# LARGE PREDATORS, PREY CARCASSES, RESOURCE PULSES, AND HETEROGENEITY IN TERRESTRIAL ECOSYSTEMS

By

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This dissertation, “Large Predators, Prey Carcasses, Resources Pulses, and Heterogeneity in Terrestrial Ecosystems,” is hereby approved in partial fulfillment of the requirements for the Degree of DOCTOR OF PHILOSOPHY IN FOREST SCIENCE.

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## Acknowledgements

Higher education is an absolute luxury.  A quick, “back-of-the-envelope” calculation of the percentage of people on planet earth that experience higher education – for any amount of time – will confirm my point.  The percentage of people creating a career in higher education is obviously much lower still.  So what?

Positions of luxury are partially obtained with another’s aid or deprivation, however incremental.  A luxurious position, therefore, obligates some acknowledgment of those who assisted such an achievement.

Specific inidividuals and institutions that supported this work are acknowledged within each paper in the Appendix.

My parents (both sets), siblings, grandparents, advisors (at all stages), committee members, and friends each deserve credit in helping me realize this accomplishment.  My wife, Amy Jane Schrank, and my sons Thomas and Dylan, deserve special mention and endless thanks because their support has been just that – extraordinary and boundless.

## Abstract

A large ungulate carcass is a significant concentration of premium resources. Understanding the flow of resources from carcasses and what factors influence carcass distribution is important to comprehending links between food-webs and ecosystem processes. This dissertation explores the ecological legacy of large ungulate carcasses in terms of their effects on soil nutrients, microbial communities, and plant quality and growth. The influence of large predators on the spatial distribution of carcasses was also explored. These studies took place across three large predator-prey systems (wolf-moose, -elk, and -deer) and employed experimental and observational analyses. Prey carcasses, whether or not they were well-utilized by predators and scavengers, created significant nutrient pulses to soils at carcass sites that lasted 2-4 growing seasons postmortem. These changes altered microbial communities and increased plant tissues quality and growth at carcass sites relative to paired control sites. Positive feedback mechanisms likely enhanced carcass effects because carcass sites become focal points for other species. The spatial distribution of carcasses was highly clustered, with carcass density depending on mortality type (wolf-killed versus starvation). Carcass density shifted significantly over time and carcass distribution was influenced by the predatory behavior of large carnivores. The chemical and physical changes at carcass sites create resource pulses and disturbances that contribute to ecosystem heterogeneity. Large predators influence ecosystem heterogeneity through carcass distribution and are thereby mechanistically linked to the maintenance of biodiversity.

# Contents

## Papers I-IV

This dissertation is based on the following papers, which will be referred to by their Roman numerals. These papers are contained in the Appendix.

I. Bump, J.K., Peterson, R.O., Vucetich, J.A., & Smith, D.W. Predator:prey body mass ratio determines magnitude of nutrient resource pulse to soils from ungulate prey carcasses. Manuscript.

II. Bump, J.K., Peterson, R.O., Vucetich, J.A., & Smith, D. W. Linking large carnivores to biodiversity in terrestrial ecosystems: wolves configure landscape heterogeneity through prey carcass distribution. Manuscript.

III. Bump, J.K., Webster, C. R., Peterson, R.O., Vucetich, J.A., Shields, J.A., & Powers, M.D. Large herbivore carcasses perforate ecological filters in forest herbaceous layers. Manuscript.

IV. Bump, J.K., Tischler, K.B., Schrank, A.J., Peterson, R.O., Vucetich, J.A. Large herbivores and aquatic-terrestrial links in southern boreal forests. Manuscript.

## Introduction

A dead mammal is a high quality resource. Consider your own elemental makeup compared to most biological tissues that return to the soil (Table 1). Adult humans possess high water content, a narrow carbon:nitrogen ratio, and relatively high nutrient concentrations compared to plant material (Table 1). These properties characterize high quality resources with significant amounts of available energy, essential nutrients, and rapid rates of decay. The same is true for pigs, rabbits, dogs, and deer – in most instances mammalian tissue trumps plant tissue in terms of resource quality and decomposition rate (Figure 1). This means that, even though ~99% of the organic resources that undergo decomposition in terrestrial ecosystems are plant derived or faecal matter (Swift et al. 1979), mammalian tissues are a top ecological and energetic prize.

**Table 1.** Chemical composition of cadaver, carcass, plant, and faecal resources

| Organic resource | H2O (%) | C:N ratio | N ($g\ kg^{-1}$) | P ($g\ kg^{-1}$) | K ($g\ kg^{-1}$) | Ca ($g\ kg^{-1}$) | Mg ($g\ kg^{-1}$) | References |
|---|---|---|---|---|---|---|---|---|
| Human cadaver | 50-75 | 5.8 | 32 | 10 | 4.0 | — | 1.0 | Tortora & Grabowski (2000) |
| Pig carcass (*Sus scrofa* L.) | 80 | 7.7 | 26 | 6.5 | 2.9 | 10 | 0.4 | DeSutter & Ham (2005) |
| Beech litter (*Fagus sylvatica* L.) | 10 | — | 12 | 1.2 | 5.0 | 17.3 | 2.1 | Vesterdal (1999) |
| Norway spruce litter (*Picea abies* L.) | 7.1 | — | 11 | 0.9 | 2.2 | 16.6 | 1.0 | Vesterdal (1999) |
| Moose pellets (*Alces alces* L.) | — | 20-23 | 2.4 | — | — | — | — | Pastor et al. (1993) |

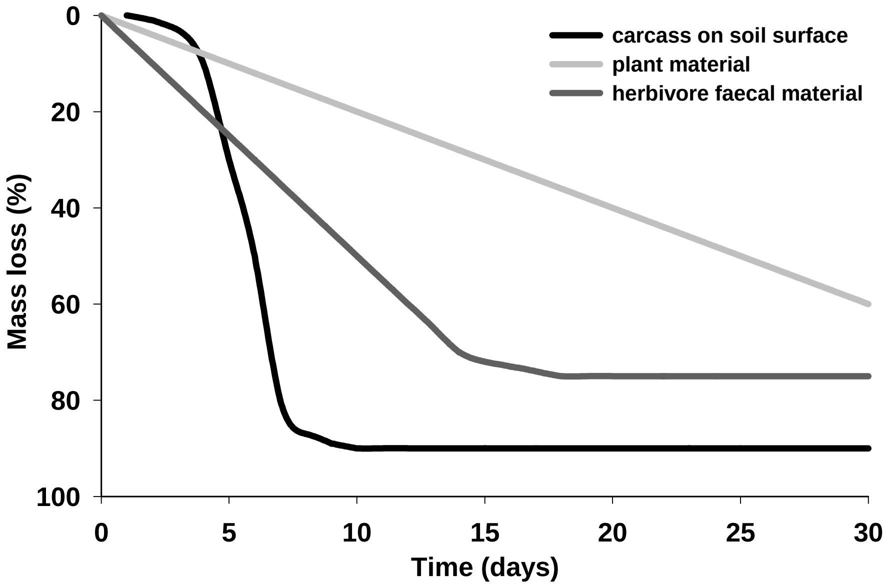

**Figure 1.** Mass loss curves generally associated with a carcass on the soils surface, plant material, and faecal material (after Carter et al. 2007). Mass loss data compiled from previous publications: carcass (Payne 1965), plant material (Wardle et al. 1994, Coleman et al. 2004); herbivore faecal matter (Putman 1983, Esse et al. 2001).

This fundamental point has long-been appreciated by community ecologists studying the quantity and quality of material that flows among organisms (Polis et al. 1997, 2004). Invertebrates, vertebrates, and microbes compete for carcass resources. Invertebrates can consume a carcass before a scavenger uses it (Putman 1978a, DeVault et al. 2004) and microbes can act as competitive consumers in food webs by releasing repellent toxins that exclude other consumers (Janzen 1977, Burkpile et al. 2006). In comparison to community ecologists, ecosystem ecologists have historically ignored or discounted organic matter input of mammalian carcasses as transient or ecologically trivial. Fundamentally, this omission is somewhat surprising given that a mammalian carcass is ~20% carbon and carbon cycling, carbon sequestration, and soil organic matter formation are vanguards of current ecological research (Fang et al. 2005, Fitter et al. 2005, Moran et al. 2005, Janzen 2006). This omission is not surprising for small carcasses (e.g., rodents) that tend to be consumed *ex situ* and often completely so that the amount of carcass material entering soils is likely negligible (Devault et al. 2003). However, large carcasses are consumed (at least partially) *in situ*, which allows carcass material to enter the soil (Coe 1973, 1978, Towne 2000) and recalcitrant tissues to remain on the soil surface (e.g., bone, hair, teeth, dessicated skin; Putman 1983).

Perhaps the appearance of most large mammal carcass sites also prevents one from recognizing that carcasses in terrestrial ecosystems could also be important to carbon and nutrient cycling, landscape heterogeneity, and biodiversity. Mammal carcasses are typically very well-used, with soft tissue resources moving up a trophic level via consumption by predators and scavengers (Wilmers et al. 2003) rather than entering decomposition pathways directly (Burkpile et al. 2006). For example, at white-tailed deer (*Odocoileus virginianus*) carcass sites in the Upper Peninsula of Michigan, USA, and at sites where wolves (*Canis lupus*) have killed moose (*Alces alces*) in Isle Royale National Park, USA, little appears to remain except bone, hair, and rumen contents (first stomach chamber). However, natural die-offs in large mammal populations, especially ungulates, are geographically widespread and declines of 70-90% are not uncommon (Young 1994). When die-offs occur predator and scavenger populations are often satiated. Such instances of high carrion abundance can lead to slow and incomplete carcass consumption, consumption of specific tissues only (e.g., visceral organs), and intact carcasses that putrefy and decompose *in situ*. Under such conditions carcasses would likely result in more intense effects on soils because a higher percentage of the carcass biomass would enter soil communities. Thus, carcass-derived materials may be particularly important when invertebrates and microbes dominate carcass decomposition or when a carcass is too large to be carried away intact by a scavenger or predator.

Recent research has begun to recognize the importance of the nutrient rich and highly labile carcasses of large mammals in terrestrial ecosystems. In the absence of predators, bison (*Bos bison*, L.), cattle (*B. Taurus*, L.), and white-tailed deer (*Odocoileus virginianus*, Boddaert) carcasses can create strong, localized disturbances that deposit nutrients at intensities that exceed other natural processes, thus altering prairie species composition and biomass (Knapp et al. 1999, Towne 2000). In the low-resource environment of the Arctic tundra, the impact of muskox carcasses on surrounding vegetation is still dramatic after 10 years (Danell 2002). The vegetation growing within and immediately adjacent to carcass sites contains high levels of nutrients as a result of growing in the enriched soil. Herbivores are attracted to patches of high-quality forage (Day and Detling 1990). Hence carcass sites become grazing sites (Towne 2000, Hobbs 2006), and the probability of repeated grazing near carcass sites initiates a positive feedback of recurrent nutrient supplementation from feces and urine deposition (Hobbs 1996). Carcass sites are also repeatedly visited by scavengers, even after soft tissues are gone, and often serve as sent-marking stations (Gray 1993, Danell 2002). These positive feedback mechanisms can expand the initial carcass impact beyond the carcass boundary for multiple years.

These studies document that carcass subsidies usually increase productivity and enhance spatial heterogeneity, the effects of which can propagate through food webs and influence species abundance and stability in complex ways (Towne 2000, Hobbs 2006). Yet, mechanisms influencing the magnitude of carcass-derived resources released to soils and carcass deposition in time and space are unexplored. Analyzing one possible mechanism, the effect of a predator (wolves) on large ungulate (white-tailed deer, elk, and moose) carcass distribution and nutrient release is the focus of one of the papers (Paper II) presented in this dissertation.

It is likely that predators, such as wolves, influence nutrient subsidies derived from prey carcasses because wolf prey items are typically well-consumed (Peterson 1977), which diverts much of the soft-tissue prey biomass that could decompose and enter soil communities. In addition to affecting the quantity of carcass-derived material entering decomposition pathways, predation patterns also affect the spatial and temporal distribution of carcasses. Wolf predation may occur in some areas of the landscape at rates greater than those for other types of mortality (e.g., starvation) and wolf predation may be more severe during certain seasons than other types of mortality. For example, wolf predation on moose typically is more severe during winter in Isle Royale National Park (IRNP) (Peterson 1977, Ballard and Ballenberghue 1997). Variable winter severity has also been identified as a significant influence on wolf predation patterns on elk in Yellowstone National Park (YNP) (Mech et al. 2001, Smith et al. 2004). Shorelines are the main kill sites for wolves in IRNP (Peterson 1977). Observations of elk before and after wolf establishment in YNP indicate that elk appear to avoid wolves in summer but do not spatially separate themselves from wolves in winter (Mao 2003). Mortality of Yellowstone's northern range elk typically occurred outside park boundaries until wolves were re-established. It now appears that more northern range elk die within YNP boundaries than prior to wolf re-establishment. Hence predation is expected to exhibit spatial and temporal patterns across the landscape and through the year. Consequently, if the carcasses of wolf prey items do indeed provide significant resource pulses to soil communities, then wolves would affect the heterogeneity of soils because wolves influence the spatial and temporal distribution of carcasses.

The first paper in this dissertation addresses whether or not prey consumption by predators eliminates or diminishes resource pulses from carcasses. The effect predation potentially has on the magnitude of carcass-derived resource pulses arguably supersedes the effect of predation on carcass spatial distribution or temporal deposition. If prey consumption by predators negates the fertilizing effects of carcass-derived resources, then the influence of predation on carcass spatial distribution or temporal deposition are moot in the context of resource pulses. Therefore, the first paper is focused on understanding the effect of predators on the magnitude of resource pulses from prey carcasses.

The second paper in this dissertation explores how large carnivores may configure landscape heterogeneity through prey carcass distribution. If carcasses produced via predation are important to above and belowground communities, and predation occurs in some locations at rates that are different than the rates for other causes of mortality (e.g., starvation, hunting), then carnivores would also affect the spatiotemporal heterogeneity of soil and plant properties. Data supporting such effects would provide empirical evidence for a mechanistic link between large carnivores and biodiversity in terrestrial ecosystems.

The third paper in this dissertation examines the disturbances and resource pulses that large ungulate carcasses create in the herbaceous layers of northern hardwood forests. The limited work on the ecological effects of large ungulate carcasses has largely focused on grassland and tundra systems (Towne 2000, Danell 2002, Hobbs 2006). The effects of large ungulate carcasses on forest ecosystems, however, are unknown. If even well-used ungulate carcasses affect forest soil, floor, and tree seedlings in the herbaceous layer, then such effects would be conservative evidence that carcasses are linked to the spatial patterns of disturbance and forest tree regeneration in a novel way.

The forth and final paper in this dissertation examines how moose carcasses can serve as a resource vector for the transfer of aquatic-derived materials into terrestrial systems. Aquatic-terrestrial links are fundamental to ecology, yet few studies have the data to assess the long-term spatiotemporal patterns of cross-habitat resource transfers. Moose are aquatic-terrestrial interface specialists (*sensu* Ballinger and Lake 2006), foraging extensively on aquatic macrophytes during spring and summer (Franzmann & Schwartz 1997), and excreting wastes and dying most frequently in terrestrial habitats (Franzmann & Schwartz 1997, Peterson 1977). Hence, moose comprise an unquantified vector for the transport of aquatic materials into terrestrial systems, which constitutes a resource loss pathway from the aquatic perspective (Leopold 1941, Likens and Bormann 1974, Loreau & Holt 2004).

## Methods

The papers presented in this dissertation combine experimental, modeling, and observational methods to examine the ecological legacy of large ungulate carcasses and the affects of predation on carcass dynamics. Detailed descriptions of methods occur in each paper. What follows here is a general summary of the methods used for each paper.

To understand the effect of predators on the magnitude of resource pulses from prey carcasses (Paper I) we used an experiment in a wolf-deer system in Michigan's Upper Peninsula, and observational analyses of the wolf-elk relationship on the Northern Range (NR) of Yellowstone National Park, USA (YNP) and of the wolf-moose relationship on Isle Royale National Park, USA (IRNP). The experiment was designed to isolate the influence of predator consumption and large vertebrate scavenging on the magnitude of resource pulses from deer carcasses. Comparisons of resource pulse between definite wolf-killed and starvation-killed elk and moose in the two national parks were used to determine the influence of predators on the magnitude of carcass-derived resource pulses in natural systems. We expected wolf-killed prey to decrease carcass-derived resource pulses because prey items in these systems are typically well utilized by wolves and scavengers. Ungulates that die from starvation may remain intact long enough to allow for putrification and significant decomposition to occur *in situ* (pers. obs.). We also expected that smaller prey items would be utilized more swiftly, resulting in significant resource pulses to be more likely and with stronger effects as the predator:prey body mass ratio decreases.

To examine how wolves may affect soil and plant heterogeneity by influencing prey carcass distribution (Paper II), we examined differences in forest (Isle Royale

National Park, USA) soil macronutrient availability, microbial biomass and composition, and plant leaf tissue nitrogen at wolf-killed moose carcass and paired control sites for ~3.5 years postmortem. We used 3654 carcass locations recorded since 1958 to see how total moose carcass density and the ratio of wolf:starvation killed carcasses changed over time and space. Additionally, differences in grassland (Yellowstone National Park, USA) soil macronutrient availability and plant leaf tissue nitrogen were compared at wolf-killed elk carcass and paired control sites for ~3.5 years postmortem.

To examine the effect of white-tailed deer carcasses on herbaceous layers and soils in a northern hardwood forest (Paper III), differences in soil macronutrient availability, ground-layer litter depth, bare soil, and spring and summer herbaceous layer species composition, were compared at deer carcass and paired control sites for 2 years postmortem. We also compared tree seedling germination, leaf tissue nitrogen, and growth between carcass and control sites to understand if carcass disturbances are linked to the successional trajectories and structural characteristics of forests (Frelich 2002, Gilliam 2007). Because predators and scavengers were not excluded from experimental plots, we expected carcasses to be well-used, with near complete consumption of soft tissues, as is most often the case in our study system. Therefore, we predicted no difference in the soil and herbaceous layer response to carcass and control treatments.

To investigate the temporal and spatial patterns of resource flux via moose from aquatic to terrestrial (Paper IV), we quantified aquatic-derived nitrogen (N) entering the terrestrial system via moose and estimated P loss from aquatic systems due to moose herbivory. We focused on N and P because these elements are typically the most limiting in temperate and boreal forests (Larsen 1980), and north-temperate and boreal lakes

(Wetzel 2001), respectively. Long-term (1958-2005) spatial patterns of the moose-mediated flux were explored by 1) analyzing carcass site clustering at island-wide and ~1km scales, and 2) comparing the likely areal extent of the aquatic resource shadow (i.e., a zone from which energy, materials, or organisms have been diverted; the location of aquatic plant removal in this study; *sensu* Polis et al.2004) to the likely areal extent of the terrestrial excretory N dispersion in summer. Carcass site locations define where aquatic resources that were incorporated into moose biomass enter consumer and detritivore communities. We expected carcass sites to be clustered due to moose habitat choice and predation patterns (Peterson 1977). Comparing the aquatic resource shadow extent to the terrestrial dispersion examines potential asymmetric spatial patterns in resource flux between donor (aquatic) and recipient (terrestrial) systems. Water depth typically limits moose foraging in aquatic habitats to near-shore, littoral zones (Franzmann & Schwartz 1997). In contrast, physical features do not generally limit where moose forage in terrestrial habitats and mortality can occur anywhere on the landscape. Consequently, we expected that the areal extent of the donor system resource shadow should be substantially less than the extent of resource dispersion in the recipient system.

## Results

### *General summary*

The typical effects large ungulate carcasses had at kill sites relative to control sites were increases in soil pH, field water, macronutrient concentrations, microbial abundance, plant tissue nitrogen, percent bare ground, tree germinant density, and tree seedling growth (Table 2). Carcasses also caused decreases in plant tissue carbon:nitrogen ratio, litter depth, and percent cover and importance value of dominant tree and herbaceous species at carcass sites versus control sites (Table 2).

Experimental exclusion of predation from carcasses indicated that in the absence of predators carcass effects may be much stronger (Paper I). There may also be a logistic relationship between predator:prey body mass ratio and the probability of a significant carcass-related effect (Figure 2). In general, soil nutrient increases at carcass sites were greater the second growing season postmortem than the first growing season, but plant tissue changes at carcass sites responded immediately, without a similar lagged response (Figure 3A). The nitrogen stable isotope differences in soils and plants at carcass sites versus control sites appear to be more persistent than the macronutrient effects, lasting beyond three growing seasons postmortem (Figure 3B). Wolf-killed prey can occur at densities much greater than for other causes of mortality and carcasses appear to be clustered in areas that favor predator hunting success (Paper II, Paper IV).

**Table 2.** Soil, bacterial, plant, and herbaceous layer characteristics that changed significantly at carcass* versus control sites

| Characteristic | Effect size (% change) |
|---|---|
| *Soil properties* | |
| pH | + 50% |
| Field water | + 200-300% |
| *Soil macronutrients* | |
| Inorganic nitrogen | + 100-900% |
| Phosphorus | + 60-300% |
| Potassium | + 50-600% |
| *Microbial abundance* | |
| Bacterial | + 30-50% |
| Fungal | + 50% |
| *Plant nitrogen* | |
| Tree leaf | + 25-100% |
| Grass | + 60-75% |
| Herbaceous leaf | + 20-40% |
| *Plant carbon:nitrogen ratio* | |
| Tree leaf | – 15-110% |
| Grass | – 30-60% |
| Herbaceous leaf | – 20-45% |
| *Dominant herbaceaous layer species* | |
| Percent cover | – 20-25% |
| Importance value | – 10-25% |
| *Hardwood forest floor* | |
| Litter depth | – 20-40% |
| Bare ground | + 40-60% |
| Germinants | + 75% |
| *Tree seedling growth* | |
| Maple ring width | + 835% |

*Includes range of mean differences from moose, elk, white-tailed deer carcasses.

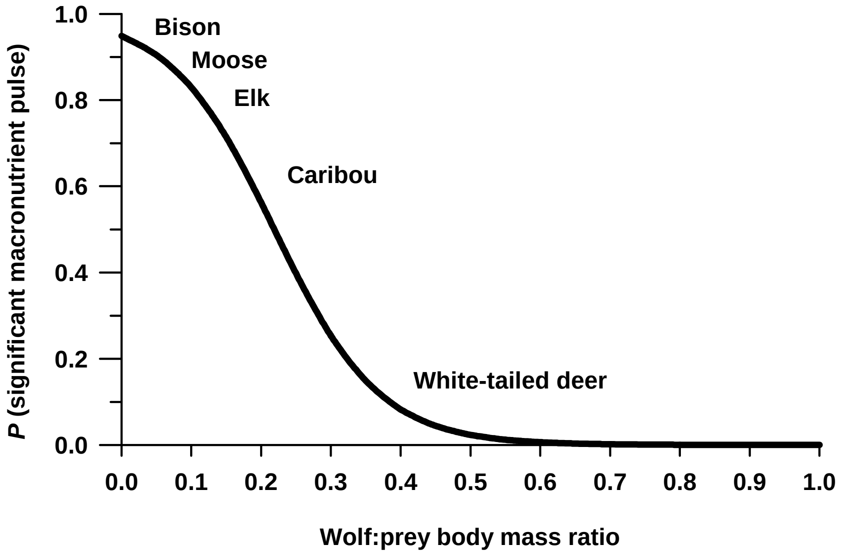

**Figure 2.** Relationship between wolf:prey body mass ratio and the probability of significant macronutrient pulses. The model is based on data from deer carcasses in Michigan's Upper Peninsula, elk carcasses in Yellowstone National Park, and moose carcasses in Isle Royale National Park, and fit using the logit transformation.

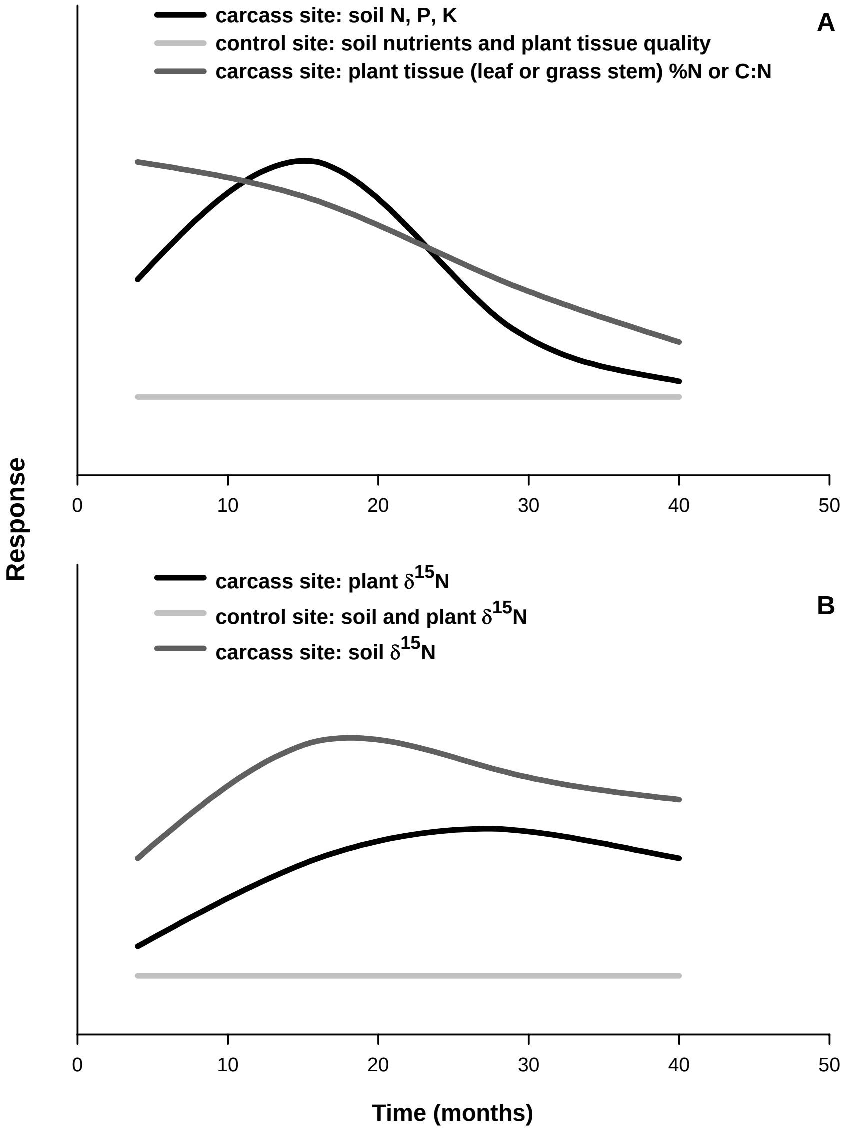

**Figure 3.** Temporal patterns of soil and plant responses typical of carcass and control sites. Soil nutrient and plant tissue quality curves (**A**) are based on data from deer carcass sites in Michigan's Upper Peninsula, elk carcass sites in Yellowstone National Park, and moose carcass sites in Isle Royale National Park. Soil and plant nitrogen stable isotope ($\delta^{15}$N) curves (**B**) are based on soil data from moose carcass sites in Isle Royale National Park and plant tissue data from deer carcass sites in Michigan's Upper Peninsula, elk carcass sites in Yellowstone National Park, and moose carcass sites in Isle Royale National Park.

