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MICROBIOLOGY

ALOHA!
Ecological paradigms from the Hawaii Ocean Time-series

The good, the bad, the ugly
Microbial metabolites and colorectal cancer

Microbial oceanography and the Hawaii Ocean Time-series programme

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Abstract | The Hawaii Ocean Time-series (HOT) programme has been tracking microbial and biogeochemical processes in the North Pacific Subtropical Gyre since October 1988. The near-monthly time series observations have revealed previously undocumented phenomena within a temporally dynamic ecosystem that is vulnerable to climate change. Novel microorganisms, genes and unexpected metabolic pathways have been discovered and are being integrated into our evolving ecological paradigms. Continued research, including higher-frequency observations and at-sea experimentation, will help to provide a comprehensive scientific understanding of microbial processes in the largest biome on Earth.

Gyres
The vast ecosystems that encompass open ocean waters of the tropical and subtropical regions of the oceans. The circular movements of the gyres, deriving from the combination of wind stress on the sea surface and the rotation of the Earth, result in physically isolated, stratified upper ocean waters that are persistently devoid of bioessential inorganic nutrients.

Biome
Contiguous habitats that share similar biogeochemical and physical properties.

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The truth is, the science of nature has been already too long made only a work of the brain and the fancy. It is now high time that it should return to the plainness and soundness of observations on material and obvious things. Robert Hooke, 1665 (REF. 1)

The ocean is an integral component of the climate system of the Earth, and it stores and transports heat, produces and consumes climate-reactive gases and regulates the hydrological cycle of the planet. Oceanic ecosystems comprise some of the largest and most complex habitats on Earth, and microorganisms — viruses, bacteria, archaea and microeukaryotes — dominate the biomass and metabolism that occur in these systems.

Contemporary marine microbial assemblages are numerically abundant and functionally diverse. Among many crucial roles, they sustain nearly one-half of global photosynthesis[2] and thus help to sequester carbon dioxide and sustain planetary habitability. Nevertheless, most marine ecosystems are grossly undersampled, so many of the details concerning the rates, pathways and controls of the carbon cycle of the oceans and cycles of related elements, including nitrogen and phosphorus, are incomplete[3]. As a result, limited information is available to assess the susceptibility and sensitivity of microbial assemblages to changes in natural and anthropogenically induced climate variability. This is especially true for the expansive open-ocean ecosystems, known as subtropical gyres, which occupy ~40% of the surface of our planet but which are remote and chronically undersampled. The North Pacific Subtropical Gyre (NPSG), which extends from approximately 15°N to 35°N latitude and from 135°E to 135°W, is the largest contiguous biome on Earth[4]. Analogous biomes are present in the South Pacific Ocean, the Indian Ocean and in both hemispheres of the Atlantic Ocean[5]. The NPSG ecosystem is very old (the present boundaries were established >10^7 years ago[6]) and was hypothesized to represent a terminal successional stage 'climax-type' community[7], with highly evolved and time-invariant plant, animal and microbial assemblages. Nevertheless, there are few places in the oceans of the world where time-resolved measurements are available for evaluating the temporal variability in microbial processes.

By the mid-1980s, increasing awareness of the importance of microorganisms to ecosystem health and function, together with recognition that the oceans are a crucial component of the global climate system, motivated the development of major field programmes that sought to understand the role of microorganisms in the oceanic carbon cycle. In 1988, the Hawaii Ocean Time-series (HOT) programme (see Further information) established Station ALOHA (A Long-term Oligotrophic Habitat Assessment) in the NPSG as a core component of the Joint Global Ocean Flux Study (JGOFS) (BOX 1).

When HOT began in October 1988, momentum was building towards the development of a detailed understanding of marine microbial assemblages, their effects on biogeochemical cycles and the sensitivities of microbially mediated processes to climate change. As most naturally occurring microorganisms were not in pure culture, taxonomic identities and metabolic characteristics were lacking and interactions among marine microorganisms had been mostly unexplored. The discovery of *Synechococcus* spp. and related novel groups of chroococcoid cyanobacteria — the so-called

Box 1 | **It's about time (series)**

Long-term, time-series studies are ideally suited to investigate complex marine microbiological processes that are affected by natural habitat variability and human-induced climate change. In 1988, the Joint Global Ocean Flux Study (JGOFS) established two ocean time series programmes: one in the North Atlantic Ocean near Bermuda (that is, Bermuda Atlantic Time-series Study (BATS)) and the other at Station ALOHA (A Long-term Oligotrophic Habitat Assessment), near Hawaii, USA (that is, Hawaii Ocean Time-series (HOT)) (FIG. 1). The overarching mission of JGOFS was to understand the physical, chemical and biological processes that govern the production and fate of biogenic materials (especially carbon) in the sea well enough to predict their influences on, and responses to, natural and anthropogenic global-scale perturbations on interannual to interdecadal timescales.

As the value of the emergent HOT and BATS data sets became evident, six additional ocean observatories with similar JGOFS scientific objectives, sampling frequencies and methods were established worldwide to provide understanding of microbial and biogeochemical processes in the sea[14]. Although the international JGOFS programme has ended, the number of coastal and open ocean observatories has continued to grow and currently includes more than 50 locations (Oceansites.org; see Further information). Although only a few of these observatories routinely track microbial processes, cross-ecosystem analysis between and among those that do has proven to be a useful tool for comparing microbial population structures and the regulation of metabolism[14,15]. For example, a metagenomic comparison of coexisting populations of *Prochlorococcus* spp. and *Pelagibacter* spp. from BATS and HOT revealed large differences in the occurrence of multiple genes that encode proteins that are involved in phosphate acquisition and metabolism. Higher representation at the phosphorus-stressed BATS station documented microbial adaptation to phosphorus scarcity[50].

picophytoplankton (that is, the 0.2–2 μm size class[8,9]) — fundamentally changed our basic understanding of the size structure of marine photosynthesis and microbial food web dynamics, specifically the central function of dissolved organic matter (DOM) cycling within the microbial loop[10]. The key roles of marine viruses were not yet known[11], and the marine omics revolution was just getting underway (BOX 2).

The HOT programme recently completed 25 years of operation, providing unique data sets replete with previously undescribed phenomena and some major surprises[12–15]. This Review presents a few of the many discoveries and highlights the value of the sustained time series sampling approaches for quantifying climate-sensitive microbial dynamics in the sea.

Habitat characteristics and seascape variability

Station ALOHA is located at 22°45′N, 158°W in deep water (4,740 m) approximately 100 km north of Kahuku Point, Oahu, Hawaii, USA, in a region that is representative of the NPSG (FIG. 1a). The habitat is characterized by warm surface waters (>24 °C) throughout the year, which create a permanent low-density cap above the stratification, which begins at approximately 60 m and effectively isolates the upper mixed layer (~0–40 m) from the remainder of the water column (FIG. 1b). Consequently, the sunlit mixed layer is chronically nutrient starved and has a near-zero nutrient concentration gradient that precludes efficient nutrient delivery from deeper in the water column (FIG. 1b). This observed separation of excess solar energy in the surface waters from inorganic nutrients below leads to conditions of extreme oligotrophy, with low-standing stocks of chlorophyll *a* (<100 ng per l), low nitrate (NO_3^-) concentrations (<10 nmol per l) and low total biomass of living microorganisms (<2 μg carbon per l). Just before the start of the HOT programme, a coordinated field study known as Plankton Rate Processes in Oligotrophic Oceans (PRPOOS; pronounced 'purpose' (REF. 16)) was organized to address the challenges of obtaining accurate microbial rate measurements in oligotrophic ecosystems. This study indicated that, despite a lack of nutrients, microbial production and growth rates in the NPSG were substantial and seemed to have been previously underestimated[17,18]. In reviewing the state of knowledge at that time, Hayward[19] termed this a "controversy with important implications", as similar habitats to the NPSG dominate our planet and greatly affect the global carbon cycle.

HOT was initially proposed as a programme of 5–10 years duration and was designed to resolve seasonal and interannual habitat variability and to understand the physical controls on biological carbon production and export, and the remineralization of organic matter. Site selection, sampling frequency and the choice of core parameters and surveillance methods were informed by previous observations in the NPSG[20–23]. However, just before the beginning of HOT, Venrick *et al.*[24] reported an unexpected decade-scale (1968–1985) doubling of euphotic zone inventories of chlorophyll (FIG. 2a). They hypothesized that increased winter winds and decreased sea-surface temperature had altered nutrient dynamics, which effectively increased the carrying capacity of the ecosystem[24]. These observations challenged the preconceived idea of long-term environmental constancy in the NPSG and suggested that a decadal (or longer) scale climate assessment would also be required to achieve the HOT programme objectives.

