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TECHNICAL NOTE

Tamsin C. F. Haslam,[1] *M.Sc. and Mark Tibbett,*[2] *Ph.D.*

Soils of Contrasting pH Affect the Decomposition of Buried Mammalian (*Ovis aries*) Skeletal Muscle Tissue

ABSTRACT: Little is known about the effect of edaphic conditions on the decomposition of buried mammalian tissues. To address this, we set up a replicated incubation study with three fresh soils of contrasting pH: a Podsol (acidic), a Cambisol (neutral), and a Rendzina (alkaline), in which skeletal muscle tissue (SMT) of known mass was allowed to decompose. Our results clearly demonstrated that soil type had a considerable effect on the decomposition of SMT buried in soil. Differences in the rate of decomposition were up to three times greater in the Podsol compared with the Rendzina. The rate of microbial respiration was correlated to the rate of soft tissue loss, which suggests that the decomposition of SMT is dependent on the microbial community present in the soil. Decompositional by-products caused the pH of the immediate soil environment to change, becoming more alkaline at first, before acidifying. Our results demonstrate the need for greater consideration of soil type in future taphonomic studies.

KEYWORDS: forensic science, CO_2-carbon, soil respiration, soil microbial biomass, decomposition, taphonomy, biodegradation, soil microbiology

The burial environment is a complex and dynamic system of interdependent chemical, physical, and biological processes. These processes influence, and are influenced by, the inclusion of a body and its subsequent decay. Experimental studies of the decomposition of human cadavers under controlled conditions have rarely been carried out (1). Field studies, occasionally using human bodies, (2–5) but more commonly animal surrogates have been used (6–9). However, knowledge of the decomposition processes and the influence of the burial environment from such studies remain limited, particularly at the soil–cadaver interface.

Soil pH can have a major effect on the rate of decomposition in soils and the composition of the decomposer communities in soils (10–12). Typically, acidic soils (pH 3.0–5.5) are dominated by fungal communities, whereas neutral soils (pH 5.5–7.5) provide conditions in which bacteria have a competitive advantage. In the case of alkaline soils (pH 7.5–9.0) fungi may dominate again, especially in the conditions left by a cadaver postputrefaction (13–16).

Given the paucity of controlled experimental evidence that considers the effects of edaphic conditions on the decomposition of mammalian cadavers (or parts thereof), we conducted an experiment to test the null hypothesis that soil type would have no effect on decomposition of skeletal muscle tissue (SMT). This we achieved by setting up a laboratory incubation experiment with three contrasting soils into which SMT was buried. The rate of SMT decomposition in the soils was measured, along with microbial biomass and respiration and changes in soil pH during the experiment. By examining the breakdown of a relatively simple substrate (SMT) in soil, our approach represents an initial attempt at investigating the complex processes associated with soils and cadavers after burial. This is not an attempt to replicate the decomposition of a whole cadaver with its enteric flora and numerous components but rather an inquiry into how edaphic differences can affect the decomposition of an important cadaveric substrate.

[1]School of Conservation Sciences, Bournemouth University, Poole, Dorset BH12 5BB, United Kingdom.
[2]Centre for Land Rehabilitation, School of Earth and Environment (M087), University of Western Australia, Crawley, WA 6009, Australia.

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Materials and Methods

A laboratory incubation study (after 17) was designed using three different types of soil of contrasting pH. These were an acidic Podzol (pH 4.6), a neutral Cambisol (Brown Earth) (pH 6.4), and an alkaline Rendzina (pH 7.8). Soils were sampled from three separate locations in the County of Dorset, on the southern coast of England. These were Middlebere Heath (NGR SY95958395) for the Podzol, East Lulworth (NGR SY86208266) for the Cambisol, and Martin Down (NGR SU19800450) for the Rendzina. These soils were chosen due to their geographical proximity (within 20 miles of each other) while having greatly contrasting levels of acidity. All the samples were collected from the *A* horizons of the soil profile. The soils were passed through a 5.6-mm sieve, following the method of Horwarth and Paul (18). This mesh size was used as Jenkinson and Powlson (19) and Ross et al. (20) have shown that sieving between 4 and 6 mm has little effect on microbial biomass size or activity. Organic lamb (*Ovis aries)* was used as mammalian SMT.

