ARTICLE

Increased intake of tree forage by moose is associated with intake of crops rich in nonstructural carbohydrates

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Abstract

Animals representing a wide range of taxonomic groups are known to select specific food combinations to achieve a nutritionally balanced diet. The nutrient balancing hypothesis suggests that, when given the opportunity, animals select foods to achieve a particular target nutrient balance, and that balancing occurs between meals and between days. For wild ruminants who inhabit landscapes dominated by human land use, nutritionally imbalanced diets can result from ingesting agricultural crops rich in starch and sugar (nonstructural carbohydrates [NCs]), which can be provided to them by people as supplementary feeds. Here, we test the nutrient balancing hypothesis by assessing potential effects that the ingestion of such crops by *Alces alces* (moose) may have on forage intake. We predicted that moose compensate for an imbalanced intake of excess NC by selecting tree forage with macro-nutritional content better suited for their rumen microbiome during wintertime. We applied DNA metabarcoding to identify plants in fecal and rumen content from the same moose during winter in Sweden. We found that the concentration of NC-rich crops in feces predicted the presence of *Picea abies* (Norway spruce) in rumen samples. The finding is consistent with the prediction that moose use tree forage as a nutritionally complementary resource to balance their intake of NC-rich foods, and that they ingested *P. abies* in particular (normally a forage rarely eaten by moose) because it was the most readily available tree. Our finding sheds new light on the foraging behavior of a model species in herbivore ecology, and on how habitat alterations by humans may change the behavior of wildlife.

KEYWORDS
Alces alces, browsing, nutritional balancing, *Picea abies*, ruminant, supplementary feeding

INTRODUCTION

Animals representing a wide range of taxonomic groups are known to select foods to achieve a nutritionally balanced diet (Dussutour et al., 2010; Felton et al., 2009; Raubenheimer et al., 2005; Robbins et al., 2007; Rothman et al., 2011; Simpson et al., 2004). Nutritional balancing occurs when an animal alters its daily food intake to approximate a specific nutritional target balance, by either selecting well-balanced food items or by combining food items with compositions that are complementary (Simpson & Raubenheimer, 2012). Such a nutritional strategy likely reflects physiological processes within the animal that are associated with fitness benefits selected for over the species' evolutionary history. For example, macronutrient balance has been shown to affect animal growth (Raubenheimer & Simpson, 1997; Simpson et al., 2004), fecundity (Lee et al., 2008), immune responses (Cotter et al., 2011), life span (Solon-Biet et al., 2015), and predation risk (Hawlena & Schmitz, 2010).

Tentative links between macronutrient balance and fitness have also been revealed in free-ranging large herbivores, such as the moose (*Alces alces* L.) (Wam et al., 2018), a model species in the development of herbivore ecology and optimal foraging theory (Belovsky, 1986). These large-bodied, long-lived herbivores inhabit northern latitudes with great seasonal fluctuations in food availability and quality, and therefore are expected to be locally or temporarily precluded from meeting their nutritional goal. For example, while populations of moose with relatively diverse natural winter diets are associated with relatively high mean calf body mass, populations whose diet diversity is kept artificially high thanks to the inclusion of agricultural crops (e.g., root vegetables), do not gain the same benefits (Felton, Holmström, et al., 2020; additional factors known to affect moose calf body mass include forage availability, weather, and maternal body condition, e.g., Ericsson et al., 2001; Holmes et al., 2021). Such crops have been bred over millennia to be rich in easily digestible nonstructural carbohydrates (NCs; sugars and starches), and as such have nutritional compositions highly divergent from what moose have evolved to eat during wintertime (Felton, Holmström, et al., 2020). Large doses of crops rich in NC can be harmful for ruminants, via changes to the rumen pH (Kahn, 2005; Schwartz et al., 1988). As a specialized browser, the natural diet of moose in the growing season consists of selected green plant material (Wam & Hjeljord, 2010). During autumn and into winter, moose gradually change their diet to include more woody plant material as the availability of green material diminishes. The composition of their rumen flora also changes in response to the less nutrient-rich diet of woody material from shrubs and trees (Van Soest, 1994). For moose in northern Europe, such plant species include, for example, the conifer *Pinus sylvestris* L. (Scots pine), and the four common broad-leaved tree species: *Populus tremula* L. (aspen), *Sorbus aucuparia* L. (rowan), *Quercus* sp. (oak), and *Salix* spp. (willow)—hereon referred to as the AROW tree species. Selection by moose among such food items appears to be influenced not only by relative availability but also by nutritional balancing (Felton et al., 2016; Wam et al., 2018).

For moose and other wild cervids who inhabit highly modified landscapes dominated by human land use, nutritionally imbalanced diets can result from the inclusion of agricultural crops. Some crops are only available through deliberate feeding. For example, cervids do not dig up root vegetables by themselves, so any inclusion of root crops in their diet is due to people supplying them within the animals' home range. Reasons for supplying crops as feed can be to enhance winter survival and reproductive success, benefit hunting and tourism, manipulate migration routes, divert animals from traffic, or reduce damage (Milner et al., 2014). However, the intended goals of supplementary feeding are rarely met, and additional unforeseen problems often arise, such as increased forest damage (Milner et al., 2014). Browsing by cervids on production trees can result in significant damage and costs to the forest owner (Pettersson et al., 2010; Storaas et al., 2001).

In this paper, we focus on the potential effects that an intake of crops rich in NC (NCC) may have on moose intake of natural forage. Based on findings from a feeding experiment with captive moose (Felton et al., 2016), we hypothesized that free-ranging moose compensate for an imbalanced intake of NC associated with the intake of crops, by increasing their use of woody material with better balanced or complementary macro-nutritional content (see *Forage nutritional composition—A background*). We furthermore hypothesized that the woody material would be sourced from the most abundant suitable tree species. The most abundant tree species in the area are, apart from *Betula* spp. (birch), the conifer trees *P. sylvestris* and *Picea abies* (Norway spruce), largely due to the extensive commercial use of planted conifer seedlings (SFA, 2018b). Thus, the feeding on crops can have repercussions not only for moose themselves, but also for forest browsing damage. We approach these research questions using DNA metabarcoding to identify plants present in fecal and rumen content from the same moose individuals during winter. We use these samples as a proxy for different feeding events. We place our findings within the context of tree forage and NCC (supplementary feed) availability in the landscape, and discuss the unintended and undesired situation when a bite of

supplied feed results in more bites on production trees, rather than fewer.

MATERIALS AND METHODS

Study areas and collection dates

This study was carried out between latitude 56° N and 59° N in the hemiboreal zone (Figure 1), with a mean annual precipitation of 700 mm, 25–100 days of annual snow cover, and a mean annual temperature of 6–9°C (1991–2020). Dominating forest types are coniferous forests with *P. abies* (46% of standing volume) or *P. sylvestris* (30% of standing volume; SLU, 2021). These conifer forests are often intermixed with naturally regenerated broadleaves, primarily *Betula pubescens* Ehrh (downy birch), *Betula pendula* Roth (silver birch), *S. aucuparia*, *P. tremula*, and *Quercus* sp. (both *Quercus robur* L. [pedunculate oak] and *Q. petrea* [Matt.] Liebl. [sessile oak]). Moose management in Sweden is organized in moose management areas (MMAs), and moose management units (MMUs). An MMA is an area supposed to host a moose population (Sandström, 2011). Each MMA is divided into several MMUs (mean MMU size 160 km^2 in

southern Sweden). We collected samples from six MMAs (Figure 1), with study area selection focused on incorporating a large variation in land use and ungulate community composition, by assessing land cover maps and hunting statistics (www.viltdata.se). Samples were collected with the help of volunteer hunting teams (for more information, see Felton, Holmström, et al., 2020). Sampled moose individuals were culled as part of the annual hunt and the data collection was not biased toward diseased individuals (Ericsson et al., 2013). No permits were required. We obtained samples from moose shot between October 14, 2014 and February 15, 2015.

