Altitudinal Variation in Mountain Gorilla (*Gorilla beringei beringei*) Plant Food Nutritional Chemistry

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Dedication

The author wishes to dedicate this thesis to all the people who helped in her meandering journey through academia. First and foremost, Dr. Shannon McFarlin, who provided not only academic support, but unwavering and unconditional care and concern throughout the last eight years. Next, to Lawrence Fatica, the author's bluntly authentic confidant throughout the oscillating trials and tribulations of graduate school. And finally, to the author's family, especially her spouse, Mark Flint, who willingly chose to join the circus.

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Abstract of Thesis

Altitudinal Variation in Mountain Gorilla (*Gorilla beringei beringei*) Plant Food Nutritional Chemistry

Virunga mountain gorillas (*Gorilla beringei beringei*) live at an ecological extreme compared to other great apes. Ranging at altitudes of ~2,330 to greater than 4,000 meters, they encounter variable vegetation zones characterized by differences in habitat and food availability, which may affect their feeding behavior and the nutritional composition of favored foods. To better understand the dietary nutritional chemistry of a subpopulation of mountain gorillas, fifteen food parts from nine different plant species in Volcanoes National Park (VNP), Rwanda were analyzed for mineral content (ash), crude protein (CP), fiber, crude fat, and gross energy (GE). Three foods present throughout the mountain gorillas' range (*Galium*, *Rubus*, and *Peucedanum spp.*) were collected at every 100-meter altitude increase up the slope of Mt. Visoke (range: 2860 – 3660 meters) to assess altitudinal variation in nutritional chemistry. Foods contain 6 to 31% CP, 22 to 55% neutral detergent fiber (NDF), 13 to 43% acid detergent fiber (ADF), 0 to 3% fat, and 4 to 26% ash content on a dry matter basis. Results reveal strong and opposite altitudinal relationships for GE (r_s = 0.625, p < 0.05) and ash content (r_s = -0.507, p < 0.05). For *Peucedanum* stems, the increase in GE with altitude is greater than what would be expected from the increase in organic matter due to the decrease in ash content, suggesting that there is a macronutrient composition change with altitude. A principal components analysis suggests that nutritional variation is primarily driven by significant differences in both GE (Z = 3.696, p < 0.05) and CP (Z = 3.127, p < 0.05) between leaves and stems, and a negative relationship between gross energy and mineral content (r_s = -0.681, p < 0.05). Given that some mountain gorillas have

been shown to prioritize consuming non-protein energy, these results suggest that the subpopulation of Virunga mountain gorillas in this study may target relatively energy-rich foods at higher altitudes.

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List of Symbols and Nomenclature

1. VNP Volcanoes National Park in Rwanda, the collection site in this study
2. CP Crude protein, the measure of all sources of nitrogen
3. GE Gross energy, the energy released as heat when a sample is combusted
4. A Weight of remaining ash in food sample
5. S Beginning sample weight prior to heating for mineral analysis
6. W_1 Original weight of the sample prior to crude fat analysis
7. W_2 Dried sample weight during crude fat analysis
8. W_3 Weight of dried sample and filter bag post-processing for crude fat
9. EE Energy equivalent or the heat of combustion of a biological sample
10. ΔT The change in temperature after sample combustion

Chapter 1: Introduction

The application of nutritional chemistry to primate foraging ecology provides insights into food choices and feeding behavior, which itself can influence social behavior, social organization, species abundance, and population distributions (Crook and Gartlan 1966; Altmann 1974; Wrangham 1979, 1987; van Schaik 1989; Oates et al. 1990; Byrne et al. 1993; Chapman et al. 2002). Primates range over a variety of different landscapes, where subtle variations in sunlight, soil composition, tree phenology, and microbial activity may significantly impact the macro- and micronutrients of plant foods (Chapman et al. 2003). To better develop standardized protocols for collecting nutritional ecology data, it is necessary to understand along what dimensions plant chemistry varies (Chapman et al. 2003). Unfortunately, difficulties in collecting and analyzing samples in remote tropical field sites has limited the number of nutritional studies aimed at determining the chemical variability of most preferred primate foods along natural ecological gradients. This study aims to begin to fill this gap in dietary research for one subspecies of endangered primate, the mountain gorilla, by analyzing a subpopulation's primary foods along an altitudinal gradient.

Chapter 2: Literature Review

Mountain gorillas (*Gorilla beringei beringei*) live at relatively high altitudes compared to other great ape species and subsist almost entirely on herbaceous vegetation (Vedder 1984; Watts 1984, 1985; Ganas et al. 2004, 2009; Rothman et al. 2006, 2007, 2014; Grueter et al. 2013). Compared to western lowland gorillas (*Gorilla gorilla*) and grauer gorillas (*Gorilla b. graueri*), mountain gorillas eat significantly less fruit and have lower dietary diversity (Doran and McNeilage 1998, 2001; McNeilage 2001; Doran et al. 2002; Rogers et al. 2004). However, they can have highly variable dietary patterns that correlate with geographic and altitudinal ranges (Ganas et al. 2004; 2009). The two mountain gorilla populations today live in Bwindi Impenetrable National Park (BINP) in Uganda and on the Virunga massif across Rwanda, Uganda, and the Democratic Republic of Congo. In contrast to BINP, the Virunga mountain gorilla's range extends to higher altitudes, has lower mean annual temperatures, less access to fruit, and lower plant diversity as is expected with increasing altitude (Butynski 1984; Watts 1984; Watts 1985; Goldsmith 2003; Nkurunungi et al. 2004; Robbins and McNeilage 2003; Sarmiento et al. 1996; Rothman et al. 2014). Previous research suggests that despite these differences in habitat and plant species, these two populations consume similar nutrient concentrations (Rothman et al. 2007; Rothman et al. 2011; Raubenheimer et al. 2015). Within VNP, eight distinct vegetation zones are distributed across the large altitudinal gradient over which Virunga mountain gorillas range, recognized by differences in local plant species composition associated with altitude (Schaller 1963; Fossey 1974; Fossey and Harcourt 1977; Plumptre 1994; Watts 1984). However, altitudinal variation in the plant nutritional chemistry of this population of mountain gorilla's most preferred foods has not been fully characterized.

Comparisons between tropical and montane primate foods suggest that nutritional quality can vary over altitude and is therefore an important aspect to consider when analyzing the nutritional chemistry of primate diets (Waterman et al. 1983; Friend et al. 1989; Körner 1989; Hikosaka et al. 2002; Rothman et al. 2014).

As one of the most informative environmental gradients, altitude can serve as a natural ecological experiment for testing evolutionary responses of both plants and animals. In high-altitude mountain environments for example, major factors influencing the distribution and productivity of organisms include wind, sun radiation, soil, and air temperature, as well as humidity and evaporation (Tranquillini 1964, Sakai and Larcher 1987, Körner 2007; Sharma et al. 2009). Temperatures at different altitudes significantly alter not only the nutritional chemistry of plant foods, but also the metabolism of animal species (Peters and Peters 1986; Hodkinson 2005). Endothermic organisms that live at high altitudes with low temperatures can reach a temperature threshold that triggers metabolic heat production, thus decreasing energy put towards foraging and digestion (Harvey and Clutton-Brock 1981; Peters and Peters 1986). Assessing the nutrient composition of foods across the entire altitudinal range of an animal enables researchers to understand whether comparable nutrition at higher altitudes can be maintained with less foraging effort (Milton and May 1976; Byrne et al. 1993). This study is the first step in that direction. Specifically, it aims to further investigate variability in the nutritional chemistry of the foods most preferred by a subpopulation of Virunga mountain gorillas over their altitudinal range.

