ARTICLES

Major viral impact on the functioning of benthic deep-sea ecosystems

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Viruses are the most abundant biological organisms of the world's oceans. Viral infections are a substantial source of mortality in a range of organisms—including autotrophic and heterotrophic plankton—but their impact on the deep ocean and benthic biosphere is completely unknown. Here we report that viral production in deep-sea benthic ecosystems worldwide is extremely high, and that viral infections are responsible for the abatement of 80% of prokaryotic heterotrophic production. Virus-induced prokaryotic mortality increases with increasing water depth, and beneath a depth of 1,000 m nearly all of the prokaryotic heterotrophic production is transformed into organic detritus. The viral shunt, releasing on a global scale ~0.37–0.63 gigatonnes of carbon per year, is an essential source of labile organic detritus in the deep-sea ecosystems. This process sustains a high prokaryotic biomass and provides an important contribution to prokaryotic metabolism, allowing the system to cope with the severe organic resource limitation of deep-sea ecosystems. Our results indicate that viruses have an important role in global biogeochemical cycles, in deep-sea metabolism and the overall functioning of the largest ecosystem of our biosphere.

Viruses are by far the most abundant 'life forms' in the world's oceans (approximately 4×10^{30} viruses)[1], exceeding prokaryotic abundance by at least one order of magnitude[1,2]. Increasing evidence indicates that viral infection may be responsible for the high mortality of autotrophic and heterotrophic organisms in surface oceans[3–5], with cascading effects on carbon cycling and nutrient regeneration[6,7]. Viral lysis of infected microbes transforms their cell contents and biomass into organic detritus (both dissolved and particulate), which can then be used again by non-infected prokaryotes (that is, viral shunt)[1,6,8]. This process supports prokaryotic heterotrophic production, but it also decreases the efficiency of the carbon transfer to higher trophic levels[6] and influences the carbon budget of the oceans, thereby modifying the amount of carbon transferred by sinking particles from the surface waters towards the ocean floor[5]. Therefore, the integration of the viral component into trophodynamic and biogeochemical models is of primary importance for an improved understanding of the function of the world's oceans[5].

Deep-sea ecosystems cover about 65% of the Earth's surface and play an important part in biomass production and biogeochemical cycles on a global scale[9]. These processes are largely mediated by benthic prokaryotes, which use organic detritus for biomass production and respiration[10]. Deep-sea ecosystems are dark and extreme environments that lack photosynthetic primary production, depend on the carbon export from the surface oceans and are characterized by severe organic nutrient limitation[9]. The biomass of all faunal components decreases exponentially with increasing water depth[11], but this does not apply to benthic prokaryotic biomass[11], which also has a relatively high metabolic rate[12]. Prokaryotic biomass, in the top 10-cm of deep-sea sediments, is approximately 160 Pg, which is equivalent to 30%–45% of the total microbial carbon on Earth[13]. The huge, nitrogen- and phosphorus-rich, prokaryotic biomass represents a potentially enormous and high quality food source for benthic consumers in deep-sea ecosystems[14], but *in situ* experiments suggest that prokaryotic biomass does not contribute significantly to the food requirements of higher trophic levels[15]. The high prokaryotic biomass in food-limited deep-sea ecosystems and the non-use of this component represent two unsolved paradoxes of the deep oceans.

Viruses in benthic deep-sea ecosystems

We report here that viral abundance in surface sediments (top 1-cm) worldwide is high at all depths, from the shelf-break down to the abyssal sediments (range, $0.10–3.53 \times 10^9\ g^{-1}$; mean, $0.96 \times 10^9\ g^{-1}$ (Supplementary Table 1), which are equivalent to $0.83–28.2 \times 10^{12}$ viruses m^{-2} with a mean of $7.65 \pm 0.32 \times 10^{12}$ viruses m^{-2}; Fig. 1a), with values similar to those reported for coastal sediments[16,17]. A high viral abundance in deep-sea sediments could be due to the supply of virioplankton adsorbed onto sinking particles[18] and/or to the high *in situ* viral production rates[16,17]. We found that the downward flux of viruses associated with settling particles to the deep sea was extremely low (Supplementary Table 2), whereas direct measurements, on the basis of different independent approaches, indicate that viral production rates in deep-sea sediments are consistently high, and that viruses are produced *in situ* (Fig. 1b and Supplementary Figs 2 and 3).

