Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soils

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1. Introduction

The decomposition of dead mammals (i.e. cadavers) in terrestrial ecosystems has received increased attention due to pressing issues in forensic science and public health. An understanding of the fate of cadavers has implications for the estimation of postmortem interval (Vass et al., 1992), location of clandestine gravesites (France et al., 1992) and transmission and degradation of causative agents (Rapp et al., 2006). Although several studies have investigated the fate of the cadaver itself (e.g. Megyesi et al., 2005), few studies have investigated the ecology of soils associated with a cadaver (gravesoils) (e.g. Fiedler et al., 2004; Rapp et al., 2006). As a consequence, the fundamental processes associated with cadaver decomposition in soil remain poorly understood. Prominent among these is the effect of temperature on gravesoil ecology.

It is well understood that temperature can have a significant effect on the decomposition of organic resource patches in soil (Swift et al., 1979), which is primarily due to the effect of temperature on microbial metabolism and abiotic chemical reactions (Paul and Clark, 1996). However, the effect of temperature on cadaver decomposition in soil, although inferred (Morovic-Budak, 1965), has yet to be investigated experimentally. Further, forensic science, the discipline that conducts the majority of investigations into...
cadaver decomposition, currently operates on the assumption that soil microbial metabolism is not associated with the early stages of cadaver breakdown (Evans, 1963). Thus, a significant gap in knowledge exists and is addressed for the first time in this study.

The current experiment was the first to investigate cadaver decomposition in a controlled setting and tested the hypothesis that higher temperature results in greater cadaver decomposition and microbial activity. We were also interested in determining if decomposition rates and temperature effects varied with soil type. Cadaver decomposition was assessed in three contrasting soils from tropical savanna ecosystems in Queensland, Australia, through the measurement of cadaver mass loss. The ecology of these gravesoils was analyzed through the measurement of carbon dioxide–carbon (CO$_2$–C) evolution, microbial biomass carbon (MBC), enzyme activities (protease, phosphodiesterase), and soil pH.

2. Materials and methods

2.1. Cadaver

Juvenile rat (Rattus rattus) cadavers (~18 g wet weight) aged 8–10 days were used as organic resource patches. Rats were killed with carbon dioxide immediately prior to burial.

2.2. Soils

Three contrasting soil types were collected from Yabulu (19°12′S, 146°36′E), Pallarenda (19°11′S, 146°46′E) and Wambiana (20°33′S, 146°08′E), Queensland, Australia. The soil at Yabulu was a Brown Sodosol (Isbell, 2002) and had a loamy sand texture. The soil at Pallarenda was a Rudosol (Isbell, 2002) and had a sand texture. The soil at Wambiana was a Grey Vertosol (Isbell, 2002) with a medium clay texture. Soil physicochemical properties and methods are presented by Carter et al. (2007a).

2.3. Experimental design

Soil (500 g dry weight) was sieved (2 mm), weighed into incubation chambers (2 l, high density polyethylene tubs: Crown Scientific, Newstead, Queensland, Australia; Product no. A80WTE+9530C), calibrated to a matric potential of −0.05 MPa, and incubated at 29 °C, 22 °C, or 15 °C for 7 days to equilibrate. This moisture regime was chosen as an approximation to 50% water-holding capacity. Following equilibration, rats were killed with carbon dioxide (CO$_2$), weighed and buried in soil on their right side at a depth of 2.5 cm. Soils associated with a cadaver will be referred to as gravesoil. In control samples soil was disturbed to simulate cadaver burial and account for any effect of soil disturbance. A sequential harvesting regime (Tibbett et al., 2004) was then implemented where cadavers and soils were destructively harvested following 7, 14, 21, or 28 days of incubation. At each harvest event designated cadavers were exhumed along with gravesoil directly surrounding the cadaver (approximately 50 g). The experiment was replicated four times resulting in a total of 288 microcosms.

