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PHOTOSYNTHETIC ACTIVITY OF INTERTIDAL MICROPHYTOBENTHIC COMMUNITIES DURING EMERSION: IN SITU MEASUREMENTS OF CHLOROPHYLL FLUORESCENCE (PAM) AND CO_2 FLUX (IRGA)[1]

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Photosynthetic microphytobenthic activity has increasingly been examined using pulse-amplitude-modulated (PAM) fluorescence techniques. Nevertheless, estimating carbon production rates from fluorescence measurements implies the establishment of reliable relationships. The aim of this study was to determine such a relationship from field measurements of both PAM fluorescence and CO_2 fluxes. Three study sites of varying sedimentary features were investigated in different seasons. Both linear and with plateau relationships were obtained between the fluorescence parameter (relative electron transport rate [rETR]) and the community-level carbon-fixation rate (gross community primary production rate [GCP] in mg $C \cdot m^{-2} \cdot h^{-1}$). The correlation calculated from the whole data set (i.e., all sites and all seasons) was very strong ($n = 106$; $r = 0.928$). Significant correlations were also obtained for light-curve parameters assessed with the two methods: P_m ($n = 8$; $r = 0.920$) and I_k ($n = 8$; $r = 0.818$). Total community-level carbon fixation for the emersion period was calculated from fluorescence measurements according to the relationship established between GCP and rETR, and between light-curve parameters, and the results were compared to the estimation obtained directly from GCP measurements. The agreement between the two estimations was quite good for both ways of calculation (with a mean discrepancy of 30% for the first one and −2% for the second one). These results suggest the potential application of PAM measurements to calculate carbon-fixation rates at large spatial and temporal scales, provided that a set of experiments coupled with CO_2-flux measurements are performed.

Key index words: **C fixation; microphytobenthos; PAM fluorescence; primary production; tidal flats**

Abbreviations: **Au, Authie; CR, community respiration rate; *D*, day length; F'_m, fluorescence maximal level under saturating light in actinic irradiance; F_t, fluorescence steady-state level under ambient light; GCP, GCP Fluo, GCP CO_2 fluxes, gross community primary production rate, estimation based on fluorescence or CO_2-flux measurements; GCP_m, maximum gross community primary production rate; *I*, incident irradiance; I_k, I_k Fluo, I_k CO_2 fluxes, saturation onset irradiance, estimated from the fluorescence or CO_2-flux measurements; I_m, theoretical maximal irradiance; IRGA, infrared gas analysis; NCP, net community primary production rate; *P*, rate of photosynthesis; PAM, pulse amplitude modulated; P_m, rate of maximum photosynthesis under saturating irradiance; rETR, relative electron transport rate; $rETR_m$, maximum relative electron transport rate; Se, Seine; So, Somme; Φ_{PSII}, effective quantum yield of the PSII**

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Benthic microalgae photosynthetic activity is the primary source of fixed carbon in shallow aquatic ecosystems and particularly in tidal flats. Tidal sediment habitats are characterized by large fluctuations in environmental parameters, and one adaptation exhibited by benthic microalgae is to migrate vertically in response to diurnal and tidal rhythms (for a review, see Consalvey et al. 2004b). Downward migration reduces wash-away of cells during immersion and grazing by predators. It may also occur during emersion in response to the elevation of light and hence may act as a behavioral process of photoacclimation. These vertical movements cause rapid changes in the amount of biomass in the photic zone of the sediment and lead to large variations in community-level photosynthetic rates (Brotas et al. 2003). Therefore, the study of intertidal benthic primary production should be carried out under in situ conditions to take into account both large environmental variability and vertical structure of the microphytobenthic community. The application of chl fluorescence as a noninvasive technique for the estimation of photosynthetic activity of microphytobenthos has increased in recent years (Hartig et al. 1998, Kromkamp et al. 1998, Barranguet and Kromkamp 2000, Perkins et al. 2001, Brotas et al. 2003, Consalvey et al. 2004a, Serôdio et al. 2005). However, fluorescence measurements are performed at very small spatial scales (vertically and horizontally), and estimation of large spatial rates from these measurements is contentious due to the vertical migrations and the patchy distribution of microphytobenthic communities (Underwood 2001). Furthermore, fluorescence methods estimate the photosynthetic electron transport rate of microphytobenthos but cannot be directly used to calculate carbon-fixation rates. Highly significant correlations between photosynthetic activity measured by fluorescence and ^{14}C-based fixation rates of natural microphytobenthos populations have already been established (Hartig et al. 1998, Barranguet and Kromkamp 2000). However, the method used for measuring primary production required the removal of microalgae from the sediment and thus could have created unrepresentative rates. Serôdio (2003) observed strong correlations between fluorescence parameters and gross photosynthetic rate determined with oxygen microelectrodes on sediment cores but noted that gross oxygen production is not as relevant as carbon fixation to quantify primary production. Thus, no significant correlation between fluorescence parameters and community-level carbon-fixation rates has been established so far for undisturbed microphytobenthic assemblages.

