Abstract
Understanding biogeochemical processes in salt marshes will help elucidate their role as essential habitats. Since microbial activity accounts for nearly all of the biogeochemical cycling that occurs in the marsh environment, monitoring bacteria and their activity is fundamental to assessing marshes as sites for biogeochemical change. In the past, this has been accomplished using approaches that estimated the average response of the entire population of micro-organisms. These studies have proven very useful for computing overall fluxes and secondary production. However, questions of diversity, population dynamics, microbial ecology, and the role of specific bacteria responsible for a biogeochemical transformation have been difficult to approach using the traditional, bulk rate techniques. The recent revolution in biochemical methods has allowed microbiologists to now identify specific groups of bacteria in a natural sample. This is done by targeting specific macromolecules in the bacterial cells such as fatty acids, proteins, and nucleic acids to characterize the various microbial members of or community independent of the other bacteria and eukaryotes present in the sample. Such studies have begun to provide information on the variety, distribution, and gene regulation of particular bacteria responsible for a given biogeochemical process.
Although a comprehensive overview of molecular techniques will not be feasible in this chapter, we shall discuss some principles of applying biochemical analysis to complex microbial communities. It is hoped the data obtained from molecular studies in marsh habitats in the future will lead to a better understanding of the linkages between the structure and the function of the microbial communities that mediate biogeochemical cycling in the environment.
1. Introduction

Marshes are sites of intense microbial activity affecting decomposition and biogeochemical cycling (Carpenter 1983, Goldhaber 1974, Van Es 1982). For example, the importance of bacteria as consumers of organic carbon in marine food webs has long been recognized (Cho 1988, Fuhrman 1980, Pomeroy 1974). This understanding has been based on classical methodologies which measure a change (typically uptake or evolution of an isotopic label) and average that change over larger areas and the entire population of bacteria to estimate an overall biogeochemical rate. These traditional approaches have generally considered the bacterial community at the sample site as a “black box” and monitored the flow of material inside and out. However, the classic methods have been unable to assess the role of different species within the complex microbial assemblage, or determine the relative contribution of any particular microbial species to biogeochemical cycling under different conditions.

At present, there is a need to understand what is going on inside the “black box” since shifts within the bacterial community will impact biogeochemical cycling in the biosphere. Historically, major changes in the bacterial community have had significant impacts on conditions for life on Earth. For example, the modern atmosphere is a direct result of the evolution of oxygenic photosynthesis by early cyanobacteria billions of years ago (Atlas 1993). The dominance of \( \text{N}_2 \) in this oxic environment illustrates the importance of present-day populations of denitrifying bacteria. In order to understand and predict how various microbial populations will affect biogeochemical cycling in marshes, some means of addressing microbial population dynamics needs to be developed.

This chapter will focus on the development of tools and techniques to approach the issues of which bacteria are inside the “black box”, which particular bacterial species is most numerous, who is changing and growing the fastest, and which traits (or genes) are being expressed in an environmental sample. Much of the research discussed here stems from work done in other parts of the marine environment and awaits application to salt marshes. This research approach is extremely important because, in regions undergoing active biogeochemical transformations (such as salt marshes), the most abundant and the fastest growing micro-organisms must be playing significant roles.

2. Definition of Molecular Methods

Bacteria generally lack defining morphological characteristics. Therefore, efforts within the last decade have focused on identifying specific molecules present within the bacteria to differentiate the various members of the microbial community. A recent example would include the direct analysis of the nucleic acids present in microbial biomass (for review, Head et al. 1998, Hugenholtz et al., 1998, Amann et al. 1995, Torsvik et al. 1996, Woese 1987). For our purposes, ”molecular methods” will be defined as any procedure that tracks a specific cellular constituent that can differentiate the various players within the microbial population. These biomarkers can include lipids, proteins, and nucleic