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Advances in monitoring environmental microbes

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Culture-independent approaches, such as next-generation sequencing and microarray-based tools, provide insight into the identity and functional diversity of microbial communities. Although these approaches are potentially powerful tools in understanding microbial structure and function, there are a number of limitations that may bias conclusions. In order to mitigate these biases, an understanding of potential biases within each stage of the experimental process is necessary. This review focuses on the biases associated with sample collection, nucleic acid extraction, processing, sequencing analyses, and Chip technologies used in microbial ecology studies.

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Introduction

The primary goal of microbial ecology is to understand the structure and function of a microbial community and detail the interactions between microbes in various environments. The inability of culture-dependent methods to assess the vast majority of environmental microbes has limited an accurate understanding of these organisms. Culture-independent methods have removed this limitation and provided access to a great wealth of phylogenetic and functional diversity contained within microbial communities. This access has enabled a sharper picture of microbial communities in a variety of settings and has enhanced our ability to harness microbes for biotechnological applications.

While culture-independent approaches have their advantages, there are a number of limitations and biases that may be introduced throughout sampling and processing of environmental samples. These biases depend on a number of individual processes, starting at collection of samples and ending with bioinformatics and conclusions. Within each step bias is introduced and carried through the pipeline, thus compounding that bias in the end result (Figure 1). Therefore, to ensure the quality of the results, we must take into account potential biases at each stage in the experimental process in order to temper our conclusions as well as take steps to mitigate that bias.

Large-scale assessments of microbial communities have utilized two distinct technologies: Sequencing-based and Chip-based approaches. In this review we examine the pipeline for molecular microbial ecology and identify some of the biases associated with each of the steps. Additionally, we compare the advantages and limitations of the use of metagenomic and gene-specific molecular technologies in the characterization of microbial communities.

Sample collection

The most fundamental step in monitoring microbial communities is obtaining a representative sample of the community. From this sample, DNA will be extracted and further used to examine the community structure and function. Within this intial step, biases that can limit the overall analysis of the results may be introduced. For example, in environments where microbial biomass is low, it may be difficult to obtain enough environmental sample to characterize the entire microbial community. Therefore, the sampling approach must be carefully considered to limit the introduction of potential bias that will affect the analysis of a microbial community.

A thorough review of prior work performed on the community of interest or related communities can provide important information (i.e. estimates of biomass, known community members, chemical parameters, etc.) to aid in designing a sampling strategy that limits the introduction of biases. Factors that must be considered in order to limit biases related to sampling methodologies include: sample type (e.g. water versus sediment), amount of sample collected, methodology for collection of samples (e.g. filter versus centrifugation), and materials used (e.g. filter type).

Sediment samples

For sediment samples, core or grab samples are often collected. During handling of the sediment samples the

