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Abstract

The repeated introduction of an organic resource to soil can result in its enhanced degradation. This phenomenon is of primary importance in agroecosystems, where the dynamics of repeated nutrient, pesticide, and herbicide amendment must be understood to achieve optimal yield. Although not yet investigated, the repeated introduction of cadaveric material is an important area of research in forensic science and cemetery planning. It is not currently understood what effects the repeated burial of cadaveric material has on cadaver decomposition or soil processes such as carbon mineralization. To address this gap in knowledge, we conducted a laboratory experiment using ovine (*Ovis aries*) skeletal muscle tissue (striated muscle used for locomotion) and three contrasting soils (brown earth, rendzina, podsol) from Great Britain. This experiment comprised two stages. In Stage I skeletal muscle tissue (150 g as 1.5 g cubes) was buried in sieved (4.6 mm) soil (10 kg dry weight) calibrated to 60% water holding capacity and allowed to decompose in the dark for 70 days at 22 °C. Control samples comprised soil without skeletal muscle tissue. In Stage II, soils were weighed (100 g dry weight at 60% WHC) into 1285 ml incubation microcosms. Half of the soils were designated for a second tissue amendment, which comprised the burial (2.5 cm) of 1.5 g cube of skeletal muscle tissue. The remaining half of the samples did not receive tissue. Thus, four treatments were used in each soil, reflecting all possible combinations of tissue burial (+) and control (−). Subsequent measures of tissue mass loss, carbon dioxide-carbon evolution, soil microbial biomass carbon, metabolic quotient and soil pH show that repeated burial of skeletal muscle tissue was associated with a significantly greater rate of decomposition in all soils. However, soil microbial biomass following repeated burial was either not significantly different (brown earth, podsol) or significantly less (rendzina) than new gravesoil. Based on these results, we conclude that enhanced decomposition of skeletal muscle tissue was most likely due to the proliferation of zymogenous soil microbes able to better use cadaveric material re-introduced to the soil.

Keywords: carbon dioxide, cemetery, forensic taphonomy, metabolic quotient, gravesoil, pH

1. Introduction

Cadaveric materials are regularly introduced to soil through natural causes such as starvation, disease, and predation (Carter et al., 2007). In addition, cadaveric materials are interred in soil to conceal evidence of fatal crime. As a consequence, the ecology of soils associated with cadaveric decomposition (gravesoils) has been applied to the investigation of terrestrial death scenes (Vass et al., 1992; Hopkins et al., 2000; Carter and Tibbett, 2003). Although the study of gravesoils has contributed to the development of valuable forensic tools to locate (Rodriguez and Bass, 1985; Carter and

Tibbett, 2003) and date (Vass et al., 1992; Carter and Tibbett, 2003; Tibbett and Carter, 2008) clandestine graves, the relationship between soil, the soil microbial biomass, and cadaveric decomposition remains poorly understood.

One particular area of gravesoil ecology that is poorly understood is the decomposition of cadaveric material following burial in pre-existing gravesoil (repeated burial: i.e. the interment of cadaveric material into soil that has been previously exposed to cadaveric material). It has long been hypothesized that repeated burial will increase the rate of cadaveric decomposition (Motter, 1898), possibly through an increase in soil microbial biomass (Janaway, 1996). Other studies have provided some support to this hypothesis by reporting that decomposition, mineralization and the soil microbial biomass can be influenced by the repeated introduction of an organic resource into the soil (Jayachandran et al., 1998). For example, the repeated application of the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) resulted in enhanced breakdown and an increase in the population of atrazine degraders in soil (Jayachandran et al., 1998). These findings are important because it contributes to the understanding of the mean residence time of atrazine and the presence of atrazine degradation by-products, which are then used to improve soil use and management. Similarly, an understanding of the repeated burial of cadaveric material will lead to enhanced methods for use in forensic science and cemetery planning. Cemetery planners would benefit from understanding repeated burial by more accurately mandating an appropriate interment time and by making more informed decisions towards the designation of soil type for cemetery use. In a forensic context, the repeated introduction of cadaveric material to soil might compromise the accuracy of soil-based estimations of postmortem interval (PMI) (Vass et al., 1992; Carter and Tibbett, 2003) and indicators of clandestine graves such as ninhydrin-reactive nitrogen (Carter et al., 2008a) and soil pH (Rodriguez and Bass, 1985; Vass et al., 1992). The implications are clear for scenarios such as cemeteries or for the disposition sites of serial killers where repeated burial in the same grave may occur.