2.4. Cadaver mass loss

Upon exhumation, cadavers were immediately frozen (−20 °C). Once frozen the cadavers were rinsed with distilled water to remove any additional soil, dried with a paper towel and weighed. Harvested soil samples were weighed into sterile culture tubes for further analysis. Soil samples were immediately stored at −20 °C. To further assess the effect of temperature on mass loss, $Q_{10}$ values were calculated for samples collected on days 7, 14, 21, and 28.

2.5. Carbon dioxide–carbon evolution

Carbon dioxide–carbon evolution was analyzed using the alkali absorption/conductivity method (Rodella and Saboya, 1999). A 30 ml vial (Crown Scientific, Newstead, Queensland, Australia; Product no. 735) (CO$_2$ trap) was filled with 20 ml sodium hydroxide (NaOH) (0.465 M) and suspended above the soil surface inside incubation chambers designated for soil sampling after 28 days. The incubation chamber was then sealed. CO$_2$ traps and the air in the incubation chamber headspace were replaced every 24 h. Upon removal from the incubation chamber the electrical conductivity of the NaOH solution inside the CO$_2$ trap was measured using a Metrohm 660 Conductometer (Herisau, Switzerland). Carbon dioxide evolution was calculated after Rodella and Saboya (1999). To further assess the effect of temperature on C mineralization, $Q_{10}$ values were calculated for CO$_2$–C rates on days 7, 14, 21, and 28.

2.6. Microbial biomass carbon

Microbial biomass carbon was estimated within 1 h of harvest using the substrate-induced respiration (SIR) technique (Anderson and Domsch, 1978) with some modifications (Lin and Brookes, 1999). Soil (5 g dry weight) was weighed into 30 ml McCartney bottles and amended with glucose solution (6 mg g$^{-1}$ soil) in order to calibrate them to 95% water-holding capacity. Following amendment with glucose solution a 6 ml vial containing 5 ml NaOH (0.1 M) (CO$_2$ trap) was placed on the soil surface and the McCartney bottle was immediately sealed. Samples were then incubated in the dark at 22 °C for 3 h or 4 h based on preliminary experiments (data not shown). After the incubation period CO$_2$ traps were removed and sealed. The NaOH solution from the CO$_2$ traps was back-titrated with HCl (0.1 M) into 5 ml BaCl$_2$ (1.0 M) and 3 drops phenolphthalein as indicator (Rowell, 1994). Microbial biomass carbon was then calculated using the equation in Anderson and Domsch (1978).

2.7. Enzyme activity

The activity of casein-hydrolyzing protease and phosphodiesterase (orthophosphodiester phosphohydrolase: EC 3.1.4.1) was assayed. Proteolytic hydrolysis of casein was measured according to Ladd and Butler (1972). Soil phosphodiesterase activity was assayed following Tabatabai (1994).
2.8. Statistical analyses

Descriptive and inferential statistics were generated using Microsoft Excel 2000 and SPSS 11.0.1 (Chicago, USA). Cadaver mass loss, MBC, enzyme activity, 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. Cadaver mass loss

Higher incubation temperatures resulted in more rapid cadaver mass loss (Figure 1). There was also a significant effect of soil type with cadaver mass loss greatest ($P < 0.001$) in Pallarenda soil. Burial resulted in a thinner, more flaccid cadaver by day 28 in all soils at all temperatures. All $Q_{10}$ values were greater than 1.0 with the highest being 7.7 (Table 1). In all treatments, the highest $Q_{10}$ values were observed during the initial 14 days of burial. $Q_{10}$ values then decreased until day 28. In some cases, $Q_{10}$ (15–22 °C) values were significantly ($P < 0.05$) greater than $Q_{10}$ (22–29 °C) values (Table 1).

