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Root microbiome modulates plant growth promotion induced by low doses of glyphosate

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Root microbiome modulates plant growth promotion induced by low doses of glyphosate

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RESUMEN

El glifosato es un herbicida de amplio espectro de uso común que inhibe la producción de aminoácidos aromáticos debido a que se une de manera reversible a la 5-enolpiruvylshikimate-3-fosfato sintasa (EPSPS). Sin embargo, el glifosato a dosis subóptimas puede inducir el crecimiento en las plantas; este fenómeno se conoce como hormesis. Los estudios de hormesis de glifosato se realizan predominantemente en condiciones libres de microbios o con microbiomas reducidos, como *in vitro* o usando sustratos autoclavados en sistemas abiertos. Existen pocos ejemplos realizados en campos agrícolas donde se podrían albergar microbiomas más diversos. Este estudio tiene como objetivo comprender el efecto del microbioma de la raíz en la promoción del crecimiento inducida por el glifosato a dosis bajas. Para alcanzar este objetivo, elegimos *Arabidopsis thaliana*, como modelo vegetal, y una comunidad bacteriana sintética (SynCom) previamente caracterizada en otros estudios. Encontramos que con la dosis de 3.6x10-6 g ae / L (dosis baja de glifosato - DBG), en la condición sin bacterias, produjo un aumento de ~ 14% en el peso seco del brote, inesperadamente, el peso seco del brote se redujo en ~ 17 % con el SynCom completo. Además, DBG enriqueció dos cepas de Firmicutes (*Paenibacillus* CL91 y *Bacillus* CL72) y dos cepas de *Burkholderia* (MF6 y MF7). Todas estas cepas corresponden a inhibidores del crecimiento de la raíz (ICR), lo que podría explicar la inhibición del crecimiento por BDG. Para entender el vínculo entre RGI y la reducción del crecimiento en BDG, armamos una nueva comunidad sintética que no incluía las cepas de ICR. Con estas nuevas comunidades se recuperó la inducción del crecimiento por BDG. Finalmente, evaluamos si las cepas ICR prevenientes un filo específico eran suficientes para cambiar la respuesta de dosis bajas de glifosato de la promoción a la inhibición del crecimiento. Descubrimos que cualquiera de los grupos de ICR era suficiente para bloquear la inducción del crecimiento y promover la inhibición del crecimiento. Nuestros resultados indican que la hormesis del glifosato depende completamente de la composición del microbioma de la raíz, específicamente de la presencia de inhibidores del crecimiento de la raíz. Dado que las cepas ICR se encuentran dispersas en la filogenia bacteriana y podrían encontrarse en los microbiomas de la raíz natural, tratar de aplicar la hormesis de glifosato en entornos agrícolas o silvestres sería difícil de lograr. Esto podría explicar el reducido número de ejemplos de hormesis de glifosato en el campo.

Palabras clave: Glifosato, hormesis, microbioma, comunidad bacteriana sintética, inhibidores del crecimiento de la raíz.

ABSTRACT

Glyphosate is a commonly used herbicide with a broad action spectrum that inhibits the production of aromatic amino acids by bonding reversibly to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). However, glyphosate at suboptimal doses can induce plant growth; this phenomenon is known as hormesis. Glyphosate hormesis studies are predominantly done in microbe-free or reduced microbiomes conditions, such as *in vitro* or using autoclaved substrates in open systems with a few examples performed in agricultural fields where more diverse microbiomes could be harbored. This study aims to understand the effect of the root microbiome on the growth promotion induced by glyphosate at low doses. To pursue this goal, we chose to use *Arabidopsis thaliana*, as a plant model, and a synthetic bacterial community (SynCom) previously characterized in other studies. We found that with a dose of 3.6x10-6 g a.e./L (low dose of glyphosate - LDG), in the no bacteria condition, produced ~14% increase in shoot dry weight, unexpectedly, shoot dry weight was reduced by ~17% with full SynCom. Additionally, LDG enriched two Firmicute strains (*Paenibacillus* CL91 and *Bacillus* CL72) and two *Burkholderia* strains (MF6 and MF7). All these strains correspond to root growth inhibitors (RGI), which could explain growth inhibition by LDG. To further test the link between RGI and the growth reduction in LDG, we assembled a new synthetic community that did not include the RGI strains. With these new community growth induction by LDG was recovered. Finally, we evaluated if RGI strains from a specific phylum were sufficient to switch the response of low doses of glyphosate from growth promotion to growth inhibition. We found that any of the groups of RGI was enough to block growth induction and promoting growth inhibition. Our results indicate that glyphosate hormesis is completely dependent on the root microbiome composition, specifically on the presence of root growth inhibitors. Since RGI strains are scattered in the bacterial phylogeny and could be found in natural root microbiomes, try to apply glyphosate hormesis in agricultural or wild environments would be tough to achieve. This could account for the reduced number of examples of glyphosate hormesis in the field.

Key words: Glyphosate, hormesis, microbiome, synthetic bacterial community, root growth inhibitors.

