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The vulnerability of red colobus monkeys to anthropogenic threats

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The vulnerability of red colobus monkeys to anthropogenic threats



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Abstract

The planet is undergoing a major loss of biodiversity, driven by a range of threats operating at various scales. Understanding species' vulnerability to these threats can help inform conservation policy and practice. This thesis combines three approaches to explore the vulnerability of red colobus monkeys (*Piliocolobus* spp.) to population declines and extinction.

First, I use correlative species distribution modelling to investigate how climate and land use determine current and future red colobus species distributions. I found that predicted loss of suitable habitat in the future varied between species (range -100% to +10%) and differed between models produced using different land use datasets. Generally, the most optimistic future for red colobus was predicted in scenarios with the highest mitigation of climate change and land use change. This highlights the importance of implementing mitigation strategies for these threats for the long-term survival of red colobus.

Second, I determine whether one intrinsic trait, niche breadth, can explain vulnerability to future habitat loss in red colobus. In the four species for which I could conduct this analysis, I found this not to be the case, suggesting that other factors, such as niche position and level of exposure to climate change and land use change, might be more important in determining the vulnerability of red colobus than niche breadth.

Third, I investigate oxidative stress as a potential mechanistic link between anthropogenic disturbance and recruitment in the Zanzibar red colobus (*Piliocolobus kirkii*) using a non-invasive approach to measure oxidative stress in urine. Given the novelty of measuring urinary oxidative stress in wild animals, I first carry out a methodological investigation. I found that time of day and the time between sample collection and freezing had some effect on oxidative stress measurements but that the collection method (pipetting from leaves vs. plastic catchpole) and the length of long-term storage time did not. This implies that oxidative stress markers are stable enough for application in field studies with consideration of a few methodological constraints. I then investigate the link between two aspects of anthropogenic disturbance (habitat degradation and tourist exposure), and four markers of oxidative stress (malondialdehyde, 8-OHdG, neopterin, total antioxidant capacity) in six groups of Zanzibar red colobus monkeys. I found that colobus experiencing higher levels of habitat degradation had higher antioxidant capacity but showed no difference in any of the other oxidative stress markers. I also found no relationship between tourist exposure and any of the oxidative stress markers. I found no link between any of the oxidative stress markers and the juvenile to adult

female ratio (a proxy of recruitment) of each group. These results suggest that the monkeys experience oxidative stress when exposed to habitat degradation but that they may prevent downstream consequences for reproduction by increasing their antioxidant response.

Overall, in this thesis, I make two substantial contributions. Firstly, I provide a critical examination of some of the methodological approaches for investigating species vulnerability to extinction using correlative and mechanistic methods. Secondly, I demonstrate that, red colobus monkeys have some capacity to cope with small-scale anthropogenic disturbance but large-scale shifts in climate and land use are likely to have detrimental effects on their survival. Conservation action should focus on tackling immediate threats, such as hunting, but should not neglect the importance and urgency of mitigation strategies for climate change and land use change.

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Author's declaration

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy.

Author contributions

Chapter 1. Introduction

This chapter is my own work and was reviewed by Alexander Georgiev, Julia Jones and Tim Davenport.

Chapter 2. Variation in niche breadth and vulnerability to climate change and land use change among red colobus species

I conceived the ideas and research questions for this chapter with Alexander Georgiev, Julia Jones, Amanda Korstjens and Philippa Gillingham. I used a dataset provided by Amanda Korstjens and supplemented by myself and Landry Green. I conducted the analyses with guidance from Alexander Georgiev, Julia Jones, Amanda Korstjens, Philippa Gillingham and Nelson Ting. I wrote the chapter and it was reviewed by everyone mentioned thus far and Tim Davenport.

Chapter 3. Methodological confounds of measuring urinary oxidative stress in wild animals

I conceived the ideas and designed the methodology for this chapter with Alexander Georgiev, Jon Blount and Tim Davenport. Alexander Georgiev, Hussein Dhirani and I carried out the sample and data collection in the field. I carried out the lab work alongside Christopher Mitchell. I conducted the analyses with guidance from Alexander Georgiev and Jon Blount. I wrote the chapter with reviews from everyone mentioned.

Chapter 4. Oxidative stress markers as indicators of anthropogenic disturbance in Zanzibar red colobus monkeys

I conceived the ideas and designed the methodology for this chapter with Alexander Georgiev, Jon Blount, Julia Jones and Tim Davenport. Alexander Georgiev, Hussein Dhirani and I carried out the sample and data collection in the field. I carried out the lab work alongside Christopher Mitchell. I conducted the analyses with guidance from Alexander Georgiev, Julia Jones, Jon Blount, Tim Davenport and James Gibbons. I wrote the chapter with reviews from everyone mentioned.

Chapter 5. Discussion

This chapter is entirely my own work and was reviewed by Alexander Georgiev, Julia Jones and Tim Davenport.

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List of acronyms

- AIC** – Akaike’s Information Criterion
- AUC** – area under the receiver operating characteristic curve
- AUC-PR** – area under the precision-recall curve
- BART** – Bayesian Additive Regression Trees
- CIMP6** – Phase 6 of the Coupled Model Intercomparison Project
- CV** – Coefficient of Variation
- DNA** – Deoxyribonucleic acid
- GCAM** – Global Change Analysis Model
- GLM** – Generalised Linear Model
- GLMM** – General Linear Mixed Effects Models
- HPLC** – High Performance Liquid Chromatography
- HPA axis** – hypothalamic–pituitary–adrenocortical (HPA) axis
- ICC** – Intra-class Correlation Coefficient
- IUCN** – International Union for the Conservation of Nature
- JCBNP** – Jozani-Chwaka Bay National Park
- MDA** – Malondialdehyde
- ML** – Maximum Likelihood
- NDVI** – Normalized Difference Vegetation Index
- OS** – Oxidative Stress
- SDM** – Species Distribution Model
- SG** – Specific Gravity
- SSP** – Shared Socio-economic Pathway
- RCP** – Representative Concentration Pathway
- TAC** – Total Antioxidant Capacity
- TSS** – True Skill Statistic
- UA** – Uric Acid
- UK** – United Kingdom
- 8-OHdG** – 8-hydroxy-2'-deoxyguanosine

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Chapter 1

Introduction



Infant Zanzibar red colobus monkey (*Piliocolobus kirkii*) in Jozani-Chwaka Bay National Park, Zanzibar

Background

Vulnerability to anthropogenic threats

Overexploitation, agricultural activity, climate change and anthropogenic disturbance are among the key global drivers of species extinction and biodiversity loss around the world (Maxwell *et al.* 2016). In recognition of this, many now consider that humanity has entered a new epoch, the Anthropocene, where human activity has become the predominant influence on climate and the environment (Crutzen and Stoermer 2000; Steffen *et al.* 2011; Malhi 2017). Assessing the vulnerability of species and ecosystems to these anthropogenic threats, and the mechanisms underlying that vulnerability, can contribute to planning effective conservation initiatives (Carwardine *et al.* 2012; Ofori *et al.* 2017).

A species' vulnerability to environmental changes is a function of three elements: exposure, sensitivity and adaptability (Williams *et al.* 2008; Foden *et al.* 2013; Ofori *et al.* 2017; Fremout *et al.* 2020). Exposure is a measure of the magnitude and rate of exposure to threats, sensitivity is the degree to which the threat affects survival and reproduction, while adaptability is the ability of the species to respond to the threat through three options: shifting their distribution, adapting locally or employing behavioural and ecological plasticity (Ofori *et al.* 2017; Wong and Candolin 2015). Sensitivity and adaptability are governed by intrinsic biological traits which make species inherently more or less vulnerable to extinction and population decline (Jones *et al.* 2003; Kamilar and Paciulli 2008; Davidson *et al.* 2012; Chichorro *et al.* 2022). Gaining knowledge of these three elements is essential to fully understand a species' vulnerability to environmental change (Ofori *et al.* 2017).

Vulnerability can be quantified in several ways (Pacifi *et al.* 2015) including distributional changes, population change (either measured directly or by using proxies), extinction probability and vulnerability indices (Foden *et al.* 2013; IUCN 2023). There are a range of methods that can be used to assess the vulnerability of a species to extinction which can be broadly grouped into correlative, trait-based and mechanistic studies (Foden *et al.* 2013). In this thesis I combine these three approaches to investigate the vulnerability of red colobus monkeys (*Ptilocolobus* spp.) to anthropogenic change. In this introduction chapter, I first give background on predicting vulnerability to environmental change using these various methods. I then present the case for oxidative stress as a potential mechanistic link between anthropogenic disturbance and gradual population decline. Next, I lay out the structure of my thesis and finally I introduce the study subjects for this research: red colobus monkeys.

Correlative approaches to assess vulnerability

Correlative methods generally employ correlative species distribution models (SDMs) to assess a species' exposure to threats and measure vulnerability in terms of distributional change under future conditions (Pacifi *et al.* 2015). SDMs estimate the relationship between observed species distribution, richness or prevalence patterns and environmental information to build spatially explicit predictions of species distribution within a geographic area of interest (Guisan and Zimmerman 2000). These models can then be applied to projections of past and future conditions or of conditions in a different geographic area allowing predictions about species distribution in time and space (Thuiller *et al.* 2005). SDMs have become increasingly common in conservation research with increased availability of robust data on species localities and the environment (Evans *et al.* 2015; Villero *et al.* 2017).

The benefits of correlative SDMs are that they are spatially explicit, can be applied to a wide range of taxa across various spatial and taxonomic scales and are relatively accessible to use, especially given the recent development of detailed open-source datasets (Evans *et al.* 2015; Pacifi *et al.* 2015). However, their main disadvantages are in the uncertainty that is introduced to the models through the underlying environmental data, through the choice of modelling procedure and through incorrect assumptions about the biotic environment of the species (Pacifi *et al.* 2015).

SDMs have been developed within the framework of the ecological niche concept (Guisan and Zimmerman 2000; Pulliam 2000; Soberón 2007; Soberón and Nakamura 2009). There are many definitions of the ecological niche and the debate about which aspect of the niche correlative SDMs represent continues (reviewed by Sillero 2011). Hutchinson (1957) defined two types of species niche: the fundamental niche as the environmental space in which individuals of a species can survive and reproduce over time, and the realised niche as the part of the fundamental niche the species actually occupies due to niche exclusion by competition. Different approaches provide estimations of different aspects of a species' niche. Correlative approaches using presences and true absences estimate something similar to the realised niche and approaches using presences only or presences and background points estimate something more similar to the fundamental niche (Fernandez *et al.* 2022). Meanwhile mechanistic models which establish a causal relationship between species distribution and predictor variables by incorporating physiological information to approximate the fundamental niche (Guisan and Zimmerman 2000; Pearson and Dawson 2003; Kearney 2006; Kearney *et al.* 2008; Morin and Lechowicz 2008; Pearman *et al.* 2008; Colwell and Rangel 2009; Kearney and Porter 2004, 2009). Most correlative approaches relate abiotic variables to observed current species distributions but exclude biotic factors such as species interactions and

dispersal limitations. However, some studies have now attempted to include biotic factors such as dispersal ability into their predictions of future range changes using correlative SDMs (Schloss *et al.* 2012; Baquero *et al.* 2021). Additionally, while correlative models have received criticism for disregarding mechanistic and causal links (Tyre *et al.* 2001), there is a growing body of evidence that observed population trends have mapped closely onto those predicted from correlative models (Gregory *et al.* 2009; Visconti *et al.* 2016). As such, correlative models may be an accurate and accessible tool for estimating species vulnerability, particularly with regard to a species' exposure to threats.

Trait-based approaches to assess vulnerability

Sensitivity and adaptive capacity are governed by intrinsic traits (Ofori *et al.* 2017) therefore trait-based approaches use species life-history, physiological, behavioural or ecological traits to estimate sensitivity and adaptive capacity to threats (Foden *et al.* 2013). They are often combined with measures of threat exposure (Foden *et al.* 2013). There has been a wealth of studies investigating which biological traits best predict extinction risk. Traits that are often linked to greater sensitivity are narrow niche breadth, high habitat specificity, small population size and small geographic range (Kamilar and Paciulli 2008). Traits often associated with greater adaptive capacity are higher reproductive rate, shorter generation length, larger population size, greater genetic diversity (Gomulkiewicz and Houle 2009) and higher dispersal capacity (Trakhtenbrot *et al.* 2005). The vulnerability of a species can then be determined by the level of each trait possessed by the species.

Trait-based approaches are commonly used due to their relatively rapid and easy application to multiple species. However, there are disadvantages to using trait-based approaches. Normally, these biological traits are proxies of sensitivity and adaptive capacity and therefore have inherent uncertainty. This is particularly the case for adaptive capacity since information on true adaptive capacity, such as dispersal ability, evolutionary capacity and phenotypic plasticity, is limited. Additionally, the threshold value of these traits at which a species is vulnerable to environmental change are unknown (Nicotra *et al.* 2015). Therefore, the choice of traits to include in the assessment, arbitrary decisions on thresholds and missing data on some traits affect estimates of vulnerability for a given species (Thomas *et al.* 2011; Foden *et al.* 2013).

Mechanistic approaches to assess vulnerability

Mechanistic models establish a causal relationship between a threat and its impact on a species (e.g. extinction, population decline) by incorporating biological mechanisms (Kearney

et al. 2008; Mantyka-Pringle *et al.* 2014). One common mechanistic approach used to assess vulnerability are mechanistic niche models. Mechanistic niche models are considered by some to be more robust than correlative SDMs and allow estimation of the fundamental niche which gives a better approximation of the suitable habitat of a species (Kearney and Porter 2004, 2009). Despite these advantages, obtaining the data required for mechanistic models is complex, costly and time-consuming and as such, mechanistic approaches tend to focus on one species at a time and are limited in the species they can be applied to (Pacifi *et al.* 2015).

Mechanistic models are developed from data collected in laboratory and field studies of demographic rates (e.g., Hinam and Clair 2008), physiology (e.g., Hinam and Clair 2008; Riddell *et al.* 2019), life history (e.g., Radchuk *et al.* 2013; Bestion *et al.* 2015), competition (e.g., Best *et al.* 2007), dispersal (e.g., Best *et al.* 2007), behaviour (e.g., Hinam and Clair 2008) and energy balance (e.g., Gallagher *et al.* 2021) among others (Pacifi *et al.* 2015). Many mechanistic studies measure physiological mechanisms linking threats and population dynamics and there is a large body of literature demonstrating the impact of anthropogenic changes on animal physiology (Madliger and Love 2015) by documenting, for example, increased energetic costs (Bradshaw *et al.* 1998), stress hormone levels, heart rate (Ellenberg *et al.* 2006) and decreased length of telomeres (Salmón and Burraco 2022).

Physiological mechanisms represent indirect mechanisms (also known as non-lethal or non-consumptive effects) through which anthropogenic change can affect a population over time (Schmitz *et al.* 2004; National Academies 2017; Pirota *et al.* 2018) as opposed to direct and immediate changes (also known as lethal or consumptive effects) to survivorship, for example, through hunting (Benítez-López *et al.* 2017) or vehicle collisions (Olgun *et al.* 2022). Most effects of anthropogenic change are not immediately or directly lethal for wildlife but instead create energetic and lost-opportunity costs (Frid and Dill 2002; Schmitz *et al.* 2004) which can accumulate spatiotemporally causing reduced individual fitness (Bradshaw *et al.* 1998; Johnson and St-Laurent 2011; Plante *et al.* 2020). The population dynamic consequences of these indirect effects on fitness can ultimately exceed those from direct sources of mortality (Creel and Christianson 2008). Therefore, investigating these indirect effects can provide valuable insight into the mechanistic link between anthropogenic threats and population dynamics (Gill *et al.* 2001). This is especially important for long-lived species for which quantifying the ultimate link between anthropogenic threats and population dynamics may take too long given the rate of biodiversity loss today. Therefore, measuring the physiological mechanistic links between disturbance and population dynamics can help to parameterise

mechanistic models of extinction risk and provide important information on species vulnerability to extinction on a timescale relevant to conservation intervention.

There is debate about the use of some physiological markers to infer population level consequences (Sutherland 2007). Glucocorticoids have been widely used to determine the physiological impact of exposure to anthropogenic disturbance (Suorsa *et al.* 2003; Martínez-Mota *et al.* 2007; Rangel-Negrín *et al.* 2009; Jaimez *et al.* 2012; Munshi-South *et al.* 2008; Tempel and Gutierrez 2004; von der Ohe *et al.* 2004). However, the link between glucocorticoid levels and the ultimate outcome of interest, fitness, is still equivocal and rarely tested directly, especially in studies of long-lived animals (Beehner and Bergman 2017; Kaisin *et al.* 2021). Using additional physiological markers to assess the impact of anthropogenic threats on fitness may therefore sharpen our ability to establish such causal links even in the short term (Beaulieu and Costantini 2014).

Oxidative stress: a potential mechanism mediating the impacts of anthropogenic threats on fitness

One useful group of biomarkers that could help to define the mechanistic link between anthropogenic threats and fitness are markers of oxidative stress. Oxidative stress occurs at the cellular level as the imbalance between reactive oxygen species produced during oxygen consumption and the antioxidant response mounted to neutralise these reactive oxygen species using endogenous and exogenous compounds (Finkel and Holbrook 2000; Balaban *et al.* 2005). When the production of reactive oxygen species overwhelms the antioxidant response, excess reactive oxygen species damage important cell components, such as lipids, proteins, and DNA (Valko *et al.* 2007). The accumulation of this damage is linked to a range of pathologies (Keaney *et al.* 2003; Bonda *et al.* 2010; Anderson and Maes 2014), advanced aging (Selman *et al.* 2012) and reproductive issues (Keskes-Ammar *et al.* 2003; Agarwal *et al.* 2012) thereby reducing fertility and survival probability (Monaghan *et al.* 2009).

Oxidative stress has clear and well-established links to health and fitness. The negative fitness consequences of high levels of OS have been shown in many species (*Geothlypis trichas*, Freeman-Gallant *et al.* 2011; *Parus major*, Helfenstein *et al.* 2010; Losdat *et al.* 2013; *Apus melba*, Bize *et al.* 2008; *Hirundo rustica*, Saino *et al.* 2011; *Phalacrocorax aristotelis*, Noguera *et al.* 2012) and has also been linked to population level trends (Beaulieu *et al.* 2013). Additionally, markers of oxidative stress have been shown to respond to various anthropogenic threats (reviewed by Beaulieu and Costantini 2014 and in Chapter 4). It is therefore possible that oxidative stress could mediate the link between anthropogenic disturbance and fitness and ultimate population declines.

Thesis overview

Aims and objectives

As I have discussed in this chapter, each method used to assess vulnerability to extinction has its own pros and cons and it is increasingly recognised that combining approaches may yield more insightful results (Keith *et al.* 2008; Pearson *et al.* 2014; Evans *et al.* 2015; Rougier *et al.* 2015; Visconti *et al.* 2016). In this thesis, therefore, I combine several approaches to assess the vulnerability to anthropogenic change of a group of endangered, long-lived mammals, the red colobus monkeys (*Piliocolobus* spp.).

My main objectives are:

- a. To determine the vulnerability of red colobus to habitat loss through climate change and land use change using correlative species distribution modelling on a pan-African scale.
- b. To investigate whether one intrinsic trait, niche breadth, can predict vulnerability of red colobus species to changes in future suitable habitat.
- c. To provide methodological insights into the use of a novel ecophysiological method (measuring urinary oxidative stress in wild animals) for studying the impact of anthropogenic threats on wildlife.
- d. To investigate oxidative stress as a potential mechanism linking anthropogenic disturbance to recruitment (a proxy of fitness) in an island endemic, the Zanzibar red colobus (*P. kirkii*).

Of the 18 species of red colobus extant today, 14 are classified as Endangered or Critically Endangered by the International Union for Conservation of Nature (IUCN) therefore, their conservation is an urgent priority. In response to the high threat of extinction facing red colobus across Africa, the Red Colobus Action Plan was created in 2021 (Linder *et al.* 2021). The Action Plan aims to unite and mobilise local and international conservation efforts for each taxa and across the red colobus range, and highlights the conservation and research priorities for each species (Linder *et al.* 2021). Assessing the threats facing red colobus is a main research priority highlighted in the Red Colobus Action Plan. Therefore, gaining a better understanding of how anthropogenic threats are affecting red colobus at different scales, the consequences this may have for their future survival, the differences in vulnerability among red colobus species, and the conservation actions that may be most appropriate to protect them are key research priorities. I aim to address these research priorities in this thesis.

Thesis outline

My thesis is divided into five chapters (Figure 1.1). This first chapter introduces the topics to be covered in this thesis. The following three chapters address my research objectives. My final chapter provides a discussion of my findings and their future application.

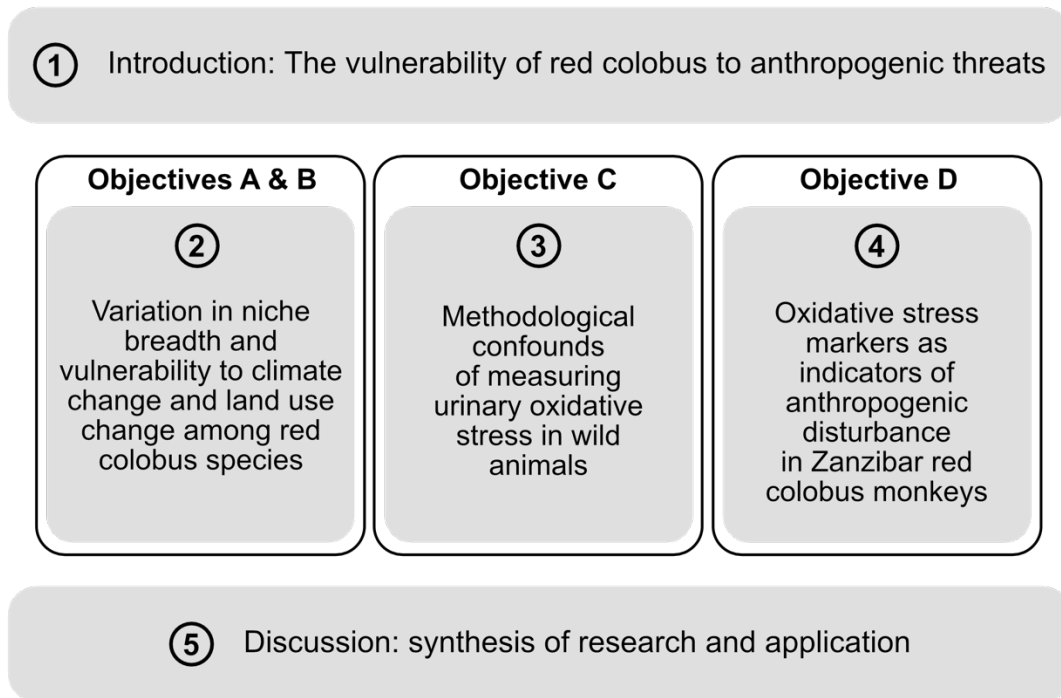


Figure 1.1: Schematic of my thesis demonstrating how different chapters contribute to the four thesis objectives. The numbers refer to the chapter numbers as described below.

Chapter 2: Variation in niche breadth and vulnerability to climate change and land use change among red colobus species

In this chapter, I explore the relationship between niche breadth and vulnerability to climate change and land use change in red colobus monkeys using a combination of ecological niche models and species distribution models. I firstly determine the niche breadth of 16 red colobus species and the pairwise niche overlap between 13 red colobus species using ecological niche modelling in environmental space. I then use Bayesian species distribution modelling to investigate the impacts of land use and climate on the distribution of four well-characterized species and investigate how their distribution is likely to change by the year 2070 under predicted climate change and land use change scenarios. The varying sample sizes between analyses is due to differences in the sizes of the datasets available. I interpret the predicted changes in suitable habitat for these four species in the context of niche breadth and niche overlap to determine whether different red colobus species are likely to respond differently to future environmental change and whether this can be predicted using niche breadth. Finally,

I also conduct a methodological investigation into the use of two land use datasets for predicting future distribution changes in red colobus monkeys.

Melvin, Z. E., Korstjens, A.H., Gillingham P.K., Landry, M.E., Ting, N., Davenport, T.R., Jones, J.P.G., & Georgiev, A. V. (in prep). Variation in niche breadth and vulnerability to climate change and land use change among red colobus species.

Chapter 3: Methodological confounds of measuring urinary oxidative stress in wild animals

The aim of this chapter is to investigate some potential methodological confounds that field researchers measuring oxidative stress non-invasively in urine are likely to encounter. I investigate the impact of four potential methodological confounds on oxidative stress measurements: 1) time of sampling; 2) environmental contamination from foliage; 3) delay between sample collection and flash-freezing in liquid nitrogen; and 4) sample storage of up to 15 months below -80°C. Since measuring urinary oxidative stress in wild animals has only been carried out once but has great potential for application to conservation questions, this chapter will be useful in guiding research design for future projects.

Melvin, Z. E., Dhirani, H., Mitchell, C., Davenport, T. R., Blount, J. D., & Georgiev, A. V. (2022). Methodological confounds of measuring urinary oxidative stress in wild animals. Ecology and Evolution, 12(7), e9115.

Chapter 4: Oxidative stress markers as indicators of anthropogenic disturbance in Zanzibar red colobus monkeys

This chapter provides the first investigation into the link between anthropogenic disturbance and oxidative stress in a wild primate. I investigate the link between two aspects of anthropogenic disturbance (habitat degradation and exposure to tourism) and oxidative stress levels in six groups of Zanzibar red colobus monkeys (*Piliocolobus kirkii*). I then investigate the relationship between group level oxidative stress and recruitment in order to determine if there might be a potential fitness consequence of elevated oxidative stress levels in this species. Oxidative stress is a promising yet underused tool for investigating the impact of humans on wildlife. As such, this research contributes new insights into the application of oxidative stress markers in conservation monitoring and provides practical conservation guidance for the endangered Zanzibar red colobus.

Melvin, Z. E., Dhirani, H., Mitchell, C., Davenport, T. R., Jones, J.P.G., Blount, J. D., & Georgiev, A. V. (in prep.). *Oxidative stress markers as indicators of anthropogenic disturbance in Zanzibar red colobus monkeys.*

Setting the scene

The impact of anthropogenic threats on primates

Primates are disproportionately vulnerable to human impacts (Bernard and Marshall 2020) for two main reasons: firstly, they are likely to experience higher levels of exposure to anthropogenic threats and secondly, they are more likely to be sensitive to, and less able to adapt to, these threats. As mostly tropical species (Kalbitzer and Chapman 2018), primates face a higher risk of exposure to climate change and land use change. Tropical species are predicted to experience a greater temperature shift under climate change than the global mean (Graham *et al.* 2016; Korstjens and Hillyer 2016; Stewart *et al.* 2020) and are predicted to experience an average of 75% reduction in range size due to climate change (Schloss *et al.* 2012). Additionally, the human population is increasing particularly rapidly in primate-range countries, which is associated with expansion of agricultural lands and loss of natural habitat (Kalbitzer and Chapman 2018). As a result, more than 90% of the forest loss between 1990 and 2020 has occurred in tropical areas, despite only 45% of the world's forested areas being in the tropics (Ometto *et al.* 2022). Therefore, primates are predicted to experience a greater change in climate and land use in the coming decades than other taxa.

Not only are primates predicted to experience higher levels of climate change and land use change than many other taxa, they are also less able to respond to these threats. Of the three options available for a species to survive exposure to human impacts (dispersal, local genetic adaptation or phenotypic plasticity (Wong and Candolin 2015)), primates are mostly only able to employ plasticity. They have little ability to shift their distribution or adapt locally due to their intrinsic biological traits such as slow life history, limited dispersal ability, social group living, short individual travel distances and restricted geographical ranges (Wong and Candolin 2015; Korstjens and Hillyer 2016). For example, of all mammal taxa, primates were found to be the least able to keep pace with geographical range shifts under climate change because of their very limited dispersal ability and larger required range shifts (Schloss *et al.* 2012). As such, plasticity in response to new conditions is the only remaining option for primate survival. Given the high vulnerability of primates to anthropogenic threats, quantifying the impacts of human-induced changes on primate populations is an urgent priority.

Red colobus (*Piliocolobus* spp.) as a study taxa

Even among primates, red colobus monkeys are a highly endangered genus. Of the 18 species extant today, 14 are classified as Endangered or Critically Endangered by the IUCN. As a result, the Red Colobus Action Plan (Linder *et al.* 2021) and Red Colobus Conservation Network, an informal association of conservationists and researchers working with red colobus across Africa and operating through the IUCN/SSC Primate Specialist Group, were formed to unify and mobilise conservation action.