Forage nutritional composition—A background

Previous research in the same study area (Felton et al., 2021) has shown that the 12 most common forage items included in the moose diet during wintertime have a very different macro-nutritional composition compared with root vegetables commonly used in supplementary feeding (Figure 2). Notably, the ratio between protein and NCs in *P. abies* forage is closer to the moose' presumed
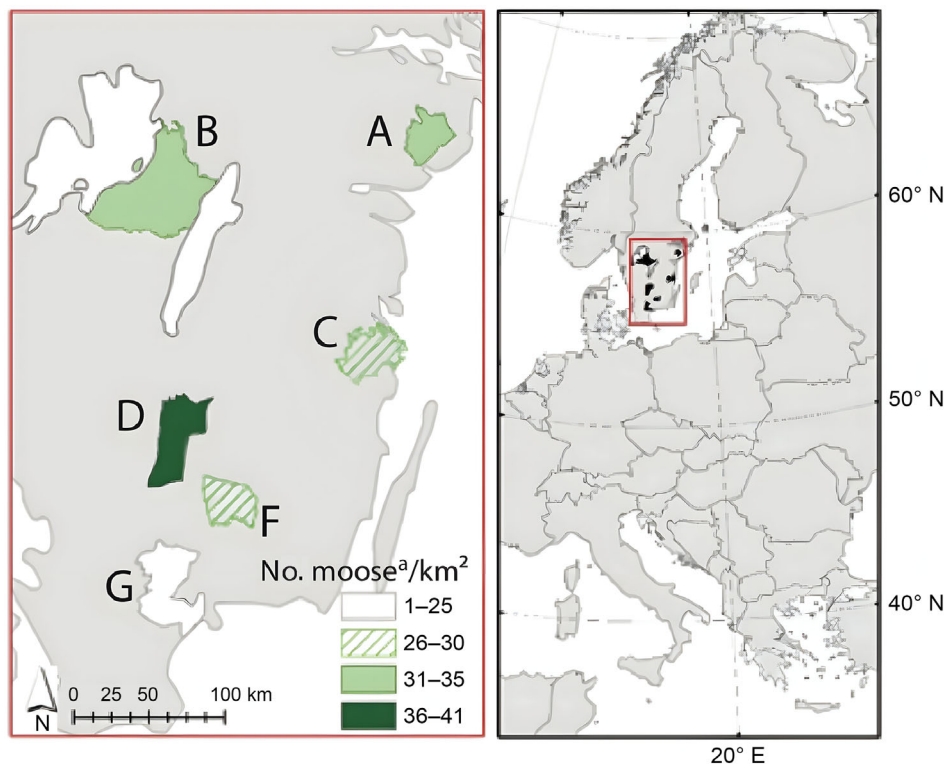
FIGURE 1 Map of the six moose management areas (MMAs) in southern Sweden used as study areas. Relative moose population density for each MMA during the study year is indicated by hunting bag statistics illustrated with a colored scale ([a]number of moose harvested in the yearly hunt per square kilometer; sourced from www.viltdata.se).
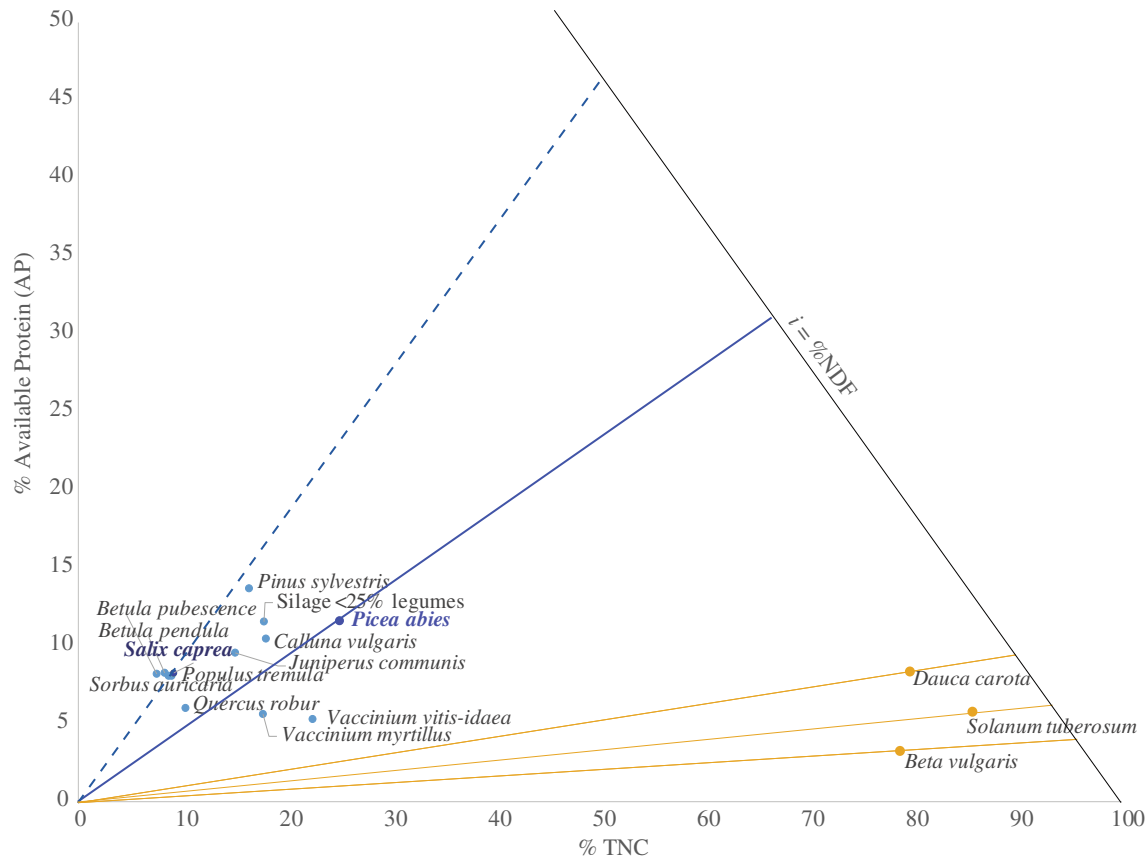
FIGURE 2　Right-angled mixture triangle (Raubenheimer, 2011) depicting the relative components of macronutrient content in 12 common moose food plants and four common supplementary feeds in Sweden, using the mean concentration of three constituents, and expressed as a percentage of total macronutrients (excluding fat) in grams of dry matter. x-axis = % total nonstructural carbohydrates (TNC). y-axis = % available protein (AP), which includes microbial nitrogen (N) but excludes N bound in the fiber fraction. i-axis (implicit axis, i.e., 100% − y-value − x-value) = % neutral detergent fiber (NDF), which is a proxy for structural carbohydrates (here: "fibers"). Increased distance from the hypotenuse means increased % fiber. Dots = composition of pooled plant samples representing each plant species. For example, the dark blue dot represents *Picea abies* twigs, with contributions of 25% TNC, 63% NDF, and 12% AP, totaling 100%. The slope of the solid blue line represents the AP:TNC ratio for *P. abies*. The dashed blue line represents the macronutrient ratio for *Salix caprea* L. (goat willow) twigs. The nutritional composition of *S. caprea* twigs appears to correspond to the wintertime nutritional target balance of moose, as identified experimentally with captive moose (Felton et al., 2016), and recently confirmed in a study of free-ranging moose (Spitzer et al., 2023). The orange dots represent three species of root vegetables (whole roots), and the orange lines represent their respective ratios. This figure is adapted from Figure 4 in Felton et al. (2021), where the description of methods can be found. Edible parts of twigs and/or needles were sampled in winter in southern Sweden. The 12 food items together represent ca 85% of total ingested dry matter by these moose populations (Felton, Holmström, et al., 2020).

