



# Dynamic soil nutrient and moisture changes under decomposing vertebrate carcasses

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**Abstract** The decomposition of animal carcasses contributes to nutrient recycling in ecosystems worldwide, including by delivering nutrients to soil. Although several studies have characterised changes in soil chemistry occurring under carcasses, many ecological studies have occurred over extended post-mortem intervals and fine-scale temporal changes in physicochemical conditions are poorly understood. We examined changes in a suite of soil physicochemical properties occurring under decomposing rabbit carcasses during summer in a grassland ecosystem. We found that carcasses lost over 90% of their starting mass and reached dry decay and skeletonization after 20 days of decomposition. Carcass temperatures were up to 15 °C higher than ambient temperatures during the active decay stage (days 3 and 5) of decomposition.

Soil moisture also increased by day 4, and this was matched with a simultaneous increase in total nitrogen and ammonium, as well increases in pH and electrical conductivity. Whereas these measures remained relatively stable as decay progressed, we found total phosphorus and phosphate continued to increase to day 20. The contrasting dynamics of N and P reflect the initial nutrient and fluid input during the rapid decay of soft tissues and intense activity of fly larvae, and the subsequent dry decay and exposure of skeletal components. Our study provides new information about the fine-scale timing of nutrient inputs and moisture and temperature changes occurring at the carcass/soil interface.

**Keywords** Decomposition · Carrion · Nitrogen · Necrobiome · Nutrient cycling · Ecosystem · Post-mortem · Ecological process · Grassland

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## Introduction

Decomposition is an ecosystem process that drives the redistribution of nutrients and energy contained in dead organic biomass (Swift et al. 1979; Benbow et al. 2019). Dead animals form a small but important fraction of terrestrial biomass (Parmenter and MacMahon 2009; Barton et al. 2019a), and their decomposition releases nutrients back into the biosphere

through autolysis, putrefaction and the action of scavengers and decomposers (Forbes and Carter 2015; Barton and Bump 2019; Benbow et al. 2019). Carcasses are colonised by small invertebrates such as flies that feed on the carcass, while bacteria accelerate the biochemical degradation of internal and external body tissues (Benbow et al. 2019). This combined action generates decomposition fluids rich in proteins, fats, and salts, as well as other material such as insect excreta and remains that enter the soil (Benninger et al. 2008; Macdonald et al. 2014). Soil biota including microbes (Strickland and Wickings 2015) and invertebrates (Szelez et al. 2016) also respond to nutrient inputs by increasing in abundance under a carcass, and subsequently ingest and/or degrade complex molecules into smaller products, thus contributing to elemental cycling in soil (Carter et al. 2007; Macdonald et al. 2014). Decomposing vertebrate carcasses therefore create a concentrated hotspot of nutrients and associated organisms termed the ‘Cadaver Decomposition Island’ (CDI) (sensu Carter et al. 2007). The CDI can persist for years, depending on the size of the carcass and its ecosystem context (Danell et al. 2002; Bump et al. 2009; Barton et al. 2016). Characterizing the soil nutrient profile under a carcass is useful for understanding how a carcass affects its surrounding environment, such as the quantity of the nutrients delivered into the soil, or the longevity of nutrients and their effects on other biota or ecosystem attributes (Parmenter and MacMahon 2009; Barton et al. 2019a; Keenan and DeBruyn 2019).

The soil under an animal carcass has been shown to change in nutrients and chemical properties over time as decomposition progresses (Parmenter and MacMahon 2009). Organic and inorganic nitrogen (N) compounds and potassium (K) and sodium (Na) associated with soft tissues (proteins, fats, body fluids) are among the first to be released during decomposition (Parmenter and MacMahon 2009; Forbes and Carter 2015). Skeletal components such as calcium (Ca) and phosphorus (P) are released more slowly and may remain in the soil for longer than other nutrients (Parmenter and MacMahon 2009). For instance, after 40 months of decomposition of a wolf-killed moose (*Alces alces*) carcass, the amount of phosphorus found in the soil was double that of control sites (Bump et al. 2009). Similarly, phosphorus in soil under kangaroo carcasses (*Macropus giganteus*) were eight times

higher than control sites after 60 months of decomposition (Barton et al. 2016).

Carrion decomposition not only affects the nutrient dynamics in the soil, but also changes the physical properties of the soil in the CDI (Carter et al. 2007; Keenan et al. 2018). Due to the alkaline and ionic nature of decomposition products, changes in the pH and electrical conductivity are expected in the soil under the carcass (Benninger et al. 2008). Moreover, the draining of liquids from the carcass can enhance the moisture content of the soil, while the metabolic activity of microbes and fly larvae feeding on the carcass can increase its internal temperature relative to ambient temperatures (Barton et al. 2019b). Carcass decomposition rates and effects on soil are strongly moderated by moisture and temperature (Benninger et al. 2008; Carter et al. 2008), which means understanding the associations between carcasses, soil nutrients and abiotic environmental variables is crucial (Carter et al. 2010; Benbow et al. 2019). The interlinked processes occurring during decomposition have typically been observed and measured by quantifying changes in temperature and moisture, concentrations of the total and soluble fractions of carbon, nitrogen and phosphorus, as well as pH and electrical conductivity (e.g. Benninger et al. 2008; Bump et al. 2009; Macdonald et al. 2014). Yet very few studies have examined these multiple parameters simultaneously from the early stage of decomposition and at short time intervals.

