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Mycophagy of White-Tailed Deer (*Odocoileus virginianus* Zimmermann) in the Boreal Forest

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Abstract: Mushrooms are a little known source of food for large herbivores, but are of high quality because of their high protein content and digestibility. Approximately 50 epigeous and hypogeous mushroom and lichen species have been identified in the diet of cervids so far using macro remains. Our main objective was to determine which mushroom species are consumed by white-tailed deer (*Odocoileus virginianus* Zimmermann) using a molecular approach. We collected 114 fecal samples from deer harvested in 2014 and 2015 on Anticosti Island (Québec, Canada), extracted total DNA from feces, and amplified fungal DNA specifically via polymerase chain reaction. Amplified fungi DNA was then sequenced with the Illumina method to identify mushroom species consumed by deer. Our results revealed that deer harvested consumed up to 4979 fungal species, including 580 species that appeared to be consumed directly. Adults tended to consume a higher mushroom diversity than juveniles, and mushroom diversity consumed by deer was much higher in 2015 than 2014. Adult females consumed a higher mushroom diversity than males, especially lactating females. Our results contribute to the understanding of the role of mushrooms and their large diversity in white-tailed deer diet.

Keywords: cervids; *Odocoileus virginianus*; mushrooms; DNA estimate of diet



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1. Introduction

For herbivores, high quality food resources are characterized by high digestibility and protein content which are driving their food selection [1,2]. Nutritional values of mushrooms are highly variable but they are generally a good source of proteins and are highly digestible [3,4]. Depending on species, growth stage and environmental conditions, crude proteins account for 4–44% of mushrooms' dry weight (Table A1) [3,4]. In comparison, dry matter of other types of forage such as grasses, forbs and shrubs contains 8–22% of crude proteins [5–7]. Although little information is available on the digestible energy of mushrooms, their gross energy content (300–400 Kcal/100 g) is comparable or higher than most forage plants [4,8]. Mushrooms are also generally more digestible (58–91%) than most plants (37–78%) [4,9,10]. The higher digestibility of mushrooms is partly related to the absence of cellulose and lignin, two components of plant cells that are difficult to digest [4]. This higher digestibility means that for the same value of crude protein and gross energy, herbivores can obtain more proteins and energy from mushrooms than from plants. Mushrooms are also a good source of water (80–90% of fresh weight), nutrients such as potassium, phosphorus, iron and selenium, as well as vitamins including thiamine and riboflavin [3,4,8]. All these elements suggest that mushrooms are a high quality resource for herbivores. Accordingly, many cervids readily select and consume mushrooms when they are available [11,12]. The contribution of mushrooms in their diet is, however, highly

variable and generally ranges between 5 and 15% on average [4]. These percentages can nonetheless vary from trace (~0%) to a majority (~80%) of the diet depending on individual, habitat and season [4,11,12].

Mycophagy of cervids and of deer in particular is, nonetheless, poorly understood. Deer appear to be opportunistic mycophages, i.e., they eat mushrooms when they encounter them while searching for other food resources or when their preferred food sources are not available [13]. Approximately 50 epigeous and hypogeous mushroom and lichen species have been identified in their diet in Europe and North America (Table A2) [9,10,14–20]. Deer also indirectly consume fungal endophyte and plant pathogen fungi present in plant tissues [14,21]. By consuming mushrooms, deer may disperse mushroom spores and thus influence the composition of communities [13].

Mycophagy by large herbivores may be influenced by individual characteristics such as age, sex, body condition and reproductive status, and by other factors favoring the presence of mushrooms such as habitat and environmental conditions in a given season or year. A more diversified diet enables individuals to better balance their intake of specific elements and thus better fulfill their nutrient requirements and regulate their intake of toxins [22]. This suggests that consuming a higher diversity of resources, including mushrooms, would be favorable to herbivores. Juveniles and adult females have a smaller digestive system than adult males and a higher metabolic rate. Therefore, juveniles and females are less efficient at digesting cellulose and fiber and should have a higher quality and more easily digestible diet than males to fulfill their nutrient requirements [23]. Moreover, juveniles and deer in poor condition generally seek resources with high protein and energy content to complete their growth [24] or rebuild their body reserves [25]. Mushrooms could be such resources providing a high source of digestible proteins and energy. As lactation induces high nutrient requirements [26], lactating females may also seek to consume a higher diversity of mushrooms than non-lactating females to better fulfill their nutrient requirements.

The diversity of mushrooms consumed by deer is expected to vary according to the habitat where they forage because some habitats are more favorable for mushroom fructifications. Old forests for instance should contain a higher diversity of mushrooms than young forests, because of a more abundant and regularly distributed root system providing a higher potential allocation of carbon to mushrooms [27]. Diversity of mushroom species is also closely associated with the diversity of host species and of substrate types [27,28]. For example, black spruces (*Picea mariana*, Mill.) grow on poor nutrient soils, thus plant/mushroom diversity in black spruce stands is generally lower than in other coniferous stands [29,30]. Conifer stands also provide a high diversity and abundance of lichens [31]. Although most lichens are poor in proteins [32], deer are expected to eat some especially as frost sets in or during winter, when other mushrooms and resources are less available [33].

Mushroom availability varies throughout the year because fructification does not occur at the same time of the year or under the same environmental conditions (e.g., humidity, temperature and light) for all species [3]. For example, hypogeous mushrooms can grow until late November in Eastern Canada, but not epigeous mushrooms [34,35]. Lichens and most polypores, on the other hand, are available throughout the year [28,31]. Therefore, deer have access to a variable diversity of mushrooms throughout the year.

Our main objective was to identify mushroom species consumed by white-tailed deer in the boreal forest. Individuals requiring a higher quality diet such as juveniles and lactating females should include a higher diversity of mushrooms in their diet because it should help them to better fulfill their nutrient needs and regulate their intake of toxins. As such, we hypothesized that (1) the diversity of mushroom species consumed by deer is highly variable from year to year because of the multiple environmental factors affecting mushroom fructification and availability; (2) individuals with higher nutrient requirements and higher metabolic rates, or that are more vulnerable to toxins, because of poor body condition, growth (juvenile) or lactation consume a higher diversity of mushrooms than

other individuals. To achieve this, we tested the effect of individual characteristics (age, sex, body condition, and female lactation status), environmental variables (stand type and age class), and date (month and year) on the diversity of mushrooms consumed by white-tailed deer in the boreal forest.

