Chemical Parameters of Decomposing Dung in Tropical Forest as Indicators of Feeding Behaviour of Large Herbivores: A Step beyond Classical Stoichiometry

Ekta Chaudhary, Pascal Jouquet, Cornelia Rumpel, Raman Sukumar

Chemical parameters of decomposing dung in tropical forest as indicators of feeding behaviour of large herbivores: A step beyond classical stoichiometry

Chaudhary E[1,2], Jouquet P[2,3], Rumpel C[3], Sukumar R[1]

[1]Centre for Ecological Sciences, Indian institute of Science, Bengaluru, Karnataka, India. [2] Indo-French Cell for Water Science (IFCWS), Indian Institute of Science, 560 012 Bengaluru, Karnataka, India. [3]Institute of Ecology and Environmental Sciences (UMR iEES Paris), Sorbonne Universités, IRD, CNRS, INRA 4 place Jussieu 75252 Paris, France. [5]

Abstract

Feeding behavior of large herbivores determines the composition of their dung and together with environmental factors the intensity of decomposition processes leading to the recycling of nutrients in tropical forests. Large herbivore dung and its decomposition has so far been characterized by stoichiometric analyses of elements such as C and N. The objective of our study was to examine the suitability of biomarker analyses and analytical pyrolysis to infer large herbivore feeding behavior and the decomposition of their dung in different environments. Our conceptual approach included exposure of fresh dung of a grazing ruminant (gaur, *Bos gaurus*) and a non-ruminant mixed-feeder (the Asian elephant, *Elephas maximus)* in two tropical forest types (dry and moist) and analysis of dung biochemical composition in two seasons (dry and wet). To this end we characterized the dungs' lignin and carbohydrate (sugar) signatures and pyrolysis products before and after 28 days of exposure.

Our results showed that stoichiometric as well as biomarker analyses were able to differentiate gaur and elephant dung independent of season and forest type, while analytical pyrolysis products did not differ between dung types. The lignin signature of fresh dung additionally indicated the forage preference of animals in different forest types and seasons. During decomposition, C and N contents decreased and the chemical composition of both dung types converged. The lignin signature of dung at the end of the experiment showed higher lignin decomposition in moist forest and wet season than dry forest and dry season. We conclude that detailed biochemical analyses can provide deeper insights into the main controls of large herbivore dung and its decomposition in tropical forests than stoichiometric analysis. In particular lignin may be a suitable indicator to investigate large herbivore feeding behavior and the environmental conditions of their habitat.

Keywords: Large herbivore ecology, dung decomposition, carbon cycling, lignin, ecosystem services, tropical forest

Introduction

The importance of large mammalian herbivores to ecosystem functioning has been highlighted in a number of studies (Owen-Smith 1988; Frank and McNaughton 1992; Olff and Ritchie 1998; Bardgett and Wardle 2010; Yessoufou et al 2013). They influence several key ecosystem processes such as turnover of nutrients (Naiman 1988), and dispersal of seeds (Sekar and Sukumar 2015). They also control plant diversity and productivity (Augustine and McNaughton 1998: Horsley et al 2003; Naiman 1988), because they have the ability to selectively feed on nutrient-rich resources (Van der Wal et al. 2004; Hobbs 1996; Guernsey et al.2015). Large herbivors can adjust their feeding behaviour depending on ressource availability (Shader et al., 2012). Between 30 and 50% of their diet consists of woody biomass. However, the understanding of the dietary choices of large herbivors is incomplete, and its assessment usually involves extensive field work and direct observation (Seloana et al., 2018). In particular the importance of different types of herbivors and their contribution to nutrient recycling in tropical ecosystems is poorly known. We hypothesised that this is due to incomplete understanding of the ecology of dung decomposition.

Initial composition of dung is mainly determined by animal's gut physiology and food preferences (Codron, Lee-Thorp, et al. 2007; Sitters et al. 2014). During decomposition of dung its composition changes, and may reflect the initial material ingested as well as the digestive processes in the intestin of different animal species along with environmental parameters. However, up to now dung decomposition of free ranging wild animals was mainly studied by stoichiometric analysis (Sitters et al. 2014), which are poorly suited to describe the nature of dung, as carbon quality changes were observed during different types of composting of cattle dung even if its total C content remained similar (Ngo et. al. 2011, 2012). Therefore, we hypothesised that the analysis of the biogeochemical signature of dung of free ranging animals at different stages of its decomposition may be a better indicator of feeding habits (ruminants vs non-ruminants) and their contribution to nutrient cycling in contrasting environments.

After deposition on soil, dung is subjected to rapid decomposition in tropical environments. Apart from initial composition, climate is also suggested as a key decomposition driver. However, some recent studies related to litter decomposition identified limitations of the climatic conditions as a driver of decomposition, especially in studies comparing decomposition across sites (Araujo and Austin 2015; McCulley, Burke, and Lauenroth 2009; Austin 2002). It was argued instead that environment, which can be described as the kind of habitat where the material is decomposing, and not climate, is a suitable factor to study the impact on decomposition (Araujo and Austin 2015). Climatic conditions such as rainfall, humidity, and temperature strongly influence the vegetation type, canopy cover and soil moisture of an area. All these factors, in turn, can be interrelated and have an aggregated or multiplicative effect on decomposition and nutrient release *in-situ* (Austin 2002). Therefore, a study of dung-soil nutrient dynamics, *in-situ*, must take into consideration the local environment in terms of climatic factors and habitat type.

In tropical forests of India, large herbivores constitute a great proportion of mammalian biomass (Karanth and Sunquist 1992). Two of the largest herbivores, elephants and gaur, are present in densities of about 3 individuals per km^{-2} each in southern India's Mudumalai forest (Varman and Sukumar 1995). Considering the defecation rate of both of these large herbivores, it can be estimated that they produce over a hundred kilograms of daily organic matter in the form of dung per square kilometre. In this study, we therefore exposed fresh dung from two contrasting herbivors, a grazing ruminant (gaur, *Bos gaurus*) and a non-ruminant mixed-feeder (the Asian elephant, *Elephas maximus)*, in two different forest types (dry and most) and analysed its elemental and biogeochemical composition (lignin, non-cellulosic carbohydrates and analytical pyrolysis) during two seasons (dry and wet). The aims of this study were to test biogeochemical parameters as indicators of (1) the contrasting feeding behaviour of large mammalian herbivores and (2) the decomposition process of their dung in contrasting environments.

Specifically, we tested the following hypotheses:

- initial dung composition is determined by animal species, forest type and season
- the chemical nature of the decomposition products depend on dung type, age, forest type and season and is better suited to investigate environmental controls of decomposition than stoichiometric characterisation

Material and Methods

Study site

The study was carried out in Mudumalai National Park (11°30′ and 11°39′N latitude, 76°27′ and 76°43′E longitude), located in Tamil Nadu, India. The park spreads over an area of 321 km^2 , most of which is at an elevation of 900-1000m ASL (Sukumar et al. 2004; Sukumar et al. 1992). During June-September, a large part of the reserve receives rains from the south-west or summer monsoon. The north-east or winter monsoon is restricted to the eastern part of the reserve during October-November. A strong rainfall gradient exists from east (600mm annually) to west (1800 mm annually) (Figure 1). Along with the rainfall gradient, the tropical forest structure and type also changes from dry thorn forest in the eastern part to dry deciduous forest (*Anogeissus-Acacia-Erthroxylon-Ziziphus type)* in the middle and to moist deciduous forest (*Lagerstroemia-Tectona-Terminalia-Dalbergia* type) in the western part of the reserve (Sukumar et al. 1992,Suresh et al 2011). Soils in Mudumalai are primarily composed of Entisols, Alfisols, Inceptisols and Mollisols (George et al 1988). The soil pH is close to neutral (6.8 to 6.2) for the entire study area (Mani et al 2018). Soil carbon, nitrogen as well all other nutrients such as K, Ca, Mg, Mn, Fe, Co, and Ni are reported to be higher in the moist deciduous forest compared to dry thorn forest (Mani et al 2018). The two largest herbivorous mammals in Mudumalai (as in other tropical forests of peninsular India) are the Asian elephant (*Elephas maximus*) and the gaur (*Bos gaurus*). Elephant density is reported to be 2.95/km^{-2} in the area whereas gaur density reaches 4.60/km^{-2} (Varman and Sukumar 1995).