In this study, we examined changes in a suite of soil physicochemical properties occurring under decomposing vertebrate carcasses (rabbit, *Oryctolagus cuniculus*). We used a controlled and replicated carcass addition experiment to quantify both the effect of carcasses on soil chemistry and the temporal dynamics in soil chemistry associated with rapid carcass mass loss during active and advanced decay. We asked the following questions:

- (1) *What abiotic changes occur under carcasses during decomposition?* Temperature and moisture are two abiotic factors that have strong effects on decomposition processes in soil, but might also be affected by carcass fluids and thermal properties.
- (2) *Are different carcass nutrients delivered into the soil at the active and advanced stages of decomposition?* As a carcass decomposes, soft

tissues (muscles, internal organs) will decay first and be consumed by fly larvae, whereas tougher tissues (bone, hair) will decay more slowly and release different nutrients into the soil at different times and in different amounts.

- (3) *Which soil chemistry variables are correlated through the decomposition process?* We predicted that several nutrients and chemical properties would change in the soil together, as large organic molecules were broken down and metabolised by microbes and fly larvae. We therefore wanted to characterise these soil nutrient combinations to identify what soil chemistry markers might be good predictors of decomposition stage.

## Methods

### Study area and experimental design

Our experiment used 25 rabbit carcasses arranged in a randomised block design (five blocks of five carcasses), in a grassland near Canberra, south-eastern Australia (Barton et al. 2019b). The grassland is dominated by the native perennial grass *Themeda australis*, has a silty-loam soil, and an A-horizon to a depth of 10–15 cm. The wider landscape is characterised by undulating low hills, with grasslands occurring on the deeper soils of valleys and plains characterised by chromosols, sodosols and kurosols (Isbell, 2002) or luvisols and solonetz (FAO 1974). For broader landscape and soil descriptions of the study area see McIntyre et al. (2010).

Carcasses were of invasive wild European rabbits (*Oryctolagus cuniculus*) killed by firearms. We did not kill the rabbits for the purpose of this study, but sourced them from a third party that performs routine and government licenced pest control operations. We used rabbits that were killed on the same night to ensure a similar time of death, and we used only whole carcasses of mature adult rabbits. We placed carcasses into refrigeration on the same night, and they remained refrigerated at 3 °C for 48 h until deployment in the field. Prior to deployment, we weighed each individual carcass to determine their starting mass.

We placed each carcass on level ground, and paired it with an adjacent control site 1-m away (Fig. S1). We

determined that a distance of 1-m between each carcass treatment and paired control was sufficient to prevent contamination and minimize differences in local soil properties. Experimental blocks were approximately 40 metres apart, and carcasses within each block were spaced approximately 5 metres from each other. We deployed all carcasses during the morning of the first day of the experiment (day 0), and took soil samples on the same day. We then destructively sampled one carcass and associated soil cores from each block ( $n = 5$ ) every 4 days until the end of the experiment at day 20, thus providing data for days 0, 4, 8, 12, 16, and 20. Previous empirical data have shown that this amount of time is sufficient to capture the majority of mass loss under similar abiotic conditions (Barton and Evans 2017).

### Temperature, rainfall, and soil moisture

We collected temperature and rainfall data using an on-site rain gauge and temperature data loggers (TC ThermoChron<sup>®</sup>) (Fig. S1). We placed a temperature logger on the soil surface at three different rabbit carcasses and their paired control sites. Loggers at carcasses were placed under the centre of mass, and loggers at control sites were placed under the grass out of direct sunlight. We programmed the loggers to collect temperature data every 30 min for the entire duration of the experiment. We summed data for every 24 h period, then divided by 48 to give a mean daily temperature. We then summed mean daily temperatures to give accumulated degree days (ADD) (Megyesi et al. 2005), which is a biologically meaningful integration of time and thermal energy underpinning insect development and other metabolic processes.

We also examined soil moisture changes at fine temporal scales using EC-5 soil moisture sensors (Decagon Devices Inc., Pullman, WA; accuracy  $\pm 1$ –2% VWC) placed at two depths (15 mm and 75 mm) at a single carcass and its paired control (Fig. S1). Two depths were chosen to detect the depth of penetration of moisture from carcasses. These data were recorded as volumetric water content (%) and collected at 10-min intervals. The data were not replicated, but did give unprecedented detail of the fine-scale moisture changes occurring under a carcass due to inputs from carcass fluids and rainfall, and assisted with the interpretation of soil chemistry and moisture changes.

## Soil sampling

We collected soil samples at days 0, 4, 8, 12, 16, 20. We lifted the carcasses off the ground and placed them into plastic bags for further assessment (Barton et al. 2019b). Soil samples were taken at each carcass and paired control site using cores (50 mm diameter) to a depth of 30 mm directly beneath each carcass. This depth has been shown previously to capture important soil chemical changes under carcasses (Macdonald et al. 2014). Three soil cores were taken for nutrient analysis, pooled on site, and then transported to the laboratory where they were allowed to air dry for several days. We took an additional core using a bulk density ring to a depth of 30 mm for determination of soil bulk density and gravimetric water content (GWC%). These samples were sealed in the field to prevent moisture loss, then transported to the laboratory.

