


An in situ approach for measuring biogeochemical fluxes in structurally complex benthic communities

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Abstract

1. The exchange of energy and nutrients are integral components of ecological functions of benthic shallow-water ecosystems and are directly dependent on in situ environmental conditions. Traditional laboratory experiments cannot account for the multidimensionality of interacting processes when assessing metabolic rates and biogeochemical fluxes of structurally complex benthic communities. Current in situ chamber systems are expensive, limited in their functionality and the deployment is often restricted to planar habitats (e.g. sediments or seagrass meadows) only.
2. To overcome these constraints, we describe a protocol to build and use non-invasive, cost-effective and easy to handle in situ incubation chambers that provide reproducible measurements of biogeochemical processes in simple and structurally complex benthic shallow-water communities. Photogrammetry tools account for the structural complexity of benthic communities, enabling to calculate accurate community fluxes. We tested the performance of the system in laboratory assays and various benthic habitats (i.e. algae growing on rock, coral assemblages, sediments and seagrass meadows). In addition, we estimated community budgets of photosynthesis and respiration by corals, rock with algae and carbonate sediments, which were subsequently compared to budgets extrapolated from conventional ex situ single-organism incubations.
3. The tests highlight the transparency (>90% light transmission) of the chambers and minimal water exchange with the surrounding medium on most substrates. Linear dissolved oxygen fluxes in dependence to incubation time showed sufficient mixing of the water by circulation pumps and no organismal stress response. The comparison to single-organism incubations showed that ex situ measurements might overestimate community-wide net primary production and underestimate respiration and gross photosynthesis by 20%–90%.
4. The proposed protocol overcomes the paucity of observational and manipulative studies that can be performed in in situ native habitats, thus producing widely applicable and realistic assessments on the community level. Importantly, the tool provides a standardized approach to compare community functions across a wide

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range of benthic habitats. We identify multiple experimental strategies, including the manipulation of stressors/factors, and discuss how the method may be implemented in a variety of aquatic studies.

KEYWORDS

3D models, community budgets, field-based measurements, incubations, photogrammetry, respiratory chambers

1 | INTRODUCTION

Anthropogenic environmental change is rapidly transforming the community composition, structure and functioning of coastal and estuarine benthic shallow-water ecosystems on a global scale (Harley et al., 2006). Processes such as primary production, calcification, organic matter (OM) remineralization and nutrient cycling are important indicators of ecosystem status, health and alteration (Cloern et al., 2016; Halpern et al., 2008; Lotze et al., 2006). However, the complexity of interactions underlying these processes requires a holistic assessment with accurate measurements of community metabolism and biogeochemical fluxes (Griffiths et al., 2017). Consequently, community-wide and standardized measurements of biogeochemical properties of benthic communities are a prerequisite for ecosystem management (Brierley & Kingsford, 2009). However, obtaining such data has proven challenging.

Currently, metabolic rates and element cycling processes of various benthic communities are mainly derived from experimental studies conducted in aquaria or mesocosm systems (Althea & Duffy, 2016; Bellworthy & Fine, 2018; Comeau, Carpenter, Lantz, & Edmunds, 2015; Russell, Thompson, Falkenberg, & Connell, 2009; Wagenhoff, Lange, Townsend, & Matthaei, 2013). In such studies, benthic communities are reconstructed *ex situ* according to information gathered during benthic field surveys. Mesocosms (continuous flow or static) provide a unique way to measure biogeochemical exchange rates under controlled conditions. These traditional methods, however, are seldom capable of accommodating the complexity and variability in natural systems (Riebesell, Fabry, Hansson, & Gattuso, 2010). Moreover, substrates and individual organisms must be actively removed and incubated *ex situ*, making this process destructive and prone to experimental artifacts. Additionally, community measurements in *ex situ* flumes and mesocosms are extremely costly, and few laboratories are capable of accommodating the complexities of natural systems.

Most community-wide *in situ* measurements of metabolic and biogeochemical processes have generally quantified spatial geochemical changes in the water column using the Eulerian (e.g. Falter, Lowe, Atkinson, Monismith, & Schar, 2008) or Lagrangian (e.g. Gattuso, Pichon, Delesalle, Canon, & Frankignoulle, 1996) flow respirometry technique. These methods involve measuring the upstream–downstream changes of the chemical properties in a parcel of water in a unidirectional flow field. This approach has difficulties in accurately tracking the movement of water parcels due to the

complex topography of many habitats, resulting in a significant margin of error (e.g. Shaw, Phinn, Tilbrook, & Steven, 2014). Moreover, water must have sufficient contact time with the substrate for its chemistry to be affected by processes of interest, limiting the resolution of geochemical measurements (Monsen, Cloern, Lucas, & Monismith, 2002).

Enclosure experiments (*ex situ* or *in situ*) associated with benthic shallow-water systems have often utilized small (<4 L volume, usually only 1 L) incubation chambers (Camp et al., 2015; Ferrier-Pagès et al., 2013; Sawall, Al-Sofyani, Banguera-Hinestroza, & Voolstra, 2014). These chambers effectively isolate a limited volume of water over individual organisms, enabling short-term incubation experiments. While providing valuable measurements of processes associated with individual organisms, community-wide responses cannot be achieved. Nonetheless, single-organism incubations are the foremost approach to extrapolate responses of representative taxa of a known habitat to community-wide budgets (e.g. Cardini et al., 2016; Eidens et al., 2014; Naumann, Jantzen, Haas, Iglesias-Prieto, & Wild, 2013).

In recent years, larger *in situ* incubation chambers (5–120 L volume) have been used across different benthic shallow-water habitats (Hughes, Atkinson, & Ansell, 2000; L'Helguen et al., 2014; Tengberg et al., 1995). These chambers enclose both the underlying substrate and overlying water to assess areal rates of production and uptake by quantifying temporal changes in the overlying water chemistry. They provide a non-invasive technique that better reflects the ambient conditions, nevertheless, these studies have often been restricted to plain two-dimensional (2D) habitats, such as sediments (e.g. L'Helguen et al., 2014; Rasheed, Wild, Jantzen, & Badran, 2006; Tengberg et al., 1995) or seagrass meadows (Silva, Santos, Calleja, & Duarte, 2005). Here, fluxes could simply be related to the covered seafloor area and the known water volume of the chamber.

The application of such systems has been notably absent for communities with structurally complex, three-dimensional (3D) structures (e.g. rocky beds, oyster banks or coral reef habitats). Photosynthesis, calcification and OM cycling, however, are all examples of processes affected by the volume and surface area of the organisms, yet no tools have been available to quantify these variables *in situ* non-intrusively. Previous studies that measured community processes on intertidal rocks (Tait & Schiel, 2010) or coral reefs (Haas et al., 2013; Yates & Halley, 2003) considered the 2D area of the seafloor alone; hence, results may have a large margin of uncertainty.

Given the above, a reliable, versatile and widely reproducible method for assessing the biogeochemical functioning of benthic

shallow-water communities in their natural environment is lacking. To address these challenges, we developed an end-to-end in situ incubation protocol that aims at measuring biogeochemical fluxes associated with whole benthic communities. The technique can be applied across a wide range of benthic shallow-water habitats, from simple (e.g. sediments) to complex (e.g. corals or rocky beds). We inform on material selection and construction processes to build cost-effective and easy to handle in situ chambers made from rigid polymethyl methacrylate cylinders. Combined with recent advances in computer-assisted photogrammetry, this approach offers a new way for accurately measuring all biogeochemical fluxes in a non-intrusive manner. We present data from laboratory tests, field incubations and, subsequently, compare our results to data from a commonly applied protocol of estimating community budgets from incubations of single organisms. Lastly, we discuss how the method may be implemented in a variety of marine and aquatic ecological studies that include standard and manipulative experimental approaches.

