Insect reproductive behaviors are important mediators of carrion nutrient release into soil

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Current declines in terrestrial insect biomass and abundance have raised global concern for the fate of insects and the ecosystem services they provide. However, the ecological and economic contributions of many insects have yet to be quantified. Carrion-specializing invertebrates are important mediators of carrion decomposition; however, the role of their reproductive activities in facilitating this nutrient pulse into ecosystems is poorly understood. Here, we investigate whether insects that sequester carrion belowground for reproduction alter soil biotic and abiotic properties in North American temperate forests. We conducted a field experiment that measured soil conditions in control, surface carrion alone, and beetle-utilized carrion treatments. Our data demonstrate that *Nicrophorus* beetle reproduction and development results in changes in soil characteristics which are consistent with those observed in surface carrion decomposition alone. Carrion addition treatments increase soil labile C, DON and DOC, while soil pH and microbial C:N ratios decrease. This study demonstrates that the decomposition of carrion drives soil changes but suggests that the behaviors of insect scavengers play an important role in the release of carrion nutrients directly into the soil by sequestering carrion resources in the ecosystem where they were deposited.

Historic declines in terrestrial insects have been documented globally1–3. Among these declines, both specialist and generalist insect populations have been effected, with Coleoptera (beetles) and Lepidoptera (butterflies and moths) experiencing elevated annual rates of decline relative to other insect taxa4. These patterns of decline are well understood in temperate regions relative to tropical ecosystems2,5; however, our knowledge of susceptible insect groups is constrained to measurements of overall insect abundances and to species in well studied taxa and ecosystems. Regardless, these observed declines have raised concern among scientists regarding the potential impact that reduced insect populations may have on ecosystems. For instance, in addition to serving as a primary food source for a variety of organisms, insects provide other ecosystem services which are valued at approximately 57 billion USD annually6. However, insect effects are often overlooked based on their relative contribution to total biomass across ecosystems, particularly in comparison to plant and microbial biomass7. Yet, research has demonstrated that insects can have strong indirect effects on soil and nutrient availability8–15. Still, there remains a large gap in the literature with respect to how less well-studied insects and their behaviors modulate soil habitat and nutrient availability.

Across ecosystems, carrion serves as a long-lasting and concentrated source of nutrients16. Although the contribution of large carrion to soil nutrients and microbial biomass is well-documented17–20, few studies investigate small vertebrate carcasses and the role of individual insect behaviors in the release of these concentrated nutrients. Rather, the majority of studies document necrophilous insect succession patterns to understand how community assemblages contribute to carcass degradation21–24, with results indicating insect activity is essential to increasing decomposition rates22,25–29. Although it is recognized that insect behaviors are important contributors to decomposition, few studies have directly quantified how specific behaviors, such as those related to mating and reproduction, may contribute to soil nutrient cycling and the microbial community.

Burying beetles (Coleoptera: Silphidae: *Nicrophorus*) are well-suited for investigating how insects modulate carcass decomposition, as they scavenge and sequester carrion belowground and utilize it as a reproductive resource30,31. Species such as *Nicrophorus orbicollis* locate carcasses during flight by detecting volatized chemical cues, and immediately following carrion discovery, a male and female burying beetle pair will collaboratively work to bury the carcass to variable depths within the soil30,32. During burial, beetle pairs will strip the fur or feathers from the carrion and roll it into a mass of meat referred to as the brood ball30–32. Beetle pairs will copulate frequently during this time33–35, while also coating the carcass with oral and anal exudates containing

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antimicrobial compounds that delay microbial-mediated decomposition. During carcass burial and preparation, females lay eggs in the surrounding soil and after approximately three to five days, eggs hatch and larvae arrive on the carcass where pairs provide biparental care to developing young. Approximately 8–11 days following larval arrival on the carcass, parental care is terminated and larvae disperse in the surrounding soil, where they pupate, then eclose as adults.

Recent research demonstrated that in forest habitats, burying beetles are able to sequester up to 75% of small vertebrate carrion (e.g., field mice) for reproduction, indicating that beetles are one of the primary insect groups engaged with facilitating the decomposition of small carrion in forest ecosystems. However, although carrion sequestration by Nicrophorine burying beetles has historically and anecdotally been considered an essential process to facilitate nutrient release into soil, recent research is the first to quantitatively support this, albeit in an artificial lab setting. Here we describe an experimental field study designed to assess whether burying beetle reproduction affects soil nutrient cycling and microbial biomass relative to surface carrion decomposition alone (i.e., no insect involvement) in a northern deciduous forest. Our objective was to determine how beetle-mediated carcass burial and utilization affects soil abiotic and biotic properties in comparison to soils with no biological input and determine whether these changes were consistent to those observed during carrion decomposition in the absence of these insect behaviors.