### *Paper summaries*

**Paper I -** Experimental exclusion of predation resulted in dramatic increases in carcass-derived nutrient pulses. Prey carcasses in natural systems resulted in significant nutrient pulses to soils, with no difference found between wolf-killed and starvation-killed carcasses. Prey carcasses may be less likely to cause significant nutrient pulses unless they are at least 3 times the mass of their predators.

**Paper II** - Wolves modulate heterogeneity in soil nutrients, soil microbes, and plant quality by clustering prey carcasses over space. Despite being well-utilized by predators, moose carcasses resulted in elevated soil macronutrients and microbial biomass, shifts in soil microbial composition, and elevated leaf nitrogen for at least 2-3 years at kill sites. Wolf-killed elk carcasses resulted in elevated soil macronutrients and leaf nitrogen for at least 3-4 years at kill sites. Wolf-killed moose were deposited in some regions of Isle Royale at up to 12× the rate of deposition in other regions. Carcass density also varied temporally, changing as much as 19-fold in some locations during the 50-year Isle Royale Moose-Wolf study period. This variation arises from spatiotemporal variation in wolf behavior and forest composition.

**Paper III** – Even well utilized white-tailed deer carcasses create unexpected disturbances that reduce the cover of dominant herbaceous layer species. Carcasses also provided pulses of limiting nutrients for at least two growing seasons postmortem. These effects increased tree seedling germination, leaf tissue quality, and growth on carcass sites compared to undisturbed sites. The spatial distribution of carcasses is influenced by predators; hence these data establish an unrecognized link between large carnivores, prey carcasses, and ecosystem processes.

**Paper IV** - Moose transfer significant amounts of aquatic-derived nitrogen to terrestrial systems, which likely increases terrestrial nitrogen availability. Aquatic-phosphorus loss due to moose herbivory may be offset by releases of phosphorus from interstitial benthic sediment waters disturbed during foraging. The areal extent of aquatic-derived nitrogen dispersion in terrestrial habitats exceeded the extent of aquatic phosphorus loss. Aquatic foraging by moose and moose carcass locations are significantly clustered at multiple scales, indicating that grey wolves and moose can create concentrated areas of resource transfer due to clustered predation and foraging patterns.

## Discussion

The papers in this dissertation support four general ideas that warrant further scientific exploration:

1) Body size relationships and functional traits of species in large mammal predator-prey systems are linked to the dynamics of energy flow and resource pulses in ecosystems (Paper I).

2) Top terrestrial predators can serve as a mechanism generating landscape heterogeneity, linking large carnivore behavior, ecosystem function, and biodiversity (Paper II).

3) The carcasses of large herbivores in forest ecosystems can create disturbances that create an important interaction between predator-prey functional traits and ecological filters (Paper III).

4) Patterns of faunal-mediated resource transfer across aquatic-terrestrial boundaries depend on species-specific functional traits, and predators can influence herbivore-controlled resource transfer between ecosystems (Paper IV).

### ***Predator-heterogeneity-biodiversity chain forged through carcass distribution***

The collective, ecological relevance of the papers in this dissertation is found within the broad question of what role do large herbivores and large carnivores play in ecosystems. The carcasses of large herbivores have been recognized as a source of disturbance in grassland ecosystems (Hobbs 2006). The papers in this dissertation demonstrate that large herbivore carcasses create disturbances in forest ecosystems as well. This is significant because forest ecosystems supporting large herbivores are circumpolar in the northern hemisphere, covering 36% of the land mass (Goodale et al. 2002). Also, research on the effects of disturbance in forest ecosystems has been dominated by large-scale, abiotic factors (e.g., fire, floods, wind, ice, landslides) and fewer biotic factors (e.g., herbivory, disease and insect outbreaks; Frelich 2002, Roberts 2004). Hence, demonstrating that herbivore carcasses serve as important disturbance mechanisms in forest ecosystems represents an advance in our understanding of large herbivore and forest ecology.

Disturbances create temporal and spatial variation in resources and ecosystem processes, i.e., heterogeneity. The papers presented in this dissertation demonstrate that large herbivore carcasses create heterogeneity in soil resources, microbial biomass and composition, and plant tissue quality, growth, and seedling germination. Microbial community composition appears to shift at carcass sites (Paper II), perhaps reflecting changes in microbial diversity since some microbes exhibit high fidelity to carcass material (Sagara 1976, 1992, 1995). Deer carcasses may also shift biodiversity in herbaceous communities in hardwood forests (Figure 4), although this result likely reflects a decrease in dominant herbaceous layer species rather than recruitment of species previously not present.

Future carcass-related research clearly needs to incorporate additional measures of biodiversity (e.g., microbial genetics) change that may be associated with carcass sites. However, it is clear that herbivore carcasses unambiguously contribute to the shifting mosaic of resources, nutrient cycling, and competitive relationship in soil communities and among plant species. This is significant because disturbance - by creating heterogeneity - is theoretically and empirically supported as a major factor in structuring ecosystems, maintaining ecosystem services, and biodiversity (Reich 1994, Frelich 2002). The "intermediate disturbance hypothesis" of species coexistence reasons that biodiversity is highest when disturbance is neither too rare nor too frequent (Grime 1973a, 1973b, Wilkinson 1999, Chesson 2000, Roxburgh et al. 2004). Competitive exclusion by dominant species results with low disturbance rates and only species capable of rapid recolonization persist with high disturbance rates (Figure. 5). The papers in this dissertation support the claim that large herbivore carcasses contribute to creating an intermediate level of disturbance in forest and grassland ecosystems.

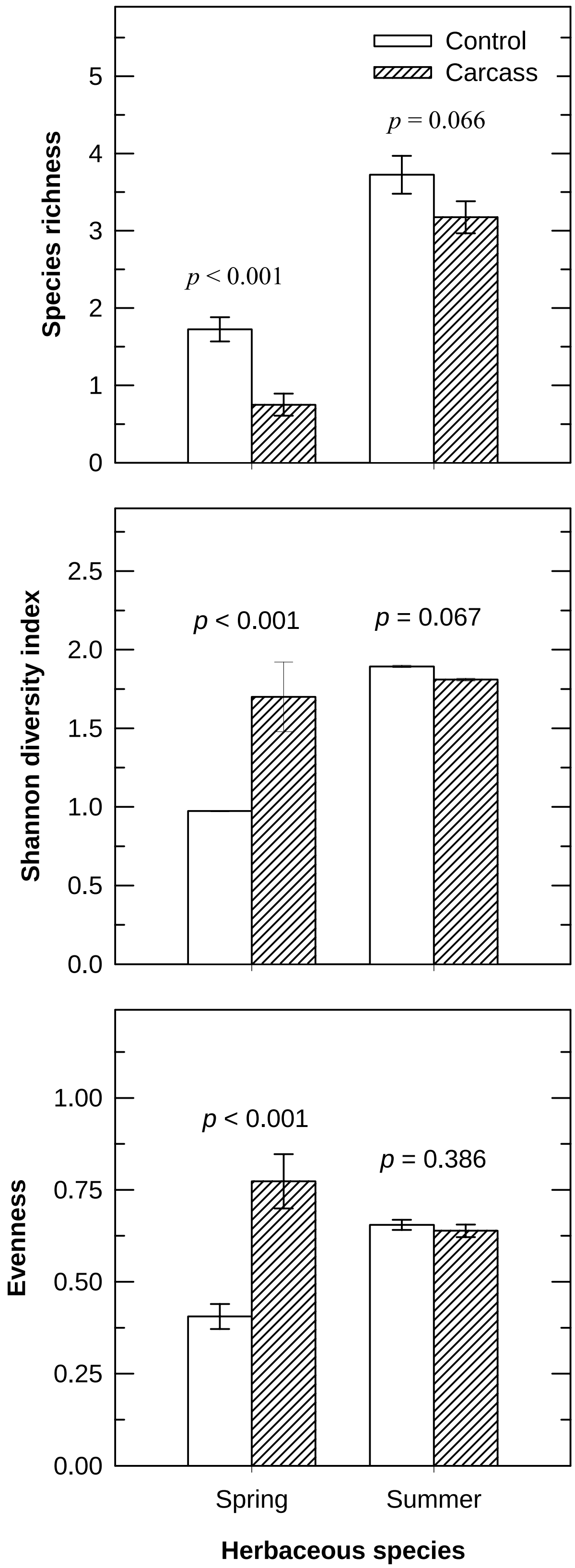

**Figure 4.** Mean ± 1SE species richness, diversity, and evenness per $m^2$ of herbaceous species by treatment type in spring and summer in a northern hardwood forest. P-values are for related signed ranks tests (Wilcoxon), alpha = 0.05, N=40.

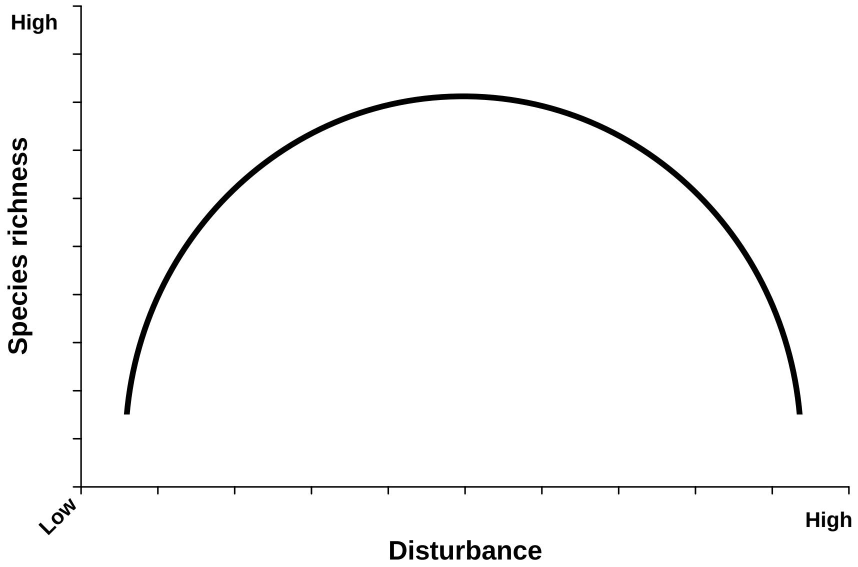

**Figure 5.** General graphical model of the intermediate disturbance hypothesis (adapted from Wilkinson 1999).

The disturbance created by the carcass of a large herbivore is affected by the presence of large herbivore predators. Predators affect both temporal and spatial characteristics of the carcass disturbance. The papers in this dissertation indicate that moose (*Alces alces,* L.) and elk (*Cervus canadensis*, Erxleben) produced via wolf predation are important to above and belowground communities (Paper II). Predation by wolves also occurs in some locations at rates that are different than the rates for other causes of mortality such as starvation and hunting (Wilmers et al. 2003, Kauffman et al. 2007). For example, wolves preferentially travel along shorelines in Isle Royale National Park (Peterson 1977), which results in moose kills being significantly clustered near water (Peterson 1977, Paper II). Similarly, landscape analysis of wolf-killed elk in

Yellowstone National Park shows that flat grasslands near streams and roads are favorable to wolf hunting success (Kauffman et al. 2007). In northern hardwood forests, wolves preferentially kill deer within pack territories, avoiding areas of territory overlap, presumably to minimize the chance of lethal interactions with wolves from other packs (Mech 1977). Hence, across three distinct systems, wolf hunting behavior affects the landscape distribution of carcasses. These wolf-carcass-landscape links demonstrate how the functional traits of species in large mammal predator-prey systems are linked to ecosystem processes, such as resource heterogeneity, through carcass distribution. These links forge a large predator-heterogeneity-biodiversity chain in terrestrial ecosystems.

Such links may be present in other systems as well. For example, lions successfully fed in areas of high prey "catchability" rather than areas of high prey density (Hopcraft et al. 2005). Hence, if carcasses at lion-kill sites result in effects similar to those observed for moose, elk, and deer carcasses then the predatory behavior of lions would be similarly linked to landscape heterogeneity.

### ***Contrasting predator-heterogeneity-biodiversity chains in marine and terrestrial systems: some key examples***

Insight into the role (i.e., functional significance) of large terrestrial carnivores can be gained by a comparison with studies of large marine predators (Bowen et al.1997). While there is utility in a comparative approach, it is important to expect that the differences in the structure and function of marine and terrestrial ecosystems could lead to distinct roles for large predators in each system. Perhaps foremost, the geometry (i.e., 2 versus 3 dimensions) of the habitat fundamentally affects foraging behavior of top predators, which influences their ecosystem-level role (Whitehead & Walde 1992). If, however, soils are properly recognized as important in the distribution of large prey by influencing the productivity and nutrient concentrations of plants (McNaughton 1988), then the habitats of terrestrial carnivores are arguably 3 dimensional as well.

In this dissertation I have presented evidence supporting the argument that large terrestrial predators physically and chemically restructure soils and plants by affecting where and when the carcasses of prey items are distributed. Similarly, some marine mammals play a significant role in the physical restructuring of the benthos (Bowen 1997). Feeding disturbances by gray whales (*Eschrichtius robustus*) and pacific walruses (*Odobenus rosmarus*) may structure benthic fauna by selectively feeding on specific species ( Fay et al. 1977, Fay & Stoker 1982, Oliver et al. 1983, Johnson & Nelson 1984, Nerini 1984, Oliver & Slattery 1985, Oliver et al. 1985). Walruses are specialized consumers of infaunal bivalves, producing many pits and furrows in soft benthic sediments as they feed (Bornhold et al. 2005, Ray et al. 2006). Gray whales also create “feeding pits” when foraging on infaunal amphipods (Nerini 1984, Oliver & Slattery 1985). Macroinvertebrates are reduced in the feeding pits created by walruses and gray whales (Oliver et al. 1985, Oliver & Slattery 1985), which may affect benthic community structure by helping to maintain early-colonizing species and creating habitat for juveniles to occupy within the mosaic of benthos where space is a limiting resource. This dynamic is analogous to the physical disturbance created by large ungulate carcasses in grassland and forest herbaceous layer communities (Towne 2000, Paper II).

Walrus and gray whale feeding also creates a biotic vector for nutrient flux from sediment pore water to the water column. Gray whales may turn over between 9 and 27% of the benthic substrate in the northern Bering Sea annually (Nerini 1984). Walruses are estimated to consume 3 million metric tons of benthic biomass annually in Beringia (Ray et al. 2006). The bioturbation and faecal deposition created by walrus feeding alone magnifies nutrient flux between the benthic and pelagic habitats by about two orders of magnitude (Ray et al 2006). In the analysis of moose as a biotic vector for the transfer of nutrients between aquatic and terrestrial habitats, it was determined the wolves likely influence where the "moose-mediated" flux occurs on the landscape (Paper IV). In comparison then, there are cases in which marine predators serve as direct vectors for nutrients between habitats, while in terrestrial systems top predators may influence the flux of nutrient between habitats more frequently through indirect mechanisms (e.g., carcass distribution and habitat choice).

Brown bears (*Ursos arctos*) are another top predator that significantly affect nutrient flux and heterogeneity in terrestrial systems. Brown bears facilitate the flow of marine-derived nutrients, especially nitrogen, into terrestrial ecosystems by consuming salmon in riparian habitats (Hilderbrand et al. 1999). Brown bears typically catch salmon and repeatedly relocate to a nearby riparian spot to consume their catch. This behavior leads to distinct areas where salmon carcasses and bear feces accumulate that are called "bear kitchens." Nitrogen cycling is altered in these bear kitchens, thereby linking top predators to the creation of biogeochemical hotspots (Holtgrieve & Schindler *unpublished*). These hotspots contribute to the heterogeneity of nutrient cycling in riparian zones.

In subalpine meadows of the Northern Rocky Mountains (USA) grizzly bears (*Ursos arctos horribilis*) dig for lily tubers. Digging by grizzly bears may be a major force in determining plant distribution and abundance in subalpine meadows (Stanford et al. *unpublished*). Nitrogen cycling and plant successional trajectories are altered in digging zones. Hence, grizzly bears are creating a disturbance that potentially affects the mosaic of plant species distribution and biodiversity in subalpine meadows. This effect on subalpine meadow heterogeneity is not unlike the effects of walrus and gray whale disturbance of benthic sediments. Further comparison of the behavior ecology of large predators between marine and terrestrial systems and across species within systems may lead to a synthetic understanding of the role of large predators in ecosystems.

### ***Prospectus***

The existence of a long-term carcass database was critical to the development of ideas, analyses, and conclusions drawn in this dissertation. The carcass database of the Isle Royale Wolf-Moose study currently contains over 4000 entries with associated attributes (sex, age, cover, etc.). The carcass database of the Yellowstone Wolf Project (begun in 1995) will soon eclipse that of the Isle Royale Wolf-Moose study (Smith *pers. comm.*). A carcass database containing 49,453 entries recorded from 1954-2000 exists for Kruger National Park in South Africa (Owen-Smith & Mills 2007). These databases offer the opportunity to study the effects of large carcasses and the influence of large predators on carcass effects in different ecosystems and across multiple biomes. This may permit a more synthetic understanding of the ecological legacy of large carcasses and the role of large predators in multiple terrestrial ecosystems.

One key question worth addressing is, “Do shifts in predator social structure alter the spatial distribution patterns of carcasses?” For example, changes in wolf-pack dominance structure, territory size and location, and the overall number of packs in an area may influence where high carcass deposition occurs. The relationship between shifting social structure could be examined by comparing carcass density and distribution among periods of key change in social structure, such as wolf pack or lion pride dominance and the formation or loss of a wolf pack or lion pride.

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# Appendix

## Paper I – Planned submission to *Oikos*

# Predator:prey body mass ratio determines magnitude of nutrient resource pulse to soils from ungulate prey carcasses

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### Abstract

Body size relationships and resource pulses are important to the structure and function of food webs. However, little data exists on the interaction between body size relationships and biotic resource pulses. Here we examine the influence of predation on nutrient resource pulses to soils derived from large ungulate carcasses. Carcass-derived nutrient pulses were examined across three wolf-prey systems in which primary prey items varied in size from white-tailed deer to elk to moose. Experimental exclusion of predation resulted in dramatic increases in carcass-derived nutrient pulses. Prey carcasses in natural systems resulted in significant nutrient pulses to soils, with no difference found between wolf-killed and starvation-killed carcasses. Prey carcasses may be less likely to cause significant nutrient pulses unless they are at least 3 times the mass of their predators. These results indicate how body size relationships and functional traits of species in large mammal predator-prey systems are linked to the dynamics of energy flow and resource pulses in ecosystems.

## Introduction

Resource pulses are important because they alter the relative strength of top-down and bottom-up forces in terrestrial and aquatic communities (Lodge et al. 1994, Ostfeld & Keesing 2000, Sears et al. 2004), may affect population dynamics and life history patterns (Southwood 1977), influence diversity gradients in food webs (Sears et al. 2004), and are valuable to conservation and predicting human disease risk (O'Donnell & Phillipson 1996, Ostfeld et al. 2006). These effects have been determined by focusing on abiotic processes and biotic events such as single species productivity, emergence, or migration that cause resources pulses. For example, ecologists have investigated how pulsed rain, oak mast, periodic cicadas, and spawning salmon influence community dynamics and ecosystem processes (Polis et al. 1997, Ostfeld & Keesing 2000, 2007, Yang 2004, Gende et al. 2002, Schindler et al. 2002, Helfield & Naimen 2006). Of the factors affecting biotic resources pulses, one of the least understood is the influence of trophic interactions.

Predator-prey relationships are key trophic interactions that move – and sometimes pulse – energy and nutrients through food webs. Recently, large ungulate carcasses have been recognized as sources of important resource pulses with lasting effects (Carter et al. 2007, Hobbs 2006). Bison (*Bos bison*) carcasses provide local pulses of limiting nutrients [nitrogen (N), phosphorus (P), potassium (K)] to soils at levels that exceed the intensity of urine and fecal deposits, thereby influencing grassland species composition, biomass, and prairie heterogeneity (Knapp et al. 1999, Towne 2000). In the low-resource environment of the Arctic tundra, the impact of muskox (*Ovibos moschatus*) carcasses on surrounding vegetation is still dramatic after 10 years (Danell 2002). The vegetation growing within and immediately adjacent to large ungulate carcasses is elevated in N as a result of growing in the enriched soil. Herbivores are attracted to patches of N-rich forage (Day and Detling 1990**,** Frank & McNaughton 1992, Yang *in press*). Hence carcass sites become grazing sites (Towne 2000), and the probability of repeated grazing near carcass sites initiates a positive feedback of recurrent nutrient supplementation from feces and urine deposition (Hobbs 1996, 2006). This positive feedback mechanism can expand the initial carcass impact beyond the carcass boundary for many years.

These and other studies (reviewed in Carter et al. 2007) are valuable because they demonstrate that large ungulate carcasses constitute an important resource pulse that links above and belowground systems. However, these studies either did not include animals that died from predation or the cause of death was unknown. Consequently, the influence of predation on carcass-derived resource pulses has not been examined. Due to variable consumption and hunting patterns, predation potentially influences carcass 1) spatial distribution, 2) temporal deposition, and 3) the magnitude of resources released. For example, elk (*Cervus canadensis*) and bison (*Bison bison*) kill site locations in Yellowstone National Park are influenced, in part, by wolf distribution and behavior (i.e., flat grasslands close to streams and roads were found to be favorable to wolf hunting success; Kaufmann et al. 2007). In the absence of predation, temporal variation in carcass deposition increases, depending mostly on winter severity and ungulate density (Wilmers & Getz 2004). It is important to examine how predation affects the magnitude of carcass-derived resources pulses because prey biomass is typically very well utilized, moving up a trophic level via predation and scavenging (Wilmers et al. 2003), rather than entering decomposition pathways directly (Burkpile et al. 2006). For example, at moose carcass sites in Isle Royale National Park, little remains beyond bone, hair, and rumen contents (Fig. 1). Hence, prey consumption by predators may eliminate or diminish resource pulses from carcasses. These observations emphasize the importance of understanding the effect of predation on carcass-derived resource pulses.

The effect predation potentially has on the magnitude of carcass-derived resource pulses arguably supersedes the effect of predation on carcass spatial distribution or temporal deposition. If prey consumption by predators negates the fertilizing effects of carcass-derived resources, then the influence of predation on carcass spatial distribution or temporal deposition are moot in the context of resource pulses. Therefore, we focused this study on understanding the effect of predators on the magnitude of resource pulses from prey carcasses. To do so, we used an experiment in a wolf-deer system in Michigan’s Upper Peninsula, and observational analyses of the wolf-elk relationship on the Northern Range (NR) of Yellowstone National Park, USA (YNP) and of the wolf-moose relationship on Isle Royale National Park, USA (IRNP). The experiment was designed to isolate the influence of predator consumption and large vertebrate scavenging on the magnitude of resource pulses from deer carcasses. Comparisons of resource pulse between definite wolf-killed and starvation-killed elk and moose in the two national parks were used to determine the influence of predators on the magnitude of carcass-derived resource pulses in natural systems. We expected wolf-killed prey to decrease carcass-derived resource pulses because prey items in these systems are typically well utilized by wolves and scavengers. Ungulates that die from starvation may remain intact long enough to allow for putrification and significant decomposition to occur *in situ* (pers. obs.). We also expected that smaller prey items would be utilized more swiftly, resulting in significant resource pulses to be more likely and with stronger effects as the predator:prey body mass ratio decreases.

## Methods

### *Deer carcass experiment*

The influence of predation on the magnitude of carcass-derived resource pulses was investigated experimentally at the Ford Center Reseach Forest, near Alberta, MI, USA: SW ¼ Section 12 T49N R34W Baraga County, MI. Experimental plots were in dry-mesic northern forests dominated by red pine, white pine, and red oak originating from natural fires. Soils were deep, well-drained sands and find sands, primarily Kalkaska and Rubicon. Bracken fern (*Pteridium aquilinum*), wintergreen (*Gaultheria* *procumbens*), blueberry (*Vaccinium angustifolium*), sweetfern (*Comptonia peregrine*), and trailing arbutus (*Epigaea repens*) were the most abundant ground cover species.

A single-factor, randomized block design was used to test for the effect of predation on the magnitude of carcass-derived resource pulses. Treatments were unmanipulated, cage-control, predator exclusion, predator inclusion, with 6 replicates each. Rectangular cages (2.4m × 1.2m × 0.7m) for cage control and predator exclusion treatment were constructed of 2.5cm square mesh wire cloth on wood frames. Cages were staked with 1.5m metal fence posts at each end. Each treatment plot in a block was within a 15m radius, and all 6 blocks were within 2km of each other.

Four, pre-treatment soil cores (4 cm diameter × 10 cm depth) were collected from each plot in late April of 2006, pooled, homogenized, weighed, and dried to a constant weight at 105ºC. Each predator exclusion and predator inclusion plot then received an intact, previously frozen road-killed deer carcass, placed laterally on the ground surface. Deer carcasses were approximately the same size (mean ± 1SD =71.6 ± 5.8kg). Estimates of carcass utilization and observations of wildlife sign and cage condition were subsequently conducted at three-day intervals for 56 days. In early September 2006 the previous coring was repeated, with the cores at each predator exclusion and predator inclusion plot taken beneath carcass remains.

Soils were analyzed for inorganic nitrogen [i.e. nitrate ($NO_3^-$) and ammonium ($NH_4^+$)], magnesium (Mg), calcium (Ca), K, and P following standard procedures (Brown 1998). Briefly inorganic N was extracted with 1N KCl and analyzed calorimetrically, a Mehlich 3 extractant was used to determine exchangeable Mg, Ca, and K levels, and a Bray P1 extractant was used to determine soil available P. While recent work has demonstrated the importance of organic nitrogen uptake in some ecosystems (Schimel and Bennet 2004), and other methods exist for indexing available N (Binkley and Matson 1983), these assays are appropriate methods for assessing gross nutrient pools available to plants and microbial communities in north, temperate forest soils (Brown 1998). Analysis of nutrient concentrations was conducted blind by Michigan State University Soil and Plant Nutrient Lab.

If availably, leaves from red oak seedlings (*Quercus rubra* <50 cm tall) were collected during the September 2006 soil sampling from each treatment plot within a block for gross nutrient analysis. Leaves of equal size were clipped at their base from actively growing plants, dried at 64 °F to a constant weight, double rinsed with distilled water, dried again, and then individually homogenized in a bearing shaker mill. Measurement of total carbon (C), total N and stable isotope ($\delta^{13}$C, $\delta^{15}$N) concentrations was performed blind on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotpe Ratio Mass Spectrometer at Michigan Technological University's Ecosystem Science Center. IAEA, USGS, and NIST certified isotopic standards were run at the beginning of each analysis. One certified standard was also run at the end of the analysis to check for calibration stability. These standards are recognized internationally, and are used to calibrate the $N_2$ reference gases which were analyzed in conjunction with every individual sample. Stable isotope values are reported in standard $\delta$ notation, and are reported on the VPDB scale for $\delta^{13}$C and on the atmospheric air scale for $\delta^{15}$N. An internal standard was run every 10 samples. Precision based on repeated measures of internal standards was +/- 0.5‰ for $\delta^{15}$N.

Macronutrient data were analyzed using one-way mixed-model (treatment and plot) repeated measures analysis of variance (ANOVA) and *a priori* planned contrast to test the hypothesis of positive carcass effects on macronutrient concentrations, with effects size greater in the predator exclusion treatment than in the predator inclusion treatment. A one-tailed test of significance is appropriate for macronutrient analyses because previous studies with carcasses have established a strong *a priori* expectation of the effect direction, reflected in the direction hypothesis (Towne 2000, Carter 2007, Bump *unpublished*). Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between sampling times is the same for each plot). Leaf quality data were analyzed using mixed-model (treatment and plot) analysis of variance (ANOVA) and *post hoc* comparisons of treatment means using the Tukey-Kramer HSD test. Brown-Forsythe and O'Brian tests were used to confirm assumptions of non-heteroscedasticity for leaf quality data.