Further experiments conducted in both *in situ* pressure and decompressed samples indicated that >99% of the prokaryotic infections are lytic, both in deep-sea sediments and at the benthic boundary layer (Supplementary Fig. 4). Deep-benthic viruses are therefore functionally active and are capable of killing the infected prokaryotes. We also estimated the burst size of prokaryotes (defined as the number of viruses that cause the lysis of the host cell) in deep-sea sediments using different independent approaches, which ranged from 11 to 106 (mean of time course experiments, 45; median, 38; Supplementary Table 3 and Supplementary Fig. 5).

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Changes in hydrostatic pressure can alter the physiological status of the prokaryotes[19–21], thus potentially influencing viral production measurements. Analyses of viral and prokaryotic production in deep-sea sediment samples at *in situ*, decompressed (at 0.1 MPa) and re-pressurized conditions (see Supplementary Methods for details) demonstrated that production rates are not significantly influenced by deep-sea sample recovery (Supplementary Figs 6 and 7), allowing the conclusion that the high viral production rates are not due to artefacts.

Combining values of viral production and viral abundance in deep-sea sediments, we conclude that deep-sea viriobenthos is a highly dynamic and active component of deep-sea ecosystems, with a viral turnover (range, 0.15–8.2 d^{-1}, mean, 1.7 ± 0.1 d^{-1}) comparable to, or higher than, that reported for highly productive aquatic ecosystems[16].

Virus-induced prokaryotic mortality

Viral abundance and production did not change significantly with water depth, even when latitude, longitude and sampling time were included as covariates in the statistical analyses (Supplementary Table 4). However, viral and prokaryotic abundance (Fig. 1a, c) covaried across a wide range of depths and environmental conditions (Fig. 2a). Also, viral production and prokaryotic heterotrophic production (Fig. 1b, d) were significantly and positively related (Fig. 2b). These positive relationships suggest a dependence of viral replication on the host abundance and metabolism, and indicate that the observed variability across depths is not due to a methodological artefact. Thus, our data confirm the emerging view that deep-sea benthic ecosystems are more dynamic than previously thought[14] and suggest that the extreme conditions of the deep oceans are not hostile for viral development.

We estimated the impact of viruses on benthic prokaryotic production, calculating the number of prokaryotes killed as the ratio between viral production and prokaryotic burst size. Using the mean burst size of the time course experiments (burst size of 45) we calculated that viruses are responsible for the abatement of 80% of the total

