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Source: Arctic, Antarctic, and Alpine Research, 49(3) : 487-500

Published By: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado

URL: https://doi.org/10.1657/AAAR0016-062
Stable isotopes and radiocarbon assess variable importance of plants and fungi in diets of arctic ground squirrels

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ABSTRACT
Arctic ground squirrels (Urocitellus parryii) rely primarily on dietary protein derived from plants to fuel gluconeogenesis during hibernation, yet fungal sporocarps may be an important, yet overlooked, protein source. Fungivory levels depend on sporocarp productivity, which varies with the dominant plant species and is higher on acidic than on non-acidic soils. To test whether these factors altered fungal consumption, we used stable isotopes to investigate arctic ground squirrel diets at two sites in northern Alaska, Toolik (primarily moist acidic tundra) and Atigun (primarily moist non-acidic tundra). Radiocarbon estimates can also indicate fungivory levels because ectomycorrhizal fungi assimilate soil-derived organic nitrogen whose ¹⁴C levels are higher than current photosynthesis. We measured radiocarbon in hair and δ¹³C and δ¹⁵N in hair, feces, ectomycorrhizal sporocarps, graminoids, and dicots. Feces were higher in δ¹³C and δ¹⁵N at Toolik than at Atigun, and fecal δ¹⁵N increased in August at Toolik, coincident with sporocarp production and fungal spores in feces. Mixing models indicated that graminoids contributed 64%, dicots 35%, and sporocarps 1% to Atigun hair protein, whereas graminoids contributed 37%, dicots 16%, and sporocarps 47% to Toolik hair protein. Acidic soils appeared to correlate with higher sporocarp production and fungivory at Toolik than at Atigun. Atigun hair resembled atmospheric CO₂ in ¹⁴C, whereas Toolik hair had higher ¹⁴C, consistent with greater fungal consumption at Toolik. Late-season sporocarps may be a key protein source for some squirrels and may provide an integrated signal of the soil organic nitrogen assimilated by ectomycorrhizal fungi.

INTRODUCTION
Arctic ground squirrels (Urocitellus parryii), the northernmost mammalian hibernator in North America, have an annual season of aboveground activity of up to 20 weeks (Sheriff et al., 2011), which includes the 6 to 10 week growing season for plants (Chapin et al., 1995). During this period, they add both fat and muscle in anticipation of overwintering (Boonstra et al., 2011; Sheriff et al., 2013). Arctic
Ground squirrels are omnivorous and consume dicots, graminoids, fungal sporocarps, carrion, insects (Holowaychuk et al., 1966; Batzli and Sobaski, 1980; McLean, 1985; Hubbs and Boonstra, 1997; Karels and Boonstra, 2000), and even small mammals such as lemmings (Boonstra et al., 1990) and juvenile snowshoe hares (O’Donoghue, 1994) (Fig. 1). Fungal sporocarp consumption by arctic ground squirrels has not been quantitatively assessed, but appears to be primarily comprised of ectomycorrhizal fungi that rely on plants such as *Betula nana*, *Salix* spp., and *Dryas* spp. for plant photosynthate. Ectomycorrhizal sporocarps are common in the Low Arctic where most ground squirrels live. Techniques to assess fungivory have included direct observations, gut content analyses, and assessment of fungal spores in fecal pellets (Pastor et al., 1996). A fourth technique, measurements of stable isotope ratios in animals, has only been used twice (McIlwee and Johnson, 1998; Flaherty et al., 2010), although it has an advantage over other techniques of directly quantifying assimilation.

Stable isotope signatures are used extensively in dietary analyses of mammals with the most common being isotope ratios of carbon (δ¹³C, expressed as δ¹³C) and nitrogen (δ¹⁵N, expressed as δ¹⁵N). The general principles have been well-characterized (Gannes et al., 1997) and rely on comparing the isotopic relationship among specific tissues (e.g., hair, blood, bone collagen, bone apatite) and potential dietary sources. In the Arctic, δ¹⁵N values are highest in ectomycorrhizal sporocarps, intermediate in monocots (graminoids), and lowest in dicots (Hobbie et al., 2009). Although these three groups have not been compared for δ¹³C in the Arctic, ectomycorrhizal sporocarps in temperate and boreal systems are generally higher in δ¹³C than co-occurring plants (Hobbie, 2005). Evaluation of fecal stable isotopes can provide seasonal information on dietary assessment because fecal δ¹³C and δ¹⁵N signatures reflect diet over a short time period (Salvarina et al., 2013). In contrast, hair δ¹³C and δ¹⁵N signatures reflect diet during the period of hair growth and primarily reflect dietary protein (Sponheimer et al., 2003a; Froehle et al., 2010).

Another isotopic technique to assess diet is radiocarbon analysis (Beavan and Sparks, 1998; Hobbie et al., 2013). Many fungi assimilate organic nitrogen from the soil into their protein, and this organic nitrogen should have a ¹⁴C:¹²C ratio (expressed as Δ¹⁴C) that is older than the current-year photosynthate incorporated by plants during photosynthesis (Hobbie et al., 2013). The bomb spike signal from thermonuclear testing in atmospheric ¹⁴CO₂ levels peaked in 1963 and has declined rapidly since (Fig. 2). If fungal amino acids are incorporated into animal protein, the Δ¹⁴C of hair from these fungivores should be older (higher) than that of atmospheric ¹⁴CO₂, and Δ¹⁴C should correlate with stable isotope indicators of fungivory. Uptake of ¹⁵N-labeled amino acids by nonmycorrhizal sedges has been demonstrated at the Arctic Long Term Ecological Research site at Toolik Lake (McKane et al., 2002). We will therefore also assess whether the dominant sedge *Eriophorum* could be a second source for soil-derived organic nitrogen in diet by testing whether sedge protein has a radiocarbon signature indicating uptake of soil-derived amino acids.