During the first 25 years of observations at Station ALOHA, we have established a long-term climatology against which to assess variability from the mean state; for example, although rates of primary production in the NPSG show predictable seasonality that is linked to solar radiation, aperiodic phytoplankton blooms are recurring features of this habitat[25–27]. Although the mechanism is not well understood, blooms seem to be linked to mesoscale eddies and Rossby waves[28–31]. These anomalies are not restricted to the concentration of chlorophyll in the well-lit regions of the upper ocean: in January 2001, an unexpected water mass, the source of which was tracked to the shelf waters off Mexico, propagated through the Station ALOHA sampling site at water depths of 300–550 m (REF. 32). A nearly identical subsurface feature was encountered during a 2013 Seaglider survey near Station ALOHA (FIG. 2b). Among other unusual characteristics, these foreign water masses had mid-depth (300–550 m) dissolved oxygen concentrations that were more than 10 standard deviations lower than the HOT climatology. With such limited observations, it remains unclear how these infrequent events might affect carbon, nutrient and microbial dynamics. Finally, the growing atmospheric inventory of carbon dioxide, which was first detected using well-calibrated time series measurements at strategic locations[33], provided the incentive for a Station ALOHA CO_2 programme (FIG. 2c). Consistent with the

Oligotrophic
A term used to describe environments that are characterized by low concentrations of growth-requiring nutrients and, consequently, low microbial biomass. Such habitats dominate the upper ocean of the large subtropical gyres. An oligotroph is the term used to describe an organism that is adapted to growing in habitats with low-nutrient conditions.

Euphotic zone
The region of the well-lit upper ocean that sustains the net production of organic matter. Often defined by the depth of penetration of sunlight, typically the depth to which 0.1% of the light intensity that is observed at the surface ocean penetrates. In clear, open ocean ecosystems, the euphotic zone can extend to 150–200 m.

Climatology
In oceanography, this term refers to the determination of the mean ecosystem state, which requires averaging of time-resolved observations of sufficient duration to adequately sample processes underlying the dominant modes of ecosystem variability.

Box 2 | **The marine omics revolution**

In 1958, Francis Crick[159] first hypothesized what has become known as the central dogma of biology — namely, the unidirectional flow of information from DNA to RNA to proteins. Soon thereafter, methods were developed to measure the concentrations (both particulate and dissolved) and dynamics (production and turnover) of these key constituents in the sea. DNA synthesis was used to track microbial growth rates, and the activities of specific enzymes were used to track energy and nutrient transformations. However, at the start of the Hawaii Ocean Time-series (HOT) programme in 1988, one could not imagine the scale and scope of the marine omics revolution that was about to take place.

The era of gene sequencing began in 1977, when novel methods were developed to determine the precise order of nucleotides within a DNA molecule[160,161]. This technology led to the first complete bacterial genome sequence 2 decades later[162]. More than 40,000 microbial genomes have been sequenced so far, with an increasing repertoire of marine representatives (Genomes online; see Further information). Whole-genome multiple displacement amplification methods have more recently been used for DNA sequencing of single cells that have been isolated from the marine environment using fluorescence-activated cell sorting, which has provided a unique opportunity to link phylogeny and metabolic potential, even in as-yet-uncultured taxa[163]. A major advance was the introduction of cultivation-independent, whole-community analysis of 16S ribosomal RNA genes as phylogenetic markers[164]. This technology enabled researchers to discover previously unknown phylotypes and to obtain quantitative assessments of microbial species diversity. With the subsequent application of massively parallel DNA tag sequencing techniques and novel computational methods, a 'rare biosphere' of marine microorganisms was discovered in the deep sea[165], although neither the metabolic state nor the ecological role of these low-rank-order abundance microorganisms has been firmly established[166]. The results of a comprehensive, global census of marine microorganisms (from the Bacteria, Archaea and Eukarya) using a standardized pyrotag sequencing approach was recently published[167].

DNA sequence analysis has also improved our understanding of biogeochemical cycles via functional gene surveys of marine habitats. Advances in DNA sequencing technologies made it possible to move from the analysis of rRNA genes to the analysis of the entire range of genes in a given habitat (known as metagenomics). In one of the first marine applications, Venter *et al.*[106] analysed several surface water samples near Bermuda and reported 1.2 million previously unknown genes. Shortly thereafter, the initial phase of the Global Ocean Sampling (GOS) survey reported additional novel sequences, including 1,700 additional new protein families[168].

Cell metabolism and its regulation are choreographed via gene expression (that is, DNA transcription and protein synthesis). The genome (or metagenome) represents the metabolic potential and sets the boundary conditions, whereas gene expression defines cellular function. The environmental metatranscriptome can be used to assess gene expression and induction intensity and is an important tool for use in environmental perturbation studies; for example, McCarren *et al.*[169] reported that selected Kyoto encyclopaedia of genes and genomes (KEGG) orthologue transcripts were enriched >500-fold just 2 hours after the addition of dissolved organic matter, which documents the ability of microorganisms to detect and respond quickly to environmental perturbations.

A high-density (19,400 unique sequences) oligonucleotide microarray (known as Microbiological Targets for Ocean Observing Laboratories (MicroTOOLS; see Further information)) that targets key functional genes of diverse marine microbial taxa from all three domains of life and from viruses has been used to quantify specific genes and transcripts[170]. Finally, a versatile robotic device, known as the Environmental Sample Processor (ESP)[171], is capable of unattended, *in situ* gene detection and quantification throughout an extended period of time. The next-generation ESP, which is currently under development, will be integrated into an autonomous underwater vehicle to explore microbial diversity and function in both space and time at Station ALOHA (A Long-term Oligotrophic Habitat Assessment) and elsewhere.

This 'explosion' of interest and investment in marine omics may have had the unintended consequence of the physiology, metabolism and ecology of marine microorganisms being somewhat ignored. Although considerable progress has been made by investments in omics-based approaches, there is now an urgent need to invest equally in new approaches to advance our understanding of microbial biogeochemistry and metabolism in the sea.

Primary production
The synthesis of organic matter from inorganic carbon. In the ocean, the vast majority of primary production is fuelled by photosynthesis.

Mesoscale eddies and Rossby waves
Physical processes that occur at spatial scales of 50–500 km and generally persist for 10–100 days. Such processes can originate from instability in the flow of currents owing to topographic features, variations in wind stress at the surface of the ocean or result from shear in the flow of waters of differing physical properties (that is, viscosity and density), such as along frontal boundaries. Such physical perturbations can propagate energy through the ocean in the form of waves or can result in the formation of isolated circulation vortices (similar to a cyclone in the atmosphere) that horizontally transport water of similar physical properties.

Biogeochemistry
The study of the interactions between biological processes and geochemical properties on Earth.

Phycobiliproteins
Proteins that are found in the photosynthetic light-harvesting complexes of various phototrophic cyanobacteria and eukaryotic algae. The proteins capture light energy and, via fluorescence events, transfer energy to photosynthetic reaction centres.

atmospheric observations at Mauna Loa Observatory, Hawaii, USA, the surface ocean CO_2 inventory is also increasing, but with greater seasonal and interannual variability[34,35] (FIG. 2c), which is leading to the condition known as ocean acidification[36].

Novel microorganisms

The three major groups of microorganisms that are now known to be numerically dominant members of the NPSG ecosystem (which are *Prochlorococcus* spp., the SAR11 clade of Alphaproteobacteria and planktonic archaea) were all discovered after the initiation of the HOT programme. Consequently, our marine microbial paradigms are changing. There are many examples in which sampling at Station ALOHA has catalysed major advances in our understanding of the role of these organisms in ocean ecology and biogeochemistry.