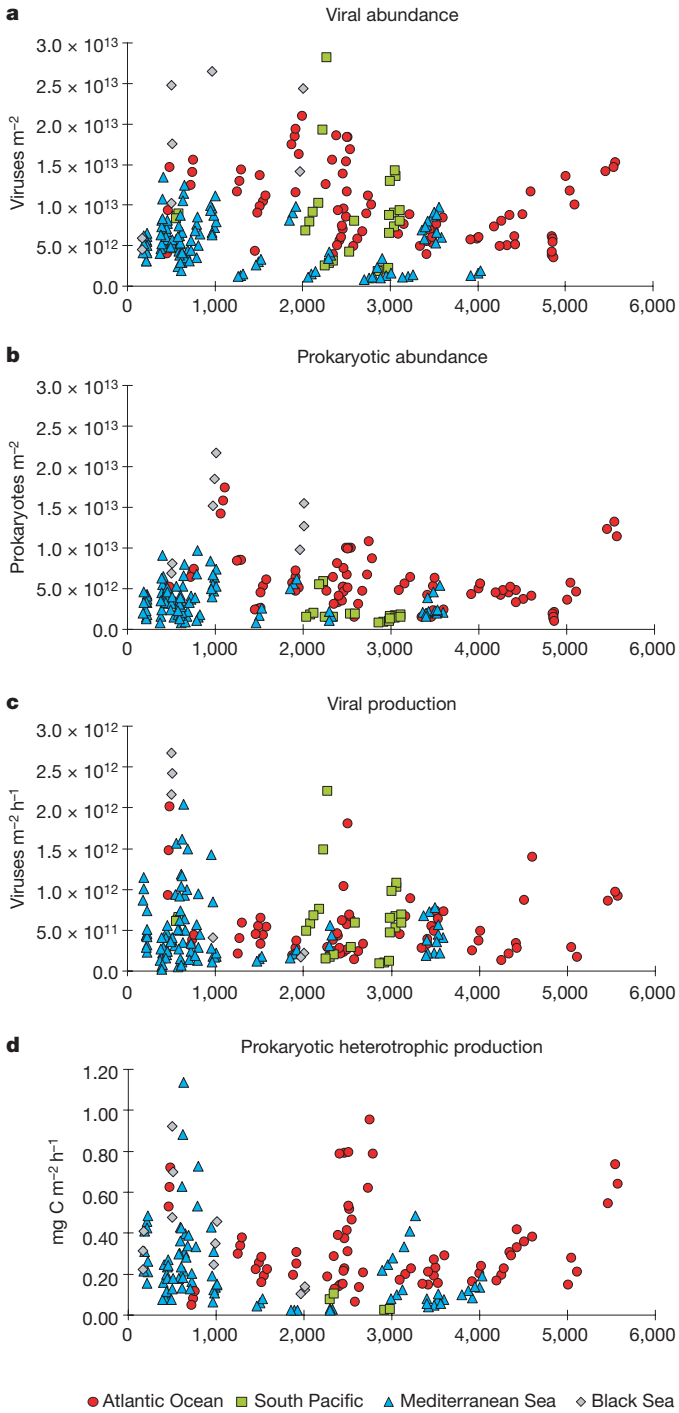

Figure 1 | Bathymetric patterns of viruses and prokaryotes in deep-sea sediments worldwide. Reported are the viral abundance (**a**; expressed as the number of viruses per m^2), the prokaryotic abundance (**b**; expressed as the number of prokaryotes per m^2), the viral production (**c**; expressed as the number of viruses produced per m^2 per h), and the prokaryotic heterotrophic production (**d**; expressed as mg C m^{-2} h^{-1}). Data originate from the Atlantic Ocean (temperate systems), the South Pacific Ocean (tropical warm systems), the western and eastern Mediterranean Sea (temperate warm systems) and the Black Sea (anoxic system).

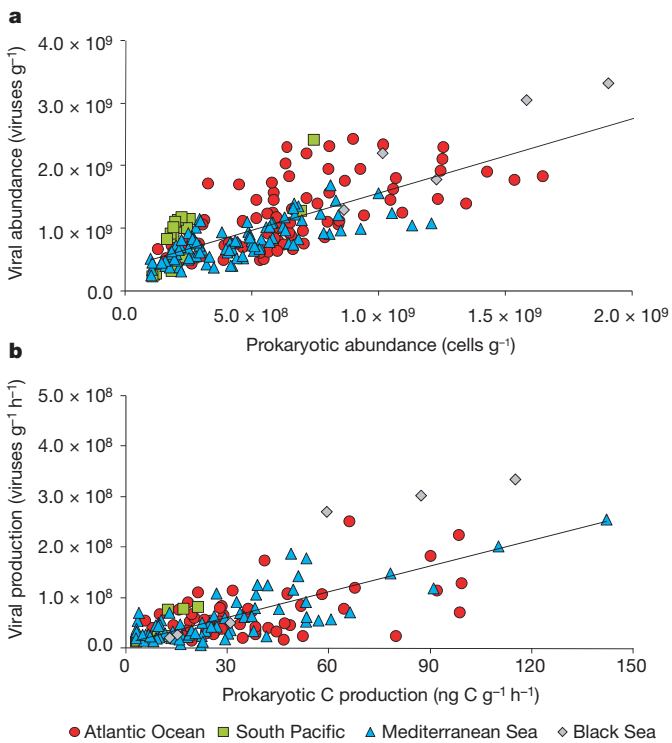

Figure 2 | Relationships between viruses and prokaryotes in deep-sea sediments worldwide. Reported are the relationship between viral and prokaryotic abundance (**a**; regression equation: $y = 1.19x + 3.63 \times 10^8$; $n = 206$, $R^2 = 0.56$, $P < 0.001$), and the relationship between viral and prokaryotic production (**b**; regression equation: $y = 1.71 \times 10^6 x + 9.89 \times 10^6$; $n = 186$, $R^2 = 0.57$, $P < 0.001$). Data originate from the Atlantic Ocean (temperate systems), the South Pacific Ocean (tropical warm systems), the western and eastern Mediterranean Sea (temperate warm systems) and the Black Sea (anoxic system).

