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A closed-chamber CO_2-flux method for estimating intertidal primary production and respiration under emersed conditions

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Abstract This paper describes a closed-chamber method for measuring CO_2 fluxes in intertidal soft sediments during periods of emersion. The method relies on closed-circuit incubations of undisturbed sediment and measurement of CO_2 exchanges using an infrared gas analyser. The method was assessed during field experiments, both in light and dark conditions, on an exposed sandy beach and in an estuary. The rates of gross community production measured under moderate irradiance (4.2 mg C m^{-2} h^{-1} on the exposed sandy beach and 35 mg C m^{-2} h^{-1} in the estuary) are in good agreement with rates reported in the literature. In conjunction with appropriate sampling strategies, this method can be useful for estimating and comparing production of intertidal areas or for assessing factors that influence production.

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Introduction

Planktonic microalgae are the major contributors to global marine primary production, but the production of the microphytobenthos can become important in shallow marine and intertidal areas (Charpy-Roubaud and Sournia 1990). It may provide as much as one third (Sullivan and Moncreiff 1988) or two thirds (Asmus 1982) of total primary production in some estuarine systems. It may even exceed phytoplankton production by a factor of ten in some intertidal habitats (Cadée and Hegeman 1974; Varela and Penas 1985). Accurate estimates of microphytobenthic production are needed for an understanding of the carbon flow in such environments. Some authors have suggested that microalgal production could be twofold higher at low tide than at high tide (Asmus 1982; Varela and Penas 1985; Pinckney and Zingmark 1991). Furthermore, in some very turbid areas, no production occurs in immersed conditions (Van Es 1982). For respiration, lower rates during emersion than during immersion might be expected, because the macrofauna has a lower activity (Asmus 1982). Changes in metabolic performance can occur during a period of emersion, owing to changes in the water content of the sediment. Holmes and Mahall (1982) showed that net photosynthesis rates are highest in water-saturated high-intertidal sediments and decline with water loss. Therefore artificial flooding, a method commonly used (e.g. Gouleau et al. 1994), is not appropriate to derive accurate estimates of intertidal primary production. Procedures capable of providing continuous measurement of sediment gas exchange under emersed conditions are then required. Measurement of epibenthic microalgal productivity under low-tide conditions (exposed to air) was previously performed using an in situ flowing-air system with CO_2-absorption columns (Pomeroy 1959) or using a $^{14}CO_2$ technique on intact sediment cores (Darley et al. 1976) and in situ (Whitney and Darley 1983).

This paper describes a closed-chamber CO_2-flux method that uses an infrared gas analyser for estimating in situ primary production and respiration of intertidal soft sediment during emersion. This method encloses sediment, phyto- and zoobenthos in order to measure fluxes for the whole community. The procedure was assessed by experiments performed in two different systems: an estuary and an exposed sandy beach in the English Channel.

Materials and methods

The chamber was constructed using a dome (16.75 l) of transparent (or opaque) Perspex fitted on a crown wheel of stainless steel. Airtightness was ensured by a silicone seal placed in a PVC groove. The crown wheel was 40 cm in diameter, 16 cm high and 1 mm thick. It was carefully pushed into the substrate so as to ensure a minimal sediment disturbance. The volume of trapped air depended on the depth of the crown wheel's insertion into the substrate and was 24.92 l when the crown wheel was pushed for 10 cm. A pump (Brailsford and Co, TD–2SA) maintained an air flow of about 2 l min^{-1} into a closed circuit that consisted of the chamber, a drying column (indicating anhydrous $CaSO_4$), a flowmeter (McMillan Co, S110) and an infrared gas analyser (LiCor Li–6251). The gas analyser, flowmeter and pump were powered by a 12-V battery. Photosynthetically active radiation (PAR, 400–700 nm) was measured inside the chamber with a quantum sensor (LiCor Li–192SA). Temperature and relative humidity in air were also measured inside the chamber using a pen-type thermo-hygrometer (LiCor Li–1400–104). Sensors were mounted on the crown wheel. The whole system (except the chamber) was placed in a portable container (Fig. 1). The chamber was connected to the system by rapid airtight connections. Environmental data (PAR, temperature and relative humidity in the enclosure) as well as the analyser data (internal temperature and CO_2 concentration) were stored on a datalogger (LiCor Li–1400). The logging frequency was 5 min for environmental data and 30 s for analyser data.

