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Abstract

Methods used to estimate community primary production and respiration in intertidal environment are still subject of controversy. Underwater community respiration (CR), net production (NCP) were calculated from simultaneous *in situ* measures of change in oxygen (O_2), dissolved inorganic carbon (DIC) and carbon dioxide (CO_2) concentration in benthic chambers performed in February, April, July and November on a *Zostera noltii* bed. The CRQ (CR_{DIC}/CR_{O2}) and CPQ (NCP_{O2}/NCP_{DIC}) varied between 0.15 and 3.07 and between 0.03 and 6.83, respectively. Carbon fluxes calculated from CO_2 measurement were greatly underestimated, representing only 0.4 to 5.9 % of fluxes estimated from DIC measurement. Indeed, CO_2 or HCO_3^- input or uptake by seagrass community affect the proportions of all the chemical components of dissolved inorganic carbon (DIC, the sum of free dissolved CO_2, carbonic acid H_2CO_3, bicarbonate and carbonate ions, HCO_3^- and CO_3^{2-}). Thus, CO_2 method is not reliable. O_2 measurement does not take into account anaerobic respiration through chemical oxidation and simultaneous O_2 and DIC measurements should be favoured to calculate CRQ and CPQ which need to be discussed in marine environment at community scale.

1. INTRODUCTION

Most of the *in situ* production studies of macrophytes have focused on the seasonal variations of macroalgae (e.g. Mann, 1972) or seagrass biomass (e.g. Auby and Labourg, 1996; Jacobs, 1979; Pérez-Lloréns and Niell, 1993). However, this approach targets a single species and measures the balance between biomass production by photosynthesis and losses by respiration and due to grazing and exportation integrated over the growing season. This does not allow for measurement of primary production at the community level and leads to a quite rough annual estimation of primary production. Closed benthic chambers were developed in order to measure community metabolism in sedimentary environments with only slight disruption of the system. This approach allows for repeatable *in situ* measurements over the year, integrating biological and physical interactions between species over short time intervals.

During the emersion period in the intertidal zone, community metabolism can be estimated by measuring CO_2 concentration changes in the air headspace of a closed benthic chamber (Migné et al., 2002). CO_2 concentrations in air (expressed in ppm) are measured with a CO_2 infrared gas analyzer. Depending on the community response intensity, the incubation duration can be very short (< 15 min), which allows the estimation of primary production and respiration. This method has been used in unvegetated sediments (e.g. Davoult et al., 2009), in seagrass beds (Ouisse et al., 2010) and also on rocky shores (Golléty et al., 2008). However, in intertidal habitats this method is limited to measurements during the emersion phase, when the sediment are directly exposed to the air.

For sediments submerged under water, the methods used to assess community metabolism in benthic chambers are all based on measuring the variations of the water column concentrations of either dissolved inorganic carbon (DIC, encompassing dissolved CO_2,

HCO_3^- and CO_3^{2-}) or dissolved oxygen. The O_2 method (Winkler titration or probe) is currently used in many marine ecosystems (e.g. Barrón et al., 2004; Buesa, 1977; Plus et al., 2001). It only measures aerobic respiration, which comprises the aerobic degradation of organic compounds and the re-oxidation of reduced products (e.g., sulfide, reduced metals, ammonium) produced by anaerobic metabolism. Changes in DIC, as calculated from pH and alkalinity variations, measure the balance of CO_2 production and consumption, and therefore, integrate both aerobic and anaerobic processes. However, while the pH can be monitored with a probe *in situ* similarly as O_2, the alkalinity measurements require the sampling of water for a titration procedure that can be considered unwieldy.

Recently, Silva et al. (2008) suggested a method to monitor the CO_2 evolution inside the closed incubation chamber during both immersion and emersion as an approach to improve our understanding of carbon cycling in intertidal benthic communities. The proposed method has been criticized and prompted discussion in the literature with respect to the appropriateness for this application (Abril, 2009; Silva and Santos, 2009). Briefly, the method is performed as follows: during immersion, water from the chamber is recirculated by a peristaltic pump at the surface and flows through an equilibrator, which allows the concentration of CO_2 to be continuously monitored in the gas phase by an infrared analyzer.

