Fecal Biomarkers in Soils Record Landscape-Scale Wild Herbivore Abundance

A. Tyler Karp[1,2,3*], James M. Russell[3], Joel O. Abraham[4], Tercia Strydom[5], A. Carla Staver[2,4]

[1]Department of Geophysical Sciences, University of Chicago, Chicago IL USA

[2]Department of Ecology and Evolutionary Biology, Yale University, New Haven CT USA

[3]Department of Earth, Environmental, and Planetary Sciences, Brown University, Providence RI USA

[4]Department of Ecology and Evolutionary Biology, Princeton University, Princeton NJ USA

[5]Scientific Services, Kruger National Park, Skukuza, South Africa

[*]Corresponding author: A. Tyler Karp (atkarp@uchicago.edu)

Key Points:

- Fecal stanols, but not sterols, were more concentrated in soils where herbivores were present than absent
- Compound distributions are related to herbivore community, but overall herbivore abundance most strongly controlled biomarker patterns
- Fecal stanol ratios increased with herbivore dung counts and have promising potential as a proxy for paleo-herbivore abundance

Abstract

In Earth history, our understanding of how large-bodied herbivores shape a variety of ecosystem processes is limited by the quality of paleoecological proxies for herbivore composition and abundance. Fecal stanols are lipids that can be produced by microbes within animal digestive systems and that could remedy this dearth of proxies. We use two multi-decadal herbivore exclosures in Kruger National Park, South Africa, to constrain whether and how biomarker signatures preserve signals of herbivore abundance. Soil samples and dung counts were collected along transects across crest, mid-slope, and sodic sites inside and outside exclosures. Soils were analyzed for steroid (sterols and stanols) concentrations and distributions. We found that stanol concentrations were significantly greater in sodic soils outside exclosures, where herbivore dung densities were greatest. By contrast, sterol concentrations did not differ between treatments. Ratios of stanol isomers to sterols, which account for both compound degradation and source, increased strongly with herbivore dung counts. Finally, while herbivore species compositions influenced steroid distributions, total herbivore abundance was their strongest predictor. Further calibration is needed, but this work provides strong preliminary evidence that wild herbivore populations are quantitatively recorded by fecal biomarker distributions.

1 Introduction

Reconstructions of terrestrial paleoecological processes have rapidly advanced through the development of lipid biomarker proxies that enable empirical reconstructions of temperature, vegetation communities, hydroclimate, and fire activity through time from marine and lake sediment cores (Inglis et al., 2022). Because lipid proxies share a generally similar taphonomy, they allow fine-scale cross-comparison of a range of ecosystem processes at a single site in a single core. However, we lack lipid proxies for many ecological processes that are known to be important in modern time and are likely important in the past – specifically, herbivory by large mammalian herbivores.

Today, large-bodied herbivores can have significant impacts on ecosystems, changing vegetation structure and composition, fire activity, and carbon cycling (Forbes et al., 2019; Karp et al., 2024; Pringle et al., 2023; Staver et al., 2021; Trepel et al., 2024). In the past, large-bodied mammals were much more diverse and likely more abundant than they are today (Manzano et al., 2023; Rowan & Faith, 2019; Smith et al., 2018) and may also have had substantial impacts on ecosystems (Karp et al., 2021), but these potential impacts are poorly characterized and have not been incorporated into most evaluations of vegetation and fire activity changes. Currently, our best sources of information about past herbivore abundances come from sites preserving mammalian fossils (Davis & Pineda Munoz, 2016), but these sites tend to be spatially and temporally disconnected from sediment archives that preserve information about past changes in terrestrial climate and vegetation. Thus, linking information about herbivore communities derived from fossils to changes in fire, vegetation, and nutrient cycling remains a challenge (Bobe et al., 2007; Du & Behrensmeyer, 2018; Faith & Lyman, 2019).

Biomarker proxies for herbivory could be a solution to these issues. Steroids are a suite of compounds that are produced by plants, animals, and decomposers in ecosystems. Sterols are primarily synthesized by plants and animals, and, though sterols can pass through the gut unaltered and are present in animal dung (Kemp et al., 2021), they are not uniquely produced by large-bodied herbivores (Prost et a., 2017). Stanols, however, are produced through the alteration of sterols either by environmental or animal gut microbiota (Bull et al., 2002). 5β-stanol isomers are mainly reduced via gut microbes (Bull et al., 2002), and to a lesser extent via microbial degradation in the environment (Gaskell & Eglinton, 1975). In contrast, 5α-stanol isomers are thought to be mainly produced via microbial degradation in the environment (particularly anerobic environments) (Bull et al., 2002), but have also been found in dung samples in elevated quantities (Harrault et al., 2019; Kemp et al., 2021). In addition, 5β- and 5α-stanols in the environment may be modified by the secondary alteration (or epimerization) from 3β to 3α isomers. Overall, 5β-stanols are generally considered diagnostic markers of fecal inputs into ecosystems, while 5α-stanol are a more ambiguous marker. Because fecal stanols can be preserved in soils and sediments for thousands of years (Arnold et al., 2021; Gallant et al., 2024), they are promising candidates for use as herbivore abundance biomarkers in the Quaternary.

Calibration work in agricultural settings has shown that these compounds can serve as proxies for the presence of domesticated herbivores (Evershed & Bethell, 1996; Prost et al., 2017; Vázquez et al., 2021; Zocatelli et al., 2017). Fecal steroids distributions in dung from both domestic and wild species can vary with functional traits like diet and digestive system (Gill et al., 2010; Harrault et al., 2019; Kemp et al., 2021). Wild herbivore communities are more diverse and include species that are functionally distinct from domesticates (including, e.g., non-ruminant megaherbivores; Hempson et al., 2017; Ripple et al., 2015), so we cannot assume that calibrations of these compounds based on agricultural contexts are accurate analogs for past settings dominated by non-domesticates. Today, the most diverse wild herbivore communities are found in subtropical African savannas (Pringle et al., 2023; Ripple et al., 2015), making them the best available modern analogs for past environments with high herbivore abundances. However, there is a strong geographical bias towards fecal sterol calibration in North American and European settings (Bull et al., 1998; Davies et al., 2022; Evershed & Bethell, 1996; Harrault et al., 2019; Prost et al., 2017; Wendt et al., 2024). To gain fecal biomarker insights about paleo herbivore abundances, we must examine the concentrations and ratios of these compounds in modern settings where intact, functionally diverse, and abundant wild herbivores are present.

Here, we evaluate whether and how biomarker signatures preserve herbivore land-use patterns in natural settings. We measured fecal sterols and stanols from soils from two multi-decadal herbivore exclosure experiments (Hlangwini and Nkhuhlu) in Kruger National Park, South Africa. If fecal stanols record wild herbivore abundance, then we expect 1) that fecal stanol concentrations will be higher in plots where herbivores are present (controls) than plots where herbivores were excluded (exclosures), and 2) that fecal stanol concentrations will be correlated with dung counts where soil samples were collected. In contrast, we do not expect sterols to be strongly related to herbivore presence, because, while they found in significant quantities in herbivore dung, phytosterols mainly derive directly from plants and cholesterol from all animals—including soil macrofauna, birds and insects (Albro et al., 1992; Jing & Behmer, 2025; Prost et al., 2017)—which were not excluded from plots.

2 Methods

2.1 Experimental design and soil sampling

The herbivore community in Kruger National Park (KNP), South Africa, is highly diverse, with 29 species of large-bodied mammalian herbivores (Pienaar, 1963). The community is dominated by impala and elephant, but includes five species of megaherbivores (Pienaar, 1963; Staver et al., 2017). We sampled soils from two longstanding herbivore exclosure experiments in KNP: the Hlangwini and Nkhuhlu exclosures (Wigley-Coetsee et al., 2022). The Hlangwini exclosure was established in 1971 (making it 51 years old at the time of sampling) (Holdo & Mack, 2014; Wigley-Coetsee et al., 2022) in the Pretoriuskop region on sandy, low nutrient soils receiving ~ 750 mm of rainfall/yr. The Nkhuhlu exclosure was established in 2002 (making it 20 years old at the time of sampling) (van Coller et al., 2013; Wigley-Coetsee et al., 2022) in the Lower Sabie region, adjacent to the Sabie River and receiving ~ 550 mm of rainfall/yr (Jacobs & Naiman,

2008). Both exclosures are on soils derived from granitic bedrock in KNP and characterized by catenas, variations in soil character down hillslopes (Khomo & Rogers, 2005). The sodic zone of the Nkhuhlu site has historically had a higher abundance of large-bodied herbivores due to its relative nutrient richness and proximity to permanent water (Jacobs & Naiman, 2008; L. M. Khomo & Rogers, 2005; Scogings et al., 2011).

Samples were collected in a nested sampling design across the two exclosures (Hlangwini=HLG and Nkhuhlu=NKH) and across two herbivore treatments (Herbivores present=CON and Herbivores excluded=EX). For each experiment and treatment, soil samples were collected at three catenal positions: crest, mid-slope, and sodic. We placed two replicate 50 m transects laterally along the catenal position for each unique combination of experiment, treatment, and catenal position. We collected two composite depth samples (0-1 cm and 0-5 cm) every 10 m along the transect. We homogenized and subsampled soils along the transect such that we produced two samples per transect, one for each depth. Soils were then dried at 40°C in a drying oven for 24 hours. This resulted in a total of 48 samples (Fig. 1).

2.2 Dung Counts

In parallel to soil sampling, we surveyed herbivore dung along the same transects. Dung counts are a widely used proxy for herbivore land use intensity (Abraham et al., 2019; Cromsigt et al., 2009; Pfeffer et al., 2018) though they are not a perfect correlate of local herbivore abundances (due, e.g., to differences in defecation rates between species) (Cromsigt et al., 2009; Pfeffer et al., 2018). Because fences require regular maintenance, dung counts also serve as a secondary confirmation of the effectiveness of exclusion treatments.

All dung piles within 2 meters on either side of the soil sampling transects were counted and identified. The herbivores in KNP have unique dung morphologies (Stuart & Stuart, 2015), such that it was possible to identify the herbivore species from which each dung pile (though species identifications based on dung morphology alone are subject to error; Spitzer et al., 2019). Where dung piles overlapped (such as in latrines or middens), the number of dung piles was estimated from the quantity of dung in a standard pile for that species. This methodology resulted in species-specific counts of herbivore dung piles along each soil sampling transect. The “total herbivores dung counts” (Fig. 2b) include small animals that are technically not excluded based on experimental design because they can fit through small holes in the fence or burrow under exclosures such as hare, porcupine, and duikers. Carnivore and omnivore dung was also counted.

2.3 Fecal steroid analysis

Sample processing generally followed Curtin et al. (2021). We ground dried soils with a mortar and pestle and weighed ~10g of sample. We extracted soluble lipids using an Accelerated Solvent Extraction System (ASE 350 ThermoScientific). Samples were flushed with 9:1 Dichloromethane:Methanol solvent at 120 °C for three 10-minute cycles. Total lipid extracts were dried and saponified with a 1 M KOH solution in Methanol:H_2O (95:5) for 3 hours at 65 °C. Neutral fractions were extracted with a 3x liquid-liquid extraction. We then ran neutral

fractions over a short $NaSO_4$ column to remove residual water from the liquid-liquid extraction. We separated the neutral fraction further into N1 "Aliphatic," N2 "Aromatic" and N3 "Polar" fractions using silica gel column chromatography with Hexane (N1), Dichloromethane (N2), and Methanol (N3). We added a cholestane internal standard to the Polar fraction, which was then analyzed for sterols and stanols. Since abundant material was extracted, samples had very high absolute amounts of steroids (~10^3-10^4 ng). Before quantification, N3 fractions were split (typically 2:1) and a split was injected from a diluted volume (typically 1 µl from 1 ml).

We identified and quantified sterols and stanols using Gas Chromatography Mass Spectrometry (GC-MS Agilent 5977B, 30 m x 250 µm x 0.25 µm TG-5MS column). We derivatized N3 Polar fractions with an on-line BSTFA derivatization modified from Wu et al. (2009), where 1:2 volume of sample to BSTFA-10% TMS were co-injected into the GC inlet at 325 °C in splitless mode. We derivatized a standard suite of cholestane, 4 sterols and 3 stanols (see Table S1; LGC Standards and Sigma-Aldrich) and ran it with this same method to establish a calibration curve each day samples were analyzed. We ran samples in SIM/Scan mode (see Table S1 for SIM ions). The initial oven temperature was set to 80°C, the oven was then ramped 12°C/min to 265°C then ramped 0.6°C/min to 288°C finally ramped 10°C/min to 300°C for a 5 min hold time. All sample concentrations were normalized to ng/g soil based on dilution, split, and grams extracted for each individual sample.

2.4 Fecal steroid ratios

Epimerization ratios are based on relative amounts of 5β3α- and 5β3β-isomers. Dominance of 5β3α-isomers has been interpreted as a signal of potential secondary degradation in the literature (Bull et al., 2002; Vázquez et al., 2021). We calculated this ratio for the coprostanol (5β3β) and epicoprostanol (5β3α) isomers:

(1)

$$\frac{5\beta,3\beta\text{-cholestanol}}{5\beta,3\beta\text{-cholestanol}+5\beta,3\alpha\text{-cholestanol}}$$

as well as for the stigmastanol (5β3β) and epistigmastanol (5β3α) isomers:

(2)

$$\frac{5\beta,3\beta\text{-stigmastanol}}{5\beta,3\beta\text{-stigmastanol}+5\beta,3\alpha\text{-stigmastanol}}$$

We calculated two other commonly derived ratios of plant fecal steroids and assessed them as potential proxies related to herbivore abundance. The 5β3(α+β)/5α3β+5β3(α+β)stigmastanol ratio (henceforth '5β/5β+α stigma') has often been proposed to examine herbivore (usually domesticate) fecal inputs to soils and sediments (Bull et al., 2002; Prost et al., 2017)

(3)

$$\frac{5\beta,3\alpha\text{-stigmastanol}+5\beta,3\beta\text{-stigmastanol}}{5\beta,3\alpha\text{-stigmastanol}+5\beta,3\beta\text{-stigmastanol}+5\alpha,3\beta\text{-stigmastanol}}$$

As a point of comparison, we also calculated the equivalent coprostanol ratio, which is not thought to be a proxy for herbivore abundance, but rather human presence or sewage (Bull et al., 2002):

(4)

$$\frac{5\beta,3\alpha\text{-cholestanol}+5\beta,3\beta\text{-cholestanol}}{5\beta,3\alpha\text{-cholestanol}+5\beta,3\beta\text{-cholestanol}+5\alpha,3\beta\text{-cholestanol}}$$

Finally, we developed two new isomer ratios based on the compounds that had significant differences between exclosure treatments as potential "herbivore abundance" proxies. First, the plant stanol to plant sterol or "PS" ratio (for 'Plant Steroid'), which was defined as

(5)

$$\frac{5\beta,3\alpha\text{-stigmastanol}+5\beta,3\beta\text{-stigmastanol}+5\alpha,3\beta\text{-stigmastanol}+5\alpha3\beta\text{-campestanol}}{5\beta,3\alpha\text{-stigmastanol}+5\beta,3\beta\text{-stigmastanol}+5\alpha,3\beta\text{-stigmastanol}+5\alpha3\beta\text{-campestanol}+\text{stigmasterol}+\beta\text{-sitosterol}+\text{Campestanol}}$$

and second, the ratio of all stigmastanol isomers to 5α-stigmastanol + stigmasterol + sitosterol or the "PU" ratio, defined as

(6)

$$\frac{5\beta,3\alpha\text{-stigmastanol}+5\beta,3\beta\text{-stigmastanol}+5\alpha,3\beta\text{-stigmastanol}}{5\alpha,3\beta\text{-stigmastanol}+\text{stigmasterol}+\beta\text{-sitosterol}}$$

2.5 Statistical analysis

All statistical analyses were conducted in R (Core, 2020). We analyzed differences in sterol and stanol concentrations, dung counts, and ratios between treatments using analysis of variance (ANOVA). We examined nested models with soil depth nested in replicate, catenal position, and treatment. Akaike Information Criterion (AICc) was used to select between combinations of nested predictor variable structure. The least complex model with $\Delta AICc \leq 2$ was selected as "best" model for the purposes of interpretation (Table S2). We used Tukey's Significance Test to evaluate differences between exclosure treatments.