Chapter 3: Materials and Methods

Study Site

Volcanoes National Park (160 km^2) is in northwestern Rwanda (1°30′S, 29°30E), and encompasses five of the eight volcanoes that make up the Virunga Massif of the Albertine Rift, the western branch of the East African Rift. The park comprises several vegetation zones defined by the physical geography of the area and the presence or absence of specific plant species (Fossey 1974; McNeilage 2001). Mt. Visoke is a volcano with a crater lake which straddles the border of northwestern Rwanda and the eastern edge of the Democratic Republic of Congo. It reaches 3,711 meters, a height at which mountain gorillas routinely climb when foraging for food. Plants analyzed in this study were sampled from 2,630 – 3,661 meters above sea level on the slopes of Mt. Visoke within VNP in Rwanda.

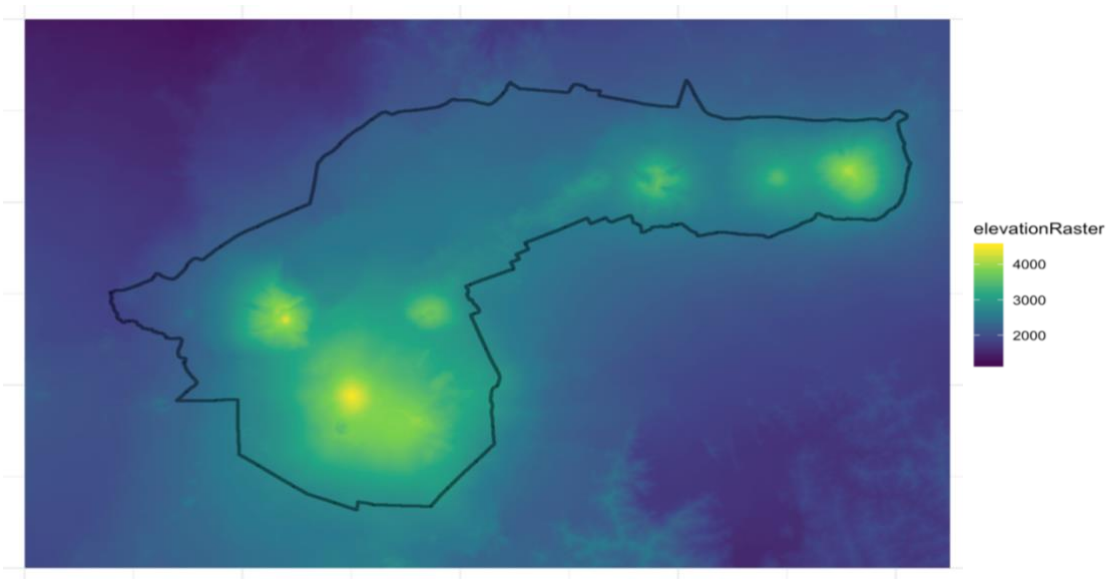

Figure 1: Volcanoes National Park, Rwanda elevation map. The parks boundaries are delineated by the black outline. Mt Visoke is the high elevation spot most right of the three southern points.

Field Methods

Fifteen different parts of nine plant species, as measured by proportional intake values by a Virunga mountain gorilla subpopulation (Watts, 1984; long-term records), were collected at the ranges and areas that individuals consumed them. These species make up approximately 90% of diet for Karisoke-monitored social groups in VNP (proportional wet weight intake for Group 4 and 5 as reported by Watts 1984; percentage of time spent foraging for Karisoke-monitored groups as reported by Grueter et al. 2013). Plant specimens (Table 1) were chosen specifically to maximize the overall percent of foods eaten by both Groups 4 and 5 as measured by proportional intake values, not necessarily to incorporate the top dietary items, as these change for different Karisoke-monitored groups.

These specific species and plat parts were opportunistically sampled by field staff of the Dian Fossey Gorilla Fund International Karisoke Research Center from the same areas that mountain gorilla groups foraged but were not necessarily from the same individual plant the gorillas were feeding on at the time. These samples were collected from the slopes of Mt. Visoke, covering an altitudinal range of 2360 to 3661 meters and within each plant's known altitudinal distribution. Additionally, *Galium spp., R. kirungensis,* and *P. linderi* were collected, when possible, at every 100-meter altitude increase up the southeastern slope of the Mt. Visoke trail leading up to the Lac de Cretère (range: 2860 m – 3660 m). These three genera were targeted because they have the largest altitudinal range of the plants chosen for this study. For each sample, around 500 grams of wet weight were placed in clean, sealed, and labeled bags and weighed to 0.01 grams within 24 hours of collection. Items were processed the same way gorillas consume them (e.g., leaves peeled from the stem, etc.) so that food samples consisted of only the portion of the plant normally ingested. Samples were dried quickly to halt plant enzymatic activities (55

– 60º C) in a portable temperature-controlled food dehydrator (Sedona Dehydrator, Tribest Corporation, Anaheim, CA). When this was not the case, samples were immediately frozen in a - 20º C freezer until they could be thawed and dried. Freezing samples prior to drying is not expected to influence results as all nutritional assays are based on dry matter and samples were re-dried prior to analyses in the laboratory.

Table 1: Number (N) of individual samples collected for plant species and parts. For example, an N of 2 means two plants were sampled for that part at that altitude or altitudinal range. Nutritional assays run for each species part are indicated by A (ash content), ADF (acid detergent fiber), NDF (neutral detergent fiber), CP (crude protein), F (fat), and gross energy (GE). An Asterix (*) denotes that at least one sample of this plant part was analyzed, but not all samples of that plant part were run for that nutritional assay due to limitations in dry matter.

Genus	Species	Family	Part	N	Altitude (m)	Nutritional Assays
Carduus	*nyassanus*	Asteraceae	Flowers	2	3268	A, ADF, NDF, CP, F, GE
Carduus	*nyassanus*	Asteraceae	Leaves	2	3268	A, ADF, NDF, CP, F, GE
Carduus	*nyassanus*	Asteraceae	Roots	2	3268	A, ADF, NDF, CP, F, GE
Carduus	*nyassanus*	Asteraceae	Stems	1	3268	A, ADF, NDF, CP, F, GE
Galium	*spp.*	Rubiaceae	Leaves, Stems	9	2860 - 3661	A*, CP, ADF*, NDF*, F*, GE
Helichryssum	*formossissimum*	Asteraceae	Flowers	1	3556	A, ADF, NDF, CP, F, GE
Helichryssum	*formossissimum*	Asteraceae	Leaves	1	3556	A, ADF, NDF, CP, F, GE
Laportea	*alatipes*	Urticaceae	Leaves	1	NA	A, ADF, NDF, CP, F, GE
Laportea	*alatipes*	Urticaceae	Stems	1	NA	A, ADF, NDF, CP, F, GE

Peucedanum	*spp.*	Apiaceae	Leaves	4	3360 - 3661	A*, CP, GE
Peucedanum	*spp.*	Apiaceae	Stems	10	2960 - 3661	A*, CP, ADF*, NDF*, F*, GE
Rubus	*spp.*	Rosaceae	Leaves	7	3160 - 3661	A, CP, ADF*, NDF*, F*, GE
Urtica	*massaica*	Urticaceae	Stems	1	2360	A, ADF, NDF, CP, F, GE
Vernonia	*adolfi-fredericii*	Asteraceae	Flowers	1	2975	A, ADF, NDF, CP, F, GE
Vernonia	*adolfi-fredericii*	Asteraceae	Young Flowers	1	2975	A, ADF, NDF, CP, F, GE
Vernonia	*adolfi-fredericii*	Asteraceae	Pith	1	2975	ADF, NDF, CP, GE
Yushania	*alpina*	Poaceae	Shoots	2	3007	A, ADF, NDF, CP, F, GE

Analytical Techniques

Chemical analyses of the food samples were carried out in the Nutrition Laboratory at the Smithsonian National Zoological Park and Conservation Biology Institute in Washington, DC. Dried samples from the field were ground to pass through a 1-mm mesh screen using a Wiley Mill® (Arthur H. Thomas Co., Philadelphia, PA). Due to restrictions on the amount of sample available for different analyses, not all samples were assayed for every nutritional component. Care was taken to make sure each species and plant part was run for fat, fiber, gross energy, crude protein, and ash content at least once, but most samples along the altitudinal gradient were not tested for fiber or fat due to limited dry mass.

