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Interactive comment on “Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries” by A. S. Wieczorek et al.

Anonymous Referee #1

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General comments.

The study by Wieczorek et al. investigates a relatively simple but relevant research question: Is chitin in soil degraded via deacetylation and chitosan, or via hydrolyzation by family 18 glycoside hydrolases (chitinases)? While the latter is generally assumed, there indeed seems to be little direct data on this, which makes the present study relevant. In addition it presents data on *chiA* diversity in the (treated) soils, and shows the shifts in the *chiA* microbial community during incubation with and without the substrates chitin and chitosan, which provides new insights into the ecology and role of some potential chitinolytic bacteria.

Overall I think the paper makes a useful contribution, but on the other hand, I don't think the study is sufficient to give a final answer to the main question. The reasons

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are that a) Only a single soil was sampled, there is no guarantee that different environmental conditions don't favor different degradation mechanisms. b) Experiments were done in 1:2.5 slurries, not in natural soil and on an overhead shaker. This introduces substantially different conditions than found in natural soil (more akin to wetlands or sediment. These limitations should be mentioned in the discussion. The study uses TRFLP and classic clone libraries. While this is no longer the latest technology, I think it is sufficient for the purpose here. I am a bit doubtful of the association of OTU with T-RFLP presented by the paper– I think this is a bit tenuous as the clone library may not represent the full diversity, and thus doing this could lead to wrong interpretations. In some cases the association is anyway equivocal (see below). I would like to see this discussed briefly.

The authors conducted additional short term experiments with GlcN additions, but not much is reported on these. In general it would have been interesting to measure GlcN, especially also in the GlcN treated samples. The methods section actually mentions analysis of sugars, but no data are reported – it is noted once that no GlcN was measured, which I think is unusual for soil - what happened there? It seems a bit of a missed opportunity that these experiments weren't performed and analyzed a bit more in-depth, and that potential chitin degradation intermediates like GlcN were not measured.

Language wise the manuscript is solid, a few issues I noted are mentioned below. Figures are likewise OK, but could be improved, a few specific comments are also found below. Overall I think this is a valid, if somewhat limited contribution.

Specific issues: The methods section seems in parts a bit incomplete: Oxygen was measured, but the method is missing from the materials and methods section. The results report on ferrous iron, materials and methods mentions only iron. Typically determination of Ferrous iron requires special precautions against rapid re-oxidation and it cannot be analyzed in an IC. Methane is mentioned with two different GC methods.

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A somewhat obscure but potentially relevant paper that was not discussed in this manuscript is the study by Makarios-Laham and Lee 1995, especially because in contrast to this study they found good degradability of chitosan (chitosan PE films, to be precise) in soil incubations.

P2164 I think a cutoff value of 50% for OTUs is extremely low, and groups chiA from ecologically very different groups together, making such a definition largely meaningless. Beier et al. 2012 e.g. used 75%, which seems more sensible. While OTUs thus defined may not correspond to the 16S rRNA phylogeny, they may nonetheless have ecological meaning.

Technical Comments:

P2157 L5. And in addition some algae.

P2159 L 25 gene marker -> marker gene

P2160 grounded -> ground

P2161 L5 “Therefore” seems to be misplaced here. L 11 – not clear for a reader what products refers to, at this point. L21 – delete “i.e., “

P2163 L25+ I think that is questionable. Did you verify this against your clones what exactly is in the “chiA like gene dataset”?

P2166 L20 “. . .within >the< same period. . .”

Figure 1: Hard to make out what is what, not clear where most control values lies. Maybe split this into more panels.

P2167 L 8-9 It is not clear what the percentage values given here refer to (% of what?)

P2169 L18 As noted above, I think the 50% cutoff is too low. Therefore conclusions regarding the coverage should also be reevaluated.

P2170 L15: could not be detected – what’s your LOD? Did you detect them in the GlcN

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treated samples? To my knowledge, GlcN is regularly detected even in untreated soils and sediments.

L 19 do you maybe mean . . . compared to that observed in . . .? The rest of the sentence implies that you observed faster degradation than Sato et al., but the first part of the sentence suggests that the faster response was observed by Sato et al.;

L21 grounded -> ground. L25 “That is likely . . .” maybe consider “This is in agreement with our expectations, as . . .”;

L25 “. . .due >to< high . . .”

P2172 L1f – in this section it’s not always clear if you refer to results in the oxic or anoxic treatment. Please revise.

L10 Under anoxic conditions accumulation of NH₄ is pretty much inevitable, as far as I know

L 10-13 “At the community level . . .” I am not sure this sentence is correct, the metabolism will certainly be different – you observed similar degradation products. You also show no data on N-sugars.

P2172 L21 According to Figure 5 OTU 3 corresponds to several TRFS, including, again, to 264 – so this association of OTU 3 with TRF 188 appears tenuous. See also OTU 1 and 2 in Fig. 5.

L21 According to Fig. 3, TRF 54 also increased in the anoxic chitin treatment – or maybe it’s 223? The colors are hard to distinguish.

Figure 2 I think the inset (panel b) is mostly redundant, the information is already available in panel a. If somewhat smarter placement of the labels can be achieved, panel a would suffice. The significance levels given are strange, however. $P \leq 0.06$ is not a typical value, and $p \leq 0.2$ is not significant. I assume $p \leq 0.05$ and $p \leq 0.01$ might be meant?

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Figure 3 – legend “were such small” -> “were so small”; “symbol”: there are no symbols in this figure.

Figure 5 legend “. . . and the numbers of organismal . . . and is given in parenthesis.” Check this sentence, something seems wrong.

P2174 L22f It might be noteworthy in this respect that Beier et al. 2011 also concluded that Actinobacteria in aquatic environments appear to rather use chitin degradation products than chitin itself.

P2175 L8 “Proteobacterial. . .”

P2175 Final conclusions: I think these should relate directly to the initially given hypotheses – the connection with the previous cellulose experiments is not really the topic of this paper.

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