target ratio (as established in previous experimental; Felton et al., 2016 and observational; Spitzer et al., 2023 studies), than the ratio between these constituents in root vegetables (Figure 2). Furthermore, the % fiber in root vegetables is much lower (8%–18%) than the % fiber in the tree forage moose normally eat during wintertime (70%–80%), as indicated by the distance between dots and the implicit axis in Figure 2.

Moose sample collection

To obtain information about the foods eaten by moose, both rumen and fecal samples were collected. Immediately after the moose was shot, a 1-L plastic airtight container was filled with rumen content. Hunters were instructed to first mix rumen contents and thereby make the composite sample representative of the whole rumen (Bergström et al., 2011; Cederlund et al., 1980). Fecal material was collected from the rectum (distal colon). Different gloves were used when collecting rumen and fecal material according to instruction, but we cannot exclude the possibility that some cross contamination occurred. Mandibles were collected for age analysis (except from calves). All samples were frozen shortly after sampling (within 0.5–1 h), and stored at −20°C. To estimate age, we sectioned one first-molar tooth and counted the

cementum annuli (Wolfe, 1969). Methods regarding all sample collection are described in detail in Felton, Holmström, et al. (2020). From the pool of samples obtained (481 rumen samples and 318 fecal samples), we limited molecular analysis to those moose individuals from which we had obtained both rumen and fecal samples, as well as complete metadata regarding place and date of harvest, sex, and age ($N = 248$).

Sample preparation and DNA extraction

From each rumen sample, a subsample was taken after defrosting and mixing. The subsample was freeze-dried in preparation for molecular analysis. The freeze-dried rumen subsamples were ground by hand, using pestle, mortar, and liquid nitrogen (Sharma et al., 2003). One randomly selected pellet per fecal sample was defrosted and crushed. Ground rumen and fecal material were placed separately in 20-mL vials and homogenized in 70% ethanol. DNA extraction and purification were automized on a QIA symphony SP platform using the DSP DNA minikit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. One template control was included for every 15 samples. DNA extracts were stored at $-20°$C.

Molecular analysis (trnL metabarcoding)

Samples were transferred to 96-well polymerase chain reaction (PCR) plates and amplified using a Qiagen Multiplex Master Mix (Appendix S1: Table S1A). "No template controls" (NTCs) were included during DNA extraction, and they were carried through to sequencing. For these samples, NTCs showed no significant amounts of PCR products and were thus excluded from the analysis. After chloroplast trnL amplification, we SPRI (Solid Phase Reversible Immobilization)-cleaned samples (2:1 SPRI to PCR) and eluted in 20 µL standard buffer solution TE (10 mM Tris–HCl (pH 8.0) and 0.1 mM EDTA). We indexed the libraries using Kapa 2× Ready-mix, with each sample being indexed with a unique i5 and i7 PCR primer for Illumina sequencing (Appendix S1: Table S1B).

Post amplification, the libraries were SPRI-cleaned at 1.7:1, before being eluted in 20 µL TE. Randomly selected libraries were then quantified on qubit fluorometer (~10% of total), before being pooled for sequencing on Illumina NextSeq at 2 × 75 bp. Libraries were sequenced to a median of 27,000 reads. After sequencing, data were pre-processed using SeqPrep2 (https://github.com/jeizenga/SeqPrep2), then processed using OBITools (Boyer et al., 2016) as in Nichols et al. (2018). We compared taxa with a local reference library that was recently built (Heinken, 2019) in order to identify more specific taxa. We refer to each identified taxa as a "molecular operational taxonomic unit" (MOTU).

Availability of the most common tree species

The ability of moose to compensate for previous imbalanced food intake by browsing on trees will depend on the availability on those trees in the landscape. *P. abies* and *P. sylvestris* together comprise 80% of standing volume in Sweden (Nilsson et al., 2022), with a large part of the remaining share represented by *Betula*. To quantify the availability of trees in our study areas, we compiled data from the Swedish National Moose Browsing Damage Inventory, which is carried out in each MMA every second year (results are freely available at https://www.skogsstyrelsen.se/abin). The inventory is done in young stands that have a mean height of 1–4 m and a size of at least 0.5 ha (for detailed description of stand selection and methodology, see Kalén et al., 2019). This stage of the forest rotation period (which we hereon refer to as "young forests") represents the habitat type with the highest concentration of trees within browsing height of moose in this region (Bergqvist et al., 2018; Wam et al., 2010), and therefore serves as a reasonable estimate of the relative availability of different tree species to our study populations of moose. In addition to recording damage, this inventory records the number of stems of *P. sylvestris*, *P. abies*, and *Betula* (including both *B. pubescens* and *B. pendula*), as well as "other production trees," present in plots of 3.5-m radius. The "other production trees" consist of species such as *Larix* sp. (larch), *S. aucuparia*, *Quercus* sp., and *Fagus sylvatica*. The count within a single plot is, for all these categories, limited to those stems that are at least as high as half of the mean height of the two tallest trees within the plot (e.g., if the mean height of the two tallest [production] trees within the plot is 2 m, stems must be at least 1 m tall to be included in the count). For our six study MMAs, we compiled inventory data (number of stems per hectare young forest per tree species and percentage of land area represented by young stands with mean height of 1–4 m) collected either in 2015 or 2016 (Appendix S1: Table S3).

Availability of NC-rich crops as supplementary feed

To estimate the availability of supplementary feed, we interviewed property owners within three MMUs located

in each of the four study areas (MMAs) A, B, F, and G (areas C and D were not included). Within each of the 12 MMUs, 11–15 smaller study sites ("tracts"; circular with radius 600 m, resulting in 113 ha) were randomly dispersed, with a minimum distance of 1 km between tracts. We contacted people owning property within these tracts (excluding properties <10 ha in size) by telephone during spring 2017 for a structured telephone survey regarding any supplementary feeding taking place (amount, type) during the three previous winter seasons (see Felton et al., 2022 for a detailed method description and results from 2016 to 2017). Here we use data concerning the winter 2014–2015, which overlaps with our moose sampling described above. We reached 57% of all relevant property owners by telephone, and 20% of them declined to participate. Here we use data from tracts ($n = 87$; range 17–27 per MMA) for which at least 25% of the total combined area of all estates relevant per tract was successfully included in the survey (see Felton et al., 2022). We compiled survey data (mean kilogram per hectare per study area) concerning sugar beet (*Beta vulgaris*), carrot (*Daucus carota*), potato (*Solanum tuberosum*), and fruit (type of fruit not specified, but most commonly apples [*Malus domestica*] are used as supplementary feed in the region).