3.2. Carbon dioxide–carbon evolution

Carbon dioxide–carbon evolution in gravesoil was greater ($P < 0.05$) than in control soil and higher temperature resulted in a higher rate of ($P < 0.05$) CO$_2$–C evolution (Figure 1). Carbon dioxide–carbon evolution from gravesoils followed a sigmoidal pattern characterized by an early lag phase that, although greater than control soils, represented a relatively slow increase in CO$_2$–C evolution. This early lag phase was followed by a period of rapid increase, followed by a period of relatively slower CO$_2$–C evolution. This slowing of CO$_2$–C evolution was most pronounced in gravesoils incubated at 29 °C or 22 °C. Carbon dioxide–carbon evolution was not affected by soil type. All $Q_{10}$ values were greater than 1.0 with the highest being 16.2 (Table 1). In all treatments, the highest $Q_{10}$ values were observed during the initial 14 days of burial. $Q_{10}$ values then decreased until day 28. In some cases, $Q_{10}$ (15–22 °C) values were significantly ($P < 0.05$) greater than $Q_{10}$ (22–29 °C) values (Table 1).

3.3. Microbial biomass carbon

MBC in gravesoil was greater ($P < 0.05$) than in control soil (Figure 2). This difference was influenced by temperature, as higher temperature resulted in greater initial increases in MBC as well as earlier peaks in MBC. Thus, peak gravesoil MBC at 29 °C was typically observed from days 7 to 14 while incubation at 22 °C or 15 °C resulted in peak gravesoil MBC from days 14 to 21. One exception was Pallarenda gravesoil incubated at 22 °C, where peak MBC was observed on day 28. A soils effect was also observed as MBC in Yabulu gravesoil was less ($P < 0.05$) than Pallarenda and Wambiana gravesoil.
Table 1. $Q_{10}$ values for mass loss and carbon dioxide–carbon (CO$_2$–C) evolution following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver in 500 g (dry weight) sieved (2 mm) soils from the Yabulu, Pallarenda, or Wambiana, Queensland, Australia incubated at 29 °C, 22 °C, or 15 °C.

<table>
<thead>
<tr>
<th>Day</th>
<th>Yabulu $Q_{10}$ (15–22 °C)</th>
<th>Pallarenda $Q_{10}$ (15–22 °C)</th>
<th>Wambiana $Q_{10}$ (15–22 °C)</th>
<th>Yabulu $Q_{10}$ (22–29 °C)</th>
<th>Pallarenda $Q_{10}$ (22–29 °C)</th>
<th>Wambiana $Q_{10}$ (22–29 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>NA</td>
<td>1.9 (0.6)</td>
<td>NA</td>
<td>3.1 (0.5)</td>
<td>NA</td>
<td>6.5 (3.9)</td>
</tr>
<tr>
<td>14</td>
<td>5.1 (2.2)</td>
<td>3.0 (0.4)</td>
<td>7.7 (2.1)</td>
<td>1.9 (0.2)</td>
<td>2.7 (0.3)</td>
<td>2.2 (0.4)</td>
</tr>
<tr>
<td>21</td>
<td>2.4 (0.4)</td>
<td>1.2 (0.1)</td>
<td>3.0 (0.1)</td>
<td>1.8 (0.1)</td>
<td>4.2 (0.5)</td>
<td>1.9 (0.3)</td>
</tr>
<tr>
<td>28</td>
<td>1.7 (0.3)</td>
<td>1.6 (0.2)</td>
<td>1.4 (0.2)</td>
<td>1.6 (0.1)</td>
<td>2.6 (0.2)</td>
<td>1.3 (0.1)</td>
</tr>
</tbody>
</table>

CO$_2$–C

<table>
<thead>
<tr>
<th>Day</th>
<th>Yabulu</th>
<th>Pallarenda</th>
<th>Wambiana</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>9.4 (0.8)</td>
<td>3.6 (0.5)</td>
<td>16.2 (2.0)</td>
</tr>
<tr>
<td>14</td>
<td>4.5 (0.6)</td>
<td>1.5 (0.1)</td>
<td>6.1 (0.7)</td>
</tr>
<tr>
<td>21</td>
<td>2.2 (0.1)</td>
<td>1.3 (0.1)</td>
<td>2.3 (0.1)</td>
</tr>
<tr>
<td>28</td>
<td>1.9 (0.1)</td>
<td>1.3 (0.1)</td>
<td>1.7 (0.1)</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent standard error where $n = 4$. Bold indicates a significant ($P < 0.05$) difference between $Q_{10}$ value within time and soil type. NA indicates inability to calculate $Q_{10}$ where mass loss values were negative (i.e. mass gain was observed).

