Improving biogeochemical knowledge through technological innovation

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The understanding of biogeochemical cycles has benefited from technological advances facilitating new kinds of measurements and observations. Satellite-borne ocean-color sensors that assess the physiological status of phytoplankton have led to improved estimates of oceanic productivity, as have micrometeorological approaches measuring terrestrial photosynthesis and respiration. The advent of satellites fitted with synthetic aperture radar (a specialized sensor used to determine inundation extent and vegetation types in wetlands) has revealed large fluxes of carbon dioxide and methane from these areas. Advances in the measurement of chemical constituents and turbulence have allowed the detection of high-resolution coupling between physical and biogeochemical processes. Genomics and proteomics – the study of genes and of an organism's complement of proteins, respectively – have revolutionized our understanding of the types of cells present in the environment and their ability to transform elements by allowing direct assessment of gene and protein sequences.

Throughout the history of science, innovative measuring techniques in combination with new theories have advanced our understanding of the natural world. The invention of the telescope allowed remotely based exploration of the solar system and modern telescopes, equipped with detectors that scan across the electromagnetic spectrum, have provided data to test the concepts and theories of cosmology and relativity; an application of x-ray crystallography led to the discovery of the structure of DNA and the subsequent revolution in molecular biology and genetics; and deep-sea drilling technologies – originally associated with petroleum and natural gas extraction – provided key support for the hypothesis of continental drift, to list just a few examples.

Our understanding of biogeochemical cycles has benefited considerably from both technical and conceptual advances. Here, we examine the important technological advances that have helped transform our knowledge of biogeochemistry in recent years and that will continue to do so as they are incorporated more extensively into ecological studies. We focus on carbon (C) because it is the major currency by which primary productivity and respiratory processes are assessed, illustrating how spaceborne remote-sensing innovations have made global-scale calculations of oceanic productivity at fine spatial and temporal scales possible, as well as the integration of inundation and vegetation dynamics into the calculation of methane (CH_4) and carbon dioxide (CO_2) exchanges in wetlands. We also discuss how adaptation of micrometeorological approaches in measuring terrestrial C balance has refined our understanding of ecosystem function. Characterization of the microbial organisms that perform many processes is integral to understanding biogeochemical cycles. It is also essential to determine the fluxes of materials among reservoirs, which requires measurements of transport processes and associated chemical constituents. We therefore discuss new analytical techniques in molecular biology and chemistry, as well as advances in the measurement of turbulent mixing required to compute fluxes.

In a nutshell:

- Technological advances have greatly facilitated the analysis of major biogeochemical cycles; examples are provided here for both terrestrial and aquatic environments
- Satellite-borne remote-sensing systems can detect phytoplankton chlorophyll concentrations and enable analysis of oceanic productivity and carbon (C) cycle dynamics over large temporal and spatial scales
- Automated, high temporal frequency observations of vegetation–atmosphere C exchanges have revolutionized our understanding of climate–biogeochemical cycle interactions in the terrestrial biosphere
- Deployment of microstructure temperature profilers and fast-response chemical sensors enables researchers to measure the exchange of materials at interfaces in aquatic environments
- Genomics and proteomics allow scientists to link the metabolic processes of previously undescribed microbes to biogeochemical processes

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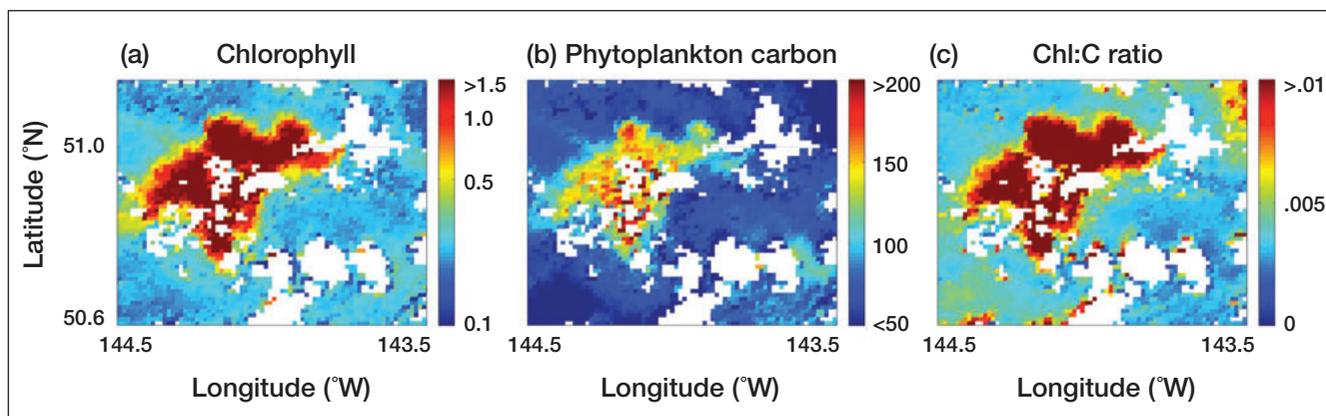
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Figure 1. *Ocean color image – from the satellite-based Sea-viewing Wide Field-of-view Sensor (SeaWiFS) – of an iron-addition experiment conducted in the northern Pacific Ocean. This image was acquired 19 days after the first iron addition (Boyd et al. 2004). (a) Chlorophyll concentration (mg m^{-3}); (b) phytoplankton C (mg m^{-3}); (c) chlorophyll-to-phytoplankton C (Chl:C) ratio.*