The aim of this study was to compare rates of photosynthesis estimated in situ from pulse-amplitude-modulated (PAM) fluorometry and CO_2 fluxes on emersed tidal flats to propose a way to calculate carbon-fixation rates from PAM measurements. CO_2 fluxes were measured in situ using a method recently developed, based on infrared gas analysis (IRGA) within a benthic chamber at a relatively large areal scale and integrating the microscale patchiness of microphytobenthos in undisturbed sediment (Migné et al. 2002). This method has been proved to be relevant to estimate the annual production rate of diverse intertidal benthic systems (Migné et al. 2004, Spilmont 2004, Spilmont et al. 2005, 2006). Three study sites, following a sedimentary gradient along the coast of the eastern English Channel, were investigated at different seasons to take into account several sources of spatial and temporal variability and to test whether the possible relationships were location- and season-specific.

MATERIALS AND METHODS

Fluorescence was measured in situ with a Diving PAM (Heinz Walz, Effeltrich, Germany) under natural ambient light. In the beginning of the experiments, three home-made supports were placed randomly in a plane surface of the sediment. Each of the supports contained an axis where it was possible to insert and block the tip of the fiber optic of the fluorometer at a constant distance (2 mm) of the biofilm and with a 60° angle to avoid possible shading. This system allowed the measurement of the effective quantum yield of the PSII (Φ_{PSII}) periodically, at the same three spots on the sediment. Φ_{PSII} was calculated according to Genty et al. (1989):

$$\Phi_{PSII} = (F'_m - F_t)/F'_m \quad (1)$$

where F_t is the fluorescence steady-state level under ambient light, and F'_m is the maximal level of fluorescence measured during a saturating pulse (0.8 s). The Φ_{PSII} can be used to calculate the relative electron transport rate (rETR) as follows:

$$\text{rETR} = \Phi_{PSII}\ \text{PAR}\ 0.5 \quad (2)$$

where PAR (in μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) is the photosynthetically active radiation (400–700 nm) measured at the sediment surface using a quantum sensor (SA-190; Li-Cor, Lincoln, NE, USA) connected to a data-logger (Li-1400; Li-Cor; 1 measurement every 15 s, averaged over 1 min). The term ''relative'' ETR is used here because the chl *a*–specific absorption coefficient, required to calculate the ''true'' ETR, could not be obtained in the sediment biofilms.

Community primary production was measured in situ by monitoring the change in CO_2 concentration in a benthic chamber (as described in Migné et al. 2002). A Perspex dome was fitted on a stainless-steel ring pushed into the sediment to about 10 cm and connected to a closed circuit of CO_2 analysis (infrared gas analyzer Li-6251; Li-Cor). The surface covered (0.126 m^2) allowed the measurement of the primary production at a relevant spatial scale to integrate the patchiness of microphytobenthos, and the slope of the partial pressure of CO_2 (μmol $\cdot$ mol^{-1}) against time (min) was used to express fluxes at the community level (mmol $\cdot$ m^{-2} $\cdot$ h^{-1}). A series of measurements were carried out at ambient light to estimate the net community primary production (NCP), and one measurement was carried out in darkness (using an opaque dome) to estimate the community respiration rate (CR). The dark incubation was always performed just after a series of light incubations to avoid any perturbation of the fluxes measured under light due to the downward migration potentially induced when darkening the sample. Short-lasting

incubations (about 7 min) were performed, which prevented temperature increase in the benthic chamber. Gross community primary production (GCP) was calculated as GCP = NCP + CR (using a single CR rate for a series of NCP rates).

Changes in GCP and rETR were followed as a function of variation in ambient light during the day exposure (from dawn to saturating light or from saturating light to dusk) to establish composite photosynthesis–irradiance (PI) curves. The rETR was measured on the three spots about every 10 min to have one value of mean rETR for each value of GCP. The relationship was described by the equation of Webb et al. (1974):

$$P = P_m[1 - \exp(-I/I_k)] \quad (3)$$

where P = GCP (in mg C · m^{-2} · h^{-1}) or rETR (mean of the three replicates), P_m = rate of maximal GCP (GCP_m) or maximal rETR ($rETR_m$), I = incident irradiance (in µmol photons · m^{-2} · s^{-1}), and I_k = saturation onset parameter. The Simplex estimation method (O'Neill 1971) was used to determine the light-curve parameters P_m and I_k (curve-fitting procedure of the ''Systat 10'' software [Systat Software Inc., Richmond, CA, USA]).

Total GCP during the measurement period (in mg C · m^{-2}) was calculated as a function of irradiance (using eq. 3 with a step of 1 min) for each date of the experiment. With this aim, a theoretical irradiance (calculated from the sinusoidal curve of daily variation of irradiance) was used as in Migné et al. (2004):

$$I(t) = I_m \sin(\pi t/D) \quad (4)$$

where I_m (in µmol photons · m^{-2} · s^{-1}) is the theoretical maximal irradiance of the day, and D (min) is the day length. The hourly mean GCP during the measurement period (in mg C · m^{-2} · h^{-1}) was then calculated for each experiment.