Figure 2. Microbial biomass carbon (μg g$^{-1}$ soil) following the burial (2.5 cm) of a juvenile rat (*Rattus rattus*) cadaver (●) and in control (no cadaver: ○) samples in 500 g (dry weight) sieved (2 mm) soils from the Yabulu, Pallarenda, or Wambiana, Queensland, Australia incubated at 29 °C, 22 °C, or 15 °C. Bars represent standard errors where $n = 4$. *Significant difference between cadaver treatments within time where *$P < 0.05$. 
Fungal hyphae were observed on the surface of all gravesoils but not observed on any control soils. The presence of fungal hyphae was most common in Wambiana gravesoil but was initially observed in Yabulu gravesoil in incubated at 29 °C. There was an apparent relationship between incubation temperature and the appearance of fungal hyphae: hyphae tended to appear earliest at 29 °C and latest at 15 °C.

3.4. Enzyme activities

Similar patterns were observed in protease activity and phosphodiesterase activity (Figures 3 & 4). Cadaver associated increases in enzyme activities were modified by temperature whereby increases in enzyme activity incubated at 29 °C took place earlier than at 22 °C, which occurred before an increase at 15 °C. Typically, higher levels of enzyme activity at 29 °C began by day 7, increases at 22 °C began by day 14, and increases at 15 °C began by day 14 (Yabulu, Pallarenda) or day 21 (Wambiana). However, no distinguishable pattern of protease activity was observed in Pallarenda soil.

Protease activity in Yabulu, Pallarenda, and Wambiana control soils was approximately 250 μg tyrosine g⁻¹ soil h⁻¹, 2750 μg tyrosine g⁻¹ soil h⁻¹, and 2500 μg tyrosine g⁻¹ soil h⁻¹, respectively (Figure 3). These levels remained constant throughout the incubation. Cadaver burial resulted in greater (P < 0.05) protease activity in all gravesoils except Pallarenda soil incubated at 15 °C. Cadaver burial led to protease activities of 2500 μg tyrosine g⁻¹ soil h⁻¹, 2800 μg tyrosine g⁻¹ soil h⁻¹, and 5650 μg tyrosine g⁻¹ soil h⁻¹ in Yabulu, Pallarenda, and Wambiana gravesoil, respectively. Thus, protease activity was also affected by soil type, as activity in Wambiana gravesoil was greater (P < 0.05) than in Yabulu and Pallarenda gravesoil.

Phosphodiesterase activity in Yabulu, Pallarenda, and Wambiana control soils was approximately 10 μg p-nitrophenol g⁻¹ soil h⁻¹, 18 μg p-nitrophenol g⁻¹ soil h⁻¹, and 60 μg p-nitrophenol g⁻¹ soil h⁻¹, respectively (Figure 4). These levels remained constant throughout the incubation. Cadaver burial resulted in greater (P < 0.05) phosphodiesterase activity in all gravesoils. This led to phosphodiesterase activities of 60 μg p-nitrophenol g⁻¹ soil h⁻¹, 50 μg p-nitrophenol...
g⁻¹ soil h⁻¹, and 100 μg p-nitrophenol g⁻¹ soil h⁻¹ in Yabulu, Pallarenda, and Wambiana gravesoil, respectively. Phosphodiesterase activity was also affected by soil type, as activity in Wambiana gravesoil was greater (P < 0.05) than in Yabulu and Pallarenda gravesoil.