We modelled the relationship between fecal biomarkers (concentrations and ratios) and dung counts using linear regression. To meet normality assumptions, we log-transformed dung counts and concentrations of all compounds and ratios, except for the 5β/5β+α stigma ratio, which met normality assumptions without transformation. AICc was used to select between linear regression and linear mixed-effects regression (Bates et al., 2015) with the experimental design (experiment | treatment | catenal position | soil depth) modeled as nested random

predictor variables and log-transformed large herbivore dung counts modeled as the fixed predictor variable. As described above, the simplest model with ΔAICc ≤ 2 was selected as "best" model (Table S3).

We tested the relationships between sterol and stanol distributions and species-specific dung counts using Canonical Correspondence Analysis (CCA) algorithms in the 'vegan' R package (Oksanen et al., 2018). This method utilizes paired multivariate data collected from each sample (*i.e.*, transect). Multivariate data on dung counts were compared with fecal biomarker multivariate data. The variance of a matrix of the fecal steroid concentrations was constrained using a matrix of dung counts for each herbivore species (Ter Braak, 1986; Legendre et al., 2011; Oksanen et al., 2018). Fecal steroid concentrations and dung counts were normalized using the Hellinger square-root transformation of proportional data (Legendre & Gallagher, 2001).

We examined whether experimental variables (*i.e.*, experiment, treatment, etc.) were also related to ordination structure using 'envfit'. We color and symbol coded samples in ordination plots by the two variables (treatment and catenal position) that were significantly correlated to the ordination (α=0.995) (Fig. 9a; Table S6). To further examine if overall community diversity and abundance metrics were related to the ordination structure, we calculated the Simpson and Shannon Diversity indices, as well as the total abundance from the dung count data for each sample. We also calculated the proportion of the dung that was from different feeding guilds (grazer, browser, mixed feeder) and digestive systems (ruminant, non-ruminant). These variables were likewise fit to the ordination using 'envfit'. Correlation vectors for the variables with correlations with $p < 0.01$ are plotted in ordination space (Fig. 9b).

2.6 Application of ratios to a previously published historical steroid dataset

To examine the applicability of the newly proposed fecal steroid ratios to sedimentary archives, we applied these metrics to a newly published fecal steroid dataset from Buffalo Ford Lake in Yellowstone National Park, USA (Wendt et al., 2024). This study includes historical elk + bison biomass estimates, which allows us to compare our new ratios and metrics to herbivore biomass through time. While these samples are not from the same location as our field calibration, re-analyzing this data with these new metrics allows us to test 1) if the relationship between ratios and herbivore abundance holds up through time rather than just across space, and 2) if ratios have the potential to be applied across a range of environmental contexts. First, after Wendt et al. (2024), we calculated bison and elk biomass the year of each historical sedimentary sample (*N*=11), using the same average species mass estimates and population data (Cook et al., 2004; Martin et al., 2018; Wendt et al., 2024). We then calculated the 5β/5β+α stigma ratio via EQ 4 and the PU ratio by applying EQ 6. However, since Wendt et al. (2024) did not measure 5α3β-campestanol, we applied a modified PS ratio (EQ 5) that excluded that compound.

We used Pearson's Product and a Kendell test to check for significant correlations. Since a relationship between fecal stanol concentrations and herbivore biomass was previously only observed during the 50 years between 1920-1970 (Wendt et al., 2024), when herbivores were

restricted to the valley around the lake in the winter, we followed the methodology in the original publication and split the datasets into two intervals (1920-1970 and 1971-2020) and analyzed them separately. For the earlier interval (1920-1970), we modelled the relationship between fecal biomarkers (concentrations and ratios) and historical herbivore biomass estimates using linear regression. We compared the AICc values between log-log and untransformed linear regressions and used the lower value to select the "best" model.

3. Results

Herbivore dung counts were higher outside than inside herbivore exclosures in Nkhuhlu and were highest in the sodic zone (Fig. 2; Table S4; Table S5; N=48, NKH:CON, $p<0.0001$; NKH:CON:Sod, $p<0.0001$), consistent with expectations. Carnivore dung was rare (only found on 20% of transects), with counts more than an order of magnitude lower than herbivore counts (total herbivore dung = 738, total carnivore/omnivore dung = 10).

We found no significant difference between total sterol and plant sterol concentrations in control and exclosure treatments (Fig. 3c-d; Table S5). However, total stanols, plant stanols, 5β3(β+α)-stanols and 5α3β-stanols were all higher in sodic zone of the control treatment relative to the other catenal positions in the controls and all catenal position in the exclosures (Fig. 3a-b; Fig. 4c-d; Table S4; N=48, $p<0.01$; see Table S5 for full report of significance for all response variables). Additionally, 5β- stanols (5β3(β+α)) concentrations were elevated in the Nkhuhlu control sodic zone samples (Fig. 4a-b; Table S4; Table S5; N=48, $p<0.0001$).

We found that the epimerization ratios, which can be influenced by steroid degradation, were higher in control plots where herbivores were present (Fig. 5a; Table S4; Table S5; *N*=48, $p=0.001$) and that the ratio between 5β3β-cholestanol and 5β3α-cholestanol ratio was slightly higher in top soil samples (Fig. 5b; Table S4; Table S5; *N*=48, $p<0.005$). No other compound concentration or ratio examined had a significant relationship to depth (Table S2).

The 5β/5β+α stigma ratio was higher in the sodic samples (Fig. 6a; Table S4; Table S5; *N*=48, $p<0.015$), and, though the ratio was slightly higher in control plots (*N*=48, $p=0.01$), treatment was not included as a predictor in the best ANOVA (Table S2). We note that the equivalent coprostanol ratio, which has been used to indicate human (or carnivore) sewage inputs, was not significantly different between any of the treatments (Fig. 6b; Table S4; Table S5; *N*=48, $p<0.1$), which suggests the stigmastanol isomer concentrations and ratios may be more robust records of herbivore abundance.

The PU ratio was defined based on the observation that, although the β-stanols may be more specific in their relationship with herbivore presence (these compounds and dung counts were an order of magnitude higher in the Nkhuhlu sodic control plots), the 5α-stanols were elevated in control sodic zone treatment where large herbivores were present. We found that PS and PU ratios were elevated in the Nkhuhlu control plots relative to exclosures and were highest in the sodic zone samples (Fig. 6c-d; Table S4; Table S5, *N*=48, all $p<0.0001$). This pattern was also present in the dung counts (Fig. 2) and differs from the 5β/5β+α stigma ratio,

which was only higher in controls and sodic samples (Fig. 6a). This may indicate that the two new ratios better reflect dung inputs than ratios currently suggested in the literature.

We explicitly tested the performance of the PS and PU ratios using regressions to examine if dung counts predict fecal steroid concentrations and ratios. For all response variables examined, the models without random effects were best (Table S3). There was no relationship between sterol concentrations and dung counts (Table S3). The plant stanol concentrations (R^2 = 0.26, *N*=48, p<0.001) and 5β3(α+β)-stigmastanol concentrations (R^2 = 0.20, *N*=48, p<0.001) increased exponentially as dung count increased exponentially (Fig. 7), as did the PS (R^2 = 0.59, *N*=48, p<0.001) and the PU (R^2 = 0.62, *N*=48, p<0.001) ratios (Fig. 8a-b) (Table S3). The 5β/5β+α stigma ratio increased exponentially as dung counts increased linearly (R^2 = 0.19, *N*=48, p<0.01) (Fig. 8c). The variance explained was higher for the PS (R^2 = 0.59, *N*=48, p<0.001) and PU (R^2 = 0.62, *N*=48, p<0.001) models than the 5β/5β+α stigma ratio (R^2 = 0.19, *N*=48, p<0.01) model.

Constrained correspondence analysis (CCA) between fecal compound concentrations and dung counts indicated that ~ 67% of the variance in the compound concentrations was constrained by the species dung counts (adjusted R^2=0.44, *N*=48, p<0.001) (Fig. 9; Table S6). A permutation test (with 999 runs) indicated that the CCA model was significant (F= 3.2926, *N* = 48, p<0.001). Only treatment (R^2= 0.16, *N*=48, p<0.001) and catenal position (R^2= 0.13, *N*=48, p<0.01) were correlated to ordination structure, and CCA1 differentiated samples most strongly by treatment (Fig. 9a). Clear separation of compound classes and sources was apparent along CCA1 and CCA2 (Fig. 9b). CCA1 separated sterols, 5β-stanols, and 5α-stanols. CCA1 values were positive for the 5β-stanols, close to zero for the 5α-stanols, and negative for the sterols. CCA2 differentiated between plant and animal sources of 5β-stanols. CCA2 values were positive for the coprostanols and negative for the 5β-stigmastanols. We found that overall community functional characteristics were weakly correlated to ordination structure (ruminants, R^2= 0.19, *N*=48, p<0.01; browsers, R^2= 0.2, *N*=48, p<0.05; mixed feeders, R^2= 0.27, *N*=48, p<0.001), and diversity metrics were not correlated to ordination structure (Table S6). However, total abundance was highly correlated to the ordination structure (R^2=0.64, *N*=48, p<0.001). The strong correlation between total dung abundance plotted in the same magnitude and direction of 5β3β-stigmastanol CCA scores (Fig. 9b).

We found that the PS and PU in lake sediments were strongly related to historical herbivore biomass between 1920-1970 (Fig. 10c, 10e; PS r=0.96, *N*=6, p=0.002; PU r=0.94, *N*=6, p=0.005). The relationship between biomass and ratio metrics was stronger than that of stanol concentrations originally observed (Fig. 10a; r=0.69, *N*=6, p=0.13). We did not find a correlation between the 5β/5β+α stigma and 1920-1970 herbivore biomass (Fig. S2; r=0.39, *N*=6, p=0.45). Neither concentrations, nor ratios, were significantly correlated with herbivore biomass between 1971-2020 (Fig. 10; Fig. S2), consistent with findings from the original study. Log-log regression models were selected over untransformed linear regressions for models with stanol concentration, PU and PS ratios as the response variables (Fig. 10d, 10f; PS r^2=0.94, *N*=6, p<0.001, RMSE = 0.006; PU r^2=0.91, *N*=6, p=0.002, RMSE = 0.007).

4 Discussion

Fecal stanols (derived from plants but modified by herbivore gut passage; Bull et al., 2002) in soils from KNP captured important information about wild herbivore abundance. Our results support key expectations (1) that fecal stanols were more concentrated in soils where herbivores were present than where herbivores were excluded, and (2) that fecal stanol concentrations increased with herbivore dung counts, a proxy for herbivore abundance. In contrast, sterols, which can be derived directly from plants or herbivores (Bull et al., 2002) were unrelated to experimental treatment or dung counts (Fig. 3c-d). This study is one of the first to examine fecal sterols and stanols in a natural setting with abundant and diverse wild herbivores, and our findings lend confidence to the use of fecal stanols as a proxy for herbivore abundance.

In the following discussion, we compare our findings with previous work on these lipids in anthropogenic settings dominated by domesticates. We explore if and how ratios capture patterns in wild herbivore abundances in time, in addition to space, by applying these ratios to a previously published fecal stanol sedimentary record from Yellowstone National Park. We discuss promising relationships between newly defined fecal biomarker ratios and wild herbivore densities to highlight pathways to build quantitative proxies for herbivores. We finish by examining limitations and uncertainties that should be addressed before these metrics can be properly implemented to reconstruct herbivore abundances.

4.1 Comparisons to other studies

Our results indicate that ambient concentrations of stanols in soils from a wild herbivore-rich environment are comparable or higher than in soils with added manure or sewage inputs examined in previous studies (Fig. 3a-b; Fig. 4). While differences in laboratory procedures can impact total concentrations, high fecal stanol concentrations in this study are consistent with a sustained high density of wild herbivores.

Stanol concentrations do not always differ between settings with and without herbivores. Previous work examining fecal biomarkers in sheep grazed and un-grazed temperate peats found no difference in 5β-stanol concentrations (Davies et al., 2022). This could reflect preservation of these compounds, which may be affected by differences in climate, herbivore community, density and soil characteristics. For example, 5α-stanols can be produced *in situ* via anerobic degradation in peat (Naafs et al., 2019), and in tropical vegetation-rich soils, 5β-stanols may be more depleted relative to 5α-stanols and sterol (Birk et al., 2011). These differences highlight the need for modern calibrations using appropriate analogs for past herbivore communities in diverse geographic and depositional settings. Indeed, the relationships defined by our work may be appropriate for reconstructing past wild herbivore abundances where they are abundant but should not be extended to reconstructing past domesticate herbivore abundances without further study.

We found that ratios of 5β3β-stanols to 5β3α-stanols (Fig. 5) and 5β-stanols to 5α-stanols (Fig. 6a-b), lower values of which are generally used to indicate potential degradation, were much lower in KNP soils than in latrines or manured fields (Elhmmali et al., 2000; Prost et al., 2017; Zocatelli et al., 2017). One potential explanation for the lower ratios is differences in the physical and chemical properties of soil, which can impact degradation processes. Soils with high clay content and negatively charged minerals tend to stabilize organic matter and result in better preservation (Wiseman & Püttmann, 2005), whereas soils in Hlangwini and Nkhuhlu are granite-derived and sandy (Holdo & Mack, 2014; Jacobs & Naiman, 2008; Staver et al., 2017), except on sodic patches that tend to be enriched in clay relative to catena crests (L. Khomo, 2008) but which nonetheless shared similar 5β to 5β3(α+β) stanol ratios with other catenal positions (Fig. 5; Table S2). Therefore, although soil mineraology likely affects preservation across very different environments, it had little to no effect on stanol perservation within the range of KNP soils examined in this study. Another explanation is that these preservational differences actually reflect different land-use histories. Our study site is a savanna that has not been used for agricultural purposes for over a century and on which large-bodied wild herbivores have been continuously present in high densities (Pienaar, 2012). Given this long history of herbivore presence, a more likely explanation for these lower isomer ratios is that the KNP the soils represent a mixture of newly deposited stanols and older, more degraded stanols.