Red colobus have a patchy distribution across the central belt of Africa from Senegal on the West Coast to the Zanzibar archipelago off the East Coast (Figure 1.2). They inhabit a variety of environments including rainforest, riparian forest, mangrove swamp, coral rag forest and dry savanna woodland (Linder *et al.* 2021; Nowak 2007). The taxonomy of red colobus has been unresolved for decades due to complex patterns of variation and allopatric distribution (Ting 2008). Taxonomic uncertainty is especially high in the central group of taxa where hybridisation may occur (Linder *et al.* 2021).

As with many primates, the main threats facing red colobus are hunting and habitat loss, degradation and fragmentation (Struhsaker 2005, 2010, Linder *et al.* 2021). Red colobus monkeys are often the first mammal species to suffer local extirpation due to hunting due to their large body size, loud presence within the forest and lack of behavioural defences to hunting (Linder and Oates 2011; Linder *et al.* 2021). Habitat loss affects all red colobus species (Linder *et al.* 2021) and has already resulted in extreme population declines for some species. For example, forest loss and degradation was almost entirely responsible for the 90% decline in the Niger Delta red colobus (*P. epieni*) over a 20-year period (Ikemeh 2015). Additionally, it is likely that climate change will become more of an issue for red colobus in the future. In a recent analysis on the vulnerability of 419 primate species to climate change, *P. gordonurum* and *P. kirkii* (both located in East Africa) were identified as some of the most vulnerable primates due to large increases in precipitation in their range (Graham *et al.* 2016). Additionally, climate change has already had detrimental effects for *P. temminckii*, which has declined by more than 50 % in last 30 years (Minhós *et al.* 2020) mostly due to severe habitat reduction and fragmentation attributed to reduced rainfall and expanding cropland (Galat-Luong and Galat 2005; Galat *et al.* 2009). As such, red colobus face high exposure to a plethora of threats that make them vulnerable to extinction.

Red colobus also have many intrinsic biological traits that are predicted to make them more vulnerable to extinction, for example they are folivorous, arboreal, forest specialists (Korstjens *et al.* 2022). However, research has shown that some red colobus species are more flexible

than previously thought (Korstjens 2019; Butynski and De Jong 2022; Korstjens *et al.* 2022). Considerable dietary and behavioural flexibility are known in *P. kirkii* (Nowak 2007; Nowak and Lee 2013), *P. rufomitratu*s (Milich *et al.* 2014; Chapman *et al.* 2015), *P. tephrosceles* (Kibaja *et al.* 2023) and *P. temminckii* (Galat-Luong and Galat 2005). This suggests that they may be able to mitigate the effects of anthropogenic threats to a certain extent through plasticity.

Given the high exposure to threats and uncertainty around whether red colobus are intrinsically more vulnerable to extinction, this taxa are an interesting group in which to investigate vulnerability to extinction. Also, given the urgent conservation concern for this threatened group of primates, this research is likely to have important practical implications for their conservation.

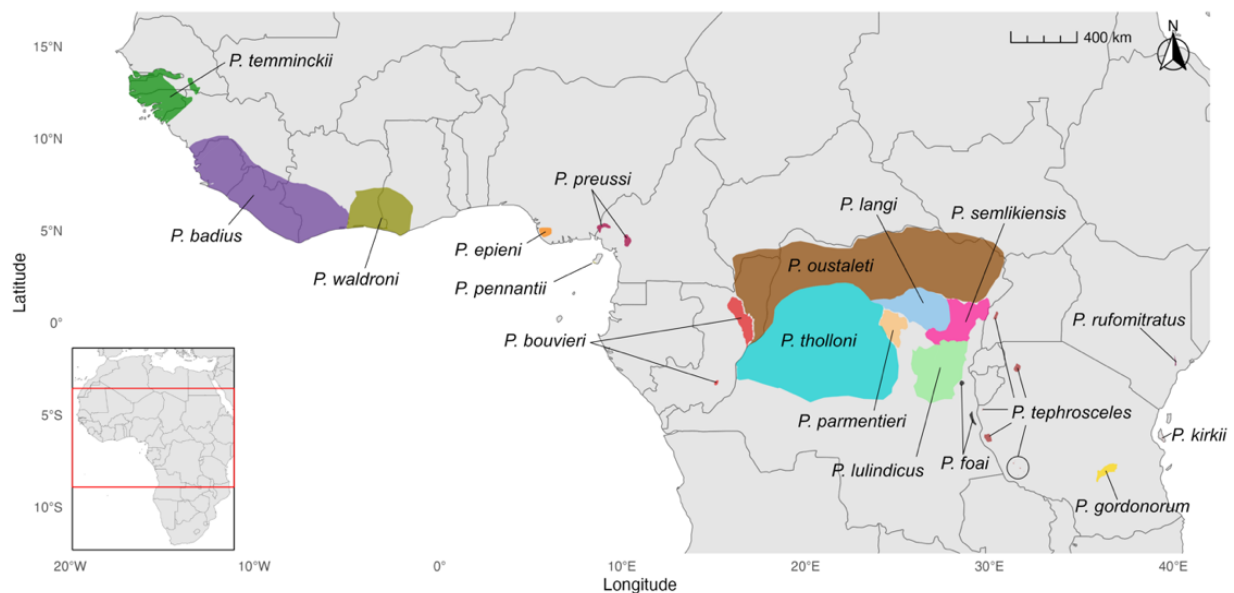


Figure 1.2: Red colobus distribution map based on species ranges from the Red Colobus Action Plan (Linder *et al.* 2021).

The Zanzibar red colobus (*Piliocolobus kirkii*) as a study species

The Zanzibar red colobus monkey is the most distinct of all the red colobus monkeys (Linder *et al.* 2021). It is endangered and endemic to Unguja, the southern island of Zanzibar, and has been legally protected in Zanzibar since 1919. The species is classified as endangered by the IUCN (Davenport, 2019) and a census carried out in 2013-15 concluded that only 5,862 individuals remain. Recruitment of colobus (the addition of individuals to the population) was low across Zanzibar compared to other red colobus species suggesting the species is in decline (Davenport *et al.* 2019). Over 85% of Zanzibar red colobus groups are found in or

adjacent to forest suggesting that, though the species can survive in degraded habitat, some form of forest is essential for their survival (Davenport *et al.* 2019).

While some hunting occurs for subsistence and as pest control (Davenport *et al.* 2019; Linder *et al.* 2021), the incidence of hunting is much lower than for other red colobus species (Siex and Struhsaker 1999; Struhsaker 2005). Therefore, the biggest threat to the Zanzibar red colobus is habitat loss, degradation and fragmentation due to unmanaged development for tourism, housing and agricultural expansion (Struhsaker 2010; Davenport 2019; Linder *et al.* 2021). The human population in Zanzibar has increased from 354,815 in 1967 to 1,889,773 in 2022 and it continues to grow at 3.7% per annum (Tanzanian National Bureau of Statistics 2022). Additionally, tourism has expanded rapidly in Zanzibar since the late 1980s with the annual number of visiting tourists rising 16-fold, from 19,368 to 311,891 between 1985 and 2014 (Tanzanian National Bureau of Statistics 2014). As a result of the increased demand placed on ecosystems by a growing local population and increased tourism, forests in Zanzibar declined by 15% between 2003 and 2018 (Omar and Cabral 2020), potentially forcing colobus monkeys to live in more degraded habitats. Thirty-one percent of the remaining approximately 5,800 colobus live outside protected areas where group sizes are significantly smaller (Davenport *et al.* 2019). As such, it is likely that there are hidden physiological costs to living in degraded habitats that are reducing individual fitness and which will ultimately lead to population decline.

In addition to the impact increased tourism has had on forest loss and degradation, direct exposure to tourists could also pose a potential threat to the Zanzibar red colobus. Jozani-Chwaka Bay National Park (50 km²) holds around 50% of all remaining Zanzibar red colobus, making this national park a stronghold for the species (Davenport *et al.* 2019). But tourism in the national park has also increased rapidly. Income from ecotourism has increased from \$100,000 per annum in 2000 to \$650,000 USD per annum today (Jozani-Chwaka Bay National Park, official records). In 2019, before the Covid-19 pandemic, over 65,000 tourists visited the national park (Jozani-Chwaka Bay National Park, official records). A portion of the income from tourism is shared with the local community meaning that there is a conservation benefit of tourism (Carius and Job 2019). However, increased tourism may put additional pressure on the red colobus. Most tourism activity is focused on 3 - 4 groups which are visited by tourists daily and tourists break the 3 m distance regulations 11% of the time (Warkentin and Georgiev, unpublished data). It is widely recognised that exposure to tourism can have pervasive and wide-reaching negative effects on wildlife. In primates alone, tourism has been shown to have caused increased risk of disease transmission (Dunay *et al.* 2018), higher levels of intraspecific aggression (Berman *et al.* 2008; El Alami *et al.* 2012; Maréchal *et al.*

2016), elevated physiological stress (Behie *et al.* 2010; Shutt *et al.* 2014) and altered activity budgets (El Alami *et al.* 2012). As such, tourism can have fitness consequences mediated through changes in physiology and behaviour. Therefore, it is important to understand the trade-off between the conservation costs and benefits of increased tourism for this endangered species.

Alongside increased tourism, Zanzibar has also experienced the expansion of its paved road network and increased volume of traffic on the roads. One of the main roads in Zanzibar, linking Zanzibar Town to the beach resorts on the east of the island, transects Jozani-Chwaka Bay National Park. This road causes 1.77% - 3.24% annual mortality from collisions in the colobus groups neighbouring the road (Olgun *et al.* 2022) despite the installation of speed bumps (Struhsaker 2010). Therefore, the increased volume of traffic in Zanzibar may pose a threat to this species, even in areas where they are protected, such as in Jozani-Chwaka Bay National Park.

The Zanzibar red colobus is an excellent study system to investigate the impact of anthropogenic disturbance on physiology, fitness and potential population decline. The Zanzibar red colobus is exposed to a wide variety of threats (Davenport 2019; Linder *et al.* 2021) and while individuals living in disturbed areas appear to be surviving, demographic data suggest that recruitment is low and that the population may be declining (Davenport *et al.* 2019). Additionally, groups living in disturbed areas are smaller in size potentially suggesting reduced fitness in response to anthropogenic disturbance (Davenport *et al.* 2019). This is despite that fact that there is relatively low hunting pressure (Struhsaker 2010) and that the Zanzibar red colobus has notable dietary and behavioural plasticity in disturbed habitats (Nowak and Lee 2013; Siex 2003). As such, there is potential for hidden physiological mechanisms linking anthropogenic disturbance to reduced fitness in this species.

Research permits and ethical approval

Formal permission to conduct fieldwork to collect behavioural and ranging data and non-invasive biological samples was obtained from the Department of Non-Renewable Natural Resources in Zanzibar. I also gained ethical approval for my research from the Bangor University Ethics Committee, UK. This research complies with the International Primatological Society guidelines for the Protection of Primate Health in the Wild.

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Chapter 2

Variation in niche breadth and vulnerability to climate change and land use change among red colobus species



Ashy red colobus monkeys (*Piliocolobus tephrosceles*) in Kibale National Park, Uganda

Abstract

Changes in climate and land use are negatively affecting biodiversity around the globe. Determining the drivers of the vulnerability of species to these changes is important for designing effective conservation strategies. Narrow niche breadth has been linked to increased extinction vulnerability across taxa. We investigated whether niche breadth was linked to variation among red colobus species' vulnerability to future habitat loss due to climate change and land use change. We also investigated whether different red colobus species had different niches and determined how the use of two different land use datasets affected predicted future changes in suitable habitat availability.

We estimated niche breadth as the standard deviation of each environmental variable in the species' geographic range. We characterised the niche of each species using kernel density estimates in environmental space and quantified niche similarity between pairs of species using Schoener's *D* and Hellinger's *I*. We tested for the significance of niche similarity between pairs of species using a niche similarity test. Finally, we estimated habitat loss between now and 2070 for four focal species for which we had sufficient presence data using species distribution models implemented using Bayesian Additive Regression Trees. We did this for four future scenarios representing varying mitigation strategies for climate change and land use change. We constructed these models using two different land use datasets to investigate the impact this had on predicted habitat loss.

We found that there was large variation in niche breadth among red colobus species and that generally, there was little niche similarity between them. We found a large variation in the loss/gain of suitable habitat between species, future scenarios and models constructed using different land use datasets (range +10% to -100%). The most optimistic scenario for the future habitat of red colobus was the scenario employing the highest mitigation for climate change and land use change, while the least optimistic was the one employing medium mitigation for land use change and no mitigation for climate change. We also showed that niche breadth was not linked to predicted habitat loss for the four species tested.

In general, red colobus species were predicted to lose suitable habitat under future climate change and land use change, though the degree of habitat loss varied between species. This variation was not predicted by the niche breadth of the species, however given that there is very little niche overlap between species, it could be that the niches of different species become relatively more or less common under future environmental change leading to varying

levels of predicted habitat loss. Future habitat loss was lower for scenarios with high mitigation of climate change and land use change reinforcing the importance of incorporating these actions into conservation management for this genus. Finally, researchers should be mindful of their choice of land use datasets for species distribution modelling and employ ground-truthing and ecological knowledge of the study species to sense-check results.

Introduction

Land use and land cover change (hereafter, land use change) is currently considered to be the most important driver of global biodiversity loss; however climate change is predicted to play an increasingly important role in the next 50 years (Newbold 2018). Species that are exposed to climate change or land use change generally have 3 options for survival: shift their distribution to a more suitable area (Chen *et al.* 2011), adapt locally, or employ behavioural and ecological plasticity (Wong and Candolin 2015). As a result, biological traits that make species less able to employ these responses make them more vulnerable to extinction (Purvis *et al.* 2000; Chichorro *et al.* 2019).

One of the most robustly supported traits associated with higher extinction vulnerability is narrow niche breadth with evidence from across species and geographic scales (Thuiller *et al.* 2005; Broennimann *et al.* 2006). Niche breadth has been linked to extinction risk in more than 80% of studies across taxa (Colles *et al.* 2009) and it was the best predictor of tolerance to habitat loss and fragmentation across mammal and amphibian species (Swihart *et al.* 2003). A recent meta-analysis identified habitat specificity (an aspect of niche breadth) as a universal predictor of extinction risk across vertebrates, invertebrates and plants (Chichorro *et al.* 2022). Additionally, temperature niche breadth has been described as a relatively easily measured and accurate predictor of vulnerability to extinction under climate change (Grinder and Wiens 2023).

However, there are other traits that could explain variation in extinction vulnerability, one of which is niche position (the position of the species' niche along each environmental axis). Some species with narrow niches are relatively successful under anthropogenic change (Frishkoff *et al.* 2015; Frishkoff *et al.* 2019). This may be the case if a species has a narrow niche in a position encompassing environmental conditions that become more common in the future (Frishkoff *et al.* 2019; Carscadden *et al.* 2020; Vela Díaz *et al.* 2020). In this case, niche breadth would not explain vulnerability to extinction. Given the divergent predictions about which traits best predict vulnerability, the link between niche breadth and extinction vulnerability requires further investigation.

One reason for inconsistent findings relating intrinsic traits to vulnerability is the taxonomic scope of the study (Sweeney and Jarzyna 2022). Comparative studies of extinction risk carried out across broad taxonomic scales may disguise differences in niche position and niche breadth within closely related taxa (Sweeney and Jarzyna 2022). For example, in a broad taxonomic study, colobines may be considered specialist due to their forest dependence and

folivorous diet, however, on closer inspection, there is great variation in the degree of forest use and diet of colobine species (Kamilar and Paciulli 2008). Therefore, comparative studies carried out on a small taxonomic scale (e.g., genus) may be beneficial for understanding how and why different species respond to differently to environmental change; knowledge that would be useful for planning targeted, practicable conservation action (Zhang *et al.* 2017; Kamilar and Paciulli 2008).

In this study we explore niche breadth and vulnerability to climate change and land use change in a single genus of primate: the red colobus monkeys (*Piliocolobus* spp.). Red colobus is an interesting taxon in which to explore the relationship between niche breadth and vulnerability to climate change and land use change for three main reasons.

Firstly, red colobus monkeys are considered one of the most threatened groups of African monkeys (Linder *et al.* 2021). All 18 recognised species of red colobus are classified as Vulnerable, Endangered or Critically Endangered, and one species (*P. waldroni*) may be extinct (Oates *et al.* 2020). A major threat facing this genus today, especially those species in central and western Africa, is hunting by humans (Linder *et al.* 2021). However, climate change and land use change are also already having negative effects on red colobus populations and are likely to worsen in the future (Ikemeh 2015; Korstjens *et al.* 2022). For example, in a recent analysis of the vulnerability of 419 primate species to climate change, *P. gordonurum* and *P. kirkii* (both found in Tanzania) were classified in the top ~5% most vulnerable species, specifically due to their small range sizes and the predicted increase in precipitation within their ranges (Graham *et al.* 2016). Furthermore, habitat loss is having a pervasive effect on all red colobus species (Linder *et al.* 2021). Decker (1994) showed that the 80% decline in numbers of *P. rufomitratu*s, a Kenyan endemic, between 1975 and 1985 was mostly due to habitat disturbance and fragmentation due to the changing river course and shifting agricultural practices. Additionally, *P. epieni*, endemic to Nigeria, declined by 90% in 20 years mainly as a result of forest loss (Ikemeh 2015; Ikemeh and Oates 2017). Therefore, understanding the response of red colobus to climate change and land use change is an urgent conservation priority.

Secondly, there is debate as to the extent to which red colobus are specialist or generalist primates, i.e., how broad their niche is. Red colobus are arboreal, forest dependent and highly folivorous (Korstjens *et al.* 2022). They have morphological adaptations for leaf digestion, but they struggle to utilise large amounts of hard-to-digest mature leaves (a common food of last resort for other colobines) (Butynski and De Jong 2022). This limits their dietary flexibility and

reduces their ability to survive in degraded or dry forests (Kamilar and Paciulli 2008; Butynski and De Jong 2022). Red colobus are also considered to have relatively low behavioural and ecological flexibility compared to other colobines and other cercopithecines in general (Korstjens 2019; Butynski and De Jong 2022) making them less able to adapt to new conditions. It has been suggested that this combination of narrow niche and limited flexibility increases their extinction risk from land use change (Harcourt 1998; Isaac and Cowlishaw 2004; Butynski and De Jong 2022) and climate change (Korstjens 2019; Butynski and De Jong 2022). However, red colobus could also be considered generalist. It could be argued that a folivorous diet indicates ecological generalism, because leaves are readily available (Kamilar and Paciulli 2008). Furthermore, red colobus may be more flexible than originally thought as considerable dietary, ecological and behavioural flexibility has been recorded in multiple red colobus species (Korstjens *et al.* 2022). For example, in *P. kirkii* (Nowak 2007; Nowak and Lee 2013), *P. tephrosceles* (Milich *et al.* 2014; Chapman *et al.* 2015; Kibaja *et al.* 2023) and *P. temminckii* (Galat-Luong and Galat 2005). Some red colobus species feed on crops where they are available (*P. kirkii*, Siex and Struhsaker 1999; *P. tephrosceles*, Kibaja 2014), and others can thrive in floristically very distinct habitats (e.g., Zanzibar red colobus in ground water forest, mangroves and coral rag forest: Siex 2003; Nowak 2007; Nowak and Lee 2013; Davenport *et al.* 2019). Given that there is evidence for both specialism and generalism in the red colobus genus and that there is debate about whether generalism or specialism portrays a survival advantage under anthropogenic change, red colobus provide an interesting study taxon to investigate how niche breadth and niche similarity between species is linked to vulnerability to future change.

Thirdly, red colobus represent a group of closely related species with presumably similar ecological niches under the phylogenetic niche conservatism theory (Harvey and Pagel 1991; Wiens *et al.* 2010). However, there is evidence for variation in niche breadth between species as, for example, the amount of folivory and habitat specialism varies between species (Korstjens *et al.* 2022; Kamilar and Paciulli 2008). As such, it is not known whether red colobus species will differ in their responses to future environmental change and whether these differences will be linked to differences in niche breadth or to ecological differences across Africa. In sum, a taxonomically focused study should be able to elucidate more nuanced patterns of response to environmental change in red colobus and thus inform more focused and relevant conservation strategies.

We employ presence-absence correlative species distribution modelling to approximate the realised niche of the red colobus species. We explore the relationship between niche breadth

and vulnerability to climate change and land use change in red colobus monkeys. First, we investigate the niche breadth of 16 red colobus species for which we had presence data. Second, we determine niche similarity for the 13 species for which we could create niche overlap models of sufficient quality. Third, for a subset of four species with sufficient presence records, we use species distribution models (SDMs) to explore which climate and land use variables contribute most to current red colobus presence and investigate how this is affected by the choice of land use datasets. Fourth, we use the SDMs for these four species to explore how suitable habitat may change under four future scenarios of climate change and land use change and for models created using different land use datasets. Fifth, we interpret these results in the context of vulnerability to climate change and land use change to address the question: are those species with narrower niches more vulnerable to habitat loss due to climate change and land use change?

Methods

Red colobus taxonomy

The taxonomy of red colobus monkeys is somewhat unresolved. They form a monophyletic clade (Ting 2008a), and there have been up to 18 recognised species (Struhsaker 2010; Mittermeier *et al.* 2013; Korstjens *et al.* 2022). We use the taxonomic classification put forth by Linder *et al.* (2021) except we recognize the two western most taxa as distinct species (*P. badius* and *P. temminckii*) following Mittermeier *et al.* (2013) as they are quite distinct from one another.

Our *Piliocolobus* presence/absence dataset

We extended the dataset created by Korstjens *et al.* (2018) and further used and described by Williams *et al.* (2021). The Korstjens *et al.* (2018) dataset provided a total of 525 sites for presence of all 15 Cercopithecoid genera found in mainland sub-Saharan Africa, which include red colobus monkeys. We supplemented the Korstjens *et al.* (2018) data with 3 presence points from Devreese (2015) and 701 presence points from a GBIF search conducted on the 05/07/2020 using genus name 'Piliocolobus'. This compiled dataset covers a time period from 1970 to present day, with most records (>80%) dating from information reported between 1990 and 2015. We removed duplicated points (23 points), museum records and GBIF points recorded as extinct (397 points) and points for which we had no definitive data for the presence/absence of red colobus (110 points). We filtered out 24 points because they fell within water bodies. We restricted our study area to -20, 45, -12, 19 degrees to exclude climatic areas we know are not suitable to red colobus, e.g., desert areas, and therefore excluded a further 140 locations that had primates but no red colobus. The resulting dataset

contained 535 locations, 359 with red colobus present and 176 without. Due to extensive deforestation and forest defaunation in Africa over the last two decades, some locations may record species presences where they are now locally extinct and therefore may fall out of the current range estimates. The species classifications of the red colobus presence points were reclassified based on the geographic range they fell within or closest to using the most recent red colobus geographic ranges (Linder *et al.* 2021, Figure 2.1). This was done so that our presence points represented the currently accepted classification of the red colobus by the IUCN Primate Specialist Group given that historic presence records may have been classified differently due to the somewhat unresolved taxonomy of the genus. Hereafter, we refer to this finalised dataset as our *Piliocolobus* presence/absence dataset. There was a lot of variation in the number of presence points per species (Figure 2.1) and two species, *P. foai* and *P. parmentieri*, were excluded from analyses because we did not have any presence points for these species.

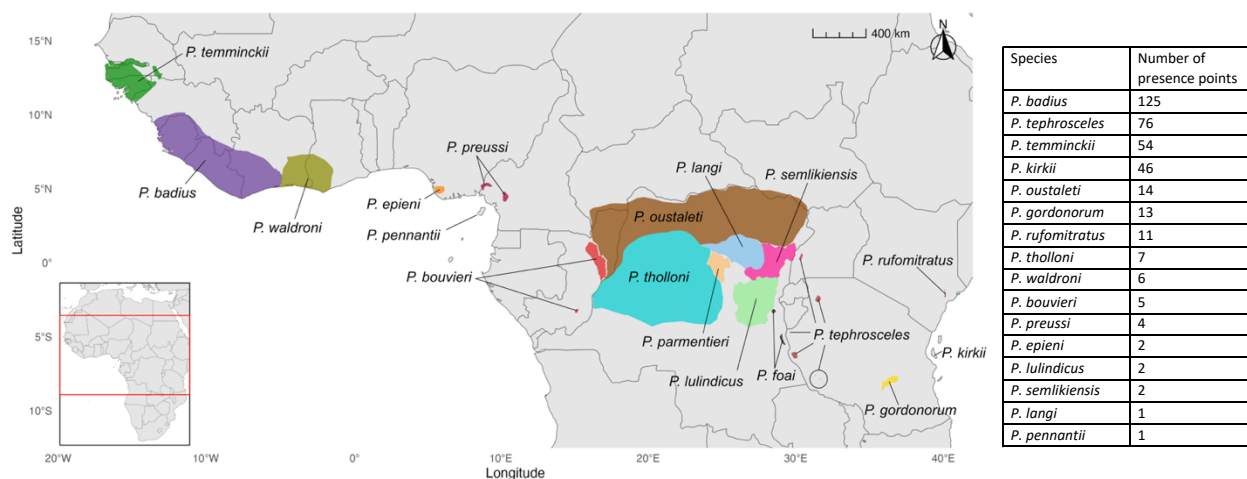


Figure 2.1: Current red colobus range estimates (Linder *et al.* 2021). The table specifies the number of presence points per species in our *Piliocolobus* presence/absence dataset.

Niche breadth and niche similarity between red colobus species

Generation of pseudo-presence points

In our *Piliocolobus* presence/absence dataset, most red colobus species did not have sufficient presence points to properly characterise their niche. Therefore, to be able to analyse niche breadth and overlap between species across the whole genus, we generated pseudo-presence points within the geographic range of each species (Linder *et al.* 2021). To avoid generating points in highly urbanised areas, we extracted the maximum 1993 Human Footprint Index (Venter *et al.* 2016) values for the true presence points of each species in our *Piliocolobus* presence/absence dataset (range = 1.25 – 30). We used the 1993 index because

this more closely matched the time period for which the true presence points were collected. We then removed areas from the geographic range of each species where the 2009 Human Footprint Index (Venter *et al.* 2016) value exceeded the maximum that the species could tolerate (i.e., the maximum value of the true presence points for the 1993 Human Footprint Index Dataset). We then randomly generated 150 points (or as many as possible given the constraints of the area and the minimum distance between points) that were at least 3 km apart in the remaining area of each species' range (Table 2.4, column 3). We used 3 km as the minimum distance between points to avoid pseudo-replication, because it is more than double the maximum mean day journey distance reported for red colobus (1040 m) and about 3 times the width of the largest mean home ranges (1 km²) (Korstjens *et al.* 2022). This dataset will subsequently be called the pseudo-presence dataset.

Environmental variables

To characterise the niche of each species, we compiled nine environmental layers: the tree cover for the year 2000 (Hansen *et al.* 2013), mean and standard deviation in NDVI (Normalised Difference Vegetation Index) from the years 1999-2019 (Copernicus Service information 2022) and six of the historical WorldClim 2.1 bioclimatic variables for 1970-2000 (BIO4 – temperature seasonality, BIO5 – maximum temperature of the warmest month, BIO7 – temperature annual range, BIO12 – annual precipitation, BIO15 – precipitation seasonality, BIO17 – precipitation of the driest quarter) (Fick and Hijmans 2017). We chose these variables because they are biologically relevant to the study species (Williams *et al.* 2021; Korstjens and Dunbar 2007) and they were not highly correlated with each other (Pearson's correlation coefficient < 0.85) (e.g. Syfert *et al.* 2013; Duan *et al.* 2014). All water bodies were removed from the raster layers using the European Space Agency global water bodies raster (ESA Land Cover CCI project team, 2016) to remove spurious NDVI values. The raster layers were resampled using the bilinear method so that they all had the same resolution and extent and were aggregated to a 3 km resolution by taking mean pixel values using the *raster* package (Hijmans 2023). A 3 km resolution was chosen to correspond with the minimum distance between pseudo-presence points in our pseudo-presence dataset.

Niche breadth and similarity between species

We measured the niche breadth for each species using the *ecospat.nichePOSNB* function in the *ecospat* package in R (Di Cola *et al.* 2017). Using this method, niche breadth is calculated as the standard deviation of each variable. This is a somewhat crude approximation of niche breadth but we chose this metric due to a lack of detailed information on diet, habitat use and ecology for many red colobus species which precluded us from characterising niche breadth

in a more biologically relevant way. We assumed equal abundance at each pseudo-presence site in our pseudo-presence dataset and used equal weighting of the 9 predictor variables. We also carried out a Pearson's correlation test to check for a correlation between niche breadth and geographic range size. We calculated geographic range size for each species using the *st_area* function in the *sf* package (Pebesma 2018; Pebesma and Bivand 2023) on the polygons for the current range estimates of each of the species in a WGS84 projection (Linder *et al.* 2021).

