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“Human-mediated” impact of ungulates on the carbon stocks of the Veluwe forest (Gelderland, The Netherlands)



MSc Ecology & Conservation

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Abstract

Greenhouse gases accumulation constitutes one of the main problems of modern society. Forests, which are the main terrestrial carbon sinks, could represent a great help to reduce the carbon footprints on regional scale. To exploit all their potential, we need a correct understanding of carbon cycling. Recent studies show that animals play a bigger role than expected in carbon dynamics. Even so, they have rarely been included in models predicting carbon cycling. In addition, while the impacts of some animals such as ungulates are affected by human presence at local scale, these effects have not been considered in studies on local carbon stocks. To cover this lack of knowledge, we analysed the impact of ungulates on local carbon stocks (soil, litter, and understory aboveground vegetation) of the Veluwe forest (Gelderland, the Netherlands), considering the influence of humans on ungulate presence and behaviour. Using camera traps located in plots at different distances from human paths (20 and 100 metres), we related differences in presence and behaviour of ungulates with the carbon quantities of the above-mentioned carbon stocks measured in the same locations of the camera traps. Because previous works have shown that ungulates change their space-use and behaviour in presence of humans, we expected fewer visits of ungulates and higher level of vigilance close (20 m) than away (100 m) from human paths. As consequence, we expected to find a negative response in the carbon stocks in the pools: with higher concentration of carbon in the aboveground vegetation, litter, and soil carbon pools close (20 m) to human paths than in the same pools away (100 m) from the paths. We found consistently fewer visits per day of ungulates close (20 m) than away (100 m) from human paths, but their behaviour was not significantly different 20 or 100 metres from human paths. We also found that in the aboveground vegetation and litter pools close (20 m) to human paths, there were higher concentrations of carbon than in the same pools away (100 m) from the paths. We did not find differences in carbon concentration in the soil carbon pools close and away from human paths, but we did find a strong positive relation between litter content and soil organic matter, which suggests an indirect effect of ungulates on soil carbon. In conclusion, our study suggests that ungulates do have an impact on the carbon stocks of the Veluwe forest and that this is affected by human presence. Therefore, when predicting carbon cycling, we should not only include effects of animals, but also consider the influence that humans can have on their habitat choice and behaviour.

Introduction

Climate change represents a main challenge of modern society (Naustdalslid, 2011). Scientists are focusing their attention on carbon cycling to find ways to store C more effectively and reduce global warming (Sedjo, 1989; Singh, 2013; Turner et al., 2009; Withey et al., 2019). According to the Kyoto protocol (UNFCCC, 1997), terrestrial carbon sinks can be used to mitigate the effect of green-house gases. Since forests represent one of the main terrestrial carbon sinks, a correct understanding of the C cycle in forest ecosystems has a key role in finding a solution to climate change. While most models predicting carbon cycling focus on the role of primary producers, recent studies show that animals have a bigger impact on carbon fluxes than expected (Schmitz et al., 2014, 2018; Tanentzap & Coomes, 2012; Wilmers & Schmitz, 2016). The lack of studies on the influence of animals on the carbon stocks might cause incorrect predictions of the carbon fluxes on regional scale and lead to the wrong conclusions.

Wild ungulates represent one of the main drivers of changes in vegetation structure and composition in forest ecosystems (Bardgett & Wardle, 2003; Bressette & Beck, 2013; Chollet et al., 2021; Gerhardt et al., 2013; Gill, 2001; Putman, 1996; Ramirez et al., 2021) affecting forest regeneration and succession (Bressette & Beck, 2013). According to Tanentzap and Coomes (2012), herbivores reduce the carbon storage of worldwide temperate forests of $40,000 \pm 60,000$ tonnes of carbon per year, by directly and indirectly affecting above- and below-ground carbon stocks (Bressette & Beck, 2013; Chollet et al., 2021; Hirst, 2021). However, their effects on the carbon pools on small spatial scales are less known, even while they might be really significant (Andriuzzi & Wall, 2017; Bardgett & Wardle, 2003; Chollet et al., 2021; Hirst, 2021; Tanentzap & Coomes, 2012). For example, Wilmers and Schmitz (2016) show that selective browsing by moose (*Alces alces*) affects litter quality, thus reducing boreal forest productivity, whereas elk's (*Cervus canadensis*) grazing is believed to increase Yellowstone's grasslands productivity; in turn, both species affect the carbon retention ability of their respective ecosystems. While several studies demonstrate the existence of direct effects of herbivores on above-ground carbon storages, e.g. via grazing and browsing, less is known about their indirect impact on below-ground carbon stocks, e.g. via trampling and affecting litter, even though evidence suggest that it might be significant (Chollet et al., 2021; Hirst, 2021; Tanentzap & Coomes, 2012) and that above- and below- ground dynamics are strictly intertwined (Andriuzzi & Wall, 2017; Bardgett & Wardle, 2003).

Ungulates like deer can directly affect below-ground carbon content by browsing, thus removing biomass, which influences litter quality and quantity that is the main source of carbon in the soil, in turn affecting soil fauna composition, and thus organic matter decomposition rates and carbon stabilization in the ground (Allombert et al., 2005; Bardgett & Wardle, 2003; García-Palacios et al., 2013; Hirst, 2021; Tanentzap & Coomes, 2012). Ungulates can also indirectly affect below-ground carbon content by trampling, which compacts the soil and changes its physical properties (Andriuzzi & Wall, 2017; Chollet et al., 2021; García-Palacios et al., 2013; Hirst, 2021; Mohr et al., 2005; Ramirez et al., 2021). Other forest ungulates like wild boar (*Sus scrofa*) can also have a great impact on soil mechanisms, aboveground vegetation growth and litter quantity (Genov et al., 2017; Liu et al., 2020; Mohr et al., 2005; Risch et al., 2010). Bioturbation activities like trampling and grubbing by wild boar can significantly affect soil properties, such as soil bulk density and moisture, altering soil fauna composition and potentially leading to an increase in soil CO₂

emissions (Cuevas et al., 2012; Liu et al., 2020; Risch et al., 2010). Soil fauna plays a key role in soil C dynamics and ungulates can affect it directly by consumption of soil plants and animals (Cuevas et al., 2012; Mohr et al., 2005; Risch et al., 2010). Mohr et al. (2001) show how the disturbance of the soil by wild boar and red deer significantly decreased the number of soil arthropods in German oak forests, and the same effect has been noticed in Hawaiian forests by wild pigs (Risch et al., 2010). Thus, although evidence suggests that ungulates such as deer and wild boar drastically affect soil C cycling, only a few studies have been focusing on their impact on soil C dynamics (Don et al., 2019; Liu et al., 2020).

Recent studies show that ungulates behaviour is strongly influenced by human presence, which can increase their vigilant behaviour, altering ungulates food intake and habitat choice (Bonnot et al., 2013; Ciuti et al., 2012; Mols et al., 2021, 2022; Möst et al., 2015; Ramirez, Jansen, et al., 2021). On top of that, different studies show that the behaviourally mediated trophic cascade triggered by humans might even overrule the one induced by predators (Ciuti et al., 2012; Mols et al., 2021; Proffitt et al., 2009), reinforcing the idea that human effects cannot be overlooked. Mols et al. (2021) already showed how recreation and hunting activities by humans affect deer space-use and vegetation growth at the Veluwe forest, the Netherlands, but if and how these effects also cascade onto the carbon content of this forest ecosystem have not been studied yet.

In this study we aim to understand the impact of humans on the small-scale effect of ungulates on the carbon content of the Veluwe forest (Gelderland, the Netherlands). We expect to find a negative relation between abundance of ungulates and proximity to human paths. In locations closer to human paths, we expect to find fewer but more vigilant ungulates along with higher concentration of carbon in the aboveground vegetation, litter, and soil carbon pools, whereas in plots farther from human paths, we expect to find more but less vigilant ungulates together with lower concentration of carbon in the studied pools. This research will increase our understanding of the dynamics of carbon stocks, analysing factors which are usually not taken into consideration when predicting the carbon content of an ecosystem and it might even help in developing conservation strategies to prevent further carbon losses on regional scales.

Methods

Study area and study design

Our study system, the Veluwe area (Gelderland, the Netherlands, Fig.1), is one of the largest connected forest area of the Netherlands. We focused our study on the northern part of the forest, and the main human settlements in the surrounding of the study area are the following: Appeldorn (52.2112° N, 5.9699° E) in the south-eastern side, Nunspeet (52.3748° N, 5.7699° E) in the northern side and Ermelo (26.5124° S, 29.9856° E) in the western side. The climate of the region is temperate maritime, with mean annual temperatures of 10.5°C ± 0.12 SE and mean annual precipitation of 850 mm ± 255 SE (29 years average, 1990-2019, De Bilt, Royal Netherlands Meteorological Inst.). The area is open to the public between sunrise and sunset and several trails allow people to walk or bike around the forest. Walking off-trails is prohibited, and only the managers of the area are allowed to use motorized vehicles on the trails.

The vegetation in the Veluwe area is dominated by deciduous and coniferous forest alternate with heather (*Calluna vulgaris*) heathlands. Our study plots were located exclusively in pine-bilberry forests, where Scots pine trees (*Pinus sylvestris*) and bilberry bushes (*Vaccinium myrtillus*) represents the main component of the flora. Kuiters and Slim (2002) reported an average of 14 ungulates per km⁻¹ in the Veluwe area, and since then the number have kept raising (Ramirez et al., 2021). In this study we focused on three deer species, red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and roe deer (*Capreolus capreolus*) and on wild boar (*Sus scrofa*), which represent the largest and most common ungulates of the area (Ramirez et al., 2021).

