Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on bacterial community composition

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Appendix A: Methods

A1 Chemical analysis

DOC concentrations (mg C L^{-1}) were determined with an organic C analyzer (Shimadzu, TOC-V CPH, Duisburg, Germany). After filtration through a 0.2 µm pre-rinsed cellulose nitrate filter, DOC was analyzed as non-purgeable C after acidification with 1% (v/v) 2 mol L^{-1} HCl. The carbon dioxide generated by catalytic high-temperature oxidation (850 °C) was measured online by infrared absorption. The quality of DOC was characterized by size exclusion chromatography with online carbon and nitrogen detection (Liquid Chromatography – Organic Carbon Detection- Organic Nitrogen Detection; LC-OCD-OND; Huber et al., 2011). Briefly, the chromatographable DOC portion of the filtered samples passes through a size-exclusion column packed with resin (Toyopearl HW 50S, volume of 250 x 20 mm). Phosphate buffer (0.029 mol L^{-1}, pH 6.5) was used as eluent at a flow rate of 1.1 ml min^{-1}. The first detector measured the absorbance at 254 nm. DOC was detected with infrared (IR) absorbance of CO_2 after ultraviolet (UV) oxidation of DOC at 185 nm in a cylindrical UV thin-film reactor (Graentzel-reactor). Fractions were identified by using fulvic and humic acid standards from the International Humic Substances Society and analyzed with a suitable software program (FIFFIKUS; DOC-Labor Huber, Karlsruhe, Germany). DOC was summarized in three fractions: high molecular weight substances (HMWS, e.g. polysaccharides), humic or humic-like substances (HS) including buildings blocks and low molecular weight substances (LMWS), which include both low molecular weight acids and low molecular weight neutral substances. HMWS and HS are known to be recalcitrant to microbial degradation (Hessen and Tranvik, 1998) and LMWS are more labile due to their low molecular weight (Saunders, 1976). The ratio between the spectral absorption coefficient (SAC in m^{-1}, at 254 nm) and the organic carbon of the humic fraction (in mg C L^{-1}) was calculated as aromaticity (so called SAC/OC: specific UV-absorption of the HS peak, L mg^{-1} m^{-1}).

A2 PLFA analysis and determination of stable carbon isotope ratios of PLFA

Total lipids were extracted from 200 ml water samples filtered over a 0.2 µm polyvinyl fluoride filter using a modified Bligh and Dyer method as described by Frostegård et al. (1991) and changed after Steger et al. (2011). After lipid fractionation on silicic acid columns (BondElut® LRC-Si, Agilent Technologies, Santa Clara, USA), the phospholipids in the polar fraction were dried under a gentle stream of nitrogen and converted to fatty acid methyl esters

(FAME) by a mild alkaline methanolysis (Guckert et al. 1985). Before analysis, the completely dried FAME fraction was dissolved in n-hexane containing 20.06 ng μL^{-1} of the fatty acid 21:0 as an internal standard. For identification and quantification of the FAMEs, a gas chromatograph coupled to a mass spectrometer (Agilent, Palo Alto, USA) was used as described in Bastida et al. (2011). One µl aliquots were injected with either splitless mode or at different split ratios of up to 1:50. FAMEs were identified by comparison of fatty acid mass spectra of standards (bacterial acid methyl ester mix, Supelco, Munich, Germany) and to the NIST MS database. The concentration of each FAME was quantified relative to the internal standard. The fatty acid nomenclature was used according to Bastida et al. (2011).

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Appendix B: Additional pulse experiment