The current study aimed to investigate the effect of repeated burial on the decomposition of skeletal muscle tissue. We tested the hypothesis that repeated burial of skeletal muscle tissue in the same soil would lead to more rapid decomposition. This was achieved using established laboratory based incubations (Tibbett et al., 2004) where skeletal muscle tissue mass loss, soil carbon dioxide–carbon (CO_2–C) respiration, microbial biomass C (MBC), metabolic quotient (qCO_2) and soil pH were measured. This work does not intend to represent the multifaceted processes associated with the decomposition of a complete cadaver. Rather, it is an initial attempt at understanding the ecology of soils associated with cadaver decomposition or other high quality ephemeral resource patches.

2. Materials and methods

2.1. Skeletal muscle tissue

Organically reared Texel × Suffolk lamb (*Ovis aries*) skeletal muscle tissue was used as the organic resource. Tissue was refrigerated (4 °C) until 1 h prior to burial, when it was kept at room temperature and prepared into 1.5 g cubes using sterile scissors and forceps.

2.2. Soil

Three contrasting soil types from Great Britain were used. These included a Brown Earth collected from Lindens Farm, East Lulworth, Dorset; a rendzina collected (0–10 cm depth) from Martin Down, Wiltshire; and a podzol collected from Hartland Moor, Dorset. The pH of these soils were 5.5 (±0.2), 7.5 (±0.1), and 4.7 (±0.1), respectively.

2.3. Experimental design

2.3.1. Stage I – initial tissue decomposition

Soil (10 kg dry weight) was sieved (4.6 mm) field fresh, placed in four polypropylene mesocosms (5 l) and calibrated to 60% water holding capacity (WHC). Soil in two of the mesocosms was amended with 150 g skeletal muscle tissue in the form of 1.5 g cubes. The rate of soil to muscle tissue was based on evidence from previous methodological work (Tibbett et al., 2004). Soil and tissue were manually mixed to randomly distribute the tissue throughout the soil. The two remaining mesocosms were used as controls and were not amended with tissue. All soils were incubated in the dark at 22 °C for 70 days. Soils were amended with distilled water every 3–4 days to maintain 60% WHC.

2.3.2. Stage II – repeated burial

Following Stage I, remaining tissue was removed from the soil. Soils were then placed (100 g dry weight at 60% WHC) in incubation microcosms (1285 ml, Merck Ltd., United Kingdom, product no. 215044808). Half of the soils were designated for a second tissue amendment, which comprised the burial (2.5 cm) of 1.5 g skeletal muscle tissue in cuboid form, as conducted in Stage I. The remaining half of the samples did not receive tissue resulting in a completely randomized 3 × 4 factorial with five replications. There were three soil treatments (Lindens Farm, Martin Down, Hartland Moor) and four tissue treatments. The tissue treatment included −/− (control soil), +/− (tissue added in Stage I only), −/+ (tissue added in Stage II only) and +/+ (tissue added in both Stages I and II). All treatments were incubated in the dark at 22 °C for 42 days. Accumulated degree days (ADDs) were calculated using 0 °C as the minimum developmental threshold (Vass et al., 1992).