An experiment was set up in which 100 g (dry weight) each of the three soils was calibrated to 60% water holding capacity (WHC) with distilled water inside sealable polyethylene bottles (1285 mL; Merck Ltd, Nottingham, U.K., product no. 215044808) and incubated with and without SMT for 42 days at 22°C. Sufficient tubs were set up to allow for six sequential (destructive) measurements to be made of SMT mass loss and soil pH, one every 7 days. Two hundred and sixteen experimental incubation containers were set up as described previously (17). For each of the three soils, 72 containers were employed and into half of these (36, selected at random) SMT (1.5 g) was buried to a depth of 2 cm

and the tubs sealed. The remaining 36 containers were left as soil-only controls. All soils were adjusted and maintained at 50% of their respective WHC and the experiment was replicated six times throughout. The air in the headspaces of all incubation containers and the CO_2 traps were changed every 48 h using a vacuum hose to remove waste gases and replenish O_2 supplies in the containers.

Twelve containers (six with SMT) were randomly selected to be harvested at 42 days and these had CO_2 respiration monitored by the use of alkaline traps (17). Carbon dioxide traps were placed in six tubs of each soil type containing soft tissue and six soil-only control containers. These were replaced every 24 h and CO_2 respiration estimate through back-titration to neutrality. After 22 days for the Cambisol and 24 days for the Podzol and Rendzina, the CO_2 traps were replaced every 48 h as microbial respiration had decreased to a level that made replacement every 24 h unnecessary.

Weekly destructive measurements were made from 12 randomly selected incubation containers, six with SMT and six soil-only controls for each soil type. For each container with SMT, the buried tissue was carefully removed with forceps and then rinsed (to remove any soil particles), dried, and weighed to establish mass loss.

To determine microbial biomass through substrate-induced respiration a glucose solution was added to the incubation containers that was equivalent to 4 mg g^{-1} soil and with a volume of water calculated to raise the WHC of the soil to 95% (21). The glucose solution was evenly distributed across the surface of the soil with a pipette. Carbon dioxide traps were suspended in the tubs, which were then resealed. After 2.5 h the traps were removed and the alkaline solution was back-titrated to neutrality and the results were used to determine the microbial biomass in each soil.

Statistical Analysis

Data were analyzed using conventional univariate techniques with proprietary statistical package SPSS (version 10, SPSS UK, Woking, Surrey, U.K.). All data were tested for normality of distribution (Shapiro–Wilk test for normality) prior to analysis of variance or the calculation of Pearson's correlation coefficients.

Results

Soil Type and Mass Loss

In all soils substantial SMT mass was lost within the first 7 days of the incubation, (39% in the Cambisol, 62% in the Rendzina, and 55% in the Podzol), after which the rate of mass loss declined (Fig. 1). After 21 days of incubation the majority of SMT decomposition had occurred, and the remaining mass of SMT was 10% in the Cambisol, 27% in the Rendzina, and 8% in the Podzol. At the end of the 6-week period there was little or no SMT left in any of the soils (Fig. 1).

The rate of SMT mass loss varied for each soil through time. There was a significant difference in the rate of SMT decomposition between all three soils for all destructive sample periods except day 35 ($p < 0.05$). A further test comparing the three soils showed that the mean values for SMT mass loss over the 6-week incubation were also significantly different for each soil type ($p < 0.05$). Percentage mass loss of SMT and microbial respiration (after 6 weeks of burial) had a very strong positive correlation (22) for all three soils. This relationship is equivalent to the aerobic catabolic efficiency as calculated by Carter and Tibbett (23). The Pearson's correlates were: Cambisol, $r = 0.988$; Rendzina, $r = 0.982$; and Podzol, $r = 0.837$.