prokaryotic heterotrophic production in global deep-sea sediments. Virus-induced prokaryotic mortality increases with water depth from 16% ± 3% (mean ± s.e.m., $n = 11$) in coastal sediments to 64% ± 3% in mesopelagic sediments (>160–1,000-m depth, $n = 41$) to 89% ± 2% in sediments beneath 1,000-m depth ($n = 67$; Fig. 3). These are conservative estimates, as the use of the mean or median burst sizes obtained from the three independent approaches (burst sizes of 37 or 33, respectively; Supplementary Table 3), or the values of burst sizes frequently used in the literature (20–30)[2,7], would result in the abatement of ~90% or more of the global prokaryotic carbon production.

These results indicate that viruses are the main agents of mortality of prokaryotes in deep-sea sediments worldwide, and determine the shunt of most of the prokaryotic carbon production into organic detritus. This finding has important implications for the comprehension of food webs and biogeochemical processes in deep-sea ecosystems. Viruses, killing most of the prokaryotes produced, drastically reduce the microbial biomass potentially available to higher trophic levels in deep-sea sediments. These results help to explain the paradox of deep-sea ecosystems in which prokaryotic biomass is largely unused by deep-sea fauna, despite severe food limitation[15].

Implications on ecosystem functioning

The amount of organic resources in the deep sea is typically extremely low (organic carbon concentrations are 10–20 times lower than in coastal systems), mostly composed of refractory compounds[22] and could represent a limiting factor for prokaryotic metabolism and turnover[23,24]. There is evidence to suggest that intracellular material released by viral lysis can be an important and readily bioavailable organic source for prokaryotes[25,26]. The significant relationship between the release of carbon from prokaryotic lysis and prokaryotic turnover in deep-sea sediments reported here (Fig. 4) suggests that a stronger viral shunt is associated with higher prokaryotic growth rates. This relationship allows us to speculate that the viral shunt, on the one hand, kills an important fraction of benthic prokaryotes and reduces the competition for available resources and, on the other, generates labile, highly bioavailable material and stimulates the metabolism of uninfected deep-sea prokaryotes. These results contribute to explaining the paradox of a fast prokaryotic turnover in food-limited, deep-sea ecosystems.

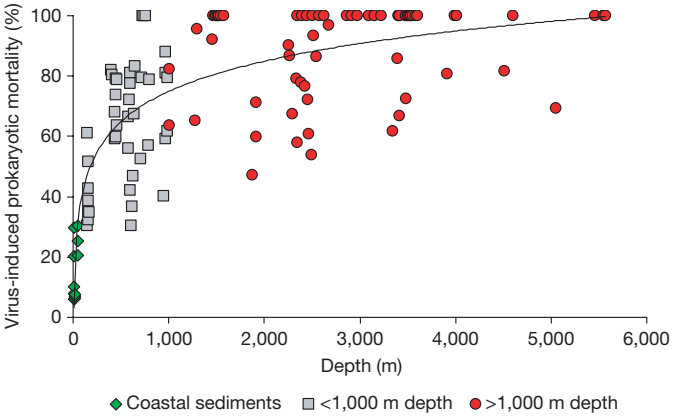

Figure 3 | Bathymetric patterns of virus-induced prokaryotic mortality. The virus-induced prokaryotic mortality was calculated as the number of cells killed divided by the total number of prokaryotes produced per hour, and expressed as a percentage. The regression equation is $y = 14.46\ln(x) - 24.98$; $n = 119$, $R^2 = 0.686$, $P < 0.001$. Data from coastal sediments (green diamonds) are derived from the literature[17,26,27]. Data for mesopelagic (grey squares), and bathyal and abyssal sediments (red circles) originate from the Atlantic Ocean (temperate systems), the South Pacific Ocean (tropical warm systems), the western and eastern Mediterranean Sea (temperate warm systems) and the Black Sea (anoxic system).