Data analysis and statistics

Metabarcoding relies on PCR, which is an inherently biased reaction, due to differences in primer binding efficiencies and guanine–cytosine (GC) content (Nichols et al., 2018). We therefore used a polymerase master mix, which has been shown to be the least biased during PCR of plant material (Nichols et al., 2018), which facilitated the use of relative read abundance (RRA) of taxa based on the proportion of a MOTU identified within a sample, as in Spitzer et al. (2021). Furthermore, because we compare RRA within plant taxa across time, any existing bias should be consistent in this regard.

For this analysis, we defined an NC-rich crop (NCC) in our winter study system as belonging to one of the four MOTUs: sugar beet, potato, Apiodeae, and Maloideae (other plant taxa could have been relevant but were not found in our data). The latter two MOTUs are on the subfamily level, and we assume (based on macroscopic observations, Felton, Holmström, et al., 2020) that these MOTUs indicate the presence of carrot and apple respectively (the latter was found in only four fecal samples). All four NCC types were used as supplementary feeds in the region (Appendix S1: Figure S1).

Previous research has indicated that diet composition does not differ significantly among age-sex classes of

moose in this region (Felton, Holmström, et al., 2020). We therefore sorted the DNA results from the 248 individual moose, of any age or sex, into three categories: Category 1 are individuals without any traces of NCC in either rumen or fecal samples ($n = 143$); Category 2 are individuals with identified NCC in fecal samples (could also be present in rumen samples) ($n = 76$); and Category 3 are remaining individuals (i.e., NCC present in rumen but not fecal samples) ($n = 29$). Experimental data suggests that the mean retention time of food particles in the alimentary canal of moose can differ between 20 and 70 h depending on the diet (Baker & Hobbs, 1987; Clauss et al., 2006, 2011). Moose rumen content can, for example, represent one or two days of ingested forage (Schwartz et al., 1987), with the feces content more likely to represent meals spanning about 2 days (Schwartz et al., 1985), but can span as many as 10 days (Hjeljord et al., 1982). Hereon, we refer to the moose in Category 2 as moose that had ingested NCC in prior feeding, because the NCC were identified in their fecal sample. Pulp from beet roots has high ruminal degradability, even in relation to other concentrate feeds commonly given to cattle (Woods et al., 2003). We therefore assume that the presence of NCC in fecal samples represents intakes that were made earlier in comparison with NCC we found in the rumen.

Items found in the rumen were likely ingested the same day as the individual was shot. However, food items rich in structural carbohydrates, such as woody twigs, have relatively low digestibility in the rumen of moose (Krizsan et al., 2018), which results in longer retention times. In roe deer, the total mean retention time was about 20 h when fed a low-fiber diet versus 30 h when fed a high-fiber diet during wintertime (Holand, 1993). We therefore consider the presence of woody items in the rumen to potentially reflect a mix between recent meals and meals ingested more than a day ago.

All statistical analyses were done in R version 4.0.2 (R Core Team, 2020). We used nonmetric multidimensional scaling (NMDS) based on the Bray–Curtis distances of relative abundance of 15 MOTUs in the rumen samples followed by a permutational multivariate ANOVA (perMANOVA, Anderson, 2001) to test whether there were differences in rumen species composition between Categories 1 and 2. Because we were interested in the potential effects of prior intake of NCC on the dietary items found in the rumen, we did not include Category 3 in this analysis. Across all the rumen samples included in Categories 1 and 2, 10 MOTUs each represented at least 1.0% of DNA, and together they represented on average 94% (SD 11%) of the rumen DNA. However, in some samples, these 10 plant species made up less than 50%. We therefore added the next five most common MOTUs that

had low percentages overall but allowed each sample's total to reach at least 50% of rumen DNA (Appendix S1: Table S2). We summed the %DNA contributed by these 15 MOTUs and calculated their individual proportions from that sum (total 100%). We also performed a test of multivariate homogeneity of group dispersions (PERMDISP2, Anderson, 2006) to check for differences in compositional diversity between the categories. Prior to the NMDS analysis, the data were square root transformed and then standardized (Wisconsin double standardization). The NMDS, perMANOVA, and PERMDISP2 were done with the metaMDS, adonis2, and betadisper functions, respectively, all in the vegan package (Oksanen et al., 2013) and with 999 permutations.

The NMDS indicated that the relative presence (see below) of the MOTU *Picea*, which we interpret as representing *P. abies*, was the major difference between moose belonging to Categories 1 and 2. We therefore tested whether the proportion of NC-rich foods in the fecal sample increases the probability of finding *P. abies* in the rumen. *P. abies* is a relatively rare forage for moose, and has potentially longer retention time in the rumen, which may exaggerate its proportion of the total DNA. For this reason, we conservatively treated the presence of *P. abies* in rumen as a binary response variable, either eaten (1) or not eaten (0), while we treated % NCC in feces as a continuous variable. As a conservative measure to capture intentional ingestion of *P. abies*, we treated *P. abies* as present in moose rumens only when the proportion of *P. abies* DNA reads corresponded to at least 1% of DNA reads in a rumen sample (i.e., $\geq 1\%$ of DNA = present [1], otherwise absent [0]).

Due to their relative similarity in macro-nutritional composition (all rich in NC), we initially combined the MOTUs, sugar beet, potato, and carrots, into a root vegetable group (apples occurred in only four fecal samples, so their effect could not be modeled). However, because we found that carrot revealed a very different pattern from the other root vegetables, we split the data and report the results separately. For comparing the effects of NC-rich (supplementary) foods with the effects of a more natural diet baseline, we also included the aggregate proportion of AROW tree species as a predictor in the analyses. These broadleaf trees have been shown to be highly selected by moose (Månsson et al., 2007).

We modeled the probability of *P. abies* presence in rumen samples as a Bayesian logistic regression with the NCC and AROW food groups in fecal samples as predictors. Because the suspected mechanism (i.e. recent feeding on *P. abies* is prompted by prior feeding on NC-rich foods) was expected to be generally true for moose across the study sites, and to allow for sufficient sample sizes, all 248 moose individuals were included in the model (see Felton et al., 2024a, 2024b). Analyses were carried out using JAGS (Plummer, 2003) via the rjags package in R (Plummer et al., 2016). We used a Bayesian framework because all estimated and derived parameters represent posterior distributions from which the probability of an effect being different from zero can be directly calculated (Johansson et al., 2018). For each coefficient, we examined the overlap of its posterior distribution with 0, that is, we calculated the probability of a coefficient being positive or negative. Subsequent interpretation is that a probability of 50% indicates a mean estimate for the coefficient of zero. Consequently, parameters where the posterior distributions largely overlap zero (the probability is close to 50%) have no predictive value and can be considered unimportant to the process being modeled (Low et al., 2016).

The differences in the effect of different predictors (e.g., does food group A have a stronger effect on *P. abies* consumption than has food group B?) can be directly quantified by subtracting the posterior distributions (A–B) within the JAGS model structure (Felton et al., 2024a, 2024b). The proportion of the resulting probability distribution which is above zero then corresponds to the probability that A > B (Aronsson et al., 2020). For all Bayesian models we used vague priors. MCMC chains (Markov chain Monte Carlo) were run for 50,000 iterations with a burn-in of 10,000 to allow for stabilization. Convergence was assessed on the basis of three chains by visual inspection of trace plots and the Gelman–Rubin diagnostic (<1.1; Gelman & Rubin, 1992). We were also interested to know whether the presence of NCC in fecal samples could predict the presence of NCC in rumens (assuming a negative relationship due to the presumed imbalance of such feed). Unfortunately, there were too few rumen samples with the NCC feed types present to statistically test this.

