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Effects of desiccation on the photosynthetic activity of intertidal microphytobenthos biofilms as studied by optical methods

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ABSTRACT

Due to the periodic exposure to air during periods of low tide, desiccation can be expected to cause important limiting effects on the photosynthetic activity of intertidal microphytobenthos biofilms. This work addresses the study of the short-term effects of desiccation on microphytobenthos using a new, simple methodological approach to non-destructively estimate the water content of muddy intertidal sediments. The method is based on the non-destructive measurement of the specular reflectance in the visible spectral region, shown to be linearly related to the water content of the uppermost 200 μm of the sediment. During air exposure, water loss by the surface sediment layers was shown to induce marked decreases in both the photosynthetic activity, as measured by the maximum quantum yield of photosystem II, F_v/F_m, and the surface microalgal biomass, as estimated from the diffusive reflectance biomass index NDVI. The effects of desiccation were largely dependent on the rate of sediment de-watering. For a same level of desiccation, samples under fast desiccation (exposed to wind of 4.2 m s^{-1}) showed much larger effects on F_v/F_m and NDVI comparatively to samples under slow desiccation (maintained under still air). By showing the rapid and significant effects of desiccation on microphytobenthos biofilm functioning, the results of this study have potentially important implications for the modelling of primary productivity of estuarine intertidal areas, as desiccation and factors inducing it may result in previously unaccounted effects on photosynthetic performance and productive biomass.

1. Introduction

The intertidal areas of estuaries are characterised by a large variability in environmental conditions caused by the exposure to air and direct sunlight during low tide periods, alternating with submersion throughout high tide. During diurnal low tide, organisms living in intertidal sediments experience potentially stressful conditions that include high light intensities, extreme low or high temperatures, high salinities and wind. These variable and extreme environmental conditions are likely to affect the communities of benthic microalgae, or microphytobenthos, which form highly dense biofilms on the surface of intertidal sediments (MacIntyre et al., 1996; Underwood and Kromkamp, 1999). During low tide, the prolonged exposure to wind and direct sunlight favours the evaporation in the uppermost layers of sediment, frequently exposing the benthic microalgae to an intense process of de-watering. Desiccation may be expected to cause important limiting effects on photosynthetic activity of microphytobenthos, as shown for other photoautotrophs such as macroalgae (Bell, 1995; Matta and Chapman, 1995; Peña et al., 1999; Hunt and Denny, 2008), lichens (Veerman et al., 2007; Heber, 2008) or cyanobacterial mats (Lüttge et al., 1995; Potts, 1999; Ohad et al., 2005).

Despite the predictable relevance of this factor for the primary productivity of benthic microalgal communities, and the large number of factors that affect the water content of intertidal sediments, such as topography, tidal regime, sediment type and climate factors varying with location and season, the available knowledge on the effects of desiccation on microphytobenthos is very limited (Holmes and Mahall, 1982; Lamontagne et al., 1989), especially if considering that the stressful effects of other factors, such as light (Kingston, 1999; Perkins et al., 2001; Serôdio et al., 2006), temperature (Blanchard et al., 1997; Guarini et al., 1997; Cohn et al., 2003) or salinity (Admiraal, 1984; Rijstenbil, 2003; Roncarati et al., 2008) have been studied in detail. References to the impact of desiccation on microphytobenthos productivity have been made in a few descriptive studies on the photosynthetic activity under *in situ* conditions, when desiccation effects could not be separated from those of other co-varying factors (Lamontagne et al., 1989; Brotas et al., 2003; Serôdio et al., 2008).

This lack of studies may be explained by the difficulty in relating biofilm photosynthetic activity to sediment de-watering, which demands for the quantification of the water content of the depth interval below the surface where photosynthesis can be carried out. The traditionally used method of determining the sediment water

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content is based on the destructive measurement of the dry weight of sediment sections (MacIntyre and Cullen, 1995; Perkins et al., 2003; Murphy et al., 2004; Jesus et al., 2006). On intertidal muddy sediments, the photic zone is very thin, typically below 250 μm (Serôdio et al., 1997, 2001; Kromkamp et al., 1998), which poses considerable difficulties in the quantification of the water content of a matching depth interval using this method. As a result, most published values of sediment water content refer to depth intervals below the surface much larger than the photosynthetically active zone (Perkins et al., 2003, Murphy et al., 2004; Jesus et al., 2006). On the other hand, it is well recognized that intertidal estuarine areas exhibit substantial heterogeneity, both spatially and temporally, and that the characterization of this variability through isolated point samples is usually time-consuming, expensive and frequently unrepresentative (Rainey et al., 2000, 2003). Considering this, recent studies have addressed the development of optical approaches, based on the measurement of diffusive reflectance, which can provide a non-invasive and non-destructive characterization of the intertidal zone (Rainey et al., 2003; Murphy et al., 2005b, 2008; Forster and Jesus, 2006).