TABLA DE CONTENIDO

ÍNDICE DE FIGURAS

RESULTS

Shoot growth promotion induced by low doses of glyphosate is lost in the presence of the full Bacterial Synthetic Community (SynCom)

Glyphosate is a commonly used herbicide that inhibits the production of aromatic amino acids by bonding reversibly to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway (Duke *et al.*, 2012). Because the enzyme EPSPS is essential in all higher plants, glyphosate has a broad action spectrum, and its use has increase since the development of resistant commercial crops (Duke *et al.*, 2012; Brito *et al.*, 2018). However, glyphosate at suboptimal doses can induce plant growth; this phenomenon is known as hormesis (Brito *et al.*, 2018). There are some examples reported in the literature in different species, in some cases, reaching 125% dry mass increase (Brito *et al.*, 2018; Velini *et al.*, 2008).

Notwithstanding these promising results, glyphosate hormesis is not a widespread phenomenon, suggesting that it depends on multiple factors that are have not been adequately investigated. Looking more closely at these studies, they were predominantly done in microbe-free or reduced microbiomes conditions, such as *in vitro* or using autoclaved substrates in open systems (Velini *et al.*, 2008; Ather *et al.*, 2017; Wagner *et al.*, 2003; Carvhallo *et al.*, 2013; Pokhrel *et al.*, 2015). There are only a few examples performed in agricultural fields (Cedergreen *et al.*, 2009; El Shahawy *et al.*, 2011), where more diverse microbiomes could be harbored (Fierer, 2017). Application of standard doses of glyphosate produces small changes in the soil microbiome composition, particularly, it affects the abundance of Actinobacteria and Proteobacteria (Duke *et al.*, 2012; Newman *et al.*, 2016). In addition, glyphosate can alter the gene expression of bacteria in the rhizosphere, reducing

carbohydrate and amino acid metabolism transcripts and enhancing protein metabolism and respiration gene transcription (Newman *et al.*, 2016).

This study aims to understand the effect of the root microbiome on the growth promotion induced by glyphosate at low doses. To pursue this goal, we chose to use *Arabidopsis thaliana*, as a plant model, and a synthetic bacterial community (SynCom) previously characterized in other studies (Finkel *et al.*, 2019a; Finkel *et al.*, 2019b). This community is composed by 185 bacterial isolates from the root endophytic compartment of healthy Arabidopsis plants (Finkel *et al.*, 2019a). The main advantage of this system is that we could have a full bacterial community and a no-bacteria control under the same conditions [12,14]. We selected 3.6x10-6 g a.e./L as a low dose of glyphosate (LDG) (Supplementary Figure S1). In the no bacteria condition, LDG produced ~14% increase in shoot dry weight. Unexpectedly, when the seedlings were exposed to the same dose and the full SynCom, shoot dry weight was reduced by ~17% compared to the full SynCom without glyphosate.

On the other hand, the main root elongation was always limited by LDG; even though, the effect was greater with the full SynCom (~2% reduction in no bacteria versus ~22% reduction with full SynCom) (Figure 1B). Overall, the growth promotion of the shoot was produced in the absence of microbiome, as reported previously, but the effect was lost in the presence of the full SynCom.