We used a PE2400 Series II CHNS/O Elemental Analyzer (PerkinElmer, Waltham, MA) to assess crude protein by determining the percent nitrogen on a dry matter basis by means of the Dumas method of molecular weight determination (Anderson and Shubin 1957). Nitrogen measurements are used as a proxy for crude protein using the conversion factor of 6.25 (Kjeldahl 1883**)**.

We measured acid detergent fiber (ADF) and neutral detergent fiber (NDF) using an ANKOM^{200} Fiber Analyzer (ANKOM, Macedon, NY) according to methods outlined by van Soest and McQueen (1973) and modified by Goering et al. (1973). NDF is defined as the residue remaining of a sample after boiling it in a neutral pH detergent solution. These fiber residues are understood to mainly contain hemicellulose, cellulose, and lignin. ADF is the residue remaining after boiling with an acidic detergent solution and is predominantly cellulose and lignin.

We evaluated total minerals in a sample by measuring the ash content on a dry matter basis. We used a Thermolyne F6018 Furnace (Hogentogler, Columbia, MD) to heat samples to 550º C for five hours. After the furnace cooled, the weight of remaining ash (*A*) was measured and compared to the beginning sample dried weight (*S*). Percent ash content on a dry matter basis was calculated with the equation:

$$\% \, Ash = \frac{A}{S} \text{ x } 100$$

We measured the percentage of crude fat in samples using an ANKOM^{XT15} fat extractor (ANKOM, Macedon, NY). Samples were weighed and heat sealed in filter bags then dried at 100º C for 3 hours. They were then repeatedly submerged in petroleum ether at a maximum temperature of 90º C to extract triglycerides and other petroleum ether soluble lipids. The sample was then dried again and weighed. Where W_1 is the original

dried weight of the sample from the field, W_2 is the dried sample weight after 3 hours at 100° C, and W_3 is the weight of the dried sample and filter bag post-processing in petroleum ether, the following equation is used to calculate the crude fiber percentage.

$$\% \, Crude \, Fiber = \frac{W_2 - W_3}{W_1} \text{X } 100$$

Finally, we assessed the gross energy of samples with a Parr 6200 isoperibol oxygen bomb calorimeter (Parr Instrument Company, Moline, Illinois). Using a standard material (benzoic acid) to determine the energy equivalent of the bomb (EE), the heat of combustion of a biological sample can be calculated by multiplying is the change in temperature after combustion (ΔT) by EE and dividing by the sample mass.

Statistical Analyses

All statistical analyses were conducted in R version 3.3.1 (R Core Team, Vienna, Austria) and RStudio version 1.0.143 (RStudio Team, Boston, MA). This study reports ranges, variances, standard deviations, and coefficients of variation for ash content, crude fat, NDF, ADF, CP, and GE of the Virunga mountain gorilla foods collected. The nutritional chemistry between different plant parts was analyzed using non-parametric Mann-Whitney U tests within and across species. Differences in nutritional components between species and parts were compared using one-way ANOVAs and Tukey Honest Significant Differences (HSD) multiple pair-wise comparisons. To analyze variation in nutritional chemistry over altitude for *Peucedanum***,** *Rubus*, and *Galium* only non-parametric tests were used since the data could not be normally transformed. Thus, the relationships between altitude and nutritional components were analyzed using the Spearman's rank correlation coefficient. A Principal Components Analysis (PCA) was

then performed to visualize the maximum amount of variation in the sample and better understand how correlated nutritional components affect this variation in terms of linearly uncorrelated variables known as principal components. Due to sample size limitations, only GE, CP, and ash content were included in the PCA. An exploratory linear regression analysis of *Peucedanum* stem ash weight and GE was also conducted to determine whether the significant relationships between these two parameters and altitude were being driven by the connection between GE and mineral content. Since minerals provide no energy, a decrease in GE is expected with an increasing ash content percentage. The linear regression of ash weight as a predictor of gross energy was a necessary analysis to explore the contribution of organic matter, such as carbohydrates, protein, and fat, to changes in GE.

Chapter 4: Results

Virguna Mountain Gorilla Nutritional Chemistry

Fifteen plant parts from nine species of plant foods (Table 1, N = 48) were analyzed for ash content, crude protein, neutral detergent fiber, acid detergent fiber, crude fat, and gross energy. Foods contained 6 to 31% crude protein, 22 to 55% neutral detergent fiber, 13 to 43% acid detergent fiber, 0 to 3% crude fat, and 4 to 26% ash content on a dry matter basis. Gross energy ranged from 3.339 to 4.686 kcal GE/g. Table 2 reports sample sizes, ranges, variances, standard deviations, and coefficients of variation across all studied food parts. The total percentage of dry matter identified by nutritional analysis ranged from 13.32%, when only crude protein was measured due to sample size restrictions, to 93.22% when ash content, CP, ADF, NDF, crude fat, and GE were all assayed. On a dry matter basis, when all six macronutrients were analyzed (N = 20), the sum of these constituents ranged from 51.26 - 92.23%, suggesting that there are more components, such as tannins and nonstructural carbohydrates (e.g., pectin and oligosaccharides) contributing to the plants' nutritional chemistries that were not measured in this study.

Table 2: Sample summary statistics

Macronutrient	**N**	**Range**	**Mean ± SD**	**Variance**	**CV**
GE (Kcal GE/g)	48	3.339 – 4.686	4.08 ± 3.79	143445.3	0.09
Ash Content (%)	40	3.62 - 25.94%	12.16 ± 5.89%	0.0035	1.44e-05
Crude Protein (%)	48	6.32 - 30.94%	17.26 ± 6.44%	0.0041	1.58e-05
Fat (%)	21	-0.4 - 3.0%	0.81 ± 1.08%	0.0001	2.64e-06
ADF (%)	22	12.55 - 42.99%	30.78 ± 9.71%	0.0094	2.38e-05
NDF (%)	22	22.32 - 54.74%	42.68 ± 8.96%	0.008	2.19e-05

Multiple one-way analyses of variance were performed to analyze the nutritional chemistry between genera. Only GE ($F(8,39) = 2.16$, $p = 0.05$) and ash content ($F(8,31) = 3.376$, $p = 0.006$) differed significantly between plants at the genus level (Fig. 2a and Fig. 2b). Subsequent Tukey HSD comparisons revealed significantly higher gross energy for

Rubus compared to *Peucedanum* (p = 0.012) with the difference between *Rubus* and *Galium* approaching significance (p = 0.053; Fig. 2a). Post-hoc tests also showed that ash content was significantly different between *Helichryssum* and *Carduus* (p = 0.024), *Rubus* (p = 0.007), and *Vernonia* (p = 0.021).