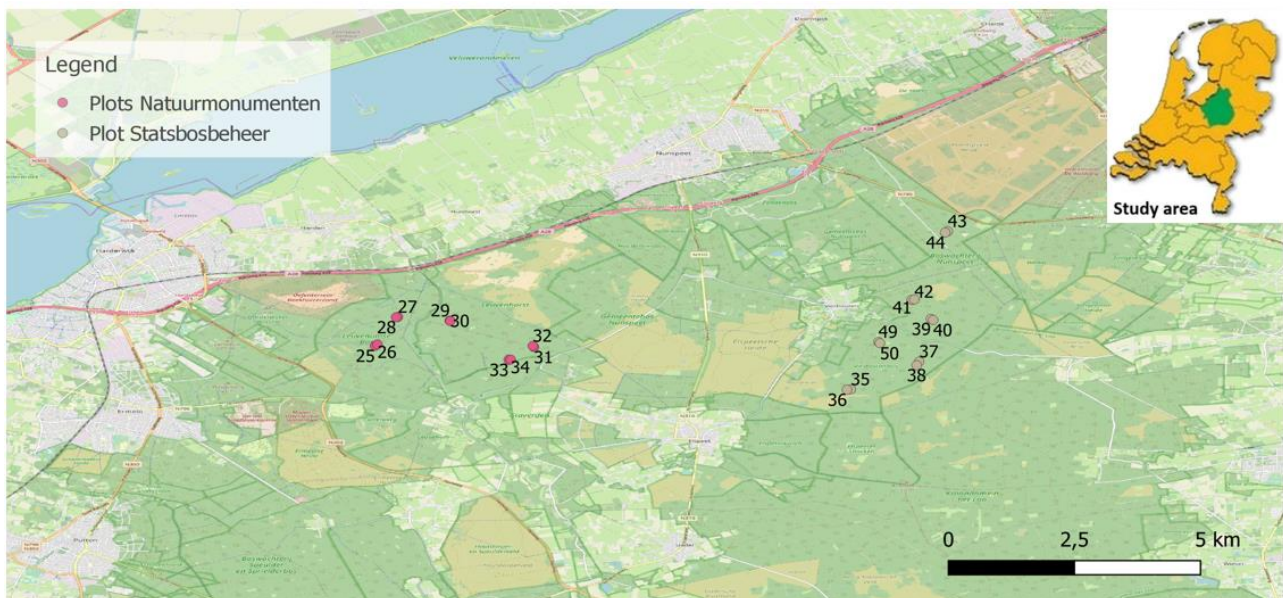


Fig.1: QGIS map of plots locations (Gelderland, the Netherlands). The pictures show the paired plot set-up of the study: uneven numbers represent plots 20 metres away from human paths, while even numbers those 100 metres away. In the legend are specified the different managements in the two locations of this study in the Veluwe forest.

We analysed a total of 22 plots (11 pairs), located in two different areas of the Veluwe forest (Fig. 1), managed by two different organizations: 12 plots were in Boswachterij Nunspeet

(2388 ha managed by Staatsbosbeheer), and the other 10 in Leuvenumse Bos (837 ha managed by Natuurmonumenten). Each pair of plots was located on a transect parallel to human paths, one 20 metres from the path and the other 100 metres away (Fig. 2). Previous studies have already showed that ungulates respond differently to these distances (Mols et al., 2021, 2022). Vegetation type (Scots pine trees and bilberry bushes) and distance to human paths (20 and 100 metres) were the main features used to select the plots.

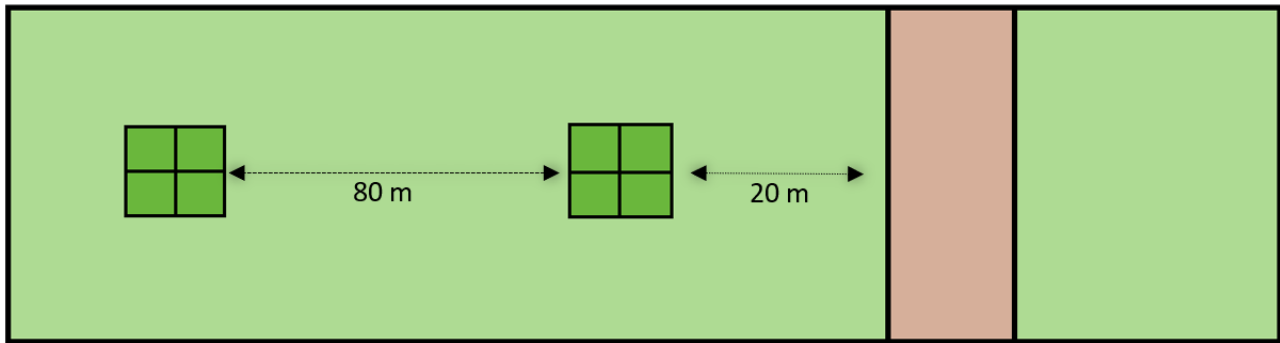


Fig. 2: paired plots set-up. The light brown stripe represents a human path, and the dark green square the paired plots 20 and 100 metres distant from the path. Each plot is divided into four subplots, see Appendix 1 for plot details.

For the purpose of this study, we assessed carbon stocks at different pools' level (understory aboveground vegetation, understory litter, and soil), in plots close (20 m) and away (100 m) from human frequented trails, which allowed us to study areas with different levels of animal presence. Using camera traps in the same locations where we assessed the carbon stocks, we related the carbon content to the presence and the behaviour of ungulates.

Camera traps

We collected data about presence and behaviour of ungulate using 22 camera traps previously located in the same locations where we assessed the carbon stocks. The recording took place from May 2020 until September 2021. Each camera trap was attached at a height of 100-120 cm on a tree, pointing on the opposite direction of the human paths. The following two types of camera traps were used: Bushnell Trophy Cam HD Agressor 2017 No Glow and Bushnell Trophy Cam HD 2013 (Appendix 2, table 1). Camera traps recorded videos of 15 seconds every time they detected a movement, both during day- and night- time. When no movements were detected, the registrations stopped. By analysing the videos with the software Boris version 8.7, we assessed the number and the species of animals visiting the plots, with a particular focus on ungulate: wild boar, red deer, roe deer and fallow deer. For all the species of deer we assessed their behaviour following an ethogram, which can be seen in Appendix 3 (table 2). We divided all the behaviours as "vigilant" or "non-vigilant" and we registered their duration to calculate the total vigilant level. We recorded the behaviour of only one individual per video, in those videos with more than one animal we chose the animal that firstly appeared in the sight of the camera. In those videos where animals were already present, we recorded the behaviour of the animal in the centre of the video. Vigilant level has been calculated only for deer species since it was too difficult to determine the behaviour of wild boar, and it is presented as a percentage of the total registered activity of the animals.

Carbon sampling

The carbon sampling took place between the 16th of March and the 17th of April 2022. We analysed a total of 22 plots, placed 1 m in front of the tree where the camera traps were previously located, so that we could measure the carbon stocks in the same areas recorded by the camera traps. All the plots were squares of 4 m x 4 m, divided in 4 subplots 2 m x 2 m (Appendix 1, Fig. 1 and 2), and characterized by the same vegetation type (Pine-bilberry forest). For each plot we recorded the number, the length and the width of dead trees, dead branches and seedlings present within the 4 m X 4 m square. To evaluate the soil structure at a plot level, we took a sample from the centre of the plot with an Edelman auger, and we measured the depth until we reached the sandy soil. For each of the 4 subplots, we took measurements of the aboveground vegetation, of the litter and of the soil. On the corner of each subplot, we placed a square 0.5 m x 0.5 m, and on this square, we cut and collected all the aboveground vegetation and the litter. For each subplot we also took a 25 cm deep sample of soil using an Edelman auger. Overall, for each plot we collected a total of 12 samples: 4 samples of aboveground vegetation, 4 of litter and 4 of soil, for a total of 264 samples. All the samples were stored into a fridge (4°C) and then analysed in the laboratory.

Laboratory analysis

Soil Organic Matter (Loss of Ignition Method)

We dried all the soil samples from each subplot at 105°C for 24 h in an electric oven. Once all the samples were dried, we took a well-mixed subsample of 8-12 g from each sample, and we measured the dry weight. All the subsamples were then placed in a muffle furnace at 420°C for 8 hours: 4 hours to reach the temperature and 4 hours at 420°C. After 8 hours in the muffle furnace all the subsamples were weighed again. The weight lost after the ignition is representative for the OM (organic matter) content of the subsample (Abella & Zimmer, 2007; Heiri et al., 2001; Hoogsteen et al., 2015), that we calculated as a percentage of the weight of the subsample before the ignition. Finally, the total SOM % (soil organic matter) of each plot was calculated as the average SOM % of each subplot. We used SOM as a proxy for the carbon content in the soil, basing on the assumption that ca. 58% of OM is carbon content (Manns et al., 2016; Navarro et al., 1993; Rutherford et al., 1992).

Litter and Aboveground Vegetation

We clipped all the litter and aboveground vegetation samples and placed in a 70°C oven inside half-open paper bags for 48-72 h until they were completely dried. Once dried, we measured the biomass of all the samples. Since different studies shows that aboveground vegetation and litter contain large amounts of carbon (Chapungu et al., 2020; Frank et al., 2004; Johnson et al., 2017; Sierra et al., 2007; J. E. Smith et al., 2013), we used their biomass as a proxy of the carbon content of these carbon pools.

Statistical Analysis

Data analysis was done with R4.1.2 (R Core Team, 2021), and the following packages were used: tidyverse (Wickham et al., 2019), dplyr (Wickham et al. 2022), ggplot2 (Wickham, 2016), cowplot (Wilke C. O., 2020), readxl (Wickham and Bryan, 2022), GGally (Schloerke B. et al., 2021), brms (Bürkner P.C., 2017), Rstan (Stan Development Team, 2022),

bayestestR (Makowski et al., 2019), tidybayes (Matthew Kay, 2022), ggpubr (Alboukadel Kassambara, 2020), viridis (Garnier et al., 2021), and car (Fox and Weisberg, 2019). (See Appendix 4, table 3, for variables' description).

