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Comment on "Role of Ammonia Oxidation in Organic Micropollutant Transformation during Wastewater Treatment": Overlooked Evidence to the Contrary

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In their critical review[1], Su, Smets, and co-authors extensively summarized studies on the role played by ammonia-oxidizing bacteria (AOB) in organic micropollutant (OMP) transformation using three levels of evidence: molecular, cellular, and community. They also comprehensively covered the abiotic reactions with the N-species formed from nitrification. We agree with the authors that some studies do support the important role played by AOB in the transformation of specific OMPs. However, we find that the authors' conclusion, "AOB are the main drivers of OMP biotransformation during wastewater treatment processes" (p. 2173), does not stand up to proper scientific scrutiny. In the following, we will present our main arguments and provide the overlooked evidence contradicting the authors' conclusion. (In the following, all page numbers and references to graphical elements refer to Su et al. 2020.[1])

Molecular level: One argument the authors used to demonstrate the dominance of AOB in OMP biotransformation was that many observed OMP biotransformation reactions could be catalyzed by AOB, including 1) hydroxylation, 2) O-dealkylation, 3) thioester oxidation, 4) dehydrogenation, and 5) nitration. However, except for nitration, all these reactions may also be catalyzed by enzymes other than ammonia monooxygenase (AMO), including monooxygenases, dioxygenases and other oxidoreductases from heterotrophs.[2, 3] Many of those enzymes commonly occur in activated sludge communities.[4, 5] Thus, detecting the same transformation products in sludge communities as in AOB pure cultures does not provide conclusive evidence for the dominant role of AOB in oxidative biotransformation of OMPs in wastewater treatment.

Cellular level: The authors state that OMPs exhibiting specific functional groups are likely transformed by AMO. However, they neglect to mention the contradicting evidence from studies using various autotrophic ammonia oxidizers.[6, 7] In those studies, seventeen OMPs were investigated that contain the functional groups listed in Table S1. Yet, only eight were transformed by the pure cultures, suggesting that AMO specificity is much higher than concluded by the authors. It is further interesting to note that the seventeen OMPs were selected because their biotransformation rates correlated with nitrification activity.[8, 9] The findings thus highlight that correlation with nitrification does not necessarily point towards causality.

Community level: The authors overlooked the evidence for OMP biotransformation contributed by microbial species other than AOB (i.e., heterotrophs) and hence did not provide a comprehensive and unbiased discussion. For instance, Men et al.[8] provide an extensive study on the role of different microbial groups using AMO inhibitors. They found that transformation by heterotrophs dominated for

44 out of 55 OMPs studied, while for only four of them AOB played a major role. Although citing this study, the authors only mention the contribution of AOB while not acknowledging the role of heterotrophs. There are more reports[10-13] not cited but demonstrating OMP biotransformation by other microbial groups. Most recently, Kennes-Veiga et al.[14] provide further evidence of efficient heterotrophic biotransformation of all the five compounds that positively correlated with nitrification in Figure 4. Thus, the statement on p. 2179 that “the abundance and significance of heterotrophic cometabolic enzymes in WWTPs is essentially unexamined, while the removal of OMPs through primary metabolism by HAB is assumed minimal” is inaccurate. Moreover, two of the three references for that statement are not supportive by contradictorily stating the equally important role of heterotrophs in OMP biodegradation.[15, 16]

Practical implications: The final suggestion (p.2183) to use bioreactors specifically enriched with ammonia oxidizers and anammox for OMP removal from secondary effluent sounds premature. Rather, conditions that support diverse microbial communities – which may overlap with conditions that support enriched ammonia oxidizers – have been shown to have the greatest efficacy for removing OMPs.[12, 17] Moreover, to our best knowledge, all cited studies on OMP transformation by ammonia oxidizers reported stable and incomplete transformation products. Whether the formation of those products coincides with reduced (eco-)toxicity is unclear. Thus, researchers repeatedly emphasized the importance of heterotrophs for complete degradation.[15]

Collectively, we find that the conclusions of this review highlighting the dominant role of ammonia oxidizers in OMP transformation in wastewater treatment do not have sound literature support and may mislead the readers.

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