Three sediment cores (sample unit: 1.9 cm^2, 10 mm depth) were randomly taken inside the chamber at the end of the experiments for the analysis of chl *a* according to the method of Lorenzen (1967).

Three sites were selected on the French coast of the English Channel: the Bay of Somme (50°13′554 N, 1°36′449 E), the Seine Estuary (49°26′841 N, 00°14′622 E), and the Bay of Authie (50°22′255 N, 1°37′525 E). They were located between mean high water of neap tide and mean tide level and were subjected twice a day to flooding (semidiurnal tidal regime, about 3 h of immersion per tidal cycle). They followed a sedimentary gradient: about 2% silt in the Bay of Somme (Migné et al. 2004), 50% in the Seine Estuary (Spilmont et al. 2006), and 80% in the Bay of Authie (J.-P. Debenay, unpublished data). Experiments were performed at different periods from August 2003 to August 2004 (Table 1).

RESULTS

Nine series of simultaneous measurements of GCP and rETR were performed (Fig. 1). Composite PI response curves were fitted according to equation 3 on data from each experiment, except the one performed in Somme in September when photosynthesis did not exhibit saturation at high light with the CO_2-fluxes method. The light-curve parameters obtained with the two methods (Table 2) were then compared. Significant correlations were obtained for P_m and I_k (Fig. 2).

$$GCP_m = 0.343\ rETR_m + 17.307 \quad (n = 8; r = 0.920; P<0.01) \quad (5)$$

$$I_k\ CO_2\ \text{fluxes} = 0.237\ I_k\ \text{Fluo} + 87.132 \quad (n = 8; r = 0.818; P<0.05) \quad (6)$$

The relationship between the fluorescence parameter (mean rETR of the triplicates) and the community-level carbon-fixation rate (GCP in mg C ·m^{-2} · h^{-1}) was analyzed for each experiment (Fig. 3). A linear relationship was obtained in September in the Bay of Somme, in November and March in the Bay of Authie, and in March in the Seine Estuary. In the other experiments, the relationship between rETR and GCP reached a plateau at high rates.

When all experiments were pooled, a strong linear correlation ($n = 106$; $r = 0.928$; $P < 0.001$) was obtained between the mean rETR of the triplicates and GCP. A conversion rate could then be calculated as the slope of the geometric mean regression forced through the origin (Fig. 4):

$$GCP = 0.744\ rETR \quad (7)$$

Two ways could then be proposed to estimate the total GCP for the emersion period from PAM measurements. The first one consisted of applying to each mean rETR value the conversion rate obtained with the whole data set (eq. 7) and then fitting the PI model to the so-estimated GCP values (eq. 3). The second one consisted of fitting the PI model on mean rETR values and converting only

TABLE 1. High-water time, period of measurements, light range, and chl *a* sediment content for each experiment performed in the three study sites.

Site	Date	High-water time		Period of measurements	Light range (µmol photons · m^{-2} · s^{-1})	Chl *a* (mg · m^{-2}) mean (SD)
		Morning	Afternoon			
Somme	17 September 2003	04:14	16:27	07:53–11:18	47–1033	224 (44)
	16 April 2004	11:05	23:30	15:57–20:45	15–1107	136 (30)
	1 August 2004	00:50		06:59–10:37	82–1154	323 (91)
			13:23	18:21–21:02	61–815	
Authie	26 August 2003		12:13	16:38–20:38	18–1186	271 (21)
	5 November 2003	09:40	22:03	15:20–17:07	35–487	189 (14)
	25 March 2004	02:16	14:33	07:04–10:50	22–599	333 (11)
Seine	27 August 2003	12:01	23:35	16:01–20:45	18–1570	205 (32)
	26 March 2004		13:23	17:34–19:06	43–526	193 (46)

