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Microbial decomposition of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil at different temperatures

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Abstract

A laboratory experiment was conducted to determine the effect of temperature (2, 12, 22 °C) on the rate of aerobic decomposition of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil incubated for a period of 42 days. Measurements of decomposition processes included skeletal muscle tissue mass loss, carbon dioxide (CO₂) evolution, microbial biomass, soil pH, skeletal muscle tissue carbon (C) and nitrogen (N) content and the calculation of metabolic quotient (qCO₂). Incubation temperature and skeletal muscle tissue quality had a significant effect on all of the measured process rates with 2 °C usually much lower than 12 and 22 °C. Cumulative CO₂ evolution at 2, 12 and 22 °C equaled 252, 619 and 905 mg CO₂, respectively. A significant correlation ($P < 0.001$) was detected between cumulative CO₂ evolution and tissue mass loss at all temperatures. Q_{10} s for mass loss and CO₂ evolution, which ranged from 1.19 to 3.95, were higher for the lower temperature range ($Q_{10}(2-12\text{ °C}) > Q_{10}(12-22\text{ °C})$) in the *Ovis* samples and lower for the low temperature range ($Q_{10}(2-12\text{ °C}) < Q_{10}(12-22\text{ °C})$) in the control samples. Metabolic quotient and the positive relationship between skeletal muscle tissue mass loss and cumulative CO₂ evolution suggest that tissue decomposition was most efficient at 2 °C. These phenomena may be due to lower microbial catabolic requirements at lower temperature.

Keywords: temperature, Q_{10} , skeletal muscle tissue, burial, decomposition, CO₂ evolution, microbial biomass, metabolic quotient, soil pH

1. Introduction

The decomposition of organic resources in soil is vital to ecosystem function. Decomposition results in the release of carbon into the atmosphere as carbon dioxide (CO₂) as well as the mineralization of essential nutrients such as nitrogen (N), phosphorus (P) and sulfur (S). The breakdown of most organic resources has been attributed to the activity of saprotrophic bacteria and fungi acting in conjunction with a variety of invertebrates (Swift et al., 1979). The activity of these organisms and thus, the rate of decomposition, can be affected by a number of factors such as temperature and resource quality.

Temperature can directly influence metabolic processes while affecting the soil microhabitat via changes in soil physicochemical properties (e.g. redox potential, soil volume) (Paul and Clark, 1996). In soils with a mesophilic microbial population an approximate doubling of activity is commonly associated with an increase of 10 °C up to

30/35 °C (van't Hoff, 1898; Conant et al., 2004). Organic resource quality, such as C:N ratio and phenol content, can determine the ease with which energy and nutrients are accessed by the decomposer community. Organic resources of high quality (i.e. low C:N ratio) typically correspond with rapid rates of decomposition and increased metabolic activity (Dilly and Munch, 1996). However, resource quality can decrease as decomposition proceeds and this can be reflected by a widening of the C:N ratio of the resource and a decrease in the metabolic activity of the decomposer population (Swift et al., 1979; Ajwa and Tabatabai, 1994).

In terrestrial ecosystems organic resources can enter the soil in the form of discrete patches such as seeds and animal cadavers. Numerous studies have been conducted in order to examine decomposition processes associated with plant-derived organic residues (Chander and Brookes, 1991; Hodge et al., 2000; Malpassi et al., 2000; Pérez-Harguindeguy et al., 2000; Coûteaux et al., 2002). However, relatively little work has focused on the decomposition of

cadavers and cadaver components (e.g. skeletal muscle tissue, hair, bone) although animals regularly die in terrestrial ecosystems (DeVault et al., 2003). Of these studies, the vast majority are concerned with the activity of arthropods (e.g. Diptera, Coleoptera, Hymenoptera) (e.g. Payne, 1965; Kocárek, 2003) and vertebrate scavengers (e.g. Cornaby, 1974; Haglund, 1997) that participate in the decomposition of cadavers on the soil surface. As a result very little is understood about the microbially mediated decomposition processes that take place following the burial, in contrast to surface deposition, of a high quality nutrient patch such as a cadaver. In fact, the extent of current knowledge is limited to the understanding that soil microorganisms can display increased biomass (substrate-induced respiration) and activity (CO_2 respiration, S^{2-} reduction, N mineralization, denitrification) in association with buried cadavers (*Sus scrofa*) (Hopkins et al., 2000).