All experimental measurements were conducted during Stage II. Tissue and soil samples were collected at intervals of 7 days using the sequential harvesting regime described by Tibbett et al. (2004). Carbon dioxide–carbon (CO_2–C) measurements were conducted using CO_2–C traps containing sodium hydroxide, which were collected at intervals of 24 h. Soil microbial biomass carbon (MBC: via substrate induced respiration), soil pH (5:1 water:soil) and metabolic quotient (qCO_2: μg CO_2–C mg^{-1} MBC g^{-1} soil h^{-1}) were measured as described in detail by Carter and Tibbett (2006). The current experiment was replicated 5 times resulting in a total of 420 microcosms.

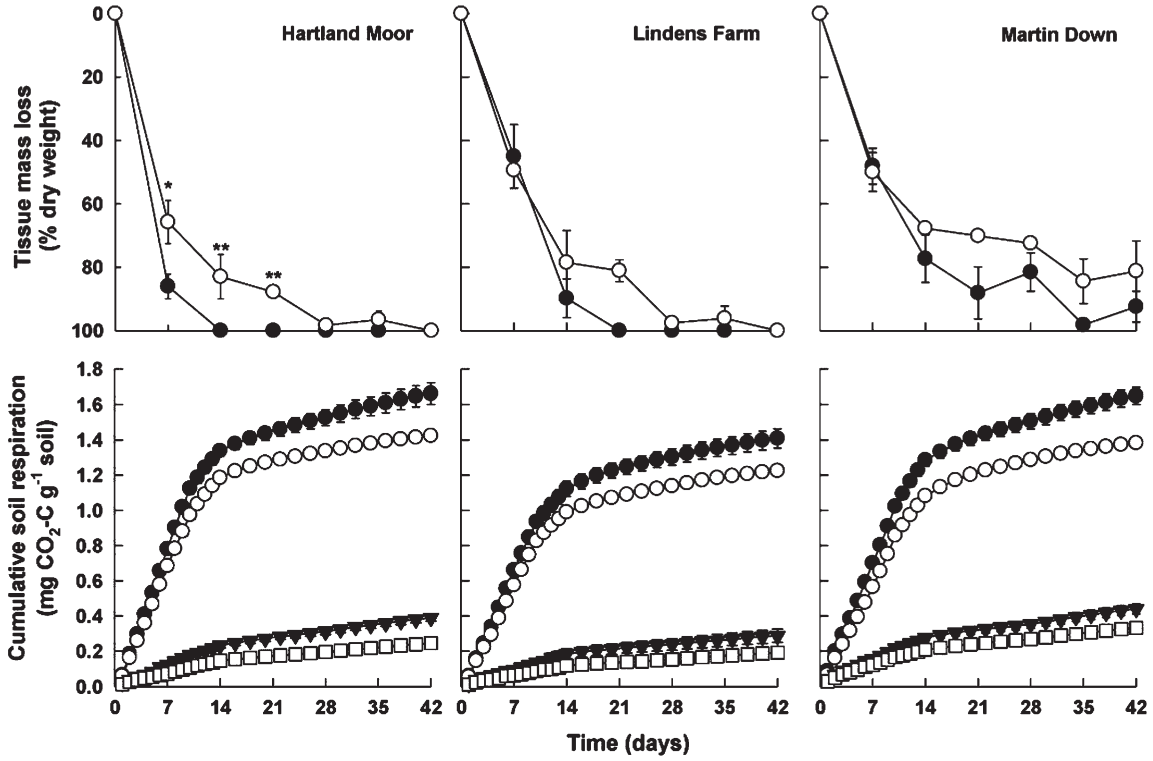


Figure 1. Mass loss (% dry weight) and cumulative soil respiration (mg CO_2–C g^{-1} soil) following burial (2.5 cm) of skeletal muscle tissue (*Ovis aries*: 1.5 g) in soil collected from Hartland Moor, Lindens Farm, or Martin Down, United Kingdom, sieved (4.6 mm), calibrated to 60% water holding capacity, and incubated in the dark at 22 °C. Data reflect measurements taken during Stage II, which followed a 70-day incubation period (Stage I). Four treatments are presented: soil that was not amended with tissue (−/−: □), soil that was amended with tissue during Stage I only (+/: −▼), soil that was amended with tissue during Stage II only (−/+: ○), and soil that was amended with tissue during both Stage I and II (+/+: ●). Bars represent standard errors where $n = 5$. Asterisks represent significant differences where * $P < 0.05$ and ** $P < 0.01$.