Consumed mushroom species were identified via DNA barcoding of feces samples, an emerging technique in the identification of food items in wildlife diet [36]. This technique consists of amplifying DNA via polymerase chain reaction using fungal specific universal primers [37], followed by next generation sequencing methods. DNA barcoding has multiple advantages as it can identify mushroom species without relying on the identification of mushroom spores. It is also less biased than morphological identification because intact DNA can be identified without having to find recognizable structures or spores in fecal samples [38]. To our knowledge, this technique has never been used to identify mushroom species in deer diet, thus we began by testing its precision in yielding a repeatable number of reads (number of times a DNA sequence was read during sequencing) in the same sample.

2. Materials and Methods

2.1. Study Area

We studied white-tailed deer (*Odocoileus virginianus* Zimmermann) mycophagy on Anticosti Island covering 7943 km² in the Gulf of St-Lawrence, Québec, Canada (49°28' N, 63°00' W) [39]. In 1896–1897, approximately 220 white-tailed deer were introduced on the island. The population rapidly increased due to the absence of predators and the deer density has been >20 deer/km² since the 1980s [39]. The island belongs to the balsam fir-white birch bioclimatic region [40] and is located at the northern limit of the white-tailed deer distribution. After many years of deer overbrowsing, vegetation on the island has changed and dominant tree species are now white spruce (*Picea glauca*, Moench), balsam fir (*Abies balsamea*, L.) and black spruce [41]. The specific boreal context of Anticosti Island and long term overbrowsing has resulted in low availability of quality forage for deer and, presumably, a high diversity of mushroom species. As such, Anticosti Island appears a great setting to study deer mycophagy and its variability among individuals. Samples were collected on deer harvested in multiple locations distributed throughout most of the island.

2.2. Data Collection

We collected pellet samples from 114 deer harvested by sport hunters during the 2014 ($n = 54$) and 2015 ($n = 52$) hunting seasons from September to November. Feces were collected directly from the gut of the animal and stored frozen at $-20\text{ }^{\circ}\text{C}$. Deer age category (adult or juvenile, i.e., 4–6-month-old) and sex were noted during sampling, and 3 body condition metrics (dressed body mass (kg), rump fat thickness (cm) and peroneus muscle mass (g)) were collected from carcasses [25]. We also evaluated the lactation status of females by looking for the presence of milk in the udder.

Three body condition metrics providing information on a different component of animal condition were combined to obtain a reliable body condition index. Body mass is considered an appropriate measure to estimate body condition fluctuations in cervids [42,43], we measured dressed body mass using a spring scale (± 0.25 kg). Rump fat thickness is known to be a good proxy of fat and energy reserves [44], we measured it using a ruler (± 0.2 cm) inserted into the fat layer at an angle of 45° from the spine at 5 and 10 cm from the base of the tail [25]. The peroneus muscle mass is correlated with protein reserves in cervids [43,45]. We measured the peroneus muscle mass with a Pesola scale (± 0.5 g) after it was extracted from the lower leg. We combined these three condition metrics using a principal component analysis to compute a global body condition index. The first three axes, respectively, explained 82.7%, 14.5% and 2.8% of the variation. We used the broken-stick criterion [46] to determine that only the first axis was significant with a higher value representing better body condition. A body condition score was then assigned to each deer sampled based on its position along that axis.

Content of feces collected at harvest should reflect deer consumption during the previous 24–48 h [47]. We defined the environmental variables (stand type and age class) associated with these feces from the location of harvest sites. Geographic coordinates were derived from locations provided by hunters. They indicated the harvest site on various maps of the hunting zones with a precision averaging about 2 km²/cm². As deer home ranges on Anticosti Island are <1 km² [48], we used a 1 km² buffer around each harvest site to determine the habitat where the deer had likely foraged before harvest and to which the content of the fecal sample was associated [25]. We assigned the sample to the most abundant stand in each buffer. Stand type and age were extracted from an ecoforestry map [49] generated in 2011 with aerial photographs taken in 2009 and providing a 30 m resolution. We combined the 61 stand types available on the map in 6 representative stand types: white spruce, black spruce, balsam fir, larch stands, bog and undetermined conifers to limit the number of potential stand types. We used two stand age classes: young stands (10–75 years) and old stands (75–120 years) to obtain two balanced age classes.

2.3. DNA Extraction and Sequencing

We lyophilized and grounded fecal samples with a 3383-L20 Thomas-Wiley Intermediate Mill grinder (Arthur H. Thomas Company, Philadelphia, PA, USA) using a 20µm sieve. We prepared each feces sample in triplicates (three 15 mg subsamples). DNA extraction was done following the DNeasy Plant kit (Qiagen, Hilden, Germany) manufacturer's instructions. Briefly, each subsample was suspended in 180 µL of lysing buffer, then it was disrupted with a 3 mm tungsten bead in a MixerMill 300 for two 90 s periods at 30 Hz. DNA was then extracted according to the manufacturer's instructions.

The eluted DNA (gDNA) was used as template for polymerase chain reaction (PCR) targeting the ITS regions of the ribosomal DNA fragment (ITS1-5.8S). Briefly, the ribosomal DNA fragments were first amplified in a two-step PCR using the Illumina fusion primers [50]. These primers contained an index sequence for tagging every sequence to a sample. Fifteen forward-indexed sequences were used in combination with 15 reverse indexed sequences to generate a total of 225 indexed combinations.

All amplicons were then purified using an Agencourt® AMPure® XP magnetic PCR clean-up system (Beckman Coulter, Brea, CA, USA), which eliminated primer dimers and small fragments. The clean PCR amplicons were quantified with the Quant-iT™ Picogreen® dsDNA assay kit (Invitrogen, Eugene, OR, USA).

