Viruses are the most abundant biological entities in the world’s oceans, and they play a crucial role in global biogeochemical cycles. In deep-sea ecosystems, archaea and bacteria drive major nutrient cycles, and viruses are largely responsible for their mortality, thereby exerting important controls on microbial dynamics. However, the relative impact of viruses on archaea compared to bacteria is unknown, limiting our understanding of the factors controlling the functioning of marine systems at a global scale. We evaluate the selectivity of viral infections by using several independent approaches, including an innovative molecular method based on the quantification of archaeal versus bacterial genes released by viral lysis. We provide evidence that, in all oceanic surface sediments (from 1000- to 10,000-m water depth), the impact of viral infection is higher on archaea than on bacteria. We also found that, within deep-sea benthic archaea, the impact of viruses was mainly directed at members of specific clades of Marine Group I Thaumarchaeota. Although archaea represent, on average, ~12% of the total cell abundance in the top 50 cm of sediment, virus-induced lysis of archaea accounts for up to one-third of the total microbial biomass killed, resulting in the release of ~0.3 to 0.5 gigatons of carbon per year globally. Our results indicate that viral infection represents a key mechanism controlling the turnover of archaea in surface deep-sea sediments. We conclude that interactions between archaea and their viruses might play a profound, previously underestimated role in the functioning of deep-sea ecosystems and in global biogeochemical cycles.

**RESULTS AND DISCUSSION**

Deep-sea sediment samples spanning depths from ca. 1000 to 10,000 m and covering a wide range of bottom-water temperatures and environmental conditions (table S1) were collected. Overall, we conducted more than 35 independent in situ and laboratory experiments and analyzed more than 480 sediment samples. To the best of our knowledge, the interactions between viruses, bacteria, and archaea in benthic deep-sea ecosystems have not previously been investigated using the high level of replication and geographic coverage of the present study.

In the top 50 cm of the sediments, fluorescence in situ hybridization analyses targeting rRNA (table S2) revealed that the number of archaea was lower than that of bacteria at all sampling sites (Fig. 1, A and B), representing 5 to 32% of the total microbial (archaeal and bacterial)
can cause lysis of host cells (1–4, 19). Rates of lytic infection (referred
to here as production of viruses after cell lysis) were high in all surface
sediments investigated, ranging from $3.6 \times 10^{12}$ to $12.6 \times 10^{12}$ viruses
m$^{-2}$ day$^{-1}$ (Fig. 1D). The depth-integrated virus abundances divided
by the respective viral production rates resulted in fast viral turnover
times, averaging 2 to 3 days, consistent with previous evidence indicat-
ing that the deep-sea virome is a highly dynamic and active component
of deep-sea ecosystems (5). Lytic viral infections release new viral progeny
(virions) along with DNA (referred to here as extracellular DNA) (24)
from the lysed host cells into the environment. The molecular method
that we used exploits this property and, to the best of our knowledge,
the first time permits quantification of and discrimination between the
impact of viral infection on either archaea or bacteria, with a sensitivity
much higher than that possible based on cell counts by fluorescence in situ
hybridization. To do so, we determined, by real-time qPCR, the number of
archaeal and bacterial 16S rRNA genes in the DNA released after cell
lysis (Fig. 3), using primers and probes selected for consistency with pre-
vious studies investigating archaeal and bacterial dynamics in deep-sea
sediments conducted worldwide (25, 26). We found that the higher the
production rate of viruses, the higher the release of 16S rRNA gene
copies in the extracellular DNA fraction (fig. S4).

To test the assumption that factors other than viruses were negligible
in causing cell lysis and in releasing 16S rRNA genes, additional
experiments based on the use of selective inhibitors of bacterial and ar-
chaean metabolism were conducted. These experiments demonstrated
that the inhibition of viral replication blocked the release of archaeal
or bacterial 16S rRNA genes (Fig. 4 and table S4). Moreover, the use
of an archaeal-specific protein synthesis inhibitor (22) resulted in
the complete cessation of virus-mediated archaeal lysis and of the accom-
panying release of archaeal 16S rRNA genes, with no effects on the viral
lysis of bacteria (Fig. 5 and table S5). Overall, the experiments of inhi-
bition of bacterial and/or archaeal metabolism indicated that nonviral
causes of mortality were negligible during the short-time incubations we
performed. Nonetheless, to exclude potential biases due to natural cell
depth over time, potentially causing release of 16S rRNA genes not due
to viral infections, we recommend avoiding incubation lasting more
than 12 hours when using cell metabolism inhibitors.

