https://lib.uliege.be https://matheo.uliege.be

Study of the winter diet quality of wild red deer populations under varying levels of predation-risk induced stress in the Bialowieza Forest.

Auteur : Origer, Marie
Promoteur(s) : Licoppe, Alain; 28756
Faculté : Gembloux Agro-Bio Tech (GxABT)
Diplôme : Master en bioingénieur : gestion des forêts et des espaces naturels, à finalité spécialisée
Année académique : 2024-2025
URI/URL : http://hdl.handle.net/2268.2/24116

Avertissement à l'attention des usagers :

Tous les documents placés en accès ouvert sur le site le site MatheO sont protégés par le droit d'auteur. Conformément aux principes énoncés par la "Budapest Open Access Initiative"(BOAI, 2002), l'utilisateur du site peut lire, télécharger, copier, transmettre, imprimer, chercher ou faire un lien vers le texte intégral de ces documents, les disséquer pour les indexer, s'en servir de données pour un logiciel, ou s'en servir à toute autre fin légale (ou prévue par la réglementation relative au droit d'auteur). Toute utilisation du document à des fins commerciales est strictement interdite.

Par ailleurs, l'utilisateur s'engage à respecter les droits moraux de l'auteur, principalement le droit à l'intégrité de l'oeuvre et le droit de paternité et ce dans toute utilisation que l'utilisateur entreprend. Ainsi, à titre d'exemple, lorsqu'il reproduira un document par extrait ou dans son intégralité, l'utilisateur citera de manière complète les sources telles que mentionnées ci-dessus. Toute utilisation non explicitement autorisée ci-avant (telle que par exemple, la modification du document ou son résumé) nécessite l'autorisation préalable et expresse des auteurs ou de leurs ayants droit.

STUDY OF THE WINTER DIET QUALITY OF WILD RED DEER POPULATIONS UNDER VARYING LEVELS OF PREDATION-RISK INDUCED STRESS IN THE BIAŁOWIEŻA FOREST

MARIE ORIGER

TRAVAIL DE FIN D'ETUDES PRESENTE EN VUE DE L'OBTENTION DU DIPLOME DE MASTER BIOINGÉNIEUR EN GESTION DES FORÊTS ET DES ESPACES NATURELS

ANNEE ACADEMIQUE 2024-2025

CO-PROMOTEURS: MARIA LOSADA & ALAIN LICOPPE

STUDY OF THE WINTER DIET QUALITY OF WILD RED DEER POPULATIONS UNDER VARYING LEVELS OF PREDATION-RISK INDUCED STRESS IN THE BIAŁOWIEŻA FOREST

MARIE ORIGER

TRAVAIL DE FIN D'ETUDES PRESENTE EN VUE DE L'OBTENTION DU DIPLOME DE MASTER BIOINGÉNIEUR EN GESTION DES FORÊTS ET DES ESPACES NATURELS

ANNEE ACADEMIQUE 2024-2025

CO-PROMOTEURS: MARIA LOSADA & ALAIN LICOPPE

Acknowledgements

This work was conducted as part of the project “*The multilevel impact of predation-induced stress in ungulates on the functioning of the temperate forest ecosystem”*, funded by the National Science Center (grant MAESTRO number 2021/42/A/NZ8/00126). This research was carried out at the Mammal Research Institute, Polish Academy of Sciences (Białowieża, Poland), which I gratefully acknowledge for hosting me. I also acknowledge the Erasmus+ programme for providing a mobility grant that enabled my stay abroad.

I would like to thank all interns from the Mammal Research Institute and Robin for the great atmosphere. Merci aussi à la promotion Forêt, et plus particulièrement à mes chipies pour la bonne ambiance. Merci Ma d’avoir été à mes côtés depuis le tout début ! Merci à ma maman et à Lucie pour le soutien sans faille et pour croire en moi, sans vous je n’en serais pas arrivée là.

I am grateful to Basia for her help in the laboratory, and the people that conducted fieldwork to collect the samples. I also thank Professor Schmidt for his ongoing support during my stay, for sharing his experience and the very helpful feedback on my thesis. Merci à Monsieur Licoppe d’avoir accepté d’être mon promoteur, ainsi que pour ses retours très encourageants.

And lastly, the biggest thank you goes to Maria. Thank you so much for your supervision, you were the best supervisor I could have wished for, always very supportive, encouraging, and fun. Thank you for everything

Table of content

Résumé

Les grands prédateurs, tels que le loup (*Canis lupus*), peuvent induire un stress chez leurs proies, entraînant des modifications comportementales. Celles-ci incluent notamment des changements dans les stratégies d'alimentation, susceptibles d'affecter la qualité du régime. Selon le *General Stress Paradigm*, les herbivores soumis à un stress chronique lié aux prédateurs ajusteraient leur comportement alimentaire en sélectionnant des aliments plus riches en glucides et plus pauvres en protéines, afin de répondre à une demande énergétique accrue. Cependant, l'impact du risque de prédation sur la qualité du régime alimentaire des herbivores, en particulier chez les grands mammifères, reste peu étudié. Les ongulés sauvages peuvent adapter leurs choix alimentaires en réponse à une combinaison de facteurs environnementaux et anthropiques. Dans les écosystèmes tempérés, cet effet peut être accentué en hiver, une saison caractérisée par une disponibilité réduite des ressources et des conditions climatiques contraignantes. J'ai étudié la manière dont la population de cerf élaphe (*Cervus elaphus*) de la forêt primaire de Białowieża (Pologne) ajuste la qualité de son régime hivernal en fonction du risque de prédation par le loup, son principal prédateur local, tout en prenant en compte les facteurs environnementaux, saisonniers et anthropiques. J'ai utilisé la spectroscopie dans le proche infrarouge (NIRS) pour analyser les variations d'indicateurs de qualité du régime (protéines brutes, fractions fibreuses et leurs ratios dérivés) dans 279 échantillons fécaux collectés à la fin de l'hiver 2023. Le risque de prédation a été estimé à l'aide d'un modèle spatial basé sur des données de présence du loup obtenues via des pièges photographiques déployés la même année. Si le risque de prédation seul n'a pas eu d'effet significatif, son interaction avec d'autres covariables, en particulier la saisonnalité, la distance à la frontière et la proximité des routes, a influencé de manière significative la qualité du régime alimentaire. Celle-ci (indiquée par la teneur fécale en protéines et les ratios protéines/fibres) augmentait avec le risque de prédation, mais cette tendance s'inversait en fin de saison d'échantillonnage, coïncidant avec le début de la saison végétative. Cela suggère que le cerf ajuste ses besoins nutritionnels en réponse au risque de prédation, mais uniquement lorsque la disponibilité de végétaux augmente. Les perturbations anthropiques ont atténué l'effet du risque de prédation sur la qualité du régime alimentaire, qui n'était détectable que lorsque les cerfs se trouvaient à distance des sources de perturbation (frontière, routes). Ces résultats soutiennent l'idée que le cerf ajuste ses choix alimentaires en réponse à une combinaison de facteurs liés à la prédation, à la saisonnalité et aux activités humaines. Ce contexte multifactoriel souligne l'importance de prendre en compte les effets interactifs lors de l'évaluation des changements alimentaires des herbivores, en particulier en lien avec le risque de prédation et durant les périodes de contrainte alimentaire. L'approche interdisciplinaire proposée ici, combinant l'analyse fécale par NIRS et la modélisation spatiale du risque de prédation à partir de pièges photographiques, apporte des éléments précieux pour la compréhension des interactions prédateurs-proies ainsi que de leurs implications plus larges sur le cycle des nutriments et la dynamique des communautés végétales en milieu forestier.

Abstract

Large predators, such as wolves (*Canis lupus*), can induce stress in their prey, causing changes in their behaviour. These include shifts in foraging strategies, which can affect diet quality. Following the General Stress Paradigm, herbivores experiencing chronic stress from predators are expected to adjust their foraging behaviour by selecting food richer in carbohydrates and poorer in proteins to meet their increased energetic demands. However, the impact of predation risk on herbivore diet quality, particularly among large mammals, remains understudied. Wild ungulates may adjust their foraging choices in response to a combination of environmental and human-related factors. In temperate ecosystems, this effect may be more pronounced during winter, a season characterised by limited forage availability and harsh climatic conditions. I investigated whether and how red deer (*Cervus elaphus*) population in the Białowieża Primeval Forest (Poland) adjusts their winter diet quality in relation to predation risk from wolves — their main predator in the area — while accounting for environmental, seasonal, and human-related factors. I used Near Infrared Spectroscopy (NIRS) to analyse the variation in diet quality indicators (including crude protein, fibre fractions, and their derived ratios) in 279 red deer faecal samples collected during late winter 2023. Predation risk was estimated using a spatial model based on wolf occurrence data obtained from camera traps deployed in the same year. While predation risk alone had no significant effect, its interaction with other covariates — particularly sampling day, distance to the border, and proximity to roads — significantly influenced diet quality. Red deer diet quality (as indicated by faecal protein content and protein-to-fibre ratios) increased with level of predation risk, however this trend was reversed at the end of the sampling season, coinciding with the onset of the vegetation season. This suggests that red deer shifted their nutritional demands in response to predation risk, but only when food choices increased. Human disturbance overrode the effects of predation risk on deer diet quality, the effect of which was only detectable when red deer were further away from disturbance sources (border, roads). These findings support the idea that red deer adjust their forage choices in response to a combination of predation, seasonal, and human-related factors. This multifactorial context highlights the importance of considering interactive effects when assessing herbivore dietary shifts, particularly in relation to predation risk and during the food-constrained season. The multidisciplinary approach proposed here, combining faecal NIRS analysis with camera trap-based predation risk modelling, provides valuable insights into predator–prey interactions, as well as their broader implications for nutrient cycling and plant community dynamics within forest ecosystems.

Introduction

Large predators, beyond the direct effects of consumption (Creel & Christianson, 2008), can induce stress in prey species, resulting in changes in their physiology and behaviour (Say-Sallaz et al., 2019; Clinchy et al., 2013). According to the General Stress Paradigm (GSP), animals under chronic stress (long-term exposure to predators) relocate energetic resources from growth and reproduction toward maintenance and survival processes (Hawlena & Schmitz, 2010b). This physiological shift is accompanied by an increase in glucocorticoid levels, which activate gluconeogenesis, converting proteins into glucose to produce readily available energy for "fight or flight" (Hawlena & Schmitz, 2010a). Consequently, chronically stressed herbivores are expected to adjust their foraging behaviour by selecting food richer in digestible carbohydrates and lower in protein (Hawlena & Schmitz, 2010b). These foraging decisions may be reflected in diet quality; however, detecting such patterns in animal excreta is challenging, as different trade-offs between predation risk and food acquisition can affect them (Verdolin, 2006; Lima & Dill, 1990). Decreased consumption of protein-rich plants may result in lower nitrogen (N) content in excreta, while increased gluconeogenesis may simultaneously elevate N excretion, leading to inconsistent levels of faecal (fN) or urinary nitrogen (Hawlena & Schmitz, 2010b). Beyond crude protein (CP) fraction, it is therefore important to consider other dietary components, such as fibre fractions and their relative proportions in faeces to more accurately assess diet quality in wild herbivores (Cupic et al., 2023; Corlatti et al., 2013).

Predator hunting modes can influence prey stress responses to predation risk. Ambush predators (e.g., lynx) often trigger stronger physiological and behavioural reactions than cursorial predators (e.g., wolves) (Rinehart & Hawlena, 2020), particularly in areas where their home ranges overlap (Schmidt et al., 2009). In Białowieża Primeval Forest (BPF, Poland), red deer have been shown to adapt their behaviour to wolves by increasing vigilance at the expense of foraging when exposed to wolf olfactory cues (Kuijper et al., 2014). In contrast, exposure to lynx cues shortened their stay (Wikenros et al., 2015), demonstrating their ability to differentiate the risk induced by both predators and modify their responses accordingly. Human activity can further modulate how red deer respond to predation risk. Recent studies have shown that ungulates avoid areas where they were previously exposed to human hunting (Ausilio et al., 2025; Bojarska et al., 2024). In BPF, the Polish-Belarusian border and the intensified human activity around it have deterred wildlife from approaching this area (Nowak, 2025). Such human disturbances may heighten the risk perceived by wild ungulates, thereby affecting their behaviour and habitat selection (Theuerkauf & Rouys, 2008). Beyond affecting ungulates' habitat selection, predation risk also shapes prey foraging behaviour and the trade-off between safety and food acquisition (Preisser et al., 2005).

In wild ungulates, several studies have demonstrated that predation risk can induce changes in foraging behaviour. In the BPF, red deer within the wolf core area increased their consumption of broadleaf trees, possibly due to heightened vigilance and more frequent head-up foraging (Churski et al., 2021). Similar behavioural shifts have been observed in other ecosystems. For instance, zebras in Hwange National Park, Zimbabwe, exhibited higher stress levels when foraging close to lions (Barnier et al., 2014), while red deer in Yellowstone National Park (YNP), USA, avoided open areas (with higher-quality forage but greater risk) in favour of forest areas (with lower food-quality, but safer from predation) following wolf reintroduction (Hernández & Laundré, 2005). Both ungulate species exhibited lower fN levels when exposed to predators, suggesting an habitat shift toward lower-quality foraging areas, likely reflecting a trade-off between predation risk and forage quality. Yet, such foraging decisions are context-dependent. During winter, a period of natural food scarcity, red deer in high-wolf density areas in YNP showed higher fN concentrations

(Christianson & Creel, 2010). This increase possibly reflected starvation-induced catabolism rather than improved forage quality. These examples highlight the need to consider both seasonal and spatial variability in forage quality when studying prey dietary responses to predation risk.

Red deer are considered intermediate feeders, both browsers and grazers (Roberts et al., 2015), but predominantly browsers in forest habitats, especially during winter, when they primarily feed on leaves and twigs of woody plants (Ratkiewicz et al., 2024). In the Biebrza Valley (eastern Poland), woody plants, both coniferous and broadleaved, comprised 86% of roe deer and 77% of red deer winter diets (Ratkiewicz et al., 2024). In the western part of BPF, coniferous forest predominates (55%), mainly consisting of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) (Jaroszewicz et al., 2019). Shrubs and coniferous browse become particularly important in late winter, as they contain higher CP levels than broadleaf trees and forbs, though they also contain higher levels of fibres and tannins, which reduce forage digestibility (Zhong et al., 2019; Obidzinski et al., 2013). Despite winter food constraints, red deer rarely rely on supplementary feeding by humans (Cupic et al., 2023; Obidzinski et al., 2013) and maintain dietary balance by adjusting the composition of their food intake to meet their nutrient demands (Ratkiewicz et al., 2024; Roberts et al., 2015). Although individual variability in winter diet quality can be high within species, environmental factors such as temperature and snow cover can still exert detectable effects on diet quality when using anonymous faecal sampling (Corlatti, 2020).

Near-infrared spectroscopy (NIRS) provides a non-destructive, rapid, and low-cost method to assess diet quality in faecal and plant samples (Evangelista et al., 2025; R. Dixon & Coates, 2009; Dryden, 2003). Although the use of fN concentration as a proxy of diet quality remains debated, due to its interaction with secondary plant metabolites, CP content, when estimated using the 6.25 nitrogen correction factor (Mariotti et al., 2008) shows a positive correlation with forage quality and digestibility when interpreted alongside other macronutrient indicators (Leslie et al., 2008). These indicators include fibre fractions: neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). NIRS is widely recognised for its utility in ungulates' diet quality studies, generally used for calibration (Tellado & Azorit, 2015; Jean et al., 2014; Showers et al., 2006; J. Kamler et al., 2004), as well as for quantifying dietary components in field-based studies on wild ungulates (Corlatti et al., 2023; Cupic et al., 2023; Hola et al., 2016; Vance et al., 2016).

In BPF, red deer are known to avoid areas with high wolf presence (Bubnicki et al., 2019) and modify their behaviour in response to perceived predation risk by increasing their vigilance (Kuijper et al., 2014). However, foraging decisions under predation risk can be overridden or buffered by habitat selection and food availability (Churski et al., 2021; Theuerkauf & Rouys, 2008). Despite these behavioural adaptations, the effect of predation risk on the diet quality of red deer remains largely unexplored, particularly during winter, the most severe and food-constrained season, when forage scarcity might play a critical role in sustaining wild ungulates (Bramorska et al., 2023).

Deer under chronic stress induced by predation risk may balance their winter foraging between carbohydrate-rich and protein-rich forage according to their nutritional demands. Understanding how predation risk shapes herbivore foraging behaviour, especially during the food-constrained period, may not only shed light on predator–prey dynamics, but also on their broader impacts on nutrient cycling and plant community structure in forest ecosystems (Hawlena & Schmitz, 2010b). This study aims to investigate the effects of wolf-induced predation risk on the winter diet quality of a free-ranging red deer population in the

BPF, by combining data from camera-trapping surveys with data from faecal NIRS analysis. Based on the GSP framework, and considering the trade-off between predation risk and food quality — possibly shaped by seasonal, environmental, and anthropogenic influences — I hypothesised that (i) red deer exposed to high predation risk will reduce diet quality towards lower protein and higher fibre content; (ii) human activity will attenuate the dietary response of red deer to predation risk, and (iii) seasonality of vegetation abundance will amplify the expected red deer dietary response to increased predation risk. I believe, this study should contribute to the development of an emerging field of NIRS analysis-based ecological research, by using macronutrient composition in faeces as a proxy for assessing foraging responses of free-living large mammals to environmental pressures at the population level.

Methods

Study area

This study was conducted in the Białowieża Primeval Forest (BPF), a European lowland temperate forest, declared a UNESCO World Heritage Site and Biosphere Reserve, spanning approximately 1,500 km² across the Polish–Belarusian border (52°41′ N; 23°49′ E, WGS84), with about one-third of its area located in Poland. BPF lies on a flat plain ranging from 135 to 202 m a.s.l. (Jaroszewicz et al., 2019), and interspersed with shallow river valleys and depressions (Kwiatkowski, 1994). The BPF is characterised by a warm-summer humid continental climate (Jaroszewicz et al., 2019). Mean daily temperature is -5 °C in January and 18 °C in July, while mean annual precipitation is 641 mm (Theuerkauf & Rouys, 2008). The BPF differs from other Western European forests by the absence of beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*). Forest stands are dominated by Scots pine (*Pinus sylvestris),* Norway spruce (*Picea abies)* and black alder (*Alnus glutionosa*) (Jaroszewicz et al., 2019). Around 50% of the Polish part of the BPF is covered by mesic and mesotrophic habitats originally typical for mixed deciduous forest. However, forest management practices have led to an increased share of coniferous cover, which now comprises around 55% of the Polish section of the BPF (Jaroszewicz et al., 2019). The Białowieża National Park (BNP) covers an area of 105 km², with 60 km² designated as strictly protected. In addition, 21 nature reserves cover an area of 120 km².

The study area covers 298 km² within the Polish section of the BPF (Figure 1). This area includes part of the extended zone of BNP (50 km² added to the park in 1996), where hunting is prohibited. The remaining area consists of managed forest, where logging or partial logging occurs, and nature reserves, where neither logging nor hunting is permitted. Nine habitat types, corresponding to phytosociological associations, are identified in the study area: fresh pine forests (*Peucedano-Pinetum*); moist pine forests (*Molinio-Pinetum*), swamp pine forests (V*accinio uliginosi-Pinetum*), mixed oak-pine forests (*Querco roboris-Pinetum*), boreal moist oak-spruce forests (*Querco-Piceetum*), fresh lime-oak-hornbeam forests (*Tilio-Carpinetum* fresh), moist lime-oak-hornbeam forests (*Tilio-Carpinetum* moist), ash-alder riparian forests (*Fraxino-Alnetum*), alder fen forest (*Ribeso nigri-Alnetum*) (Matuszkiewicz et al., 2022). The main rivers in the BPF are Narewka, Orłówka, and Hwoźna, which flow through sandy and clay-sandy soils (Kwiatkowski, 1994) and a mosaic of swampy terrain, dense forests, marshes, and open meadows (Jaroszewicz et al., 2019). Human settlements in the region are scattered and generally low populated (ranging from 9 to 1,620 people per village), with most inhabitants concentrated in the Bialowieza district (10.08 people/km²; (*Białowieża (Rural Commune, Poland)*, n.d.). A 186 km-long and 5.5 m-high wall was recently constructed along the Polish-Belarusian border, where intense military activity has occurred since its construction in 2022, impacting biodiversity (Nowak, 2025). Moreover, supplementary feeding sites for wild ungulates are uniformly distributed across BPF (Figure 1).