### *Moose and elk carcass site analysis*

Comparison of macronutrient resource pulses between definite wolf-killed and starvation-killed elk on the NR of YNP and moose in IRNP was done to determine the influence of predators on magnitude of carcass-derived resource pulses in natural systems. Wolf-killed and starvation-killed animals in these systems serve as natural analogs to the predator inclusion and predator exclusion treatments in the deer carcass experiment.

Moose likely colonized Isle Royale sometime between 1905 and 1913, followed by wolves in the late 1940s. Island moose and wolf populations have fluctuated dramatically during the past half-century in what appears to be 2-decade intervals with significant predation, food, and weather effects on moose. Introduced disease (canine parvovirus) has significantly reduced wolves at one point and parasites (*Dermacentor albipictus*) likely affect moose levels (Peterson 1977). Moose are the primary component (~85%) of wolf diets on Isle Royale (Thurber & Peterson 1993). Wolves were extirpated from the YNP in the 1930s, reintroduced in 1995, and have since increased from 14 wolves in three packs to 172 wolves in 11 packs in 2007 in the Greater Yellowstone Ecosystem (Smith *unpublished*). Elk are the primary component (92%) of wolf diets in YNP (Smith et al. 2004). Details of the YNP and IRNP study areas have been previously described in (Smith et al. 2004) and (Peterson 1977), respectively.

Carcass locations were determined in winter during aerial surveys, with subsequent ground inspection and necropsy. Wolf-killed moose and elk are distinguished readily from other mortality causes. Kills were classified as wolf-caused when wolves were observed making the kill, or evidence supported wolves as the cause (e.g. wolves feeding on fresh carcass, presence of chase tracks, signs of struggle). Starvation-killed moose and elk are identified as intact carcasses, without evidence supporting wolves as the cause of mortality. Starvation killed moose and elk are annually rare for average years in both IRNP and YNP.

In spring (April-May) from 2004-2007 soils were sampled at 20 different moose carcass sites in IRNP (wolf-killed $n = 15$ , starvation-killed $n = 5$) and at 29 elk carcass sites in YNP (wolf-killed $n = 14$ , starvation-killed $n = 15$). All carcasses died in the previous winter and carcass sites of both mortality types were sampled in all years. Four soil cores (4 cm diameter × 10 cm depth) were extracted beneath the rumen remains at each carcass center (hereafter carcass) and duplicate pairs of cores (hereafter control) were extracted 3-9 m out from the center in opposite directions, perpendicular to the slope gradient. The distance between carcass and control cores was determined based on the spread of hair and rumen remaining at carcass sites. A 3-9 m spacing ensured that hairmats, rumen or other carcass remains did not enter control cores. This paired sampling design minimized sight affects on carcass versus control comparisons, and controlled for variation in annual climate between sampling times. Core carcass area was estimated as the area of an ellipse with major and minor axis measured from the spread of hair and rumen at kill sites. Soil preparation and macronutrient analysis followed methods described for the deer carcass experiment.

Macronutrient data were analyzed using matched-pairs analysis to test the expectation of positive carcass effects on soil macronutrient concentrations and to test if effects varied between mortality type. Brown-Forsythe and O'Brian tests were used to confirm assumptions of non-heteroscedasticity. Soil nitrate, ammonium, phosphorus, and potassium were log transformed prior to analysis to meet assumptions of non-heteroscedasticity. Macronutrient data are presented in original units, back-transformed for interpretation.

### *Effect of predator:prey body mass ratio*

We used logistic regression (maximum likelihood by iterative fitting) to test the hypothesis that the probability of a significant macronutrient pulse is related to the wolf:prey body mass ratio and wolf pack size. Wolf:prey body mass ratios were 0.1, 0.2, and 0.6 for IRNP, YNP, and Michigan's Upper Peninsula respectively, based on mean weights of wolves and deer, elk, and moose in each system (Peterson 1977, Smith *unpublished*). Mean wolf pack sizes were 5, 8, and 4.5 for IRNP, YNP, Michigan's Upper Peninsula, respectively (Potvin et al. 2005, Vucetich & Peterson 2004, Smith *unpublished*). Bias corrected effect sizes were calculated (Dunlop et al. 1996) to test for differences between wolf:prey body mass ratio and the effect size of significant resource pulses.

## Results

### *Deer carcass experiment*

Ravens (*Corvas corax*) began scavenging carcasses on predator inclusion plots within 3 days. Tracks and scats indicated that wolves and coyotes (*Canis latrans*) located and began utilizing the same carcasses within 9 days. Soft tissues were >99% consumed (gone) by day 12. In each block predator inclusion carcasses were dismembered and dragged >10 m beyond the block center. Hair mats were the only carcass remains that appeared to remain on the treatment plot. Unsuccessful efforts to dig under predator exclusion cages were apparent in one block. Predator exclusion carcasses decayed *in situ*, without apparent scavenging from vertebrates larger than ~200g (i.e., cages did not exclude mice, shrews, and weasels).

Macronutrients ($NO_3^-$, $NH_4^+$, Mg, Ca, K, P) were 0.5-210 times higher postmanipulation in soils with predator exclusion ($F_{3,\,23} = 5.1\text{-}30.1$, $P < 0.0001\text{-}0.007$, Fig. 1). Mean (± SE) soil pH rose significantly ($F_{3,\,23} = 17.3$, $P < 0.0001$) from 3.8±0.08 to 5.7±0.11 and soil field water was 2-3 times higher ($F_{3,\,23} = 59$, $P < 0.0001$) beneath carcasses with predator exclusion. Soil macronutrient pulses and physical changes were not present for unmanipulated, cage control, and predator inclusion treatments (Fig. 2). Results were not affected by block location ($F_{5, 23} < 1.1$, $P > 0.4$).

Oak seedlings were present within 0.5m of each treatment at 4 of the 6 blocks. Leaves from plants growing in proximity to carcasses within the predator exclusion treatment exhibited nearly double the N found in leaves from the other treatments ($F_{3, 15} = 9.3$, $P < 0.004$), which improved gross forage quality by significantly decreasing the foliar C:N by 30% on average compared to other treatments ($F_{3, 15} = 5.7$, $P < 0.018$, Fig. 3). Foliar stable isotope values (grand mean ± SE: $\delta^{13}C = -30.61 \pm 0.24$, $\delta^{15}N = -5.33 \pm 0.38$) were the same across treatments ($F_{3, 15} < 0.36$, $P > 0.77$). Foliar results were not affected by block location ($F_{5, 23} < 1.1$, $P > 0.3$).

*Moose and elk carcass site analysis*

$NO_3^-$ and $NH_4^+$ measurements were combined (i.e., inorganic N) because $NO_3^-$ levels were not detectable in >50% of the soil samples from IRNP. Soils from moose carcass sites in IRNP were 0.2-4.2 times higher in Ca, K, P, and inorganic N compared to control sites ($t_{20} = 3.08$-$5.41$, $P < 0.0001$-$0.006$, Fig. 3), and levels of K, P, and inorganic N were 0.4-8 times higher for the same comparison in YNP soils ($t_{29} = 4.06$-$7.27$, $P < 0.0001$-$0.004$, Fig. 4). In YNP the carcass-derived K pulse was 3.2 times higher from prey that died from starvation compared to prey that was killed by wolves ($F_{1, 28} = 7.0$, $P = 0.006$, Fig. 5), and in IRNP the inorganic N pulse was 1.5 times higher for the same comparison ($F_{1, 28} = 6.5$, $P = 0.02$, Fig. 5).

### *Effect of predator:prey body mass ratio*

The probability of a significant macronutrient pulse increases with decreasing predator:prey body mass ratio ($X^2_1 = 10.06$, $P = 0.0015$, Fig. 6). The odds of a wolf-killed moose in IRNP resulting in significant macronutrient pulses were 4 times the odds of a wolf-killed elk in YNP resulting in a significant nutrient pulse, and 780 times greater than a deer in the predator-inclusion treatment. Mean wolf pack size did not affect the probability of a significant macronutrient pulse ($X^2_1 = 1.53$, $P = 0.21$). No difference was found between the mean effect size of significant resource pulses in IRNP and YNP ($X^2_1 = 0.1$, $P = 0.75$, grand mean ± SE = 2.77±1.7).

## **Discussion**

Predation appears to be an important factor influencing the magnitude of carcass-derived nutrient pulses, depending on the relative sizes of predator and prey, and the nutrient type. The experimental exclusion of predation results in a dramatic increase in carcass-derived nutrient pulses and aboveground plant effects (Fig. 2, Fig. 3). Large carcasses in natural systems result in significant nutrient pulses (Fig. 4), with few differences between mortality types (Fig. 5). Prey carcasses likely have little chance of causing a significant nutrient pulse unless they are at least 3 times the mass of their predators (Fig. 6). There are good reasons to consider the logistic model describing the relationship between wolf:prey body mass ratio and the probability of a significant nutrient pulse (Fig. 5) as rationally sound, with some obvious exceptions.

Consider that small prey, such as beaver (*Castor canadensis*) or snowshoe hair (*Lepus americanus*), are consumed entirely by wolves (Peterson 1977). Medium-sized prey, such as whitetail deer, are quickly (often in <1 day) consumed by predators and scavengers, frequently dismembered and easily scattered (Huntzinger 2006). Moose, elk, and similarly sized large prey (e.g., bison, muskox) take longer to consume, are less easily moved about, and the volume of their rumen (first stomach chamber) is greater than that of deer. Intestine volume is important because rumen contents are nearly ubiquitous at carcass locations, hardly ever consumed by predators, and are important to carcass-derived nutrient pulses (Bump *unpublished*). Consequently, prey carcasses that are large enough to remain in one location and that are not consumed will more likely result in significant resource pulses. However, ungulate carcasses of any size can result in significant nutrient pulses in exceptional cases such as 'die-offs' (Young 1994), in which predators and scavengers are satiated, and decomposition occurs *in situ*.

Natural die-offs in large mammal populations are geographically widespread and not uncommon (Young 1994). Declines of 70-90% in large mammal populations are not uncommon; declines of >90% are rare (Young 1994, Erb and Boyce 1999, Young 1999). Herbivore die-offs are more often attributed to starvation and predator die-offs are more often attributed to disease. When die-offs occur predator and scavenger populations are often satiated. This can lead to slow and incomplete consumption, high-grading (consumption of specific organs only), and intact carcasses that putrify and decompose in situ. For example, the deer population in the Upper Peninsula of Michigan declined ~35% over the 1995-1996 winter (Langenau 1996). The moose population in Isle Royale National Park declined ~52% over the same winter (Peterson *unpublished*).

Decomposing, intact moose and deer carcasses were observed in each system Peterson *unpublished,* Beyer *unpublished).* Under such situations deer carcasses would likely result in nutrient pulses similar to those observed for the predator exclusion treatment (Fig. 2).

Size relationships are important in structuring trophic linkages within foodwebs in both marine and terrestrial ecosystems (Woodward et al 2005). Predator-prey size relationships are important in biomass flux through food webs (Owen-Smith and Mills 2008), although this work focused on resources flux from prey to predators. In general, larger herbivores immobilize nutrients for longer than smaller herbivores, which results in slower return time for nutrients. This size-related effect can result in consumer-driven resource dynamics. Our results demonstrate that predator-prey size relationships are also an important factor in carcass-derived resource pulses. This finding is important to understanding the energy and resource flow in systems with and without large carnivores. For example, in areas where wolves and deer are present, materials and energy at the large herbivore trophic level are transferred up a trophic level via predation. In areas where large predators have been extirpated, a larger fraction of energy and material at the herbivore trophic level enters decomposition pathways. For instance, experimental deer carcasses in a prairie ecosystem that did not die from predation resulted in significant nutrient pulses even when scavenged (Towne 2000). Hence, the presence of predators and the size of their prey are important to nutrient recycling rates and above-belowground links via carcass resources pulses. Predators may slow the cycling of some nutrients in some ecological networks by retaining limiting elements in predator biomass (Woodward et al. 2005).

The influence of predator-prey size relationships on the magnitude of carcass-derived nutrient pulses is important to understanding the ecological role of large carnivores.  Other research demonstrates that even wolf-killed moose and elk carcasses result in higher soil microbial biomass, shifts in microbial composition, and elevated leaf or grass nitrogen for at least 2-3 years at kill sites, and that carcass spatial and temporal distribution depends importantly on wolf hunting behavior (Bump *unpublished*). Consequently, when large prey items are available, wolves influence resource heterogeneity, which is an important mechanism in the maintenance of biodiversity (Reice 1994).  Our results indicate that the role top terrestrial predators serve in generating landscape heterogeneity and thereby carnivore-biodiversity links, likely depends on predator:prey body size relationships.  If large prey items are available, then top predators are more important in carcass-derived resource pulse dynamics and the generation of above and belowground heterogeneity than if only relatively smaller prey are available.  Predator social behavior (i.e., group or asocial living) is also likely to be important to carcass-derived resource pulses due to variable consumption rates among different degrees of predator sociality.  Thus far no comparisons have been made of carcass-derived resource pulse dynamics among top terrestrial carnivores.

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## Figure legends

**Figure 1.** Photographs of wolf-killed moose on Isle Royale National Park (A) and the same carcass three days later (B). All that remains in photograph (B) is hair (grey) and rumen contents (green); hide has been consumed and bones have been scattered.

**Figure 2.** Indices of macronutrient availability in soils before and after experimental deer carcass manipulation. Error bars show mean ± SE. Note different *y* axis scales.

**Figure 3.** Foliar nitrogen content (**A**) and carbon:nitrogen ratio (**B**) in oak leaves growing on experimental deer carcass treatments. Error bars show mean ± SE. Bars labeled with different lower case letters are significantly different ($P < 0.05$).

**Figure 4.** Indices of macronutrient availability in soils from wolf-killed moose or elk carcass sites (solid circles) and paired control sites (open circles) in Isle Royale and Yellowstone National Parks. Error bars show mean ± SE. Note different *y* axis scales. Delta ($\Delta$) values indicate mean difference for carcass versus control comparisons that were significantly different ($P < 0.05$).

**Figure 5.** Comparison of mean carcass-control macronutrient differences between mortality types in Yellowstone (**A**) and Isle Royale (**B**) national parks. Error bars show mean ± SE. Note different *y* axis scales. Asterisks (*) indicate significant ($P < 0.05$) differences between starvation versus wolf mortality.

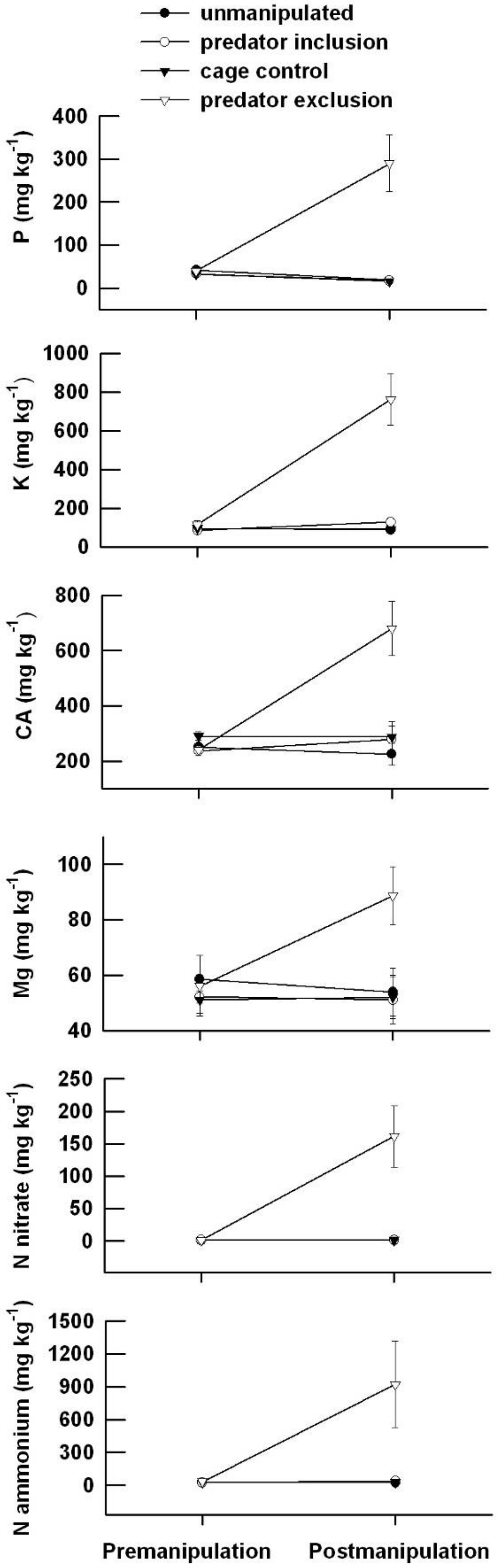
**Figure 6.** Relationship between wolf:prey body mass ratio and significant macronutrient pulses for deer carcasses in Michigan's Upper Peninsula (*circles*), elk carcasses in Yellowstone National Park (*diamonds*), and moose carcasses in Isle Royale National Park (*triangles*). Points with same wolf:prey body mass ratio are jittered to show sample sizes. Each point represents a distinct macronutrient comparison between carcass and control treatments. The model is fit using the logit transformation.

## Figures

**Figure 1**

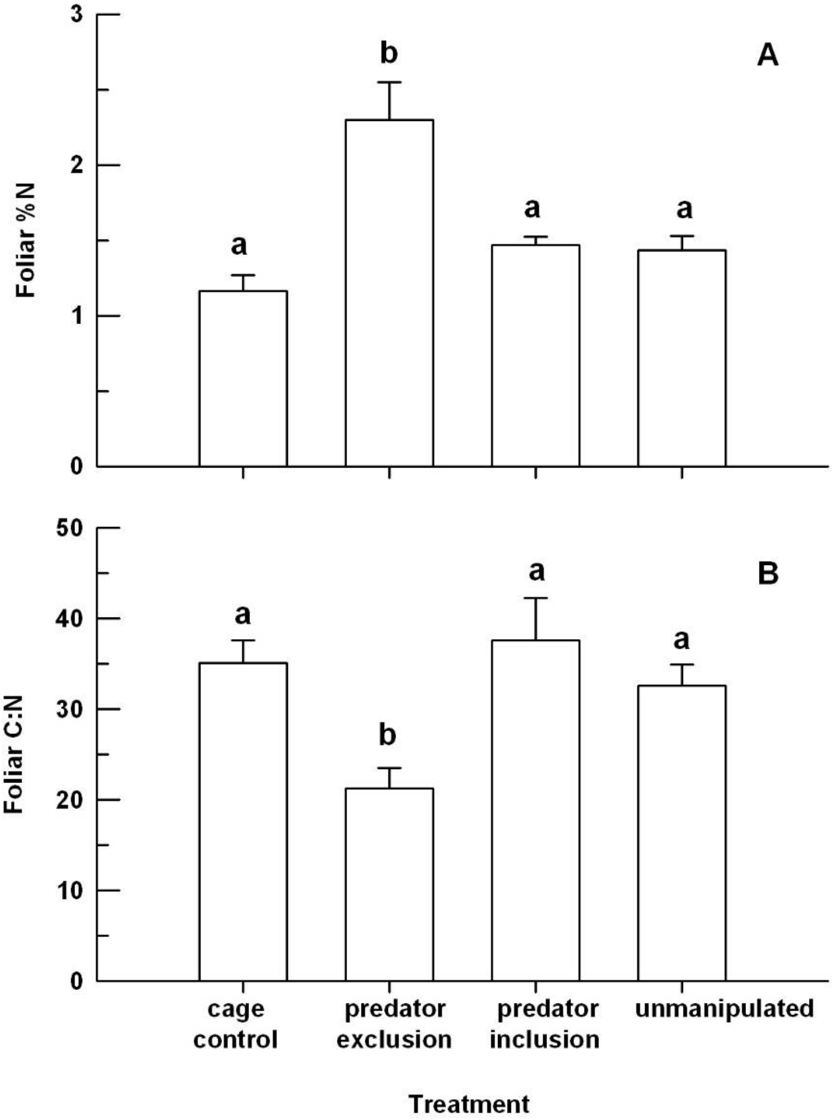
**Figure**

**2**



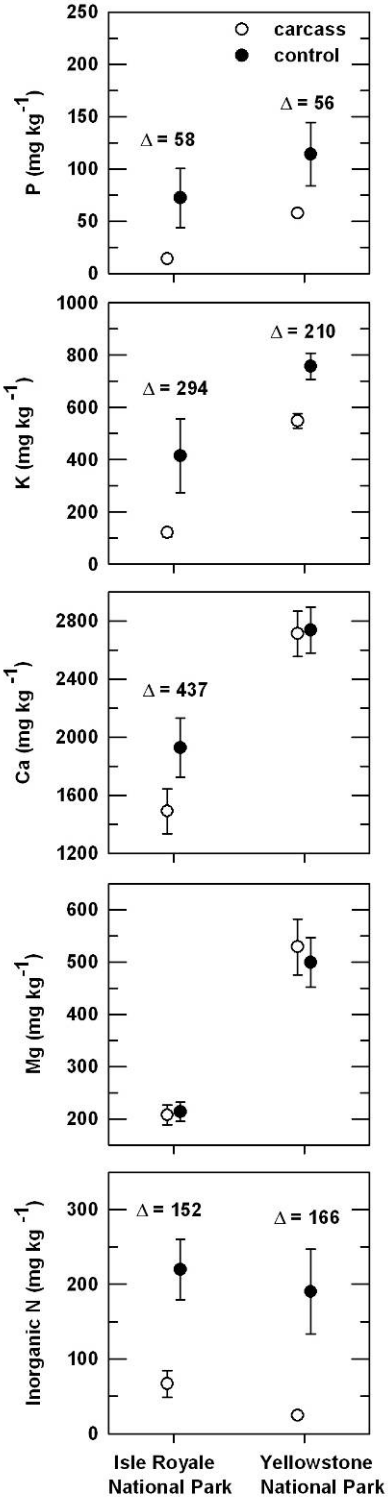
unmanipulated
predator inclusion
cage control
predator exclusion
P (mg kg$^{-1}$)
400
300
200
100
0
K (mg kg$^{-1}$)
1000
800
600
400
200
0
CA (mg kg$^{-1}$)
800
600
400
200
Mg (mg kg$^{-1}$)
100
80
60
40
N nitrate (mg kg$^{-1}$)
250
200
150
100
50
0
N ammonium (mg kg$^{-1}$)
1500
1200
900
600
300
0
Premanipulation
Postmanipulation

Figure 3



A
Foliar %N
0
1
2
3
a
b
a
a
B
Foliar C:N
0
10
20
30
40
50
a
b
a
a
cage control
predator exclusion
predator inclusion
unmanipulated
Treatment

Figure 4



○ carcass
● control
P (mg kg$^{-1}$)
250
200
150
100
50
0
Δ = 58
Δ = 56
K (mg kg$^{-1}$)
1000
800
600
400
200
0
Δ = 294
Δ = 210
Ca (mg kg$^{-1}$)
2800
2400
2000
1600
1200
Δ = 437
Mg (mg kg$^{-1}$)
600
500
400
300
200
Inorganic N (mg kg$^{-1}$)
300
200
100
0
Δ = 152
Δ = 166
Isle Royale National Park
Yellowstone National Park

**Figure 5**

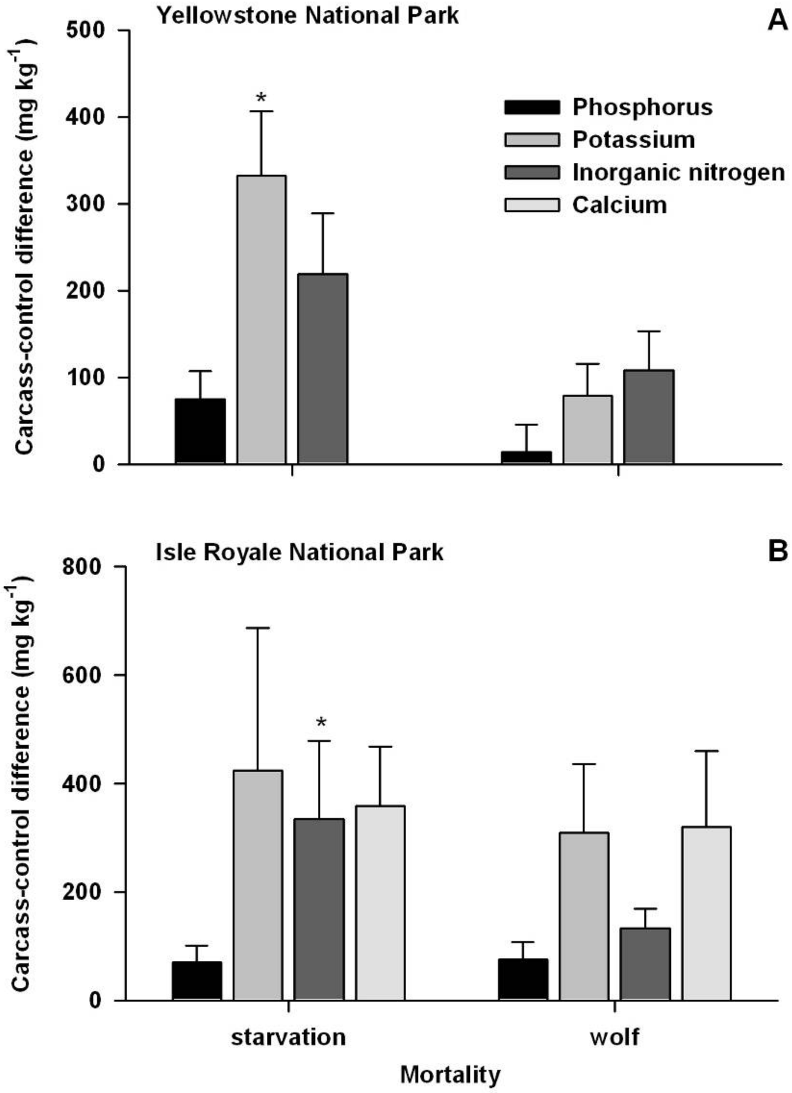
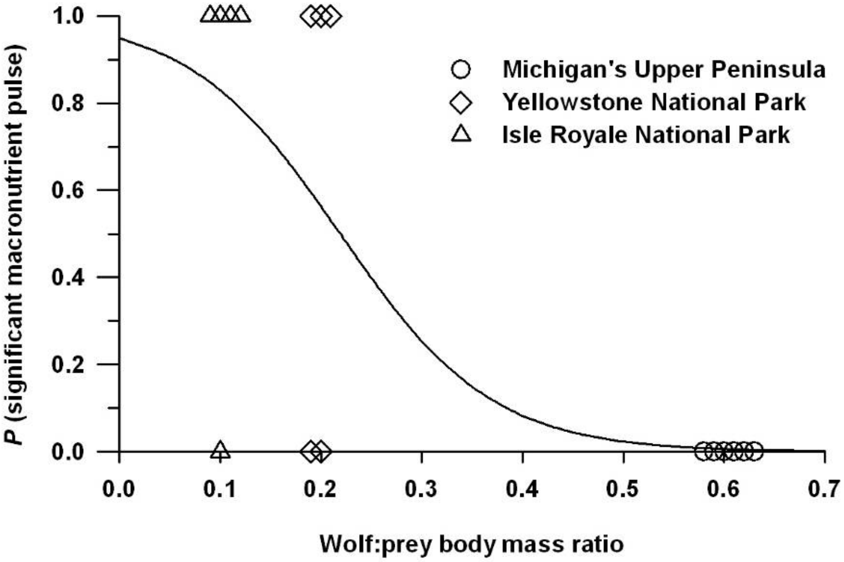

Yellowstone National Park
A
Carcass-control difference (mg kg$^{-1}$)
500
400
300
200
100
0
*
Phosphorus
Potassium
Inorganic nitrogen
Calcium
Isle Royale National Park
B
Carcass-control difference (mg kg$^{-1}$)
800
600
400
200
0
*
starvation
wolf
Mortality

**Figure 6**



1.0
0.8
0.6
0.4
0.2
0.0
P (significant macronutrient pulse)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Wolf:prey body mass ratio
○ Michigan's Upper Peninsula
◇ Yellowstone National Park
△ Isle Royale National Park

## Paper II – Submitted to *Proceedings of the National Academy of Sciences, USA*

# Linking large carnivores to biodiversity in terrestrial ecosystems

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## Abstract

Some studies support a positive correlation between the presence of large, terrestrial carnivores and the maintenance of biodiversity, but mechanistic links between top terrestrial predators and biodiversity remain poorly understood. In terrestrial communities biodiversity is strongly related to soil resource heterogeneity. Here we demonstrate that large carnivores configure landscape heterogeneity through prey carcass distribution. A 50-year record comprised of >3600 moose carcasses from Isle Royale National Park, USA, showed that wolves modulate heterogeneity in soil nutrients, soil microbes, and plant quality by clustering prey carcasses over space. Despite being well-utilized by predators, moose carcasses resulted in elevated soil macronutrients and microbial biomass, shifts in soil microbial composition, and elevated leaf nitrogen for at least 2-3 years at kill sites. For wolf-killed prey in Yellowstone National Park, USA, elk carcasses resulted in elevated soil macronutrients and leaf nitrogen for at least 3-4 years at kill sites. Wolf-killed moose were deposited in some regions of the study landscape at up to 12× the rate of deposition in other regions. Carcass density also varied temporally, changing as much as 19-fold in some locations during the 50-year study period. This variation arises from spatiotemporal variation in wolf behavior and forest composition. This study identifies a top terrestrial predator as a mechanism generating landscape heterogeneity, demonstrating reciprocal links between large carnivore behavior, ecosystem function, and biodiversity.

