# Shedding light on detritus: Interactions between invertebrates, bacteria and substrates in benthic habitats

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**Publication date**
2013

# *Chapter 5*

## Solar radiation shapes bacterial functional diversity in sediments

*Manuscript under review:*
Hunting, E.R., C. White, M. van Gemert, D. Mes, E. Stam, H.G. van der Geest, M.H.S. Kraak and W. Admiraal. **Solar radiation shapes bacterial functional diversity in sediments**.

**Abstract:** Solar radiation is known to influence the species composition of bacterial communities. UV radiation can directly affect bacteria or alter the composition of organic matter, rendering available substrates for bacteria. However, effects of solar radiation on bacterial communities residing in sediments remain completely unexplored. This study investigated the influence of mimicked solar radiation on bacterial functional diversity in laboratory sediments. Two different organic matter sources, labile and recalcitrant organic matter (OM), were used and metabolic diversity was measured with Biolog GN. Radiation exerted strong negative effects on the metabolic diversity in the treatments containing recalcitrant OM, more than in treatments containing labile OM. The functional composition differed significantly between the treatments. Our findings demonstrate that a combined effect of light and OM shapes the functional composition of microbial communities developing in sediments, and acts as an important sorting mechanism for bacterial communities in wetlands.

***Keywords:*** *Organic matter quality, Solar radiation, benthic bacterial communities, bacterial metabolic diversity*

Several studies have demonstrated that solar radiation may affect bacterial communities (e.g. Baldy et al. 2002; Piccini et al. 2009; Zepp et al., 2011). Especially UV radiation exerts negative effects on bacterial communities due to its detrimental effects on DNA (e.g. Santos et al. 2012a), but solar radiation may also change the chemical composition and palatability of organic compounds by photodegradation (e.g. Engelhaupt et al. 2002; Sulzberger and Durisch-Kaiser, 2009). Such changes in the chemical composition of OM may subsequently cascade towards shifts in bacterial community composition due to the interplay between bacterial resource niches (i.e. the type of substrates that are utilized) and available resources (e.g. Salles et al. 2009). This suggests that the functional composition of bacterial communities may also change when exposed to solar radiation.

Several studies have demonstrated that photolytic changes of OM can result in enhanced bacterial production (e.g. Wetzel et al., 1995), and Santos et al. (2012b) indeed provided some evidence that UV-B radiation can induce shifts in the functional composition of bacterioplankton communities. However, potential effects of solar radiation on bacterial communities residing in sediments remain completely unexplored. This study therefore aimed to investigate the effect of solar radiation on the functional composition of bacterial communities in shallow aquatic sediments, and compared the effect of light and dark incubation on bacterial metabolic diversity in sediment microcosms. Since composition of the available OM is one of the main drivers of bacterial community composition (e.g. Hättenschwiler and Vitousek 2000; Myers et al 2001; Baldy et al., 2002; Docherty et al., 2006), we assessed the effects of light exposure on bacterial communities inhabiting either a labile or a recalcitrant OM source.

**Methods**

*Sediment microcosms*

Freshly collected stinging nettle, *Urtica dioica*, was used as a labile OM source, and intact peat collected from natural peatlands was used as recalcitrant OM source. Both nettle and peat were frozen in liquid nitrogen and thoroughly ground in a pestle and mortar. Quartz sand (0.1-0.5 mm; Dorsilit, Eurogrit, Papendrecht, The Netherlands) was mixed with either the labile or recalcitrant OM source (95:5 weight ratio sand:OM-source with final dry weight OM concentrations of 0.63% and 0.52% for labile and recalcitrant OM, respectively), and then autoclaved. 5 mL of sediment was subsequently added to each of the 5 replicate microcosms per treatment (plastic round vials (Greiner Bio-one, Germany): 27 mm diameter, 5 cm height), resulting in ~1 cm sediment layer. Each microcosm received 2 cm of overlying water (Dutch Standard Water, DSW; deionized water with 200

mg/L $CaCl_2 \cdot 2H_2O$, 180 mg/L $MgSO_4 \cdot H_2O$, 100 mg/L $NaHCO_3$ and 20 mg/L $KHCO_3$; pH = 8.2 ± 0.2). A mixture of sediment pore-water and surface water collected from 2 different natural wetland systems was added as bacterial inoculum.