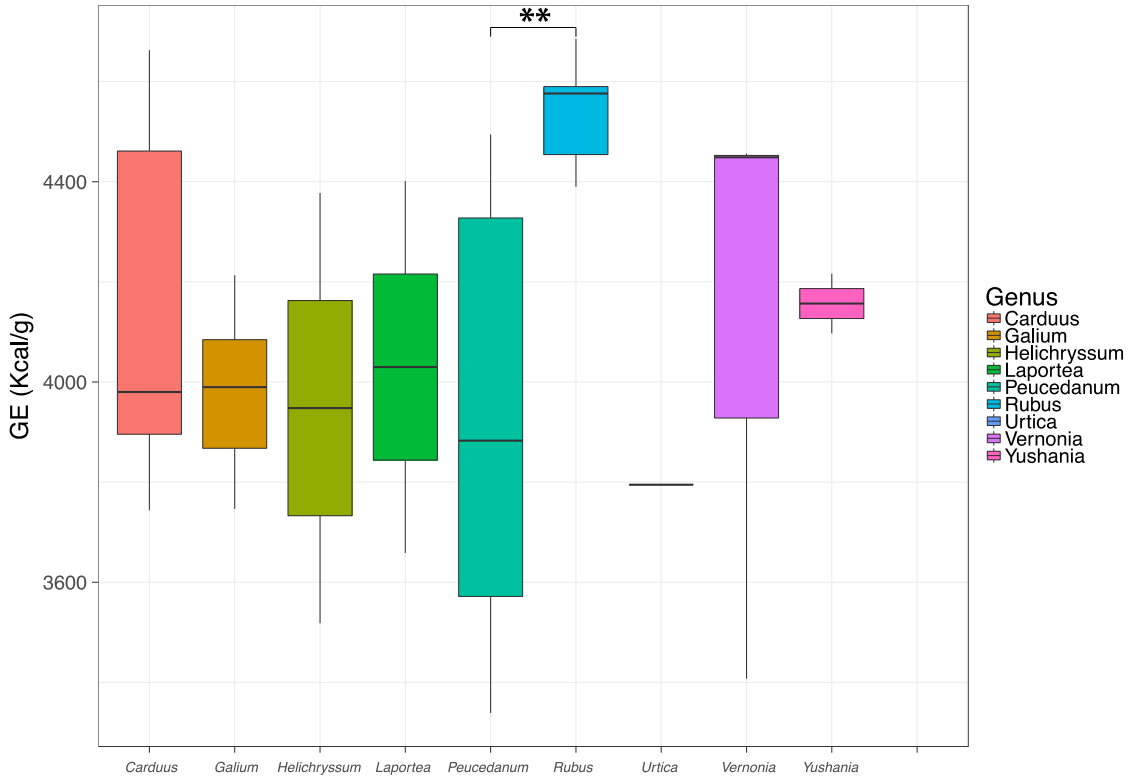

Figure 2a: GE (Kcal/g) by genus showing significant differences between *Rubus* and *Peucedanum* (p = 0.012) with *Rubus* and *Galium* approaching significance (p = 0.053). These boxplots report minimum, quartile one, median, quartile three, and maximum values.

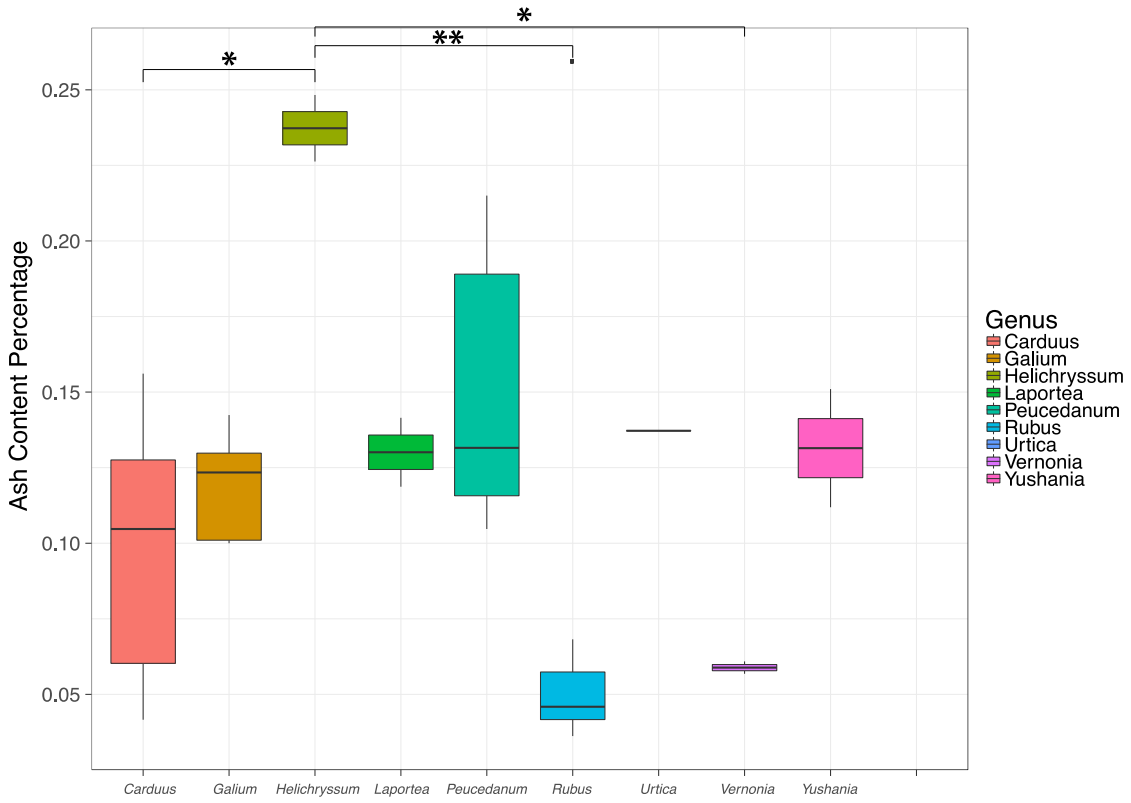

Figure 2b: Ash content percentage by genus showing significant differences between *Helichryssium* and *Carduus* ($p = 0.024$), *Rubus* and *Helichryssium* ($p = 0.007$), and *Helichryssium* and *Vernonia* ($p = 0.021$). These boxplots report minimum, quartile one, median, quartile three, and maximum values.

Nutritional Contribution of Different Plant Parts

Plant parts were significantly different in their nutritional chemistry profiles (Fig. 3a and 3 b). Mann-Whitney U tests indicated that leaves were significantly higher than stems in GE ($Z = 3.6955$, $p < 0.05$) and CP ($Z = 3.1269$, $p < 0.05$), but not ash content ($Z = -1.5689$, $p = 0.12$), NDF ($Z = -1.3887$, $p = 0.16$), ADF ($Z = -1.3887$, $p = 0.16$) or fat ($Z = 1.1742$, $p = 0.24$). A principal components analysis of *Galium*, *Rubus*, and *Peucedanum* plant parts shows that the first principal component (PC1) encompasses the most separation between plant parts representing 73.17% of variation (Fig. 4). Loadings on the principal components analysis illustrate that spacing on PC1 is primarily driven by differences in

GE and ash content (Fig. 4, Table 3a and 3b). However, these conclusions are restricted as only GE, ash content, and CP were included in the principal components analysis due to limitations in dry matter sample size.