## Text

When carnivores affect ecosystem processes and biodiversity it is generally thought that they do so primarily by their effects on the population dynamics and behavior of large herbivores (1-5). However, large, terrestrial carnivores might affect ecosystems processes and biodiversity in an entirely different way. We illustrate how a large carnivore can affect ecosystems by impacting landscape heterogeneity. If carnivores influence the distribution of carcasses that result from predation, they would also affect the spatiotemporal heterogeneity of soil and plant properties. To be true, carcasses produced via predation would have to be important to above and belowground communities, and predation would have to occur in some locations at rates that are different than the rates for other causes of mortality (e.g., starvation, hunting). Data supporting such effects would provide empirical evidence for a mechanistic link between large carnivores and biodiversity in terrestrial ecosystems. This would be important because it would add to the few, widely cited empirical studies supporting a positive correlation between the presence of large, terrestrial carnivores and the maintenance of biodiversity (1). Here, we provide this kind of evidence.

In terrestrial communities biodiversity is strongly related to soil heterogeneity (6-10). Soil heterogeneity is an important determinant of soil diversity (9, 10), which causes patchiness of soil resources, which then influences aboveground diversity and overall ecosystem function (11, 12). Biotic interactions affect the heterogeneity of soil resources frequently through plant-soil associations and invertebrate soil fauna (7, 13). The effects of large herbivores on soil heterogeneity are less encompassing, and are typically characterized by indirect feedbacks between selective herbivory and leaf litter quality, and nutrient-rich patch generation through feces and urine deposition (14). Recently however, the nutrient rich and highly labile carcasses of large ungulates have been recognized as being consequential in the generation of landscape heterogeneity (reviewed in 15). In the absence of predators, bison (*Bos bison*), cattle (*B. taurus*) and deer (*Odocoileus virginianus*) carcasses can provide local nutrient pulses at intensities that exceed other natural processes, thus influencing plant composition and biomass (16). But the effects of predators and scavengers on carcass spatial distribution, temporal deposition, and the magnitude of nutrients released due to variable carcass consumption are unknown.

While carcasses produced by means other than predation (e.g., starvation, disease, vehicle collisions, hunter deposited gut piles) may remain intact long enough to putrefy and decompose largely in place, carcasses from predation are typically very well utilized, moving up a trophic level via consumption by predators and scavengers (17). For example, at sites where wolves (*Canis lupus*) have killed moose (*Alces alces*) in Isle Royale National Park, USA, little appears to remain except bone, hair, and rumen contents (first stomach chamber; supporting information Fig. S6). Such appearances likely prevent one from recognizing that carcasses produced via predation could also be important to soil and plant heterogeneity as are carcasses produced in other ways. Moreover, large predators partly determine the spatiotemporal distribution of carcasses on the landscape, which can result in distinct kill-site clustering and carrion availability patterns (17, 18). Hence, observing that carcasses produced via predation are distributed in distinct patterns and subsequently affect soil resources, would be evidence that carnivores are linked to heterogeneity in a novel way. Thus far no data support such a link.

We show how wolves affect soil and plant heterogeneity in distinct ecosystems by influencing prey carcass distribution. Differences in forest (Isle Royale National Park, USA) soil macronutrient availability, microbial biomass and composition, and plant leaf tissue nitrogen were compared at wolf-killed moose carcass and paired control sites for ~3.5 years postmortem. We used 3654 carcass locations recorded since 1958 to see how total moose carcass density and the ratio of wolf:starvation killed carcasses changed over time and space. Additionally, differences in grassland (Yellowstone National Park, USA) soil macronutrient availability and plant leaf tissue nitrogen were compared at wolf-killed elk carcass and paired control sites for ~3.5 years postmortem.

## Results

We measured nitrogen, phosphorus, and potassium levels at paired sites (carcass and control) because these macronutrients are generally limiting to primary productivity in boreal and temperate systems (14). Soils at carcass sites had 100-600% more inorganic nitrogen ($NH_4^+$ and $NO_3^-$, $F_{1, 51} = 20.1$, $P < 0.0001$), phosphorus ($F_{1, 49} = 18.1$, $P < 0.0001$), and potassium ($F_{1, 46} = 10.1$, $P = 0.0027$) relative to surrounding control sites for several growing seasons (Fig. 1). Differences between carcass sites and control sites exhibited a temporal pattern of initial increase and subsequent decrease (Fig. 1). This pattern may reflect either a lagged soil response to decomposing carcass remains or (and) positive macronutrient feedbacks at carcass sites. Positive feedbacks would occur because carcass sites are nutrient and energy focal points, receiving exuviae and puparia materials from dead invertebrates, and fecal and urine deposition from scavengers, grazers, and predators (16). Such activity means that although these intense macronutrient effects are likely restricted to the core area encompassing carcass remains at kill sites (which is on average 9 $m^2$ for wolf-killed moose on Isle Royale), a single carcass could be ecologically important at larger scales.

Nutrient inputs like those in Fig. 1, typically cause rapid microbial growth in soil communities, which then mobilize organic detritus into plant-available forms (7). Soil microbial communities can influence important ecosystem functions such as plant biodiversity and productivity (7-10). Carcass sites had, on average, a 38% higher total abundance of bacterial and fungal phospholipids fatty acids (PLFAs) versus control sites ($F_{1, 27} = 11.9$, $P = 0.0018$). Bacterial PLFAs were 30-50% more abundant at carcass compared to control sites, and appeared to track the macronutrient availability patterns postmortem (Fig. 2A). Fungal PLFAs were 50% more abundant at carcass compared to control sites at the latest postmortem sampling (Fig. 2B). The fungal-to-bacterial ratio increased significantly from 0.15 to 0.66 (Fig. 2, $F_{3, 14} = 6.1$, $P = 0.0071$) in the carcass plots compared to control plots with time. Fungal scavengers likely increased over time by preying upon what had been an earlier abundance of bacteria, which explains the shift in the microbial community composition (Fig. 2).

Belowground nutrient and microbial processes are important ecological drivers of aboveground community structure and functioning (8). Increased microbial abundance at carcass sites (Fig. 2) could improve resource availability for plants within the carcass footprint. We measured foliar nitrogen, which strongly regulates photosynthetic potential, in large-leaf aster plants (*Aster macrophyllus*), an important herbaceous species in many boreal systems (19). We found that foliar nitrogen levels were 33%, 22%, and 20% higher in plants growing on carcass sites compared to control sites ($F_{1,39} = 22.6$, $P < 0.0001$) for the first three growing seasons postmortem (Fig. 3A). Mean foliar carbon-to-nitrogen ratio decreased 25% over the first three growing seasons ($F_{1,39} = 13.6$, $P = 0.0007$), indicating higher aggregate leaf tissue quality at carcass sites. To assess whether asters likely assimilated organic nitrogen (as other plants do, 20) released directly from carcasses, we measured stable nitrogen isotope ($\delta^{15}N$) values of foliage and soils at carcass compared to control sites (21). Aster foliage and soils from carcass sites had elevated $\delta^{15}N$ compared to control sites at least half the time, but lagged in response to carcass effects (Fig. 3B, $F_{1,46} = 5.5$, $P = 0.023$; Fig. 3C, $F_{1,45} = 15.5$, $P = 0.0003$). This pattern may reflect slower decomposition of some isotopically heavy, recalcitrant carcass remains (e.g., bone or hair). Moose remains (i.e., bone, hair, rumen) show enriched $\delta^{15}N$ relative to their plant diet (mean $\delta^{15}N_{\text{moose remains}} = 1.12‰$, $N = 18$) due to preferential retention of heavier isotopes in consumers. The increased $\delta^{15}N$ in soils and foliage at carcass sites suggests that a carcass-derived nitrogen source leads to higher available nitrogen in soils, resulting in increased plant nitrogen assimilation (Fig. 3). These results indicate that the belowground effects of carcass-derived nutrients can be used in plant growth for three growing seasons postmortem, which may influence aboveground trophic interactions. For example, large herbivores are attracted to patches of nitrogen-rich forage (14). Hence carcass sites become foraging sites (16), and the probability of repeated foraging within and around carcass sites initiates a positive feedback of recurrent nutrient supplementation from feces and urine deposition.

Rates of moose mortality vary across time and space in response to decadal scale (or longer) habitat changes such as forest fires and predator dynamics such as variable pack number and changes in pack social structure (22). Temporal variation was investigated by comparing the carcass data in two, 24-year periods: 1958-1982 and 1983-2006). Carcass density changed as much as 19-fold for various areas of the island between the two time periods (Fig. 4A, 4B). These spatiotemporal changes are attributable to shifts in moose habitat selection arising from shifts in forest composition following extensive forest fires in 1936 and subsequent forest succession. The largest burn area was in the middle of the island (22), which is where temporal changes in carcass density are most pronounced (Fig. 4A, 4B).

We separated the influence of predation on carcass location from total carcass density by measuring the ratio of wolf-killed to starvation-produced carcasses across the island at island-wide and 1-km$^2$ scales. In some areas wolf-killed moose were 12 times more common than starvation-killed moose (Fig. 4C). The distribution of wolf-killed moose showed a striking degree of clustering at the island-scale, with less than a 0.1% likelihood that the clustering of wolf-killed moose carcass sites resulted from random chance (Fig. 4C, 4D; Getis-Ord Gi z-score = 4.13 standard deviations; 22). The ratio of a wolf-killed moose compared to a starvation-killed moose occurring in some areas declined as much as 9-fold and increased in other areas by up to 5-fold between time periods (Fig. 4D). At a 1-km$^2$ scale, wolf-killed carcass 'hot spots' are up to 7 times more tightly clustered than 'cold spots' of low carcass density (local Getis-Ord Gi* z-scores ranged from -4.02 to 27.6 standard deviations, Fig. S7). Wolves preferentially travel along shorelines (22) which results in high-density predation zones in close proximity to water, such as a river drainage, an isthmus, a harbor, and a peninsula (Fig. 4E-4H, respectively). Changes in wolf pack number and subsequent territory shifts may explain the absence of the high wolf:starvation killed region along a river drainage illustrated in Fig. 4E during the second 25-yr period of the study (22). These patterns illustrate how wolves directly influence the spatiotemporal distribution of carcasses.

We observed similar responses for equivalent nutrient analysis of soils and grasses at wolf-killed elk carcass sites in Yellowstone National Park, USA. Elk carcass sites had 100-700% more inorganic nitrogen ($F_{1, 105} = 18.6$, $P < 0.0001$), phosphorus ($F_{1, 105} = 17.6$, $P < 0.0001$), and potassium ($F_{1, 105} = 6.4$, $P = 0.0013$) relative to surrounding control sites for several growing seasons (Fig. 5 A-C). Grass tissue nitrogen levels were 18-80% higher in plants growing on carcass sites compared to control sites ($F_{1, 43} = 17.7$, $P < 0.0001$) for the first 4 growing seasons postmortem (Fig. 5D). This grassland ecosystem result is substantiating because elk carcass distribution is influenced by wolf hunting, which can create clustering of carcass-effects (i.e, flat grasslands close to streams and roads were found to be favorable to wolf hunting success, 24).

## Discussion

The varying spatial structure of wolf-killed moose carcasses (Fig. 4C-4H) is evidence of a mechanistic link between a large carnivore's predatory behavior and heterogeneity in soil nutrients, microbial communities, and producer tissue quality (Figs. 1-3). These results indicate an important way by which large terrestrial predators can influence spatiotemporal dynamics of ecosystem processes, including the landscape mosaic of nutrient cycling, biodiversity, and species interactions. Even well utilized large-ungulate carcasses that are produced in winter cause substantial resource ‘hot spots’ in grassland and southern boreal forest and soils, with effects on belowground communities and aboveground producers (Figs. 1-5). These predator-mediated effects occur on the scale of other important factors in forest and grassland ecosystems (e.g., tip-up mounds, lightening strikes, nurse trees, seeps, mineral licks, wallows, ant-hills). At the landscape scale, long-term carcass deposition patterns could influence forest dynamics by shifting competitive relationships among tree seedlings through changes in the nutrient concentrations in their growth environment, thereby affecting subsequent growth, survival, and reproduction (7, 25, 26). The slower growth rates and longer life spans of trees relative to moose (~1-2 orders of magnitude) increase the chance that an individual tree will benefit from a carcass ‘hotspot’ in its lifetime. The cumulative landscape effects of repeated carcass deposition in areas of high kill density remain unexamined.

The results we observed in forest and grassland ecosystems are likely to occur elsewhere, where large carnivore-ungulate relationships are intact. For example, in the low-resource environment of the Arctic tundra, the impact of a muskox (*Ovibos moschatus*) carcass on surrounding vegetation was still dramatic after 10 years (27), which emphasizes that carcass-effects may last longer in some systems. Systems with intact large carnivore-ungulate prey relationships exist, albeit often over a reduced range, on all continents except Australia and Antarctica (14).

Nearly all wild ungulates are hunted by humans, which results in carcass distribution patterns significantly different than those created by wild carnivores. Hunter-kills favor scavengers with large feeding radii by arriving in super abundant pulses and with less dispersion than wild predator-kills (17, 18). Domestic ungulate carcasses are unlikely to produce effects similar to wild carcasses because domestic animals are removed and government agencies in the USA forbid leaving domestic carcasses on public rangelands, where they may attract large carnivores and scavengers (28). These anthropogenic particulars emphasize the importance of understanding large animal carcass dynamics in the context of conservation.

This study contributes to an emerging awareness about how carcasses of vastly different sizes, from whales to salmon to cicadas, may have significant and lasting effects in diverse ecological systems (15, 16, 29-31). When large terrestrial carnivores affect ecosystem processes and biodiversity, it is typically believed that the mechanism involves strong species interactions (e.g., trophic cascades 1-5). This study demonstrates a new mechanism whereby carnivores affect biodiversity by creating ecosystem heterogeneity at multiple scales.

Some have argued that carnivore restoration is justified because it promotes natural biodiversity processes (1). However, such arguments have been weak because too little is known about how carnivores affect biodiversity (1). In this way our paper strengthens such arguments for carnivore restoration. These findings may also represent a novel justification for carnivore restoration. Specifically, the connections we discovered are strong, yet unexpected because carnivores and soil heterogeneity are seemingly unrelated. Such connections can inspire awe and wonder, which creates values that powerfully motivate conservation (32).

## Methods

A complete description of carcass locations, determining causes of mortality, sampling, and soil and plant analysis is provided in Supporting Information (SI Text). Briefly, moose and elk carcass locations were determined in winter during aerial surveys, with subsequent ground inspection and necropsy, and in spring and summer through extensive hiking. Wolf-killed moose and elk are distinguished readily from other mortality causes based on direct observations and field evidence. A paired soil and plant sampling design minimized site effects on carcass versus control comparisons, and controlled for variation in annual climate between sampling times. Macronutrient, PLFA, and stable isotope analysis of soils and plants was conducted blind following standard procedures detailed in SI Text. Results were analyzed using mixed-model repeated measures analysis of variance (ANOVA) to test the expectation of positive carcass effects on soil macronutrient concentrations (N, P, K), total microbial biomass, bacterial and fungal PLFAs, fungal-to-bacteria ratios, $\delta^{15}N$ concentrations, and plant tissue %N and $\delta^{15}N$ concentrations. Planned contrasts were done at each sampling time to evaluate the magnitude of the carcass effect at 4-, 16-, 28-, and 40-months postmortem. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between any pair of times is the same). The influence of wolves on the spatiotemporal moose carcass distribution was analyzed using a map algebra approach (i.e., the ratio of wolf-killed to starvation-produced carcasses) in a geographic information system (ArcGIS, ESRI™). This is the most parsimonious method available given the long-term point location data set. High and low density carcass clustering was analyzed using global and local indicators of spatial association (i.e., Getis-Ord general G, local Getis-Ord Gi*).

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## Figure legends

**Fig. 1.** Indices of macronutrient availability in soils from wolf-killed moose carcass sites (solid circles) and paired control sites (open circles) at 4-, 16-, 28-, 40-months postmortem. (**A**) Inorganic nitrogen (nitrate + ammonium), (**B**) phosphorus, and (**C**) potassium. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each postmortem sampling time. Error bars show mean ± SE (some are too small to be seen). Note different *y* axis scales.

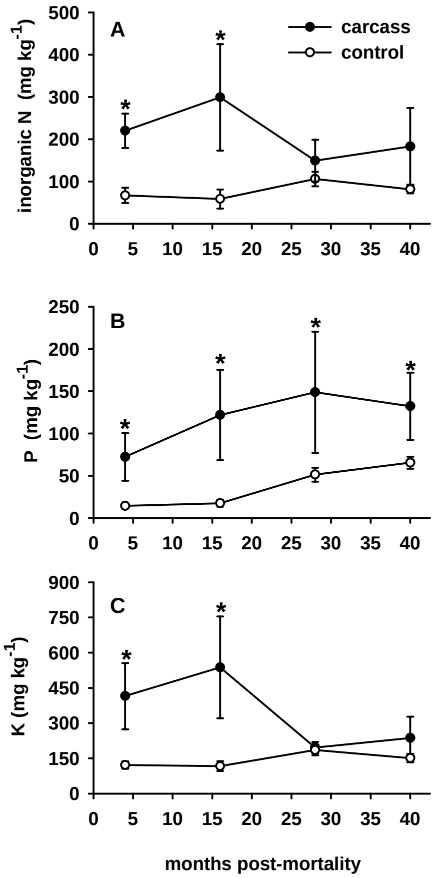
**Fig. 2.** Soil bacterial (**A)** and fungal (**B**) phospholipids fatty acids (PLFAs) from wolf-killed moose carcass sites (solid circles) and paired control sites (open circles) at 4-, 16-, 28-, 40-months postmortem. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each postmortem sampling time. Error bars show mean ± SE (some are too small to be seen). Note different *y* axis scales.

**Fig. 3.** Foliage nitrogen content (**A**), foliage $\delta^{15}N$ (**B**), and soil $\delta^{15}N$ (**C**) from wolf-killed moose carcass sites (solid circles) and paired control sites (open circles) at 4-, 16-, 28-, 40-months postmortem. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each postmortem sampling time. Error bars show mean ± SE. Note different *y* axis scales.

**Fig. 4.** Distribution maps of moose carcasses in Isle Royale National Park, USA. (**A**) Carcass density from 1958-1982. (**B**) Change in carcass density in 1983-2006 compared to 1958-1982. (**C**) Ratio of wolf:starvation killed moose density 1958-1982. Values > 1 indicate areas where wolf-killed moose are more numerous and values < 1 indicate areas where starvation-killed moose are more numerous (values of 1 indicate equal occurrence). NA indicates regions in which both wolf- and starvation-killed moose were not coincident. Land areas adjacent to lower case letters e-h correspond to panels E-H. (**D)** Change in wolf:starvation killed moose density between 1983-2006 compared to 1958-1982. (**E-H**) Areas of high wolf:starvation killed moose – a river drainage, an isthmus, a harbor, and a peninsula, respectively. Note increasing scales from E-H and that ratio density is expressed per hectare. NA is the same as in panel C.

**Fig. 5.** Indices of macronutrient availability in soils and grasses from wolf-killed elk carcass sites (solid circles) and paired control sites (open circles) at 4-, 16-, 28-, 40-months postmortem in Yellowstone National Park. (**A**) Inorganic nitrogen (nitrate + ammonium), (**B**) phosphorus, (**C**) potassium, and (**D**) grass % nitrogen. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each postmortem sampling time. Error bars show mean ± SE (some are too small to be seen). Note different *y* axis scales.

**Figure 1**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carcass
control
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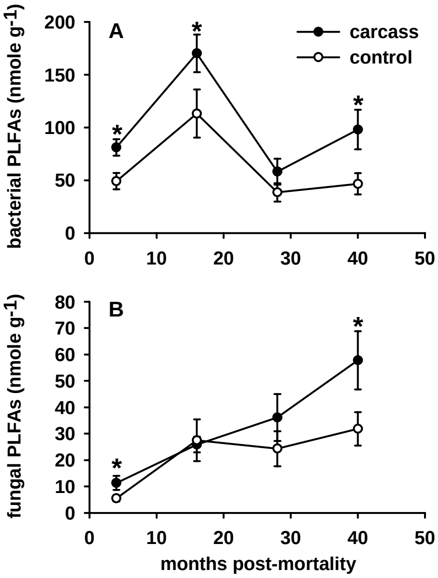
0
100
200
300
400
500
B
P (mg kg$^{-1}$)
0
50
100
150
200
250
C
K (mg kg$^{-1}$)
0
150
300
450
600
750
900
0 5 10 15 20 25 30 35 40
*
months post-mortality

**Figure 2**


A
B
carcass
control
bacterial PLFAs (nmole g-1)
fungal PLFAs (nmole g-1)
200
150
100
50
0
80
70
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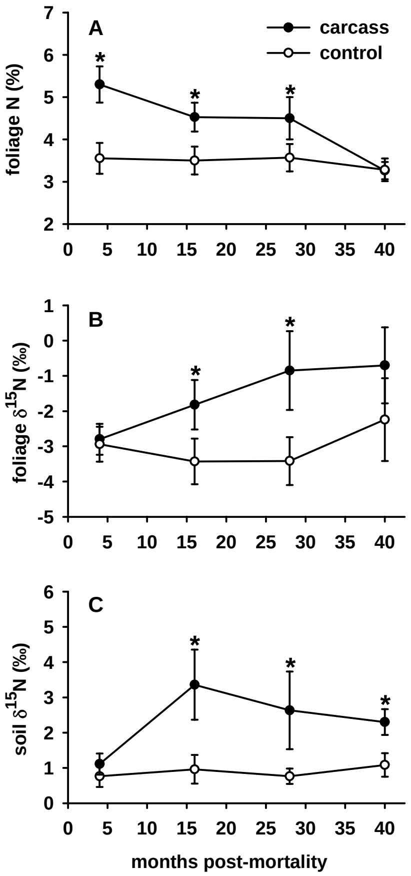
50
40
30
20
10
0
0
10
20
30
40
50
months post-mortality
*

**Figure 3**


A
B
C
carcass
control
foliage N (%)
foliage $\delta^{15}$N (‰)
soil $\delta^{15}$N (‰)
months post-mortality
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5
10
15
20
25
30
35
40
*

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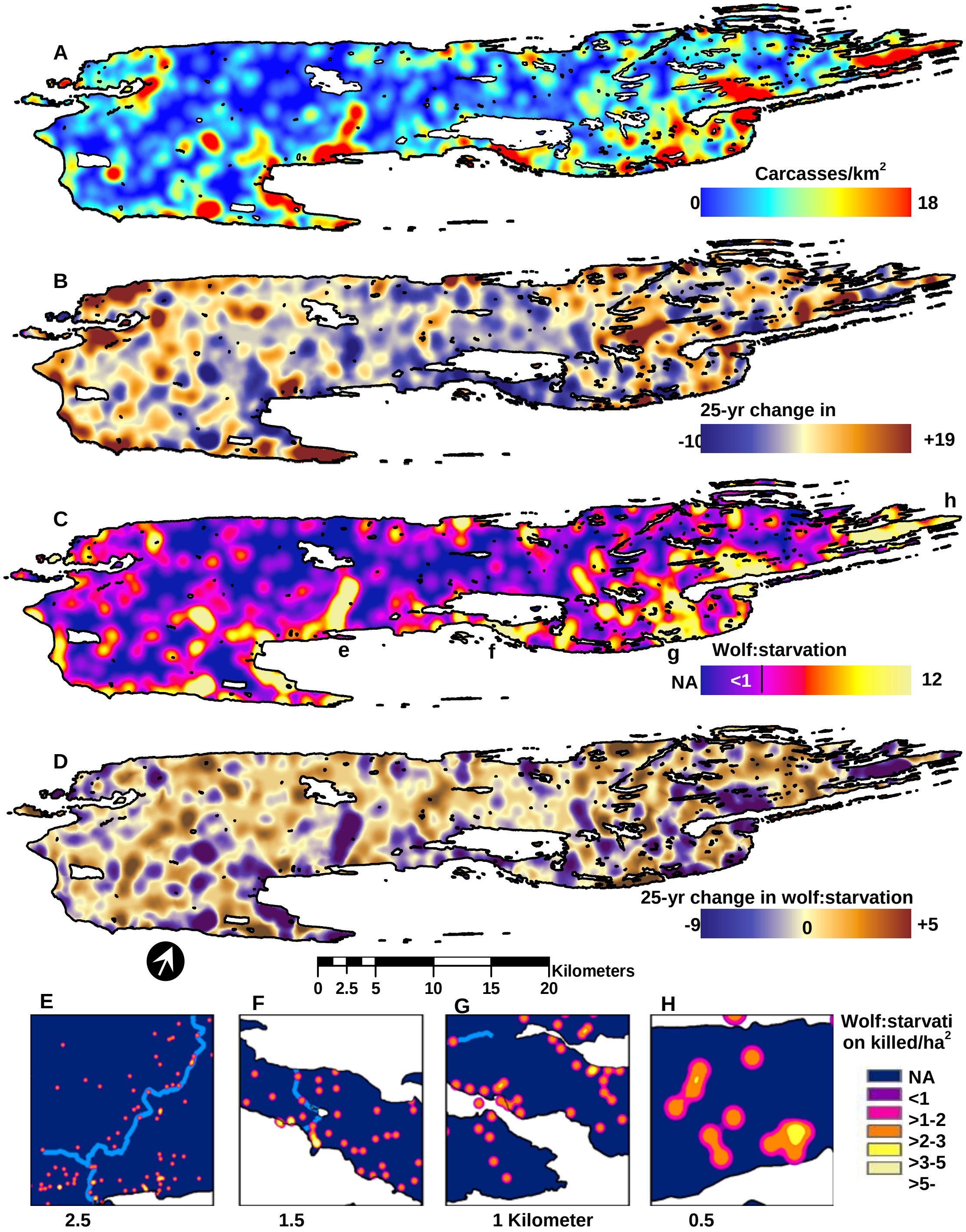

A
Carcasses/km$^2$
0
18
B
25-yr change in
-10
+19
C
h
e
f
g
Wolf:starvation
NA
<1
12
D
25-yr change in wolf:starvation
-9
0
+5
Kilometers
0
2.5
5
10
15
20
E
F
G
H
2.5
1.5
1 Kilometer
0.5
Wolf:starvation killed/ha$^2$
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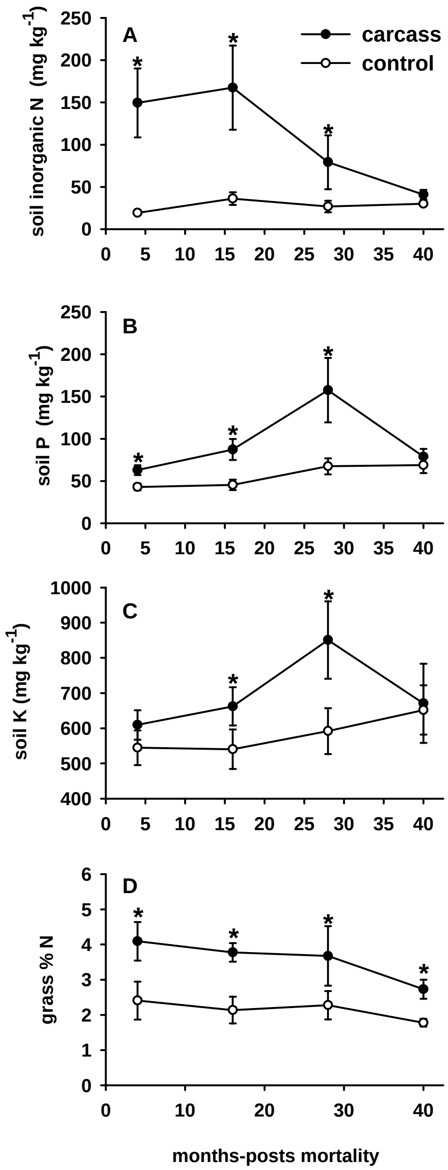
<1
>1-2
>2-3
>3-5
>5-

## Figure 5


A
B
C
D
carcass
control
soil inorganic N (mg kg$^{-1}$)
soil P (mg kg$^{-1}$)
soil K (mg kg$^{-1}$)
grass % N
0
50
100
150
200
250
400
500
600
700
800
900
1000
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
40
*
months-posts mortality

## Supporting Information

### SI Text

### Carcass locations

Moose carcass locations from 1958-2006 ($N$=3654) were determined in winter during aerial surveys, with subsequent ground inspection and necropsy, and in spring and summer through extensive, island-wide hiking (1, 2). Wolf-killed moose are distinguished readily from other mortality causes. Kills were classified as wolf-caused when wolves were observed making the kill, or evidence supported wolves as the cause (e.g. wolves feeding on fresh carcass, presence of chase tracks, signs of struggle).