To calculate niche similarity between each pair of species, we carried out a similarity test developed by Broennimann *et al.* (2012) on our pseudo-presence dataset. First, the niche of each species was characterised in environmental space (Broennimann *et al.* 2012). This method, firstly, reduces the number of environmental variables to two using a principal components analysis, secondly, projects the principal components analysis scores of the species distribution onto a grid of cells and, thirdly, estimates the density of occurrences for each species in each grid cell using kernel density smoothing. We excluded the 5% lowest environmental density and species density values when characterising each species' niche to remove outlying data. Characterising the niche in environmental space in this way avoids the necessary assumptions of quantifying the niche in geographic space using species distribution modelling approaches (Broennimann *et al.* 2012). Based on visual inspection of the kernel densities, three species' niche estimates (*P. gordonorum*, *P. pennantii*, *P. tephrosceles*) suffered from a methodological artefact whereby the kernel is set incorrectly and the optimal environments are seemingly along the edge of the kernel where there is little to no data. As such, these three species were excluded from subsequent analyses (see Supplementary Material S2.1). Second, niche overlap between pairs of species was quantified using Schoener's D and Hellinger's I (Schoener 1968; Warren *et al.* 2008). These two metrics range from 0 representing no similarity to 1 representing high similarity. Finally, we carried out a niche similarity test (or background test) to assess whether the niches of pairs of species were significantly more similar than expected by chance given the set of available environments in their broad geographic regions (Warren *et al.* 2008). We chose the similarity test instead of the equivalency (or identity) test because the equivalency test often shows species to have different niches purely because different environments are available to them rather than them having different environmental preferences. The similarity test corrects for the differential availability of habitats between the two species and therefore rejecting the null hypothesis means that the species are more or less similar in their environmental distributions than expected given the available habitat in their respective regions (Warren *et al.* 2021). The similarity tests were carried out using 200 background points randomly generated without a

minimum distance restriction in a 50km radius of the pseudo-presence points. We used 1000 simulations to generate the null distribution and test for statistical significance in niche similarity. These analyses were carried out using the *ENMTools* package (Warren *et al.* 2021).

We then tested whether observed niche similarities between species can be explained by phylogenetic relationship. We first created a phylogenetic tree that included 10 out of the 18 red colobus species (*P. badius*, *P. bouvieri*, *P. epieni*, *P. langi*, *P. kirkii*, *P. oustaleti*, *P. preussi*, *P. rufomitratu*s, *P. temminckii*, *P. tholloni*) (Supplementary Material S2.2). The other 8 species were not included in the phylogenetic tree due to either a lack of sequence data and/or lack of presence data to quantify niche breadth. Mitochondrial sequence data from Ting (2008b) were aligned (with olive colobus, *Procolobus verus*, included as an outgroup) in Geneious for a 4,092 base pair dataset encompassing the regions spanning the ND3, ND4, and ND5 genes. A maximum likelihood tree was inferred using the RAxML plugin in Geneious with a GTR G+I model. We then fitted an ultrametric chronogram to the phylogenetic tree using a penalized maximum likelihood method to estimate divergences times in the *ape* package (Paradis and Schliep 2019). We tested for a relationship between divergence time and niche similarity (in this case measured using D and I statistics) using an age range correlation analysis (Fitzpatrick and Turelli 2006) implemented in the *phyloclim* package (Heibl and Calenge 2013). The age-range correlation analysis calculates overlap scores at each node in the phylogeny using nested averages of the overlaps among all the pairs of species across the two clades descending from the node. The overlap score of a node represents the average overlap between species after a given time since speciation (Fitzpatrick and Turelli 2006). To estimate the significance of the relationship, we obtained the slope of node age against niche similarity and compared this to the distribution of 10,000 slope and intercept values generated using Monte Carlo resampling of the niche overlap matrix under the null hypothesis of no phylogenetic signal (Cardillo and Warren 2016; Kolanowska *et al.* 2017).

Current and Future Red Colobus Distributions

Presence and absence data

In this analysis, we used the true location data from our *Piliocolobus* presence/absence dataset described above. We restricted this analysis to include only the four species for which we had more than 45 presence points (*P. badius*: N = 125, *P. kirkii*: N = 46, *P. tephrosceles*: N = 76, *P. temminckii*: N = 54). For each of these species, absence points were classified as all locations in our *Piliocolobus* presence/absence dataset where that species did not occur but where at least one other cercopithecoid did occur (this could be another *Piliocolobus* species or any other African cercopithecoid). We included only locations where at least one

other primate was reported to occur to limit our models to consider only locations that could potentially be inhabited by a primate. The alternative approach would be to select pseudo-absences across the landscape outside of the presence-locations (as is more typically done in SDMs). We decided against this because we wanted to understand red colobus niches at a more detailed scale. Using pseudo-absences across the landscape can lead to higher fits for the SDM but it tells us less about a specific species' habitat preference and more about general primate preferences, as many primates have a comparable fundamental preference for forested areas. Our approach means that our models may show a less strong fit because highly unsuitable areas are not considered, but it tells us more about how each specific red colobus species differs from a generic African monkey. It also means that we only included locations where primate surveys have been carried out, so we can be relatively confident that the species is absent, with the caveat that complete knowledge of true absence is difficult to achieve.

Environmental variables to build SDMs: climate and land use

We constructed our models using nine environmental variables covering climate and land use. To assess the impact of climate on red colobus distribution, we used the same six WorldClim v2.1 bioclimatic variables described above. We used the historical WorldClim dataset (average across 1970-2000) at 2.5 minutes resolution (~4.5 km at the equator), instead of the 3km data described above, to harmonise with the available land use data.

To assess the impact of land use on red colobus distribution, we characterised the proportional coverage of forest, cropland and grassland. We tested two land use datasets: Global Change Analysis Model (GCAM) v5 (Chen *et al.* 2020) and Chen *et al.*'s (2022) land use dataset (hereafter called the Chen dataset). We selected these datasets because they are some of the highest resolution available (0.05° (or ~5.5 km at the equator) and 1 km respectively). Coarse resolution land use datasets, even those at 10 km resolution, limit the ability to investigate the impacts of land use on species distribution given that land use change often occurs at small scales and even small changes in land use can have big impacts on biodiversity (Li *et al.* 2017; Carvalho *et al.* 2021). Additionally, some red colobus species have such restricted geographic ranges that land use data at a 10 km resolution may mask variation in land use within species ranges. Furthermore, these two datasets project land use into the future on a timescale comparable to the WorldClim dataset using a range of scenarios comprising Shared Socioeconomic Pathway (SSP) – Representative Concentration Pathway (RCP) combinations. Shared socioeconomic pathways (SSPs) represent alternative plausible future scenarios of aspects of society including demography, economy, policy, technology and

the environment (Kriegler *et al.* 2012; Popp *et al.* 2017; Riahi *et al.* 2017; O'Neill *et al.* 2017). Representative Concentration Pathways (RCPs) use radiative forcing to indicate future climate change possibilities (van Vuuren *et al.* 2011). Radiative forcing is the difference between the incoming and outgoing radiation (Hansen *et al.* 1997) which is affected by greenhouse gas levels in the atmosphere, among other things. The wide range of future scenarios available for these datasets meant we could select the future scenarios we wanted to investigate.

For the GCAM dataset, we used the model mean dataset which represents the mean across five General Circulation Models (i.e., mathematical models to simulate the Earth's climate system): GFDL-ESM2M, HadGEM2-ES, IPSL-CM5A-LR, MIROC5 and NorESM-M. We did this to capture variation in climatic predictions. To represent current land use, we used the mean of the 2015, 2020 and 2025 datasets for the SSP2-4.5 scenario. We used this average because the GCAM model does not provide a definitive current land use estimate and we chose this scenario as it represents a “middle of the road” scenario. We resampled the rasters using the bilinear method implemented using the *raster* package (Hijmans 2023) to match the WorldClim variables' resolution, projection and grid. We then reclassified the land use types into broader classifications relevant to this study using the classification system suggested in Chen *et al.* (2020) (Table 2.1). The GCAM land use dataset consists of 33 layers, one layer for each land use type. Each pixel value represents the proportional area of that pixel made of that land use type. Therefore, to reclassify the land use types, we summed the pixel values across the layers in each grouping. As such, the pixel values in our land use types represent the proportion of the pixel that is made up of forest, grassland or cropland.

Table 2.1: Land classification among the GCAM dataset and the one used in this study

Land classification in our study	Land classification in GCAM
Forest proportion	Needleleaf evergreen tree - temperate Needleleaf evergreen tree – boreal Needleleaf deciduous tree - boreal Broadleaf evergreen tree - tropical Broadleaf evergreen tree - temperate Broadleaf deciduous tree - tropical Broadleaf deciduous tree - temperate Broadleaf deciduous tree - boreal
Grassland proportion	C3 Arctic C3 Grass

	C4 Grass Broadleaf evergreen shrub - temperate Broadleaf deciduous shrub - temperate Broadleaf deciduous shrub- boreal
Cropland proportion	Corn: rainfed Corn: irrigated Wheat: rainfed Wheat: irrigated Soybean: rainfed Soybean: irrigated Cotton: rainfed Cotton: irrigated Rice: rainfed Rice: irrigated Sugar crop: rainfed Sugar crop: irrigated Other crop: rainfed Other crop: irrigated Bioenergy crop: rainfed Bioenergy crop: irrigated

For the Chen dataset (Chen *et al.* 2022), we used the current land use layer from 2015 to build the SDMs. This dataset assigns a land use category to each pixel, so to calculate proportional land use for each pixel we aggregated the pixels to 5km resolution and calculated the proportional coverage of forest, cropland and grassland in the aggregated pixels. We then resampled the raster using the bilinear method to match the WorldClim variables.

None of the variables (both land use and climatic) were highly correlated with each other (Pearson's correlation coefficient < 0.85) (e.g. Syfert *et al.* 2013; Duan *et al.* 2014). All variables were cropped to the study area.

Species distribution models

We extracted the variable values for the presence and absence points using the *gridRecords* function in the *fuzzySim* package (Barbosa 2015). This function returns the centroid latitude and longitude of each pixel containing a presence or absence point, the extracted value for that pixel for each variable and whether the species was present or absent in that pixel. Pixels

that contain at least one presence point were classified as a species presence and pixels that contain at least one absence point and no present points were classified as species absence. We used this method because it removes duplicates within each pixel and thins the presence points with a distance equal to the raster pixel size to reduce the spatial autocorrelation. The resulting number of presence and absence points for each species is given in Table 2.2.

Table 2.2: *The number of presence and absence points for each of the four focal red colobus species using the gridRecords function on our Piliocolobus presence/absence dataset. The absence points are the collective locations of all other cercopithecoid species in the study area.*

Species	Number of presence points	Number of absence points
<i>P. badius</i>	64	366
<i>P. kirkii</i>	23	407
<i>P. temminckii</i>	44	386
<i>P. tephrosceles</i>	66	364

We built our SDMs using a promising new method for species distribution modelling: Bayesian Additive Regression Trees (BART). We did this using the package *embarcadero* in R, a wrapper for the *dbarts* package (Carlson 2020; Dorie 2020; Baquero *et al.* 2021; Carlson *et al.* 2022). BART is an alternative tree method that uses a Bayesian approach to estimate the probability of species presence based on decision trees that explain different components of variance in the outcome variable. BART performs comparably to other classification and regression tree methods (Chipman *et al.* 2010), but being a Bayesian method, BART benefits from providing posterior distributions on predictions, allowing us to assess the uncertainty of the model and create partial dependence plots.

We built three sets of BART models using the function defaults containing different combinations of predictor variables: 1) GCAM and WorldClim, 2) Chen and WorldClim, and 3) WorldClim only. Each set contained four models – one per study species (12 models total).

Model validation

We validated these models using a 5-fold block-cross validation approach with a block size of 50 km² (10km² for *P. kirkii* because their range is restricted to the island of Unguja in Zanzibar, total area = 1666 km²) using the R package *BLOCKCV* (Valavi *et al.* 2018). Block-cross validation partitions the study area into spatial blocks of a specified size and assigns each block to a fold. It then iterates through the folds, running the model using the data outside of the fold and then evaluates the predictive capacity of the model using the data in the fold. This allows the data to be used in both the training and testing datasets. We evaluated model

performance using the area under the receiver operating characteristic curve (AUC) statistic, the area under the precision-recall curve (AUC-PR) statistic, which can be more informative than the AUC on imbalanced datasets, and the True Skill Statistic (TSS). Both AUC statistics assess a model's discrimination performance where a value of 1.0 indicates a model that perfectly predicts a given species' distribution and contains predictors that strongly predict species presence. A value of 0.5 indicates high levels of model uncertainty and the predictors that have little predictive ability (Landis and Koch 1977). The TSS measures classification performance, i.e., the proportion of correctly classified presences and absences. These statistics were generated using the R package *ModEvA* (Barbosa *et al.* 2013) and were averaged across the 5 block-cross validation folds for each species. To investigate which variables had the biggest influence on determining where species were present or absent we created variable importance plots and to visualise the relationship between each predictor and species presence we created partial dependence plots.

Predicted suitable habitat for 2070

We projected each SDM to the year 2070 to calculate predicted habitat loss or gain for each species under future climate change and land use change. For the future projections, we used four future scenarios represented by Shared Socioeconomic Pathway (SSP) – Representative Concentration Pathway (RCP) combinations. Phase 6 of the Coupled Model Intercomparison Project (CIMP6) recommend coupling SSP-RCP scenarios to make research more comparable across studies and recommend some of the most likely scenarios as high priority SSP-RCP couplings for future research. In this study we focus on the four Tier 1 coupling scenarios designated by CIMP6: SSP1-2.6, SSP2-4.5, SSP3-7.0 and SSP5-8.5 (Table 2.3; van Vuuren *et al.* 2014). For the GCAM land use dataset, SSP3-7.0 was not available so we used the SSP3-6.0 scenario instead, meaning that the radiative forcing was set to 6.0 W/m² instead of 7.0 W/m².

For each scenario, the future climate was characterised by taking the mean of the 2070 WorldClim datasets across four General Circulation Models: GISS-E2-1-H, INM-CM4-8, IPSL-CM6A-LR and MIROC6. These were chosen because they had data available for all the future scenarios and represent distantly related models as recommended by Sanderson *et al.* (2015). To characterise future land use, we took the mean across four years (years 2065, 2070, 2075, 2080) for each of the two land use datasets (GCAM dataset and Chen dataset). For the GCAM datasets, we used the downloadable model means of the five General Circulation Models as we did for the scenario of 'current conditions'. We processed the future

conditions datasets in the same way as their respective current conditions datasets as described above.

Table 2.3: Description of the four scenarios (which represent combinations of the Shared Socio-Economic Pathways (SSP) and the Representative Concentration Pathways (RCP)) used to project red colobus distribution in 2070 under climate change and land use change. Summarised from O'Neill et al. 2014, van Vuuren et al. 2014, Popp et al. 2017. CO² = carbon dioxide, ppm = parts per million, W/m² = watts per metre squared.

Scenario combination	Description
SSP1-2.6 (SSP1 + RCP2.6)	This is the most optimistic scenario in which climate change and land use change are highly regulated. As such, the rate of change in radiative forcing declines, resulting in 2.6 W/m ² radiative forcing by 2100 (equivalent CO ² concentration of 450ppm) and land use change slows in comparison to previous decades.
SSP2-4.5 (SSP2 + RCP4.5)	This is an intermediate scenario with moderate regulation of climate change and land use change. As a result, this scenario represents a stabilising rate of change in radiative forcing with 4.5 W/m ² radiative forcing by 2100 (equivalent CO ² concentration of 650ppm) and continuing land use change but declining over time.
SSP3-7.0 (SSP3 + RCP7.0)	This is the worst-case scenario in terms of land use change. This scenario represents little regulation of climate change and land use change leading to 7.0 W/m ² radiative forcing by 2100 (equivalent CO ² concentration of 850ppm) and continued deforestation.
SSP5-8.5 (SSP5 + RCP5.8)	This is worst-case scenario in terms of climate change. This scenario represents a rising rate of change in radiative forcing with 8.5 W/m ² radiative forcing by 2100 (equivalent CO ² concentration of 1350ppm). Additionally, land use is incompletely regulated so that land use change still occurs but declines over time.

Quantifying change in suitable habitat between now and 2070

After projecting our models to predict 'probability of presence' values (i.e., suitability value) for all sites in the year 2070, we translated the resulting probability value into a binary value of presence or absence using a cut-off probability value. The cut-off probability value for predicted presence versus absence was determined based on the suitability threshold that produced the greatest fit in each species' current-conditions model (i.e., maximised true skill statistic in each model) (Pinto-Ledezma and Cavender-Bares 2021). We did this using the *optiThresh* function in the *modEvA* package (Barbosa et al. 2013). We then calculated the

loss or gain of predicted areas of presence between the current time period and 2070. We did this within the current geographic range of each species and for 100 km, 250 km and 500 km buffers around the range for *P. badius*, *P. temminckii* and *P. tephrosceles*, to allow for potential migration scenarios. For *P. kirkii*, instead of using these buffers, we calculated the predicted habitat change for the island of Unguja where this species is endemic, under the assumption that colobus will not be able to migrate from this island.

We then quantified each species' vulnerability to future climate change and land use change as the percentage change in the area of predicted presence between the present and 2070 for the current geographic range and the buffer zones calculated as (Zhang *et al.* 2017):

$$\frac{AreaFuture - AreaCurrent}{AreaCurrent} * 100$$

All statistical analyses were conducted in RStudio (RStudio Team 2020) and code is available on my GitHub. The code is currently private and invitations have been sent to examiners separately.

Results

Ecological niche breadth and similarity between red colobus species

Niche breadth varied greatly among the 16 species (range 8.2 – 90.2; Table 2.4). *P. badius* had the broadest niche while *P. langi* had the narrowest niche (Table 2.4). Niche breadth was not correlated with geographic range size (Pearson's $r(14) = 0.252$, $p\text{-value} = 0.35$).

Most pairs of species exhibited low niche similarity based on both Schoener's *D* and Hellinger's *I* (Table 2.5). Only eight pairs of species for Schoener's *D* and six pairs of species for Hellinger's *I* had niches that were more similar than expected by chance (Table 2.5). In general, niche similarity was significantly greater than expected by chance between species found in the central African group (*P. bouvieri* - *P. langi*, *P. langi* - *P. semlikiensis*, *P. langi* - *P. tholloni* and *P. thollonii* - *P. semlikiensis*); between *P. badius*, *P. waldroni* and *P. kirkii* (all three pairwise comparisons) and between *P. oustaleti* (in the central African group) and *P. epieni* (found in Nigeria) (Table 2.5).

There was no phylogenetic signal in the niche similarity among red colobus species: the age-range correlation was not significant for niche overlap measured by either *D* or *I* (Table 2.6).

Table 2.4: The niche breadth for each red colobus species calculated using randomly generated pseudo-presence points within the geographic range of each species and equal weighting of 9 environmental variables and the number of pseudo-presence points per species in the pseudo-presence dataset. This dataset was used to calculate niche breadth and characterise the niche of each species.

Species	Niche breadth mean	Number of pseudo-presence points
<i>P. badius</i>	90.2	150
<i>P. temminckii</i>	66.3	150
<i>P. tephrosceles</i>	29.5	150
<i>P. waldroni</i>	28.8	150
<i>P. tholloni</i>	27.6	150
<i>P. pennantii</i>	24.9	13
<i>P. lulindicus</i>	24.1	150
<i>P. gordonorum</i>	23.2	150
<i>P. semlikiensis</i>	22.2	150
<i>P. oustaleti</i>	20.8	150
<i>P. epieni</i>	20.4	127
<i>P. kirkii</i>	19.4	35
<i>P. preussi</i>	13.9	150
<i>P. bouvieri</i>	11.7	150
<i>P. langi</i>	9.4	150
<i>P. rufomitratu</i>	8.2	32

Table 2.5: Niche similarity between 13 red colobus species based on the Schoener's *D* (above the diagonal) and Hellinger's *I* (below the diagonal) statistics with corresponding *p*-values for the similarity test in brackets below each estimate. Values highlighted in red are those that fell significantly above the 1000 randomised values, indicating significant niche similarity (alpha level = 0.05). The red colobus species are ordered from west to east in terms of the west-most boundary of their geographic range.

	<i>P. temminckii</i>	<i>P. badius</i>	<i>P. waldroni</i>	<i>P. epieni</i>	<i>P. preussi</i>	<i>P. bouvieri</i>	<i>P. tholloni</i>	<i>P. oustaleti</i>	<i>P. langi</i>	<i>P. lulindicus</i>	<i>P. semlikiensis</i>	<i>P. rufomitratu</i>	<i>P. kirkii</i>
<i>P. temminckii</i>		0.003 (0.329)	0 (0.453)	0 (0.476)	0 (0.481)	0 (0.464)	0 (0.470)	0 (0.464)	0 (0.468)	0 (0.461)	0 (0.472)	0.010 (0.229)	0 (0.250)
<i>P. badius</i>	0.008 (0.328)		0.350 (0.043)	0.126 (0.194)	0.115 (0.277)	0.102 (0.261)	0.236 (0.148)	0.431 (0.114)	0.050 (0.168)	0.087 (0.311)	0.114 (0.160)	0.035 (0.304)	0.524 (0.018)
<i>P. waldroni</i>	0 (0.449)	0.588 (0.034)		0.065 (0.470)	0.036 (0.477)	0 (0.413)	0.008 (0.290)	0.207 (0.254)	0 (0.464)	0 (0.373)	0 (0.462)	0.006 (0.332)	0.509 (0.012)
<i>P. epieni</i>	0 (0.455)	0.318 (0.190)	0.136 (0.470)		0.072 (0.215)	0.023 (0.299)	0.077 (0.215)	0.314 (0.039)	0 (0.413)	0.007 (0.302)	0.048 (0.193)	0 (0.476)	0 (0.356)
<i>P. preussi</i>	0 (0.457)	0.217 (0.327)	0.060 (0.498)	0.140 (0.236)		0 (0.431)	0.086 (0.177)	0.081 (0.190)	0 (0.466)	0.008 (0.461)	0 (0.454)	0 (0.480)	0.005 (0.253)
<i>P. bouvieri</i>	0 (0.453)	0.223 (0.248)	0 (0.409)	0.028 (0.337)	0 (0.406)		0.321 (0.081)	0.241 (0.193)	0.453 (0.038)	0.230 (0.128)	0.465 (0.052)	0 (0.447)	0 (0.423)
<i>P. tholloni</i>	0 (0.466)	0.268 (0.209)	0.012 (0.306)	0.175 (0.236)	0.161 (0.167)	0.553 (0.058)		0.293 (0.122)	0.257 (0.012)	0.376 (0.135)	0.506 (0.042)	0 (0.476)	0 (0.471)
<i>P. oustaleti</i>	0 (0.459)	0.604 (0.133)	0.268 (0.345)	0.536 (0.043)	0.101 (0.251)	0.394 (0.185)	0.372 (0.167)		0.090 (0.165)	0.151 (0.168)	0.235 (0.121)	0 (0.469)	0.241 (0.100)
<i>P. langi</i>	0 (0.456)	0.121 (0.158)	0 (0.447)	0 (0.381)	0 (0.437)	0.555 (0.079)	0.500 (0.007)	0.158 (0.166)		0.218 (0.180)	0.496 (0.023)	0 (0.466)	0 (0.459)
<i>P. lulindicus</i>	0 (0.451)	0.124 (0.354)	0 (0.371)	0.009 (0.323)	0.010 (0.450)	0.314 (0.175)	0.537 (0.151)	0.171 (0.212)	0.350 (0.194)		0.509 (0.050)	0 (0.468)	0 (0.450)
<i>P. semlikiensis</i>	0 (0.456)	0.189 (0.172)	0 (0.460)	0.059 (0.229)	0 (0.436)	0.592 (0.090)	0.680 (0.053)	0.294 (0.171)	0.689 (0.023)	0.659 (0.069)		0 (0.475)	0 (0.469)
<i>P. rufomitratu</i>	0.176 (0.222)	0.138 (0.273)	0.008 (0.347)	0 (0.459)	0 (0.451)	0 (0.441)	0 (0.465)	0 (0.456)	0 (0.444)	0 (0.457)	0 (0.454)		0.022 (0.429)
<i>P. kirkii</i>	0 (0.247)	0.682 (0.014)	0.652 (0.018)	0 (0.339)	0.009 (0.264)	0 (0.414)	0 (0.464)	0.281 (0.157)	0 (0.443)	0 (0.440)	0 (0.461)	0.086 (0.411)	

Table 2.6: Results of the age-range correlation analyses of phylogenetic signal in niche similarity measured by Schoener's *D* (*D*) and Hellinger's *I* (*I*) using 10000 randomisation tests under Monte Carlo resampling. *f* (greater) is the fraction of randomized datasets with intercept and slopes greater than the observed data and the corresponding *P*-values (*P*) are calculated as $2 * \min(f, 1 - f)$.

	Intercept	f (greater)	Slope	f (greater)
D	0.006	0.491 (<i>P</i> = 0.982)	-0.014	0.343 (<i>P</i> = 0.685)
I	0.016	0.485 (<i>P</i> = 0.969)	-0.040	0.353 (<i>P</i> = 0.705)

Current and Future Red Colobus Distributions

Model validation

We ran models only for the four species for which we had sufficient presence points to build robust SDMs. The predictive accuracy of the models estimated by block-cross validation was consistently above the typically accepted performance thresholds (AUC = 0.7, AUC-PR = 0.7, TSS = 0.5, Landis and Koch 1977) (Table 2.7).

Table 2.7: Mean AUC, AUC-PR and TSS across the 5 folds of the block cross validation for each of the models. AUC = area under the receiver operating characteristic curve statistic, AUC-PR = area under the precision-recall curve which can be more informative than the AUC on imbalanced datasets and TSS = True Skill Statistic. Typically accepted performance thresholds are AUC = 0.7, AUC-PR = 0.7, TSS = 0.5 (Landis and Koch 1977).

Modelling approach	Species	AUC	AUC-PR	TSS
WorldClim only	<i>P. badius</i>	0.97	0.81	0.82
	<i>P. kirkii</i>	1	0.93	0.90
	<i>P. temminckii</i>	0.98	0.76	0.88
	<i>P. tephrosceles</i>	0.96	0.76	0.81
GCAM and WorldClim	<i>P. badius</i>	0.97	0.79	0.85
	<i>P. kirkii</i>	1	0.98	0.88
	<i>P. temminckii</i>	0.97	0.79	0.86
	<i>P. tephrosceles</i>	0.98	0.85	0.83
Chen and WorldClim	<i>P. badius</i>	0.97	0.79	0.82
	<i>P. kirkii</i>	1	0.93	0.86
	<i>P. temminckii</i>	0.97	0.79	0.87
	<i>P. tephrosceles</i>	0.96	0.76	0.82

Variable importance and relationship with species presence

Across the 4 species of red colobus for which we had sufficient presence points, we found that different climatic and land use variables were most important for different species, however all variables had similar values for variable importance within a given model (range 0.1 – 0.2, the maximum difference in importance between the least and most important variable in a model was 0.04, Figure 2.2). Precipitation seasonality (BIO 15) was most often the most important predictor and temperature seasonality (BIO 4) was most often the least important predictor. In the GCAM and WorldClim models, proportion of grassland was generally quite an important predictor whereas in the Chen and WorldClim models it was the least important of the three land use predictors. In general, for the Chen and WorldClim models, the land use variables were found to be of lower importance whereas for the GCAM and WorldClim models, they were of higher importance. The relationship between each variable and the predicted outcome varied by species and land use dataset used. In both the GCAM and WorldClim models and the Chen and WorldClim models, some species were shown to prefer areas with a higher proportion of cropland and grassland, but the confidence intervals around each relationship were large (Supplementary Material S2.3).