In the current study, we used mammalian skeletal muscle tissue as a high quality nutrient patch in soil. We aim to examine the effect of temperature on the decomposition of skeletal muscle tissue in soil. This work represents an initial attempt at constructing a model that may be used to understand the multifaceted processes associated with the decomposition of buried cadavers or complex resource patches of similarly high quality. A fundamental understanding of cadaveric decomposition can begin to be achieved by examining the breakdown of a relatively uniform organic source of nutrients and energy, such as skeletal muscle tissue in soil. To this end we tested the hypothesis that an increase in temperature will increase skeletal muscle tissue decomposition in soil at a Q_{10} value of 2. This was achieved through the measurement of skeletal muscle tissue mass loss, CO_2 evolution, microbial biomass, soil pH, and tissue C:N ratio. In addition, microbial metabolic efficiency was evaluated by calculating metabolic quotient ($q\text{CO}_2$).

2. Materials and methods

2.1. Soil and skeletal muscle tissue

A sandy loam (61% sand, 18% silt, 21% clay) soil (Brown Earth) of the Fyfield series collected from Lindens farm, East Lulworth, Dorset, England (NGR SY 862208266) was sampled at 0–20 cm with a spade. Soil biophysicochemical characteristics of this soil have been previously reported including, organic C (2.1%), microbial biomass C ($347 \mu\text{g g}^{-1}$ soil), total N (0.15%) and pH (6.4) (Tibbett et al., 2004). Organic texel+suffolk lamb (*Ovis aries*) skeletal muscle tissue was used as the organic resource. Organic skeletal muscle tissue was chosen in order to remove the affect of antibiotics in the tissues. Tissue samples were carefully selected due to the intrinsic heterogeneity of skeletal muscle tissue (Tortora and Grabowski, 2000). Skeletal muscle tissue lacking integument and visible fat was sampled from the leg and stored at 4 °C for 24 h prior to burial. Skeletal muscle tissue samples were then cut into cuboid pieces (1.5 g) in preparation for burial. This was conducted in a sterile laminar flow cabinet using a sterilized scalpel.

2.2. Incubation of soil microcosms

Field fresh soil was sieved (4.6 mm), weighed to 100 g (dry weight) and calibrated to 60% water holding capacity (WHC) inside sealable polyethylene bottles (1285 ml, Merck Ltd, United Kingdom, product no. 215044808). These will be referred to as soil microcosms. Soil microcosms were placed in the dark at 2, 12 or 22 °C for 48 h in order to equilibrate and were grouped according to replicate number. Following equilibration a skeletal muscle tissue sample (1.5 g) was buried in the soil at a depth of 2.5 cm. Soil microcosms containing muscle tissue will be referred to as Ovis samples. The tissue burial procedure was also conducted in control samples (soil without tissue) in order to simulate the burial process and account for any effect caused by soil disturbance. The experiment was replicated six times. Each treatment was set-up with sufficient replicates for six sequential harvest events resulting in a total of 216 microcosms.

2.3. Skeletal muscle tissue mass loss

Skeletal muscle tissue samples were destructively harvested at intervals of 7 days over a period of 42 days (Tibbett et al., 2004). Following harvest, tissue was rinsed with distilled water, dried and weighed gravimetrically.

2.4. Carbon dioxide evolution

To determine the amount of CO_2 respired from the soil and decomposing tissue, 10 ml of sodium hydroxide (NaOH) (0.3 M) solution was placed in 20 ml vials (CO_2 traps) and suspended in the soil microcosms. The soil microcosms were then sealed. CO_2 traps and the air in the soil microcosms were replaced at intervals of 24 h for a period of 42 days. The NaOH solution from the CO_2 traps was back-titrated with HCl (0.1 M) into 10 ml BaCl_2 (1.0 M) and six drops phenolphthalein as indicator (Rowell, 1994). A temperature coefficient (Q_{10}) was used to assess the difference in biological activity at 10 °C intervals.

2.5. Microbial biomass C

Soil microbial biomass C (C_{mic}) was estimated in soils harvested on day 21 and day 42 using the substrate induced respiration (SIR) technique (Anderson and Domsch, 1978) with some modifications by Lin and Brookes (1999): glucose was added to the soil in solution in order to calibrate the soil to 95% WHC. Soil water content can have a great effect on SIR rate (West and Sparling, 1986) and, therefore, 95% WHC was chosen to allow for the distribution of glucose throughout the soil matrix. No problems associated with the limitation on the availability of O_2 were anticipated as a 110% WHC has been successfully used (Lin and Brookes, 1999). Preliminary testing demonstrated that the peak flush of microbial CO_2 occurred after 2.5 h of incubation at 22 °C. The glucose concentration that resulted in maximum CO_2 evolution was 4 mg glucose gram^{-1} soil. The soil was amended with glucose solution following tis-

sue harvest and microbial biomass C ($\mu\text{g C g}^{-1}$ soil) was calculated after Anderson and Domsch (1978).