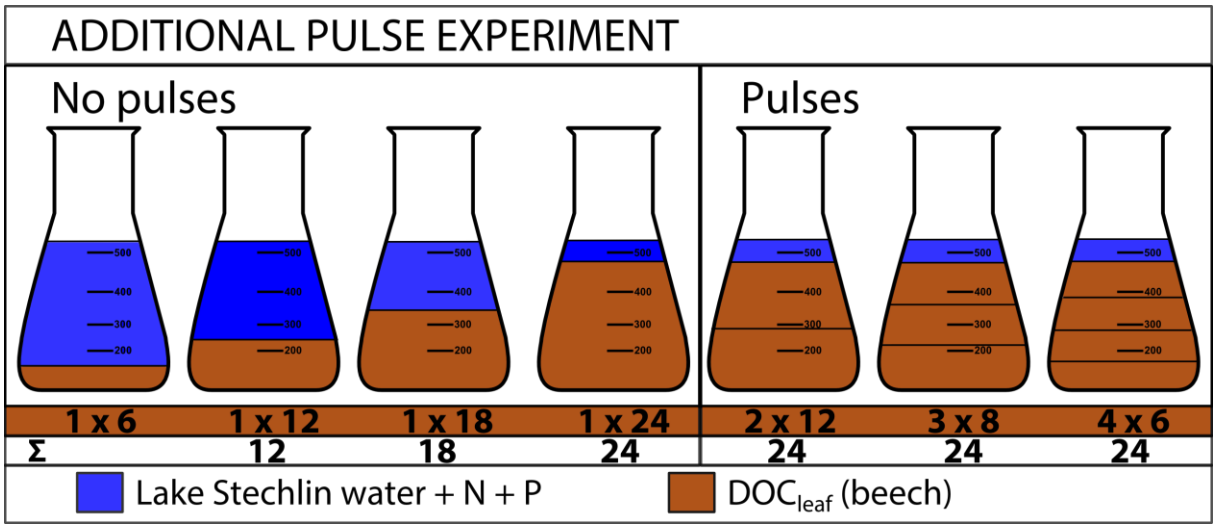

Figure B1: Experimental set-up of the additional pulse experiment showing the two parts with and without pulses.

Experimental set-up

We performed an additional pulse experiment to test whether pulsing in DOC addition has an influence on bacterial DOC consumption. We used lake water and the natural bacterial community plus nutrients (oligotrophic Lake Stechlin in northern Germany; for more details see Allgaier and Grossart 2006) and a single DOC source (beech DOC_{leaf}). The DOC_{leaf} was added at the beginning of the experiment without any pulses, but at increasing DOC concentrations. DOC concentrations increased by 6 mg C L^{-1} from 6 to 24 mg C L^{-1} in 250 ml Erlenmeyer flasks (see figure1). Additionally, we performed three incubations with the same total DOC concentrations but with an increasing number of pulses (see figure 1). All incubations were done in triplicates for 12 days under the same conditions as in the main experiment. We measured DOC concentrations (procedure described in the methods section of the manuscript) and calculated DOC consumption after 12 days.

Results

The DOC consumption increased with increasing DOC concentrations (from 45% in treatment 1x6 to 58% in 1x12, 64% in 1x18, and 67% in treatment 1x24, respectively) but were the same (67% in treatment 1x24 and 69% in treatment 4x6) for the 24 mg C L^{-1} treatment without pulses and in all other pulsed incubations with the same total DOC

concentrations (Fig. A1). The treatments without pulses were significantly different (Kruskal-Wallis; df = 3 and p = 0.016). In contrast, treatments with varying DOC pulses were not significantly different (Kruskal-Wallis; df = 3 and p = 0.059). This confirms that the pulses had no influence on bacterial DOC consumption, whereas differences in DOC concentrations significantly affected it.