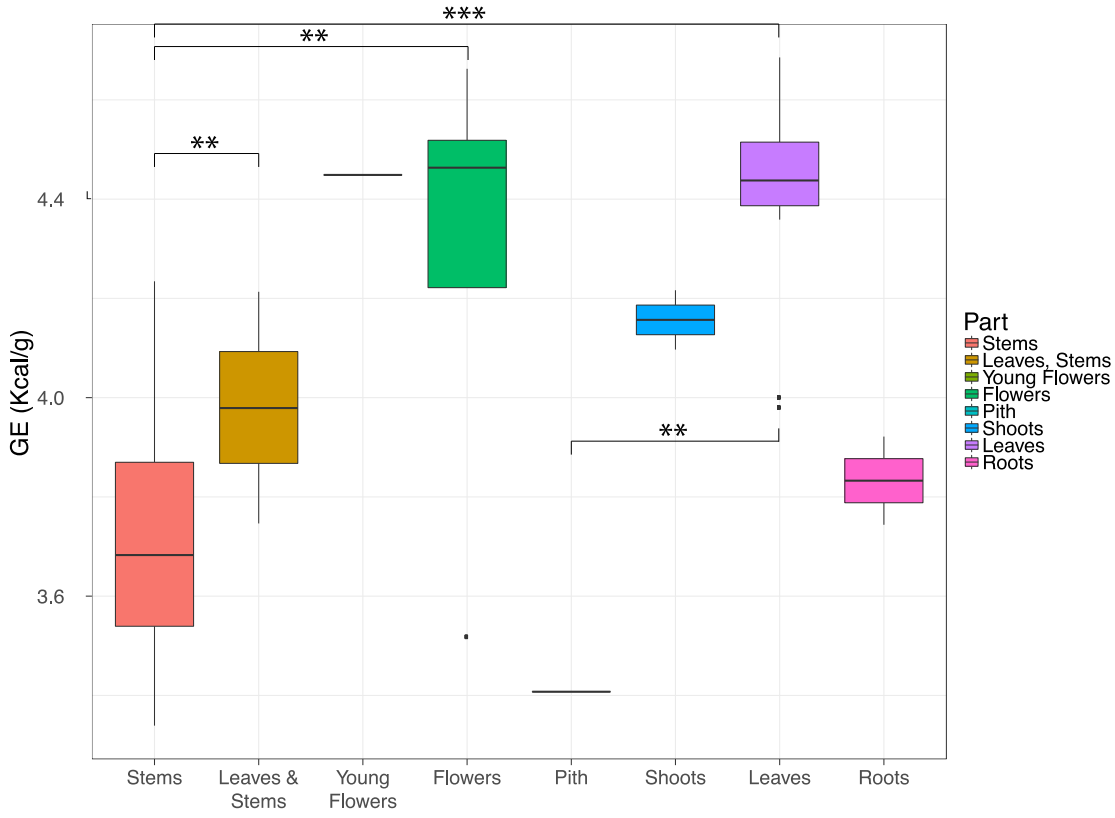

Figure 3a: GE (Kcal/g) by plant part showing significant differences between stems and leaves/stems (p = 0.003), flowers (p = 0.007), leaves (p = 0.0000001) as well as between leaves and pith (p = 0.006). These boxplots report minimum, quartile one, median, quartile three, and maximum values.

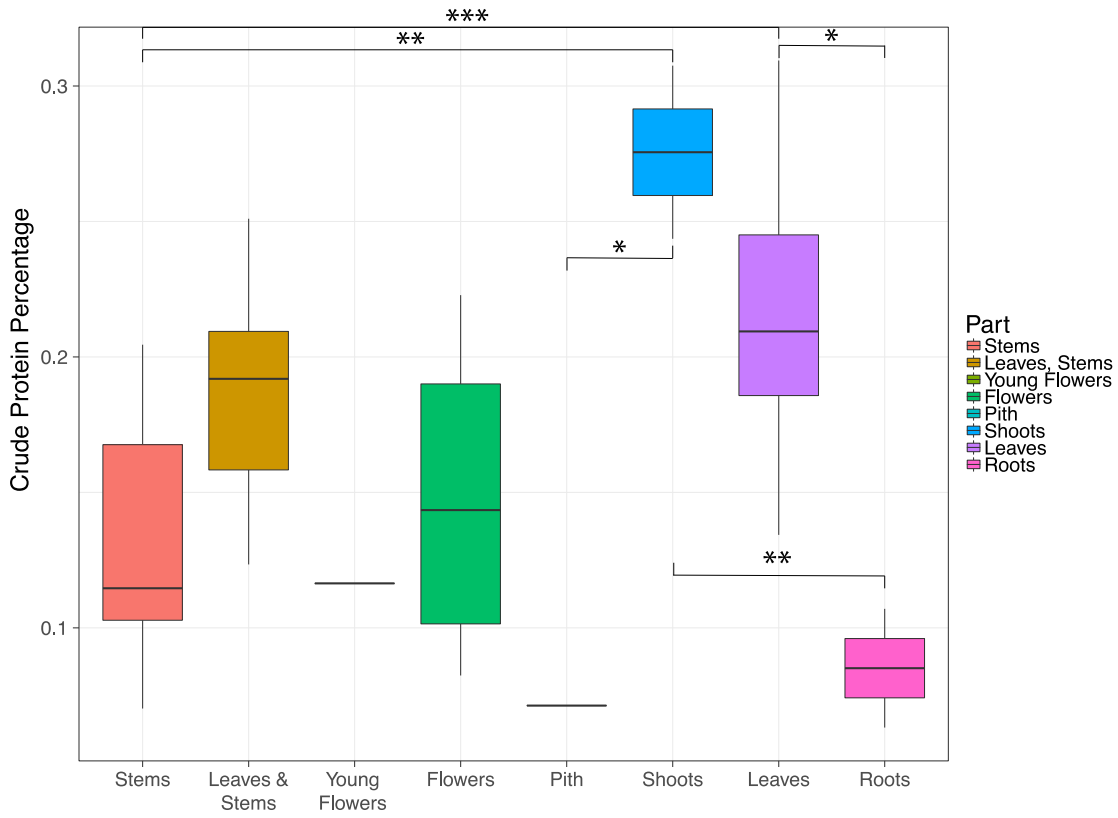

Figure 3b: Crude protein percentage by plant part showing significant differences between stems and leaves (p = 0.001), as well as shoots and stems (p = 0.006), pith (p = 0.022), and roots (p = 0.006) and between roots and leaves (p = 0.016). These boxplots report minimum, quartile one, median, quartile three, and maximum values.

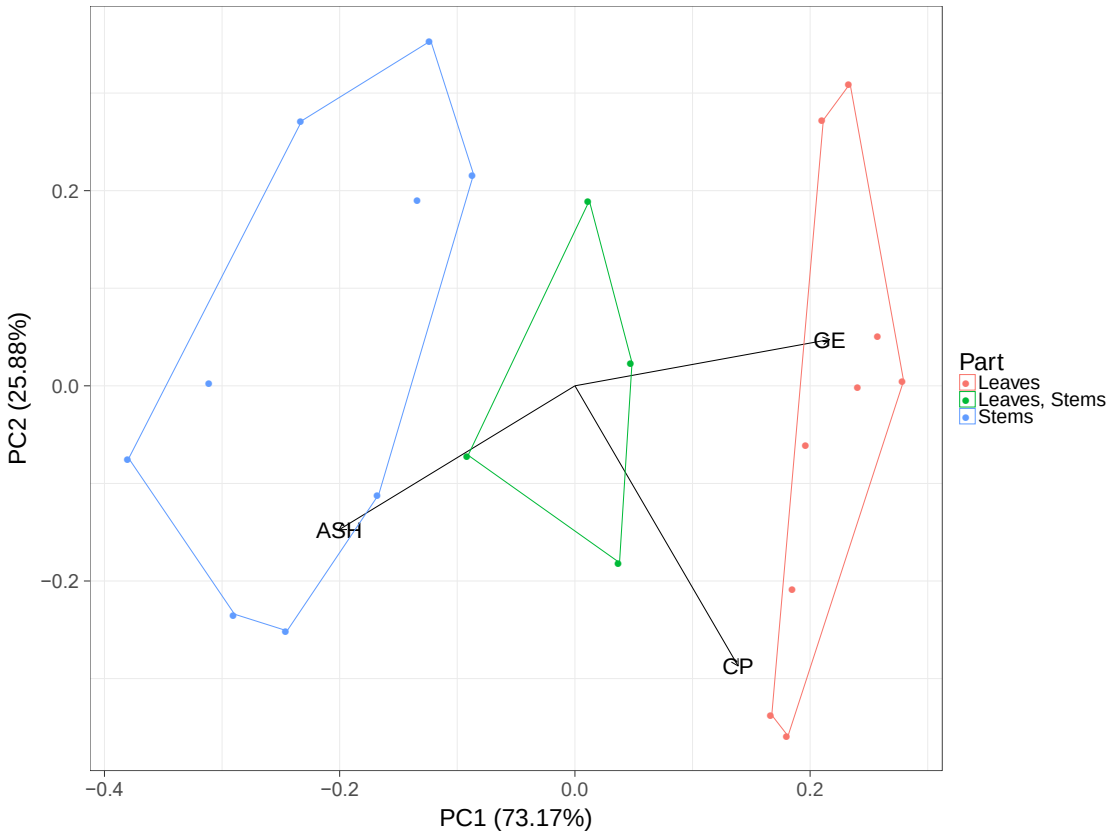

Figure 4: Principal components analysis with crude protein, ash content, and gross energy as input variables, showing the separation of plant parts.