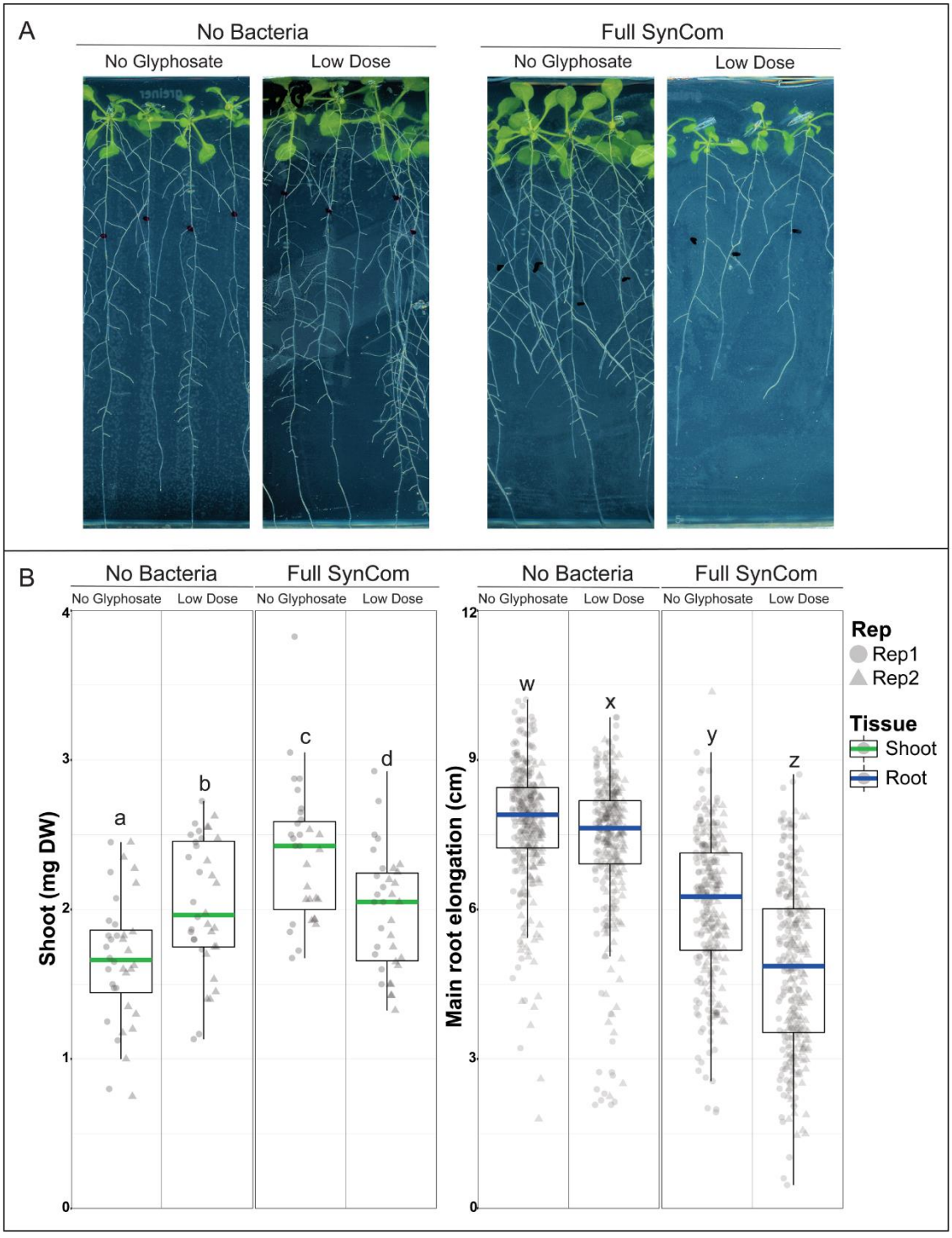

Figure 1. Shoot growth promotion induced by low doses of glyphosate is lost in the presence of the full Bacterial Synthetic Community (SynCom). (A) Twelve-day old seedlings in half MS media with or without SynCom and a low dose of glyphosate (3.6x10-6 g a.e./L). (B)

Boxplot for shoot dry weight and main root elongation. Letters over the bars indicate significant differences (Tukey's HSD $p < 0.05$). Eighteen plates were used per treatment in two replicas. Each plate contained 8-10 seedlings

Low doses of glyphosate produce small changes in microbiome composition of agar and root

To understand the effect of low doses of glyphosate in the synthetic community, the agar and root microbiomes were characterized using metagenomics of the V3-V4 region of the 16S gene. The sequences obtained were merged and mapped directly against the 16S sequences for all members of the synthetic community with zero mismatches to identify the corresponding isolate (Finkel *et al.*, 2019a). Constrained Analysis of Principal Coordinates (CAP) showed that CAP1 separates the fractions (agar and root) and it represents the 96.37% of the variance, while the CAP2 represents the separation between glyphosate treatments but only account for 1.98% of the variance (Figure 2A). Even though the effect of LDG over the microbiome was small was still statistically significant. Proteobacteria had a slightly increase in the agar, while Firmicutes were increased in the root, and Actinobacteria dropped in both fractions (Figure 2B).

To identify specific isolates affected, a model was created using DESeq (Bioconductor) (Tsolakidou *et al.*, 2019) that compare low dose versus no glyphosate in each fraction. Most of the differentially abundant isolates were found enriched in the LDG condition in the root without direct relation with phylogeny. Two strains of Firmicutes (one *Paenibacillus* and one *Bacillus* strain), were enriched in LDG with more than two log2 fold change (Figure 2C), which correspond to the increased in Firmicutes observed in the phylograms (Figure2B). Leaving aside these specific strains, most of the synthetic community remained unchanged.

Low doses of glyphosate enrich Root Growth Inhibitor strains

In a previous study, Finkel *et al.* (2019a) explored the role of different members of the synthetic community on root development. To this end, they planted A. thaliana seedlings in mono-association with each member of the SynCom and recorded the main root elongation. Isolates that generated less than 3cm of elongation were considered Root Growth Inhibitors (RGI). RGI strains were found across all the community in all the phyla, except in Bacteroidetes (Finkel *et al.*, 2019a). To explore a link between RGI strains and the reduction in growth produce by LDG, we constructed a phylogenetic tree using 47 gen markers from the 185 isolates, and we mapped the main root elongation in mono-association (outer ring Figure 2D). We found that the two highly enriched strains in LDG (*Paenibacillus* CL91 and *Bacillus* CL72) correspond to RGI; additionally, two *Burkholderia* strains (MF6 and MF7) are also enriched (Figure 2D). Other non-RGI were also enriched in LDG, even so growth reduction may not be related to those strains.