This work addresses the short-term effects of desiccation on the photosynthetic activity of intertidal microphytobenthos biofilms, based on a new, simple methodological approach to non-destructively estimate the water content of the uppermost, photosynthetically active layers of the sediment. This method is based on the measurement of the specular reflectance of visible light emitted from intact sediment samples, shown to be linearly related to the relative water content of the uppermost 200 μm. Unlike diffusive reflectance, which measures the light that after inciding on a surface is scattered in a wide range of directions and is related to the absorption characteristics of the materials, specular reflectance regards the light that is reflected in a mirror-like way and is related to the surface reflection properties (smoothness versus roughness). Measurements of water content can thus be obtained virtually non-invasively, enabling the simultaneous measurement of photophysiological parameters, as those based on other optical techniques such as pulse amplitude modulated (PAM) fluorometry or hyperspectral diffusive reflectance analysis. The effects of desiccation on biofilm photosynthesis were studied by simultaneously measuring changes in relative water content and in the photosynthetic activity (using PAM fluorometry) and surface microalgal biomass (using diffusive reflectance spectral analysis).

2. Materials and methods

2.1. Sampling

Undisturbed sediment samples were collected on Vista Alegre (40° 35′ N, 8° 41′ W), an intertidal mudflat in the Ria de Aveiro, west coast of Portugal. Sampling was carried out during diurnal low tide periods. Sampled sediments are composed by fine grains (97% particles < 63 μm) and are colonized by microalgal communities dominated by diatoms (Serôdio et al., 2008). The samples were collected using plexiglas corers (1.9 and 3.6 cm internal diameter) and taken to the laboratory where they were maintained in water collected in the sampling site until measurements were carried out. All experiments were carried out in the laboratory under constant conditions of temperature (20 °C) and relative air humidity (60%).

2.2. Spectral reflectance

Specular and diffusive reflectance spectra were measured using a fiberoptic spectrometer (USB2000-VIS–NIR, grating #3, Ocean Optics, Duiven, The Netherlands). Spectra were recorded over the 350–1000 nm bandwidth, using a 400 μm-diameter fiberoptic (QP400-2-VIS–NIR-BX, Ocean Optics). Samples were illuminated with white light, provided by a halogen lamp (Quartzline DDL 150 W, General Electric, USA) in a fiberoptic illuminator (Olympus Highlight 3000 Cold Light Source Illuminator, Hamburg, Germany). To ensure the exposure of the sample to light in the 750 nm range (necessary for computation of the NDVI index, see below), the infrared filter present by default in the illuminator was removed. Specular and diffusive spectra were normalized to the spectrum reflected by a reference (WS-1-SL Spectralon Reference Standard, Ocean Optics). A dark reflectance spectrum measured was subtracted to both spectra to account for the dark current noise of the spectroradiometer. Sample and reference spectra were measured under a constant irradiance of 100 μmol m^{-2} s^{-1}.

Specular reflectance spectra were measured by illuminating the sediment surface with a beam of collimated white light incident at a 45° angle and by measuring the spectra of the reflected light also at a 45° exit angle. The relative position of the fiberoptics used to illuminate the sample and to collect the reflected light were maintained fixed using a custom-made adaptor (Fig. 1). Before each measurement, the distance between the sample surface and the fiberoptics was adjusted using a micromanipulator (MM33, Märtzhäuser, Germany) (Fig. 1). This adjustment was often necessary due to the contraction of the sediment due to de-watering and of the consequent increase of the distance between the sediment surface and the fiberoptics.

Diffusive reflectance spectra were measured to estimate the surface microalgal biomass, using the biomass index NDVI (normalized difference vegetation index; Rouse et al., 1973). For this purpose, the spectroradiometer fiberoptics was positioned perpendicularly to the sediment, at a fixed distance set to match the view field with the total area of the sample. NDVI was calculated by:

$$NDVI = (R_{d,750} - R_{d,675}) / (R_{d,750} + R_{d,675}) \quad (1)$$

where $R_{d,750}$ and $R_{d,675}$ represent the average diffusive reflectance in the intervals of 749.73–750.39 nm and 674.87–675.55 nm, respectively.

2.3. Photosynthetic activity

The photosynthetic activity of microphytobenthos samples was measured non-destructively using PAM fluorometry (Schreiber et al., 1986). Variable chlorophyll fluorescence was measured using a fluorometer comprising a computer-operated PAM-Control Unit

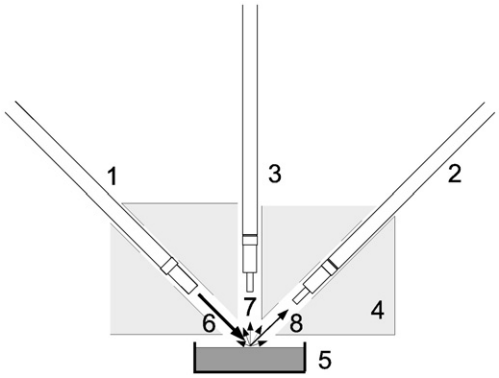

Fig. 1. Schematic diagram of the experimental setup used to measure specular and diffusive reflectance on undisturbed microphytobenthos biofilms. 1. Illuminator fiberoptics delivering incident white light provided by a halogen lamp. 2. Spectroradiometer fiberoptics collecting specular reflectance used to estimate the relative water content of the sample. 3. Spectroradiometer fiberoptics collecting diffusive reflectance used to calculate the biomass index NDVI. 4. Cross section of the custom-made adapter used to maintain the relative positions of the fiberoptics and the sample. 5. Sediment sample, connected to a micromanipulator used to control its vertical position relatively to the fiberoptics. 6. Incident light beam. 7. Diffusive reflectance (small arrows). 8. Specular reflectance.