Experimental procedure

The field work was carried out in two contrasting forest types, namely, the dry thorn forest and the moist deciduous forest of Mudumalai during the wet (July-August 2015) and dry seasons (December 2015-February 2016). Fresh dung was collected and three replicates of each treatment (i.e. animal type, season, forest) were set up for 28 days. Three replicates were chosen as minimum requirement for statistical analyses due to limited time and budget available for chemical analyses. We tested the suitability of this approach with the power.anova.test function (Microsoft Excel). Using the C content as an example, this analyses showed that n = 3.14 are necessary for a power of the test to equal 0.8. Therefore, with three replicates per treatment and a balanced dataset, our experimental design may be appropriate. For each set up we used 800 cm^3 of dung. This volume was decided based on the average size of elephant boli dimensions. The first collection of dung was made from fresh dung i.e. at day 1 and the second collection was post exposure at day 28. A portion of dung sample (20g) was collected dried at 45°C in an oven and powdered to 2 mm for chemical analysis. Both gaur and elephant fresh dung were set up for soil microbial and macro fauna decomposition for 28 days. To maintain the heterogeneity of dung material, composite samples were made from the periphery and the centre of the dung pat/boli.

Chemical analyses

Total C and N contents were measured using the combustion method with a Leco CHN analyser (LECO Corp, St Joseph, MI). The amount of lignin was quantified using gas chromatography after hydrolysing the sample with alkaline cupric oxide in alkaline solution at high temperature (Hedges and Ertel, 1985). CuO oxidation products, the lignin monomers, were purified on C18 columns using solid phase extraction. After derivatisation, they were analysed using a HP gas chromatograph (HP GC 6890) equipped with a flame ionisation detector (FID) and an SGE BPX-5 column (50 m length, 0.25 mm inner diameter, 0.32 µm coating). We determined single-ring phenol compounds such as V (vanillyl), S (syringyl) and C (p-coumaryl) along with their acid, aldehyde and ketone side chains. For convenience, hereafter we will use LigV, LigS and LigC for V (vanillyl), S (syringyl) and C (p-coumaryl) respectively. The total amount of lignin was estimated as the sum of LigV, LigS and LigC compounds. Non-cellulosic sugars were determined using a protocol established by Rumpel and Dignac (2006) and modified by Eder et al. (2010). Briefly, monosaccharides were released after hydolysis with 4M Trifluoric acid and derivatised by transformation into acid alditols. For measuring total monosaccharide content, we used a HP gas chromatograph (HP GC 6890) equipped with flame ionisation detector (FID). Individual neutral sugar released from the dung sample was calculated as total ion currency of the internal standard myoinositol. The total non-cellulosic sugar was measured as a sum of 8 monosacharides namely, rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose.

Dung molecular composition was also analysed using analytical pyrolysis, which gives information about the relative contribution of polysaccharide-derived, lignin-derived, aliphatic-derived, N-containing and unspecified compounds. A coil probe pyrolyser (CDS Pyroprobe 5150) was used, coupled to a Hewlett Packard HP-5890 gas chromatograph coupled with a Hewlett Packard HP-5889 mass spectrometer (electron energy 70 eV). The protocol for this method was followed as mentioned in (Ngo et al. 2011). Peaks in the spectrometer were identified as different compounds depending on their mass spectra (Table S2). Peak area was integrated on the total ion current (TIC) trace with the help of GC ChemStation program (Agilent Technologies).

Statistical analyses

A Levene's test was initially conducted for verification of the homogeneity of variance (Levene, 1960). Linear model was used to determine the statistically significant predictors (animal species, forest type, season, day) affecting the chemical composition of dung (carbon, nitrogen, sugar and lignin contents). The global model considered for linear regression was: *element ~ animal + season + forest type + day + day: season+ day: forest type.* The interaction term for day and season as well as for day and forest were considered with the assumption that forest canopy and rainfall (season) from day1 to day 28 can affect dung moisture which, in turn, can impact dung biochemical properties. The significant predictors were ranked in descending order of their relative importance, on the basis of their t-value. Further, Principal Component Analyses (PCA) were carried out to differentiate gaur and elephant dung composition from day 1 to 28 using the C, N, sugar and lignin contents, pyrolysis and lignin signatures results. All statistical calculations were carried out using R (R Development Core Team, 2008) with ggplot2 and Ade4 packages. Differences among treatments were declared at the P *value* < 0.05 level of significance.

3. Results

3.1 Elemental composition, lignin content and sugar content of large herbivore dung during its decomposition

Elephant and gaur dung considerably differed in their chemical composition especially at day 1. The average C concentration of fresh elephant dung (mean ~408 mg/g) was higher than of gaur dung (mean ~389 mg/g). Conversely, N content was found to be considerably higher in GAUR (mean~16 mg/g) than in ELEPH (mean ~11 mg/g). These differences resulted in lower C:N ratios for GAUR than ELEPH. ELEPH also showed higher lignin concentration than in GAUR (39 mg/gC against 25mg/gC) while the opposite was true for sugar content. In table 1, we show mean values of the four biochemical markers measured in the two seasons for elephant and gaur dung for day1 i.e. fresh dung and for day 28 i.e. decomposed dung. Splitting the initial dung biochemical concentration season wise (see table 1) we found that the carbon and carbon polymer, lignin is higher in dry season of fresh dung whereas nitrogen is lower in dry season. Over the course of the 28 day experiments, we found that unlike carbon, nitrogen or sugar, lignin reduced drastically in elephant samples especially in dry season from ~46mg/g in fresh dung to ~30mg/g in decomposed dung, while remaining largely unaffected in other season and gaur samples.

3.2 Relative importance of environmental factors for determining dung composition

The relative importance of the factors determining dung composition is shown in table 1. Animal type was found to be the most important predictor influencing all the chemical variables. Other than the animal type, only the age of dung significantly influenced dung composition for more than one dependent variable, namely, the C and N contents. The lignin content was the most sensitive chemical variable since it was influenced by the forest type, animal species, season and dung age ($P < 0.05$ in all cases).

The two most important predictors of dung composition, namely, animal type and age of dung, were used for differentiating dung samples (Figures 2a, b). At day 1, ELEPH and GAUR were clearly differentiated in the PCA plane, mainly along the first axis that explained 44.6% of the total variability. Elephant dung was characterized by higher carbon and lignin contents (Fig. 2b). The specific chemical fingerprints of elephant and gaur dung were lost after 28 days. Figure 3a shows that ELEPH was also characterized by higher total C content than in GAUR ($P < 0.05$, Figure 3a) while GAUR was enriched in N compared to ELEPH ($P < 0.05$, Figure 3c. Irrespective of the animal type, the C content significantly decreased from day 1 to day 28 (Figure 3b), while no significant difference in N content was measured ($P > 0.05$, data not shown). Other than for animal type and day, the C and N contents were unaffected by the other factors such as the season and forest types ($P > 0.05$ in all cases). The lignin content was significantly influenced by the animal type as well as the season and the interactive effect of the forest type and day ($P < 0.05$ in all cases, Table 2). The lignin content was higher in ELEPH than in GAUR and it was also more important during the dry season as compared to wet season (Figure 4a,b). The lignin content decreased from day 1 to 28 in wet forest while it remained unaffected in dry forest (figure 4c). Unlike lignin, the sugar content varied only between animal type and it was found to be more concentrated in gaur than in elephant dung ($P < 0.05$) (Figure 5).

