

## Response to recommenders – Revision 2

We thank the recommenders again for their time and their constructive remarks. The paper was revised and the tracked change version can be found at this link (file too large for upload on PCI website – limit size at 5Mb): <https://1drv.ms/b/s!AhEwQWtXbYPTi7NwTxCrXjBjgaYMeQ?e=W5HX06>

There are still one minor comments on your response: could you indicate in the result section your analysis of index hopping which was lower than 0.01 % ? For the rest, all your responses are accepted (except discussion shortening by eliminating results - see comment of new reviewer).

Thanks for reviewing and supervising our submission. We have included the following sentence in the text: The index hopping rate was assessed using our positive controls and the values for this sequencing run were < 0.01% as expected for the Illumina platform

## Reviewer 3

### GENERAL COMMENTS

As the previous reviewer indicated, this study is well done. Furthermore, the authors have adequately responded to all of the previous reviewer's questions. In my opinion, this manuscript is acceptable for publication with some modifications:

Thanks for your time reviewing our paper and for the constructive comments. Our responses are written below in blue and the changes are highlighted in yellow in the manuscript.

Firstly, there are numerous grammatical errors and other minor points that I have indicated in my line-by-line specific comments.

Secondly, although I do not believe that it is intentional, the authors are over-selling the novelty of their findings on the relationship of seed microflora and seedling phenotype. The phenotypes that they assessed were "normal" and "abnormal". The latter could be considered to be diseased/unhealthy. Hence, they are stating that seed microflora is linked to seedling health – something that is well known if you consider the science of Plant Pathology. They should find another way to report this finding. Maybe it should be something that is expected and reassuring. Indeed, they could have also observed growth promotion of seedlings, but they did not measure length or fresh weight, so they did not have objective criteria for this classification. In any case, that would not have been novel either.

Thanks for this comment, we agree and in our opinion this finding is definitely not the most novel compared to other results linked to seed core taxa, phenotype modulation in a community context or the precise manipulation of both seed and seedling microbiota using SynComs. We have rewritten significant parts of the abstract and discussion to better highlight these original results.

Thirdly, the discussion is long and does not get to the point. It would be much appreciated if the first part of the discussion were not just a re-statement of the major findings; could they indicate the significance and novelty of their results, for example? It would also be interesting for the authors to mention the extent to which their results could be generalized and what are the eventual practical applications.

Thanks for this comment, we have rewritten some parts of the discussion to remove results and highlight the significance and novelty of the results. For instance, at the beginning:

This study demonstrates that a precise manipulation of the seed and seedling bacterial community is possible through SynCom inoculations. The main achievements of this experiment rely on the successful reconstruction of a richness gradient (6, 8 and 12 strains)

on both seeds and seedlings and on the strong reduction in the natural variability of microbiota structure (beta-dispersion).

The second part of the discussion was completely reorganized and shortened to address your comments.

## SPECIFIC COMMENTS

### Abstract

L 33-34. The authors state « Altogether, these results show that SynCom inoculation can effectively manipulate seed and seedling microbiota diversity and modulate plant phenotypes.” The phenotypes they measured were “normal” and “abnormal”. “Abnormal” seedlings were described by traits that are coherent with “disease” or poor health. Hence, the authors are claiming that inoculation of seeds can lead to diseased seedlings. That seems to be a basic concept in plant pathology. What is the new information concerning the relationship between microflora and seedling health?

Thanks for this comment. We agree that the effect of detrimental seed-borne taxa on seedling phenotypes is not novel and should not be highlighted in this way. For us, the originality of the paper relies on the fact to study an assemblage of several strains rather than single strains to better understand the early stages of plant microbiota assembly and the role of microbial interactions in disease expression. This approach represents an interesting tool for the scientific community to better establish causality between plant microbiota composition and phenotypes. We rephrased some sections of the abstract and discussion to shift the focus from the modulation of plant phenotypes. As for the terminology of “Normal” and “Abnormal” seedlings, this is the nomenclature used in the standard protocol of the International Seed Testing Association to characterize seed vigor. We used this terminology to be in agreement with the seed quality literature.

### Abstract:

Altogether, these results show that SynCom inoculation can effectively manipulate seed and seedling microbiota diversity and thus represents a promising tool to better understand the early stages of plant microbiota assembly.

### End of Discussion:

Altogether, these results indicate that this synthetic ecology approach is valuable to better understand the assembly of plant microbiota in early life stages and the context dependency of disease expression in a community setting. This approach permitted identifying several seed core bacteria with detrimental effects on germination and seedlings but also to characterize the transmission success of diverse bacterial strains (i.e increasers, stable, decreaseers) to seedlings in three community contexts.