3.5. Soil pH

Cadaver burial resulted in an increase of pH to a peak between 8 and 8.1, regardless of basal pH (Figure 5). This increase was apparently affected by temperature with greater temperature resulting in earlier peaks in pH. In addition, greater decreases in pH in Yabulu and Pallarenda gravesoil were observed at 29 °C on day 28 at the end of the incubation.

4. Discussion

These data show that cadaver decomposition was more rapid at higher temperature, which corresponded with higher levels of soil microbial activity, microbial biomass, protease activity, and phosphodiesterase activity. A temperature effect was observed where peaks or differences in decomposition that occurred at higher temperature would occur at later sample periods at lower temperature. These findings are consistent with the effect of temperature on the decomposition of skeletal muscle tissue (Ovis aries) (Carter and Tibbett, 2006) and other organic resources such as sewage sludge (Albiach et al., 2000) and pine (Pinus halepensis) needle litter (Coûteaux et al., 2002). The temperature effect on soil respiration, microbial biomass and enzyme activities might indicate that the soil microbial biomass plays a significant role in the decomposition of cadavers following burial in soil.

The current Q10 values were similar to Q10 values associated with the decomposition of skeletal muscle tissue (Carter and Tibbett, 2006). Similar patterns in relation to temperature range were evident between the two studies with the lower temperature range, Q10 (15–22 °C), reporting larger values than Q10 (22–29 °C). This is most likely because 15 °C is outside of the optimal growth range for mi-
croorganisms in these tropical soils. Some \( Q_{10} \) values in the current study far exceed some of those reported previously for mass loss and \( \text{CO}_2 \)-C respiration. This might reflect large-scale protein utilization, which provides evidence that resource availability plays a crucial role in microbial respiratory response (Liu et al., 2006). That the current \( Q_{10} \) values were two to three times higher than those observed with the decomposition of plant litter (e.g. Coûteaux et al., 2002) might demonstrate the significant effect of resource quality on microbial metabolism. As quality (i.e. resource availability) decreases, so do \( Q_{10} \) values.

At present, it is not possible to determine whether soil-borne or cadaver-borne organisms were the primary mediators of decomposition. It is well-established that a cadaver contains a significant number of enteric microorganisms (Wilson, 2005), which play an important role in cadaver breakdown (Clark et al., 1997; Gill-King, 1997). Investigating this relationship would represent a significant contribution to traditional dogma (Evans, 1963), which states that that aerobic soil and/or dermal microorganisms are not active during the early stages of cadaver decomposition and only play a significant role in cadaver decomposition in the latter stages of decomposition when bone, desiccated tissue and hair represent the primary sources of carbon. Considering that a fresh cadaver is a high quality resource, it seems unlikely that cadaver burial in soil would not trigger the activity of soil microorganisms.

A sigmoidal pattern of cadaver decomposition, observed most clearly here via \( \text{CO}_2 \)-C evolution, is consistent with several previous cadaver decomposition studies (see Carter et al., 2007b). This sigmoidal pattern comprised three general phases that, in order, comprised a lag phase, a period of rapid decomposition, and a final slowing down of decomposition. The initial lag phase was characterized by initial increases in microbial activity, microbial biomass, protease activity and phosphodiesterase activity. This phase might also have been associated with an initial increase in ammonification, as indicated by an increase in soil pH (see Hopkins et al., 2000). This period likely represented the initial establishment of the decomposer population. The lag phase was followed by the period of most rapid decomposition, which was characterized by the maintenance of elevated microbial activity, microbial biomass, enzyme activity and pH. This period might represent the proliferation of

Figure 5. pH of sieved (2 mm) soils from Yabulu, Pallarenda, or Wambiana, Queensland, Australia following the burial (2.5 cm) of a juvenile rat (Rattus rattus) cadaver (●) and in control (no cadaver: ○) samples incubated at 29 °C, 22 °C, or 15 °C. Bars represent standard errors where \( n = 4 \). *Significant difference between treatments within time where ***\( P < 0.001 \).
cadaver utilizing, possibly zymogenous, microorganisms. The third period of decomposition was characterized by a slowing of cadaver decomposition and a gradual decrease in microbial activity and microbial biomass. This probably resulted from the depletion of readily available nutrients and a shift in the microbial community from zymogenous to a more autochthonous community.

