

Online resource 3

This files contains images of benthic and hyporheic biofilm cells captured during a microscopic investigation. Cells were extracted and stained with DAPI to discriminate between bacteria and natural autofluorescence of the algal cells (details available in the main text).

Title:Disentangling effects of multiple agricultural stressors on benthic and hyporheic nitrate uptake

Biogeochemistry

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Acquisition of the images

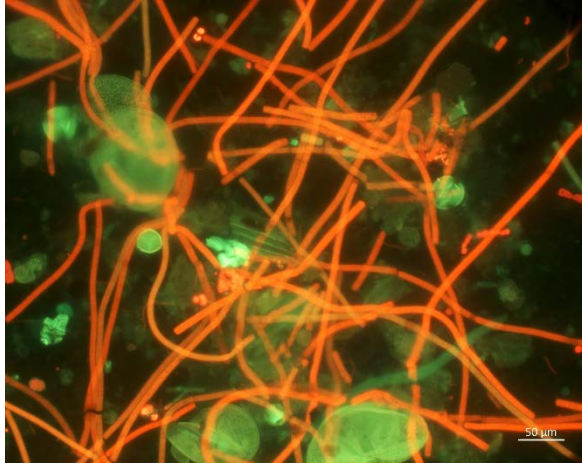
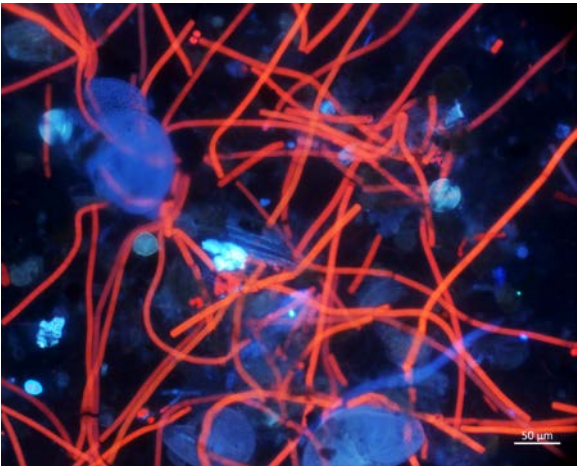
A microscopic investigation was performed using a Zeiss AxioImager.Z2 epifluorescence microscope equipped with an HXP R 120W/45C UV Hg-vapor lamp, Colibri.2 LED illuminations and the following fluorescence filters: DAPI (365/10 nm excitation, 420 LP emission, FT 395 Beam Splitter), Alexa488 (472/30 excitation, 520/35 emission, 495 Beam Splitter), and Alexa594 (562/40 excitation, 624/40 emission, 593 Beam Splitter). The filter sets discriminated clearly between DAPI (365 nm), and natural autofluorescence of the diatom cells. Imaging was done with 100X oil objective numerical aperture N:A 1.4. The software for image acquisition allows for overlapping images acquired successively with different filter sets (Zen software from Carl Zeiss).