In addition to the findings obtained from the study of the bacterial
and archaeal cell metabolism, independent evidence of the importance
of archaeal viruses in infecting and killing archaea was provided by the
increase over time in the relative abundance of DNA sequences of ar-
chaean viruses, including sequences affiliated to the known putative
viruses infecting MG-I Thaumarcheota (for example, Thaumarchaeo-
ta phage AAA160-J20 and thaumarchaeal putative virus Oxic1_7)
(fig. S5 and Supplementary Methods) (27, 28). All of these results
support the contention that viruses are a major cause of mortality
for benthic deep-sea archaea and bacteria (5). However, we also note
that adequate precautions should be taken and controls performed
when analyzing natural systems using our newly developed approach,
particularly when bacterial and archaeal mortality might be caused by
other factors.

The accuracy of quantitative estimates of 16S rRNA gene copies of
natural microbial assemblages could be affected by selective amplification
of PCR products (29). To minimize the effect of primer selectivity, we
used primer sets with the widest coverage for bacterial and archaeal taxa
(table S2) (30). Moreover, the diversity of 16S rRNA genes contained
within the intracellular and extracellular DNA was similar (figs. S2 and
S6, A and B) as well as the diversity of the bacterial and archaeal sequences
contained within these two metagenomes (that is, the intracellular and

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extracellular DNA pools; fig. S6, C and D). This indicated that we were assessing the same components of the intracellular and extracellular DNA pools and could make reliable estimates of the diversity and the number of 16S rRNA genes released by viral lysis. The presence of multiple genomes per cell (polyploidy), documented in certain bacteria (31) and archaea (Euryarchaeota) (32), should also be taken into account to accurately convert the number of 16S rRNA gene copies into cell abundance. However, in our samples Euryarchaeota had a negligible representation, thereby indicating that polyploidy did not have a significant effect on the estimates of virus-induced archaeal mortality.

We estimated that viral infections were responsible for the abatement of 1.0 to 2.2% day$^{-1}$ (on average 1.6% day$^{-1}$) of the bacterial abundance and 2.3 to 4.3% day$^{-1}$ (on average 3.2% day$^{-1}$) of the archaeal abundance in deep-sea sediments (Fig. 6). Therefore, despite the number of archaea in the sediments being much lower than that of bacteria, the impact of viruses on archaea was on average significantly higher. These findings were consistent along the vertical profile of the sediments down to 50-cm depth, across all oceanic regions investigated, and were not affected by pressure conditions (fig. S7). These values, once combined, are consistent with estimates of the overall virus-induced mortality rates determined at similar depths worldwide (5). The overall mortality rates of archaea plus bacteria (on average 2.6 ± 0.6 × 10$^6$ killed cells per gram per day) were very similar to those obtained by dividing the viral production by the average burst size reported for deep-sea sediments (that is, 45 viruses released per lysed cell, resulting in 2.1 ± 0.4 × 10$^6$ killed cells per gram per day) (5). Moreover, the viral production divided by the rates of lysis we derived from the release of 16S rRNA gene copies resulted in values of burst size (mean, 48;
range, 17 to 187) almost identical to those previously reported by Danovaro et al. (5), providing further evidence of the reliability of the results presented here.