Table 3a: PCA output

	PC1	PC2	PC3
Standard Deviation	1.4816	0.8811	0.16905
Proportion of Variance	0.7317	0.2588	0.00953
Cumulative Proportion	0.7317	0.9905	1.00000

Table 3b: PCA loadings

	PC1	PC2	PC3
Gross Energy	-0.6641334	0.1454763	-0.7333235
Crude Protein	-0.4255314	-0.8800495	0.2107982
Ash Content	0.6146949	-0.4520503	-0.6463751

Altitudinal Variability in Nutritional Composition

Results show a strong positive relationship between GE and altitude when all available plant samples (species-part combination) were included in the analysis (r_s = 0.449, $p < 0.05$; Fig. 5). This same relationship holds true for *Peucedanum* ($r_s = 0.811$, $p <$

0.05) and *Galium* (r_s = 0.817, $p < 0.05$) when analyzed separately (Fig. 5), though no other species from this study were significant. Ash content was also significantly negatively related to increasing altitude (r_s = -0.507, $p < 0.05$), but only when *Galium*, *R. kirungensis*, and *P. linderi* were analyzed together as a separate group targeting sampling at every 100-meter increase in altitude (Fig. 7). This relationship was only significant for *Peucedanum* when these three genera were analyzed separately (r_s = -0.704, $p < 0.05$; see Fig. 8a for *Peucedanum* GE and ash content over altitude. According to a separate regression analysis between *Peucedanum* stem GE and ash content (R^2 = 0.822, F (1, 7) = 32.41, $p < 0.05$; Fig. 8b), for every 0.1 g decrease in ash content, GE increases by 1.03 kcal/g. An increase of only 0.4 – 0.45 kcal/g would suggest that the GE increase over altitude is being driven by a change in ash content alone instead of the composition of the organic matter. However, since this increase is much larger, the implication is that the proportional content of organic matter (e.g., carbohydrates, protein, fat) changes over altitude and the change in GE is not driven entirely by the change in mineral content.

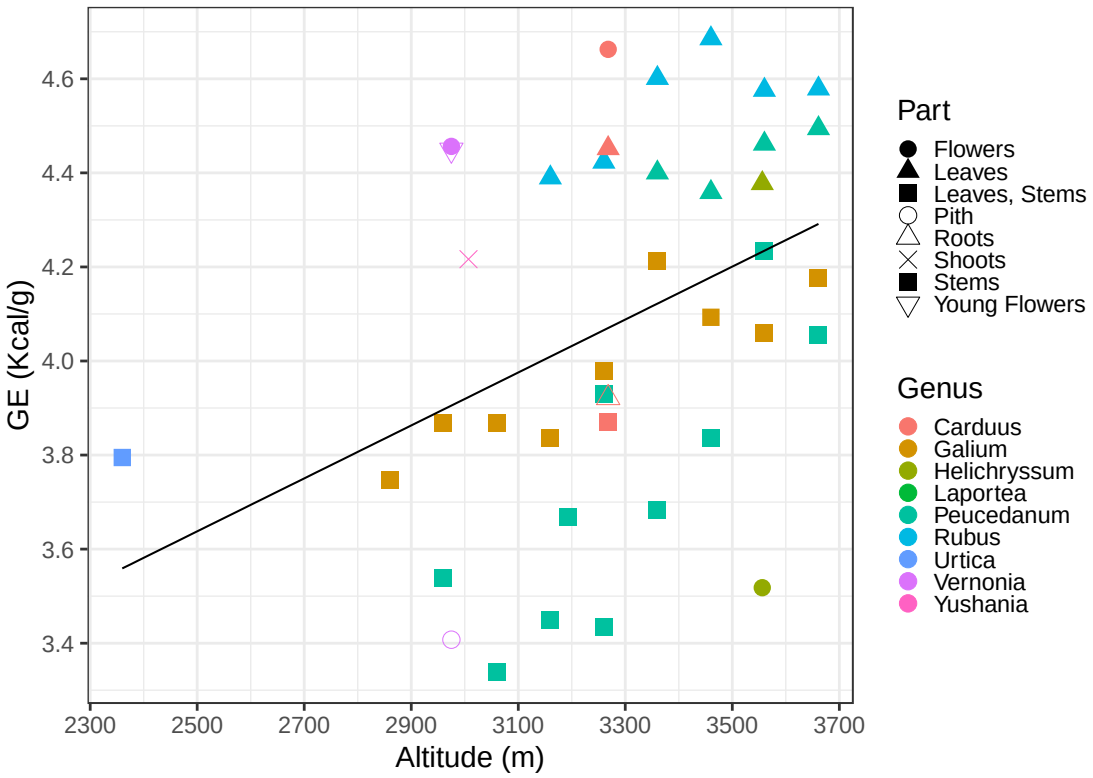

Figure 5: Kcal GE/g over altitude ($r_s = 0.449$, $p < 0.05$).

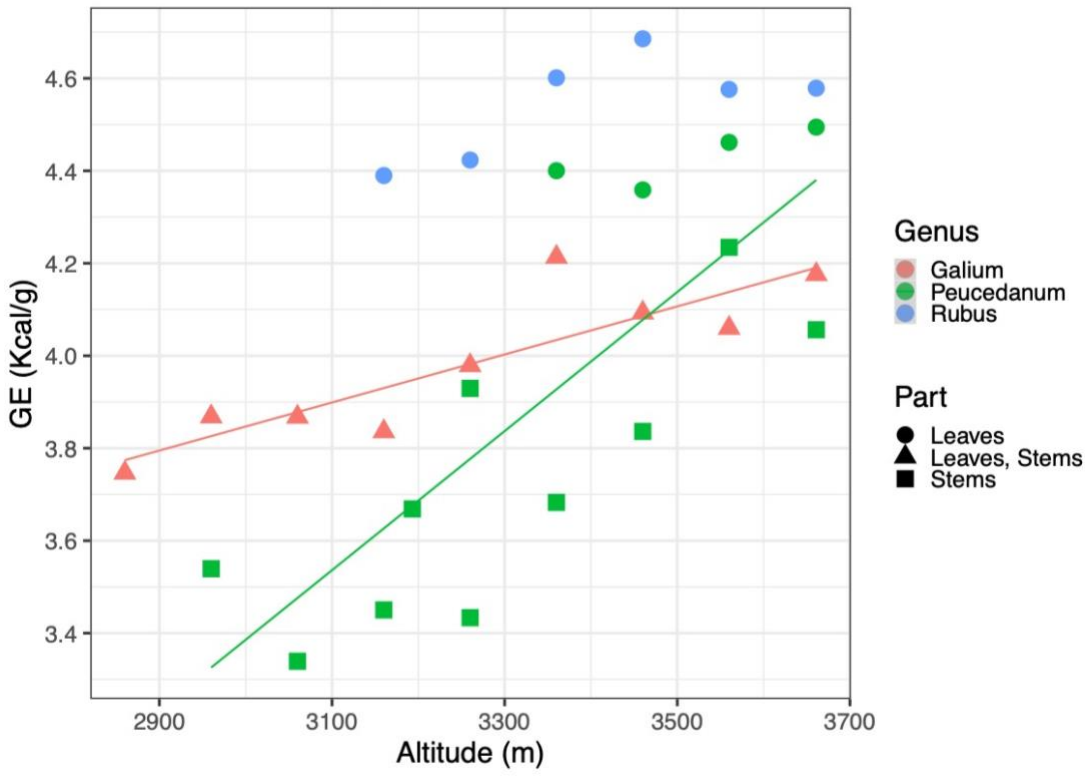

Figure 6: Kcal GE/g over altitude using only *Galium*, *Peucedanum*, and *Rubus* ($r_s = 0.625$, $p < 0.05$). Separately, only *Peucedanum* ($r_s = 0.811$, $p < 0.05$) and *Galium* ($r_s = 0.817$, $p < 0.05$) are significant.

