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Rebuttal to Dominant decomposition pathways in pit latrines: a commentary
Anaerobic digestion is the dominant pathway for pit latrine decomposition and is limited by intrinsic factors.
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We appreciate the effort that the authors of the commentary made to read our paper and reflect on these results and the consequences for sanitation for the poor. However, we feel that the concerns raised by the authors of the commentary can be resolved, which we aim to do with the reasoning below. The commentary addresses three main points:

1. There was an oversight in our analysis to consider that the loss of organic material between stool and surface layer was entirely due to anaerobic digestion and we did not allow for the possible contribution of aerobic processes.

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2. The possible use of added water to accelerate decomposition may be problematic for other reasons.
3. We have overestimated the contribution of latrines to global greenhouse gas emissions.

We will consider these three main points in turn.

1. *There was an oversight in our analysis to consider that the loss of organic material between stool and surface layer was entirely due to anaerobic digestion and we did not allow for the possible contribution of aerobic processes.*

We have studied the commentary and the alternative approach suggested by the authors, but we (still) feel that our own analysis, which was based on experimental data obtained in situ and under defined laboratory conditions, is correct. *While we do not rule out a contribution from*

aerobic decomposition, the fact that the actual decomposition measured in situ (which incorporates the whole of the latrine including the surface) compares so closely to the potential degradation as estimated from the laboratory decomposition of the top layer (again including surface material) to us argues strongly that anaerobic decomposition is the major pathway. If aerobic decomposition had played a *major* part we would have seen a difference between the two sets of data as the potential decomposition would have been expected to be much less than the actual.

The authors' argument rests on the hypothesis that there is some rapid aerobic decomposition of fresh stools at the surface of the latrine and they quote several papers which describe or relate to this theory. Of these papers there are three, Nwaneri *et al.* (2008), Byrne *et al.* (2017), and Brouckaert *et al.* (2013), which provide experimental data; the other does not present new data but includes a preliminary report of the Nwaneri study (Buckley *et al.* 2008). Brouckaert *et al.* (2013) use a model in which the available chemical oxygen demand (COD) is characterized according to the biodegradable (organic) and non-biodegradable (organic or ash) fraction and disregard the surface degradation because they feel that the data that they use do not provide any information that could be used to distinguish between the degradation under aerobic (which they assume to be important) or anaerobic conditions.

These papers represent an early attempt to explore the decomposition processes in pit latrines and for that the authors are to be commended. However, unfortunately none of these papers gives sufficient experimental detail or evidence to justify the interpretation or reanalysis of our data offered by the commentary authors.

The hypothesis for aerobic digestion advanced by Nwaneri *et al.* (2008) rests on their observation that the COD content of the 'pit surface layer' is much less than fresh faeces. In our view the information presented in the paper is insufficient to draw this conclusion. The authors do not say where their samples at the surface were taken from, and to what depth, so it is unclear what they mean by 'surface layer' (other than <0.5 m). From the preliminary work cited by Buckley *et al.* (2008) it appears that the samples were taken during manual emptying of the pits and there may well have been mixing of different layers. Depth is important for two reasons. Firstly, the surface layer, which is likely to be 'aerobic', is likely to be very shallow and difficult to isolate from deeper material, and secondly it is an indicator of age of material and thus the rapidity of the processes undergone. Without knowing the depth of material sampled it is therefore very difficult to

state whether it represents changes occurring at the very surface. If deeper material had been included, as seems likely if manual emptying was used, then that could partially explain the lower COD just on the basis of its age. Indeed Nwaneri *et al.* (2008) state that aerobic degradation is occurring in the very topmost layer of fresh material, before it gets overlaid by new material, but also say this layer is too small to take samples from and that once it is overlaid anaerobic digestion takes over.

In our research we used a sampling device (for details refer to Torondel *et al.* 2016) that enabled us to take samples every 20 cm. The samples (0–20 cm, 20–40 cm, etc) were not mixed with layers above or below and each of the layers were analysed separately. This is different from procedures described by others.

Likewise we do not feel that the paper by Byrne *et al.* (2017) can be used in support of the commentary authors' arguments. Firstly, it was a study of pour-flush latrines where at least 1.5 litres of water were added together with the stools, so it is not comparable to the typical dry latrines we studied in Tanzania. Secondly, again no details were given of where and to what depth the samples of latrine sludge were taken. Thirdly, the data used to support rapid early aerobic digestion were obtained in a CSTR (completely stirred tank reactor) test by measuring gas production, but the authors do not provide any information on the conditions used for this test or what gas was measured. Nor do they specify the conditions of their short-term biodegradability test. We do not know if they were aerobic or anaerobic.