Using this method, Silva et al. (2008) measured a community metabolism greatly lower during immersion than during emersion in the *Zostera noltii* bed of the Ria Formosa Lagoon. These results were completely the opposite of the results described in two recently published studies for other *Zostera noltii* beds, which were based on DIC measurements during the immersion period and measurements of CO_2 in air during the emersion periods. Thus, Clavier et al. (2011) and Ouisse et al. (2011), who studied the *Z. noltii* beds in the Banc d'Arguin and

in Brittany, respectively, both measured up to 7 fold higher community metabolism during immersion than during emersion. Practically, CO_2 represents only a small part of the DIC pool that is in dynamic equilibrium with the other forms of inorganic carbon in seawater. The uptake or release of one of the DIC components during photosynthesis and respiration, respectively, will shift the equilibrium and thus affect the proportions of all the others (see Fig. 1 in Abril, 2009). Thus, investigators must either take care to measure sufficient chemical variables to fully characterize the carbon dioxide system, or directly measure total DIC (Dickson et al., 2007). Collectively, the discussion in the literature (Abril, 2009; Silva and Santos, 2009) and the contrasting results obtained according the different methods (Silva et al., 2008; Clavier et al., 2011; Ouisse et al., (2011) clearly demonstrate that this is an important subject of controversy which can lead to opposite conclusions on the role of benthic communities in the carbon cycle in coastal areas. Nevertheless, to the best of our knowledge there is no experimental study where these two methods (DIC and CO_2) have been applied simultaneously and compared. In this context, the main aim of this study was to evaluate simultaneously these two methodologies during *in situ* measurements of intertidal community production and respiration during immersion in seagrass beds. In addition, considering that temperature is one of the main parameters controlling community metabolism (Touchette & Burkholder 2000 for review) and also influences the partitioning of CO_2 in the DIC pool, we performed our analyses during different seasons.

2. MATERIAL AND METHODS

2.1. Study site

The study was carried out on an intertidal *Zostera noltii* bed located near Roscoff (48°N41.735, 3°W57.653, Western English Channel, France). The seagrass bed (covering

around 700 m^2) is located below the mean low water neap tide level (3.30 m above chart datum) and is exposed to high daily water level variations, which influence light availability.

2.2. Benthic chamber

Three benthic chambers were installed by SCUBA-diving. Each was made of a crown wheel of stainless steel and an acrylic hemisphere, covering a surface area of 0.071 m^2 and enclosing a volume of 10.5 L of water. The crown wheels were pushed down to 10 cm sediment depth and sealed with clear (for net community production measurements, NCP) or dark (for community respiration measurements, CR) hemispheres using wing nuts. Watertightness was ensured by a silicone seal placed in a PVC groove at the top of the crown wheel. One of the three chambers was linked to the boat (for the continuous measurement of O_2, pH and CO_2), flowing being ensured by a peristaltic pump (flow-through chamber). The seawater inside the two other chambers was mixed by autonomous stirrers. A sample of seawater was withdrawn from each of the three chambers by 100 ml syringes at the beginning and at the end of incubations (2-point method). Dissolved oxygen concentration and pH were immediately measured on a subsample using a luminescent/optical dissolved oxygen probe with built-in temperature sensor (LDO101, accuracy ± 0.2 mg l^{-1}) and a combination pH probe with a gel-filled double junction reference and built-in temperature sensor (PHC101, accuracy ± 0.002, NBS scale, previously calibrated). The rest of the sample was passed through a cellulose acetate membrane filter (0.8 µm) and stored pending potentiometric laboratory determination of total alkalinity (Millero et al., 1993) on 3 subsamples of 20 ml the following day. Dissolved oxygen concentration, pH and CO_2 concentration were also continuously monitored inside the flow-through chamber. O_2 concentration and pH were measured using LDO101 and PHC101 probes and data were recorded with a 1 min frequency using a multi data logger (HQ40D, Hach Lange Ltd, Loveland USA). CO_2 concentration was measured

using coupled closed water and air circuits, following Silva et al (2008). The gas exchange column (MiniModule® Membrane Contactor, Celgard, USA) allows the water pumped from the chamber to equilibrate the gas (CO_2) partial pressure between air and water. Changes in air CO_2 concentration (ppm) were measured with a CO_2 infrared gas analyzer (LiCor Li-800) and data were recorded (data logger, LiCor Li-1400) with a 15 s frequency.