### Soil nutrient analysis

In late May-early June of 2004 soils were sampled at 17 wolf-killed moose carcass sites selected indiscriminantly, of which 12 died in the winter of 2003-2004 and 5 died during the winter of 2002-2003. Each site was subsequently sampled in spring of 2005 and 2006, thereby providing a postmortem chronology spanning ~3.5 years. At the time of sample collection, predators and scavengers had removed most of the soft tissue. Duplicate soil cores (4 cm diameter × 10 cm depth) were extracted beneath the rumen remains at each carcass center (hereafter carcass; Fig. S6) and duplicate cores (hereafter control) were extracted 6 m out from the center in opposite directions, perpendicular to the slope gradient. The distance between carcass and control cores was determined based on the spread of hair and rumen remaining at carcass sites; 6 m spacing ensured that hairmats, rumen or other carcass remains did not enter control cores. This paired sampling design minimized sight effects on carcass versus control comparisons, and controlled for variation in annual climate between sampling times. Core carcass area was estimated as the area of an ellipse with major and minor axis measured from the spread of hair and rumen at kill sites.

Soils were dried and analyzed for inorganic nitrogen (N) (i.e. $NH_4^+$ and $NO_3^-$), phosphorus (P), and potassium (K) following standard procedures (3). Inorganic N was extracted with 1N KCl and analyzed calorimetrically, a Bray P1 extractant was used to determine soil available P, and a Mehlich 3 extractant was used to determine exchangeable K levels. While recent work has demonstrated the importance of organic nitrogen uptake in some ecosystems (4), and other methods exist for indexing available N (5), these assays are appropriate methods for assessing gross nutrient pools available to plants in north, temperate forest soils (1). Analysis of nutrient concentrations was conducted blind by Michigan State University Soil and Plant Nutrient Lab.

Macronutrient data were analyzed using mixed-model repeated measures analysis of variance (ANOVA) to test the expectation of positive carcass effects on macronutrient concentrations. Planned contrasts were done at each sampling time to evaluate the magnitude of the carcass effect at 4-, 16-, 28-, and 40-months postmortem. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between any pair of times is the same).

### Soil microbial analysis

For each postmortem sampling period (i.e., 4-, 16-, 28-, 40-months), five carcass sites ($N$=20) were selected randomly for microbial analysis. In spring of 2005 and 2006, four subsample soil cores (4 cm diameter × 5 cm depth) were extracted from carcass and control plots at kill-site locations. Subsample cores were immediately homogenized and then frozen for blind phospholipid fatty acid (PLFA) analysis at Microbial Insights, Inc. (2005 samples) and the Balser Soil Microbial Laboratory at the University of Wisconsin (2006 samples). Duplicate samples were sent to each lab to correct for extraction differences between labs. PLFA analysis provides information on microbial community composition by evaluating the specificity of phospholipid membrane structure in functional and taxonomic groups of microbes (6). This methods permits quantitative measurement of total microbial biomass, and bacterial and functional groups, thereby permitting a description of the entire microbial community with information obtained about viable biomass concentrations, community composition, and metabolic status. In this study, the PLFA method is superior to other microbial census techniques such as substrate-induced respiration (SIR) and BIOLOG plates because of better microbial community resolution and non-selectivity (6).

PLFA data were analyzed using mixed-model repeated measures analysis of variance (ANOVA) to test the expectation of positive carcass effects on total microbial biomass, bacterial and fungal PLFAs, and fungal-to-bacteria ratios. Planned contrasts were done at each sampling time to evaluate the magnitude of the carcass effect at 4-, 16-, 28-, and 40-months postmortem. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between any pair of times is the same).

***Soil isotope analysis***

For each postmortem sampling period i.e., 4-, 16-, 28-, 40-months, 11, 7, 8, and 6 carcass sites were randomly selected for stable N isotope ($\delta^{15}N$) concentration analysis. Soil subsamples (*N*=32) were homogenized in a bearing shaker mill and analysis was performed blind on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer at Michigan Technological University's Ecosystem Science Center. IAEA, USGS, and NIST certified isotopic standards were run at the beginning of each analysis. One certified standard was also run at the end of the analysis to check for calibration stability. These standards are recognized internationally, and are used to calibrate the $N_2$ reference gases which were analyzed in conjunction with every individual sample (7). Stable isotope values are reported in standard δ notation, and are reported on the atmospheric air scale for $\delta^{15}N$. An internal standard was run every 10 samples. Precision based on repeated measures of internal standards was +/- 0.5‰ for $\delta^{15}N$.

Soil isotope data were analyzed using mixed-model repeated measures analysis of variance (ANOVA) to test the expectation of positive carcass effects on $\delta^{15}N$ concentrations. Planned contrasts were done at each sampling time to evaluate the magnitude of the carcass effect at 4-, 16-, 28-, and 40-months postmortem. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between any pair of times is the same).

### *Moose carcass isotope analysis*

The total N and $\delta^{15}N$ concentrations in hair, bones, and rumen contents collected from 6 carcass sites were measured on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer at Michigan Technological University's Ecosystem Science Center following specifications described above for soils (7).

### *Aster leaf tissue analysis*

This herbaceous perennial groundcover species is native throughout much of the eastern and central range of moose in North America. It is important as one of the first forage species consumed by moose in spring and is consumed throughout summer (8). Large-leaf aster is well suited as a representative to examine the effect of ungulate carcasses on herbaceous and tree seedling leaf tissues because it is a near ubiquitous understory species on Isle Royale. Moose on Isle Royale consume large leaf aster with conspicuous patchiness, grazing every aster in an area of several square meters (8).

In early June of 2006 plants were collected at 36 carcass sites, including all sites sampled for soils analysis. Sampled plants were located as close as possible to carcass and control soil core holes; usually within 10cm and always within 100cm. For each site carcass and control leaves of equal size were clipped at their base from actively growing plants, dried at 64 °F to a constant weight, double rinsed with distilled water, dried again, and then individually homogenized in a bearing shaker mill. Measurement of total carbon, total N and $\delta^{15}N$ concentrations was performed blind on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and

Deltaplus Continuous Flow-Stable Isotope Ratio Mass Spectrometer at Michigan Technological University's Ecosystem Science Center following specifications described above for soils (7).

Aster leaf tissue data were analyzed using mixed-model repeated measures analysis of variance (ANOVA) to test the expectation of positive carcass effects on total N and $\delta^{15}$N concentrations, and negative effect on carbon-to-nitrogen ratio. Planned contrasts were done at each sampling time to evaluate the magnitude of the carcass effect at 4-, 16-, 28-, and 40-months postmortem. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between any pair of times is the same).

### ***Moose carcass distribution analysis***

Mortality patterns are expected to shift on decadal or longer time scales in response to habitat changes such as forest fires and predator dynamics such as variable pack number and changes in pack social structure (1). Therefore, temporal change in mortality patterns was investigated by dividing the carcass data into two, 24-year periods: 1958-1982 and 1983-2006. This was done for the entire carcass data set and the investigation of the ratio of wolf:starvation killed carcasses only (below). This permitted an assessment of temporal change within the entire data set and an explicit assessment of how the influence of wolves on the spatial distribution of moose carcasses changed over time.

The influence of wolves on moose carcass distribution was analyzed using a map algebra approach (9) in a geographic information system (ArcGIS, ESRI™). This is the most parsimonious method available given the long-term point location data set. First, two carcass density maps were created: one for wolf-killed moose and one for starvation-killed moose. Of the moose carcass locations recorded from 1958-2006 ($N$=3654), mortality was known with high confidence to be caused by wolves for 939 individuals, and by starvation for 577 individuals. The number of carcasses per $km^2$ was calculated for each carcass location by mortality type. The 1-km scale is representative of the scale of moose-wolf predation events (1). Second, the density map created for wolf-killed moose was divided by the density map created for starvation-killed moose ($\text{Mortality}_{\text{wolves}} \div \text{Mortality}_{\text{starvation}}$), yielding a map surface illustrating the relative likelihood of wolf-killed moose to starvation-killed moose across the landscape. Values > 1 indicate areas where carcass distribution is more influenced by wolves and values < 1 indicate where carcass distribution is more influenced by moose (values of 1 indicate equal influence).

High and low density carcass clustering was analyzed using global and local indicators of spatial association (i.e., Getis-Ord general G, local Getis-Ord Gi*; 10, 11). The Getis-Ord General statistic summarizes spatial autocorrelation for the entire island, while the local Getis-Ord Gi* statistic assess autocorrelation within a ‘neighborhood’ of locations within a search radius. Hence, the Getis-Ord general G statistic tests the hypothesis that there is no spatial clustering over the entire island and the Getis-Ord Gi* statistic identifies areas of density relatedness and significant clustering at the 1-km scale, i.e. ‘hot- and cold-spots’ (10, 11). Each test produces Z-scores, which if less than -1.96 or greater than 1.96 (i.e., one standard deviation) are considered significant at alpha=0.05. The higher (or lower) the Z-score, the stronger the intensity of the clustering. A Z-score near zero indicates no apparent clustering within the study area. A positive Z-score indicates clustering of high values. A negative Z-scores indicates clustering of low values. We used Euclidian distance and inverse distance squared methods in the clustering analysis.

Results are presented with island-scale maps that depict carcass density in the first time period and relative change in the second time period for all carcass locations and the ratio of wolf:starvation killed moose . The ratio maps of wolf:starvation killed moose are also presented per hectare for four areas at various scales because carcass sites create noteworthy biological activity at a 1-hectare scale. Carcass sites are nutrient and energy focal points, receiving exuvia and puparia materials from dead insects, feathers from avian scavengers, and fecal and urine deposition from scavengers, grazers, and predators. Consequently, a single moose carcass is ecologically important on the scale of at least 1-hectare, even though the intense macronutrient effects are likely restricted to the core area encompassing carcass remains at kill sites.

### ***Yellowstone soil and grass samples***

Beginning in 2005, a parallel carcass study began examining wolf-killed elk carcass sites ($N = 19$) on the northern range of Yellowstone National Park. Locating carcasses, and the collection and analysis of soil nutrient and grass tissue quality followed the methods described above for Isle Royale soils and aster leaf tissue, with the exception that in the first year of data collection (2005) soils were sampled with a 2.5-cm-diameter probe.

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**Figure S6**

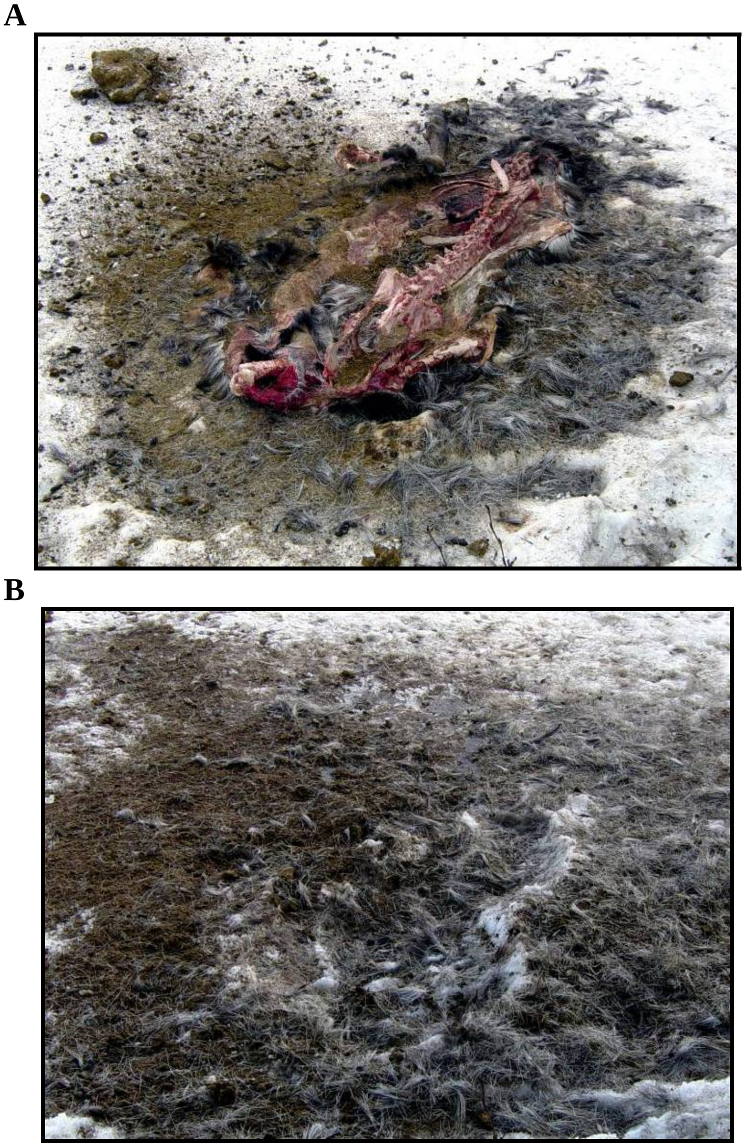

**Fig. S6.** Photographs of wolf-killed moose on Isle Royale (A) and the same carcass three days later (B). All that remains in photograph (B) is hair (grey) and rumen contents (dark green); hide has been consumed and bones have been scattered.

**Figure S7**

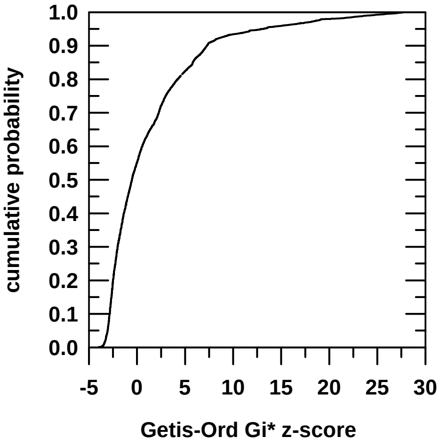

**Fig. S7.** Carcass clustering at the 1-km$^2$ scale: cumulative probabilities of local spatial statistic z-scores calculated for wolf-killed moose carcass locations (1958-2006; *n*=939) on Isle Royale National Park (Lake Superior, USA). Z-scores less than -1.96 or greater than 1.96 (i.e., one standard deviation) are considered significant at alpha=0.05. Positive Getis Ord Gi* z-scores indicate clustering of high density carcass sites and negative values indicate clustering of low density carcass sites. The greater range of positive scores z-scores indicates that high density ('hot spots') carcass areas were more strongly associated with one another than with low density carcass areas ('cold spots').

## Paper III – Submitted to *Ecology*

# Large ungulate carcasses perforate ecological filters in forest herbaceous layers

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### Abstract

Ecological filters are important in the function of ecosystems. Herbaceous layers significantly influence temperate forest ecosystems by filtering tree species during the early life history stages of tree reproduction. How tree species successfully pass through this ecological filter is an important, unanswered question in forest ecology. We experimentally tested the effects of large ungulate carcasses on the filtering function of herbaceous layers. We found that even well-utilized carcasses create unexpected disturbances that reduce the cover of dominant herbaceous layer species. Carcasses also provided pulses of limiting nutrients for at least two growing seasons postmortem. These effects increased tree seedling germination, leaf tissue quality, and growth on carcass sites compared to undisturbed sites. The spatial distribution of carcasses is influenced by predators; hence these data establish an unrecognized link between large carnivores, prey carcasses, and ecosystem processes. These results support a novel understanding of disturbance by large herbivores in forest ecosystems by demonstrating an important interaction between predator-prey functional traits and ecological filters.

**Keywords:** forest ecology, disturbance, heterogeneity, patch dynamics, resource pulses, spatial pattern, biodiversity, regeneration niche, trophic cascades, predator-prey

## Introduction

Despite its small stature, the herbaceous layer is proving to be of large importance to the structure and function of forest ecosystems (Gilliam 2007). Forest biodiversity, energy flow, nutrient cycling, and regeneration are significantly affected by the composition of herbaceous layers (Siccama et al. 1970, Gilliam & Roberts 2003a, Roberts 2004, Whigham 2004, Gilliam 2006). Herbaceous species outnumber tree species nearly 6:1 in most forests (Gilliam 2007). Although the herbaceous layer represents less than 1% of temperate forest biomass, it can contribute up to 20% of the foliar litter to the forest floor (Muller 2003). Herbaceous litter also generally exhibits higher concentrations of limiting nutrients and can decompose twice as rapidly as tree litter (Muller 2003), thereby affecting energy flow and nutrient cycling to a degree that is disproportional to its relative biomass in forest ecosystems (Gilliam 2007).

One way in which the herbaceous layer is linked to overstory development is by significantly influencing the early life history stages of tree reproduction. Tree seeds must first penetrate the herbaceous layer to reach the forest floor, germinate, and then grow above the herbaceous layer to potentially reach the overstory. Hence, the herbaceous layer can act as an ecological filter, selectively sifting tree seeds falling to and seedlings growing from the forest floor (George & Bazzaz 1999a, b, 2003, Coomes et al. 2005, Dearden & Wardle 2008). Under some conditions, persistent monodominant herbaceous layers develop that are nearly impenetrable (Royo & Carson 2006). Large scale disturbances (e.g., fire) permit tree species to "escape" this ecological filter but can also create heavy mortality among seedlings (Frelich 2002). Smaller scale, more frequent mechanisms that simultaneously reduce filtering effects by the herbaceous layer and positively influence early stages of tree reproduction remain unidentified. Here we show that even well-consumed large ungulate carcasses create unexpected disturbances that perforate dense herbaceous layers and provide nutrient pulses that increase tree germination and growth on carcass sites relative to undisturbed sites.

Forest disturbance ecology has largely focused on large-scale, abiotic factors (e.g., fire, floods, wind, ice, landslides) and fewer biotic factors (e.g., herbivory, disease and insect outbreaks; Frelich 2002, Roberts 2004). The role of large ungulates in forest disturbance is characterized by cases of sustained over-browsing, which can reduce plant cover and diversity, alter biogeochemical cycling, and shift future overstory composition (Côté et al. 2004). Recently, however, the nutrient rich and highly labile carcasses of large ungulates have been recognized as important disturbances in other systems (Hobbs 2006, Carter et al. 2007). Bison (*Bos bison*, L.), cattle (*B. Taurus*, L.), and white-tailed deer (*Odocoileus virginianus*, Boddaert) carcasses can create strong, localized disturbances that deposit nutrients at intensities that exceed other natural processes, thus altering prairie species composition and biomass (Towne 2000). These grassland effects persisted 5 years postmortem, and in the low-resource environment of the Arctic tundra, the impact of a muskox (*Ovibos moschatus*, Zimmerman) carcass on surrounding vegetation was still dramatic after 10 years (Danell et al. 2002). These studies emphasize that carcass-disturbances may last longer in some systems. The effects of large ungulate carcasses on forest ecosystems, however, are unknown.

Large ungulate carcasses are typically very well-used, with soft tissue resources moving up a trophic level via consumption by predators and scavengers (Wilmers et al. 2003) rather than entering decomposition pathways directly (Burkpile et al. 2006). For example, at deer carcass sites in the Upper Peninsula of Michigan, USA, little appears to remain except bone, hair, and rumen contents (first stomach chamber; Figure S1). Such appearances likely prevent one from recognizing that carcasses in forest ecosystems could also be important to soil, herbaceous, and overstory layer disturbance dynamics. However, natural die-offs in large mammal populations, especially ungulates, are geographically widespread and declines of 70-90% are not uncommon (Young 1994). When die-offs occur, predator and scavenger populations are often satiated. Such instances of high carrion abundance can lead to slow and incomplete carcass consumption, consumption of specific tissues only (e.g., visceral organs), and intact carcasses that putrefy and decompose *in situ*. Under such conditions, carcasses would likely result in more intense nutrient pulses and disturbances because a higher percentage of the carcass biomass would enter soil communities. Therefore, if even well-used ungulate carcasses affect forest soil, floor, and tree seedlings in the herbaceous layer, then such effects would be conservative evidence that carcasses are linked to the spatial patterns of disturbance and forest tree regeneration in a novel way. Thus far no data support such a link.

In this study we experimentally examined the effect of white-tailed deer carcasses on herbaceous layers and soils in a northern hardwood forest in the Upper Peninsula of Michigan, USA. Differences in soil macronutrient availability, ground-layer litter depth, bare soil, and spring and summer herbaceous layer species composition, were compared at deer carcass and paired control sites for 2 years postmortem. We also compared tree seedling germination, leaf tissue nitrogen, and growth between carcass and control sites to understand if carcass disturbances are linked to the successional trajectories and structural characteristics of forests (Frelich 2002, Gilliam 2007). Because predators and scavengers were not excluded from experimental plots, we expected carcasses to be well-used, with near complete consumption of soft tissues, as is most often the case in our study system (Figure S1). Therefore, we predicted no difference in the soil and herbaceous layer response to carcass and control treatments.

## Materials and methods

### *Field site and carcass experiment*

The effect of deer carcasses on herbaceous layers was investigated experimentally at the Ford Center Reseach Forest, near Alberta, MI, USA: Section 18, T49N-R33W Baraga County, MI. Experimental plots were in a mesic northern hardwoods forest on an outwash plain dominated by sugar maple (*Acer saccharum*, L.), with sandy loam soils (primarily Allouez). Trout lily (*Erythronium americanum*, L.) and spinulose shield fern (*Dryopteris austriaca* var. *spinulosa*, Jacquin) are the most abundant herbaceous species in the spring and summer, respectively. Disturbance history has been windthrow dominated after cutover in the late 1800s and early 1900s. To minimize potential differences in available light reaching the herbaceous stratum, plots were located under a homogenous canopy, containing no large gaps, and with continuous cover during the experiment.

A two-factor, randomized block design was used to test for effects of carcasses and deer herbivory on forest soil macronutrients, herbaceous layer composition, forest floor cover, and tree seedling germination, tissue quality, and growth. Deer herbivory was added as a factor because herbivory by locally abundant deer may mask carcass disturbance effects. Hence, treatments were unmanipulated, deer exclusion-control, carcass-disturbance, and deer exclusion-carcass-disturbance, with 10 replicates each. Cyndrical, open-top cages (1.5m × 2m diameter) for exclusion-control and exclusion-carcass-disturbance treatments were constructed of 15.24cm square, wire mesh staked with 1m metal bars at two opposing points. Cages were erected after carcasses were consumed. Each treatment plot in a block was within a 25m radius, and all blocks were within 1 km of each other.

Pre-treatment soil sampling (described below) was done in late April 2005 and then each carcass-disturbance and exclusion-carcass-disturbance plot received an intact, road-killed deer carcass that was previously frozen. Carcasses were placed laterally on the ground surface, centered in plots. Deer carcasses were approximately the same size (mean ± 1SD = 56 ± 9kg, $N = 40$). Daily observations and surveys of wildlife sign were subsequently conducted until soft tissues were >95% consumed and then deer exclusion cages were erected.

### ***Soil macronutrients***

We measured nitrogen (N), phosphorus (P), and potassium (K) levels because these macronutrients are generally limiting to primary productivity in boreal and temperate systems (Frelich 2002). Soils were collected before treatment in late April 2005, and after treatment in early September 2005 and 2006. At each sampling time, four soil cores (4cm diameter × 10cm depth) were collected within 1m of the center of each plot, pooled, homogenized, weighed, and dried to a constant weight at 105ºC. A random sub-sample of soils from 5 blocks was analyzed for inorganic nitrogen [i.e. nitrate ($NO_3^-$) and ammonium ($NH_4^+$)], magnesium (Mg), calcium (Ca), K, and P following standard procedures (Brown 1998). Briefly inorganic N was extracted with 1N KCl and analyzed calorimetrically, a Mehlich 3 extractant was used to determine exchangeable Mg, Ca, and K levels, and a Bray P1 extractant was used to determine soil available P. While recent work has demonstrated the importance of organic nitrogen uptake in some ecosystems (Lipson & Näshholm 2001), and other methods exist for indexing available N (Binkley & Matson 1983), these assays are appropriate methods for assessing gross nutrient pools available to plants and microbial communities in north, temperate forest soils (Brown 1998). Analysis of nutrient concentrations was conducted blind by Michigan State University Soil and Plant Nutrient Lab. Macronutrient data were analyzed using one-way mixed-model (carcass and herbivory exclusion treatments) repeated measures analysis of variance (ANOVA) and *a priori* planned contrasts to test the hypothesis of positive carcass effects on macronutrient concentrations. Brown-Forsythe and O'Brian tests were used to confirm assumptions of circularity (i.e., the variance of the *difference* of observations between sampling times is the same for each plot; Gotelli & Ellison 2004).