We were not successful in amplifying fungal DNA from every sampled deer and for some of them we could only amplify fungal DNA for one or two subsamples. In the end, we obtained amplicons from 238 (137 in 2014 and 101 in 2015) out of 342 subsamples. The successfully amplified subsamples came from 106 individual deer out of the 114 sampled. Tagged amplicon samples (with differing multiplex identifiers or indexes) were then pooled in equimolar amounts of 4 ng DNA per sample. Final quantification of the pool, verification of removal of primer artifact, and amplicon quality check were performed with the Agilent 2100 BioAnalyzer system (Agilent Technologies, Santa Clara, CA, USA). In total, 75 ng of pooled DNA samples were sent to the Genomic Sequencing and Genotyping Platform of the Centre Hospitalier de l'Université Laval Research Centre (RCCHUL, Québec, QC, Canada) which performed the Illumina paired-end 300 Illumina sequencing.

2.4. Bioinformatic Analyses

A stringent treatment of Illumina DNA sequences was executed to prevent formation of a disproportionate number of fictitious Molecular Operational Taxonomic Units (MOTU), our proxies for fungal species, and to produce a credible and biologically relevant number of MOTUs [51,52]. Analyses were done as described in Christopherson et al. [53]. Briefly, sequence assembly was done using PANDASeq [54] and was then filtered and trimmed with IllumiCut [55]. Sequences with homopolymers longer than 9 bp were removed and HomopRemover reads shorter than 120 bp were discarded [56]. Dereplication on the full length of the set of sequences was performed before construction of clusters with MOTHUR

v.1.28.0 [57]. The sequence set was then organized into clusters with USEARCH 64 bit [58] with a sequence similarity threshold of 97% [59] to agglomerate DNA reads and create MOTUs of fungal species eaten by deer, the most abundant sequence types serving as cluster seeds. The chimera checker UCHIME v.4.2 [60] was used on subsequent de novo and database mode with chimera-free reference fungal database (EmerenciaID available at <http://www.emerencia.org/fungalitspipeline.html> accessed 17 August 2016) [61] under default parameters to detect and discard potential chimeric sequences from the dataset.

Representative sequences, which are the most frequent sequence in each MOTU, were extracted and then screened against relevant databases using local BLAST v.2.2.28+ or the boosted translated BLAST program USEARCH v.6.0.307 [60,62,63]. The 25 top best BLAST hits were sought in databases by a BLASTn or USEARCH search, setting the minimum identity and query coverage parameters to 80% and 70%, respectively. The output file was then trimmed to remove MOTUs with less than 10 reads and MOTUs with the same Genbank reference numbers and sequence similarity above 95% were fused. All MOTUs that were microfungi (including endophytes and epiphytes), coprophilous fungi and rumen anaerobic fungi were removed from the list as they were not the object of this study. Remaining MOTUs were classified as epigeous fungi, hypogeous fungi, polypores or lichens.

2.5. Statistical Analyses

To minimize the impact of fungal contamination by spores and to make sure we only considered mushroom species that appeared to have been directly consumed by white-tailed deer, we only considered species with more than 500 reads across all samples of a given year in our assessment of the overall diversity of macroscopic mushroom species [64]. After this threshold, we considered a species to have been consumed by an individual deer when a read was found in its feces samples.

From the 106 individuals for which we could identify mushroom species in their feces, we retained a total of 95 (47 in 2014 and 48 in 2015; Table 1) for which we had all the necessary data for statistical analyses. We analyzed the effects of deer age (juvenile vs. adult), body condition index, forest stand type and age, as well as the month and year of harvest on the diversity of mushrooms consumed by deer separately for males and females because their foraging behavior and constraints likely differ during the fall. In addition, we tested the effect of lactation on the diversity of consumed mushroom species among adult females. For each analysis (males, females and adult females), we listed all biologically relevant combinations of the aforementioned variables in linear models (Tables A3–A5) and used the Akaike information criterion adjusted for small sample size (AICc) to select the best models ($\Delta AICc < 2$) [65]. We present the effects of the explanatory variables included in the best models with their 95% confidence intervals.

Table 1. Sample size per year, sex and age class for individual white-tailed deer harvested on Anticosti Island (Quebec, Canada) in 2014 and 2015 and retained in the statistical analyses of mushroom species diversity.

Sample Size	Males		Females		Total
	Juveniles	Adults	Juveniles	Adults	
2014	0	15	2	30	47
2015	5	8	3	32	48
Total	5	23	5	62	95

3. Results

3.1. Consumed Mushroom Species

We obtained >26 million mushroom DNA sequence reads which clustered in 4979 MOTUs, our proxy for mushroom species. Fifty-six percent of these species were microscopic (52% endophytes and 4% coprophilous species), reflecting indirect consumption by white-

tailed deer, and 44% ($N = 2184$) were macroscopic, reflecting direct and indirect (spores for example) consumption. From these, we estimated that deer directly consumed a total of 580 macroscopic mushroom species based on the threshold of 500 total reads needed in at least one of the studied years. Deer sampled in 2015 consumed a higher mushroom diversity (424 species) than deer sampled in 2014 (342 species; Figure 1; Tables S1 and S2). The two most consumed genera/groups were the *Cortinarius* and armillarioids (*Armillaria*, *Pholiota* and *Hypholoma*; Tables 2 and 3). Three mushroom species, *Cantharellus lutescens*, *Hypholoma capnoides* and *Lactarius deliciosus*, had the highest number of reads and the most frequent occurrence in deer feces (Tables 2 and 3; Tables S1 and S2). The species accumulation curve (Figure 1) revealed that an asymptote of the diversity of mushroom species in the diet of white-tailed deer was reached with approximately 40 fecal samples.