## Laboratory processing of samples

Each rabbit carcass was weighed prior to deployment (start mass (g)) and after collection (end mass (g)). Carcasses were oven dried at 45 °C after collection until mass was stable, and then weighed again to give dry mass. We used all soil samples ( $n = 5$ ) from each sampling time (day 0, 4, 8, 12, 16, 20). We determined gravimetric water content and soil bulk density by weighing on return to the laboratory, then drying at 105 °C until a constant mass was achieved (Rayment and Higginson 1992). Soils cores collected for chemical analysis were homogenized by light grinding with a mortar and pestle to reduce the size of aggregates, removal of extraneous organic matter and invertebrates, with the soil then passed through a 2 mm sieve. Electrical conductivity (EC) and pH were measured on 1:5 soil to water extracts using a TPS WP-81m. Total carbon (C) and total nitrogen (N) were determined with Dumas dry combustion and conductometric analysis (Vario Max CNS, Elementar, Germany) (Matejovic 1997). Total phosphorus (P) was determined after Kjeldahl digestion at 350°C. Ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) were determined following a 1:10 soil to 2 M KCl extraction. Orthophosphate ( $\text{PO}_4^{3-}$ ) was determined following a 1:40 soil to 0.5 M sodium bicarbonate (pH 8.5) extraction. Analyte concentrations were then determined colourimetrically using flow injection autoanalysis (Lachat

Instruments, Milwaukee, Wisconsin, USA)(Diamond 2006).

## Statistical analysis

We quantified the effect of time (day of experiment) on carcass wet mass using a linear mixed model. We fitted time as a categorical fixed effect (6 levels) and experimental block as a random effect.

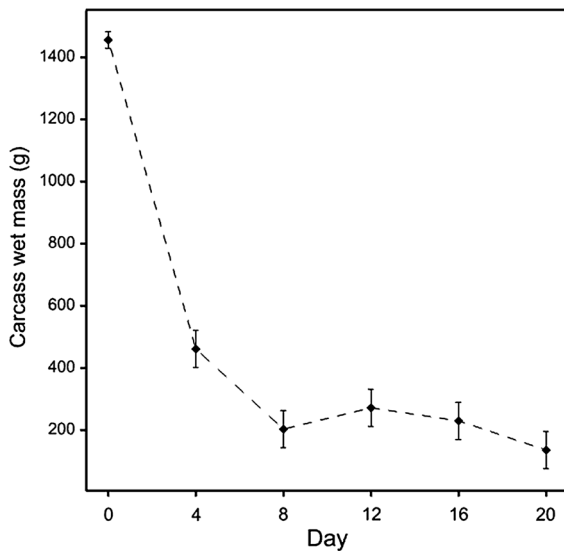
We examined the fine-scale soil moisture changes (as % volumetric water content) recorded by our probes at two depths by first calculating the 12-h moving average of the soil moisture content recorded every 10 min. We plotted these data with recorded rainfall to assess the temporal dynamics of soil moisture. We also calculated the 12-h moving average of half-hourly temperature measurements recorded under carcasses and controls with the thermochron data loggers.

We used linear mixed models to quantify the effects of time, carcass addition, and their interaction on each of the soil chemistry measures (C, N, C:N, P,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ , EC, pH, GWC%). We fitted experimental block as a random effect to account for potential spatial effects, and conducted all our models using Genstat 18 (VSN International Ltd. 2016).

Finally, we wanted to explore the correlations among the soil physicochemical properties we measured, and how these changed during decomposition. We therefore performed a principal components analysis (PCA) of eight soil variables (GWC%, pH, EC, C:N ratio, P,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$ ) to quantify co-linearity among variables and identify what suite of variables changed together or independently (Gotelli and Ellison 2004). We used a correlation matrix of the data as the variables were quantified using different methods and units, and performed this analysis using PC-Ord 7 (McCune and Mefford 2018).

## Results

We found a strong negative effect of time on the proportion of carcass mass remaining ( $F = 202.74$ ,  $P < 0.001$ ). Carcasses reduced in mass by approximately 90% over 20 days of decomposition, with more than 60% lost in the first 4 days (Fig. 1). Colonisation of carcasses by flies was observed during the first few hours, with bloat occurring within the first



**Fig. 1** The proportion of carcass mass remaining over 20 days of decomposition. Means are model estimates with standard errors ( $n = 5$  at each time point)

day. Active decay progressed rapidly, and larval masses were obvious at day 4, with substantial and obvious cavities in carcasses. Advanced decay was reached by day 8, and many larvae had developed into pupae and active larvae were less abundant. Carcasses at day 12 entered the dry decay stages and appeared as largely fur, skin and bones only. At this stage, the relative dominance of arthropods switched to beetles (adults and larvae).

Carcass temperatures reached higher maximums than control sites, and were up to 15 °C warmer during days 3–5 when carcasses were undergoing active decay and had large active larval masses (Fig. 2a) (see also Fig. S2). Soil volumetric water content (VWC%) under carcasses at a depth of 15 mm had a notable increase during day 3 of decomposition, and was subsequently always higher than the control sites (Fig. 2b). Soil moisture did not show any change at carcasses at a depth of 75 mm, but did respond to a rainfall event at day 12.