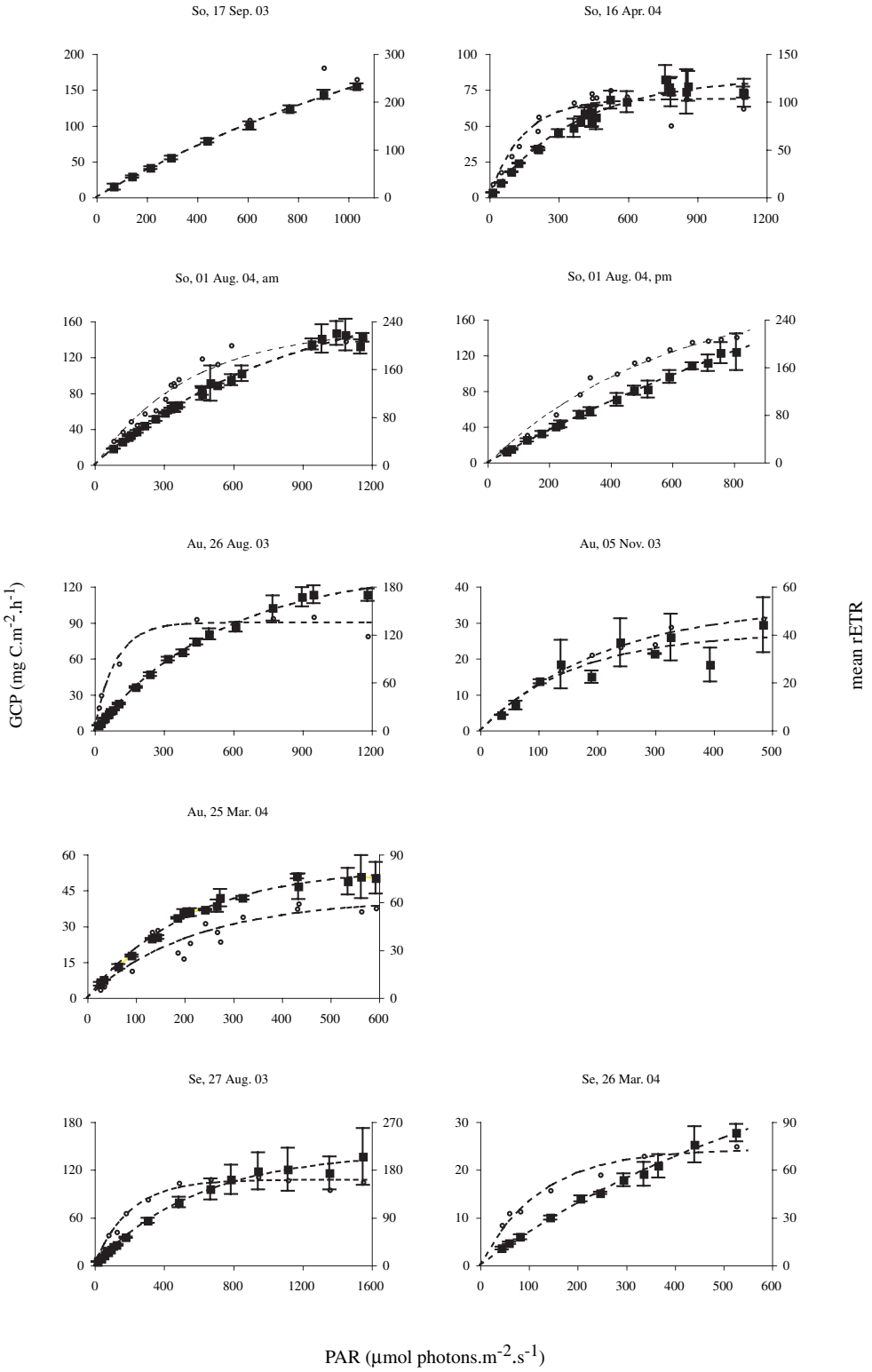

FIG. 1. Gross community primary production (GCP) in mg C · m^{-2} · h^{-1} (open dots, left axis) and mean relative electron transport rate (mean rETR) ± SD (squares, right axis) measured simultaneously under varying incident irradiance (PAR in µmol photons · m^{-2} · s^{-1}) on the three study sites (So, Somme; Au, Authie; Se, Seine). Dotted lines show the composite light curves fitted according to the model of Webb et al. (1974).

TABLE 2. Light-curve parameters obtained with fluorescence ($rETR_m$ and I_k) and CO_2-flux (GCP_m and I_k) measurements according to the model of Webb et al. (1974), number of data, and determination coefficient on the three study sites (So, Somme; Au, Authie; Se, Seine).

	Fluorescence				CO_2 fluxes			
Experiment	$rETR_m$	I_k	n	r^2	CGP_m	I_k	n	r^2
So, 16 April 2004	125.02	378.77	23	0.996	68.36	146.40	15	0.987
So, 1 August 2004 (morning)	298.57	880.22	24	0.998	152.27	405.47	18	0.993
So, 1 August 2004 (afternoon)	552.74	1951.12	16	0.999	193.32	579.71	13	0.997
Au, 5 November 2003	40.57	162.63	10	0.972	34.89	217.56	9	0.998
Au, 25 March 2004	81.63	209.45	18	0.998	41.01	216.84	16	0.973
Au, 26 August 2003	201.45	557.33	17	0.999	89.76	90.99	7	0.993
Se, 27 August 2003	212.71	597.71	15	0.998	107.10	202.32	13	0.996
Se, 26 March 2004	157.45	704.53	11	0.999	24.22	125.14	7	0.995

