G64 Microbial Processes in Soils Associated With Skeletal Muscle Tissue and Cadaver Decomposition at Different Temperatures

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After attending this presentation, attendees will understand that the examination of soil microbiological and biochemical processes can provide insight into how temperature can affect the decomposition of cadavers and cadaver components in soil.

This presentation will impact the forensic community and/or humanity by demonstrating the soil microbial community has the potential to provide a basis for the estimation of postmortem and/or post burial interval.

Forensic taphonomy, and forensic science in general, has benefited greatly from the application of biological sciences. For example, the use of entomological principles and practices has led to an increased understanding of cadaver decomposition while providing an efficient and effective means to estimate postmortem and/or postburial interval. Considering that the decomposition of most resources in terrestrial ecosystems is due to the activity of soil microorganisms (often acting in conjunction with a variety of invertebrates) microbial processes in soils have been given little consideration. The microbiota are responsible for the regulation of nutrient transformation and storage in soils. As a result, all organic matter placed in the soil is acted upon (eventually) by soil microorganisms prior to being recycled into the wider ecosystem. The soil microbial biomass is a dynamic population, which can respond rapidly to environmental conditions (e.g., temperature, moisture) and the introduction of fresh substrates. These stimuli may result in a population adapted for specific circumstances.

There are a number of well established microbiological and biochemical methods for studying structural and functional characteristics of soil microbial communities. For example, substrate-induced respiration (SIR) and chloroform-fumigation incubation can be used to estimate the soil microbial biomass while fatty acid methyl ester and DNA analysis can be used to identify the taxa that make up the soil microbial community. Functional processes may be examined through measurements of CO_2 respiration, enzyme activity and nitrogen mineralization, among others. In this study we have applied a number of these methods (mass loss, microbial CO_2 respiration, SIR, enzyme assays) to determine whether temperature affects microbially mediated decomposition of skeletal muscle tissue (Ovis aries) and cadavers (Rattus rattus) in soils.

In experiment 1, skeletal muscle tissue (Ovis aries: 1.5 g) was incubated in soil microcosms at 2 °C, 12 °C and 22 °C in a sandy loam soil (100 g) from Dorset, England. Tissue mass loss was measured gravimetrically at seven day intervals over a period of 42 days. Microbial CO_2 respiration was measured every 24-48 hours using the alkali (0.3M NaOH) absorption method. Soil microbial biomass was estimated on day 21 using the SIR technique. Mass loss and SIR samples were collected using a destructive, sequential harvesting program.

In experiment 2, juvenile cadavers (Rattus rattus: ~20 g) were buried in a sandy loam soil (500 g) from tropical Queensland, Australia and incubated at 15 °C, 22 °C and 29 °C. Soil enzyme activities (arylsulphatase, dehydrogenase, phosphodiesterase, protease) were assayed on day 21 using standard soil enzymological techniques.

Experiment 1 demonstrated that each 10 °C increase resulted in an increase in the rate of tissue mass loss. These differences were maintained until day 42, when tissue incubated at 12 °C and 22 °C displayed similar levels of mass loss ($P = 0.266$). This may be due to the loss of readily available substrate (tissue) whereby the remaining tissue represents a more recalcitrant form of organic matter. The rapid utilisation of readily available nutrients was suggested by a flush of CO_2 following burial at 12 °C and 22 °C. Microbial CO_2 respiration gradually decreased following this flush. Test samples (soil with tissue) always demonstrated greater respiration rates than control samples (soil without tissue). Test samples incubated at 22 °C demonstrated greater levels of CO_2 respiration than samples incubated at 12 °C until day 23 ($P = 0.267$). Microbial CO_2 respiration at 2 °C was less than the other temperatures until day 42 ($P = 0.052$). A direct relationship was demonstrated between tissue mass loss and microbial CO_2 respiration (22 °C: $r = 0.690$***, 12 °C: $r = 0.810$***, 02 °C: $r = 0.836$***). Thus, the measurement of microbially respired CO_2 can provide a basis on which to accurately predict soft tissue decomposition in soil. This can be achieved by calculating the amount of CO_2-C respired. Microbial biomass estimations demonstrated no differences between test and control samples incubated at 2 °C ($P = 0.139$) or 12 °C ($P = 0.088$). Test samples incubated at 22 °C demonstrated a greater microbial biomass than control samples incubated at the same temperature ($P = 0.002$). This suggests

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that temperature is able to control growth of the soil microbial biomass even in the presence of a highly utilisable substrate.

In experiment 2 test samples always displayed greater protease and phosphodiesterase activity than control samples. Protease activity in test samples incubated at 15 °C were less than in test samples incubated at 22 °C (P = 0.013). Phosphodiesterase activity in test samples incubated at 15 °C was greater than in test samples incubated at 22 °C (P = 0.010). These results demonstrate that cadaver burial can bring about an increase in the activity of some enzymes commonly associated with soil microbial communities. The activity of these enzymes seems to be influenced by temperature. Arylsulphatase and dehydrogenase results did not respond to the presence of the cadaver.

The decomposition of the organic matter used in these experiments may be attributed to increased microbial biomass and/or enzyme activity. These decomposition processes can be greatly influenced by temperature. Soil microorganisms can play a rapid and substantial role in the decomposition of skeletal muscle tissue buried in soil. The decomposition of this relatively simple substrate can be accurately predicted through the estimation of C mineralization (CO_2-C) and assimilation. This method may also assist in the study of the decomposition of amorphous materials such as blood and hair. The decomposition processes examined in the current work were most likely carried out by a diverse community of microorganisms. One particular feature of the taxa that comprise soil microbial communities is that they commonly exhibit a succession whereby a change in the composition of the community takes place over time as a result of change in environmental conditions or substrate. We believe that continued research into soil microbial succession in grave soils may possibly provide a basis for the estimation of postmortem and/or postburial interval.

Forensic Taphonomy, Temperature, Soil Microbial Community

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