2 | MATERIALS AND METHODS

2.1 | Construction of benthic incubation chambers

Benthic chambers were constructed from 5-mm-thick polymethyl methacrylate (PMMA) cylinders (diameter: 0.50 m, height: 0.39 m)

with a removable, gas-tight lid of the same material (0.40 m total height) (Figure 1; additionally, see Supplementary Material for a detailed overview of all parts required for the construction, prices and vendors, as well as detailed design and assembly schematics in Supporting Information Figures S1–S3). The size was chosen to accommodate large communities with rigid features while still being easily handled by divers. The chambers are open at the bottom to be mounted over natural benthic communities. On soft-bottom substrates, the chambers are inserted into the ground down to 5 cm to seal off the incubation medium from the surrounding seawater. On hard-bottom substrates, where the chambers cannot be inserted into the ground, wide (20 cm) PVC skirts (Supporting Information Table S1) attached 5 cm above the base are used to minimize water intrusions. The lid-top can be fastened onto the chamber using M5 × 0.8 mm wing-head PVC bolts (Supporting Information Table S1). A silicone O-ring (Supporting Information Table S1) sits in a gland to create a gas-tight seal between chamber and lid. Once mounted correctly, the chambers enclose a theoretical volume of 66.8–76.7 L of water (depending on the depth of insertion into the ground) and cover 0.2 m² of the seafloor.

Due to their flat surface, lids can be easily modified to accommodate measurement and sampling requirements for the desired experiment. In the setup presented here, all chambers were equipped with autonomous recording dissolved oxygen (DO) sensors (HOBO U26, Onset Computer Corporation, Cape Cod, USA; precision 0.02 mg/L,

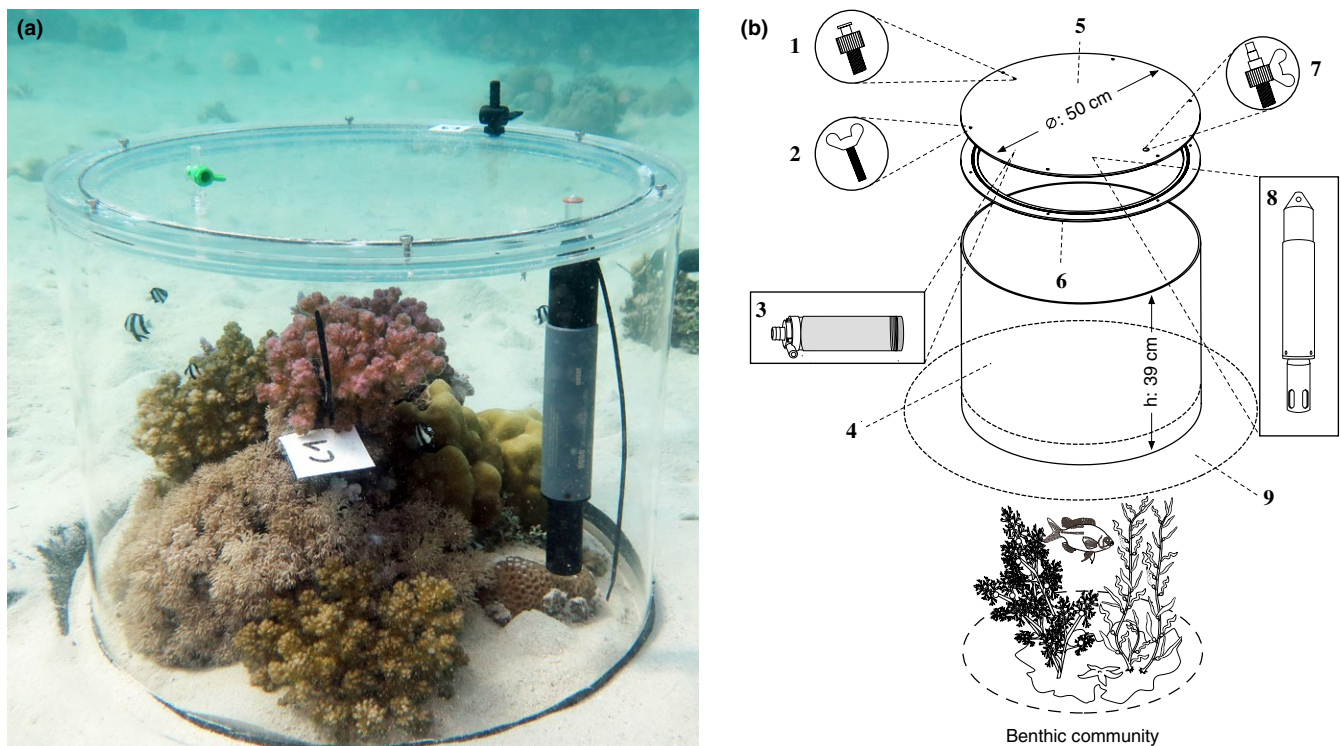


FIGURE 1 Benthic incubation chamber deployed in situ over a natural coral reef community (a), and general layout of the chamber (b). (1) One one-way stopcock female to male luer-lock; (2) Six wing-head bolts to fasten lid; (3) Adjustable flow control circulation pump; (4) Main chamber body made from polymethyl methacrylate cylinders (50 cm diameter, 39 cm height); (5) Removable lid made from polymethyl methacrylate (0.5 cm thickness); (6) O-ring; (7) Stopcock ¼ inch with hose barb adapter; (8) Temperature and dissolved oxygen recorder (HOBO U26); (9) PVC skirt. Note: The circulation pump in A is behind the tubular oxygen sensor

accuracy \pm 2%, automatic temperature and pressure compensated and salinity corrected), and HOBO Pendant[®] light loggers (Onset Computer Corporation).

Furthermore, the chambers were equipped with two sampling ports: (a) One one-way stopcock female to male luer-lock (Supporting Information Table S1); (b) one stopcock $\frac{3}{4}$ inch with barbed hose adapter (Supporting Information Table S1). Among others, these sampling ports offer the possibility for controlled sampling of many biological and chemical variables, such dissolved organic carbon (DOC), dissolved inorganic nutrients, dissolved inorganic carbon (DIC), total alkalinity (TA), bacterial or viral samples.

All chambers were equipped with individual water circulation pumps to ensure that withdrawn water samples for later analysis are representative of the entire volume and to avoid the development of chemical gradients (Supporting Information Figure S4). An adjustable flow control further allowed to mimic hydrodynamic conditions under ambient conditions. For this, 6 V DC motors (300 mA, 2 W) with a magnetic impeller (Supporting Information Table S1) were powered by four 1.5 V AA mignon cells (2600 mAh) (Supporting Information Table S1). A low voltage pulse-width modulation (PMW) speed controller (Supporting Information Table S1) enables stepless controllable flow rates of 0.2–2 L/min. All electronic parts were encapsulated in a PVC tube (40 mm inner diameter, 150 mm length) (Supporting Information Table S1) with the pump on one end, and with a screw cap and a silicone O-ring (Supporting Information Table S1) at the back. The screw cap allows easy access to the electronics to exchange batteries, switching on/off of pumps and to conduct maintenance works. The pump at the front end was secured and sealed off with non-toxic marine grade epoxy resin (Supporting Information Table S1).

2.2 | General procedures: chamber deployment, water sampling and cleaning

2.2.1 | Chamber deployment

The chambers can be deployed easily by two people either snorkelling or SCUBA diving depending on the study site. Chambers are first installed without their lids for easier manoeuvrability and to minimize pressure and wave-induced disturbance to the benthic substrate. The 39-cm-high chambers are carefully positioned over the benthic community of interest, and pushed into the sediment to a depth of 5 cm, thus enclosing a 34-cm-high water column. The wide PVC skirts attached to the chambers are placed onto the substrate and weighed down by a heavy chain, rocks or rubble to further prevent water exchange. If the substrate does not allow full insertion of the chamber, the PVC skirt will sufficiently isolate the chamber from the surrounding water (see results of leakage testing below). At least 1 hr of time should be given to let sediments settle before proceeding. Subsequently, the lid, equipped with pumps and sensors, is fitted to the chamber and secured in place with six wing-head bolts. The incubations start by closing the chambers' lid and sampling ports.

