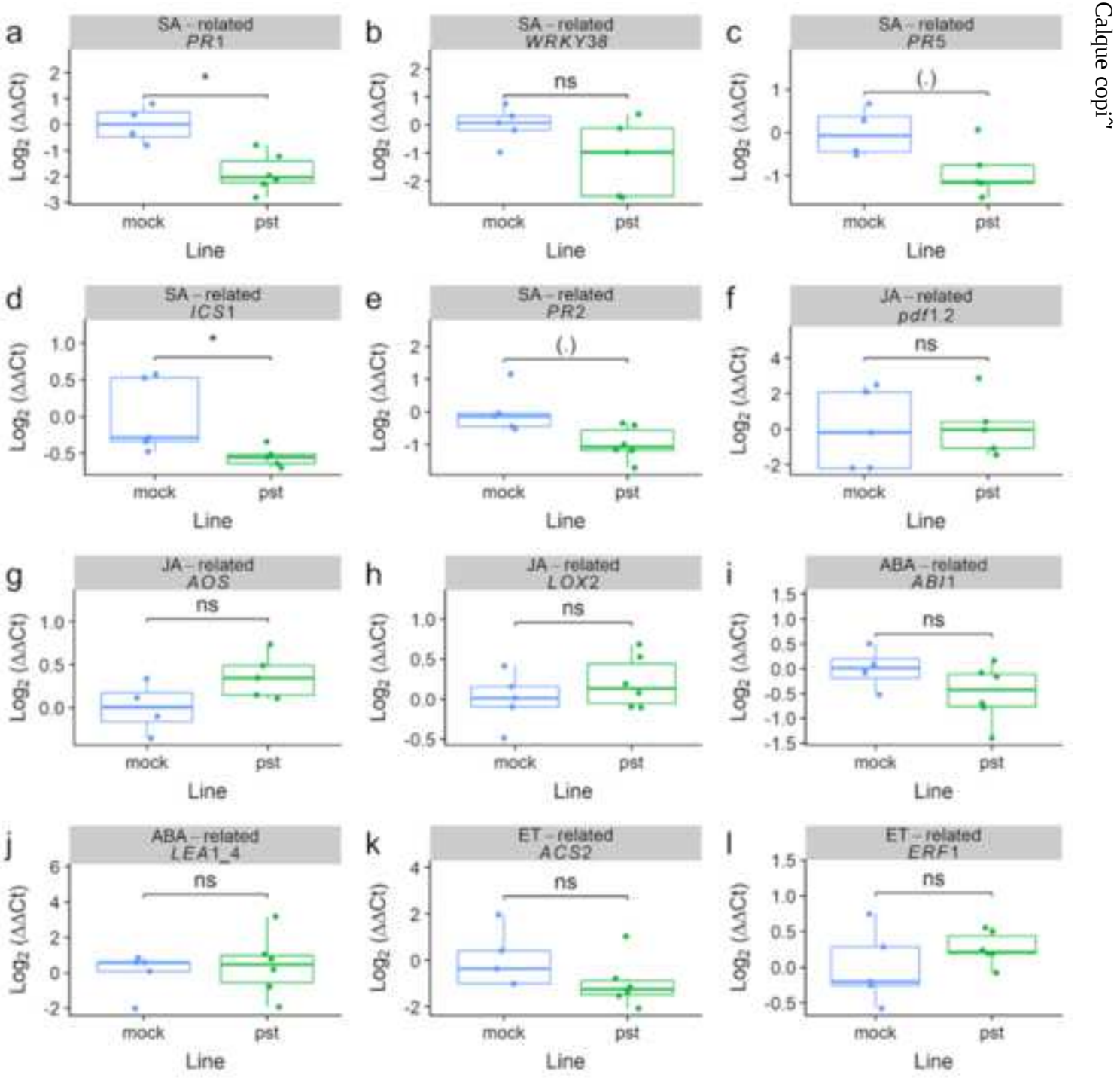

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Fig.4

