

## ECOLOGY

CO<sub>2</sub> leakage alters biogeochemical and ecological functions of submarine sands

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Subseabed CO<sub>2</sub> storage is considered a future climate change mitigation technology. We investigated the ecological consequences of CO<sub>2</sub> leakage for a marine benthic ecosystem. For the first time with a multidisciplinary integrated study, we tested hypotheses derived from a meta-analysis of previous experimental and in situ high-CO<sub>2</sub> impact studies. For this, we compared ecological functions of naturally CO<sub>2</sub>-vented seafloor off the Mediterranean island Panarea (Tyrrhenian Sea, Italy) to those of nonvented sands, with a focus on biogeochemical processes and microbial and faunal community composition. High CO<sub>2</sub> fluxes (up to 4 to 7 mol CO<sub>2</sub> m<sup>-2</sup> hour<sup>-1</sup>) dissolved all sedimentary carbonate, and comigration of silicate and iron led to local increases of microphytobenthos productivity (+450%) and standing stocks (+300%). Despite the higher food availability, faunal biomass (−80%) and trophic diversity were substantially lower compared to those at the reference site. Bacterial communities were also structurally and functionally affected, most notably in the composition of heterotrophs and microbial sulfate reduction rates (−90%). The observed ecological effects of CO<sub>2</sub> leakage on submarine sands were reproduced with medium-term transplant experiments. This study assesses indicators of environmental impact by CO<sub>2</sub> leakage and finds that community compositions and important ecological functions are permanently altered under high CO<sub>2</sub>.

## INTRODUCTION

The atmosphere takes up large amounts of CO<sub>2</sub> from anthropogenic sources, resulting in global warming and increasing dissolution of CO<sub>2</sub> into seawater, with detrimental consequences for the ocean ecosystems (1). Ocean acidification is predicted to decrease seawater pH by 0.2 to 0.4 units by 2100 at unchanged rates of CO<sub>2</sub> emissions (2). To meet the international goal of limiting global warming to 1.5°C, the use of fossil fuels would have to end before 2040 (3) and may need to be complemented by mitigation technologies. One way to reduce industrial emissions is CO<sub>2</sub> capture and storage (CCS) in the subsurface, which includes subseabed reservoirs (4). This new maritime mitigation technology causes a need for assessments of ecological risk, especially from potential CO<sub>2</sub> leakage (5, 6). Besides reducing effectiveness of the technology, CO<sub>2</sub> leakage from subseafloor reservoirs could lead to extreme pore- and seawater acidification, with pH substantially lower than 7 (7), and thereby negatively affecting the local ecosystem. Current knowledge of high-CO<sub>2</sub> effects on marine ecosystems is mostly based on assessing the vulnerability of individual specimens and mesocosm communities to artificially enhanced CO<sub>2</sub> levels in seawater (8, 9). However, knowledge on long-term ecosystem-level responses and assessment of adaptation and resilience of communities are limited (10, 11). Thus, a crucial question

remains whether CO<sub>2</sub> leaks can locally lead to profound and persistent changes of element cycling, as well as to negative effects on ecosystem functions and services, including biodiversity and productivity. This question calls for field studies of naturally complex, dynamic ecosystems under long-term high-CO<sub>2</sub> exposure (for example, caused by volcanic degassing) (12, 13). To our knowledge, this study is the first synchronous assessment (that is, occurring at the same time and place) of high-CO<sub>2</sub> effects covering all trophic levels from microbes to macrofauna in submarine sands. Sands make up a substantial proportion of shelf seas and play a critical role as biogeochemical filters at the land-sea boundary (14). We investigated for over 2 years the impact of CO<sub>2</sub> degassing on benthic biogeochemistry and community structure from microbes to macrofauna, focusing on carbon cycling (primary productivity and organic matter remineralization). In addition, we transplanted sediments between CO<sub>2</sub>-vented and nonvented sites to assess the immediate effects of changing CO<sub>2</sub> levels within a year and to test whether we could reproduce the natural patterns. On the basis of a meta-analysis of previous high-CO<sub>2</sub> impact studies, we derived and tested the following hypothesis: CO<sub>2</sub> leakage enhances benthic primary production but negatively affects ecosystem functional diversity, with consequences for the benthic food web and carbon fluxes.

## RESULTS

## Identification of natural analog sites for the leakage scenario

The Aeolian archipelago in the southern Tyrrhenian Sea is a ring-shaped volcanic arc (fig. S1A), composed of 7 islands and 10 seamounts, associated with the Peloritanean-Calabrian orogenic belt (15). Panarea, the smallest (3.3 km<sup>2</sup>) Aeolian island, represents the emergent part of a wide stratovolcano more than 2000 m high and 20 km across (16). In 2011, we surveyed a number of CO<sub>2</sub>-vented sites around Panarea to identify those to be used as “natural laboratories” to assess pure CO<sub>2</sub> effects, finally selecting the eastern side of Basiluzzo Islet (a rhyolitic dome northeast to Panarea). Two sites (fig. S1B) best fulfilled the “natural laboratory”

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criteria: (i) continuous, dispersed degassing of CO<sub>2</sub> through sand causing low pH; (ii) similar oxygen availability and negligible coemission of toxic substances or microbial energy sources such as sulfide and methane; and (iii) no significant temperature anomalies from hydrothermalism. The

selected “CO<sub>2</sub>-R” and “CO<sub>2</sub>-G” sites showed comparable environmental conditions to the reference (Table 1), and rather evenly distributed gas leakage (fig. S1D; density of two to three gas bubble strings per m<sup>2</sup>). The reference site (REF) showed no gas emissions (fig. S1C).

**Table 1. Main environmental characteristics of sampling sites at Basiluzzo Islet (Panarea Island, Italy).** na, not available.

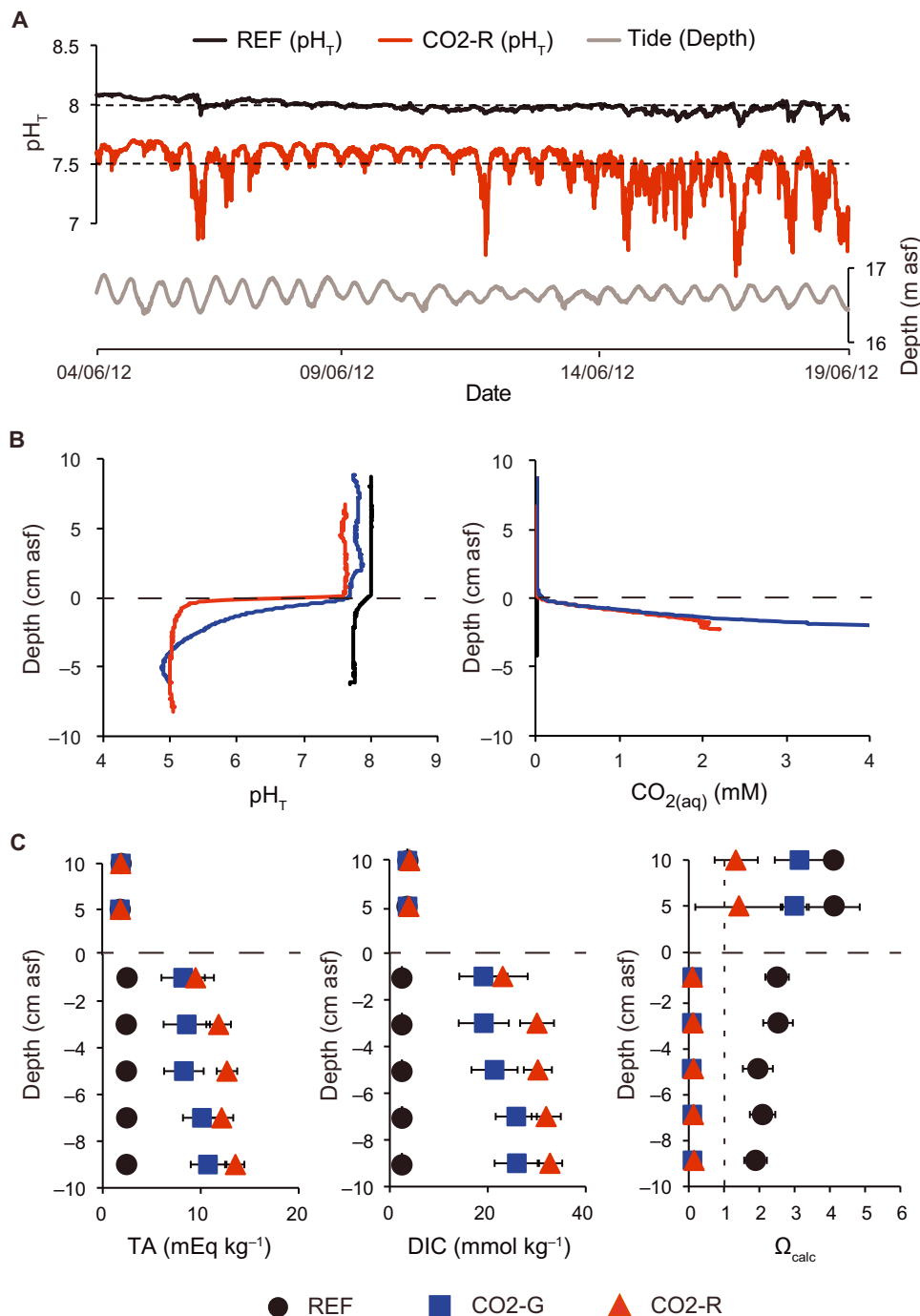
Site		REF	CO <sub>2</sub> -G	CO <sub>2</sub> -R
	Coordinates	N 38°39.827'	N 38°39.820'	N 38°39.749'
		E 15°07.118'	E 15°07.137'	E 15°07.123'
	Water depth	m	14–17	21
	Area <sup>†</sup>	m <sup>2</sup>	100	35
	Seagrass meadows	<i>Posidonia oceanica</i>	<i>Posidonia oceanica</i>	<i>Posidonia oceanica</i>
Bottom water properties (10 cm asf)	Temperature <sup>‡</sup>	°C	18.8–19.5	18.8–19.0
	Salinity	‰	38	38
	O <sub>2</sub> <sup>§,***</sup>	μmol liter <sup>-1</sup>	243 (±9)	na
	ORP <sup>§,***</sup>	mV	245 (±75)	na
	pH <sub>T</sub> <sup>¶</sup>		7.9	7.8
	DIC <sup>¶</sup>	mmol liter <sup>-1</sup>	2.1 (±0.1)	2.3 (±0.1)
	TA <sup>¶</sup>	mEq kg <sup>-1</sup>	2.3 (±0.1)	2.3 (±0.1)
	Ω <sub>calc</sub> <sup>  </sup>		4.0 (±0.2)	3.2 (±0.9)
	Si(OH) <sub>4</sub> <sup>††</sup>	μmol liter <sup>-1</sup>	2.1 (±1.0)	2.7 (±1.8)
	PO <sub>4</sub> <sup>3-††</sup>	μmol liter <sup>-1</sup>	0	0.3 <sup>††</sup>
	NH <sub>4</sub> <sup>††</sup>	μmol liter <sup>-1</sup>	4.9 (±1.3)	1.8 (±1.5)
	NO <sub>2</sub> <sup>-</sup> /NO <sub>3</sub> <sup>-††</sup>	μmol liter <sup>-1</sup>	0.4 (±0.1)	0.8 (±0.4)
	Fe <sup>††,*</sup>	μmol liter <sup>-1</sup>	0.1 (±0.02)	0.2 <sup>††</sup>
	Mn <sup>††</sup>	μmol liter <sup>-1</sup>	0	0.5 <sup>††</sup>
Sediment properties (0–10 cm layer)	Color		Gray	Gray
	Median grain size		Coarse sand	Coarse sand
	Porosity <sup>§§</sup>	%	38–44	40–42
	Carbonate content <sup>¶¶,***</sup>	mg g <sup>-1</sup>	9.34 (±1.13)	0.04 (±0.02)
Porewater fluxes	Porewater pH <sub>T</sub> <sup>   </sup>		7.5–7.4	5.5–5.4
	Gas bubbling		No	Yes
	CO <sub>2</sub> content	%	—	90–97
	Gas flow <sup>†††</sup>	Liter m <sup>-2</sup> hour <sup>-1</sup>	—	80
	Porewater flow <sup>†††</sup>	Liter m <sup>-2</sup> day <sup>-1</sup>	11–69	12–45
	DIC flux <sup>§§§,***</sup>	mol m <sup>-2</sup> day <sup>-1</sup>	0.0–0.2	2.4–13.8
	Si(OH) <sub>4</sub> <sup>§§§,***</sup>	mmol m <sup>-2</sup> day <sup>-1</sup>	0.0–0.9	10.0–41.7
			17.6–28.2	