Fig. 1 Container with circuit of CO_2 analysis, data logger and enclosure with irradiance and temperature/humidity captors in the field

The infrared gas analyser was calibrated in the field just prior to each experiment. Calibration was performed using CO_2-free air (CO_2 being trapped by soda lime), and air of known pCO_2 (353.4 ppm). Experiments were carried out in ambient light and darkness, to estimate net community primary production (NCP) and community respiration (CR). Light and dark incubations were performed successively.

Carbon dioxide flux (ΔCO_2 mmol mol^{-1} min^{-1}) was calculated by regressing CO_2 concentration (μmol mol^{-1}) on time during light or dark incubations. Fluxes were expressed at the community level (mmol m^{-2} h^{-1}) taking into account the volume of the enclosure (24.92 l) and the sediment surface (0.126 m^2). Gross community primary production (GCP) was estimated as follows: $GCP = NCP - CR$.

Four series of incubations were performed: three on muddy-sand substrate at Le Crotoy in the estuary of the River Somme (50°13′ 554 N, 1°36′ 449 E, in August 2000) and one on the exposed sandy beach of Wimereux (50°45′ 905 N, 1°36′ 397 E, in October 2000). The biomass of macrozoobenthos in these sites, expressed as ash-free dry weight, was, respectively, about 10 g and < 1 g AFDW m^{-2}, and the sediment chlorophyll-*a* content was, respectively, about 200 mg and 2.5 mg m^{-2}. Irradiance (PAR in μmol m^{-2} s^{-1}) and temperature (°C) prevailing in the enclosure during these incubations are given in Table 1.

Results

During each incubation, CO_2 concentration changed linearly after a period of stabilization (1–9.5 min); this period was longer in incubations in the dark than in the light (Fig. 2). The slope of CO_2 concentration versus time was calculated from the linear portion of each recording; the coefficients of determination were between 0.937 and 0.998 (Table 1).

A negative net flux of CO_2 (i.e. net consumption of CO_2) was measured in the two study sites under moderate irradiance (Table 1), but the magnitudes of the respiration and of the gross primary production were different, being, respectively, 22- and 8-fold greater in Le Crotoy than in Wimereux.

Different responses were observed in the three incubations performed under different levels of irradiance in Le Crotoy (Table 1). The slope of CO_2 concentration versus time was about 50% higher under high light (1242 μmol m^{-2} s^{-1}) than under moderate light (832 μmol m^{-2} s^{-1}). The community respiration after the incubation under high light was also higher than that measured after the incubation carried out under moderate light. CR in the second dark incubation (performed at a temperature of about 25 °C) was twofold higher than that of the first incubation (performed at a temperature of about 22 °C). Then, the estimation of gross primary production remained greater for the incubation under high light (about twofold greater than that under moderate light). A positive net flux of CO_2 (i.e. net production of CO_2) was observed under low light (37 μmol m^{-2} s^{-1}), similar to the rate of community respiration (Fig. 2).

