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A simple, user friendly tool to readjust raw PAM data from field measurements to avoid over- or underestimating of microphytobenthos photosynthetic parameters

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ABSTRACT

Intertidal mudflats are among the most productive ecosystems and microphytobenthic (MPB) biofilms play a key role in primary production. MPB primary production varies at short spatial and temporal scales. Accurate measurements thus require rapid non-intrusive methods like pulse amplitude modulate (PAM) fluorescence. However, the effect of granulometry and chl *a* concentration profile in light attenuation on irradiance and on fluorescence signal in the photic layer need to be taken into account when primary production is estimated using PAM. We propose a tool to readjust raw photosynthetic parameters ($rETR_{max}$, α, I_k) estimated from PAM measurements on the field, to avoid over- or underestimation. To develop the tool, we used models previously designed by Kühl and Jørgensen (1992), Serôdio (2004) and Forster and Kromkamp (2004) by integrating the chl *a* distribution profiles and sediment granulometry from pure sand to pure mud. The sensitivity of the correction to sediment granulometry and the shape of chl *a* profile were evaluated theoretically using a typical fluorescence data set obtained using PAM measurements. Our results confirm the importance of accounting for both the chl *a* profile and sediment granulometry when estimating a light attenuation coefficient. We show that, with the same chl *a* profile, the photosynthetic parameters are more underestimated in mud than in a sandy environment. Thus, granulometry and the chl *a* profile need to be systematically quantified and used to correct raw data measured in field studies using PAM before estimating photosynthetic parameters. The numerical tool is available as an e-document that is simple and easy to apply to any PAM data.

1. Introduction

Littoral areas of lakes and coastal seas are among the most productive ecosystems in the world, and their production far exceeds that of open oceans (Geider et al., 2001). One of the main primary producers in these regions is the microalgae that develop in the euphotic zone of many types of benthic substrates (Underwood and Kromkamp, 1999; Aberle-Malzahn, 2004). The importance of microphytobenthic primary production (PP) is similar to that of phytoplankton (Underwood and Kromkamp, 1999) where ~90% of production is consumed or recycled to maintain local heterotrophic metabolism (Cloern et al., 2014). Many authors consider that the productivity and biomass of microphytobenthos (MPB) are greater than those of phytoplankton on intertidal mudflats (De Jonge and Van Beuselom, 1992; Lucas and Holligan, 1999; Guarini et al., 2000; Kang et al., 2015). Moreover, these microphytobenthic communities, which include assemblages of diatoms, green algae, and cyanobacteria (Admiraal et al., 1985) also have major ecological implications as ecosystem engineers (Sutherland et al., 1998; Tolhurst et al., 2006; Lubarsky et al., 2010), as trophic support for benthic fauna locally (Herman et al., 2000), but also after exports to adjacent habitats of intertidal mudflats (Ubertini et al., 2012; Kang et al., 2015).

The primary production and standing stocks of MPB biofilms inhabiting intertidal sediments vary at several spatial and temporal scales (Blanchard et al., 2001; Orvain et al., 2012). Changes can vary from very short to long time scales linked to tidal rhythm, daily photoperiod, spring/neap cycles and seasonal cycles (Taylor, 1964; Pinckney and Zingmark, 1991; Blanchard et al., 2001). Primary production also displays a high degree of spatial variability from high-resolution patchy distribution related to the intrinsic autoecology of biofilms (Weerman et al., 2011) to benthic fluxes regulated by macrofauna, biogeochemical components affecting organic matter, and release of nutrients (Thrush et al., 2013). But primary production is also influenced by mesoscale patterns related to the morphodynamics of estuarine landscapes and