†Patch of bare sediment within seagrass bushes. ‡Average temperatures in 2011 to 2013 measured in situ with SEAGUARD at 30 cm asf. §Average (±SD;  $n = 4000$ ) of 2012 data collected in situ with RBR sensors over 15 days at 2 cm asf. ¶Average of 2011 to 2013 measurements ( $n = 9$ ). ||Calculated using R package seacarb v 3.0.11; input variables, pH<sub>T</sub> and TA (for details, see table S1). ††Average (±SD;  $n = 3$ ) of 2013 data; one sample available for PO<sub>4</sub><sup>3-</sup>; one sample available for dissolved Fe and Mn at CO<sub>2</sub>-G. †††No replicates available. §§Average porosity assessed from sediment samples collected in 2011 to 2013 ( $n = 3$ ). ¶¶Average (±SD;  $n = 8$ ) of CaCO<sub>3</sub> content in 0- to 2-cm and 4- to 6-cm layers for 2012 and 2013. |||Average at top (0 to 2 cm) and bottom (8 to 10 cm) layers of sediment profile in 2011 to 2013 (for details, see table S1). ††††At seafloor during low tide. †††††Range (2012 to 2013) of porewater efflux. §§§§Range of fluxes measured in 2013 ( $n = 6$ ). \* $P < 0.05$ , \*\*\* $P < 0.001$ ; Welch's  $t$  test between REF and CO<sub>2</sub>-R.

**Gas and bottom water chemistry**

The gas bubbles emanating at CO<sub>2</sub>-R and CO<sub>2</sub>-G consisted mainly of CO<sub>2</sub>, with traces of CO and CH<sub>4</sub> (0.32 and 0.01 parts per million, respectively). Tidal variations in venting, with enhanced CO<sub>2</sub> leakage during low tide, caused peaks in pH<sub>T</sub> (total scale; Fig. 1A). The CO<sub>2</sub> emission rates

during low tide were 6.6 mol CO<sub>2</sub> m<sup>-2</sup> hour<sup>-1</sup> at CO<sub>2</sub>-R and 4.2 mol CO<sub>2</sub> m<sup>-2</sup> hour<sup>-1</sup> at CO<sub>2</sub>-G. Bottom water oxidation-reduction potential (ORP) at CO<sub>2</sub>-R was significantly lower than at REF (Table 1), being driven by effluxes of Fe<sup>2+</sup>-enriched porewater at the vented sites (see section below). Bottom water O<sub>2</sub> concentration varied slightly with the light



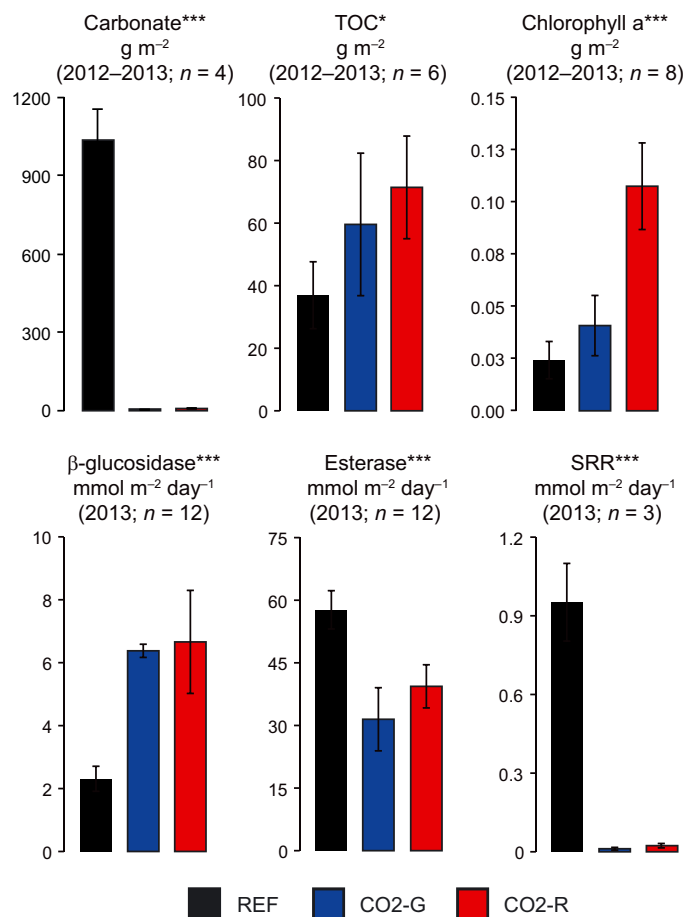
**Fig. 1. Chemico-physical conditions.** (A) Daily variation of bottom water pH<sub>T</sub> over 15 days of in situ measurements with RBR sensors (dashed black line represents the average) at REF and CO<sub>2</sub>-R sites and the tide shown as variation in water depth (right y axis) of the CO<sub>2</sub>-R site in 2012. (B) Spatial variation of pH<sub>T</sub> and dissolved CO<sub>2</sub> [CO<sub>2(aq)</sub>] at REF, CO<sub>2</sub>-G, and CO<sub>2</sub>-R at high resolution below and above sediment surface (asf; spatial scale, 200 μm). The profiles were obtained with in situ microsensors in 2012. (C) Bottom water and porewater profiles for total alkalinity (TA), dissolved inorganic carbon (DIC), and saturation of calcite (Ω<sub>calc</sub>); dashed line is the Ω<sub>calc</sub> state threshold for carbonate dissolution. TA and DIC are average data (±SD; n = 6) of samples collected during campaigns 2012 to 2013. Ω<sub>calc</sub> was calculated from pH<sub>T</sub> and TA data.

period due to photosynthesis, with the average being higher at CO<sub>2</sub>-R than at REF (Table 1).

The bottom water pH<sub>T</sub> measured at 5 to 10 cm above seafloor (asf) was, on average, 7.9 at REF and 6.6 to 7.7 at CO<sub>2</sub>-R (Fig. 2B), compared to 7.5 to 7.9 at CO<sub>2</sub>-G (table S1). Saturation states of calcite ( $\Omega_{\text{calc}}$ ) and aragonite ( $\Omega_{\text{ara}}$ ) were lower at the vented sites than at REF, but always >1 (Fig. 1C and table S1). Only marble tiles exposed to the vented seafloor partially dissolved within 1 year ( $n = 6$ ; dissolution rates, 0.02 to 9.94 mg day<sup>-1</sup>), whereas those with a distance >85 m away from the venting area center (CO<sub>2</sub>-R) did not show dissolution ( $n = 42$ ). Nutrient levels (phosphate, ammonium, nitrite, and nitrate) in the bottom water did not differ significantly between the CO<sub>2</sub>-impacted sites and REF (Table 1). The silicate content was also similar, with 1.2 to 1.8  $\mu\text{M}$  in 2012 and 1.7 to 3.5  $\mu\text{M}$  in 2013. However, iron and manganese bottom water concentrations increased at the CO<sub>2</sub>-vented sites and were highest at CO<sub>2</sub>-R (Table 1).