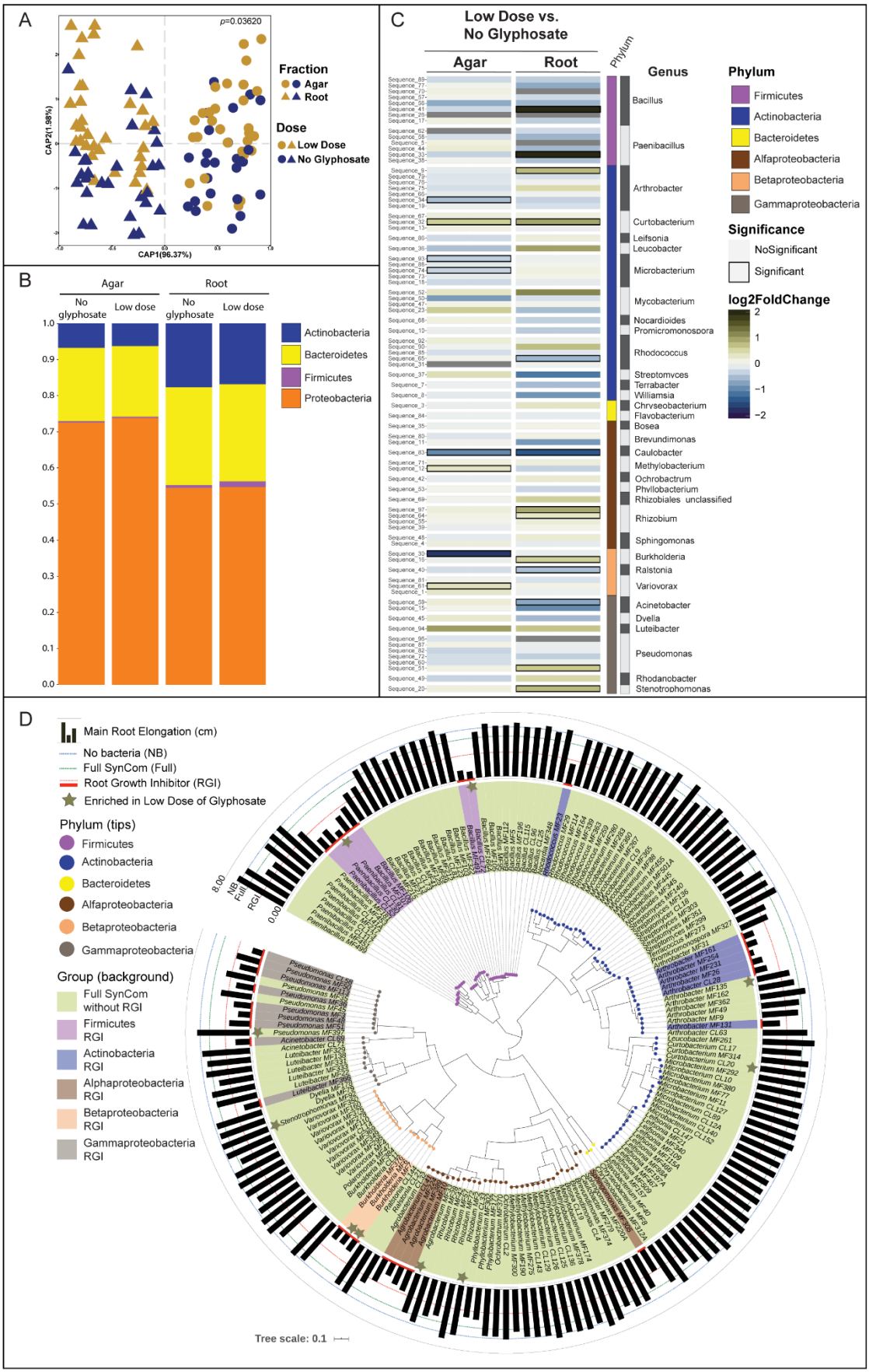
A
p=0.03620
CAP2(1.98%)
CAP1(96.37%)
Fraction
Agar
Root
Dose
Low Dose
No Glyphosate
B
Agar
Root
No glyphosate
Low dose
No glyphosate
Low dose
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Actinobacteria
Bacteroidetes
Firmicutes
Proteobacteria
C
Low Dose vs.
No Glyphosate
Agar
Root
Phylum
Genus
Bacillus
Paenibacillus
Arthrobacter
Curtobacterium
Leifsonia
Leucobacter
Microbacterium
Mycobacterium
Nocardioides
Promicromonospora
Rhodococcus
Streptomyces
Terrabacter
Williamsia
Chryseobacterium
Flavobacterium
Bosea
Brevundimonas
Caulobacter
Methylobacterium
Ochrobactrum
Phyllobacterium
Rhizobiales unclassified
Rhizobium
Sphingomonas
Burkholderia
Ralstonia
Variovorax
Acinetobacter
Dyella
Luteibacter
Pseudomonas
Rhodanobacter
Stenotrophomonas
Phylum
Firmicutes
Actinobacteria
Bacteroidetes
Alfaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Significance
NoSignificant
Significant
log2FoldChange
2
1
0
−1
−2
D
Main Root Elongation (cm)
No bacteria (NB)
Full SynCom (Full)
Root Growth Inhibitor (RGI)
Enriched in Low Dose of Glyphosate
Phylum (tips)
Firmicutes
Actinobacteria
Bacteroidetes
Alfaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Group (background)
Full SynCom without RGI
Firmicutes RGI
Actinobacteria RGI
Alphaproteobacteria RGI
Betaproteobacteria RGI
Gammaproteobacteria RGI
8.00
NB
Full
RGI
0.00
Tree scale: 0.1

Figure 2. Low doses of glyphosate enrich Root Growth Inhibitor strains. (A) Constrained Analysis of Principal Coordinates (CAP) scatterplots showing the effect the low dose of glyphosate (LD) within agar and root fractions. PERMANOVA p-value from glyphosate dose is presented. (B) Phylograms representing the microbiome composition for each fraction and glyphosate treatment. (C) Low dose glyphosate enrichment patterns across the two fractions. Each row represents a unique V3V4 sequence from the SynCom isolates. The heatmaps are colored by log2 fold changes. LDG enrich sequences are presented in green gradient, while no glyphosate enrich sequences are presented in blue gradient. Comparisons with q-value < 0.05 are contoured in black. (D) Phylogenetic tree of 185 members included in the synthetic community (SynCom). The outer ring displays the main root elongation produce of each isolate in mono-association. Eighteen plates were used per treatment in two replicas. Each plate contained 8-10 seedlings.