We found no effect of carcass addition on total carbon (Fig. 3a, Table 1), but a positive effect on total nitrogen, which increased by approximately 40% at day 4, and was then relatively stable (Fig. 3b). This was reflected by soil C:N ratio (Fig. 3c). We also found an interaction between carcass addition and time for soil total phosphorous (Table 1), which continued to increase following carcass addition

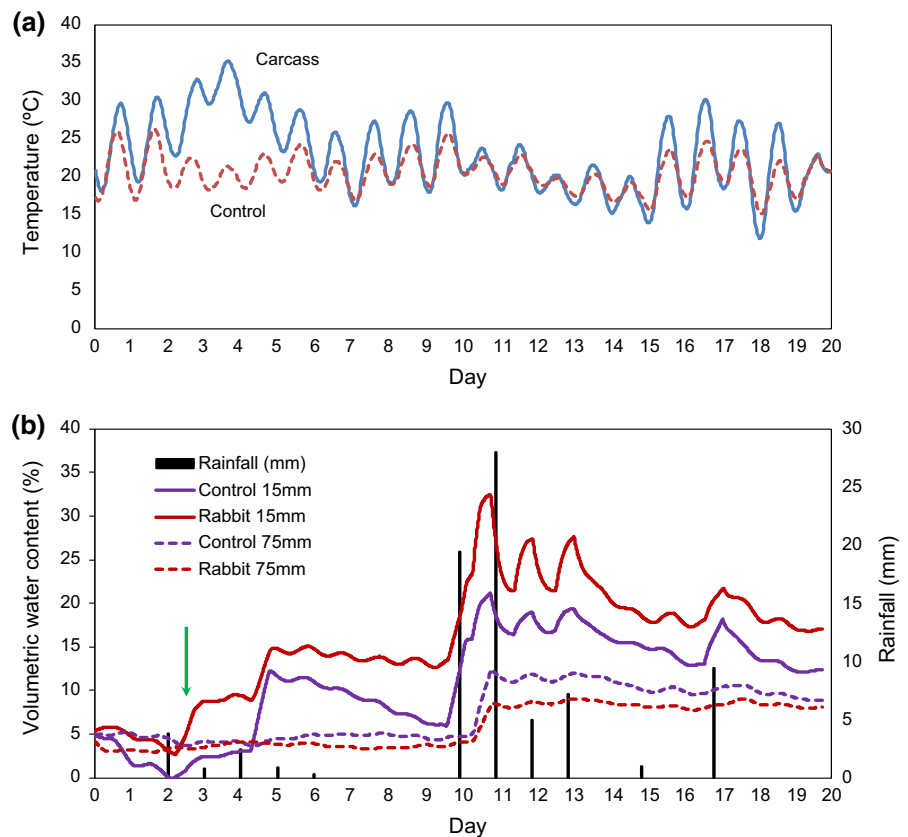
(Fig. 3d). We also found a significant effect of carcass addition on soluble forms of nitrogen and phosphorous, as well as pH, EC and GWC% (Table 1). Ammonium concentrations were approximately 20 times higher than control levels from day 4, and remained high (Fig. 4a). However, nitrate did not change under carcasses, but did increase slightly at controls following rainfall at day 12 (Fig. 4b). Phosphate continued to increase in concentration under carcasses for the duration of the experiment (Fig. 4c). Soil pH and EC increased under carcasses from day 4 (Fig. 4d, e), and soil gravimetric water content (GWC%) also increased at day 4 and remained higher than controls for the duration of the experiment (Fig. 4f).

Principal components analysis reduced the seven soil variables down to three new axes explaining 97% of the total variation (Table 2). The first axis explained 80% of total variation, and had positive correlations with  $\text{NH}_4^+$ , pH, and EC, and a negative correlation with C:N ratio. The second axis had a strong positive correlation with  $\text{NO}_3^-$ , whereas the third axis had positive correlations with total and available phosphorus. When plotted as ordinations, the control samples were clearly separated from the carcass samples by the first principal component (elevated nitrogen, pH, EC), and further separation of carcass samples along the third principal component (increasing phosphorus over time, Fig. 5). There was no informative separation of sample times along the second principal component ( $\text{NO}_3^-$ , Fig. S3). Information about nitrogen and phosphorous therefore indicated that distinct decomposition stages are able to be detected in the soil profile.

## Discussion

We set out to quantify changes in soil physicochemical properties under decomposing vertebrate carcasses during active and advanced decay. Our choice to sample at intervals of 4 days has enabled us to reveal the dynamics of nutrient inputs occurring at relatively short time intervals, including the different dynamics of nitrogen and phosphorous. This is a key point of difference from other soil and carcass decomposition studies. Below we discuss the patterns we observed and the processes causing them.

**Fig. 2 a** Changes in temperature under rabbit carcasses and nearby controls expressed as the 12 h moving average of data collected at 30-minute intervals. Higher temperatures were obvious under carcasses during days 3–5 of decomposition. **b** Changes in soil volumetric water content expressed as a 12 h moving average of data collected at 10-minute intervals under a rabbit carcass and nearby control at two depths (15 mm and 75 mm). Carcass effects are evident only at 15 mm depth, with greater relative moisture input occurring during day 3 (arrow). Only moisture from a major rainfall event penetrated to 75 mm during day 11



