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Interactive comment on "Physical constraints for respiration in microbial hotspots in soil and their importance for denitrification" *by* Steffen Schlüter et al.

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We are grateful to the reviewer for the positive feedback and constructive suggestions. In the new version of the manuscript we put more emphasis on the description of hotspot architectures already in the introduction and carve out our hypothesis more clearly that the separation distance between hotspots governs local oxygen supply which in turn regulates growth and the aerobic/anaerobic transition. In the method section we now determine the average spacing between hotspots in the random architecture (6mm) and layered architecture (1mm).

The revised paragraph in the Introduction on the objectives/hypothesis reads now as

follows:

The objective of the present study was to study the interplay between microbial activity and physical diffusion in controlling aerobic and anaerobic respiration for different spatial distributions of hotspots. We embedded uniform artificial hotspots inoculated with denitrifying pure cultures (Schlüter et al., 2018) in sterile sand in two different architectures: either densely packed in two layers with minimal distance between hotspots within a layer or distributed randomly with maximum spacing between individual hotspots. We hypothesized that the competition for oxygen would depend on this separation distance between the hotspots, which in turn would control microbial cell growth and O2 consumption and thus affect the timing of the aerobe-anaerobe transition in respiration, i.e. the onset of denitrification. Further, by placing hotspots inoculated with complete (P. denitrificans) and truncated (A. tumefaciens) denitrifiers in distinct horizontal layers instead of a signal strain in all hotspots, we expected to see interactions between these two different denitrification regulatory phenotypes with respect to overall N2O turnover. Moreover, we incubate the different hotspot architectures at three different water saturations to constrain the overall oxygen replenishment from the headspace. With this full factorial experimental setup we wanted to address the question how the overall oxygen supply (regulated by saturation) and local oxygen supply (regulated by hotspot distribution) in concert affect denitrification kinetics, total N-gas release and product stochiometry.

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