Growth promotion induced by low doses of glyphosate is recovered when RGI strains were drop out from the community

To further test the link between RGI and the growth reduction in LDG, we assembled a new synthetic community that did not include the RGI strains. This new Syncom was composed of all isolates with green background in Figure 2D. As in previous experiments, LDG induced growth in the no bacteria control and reduced growth with the full SynCom. However, when the RGI strains were not incorporated (Full-RGI SynCom), shoots were larger than the no glyphosate treatment (Figure 3). Surprisingly, the rosettes had more dry weight than any other treatment, showing that growth promotion induced by the Full-RGI SynCom and by the low dose of glyphosate possibly have distinct mechanisms.

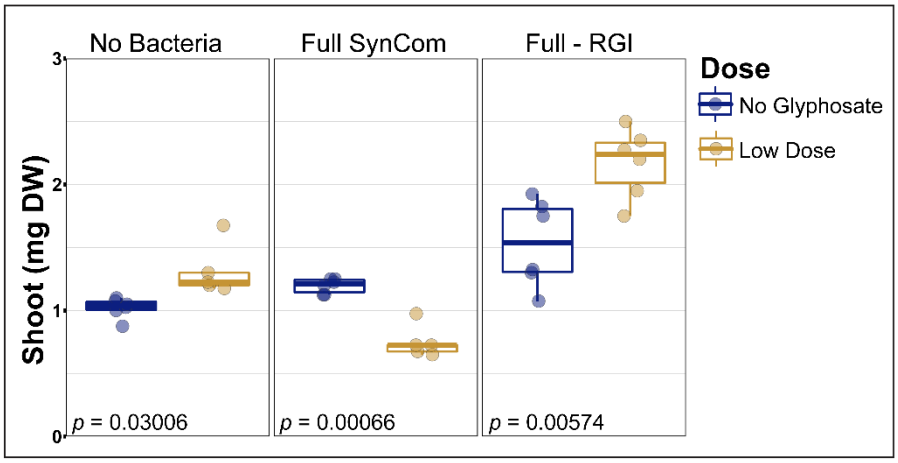

Figure 3. Growth promotion induced by low doses of glyphosate was recovered when RGI strains were drop out from the community. Each panel shows the effect of the low dose glyphosate with different bacterial treatments. The middle panel represents the full 185 member SynCom, while the community in the right panel does not include any root growth inhibitor strains (Full-RGI). FDR-corrected p-values are shown within each plot. Six plates were used per treatment. Each plate contained 8-10 seedlings.

RGI strains from different phyla are sufficient to reduce growth with low doses of glyphosate

Finally, we evaluated if RGI strains from a specific phylum were sufficient to switch the response of low doses of glyphosate from growth promotion to growth inhibition. We use the SynCom without RGI as a base (Full-RGI), and then we put back each specific group of RGI from each phylum. Any of the groups of RGI was enough to block growth induction and promoting growth inhibition, with the exception of Betaproteobacteria.