3.3 *Lignin quality*

The specific lignin signatures i.e. LigV, LigS, LigC and total lignin of GAUR and ELEPH at day 1 are decipted in Fig. 6.a and Table S1 (Supplemenary material). Treatments were mainly differentiated along the first and second axes, which explained approximately 78% and 21% of the total variability, respectively. In general, along the two principal axes, we found GAUR and ELEPH to have unique lignin signature except one treatment, i.e. wet season wet forest where both the dung sample show similar signature. Within gaur dung, the samples from different season and forests could not be differentiated in the PCA plane, ELEPH differed across forest types and seasons. Forest type led to differentiation of ELEPH along the first axis, with wet forest positively and dry forest negatively correlating with lignin parameters. On the other hand, the influence of the season was positively distributed on the second axis.

Just like day1, a second PCA was carried out from the same variables at day 28. The first two axes of the PCA explained 98% of the total variability (Fig. 6). LigV, LigS and LigC and the lignin contents were distributed along the first axis, while their distribution along the second axis was mainly influenced by the LigC content. GAUR samples were found to be all clumped together with no clear difference among treatments, while ELEPH were clearly differentiated from each other on the second axis of the PCA. ELEPH in dry forest for both seasons was associated with a higher coumaryl content, in comparison with dung from wet season.

3.3.4 Pyrolysis analyses

Fig 7 shows a PCA results of analytical pyrolysis for dung samples of ELEPH and GAUR at day 1 and 28. The first two axes of the PCA explained 77% and 74% of the total variability, for day 1 and 28 respectively. We found the day 1 samples of both the dung types exhibit a somewhat unique identity on PCA planes, however at day 28 the overlap in ELEPH and GAUR signatures increases greatly in comparison to day 1.

Discussion

Herbivore feeding ecology and initial dung composition

Both classical stoichiometric and biochemical analyses reveal a strong difference in fresh dung composition between gaur and elephant samples. As expected, total C and N varied between the two herbivore species. Cordon et al (2007) showed that the fresh dung nitrogen is negatively correlated with the amount of graze and positively correlated with the amount of browse, which implies that we would have expected higher nitrogen in elephant dung. However, in our results, GAUR samples were richer in N in comparison with ELEPH because the above conclusion are valid for only bovids and ruminants. In ELEPH, a non-ruminant, C from the browse especially in polymer forms such as lignin will remain undigested while in GAUR's ruminating gut C is well digested and N is mostly rejected. This result is also in line with the higher lignin content recorded for ELEPH, indicating that they contained undigested structural components, resulting in a higher C:N ratio (Sitters et al 2014). These results also confirm the general assumption that higher C:N ratio in diet is associated with higher body mass, ascribed to a fall in diet quality with an increase in body size (Edwards 1991; Codron et al. 2007; de Iongh et al. 2011, Owen- Smith, 1992). In general, our results are similar to those reported in African savannah studies, albeit with slightly higher (in case of savannahs) absolute values (Sitters et al. 2014), perhaps due to differences in plant species being consumed in African savannahs and Indian forests.

Further, the analysis of lignin and sugar in dung evidenced a significant difference in ELEPH and GAUR. But unlike the analysis of the C and N contents, the proportion of lignin was influenced by the environmental variables (i.e., the seasons and forest types), most likely reflecting the forage preference of animals, which may change between habitat type and seasons. Indeed, elephant primarily graze on perennial grasses such as *Themeda* and bamboo species and browse on *Acacia* spp., *Kydia* and *Ziziphus* trees in Mudumalai (Baskaran 2010). Acid detergent lignin (ADL) estimation for such browse species from Africa show that they contain about 7 to 21% of ADL. Conversely, the diet of gaur is dominated by *Heteropogon sp.*, *Bothriochloa* sp and *Themeda*, which are much less lignified with ~5% ADL (Lowry et al 2002; Codron, Lee-Thorp, et al. 2007). Unlike gaur, elephant foraging also varies between seasons and habitats. For instance, in Mudumalai forest, elephants have been reported to feed on lignin rich species such as Acacia (data from various African *Acacia* spp. suggest ~ 10% ADL, Lowry et al., 2002) in dry habitat and during the dry season while preferring to feed on grass species like *Bothriochloa* sp. (5.3% ADL) during the wet season (Lowry et al 2002). The ADL values reported here are from fresh plant leaves but the values we measured in dung were concentrated per gram of dung sample, as other digestible components of carbon were digested by elephant. Therefore, smaller differences in lignin content among plants such as Acacia spp. and *Heteropogan* and *Bothriochloa* were likely to be exaggerated in dung samples. Conversely, lignin results of GAUR showed that its quality (LigV, LigS and LigC) and quantity (LigV+LigS+ LigC) actually remained constant across seasons and forests, most likely because gaurs are selective grass feeders (Ahrestani 2009).

Relative importance of the dung initial composition and environmental factors on decomposition

Litter-based studies have considered loss of mass and C, N, P changes as decomposition indicators showing that at a global or regional scale, environment is considered to be the dominant controlling factor of decomposition rates (Meentemeyer 1978; Parton, Stewart, and Cole 1988; Wall et al. 2008; Bradford et al. 2016). However, at local or smaller scales, litter quality takes over as the predominant factor (Swift et al., 1979). Despite distinct differences in forest type in our study area (e.g. 600-900 vs. 1300-1800 mm of rainfall between the dry thorn and moist deciduous forests), the environment did not stand out as an important variable differentiating the C and N content of initial dung. Conversely, the dissimilarity in initial dung composition between elephant and gaur (Edwards 1991) was the dominating factor influencing the C and N contents after 28 days of field exposure. This result is in accordance with Sitters et al. (2014) who showed that the initial stoichiometric composition of mixed feeders such as elephant and grazers such as African buffalo (*Syncerus caffer*), hartebeest (*Alcelaphus lichtensteinii*) and reedbuck (*Redunca redunca*) dung determines the rate of nutrient release during decomposition. Apart from initial dung composition, our study also shows that the age of dung was an important predictor of its stoichiometric composition at the end of field exposure. However, we would also like to stress on the limitations of the stoichiometric approach for characterizing dung (de-) composition in tropical ecosystems since the carbon and nitrogen content in dung was not explained by the forest type and season. These two parameters had an influence on the lignin concentration in fresh dung samples and its decomposition. Lignin, a group of complex aromatic polymers is resistant to enzymatic degradation. Higher the lignin concentration, lower will be the access of soil microbes to labile carbon (Pauly et al 2008). In fact, lignin is also known to impede the degradation of several other compounds that are locked in lignin linkages in plant cell wall (Gallo et al 2006). Thus, the concentration of lignin may control the rate of decomposition of organic matter, further affecting the nutrient cycling, especially the carbon turnover (Potter and Klooster 1997).

Changes in dung composition during decomposition

Over the time span of the experiments, carbon was lost readily from the dung, also shown by several other dung decomposition studies (Aarons et al. 2009; Williams and Haynes 1995; Yoshitake, Soutome, and Koizumi 2014). However, total carbon analysis does not inform us about the mechanism of carbon reduction – whether respired into the atmosphere, fixed in the soil as a recalcitrant, dissolved and leached into the soil, or broken into a simpler compound and incorporated in the soil (Menéndez, Webb, and Orwin 2016). We adopted a novel approach to understand dung decomposition by further looking at total sugars (sum of non-cellulosic sugars) and total lignin (sum of single- ring phenol compounds such as V (vanillyl), S (syringyl) and C (p-coumaryl)). Over the course of the experiment, the total sugar remained constant across treatments, but lignin was lost in wet forest treatments while remaining unaffected in the dry forest. This lignin change in wet forest can be attributed to optimal moisture conditions. Litter decomposition studies propose soil moisture to be the primary driver of lignin decomposition providing suitable conditions for lignin-degrading microorganisms (Otto and Simpson 2006; Osono 2007).

Even though we emphasised the importance of substrate quality in determining decomposition rates in our study, we also show that this distinctiveness of the substrate composition may be lost within a span of few weeks in tropical dung systems (fig 1 and 7). We argue that considering initial substrate composition as a decomposition predictor may be a time-dependent variable, especially when considering dung-soil nutrient dynamics.

