

## RC2:

### Major comments

This is an interesting research paper of two compared microbial approaches for characterizing some types of endophytic fungi with pioneer grass *Miscanthus condensatus*. The data indicate that seven orders of DSE were found in the culture method and some isolates have the ability to improve rice growth, especially in extremely low pH condition. Sun and colleague also conduct pot experiments to verify their hypotheses. It's an interesting study, but there are a few issues, which I detail below. The paper could be accepted before the authors make some revisions.

Re: thanks for your relatively positive comments.

First, this paper still needs more supportive references added to the material and methods. When authors do the root surface sterilization, they use 70% ethanol for 1min, which will kill most of the microbes including microbes that are good for plant growth and bad for plant growth. After several sterilization steps, the pure cultures just begin. There are some standard ways or currently- approved approaches to isolate rhizosphere microbiome (like Joseph et al., 2015). I strongly recommend authors give more references to support that the method they have chosen is scientific or recognized.

Re: thank you, we have added some references in this section to validate it is scientifically correct.

Second, the authors have conducted the MiSeq sequencing for identification. It's great. However, I have not found enough analyses about the sequencing data. Why not put the data together into the whole paper? I believe the results and discussion sections could be more abundant.

Re: thank you. This study combined both sequencing and isolation methods. Also, the inoculation is included. To put many figures in the main text would dilute the whole results. Anyhow, the suggestion is valuable. Thank you.

### Minor comments

L48 *Miscanthus condensatus* is the subject this research paper focuses on. So add the species name as key words.

Re: I have added, thank you.

L62 which is characterized by

Re: It has been revised, accordingly. Thank you.

L66 These two references only represent the ecosystem that heavy metal pollution influenced. Add more references to show other different ecosystems.

Re: I have add another reference about the effects of DSE on acidic mine soil.

L72 add more references for i) part.

Re: I have added, thanks.

L143 simplify the wording, here are two “it”.

Re: It has been revised, accordingly. Thank you.

L144 how to know 60% of vegetation was affected, add more references to prove or be more specific to describe how to measure this metric.

Re: I have add two references, and they measured the damage rate of vegetation by satellite images (SPOT-2/HRV-XS and TERRA/ASTER).

L151 maybe abundant is not an appropriate word.

Re: It has been revised, accordingly. Thank you.

L160 it's not a clear expression, remove the parenthesis, like “Mount Oyama is an xxx, located in the center of xxx”.

Re: It has been revised, accordingly. Thank you.

L165 please check the units.  $\text{Ca}^{2+}$  cmol kg<sup>-1</sup>, not cmolc kg<sup>-1</sup>?

Re: It was written as cmolc kg<sup>-1</sup> in the reference, maybe spelling errors, I have revised, thanks.

L167 remove the number after the decimal point.

Re: It has been revised, accordingly. Thank you.

L170-171 how divide three healthy specimens into two portions? it is not reasonable.

Re: I didn't express clearly. Three healthy specimens were collected, and then individual roots of each specimen were divided into two portions for different experimental purpose.

L177 remove the sentence “which are xxx with the roots of plants”.

Re: It has been revised, accordingly. Thank you.

L184 upper case Cornmeal.

Re: It has been revised, accordingly. Thank you.

L175-L190 the whole part lacks supportive references.

Re: Thank you for your kind comments. Isolation was performed by modifying the method of Sahu, et al., (2022). Detailedly, the requirement of surface sterilization protocol is different according to different host plant. The sterilant solution, concentration and exposure time could be optimized based on host plant and tissue type. In the present study, the sterilant solution, concentration and exposure time were optimized by a series of gradient experiments (single factor and multilevel design). Based on these pre-experiment (data not shown in this paper), we successful realized the isolation of endophytic fungi (with high diversity but no surface contamination) for this study and future use.

L181 I am not sure 70% ethanol is good enough.

Re: Ethanol acts as lipid solvent and protein denaturant, it is commonly used at con concentration of 60%-85% as a surface sterilant. However, by and large, in the ethanol-sodium hypochlorite combination, the concentration and exposure of ethanol are constant. Around 70%-75% ethanol is used for 0.5-1 min. Since NaOCl is a strong oxidative agent, a lower concentration with short exposure is generally given.

L187 why two weeks incubation? Add more description about the reasons.

Re: The plates were checked every second day for fungal growth. Endophytic fungi were considered have a relatively slow growth rate (about 10-15 mm of radial growth in 7 days). DSE were the most prevalent with the greatest number of isolates. Other taxa were isolated in much smaller numbers. *Mortierella*, *Trichoderma*, *Penicillium*, etc, were mostly isolated within 5-7 days of placing roots in the medium. Generally, 10 days are required to establish DSE colonization of the host plant (Actually, 2-3 weeks has often been used in many other studies for short-term growth evaluation of host plants using DSE). So in this study we incubated 2 weeks for establishing DSE colonization of the host plant.

L188-L190 add more detailed information about incubation.

Re: I have added, thank you.

L208 “November samples” is not an appropriate phrase. It’s confusing.

Re: I have revised, thank you.

L227-L229 shorten this sentence. It’s tedious.

Re: It has been revised, accordingly. Thank you.

L230 & L234 add reference after website.

Re: This is used very often, we guess the website is also kind of reference. We could add it if reviewer insist.

L238 which three dominant isolates?

Re: I have added, thank you.

L240 remove replicate.

Re: Sorry not clear here, i guess replicate is OK here.

L240 thus totaling is not an appropriate wording.

Re: sorry, we have corrected to “total” and changed the sentence as “and thus total 48 experimental pots in the study”.

L246 suface- what? One more space.

Re: It has been revised, accordingly. Thank you.

L275 Results and Discussions

Re: It has been revised, accordingly. Thank you.

L283 change 2 to two. Please go over the whole paper like this mistake (like L297 change 7 to seven).

Re: It has been revised, accordingly. Thank you.

L337 sentence-initial needs some space.

Re: It has been revised, accordingly. Thank you.

L345 inter- and intra-cellularly.

Re: It has been revised, accordingly. Thank you.

L414 they? Re-write this sentence.

Re: Sorry, it is lengthy, we have separated it. Thank you.

**References:**

L459 L462 L464 L466 L470 and others, please uniform the journal name refer to the journal's requirements.

Re: It has been revised, accordingly. Thank you.

L453 L531 L532 species name should be italic.

Re: It has been revised, accordingly. Thank you.

L489 no pages?

Re: It has been revised, accordingly. Thank you.

L542 journal name?

Re: Plant Soil. It is short for the name of journal Plant and Soil.

L548 350 C?

Re: It has been revised to 350 °C. Thank you.

L549 Soil Biology? And no doi.org is listed.

Re: It has been revised to **Soil Biol Biochem 57, 513-523. DOI: 10.1016/j.soilbio.2012.10.033. Thank you**

L606 Al?Fe ?

Re: Sorry, it has been revised to make it clear. Thank you.

### **Figures:**

L691 legend is needed for the figure.

Re: It has been revised, accordingly. Thank you.

L693 I don't know which one picture represents which pH conditions. Add the pH label in the figure and list the pH values.

Re: We have added the pH label in the figure and list the pH values.

L709 please add "\*" and "\*" means what?

Re: we have added the details. Thank you.