We found the environmentally altered 5α3β-stigmastanol in higher overall concentrations than 5β-stigmastanol isomers in all soils examined here, including those where herbivores were present (Fig. 4; Fig. 6a). Although the magnitude of these isomer ratios indicates higher potential for degradation of 5β-isomers and sterols compared to the published literature (Vázquez et al., 2021), we note that 5α-stanols concentrations were still significantly elevated in soils where herbivores were present (Fig. 4c-d). Various stanol compounds had elevated levels in KNP sodic soils of herbivore-present controls versus herbivores exclusions, including total stanols, plant stanols, the 5α- and 5β-isomers of all stanols, and the 5α- and 5β-isomers of stigmastanol (Fig. 3a-b; Fig. 4). While the 5α-isomer has been tied to aerobic degradation of sterols in the environment (Bull et al., 2002), 5α-stanols are also found in dung samples of herbivores (specifically wild herbivores) in significant quantities (Kemp et al., 2021; Prost et al., 2017). We propose that these 5α-isomers are sensitive signals of herbivore abundance even though their reduction takes place outside the gut. It is possible that dung itself creates a microenvironment for increased production of 5α-stigmastanol from sterols, either due to the high concentration of nutrients or the unique microbial community living on and in the dung itself (Bol et al., 2000; Sukhum et al., 2021; Sun et al., 2024).

Previous studies have suggested that the 5β/5β+α stigma ratio (EQ. 3), or minor variations of this ratio, may be applied as a proxy for shifts in herbivore (mostly domesticate) presence in sedimentary records (Li et al., 2024; Prost et al., 2017; Vázquez et al., 2021). Although we found that this ratio to be elevated in sodic soils where herbivores were present (Fig. 5a), our results indicate that this may not be the best way to capture signals of herbivore abundance. We found the relationship between the 5β/5β+α stigma ratio and dung counts was linear-log (Fig. 8c; $R^2 = 0.19$, $p < 0.005$). Since dung counts are a widely accepted proxy for

herbivore abundance (Ahrestani et al., 2018; Hema et al., 2017; Pfeffer et al., 2018), this relationship indicates that the sensitivity of the ratio decreases as herbivore abundance increases. Additionally, we found no relationship between the sedimentary 5β/5β+α stigma ratio and historical herbivore biomass in the Yellowstone dataset (Fig. S2). Thus, alternative ratios might better capture the relationship between stanols, dung counts, and herbivore abundance.

4.2 New proxy ratios

We used the differences in sterol and stanol distributions observed in control and exclosure plots to define two new ratios and used regressions with dung counts to evaluate the responses of these new ratios to herbivore abundances. First, we proposed the PS ratio (EQ 5), based on the observation that places where herbivores were present versus where they were absent had similar plant sterol concentrations but had higher plant stanols concentrations. Second, we proposed the PU ratio (EQ 6), which includes 5α3β-stigmastanol in both the numerator and the denominator to account for its being elevated where herbivores are present, but with a potential for a more general aerobic environmental origin noted by other studies (Bull et al., 2002; Evershed et al., 1997; Vázquez et al., 2021)(Bull et al., 2002; Evershed et al., 1997; Vázquez et al., 2021). Our results indicate that both the PS (R^2 = 0.59, p<0.0001) and the PU (R^2 = 0.62, p<0.0001) ratios have potential as a proxy for wild herbivore abundance (Fig. 8a-b). A log-log model accurately described the relationship between fecal steroid ratios and herbivore dung counts.

We evaluated whether biomarkers captured spatial heterogeneity in herbivore abundance at scale finer than treatment. Herbivore spatial patterns can vary substantially. For example, herbivores tend to aggregate in high densities around permanent water sources like rivers and watering holes, particularly during the dry season (Pringle et al., 2023). Additionally, grazing lawns and high nutrient sites provide preferred fodder and thus tend to be more intensely used (van Coller et al., 2013; Hempson et al., 2015; McNaughton, 1984). This heterogeneity was reflected in both dung counts and in biomarkers: dung abundances differed by several orders of magnitude across transects, with the highest dung densities on nutrient-rich sodic soils (Fig. 2). Thus, herbivore abundances are not evenly distributed across landscapes but are instead highly skewed. Order-of-magnitude differences in herbivore land-use across landscapes require a ratio and model that can accurately capture this right-skewed distribution. We find that the PU and PS ratios (Fig. 8a-b), as well as stanol concentrations generally (Fig. 7), accurately captured both the spatial heterogeneity of and order of magnitude (*i.e.,* log-log) differences in herbivore abundance at the landscape scale.

Notably, we find this relationship not only across both space in South African soils (Fig. 8), but also across time recorded in sediments from a North American Lake (Fig. 10). However, there is less variance in the sedimentary dataset. This suggests that some of the heterogeneity in herbivore abundance observed between soils sampled within a landscape may be integrated across space and time in sedimentary records. Indeed, the log-log models between sedimentary ratios and historical herbivore densities (Fig. 10d,f; PS r^2=0.94, *N*=6, p<0.001, RMSE = 0.006; PU

463 r^2=0.91 , *N*=6, p=0.002, RMSE = 0.007) capture more of the variance than the landscape scale
464 models between soil ratios and dung counts (Fig. 8a-b; PS r^2=0.59, *N*=48, p<0.0001, RMSE =
465 0.10; PU r^2=0.62 , *N*=48, p<0.0001, RMSE = 0.06). It is possible that the tighter relationship in
466 the lake sediments is in part due to the smaller number of measurements compared to the soils
467 (*N*=6 versus *N*=48).
468
469 The distribution of specific herbivore species may also impact fecal biomarker
470 distributions and ratios. Relative amounts of sterols and stanols in wild herbivore dung are
471 related to differences in both diet as well as the digestive systems of specific species, among
472 other factors (Gill et al., 2010; Kemp et al., 2021; Prost et al., 2017). Ratios between different
473 compounds have been used to differentiate between pigs, cattle, and horses in archeological
474 settings (Prost et al., 2017; Shah et al., 2007; Vázquez et al., 2021). For example, non-ruminant
475 horses could be distinguished from ruminant sheep and cows and omnivorous pigs could be
476 distinguished from other herbivore domesticates based on their steroid distributions (Harrault
477 et al., 2019; Prost et al., 2017). Notably, previous work indicated that digestive system strongly
478 influenced fecal steroid distributions in dung (Kemp et al., 2021). We therefore evaluated the
479 potential for fecal steroid distributions to provide information about the species composition of
480 herbivore communities (Fig. 9). We found that wild herbivore community composition and
481 compound distributions were related (Fig. 9a; R^2=0.44; p<0.001). Herbivore functional traits
482 were also related to steroid distributions (Fig. 9b; ruminants, R^2= 0.19, p<0.01; browsers, R^2=
483 0.2, p<0.05; mixed feeders, R^2= 0.27, p=0.001), but these relationships explained relatively little
484 variance. Instead, we found the sites with the greatest overall abundance of herbivores were
485 dominated by β-stanols, specifically 5β3β-stigmastanol (Fig. 9b; R^2= 0.64, p<0.001). We note
486 that the sites that have the highest overall herbivore abundance and 5β3β-stigmastanol
487 concentrations were dominated by impala (mixed feeding ruminants), which all plot together in
488 ordination space (Fig. 9). This makes it difficult to determine if the dominance of stigmastanol
489 isomers in these samples is truly related to animal functional traits or just due to the high
490 overall dung inputs.
491
492 While our results do not rule out the possibility that fecal steroid distributions preserve
493 information about past herbivore functional communities, the overall abundance of herbivores
494 may have a stronger impact on steroid distributions than which herbivores are present in
495 depositional settings with order of magnitude differences in herbivore landscape-use. The
496 interpretation that the PU and PS ratio mainly track overall herbivore abundance rather than
497 species composition is also supported by the strong relationship observed between these ratios
498 in historical sediments from Yellowstone National Park and records of historical herbivore
499 biomass (Fig. 10d, Fig. 10f). Both archives capture dung inputs from wild herbivores, but the
500 species in communities in Kruger and Yellowstone are completely distinct. Together, this data
501 strongly suggests that the PU and PS ratios have utility as an herbivore abundance proxy across
502 multiple geologic archives, communities, and environmental contexts.

503 4.3 Limitations and future work

Our work supports the use of stanols to infer wild animal abundances in natural settings and especially for use of the PU and PS ratios as a proxy for herbivore abundance. Nevertheless, we caution that more work is needed before these metrics can be quantitatively applied to reconstruct herbivore abundance from sediments without independent estimates.

First, this study only examines fecal steroids across two similar sites that do not capture the full extent of herbivore variability across KNP, much less other savannas or other biomes with different environmental conditions and herbivore densities. Although our application of the proxy to Yellowstone sediments suggests promise, to fully constrain how herbivore abundance, community composition, and other factors influence steroids in mixed sedimentary records, it would be helpful to expand this calibration to measure fecal biomarkers in soils across sites with a wider range of herbivore abundances and community compositions.

Second, we only sampled these sites once. Although soils likely represent a temporally integrated record of herbivore populations (though probably biased towards more recent inputs), dung counts do not capture the inter- or even intra-annual variability of herbivore-landscape use (Hema et al., 2017; Pfeffer et al., 2018). Repeated surveys of both dung and fecal steroids would help to resolve temporal variability in signals of herbivores. Studies that experimentally spike a setting with dung or herbivores (Bull et al., 1998; Mutillod et al., 2024) may also be helpful to test how competing fecal steroid fluxes of additions and degradation balance through time.

Third, additional geochemical and ecological information may improve the use of fecal lipids to characterize shifts in herbivore abundances or community composition. For this study, we focused on eleven compounds that are most commonly measured in sedimentary records and modern dung samples (Bull et al., 2002; Kemp et al., 2021; Prost et al., 2017). However, many studies of dung samples advocate for measuring more compounds, such as bile acids, to increase statistical power to differentiate between herbivore species and functional characteristics (Harrault et al., 2019; Kemp et al., 2021; Prost et al., 2017). Additionally, measuring fecal steroids alongside other proxies for paleo-herbivore shifts, such as dung fungus spores or ancient DNA (Baker et al., 2016; Curtin et al., 2021; Davies et al., 2022; Ekblom & Gillson, 2010), may yield insights into potential biases of each proxy and provide a greater understanding of changing herbivore communities and abundances through time.

Fourth, understanding the mechanism by which steroids are transported into sediments will be critical to accurately interpreting PU and PS ratios as an herbivore abundance proxies. Recent work on lake surface sediments from China indicates the spatial variability of wild and domesticate fecal steroid signals can be recorded in sedimentary records (Li et al., 2024). However, in Yellowstone sediments, strong relationships between steroid concentrations, ratios, and herbivore abundance were only present during the 1920-1970 interval when herbivore populations were in greater proximity to the lake in the winter (Coughenour & Singer, 1996; Meagher, 1989; Wendt et al., 2024). This suggests that records may reflect shifts in herbivore landscape use patterns, rather than absolute abundances. For example, in a subtropical setting such as Kruger, an increase in aridity or seasonality could result in herbivore

548 populations aggregating around water sources (i.e., lakes and rivers), which could give the
549 appearance of increasing herbivore abundance that only reflects local densities in the dry
550 season or during dry periods. Explicit source-to-sink studies and experiments are needed to test
551 hypotheses for different to transport mechanisms and biases, and these should be conducted
552 across a wide range of environmental contexts.
553
554 Finally, more work is needed to understand how degradation and other taphonomic
555 processes may alter steroid concentrations and distributions between their soil source and
556 proxy archives such as lake sediments. While our work indicates stanol-to-sterol ratios can
557 distinguish between herbivore inputs at the landscape level, there is a large body of evidence
558 showing that these ratios are sensitive to redox conditions in sediments (Li et al., 2024;
559 Nishimura & Koyama, 1977; Wakeham, 1989). Additionally, different thermodynamic stabilities
560 of the isomers may lead to lower preservation potential of β-stanols in tropical environments
561 (Birk et al., 2011; Mackenzie et al., 1982). Though sedimentary conditions may impact these
562 ratios, additional metrics or analyses may be able to correct for alterations to these ratios
563 associated with in-situ production of stanols, and our application of PU and PS ratios to
564 sediments in Yellowstone indicates that at least under some circumstances these issues are not
565 insurmountable.

566 **5 Conclusion**

567 This work provides a foundation for the use of fecal steroids as a geochemical proxy for
568 wild animal abundances in natural settings. We found that stanols were more concentrated in
569 plots where herbivores were present and in areas where herbivores were abundant. We also
570 found that, although wildlife species composition did impact compound distributions, the main
571 determinant of compound distributions was total herbivore abundance. We defined two new
572 stanol-to-sterol ratios based on our observations of compound concentrations and found that
573 both were strongly predicted by large herbivore dung counts, a common proxy for herbivore
574 abundance. These ratios may thus serve as a relative or even a quantitative proxy for paleo-
575 herbivore abundance, though we caution that more calibration work is needed to address
576 several taphonomic factors before they can be applied with confidence to sediments.
577 Altogether, fecal steroids may provide a means to examine herbivore abundance and ecological
578 impacts when used alongside other proxies in the same sediment records. Ultimately, fecal
579 steroids may promote an improved understanding of ecosystem processes, disturbances, and
580 feedbacks in past ecosystems and their response to past climate changes.