![FIGURE 1. Arctic ground squirrel consuming (left) a *Leccinum* sporocarp or (right) grasses. In the left picture, woody dicots (*Betula* at upper left, *Dryas* immediately below the sporocarp) are visible along with graminoids such as *Eriophorum* and *Carex*. Left photo from http://en.wikipedia.org/wiki/Arctic_ground_squirrel; right photo by Art Scheiber.](https://bioone.org/journals/Arctic,-Antarctic,-and-Alpine-Research)
We investigated the relative importance of fungivory versus herbivory using several different techniques in two populations (Toolik and Atigun) of arctic ground squirrels located about 22 km apart in the foothills of the Brooks Range in northern Alaska. We measured $\delta^{15}$N and $\delta^{13}$C signatures of plants, sporocarps, and hair to determine consumption of graminoids, dicots, and fungal sporocarps, and then used mixing models on hair isotopes to determine consumption of graminoids, dicots, and fungal sporocarps in these two populations. Other factors that could influence diet or isotopic signatures were addressed using multiple regressions on hair and fecal isotopes. The short turnover time of fecal isotopes could allow a seasonal signal of fungivory to be detected because the fruiting of the sporocarps is restricted to the second half of the growing season. In addition, we measured radiocarbon in hair for possible signals of soil-derived organic nitrogen, and examined fecal samples from the two sites for the presence of fungal spores and other dietary items.

We hypothesized that these sporocarps may be a key protein-rich food source for arctic ground squirrels based on evidence of fungivory in other rodents (Johnson and Packer, 1967) and on one record of fungal consumption by arctic ground squirrels (Mayer, 1953). We also hypothesized that arctic ground squirrels from Toolik would have higher levels of fungivory than Atigun because of positive relationships between sporocarp production and low pH (Rao et al., 1997; Högberg et al., 2007; Soudzilovskaia et al., 2015). We addressed this hypothesis through the multiple methods mentioned above. Specifically, our overarching hypothesis predicted that fungivory would be higher at Toolik than Atigun as indicated by (1) mixing models, (2) radiocarbon estimates, (3) hair and fecal $\delta^{13}$C and $\delta^{15}$N levels, and (4) fecal fungal spores presence.

**Materials and Methods**

**Site Description**

Tissues of arctic ground squirrels and of potential dietary items (plants and fungal sporocarps) were collected at two sites in the northern foothills of the Brooks Range in Alaska. The Toolik site is located near the Toolik Field Station along the Dalton Highway, 254 km north of the Arctic Circle ($68°37′40″N, 149°35′41″W$) at an elevation of 720 m. The Atigun site is located 22 km south of Toolik Field Station along the Dalton Highway in the Atigun river drainage ($68°26′58″N, 149°21′43″W$) at an elevation of 804 m. Both sites are underlain by continuous permafrost with a depth of summer thaw that is generally 1–2 m where squirrels are present. However, the origin of the soils at the sites differ, with the acidic Toolik soil derived from glacial till, whereas the alkaline soil at the Atigun site was originally a wind-blown sand dune (Hamilton, 1979; Brown and Kreig, 1983). Vegetation at Atigun generally corresponds to non-acidic tundra, whereas vegetation at Toolik consists of moist-acidic or dry-acidic shrub tundra (Walker et al., 1981).

At the Toolik site, described in Walker and Maier (2008) and Hobbie et al. (2005), ground squirrel burrows are in well-drained soils along a 2 km strip of the east side of Toolik Lake, with some burrows in the field station area. The vegetation is dry-acidic and moist-acidic shrub tundra. Most burrows are in prostrate-shrub tundra with some burrows in erect-shrub tundra and barren areas around the Toolik station. Dominant species in the prostrate-shrub tundra include Arctostaphylos alpina, Betula nana, Cassiope tetragona, Dryas integrifolia, Dryas octopetala, Empetrum nigrum, Ledum palustre ssp. decumbens, Salix phlebophylla, Salix rotundifolia, Salix pulchra, Vaccinium uliginosum, Vaccinium vitis-ideae, graminoids, and mosses. Erect-shrub tundra is dominated by Betula nana, Rubus chamaemorus, Salix pulchra, graminoids, and mosses.

At the Atigun site, previously described in Walker et al. (1981) and Sheriff et al. (2011), ground squirrel burrows are in well-drained soil, primarily on the east side of the Dalton Highway. Vegetation cover data were collected during August 2008 at the Atigun study site (Shamhart, 2010). The non-acidic tundra is dominated by medium-height willows, Dryas octopetala, Rhododendron lapponicum, Arctostaphylos alpina, and Vaccinium uliginosum.
Collection of Plants and Fungi

Isotopic data on plants at Toolik were taken from previously archived values collected in 2000 (<http://ecosystems.mbl.edu/ARC/terrestrial/biomass/index.html>, file lghshten). We also compared these data against archived 2012 harvest data from Toolik (Hobbie and Moore, 2017) and observed that 2000 and 2012 data for graminoids were essentially identical (differing by 0.0‰ for \( \delta^{13}C \) and by 0.2‰ for \( \delta^{15}N \)). DICs were 0.9‰ lower in \( \delta^{13}C \) and 0.5‰ higher in \( \delta^{15}N \) in 2012 than in 2000. At Atigun, roots, leaves, and other plant components were collected in 2007 and 2008 from known or likely food items of arctic ground squirrels. These consisted of shrub leaves of *Arctostaphylos rubra*, *Dryas octopetala*, *Betula nana*, *Salix* spp., and *Vaccinium uliginosum*; forb leaves of *Oxytropis* sp., *Hedysarum* sp., and *Artemisia* sp.; berries of *Arctostaphylos rubra*, *Empetrum nigrum*, *Shepherdia canadensis*, and *Vaccinium uliginosum*; and roots of *Dryas*, *Epilobium*, *Hedysarum*, and *Oxytropis*. The graminoids *Carex bigelowii* and *Eriophorum angustifolium* were collected at both Toolik and Atigun, with the addition of *Eriophorum vaginatum* at Toolik and unidentified graminoids at Atigun. The dicots at Toolik consisted of *Andromeda polifolia*, *Betula nana*, *Cassiope tetragona*, *Dryas integrifolia*, *Ledum palustre*, *Rubus chamaemorus*, *Vaccinium uliginosum*, and *Vaccinium vitis-idaea*. Fungal sporocarps were opportunistically collected in 2008 in both the Atigun River drainage and the Toolik Lake study areas but were not necessarily located within burrow areas. Plants and sporocarps were dried in a food dehydrator at Toolik Field Station and ground at the University of New Hampshire.