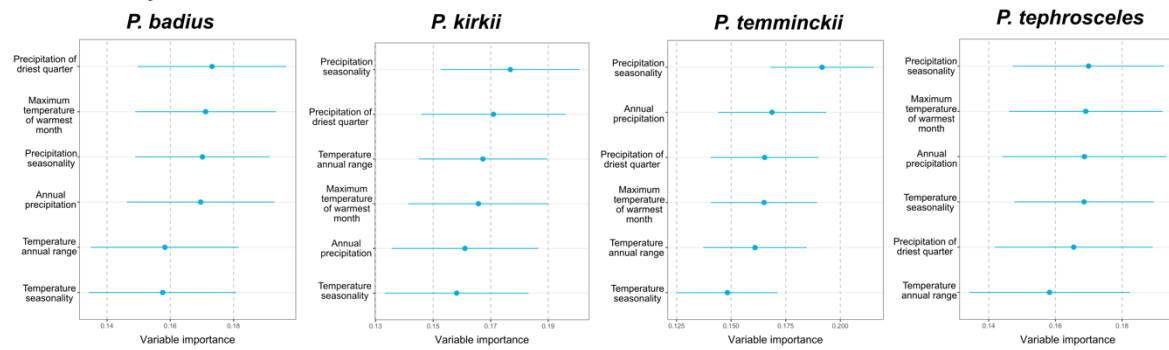
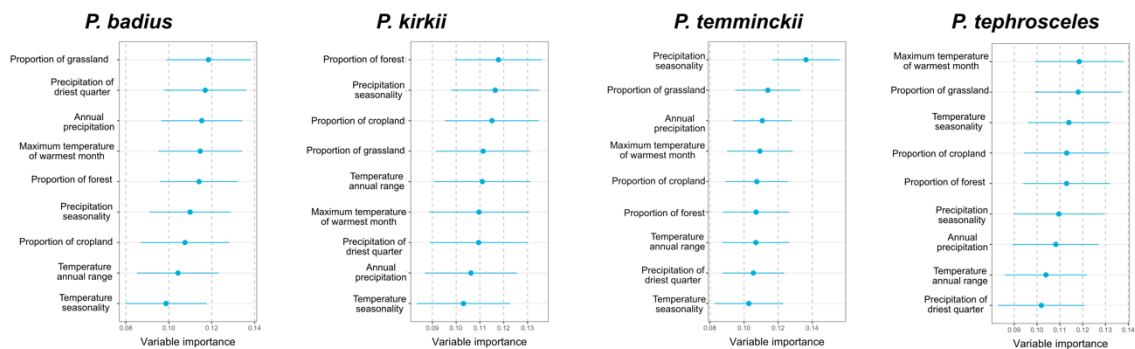
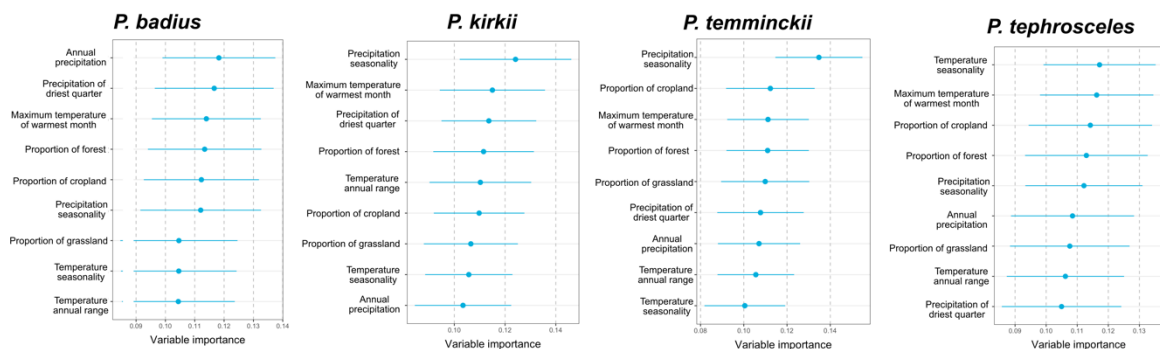
WorldClim only models:**GCAM and WorldClim models:****Chen and WorldClim models:**

Figure 2.2: Variable importance for the Bayesian Additive Regression Tree Species Distribution Models for each of the 4 species of red colobus for which we had sufficient presence points. Variable importance is measured as the number of times a variable is used to split sites into presence and absences in the underlying decision trees. The lines represent standard deviations based on model permutations.

Predicted suitable habitat change between now and 2070

In general, the predicted pattern of future suitable habitat for each species in each scenario differed between models built using different datasets (Figure 2.3, Supplementary Material S2.4). The Chen and WorldClim models gave more similar predictions to the WorldClim only

models than the GCAM and WorldClim models did (Figure 2.3). In one particular case, there was a large discrepancy between the prediction from the WorldClim only models and GCAM and WorldClim models, where the percentage loss of suitable habitat in *P. kirkii*'s current range was 100% loss for the GCAM and WorldClim model and 0% loss for both the Chen and WorldClim and WorldClim only models.

In general the smallest losses or biggest gains in area of predicted presence were found for the best-case scenario for both land use and climate change (scenario SSP1-2.6) and the biggest losses or smallest gains were seen for the scenario with no mitigation of climate change and medium mitigation of land use change (SSP5-8.5) (Table 2.3). However, there were some exceptions to this general rule for the GCAM model set whereby, for *P. temminckii* and *P. tephrosceles*, scenario SSP1-2.6 provided the worst future scenario (smallest gain and biggest loss in suitable habitat respectively) and SSP2-4.5 provided the best future scenario (the biggest gain and smallest loss in suitable habitat respectively) (Figure 2.3).

P. badius were predicted to lose suitable habitat in their current range (range -2.9% and -22.7%) for all scenarios and model sets (Figure 2.3a-c). Most of this loss occurred in the south-eastern part of their current range (Figure 2.3a-c). Similarly, they were predicted to lose suitable habitat in the buffer areas (area around the current range where red colobus could disperse) in all but one scenario where there was a small increase in predicted area of suitable habitat (+0.5% for SSP1-2.6 in the WorldClim only model set, Figure 2.3a).

The *P. kirkii* models showed mixed results. Under the WorldClim only and Chen and WorldClim models, *P. kirkii* did not lose any suitable habitat from its current range under any future scenario but in the GCAM and WorldClim model they lost 80% of their suitable habitat under the SSP1-2.6 scenario and 100% under the other three future scenarios (Figure 2.3d-f). On the island more generally (representing potential dispersal), both the Chen and WorldClim and WorldClim only models (Figure 2.3d,f) predicted similar percentage loss of suitable habitat across the range of future scenarios (range 16.7% to 45.8% loss). Both models predicted that suitable habitat would generally be lost from the north-western part of the island. However, the GCAM and WorldClim model predicted much higher losses of suitable habitat across the island (49.1% to 83% loss), mainly occurring in the centre of the island, leaving pockets of remaining suitable habitat along the coastline (Figure 2.3e).

P. temminckii was the only species that was predicted to increase its area of presence under future change (Figure 2.3g-i). In every scenario and model set, this species showed an

increase in the predicted area of presence within their current range. The percentage increase in area did not vary much between scenarios and model sets, with the increase being 2-4% for all but one case: SSP2-4.5 for the GCAM and WorldClim model which predicted a 10% increase in area (Figure 2.3h). All the increased area occurred in the south-eastern part of their current range, the only area currently unsuitable for them. Within the buffer areas, the change in predicted area of suitable habitat was more mixed. The WorldClim only model predicted that the area of suitable habitat would generally decline (Figure 2.3g), meanwhile the GCAM and WorldClim model predicted that the area of suitable habitat would increase in all scenarios and buffer sizes (Figure 2.3h). Most of this increase was to the north-east of the current species range. For the Chen and WorldClim model, there were mixed results with some scenarios showing increased predicted area of suitable habitat in some buffers, and others showing a decrease (Figure 2.3i).

P. tephrosceles were predicted to lose area of suitable habitat from its current range (range 9.5% to 42.4% loss) in all but one scenario (SSP3-6.0 in the GCAM and WorldClim model, +9.1%) (Figure 2.3j-l). In all cases, there was a predicted loss of suitable habitat in the southern half of its current range. In the buffer areas, all three models predicted a reduction in suitable habitat for all scenarios (range 6.2% to 60.8% loss) (Figure 2.3j-l). Again, in the buffer areas, this predicted loss was mostly in the southern half of the area.

Based on maps of the changes in proportional forest cover between now and 2070 (Supplementary Material S2.5), areas of high predicted forest loss do not align with areas predicted to become unsuitable for red colobus, suggesting that geographic changes in forest cover are not driving the changes in the area of suitable habitat we predicted for red colobus.

Comparing red colobus species' niche breadth, niche overlap and response to environmental change

Of the four species we investigated, *P. tephrosceles* was predicted to have the largest losses in suitable habitat in their current range under future climate change and land use change (range +9.1% to -42.4%) followed by *P. badius* (range -2.9% to -22.7%). *P. kirkii* could be classified as suffering the largest or third largest loss, with the GCAM and WorldClim model predicting up to 100% loss of habitat in their current range and the other two models predicting no loss of habitat in their current range. Finally, *P. temminckii* was the only taxon predicted to gain suitable area under future environmental change (2-4% within its current range). Regardless of whether *P. kirkii* is classified as having the largest or third largest loss of habitat in its current range, the change in available habitat in the future was not linked to niche breadth

among red colobus species (Table 2.4, column 2) nor with the size of their current geographic range.

Of these four species, we were able to determine niche overlap for three of them (*P. tephrosceles* was excluded based on visual inspection of the niche estimate kernel densities). *P. badius* and *P. kirkii* showed significant niche overlap whereas *P. temminckii* did not show niche overlap with either of these species. *P. temminckii* was predicted to gain area of suitable habitat under future change whereas *P. kirkii* and *P. badius* were predicted to lose suitable habitat or experience no change. This may reflect a decline in the availability of habitat that falls within the niche of both *P. kirkii* and *P. badius* and an increase in the availability of habitats within *P. temminckii*'s niche.