2.3. Sampling strategy

Carbon and oxygen fluxes were measured over an immersion period during spring tides in April, July and November 2009 and February 2010. On each occasion, short incubations (ca 45-60 min) were performed simultaneously in the 3 benthic chambers: n successive incubations at ambient light (n =1 in February, 3 in April, 4 in July and 2 in November) to measure NCP and one incubation in darkness on the next incoming tide to measure CR. Benthic chambers were opened between successive incubations to restore ambient conditions but crown wheels were maintained at the same places.

Incident photosynthetically available radiation (µmol quanta m^{-2} s^{-1}) was measured near the benthic chambers using an ultra-miniature MDS-MKV/L sensor (Alec Electronics™) and was recorded with a 1 min frequency.

2.4. Community respiration and production calculation

For the three benthic chambers, community respiration (CR, dark incubation) and net production rates (NCP, light incubation) were estimated from the difference between final and initial concentrations in either dissolved inorganic carbon (DIC) or O_2 concentrations, both corrected for temperature change. CR and NCP were expressed in mmol m^{-2} h^{-1}.

The DIC concentrations were calculated from the pH, total alkalinity (TA), temperature and salinity using CO_2sys (calculation routine in MS Excel, Pierrot et al., 2006), the dissociation constant from Mehrbach et al. (1973) and refit by Dickson and Millero (1987). Total alkalinity (TA) did not differ between the beginning and the end of each incubation (Wilcoxon paired test, n = 12, p = 0.10 and n = 11, p = 0.45 for dark and last light incubations, respectively).

In the benthic chamber equipped with flow-through gas equilibrator and meters, pH, O_2 and CO_2 changes were also calculated as the slopes of the linear regressions between each measured parameter and incubation time. CO_2 concentrations in air were converted to CO_2 concentrations in water using the solubility coefficients given by Weiss (1974). The theoretical part of dissolved CO_2 in DIC pool was also calculated using CO2sys (Pierrot et al., 2006).

Differences between carbon community fluxes estimated from CO_2 measured and calculated and differences between 2-point and continuous estimation of DIC and oxygen fluxes were tested using Wilcoxon paired tests on independent observations (CR and highest NCP fluxes).

Mean community respiratory ($CRQ = CR_{DIC}/CR_{O2}$) and photosynthetic ($CPQ = NCP_{O2}/NCP_{DIC}$) quotients were calculated as the slope of the regression lines between CR_{DIC} and CR_{O2} and between NCP_{O2} and NCP_{DIC} respectively using only independent observations (CR and highest NCP fluxes estimated from the 2-point method in the three benthic chambers). Since both variables were affected by natural variability and measurement errors and were not independent of the other, the geometric mean regression was applied (Ricker, 1973) to calculate the mean and standard deviation. The regressions have been forced through

the origin since their intercepts were not significantly different from zero (test based on Student's law).

3. RESULTS

The time courses of pH and O_2 concentration showed that these variables varied linearly with time during the incubations (see an example in Fig. 1). On the whole, the continuous and 2-point methods provided similar estimations of fluxes in the flow-through chamber for both DIC and O_2 (Fig. 2). The Wilcoxon paired test performed on independent observations showed no significant differences ($p > 0.05$) either for DIC or oxygen fluxes.

Carbon and oxygen fluxes estimated by the 2-point method were similar in the three chambers (Fig. 3). Carbon and oxygen fluxes highlighted quite similar trend but with different magnitude and the average ratio between oxygen and carbon fluxes did not depart from the ratio 1:1 (Fig. 4). The community respiratory quotient (CR_{DIC}/CR_{O2}) varied from 0.15 to 3.07 and the mean was 0.95 (± 0.22, n = 12). The absolute value of NCP_{DIC} was always higher than the NCP_{O2} and the community photosynthetic quotient (NCP_{O2}/NCP_{DIC}) varied from 0.03 in July to 6.83 in April (exceptional value) and the mean calculated on independent observations (i.e. one incubation per immersion period) was 0.42 ± 0.27 (n = 11).