Hair and Feces Collection

Arctic ground squirrels were trapped using Tomahawk live traps baited with carrots during the spring, summer, and fall of 2007, 2008, 2011, and 2012 at the Toolik and Atigun study sites. Traps were set between 8:00 and 10:00 a.m. and checked within 2 h. Captured squirrels were brought to Toolik Field Station and anesthetized using isoflurane mixed with oxygen. The animals were then weighed, sexed, measured, and tagged with Monel #1 tags (National Band and Tag Co., Newport, Kentucky, U.S.A.) in both ears (first capture only). Hair was collected from 7 June to 8 September 2007, from 18 April to 30 August 2008, and from 5 April to 8 August 2011. Hair samples were collected by shaving a 4 cm² patch on the abdomen while under anesthesia. In 2008 and 2012, a fresh fecal sample was collected either in the lab prior to anesthetization or in the field from below the trap, which had been previously cleared. Fecal samples were placed on ice for 4–6 h prior to being stored at –20 °C. Squirrels were trapped from 24 to 30 August 2008 and from 9 May to 8 September 2012. The animals were held overnight and returned to the burrows where they were captured. All live animal procedures were approved by the Institutional Animal Care and Use Committees of the University of Fairbanks and the University of New Hampshire (UAF IACUC #06-25 and #08-59, UNH IACUC #080904A).

Microscopic Analysis of Feces

Freeze-dried fecal samples were weighed, and deionized water added at 110 µL mg⁻¹ fecal material to normalize dilution. Samples were ground to a slurry by micropestle and frozen at –20 °C for 24 h. For microscopic examination, samples were thawed and 50 µL dropped onto a slide. A drop of Melzer’s reagent was added to the slurry to detect amyloid reactions, and a 24 × 24 mm cover slip was placed on the slide. Each sample was completely scanned at 100× magnification in approximately 16 objective passes across the slide, progressing from the top to the bottom. To identify potential dietary items or spores, magnification was increased to 400× or 1000×. Spores or spore-like structures were categorized into morphological groups, based on their size, shape, and surface features (Castellano et al., 1989; Colgan et al., 1997). The quantity of each morphological type and other features of interest were documented for each sample, and for most samples the relative dominance of plant, fungal, and insect remains was recorded. Spore counts were used as a measure of relative abundance on a given slide (not between slides) because, although weight-appropriate amounts of distilled water were added to each sample, the feces varied widely in their consistency, and some were quite concentrated in fecal material whereas others appeared much more dilute.

Stable Isotope Analysis

At the University of New Hampshire Stable Isotope Lab, foliage, sporocarps, and fecal samples were ground to a powder, and hair was clipped into small pieces. Foliage, sporocarps, hair, and fecal samples were weighed to 1 µg precision into tin cups. Hair samples were 1.0–1.5 mg, and other samples were 4–5 mg. The \( \delta^{15}N \) and \( \delta^{13}C \) values were determined using a Costech 4010 Elemental Analyzer and a Delta XP Mass Spectrometer with a precision on duplicate samples of 0.2‰.

Lab standards of sporocarps, tuna muscle, apple leaves (NIST1515), and pine needles (NIST1575a) were included with each run. At least 15 standards and two sample duplicates were included with each run of 28
samples. All data were normalized and corrected for drift and linearity.

Radiocarbon Analysis

Five hair samples of individual adults at Toolik and two at Atigun were analyzed for $^{14}C$ content in animals collected in 2008. To provide a well-integrated site average, an additional six samples from Toolik and five samples from Atigun that consisted of 3–4 individuals each were also analyzed. These samples were from either 2008 or 2011. Hair was converted to graphite targets at the University of Florida, and then analyzed at the Keck-Carbon Cycle AMS facility at the University of California at Irvine.

Isolating Eriophorum Protein for $^{14}C$ Analyses

We isolated Eriophorum protein from archived samples and compared its $\Delta^{14}C$ values against atmospheric $CO_2$, because $^{14}C$ differences from atmospheric $CO_2$ indicate some assimilation of soil-derived organic nitrogen. Archived foliage collected at Toolik Field Station in 1986, 1995, and 2000 from the Ecosystems Center, Marine Biological Laboratory (U.S.A.) was used. Isolation of protein and structural carbon followed Hobbie et al. (2013). Ground foliage and the barley standard FIRI-G were initially extracted three times with hexanes (30 min at 70 °C) to remove non-polar compounds, followed by three extractions with 80% ethanol (30 min at 100 °C) to remove soluble polar compounds, mainly carbohydrates. The remaining solid was hydrolyzed once with 6 M hydrochloric acid at 110 °C for 24 h. Hydrolysate was filtered, and soluble compounds were purified to amino acids using cation-exchange resin. The amino acids were eluted and then dried down as the protein fraction. The remaining, insoluble material following acid hydrolysis was extracted at 100 °C for 2 h with 0.2 M sodium hydroxide to remove residual proteins and the remaining solid material was considered the structural fraction. The protein and structural fractions were then sent to the University of Florida, converted to graphite targets, and analyzed for $^{14}C$ at the Keck-Carbon Cycle AMS facility at the University of California at Irvine in 2013.

Estimating Diet Composition from Isotopic Signatures

We used two statistical approaches to examine the controls over dietary patterns of arctic ground squirrels. In one approach, isotopic mixing models developed over the past 15 years were used to partition the three sources of graminoids, dicots, and sporocarps, using isotopic information on hair (Semmens et al., 2009).