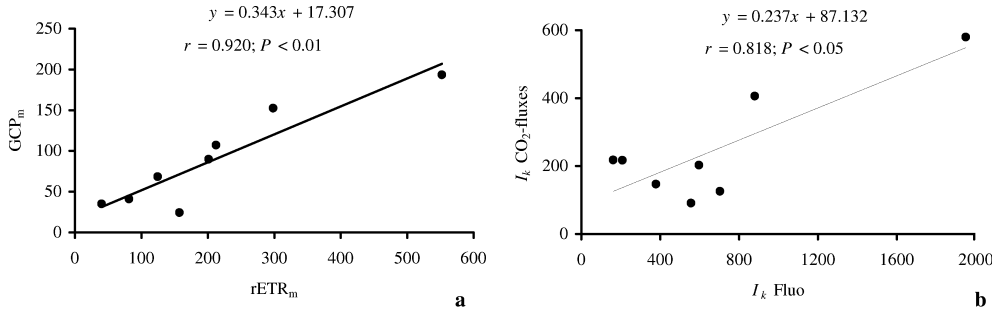

FIG. 2. Correlation and predictive regression between the photosynthesis–irradiance (PI) parameters (a) P_m and (b) I_k estimated from the fluorescence and the CO_2-flux measurements. GCP_m, maximum gross community primary production rate; I_k Fluo, saturation onset irradiance estimated from fluorescence measurements; I_k CO_2 fluxes, saturation onset irradiance estimated from CO_2-flux measurements.

the light-curve parameters according to the relationships previously established (eqs. 5 and 6). These two ways were applied to the eight experiments for which the PI models were fitted, and the results were compared to the estimation obtained directly from GCP measurements. The agreement between the two methods was quite good for both ways of calculation, but better for the second one (Fig. 5). The first way always overestimated the rate of production, with a mean discrepancy of 30%, whereas the second one either overestimated or underestimated it, with a mean discrepancy of −2%.

DISCUSSION

In the present study, we monitored short-term variations in microphytobenthic photosynthesis in situ using both fluorescence and CO_2-flux measurements and established two ways of converting rETR measurements into community carbon-fixation rates, valuable for three sites of varying sedimentary features (from 2% to 80% silt). To our knowledge, this represents the first attempt to study the correlation between fluorescence parameters and community-level carbon-fixation rates using nondestructive techniques on tidal flats directly in the field.

The use of PAM measurements to calculate absolute production rates of microphytobenthos communities in terms of carbon was achieved in two previous studies. First, Hartig et al. (1998) showed a highly significant correlation between ^{14}C- and fluorescence-based production rates measured on a suspension of the motile fraction of a microphytobenthos community. Second, Barranguet and Kromkamp (2000) established a conversion factor allowing estimation of carbon fixation from ETR measurements made on intact sediment cores. Their results also spanned all seasons and varying sedimentary features (from 5% to 43% silt), and they did not observe variation in the conversion factor between ETR and chlorophyll-specific carbon-fixation rates. However, in these two cases, fluorescence-based estimates gave a prediction of only potential primary production as carbon-fixation rates were estimated with techniques that eliminated the physical and chemical microgradients present within the photic zone of intact sediments.

Since the pronounced short-term variability in sediments' environmental factors occurring on tidal flats may control microphytobenthic photosynthesis (especially through the migratory behavior of microalgae), in situ studies are required to estimate relevant rates at the community level. To take into account the vertical migrations of diatoms within a biofilm, Perkins et al. (2001, 2002) estimated both ETR (derived from fluorescence data) and primary production (measured by ^{14}C incorporation) on intact sediment cores, but they failed to establish a

