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Mortality hotspots: Nitrogen cycling in forest soils during vertebrate decomposition

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ABSTRACT

Decomposing animals alter soil biogeochemical cycles, and these natural ephemeral nutrient patches (or 'hotspots') are important for maintaining landscape heterogeneity and enriching local biodiversity. Soil nitrogen (N) enrichment associated with decomposing animals has been documented, but to date an integrated systems-level understanding of the fate and rates of N compound transformations is lacking. The goal of this study was to develop a comprehensive view of temporal changes in N biogeochemical cycling during vertebrate decay. Vertebrate decomposition significantly altered soil N cycling, and was divided into three main biogeochemical phases based on soil chemistry. Phase one included initial and early decay, distinguished by oxic soils with low, background carbon and N cycling rates. Fluid release and insect colonization during active and advanced decay, defined as phase two, stimulated soil microbial communities, particularly those able to degrade phospholipids and nucleic acids. This resulted in anaerobic soils, 250 times greater ammonium and ten times greater carbon dioxide than background, and the highest ^{15}N-enrichment rates. The final biogeochemical phase, encompassing the early and late skeletal stages, was characterized by enhanced nitrification and denitrification as evidenced by significantly elevated nitrate, dissolved organic nitrogen, and enhanced nitrous oxide release. As a result of decay and multiple synchronous processes, soil δ^{15}N was enriched by 6–10‰ above background, demonstrating the influence of decay on soil isotopic signatures. This work provides a systems-level synthesis of N redistribution during animal decay and has significant implications for our understanding of nutrient turnover rates and dynamics in terrestrial ecosystems.

1. Introduction

The deposition and breakdown of dead animals (carrion or carcasses) form isolated and ephemeral nutrient resource patches, or "hotspots" (McClain et al., 2003; Carter et al., 2007; Aitkenhead-Peterson et al., 2012), and provide an important source of critical, and often limiting, nutrients (Danell et al., 2002; Barton et al., 2013; Benbow et al., 2016; Wheeler and Kavanagh, 2017). These pulses of organic matter result in the release of carbon, phosphorus, nitrogen, and sulfur, stimulating micro-and macrofauna proximal to the decaying material (Carter et al., 2007) as well as scavengers and insects sourced from distal areas (DeVault et al., 2003). For vertebrates, decomposition results in a succession of decay stages, each distinguished by visible changes to the carcass and colonizing insects (Payne, 1965; Mann et al., 1990; Vass et al., 1992). Concurrently, a succession of microbial populations occurs both internally within the carcass and externally in the soil (Dent et al., 2004; Wardle et al., 2004; Hauther et al., 2015; Crippen et al., 2016; DeBruyn, 2016; Javan et al., 2016; DeBruyn and Hauther, 2017). Animal decay can also have significant impacts on nearby plant communities (Towne, 2000; Danell et al., 2002; Bump et al., 2009b).

In terrestrial ecosystems, it is increasingly recognized that depolymerization of organic molecules is the rate limiting step in N cycling (Schimel and Bennett, 2004). The release of significant quantities of nitrogen (N) and carbon (C) rich compounds to the soil during carcass decay induces a series of soil chemical changes including shifts in pH, conductivity, gaseous emissions (e.g., CO_2, N_2O, H_2S, CH_4), and ionic concentrations (e.g., NH_4^+, NO_3^-, PO_4^{3-}, Ca^{2+}) (Towne, 2000; Melis et al., 2007; Benninger et al., 2008; Cobaugh et al., 2015). Proximal plant growth and N-uptake is generally inhibited due to significant changes to soil physical chemistry, including the establishment of anoxic soils, and accumulation of toxic levels of ammonia, ammonium,

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and urea (Miller and Cramer, 2005). Therefore, the soil chemical changes predominantly reflect: (1) the input of carcass-derived organics and their decomposition products, and (2) *in situ* N and C cycling by carcass- and soil-sourced microbial communities or abiotic processes (i.e. non-biologically mediated volatilization, erosion, leaching). Nitrogen cycling in particular is altered in these hotspots (Melis et al., 2007) in response to breakdown of proteins from the carcass, as evidenced by elevated protease activities and increased amino acid and peptide concentrations (Hopkins et al., 2000; Macdonald et al., 2014). Abundances of microbial nitrogenase-encoding genes declined, suggesting a possible decrease in N fixation capacity (Moreno et al., 2011). Substantial increases in ammonia concentrations without a subsequent increase in nitrate suggests nitrification inhibition (Hopkins et al., 2000; Cobaugh et al., 2015). The shifts in soil chemical composition surrounding decomposing carcasses are profound enough that they have been used to estimate the postmortem interval, providing a key resource for forensic scientists (Vass et al., 1992; Benninger et al., 2008; Aitkenhead-Peterson et al., 2012).

