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Peer Review File

Organic pollutant-induced long-distance ROS signaling drives plant systemic acquired acclimation via rhizomicrobiota

Corresponding Author: Professor Xiangyang Yu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript reports a novel study on organic pollutant-induced long-distance ROS signaling drives plant acquired acclimation via rhizosphere bacteria. The manuscript was generally well organized. Some issues should be concerned and improved.
1.The second paragraph of Introduction: How about the response of foliar/phyllosphere microbiome to organic pollutants? Is it possible that the immigration of foliar/phyllosphere microbes to rhizosphere?
2. Lines 109-110: Please give the full names for B. rapa, Thia, etc.
3. Lines 110-112: Did you analyze the metabolic intermediates of the targeted organic pollutants? Is it possible that the transfer/translocation of intermediates after their metabolism in vivo? Please confirm the exclusion of pollutants and their intermediates.
4. Lines 134-141: Please explain why only foliar-sprayed with Thia to investigate the functions of strains and synCom?
5.Lines 173-177: how to differentiate the decreasing amount of metabolites by secretion (down-regulated) or consumption?
6.How about the ROS contents and expression of RBOH gene clusters in leaves and shoots? How to confirm the long-distance transmission of ROS from leave to shoots?
7.Why not use some inhibitors to reduce ROS content in roots to verify its effect on plant carbon efflux?
8.Please check the expression, such as H2O2 and hydrogen peroxide to be consistent.
9.Lines 296-312: How to exclude other signaling (except ROS) which might play important role in response to organic pollutants and recruiting rhizosphere bacteria? Please give simple discussion.
10.Lines 369-370: How to exclude the metabolites from microbes (especially endophytes)? Please give simple explanation.
11.Any specific responses of Sphingomonas sp. LSS1 and Lysobacter sp. LSS2 to ROS and organic pollutants?

Reviewer #2

(Remarks to the Author)
In this manuscript the authors have worked hard to identify the mechanism by which foliar pollutants affect the rhizomicrobiota. The authors have concluded that the pollutants increase ROS and generate ROS wave, systemic signaling that involves Ca2+ signals. The signal reach to the roots where local ROS is produced and affect the membrane stability to release carbon compounds which recruit rhizomicrobiota and also attenuating NO signal that loosen the cell wall and allow colonization of rhizomicrobiota.
I found this manuscript to be interesting, well written and timely. I do however have a major concern regarding the results and interpretation of the ROS signal that lead to recruitment and colonization of the rhizomicrobiota. The authors have shown in figure 5 the colonization is dependent on NO production and it is reduced by inhibiting of NO production. It would be more convincing if the authors will also show similar experiments for ROS and their inhibition or enhancement. Thus, linking it directly to the ROS signal propagation.

Reviewer #3

(Remarks to the Author)
The manuscript titled "Organic pollutant-induced long-distance ROS signaling drives plant systemic acquired acclimation