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Open Research

The biomarker and dung count data used for in this study are available in Dryad for reviewers at the following link:
http://datadryad.org/stash/share/RndQi_YKC0GxS3aSHiKQXwq7SH1xkEnlfB_WF23rd98
If the manuscript is accepted for publication: Data will be available at:
https://doi.org/10.5061/dryad.5x69p8dfw

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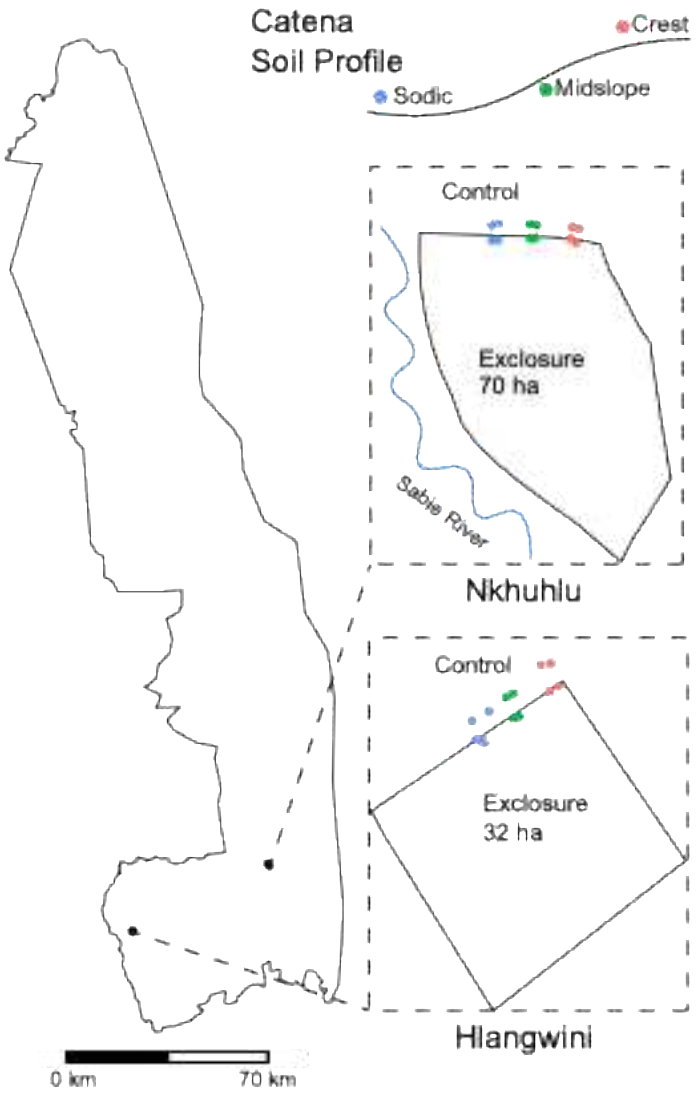


Figure 1: Experimental and sampling design. Locations of exclosures are shown on map of Kruger National Park. Samples were collected from 24 transects in a nested design arranged as shown in the figure. Two replicate transects were sampled for each region of the catenal position, each treatment, and each experiment. Samples were collected at two depths per transect 0-1 cm and 0-5 cm, resulting in a total of 48 samples. The catenal position is shown from the side indicating the general landscape relief. The two exclosure experiments are shown in the insets and the location of sampling transects are indicated by the points.

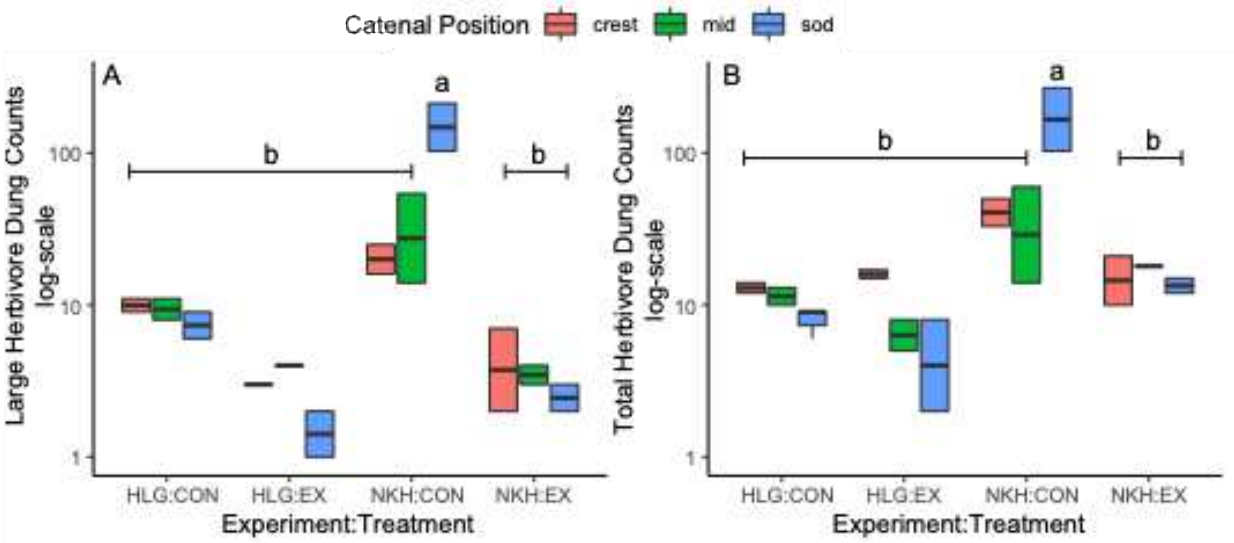


Figure 2: Dung counts across experiment, treatments, and catenal position. For all boxplots, "a" and "b" notation indicate significance at lowest nested level. a) Dung counts of large (>10 kg) herbivores only. Tukey's Significance Test (NKH-HLG, p= 2.8e-06; NKH:CON-all, p<0.0001; NKH:CON:Sod-all, p<0.0001). b) Dung counts of all herbivores. Tukey's Significance Test (NKH-HLG, p= 1.21e-05; NKH:CON-all, p<0.0001; NKH:CON:Sod-all, p<0.0001).

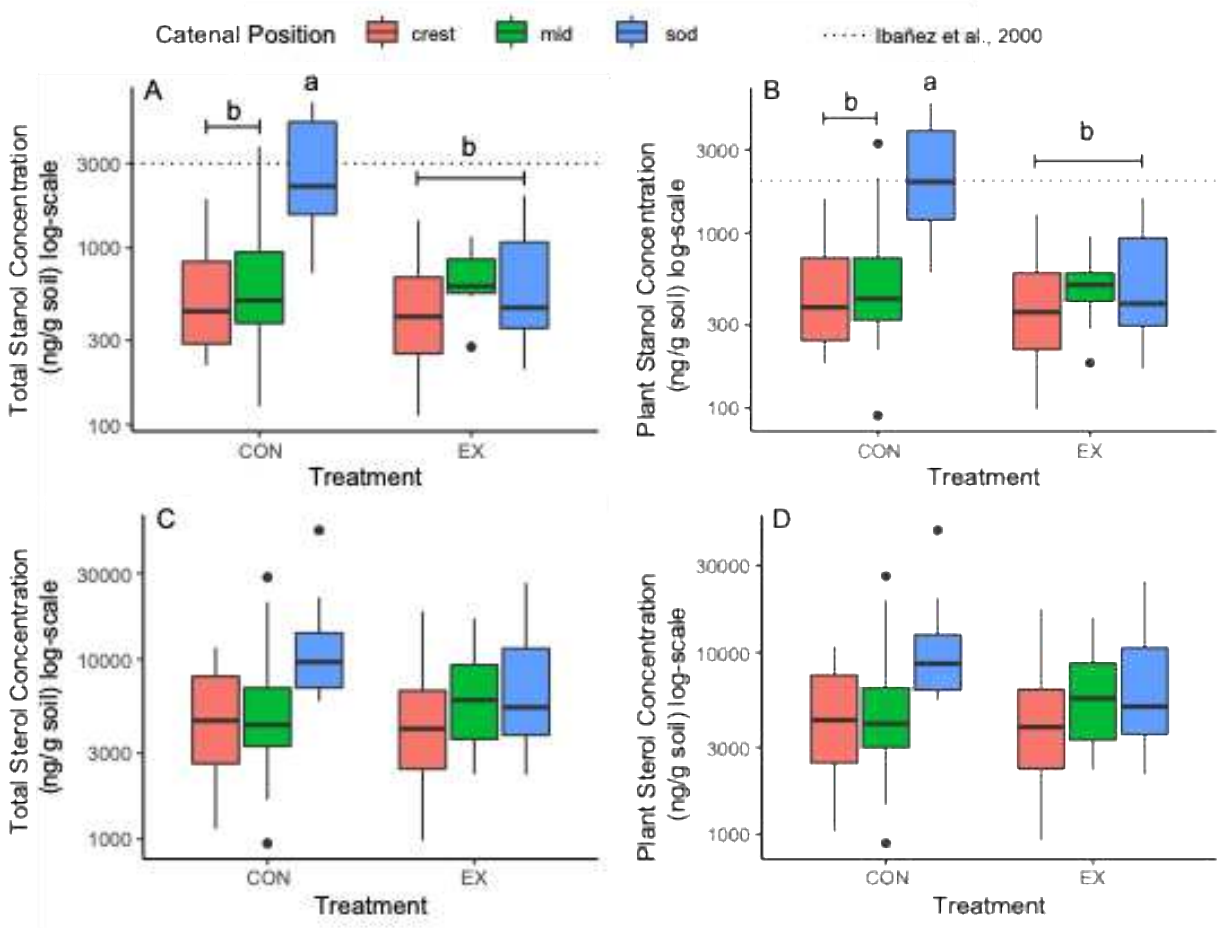


Figure 3: Stanol and Sterol concentrations across treatments and catenal position. For all boxplots, "a" and "b" notation indicate significance at lowest nested level. Dotted line shows the values for manured soils reported in (Ibañez et al., 2000). a) Total stanol concentrations. Tukey's Significance Test (CON-EX, p=0.005; CON:Sod- CON:Mid, p<0.01; CON:Sod- EX:Sod p=0.002; CON:Sod- all else, p<0.0001). b) Plant stanol concentrations. Tukey's Significance Test (CON-EX, p= 0.005; CON:Sod- CON:Mid, p=0.015; CON:Sod- EX:Sod p=0.003; CON:Sod- all else, p<0.0001). c) Total sterol concentrations, no significant difference (p>0.2) between any variables. d) Plant sterol concentrations, no significant difference (p>0.2) between any variables.

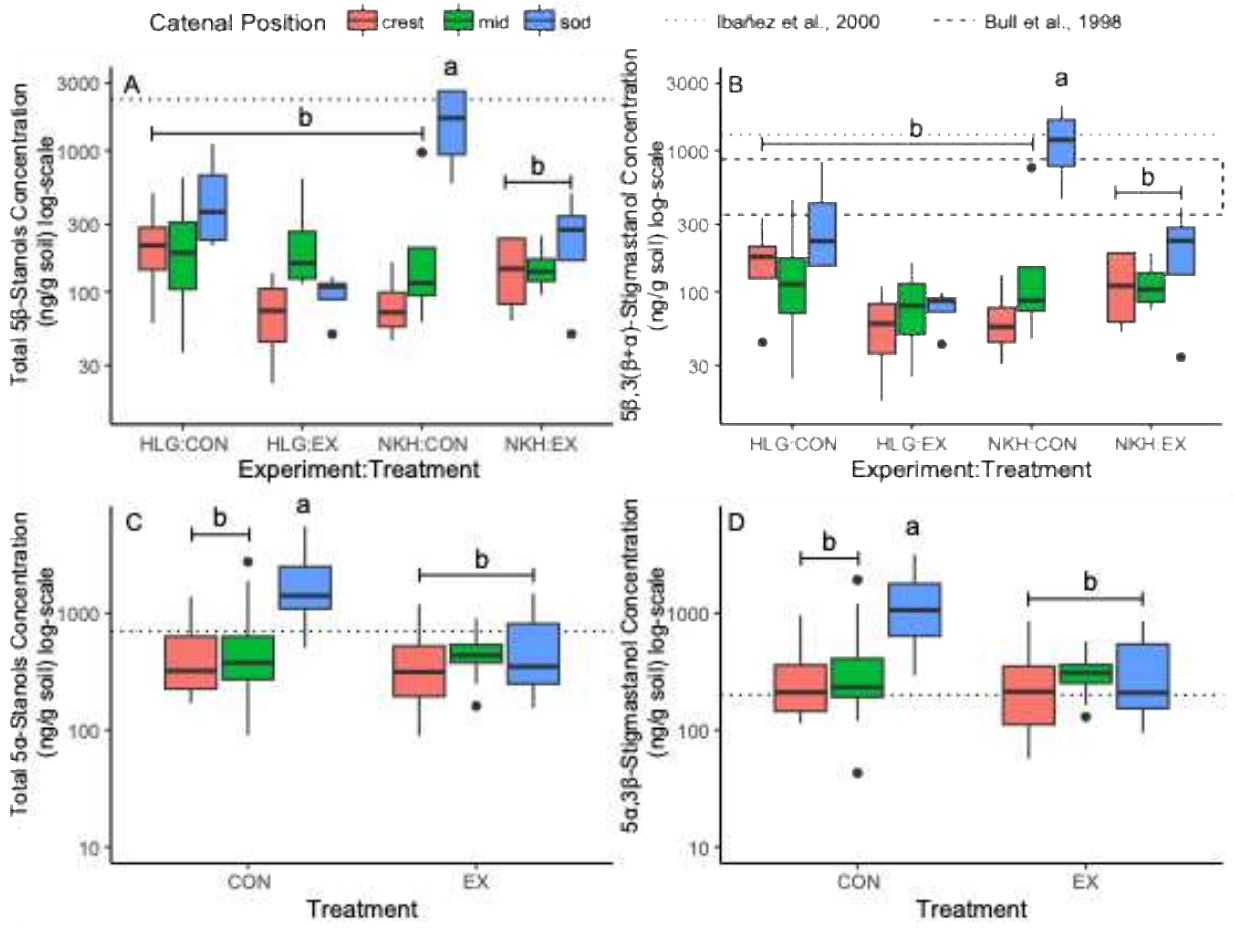


Figure 4. Stanol isomer concentrations across experiments, treatments, and catenal position. For all boxplots, "a" and "b" notation indicate significance at lowest nested level. Dotted line shows the values for manured soils reported in (Bull et al., 1998; Ibañez et al., 2000). a) Total 5β-stanol concentrations. Tukey's Significance Test (NKH:EX-NKH:CON, p=0.009, HLG:EX-NKH: CON, p=0.004, NKH: CON:sod-all, p<0.001). b) Total 5β-stigmastanol concentrations. Tukey's Significance Test (NKH-HLG, p=0.02; NKH:EX-NKH: CON, p=0.008, HLG:EX-NKH: CON, p= 0.001, NKH: CON:sod-all, p<0.001). c) Total 5α-stanol concentrations. Tukey's Significance Test (EX-CON, p=0.01; CON:sod-all, p<0.01). d) Total 5α-stigmastanol concentrations. Tukey's Significance Test (EX-CON, p=0.01; CON:sod-all, p<0.007)