Results
Soil abiotic characteristics. Principal coordinates analysis indicated that there was strong separation in soil abiotic characteristics between carcass addition study plots and control treatments along axis 1 (Fig. 1). The PCoA explained 89% of the total variation in soil abiotic characteristics, with 58% of the variation explained by axis 1. Axis 1 was largely explained by the covariances of soil DON, DOC and DOC:N, while soil NO$_3^-$ covaried with axis 2. PERMANOVA indicated that the observed separation between carrion addition treatments and the control within the ordination was significant ($F_{2,23} = 5.02; R^2 = 0.32; P < 0.001$), as both the carcass only (CO) and carcass plus burying beetle (CB) treatments exhibited significantly different soil abiotic characteristics as compared to the control plots ($P < 0.01$). There was no difference in soil abiotic characteristics between the carcass only and carcass burying beetle treatments.

Subsequent analysis of variance indicated an effect of treatment on soil pH ($F_{2,21} = 91.03; P < 0.001$), with Tukey’s mean separation indicating that the burying beetle plots were significantly more basic than both the carcass only ($P < 0.01$) and control treatments ($P < 0.001$), while carcass only treatments were significantly more basic than the control soil ($P < 0.001$; Table 1). However, the observed difference between the carcass only and carcass burying beetle treatments was relatively small relative to the difference between the control and carrion addition treatments (Table 1). Treatment did not influence soil moisture ($F_{2,21} = 1.44; P = 0.26$), and there was no effect of treatment on soil inorganic N levels.
Soil C mineralization, an index of bioavailable C, differed among treatments ($F_{2,21} = 4.93; P < 0.05$) (Table 1, Fig. 2). Specifically, the burying beetle treatments exhibited a significantly greater labile C pool compared to the controls ($P < 0.05$). Soil dissolved organic carbon (DOC; non-fumigated samples) also significantly differed among treatments ($F_{2,23} = 17.59; P < 0.001$), with both the CO ($P < 0.01$) and CB ($P < 0.001$) treatments exhibiting greater DOC than controls (Table 1, Fig. 2). With respect to dissolved organic nitrogen (DON), carcass addition plots exhibited significantly greater levels of DON than the controls ($F_{2,21} = 21.87; P < 0.001$; Tukey HSD test: $P < 0.001$) (Table 1, Fig. 2). These changes in DOC and N resulted in significant decreases in the ratio between DOC:N ($F_{2,21} = 114.38; P < 0.001$) in the CO ($P < 0.01$) and CB treatments (2018: $P < 0.05$) compared to controls.

The effect of treatment on total soil C:N ratio was consistent with these findings, with the controls exhibiting significantly greater soil C:N ratios relative to the CO ($P < 0.05$) and CB treatments ($P < 0.01$). However, there was no difference in total C ($F_{2,21} = 0.19; P = 0.98$) or N ($F_{2,21} = 1.86; P = 0.18$) among treatments.

**Microbial biomass and community composition.** Analysis of variance indicated microbial biomass N was greater ($F_{2,21} = 28.48; P < 0.001$) within both the CO and CB treatments relative to the controls ($P < 0.001$). Microbial biomass C did not differ among treatments ($F_{2,21} = 0.997; P = 0.386$) resulting in an MBC:N ratio that was significantly reduced in both the CO and CB treatments relative to the controls ($P < 0.001$).

### Table 1. Soil characteristics (means ± 1SE; n: C = 8, CO = 8, CB = 8). Effects of treatment were tested with a one-way ANOVA. Treatment level differences were determined by Tukey-pairwise comparisons. * significant at $P<0.05$; **$P<0.01$; ***$P<0.001$ relative to the control. ● significant at $P<0.05$ relative to carcass only treatment. Bolded $P$-values indicate a significant ANOVA result.

| Treatment                  | Control (C)       | Carcass only (CO) | Carcass burying beetle (CB) | $P$-value |
|----------------------------|-------------------|-------------------|-----------------------------|-----------|
| pH                         | 4.9 ± 0.07        | 6.2 ± 0.09***     | 6.7 ± 0.13***●             | <0.001    |
| Moisture                   | 0.56 ± 0.06       | 0.66 ± 0.08       | 0.68 ± 0.04                 | 0.260     |
| Labile C (µg C g⁻¹ soil)   | 2456 ± 308        | 3520 ± 735        | 4402 ± 479*                 | 0.018     |
| DOC (µg g⁻¹ soil)          | 512 ± 63          | 1570 ± 704**      | 2513 ± 424***               | <0.001    |
| DON (µg g⁻¹ soil)          | 73 ± 8            | 1473 ± 114***     | 1582 ± 103***               | <0.001    |
| DOC:DON                    | 8.1 ± 0.34        | 1.1 ± 0.39**      | 1.8 ± 0.27**                | <0.001    |
| NH₄⁺ (µg N g⁻¹ soil)       | 43.9 ± 3.9        | 40.8 ± 3.9        | 40.7 ± 4.9                  | 0.820     |
| NO₃⁻ (µg N g⁻¹ soil)       | 4.05 ± 1.08       | 3.79 ± 0.89       | 4.51 ± 1.41                 | 0.904     |
| Total C (%)                | 11.8 ± 0.88       | 11.0 ± 1.04       | 11.6 ± 0.86                 | 0.980     |
| Total N (%)                | 0.58 ± 0.06       | 0.69 ± 0.07       | 0.73 ± 0.05                 | 0.180     |
| C:N                        | 21.2 ± 1.28       | 17.02 ± 0.80*     | 16.1 ± 0.85**               | 0.004     |
| MBC (µg g⁻¹ soil)          | 2409 ± 102        | 2469 ± 346        | 2972 ± 396                  | 0.385     |
| MBN (µg g⁻¹ soil)          | 306 ± 35          | 1301 ± 279***     | 1632 ± 190***               | <0.001    |
| MBCN                       | 10.1 ± 1.18       | 2.5 ± 0.38***     | 2.1 ± 0.18***               | <0.001    |

