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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 #2

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The manuscript "Microbial responses to chitin and chitosan in oxic and anoxic agricultural soil slurries" by A. S. Wieczorek et al. focuses on the question whether chitin in an agricultural soil is degraded via hydrolisis to N,N'-diacetylchitobiose and oligomers of N-acetylglucosamine or deacetylation to chitosan. Furthermore, the study aims to identify chitinolytic taxa under oxic and anoxic conditions by analysis of chitinase genes belonging to subfamily A (GH18). The topic of this study is of high relevance as chitin is a globally important biopolymer and microbially preferred degradation pathways have not been identified for different terrestrial environments until now.

The authors identified previously unknown chiA genotypes and potential chitinolytic taxa in their soil slurry incubations. The main hypothesis '(i) that chitin in soil is not primarily hydrolyzed via deacetylation to chitosan' remains unverified. This is mainly

due to fact that the authors do not clearly address questions about the transferability of their results to in situ conditions or other soils. The applied methods are overall appropriate. However, the materials and methods section is missing some information, i.e. quantification of oxygen and ferrous iron. The description of soil slurry incubations could be improved, e.g. better overview of the different treatments, information about unsupplemented controls, rationale behind the chosen concentrations. Latter is especially important for questions about their environmental relevance and if the amount of applied chitosan did result in (foreseeable) toxicity. A statement about this should be implemented in the text. Lastly, I have concerns about the applied chiA genotype difference criterion of 50% amino acid dissimilarity.

The paper makes a solid contribution but I definitely see room for improvement.

More specific comments below:

P.3 l.21: Toxic at which concentrations? In this context: is it environmentally relevant?

P.5 l.12: 'can differentially impact on the stimulation' change to 'can differentially impact the stimulation'

P.5 l.1: I would avoid the term 'classic' in this context.

P.6 l.11 Did the authors mean 'soil with oxic or anoxic water'?

P.6 l.24 Please specify? How large were these crystals? Why were these crystals not ground?

P.7 l.7 Why did the authors choose these concentrations? Are they environmentally relevant? Would an inhibition of microbial activity by chitosan toxicity (at which concentration?) have been expected in this set up?

P.7 l.7-8 Concentrations in your treatments are not easily understandable, i.e. three treatments and two concentration levels. The reader has to go back in the text in order to understand. Consider rephrasing.

P.7-8 How were oxygen and ferrous iron quantified?

P.10 l.24 I consider the chosen threshold value of 50% amino acid dissimilarity as too high - even for a functional marker gene. What is the (ecological) rationale behind this grouping? I don't see an incongruency to organismal phylogenies as a good reason here. For example, Cretoiu et al. (2012) chose a difference criterion of 20% which seems more appropriate for diversity estimations. Are the authors sure that they did not miss some important messages here?

P.12 l.17 Please rephrase to 'unsupplemented controls' and add this information to your figures and materials and methods section.

P.15 l.17-20 I have doubts that rarefaction analysis at such OTU cut off values provide meaningful information about genotype richness.

P. 19 l.26 and P.21 l.17 The authors should be more precise about what they consider as 'high similarity' and 'distantly related'.

P.21 l.25 Which experimental conditions?

Fig. 4 Why did the authors choose significance levels of $p \leq 0.06$ and $p \leq 0.2$, instead of conservative values $p \leq 0.05$ and $p \leq 0.1$?

Interactive comment on Biogeosciences Discuss., 11, 2155, 2014.