Reviewer #1: Comments:  
The manuscript entitled "Biological manganese oxidation in biofilms from oxygen-supplemented biological activated carbon (BAC) filters"(MS#: WR80060) presents an investigation into manganese-oxidizing bacteria (MnOB) within biofilms sampled from a full-scale oxygen-supplemented biological activated carbon (BAC) filter used for Mn removal from wastewater. The study focuses on the microbial community composition, Mn oxidation efficiency, and the characteristics of biogenic Mn oxides (MnOx) formed. The identification of novel MnOB species and their potential role in the formation of MnOx adds novelty to the research. Overall, this paper appears to be a valuable contribution to the field of water treatment and environmental science. I think there is room for improvement, as outlined below under MAJOR ISSUES and MINOR POINTS:  
  
MAJOR ISSUES  
1) ON EXPERIMENTAL DESIGN  
a) In general, the experimental design is sound, and the authors managed to articulate their ideas and findings neatly. Since the existential state of manganese is relevant to the environmental conditions, in addition to VSS and pH measurement, it might be beneficial to monitor other relevant parameters such as dissolved oxygen concentration throughout the incubation period.  
b) Increasing the duration of the incubation period beyond 42 days may allow for a more complete assessment of Mn oxidation activity and the development of Mn oxides. This extended timeframe could capture additional dynamics and provide a more comprehensive picture.  
  
2) ON RESULTS & DISCUSSIONS  
a) From the SEM, it is not clear to confirm that MnCO3 slurry is aggregated around. EDS is required to prove that.  
b) It would be beneficial if the paper discussed the potential mechanisms and metabolic pathways (using multi-omics or gene abundance analysis via qPCR/RT-qPCR) involved in Mn oxidation by the identified microbial community.  
c) Additionally, further studies could explore the long-term stability and performance of MnOB in engineered water treatment systems.  
  
MINOR ISSUES  
1) ON WRITING FLAWS  
The manuscript is well-written overall. However, there are several orthographic, terminology-related, as well as content-oriented issues. The first ten issues I noticed are as follows:  
a) "... the experimental bottles that was..." --> " bottles that were"  
b) "266 -fold "-->"266-fold"  
c) "which relative abundance decreased..." -->" whose relative abundance decreased …"  
d) "… different inoculum composition"-->"… different inoculum compositions"  
e) "… Rhodococcus and Rhizobiales"-->""… Rhodococcus, and Rhizobiales"  
f) " Biogenic MnOx are frequently…"-->" Biogenic MnOx is frequently…"  
Note: I also recommend the authors run a deep grammar check, particularly focusing on the use of particles. (Grammarly is a good tool for that.)  
2) ON VISUAL ITEMS  
All elements used in the figure S2 should be original (made by the authors).  
  
FINAL REMARKS  
Overall, I think this work has enough novelty and is potentially impactful, although there are some technical issues to be sorted out. Thus, I DO SUPPORT the publication of this manuscript upon MAJOR REVISION. I hope that this report will help the authors amend their work for publication.

The study collected biofilm from a full-scale BAC biofilter which removes Mn as a contaminant. These biofilm samples were then cultivated in laboratory conditions with MnCO3 as the only nutrient source. The growth of bacteria within the cultures was investigated by 16S rRNA marker gene sequencing, with a specific focus on Mn oxidizing bacteria, and the formation of Mn oxides was examined by ICP-OES, SEM, and XRD. The introduction included an in-depth background on Mn oxidation in biofilters generally, but lacked detail regarding Mn oxidation in reuse water treatment plants, or indeed any background on reuse biofiltration at all. The methods are detailed and clear. The inclusion of QIIME 2 scripts and R markdown are specifically appreciated. Mn oxides were observed to form in laboratory cultivated biofilms and putative Mn oxidizing genera/families were identified. While based on previously published studies, the method used to enrich a community of multiple bacteria (including MnOB) is novel compared to the majority of previously published studies which focus on isolation and cultivation of single bacteria species. However, the remainder of the results lack novelty. Many of the taxa identified in the lab cultures have previously been shown to do Mn oxidation either in vitro, and/or found in abundance in Mn oxidizing biofilters. There have also been several previous studies which have investigated the Mn oxidizing microbiome in BAC biofilters and, aside from the laboratory cultivation, this study does not provide significant new insights. This study is also lacking in-depth analysis of the microbiome community, including alpha and beta diversity analysis, which is routine in microbiome studies. The novelty of the paper could be improved by focusing on the role of biological Mn oxidation in biofilters as part of wastewater reuse treatment plants, which is not as well studied as biological Mn oxidation in drinking water treatment plants from surface/groundwater sources.