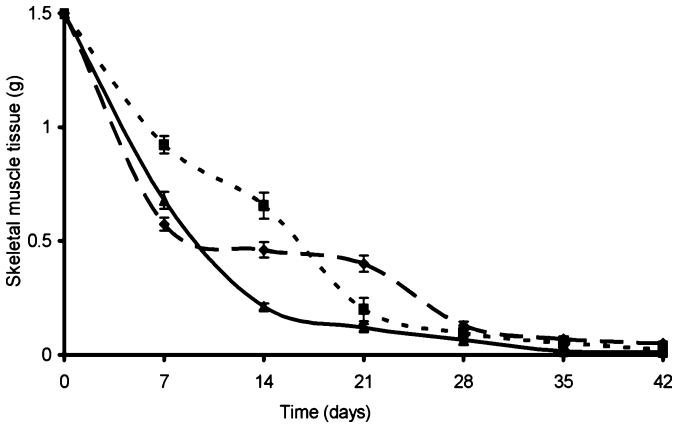

FIG. 1—*Decomposition of skeletal muscle tissue (1.5 g) as measured by mass loss for three soils: acidic Podsol (solid line with triangles), neutral Cambisol (Brown Earth) (dotted line with squares), and alkaline Rendzina (dashed line with diamonds). Bars equal ± SE,* n = *6.*

Soil Type and Microbial Respiration Rates

Basal respiration of the unamended control soils remained reasonably stable throughout the incubation. Mean basal respiration decreased in order Rendzina (3.4 μg/g soil/h) > Podsol (2.4 μg/g soil/h^{-1}) > Cambisol (1.5 μg/g soil/h) (Fig. 2).

The general response curve for CO_2 respiration in the presence of SMT was similar in all soils with a large increase in the first 2–4 days leading to a maximum rate of respiration that varied only slightly between the three soils: Rendzina (17.93 μg/g soil/h), Podzol (17.87 μg/g soil/h), and Cambisol (17.34 μg/g soil/h). After these early peaks, the rate of CO_2 evolution decreased overall but with different patterns of decline for each soil (Fig. 2).

Soil Type and pH

All soils amended with SMT became more alkaline after the first week of incubation and reached similar maximum values of pH 8.2–8.5 (Fig. 3). The more acidic the native soil the greater the change in pH through the course of the incubation. Hence, the Rendzina had the narrowest range, pH 7.1–8.2; followed by the Cambisol pH 5.1–8.5; with the greatest variation shown by the Podzol, which rose from pH 4.6 to 8.2. Changes in soil pH exhibited a sigmoidal characteristic in all soils which returned to pH levels approaching their starting values by the end of the incubation.

Microbial Biomass and Soil Type

Microbial biomass as measured by substrate-induced respiration showed large differences between the three soils. Microbial biomass increased in the order Cambisol < Podsol < Rendzina. In the early weeks of the incubation the introduction of SMT increased the size of the microbial population in the Rendzina (Fig. 4), decreased the size of the microbial population in the Podsol, and had little effect on the Cambisol. In the latter weeks of the incubation the microbial biomass was less in the soils in which SMT had been buried.

