



Review

Potential applications of soil microbial ecology and next-generation sequencing in criminal investigations



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ABSTRACT

The complex relationships between the changes in microbial community profiles and postmortem interval (PMI) estimates have recently been discussed in the forensic literature. Edaphic, necrobiomic microorganisms at the cadaver-soil interface construct multi-species communities that change in richness and activity when the host body dies and begins to decompose. Characterization of these dynamic changes has been made possible by current advances in high throughput, next-generation platforms. The effectiveness of these metagenomic technologies is that they pride the foundations of a framework for identification of grave sites and the determination of postmortem timelines, or “microbial clocks.” The proposed clocks may help substantiate the estimation of PMI. Studies have demonstrated the differences between soils collected at grave sites and control soils which may be useful in identifying clandestine grave sites. In this review is the discussion of the recent and formative findings involving sequencing applications of soil microbial communities relating the differences in taxon richness and abundance patterns as molecular tools with broad and important applications in forensics.

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1. Introduction

The history of soil ecology research is vast and extensive. Much of the research in the field of soil ecology has been conducted for the benefit of agricultural developments or to study the deleterious impact of environmental stressors on edaphic ecosystems. However, genomic studies of the abundance and activity of soil necrobiomic microbial communities associated with decaying

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human and animal cadavers, along with the underlying soils, have steadily advanced to the forefront of applied ecological research (Can et al., 2014; Hyde et al., 2013; Metcalf et al., 2013; Pechal et al., 2013, 2014). Recent emphases on decomposition ecology have yielded robust investigations of the soil ecological necrobiome, namely the macro- and micro-organisms associated with decaying heterotrophic biomass (Benbow et al., 2013).

Cadaver decomposition is a complex succession of chemical, biological and geochemical changes within the carcass that directly affects the niche, functions and ecology of the micro-organisms in the neighboring soil (Finley et al., 2014). Microbes generally do not live in single species communities (Wooley et al., 2010). Accordingly, necrobiomic microorganisms at the cadaver-soil interface reside in multi-species communities that rapidly and measurably change in abundance and activity when the host body dies. The microorganisms associated with the necrobiomic communities have been referred to as the epinecrotic microbial communities of the decomposing carcass (Pechal et al., 2014). These communities have recently been proposed to be useful for forensic investigations, and a laboratory study using mice carcasses tracked changes in the soil beneath the remains and demonstrated measurable shifts in the community (Metcalf et al., 2013). Traditional forensic decomposition studies have focused mainly on the macroscopic, observable postmortem evidence such as animal scavengers or entomological developmental stages. However, there is a growing awareness that the microorganisms on a dead host and in the soil beneath it could potentially be used as evidence in criminal investigations. The goal of this paper is to review recent trends in soil microbial ecology and how new technologies may open up new lines of applications in the forensic sciences – a novel and promising line of inquiry for soil ecologists. A second goal is to discuss next-generation approaches to research into this area of soil microbial ecology for application in carrion decomposition studies.

Decomposition is a fundamental process in ecosystem function and energy flow where nutrients are recycled into the ecosystem food cycle. Recent microbial ecology literature suggests that microbial communities exhibit a wide range of successional patterns depending on environmental conditions (Shade et al., 2013). For example, studies have demonstrated that microbial communities change directionally on the surface of leaves (Redford and Fierer, 2009) and in seasonal patterns in surface waters of aquatic systems (Gilbert et al., 2012). Likewise, studies have shown that microbial succession is a characteristic of carrion decomposition (Metcalf et al., 2013; Pechal et al., 2014), as demonstrated by high-throughput, next-generation sequencing techniques. Past sequencing technology was limited to culturable microbial taxa. Therefore, next-generation approaches are opening up new lines of inquiry within the microbiological sciences, including soil microbial ecology. However, current sequencing technology is limited by the sheer size of the genomes sequenced, where whole-genome sequencing is limited to 10^9 bases per single run (El-Metwally et al., 2013). An entire soil metagenome may approach an estimated 10^{15} base pairs (Delmont et al., 2011). Notwithstanding, an attempt to monitor the changes in the soil epinecrotic signatures during decomposition provides an innovative molecular tool for criminal investigators for the estimation of the PMI or identifying clandestine grave sites.