2.6. Metabolic quotient ($q\text{CO}_2$)

The microbial metabolic quotient ($q\text{CO}_2$) was determined by dividing the CO_2 evolution rate ($\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil h^{-1}) by C_{mic} ($\text{mg C}_{\text{mic}} \text{g}^{-1}$ dry soil) (Dilly and Munch, 1998). The CO_2 evolution rate from 24 h prior to the estimation of C_{mic} was used in the calculation. The value of $q\text{CO}_2$ can be used to determine the efficiency with which C is utilized by the soil microbial biomass (Dilly and Munch, 1998).

2.7. Skeletal muscle tissue and soil analysis

The C and N content of fresh tissue and tissue harvested at each temperature on day 35 was measured using dry combustion chromatography (Carlo-Erba EMASyst 1106). The pH of soil directly surrounding the tissue was measured on days 7, 14, 28 and 35 using a 1:2.5 soil:water (w:v) suspension.

2.8. Statistical analysis

Descriptive and inferential statistics were carried out using Microsoft Excel 2000 and SPSS 11.0.1, respectively. For data that passed preliminary tests for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test) one-way ANOVAs were conducted comparing difference in both time and temperature for mass loss, CO_2 evolution and soil pH. For microbial CO_2 evolution data, a repeated measures ANOVA was conducted; as this data met all the assumptions and experimental criteria for repeated measures analysis (Webster and Payne, 2002). Subsequently, skeletal muscle tissue mass loss, soil pH and CO_2 respiration data were analyzed using Tukey's HSD post hoc test. Pearson's correlation coefficient and linear regression analysis was used to test for a relationship between skeletal muscle tissue mass loss and CO_2 evolution. Where data did not pass the preliminary tests required for parametric analysis, non-parametric statistics were generated. Mann-Whitney U -tests were conducted for microbial biomass, Q_{10} s, $q\text{CO}_2$ and C:N ratio data.

3. Results

3.1. Skeletal muscle tissue mass loss

A 10 °C increase in temperature resulted in increased skeletal muscle tissue mass loss at each harvest except on day 42 in samples incubated at 12 and 22 °C (Figure 1). Tissue samples incubated at 12 and 22 °C lost 60 and 80% of mass during the initial 14 days of burial. A loss of 20% of mass at 2 °C took place during the first 7 days of burial. These rapid rates of mass loss were followed by a more gradual rate of decomposition. The relationship between mass loss and time was described by a cubic regression equation (Figure 1). The mean Q_{10} of tissue mass loss ($Q_{10}\text{-ML}$) between 2 and 12 °C at the end of the incubation (day 42) was greater than $Q_{10}\text{-ML}$ (12–22 °C) ($P < 0.05$) (Table 1).

3.2. Carbon dioxide evolution

The burial of skeletal muscle tissue in soil incubated at 12 and 22 °C resulted in an immediate flush of CO_2 evolution that peaked on day 2 (Figure 2(a)). This flush was not detected in Ovis samples incubated at 2 °C. Instead, an immediate decrease in CO_2 evolution followed by a gradual increase took place. The immediate decrease in CO_2 evolution was analogous to the pattern of microbial activity in the control samples (Figure 2(a)). Ovis samples incubated at 22 °C generated CO_2 at a greater rate than Ovis samples incubated at 12 °C until day 23 when both declined at a similar rate. By day 42 all Ovis samples evolved CO_2 at an equal rate (Figure 2(a)). Carbon dioxide evolution in control samples incubated at 22 °C was greater than in control samples incubated at 2 and 12 °C (Figure 2(a)). Control samples incubated at 12 °C generated CO_2 at a rate greater than control samples incubated at 2 °C every day except day 23, day 41 and day 42.

A 10 °C increase in temperature resulted in an increase in cumulative CO_2 evolution in Ovis and control samples (Figure 2(b)). The $Q_{10}\text{-CO}_2$ in Ovis samples was greater between 2°C and 12 °C than between 12 and 22 °C (Table 1). Conversely, mean $Q_{10}\text{-CO}_2$ in control samples was greater between 12 and 22 °C than between 2 and 12 °C.