RESULTS

Availability of the most common tree species

The total count of *P. abies* within browsing height of moose across all study areas (1312 trees/ha young forest) was more than three times higher than the presence of *P. sylvestris* (381 trees/ha). The total number of *Betula* sp. stems was higher than both conifers (4956 trees/ha). These densities were calculated as a mean across inventoried production stands (by the national moose browsing

inventory), in each of the six MMAs. The density of trees of *P. abies*, *P. sylvestris*, and *Betula* sp. differed among the six study areas (Appendix S1: Figure S1, Table S3). The proportion of land area representing production stands, with mean height of 1–4 m, was on average 8% (range 4%–16% among study areas; Appendix S1: Table S3).

Availability of NC-rich crops as supplementary feed

Results from the telephone survey showed large differences among study areas in how much NCC were made available to wildlife by local land owners (Appendix S1: Figure S1). Supplementary feeding with NCC was most common in study area G: in total, 47,361 kg root vegetables (97.3% sugar beet, 2.5% carrot, 0.2% potato) were made available here during the winter, in a survey area of 3051 ha, resulting in a mean of 265 kg/ha (Appendix S1: Figure S1). In the other MMAs, the availability of NCC was much lower, ranging between 0 and 21 kg/ha. Note that the sampled moose in this study ranged across much larger areas than the areas covered by this telephone survey, so the results should be seen only as an indication of differences among MMAs and of the overall much lower availability of NCC than conifer forage across the landscape.

Overall description of plants identified in rumen and fecal samples

We found a total of 169 MOTUs in rumen and fecal samples combined. However, many of these MOTUs were detected in very low relative abundance, and the number of MOTUs with at least 1% of DNA was only 15 (Figure 3). A similar number of MOTUs were found in rumen samples (133) as in fecal samples (132). The proportions of the MOTUs with at least 1% of DNA was also similar between rumen samples (12 MOTUs) and fecal samples (16 MOTUs). The MOTU *Pinus* was dominant in both data sets (52% in rumen and 54% in fecal), followed by *Vaccinium* and *Calluna vulgaris* (Figure 3). *Picea* was the fourth most prevalent MOTU in rumen samples (5%) and the eighth most prevalent MOTU in fecal samples (2%). *Salix* contributed to 5% of DNA in both data sets.

Within the data set ($N = 248$ moose individuals), we found NC-rich crops present in 107 individuals (either in rumen or fecal sample or both), spanning all six MMAs. However, the six MMAs were not evenly represented in our two categories of moose based on the presence of NC-rich crops in our samples (Appendix S1: Figure S2). Of the identified NCC in rumen samples, carrot (*Apioideae*) was the most encountered, with the maximum per sample being as high as 54% of the DNA. Across fecal samples from moose in Category 2, the mean
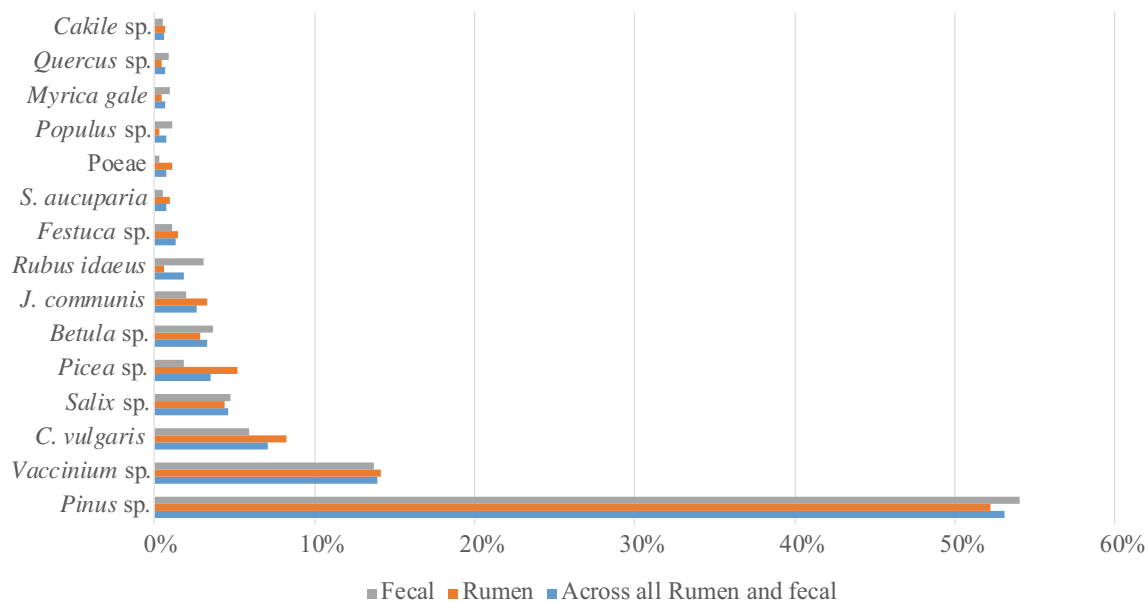
FIGURE 3   Percent of DNA of molecular operational taxonomic units (MOTUs) found in rumen and fecal samples sourced from moose ($N = 248$ individuals; 496 samples) in southern Sweden (see Figure 1) October 2014–February 2015. MOTUs are sorted according to their relative presence in the overall mean (blue) across all rumen and fecal samples, and includes those that contribute with at least 1% of total DNA in this combined data set. The 15 MOTUs included in this figure together represented 95%–96% of the DNA in samples.

proportion of carrot was only 1.8% of DNA, and the mean proportion of beet (*B. vulgaris*) was 0.14% of DNA (Appendix S1: Table S4). It is possible that NCC are more digestible than other forage items and will lead to fewer traces of DNA in the fecal samples.

Composition of MOTUs found in rumen samples (NMDS)

The NMDS analysis (Figure 4) required three dimensions to converge and to achieve an acceptable stress level (0.17). There was a significant ($p = 0.004$) difference in rumen plant composition between moose individuals belonging to the two different categories according to the perMANOVA. In other words, the moose that had included NCC in prior feeding had significantly different plant composition in their rumen than moose that had not eaten NCC at all. As illustrated by the NMDS, most of this difference was allocated along the first dimension, which showed its strongest correlation with the proportion of *Picea* (Figure 4). *Pinus* showed no such relationship. In addition, there was a significantly ($p = 0.04$) larger dispersion among the rumen samples of moose that had eaten NCC according to the PERMDISP2 test (see confidence ellipses around the centroids in Figure 4), indicating a larger compositional diversity in this category, despite this sample being considerably smaller.

## Probability of finding *P. abies* in rumen

We found that even small proportions of sugar beet and potatoes in fecal samples, indicating prior intake, strongly predicted the presence of *P. abies* (Figure 5; Bayesian logistic regression: 99.9% of the posterior distribution >0; Table 1), whereas this effect was very weak for carrots (Apioideae; 63% of the posterior distribution >0; Table 1), and the AROW species food group (67% of the posterior distribution >0; Table 1). We also found a 99.8% probability that the effect of sugar beets and potatoes on *P. abies* consumption was higher than that of carrots or AROW (on average, 60.4 percentage points higher compared with carrots and 72.7 percentage points higher compared with AROW).

DISCUSSION

Using fecal and rumen samples from the same moose individuals as a proxy for different feeding events, we found a relationship between the composition of rumen content and the ingestion of crops rich in NCs.