**Figure 2.** (A) Percent increase in each soil nutrient relative to the control and (B) changes in soil nutrient levels according to treatment. Dark grey indicates the control, gray indicates the carcass only treatment, and light grey indicates treatments with both carrion and burying beetles. Nutrient abbreviations: Dissolved organic nitrogen (DON), Dissolved organic carbon (DOC) and Labile Carbon (Labile C). Boxplot elements are as follows: center line, median; box limits, upper and lower quartiles; whiskers, 1.5 × interquartile range; points, outliers. Levels of significance relative to the control is indicated as follows: * significant at $P<0.05$; ** $P<0.01$ and *** $P<0.001$. 

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Microbial community composition was not different across treatments ($F_{2,21} = 1.28; P = 0.297$; Supplementary Fig. S1 & Table S1) nor was there a difference in the relative biomass of individual microbial groups or the fungal:bacterial biomass ratio (Table 2).

### Discussion

The rate of carrion decay and break-down is facilitated by necrophilous insect groups21,23, and these activities provide a localized, nutrient pulse to the soil16,44. Our hypothesis that burying beetles would affect soil characteristics in response to their reproductive activities was not supported. Rather, the results of our study demonstrate that carrion attributes drive soil characteristics in response to decomposition. However, the reproductive activities of burying beetles and consumption of the carcass by developing larvae did not negate the benefits of carrion decomposition, as the changes in soil characteristics within the burying beetle treatment were consistent with those observed during surface carrion decomposition alone. Taken together, these findings indicate that the carrion decomposition process drives alterations in soil characteristics, and that burying beetle reproduction does not appreciably reduce carrion contributions to soils. For instance, we found that there was an increase in soil pH (pH > 6) and dissolved organic C and N in carrion addition plots, regardless of burying beetle utilization of the carcass for reproduction and larval growth. Changes in soil pH during carrion decomposition are well-documented18,20,45, with increases associated with the influx of by-products released during tissue deterioration, most commonly driven by increases in soil ammonium25,46,47. Although we did not observe increased soil NH$_4^+$ in the carrion only (CO) nor the burying beetle (CB) treatments, the observed increases in dissolved organic C and N align with nutrient profile changes observed in Keenan et al. (2018). These findings indicate that although the carrion was consumed by burying beetle larvae in the burying beetle treatment, the observed abiotic changes were consistent with microbially mediated carrion decomposition on the soil surface and still result in a net contribution of nutrients to the soil. However, the release of nutrients between these two treatments is likely facilitated through differing metabolic pathways, with insect frass within the burying beetle treatment potentially serving as the primary source of N relative to a naturally decomposing carcass34,48,49.

Insect necrophilous behaviors co-occur with shifts in microbial community abundances in response to the stage of decomposition57,58. However, we did not observe any differences in microbial abundance and biomass in response to carrion only or burying beetle treatments. Rather, there was a significant reduction in microbial biomass C:N ratio from 8:1 within the control to approximately 2:1 in the carrion addition treatments, which is lower than ratios typically reported in soils without carrion inputs45. Microbial biomass N increased within these treatments, while microbial biomass C did not differ. The increase in microbial biomass N is likely explained by the influx in soil N within both the carrion only and burying beetle treatments. Following nutrient release within these treatments, microbes likely immobilize N and differentially store nutrients which would alter their biomass C:N ratios52–55. Additionally, the greater abundance of bacteria across our plots relative to insect larvae may also contribute to these low ratios, as bacteria tend to exhibit lower C:N ratios (4:1 to 10:1) than those observed in fungi (8:1 to 29:1)56. This data indicates that the incorporation of carrion in ecosystem landscapes, and the sequestration and consumption of these carcass by burying beetles for reproduction and larval growth, create nutrient hotspots in the soil which are utilized during microbial metabolism. However, further studies are required to provide increased resolution regarding the associated stoichiometric ratios of carrion associated microbial communities, and the role of necrophilous groups in isolating carrion and creating nutrient hotspots in the soil.