### Introduction

L 53-54. Perhaps the authors are unaware of the work around the “pink-pigmented-facultative-methylotrophs” (PPFMs) that peaked about a decade ago and led to publications such as the 2 listed below. PPFMs were found to be ubiquitous on aerial parts and seeds of plants. The hormones they produce had stimulatory effects on seed germination.

- Raja et al 2019. Current Science 117 :2052-2058 DOI 10.18520/cs/v117/i12/2052-2058
- Kumar et al 2019. Biologia 74:287-308 DOI 10.2478/s11756-019-00190-6

Thanks for this comment, we have now rewritten this part of the introduction as follows: Several individual seed endophytic bacteria, such as methylotrophic taxa, have been reported to promote stress tolerance and germination (Kumar et al. 2019, Raja et al. 2019). However, the research is still scarce on the influence of complex seed microbial communities on germination and seedling phenotypes or on the role of microbial interactions in the observed phenotypes (Lamichhane et al. 2018).

L 78-79. "The contribution of the plant microbiota on host nutrition or resistance to pathogens have been ..." There are grammar mistakes. Change to: "The contribution of the plant microbiota TO host nutrition or resistance to pathogens HAS been....."

Done

L 93. Could the authors be more specific about what they mean by "Characterize the transmission"? What does this mean exactly?

Thanks, we tried to improve the wording of this objective as follows:

Monitor the transmission of individual seed-borne bacteria and synthetic bacterial communities from seed to seedlings (here on radish plants) using *gyrB* amplicon sequencing

L 95-96. This is confusing and vague "Determine whether individual seed-borne bacteria or synthetic bacterial communities can impact seedling phenotype". Could they say "Test the hypothesis that individual seed-borne bacteria and/or synthetic bacterial communities have significant effects on seedling phenotype." ?

We changed the sentence using the reviewer's suggestion, thanks.

## Methods

L 115. Change to "This technique permits ISOLATION AND CHARACTERIZATION OF both the endophytic" (Note that this is a very common mistake for non-native English speakers. The verb "permit" should be followed by an object, i.e. a noun and not a verb)

Thanks, we corrected the sentence

L 120. Change to "10% TSA" (and throughout the manuscript)

Done

L 105-132. At the end of this section, it would be useful to describe the set of strains that were selected. 12 strains, right? It is confusing to need to wait until the next section to discover this information.

Thanks for this comment. We went back and forth on where the strain selection should be presented (Methods or Results) in the text. Since the strain selection is based on data analysis using the Seed Microbiota database and is justified through the presentation of figures (Figure 1), we think that it is a better option to present the set of strains in the first part of the Result section. We hope the reviewer will understand this rationale.

L 142. "Endophyte" (eliminate the "s")

Done

L 169-170. The authors state "A seedling was considered abnormal if at least 50% of the cotyledons or leaves were necrotic or rotten ..." Given that radishes have 2 cotyledons, this sentence means that if 1 of the cotyledons was necrotic or rotten, they considered it abnormal. Better to state it like that. Concerning leaves, after four days, how many leaves did the seedlings have? It would be useful to indicate and then rephrase the criteria for abnormality in terms of the numbers of leaves and cotyledons.

Thanks for this comment. At this stage (4 days), only the cotyledons are present, so we rephrased the sentence like this:

A seedling was considered abnormal if at least one of the cotyledons was necrotic or rotten, if the hypocotyl or epicotyl were deformed, or if the root system was absent, stunted or rotten.

L 168-171. To assess the effect of the seed microflora on seedling phenotype, did the authors determine the fresh weight of the seedlings? This would have been a very objective criterion.

Thanks, we agree but we would have preferred to characterize dry biomass. Unfortunately, microbiota analysis (immediate storage at -80°C of fresh tissues) is not compatible with dry biomass measurements (drying at 100°C for 2 days). Plant fresh biomass is known to be

unreliable and poorly correlated to dry biomass. So, we have decided to perform only the international protocols relevant for seed and seedling quality (and seed companies) that has been created by the International Seed Testing Association (ISTA) through the assessment of emergence rates and proportion of abnormal phenotypes.