Specifically, even small proportions of starch- and sugar-rich feed (sugar beet and potato) in the fecal matter, indicating previous intake, strongly predicted the probability of moose consuming *P. abies* (Figure 5). The pattern we observed could not be explained by conventional food quality paradigms, for example, that the moose would ingest more *P. abies* forage after/when eating crops because it is of "higher quality." Relative to the crops eaten, *P. abies* forage is far lower in energy and higher in fiber (Figure 2, see also Felton et al., 2021; Widén et al., 2022), and thus conventionally classified as a so-called "low quality" food item. Instead, we suggest that the nutritional balancing hypothesis can explain our observation.

All animals—regardless of whether they are ruminants or not—must choose what to eat and how much, but these choices are limited by what is available at various spatial and temporal scales. Decisions are governed in part by evolved regulatory mechanisms, such as feedbacks between the gut, brain, and taste receptors (Ellsworth et al., 2013; Provenza, 1995; Simpson & Raubenheimer, 2012). To date, many species of animals have been found to select foods to achieve a nutritionally balanced diet, and the moose does not appear to be an exception. In fact, research has shown that the preferred nutritional target for moose in Sweden during wintertime appears to be close to the composition of twigs of *Salix* spp. (Felton et al., 2016, 2021; Spitzer et al., 2023), and that compared with NC-rich crops, ingestion of any of the common woody forage plants would take the moose closer to their target balance (Figure 2). Complementing the consumption of NC-rich food items with items closer to this macro-nutritional target ratio therefore appears to be a suitable response by the moose. Furthermore, our assessments showed that while the availability of young forests and conifer forage trees (particularly spruce) was high, the overall availability of NCC as supplementary feed was low (Appendix S1: Figure S2, Table S3). This supports the interpretation that moose balanced an intake of relatively rare and imbalanced NCC by ingesting woody material from common tree species, rather than the other way around. Compensatory feeding due to nutritional imbalance is a phenomenon that has been observed in a variety of taxa (Lambert & Rothman, 2015; Lee et al., 2004; Simpson et al., 2015). Often these patterns are revealed by researchers conducting controlled experiments. Examples of such experiments pertain to moose (Felton et al., 2016), red deer (Miranda et al., 2015), and white-tailed deer (Timmons et al., 2010). Our results indicate that these findings are not limited to captive settings, but are mirrored by wild populations operating at landscape scales.
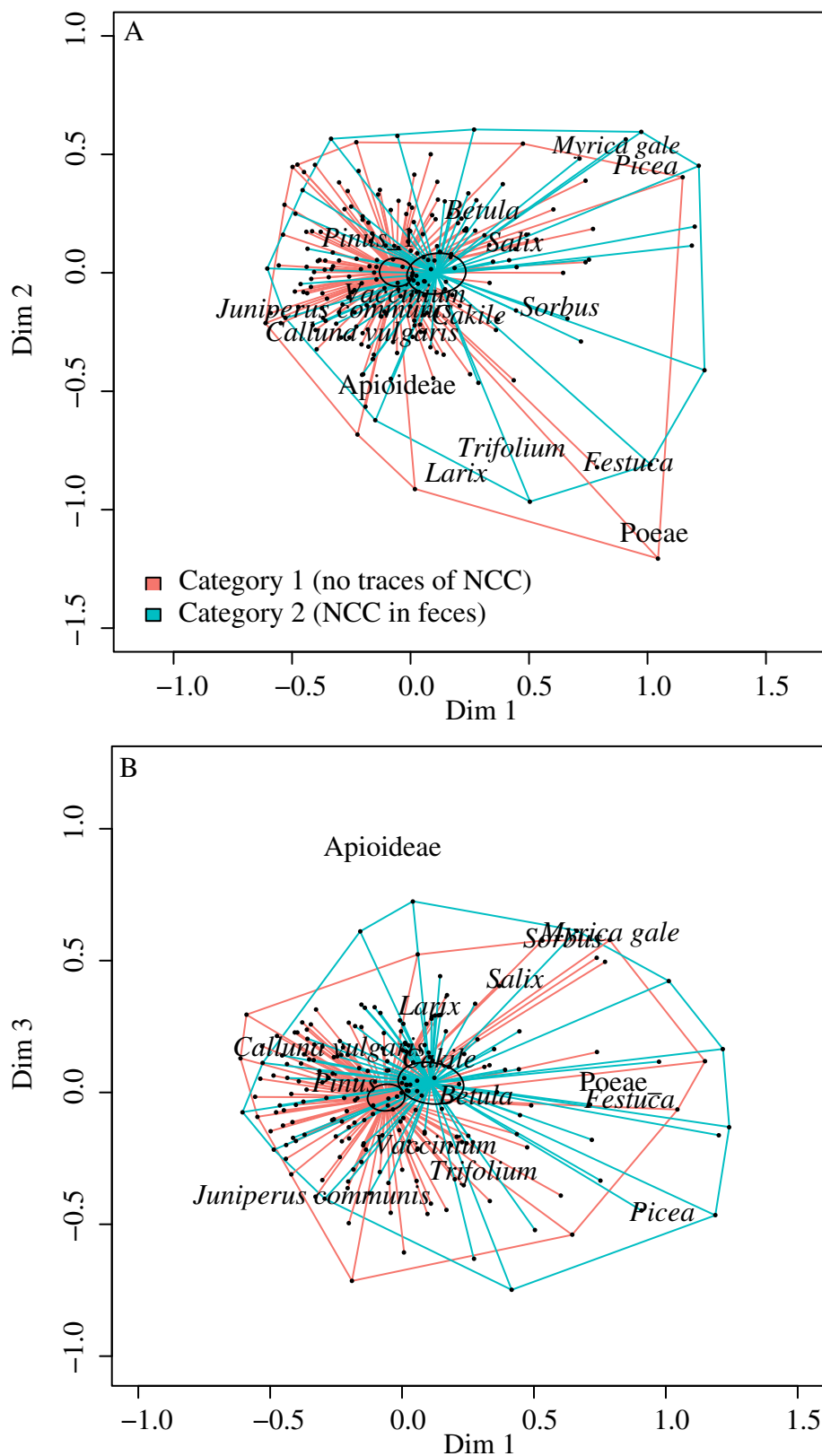
FIGURE 4 Nonmetric multidimensional scaling of the plant compositions of moose rumen samples, sampled during winter. (A) Dimensions 1 versus 2; (B) Dimensions 1 versus 3. Points show the position of the individual rumen samples in ordination space while the polygons show the distribution limits of the two moose categories: Category 1 (red)—Moose individuals without any traces of any crops rich in nonstructural carbohydrates (NCC) in either rumen or fecal samples ($N = 143$); Category 2 (blue)—Individuals with NCC in fecal samples ($N = 76$). Ellipses around the centroids are 95% CIs.
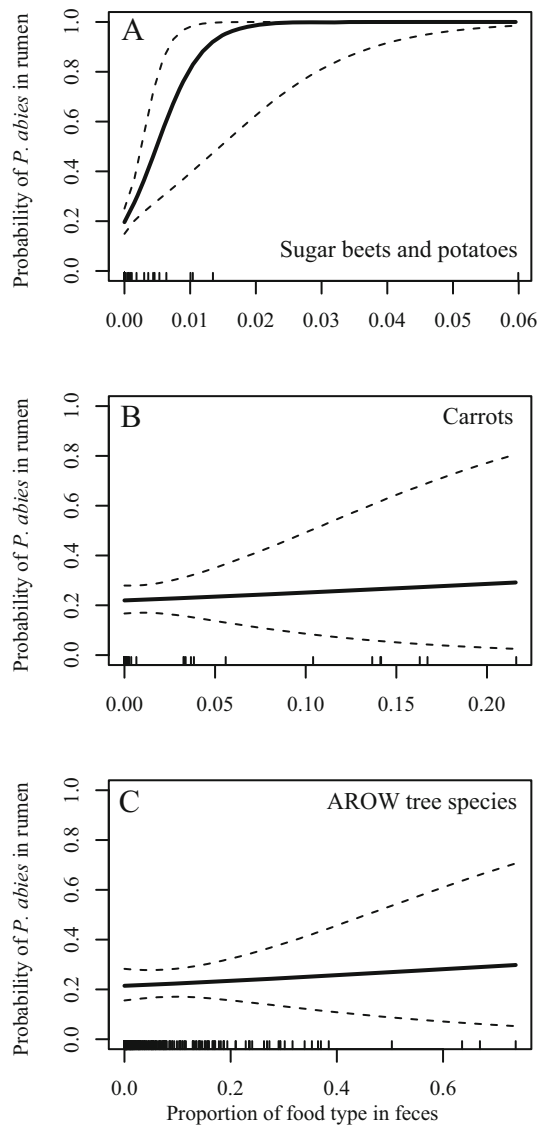
FIGURE 5 The probability of *Picea abies* being present in moose rumens depending on the previous consumption of different food groups: (A) sugar beets and potatoes, (B) carrots, and (C) AROW tree species. Solid lines represent the medians of the posterior distribution, and dashed lines indicate the 95% credible intervals. The rug bars on the *x*-axis represent the proportions of the different food groups in the fecal samples. Please note the different data ranges on the *x*-axis.