2.4. Statistical analyses

Descriptive and inferential statistics were generated using Microsoft Excel 2000 and SPSS 11.0.1 (Chicago, USA). Skeletal muscle tissue mass loss, MBC, qCO_2 and soil pH data were analyzed using a univariate analysis of variance. Carbon dioxide–carbon evolution data was analyzed using a repeated measures ANOVA following rank transformation.

3. Results

3.1. Mass loss

Repeated burial resulted in greater mass loss in all soils (Figure 1). However, this effect was not observed after day 21. Tissue decomposition also differed between soil type, as decomposition in Hartland Moor and Lindens Farm soil was greater than in Martin Down soil.

3.2. Soil respiration

Tissue burial during Stage II (−/+, +/+) resulted in significantly greater CO_2–C evolution relative to other tissue treatments, regardless of soil type (Figure 1). Furthermore, repeated burial resulted in the greatest rates of CO_2–C evolution in all soils. The evolution of CO_2–C following repeated burial (+/+) was greater ($P < 0.05$) than following −/+ from day 8 (Hartland Moor), day 6 (Lindens Farm), or day 3 (Martin Down). CO_2–C evolution following +/− was greater ($P < 0.05$) than in control soils from day 10 (Hartland Moor) or day 18 (Lindens Farm, Martin Down).

3.3. Microbial biomass carbon

In all soils, peak levels of MBC were observed on day 14, which was followed by a decrease on day 21 (Figure 2). The burial of tissue in soil during Stage II (+/+, −/+) resulted in a significant ($P < 0.05$) increase in MBC. A significant difference between +/+ and −/+ was only observed in Martin Down on day 21 (+/+ < −/+) and 28 (−/+ < +/+). In contrast, tissue burial in Stage II had no effect on MBC in Hartland Moor or Lindens Farm.

3.4. Metabolic quotient (qCO_2)

The burial of tissue during Stage II (+/+, −/+) resulted in a significantly ($P < 0.05$) greater qCO_2 in all soils (Figure 2). This effect was greatest on day 7 and persisted until day 28 (Hartland Moor, Martin Down) or day 35 (Lindens Farm). A difference between +/+ and −/+ was observed during the initial 14 days of incubation in Lindens soil. However, this effect was only observed on day 35 and day 42 in Hartland Moor soil. No consistent effect of repeated burial on qCO_2 was detected in Martin Down soil, as significant differences between +/+ and +/− were observed on days 7, 21, 35, and