The necrobiome is comprised of organisms that include bacteria fungi, protists, invertebrates and vertebrates (Benbow et al., 2013). A pivotal study using replicate pigs documented two major findings in the microbial component of the necrobiome: (1) bacterial communities change during the decomposition process and (2) the sequence of the apparent changes could potentially be used to formulate time of death estimates in forensic investigations (Pechal et al., 2014). Furthermore, findings from the

Human Microbiome Project (HMP) demonstrated that although human bacterial community structure displays minimal variability during an adult lifespan, it differs considerably among individuals (Turnbaugh et al., 2007). These findings indicate that conceivably the individualized microbial community's fingerprint has applications in forensic criminal investigations beyond the other communities of the necrobiome (i.e., necrophagous insects and scavengers) that has been traditionally used during investigations.

Necrophagous insect taxa are the predominant eukaryotic promoters of vertebrate cadaver decomposition (Benbow et al., 2013; Matuszewski et al., 2010; Payne, 1965; VanLaerhoven, 2010). One limitation to these approaches is that postmortem observations do not change continuously (Lv et al., 2014). For example, blow fly larvae are useful until the advanced decay stage of decomposition, which can occur as soon as 10–14 days after death in warmer months (Payne, 1965). Also, once the fly larvae pupate, it is more difficult to estimate the PMI. Thus, animal consumption and entomological evidences are not as effective in long-term PMI determinations (Lv et al., 2014). A roadmap and framework to unify basic and applied research for understanding the ecological, evolutionary and genetic mechanisms occurring during cadaver decomposition has been proposed (Tomberlin et al., 2011) that addresses the potential role of microbes during the carrion decomposition process. Likewise, in this review, a discussion of how high throughput, next-generation metagenomic sequencing may become more widely used in forensics, by first providing a critical examination of a conceptual framework and then providing more details into the processes and considerations important for such applications. Then this will provide suggestions for future research to formulate tools using the individualized microbial community's fingerprint for forensic investigations.

2. Epinecrotic soils

Soil epinecrotic microbial communities, the microorganisms on and/or in decomposing heterotrophic biomass, has recently garnered much forensic research interest. Soils are extremely heterogeneous terrestrial ecosystems that contain highly complex composites of layers of both organic and inorganic molecules. These layers are made up of both living and the remnants of decomposing animals, plants, bacteria, fungi and other micro-organisms (Turbé et al., 2010). Edaphic microorganisms such as algae, bacteria and fungi form the majority of the soil biomass and are ubiquitous in soils. These microorganisms represent a large portion of the Earth's living biomass, with between 10^6 and 10^7 grams of microbial biomass per square meter of surface soils (Baldrian et al., 2012).

The study of soil microbial ecosystems is hampered not only by the heterogeneity of soil but also by the sheer number of microbial cells and diversity of distinct taxa per gram of soil. Studies have estimated that the number of species of bacteria per gram of soil varies between 2000 and 8.3 million cells depending on the soil type (Roesch et al., 2007). Approximately 80% of edaphic bacteria are located in the pores between soil particles, free or attached to particle surfaces such as the ultrathin water films surrounding soil particles (Stotzky, 1997; Ranjard and Richaume, 2001). Another immensely diverse group of edaphic decomposers are fungi. Fungi are one of the major decomposers in virtually all terrestrial environments and are implicated to be a large contributor to vertebrate decomposition on soil (Killham, 1994; Parkinson et al., 2009).

Several environmental factors define the microbial niche and how this niche influences the dominance of edaphic bacteria and fungi. The initial pH of the soil can have an effect on the predominant microbial decomposer and the rate of decomposition (Haslam and Tibbett, 2009; Killiam, 2004; Wilson-Taylor, 2012).

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