Prochlorococcus *spp.* Johnson and Sieburth[9] provided the first electron microscopic evidence for novel prokaryotic phototrophs, which they termed 'type II cells' to distinguish them from *Synechococcus* spp. Nearly a decade later, just as we were preparing for the first HOT cruise (HOT-1; October 1988), *Prochlorococcus* spp. was discovered in the North Atlantic Ocean[37]. This novel, abundant group of unicellular cyanobacteria lacks phycobiliproteins but contains unusual divinyl chlorophyll *a* and chlorophyll *b* pigments[37]. Indeed, it was their unique pigment-based flow cytometric signature (red fluorescence at 660–700 nm when excited by blue light at 488 nm) that led to this important discovery (FIG. 3a,b). Soon afterwards, it was shown that *Prochlorococcus* spp. was the dominant oxygenic phototroph in all tropical and subtropical marine environments[38], including Station ALOHA[39,40]. Whereas *Prochlorococcus* spp. cell

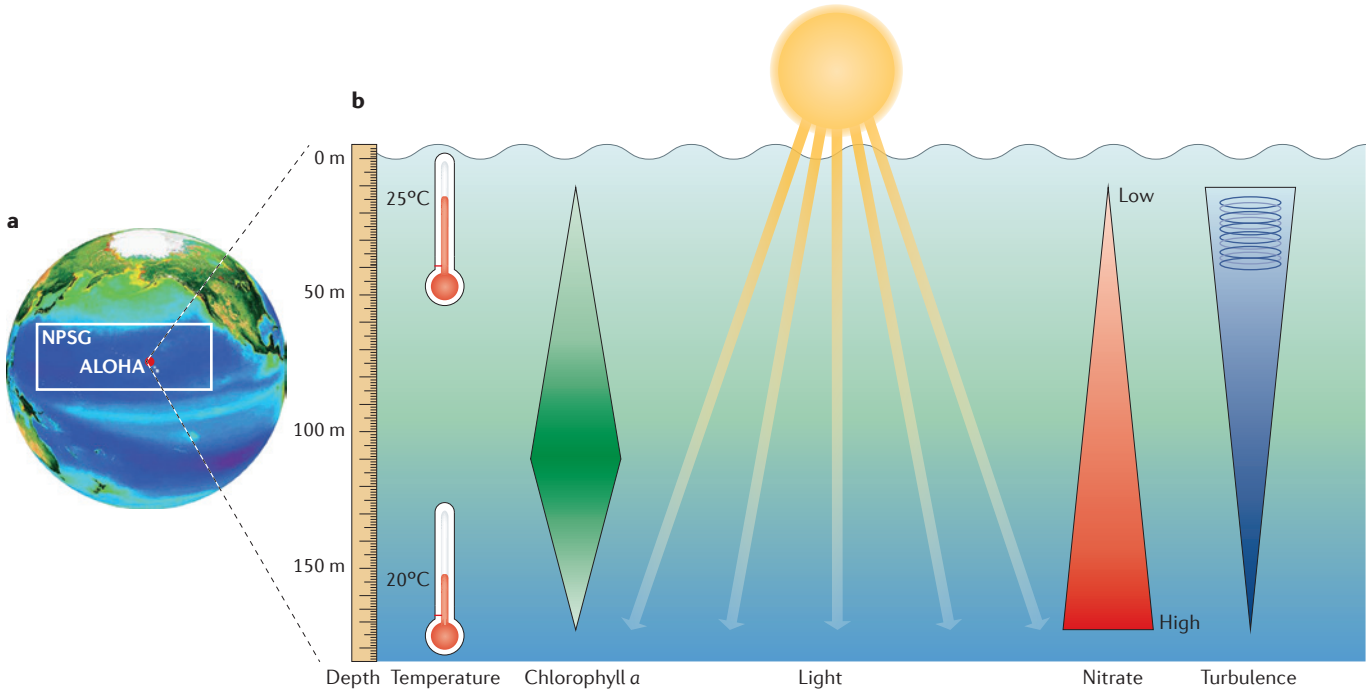

Figure 1 | **Station ALOHA habitat characteristics. a** | Location of Station ALOHA (A Long-term Oligotrophic Habitat Assessment) in the North Pacific Subtropical Gyre (NPSG) depicted on a Sea-viewing Wide Field-of-view Sensor (SeaWiFS) map of ocean colour (see Further information) showing the low concentrations of chlorophyll *a* (dark blue) that surround the site. **b** | The schematic shows the general habitat characteristics at Station ALOHA based on the 25 year climatology. This is an extremely oligotrophic environment that is characterized by low-standing stocks of chlorophyll (the subsurface chlorophyll peaks at ~105 m) and nitrate concentrations (note that primary production peaks where light is high but nutrients (such as nitrate) are nearly absent). Light that is sufficient for photosynthesis penetrates to at least 175 m. Temperature and the amount of turbulent mixing are also shown.

abundance is highest in the upper mixed layer and decreases with depth, divinyl chlorophyll *a* concentrations and fluorescence per cell both increase with increasing water depth (FIG. 3a), which is a feature that is indicative of photoadaptation.

It soon became evident that the *Prochlorococcus* spp. assemblage was actually a mixture of many genetically and physiologically distinct subpopulations, known as ecotypes[41], which leads to complex niche partitioning in time and space[42,43]. A temporal (2003–2008) analysis of two high-light-adapted and three low-light-adapted *Prochlorococcus* spp. ecotypes at Station ALOHA revealed depth-stratified assemblages throughout most of the 5 year observation period[44] (FIG. 3c). If ecotype stratification is disrupted by stochastic mixing, it is quickly re-established[44]. A recent laboratory study revealed that the dominant Station ALOHA *Prochlorococcus* spp. strain (MIT 9313) grew faster and achieved a higher yield when it was co-cultured with selected heterotrophic bacteria that were isolated from Station ALOHA[45]. It was recently reported that *Prochlorococcus* spp. produces extracellular lipid vesicles that contain nutrients, proteins and nucleic acids[46]. Furthermore, this study showed that the vesicles can support the growth of heterotrophic bacteria, which demonstrates a potential pathway for carbon and energy exchange. Mutualistic interactions among different types of marine microorganisms seem to be key to fuelling productivity and regulating biomass, even in a relatively diffuse growth medium like seawater.

As *Prochlorococcus* spp. genomes became available[47], it was shown that each ecotype shares a relatively small common set of genes (~1,350 genes) and a variable number of additional genes[48]. As each new isolate is sequenced (n = 12 genomes, as of 2007), the size of the core genome decreases slightly (1,273 genes), whereas the pan-genome (which is the sum of all of the unique genes) increases (5,736 genes[49]). Analysis of field samples that were collected at Station ALOHA indicated that 974 *Prochlorococcus* spp. genes occurred at <0.25 copies per cell, which is suggestive of genetic microadaptation[50]. However, as most of these genes have unidentified functions, the ecological relevance of these findings remains unknown. The more recent single-cell genome sequencing of naturally occurring *Prochlorococcus* spp. cells has provided additional insights: analysis of just ten cells from the tropical Pacific Ocean added 394 new genes to the ever-growing pan-genome[51]. With an estimated global abundance of 2.8×10^{27} *Prochlorococcus* spp. cells[52], the genetic and physiological variability of this group is likely to be extraordinary and may help to explain why *Prochlorococcus* spp. is the most abundant phototroph on our planet.

SAR11. From a single water sample that was collected at 3 m depth on HOT-2 (December 1988), Schmidt *et al.*[53]

Genetic microadaptation
Selective evolutionary changes in the genetic content of closely related microorganisms.