Conclusions

This study reveals that the classical stoichiometric analysis might not be sufficient in understanding the factors determining large herbivore feeding behavior and dung decomposition in tropical forest. While non-cellulosic sugars and analytical pyrolysis of dung did not differentiate animal type or environment, we found that lignin parameters can vary even within species and dung decomposition in different habitats. We show that lignin decomposition is slower in dry forest and dry season as compared to moist forest and wet season but such different behaviour was not seen in stoichiometric parameters such as carbon or nitrogen. Therefore, we suggest that the analyses of lignin biomarkers of large herbivore dung may provide detailed information about their feeding behaviour and environmental factors characterising their habitat.

Acknowledgements:

We thank Mani and Madan for their assistance in the field and Valerie Pouteau for her help with chemical analysis. Prof. H. N. Nagaraja and Ankur Shringi provided statistical advice for this study. We thank the Tamil Nadu Forest Department for research permissions to work at Mudumalai, and Wildlife Crime Control Bureau, Ministry of Environment and Forests, New Delhi, for permitting dung samples to be taken outside India for the analysis. This study was funded by the Ministry of Environment and Forests, Government of India, the Institute of Research for Development IRD, France and the National Center for Scientific Research CNRS, France. RS was a JC Bose National Fellow during the tenure of this study.

References

Aarons, S.R., O'Connor, C.R., Hosseini, H.M., Gourley C.J.P. 2009. Dung Pads Increase Pasture Production, Soil Nutrients and Microbial Biomass Carbon in Grazed Dairy Systems. Nutrient Cycl. Agr. 84, 81–92.

Ahrestani, F. S. 2009. Asian Eden: Large Herbivore Ecology in India.

Anderson, J.M., Coe, M.J. 1974. Decomposition of Elephant Dung in an Arid, Tropical Environment. Oecologia 14, 111–125.

Araujo, P.I., Austin, A.T. 2015. A Shady Business: Pine Afforestation Alters the Primary Controls on Litter Decomposition along a Precipitation Gradient in Patagonia, Argentina. J. Ecol. 103, 1408–20.

Augustine, D.J., McNaughton, S.J. 1998. Ungulate Effects on the Functional Species Composition of Plant Communities: Herbivore Selectivity and Plant Tolerance. J. Wildlife Manag. 62, 1165.

Augustine, D.J., McNaughton, S.J., Frank, D.A. 2003. Feedbacks between Soil Nutrients and Large Herbivores in a Managed Savanna Ecosystem. Ecol. Appl. 13, 1325–1337.

Austin, A.T. 2002. Differential Effects of Precipitation on Production and Decomposition along a Rainfall Gradient in Hawaii. Ecology 83, 328.

Bardgett, R.D., Wardle, D.A. 2003. Herbivore-Mediated Linkages between Aboveground and Belowground Communities. Ecology, 84, 2258–2268.

Bertone, M.A., Green, J.T., Washburn, S.P., Poore, M.H., Watson, D.W. 2006. The Contribution of Tunneling Dung Beetles to Pasture Soil Nutrition. Forage Graz. Land, 4, 1-12.

Bradford, M.A., Berg, B., Maynard, D.S., Wieder, W.R., Wood, S.A. 2016. Understanding the Dominant Controls on Litter Decomposition. J. Ecol. 104, 229–38.

Codron, D., Lee-Thorp, J.A., Sponheimer, M., Codron, J. 2007. Nutritional Content of Savanna Plant Foods: Implications for Browser/Grazer Models of Ungulate Diversification. Europ. J. Wildlife Res., 53, 100–111.

Codron, D., Lee-Thorp, J.A. Sponheimer, M. Codron, J., De Ruiter, D., Brink, J.S. 2007. Significance of Diet Type and Diet Quality for Ecological Diversity of African Ungulates. J. Animal Ecol. 76, 526–537.

Coe, M. 1977. The Role of Termites in the Removal of Elephant Dung in the Tsavo (East) National Park Kenya. African J. Ecol., 15, 49–55.

Eder, E., Spielvogel, S., Kölbl, A., Albert, G., Kögel-Knabner, I. 2010. Analysis of hydrolysable neutral sugars in mineral soils: improvement of alditol acetylation for gas chromatographic separation and measurement. Org. Geochem., 41, 580-585.

Edwards, P.B. 1991. Seasonal Variation in the Dung of African Grazing Mammals, and Its Consequences for Coprophagous Insects. Funct. Ecol. 5, 617.

Fincher, G.T., Monson, W.G., Burton, G.W. 1981. Effects of Cattle Feces Rapidly Buried by Dung Beetles on Yield and Quality of Coastal Bermudagrass. Agron. J., 73, 775-779.

Frank, D.A., McNaughton, S.J. 1992. The Ecology of Plants, Large Mammalian Herbivores, and Drought in Yellowstone National Park. Ecology, 73, 2043–58.

Freymann, B.P., Buitenwerf, R., DeSouza, O., Olff, H. 2008. The Importance of Termites (Isoptera) for the Recycling of Herbivore Dung in Tropical Ecosystems: A Review. Europ. J. Entomol. 105, 165.

Gallo M.E. Sinsabaugh R.L., Cabaniss S.E. 2006. The role of ultraviolet radiation in litter decomposition in arid ecosystems. Appl. Soil Ecol. 34, 82–91.

Guernsey, N.C., Lohse, K.A., Bowyer, R.T., 2015. Rates of decomposition and nutrient release of herbivore inputs are driven by habitat microsite characteristics. Ecol. Res., 30, .951- 961.

Hättenschwiler, S., Bracht Jørgensen, S. 2010. Carbon Quality rather than Stoichiometry Controls Litter Decomposition in a Tropical Rain Forest: Decomposition in a Tropical Rain Forest. Journal Ecol., 98, 754–63.

Horsley, S.B., Stout, S.L. DeCalesta, D.S. 2003. White-tailed deer impact on the vegetation dynamics of a northern hardwood forest. Ecol. Appl. 13, 98–118.

de Iongh, H. H., de Jong, C. B., van Goethem, J., Klop, E., Brunsting, A.M.H., Loth, P.E., a Prins, H.H.T. 2011. Resource Partitioning among African Savanna Herbivores in North Cameroon: The Importance of Diet Composition, Food Quality and Body Mass. J. Trop. Ecol. 27, 503–13.

Ullas, K.K., Sunquist, M.E. 1992. Population Structure, Density and Biomass of Large Herbivores in the Tropical Forests of Nagarahole, India. J. Trop. Ecol. 8, 21–35.

Kazuhira, Y., Hdeaki, K., Takuro, K., Toshiharu, A. 1991. Nitrogen Mineralization and Microbial Populations in Cow Dung, Dung Balls and Underlying Soil Affected by Paracoprid Dung Beetles. Soil Biol. Biochem. 23, 649–653.

Lowry, J.B., Kennedy, P.M Conlan, L.L. 2002. Lignin in the cell fraction of tropical forages. J. Sci. Food Agri., 82, 370–74.

McCulley, R.L., Burke, I. C. Lauenroth, W.K. 2009. Conservation of Nitrogen Increases with Precipitation across a Major Grassland Gradient in the Central Great Plains of North America. Oecologia 159, 571–81.

Meentemeyer, V. 1978. Macroclimate and Lignin Control of Litter Decomposition Rates. Ecology 59, 465–472.

Menéndez, R., Webb, P., Orwin K.H. 2016. Complementarity of Dung Beetle Species with Different Functional Behaviours Influence Dung–soil Carbon Cycling. Soil Biol. Biochem. 92, 142–48.

Naiman, R.J. 1988. Animal Influences on Ecosystem Dynamics. BioSci. 38, 750–52.

Ngo, P.T., Rumpel, C., Dignac, M.-F., Billou, D., Duc, T.T., Jouquet, P. 2011. Transformation of Buffalo Manure by Composting or Vermicomposting to Rehabilitate Degraded Tropical Soils. Ecol. Engin. 37, 269–76.