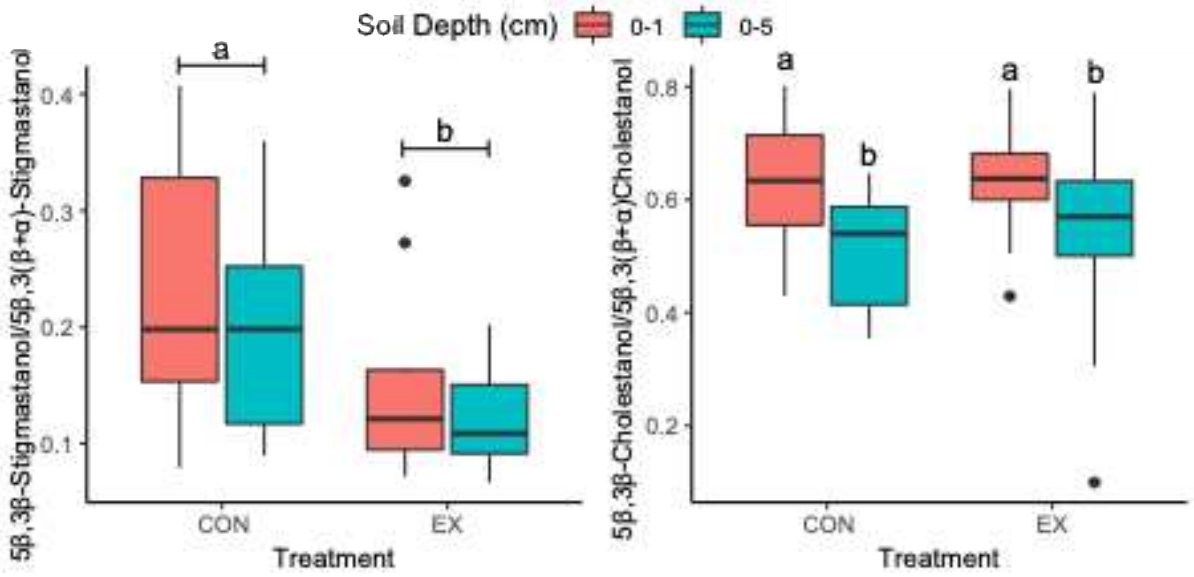
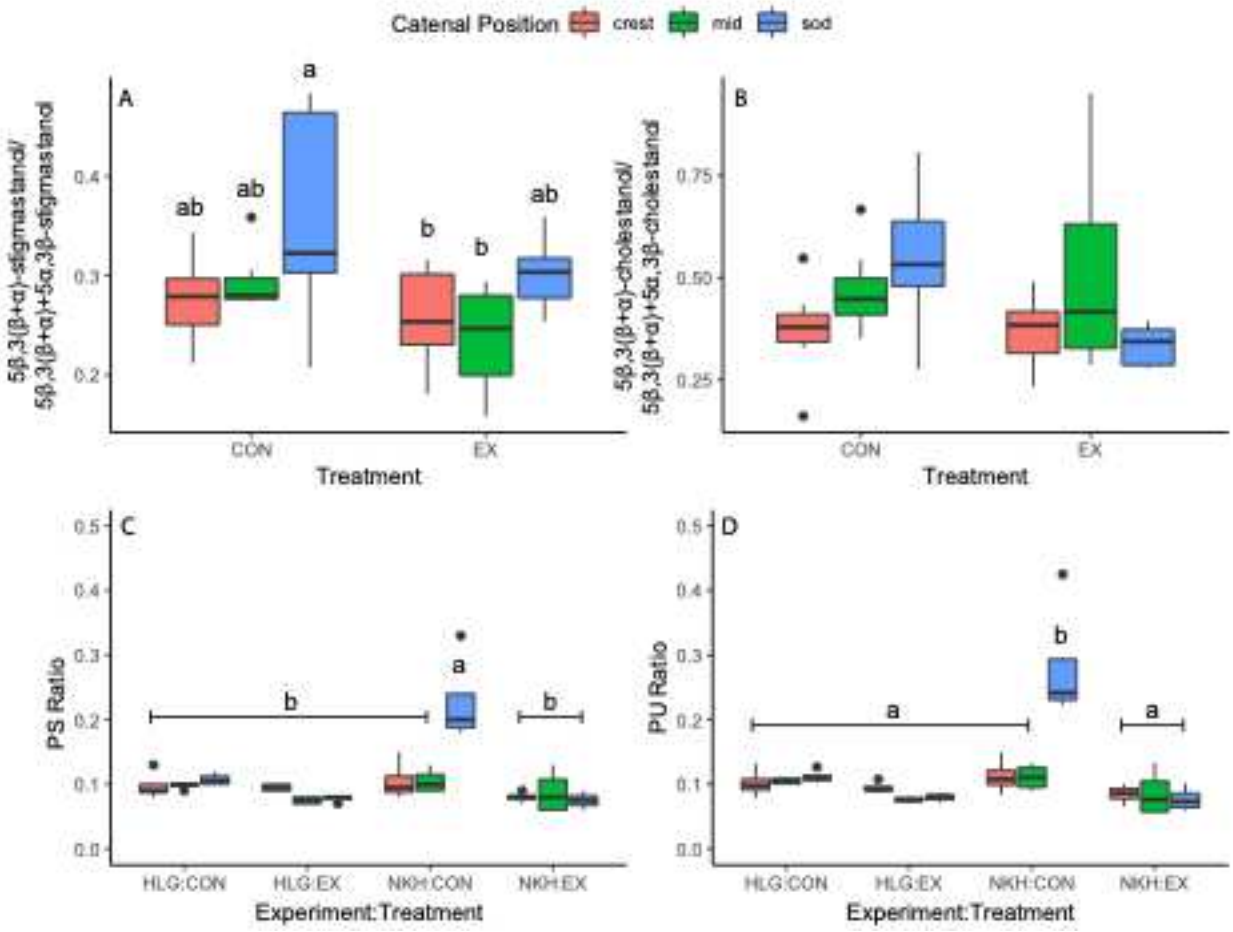


Figure 5. 5β3β to 5β3(α+β) epimerization stanol isomer ratios across soil depths. For all boxplots, "a" and "b" notation indicate significance. a) 5β3β-stigmastanol to 5β3(α+β)-stigmastanol. (Tukey's Significance Test, con-ex=0.00; 0-1 cm – 0-5 cm p>0.2). b) 5β3β-cholestanol to 5β3(α+β)-cholestanol (Tukey's Significance Test, 0-1 cm – 0-5 cm p=0.005)



860
861 **Figure 6.** Stanol isomer ratios with proxy potential across experiments, treatments, and catenal
862 position. For all boxplots, "a" and "b" notation indicate significance at lowest nested level. a)
863 5β3(α+β)/5α3β+5β3(α+β)-stigmastanol ratio. Tukey's Significance Test (Sod-all, $p<0.015$). b)
864 5β3(α+β)/5α3β+5β3(α+β)-cholestanol ratio. No significant difference between between any
865 variables ($p>0.1$). c) PS Ratio. Tukey's Significance Test (NKH-HLG, $p= 0.007$; NKH:CON-all,
866 $p<0.0001$; NKH:CON:Sod-all, $p<0.0001$). d) PU Ratio. Tukey's Significance Test (NKH-HLG, $p=$
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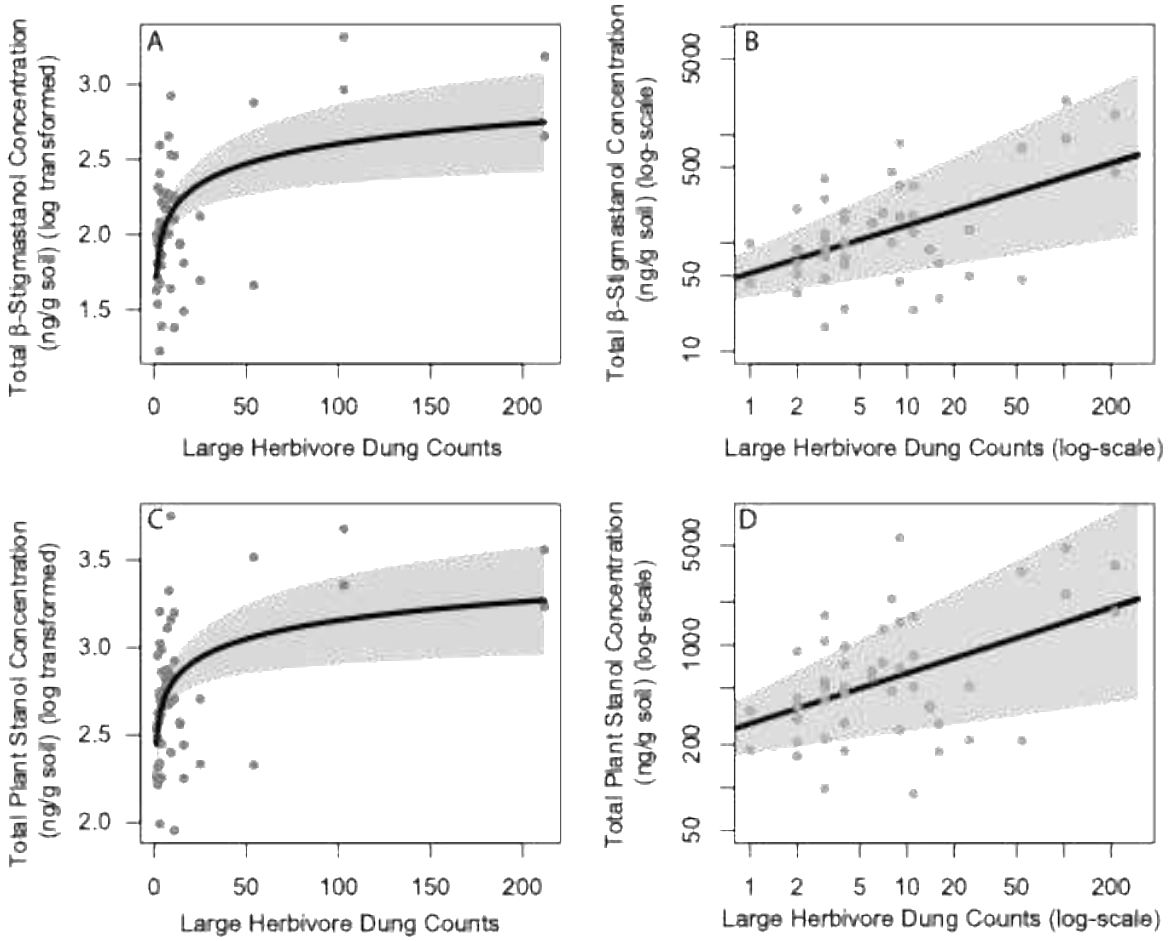
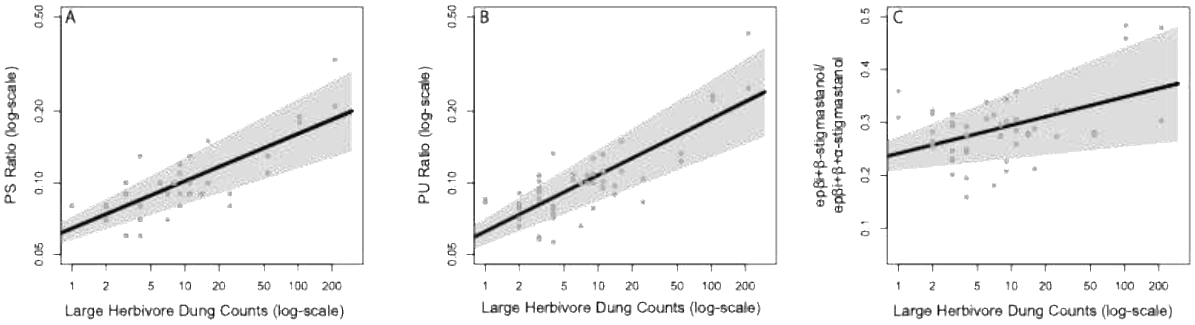


Figure 7. Response of stanol concetrations to dung counts. a-b and c-d show the same data, but are different graphical representation of the log-log relationships. a) Linear plot of log transfomed total 5β-stigmastanol concentrations ~ large herbivore dung counts. b) Log-Log plot of total 5β-stigmastanol concentrations ~ large herbivore dung counts: $y= 52.4x^{0.442}$. Adjusted $R^2 = 0.26$, p=0.0001. RMSE=0.40 c) Linear plot of log transfomed total plant stanol concentrations ~ large herbivore dung counts. d) Log-Log plot of total plant stanol concentrations ~ large herbivore dung counts: $y= 280.7x^{0.354}$. Adjusted $R^2 = 0.20$, p= 0.0009. RMSE=0.38.



879 **Figure 8.** Response of potential herbivore proxy stanol ratios to dung counts. a) Log-Log plot of
880 PS Ratio ~ large herbivore dung counts: $y=0.0645x^{0.198}$ Adjusted $R^2 = 0.59$, p= 1.274e-10. RMSE=
881 0.09. b) Log-Log plot of PU Ratio ~ large herbivore dung counts: $y= 0.0625x^{0.237}$. Adjusted R^2 =
882 0.62, p= 1.755e-11. RMSE= 0.10. c) Linear-Log plot of 5β/5β+α-Stigmastanol Ratio ~ large
883 herbivore dung counts: $y=x^{0.053} + 0.241$. Adjusted $R^2 = 0.19$, p= 0.001258. RMSE= 0.06.
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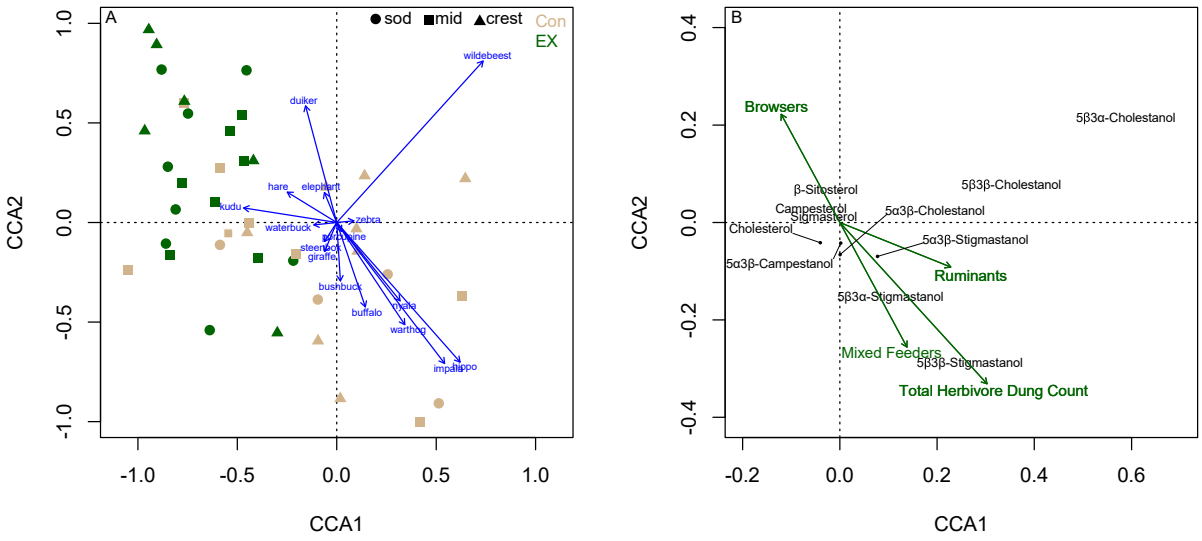