Carbon dioxide accumulated in Ovis samples over intervals of 7 days was plotted as a function of tissue mass loss at intervals of 7 days (Figure 3). A significant correlation was detected between skeletal muscle tissue mass loss and cumulative CO_2 evolution at 2 °C (Pearson's $R = 0.803$; $P < 0.001$), 12 °C (Pearson's $R = 0.728$; $P < 0.001$) and 22 °C (Pearson's $R = 0.749$; $P < 0.001$) (Figure 3).

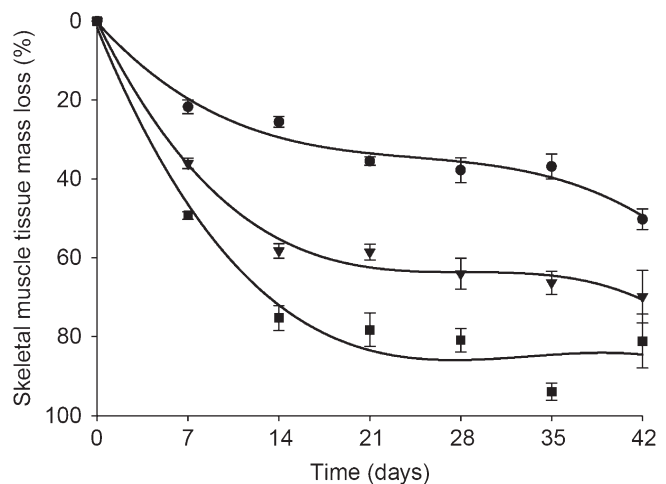


Figure 1. Mass loss of a 1.5 g cube of skeletal muscle tissue (*Ovis aries*) following burial (2.5 cm) in a sandy loam soil of the Fyfield Series from Lindens farm, East Lulworth, Dorset, England incubated at 2 °C (●), 12 °C (▼), and 22 °C (■). Curves represent cubic polynomial equation. Equations are as follows: 2 °C ($y = 0.34 + 3.63x + -0.14x^2 + 0.002x^3$; $r^2 = 0.88$); 12 °C ($y = 0.27 + 6.66x + -0.23x^2 + 0.003x^3$; $r^2 = 0.90$); 22 °C ($y = 1.77 + 8.0x + -0.25x^2 + 0.003x^3$; $r^2 = 0.91$). Bars represent standard errors where $n = 6$.

Table 1. Temperature coefficients (Q_{10}) of skeletal muscle tissue (*Ovis aries*) mass loss (Q_{10} -ML) and cumulative CO_2 evolution (Q_{10} - CO_2) following the burial (2.5 cm) of a 1.5 g cube of skeletal muscle tissue in a sandy loam soil (100 g dry weight) of the Fyfield series from Lindens farm, East Lulworth, Dorset, England.

Day	Q_{10} -ML		Q_{10} - CO_2			
	(2-12 °C)	(12-22 °C)	(2-12 °C)		(12-22 °C)	
			Ovis	Control	Ovis	Control
7	1.74 ^{abc} (0.20)	1.37 ^{ab} (0.03)	3.95 ^z (0.27)	1.90 ^{vw} (0.22)	1.86 ^{vw} (0.03)	2.08 ^{wx} (0.11)
14	2.31 ^c (0.13)	1.30 ^{ab} (0.08)	3.15 ^y (0.19)	1.92 ^{vw} (0.16)	1.92 ^{vw} (0.05)	2.29 ^x (0.12)
21	1.65 ^{abc} (0.05)	1.34 ^{ab} (0.06)	2.83 ^y (0.14)	1.81 ^{vw} (0.10)	1.77 ^{vw} (0.08)	2.19 ^x (0.08)
28	1.79 ^{abc} (0.26)	1.27 ^{ab} (0.05)	2.74 ^y (0.14)	1.75 ^{vw} (0.08)	1.60 ^{vw} (0.10)	2.23 ^x (0.08)
35	1.86 ^{bc} (0.17)	1.44 ^{ab} (0.09)	2.66 ^y (0.15)	1.75 ^{vw} (0.07)	1.52 ^{vw} (0.10)	2.24 ^x (0.08)
42	1.42 ^{ab} (0.19)	1.19 ^a (0.11)	2.48 ^y (0.14)	1.72 ^{vw} (0.05)	1.48 ^v (0.10)	2.22 ^x (0.07)
Mean	1.80 (0.12)	1.32 (0.03)	2.97 (0.22)	1.69 (0.08)	1.81 (0.03)	2.21 (0.03)

a, b, c denote differences ($P < 0.05$) in Q_{10} -ML between temperature. v, w, x, y, z denote differences ($P < 0.05$) in Q_{10} - CO_2 between temperature and tissue treatments. Standard errors are presented in brackets where $n = 6$.