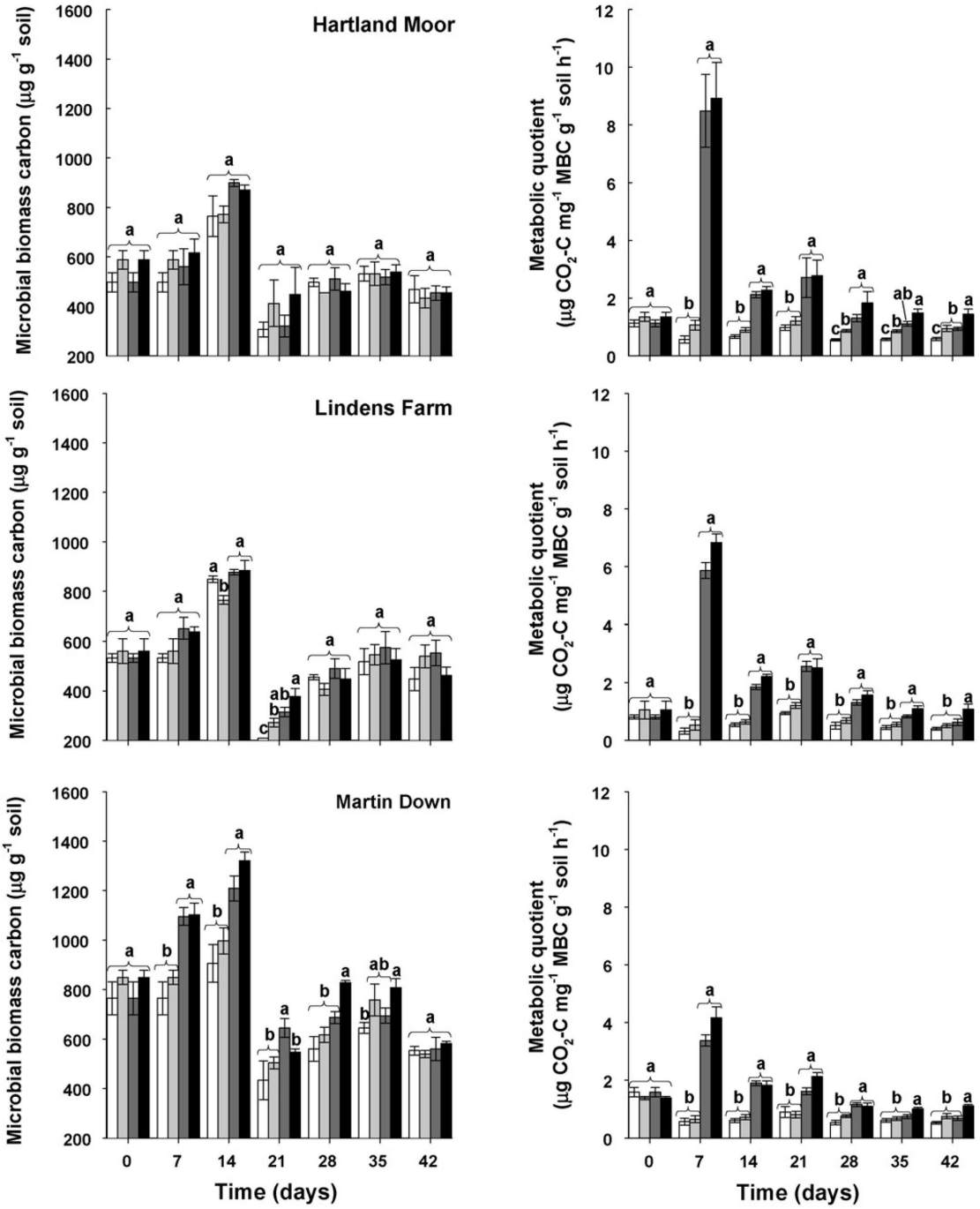
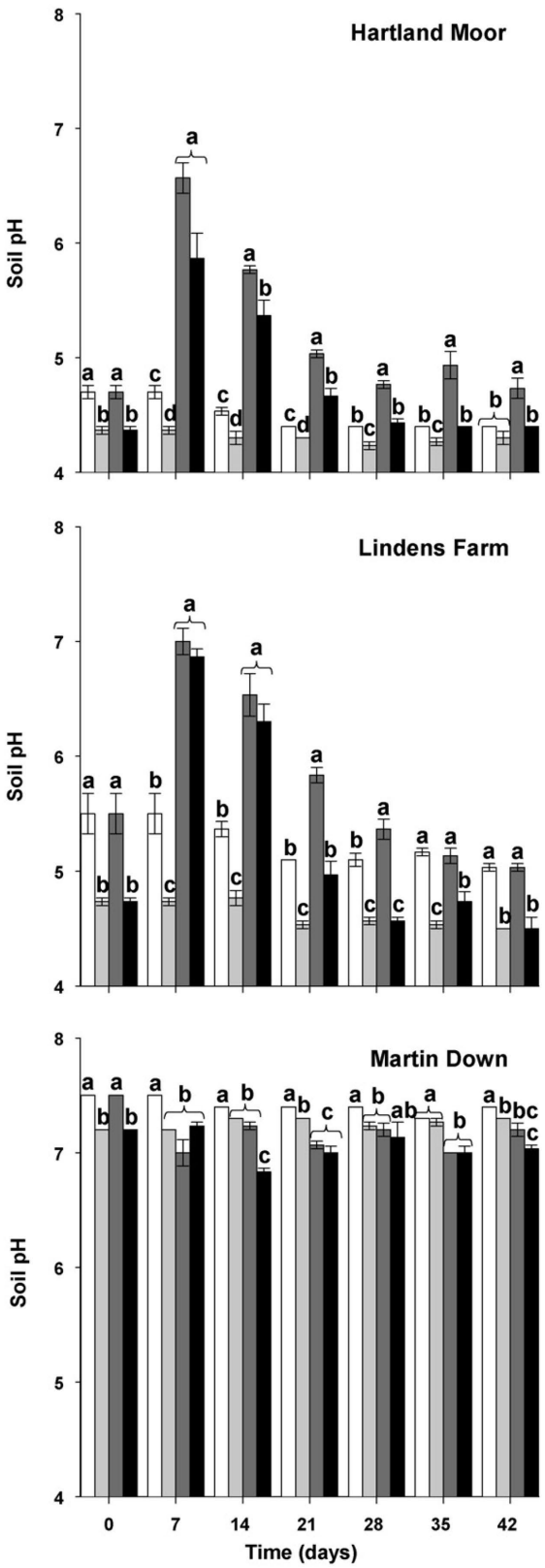