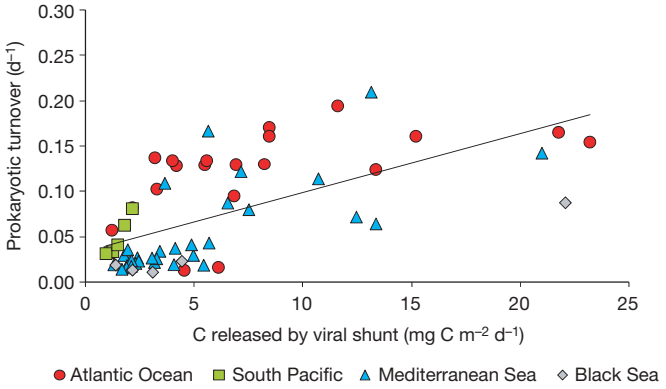

Figure 4 | Relationship between carbon released by viral lysis of prokaryotic biomass (viral shunt) and by prokaryotic turnover. Prokaryotic turnover was expressed per day. The regression equation is: $y = 0.0065x + 0.034$; $n = 63$, $R^2 = 0.391$, $P < 0.01$. Data originate from the Atlantic Ocean (temperate systems), the South Pacific Ocean (tropical warm systems), the western and eastern Mediterranean Sea (temperate warm systems) and the deep Black Sea (anoxic system).

The release of labile organic material from killed prokaryotic cells has important implications for carbon cycling and nutrient regeneration on a global scale. We estimate that the viral shunt, on a global scale, can release from ~0.37 Gt C yr^{-1} (the most conservative estimate on the basis of the highest value of burst size) to 0.63 Gt C yr^{-1} (if a burst size of 45 is used). Therefore, the viral shunt is an essential source of labile organic compounds to deep-sea ecosystems. Using conservative conversion factors[14], we estimate that the organic carbon supplied by viral shunt contributes to around 35% of the total benthic prokaryotic metabolism, increases their turnover and promotes the recycling of key elements (including nitrogen and phosphorus associated with prokaryotic biomass). Given the global relevance of biogeochemical processes occurring in deep-benthic ecosystems[10], our results indicate that the deep-sea viral shunt is a crucial process, so far neglected, which has to be included in global models of carbon, nitrogen and phosphorus cycling and nutrient flows.

We conclude that viral infection has an important part in the functioning of the largest ecosystem of the biosphere by controlling benthic prokaryotic biomass (top down, predatory control), by stimulating prokaryotic metabolism (bottom-up mechanism), and by accelerating biogeochemical processes.

METHODS SUMMARY

We collected a data set of 232 deep-sea sediment samples and measured the impact of viruses on deep-sea benthic prokaryotes and biogeochemical cycles. Our data cover latitudes from 79° N to 34° S, and all depths from 165 m to 5,571 m (sampling location and details on the data set is provided in Supplementary Table 1 and Fig. 1), and include deep-sea sites spanning a wide range of bottom-water temperatures and trophic conditions. Measurements of viral and prokaryotic abundance and production were carried out synoptically on the same samples and by using the same protocols for the entire data set, thus ensuring methodological consistency.

Viral and prokaryotic abundances in deep-sea sediments were determined by epifluorescence microscopy using highly sensitive fluorochromes. For the determination of viral production, two different procedures were applied and compared. The first is based on a dilution technique of samples with virus-free sea water, and allows the determination of viral production on the basis of the increase in viral number over time. The second is based on the use of ^{3}H-thymidine, and allows the determination of viral production through the assessment of the incorporation rates of radiolabelled substrates into viral genomes. Radiolabelled substrates were also used to determine prokaryotic heterotrophic carbon production. To substantiate the measurements of viral and prokaryotic heterotrophic carbon production carried out at 0.1 MPa, further experiments were carried out under *in situ* pressure conditions using *ad hoc* high pressure sampling devices. The ratio between viral production and prokaryotic burst size (estimated using three independent approaches) was used to estimate the