For each sampling date, the metabolism was greatly lower when estimated from CO_2 concentration measurements, representing only from 0.4 to 5.9 % of the fluxes estimated by DIC measurements (Fig. 5 and Table 1). The carbon flux underestimations from the *in situ* CO_2 direct measurements were not different than expected from calculated $CO_2^{(t)}$ rates (Wilcoxon paired test, n = 4, p = 0.25 and n = 4, p = 0.63 for dark and last light incubations, respectively).

4. DISCUSSION

4.1. Two-point samplings versus continuous measurements

NCP and CR calculated from measurements at the beginning and the end in the flow-through benthic chamber were similar to those calculated from continuous measurements. This is explained by the fact that pH and O_2 concentration varied linearly with time as the incubation time was short enough to prevent large changes in environmental conditions. Indeed, a longer duration of enclosure can potentially lead to CO_2 depletion or severe O_2 oversaturation which might inhibit photosynthesis (Noël et al., 2010) and enhance photorespiration (Heber et al., 1996). Furthermore, prolonged enclosure may induce temperature increase that might enhance respiration (del Giorgio and Williams, 2005). Although continuous pH, O_2 and temperature measurements are recommended, for short incubations, the measure of these variables only at the beginning and at the end of the incubation is often acceptable to obtain a correct measure of the rates of the community metabolism. However, the length of incubation periods that can lead to non-linear behaviour in time courses depends on the community structure isolated in the chamber. Therefore, it is important to check the appropriateness of the length of the incubation period prior to applying two-point measurements.

4.2. Flow-through vs. stirrer motion

Water motion is known to play an important role in controlling the rates of benthic metabolism by modifying the diffuse boundary layer and this has been studied for seagrasses (Larkum et al., 1989; (Fonseca and Kenworthy, 1987). In the present study, two different methods have been used to ensure water motion inside the benthic chambers (pumping circuit or stirrer), but the water flow was not measured. However, as the O_2 and DIC fluxes were

similar in the two types of benthic chambers, we conclude that both type of mixing were equivalent and probably the interior flow velocities were comparable.

4.3. Oxygen vs DIC measurements

The annual mean CRQ is in the range of values reported in the literature for seagrass beds (Barrón et al., 2006; Clavier et al., 2011). CRQ can vary where aerobic and anaerobic heterotrophic and autotrophic organisms co-exist, such as in the sediments of the seagrass beds. Organic matter decomposition with a concurrent CO_2 production can occur without oxygen as the terminal electron acceptor, because this process may proceed through pathways of nitrate, manganese, iron and sulfate respiration (Jorgensen, 1977), while fermentation processes also produce CO_2 without oxygen uptake. The annual mean CPQ is also in the range of literature data for marine algae (Burris, 1981) and for seagrass communities (Barrón et al., 2006). Although *Zostera noltii* may have a C4-like metabolism with no photorespiration (Jiménez et al., 1987), benthic microalgae from the sediment and epiphytes may exhibit some photorespiration process. This process can favour primary producers by removing excess products of the light reaction (i.e., ATP, NADPH), and limiting damage to the photosynthetic apparatus during period of high dissolved O_2 and low CO_2 and under high light intensity (Heber et al., 1996). Photorespiration implies that at least 3 oxygen molecules will be consumed per molecule of carbon dioxide used, decreasing the CPQ at the community level. The imbalance between O_2 and DIC can be also attributed to exchange between leaves and roots (Borum et al., 2006 and references therein) and to radial loss of O_2 from roots into the rhizosphere (Ribaudo et al 2011). This process maintain oxic conditions around roots and may thus provide protection against accumulation of reduced toxic compounds from surrounding sediment (Marbà et al., 2006 and references therein). The importance of this transport varies

seasonally, with a maximum in summer when sediment respiration rates are highest (Ribaudo et al., 2011).