■ Primary productivity and C dynamics in aquatic and terrestrial ecosystems

Central to the biogeochemistry of C is the fixation of CO_2 through photosynthesis – that is, primary productivity. Although this process has been studied in individual plants or small samples of phytoplankton for decades, temporal and spatial variability of primary productivity has made regional and global estimates difficult to make with sufficient accuracy. Yet these estimates are critical for understanding interactions between the C cycle and changes in climate. Recent advances in remote sensing and micrometeorological techniques have revolutionized the ability to determine oceanic and terrestrial productivity. When combined with large-scale experimental manipulations, these techniques have led to an improved understanding of the ecological and biophysical mechanisms that control productivity. Wetlands process considerable amounts of C, some of which is either reduced to CH_4 or emitted as CO_2. However, the extensive and remote areas covered by tropical and northern wetlands, and their spatial and temporal heterogeneity, complicate the assessment of these ecosystems' roles in C dynamics on a regional scale. Innovations in remote sensing have greatly helped in addressing these challenges.

Ocean-color remote sensing

The chlorophyll content, or "green-ness", of a water body provides an index of its primary productivity. With the launch, in the 1990s, of satellite-borne sensors capable of measuring the faint signals of blue and green light emitted from the oceans, it became possible to estimate oceanic chlorophyll concentrations globally (McClain 2009). Recent algorithms have enabled calculation of backscattering of underwater light by particulate matter suspended in the ocean, a measure of phytoplankton C (Behrenfeld *et al.* 2005). The ability to determine chlorophyll content and C concentrations independently allows estimation of the chlorophyll-to-phytoplankton C (Chl:C) ratio; laboratory

observations indicate that this ratio varies as a function of light and nutrient levels. It is therefore now possible to assess phytoplankton physiological status on global scales, a key step toward developing mechanistic models for the estimation of net primary production (Behrenfeld *et al.* 2005).

As an example, we describe an application of these new tools in studying the linkages between phytoplankton and biogeochemical processes in the waters of the sub-Arctic Pacific Ocean. Because of the region's high nitrogen (N) and phosphorus (P) levels, phytoplankton should be abundant; however, the area is characterized by relatively low chlorophyll levels – iron limitation is thought to be the causal factor (Miller *et al.* 1991). Satellite imagery collected at a site after a large-scale iron-addition experiment indicated increased concentrations of chlorophyll and phytoplankton C (Figure 1). In particular, the observed Chl:C ratio increase was consistent with the idea that the addition of iron reduces nutrient stresses on phytoplankton, enabling more energy to be used to increase light-harvesting capabilities. Other researchers have used the signal from chlorophyll fluorescence to assess the physiological state of phytoplankton and its relationship to iron stress (Behrenfeld *et al.* 2009).

Eddy covariance measurements in terrestrial systems

Through the photosynthetic process, plants take up CO_2 and release water vapor (H_2O) and oxygen (O_2) to the atmosphere. Conversely, respiration by plants and decomposition of organic C in soils by microorganisms release CO_2 to the atmosphere. The difference between photosynthesis and respiration determines whether vegetation in a particular area is a sink or source of CO_2.

Eddy covariance measurements take advantage of the fact that when wind passes across vegetated surfaces, turbulent eddies cause vertical fluxes, such that gases are exchanged between the atmosphere above the plant canopy, within the canopy, and along the ground's surface (Figure 2). By rigging towers with sensors capable of taking high-frequency measurements of wind speed/direc-

tion and airborne CO_2 concentrations within and immediately above the plant canopy, the net flux of CO_2 (ie the difference between photosynthesis and respiration) into or out of an ecosystem may be estimated.

The use of eddy covariance to measure C exchange between vegetation and the atmosphere demonstrates how technology from one specialty can aid in the development of another specialty. Originally developed and applied in micrometeorology, eddy covariance tower sites are now found at numerous locations worldwide. Important benefits of this technique (summarized in Baldocchi *et al.* 1988) include: (1) the ability to conduct in situ measurements with minimal damage to the surrounding vegetation; (2) automated measurement and data-handling capability, enabling continuous, high-frequency observations of vegetation–atmosphere C exchanges; and (3) information on C exchange over areas measuring tens to hundreds of square kilometers, rather than tens to hundreds of square meters typical of plot-scale studies. Of course, no technique is without limitations. Eddy covariance estimates of C exchange involve a host of assumptions estimating photosynthesis and respiration from atmospheric CO_2 concentrations, and low wind speeds can cause measurement problems (Baldocchi *et al.* 1988).