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886 **Figure 9.** Canonical Correspondence Analysis of fecal steroid relative abundances and species
887 relative dung counts. Note compound scores are smaller than sample scores, so we zoom in
888 and plot a smaller axes range for B to emphasize how the compounds sperate out on CCA1 and
889 CCA2. a) Sample scores and biplot arrows for species from the dung count dataset. Symbols
890 represent the catenal position the sample is from and colors represent if the sample was a
891 control (herbivores) or exclosure (no herbivores). b) Compound scores and 'envfit' correlation
892 of the total herbivore dung counts, proportion browser dung, proportion mixed feeder dung,
893 proportion ruminant dung for each sample.
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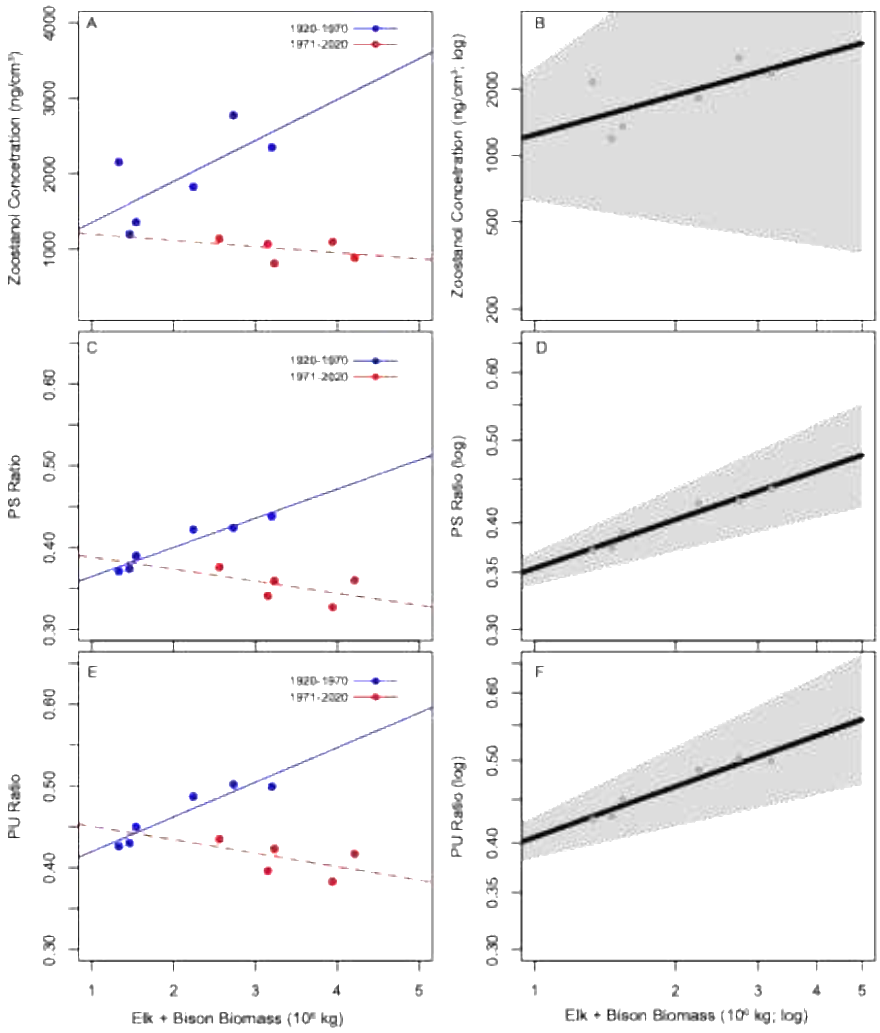

Figure 10. Application of PU and PS ratios to previously published sedimentary steroid record from Buffalo Ford Lake (Yellowstone National Park, USA) compared to historical Yellowstone herbivore biomass data. Steroid concentrations and biomass estimates are from Wendt et al. (2024). Blue series are samples with from 1920-1970). Red series are samples from 1971-2020. Panel A is reproduced from Wendt et al. (2024) Figure S3. a) Zoostanol (5β3(β+α)-stigmastanol+5β3(β+α)-cholestanol) concentrations ~ historical Yellowstone herbivore biomass: Blue r=0.69, p= 0.13, Red: r =-0.37, p= 0.54 b) Log-Log plot of Zoostanol (5β3(β+α)-stigmastanol+5β3(β+α)-cholestanol) concentrations ~ historical Yellowstone herbivore biomass from 1920-1970: y= $1242x^{0.595}$ Adjusted R^2 = 0.30, p= 0.15. RMSE= 0.10. c) Log-Log plot of PS Ratio ~ historical Yellowstone herbivore biomass: Blue r=0.96, p= 0.002, Red: r=-0.52, p=0.36 d) Log-Log plot of PS Ratio ~ historical Yellowstone herbivore biomass: y= $0.353x^{0.190}$ Adjusted R^2 = 0.94, p= 0.0009. RMSE= 0.006. c) Log-Log plot of PU Ratio ~ historical Yellowstone herbivore biomass: Blue r=0.94, p= 0.005, Red r=-0.52, p=0.37 f) Log-Log plot of PU Ratio ~ historical Yellowstone herbivore biomass: y= $0.406x^{0.198}$. Adjusted R^2 = 0.91, p= 0.002. RMSE= 0.008.

Figure 1.

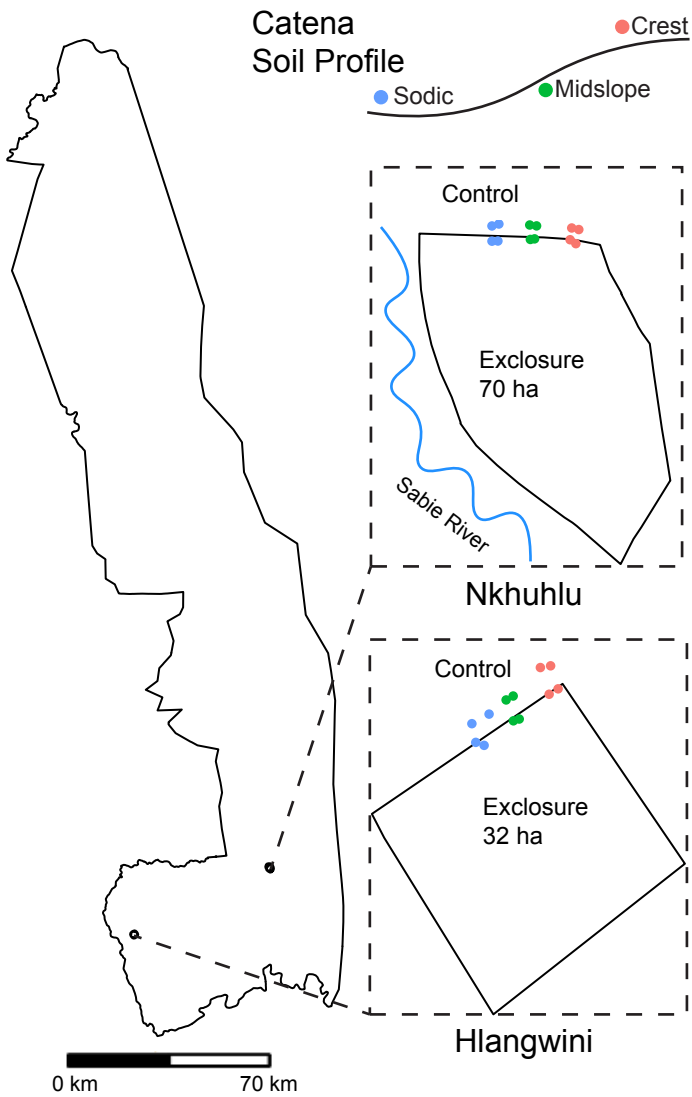
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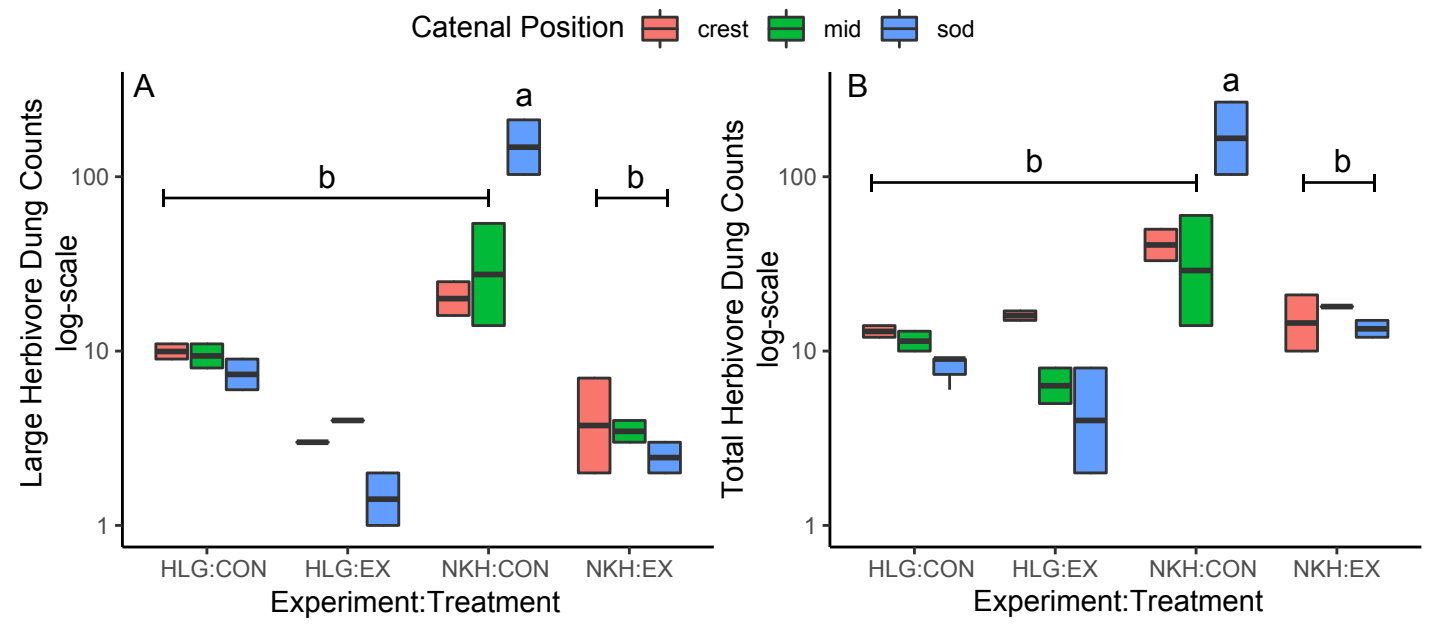
Figure 2.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Large Herbivore Dung Counts
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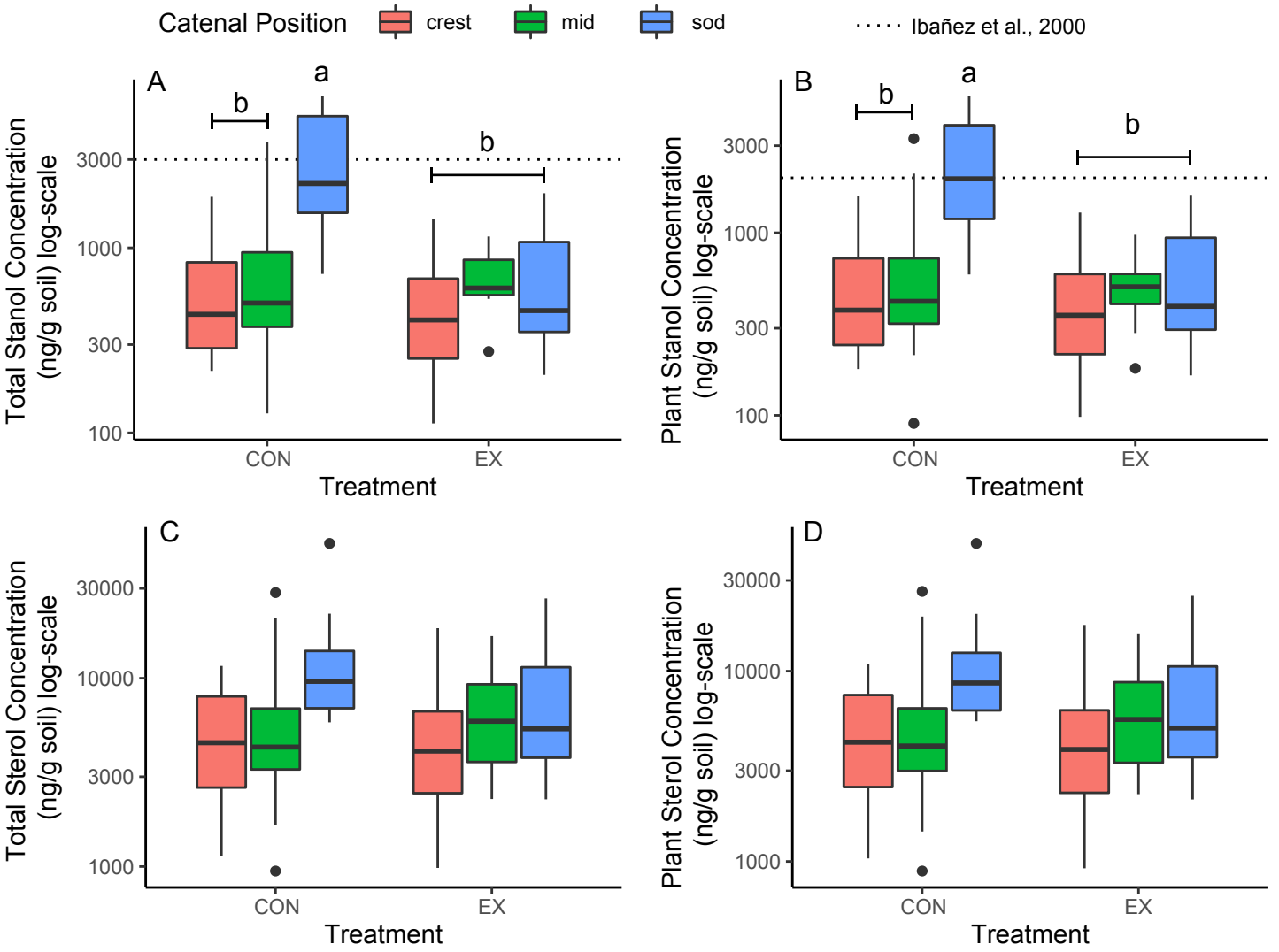
HLG:EX
NKH:CON
NKH:EX
Experiment:Treatment