### ***Herbaceous layer composition and forest floor cover***

In May and August of 2005 and 2006 herbaceous layer composition (aerial percent cover $m^{-1}$, including bare ground) was measured for all species <50cm tall on all plots. To reduce observer bias a singular observer performed ocular estimates. To ensure positive identification, plots were later revisited to identify unknown species in flower. Species importance values [IV = (relative percent cover + frequency)/2], were used to asses differences in the relative dominance of herbaceous species between treatments. Individual importance values were calculated for each species present on at least 50% of sample plots and remaining species were pooled into a single category. Percent cover and importance value data were analyzed using matched-pairs analysis to test the hypothesis of no difference between treatment plots within each year. Alpha level ($\alpha$=0.006) was Bonferroni adjusted to account for multiple comparisons. Normal probability plots and Levene tests were used to confirm assumptions of normality and homoscedasticity, respectively.

### ***Tree seedling tissue quality, germination, and growth***

We measured foliar nitrogen, which strongly regulates photosynthetic potential (Messaoud & Houle 2006), in sugar maple seedlings (<50cm tall), the dominant seedling and overstory species present. To assess whether maple seedlings likely assimilated organic nitrogen (as other plants do; Lipson & Näshholm 2001) released directly from carcasses, we measured stable nitrogen isotope ($\delta^{15}N$) values of foliage for treatments with and without carcasses (Fry 2006). Leaves from sugar maple seedlings were collected during early June 2005-2007 from each treatment for measurement of total carbon (C), total N (dry mass) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) concentrations. Leaves of equal size were clipped at their base from actively growing plants, dried at 64 °F to a constant weight, double rinsed with distilled water, dried again, and then individually homogenized in a bearing shaker mill. Analysis was performed blind on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Deltaplus Continuous Flow-Stable Isotpe Ratio Mass Spectrometer at Michigan Technological University's Ecosystem Science Center. IAEA, USGS, and NIST certified isotopic standards were run at the beginning of each analysis. One certified standard was also run at the end of the analysis to check for calibration stability. Stable isotope values are reported in standard δ notation, and are reported on the VPDB scale for $\delta^{13}C$ and on the atmospheric air scale for $\delta^{15}N$. An internal standard was run every 10 samples. Precision based on repeated measures of internal standards was +/- 0.25‰ for $\delta^{13}C$ and 0.5‰ for $\delta^{15}N$.

Sugar maple seed mast was high in 2006, hence seedling germination and summer survival was measured in 2007. A single observer conducted double-counts of germinants in a 0.25 $m^2$ area centered on each treatment plot in late May and mid-August. Germinant density for each sampling time was averaged for each treatment and is expressed per $m^2$. After leaf-fall in 2007, available maple seedlings (height <50cm) from each plot (i.e., within 0.5m of the center) were collected for growth analysis. Seedlings were air dried and stems were then cut 3cm above the root collar. Terminal stem surfaces were sanded to reveal cellular structure and help identify false and light rings. Annual ring widths were measured to the nearest 0.01mm using a binocular microscope and sliding-stage micrometer. Widths were measured twice, blind to treatment, and then averaged.

Tissue quality, stable isotope, and germination data were analyzed using statistical methods described for soil macronutrients. Ring width data were analyzed using a paired *t*-test to test the hypothesis of zero difference between mean ring widths for two years pre- and post-treatment. Normal probability plots and Levene tests were used to confirm assumptions of normality and homoscedasticity, respectively.

## Results

Direct observation and field sign (scat and tracks) indicated that predators (grey wolves, *Canis lupus*, L.; coyotes, *Canis latrans*, L.) and vertebrate scavengers (e.g., red fox, *Vulpes vulpes*, L.; ravens, *Corvus corax*, L.; bald eagles, *Haliaeetus leucocephalus*, L.) visited carcass plots, removing >95% of the soft tissue within 3 weeks of carcass additions. The core area (convex polygon) encompassing carcass remains and disturbed by predator and scavenger activity averaged 6.25m$^2$ ($N = 40$). Deer herbivory did not affect the response of any soil, ground, or herbaceous layer attribute to treatments. Consequently, results presented here are from a single factor analyses with pooled results. Results for unmanipulated and exclusion-control treatments were pooled into one treatment (hereafter control) and results for carcass-disturbance and exclusion-carcass-disturbance treatments pooled into one treatment (hereafter carcass).

Prior to treatment no difference in macronutrients (P, K, Mg, Ca, $NO_3^-$, $NH_4^+$) existed between treatment plots (Fig. 1). At 3 and 15 months after treatment, soils at carcass sites had 80% and 60% more phosphorus, respectively ($F_{1, 56} = 11.6$, $P = 0.001$), 52% and 33% more potassium ($F_{1, 56} = 5.7$, $P < 0.020$), 895% and 208% more nitrate ($F_{1, 56} = 5.8$, $P < 0.019$), and 298% and 137% more ammonium ($F_{1, 56} = 18.1$, $P < 0.022$) relative to adjacent control sites. (Fig. 1A, B, E, F). Differences between carcass sites and control sites exhibited a temporal pattern of initial increase and subsequent decrease (Fig. 1).

Dominant spring and summer herbaceous species responded negatively to the carcass additions for two growing seasons after treatment ($P = 0.0002$ to $<0.0001$; Table 1). The decrease in percent cover (20-22%) and importance value (9.5-24%) of dominant herbaceous species on carcass sites relative to control sites was highest the first growing season after treatment (Fig. 2). With exception of fern importance values, these effects persisted during the second growing season after treatment (Fig. 2). No difference was found between treatments for all other summer herbaceous species (Table. 1). Coverage of the dominant tree seedling decreased with moderate significance ($P$=0.008) the first growing season after treatment (Table 1). Decreases in spring litter depth ($F_{3, 156} = 28.8$, $P < 0.001$) and increases in percent bare ground ($F_{3, 156} = 19.7$, $P < 0.003$) on carcass sites compared to control sites presumably led to increased maple seedling germination on carcass sites (Fig. 3, $F_{1, 77} = 50.0$, $P < 0.001$).

Foliar nitrogen levels were 101% and 24% higher in plants growing on carcass sites compared to control sites ($F_{1, 57} = 34.3$, $P < 0.0001$) for the first two growing seasons after treatment (Fig. 4A), indicating higher photosynthetic potential for plants on carcass sites. Mean foliar carbon-to-nitrogen ratio decreased 50% over the first growing season (Fig. 4C, $F_{1, 57}$ = 57, $P < 0.0001$), indicating higher aggregate leaf tissue quality at carcass sites. Maple foliage from carcass sites had elevated $\delta^{15}$N compared to control sites during all three growing seasons after treatment, but lagged in response to carcass effects (Fig. 4B, $F_{1, 57}$ = 38.1, $P < 0.0001$). Tree ring width of maple seedlings two years before treatment did not differ between carcass and control plots (*t*-ratio = 1.39, df = 37, $P = 0.17$), but for two years after treatment seedlings on carcass plots exhibited increased ring widths compared to control plots after treatment (*t*-ratio = 7.27, df = 37, $P < 0.0001$, Fig. 5).

## Discussion

Contrary to our expectation, even well-utilized large herbivore carcasses created disturbances that reduced the cover of dominant herbaceous layer species in spring and summer (Table 1, Fig. 2). Carcass disturbance resulted in significant pulses of growth-limiting nutrients for at least two growing seasons postmortem (Fig. 1). These effects resulted in higher tree seedling germination, foliar N, and growth at carcass sites compared to control sites (Figs. 3-5). These data support a novel understanding of disturbance by large herbivores in forest ecosystems. Large ungulate carcasses effectively perforated the herbaceous layer filter that can differentially influence seed predation, germination, and seedling growth and survival (*sensu* George & Bazazz 1999a, b, 2003). This is important because mechanisms that structure seedling development ultimately determine future forest composition by influencing the trajectory, rate, and spatial patterns of regeneration (Grubb 1977, Frelich 2002).

The growth rate of tree seedlings is often correlated with light availability (George & Bazazz 2003, Delagrange et al. 2004, Gilliam 2007). Other experimental work has demonstrated that when dominant fern cover in deciduous forests is decreased, light levels below the fern layer increase, resulting in higher seedling germination, growth, and survival of specific species (George & Bazazz 1999a, b). *Acer sp.* seedling germination patterns, in particular, are positively correlated with photosynthetic photon flux density (Messaoud & Houle 2006). Higher light levels, although not measured here, likely occurred at carcass disturbed sites because the cover of dominant species decreased (Table 1). Increases in light availability due to carcass disturbance almost certainly contributed to the strong growth response of maple seedlings on carcass sites (Fig. 5). Maple seedling cover decreased on carcass sites the first season following treatment (Table 1). This change suggests that a reduction in competition for light and nutrients among maple seedling conspecifics may also contribute to the seedling foliar N and growth response (Figs. 4, 5). Increased foliar N for carcass site seedling would enhance photosynthetic rates compared to control site seedlings at any given light level because foliar N is strongly related to carbon fixation (Elvir et al. 2005). The total disturbance effect of large ungulate carcasses, therefore, appears to be at least threefold. Carcasses provide nutrient pulses, likely increase light availability, and alter competitive relationships in forest herbaceous layers.

The changes in soil, leaf litter, species cover, and plant foliage at carcass sites create heterogeneity of important resources such as nutrients, light, and space. Resource heterogeneity is a mechanism that likely provides unique regeneration niches for trees and maintains coexistence in competing plant species (Grubb 1977, Pacala & Tilman

1994, George & Bazazz 1999a, b, Chesson 2000, Catovsky et al. 2002). For example, spatial heterogeneity in microenviroments led to large differences in growth and survival of dominant tree species in southern Appalachian forests (Beckage & Clark 2003). In a tallgrass prairie system, the pulse of resources released from bison carcass resulted in differences in species composition between disturbed and undisturbed patches that persisted at least five years postmortem, thereby contributing to grassland heterogeneity (Towne 2000). Our results suggest that carcass-derived resources in forests may shift competitive relationships among species with different resource use efficiencies, thereby affecting subsequent growth, survival, and reproduction. It is important to recognize that the disturbance observed at carcass sites occurs at a spatial scale similar to other factors (e.g., pit and tip-up mounds, nurse logs) that promote the maintenance of species diversity in temperate forest communities (Harmon and Franklin 1989, Beatty 2003, Christie and Armesto 2005). Hence, in addition to perforating the physical filter created by herbaceous foliage, large herbivore carcasses create biogeochemical “hotspots” (Fig. 1) in forest ecosystems that may help maintain tree and herbaceous species biodiversity by creating resource heterogeneity.

The soil response to carcass remains (Fig. 1) may include the effects of positive macronutrient feedbacks at carcass sites. Positive feedbacks occur because carcass sites are nutrient and energy focal points, receiving exuviae and puparia materials from dead invertebrates, and fecal and urine deposition from scavengers, large herbivores, and predators (Carter et al. 2007). For example, bird droppings whitewashed course woody debris and leaf litter at carcass sites. Such activity means that although the intense N, P, and K effects are likely restricted to the core area encompassing carcass remains at kill sites, a single carcass could be ecologically important at larger scales by influencing the landscape mosaic of herbaceous layer and forest overstory links (Gilliam and Roberts 2003c).

The lagged response in foliage $\delta^{15}N$ on carcass sites (Fig. 4B) may reflect slower decomposition of some isotopically heavy, recalcitrant carcass remains (e.g., bone or hair). The bones and hair of large herbivores show enriched $\delta^{15}N$ relative to their plant diet due to preferential retention of heavier isotopes in consumers (Fry 2006). The increased $\delta^{15}N$ in foliage at carcass sites suggests that a carcass-derived N source leads to higher available N in soils, resulting in increased plant N assimilation (Fig. 4), which may influence aboveground trophic interactions. For example, herbivores are attracted to patches of nitrogen-rich forage (Day and Detling 1990). Hence carcass sites become foraging sites (Towne 2000, Danell et al. 2006), and the probability of repeated foraging within and around carcass sites initiates a positive feedback of recurrent nutrient supplementation from frass, feces, and urine deposition. Deer herbivory was not a significant factor in plant response to carcass disturbance, perhaps because of predator and scavenger activity at carcass sites. Increased invertebrate herbivory of plants growing on carcass sites remains unexamined.

When natural die-offs in large ungulate populations occur, carcass distribution can exhibit strong spatial patterns. For example, during winter deer in northern hardwood forests typically congregate in cedar and hemlock stands known as “deeryards” (Halls 1984). Such aggregations can create areas of high carcass density when die-offs occur. Declines of ~20-30% in Michigan’s Upper Peninsula deer population (~100,000-200,000 individuals) have been documented during the winters of 1955/56, 1978/79, 1985/86, 1993/94, and 1995/96 (Langenau 1996, Doepker et al.1996). In such years, we would expect the deer carcass effects demonstrated here (Table 1, Figs. 1-5) to be highly aggregated on the landscape. In mild Upper Peninsula winters ~35,000 deer die due to starvation, ~70,000 in moderate winters (Michigan Department of Natural Resources 2008). The potential long-term consequences of repeated carcass deposition on soils and herbaceous layers in areas of high mortality, such as deeryards, remain unexamined.

These results also indicate an important way by which the predators of large herbivores can influence the landscape mosaic of nutrient cycling and species interactions. If carnivores affect the distribution of carcasses that result from predation, they would also affect the spatiotemporal heterogeneity of soil and plant properties. Recent research in southern boreal forests and grasslands in North America indicates that moose (*Alces alces,* L.) and elk (*Cervus canadensis*, Erxleben) carcasses produced via wolf predation are important to above and belowground communities (Bump *unpublished*). Predation by wolves also occurs in some locations at rates that are different than the rates for other causes of mortality such as starvation and hunting (Bump *unpublished*, Wilmers et al. 2003, Kauffman et al. 2007). For example, wolves preferentially travel along shorelines in Isle Royale National Park (Peterson 1977), which results in moose kills being significantly clustered near water (Peterson 1977, Bump *unpublished*). Similarly, landscape analysis of wolf-killed elk in Yellowstone National Park shows that flat grasslands near streams and roads are favorable to wolf hunting success (Kauffman et al. 2007). In northern hardwood forests, wolves preferentially kill deer within pack territories, avoiding areas of territory overlap, presumably to minimize the chance of lethal interactions with wolves from other packs (Mech 1977). Hence, across three distinct systems, wolf hunting behavior affects the landscape distribution of carcasses. These wolf-carcass-landscape links demonstrate how the functional traits of species in large mammal predator-prey systems are linked to ecosystem processes, such as resource heterogeneity, through carcass distribution.

Humans also hunt nearly all wild ungulates, which results in spatial and temporal carcass distributions patterns that are different than those created by wild carnivores. Carcasses and gut piles from hunter-kills favor scavengers with large feeding radii by arriving in super abundant pulses and with less dispersion than wild predator-kills (Wilmers et al. 2003, Wilmers & Getz 2004). Through hunting harvest, wounding loss, and vehicle collisions humans killed ~2.5-4 times more deer than wolves in Michigan's Upper Peninsula (~61,000 human caused vs. ~15,000-25,000 by wolves, Michigan Department of Natural Resources 2008). Large ungulate mortality due to vehicle collisions is widespread and exhibits distinct spatial patterns (Romin & Bissonette 1996). These anthropogenic particulars emphasize that the important ecological effects of large animal carcasses can be significantly affected by human behavior as well as the hunting behavior of wild predators.

This study contributes to a growing recognition that carcasses of vastly different sizes, from whales to salmon to cicadas, have significant and lasting effects in diverse ecological systems (Towne 2000, Smith & Baco 2003, Yang 2004, Helfield & Naimen 2006, Carter et al. 2007). The effect of deer carcasses on forest herbaceous layers demonstrated here expands this awareness significantly because large herbivores are nearly ubiquitous in temperate and boreal forests (Danell et al. 2006). Large herbivore carcasses, therefore, can be understood as biotic disturbance mechanisms with important ecological effects across multiple biomes.

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## Tables

**Table 1.** Mean (±SE) percent cover of herbaceous layer species

| | | Treatment | | Paired analysis* | |
|---|---|---|---|---|---|
| Species | Year | Carcass | Control | *t*-ratio | *P*-value |
| Percent cover | | | | | |
| *Erythronium americanum*[†] | 2005 | 4.3±0.5 | 26.5±3.8 | -5.90 | <0.0001 |
| (trout lily) | 2006 | 0.5±2.0 | 14.0±2.6 | -5.15 | <0.0001 |
| *Dryopteris spinulosa*[‡] | 2005 | 8.2±1.8 | 28.6±3.8 | -6.07 | <0.0001 |
| (spinulose shield fern) | 2006 | 3.4±0.9 | 8.4±1.5 | -3.22 | 0.002 |
| *Acer saccharum*[§] | 2005 | 9.4±0.9 | 14.7±1.6 | -2.76 | 0.008 |
| (sugar maple) | 2006 | 17.5±2.6 | 21.7±3.0 | -1.20 | 0.238 |
| All other species[¶] | 2005 | 9.1±1.4 | 13.4±2.3 | -4.25 | 0.049 |
| | 2006 | 17.3±2.6 | 17.6±3.3 | -0.09 | 0.927 |

*Comparisons of the difference between matched pairs of treatment plots (*N*=40 for each test; Bonferroni adjusted α=0.006). [†]Dominant spring herb. [‡]Dominant summer herb. [§]Dominant tree seedling. [¶]Includes summer herb species only.

## Figure legends

**Figure 1** Indices of nutrient availability in soils from white-tailed deer carcass sites (solid circles) and paired control sites (open circles) before carcass treatment (May ’05) and at the end of two growing seasons after treatment (Sept. ’05, Sept. ’06). (**A**) Ammonium, (**B**) nitrate, (**C**) calcium, (**D**) magnesium, (**E**) potassium, and (**F**) phosphorus. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each postmortem sampling time. Error bars show mean ± SE (some are too small to be seen). Note break in *x* axis and the different *y* axis scales.

**Figure 2** Comparison of mean white-tailed deer carcass-control differences in percent cover (**A**) and importance value (**B**) for dominant spring (lily) and summer (fern) herbaceous species. Reference line is at 0, indicating no difference between treatments. Error bars show mean ± SE. Note break in *x* axis scales. Asterisks (*) indicate a mean difference significantly below 0 ($P < 0.05$).

**Figure 3** Contrasting white-tailed deer carcass (**A**) and control (**B**) sites in spring after heavy sugar maple mast in previous fall season. Mean litter depth (triangles), bare soil (circles), and sugar maple seedling germination (squares) on deer carcass and control sites (**C**). Significant ($P < 0.05$) differences exist between carcass and control treatments exist at each sampling time.

**Figure 4** Foliar nitrogen content (**A**), $\delta^{15}$N (**B**), and carbon:nitrogen ratio (**C**) from white-tailed deer carcass (solid circles) and paired control sites (open circles) in sugar maple leaves for three growing seasons after treatment. Asterisks (*) indicate significant ($P < 0.05$) differences for planned contrasts between carcass and control sites at each sampling time. Error bars show mean ± SE. Note different *y* axis scales.

**Figure 5** Mean white-tailed deer carcass-control differences in tree ring width for sugar maple seedlings growing on carcass and paired control sites. Reference line is at 0, indicating no difference between treatments. Error bars show mean ± SE. Arrow indicates start (2005) of experimental treatment.

# Figures

**Figure 1**

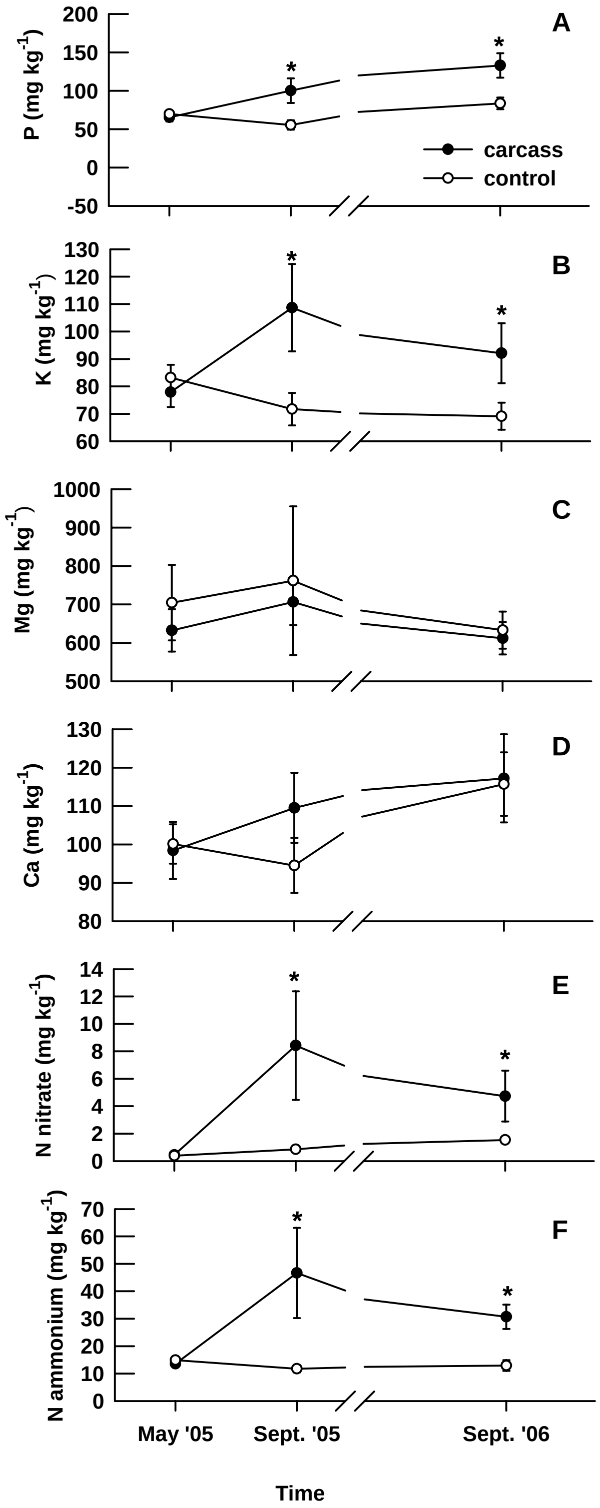


**Figure 2**

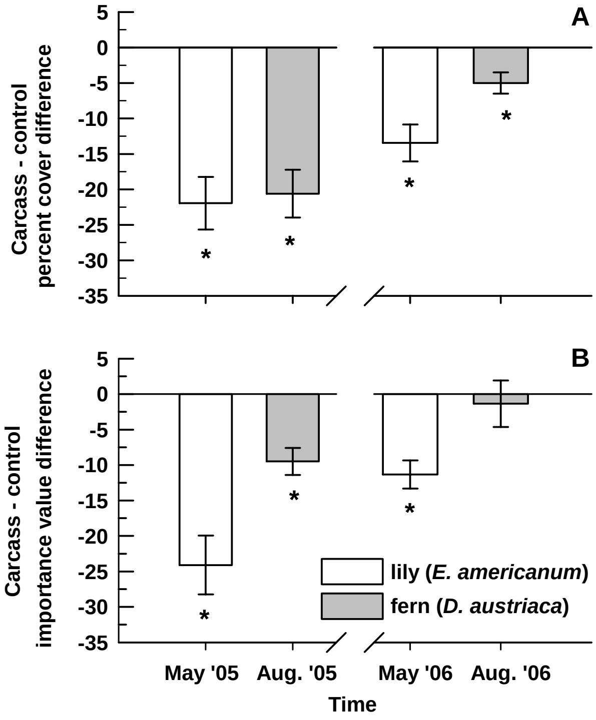
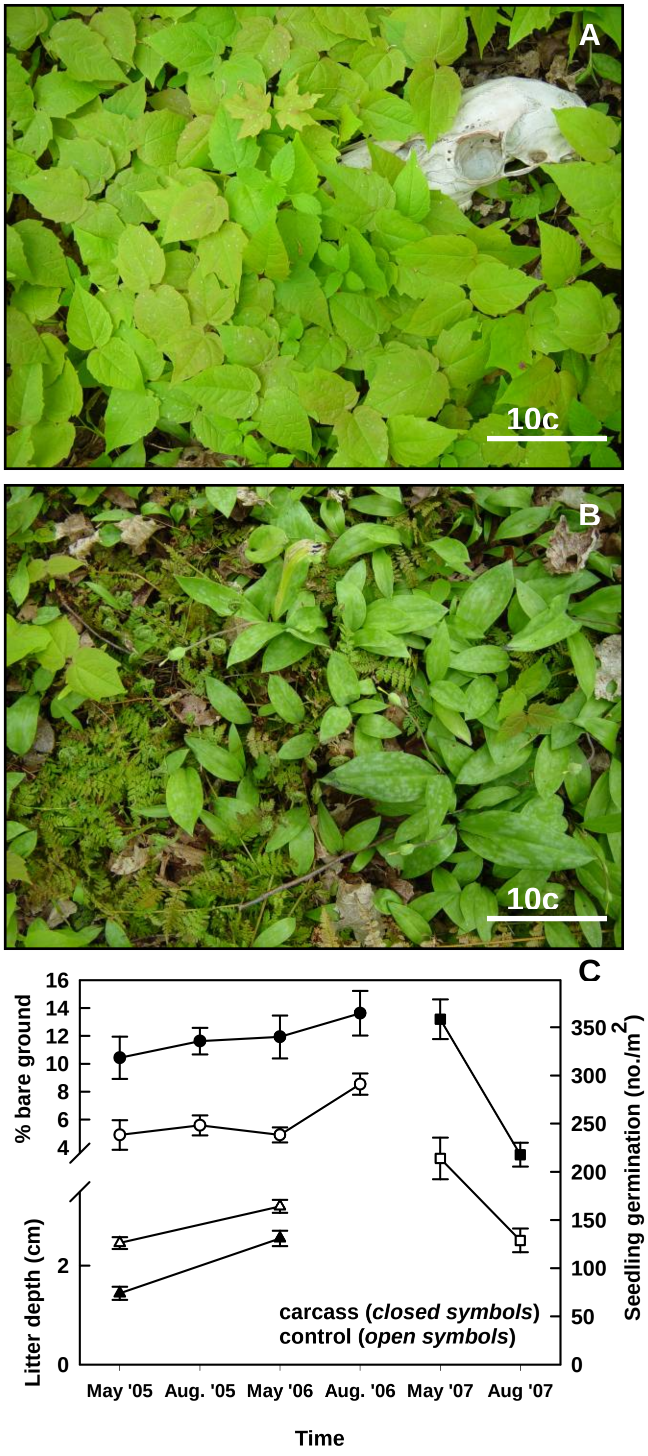
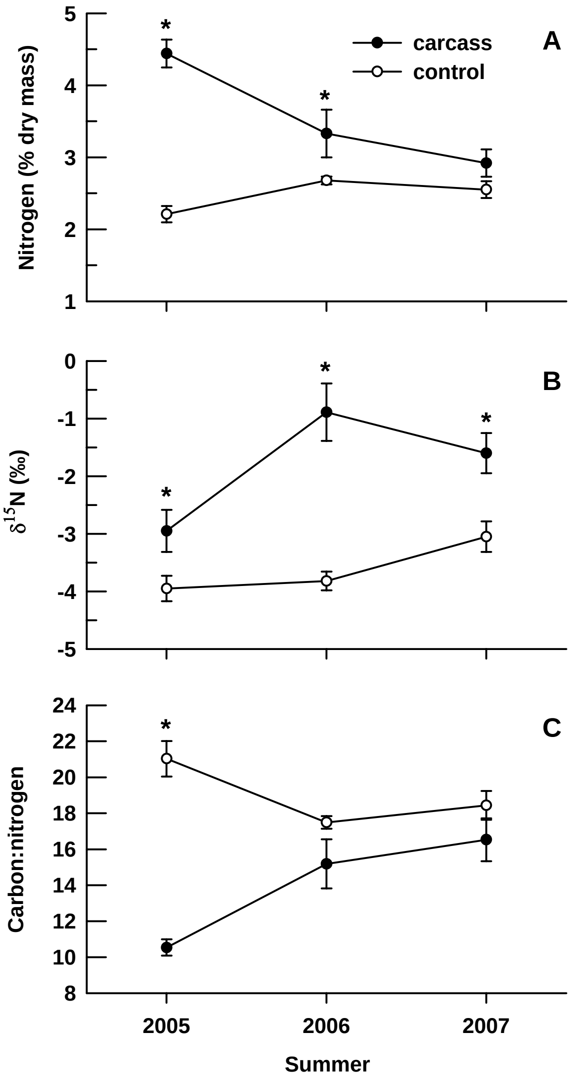

A
B
Carcass - control percent cover difference
Carcass - control importance value difference
5
0
-5
-10
-15
-20
-25
-30
-35
*
lily (*E. americanum*)
fern (*D. austriaca*)
May '05
Aug. '05
May '06
Aug. '06
Time

**Figure 3**


A
10c
B
10c
C
% bare ground
16
14
12
10
8
6
4
Litter depth (cm)
2
0
Seedling germination (no./m$^2$)
350
300
250
200
150
100
50
0
carcass (*closed symbols*)
control (*open symbols*)
May '05
Aug. '05
May '06
Aug. '06
May '07
Aug '07
Time

**Figure**

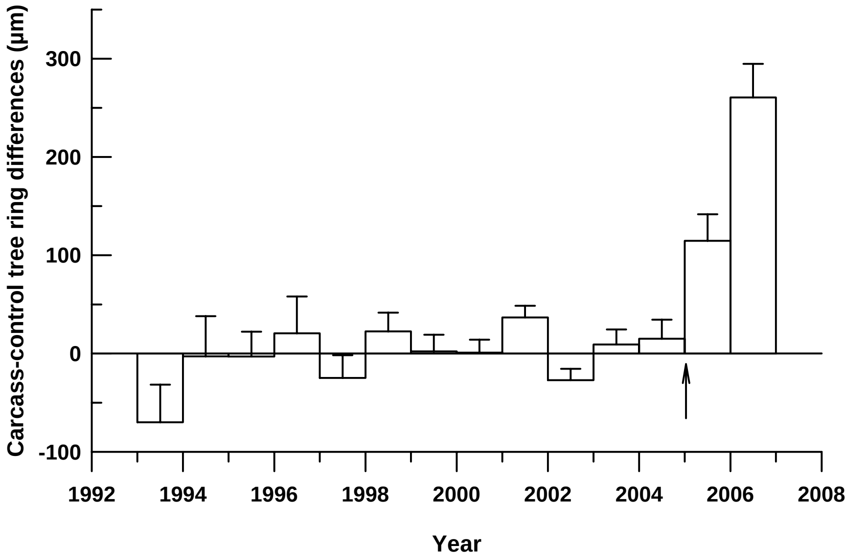
**4**



A
carcass
control
Nitrogen (% dry mass)
5
4
3
2
1
*
*
B
$\delta^{15}N$ (‰)
0
-1
-2
-3
-4
-5
*
*
*
C
Carbon:nitrogen
24
22
20
18
16
14
12
10
8
*
2005
2006
2007
Summer

**Figure**

**5**



Carcass-control tree ring differences (µm)
300
200
100
0
-100
1992
1994
1996
1998
2000
2002
2004
2006
2008
Year

## Supplementary Information

**Figure S1** Photograph of a one week-old, scavenged white-tailed deer carcass (**A**).

Photographs of a deer carcass site in spring (**B**) and early summer (**C**).