2.2.2 | Water sampling

Water samples for water chemistry parameters (e.g. DOC, DIC, DIN, TA, etc.) or other biological variables (e.g. eDNA) can be withdrawn from one of the two sampling ports at any time of the incubation. Small water samples can be withdrawn with syringes from the luer-lock valve. Larger volumes of water can be withdrawn from the barbed stopcock. We recommend opening both sampling ports when withdrawing a water sample to avoid a reduced pressure inside the chamber and to replace the withdrawn volume of water by water from outside the chamber rather than by sediment pore water. Considering that 200 ml of water is sufficient for the concurrent measurement of inorganic nutrients, TA, DIC, DOC and fluorescent dissolved OM, a water exchange during sampling of 0.26% is negligible. Furthermore, the dilution of the water body inside the chambers with surrounding seawater can be easily corrected for mathematically. Additionally, the two sampling ports are positioned opposite of each other on the lid, reducing the risk of sampling water from the inlet valve given sufficient mixing by the pumps.

2.2.3 | Cleaning procedures

After field deployment, the incubation chambers can be rinsed with fresh water. Wing-head screws, valves and other attached parts can be removed and cleaned separately. All materials of the presented setup can be washed with 4% HCl solution if necessary (e.g. for reliable DOC measurements). We recommend rinsing with deionized water before long-term storage.

2.3 | Considerations for calculating accurate community fluxes

2.3.1 | Surface area and volume quantification of structurally complex habitats

To evaluate physiological and biogeochemical processes of complex habitats, it is crucial to determine the volume and surface area of the enclosed benthic communities. Here, we quantified the 3D surface area and volume of a community in a chamber by photogrammetric techniques involving computer modelling discussed in Lavy et al. (2015) and Gutierrez-Heredia, Benzoni, Murphy, and Reynaud (2016). The use of cloud computing and the availability of freeware make this tool widely accessible. Briefly, around 100 digital photographs are taken in situ from multiple angles of each community with a digital camera with an underwater housing (Figure 2a). Using the software ReCap[®] (Autodesk Inc.), raw pictures can be uploaded into a cloud-based interface for further processing, thereby reducing the computational requirements of the users' machine. ReCap[®] automatically produces a digital 3D model from the photographs that can be downloaded within 1 hr and opened with the software interface. The coloured model can be used for the determination of the community composition (Figure 2b). From the mesh model (Figure 2c), the 3D surface area and volume are calculated using internal tools of the same software.

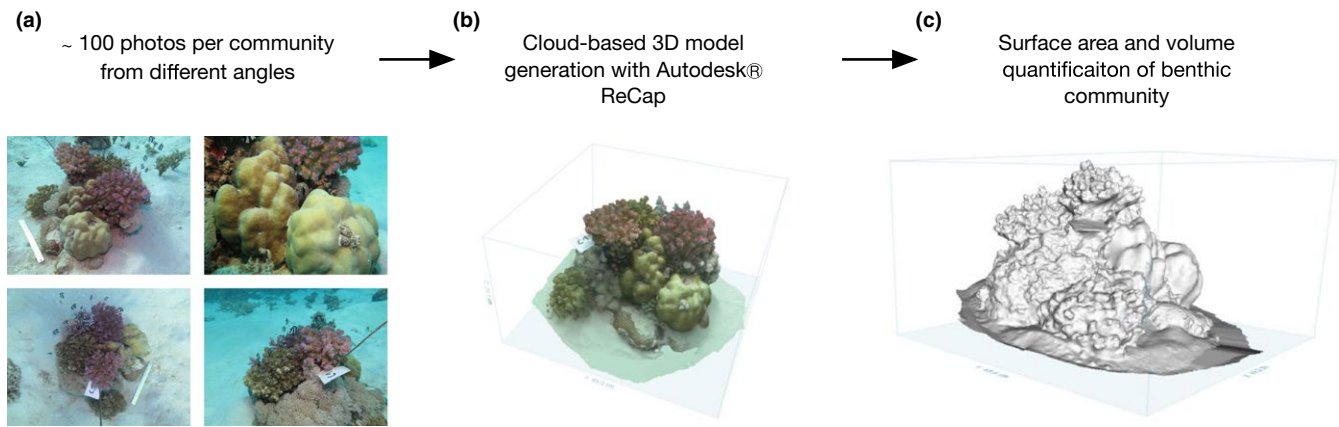


FIGURE 2 Simplified workflow to generate 3D models for the assessment of community structure, surface area and volume of structurally complex benthic communities. (a) Around 100 photos are taken of the benthic community from different angles with a simple underwater digital camera (i.e. Canon PowerShot G12). (b) Pictures are uploaded for a cloud-based 3D model generation with the software ReCap® (Autodesk Inc.). Coloured 3D models based on pictures are used for assessments of community structure and composition. (c) Software-integrated tools enable surface area and volume quantification

The exact water volume (V) in each chamber during an incubation is calculated by subtracting the measured community volume (V_C) as well as the volume occupied by the sensors (V_S) and the pump (V_P) from the theoretical volume (V_T) enclosed by the chamber above the sediment line:

$$V = V_T - V_C - V_S - V_P$$

2.3.2 | Calculating community fluxes from discrete water samples and continuous measurements

Community fluxes of water chemistry parameters can be calculated from discrete water samples or from continuous measurements by sensors (e.g. dissolved oxygen loggers). All fluxes should be normalized to incubation water volume (V ; in L), incubation duration (I_T ; in h) and the surface area (SA ; in m^2) of the enclosed benthic community. Community fluxes for a water chemistry variable (Var) can be calculated from discrete start-end point sampling as:

$$Var(t) = \left[\frac{(\Delta Var) \cdot V}{I_T \cdot SA} \right]$$

For variables that can be continuously measured (e.g. dissolved oxygen) community fluxes of monotonic metabolic processes (e.g. photosynthesis and respiration) can be calculated from linear regressions. Here, the slope (m) of a linear regression is used to calculate flux rates as:

$$Var(t) = \left[\frac{m \cdot V}{SA} \right]$$

As full time series are often nonlinear at times (e.g. the rate of O_2 initially decelerates as the organisms equilibrate after handling), calculations using the full time series can result in underestimating metabolic rates. Truncating the data to exclude these nonlinearities manually is subjective and difficult to reproduce. Therefore, we recommend using the “LOLINR” package for R (Olito, White, Marshall, & Barneche, 2017), which provides a flexible toolkit to implement local

linear regression techniques to estimate biological rates from linear and nonlinear time-series data. Local linear regressions enhance the accuracy by estimating the slope of a linear subset of the time series as defined by linearity metrics underpinning the function (details in Olito et al., 2017).

2.4 | Performance tests on various substrates under laboratory and in situ conditions

2.4.1 | Laboratory assays for leakage testing and light transmission

To ensure that a watertight seal can be created around benthic communities, chambers were secured on different substrates in 200-L holding tanks in the laboratory according to the protocol above. Six replicate chambers were placed on coarse reef sand (1–3-mm-grain size) and six on coarse rock/rubble pieces (c. 5 cm) respectively. After closing the chambers, red food colour dye Allura Red (Red 40) was injected into the chambers via the luer-lock valve. All chambers were equipped with temperature, light and DO loggers and the circulation pumps were switched on to simulate field deployment conditions. An initial 50-ml aliquot of water was withdrawn from each chamber and from the surrounding tank water (background control) using a syringe for initial concentrations. Each chamber was then left in the holding tank for 6 hr before another water sample was collected from inside the chamber and the surrounding water. A Trilogy Laboratory Fluorometer with an absorbance module (Trilogy 7200-050) was used to measure the absorbance of light at 504 nm by each sample to determine if any dye had transferred from inside the chambers to the surrounding tank water. A series of volumetric dilutions made from a stock solution of known Allura Red concentrations (7-point calibration) was used to determine the concentration of Allura Red in a sample.