Table 1 Light conditions (*PAR*: photosynthetically active radiation in μmol m^{-2} s^{-1}) and temperature (*T* in °C), both expressed as mean ± SD, with sample size in parenthesis; slopes of CO_2 concentration (CO_2 flux in μmol mol^{-1} min^{-1}), sample size (*n*) and coefficient of determination (R^2); CO_2 flux (ΔCO_2 in mmol m^{-2} h^{-1}) and estimations of gross primary production (mmol m^{-2} h^{-1}) from light and dark incubations performed at Le Crotoy and Wimereux

	PAR (μmol m^{-2} s^{-1})	*T* (°C)	CO_2 flux (μmol mol^{-1} min^{-1})	*n*	R^2	ΔCO_2 (mmol m^{-2} h^{-1})	GCP (mmol m^{-2} h^{-1})
Incubations/light							
Le Crotoy							
25 Aug (10.05 a.m.–10.25 a.m.)	832 ± 41(20)	22.1 ± 1.0(5)	–3.53	39	0.996	–1.87	2.95
25 Aug (11.15 a.m.–11.45 a.m.)	1242 ± 43(29)	26.9 ± 1.9(7)	–6.8	55	0.998	–3.61	5.77
28 Aug (8.00 p.m.–8.25 p.m.)	37 ± 14(31)	17.4 ± 0.1(5)	4.27	45	0.937	2.27	0.00
Wimereux							
26 Oct (4.00 p.m.–4.40 p.m.)	545 ± 127(39)	19.5 ± 0.4(8)	–0.57	66	0.954	–0.3	0.35
Incubations/dark							
Le Crotoy							
25 Aug (10.55 a.m.–11.10 a.m.)	0	22.0 ± 0.1(3)	2.02	29	0.992	1.07	0
25 Aug (11.50 a.m.–12.20 a.m.)	0	24.9 ± 1.2(7)	4.06	51	0.990	2.16	0
28 Aug (7.25 p.m.–7.55 p.m.)	0	17.8 ± 0.3(7)	3.93	45	0.992	2.09	0
Wimereux							
26 Oct (4.45 p.m.–5.20 p.m.)	0	14.7 ± 1.2(8)	0.09	52	0.989	0.05	0

Discussion

These results demonstrate that the closed-chamber method can be conveniently used to measure the net primary production and respiration of emersed sediments. Mixing in the chamber atmosphere was efficient and no chamber leakage (which would allow exchange with outside gas) was detected. Linear variations in CO_2 concentration measured under light and dark conditions during the incubations are consistent with the present knowledge of gas fluxes in intertidal sediment. In the productive system (muddy-sand substrate, Le Crotoy) no photosynthetic-rate decrease was observed with declining CO_2 concentration in the enclosure, despite a relatively long time of incubation (20–30 min) and a final pCO_2 as low as 200 ppm. In the closed chamber, temperature increased rapidly in the sun. Nevertheless, no photosynthetic-rate variation was observed with this variation in temperature, even for the greatest temperature increase (5.35 °C for 30 min) measured in August just before midday. To obtain the linear trend, a longer stabilization period was required in the incubations under dark conditions than under light conditions. Air mixing in the chamber was not considered a possible cause of the phenomenon. Some other cause, such as physiological processes in organisms living in the sediment, must be considered: fixation of carbon dioxide can be observed during a short period of darkness just after a period of light. The duration of incubation must then be long enough to reach the physiological steady state of the organisms and thus allow the linear portion of the curve to be established. But it must also be short enough to reduce changes in ambient conditions in the enclosure (CO_2 concentration, temperature, irradiance, relative humidity) that could influence rates of metabolism. To avoid such changes, some authors (Nay et al. 1994; MacIntyre et al. 1996; Streever et al. 1998) pointed out