via rhizomicrobiota" submitted by Yong Li et al., elucidates the critical role of long-distance reactive oxygen species (ROS) signaling in promoting the recruitment and colonization of beneficial rhizobacteria, which facilitates plant systemic acclimation under local organic pollutant stress. Although the novelty of long-distance ROS signaling within plant systems is challenging for the reviewer to evaluate, the research topic is significant. The experimental volume is relatively substantial, and the writing style is standardized. Specific comments are as follows:
1. Line 86: “In addition, Nitric oxide (NO), an intrinsic gasotransmitter, acts as a vital player of ROS in plant stress responses." Please provide references and further elaborate on the current research progress regarding NO in the plant ROS response system.
2. Figure1: It is recommended that the full names of the abbreviations for each treatment group be included in the figure caption.
3. Line 120-127: These sentences are confusing. How were the 91 genera that responded consistently across treatments identified? How many samples were used to calculate their relative abundance—in all the samples, only the control group, or certain treatment groups? Additionally, Figure 1c is also unclear. The total percentage of up, down, and unchanged genera in Figure 1c is 100%. How many genera were involved in this analysis? All of them, or just the previously mentioned 91 or 56 genera? Why not analyze all the genera? What statistical test was employed to determine the upregulation and downregulation?
4. Line 132: How many strains were inoculated? Was the amount of inoculation greater or lesser than the number of plants recruited under the influence of organic pollutants?
5. Lines 147-148: Should water or 0.1% polysorbate-80 be considered the control group?
6. Section 2.3: The term “carbon effluxes” is used to refer to increased root secretions in the organic pollutant treatment group, seemingly implying that these root secretions primarily serve as a carbon source for plants. Please explain how these carbon sources selectively induce the proliferation of beneficial bacteria in the rhizosphere rather than promoting the growth of all bacteria.
7. Lines 174-177: Since most bacteria can utilize a wide variety of organic substances, it is not surprising that these two bacterial strains can consume these substances, and other bacteria may do the same.
8. If, as stated by the authors, the main function of ROS is to increase membrane permeability, leading to carbon efflux, what are the primary substances being excreted? Are these tissue fluids or secretions synthesized by plants in response to organic pollution? Are the increases in secretions uniform across different types of organic pollutants?
9. Lines 287-289: The authors speculate that the increased colonization of LSS1 and LSS2 results from cell membrane relaxation. How can the recruitment effect of rhizosphere secretions be ruled out? Does the increase in selectivity due to changes in cell membrane relaxation enhance the colonization of beneficial rhizosphere bacteria, or does it also apply to non-beneficial bacteria?
10. Lines 300-302: Does the ROS long-distance transport system also play a role when plants encounter other stimuli, as shown in other studies?
11. Section 4.2.1: Why was the Thia treatment group selected?
12. Section 4.2.2: Which has a greater degradation effect on Thia: LSS1 and LSS2 or the mixed complex flora recruited by the plants?
13. Figure 1e: Please provide error bars.
14. Figure 5j: It is suggested to add an explanation of how differences between the photos of different groups were observed.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Authors revised the manuscript according to the comments and suggestion of reviewers.
It is suggested to be accepted.

Reviewer #2

(Remarks to the Author)
The authors have successfully addressed all my comments from the previous version of the manuscript.

Reviewer #3

(Remarks to the Author)
No other comments.The revisions have addressed the raised concerns and enhanced the overall quality of the study.

Response to Reviewers

Reviewer: 1

Comments:

The manuscript reports a novel study on organic pollutant-induced long-distance ROS signaling drives plant acquired acclimation via rhizosphere bacteria. The manuscript was generally well organized. Some issues should be concerned and improved.

Response: Thank you for your positive feedback and recommendation. We have made the changes as recommended and are looking forward to having our manuscript accepted for publication.

1. The second paragraph of Introduction:

How about the response of foliar/phyllosphere microbiome to organic pollutants?

Response: Thank you for your suggestion. The effect of the response of phyllosphere microbiome to organic pollutants has been added in the part of Introduction. These organic pollutants can not only regulate plant metabolism but also affect plant microbiota community. Studies have shown that the pesticide N-(3,5-dichlorophenyl) succinimide increases the abundance of *Acinetobacter* while decreasing the abundance of *Stenotrophomonas* and *Flavobacterium* in the tobacco phyllosphere[14]. Fungicide enostroburin has been observed to elevate the abundance of *Pantoea* in the wheat phyllosphere.

Please see Line 61-67.

Is it possible that the immigration of foliar/phyllosphere microbes to rhizosphere?

Response: Thank you for your question. Based on a thorough review of the existing literature, we acknowledge that pesticide exposure does indeed affect both phyllosphere and rhizosphere microbes. However, there remains a notable lack of evidence concerning the migration of phyllosphere microbes to the rhizosphere. Our future studies will focus on elucidating the impact of phyllosphere microbes on plant resistance to organic pollutants, as well as exploring their migration patterns within plants.

Please see Line 484-486.