### Porewater chemistry

At REF, the pH<sub>T</sub> decreased slightly with increasing sediment depth at REF, from 7.9 (sediment surface) to 7.7 [2.0 cm below seafloor (bsf)]



**Fig. 2. Biogeochemical conditions at investigated sites.** All data are integrated (summed up) over the 0- to 5-cm sediment layer, except for extracellular enzymatic activity ( $\beta$ -glucosidase and esterase) data, which are integrated over the 0- to 2-cm layer (error bars are  $\pm$ SD; year and number of sampling are given in each plot). TOC, total organic carbon; SRRs, sulfate reduction rates. Stars indicate significant differences between sites [analysis of variance (ANOVA); \* $P < 0.05$ , \*\*\* $P < 0.001$ ; for details, see table S2C].

and then remained constant (Fig. 1B and table S1). In contrast, at CO<sub>2</sub>-G and CO<sub>2</sub>-R, a pH<sub>T</sub> of ca. 5.5 was reached already at 2.5 and 0.5 cm bsf, respectively (Fig. 1B and table S1). CO<sub>2(aq)</sub> increased rapidly with sediment depth, from 0.02 mM (sediment surface) to 5.3 mM (2 cm bsf) at both vented sites. O<sub>2</sub> penetrated to 2 cm bsf at REF and CO<sub>2</sub>-G sediments and to ca. 1 cm bsf at CO<sub>2</sub>-R (fig. S2A). ORP was constant at REF and decreased at CO<sub>2</sub>-G and CO<sub>2</sub>-R (to  $-50$  mV within 1 cm bsf; fig. S2A). No sulfide was detected in the subsurface porewaters, but peaks of a few micromolar were measured directly at the sediment surface at REF and CO<sub>2</sub>-G and to a lesser extent at ca. 0.5 cm bsf at CO<sub>2</sub>-R (fig. S2A). Hydrogen concentrations were below 1  $\mu\text{M}$  (detection limit, 0.3  $\mu\text{M}$ ) and constant down to 5 cm bsf at all sites (fig. S2A). Together, the chemical gradients indicated that CO<sub>2</sub>-R was more strongly vented than CO<sub>2</sub>-G.

In REF sediments, porewater total alkali (TA) was constant but increased substantially with depth at the CO<sub>2</sub>-vented sites (Fig. 1C).  $\Omega_{\text{calc}}$  and  $\Omega_{\text{ara}}$  decreased to <1 at the vented sites, whereas they remained around 2 and 1 at REF, respectively (Fig. 1C and table S1). Porewater at the vented sites was significantly enriched in silicate, iron, manganese, and, somewhat, phosphate (fig. S2B). Fe<sup>2+</sup> was almost absent from REF porewaters, whereas it reached 0.5 to 1 mM at the vented sites, explaining the ORP dynamics in porewater and bottom waters and also the enhanced bottom water concentrations. In contrast, B, Ca, Na, Mg, Sr, Li, and K concentrations were similar at REF and CO<sub>2</sub>-vented sites.

### Sediment grain size, carbonate, and elemental composition

All three sites were dominated by coarse sand with similar porosity and grain size distribution (Table 1). Concurrent with the observed undersaturation in calcite and aragonite in porewaters of the vented sites, the solid-phase carbonate content was about 100 to 200 times lower compared to those in REF sediments (Table 1 and Fig. 2). In accordance with the high porewater Fe concentration, also solid Fe was elevated at the vented sites (3.4 mg g<sup>-1</sup> at CO<sub>2</sub>-R versus 0.4 mg g<sup>-1</sup> at REF). Total organic carbon (TOC) was low (<0.1%) but approximately twofold higher in the surface sediments of CO<sub>2</sub>-R and CO<sub>2</sub>-G compared to REF (Fig. 2). Total organic nitrogen (TN) was also very low (<0.2  $\mu\text{g mg}^{-1}$ ) at all three sites, leading to a C/N ratio of ca. 4 to 7.5 in the surface sediments. Both TOC and TN were higher in 2013 than in 2012 (table S2A).

### Fluxes and remineralization rates

Benthic chambers placed in between the bubble streams at the CO<sub>2</sub>-vented sites showed a decreasing pH in the enclosed water bodies with time, together with a substantial efflux of dissolved organic carbon (DIC) and silicate from the sediment (table S3). In comparison, at REF, no effluxes of silicate or DIC were detected, and the chamber water pH<sub>T</sub> remained stable at 8.0 in incubations of up to 5 hours.

Benthic chamber measurements at the vented sites showed similar advective fluid flow rates as those at REF (Table 1). On the basis of ORP signals, porewater iron concentrations, and fluid flow rates, it is likely that a substantial iron efflux occurred at the vented sites. Thus, respiration rates were corrected for potential oxygen consumption by purely chemical Fe<sup>2+</sup> oxidation, which amounted to 1 to 7% of the total oxygen consumption at the vented sites. At the time of chamber deployments, the seafloor at all sites showed net oxygen consumption, and respiration always exceeded photosynthetic O<sub>2</sub> production even during daytime. However, both oxygen respiration and production were substantially higher at the vented sites compared to REF (Table 2). Diffusive oxygen fluxes calculated from microprofiler measurements (fig. S2) were <10% of the total fluxes but were also higher at CO<sub>2</sub>-R (2.6 mmol m<sup>-2</sup> day<sup>-1</sup>) than at REF (1.7 mmol m<sup>-2</sup> day<sup>-1</sup>).

**Table 2. Benthic oxygen fluxes.** Oxygen exchange (transparent chamber; net O<sub>2</sub> flux), oxygen respiration (masked chamber; O<sub>2</sub> respiration), and oxygen production [GPP = net O<sub>2</sub> flux + (O<sub>2</sub> respiration)] rates obtained from benthic chambers deployed in 2013; oxygen production-to-respiration (GPP/R) ratio, respiration per unit of total biomass (R/B<sub>Total</sub>), and respiration per unit of heterotroph biomass (bacteria and animals; R/B<sub>Heterotrophs</sub>). Benthic masked chambers rates ( $n = 2$ ) and average (mean with  $\pm$ SD in parenthesis), maximum (Max), and minimum (Min) rates of transparent chambers ( $n = 3$  to 4) and O<sub>2</sub> production ( $n = 6$  to 8) are given. nt, not tested for significance level; ns, not significant ( $P > 0.05$ ).

		Net O <sub>2</sub> flux (daylight) <sup>ns</sup>	O <sub>2</sub> respiration (masked) <sup>nt</sup>	O <sub>2</sub> production**	GPP/R	R/B <sub>Total</sub>	R/B <sub>Heterotrophs</sub>
		mmol m <sup>-2</sup> day <sup>-1</sup>	mmol m <sup>-2</sup> day <sup>-1</sup>	mmol m <sup>-2</sup> day <sup>-1</sup>		day <sup>-1</sup>	day <sup>-1</sup>
REF	Mean	-7 (6)	na	10 (8) <sup>†</sup>	0.6	0.06/0.14	0.07/0.18
	Max	-11	-23	21			
	Min	-2	-10	0			
CO2-G	Mean	-58 (63)	-188 <sup>†</sup>	130 (63)	0.7	1.13	2.06
	Max	-151	na	173			
	Min	-15	na	37			
CO2-R	Mean	-18 (6)	na	55 (37) <sup>§</sup>	0.8	0.16/0.49	0.41/1.14
	Max	-24	-106	96			
	Min	-11	-38	15			

<sup>†</sup>Average ( $\pm$ SD;  $n = 6$ ) of O<sub>2</sub> production calculated from each Net O<sub>2</sub> flux using O<sub>2</sub> respiration from both masked chambers. <sup>‡</sup>At CO2-R, only one masked chamber was available. <sup>§</sup>Average ( $\pm$ SD;  $n = 8$ ) of O<sub>2</sub> production calculated from each O<sub>2</sub> flux using O<sub>2</sub> respiration from both masked chambers. <sup>\*\*</sup> $P < 0.01$  (Welch's  $t$  test between REF and CO2-R).

Standard proxies for microbial activities were also influenced by high CO<sub>2</sub>. The  $\beta$ -glucosidase hydrolytic activity measured at substrate saturation ( $V_{\max}$ ) was significantly higher at CO2-G and CO2-R than at REF, whereas the aminopeptidase and esterase activities were significantly lower at the vented sites (Fig. 2 and fig. S3). No significant differences between sites were observed in chitinase activity (fig. S3). SRRs determined in vitro were substantially higher at REF than at both vent sites (Fig. 2). Its contribution to aerobic benthic respiration was 16% at REF and 0.04% at the vent sites.

### Microbial community patterns

Benthic diatoms (Bacillariophyceae) dominated the microphytobenthos in the surface sediment layer ( $95 \pm 4\%$  of cells), and their abundances were up to three times higher at CO2-R than at CO2-G and REF. Abundances were ca. 50% lower in 2012 than in 2013 at all sites:  $4709 \pm 636$  cells cm<sup>-2</sup> and  $8932 \pm 560$  cells cm<sup>-2</sup> at CO2-R,  $1552 \pm 158$  cells cm<sup>-2</sup> and  $6079 \pm 973$  cells cm<sup>-2</sup> at CO2-G, and  $1744 \pm 150$  cells cm<sup>-2</sup> and  $4909 \pm 218$  cells cm<sup>-2</sup> at REF, respectively (table S2A). CO2-R also showed the highest content of chlorophyll a (Chl a) pigments (Fig. 2). Chl a made up 73 to 95% of the chloroplastic pigment equivalents (CPEs), indicating that the pigments originated mostly from living cells. At all sites, Chl a decreased with sediment depth, with 33 to 50% concentrated in the top 2 cm, and was positively correlated with TOC (Pearson's  $R = 0.4967$ ;  $P < 0.001$ ;  $n = 63$ ).

In contrast, total bacterial cell abundances were similar at all sites (Fig. 3A). The highest abundances occurred in the upper sediment layer (ca.  $0.7 \times 10^9$  cells ml<sup>-1</sup>) and decreased with sediment depth. Bacteria dominated [54 to 71% of 4',6-diamidino-2-phenylindole (DAPI)-stained cells] over Archaea (3 to 1% of DAPI-stained cells).