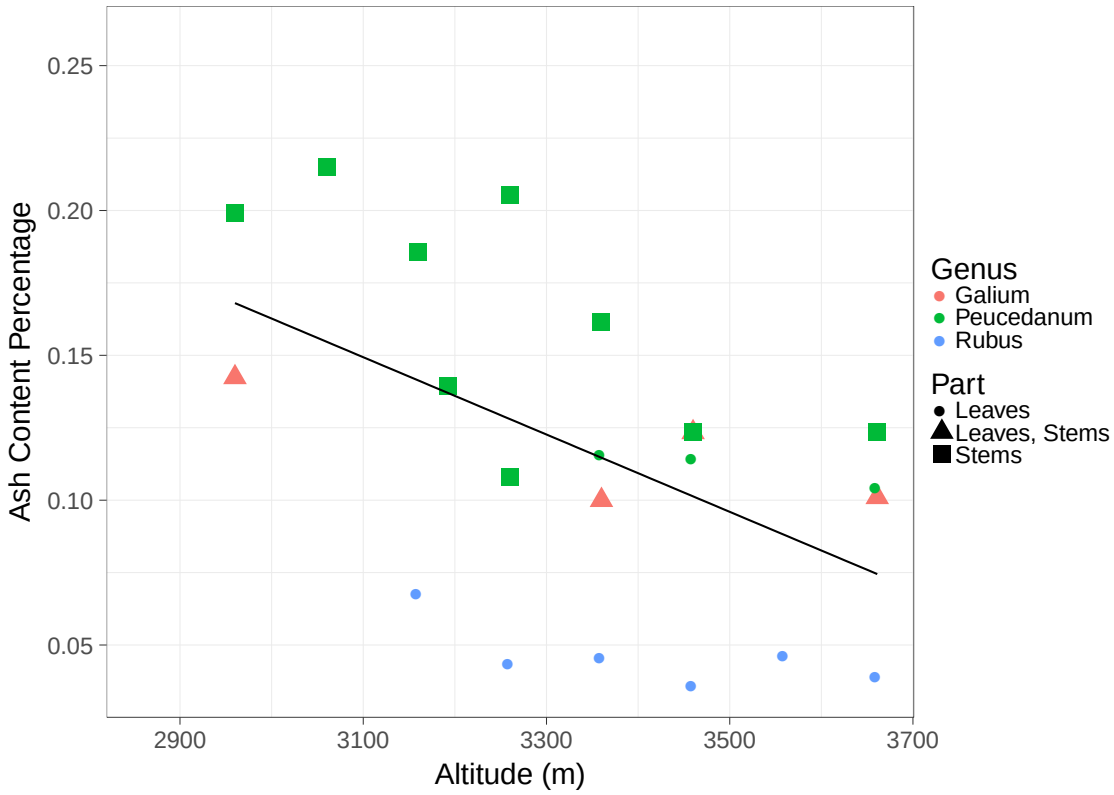

Figure 7: Ash percentage over altitude using only *Galium*, *Peucedanum*, and *Rubus* ($r_s = -0.507$, $p < 0.05$). The black line represents all three genera analyzed together. When genera are analyzed separately, the only significant relationship is *Peucedanum* ($r_s = -0.704$, $p < 0.05$; fit line not shown).

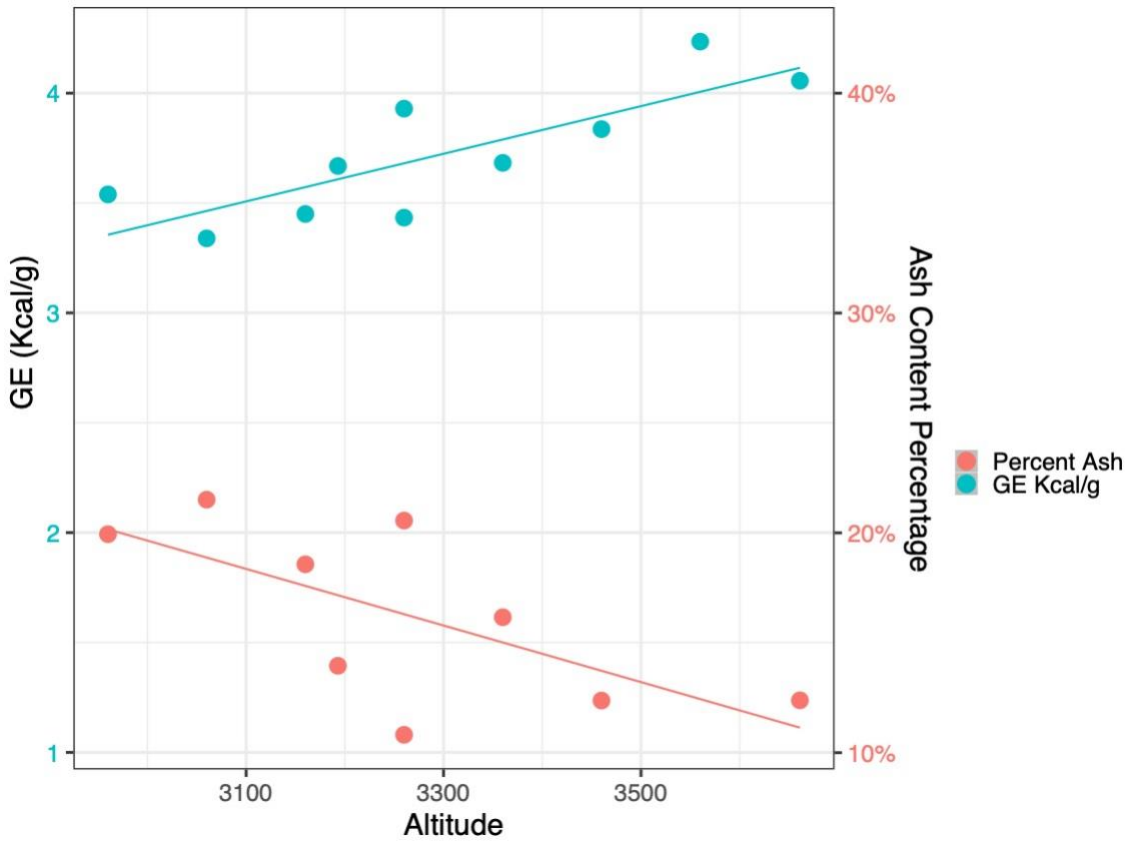

Figure 8a: *Peucedanum* stem ash percentage (r_s = -0.644, $p < 0.05$) and GE (r_s = 0.796, $p < 0.05$) both plotted over increasing altitude. As the figure illustrates, GE significantly increases as ash content significantly decreases.