Thus measuring O_2 fluxes and assuming a community quotient equal to 1 can overestimate community carbon fluxes when photorespiration occurs or underestimate it when anaerobic metabolism or radial O_2 loss occurs. Hence, for obtaining a complete picture of community metabolism O_2 and DIC fluxes need to be measured simultaneously and CPQ and CRQ need to be interpreted in view of these above-mentioned processes.

4.4. Calculation of community metabolism using dissolved CO_2 versus DIC measurements

During all samplings, the NCP_{CO2} and CR_{CO2} rates measured from monitoring dissolved CO_2 concentrations were dramatically lower than their corresponding rates calculated from DIC measurements (NCP_{DIC} and $CR_{DIC,}$ respectively). Thus, the rates measured from dissolved CO_2 measurements only represented 0.4 to 5.9 % of the DIC fluxes (Table 1). Hence, now with experimental data we clearly show that directly using *in situ* measures of dissolved CO_2 for calculating carbon fluxes results in severe underestimation and thus confirm the theoretical considerations described by Abril (2009). As a matter of fact, the rates calculated from variations of dissolved CO_2 (NCP_{CO2} and CR_{CO2}) were very similar to the theoretically expected $CO_2^{(t)}$ rates, which correspond to the predicted rates of variation of dissolved CO_2 based on the corresponding NCP_{DIC} or CR_{DIC} values and considering the theoretical proportion of CO_2 in DIC according pH and temperature. This is striking even if we consider that CO_2 concentration was measured at the surface without taking into account the effect of pressure changes with the tide nor the temperature difference between water and the equilibrator (Takahashi et al., 1993), and the CO_2 removal efficiency is approximately 75 % according to the manufacturer equilibrator's data. When the variations of dissolved CO_2

observed in the present study would be used as a direct proxy for community metabolism, one might suppose the community metabolism during immersion to be dramatically lower than during emersion, as was concluded by Silva et al. (2008) for the *Z. noltii* beds in the Ria Formosa Lagoon. However, this is clearly not correct due to the problem of severe underestimation. Actually at the same site in our previous study using measurements of DIC during and CO_2 concentrations in air during emersion we showed that community metabolism was estimated to be 7 fold higher during immersion than emersion in this *Zostera noltii* bed (Ouisse et al., 2011). This was-attributed to a possible nutrient limitation and an important self-shading of the community by seagrass leaves during emersion. Indeed, the leaf superimposition preserved the community against desiccation but decreased the capacity to harvest light.

While IRGA technique (direct CO_2 measure) is sensitive and has been proved to be a powerful method to investigate community metabolism during emersion in many intertidal studies in seagrass bed (Ouisse et al., 2010), in rocky shore (Golléty et al., 2008) or in others sediment systems (e.g. Davoult et al., 2009; Hubas et al., 2006; Migné et al., 2011; Migné et al., 2004), its utilization in coupled water-air circuit greatly underestimates community metabolism during immersion. The carbon fluxes reported under submerged conditions by Silva et al. (2008) using direct CO_2 measurements need to be reassessed by coupling these measures with measures of pH or alkalinity in order to describe the carbon dioxide system and estimate the DIC fluxes during immersion periods.

5. CONCLUSIONS

Incubations in closed benthic chambers take are instrumental for measuring community metabolism as it takes the interactions between species into account under natural conditions. This comparative study highlights that the two-point sampling at the beginning and end of the

incubation is an adequate method to measure the community fluxes during short incubations. It also confirms that the continuous measure of dissolved CO_2 alone (i.e. without pH or alkalinity) is inappropriate to assess carbon community fluxes in marine environments during immersion and need to be combined with alkalinity or pH to achieve carbon fluxes. Even if the O_2 method seems to be handy and easy to use, the assumption of CRQ or CPQ equal to 1 can lead to misinterpretation of carbon fluxes in coastal environment. Oxygen and DIC methods are complementary and need to be used simultaneously to discuss CRQ and CPQ values. The difference over the course of the year between measured and theoretically assumed values of these quotients (ratio of 1:1) and the total alkalinity variations can give some information about anaerobic mineralization of organic matter in the seagrass bed.

