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Sediment disturbance negatively impacts methanogen abundance but has variable impacts on total methane emissions.

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Methane emissions from aquatic ecosystems are increasingly recognized as substantial, yet variable, contributions to global greenhouse gas emissions. This is in part due to the challenge of modelling biologic parameters that affect methane emissions from a wide range of sediments. For example, the impacts of fish bioturbation on methane emissions in the literature have been shown to result in a gradient of reduced to enhanced emissions from sediments. However, it is likely that variation in experimental fish density, and consequently the frequency of bioturbation by fish, impacts this outcome. To explore how the frequency of disturbance impacts the levels of methane emissions we quantified greenhouse gas emissions in sediment microcosms treated with various frequencies of mechanical disturbance, analogous to different levels of activity in benthic feeding fish. Greenhouse gas emissions were largely driven by methane ebullition and were highest for the intermediate disturbance frequency (disturbance every 7 days). The lowest emissions were for the highest frequency treatment (3 days). In terms of total microbial community, no statistical difference was observed in the total community structure of any disturbance treatment (0, 3, 7, 14 days) or sediment depth (1 cm, 3 cm) measured. Looking specifically at methanogenic Archaea however, a shift towards greater relative abundance of a putatively oxygen-tolerant methanogenic phylotype (*ca.* Methanothrix paradoxum) was observed for the highest frequency treatments and at depths impacted by disturbance (1 cm). Notably, quantitative analysis of *ca.* Methanothrix paradoxum demonstrated no change in abundance, suggesting disturbance negatively and preferentially impacted other methanogen populations, likely through oxygen exposure. This was further supported by a linear decrease in quantitative abundance of methanogens (assessed by qPCR of the *mcrA* gene), with increased disturbance frequency in bioturbated sediments (1 cm) as opposed to those below the zone of bioturbation (3 cm). However, total methane emissions were not simply a function of methanogen populations and were likely impacted by the residence time of methane in the lower frequency disturbance treatments. Overall, this work contributes to understanding how animal behavior may impact variation in greenhouse gas emissions and provides insight into how frequency of disturbance may impact emissions.