Nichols, E., Spector S., Louzada J., Larsen, T. Amezquita, S., Favila M.E. 2008. Ecological Functions and Ecosystem Services Provided by Scarabaeinae Dung Beetles. Biol. Conservation 141, 1461–74.

Osono, T. 2007. Ecology of Ligninolytic Fungi Associated with Leaf Litter Decomposition. Ecol. Res. 22, 955–74.

Otto, A., Simpson, M.J. 2006. Evaluation of CuO Oxidation Parameters for Determining the Source and Stage of Lignin Degradation in Soil. Biogeochem., 80, 121–42.

Owen-Smith, R. N. 1992. Megaherbivores: the influence of very large body size on ecology. Cambridge university press.

Parton, W.J., Stewart, J.W.B. Cole, C. V. 1988. Dynamics of C, N, P and S in Grassland Soils: A Model. Biogeochem., 5, 109–131.

Paul, E.A.,. 2007. Soil Microbiology, Ecology, and Biochemistry. 3rd ed. Amsterdam , Boston: Academic Press.

Pauly M., Keegstra K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. Plant J. 54, 559–568.

Potter C.S., Klooster S.A. 1997. Global model estimates of carbon and nitrogen storage in litter and soil pools: Response to changes in vegetation quality and biomass allocation. Tellus B Chem. Phys. Meteorol., 49, 1–17.

Rumpel, C., Dignac, M.F. 2006. Gas chromatographic analysis of monosaccharides in a forest soil profile: Analysis by gas chromatography after trifluoroacetic acid hydrolysis and reduction–acetylation. Soil Biol. Biochem., 38, 1478-1481.

Sekar, N., Sukumar, R. 2015. The Asian Elephant Is amongst the Top Three Frugivores of Two Tree Species with Easily Edible Fruit. J. Trop. Ecol. 31, 385–94.

Seloana, M.Q., Potgieter, J.J., Kruger, M.J. 2018. Feeding patterns of elephants at the Atherstone Collaborative Nature Reserve. African J. Ecol., 56, 445-454.

Sitters, J., Maechler, M.-J., Edwards, P.J., Suter, W., Venterink, H.O. 2014. Interactions between C : N : P Stoichiometry and Soil Macrofauna Control Dung Decomposition of Savanna Herbivores. Funct. Ecol., 28, 776–86.

Sukumar, R. 2003. *The Living Elephants: Evolutionary Ecology, Behavior, and Conservation*. New York: Oxford University Press.

Sukumar, R., Dattaraja, H.S. Suresh, H.S., Radhakrishnan, J., Vasudeva, R. Nirmala, S. 1992. Long-Term Monitoring of Vegetation in a Tropical. Current Sci. 62, 608-616

Sukumar, R., Suresh, H.S., Dattaraja, H.S., John, R., Joshi, N.V. 2004. *Mudumalai Forest Dynamics Plot, India*. Tropical forest diversity and dynamism: Findings from a large-scale plot network. Chicago, IL: University of Chicago Press.

Swift, M.J., Heal O.W., Anderson, J.M. 1979. Decomposition in Terrestrial Ecosystems. Vol. 5. Univ of California Press.

Van Der Wal, R., Bardgett, R.D., Harrison, K.A., Stien, A., 2004. Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. Ecography, 27, 242-252.

Varman, K.S., Sukumar R. 1995. The Line Transect Method for Estimating Densities of Large Mammals in a Tropical Deciduous Forest: An Evaluation of Models and Field Experiments. J. Biosci. 20, 273–87.

Wall, D.H., Bradford, M.A., St. John, M.G., Trofymow, J.A., Behan-Pelletier, V., Bignell, D.E., Dangerfield, J.M. 2008. Global Decomposition Experiment Shows Soil Animal Impacts on Decomposition Are Climate-Dependent. Global Chan. Biol., 14, 2661–2677.

Wolters, V., 2000. Invertebrate control of soil organic matter stability. Biol. Fert. Soils, 31, 1-19.

Williams, P.H., Haynes, R.J. 1995. Effect of Sheep, Deer and Cattle Dung on Herbage Production and Soil Nutrient Content. Grass Forage Sci. 50, 263–271.

Yessoufou, K., Davies, T.J., Maurin, O., Kuzmina, M., Schaefer, H., Bank, M., Savolainen, V., 2013. Large herbivores favour species diversity but have mixed impacts on phylogenetic community structure in an African savanna ecosystem. J. Ecol., 101, 614- 625.

Yoshitake, S., Soutome, H., Koizumi H. 2014. Deposition and Decomposition of Cattle Dung and Its Impact on Soil Properties and Plant Growth in a Cool-Temperate Pasture. Ecol. Res. 29, 673–84.

Table 1. Dung C, N, total lignin and total sugar concentrations (mean and SE) for elephant and gaur dung across two seasons (wet and dry) and day 1 and day 28. Differences between dung C, N, total lignin and sugar wee determined with one-way Anova followed by HSD post hoc test. Different letters in superscript signify statistically significant mean values ($p<0.05$).

Animal	**Season**	**Day**	**Carbon**	**Nitrogen**	**Total Lignin**	**Total sugar**
				mg g^{-1}	***mg g^{-1} C***	***mg g^{-1}***
Elephant	**Dry**	**1**	415.83^{a}	11.30^{bc}	45.93^{a}	0.23^{a}
Elephant	**Dry**	**28**	375.67^{abc}	9.47^{c}	29.64^{b}	0.39^{a}
Elephant	**Wet**	**1**	401.50^{ab}	10.78^{c}	32.13^{b}	0.34^{a}
Elephant	**Wet**	**28**	340.67^{cd}	11.46^{bc}	27.21^{bc}	0.38^{a}
Gaur	**Dry**	**1**	401.83^{ab}	16.49^{a}	28.32^{b}	0.29^{a}
Gaur	**Dry**	**28**	364.17^{bc}	13.81^{abc}	27.25^{bc}	0.19^{a}
Gaur	**Wet**	**1**	377.67^{abc}	16.12^{abc}	22.80^{cd}	0.28^{a}
Gaur	**Wet**	**28**	305.17^{d}	$12.75a^{abc}$	19.94^{d}	0.18^{a}

Table 2. Ranking predictors (animal, season, forest, day and the two interaction terms) in their order of importance based on t values explaining the C, N, lignin and sugar contents in dung (in %). Only significant predictors are shown in the table, ranked in descending order of t values.

Carbon	**Nitrogen**	**Lignin**	**Sugar**
Day $t = 5.12$, $P < 0.001$	Animal species $t = 6.49$, $P < 0.001$	Forest type $t = 4.59$, $P < 0.001$	Animal species $t = 2.651$, $P = 0.01$
Animal species $t = 3.18$, $P = 0.001$		Animal species $t = 3.65$, $P < 0.001$	
		Day change in wet forest $t = 3.625$, $P = 0.001$	
		Season $t = 3.082$, $P = 0.003$	

List of figures

Fig.1. Location of Mudumalai National Park in southern India. The shades of blue represent the three forest types found across the park. Stars represent the two forest type where the study was carried out. Sampling was done during the wet and dry seasons (in green and orange boxes, respectively).

Fig 2. PCA differentiating gaur (Gau, in blue) and elephant (Ele, in red) dung based on their carbon (C), nitrogen (N), lignin and sugar contents at day 1 (fig.a) and 28 (fig.b) and their correlation circle diagrams for day1 (c) and day 28 (d).

Fig.3. Boxplots showing differences in (a) total carbon (mg g^{-1}) in elephant (E) and gaur (G) dung samples, (b) total carbon (mg g^{-1}) in dung at day 1 and 28, and (c) total nitrogen (mg g^{-1}) in elephant and gaur dung samples. Values are presented for only statistically different predictors. All values are reported at statistical significance of P value < 0.05 ('*' at P < 0.05 and '***' at P < 0.001). n = 24 in all cases.