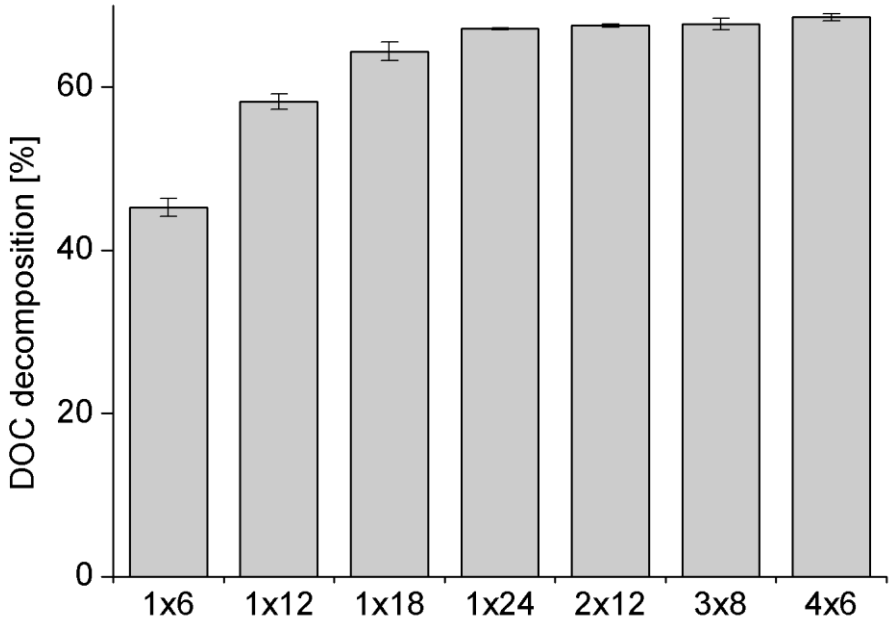

Figure B2. DOC consumed (mean ± standard deviation; in %) after 12 days in the four treatments with increasing DOC (1x6, 1x12, 1x18, 1x24) and in the three treatments with pulsed DOC input (2x12, 3x8, 4x6).

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Appendix C: Liquid Chromatography with Organic Carbon Detection (LC-OCD) of illuminated and non-illuminated DOC_{leaf} without microorganisms.

Experimental set-up

We incubated beech leachate DOC (DOC_{leaf}) in sterile conditions to test whether the presence of light in our incubation has an influence on DOC quality. Therefore, we set up 250 ml Erlenmeyer flasks with beech DOC_{leaf} at 7 mg C L^{-1} diluted with double distilled water. We incubated three Erlenmeyer flasks in light comparable to the incubation conditions of the main experiment and three Erlenmeyer flasks in complete darkness. After one week we froze the samples until further chemical analysis. The samples were analysed with the Liquid Chromatography - Organic Carbon Detection - Organic Nitrogen Detection (LC-OCD-OND) as described in the method section of the manuscript.

Results

We detected no significant differences between the light and dark treatments (Fig. B1). Upon illumination, there was a slight decrease in HS and an accompanied increase in LMWS. In general, however, the quality revealed by LC-OCD did not change by illumination when comparing the start (t_0) with the end of the incubation (Mann-Whitney U; $p > 0.05$ for t_0 vs. t_7 non-light and for t_0 vs. t_7 light) and in the light and dark treatment at the end of the experiment (Kruskal-Wallis; $p > 0.05$ for dark vs. light).

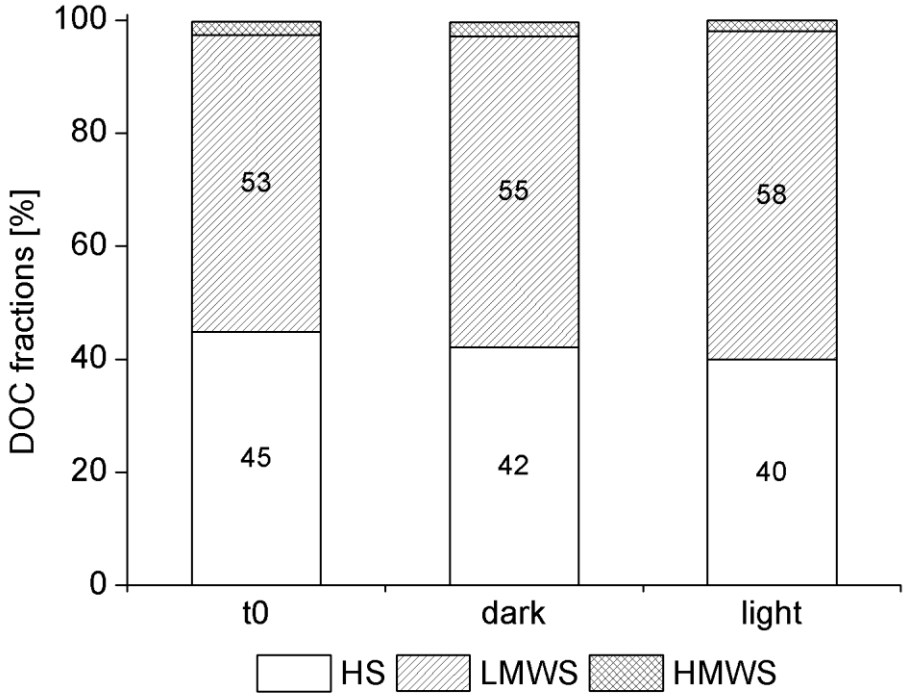

Figure C1. Percentage of measured DOC fractions at the start (t_0) and end (t_7) of the incubation in both dark and light treatment. According to Huber et al. (2011) we claimed the fractions HS which represent humic like substances and building blocks, LMWS consist of low molecular weight substances and HMWS depict high molecular weight substances.