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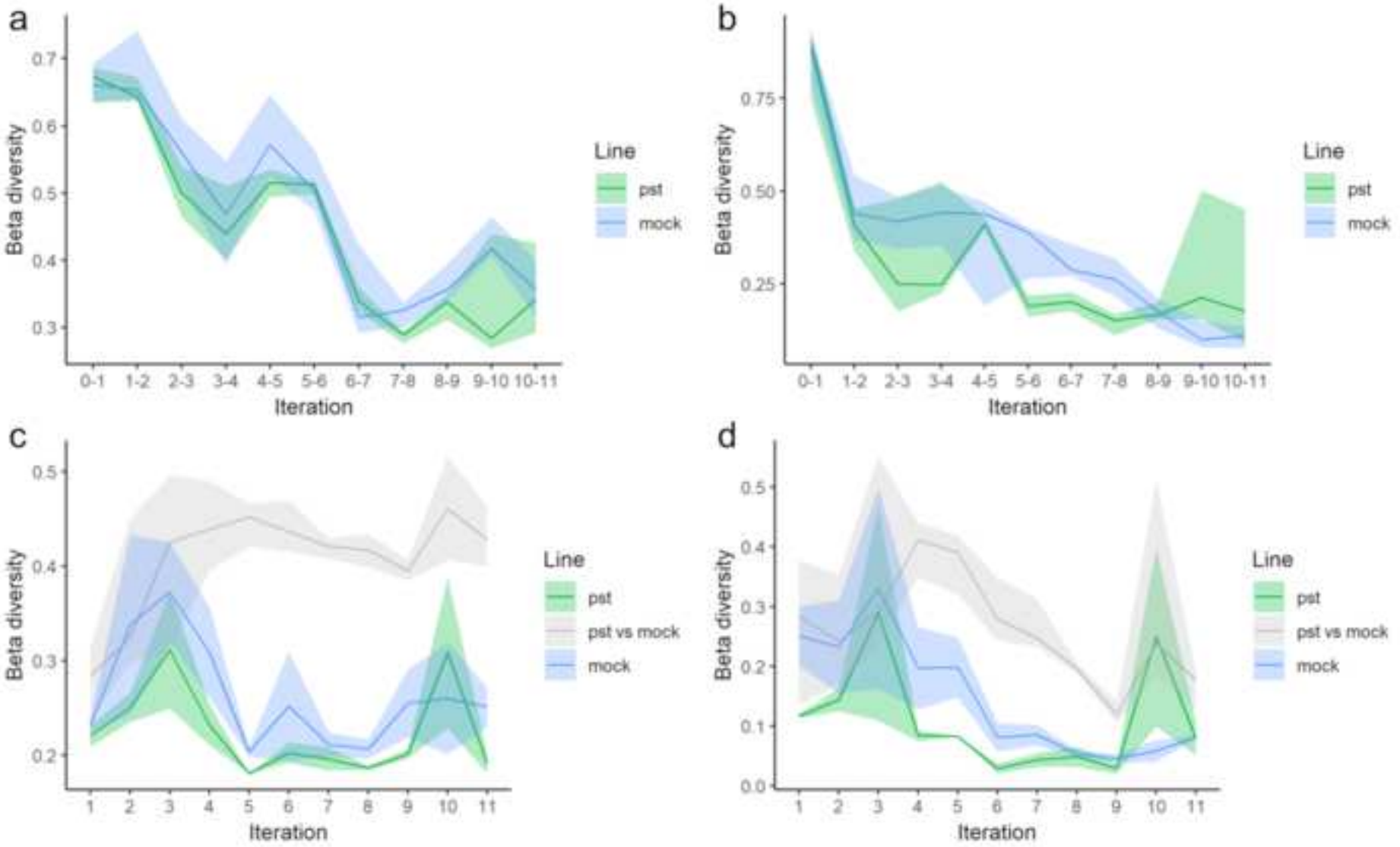