Fig. 4 Boxplots showing differences in (a) total lignin (mgg^{-1}C) in elephant and gaur dung samples (n=24), (b) total lignin (mgg^{-1}C) in dung for the dry and wet seasons (n=24). (c) total lignin (unit) in the wet and dry forests at 1 or 28 days (n = 12) Values are presented for only statistically different predictors. All values are reported at statistical significance of P value < 0.05 ('*' at P < 0.05, '**' at P < 0.01 and '***' at P < 0.001).

Fig.5. Boxplots showing differences in sugar contents (mgg^{-1}) in elephant and gaur dung samples All values are reported at statistical significance of P value < 0.05 ('*' at P < 0.05). n =24

Fig.6. PCA showing the lignin signatures of elephant (e, in red) and gaur (e, in blue) dung samples at day 1 (a) and 28 (b) for dry and moist(moi) forest in dry and wet season . The arrows within the ordination plane represent the specific lignin signature V (vanillyl), S (syringyl) and C (p-coumaryl) and total lignin contents (V+S+C) included in the analysis

Fig.7. PCA differentiating gaur (Gau, in blue) and elephant (Ele, in red) dung based on pyrolysis results that included, polysaccharides, lignin, nitrogen, and unknown parent compounds, at day 1 (a) and 28 (b) and their correlation circle diagrams for day1 (c) and day 28 (d).

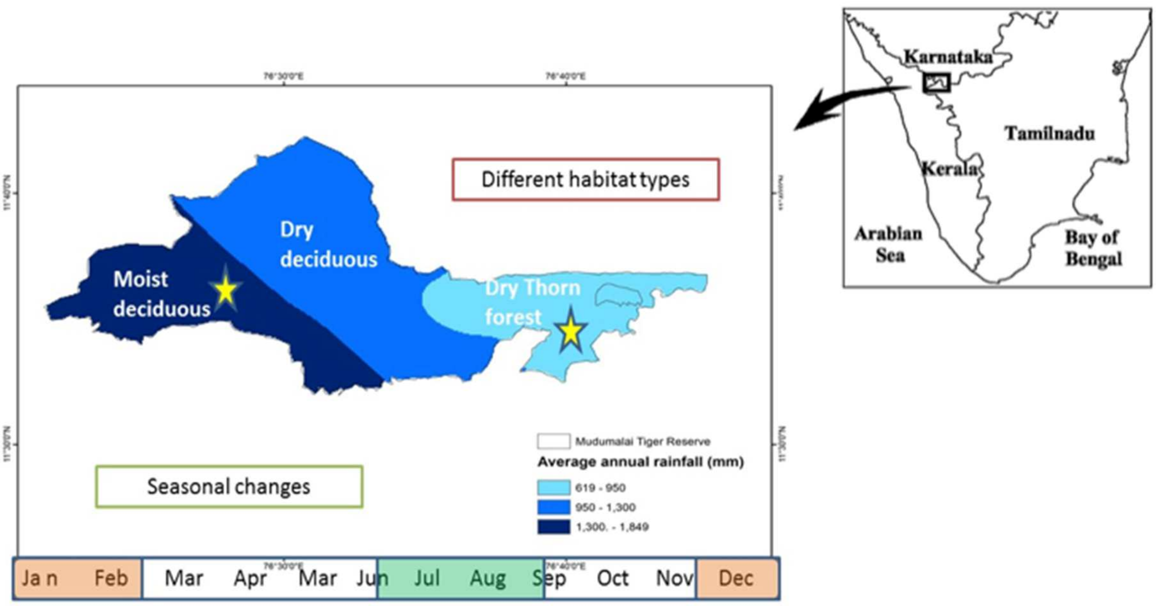


Fig.1. Location of Mudumalai National Park in southern India. The shades of blue represent the three forest types found across the park. Stars represent the two forest type where the study was carried out. Sampling was done during the wet and dry seasons (in green and orange boxes, respectively).

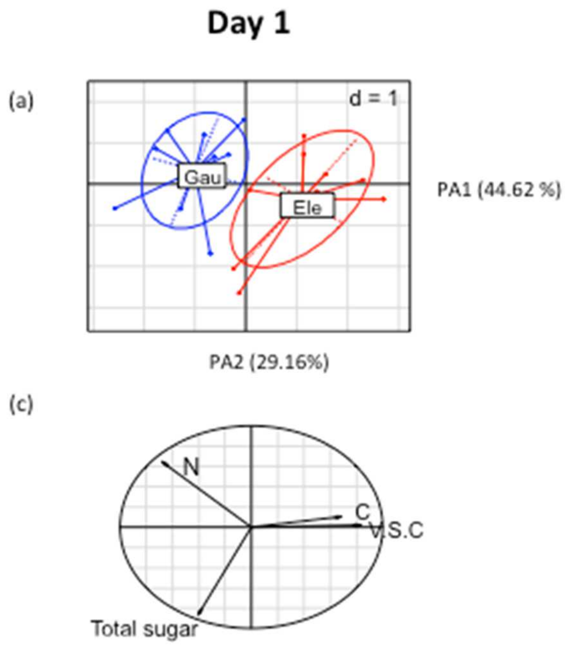


Fig 2. PCA differentiating gaur (Gau, in blue) and elephant (Ele, in red) dung based on their carbon (C), nitrogen (N), lignin and sugar contents at day 1 (fig.a) and 28 (fig.b). PA1 and PA2 are the first two principal axis that explain the variation (mentioned as percentage) Fig c and d are correlation circle diagrams for day1 and day 28 respectively.

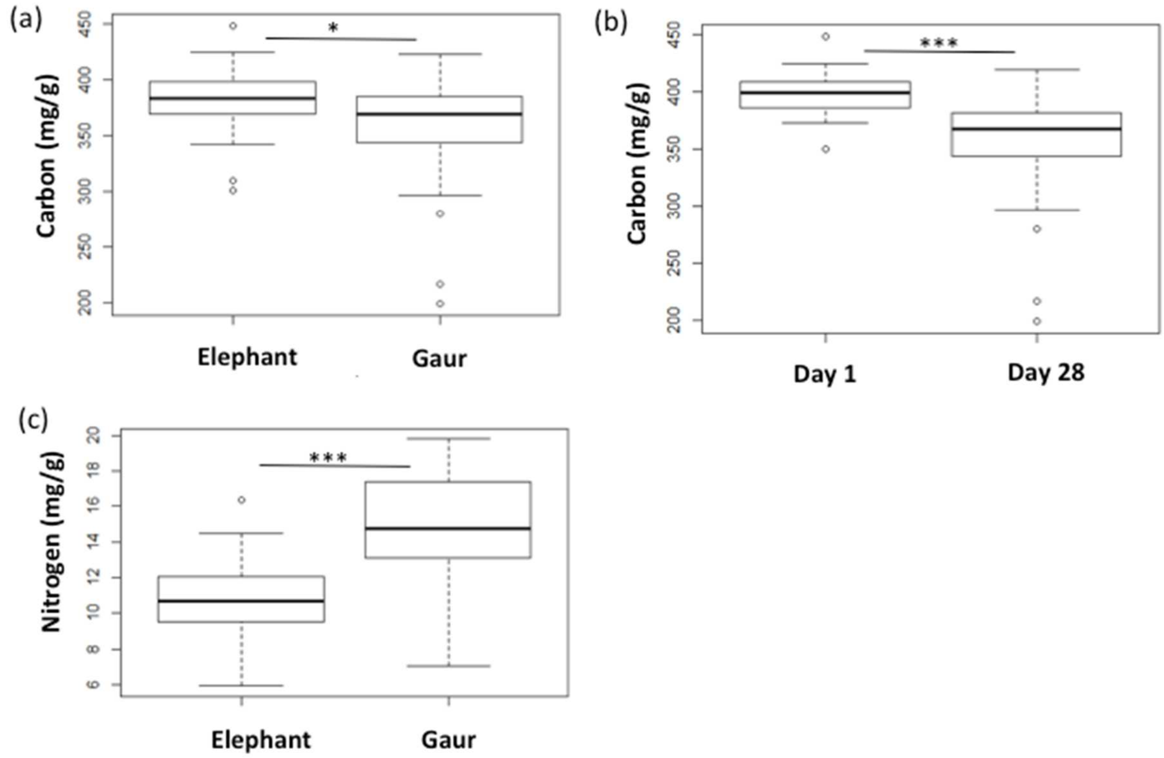


Fig.3. Boxplots showing differences in (a) total carbon (mg g^{-1}) in elephant (E) and gaur (G) dung samples, (b) total carbon (mg g^{-1}) in dung at day 1 and 28, and (c) total nitrogen (mg g^{-1}) in elephant and gaur dung samples. Values are presented for only statistically different predictors. All values are reported at statistical significance of P value < 0.05 ('*' at $P < 0.05$ and '***' at $P < 0.001$). n = 24 in all cases.