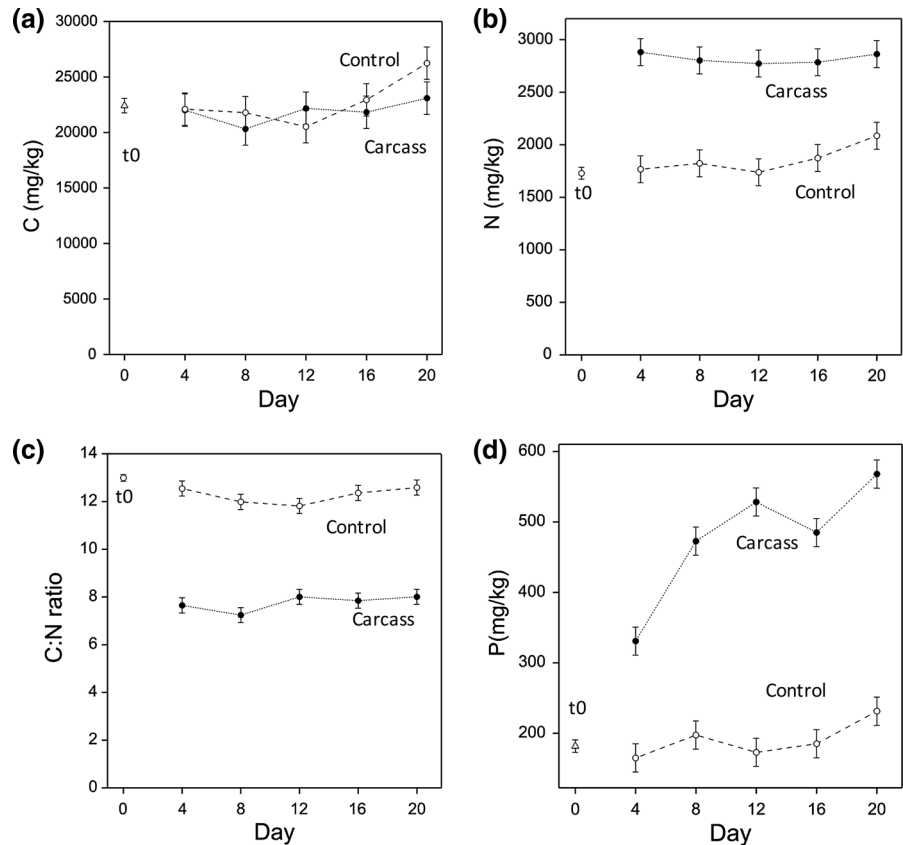
### Decomposition rate and abiotic effects

The decomposition of the rabbit carcasses was very rapid due to the high temperatures that occurred during the experiment. We observed the arrival of flies during the start of the experiment, and observed active larval masses by day 4 (Barton et al. 2019b). This coincided with the peak in carcass temperatures during days 3–5, which is likely due to the metabolic activity of both the larvae and the proliferation of bacteria (Turner and Howard 1992). We deliberately excluded vertebrate scavengers from our carcasses, which will often consume small carcasses very quickly, including the skin, ligaments, hair, and bones (Cunningham et al. 2018). Yet we found that the mass loss of the carcasses reached over 90%. This highlights the impressive functional role of flies in carrion removal from landscapes, a finding also reported elsewhere (Payne 1965; Pechal et al. 2014; Barton and Evans 2017).

The moisture input from carcasses occurred mostly during day 3, and was then consistently higher than control sites. This indicates that fluid leakage occurred

after bloat and the onset of active decay and insect activity, but also suggests the microenvironments created by the carcasses appears to maintain stable moisture levels. In our hot and dry study environment, a stable moisture microenvironment is likely to be important for the persistence of insect and microbial activity, and therefore the continued decomposition of the carcass and subsequent nutrient recycling and dispersal. Our detection of soil moisture changes contrasts with findings reported by Benninger et al. (2008), who reported no effect of carcasses on soil moisture despite their use of much larger pig *Sus scrofa* carcasses. The soil moisture sensors indicated clear changes at 15 mm depth, but little change at 75 mm. This indicates that nutrient inputs to the soil occurred at depths shallower than 75 mm. This differs from a study of guinea pig carcasses (620 g, *Cavia porcellus*) which found changes extended to 14 cm into sandy soil (Bornemissza 1957), and elephant carcasses (*Loxodonta africana*, 1629 kg) that extended to 40 cm into sandy loam soil (Coe 1978). The soil moisture and temperature peaks that we found

**Fig. 3** Changes in **a** total carbon, **b** total nitrogen, **c** C:N ratio, and **d** total phosphorous in soil under carcasses and at control sites measured at 4-day intervals. Means are model estimates with standard errors ( $n = 5$ )



during active decay reinforces the idea of the ‘CDI’ effect that also extends to abiotic factors (Carter et al. 2007). However, both environmental context and abiotic factors, as well as decomposition rate and soil type, likely interact to determine the magnitude of the soil moisture and temperature dynamics occurring under decomposing carcasses.

#### Soil macronutrients

Soil total carbon did not change during decomposition of our rabbit carcasses, which contrasts with the result of Keenan et al. (2018), who reported significant changes in carbon between decay stages of beaver carcasses. The absence of soil carbon changes in our study might be due to the extremely rapid evolution of  $\text{CO}_2$  or  $\text{CH}_4$  resulting from the microbial processing of organic matter, potentially offsetting inputs from the carcasses. However, we remain unsure about why carbon was not elevated during active decay after initial large inputs of carcass fluids and other nutrients increased in the soil profile.