Figure 2.3a: *P. badius* - WorldClim only models

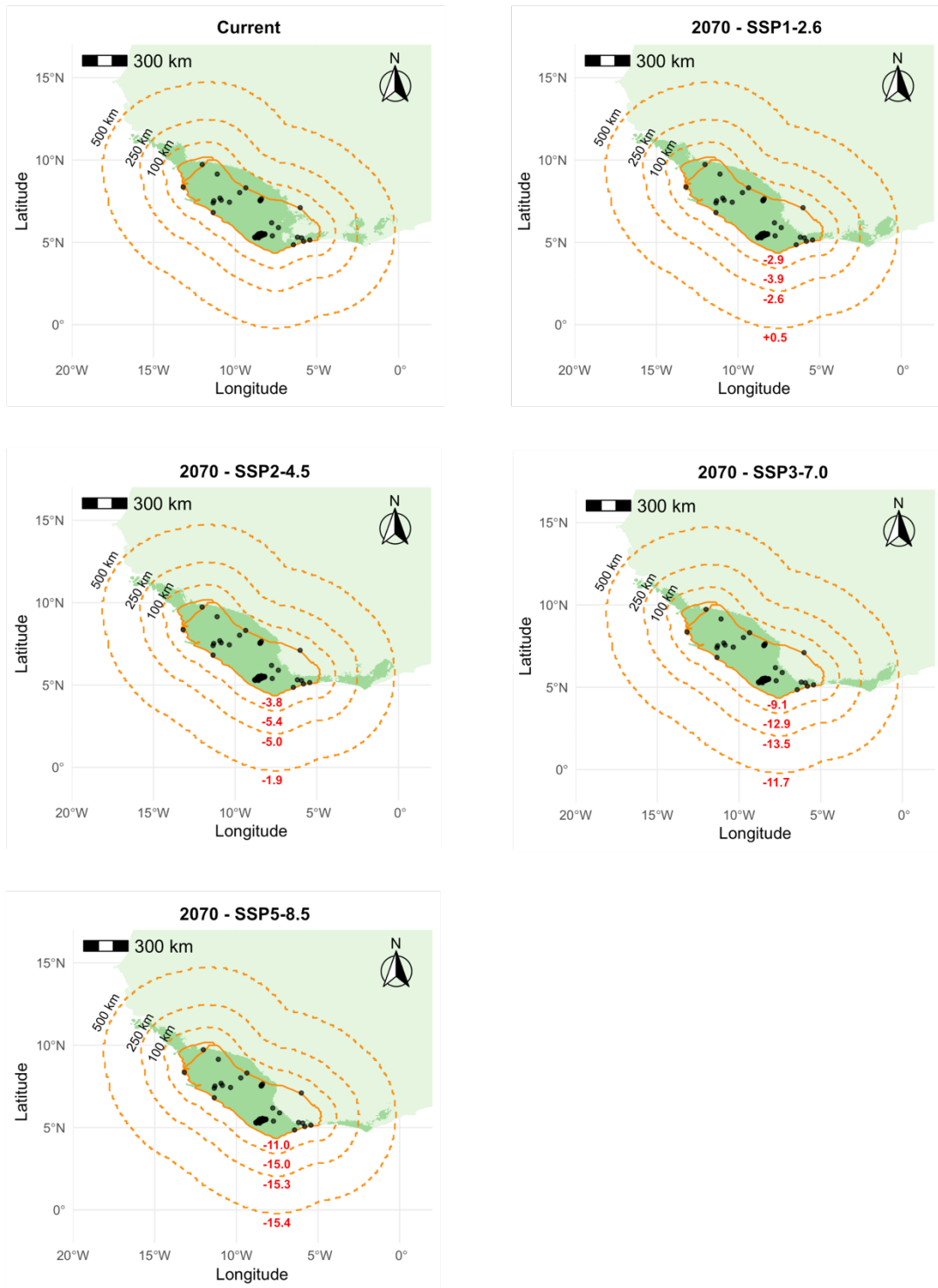


Figure 2.3b: *P. badius* - GCAM and WorldClim models

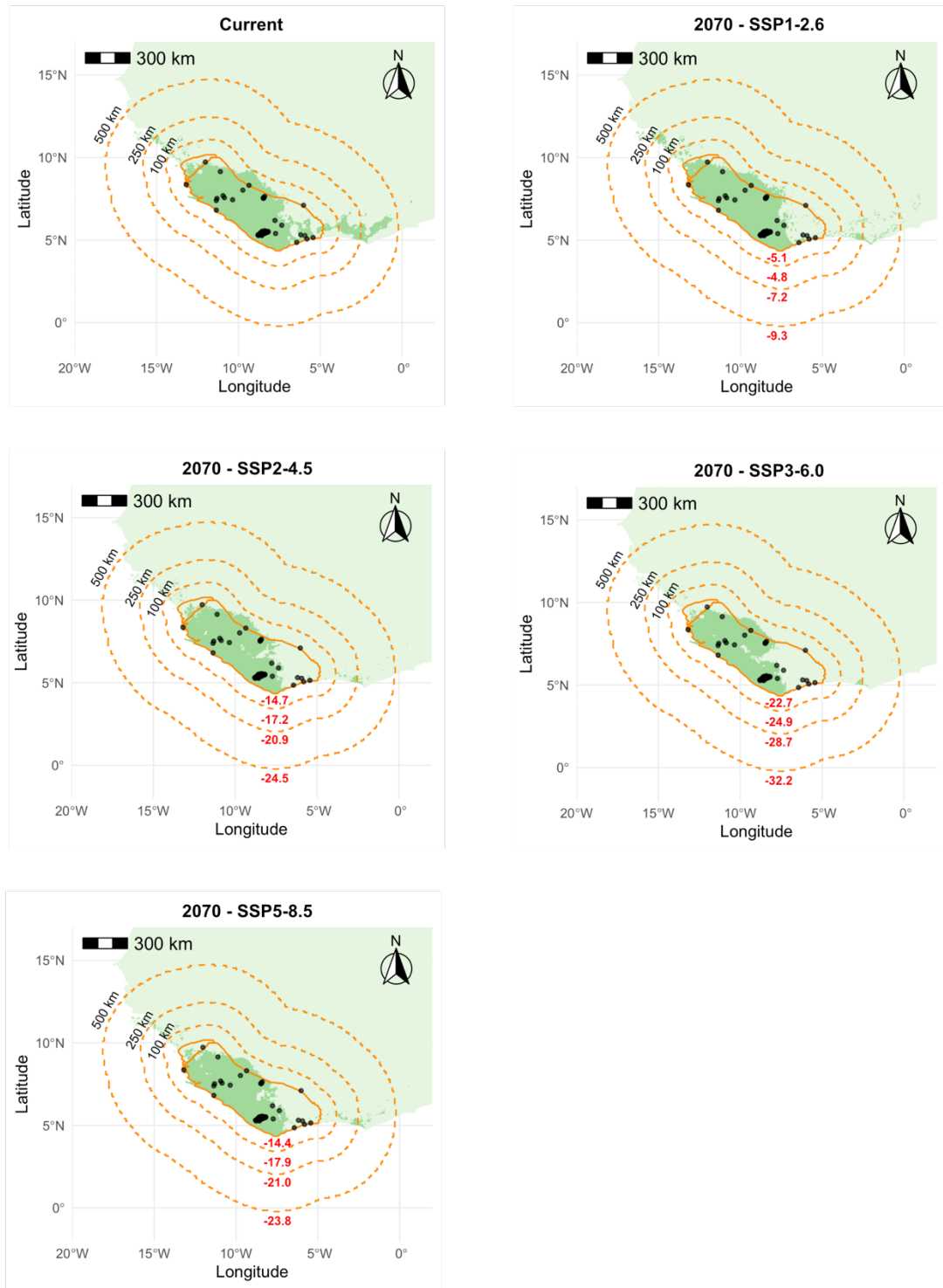


Figure 2.3c: *P. badius* - Chen and WorldClim models

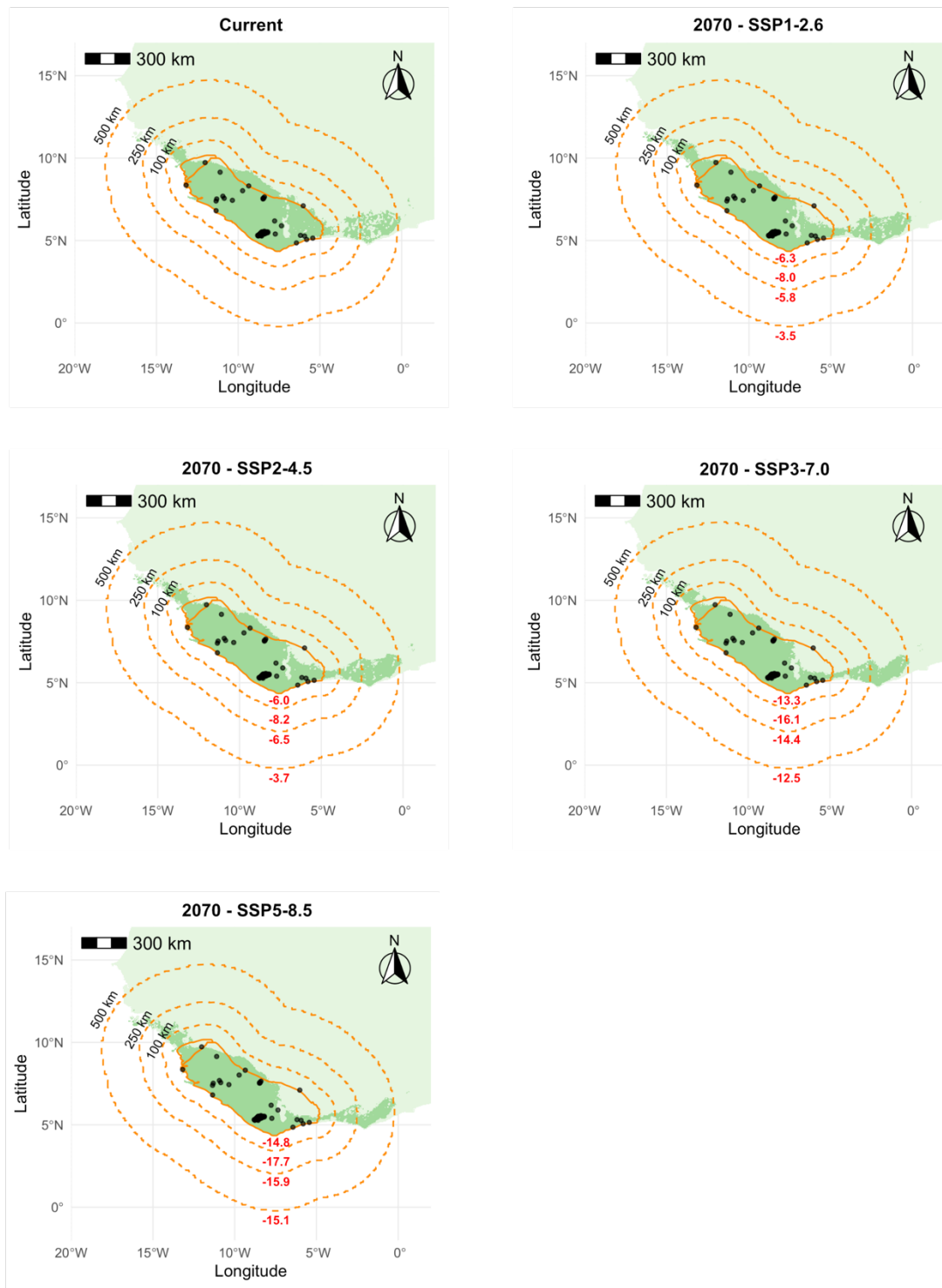


Figure 2.3d: *P. kirkii* - WorldClim only models

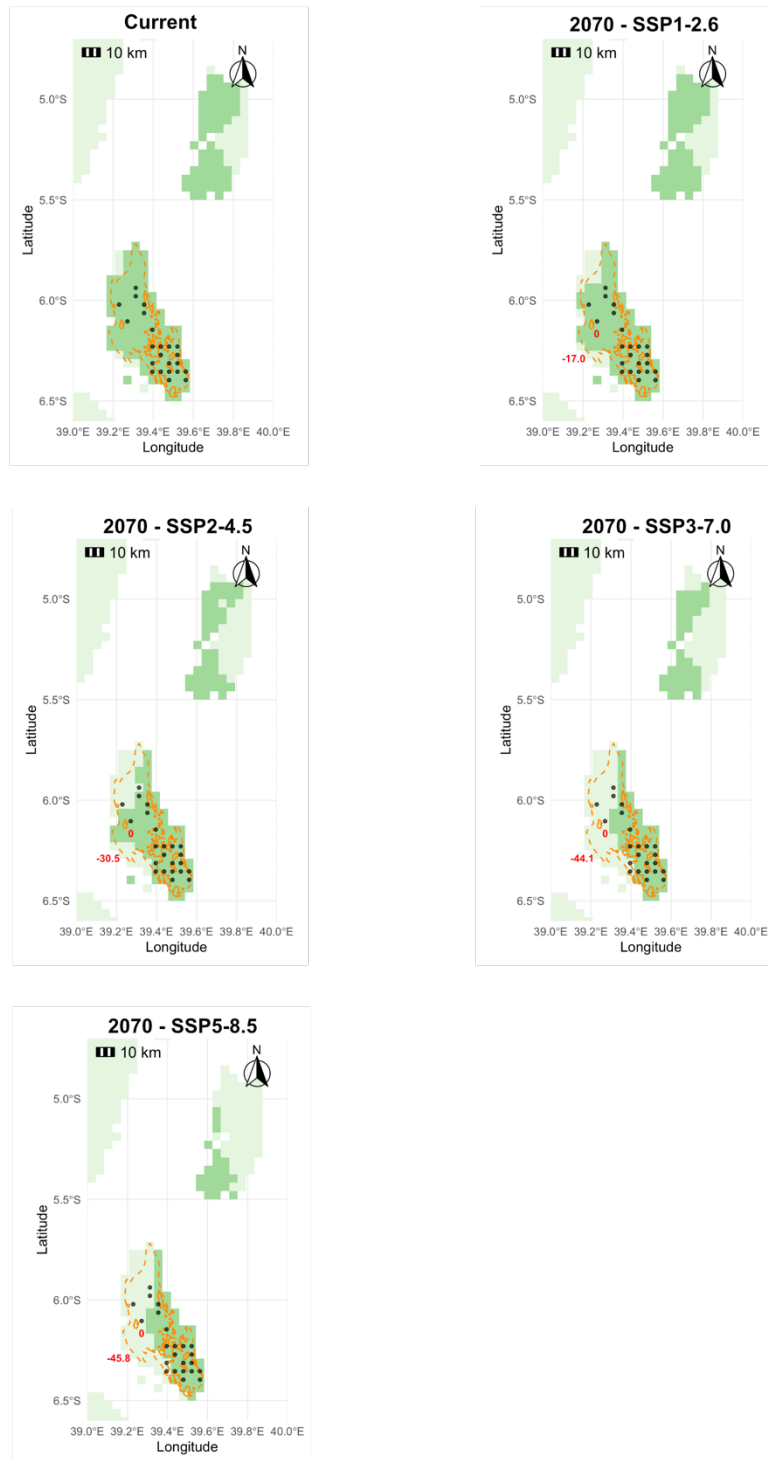


Figure 2.3e: *P. kirkii* - GCAM and WorldClim models

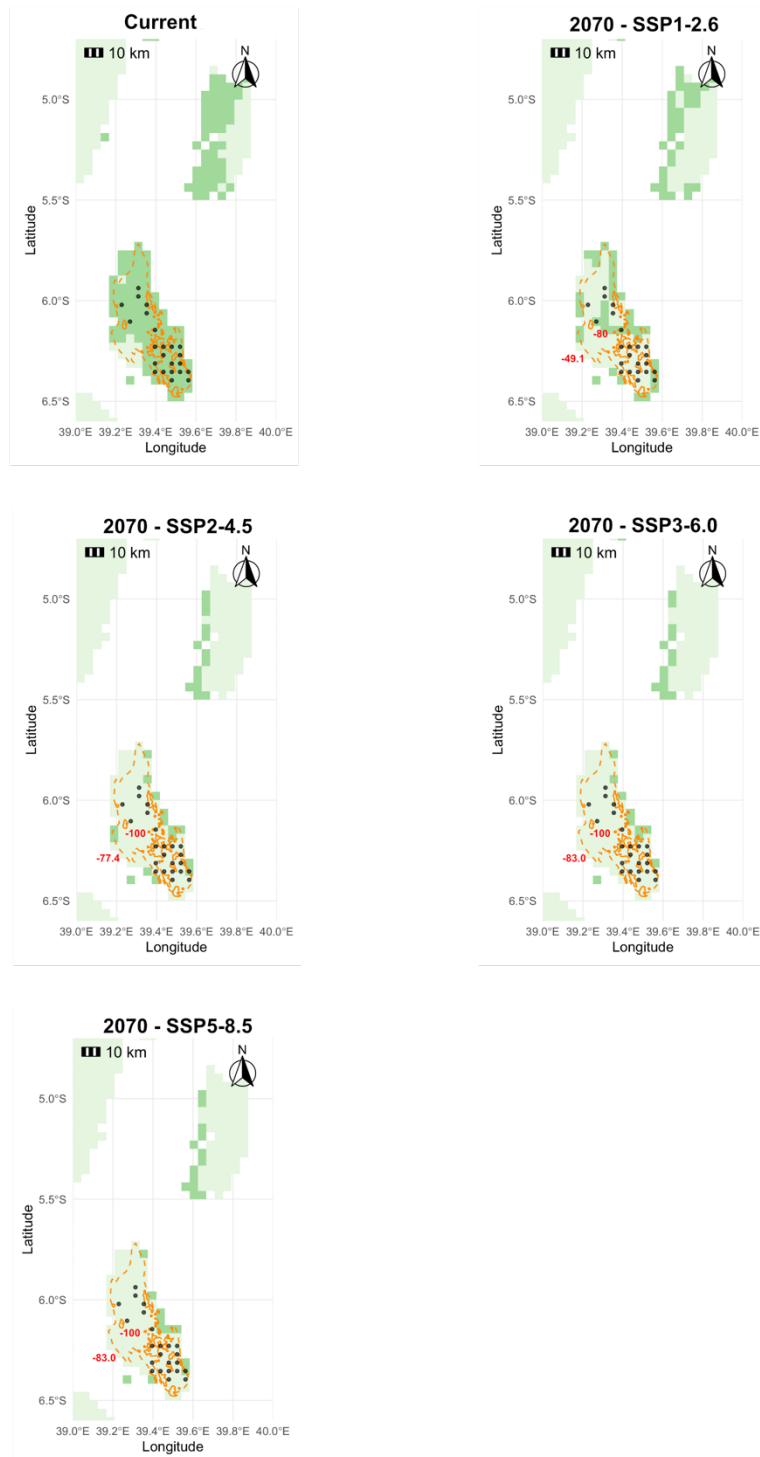


Figure 2.3f: *P. kirkii* - Chen and WorldClim models

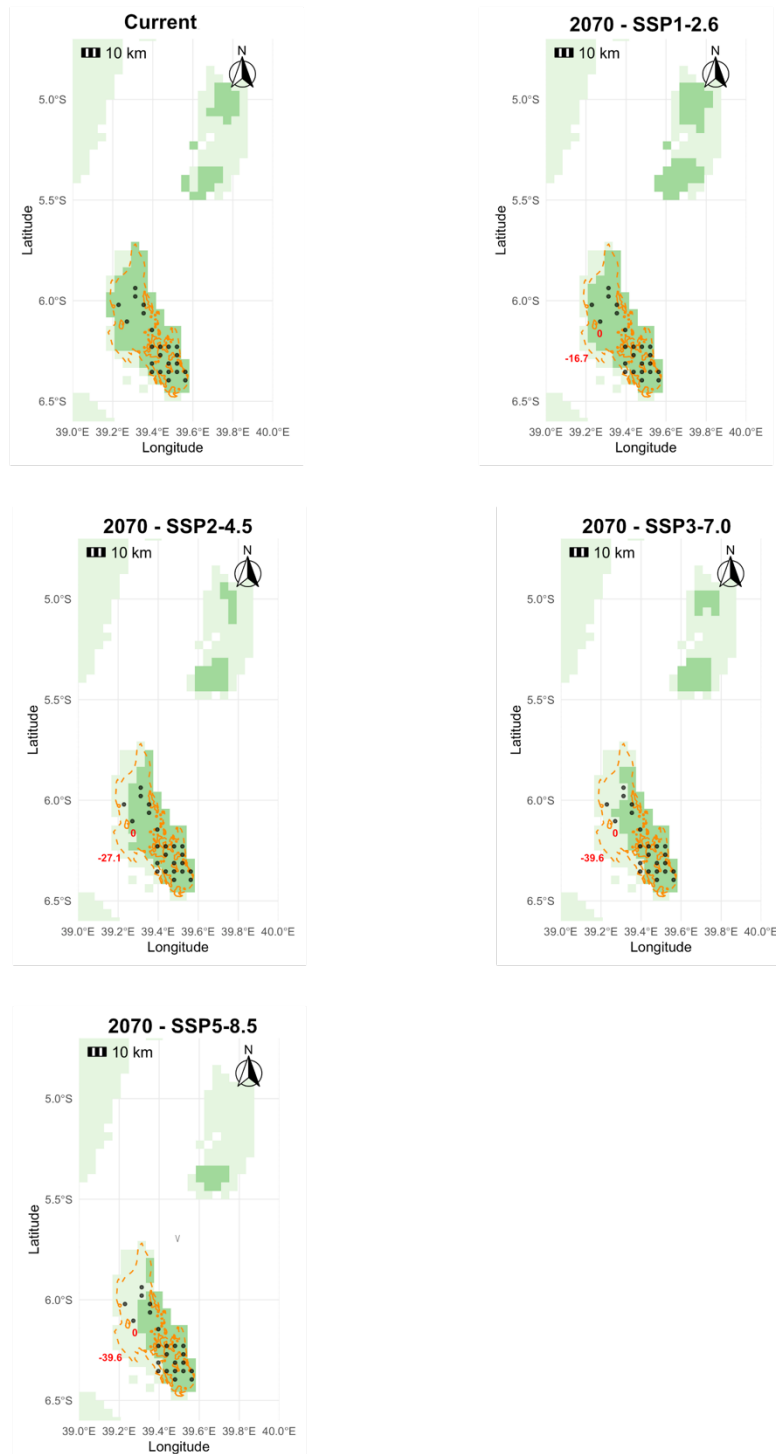


Figure 2.3g: *P. temminckii* - WorldClim only models

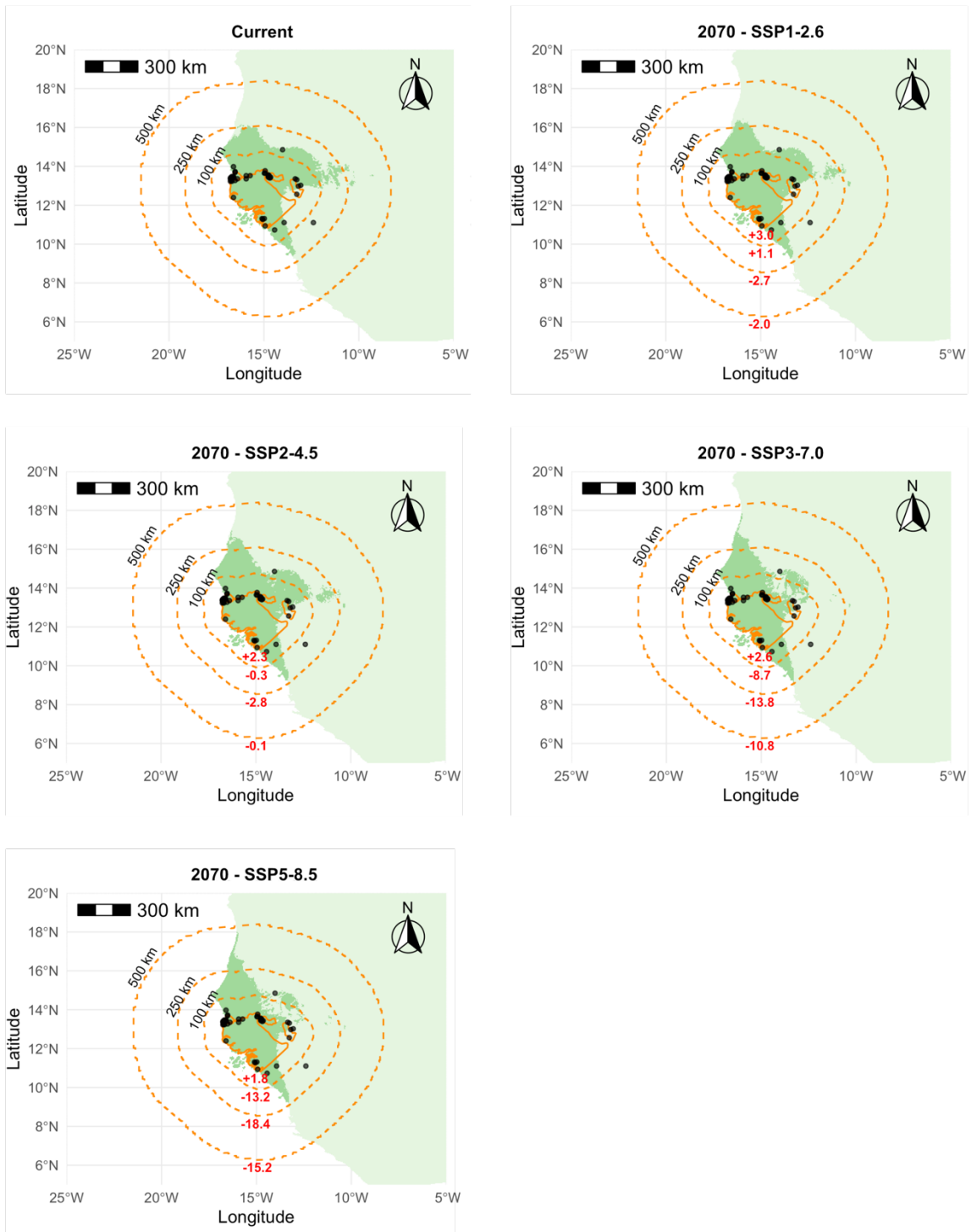


Figure 2.3h: *P. temminckii* - GCAM and WorldClim models

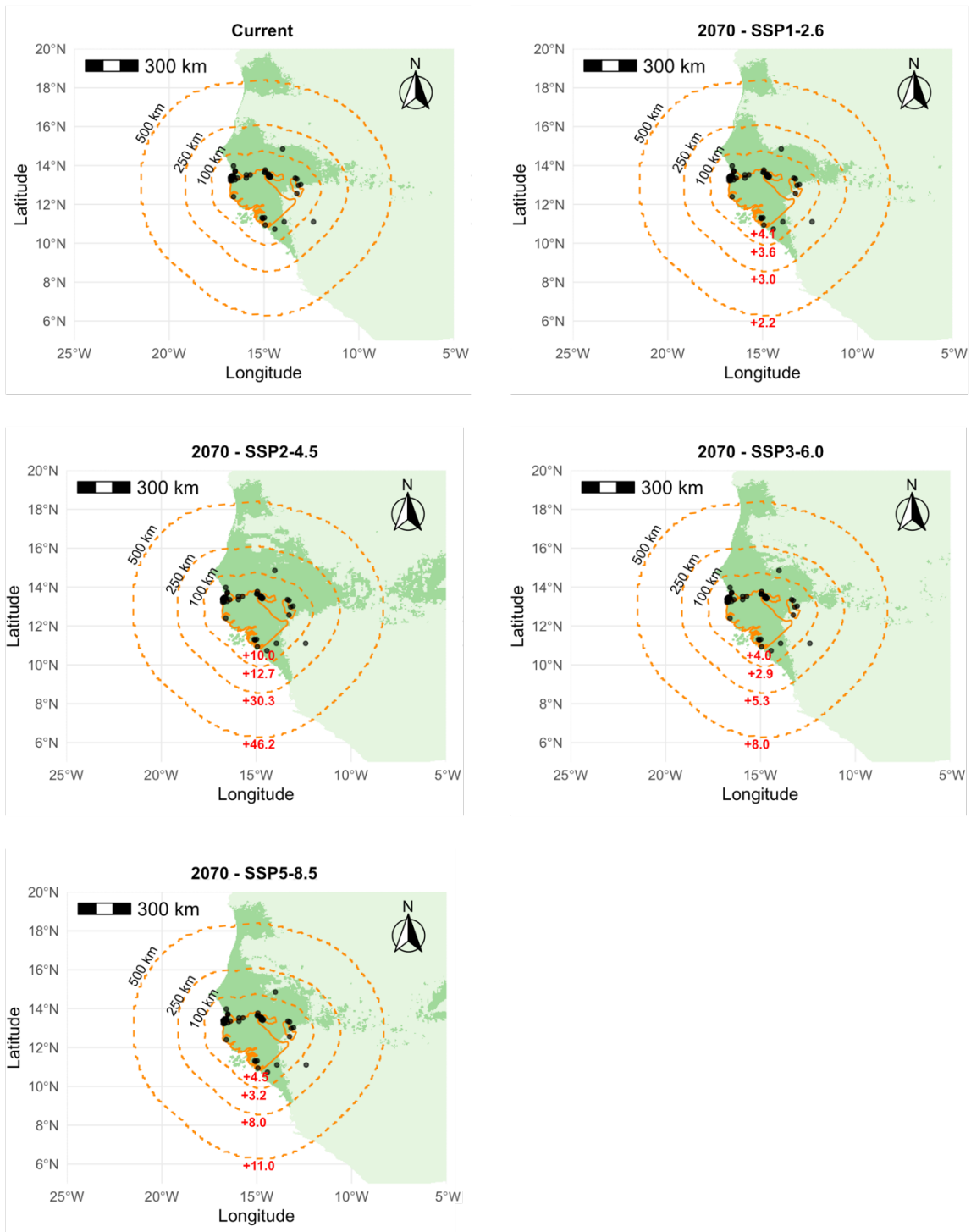


Figure 2.3i: *P. temminckii* - Chen and WorldClim models

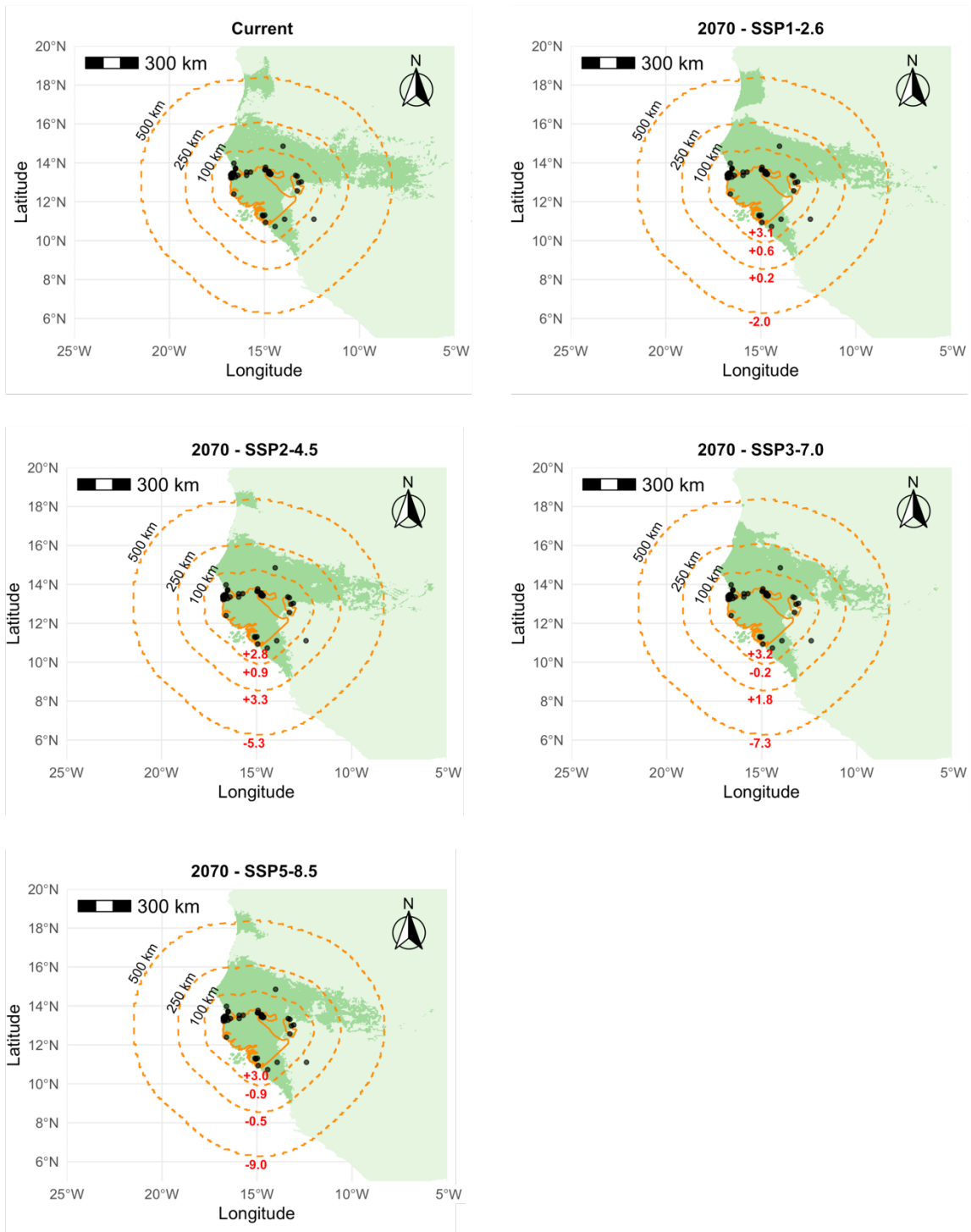


Figure 2.3j: *P. tephrosceles* - WorldClim only models

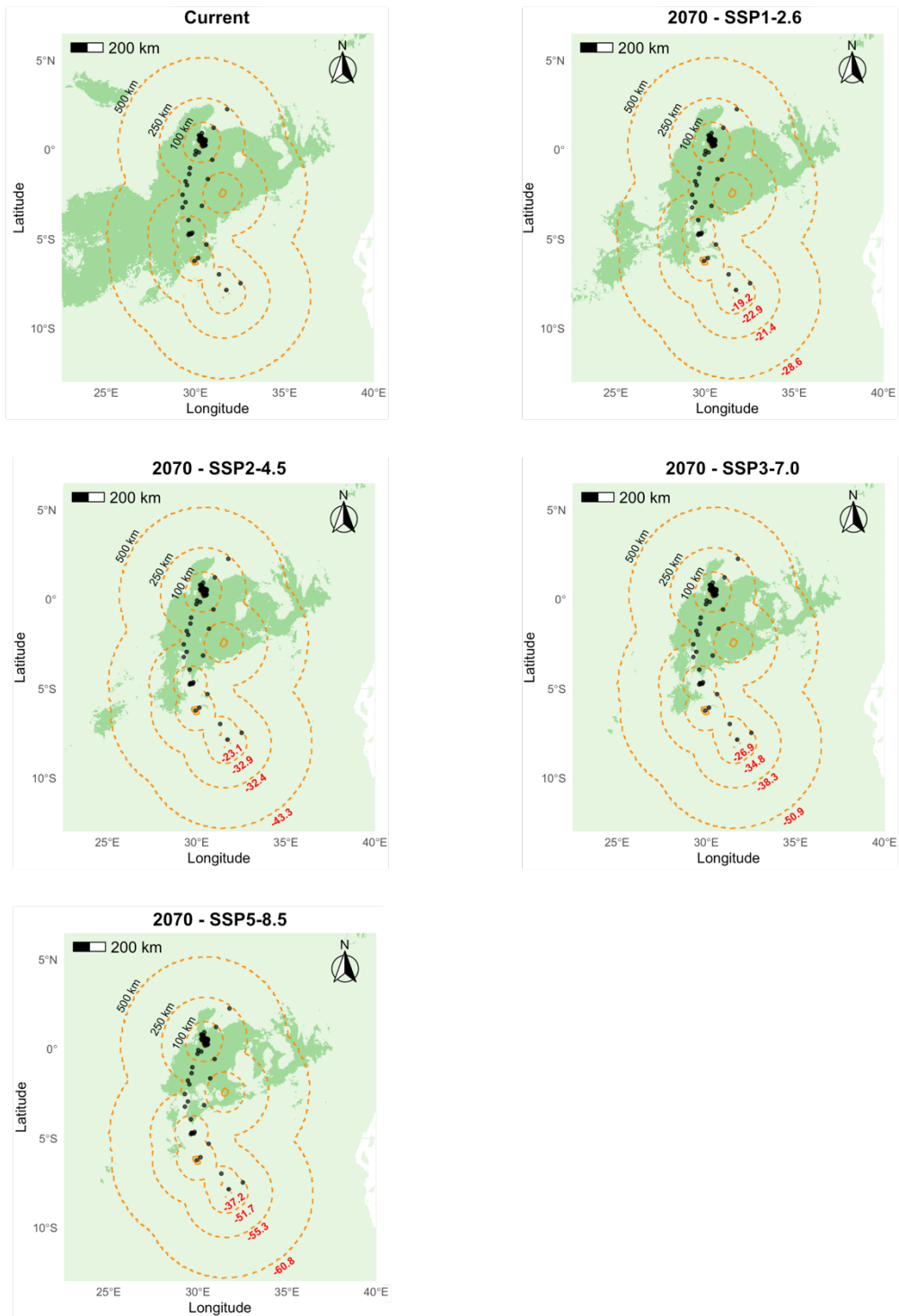


Figure 2.3k: *P. tephrosceles* - GCAM and WorldClim models

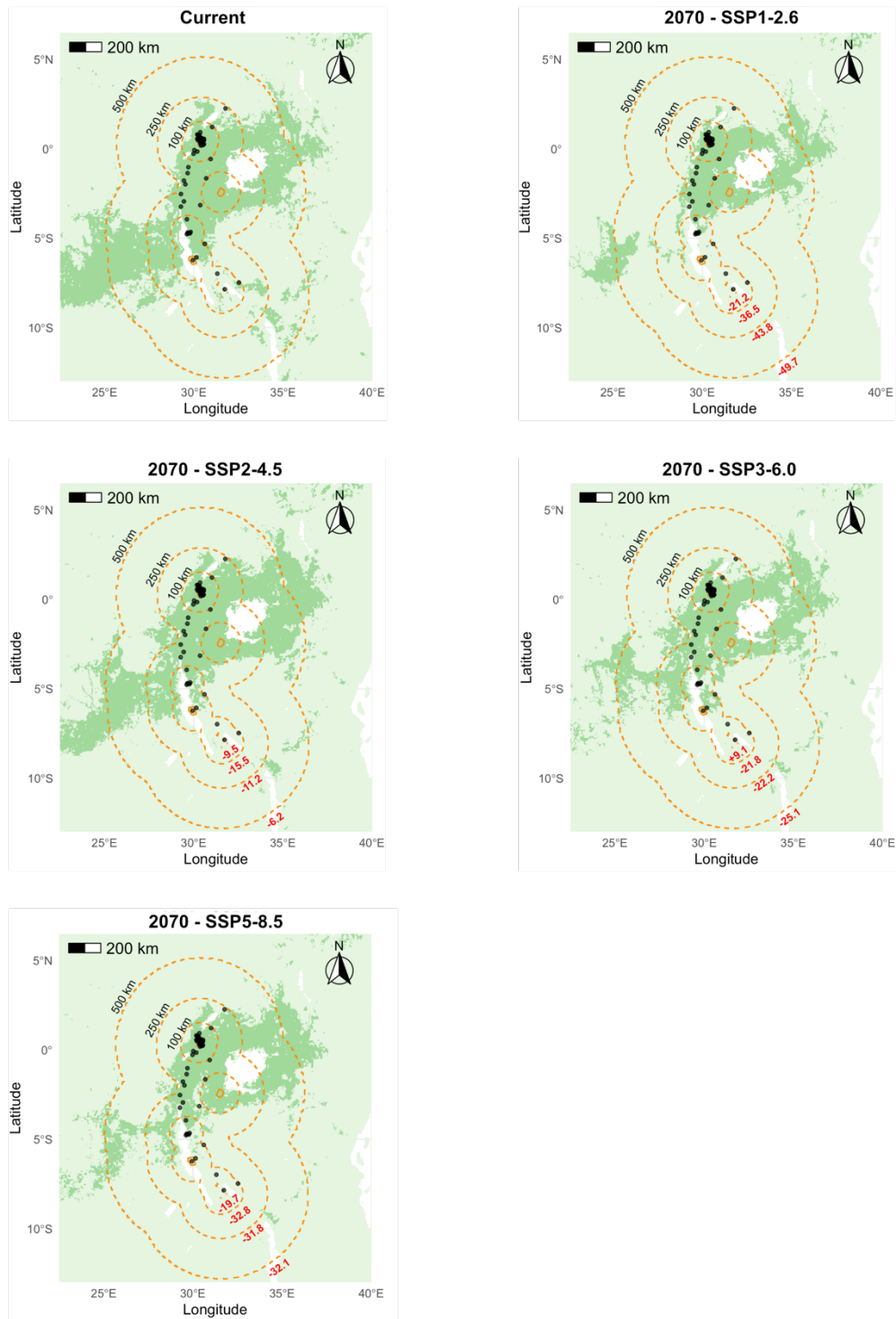


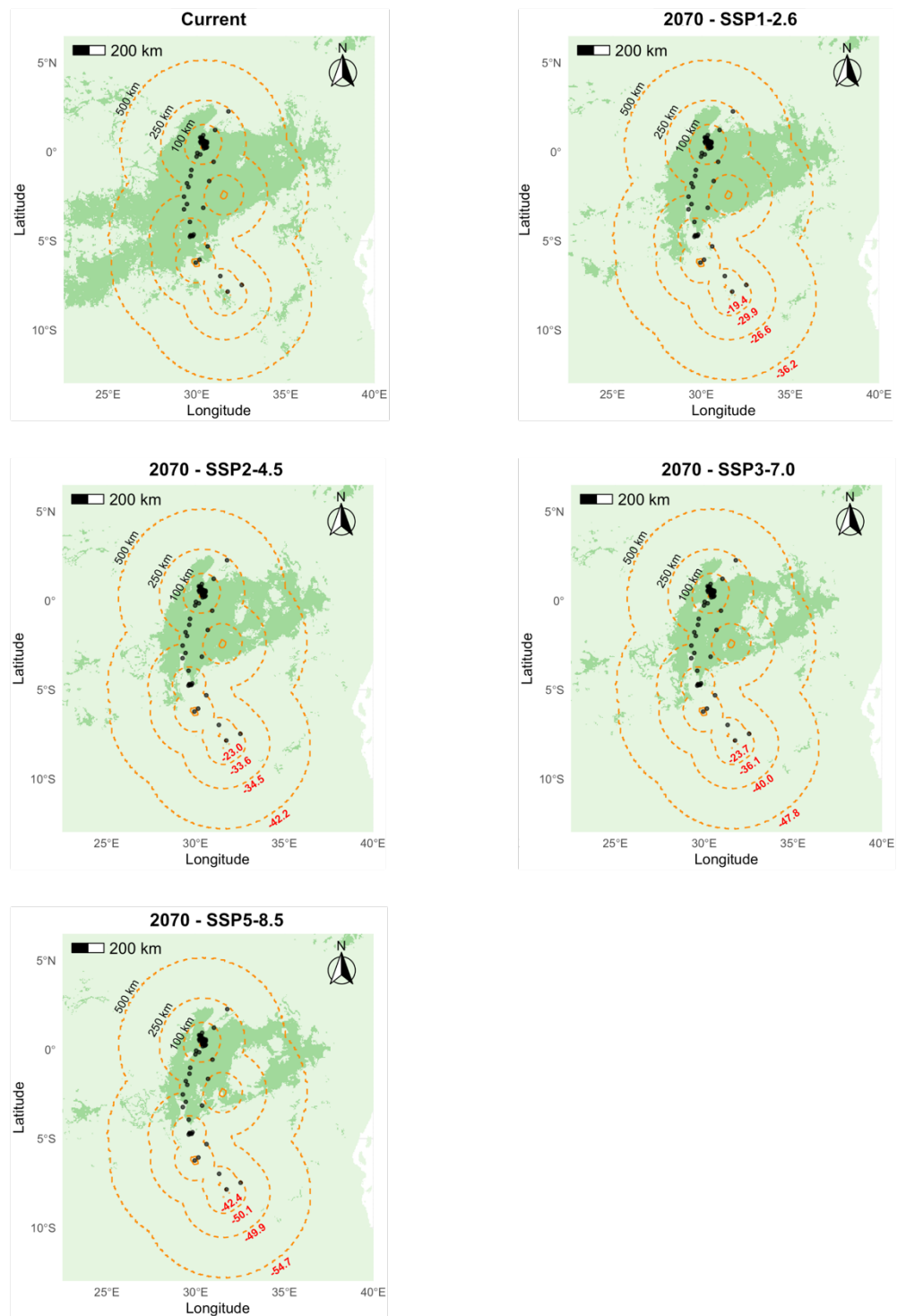
Figure 2.31: *P. tephrosceles* - Chen and WorldClim models

Figure 2.3: Predicted presence of each species in the present and in 2070 under four SSP-RCP scenarios. The areas of dark green are areas of predicted presence and areas of light green are areas of predicted absence using a binarization procedure for predicted probability

of occurrence using a threshold that maximises the true skill statistic. White areas represent bodies of water. The area denoted by a solid orange line represents the current species range and the dashed orange lines represent 100km, 250km and 500km migration buffers. In the case of *P. kirkii*, the species is endemic to the island of Unguja and therefore is assumed to not be able to migrate beyond the coastline of that island. Therefore, this coastline is used as the migration buffer for this species. Black points represent current presence points used to create the species distribution models. The numbers in red show the percentage change in area of predicted presence between the current scenario and each future scenario for the current species range and the buffer areas.

Discussion

In this study, we used data on the current geographic ranges of 16 of the 18 recognised red colobus species (*Piliocolobus* spp.) to evaluate niche breadth and niche overlap between species. We also used a dataset of true presences and absences for four red colobus species to evaluate the drivers of current species distribution and the potential changes in suitable habitat under future climate change and land use change using SDMs. We found high variation in niche breadth between red colobus species and that there was generally very little similarity in niches between species apart from a few pairings, some of which were geographically distant from each other. We found that the choice of land use dataset influenced the order of importance of variables in SDMs and led to different predictions for future loss or gain of suitable habitat for some species. We found that climatic variables were generally more important in predicting red colobus distributions but this varied depending on the land use dataset used. We showed that *P. tephrosceles*, *P. kirkii* and *P. badius* are predicted to lose suitable habitat under climate change and land use change in the future, whereas *P. temminckii* is predicted to gain suitable habitat. Finally, we demonstrated that niche breadth did not explain predicted changes in suitable habitat across species, although this was based on a very limited number of species.

There was large variation in niche breadth and little similarity between red colobus species' niches

We found a large variation in niche breadth among red colobus species. The large variation in niche breadth may be surprising given that red colobus are generally considered highly specialised (Korstjens *et al.* 2022). The species with the broadest niches were *P. badius* and *P. temminckii*, two closely related species, followed by *P. tephrosceles*. Evidence from field studies suggests that two of these species have habitat, dietary and behavioural flexibility: *P. tephrosceles* (Milich *et al.* 2014; Chapman *et al.* 2015; Kibaja *et al.* 2023) and *P. temminckii*

(Galat-Luong and Galat 2005). These field studies may provide some anecdotal support for the broad niches we identified in these species. However, *P. kirkii* could be considered one of the most flexible red colobus species (Nowak 2007; Nowak and Lee 2013) and yet had the 12th broadest niche out of the 16 species we investigated. As such, niche breadth may not fully capture the potential for red colobus to employ behavioural and ecological plasticity in response to environmental change. Additionally, we used a relatively crude estimate of niche breadth due to the lack of detailed information on the ecology of many red colobus species. As such, if this analysis were repeated with a different niche estimate, based on diet or habitat use for example, the results may be different. This would be an interesting and worthwhile research topic if datasets become more complete for the understudied red colobus species. Nonetheless, this study demonstrates that some red colobus species may be more flexible than previously thought.