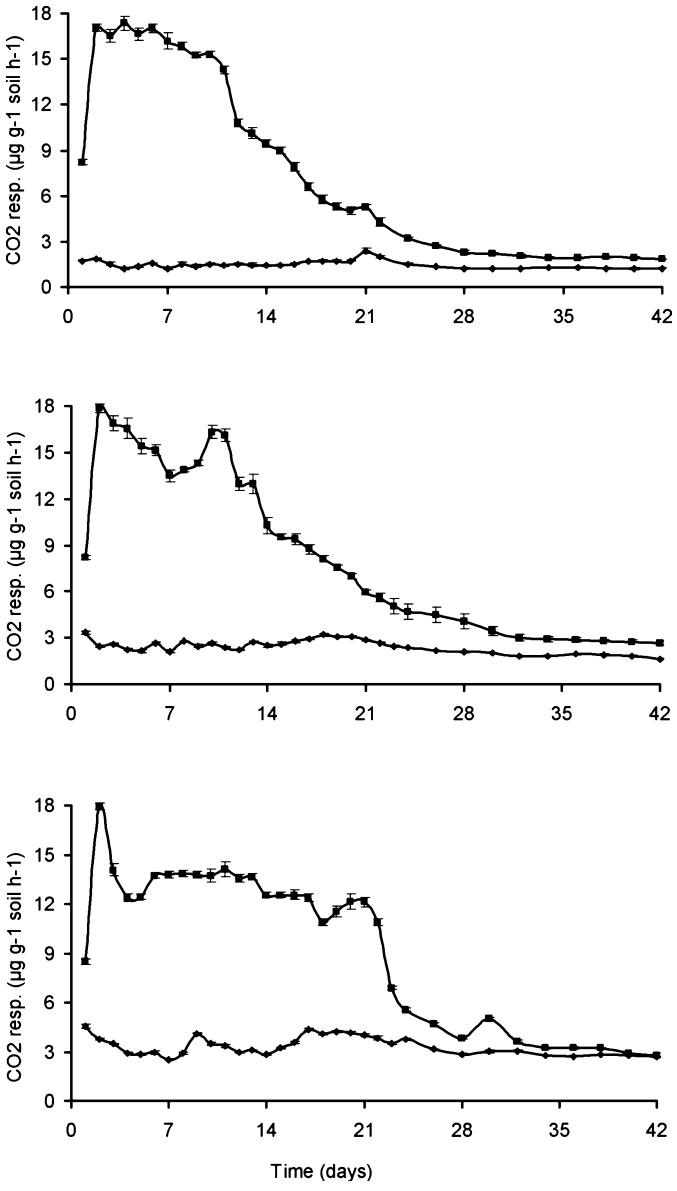

FIG. 2—*Carbon dioxide respiration over 42 days for three soils: Cambisol (Brown Earth) (top), Podsol (middle), Rendzina (bottom). Lower lines with diamonds represent basal respiration and higher lines with squares represent respiration after the burial of skeletal muscle tissue. Bars equal ± SE,* n = *6.*

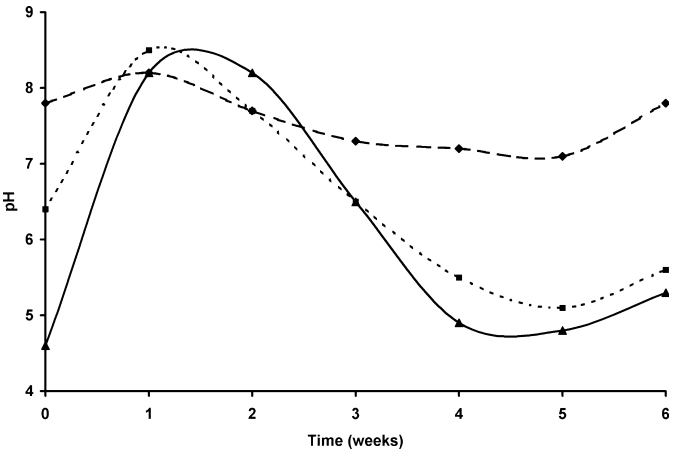

FIG. 3—*pH change after the introduction of skeletal muscle tissue (1.5 g) to soil microcosms containing acidic Podsol (solid line with triangles), neutral Cambisol (Brown Earth) (dotted line with squares), and alkaline Rendzina (dashed line with diamonds).* n = *6.*

Discussion

Soil type had a considerable effect on the decomposition of SMT buried in soil. Differences in the rate of decomposition (SMT mass-loss) were over three times greater in the Podsol compared with the Rendzina after 21 days. This result has major implications for forensic taphonomy as little consideration has been given to the type of soil that a buried cadaver (or part thereof) is found in, and this question must now be pursued with vigor. Although important, the findings of this study do not simulate the burial of a whole cadaver but are more in keeping with the likely decomposition dynamics of decapitated peripheral parts of body (in the size range of limbs to digits). Nonetheless, for a given point in time (time since burial), and when all other factors remain equal, the decomposition of SMT can be strongly affected by soil type.