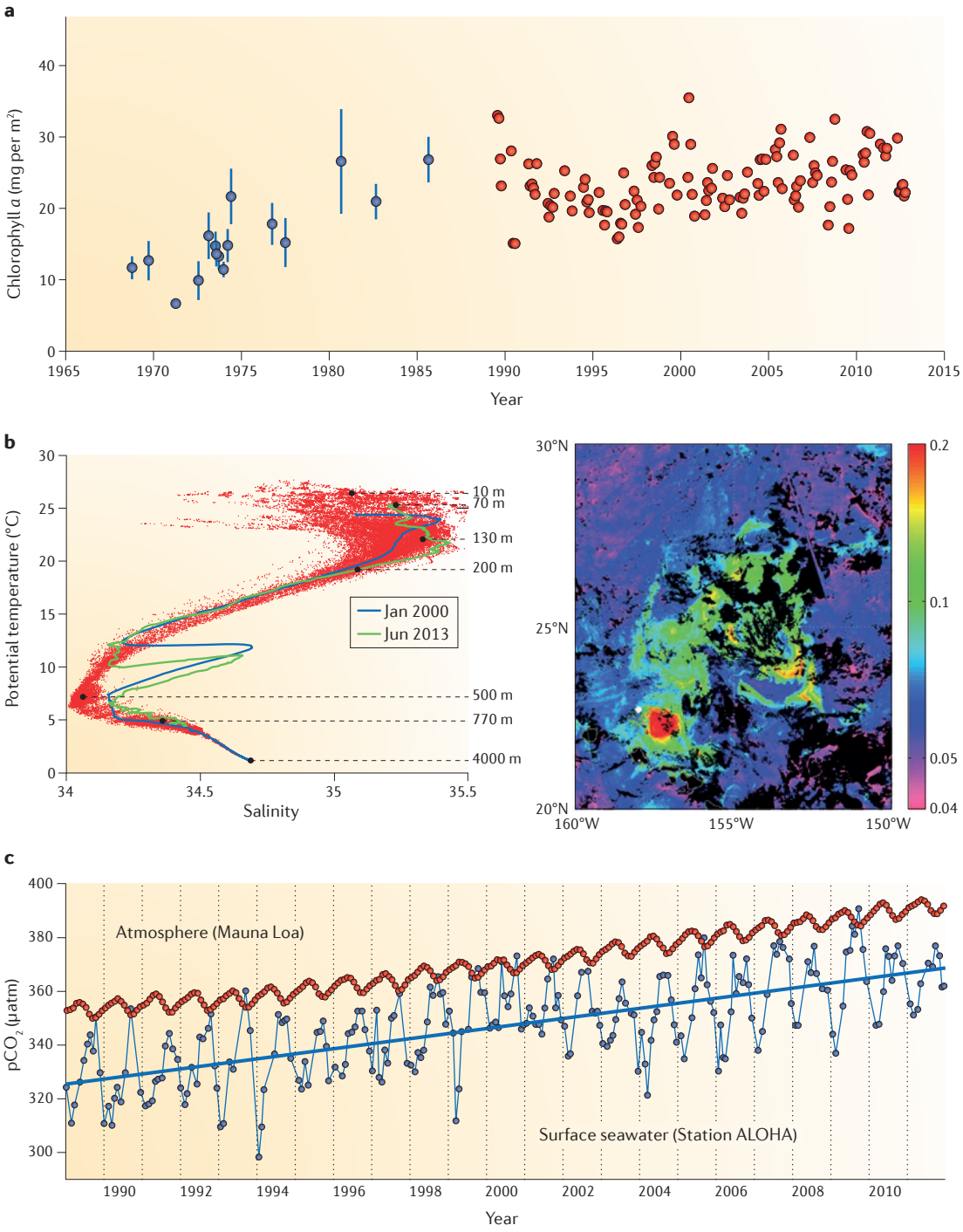

Figure 2 | **Selected examples of temporal variability in the NPSG.** **a** | Euphotic-zone depth (0–200 m)-integrated chlorophyll *a* concentrations from 1968 to 2013, showing a >twofold step increase mid-record just before the start of the Hawaii Ocean Time-series (HOT) era, based on observations presented by Venrick *et al.*[24] (blue) combined with HOT programme data (red). **b** | Anomalous subsurface water masses at Station ALOHA (A Long-term Oligotrophic Habitat Assessment) have been observed only twice during the 25 year observation period — in January 2000 and in June 2013. The left-hand panel shows a temperature versus salinity plot, which depicts the climatology (in red) and the two anomalies. The right-hand panel shows a satellite-based chlorophyll *a* (mg per m^3) image of the region north of Hawaii, which shows a major phytoplankton bloom near Station ALOHA (white symbol at 22°45′N, 158°W) in July 2005. **c** | Secular increase in the pCO_2 of the atmosphere (red) and upper ocean (blue) during the HOT era. Left-hand side of part **a** of the figure from Venrick, E.L., McGowan, J.A., Cayan, D.R. & Hayward, T.L. Climate and chlorophyll *a*: long-term trends in the central North Pacific Ocean. *Science* **238**, 70-72 (1987). Modified with permission from AAAS. The satellite-based chlorophyll *a* image in part **b** is provided by J. Nahorniak, Oregon State University, USA, using AQUA MODIS L2 ocean colour data that is publicly available from the NASA Ocean Biology Processing Group website (see Further information).

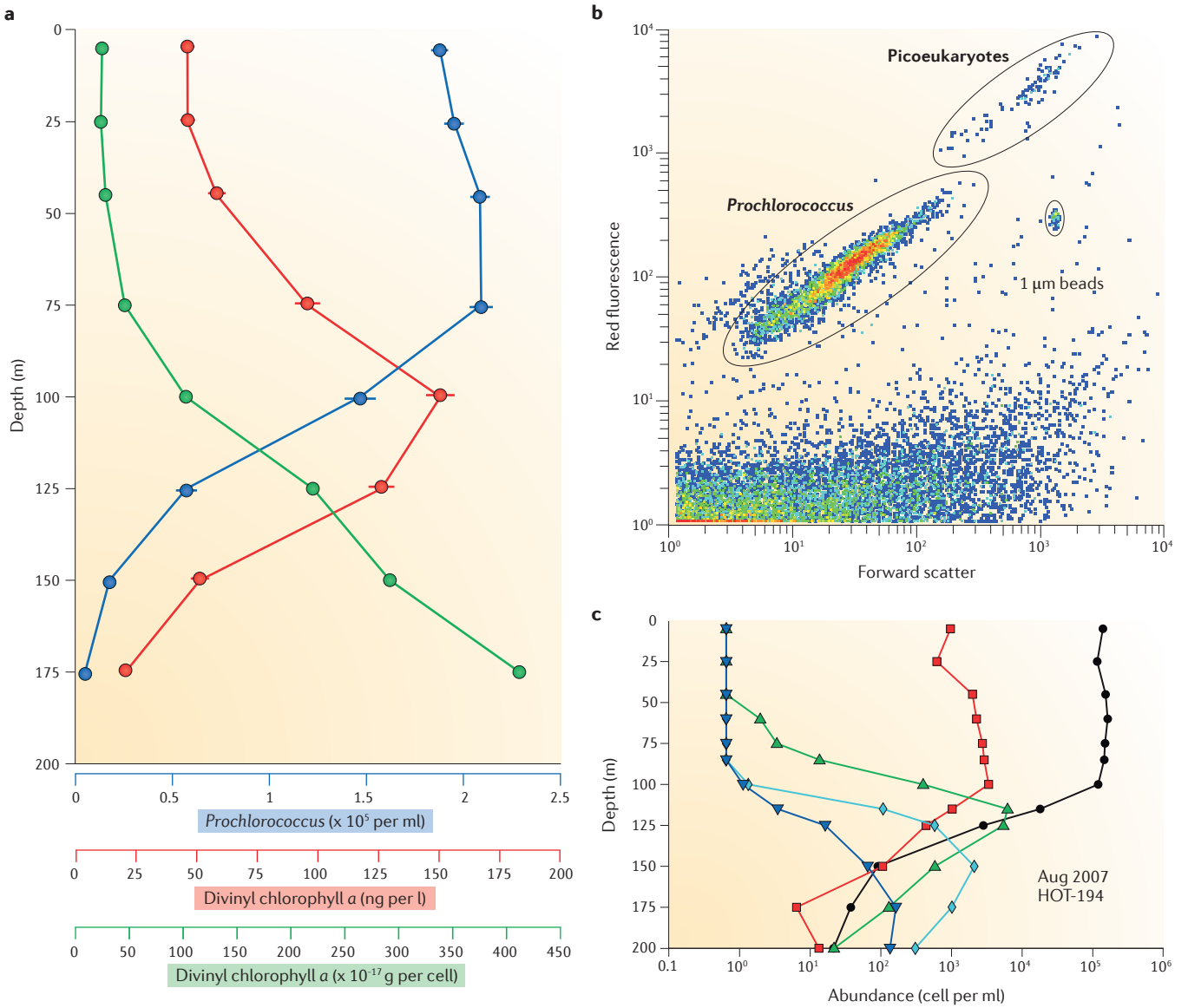

Figure 3 | ***Prochlorococcus* spp. distributions and dynamics.** **a** | Vertical profile of mean (±SE; n = 63) *Prochlorococcus* spp. cells, mean (±SE, n = 63) divinyl chlorophyll *a* concentrations (red) and divinyl chlorophyll *a* per cell (green) at Station ALOHA (A Long-term Oligotrophic Habitat Assessment) for the period October 2005–December 2011, which shows a light-dependent change in chlorophyll (known as photoadaptation). **b** | Representative flow cytometric signature of red autofluoresence versus forward scatter for *Prochlorococcus* spp. collected at 143 m at Station ALOHA . **c** | Depth profiles of *Prochlorococcus* spp. ecotypes at Station ALOHA for August 2007. The ecotypes that were tracked were: MIT9312 (black), MED4 (red), NATL (green), SS120 (light blue) and MIT9313 (dark blue). Part **c** of the figure adapted from REF. 44, Nature Publishing Group.

used 16S rRNA shotgun gene cloning and sequencing to explore microbial diversity at Station ALOHA. They identified rRNA genes from cyanobacteria (*Synechococcus*-like), eukaryotes and proteobacteria, including three alphaproteobacterial sequences (termed ALO-21, ALO-38 and ALO-39)[53]. This group of alphaproteobacterial sequences was nearly identical to the novel SAR11 clones that were reported by Giovannoni *et al.*[54] from the Sargasso Sea just 1 year earlier, which thereby provided the first evidence for a cosmopolitan (that is, Atlantic Ocean and Pacific Ocean) distribution of SAR11. It was subsequently reported that the SAR11 clade is the dominant group of microorganisms in the global ocean and that it accounts for up to 50% of the total surface-water microbial community and 25% of the subeuphotic zone microbial community[55].