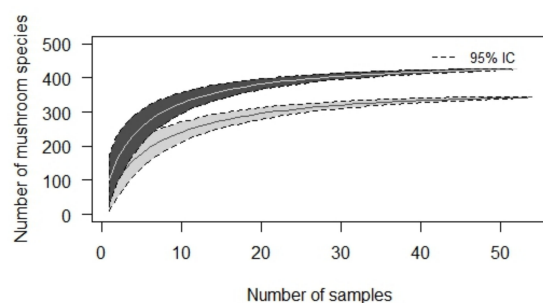


Figure 1. Cumulative number of macroscopic mushroom species (with >500 reads total) found in white-tailed deer feces on Anticosti Island (Quebec, Canada) according to the number of fecal samples in 2014 (light gray) and 2015 (dark gray).

Table 2. List of the 10 macroscopic mushroom species with the highest total number of reads in the 238 subsamples of white-tailed deer feces collected on Anticosti Island (Quebec, Canada) in 2014 and 2015.

Species	Total Reads
<i>Hypholoma capnoides</i>	1,111,546
<i>Lactarius deliciosus</i>	946,384
<i>Cantharellus lutescens</i>	837,572
<i>Armillaria ostoyae</i> ¹	331,324
<i>Suillus pictus</i>	305,891
<i>Russula nauseosa</i> ¹	304,270
<i>Suillus bresadolae</i>	299,284
<i>Cortinarius alboviolaceus</i>	263,161
<i>Paxillus involutus</i>	259,158
<i>Craterellus tubaeformis</i>	257,369

¹ Species only found in 2015.

Table 3. List of the 10 macroscopic mushroom species most frequently found in the 238 subsamples of white-tailed deer feces collected on Anticosti Island (Quebec, Canada) in 2014 and 2015.

Species	Total Occurrence
<i>Lactarius deliciosus</i>	199
<i>Cantharellus lutescens</i>	159
<i>Hypholoma capnoides</i>	155
<i>Cortinarius alboviolaceus</i>	144
<i>Craterellus tubaeformis</i>	122
<i>Russula cessans</i>	108
<i>Suillus bresadolae</i>	106
<i>Hebeloma velutipes</i>	106
<i>Cortinarius caninus</i>	105
<i>Suillus</i> sp.	98

3.2. Effects of Intrinsic and Extrinsic Variables on the Diversity of Mushrooms in Deer Diet

Females consumed a higher diversity of mushrooms than males (Intercept_{female as reference} = 80.8, $t_{\text{value}} = 21.4$, CI95% = 73.4: 88.2; $\beta_{\text{male}} = -16.5$, $t_{\text{value}} = -2.4$, CI95% = -30.1: -2.9), and males were in lower body condition than females during the fall sampling period (Intercept_{female as reference} = 0.3, $t_{\text{value}} = 1.9$, CI95% = -0.1: 0.7; $\beta_{\text{male}} = -1.2$, $t_{\text{value}} = -3.7$, CI95% = -1.9; -0.6). Therefore, we performed sex-specific model selection. Model selection for both sexes produced similar results with the best models including age and year. In both cases, a second model was within a $\Delta\text{AICc} < 2$ (Tables A3 and A4) but the inclusion of the additional variable (body condition) did not significantly improve the model likelihood, thus we present only the result of the most parsimonious model. For both males and females, the selected models indicated that adults consumed a higher diversity of mushrooms than juveniles and that the diversity of consumed mushrooms was higher in 2015 than in 2014 (Table 4). The best model for adult females (Table A5) suggested that lactating females consumed a higher mushroom diversity than non-lactating females (Table 5).

Table 4. Explanatory variables (category), estimates (β), standard errors (SE), t -values (t) and confidence intervals (CI5% and CI95%) of the best models assessing the effects of age, lactation status and year on the diversity of mushrooms consumed by male and female white-tailed deer on Anticosti Island (Québec, Canada). Intercept categories are adult and 2014.

Models	Explanatory Variables	β	SE	t	CI5%	CI95%
Females						
Age + Year	Intercept	65.4	5.1	12.9	55.5	75.4
	Age (juvenile)	-29.4	13.2	-2.2	-55.2	-3.6
	Year (2015)	33.7	6.9	4.9	20.1	47.2
Males						
Age + Year	Intercept	60.1	5.7	10.6	49.0	71.1
	Age (juvenile)	-29.2	12.5	-2.3	-53.6	-4.7
	Year (2015)	20.3	9.6	2.1	1.5	39.1

Table 5. Explanatory variables (category), estimates (β), standard errors (SE), t -values (t) and confidence intervals (CI5% and CI95%) of the best models assessing the effects of age, lactation status and year on the diversity of mushrooms consumed by adult female white-tailed deer on Anticosti Island (Québec, Canada). Intercept categories are non-lactating and 2014.

Models	Explanatory Variables	β	SE	t	CI5%	CI95%
Adult females						
Year + Lactation	Intercept	51.1	8.1	6.3	35.2	67.0
	Year (2015)	37.0	8.6	4.3	20.3	53.8
	Lactation (lactating)	19.2	9.0	2.1	1.6	36.8

4. Discussion

4.1. Consumed Mushroom Species

Approximately 100 macroscopic mushroom species had been observed on Anticosti Island before (Table A6) [40,66]. Our study brought that number to at least 580 mushroom species that appear to be consumed directly by white-tailed deer, most of which had never been listed in their diet before. In most previous studies, mushrooms were considered as one category and could not be identified at the species level [67–70]. To our knowledge, we also report for the first time the potential consumption of >2500 endophyte and coprophilous mushroom species by deer. Consumption of these species was expected because deer consume endophytes inside plant tissues and coprophilous species spores on plants, but not to this extent. Although we expected to detect more DNA sequence reads from macroscopic than microscopic mushrooms because directly eaten mushroom species have a larger biomass and, therefore, should have produced more DNA reads than species eaten

indirectly, we found many more microscopic than macroscopic species in deer feces. This result is a clear demonstration of the omnipresence of microscopic species in the environment. Based on the species accumulation curve, our estimation of the overall diversity of mushrooms consumed by white-tailed deer on Anticosti Island is representative and the inclusion of the 104 subsamples that could not be amplified would have had a limited impact on the total number of mushroom species detected. The diversity of mushroom species consumed by males and juveniles could, however, be underestimated because the number of samples analyzed for these segments of the population was below the approximately 40 samples required to reach the asymptote for the number of mushroom species.