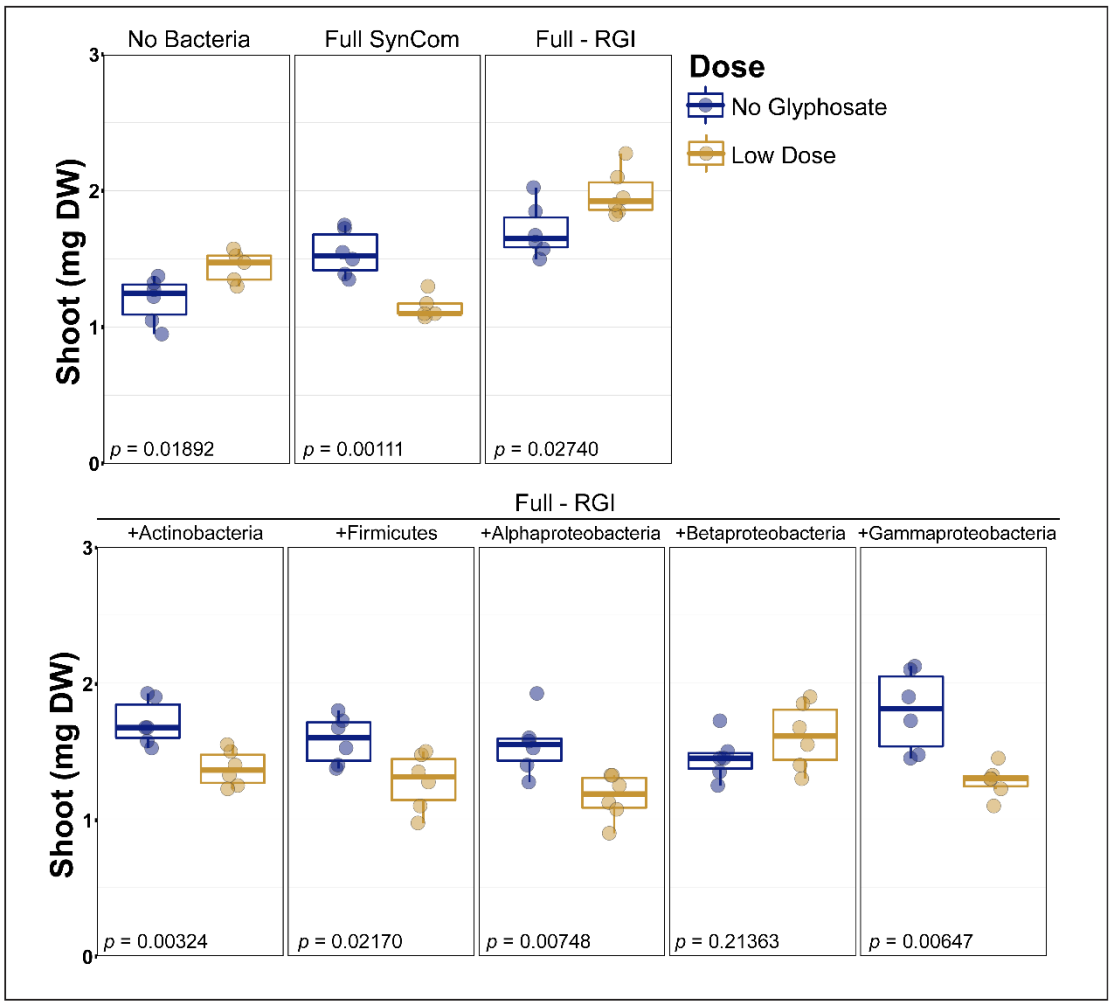

Figure 4. RGI strains from different phyla are sufficient to reduce growth with low doses of glyphosate. Each panel shows the effect of the low dose glyphosate with different bacterial treatments. Top panels represent the full 185 member SynCom and the SynCom without root growth inhibitor strains (Full-RGI). Bottom panels indicate the outcome after including a specific RGI group with the Full-RGI community. FDR-corrected p-values are shown within each plot. Six plates were used per treatment. Each plate contained 8-10 seedlings.

DISCUSION

We were able to observe growth promotion induced by low doses of glyphosate as previously reported *(Brito et al., 2018)*. At recommended doses, glyphosate inhibits growth by blocking the activity of the EPSPS enzyme resulting in the reduction of cyclic amino acid production *(Duke et al., 2012)*. However, an opposite effect was observed in soybean exposed to low doses, plants had higher levels of cyclic amino acids and other aromatic compounds downstream EPSPS, especially, tryptophan which increased 80% respect to the control (Silva *et al.*, 2016). This can show that LDG could up-regulate the production of EPSPS and the pathway downstream. In Arabidopsis, overexpressing of exogenous EPSPS, in absence of glyphosate, has been correlated with dry mass gain and higher auxin content (Fang *et al.*, 2018), so a link could be stablished with LDG, increase expression of EPSPS and growth promotion, nonetheless further analysis of transcriptomics and metabolomics need to be performed. A critical point to notice is that fitness enhancement by overexpression of EPSPS have been observed *in vitro* or in greenhouse experiments for various species, but contradictory results were found in field experiments (Yang *et al.*, 2017), similar to what have been reported about glyphosate hormesis.

To understand the effect of root microbiome over glyphosate hormesis, we employed a previously characterize SynCom (Finkel *et al.*, 2019a; Finkel *et al.*, 2019b). Low doses of glyphosate produced small changes in the composition of the root microbiome, as previously report for higher doses (Duke *et al.*, 2012). Assembly of root microbiome is in part dependent of root exudates, specifically on aromatic organic acids and amino acids (Zhalnina *et al.*, 2018;

Sasse *et al.*, 2018). Since, LDG could be related to alter production of cyclic compounds and tryptophan (Silva *et al.*, 2016), this could account for the changes in the microbiome.

Our results indicate that glyphosate hormesis is completely dependent on the root microbiome composition, specifically on the presence of root growth inhibitors. Since RGI strains are scattered in the bacterial phylogeny and could be found in natural root microbiomes, try to apply glyphosate hormesis in agricultural or wild environments would be tough to achieve. This could account for the reduced number of examples of glyphosate hormesis in the field. We expect that plants that overexpress EPSPS grown with the synthetic communities designed in this study will have the same range of responses that we found with low doses of glyphosate.

Bacterial root growth inhibition could arise from different mechanisms like production of toxic compounds (Hogenhout *et al.*, 2008), presence of pathogen-associated-molecular-pattern (PAMP) or microbe-associated molecular pattern (MAMP) (Teixeira *et al.*, 2019; Pel *et al.*, 2012), and production of auxin and auxin-like compounds (Finkel *et al.*, 2019a). All these mechanisms are widespread in all the bacterial phyla and one strain could harbor more than one (Finkel *et al.*, 2019a). Various isolates from the 185-member SynCom have been identified as auxin/auxin-like producers (Finkel *et al.*, 2019a), also, tryptophan-dependent IAA synthesis pathways were found in 82.2% of 7282 bacterial genomes associated with plant root environments (Zhanh *et al.*, 2019). Increment of tryptophan concentration in the root and root proximities are related to enhance production of auxin/auxin-like compounds by different bacteria that could result in enhance growth inhibition (Jaeger *et al.*, 1999; Sarwar *et al.*, 1995). This could clarify why was necessary to drop up all root growth inhibitor isolates from the community to complete recover grown induction.

Using a bottom-up approached, we were able to assemble different synthetic communities that modulated the plant response to low doses of glyphosate, from growth inhibition to growth promotion, depending on the presence of root growth inhibitors. Complementary experiments are essential to link tryptophan overproduction induce by low doses of glyphosate and growth inhibition.