3.3. Microbial biomass C

Generally, greater C_{mic} was detected in the Ovis samples. However, these concentrations were only significantly greater than control samples on day 21 at 22 °C ($P < 0.05$) and day 42 at 12 °C ($P < 0.01$) (Table 2). An increase in temperature also resulted in increased C_{mic} but these differences

were not significant. All samples contained less C_{mic} on day 42. This decrease was not significant.

Interestingly, the Ovis samples contained fungal hyphae on the soil surface directly above the buried tissue. In Ovis samples incubated at 22 °C the hyphae became macroscopically observable from day 5 to day 10. At 12 °C fungal hyphae were observed on the surface of the Ovis samples from day 9 to day 21. At 2 °C the hyphae were observed from day 25 to the end of the incubation.

3.4. Metabolic quotient ($q\text{CO}_2$)

Tissue burial resulted in an increased $q\text{CO}_2$ (Table 2). On day 21 $q\text{CO}_2$ of Ovis samples incubated at 12 or 22 °C was greater than in Ovis samples incubated at 2 °C. The $q\text{CO}_2$ of control samples on day 21 followed the pattern 22 > 12 > 2 °C. By day 42 $q\text{CO}_2$ had decreased in all samples. This decrease was significant ($P < 0.05$) in Ovis samples. Temperature did not affect the $q\text{CO}_2$ of Ovis samples on day 42 (Ta-

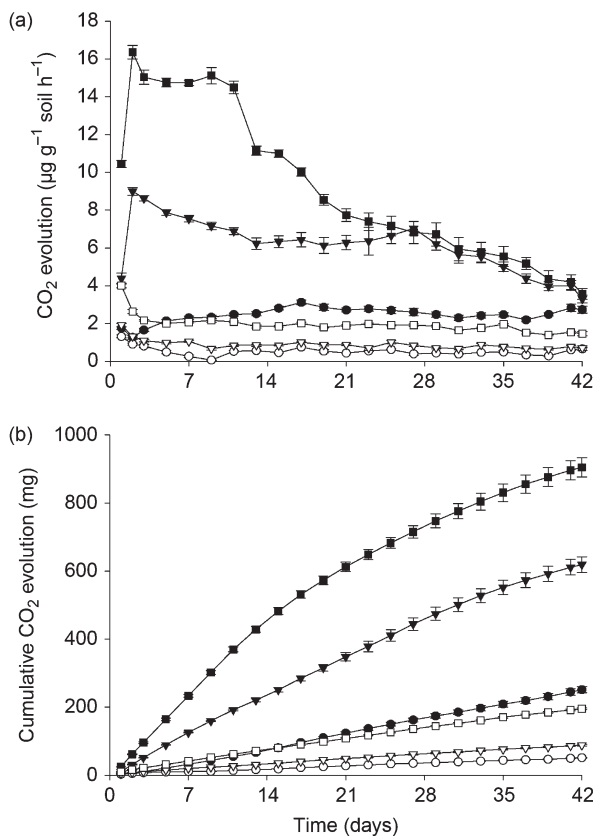


Figure 2. Daily (a) and cumulative (b) CO_2 evolution following the burial (2.5 cm) of a 1.5 g cube of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil of the Fyfield Series from Lindens farm, East Lulworth, Dorset, England incubated at 2 °C (●), 12 °C (▼), and 22 °C (■). Unfilled symbols represent CO_2 evolution in control (soil without tissue) samples. Bars represent standard errors where $n = 6$.