In combination with ground- and satellite-based observations and experiments, the eddy covariance technique has provided a wealth of data on the patterns and processes regulating C uptake and storage in the terrestrial biosphere (Law *et al.* 2002). Eddy covariance data have also been invaluable in developing state-of-the-art, global-scale models of the C cycle (Dolman *et al.* 2003) and in testing theories about the C balance of terrestrial ecosystems. For example, Luyssaert *et al.* (2008) found that old-growth forests sequester atmospheric CO_2 at rates similar to those of young forests. This result conflicts with a long-held theory that old-growth forests become "carbon neutral" as inputs of C through photosynthesis are balanced by C outputs via plant and microbial respiration.

Free-air CO_2 enrichment (FACE)

A critical innovation in the field of elevated CO_2 research was the development of free-air CO_2 enrichment (FACE) technology (Figure 3), the key feature of which enables the enrichment of atmospheric CO_2 without modification of other environmental variables. At FACE sites, pure CO_2 gas is mixed with ambient air to artificially elevate atmospheric CO_2 levels (Pepin and Korner 2002). FACE sites contain many instruments measuring wind speed/direction and CO_2 concentrations, enabling rapid adjustments in the rate at which CO_2 is introduced into experimental plots.

With a substantial commitment from governments to fund FACE experiments, studies have extended over a decade in intact ecosystems, providing insights about whole-ecosystem responses to rising CO_2 levels. The unexpected outcome of these long-term experiments is
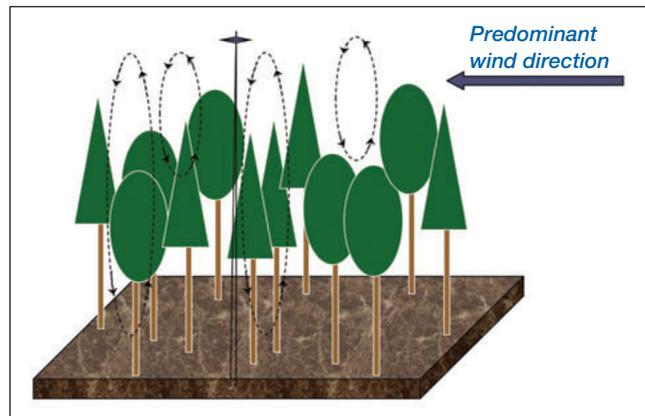

Figure 2. The eddy flux technique is based on air movement across plant canopies. Because the canopy surface is "rough", air tumbles through the plant canopy to the soil surface, creating eddies (dashed arrows). During the day, photosynthesis exceeds respiration, depleting these air masses of CO_2. At night, only respiration occurs, increasing the concentration of CO_2 in the air masses. For any measurement interval, the difference in the concentration of CO_2 just above the plant canopy and in the background atmosphere determines whether an ecosystem is a source or sink for atmospheric CO_2. The tower in the center of the simulated forest canopy is equipped with state-of-the-art sensors measuring wind speed, wind direction, CO_2 concentrations, and a host of other biophysical attributes every few seconds.

the variety of ecosystem responses to elevated CO_2. Indeed, the response of primary productivity to elevated CO_2 spans a gradient, from experiments in which (1) there is no response to elevated CO_2 alone (Korner *et al.* 2005) to experiments in which (2) the response is initially high but then declines to levels observed under ambient CO_2 (Reich *et al.* 2006; Seiler *et al.* 2009), or (3) the response is sustained through time (Finzi *et al.* 2006). Increases in primary production associated with high CO_2 concentrations require sufficient availability of other resources, and much of the variation in net primary productivity in response to CO_2 enrichment can be tied to nutrient limitation (Finzi *et al.* 2001; Reich *et al.* 2006).

In an era of rising atmospheric CO_2 concentrations, increased transport and deposition of reactive nitrogen, warming temperatures, and shifting quantities and patterns of precipitation, it is important to study the effects of multiple changes simultaneously. As so-called multifactor global-change experiments increase in number and scope, it will be interesting to see whether and how theories developed from single-factor experiments (eg CO_2 fertilization) will apply in a multivariable context. Emerging technologies, such as eddy covariance, along with novel methods for rapidly assessing nutrient pools and fluxes, will be needed to maximize the benefits of these experiments.