Due to the relatively small sample size of this study and to avoid Error Type I by modelling different linear mixed models, the different response variables of this model have been analysed together with a Bayesian Multivariate Mixed Model, with a normal distribution, which use the Markov Chain Monte Carlo approach, and it allows for interactions between response variables. It can be mathematically described by the following model:

$$\begin{bmatrix} Ab \\ Li \\ OM \\ Vis \\ Vig \end{bmatrix} \sim MVNORM \left(\begin{bmatrix} \mu_{Ab} \\ \mu_{Li} \\ \mu_{OM} \\ \mu_{Vis} \\ \mu_{Vig} \end{bmatrix}, \Sigma \right)$$

This model can be seen as a construct of the following five equations:

$$\begin{aligned} \mu_{Ab} &= \alpha_{Ab} + U + \beta_{DP} + \varepsilon_{Ab} \\ \mu_{Li} &= \alpha_{Li} + U + \beta_{DP} + \varepsilon_{Li} \\ \mu_{OM} &= \alpha_{OM} + U + \beta_{DP} + \varepsilon_{OM} \\ \mu_{Vis} &= \alpha_{Vis} + U + \beta_{DP} + \varepsilon_{Vis} \\ \mu_{Vig} &= \alpha_{Vig} + U + \beta_{DP} + \varepsilon_{Vig} \end{aligned}$$

Box 1: definition of the variables used in the previous equations

Ab = Aboveground Vegetation	Li = Litter
OM = Soil Organic Matter	Vig = Vigilant Level
Vis = Visits per Day of animals	MVNORM = Multivariate Normal Model
DP = Distance to Path	Σ = variance
U = Random Intercept = Location	μ = mean
β = Intercept	α = Slope
ε = Residuals	
(See Table 1 for values)	

To compare the results of the Bayesian analysis with a more common frequentist model we also run a linear mixed model for each of the five response variables: "Aboveground Vegetation", "Understory Litter", "Soil Organic Matter", "Visits per Day" and "Vigilant Level". For each linear mixed model, we use "Distance to Path" (20 and 100 metres) as the only explanatory variable and "Location" ("Natuurmonumenten" and "Statsbosbeheer") as random effect. This method, however, does not consider correlations between response variables and provides less information than the Bayesian Multivariate Mixed Model (see Appendix 9, table 4, for frequentist models results.).

Before working on the frequentist and the Bayesian models we made the following transformations, based on the raw distribution of the variables (Appendix 5, fig. 3).

“Aboveground Vegetation” and “Visits per Day” raw data distribution were both right skewed, but after log-transforming them, we considerably improved their distribution (Appendix 5, fig. 4). “Soil Organic Matter” and “Vigilant level” data were reported in percentages, and they presented an unregular distribution (Appendix 5, fig. 3), so we did an arcsine squared root transformation which homogenized the variance (Appendix 5, fig. 4). “Litter” data were not transformed. Since we were assuming a Gaussian distribution and the different variables had different scales, we standardized all the continuous parameters of the model after the transformations.

For each response variable (“Visits per Day”, “Vigilant Level”, “Aboveground Vegetation”, “Litter”, and “OM”) we set up a linear mixed model with a Gaussian distribution using “Distance to Path” as explanatory variable and “Location” as random intercept. Even if “Location” has only two levels (Appendix 4, table 3), we still decided to treat it as a random effect since we were not interested in its particular values as a fixed effect, but we were just interested in checking for its impact. According to Gomes (2021), the presence of random effects with less than five levels does not influence fixed effects estimations, so we felt confident that we would have not altered the analysis by using “Location” as random effect.

After setting the five linear mixed models, we set weakly informative priors for each response variable. Differently from frequentist analyses, Bayesian models need to include prior information, which represent the expected probabilities of different values before collecting and analysing the data, and which can be non-, weakly- or fully- informative. According to Lemoine (2019) ecologists should consider weakly informative priors as “default” in Bayesian analysis. After setting the priors distribution, we combined the five models together in a multivariate mixed model, using the `brm()` function of the `brms` package, that allowed us to investigate the correlations between the response variables. Afterwards, we checked for the fitness of the model parameters by using the `pp_check()` function of the `bayesplot` package, which gives a graph that compares the posteriors distribution with the observed data distribution (Appendix 6, fig. 5). The posterior distribution updates the prior distribution using the observed data applying the Bayes’ theorem (see equation below). If the observed data distribution is within the range of the posterior distribution, such as in Appendix 6 (figure 5), then the model gives a valid prediction of the reality.

$$P(A|B) = \frac{P(B|A) \cdot P(A)}{P(B)}$$

Box 2: definition of the variables used in the previous equation.

P (A | B) = probability of A occurring when B is true;
P (A) = probability of A occurring independently from B;
P (B) = probability of B occurring independently from A.

By investigating the Rhat, the Bulk_ESS and the Tail_ESS values, we also checked for the convergence of the different chains. Eventually, we calculated predictions for each response variable and, after de-standardizing the predictions, we plotted them against the actual value, confirming again the goodness of fit of the model (Fig. 3).

The Probability of Direction (pd) value of the response variables was given in the posterior table obtained by the `describe_posterior()` function of the `bayestestR` package. `Describe_posterior()` function gives the pd value only for the response variables and to obtain these values also for the residual correlations we used the `hypothesis()` function. R^2 values were calculated with `bayes_R2()` function of `brms`.

In the result section, we will refer to values with CI (Credibility Interval) not overlapping with zero and with pd value (Probability of Direction) higher than 95% as significant. Values with the CI only slightly overlapping with 0 and with pd value close to 95% or in general higher than 90% will be considered as almost significant, while all the other values are considered as not significant (Makowski et al., 2019). However, low pd values only gives uncertainty about the direction of a relation but does not tell for certain that there are no relationships (Makowski et al., 2019).

Results

All the main results of this analysis can be seen in Table 1, while the graphs below (Fig.3) show the fitted against the predicted values for all the five response variables and gives more information about the fitness of the model. See Appendix 7 (Fig. 6) for the graphic output of the Markov Chain Monte Carlo approach. The results of the frequentist analysis are in accordance with the Bayesian results and show significantly more visits per day of ungulates together with significant higher values of C concentration in the aboveground vegetation and litter carbon pools in plots 20 metres away from human path than in those 100 m away. The table with the frequentist results can be seen in Appendix 9 (Table 4).

Actual vs Predicted values at different distance from human paths

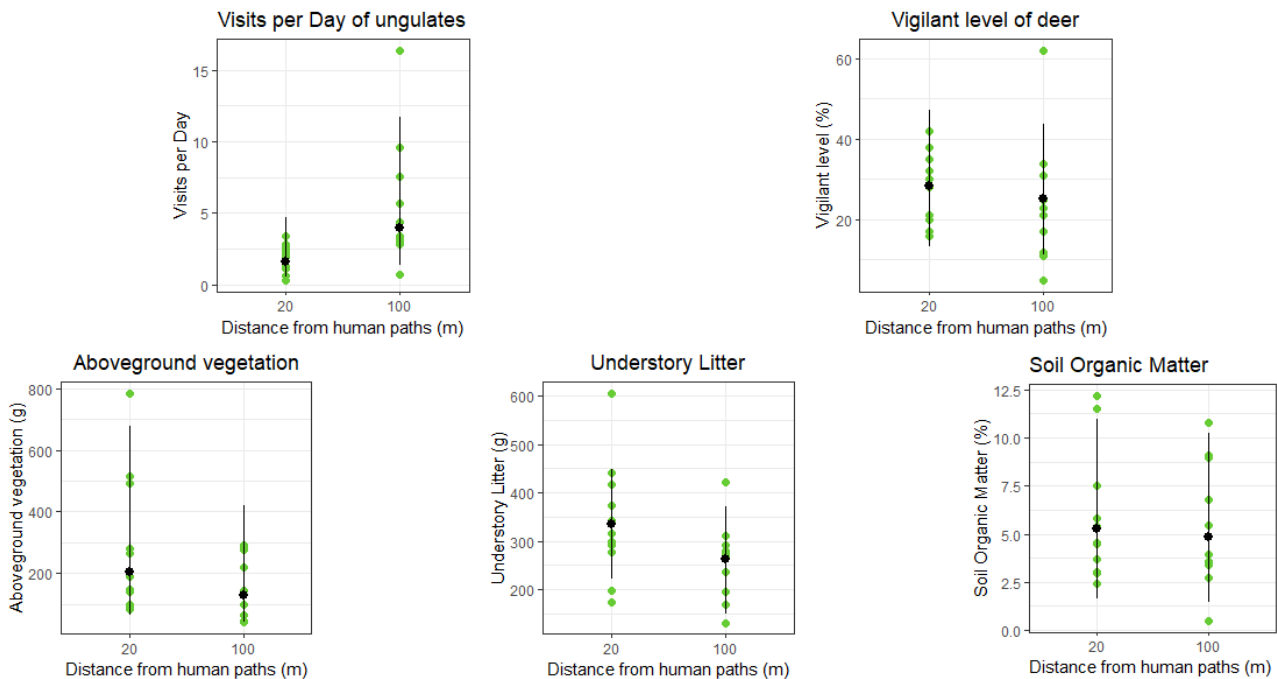


Fig. 3: Predicted vs Actual values for all five response variables. The green points represent the actual values while the black points the estimate of possible data predicted by R Studio based on the actual values. The black line shows the range of the predicted values. If the actual values are within the predicted range the model is considered to have a good fit.

Table 1: the table shows the value of the Estimates, Credibility Interval (CI), Probability of Direction (pd) and R² for fixed effects, residual correlations, and random effects. The estimates of distance to path 20m are equal to the difference between distance path 20 – distance path 100. The intercept represents distance to path 100 m. (*Statsbosbeheer)

Response	Fixed	Estimate	95% CI	pd	R ²
Aboveground	Intercept	-0.31	[-1.73, 1.16]	69%	72%
=	Distance to path 20 m	0.57	[0.10, 1.04]	99%	=
Litter	Intercept	-0.35	[-1.40, 0.68]	79%	16%
=	Distance to path 20 m	0.68	[-0.10, 1.46]	96%	=
Organic Matter	Intercept	-0.11	[-1.52, 1.30]	57%	51%
=	Distance to path 20 m	0.13	[-0.47, 0.71]	67%	=
Visit per Day	Intercept	0.51	[-0.67, 1.71]	85%	36%
=	Distance to path 20 m	-1.01	[-1.69,-0.30]	100%	=
Vigilant Level	Intercept	-0.11	[-1.28, 1.05]	59%	10%
=	Distance to path 20 m	0.21	[-0.65, 1.03]	69%	=
Residual correlations		Estimate	95% CI	pd	
	Aboveground * Litter	-0.19	[-0.51, 0.14]	83%	
	Aboveground * Organic Matter	-0.14	[-0.47, 0.21]	76%	
	Litter * Organic Matter	0.56	[0.27, 0.77]	100%	
	Aboveground * Visit per Day	-0.26	[-0.57, 0.08]	90%	
	Litter * Visit per Day	0.32	[-0.01, 0.61]	94%	
	Organic Matter * Visit per Day	0.09	[-0.26, 0.41]	66%	
	Aboveground * Vigilant Level	0.32	[-0.02, 0.61]	94%	
	Litter * Vigilant Level	-0.01	[-0.34, 0.32]	52%	
	Organic Matter * Vigilant Level	0.01	[-0.33, 0.34]	52%	
	Visit per Day * Vigilant Level	-0.19	[-0.51, 0.15]	83%	
Response	Random Effects	Estimate	95% CI		
sd (Aboveground)	Location: SBB*	1.86	[0.55, 5.22]		
sd (Litter)	Location: SBB	0.82	[0.02, 3.44]		
sd (Organic Matter)	Location: SBB	1.73	[0.47, 4.82]		
sd (Visit per Day)	Location: SBB	1.07	[0.05, 3.84]		
sd (Vigilant Level)	Location: SBB	0.96	[0.04, 3.65]		

Presence and behaviour of ungulates

Plots closer to human paths (20 m) have a lower visits per day of ungulates (1.81 ± 0.30 SE) than plots 100 m away (5.46 ± 1.32) (Fig. 4). We can confirm this difference by looking at the Credibility Interval, which does not overlap with 0, and at the pd value of 100% (Table 1), which is the strongest result of the model. However, by looking at the R² value (Table 1), it seems that distance to path only partially explains the variance in visits per day between different distances.