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tidal bars and flats (Fagherazzi et al., 2014) and large scale changes related to sediment composition, salinity, nutrient inputs linked to river flows (Benyoucef et al., 2014) and shear stress (Fagherazzi et al., 2014). Among all these sources of variability, light is the main factor influencing primary production (Underwood and Kromkamp, 1999). There are major spatial and temporal gradients in the availability of light in MPB habitats that control primary production. Steep irradiance gradients occur across the surface of the sediment bed that depends on grain size (Kühl et al., 1994) and on the relative proportion of silt and sand. Intertidal sites are subject to varying patterns of diel lightning periods mediated by periods of tidal immersion (Underwood and Kromkamp, 1999). Such changes are accompanied by a variation in the spectral quality of light in sediments (Kühl and Jørgensen, 1992). Irradiance is further modified by increased attenuation of light at depth due to the presence of microalgal biofilms in the surface layers of the sediments (Ploug et al., 1993; Kühl and Jørgensen, 1994). To cope with light variability, the majority of epipelic microphytobenthos are mobile and undertake rhythmic vertical migration linked to both the diel and tidal cycles (Taylor, 1964; Baillie and Welsh, 1980; Edgar and Pickett-Heaps, 1984; Mitbavkar and Anil, 2004). In intertidal sediments, this motility has been hypothesized to be a strategy developed by MPB biofilms to colonize the illuminated surface when light is available (using upward migration). Downward MPB migration occurs during high exposure (tidal diurnal emersion), as a strategy to avoid photoinhibition due to high light exposure and potential saturation of photosystems (Admiraal, 1984) and to capture remineralized nutrients concentrated in deeper sediment layers (Orvain et al., 2003). The level of light at the surface of intertidal mudflats is very much higher than the highest possible level in the phytoplanktonic photic layer in the water column, the migratory strategy could thus be the main adaptation to limit impairment due to excessive light. Especially compared with other forms of physiological adaptation of diatom cells, such as modifications in internal chl *a* concentration, pigment composition, the number of active reactional centers, the size of the light harvesting cross section, or activation of xanthophyll cycle (Serôdio et al., 2012; Cartaxana et al., 2013). In diatoms, this mobility is associated with the excretion of extracellular polymeric substances (Decho, 2000), primarily glycoproteins, which can also be used by bacteria, meiofauna and macrofauna as carbon sources (Middelburg et al., 2000) and reinforce the importance of microphytobenthos as a food web support.

Because of high variability at short spatial and temporal scales, accurate measurements of primary productivity require rapidly repeated spatially and temporally close measurements while avoiding disturbances in the microscopic gradient of the photic zone under the air-sediment interface (Kühl and Jørgensen, 1994). However, traditional primary production measurements using labeled ^{14}C carbon cannot be used without disturbing natural assemblage and resuspending them in incubators for experiments longer than 1 h (Blanchard et al., 1996; Underwood and Smith, 1998). The turbidity and shading effects of algae make the control of light and its availability for algal cells difficult to accurately estimate in incubators. Moreover, the typical high-resolution variability of benthic primary producers and processes under the influence of natural microscopic gradients in the photic zone cannot be measured by such techniques. For this reason, there has been an increase in research on rapid non-intrusive methods using oxygen electrodes (Serôdio, 2003) and pulse amplitude modulated (PAM) fluorescence (Kromkamp et al., 1998; Serôdio, 2003; Forster and Kromkamp, 2004; Jesus et al., 2006), which exploits the optical properties of chlorophyll *a* pigments (chl *a*) for rapid and remote detection of the MPB photosynthetic activity in these fragile environments (Jesus et al., 2006). This technique has considerable advantages, such as the rapidity and non-intrusive nature of the measurements that facilitate adaptation to the degree of temporal and spatial variability of the MPB communities (Serôdio, 2004). PAM techniques are easily deployed in the field, explaining why there is extensive literature on the use of PAM fluorometers (Walz, Germany) in studies of MPB communities (Serôdio et al., 1997, 2007; Kromkamp et al., 1998; Underwood and Kromkamp, 1999; Barranguet and Kromkamp, 2000; Serôdio and Catarino, 2000; Perkins et al., 2001, 2011; Forster et al., 2006; Vieira et al., 2013; Juneau et al., 2015).