In addition, moose need to maintain a rumen environment beneficial for their rumen flora. Ingestion of too much starch or sugar can result in a shift in the rumen pH, which in turn can result in ruminal acidosis and deterioration of the microbial flora if pH cannot be corrected by diet choice (Keunen et al., 2002). Moose should be particularly sensitive to this during winter, as the composition of their rumen flora at this time is more specialized to digest relatively nutrient-poor winter diet of woody material with high-fiber contents

(Van Soest, 1994), and the moose' metabolism is correspondingly relatively low (the so-called hidden hibernation, Arnold, 2020). The sudden intake by a moose of sugar-rich food items during wintertime may therefore provide an extra strong signal, leading to a more urgent need to compensate for its off-target state: by either picking the nearest forage plant (e.g., *P. abies*), or spending time searching for a more preferred plant (e.g., *P. sylvestris* or AROW). In general, the more divergent an ingested food type is to the normal diet, the stronger such "off-target" signals should be within the body of a consumer (Berthoud et al., 2012), with the signals helping the individual to get closer to its preferred nutritional target by influencing food choices (Raubenheimer et al., 2022).

Another potential explanation that can act in parallel with the above is the interplay between nutrients and potentially toxic chemicals (Behmer et al., 2002; Nersesian et al., 2012). The ability of a herbivore to tolerate plant secondary metabolites (PSMs) can be linked to nutrient supply, due to the nutritional costs involved in detoxification (Illius & Jessop, 1995). It is possible that the high amounts of macronutrients provided by the NC-rich crops in our study enhanced the moose's ability to tolerate the specific PSMs in *P. abies*, which seem to be partly responsible for the low consumption of this plant species by moose under most circumstances (e.g., higher concentrations of total phenols and condensed tannins in *P. abies* forage compared with *P. sylvestris*, Stolter et al., 2009). Thereby, a plant that normally is chemically well defended, such as *P. abies*, can, after the ingestion of NC-rich crops, become more tolerated.

Based on the above, it may seem strange that moose would ingest starch- and sugar-rich food items in the middle of winter if such items cause an upset to their digestive system. The qualitative resource-constraint hypothesis (Cotter et al., 2011) may help us understand this behavior. The macronutrient requirements needed to satisfy some pairs of biological traits of an animal may be noncomplementary, such that no single blend of ingested nutrients can optimally satisfy both traits (Cotter et al., 2011; Raubenheimer et al., 2022). While the ingestion of NC-rich feeds may appear counterproductive with respect to the moose' rumen environment, the extra energy may help fulfill another demand, for example, thermoregulatory requirements (Guo et al., 2018). The optimal dietary balance may also vary among individuals and across different life stages, leading to different nutrition-mediated trade-offs depending on the circumstance (Raubenheimer et al., 2022). We emphasize that the proportions of NC-rich crops found in rumen samples were generally low (average < 5% of DNA; although as much as 54% was observed). Further research is needed to identify at which level of intake, and over which

TABLE 1 Results from Bayesian logistic regression used to predict the probability of *Picea abies* presence in rumens.

Parameter	Posterior distribution	95% credible interval	Effect probability
Intercept	−1.46 ± 0.20	−1.86 to −1.08	...
Sugar beets and potato	296.35 ± 114.64	94.94 to 540.0	0.999
Carrots	1.37 ± 6.06	−11.32 to 12.52	0.633
AROW	0.56 ± 1.41	−2.31 to 3.23	0.668

Note: The table shows the mean ± SD of the posterior distribution for model coefficients and their 95% credible interval. For each coefficient, the proportion of the posterior distribution is shown as the "effect probability" (1 = complete certainty; 0.5 = complete uncertainty), that is, the probability that the effect of the parameter on the presence of *P. abies* in moose rumens is in the direction of the sign (positive or negative) in front of the coefficient. AROW stands for the broad-leaved trees *Populus tremula*, *Sorbus aucuparia*, *Quercus* sp., and *Salix* sp.

duration, the inclusion of NC-rich crops in winter diets becomes negative for the long-term health of the moose.

The observed proportions of *P. abies* in our samples were also generally low (average 2% and 5% of DNA in fecal and rumen samples, respectively). It is normal for *P. abies* to play a minor role in the diet of moose and other deer in Sweden (Månsson et al., 2007), while *P. sylvestris* is a staple food for many populations (Cederlund et al., 1980; Spitzer, 2019). This raises the question as to why the moose compensates for an imbalanced meal with forage from *P. abies*, and not another forage species? Analyses of the macro-nutritional composition of 12 common winter forages for moose in this region indicate that forage from any tree during winter would provide an acceptable complement, due to the large differences in NC and fiber content between these woody plants and root vegetables (Figure 2). We suggest that the much higher abundance of *P. abies* within the browsing height of moose in this region influences the pattern we observed, due to cost efficiency. The availability of young *P. abies* trees was three times higher than the availability of young *P. sylvestris* trees in our study areas. The argument that the relative availability of plant species is a strong driver behind the observed foraging pattern is supported by the fact that study area C stands out in our data: in that area, the availability of *P. sylvestris* within browsing height was highest (Appendix S1: Figure S1), and this is also where rumen samples contained the highest proportion of *P. sylvestris*. In areas where *P. sylvestris* is also readily available, the moose likely compensate with this species too.