BPF hosts a great biodiversity, comprising 12,000 animal species, of which 59 are mammals (Jaroszewicz et al., 2019). The diverse community of large mammals is mainly represented by wild ungulates, such as red deer (*Cervus elaphus*), roe deer *(Capreolus capreolus*), moose (*Alces alces*), European bison (*Bison bonasus bonasus*), and wild boar (*Sus scrofa*). Among them, red deer is the most abundant ungulate species in the area (Bubnicki et al., 2019; Borowik et al., 2013) and the preferred prey of wolves (Jędrzejewska et al., 2002; Jędrzejewski et al., 2000). Two large carnivores, the grey wolf (*Canis lupus*) and the Eurasian lynx (*Lynx lynx)*, shape the landscape of fear in the BPF (Bubnicki et al., 2019). Other native carnivores include the red fox (*Vulpes vulpes*), European badger (*Meles meles*), pine marten (*Martes martes*), stone marten (*Martes foina*), least weasel (*Mustela nivalis*), stoat (*Mustela erminea*), and European polecat (*Mustela putorius*). The raccoon dog (*Nyctereutes procyonoides*), now widespread, is an introduced species (Jędrzejewski et al., 2000).

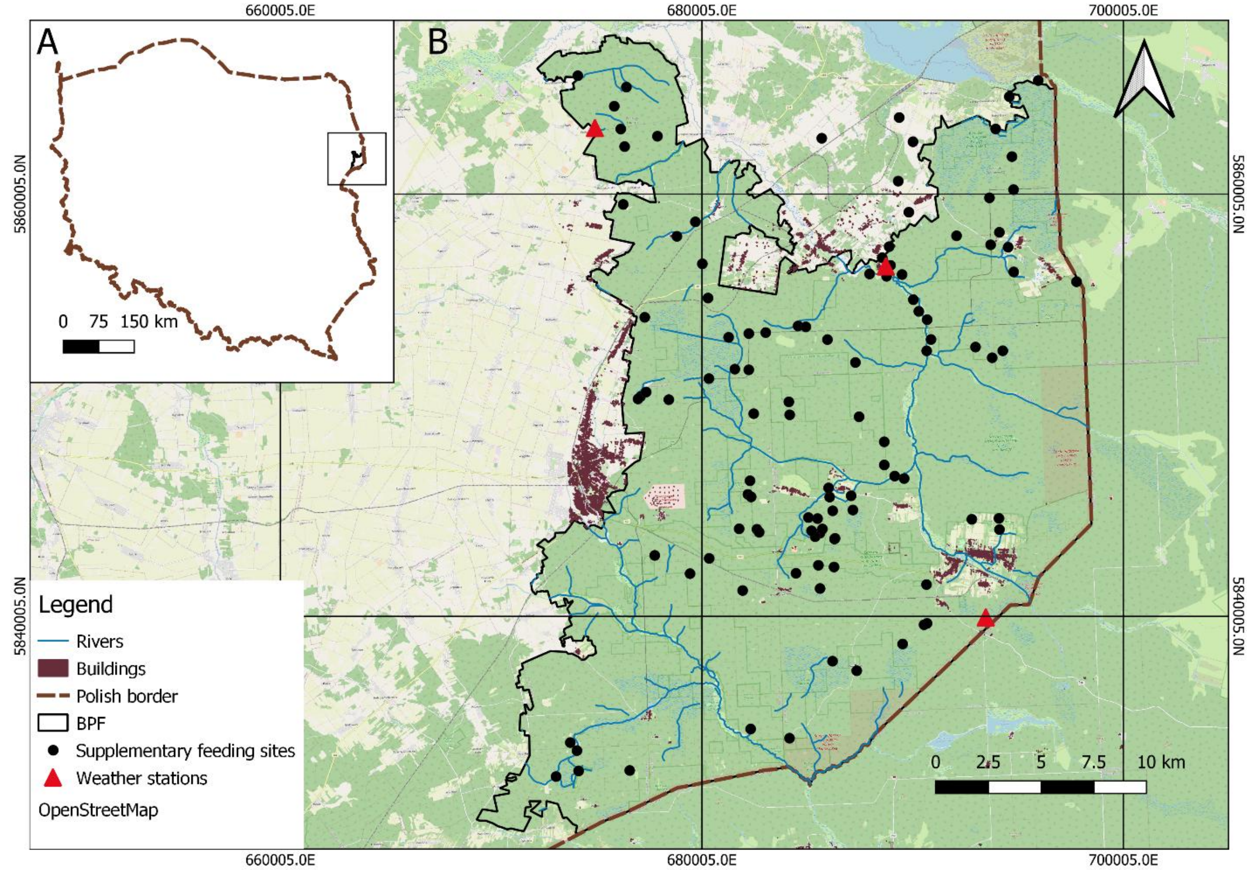

Figure 1*: Map of the study area in Białowieża Primeval Forest (WGS84/ UTM zone 34N), Poland (layer: https://mapy.geoportal.gov.pl/wss/service/PZGIK/PRG/WFS/AdministrativeBoundaries), showing the location of rivers, buildings (layers: https://www.openstreetmap.org, OpenStreetMap, CC-BY-SA), supplementary feeding sites (layer: data from State Forests register combined with MRI PAS records), and meteorological stations for climate data (layer: www.ibles.waw.pl, licence CC BY 4.0 PL). Created with QGIS desktop 3.28.11 (QGIS Development Team, 2025).*

Field procedures

Sample collection

Red deer faecal samples (n = 325 pellet groups) were collected in winter 2023, between March 1st and April 3rd, after the end of the hunting season (February 28th), and covering a surface area of 298 km² (Figure 2). Weather conditions were generally snowy (snow depth: 0.66 ± 0.15 mm of water equivalent in March 2023) (Hersbach et al., 2020), dry (precipitation: 1.9 ± 3.0 mm/day in March 2023), and cold (temperature: 3.9 ± 4.2°C in March 2023) throughout the sampling season. The sampling protocol involved visiting previously mapped forest and gap plots. Upon arrival, two-person teams searched for animal paths along the edges of gaps and from the forest roads, tracking red deer into the forest. When fresh deer tracks were detected, they were followed until fresh faeces were located. GPS location was recorded, and faeces were classified as fresh (black, shinny, pointed pellets) or very fresh (pellets coated with gut mucus). Complete pellet groups (at least 20 pellets) were collected per location. Faeces found along the same animal path and with similar freshness, but located up to 200 m apart, were assumed to belong to different individuals based on the average daily defecation rate for red deer (Vala & Ernst, 2011) and their average daily movement distance (Pépin et al., 2004). In BPF, the estimated average daily movement range for red deer is 1 km² (J. F. Kamler et al., 2007). All faecal samples were collected using laboratory gloves and placed in Ziplock bags labelled with the date and sample number. Samples were stored in hand coolers with ice packs during field sampling and subsequently transported in an insulated cooler to the laboratory, where they were frozen at -20 °C until further analysis (Tellado & Azorit, 2014).

Predation risk

Sixty camera traps were deployed along the forest roads for carnivore monitoring in summer 2023, between July 3rd and September 15th, covering the entire study area (Figure 2). This annual carnivore monitoring based on camera trapping has been conducted by the Mammal Research Institute, Polish Academy of Sciences (MRI PAS) since 2016, generally in summer, after the breeding season. Camera traps (Browning BTC-PATRIOT-FHD with long-range invisible infrared flash) were set to record 30-second videos upon trigger, with a 1-second delay between recordings, and operating during day and night. Cameras were placed next to the forest roads to maximise large carnivore observations and were inspected biweekly (at least three times) during the deployment period. Out of 42,459 total recordings, 774 captured 1,082 individual wolf observations (mean ± SD: 1.4 ± 1.1 observations per event). These data were used to estimate the spatial variation in predation risk from wolves within BPF.

A predictive model of the expected number of wolves using a given grid cell was developed following the methodology described by Bubnicki et al. (2019). To develop this model, only wolf observations from the 2023 camera-trapping survey were used. For each 0.25 km² grid cell, the number of wolves using the cell during the sampling period was estimated using Binomial-Poisson N-mixture models, which are well suited for spatially replicated count data collected over multiple surveys at each site. Unlike the original study, no covariates were included in the model to estimate wolf space use of the landscape. The model output was interpreted as the predation risk perceived by prey species, reflecting the spatial probability of predator encounters. The predicted predation risk from wolves within BPF ranged from 1.7-7.2 individuals per grid cell (Figure 2).

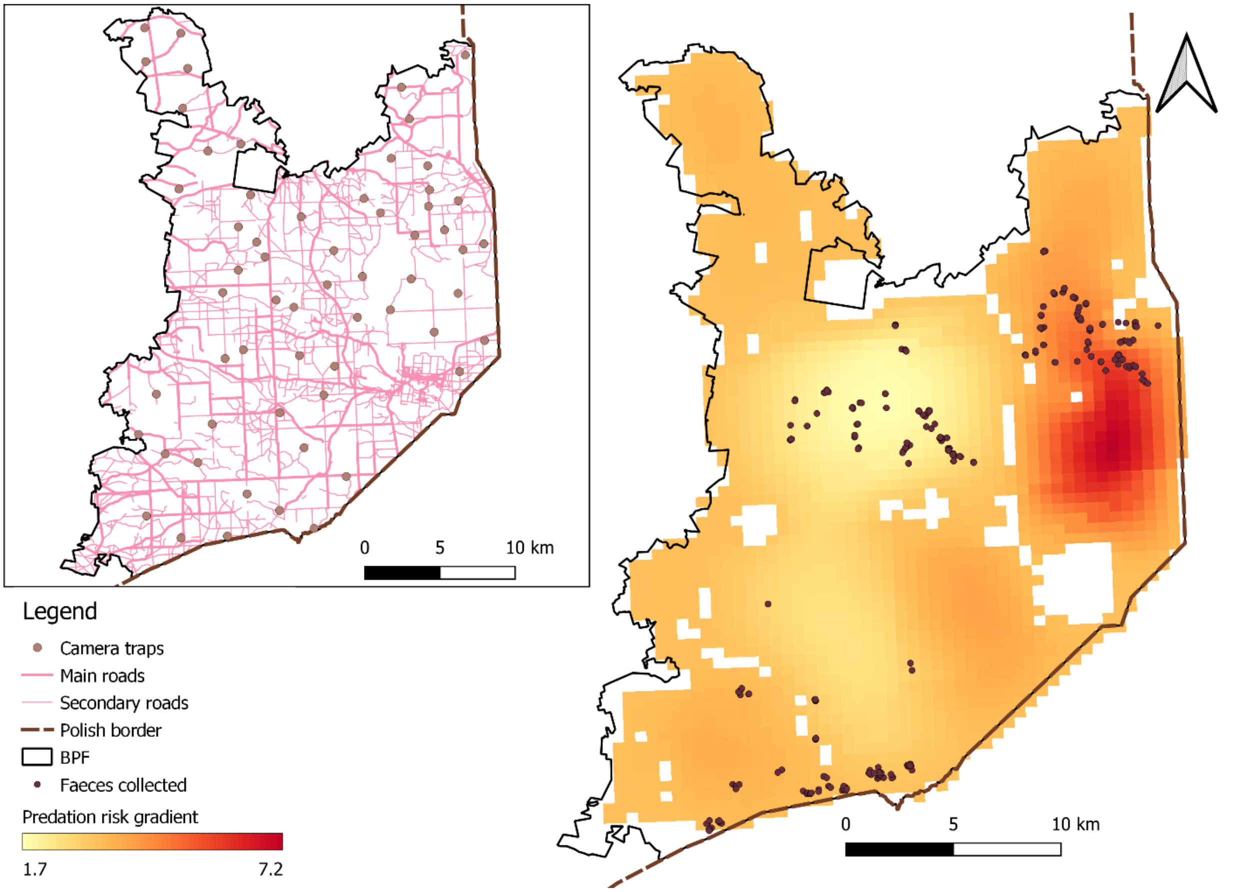

***Figure 2**: Map of the predicted spatial gradient of predation risk (adapted from Bubnicki et al., 2019) with the locations of the red deer faecal samples analysed (n = 294). The inset map shows the camera-trapping setup (n = 60 cameras) used for wolf monitoring in 2023, deployed along forest roads in the BPF (layer: https://www.openstreetmap.org, OpenStreetMap, CC-BY-SA).*

Lab procedures

Sample preparation

The 325 faecal samples were stored at -20°C in a freezer until processing. They were then oven-dried at 60°C until reaching a constant weight. After drying, samples were ground in a SM-450 mill (MRC Labs, Israel) for two minutes and 30 seconds for further dietary analysis. The ground samples were stored in dry conditions (paper envelopes inside hermetically sealed containers with silica gel) until analysis.

NIRS analysis

Near-infrared spectroscopy (NIRS) is an increasingly recommended chemometric technique for quantifying various nutritional constituents in herbivore faecal samples (R. Dixon & Coates, 2009). Spectral data were acquired in the near-infrared (NIR) region (4000 – 14286 cm^{-1}) using an Antaris II FT-Near Infrared Analyzer (Thermo Fisher Scientific Inc., USA). Dried and ground samples were placed in a 5 cm diameter sampling cup equipped with a quartz window and an internal plunger to ensure consistent filling of the cup. For each sample (n = 325), three spectra were collected (Figure 3). Each spectrum was generated by scanning the sample 32 times at a spectral resolution of 8 cm^{-1}, using a sample cup spinner to ensure uniform scanning across the sample surface. The sampling cup was carefully cleaned with a soft brush between samples. Every 15 samples, the entire container was washed with deionised water and air-dried. All scanning procedures were conducted using the OMNIC software (version 9 for Windows; Thermo Fisher Scientific Inc., USA).

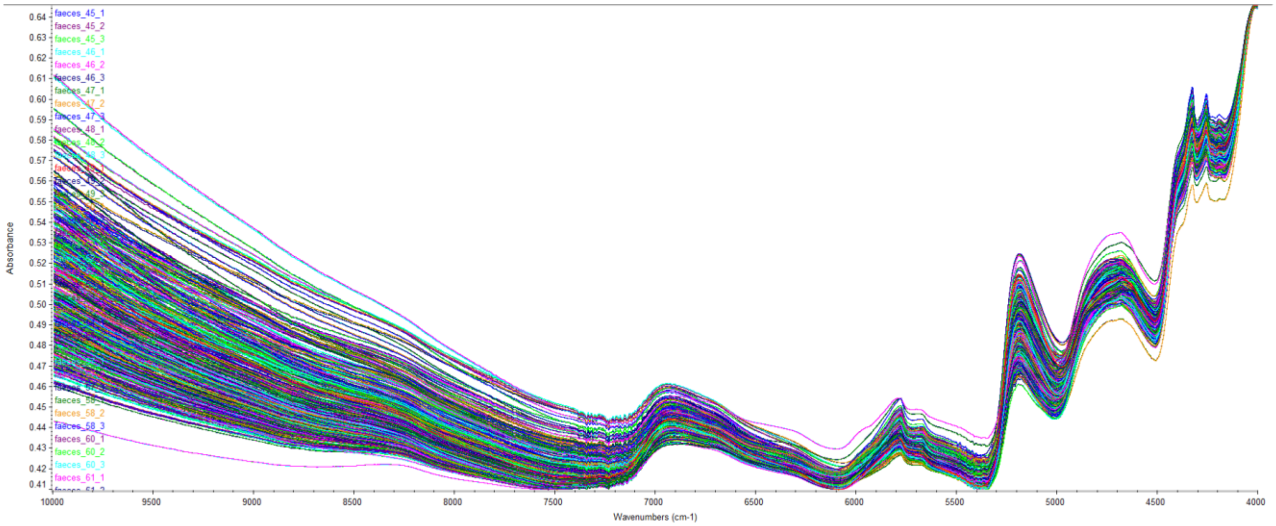

***Figure 3**: Near-infrared spectra obtained from 325 red deer faecal samples (three scans per sample). Each line corresponds to a single scan. The spectra illustrate the variability among samples prior to calibration and further analysis.*

NIRS calibration

An independent calibration model was developed for each of the four dietary components analysed: Crude Protein (CP) as indicator of high-quality forage, neutral detergent fibre (NDF) as indicator of total structural fibre (hemicellulose, cellulose, lignin), acid detergent fibre (ADF) as indicator of less-digestible fibre (cellulose, lignin), and acid detergent lignin (ADL) as indicator of non-digestible fibre (lignin) (Dryden, 2003). Thirty-one reference samples were randomly selected and analysed by Nuscana Biotechnical laboratory (Poland), following standard protocols for CP (Kjeldahl titration method, raw calculation by applying correction factor: 6.25), NDF (ISO 16472:2006, Application note C.Gerhardt), ADF and ADL (PN ISO 13906:2009 AOAC Official method 973.18, Application note C.Gerhardt). The 31 samples used for calibration were excluded from the statistical analysis. Calibration and quantification procedures were performed using the TQ Analyst software (version 9 for Windows; Thermo Fisher Scientific Inc., USA).

To assess the performance of each calibration model, the following metrics were considered: the root-mean square error of calibration (RMSEC), prediction (RMSEP), and cross-validation (RMSECV), as well as the correlation coefficient (r) of calibration, validation and cross-validation, following previous NIRS calibrations using red deer faeces (Tellado et al., 2015). The best model was selected based on the highest correlation coefficient and the lowest root-mean square error values. The range error ratio (RER = range of reference values/RMSEP) was also calculated to assess optimal calibration of selected models. A RER between 4 and 8 indicates suitability for screening purposes, values between 8 and 12 suggest the model can be used for quality control, and values above 12 indicate that the calibration is adequate for quantification (Tellado et al., 2015; Millmier et al., 2000).

Partial least squares (PLS) regression was used to obtain all calibration equations, as it is the most commonly used in NIRS calibration. PLS is particularly suited for handling overlapping absorbance peaks and correlations among spectral bands at different wavelengths. The optimal number of PLS factors was determined using the predicted residual error sum of squares (PRESS) diagnostic, which identifies the number of factors that minimises RMSECV. Potential outliers among reference values were assessed during calibration using Dixon's test ($n \leq 30$) (W. J. Dixon, 1950).

For NDF, ADF, and ADL, the second derivative was used as a spectral pre-treatment, and each corresponding model used three PLS factors. For CP, the best results were obtained using the first derivative pre-treatment and five PLS factors. The number of samples used for calibration and validation, respectively, was: CP (18/13), NDF (17/14), ADF (15/16), and ADL (16/15). All independent PLS calibration models included one spectrum per reference standard sample (Dryden, 2003). Results from all calibration models are presented in Table 1.