We found differing patterns of nitrogen and phosphorus inputs to the soil. Protein is the main source of nitrogen from carrion, and these large molecules are broken down via proteolysis into smaller proteoses, peptones, polypeptides and amino acids (Macdonald et al. 2014). Inputs of nitrogen may be released into the soil following bloat and the onset of active decay when autolysis and fluid leakage occur, as well as during active tissue putrefaction, liquefaction and disintegration stages. We found that total nitrogen peaked at day 4 of decomposition, which was during active decay, and so was likely the product of autolysis and fluid leakage. This was similar for the inorganic fraction ( $\text{NH}_4^+$ , ammonium), but which was also likely contributed to by fly larvae activity and excretion (Chapman 1998). Given the highly labile nature of ammonium and its rapid uptake by plants (Swift et al. 1979), our measure of N might be an underestimate, further underscoring the remarkable concentrations generated by decomposing carcasses. Of note was the absence of elevated nitrate ( $\text{NO}_3^-$ ) levels under our rabbit carcasses. This also contrasts with the study by

**Table 1** Summary of linear mixed models testing for the effects of time (Day), treatment (Treat), and their interaction (Day × Treat) on soil attributes

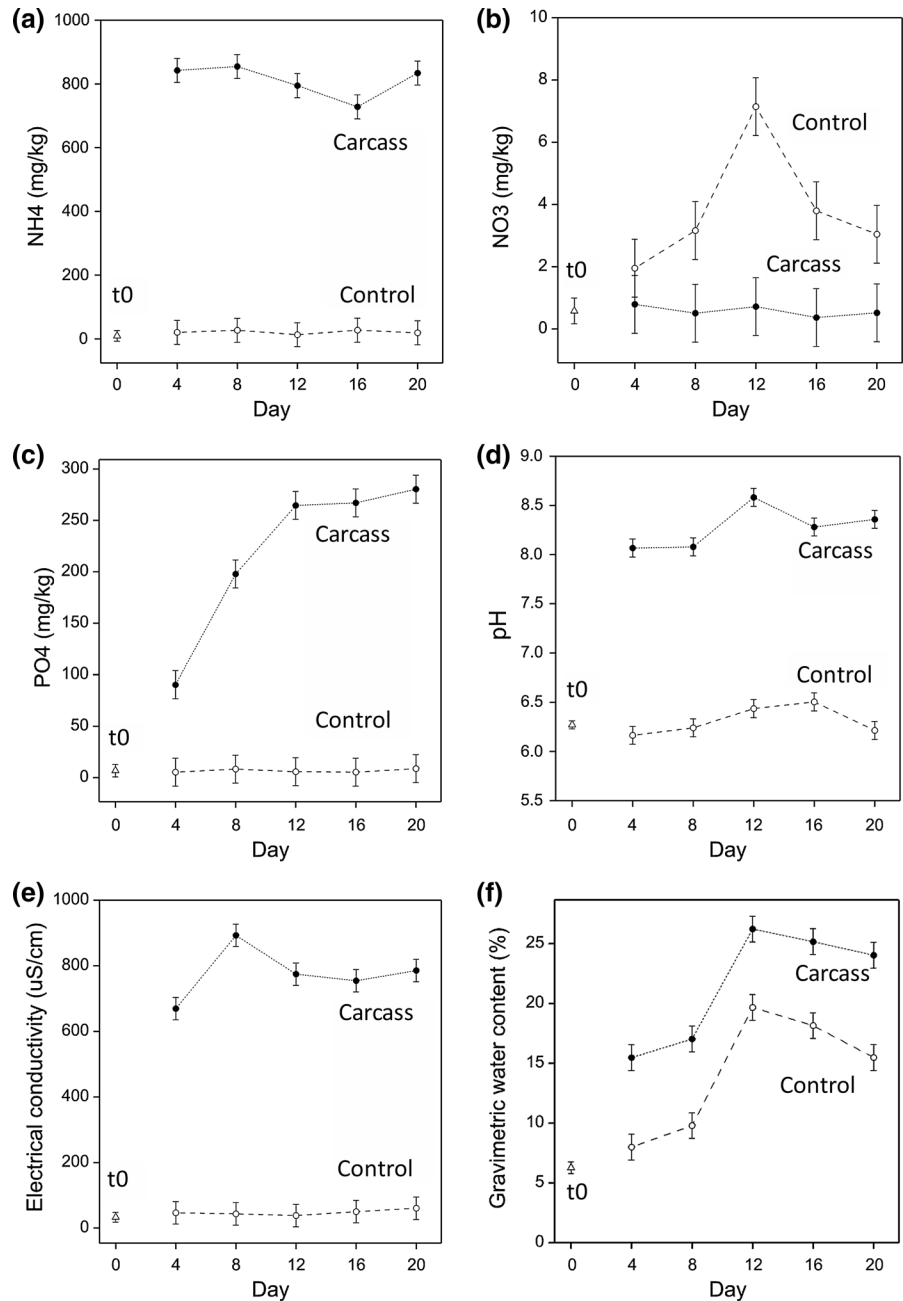
Fixed term	d.f.	F statistic	p value
<b>C:N ratio</b>			
Day	5	60.66	< 0.001
Treat	1	509.1	< 0.001
Day × Treat	4	0.88	0.483
<b>C</b>			
Day	5	1.53	0.194
Treat	1	0.82	0.369
Day × Treat	4	0.74	0.571
<b>N</b>			
Day	5	15.78	< 0.001
Treat	1	141.46	< 0.001
Day × Treat	4	0.5	0.735
<b>P</b>			
Day	5	50.46	< 0.001
Treat	1	513.19	< 0.001
Day × Treat	4	6.9	< 0.001
<b>NH<sub>4</sub><sup>+</sup></b>			
Day	5	78.69	< 0.001
Treat	1	1102.27	< 0.001
Day × Treat	4	0.98	0.424
<b>NO<sub>3</sub><sup>-</sup></b>			
Day	5	3.89	0.004
Treat	1	30.39	< 0.001
Day × Treat	4	2.23	0.077
<b>PO<sub>4</sub><sup>3-</sup></b>			
Day	5	54.79	< 0.001
Treat	1	616.42	< 0.001
Day × Treat	4	16.83	< 0.001
<b>pH</b>			
Day	5	88.48	< 0.001
Treat	1	1153.72	< 0.001
Day × Treat	4	1.81	0.139
<b>EC</b>			
Day	5	83.67	< 0.001
Treat	1	1129.92	< 0.001
Day × Treat	4	2.82	0.033
<b>GWC%</b>			
Day	5	112.91	< 0.001
Treat	1	117.22	< 0.001
Day × Treat	4	0.24	0.915