Presence and Behaviour at different distance from human paths

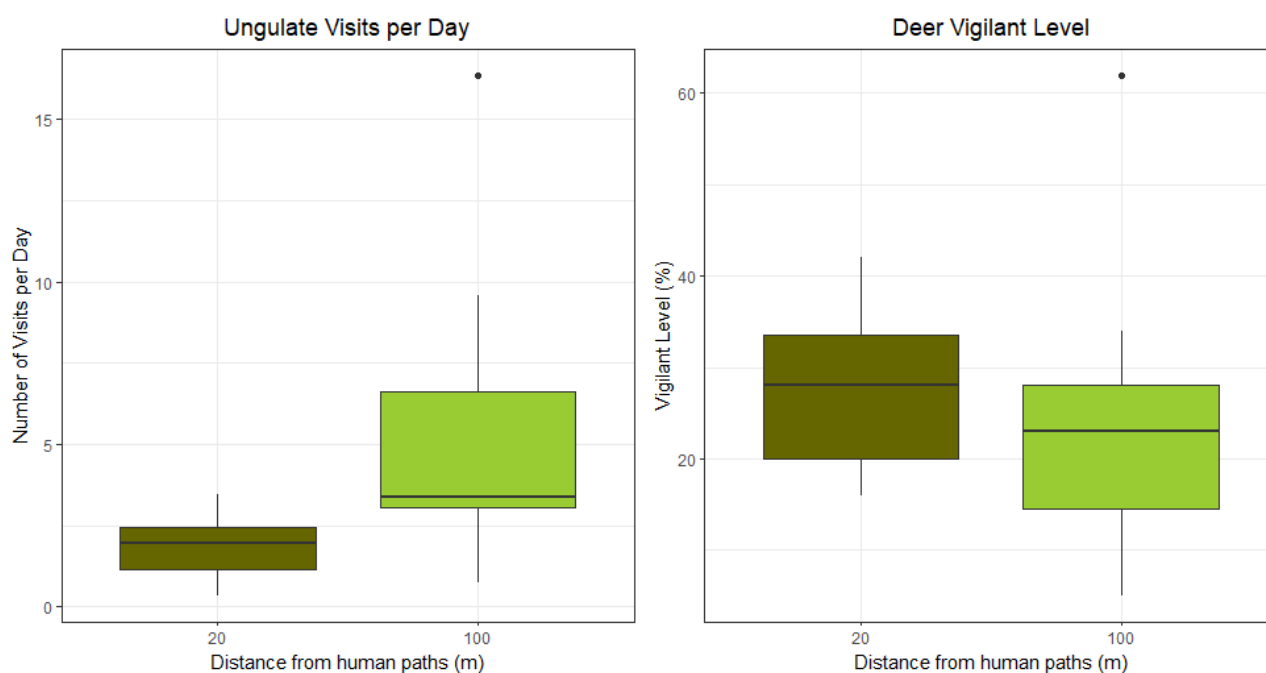


Fig.4: The boxplots show the average visit per day of deer and wild boars (left) and the different vigilant level of deer (right) of the collected data at different distances from human paths (right).

Results of the vigilant level appear to be weaker and not significantly different between plots 20 metres and 100 metres from human paths (Fig. 4). Ungulates are estimated to be more vigilant 20 metres (27.81 ± 2.70 SE % vigilant level) than 100 metres away (24.18 ± 4.60 % vigilant level) from human paths, but this difference is weakly confirmed by a pd of 69% (Table 1). Also, the R^2 is low (Table 1), suggesting that there must be other factors explaining the variance in the vigilant level between different distances.

Carbon Stocks

The carbon content of the aboveground vegetation and litter carbon pools were significantly higher at plots 20 metres away from human paths than in those plots 100 metres away. While the difference in the carbon content of the soil carbon pool at different distance from human paths was not significant (Table 1).

Aboveground Vegetation

Plots 20 m from human paths have higher concentration of aboveground vegetation content (279.90 ± 68.04 SE g per sample unit, - s.u. = 0.25 m^2 -) than plots 100 m away (165.14 ± 32.55 g per s.u.) (Fig. 5). This result is confirmed by the smaller Credibility Interval of the model (Appendix 7, Fig. 6), which does not overlap with 0, and by a pd value of 99% (Table 1). Based on the relatively high R^2 value (76 %; Table 1), distance to path explains variability in Aboveground Vegetation content better than it explains variance of any other variables of the model.

Carbon Stocks at different distances from human paths

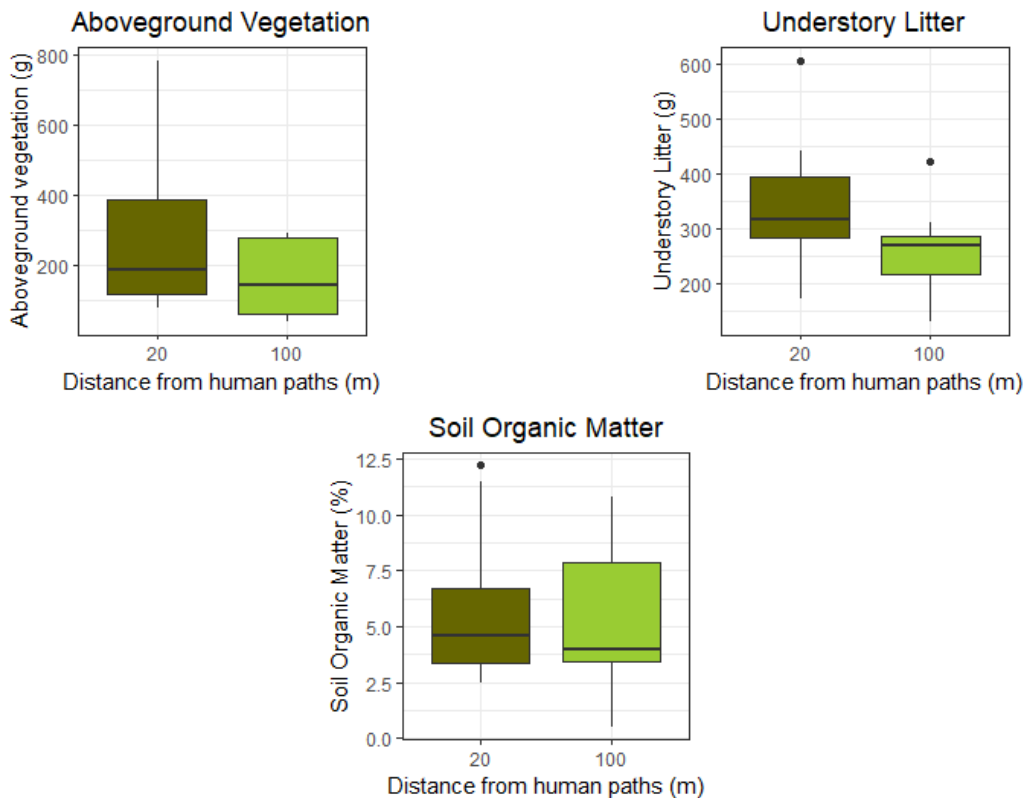


Fig. 5: the boxplots show the average Aboveground Vegetation (top-left), Understory Litter (top-right) and Soil Organic Carbon (down) of the collected data at different distances from human paths. The values of Aboveground Vegetation and Understory Litter are expressed in grams per sample unit (s.u.=0.25 m²), while the values of Soil Organic Matter is expressed in percentages per sample unit (s.u.= 240.53 cm³)

Understory Litter

Plots 20 m away from human paths have higher litter content (339.49 ± 36.35 SE g per sample unit, - s.u.= 0.25 m² -) than plots 100 m away (258.50 ± 23.40 g per s.u.). The Probability of Direction confirm this difference with a value of 96%, which we can define significant, however the Credibility Interval slightly overlap with zero (Appendix 7, Fig. 6) and the R² value is weak (Table1).

Soil Organic Matter

Plots 20 m away from human paths show higher concentration of SOM (5.79 ± 1.01 SE % per s.u., - s.u. = 240.53 cm³ -) than plots 100 m away from human paths (5.34 ± 0.96 % per s.u.). The pd value is only equal to 67% and the CI overlap with 0 with a wide range (Appendix 7, Fig. 6), hence we cannot statistically confirm the difference. Moreover, the R² value (51%; Table 1) suggests that only half of the variation in soil organic matter content is explained by the distance from human paths.

Relation between carbon stocks, behaviour and abundance of ungulate

Figure 6 shows the significant correlations between the raw data of our response variables, while the graphic visualization of the non-significant correlation between the other response

variables can be seen in the Appendix 8 (Fig. 7). With a positive estimate of 0.54, the one between Litter and SOM is the strongest relation between the response variables of the model (Table 1). This result is strongly supported by a pd value of 100% and a narrow Credibility Interval which does not overlap with 0 (Table 1). Litter is also positively correlated with the number of visits per day of ungulates (Table 1). The CI of this correlation only slightly overlap with 0 and the pd value is almost significant (Table 1). All the other residual correlations of litter are not significant.