***Table 1**: Goodness of fit statistics to assess calibration model quality: root-mean square error of calibration (RMSEC), prediction (RMSEP), and cross-validation (RMSECV), with their respective correlation coefficient (r), and the range error ratio (RER).*

Components	Calibration		Validation		Cross-validation		Suitability	NIR (4000-14286 cm^{-1})
	r	RMSEC	*r*	RMSEP	*r*	RMSECV	RER	Spectral regions (cm^{-1})
CP	0.9844	0.263	0.9847	0.257	0.9308	0.546	19.13	4871.31 - 4801.88 4146.20 - 4126.92
NDF	0.9651	2.70	0.5908	7.54	0.7474	6.88	4.40	9287.50 - 9264.35 9673.19 - 9657.76 9719.47 - 9700.19
ADF	0.9566	2.07	0.5454	5.71	0.7585	4.75	4.39	9966.32 - 9954.75 8955.80 - 8932.66
ADL	0.9520	1.18	0.7185	2.93	0.6625	2.94	10.09	9665.48 - 9650.05 9553.62 - 9518.91 9245.07 - 9229.64

Statistical analysis

All statistical analyses were conducted using R software version 4.5.0 for Windows (R Core Team, 2021). To detect potential outliers among the three replicates per sample, a Dixon's Q test was applied using the dixon.test() function from the {outliers} R package (Komsta, 2022). This test is recommended for small sample sizes with normally distributed data (J. N. Miller & Miller, 2010). A total of 22 replicates for CP, 12 for NDF and ADF, and 16 for ADL were identified as outliers and excluded from the statistical analysis. The remaining replicates were averaged to obtain a single representative value per sample for each dietary component. The following protein-to-fibre ratios were calculated: CP/NDF (as a proxy of the proportion of protein relative to total fibre fraction) and CP/ADF (as a proxy of the proportion of protein relative to less-digestible fibre fraction). Additionally, the cell wall digestibility (CWD) index was estimated for each sample as following the equation (Tellado & Azorit, 2014):

$$CWD = 100 - (ADL/ NDF*10) \tag{1}$$

High CWD index values are associated with higher proportions of hemicellulose and cellulose relative to lignin content (indicating higher digestibility of the forage), whereas low CWD index values result from higher lignin content relative to hemicellulose and cellulose (indicating lower digestibility) (Tellado & Azorit, 2014).

Seven independent linear mixed models (LMMs) were fitted using the lme() function from the {nlme} R package (Pinheiro et al., 2025; Pinheiro & Bates, 2000). The respective response variables were the four dietary components (CP, NDF, ADF, ADL), the two protein-to-fibre ratios (CP/NDF, CP/ADF), and the digestibility index (CWD). Location of the faeces collected (GPS coordinates) was buffered within a 200 m radius (0.126 km²) and included as a random factor (n = 81 groups).

The focal predictor of the red deer dietary responses was the predation risk, included as a fixed effect in the LMMs. To control for potential human disturbance, the distance to the border, the nearest main road (meters), and their respective interactions with predation risk, as well as distance to the nearest building (meters), were also included as fixed effects. To account for spatial differences in food availability, the dominant habitat type (Matuszkiewicz et al., 2022) within a 1 km² buffer area around each sample location, distance to the nearest river and the nearest feeding site (meters) were added as fixed effects. The chosen buffer area (1 km²) corresponds to the average daily home range of red deer in the BPF (J. F. Kamler et al., 2007).

Temporal variation was accounted for by including the sampling day (n = 16), counted as consecutive days from the start of the sampling period (following the end of the hunting season), and its interaction with predation risk. Additional fixed effects included sample freshness at the time of collection (categorized as fresh *vs*. very fresh), air temperature 5 cm above ground (°C) on the sampling day, and precipitation (mm) on the day before faeces collection. Freshness and temperature were included to control for sample degradation, while precipitation was considered due to its potential influence on deer foraging behaviour. Climatic data were obtained from three meteorological stations (Figure 1) belonging to the Forest Research Institute (the data were obtained and prepared as part of the "LIFE+ ForBioSensing PL: Comprehensive monitoring of stand dynamics in the Białowieża Forest using remote sensing data" project, co-financed by the European Commission under the LIFE+ European Union financial instrument (contract no.: LIFE13 ENV/PL/000048) and the National Fund for Environmental Protection and Water Management (contract no.: 485/2014/WN10/OP-NM-LF/D) and data from the nearest station were assigned to each sample location (Boczoń & Sałachewicz, 2022).

Some samples were excluded from the analysis for the following reasons: missing predation risk value (n = 2), sample freshness not rated as “fresh” or “very fresh” (n = 6), or location >4.5 km from the nearest feeding site and identified as outliers using Rosner's test (n = 7), with the rosnerTest() function from the {EnvStats} R package (Millard, 2013). The final dataset analysed included 279 samples.

All continuous variables were standardised (mean = 0, SD = 1) to allow comparison of effect sizes and model convergence reasons. Global models included all above-mentioned covariates and were fitted using the maximum likelihood (ML) method. Multicollinearity was assessed using the vif() function from the {car} R package (Fox & Weisberg, 2019); all variance inflation factor (VIF) values were below 10 (or even below five for most of the variables). Model selection was performed using the stepAIC() function from the {MASS} R package (Venables & Ripley, 2002), applying both forward and backward selection. Final selected models were fitted using the restricted maximum likelihood (REML) method.

Results

In total, 294 samples were collected across an area of 298 km², where predation risk values ranged from 1.7 to 7.2 within grid cells sized 0.25 km². After removing outliers, the effect of predation risk on winter deer diet quality was assessed in 279 of the 294 faeces analysed via NIRS. Among the dietary indicators, crude protein (CP) showed the least variation (standard deviation (SD) = 1.70), whereas total fibre fraction (NDF) showed the greatest (SD = 4.5). However, none of the independent dietary components or their derived ratios varied significantly with predation risk when considered alone (Tables 2 and 3). The effect of predation risk on winter deer diet quality was only detected when examined in interaction with other influencing factors (such as sampling period, distance to the border, distance to the nearest road). Detailed results for each dietary component and ratios are described below.

CP

Crude protein (CP) content ranged from 5.02 to 17.26 % dry matter (DM), showing the lowest variability among all dietary indicators (mean ± SD = 11.01 ± 1.70 %DM). Linear mixed model (LMM) results indicated that faecal CP (%DM) significantly increased during the sampling period (March 1st–April 4th, 2023; 16 days) following the end of the hunting season on February 28th (see Table 2a, Figure S1 in the Supplementary Materials). Faecal CP content also increased with greater distance to the nearest main road (Figure S2). While predation risk did not have a direct effect on CP content, an indirect effect was observed through significant interactions with environmental variables: faecal CP content increased with predation risk further away from the state border (Figure 4) and decreased with increasing predation risk in early spring (Figure 5). Other potential confounding factors showed marginal effects, including a positive trend with distance to the nearest building ($p = 0.076$). Additionally, CP content in red deer faeces marginally declined with precipitation on the day before sampling ($p = 0.065$) and showed a marginal variation based on sample freshness ($p = 0.062$; Table 2a).

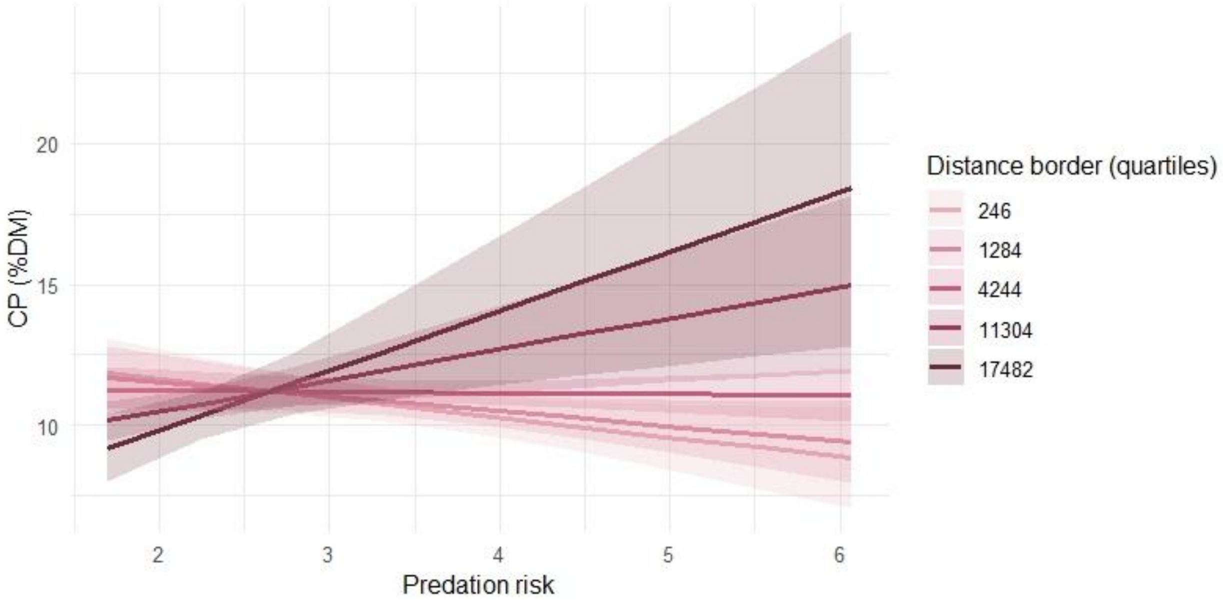

Figure 4*: Predicted values from linear mixed model showing the variation of crude protein content (CP % dry matter) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell) interacting with the distance to the state border (meters). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. The distance to the state border was grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

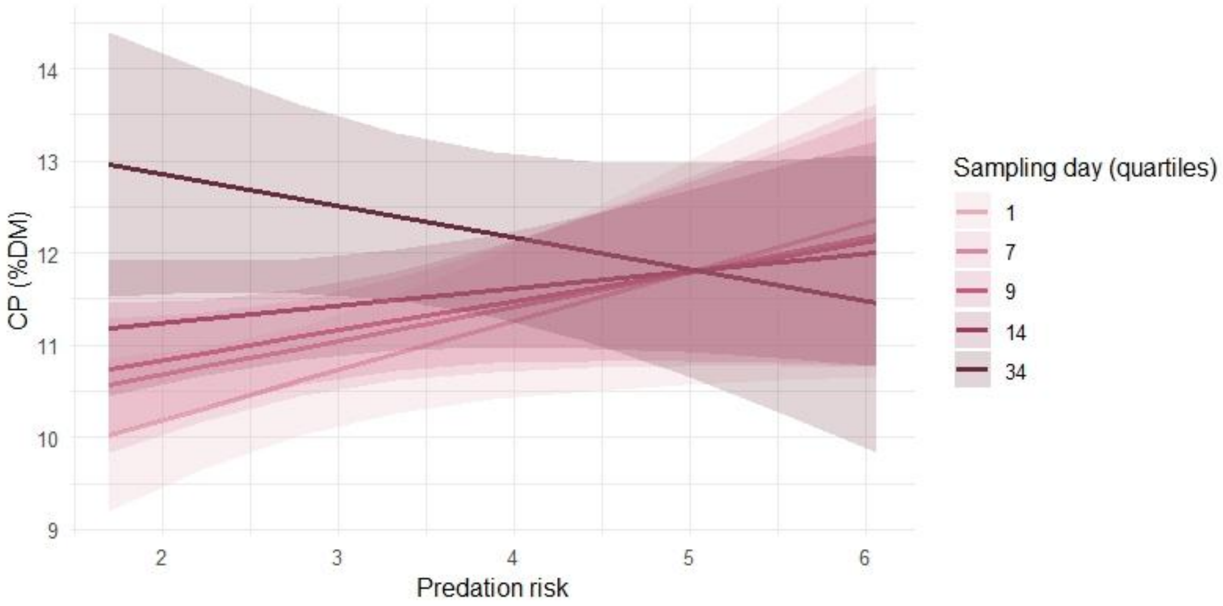

Figure 5*: Predicted values from linear mixed model showing the variation of crude protein content (CP % dry matter) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell), interacting with the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

NDF

Neutral detergent fibre (NDF) content ranged from 43.85 to 72.41 %DM in red deer faeces and showed the highest variability among all dietary indicators (mean ± SD = 57.31 ± 4.5 %DM). The second LMM revealed a significant increase in faecal total fibre content with precipitation on the day preceding sampling (Table 2b; Figure S3). The predation risk showed no significant effect on the NDF variation in red deer faeces, and none of the potential interactions with predation risk (sampling period, distances to the state border, nearest building, or nearest road) were selected. The faecal freshness showed a marginal negative effect on the NDF content (*p* = 0.062; Table 2b).

ADF

Acid detergent fibre (ADF) content ranged from 33.97 to 57.38 %DM and averaged 45.45 ± 4.27 %DM. The third LMM results showed no direct effect of predation risk on the content of the less-digestible fibre fraction, whereas the interaction between predation risk and the sampling period had a significant effect (Table 2c). During the first weeks of sampling at the end of winter, ADF content in red deer faeces showed a decrease with higher predation risk levels. As the sampling period progressed, this negative effect on ADF content weakened and ultimately reversed toward the early spring (onset of vegetation season), showing a significant increase in high predation risk areas (Figure 6). The faecal ADF content also decreased with the distance to the state border (Table 2c; Figure S4). Additionally, this less-digestible fibre content increased with precipitation the day prior to sampling (Figure S5).

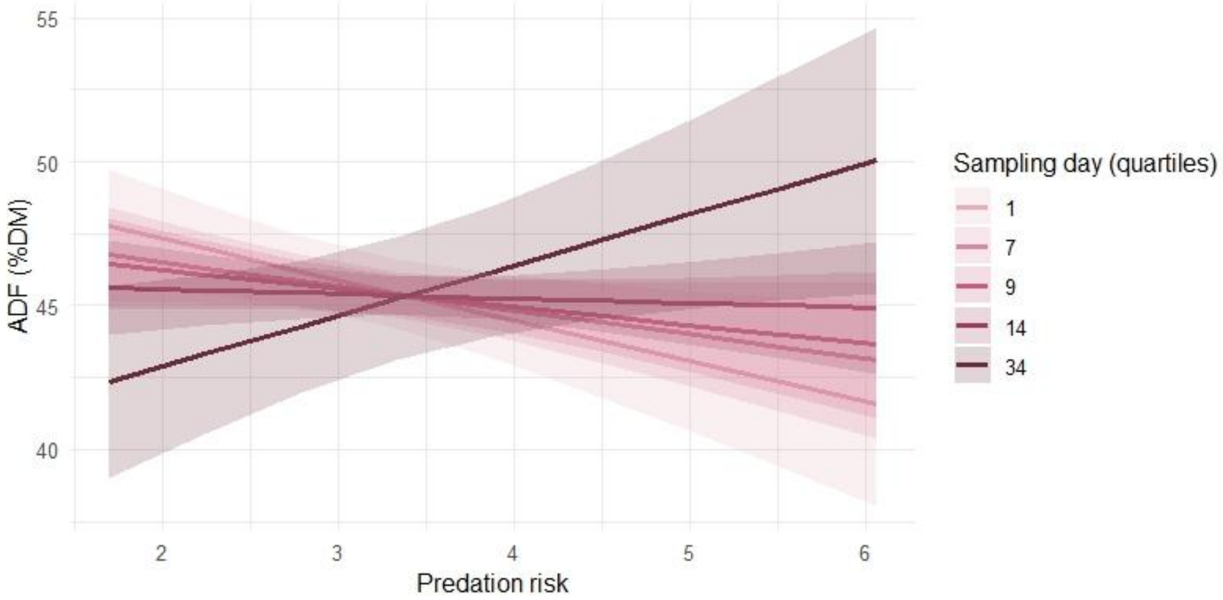

Figure 6*: Predicted values from linear mixed model showing the variation of acid detergent fibre (ADF) content in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell) over the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

ADL

Acid detergent lignin (ADL) content varied between 18.35 and 28.76 %DM and averaged 23.61 ± 2.12 %DM. The results of the fourth LMM indicated a decrease in faecal ADL content with the increasing distance to the nearest river (Table 2d; Figure S6), and to the nearest building (Figure S7). The faecal freshness showed a negative influence on the lignin content in the red deer faeces (Table 2d); very fresh samples contained less ADL. Additionally, ADL content increased with the temperature 5 cm above the ground (Figure S8), an environmental effect possibly associated with the sample freshness. Although predation risk alone had a marginally negative effect on ADL content in red deer faeces (*p* = 0.068), a significant interaction with the sampling period revealed an increasingly negative impact of predation risk on lignin content at the end of sampling period, coinciding with the onset of the vegetation season (Figure 7).

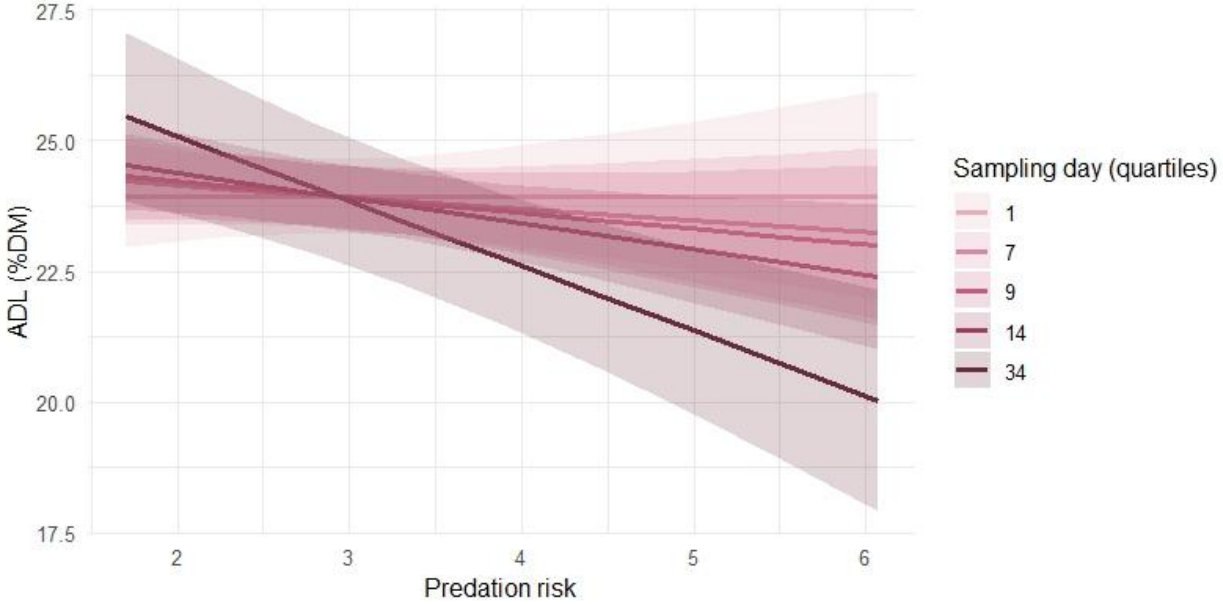

***Figure** 7: Predicted values from linear mixed model showing the variation of acid detergent lignin (ADL) content in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25km² grid cell) over the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

CP/NDF

The crude protein to neutral detergent fibre ratio (CP/NDF) varied between 0.09 and 0.34 and averaged 0.19 ± 0.03 among all samples. The CP/NDF ratio increased with the distance to the nearest building (Figure S9) and over the winter sampling period (Figure S10). Very fresh samples showed higher CP/NDF ratios than fresh faeces (Table 3a). The ratio of protein to fibre decreased with increasing precipitation the day before the sample was collected (Figure S11). While predation risk only had a marginally negative effect (*p* = 0.088), significant interaction effects with other variables have been detected: the protein-to-fibre ratio increased with predation risk and increasing distance to the state border (Figure 8), whereas it decreased with higher predation risk levels later in the sampling period, but generally increased during first weeks of sampling period (Figure 9). The distance to the state border and to the nearest road both showed a marginally positive effect on the CP/NDF ratio (border: *p* = 0.085, road: *p* = 0.095; Table 3a).