Keenan et al. (2018), who found higher nitrate levels under beaver carcasses during dry and skeletal stages of decay. Our study, however, did not follow through to complete skeletonization, and so may not have progressed for sufficient time for us to detect elevated nitrate. We suggest there was also potential for inhibition of the nitrification process during early and active decay in our study. The nitrification process involves the biological oxidation of ammonium to nitrites, and then to nitrates, and is driven by microbes (Swift et al. 1979). Inhibition of soil microbes can occur with high pH, low oxygen, and high concentrations of ammonium (Keenan et al. 2018). Our results showed both pH and ammonium levels were very high in the soils throughout our study, and so was likely to be limiting the microbial nitrification process.

We found that phosphorus increased at day 4 of the experiment, reflecting the phosphorus contained in the decomposition fluids. However, levels of total phosphorus, and particularly soluble phosphate, continued to increase through to day 20 of the experiment. The more gradual increase in phosphorus relative to nitrogen might be explained by slower lipid breakdown compared with proteins (Bickel and Tang 2010), with proteins readily metabolized by soil microbes (Macdonald et al. 2014). However, both total phosphorus and phosphate increased over time, suggesting it continued to enter the soil profile after carcass proteins and fluids had stopped. Phosphorus is found in all animal tissues, but is more concentrated in bones as calcium phosphate (Subalusky et al. 2017; Barton et al. 2019b). The gradual increase in soil phosphorus that we recorded is probably indicative of the skeletal bones becoming exposed, and then entering the soil profile with the assistance of larger insects and rain. Initial quantities of phosphorus entering the soil from the carcasses are largely in organic forms, and are mineralised into inorganic forms by soil microbes (Swift et al. 1979). The rapid rise in phosphate that we detected indicates that mineralisation of the organic phosphates occurred very rapidly, and this was far beyond the uptake capacity of nearby plants. The extended elevated levels of soil phosphorus reported in other studies (Barton et al. 2016; Bump et al. 2009) suggests that carcasses may continue to drive localised phosphorus cycling for many months.



**Fig. 4** Changes in soil attributes occurring at 4 day intervals during decomposition of rabbit carcasses, including concentrations of **a** ammonium, **b** nitrate, **c** phosphate, **d** pH, **e** electrical conductivity, and **f** gravimetric water content. Means are model estimates with standard errors ( $n = 5$ )



### Linking patterns and processes

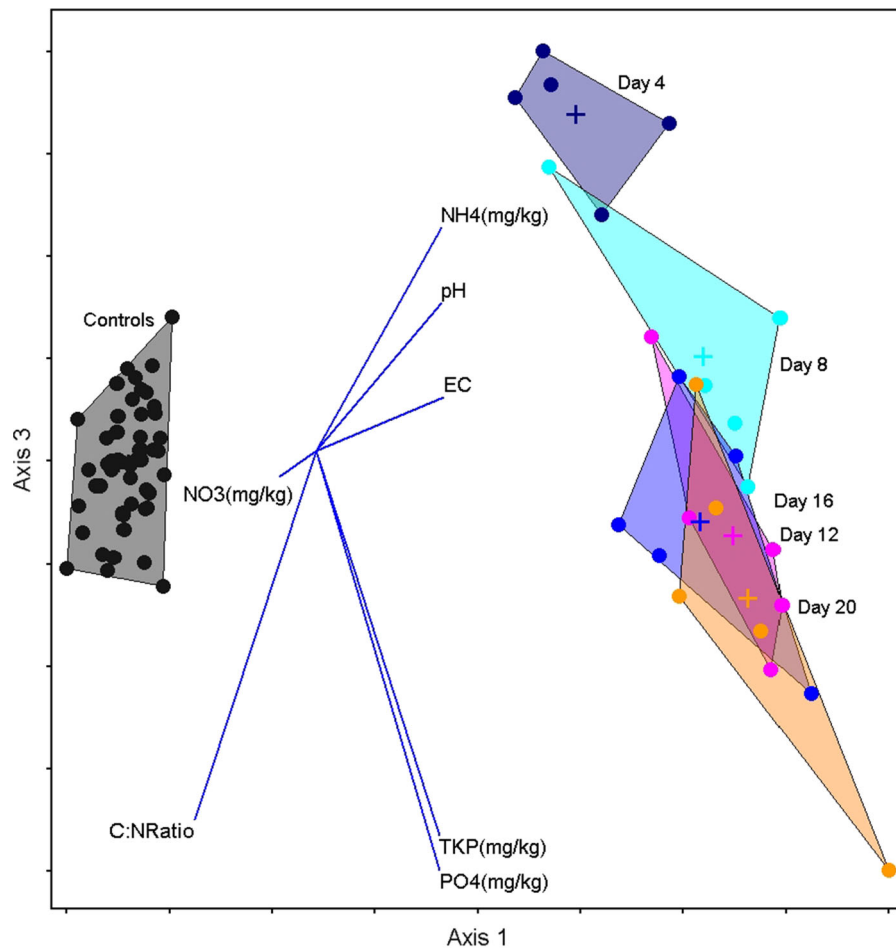
The combined soil changes that occur during active decomposition has, so far, received little attention (but see Keenan et al. 2018). We found strong correlations among soil parameters according to decay stage, with day 4 (active decay) characterized by elevated ammonium and pH, whereas Day 8 (advanced decay)