Remote sensing of wetlands

Wetlands cover extensive areas, are widely dispersed across Earth, and include a diversity of plant communi-

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Figure 3. *Deployment of FACE instrumentation. (a) The FACE plots at the Duke Forest in North Carolina, where loblolly pine (Pinus taeda) and hardwood trees have received elevated concentrations of atmospheric CO_2 for the past 14 years. (b) A close-up of the array of towers and stacked tubing from which the CO_2 is dispersed. (c) A view of the onsite liquid CO_2 storage tanks and the array of diffusers required for the conversion to gaseous form. Pure CO_2 gas is pumped to each of four elevated CO_2 plots, where it is mixed with ambient air to reach the target concentrations of 590 parts per million (ppm), an amount 200 ppm above present-day concentrations. Four additional plots serve as controls, three of which are fully instrumented.*

ties. However, wetland habitats – particularly those in tropical and northern regions – are not well characterized in terms of their ecological importance, in part because of difficulties in determining the varying extent and timing of inundation among the diversity of vegetative cover types. Fortunately, the past two decades have seen the development of a new suite of optical and microwave remote-sensing systems and analysis algorithms that largely overcome these obstacles (Melack 2004). An especially promising approach is synthetic aperture radar (SAR), a technique that transmits and receives microwave signals from and to a sensor and, therefore, allows for accurate classification of digital radar images into various vegetative classes and by inundation status. With the advent of SAR-equipped satellites, studies of inundation and wetland vegetation worldwide have become feasible. Complementary use of aircraft and satellite-borne sensors of a few to hundreds of bands of visible light allow detection of chlorophyll in plants and algae (Melack and Gastil 2001; Novo *et al.* 2006).

Recent applications of remote sensing to wetland studies in Amazonia provide excellent examples of how these new techniques are advancing biogeochemical understanding (Melack *et al.* 2009). In particular, Hess *et al.* (2003) developed a method of classifying vegetative–hydrological states using SAR mosaic images captured by the Japanese Environmental Resource Satellite and validated their results using high-resolution digital videography (Figure 4). Such vegetative–hydrological classification schemes are essential for studying wetland biogeochemistry. Because CH_4 is produced predominantly in anoxic environments associated with flooded habitats, Melack *et al.* (2004) were able to calculate regional rates of CH_4 emission by combining habitat-specific field measurements with passive and active microwave data that revealed temporal variations in the extent of both flooding and vegetation. On the basis of extensive field data and measurements of inundation areas derived from remotely sensed data, Richey *et al.* (2002) reported that CO_2 evasion (eg outgassing from water to atmosphere) from lakes, rivers, and wetlands in the Amazon is an important term in the C balance for the Amazon Basin. Methane and CO_2 are important greenhouse gases, and improved understanding of their evasion from large tropical wetlands, such as those in the Amazon, is a key component in predicting the role of the C cycle in global warming. A clever combination of satellite-derived observations of gravitational anomalies associated with flooding and atmospheric CH_4 concentrations illustrates the promise for further advances in understanding global-scale CH_4 fluxes made possible by new measurement tools (Bloom *et al.* 2010).

■ Linking physical and biogeochemical processes in aquatic systems

Crucial to studies of biogeochemical cycles is the determination of fluxes of materials between sediments, water, and the atmosphere and within these reservoirs, and calculation of these fluxes requires measurements of transport processes and associated chemical constituents. Over the past two decades, major advances in measuring and modeling aquatic systems' physical processes – active in the dispersion and advection of gases, solutes, and particles – have been made, but these innovations have yet to be routinely incorporated within biogeochemical studies.

Improved understanding of turbulence has been critical to recent advances in measuring transport processes. Turbulent flows comprise three-dimensional eddies, with smaller eddies embedded in larger ones and with variations in eddy velocity, in part dependent upon eddy size. Turbulence is important because it induces fluxes across boundaries. Profiles of turbulence may be acquired with shear probes (Moum *et al.* 2008) or by millimeter-scale measurements of temperature (MacIntyre *et al.* 1999). Time series of turbulence can be obtained with acoustic Doppler velocimeters or acoustic Doppler current profilers (Figure 5). Long-term deployments of arrays of high-resolution, fast-response temperature sensors permit identification of regions of enhanced turbulence, sizes of turbulent eddies, and time series of eddy diffusivity.

Coupling measures of turbulence with estimates of biogeochemical fluxes can be accomplished by indirect or direct methods. In the indirect approach, either a mass transfer

coefficient or an eddy diffusivity coefficient is measured, and fluxes are computed as the product of the coefficient and a concentration gradient. The coefficient of eddy diffusivity is computed either based on fluxes of heat and tracers, or from measurements of the small-scale temperature or velocity fluctuations caused by turbulence (MacIntyre *et al.* 1999; MacIntyre *et al.* 2009). Fluxes at the benthic boundary layer and at the air–water interface are computed by way of mass transfer coefficients (Lorke *et al.* 2003; Laurion *et al.* 2010). Alternatively, a direct approach, known as eddy covariance and discussed above in a terrestrial-system application, involves simultaneous measurements of fluctuating solute concentrations and vertical velocities. This approach is being applied in lakes, estuaries, and coastal waters, and has been used successfully in studies of oxygen flux across the benthic boundary layer (Berg *et al.* 2003) and of gas flux across the air–water interface (Edson *et al.* 2008).