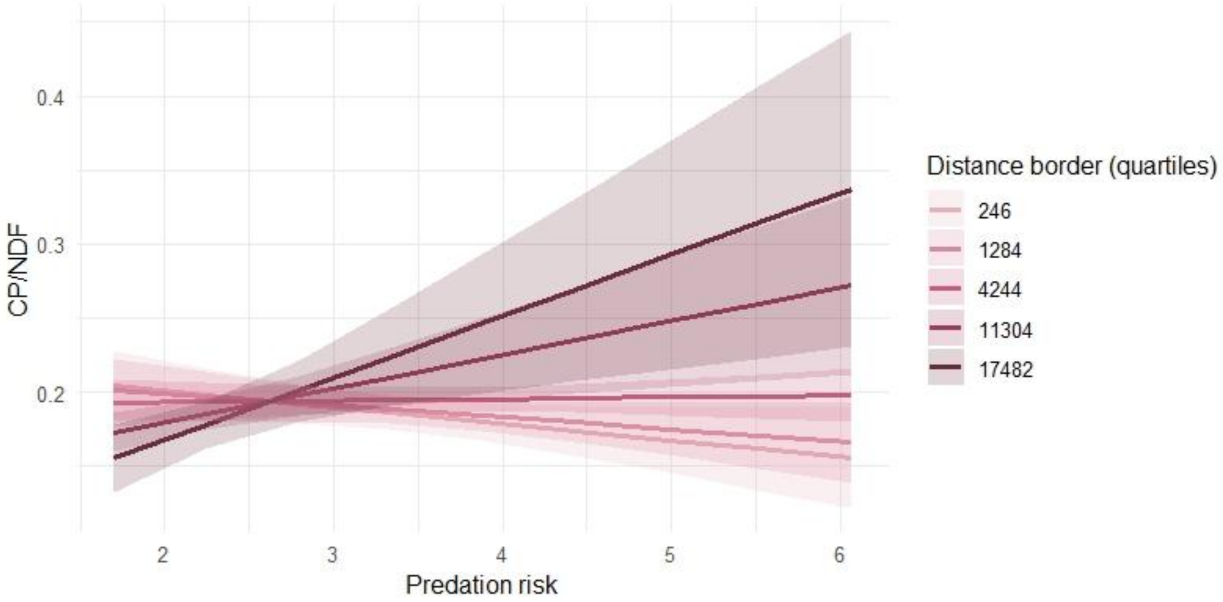

Figure 8: Predicted values from linear mixed model showing the variation of the crude protein to neutral detergent fibre ratio (CP/NDF) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell), interacting with the distance to the state border (meters). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. The distance to the border was grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.

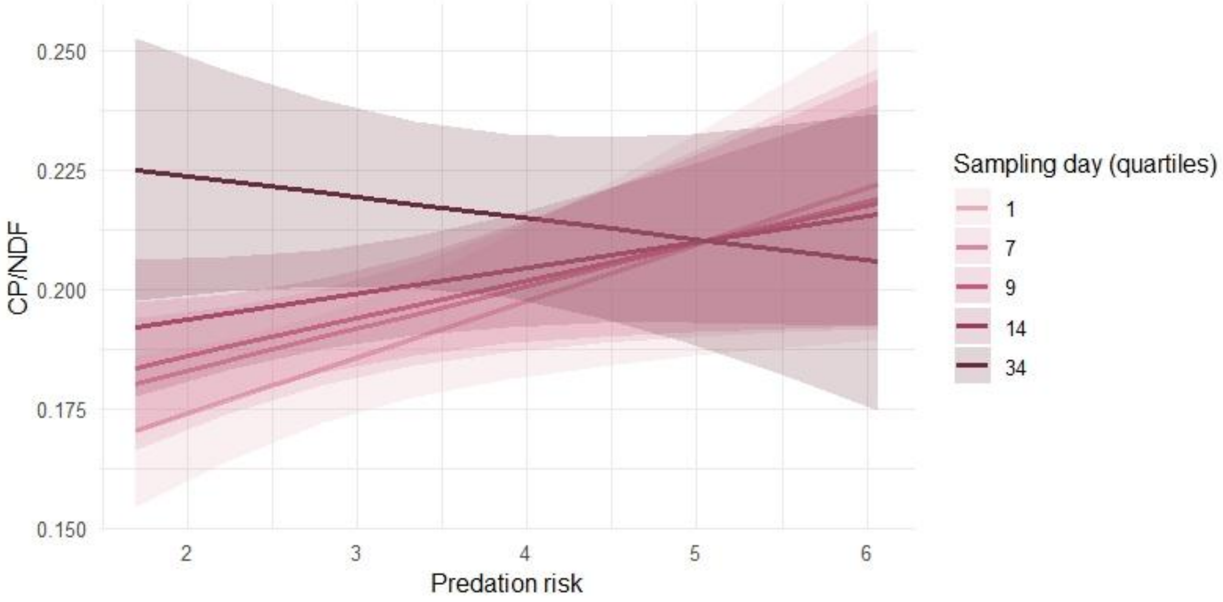

Figure 9: Predicted values from linear mixed model illustrating the variation of the crude protein to neutral detergent fibre ratio (CP/NDF) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell) over the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.

CP/ADF

The protein to acid detergent fibre ratio (CP/ADF) ranged from 0.11 to 0.39 and averaged 0.24 ± 0.05. The sixth LMM results indicated that the relative CP content to ADF in red deer faeces increased later in the winter sampling period (Figure S12) and decreased as precipitation increased the day before sampling (Figure S13). The CP/ADF increased marginally with the increasing distance to the Polish state border and the nearest road ($p = 0.051$; Table 3b). While predation risk had no direct effect on the CP to ADF ratio, significant interactions were observed with distance to the nearest road and over the sampling period: CP/ADF decreased with higher predation risk farther from roads (Figure 10) and later in the sampling period, coinciding with onset of the vegetation season (Figure 11).

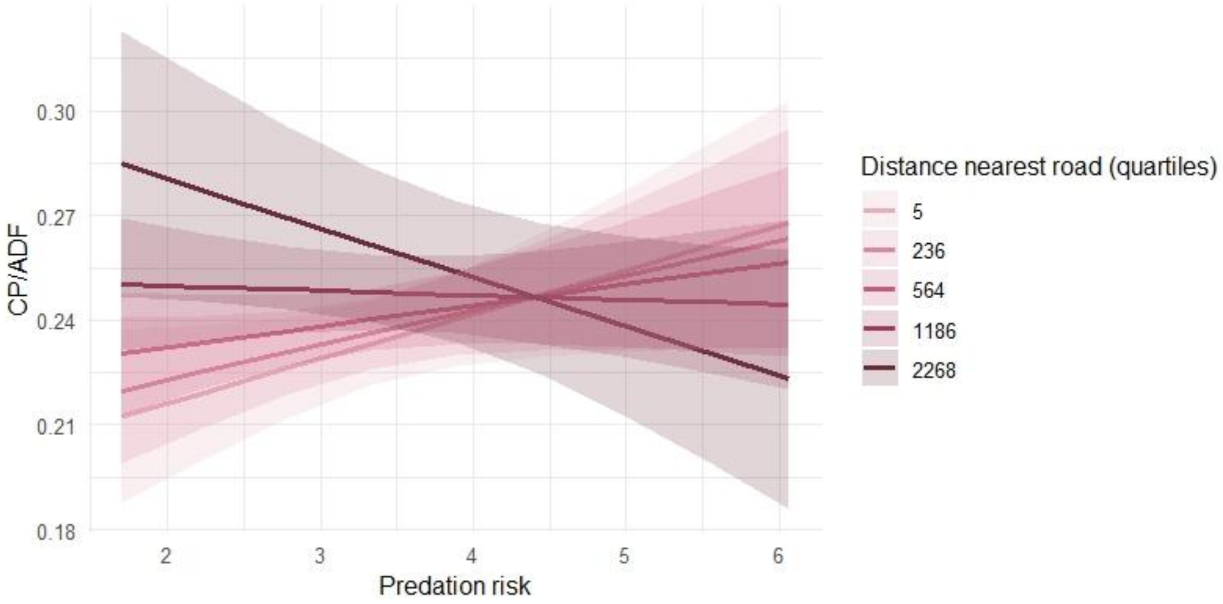

__Figure 10__: Predicted values from linear mixed model showing the variation of the crude protein to acid detergent fibre ratio (CP/ADF) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell), interacting with the distance to the state border (meters). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. The distance to the nearest road was grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.

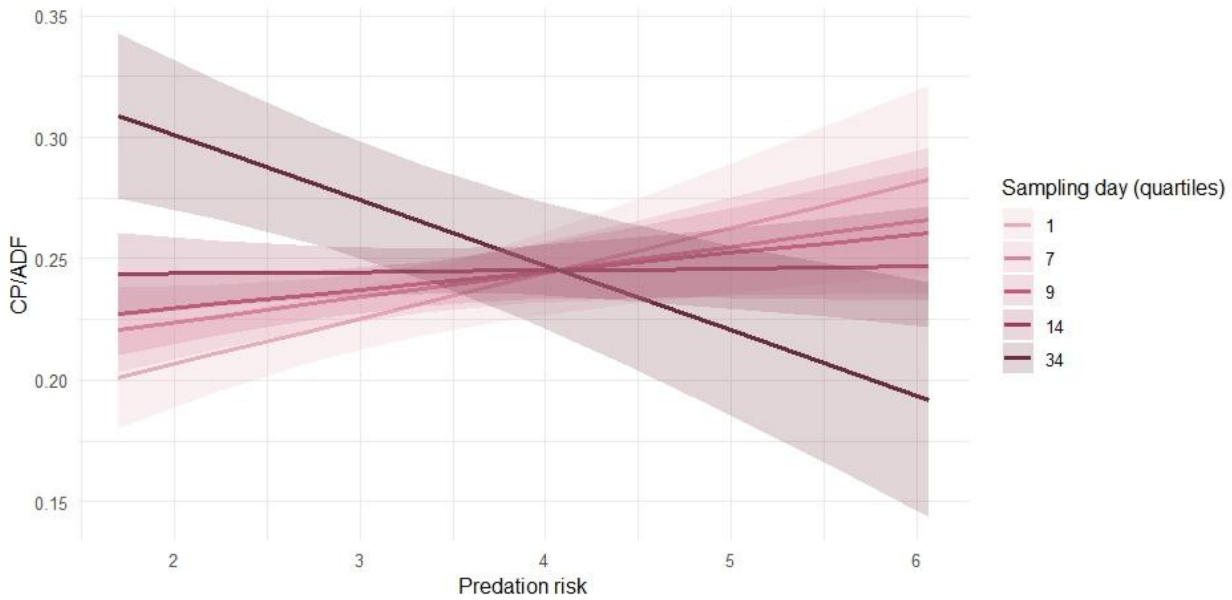

***Figure 11**: Predicted values from linear mixed model illustrating the variation of the crude protein to acid detergent fibre ratio (CP/ADF) in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell), over the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

CWD

Cell wall digestibility (CWD) index varied between 94.89 and 96.82 and averaged 95.87 ± 0.36. The CWD index decreased when the distance to the state border increased (Figure S14). Conversely, this index increased with the distance to the nearest building (Figure S15). The increase of temperature 5 cm above the ground led to the decrease of CWD index in the red deer faeces (Figure S16). Although predation risk did not have a direct effect on the faecal CWD index, its interaction with the sampling period had a significant positive effect: CWD index increased with predation risk at the end of the sampling period, coinciding with the onset of the vegetation season (Figure 12). Additionally, the interaction between predation risk and the distance to the nearest road had a marginal effect on CWD index (*p* = 0.056, Table 3c): it increased at locations with higher predation risk and greater distance to the nearest road.

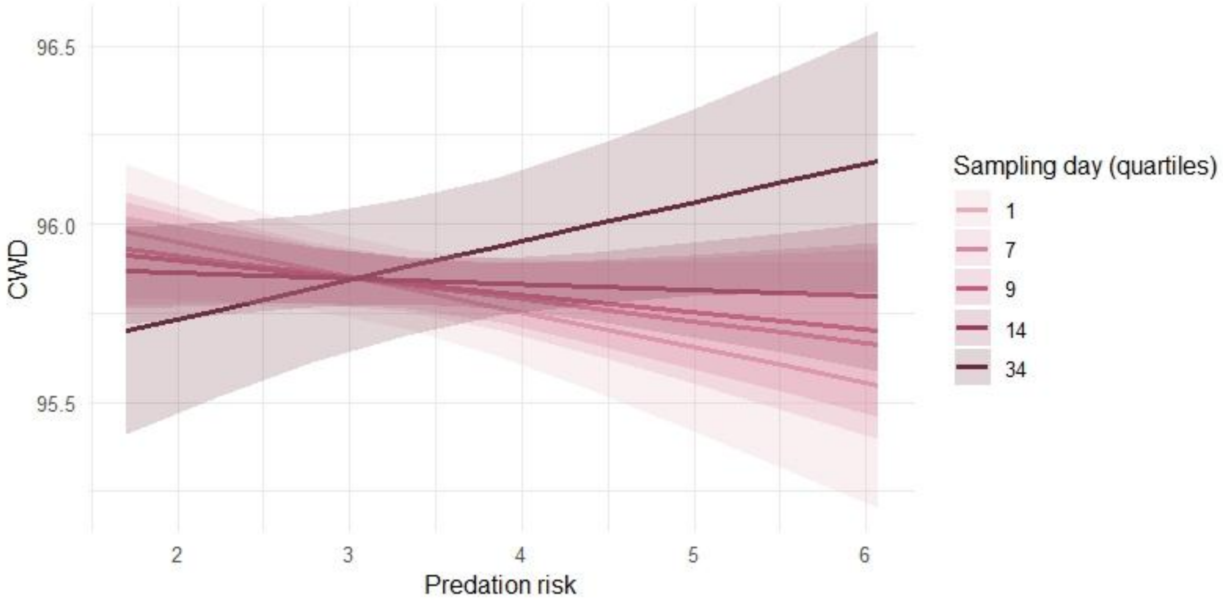

Figure 12*: Predicted values from linear mixed model illustrating the variation of the cell wall digestibility (CWD) index in red deer faeces in response to predation risk from wolves (ranging from 1.7 to 7.2 individuals per 0.25 km² grid cell), over the sampling period (days). Deer faecal samples (n = 279) were collected within a 200 m radius at 81 locations (random intercept) in the Białowieża Forest. Sampling days (n = 16) were grouped into four quartiles for visualisation of the trends estimated separately for each group and represented by different coloured lines.*

Table 2*: Results of linear mixed models analysing the effect of predation risk, estimated as the spatial probability of wolf encounters, on the variation in the winter diet quality of red deer. Dietary components assessed included (a) crude protein (CP), (b) neutral detergent fibre (NDF), (c) acid detergent fibre (ADF), and (d) acid detergent lignin (ADL), all expressed as percentages of dry matter (% DM) and averaged from replicates obtained via near-infrared spectroscopic analysis of 279 faecal samples. Faeces were collected within a 200 m radius buffer area at 81 locations (included as a random factor, in italics) in the Białowieża Forest, Poland.*

Response variable	Effects	Variance	SD	Estimate	SD	Wald Chi-square	d.f.	*p*-value
(a) CP	Intercept			11.379	0.271	1765.968	1	< 0.001
	Predation risk			0.275	0.211	1.695	1	0.193
	Distance border			0.581	0.318	3.338	1	0.068
	Distance nearest road			0.332	0.162	4.190	1	0.041
	Sampling day			0.343	0.126	7.403	1	0.007
	Distance nearest building			0.306	0.172	3.155	1	0.076
$R^2 m$ = 0.132	Freshness			0.382	0.205	3.487	1	0.062
$R^2 c$ = 0.324	Precipitation			-0.242	0.131	3.397	1	0.065
AICc = 1077	Predation risk*distance border			0.922	0.324	8.085	1	0.004
	Predation risk*sampling day			-0.220	0.098	5.093	1	0.024
	Location (random)	0.585	0.765					
(b) NDF	Intercept			57.845	0.443	17019.434	1	< 0.001
	Predation			-0.505	0.331	2.330	1	0.127
	Distance nearest road			0.539	0.339	2.531	1	0.112
$R^2 m$ = 0.106	Distance nearest river			-0.475	0.339	1.957	1	0.162
$R^2 c$ = 0.232	Freshness			-1.014	0.544	3.475	1	0.062
AICc = 1612	Precipitation			1.054	0.312	11.384	1	0.001

	Location (random)	2.653	1.629					
(c) ADF	Intercept			45.363	0.329	19066.841	1	< 0.001
	Predation risk			-0.436	0.470	0.861	1	0.354
	Distance border			-1.511	0.484	9.759	1	0.002
	Sampling day			-0.045	0.357	0.016	1	0.899
	Distance nearest feeding site			-0.498	0.349	2.039	1	0.153
R^2m = 0.126	Precipitation			1.158	0.372	9.682	1	0.002
R^2c = 0.290	Temperature on the ground			-0.712	0.442	2.595	1	0.107
AICc = 1583	Predation risk*sampling day			0.795	0.259	9.456	1	0.002
	Location (random)	3.190	1.786					
(d) ADL	Intercept			23.774	0.313	5787.719	1	< 0.001
	Predation risk			-0.432	0.237	3.323	1	0.068
	Distance border			0.328	0.345	0.904	1	0.342
	Sampling day			-0.119	0.170	0.490	1	0.484
	Distance nearest river			-0.582	0.187	9.743	1	0.002
R^2m = 0.296	Distance nearest building			-0.687	0.203	11.480	1	0.001
R^2c = 0.473	Freshness			-0.736	0.237	9.675	1	0.002
AICc = 1146	Temperature on the ground			0.872	0.181	23.188	1	< 0.001
	Predation risk*distance border			-0.545	0.393	1.922	1	0.166
	Predation risk*sampling day			-0.311	0.116	7.147	1	0.008
	Location (random)	0.877	0.936					

Table 3*: Results of linear mixed models analysing the effect of predation risk, estimated as spatial probability of wolf encounters, on variation in the winter diet quality of red deer. Dietary indicators assessed included (a) crude protein relative to neutral detergent fibre ratio (CP/NDF), (b) crude protein relative to acid detergent fibre ratio (CP/ADF), and (c) cell wall digestibility (CWD) index, all based on dietary components analysed via near-infrared spectroscopy in 279 faecal samples. Faeces were collected within 200 m radius around 81 locations (included as a random factor, in italics) in the Białowieża Forest (Poland).*

Response	Effects	Variance	SE	Estimate	SD	Wald Chi-square	d.f.	*p*-value
(a) CP/NDF	Intercept			0.198	0.005	1580.836	1	< 0.001
	Predation risk			0.007	0.004	2.897	1	0.089
	Distance border			0.010	0.006	2.951	1	0.086
	Distance nearest road			0.005	0.003	2.784	1	0.095
	Sampling day			0.006	0.002	6.955	1	0.008
$R^2m = 0.166$	Distance nearest building			0.006	0.003	4.073	1	0.044
$R^2c = 0.328$	Freshness			0.010	0.004	6.220	1	0.013
AICc = -1054	Precipitation			-0.008	0.002	9.411	1	0.002
	Predation risk*distance border			0.016	0.006	7.279	1	0.007
	Predation risk*sampling day			-0.004	0.002	5.188	1	0.023
	Location (random)	0.001	0.013					
(b) CP/ADF	Intercept			0.242	0.004	3012.456	1	< 0.001
	Predation risk			0.005	0.005	0.859	1	0.354
	Distance border			0.009	0.005	3.817	1	0.051
	Distance road			0.007	0.004	3.814	1	0.051
	Sampling day			0.008	0.004	4.334	1	0.037
$R^2m = 0.156$	Freshness			0.009	0.006	2.602	1	0.107

R^2c = 0.281	Precipitation			-0.012	0.004	8.974	1	0.003
AICc = -875	Temperature on the ground			0.007	0.005	2.152	1	0.142
	Predation risk*sampling day			-0.011	0.003	17.898	1	< 0.001
	Predation risk*distance nearest road			-0.007	0.003	6.211	1	0.013
	Location (random)	0.001	0.016					
(c) CWD index	Intercept			95.840	0.032	8785100.000	1	< 0.001
	Predation risk			-0.036	0.044	0.645	1	0.422
	Distance border			-0.141	0.049	8.192	1	0.004
	Distance road			0.001	0.035	0.001	1	0.982
	Sampling day			0.014	0.032	0.198	1	0.657
R^2m = 0.157	Distance nearest building			0.077	0.039	3.977	1	0.046
R^2c = 0.477	Temperature on the ground			-0.117	0.032	13.621	1	<0.001
AICc = 204	Predation risk*sampling day			0.052	0.022	5.504	1	0.019
	Predation risk*distance nearest road			0.052	0.027	3.641	1	0.056
	Location (random)	0.045	0.211					

Discussion

The results of this study provided no support for the major hypothesis that predation risk from wolves may have a direct effect on red deer diet quality in the BPF. However, it suggests that they responded to it by altering the quality of food consumed, following the changes in seasonal food availability and the level of human disturbance.