Efforts to cultivate SAR11 were eventually successful[56], and a few years later, the genome of '*Candidatus* Pelagibacter ubique' was sequenced[57]. *Ca.* Pelagibacter ubique has a streamlined genome, which contains the smallest number of genes (n = 1,354) for any free-living organism. Comparative genome analyses of seven diverse strains showed that 705 genes were shared in a conserved core genome and that an additional 1,853

genes were present in the flexible pan-genome[58], which suggests that there is extensive ecotypic differentiation among SAR11 lineages. Streamlining of the *Ca.* Pelagibacter ubique genome has resulted in obligate auxotrophy for reduced sulphur, glycine and selected vitamins and an unexpected capacity for demethylation and one-carbon metabolism[59–63].

Eiler *et al.*[64] used a quantitative PCR assay to enumerate SAR11 throughout the water column (0–4,000 m) at Station ALOHA over a 3 year period (2004–2007). On average, SAR11 represented 36% of total 16S rRNA gene copies, and the highest proportions were in the euphotic zone (0–175 m) during the winter (mean = 44%). Assuming that each cell had a single copy of the rRNA gene, SAR11 cell abundances at Station ALOHA ranged from 0.3×10^5 cells per ml to 6.3×10^5 cells per ml. The numerical dominance of SAR11 at Station ALOHA and elsewhere raises important questions about evolutionary adaptation to life in the sea. In this regard, high intraspecific recombination contributes to adaptive physiological diversification[65], which possibly increases resource partitioning and reduces competition but maintains clade dominance[66]. As with *Prochlorococcus* spp., the interdependence of SAR11 on the metabolism of other microorganisms seems to be an advantageous evolutionary strategy for growth in remineralization-intensive habitats, such as at Station ALOHA.

Archaea. When the HOT programme began, only two major evolutionary domains of life were recognized in the sea: bacteria and eukaryotes. Although archaea (previously known as archaebacteria) had been discovered as a distinct monophyletic lineage a decade earlier using rRNA sequence analysis[67], they were thought to exist only in extreme habitats, thereby precluding them from the open sea. This understanding changed radically in 1992, following two independent reports of novel archaea in marine plankton[68,69]. Investigation of diverse marine habitats, including Station ALOHA, suggested that the archaea belonged to two main groups (that is, Crenarchaeota and Euryarchaeota), but a third archaeal phylum — Thaumarchaeota — has more recently been proposed[70,71]. The Thaumarchaeota is a highly diversified and abundant group of organisms that includes the marine group I (MGI) archaea[71]. Using rRNA-targeted, fluorochrome-labelled polynucleotide probes (that is, poly fluorescence *in situ* hybridization (FISH)) developed by DeLong *et al.*[72], the distributions of bacteria and archaea were investigated at Station ALOHA over a 15 month period[73]. MGI archaea were nearly absent (<5% of total cells) in surface waters (0–100 m) but were approximately equal in abundance to bacteria below 1,000 m (REF. 73). This high proportion of MGI archaea throughout the expansive deep-sea environment showed that they may be one of the most abundant groups of microorganisms in the sea — on a global basis, they are estimated at 10^{28} cells, despite being mostly unknown only a decade earlier.

The discovery of marine archaea raised important questions concerning their ecological niche (or niches). Several lines of evidence indicate that the MGI archaea are chemolithoautotrophic; for example, enrichment cultures or isolates of several representatives of the Thaumarchaea, including *Cenarchaeum symbiosium*[74,75] and *Nitrosopumilus maritimus*[76,77], are capable of ammonia-based chemolithoautotrophy (see Nitrogen cycle section).

New metabolic pathways

Marine microorganisms are the catalysts that are responsible for solar energy capture and for sustaining the cycles of the major elements, such as carbon, nitrogen, phosphorus and sulphur. Since the initiation of the HOT programme, and in many cases owing to research that has been conducted at Station ALOHA, new metabolic pathways have been discovered, many of which have potentially important ecological consequences.

Phototrophy. The process of photosynthesis, whereby solar energy is captured by chlorophyll-containing green plants and stored as chemical bond energy in organic molecules, is the fundamental ecological process that sustains all life in the sea. Hence, the discovery of a novel proteorhodopsin-based pathway of ocean phototrophy[78] was both remarkable and surprising. The proteorhodopsin genetic pathway was shown to belong to an uncultivated member of the marine Gammaproteobacteria, known as SAR86 (REF. 78). The protein was cloned and functionally expressed in *Escherichia coli*, which confirmed that this proteorhodopsin was an active, light-driven proton pump. The proteorhodopsin-based phototrophy hypothesis was further supported by the demonstration of photochemical activity in bacterial membrane preparations of samples that were collected from Monterey Bay, California, USA[79]. In this study, the authors also detected the presence of proteorhodopsin genes in bacterioplankton DNA extracts from various marine environments, including Station ALOHA[79]. When Station ALOHA proteorhodopsin genes were expressed in *E. coli*, the action spectra matched the depth of sample origin[79]. Such spectral tuning would optimize energy capture by proteorhodopsin phototrophy under *in situ* conditions. These discoveries led to the hypothesis that proteorhodopsin phototrophy may have an important and previously overlooked role in solar energy capture in the sea[78,80].

The marine proteorhodopsin family of proteins is extremely diverse, as are the microorganisms that contain them[81,82]. This has presented a challenge for determining the potential physiological and ecological roles of proteorhodopsin expression. Martinez *et al.*[83] documented the marine picoplankton proteorhodopsin photosystem by expression and photophosphorylation (that is, ATP production) in recombinant *E. coli* cells. Similarly, Walter *et al.*[84] showed that green-light-powered *E. coli* (that had been transformed with the SAR86 proteorhodopsin system) created a proton motive force that was sufficient to power the flagellar motor and sustain motility, even when conventional metabolism was stressed by oxygen depletion or inhibitors of respiration. They suggested that proteorhodopsin phototrophy may be a mechanism for survival under environmental stress. Indeed, proteorhodopsin phototrophy has been shown

Chemolithoautotrophic
A term used to describe organisms that use sources of chemical energy, rely on inorganic compounds (for example, H_2O and H_2S) for reducing power and assimilate inorganic carbon for cellular growth.

Proteorhodopsin
A photoactive, transmembrane protein that functions as a light-driven proton pump. Different forms of the protein differ in their light-absorption characteristics, which enables the absorption of light energy from different regions of the visible light spectrum. In the ocean, proteorhodopsin is found among diverse members of the Bacteria, Archaea and Eukarya.

to increase the long-term survival in carbon-limited cultures for both copiotrophic marine bacteria (such as Vibrio sp. AND4 (REF. 85)) and oligotrophic marine bacteria (such as *Ca*. Pelagibacter ubique[86]). Gomez-Consarnau *et al.*[87] were the first to report that light had a positive influence on the cell yield of the proteorhodopsin-containing marine bacterium, *Dokdonia* sp. (strain MED 134), compared with cells that were grown in darkness. The authors concluded that proteorhodopsin-based phototrophy may supply an added source of energy for growth in oligotrophic marine habitats[87]. Subsequent laboratory experiments using this strain examined possible light-induced transcriptional responses[88]. As before, growth rates and cell yields were higher when exposed to light. Furthermore, in addition to the proteorhodopsin photosystem, many genes were upregulated in the presence of light, including genes for retinal biosynthesis, ATP synthesis, membrane transporters and other light-sensing systems (for example, cryptochrome[88]). However, an estimation of the total solar energy capture in the sea by this novel pathway remains to be determined[80].

Another major unexpected discovery was the widespread occurrence of aerobic anoxygenic phototrophs (AAPs) in the tropical Pacific Ocean[89]. Subsequent field investigation showed that their probable niche may be as facultative photoheterotrophs[90]. In this regard, the AAPs contribute to the broad range of microorganisms that can simultaneously use solar energy and DOM to drive the major biogeochemical cycles in the sea[91]. The *puf* genes, which code for AAP pigment synthesis and the unique light-harvesting and reaction centre complexes seem to be broadly distributed in marine bacterioplankton, including those at Station ALOHA[92], but their quantitative role in the ocean energy budget remains uncertain[80]. On the basis of a metatranscriptomic survey of the upper ocean at Station ALOHA, Frias-Lopez *et al.*[93] found that the major forms of phototrophic metabolism (for example, photosynthesis, AAP-mediated photoheterotrophy and proteorhodopsin-mediated phototrophy) were among the most highly expressed of all gene clusters.