In general, we found very little similarity between the niches of red colobus species and no phylogenetic pattern in niche overlap within the genus. Again, this was somewhat surprising given that closely related species are assumed to have similar ecological niches under the phylogenetic niche conservatism theory (Harvey and Pagel 1991; Wiens *et al.* 2010). We found significant niche similarity between some of the species in the central African group and between *P. badius* and *P. waldroni*, which is to be expected given their close geographic and phylogenetic relationships (Ting 2008a; Linder *et al.* 2021). However, a more surprising result was the significant niche similarity between *P. kirkii* and each of *P. badius* and *P. waldroni*. *P. kirkii* is distantly phylogenetically related to *P. badius* and is endemic to the coastal forests of Tanzania in East Africa while *P. badius* and *P. waldroni* are found in West Africa. The niche similarity test that we used calculates niche similarity while accounting for the habitats available to the species. As such, we can conclude that the significant niche similarity between *P. kirkii* and each of *P. badius* and *P. waldroni* was not merely due to the species having the same habitats available to them. Therefore, this surprising pattern of niche similarity may be because these three species are all adapted to coastal habitats or that the niches of *P. badius* and *P. temminckii* are so broad that they encompass the niche of *P. kirkii*.

We found no correlation between niche breadth and geographic range size for the 16 species we studied. Niche breadth has been positively correlated with range size across a variety of taxa (Slatyer *et al.* 2013). This is especially true when niche breadth is calculated using data extracted from species geographic ranges because species with larger geographic ranges tend to cover a larger range of each environmental variable. However, we found no such relationship in this study.

Overall, our results show that red colobus monkeys are more ecologically diverse than previously expected. However, we would like to caveat these conclusions with two limitations in how we estimated niche breadth. Firstly, we calculated a static measure of niche breadth, i.e., we calculated it using a snapshot of presence/absence data rather than measures of population growth or decline. As such, our niche breadth estimates could encompass environmental data from sink populations, where the population is not growing or is in decline (Carscadden *et al.* 2020). This issue is exacerbated in long-lived and species with poor dispersal ability, such as red colobus (Wong and Candolin 2015; Korstjens and Hillyer 2016), because their current geographical distribution is likely a response to past conditions rather than current conditions and their limited dispersal ability means that they may be trapped in areas of poor suitability (Bohner and Diez 2020; Pagel *et al.* 2020). As such, the niche breadths quantified in this study may be an overestimation of the true niche breadth of red colobus species. Secondly, the niche modelled in this study represents the realised niche of the species rather than the fundamental niche as it is based on the current geographic range of each species. As such, the species' niches could be broader than our results suggest.

Species distribution models created using different land use datasets produced different results

We found differences in variable importance for models created using two different land use datasets: the Global Change Analysis Model (GCAM) v5 dataset (Chen *et al.* 2020) and the Chen dataset (Chen *et al.* 2022). For each model, all variables had relatively similar levels of importance. This is likely because we selected variables that are known to be important in determining the distribution of red colobus species. However, there were differences in which variables were considered more or less important for predicting suitable habitat for each species depending on the datasets used to build the model. The GCAM and WorldClim models generally showed land use variables to be more important than they were in the Chen and WorldClim models.

We also found that models created using different land use datasets produced different predictions for suitable habitat in the year 2070. We found that predictions from the Chen and WorldClim models and the WorldClim only models were more similar to each other than to the GCAM and WorldClim models. The differences in predicted future suitable habitat between different land use datasets was particularly prominent for *P. kirkii*. Under the WorldClim only and Chen and WorldClim models, *P. kirkii* did not lose any suitable habitat from its current range under any future scenario meanwhile in the GCAM and WorldClim model, the species

lost 80% of suitable area under the SSP1-2.6 scenario and 100% under the other three future scenarios. Overall, there was no particular trend across species showing that one dataset consistently predicted more (or less) suitable habitat in the future making it difficult to determine which land use dataset may be more or less reliable in this context.

The discrepancies in the predictions made using models created from different land use datasets may arise from differences in the way that the land use categories were classified by different datasets. The creation of categorical land use datasets, such as those used in this study, involves imposing discrete land-cover classes onto continuous remote sensing data, potentially leading to classification errors and a loss of information on gradual changes in land use (Goodchild *et al.* 1992; Bradley and Fleishman 2008; Gillespie *et al.* 2008). Such errors and missing information can lead to a higher rate of error when predicting future suitable habitat using SDMs, as we observed in this study. Different land use datasets are designed for the purpose of the specific study or mapping campaign they are created for and therefore use their own set of classification methods (Bradley and Fleishman 2008). This can lead to large differences in land use classifications between datasets and it means that a particular dataset may not properly capture the spatial variation in land use in a way that is relevant or thematically detailed enough for the focal species' ecology (Bradley and Fleishman 2008). These issues may have been further exacerbated in this study because we grouped land use categories into overarching categories. We did this because we deemed it would be easier to interpret the results and make biologically meaningful conclusions from them, however, by doing this, information on the spatial variation in land use may have been lost even further. This could explain some of the discrepancies seen in the results of models built using different land use datasets. Generally, continuous remotely sensed datasets, or in the very least categorical datasets with a large number of categories, likely lead to more robust and better-performing SDMs than categorical land use datasets (Bradley and Fleishman 2008; Cord and Rödder 2011; Cord *et al.* 2014). As such, using remote sensing datasets might be a better option for modelling current red colobus distributions but the prediction of future changes in distribution based on land use will require the use of categorical datasets because future emitted radiation (recorded through remote sensing) cannot be predicted. Therefore, the best option in this case would be to select a categorical land use datasets that are thematically detailed enough and relevant to the study system.

Although researchers are likely aware of the fact that there are differences between land use datasets, the effect different categorical land use datasets have on the predictions of SDMs has not been explicitly studied as we have here. New land use products are validated against existing products or ground-truthed datasets when created to ensure consistency and

accuracy, as was done for the two land use datasets used in this study (Chen *et al.* 2020; Chen *et al.* 2022). However, most validations of global datasets are carried out at global or regional scales which may miss some small-scale discrepancies that could have a large effect on the predictions made using SDMs. One study compared the accuracy and spatial correspondence of three 10 m resolution land use datasets (Dynamic World, World Cover and Esri Land Cover) (Venter *et al.* 2022). They found large inaccuracies between datasets that varied by spatial scale (global vs. regional) and that different datasets had spatial and thematic biases. Similarly, we demonstrated some large discrepancies between SDMs created using different land use datasets. *P. kirkii* was predicted to lose all (GCAM and WorldClim model) or none of its current suitable habitat (Chen and WorldClim and WorldClim only models) depending on the choice of land use dataset. This was despite high levels of accuracy and consistency with existing datasets demonstrated in the initial validation studies (Chen *et al.* 2020; Chen *et al.* 2022) and high validation scores we achieved in this study for each model using commonly used indices, such as AUC and TSS. As such, our study highlights the need to carry out a critical examination of land use datasets on a geographic scale relevant to the study species. We recommend that researchers carry out a visual inspection of land use datasets in key areas for the focal species for which they have ground-truthing data and apply ecological knowledge of the study species to identify suspicious results. This will allow researchers to identify issues with land use datasets that might otherwise be missed. Researchers should select datasets that characterise land use accurately in their study areas and in a way that is relevant to the study species (Cord *et al.* 2014; Venter *et al.* 2022). Regional or country-level land use datasets may provide more accurate estimates of land use that can be validated through ground-truthing more easily. They may also provide more nuanced classification of land use types based on local ecology and geography that will be more relevant to the study species. However, these are not available for all regions of interest and may not apply to large-scale studies.

Climate had a bigger influence on red colobus distributions than land use but this was likely due to methodological artefacts

Overall, we found that climatic factors tended to be more important in predicting red colobus presence/absence than land use variables. In the GCAM and WorldClim models, proportion of grassland was generally quite an important predictor and, in both the GCAM and WorldClim models and Chen and WorldClim models, the partial dependence plots showed that some species preferred areas with a higher proportion of cropland and grassland. These results are surprising. Based on our knowledge of the ecology of red colobus, we know that some species use cropland habitat regularly (e.g. *P. kirkii*: Siex 2003, Nowak 2007; Nowak and Lee 2013,

Davenport *et al.* 2019) but most red colobus are not able to survive in cropland- or grassland-dominated landscapes and are pushed into forest refuges when exposed to humans (Grubb and Powell 1999; Luiselli *et al.* 2015; Galat-Luong and Galat 2005; Mayhew *et al.* 2020). Additionally, we know that habitat loss is an important threat to primate species (Estrada *et al.* 2017) and red colobus species specifically (Ikemeh 2015; Linder *et al.* 2021; Korstjens *et al.* 2022). For example, a 90% decline in the Niger Delta red colobus (*P. epieni*) over a 20-year period has been mostly attributed to forest loss and degradation (Ikemeh 2015). Therefore, these results require further explanation.

These counter-intuitive results most likely arose from artefacts in the methodology used in this study. It is likely that climatic factors were shown to be more important than land use because we used presence points of other cercopithecine primates (including the other red colobus species) as absence points in each of our species' models. As such, land use may have been less important than climate because most of our absence points fall in forested areas where cercopithecine species have been recorded. Therefore, there may be a general lack of variation in land use between presence and absence points, meaning land use information does not contribute much to the model. Climate is more likely to vary across forests where primates are present and therefore contributed more explanatory variation to the models.

The fact that some species preferred areas dominated by cropland and grassland may be explained by the fact that this indicates a preference relative to other cercopithecine species and not an absolute preference. Again, the absence points for each species were the presence points of other cercopithecine primates (including the other red colobus species). Therefore, for any given variable, if a species prefers higher values of that variable, it means that they prefer higher values compared to other African primate species, not that they have an absolute preference for high values. In this context, the results of this study would suggest that some red colobus "prefer" grassland and cropland more than other species do. However, colobines are known to be more forest-dependent than most other cercopithecids (Korstjens 2019). Therefore, this result is likely to be due to one of three other methodological constraints.

Firstly, this result could arise from a misclassification of land use categories in the land use datasets, as is common in categorical land use datasets (Goodchild *et al.* 1992; Bradley and Fleishman 2008; Gillespie *et al.* 2008). We carried out a visual inspection of the GCAM and Chen datasets for Kibale National Park, Uganda (0.4364° N, 30.3667° E), and for Jozani-Chwaka Bay National Park, Zanzibar (6.233°S, 39.404°E), for which we have detailed knowledge of the habitat types (Supplementary Material S2.6). We found that for Kibale

National Park, much of the forested area was incorrectly classified as grassland for the GCAM dataset and for Jozani-Chwaka Bay National Park, much of the forested area was classified as cropland in the Chen dataset. Therefore, it is possible that misclassification of land use categories in the datasets could be responsible for predicting that some red colobus species prefer areas with high proportions of cropland and grassland. This further highlights the need for careful critical examination of land use datasets on a scale relevant to the ecology of the species.

Secondly, by grouping smaller categories in the GCAM and Chen datasets together to form the cropland and grassland categories, we may have lost nuanced information on land use which could have better-explained red colobus distribution. For example, areas classified as cropland could vary from tree plantations, which red colobus may be able to use, to rice fields which red colobus would not. By grouping these together under “cropland”, we may have created models that predict red colobus to be found in cropland in general which would not be the case on the ground. Unfortunately, the land use datasets used here, as is the case with most categorical land use datasets, did not include the level of thematic detail needed to differentiate types of cropland in a way that is relevant to red colobus ecology, e.g., tree structure. As mentioned earlier, land uses datasets are designed for a specific purpose and therefore it is often difficult to retrofit their classification systems to suit the ecology of the species in a different study (Bradley and Fleishman 2008). Therefore, it may be that a lack of thematic detail in the land use classifications lead to the models predicting that some red colobus species prefer cropland and grassland.

Thirdly, these spurious results may have arisen from a discrepancy between the timing of data gathering used to generate the land use dataset and data gathering for our presence/absence colobus dataset. We created our models using land use data from 2015-2025, while the majority (>80%) of presence/absence records date between 1990 and 2015. Most of our presence points are from national parks where there would have been the highest level of habitat protection which should mean that few points would have experienced changes in land use. However, given the rapid loss of forest across the red colobus range in the last few decades, it is possible that locations in our presence/absence colobus dataset may record species presences in cropland, where forest would have stood at the time the presence was recorded. Therefore, this may also explain why our models predicted that red colobus prefer areas with a high proportion of cropland and grassland. This may be less of an issue for the WorldClim data because the current scenario for that dataset represents 1970-2000 which covers the time period that most of the presence points were collected.

Overall, although our results suggest that red colobus distributions are mostly influenced by climate rather than by land use, this is most likely due to the methodology used here and error within the datasets used rather than a true result and should therefore be interpreted with caution. This conclusion has important implications for SDM studies that rely on global land use products more generally, as these studies are likely to suffer from these three common methodological artefacts but rarely discuss or account for them explicitly.

Three out of four red colobus species were predicted to lose suitable habitat under future climate change and land use change

The future predicted distribution of red colobus varied greatly between species and input datasets, ranging from a 10% gain in suitable habitat to a 100% loss. *P. badius* and *P. tephrosceles* lost suitable habitat from their current range and dispersal buffers in almost all scenarios (up to 22.7% loss for *P. badius* and 42.4% loss for *P. tephrosceles*). *P. kirkii* showed varied results based on the land use dataset used to build the models, ranging from no loss of suitable habitat from their current range under any future scenario to a loss of 80% of suitable area under the SSP1-2.6 scenario and 100% under the other three future scenarios in the GCAM and WorldClim model. Meanwhile, *P. temminckii* generally showed an increase in suitable habitat in their current range by 2070 (around 2-4%).

Based on our results, *P. badius*, *P. tephrosceles* and *P. kirkii* can be considered vulnerable to climate change and land use change given that they were generally predicted to suffer a loss in suitable habitat in the future due to exposure to these threats. This is particularly concerning for *P. tephrosceles* and *P. kirkii* given their already restricted geographic ranges. Our finding that *P. temminckii* were predicted to expand their range under future environmental change was surprising. Empirical observations have demonstrated that *P. temminckii* have declined by more than 50 % in last 30 years (Minhós *et al.* 2020) mostly due to severe habitat reduction and fragmentation attributed to reduced rainfall and expanding cropland, particularly along the north and eastern edges of their range (Galat-Luong and Galat 2005; Galat *et al.* 2009). One explanation for our result is that *P. temminckii* have developed adaptations to allow them to survive in human-dominated landscapes and in climates that are likely to become more common under climate change. Galat-Luong and Galat (2005) recorded dietary, locomotive and social adaptations in this species said to help them to cope with changes in their habitat so it is possible that *P. temminckii* have the ecological and behavioural flexibility to allow them to increase their range under environmental change. As such, they may be currently occupying habitats that would be unsuitable for other red colobus species and which may

become more common in the future. However, an alternative explanation, is that static SDMs, such as the ones used in this study, only capture a snapshot of species presence and do not incorporate information on demographic trends. As such, the *P. temminckii* populations used in our models may be surviving in sink populations in suboptimal habitats, such as grassland and croplands, and are likely to become locally extinct in the future. As mentioned earlier, static models may overestimate the niche of a species (Carscadden *et al.* 2020), therefore we may be overestimating their suitable habitat in the future. A final explanation for this result is that there is a difference in the timing of data collection for the presence points and environmental datasets used to build our models meaning some of the points in our datasets may fall in areas classified as land use types and climates that are not tolerable to red colobus populations anymore. As a result, our models may have incorrectly predicted areas of suitable habitat in the future.

For all four red colobus species, the dispersal buffer areas generally did not gain sufficient suitable habitat to buffer against the loss of habitat in their current range. In addition, the limited dispersal ability of red colobus, given that they are primates (Wong and Candolin 2015; Korstjens and Hillyer 2016), means that they are unlikely to be able to utilise any newly suitable areas without assisted translocation. Therefore, movement to newly created suitable habitat is unlikely to be a viable solution for red colobus survival and as such conservation efforts should focus on protecting suitable habitat within the current range of each species.

Generally, the smallest losses or biggest gains in area of predicted presence (depending on the species) were found for the best-case scenario for climate change and land use change (scenario SSP1-2.6). The biggest losses or smallest gains (depending on the species) were predicted for a scenario with the biggest increase in CO² emissions and medium regulation of land use change (SSP5-8.5). This shows how important implementing mitigation for climate change and land use change is for the future of the red colobus genus. Climate change and land use change are just two of a suite of anthropogenic threats facing red colobus. Some red colobus species, especially those in central and western Africa, are highly threatened by hunting (Linder *et al.* 2021). Red colobus monkeys are particularly vulnerable to hunting among mammals due to their large body size, loud presence within the forest and lack of behavioural defences to hunting (Linder and Oates 2011; Linder *et al.* 2021). As a result, they are often the first mammal to experience local extirpation by hunting (Linder *et al.* 2021). As such, it is likely that hunting may cause local extinction of red colobus populations before climate change and land use change take effect. Managing more pressing threats, such as hunting, will give red colobus the time and space to respond to future climate change and land

use change effectively, but the urgency of implementing mitigating actions for climate change and land use change should not be overlooked. As shown in this study, they are likely to have pervasive effects on red colobus survival. Furthermore, climate change related food insecurity and habitat loss and fragmentation associated with land use change are predicted to exacerbate hunting further (Galán-Acedo *et al.* 2019; Chapman and Peres 2021). As such, a step towards curbing hunting of red colobus by humans may be to mitigate climate change and land use change.

It is important to note that these analyses were conducted on just four red colobus species for which we had sufficient presence data. Given the spatial variability in climate change and land use change across Africa, the fact that the four species studied had different responses to environmental change and the fact that the 13 species studied have dissimilar niches, the response of these four red colobus species may not reflect that of other red colobus species. This is a particular issue for Central African species for which we have limited data. We suggest that future research efforts focus on collecting data on presence and exposure to threats for understudied species, as has been identified as a research priority in the Red Colobus Action Plan (Linder *et al.* 2021), to strengthen our understanding of their vulnerability to climate change and land use change.

Niche breadth was not linked to predicted changes in suitable habitat

Although we found large variation in niche breadth among red colobus species, there was no relationship between niche breadth and predicted change in suitable habitat for the four species for which we could conduct this analysis. Therefore, it is possible that a different intrinsic trait or level of exposure to threats may be driving patterns of extinction risk among red colobus species. We found very little niche overlap between red colobus species, indicating that different red colobus species occupy different niches. Additionally, of the four species analysed, we were able to determine niche similarity for three of them. *P. badius* and *P. kirkii* had statistically similar niches and both were predicted to lose suitable habitat meanwhile *P. temminckii*'s niche did not overlap with the other two and was predicted to gain habitat in the future. Taking these two results together, it may be that niche position, in combination with the degree of environmental change, may better predict future loss or gain of habitat. Under the niche position hypothesis, species with niches encompassing environmental conditions that become more common under environmental change may experience a gain in suitable habitat, even though their niche may be narrow (Thuiller *et al.* 2005; Rocha *et al.* 2018). As such, it may be that the environmental conditions within *P. temminckii*'s niche become more common in the future, while the environmental conditions of

the shared niche of *P. badius* and *P. kirkii* become less common, regardless of the breadth of the niche of the species. While this is an interesting hypothesis, these results should be taken with caution as they are based on a very limited number of species (N = 3). A future study investigating the role of niche breadth and niche position on predicted change in suitable habitat for red colobus based on more data (e.g., Thuiller *et al.* 2005) would be worthwhile.

Implications for conservation and future research

We have provided a novel investigation into the effect of choice of land use dataset on species distribution model outcomes. We recommend that future studies carry out a critical examination of datasets to optimise their match with the study's aims, the ecology of the study species and with ground-truthed land classifications in the study area. Researchers should provide a clear description of the pitfalls and caveats of the datasets they are using, particularly if the research is to be incorporated into conservation plans where knowledge of the margins of error is particularly important.

We demonstrate that red colobus species are more ecologically diverse than previously assumed and can inhabit a broad range of niches. As such, one red colobus species cannot be assumed to be an ecological surrogate for another and conservation management should be addressed at a species as well as a genus level. Red colobus species respond differently to future climate change and land use change, but *P. tephrosceles*, *P. kirkii* and *P. badius* are all predicted to lose habitat. Our findings are particularly concerning for *P. tephrosceles* and *P. kirkii* because of their already restricted ranges. We recommend that for *P. tephrosceles*, conservation efforts should focus on preserving the current range of this species, particularly in the southern half of their range where most of their suitable habitat is likely to be lost. Additionally, the creation of forest corridors between forest patches would allow this species to migrate to areas with more favourable conditions should they experience environmental change. For *P. kirkii*, most of their suitable habitat is predicted to be lost from the north-western part of Unguja where very few individuals remain today (Davenport *et al.* 2019), therefore conservation efforts would be best placed in bolstering protection for areas of suitable habitat in the southern part of the island.

The variation in niche breadth we observed did not explain differences in predicted habitat loss or gain, although this was based on a very limited number of species. Further collection of data on the presence/absence of red colobus should be a research priority, as has already been recommended in the Red Colobus Action Plan (Linder *et al.* 2021). This would greatly

improve our ability to accurately model the current and future suitable habitat of red colobus and to investigate the drivers of vulnerability of red colobus to future change.

We demonstrate that in the best-case scenario where climate change and land use change mitigation measures are successfully carried out, all red colobus species fare better. While red colobus face more immediate threats such as hunting, we should not neglect the importance and urgency of implementing mitigation for climate change and land use change. The combined management of long-term and immediate threats is essential to give the red colobus the best chance of survival in the future.

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Chapter 3

Methodological confounds of measuring urinary oxidative stress in wild animals



Me, when I finally managed to get my first shipment of urine samples back to the UK in liquid nitrogen.

Abstract

Biomarkers of oxidative stress (OS) are useful in addressing a wide range of research questions, but thus far, they have had limited application to wild mammal populations due to a reliance on blood or tissue sampling. A shift towards non-invasive measurement of OS would allow field ecologists and conservationists to apply this method more readily. However, the impact of methodological confounds on urinary OS measurement under field conditions has never been explicitly investigated. We combined a cross-sectional analysis with a field experiment to assess the impact of four potential methodological confounds on OS measurements: 1) time of sampling, 2) environmental contamination from foliage; 3) delay between sample collection and flash-freezing in liquid nitrogen; 4) sample storage of up to 15 months below -80°C . We measured DNA oxidative damage (8-hydroxy-2'-deoxyguanosine, 8-OHdG), lipid peroxidation (malondialdehyde, MDA), Total Antioxidant Capacity (TAC) and uric acid (UA) in 167 urine samples collected from wild Zanzibar red colobus (*Piliocolobus kirkii*). We found that MDA was higher in samples collected in the morning than in the afternoon but there were no diurnal patterns in any of the other markers. Contamination of samples from leaf surfaces and time frozen at -80°C for up to 15 months did not affect OS marker concentrations. Freezing delay did not affect OS levels cross-sectionally, but OS values from individual samples showed only moderate to good consistency and substantial rank order reversals when exposed to different freezing delays. We recommend that the impact of storage time on OS marker concentrations and diurnal patterns of OS markers be considered when designing sampling protocols. However, given the high stability we observed for four OS markers subject to a variety of putative methodological confounds, we suggest that urinary OS markers provide a valuable addition to the toolkit of field ecologists and conservationists within reasonable methodological constraints.

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Introduction

Non-invasive techniques have revolutionised the field of ecophysiology by, firstly, reducing the adverse effects of field research on animal welfare and behaviour, and, secondly, limiting the confounding effects of the stress of capture and restraint on biomarker measurements (Nwunuji *et al.* 2014; Costantini *et al.* 2017). This has allowed us to address new questions in previously inaccessible study systems (Narayan 2013; Behringer and Deschner 2017). But while non-invasive methods are commonly applied to study animal energetics (Emery Thompson 2017), endocrinology (McCormick and Romero 2017) and, more recently, immune function (Behringer *et al.* 2017; Tombak *et al.* 2020), non-invasive studies of oxidative stress physiology in the wild are rare (Thompson González *et al.* 2020).

Oxidative stress (OS) is caused by the imbalance between the production of reactive oxygen species and the production, dietary intake and repairing action of antioxidants. Oxidative stress can damage cellular DNA, proteins and lipids (Valko *et al.* 2007). This can have negative consequences for health, reproduction and survival (Bize *et al.* 2008; Saino *et al.* 2011; Sebastiano *et al.* 2017) making OS markers highly relevant to individual fitness. Additionally, oxidative stress is universal to aerobic organisms, meaning that OS can be measured in a wide range of taxa (Beaulieu and Costantini 2014). Uniquely among physiological markers, both the damage and protection aspects of OS can be measured separately, meaning that OS markers can give us unparalleled insights into both the costs facing an organism and an organism's ability to cope with these costs (Beaulieu and Costantini 2014). These properties of OS markers make them particularly suitable tools for the study of life history trade-offs (Monaghan *et al.* 2009; Speakman *et al.* 2015; Blount *et al.* 2016; Thompson González *et al.* 2020) and the impacts of anthropogenic disturbance (Semeniuk *et al.* 2009). Indeed, many studies have investigated these topics in wild animals but have relied exclusively on blood or tissue samples to quantify OS (e.g. *Ovis aries*: Nussey *et al.* 2009; Christensen *et al.* 2016; *Mungos mungo*: Vitikainen *et al.* 2016; *Macaca mulatta*: Georgiev *et al.*, 2015a; Georgiev *et al.*, 2015b; *Dasyatis americana*: Semeniuk *et al.* 2009; *Mandrillus sphinx*: Beaulieu *et al.* 2014). The reliance on invasive sampling has thus precluded the broader adoption of OS markers in field research, especially in the case of large, endangered, and difficult-to-capture animals.

A shift to a non-invasive approach for quantifying OS will allow us to study a wider range of animals, address research questions requiring longitudinal measurements and reduce the risk posed to study species. Urinary OS markers provide a promising alternative to blood and tissue sampling for field studies. Markers of OS are more stable in urine than in blood

(Il'yasova *et al.* 2012), and have been used in clinical research since at least the 1980s (Cathcart *et al.* 1984). More recently, urinary OS markers have been applied in studies of captive animals in laboratory and zoo settings (Cho *et al.* 2009; Marchal *et al.* 2013; Costantini *et al.* 2021) but thus far, only one study has measured OS in wild animals via non-invasive sampling (Thompson González *et al.* 2020). In their study of wild chimpanzees, Thompson González *et al.* (2020) showed that MDA-TBARS (a marker of lipid peroxidation) was higher while Total Antioxidant Capacity was lower later in the day and they found a weak negative relationship between storage time and MDA-TBARS. However, a more explicit investigation of a broader range of methodological confounds of measuring OS in the field is essential to aid the planning of robust field research using OS markers in the future.

In this study we therefore aimed to explicitly examine four major confounds that can affect the interpretation of OS values in opportunistically collected urine samples in a remote field setting: 1) time of sampling; 2) environmental contamination from foliage during sample collection; 3) a delay between sample collection and flash-freezing in liquid nitrogen; and 4) prolonged sample storage at or below -80°C .

First, any circadian variation in marker excretion can be problematic when relying on imbalanced datasets that are characteristic of opportunistic sampling. Evidence of diurnal variation in OS is mixed and differs between markers and sample media, both in humans (Valencia *et al.* 2001; Singh *et al.* 2004; Alajbeg *et al.* 2017) and chimpanzees (Thompson González *et al.* 2020). Therefore, an assessment of diurnal variation in multiple OS markers in an additional species, in particular a non-ape species, will provide a valuable addition to this literature and aid in determining the potential impact of imbalanced sampling on analysis of OS markers.

Second, we also examined the effect of possible environmental contamination on OS marker levels. Evidence for significant and consistent impacts of environmental contaminants on urinary biomarkers is mixed, therefore a marker-specific assessment of such confounds is usually necessary (Schwartz and Granger 2004; Heistermann and Higham 2015; Braga Goncalves *et al.* 2016; Higham *et al.* 2020). Environmental contamination of samples can be introduced through a variety of ways, for example, mixing with soil, faeces or contact with vegetation. Contamination of samples through contact with vegetation is one of the most difficult sources of contamination to avoid because the collection of urine directly from the stream of a wild animal is not always possible and samples are regularly collected from the surface of vegetation (Higham *et al.* 2011; Surbeck *et al.* 2012; Fedurek *et al.* 2016; Rincon *et al.* 2019; Thompson González *et al.* 2020). Although the potential effects of leaf-borne

contaminants on urinary biomarker measurement has been successfully ruled out for some metabolites (testosterone and creatinine: Muller and Wrangham 2004; Marshall and Hohmann 2005; estrone conjugates: Knott 2005), this is yet to be confirmed for OS markers.

Third, we assessed the effect of a delay between sample collection and flash-freezing in liquid nitrogen, which is often unavoidable and difficult to standardise at remote field sites. Markers of oxidative status are generally considered stable during short-term storage before freezing (blood: at 0–4°C for 40 hours and at 21–22°C for 40 hours, Koracevic *et al.* 2001; 4°C for up to 24 hours, Nussey *et al.* 2009; 3 hr to 48 hr at 4°C and 20°C, Jansen *et al.* 2013a; urine: 20°C for 26 hours, Lee and Kang 2008; 4°C and 25°C for 24h, Matsumoto *et al.* 2008). While these results are promising, whether the same degree of urinary marker stability would be retained at the higher ambient temperatures often found in field conditions in the tropics requires evaluation.