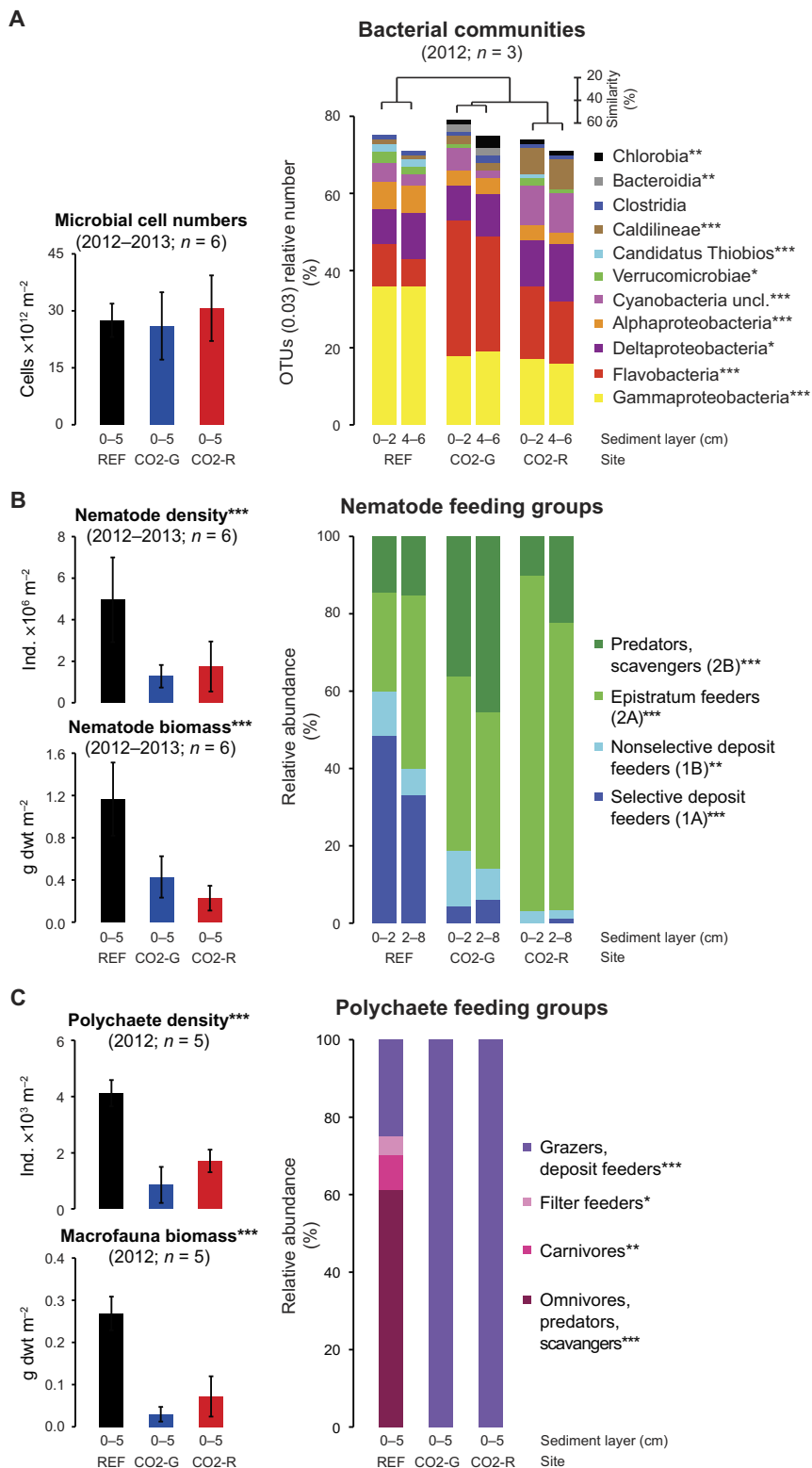
With 454 massively parallel tag sequencing (MPTS), we recovered a total of 9674 bacterial operational taxonomic units (OTU<sub>S0,03</sub>) from all sediment layers combined. At the class level, bacterial communities of the vented sites were dominated by Flavobacteria, Gammaproteobacteria,

Deltaproteobacteria, Caldilineae, and unclassified Cyanobacteria (Fig. 3A). Functional group analysis (table S4) showed that CO<sub>2</sub> leakage stimulated primary producers (that is, Cyanobacteria and Chlorobia), aerobic and anaerobic organic matter-degrading bacteria (that is, Flavobacteria and Caldilineae), some metal-reducing bacteria (that is, Desulfuromonadales), and some ferrotrophic bacteria (that is, Rhodobacteraceae). Concurrent with the negative impact of high CO<sub>2</sub> on SRR, the relative sequence abundances of sulfate reducers (that is, Desulfobacterales) were reduced, as well as those of sulfur oxidizers (that is, *Candidatus Thiobios*) and nitrifiers (that is, *Nitrospira*, *Nitrosospira*, and *Nitrosococcus*).

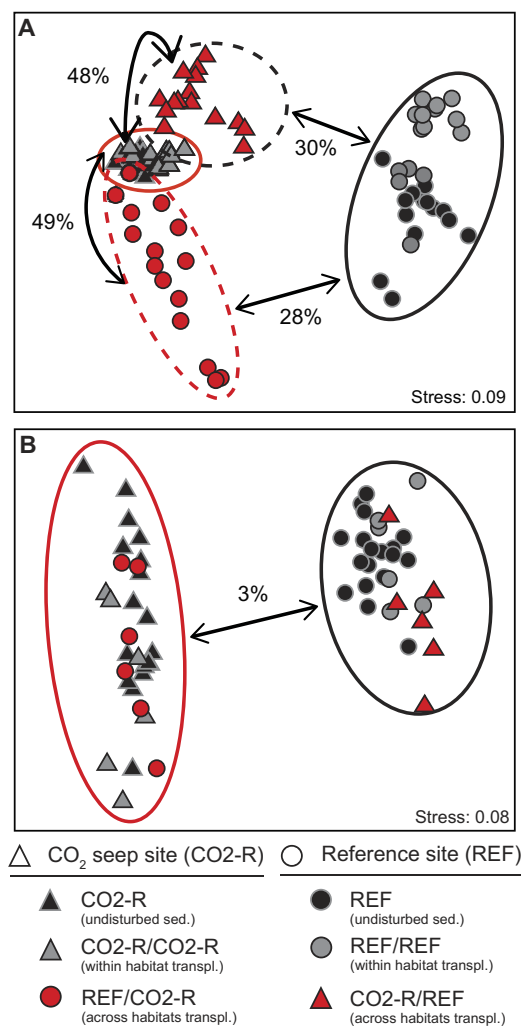
pH and DIC were the main environmental parameters influencing the bacterial community structure, explaining more than 35% of the variance in OTU composition for all data sets [that is, amplified ribosomal intergenic spacer analysis (ARISA) and MPTS; table S5A]. The three sites differed in bacterial community composition by 58 to 74% (that is, ARISA; fig. S4A). The principal source of variability was associated with the differences in CO<sub>2</sub> flux and associated parameters (table S6).

### Benthic invertebrate communities

Total meiofauna density was much higher at REF than at the vented sites [ $1019 \pm 354$  individuals (Ind.) m<sup>-2</sup> versus  $407 \pm 237$  Ind. m<sup>-2</sup>], which was mirrored in all taxa: nematodes (Fig. 3B) and copepods, which dominated, but also nauplii, polychaetes, and tardigrades (table S7). Total meiofauna and nematode abundances decreased rapidly along the sediment profile (ANOVA;  $P < 0.001$ ;  $F_{3,71} = 19.0$  and  $F_{3,71} = 11.6$ , respectively), more steeply at the CO<sub>2</sub>-vented sites than at REF. At REF, this gradient was not reflected in nematode biomass, conversely at CO<sub>2</sub>-vented sites, the biomass was significantly higher in the top layer (0 to 2 cm) than in the other layers (ANOVA;  $P < 0.001$ ;  $F_{3,20} = 6.6$  for CO2-G and  $F_{3,20} = 12.7$  for CO2-R). Community structure for meiofauna at the higher taxon level differed significantly between the three sites (tables S6 and S7) but was even more different at the nematode species level (table S7 and Fig. 4B). Year-to-year differences in the nematode



**Fig. 3. Community composition of studied sampling site (top 5 cm of sediments).** (A) Microbial cell numbers and bacterial community structure, as described by 454 MPTS, showing relative number of sequences for dominant bacterial classes (that is, OTUs > 0.1%) clustered according to similarity [based on the Bray-Curtis distance matrix, surface, and subsurface layer; analysis of similarities (ANOSIM);  $R = 0.948$ ;  $P < 0.001$ ]. (B) Nematode density and biomass and relative abundance of nematode feeding groups. (C) Polychaete density, macrofauna biomass, and relative abundance of polychaete feeding groups. Error bars are  $\pm$ SD; year and number of sampling are given in each plot; stars indicate significant differences between one or both CO<sub>2</sub>-vented sites and the REF (ANOVA; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ; for details, see table S2E). Ind., individuals; dwt, dry weight; uncl., unclassified.



**Fig. 4. Effect of medium-term transplantations on benthic community composition.** Nonmetric multidimensional scaling ordination plot (based on the Bray-Curtis dissimilarity matrix) of bacterial (ARISA-OTUs-based) and nematode (genus level) community structure for undisturbed sediment (sed.), within-habitat, and across-habitat transplants (transpl.). **(A)** Bacterial community of REF sediment (top, 10-cm layer) transplanted into CO<sub>2</sub>-R sediment (REF/CO<sub>2</sub>-R) was significantly different from the source community after 1 year (ANOSIM;  $R = 0.982$ ;  $P < 0.001$ ). The bacterial community of CO<sub>2</sub>-R transplanted into REF sediment (CO<sub>2</sub>-R/REF) was also significantly different to that of the original site (ANOSIM;  $R = 0.961$ ;  $P < 0.001$ ). Both communities that were transplanted within their own habitat remained similar to the undisturbed ones. **(B)** Nematode community of the 0- to 4-cm sediment horizon 1 year after transplantation was significantly different in across-habitat transplants (ANOSIM;  $R = 0.723$ ;  $P < 0.001$ ).

community structure at each site accounted for only 8.1% of the variance and could be explained by shifts in relative abundance of the three dominating species at each site, together explaining 40 to 80% of the annual variance (table S6). Most dominant species at REF (that is, >5% relative abundance; table S7) occurred also at the vented sites, but only with <0.5%. Instead, *Microloaimus compridus*, *Microloaimus honestus*, and *Oncholaimus campylocercoides* became highly dominant at both vent sites with >8% (table S7). Generally, the most abundant nematode species at vent sites were rare at REF (0.05 to 0.17% relative abundance). The abundance of selective deposit feeders (1A)

decreased at the vent sites, which were instead dominated by predators and scavengers (2B; CO<sub>2</sub>-G) or epistratum feeders (2A; CO<sub>2</sub>-R; Fig. 3B). This finding is in line with the highest diatom densities at CO<sub>2</sub>-R. pH was the most influencing environmental parameter for the nematode assemblage structure over the whole sediment profile, explaining more than 40% of the variance (table S5A).