Kirchman and Hanson[94] recently published a theoretical cost versus benefit analysis of the bioenergetics of proteorhodopsin- and AAP-based phototrophy in the sea, which is based on data from laboratory studies of model organisms as *in situ* energy flux data are not available. Consequently, the quantitative ecological relevance of these novel phototrophic pathways is unknown and is a contemporary challenge for the discipline of microbial oceanography[80].

Carbon cycle. Approximately one-half of global primary production (an amount equivalent to 500×10^{15} g C per year) derives from the photosynthetic activities of marine microorganisms[2]. A major fraction (>75%) of marine productivity occurs in the expansive ocean gyres, including Station ALOHA. However, accurate field estimations of gross primary production (GPP), autotrophic respiration (AR), heterotrophic respiration (HR), total microbial community respiration (CR) (where CR = AR + HR), net primary production (NPP; where NPP = GPP – AR), net community production (NCP; where NCP = GPP – CR) and new and export production are difficult to achieve with our current understanding of these ecological processes and the limitations of contemporary methodologies[95].

The HOT programme has acquired the longest record of ^{14}C-bicarbonate-based primary production in the NPSG (FIG. 4a–c). Despite vanishingly small concentrations of inorganic nutrients and relatively low phytoplankton biomass, the ^{14}C results indicate moderate productivity (>0.5 g carbon per m^2 per day) (FIG. 4c), with a detectable seasonal cycle, where peak productivities occur throughout the early summer (FIG. 4a–c). However, the ^{14}C-based measurement of primary production does not account for all light-dependent inorganic carbon fixation that occurs during the incubation period or for carbon losses owing to respiration, grazing and other processes. As a result, the ^{14}C method provides only a lower constraint on GPP.

Export production at Station ALOHA has been estimated using sediment traps[96,97] (FIG. 4d). Annual export production, scaled to our best estimates of GPP[98], is a few percent at most, which reflects a remineralization-intensive ecosystem in which nutrients are cycled <30–50 times within the euphotic zone (0–150 m) before being removed from the system. Under these conditions, the captured solar energy is locally dissipated by the mostly microbial-based food web, and CR is nearly indistinguishable from GPP. The theoretical and ecological roles of proteorhodopsin-based phototrophy and photoheterotrophy, with regard to our current paradigm of NCP (that is, GPP – CR), have not yet been considered. These carbon- and oxygen-based concepts may ultimately need to be redefined in terms of energy flow, although we currently lack reliable field methods to do so[80].

Particulate matter export must be quantitatively linked to new nutrient delivery over long periods of time (months to years) in order to sustain NCP at Station ALOHA. This comparison has traditionally been attempted using nitrogen concentrations and fluxes[99]; however, current estimates of NO_3^- delivery by eddy diffusion at Station ALOHA are less than 25% of the estimated requirement[100]. The remaining nitrogen deficit (~100 mmol N per year) is thought to be supplied by aperiodic, event-driven vertical transport of NO_3^-, which results from mesoscale eddy entrainment, N_2 fixation (see the Nitrogen cycle section), phytoplankton vertical migrations, atmospheric deposition of fixed nitrogen or other mechanisms[100–103].

Nitrogen cycle. Nitrification and N_2 fixation were both discovered more than a century ago, but novel aspects of these microbial processes continue to emerge at Station ALOHA[104]. Nitrification is a key process in the deep sea and transforms the most reduced form of nitrogen (that is, ammonia (NH_3)) to the most oxidized and dominant form (that is, NO_3^-). The pioneering research of Von Brand *et al.*[105] established this basic understanding and led to the eventual isolation of ammonia-oxidizing bacteria (AOB) and nitrite (NO_2^-)-oxidizing bacteria (NOB). These microorganisms were thought to be

Copiotrophic
A term used to describe an organism that is adapted to growth in habitats where nutrient concentrations are high. Such habitats are rare in the open sea but can occur at microscales, such as those in proximity to sources of organic matter (that is, living cells and detritus).

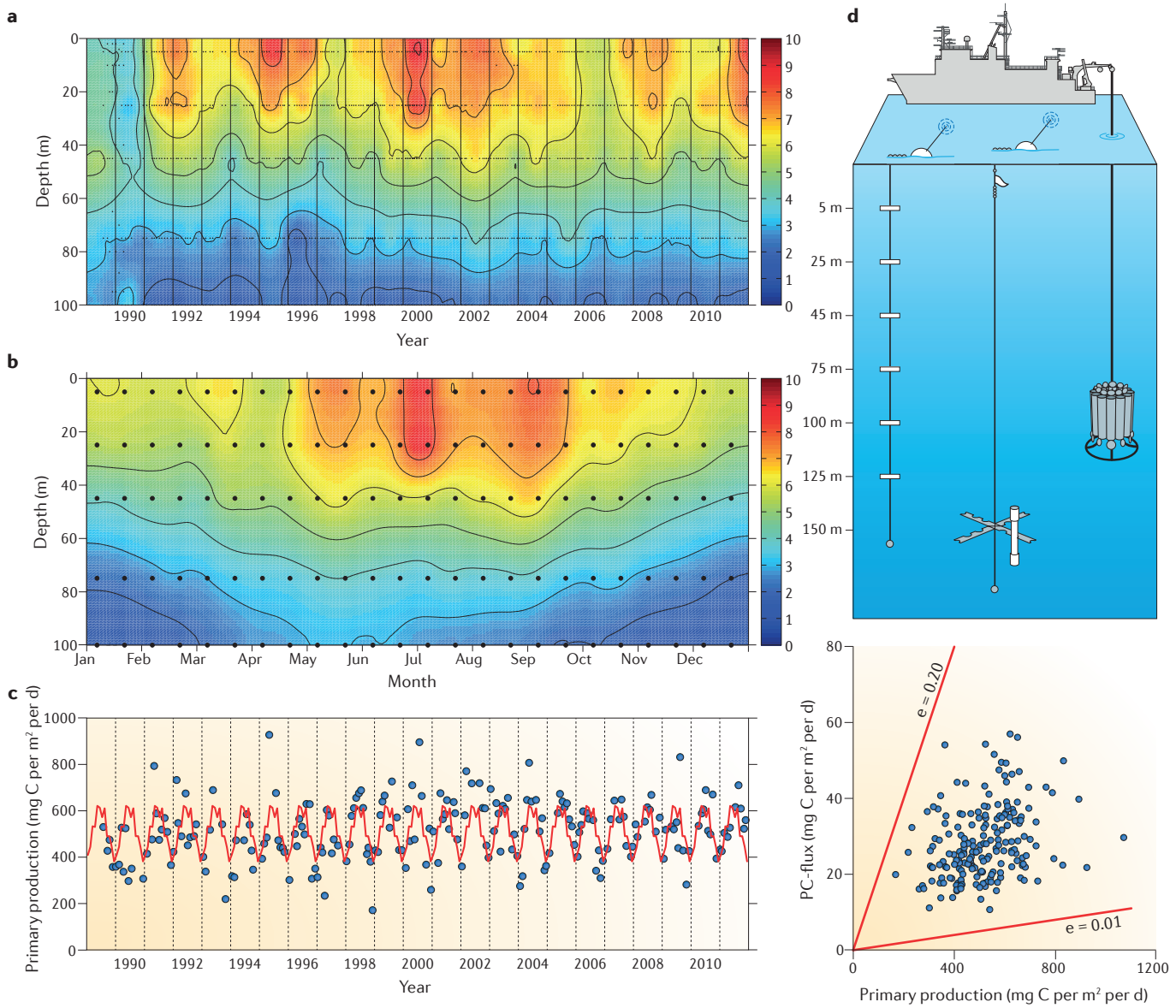

Figure 4 | **Temporal and depth variability in primary production at Station ALOHA.** **a** | Contour plot of upper water column (0–100 m) primary production (^{14}C-based; mg C per m^3 per day) based on approximately monthly observations throughout a 23 year period. **b** | Annual production climatology (mg carbon per m^3 per day). **c** | Individual euphotic zone depth-integrated (0–200 m) primary production estimates shown with annual climatology (red line) to show both seasonal and interannual variations. **d** | The diagram shows the free-drifting primary production and sediment trap arrays that were used to collect the data shown. Particulate carbon (PC) export (sediment traps at 150 m reference depth) versus depth-integrated (0–150 m) primary production with export ratio (e = flux/production) contours of 0.01 and 0.20.