Light transmission through 0.5-mm-thick polymethyl methacrylate cylinders was tested with a LAMBDA 1050 UV/Vis/NIR Spectrophotometer (PerkinElmer) at a 1-nm interval from 250 nm–750 nm wavelength. In addition, light transmission was assessed in situ by comparing the data from light loggers deployed inside and outside of the chambers (details are described in the supplementary material).

2.4.2 | Incubations of simple and structurally complex benthic substrates

To validate the versatility of the system on various substrates and conditions, incubations for DO production measurements were carried out on: (a) hard-bottom communities: that is, algae growing over a reef framework at 5 m water depth; (b) hard-bottom communities on sand: that is, assemblages of coral colonies on sand at 5 m water depth; (c) carbonate sands at 5-m water depth in a reef lagoon and (d) mixed species seagrass meadows at 1.5-m water depth. In addition, to sensitize for the correct use of the chambers (i.e. activation of pumps, securing chambers with weights), we carried out incubations of coral colonies on sand with and without an active circulation pump system, and incubations of mixed species seagrass meadows at 1.5-m water depth under high wave conditions with and without additional weights to secure the incubation chambers on the substrate. All incubations were performed concurring to the protocol above. Incubations lasted for 90–120 min. Incubations of different communities were carried out during different seasons in 2017, and absolute values should, therefore, not be compared. As any stress response from the organisms, water intrusions from the surrounding medium or non-sufficient mixing by the pumps results in nonlinear DO production rates, the “quality” of the incubations was assessed by evaluating the linearity of DO fluxes corrected for background seawater metabolism and normalized to community surface area. For this, the overall linearity in DO production of each incubation was tested by Pearson correlations of the full time series. In addition, we quantified the number of data points (given in % of the total number of data points of the full time series) that met the model assumptions for the local linear regressions. As recommended by the model developers, model assumptions are met if 20% of the data points follow a strictly linear trend. Hence, selected model parameters were $\alpha = 0.2$, with a linearity metric = L% (details of the model in Olito et al., 2017).

2.5 | Case study: Coral reef community budgets of primary production and comparison to ex situ single-organism incubations

To illustrate the applicability over simple (i.e. sediments) and structurally complex (i.e. coral and algae) communities, we conducted a case study at a coral reef in the Central Red Sea. Field tests were carried out at Abu Shosha reef located on the west coast of Saudi Arabia (22°18'16.3"N; 39°02'57.7"E) in July 2017. The reef is characterized by a patchwork of coral- and algae-dominated communities that are distributed on an area of coarse carbonate reef sand at

a water depth of 4–7 m. For the incubations, four coral-dominated communities surrounded by sediment (>40% coral cover, but <10% algal cover), four rocky communities overgrown by heterogeneous assemblages of algae surrounded by sediment (hereafter “algae-dominated”; >40% algae, but <10% coral cover) and carbonate sediments (hereafter “sediments”) were selected haphazardly with a minimum distance of 3 m. All communities were incubated for measurements of net primary production (NPP) and respiration (R) according to the protocol above (detailed procedures for the case study can be found in the supplementary material). To estimate gross primary production (GPP), each R measurement was added to its corresponding NPP measurement ($NPP + |R| = GPP$), assuming that respiration rates in the light were equal to those derived from dark incubations (covered chambers) during the day (Bidwell, 1977). O_2 fluxes were converted into carbon (C) fluxes assuming a theoretical molar ratio of $CO_2:O_2 = 1$ (Clavier, Boucher, & Garrigue, 1994). For comparison with other benthic systems and methods, resulting contributions were expressed in $mmol\ C\ m^{-2}\ h^{-1}$.

Subsequently, in situ community measurements were compared to ex situ community budgets estimated from single-organism incubations—a commonly applied technique to measure community budgets of structurally complex habitats, such as coral reefs. Community budgets from single-organism incubations were estimated by an adapted protocol from Cardini et al. (2016). The details for the ex situ incubations are described in the supplementary material. Briefly, organisms representing the dominant functional components from benthic chambers during in situ incubations were incubated individually for NPP, R and GPP rates in the laboratory. Afterwards, ex situ fluxes of single-organism incubations were extrapolated to the metabolism in in situ chambers, considering the specific 3D area covered by the respective functional groups from the chambers' communities and the volume of seawater included in the chambers.

3 | RESULTS

3.1 | Leakage testing

No significant difference between start ($1.32 \pm 0.05\ \mu\text{mol/L}$) and end ($1.25 \pm 0.04\ \mu\text{mol/L}$) concentration of Allura Red dye inside the incubation chambers was detected on coarse reef sands (mean concentrations reduced by 5.3% within 6 hr; two-tailed *t*-test: $p\ |t| = 0.2722$) (Figure 3). On rock/rubble, the concentration of Allura Red was significantly reduced by 12.4% within 6 hr (from 1.37 ± 0.04 to $1.20 \pm 0.03\ \mu\text{mol/L}$ respectively; two-tailed *t*-test: $p\ |t| = 0.0057$), indicating minimal water exchange under laboratory conditions (Figure 3).

3.2 | Light

Spectral analysis showed a light transmission through the chamber material averaging 92% within PAR (photosynthetic active radiation; 400–700 nm) (Figure 4a), while light within the UV range was attenuated (16% and 1% transmission for UVA and UVB respectively).

During field deployment, on average 9.4% reduced light availability within PAR was measured (90.6% transmission) for incubation chambers compared to outside of the chambers at the same time and water depth (Figure 4b).

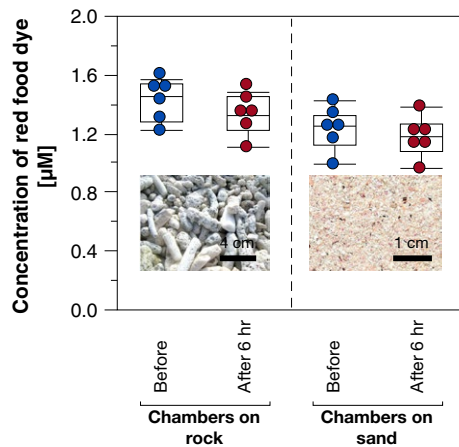


FIGURE 3 Leakage test of incubation chambers on different substrates. Six replicate chambers each were placed in large holding tanks on rock/rubble pieces (c. 5 cm) and coarse reef sand (1–3 mm), and red food dye was injected into the chambers. The concentrations of Allura Red dye (determined by absorbance of light at 504 nm) was measured from samples in the chambers after adding the dye (referred to as “Before”) and after 6 hr of incubation (referred to as “After 6 hr”). Boxplots showing the median (line across a box), quartiles (upper and lower bounds of each box) and extremes (upper and lower whisker). The ends of the whisker are set at $1.5 \times \text{IQR}$ above the third quartile (Q3) and $1.5 \times \text{IQR}$ below the first quartile (Q1). Scale bars are given in each picture