Perhaps the most compelling evidence of the importance of temperature on cadaver decomposition, at least from a practical (forensic) perspective, is that the final phase of decomposition was only observed at 29 °C and 22 °C and not at 15 °C, which serves to highlight the important effect of temperature on cadaver breakdown and resource availability. Interestingly, peak protease activity was associated with late phase decomposition. Similar patterns have been observed during the decomposition of leaf litter (Dilly and Munch, 1996; Nannipieri et al., 1983) and might be due to the release of labile organic materials upon the death of the microbial biomass (Nunan et al., 2000). This might signal a second change in microbial community structure: the proliferation of zymogenous microorganisms in response to the introduction of dead microbial cells (e.g. Nunan et al., 2000). This late peak in protease activity may also have implications for the study of prion protein degradation in soil (see Rapp et al., 2006) particularly given the general lack of temperature responsiveness, most notable in the Pallarenda soil.

Several effects of soil type were observed during cadaver decomposition. First, cadaver mass loss was greatest in Pallarenda soil, which is consistent with other observations of cadaver decomposition in sandy soils (Fiedler et al., 2004; Forbes et al., 2005). Rapid mass loss (fresh weight) was probably due to rapid desiccation resulting from a greater rate of gas diffusion in a coarse-textured soil. Differences in protease activity between soils might be due to the relatively high basal level of N in Wambiana soil, which could result in little need for the breakdown of proteinaceous materials by soil microorganisms. In contrast, phosphodiesterase activity in Wambiana gravesoil was greater ($P < 0.05$) than in Yabulu and Pallarenda gravesoil. The reason for this is unclear but may reflect great microbial demand for P in a potentially P fixing (clay) soil (see Carter et al., 2007a, and discussion in Tibbett, 2002), or alternatively may simply reflect different microbial community function between the soils.

Soil type, however, did not affect the dynamics of gravesoil pH but did affect the magnitude of the change. In each case pH rose to approximately pH 8, a remarkable change of almost 5 pH units in 7 days for Yabulu but closer to 2 pH units for Wambiana. The increase of pH following cadaver burial has been attributed to the accumulation of ammonium (Hopkins et al., 2000) and observed in association with human (Vass et al., 1992), dog (Canis familiaris) (Reed, 1958), and pig (Sus scrofa) (Hopkins et al., 2000) cadavers as well as skeletal muscle tissue from lamb (O. aries) (Carter and Tibbett, 2006). The decrease in pH observed in some gravesoils incubated at 29 °C or 22 °C could be the result of soil returning to its basal pH due to the microbial utilization of base cations. This might reflect more complete decomposition that has been observed in association with the decomposition of human (Vass et al., 1992) and bovine (Bos bison) (Towne, 2000) cadavers over periods longer than 28 days.

The current study demonstrates that microbial activity is a major contributor to cadaver decomposition in soil and shows that the persistence of a cadaver in soil can be influenced by temperature and soil type. These findings contradict the long-standing assumption that soil type has little effect on cadaver breakdown (Mant, 1950) but does not yet completely address the assumption that soil microorganisms do not participate in the early stages of cadaver decomposition (Evans, 1963). This new insight has implications for applied soil ecology, particularly when used in forensic science. Soils are typically used by forensic science to link objects and persons with crime scenes. However, many cadaver decomposition studies have shown that processes in soil can help to locate clandestine graves (Carter et al., 2008) and estimate time since death (Vass et al., 1992). The current laboratory conditions have shown that different temperature-based models for predicting cadaver decomposition might differ between soils. The precision of these applications will only be improved through the investigation of cadaver decomposition in field and laboratory conditions.

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