To be sustainable over time, the rates of virus-induced mortality of bacteria and archaea should be balanced by cellular turnover. The turnover times of archaea and bacteria determined on the basis of C production rates (fig. S8 and table S6) were similar (11 to 23 days for archaea and 12 to 27 days for bacteria). These values were unaffected by pressure conditions (fig. S9) and were consistent with results available from deepwater masses (range, 20 to 100 days) (33) and deep-sea sediments (range, <2 to 67 days) (22). Notably, the turnover times of archaea, estimated by dividing the virus-induced cell mortality rates by the abundance of archaeal cells (12 to 22 days), were roughly equivalent to those determined by the radiolabeling experiments. In contrast, the turnover times of bacteria estimated from virus-induced mortality rates were significantly longer (31 to 52 days) than those calculated on the basis of radiolabeled substrate incorporation. This means that viral infection is responsible for the abatement of almost the entire biomass production of benthic archaea, whereas factors other than viruses (for example, predation or grazing by benthic consumers) may be responsible

Fig. 3. Release of bacterial and archaeal genes due to viral lysis. The panel shows the release of viruses from killed archaea and bacteria (left column) and consequent shift of the 16S rRNA genes from bacteria (central column) and archaea (right column) to the extracellular DNA pools during time-course experiments conducted on deep-sea sediments from the Atlantic Ocean (first row), Mediterranean Sea (second row), Arctic Ocean (third row), and Pacific Ocean (bottom row). Reported are means ± SDs at the different time intervals, as well as the equation and $R^2$ value of each line of best fit.

Viral abundance | Bacterial 16S rDNA | Archael 16S rDNA

![Fig. 4. Effect of the inhibition of archaenal and bacterial metabolism. Reported are, from left to right, viral abundance, bacterial 16S rRNA gene copies in the extracellular DNA, and archaenal 16S rRNA gene copies in the extracellular DNA, during time-course incubations of untreated (control, blue bars) and treated (light blue bars, following addition of GC7 plus antibiotics to inhibit archaenal and bacterial metabolism) deep-sea sediment samples. Reported are average values and SDs. Asterisks indicate the significant increase (**P < 0.01) in the abundance of viruses and the number of bacterial or archaenal 16S rRNA gene copies after 12 hours of incubation in untreated (control) samples. ns, no significant differences occurring over time in treated samples; rDNA, ribosomal DNA.](image1)

Viral production | Bacterial 16S rDNA | Archael 16S rDNA

![Fig. 5. Effect of the selective inhibition of archaenal metabolism. Reported are, from left to right, viral production, bacterial 16S rRNA gene copies in the extracellular DNA, and archaenal 16S rRNA gene copies in the extracellular DNA, during time-course incubations of untreated (controls, blue bars) and treated (light blue bars, following addition of GC7 to selectively inhibit archaenal metabolism) deep-sea sediment samples. Reported are average values and SDs. Asterisks (***P < 0.01; **P < 0.001) indicate a significant decrease after 12 hours of incubation for viral production rates in treated samples, a significant increase in the number of both bacterial and archaenal 16S rRNA gene copies in control samples, or a significant increase in the number of bacterial only 16S rRNA gene copies in treated samples. ns, no significant increase in archaenal 16S rRNA gene copies occurring over time in treated samples.](image2)

for the removal of a larger fraction of the biomass produced by bacteria than by archaea.

Our results, based on massive sequencing analyses of the 16S rRNA genes released by lysed bacteria and archaea, provided new insight on the role of viral infections in the microbial biodiversity and functioning of the deep oceans (5, 34). We found that, in all investigated deep-sea ecosystems, the viral impact occurred primarily on specific taxa belonging to MG-I Thaumarchaeota (fig. S10), suggesting that the viral impact is highest on the most represented archaeal group inhabiting surface deep-sea sediments (11–13).

Our findings also indicated that C production of benthic bacterial assemblages was almost entirely due to heterotrophic metabolism, whereas more than 60% of the C production of benthic archaenal assemblages was due to chemosynthetic processes (fig. S8). In oxygenated surface deep-sea sediments, chemosynthesis is largely dependent on the oxidation of ammonia (1 mol of CO₂ fixed to 10 mol of NH₄⁺ oxidized) (20), which is supplied by microbial heterotrophic metabolism (22, 35). In line with this, we found a significant relationship between chemosynthetic and heterotrophic C production (Fig. 7A). In turn, our results confirm previous evidence (5) that the labile organic C released by virus-induced cell lysis can stimulate the heterotrophic metabolism of uninhibited deep-sea archaea and bacteria in the deep sea (Fig. 7B).