Macrofauna was dominated by polychaetes at all sites, with relative abundances of  $71 \pm 8\%$  (REF),  $69 \pm 45\%$  (CO<sub>2</sub>-G), and  $45 \pm 36\%$  (CO<sub>2</sub>-R). Polychaete abundances, as well as the whole macrobenthos biomass, were substantially lower at the vent sites compared to REF (Fig. 3C). The polychaete community structure also differed significantly between REF and the two vent sites (tables S6 and S7 and fig. S4C). At the vent sites, all polychaetes were grazers or deposit feeders, whereas at REF, filter feeders, carnivores, and omnivores also occurred (Fig. 3C).

### Sediment transplantation

Sediment was transplanted between REF and CO<sub>2</sub>-R, and after 1 year, sediment parameters, microbial activities, nematode density, and bacterial and nematode community structure were compared. In REF/CO<sub>2</sub>-R, porewater DIC, TA, silicate, and iron increased, and pH decreased, whereas the opposite trend occurred in CO<sub>2</sub>-R/REF (fig. S3 and table S8). Both pH and carbonate content of CO<sub>2</sub>-R/REF remained significantly lower than those of REF after 1 year (table S8 and Table 1). Still, the carbonate content increased 35-fold in the 0- to 2-cm sediment layer and doubled in the 4- to 6-cm sediment layer. In contrast, carbonates dissolved in REF/CO<sub>2</sub>-R both in the 0- to 2-cm sediment layer and in the 4- to 6-cm sediment layer (table S8).

As to microbial activities, the  $\beta$ -glucosidase activity increased in REF/CO<sub>2</sub>-R, whereas aminopeptidase and esterase activities decreased (fig. S3). SRR also decreased in REF/CO<sub>2</sub>-R and did not recover in CO<sub>2</sub>-R/REF (fig. S3). Chl a increased in REF/CO<sub>2</sub>-R and decreased somewhat in CO<sub>2</sub>-R/REF (fig. S3).

It took a year until the cross-transplanted bacterial and nematode communities resembled the respective background communities (Fig. 4). However, the nematode density decreased significantly in REF/CO<sub>2</sub>-R but did not increase in CO<sub>2</sub>-R/REF (fig. S3). pH and DIC were the main environmental factors responsible for the observed shifts in bacterial and nematode community structure in the transplanted sediments (fig. S5B).

### DISCUSSION

Here, we focused on permeable sandy marine ecosystems that occupy large areas of the continental shelves, the target areas for submarine CCS (7). To assess potential ecological risks from CO<sub>2</sub> leakage (5), we synchronously investigated the geochemical phenomena of CO<sub>2</sub> leakage and its effects on community function and composition including different benthic size classes and trophic groups from microbes to macrofauna. Moreover, our multidisciplinary integrated approach allowed us to test the main hypothesis derived from a meta-analysis of previous experimental and in situ studies (Table 3): CO<sub>2</sub> leakage locally enhances primary production in sandy sediments, but it negatively affects ecosystem functional diversity, with consequences for the benthic food web and carbon fluxes.

### Geochemical phenomena of CO<sub>2</sub> venting

Here, we compared the geochemical characteristics of a nonvented REF, representing the natural baseline, with two different CO<sub>2</sub>-vented sites, CO<sub>2</sub>-R and CO<sub>2</sub>-G, of similar hydrological and sedimentological characteristics (Table 1 and fig. S1). CO<sub>2</sub> leakage was identified visually as

**Table 3. Summary of CO<sub>2</sub> impact on benthic organisms and processes at the Basiluzzo Islet sites (soft sediments) and comparison with available benthic data from other shallow natural CO<sub>2</sub> vents (soft and rocky seafloor) at Ischia, Vulcano, and Papua New Guinea.** Significant deviations from the REF are described by upward (enhancing) or downward (declining) arrows, respectively, or by + for changes in community structure. 0, neutral; PNG, Papua New Guinea; OC, organic carbon; EEA, extracellular enzymatic activity; Undist., undisturbed sediments at CO<sub>2</sub> vents; Transpl., medium-term (1 year) transplanted sediments from reference to CO<sub>2</sub>-impacted site (REF/CO<sub>2</sub>-R). For references and detailed description of CO<sub>2</sub> affects on marine environments at natural CO<sub>2</sub> vents, see table S9.

		Basiluzzo		Ischia	Vulcano	PNG
		Undist.	Transpl.			
Invertebrates	Community structure	+	+	+	na	+
	Abundance	↓	↓	↑↓	na	↓
	Biomass	↓	na	na	↓	↓
Seagrass	Density	0	na	↑/0	↓	↑
	Biomass	na	na	na	↓	↑
	Photosynthetic activity	na	na	0	↑	↑
Macroalgae	Community structure	na	na	+	na	+
	Community structure	+	na	na	+	na
Microphytobenthos	Abundance	↑	na	na	↑	na
	Biomass	↑	↑	na	↑	na
Bacteria	Community structure	+	+	na	+	+
	Abundance	0	0	na	na	na
Primary production and OC remineralization	Oxygen production	↑	na	na	na	na
	Oxygen respiration	↑	na	na	na	na
	SRR	↓	↓	na	na	na
OC degradation	β-glucosidase (EEA)	↑	↑	na	na	na
	Esterase (EEA)	↓	↓	na	na	na
Nutrients flux	Silicate, iron	↑	↑	na	na	na

escaping gas bubbles of >90% CO<sub>2</sub> content (fig. S1D). Similar to this natural analog, CO<sub>2</sub> upward migration through subseafloor sediment strata in the case of CCS leakage would result in the dissolution of the gas, leading to subsequent reactions with porewater and sediments, so that only a fraction of the gas would escape to the water column (17). As a consequence of CO<sub>2</sub> dissolution, porewater pH and carbonate saturation would decrease, whereas DIC and TA will increase (18). We recorded all of these geochemical phenomena at the Basiluzzo vent sites: CO<sub>2</sub> venting through the sandy sediments resulted in a loss of solid-phase carbonate and a decrease in porewater pH (Table 1, Fig. 1B, and table S1), as well as in emission of acidified porewaters to the water column (Fig. 1A and table S3). Hence, the long-term geochemical consequence of CO<sub>2</sub> leakage through marine sediments would be the local decline of buffering capacity and a reduction of the mineral carbon sink (Fig. 5).

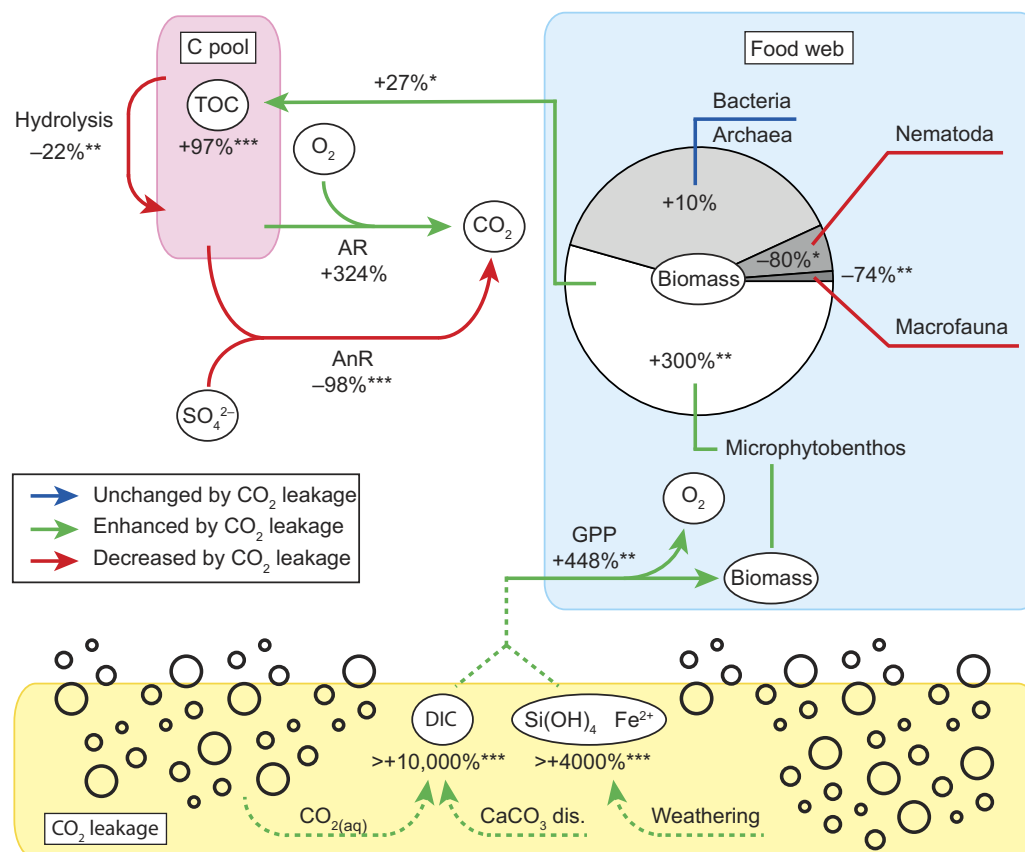
The CO<sub>2</sub>-enriched porewater fluids at Basiluzzo did not contain elevated sulfide or methane concentrations typically associated with hydrothermalism. Boron, found at high concentrations in Panarea hydrothermal fluids (19), showed typical seawater concentrations in the Basiluzzo porewaters. The temperature anomaly in the surface sediments at the vent sites was negligible. However, we recorded some

ORP dynamics in the bottom water above the vents, as well as substantially elevated iron, manganese, and silicate concentrations in the porewaters, derived from subseafloor hydrothermal and/or CO<sub>2</sub> reactions with the bedrock and overlying sands. Together with the high CO<sub>2</sub> fluxes, these high iron and silicate concentrations apparently enhanced productivity of the microphytobenthos. In an analog CCS leakage scenario, the CO<sub>2</sub> co-leakage will depend on the type of geological reservoir, and these could include mineral products of weathering from CO<sub>2</sub> exposure as well as hydrocarbons (18).