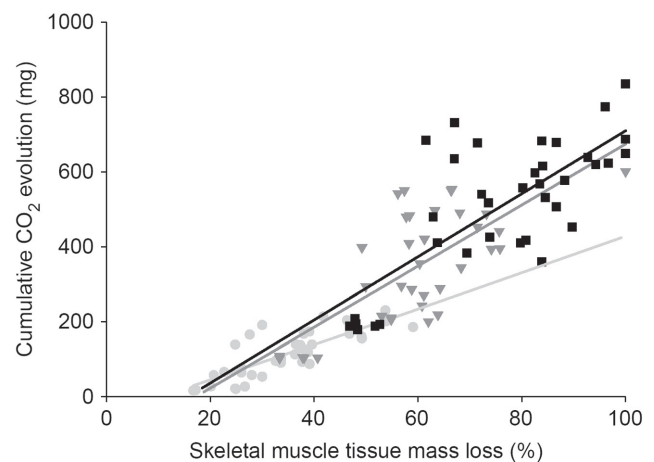


Figure 3. Correlation between CO_2 evolution and skeletal muscle tissue (*Ovis aries*) mass loss following burial (2.5 cm) in a sandy loam soil of the Fyfield Series from Lindens farm, East Lulworth, Dorset, England incubated at 2 °C (●), 12 °C (▼), and 22 °C (■). Equations are as follows: 2 °C ($y = -49.49 + 4.71x$; $r^2 = 0.64$); 12 °C ($y = -140.41 + 8.15x$; $r^2 = 0.53$); 22 °C ($y = -131.24 + 8.41x$; $r^2 = 0.56$).

Table 2. Microbial biomass C (C_{mic}) ($\mu\text{g g}^{-1}$ soil) and metabolic quotient ($q\text{CO}_2$) ($\mu\text{g CO}_2\text{-C mg}^{-1} C_{mic} \text{ h}^{-1}$) in a sandy loam soil of the Fyfield series from Lindens farm, East Lulworth, Dorset, England following the burial (2.5 cm) of a 1.5 g cube of skeletal muscle tissue (*Ovis aries*) (Ovis) and without skeletal muscle tissue (control).

Measure	Temperature (°C)	Sample	Day	
			21	42
C_{mic}	2	Ovis	781 (47)	646 (44)
		Control	699 (30)	646 (65)
	12	Ovis	787 (28)	775 (29)**
		Control	717 (25)	693 (18)
	22	Ovis	852 (17)*	810 (34)
		Control	764 (11)	758 (33)
$q\text{CO}_2$	2	Ovis	1.95 (0.15) ^d	1.20 (0.14) ^c
		Control	0.39 (0.03) ^a	0.29 (0.05) ^a
	12	Ovis	4.67 (0.53) ^e	1.15 (0.08) ^c
		Control	0.70 (0.08) ^b	0.28 (0.05) ^a
	22	Ovis	5.25 (0.35) ^e	1.20 (0.10) ^c
		Control	1.35 (0.05) ^c	0.53 (0.06) ^b

*($P < 0.05$) and **($P < 0.010$) represent differences in C_{mic} within temperature treatments. Letters represent differences ($P < 0.05$) in $q\text{CO}_2$ between treatments. Standard errors are presented in brackets where $n = 6$.

ble 2). On day 42 the $q\text{CO}_2$ of control samples incubated at 22 °C was greater than the $q\text{CO}_2$ of control samples incubated at 2 and 12 °C (Table 2).

3.5. Skeletal muscle tissue and soil analysis

The C:N ratio of fresh, unburied skeletal muscle tissue was 5.5:1 (± 0.4). On day 35 the C:N ratio of tissue became wider with each 10 °C increase in temperature (2, 12 and 22 °C was 3.8:1 (± 0.1), 5.8:1 (± 0.2), and 12.3:1 (± 1.8), respectively).

Tissue burial resulted in an increase in soil pH from 6.4 to a maximum of 7.9 (Figure 4). The speed of pH change was related to temperature with higher temperatures resulting in more rapid change. Soil pH in Ovis samples at 22 °C began to decline after day 14. The pH of Ovis soils incubated at 2 °C increased until day 35 when it reached 7.9. A similar pH was detected on day 14 in Ovis samples incubated at 12 °C. The pH of control samples did not significantly change during the incubation.