The PAM method relies on measurements of the fluorescence emitted by MPB in response to light pulses. After a period of darkness imposed on the MPB sample (between 5 and 10 min depending on the study), the minimum level of fluorescence (F_0) is recorded. Then, in response to a light saturating flash, the maximum level of fluorescence (F_M) is recorded. After which increasing the pulse of actinic light (I) with a time lag (e.g. lasting 30 s) makes it possible to measure a series of steady-state fluorescence level $F_S(I)$ and new maximum levels $F_M'(I)$. Using these fluorescence values, the light level (I) and an ETR factor, the electron transport rate (ETR) in photosystem II (PSII), which equals the product of apparent or effective photochemical efficiency, can be calculated: $ETR(I) = [(F_M'(I) - F_S(I)) / F_M'(I)] \times I \times$ ETR factor. Since the percentage of photons absorbed by active Photosystem II (PSII) is debatable and can differ among species according to Johnsen and Sakshaug (2007) and Schreiber et al. (2012), the ETR factor is not considered as a constant value. During the first steps of data treatment of PAM results, ETR can be expressed in relative form: $rETR(I) = [(F_M'(I) - F_S(I)) / F_M'(I)] \times I$. Photosynthetic parameters ($rETR_{max}$, α, I_{opt} and I_k) can therefore be estimated by adjusting the rETR/I curves to photosynthetic non-linear models: for instance either the Webb et al. (1974) model, when there is no decrease in rETR at high levels of I, or the Eilers and Peeters (1988) model, when there is an apparent decrease in rETR at the highest I. Although these method do not allow direct access to primary production measurements, many studies have shown that it is possible to use the fluorescence approach to estimate primary production as accurately as with other traditional incubation methods, such as carbon incorporation or oxygen release measurements (Hartig et al., 1998; Barranguet and Kromkamp, 2000; Serôdio, 2003; Morris and Kromkamp, 2003; Serôdio et al., 2007).

However, PAM measurements require cautious interpretation especially because of the micro-heterogeneity of the benthic habitat, which has marked effects on light and fluorescence attenuation in sand and mud particles (Kühl and Jørgensen, 1994) and vertical profiles of the MPB biomass (Vieira et al., 2013), but also because of the microtopography that affects incident light at the surface. Thus PAM measurements are actually affected by light attenuation, which in turn, is mainly dependent on the vertical profile of chl *a* concentration (self-shading by the MPB positioned in the upper layers), their migration behavior, and grain size (Kühl and Jørgensen, 1994; Forster and Kromkamp, 2004; Serôdio, 2004), but also by minor factors like the effect of the different composition of pigment in the MPB species assemblage (diatoms, cyanobacteria, euglenoids) on spectral radiation, the presence of pheopigment, which are breakdown products of chl *a*, and the presence of water (Kühl and Jørgensen, 1994). Perkins et al. (2011) argued that the application of chlorophyll fluorescence to MPB biofilms is complex because of the signal emanating from subsurface cells, vertical cell migration in the sediment matrix, high regulation capacity, chlororespiration in the dark, and the effects of the physical structure of the sediment/biofilm matrix (light attenuation caused by the sediment matrix). Due to light attenuation in the sediment, the level of irradiance received by the photosynthetic cells in their vertical position in the sediment photic layer is attenuated. Conversely, the attenuation also affects the fluorescence returned by cells and measured at the surface of the sediment. For these reasons, raw field measurements underestimate the actual level of fluorescence produced. Serôdio (2004) and Forster and Kromkamp (2004) demonstrated that it is possible to calculate light and fluorescence attenuations during PAM measurements. These two studies agreed that 40% of the error in estimations of photosynthetic parameters occurs between measured and corrected values. These models were applied in case studies (scenarios) by simulating various vertical migratory patterns with the microscopic profile of chl *a* biomass. However, the granulometry of the sediment