### Effects of CO<sub>2</sub> venting on primary production and microphytobenthos

The CO<sub>2</sub>-vented sites had higher microphytobenthos standing stocks, higher Chl a content, and more TOC, mostly due to an enhanced productivity of benthic diatoms (Figs. 2 and 5), especially where CO<sub>2</sub> leakage was the highest (CO<sub>2</sub>-R). This effect was reproduced by transplantation of reference sands to CO<sub>2</sub>-R, showing increased Chl a content after 1 year (fig. S3). Noncalcifying benthic primary producers are likely to profit from high CO<sub>2</sub> (Table 3) as a result of the reduction in the energy costs for carbon concentration mechanisms (20). In addition, the higher availability of nutrients (especially silicate and iron, but also phosphorus) in





**Fig. 5. Synthesis scheme summarizing the effects of CO<sub>2</sub> leakage on the benthic food web.** Values depict the percentage of increased or decreased standing stock or activity in CO<sub>2</sub>-vented sediments relative to REF [(CO<sub>2</sub>-R/REF) - 1]. Continuous and dashed arrows show biotic and abiotic processes, respectively. C pool, detrital carbon pool; AR, aerobic respiration; AnR, anaerobic respiration; GPP, gross primary production; CaCO<sub>3</sub> dis., CaCO<sub>3</sub> dissolution. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 (Welch's *t* test; *n* = 3 to 6). Average data of 2012 and 2013 sampling campaigns are shown, except for macrofauna/polychaete data, which are the average of 2012, and for AR and GPP data, which are the average of 2013.

the upward migrating fluids at the vent sites may stimulate microphytobenthos growth. Similar effects were recorded previously along a natural CO<sub>2</sub> gradient at Volcano Island (Italy), where the microphytobenthos was found to be promoted by CO<sub>2</sub> leakage, showing a twofold increase in Chl *a* concentrations and a two to four times higher diatom abundance under high CO<sub>2</sub> (21). As to specific effects on benthic diatom genera, a previous study at Basiluzzo (22) suggested that the diatom genera *Fragilaria*, *Diploneis*, and *Amphora* are favored by CO<sub>2</sub> leakage. *Fragilaria* was the most abundant diatom taxon at CO<sub>2</sub>-R and was observed to form colonies on the surface sands, likely as a response to the combination of CO<sub>2</sub> and nutrient enrichment by pore-water advection. This dominance of chain-forming diatoms at CO<sub>2</sub> vent sites has been also reported in other coastal areas (21, 23). Furthermore, *Diploneis* seems to be an opportunistic genus that becomes more competitive in the presence of environmental stress (24). This genus, as well as members of *Amphora*, was represented by larger cells with heavily silicified frustules at CO<sub>2</sub>-R, compared to REF. This increase in microphytobenthos standing stock was also reflected in significantly higher oxygen production at CO<sub>2</sub>-R compared to REF (Table 2). The CO<sub>2</sub> leakage caused a higher primary production to respiration ratio, keeping more carbon fixed in microphytobenthos biomass and in total organic carbon. Our results suggest that this effect is also due to reduced grazing pressure and altered microbial community function, as discussed below.

### Effects on faunal community biomass and composition

Our study shows that CO<sub>2</sub> leakage led to a significant decline in abundance and biomass and a change in community composition for meiofauna and macrofauna (Fig. 3, B and C). Being the most abundant taxa at all target sites, nematodes and polychaetes were particularly affected (fig. S4, B and C). Previous studies using benthic mesocosms and laboratory experiments found that acute CO<sub>2</sub> leakage exposure changed macrofaunal or meiofaunal community abundance, biomass, and composition as a result of seawater acidification (duration of experiments, maximum of 20 weeks; pH levels, ≥5.6) (11, 25–28). Specific experiments focusing on nematode communities found no negative effects on abundance, composition, or diversity at pH ≥ 6 (28–30). For this highly abundant meiofaunal taxon, decreases in density (29, 31, 32) or an increasing mortality based on changed morphometrics (33) only occurred when seawater pH is <6. In our study of naturally CO<sub>2</sub>-vented sands, we detected substantial long-term effects on nematodes already below a porewater pH of 7. This effect was reproduced by the experimental transplantations, which lead to a significant decrease of nematode density and a shift in community structure as a direct consequence of CO<sub>2</sub> leakage (fig. S3 and Fig. 3B). The nematode community did not fully recover to background density within 1 year. Furthermore, for several taxa of meiofauna and macrofauna, we show that these CO<sub>2</sub> effects persist under long-time exposure and are not overcome by adaptation and community change. Our results confirm

previous findings on epibenthic macrofauna communities from other CO<sub>2</sub> vents (Table 3) and indicate that few invertebrate taxa can cope with high CO<sub>2</sub>. Particularly, opportunistic species with short life spans and capable of rapid colonization in strongly disturbed habitats seemed to tolerate the extreme and chronic high partial pressure of CO<sub>2</sub>/low-pH conditions. These include polychaete species of the *Capitella* clade, some spongiids, the interstitial hesionid *Microphthalmus tyrrhenicus*, the paraonid *Aricidea cerrutii*, and the nematode species *Microaimus compridus*, *Microaimus honestus*, *Oncholaimus campylocercoides*, and *Daptonema microspiculum*. The polychaete and nematode communities also showed a strong trophic shift, being more diversified under the baseline pH conditions (Fig. 3, B and C). Hence, our observations confirm that despite the additional energy availability due to the high microphytobenthic production at the CO<sub>2</sub>-vented sites, the associated benthic communities are negatively affected by high CO<sub>2</sub>, with declining densities and loss of functional diversity as main consequences.

### Effects of elevated CO<sub>2</sub> levels on microbial communities

In contrast to the faunal communities, we did not detect a significant change of bacterial or archaeal densities in CO<sub>2</sub>-vented sands compared to REF (Fig. 3A). Some bacterial taxa, like Oceanospirillaceae, did not show differences in relative sequence abundance between vent and REFs and, hence, seem not to be affected by CO<sub>2</sub> leakage (table S4). However, we detected an overall substantial shift in community composition already at the phylum and class levels, which was increasingly pronounced at increasing taxonomic resolution (fig. S4A). Previous studies using natural leakage analogs also found impacts on both microbial compositions already at pH < 7.7 (Table 3).

Here, the most striking change in community composition was the decline of relative abundances of Gammaproteobacteria by 50%. Members of this group typically dominate marine sediments but seem unable to cope well with high CO<sub>2</sub>, as previously reported from pelagic mesocosms (34–37), sedimentary CO<sub>2</sub> vents (38, 39), and sponge and coral associates (40). In contrast, the Flavobacteriaceae, a bacterial family relevant in the degradation of marine algal organic matter (41), are not negatively affected by high-CO<sub>2</sub> exposure and low pH according to our study, as well as to laboratory experiments (34, 42) and previous observations on natural CO<sub>2</sub>-vented sites (40, 43). As for the anaerobic bacteria inhabiting the deeper, more acidified sediments, the Caldilineales, a group involved in organic matter degradation under anaerobic and low-pH conditions (44, 45), were favored at the vented sites. Conversely, the relative sequence numbers of anaerobic bacteria, including sulfate reducers (order Desulfobacterales) and nitrifiers (genus *Nitrospira*, *Nitrospina*, and *Nitrosococcus*), decreased significantly at the CO<sub>2</sub> vents. Despite the high Fe<sup>2+</sup> availability in porewaters, no mats of iron-oxidizing bacteria were observed, and typical iron oxidizers were missing in the acidified sands. In the transplantations, the significant shift in bacterial community occurring after 1 year (Fig. 4A) was in line with the long-term changes but was not detectable in short-term incubations (that is, 2 weeks; data not shown). Transplantation from the vent site to the REF showed incomplete recovery within a year.

### Consequences of CO<sub>2</sub> leakage on local food webs and carbon fluxes

Acidification influences all cellular processes, including enzyme kinetics and membrane potentials, but different species are differently adapted to high CO<sub>2</sub> levels. Our study shows significant, long-lasting effects of high CO<sub>2</sub> on benthic biomass and composition, which alter biogeochemical functions at the ecosystem level (Fig. 5). Integrated

with findings from previous experiments and field studies, we prove that these are consistent indicators of high-CO<sub>2</sub> effects across different ecosystem types, organism size classes, and ecological functions.

Our study detected a substantial increase in microphytobenthos primary production and standing stock in relation to CO<sub>2</sub> seepage and the migration of nutrients such as silicate and iron. We expected this to compensate the metabolic costs of adaptation to high CO<sub>2</sub> for the infaunal communities (46), thus favoring high faunal biomasses of a few adapted types. Instead, both meiofauna and macrofauna communities significantly declined in biomass (that is, by up to 90%). Although replacement of typical species of shallow sandy sediments by opportunistic species with different trophic functionalities occurred (Fig. 3, B and C), this did not lead to similar levels of faunal density and biomass. Furthermore, we found a long-lasting shift in microbial community composition and function. The high-CO<sub>2</sub> venting caused a decrease in the whole hydrolytic capacity of the benthic communities, as revealed by measurements of the potential activity of aminopeptidases and esterases (Fig. 2 and fig. S3). These results match those on coastal sediments in mesocosms (47). Only the β-glucosidase responded by increased hydrolytic activities, as reported in previous experiments with bacterioplankton (48–51) and here also with transplant experiments. This enzyme, responsible for polysaccharide degradation, may be enhanced as a consequence of the higher microphytobenthic productivity (22). Furthermore, anaerobic remineralization by sulfate reducers was almost fully repressed in the vented sites (Fig. 2 and fig. S3), matching the substantial decline in sulfate reducer sequences. A sensitivity of sulfate-reducing bacteria to high CO<sub>2</sub> was also previously observed at CO<sub>2</sub>-vented sediments off Papua New Guinea (52). Other functional groups affected by CO<sub>2</sub> based on relative sequence abundance were sulfite oxidizers and nitrifiers.