4. Discussion

The decomposition of skeletal muscle tissue was characterized by an increase in CO_2 evolution and an initial period (7–14 days) of rapid mass loss. These phenomena were apparently regulated by temperature and are similar to the decomposition dynamics of other organic nutrient patches of high quality such as earthworm residues (Hodge et al., 2000), sewage sludge (Clark and Gilmour, 1983; Díaz-Burgos et al., 1993; Ajwa and Tabatabai, 1994), manure (Ajwa and Tabatabai, 1994) and plant material (Dilly and Munch, 1996). At 12 and 22 °C the rate of mass loss and CO_2 evolution slowed following the initial period of rapid decomposition. This is likely due to a decrease in readily available nutrients (Ajwa and Tabatabai, 1994) after an initial flush of microbial activity based on a zymogenous response to highly decom-

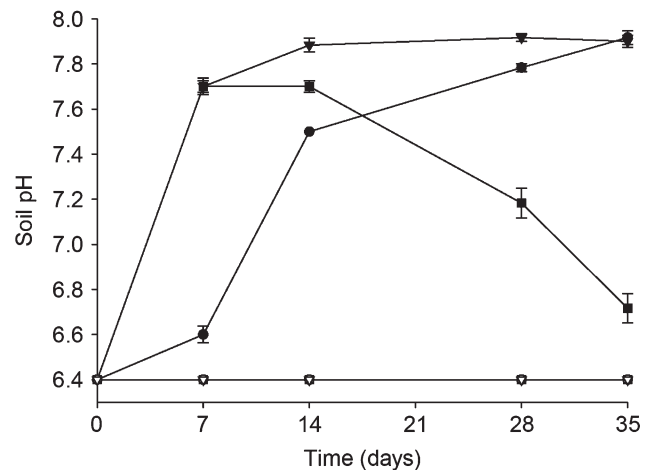


Figure 4. Soil pH following the burial (2.5 cm) of a 1.5 g cube of skeletal muscle tissue (*Ovis aries*) in a sandy loam soil of the Fyfield series from Lindens farm, East Lulworth, Dorset, England incubated at 2 °C (●), 12 °C (▼), and 22 °C (■). Unfilled symbols represent soil pH in control (soil without tissue) samples. Soil pH was not measured on day 21. Bars represent standard errors where $n = 6$.

posable substrates such as blood and free proteins (Ajwa and Tabatabai, 1994). This is reflected by a widening of the C:N ratio of tissue buried at 22 °C and, although there was no significant change in C:N ratio, a decrease in the percentage of C and N (data not shown) in tissue samples incubated at 12 °C. The shorter period of rapid mass loss and lack of an immediate flush of CO_2 at 2 °C (also displayed in control samples) has been previously observed in a temperate soil incubated at a psychrotrophic temperature (4 °C) (Winkler et al., 1996). These characteristics may reflect the inability of a mesotrophic population to immediately access nutrients at a low temperature. This may be due to the inhibition of the expression of hydrolytic enzymes responsible for the catalysis of compounds containing organic N (Frankenberger and Tabatabai, 1991) and might also explain the increase in the C:N ratio of tissue buried at 2 °C. The gradual increase in CO_2 evolution following tissue burial at 2 °C is consistent with that shown for the decomposition of a readily available nutrient source (holocellulose) at low temperature (4 °C) (Nicolardot et al., 1994) and, along with the mass loss and CO_2 data at mesotrophic temperatures, supports the idea that the soil microbial biomass can utilize skeletal muscle tissue as a source of nutrients and energy during early stages of cadaveric decomposition.

We accept the hypothesis that an increase in temperature will increase skeletal muscle tissue decomposition in soil at a Q_{10} relationship of two based on Q_{10} -ML (2–12 °C) and Q_{10} - CO_2 (12–22 °C) data. However, Q_{10} -ML (12–22 °C) was approximately 1.3 and Q_{10} - CO_2 (2–12 °C) was closer to three. These results show that low temperature induced a low microbial energy requirement for catabolism. Interestingly, Q_{10} - CO_2 in control samples showed the opposite trend (i.e. increase of Q_{10} - CO_2 with increase in temperature). This might mean that respiration was limited at low temperature either due to nutrient availability and/or the cold-induced repression of hydrolytic enzymes involved in

decomposition. Both of these trends have been previously observed (Kirschbaum, 1995; Winkler et al., 1996; Reichstein et al., 2000; Conant et al., 2004). The Q_{10} -ML ranged from 1.19 to 2.31, which is similar to the influence of temperature on the decomposition of plant litter in several terrestrial ecosystems (Andren and Paustian, 1987; Gholz et al., 2000; Couëteux et al., 2002). A change in the quality of the nutrient patch would account for the decrease of Q_{10} s over time. Readily available nutrients were likely utilized at a greater rate at high temperature. Thus, a greater amount of available nutrients would be present at lower temperatures for a longer period of time and the lower Q_{10} s observed would be expected if process rates positively correlate to available nutrients (see Winkler et al., 1996).