prokaryotic mortality induced by viruses. Finally, the relative importance of lysogenic infections was investigated by time course experiments of deep-sea samples previously treated with specific agents inducing the lytic cycle.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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1. Suttle, C. A. Viruses in the sea. *Nature* **437,** 356–361 (2005).
2. Weinbauer, M. G. Ecology of prokaryotic viruses. *FEMS Microbiol. Rev.* **28,** 127–181 (2004).
3. Proctor, L. M. & Fuhrman, J. A. Viral mortality of marine bacteria and cyanobacteria. *Nature* **343,** 60–62 (1990).
4. Suttle, C. A., Chan, A. M. & Cottrell, M. T. Infection of phytoplankton by viruses and reduction of primary productivity. *Nature* **347,** 467–469 (1990).
5. Suttle, C. A. Marine viruses—major players in the global ecosystem. *Nature Rev. Microbiol.* **5,** 801–812 (2007).
6. Fuhrman, J. A. Marine viruses and their biogeochemical and ecological effects. *Nature* **399,** 541–548 (1999).
7. Wommack, K. E. & Colwell, R. R. Virioplankton: viruses in aquatic ecosystems. *Microbiol. Mol. Biol. Rev.* **64,** 69–114 (2000).
8. Wilhelm, S. W. & Suttle, C. A. Viruses and nutrient cycles in the sea. *Bioscience* **49,** 781–788 (1999).
9. Gage, J. D. & Tyler, P. A. *Deep-Sea Biology: A Natural History of Organisms at the Deep Sea Floor* (Cambridge Univ. Press, 1991).
10. Jahnke, R. A. The global ocean flux of particulate organic carbon: areal distribution and magnitude. *Glob. Biogeochem. Cycles* **10,** 71–88 (1996).
11. Rex, M. A. *et al.* Global bathymetric patterns of standing stock and body size in the deep-sea benthos. *Mar. Ecol. Prog. Ser.* **317,** 1–8 (2006).
12. Yayanos, A. A. Microbiology to 10,500 meters in the deep sea. *Annu. Rev. Microbiol.* **49,** 777–805 (1995).
13. Whitman, W. B., Coleman, D. C. & Wiebe, W. J. Prokaryotes: The unseen majority. *Proc. Natl Acad. Sci. USA* **95,** 6578–6583 (1998).
14. Dell'Anno, A. & Danovaro, R. Extracellular DNA plays a key role in deep-sea ecosystem functioning. *Science* **309,** 2179 (2005).
15. Witte, U. *et al. In situ* experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature* **424,** 763–766 (2003).
16. Danovaro, R. *et al.* Viriobenthos in freshwater and marine sediments: a review. *Freshwat. Biol.* **53,** 1186–1213 (2008).
17. Siem-Jørgensen, M., Glud, R. N. & Middelboe, M. Viral dynamics in a coastal sediment: seasonal pattern, controlling factors and relations to the pelagic-benthic coupling. *Mar. Biol. Res.* **4,** 165–179 (2008).
18. Mari, X., Kerros, M.-E. & Weinbauer, M. G. Virus attachment to transparent exopolymeric particles along trophic gradients in the Southwestern Lagoon of New Caledonia. *Appl. Environ. Microbiol.* **73,** 5245–5252 (2007).
19. Tamburini, C., Garcin, J., Ragot, M. & Bianchi, A. Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean. *Deep-Sea Res. II* **49,** 2109–2123 (2002).
20. Tamburini, C., Garcin, J. & Bianchi, A. Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea. *Aquat. Microb. Ecol.* **32,** 209–218 (2003).
21. Bartlett, D. H. Pressure effects on *in vivo* microbial processes. *Biochim. Biophys. Acta* **1595,** 367–381 (2002).
22. Druffel, E. R. M. & Robinson, D. H. Is the deep sea on a diet? *Science* **284,** 1139–1140 (1999).
23. Deming, J. W. & Barross, J. A. in *Organic Geochemistry: Principles and Applications* (eds Engel, M. H. & Macko, S.A.) 119–144 (Plenum, 1993).
24. Danovaro, R., Della Croce, N., Dell'Anno, A. & Pusceddu, A. A depocenter of organic matter at 7800-m depth in the SE Pacific Ocean. *Deep-Sea Res. I* **50,** 1411–1420 (2003).
25. Noble, R. T. & Fuhrman, J. A. Breakdown and microbial uptake of marine viruses and other lysis products. *Aquat. Microb. Ecol.* **20,** 1–11 (1999).
26. Corinaldesi, C., Dell'Anno, A. & Danovaro, R. Viral infection plays a key role in extracellular DNA dynamics in marine anoxic systems. *Limnol. Oceanogr.* **52,** 508–516 (2007).
27. Mei, M. L. & Danovaro, R. Virus production and life strategies in aquatic sediments. *Limnol. Oceanogr.* **49,** 459–470 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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METHODS