Microbially-mediated decomposition can lead to changes in stable isotopic ratios (specifically $\delta^{15}N$ and $\delta^{13}C$) due to equilibrium and kinetic fractionation effects (Ehleringer and Cerling, 2002), and these ratios are frequently used to infer soil organic matter sources and turnover times. Prior studies of plant litter decomposition demonstrated an overall increase in $\delta^{15}N$ on annual to decadal time-scales (Connin et al., 2001; Asada et al., 2005; Billings and Richter, 2006), with the potential for an increase or decrease in $\delta^{15}N$ of organic matter during initial decay (Craine et al., 2015). Several studies observed enhanced foliar $\delta^{15}N$ in forest ecosystems as a result of carcass enrichment (Koyama et al., 2005; Bump et al., 2009a), indicating that soil biogeochemical N transformations are occurring. However, the potential for vertebrate decomposition hotspots to influence soil isotopic composition is understudied. In particular, since vertebrate decomposition proceeds on significantly shorter time-scales compared to plant litter (e.g., hours to months), there is uncertainty if animal and plant hotspots exhibit similar patterns with respect to $\delta^{15}N$ isotopic enrichment. Diverse N cycling processes have the potential to influence soil stable isotopic composition (Hogberg, 1997; Robinson, 2001; Billings and Richter, 2006; Craine et al., 2015), and many are known to be active during vertebrate decomposition, particularly gaseous emission of N-phases. Therefore, we expected to observe an increase in soil $\delta^{15}N$ reflecting: (1) the input of carcass-derived and isotopically-enriched fluid; and (2) increased gaseous emissions. However, these changes are expected to be short-lived, with soils returning towards background stable isotopic composition during the skeletal phase.

While it is well accepted that a decomposing vertebrate carcass provides a pulse of N-rich organics to the ecosystem, most studies of carrion hotspots to date lack the temporal resolution or only measure a small subset of parameters. Thus the timing and magnitude of specific N transformations during decomposition is poorly constrained (Barton et al., 2013). This leaves gaps in our understanding of the fate of carcass-derived N, and restricts our ability to scale these hotspot events to ecosystems (for example, estimating impacts of mass mortality events). Therefore, the goal of this study was to comprehensively evaluate the consequences of carrion deposition on soil N cycling, focusing on the early (less than 6 months) stages of decay. These results provide novel insights into the biogeochemical cycling of N and C in soils when stimulated by animal decomposition, important for developing carrion nutrient budgets. We present a systems-level characterization of mortality hotspot evolution by combining soil physical chemical, microbial, and isotopic data. These results help to resolve the temporal changes in N processing during decay, provide important insights into the contributions of vertebrate decay to local biogeochemical cycling, and reframed the stages of decay into three distinct biogeochemical phases.

2. Materials and methods

2.1. Field experimental location and layout

The decomposition experiment was conducted during July and August 2016, at the Oak Ridge Arboretum, a University of Tennessee Forest Resources Research and Education Center, located in Oak Ridge, TN, USA. The soil is part of the Fullerton-Pailo Complex, characterized as Typic Paleudult (Soil Survey Staff). The soil parent material is part of the Knox Group, consisting of cherty limestone and dolomite with some interbedded sandstone units (Montañez and Read, 1992). During the field trial, air temperatures ranged from 15 to 35 °C. Additionally, East Tennessee experienced severe drought, receiving only 15.2 cm of rain during the experiment, 13 cm below average.

Nuisance beaver carcasses (*Castor canadensis*) obtained from the USDA APHIS in East Tennessee were captured using Conibear body gripping traps, leaving the beaver hide intact. Traps were checked daily. Beaver carcasses were stored at −20 °C until initiating the decomposition experiment (maximum freezing time of 1 year). A total of five beavers (13.5–23 kg each) were used (Fig. S1). The sex and age of the beavers were unknown. Because all animals used in this study were salvaged carcasses, no IACUC approval was needed. While still frozen, beavers were placed inside scavenger prevention wire cages (1.19 × 0.74 × 0.81 m), allowing direct contact with the underlying soil, but minimizing access by large scavengers (e.g., raccoons and vultures). Polyvinyl chloride (PVC) plastic collars (20 cm diameter x 14 cm height) were inserted into the soil adjacent to each enclosure at a depth of ~6 cm (leaving a ~7 cm-tall headspace) to enable sampling of gas fluxes (CO_2, N_2O, CH_4) at each decomposition stage. Collars were left *in situ* during the course of the experiment. Enclosures were arrayed radially around central location at a minimum distance of 3 m from the next enclosure, an adequate distance to ensure isolated hotspots (Danell et al., 2002; Aitkenhead-Peterson et al., 2012). Three additional beavers were placed in a scavenger prevention enclosure on a plastic tray to trap decomposition fluid. During active decay, decomposition fluids were collected from the tray and frozen (−20 °C) until further analyses.

2.2. Soil sample collection and instrumentation

Sampling intervals that captured key points in decomposition were selected prior to beginning the field experiment and were based on a previous study of decomposition (Cobaugh et al., 2015). Target decomposition periods were: initial (immediately after placement), early (bloat, Days 2–4), active (evidence of rupture and fluid release, Days 6–8), advanced (approximately 80% of initial mass lost, Day 11), early skeletal (exposure of bones and teeth; some mummified soft tissue, Day 40), and late skeletal (extended period after decay, Day 135). At each sampling time, photographs and notes were taken of each beaver, recording smells, insects, and stage of decomposition for each region of the carcass (Fig. 1). Decomposition progressed rapidly, with active decomposition reached after 1 week. At each sampling time, 5 cores from the top 5 cm of soil beneath carcasses (the A horizon) were collected using 3 cm diameter augers. Augers were cleaned with deionized water and 70% ethanol between each sample. Control soil (top 5 cm) was collected from 5 locations, 4–5 m away from each decaying beaver, and pooled to generate a single composite control soil sample for each time point, except for the initial samples, which represent three discrete control samples.

Soil moisture, temperature, and electrical conductivity sensors (Decagon Devices; GS3, RT) were placed underneath each carcass, and temperature sensors were inserted posteriorly inside each carcass to record hourly values. At each soil sampling time, soil dissolved oxygen content was measured using a multiparameter Orion device calibrated