A major challenge in linking calculations of transport with biogeochemical processes is obtaining the biological or chemical measurements at a frequency appropriate to merge with the physical data. However, recent advances in sensor technology, such as the availability of in situ nitrate (NO_3^-) analyzers or optical oxygen sensors (Figure 5), are beginning to alleviate this problem. Profiling or moored fluorometers, which measure chlorophyll concentrations and states of fluorescence, when used with in situ temperature and light sensors or temperature microstructure profilers, have the potential to integrate stratification dynamics and variability of light climate for continuous estimates of primary productivity.

New approaches that combine rapid bench-top or in situ measurements of the ultraviolet (UV)–visible or fluorescence spectrum with multivariate statistical techniques hold considerable potential for quantifying the C sources for aquatic ecosystems (McKnight *et al.* 2001; Hassouna *et al.* 2007). One such approach, fluorescence spectroscopy, subjects an intact water sample to a range of UV wavelengths while recording emission over a range of wavelengths. The approach has been used to distinguish algal from terrestrial vegetation sources of dissolved organic matter in streams and lakes (McKnight *et al.* 2001), and to link dissolved organic matter sources to changes in microbial community structure (Nelson 2009). Another analytical development poised to transform the ability to measure metabolic processes at high spatial and temporal resolution is in situ measurements of stable isotopes in CO_2 and H_2O (Bowling *et al.* 2003).

Continuous measurements are enabling the observation of biogeochemical and ecological properties at rates sufficient to test theories of ecosystem change. Regime shifts are one form of ecosystem change where switches between alternate states result in different ecosystem properties, and theory suggests that regime shifts are preceded by changes in system variability (Scheffer *et al.* 2009). High-frequency observations of ecosystem components offer a new potential for evaluating hypotheses about variance properties in relation to regime shifts, through sensors that measure attributes of productivity, biomass, and respiration (Cole *et al.* 2000).

Figure 4. *Synthetic aperture radar (SAR)-derived vegetative cover and inundation for a reach of the Solimões River in the central Amazon Basin, Brazil (modified from Hess* et al. *2003).*

■ Roles of genomics

By allowing direct assessment of the identity and spatio-temporal dynamics of gene and protein sequences in the environment, genomics and proteomics have revolutionized our understanding of the types of microbial cells present in the environment and their potential role in biogeochemical cycles. The age of environmental genomics began with work demonstrating that the diversity of microbial ribosomal gene sequences in the environment far surpassed diversity estimates from microscopic and culture-based methods (Stahl *et al.* 1985). Sequence-based methods of community fingerprinting (Liu *et al.* 1997) and phylogenetic staining (Giovannoni *et al.* 1988) have since allowed tracking of natural, uncultivated microbial populations through time and space, and have been used widely to develop correlational linkages between microbial community structure and biogeochemical processes in diverse environments (Nelson 2009).