Figure 3.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Plant Stanol Concentration (ng/g soil) log-scale
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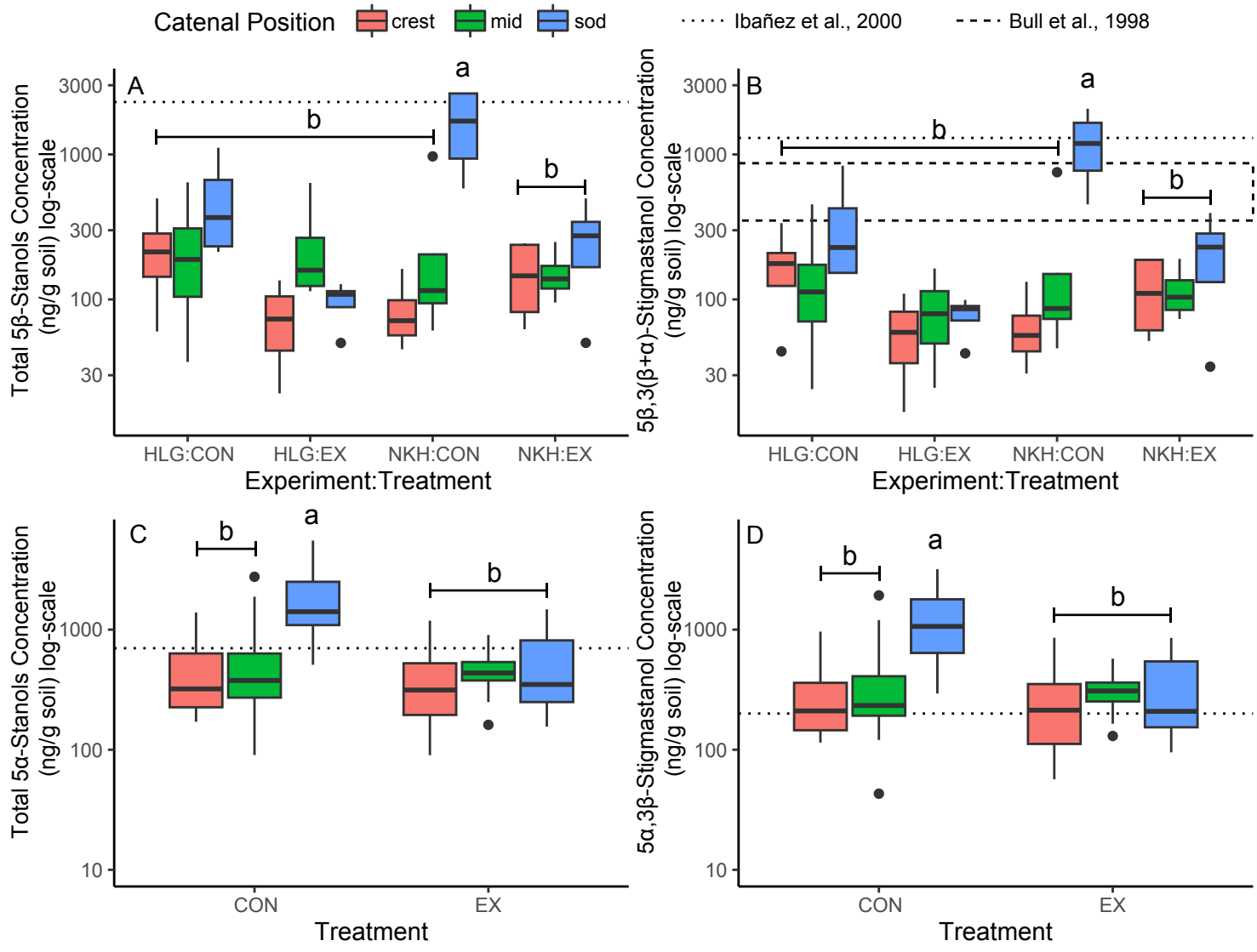
Figure 4.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Total 5β-Stanols Concentration (ng/g soil) log-scale
5β,3(β+α)-Stigmastanol Concentration (ng/g soil) log-scale
Total 5α-Stanols Concentration (ng/g soil) log-scale
5α,3β-Stigmastanol Concentration (ng/g soil) log-scale
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300
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HLG:CON
HLG:EX
NKH:CON
NKH:EX
Experiment:Treatment
CON
EX
Treatment

Figure 5.

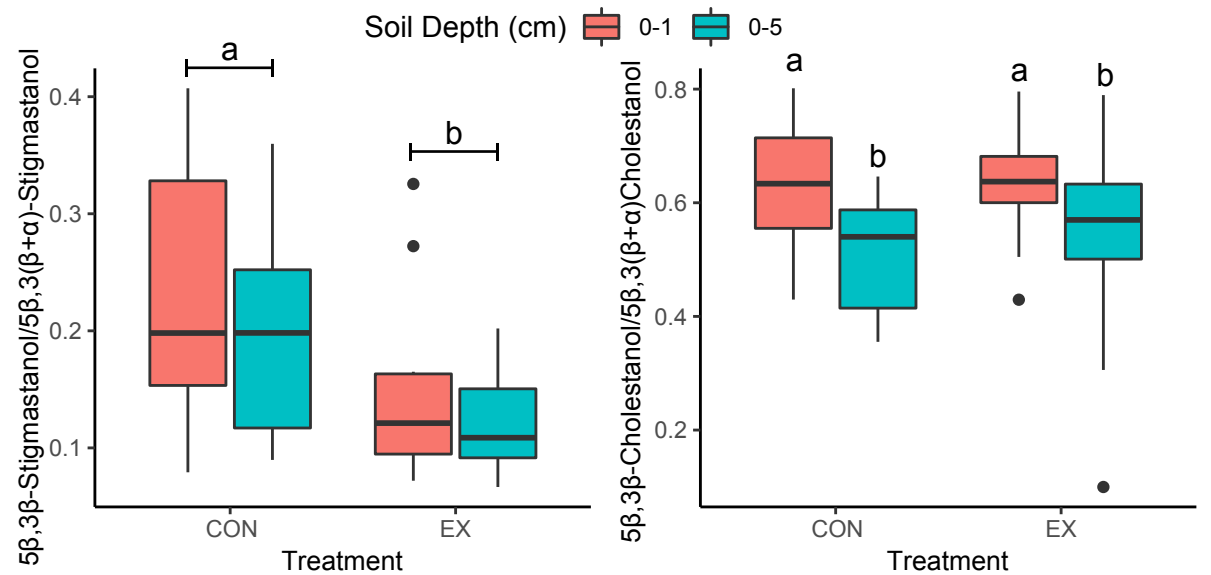

Soil Depth (cm)
0-1
0-5
a
b
0.4
0.3
0.2
0.1
5β,3β-Stigmastanol/5β,3(β+α)-Stigmastanol
CON
EX
Treatment
a
b
a
b
0.8
0.6
0.4
0.2
5β,3β-Cholestanol/5β,3(β+α)Cholestanol
CON
EX
Treatment

Figure 6.

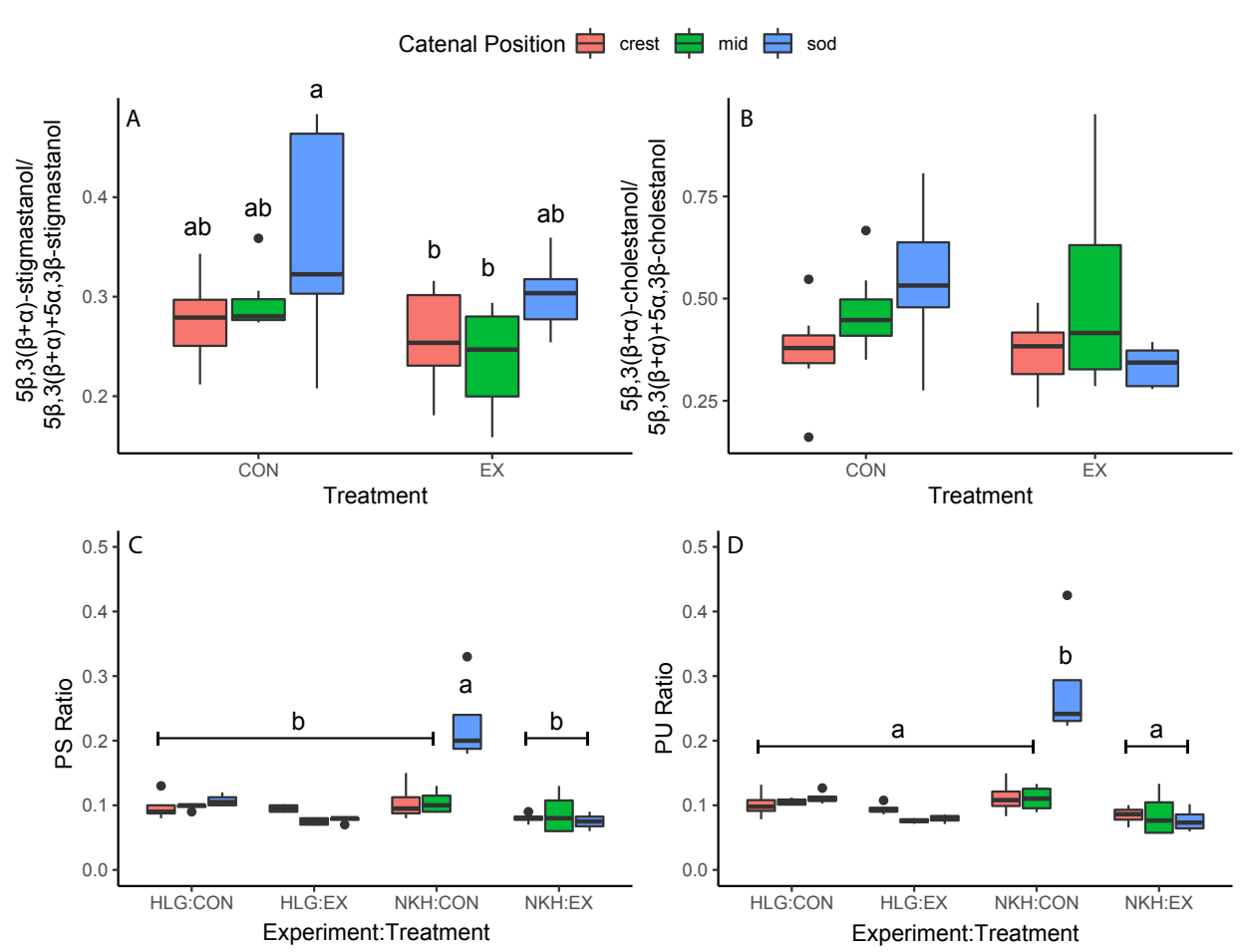

Catenal Position
crest
mid
sod
A
5β,3(β+α)-stigmastanol/
5β,3(β+α)+5α,3β-stigmastanol
0.4
0.3
0.2
a
ab
ab
b
b
ab
CON
EX
Treatment
B
5β,3(β+α)-cholestanol/
5β,3(β+α)+5α,3β-cholestanol
0.75
0.50
0.25
CON
EX
Treatment
C
PS Ratio
0.5
0.4
0.3
0.2
0.1
0.0
b
a
b
HLG:CON
HLG:EX
NKH:CON
NKH:EX
Experiment:Treatment
D
PU Ratio
0.5
0.4
0.3
0.2
0.1
0.0
a
b
a
HLG:CON
HLG:EX
NKH:CON
NKH:EX
Experiment:Treatment

Figure 7.

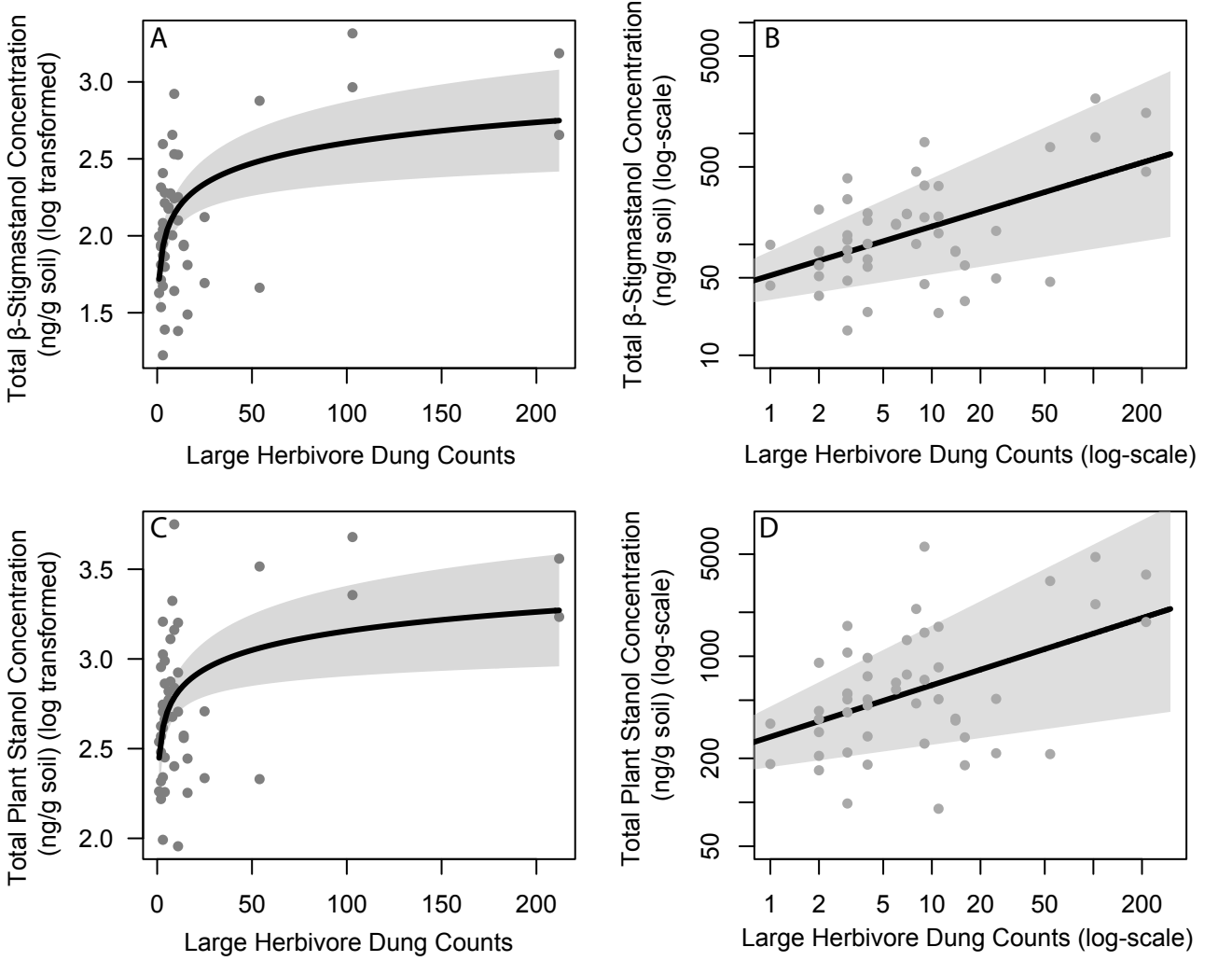
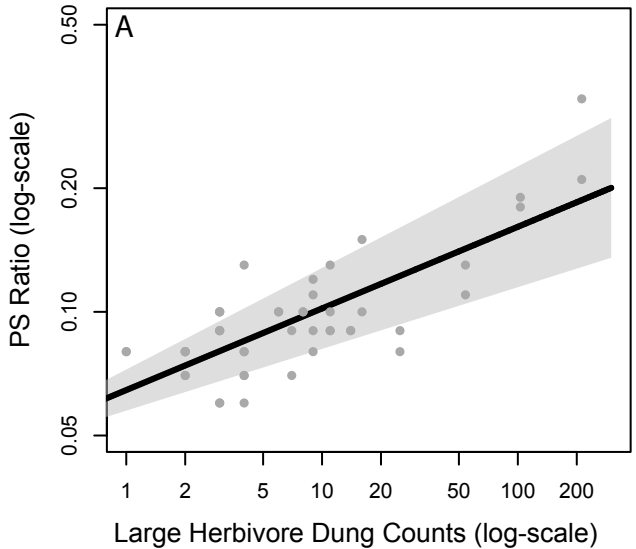
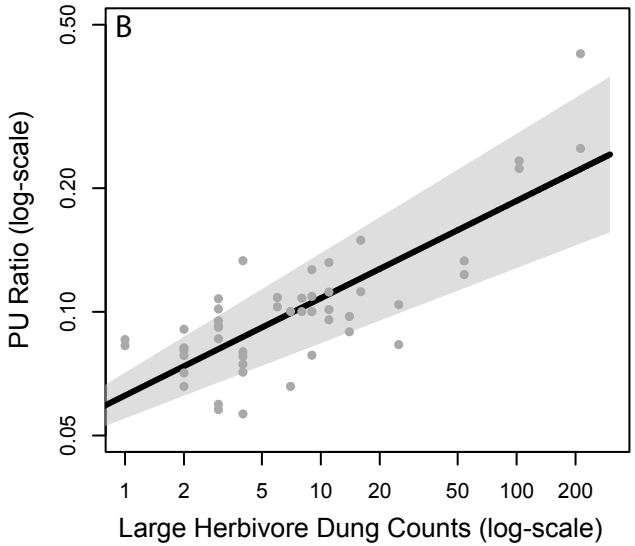