## Paper IV – Planned submission to *Journal of Animal Ecology*

# Large herbivores and aquatic-terrestrial links in southern boreal forests

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*Running Headline:* Aquatic-terrestrial resource flux by moose

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## Summary

**1.** Understanding the consequences of cross-habitat resource flux requires knowledge of the flux's spatial and temporal dynamics, yet few studies exist with sufficient long-term data to provide such information. Long-term study of the moose *Alces alces* (L.) population in Isle Royale National Park (Lake Superior, USA) provides an opportunity to examine the patterns of resource flux from aquatic to terrestrial habitats over ~50-yrs.

**2.** We analyzed the spatiotemporal dynamics of aquatic-derived nitrogen (N) that moose transfer to terrestrial systems and the potential aquatic system phosphorus (P) loss due to moose herbivory by using excretion models, foraging parameters, moose densities, and Isle Royale moose carcass locations (*n*=3616) collected from 1958-2005.

**3.** Moose transfer significant amounts of aquatic-derived N to terrestrial systems, which likely increases terrestrial N availability. Aquatic-P loss due to moose herbivory may be offset by releases of P from interstitial benthic sediment waters disturbed during foraging. The areal extent of aquatic-derived N dispersion in terrestrial habitats exceeded the extent of aquatic P loss.

**4.** Aquatic foraging by moose and moose carcass locations are significantly clustered at multiple scales, indicating that grey wolves *Canis lupus* (L.) and moose can create concentrated areas of resource transfer due to clustered predation and foraging patterns.

**5.** This study demonstrates that patterns of faunal-mediated resource transfer depend on species-specific natural history, and that predators in this system influence herbivore-controlled resource transfer between ecosystems. Given the circumpolar extent of moose, they constitute an important, unquantified aquatic-terrestrial resource vector in boreal systems.

*Keywords*: riparian, food webs, susbsidies, trophic cascade, hotspots.

## INTRODUCTION

Integrating organism, population, community, and ecosystem perspectives requires quantifying patterns and consequences of resource flow (flux) across habitat boundaries (Leopold 1941, Likens and Bormann 1974, Polis et al. 1997, Loreau and Holt 2004, Polis et al. 2004). Ecologists hypothesize that resource subsidies affect species interactions and the stability of ecosystems (Huxel and McCann 1998, McCann et al. 2005), primary productivity (Odum et al. 1979, Polis et al. 1997), and biological diversity patterns (Moore et al. 2004, Croll et al. 2005). Flux between habitats depends importantly on adjoining physical characteristics and biological vectors. The specific physiology and behavior of a biotic vector can significantly influence multiple aspects of exchange across habitat boundaries (e.g., Ben-David et al. 2005). Studies addressing these ideas often focus on faunal-mediated nutrient and energy fluxes between aquatic and terrestrial habitats, for instance marine to terrestrial via salmon, birds, and river otters (Naiman et al. 2002, Ben-David et al. 2005, Croll et al. 2005) and river or lake to riparian zone via aquatic insects (Sabo and Power 2002a, Knight et al. 2005). Understanding such resource flow dynamics requires knowledge of the flux's: 1) magnitude and quality, 2) mediating agents, 3) consequences, 4) temporal aspects and, 5) spatial extent. The first four of these flux aspects are relatively well explored (reviewed in Polis et al. 2004, Ballinger and Lake 2006), compared to a lack of knowledge about long-term spatiotemporal dynamics of resource flow across aquatic-terrestrial boundaries (but see Ben-David et al. 2005 for an exception).

Long-term study of the moose *Alces alces* (L.) population in Isle Royale National Park (544 km$^2$ archipelago in Lake Superior, USA; 48°N, 89°W) provides an opportunity to examine the long-term patterns of resource flow from aquatic to terrestrial habitats over multiple decades. Moose are aquatic-terrestrial interface specialists (*sensu* Ballinger and Lake 2006), foraging extensively on aquatic macrophytes during spring and summer (Franzmann & Schwartz 1997), and excreting wastes and dying most frequently in terrestrial habitats (Franzmann & Schwartz 1997, Peterson 1977). Hence, moose comprise an unquantified vector for the transport of aquatic materials into terrestrial systems, which constitutes a resource loss pathway from the aquatic perspective (Leopold 1941, Likens and Bormann 1974). As submerged macrophytes mature, partial senescence of leaves occurs, leaching nutrients. 60-85% of total phosphorus (P) readily leaches within days, most within hours, contributing to internal lake P supply rate (Wetzel 2001). Aquatic resources ingested by moose are either deposited as urine and feces on the landscape or are incorporated into moose biomass. Significant soil and terrestrial plant responses to urine and fecal returns are well-quantified for large herbivores (McNaughton 1985, Hobbs 1996, Frank 1998, Olofsson et al. 2004), including moose on Isle Royale (Pastor et al. 1993). Large herbivore carcasses are typically considered a resource for secondary consumers (e.g., Wilmers et al. 2003), but can also directly contribute to detritivore and producer communities (Danell et al. 2002). Consequently, aquatic-derived resources can flow through either moose excretion or biomass pathways and enter consumer, producer, or detritivore communities.

Boreal forests are well suited for examining spatial patterns of resource flow across aquatic-terrestrial boundaries. The boreal forest is a large circumpolar biome and covers ~28% of the North American continent north of Mexico (Larsen 1980). Moose are the largest member of the deer family (Cervidae), and their distribution parallels that of the boreal forest (Franzmann & Schwartz 1997). Adult moose carcasses represent substantial (270-550 kg), highly concentrated pools of labile nutrients available to predators (e.g. grey wolves *Canis lupus,* L.), scavengers (Wilmers et al. 2003), and detritivore communities (Burkepile et al. 2006). Generally, boreal soils are relatively young (<10,000 yr.), nutrient-poor spodosols (Larsen 1980). Aboveground net primary productivity (NPP) in boreal forests is typically less than in lower latitude forests; approximately 380 g $m^{-2}$ $yr^{-1}$ (Saugier et al. 2001). Lentic systems in boreal regions, however, exhibit more variable annual productivity, typically ranging between 5-300 g $m^{-2}$ $yr^{-1}$ (Wetzel 2001). The ecological importance of resource transfers is thought to be positively related to asymmetrical productivity between ecosystems (Polis et al. 1997). Hence, the variability in productivity of boreal aquatic systems and the ubiquitous presence of a proficient resource vector via moose, emphasize the importance of understanding the dynamics of aquatic-terrestrial resource transfer for this biome.

Here we investigate temporal and spatial patterns of resource flux via moose from aquatic to terrestrial habitats of the Isle Royale ecosystem. First, we quantified daily and annual aquatic-derived nitrogen (N) entering the terrestrial system via moose. Second, we estimated annual P loss from aquatic systems due to moose herbivory. We focused on N and P because these elements are typically the most limiting in temperate and boreal forests (Larsen 1980), and north-temperate and boreal lakes (Wetzel 2001), respectively.

Lastly, long-term (1958-2005) spatial patterns of the moose-mediated flux were explored by 1) analyzing carcass site clustering at island-wide and ~1km scales, and 2) comparing the likely areal extent of the aquatic resource shadow (i.e., a zone from which energy, materials, or organisms have been diverted; the location of aquatic plant removal in this study; *sensu* Polis et al.2004) to the likely areal extent of the terrestrial excretory N dispersion in summer. Carcass site locations define where aquatic resources that were incorporated into moose biomass enter consumer and detritivore communities. We expected carcass sites to be clustered due to moose habitat choice and predation patterns (Peterson 1977). Comparing the aquatic resource shadow extent to the terrestrial dispersion examines potential asymmetric spatial patterns in resource flux between donor (aquatic) and recipient (terrestrial) systems. Water depth typically limits moose foraging in aquatic habitats to near-shore, littoral zones (Franzmann & Schwartz 1997). In contrast, physical features do not generally limit where moose forage in terrestrial habitats and mortality can occur anywhere on the landscape. Consequently, we expected that the areal extent of the donor system resource shadow should be less than the extent of resource dispersion in the recipient system.

## METHODS

### Aquatic to terrestrial nitrogen flux via moose excretion

We partitioned excretory nitrogen (N) returns of aquatic origin from biomass returns because these distinct resource pathways operate on separate timescales (i.e., excretion always occurs daily versus variable mortality occurrence) and excretory returns surpass biomass returns in most years. Estimation of daily excretory N flux from aquatic to terrestrial habitats via moose was based on N excretion models developed by Hobbs (1996, 2006, Appendix A). The total daily N added to an environment through ungulate urinary and faecal excretion can be expressed as a function of body mass and plant N content (Hobbs 2006). Total daily excretory N ($N_t$, g day $^{-1}$ individual $^{-1}$) returned to the foraging system per moose is expressed as,

$$N_t = F_p + U_p + F_e + U_e \qquad \text{eqn 1}$$

where $F_p$, $U_p$, $F_e$, $U_e$, represent faecal N of plant origin, urinary N of plant origin, endogenous faecal N, and endogenous urinary N respectively, as defined by Hobbs (2006; Appendix S1). Hobbs' (2006; Appendix S1) suite of excretory functions rely on intake rates as a function of biomass and an empirical relationship measured between dry matter digestibility and plant N content developed for elk diets in Colorado (Hobbs 2006). To better represent our study system we used a range of known moose summer intake rates (2.6-3.5 % of body mass per day in dry matter; p 450 in Franzmann & Schwartz 1997) and the range of plant N content reported for summer terrestrial and aquatic forage on Isle Royale (0.72-3.66% dry matter; Tischler 2004) . Body mass used was the whole weight mean (408.25kg) for cows (*n*=2) and bulls (*n*=3) measured for Isle Royale moose in winter (Peterson 1977).

To determine the proportion of $N_t$ (Eq. 1) that was of aquatic origin we relied upon recent estimates of the aquatic fraction of summer moose diets [0.14-0.37; range for estimates based on stable carbon and nitrogen isotope analysis (Tischler 2004)], a finding comparable to a previous estimate of 0.18 (Belovsky & Jordan 1978). Consequently, if we let $A$ indicate the aquatic diet fraction range (0.14-0.37), then N of aquatic origin

($N_{aquatic}$ g day$^{-1}$ individual$^{-1}$) excreted when moose are feeding on aquatic macrophytes is estimated by,

$$N_{aquatic} = N_t \times A \qquad \text{eqn 2}$$

and would constitute an ecologically meaningful estimate of the daily N individual moose transfer from aquatic to terrestrial systems. The equation (2) calculation assumes moose excretory returns directly to aquatic systems are negligible, which is initially reasonable given that aquatic feeding bouts average 42.8 min day$^{-1}$ on Isle Royale, digestive retention times typically exceed 12 hours, and moose in summer spend about 5× more time per day on land than foraging in aquatic habitats on Isle Royale (Belovsky & Jordan 1978). Additionally, in 65 hours of observing moose in lakes, defecation was never observed, while in 48 hours of terrestrial observation, defecation was frequently observed (Belovsky & Jordan 1978). In calculating $N_{aquatic}$ we held intake rate constant at 3.1% of body mass per day in dry matter (i.e., the median of the range in summer intake rates reported for moose in Franzmann & Schwartz 1997).

Terrestrial nutrient fluxes are often most informative and comparable when done for populations on a per unit area per year basis (Hobbs 2006). Therefore, per unit area total summer excretory N additions of aquatic origin ($N_{flux}$ kg ha$^{-1}$ yr$^{-1}$) for moose densities (0-10 individuals km$^{-2}$) found in North America (Franzmann & Schwartz 1997) were estimated as,

$$N_{flux} = \left(N_{aquatic} \times P \times D\right) \div S \qquad \text{eqn 3}$$

where $P$ is moose population density, $D$ is the number of days aquatic foraging typically occurs on Isle Royale (we used 108 days; Belovsky & Jordan 1978) and $S$ the mean summer core-area size (0.4km$^2$; Dussault et al. 2005).

The relative importance of $N_{flux}$ was explored by comparison to annual terrestrial N cycling components: net N mineralization [20 kg ha$^{-1}$ year $^{-1}$ measured in 3 upland forests of aspen-birch-spruce, birch-spruce, and birch-spruce-fir by Pastor et al. (1993) on Isle Royale], dissolved inorganic N runoff [1.6 kg N ha$^{-1}$ year $^{-1}$ reported maximum for undisturbed boreal forests (Wetzel 2001)], and atmospheric deposition [3 kg N ha$^{-1}$ year $^{-1}$ reported for Isle Royale (Stottlemyer and Toczydlowski 1995)]. $N_{flux}$ was expressed as a percentage of these terrestrial N cycling components.

## Aquatic phosphorus loss due to moose herbivory

Estimation of the total P loss from the littoral zone in aquatic systems due to moose herbivory was based on aquatic consumption rates on Isle Royale and P concentration in aquatic macrophytes. P loss ($P_{loss}$ kg yr $^{-1}$ individual$^{-1}$) was calculated as,

$$P_{loss} = I \times C \times D \qquad \text{eqn 4}$$

where $I$ is daily aquatic forage consumption [we used the range 0.655-1.081 kg dry matter day$^{-1}$ moose$^{-1}$ reported for Isle Royale moose (Belovsky & Jordan 1978)], $C$ the concentration of P in aquatic macrophytes [we used a range of 0.19-0.75% dry mass for regional aquatic species and those known to be consumed by moose (Gerloff & Krombholtz 1966)], and $D$ is the same as defined for Eq. (3).

The relative importance of $P_{loss}$ was explored by comparison to the summer internal P supply rate (240 kg wk $^{-1}$) calculated for Shagawa Lake, MN in 1972 (Larsen et al. 1981). Internal P supply rates have not been determined for Isle Royale lakes to our knowledge. Shagawa lake was the best proxy found for Isle Royale interior lakes: a small, shallow, soft-water glacial lake, lying in a rock-scoured basin of the boreal forest region of northeast MN, about ~128km west of Isle Royale at the same latitiude, and exhibiting limnological properties within the range of Isle Royale interior lakes (Larsen et al. 1981).

## Long-term flux dynamics on Isle Royale

Total annual N flux and aquatic P loss due to moose herbivory on Isle Royale for 1915-2005 was calculated using modified $N_{flux}$ (Eq. 3) and $P_{loss}$ (Eq. 4) expressions: annual adult and yearling moose population estimates substituted for $P$ and the terrestrial dispersion area (100 km$^2$; justification below) replaced $S$ for the N calculation. Detailed methods for annual moose censuses since 1958 have been reported elsewhere (Vucetich & Peterson 2004). Moose population estimates prior to 1958 are less reliable and are included to illustrate the general trend only (Peterson 1977).

## Spatial dynamics of aquatic to terrestrial resource flux via moose

The location of where aquatic-derived resources that are incorporated into moose biomass enter secondary consumer or detritivore pathways was analyzed using moose carcass locations collected from 1958-2005 (*n*=3616). Carcass locations were determined in winter during aerial surveys, with subsequent ground inspection, and in spring and summer through extensive, island-wide hiking (Peterson 1977, Vucetich & Peterson 2004). The number of carcasses within a 1km radius was calculated for each carcass location. High and low density carcass clustering was then analyzed using global and local indicators of spatial association (i.e., Getis-Ord general G, local Getis-Ord Gi* and Local Moran's I, Fortin and Dale 2006) in a geographic information system (ArcGIS, ESRI™). The Getis-Ord General statistic summarizes spatial autocorrelation for the entire study area, while the local Getis-Ord Gi* and Local Moran's I statistics assess autocorrelation within a 'neighborhood' of locations within a search radius. Hence, the global spatial statistics permit summarizing the spatial pattern over the entire island and the local statistics identify areas of density relatedness and significant clustering at the 1km-scale, i.e. 'hot- and cold-spots' (Fortin and Dale 2006). We used Euclidian distance and inverse distance squared methods in the clustering analysis.

We compared the potential area of resource loss to the area of resource gain by approximating the probable littoral zone area from which aquatic macrophyte material was removed [i.e., resource shadow; *sensu* Polis et al. (2004)] in the aquatic systems to the probable excretory dispersion in the terrestrial system. The areal extent of seasonal aquatic resource shadow was estimated by dividing the mean daily aquatic forage consumption [0.655-1.081 kg dry matter day$^{-1}$ moose$^{-1}$ reported for Isle Royale moose (Belovsky & Jordan 1978)] by an average of aquatic forage consumption per m$^{2}$ obtained from two studies of moose on Isle Royale [0.073 kg dry matter yr$^{-1}$ moose$^{-1}$ (Belovsky & Jordan 1978, Qvarnemark and Sheldon 2004)]. Thus, the aquatic resource shadow equaled 9-15 $m^2$ $day^{-1}$ $moose^{-1}$, which was then multiplied by the number of days that moose forage on aquatic plants [i.e. *D* in Eq. (3); 108] and annual moose population estimates (described above) to yield annual approximations of the aquatic resource shadow.

The comparative areal extent of aquatic resource transfer through excretion in the recipient terrestrial systems was estimated using the mean summer core-area size (0.4 ± 0.1 $km^2$; Dussault et al. 2005), and observations of summer congregation of moose near inland lakes on Isle Royale (Peterson 1977). The core-area radius multiplied by total length of inland lake shoreline available for moose foraging [i.e., 276 km ×0.36km = ~100 $km^2$ (10,000 ha)] provides an ecologically meaningful estimate of the maximum spatial extent that aquatic-derived resources are most likely distributed over the terrestrial landscape via moose.

## RESULTS

### Aquatic to terrestrial nitrogen flux via moose excretion

The summer total daily N ($N_t$) excreted by Isle Royale moose increases as intake rate and forage N concentration rise, with a mean (±1SD) on Isle Royale of 283±37 g N $day^{-1}$ $moose^{-1}$ (Fig. 1A). $N_t$ is more strongly influenced by plant N concentration than intake rate because faecal N excretion increases linearly and urinary N excretion quadratically with forage N concentrations (Hobbs 1996, 2006). Similarly, the summer daily N of aquatic origin ($N_{aquatic}$) excreted by moose increases as the aquatic diet fraction and forage N concentration rise, with a mean (± SD) on Isle Royale of 78±12 g N $day^{-1}$ $moose^{-1}$ (Fig. 1B). Again, $N_{aquatic}$ is more strongly influenced by plant N concentration than the aquatic diet fraction. The aquatic diet fraction range used (i.e., $A$ in Eq. (2) = 0.14-0.37) in calculating $N_{aquatic}$ is conservative; the 95% CI reported by Tischler (2004) for this range is 0.09-0.57. Hence, $N_{aquatic}$ could be substantially lower or higher for individual moose. The total summer excretory N of aquatic origin ($N_{flux}$) transferred to terrestrial systems via moose on Isle Royale was (mean ± SD) 1.2±0.2 kg N $ha^{-1}$ $yr^{-1}$, constituting 6%, 75%, and 40% of net annual N mineralization, annual N runoff, and annual atmospheric N deposition, respectively (Fig. 2). At high moose densities, which often occur in riparian habitats (Peterson 1977), $N_{flux}$ may be ~3× higher (Fig. 2).

### Aquatic phosphorus loss due to moose herbivory

The mean (± SD) total summer P loss from aquatic systems due to moose herbivory ($P_{loss}$) on Isle Royale was 31±6.7 kg P $yr^{-1}$ $moose^{-1}$, constituting 0.9% of the internal P supply rate (Fig. 3). We consider these conservative estimates of the relative importance because $P_{loss}$ was compared to internal P supply rates for a lake (i.e., Shagawa Lake, MN) with historical anthropogenic P inputs from local, municipal waste water (Larsen et al. 1981), which has never been the case for Isle Royale lakes.

### Long-term flux dynamics on Isle Royale

Over the past century terrestrial N gain and aquatic P loss via moose have exhibited 10-fold and 3-fold fluctuations, respectively, before and after wolf establishment (Fig. 4). Mean (± SD) annual N gain for terrestrial habitats for1958-2005 was 1.06±0.4 kg N $ha^{-1}$ $yr^{-1}$. Mean (± SD) annual aquatic P loss via moose for 1958-2005 was 906±336 kg $yr^{-1}$.

### Spatial dynamics of aquatic to terrestrial resource flux via moose

Long-term carcass location patterns indicate that aquatic resources incorporated into moose biomass enter consumer and detritivore pathways across the island, with obvious areas of high and low resource transfer (Fig. 5A). Terrestrial dispersion of aquatic-derived excretory resources transferred via moose is likely highest in riparian zones and within ~0.5km of lake shorelines (Fig. 5B). The mean (±SD) extent of seasonal aquatic resource shadow (~1.2±$\text{km}^2$, range=0.4-3.3 $\text{km}^2$) was less than the scope of terrestrial distribution (~100 $\text{km}^2$). However, the effects of littoral zone foraging by moose probably extend to pelagic zones, depending on lake mixing dynamics (Wetzel 2001).

A positive General G value indicated that high density moose carcass sites were clustered together on an island-wide scale (Observed General G = 0.1E-5, Expected General G <0.1E-5, General G variance <0.1E-5). Hence, there was less than a 0.1% likelihood ($\alpha = 0.05$) that the clustering of high density moose carcass sites was from the result of random chance (z-score = 4.13 standard deviations).

Together, the Getis-Ord Gi* and Local Moran's I z-scores indicated that there was significant clustering of high density and low density carcass areas (Fig. 6a) and that sites of similar density were most often located adjacent to each other at local scales (Fig. 6b; ~1km). The positively skewed z-score distributions (Fig. 6a) indicated that high density ('hot spots') carcass sites were more strongly associated with one another than with low density carcass sites ('cold spots'), and that similar carcass density values were more strongly associated (Fig. 6b).

## DISCUSSION

The range in relative importance of $N_{flux}$ as compared to net annual N mineralization (0%-25%; Fig. 2), illustrates that the relative importance of the moose-mediated N flux is strongly dependent on moose biomass and terrestrial system net N mineralization. For example, Stottlemyer et al. (1995) found annual net N mineralization rates under dominant boreal tree species [i.e., white spruce *Picea glauca* (Moench) and paper birch *Betula papyrifera* (Marsh)] on Isle Royale to be consistently below half of the rate used in this analysis (20 kg ha$^{-1}$ year $^{-1}$ from Pastor et al. 1993), which would at least double the percentage of net annual N mineralization (and other N cycle components) that $N_{flux}$ constitutes. Comparing $N_{flux}$ to other important N cycle components on Isle Royale (e.g., total plant uptake, alder and cyanobacteria N-fixation, organic N availability) would, if data were available, refine our understanding of the relative importance of $N_{flux}$. However, our $N_{flux}$ estimate (mean± SD = 1.2±0.2 kg N ha$^{-1}$ yr$^{-1}$) is similar to N fixation inputs by mosses that are argued to be important to boreal forests elsewhere (Zackrisson et al. 2004)

Most North American moose population densities are <1 moose km$^{-2}$ (Franzmann & Schwartz 1997) and Isle Royale densities generally range ~1-6 individuals km$^{-2}$ (Vucetich & Peterson 2004). However, $N_{flux}$ could be substantially higher in areas where animals are concentrated in preferred foraging and resting sites. For example, over 3 individual moose h$^{-1}$ have been recorded visiting Isle Royale mineral licks, all of which are near inland lakes (Risenhoover & Peterson 1986) and as many as 9 different moose have been observed simultaneously foraging in an area less than 1 km$^{-2}$, on a single lake at Isle Royale (Peterson 1995). Under such conditions, the total summer excretory N of aquatic origin ($N_{flux}$) transferred to terrestrial systems via moose could approach ~4.5 kg N $ha^{-1}$ $yr^{-1}$, constituting 23%, 150%, and 282% of net annual N mineralization, annual N runoff, and annual atmospheric N deposition, respectively (Fig. 2).