STAR METHODS

Experimental model and subject details

In vitro plant growth conditions.

Arabidopsis thaliana Columbia – 0 (Col-0) seeds were surface-sterilized for 10min with 70% bleach + 0.2% Tween-20, rinsed three times with sterile distilled water, and stratified at 4 °C for two days at dark. Between 40 to 50 seeds were sowed on vertical square plates with half- strength MS supplemented with 0.5g/L MES + 5g/L sucrose + 10g/L Bacto-agar with a final pH of 5.6-5.7. After 7 days, 10 seedlings were transfer to vertical square plates with half-strength MS supplemented with 0.5g/L MES + 10g/L Bacto-agar, with or without the synthetic community (SynCom) and the different glyphosate treatments with a final pH of 5.6-5.7. For the dose standardization experiment, 3.6x10-3 and 3.6x10-6 g a.e./L were assessed; for all the rest of the experiments, 3.6x10-6 g a.e./L was used a low dose of glyphosate (LDG). Each experiment included a no glyphosate control. Plates were set randomly in a growth chamber with a 16-h dark/8-h light regime at 21 °C day/18 °C night for 12 days.

Bacterial culture and plant-inoculation.

The bacterial isolates used here were previously obtained and sequenced in a previous study [29]. Seven days before each experiment, glycerol stocks from each isolate were inoculated in 400µL KB medium in a 96 deep well plate. The plates were incubated at 28°C at 250 rpm. After 5 days, 40µL of the liquid culture were transfer to a new 96-well plate with fresh 400µL KB medium and grow under the same conditions for 2 days. Ultimately, 7-day and 2-day liquid culture were combined for each isolate. This procedure accounts for variable growth rates and aims to secure that non-stationary cells are present in the final inoculum, as previously

describe by (Finkel *et al.*, 2019a). The number of cells for each isolate was normalized to a optical density at 600nm (OD600) equal to 1 (Infinite M200 Pro plate reader, TECAN) in the final pool. The mixed culture was washed three times with with 10 mM MgCl2 to remove media and debris. Then, the washed mixed culture was diluted to a final OD600 of 0.2, and 100 µL of the inoculum was spread on 10 X 10 cm vertical square agar plates with the corresponding medium prior to transfer the seedlings.

Method details

DNA extraction.

Roots were pooled from 6-8 plants for each plate and placed in 2.0 ml Eppendorf tubes with three sterile glass beads. Then, the samples were wash three times with sterile distilled water and frozen with liquid nitrogen. Roots were lyophilized for 48 hours (Labconco freeze dry system) and pulverized (tissue homogenizer MPBio). Agar from each plate was collected in 60 ml syringes with sterilized Miracloth (Millipore) at the tip and store at -20 °C. After one week, syringes were thawed at room temperature and then gently pressed through the Miracloth into 50 ml tubes. The liquid samples were centrifuged at 4200rpm for 20 min and the supernatant was discarded. The remaining liquid with the pellet was transferred into a 2.0 ml Eppendorf tube, centrifuged, all supernatant was removed, and the pellet stored at -80 °C. DNA from root samples and pellets from agar were carried out using 96-well-format DNeasy PowerSoil Kit (Qiagen) following the manufacturer's instruction. Samples were randomized in the plates and maintained throughout library preparation and sequencing.

Library preparation and sequencing.

Library preparation were done accordingly to Gohl *et al.*, (2016) using a dual index approached. V3-V4 region of the bacterial 16S rRNA gene were amplified using the primers

338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reaction was performed as follows: 1.5 µL of 10 µM of each primer, 1 µL of 10 µM mitochondrial PNA, 1 µL of 10 µM plastid PNA, 6.5 µL PCR grade water, 12.5 µL Kappa Mastermix and 1 µL gDNA template; temperature cycling: 3 min. 95° C, 20 cycles of 15 sec. 95° C, 15 sec. 78° C, 15 sec. 50° C and 15 sec. 72° C. PCR reaction were made in triplicate and amplification was checked in 1.5% agarose gel at 100 V for 35 min. The triplicate reactions were pooled and purified using AMPure XP magnetic beads (Beckman Coulter) and quantified with Qubit BR DNA assay (Invitrogen). Libraries were pooled in equal amounts and then diluted to 10 pM for sequencing on an Illumina MiSeq instrument using a 600-cycle V3 chemistry kit.

Amplicon sequence data processing.

Reads with 100% correct primer sequences were merged (MT-Toolbox) (Yourstone *et al.*, 2014) and quality filtered (Sickle) (Joshi *et al.*, 2011) for Q-score > 20. The merge sequences were globally aligned to the 16S rDNA sequences of the 185 isolates in the SynCom (USEARCH v7.1090) (Edgar, 2010) and were classified into 97 unique sequences (USeq). A USeq is a cluster of 100% identical sequences coming from a single or multiples isolates, as previously done by Finkel *et al.* (2019a). Arabidopsis organellar and known bacterial contaminants were remove using the option 'usearch_global' at a 98% identity threshold (USEARCH v7.1090). Mapped sequences were used to produce an abundance table. The table were processed and analyzed with functions from the ohchibi package (https://github.com/isaisg/ohchibi).

