Knowing your neighbourhood – The effects of Epichloë endophytes on foliar fungal assemblages in perennial ryegrass in dependence of season and land-use intensity

Reviewer comments:

1.- Please correct genus name on line 58 “Altanaria”.

2.- Please clarify the meaning of “which integrates the most common practices, such as mowing, grazing, and fertilization, into one index” on line 95.

3.- Please correct name of technique “immunoplot” on line 109

4.- Are they a special kind of tubes? on line 112-113 “2.5 ml reaction tubes”.

5.- it is very important to clarify the sentence on lines 112-114. It is recommended to store samples at -20 celsius degrees ONLY if they are processed almost immediately. Please clarify this because many readers might consider store samples at -20 celsius as the norm. Also, you can rewrite this sentence based on the next reference: Conducting a Microbiome Study Goodrich, Julia K. et al. Cell , Volume 158 , Issue 2 , 250 - 262

6.- The methods and materials section is confusing regarding the number of plants sampled. I understood you sampled 20 plants x 80 sites x 2 seasons, so it is 3200 plants but in line 119 you say “2147 sampled L. perenne plants”. Please correct or clarify.

7.- On lines 124-126 you mention that “For each grassland and season, we randomly selected one immuno-positive (Epichloë-infected, E+) and one immuno-negative (Epichloë-free, E-) plant sample, resulting in a total of 68 E+ and 68 E- samples”. Choosing only one plant per grassland and season is not statistically robust to support your conclusions. You should have chosen at least 3 plants. Please clarify or justify.

8.-Regarding lines 129-130, which samples you used for doing the endophytes analyses? I inferred you used the same material from the E+ and E- plants but the sentence is not clear. “For analyses of the foliar fungal assemblages by Next Generation Sequencing (NGS), 136 leaf blades were cut from the remaining material of the collected grass tillers in the laboratory”. Please clarify.

9.- On line 185-186, the next sentence “We found a total of 247 fungal OTUs associated within 128 L. perenne leaves and identified 33 genera” is not consistent with what is explained on lines 129/130. Please read point 8.

10.- On Results section, on lines 187-188, 8% of the OTUs could not be identified at the phylum level. It is very surprising as for sequencing processing you mention a very stringent method. Could be the primers you used? Please rewrite addressing this concern.

11.- On Results section, I think it is very short and basically does not present a good description of what you show on tables and figures. Please explain better.

12.- Please correct the genus name “Altanaria” and “Pencillium” on line 208.

13.- I disagree with the next sentence on Discussion section: “The environmental context, including soil, vegetation, surrounding landscape, weather and climate could all be responsible for differences in the foliar fungal assemblages among the three regions”.

You did not sampled the same number of plants as explained on lines 162-167: “Sequence processing revealed 247 fungal OTUs, represented by 4.907.006 quality-filtered ITS1 sequence reads, in 128 processed samples (63 E+ and 65 E-) from 19 sites in the region ALB (spring: 7, summer: 7, both season: 5), 46 samples (spring: 24, summer:22); 14 sites in the region HAI (spring: 10, summer: 2, both season: 2), 30 samples (spring: 22, summer: 8) and 19 sites in the region SCH (spring: 4, summer: 6, both season:9), 52 samples (spring: 23, summer: 29)”. You must rewrite your discussion assessing this. For me it is very confusing.

My main concern is that you do not have a solid foundation to present your conclusion based on the information on the manuscript. It can be improved. The one figure is missing is the one presenting the “OTUs sampling curves” where you should show that despite not having the same number of samples to compare, you obtained the maximum number of OTUs per sample, so you are not missing biodiversity and richness data and your conclusions are valid in the context of your study.