Potential food sources were classified into three discrete categories: graminoids, dicots, and fungi. Isotopic signatures for each category were then estimated by averaging potential food sources within each category. The Bayesian mixing model MixSIAR, as implemented within MixSIAR GUI 1.0 (Stock and Semmens, 2013), was used to identify the probability distributions of the contributions of these three food resources in the diet of Toolik and Atigun squirrels. MixSIAR estimates the probability distributions of the contributions of each source to a mixture and includes the uncertainty associated with multiple sources, isotope signatures, and tissue-diet discrimination (Semmens et al., 2009). The estimated median contribution is given for comparative purposes. The input parameters included in the mixing model were the isotopic values of consumers (hair), isotopic values of potential food sources (measured in this study), and tissue-enrichment factors (change in isotope signature from diet to tissue) and their associated standard deviations. Rat feeding trials in Kurle et al. (2014) provided a hair-diet $^{13}C$ enrichment factor of $3.3 \pm 0.4\%o$. This value was nearly identical to the average $^{13}C$ enrichment of 3.2 $\pm$ 0.3‰ for hair relative to diets of five mammalian herbivores (Sponheimer et al., 2003a) and $^{13}C$ enrichment of 3.6 $\pm$ 0.3‰ in bats (Salvarina et al., 2013). We used a value of $3.3 \pm 1.0\%o$ for $^{13}C$ enrichment in our MixSIAR modeling. $^{15}N$ enrichment for hair relative to a diet of $1.9 \pm 0.1\%o$ was calculated for rats from five different diets in Caut et al. (2008) and a value of $3.3 \pm 1.0\%o$ estimated from Sponheimer et al. (2003b), from five mammalian herbivores. We used a value for $^{15}N$ enrichment of $1.9 \pm 1.0\%o$, because the digestive systems of arctic ground squirrels are presumably more similar to those of rats than to the herbivores of the Sponheimer study. The Gelman-Rubin, Heidelberger-Welch, and Geweke diagnostic tests were used to confirm that the model had converged (Hata et al., 2015). Markov Chain Monte Carlo parameters were set as follows: number of chains = 3, chain length = 100,000, burn in = 50,000, thin = 50.

Statistical Analyses on Hair and Feces

In another approach, we used mixed multiple regression models to examine how isotopic patterns in hair or feces correlated with year, season, sex, age class, or chemical composition. Patterns in $\delta^{15}N$ and $\delta^{13}C$ signatures in hair and feces were analyzed using stepwise multiple regression in JMP (SAS Institute, Middleton, Massachusetts, U.S.A.). The $\delta^{15}N_{\text{hair}}$ and $\delta^{13}C_{\text{hair}}$ signatures were analyzed as dependent variables in separate regression models, and each model included sex, age class, year, and site as independent variables. Categories for sex were male and female; censored males and animals of unknown sex were excluded.
from regression analyses. Age classes were adults, yearlings, juveniles, and animals of unknown age. Years were 2007, 2008, or 2011, and sites were Toolik and Atigun.

The carbon and nitrogen isotope signatures of fecal samples were analyzed for 2008 and 2012 using stepwise multiple regressions for \( \delta^{13}N_{\text{feco}} \) and \( \delta^{13}C_{\text{feco}} \). Independent variables for fecal analyses were site, year, date (day of year divided by 100), log_{10} C/N, the interaction of date and site, and either \( \delta^{13}N_{\text{feco}} \) (if \( \delta^{13}C_{\text{feco}} \) was the dependent variable) or \( \delta^{13}C_{\text{feco}} \) (if \( \delta^{13}N_{\text{feco}} \) was the dependent variable). To account for possible non-linear relationships with the date, date was included as up to a third-order polynomial. Because other compound classes such as lipids and carbohydrates are higher in C/N and lower in \( \delta^{13}C \) than protein, their possible influence on \( \delta^{13}C_{\text{feco}} \) was accounted for by including C/N as an explanatory variable in feces. We examined the potential for seasonality in fecal isotopes by including the date of fecal collection as a continuous variable. In stepwise regression analyses, the model formulations that minimized the values of AICc (Akaike Information Criteria with a correction for sample size) were selected (Carleton et al., 2008). In addition, we also compared \( \delta^{13}N, \delta^{13}C, \) and C/N of fecal samples between Atigun and Toolik using \( t \)-tests.

**RESULTS**

**Estimating Fungivory and Herbivory from Stable Isotope Patterns in Hair and Food Sources**

Potential food sources at Toolik and at Atigun were separated into the three categories of fungi, graminoids, and dicots. For each site, stable isotope values within each category were averaged and used in subsequent analyses (Table 1). Isotopic data by taxa on edible fungi are given in Table 1, and they averaged –25.2 ± 0.1‰ for \( \delta^{13}C \) and 4.0 ± 0.1‰ for \( \delta^{15}N \) at Toolik and –25.3 ± 0.1‰ for \( \delta^{13}C \) and 6.4 ± 1.0‰ for \( \delta^{15}N \) at Atigun. Isotopic data for plants by taxa at Toolik separated clearly into graminoid and dicot groupings (Fig. 3); average \( \delta^{13}C \) and \( \delta^{15}N \) values for graminoids were –25.6 ± 0.3‰ and 1.7 ± 0.4‰, respectively, whereas average \( \delta^{13}C \) and \( \delta^{15}N \) values for dicots were –28.2 ± 0.4‰ and –4.3 ± 1.0‰, respectively. Dicots at Atigun included *Arctostaphylos rubra*, *Artemisia sp.*, *Betula nana*, *Dryas octopetala*, *Hedysarum sp.*, *Oxytropis sp.*, *Salix spp.*, *Shepherdia canadensis*, and *Vaccinium uliginosum*. Isotopic data for plants at Atigun are in online Appendix Table A1.

At Toolik, the hair \( \delta^{13}C \) and \( \delta^{15}N \) signatures averaged –23.52 ± 0.07‰ and 4.53 ± 0.13‰ (n = 53), respectively, whereas at Atigun the average signatures were –24.66 ± 0.05‰ and 0.95 ± 0.08‰ (n = 108), respectively. Results for individuals are shown in Figure 4 by site and year. The C:N ratio of hair averaged 2.906 ± 0.007 at Toolik and 2.899 ± 0.006 at Atigun (\( t \)-test, \( P = 0.494 \)).