The indirect benefits of insect driven decomposition of carrion are often underappreciated and understudied relative to other detrital inputs. As carrion size decreases, invertebrates are increasingly likely to utilize the resource for their own life histories57,58. Indeed, research suggests that the activities of insect scavengers play an essential role in preventing vertebrate scavengers from removing carrion from the ecosystem, as the proportion of carrion removed by vertebrate scavengers is greatly reduced in warm weather (from 65 to 16–20%) when invertebrates are active57,59,60. Burying beetles alone can sequester greater than 65% of small carrion in forest ecosystems, compared to 10–35% by vertebrate scavengers57,61. In this context, our study indicates that at the ecosystem scale, insects which sequester and isolate resources from other scavengers can play a significant role in creating nutrient hotspots where an organism died. When we consider insect groups such as the burying beetle (75 species in Northern Hemisphere), that can sequester vertebrate carcasses ranging in size from 4 to 210 g31, and are distributed across North America with temporal and phenological shifts in activity patterns31,62,63, the potential nutrient input from carrion sequestration can be significant. For example, in NH forests alone there are five burying beetle species active May–September. If we presume 6% death rate per week64 of small mammals (~35 g), captured across two weeks in the summer65, 75% of which are used by burying beetles61, while assuming this mortality and capture rate is consistent across 16 weeks of activity, their behaviors could contribute up to

| Treatment                  | Control (C) | Carcass Only (CO) | Carcass Burying Beetle (CB) | P-value |
|----------------------------|-------------|-------------------|-----------------------------|---------|
| Total microbial biomass    | 185 ± 26    | 235 ± 38          | 258 ± 33                    | 0.298   |
| Fungi                      | 29.92 ± 3.59| 38.7 ± 4.79       | 40.9 ± 5.08                 | 0.214   |
| Bacteria                   | 141 ± 21    | 199 ± 31          | 180 ± 26                    | 0.306   |
| Fungi:bacteria Ratio       | 0.225 ± 0.02| 0.226 ± 0.02      | 0.219 ± 0.01                | 0.96    |

Table 2. Soil microbial biomass as estimated by phospholipid fatty acid (PLFA) analysis (nmol g$^{-1}$ dry soil) (means ± 1SE; n: C = 8, CO = 8, CB = 8). Effects of treatment were tested with a one-way ANOVA. * significant at $P<0.05$ relative to the control.
2.37 g DON or 2.14 g DOC m⁻² y⁻¹. This is a conservative estimate given these calculations do not account for utilization of carcasses of greater mass.

Given the limitations of field-based studies that examine ecosystem interactions and the limited nature of our understanding of invertebrate decomposer roles in modulating soil properties, future studies would do well to further investigate the relationships among invertebrate scavengers and soil nutrients. As our study prevented invertebrate activities on surface carrion treatments, we were unable to draw conclusions regarding the significance of burying beetle reproduction and larval development on soil nutrients relative to other invertebrate scavenger activities. Future studies could further investigate the role that carrion sequestration by burying beetles, larval activities, and larval number and/or mass, play in creation of nutrient hotspots and how it influences the lateral and vertical spread of nutrients relative to surface-level carrion decomposition. Additionally, it would be informative to measure the proportion of carrion nutrients that are retained within larval biomass versus released into the soil via metabolic pathways such as insect frass, elucidating the chemical and nutrient characteristics of larval frass within scavenging insect groups.

Methods

Burying beetle collection and maintenance. *Nicrophorus orbicollis* was captured in the summer of 2018 at the University of New Hampshire’s (UNH; Durham, NH, USA) forest sites located at Kingman and Woodman Farms using 5-gallon above-ground pitfall traps baited with aged chicken liver⁹⁶,⁹⁷. Wild-caught beetles were maintained in the lab for approximately 2 weeks in solitary acrylic containers (Pioneer Plastics, 109.53 mm × 57.15 mm × 44.45 mm) filled with moist peat. Beetles were maintained under a 14:10 day:night light cycle, provided water ad libitum, and fed raw pork loin twice weekly.