2. Lines 109-110: Please give the full names for B. rapa, Thia, etc.

Response: Thank you for your suggestion and it has been revised.

Please see Line 115-117.

3. Lines 110-112: Did you analyze the metabolic intermediates of the targeted organic pollutants? Is it possible that the transfer/translocation of intermediates after their metabolism in vivo? Please confirm the exclusion of pollutants and their intermediates.

Response: Thank you for your suggestion. We conducted measurements of metabolic intermediates of these organic pollutants based on LC-QTOF/MS after two weeks of exposure. We detected some key metabolic intermediates, such as clothianidin from thiamethoxam, tebuconazole-tert-butylhydroxy from tebuconazole and 6-ethyl-o-toluidine from acetochlor, all of which were detected

in the vegetable shoots but not in the plant roots or rhizosphere soil. The above results rule out the possibility of direct stimulation of the rhizosphere environment by these organic pollutants or their intermediates.

Please see Line 134-139 and 676-680, and Figure S1.

4. Lines 134-141: Please explain why only foliar-sprayed with Thia to investigate the functions of strains and synCom?

Response: Thank you for you suggestion and it has been revised. No residues of these organic pollutants or their key intermediates were detected in roots or soil (Figure S1), which rules out the possibility of direct stimulation of the rhizosphere environment by these organic pollutants or their intermediates. This indicates that different pollutants, acting as stress factors, indirectly induce changes in the rhizosphere bacterial community, and the reshaped microbial community may subsequently regulate plant health. Additionally, compared to persistent organic pollutants, Phen and Trich, exposure of plants to pesticides is more common in agricultural production due to its widespread use. We selected the enriched genera *Sphingomonas* and *Lysobacter*, along with Thia, as representatives to assess their effects on organic pollutant degradation and plant growth.

Please see Line 134-143.

5. Lines 173-177: how to differentiate the decreasing amount of metabolites by secretion (down-regulated) or consumption?

Response: Thank you for your question. The issue you have raised is indeed very important. When hydroponic plants are co-cultured with bacterial strains, the metabolites in the culture solution may originate from either the plant or the bacteria. This poses a challenge in differentiating whether the decreasing amount of metabolites is due to reduced secretion by the plant or consumption by the bacteria. To address this challenge, in this study, we first collected root exudate solutions from plants exposed to pesticides and used these solutions to culture bacterial strains. Subsequently, we employed exometabolomics to measure changes in metabolites during the strain cultivation process. The metabolites that exhibited significant decreases in abundance in the solution are likely to have been consumed by the strains.

Please see Line 195-208.

6. How about the ROS contents and expression of RBOH gene clusters in leaves and shoots?

Response: Thank you for your question. ROS contents and expression of *RBOH* gene clusters in leaves and stems have been added in the revised paper.

ROS in leaves began to increase significantly at 3 h post-exposure, while ROS in stems and roots exhibited delayed responses, with significant increases beginning at 6 hours after foliar Thia treatment (Student's *t*-test, $P < 0.05$; Figure 3c).

Foliar exposure of Thia for 6 h significantly upregulated the expression of 6, 4 and 4 *BrRBOH* members in leaves, stems, and roots, respectively, compared to the corresponding tissues from the pre-exposure group (Student's *t*-test, $P < 0.05$;

Figure 3f, S9) and other detected *BrRBOHs* showed a slight decrease or no change in expression in different comparisons (Figure 3f, S9). Notably, among these upregulated genes, *BrRBOH03* exhibited an increase of 4.18 ~ 6.28 times in the whole plants exposed to Thia, compared to the pre-exposure group (Student's *t*-test, $P < 0.05$; Figure 3f, S9). The expression of *BrRBOH07*, *BrRBOH08* and *BrRBOH11* was additionally increased in the stems of Thia-treated plants and the expression of *BrRBOH06*, *BrRBOH12*, and *BrRBOH13* was additionally increased in the roots of Thia-treated plants, exhibiting an increase of 1.75 ~ 7.78 times, compared to the corresponding tissues from the pre-exposure group (Student's *t*-test, $P < 0.05$; Figure 3f, S9). The activation of *BrRBOH* genes, accompanied by the accumulation of ROS and Ca^{2+} in roots, indicates the involvement of BrRBOHs in the long-distance transmission of ROS upon foliar treatment with organic pollutants.