Fourth, we also considered how duration of frozen storage affects OS markers. Field studies of wild animals are often conducted in remote locations with limited access to laboratory equipment meaning samples are often stored for months or years before analysis. Additionally, length of time in storage is a particularly difficult confound to standardise because samples are normally collected over a long period of time and are assayed in the lab in one or several batches. Therefore, ensuring stability of OS markers during storage is necessary to reliably compare samples. OS markers in blood have been found to be stable in long-term storage after freezing for up to 2 years (-20°C for 1 month, Koracevic *et al.* 2001; -20°C, -80°C and -196°C for 12 months, Jansen *et al.* 2013b; -80°C for 60 months, Jansen *et al.* 2017). However, the long-term stability of OS markers in urine is less certain and seems to vary by marker. Urinary MDA levels have been showed to decline over long-term frozen storage (30 days at -20°C, Martinez and Kannan 2018; 1-10 years at -30°C, Thompson González *et al.* 2020) while other markers remained stable (800 days at -80°C, Matsumoto *et al.* 2008; 30 days at -20°C, Martinez and Kannan 2018; 1-10 years at -30°C, Thompson González *et al.* 2020). The extent to which such declines would be observed in other urinary OS markers and the timeline of such effects require further study.

We investigated the effect of putative methodological confounds on OS marker levels in the Zanzibar red colobus (*Piliocolobus kirkii*), an endangered primate for which no physiological data have ever been reported either from the wild or from captivity. The redox status of an organism is the result of a complex cascade of processes and therefore it is necessary to measure multiple markers representing different aspects of these processes to properly capture the OS an animal is facing (Speakman *et al.* 2015). We chose 4 complementary OS

markers representing different aspects of the oxidative status of the animal; two markers of oxidative damage: 8-hydroxydeoxyguanosine (8-OHdG), a marker of DNA oxidative damage; malondialdehyde (MDA), a marker of lipid peroxidation, and two markers of antioxidant capacity: Total Antioxidant Capacity (TAC) and uric acid (UA). These markers are known to be stable, represent system-wide levels of OS and have a variety of commercial assays available to test for them. Because of this, these markers have been used to measure OS in a variety of contexts, for example, in studies of wildlife conservation (French *et al.* 2017), life history (Christensen *et al.* 2016, Thompson González *et al.* 2020), behavioural ecology (Georgiev *et al.*, 2015a; Georgiev *et al.*, 2015b, Beaulieu *et al.* 2014) and socioecology (Costantini *et al.* 2017). Therefore, our choice of markers represents a useful marker set for ecologists and conservationists.

We examined how the concentrations of these four markers were affected by 1) time of day; 2) environmental contamination from leaf surfaces; 3) sample freezing delays (time between sample collection and flash-freezing in liquid nitrogen); and 4) time elapsed between freezing and laboratory analysis. We did not have clear expectations regarding the presence of diurnal variation in marker values given the lack of consistent patterns in previous studies nor did we have predictions about the direction of the effect of environmental contamination on marker concentrations given that there is no previous research on this topic. We expected that longer freezing delays and longer time spent frozen would be linked to decreased levels of all OS markers because these markers are expected to degrade over time.

Materials and Methods

Study site and subjects

We sampled 40 wild Zanzibar red colobus (5 adult males, 35 adult or subadult females) from three groups in and around the edges of Jozani-Chwaka Bay National Park, Zanzibar (6.233°S, 39.404°E). The Zanzibar red colobus is endemic to the island of Unguja where there are ca. 6,000 individuals, 50% of which are found at this national park (Davenport *et al.* 2019). The subjects of this study are exposed to high levels of habitat disturbance and human activity from roads, tourism and nearby villages and farms (Siex and Struhsaker 1999; Georgiev *et al.* 2019; Olgun *et al.* 2021). They do not receive provisioned food. The mean maximum and minimum temperature between February 2019 and February 2020 at Jozani-Chwaka Bay National Park was 34.5 °C and 21.7°C respectively (Zanzibar Red Colobus Project, unpublished data). The mean daily temperature variation was 12.7°C.

Urine sampling and storage

We opportunistically collected 225 urine samples from the 40 colobus (mean 6.5 samples per individual, range 1-29) typically between 7:00 – 18:00 h over a period of 12 months (August 2018 - September 2019). We collected samples immediately after excretion from identified individuals which could be distinguished using facial markings and other distinguishing features (e.g., scars, injuries, posture/shape). We either caught urine midstream using a plastic bag on the end of a catchpole or pipetted fresh urine splatter from the leaves. We only collected samples, which were not visibly contaminated with faeces or detritus. Samples were carried in the dark and on ice in insulated lunchboxes until flash-freezing in liquid nitrogen was possible later the same day. The samples were then transported to the UK in dry shippers below -150°C. Once at the laboratory, all samples were stored in -80°C freezers until assaying.

Freezing delay experiment

To examine the effect of varying delays to flash-freezing of urine samples on OS marker measurement, we conducted a field experiment with seven urine samples. Upon collection of the sample, we briefly mixed and aliquoted each sample into four tubes and stored them on ice in an insulated lunch box as described above. Upon return to the field base, we flash froze the first sample in liquid nitrogen (mean time since collection = 51 minutes, range 31 - 82 minutes) then the other aliquots were stored in the lunch box until freezing at 2-hour intervals after the first (mean time between collection and freezing for the second aliquot was 169 minutes (range 151 - 202 minutes), 289 minutes for the third aliquot (range 271 - 322 minutes) and 413 minutes for the fourth aliquot (range 382 - 442 minutes).

Oxidative stress marker analysis

We measured four markers of OS in all urine samples: a marker of DNA oxidative damage (8-OHdG), a marker of lipid oxidative damage (MDA) and two markers of antioxidant capacity (TAC and UA). 8-OHdG concentration was measured using the Invitrogen DNA Damage Competitive ELISA kit (catalogue number: EIADNAD). The concentration of MDA was measured using High Performance Liquid Chromatography (HPLC) with no sample dilution. The concentration of TAC was measured using the Cayman Chemical Antioxidant Assay Kit (catalogue number: 709001). The concentration of UA was measured using the Cayman Chemical Uric Acid Assay Kit (catalogue number: 700320). All assays were carried out as per the manufacturer's instructions and to obtain values within the sensitivity range of the assay, we diluted samples 1:150 for 8-OHdG, 1:100 for TAC and 1:200 or 1:400 for UA. We adjusted the concentrations of all markers for urine dilution using specific gravity measured in the undiluted samples (Anestis *et al.* 2009). For 8-OHdG, TAC and UA, we assayed each sample

in duplicate within the same plate and we repeated two samples across all plates as inter-assay controls. The intra-assay coefficients of variation (CVs) were 9.8% for 8-OHdG, 7.2% for TAC and 4.5% for UA. The inter-assay coefficients of variation (CVs) were 11.7% for 8-OHdG, 24.7% for TAC and 14.8% for UA. Due to the high inter-assay CV for TAC, we included plate as a random effect in the mixed model. To estimate the repeatability of measurement of HPLC, we analysed 20 samples in duplicate which had an average CV of 4.22%.

We removed all samples for which specific gravity could not be measured due to values falling outside the detection range of the specific gravity metre (range 1 - 1.05) (N = 56). Two samples were removed because they had a low specific gravity (<1.004) which was leading to inflated marker concentrations (Thompson González *et al.* 2020). We removed 43 8-OHdG measurements, 1 MDA measurement, 11 TAC measurements and one UA measurements due to having a CV > 15%. Some samples were assayed for some markers and not others due to small sample volume and budgetary constraints.

Statistical analyses

Cross-sectional analysis: testing the effects of environmental contamination, time of day and duration of sample storage before and after freezing on OS marker measurement

We used a systematic model selection method in which we constructed a set of candidate GLM models to investigate the impact of the four methodological confounds (method of collection (two levels: plastic (n = 135), leaves (n = 32)), time of day (decimal hours past midnight), freezing delay (decimal hours) and time frozen (decimal weeks)) on each OS marker. Because we did not have *a priori* predictions about the effects, each set of candidate models consisted of all combinations of the covariates and a null model containing only the random effects. Collection method was not included in the TAC and UA models because all assayed samples were collected on plastic. In all models, individual monkey ID was included as a random effect to account for multiple sampling of individuals. Plate was also included as a random effect in the TAC models due to these assays having high inter-plate CVs. Finally, the number of freeze-thaw cycles was included as a fixed effect in the MDA model because 10 samples had 2 additional freeze thaw cycles. This was included as a fixed effect instead of a random effect because it had only two levels. The number of freeze-thaw cycles of samples assayed for the remaining three markers was the same so it was not necessary to account for statistically. For a full list of model structures please see Supplementary Materials S3.1. The models were constructed using the *lme4* package in R (Bates *et al.* 2015). We checked model residuals for normality and homogeneity by visual inspection of qqplots and scatterplots of fitted values versus standardised residuals respectively. We log-transformed 8-OHdG, MDA

and UA measurements to homogenise and normalise the residuals. Collinearity was not an issue in these models (Variance Inflation Factors < 3.0; *car* package, Fox and Weisberg 2019). The candidate models in each set were ranked based on AIC_c (Akaike's Information Criterion corrected for small sample size bias) to select the most parsimonious model with the lowest AIC_c value and highest AIC_c model weight. In each set of candidate models, more than one model had support ($\Delta(Q)AIC_c < 2$) and therefore we carried out multi-model inference using model averaging with shrinkage in the *AICcmodavg* package in R (Mazerolle 2020). Model averaging with shrinkage calculates weighted averages of the estimates based on all candidate models whereby models not containing the variable of interest are assigned a value of 0 for the β and variance. This is considered more robust method of model averaging than only averaging the models containing the variable of interest (Burnham and Anderson 2002, Cade 2015).

Diurnal variation in OS markers

In addition to examining the effect of time of day on OS marker levels cross-sectionally as described above, we identified matched pairs of samples that were collected in the morning (before 12 pm, mean = 09:24 h) and the afternoon (after 12 pm, mean = 14:57 h) from the same individual on the same day (nine pairs). If there were multiple samples in the morning or afternoon from the same individual, we averaged the marker concentrations across these samples. The average difference in collection time between morning and afternoon samples was 5 hours and 36 minutes. We tested for differences between morning and afternoon urinary MDA levels using a Wilcoxon matched-pairs test. This analysis was only carried out for MDA because there were not enough measurements of the other markers to conduct a matched-pairs analysis following the exclusion of samples with an intra-sample CV above 15%. We also calculated the coefficient of variation each for samples collected in the morning and for samples collected in the afternoon across the 12-month dataset for all markers. We compared these CV values to determine whether OS markers are more variable in the morning or in the afternoon. These analyses were conducted on samples collected on plastic only.

Field experiment testing the effect of freezing delay on OS marker levels

To investigate how consistent OS measurements were across 2-hour freezing delay increments we calculated Kendall's concordance coefficients for each marker to investigate changes in rank order of samples following different freezing delays and we calculated the intra-class correlation coefficient (ICC) for each marker based on a single-rating, absolute agreement, two-way mixed-effects model. These analyses were carried out using *DescTools* (Signorell *et al.* 2021) and *irr* (Gamer *et al.* 2019) respectively. To interpret the results of the ICC, we used commonly accepted cut-off values for qualitative ratings of agreement where

less than 0.5 = poor reliability, 0.5-0.75 = moderate reliability, 0.75-0.9 = good reliability and 0.9 and above = excellent reliability (Koo and Li 2016). Additionally, for illustrative purposes, we calculated the percentage change in OS concentration from time 0 for each freezing delay interval for each sample. All these samples were collected on plastic. This analysis was only conducted for MDA and 8-OHdG due to budgetary constraints. After excluding samples as described above, we analysed seven sets of aliquots for MDA and four for 8-OHdG because three of the sets of 8-OHdG aliquots were incomplete.

All analyses were carried out using R Studio (RStudio Team, 2020).

Results

The final analytic dataset consisted of 108 8-OHdG measurements, 167 MDA measurements, 100 TAC measurements and 103 UA measurements collected between 07:15 and 17:54 h over 47 non-consecutive days. The freezing delay between sample collection and storage in liquid nitrogen ranged from 5 to 520 minutes (mean \pm SE = 108 ± 6.7 minutes). Samples were kept frozen for a mean \pm SE of 154 ± 9 days for 8-OHdG (range = 64 - 440 days), 131 ± 9 days for MDA (range = 17 - 401 days), 761 ± 11 days for TAC (range = 664 - 1039 days) and 772 ± 12 days for UA (range = 667 - 1044 days) between collection in the field and assaying in the laboratory.

Cross-sectional analysis: testing the effects of environmental contamination, time of day and duration of sample storage before and after freezing on OS marker measurement

Model selection identified 13, 11, 7 and 7 plausible models ($\Delta(Q)AICc < 2$) for 8-OHdG, MDA, TAC and UA respectively (Supplementary Materials S3.1). Multi-model averaging with shrinkage showed OS marker concentration was not affected by any of the investigated methodological confounds (Table 3.1).

Table 3.1: The model-averaged estimates and confidence intervals with shrinkage for each model parameter.

	Model- averaged estimate with shrinkage	Unconditional SE	95% Unconditional confidence interval
log(8-OHdG (ng/ml corr. SG))			
N = 108			
Method of collection	-0.16	0.12	-0.4, 0.08
Length of time frozen	0	0	0,0
Time of day	-0.01	0.01	-0.03, 0.02
Freezing Delay	0.01	0.02	-0.03,0.05
log(MDA (μM corr. SG))			
N = 167			
Method of collection	-0.03	0.06	-0.16, 0.09
Length of time frozen	0	0	0, 0.01
Time of day	-0.02	0.01	-0.04, 0.01
Freezing Delay	0	0.01	-0.02, 0.02
TAC (mM corr. SG)			
N = 100			
Length of time frozen	0.09	0.07	-0.04, 0.23
Time of day	-0.23	0.28	-0.78, 0.33
Freezing Delay	0.11	0.33	-0.54, 0.77
log(UA (μM corr. SG))			
N = 103			
Length of time frozen	0	0	0, 0.01
Time of day	-0.01	0.02	-0.04, 0.02
Freezing Delay	0.01	0.02	-0.03, 0.04

Diurnal changes in OS

MDA concentrations were higher in samples collected in the morning than in the afternoon (N = 9, V = 41, p-value = 0.02734, Figure 3.1). Across the entire dataset (morning N = 78, afternoon N = 57), 8-OHdG was more variable in samples collected in the morning (morning: CV = 45.9%, afternoon: CV = 36.9%) whereas MDA was more variable in samples collected in the afternoon than those collected in the morning (morning: CV = 44.2%, afternoon: CV =

54.2%). Marker concentrations were equally variable in the morning and the afternoon for both TAC and UA (TAC: morning: CV = 33.9%, afternoon: CV = 29.3%; UA: morning: CV = 40.9%, afternoon: CV = 43.2%).

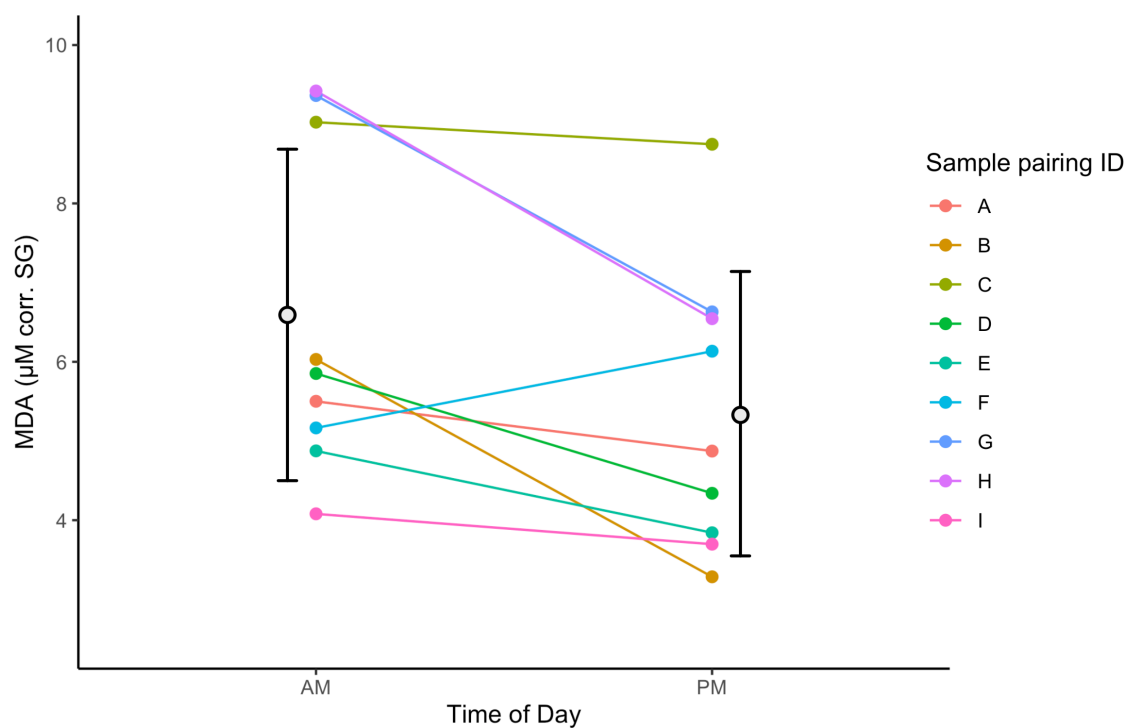


Figure 3.1: Difference in MDA concentration between pairs of samples collected from the same individual on the same day in the morning (before midday) and in the afternoon (after midday). The black open circles and lines represent the mean MDA concentration and standard deviation for morning and afternoon samples.

Experimental test of the effect of freezing delay on OS

Across the four freezing delay increments, 8-OHdG levels were more variable than MDA levels (8-OHdG percentage change: median = 20.9%, minimum = 8.8%, maximum = 42.2%; MDA percentage change: median = 11.5%, minimum = 1.1%, maximum = 60.8%), with MDA only exceeding $\pm 30\%$ for one measurement (Figure 3.2a). Similarly, the mean CV across freezing delays was 18.5% for 8-OHdG (4 samples) and 9.9% for MDA (7 samples). The Kendall's coefficients of concordance (W) between successive freezing delays were significant (8-OHdG: $W = 0.7$, $\chi^2 = 8.4$, $df = 3$, $p = 0.038$; MDA: $W = 0.76$, $\chi^2 = 18.21$, $df = 6$, $p = 0.006$) and indicate moderate levels of concordance. The intraclass correlation coefficients demonstrate good reliability for 8-OHdG measurements and good reliability for MDA measurements across freezing delay steps (*sensu* Koo and Li 2016) (Table 3.2). Despite having moderate to good reliability between freezing delay steps, there was frequent rank order changes between the freezing delay steps (Figure 3.2b).

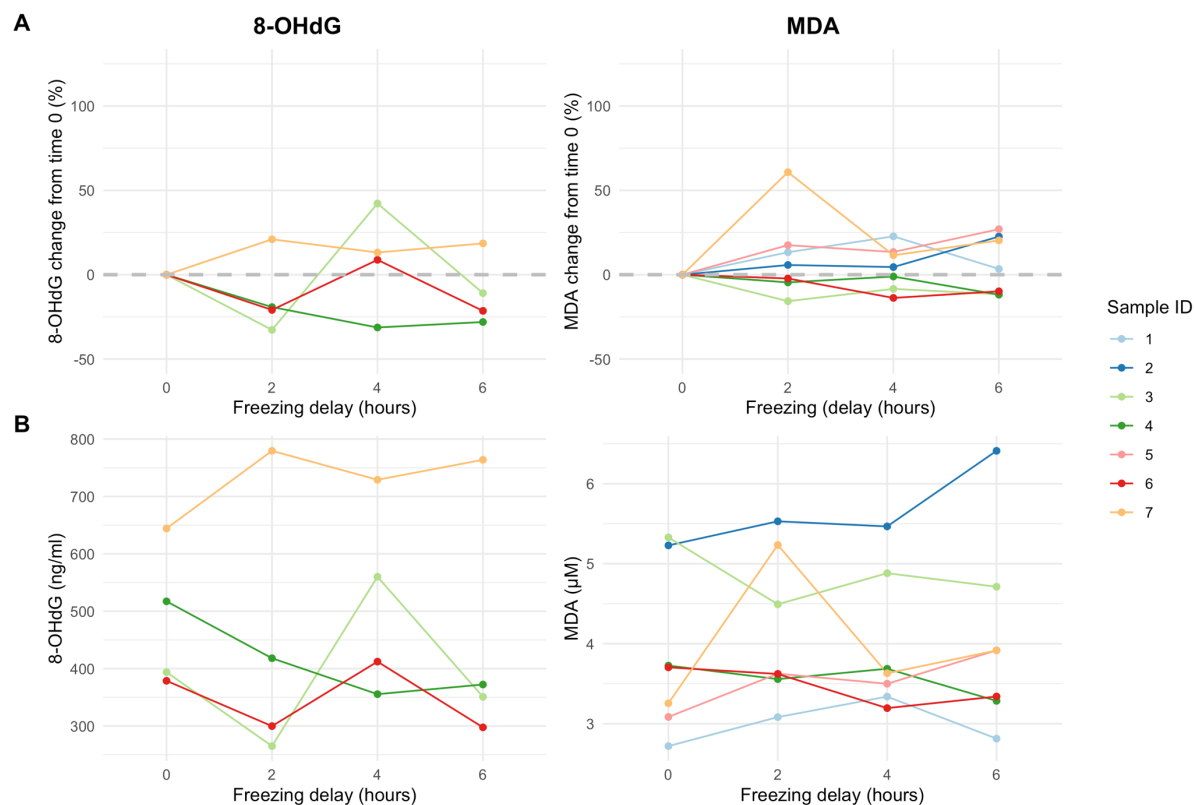


Figure 3.2: Effect of freezing delay on OS marker measurement (8-OHdG and MDA, both corrected for specific gravity): (a) Percentage change relative to values of time 0 controls; and (b) absolute value change. Controls were frozen as soon as possible after collection and then aliquots were frozen following 2, 4 and 6-hour delays. Sample numbers 1, 2 and 5 are missing in for 8-OHdG due to failed measurements in some aliquots.

Table 3.2: Results of Intraclass Correlation Coefficient (ICC) calculation using a single-rating, absolute agreement, 2-way mixed-effects model.

	N	ICC	95% Confidence Interval		F test	
			Lower Bound	Upper Bound	Value	Significance
8-OHdG	4	0.81	0.41	0.98	16	< 0.001
MDA	7	0.80	0.52	0.96	15.8	< 0.001

Discussion

In this study we demonstrate that OS markers are generally robust to methodological confounds that are common in field research. However, the reliability of some OS estimates may be affected by the duration of sample storage before freezing and the time of day the

sample was collected. We highlight some potential confounds that future studies should consider and provide practical recommendations for the measurement of OS in urine collected non-invasively and opportunistically from wild animals. Finally, we discuss the potential for use of urinary OS markers in field research.

Diurnal variation

While 8-OHdG, TAC and UA did not exhibit a pronounced diurnal variation in Zanzibar red colobus urine samples, MDA was higher in the morning than in the afternoon. This result was only apparent in the matched-pairs analysis and not in the cross-sectional analysis. Following the removal of samples with intra-sample CVs above 15%, we did not have enough OS concentration measurements to conduct a matched-pairs test for 8-OHdG, TAC and UA. Therefore, diurnal patterns could be present in these three markers if a matched-pairs analysis were conducted in the future. Evidence for diurnal patterns in OS markers is mixed, with some studies showing no diurnal variation (urine: *Homo sapiens*, Grew *et al.* 2014; blood: *Fregata magnificens*, Sebastiano *et al.* 2017; *Acinonyx jubatus*, Costantini *et al.* 2017) and some showing varying diurnal patterns even for the same markers (blood: *Homo sapiens*, Valencia *et al.* 2001; Kanabrocki *et al.* 2002; Singh *et al.* 2004; saliva: *Homo sapiens*, Alajbeg *et al.*, 2017; Watanabe *et al.* 2019; urine: *Homo sapiens*, Kanabrocki *et al.* 2002; Miwa *et al.* 2004; *Pan troglodytes*, Thompson González *et al.* 2020). One could argue that these conflicting conclusions may be due to the analytical procedures used. Where the analysis has been conducted on urine, variation in urine concentration could mask true diurnal patterns in production because urine concentration varies widely throughout the day. Some studies adjust OS measurements for urine concentration using specific gravity or creatinine (Thompson González *et al.* 2020) while others do not (Kanabrocki *et al.* 2002). We adjusted for urine concentration using specific gravity in this study, but still found conflicting results to those of Thompson González *et al.* (2020), who demonstrated, in wild chimpanzees, that MDA increased, and TAC decreased throughout the day. Therefore, the lack of a consistent diurnal pattern in OS markers in this study and others suggests that there may be a third variable driving OS marker concentrations that is associated with circadian rhythms but varies between sites, species and sample sets within the same study system. Based on this, we suggest that urinary OS measurements should always be adjusted for urine concentration and each dataset should be examined for diurnal patterns as a precaution. If possible, researchers should collect urine samples consistently at one time of day unless the study design dictates otherwise.

Environmental contamination

We found that contact with leaves had no effect on OS measurements for any of the markers we studied. This lack of effect of leaf contamination is similar to that which has been observed in urinary steroid measurements (Muller & Wrangham 2004; Marshall & Hohmann, 2005; Knott 2005). This result suggests that urinary OS biomarkers are sufficiently stable to be collected from either plastic or leaf surfaces, which would give flexibility in sample collection method to field biologists that may not always succeed in placing a plastic sheet underneath their study subjects with full precision and perfect timing. We would, however, recommend additional testing of the impact of collection method on urinary OS marker concentration using an experimental approach following Muller & Wrangham (2004) and Knott (2005).

Storage time before freezing

Our cross-sectional analysis showed that freezing delay up to 520 minutes did not have a significant effect on any of the four OS markers that we measured. Our study agrees with previous field research cross-sectionally testing the effect of freezing delay on blood OS marker concentrations (Nussey *et al.* 2009; Costantini *et al.* 2017). However, our experimental analysis of MDA and 8-OHdG measurements showed that values of these markers were highly variable and inconsistent across freezing delays of up to 360 minutes, more than has been observed in clinical OS studies (Lee and Kang 2008; Matsumoto *et al.* 2008) and other urinary biomarkers (e.g., urinary neopterin: Heistermann and Higham 2015). This discrepancy might be due to a difference in ambient temperature at our tropical field site and in the lab conditions used in these studies. The difference in results between our cross-sectional and experimental study suggests that freezing delay may not be an issue for higher-level comparisons of OS between groups of samples, but that comparisons between individual samples exposed to different freezing delays may not be informative. An investigation of the rank orders of individual samples is missing from the previous studies of freezing delay and urinary OS measures (Lee and Kang 2008; Matsumoto *et al.* 2008) and therefore these fine-scale differences between samples may have been overlooked. Future research should consider the potential confounding effects of storage on OS measurements, especially if individual samples are to be compared.

We found that for both 8-OHdG and MDA, OS concentration oscillated across freezing delay steps where we would expect a steady increase in damage. Even though samples were mixed using a pipette prior to aliquoting, we suspect this oscillation might be caused by drawing the four urine sub-samples from a heterogenous urine sample, rather than due to lab measurement error, as that was low (CV <15%). Therefore, there may be additional

confounding factors affecting the reliable comparison of OS measurements from individual samples and we advise that spot-sampling (whereby single samples are taken to represent the concentration for a unit of analysis e.g. individuals, sites, periods) should be avoided. Instead, multiple samples should be taken to calculate mean OS concentrations for each unit of analysis.

MDA was more stable across the four freezing delay steps than 8-OHdG. In fact, using a well-accepted CV cut-off value of 15%, most of the 8-OHdG measurements would be deemed unreliable if the aliquots were treated as measurement replicates. There is no indication in the literature that 8-OHdG is a less stable molecule than MDA (Cooke *et al.* 2008) so the difference in variability between the markers most likely comes from the methods we used to quantify them (ELISA for 8-OHdG and HPLC for MDA). While ELISAs have been widely used to measure 8-OHdG in urine samples, they are subject to greater measurement variability (Barregard *et al.* 2013) than chromatographic approaches such as HPLC (Graille *et al.* 2020). An ELISA is adequate for comparing relative urinary OS levels between groups (where multiple samples contribute to a mean estimate) but not to reliably measure exact concentrations of markers (Yoshida *et al.* 2002; Cooke *et al.* 2008). Researchers should be aware of the analytical limitations of different methods and select the most appropriate method to address their question. For example, if individual samples need to be compared, HPLC approaches, which have higher specificity and sensitivity, would be more desirable.