Soil chemical analyses. Soil moisture was determined gravimetrically by drying subsamples (~ 5 g) at 60 °C for 48 h. Soil pH was evaluated using a digital pH probe (Cole-Palmer, Vernon Hills, IL) in a 1:10 soil: water suspension. Inorganic N was determined by extracting soil with 2 M KCl (1:5 wt:vol), filtering (40 Watman filters), and quantifying the concentration of nitrate (\( \text{NO}_3^- \)) and ammonium (\( \text{NH}_4^+ \)) calorimetrically using a multi-detection microplate reader (Synergy™ HT, BioTek Instruments, Inc., Part #7091000, Winooski, Vermont, USA) at 540 and 640 nm, respectively. Nitrate quantification was determined by the vanadium (III) reduction reaction⁹⁶ while ammonium was determined using the indophenol-blue method⁹⁷. The detection limits for both \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) was 0.1 ppm⁹⁷. Total soil C and N were determined by dry combustion of finely ground samples using a Costech C/H/N/S Elemental Analyzer. Labile C (carbon mineralization) was estimated using a 30-day incubation with soil subsamples (10 g) sealed in 0.933 L Mason jars incubated at 25 °C. Headspace samples were collected daily to determine atmospheric \( \text{CO}_2 \) concentrations using a LI-COR infrared gas analyzer (Model LI-6252, LI-COR Biosciences, Lincoln, NE). Jars were flushed with \( \text{CO}_2 \)-free air following each headspace sampling to maintain \( \text{O}_2 \) levels.
Microbial analyses. Microbial biomass C and N were determined on 0.5 M K₂SO₄ soil extracts (1:3 wt:vol) following chloroform fumigation. Dissolved C and N concentrations in the extracts were determined using thermal oxidation with near infrared carbon detection followed by chemiluminescence nitrogen detection on a Shimadzu TOC-L with an attached TNM-L unit.

Microbial community composition was determined using phospholipid fatty acid (PLFA) analysis. Microbial lipids were extracted from 1 g of sieved, root-free freeze-dried soil that had been stored at − 80 °C until analyses began. Lipids were extracted by utilizing a single-phase solvent (chloroform) combined with phosphate buffer which was based on a modified Bligh and Dyer (1959) extraction procedure. This technique extracts lipids from viable microorganisms captured at the time of sampling. Lipid extracts were fractionated on silicic acid columns into neutral, glycol- and polar lipids, with only polar lipids collected. Following collection, polar lipids were methylated with 0.2 M methanolic KOH solution to form fatty acid methyl esters (FAMEs). FAMEs were dried and reconstituted in hexane for quantification on a Varian 3800 GC-FID (Varian, Inc., Walnut Creek, CA). FAME peaks were compared against a standard library of FAMES and based on retention time data of the known standards. Peak area concentrations were converted to nmol PLFA g⁻¹ dry soil based on the peak area of its matching standard peak. The polyenoic unsaturated fatty acids, 18:2w6 and 18:1w9c, were considered fungal biomarkers. Branched, saturated gram-positive fatty acids of i15:0, a15:0, i16:0, i17:0 and a17:0 as well as the monoenoic and cyclopropane unsaturated gram-negative fatty acids of 16:1w7c, 16:1w7t, 18:1w7c and cy19:0 were considered part of the total bacterial biomass. Total bacterial biomass was also represented by 15:0, which was considered a general bacterial marker to complete the bacterial assessment.

Statistical analyses. All data analyses were performed in R version 3.5.3 (R Core Team, 2019). To assess the multivariate response of soil abiotic characteristics (pH, moisture, inorganic N, total C:N ratio, DON, DOC and DOC:N ratio) to treatment, a principal coordinate analysis (PCoA) was conducted with treatment means in the package ape. To determine whether the visualized separation of treatments was significant, a Permutational Analysis of Variance (PERMANOVA) with Euclidean distance was conducted using the package vegan. The function betadisper was used to determine whether data met the assumption of treatment homogeneity.

Following a significant result within the PERMANOVA, pairwise comparisons amongst treatment groups were conducted with the function betadisper. A Mantel test was performed with the package vegan to determine whether abiotic soil characteristic relationships with the PLFA community were significantly correlated and driving the changes in the microbial community composition matrix within each treatment, a Mantel test was performed with the package vegan. All treatments had a sample size of eight each.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability
R code utilized in this study is available upon reasonable request from the corresponding author.

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References
1. Hallmann, C. A. et al. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE* **12**, e0185809 (2017).
2. Sánchez-Bayo, F. & Wyckhuys, K. A. G. Worldwide decline of the entomofauna: a review of its drivers. *Biol. Conserv.* **232**, 8–27 (2019).
3. van Klink, E. et al. Meta-analysis reveals declines in terrestrial but increases in freshwater insect abundances. *Science* **368**, 417–420 (2020).
4. Losey, E. J. & Vaughan, M. The economic value of ecological services provided by insects. *Bioscience* **56**, 311 (2006).
5. Bar-On, Y. M., Phillips, R. & Milo, R. The biomass distribution on Earth. *Proc. Natl. Acad. Sci. USA* **115**, 6506–6511 (2018).

6. Yang, L. H. & Grafton, C. Insects as drivers of ecosystem processes. *Curr. Opin. Insect Sci.* **2**, 26–32 (2014).

7. Hunter, M. D. Insect population dynamics meets ecosystem ecology: effects of herbivory on soil nutrient dynamics. *Agric. For. Entomol.* **3**, 77–84 (2001).