Perhaps the strongest demonstration of the power of genomics to revise assumptions about ecosystem biogeochemistry came with the discovery of abundant and diverse genes that code for proteorhodopsin and nitrogenase in free-living oceanic microbial communities (Beja *et al.* 2000; Zehr *et al.* 2001). The occurrence of diverse and abundant marine bacterioplankton lineages – contain-
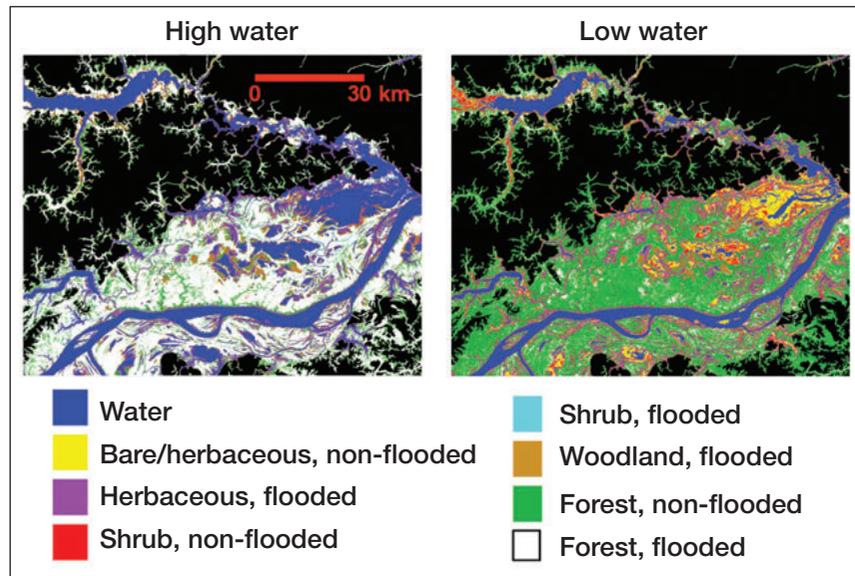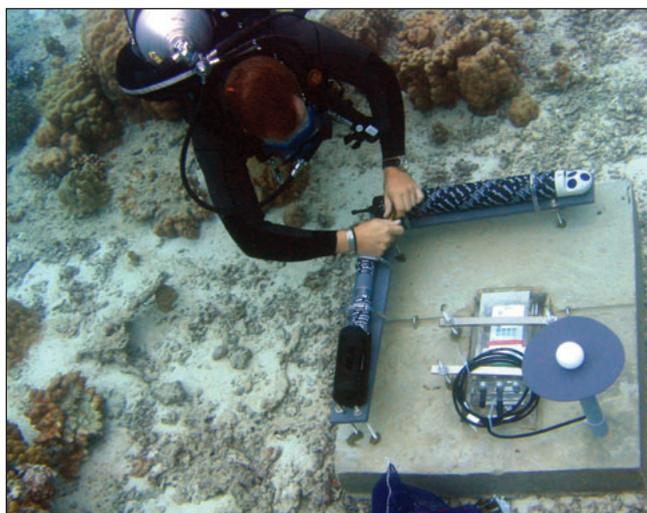
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Figure 5. *Diver installing acoustic Doppler profiler, optical oxygen sensor, and irradiance sensor for benthic metabolism studies in Moorea, French Polynesia.*

ing genes coding for proteorhodopsin and other anoxygenic light-harvesting pigments – suggests that there is a great deal more photosynthesis and mixotrophic metabolism in aquatic environments than previously detected. The presence of nitrogenase in diverse unicellular microbes suggests that nitrogen fixation may be far more widespread and important as a nutrient source in the oceans, ultimately altering interpretations of C flow in aquatic habitats (Karl 2002). These studies were followed by others verifying that the indicated biogeochemical processes were indeed important at large scales, leading to the revision of open-ocean biogeochemistry models (Beja *et al.* 2001; Montoya *et al.* 2004). Through this process of functional gene discovery and subsequent biogeochemical verification, genomic approaches will most likely continue to expand the understanding of biogeochemical cycles.

The development of metagenomic (the collective genetic sequence of all organisms in a sampled habitat) approaches has made functional genomic snapshots of ecosystems possible. In extreme microbial ecosystems, such as acid mine drainages and hydrothermal vents, this approach has permitted mechanistic detailing of biogeochemical cycles and the involvement of specific microbial taxa in the various cycles (Reysenbach and Shock 2002; Tyson *et al.* 2004). In more diverse systems, such as the open ocean or human gut, these datasets clarify the relative abundance of different taxa and functional genes and allow for reconstruction of community metabolic pathways and modeling of biogeochemical interactions among consortia (DeLong *et al.* 2006; Gill *et al.* 2006). Metagenomes have now been successfully complemented with other "post-genomic" approaches, including the use of transcriptomic (ie collective messenger RNA sequence data) and proteomic (ie collective protein sequence data) methods to clarify the differences between genetic potential and actively expressed metabolic products (Frias-Lopez *et al.* 2008; Sowell *et al.* 2009).

Methods are rapidly emerging to link genomic data directly to biogeochemical processes. Stable isotope probing permits separation of community nucleic acids based on incorporation of labeled substrates, allowing metagenomic or transcriptomic characterization of a select subset of genomes associated with specific biogeochemical processes (Kalyuzhnaya *et al.* 2008). New sequencing technologies have reduced the cost and increased the efficiency of gene sequencing, permitting researchers to develop and analyze multiple metagenomes and transcriptomes along environmental or temporal gradients (Willner *et al.* 2009). In the coming decades, genomics and related fields in systems biology (eg proteomics and metagenomics) will take center stage in defining ecosystem biogeochemical dynamics, developing management strategies, and predicting ecological responses to global change.

■ Future challenges and opportunities

Challenges that result from technological advances, such as those discussed here, include the costs of supporting the continued operation of remote-sensing and data systems, maintaining long-term, whole-ecosystem experiments, and sustaining state-of-the-art analytical facilities. The reward of doing so is an increased capacity to measure and model coupled biogeochemical cycles. One essential element in this increased capacity is, and will be, the invention and application of new technologies. As members of the ecological scientific community continue to learn how to distribute and creatively use the ever-increasing volume and kinds of data on biogeochemical processes and fluxes, the resulting integrated analyses will enhance our understanding of ecosystems and their short-term variability and long-term changes. With the resulting increased understanding, ecologists will be better able to inform policies that influence human societies and their environment.