*Experimental set up*

To mimic natural conditions and exposure to solar radiation, we incubated the samples at 15°C under a dark:light regime of 12h:12h. We used mercury lamps (Arcadia-D3, Redhill, United Kingdom: 160W; luminous flux 1900 lm) that, in addition to emission of visible light (400-800nm), emit UV radiation (UV-B 1.75 $W.m^{-2}$ at 310 nm; UV-A 10 $W.m^{-2}$ at 365 nm). These intensities of UV-radiation are commonly registered in temperate areas (Kelly et al., 2003). The duration of the incubation was 5 days. An additional set (n=5) of microcosms of both OM types was incubated in the dark as control. This yielded a total of four treatments, consisting of: **1)** labile OM in light; **2)** labile OM in the dark; **3)** recalcitrant OM in light; and **4)** recalcitrant OM in the dark. Each treatment was replicated 5 times. After 5 days, bacterial metabolic diversity was determined as described below.

*Community metabolic diversity (CMD)*

Community metabolic diversity (CMD) in the sediment was assessed by community level physiological profiling (CLPP) using Biolog® GN microplates containing 95 unique single substrates (Biolog, Inc., Hayward, USA; Garland and Mills 1991). Biolog GN plates are comprised of simple, common substrates (e.g. Sucrose, Mallose and Citric Acid), and do not include recalcitrant substrates nor specific substrates typical of the OM used in this study. It is therefore impossible to directly relate substrate utilization profiles to the actual functioning of the developed bacterial communities. Nonetheless, the number of substrates used can serve as a proxy of the metabolic diversity of the bacterial community, and differences in utilization profiles indicate that functionally distinct bacterial communities can develop depending on treatment (Garland 1999). CMD was determined after 5 days of incubation. Pore water was sampled by pipetting 1 mL of the sediment top layer, while preventing sampling of the overlying water. Samples were subsequently diluted 30x with DSW and distributed over the 96 Biolog® GN wells. Plates were incubated for 36h at 37°C and utilization patterns of 95 different single carbon sources were measured at 490 nm using an automated microplate reader (VERSAmax tunable microplate reader, Molecular Devices, Sunnydale, USA). This data was used to calculate the CMD (Garland 1997) using a threshold absorbance of 0.15, and analyzed with a two-way ANOVA and Tukey-HSD post hoc test. To relate the bacterial functional composition to the four

treatments, utilization patterns of the 95 carbon sources were analyzed using a Bray-Curtis-based cluster analysis and a two-way ANOSIM (Hammer et al. 2001).

**Results**

Effects of light on the metabolic diversity of the different treatments are presented in Figure 1. No significant difference was observed between the light exposure and the control dark incubation in the sediments containing labile organic matter. In contrast, light exposure significantly reduced the community metabolic diversity (CMD) in the treatments containing recalcitrant DOM (two-way ANOVA, Tukey HSD, p=0.004; Fig. 1).

In addition to the number of substrates used by the bacterial community, we assessed which set of substrates was used to compare the functional composition of the communities that developed during the incubation. A two-way Analysis of Similarity (two-way ANOSIM) results revealed that the bacterial resource niches differed significantly between treatments depending on both radiation and organic matter type (two-way ANOSIM: Light R=0.536, p=0.0007; OM R=0.302, p=0.0146, respectively) (Fig. 2), showing that the developed bacterial communities were functionally distinct.

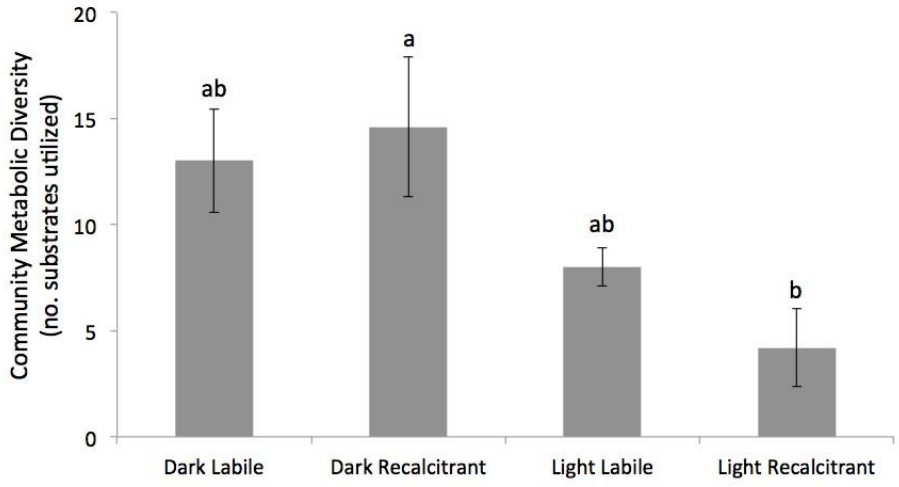


*Fig. 1: Mean (± S.E.) community metabolic diversity (CMD) of the four treatments after five days incubation. Bars with the same letters are not significantly different (two-way ANOVA with Tukey's HSD post hoc test, n=5, p<0.05).*