Line 42: Burkholderiaceae and Pirellulaceae are at the family level, not the genus level

Line 65 - 67: What do the authors mean by ‘type strains’? The reference included here (Zhou and Fu, 2020) states several hundred Mn oxidizing microorganisms have been identified, the majority of which are bacteria. This seems to directly contradict what the authors have written.

Line 66 - 67: It would be beneficial to add here that these phyla were formerly named Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria as these names are more commonly used and understood, and also align with the names used in the reference. I do however appreciate the author’s use of the recently changed nomenclature.

Line 77 - 81: I disagree with these statements. Biological Mn oxidation in water treatment biofilters is a well established practice used in Europe and North America, particularly in ground water sources (See Mouchet, 1992, DOI: 10.1002/j.1551-8833.1992.tb07342.x, Tobiason et al., 2016, DOI: 10.1007/s40726-016-0036-2). Abiotic formation of MnOx in conventional treatment plants through the presence of strong oxidants within filters is also a well established practice.

Line 81 - 82: MnOx can deposit within distribution systems at much lower concentrations than 0.1 mg/L (See Li et al. 2019, DOI: 10.1016/j.watres.2019.114897).

Line 85 - 87: This is not entirely correct. Yes, MnOB oxidize soluble Mn2+ to its particulate form, but the Mn oxide particles are not (in the majority) removed by backwashing. Unlike Fe oxides, Mn oxides mainly adhere to the surface of the media, and continue to accumulate there over time. This accumulation of Mn on the media surface was observed in the reference stated here (Bernstein et al. 2022) in which the amount of Mn present on the media surface increased with the length of time the media was in use. See also Breda et al. 2016 DOI: 10.2166/aqua.2016.093 and McCormick et al. 2022 DOI: 10.1039/d2ew00568a.

Lins 90 - 92: The following studies have described the MnOB subpopulations in drinking water GAC/BAC biofilters and may be a beneficial resource for the authors: Hu et al. 2020, DOI: 10.1021/acs.est.9b07143, McCormick et al. 2021, DOI: 10.1016/j.watres.2021.117793, Keithley et al. 2023, DOI: 10.1016/j.watres.2023.119587, Chen et al. 2023, DOI: 10.1016/j.jhazmat.2023.131877, McCormick et al. 2023, DOI: 10.1016/j.watres.2023.120515.

Line 192 - 193: The authors state SILVA v.138 was used for taxonomic classification, however there are several instances throughout the paper where they use taxonomic classification that is different from SILVA v.138 (ie. Pseudomonadata instead of Proteobacteria, Betaproteobacteriales instead of Burkholderiales). Figure S3 is particularly challenging as it combines both Pseudomonadata and Bacteroidota in the same figure as Proteobacteria and Bacteroidetes, which both refer to the same phyla but by different names. This is confusing and makes it difficult to compare the current study with previously published literature, specifically the mixture of NCBI taxonomy, previous versions of SILVA taxonomy, and the Genome Taxonomy Database taxonomy recently adopted by SILVA in version 138. (I understand previous versions of SILVA/GTDB have used Betaproteobacteriales, however the current version (138) uses Burkholderiales) I strongly encourage the authors to choose a consistent taxonomic nomenclature throughout the paper, or state clearly in the methods section why a mixture of nomenclature was used.

Line 285 - 289: This seems to state that the observed Mn oxides were likely formed abiotically, and yet lines 221 - 223 seems to state that the same nanoflower birnessite Mn oxides were biologically formed. Which is it? Please clarify.

Lines 308 - 320: Given that the ICP-OES data indicates more than half of the Mn in the January samples was insoluble (and therefore likely a biologically formed Mn oxide as suggested by the authors) and nanoflower structures similar to those present in the September samples were also observed, it is surprising that the XRD data did not identify biologically formed birnessite. Can the authors provide more detail or additional hypotheses as to why birnessite was not observed by XRD?