In regression models, site was the dominant factor controlling both \( \delta^{13}C \) (88% of variance) and \( \delta^{15}N \) (96% of variance), with Toolik higher than Atigun in both parameters. Age class explained 10% of variance in \( \delta^{13}C \), with adults (n = 77) averaging 0.35‰ higher than yearlings (n = 15), juveniles (n = 48), or animals of unknown age (n = 21). Sex explained 1% of variance in \( \delta^{15}N \), with males (n = 84) 0.35‰ higher than females (n = 77). Year explained 2% of \( \delta^{13}N \) variance, with 2011 (n = 68) 0.84‰ higher than 2007 (n = 7) and 0.4‰ higher than 2008 (n = 84) (Table 2). Given the primary importance of site in controlling stable isotope patterns, we grouped all age classes from both sexes when using isotope mixing models to explore diet.

### TABLE 1

Isotopic values of graminoids, dicots, and taxa of edible sporocarps by taxa at Atigun and Toolik. Data are means ± se.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Atigun</th>
<th>Toolik</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \delta^{13}C ) ± se (%)</td>
<td>( \delta^{13}N ) ± se (%)</td>
</tr>
<tr>
<td>Boletaceae</td>
<td>–25.3 ± 0.1</td>
<td>8.7 ± 0.3</td>
</tr>
<tr>
<td><em>Boletus</em></td>
<td>–24.9 ± 0.1</td>
<td>9.8 ± 0.1</td>
</tr>
<tr>
<td><em>Leccinum</em></td>
<td>–25.0 ± 0.2</td>
<td>6.6 ± 2.4</td>
</tr>
<tr>
<td><em>Laccaria</em></td>
<td>–25.2 ± 0.1</td>
<td>2.8 ± 1.9</td>
</tr>
<tr>
<td><em>Lactarius</em></td>
<td>–25.7 ± 0.1</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td><em>Russula</em></td>
<td>–25.6 ± 0.3</td>
<td>5.8 ± 1.3</td>
</tr>
<tr>
<td>average</td>
<td>–25.3 ± 0.1</td>
<td>6.4 ± 1.0</td>
</tr>
<tr>
<td><em>Graminoids</em></td>
<td>–25.8 ± 0.7</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td><em>Dicots</em></td>
<td>–28.2 ± 0.2</td>
<td>–3.8 ± 0.6</td>
</tr>
</tbody>
</table>
Dietary patterns were estimated using the MixSIAR model. Dietary sources were aggregated into three categories of graminoids, dicots, and fungi because the different taxa within these sources clustered in similar isotopic space. Average (±sd) contribution to hair from dicots, graminoids, and fungi was 0.134 ± 0.043, 0.366 ± 0.085, and 0.496 ± 0.065 at Toolik and 0.366 ± 0.046, 0.593 ± 0.056, and 0.041 ± 0.025 at Atigun, respectively (Table 3, complete MixSIAR results are in online Appendix Table A3).

**Radiocarbon in Hair and Eriophorum**

Toolik hair averaged 27% higher in Δ14C than Atigun hair (66.9 ± 6.3‰, n = 12 versus 40.2 ± 3.2‰, n = 7, t-test assuming unequal variance, p = 0.002). Toolik hair averaged somewhat higher than atmospheric CO2 in Δ14C and was more variable (F-test on Δ14C, p = 0.027), whereas Atigun hair was slightly below atmospheric CO2 (Fig. 5). Eriophorum protein was slightly higher in Δ14C than structural carbon, whereas Eriophorum structural carbon resembled atmospheric CO2 in Δ14C (Table 4). Extracts of the barley standard resembled the known bulk values for the barley of 102.5%, with barley protein at 95.9‰ and 98.4‰ and barley structural carbon at 95.9% and 101.0‰.

**Stable Isotope Patterns in Feces**

Means of δ15N, δ13C, and log C/N at the two sites were compared for both 2008 and 2012 in Table 5, and regression models for samples are given in Table 6. In 2008, feces were higher in δ15N at Toolik (n = 3) than at Atigun (n = 31) and were similar in δ13C and in C/N at the two sites. In regression models, location and the cube of the date were significant factors for δ13C, whereas site, year, date, the square of the date, the log C/N, and the interaction of location and date were significant factors for δ15N (Table 6). The positive correlation of date and the square of date, and the interaction of date and the Toolik site reflected increased fecal δ15N toward the end of the growing season at Toolik but not at Atigun (Fig. 6).

The δ15N and δ13C increased in Toolik in the fall. This paralleled a decrease in C/N for Toolik samples but not Atigun samples collected later in the growing season, presumably reflecting a shift in diet at Toolik to lower C/N sources such as fungi.

In a stepwise regression model, site, the cube of date, and the interaction of site and date significantly affected fecal C/N (Table 7; complete regression results in online Appendix Table A5). The cube of date accounted for 72% of explained variance, and location times date accounted for 21%. Atigun C/N was about 2 lower than Toolik C/N.

**Microscopic Analysis of Feces**

We examined 21 fecal samples at Toolik and 22 at Atigun, all from 2012. Collections were equally...
TABLE 2
Stepwise multiple regression analyses of arctic ground squirrel hair δ¹³C and δ¹⁵N as correlated with site, sex, year, and age class. The % variance explained (%Var) for each of the parameters is also given. Adjusted $r^2$ for δ¹³C was 0.570 and adjusted $r^2$ for δ¹⁵N was 0.824 ($P < 0.001$ for both, $n = 161$). Data are means ± se. For age classes, Y = yearling, J = juvenile, A = adult, U = unknown age; for years, 07 = 2007, 08 = 2008, and 11 = 2011. Full regression analyses are given in online Appendix Table A2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%Var</th>
<th>Value ± se</th>
<th>$P$</th>
<th>%Var</th>
<th>Value ± se</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>—</td>
<td>—24.10 ± 0.04</td>
<td>&lt;0.001</td>
<td>—</td>
<td>2.59 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site (Atigun)</td>
<td>88.4</td>
<td>—0.55 ± 0.04</td>
<td>&lt;0.001</td>
<td>96.4</td>
<td>—1.88 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age Class (Y&amp;U&amp;J-A)</td>
<td>10.2</td>
<td>—0.17 ± 0.04</td>
<td>&lt;0.001</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age Class (U&amp;J-Y&amp;A)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.4</td>
<td>0.11 ± 0.07</td>
<td>0.099</td>
</tr>
<tr>
<td>Year (11-08&amp;07)</td>
<td>1.4</td>
<td>—0.07 ± 0.04</td>
<td>0.096</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Year (07-11)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.9</td>
<td>—0.42 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.1</td>
<td>—0.19 ± 0.06</td>
<td>0.004</td>
</tr>
</tbody>
</table>