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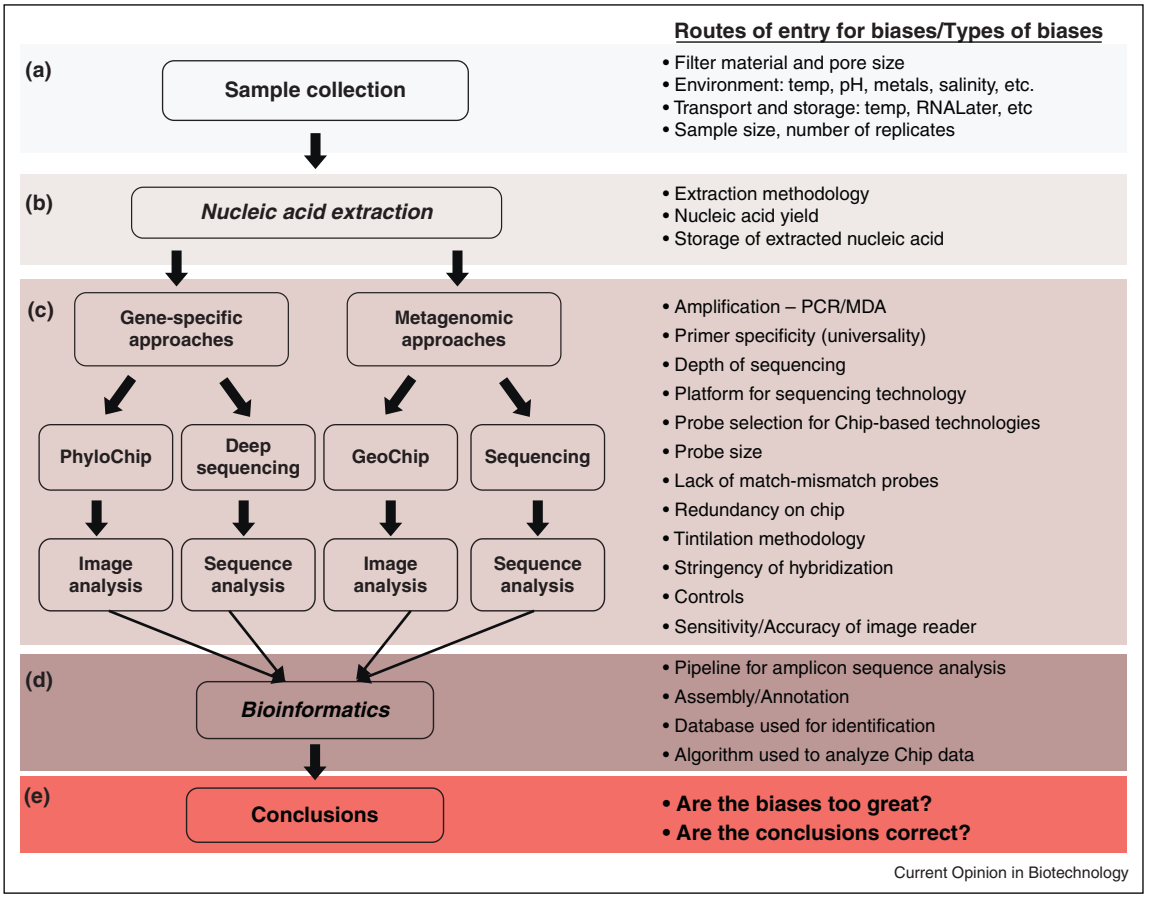
Figure 1



Pipeline of individual processes associated with culture-independent approaches. Within steps A–E, biases are introduced and carried through, resulting in compounding bias. Biases introduced at earlier stages are further amplified by the end of the pipeline. Light color represents little bias and darker color represents increase in the number of bias present. **(a)** Sample collection is the initial step in culture-independent approaches; **(b)** extraction of nucleic acids; **(c)** molecular techniques and analyses associated with culture-independent approaches; **(d)** bioinfomatics; and **(e)** conclusions.

microbial community may be exposed to conditions different from their natural environment. Ideally, samples should be immediately stored at −80 °C until nucleic acids are extracted. This is especially important if RNA is the desired product. Another concern with processing sediment samples is the presence of substances such as humic acids. If humics are extracted along with DNA or present with the sample, they can potentially interfere with downstream applications like PCR [1,2,3•,4•,5,6]. Additionally, cleanup methods may result in loss of DNA within the sample [7,8].

Water samples

Water samples are either collected using filtration or centrifugation. Selection of collection methods is generally dependent on the sample volume required. For example, if large volumes of water are required, filtration is preferable due the relative ease of filtering large volumes of water and the difficulty of transporting large volumes of water to be centrifuged.

A potential source of bias associated with filtration-based sampling methods is the selection of the filter membrane for collection of cells and extraction of DNA [9–11]. There are a number of membranes available, each with its own advantages and disadvantages. No matter what filter is used, one of the greatest concerns associated with DNA extraction from filters is the ability to recover the cells and nucleic acids from the filter material. One concern is the presence of non-biological contaminants, such as metals, in the water. If these materials are sorbed to the filter, there is a possibility that nucleic acids and lipids may be degraded or destroyed. Additionally, sorption of nucleic acids to the filter material could potentially bias results. For some filters, cells may adhere to the material thus limiting the overall yields of DNA as well as

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