had relatively higher values for electrical conductivity. This is indicative of key decomposition processes, including rapid mass loss and the leaching of body fluids containing cations (i.e.  $\text{NH}_4^+$ ) and salts (ions) of sodium (Na) and potassium (K) into the soil, as well as the excretion of ammonium by large active larval masses (Barton et al. 2019b). Later decay stages from day 12 onwards were characterized by higher relative

**Table 2** Summary of principal components analysis—variable loadings for three principal components summarizing seven soil parameters

Soil measure	PC1	PC2	PC3
C:N ratio	− <b>0.624</b>	− 0.115	0.184
Electrical conductivity ( $\mu\text{S}/\text{cm}$ )	<b>0.385</b>	− 0.021	0.175
pH	<b>0.449</b>	− 0.048	0.063
$\text{NH}_4^+$ (mg/kg)	<b>0.510</b>	− 0.030	− 0.020
$\text{NO}_3^-$ (mg/kg)	− 0.028	<b>0.990</b>	0.020
$\text{PO}_4^{3-}$ (mg/kg)	0.005	− 0.034	<b>0.699</b>
P (mg/kg)	0.040	0.046	<b>0.665</b>
Percentage variation	80.470	13.570	3.300

Important loadings are shown in bold



**Fig. 5** Ordination of the first and third principal components showing soil samples taken from control sites at day 0, and carcasses as days 4, 8, 12, 16, and 20 of decomposition. Soil

phosphorus concentrations, and appears to be indicative of advanced and dry decay when bone and lipid degradation are occurring (Benninger et al. 2008; Parmenter and MacMahon 2009; Keenan et al. 2018). Our findings are broadly similar to the soil nutrient

variables correlated with each axis are shown, with longer vectors indicating stronger correlations, see Table 2)

conditions described by Keenan et al. (2018), and suggests that carcass decomposition effects on soil may be generally predictable across different carcass species and different biomes. This is important because the few studies on soil responses to carcass

decomposition are from distinct ecosystems, including: bison (*Bison bonasus*) in a forest ecosystem (Melis et al. 2007), bison (*Bos bison*) in a prairie ecosystems (Towne 2000), deer (*Odocoileus virginianus*) in a forest ecosystem (Bump et al. 2009), kangaroos (*Macropus giganteus*) in grassy woodland (Macdonald et al. 2014; Barton et al. 2016), and rats (*Rattus norvegicus*) in a shrub-steppe ecosystem (Parmenter and MacMahon 2009). Synthesis across ecosystems is lacking, but evidence is now accumulating for a general and predictable sequence of carcass nutrient transfer to soils (Parmenter and MacMahon 2009; Keenan et al. 2018).

### Implications and conclusions

From an ecosystem perspective, only a small fraction of carcass nutrients enter the soil compared with the nutrients that are consumed by flies (Barton et al. 2019b) or scavenged by vertebrates (Peisley et al. 2017). Nevertheless, the nutrients are often very concentrated, generate a mosaic of nutrient hotspots through a landscape, and contribute to the maintenance and soil fertility and heterogeneity (Towne 2000; Bump et al. 2009; Barton et al. 2013). The abundance and distribution of even small carcasses could have an important role in ecosystems. Nutrients delivered into soil create a hotspot of fertility that may last for several years for larger carcasses such as kangaroos (Barton et al. 2016) or deer (Bump et al. 2009). For smaller carcasses, the effect will be proportionally smaller, but still might be noteworthy for nutrient poor soils (Macdonald et al. 2014). In Australia, rabbits are an invasive pest and are controlled by shooting, poisoning, and biological agents (myxoma virus, calicivirus) across vast areas (Ratcliffe et al. 1952; Saunders et al. 2010). Dead animals are often left in situ and their carcasses return some nutrients into the soil. In the context of our study area, and in much of southeastern Australia where rabbit populations are established, the contribution of rabbit carcasses to biogeochemical cycling is likely to be significant. For example, Barton et al. (2019a) give an example of 50 rabbit carcasses per km<sup>2</sup> per year delivering approximately 10 kg of nutrients and moisture into the soil, albeit very localised at carcass sites. This number is likely to be significantly higher following culling events, or the introduction of biological control agents, possibly providing ecologically important nutrient pulses.

Our study has shown how multiple soil parameters change during the early and advanced stages of decomposition, including the sequential inputs of moisture, salts, nitrogen, and phosphorus. These results provide new insight into the fine-scale temporal processes occurring under carcasses from an Australian context, and corresponds with the decomposition phases associated with rapid ‘soft’ tissue breakdown and fly activity, and ‘hard’ tissue breakdown of keratinous tissues and skeletal remains. Our study provides new information about the fine-scale timing of nutrient inputs and underpinning processes occurring at the carcass/soil interface.

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