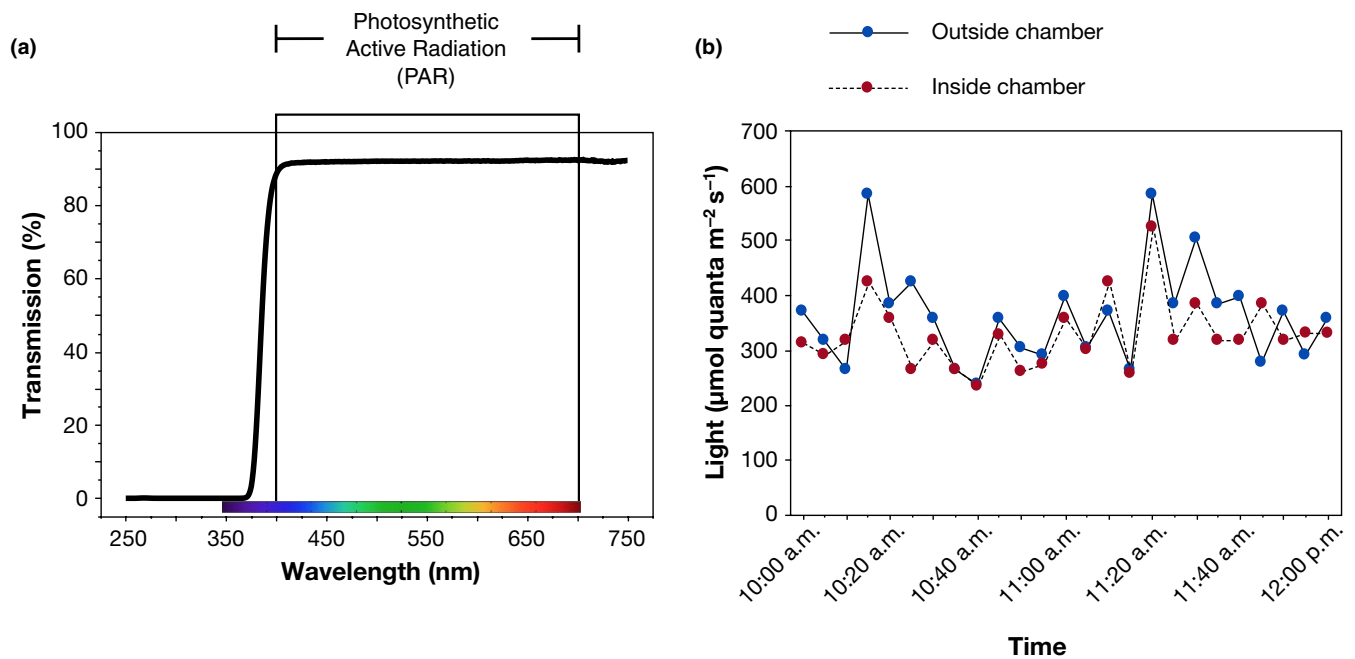


FIGURE 4 Spectral transparency of benthic incubation chambers. (a) Transmittance of the polymethyl methacrylate material (0.5 mm thickness) measured with a spectrophotometer (UV-2450, Shimadzu Corporation) at a 1-nm wavelength interval. (b) Simultaneous measurements of light (photosynthetic active radiation spectra) inside and outside of one incubation chamber deployed at 5 m water depth in June 2017

3.3 | Incubations of hard-bottom and soft-bottom communities

Incubations of both structurally complex (i.e. “rocks with algae” and “corals on sand”) and simple (i.e. “carbonate reef sediments” and “sea-grasses”) habitats revealed a production of DO over time (Figure 5a). Incubation chambers that were deployed on hard-bottom substrates (i.e. rocks with algae) had detectable water intrusions from outside the chamber, resulting in an average linearity of the DO production of $r^2 = 0.916 \pm 0.032$ (Pearson correlation; $n = 4$), where 20%–48% of the data points could be included in the local linear regressions by the *LolinR* function. Incubations that took place on sediments (i.e. corals on sand, carbonate sediments and seagrasses), showed a more linear DO production ($r^2 = 0.994 \pm 0.002$; Pearson correlation; $n = 12$), where 51%–90% of the data points could be included in the local linear regressions.

Incubations of coral-dominated communities on sand with and without active circulation pumps highlighted the necessity of the pumps in mixing the water column within the chambers (Figure 5b). The lack of active circulation pumps resulted in nonlinear DO production ($r^2 = 0.721 \pm 0.114$; Pearson correlation; $n = 3$), where only 15%–22% of the data points could be included in the local linear regressions. In contrast, sufficient water circulation with active pumps resulted in a linear DO production ($r^2 = 0.997 \pm 0.002$; Pearson correlation; $n = 4$), where 58%–88% of the data points could be included in the local linear regressions.

In an intertidal habitat with high wave action, chambers without additional weights on the PVC skirts were shaken by the water movement, resulting in a nonlinear DO production ($r^2 = 0.689 \pm 0.211$; Pearson correlation; $n = 4$); <20%–50% of the data points included in