■ References

Baldocchi DD, Hicks BB, and Meyers TP. 1988. Measuring biosphere–atmosphere exchanges of biologically related gases with micrometeorological methods. *Ecology* **69**: 1331–40.

Behrenfeld MJ, Boss E, Siegel DA, and Shea DM. 2005. Carbon-based ocean productivity and phytoplankton physiology from space. *Global Biogeochem Cy* **19**, doi:10.1029/2004GB002299.

Behrenfeld MJ, Westberry TK, Boss ES, *et al.* 2009. Satellite-detected fluorescence reveals global physiology of ocean phytoplankton. *Biogeosciences* **6**: 779–94.

Beja O, Aravind L, Koonin EV, *et al.* 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. *Science* **289**: 1902–06.

Beja O, Spudich EN, Spudich JL, *et al.* 2001. Proteorhodopsin phototrophy in the ocean. *Nature* **411**: 786–89.

Berg P, Roy H, Janssen F, *et al.* 2003. Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy correlation technique. *Mar Ecol-Prog Ser* **261**: 75–83.

Bloom AA, Palmer PI, Fraser A, *et al.* 2010. Large-scale controls of methanogenesis inferred from methane and gravity spaceborne data. *Science* **327**: 322–25.

Bowling D, Sargent S, Tanner B, and Ehleringer J. 2003. Tunable diode laser absorption spectroscopy for stable isotope studies of ecosystem–atmosphere CO_2 exchange. *Agr Forest Meteorol* **118**: 1–19.

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Boyd PW, Law CS, Wong CS, *et al.* 2004. The decline and fate of an iron-induced subarctic phytoplankton bloom. *Nature* **428**: 549–53.

Cole JJ, Pace ML, Carpenter SR, and Kitchell JF. 2000. Persistence of net heterotrophy in lakes during nutrient addition and food web manipulation. *Limnol Oceanogr* **45**: 1718–30.

DeLong EF, Preston CM, Mincer T, *et al.* 2006. Community genomics among stratified microbial assemblages in the ocean's interior. *Science* **311**: 496–503.

Dolman AJ, Schulze ED, and Valentini R. 2003. Analyzing carbon flux measurements. *Science* **301**: 916–17.

Edson JB, DeGrandpre MD, Frew N, and McGillis WR. 2008. Investigations of air–sea gas exchange in the CoOP Coastal Air–Sea Chemical Exchange Project. *Oceanography* **21**: 34–45.

Finzi AC, Allen AS, DeLucia EH, *et al.* 2001. Forest litter production, chemistry, and decomposition following two years of free-air CO_2 enrichment. *Ecology* **82**: 470–84.

Finzi AC, Moore JP, DeLucia EH, *et al.* 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. *Ecology* **87**: 15–25.

Frias-Lopez J, Shi Y, Tyson GW, *et al.* 2008. Microbial community gene expression in ocean surface waters. *P Natl Acad Sci USA* **105**: 3805–10.

Gill SR, Pop M, DeBoy RT, *et al.* 2006. Metagenomic analysis of the human distal gut microbiome. *Science* **312**: 1355–59.

Giovannoni SJ, Delong EF, Olsen GJ, and Pace NR. 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. *J Bacteriol* **170**: 720–26.

Hassouna M, Theraulaz F, and Massiani C. 2007. Direct estimation of nitrate, total and fractionated water extractable organic carbon (WEOC) in an agricultural soil using direct UV absorbance deconvolution. *Talanta* **71**: 861–67.

Hess LL, Melack JM, Novo EMLM, *et al.* 2003. Dual-season mapping of wetland inundation and vegetation for the central Amazon Basin. *Remote Sens Environ* **87**: 404–28.

Kalyuzhnaya MG, Lapidus A, Ivanova N, *et al.* 2008. High-resolution metagenomics targets specific functional types in complex microbial communities. *Nat Biotechnol* **26**: 1029–34.

Karl DM. 2002. Microbiological oceanography: hidden in a sea of microbes. *Nature* **415**: 590–91.

Korner C, Asshoff R, Bignucolo O, *et al.* 2005. Carbon flux and growth in mature deciduous forest trees exposed to elevated CO_2. *Science* **309**: 1360–62.

Laurion I, Vincent WF, MacIntyre S, *et al.* 2010. Variability in greenhouse gas emissions from permafrost thaw ponds. *Limnol Oceanogr* **55**: 115–33.

Law BE, Falge E, Gu L, *et al.* 2002. Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. *Agr Forest Meteorol* **113**: 97–120.