Figure legend Online resource 3

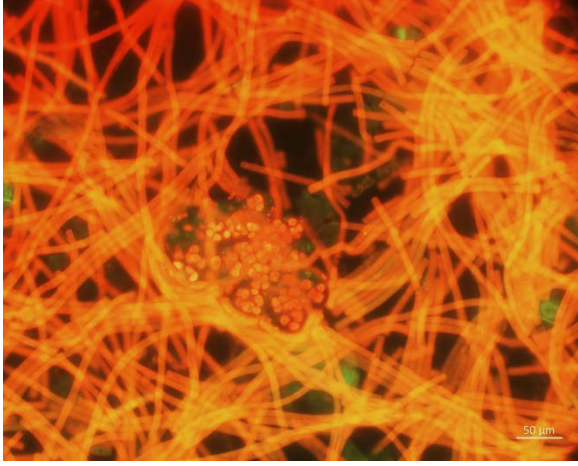
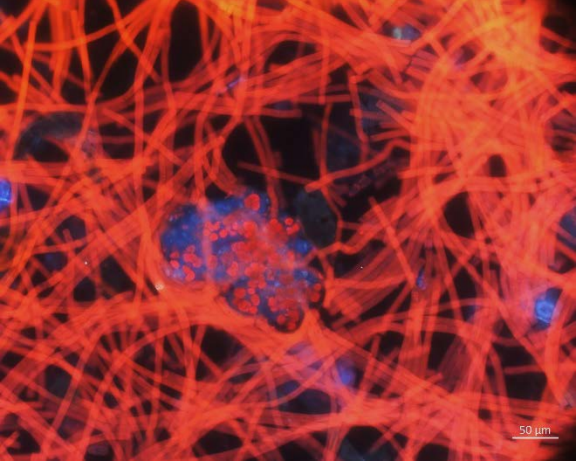
Figures on slide 4-5: Representative fluorescence microscopy images of benthic and hyporheic biofilm samples. At the end of each experiment, cells were chemically fixed, separated from the solid particles by mild sonication and distributed on 0.22 μm pore size polycarbonate filters. Prior to microscopic investigation, filters containing cells were stained with DAPI to discriminate between non-photosynthetic, mainly bacterial cells (blue) and the photoautotrophs based on their natural autofluorescence (red-orange or green). Overlay images of both DAPI and autofluorescence lead to purple-colored cells. While benthic samples lead to a high abundance of photoautotrophs, mainly with a filamentous morphology, the hyporheic samples were generally characterized by low cellular abundances, mostly formed by bacterial cells, and a very reduced abundance of photoautotrophs.

Benthic

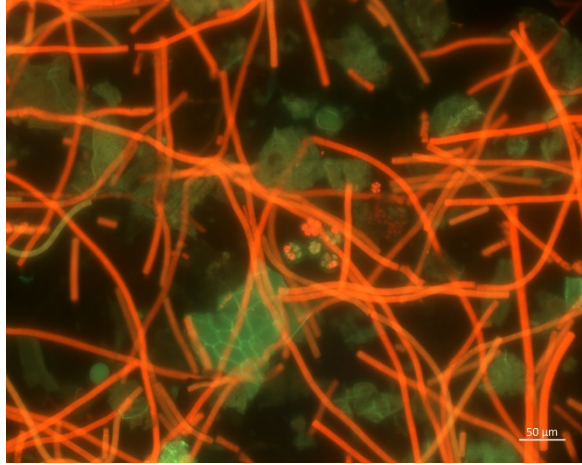
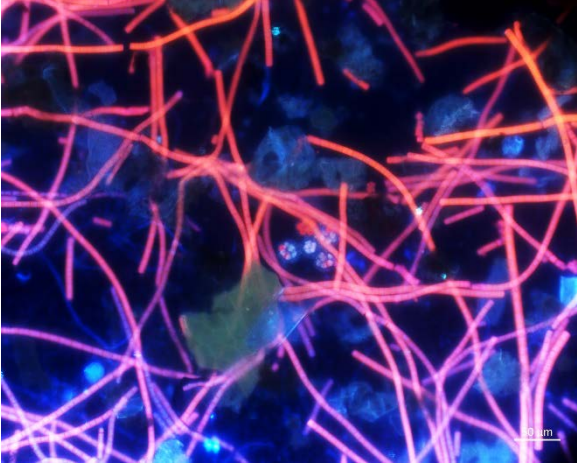
Fine sediment Benth 8_6