Please see Line 219-222 and 230-246.

How to confirm the long-distance transmission of ROS from leave to shoots?

Response: Thank you for your question. In the study, we found that plant leaves sensed organic pollutants (e.g. herbicide, insecticide, fungicide, polycyclic aromatic hydrocarbon, and polychlorinated biphenyl) to generate ROS, followed by the occurrence of long-distance ROS wave from leaves to roots via Ca^{2+}-RBOH-ROS signaling module. Inhibition of Ca^{2+} channels with La^{3+} in leaves blocked ROS accumulation in stems and roots after foliar exposure of Thia (Student's *t*-test, $P > 0.05$; Figure 3e). These results suggest that foliar exposure of organic pollutants triggers Ca^{2+}-dependent ROS signaling transmission from leaves to stems and roots.

Please see Line 226-229.

7. Why not use some inhibitors to reduce ROS content in roots to verify its effect on plant carbon efflux?

Response: Thank you for your suggestion. In the revised manuscript, we added ROS inhibitor NAC to reduce ROS content in roots in order to verify its effect on plant carbon efflux.

Our findings revealed that the biomass of the dried root exudates significantly increased in H_2O_2- and linoleic acid-treated groups, exhibiting 1.26 and 1.21 times higher values than that of the control group, respectively (ANOVA, Tukey's test, $P < 0.05$; Figure 4f). The increase in root exudates observed under H_2O_2- and linoleic acid treatment was comparable to that in the Thia-treated group (Figure 2a). The increase of biomass of the dried root exudates under H_2O_2- and Thia-treated groups was also inhibited by adding NAC (Figure 5f).

Please see Line 271-277.

8. Please check the expression, such as H_2O_2 and hydrogen peroxide to be consistent.

Response: Thank you for your suggestion. We have chosen to use the term "H_2O_2" consistently throughout the manuscript.

9.Lines 296-312: How to exclude other signaling (except ROS) which might play important role in response to organic pollutants and recruiting rhizosphere bacteria? Please give simple discussion.

Response: Thank you for your suggestion, and we have incorporated it into our revision. There exist various long-distance signals within plants, such as ROS and phytohormones. It has been reported that plants mediate the reshaping of rhizosphere microbiota through salicylic acid in response to biotic stress. In the present study, we conducted a series of experiments to elucidate the ubiquitous role of plant long-distance ROS signaling in driving the recruitment and colonization of beneficial rhizobacteria for the establishment of plant systemic acclimation upon local organic pollutant stress. Indeed, we have not excluded the potential roles of other signaling pathways in this process.

Please see Line 422-428.

10. Lines 369-370: How to exclude the metabolites from microbes (especially endophytes)? Please give simple explanation.

Response: Thank you for your suggestion. Root exudates serve as a crucial link in the interaction between plants and rhizosphere microbes. In soil-plant systems, the composition of rhizosphere metabolites is relatively complex, encompassing root exudates, microbial metabolites, and soil components. To eliminate the influence of microbial metabolites and soil components, hydroponic conditions were employed to analyze the effect of organic pollutants on plant root exudates. It is worth noting that, although hydroponically-grown plants may contain endophytes, the short duration of our experiment (48 hours of organic pollutant exposure) could avoid, to some extent, the influence of endophytes on root exudate release.

Please see Line 163-168.

11. Any specific responses of Sphingomonas sp. LSS1 and Lysobacter sp. LSS2 to ROS and organic pollutants?

Response: Thank you for your feedback. Our findings reveal that either Strain LSS1 nor LSS2 displayed chemotaxis towards H2O2, thereby eliminating the possibility of ROS playing a direct role in the recruitment and colonization of these bacterial species (Figure S11).