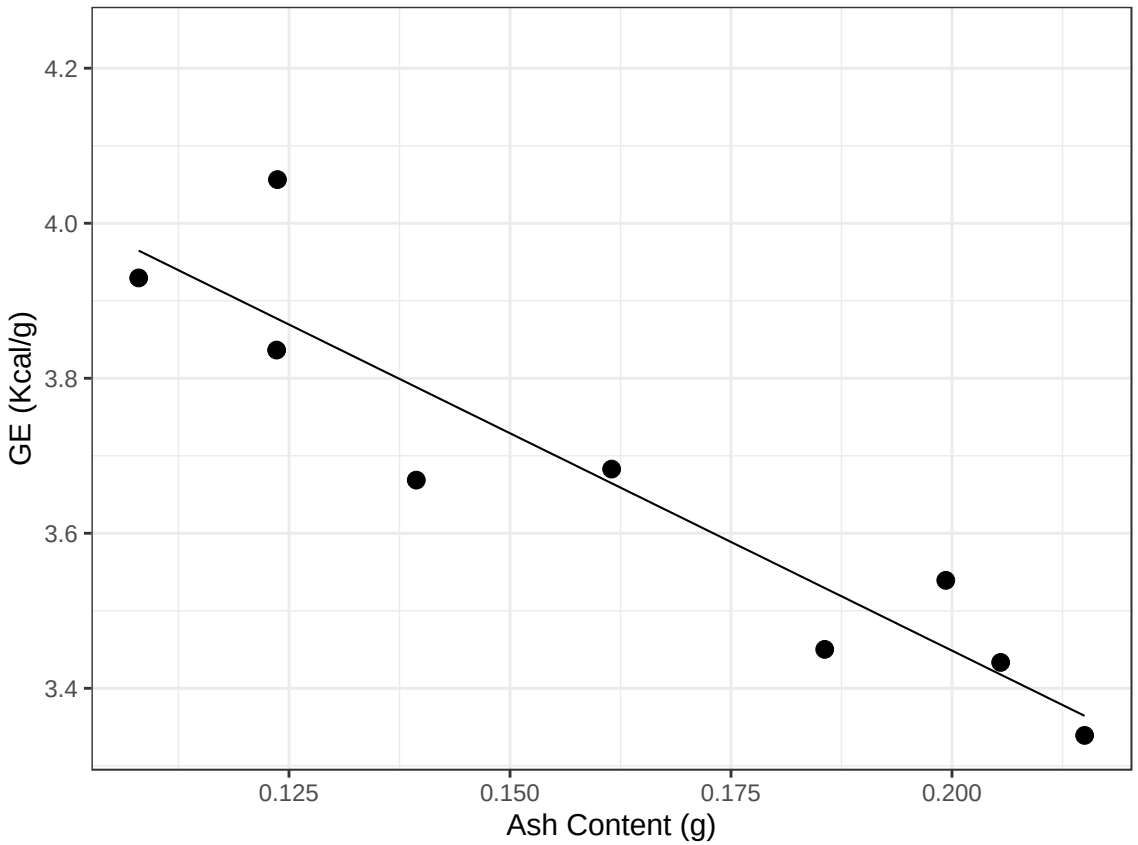

Figure 8b: A separate regression analysis ($R^2 = 0.822$, F (1, 7) = 32.41, $p < 0.05$) shows that for every 0.1 g decrease in ash content, GE increases by 1.02578 kcal/g, implying that the proportional content of organic matter (e.g., carbohydrates, protein, fat) changes over altitude and the significant change in GE is not driven entirely by the change in ash content.

Chapter 5: Discussion and Conclusion

Our findings regarding the nutritional chemistry of Virguna mountain gorilla foods are comparable to prior studies of gorilla nutritional ecology in terms of macronutrient values (Watts 1984; Ganas et al. 2004, 2009; Grueter et al. 2013; Rothman et al. 2006, 2007, 2008, 2014). However, this study adds important new knowledge regarding variability in the nutritional content of primate foods over an altitudinal gradient. Given that previous studies have found that nutritional quality can differ significantly between montane and tropical environments inhabited by mountain gorillas (Rothman et al. 2014), the elevation range of this subpopulation represents the perfect natural experiment by which to better understand other ecological factors that may influence the nutritional geometry of mountain gorillas. Though conclusions from this study are limited by sample size, our findings indicate that the nutritional chemistry of a subpopulation of Virunga mountain gorilla foods varies significantly with altitude within VNP, Rwanda.

With increasing altitude, mountain gorilla plant foods included in this study show significant increases in gross energy (GE) and decreases in ash content. However, since sample size limited our ability to quantify the content of all macronutrients in every sample, the exact mechanisms by which GE is increasing is not yet well understood. Further, some of these genus-level differences in GE may be due to experimental design, as *Galium*, *Rubus*, and *Peucedanum* were targeted for sequential altitudinal sampling and thus were represented by a higher number of samples. Ash content genus-level distinctions may be due to differences in plant part mineral content as *Rubus* leaves are particularly low in ash content while *Helichryssium* leaves and flowers are relatively high (Fig. 2b). Nevertheless, a possible explanation for the finding that GE increases with altitude is that increased

sunlight at higher altitudes boosts the rate of photosynthesis and therefore glucose production in plant leaves (Golley 1961; Bliss 1962; Gates 1965; Smith 1967). Unsurprisingly then, leaves are higher in GE compared to stems. These plant part distinctions seem to be driving most of the variation on PC1. In theory then, it is not surprising that ash content decreases as GE increases with elevation. Ash content percentage on a dry matter basis measures the amount of mineral that remains after removing water and organic matter from the sample, which includes fat, protein, and carbohydrates such as sugar. These nutritional components contribute to gross energy, so as the percent ash content increases, GE will most likely decrease, as shown on PCA loadings (Fig. 2, also see Fig. 8).

Virunga mountain gorillas predominantly subsist on herbaceous vegetation compared to the more seasonally frugivorous population of mountain gorillas in BINP and other gorilla populations throughout Africa (Butynski 1984; Goldsmith 2003; Nkurunungi et al. 2004; Robbins and McNeilage 2003; Sarmiento et al. 1996; Rothman et al. 2014). Fruits are a major dietary contributor of nonstructural carbohydrates and are therefore relatively high in non-protein energy compared to protein-rich leaves (Mundy et al. 1998; O'Brien et al. 1998; Robbins 2012). Previous work on BINP mountain gorilla diets has found that they prioritize non-protein energy and consume excess protein when carbohydrate-rich fruits are not available (Rothman et al. 2011). Given these findings and the paucity of available fruit, Virunga mountain gorillas' protein intake likely is well above requirements. They may therefore feed on relatively energy-rich plant parts at high altitudes, especially if these plants are also available at higher masses. The combination of both a high-energy food and enough mass to feed an entire social group would be enough

to entice mountain gorillas to range more frequently at higher elevations. However, there could be a tradeoff between energy and food mass, especially if plant foods at a lower altitude can offer enough food for a social group to forage for an extended period. Overall, these results suggest that gross energy content in foods may affect ranging patterns and social behavior of the Virguna mountain gorillas in this subpopulation of habituated individuals (Crook and Gartlan 1966; Altmann 1974; Wrangham 1979, 1987; van Schaik 1989; Oates et al. 1990; Byrne et al. 1993; Chapman et al. 2002).

As habitats become progressively degraded and destroyed, endangered primates are pushed to the limits of their ecological niches and forced to inhabit increasingly extreme environments. It is therefore critical to analyze the nutritional chemistry of these remaining habitats to better comprehend the nutritional requirements and limitations of threatened non-human primate species. For Virunga mountain gorillas, which already exist at an ecological extreme, a better understanding of food choice in relation to natural gradients such as altitude will better inform conservation management policies as climate change and increased anthropological contact affect their feeding ecology, social interactions, and ranging behaviors. However, further studies are needed to investigate the mechanisms of gross energy variation over the Virunga mountain range, especially given mountain gorillas' proclivity to prioritize energy-rich foods high in nonstructural carbohydrates (Rothman et al. 2011). To accomplish this, next steps include more comprehensive sampling of key foods for gorilla groups across the Virunga massif to measure fat, carbohydrates, and protein to investigate variation in the components of gross energy over altitude.

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