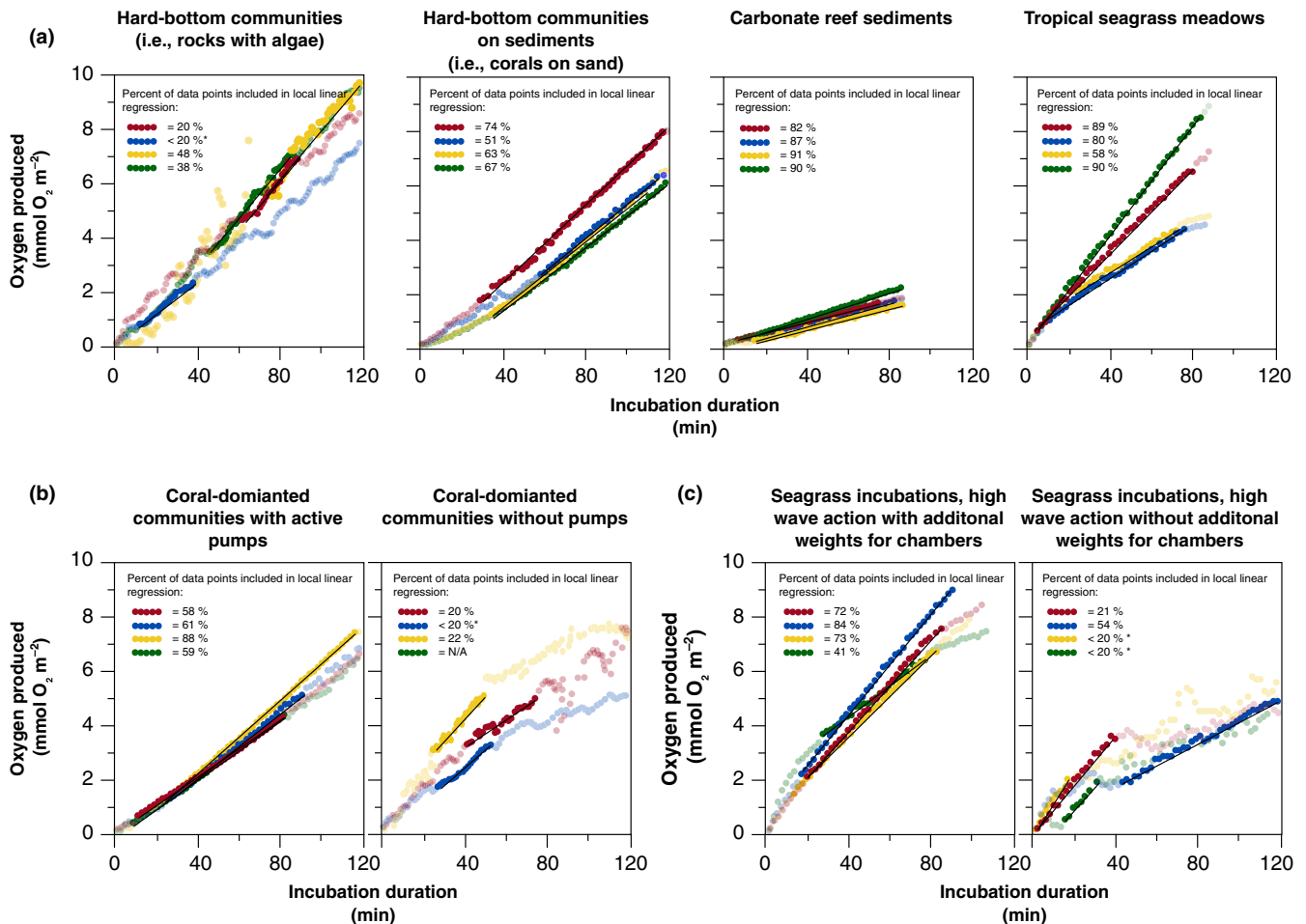


FIGURE 5 Dissolved oxygen production during incubations of various substrates (a), with/without active circulation pumps (b) and under high wave action in an intertidal zone with/without additional weights (c). Shown is the production of dissolved oxygen over time (incubation periods ranging from 90 to 120 min), corrected for seawater background metabolism and normalized to the surface area of the incubated substrate. Points of different colours represent independent replicate incubations of the respective substrate. Opaque points with lines (●, ●, ● and ●) represent data points of incubations that were included by the “LoLINR” package for R (Olito et al., 2017) in a local linear regression for dissolved oxygen calculations. The amount of points included in the local linear regression is given in per cent in each plot. As recommended by Olito et al. (2017), an asterisk indicates that <20% of the data points followed a linear trend, hence model assumptions were not met. Transparent points (●, ●, ● and ●) represent all data point of the respective incubation that did not follow a linear trend

local linear regressions) due to water intrusions (Figure 5c). In contrast, 5-kg heavy chains on the PVC skirts secured the chambers sufficiently onto the substrates, resulting in linear DO production rates ($r^2 = 0.982 \pm 0.009$; Pearson correlation; $n = 4$; 41%–84% of the data points included in local linear regressions).

3.4 | Community-wide budgets for photosynthesis and respiration in coral reef habitats

In situ incubations revealed a significant difference between benthic coral reef habitats (i.e., coral-dominated vs. algae-dominated vs. sediments) for NPP, R and GPP (Figure 6a; detailed statistical test results in Supporting Information Table S3 of the supplementary material). Algae-dominated communities exhibited three- and ninefold higher NPP rates ($8.99 \pm 1.18 \text{ mmol C m}^{-2} \text{ h}^{-1}$) compared to coral-dominated communities and sediments respectively (3.29 ± 0.23 and $0.98 \pm 0.41 \text{ mmol C m}^{-2} \text{ h}^{-1}$), while respiration rates were 90% higher in algae-dominated

communities ($-6.46 \pm 0.32 \text{ mmol C m}^{-2} \text{ h}^{-1}$) compared to both coral-dominated communities and sediments ($-3.37 \pm 0.26 \text{ mmol C m}^{-2} \text{ h}^{-1}$, and $-3.40 \pm 0.31 \text{ mmol C m}^{-2} \text{ h}^{-1}$ respectively). This resulted in 2.3-fold higher GPP values ($15.45 \pm 1.32 \text{ mmol C m}^{-2} \text{ h}^{-1}$) of algae-dominated communities, compared to communities dominated by corals ($6.65 \pm 0.47 \text{ mmol C m}^{-2} \text{ h}^{-1}$) and 3.5-fold higher GPP values as compared to sediments ($4.38 \pm 0.15 \text{ mmol C m}^{-2} \text{ h}^{-1}$).

We assessed the performance of in situ incubations by monitoring that the temporal patterns of DO concentrations remained linear during incubations. The average correlation coefficient from all light incubations was $r^2 = 0.996 \pm 0.002$ (Pearson correlation; $n = 12$; $p < 0.0001$ in all cases) and $r^2 = 0.991 \pm 0.004$ (Pearson correlation; $n = 12$; $p < 0.0001$ in all cases) for all dark incubations, with 73%–90% and 69%–91% of the data points included in the local linear regressions respectively. In an exemplary chamber with a coral-dominated community, DO increased from 5.12 to 7.84 mg/L during the light showing a highly linear relationship

(Pearson correlation: $r^2 = 0.997$, $p < 0.0001$) to the incubation time (Figure 6b). Furthermore, the water temperature inside the chamber increased slightly by 0.4°C during the incubation (from 32.0 to 32.4°C. In contrast, during the 2-hr dark incubation period of

the same community, DO decreased from 5.00 to 2.02 mg/L within 2 hr (Pearson correlation: $r^2 = 0.993$, $p < 0.0001$) (Figure 6c). The water temperature remained stable (31.9°C before and after the incubation).

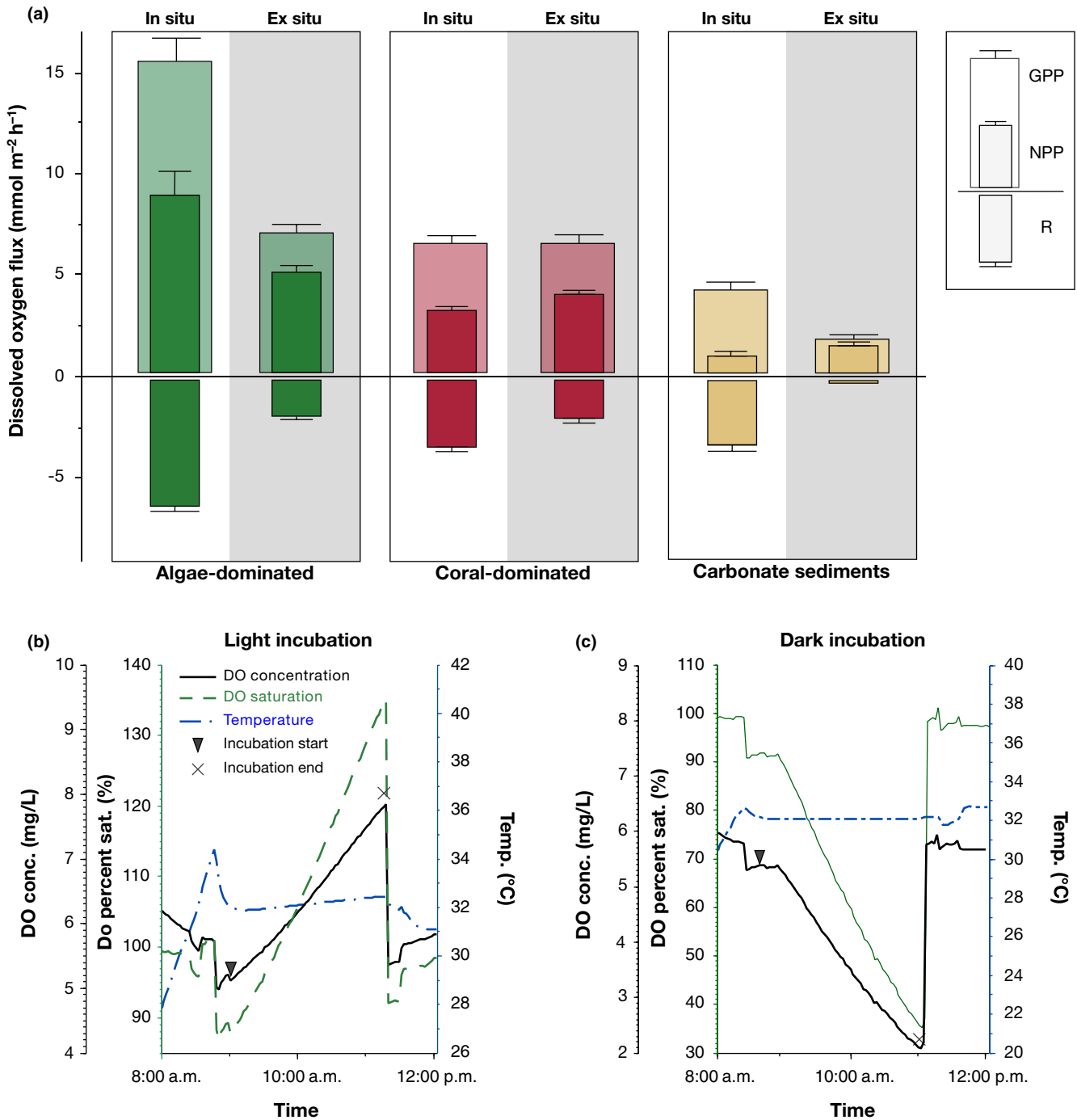


FIGURE 6 Community budgets of dissolved oxygen fluxes from in situ benthic chamber incubations and ex situ single-organism measurements of algae- and coral-dominated reef habitats and carbonate reef sediments. Community fluxes of dissolved oxygen (DO) of the respective native communities (in situ) and estimated budgets from single-organism incubations (ex situ) (a). Sensor readings of DO and temperature (Temp.) during 2-hr light (b) and dark (c) incubations of one representative coral-dominated community. GPP, Gross primary production; NPP, Net primary production; R, Respiration; DO conc., Dissolved oxygen concentration; DO Percent Sat., Dissolved oxygen per cent saturation. Values of (a) are given as means ($n = 4$) \pm SE