Overall, *Cortinarius* was the mushroom group most frequently consumed and the group with the highest number of consumed species (Tables S1 and S2). This was somewhat expected since *Cortinarius* is a varied group commonly found in conifer stands [28]. The second most frequently consumed group was armillarioids, which are relatively large mushrooms growing in easily detectable and recognizable clumps [28]. These groups were followed by *Lactarius*, boletoids, chantarelloids and *Russula*, all rich in protein contents suggesting that deer may use them because of their high protein content [30,71,72] (Table A1, Tables S1 and S2). The mushroom species most frequently consumed by white-tailed deer on Anticosti Island was *Lactarius deliciosus* containing approximately 20% of crude protein content. A species of chanterelle (*Cantharellus lutescens*), a group containing a higher protein content than most other mushroom species, was also consumed frequently [5–75]. *Hypholoma capnoides* was also a species frequently consumed by deer. A limit of all metabarcoding studies of diet, however, is that the relation between what is really eaten and what is quantified in the feces is unknown. Mushroom digestibility is high and varies with species [76], thus DNA from easily digestible species may be underrepresented in the DNA present in feces.

4.2. Effects of Intrinsic and Extrinsic Variables on the Diversity of Mushrooms in Deer Diet

As expected, year was one of the main factors determining the number of species consumed by deer as those harvested in 2015 consumed 34–73% more mushroom species than deer harvested in 2014. There were likely more mushroom species available for deer on Anticosti Island in 2015 than in 2014. Results from mushroom inventories by mycologists conducted in 2014 and 2015 in comparable environments near Sept-Îles, about 150 km from Anticosti Island, suggested a higher availability of mushrooms in 2015 when 422 species were found compared to 347 species in 2014 (Tables S3 and S4). Fruiting abundance of mushrooms can vary largely among years because it is affected by several environmental factors, such as humidity, light and temperature [3].

Our hypothesis that individuals with higher nutrient requirements, as well as those with smaller digestive systems and higher metabolic rates should consume more mushroom species than others to better meet their nutrient needs and regulate their intake of toxins was partly supported. Females consumed a higher diversity of mushroom species than males and lactating females consumed a higher diversity of mushrooms than non-lactating females. The difference between males and females, however, could be linked to the behavioral changes occurring in males before and during the rut when they reduce food intake [77]. As such, males could have simply foraged less and thus consumed fewer mushroom species than females during the sampling period. This could also be associated with the poorer body condition of males compared to females during that period. On the other hand, the consumption of mushrooms by juvenile deer diverged from our expectations as they consumed a lower diversity of mushrooms than adults even if they need more proteins and energy for growth and a higher quality more digestible diet because of their smaller digestive system [22,23,78]. This may occur because adults are more experienced than juveniles in finding and consuming mushrooms and may thus have learned to utilize more mushroom species. As mentioned above, we cannot exclude that the lower diversity of mushrooms found in male and juvenile fecal samples could simply be related to the lower sample size for these segments of the population. Although body condition entered the top

models, we did not find any relation between body condition index and the diversity of mushrooms consumed by deer. Dominant stand type and age in deer habitat, as well as month during the fall did not seem to affect the diversity of mushroom species consumed by deer. The precision of the locations of harvest sites used to define habitat was, however, limited because coordinates were derived from maps based on information provided by hunters. This could have reduced our capacity to identify relationships between stand type and age, and the diversity of mushroom species. The 1 km buffer used around harvest sites to define stand characteristics should, however, have reduced the impact of this limitation.

5. Conclusions

Our finding of the probable consumption of 580 mushroom species by white-tailed deer suggest that mycophagy is an overlooked component of the ecology of white-tailed deer in the boreal forest. Furthermore, these mushroom species have the potential to influence the growth and distribution of other mushroom and plant species according to where deer disperse their spores. We also showed that white-tailed deer mycophagy is highly variable, even within two successive years, suggesting that mycophagy is probably partly opportunistic and largely dependent on mushroom availability. Our results also suggest that even if deer consume mushrooms opportunistically, they could seek to consume a higher diversity of mushrooms when they have higher nutrient requirements such as for lactating females for instance. Finally, our results suggest that experience could play a role in deer mycophagy because adults consumed a higher diversity of mushrooms than juveniles, but this would need to be confirmed with additional sampling of juveniles' diets.

White-tailed deer mycophagy may be more frequent on Anticosti Island than in other parts of their distribution because of the limited availability of preferred resources such as broadleaf trees and herbaceous plants resulting from many years of overbrowsing on the island [39]. White-tailed deer could thus consume fewer mushroom species when broadleaf forage is not limited and where availability of mushrooms is lower. On the other hand, mycophagy could be a significant source of essential nutrients and proteins in areas where overabundant deer populations decreased the availability of high quality forage. In any case, our results suggest that mycophagy is largely underestimated in the description of the diet of white-tailed deer and probably other cervids. More efforts should be allocated to better understand the implications of mycophagy in the acquisition of resources by herbivores.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12091247/s1>, Table S1: Macroscopic mushroom species consumed by white-tailed deer harvested in 2014 on Anticosti Island (Québec, Canada). Included are the number of times their DNA sequences were obtained during sequencing of all samples from that year (number of reads) and the number of samples in which they were found (occurrence), Table S2: Macroscopic mushroom species consumed by white-tailed deer harvested in 2015 on Anticosti Island (Québec, Canada). Included are the number of times their DNA sequences were obtained during sequencing of all samples from that year (number of reads) and the number of samples in which they were found (occurrence), Table S3: Bulletin du Cercle des mycologues de Sept-îles, Volume 15, Numéro 2, Octobre 2014, Table S4: Bulletin du Cercle des mycologues de Sept-îles, Volume 16, Numéro 2, Octobre 2015.