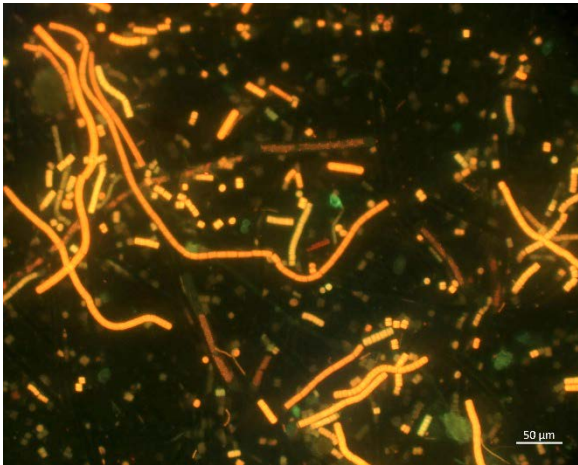
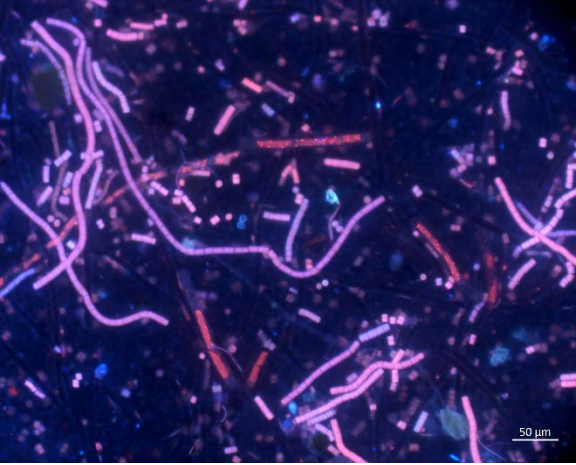
Light and Phosphorous Benth 16_3



Control Benth 13_3

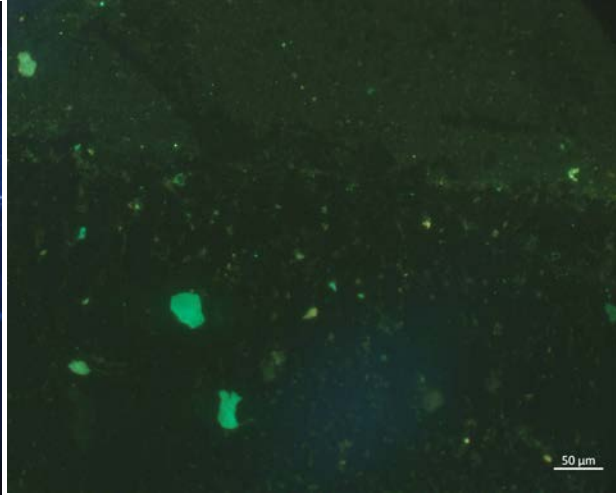
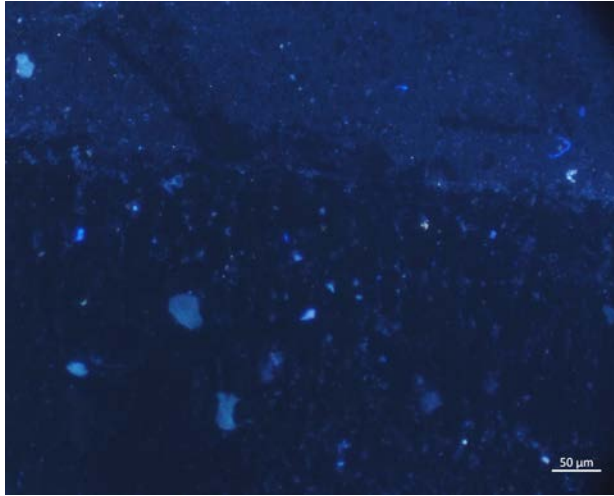