3.5 | Comparison of in situ and ex situ estimates of community-wide oxygen fluxes

The comparison of community oxygen fluxes between in situ and ex situ incubations revealed significant differences between these methods (Figure 6, A; detailed statistical test results in Supporting Information Table S3). Community fluxes derived from ex situ incubations showed significantly lower R rates for each of the three habitats compared to in situ incubations (25%, 62% and 92% lower for coral, algae and sediments respectively). Furthermore, ex situ incubations overestimated NPP by 35% in the case of coral-dominated communities, and by 82% in sediments. While GPP among the two methods was similar in coral-dominated communities, it was underestimated by ex situ incubations by >50% both in algae and sediments. As a consequence, ex situ extrapolations were not able to resolve differences in community metabolism between algae and coral-dominated communities.

4 | DISCUSSION

Most marine and aquatic ecosystems are presently under threat due to the combined effects of anthropogenic perturbations (Halpern et al., 2008). Understanding and predicting the responses of these ecosystems to stress requires considering processes across all levels of biological organization (Brierley & Kingsford, 2009). While current knowledge is advancing rapidly on scales ranging from molecular to whole-organism levels, our understanding of responses of whole communities in their natural environment is limited due to logistical and methodological constraints. In particular, measuring responses of communities with complex, three-dimensional structures is challenging, as many biogeochemical variables are affected by the volume and surface area of the organisms, with no tools available to quantify, non-intrusively, these variables in situ. Thus, it is crucial to develop a tool to accurately assess biogeochemical processes central to the ecological success of structurally complex benthic communities in situ. We have demonstrated that reliable and reproducible metabolic data can be generated using a combination of freely accessible photogrammetry tools and low-cost, easily deployable incubation chambers.

4.1 | Advantages of the new in situ chamber incubation method

A major advantage of the here-described protocol is the ability to measure metabolic and biogeochemical processes in various benthic habitats, with both simple and complex structures. In the existing literature, a multitude of in situ incubation chambers are presented. Especially abundant are systems for the incubation of bare sediments (e.g. L'Helguen et al., 2014; Rasheed et al., 2006; Tengberg et al., 1995) or seagrass meadows (Silva et al., 2005), while approaches targeting hard-bottom communities (e.g. coral assemblages or rocks with algae) are scarce (but see Tait & Schiel, 2010; Haas et al., 2013).

In addition, most chambers are purpose-built for one specific study and community type, limiting the ability to compare ecosystem functions across a wide range of benthic habitats.

Both the laboratory and field assays demonstrated that the here-presented system could be deployed successfully on sediments, seagrass meadows, rocky habitats and coral assemblages—some of the most conspicuous benthic shallow-water habitats (Diaz, Solan, & Valente, 2004). Thereby, a combination of hardware features and the post-processing of data renders the approach extremely versatile. The use of PMMA cylinders offers multiple advantages, such as that they are commercially available in large sizes and at low costs. Particularly hard-bottom communities (e.g. branching coral colonies) require chambers that are large enough to be mounted over rigid features (e.g. coral branches). The size of 50 cm diameter and 40 cm height has, thereby, proven more versatile over systems using small chambers that cover less surface area (e.g. Migné, Trigui, Davoult, & Desroy, 2018; Silva et al., 2005), or dome-shaped chambers (e.g. Smith, Böhlke, Repert, & Hart, 2009) that are restricted in their height in the peripheries. The removable lid maintains the good manoeuvrability underwater that, usually, goes along with smaller devices only (but see Haas et al., 2013). As the chambers are constructed entirely from PMMA, incubated communities are not subjected to shading by opaque materials (e.g. as used for frames in Haas et al., 2013 for stabilization), and the light transmission (90%–92% within the PAR spectrum) exceeds values reported for flexible systems made from polyvinyl chloride (Camp et al., 2015). Thereby, the risks to underestimate processes related to photosynthesis are reduced. In addition, PMMA is durable and can be acid-washed, as needed for precise measurements of DOC and related compounds. The large, rigid and level surfaces of the chambers allow easy customization, permitting multiple sensors and additional device or sampling port attachments that can promote to use the system for numerous experimental strategies. As such, the dual two-way valve mechanism supports additions and removals of small and large amounts of water to be made to and from the chambers without disturbance of the incubation.

A major consideration when deploying benthic chambers is the mixing of the enclosed water. Despite its importance, water circulation systems are often disregarded as the individual power supply and a watertight encapsulation is deemed challenging. We present custom-made, rechargeable pumps that ensure constant mixing within the chambers. The water movement simulates as closely as possible the hydrodynamic conditions of the undisturbed benthic substrate and ensures that water phase measurements reflect the average conditions inside the chamber. The correct alignment of the pump's outflow and the adjustable flow control creates a unidirectional current flow in round chambers, resulting in a radial pressure gradient that simulates sediment percolation as generated by natural flow-topography interactions (Glud, Forster, & Huettel, 1996; Huettel & Rusch, 2000; Rasheed, Wild, Franke, & Huettel, 2004).

Besides the hardware, one of the most important features of the here-presented approach is the use of cloud-based photogrammetry tools. The freely available software enables, for the first time,

non-intrusive assessments of the composition, volume and surface area of benthic communities (Gutierrez-Heredia et al., 2016; Lavy et al., 2015). This step is essential to relate changes in the water chemistry to the benthic community of interest and to make results widely comparable across regions and ecosystems.

Lastly, an important advantage of the system is that all materials required to build the incubation chambers are commercially available online at relatively low cost (c. 250 USD per chamber—including pumps, excluding sensors) (Supporting Information Table S1). In combination with minimal construction efforts (i.e. simple drilling, gluing, etc.) and an available protocol, the system can be used with high replication and at high temporal and spatial resolution without the need for costly infrastructure during deployment and sampling. Thereby, the system can increase the sampling scale of in situ measurements and facilitates precise, standardized flux measurements across many different benthic habitats.