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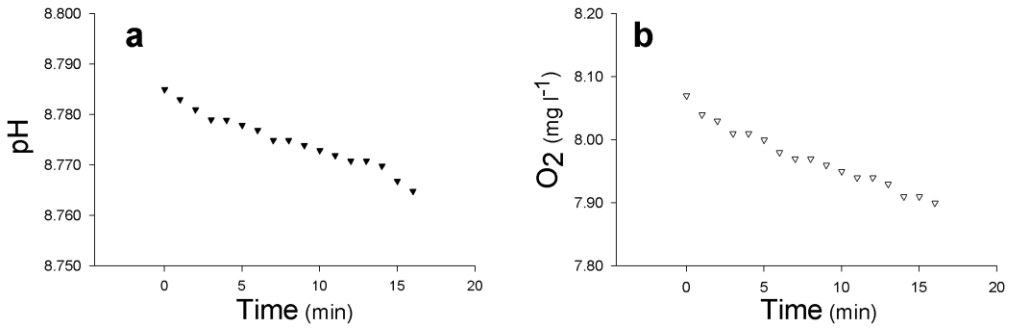

Figure 1: Examples of the time courses of (a) pH and (b) O_2 concentration (in mg l^{-1}) during a dark incubation, both parameters have been corrected for temperature change.

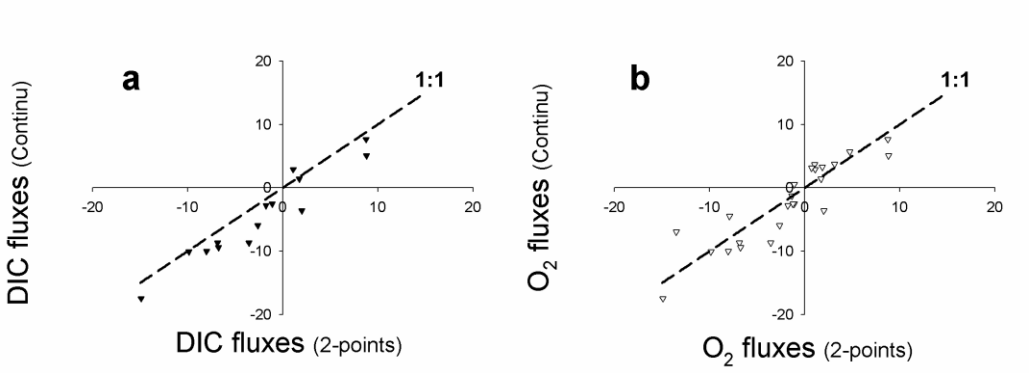

Figure 2: Comparison of (a) DIC (mmol m^{-2} h^{-1}) and (b) O_2 (mmol m^{-2} h^{-1}) fluxes calculated from continuous monitoring and two-point methods in the flow-through chamber