These findings provide new evidence for a possible link between viral infections and chemosynthetic production in the microbial food web. Viral-mediated mortality of bacteria and archaea may stimulate heterotrophic metabolism. This, in turn, may enhance nitrogen regeneration processes, supporting ammonia-dependent chemosynthetic production. This biological feedback could have important consequences for C and N cycling. Assuming that the entire organic matter pool released by viral lysis is used by heterotrophic metabolism, and considering that 1 mol of ammonia is produced for every 1 mol of CO₂ released (based on archaea and bacteria C/N ratio of 4:1), our results suggest that this process can provide 30 to 60% of the ammonia required to sustain chemosynthetic production of archaea. The relevance of this process indicates that viral infections can be one of the main drivers of chemosynthetic production in deep-sea sediments, but it can also be hypothesized that a large fraction of ammonia, as well as other compounds supporting archaenal metabolism, derives from other heterotrophic processes (for example, from benthic fauna).

Despite the numerical dominance of bacteria, the C released by virus-killed archaea contributes 15 to 30% of the total C released by viral lysis in surface deep-sea sediments. Viral lysis of archaea and bacteria is estimated to release 0.37 to 0.63 GtC year⁻¹ globally (5). The viral lysis of archaea alone is equivalent to ~0.08 to 0.14 GtC year⁻¹ in the top 1 cm, reaching ~0.3 to 0.5 GtC year⁻¹ in the top 50 cm of the sediments when extrapolated to a global scale. We show here for the first time the crucial
role of viruses in controlling archaeal dynamics and therefore the functioning of deep-sea ecosystems, and suggest that virus-archaea interactions play a central role in global biogeochemical cycles.

**MATERIALS AND METHODS**

**Study area and sampling strategy**

Deep-sea sediment samples were collected in 2009–2011, during five independent oceanographic cruises conducted in the Atlantic Ocean, Arctic Ocean, and Pacific Ocean and the Mediterranean Sea (table S1). At each sampling station, surface (the top 1 cm) and subsuperficial sediment samples, down to 50-cm depth, were collected from independent cores (n = 3) using either multiple corers or a remotely operated vehicle (ROV). The experimental setup, which was used for all of the sampling areas investigated, consisted of 12-hour incubations of mesocosms maintained in the dark and in situ temperature conditions. Additional parallel incubations at in situ pressure were also carried out.

**Abundance of bacteria, archaea, and viruses and viral production**

The abundance of bacteria and archaea was determined by epifluorescence microscopy using the CARD-FISH technique (36), whereas viral abundances were determined after staining with SYBR Green I (37). The viral production in the deep-sea sediment samples was determined by the dilution technique (that is, a dilution-based procedure) through the measurement of the increase in viral abundance over time (5, 38, 39). This technique is the most widely used for benthic environments (thus enabling a comparison with available data), and it has the advantage of minimizing the impact of protozoa and/or fauna grazing on bacteria and archaea during the incubations (5, 38, 39). Details are reported in the Supplementary Materials.

**Viral impact on archaea and bacteria**

The same experimental mesocosms used for the determination of viral production were used to selectively quantify the number of archaea and/or bacteria killed by viruses, as follows. Sediment subsamples were incubated in the dark at the in situ temperature and collected at the beginning and after 1 to 3, 3 to 6, and 12 hours of incubation. Extracellular DNA was recovered from sediments collected at each time interval of incubation (40). Real-time qPCR analyses were used to quantify the number of 16S rRNA gene copies contained in the recovered extracellular DNA fraction for each time interval of incubation. The quantification of archaeal and bacterial 16S rRNA gene copies contained within the extracellular DNA was performed using primers and probes selectively targeting archaea (41) or bacteria (table S2 and Supplementary Materials) (42). The number of copies of 16S rRNA genes was calculated on the basis of the template length in base pairs (bp) and an average weight of 650 Da bp−1. The rates of release of 16S rRNA gene copies from archaea and bacteria killed by viruses were determined in the extracellular DNA pools from the linear regression analysis of the number of 16S rRNA gene copies over time. These rates were then converted into bacterial and archaeal cells killed by viruses assuming a value of 1.7 copies of 16S rRNA genes per cell, both for bacteria and archaea. This conversion factor represented the average number of 16S rRNA gene copies per archaeal cell provided by the Ribosomal RNA operon copy number Database (rrnDB) (http://rrndb.unms.med.umich.edu/) (43). This value was lower than the 16S rRNA gene copy number of bacteria (on average 4.2 copies per cell considering all bacterial genomes in the rrnDB) and slightly higher than that of all currently known Thaumarchaeota, including
those inhabiting deep-sea sediments (1 copy per cell) (16, 43, 44). Thus, our estimates of the mortality rates of archaea were conservative, because the relative impact of viruses on archaea could be even higher using one copy per cell as a conversion factor.