8. Nichols, E. et al. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biol. Conserv.* **141**, 1461–1474 (2008).

9. Lobry de Bruyn, L. A. & Conacher, A. J. The role of termites and ants in soil modification: a review. *Aust. J. Soil Res.* **28**, 55–93 (1990).

10. Shukla, R. K., Singh, H., Rastogi, N. & Agarwal, V. M. Impact of abundant *Pheidole* ant species on soil nutrients in relation to the food biology of the species. *Appl. Soil Ecol.* **7**, 15–23 (2013).

11. López-Hernández, D. Nutrient dynamics (C, N and P) in termite mounds of *Nasutitermes ephratae* from savannas of the Orinoco llanos (Venezuela). *Soil Biol. Biochem.* **33**, 747–753 (2001).

12. Nkem, J. N., Lobry de Bruyn, L. A., Grant, C. D. & Hulugalle, N. R. The impact of ant bioturbation and foraging activities on surrounding soil properties. *Pedobiologia* **44**, 609–621 (2000).

13. Jouquet, P., Traoré, S., Chossai, C., Hartmann, C. & Bignell, D. Influence of termites on ecosystem functioning. Ecosystem services provided by termites. *Eur. J. Soil Biol.* **47**, 215–222 (2011).

14. Frost, C. J. & Hunters, M. D. Insect canopy herbivory and frass deposition affect soil nutrient dynamics and export in oak mesocosms. *Ecology* **85**, 3335–3347 (2004).

15. Calderon-Cortes, N. et al. Ecosystem engineering and manipulation of host plant tissues by the insect borer *Oncideres albomarginata chamela* *J. Insect Physiol.* **48**, 124–136 (2016).

16. Barton, P. S., Cunningham, S. A., Lindemann, D. B. & Manning, A. D. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia* **171**, 761–772 (2013).

17. Danell, K., Axs, D. B. & Bräthén, K. A. Effect of muskox carcasses on nitrogen concentration in tundra vegetation. *Arctic* **55**, 389–392 (2002).

18. Melis, C. et al. Soil and vegetation nutrient response to bison carcasses in Białowieża primeval forest Poland. *Ecol. Res.* **22**, 807–813 (2007).

19. Bump, J. K., Peterson, R. O. & Vucetich, J. A. Wolves modulate soil nutrient heterogeneity and foliar nitrogen by configuring the distribution of ungulate carcasses. *Ecology* **90**, 3159–3167 (2009).

20. Benninger, L. A., Carter, D. O. & Forbes, S. L. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Sci. Int.* **180**, 70–75 (2008).

21. Anderson, G. S. & VanLaerhoven, S. L. Initial studies on insect succession on carrion in Southwestern British Columbia. *J. Forensic Sci.* **41**, 617–625 (1996).

22. Matuszewski, S., Bajerlein, D., Konwerski, S. & Szpila, K. Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: succession of carrion fauna. *Forensic Sci. Int.* **207**, 150–163 (2011).

23. Barton, P. S., Evans, M. J., Pechel, J. L. & Benbow, M. E. Necrophilous insect dynamics at small vertebrate carrion in a temperate eucalypt woodland. *J. Med. Entomol.* **34**, 964–973 (2017).

24. Benbow, M. E., Lewis, A. J., Tomberlin, J. K. & Pechal, J. L. Seasonal necrophagous insect community assembly during vertebrate carrion decomposition. *J. Med. Entomol.* **50**, 440–450 (2013).

25. Payne, J. A. A summer carrion study of the baby pig *Sus Scrofa* Linnaeus. *Ecology* **46**, 592–602 (1965).

26. Kočárek, P. Decomposition and Coleoptera succession on exposed carrion of small mammal in Opava, the Czech Republic. *Eur. J. Soil Biol.* **39**, 31–45 (2003).

27. Borzemissza, G. F. An analysis of arthropod succession in carrion and the effects of its decomposition on the soil fauna. *J. Zool.* **5**, 1–12 (1955).

28. Parmenter, R. R. & MacMahon, J. A. Carrion decomposition and nutrient cycling in a semiarid shrub–steppe ecosystem. *Ecol. Monogr.* **79**, 637–661 (2009).

29. Pechal, J. L., Benbow, M. E., Crippen, T. L., Tarone, A. M. & Tomberlin, J. K. Delayed insect access alters carrion decomposition and necrophagous insect community assembly. *Ecosphere* **5**, 1–21 (2014).

30. Fukowski, E. Ökologische untersuchungen an necrophorus. *Z. Morphol. Oekol.* **27**, 518–586 (1933).

31. Scott, M. P. The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**, 595–618 (1998).

32. Milne, I. J. & Milne, M. The social behavior of burying beetles. *Sci. Am.* **235**, 84–89 (1976).

33. Müller, J. K. & Eggert, A. K. Paternity assurance by ‘helpful’ males: adaptations to sperm competition in burying beetles. *Behav. Ecol. Sociobiol.* **24**, 245–249 (1989).