Accordingly, the excretory N transferred from aquatic to terrestrial habitats via moose (Figures 1B, 2, & 4) likely increases terrestrial N availability within moose summer core areas on Isle Royale. Terrestrial ecosystems exhibit a dichotomous N cycling response to large mammal herbivory depending on feedbacks between forage tissue chemistry and soil properties (reviewed in Pastor et al. 2006). Pastor et al. (2006) argue that forage N concentrations are critical in determining nutrient accelerating or decelerating (*sensu* Ritchie et al. 1998) scenarios based on observations that faecal N excretion increases linearly and urinary N excretion quadratically with forage N concentrations (Hobbs 1996, 2006). The resulting critical N concentration in forage is ~1.5% dry mass, above which mammalian herbivores excrete N primarily as urea to remove excesses, below which N is excreted primarily in feces to maximize N retention (Pastor et al 2006). Urea-derived N is readily available to plants, but faecal-N is less so and can lead to slower N mineralization rates than found in soil humus alone (Pastor et al. 1993).

These insights lead us to propose that moose seasonally increase terrestrial N cycling when foraging on N-rich aquatic macrophytes, which, if accurate, contrasts with the depression of soil N mineralization found previously on Isle Royale (Pastor et al. 1993, 2006). The basis for this suggestion is the observation that two N-rich aquatic genera, *Potamogeton sp.* and *Myriophyllum sp.*, are readily consumed by moose and dominate shallow aquatic habitats on Isle Royale (Qvarnemark and Sheldon 2004).

Tischler (2004) found the mean N-content of *Potamogeton sp.* (*n*=28) and *Myriophyllum sp.* (*n*=5) was 2.45% and 2.24% dry mass, respectively, compared to 1.3% for combined summer and winter terrestrial forage sampled (*n*=182). Aquatic foraging, therefore, would cause moose to excrete excess N primarily as urea in riparian zones and within ~0.5km of lakeshores (Fig. 5B). In evaluating this perspective on the functional role of moose, it is important to note that the timing, duration, and intensity of aquatic foraging by moose is variable, ranging from the few months observed in our system to the year-round use of aquatic plants reported by MacCracken (1993) on the Copper River Delta, Alaska. Hence, the degree to which moose increase terrestrial N cycling with aquatic N inputs would vary with the aquatic foraging dynamics of specific moose populations.

From the aquatic perspective, submerged macrophytes are important because they significantly affect zooplankton and fish distributions (Weaver et al. 1997), foraging strategies (Dionne & Folt 1991), community assemblages (Chick & Mcivor 1994), lake nutrient cycling, and primary productivity (Carpenter & Lodge 1993). Littoral zone macrophytes and their attached epiphytic microflora have been conceptualized as a ‘metabolic sieve’ because they intercept a significant portion of the chemical load in runoff entering lakes (Wetzel 2001). In doing so, macrophytes heavily influence the connectivity of nutrient cycling along the riparian-littoral-pelagic continuum (Wetzel 2001). Submerged macrophytes obtain most of their P from interstitial sediment waters, where concentrations are orders of magnitude greater than in overlying water (Carpenter & Lodge 1993). Because active and senescent macrophytes both readily leach P they can significantly modify phosphorus loadings and budgets in aquatic ecosystems (Carpenter & Lodge 1993). The significant influence of consumers (zooplankton, benthic invertebrates, and fish) have on internal P cycling has been recognized (reviewed by Vanni 2002), but such analyses have not included moose. Research on aquatic foraging by moose has been dominated by an individual-physiological perspective (e.g., Belovsky & Jordan 1978, Risenhoover & Peterson 1986, MacCracken et al. 1993, Franzmann & Schwartz 1997). The potential direct (e.g. P loss; Fig. 3) and indirect effects of moose populations on aquatic ecosystems require more scrutiny (but see Qvarnemark and Sheldon 2004 for effects on aquatic plant diversity). For example, turbation of lake sediments by moose while foraging for aquatic plants certainly releases P from interstitial sediment waters. Therefore, internal lake P supply rates could hypothetically increase, producing a net positive effect on lake P cycling despite the P loss due to moose herbivory (Figures 3 & 4). Testing such a hypothesis is necessary to understand feedbacks between animal populations and ecosystem processes across ecological interfaces (Polis et al. 2004).

In this study we did not quantify the portion of aquatic forage incorporated into moose biomass. To do so is complicated by different assimilation efficiencies (e.g., due to tannin content of tree species) and availabilities of aquatic and terrestrial forages (Belovsky & Jordan 1978). Even so, where moose consume aquatic vegetation, some fraction of moose biomass is undeniably aquatic in origin. Thus, moose link seasonal aquatic system productivity with terrestrial predator, scavenger, and detritivore communities. The analysis of long-term carcass spatterns (Figures 5 & 6) indicates where such food web linkages occur on the landscape and is a prerequisite to understanding the effects of aquatic subsidies (Polis et al. 2004). Isle Royale moose carcasses, despite being very well-utilized by wolves and scavengers, increase soil macronutrients and microbial biomass, shift soil microbial composition, and elevate leaf nitrogen for at least 2-3 years in plants at kill sites (Bump et al. unpublished). The moose carcass location pattern reveals that the transfer of aquatic subsidies via moose biomass to upper trophic levels and detritivore communities is clustered over time, at multiple scales (Fig. 6). The known mechanisms influencing carcass 'hot' and 'cold' spots include island-scale habitat features and smaller scale predation patterns.

Fire patterns and differential regeneration of balsam fir *Abies balsamea* (L.), an ecologically important winter-browse species, correlate with the typical island-wide spatial pattern in moose density. The highest moose densities (~5.4 individuals km$^{-2}$) are at the east end, low densities mid-island in major 1936 burn areas (~0.8 individuals km$^{-2}$), and moderate densities (~1.8-3.4 individuals km$^{-2}$) at the west end (Vucetich and Peterson 2004). The direct influence of wolves on the spatial distribution of carcasses, and thereby patterns in the moose-mediated resource flux, is readily observable. Wolves kill moose in some regions of the landscape at up to 12× the rate of other regions (Bump et al. unpublished). On Isle Royale, kills of calves are usually near shorelines, in heavy cover, while adult kills appear more randomly distributed (Peterson 1977, Stephens and Peterson 1984). In summer, cow moose and calves are significantly associated (~2× more likely than bulls) with human camp grounds (and no wolves), which are almost all situated on shorelines (Stephens and Peterson 1984). Calves may not consume aquatic forage directly, but are nonetheless supported by aquatic forage insofar as it sustains lactating cows. Moose densities in winter, which are important for assessing where aquatic resources incorporated into moose biomass are distributed, averaged 415% greater on small islets than on the main island (Stephens and Peterson 1984). Hence, moose habitat selection reflects tradeoffs between acquiring essential resources and avoiding predators (Stephens and Peterson 1984, Dussault et al. 2005), which results in excretion and carcass spatial patterns (Figures 5 & 6).

Current knowledge of Isle Royale wolf-moose population dynamics indicates that wolves probably influence long-term moose biomass, and thereby the mean aquatic-terrestrial resource flux via moose, but have little effect on the interannual variation of this resource flux. Isle Royale's *mean* wolf abundance is a significant determinant in the *mean* moose abundance (Vucetich and Peterson 2004). However, year-to-year variation in moose population growth rate is largely unexplained, and appears more heavily influenced by abiotic (weather) and bottom-up (food) factors than by top-down factors such as short-term fluctuations in wolf abundance (Vucetich and Peterson 2004). Introduced disease (canine parvovirus) significantly reduced wolves at one point and parasitic ticks *Dermacentor albipictus* (Packard) likely affect moose levels (DelGiudice et al. 1997). Thus, the cascading effect of wolves, weather, and disease on the magnitude of aquatic to terrestrial resource flow via moose biomass is not easily predicted one year to the next.

Our results indicate that moose constitute an important aquatic-terrestrial resource vector in boreal systems. Future refinement of the first approximations made in this study may result in different modeling parameters and flux estimates, but such improvements will not likely alter the qualitative importance of our conclusions. Providing moose exhibit aquatic foraging, and excrete and die predominantly on land, they will constitute a resource vector conferring a net influx to terrestrial habitats and efflux from aquatic habitats within their range. Resource dispersion in the recipient system will typically be more extensive than the donor system resource shadow due to the landscape-level terrestrial range of moose and depth-restricted extent of their littoral zone foraging. However, repeated use of specific areas and clustering of moose carcasses over time can create highly concentrated areas of resource transfer in recipient systems. The consequences of the resource flux estimated here require further study, especially the possible effects of moose on aquatic ecosystems: the direct net influence on water chemistry, lake sediment disturbance, and the potential indirect effects on aquatic food webs (i.e., phytoplankton, zooplankton, fish populations) are unresolved. Wolves influence moose populations, habitat use, and carcass distribution; in doing so, they also impact aquatic-terrestrial resource subsidies transferred via moose.

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## Figure legends

**Fig. 1** Nitrogen excretion by moose (*Alces alces*) in Isle Royale National Park (Lake Superior, USA). Open plot sections indicate best estimates for Isle Royale moose based on mean ± 1SD of model parameters. (a) Summer total daily nitrogen ($N_t$) excreted as a function of summer intake rate and the plant nitrogen concentration range exhibited for summer terrestrial and aquatic forage. (b) Summer daily nitrogen of aquatic origin ($N_{aquatic}$) excreted as a function of the summer aquatic diet fraction and the plant nitrogen concentration range exhibited for summer terrestrial and aquatic forage.

**Fig. 2** Total summer excretory nitrogen of aquatic origin ($N_{flux}$) transferred to terrestrial systems via moose (*Alces alces*) as a function of North American moose population densities and the summer daily nitrogen of aquatic origin ($N_{aquatic}$) excreted by moose. Open plot section indicates best estimate for Isle Royale moose based on mean ± 1SD of model parameters. Additional y-axes indicate relative importance by expressing $N_{flux}$ as a percentage of annual net nitrogen mineralization, annual forest runoff, and annual atmospheric deposition. See methods for values of these nitrogen cycling components used for comparison.

**Fig. 3** Total summer phosphorus loss from aquatic systems due to moose (*Alces alces*) herbivory ($P_{loss}$) in Isle Royale National Park (Lake Superior, USA), as a function of consumption and the plant phosphorus concentration. Open plot section indicates best estimate for Isle Royale moose based on mean ± 1SD of model parameters. Additional y-axes indicate relative importance by expressing $P_{loss}$ as a percentage of internal lake P supply rates. See methods P supply rate used for comparison.

**Fig. 4** Long-term excretory nitrogen of aquatic origin transferred to terrestrial systems (left y-axis) and total aquatic phosphorus lost from lakes (right y-axis) via adult and yearling moose (*Alces alces*) on Isle Royale National Park (Lake Superior, USA). Shaded error bars since 1958 indicate 95% CI. Data prior to 1958 should be considered illustrative of a general trend only.

**Fig. 5** Long term moose (*Alces alces*) carcass and aquatic-terrestrial resource flux distribution in Isle Royale National Park (Lake Superior, USA). (a) Moose carcass (*n*=3616) density map 1958-2005. Island white areas = 0 carcass density. (b) Aquatic-derived resource dispersion in the recipient terrestrial system. Buffer zone around interior lakes indicates probable mean summer core areas of moose foraging on aquatic macrophytes.

**Fig. 6** Cumulative probabilities of local spatial statistic z-scores calculated for moose (*Alces alces*) carcass location densities (1958-2005; *n*=3616) in Isle Royale National Park (Lake Superior, USA). Z-scores less than -1.96 or greater than 1.96 (i.e., one standard deviation) are considered significant at alpha=0.05. Vertical reference lines mark one standard deviation. Horizontal reference lines mark where the probability functions intersect vertical reference lines. (a) Getis Ord Gi* z-scores indicate a third of carcass sites are located in areas of high (32%) or low (31%) carcass site density (i.e., hot or cold spots respectively). (b) Local Moran's I z-scores indicate 4% of carcass sites exhibit carcass densities dissimilar to adjacent areas, while about half (52%) of carcass sites are located in areas of similar carcass density.

Figure 1

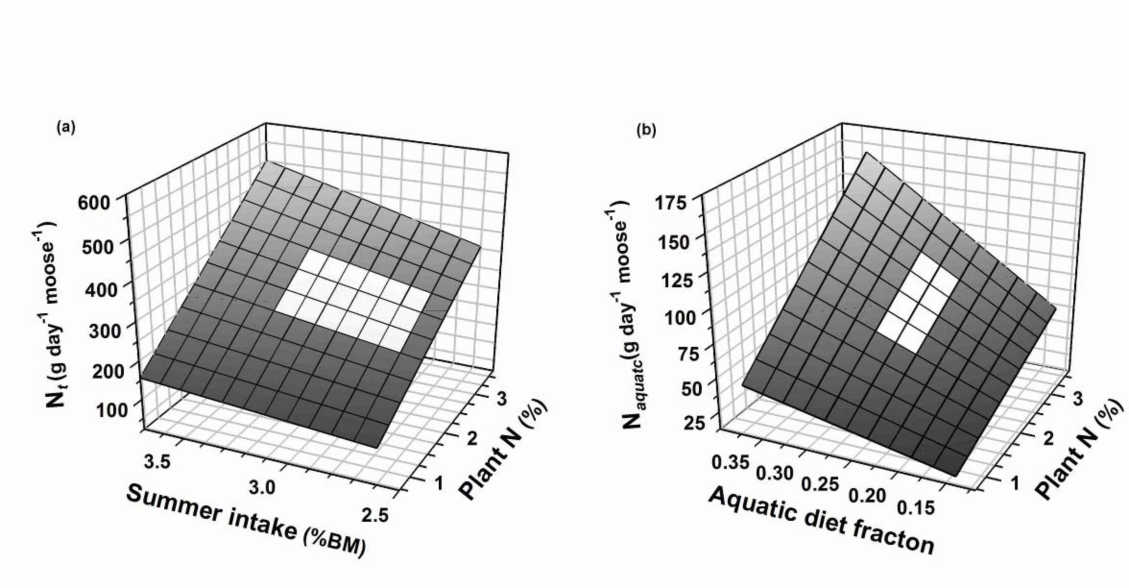
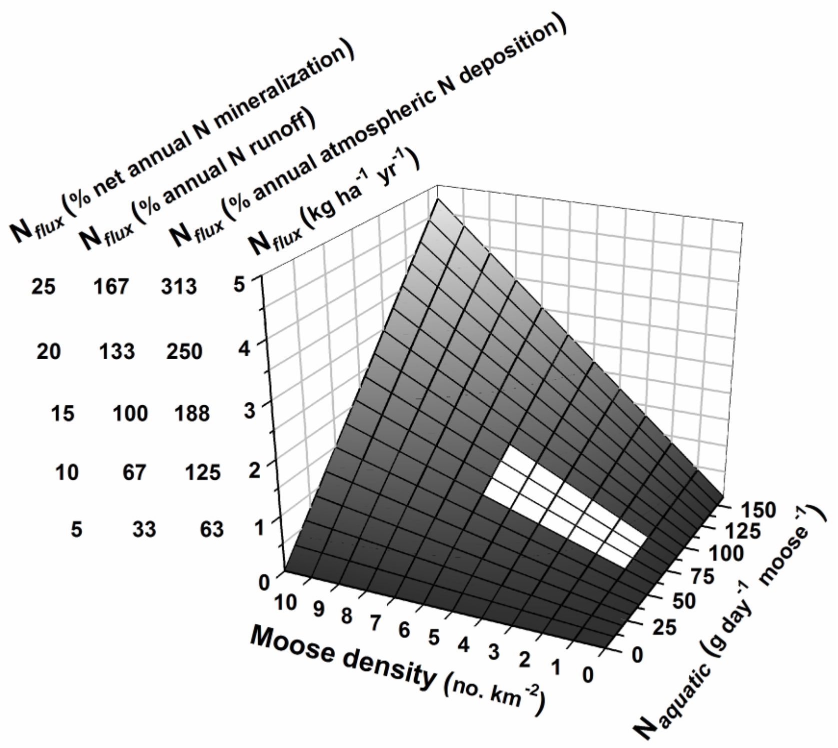

(a)
600
500
400
300
200
100
$N_t$ (g day$^{-1}$ moose$^{-1}$)
3.5
3.0
2.5
Summer intake (%BM)
1
2
3
Plant N (%)
(b)
175
150
125
100
75
50
25
$N_{aquatc}$(g day$^{-1}$ moose$^{-1}$)
0.35
0.30
0.25
0.20
0.15
Aquatic diet fracton
1
2
3
Plant N (%)

Figure 2



N flux (% net annual N mineralization)
N flux (% annual N runoff)
N flux (% annual atmospheric N deposition)
N flux (kg ha-1 yr-1)
25 167 313 5
20 133 250 4
15 100 188 3
10 67 125 2
5 33 63 1
0
10 9 8 7 6 5 4 3 2 1 0
Moose density (no. km-2)
0 25 50 75 100 125 150
N aquatic (g day-1 moose-1)

Figure 3

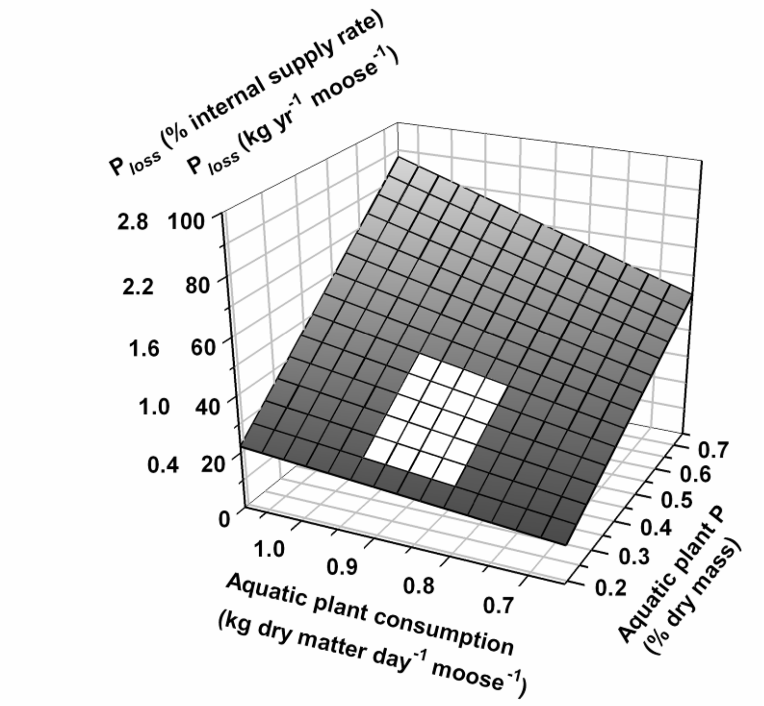
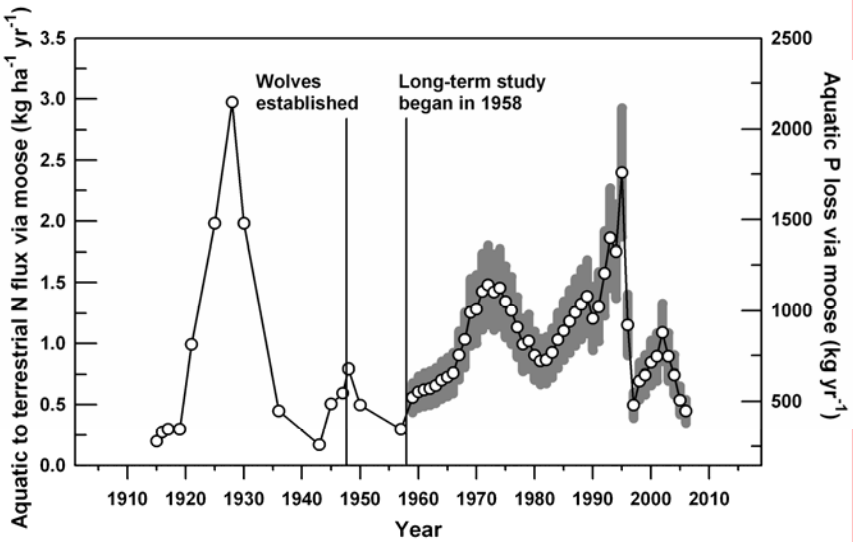

P loss (% internal supply rate)
P loss (kg yr-1 moose-1)
2.8
2.2
1.6
1.0
0.4
100
80
60
40
20
0
1.0
0.9
0.8
0.7
Aquatic plant consumption
(kg dry matter day-1 moose-1)
0.7
0.6
0.5
0.4
0.3
0.2
Aquatic plant P
(% dry mass)

Figure 4



Aquatic to terrestrial N flux via moose (kg ha$^{-1}$ yr$^{-1}$)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Wolves established
Long-term study began in 1958
2500
2000
1500
1000
500
Aquatic P loss via moose (kg yr$^{-1}$)
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
2010
Year

Figure 5

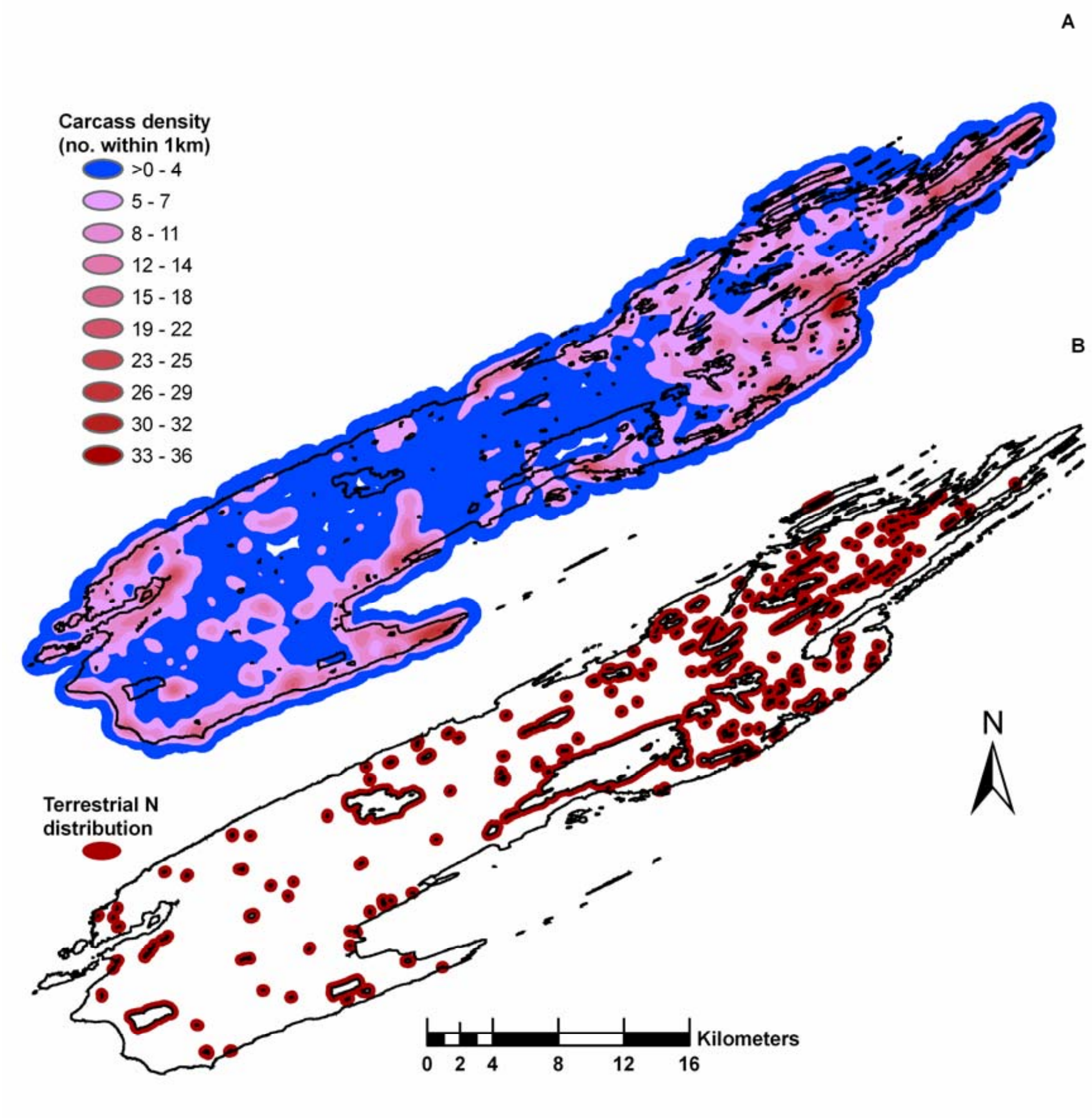
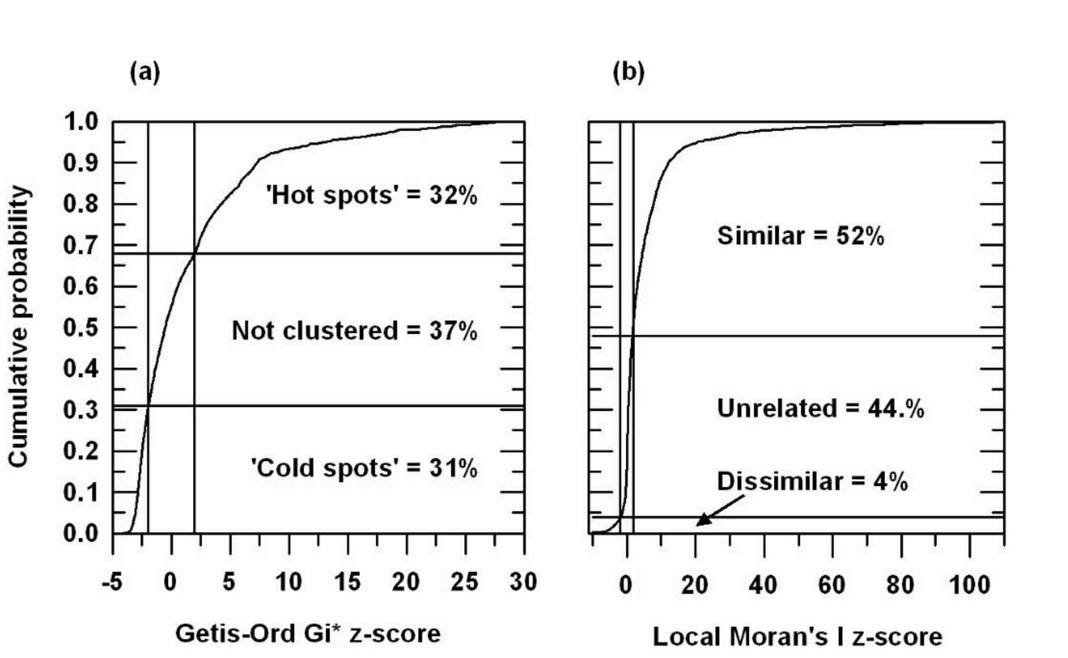

A
Carcass density
(no. within 1km)
>0 - 4
5 - 7
8 - 11
12 - 14
15 - 18
19 - 22
23 - 25
26 - 29
30 - 32
33 - 36
B
Terrestrial N
distribution
N
0
2
4
8
12
16
Kilometers

Figure 6


(a)
(b)
Cumulative probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
'Hot spots' = 32%
Not clustered = 37%
'Cold spots' = 31%
-5
0
5
10
15
20
25
30
Getis-Ord Gi* z-score
Similar = 52%
Unrelated = 44.%
Dissimilar = 4%
0
20
40
60
80
100
Local Moran's I z-score