TABLE 3
Median assimilation by arctic ground squirrels at Toolik versus Atigun with 95% Bayesian credible intervals for dicots, graminoids, and fungi. This was estimated from MixSIAR analyses on δ¹³C and δ¹⁵N values of these three sources. Complete MixSIAR results are in online Appendix Table A3.

<table>
<thead>
<tr>
<th>Diet source</th>
<th>Toolik</th>
<th>Atigun</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicots</td>
<td>0.134 (0.056–0.226)</td>
<td>0.368 (0.275–0.453)</td>
</tr>
<tr>
<td>Graminoids</td>
<td>0.366 (0.207–0.540)</td>
<td>0.593 (0.486–0.701)</td>
</tr>
<tr>
<td>Fungi</td>
<td>0.496 (0.361–0.620)</td>
<td>0.040 (0.001–0.092)</td>
</tr>
</tbody>
</table>

FIGURE 5. Radiocarbon values for arctic ground squirrel hair, with individual Toolik and Atigun samples indicated with a lowercase “t” (6 samples) or “a” (2 samples). Pooled samples from 3 to 4 individuals each are indicated with an uppercase “T” (6 samples) or “A” (5 samples). Northern hemisphere (NH) atmospheric ¹⁴CO₂ levels are also plotted by year, data from Hua et al. (2013).
DISCUSSION

Fecal Isotopes and Diet

Stable isotope ratios in feces reflect recent food consumption and accordingly provide information complementary to the long-term and integrated record provided by ratios in hair (Flaherty et al., 2010). Whereas isotopes in feces reflect the overall recent diet, those in hair primarily reflect dietary protein when the hair grew, because metabolic routing means that much of animal protein is derived directly from dietary amino acids (Hobbie, 2017). The few fecal samples from arctic ground squirrels at Toolik in 2008 were higher in $\delta^{15}N$ than the Atigun samples (Table 5), corroborating our evidence of greater fungal consumption by Toolik animals. The larger data set of 2012 supported this interpretation, because there was a

**TABLE 4**

*Eriophorum* radiocarbon in structural and protein component and estimated ages of those components.

<table>
<thead>
<tr>
<th>Year</th>
<th>Structural $\Delta^{14}C$ ± error (%)</th>
<th>Age (years)</th>
<th>Protein $\Delta^{14}C$ ± error (%)</th>
<th>Age (years)</th>
<th>Atmospheric $\Delta^{14}CO_2$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>215.1 ± 2.3</td>
<td>1–2</td>
<td>235.2 ± 2.7</td>
<td>3</td>
<td>191.1</td>
</tr>
<tr>
<td>1995</td>
<td>110.3 ± 2.5</td>
<td>0</td>
<td>128.7 ± 2.3</td>
<td>1</td>
<td>115.5</td>
</tr>
<tr>
<td>2000</td>
<td>89.8 ± 2.2</td>
<td>0</td>
<td>91 ± 2.3</td>
<td>0</td>
<td>87</td>
</tr>
</tbody>
</table>

**TABLE 5**

$\delta^{15}N$, $\delta^{13}C$, and the log of C/N of arctic ground squirrel feces collected at Atigun and Toolik in 2008 ($n = 31$ and 3, respectively) and 2012 ($n = 51$ and 28, respectively). Samples in 2008 were collected from 24 to 31 August, and samples in 2012 were collected from 9 May to 8 September. The sites were compared using $t$-tests. Data are means ± se.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Year</th>
<th>Atigun</th>
<th>Toolik</th>
<th>$t$-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{15}N$ (%)</td>
<td>2008</td>
<td>0.27 ± 0.23</td>
<td>6.39 ± 0.93</td>
<td>0.017</td>
</tr>
<tr>
<td>$\delta^{13}C$ (%)</td>
<td>2008</td>
<td>−28.04 ± 0.13</td>
<td>−28.07 ± 0.26</td>
<td>0.929</td>
</tr>
<tr>
<td>Log, C/N</td>
<td>2008</td>
<td>2.74 ± 0.04</td>
<td>2.59 ± 0.11</td>
<td>0.292</td>
</tr>
<tr>
<td>$\delta^{15}N$ (%)</td>
<td>2012</td>
<td>0.13 ± 0.12</td>
<td>2.51 ± 0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\delta^{13}C$ (%)</td>
<td>2012</td>
<td>−28.18 ± 0.08</td>
<td>−27.33 ± 0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Log, C/N</td>
<td>2012</td>
<td>2.72 ± 0.05</td>
<td>2.81 ± 0.08</td>
<td>0.351</td>
</tr>
</tbody>
</table>

**TABLE 6**

Effects of site, year, date of sampling, log of C/N of arctic ground squirrel feces collected in 2008 and 2012 ($n = 109$). For $\delta^{13}C$ and $\delta^{15}N$, adjusted $r^2 = 0.281$ and 0.700, respectively, $p < 0.001$ for both. %Var is the % of variance explained by a specific factor. Date unit is 100 days, e.g., July 12 would be 1.94. Stepwise regression tests to minimize AICc are in online Appendix Table A4.