34. House, C. M. et al. The evolution of repeated mating in the burying beetle *Nicrophorus viviparus*. *Evolution* **62**, 2004–2014 (2008).

35. Pettinger, A. M., Steiger, S., Müller, J. K., Sakaluk, S. K. & Eggert, A. K. Dominance status and carcass availability affect the outcome of sperm competition in burying beetles. *Behav. Ecol.* **22**, 1079–1087 (2011).

36. Arce, A. N., Johnston, P. R., Smiseth, P. T. & Rozen, D. E. Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *J. Evol. Biol.* **25**, 930–937 (2012).

37. Hall, C. L. et al. Inhibition of microorganisms on a carrion breeding resource: the antimicrobial peptide activity of burying beetle (Coleoptera: Silphidae) oral and anal secretions. *Environ. Entomol.* **40**, 669–678 (2011).

38. Duarte, A., Welch, M., Swannack, C., Wagner, J. & Kilner, R. M. Strategies for managing rival bacterial communities: lessons from burying beetles. *J. Anim. Ecol.* **87**, 414–427 (2018).

39. Trumbo, S. T. Reproductive benefits and the duration of paternal care in a biparental burying beetle *Nicrophorus orbicollis*. *Behaviour* **117**, 82–105 (1991).

40. Trumbo, S. T. Fate of mouse carcasses in a Northern Woodland. *Ecol. Entomol.* **41**, 737–740 (2016).

41. Hackbarth, W. W., Freeman, L., Payton, M. & Peterson, B. C. Burying beetle (Coleoptera: Silphidae: *Nicrophorus fabricius*) brood improving improves soil fertility. *Coleopt. Bull.* **74**, 427–433 (2020).

42. Benbow, M. E. et al. Nicrophorinae framework for bridging decomposition ecology of autotrophically and heterotrophically derived organic matter. *Ecol. Monogr.* **89**, 1–26 (2018).

43. Carter, D. O., Yellowlees, D. & Tibbett, M. Cadaver decomposition in terrestrial ecosystems. *Naturwissenschaften* **94**, 12–24 (2007).

44. Carter, D. O., Yellowlees, D. & Tibbett, M. Temperature affects microbial decomposition of cadavers (*Rattus rattus*) in contrasting soils. *Appl. Soil Ecol.* **40**, 129–137 (2008).

45. Meyer, J., Anderson, B. & Carter, D. O. Seasonal variation of carcass decomposition and grave soil chemistry in a cold (Dfa) climate. *J. Forensic Sci.* **58**, 1175–1182 (2013).

46. Metcalfe, J. L. et al. Microbial community assembly and metabolic function during mammalian corpse decomposition. *Science* **351**, 158–162 (2016).

47. Chen, Y. A., Forschler, B. T. & Nielsen, U. Elemental concentrations in the frass of saproxylic insects suggest a role in micronutrient cycling. *Ecosphere* **7**, 1–13 (2016).

48. Couture, J. J. & Lindroth, R. L. Atmospheric change alters frass quality of forest canopy herbivores. *Arthropod. Plant. Interact.* **8**, 33–47 (2014).
50. Metcalfe, J. L. et al. A microbial clock provides an accurate estimate of the postmortem interval in a mouse model system. Elife 2013, 1–9 (2013).

51. Xu, S., Liu, L. L. & Sayer, E. J. Variability of above-ground litter inputs alters soil physicochemical and biological processes: a meta-analysis of litterfall-manipulation experiments. Biogesencesciences 10, 7423–7433 (2013).

52. Wilson, W. A. et al. Regulation of glycogen metabolism in yeast and bacteria. FEMS Microbiol. Rev. 34, 952–985 (2010).

53. Ackerbergova, L. & Nahalka, J. Polyphosphate—an ancient energy source and active metabolic regulator. Microb. Cell Fact. 10, 1–14 (2011).

54. Kornberg, A. Inorganic polyphosphate: toward making a forgotten polymer unforgettable. J. Bacteriol. 177, 491–496 (1995).

55. Wilkinson, J. E. Carbon and energy storage in bacteria. J. Gen. Microbiol. 32, 171–176 (1963).

56. Paul, E. A. Soil Microbiology, Ecology and Biochemistry (Academic Press, Cambridge, 2014).

57. Devault, T. L., Brisbin, I. L. Jr. & Rhodes, O. E. Jr. Factors influencing the acquisition of rodent carrion by vertebrate scavengers and decomposers. Can. J. Zool. 82, 502–509 (2004).

58. Devault, T. L., Rhodes, O. E. Jr. & Shivik, J. A. Scavenging by vertebrates: behavioral, ecological and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. Oikos 102, 225–234 (2003).