L 180. Change to “bacterial communities”

Done

L 188. “PCR cycling conditions were done with an initial” Change to “PCR cycling conditions constituted an initial”

Done

L 220-222. For the Fisher exact test on proportions, did the authors use transformations to even-out the data distribution? This is recommended. See:

<https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1890/10-0340.1>

Thanks for this comment, our wording was confusing because the graphical representation is shown as proportions but the statistical analysis was performed on the counts of the number of normal or abnormal seedlings using the Fisher's Exact Test for Count Data. We modified the sentence, like this: The statistical tests performed to compare the number of normal and abnormal seedling phenotypes between control and inoculated seeds (single strain or SynCom) was done using a Fisher exact test for count data.

## Results

L 281 and beyond. Concerning the expression of population density on seedlings, how was the mean calculated? Did the authors calculate the log values for each seedling and then take the mean – or vice versa? This is not described in the methods.

Thanks, we have now added the following sentence to describe our approach:

For graphical representations, we calculated the log values of bacterial population density for each sample separately and then calculated the descriptive statistics of each condition to represent them as box plots.

Secondly, I am not sure that stating a population size as, for example, 5.2 log CFU per seedling is recommended nor is it good use of English. I think that the formal way to state population sizes in the text is – for this example –  $1.58 \times 10^5$  CFU per seedling. Also, the authors should be consistent: CFU/seedling or CFU per seedling or CFU by seedling (X-axis of Fig 2)...., but for most publications the recommended format in the text is CFU seedling<sup>-1</sup>. I am sorry that this seems to be nitpicking details.

Thanks for this comment, we changed the format as CFU seedling<sup>-1</sup> in the text and figures.

L 290-291 “The control seedlings that originated from surface-sterilized seeds were below detection limit or close to 2 log CFU/seedling....” No. The seedlings were not below the detection limit. The bacterial densities on the seedlings were below the detection limit.

Thanks, we rephrased the sentence: The bacterial densities on the control seedlings that originated from surface-sterilized seeds were below detection limit or close to 2 log CFU seedling<sup>-1</sup>

L 294. For the title of Fig. 2, what does “high” colonization mean? Relative to what? Could you be more straightforward (ex: bacteria with population densities > 10e7 CFU/seedling)?

Thanks, we clarified the title: High seedling bacterial colonization (bacterial population densities > Log 7 CFU seedling<sup>-1</sup>) in the different inoculation treatments (n=30 seedlings per condition). CFU: Colony Forming Unit. The different letters represent the results of a post-hoc Tukey HSD test.

L 326. "It was confirmed that SynCom inoculation enabled to reconstruct a diversity gradient" "Enabled" is like "permit", above. It must be followed by a noun or a participle as an object. "... enabled the reconstruction of a diversity gradient"

Done

L 330. Change to "likely TO BE endophytic bacteria"

Done

L 331-334 "The microbiota comparison of native and surface-disinfected seeds suggests that remaining endophytes are mainly dominated by *Pseudomonas* species and *Pantoea agglomerans* likely have high epiphytic abundance (Figure S2)." I do not understand this sentence. It might be for grammatical reasons rather than for concepts.

Thanks, we rephrased the sentence:

The microbiota comparison of native and surface-disinfected seeds suggests that seed endophytes still present after disinfection are dominated by *Pseudomonas* species and *Pantoea agglomerans*.

L 421 "The 12-strains SynCom.." When nouns are used as adjectives, they are in the singular form, i.e. "12-strain SynCom".

Done

L 429 Fig 7. "B) Photography of the" Change to "Image of the"

Done

L 435-436. The authors state "The seedling phenotype was a significant driver of seedling microbiota". How do they know cause and effect? If this were a study on disease causation, for the same experiment the conclusion would have been that the microflora affected seedling phenotype. If the authors want to be neutral about cause and effect, they could say that there was a significant correlation between microflora and seedling phenotype. Likewise, the legend of Figure 8 should be changed to reflect this neutrality.

Thanks for this comment, we rephrased the sentence and the figure legend to be more neutral: Microbiota structure was significantly distinct between normal and abnormal seedlings ( $R^2=3.7\%$ ).

Figure 8: A) Bacterial community structure (stress=0.17) and B) strain relative abundance of the 12-strain SynCom in normal and abnormal seedlings.

L 456. Correct the grammar (enabled to...)

Done

L 458 Change "allowed to strongly reduce the" to "led to a strong reduction in the"

Done

L 460. Correct the grammar (permits to...)

Done

L 476. Change "On the opposite" to "In contrast, "

Done

L 514 "bacterium" (and not "bacteria")

Done

L 515. Here the authors list the various life styles "(endophytes, epiphytes, saprotrophs, pathogenic)". All of these words should be in the same form, either as adjectives or as nouns. Be consistent.

Done

L 521 "one report indicate". Use the singular form of the verb.

Done