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Enzymatic biofilm digestion in soil aggregates facilitates the release of particulate organic matter by sonication

Dear Editor and Authors,

I am writing regarding the second review of Manuscript “soil-2015-87-manuscript-version3.pdf”

In my opinion the paper has been much improved, and the responses to my comments are well reasoned. I think this is an interesting development in an exciting area of soil science. However, before I would recommend publication, I do think there are some important changes that a) have not been done as it is claimed (presumably just file version errors), but b) do need to be done.

This is to refine the clarity of the message in the Author’s well-reasoned ‘new line of argument’ and to clarify how NH_4NO_3 may have explained the deviation from the expected results relative to the control (which taken collectively would mean results do not contradict the aggregate stabilisation mechanism proposed by the authors).

Attending to some specific responses:

Authors [8] We now write: “Decreased POC release in E1 could be explained by pre-incubation of soil aggregates given 0.2 mM NH_4NO_3 and further addition of NH_4NO_3 with enzyme application. Redmile-Gordon et al. (2015) proposed that low C/N ratios of substrates available to soil microorganisms reduces cell specific EPS production rates, and may trigger microbial consumption of EPS to acquire C for cell-growth. The observations leading to this proposed dynamic were also found by addition of NH_4NO_3 . In the present study, NH_4NO_3 was applied with all treatments including the control (which also received no C from enzyme provision). The resulting lowest C/N ratio in the control soils may itself have decreased the EPS, contributing to the higher than expected release of POM from the control soil with sonication at 50 J mL⁻¹, and the break in the trend for increasing POM release with increasing enzyme addition.”

MRG: The above is fine, but, the actual messages in manuscript version 3 are fragmented.

On the contrary to what is suggested above, the paragraph above is not what appears in the latest version of the manuscript [lines 421 to 435]. This might be ok if the section clearly retained the same meaning. But the narrative is not clear. I suggest the authors present the (uninterrupted) paragraph that they state has been written above. The manuscript would read more easily if it was done as claimed above, and with the interfering sentences about enzymes being used as a carbon source grouped together, removed, and placed in a subsequent paragraph. Thus dealing with the impact of N first.

Accordingly, I suggest:

1) delete distracting line over 418-419: "Whereas E4 matches the forecast of releasing more POM than the control scenario" (this is a repeated message – It is already clear that E4 is in line with the model).

2) to move the distracting but important statement currently at line 429-431 to a subsequent paragraph (see later suggestion) "Enzyme C in E1 to E4 could be used as microbial C source. The addition of SOC is known to lead to soil aggregate stabilization (Watts et al., 2005; Tang et al., 2011) and withdraw the effect of reduced C/N ratio."

3) Please also correct the above sentence as is not strictly correct, - i.e. the cited authors do not 'Add SOC', and more importantly this changes the C/N ratio, (not removing its significance). The Authors do not state the resulting C/N for E1, and this is fine, but it cannot be presumed that it 'withdraws the effect of C/N ratio'. It changes the C/N ratio of labile substrate... but to what? For simplicity in addition to moving it I suggest it is just changed to:

"Enzyme C in E1 to E4 could be used as microbial C source. The addition of C increases the C/N ratio and has been shown to lead to soil aggregate stabilization (Watts et al., 2005; Tang et al., 2011)."

And then

4) delete 'in contrast, the retention of' at line 431: As now there will be no need for 'in contrast' as the narrative is not confounded by the additional statement found in the present version of the manuscript [v3] at lines 429-431. 'The retention of' is also confusing, I think I know what is in mind, but it is still confusing and makes the discussion unclear.

i.e. [lines 431 435] ~~In contrast, the retention of~~ the lowest C/N ratio in the control soils may itself have sustained EPS consumption and repressed reconstruction of the EPS, contributing to the higher than expected release of POM from the control soil with sonication at 50 J mL⁻¹ and the break in the trend for increasing POM release with increasing enzyme addition.

Also) Paragraph break needed around line 435. I think 1) Delete 'However'. 2) continue with 'Decay rates of enzymes... ...carbon source' 3) insert *"Enzyme C in E1 to E4 could be used as microbial C source. The addition of C increases the C/N ratio and has been shown*

to lead to soil aggregate stabilization (Watts et al., 2005; Tang et al., 2011).” Then perhaps 4) continue with “Under certain conditions POM carbon...

I hope you find these suggestions helpful.

Next follows another example where the manuscript has not been changed as described (which makes things a little difficult for me).

[9] Further “Probably high enzyme concentrations dissolve biofilm structures that remain part of the coarse POM at low enzyme treatment, which results in underestimation of E4 POC release.” was added in this paragraph.

Searches for components of the sentence in the script yields no results – I also do not know what this sentence means and I do suggest it is not re-added...

Please make minimal changes and document them fully.

Authors [12] “The applied enzymes have no relevant mass input to extractable POM”

Agreed.

Minor corrections:

*line 67 and 73 Typo’s ? (consider removing full stops?)
217 – ‘silT’? (though I do like ‘silky’ as a description)
Results: Paragraph formatting (spaces) needs attention.*

It has been a pleasure to learn of your work.

Best wishes and regards,

Marc