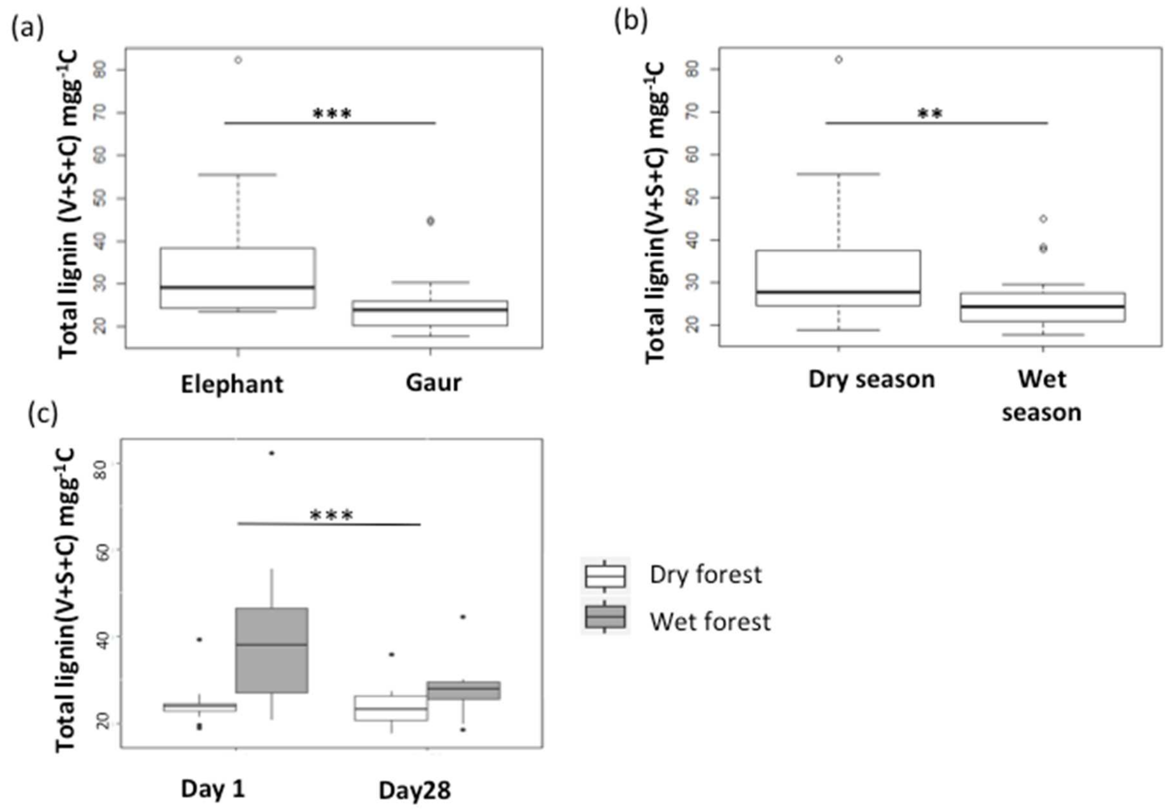


Fig. 4 Boxplots showing differences in (a) total lignin (mgg^{-1}C) in elephant and gaur dung samples (n=24), (b) total lignin (mgg^{-1}C) in dung for the dry and wet seasons (n=24). (c) total lignin (unit) in the wet and dry forests at 1 or 28 days (n = 12) Values are presented for only statistically different predictors. All values are reported at statistical significance of *P* value < 0.05 ('*' at *P* < 0.05, '**' at *P* < 0.01 and '***' at *P* < 0.001).

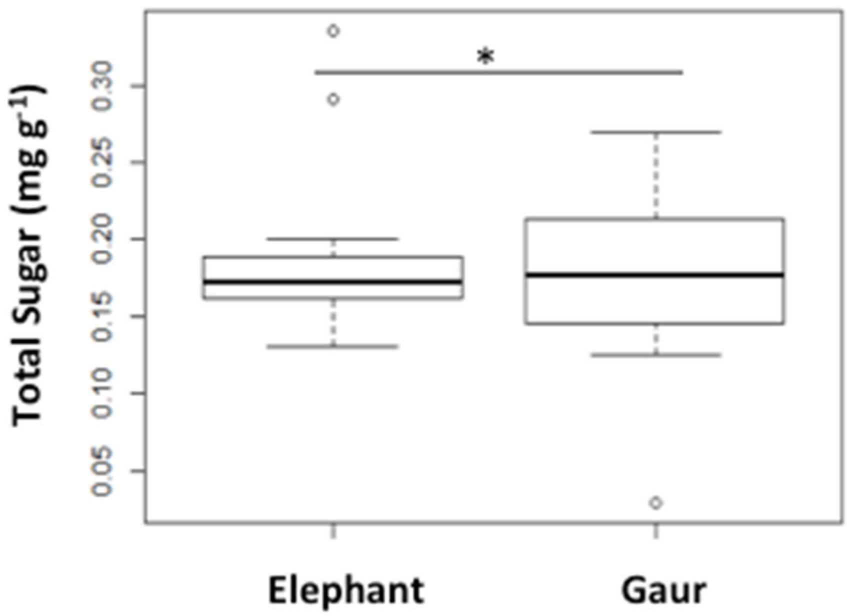


Fig.5. Boxplots showing differences in sugar contents (mgg^{-1}) in elephant and gaur dung samples All values are reported at statistical significance of P value < 0.05 (‘*’ at P < 0.05). n =24

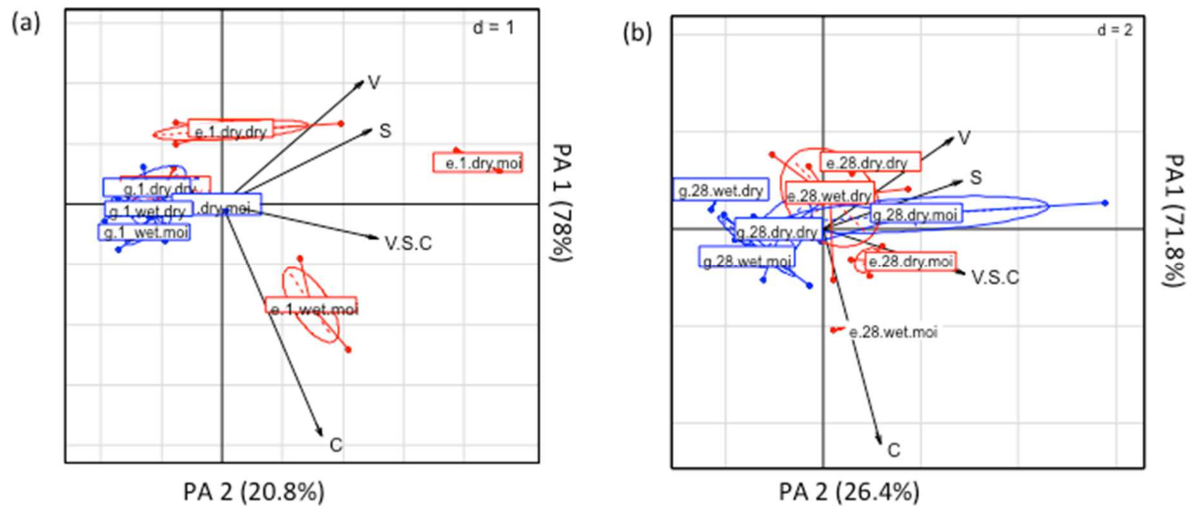


Fig.6. PCA showing the lignin signatures of elephant (e, in red) and gaur (e, in blue) dung samples at day 1 (a) and 28 (b) for dry and moist(moi) forest in dry and wet season . PA1 and PA2 are the first two principal axis that explain the variation (mentioned as percentage). The arrows within the ordination plane represent the specific lignin signature V (vanillyl), S (syringyl) and C (p-coumaryl) and total lignin contents (V+S+C) included in the analysis.