Please see 329-332.

Reviewer #2 (Remarks to the Author):

1) In this manuscript the authors have worked hard to identify the mechanism by which foliar pollutants affect the rhizomicrobiota. The authors have concluded that the pollutants increase ROS and generate ROS wave, systemic signaling that involves Ca2+ signals. The signal reach to the roots where local ROS is produced and affect the membrane stability to release carbon compounds which recruit rhizomicrobiota and also attenuating NO signal that loosen the cell wall and allow colonization of rhizomicrobiota. I found this manuscript to be interesting, well written and timely.

Response: Thank you for your positive feedback and recommendation. We have made the changes as recommended and are looking forward to having our manuscript accepted for publication.

2) I do however have a major concern regarding the results and interpretation of the ROS signal that lead to recruitment and colonization of the rhizomicrobiota. The authors have shown in figure 5 the colonization is dependent on NO production and it is reduced by inhibiting of NO production. It would be more convincing if the authors will also show similar experiments for ROS and their inhibition or enhancement. Thus, linking it directly to the ROS signal propagation.

Response: Thank you for your suggestion. We have conducted an additional experiment to investigate the effect of ROS and its inhibition NAC on the colonization behavior of the rhizomicrobiota. We placed the plants treated with ROS and SNP, along with their inhibitor-treated counterparts, into a solution containing RFP-labeled LSS1 and LSS2 to investigate the effects of ROS and SNP on bacterial colonization behavior. Observation under a fluorescence inverted microscope revealed that the roots of plants treated with Thia, H_2O_2, and SNP showed more red fluorescence compared to the control, with the CFUs of the strains being approximately 1.85 to 2.59 times higher (Figure 5j, k; Figure S10). The increase of colonization under Thia-, H_2O_2- and SNP-treated groups was inhibited by further adding NAC or cPTIO (Figure 5k). Therefore, it can be concluded that ROS-mediated NO increase promotes cell wall relaxation via increasing cellulase activity and facilitates the colonization of rhizosphere beneficial bacteria.

Please see Line 322-334.

Reviewer #3 (Remarks to the Author):

The manuscript titled "Organic pollutant-induced long-distance ROS signaling drives plant systemic acquired acclimation via rhizomicrobiota" submitted by Yong Li et al., elucidates the critical role of long-distance reactive oxygen species (ROS) signaling in promoting the recruitment and colonization of beneficial rhizobacteria, which facilitates plant systemic acclimation under local organic pollutant stress. Although the novelty of long-distance ROS signaling within plant systems is challenging for the reviewer to evaluate, the research topic is significant. The experimental volume is relatively substantial, and the writing style is standardized. Specific comments are as follows:

Response: Thank you for your positive feedback and recommendation. We have made the changes as recommended and hope that our manuscript will be accepted for publication.

1. Line 86: “In addition, Nitric oxide (NO), an intrinsic gasotransmitter, acts as a vital player of ROS in plant stress responses." Please provide references and further elaborate on the current research progress regarding NO in the plant ROS response system.

Response: Thank you for your feedback. A reference and current research progress regarding NO in the plant ROS response system have been added in the revised manuscript.

“In addition, nitric oxide (NO), an intrinsic gasotransmitter, acts as a vital player

of ROS in plant stress responses[24]. ROS can affect cellular NO concentrations by fine-tuning S-nitrosoglutathione reductase activity, thereby activating protection against oxidative stress[25]. NO can regulate cellular ROS levels by enhancing the activity of antioxidant enzymes such as catalase and superoxide dismutase under stress conditions[26].”

Please see Line 89-94.

2. Figure 1: It is recommended that the full names of the abbreviations for each treatment group be included in the figure caption.

Response: Thank you for your suggestion. It has been revised and please see the caption of Figure 1.

“Figure 1 Effects of leaf-exposure organic pollutants on plant rhizosphere bacterial communities and feedback of beneficial bacteria to plants. Organic pollutants included thiamethoxam (Thia), tebuconazole (Teb), acetochlor (Ace), phenanthrene (Phen), and trichlorobiphenyl (Trich).”