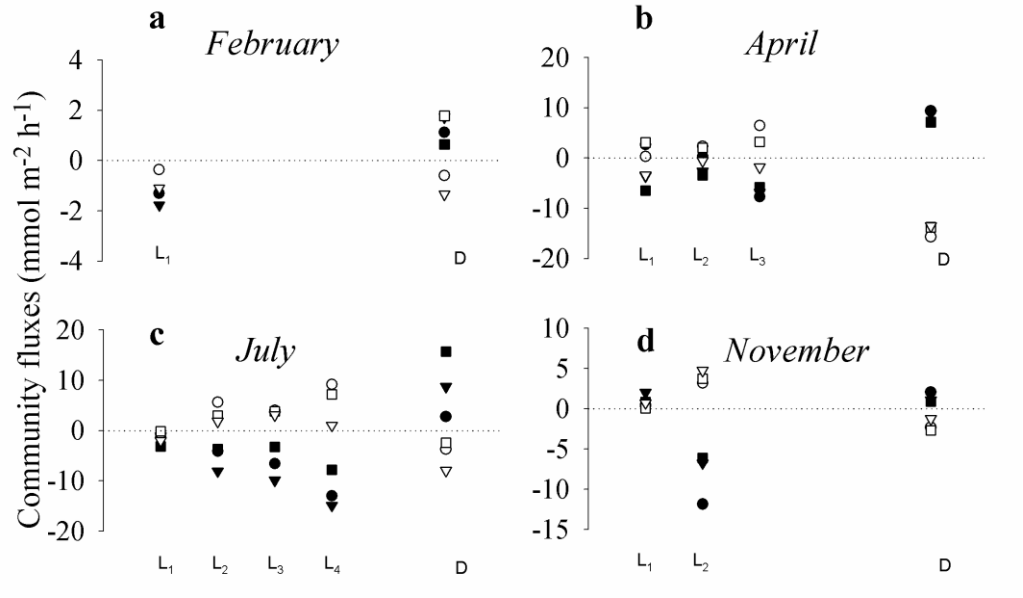

Figure 3: Community net production and respiration (mmol m^{-2} h^{-1}) estimated from the two-point method as DIC (closed symbol) and O_2 (open symbol) fluxes in the three benthic chambers. The fluxes estimated in the flow-through benthic chamber are indicated by triangles. L_1, L_2, L_3, L_4 and D indicate successive light and dark incubations in (a) February, (b) April, (c) July and (d) November. Light conditions during incubations are given in Table 1.

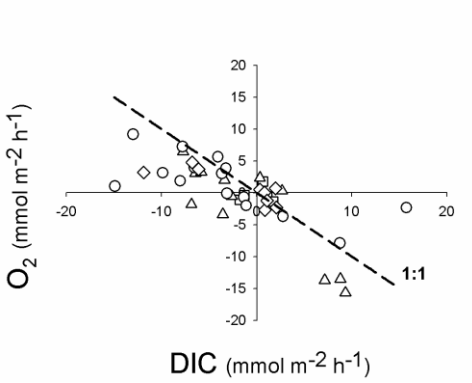

Figure 4: Cross-plot of O_2 and DIC (mmol m^{-2} h^{-1}) fluxes estimated from the 2-point method in the three benthic chambers in February (square), April (triangle), July (circle) and November (diamond).