Mesopelagic
A term used to describe the vertical region of the ocean that encompasses the mid-depth (generally ~200–1000 m) waters that lie between the well-lit euphotic zone and the deep bathypelagic waters. The mesopelagic waters are characterized by vanishingly low light and pronounced gradients in temperature and nutrients.

slow-growing and to live as chemolithoautotrophs on reduced inorganic nitrogen that is ultimately supplied to the deep sea via exported particulate and dissolved matter. The rate-limiting step in nitrification — the oxidation of NH_3 — is catalysed by the enzyme ammonia monooxygenase, which is encoded, in part, by the *amoA* gene. The discovery of homologous *amoA* genes in archaea[106,107] from marine metagenomic surveys led to the hypothesis that ammonia-oxidizing archaea (AOA) may have an important role in nitrification[108].

In the few years since the discovery of AOA, there has been extensive field investigation of the distribution, abundance, metabolism and possible ecological relevance of AOA worldwide, including at Station ALOHA[109–112]. Archaeal *amoA* genes are ubiquitous and exceed bacterial *amoA* genes, especially in the mesopelagic zone (~200–1,000 m). However, the exact role of archaea in the marine carbon and nitrogen cycles is unresolved and may be site-specific; for example, despite relatively low gene abundances in the upper ocean (<10^4 copies per l), transcription of *amoA* seems to peak at the boundary between the euphotic zone and the mesopelagic zone (~150–175 m)[112]. Such results suggest that the lower euphotic zone is a region of active nitrification,

which is consistent with direct measurements of nitrification at Station ALOHA[113–115]. It also seems that *Nitrospina*-like NOB microorganisms are abundant at Station ALOHA[110] and are presumably active in the coupled oxidation of NO_2^- to NO_3^-.

Like many other ecological processes, our current knowledge of marine archaeal physiology and metabolism is based on very few model systems[75,76,116,117]. Kinetic adaptation by AOA to low NH_3 concentrations[116] may confer a competitive advantage over AOB and could help to explain the relatively high abundance of archaea in the mesopelagic zone of Station ALOHA[73]. However, the discovery of AOA has not yet solved the puzzle of why there seems to be a 'division of labour' or resource diversification in the two-step process of nitrification (that is, the conversion of NH_3 to NO_2^- and the conversion of NO_2^- to NO_3^-). Unless new groups of nitrite-oxidizing archaea are also discovered, the second and much lower energy-yielding step of nitrification must be dependent on the activities of NOB. In laboratory culture, *N. maritimus* quantitatively converts NH_3 to NO_2^- (REF. 76), but we rarely observe a build-up of NO_2^- in the deep sea (although trace amounts of NO_2^- are detectable at Station ALOHA[118]), so AOA and NOB activities must be tightly coupled. Alternatively, it is possible that a complete chemolithoautotrophic ammonia oxidizer (an organism that converts NH_3 to NO_3^- — a process known as comammox) as postulated by Costa *et al.*[119], may eventually be discovered at Station ALOHA.

It is important to consider the bioenergetics of deep-sea nitrification. Aphotic zone oxidation of imported reduced nitrogen (for example, by sinking particulate matter) provides a potential energy source for 'dark' chemolithoautotrophy but does not support primary production from a bioenergetics perspective, as the energy that is released during nitrification is most probably derived from solar energy that is initially captured via photosynthesis. Also, the oxygen that is required to oxidize both NH_3 and NO_2^- must be restored via photosynthesis, so net positive 'primary production' in the deep sea is impossible to achieve, given our current understanding of ecological energetics. From an evolutionary perspective, it seems that the organisms that are responsible for NH_3 and NO_2^- oxidation would have evolved strategies to supplement their energetic demands via heterotrophic (or mixotrophic) metabolism, rather than relying on obligate chemolithoautotrophy.

N_2 fixation is another key process in the marine nitrogen cycle. The fixed nitrogen-starved oligotrophic gyres provide ideal habitats for the selection of N_2-fixing microorganisms that would shift nutrient limitation to another essential element, such as phosphorus or iron[120]. An encounter with a major *Trichodesmium* spp. bloom near Station ALOHA during HOT-9 (Aug 1989) challenged us to establish a quantitative assessment of N_2 fixation as a source of new nitrogen and its coupled effects on carbon and phosphorus cycles[101]. When the HOT programme began, N_2 fixation was thought to be restricted to only a few taxa, and most of the field work focused on the colony-forming cyanobacterium, *Trichodesmium* spp. (REF. 121). This paradigm changed with the discovery of the *in situ* transcription of novel nitrogenase (*nifH*) genes in the 0.2–10 μm size class of particles at Station ALOHA[122]. The *nifH* gene fragments were amplified, cloned and sequenced to reveal two major groups of unicellular cyanobacteria (known as UCYN-A and UCYN-B; UCYN-B is closely related to *Crocosphaera watsonii* strain WH 8501 (REF. 122)). Unicellular microorganisms seem to be responsible for most of the N_2 fixation at Station ALOHA[123,124], which has led to a reassessment of the role of N_2 fixation in supporting new production and export production at Station ALOHA[104]. However, in contrast to other marine planktonic lineages that show a high degree of genetic and ecotype diversification, metagenomic sequence analysis of UCYN-B at Station ALOHA revealed nearly complete homology to the *C. watsonii* strain WH 8501 that was isolated from the North Atlantic Ocean 3 decades earlier[125]. Zehr and colleagues more recently used metagenomic analyses of flow cytometrically sorted UCYN-A cells from Station ALOHA to further investigate the metabolism and adaptation of the organism[126]. Unlike *Crocosphaera* spp. and *Trichodesmium* spp., UCYN-A has a streamlined genome (1.44 Mb) that lacks genes for oxygenic photosystem II, a complete tricarboxylic acid cycle and pathways for many key biosynthetic precursors[127]. Nonetheless, UCYN-A retains a light-harvesting capacity and the ability to fix N_2 (REF. 127). A major breakthrough in interpreting the reduced metabolic capacity of this organism came from targeted flow cytometric sorting coupled with isotopic and genetic analyses of naturally occurring UCYN-A cells. Those analyses showed that UCYN-A exists in a mutualistic, symbiotic association with a unicellular eukaryotic alga: '*Candidatus* Atelocyanobacterium thalassa' (REF. 128).

Assessments of the role of N_2 fixation as a source of new production at Station ALOHA include *nifH* gene surveys, phylotype-specific *nifH* expression and rates of N_2 fixation using an isotopic tracer[129,130]. The diazotroph community structure and function vary considerably in time and space, and peak N_2 fixation rates occur in near-surface waters in summer when temperatures are >25.2 °C and during periods of positive sea surface height anomalies, as reflected in satellite altimetry[124]. This suggests that mesoscale processes, especially anticyclonic eddies, may select for diazotrophs[26,31,124]. Based on a 3 year (2005–2007) observation period, upper ocean (0–100 m) rates of N_2 fixation averaged 111 ± 66 μmol N per m^2 per day, with ~77% of the total rate supported by the <10 μm size fraction[124]. This value is ~37% of the measured particulate nitrogen export during this same period, which emphasizes the contemporary role of N_2 fixation in the Station ALOHA nitrogen cycle. However, a serious potential flaw has recently been reported in the routine application of the $^{15}N_2$-tracer technique, which suggests that rates of N_2 fixation may have been underestimated[131]. At Station ALOHA, the revised rates are more than double previous estimates[132], which suggests that N_2 fixation, rather than NO_3^- flux, may be the most important source of new nitrogen in this ecosystem.

Diazotroph
A dinitrogen (N_2)-fixing microorganism. The ocean contains diverse assemblages of diazotrophs, which include microscopic single-celled organisms and larger filamentous forms. These organisms rely on diverse metabolisms and are frequently found in symbiosis with other planktonic prokaryotes and eukaryotes. Diazotrophs seem to be most abundant in the upper ocean, where nutrient concentrations (specifically inorganic nitrogen) are low and sunlight, which is their primary energy source, is plentiful.

One of the more predictable, but still unexplained, phenomena at Station ALOHA is the so-called summer export pulse (SEP) of fresh organic matter to the seabed in late summer when surface ocean conditions are well stratified and least likely to receive a resupply of new nutrients[97]. Analysis of the 12 year deep-ocean sediment trap record revealed that the SEP was a result of N_2 fixation via diatom–diazotroph symbiosis as the source of new nitrogen, coupled with near-surface aggregation of the relatively large diatom cells and rapid export to the deep sea. It was hypothesized that photoperiod — specifically, a rapid decrease in day length following the second passing of the sun on 5 July at the latitude of Station ALOHA (22°45′N) — functions as the predictable trigger for this important cascade of biological, ecological and biogeochemical processes[97].