The initial apparent high rate of decomposition of SMT may be through the loss of moisture from the freshly cut material. Desiccation may play a major role in the mass loss of cadavers (14) and this is in itself not biodegradation but does account for significant loss of mass in contrived experimental, and natural, settings.

While SMT will contain its own microbial population and internal autolytic enzymes, rapid colonization of the SMT is likely to occur from the heterotrophic microbial population in each soil (23,24). Our data suggest that the microbial biomass in each soil responded differently to the introduction of the SMT, which is effectively a package of moisture and nutrients (14,24,25). The initial microbial composition of a soil is known to be pH dependent and the SIR response indicated that the composition of the microbial population differed between the soils. The variable responses of the microbial biomass in the soil may be as a result of the differences in the efficiency of the microbial degradation of SMT in each soil and the exhaustion of the SMT substrate seems to have resulted in a smaller microbial biomass or at least one not adapted to a rapid glucose response. It is also possible that the release of acetic acids, oxalic acid, and phenolic compounds from the later stages of decomposition may have inhibited microbial growth in the later stages of the experiment in the amended soil (26,27). The small changes in the microbial biomass for the Cambisol are in keeping with a previous experiment conducted in this soil type (23). The general patterns of microbial respiration are similar to those found in similar studies with SMT (17,23,28). Where whole cadavers have been used, the initial respiratory response seems to be slower to peak than for SMT alone (29). As decomposition begins, autolysis causes cellular enzymes, such as lipases, amylases, and proteases, to degrade the cell walls of the substrate. This process may accelerate subsequent decomposition by the soil biomass, contributing to the initial respiration flush.

Previous studies have noted an increase in pH around buried SMT and carcasses (5,9,23,29,30). This initial rise in pH may have been caused by the ammonification of proteins and other organic nitrogen sources from the flesh substrate (23,28,31,32). In addition the SMT, which contains a high proportion of myofibril proteins, would have provided a localized surplus of amino-nitrogen for ammonification. A number of base-forming cations, such as potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) are present as essential elements of cellular composition, and the mineralization of these cationic species may also have contributed to the rise in pH. This was greatest for the Podsol and smallest for the Rendzina.