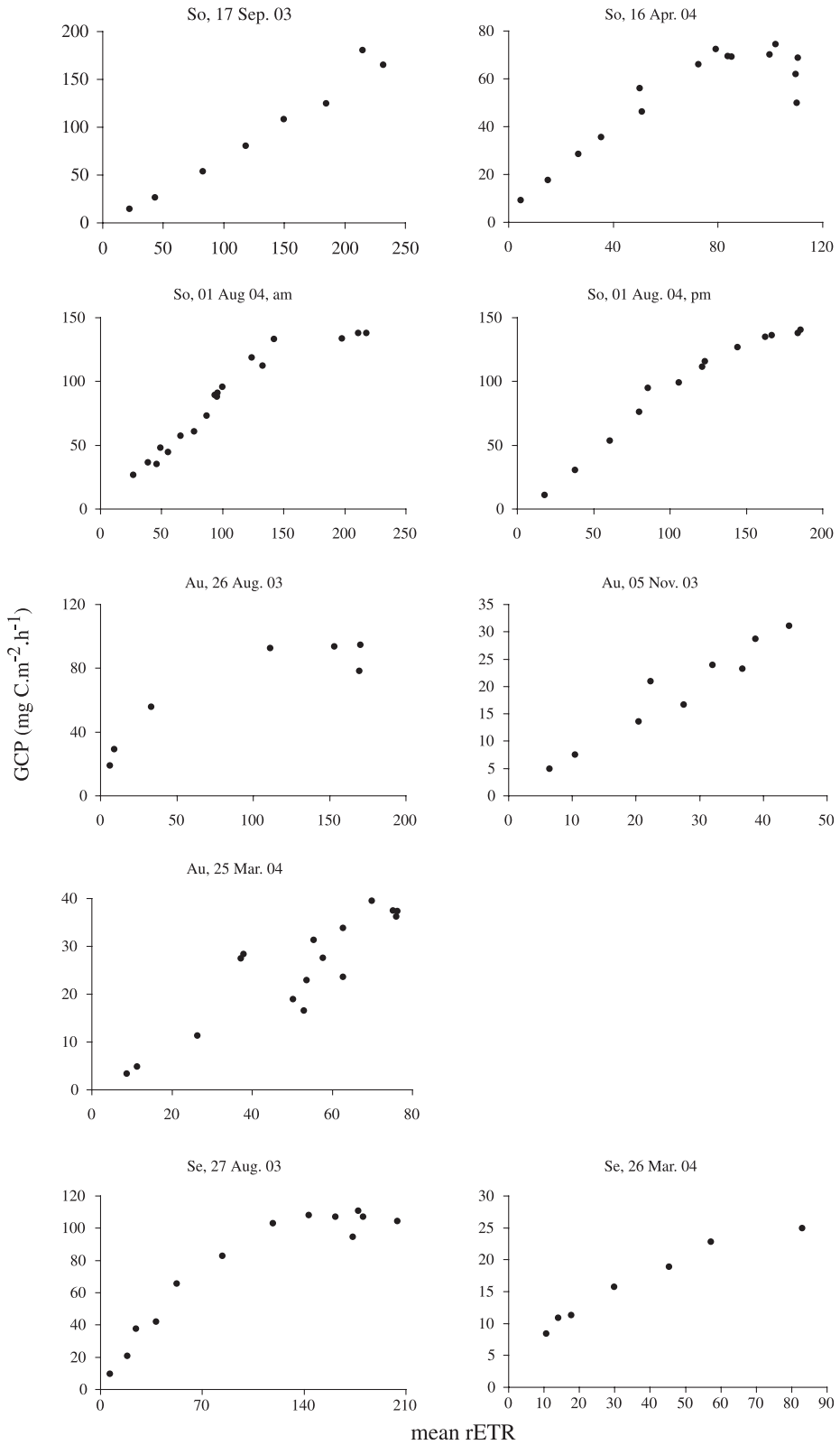

FIG. 3. GCP (in mg $C \cdot m^{-2} \cdot h^{-1}$) plotted against mean rETR at each site (So, Somme; Au, Authie; Se, Seine) and date. GCP, gross community primary production; mean rETR, mean relative electron transport rate.

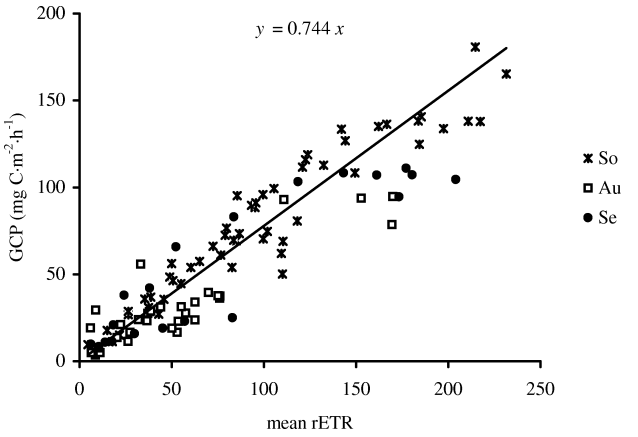

FIG. 4. GCP (in mg $C \cdot m^{-2} \cdot h^{-1}$) plotted against mean rETR for all sites and dates (So, Somme; Au, Authie; Se, Seine) and conversion rate as the slope of the geometric mean regression forced through the origin ($n = 106$; $r = 0.928$; $P < 0.001$). GCP, gross community primary production; mean rETR, mean relative electron transport rate.