This does however raise the related question as to why the moose did not try to compensate with *Betula*, even though *Betula* twigs also provide moose with well-balanced forage (Figure 2). *Betula* trees are readily available in all study areas, but the proportion of *Betula* in the diet was not a significant explanatory factor in our analysis (Figure 4). Interestingly, *Betula* trees are often an enigma in moose research. For example, studies from southern Sweden and Norway have found that *Betula* is

underrepresented in the diet of moose populations (Felton, Holmström, et al., 2020; Wam et al., 2018; Wam & Hjeljord, 2010), compared with what has been recorded for moose populations in the north of Sweden (e.g., Spitzer et al., 2023). Likewise, a recent study found that only a very small proportion of the available *Betula* forage in southern Sweden was used by local moose populations (Ara et al., 2022). Why these moose populations do not make greater use of this abundant food resource requires further research, particularly with respect to possible structural or chemical deterrents.

It remains similarly unclear why the concentration of carrot in fecal samples did not predict the probability of finding *P. abies* in the rumen, while the concentration of sugar beets and potato did (Figure 5); this is despite all three being root vegetables that are rich in easily digestible carbohydrates (Felton et al., 2021). One possible explanation is that the carrot-eating moose balanced their diet with dwarf shrubs (*Ericaceae*) instead of with *P. abies*. We suggest this because a previous study of these populations found that the moose located where carrot was commonly provided as supplementary feed, and which had carrot most prevalent in their diets (study area G), also consumed higher proportions of dwarf shrub than other moose populations (Felton, Holmström, et al., 2020).

A source of potential error in our study stems from the digestibility of some items and their trnL copy number. We used chloroplast DNA as the marker to identify plant taxa, and only very small amounts of DNA from root vegetables were found in the rumen samples. Unfortunately, we do not know whether these small readings were in fact due to limited root vegetable consumption (due to sporadic opportunistic intake linked to availability), or from the digestibility of these food items, or their trnL copy number. However, the overall dietary pattern we observed in this study closely mirrors what has been reported regarding the moose populations' dietary consumption in this area using other methods (e.g., macrohistology, Felton, Holmström, et al., 2020).

Second, we expect there to be large variation in time span between meals identified in rumen and fecal data among individuals in our study. We had no data on exactly when different forages were ingested, nor their respective retention time. Importantly, the pattern observed cannot be explained entirely by the potentially longer retention time of *P. abies* in the digestive system of the moose (see *Data analysis and statistics*). If the presence of *P. abies* in rumen was only due to retention time, we should see approximately equal proportions of this tree species in rumen and fecal samples. There is also a potential mismatch between our data and the expected time of nutrient balancing regulation. The time period of regulation in moose has not been established, but a feeding experiment with captive moose suggests that they may regulate their macronutrient intake on a 24-h basis (Felton et al., 2016).

Implications for forest and wildlife management

Our results show that the relative concentrations of NC-rich crops in the fecal matter of a moose predict the presence of *P. abies* in the rumen with significant confidence. Inappropriate supplementary feeding of game animals (using NC-rich crops) could therefore have implications for forestry, in terms of potential damage to the production trees. Because free-ranging moose are not known to dig up root vegetables, we can be confident that access to the sugar beets and potatoes was via (intended or unintended) supply by humans. Most of these crops are also harvested prior to the period in which we collected moose samples. Even though supplementary feeding can benefit the animals in terms of increased winter survival and reproduction, unintended side effects are prevalent (Milner et al., 2014). Other means of increasing forage availability for local game populations, for example, retaining and creating sources of natural forage such as trees and shrubs, come with fewer negative side effects and at the same time a large number of biodiversity and ecosystem service co-benefits. We therefore suggest that maintaining a diversity and abundance of native forage plants offers a more promising landscape-scale solution for successfully combining forestry with healthy cervid populations and additional societal values.

Even though relatively small amounts of *P. abies* may be eaten per moose individual, there could be significant total browsing pressure on these trees in an area, through the combined browsing pressure of several coexisting cervid species. For example, even though *P. abies* is also generally avoided as a forage by fallow deer, they do browse significantly more often on *P. abies* in southern Sweden when it is located close to supplementary feeding sites, especially if there is a limited availability of alterative forage in the field layer (Garrido et al., 2014). If we adopt a longer term perspective, speculative concerns can be raised as to whether the continued supplementary feeding of agricultural crops to wild cervids will habituate them to a higher consumption of *P. abies* than would otherwise occur. This outcome may likewise be enhanced by the artificially high prevalence of *P. abies* in southern Sweden due to its preferential use in forestry (Felton, Petersson, et al., 2020). Of related concern, recent observations in Finland indicate that moose are decreasing the amount of *P. sylvestris* and increasing the amount of *P. abies* in their diets (Anonymous, 2016). In some regions *P. abies* is already under pressure from cervids, due to mature trees often being debarked by red deer (Jarnemo et al., 2014). Interestingly, agricultural crops also play a role in that context, as *P. abies* stands located close to fields of rapeseed plants (a crop rich in protein and energy) suffer significantly more bark stripping damage than stands located at greater distances from these crops (Jarnemo et al., 2022). Even minor additional sources of pressure on *P. abies* stands would not be welcomed by forest owners. In Sweden, the Forest Agency has a set goal of keeping browsing damage on young *P. abies* trees below 1% of stems on a yearly basis (SFA, 2018a). The current levels of damage to *P. abies* trees within browsing height is 1.3% in the southern part of Sweden (SFA, 2022), already exceeding the Agency's goal.

However, we note that an increased amount of *P. abies* biomass consumed by moose as observed in this study does not necessarily result in the damage of economic concern. Browsing damage is usually defined as involving the consumption of the top shoot, bark peeling, or stem breakage (SFA, 2022). Moose can obtain biomass from browsing on side shoots, but such consumption is not generally noted as damage. Further research needs to be done to identify whether the level of *P. abies* consumption observed in this study results in damage or not.

CONCLUSIONS

By studying the content of rumen and fecal material from the same moose individuals inhabiting the southern Swedish rural landscape, we found indications of increased intake of *P. abies* to be induced by the inclusion of crops rich in NCs in their diet. We suggest three nonexclusive physiological explanations to this pattern: (1) nutrient balancing in general; (2) the specific circumstances of

the moose rumen environment; and (3) the interplay between macronutrients and detoxification. Our findings shed new light on the nutritional ecology of a large free-ranging ruminant, and on how habitat alterations such as supplementary feeding may change the foraging behavior of wildlife. Combined with earlier findings that the inclusion of NC-rich items in the diets of moose is associated with an imbalanced nutritional state in the rumen (Felton et al., 2021), and that the addition of such items in the moose' diet does not necessarily correspond to a fitness benefit (Felton, Holmström, et al., 2020), our results lead us to conclude that the provision of some supplementary feed has the potential to result in lose–lose outcomes for both moose health and forestry goals.

AUTHOR CONTRIBUTIONS

Annika M. Felton, Adam Felton, Jonas Malmsten, and Hilde K. Wam conceptualized the idea. Annika M. Felton, Jonas Malmsten, Robert Spitzer, Ruth V. Nichols, Erik Löfmarck, and Jonas Malmsten developed the methodology. Annika M. Felton, Robert Spitzer, Ruth V. Nichols, Erik Löfmarck, and Brendan L. O'Connell conducted the investigation. Annika M. Felton, Per-Ola Hedwall, and Robert Spitzer conducted formal analysis. Annika M. Felton wrote the original draft. Annika M. Felton, Robert Spitzer, David Raubenheimer, Per-Ola Hedwall, Adam Felton, Ruth V. Nichols, Brendan L. O'Connell, Jonas Malmsten, Erik Löfmarck, and Hilde K. Wam interpreted the results, and reviewed/edited the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Annika M. Felton administrated the project and acquired the funding.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.