Interaction with the sampling date significantly influenced all dietary indicators on red deer faeces, except total fibre (NDF). This supports the hypothesis that the effect of predation risk is influenced by seasonality, especially during winter, when food availability is constrained and the weather conditions are severe (Bramorska et al., 2023; Morosinotto et al., 2017). Crude protein content and CP/NDF ratio in deer faeces also varied with the predation risk when considering the distance to the state border, while the CP/ADF ratio was affected by the predation risk when interacting with the distance to the main roads. These results highlight the strong effect of human disturbance in shaping red deer perception of predation risk (Ausilio et al., 2025) and, thus, their diet quality. Previous studies have already suggested that large herbivores may adapt their behavioural responses to complex interactions between environmental factors such as predation risk (Kuijper et al., 2014), human disturbance (Theuerkauf & Rouys, 2008), and weather conditions (Li, 2013). Interactive effects of predation risk were also found with prey density in tadpoles (Guariento et al., 2015), climate in snails (L. P. Miller et al., 2014), or supplementary feeding in owls (Morosinotto et al., 2017). Small mammals modify their foraging patterns to the perceived risk of predation according to habitat (forest *vs*. open), available shelter, and predator type (cursorial *vs*. ambushing) (Kelleher et al., 2021).

Influence of the seasonality

The sampling period appeared to be an important factor in winter red deer diet quality, especially for the protein content. As the season progressed, the faecal CP content increased, reflecting the increase of diet quality. This might be related to the onset of the vegetation season, with the appearance of early spring plants and the first leaves on twigs (Faliński, 1986). During winter, red deer in the BPF primarily feed on coniferous trees, such as Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) (Gębczyńska, 1980), and a high faecal content of Norway spruce needles in ungulates has been suggested to indicate a low diet quality (J. Kamler & Homolka, 2011). When spring begins, the protein content of twigs increases, while the total fibre content decreases (Palo et al., 1985), indicating improved forage quality at the transition from winter to spring (J. Kamler & Homolka, 2011; Chen et al., 1998; Palo et al., 1985). These seasonal changes in plant nutritional composition heighten the quality and digestibility of forage for wild ungulates (Čupić et al., 2021; Palo et al., 1985). However, this seasonal balance in red deer diet quality might also reflect a decrease in human-induced stress, as the sampling period began right after closing the hunting season (February 28th). Hunting is known to induce stress in red deer, which leads them to adjust their behaviour (Ausilio et al., 2025; Bojarska et al., 2024; Cromsigt et al., 2013). Therefore, the observed increase in CP over the sampling period may reflect animals' gradual adaptation to the release from hunting pressure. Following the GSP concept (Hawlena & Schmitz, 2010b), human-induced stress (either hunting-related or stemming from other anthropogenic factors) could induce dietary shifts in animals. Therefore, seasonal variation in protein and fibre content in red deer diet may likely reflect two competing, but not mutually exclusive scenarios: (1) red deer balance the quality of their diet under chronic predation stress according to winter food constraints, and (2) the confounding influence of human pressure on perceived predation risk may weaken as human activity declines.

Influence of the interaction of predation risk and seasonality

According to the above, we can expect increased protein-to-fibre ratios in red deer diets toward the early spring. However, while faecal CP content increased over the sampling period, when considering the interaction with predation risk, deer reduced their protein intake under high predation risk only at the beginning of spring, coinciding with the onset of the vegetation season (early April). This may suggest that while the malnourished red deer emerging from winter food scarcity may generally seek protein-rich food to rebuild their depleted body reserves, they may also be forced to shift again to low-protein food when exposed to high predation risk. Therefore, red deer seem to adjust their foraging behaviour according to perceived predation risk, primarily when higher-quality and more abundant food is available. The trends of the CP/NDF and CP/ADF ratios were similar to that of CP content, suggesting that these protein-to-fibre ratios are mainly driven by variation in CP intake. Conversely, faecal ADF content increased with predation risk at the end of the sampling period, likely illustrating a dietary shift to lower-quality food. In contrast, the CWD index also increased under higher predation risk in early spring, indicating a shift to more digestible forage, with lower lignin content relative to total fibre. Consistently, faecal ADL content itself exhibited a stronger decrease with increasing predation risk in early spring. This suggests that deer foraged on plants with lower ADL content when exposed to higher levels of predation risk. Such a spatiotemporal pattern reflects a shift toward a higher-quality diet, characterised by greater digestibility and higher energy available (Čupić et al., 2021), to meet the elevated energetic demands under predator presence. These results align with previous studies reporting decreased fN levels under predation risk-induced stress (Barnier et al., 2014; Hernández & Laundré, 2005), and support the prediction of the GSP framework (Hawlena & Schmitz, 2010b). The seasonal decline in faecal ADL excretion might also reflect the onset of the vegetation season (T. Kaminski, personal communication, July 2025) when green plant tissues, which typically contain lower lignin, become more available (Alves et al., 2021; Novaes et al., 2010). Overall, these results suggest that red deer adjust their winter diet in response to predator presence, optimising forage quality to meet seasonal energetic demands, particularly in the pre-reproductive period (Corlatti et al., 2013; Brown et al., 1999).

Influence of human-related factors and their interaction with predation risk

As hypothesised, human activity related to the state border outweighed the influence of predation risk on red deer winter diet quality, as the latter was evident only farther away from the state border, where CP content and CP/NDF ratios clearly increased with predation risk. This pattern may result from intense human activity near the border —characterised by unpredictable stressors such as gunshots, heavy traffic and barking dogs related to military activity—which may override the impact of predation risk (Nowak, 2025). The spatially predictable but temporarily unpredictable nature of these stressors increases the human threat perceived by ungulates (Cromsigt et al., 2013). Such persistent human-induced stress near the border may be stronger than the predator-induced stress (Ausilio et al., 2025; Proudman et al., 2021; Zbyryt et al., 2018; Theuerkauf & Rouys, 2008), thus preventing a diet shift induced by predation risk. This spatial trend may reflect heightened protein requirements to meet metabolic demands, particularly toward the end of winter, when deer can lose up to 50% of their body weight (Nasiadka et al., 2016).

When considering the effect of proximity to main roads, it was surprisingly opposite to the influence of the border zone. The CP/ADF ratio decreased with increased predation risk at greater distances from roads but increased when faeces were collected closer to roads. This pattern indicates a shift toward lower-quality diet, containing more fibre than protein, when red deer foraging further away from human areas under high perceived predation risk. In contrast, close to roads, deer may need to increase their protein-to-fibre intake when exposed to predation risk to offset the combined stress from both humans and predators. This may be particularly related to the fact that wolves prefer to travel on forest roads, even those with high traffic levels (Bojarska et al., 2020). Conversely, farther from the roads, deer responded to higher predation risk by decreasing their protein-relative-to-fibre intake, consistent with my predictions of the GSP framework.

When examining the effect of the distance to human areas alone, the CP content in faeces increased with distance from main roads, while both the CP/NDF ratio and the CWD index increased with greater distance from buildings. These findings indicate a decline in diet quality when red deer forage closer to human infrastructure. Consistently, ADL content decreased with increasing distance from buildings, further suggesting that diet quality is higher farther from human settlements. These results highlight the strong influence of human disturbances in shaping red deer's perception of risk and their foraging behaviour (Ausilio et al., 2025; Bojarska et al., 2024; Cromsigt et al., 2013). It also stands in contrast with the 'human shield' concept which posits that human-dominated (predator-free) areas may provide refuge for prey offering improved nutritional quality of forage (Berger, 2007; Hebblewhite et al., 2005).

Influence of the climate

Climatic conditions also appeared to influence red deer diet quality. On days following higher rainfall, the faeces contained more fibre relative to protein as reflected by lower CP/NDF and CP/ADF ratios, and higher NDF and ADF values. This pattern likely reflects behavioural changes in wet conditions: deer tend to spend more time bedding and ruminating during rainy days (Li, 2013), and seek denser forest cover to avoid wetting their fur, which can cause heat loss (Mysterud & Østbye, 1999) — a behaviour likely even more important in winter when temperatures are low. Faecal ADL content increased at higher temperatures, while CWD index decreased. This is counterintuitive, as a seasonal warming trend toward the end of the sampling period should lead to lower ADL and higher digestibility in deer forage. A plausible explanation is that lignin, being highly resistant to environmental degradation, becomes proportionally more concentrated (%DM) in less fresh faeces at higher temperatures, as more labile components (lipids, protein, carbohydrates) are lost through microbial degradation. In Poland, red deer densities are strongly linked to winter temperatures and forest cover (Borowik et al., 2013), suggesting that climate may amplify the top-down control exerted by large predators on prey foraging (L. P. Miller et al., 2014), with potential consequences for ungulates' diet quality.

Influence of other environmental factors

The habitat type and the distance to the nearest feeding site were included in the global models in order to account for resource availability, an important influence on ungulate diet quality (Bramorska et al., 2023; Riesch et al., 2022; Theuerkauf & Rouys, 2008). However, habitat type was never retained in the final models. This may be due to the inclusion of other spatial covariates or the spatial random effect, which likely capture most of the variation associated with habitat characteristics. In winter, resource availability may be relatively uniform across habitat types in BPF (Theuerkauf & Rouys, 2008), reducing the potential influence of habitat composition on red deer diet quality (Gębczyńska, 1980). As for feeding sites, their relatively homogeneous distribution across the study area may have limited their explanatory power. Additionally, previous studies suggest that red deer do not heavily rely on supplementary feeding during winter (Cupic et al., 2023; Obidzinski et al., 2013), unlike European bison, which are grazers that strongly rely on supplementary feeding in BPF (Bramorska et al., 2023).

Considering other environmental variables, faecal ADL content was higher when faeces were collected closer to rivers, an unexpected result as riparian areas are typically richer in herbaceous plants (Foley-

Congdon et al., 2024) and characterised by highly-productive open areas where red deer prefer to forage (Riesch et al., 2022). This counterintuitive pattern may be explained by the abundance of various grass species in the meadows along river valleys (especially reed *Phragmites australis* commonly covering large areas of wetlands), which have relatively high lignin content (15-22%DM) (Waliszewska et al., 2021). Dried stems of these plants are still abundant during winter and despite their low nutritional values may constitute attractive forage for ungulates. In BPF, red reed diet is mainly dominated on woody plants such Betulaceae, *Rubus*, Salicaceae, *Quercus* (Churski et al., 2021). Therefore, their winter diet may rely more on woody parts of these plants, also present in these riparian areas, which typically contain higher lignin levels, ranging from 14.5-25 %DM (Lourenço & Pereira, 2018).

Recommendations

Future studies should aim to incorporate direct data on food availability, for example, through plant surveys during the sampling period. Comparing the nutritional composition of available plants with that of red deer faeces would provide a more comprehensive understanding of diet selection and forage quality. Micro histological or DNA-based analyses on faeces (Churski et al., 2021) could help identify the specific plant species consumed by red deer during winter, a season characterised by food constraints. Meanwhile, NIRS remains a valuable tool for detecting differences in the nutritional quality of the selected plant tissues. Replicating this study during the peak of vegetation season (summer) would be especially insightful, as greater plant diversity and abundance would allow red deer to be more selective in their foraging decisions. Given that predation risk effects were already detected within the relatively constrained winter diet, stronger effects might be expected during summer, when dietary choices are broader. Future studies might consider species life-history traits, such as sex and age, as they may influence red deer foraging behaviour, not only due to differences in body size but also because of varying perceptions of predation risk from wolves, which preferentially target young and female individuals (Jędrzejewski et al., 2000). Additionally, including the predation risk induced by lynx — an ambush predator known to trigger different behavioural responses in ungulates (Wikenros et al., 2015) — could provide further valuable insights.

Conclusions

Although variation in winter diet quality of deer was narrow — likely due to seasonal food scarcity — significant effects of predation risk could still be detected, but only when considering its interactions with seasonal and human-related factors. Notably, the interaction between predation risk and seasonality provided insights into how winter severity and food constraints influence red deer's dietary responses to predation risk. Despite food availability and quality likely increased at the beginning of spring, deer shifted to foraging on plants higher in fibre content relative to protein. This supports the GSP concept that herbivores exposed to chronic predator-induced stress tend to select plants richer in fibre rather than in protein, suggesting a dietary shift toward low-quality food. Additionally, a strong effect of human disturbance on red deer responses to predation risk was evident, with human-induced stress appearing to override the influence of predation risk-induced stress.

This study contributes to a deeper understanding of predator-prey interactions, and their ecological consequences, particularly regarding the nutrient cycling across trophic levels. Herbivores under chronic predation-risk stress may play a crucial role in shaping the plant community composition and ecosystem functioning. Future studies should incorporate data on the nutritional composition of available plants to gain a more comprehensive understanding of red deer diet quality. Moreover, replicating this study during summer, when food availability peaks and red deer can be more selective, could reveal a stronger effect of predation risk on their diet quality.

Personal contribution

I carried out the laboratory work, including sample preparation and Near-infrared spectroscopy. I performed the calibration procedure to quantify the diet quality indicators and conducted the statistical analysis.

References

Alves, M., Martins, R. T., & Couceiro, S. R. M. (2021). Breakdown of green and senescent leaves in Amazonian streams: A case study. *Limnology*, *22*(1), 27–34. https://doi.org/10.1007/s10201-020-00626-y

Ausilio, G., Wikenros, C., Sand, H., Devineau, O., Wabakken, P., Eriksen, A., Aronsson, M., Persson, J., Mathisen, K. M., & Zimmermann, B. (2025). Contrasting risk patterns from human hunters and a large carnivore influence the habitat selection of shared prey. *Oecologia*, *207*(7). https://doi.org/10.1007/s00442-025-05742-z

Barnier, F., Valeix, M., Duncan, P., Chamaillé-Jammes, S., Barre, P., Loveridge, A. J., Macdonald, D. W., & Fritz, H. (2014). Diet quality in a wild grazer declines under the threat of an ambush predator. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1785), 20140446. https://doi.org/10.1098/rspb.2014.0446

Berger, J. (2007). Fear, human shields and the redistribution of prey and predators in protected areas. *Biology Letters*, *3*(6), 620–623. https://doi.org/10.1098/rsbl.2007.0415

Białowieża (Rural Commune, Poland)—Population Statistics, Charts, Map and Location. (n.d.). Retrieved 25 July 2025, from https://citypopulation.de/en/poland/podlaskie/admin/powiat_hajnowski/2005022__bia%C5%82owie%C5%BCa/

Boczoń, A., & Sałachewicz, A. (2022). *3. Climatic conditions of Białowieża Forest*.

Bojarska, K., Gerber, N., Herzog, S., Isselstein, J., Meiβner, M., Riesch, F., Signer, J., Van Beeck Calkoen, S., Zetsche, M., & Balkenhol, N. (2024). Red deer responses to drive hunts are related to habitat and landscape characteristics. *The Journal of Wildlife Management*, *88*(5). https://doi.org/10.1002/jwmg.22583

Bojarska, K., Sulich, J., Bachmann, S., Okarma, H., Theuerkauf, J., & Gula, R. (2020). Opportunity and peril: How wolves use a dense network of forest roads. *Mammalian Biology*, *100*(2), 203–211. https://doi.org/10.1007/s42991-020-00014-0

Borowik, T., Cornulier, T., & Jędrzejewska, B. (2013). Environmental factors shaping ungulate abundances in Poland. *Acta Theriologica*, *58*(4), 403–413. https://doi.org/10.1007/s13364-013-0153-x

Bramorska, B., Kowalczyk, R., Kamiński, T., & Borowik, T. (2023). Linking winter severity to space use of European bison around feeding sites in Białowieża Primeval Forest (NE Poland). *European Journal of Wildlife Research*, *69*(4). https://doi.org/10.1007/s10344-023-01690-2

Brown, J. S., Laundre, J. W., & Gurung, M. (1999). The Ecology of Fear: Optimal Foraging, Game Theory, and Trophic Interactions. *Journal of Mammalogy*, *80*(2), 385–399. https://doi.org/10.2307/1383287

Bubnicki, J. W., Churski, M., Schmidt, K., Diserens, T. A., & Kuijper, D. P. (2019). Linking spatial patterns of terrestrial herbivore community structure to trophic interactions. *eLife*, *8*. https://doi.org/10.7554/elife.44937

Chen, H., Ma, J., Li, F., Sun, Z., Wang, H., Luo, L., & Li, F. (1998). Seasonal composition and quality of red deer Cervus elaphus diets in northeastern China. *Acta Theriologica*, *43*, 77–94. https://doi.org/10.4098/at.arch.98-6

Christianson, D., & Creel, S. (2010). A nutritionally mediated risk effect of wolves on elk. *Ecology*, *91*(4), 1184–1191. https://doi.org/10.1890/09-0221.1

Churski, M., Spitzer, R., Coissac, E., Taberlet, P., Lescinskaite, J., Van Ginkel, H. A. L., Kuijper, D. P. J., & Cromsigt, J. P. G. M. (2021). How do forest management and wolf space-use affect diet composition of the wolf's main prey, the red deer versus a non-prey species, the European bison? *Forest Ecology and Management*, *479*, 118620. https://doi.org/10.1016/j.foreco.2020.118620

Clinchy, M., Sheriff, M. J., & Zanette, L. Y. (2013). Predator-induced stress and the ecology of fear. *Functional Ecology*, *27*(1), 56–65. https://doi.org/10.1111/1365-2435.12007

Corlatti, L. (2020). Anonymous fecal sampling and NIRS studies of diet quality: Problem or opportunity? *Ecology and Evolution*, *10*(12), 6089–6096. https://doi.org/10.1002/ece3.6354

Corlatti, L., Bassano, B., Valencak, T. G., & Lovari, S. (2013). Foraging strategies associated with alternative reproductive tactics in a large mammal. *Journal of Zoology*, *291*(2), 111–118. https://doi.org/10.1111/jzo.12049

Corlatti, L., Palme, R., Valencak, T. G., & Gomez, K. M. (2023). Season-dependent impact of forage quality on stress in alpine chamois. *Ecology and Evolution*, *13*(5). https://doi.org/10.1002/ece3.10045

Creel, S., & Christianson, D. (2008). Relationships between direct predation and risk effects. *Trends in Ecology & Evolution*, *23*(4), 194–201. https://doi.org/10.1016/j.tree.2007.12.004

Cromsigt, J. P. G. M., Kuijper, D. P. J., Adam, M., Beschta, R. L., Churski, M., Eycott, A., Kerley, G. I. H., Mysterud, A., Schmidt, K., & West, K. (2013). Hunting for fear: Innovating management of human–wildlife conflicts. *Journal of Applied Ecology*, *50*(3), 544–549. https://doi.org/10.1111/1365-2664.12076

Čupić, S., García, A. J., Holá, M., & Ceacero, F. (2021). Evaluation of factors inducing variability of faecal nutrients in captive red deer under variable demands. *Scientific Reports*, *11*(1), 2394. https://doi.org/10.1038/s41598-021-81908-y

Cupic, S., Jezek, M., & Ceacero, F. (2023). Are they both the same shit? Winter faeces of roe and red deer show no difference in nutritional components. *Journal of Forest Science*, *69*(3), 114–123. https://doi.org/10.17221/19/2023-JFS

Dixon, R., & Coates, D. (2009). Review: Near Infrared Spectroscopy of Faeces to Evaluate the Nutrition and Physiology of Herbivores. *Journal of Near Infrared Spectroscopy*, *17*(1), 1–31. https://doi.org/10.1255/jnirs.822

Dixon, W. J. (1950). Analysis of Extreme Values. *The Annals of Mathematical Statistics*, *21*(4), 488–506. https://doi.org/10.1214/aoms/1177729747

Dryden, G. (2003). *Near infrared reflectance spectroscopy: Applications in deer nutrition*.