Relations between collected data with a significant residuals correlation

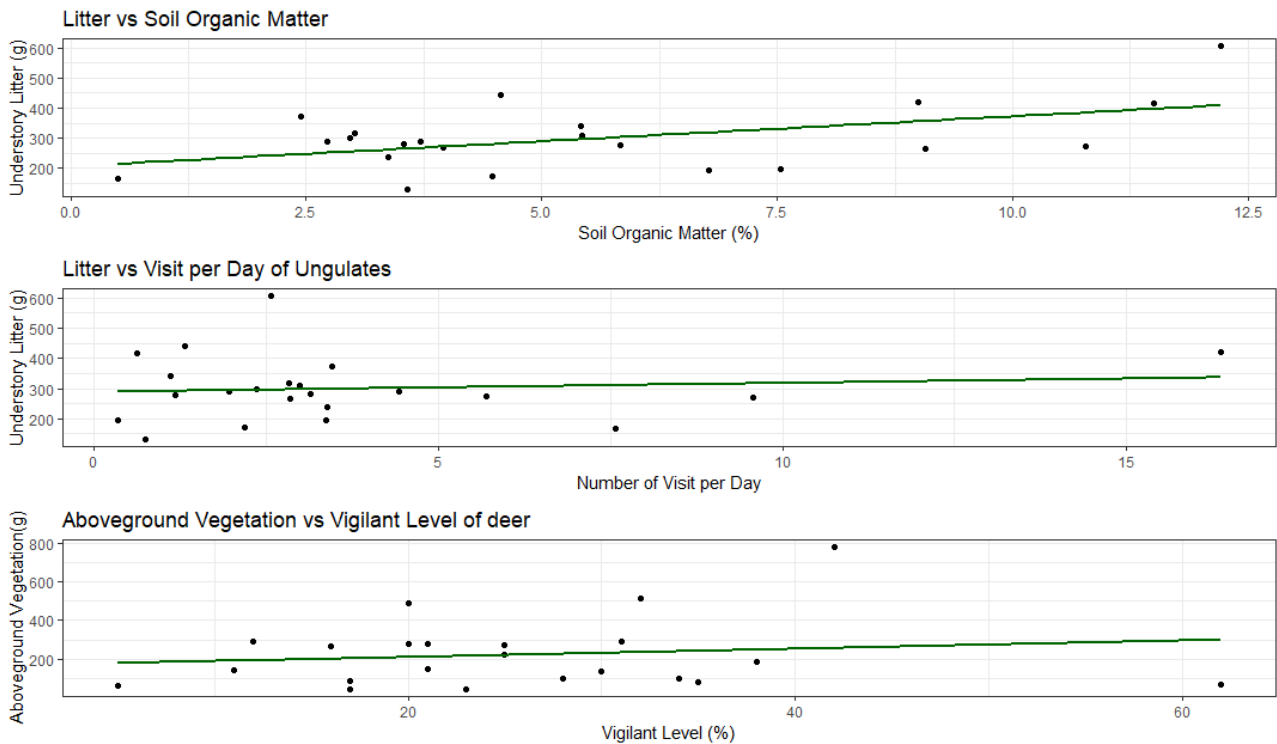


Fig. 6: significant correlations between the raw data of the analysed response variables, see Appendix 6.6 (fig. 8) for the correlations of the other response variables.

Aboveground Vegetation is positively correlated with Vigilant Level (Fig.6), with an estimate of 0.34 (Table 1). Even if the CI marginally overlap with 0, we can confirm the positive direction of this correlation by looking at the pd value (94 %; Table 1). Aboveground Vegetation is also negative correlated with number of visits per day, but with a pd value of 90 % and the CI slightly overlapping with zero (Table 1), we can refer to this relation only as almost significant.

Ultimately, the residuals of Soil Organic Matter are significantly related only with Litter content (see above). The second and third strongest correlations of OM are respectively the negative relation with aboveground vegetation and the positive with visits per day (Appendix 8, Fig. 7), but the pd values and the CI do not confirm the directions (Table 1).

Location

Location has been treated separately as a random effect and the results show that plots in Staatsbosbeheer (Fig. 1) have significant higher values for all the response variables analysed in the model (Table 1). However, even if the CI never overlap with 0, the range is very wide for all the parameters of the model, and so we cannot really consider the estimates of the model as very precise. Generally, it is possible to say that there is a significant difference in number of animals, in their vigilant behaviour and in the C stocks between the two Locations.

Discussion

Overall, this study suggests that ungulates and humans impact carbon stocks at local spatial scales. We found fewer ungulates visits close than further away from human paths and they tended to be more vigilant closer to human roads. In line with this difference in presence and behaviour, carbon stocks differed significantly between different distances from human paths, with greater quantities of carbon content closer to the paths where less animals are present. This effect was stronger for the aboveground vegetation carbon pool, followed by the understory litter and soil carbon pools, which was the only carbon pool without significant differences at different distances from human paths. Moreover, we found a strong positive relation between litter and OM, which suggests that ungulates indirectly affect soil organic matter with their impact on litter quantity.

We found that fewer ungulates frequent areas closer to human trails, and this is in accordance with the results of Mols et al. (2021) in the Veluwe forest and with other studies around the globe (Bonnot et al., 2013; Proffitt et al., 2009). These findings have been extended also to large carnivores, such as mountain lion (*Puma concolor*) (Suraci et al., 2019), and meso-carnivores such as badgers (*Meles meles*) (Clinchy et al., 2016), which also show changes in behaviour and space use in presence of humans. These results suggest that humans impact the space use of ungulates and that ungulates tend to avoid areas closer to humans. On the other hand, ungulates behaviour was not significantly affected by the presence of humans in the Veluwe forest. These results are in contrast with the findings of other studies which show a vigilant response of ungulates to the presence of humans, yet this response was mainly related to humans walking off-trails (Miller et al., 2006; Stankowich, 2008; Taylor & Knight, 2003), which in the Veluwe forest is strictly forbidden. Stankovich (2008) also suggested that ungulates response to humans is related to the natural history of the territory and to the level of accustomization of the local population towards human presence. Furthermore, Ciuti et al. (2012) found that elk (*Cervus canadensis*) reduce foraging time in presence of humans, and the same was found for large carnivores (Smith et al., 2017). Defining the behaviour of an animal can be done analytically but still it is open to interpretations, and perhaps, for this kind of research, it would be more effective recording the foraging time at different distances from human paths rather than classifying vigilant and non-vigilant behaviours.

Aboveground vegetation is the carbon pool with the greatest differences in carbon content at different distances from human paths in the Veluwe forest. We found higher concentration of carbon in plots closer to human paths which are also the plots less visited by ungulates. These results make the aboveground vegetation carbon pool the one mainly affected by ungulates in our study area. These findings are backed up by different studies in different ecosystems which also show a negative impact of ungulates on aboveground vegetation content and sapling performance (Gill, 2001; Haffey & Gorchoy, 2019; Mols et al., 2021; Putman, 1996; Tanentzap & Coomes, 2012). The effect of ungulates on aboveground vegetation content can be related to the direct impact that browsing has on the vegetation (Gill, 2001). The positive relation between aboveground vegetation content and vigilant level points out the indirect role played by humans in the vegetation structure on local scale. Indeed, if animals are more vigilant, they will spend less time foraging and the vegetation will grow higher.

The understory litter carbon pool also presents greater quantities of carbon close to human paths than away, suggesting an impact of ungulate also on this carbon pool in the Veluwe forest. Other studies show how deer exclusion increases litter biomass (Bressette et al., 2012; Haffey & Gorchov, 2019; Ramirez et al., 2021). According to Bressette (2012), trampling activities increase litter decomposition rate and Johnson (1995) shows that when the foraging is scarce, deer consume leaf matter. Grubbing by wild boars also is likely to reduce litter biomass and the reduction of aboveground vegetation biomass, due to the impact of browsing, is likely to have the same effect. However, we did not find a positive relation between aboveground vegetation and litter biomass, maybe due to the fact that we only calculate the biomass of understory aboveground vegetation excluding canopy cover. Moreover, several studies demonstrate an impact of ungulates' browsing on litter quality (Bardgett & Wardle, 2003; Chollet et al., 2021; Harrison & Bardgett, 2003; Hirst, 2021; Tanentzap & Coomes, 2012), which in turn can negatively affect the nutrient cycling with possible negative outcome for the soil carbon stock, but we did not test for this effect in our research.

We did not find a significant difference in soil carbon content at different distances (20 and 100 m) from human paths, which make the soil carbon pool the one less affected by ungulates in our study. However, we found a strong positive relation between litter content and SOM, suggesting an indirect effect of ungulates on the soil carbon stock by affecting litter content. This positive relation supports the idea that litter biomass mediates the cascading effects between above- and below- ground (Bressette et al., 2012), accordingly to the theory that above- and below- ground dynamics are strictly intertwined (Andriuzzi & Wall, 2017; Bardgett & Wardle, 2003). Most of the studies relate the indirect effects of ungulates on soil carbon stock to their negative impact on litter quality and quantity which reduce the amount of nutrients reaching the soil and thus the soil carbon content (Bardgett & Wardle - 2003; Chollet et al., 2021; Harrison & Bardgett, 2003; Hirst, 2021; Tanentzap & Coomes, 2012). Ungulates can also indirectly affect SOM by reducing the amount of soil fauna (Allombert et al., 2005; Andriuzzi & Wall, 2017; Cuevas et al., 2012; Mohr et al., 2005; Ramirez et al., 2021; Risch et al., 2010) which has a key role in soil carbon dynamics. Moreover, bioturbation activities such as trampling and grubbing can significantly affect soil properties, such as soil bulk density and moisture, potentially leading to an increase in soil CO₂ emissions (Cuevas et al., 2012; Liu et al., 2020; Risch et al., 2010). This has been demonstrated by Risch et al. (2010), who show that wild boar grubbing significantly increases soil carbon emission rates in Swiss hardwood forests. According to Tanentzap and Coomes (2012), when given enough time, natural systems can offset the negative impact of herbivory on the carbon stocks, and this might explain why we did not find differences in carbon content in the soil carbon pool at different distances from human paths.