59. Devault, T. L. & Rhodes, O. E. Identification of vertebrate scavengers of small mammal carcasses in a forested landscape. Acta Theriol. 47, 185–192 (2002).

60. Smith, J. R., Laatsch, L. J. & Beasley, J. C. Spatial complexity of carcass location influences vertebrate scavenger efficiency and species composition. Sci. Rep. 7, 1–8 (2017).

61. Wilson, D. S. & Fudge, J. Burying beetles: intraspecific interactions and reproductive success in the field. Ecol. Entomol. 9, 195–203 (1984).

62. Keller, M. L., Howard, D. R. & Hall, C. L. Spatiotemporal niche partitioning in a specious silphid community (Coleoptera: Silphidae). Sci. Nat. 106, 1–12 (2019).

63. Owings, C. G. & Picard, C. I. Temporal survey of a carrion beetle (Coleoptera:Silphidae) community in Indiana. Proc. Indiana Acad. Sci. 124, 124–128 (2015).

64. Snyder, D. P. Survival rates, longevity, and population fluctuations in the white-footed mouse, Peromyscus leucopus Southeastern Michigan. Museum Zool. University Michigan 95, 1–33 (1956).

65. Stephens, R. B., Hocking, D. J., Yamasaki, M. & Rowe, R. J. Synchrony in small mammal community dynamics across a forested landscape. Ecoscience 40, 1198–1209 (2007).

66. Leasure, D. R., Rupe, D. M., Phillips, E. A., Opine, D. R. & Haxel, G. R. Efficient new above-ground bucket traps produce comparable data to that of standard transects for the endangered American Burying Beetle, Nicrophorus americanus Olivier (Coleoptera: Silphidae). Coleopt. Bull. 66, 209–218 (2012).

67. Bedick, J. C., Ratcliffe, B. C. & Higley, L. G. A new sampling protocol for the endangered American Burying Beetle, Nicrophorus americanus Olivier (Coleoptera:Silphidae). Coleopt. Bull. 58, 57–70 (2004).

68. Trumbo, S. T. Reproductive success, phenology and biogeography of burying beetles (Silphidae, Nicrophorus). Am. Midl. Nat. 124, 1–11 (1990).

69. Trumbo, S. T. Nesting failure in burying beetles and the origin of communal associations. Evol. Ecol. 9, 125–130 (1995).

70. Bray, R. S. & Hendrix, S. A. Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium (III) reduction with chemiluminescence detection. Anal. Chem. 61, 2715–2718 (1989).

71. Sims, G. K., Ellsworth, T. R. & Mulvany, R. L. Microscale determination of inorganic nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26, 303–316 (1995).

72. Contosta, A. R., Frey, S. D. & Cooper, A. B. Seasonal dynamics of soil respiration and N mineralization in chronically warmed and fertilized soils. Ecosphere 2, 1–24 (2011).

73. Vance, E. D., Brooks, P. C. & Jenkinson, D. S. Microbial biomass measurements in forest soils: the use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biol. Biochem. 19, 697–702 (1987).

74. Vance, E. D., Brooks, P. C. & Jenkinson, D. S. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707 (1987).

75. Brooks, P. C., Landman, A., Pruden, G. & Jenkinson, D. S. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842 (1985).

76. Bligh, E. G. & Dyer, W. J. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917 (1959).

77. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).

78. Guckert, J. B., Antworth, C. P., Nichols, P. D. & White, D. C. Phospholipid, ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. FEMS Microbiol. Lett. 31, 147–158 (1985).

79. Bardgett, R. D., Hobbs, P. J. & Frostegård, A. Changes in soil fungal/bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biol. Fertil. Soils 22, 261–264 (1996).

80. Bäath, E. The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microb. Ecol. 45, 373–383 (2003).

81. Ekulund, F., Olsson, S. & Johansen, A. Changes in the succession and diversity of protozoan and microbial populations in soil with a range of copper concentrations. Soil Biol. Biochem. 35, 1507–1516 (2003).

82. Leckie, S. E., Prescott, C. E., Grayston, S. J., Neufeld, I. D. & Mohn, W. W. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. Microb. Ecol. 35, 29–40 (2004).

83. Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35, 526–528 (2019).

84. Oksanen, J. & et al. Vegan: community ecology package (2019). http://cran.r-project.org/.

85. Hervé, M. RVAideMemoire: Testing and plotting procedures for biostatistics version 0.9-75 from CRAN. R package version 0.9-75 (2020).

86. Fox, J. & Weisberg, S. An R Companion to Applied Regression (SAGE Publications Inc, Thousand Oaks, 2018).

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Author contributions
B.K.W.K. conducted the field experiment, produced and analyzed all data, created the figures, and wrote up the paper with input from all authors. S.F. provided methodological, experimental design, and manuscript feedback.
D.R.H. co-conceived the project and provided manuscript feedback. C.L.H. conceived and designed the project and provided manuscript feedback. All authors read, commented on and approved the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**

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