Liu WT, Marsh TL, Cheng H, and Forney LJ. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microb* **63**: 4516–22.

Lorke A, Muller B, Maerki M, and Wuest A. 2003. Breathing sediments: the control of diffusive transport across the sediment–water interface by periodic boundary-layer turbulence. *Limnol Oceanogr* **48**: 2077–85.

Luyssaert S, Schulze ED, Borner A, *et al.* 2008. Old-growth forests as global carbon sinks. *Nature* **455**: 213–15.

MacIntyre S, Flynn KM, Jellison R, and Romero JR. 1999. Boundary mixing and nutrient flux in Mono Lake, California. *Limnol Oceanogr* **44**: 512–29.

MacIntyre S, Fram JP, Bettez ND, *et al.* 2009. Climate-related variations in mixing dynamics of an Alaskan arctic lake. *Limnol Oceanogr* **54**: 2401–17.

McClain CR. 2009. A decade of satellite ocean color observations. *Ann Rev Mar Sci* **1**: 19–42.

McKnight DM, Boyer EW, Westerhoff PK, *et al.* 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnol Oceanogr* **46**: 38–48.

Melack JM. 2004. Remote sensing of tropical wetlands. In: Ustin S (Ed). Manual of remote sensing. New York, NY: John Wiley and Sons.

Melack JM and Gastil M. 2001. Airborne remote sensing of chlorophyll distributions in Mono Lake, California. *Hydrobiologia* **466**: 31–38.

Melack JM, Hess LL, Gastil M, *et al.* 2004. Regionalization of methane emissions in the Amazon Basin with microwave remote sensing. *Glob Change Biol* **10**: 530–44.

Melack JM, Novo EMLM, Forsberg BR, *et al.* 2009. Floodplain ecosystem processes. In: Gash J, Keller M, and Silva-Dias P (Eds). Amazonia and global change. Washington, DC: American Geophysical Union.

Miller CB, Frost BW, Wheeler PA, *et al.* 1991. Ecological dynamics in the sub-arctic Pacific, a possibly iron-limited ecosystem. *Limnol Oceanogr* **36**: 1600–15.

Montoya JP, Holl CM, Zehr JP, *et al.* 2004. High rates of N_2 fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* **430**: 1027–31.

Moum JN, Nash JD, and Klymak JM. 2008. Small-scale processes in the coastal ocean. *Oceanography* **21**: 22–33.

Nelson CE. 2009. Phenology of high-elevation pelagic bacteria: the roles of meteorologic variability, catchment inputs and thermal stratification in structuring communities. *ISME J* **3**: 13–30.

Novo EMLM, Barbosa CCF, Freitas RM, *et al.* 2006. Seasonal changes in chlorophyll distributions in Amazon floodplain lakes derived from MODIS images. *Limnology*, doi:10.1007/s10201-006-0179-8.

Pepin S and Korner C. 2002. Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests. *Oecologia* **133**: 1–9.

Reich PB, Hobbie SE, Lee T, *et al.* 2006. Nitrogen limitation constrains sustainability of ecosystem response to CO_2. *Nature* **440**: 922–25.

Reysenbach AL and Shock E. 2002. Merging genomes with geochemistry in hydrothermal ecosystems. *Science* **296**: 1077–82.

Richey JE, Melack JM, Aufdenkampe AK, *et al.* 2002. Outgassing from Amazonian rivers and wetlands as a large tropical source of atmospheric CO_2. *Nature* **416**: 617–20.

Scheffer M, Bascompte J, Brock WA, *et al.* 2009. Early warning signals for critical transitions. *Nature* **461**: 53–59.

Seiler TJ, Rasse DP, Li JH, *et al.* 2009. Disturbance, rainfall and contrasting species responses mediated aboveground biomass response to 11 years of CO_2 enrichment in a Florida scrub-oak ecosystem. *Glob Change Biol* **15**: 356–67.

Sowell SM, Wilhelm LJ, Norbeck AD, *et al.* 2009. Transport functions dominate the SAR11 metaproteome at low-nutrient extremes in the Sargasso Sea. *ISME J* **3**: 93–105.

Stahl DA, Lane DJ, Olsen GJ, and Pace NR. 1985. Characterization of a Yellowstone hot-spring microbial community by 5S ribosomal-RNA sequences. *Appl Environ Microb* **49**: 1379–84.

Tyson GW, Chapman J, Hugenholtz P, *et al.* 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature* **428**: 37–43.

Willner D, Thurber RV, and Rohwer F. 2009. Metagenomic signatures of 86 microbial and viral metagenomes. *Environ Microbiol* **11**: 1752–66.

Zehr JP, Waterbury JB, Turner PJ, *et al.* 2001. Unicellular cyanobacteria fix N_2 in the subtropical North Pacific Ocean. *Nature* **412**: 635–38.

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