According to the ecology of fear theory, in presence of a threat, herbivores will change the space-use of their ecosystem and reduce the feeding efforts in favour of a more vigilant behaviour, and this can trigger a cascading effect which can lead to dramatic changes in the vegetation structure and composition (Bonnot et al., 2013; Laundre et al., 2010; Ripple & Beschta, 2004; Strickland et al., 2013; Zantede & Clinchy, 2019). This theory is usually related to wild predators, however, recent study show that ungulates behaviour is strongly influenced by the presence of humans (Ciuti et al., 2012; Mols et al., 2021; Möst et al., 2015), and the behaviourally mediated trophic cascade triggered by humans might even overrule the one induced by predators (Ciuti et al., 2012; Mols et al., 2021; Proffitt et al., 2009). For

example, Profitt et al. (2009) show that some ungulates such as elk (*Cervus canadensis*) respond to human disturbance even more than to predator disturbance, backing the idea that humans are seen as apex predators by most of the animals. Our results support the hypothesis that the fear of human can trigger a trophic cascade. Indeed, in those plots closer to human paths, ungulates are fewer and more vigilant. They spend less time browsing and trampling and, according to the mechanisms explained before, they reduce their impact on the different carbon stocks, in turn enhancing the carbon stored in the carbon pools close to human trails. Thus, we found that the behaviourally mediated trophic cascade triggered by humans in the Veluwe forest does not stop at the vegetation structure and composition, but it goes deeper influencing the carbon stocks. Therefore, animals' impact on the carbon stocks of an ecosystem should not be overlooked, and human influence should be considered when studying ecology of fear and the following cascading effects on the local scale.

As it is shown in the study of Schmitz (2018), different animals can have different impact on the carbon cycling of their respective ecosystems, both aquatic and terrestrial. For example, on the western coast of North America conservation efforts recovered sea otters (*Enhydra lutris*) population, enhancing kelp forest carbon uptake by 1100%, by top-down regulation on sea urchins (Estes et al., 1998; Schmitz et al., 2018; Wilmers et al., 2012). However, in some areas of the Alaskan coastline, sea urchins' population re-flourished to the detriment of kelp forest biomass, due to a diet shift of killer whales (*Orcinus orca*) on sea otters (Estes et al., 1998). Likewise, in North America, elk (*Cervus canadensis*) grazing increased grassland productivity, whereas moose (*Alces alces*) reduced boreal forest productivity by browsing on deciduous trees, and the reintroduction of wolves (*Canis lupus*) in the two different ecosystems mitigated both effects (Wilmers & Schmitz, 2016). These results suggest that the impact of animals on the carbon stocks can have a great variation, both positive and negative. Plus, the cascading effect of a predator can be easily reversed by another predator and even the same predator can have opposite effects on different ecosystem types. Thus, when predicting the C cycling, it is not enough to generalise the results of a study on different ecosystems, but each case should be analysed separately, also considering the different response of herbivores to predation risk (Atkins et al., 2019).

Taking this into account, we believe that future studying should extend this research toward different areas of the world, to see how the carbon stocks of different ecosystems are influenced by different animals and different level of human influence. Additionally, future research should not only expand the areal of the study comparing differences and similarities, but they should also increase the number of protagonists involved. For example, we know that soil fauna plays a major role in soil dynamics, such as OM decomposition and carbon stabilization (Fahey et al., 2013; Filser et al., 2016; Fox et al., 2006; García-Palacios et al., 2013; Vetter et al., 2004), and we also know that ungulates can directly and indirectly affect the amount of soil fauna (Allombert et al., 2005; Andriuzzi & Wall, 2017; Cuevas et al., 2012; Mohr et al., 2005; Ramirez et al., 2021; Risch et al., 2010) and their decomposing ability (Chollet et al., 2021), in turn possibly affecting soil carbon dynamics. Soil fauna can directly impact soil organic matter by regulating litter and soil decomposition rates and by bioturbation and humification activity, and indirectly by stimulating microbial activity (Filser et al., 2016; Fox et al., 2006). According to Filser et al. (2016), carbon cycling models which do not consider the activity of soil fauna lose most of their potential predictive power. Therefore, it would be interesting to see whether soil fauna

abundance change between different distances from human paths, and how these differences are related to the carbon stocks. Moreover, since a few years wolves are back in the Veluwe forest, and even if their impact on the ecosystem might not be still as great as the one of humans, it would be interesting to analyse the landscape of fear that they are creating and the behaviourally mediated trophic cascade which could rapidly be re-established (Atkins et al., 2019). This kind of research would allow us to study whether human-induced fear also affects predators' behaviour triggering a further trophic cascade (Smith et al., 2017; Suraci et al., 2019; Zanello & Clinchy, 2019), and see how ungulates are adapting their habitat choices between humans and wolves and how this is influencing the ecosystem and eventually the carbon stocks. Also, if it is true that, when given enough time, natural system can offset the negative impact of herbivory on the C stocks (Tanentzap and Coomes, 2012), it would be interesting to study how long it would take for the C stocks of the Veluwe forest to recover after the exclusions of ungulates.

In conclusion, the results of this research suggest that, when studying carbon cycling, we should include the impact of animals on the carbon content, considering that human recreation activities can trigger a trophic cascade which impacts the carbon stocks. Additionally, in the case of the Veluwe forest, managers should be aware of the negative impact that ungulates can have on the carbon stocks and operate a population control in accordance with that. Alongside with the already existing practice of culling, population control could be implemented by favouring the reproduction success of the wolves already present in the area. Also, wolves' territory could be extended in those parts of the Veluwe forest where they are still not present, such as the two locations of this case study. Reducing the number of ungulates in the Veluwe forest could help increase the amount of carbon stored above and below ground, maximizing the carbon retention ability of the forest and mitigating the green-house gases on the regional scale as it is proposed in the Kyoto protocol (UNFCCC, 1997).

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Appendix

1. Plot set-up

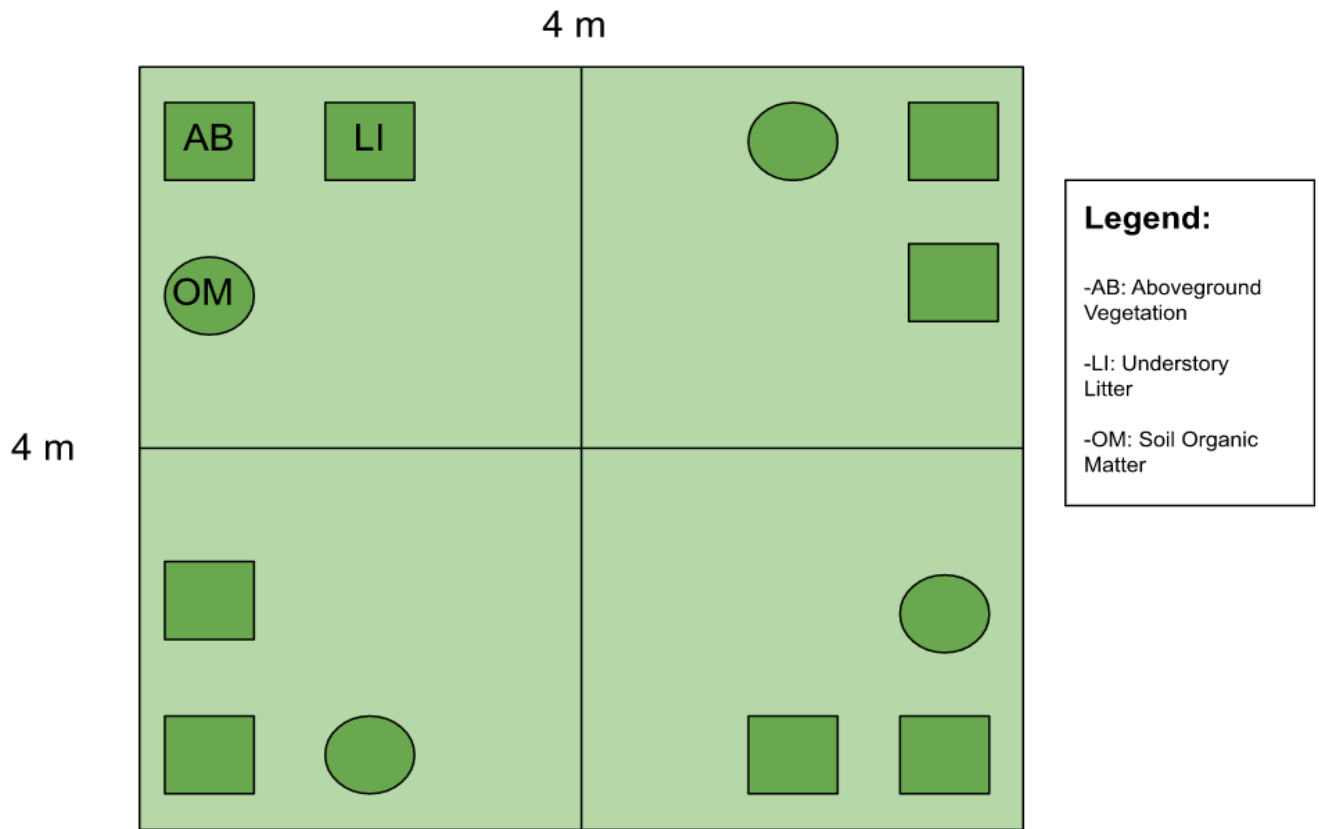


Fig.1: plot set up. Each plot (4x4m) was divided in 4 subplots (2x2m). For each subplot I took samples of Aboveground Vegetation, Understory Litter and Soil Organic Matter. The data of each plot represents the mean of the measurements of each subplot.



Fig.2: picture of a 4x4 m plot. On the bottom right there are some of the collected samples.

2. Camera traps set-up

Table 1: set-up of the two types camera traps used in the Veluwe forest.

Camera types	Bushnell Trophy Cam Aggressor HD 2017	Bushnell Trophy Cam HD 2013
Mode	Hybrid	Day
Image size	HD	HD
Image format	Full screen	Full screen
LED control	High	High
Video size	1280x720	1280x720
Interval	0.6s	1s
Sensor level	High	High
NV Shutter	Auto	Auto
Camera mode	24 hrs	24 hrs
Video length	15s	15s
Field scan	Off	Off
Video sound	On	On

3. Ethogram

Table 2: Ethogram description of vigilant and non-vigilant behaviours.

Behavior	Description
Non-vigilant behaviours	
unknown behaviour	Behaviour which does not fit in any category or is unclear
presence	If an animal is present which we do not study behaviour on
social interaction	Interaction between animals
scratching	Animal is scratching
other non-vigilant	Everything not fitting in another non-vigilant category
rutting	Rutting animal
lying	Lying on the ground
walking	
foraging	Searching for food. Also, if the animal is walking during foraging
Vigilant behaviours	
sudden rush due to camera	Sees camera trap and runs away
running	
visual vigilant	Head above shoulders, looking around and chewing
looking into camera	
unknown vigilant	Everything not fitting in another vigilant category
sudden rush	Running away, not seeing camera trap
auditory vigilant	Head above shoulders, looking around and not chewing
other vigilant	Behaviour which does not fit in any category or is unclear
vigilant while walking	

4. Variables of the study

Table 3: description of all the variables used in the model.