Owing to these and other discoveries, we currently have a fundamentally different understanding of the new versus regenerated production paradigm compared with the model that was developed by Dugdale and Goering[133], which existed at the start of the HOT programme. N_2 fixation to NH_3 is now known to supply >50% of the new nitrogen, and much of the NO_3^- in the upper portion of the water column may be derived from NH_3 recycling via local nitrification rather than from upwelling of deep waters[104,113,134,135]. Furthermore, a decade (or longer) scale oscillation between N_2 fixation-favourable and -unfavourable conditions may be triggered by the N/P stoichiometry of available nutrients, with selection for or against N_2-fixing microorganisms[13,120,136]. This hypothesized alternation of ecosystem states can only be tested using time series observations across relevant timescales (that is, multiple decades). Indeed a recent analysis of nitrogen isotopic ratios preserved in skeletons of long-lived deep-sea corals that were collected near Hawaii indicated that the recent increase in N_2 fixation that was observed at Station ALOHA may be part of a longer centennial-scale trend of increased N_2-based export production[137].

Phosphorus cycle. At the start of the HOT programme, the marine phosphorus cycle was thought to be well-understood and relatively simple, especially compared with the cycles of carbon, nitrogen and sulphur. However, recent reports of sulphur substitution for phosphorus in phosphorus-stressed microbial assemblages[138], quantitative assessments of the role of dissolved organic phosphorus (DOP)[139] and the discovery of a phosphorus redox cycle in the open sea[140] have stimulated renewed interest in the role of phosphorus in microbial oceanography.

DOP is a diverse mixture of molecules and compounds that, in the surface waters at Station ALOHA, generally exceed the concentrations of inorganic phosphate by several-fold[141]. Although poorly characterized, DOP is known to include phosphate esters (C–O–P) and organophosphonates (C–P). C–P compounds are ancient molecules that, until recently, were not thought to be important in the contemporaneous marine phosphorus cycle[142]. However, detection of C–P compounds in marine DOP[143,144] and the discovery of C–P catabolism genes in the Global Ocean Sampling (GOS) metagenomic library[145] suggest that they may have important physiological and ecological roles. Furthermore, most C–P compounds contain phosphorus in a lower oxidation state than the +5 value that characterizes all C–O–P compounds[146]. Consequently, it is likely that phosphorus metabolism in the sea is characterized by a cascade of microbial oxidation–reduction reactions that have bioenergetic and ecological consequences that are analogous to the microbial carbon, nitrogen and sulphur cycles[140].

Pioneering research at Station ALOHA documented the aerobic production of methane (CH_4), which is a potent greenhouse gas, from catabolism of methylphosphonate (MPn; the simplest organophosphonate)[147]. Subsequent laboratory-based studies confirmed the ability of the marine N_2-fixing cyanobacterium *Trichodesmium* spp. to efficiently grow on MPn as a sole source of phosphorus and to quantitatively convert MPn to CH_4 (REF. 148). At the time of this discovery, the ecological importance of MPn metabolism was unclear, as there was no known natural source of MPn in the biosphere. However, the recent discovery of a novel MPn biosynthetic pathway in the marine NH_3-oxidizing thaumarchaeote *N. maritimus*[149] provided new information about possible sources of phosphonates in the sea. To link this pathway back to the marine environment, the authors screened the GOS database and discovered that key genes in the MPn biosynthesis pathway were both ubiquitous and abundant[149]. The recent report of MPn-dependent growth of SAR11 with stoichiometric conversion to CH_4 (REF. 150) supports the hypothesis of aerobic production of CH_4 at Station ALOHA[147]. The ecological importance of C–P biosynthesis and turnover and the bioenergetics of environmental phosphorus reduction–oxidation reactions are topics of current investigation at Station ALOHA.

Lessons learned and future prospects

With each additional year of data, time series measurement programmes become more valuable for understanding natural ecosystem variability, controls on microbial rates and processes and the potential effects of human-induced climate change. However, most ocean time series programmes that were established in the latter half of the twentieth century have already been terminated[151], and once a programme ends, there is little incentive to restart it. A key question is: "how long is necessary to develop an accurate and comprehensive ecological understanding and prognostic modelling capability?" Rudnick and Davis[152] demonstrated that, for systems in which the signal-to-noise ratio is low, short time series records (<20 years) are insufficient to statistically resolve recurrent trends. As Wunsch[153] lamented, "sometimes there is no alternative to uncertainty except to await the arrival of more and better data." Although some ecological regimen shifts may be predicted by leading indicators as crucial transition points are approached[154], unambiguous detection of climate change effects on ocean productivity in oligotrophic gyres will probably require at least 30–40 years

Stoichiometry
The proportions of specific nutrient elements that are found in organic compounds or dissolved in seawater. Variance in the relative proportions of these elements provides insights into specific ecological and biogeochemical processes; for example, nitrogen fixation supplies fixed cells with nitrogen but consumes phosphorus from seawater, which results in a shift in the relative proportions of these two elements in cellular material and in seawater.

of observations to discern a trend from natural ecosystem variability[155,156]. This sobering assessment of the scale of the challenge incentivizes ocean observation programmes, such as HOT, that are already in their third decade of operation.

The discipline of microbial oceanography has experienced remarkable growth during the past 25 years. The discoveries of novel microorganisms, unexpected metabolic processes and fundamental ecological and evolutionary relationships have challenged old paradigms and created new fundamental research opportunities. Observations and experimentation at Station ALOHA have contributed considerably to this increasing body of knowledge in ways that could never have been predicted when the HOT programme began in 1988. Future time series programmes might benefit from a few lessons learned. First, a multidisciplinary approach is essential: physics (including optics), biogeochemistry and ecology are key to understanding the controls on microbial diversity, metabolism and population interactions. Second, consistent high-quality measurements using certified reference materials, if available, are the bedrock of any time series observation programme. New methods and technologies should be encouraged, but standardization and consistency must be carefully evaluated and documented before novel approaches are used. Effective data-management and data-sharing policies enrich the value of a time series programme and receive support from the broader scientific community. Leveraged resources and assets, both financial and intellectual, are absolutely essential for sustaining time series programmes. Last, it is crucial to link hypothesis-testing field experimentation to the collection of time series data. Ultimately, the creation and dissemination of scientific knowledge must be the driving force if a time series programme is to remain vibrant and relevant.

The HOT programme provided a foundation for the establishment of the Center for Microbial Oceanography: Research and Education (C-MORE; see Further information) in 2006. The improved understanding of the NPSG as a result of HOT programme observations at Station ALOHA motivated the establishment of a science and technology centre that is focused on developing new conceptual models and theory- and hypothesis-testing field experimentation. On 1 July 2014, the Simons Collaboration on Ocean Processes and Ecology (SCOPE; see Further information) was established to further improve our basic understanding of microbial, biogeochemical and ecological processes at Station ALOHA, especially the mechanistic understanding of high-frequency (hours to weeks) variability and the quantitative assessments of solar energy capture and nutrient transformations in the sea. A recent study at Station ALOHA showed oscillating diel rhythms of gene transcription that were staggered in time among the different phototrophic and heterotrophic microbial species[157]. These recurrent waves of species-specific metabolism suggest the existence of complex, but well-coordinated, pathways for matter and energy flow in the sea.

Finally, it is impossible to predict or even fully comprehend things that are yet unknown in the magnificent marine microbial world, but as Louis Pasteur proclaimed in a public lecture on 7 December 1854, "Dans les champs de l'observation le hazard ne favourise que les spirits prepares" ("In the fields of observation, chance favours only the prepared mind"). The HOT programme assembled a team of well-prepared interdisciplinary scientists during an unprecedented period of discovery in the discipline of microbial oceanography. However, many basic ecological questions remain only partially answered[158]. It is expected that the HOT, C-MORE and SCOPE programmes will continue to be sources of novel discovery and great promise well into the future.

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Competing interests statement
The authors declare no competing interests.

FURTHER INFORMATION
Center For Microbial Oceanography: Research and Education (C-MORE): http://cmore.soest.hawaii.edu
Genomes Online: http://www.genomesonline.org/cgi-bin/gold/index.cgi
Hawaii Ocean Time-series (HOT) programme: http://hahana.soest.hawaii.edu/hot/hot_jgofs.html
Microbiological Targets For Ocean Observing Laboratories (Microtools): https://sites.google.com/site/microtoolsii/
NASA Ocean biology Processing Group: https://earthdata.nasa.gov/data/data-centers/obpg
Oceansites.org: http://www.oceansites.org
Sea-viewing Wide Field-of-view Sensor (seaWiFS) map of ocean colour: oceancolor.gsfc.nasa.gov/SeaWiFS/
Simons Collaboration on Ocean Processes and Ecology (SCOPE): http://scope.soest.hawaii.edu

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