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Appendix A

Table A1. Crude protein content (% of dry weight) of boletoids, armillarioids, hypogeous fungi, chanterelloids, *Lactarius*, *Russula* and *Cortinarius* species.

Species	Groups	Crude Protein (% of Dry Weight)	References
<i>Tuber Hafizi</i>	Hypogeous	19.2	[79]
<i>Tuber nivea</i>	Hypogeous	16.0	[79]
<i>Tuber Boudieri</i>	Hypogeous	15.8	[79]
<i>Tuber claveriy</i>	Hypogeous	15.0	[79]
<i>Ophiocordyceps sinensis</i>	Hypogeous	38.6	[80]
<i>Cordyceps militaris</i>	Hypogeous	29.7	[80]
<i>Armillaria mellea</i>	Armillarioids	21.1	[81]
<i>Lactarius volemus</i>	<i>Lactarius</i>	25.2	[81]
<i>Agaricus arvensis</i>	<i>Agaricus</i>	56.3	[71]
<i>Armillariella mellea</i>	Armillarioids	22.3	[71]
<i>Boletus edulis</i>	Boletoids	33.1	[71]
<i>Craterellus cornucopioides</i>	Chanterelloids	22.3	[71]
<i>Cantharellus cibarius</i>	Chanterelloids	18.7	[71]
<i>Lactarius deliciosus</i>	<i>Lactarius</i>	29.8	[71]
<i>Agaricus campestris</i>	<i>Agaricus</i>	18.6	[72]
<i>Armillaria mellea</i>	Armillarioids	16.4	[72]
<i>Suillus mediterraneensis</i>	Boletoids	24.3	[72]
<i>Boletus reticulatus</i>	Boletoids	22.6	[72]
<i>Boletus edulis</i>	Boletoids	21.1	[72]
<i>Boletus erythropus</i>	Boletoids	20.9	[72]
<i>Boletus armeniacus</i>	Boletoids	18.3	[72]
<i>Boletus aereus</i>	Boletoids	17.9	[72]
<i>Suillus variegatus</i>	Boletoids	17.6	[72]
<i>Suillus granulatus</i>	Boletoids	16.5	[72]
<i>Cantharellus cibarius</i>	Chanterelloids	35.8	[72]
<i>Lactarius salmonicolor</i>	<i>Lactarius</i>	37.3	[72]
<i>Lactarius deliciosus</i>	<i>Lactarius</i>	20.2	[72]
<i>Russula delica</i>	<i>Russula</i>	50.6	[72]
<i>Russula olivacea</i>	<i>Russula</i>	16.8	[72]
<i>Russula cyanoxantha</i>	<i>Russula</i>	16.8	[72]
<i>Boletus armeniacus</i>	Boletoids	18.3	[73]
<i>Suillus variegatus</i>	Boletoids	17.6	[73]
<i>Boletus impolitus</i>	Boletoids	16.0	[73]
<i>Cortinarius praestans</i>	<i>Cortinarius</i>	14.6	[73]
<i>Tuber Zubaidi</i>	Hypogeous	27.2	[74]
<i>Tuber Gibaah</i>	Hypogeous	25.0	[74]
<i>TuberKolehissi</i>	Hypogeous	19.6	[74]

Table A2. Fungal species (including lichens) consumed by different deer species in Europe and North America based on various methods.

Genus and Species	Deer Species	Methods	References
<i>Daedalia confragosa</i>	<i>Odocoileus virginianus</i>	Behavioral observation	[14]
<i>Panus stipticus</i>	<i>Odocoileus virginianus</i>	Behavioral observation	[14]
<i>Polypores elegans</i>	<i>Odocoileus virginianus</i>	Behavioral observation	[14]
<i>Scleroderma vulgare</i>	<i>Odocoileus virginianus</i>	Behavioral observation	[14]
<i>Stereum rameale</i>	<i>Odocoileus virginianus</i>	Behavioral observation	[14]
<i>Polypores arcularius</i>	<i>Odocoileus virginianus</i>	Rumen samples	[18]
<i>Usnea</i> sp.	<i>Odocoileus virginianus</i>	Rumen samples	[18]
<i>Boletoids subaureus</i>	<i>Odocoileus hermianus</i>	Stomach contents	[10]
<i>Clitocybe gigantea</i>	<i>Odocoileus hermianus</i>	Stomach contents	[10]
<i>Morchella esculenta</i>	<i>Odocoileus hermianus</i>	Stomach contents	[10]
<i>Russula atropurpurea</i>	<i>Odocoileus hermianus</i>	Stomach contents	[10]
<i>Usnea barbata</i>	<i>Odocoileus hermianus</i>	Stomach contents	[10]
<i>Amanita muscaria</i>	<i>Odocoileus hermianus</i>	Behavioral observation	[17]
<i>Boletoids oriantiachus</i>	<i>Odocoileus hermianus</i>	Behavioral observation	[17]
<i>Cortinarius</i> sp.	<i>Odocoileus hermianus</i>	Behavioral observation	[17]
<i>Russula emetica</i>	<i>Odocoileus hermianus</i>	Behavioral observation	[17]
<i>Agaricus</i> sp.	<i>Odocoileus hermianus</i>	Rumen samples	[9]
<i>Rhizopogon evadens</i>	Deer in North America	Fecal inoculant	[20]
<i>Rhizopogon fuscorubens</i>	Deer in North America	Fecal inoculant	[20]
<i>Rhizopogon occidentalis</i>	Deer in North America	Fecal inoculant	[20]
<i>Rhizopogon salebrosus</i>	Deer in North America	Fecal inoculant	[20]
<i>Suillus brevipes</i>	Deer in North America	Fecal inoculant	[20]
<i>Suillus tomentosus</i>	Deer in North America	Fecal inoculant	[20]
<i>Suillus umbonatus</i>	Deer in North America	Fecal inoculant	[20]
<i>Thelephora americana</i>	Deer in North America	Fecal inoculant	[20]
<i>Thelephoraceae</i> sp.	Deer in North America	Fecal inoculant	[20]
<i>Tomentella subilicina</i>	Deer in North America	Fecal inoculant	[20]
<i>Elaphomyces anthracinus</i>	Deer in North America	Behavioral observation	[15]
<i>Elaphomyces granulatus</i>	Deer in North America	Behavioral observation	[15]
<i>Rhizopogon roseolus</i>	Deer in North America	Behavioral observation	[15]
<i>Armillarias ventricosa</i>	Deer in North America	-	[16]
<i>Clavaria</i> sp.	Deer in North America	-	[16]
<i>Lactarius</i> sp.	Deer in North America	-	[16]
<i>Suillus granulatus</i>	Deer in North America	-	[16]
<i>Amanita</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Boletuss</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Calvatia</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Coprinus</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Cortinarius</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Elaphomyces</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Elaphomyces virgatosporus</i>	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Entoloma</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Hypholoma</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Inocybe</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Lycoperdon</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Macrolepiota</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Pluteus</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Russula</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Suillus</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]
<i>Xerocomus</i> sp.	<i>Capreolus capreolus</i>	Organs and feces	[19]

