

***Interactive comment on “Acute glyphosate exposure does not condition the response of microbial communities to a dry–rewetting disturbance in a soil with long history of glyphosate–based herbicides” by Marco Allegrini et al.***

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Anonymous Referee #1 Received and published: 22 April 2020 General comments: In this manuscript, the authors evaluate the potential conditioning effect of an acute glyphosate exposure (first imposed perturbation) on the response of soil microbial communities to a single dry-rewetting event (second imposed perturbation), in soils with a long history of exposure to glyphosate-based herbicides. The topic under study is rel-

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evant, the hypothesis is sound, and the experimental design is suitable for the aim of the study. In addition, the manuscript is concise, well written and organized; therefore I recommend its publication after some minor revisions.

Technical corrections: L27-28: The phrase “(e.g. pesticides)” is repeated in both sentences; maybe it’s not necessary to mention it twice. AC: We agree with the comment. The phrase will be removed in line 27.

L124-125 and Table 2: I’m not sure that this table is really necessary here. Maybe the information of R2 and %Efficiency could be simply put in a sentence in the methods section? I suggest a brief sentence, like: “The efficiencies of qPCR assays were 84.1% (amoA-AOB), 78.57% (amoA-AOA), 91.07% (total bacteria 16S rRNA) and 93.67% (Actinobacteria 16S rRNA); and R2 values were  $\geq 0.99$  in all assays”. AC: We agree with the suggestion. Table 2 will be removed and the information will be inserted in the text in the same way as suggested by the reviewer.

L145-146: I’m not sure that I’m getting this right. How does the lack of interaction between the two disturbances support the absence of a PICT response? Can you briefly clarify what a clear PICT response would be? Is it possible that even if there was a PICT response, there wasn’t interaction with the second perturbation (desiccation)? AC: Changes in microbial communities associated with the development of a greater tolerance (PICT) to a pesticide in the field (chronic exposure) might, at the same time, conceal a higher sensitivity in the response to other perturbations (a “cost of tolerance” when adapting to an environmental stress; Clements and Rohr, 2009). Thus, if no PICT response was observed in the studied soil after long exposure in the field (Allegrini et al., 2015), it could be expected that a single glyphosate application to microcosms (acute exposure) would have no effect at all in the structure of the microbial community and, consequently, no conditioning effect of this acute glyphosate exposure should be observed on the response to a secondary perturbation (dry-rewetting in this case). The absence of conditioning effect is consistently reflected in the non-significant interaction term of ANOVA. However, it is important to mention that even if a PICT response would

have been observed in this soil, the higher tolerance could have associated costs in the response to only some environmental stresses (e.g., to stresses caused by other xenobiotics but no to a dry-rewetting stress). Thus, a non-significant interaction could be also observed for a soil in which a PICT has been detected. Based on this argument, we consider that the absence of interaction in our study is not a conclusive result supporting the absence of a PICT response. In other words, the result we observed in the microcosm assay (no conditioning effect of an acute glyphosate exposure to dry-rewetting response) is an expected result for a soil in which no PICT was observed (as explained above) but it cannot be considered a supporting evidence of the absence of a PICT response in this soil. We will remove the phrase “supporting the absence of a PICT response” in line 146. Also we will introduce the concept of “cost of tolerance” after line 142, as explained before in response to the reviewer comment.

L147 and L155: Can you please check Table numbers? I believe it's Table 3. AC: As indicated by the reviewer it is Table 3 and not Table 2.

L152: “does not necessarily result” AC: Ok, the error will be corrected.

L161: Tables 3 and 4. AC: Ok, the error will be corrected.

L173: Maybe “even though” instead of “even that”? AC: We agree with the suggestion. Figure 3: I'm sorry, what do lowercase letters mean? Replicates within treatments sometimes have different lowercase letters, e.g., CD/SG\_a, CD/SG\_b and CD/SG\_c. AC: Lowercase letters were used to identify the different replicates within treatments.

Anonymous Referee #2 Received and published: 15 May 2020 The response of microbial communities to different perturbations is of great interest for designing sustainable farming practices (either tactic or strategic). Particularly the long term effect of GBHs is relevant in no-till agricultural systems, and the dry-wetting effects are important in rain-fed agriculture. This research explores in a microcosm experiment the effect of GBHs and dry-rewetting perturbations on soil microbial communities, but the interaction effect was not clear. Despite sound methods were used in the present work, deeper stud-

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ies are needed and can be addressed with new research techniques like microbiome sequencing and also by repeated cycles of dry-rewetting to address more clearly the ecological impact (eg. resilience, resistance to disturbance). The manuscript is appropriate for publishing in SOIL. Some minor corrections are needed:

1- Check references: year in text is different from the year in References list a. Line 40 and 148: Evans and Wallenstein, 2011 or 2012? b. Line 87: Zabaloy et al 2016 is not in Reference list c. Line 89: Pfeiffer et al 2013 or 2014? d. Line 144: Clements and Rohr 2009 or 2008? e. Line 151: Franzluebbers et al 1995 or 1994? AC: All references were checked and the modifications will be introduced as indicated by the reviewer.

2- As Reviewer #1 suggests, the concept of PICT response and the absence of interaction could be explained with more detail. AC: We agree with the need of clarification of this concept. Please see the response to the third comment of Reviewer 1 (L145-146).

3- Line 58: how many years is “long term”? Despite described in Allegrini et al 2015, please indicate in the text. AC: With long-term we refer to a history of more than 20 years. We will introduce it in the text as suggested by the reviewer.

4- Line 48: change quantitae by quantity. AC: The change will be introduced in the text as indicated by the reviewer.

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