Figure 2. Microbial biomass carbon (µg g^{-1} soil) and metabolic quotient (qCO_2: µg CO_2–C mg^{-1} microbial biomass C g^{-1} h^{-1}) following burial (2.5 cm) of skeletal muscle tissue (*O. aries*: 1.5 g) in soil collected from Hartland Moor, Lindens Farm, or Martin Down, United Kingdom, sieved (4.6 mm), calibrated to 60% water holding capacity, and incubated in the dark at 22 °C. Data reflect measurements taken during Stage II, which followed a 70-day incubation period (Stage I). Four treatments are presented: soil that was not amended with tissue (−/−: □), soil that was amended with tissue during Stage I only (+/−: ■), soil that was amended with tissue during Stage II only (−/+: ■), and soil that was amended with tissue during both Stage I and II (+/+: ■). Bars represent standard errors where $n = 5$. Letters represent significant ($P < 0.05$) differences between treatments within time.

42. A significant ($P < 0.05$) difference in qCO_2 between +/− and −/− was rare. This phenomenon was only observed in Hartland Moor soil on days 28 and 35. Differences between soils were also uncommon: the qCO_2 of +/+ and −/+ in Hartland Moor on day 7 was greater than in Lindens, which was greater than in Martin Down.

3.5. Soil pH

The burial of tissue in Hartland Moor and Lindens Farm during Stage II (+/+, −/+) resulted in a significant ($P < 0.05$) increase in soil pH, which was followed by a gradual decrease toward basal pH levels (Hartland Moor: pH 4.7; Lindens Farm: pH 5.5) (Figure 3). In contrast, the pH of Martin



Down following tissue burial during Stage II (+/+, −/+) was consistently less than in control soil. Soil pH following repeated burial was less than following −/+ in all soils. This effect was observed from day 14 or from day 21 until the end of the incubation in Hartland Moor soil and Lindens Farm soil, respectively. This effect was also observed in Martin Down, but only on day 14. Soil pH also differed between soil types. The pH of Martin Down was greater than Hartland Moor and Lindens Farm, regardless of issue treatment.