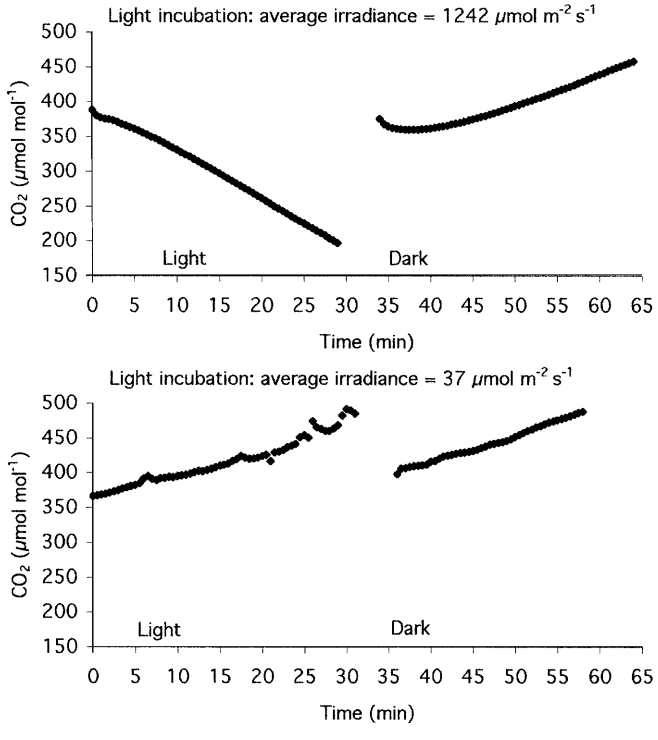

Fig. 2 Examples of variation of CO_2 concentration in light and dark incubations at the station of Le Crotoy (recording frequency is 30 s)

the need for very short incubations (a few minutes) in measuring CO_2 flux by enclosure methods. Whitney and Darley (1983) approached this problem by circulating a large volume of air while monitoring CO_2 fluxes. Their incubations, carried out in an enclosure with an adjustable volume of 7 to 14 l, were of 20 min duration. In future measurements performed with the apparatus presented here (volume about 25 l), incubation time could be reduced under transparent enclosure for highly productive systems, but should be limited to about 20 min in low productive systems and in incubations under dark conditions. Reducing the incubation time to 10 min, the temperature increase in the chamber might be reduced to 1.84 °C for the incubation performed in August just before midday.

Net community production differed in the three incubations in Le Crotoy, showing changes in benthic metabolism with irradiance. Many investigators have found that subsaturating light level is the major factor accounting for variability in microphytobenthic primary productivity within a given intertidal habitat, temperature being expected to influence primary productivity only in sediments exposed to high irradiance (MacIntyre et al. 1996). In contrast, Blanchard and Guarini (1996) demonstrated the determining influence of mud temperature on microphytobenthic production in intertidal mudflats. They suggested that the hourly variation of incident irradiance does not affect the photosynthetic capacity of microphytobenthos directly, but indirectly through heating of the mud.

However, the correlation of productivity with irradiance depends both on the range of light intensities observed and the irradiance at which photosynthesis is saturated. When photosynthesis–irradiance curves have been constructed for intact sediment, the integral primary production of the photic zone has been found to saturate at irradiances between 100 and 1260 μmol m^{-2} s^{-1} (MacIntyre et al. 1996), although some curves do not appear to show saturation (Whitney and Darley 1983).

The lowest irradiance tested in Le Crotoy (37 μmol m^{-2} s^{-1}) was below the compensation irradiance. Above this compensation irradiance, NCP could be expected to increase with increasing irradiance until the saturation intensity was reached. Nevertheless, differences in gross primary production observed between the two others incubations (performed at irradiances of 832 and 1242 μmol m^{-2} s^{-1}) may also have been caused by daily changes in the temperature which increased in the incubation performed at higher irradiance (from 22.1 °C at moderate irradiance to 26.9 °C at high irradiance).

The rate of community respiration exhibited a large difference in the two successive measurements performed in Le Crotoy. This variation may also be explained by a shift in temperature or be related to the variation of gross primary production. Using a stepwise multiple-regression analysis, Van Es (1982) showed that temperature was of prime importance as a factor in community respiration. In the tidal flats that he investigated, temperature alone explained 50% of the observed variation in community respiration. In contrast, other variables, including the rate of primary production, were much less important.