Evangelista, C., Contò, M., Basiricò, L., Bernabucci, U., & Failla, S. (2025). Near-Infrared Spectroscopy for Assessing the Chemical Composition and Fatty Acid Profile of the Total Mixed Rations of Dairy Buffaloes. *Applied Sciences*, *15*(6), 3211. https://doi.org/10.3390/app15063211

Faliński, J. B. (1986). *Vegetation dynamics in temperate lowland primeval forests. Vol. 2*. dr W. Junk Publishers. https://www.rcin.org.pl/dlibra/publication/5858/edition/245

Foley-Congdon, E., Jellinek, S., Chee, Y. E., & Greet, J. (2024). Revegetated riparian areas are dominated by weeds, and lack structural diversity and natural recruitment: Lessons for restoration practice. *Restoration Ecology*, *32*(4). https://doi.org/10.1111/rec.14098

Fox, J., & Weisberg, S. (2019). *An R Companion to Applied Regression* (Third). Sage. https://www.john-fox.ca/Companion/

Gębczyńska, Z. (1980). Food of the roe deer and red deer in the Białowieża Primeval Forest. *Acta Theriologica*, *25*(40), 487–500. https://doi.org/10.4098/AT.arch.80-44

Guariento, R. D., Carneiro, L. S., Jorge, J. S., Borges, A. N., Esteves, F. A., & Caliman, A. (2015). Interactive effects of predation risk and conspecific density on the nutrient stoichiometry of prey. *Ecology and Evolution*, *5*(21), 4747–4756. https://doi.org/10.1002/ece3.1740

Hawlena, D., & Schmitz, O. J. (2010a). Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences*, *107*(35), 15503–15507. https://doi.org/10.1073/pnas.1009300107

Hawlena, D., & Schmitz, O. J. (2010b). Physiological Stress as a Fundamental Mechanism Linking Predation to Ecosystem Functioning. *The American Naturalist*, *176*(5), 537–556. https://doi.org/10.1086/656495

Hebblewhite, M., White, C. A., Nietvelt, C. G., McKenzie, J. A., Hurd, T. E., Fryxell, J. M., Bayley, S. E., & Paquet, P. C. (2005). Human activity mediates a trophic cascade caused by wolves. *Ecology*, *86*(8), 2135–2144. https://doi.org/10.1890/04-1269

Hernández, L., & Laundré, J. W. (2005). Foraging in the 'landscape of fear' and its implications for habitat use and diet quality of elk Cervus elaphus and bison Bison bison. *Wildlife Biology*, *11*(3), 215–220. https://doi.org/10.2981/0909-6396(2005)11[215:FITLOF]2.0.CO;2

Hersbach, H., Bell, B., Berrisford, P., Hirahara, S., Horányi, A., Muñoz‐Sabater, J., Nicolas, J., Peubey, C., Radu, R., Schepers, D., Simmons, A., Soci, C., Abdalla, S., Abellan, X., Balsamo, G., Bechtold, P., Biavati, G., Bidlot, J., Bonavita, M., … Thépaut, J. (2020). The ERA5 global reanalysis. *Quarterly Journal of the Royal Meteorological Society*, *146*(730), 1999–2049. https://doi.org/10.1002/qj.3803

Hola, M., Jezek, M., Kusta, T., & Cerveny, J. (2016). Evaluation of winter food quality and its variability for red deer in forest environment: Overwintering enclosures vs. Free-ranging areas. *CENTRAL European Forestry Journal*, *62*(3), 139–145. https://doi.org/10.1515/forj-2016-0018

Jaroszewicz, B., Cholewińska, O., Gutowski, J. M., Samojlik, T., Zimny, M., & Latałowa, M. (2019). Białowieża Forest—A Relic of the High Naturalness of European Forests. *Forests*, *10*(10), 849. https://doi.org/10.3390/f10100849

Jean, P.-O., Bradley, R. L., Giroux, M.-A., Tremblay, J.-P., & Côté, S. D. (2014). Near Infrared Spectroscopy and Fecal Chemistry as Predictors of the Diet Composition of White-Tailed Deer. *Rangeland Ecology & Management*, *67*(2), 154–159. https://doi.org/10.2111/REM-D-13-00112.1

Jędrzejewska, W., Schmidt, K., Theuerkauf, J., Jędrzejewska, B., Selva, N., Zub, K., & Szymura, L. (2002). Kill Rates and Predation by Wolves on Ungulate Populations in Bialowieza Primeval Forest (Poland). *Ecology*, *83*(5), 1341. https://doi.org/10.2307/3071948

Jędrzejewski, Wł., Jędrzejewska, B., Okarma, H., Schmidt, K., Zub, K., & Musiani, M. (2000). Prey Selection and Predation by Wolves in BiaŁowieŻa Primeval Forest, Poland. *Journal of Mammalogy*, *81*(1), 197–212. https://doi.org/10.1644/1545-1542(2000)081<0197:PSAPBW>2.0.CO;2

Kamler, J. F., Jędrzejewska, B., & JĘdrzejewski, W. (2007). Factors affecting daily ranges of red deerCervus elaphus in Białowieża Primeval Forest, Poland. *Acta Theriologica*, *52*(2), 113–118. https://doi.org/10.1007/bf03194206

Kamler, J., & Homolka, M. (2011). Needles in faeces: An index of quality of wild ungulate winter diet. *Folia Zoologica*, *60*(1), 63–69.

Kamler, J., Homolka, M., & Čižmár, D. (2004). Suitability of NIRS analysis for estimating diet quality of free-living red deer *Cervus elaphus* and roe deer *Capreolus capreolus*. *Wildlife Biology*, *10*(3), 235–240. https://doi.org/10.2981/wlb.2004.021

Kelleher, V., Hunnick, L., & Sheriff, M. J. (2021). Risk-induced foraging behavior in a free-living small mammal depends on the interactive effects of habitat, refuge availability, and predator type. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.718887

Komsta, L. (2022). *outliers: Tests for Outliers* (Version R package version 0.15) [Software]. The R Foundation. https://doi.org/10.32614/cran.package.outliers

Kuijper, D. P. J., Verwijmeren, M., Churski, M., Zbyryt, A., Schmidt, K., Jędrzejewska, B., & Smit, C. (2014). What Cues Do Ungulates Use to Assess Predation Risk in Dense Temperate Forests? *PLoS ONE*, *9*(1), e84607. https://doi.org/10.1371/journal.pone.0084607

Kwiatkowski, W. (1994). Vegetation landscapes of Białowieża Forest. *Phytocoenosis*, *6*, 35–37.

Leslie, D. M., Bowyer, R. T., & Jenks, J. A. (2008). Facts From Feces: Nitrogen Still Measures Up as a Nutritional Index for Mammalian Herbivores. *The Journal of Wildlife Management*, *72*(6), 1420–1433. https://doi.org/10.2193/2007-404

Li, Z. (2013). Sex-Age Related Rumination Behavior of Père David's Deer under Constraints of Feeding Habitat and Rainfall. *PLoS ONE*, *8*(6), e66261. https://doi.org/10.1371/journal.pone.0066261

Lima, S. L., & Dill, L. M. (1990). Behavioral decisions made under the risk of predation: A review and prospectus. *Canadian Journal of Zoology*, *68*(4), 619–640. https://doi.org/10.1139/z90-092

Lourenço, A., & Pereira, H. (2018). Compositional Variability of Lignin in Biomass. In M. Poletto (Ed.), *Lignin—Trends and Applications*. InTech. https://doi.org/10.5772/intechopen.71208

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting Nitrogen into Protein—Beyond 6.25 and Jones' Factors. *Critical Reviews in Food Science and Nutrition*, *48*(2), 177–184. https://doi.org/10.1080/10408390701279749

Matuszkiewicz, J. M., Paluch, R., Szulc, A., Mielcarek, M., Lisiewicz, M., Kuberski, Ł., & Stereńczak, K. (2022). *Identification and mapping of forest communities of the Białowieża Forest using remote sensing data* (pp. 310–325).

Millard, S. P. (2013). *EnvStats: An R Package for Environmental Statistics*. Springer New York. https://doi.org/10.1007/978-1-4614-8456-1

Miller, J. N., & Miller, J. C. (2010). *Statistics and chemometrics for analytical chemistry* (6th ed). Prentice Hall/Pearson.

Miller, L. P., Matassa, C. M., & Trussell, G. C. (2014). Climate change enhances the negative effects of predation risk on an intermediate consumer. *Global Change Biology*, *20*(12), 3834–3844. https://doi.org/10.1111/gcb.12639

Millmier, A., J. Lorimor, C. Hurburgh Jr., C. Fulhage, J. Hattey, & H. Zhang. (2000). Near-infrared sensing of manure nutrients. *Transactions of the ASAE*, *43*(4), 903–908. https://doi.org/10.13031/2013.2986

Morosinotto, C., Villers, A., Varjonen, R., & Korpimäki, E. (2017). Food supplementation and predation risk in harsh climate: Interactive effects on abundance and body condition of tit species. *Oikos*, *126*(6), 863–873. https://doi.org/10.1111/oik.03476

Mysterud, A., & Østbye, E. (1999). Cover as a Habitat Element for Temperate Ungulates: Effects on Habitat Selection and Demography. *Wildlife Society Bulletin (1973-2006)*, *27*(2), 385–394. JSTOR.

Nasiadka, P., Kowalski, M., Sadowski, B., Wojtan, R., & Merta, D. (2016). Charakterystyka masy tuszy i poroży byków jeleni (*Cervus elaphus* L.) bytujących w Lasach Spalskich i Lasach Gostynińsko−Włocławskich w Polsce centralnej. *Sylwan 160 (9): 777-788*. https://doi.org/10.26202/SYLWAN.2016031

Novaes, E., Kirst, M., Chiang, V., Winter-Sederoff, H., & Sederoff, R. (2010). Lignin and Biomass: A Negative Correlation for Wood Formation and Lignin Content in Trees. *Plant Physiology*, *154*(2), 555–561. https://doi.org/10.1104/pp.110.161281

Nowak, K. (2025). *Understanding and Mitigating the Ecological Footprint of State Border Barriers*. Zenodo. https://doi.org/10.5281/ZENODO.15005820

Obidzinski, A., Kieltyk, P., Borkowski, J., Bolibok, L., & Remuszko, K. (2013). Autumn-winter diet overlap of fallow, red, and roe deer in forest ecosystems, Southern Poland. *Central European Journal of Biology*, *8*(1), 8–17. https://doi.org/10.2478/s11535-012-0108-2

Palo, R. T., Sunnerheim, K., & Theander, O. (1985). Seasonal variation of phenols, crude protein and cell wall content of birch (Betula pendula Roth.) in relation to ruminant in vitro digestibility. *Oecologia*, *65*(3), 314–318. https://doi.org/10.1007/bf00378904

Pépin, D., Adrados, C., Mann, C., & Janeau, G. (2004). Assessing real daily distance traveled by ungulates using differential GPS locations. *Journal of Mammalogy*, *85*(4), 774–780. https://doi.org/10.1644/ber-022

Pinheiro, J., & Bates, D. (2000). *Mixed-Effects Models in S and S-PLUS*. Springer-Verlag. https://doi.org/10.1007/b98882

Pinheiro, J., R Core Team, & Bates, D. (2025). *nlme: Linear and Nonlinear Mixed Effects Models* [Dataset]. The R Foundation. https://doi.org/10.32614/cran.package.nlme

Preisser, E. L., Bolnick, D. I., & Benard, M. F. (2005). Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology*, *86*(2), 501–509. https://doi.org/10.1890/04-0719

Proudman, N. J., Churski, M., Bubnicki, J. W., Nilsson, J.-Å., & Kuijper, D. P. J. (2021). Red deer allocate vigilance differently in response to spatio-temporal patterns of risk from human hunters and wolves. *Wildlife Research*, *48*(2), 163. https://doi.org/10.1071/wr20059

Ratkiewicz, M., Coissac, E., Swislocka, M., Czajkowska, M., Kowalczyk, R., Czernik, M., & Taberlet, P. (2024). Winter diet overlap among moose, roe deer and red deer in coniferous and mixed forests depends on snow cover depth. *Forest Ecology and Management*, *556*. https://doi.org/10.1016/j.foreco.2024.121710

Riesch, F., Wichelhaus, A., Tonn, B., Meißner, M., Rosenthal, G., & Isselstein, J. (2022). Grazing by wild red deer can mitigate nutrient enrichment in protected semi-natural open habitats. *Oecologia*, *199*(2), 471–485. https://doi.org/10.1007/s00442-022-05182-z

Rinehart, S., & Hawlena, D. (2020). The effects of predation risk on prey stoichiometry: A meta-analysis. *Ecology*, *101*(7), e03037. https://doi.org/10.1002/ecy.3037

Roberts, C., Westbrooke, M., Florentine, S., & Cook, S. (2015). Winter diet of introduced red deer (*Cervus elaphus*) in woodland vegetation in Grampians National Park, western Victoria. *AUSTRALIAN MAMMALOGY*, *37*(1), 107–112. https://doi.org/10.1071/AM14013

Say-Sallaz, E., Chamaillé-Jammes, S., Fritz, H., & Valeix, M. (2019). Non-consumptive effects of predation in large terrestrial mammals: Mapping our knowledge and revealing the tip of the iceberg. *Biological Conservation*, *235*, 36–52. https://doi.org/10.1016/j.biocon.2019.03.044

Schmidt, K., Jędrzejewski, W., Okarma, H., & Kowalczyk, R. (2009). Spatial interactions between grey wolves and Eurasian lynx in Białowieża Primeval Forest, Poland. *Ecological Research*, *24*(1), 207–214. https://doi.org/10.1007/s11284-008-0496-y

Showers, S. E., Tolleson, D. R., Stuth, J. W., Kroll, J. C., & Koerth, B. H. (2006). Predicting diet quality of white-tailed deer via NIRS fecal profiling. *Rangeland Ecology & Management*, *59*(3), 300–307. https://doi.org/10.2111/04-069.1

Tellado, S., & Azorit, C. (2014). An integrative assay to quantify the nutritional quality of the selected diet of two Mediterranean free-living deer by faecal-FT-NIRS. *Animal Production Science*, *55*(1), 11. https://doi.org/10.1071/AN13064

Tellado, S., & Azorit, C. (2015). An integrative assay to quantify the nutritional quality of the selected diet of two Mediterranean free-living deer by faecal-FT-NIRS. *Animal Production Science*, *55*(1), 11. https://doi.org/10.1071/AN13064

Tellado, S., Orpez, R., Muñoz-Cobo, J., & Azorit, C. (2015). Fecal-FT-NIRS as a noninvasive tool for assessing diet quality of mediterranean deer. *Rangeland Ecology & Management*, *68*(1), 92–99. https://doi.org/10.1016/j.rama.2014.12.003

Theuerkauf, J., & Rouys, S. (2008). Habitat selection by ungulates in relation to predation risk by wolves and humans in the Białowieża Forest, Poland. *Forest Ecology and Management*, *256*(6), 1325–1332. https://doi.org/10.1016/j.foreco.2008.06.030

Vala, Z., & Ernst, M. (2011). Red deer density in the air-polluted area of forest ecosystems in the Krušné hory Mts. - Klášterec nad Ohří Forest District. *Journal of Forest Science*, *57*(2), 59–63. https://doi.org/10.17221/57/2010-JFS

Vance, C. K., Tolleson, D. R., Kinoshita, K., Rodriguez, J., & Foley, W. J. (2016). Near Infrared Spectroscopy in Wildlife and Biodiversity. *Journal of Near Infrared Spectroscopy*, *24*(1), 1–25. https://doi.org/10.1255/jnirs.1199

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed). Springer.

Verdolin, J. L. (2006). Meta-analysis of foraging and predation risk trade-offs in terrestrial systems. *Behavioral Ecology and Sociobiology*, *60*(4), 457–464. https://doi.org/10.1007/s00265-006-0172-6

Waliszewska, B., Grzelak, M., Gaweł, E., Spek-Dźwigała, A., Sieradzka, A., & Czekała, W. (2021). Chemical characteristics of selected grass species from Polish meadows and their potential utilization for energy generation purposes. *Energies*, *14*(6), 1669. https://doi.org/10.3390/en14061669

Wikenros, C., Kuijper, D. P. J., Behnke, R., & Schmidt, K. (2015). Behavioural responses of ungulates to indirect cues of an ambush predator. *Behaviour*, *152*(7–8), 1019–1040. https://doi.org/10.1163/1568539x-00003266

Zbyryt, A., Bubnicki, J. W., Kuijper, D. P. J., Dehnhard, M., Churski, M., & Schmidt, K. (2018). Do wild ungulates experience higher stress with humans than with large carnivores? *Behavioral Ecology*, *29*(1), 19–30. https://doi.org/10.1093/beheco/arx142

Zhong, L., Zhang, W., Yang, M., Wu, S., Zhi, X., & Zhang, M. (2019). Winter diet variation and overlap of sympatric red deer and sika deer in northeast China. *Polish Journal of Ecology*, *67*(4), 354–366. https://doi.org/10.3161/15052249PJE2019.67.4.007

Supplementary materials

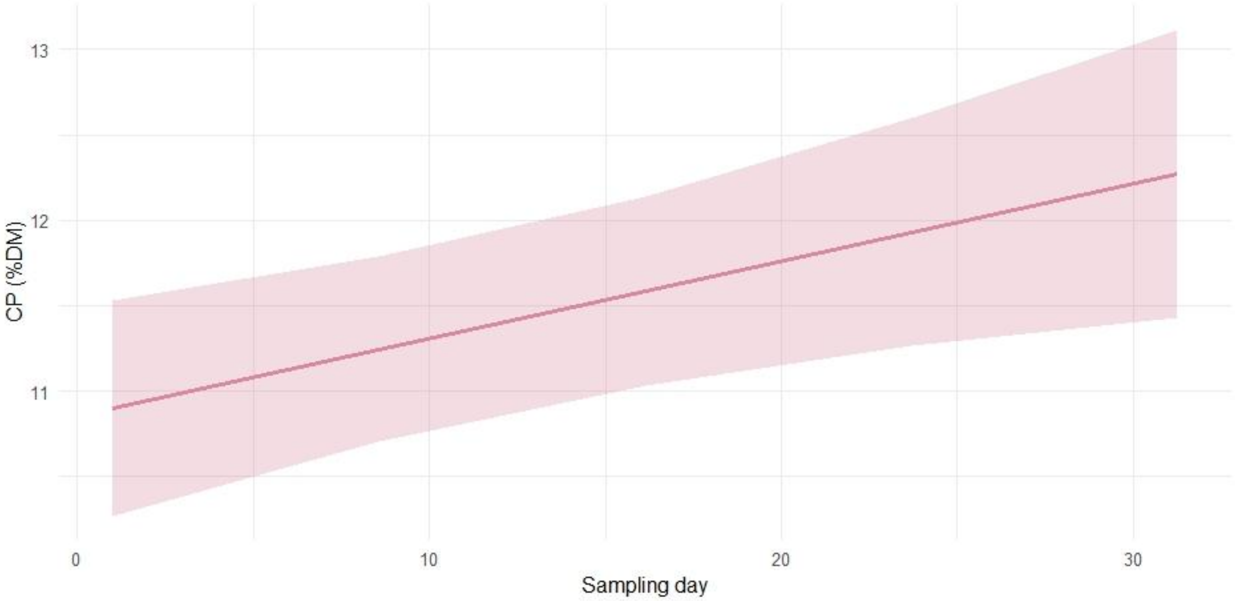

Figure S1: *Predicted values from linear mixed model illustrating the variation of crude protein content (CP % dry matter) in red deer faeces in response to the sampling period (days). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