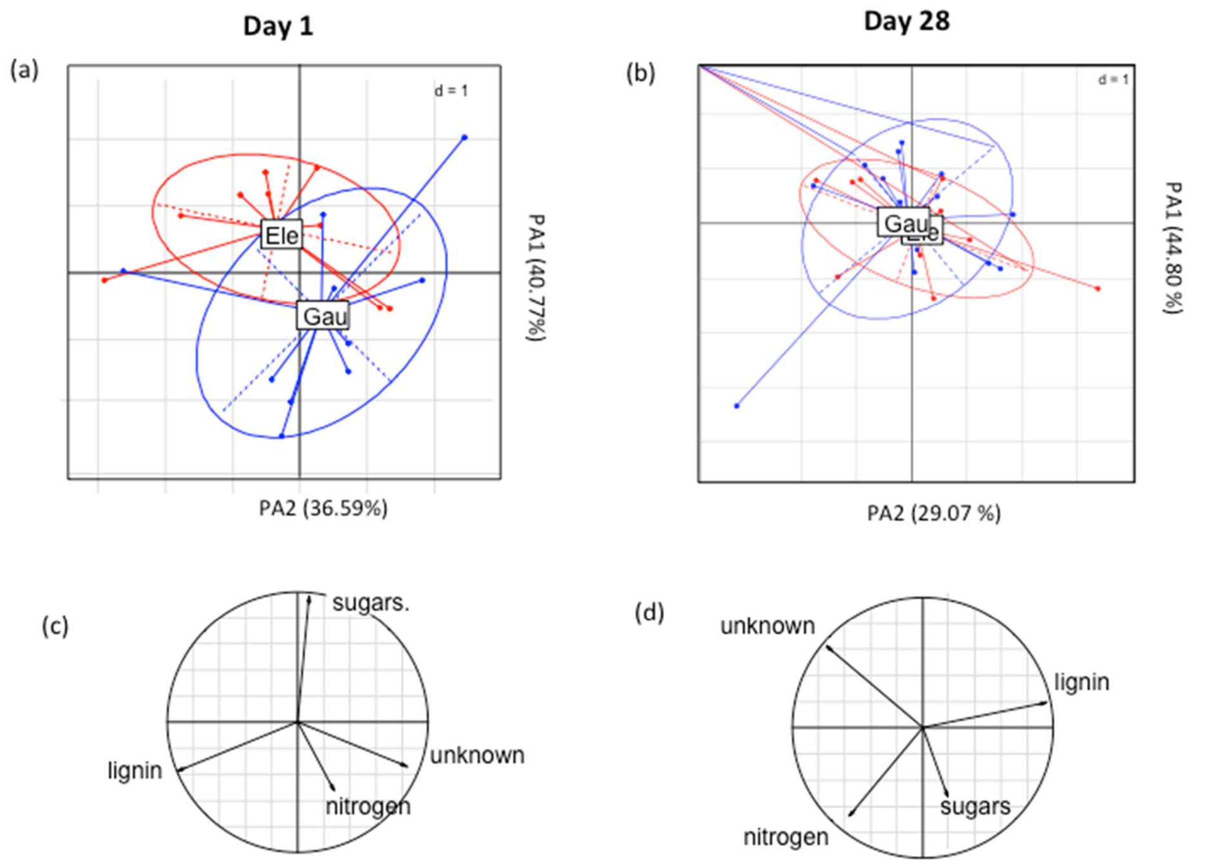
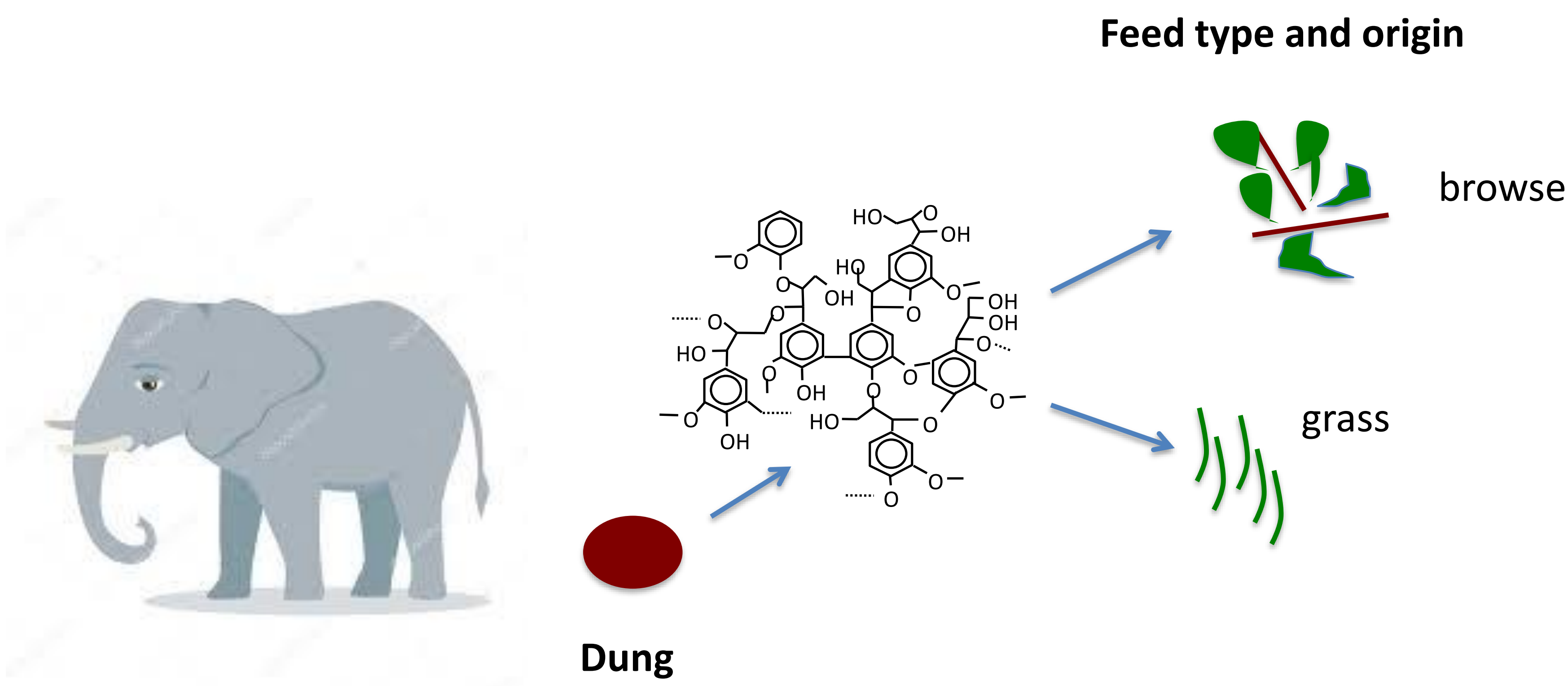


Fig.7. PCA differentiating gaur (Gau, in blue) and elephant (Ele, in red) dung based on pyrolysis results that included, polysaccharides, lignin, nitrogen, and unknown parent compounds, at day 1 (a) and 28 (b) and their correlation circle diagrams for day1 (c) and day 28 (d).


Feed type and origin
browse
grass
Dung