<table>
<thead>
<tr>
<th>Term</th>
<th>%Var</th>
<th>Value ± se (%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>—</td>
<td>−27.75 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site (Atigun)$^a$</td>
<td>81.8</td>
<td>−0.38 ± 0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$^b$(Date − 1.94)$^3$</td>
<td>18.2</td>
<td>−1.33 ± 0.45</td>
<td>0.004</td>
</tr>
<tr>
<td>$\delta^{13}C$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>—</td>
<td>1.82 ± 1.54</td>
<td>0.240</td>
</tr>
<tr>
<td>Site (Atigun)$^a$</td>
<td>69.6</td>
<td>−1.62 ± 0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Year (2008)$^b$</td>
<td>6.3</td>
<td>0.54 ± 0.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Date</td>
<td>6.7</td>
<td>1.51 ± 0.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(Date − 1.94)$^2$</td>
<td>9.9</td>
<td>3.90 ± 0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log, C/N</td>
<td>3.9</td>
<td>−1.21 ± 0.38</td>
<td>0.002</td>
</tr>
<tr>
<td>Site$^a$ × Date$^c$</td>
<td>3.6</td>
<td>−1.00 ± 0.33</td>
<td>0.003</td>
</tr>
</tbody>
</table>

$^a$Atigun would have the value added, Toolik would have the value subtracted.

$^b$Mean of date values was 1.94; the cube of the date was significant for $\delta^{13}C$ and the square of the date was significant for $\delta^{15}N$.

$^c$2008 would have the value added, 2012 would have the value subtracted.
significant interaction of date and site for δ^{15}N (Table 6), and late-season samples from Toolik were generally high in δ^{15}N (Table 6 and Fig. 7), low in C/N (Table 7), and also had abundant fungal spores, unlike early-season samples (online Appendix Table A6). Fungal sporocarps are low in C/N (Hasselquist and Högberg, 2014) relative to arctic plants at Toolik.

Insect parts in some of the feces from both locations indicated that we did not have isotopic data on all potential food sources. Insect consumption has yet to be quantified in arctic ground squirrels, but small percentages of insects by volume have been recorded in related species of ground squirrels such as in the golden-mantled ground squirrel (Callospermophilus lateralis) in coniferous forests (Tevis, 1953; percentages were 1% in spring and fall, rising to 16% in summer) and the desert-dwelling antelope squirrel (Ammospermophilus leucurus) (~10%; Bradley, 1968).

Arthropod consumption was not modeled in MixSIAR because we lacked data on the appropriate isotopic end members for this potential food source. Published work indicated that insects are generally higher in δ^{15}N and δ^{13}C than plants (Ikeda et al., 2010; Hyodo, 2015). However, ongoing work in other biomes indicates that arthropods are intermediate between plants and fungi in isotopic composition (personal communication, R. Stephens), and the dominant dietary component in feces was not a factor in the AICc-selected regression analyses on fecal δ^{15}N, δ^{13}C, and C/N (online Appendix Table A7). Because both arthropods and fungi appear higher in δ^{15}N and δ^{13}C than plants, including arthropods in mixing models would necessarily reduce the estimated dietary contribution of fungi. The maximum volume of animal matter (5%) recorded in stomachs of Toolik

![FIGURE 6. Fecal isotope patterns in 2012 plotted against day of year of collection. Toolik samples are indicated by clear circles and Atigun samples by filled triangles. (A) δ^{15}N; (B) δ^{13}C; (C) C/N.](https://bioone.org/journals/Arctic,-Antarctic,-and-Alpine-Research)

![FIGURE 7. The relative effect of date and site on fecal δ^{15}N at Atigun and Toolik, illustrating the greater seasonality in the δ^{15}N signal at Toolik. The graph was computed by combining the effect on fecal δ^{15}N of site, date, site × date, and date squared as given in the multiple regression analysis in Table 6.](https://bioone.org/journals/Arctic,-Antarctic,-and-Alpine-Research)
Interpreting Stable Isotope Patterns

Several of the independent variables in addition to site correlated significantly with $\delta^{13}C$. In the regression analysis (Table 2), the calculated 0.35‰ $^{13}C$ enrichment of adults relative to other animals presumably reflected the reported $^{13}C$ depletion in milk of nursing mothers relative to the diet caused by the high content of $^{13}C$-depleted milk lipids (Miller et al., 2008).

Hair samples from Toolik were sufficiently high in $\delta^{15}N$ and $\delta^{13}C$ to suggest a relatively large contribution of sporocarps to dietary protein, given the high $\delta^{15}N$ and $\delta^{13}C$ in sporocarps (Table 1) relative to graminoids and dicots (Fig. 3). In contrast, Atigun hair samples appeared to incorporate little fungal protein. These results were supported by the MixSIAR analyses that indicated 30–62% contribution of fungi at Toolik and 0–4% contribution of fungi at Atigun (Table 3).

The half-life of formation of hair carbon in rodents is 40–50 days (Tieszen et al., 1983; Caut et al., 2008), and the carbon of course persists for longer. Arctic ground squirrels molt in the spring and grow underwool in late summer, but it was not possible to determine when hair had molted. Hair sampled in April and May most likely reflected carbon and nitrogen that were assimilated in the prior season from the late summer molt (August–September), with hair sampled from the beginning of July reflecting current year diet from the early molt (May–July). Males molt in late May and June, and females in June and July after they wean their young (Butterworth, 1958), thus some sexual differences in molting time may influence isotopic patterns in hair, such as the small (0.4‰) depletion in $^{15}N$ in females relative to males (Table 2).