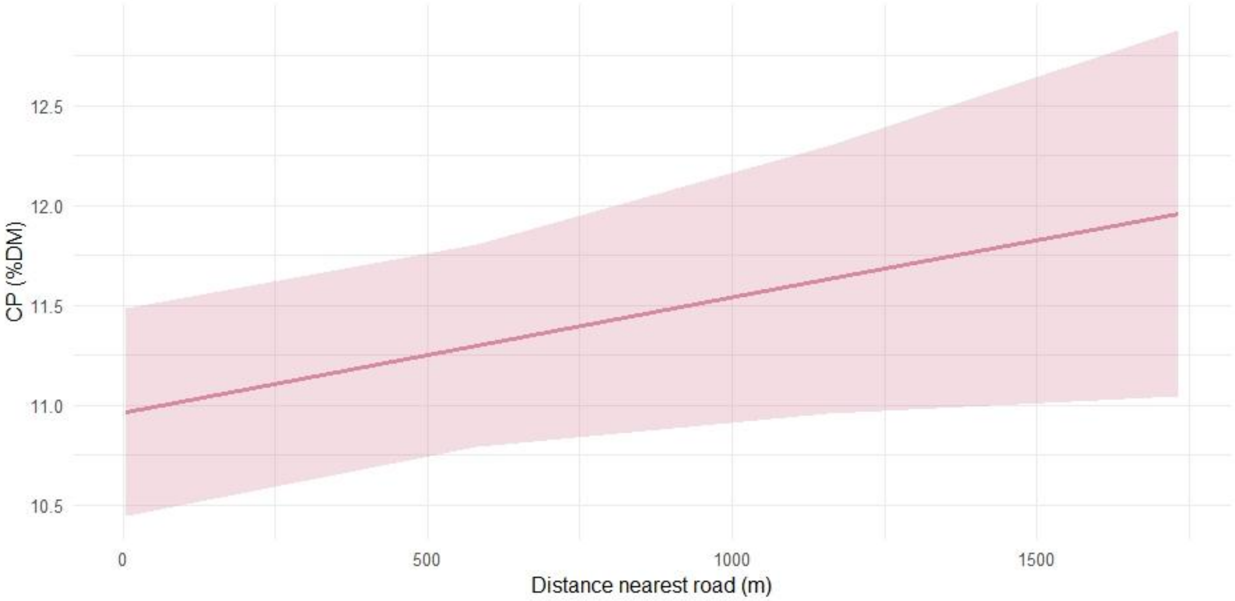

Figure S2*: Predicted values from linear mixed model illustrating the variation of crude protein content (CP % dry matter) in red deer faeces in response to the distance to the nearest road (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

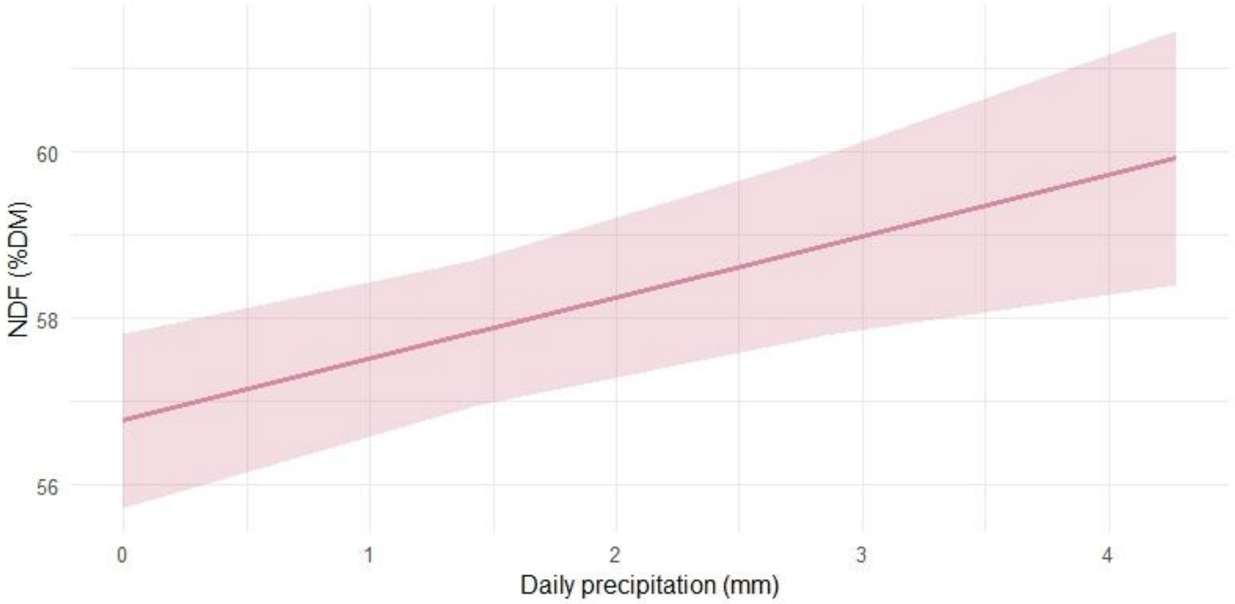

Figure S3: *Predicted values from linear mixed model illustrating the variation of neutral detergent fibre content (NDF % dry matter) in red deer faeces in response to precipitation the day before sampling (millimetres). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

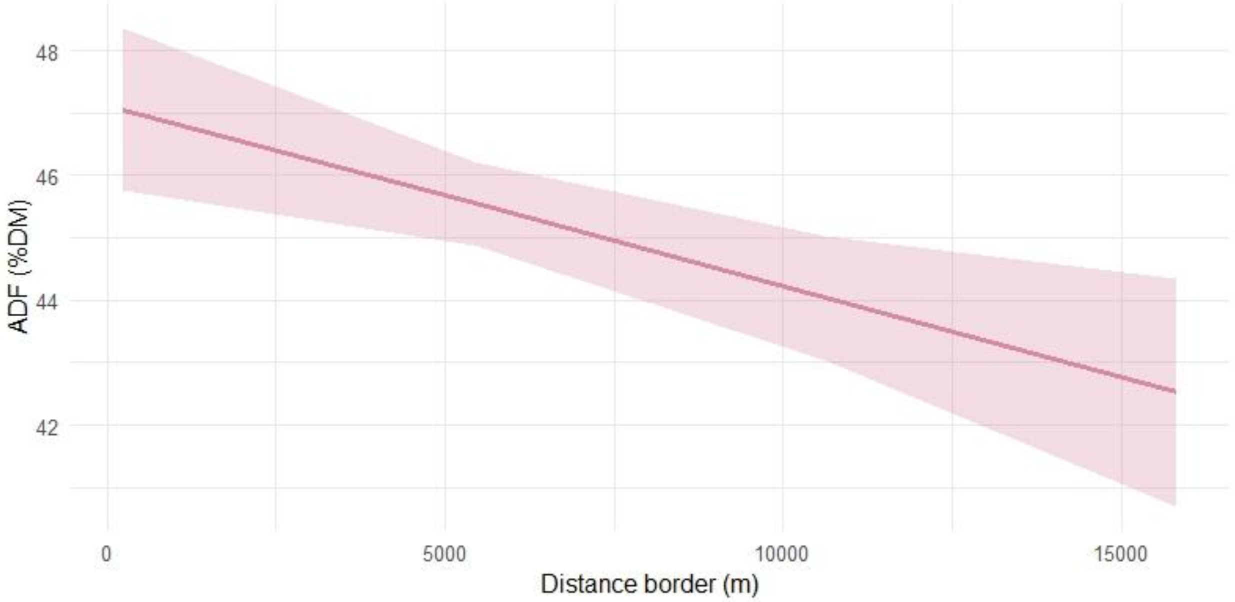

Figure S4: *Predicted values from linear mixed model illustrating the variation of acid detergent fibre content (ADF % dry matter) in red deer faeces in response to the distance to the state border (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

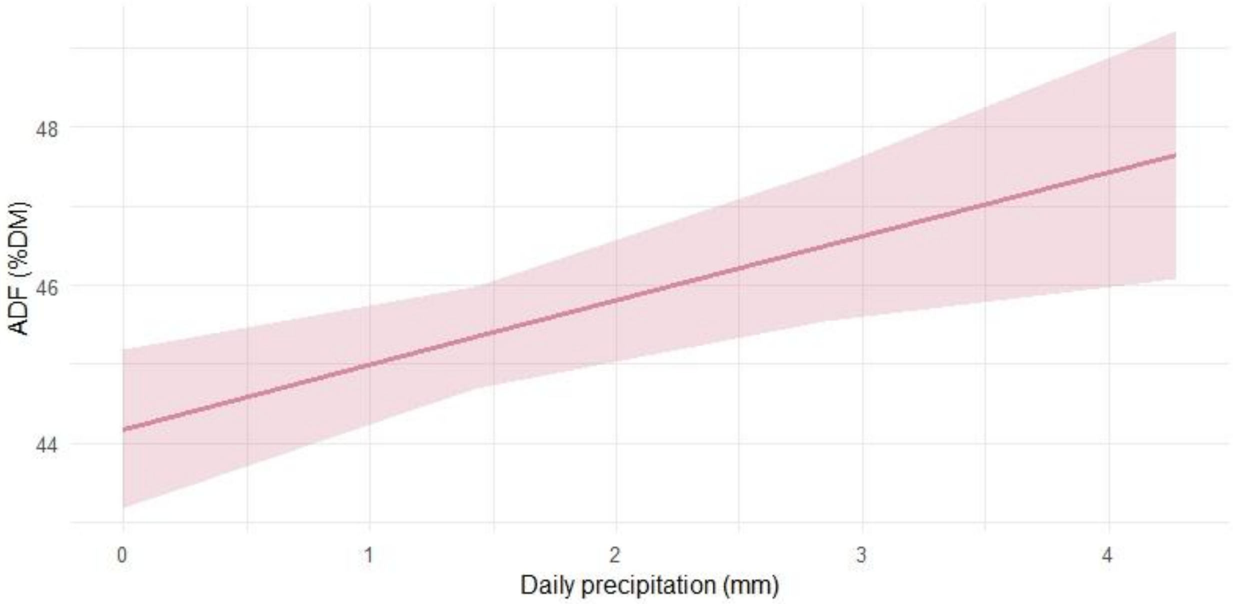

Figure S5*: Predicted values from linear mixed model illustrating the variation of acid detergent fibre content (ADF % dry matter) in red deer faeces in response to precipitation the day before sampling (millimeters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

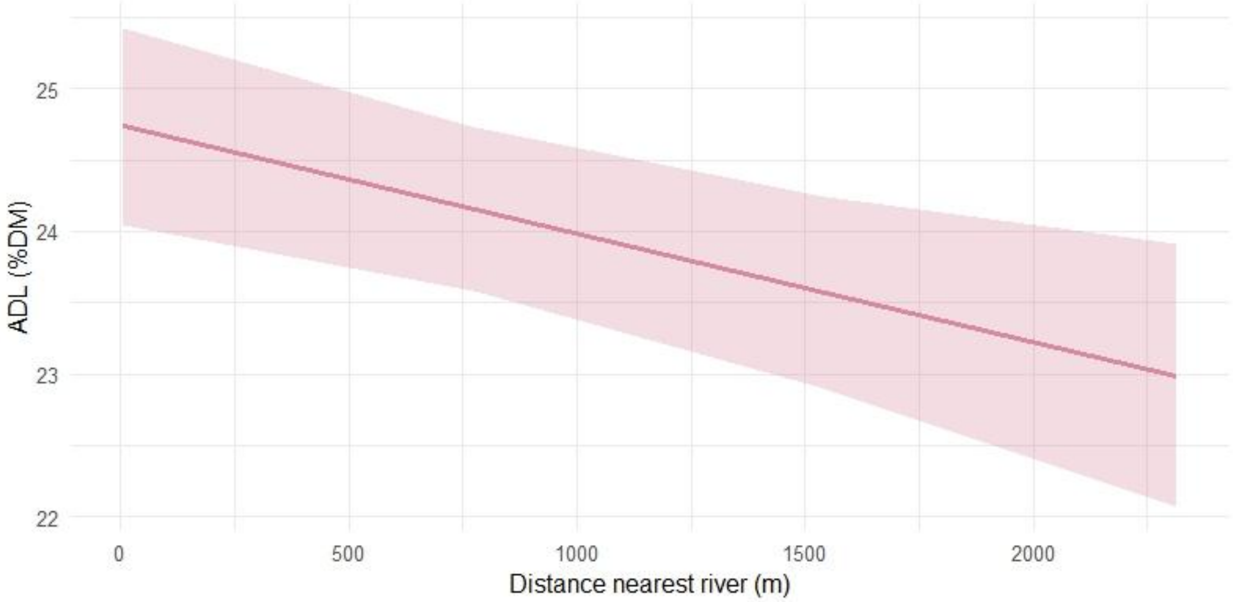

Figure S6*: Predicted values from linear mixed model illustrating the variation of acid detergent lignin content (ADL % dry matter) in red deer faeces in response to the distance to the nearest river (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

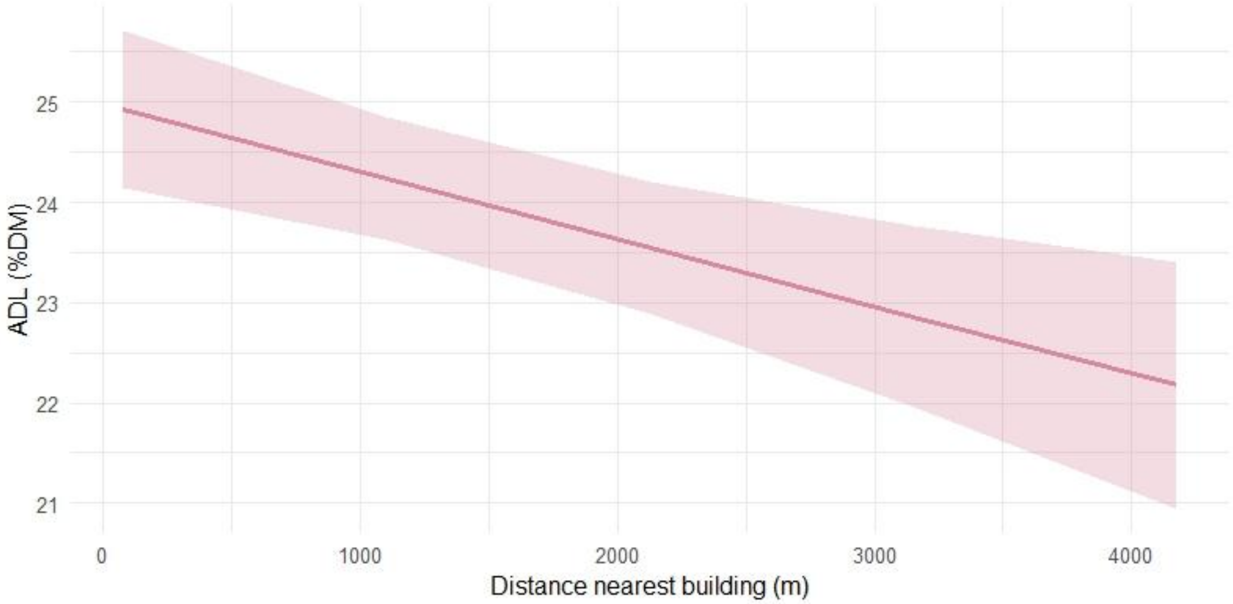

***Figure S7**: Predicted values from linear mixed model illustrating the variation of acid detergent lignin content (ADL % dry matter) in deer faeces in response to the distance to the nearest building (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

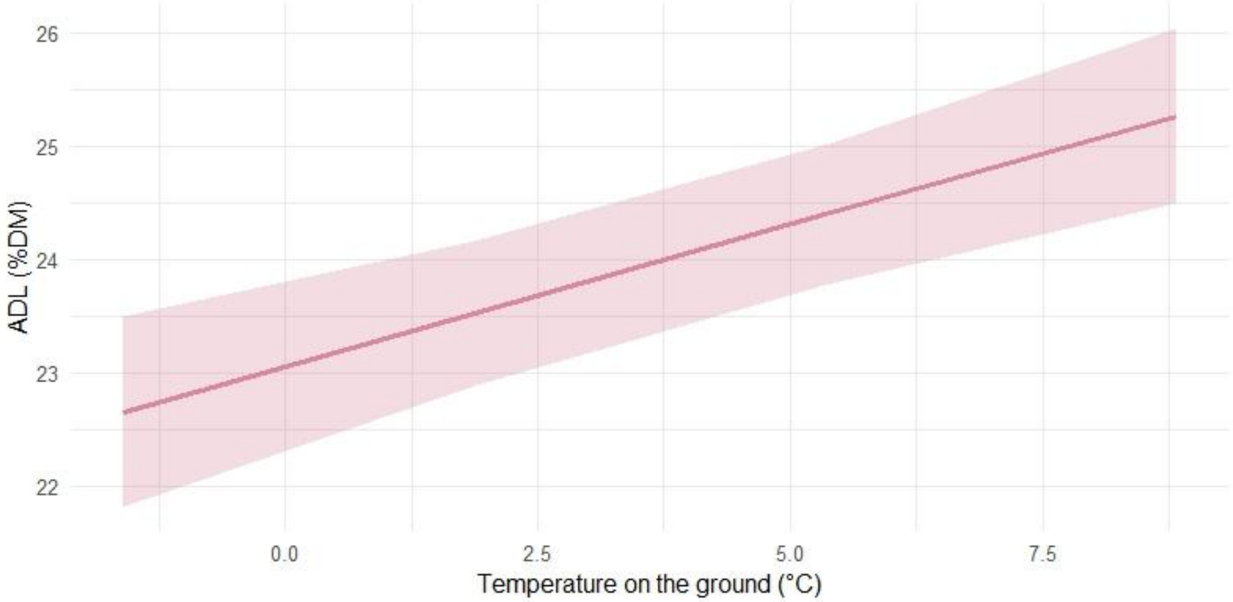

***Figure S8**: Predicted values from linear mixed model illustrating the variation of acid detergent lignin content (ADL % dry matter) in deer faeces in response to the temperature 5 cm above the ground (degree Celsius). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