Line 322: “Putative” is the accurate description, brackets are not required.

Line 326: Suggest changing “feeding” to “MnCO3 was available as a nutrient source” or similar, this is more accurate and less informal.

Line 332 - 336: It may be beneficial for the authors to mention that the abundance of the phylum Nitrospirae also decreases in the cultivated biofilm samples, which is not surprising as any ammonia/nitrite present in the inoculum sample would rapidly be used up. This is additionally relevant due to the well established relationship between oxidation of ammonia/nitrite and oxidation of Mn in biofiltration.

Line 337 - 338: Are the taxa in Fig. 4 individual ASVs or were they concatenated to genus/family/order levels? If they are individual ASVs they should not be described as ‘groups’ in this section. Clarify in the text with additional detail.

Line 345 - 346: This is not entirely correct. The study referenced here (Zhao et al. 2020) did observe strong evidence suggesting a possible link between the abundance of Hyphomicrobium and Mn removal in one of the filters, but this data was correlative, not causative. They did not have sufficient data (such as transcriptomics, proteomics, FISH) to definitively confirm that Hyphomicrobium was responsible for Mn oxidation.

Line 360 - 365: The colour coded arrows do improve the clarity of the figure, however the lack of a scale for relative abundance makes this figure less informative. Yes, the bars are changing in size, but is it by 0.001% or 1%? A scale is required.

Lines 355 - 356 refer to groups rarely associated with Mn oxidation, but in Lines 356 - 369 the authors reference several studies in which Burkholderiaceae was observed to do Mn oxidation. This is confusing and requires clarity. Also, Burkholderiaceae is a family, not a genus as stated on Line 367, and members of Burkholderiaceae have been observed to do Mn oxidation in additional studies (see Akob et al. 2014, DOI: 10.1128/AEM.01296-14, Yang et al. 2013, DOI: 10.1371/journal.pone.0073778, Cahyani et al. 2009, DOI: 10.1007/s00374-008-0337-8)

Line 370: See previous comment regarding the mixture of NCBI and Silva taxonomic nomenclature, is it Actinomycetes or Corynebacteriales?

Lines 279 - 382: I do not think the authors have provided sufficient data to support the suggestion that the increased abundance of Ellin6067 is due to oxidation of ammonia from biomass decay, especially since their own VSS data suggests that biomass increased over the 42 days.

Line 394 - 396: Hydrogenophaga was not included in the core microbiome discribed in Lines 338 - 341. Additionally, it is difficult to determine what a ‘relevant difference’ is from Fig 4. because there is no scale present for the relative abundances.

Line 390 - 403: In lines 228 - 229 the authors suggest the 7 day shut down of the filters could have altered the communities of the September and January IB, but these two microbiomes have been given only a cursory comparison in this section. I was expecting a more in-depth analysis, specifically including details on alpha and beta diversity which are commonplace for studies on microbiome analysis. Given the small sample sizes statistical analysis would not be appropriate, but comparisons between alpha and beta diversity could be made more generally, as has been done with data in Fig 4. I strongly suggest the authors provide additional diversity data in future versions of the paper to improve it’s impact and benefits for readers.

Line 405 - 419: This seems more like an extended introduction rather than an informative results and discussion section.

Lines 449 - 451: This statement is incorrect. Of the taxa listed in Fig 4 with green arrows in one or both September/January samples, half have been either shown to be MnOB in vitro, and/or identified in Mn oxidizing biofilters ( Akob et al. 2014, DOI: 10.1128/AEM.01296-14, Yang et al. 2013, DOI: 10.1371/journal.pone.0073778, Cahyani et al. 2009, DOI: 10.1007/s00374-008-0337-8, Mouchet, 1992, DOI: 10.1002/j.1551-8833.1992.tb07342.x, Sly et al. 1988, DOI: 10.1016/s0723-2020(88)80051-1, Marcus et al. 2017, DOI: 10.1111/1758-2229.12508, McCormick et al. 2023, DOI: 10.1016/j.watres.2023.120515, McCormick et al. 2021, DOI: 10.1016/j.watres.2021.117793). Additionally, of the 8 taxa identified as ‘dominant bacteria’, only Pirellulaceae Pir 4 lineage has not previously been identified as MnOB or present in a Mn oxidizing biofilter.