Fine sediment + Light and Phosphorous Benth 23_5

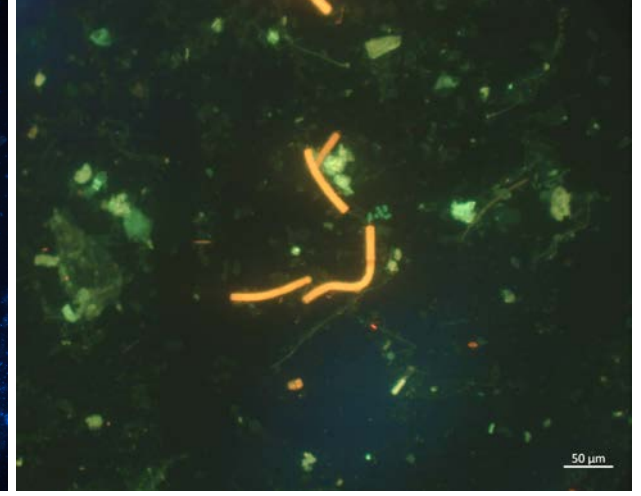
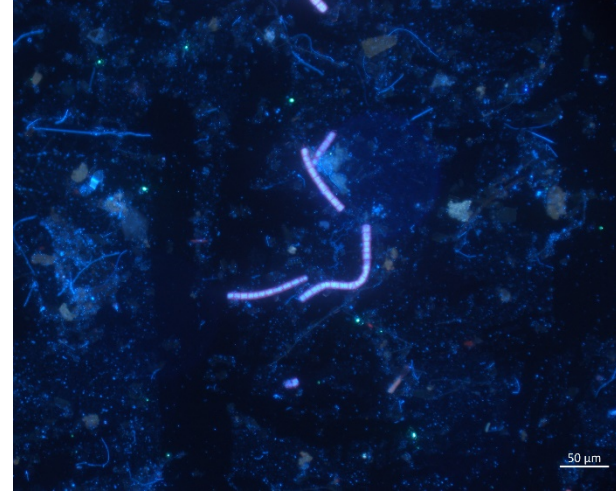


Hyporheic

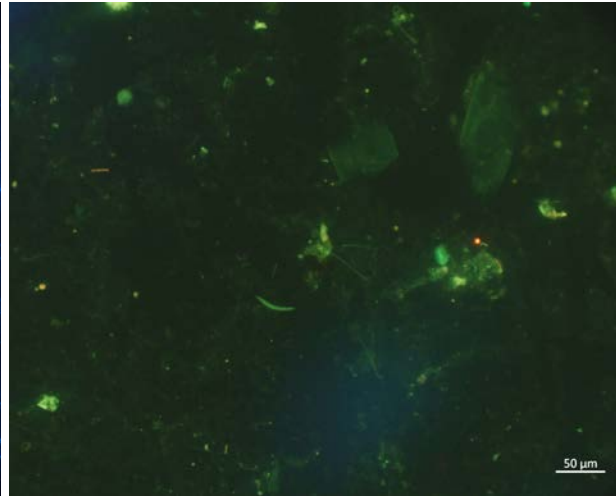
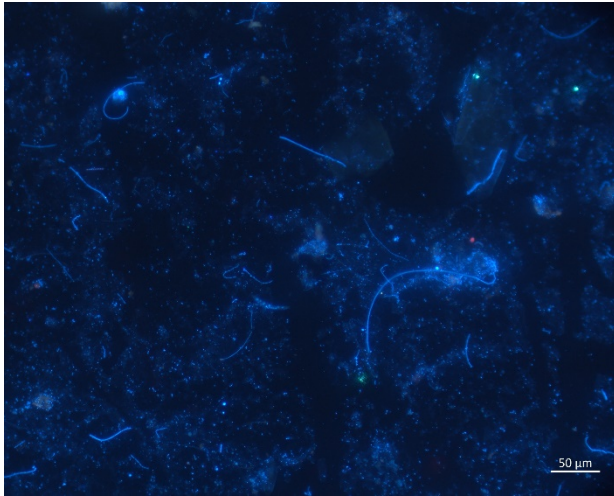
Fine sediment Hypo 8_4



Light and Phosphorous Hypo 16_2



Control Hypo 7_2



Fine sediment + Light and Phosphorous Hypo 23_3