3. Line 120-127: These sentences are confusing.

How were the 91 genera that responded consistently across treatments identified? How many samples were used to calculate their relative abundance—in all the samples, only the control group, or certain treatment groups? Additionally, Figure 1c is also unclear. The total percentage of up, down, and unchanged genera in Figure 1c is 100%. How many genera were involved in this analysis?

All of them, or just the previously mentioned 91 or 56 genera? Why not analyze all the genera?

What statistical test was employed to determine the upregulation and downregulation?

Response: Thank you for your question. In the revised manuscript, the difference of bacterial composition at genus level was re-analyzed. Across all samples, a total of 650 genera were identified. Linear discriminant analysis effect size (LEfSe) with Wilcoxon rank-sum test was used to compare genera between the organic pollutants and control groups, with taxa considered significantly different only when $P < 0.05$ and LDA effect size > |2.5|. Among these genera, 138 were present in the pollutant-treated groups compared to the control group (Table S1). Among these genera, 37 to 48 exhibited significantly increases, while 27 to 39 showed decreases across the different pollutant groups compared to the control (Figure 1c-d). Treatment with organic pollutants led to an increase in 19 genera, with *Sphingomonas* and *Lysobacter* exhibiting the highest average relative abundances among all samples (Figure 1e-f).

Figure 1c has been updated based on the results obtained from all genera. The average relative abundance of the genera across all samples was calculated for Figure 1e.

Please see Line 126-133 and Figure 1 c and e.

4. Line 132: How many strains were inoculated? Was the amount of inoculation greater or lesser than the number of plants recruited under the influence of organic pollutants?

Response: Thank you for your question. We have included the information regarding the amount of inoculation. In this study, The root of plant seedlings was separately inoculated with 1.0 mL of LSS1 (OD_{600nm} = 0.5), LSS2 (OD_{600nm} = 0.5) and their proportional synthetic microbial community (SynCom), while the control group was treated with 1.0 mL sterile water. Generally, the inoculation dose used in this study was designed to exceed the number of natural rhizosphere strains that plants recruit under stress conditions. The inoculation protocol employed in this study aligns with those used in numerous other studies investigating the effects of bacteria on plant physiological metabolism (Nature Plants 7, 644-654).

Please see Line 145-147.

5. Lines 147-148: Should water or 0.1% polysorbate-80 be considered the control group?

Response: Thank you for your feedback. The control plants were leaf-sprayed by water containing 0.1% polysorbate-80.

Please see Line 168-170.

6. Section 2.3: The term “carbon effluxes” is used to refer to increased root secretions in the organic pollutant treatment group, seemingly implying that these root secretions primarily serve as a carbon source for plants. Please explain how these carbon sources selectively induce the proliferation of beneficial bacteria in the rhizosphere rather than promoting the growth of all bacteria.

Response: Thank you for your feedback and it has been revised. In our study, we

observed that in responding to organic pollutant exposure, plants could increase the release of plant carbon, including various primary and secondary metabolites. These plant carbon can affect the rhizosphere microbial community in multiple ways. Primary metabolites such as amino acids and organic acids provide universal carbon sources for rhizosphere bacteria. Additionally, certain secondary metabolites, including phenolic acids, flavonoids, and glucosinolates, not only exhibit antibacterial activity but also selectively recruit specific beneficial bacteria in the rhizosphere. Rhizosphere bacterial community has revealed that both *Sphingomonas* and *Lysobacter* were specifically recruited to the rhizosphere by stressed plants, rather than all genera. We found that both *Sphingomonas* sp. LSS1 and *Lysobacter* sp. LSS2 displayed a strong ability to chemotax toward and utilize root exudates from stressed plants, leading to their enrichment in the rhizosphere.

Please see Line 433-441.

7. Lines 174-177: Since most bacteria can utilize a wide variety of organic substances, it is not surprising that these two bacterial strains can consume these substances, and other bacteria may do the same.