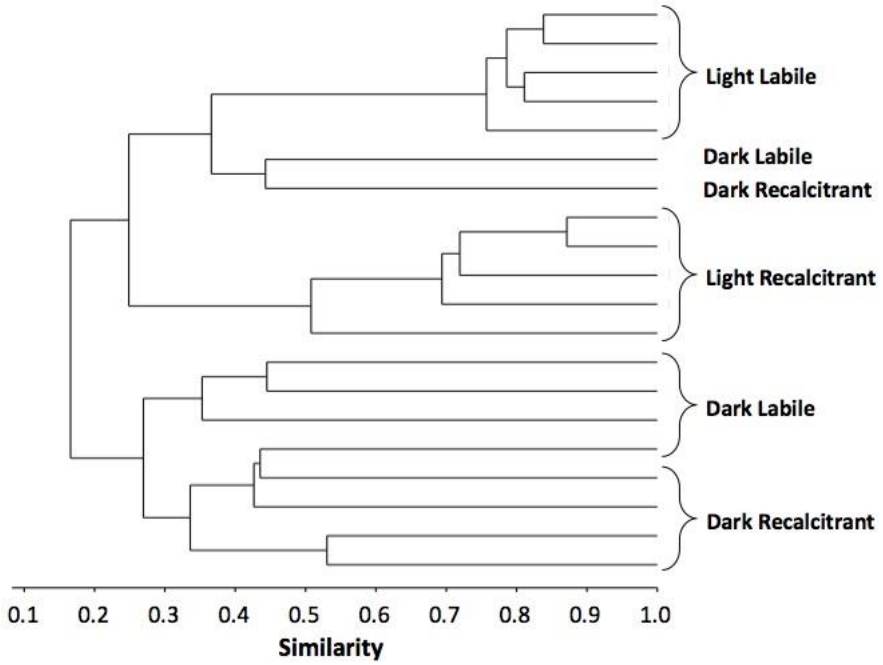


*Fig. 2: Bray-Curtis-based dendrogram representing level of similarity between sets of substrates used by the bacterial communities after five days incubation at four different conditions.*

**Discussion**

Radiation diminished the number of organic substrates used and reduced the similarity of substrate use between bacterial communities. This was most evident when peat was used as substrate, and this might have been provoked by either visible light and/or UV. The potential detrimental effects of UV radiation on e.g. DNA and enzymes (e.g. Santos et al. 2012a) are typically held responsible for this negative effect, but we speculate that compounds liberated during radiation mediated degradation of recalcitrant OM negatively affected some members of the bacterial community. It has been demonstrated that photo-degradation of OM creates useful low molecular weight compounds, as well as toxic hydrogen peroxide and various free radicals (e.g. Mopper and Zhou, 1990; Scully et al., 1996). Substituted organic molecules and aromatic products may also from during this process (Mill et al., 1980). Recalcitrant OM, in contrast to labile OM, contains substantial amounts of aromatic compounds (e.g. humic acid) that are known to strongly absorb UV-B (Zepp

et al., 1985). Solar radiation is composed of visible light and UV-radiation, and therefore more likely to affect the chemical composition of recalcitrant OM than labile OM, explaining why in the present study radiation effects were most prominent on peat. Thus, although the importance of these indirect effects of photo-degradation or photo-activation for bacterial community structure and productivity cannot be extrapolated to natural substrates and natural radiation, our data support the notion that the effect of solar radiation differs depending on the structural composition of the organic matter (cf. Tranvik and Kokolj 1998; Engelhaupt et al., 2002; Docherty et al., 2006; Köhler et al., 2012). More importantly, the present study showed that the interaction of bacterial community metabolism with radiation and recalcitrance of organic substrates may occur at the boundary of sediment and water, a prominent habitat in mudflats and wetlands. This suggests that both solar radiation and OM composition are important drivers shaping bacterial communities in shallow benthic environments, demonstrating that solar radiation is a currently overlooked, but important sorting mechanism (Mann and Wetzel 1995; Santos et al. 2012b) in wetlands.

In conclusion, solar radiation alters and diminishes the metabolic diversity of bacterial communities in peat containing sediments, and that solar radiation shapes the functional composition of bacterial communities in shallow wetland sediments.