Together, the observed CO<sub>2</sub>-leakage effects had consequences on carbon remineralization per biomass (R/B). This ratio was higher in the vented sands, indicating that more organic carbon needs to be remineralized per unit of biomass, compared to the reference (Table 3 and Fig. 5). Similarly, an altered food web structure and an impaired carbon cycling have been recently reported for soils affected by natural CO<sub>2</sub> leakages (53). The results of the transplantation were in full accordance with the observed field patterns, showing basic CO<sub>2</sub> effects on community biomass, composition, and biogeochemical function that will not be overcome by long-term adaptation of the involved species. Rather, the selection of opportunistic or tolerant species caused long-term deviation from reference ecosystem-level functions in terms of productivity, standing stock, and remineralization rates. There was an overall increase of productivity but also a higher respiration rate per standing stock, thus decreasing the biological carbon sink function. Furthermore, the quantitatively more relevant geochemical carbon sink was weakened by the carbonate dissolution and by the long-term loss of buffering capacity due to CO<sub>2</sub> leakage.

### CONCLUSION

Our study shows that CO<sub>2</sub> leakage substantially changed the carbonate chemistry in permeable sandy sediments, increasing mineral weathering and nutrient flux (for example, iron and silicate). This led to local shifts in bacterial communities and enhanced microphytobenthos growth but also to a decline in benthic meiofauna and macrofauna density and composition. Together, CO<sub>2</sub> leakage altered the ecosystem functions in terms of remineralization and carbon transfer along the food web. Hence, there is a substantial risk that CO<sub>2</sub> leakage from submarine CCS sites may locally lead to negative impacts on the ecosystem and the function

of the seafloor as carbon sink, when globally, it would help mitigate the detrimental consequences of climate change and acidification on ocean ecosystems. CO<sub>2</sub> leakage from submarine CCS would be very difficult, if not impossible, to stop. Therefore, site selection and spatial planning for marine CCS should include the environmental assessment of habitats, community composition, and ecological functions, including the resilience of local marine species, to mitigate ecological risks.

## MATERIALS AND METHODS

### Site selection

Locations with natural subseafloor CO<sub>2</sub> venting are studied as analogs to assess the impacts of CO<sub>2</sub> leakage as a risk of CCS technology for marine ecosystems (12). Natural CO<sub>2</sub>-vented sediments occur in active volcanic/tectonic regions and are driven by hydrothermal circulations (12). The selection of purely CO<sub>2</sub>-vented sediments for studies of high-CO<sub>2</sub> effects is crucial to reduce confounding factors, such as presence of gases representing microbial energy sources (that is, H<sub>2</sub>, H<sub>2</sub>S, and CH<sub>4</sub>), toxic elements (that is, metals), and temperature anomalies (54, 55). For example, some of the Mediterranean CO<sub>2</sub> vents, such as the one in Levantine Bay of Volcano Island and some at Panarea, show high temperatures around 95°C and a relatively high H<sub>2</sub>S gas content of ca. 2%, complicating the isolation of pure CO<sub>2</sub> effects at venting sites by favoring growth of extremophilic and thiotrophic microbial communities (56, 57). Before our study, we evaluated different CO<sub>2</sub>-vented habitats around Panarea Island to choose appropriate study sites for investigating the pure effects of CO<sub>2</sub> leakage and seawater acidification on benthic life. During the first expedition (29 May to 10 June 2011), nine gas emission sites and two potential REFs were explored by diving in water depths of around 20-m depth. Larger areas were surveyed by manta towing (58). The divers sampled sediments and water asf for pH and sulfide measurements, measured temperature, and took documentary pictures and videos. At the study sites off Basiluzzo Islet, shallow sand flats were interspersed with seagrass meadows at 14- to 21-m depth. The CO<sub>2</sub>-impacted sites studied here were identified visually by the ebullition of CO<sub>2</sub> from the seafloor. We found the east of Basiluzzo Islet to be suitable as a submarine natural analog to assess biogeochemical and ecological consequences of CO<sub>2</sub> leakage because of the purity of the gas escaping from the seafloor, combined with the absence of cofounding hydrothermal effects altering microbial communities (Table 1). The experimental fieldwork was carried out in 2012 (02 to 21 June) and 2013 (01 to 14 June).

### Data analysis

pH as a key variable for high CO<sub>2</sub> is presented in pH<sub>T</sub>. We calibrated all sensors with commercial National Bureau of Standards buffers and corrected these to pH<sub>T</sub> by subtracting 0.13 units, as described in Zeebe and Wolf-Gladrow (59). To assess significant differences between two or more independent data sets across the different sites, we used Welch's *t* test (for example, sensors network and metadata analysis) or different ANOVAs (for example, biogeochemical and microbiological data and functional groups) (for details, see Statistical Analyses in the Supplementary Materials and Methods).

### Chemical analyses of vent gases and fluids, seawater, porewater, and sediments

#### Gases, seawater, and vent fluids

Gas bubbles were sampled by scuba divers using exetainers and gas-collecting tubes made from glass, and analyzed via gas chromatography

for CO<sub>2</sub>, O<sub>2</sub>, Ar, N<sub>2</sub>, CH<sub>4</sub>, C<sub>2</sub>H<sub>6</sub>, C<sub>3</sub>H<sub>8</sub>, COS, SO<sub>2</sub>, and H<sub>2</sub>. Hydrogen sulfide concentrations of these bubbles were determined by electrochemical sensor measurements directly after sampling (for details, see the Supplementary Materials). Seawater was sampled with a 5-liter Niskin bottle at ca. 30 cm asf and with 50-ml glass syringes at 5 to 10 cm asf. Benthic chamber water samples (that is, mixture of overlying bottom water and porewater) were collected with 50-ml glass syringes directly connected to benthic chambers at different times and analyzed for pH, nutrient [NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>, and Si(OH)<sub>4</sub>] concentration, DIC, and TA. Samples for CH<sub>4</sub> concentration in porewaters were filled into evacuated and preweighed glass containers with 2 to 3 NaOH pellets for subsequent analysis via gas chromatography (Focus GC, Thermo Fisher Scientific) (60).

#### Porewater and sediment geochemistry

Profiles of pH, DIC, TA, and nutrient [NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>+NO<sub>2</sub><sup>-</sup>, and Si(OH)<sub>4</sub>], sulfide, and Fe/Mn concentrations in the sediments were assessed by extracting porewater at 2-cm depth intervals with a TUBO device and with Rhizons (SMS type MOM, 19.21.21F; mean pore size, 0.15 μm; Rhizosphere Research Products) attached to 10-ml syringes. From three replicate push cores per site and per year, samples (2-cm intervals, maximum length up to 10 cm) were preserved for analyses of granulometry, porosity, TOC, TN, CPE (including Chl *a*), and calcium carbonate (CaCO<sub>3</sub>) content. The samples were preserved and analyzed as described in Böer *et al.* (61) (for details, see the Supplementary Materials). Chl *a* concentrations were converted to microphytobenthos biomass using a 1:40 Chl *a*-to-C biomass ratio (62).

### In situ measurements

#### Time-lapse recordings

To monitor gas flow visually in situ, time-lapse photography was conducted (videos available at <https://doi.pangaea.de/10.1594/PANGAEA.825241>) (for details, see the Supplementary Materials).

#### Oceanographic measurements

A SEAGUARD recording current meter (Aanderaa Data Instruments) was used in 2011, 2012, and 2013 to monitor for 24 to 72 hours current velocity, temperature, salinity/conductivity, pressure, turbidity, and oxygen concentrations within the water column at about 30 cm asf.

#### Bottom water chemistry loggers

At five selected sites, the pH, oxygen (O<sub>2</sub>), ORP, and pressure (tides) were measured using five RBR loggers (RBR-Datalogger XR-420 D; RBR; [www.rbr-global.com](http://www.rbr-global.com)) with the sensors at 2 cm asf.

#### Porewater chemistry

A sediment microsensors profiler was equipped with sensors for pH (Microelectrodes Inc.) (63), O<sub>2</sub> (64), CO<sub>2(aq)</sub> (Microelectrodes Inc.), ORP (a Pt wire, exposed tip is 50 μm thick and 0.5 mm long), temperature (Pt100; UST Umweltsensortechnik GmbH), H<sub>2</sub> (Unisense), and H<sub>2</sub>S (65). The profiler was deployed at CO<sub>2</sub>-R, CO<sub>2</sub>-G, and REF in 2012. The CO<sub>2</sub> sensors were damaged during the measurement at ca. 2 cm bsf at CO<sub>2</sub>-R; all other sensors worked successfully.

#### Diffusive oxygen fluxes

Diffusive oxygen fluxes were calculated from the in situ oxygen micro-profiles using Fick's law of diffusion as described previously (66).

#### Total oxygen uptake rates

Benthic chambers were inserted into the seafloor to measure total fluxes of oxygen, nutrients, pH, and DIC within a defined sediment and seawater volume. At CO<sub>2</sub>-vented sites, the chambers were deployed between bubble streams to avoid the formation of internal headspace. We repeatedly subsampled the overlying water with syringes during daylight from transparent and masked chambers to assess

oxygen exchange (net O<sub>2</sub> flux) and oxygen respiration, respectively. Oxygen production, that is, gross primary production (GPP), was estimated as net O<sub>2</sub> flux + (O<sub>2</sub> respiration). To correct for anoxic fluid efflux from the seafloor, bags were attached to each chamber to measure the cumulative incubation volume (further information in the Supplementary Materials). Because of relatively high respiration rates, only incubations <6 hours were used to calculate oxygen fluxes.