4. Discussion

The repeated burial of skeletal muscle tissue led to more rapid decomposition as measured by tissue mass loss and CO_2–C evolution. This is most likely due to a change in the function of the soil microbial community to the availability of skeletal muscle tissue. This adaptation enhanced the decomposition of skeletal muscle tissue in simulated gravesoil relative to control soil and was likely due to a greater population of zymogenous microorganisms following repeated burial. Thus, we accept our hypothesis, and the hypothesis of Motter (1898), that repeated burial will result in enhanced decomposition of cadaveric material. Enhanced decomposition following repeated application of an organic resource is not a new finding. This phenomenon has been repeatedly observed in association following repeated application of atrazine (Abdelhafid et al., 2000). The current results might reflect the concept that "resource selects community" (Beijerinck, 1913) while providing further evidence that cadaveric decomposition following burial can be driven, in part, by the soil microbial biomass (Fiedler et al., 2004; Carter and Tibbett, 2006).

It seems likely that soil type can significantly enhance or restrict the decomposition of cadaveric material (Fiedler and Graw, 2003; Forbes et al., 2005) and it is clear from our study that the decomposition rates of skeletal muscle tissue vary according to soil type. Furthermore, the differences in mass loss, MBC, CO_2–C, and soil pH between soils show that biological and chemical properties do not necessarily converge following repeated amendment of muscle tissue. A similar finding has been observed following repeated diesel contamination (Bundy et al., 2002). These observations support a view that contrasting soils and soil microbial populations have differing intrinsic abilities of decomposing cadaveric resources.

One parameter that was consistent across all three soils was a large increase in MBC and qCO_2 after the burial of tissue in Stage II of the experiment, particularly during the initial 14 days of incubation. It is possible that this represents a large decrease in efficiency in the indigenous population of muscle tissue degraders and a period of functional change

Figure 3. Soil pH following burial (2.5 cm) of skeletal muscle tissue (*O. aries*: 1.5 g) in soil collected from Hartland Moor, Lindens Farm, or Martin Down, United Kingdom, sieved (4.6 mm), calibrated to 60% water holding capacity, and incubated in the dark at 22 °C. Data reflect measurements taken during Stage II, which followed a 70-day incubation period (Stage I). Four treatments are presented: soil that was not amended with tissue (−/−: □), soil that was amended with tissue during Stage I only (+/−: ■), soil that was amended with tissue during Stage II only (−/+: ■), and soil that was amended with tissue during both Stage I and II (+/+: ■). Bars represent standard error of 5 laboratory replicates. Letters represent significant ($P < 0.05$) differences between treatments within time.

resulting from repeated burial. This is reflected by the significant decrease in MBC between days 14 and 21, which might indicate a significant decrease in the zymogenous microbial community resulting from the depletion of readily available skeletal muscle tissue. Alternatively, it may reflect other abiotic fluxes in the soil during decomposition that can alter apparent metabolic quotient and perhaps suggest it is not a useful parameter in this study (see Wardle and Ghani, 1995). It seems unlikely that the soils previously exposed to muscle tissue are greatly inefficient at its degradation.

One interesting, albeit incidental, finding was the decrease in soil pH over time. The present work represents one of the few cadaver decomposition studies that has reported a decrease in pH during the decomposition of cadaver components (Vass et al., 1992; Towne, 2000; Danell et al., 2002; Tibbett, 2008). This is probably because these are the only studies to monitor the pH of gravesoil over an extended period. The breakdown of skeletal muscle tissue, like complete cadavers (Vass et al., 1992; Hopkins et al., 2000; Carter et al., 2008b), results in an initial increase in pH (Carter and Tibbett, 2006), which is likely due to an increase in the concentration of ammonium (Hopkins et al., 2000). The decrease of soil pH to below basal levels might be due to a decrease in the concentration of ammonium and an increase in the concentration of nitrate. This might offer a further explanation for the ecological succession of the postputrefaction fungi (Sagara, 1995; Sagara et al., 2008) observed in association with carcasses (see Tibbett and Carter, 2003). These fungi fruit in a sequence that has been grouped into an Early Phase, where fungi fruit in alkaline soil containing elevated levels of ammonium, and a Late Phase where fungi fruit in response to slightly acidic soil and nitrate (Sagara et al., 2008).