We do not consider it reasonable to assume that because stools are exposed to air briefly at the surface of the latrine any decomposition which occurs there will be aerobic. It is quite possible and indeed likely that some pathways, particularly hydrolysis of large complex polysaccharides, which is likely to be mediated by extracellular enzymes, will carry on as in the gut even in the presence of oxygen. Further, the length of time for which stools are exposed to air is likely to be quite short as typically 4–5 people will be using the latrine each day, and as the surface of latrines is often quite fluid then the stools may not 'sit' on top for very long before becoming part of the body of the latrine material, where it is generally agreed that conditions will be anaerobic. Whether this length of time is sufficient for processes in faeces to switch from anaerobic (as in the gut) to aerobic is open to doubt.

The commentary authors cite Torondel *et al.* (2016) and Byrne *et al.* (2017) as having demonstrated 'aerobic microbial diversity' to support the 'aerobic hypothesis'. This is not

justified. Torondel *et al.* (2016) make it clear that the dominant organisms in Tanzanian pit latrines such as the Firmicutes (66%) are derived from faeces and are anaerobes and facultative anaerobes. It is important to mention here that the latrines described in our paper are included in the latrines studied in the paper by Torondel *et al.* (2016). Byrne *et al.* (2017) state that both aerobic and anaerobic bacteria were identified but do not give examples of the former in the results and the organisms listed in their figure and highlighted in the results seem mainly to belong to anaerobic phyla. Furthermore, they show that the overall microbial composition in the pit does not really vary if one compares samples taken from the front (near the inlet) or the back of the latrines and they state that 'Clearly, as biodegradation occurs in standing pits, the microbial community shifts to populations that are presumably active in degradation'. Also, it is important to be aware that both studies are based on DNA analysis (presence of microorganisms) and not RNA (activity of microorganisms).

We recently found a paper by Nakagiri *et al.* (2017) that reports on prevailing redox potentials in pit latrines (in Uganda). They report that the conditions in 95% of the latrines that they studied were anoxic (ORP < +50 mV) and the major part was working in anaerobic fermenting conditions (ORP -199 to -51 mV). In fact less than 4% of the surfaces of the latrines under study in that paper were aerobic. Unfortunately at the time we were not able to measure the ORP in the latrines included in our study. The fact that the anaerobic conditions prevail is backed up by observations that we needed to keep our top samples strictly anaerobic during transport from the pit latrines to our laboratory. Methanogenic archaea are sensitive to oxygen and usually slow-growing microorganisms. When handled with care, samples with ample biodegradable organic matter present showed immediate methane formation. This immediate methane formation would not have been observed if these top samples were predominantly aerobic.

We appreciate the effort that the authors made in analysing our data, recalculating the contribution of aerobic processes and presenting the outcome in a modified table. However, the authors seem to have made a false assumption in retabulating our results that our 'top layer' represents the layer of fresh material where aerobic digestion of stools is supposed to occur. We used a sampling device that enabled us to separate different layers of 20 cm each, so in fact the top layer represents a layer 20 cm deep and the vast majority of the material will be sub-surface and anaerobic. The difference in COD values

between stool and top layer in our view cannot therefore be completely ascribed to aerobic processes as the authors suggest.

Overall, having examined the papers cited by the commentary authors in support of this role as outlined above we do not find sufficient evidence to justify the interpretation or reanalysis of the data presented in the commentary.

2. *The possible use of added water to accelerate decomposition may be problematic for other reasons.*

We think it is premature for the commentary authors to raise potential objections to the use of water to accelerate latrine material decomposition. We feel it was a perfectly reasonable suggestion to make based on our evidence. We recognize that it will have to undergo further laboratory and field exploration, and it would be unfortunate if such work and potentially beneficial innovations were deterred because of hypothetical concerns at this stage.

3. *We have overestimated the contribution of latrines to global greenhouse gas emissions.*

We respectfully disagree with the commentary authors. Our samples used to measure biogas production were from the top 20 cm of the latrine, comprising fresh, recent and partly decomposed material. It represents the 'feed layer' for the latrine, i.e. the material which is going to undergo breakdown. Therefore we can confidently say that our data do represent the full potential biogas production from latrines, and this was borne out by our in situ measurements. So we do not see any need to make adjustments for a proportion of aerobic digestion, especially as we consider, as outlined above, that there is insufficient evidence from the literature that it occurs and that our own evidence strongly supports anaerobic digestion as the main pathway.

The commentary authors then go on to argue that if their assumptions about aerobic digestion are correct then a better approach to mitigate sanitation greenhouse gas contributions would be to integrate latrines with a safely managed sanitation service including some kind of off-site passive aerobic treatment. We would argue that this is not likely to deliver such significant improvements as direct aerobic on-site treatment for two reasons. First, pits are usually only emptied when they are full, by which time considerable anaerobic digestion will have occurred. Second, off-site treatment is dependent on the availability of affordable and reliable emptying services, which in many developing countries are not present to the extent required.

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