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Methods: Traditional and Molecular

MICROBIAL ECOLOGY

Accurate Estimation of Microbial Loop Processes and Rates

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Abstract. Our conceptual models of microbial food webs in aquatic ecosystems provide a unifying hypothesis for the design and conduct of field studies. Our ability to provide a rigorous test of these hypotheses, however, relies to a large extent on the availability of precise and accurate methods. Although considerable progress has been made over the past two decades, unambiguous resolution of in situ microbial rates and processes will probably require improved or novel methodologies.

Introduction

Success in most modern fields of science requires a careful integration of both intellectual and technical efforts. The study of microbial ecology is no exception. In the 20 years that have passed since Pomeroy [2] first provided the intellectual framework for studies of the aquatic microbial food web, numerous attempts have been made to obtain accurate estimation of the standing stocks and turnover rates of individual components of the microbial loop. Nevertheless, the diversity of microorganisms and their microhabitats and the existence of complex biological interactions continues to overwhelm the capabilities of existing analytical methods. Furthermore, ecologists frequently refer to "carbon and energy flux" as though they are obligatorily coupled. Few methods currently exist for the measurement of energy flux, and only limited field data are available on this parameter. We clearly need coevolving ecological paradigms and methodologies.

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The two most fundamental measurements necessary to describe microbial communities in nature are the standing stocks of the individual carbon pools and the rates of biomass production and loss (Fig. 1). Unfortunately, these parameters cannot be measured with the precision or accuracy that is often required for modern hypothesis testing. For example, most current methods rely upon the use of poorly constrained extrapolation factors to convert estimates of cell numbers or uptake of radiolabeled molecules into meaningful units of carbon or energy. These extrapolations further degrade the precision and accuracy of the initial measurements. Recent

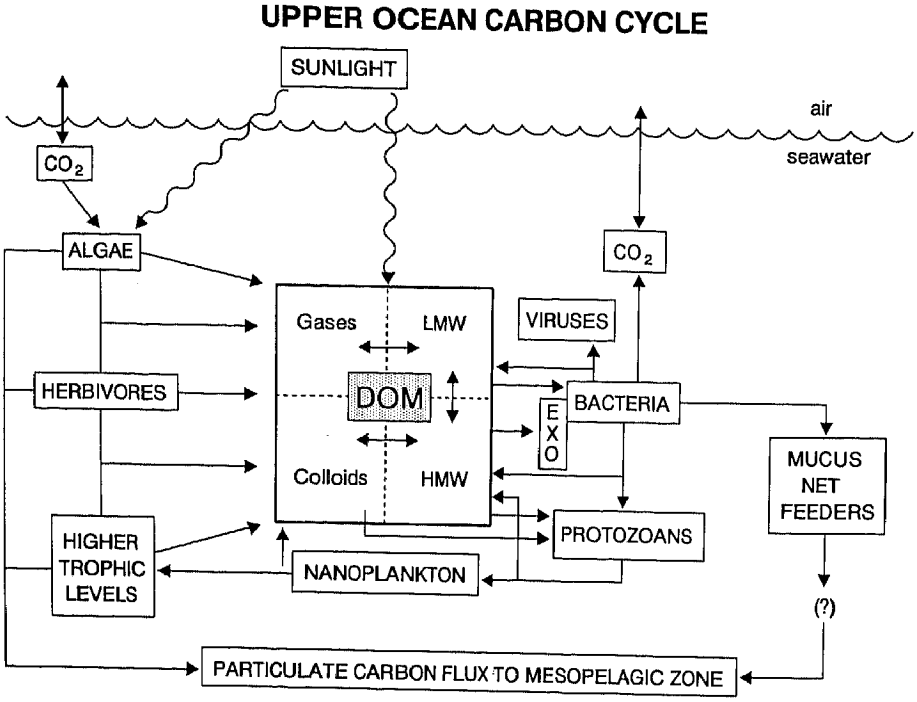

Fig. 1. Schematic representation of the upper ocean carbon cycle depicting the complex physical, chemical, and biological interactions that are known to exist. The microbial loop, pictured on the right of the dissolved organic matter (DOM) box, is hypothesized to be a central component of the carbon cycle. However, the rates of DOM production, interconversion and utilization, the rates of bacterial production and loss (from combined effects of protozoan grazing and mucus net feeder activities, viral lysis, and death), and the efficiency of conversion of DOM to bacterial biomass are not well known for most ecosystems.