4.2 | Lessons learned from pilot deployments

The field deployment of the autonomous operating chambers provided in situ metabolic data from various benthic shallow-water habitats. The chambers performed consistently when deployed on soft-bottom substrates. On sediments and seagrass meadows, community-wide DO production rates were highly linear over time, and no significant water intrusions were detected. The large PVC skirts around the chambers maintained a good seal from the surrounding water on most substrates. Yet, the linearity of DO fluxes was slightly reduced as some water intrusions were detected on rocky substrates, despite that reliable DO production rates were calculated by local linear regressions. For future considerations, we recommend additional soft edges (e.g. thick closed-cell polystyrene foam) at the bottom of the chambers that, if compressed, fill irregularities in the substrate, forming a good seal between the rock and chamber (Tait & Schiel, 2010). DO production rates during field tests were not linear when the circulation pumps were either switched off (indicator for stratification within the chambers) or when chambers were not secured to the substrates with weights in an intertidal habitat with strong waves (indicator for water intrusions). These tests highlight that special attention should be paid to use and deploy the chambers correctly to achieve reliable measurements. All incubations had in common that the rate of O₂ production initially decelerates as the organisms equilibrate after handling and closing the chambers. Thereby, the use of the *LOLINR* package for local linear regressions offers the advantage to use non-linear time-series data for calculating monotonic metabolic processes (Olito et al., 2017). We further highlight the importance of including continuous monitoring of variables (e.g. dissolved oxygen). Such measurements allow identifying seawater intrusions and improper mixing in the chambers, thereby inform on possible confounding factors for other non-continuously monitored variables. If sensors for continuous measurements cannot be used for a variable of interest, water samples should be taken after an initial equilibration period of 10–20 min.

We highlight the versatility of the chambers in a case study calculating community-wide photosynthesis and respiration budgets of different coral reef communities of the Central Red Sea. The standardized protocol enabled a direct comparison between structurally complex (i.e. coral- and algae-dominated reef communities) and more simple, planar (i.e. sediments) habitats. Thereby, oxygen fluxes of sediments were in the same order of magnitude to previous studies from the Red Sea (e.g. Wild et al., 2009) and elsewhere (e.g. Rasheed et al., 2004) that used small (5–6 L) stirred benthic chambers. A direct comparison to values derived from in situ incubations of complex habitats (i.e. coral- and algae-dominated communities) is not possible, as no comparable approach has been applied before. Consequently, we related resulting oxygen fluxes from our in situ approach to estimated community budgets from ex situ single-organism incubations from the same reef. While in the same order of magnitude, an apparent trend was detected: Community budgets from ex situ single-organism incubations, as a conventional technique, tended to overestimate NPP, while community-wide R and GPP rates were underestimated by up to 90%. This tendency is also evident when comparing our findings to previous coral reef community budgets from single-organism incubations (e.g. Cardini et al., 2016; Naumann et al., 2013). Using this traditional ex situ approach, communities may be disturbed, and cryptic habitats, and particularly cracks and crevices within the reef matrix are not considered. Hence, studies of benthic fluxes in the natural environment result in more realistic estimates, since cryptic habitats encompass about 60%–75% of the total surface area of the reef (Richter & Wunsch, 1999). The organisms inhabiting the cryptic spaces (e.g. sponges, bryozoans and tunicates) are generally not included in ex situ budgets, however, they metabolize organic matter in the order of 15%–30% of the gross production of a coral reef (reviewed in de Jongh & Van Duyl, 2004; Maldonado, Ribes, & Duyl, 2012).

During all experiments, all system components proved extremely reliable. Importantly, the “plug and play” design of system enabled two divers to deploy up to eight chambers and to start the incubations within 45 min of dive time. An additional dive time of around 5–10 min will allow taking a sufficient number of pictures from each community for a later 3D model generation.

Before the final experimental application of chambers, the users should identify the optimal incubation time, and biomass to water volume ratio, to avoid anoxic or hypoxic conditions during incubations of communities of interest. Hence, due to the large size of the selected coral reef communities of the case study, incubations were already terminated after 2 hr. At this time, the O₂ saturation in the chambers reached values of 135% during light, and 35% during dark incubations. The black PVC sleeves for each chamber provided a way to measure dark respiration rates during the day, that can be considered similar to respiration rates during the night (Bidwell, 1977). Sleeves are not needed for respiration measurements during the night.

4.3 | Limitations of benthic in situ incubations

Overall, no direct limitations regarding deployment, replication and reproducibility of in situ incubations were observed during our field

testing. However, some general limitations with benthic chambers include: (a) Depending on the size of the community relative to the water volume, measurements can only be conducted for a few hours out of the day, making daily rates challenging to obtain. Longer incubations would require replacing the seawater periodically to maintain natural conditions (e.g. as in Tait & Schiel, 2010); (b) Although the chambers are among the largest currently available, size constraints still limit incubations to relatively small assemblages and results may not necessarily reflect all aspects of fully sized communities; (c) PMMA effectively removes most UV radiation, hence, the chambers possibly affect measured rates of photosynthesis for very shallow waters, where UV may inhibit some photosynthetic processes; (d) Some environments may be too energetic for using enclosures or do not enable to achieve a good isolation of the water. Additional weights, soft edges on the bottom and more extended PVC flaps on the sides may improve the sealing on hard substrates. We recommend the execution of a leakage test when using the chambers in a new location/type of substrate; and (e) Large incubation chambers provide important information about community-wide metabolic and biogeochemical fluxes; however, individual incubations may be necessary to complement ecological information about the contribution of a particular group or taxa to the community functioning.

4.4 | Potential use in marine and aquatic research

Although this study focused on tropical benthic shallow-water communities, the method can easily be applied to any benthic community of interest that is accessible from land or within the limits of a scientific diving operation. As a descriptive tool at the community level, the chambers offer a new way to achieve reliable data of biogeochemical functioning in response to natural and anthropogenic changes, as synergistic and antagonistic interactions between organisms are not considered when organisms are incubated separately. We highlight the possibility to measure multiple parameters simultaneously (through sensors and water sampling) that can help linking responses that have often been assessed independently only, or where high species diversity and biotic interactions shape ecosystem functions.

Importantly, the presented approach can easily be used in experimental and manipulative strategies to quantify community functional responses to relevant global and local disturbances. While not pursued in the initial construction phase, the large rigid surfaces and the individual power supply of each chamber can support additional accessories, such as peristaltic dosing pumps, immersion heaters or pH flow feed-back control systems as applied previously (Kline, Kuntz, Breitbart, Knowlton, & Rohwer, 2006; Marker et al., 2010). These developments should not be considered as a limitation to the existing approach but rather highlight the advantages of such an adaptable system.

Lastly, flux measurements with benthic chambers can provide a practical tool in quantifying gas (e.g. methane, nitrous oxide, carbon dioxide) emissions from, and carbon sequestration capacity by (known as "blue carbon") highly productive benthic ecosystems (e.g. seagrass meadows or algal beds) (reviewed in Howard, Hoyt,

Isensee, Telszewski, & Pidgeon, 2014). This information may be critical for assessing the capacity these ecosystems have for mitigating climate change (e.g., McGlathery, Anderson, & Tyler, 2001; Yarbro & Carlson, 2008).

5 | CONCLUSION

The here-presented incubation chambers offer an easy and cost-effective sampling solution that overcomes some challenges of conventional respirometry chambers. By allowing non-destructive, in situ metabolic and biogeochemical analysis of both simple and structurally complex benthic communities, the presented chambers offer a versatile platform for novel applications and experimental strategies. Measuring the effects of community interactions underlying benthic–pelagic biogeochemical processes may rapidly advance our understanding of ecosystem functioning and complement findings from aquaria and mesocosm studies. In doing so, the platform can transcend the boundaries of current research in multiple directions: (a) Providing a standardized method that can be applied across multiple habitats; (b) Measuring the response of whole communities, rather than from isolated organisms; (c) Overcoming the shortage of observational and manipulative studies carried out directly in the field, thus producing widely applicable and realistic findings; and (d) Enhancing our mechanistic understanding of how environmental drivers modulate particular processes across different habitats.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

F.R., C.W., S.C., N.R., B.K., R.C. and B.H.J. conceptualized and designed research. F.R., N.R., H.A. and Y.C.E-K. performed research, and F.R., N.R. and Y.C.E-K. analysed data. F.R., C.W., S.C. and N.R. wrote original draft of the manuscript. All authors read and approved the final manuscript.

DATA ACCESSIBILITY

The data used in this study are available at Dryad Digital Repository <https://doi.org/10.5061/dryad.80c1rq2> (Roth et al., 2019).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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