Table A3. Selection of linear models, based on Akaike criterion, explaining mushroom diversity consumed by all male white-tailed deer on Anticosti Island, Québec, Canada. For each model, the number of estimated parameters (K), the second order Akaike criterion adjusted for small sample size (AICc), the delta AICc and weight (AICcWt), as well as the log-likelihood are given. Tested models include various combination of deer age (juvenile vs. adult), body condition index, forest stand type and age, as well as the month and year of harvest.

Models	K	AICc	Delta_AICc	AICcWt	LL
Age + Condition + Year	5	257.84	0	0.44	−122.56
Age + Year	4	258.82	0.98	0.27	−124.54
Null	2	260.19	2.34	0.14	−127.85
Year	3	261.62	3.78	0.07	−127.31
Age + Month + Year	6	262.84	5	0.04	−123.42
Condition + Year	4	263.94	6.1	0.02	−127.1
Stand_Age + Year	4	264.16	6.32	0.02	−127.21
Month + Year	5	265.56	7.72	0.01	−126.42
Stand_type + Year	6	266.24	8.4	0.01	−125.12
Condition + Month + Year	6	268.32	10.47	0	−126.16
Stand_type + Stand_Age + Year	7	269.77	11.93	0	−125.09
Condition + Stand_type + Stand_Age + Year	8	273.30	15.46	0	−124.86
Month + Stand_type + Stand_Age + Year	9	277.62	19.78	0	−124.81
Condition + Stand_type + Stand_Age + Month + Year	10	282.02	24.18	0	−124.54

Bold letters indicate best models.

Table A4. Selection of linear models, based on Akaike criterion, explaining mushroom diversity consumed by all female white-tailed deer on Anticosti Island, Québec, Canada. For each model, the number of estimated parameters (K), the second order Akaike criterion adjusted for small sample size (AICc), the delta AICc and weight (AICcWt) as well as the log-likelihood are given. Tested models include various combination of deer age (juvenile vs. adult), body condition index, forest stand type and age, as well as the month and year of harvest.

Models	K	AICc	Delta_AICc	AICcWt	LL
Age + Year	4	643.59	0	0.49	−317.47
Age + Condition + Year	5	645.41	1.82	0.20	−317.22
Year	3	646.37	2.77	0.12	−319.99
Age + Month + Year	6	647.75	4.15	0.06	−317.17
Condition + Year	4	647.94	4.35	0.06	−319.65
Stand_Age + Year	4	648.50	4.91	0.04	−319.93
Month + Year	5	650.73	7.13	0.01	−319.87
Condition + Month + Year	6	652.60	9.01	0.01	−319.6
Stand_type + Year	8	652.93	9.34	0	−317.23
Stand_type + Stand_Age + Year	9	654.51	10.92	0	−316.68
Condition + Stand_type + Stand_Age + Year	10	656.97	13.38	0	−316.52
Month + Stand_type + Stand_Age + Year	11	659.74	16.15	0	−316.47
Condition + Stand_type + Stand_Age + Month + Year	12	662.56	18.96	0	−316.39
Null	2	663.25	19.65	0	−329.53

Bold letters indicate best models.

Table A5. Selection of linear models, based on Akaike criterion, explaining mushroom diversity consumed by adult female white-tailed deer on Anticosti Island, Québec, Canada. For each model, the number of estimated parameters (K), the second order Akaike criterion adjusted for small sample size (AICc), the delta AICc and weight (AICcWt) as well as the log-likelihood are given. Tested models include various combination of deer body condition index and lactation status, forest stand type and age, as well as the month and year of harvest.

Models	K	AICc	Delta_AICc	AICcWt	LL
Year + Lactation	4	454.95	0	0.52	−223
Stand_Age + Year + Lactation	5	456.51	1.56	0.24	−222.52
Condition + Year + Lactation	5	457.43	2.48	0.15	−222.98
Month + Year + Lactation,	6	459.34	4.39	0.06	−222.62
Condition + Month + Year + Lactation	7	461.88	6.93	0.02	−222.5
Stand_type + Year + Lactation	8	463.26	8.31	0.01	−221.74
Stand_type + Stand_Age + Year + Lactation	9	465.62	10.67	0	−221.38
Condition + Stand_type + Stand_Age + Year + Lactation	10	468.67	13.72	0	−221.28
Month + Stand_type + Stand_Age + Year + Lactation	11	471.81	16.86	0	−221.13
Condition + Stand_type + Stand_Age + Month + Year + Lactation	12	474.97	20.02	0	−220.89
Year	3	551.51	96.56	0	−272.53
Condition + Year	4	552.06	97.11	0	−271.64
Stand_Age + Year	4	552.97	98.02	0	−272.1
Condition + Month + Year	6	555.54	100.59	0	−270.93
Month + Year	5	555.93	100.98	0	−272.37
Stand_type + Year	7	559.61	104.66	0	−271.66
Stand_type + Stand_Age + Year	8	560.63	105.68	0	−270.82
Condition + Stand_type + Stand_Age + Year	9	561.22	106.27	0	−269.7
Condition + Stand_type + Stand_Age + Month + Year	11	565.81	110.86	0	−268.97
Month + Stand_type + Stand_Age + Year	10	566.1	111.15	0	−270.66
Null	2	568.04	113.09	0	−281.91

Bold letters indicate best models.