The new system described in the present paper was appropriate for measuring fluxes of CO_2 in the two selected environments, an exposed sandy beach and an estuary. The method clearly separated different systems based on CO_2 flux, even if further measurements are necessary to assess the effects of environmental factors. The rates of gross community production measured under moderate irradiance (4.2 mg C m^{-2} h^{-1} at Wimereux and 35 mg C m^{-2} h^{-1} in the Somme estuary) are in good agreement with rates measured by different methods and reported in the literature for shallow-water marine habitats: an annual range of 1 to 21 mg C m^{-2} h^{-1} in sandy sediments of the Gulf of Fos (in situ O_2 incubations; Plante-Cuny and Bodoy 1987) and 10 to 115 mg C m^{-2} h^{-1} in the Ems-Dollar estuary (laboratory ^{14}C incubations; Colijn and De Jonge 1984). One needs, however, to be aware that meaningful comparisons are still and will remain difficult, owing to the sharp differences between the techniques used to measure productivity and the huge variability of environmental parameters at tidal or seasonal scales.

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References

Asmus R (1982) Field measurements on seasonal variation of the activity of primary producers on a sandy tidal flat in the northern Wadden Sea. Neth J Sea Res 16:389–402

Blanchard G, Guarini JM (1996) Studying the role of mud temperature on the hourly variation of the photosynthetic capacity of microphytobenthos in intertidal areas. C R Acad Sci (Paris) 319:1153–1158

Cadée GC, Hegeman J (1974) Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. Neth J Sea Res 8:260–291

Charpy-Roubaud C, Sournia A (1990) The comparative estimation of phytoplanktonic, microphytobenthic and macrophytobenthic primary production in the oceans. Mar Microbial Food Webs 4:31–57

Colijn F, De Jonge VN (1984) Primary production of microphytobenthos in the Ems-Dollar estuary. Mar Ecol Prog Ser 14:185–196

Darley WM, Dunn EL, Holmes KS, Larew HG (1976) A ^{14}C method for measuring epibenthic microalgal productivity in air. J Exp Mar Biol Ecol 25:207–217

Gouleau D, Blanchard G, Cariou-LeGall V (1994) Production potentielle et consommation d'oxygène sur une vasière intertidale au cours d'une émersion. Vie Milieu 44:109–115

Holmes RW, Mahall BE (1982) Preliminary observations on the effects of flooding and dessication upon the net photosynthetic rates of high intertidal estuarine sediments. Limnol Oceanogr 27:954–958

MacIntyre HL, Geider RJ, Miller DC (1996) Microphytobenthos: the ecological role of the "secret garden" of unvegetated,

shallow-water marine habitats. I. Distribution, abundance and primary production. Estuaries 19:186–201

Nay SM, Mattson KG, Bormann BT (1994) Biases of chamber methods for measuring soil CO_2 efflux demonstrated with a laboratory apparatus. Ecology 75:2460–2463

Pinckney J, Zingmark RG (1991) Effects of tidal stage and sun angles on intertidal benthic microalgal productivity. Mar Ecol Prog Ser 76:81–89

Plante-Cuny MR, Bodoy A (1987) Biomasse et production primaire du phytoplancton et du microphytobenthos de deux biotopes sableux (Golfe de Fos, France). Oceanol Acta 10:223–237

Pomeroy LR (1959) Algal productivity in salt marshes of Georgia. Limnol Oceanogr 4:386–397

Streever WJ, Genders AJ, Cole MA (1998) A closed-chamber CO_2 flux method for estimating marsh productivity. Aquat Bot 62:33–44

Sullivan M, Moncreiff C (1988) Primary production of edaphic algal communities in a Mississippi salt marsh. J Phycol 24: 49–58

Van Es FB (1982) Community metabolism of intertidal flats in the Ems-Dollard Estuary. Mar Biol 66:95–108

Varela M, Penas E (1985) Primary production of benthic microalgae in an intertidal sand flat of the Ria Arosa, NW Spain. Mar Ecol Prog Ser 25:111–119

Whitney DE, Darley WM (1983) Effect of light intensity upon salt marsh benthic microalgal photosynthesis. Mar Biol 75:249–252