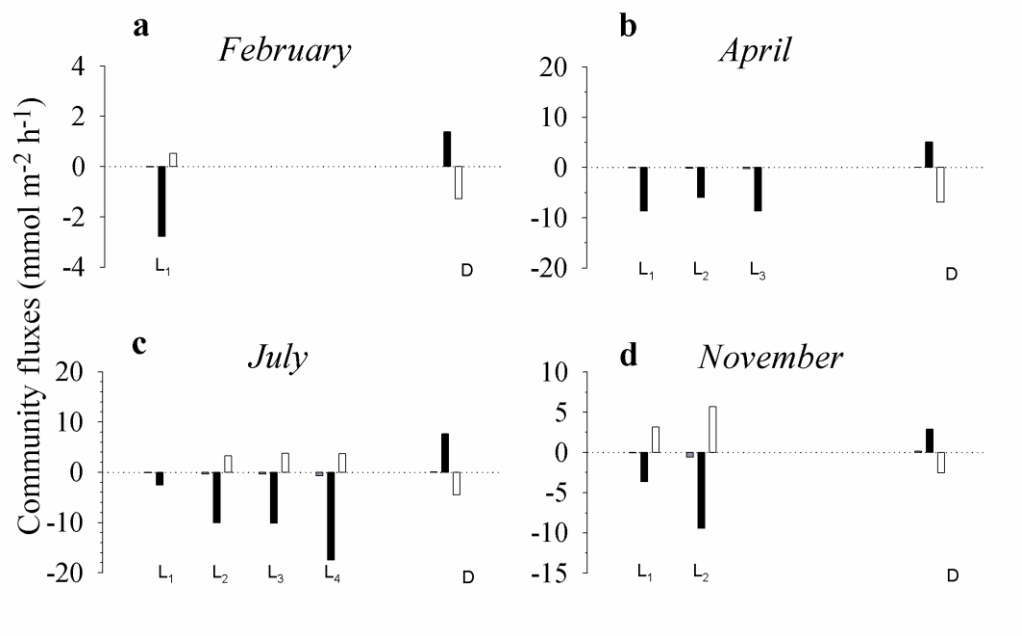

Figure 5: The community net production rates NCP_{CO2} (grey bars), NCP_{DIC} (black bars) and NCP_{O2} (white bars) calculated from continuous measurements of CO_2, DIC, and O_2, respectively in the flow-through benthic chamber incubated under ambient light conditions (see Table 1). The community respiration rates CR_{CO2} (grey bars), CR_{DIC} (black bars) and CR_{O2} (white bars) calculated from continuous measurements of CO_2, DIC, and O_2, respectively in the flow-through benthic chamber during the dark conditions. L_1, L_2, L_3, L_4 and D indicate successive light and dark incubations, respectively, during the measurements in (a) February, (b) April, (c) July and (d) November. Values and environmental conditions are presented in the Table 1. All rates are expressed in mmol m^{-2} h^{-1}

Month	Date		Light exposure	Temperature	Initial pH	DIC	$CO_2^{(t)}$	CO_2	O_2
			µmol quanta m^{-2} s^{-1}	*°C*		--------	*mmol m^{-2} h^{-1}*		--------
February									
	02/13/09 09:30 AM	L_1	118 ± 60	8.7 ± 0.0	7.950	-2.77	-0.45	-0.02	0.52
	02/13/09 11:30 AM	D		8.3 ± 0.6	8.275	1.39	0.08	0.01	-1.28
April									
	04/24/09 09:00 AM	L_1	81 ± 18	12.0 ± 0.0	8.269	-8.66	-0.35	-0.03	NA
	04/24/09 10:00 AM	L_2	226 ± 97	12.1 ± 0.1	8.328	-5.92	-0.24	-0.14	NA
	04/24/09 10:45 AM	L_3	482 ± 128	12.6 ± 0.1	8.380	-8.67	-0.30	-0.26	NA
	04/24/09 02:30 PM	D		14.5 ± 0.7	8.747	5.04	0.05	0.03	-6.90
July									
	07/27/09 11:20 AM	L_1	119 ± 55	16.1 ± 0.1	8.442	-2.53	-0.05	-0.10	NA
	07/27/09 12:30 PM	L_2	244 ± 58	16.4 ± 0.1	8.470	-10.02	-0.16	-0.36	3.26
	07/27/09 01:20 PM	L_3	297 ± 173	16.6 ± 0.2	8.464	-10.09	-0.19	-0.36	3.74
	07/27/09 02:15 PM	L_4	1119 ± 305	18.5 ± 0.3	8.525	-17.45	-0.32	-0.72	3.69
	07/27/09 06:30 PM	D		18.3 ± 0.3	8.785	7.66	0.06	0.07	-4.47
November									
	11/17/09 09:30 AM	L_1	54 ± 30	12.3 ± 0.1	8.134	-3.62	-0.28	-0.04	3.14
	11/17/09 10:30 AM	L_2	229 ± 130	12.4 ± 0.1	8.211	-9.41	-0.56	-0.55	5.68
	11/17/09 02:00 PM	D		13.1 ± 0.1	8.294	2.90	0.15	0.13	-2.51

Table 1: The community net production (NCP) and respiration rates (CR) measured during successive light (L_1, L_2, L_3, L_4) and dark incubations (D), respectively (cf. Fig. 5) in the flow-through benthic chamber in February, April, July and November. The DIC column lists NCP_{DIC} or CR_{DIC}-values calculated from variations of DIC. The $CO_2^{(t)}$ column corresponds to a predicted rate of variation of dissolved CO_2 based on NCP_{DIC} or CR_{DIC}-values and considering the theoretical proportion of CO_2 in DIC according pH and temperature. The CO_2 column lists NCP_{CO2} or CR_{CO2}-values calculated directly from the measured variations of dissolved CO_2. The O_2 column lists NCP_{O2} or CR_{O2}-values calculated directly from the measured variations of dissolved O_2. Light intensity (mean ± SD) near the benthic chamber and temperature (mean ± SD) inside the benthic chamber have measured for each incubation. NA indicates non available data.