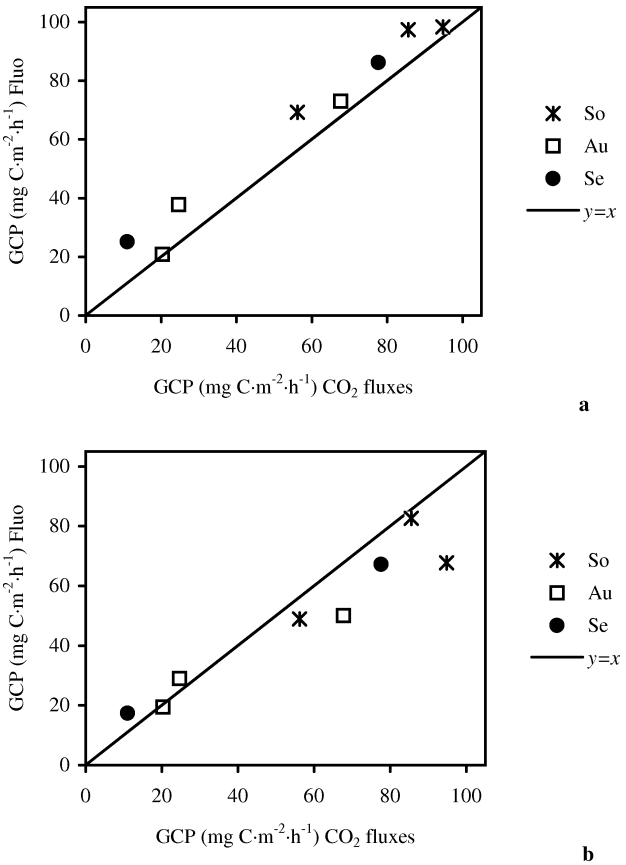

FIG. 5. Production rates for the emersion period (in mg $C \cdot m^{-2} \cdot h^{-1}$): values based on fluorescence measurements as a function of values based on CO_2-flux measurements in the three study sites (So, Somme; Au, Authie; Se, Seine). Production rates based on fluorescence measurements were calculated according to the two proposed ways: (a) using the conversion factor for each value of mean rETR and (b) converting the photosynthesis–irradiance (PI) parameters (see text). GCP Fluo, gross community primary production rate, estimation based on fluorescence measurements; GCP CO_2 fluxes, gross community primary production rate, based on CO_2-flux measurements.

correlation. The reasons for this negative result may include methodological aspects, such as the need for a long dark incubation for the ^{14}C uptake in intact cores, which is likely to affect considerably the migratory behavior of microalgae (Serôdio 2003). In the present study, in situ CO_2-flux measurements within a benthic chamber allowed us to consider primary production at the community level under natural environmental conditions. With this method, placing the sample in the dark was also necessary to assess the gross primary production, but since it was always performed at the end of the series of incubations, the impact on the vertical structure of the biofilm did not affect the underlight measurements. Nevertheless, the assumptions behind the calculation of a series of gross primary production rates with a single measurement of respiration rate by placing the sediment in darkness were (i) an equivalent CR rate under light and dark conditions, and (ii) a constant CR rate throughout the period of measurement. These assumptions were certainly not true, as respiration rates are higher under light conditions (photorespiration and heterotrophy) and because respiration rates are mainly controlled by temperature, which is likely to exhibit great variability during an emersion period.

Various types of rETR–GCP relationships. In the detailed analysis of each experiment of the present survey, rETR–GCP relationships were either linear or with a plateau. Linear relationships obtained between one biomass-dependent variable (GCP) and one basically biomass-independent variable (rETR) implied that surface biomass was fairly constant or covaried with irradiance during measurements. Deviations from linearity at high irradiances, observed in the second type of relationship, could be explained both by physiological and behavioral responses of the microphytobenthos. Possible physiological explanations might be the existence of alternative electron sinks (e.g., the Mehler reaction or photorespiration) or limitation by Calvin cycle reactions (Flameling and Kromkamp 1998). In addition, some cells exhibited behavioral response to high irradiances, migrating downward to a depth where light was strongly attenuated, and then attained a higher Φ_{PSII} (Underwood et al. 1999, Perkins et al. 2001, 2002). However, PAR measured at the surface of the sediment was used for the calculation of the rETR (see eq. 2), which resulted in its overestimation (Consalvey et al. 2005). The overestimation of rETR can be expected to increase toward the end of the low-tide period, following sediment desiccation and increasing particle compaction leading to higher light attenuation (Brotas et al. 2003). Furthermore, the models of Forster and Kromkamp (2004) or Serôdio (2004) showed that the Φ_{PSII} as measured at the sediment surface

(apparent Φ_{PSII}) was higher than the inherent photosynthetic efficiency of algal cells in the assemblage. This finding, obtained from numerical simulations and experimentally validated (Serôdio 2004), was due to the influence of fluorescence emitted from deeper sediment layers and was most pronounced at high irradiances. The overestimation of Φ_{PSII} is exacerbated by nonphotochemical quenching, which affects especially the cells at the surface layer and leads to an increase in the signal contribution from deeper in the sediment (Forster and Kromkamp 2004). In the present study, deviations from the linearity at high light might be caused mainly by the difference in the depth intervals monitored by fluorescence and CO_2-flux measurements. Under low irradiances, photosynthesis was restricted to the layers of the sediment near the surface; the photic zone then corresponded to the depth monitored by both the fluorescence and CO_2-flux measurements. In contrast, under high irradiances, CO_2-flux measurements did integrate photosynthetic rates taking place at depths not reachable by the fluorescence method. Indeed, the photic zone under high irradiances (>0.2 mm depth for silt-clay sediment, Mac Intyre et al. 1996) was larger than the depth detected by fluorescence, which was limited to the layers near the surface of the sediment: 100–200 μm according to Barranguet and Kromkamp (2000), <150 μm according to Kromkamp et al. (1998), and <270 μm according to Serôdio et al. (1997). Furthermore, microphytobenthos could exhibit micromigration patterns in the uppermost layer of the sediment (Kromkamp et al. 1998), and it has been suggested that a rapid turnover of cells at the sediment surface during high-light periods could optimize the productivity of the biofilm (Consalvey et al. 2004b). In the same way, the higher values of I_k obtained in summer with the fluorescence measurements compared to those obtained with the CO_2-flux measurements (Table 2) could be explained by the differences in the depth monitored by the two methods—fluorescence measurements detecting mainly the response of surface high-light-adapted cells. Because oxygen microelectrodes allow the measurement of vertical profiles of photosynthesis, Serôdio (2003) successfully compared fluorescence measurements with photosynthesis rates integrated over different depth intervals. He showed that the pattern of vertical variation in the correlation between fluorescence and O_2 measurements varied with the incident irradiance. Under high irradiances (>900 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}), the correlation was higher when only superficial layers (0–50 μm) were included for the depth integration of photosynthesis rates, decreasing as more layers were considered.