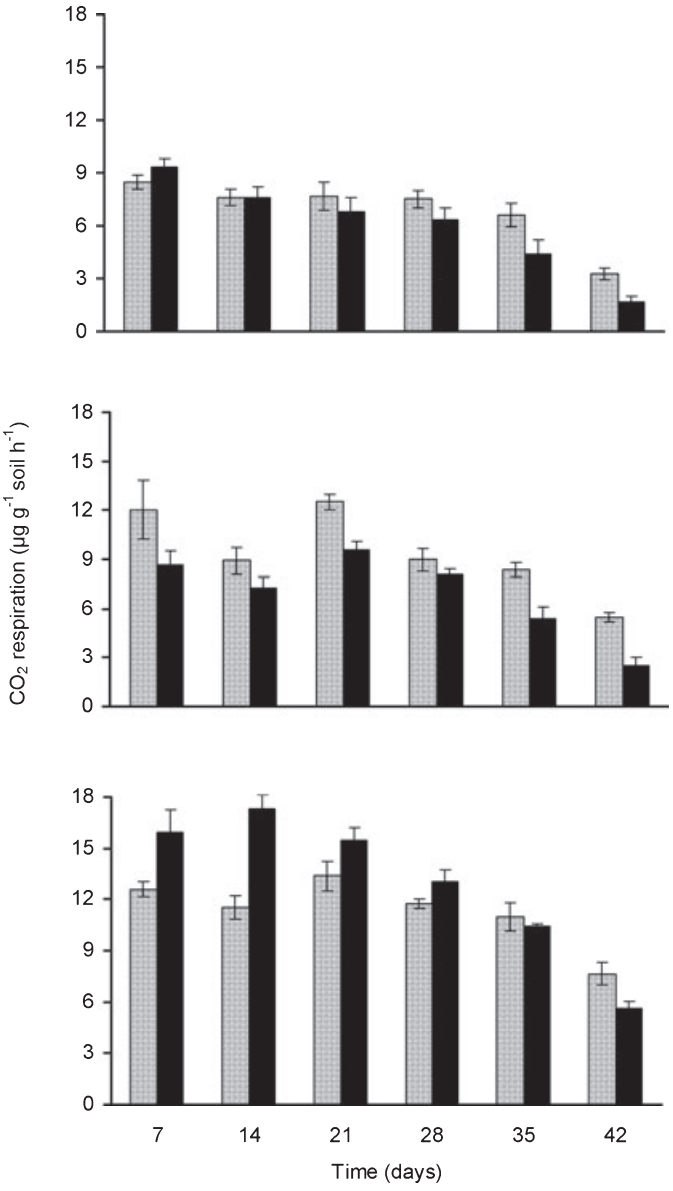

FIG. 4—*Soil microbial biomass estimated by substrate-induced respiration at weekly intervals over 42 days for three soils: Cambisol (Brown Earth) (top), Podsol (middle), Rendzina (bottom). Gray columns represent control soils and black columns represent soil in which skeletal muscle tissue (1.5 g) has been buried. Bars equal ± SE,* n = *6.*

The differences in pH change between the soils may be due to differences in the buffering capacity of the soils, that is, the ability of the soil to resist changes in pH. Generally the higher the cation exchange capacity (CEC) of the soil, the greater its buffering capacity. Although the CEC of the soils was not determined, it is generally understood that a Rendzina would have a higher CEC and a Podsol a lower CEC (33,34).

There may be several reasons for the fall in pH in all the amended soils, to a level below that of the control soils. Ammonification is the first step in the mineralization of organic nitrogen and is followed by the nitrification of ammonia by enzymatic oxidation to nitrate, which is often termed an acidifying reaction (35). Nitrification can be retarded in acidic mineral soils which lack an abundance of exchangeable base-forming cations. This would help to explain why the pH of the amended Rendzina fell below the starting pH almost 2 weeks before the Podzol but does not explain why the pH of the amended Cambisol only fell below that of the control after 4 weeks. Vass et al. (26) suggested that continual degradation of soft tissue produces nonmineralized decompositional by-products, such as acetic acid, phenolic compounds, and fatty acids, which are deposited into the soil solution. These organic compounds effectively reduce pH and may have inhibited subsequent decomposition and microbial activity. Vass et al. (27) found that oxalic acid was also a by-product of muscle tissue decomposition and its deposition would also have lowered the pH values of the soils.

As the soft tissue was attacked by the soil biomass, decompositional by-products caused the pH of the immediate soil environment to change. All the soils became more alkaline at first, before acidifying. The degree of change in pH, which was shown to be different for each soil, is dependent on the buffering capacity and the CEC of the soil. These parameters are controlled, to some extent, by the initial pH of the soil. The changes in the rate of soft tissue mass loss and microbial respiration, demonstrated the ability of the soil biomass to adapt to changes in pH. This was likely to have been achieved by two processes. The most probable was a functional change, in which different sections of the microbial community, able to tolerate a higher pH, thrived at the expense of others. It is also possible that efficiency changes, in which the community adapted, to attack the available substrate, were involved in the decomposition of the soft tissue.

The implementation of soil-based analyses can only serve to improve our knowledge of burial environments and human decomposition rates. Assessment of the burial environment at a chemical and microbial level can have enormous benefits in identifying decompositional variables, associated with soil dynamics and function (e.g., 13,26,36). This is essential, if biodegradation phenomena are to be more fully understood beyond the effect of scavengers and insects. New research methodologies involving applications of established techniques in the soil-based sciences can, and are, being developed for further taphonomic research (37). In the future, it is hoped, the results and improved techniques learned from such studies will be incorporated, in a forensic setting, to more readily locate and date graves and aid in the individuation of remains.

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Additional information and reprint requests:
Mark Tibbett, Ph.D.
Centre for Land Rehabilitation, School of Earth and Environment
University of Western Australia
WA 6009, Australia
E-mail: Mark.Tibbett@uwa.edu.au