#### Sulfate reduction rates

SRRs, as a proxy of anaerobic microbial respiration, were measured ex situ on sediments collected with push corers and sliced in 2-cm intervals (67).

#### Extracellular enzymatic activities

EEAs were analyzed by incubating the top 2 cm of the sediments with substrates for β-glucosidase, chitinase, leucine aminopeptidase, and esterase (see further details in the Supplementary Materials) (61).

#### Calcite dissolution rates (marble tiles)

The corrosion of CaCO<sub>3</sub> structures by CO<sub>2</sub> was determined from the weight loss of marble tiles exposed at 0.5 m asf at REF CO<sub>2</sub>-G and CO<sub>2</sub>-R from June 2012 to June 2013. Once retrieved, the marble tiles were dried and weighed, and the dissolution rates were estimated as the difference in weight between pre- and postdeployment tiles, divided by exposure time (362 to 364 days; for further details, see the Supplementary Materials).

### Microbiological analyses

#### Sample collection and DNA extraction

Samples from 0 to 2 cm bsf were obtained by 20 Sarstedt tubes (50 ml), and three push cores were collected for additional sections between 0 and 10 cm bsf per site and per year. Samples were kept frozen at -20°C until subsequent analyses. DNA was extracted from 1 g of sediment per sample using the FastDNA SPIN Kit for Soil (Qbiogene), including an additional heating step to increase yield and final elution of the DNA in tris-EDTA buffer.

#### Bacterial community structure

The high-throughput fingerprinting technique ARISA [according to Ramette (68)] was applied to all sediment samples (0 to 2 cm bsf layer,  $n = 23$ ; 2 to 4 cm, 4 to 6 cm, 6 to 8 cm, and 8 to 10 cm bsf layers,  $n = 3$ , per site and per year). In addition, 454 MPTS [according to Sogin *et al.* (69)] was used for the 0 to 2 and 4 to 6 cm bsf layers collected in 2012 ( $n = 3$ ) (for details, see the Supplementary Materials).

#### Total microbial and microphytobenthos cell counts

Sediment samples were fixed in 2% (for microbes) and 4% (for microphytobenthos) buffered formaldehyde/seawater and stored at 4°C until subsequent analysis. Microbial abundance was estimated by epifluorescence microscopy after staining with acridine orange (61). Catalyzed reporter deposition fluorescence in situ hybridization was applied for bacterial and archaeal cell enumeration. For microphytobenthos, only viable cells were counted under an inverted light microscope (Leica Microsystems AG) using 32× to 40× objective (final magnification, ×320 to ×400) (see the Supplementary Materials).

### Fauna sampling and analysis

Meiofauna samples (size class, 0.032 to 1 mm) were collected with pre-cut (2-cm horizons) and taped push cores with an inner diameter of 4.7 cm in 2011 ( $n = 3$  per site; upper 8 cm) or 5 cm in 2012 and 2013 ( $n = 3$  per site; upper 8 cm). Samples were fixed and preserved in 4% buffered formaldehyde/seawater. Macrofauna samples (size class, >1 mm) were collected with push corers (inner diameter of 6.4 cm;  $n = 5$  per site; upper 5 cm). Meiofauna and macrofauna organisms

were identified to phylum or class level under a stereoscopic microscope. All meiofaunal nematodes and macrofaunal polychaetes were further identified to species level and allocated to functional feeding groups (see the Supplementary Materials). Depending on reproduction rate and tolerance to disturbance, nematodes were also allocated to a colonizer-persister (cp) category based on Bongers *et al.* (70): cp-2, short generation time, high reproduction rate, and very tolerant to disturbances; cp-3, characteristics intermediate to cp-2 and cp-4 and relatively sensitive to disturbances; cp-4, long generation time and sensitive to pollutants; and cp-5, long life span, low reproduction rate, and very sensitive to pollutants and other disturbances. The nematodes' maturity index was determined as an ecological measure of environmental disturbance (see the Supplementary Materials) (70).

### Sediment transplantation experiments

Sediments were transplanted in situ within and between REF and CO<sub>2</sub>-R sites: (i) reimplanted at the same site (within habitat: REF/REF and CO<sub>2</sub>-R/CO<sub>2</sub>-R) to control for transplantation effects, and (ii) reimplanted to the other habitat type (across habitat: REF/CO<sub>2</sub>-R and CO<sub>2</sub>-R/REF) to assess the effect of the different environmental setting. Samples for microbial community analyses (that is, ARISA) were collected immediately after transplantation ( $n = 3$  per treatment), after 2 weeks ( $n = 1$  per treatment), and after 1 year ( $n = 3$  per treatment). Samples for porewater composition, sediment geochemistry, microbial activity, and nematode abundance and community structure were collected 1 year after transplantation ( $n = 3$  per treatment). Samples were taken and analyzed as described in the above sections, except for nematodes that were identified at genus level.

### SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/4/2/eaao2040/DC1>

Supplementary Materials and Methods

fig. S1. Maps and seafloor images of the sampling area.

fig. S2. Bottom water and porewater chemistry.

fig. S3. Biogeochemistry of medium-term transplants and undisturbed sediments.

fig. S4. Similarity between benthic communities at investigated sites.

table S1. Bottom water and porewater chemistry at the study sites off Basiluzzo Islet.

table S2. Outcome of one-way ANOVA.

table S3. pH<sub>T</sub> change and in situ fluxes obtained from benthic chambers deployed in 2013.

table S4. Change in relative sequence number of highly abundant, putative functional groups of bacteria based on 454 MPTS OTUs annotation (OTU > 0.1%).

table S5. Outcome of distance-based multivariate regression analysis.

table S6. Outcome of permutational ANOVA for environmental variables (Env. Setting), bacterial community structure (ARISA), and fauna community structure (that is, nematode species, meiofauna higher taxon, polychaete species, and macrofauna higher taxon).

table S7. Relative abundances of macrofauna taxa, polychaete species, meiofauna taxa, and nematode species at the three investigated sites.

table S8. Differences in porewater and sedimentary environmental settings in medium-term transplant experiments ( $n = 3$ ).

table S9. Overview of CO<sub>2</sub>/pH impacts on benthic organisms at natural CO<sub>2</sub> vents, as observed in previous studies.

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**Acknowledgments:** The authors would like to thank B. Merkel (TU Freiberg) for the sediment element analysis in 2011, F. Italiano (Istituto Nazionale di Geofisica e Vulcanologia (INGV) Palermo) for the vent fluid analysis in 2011, S. Beaubien and S. Lombardi (UniRoma1) for the on-site analysis of H<sub>2</sub>S gas in 2012, N. Bigalke (GEOMAR) for the estimation of the gas emission rates, M. Haeckel and R. Surberg (GEOMAR) for the porewater element analysis, J. Gil [Centre d'Estudis Avançats de Blanes, Consejo Superior de Investigaciones Científicas (CEAB-CSIC)] for his help in identifying some problematic polychaetes, and D. Wolf-Gladrow for the helpful discussions of the carbonate system. The authors are also grateful for the technical support by M. Meiners, E. Weiz-Bersch, M. Alisch, W. Stiens, and R. Stiens (HGF-MPG Joint Research Group on Deep Sea Ecology and Technology); help in field work and laboratory activities by N. Viaene, B. Beuselinck, A. Van Kenhove, G. De Smet, F. Sedano Vera, N. De Jesr, and L. Lins (UGent Marine Biology); scientific diving assistance by B. Unger, M. Schneider, H. Kuhfuss, and A. Eich (HYDRA Institute for Marine Sciences); and logistic support by A. Fogliuzzi (Amphibia). **Funding:** This work was funded by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement number 265847 [Sub-seabed CO<sub>2</sub> storage: Impact on Marine Ecosystems (ECO2)] and supported by the Max Planck Society and by the Flemish Fund for Scientific Research (grant number 1242114N). This study is also a contribution of D.M. to the research project MarSymBionics (reference number CTM2013-43287-P), funded by the Spanish "Agencia Estatal de Investigación" (AEI), and PopCOmics (CTM2017-88080), funded by the AEI and the European Funds for Regional Development (FEDER) and to the Consolidated Research Group on Marine Benthic Ecology (2014SGR120) of the Generalitat de Catalunya. **Author contributions:** This study was designed by A.B. and A.R. Sampling activities and in situ experiments were performed by M.W., C.L., K.G., S.M., M.M., D.d.B., F.W., and A.R. M.M., D.d.B., and F.W. analyzed the in situ measurements. M.M. and G.W. analyzed the microbial and biogeochemical data. K.G. and D.M. analyzed the meiofauna and macrofauna. T.C. and C.D.V. analyzed the microphytobenthos. C.L. and M.W. analyzed the videos and images of the study sites. A.B., A.V., D.d.B., and M.W. contributed reagents/materials/analysis tools. The paper was written by M.M., K.G., and A.B. with contributions and final approval of all co-authors. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data were archived on the PANGAEA database: doi.pangaea.de/10.1594/PANGAEA.871453. Porewater element composition can be found at doi.pangaea.de/10.1594/PANGAEA.847916, and sediment element composition at doi.pangaea.de/10.1594/PANGAEA.847825. MPTS sequences were deposited on the European Nucleotide Archive under accession number PRJEB21026. The sequences were archived using the brokerage service of the German Federation for Biological Data (71). All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 29 June 2017

Accepted 5 January 2018

Published 7 February 2018

10.1126/sciadv.aao2040

**Citation:** Molari, K. Guilini, C. Lott, M. Weber, D. de Beer, S. Meyer, A. Ramette, G. Wegener, F. Wenzhöfer, D. Martin, T. Cibic, C. De Vittor, A. Vanreusel, A. Boetius, CO<sub>2</sub> leakage alters biogeochemical and ecological functions of submarine sands. *Sci. Adv.* **4**, eaao2040 (2018).



## CO<sub>2</sub> leakage alters biogeochemical and ecological functions of submarine sands

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*Sci Adv* 4 (2), eaao2040.  
DOI: 10.1126/sciadv.aao2040

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