Variables	Description
Plot	Plot numbers
DistPath	Distance from human paths (20 and 100 m)
Location	NM = Natuurmonumenten, SBB=Statsbosbeheer
DryW	Total dry weight of earth samples to measure Soil Organic Matter (g)
OM	Total weight of Soil Organic Matter (g)
OM_perc	Percentage of OM in the total dry weight of earth (%)
Litter	Understory Litter measured in each plot
Aboveground	Aboveground Vegetation measured in each plot
SampleDepth	Depth of the ground until sandy soil
Litter_cm	Depth of the litter layer in the soil
OM_cm	Depth of organic matter layer in the soil
Sandsoil (cm)	Depth in the ground where sandy soil start
visitXday	Average of ungulates visiting each plot per day
Vig_perc	Percentage of vigilant level of all the recorded activities of ungulates
Nvig_perc	Percentage of non-vigilant level of all the recorded activities of ungulates
Log_visit	Log transformation of visitXday
Vig_perc2	Arcsine squared root transformation of Vig_perc
Om_perc2	Arcsine squared root transformation of OM_perc
Log_AB	Log transformation of Aboveground
AB_z	Standardized log_AB
LI_z	Standardized Litter
OM_z	Standardized OM_perc2
Log_vXd_z	Standardized Log_visit
VP2_z	Standardized Vig_perc2

5. Distribution raw data before and after the transformation

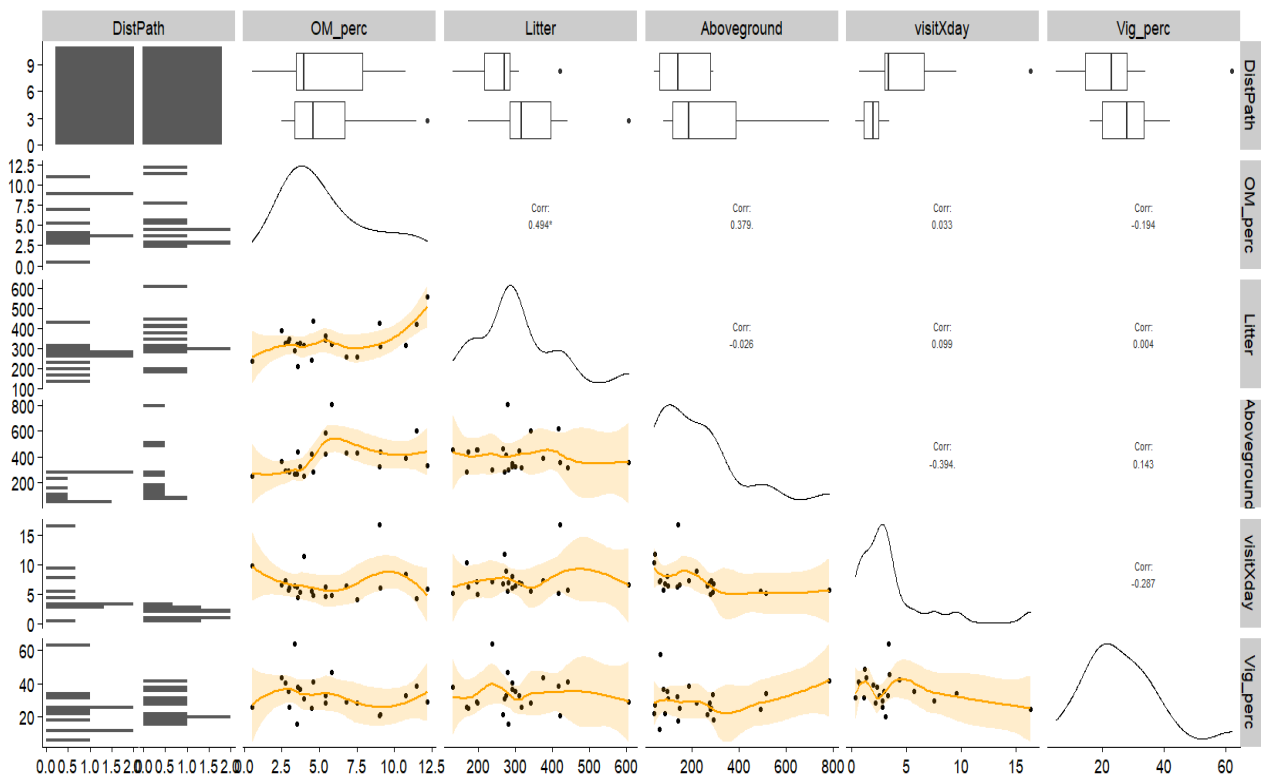


Fig. 3: Distribution of raw data and correlations. Look table 2 for variables name.

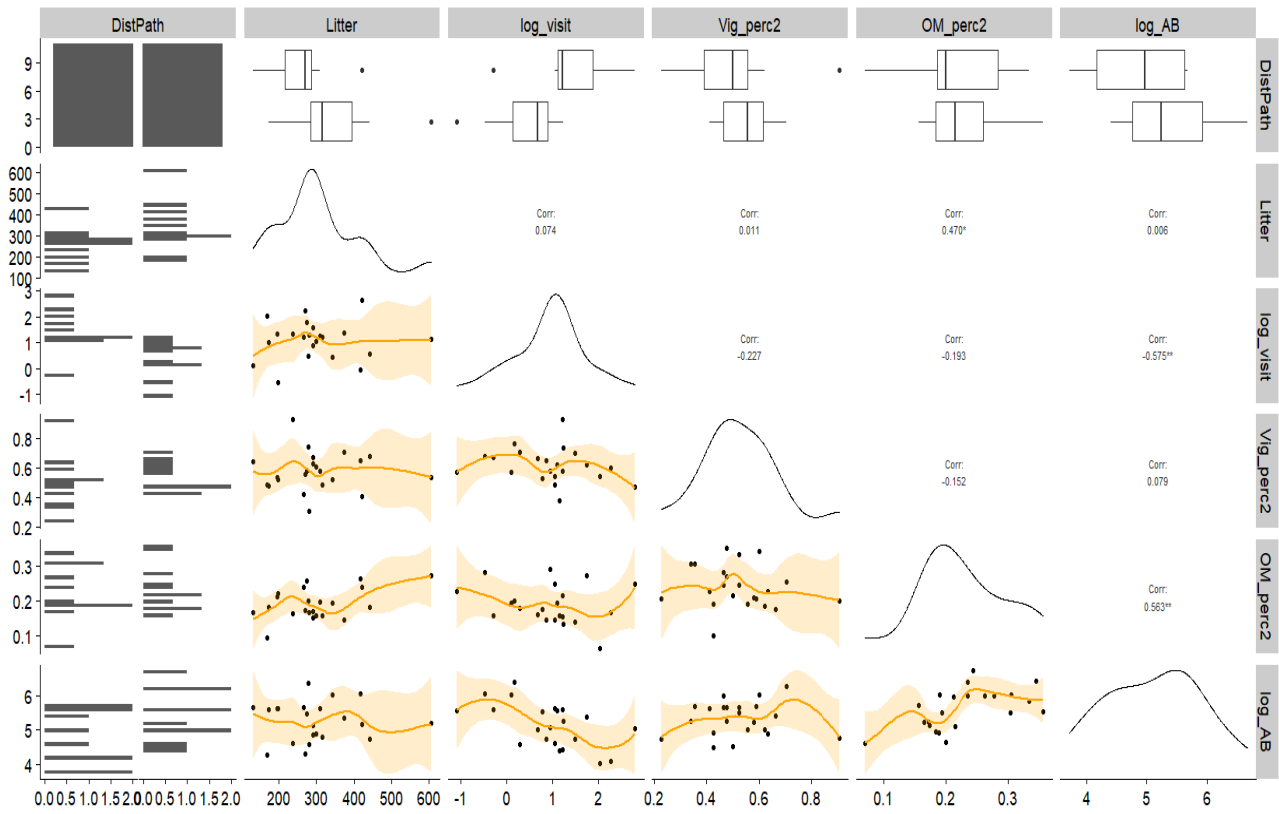


Fig. 4: Distribution and correlations of data after transformations. Look table 2 for variables name.

6. Posterior Check

Posterior Check

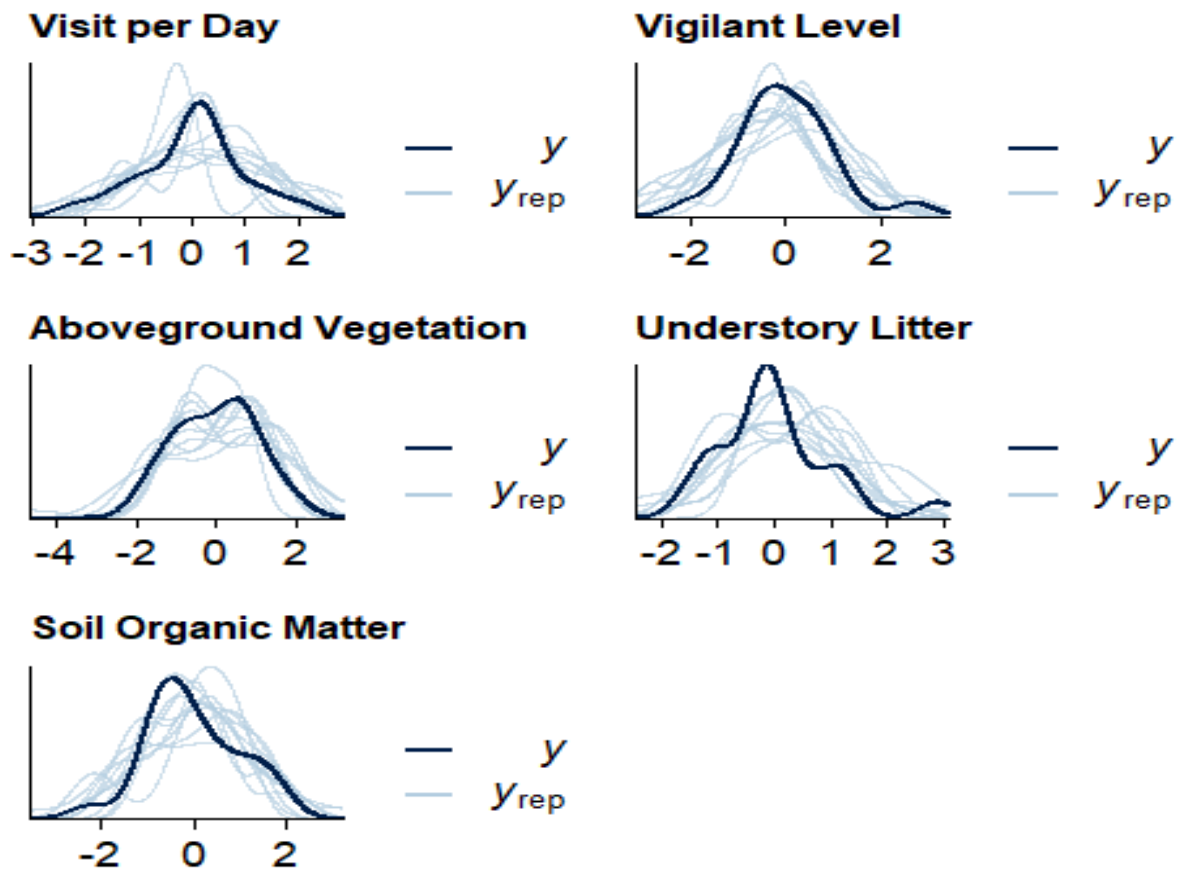


Fig. 5: Posterior distribution. Y = actual values, Y_{rep} = posterior values. The actual values fit within the posterior values, so the model has a good fit.