Response: Thank you for your question and it has been revised. This plant-derived carbon can affect the rhizosphere microbial community in multiple ways. Primary metabolites such as amino acids and organic acids could serve as universal carbon sources for rhizosphere bacteria. Additionally, specific secondary metabolites not only exhibit antibacterial activity but also selectively recruit specific beneficial

bacteria in the rhizosphere. Rhizosphere bacterial community has revealed that both *Sphingomonas* and *Lysobacter* were specifically recruited to the rhizosphere by stressed plants, rather than all genera. We found that both *Sphingomonas* sp. LSS1 and *Lysobacter* sp. LSS2 displayed a strong ability to chemotax toward and utilize root exudates from stressed plants.

Additionally, exometabolomics analysis revealed that these strains exhibited distinct preferences for specific root exudate metabolites. Strain LSS1 preferred to consume small-molecule organic acids (citric acid, quinic acid, and pipecolic acid), whereas Strain LSS2 favored lysophosphatidylcholines and vitamins (riboflavin and pantothenic acid). The differences in the utilization of root exudates by these two strains suggest that they are more likely to coexist rather than compete within the rhizosphere.

Please see Line 198-206 and 441-443.

8. If, as stated by the authors, the main function of ROS is to increase membrane permeability, leading to carbon efflux, what are the primary substances being excreted?

Response: Thank you for your feedback. In this study, to mitigate the influence of microbial metabolites and soil components, we employed hydroponic conditions to analyze the effect of organic pollutants on plant root exudates. Metabolomic analysis using LC-QTOF/MS revealed that the metabolic profiles of root exudates were significant changed upon foliar exposure of organic pollutants (PCA, PERMANOVA, $P < 0.05$; Figure S3). A total of 262 metabolites showing

significant changes were identified (Student's *t*-test with $P < 0.05$, PLS-DA with VIP > 1; Figure S4, Table S2). Among these metabolites, 239-259 metabolites were significantly increased in treatments with different organic pollutants, compared to the control group. A total of 215 metabolites increased consistently, accounting for 82.1% of total metabolites, despite some variation in the degree of increase (Figure 2b-c). These metabolites mainly included primary and secondary metabolites, such as amino acids, organic acids, fatty acids, lysophosphatidylcholines, nucleosides, vitamins, phenolic acids, flavonoids, and alkaloids (Figure 2d).

Please see Line 173-183.

Are these tissue fluids or secretions synthesized by plants in response to organic pollution?

Response: Thank you for your feedback. Our findings indicate that ROS stimulated the release of plant carbons, including primary and secondary metabolites, into rhizosphere. Consequently, it can be inferred that while these metabolites originate from plant roots, their increased presence is not necessarily indicative of augmented synthesis within the plants in response to pesticide exposure.

Are the increases in secretions uniform across different types of organic pollutants?

Response: Thank you for your question. A total of 215 metabolites increased consistently, accounting for 82.1% of total metabolites, despite some variation in the degree of increase (Figure 2b-c). These metabolites mainly included primary

and secondary metabolites, such as amino acids, organic acids, fatty acids, lysophosphatidylcholines, nucleosides, vitamins, phenolic acids, flavonoids, and alkaloids (Figure 2d). These findings suggest that foliar exposure of different organic pollutants could consistently enhance the release of a large quantity of small-molecule organic carbon from roots.

Please Line 179-185.

9. Lines 287-289: The authors speculate that the increased colonization of LSS1 and LSS2 results from cell membrane relaxation.

How can the recruitment effect of rhizosphere secretions be ruled out?

Does the increase in selectivity due to changes in cell membrane relaxation enhance the colonization of beneficial rhizosphere bacteria, or does it also apply to non-beneficial bacteria?