Data on stomach and cheek pouch contents of arctic ground squirrels near Atqasuk (70°29′N, 157°25′W) in northern Alaska from Batzli and Sobaski (1980) provide detailed direct measurements of arctic ground squirrel diets for July and August to compare against the current estimates. In their study, herbaceous dicots (47%), seeds (18%), monocots (8.5%), and Equisetum (8%) were the primary food sources. Fungal consumption was not mentioned, but the animal matter (apparently insects) contribution to stomach contents was 4.9% in May–June, 0.4% in July, and 1.3% in August. In parallel choice experiments of individual plant taxa, highest average consumption of offered foods was for Equisetum (75%, one taxon), followed by forbs (62 ± 22%, 34 species), deciduous shrubs (46 ± 17%, 8 species), monocots (17 ± 16%, 10 species), and evergreen shrubs (13 ± 11%, 5 species) (values ± sd) (Batzli and Sobaski, 1980). The Atqasuk site was similar in some respects to Atigun, with most burrows located in sandy areas or river bluffs, and the species compositions of forbs and grasses indicate non-acidic tundra at both sites (personal communication, Donald Walker). However, the diet estimates from stomach contents (Atqasuk) and from isotopes in hair (Atigun) are rather different, but that is hardly surprising given probable wide differences among plants in protein content as well as inherent site differences between the northern Alaskan coastal plain (Atqasuk) and the Brooks Range foothills (Atigun).

Interpreting Radiocarbon Results

Toolik hair averaged 27‰ higher in $\Delta^{14}C$ than Atigun hair, indicating that Toolik squirrels incorporated older carbon than Atigun squirrels. We interpret this difference to reflect the incorporation into food sources of old, soil-derived amino acids that were first synthesized when $^{14}C$ content of the atmosphere was considerably higher. Because arctic ground squirrels...
primarily consume plant matter (Batzli and Sobaski, 1980), we considered the possibility that protein from non-mycorrhizal plants may themselves preserve a 14C signal of soil organic nitrogen. The limited data on Eriophorum radiocarbon indicated that protein carbon of some plants can be slightly older than structural carbon, by 0–3 years (Table 4). However, Eriophorum is not a significant dietary source for soil-derived organic nitrogen, as these ages are insufficient to account for the 14C enrichments seen in arctic ground squirrels at Toolik. This indicated that Toolik squirrels incorporated some old organic nitrogen into hair, presumably via fungi, and that the low 14C levels in Atigun squirrels reflected little fungal consumption. These results pointed to the probable persistence of organic nitrogen compounds in the soil, their incorporation into ectomycorrhizal fungi (Tibbett et al., 1998; Clemmensen et al., 2008; Hobbie et al., 2013), and the subsequent assimilation of that organic nitrogen by fungivorous mammals.

**Implications**

The large differences in isotope values between the two arctic ground squirrel locations analyzed in this study indicate that the two populations choose food based on availability. This feeding strategy should allow arctic ground squirrels to adapt to changing conditions despite the fact that forbs, one of their important food sources (Hollowaychuk et al., 1966; Batzli and Sobaski, 1980), are expected to decrease across the Arctic with climate change (Chapin et al., 1995). However, the current effects of climate change specifically at Toolik show a warming in the fall but later snowmelt in the spring (Cherry et al., 2014). Expanding ectomycorrhizal shrub habitat in the Arctic (Myers-Smith et al., 2011) and at Toolik may increase arctic ground squirrel fungivory as ectomycorrhizal sporocarps become more common.

Ectomycorrhizal plants at Toolik and Atigun could provide sugars for the 13C-enriched ectomycorrhizal sporocarps that may be consumed by arctic ground squirrels. However, the proportion of ectomycorrhizal plants appeared to differ; heath, shrub, and tussock vegetation types at Toolik had more *Betula* and less *Salix* (Shaver and Chapin, 1991), whereas vegetational quadrats at Atigun indicated between 1% and 20% *Dryas*, 4% and 16% *Salix*, 1% and 9% *Arctostaphylos*, and <1% *Betula* (Shamhart, 2010). The lesser proportion of *Betula* may translate into less sporocarp production at Atigun than at Toolik, although the substrate itself (glacial till at Toolik, dune sand at Atigun) may be the controlling factor. No data are available on the relative sporocarp productivity of *Betula nana* systems versus *Salix* spp. systems in the Arctic, or on the influence of substrate on sporocarp productivity. However, ectomycorrhizal sporocarp productivity decreases with increasing pH in forest communities (Rao et al., 1997; Högberg et al., 2007), and soil pH at Atigun should be higher than at Toolik because of the contribution of eroding limestone to Atigun (Walker et al., 1981). The proportion of fungi in diets of arctic ground squirrels probably integrates sporocarp productivity. Protein assimilation from sporocarps may be particularly important because hibernation in arctic ground squirrels requires gluconeogenesis from lean muscle mass, unlike in other squirrel hibernators (Boonstra et al., 2011). Thus, protein demand is likely higher than in other squirrel species. The clear elevation of Δ14C signatures of arctic ground squirrels at Toolik relative to Atigun indicated that ectomycorrhizal fungi mobilized and assimilated considerable soil organic nitrogen that could then be transferred to animals as amino acids. Arctic ground squirrels are effectively functioning then as integrators of the organic nitrogen cycled through ectomycorrhizal fungi, and may prove useful in detecting if soil warming in the Arctic is increasing the mobilization of soil-derived nitrogen.

**Data Accessibility**

Data will be archived at the website of the Arctic Long-Term Ecological Research site at http://ecosystems.mbl.edu/ARC/datacatalog.html, and will be searchable by subject (title, abstract, or keywords), owner, spatial criteria, or taxonomic criteria.

The following appendices are available online for this article: Table A1 (isotopic values of plant food items at the Atigun site from samples collected in 2007 and 2008); Table A2 (stepwise multiple regressions of hair δ13C and δ15N); Table A3 (MixSIAR results for dietary estimates of consumption by arctic ground squirrels of dicots, graminoids, and fungi at Toolik and Atigun); Table A4 (stepwise multiple regression of fecal δ13C and δ15N); Table A5 (stepwise multiple regressions of fecal C/N); Table A6 (fungal taxa recorded and other observations in fecal samples from Atigun and Toolik); and Table A7 (fecal δ13C, δ15N, and C/N for samples that also had information on dietary components from microscopy).

**Acknowledgments**

We thank Rebecca Rowe, Ryan Stephens, Andy Maguire, and several anonymous reviewers for comments on previous versions. This study was supported by the NSF LTER network and grants from U.S. National Science Foundation OPP-1108074 and IOS-1147232.
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*MS submitted 26 September 2016
MS accepted 12 June 2017*