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Fig.5

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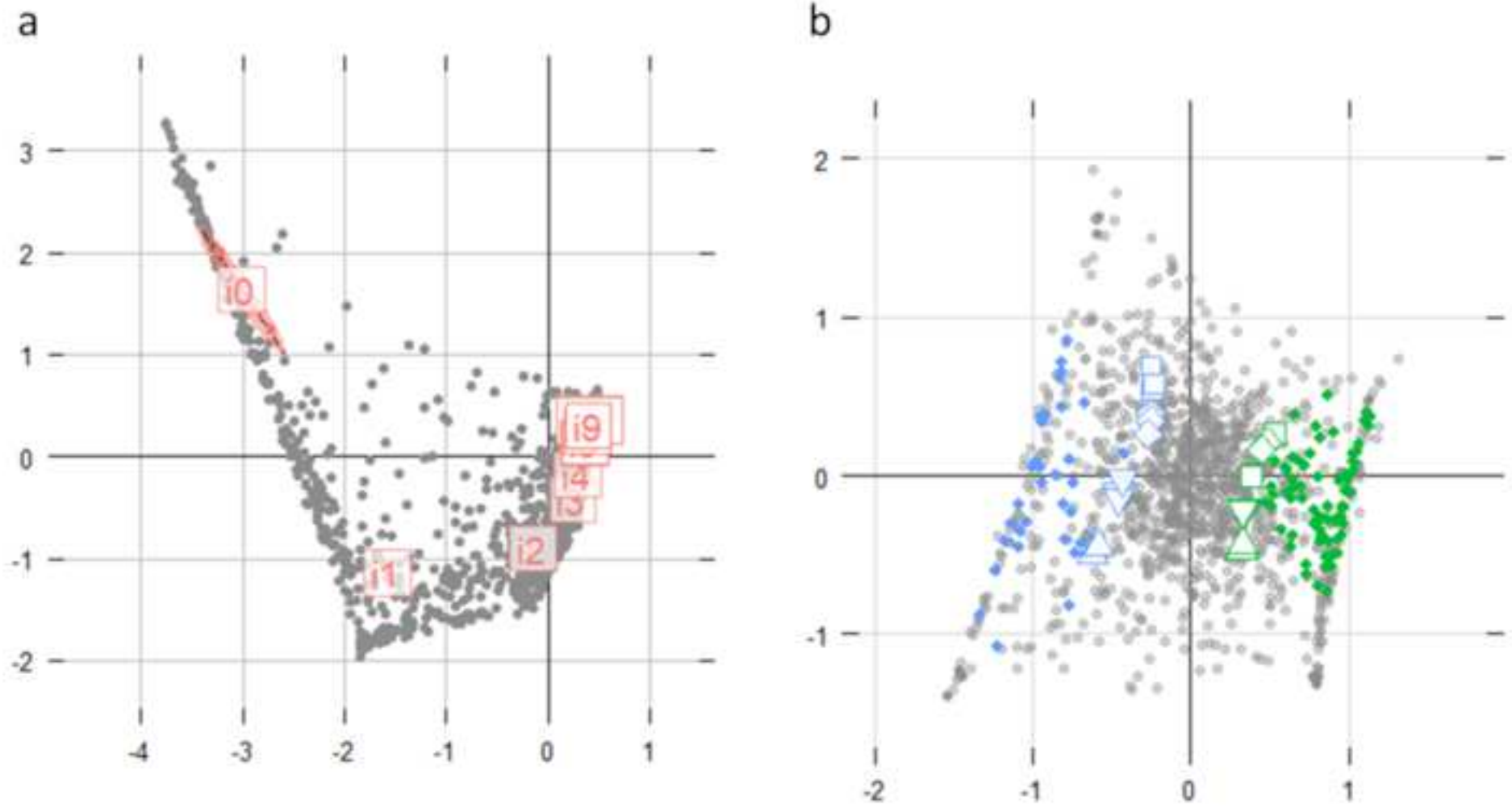