Table A6. Fungal species and genus (including lichens) observed on Anticosti Island (Québec, Canada) according to the Anticosti Island monograph and personal observations of Danièle Morin [40,66].

Species and Genus	Reference
<i>Agaricus arvensis</i>	[40]
<i>Albatrellus confluens</i>	[40]
<i>Aleuria aurantia</i>	[40]
<i>Amanita muscaria</i>	[40]
<i>Auricularia auricula-judae</i>	[40]
<i>Cantharellus cibarius</i>	[40]
<i>Clavariadelphus</i> sp.	[40]
<i>Clavulinopsis fusiformis</i>	[40]
<i>Coprinus atramentarius</i>	[40]
<i>Coprinus comatus</i>	[40]
<i>Cortinarius alboviolaceus</i>	[40]
<i>Cortinarius armillatus</i>	[40]
<i>Cortinarius violaceus</i>	[40]
<i>Craterelle tubaeformis</i>	[40]
<i>Geastrum</i> sp.	[40]
<i>Gyromitra</i> sp.	[40]
<i>Helvella crispa</i>	[40]
<i>Hydnum repandum</i>	[40]
<i>Hygrocybe coccinea</i>	[40]
<i>Hygrophoropsis aurantiaca</i>	[40]
<i>Hypomyces lactifluorum</i>	[40]
<i>Hypsizyguus ulmarius</i>	[40]
<i>Inocybe</i> sp.	[40]
<i>Laccaria laccata</i>	[40]
<i>Lactarius deterrimus</i>	[40]

Table A6. Cont.

Species and Genus	Reference
<i>Lepiota</i> sp.	[40]
<i>Lycoperdon perlatum</i>	[40]
<i>Lycoperdon pyriforme</i>	[40]
<i>Marasmius scorodoni</i>	[40]
<i>Mutinus caninus</i>	[40]
<i>Neolepta irregularis</i>	[40]
<i>Paxillus involutus</i>	[40]
<i>Paxillus</i> sp.	[40]
<i>Pleurocybella porrigens</i>	[40]
<i>Pleurotus ostreatus</i>	[40]
<i>Russula decolorans</i>	[40]
<i>Russula emetica</i>	[40]
<i>Sarcodon imbricatus</i>	[40]
<i>Suillus cavipes</i>	[40]
<i>Suillus grevillei</i>	[40]
<i>Tremella foliacea</i>	[40]
<i>Tremellodon gelatinosum</i>	[40]
<i>Tremiscus helvelloides</i>	[40]
<i>Tricholoma</i> sp.	[40]
<i>Xeroconus badius</i>	[40]
<i>Alectoria sarmentosa</i>	[66]
<i>Amanitopsis</i> sp.	[66]
<i>Arthonia swartziana</i>	[66]
<i>Biatora campestris</i>	[66]
<i>Biatora decipiens</i>	[66]
<i>Biatora sanguineo-atra</i>	[66]
<i>Biatora uliginosa</i>	[66]
<i>Boletinus</i> sp.	[66]
<i>Boletus</i> sp.	[66]
<i>Bryopogon jubata</i>	[66]
<i>Buellia parasema</i>	[66]
<i>Cetraria islandica</i>	[66]
<i>Chone unfindibulformis</i>	[66]
<i>Cladonia gracilis</i>	[66]
<i>Cladonia turgida</i>	[66]
<i>Clavaria aurea</i>	[66]
<i>Clitocybe laccata</i>	[66]
<i>Collema flaccidum</i>	[66]
<i>Collybia</i> sp.	[66]
<i>Exoascus</i> sp.	[66]
<i>Fistulina</i> sp.	[66]
<i>Fomes fomentarius</i>	[66]
<i>Giromitra</i> sp.	[66]
<i>Graphis scripta</i>	[66]
<i>Heterothecium grossum</i>	[66]
<i>Heterothecium pezizoideum</i>	[66]
<i>Heterothecium sanguinarium</i>	[66]
<i>Hirneola</i> sp.	[66]
<i>Lactarius piperatus</i>	[66]
<i>Lecanora prinigna</i>	[66]
<i>Lecanora surfusca</i>	[66]
<i>Lecidea enteroleuca</i>	[66]
<i>Lenzites betulina</i>	[66]
<i>Lycoperdon</i> sp.	[66]
<i>Morchella deliciosa</i>	[66]
<i>Nephroma levigatum</i>	[66]
<i>Pannaria brunnea</i>	[66]
<i>Pannaria lepidiota</i>	[66]
<i>Pannaria macounii</i>	[66]

Table A6. Cont.

Species and Genus	Reference
<i>Pannaria mycophylla</i>	[66]
<i>Parmelia saxatilis</i>	[66]
<i>Peltigera aptosa</i>	[66]
<i>Peltigera canina</i>	[66]
<i>Pertusaria multipunctata</i>	[66]
<i>Peziza odorata</i>	[66]
<i>Placodium aurantiacum</i>	[66]
<i>Placodium elegans</i>	[66]
<i>Pleurotus</i> sp.	[66]
<i>Pluteus cervinus</i>	[66]
<i>Polyporus versicolor</i>	[66]
<i>Ramalina calicaris</i>	[66]
<i>Ramalina polymorpha</i>	[66]
<i>Sphaerophorus globiferous</i>	[66]
<i>Stereocaulon pileatum</i>	[66]
<i>Stereocaulon tomentosum</i>	[66]
<i>Sticta pulmonaria</i>	[66]
<i>Sticta scorbiculata</i>	[66]
<i>Theloshistes polycarpus</i>	[66]
<i>Umbilicaria hyperborea</i>	[66]
<i>Usnea barbata</i>	[66]

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