

Denitrification in oxic soils: quantifying the contribution of anoxic microsites to large-scale nitrate removal.

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Microbial denitrification is a critical process in natural subsurface, driving nitrogen cycling and organic carbon turnover while contributing to the removal of the widespread nitrate contamination. While traditionally considered an anaerobic process, denitrification has been observed in oxic environments, suggesting the presence of anoxic microsites that facilitate anaerobic metabolism within otherwise oxygen-rich surroundings. These microsites often evade detection by bulk oxygen measurements, leaving their spatiotemporal dynamics and contribution to denitrification poorly understood. This knowledge gap largely stems from the methodological challenges of observing coupled oxygen and microbial dynamics at the microscale (microns to millimeters) in natural subsurface environments.

To address these challenges, we simulated the wetting of 3 cm of sandy soil using a microfluidic device integrated with a transparent planar oxygen sensor. Wide-field and fluorescent time-lapse microscopy were employed to track the spatially heterogeneous growth of a facultative denitrifier (*P. veronii* 1YdBTEX2) alongside oxygen concentration dynamics at the microscale. Additionally, nitrate concentrations in the device outflow were analyzed to quantify the overall denitrification rate in the microfluidic device with an ad hoc spectrophotometric method, indicating anaerobic respiration.

Our results revealed that microbial colonization closely correlated with the formation of oxygen-depleted zones. Despite the pore space remaining oxic at the bulk scale throughout the experiment (72 hours), oxygen-depleted "hot-moments", or anoxic microsites, occupied up to 10% of the pore space, providing conditions suitable for anaerobic nitrate respiration. Remarkably, nitrate concentrations in the effluent decreased from 0.5 mM to nearly zero after 50 hours, demonstrating efficient denitrification despite the limited spatial extent of anoxic zones. Contrary to conceptual models predicting reduced activity in previously oxic regions, our findings showed that denitrification peaked during maximum oxygen consumption. This suggests that simultaneous increases in aerobic and anaerobic volumes promote the persistence of anoxic microsites and sustain denitrification in oxic soils.