Skeletal muscle tissue represented a high quality nutrient patch capable of supporting the growth of the soil microbial biomass at mesotrophic temperatures. This is not surprising considering that the soil microbial biomass can act as a sensitive indicator of change in nutrient status and temperature (Wardle, 1992). It is important to note that the use of SIR on soil subjected to organic amendments can lead to an overestimation of C_{mic} (Sparling et al., 1981). However, the clear temperature effect and the increase in C_{mic} associated with skeletal muscle tissue of relatively low quality after 42 days of burial at 12 °C leads to the belief that the observed increases in C_{mic} are accurate.

Metabolic quotient has previously been used to indicate the efficiency in which organic C is utilized by the soil microbial biomass (Anderson and Domsch, 1990; Dilly and Munch, 1998). Increased qCO_2 at 12 and 22 °C represented a less efficient utilization of C (Dilly and Munch, 1998). Using qCO_2 one would conclude that incubation at 2 °C resulted in the most efficient tissue decomposition. An alternative assessment of tissue decomposition efficiency is the relationship between CO_2 evolved and tissue mass loss. Tissue mass loss is significantly correlated ($P < 0.001$) to cumulative CO_2 evolution at all temperatures and a reduced slope at 2 °C supports the concept that temperature reduced the rate of decomposition and increased the associated metabolic efficiency.

Although the soil microbial biomass includes bacteria, fungi, yeasts, algae, protozoa (Sakamoto and Oba, 1994; Savin et al., 2001) the current experimental design requires the soil microbial biomass to be regarded as a single entity. However, the proliferation of fungal hyphae in association with skeletal muscle tissue was an interesting observation. This increase in fungal biomass was similar to that in association with N-rich alfalfa meal, in contrast to N-poor wheat straw or starch (Mamilov et al., 2001). The macroscopic presence of fungi, particularly fruiting structures, in intimate association with cadaveric decay has been reported in many regions around the world (Tibbett and Carter, 2003). These fungi are known as postputrefaction fungi (Sagara, 1995) or taphonomic mycota (Carter and Tibbett, 2003) and tend to fruit in a successional sequence due, in part, to the availability and form of N (e.g. NH_4^+ , NO_3^-) (Tibbett and Carter, 2003). While it is unknown if the hyphae observed in the current study represented recognized postputrefaction fungi, it is important to note the

significant effect that temperature had on the proliferation and persistence of the hyphae as well as the growth of the soil microbial population in general.

It has long been known that cadaver decomposition can result in increased soil pH (Reed, 1958) and this has been attributed to an accumulation of NH_4^+ (Hopkins et al., 2000). In addition, C and N mineralization liberates acids (H_2CO_3 , HNO_3) and massive C mineralization can result in anaerobic micro-sites inducing low redox potential and increasing pH values. However, few studies have measured soil pH over the course of cadaveric decomposition (Vass et al., 1992). The decrease in pH of soils in the *Ovis* samples incubated at 22 °C during the latter stages of the incubation could be the result of the soil reverting back to its natural pH because of the utilization of base cations by the soil microbiota. This fall in pH is in keeping with more complete decomposition at the higher temperature and is similar to findings from related research (Vass et al., 1992). The patterns of pH change observed in the absence of an enteric flora suggest that the soil microbial biomass may have contributed to the changes in pH observed in the presence of whole cadavers in other studies (Rodriguez and Bass, 1985; Vass et al., 1992; Hopkins et al., 2000).

The current results show that skeletal muscle tissue can be immediately used as a source of nutrients by the soil microbial biomass and this utilization can be greatly affected by temperature. There was an apparent "temporal wave" throughout our data that represented the slowing down of decomposition processes at lower temperatures. Where peaks or differences in skeletal muscle tissue mass loss, CO_2 evolution, C_{mic} , pH and the macroscopic presence of fungal hyphae occurred at 22 °C at one sample period, they would occur at later sample periods in lower temperature incubations. However, the effects were more subtle than a uniform slowing down of process rates because apparent changes to the metabolic function of the microbial population also occurred. It is currently unknown if these patterns would apply to a complete cadaver with its enteric microflora and numerous components such as skin, bone and hair. Regardless, carnivore and herbivore cadavers represent sources of sequestered nutrients and much more detailed work is required to understand the processes associated with the transfer of energy and nutrients from the herbivore subsystem to the decomposer subsystem.

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