To test the hypothesis that the 16S rRNA gene release into the extracellular DNA pool was related to the possibility that metabolically active bacterial and archaeal cells were infected and lysed by viruses, additional time-course experiments were conducted by incubating deep-sea sediment samples with GC7 (N'-guanyl-1,7-diaminoheptane; final concentration of 1 mM) to selectively inhibit archaeal metabolism (22, 45–47) or with GC7 plus different antibiotics [chloramphenicol (0.3 mg ml⁻¹), tetracycline (0.05 mg ml⁻¹), rifampicin (0.05 mg ml⁻¹), and streptomycin (1 mg ml⁻¹)] to also inhibit bacterial metabolism (47–49). These concentrations were sufficient to inhibit both archaeal and bacterial metabolism, as revealed by synoptic incorporation experiments with radiolabeled substrates (both ³H-leucine and ¹⁴C-bicarbonate).

Bacterial and archaeal metabolism using radiolabeled substrates

For heterotrophic or chemosynthetic C production determinations, sediment subsamples were added with 0.2-µm prefiltred seawater (collected at the sediment water interface of each station), containing ³H-leucine (68 Ci mmol⁻¹; final concentration of 0.5 to 1.0 µM) or ¹⁴C-bicarbonate (58 mCi mmol⁻¹; final concentration of 12 µCi ml⁻¹), respectively (5, 22). The relative contribution of bacteria and archaea to the overall heterotrophic and chemosynthetic C production was assessed by parallel experiments using selective inhibitors of microbial metabolism (see the Supplementary Materials).

Effects of pressure on the estimates of archaeal and bacterial mortality

Analyses of viral and archaeal plus bacterial production in deep-sea sediment samples collected from the Mediterranean Sea were conducted at both in situ and atmospheric (that is, 0.1 MPa) pressure to test for differences in the estimates of virus-induced mortality of archaea and bacteria. Moreover, we independently checked for the presence of possible artifacts due to the manipulation of the sediment during the sampling by determining the ratios between the numbers of copies of 16S rRNA genes recovered in the extracellular DNA pool and the total archaeal plus bacterial abundance for all sediment samples collected at the different depths.

Identification of archaea lysed by viruses and viruses responsible for their mortality

To identify the main taxa of archaea lysed by viruses at each sampling site, 16S rRNA gene sequences contained within the extracellular DNA pools at the beginning and after 12 hours of incubation were analyzed by high-throughput sequencing. The bacterial and archaeal 16S rRNA genes contained within the extracellular DNA pools were amplified (30) and sequenced on a 454 FLX Titanium platform. Metagenomic analyses conducted on the free viruses recovered from deep-sea sediment samples at the beginning of the time-course experiments and after 12 hours (that is, after the viral infection events) were used to identify archaeal viruses causing cell mortality. Viruses were dislodged from the sediment using a physical-chemical procedure (50), and viral DNA, which was extracted and purified according to standard protocols, was sequenced on a 454 FLX Titanium platform (see the Supplementary Materials).
A. Klindworth, E. Pruesse, T. Schweer, J. Peplies, C. Quast, M. Horn, F. O. Glöckner,
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R. C. Edgar, MUSCLE: Multiple sequence alignment with high accuracy and high speed.
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Virus-mediated archaeal hecatomb in the deep seafloor

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