Sampling sites and sampling strategy. Sampling was carried out in the Atlantic Ocean (~30% of the investigated sites), the Pacific Ocean (~25%), the Mediterranean Sea (~42%) and the Black Sea (~3%). Overall, 38% of the sampling sites were located between depths of 165 m and 1,000 m, 35% were between depths of 1,000 m and 3,000 m, and 27% were between depths of 3,000 m and 5,571 m. Details of the materials and methods used in the present study are reported in the Supplementary Information.

Viral abundance, production and lysogenic fraction. For the determination of viral abundance, fresh and unpreserved sediments were processed immediately after retrieval to minimize viral loss due to the use of preservatives (for example, formaldehyde)[28–30]. Viral abundances were determined by epifluorescence microscopy after extraction of benthic viruses from the sediments and subsequent staining with SYBR Green I (ref. 29). Viral abundance was normalized to gram of sediment dry weight after desiccation (60 °C, 24 h) and converted in surface units (viruses m^{-2}), assuming a constant value of 1.8 g cm^{-3} for the sediment dry bulk density.

Viral production in deep-sea sediment samples was determined by the dilution technique[27,31] (see Supplementary Methods for details). This technique has been the most widely used so far in benthic environments (thus enabling a comparison with available data) and has the advantage of minimizing the impact of protozoan and/or faunal grazing during the incubations[16]. The reliability of the protocol was tested by comparing the results of viral production obtained using different sediment dilutions (1:2, 1:5, 1:10 (v/v) of sediment versus virus-free sea water; see Supplementary Methods), and by comparing the results of viral production acquired by the dilution technique with those obtained by the incorporation of ^{3}H-thymidine by deep-sea viruses[32,33]. All incubations were carried out in the dark and at *in situ* temperature. The effects of pressure were tested for both techniques (dilution and ^{3}H-thymidine incorporation) by comparing viral production from decompressed samples (0.1 MPa) with samples maintained at *in situ* pressure (Supplementary Figs 3 and 6). To estimate the lysogenic fraction among natural assemblages of deep-sea prokaryotes, we used one of the most widely used induction agents (mitomycin carbon[27,34]; Supplementary Methods). Deep-sea sediment samples were inoculated with mitomycin carbon and incubated at *in situ* pressure and temperature. Decompressed samples (0.1 MPa) were used as a control (Supplementary Fig. 4).

Prokaryotic abundance, biomass and production. Prokaryotic abundance in deep-sea sediments was determined from the same sediment samples used for the viral counts. Prokaryotic cells were extracted from the sediments according to standard procedures, stained with SYBR Green I and counted by epifluorescence microscopy [27–29]. For the determination of the prokaryotic biomass, the cell biovolume was converted into carbon content assuming 310 fg C μm^{-3} as a conversion factor[24,35]. Prokaryotic abundance and biomass were normalized to sediment dry weight after desiccation (60 °C, 24 h). Prokaryotic heterotrophic production in deep-sea sediments was determined by ^{3}H-leucine incorporation (up to 3 h of incubation in the dark at *in situ* temperature)[24]. The effect of pressure on benthic prokaryotic heterotrophic production was tested using ^{3}H-thymidine incorporation[36], synoptically with tests conducted for viral production and compared to decompressed and non-decompressed samples (Supplementary Fig. 7).