7. Markov Chain Monte Carlo (MCMC) approach

Graphical display of the Markov Chain Monte Carlo approach

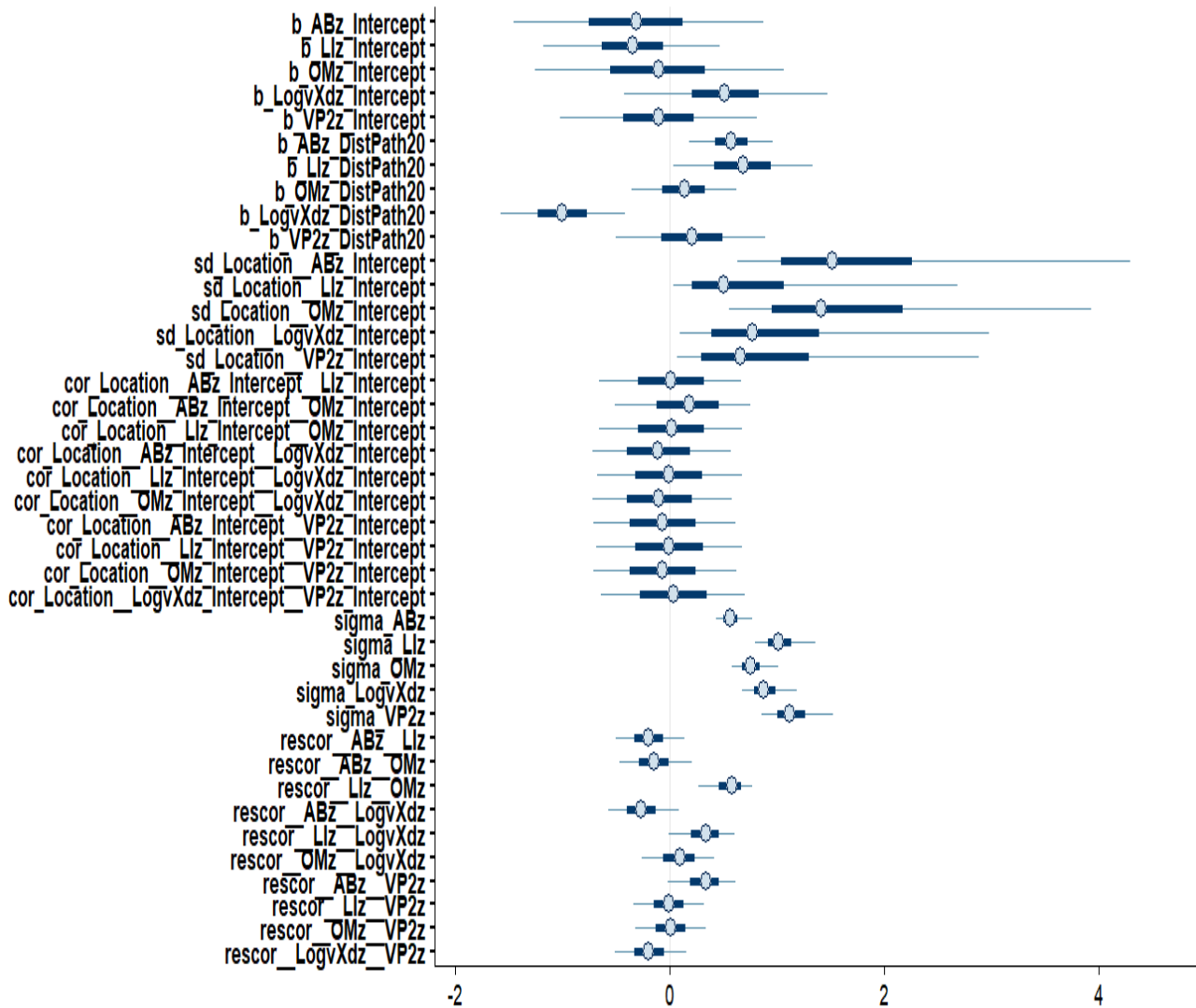


Fig. 6: graphic visualization of the MCMC approach. All the analysed parameters of the model and their interactions are on the y axis. Horizontal lines represent the Credibility Interval of the parameter, the point represent the estimates. When the horizontal lines do not overlap with 0 (vertical line) the results are considered significant. Look table 2 for variables name.

8. Correlation between response variables

Relations between collected data with non-significant residuals correlation

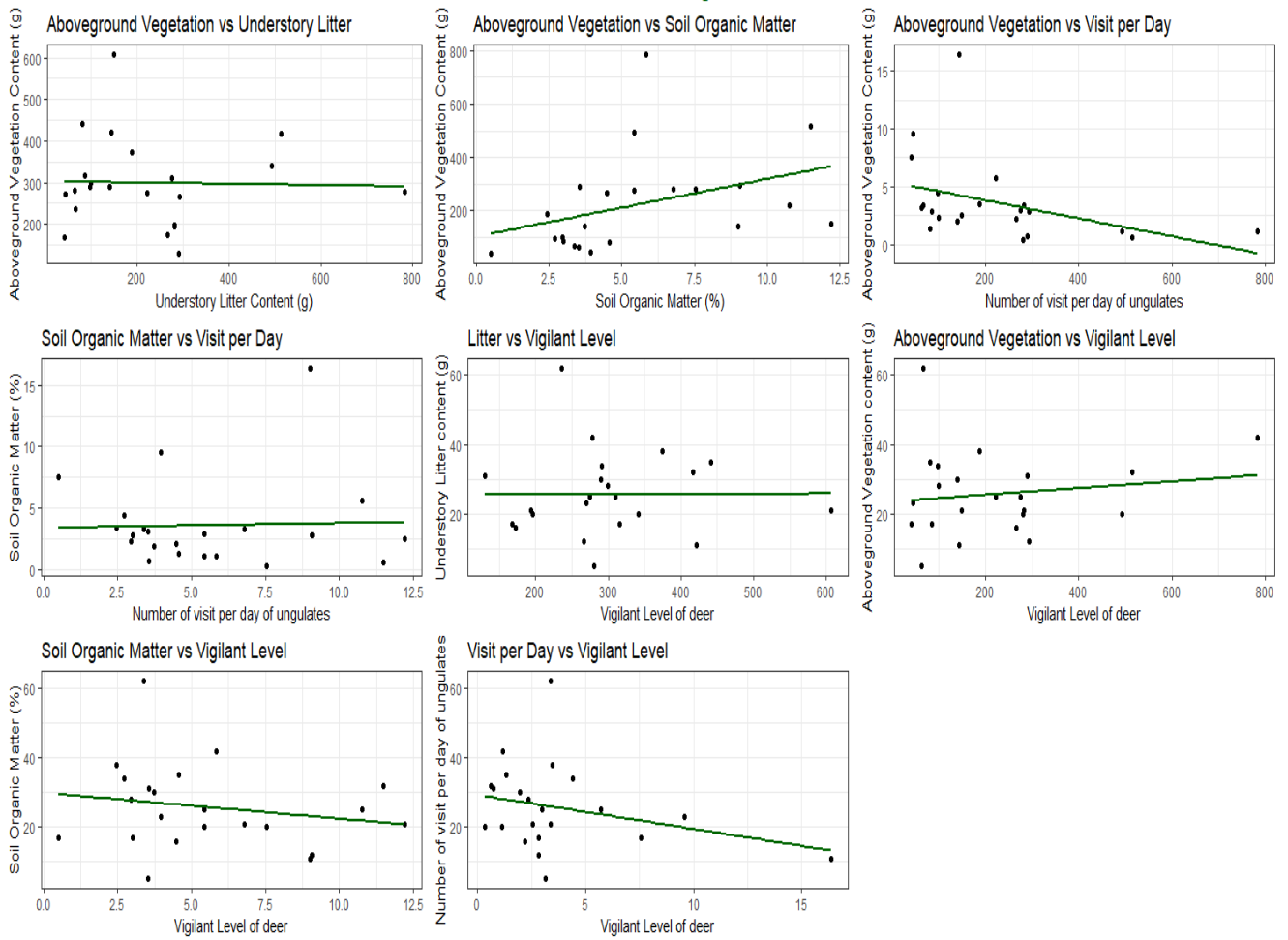


Fig.7: the graphs show the relations between collected data of the response variables with non-significant residuals correlations.

9. Frequentist model results

Table 4: The table shows the results of five linear mixed model: $y \sim \text{Distance to path} + (1|\text{Location})$, where y represents the different response variables. The results of these frequentist models are in accordance with the results of the Bayesian model.

Response Variables	Estimate \pm SE	p-value
Visit per day	-1.1303 \pm 0.3377	0.00339 **
Vigilance level	0.3139 \pm 0.4313	0.475
Aboveground Vegetation	0.6256 \pm 0.2176	0.00968 **
Litter	0.7550 \pm 0.4030	0.0757 .
Organic Matter	0.1847 \pm 0.2960	0.540