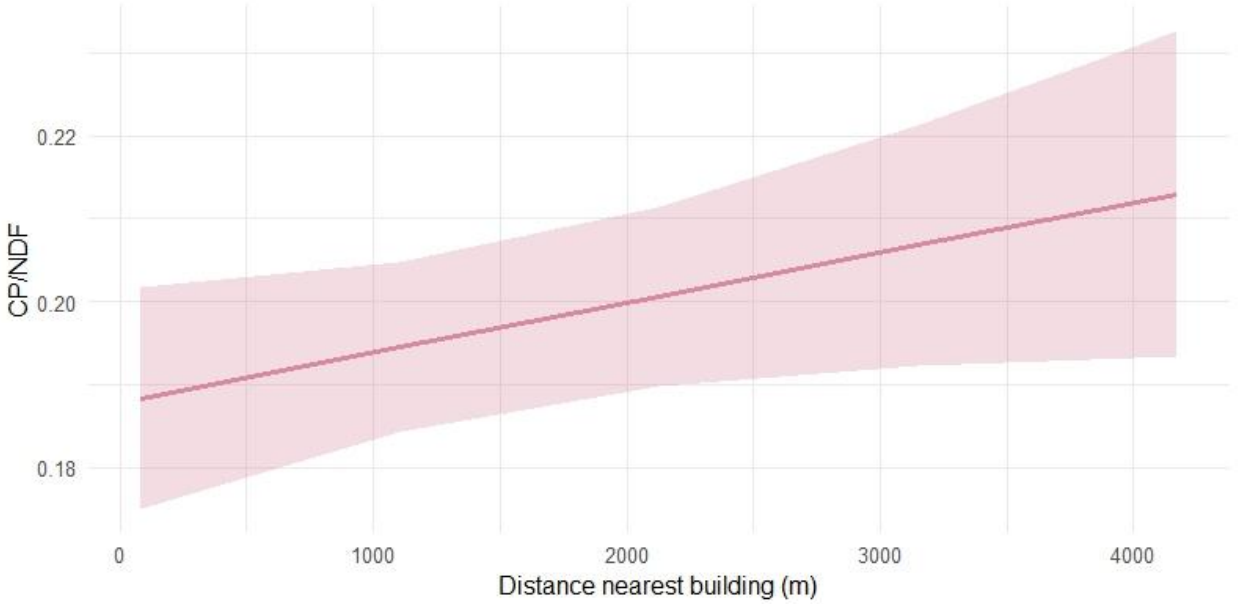

Figure S9: *Predicted values from linear mixed model illustrating the variation of the crude protein to neutral detergent fibre ratio (CP/NDF) in red deer faeces in response to the distance to the nearest building (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

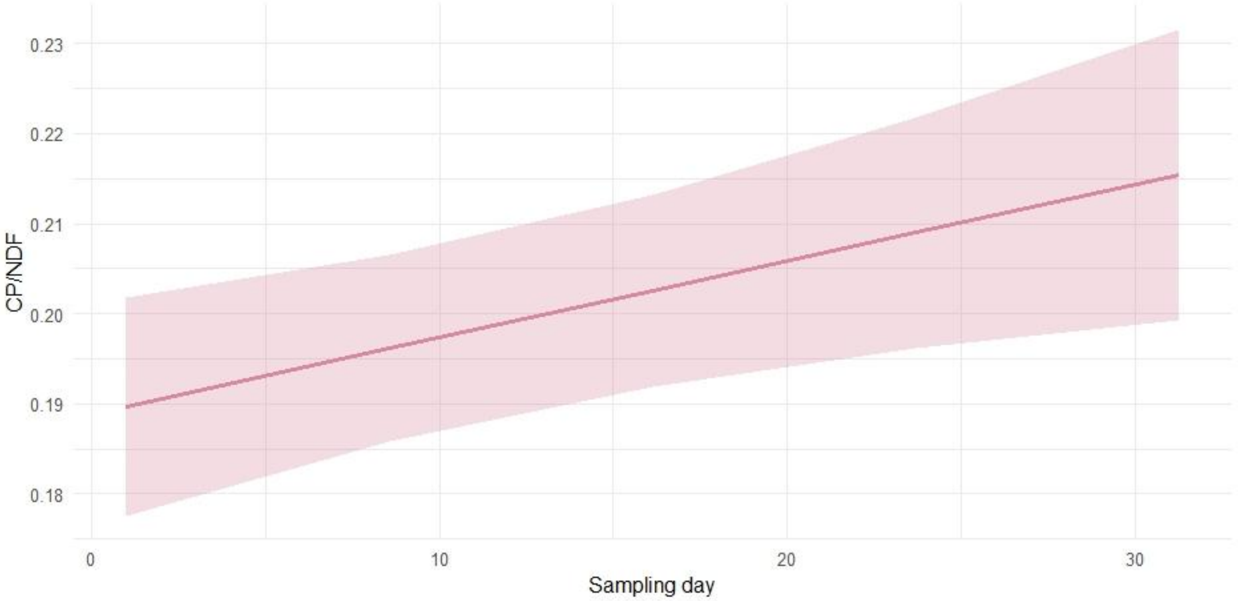

Figure S10: *Predicted values from linear mixed model illustrating the variation of the crude protein to neutral detergent fibre ratio (CP/NDF) in red deer faeces in response to the sampling period (days). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

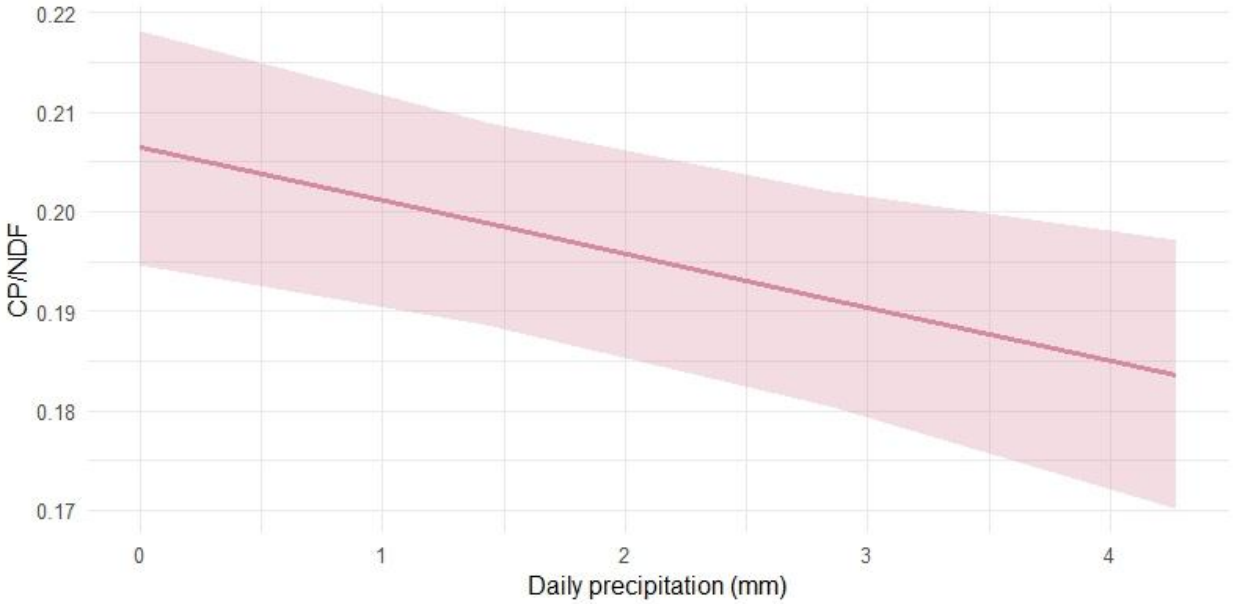

***Figure S11**: Predicted values from linear mixed model illustrating the variation of the crude protein to neutral detergent fibre ratio (CP/NDF) in red deer faeces in response to precipitation the day before sampling (millimeters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

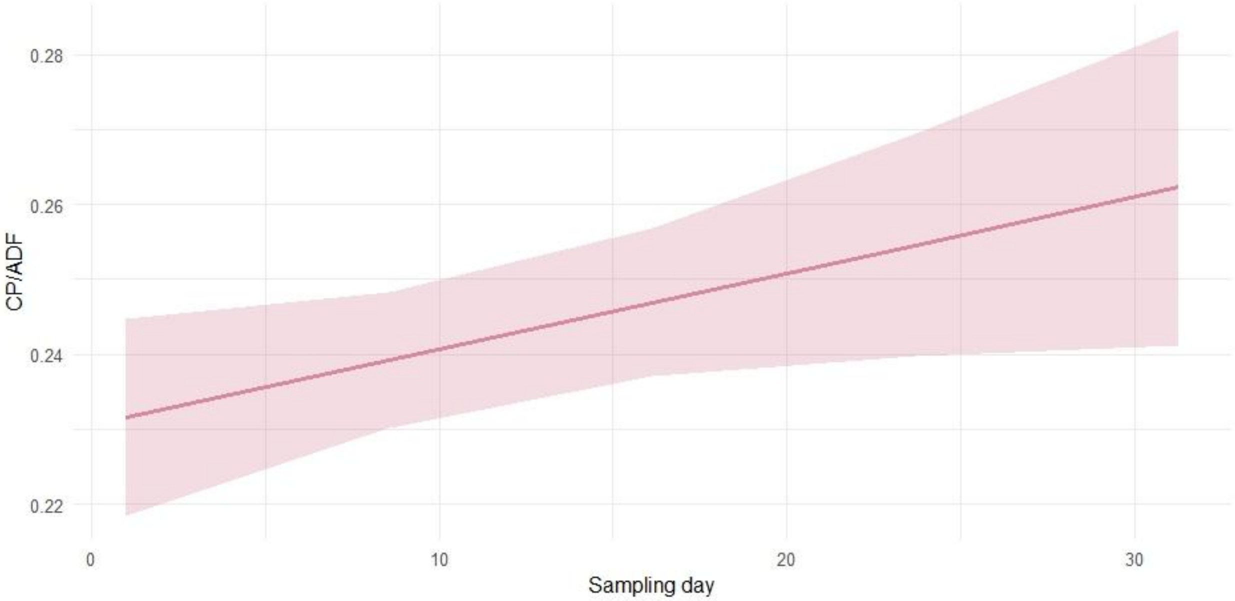

***Figure S12**: Predicted values from linear mixed model illustrating the variation of the crude protein to acid detergent fibre ratio (CP/ADF) in red deer faeces in response to the sampling period (days). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

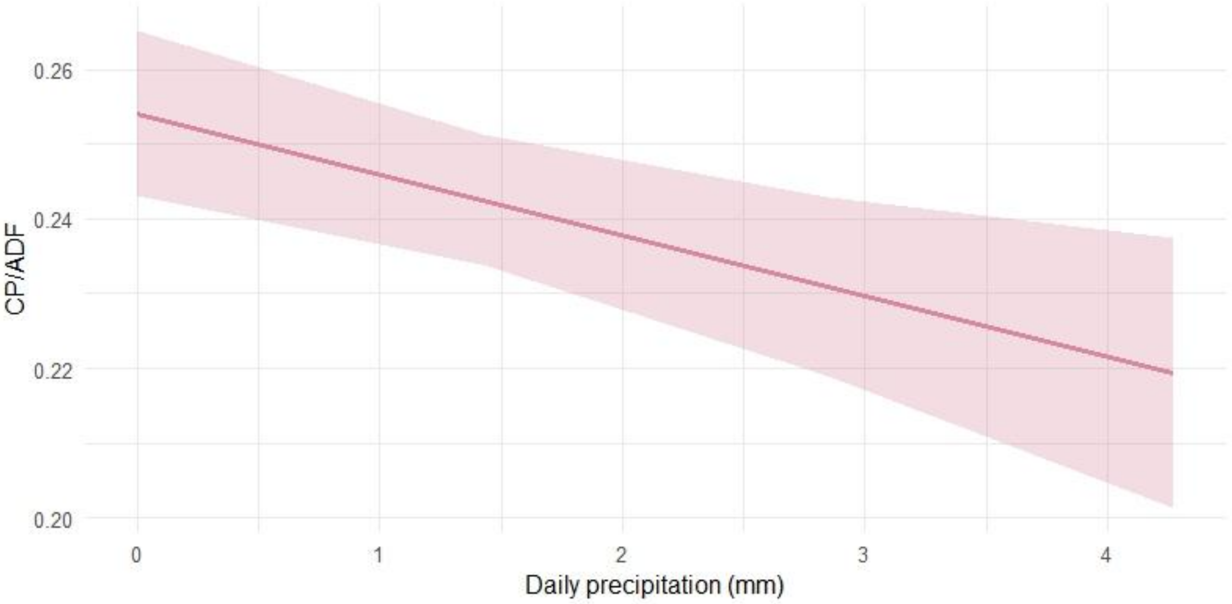

***Figure S13**: Predicted values from linear mixed model illustrating the variation of the crude protein to acid detergent fibre ratio (CP/ADF) in red deer faeces in response to precipitation the day before sampling (millimeters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

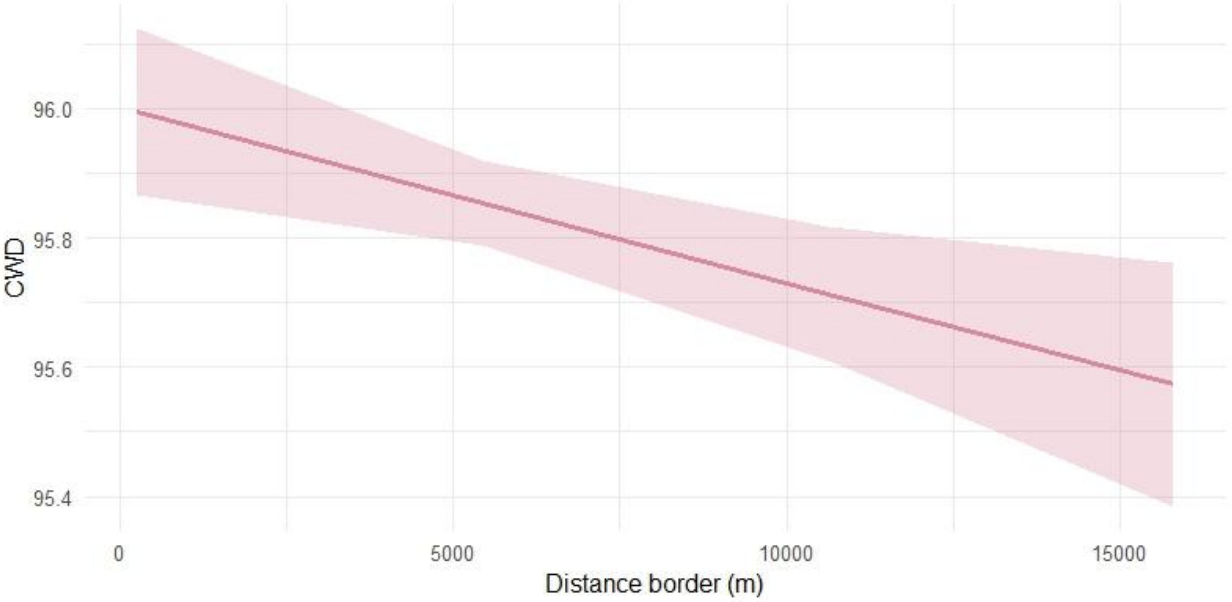

***Figure S14**: Predicted values from linear mixed model illustrating the variation of the cell wall digestibility index (CWD) in red deer faeces in response to the distance to the state border (meters). Data inlcluded 279 faecal samples collected around 81 locations in the Białowieża Forest.*

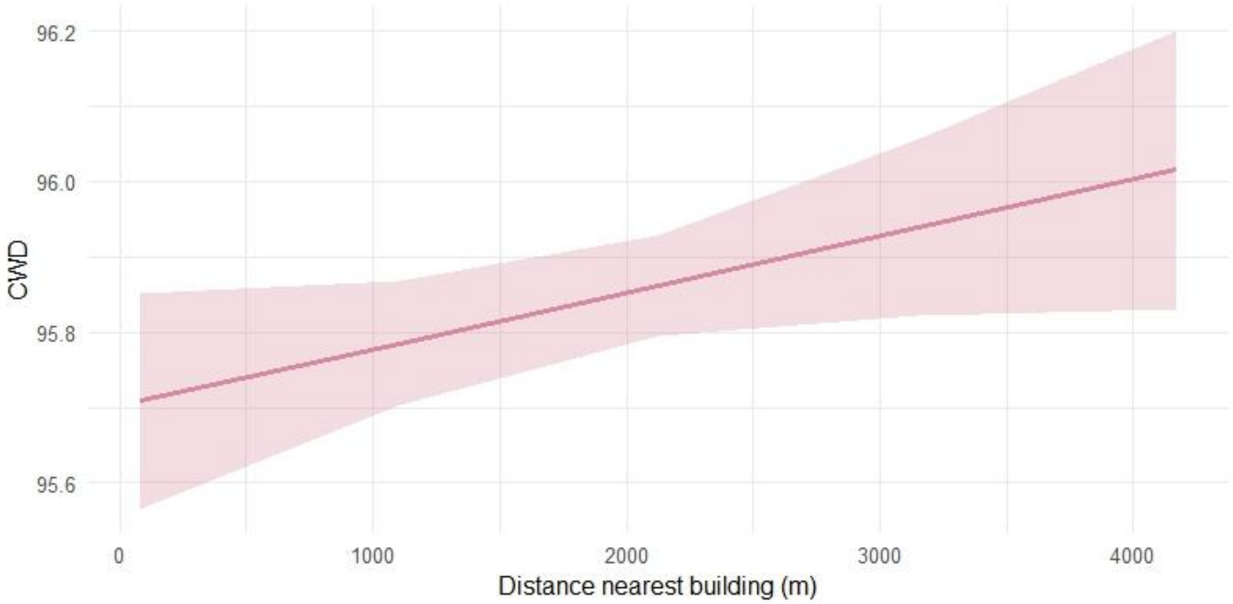

Figure S15: *Predicted values from linear mixed model illustrating the variation of the cell wall digestibility index (CWD) in red deer faeces in response to the distance to the nearest building (meters). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*

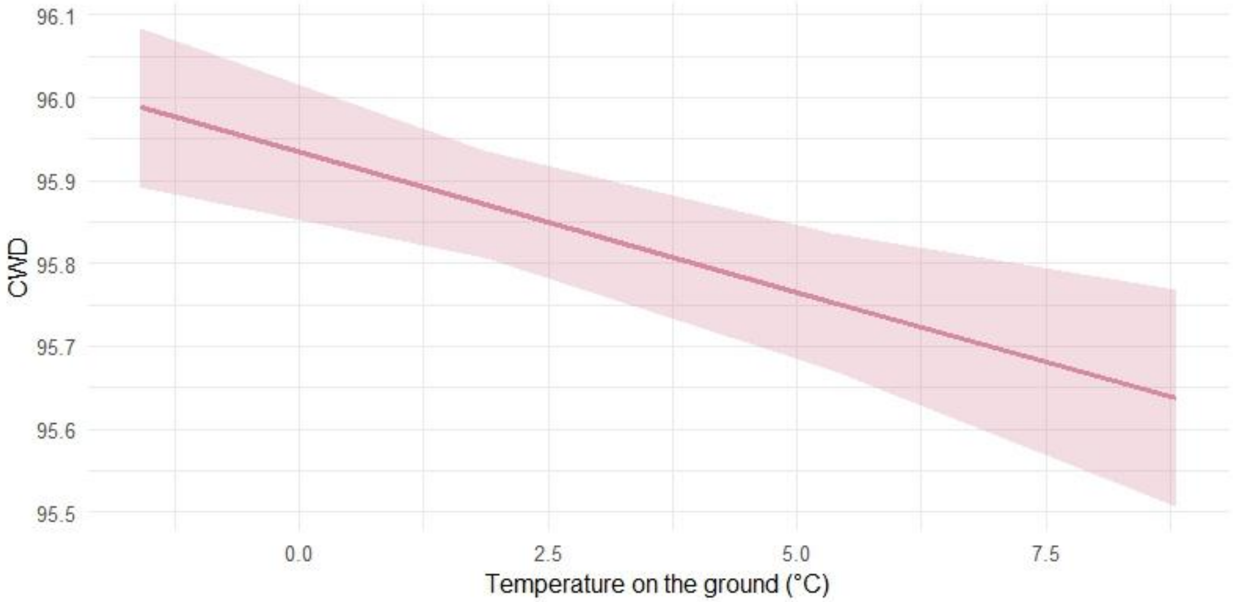

Figure S16: *Predicted values from linear mixed model illustrating the variation of the cell wall digestibility index (CWD) in red deer faeces in response to the temperature 5 cm above the ground (degree Celsius). Data included 279 faecal samples collected around 81 locations in the Białowieża Forest.*