Effects of pressure on viral and prokaryotic production. In 2005 and 2007, two series of independent experiments were conducted to test the effects of pressure on different microbial variables (that is, viral production, lysogenic fraction and prokaryotic heterotrophic production). In 2005, 500-ml high-pressure bottles were used for the sampling of the water–sediment interface (collected at 2,474 and 3,526 m depth). Samples were incubated at *in situ* pressure and temperature conditions[19,20] with special attention to maintain the same pressure during the entire experiment (recovery, transfer and incubations). In 2007, sediment samples were collected at 3,500 m depth by means of a multiple corer, retrieved on board, immediately transferred in sterilized syringes and re-pressurized in high-pressure bottles, kept in the dark and under *in situ* temperature conditions for subsequent viral and prokaryotic production measurements (see details in Supplementary Methods). All variables were determined by time course experiments and, at each sampling time, aliquots of the incubated samples were withdrawn directly from the pressurized experimental system (see Supplementary Methods). For all experiments, extra samples were collected, using the same sampling equipments without pressurization for evaluating the effect of incubation at 0.1 MPa on all of the measured microbial variables.

Virus-induced prokaryotic mortality. The number of prokaryotes killed by viruses per gram of sediment per hour was calculated as the ratio between viral production (as the number of benthic viruses produced per gram of sediment per hour) and burst size (as the number of viruses released by cell lysis because of viral infection). Prokaryotic burst size was estimated using three independent approaches: (1) from time course experiments in which the increase of viral abundance with time was divided by the number of prokaryotic cells killed; (2) by applying a regression analysis between cell biovolume and burst size derived from the literature[37]; and (3) using transmission electron microscopy for counting the number of phages in deep-sea benthic prokaryotic cells[38]. Details on the procedures are reported in Supplementary Information. The virus-induced prokaryotic mortality was calculated as the number of cells killed divided by the total number of prokaryotes produced per hour, and expressed as a percentage[27].

Statistical analyses. To test whether the investigated benthic microbial variables were influenced by water depth, latitude, longitude or sampling period, we carried out a multivariate multiple regression analysis. All analyses were done with the routine distance-based multivariate analysis for a linear model (DISTLM) forward, and the effects of depth, sampling time, longitude and latitude were included as covariates in the analyses. *P* values were obtained with 4,999 permutations of residuals under the reduced model[39].

28. Helton, R. R., Liu, L. & Wommack, K. E. Assessment of factors influencing direct enumeration of viruses within estuarine sediments. *Appl. Environ. Microbiol.* **72,** 4767–4774 (2006).
29. Danovaro, R., Dell'Anno, A., Trucco, A., Serresi, M. & Vanucci, S. Determination of virus abundance in marine sediments. *Appl. Environ. Microbiol.* **67,** 1384–1387 (2001).
30. Wen, K., Ortmann, A. C. & Suttle, C. A. Accurate estimation of viral abundance by epifluorescence microscopy. *Appl. Environ. Microbiol.* **70,** 3862–3867 (2004).
31. Wilhelm, S. W., Briden, S. & Suttle, C. A. A dilution technique for the direct measurement of viral production: a comparison in stratified and tidally mixed coastal waters. *Microb. Ecol.* **43,** 168–173 (2002).
32. Steward, G. F., Wikner, J., Smith, D. C., Cochlan, W. P. & Azam, F. Estimation of virus production in the sea: 1. Method development. *Mar. Microb. Food Webs* **6,** 57–78 (1992).
33. Fuhrman, J. A. & Noble, R. T. Viruses and protists cause similar bacterial mortality in coastal seawater. *Limnol. Oceanogr.* **40,** 1236–1242 (1995).
34. Weinbauer, M. G., Brettar, I. & Höfle, M. G. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic marine waters. *Limnol. Oceanogr.* **48,** 1457–1465 (2003).
35. Fry, J. C. in *Methods in Aquatic Bacteriology* (ed. Austin, B.) 27–72 (John Wiley & Sons, 1990).
36. Fuhrman, J. A. & Azam, F. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar. Biol.* **66,** 109–120 (1982).
37. Weinbauer, M. G. & Höfle, M. G. Size-specific mortality of lake bacterioplankton by natural virus communities. *Aquat. Microb. Ecol.* **15,** 103–113 (1998).
38. Weinbauer, M. G., Fuks, D. & Peduzzi, P. Distribution of viruses and dissolved DNA along a coastal trophic gradient in the Northern Adriatic Sea. *Appl. Environ. Microbiol.* **59,** 4074–4082 (1993).
39. Anderson, M. J. *DISTLM v.5: a FORTRAN Computer Program to Calculate a Distance-Based Multivariate Analysis for a Linear Model* (Department of Statistics, Univ. Auckland, 2004).