Response: Thank you for your question. In this section, we wish to clarify that it is the relaxation of the cell wall (rather than the cell membrane) that could facilitate the colonization of LSS1 and LSS2. Indeed, the colonization behavior of these bacteria towards plant roots could be influenced by the selective enrichment facilitated by root exudates and cell wall relaxation. Firstly, we observed that plants exposed to organic pollutants increase the release of plant-derived carbon fluxes, including various primary and secondary metabolites. This plant-derived carbon can affect the rhizosphere microbial community in multiple ways. Primary metabolites such as amino acids and organic acids could serve as universal carbon

sources for rhizosphere bacteria. Additionally, specific secondary metabolites, including phenolic acids, flavonoids, and glucosinolates, not only exhibit antibacterial activity but also selectively recruit specific beneficial bacteria in the rhizosphere. Rhizosphere bacterial community has revealed that both *Sphingomonas* and *Lysobacter* were specifically recruited to the rhizosphere by stressed plants, rather than all genera. When the root cell wall becomes loosened, these bacteria recruited by stressed plants could more easily facilitate colonization in plant roots.

Please see Line 433-443 and 465-468.

10. Lines 300-302: Does the ROS long-distance transport system also play a role when plants encounter other stimuli, as shown in other studies?

Response: Thank you for your question. It has been revised.

"It was found that foliar exposure to organic pollutants not only induced local ROS burst in leaves, but also boosted ROS accumulation in other organs (e.g., stems and roots), suggesting that ROS may be a systemic signaling in this process. In response to stimuli such as light or wounding, plants exhibit systemic ROS accumulation within minutes[34]. However, in this study, ROS waves in stems and roots also occurred but delayed, occurring 3-6 hours after foliar treatment with organic pollutants. Unlike the immediate cellular injury caused by light or wounding stress, plants may take longer to absorb and to initiate stress responses. Cadmium stress could induce a ROS burst in the tips of the exposed roots and trigger systemic ROS

signaling to the unexposed roots[35].”

Please see Line 354-363.

11. Section 4.2.1: Why was the Thia treatment group selected?

Response: Thank you for your question. It has been revised.

No residues of these organic pollutants or their key intermediates were detected in roots or soil (Figure S1), which rules out the possibility of direct stimulation of the rhizosphere environment by these organic pollutants or their intermediates. This indicates that different pollutants, acting as stress factors, indirectly induce changes in the rhizosphere bacterial community, and the reshaped microbial community may subsequently regulate plant health. Additionally, compared to persistent organic pollutants, Phen and Trich, exposure of plants to pesticides is more common in agricultural production due to its widespread use. We selected the enriched genera *Sphingomonas* and *Lysobacter*, along with Thia, as representatives to assess their effects on organic pollutant degradation and plant growth. Representative strains, *Sphingomonas* sp. LSS1 and *Lysobacter* sp. LSS2, were isolated from Thia-treated rhizosphere soil (Figure S2).

Please see Line 134-144.

12. Section 4.2.2: Which has a greater degradation effect on Thia: LSS1 and LSS2 or the mixed complex flora recruited by the plants?

Response: Thank you for your question. SynCom showed the greatest degradation

effect on Thia, with reductions in pollutant concentration of about 38.8% compared to controls, followed by Strain LSS1 (25.4%) (ANOVA, Tukey's test, $P < 0.05$; Figure 1j). No degradation effect was observed for Strain LSS2 (ANOVA, Tukey's test, $P > 0.05$).

Please see Line 153-157.

13. Figure 1e: Please provide error bars.

Response: Thank you for your suggestion. It has been revised and please see Figure 1e.

14. Figure 5j: It is suggested to add an explanation of how differences between the photos of different groups were observed.

Response: Thank you for your question. We have made the necessary revisions. Observation under a fluorescence inverted microscope revealed that the roots of plants treated with Thia, H_2O_2, and SNP showed more red fluorescence compared to the control, with the CFUs of the strains being approximately 1.85 to 2.59 times higher (Figure 5j, k; Figure S10). The increase of colonization under Thia-, H_2O_2- and SNP-treated groups was inhibited by further adding NAC or cPTIO (Figure 5k).

Please see Line 324-329.