Beta diversity was analyzed with a Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis dissimilarity calculated from the relative abundance matrices. The Fraction:Dose interaction analysis was perform constraining for the replica effect. Also, a

Permutational Multivariate Analysis of Variance (PERMANOVA) was perform using the adonis function from vegan package v2.5-3 (Oksanen et al, 2015).

To establish the enrichment profiles in the comparison Low Dose of Glyphosate vs. No Glyphosate from each fraction, we employed the package DESeq2 v1.22.1 (Love *et al.*, 2014) to run the model: Abundance ~ Dose + Rep using the USeq count table. A USeq was considered statistically significant if it had a false discovery rate (FDR) adjusted p-value < 0.05.

Phylogenetic tree.

The phylogenetic tree of the SynCom isolates was previously construct by Finkel *et al.* We selected the same 47 markers and the same approached to create a super alignment and to infer the phylogeny utilizing the WAG model of evolution (FastTree v2.1) (Price *et al.*, 2010). Then, we used the web-based tool (https://itol.embl.de/) to visualize the tree and to add the information of main root elongation from each isolate in mono-association available in supplementary data S4 (https://www.biorxiv.org/content/10.1101/645655v1.supplementary-material) by Finkel *et al.* (2019a).

Quantification and statistical analysis

Growth assessment.

Shoot and root growth were measure after twelve days post-transferring to the specific media as described in "*In vitro* plant growth conditions" section. For main root elongation, plates were imaged using a document scanner, and primary root length from each plant was measured using ImageJ. Shoot were harvest for dry weight. Six to eight shoots, from one plate, were put in a preweighed 2.0 ml Eppendorf tubes and placed in an oven at 60° C for 72h when the weight of the tubes was stable. To calculate the dry weight, the initial weight

of the tube was subtracted from weight of the tube with the shoot after 72h and divided to the number of shoots placed in each tube. For the experiment presented in Figure 1, main root elongation and shoot dry weight were assessed, while for dose standardization (Supplementary Figure S1) and drop-out experiments (Figure 3 and Figure 4) only shoot dry weight were used.

Statistical analysis.

Analyses of variance (ANOVA), controlling for the replicate effect, was used for Figure 1B and S1. Differences between treatments were shown using the confidence letter display (CLD) derived from the Tukey's post hoc test (package emmeans)

Statistical analysis used in Figure 2 are explain in "Amplicon sequence data processing" section.

For Figure 3 and 4, differences between No Glyphosate and Low Dose were analyzed using Student's t-test and adjusting the p-values for multiple testing using false discovery rate (FDR) performed in R.

Number of replicates are given in the respective figure legends.

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SUPPLEMENTAL INFORMATION

Supplementary Figure S1

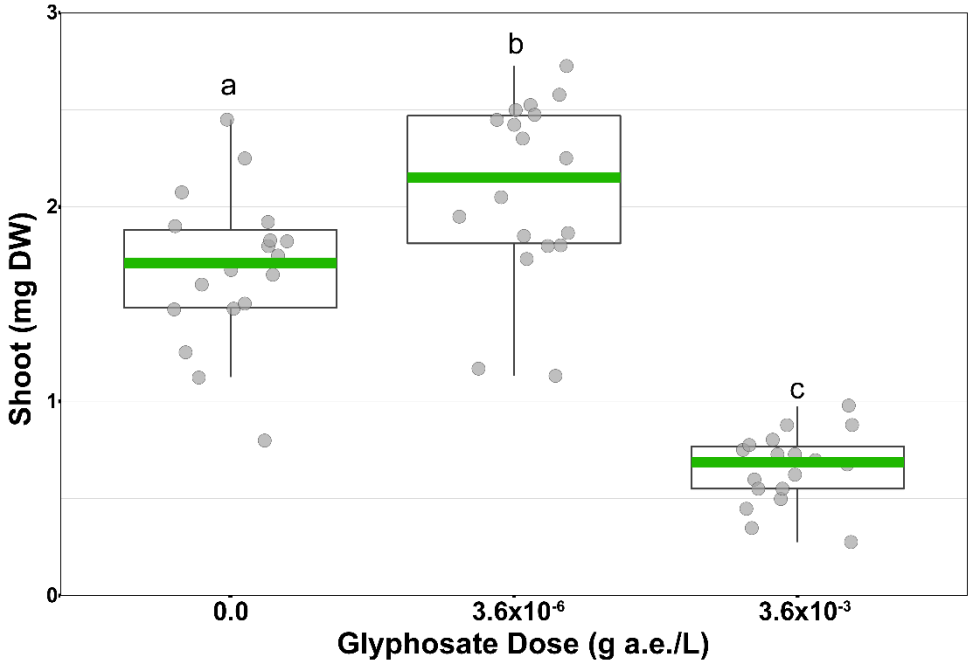

Figure S1. Glyphosate dose standardization. 7-day all seedlings were transfer to half-strength MS media without glyphosate (0.0) or amendment with 3.6x10-6 and 3.6x10-3 g a.e./L. and harvested after 12 days. Boxplot for shoot dry weight shows that 3.6x10-6 g a.e./L induce growth compare to no-glyphosate, while 3.6x10-3 g a.e./L produce toxic effects. Letters over the bars indicate significant differences (Tukey's HSD $p < 0.05$). Eighteen plates were used per treatment in two replicas. Each plate contained 8-10 seedlings.en subtítulos.

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