The depth of light penetration into the sediment (as well as the depth detected by fluorescence) varies both with the granulometry and the organic content of the sediment. Mac Intyre et al. (1996) reported values for the depth of light penetration (i.e., the depth of 1% surface light level) ranging from 0.2 mm for silt-clay to 13.2 mm for sand, with a mean particle size of 330 μm. The overestimation of apparent Φ_{PSII} with respect to inherent Φ_{PSII} also varies as a function of the sediment characteristics and the density of the biofilm (Forster and Kromkamp 2004). The proportion of epipelic diatoms undergoing migration also depends on the sediment type, being larger in silty than in sandy sediments (Underwood 2001). Another factor that could contribute to the variable patterns observed from one experiment to another is that the time lag between tidal exposure (or immersion) and measurements varied (Table 1). This is critical since the surface biomass of microphytobenthos increases rapidly over the first period of light exposure (and decreases close to the period of immersion; Perkins et al. 2001) and thereafter influences community production (Spilmont et al. 2007). The effects of all these features are inseparable, and the variable irradiance threshold for the deviation from the linearity according to the study site could not be explained.

Linear relationships between rETR and GCP were obtained in experiments performed at lower irradiances (<600 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}) in March and November, but also under irradiances as high as 1033 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} in September. This experiment, for which the PI model could not be fitted for the CO_2-fluxes method, was carried out under sharp temperature changes (the temperature at the sediment surface increased 3.7°C $\cdot$ h^{-1}), and the temperature effect could have overshadowed the role of light (Migné et al. 2004).

Calculation of community carbon-fixation rates from fluorescence measurements. Despite the occurrence of deviations from the linearity at high irradiances in some experiments, and despite the differences in sedimentary features and sediment chl *a* content between sites and seasons, the linear correlation between the fluorescence parameter and the community-level carbon-fixation rate, calculated on the whole data set, was very strong. Nevertheless, the depth integration of production rates and the variability in the photic depth and surface biomass likely represent a source of error in the estimation of GCP from fluorescence data. Indeed, the use of this conversion factor led to overestimation of the carbon production. Another way to estimate the carbon-fixation rate from fluorescence measurements would be to use relationships between light-curve parameters. Contrary to the study by Barranguet and Kromkamp (2000), we obtained significant correlations between light-curve parameters (P_m and I_k) determined by both methods, although data from all sediment types and seasons were pooled. The establishment of composite PI curves from in situ measurements, if not relevant for strictly physiological interpretation, presented the advantage of integrating the physiological and migratory respon-

ses of the microphytobenthos to the environmental changes (such as sediment desiccation and increasing sediment compaction) occurring during low tide. That integration allowed the estimation of realistic budgets at the community level. This second way of conversion was tested in the present study and gave better results, as shown by the comparison of the production rate calculated for the emersion period.

To estimate community carbon-fixation rates from PAM measurements, a set of coupled experiments appear to be necessary. For example, to estimate the annual primary production rate of a selected station, we suggest building composite PI curves both from fluorescence and CO_2-flux measurements at each season to establish the relationship between photosynthetic parameters and building intermediate curves from PAM measurements only for an accurate estimation of the annual variations. Better estimation could be obtained if these measurements were coupled with a precise determination of the microphytobenthos biomass in the photic zone because it is a key factor in determining the absolute rate of photosynthesis of the biofilm—this absolute rate of photosynthesis (chl *a*–normalized GCP) being more likely to be compared to rETR, which is basically a biomass-independent parameter. But even if chl profiles within the sediment could be accurately measured using liquid-nitrogen freezing, the calculation of the chl *a*–normalized GCP would imply the more complex determination of the actual photic zone. In the present survey, carbon-fixation rates could be established at the community level from in situ fluorescence measurement with a reasonable accuracy without the need for a complex assessment of the microphytobenthos biomass in the photic zone or the actual light experienced by the algae in the sediment. Nevertheless, measurements were restricted to relatively short periods with probable covariation in surface biomass and light, and fluorescence-based estimation of production remains critical when surface biomass varies under constant irradiance.

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