A
Total β-Stigmastanol Concentration (ng/g soil) (log transformed)
3.0
2.5
2.0
1.5
0
50
100
150
200
Large Herbivore Dung Counts
B
Total β-Stigmastanol Concentration (ng/g soil) (log-scale)
5000
500
50
10
1
2
5
10
20
50
200
Large Herbivore Dung Counts (log-scale)
C
Total Plant Stanol Concentration (ng/g soil) (log transformed)
3.5
3.0
2.5
2.0
0
50
100
150
200
Large Herbivore Dung Counts
D
Total Plant Stanol Concentration (ng/g soil) (log-scale)
5000
1000
200
50
1
2
5
10
20
50
200
Large Herbivore Dung Counts (log-scale)

Figure 8.


A
PS Ratio (log-scale)
0.05
0.10
0.20
0.50
1
2
5
10
20
50
100
200
Large Herbivore Dung Counts (log-scale)


B
PU Ratio (log-scale)
0.05
0.10
0.20
0.50
1
2
5
10
20
50
100
200
Large Herbivore Dung Counts (log-scale)

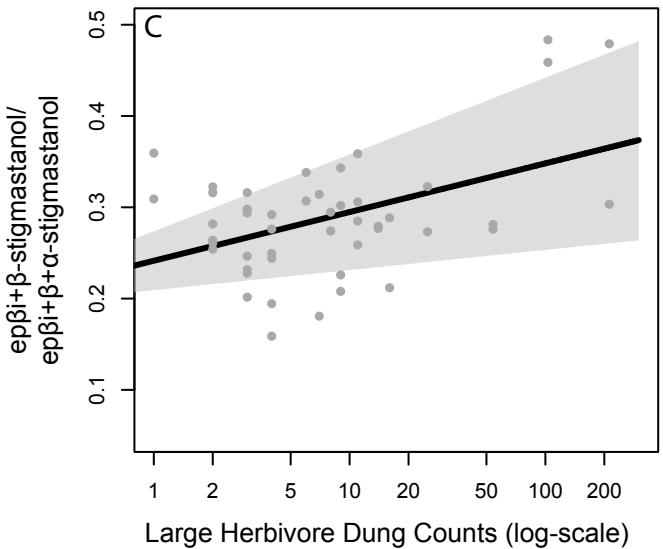
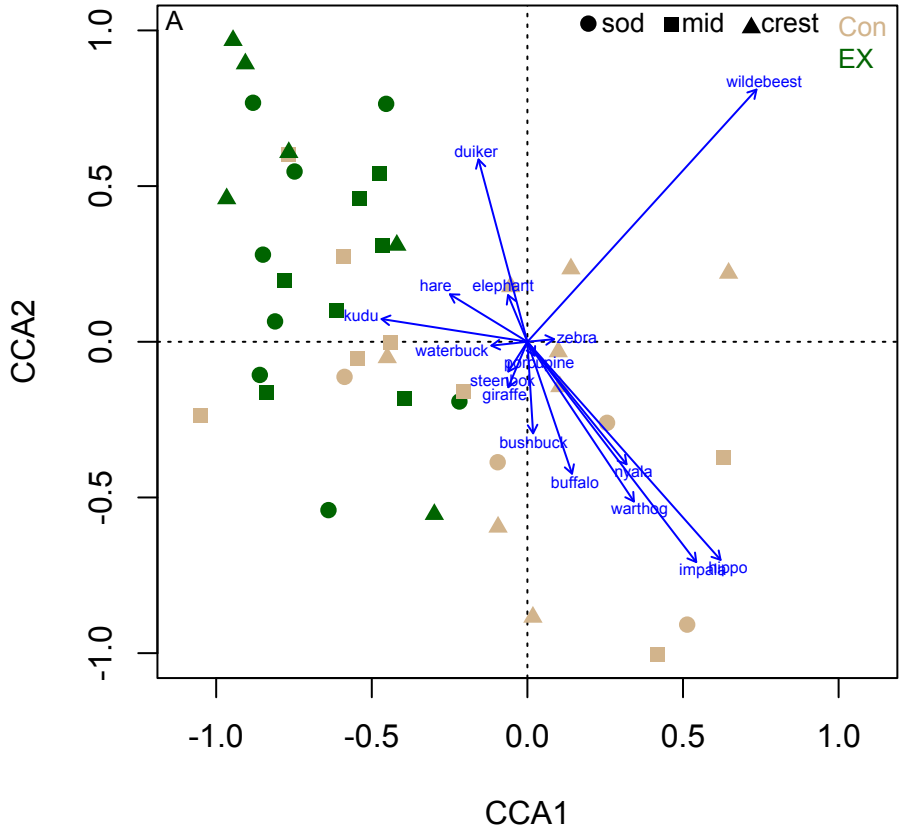

C
epβi+β-stigmastanol/
epβi+β+α-stigmastanol
0.1
0.2
0.3
0.4
0.5
1
2
5
10
20
50
100
200
Large Herbivore Dung Counts (log-scale)

Figure 9.


A
sod
mid
crest
Con
EX
wildebeest
duiker
hare
elephant
kudu
zebra
waterbuck
porcupine
steenbok
giraffe
bushbuck
buffalo
nyala
warthog
impala
hippo
CCA2
CCA1
1.0
0.5
0.0
-0.5
-1.0

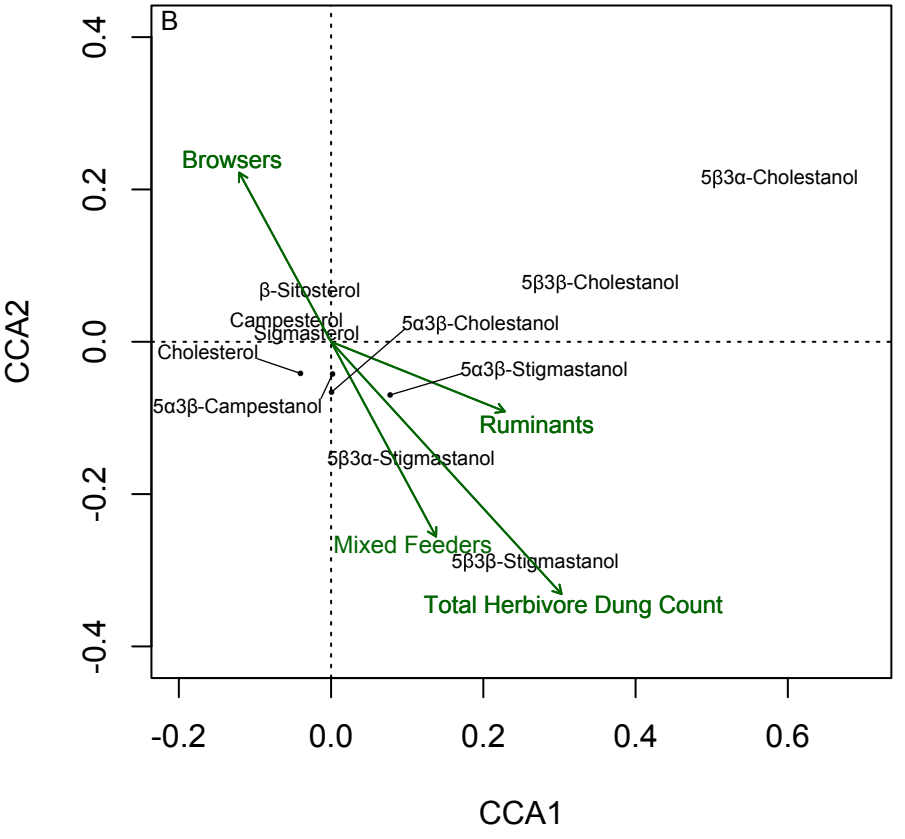
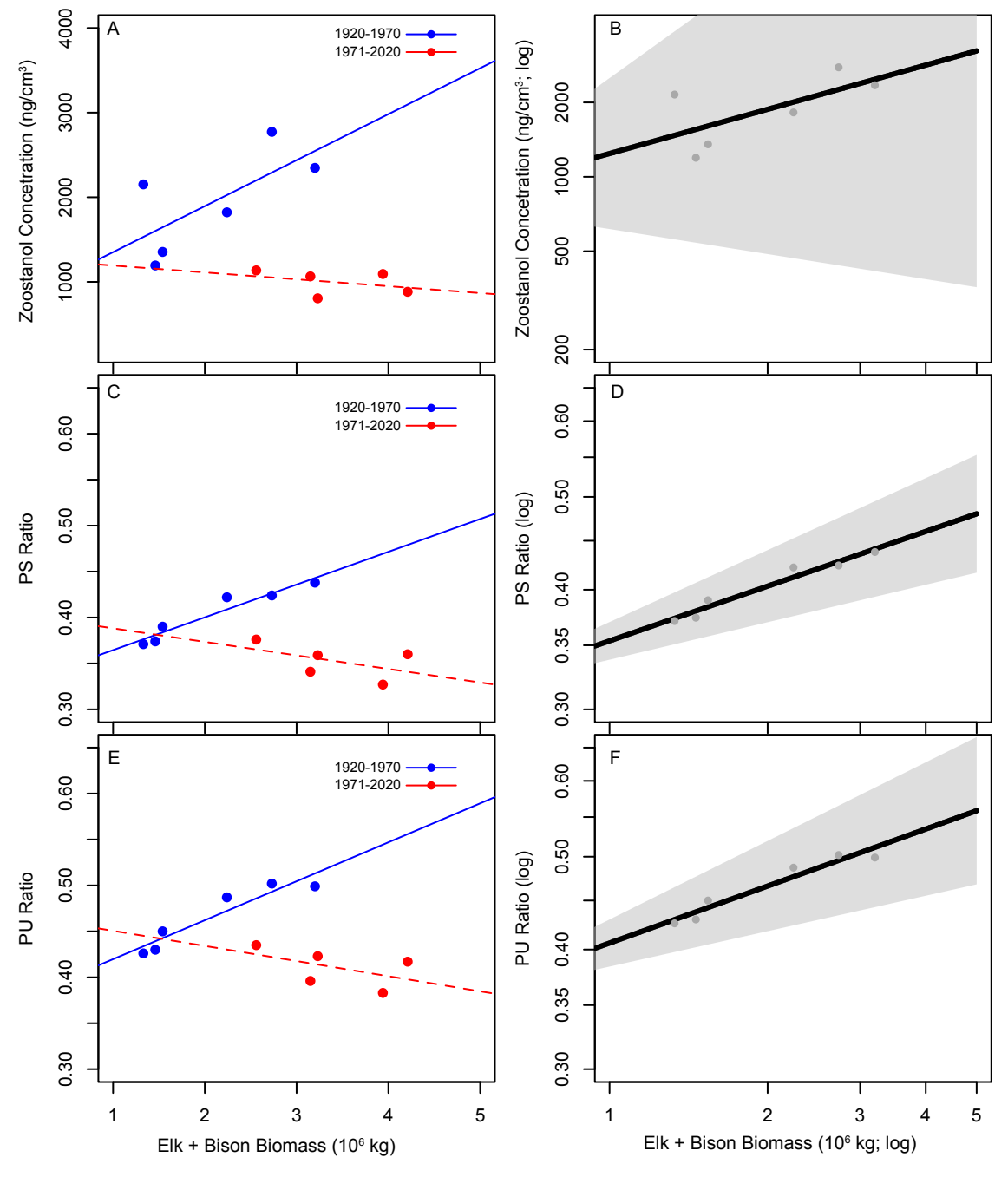

B
Browsers
5β3α-Cholestanol
β-Sitosterol
5β3β-Cholestanol
Campesterol
5α3β-Cholestanol
Stigmasterol
Cholesterol
5α3β-Stigmastanol
5α3β-Campestanol
Ruminants
5β3α-Stigmastanol
Mixed Feeders
5β3β-Stigmastanol
Total Herbivore Dung Count
CCA2
CCA1
0.4
0.2
0.0
-0.2
-0.4
0.6

Figure 10.


A
1920-1970
1971-2020
Zoostanol Concetration (ng/cm³)
1000
2000
3000
4000
B
Zoostanol Concetration (ng/cm³; log)
200
500
1000
2000
C
PS Ratio
0.30
0.40
0.50
0.60
D
PS Ratio (log)
0.30
0.35
0.40
0.50
0.60
E
PU Ratio
0.30
0.40
0.50
0.60
F
PU Ratio (log)
0.30
0.35
0.40
0.50
0.60
1
2
3
4
5
Elk + Bison Biomass (10⁶ kg)
Elk + Bison Biomass (10⁶ kg; log)