Table 1. Bacterial enumeration precision estimation for Hawaiian waters

Method	Precision (CV)	Reference
Epifluorescence microscopy[1]	range 5–30%	D. Bird, unpublished data from Hawaii Ocean Time-series Program, cruise HOT-4
Flow cytometry[2]	mean 1.4%	M. Landry et al. [1]

[1] Three separate filters, count 30 fields, field count CV = 20–60%
[2] Replicate samples

advances in flow cytometry as an alternative to direct microscopy for enumerating bacteria from aquatic habitats may offer numerous advantages, including improved precision and accuracy (Table 1). Furthermore, methods now exist for the direct measurements of carbon pools and fluxes at ecologically-relevant concentrations and rates (Table 2). These direct measurements eliminate the need for uncertain and variable extrapolation factors; an obvious advantage in quantitative ecological studies.

Table 2. Precision and accuracy estimates for direct chemical measurements of microbial loop elements at Station ALOHA

Parameter	Method	Ambient concentration (0–100 m)	Precision[a]	Certified standards available
ΣCO_2	coulometry (SOMMA)	~2,000 μmol kg^{-1}	0.05% (±1 μmol kg^{-1})	yes
DOC	high temp. catalytic oxidation	80–120 μmol kg^{-1}	1% (±1 μmol kg^{-1})	no
O_2	computer-assisted micro-Winkler	~225 μmol kg^{-1}	0.07% (±0.15 μmol kg^{-1})	yes
NO_3	chemiluminescence	1–10 nmol kg^{-1}	1% (<1 nmol kg^{-1})	yes
PO_4	MAGIC	5–50 nmol kg^{-1}	1–3% (<0.5 nmol kg^{-1})	yes

[a] Numbers in parentheses indicate typical concentrations that are able to be reliably measured using the currently available methods

Because tritiated thymidine uptake has been used so extensively in aquatic microbial ecology, I would like to make a few comments regarding that method. Nonspecific catabolic labeling of macromolecules during the assimilation of tritiated thymidine, once thought not to exist, is now recognized as an ubiquitous ecological phenomenon. This effect, however, must still be considered to be a second-order criticism of the use of thymidine uptake as a measure of heterotrophic bacterial production compared to the more serious limitations of unknown isotope dilution, variable per capita uptake rates, and the ability of starved, nongrowing cells to assimilate thymidine. Furthermore, extrapolation factors derived over the past several years with similar methods have a range in excess of 100-fold, and it now appears almost certain that the conversion factor is a variable, habitat-dependent parameter. One might logically ask whether this or other laboratory-based methods of bacterial metabolism and biosynthesis can be used reliably in ecological studies and, if not, why not?

The use of "modern" nucleic acid-based measurements in microbial ecology seems to be a logical extension of their application to problems in other subdisciplines of microbiology. However, like most new approaches, the potential, which in this case appears to be excellent, may never be fully realized. We simply do not have enough experience or data either to embrace or to reject any of these specific approaches at the present time. There are several known limitations, however, that should be emphasized. First, nearly all of the nucleic acid-based methods rely on efficient DNA (or RNA) extraction from sample materials and error-free amplification by the polymerase chain reaction (PCR). The validity of these assumptions is neither guaranteed nor expected. Interference from nucleic acids contained in nonviable or moribund cells and from detrital ("nonliving") pools that sometimes dominate the total nucleic acid pool in nature, also need to be carefully evaluated. In promoting nucleic acid-based measurements in microbial ecology one needs to be cognizant of the potential "mismatch" between taxonomy or genotype and physiological ecology. The mere detection of target genes, rather than gene prod-

ucts, does not necessarily imply gene expression; these methods reveal potential rather than in vivo activities. Furthermore, it is well known that selected metabolic traits are broadly dispersed with little taxonomic coherence. Finally, because of the design of most nucleic acid-based measurement studies the precision and accuracy of the resultant data are largely unknown and difficult to determine.

In order to elicit a healthy debate on the application of nucleic acid-based methods, one might ask the following questions: (1) Can the extensive ecological theory on species diversity in animal and plant communities be directly applied to oceanic microbial assemblages? Is new theory required? and (2) Does bioelement cycling research (e.g., studies of the microbial loop) require a comprehensive species list and, if so, at what cost and level of effort?

Summary and Future Prospectus

- Development and broad distribution of standard reference materials for use in microbial food web studies.
- Greater reliance on direct chemical measurements to estimate C, N, P, and O_2 fluxes.
- Improvement of modeling efforts to constrain biogeochemical mass and energy fluxes.
- Development of autonomous and remote sensing methods for microbial food web processes.

References

1. Landry M, Kirshtein J, Monger B (1993) Quantitative enumeration of paraformaldehyde preserved *Prochlorococcus* by flow cytometry. Signal Noise 6:3
2. Pomeroy LR (1974) The ocean's food web: a changing paradigm. BioScience 24:499–504