The current results have a number of implications for applied gravesoil ecology. The planning of cemeteries should consider soil type and length of interment due to the inefficient decomposition of cadavers in some soils (typically clays) (Santarsiero et al., 2000; Fiedler et al., 2004). An understanding of the dynamics associated with the regular amendment of soil with a cadaveric resource will likely lead to the designation of soil better suited for cemetery use. In turn, this should lead to more complete decomposition and alleviate problems associated with the formation of adipocere (e.g. Fiedler et al., 2004), proliferation of pathogens and contamination of groundwater (e.g. Santarsiero et al., 2000).

These results also might benefit forensic science. Physical, chemical and biological characteristics of soils are most commonly used by forensic science as a tool to link a suspect and/or victim to a scene (Horswell et al., 2002; Lerner et al., 2006). However, soils have begun to receive increased attention as a tool to locate clandestine graves (Rodriguez and Bass, 1985; Carter and Tibbett, 2003, 2006; Tibbett and Carter, 2008) and estimate postmortem interval (Vass et al., 1992; Carter and Tibbett, 2003; Tibbett et al., 2004). The current data have significant implications for both of these applications. The use of soil pH was first proposed in the mid-1980s as a means to locate clandestine graves (Rodriguez and Bass, 1985). However, this, and more recent proposals (Carter and Tibbett, 2006), based this tool on the formation of alkaline conditions during cadaver breakdown. The current data show that an extended period of decomposition (>28 days, >616 ADDs) can be associated with gravesoil that is more acidic than basal levels. Thus, rather than elevated alkalinity, pH change (alkaline first, then acid) is likely to be related to burial time and in all likelihood be driven by the nitrogen cycle of the gravesoil. Further work is required here and pH change and nitrogen dynamics may have great potential as temporal markers of gravesites.

Soil-based estimations of postmortem interval (Vass et al., 1992; Carter and Tibbett, 2003) might be significantly affected by the current data. Repeated burial may compromise each of the methods proposed by Vass et al. (1992) because the pH of gravesoil following repeated burial differs from that following a single amendment. Thus, it is likely that the concentrations of nutrients and fatty acids also differ following repeated introduction or burial. A similar concern about the dynamics of aboveground insect communities and cadaver decomposition has been recently demonstrated by forensic entomologists (Shahid et al., 2003; Schoenly et al., 2005). This research must be conducted and properly disseminated to the scientific community if it is to be used in courts of law, such as those in the USA, where lack of peer-reviewed publication can preclude the admissibility of scientific methodology (Kiely, 2006).

Future work into the forensic application of gravesoil ecology should also incorporate accumulated degree days (ADDs: sum of average daily temperature). This method of measuring time has been in use since 1735 (de Reaumur, 1735) and has been of particular interest to crop entomologists (Arnold, 1959; Arnold, 1960) since the middle of the 20th century. Thus, it is not surprising that ADDs represent a fundamental component of the forensic application of entomology (see Higley and Haskell, 2001). The measurement of ADDs will help facilitate the comparison of cadaver decomposition between ecosystems and laboratories. However, accurate measures of ADDs require the determination of a minimum developmental threshold (i.e. base temperature) which, at this point, is lacking. Vass et al. (1992) used a base temperature of 0 °C although soil microbial activity can occur in soils at sub-zero temperatures, but does not respond much below 2–4 °C. In addition, extracellular enzyme activity can occur at temperatures as low as −20 °C (see Tibbett, 2002; Margesin et al., 2007). These phenomena must be considered when developing soil ecology as a means to estimate postmortem interval and locate clandestine graves.

In conclusion, the repeated introduction of skeletal muscle tissue led to an enhanced rate of decomposition, which was not associated with an increase in soil microbial biomass. This is most likely due to a change in the function of the soil microbial community. Further, the biological and chemical properties of these soils did not converge the following repeated amendment of skeletal muscle tissue, which might indicate that contrasting soils and soil microbial populations have differing intrinsic abilities of decomposing cadaveric resources. It was apparent that soil type can significantly enhance or restrict the decomposition of cadaveric material following burial.

Acknowledgments

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