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Fig.6

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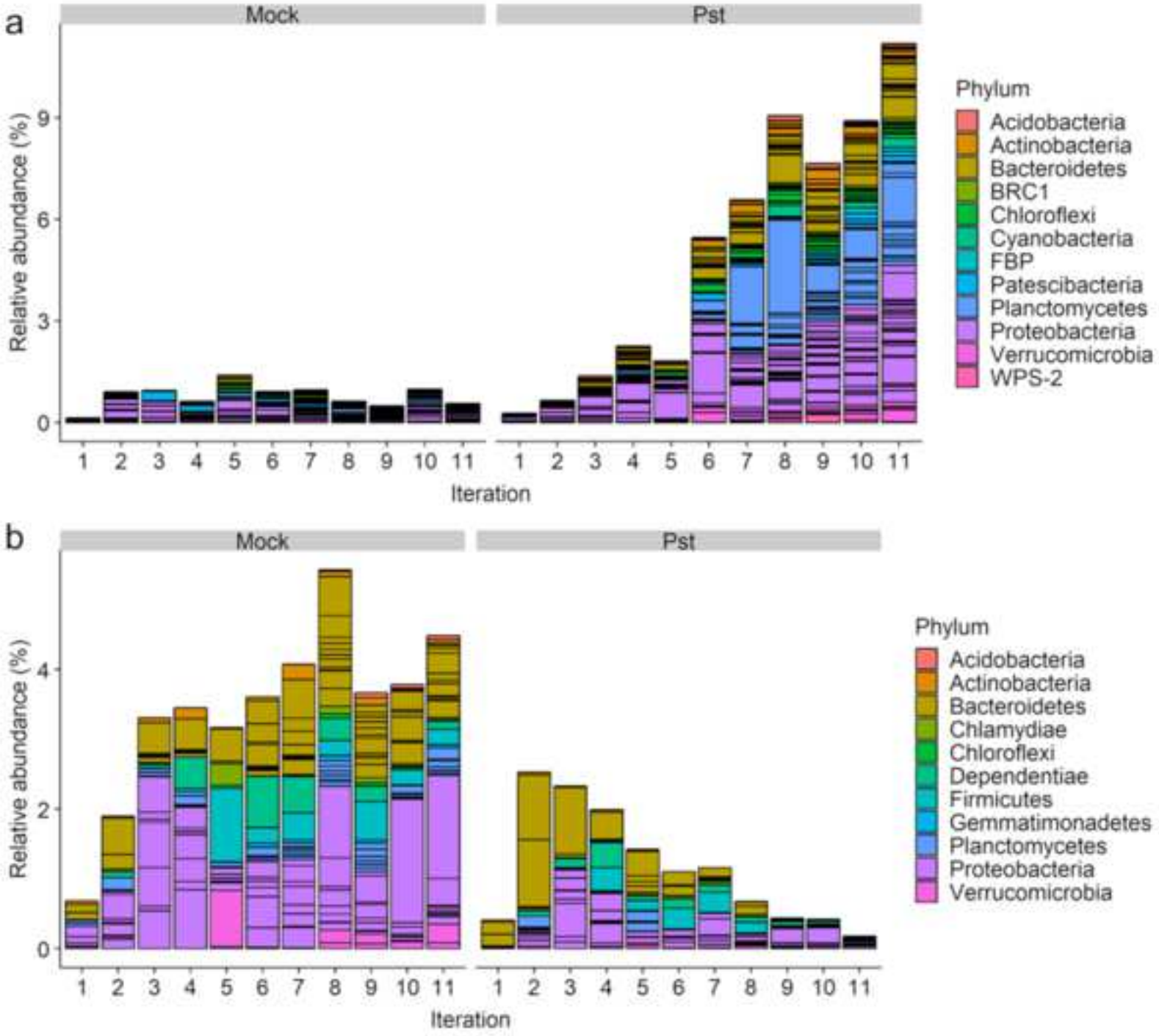

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Supplementary Material

ESM-1.pdf