Sample storage after freezing

In our correlational analysis, all OS marker measurements remained stable across frozen storage times, which is supported by previous research demonstrating high levels of stability of various OS markers during storage below -20°C for over one year (blood: Koracevic *et al.* 2001; Jansen *et al.* 2013b; Costantini *et al.* 2017; Jansen *et al.* 2017; Rubio *et al.* 2018; urine: Matsumoto *et al.* 2008; Martinez and Kannan 2018; Thompson González *et al.* 2020). Our result must be caveated with the fact that we only tested the effect of duration of storage at -80°C up to 15 months for MDA and 8-OHdG, and for up to 35 months for TAC and UA. Other studies have demonstrated degradation of OS markers over different time periods and at different temperatures. For example, Martinez and Kannan (2018) found a 40% decrease in MDA in human urine samples after 30 days of storage at -20°C and Thompson González *et al.* (2020) who demonstrated a weak negative effect of storage time (1-10 years) at -30°C on MDA-TBARS in urine from wild chimpanzees. Therefore, we still recommend that samples should, ideally, be stored at -80°C and for as short a time as is possible and that the potential effect of time in storage on marker values should be examined for each dataset. However, our

result is promising for future studies utilising OS markers, both in field and laboratory environments.

Conclusions

The application of non-invasive OS measurement in the field will strengthen our ability to address exciting theoretical and applied questions, for example the role of OS in life-history trade-offs, development, aging, reproduction and the effects of anthropogenic disturbance and environmental conditions on wild animal physiology, health and fitness. The redox system is highly conserved across taxa and plays an important role in many biological processes. Therefore, it can be studied in a wide variety of contexts and study systems, something that will only be aided by being able to study natural populations in a non-invasive way. Additionally, being able to investigate these questions in populations engaging in natural social interactions and facing natural resource restrictions will make the insights more pertinent than those that might be gained through laboratory studies.

However, applying physiological methods in the field increases the potential for methodological confounds (e.g., from environmental contamination, differences in freezing delays, heterogeneous urine samples or assay method). Here we demonstrated a high level of stability of four urinary OS markers in response to four common methodological confounds of field research. In general, we advise that future studies should be mindful of the potential for diurnal patterns in OS markers and of the potential confounding effects of storage on OS measurements. In particular, we advise that markers of OS concentrations should be adjusted for urine concentration, samples should be collected at one time of day and samples should be stored at -80°C for the shortest time possible. Additionally, researchers should avoid spot-sampling to ensure the reliability of their results. Nevertheless, our results provide encouraging evidence that these markers are sufficiently stable to conduct a robust study of OS in non-invasively collected urine samples from wild animals within reasonable methodological constraints.

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Chapter 4

Oxidative stress markers as indicators of anthropogenic disturbance in Zanzibar red colobus monkeys



Adult male Zanzibar red colobus monkey (*Piliocolobus kirkii*) in Jozani-Chwaka Bay National Park, Zanzibar

Abstract

Anthropogenic disturbance places wildlife into evolutionarily novel conditions which can result in changes to aspects of physiology, for example oxidative stress. Markers of oxidative stress can provide information on the effect of disturbance before population level changes occur, due to their close link to health and reproduction. We investigated the relationship between two measures of anthropogenic disturbance (habitat degradation and exposure to tourists) and oxidative stress levels in the Zanzibar red colobus (*Piliocolobus kirkii*). We measured three markers of oxidative damage (8-OHdG, MDA, neopterin) and one marker of antioxidant capacity (TAC) in urine samples collected non-invasively from six wild colobus groups experiencing a range of habitat degradation and tourist exposure. We also investigated the link between oxidative stress and the juvenile: adult female ratio of each group (as a proxy for recruitment). Monkeys experiencing high levels of habitat degradation had elevated antioxidant capacity but did not differ in their levels of oxidative damage. There was no association between any of our markers of oxidative stress and tourist exposure or recruitment. We suggest that monkeys are upregulating their levels of antioxidant capacity in response to habitat degradation, thereby successfully mitigating oxidative damage, and, as a result, do not suffer changes to recruitment. We conclude that protecting forest habitat is the main intervention needed to reduce the impact of anthropogenic disturbance on Zanzibar red colobus physiology. Additionally, focusing tourism on a small number of highly habituated groups would be beneficial as we speculate that the lack of a relationship between oxidative stress and tourist exposure in this study is because of habituation to people. Non-invasive urine sampling of oxidative stress markers could have wider application in conservation monitoring.

Introduction

Due to increasing anthropogenic disturbance, wildlife across the world is experiencing evolutionarily novel conditions (Sih *et al.*, 2011). While some species can survive with anthropogenic disturbance, others may struggle to adapt, leading to population declines (Brashares *et al.*, 2001, French *et al.*, 2011), range shifts (Larsen, 2012) and extinctions (Wan *et al.*, 2019). Physiological changes resulting from anthropogenic disturbance can indicate an animal's capacity to cope with new conditions (Beaulieu and Costantini, 2014), and is often detectable before witnessing changes at the population level (e.g., changes in birth or death rates) (Madliger and Love, 2014). Therefore, the information gained from physiological markers may allow early detection of future population declines and allow conservationists to intervene.

The physiological impacts of anthropogenic disturbance have been most widely studied using glucocorticoids, which increase when the hypothalamic–pituitary–adrenocortical (HPA) axis is activated in response to stressors (Sapolsky *et al.*, 2000). However, evidence for the link between glucocorticoids and anthropogenic disturbance is mixed. Some studies have found a positive relationship (Suorsa *et al.*, 2003; Martínez-Mota *et al.*, 2007; Rangel-Negrín *et al.*, 2009; Jaimez *et al.*, 2012), some have found a negative relationship (Munshi-South *et al.*, 2008), and some have found no relationship at all (Tempel and Gutierrez, 2004; von der Ohe *et al.*, 2004). Beyond this, a potentially more important issue persists: the link between chronically elevated glucocorticoid levels and the ultimate outcome of interest, fitness, is still equivocal (Beehner and Bergman, 2017, Kaisin *et al.*, 2021). This makes it difficult to make inferences about the population level impacts of disturbance using glucocorticoids, leaving room for improvement in our conservation physiology toolbox (Beaulieu and Costantini, 2014).

Markers of oxidative stress (OS) could fill this gap for two main reasons. Firstly, OS is responsive to anthropogenic disturbance. OS is the imbalance between levels of reactive oxygen species produced during oxygen consumption and the organism's antioxidant response (Finkel and Holbrook, 2000; Balaban *et al.*, 2005). Increased production of reactive oxygen species can result directly from physiological (e.g., immune cell activity), chemical (e.g., pollution or pesticide exposure) or physical mechanisms (e.g., heat), or from metabolic upregulation, for example in response to physical activity or activation of the HPA-axis (Beaulieu and Costantini, 2014). Exposure to anthropogenic disturbance has the potential to increase oxidative stress through any one of these mechanisms. There is evidence for the link between anthropogenic disturbance and OS, although the direction of the effect varies by study system, marker and type of disturbance (Table 4.1). Secondly, OS has well established links with health and fitness, meaning it could mediate the link between anthropogenic

disturbance and population declines (Beaulieu *et al.*, 2013; Beaulieu and Costantini, 2014). During OS, excess reactive oxygen species can damage important cell components, such as lipids, proteins, and DNA (Valko *et al.*, 2007). The accumulation of this damage is linked to a range of pathologies (Keaney *et al.*, 2003; Bonda *et al.*, 2010; Anderson and Maes, 2014), accelerated aging (Selman *et al.*, 2012) and reproductive complications (Keskes-Ammar *et al.*, 2003; Agarwal *et al.*, 2012) thereby reducing fertility and survival probability, in other words, fitness (Monaghan *et al.*, 2009). The negative fitness consequences of OS have been demonstrated in many species (*Geothlypis trichas*, Freeman-Gallant *et al.*, 2011; *Parus major*, Helfenstein *et al.*, 2010; Losdat *et al.*, 2013; *Apus melba*, Bize *et al.*, 2008; *Hirundo rustica*, Saino *et al.*, 2011; *Phalacrocorax aristotelis*, Noguera *et al.*, 2012). There is also evidence that individual oxidative status is related to population trends in Gentoo (*Pygoscelis papua*) and Adélie penguins (*Pygoscelis adeliae*) (Beaulieu *et al.*, 2013). As such, OS markers have great potential for understanding how environmental conditions may affect individual fitness and population trends (Beaulieu *et al.*, 2013). However, to our knowledge, only one study has jointly investigated the impact of anthropogenic disturbance on OS and the subsequent fitness consequences in a wild animal (Marine iguana, *Amblyrhynchus cristatus*, French *et al.* 2017).

This study aims to contribute to this knowledge gap by investigating these relationships in the Zanzibar red colobus monkey (*Piliocolobus kirkii*). The Zanzibar red colobus monkey is an endangered primate, endemic to the island of Unguja in Zanzibar (Davenport, 2019), and is highly threatened by habitat loss and degradation. Between 2003 and 2018, Zanzibar's forest cover declined by 15% (Omar and Cabral 2020) due to human population growth, agricultural expansion and unmanaged development for tourism and housing (Struhsaker 2010, Linder *et al.*, 2021). 31% of the remaining approximately 5,800 red colobus live outside protected areas where deforestation is particularly prevalent (Davenport *et al.*, 2019). Although the species appears to be able to survive with these disturbances, recruitment is low across the island and group sizes are smaller in more disturbed areas suggesting that there may be hidden physiological costs to exposure to habitat degradation (Davenport *et al.*, 2019). While there is no explicit study of the impact of anthropogenic habitat degradation on OS, we propose two mechanisms by which habitat degradation could lead to OS. Firstly, through changes in food availability which could increase metabolically intensive foraging behaviour or reduce the availability of dietary antioxidants. The link between natural habitat condition and OS via these mechanisms has been demonstrated in wild common scale-backed antbirds (*Willisornis poecilinotus*) (Gomes *et al.*, 2014) and Seychelles warblers (*Acrocephalus sechellensis*) (van de Crommenacker *et al.*, 2011). Secondly, through activation of the HPA-axis due to exposure to psycho-social stressors associated with habitat degradation (Costantini *et al.*, 2011). For example, increased intra-specific aggression in response to food competition in areas of high

habitat degradation has been observed in the Zanzibar red colobus (Siex, 2003), which could lead to OS.

Another disturbance facing the red colobus, that could lead to OS, is tourist exposure. Half of the remaining Zanzibar red colobus are found in Jozani-Chwaka Bay National Park (JCBNP), the stronghold for the species (Davenport *et al.*, 2019). However, JCBNP receives over 65,000 tourists annually (JCBNP, official records 2019), many of whom get very close to the monkeys, breaking the 3m distance regulations some 11% of the time (Warkentin & Georgiev, unpublished data). Tourist exposure has been shown to affect OS in wild animals (Table 4.1), but the mechanisms are unknown. We suggest three possible mechanisms by which tourist exposure could affect OS. Firstly, through activation of the HPA-axis which has been demonstrated in a variety of primate species (*Trachypithecus geei*, Sarmah *et al.*, 2017; *Alouatta pigra*, Behie *et al.*, 2010; *Gorilla gorilla*, Shutt *et al.*, 2014). Secondly, through elevated immune activity due to increased disease transmission (Dunay *et al.*, 2018) in tourist-exposed primates. Thirdly, through increased energy expenditure due to behavioural changes in response to tourist exposure, such as increased intraspecific aggression (Berman *et al.*, 2007; Semeniuk and Rothley, 2008; Majolo *et al.*, 2013; Maréchal *et al.*, 2016).

We investigate the effect of habitat degradation and tourist exposure on OS in six groups of Zanzibar red colobus using non-invasive urinary OS markers. We predict that red colobus groups exposed to higher levels of habitat degradation and tourism will have higher levels of oxidative damage and lower levels of antioxidant protection than groups exposed to lower levels of these disturbances. We also predict that groups with higher levels of oxidative damage and lower levels of antioxidant capacity will have lower levels of recruitment.

Table 4.1: A selection of case studies demonstrating the varied impacts of anthropogenic disturbance on OS across study systems.

Change in production of ROS	Type of disturbance	Study system	Citation
Increased oxidative damage	Chemical pollution	Hybrid sparrow, (<i>Passer domesticus</i> × <i>Passer hispaniolensis</i>)	Amri <i>et al.</i> , 2017
		House sparrow, <i>Passer domesticus</i>	Herrera-Dueñas <i>et al.</i> , 2017
	Tourism	White stork, <i>Ciconia ciconia</i>	Tkachenko and Kurhaluk, 2013
		Sting ray, <i>Dasyatis americana</i>	Semeniuk <i>et al.</i> , 2009
		Marine iguana, <i>Amblyrhynchus cristatus</i>	French <i>et al.</i> , 2017
	Reduced food availability	Northern Bahamian Rock Iguana, <i>Cyclura cychlura</i>	Webb, 2020
	Forest gaps (natural)	Seychelles warbler, <i>Acrocephalus sechellensis</i>	van de Crommenacker <i>et al.</i> , 2011
No change in oxidative damage	Chemical pollution	Common scale-backed antbird, <i>Willisornis poecilinotus</i>	Gomes <i>et al.</i> , 2014
		Blue tit, <i>Cyanistes caeruleus</i>	Salmón <i>et al.</i> , 2018
		Great tit, <i>Parus major</i>	Salmón <i>et al.</i> , 2018
		House sparrow, <i>Passer domesticus</i>	Salmón <i>et al.</i> , 2018
	Metal pollution	Tree sparrow, <i>Passer montanus</i>	Salmón <i>et al.</i> , 2018
		White stork, <i>Ciconia ciconia</i>	de la Casa-Resino <i>et al.</i> , 2015
Light pollution	Great tit, <i>Parus major</i>	Raap <i>et al.</i> , 2016	
Increased antioxidant activity	Chemical pollution	Blue tit, <i>Cyanistes caeruleus</i>	Salmón <i>et al.</i> , 2018
		Great tit, <i>Parus major</i>	Salmón <i>et al.</i> , 2018
		House sparrow, <i>Passer domesticus</i>	Salmón <i>et al.</i> , 2018
		Tree sparrow, <i>Passer montanus</i>	Salmón <i>et al.</i> , 2018
	Urbanisation	House sparrow, <i>Passer domesticus</i>	Herrera-Dueñas <i>et al.</i> , 2017
		White stork, <i>Ciconia ciconia</i>	Tkachenko and Kurhaluk, 2013
	Metal pollution	White stork, <i>Ciconia ciconia</i>	de la Casa-Resino <i>et al.</i> , 2015
		Great tit, <i>Parus major</i>	Rainio <i>et al.</i> , 2013
		Blue tit, <i>Cyanistes caeruleus</i>	Rainio <i>et al.</i> , 2013
		Pied flycatcher, <i>Ficedula hypoleuca</i>	Rainio <i>et al.</i> , 2013
	Plastic pollution	Striped red mullet, <i>Mullus surmuletus</i>	Alomar <i>et al.</i> , 2017
	Decreased antioxidant activity	Chemical pollution	Hybrid sparrow, (<i>Passer domesticus</i> × <i>Passer hispaniolensis</i>)
Urbanisation		White stork, <i>Ciconia ciconia</i>	Tkachenko and Kurhaluk, 2013
Tourism		Sting rays, <i>Dasyatis americana</i>	Semeniuk <i>et al.</i> , 2009
No change in antioxidant activity	Tourism	Marine iguanas, <i>Amblyrhynchus cristatus</i>	French <i>et al.</i> , 2017
	Reduced food availability	Seychelles warbler, <i>Acrocephalus sechellensis</i>	van de Crommenacker <i>et al.</i> , 2011
	Urbanisation	Eurasian kestrels, <i>Falco tinnunculus</i>	Wemer <i>et al.</i> , 2021
	Plastic pollution	Striped red mullet, <i>Mullus surmuletus</i>	Alomar <i>et al.</i> , 2017
	Light pollution	Great tit, <i>Parus major</i>	Raap <i>et al.</i> , 2016

Methods

Study site and subjects

We collected urine samples from 50 Zanzibar red colobus in six groups in and around JCBNP, Zanzibar (6.233°S, 39.404°E) (Figure 4.1) from September 2018 to March 2020 (Supplementary Material S4.1). The groups were differentially exposed to habitat degradation and tourism (Figure 4.2a, Supplementary Material S4.1). The habitats of the groups ranged from mostly undegraded habitat to highly degraded habitat. The undegraded habitat comprised of natural forest with some areas showing evidence of historical logging, secondary growth and some invasive species. Meanwhile, the highly degraded habitat consisted of an agricultural matrix of orchards, open vegetable plots and pine plantations. Most tourism activity was focused on 3 - 4 groups (two of which are included in this study) which were visited by tourists on a daily basis, either guided by the official national park staff or by unofficial guides. None of the colobus groups were provisioned with food. Groups residing on the periphery of the national park face mortality from hunting by dogs (Georgiev *et al.*, 2019) and 1.8% - 3.2% annual mortality from collisions with vehicles on the main road transecting their home ranges (Olgun *et al.* 2022) (Figure 4.1). Not all groups were studied for the entire study period (Supplementary Material S4.1).

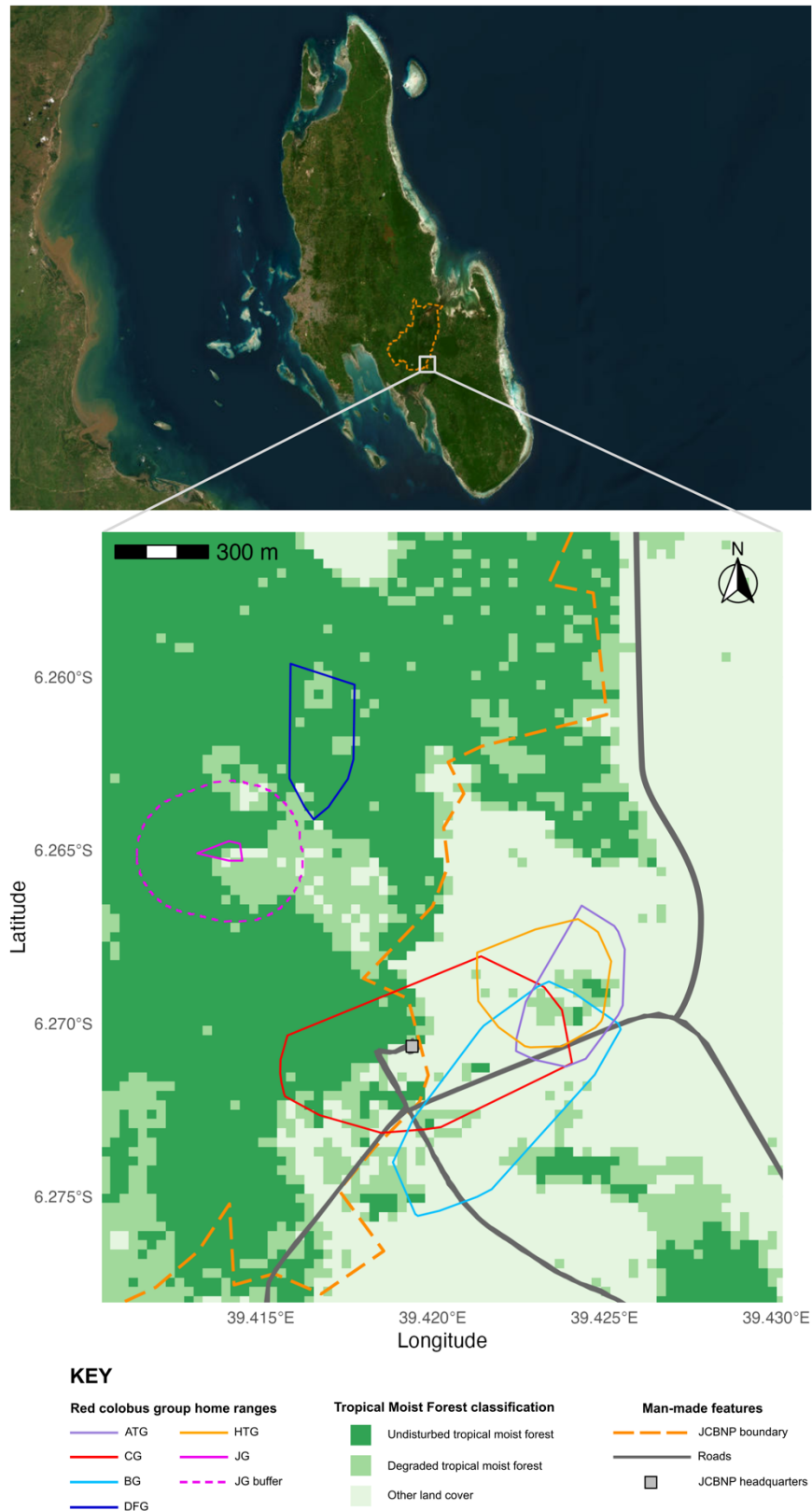


Figure 4.1: Map showing the home range of each study group of red colobus on the island of Unguja in Zanzibar. DFG = Deep Forest Group, JG = Joshi Group, ATG = Apple Tree Group, HTG = Half Tail Group, BG = Baobab Group, CG = Camp Group. Home ranges were created

using a 99% minimum convex polygon based on ranging data collected from each group (2 - 96 follow days). JG's range was based on just 2 follow days of data and therefore we created a buffer around the estimated range to match the average range area of the other 5 groups. This is shown on the map as JG buffer. The raster layer of level of habitat degradation is from the Tropical Moist Forest dataset (Vancutsem *et al.* 2021). The JCBNP boundary is from the Protected Planet: The World Database on Protected Areas (UNEP-WCMC 2023).

Habitat degradation

We defined exposure to habitat degradation as the proportion of each home range that was classified as 'degraded tropical moist forest' or 'other land cover' (i.e., not undisturbed tropical moist forest) in the Tropical Moist Forest dataset (Vancutsem *et al.*, 2021). First, we calculated the home range of each group using a 99% minimum convex polygon in the *adehabitatHR* package (Calenge, 2006) based on GPS data collected for each group between July 2018 and September 2022 (Figure 4.1). The number of follow days with GPS data varied between groups (from two to 96 follow days). Joshi Group's home range was based on just two days of GPS data meaning that the extent of this group's home range was likely underestimated. To overcome this limitation, we created a buffer around Joshi Group's home range that was the same area as the mean of the other five colobus groups (0.192 km²) using the *st_buffer* function in the *sf* package (Pebesma, 2018) (Figure 4.1). To check the impact of this decision on our results, we also ran all the subsequent analyses using the original home range and it did not change the results (Supplementary material S4.5). We thus present the results for the buffered home range. After establishing the home range of each group, we reclassified the Tropical Moist Forest dataset into degraded vs. forest habitat by combining the 'degraded tropical moist forest' and 'other land cover' categories. We did this because undisturbed forest is important for this species (Davenport *et al.*, 2019). We then calculated the proportion of each home range area classified as degraded under our reclassification scheme using the *extractr* package (Baston, 2022) (Figure 4.2).

Tourism

We measured tourist exposure by recording the presence or absence of tourists with each group at 15-minute intervals during follow days (number of follow days per group in Figure 4.2a). We then calculated the daily proportion of observations with tourists present and took the mean across follow days (Figure 4.2a).

Urine sampling and storage

We collected 423 urine samples across the six groups during the study period. We collected urine samples opportunistically while following the monkeys throughout the day (typically between 07:00 – 18:00 h); excluding samples that were visibly contaminated with faeces or detritus. Where possible, we identified the individuals we sampled using distinguishing features such as facial markings, scars, injuries and posture/shape (N = 366 samples), if not, we simply noted their age-sex class (adult male, adult female or immature). Urine samples were caught midstream using a plastic bag on the end of a catchpole or were pipetted from urine splatter on leaves. We have previously shown that collecting urine from leaves does not significantly affect OS marker values (Melvin *et al.*, 2022). In the field, samples were carried in the dark and on ice in cool-packs until flash-freezing in liquid nitrogen later the same day at the field base. They were transported to the UK in dry shippers below -150°C and, once at the laboratory, all samples were stored at -80°C until assaying.

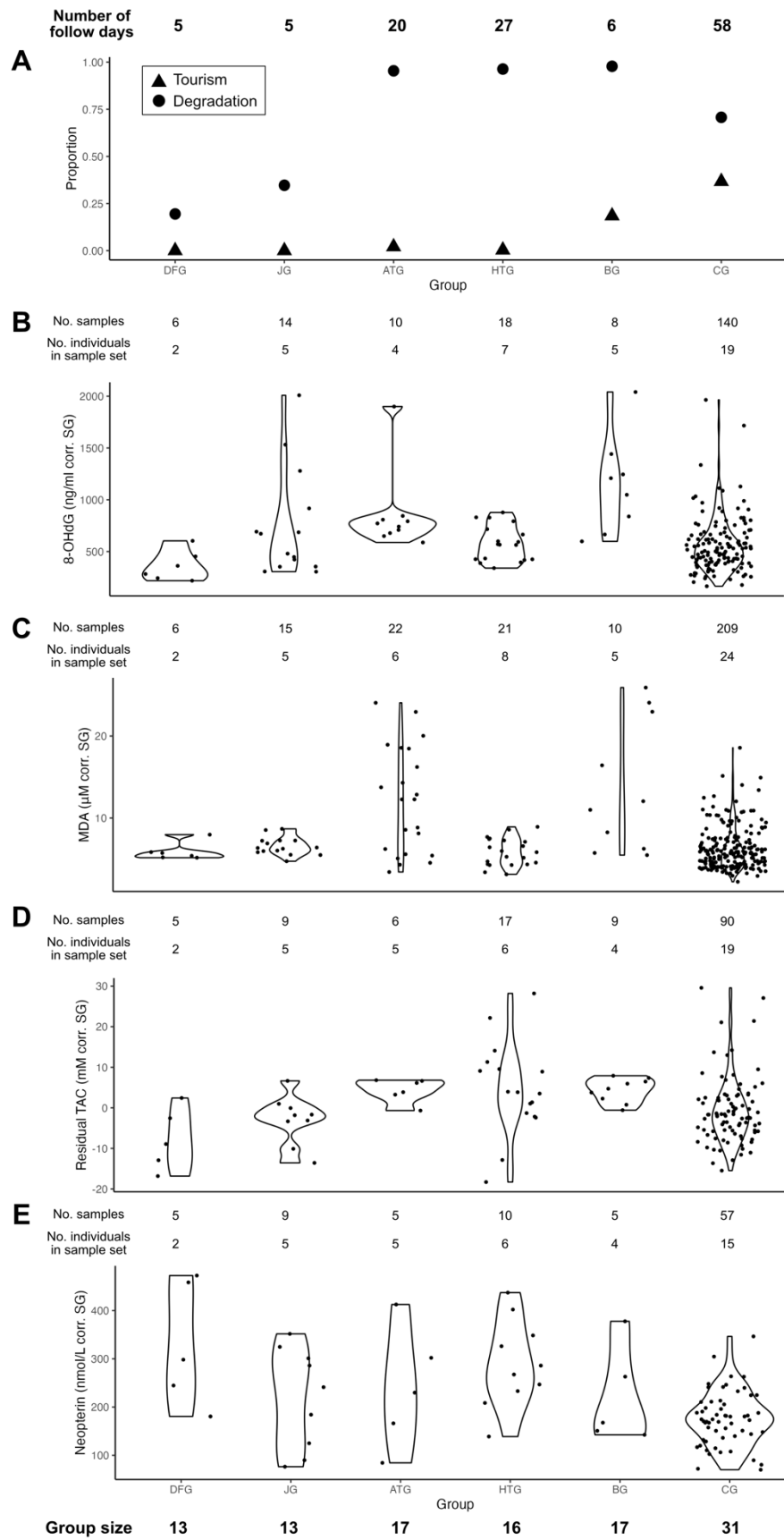


Figure 4.2: A: The mean daily proportion of 15-minute interval observations with tourists present and the number of follow days that this data is based on. The proportion of the home

range classified as degraded in the Tropical Moist Forest dataset for each group is also shown. **B-E:** Violin plots of the sample set for each OS marker split across study groups. Also shown are the total group sizes of each group, the number of samples in the set for each marker and the number of individuals in the sample set for each marker. DFG = Deep Forest Group, JG = Joshi Group, ATG = Apple Tree Grove Group, HTG = Half Tail Group, BG = Baobab Group, CG = Camp Group.

OS marker selection

We measured four markers of oxidative stress in the urine samples: three markers of oxidative damage (a marker of lipid oxidative damage, malondialdehyde, or MDA; a marker of DNA oxidative damage, 8-OHdG; and a marker of T helper cell 1 derived cellular immune activation, neopterin) and one marker of antioxidant capacity (Total Antioxidant Capacity, or TAC). These markers are known to be stable, represent system-wide levels of OS and have a variety of commercial assays available to test for them.

Malondialdehyde (MDA) is a product of the decay of unstable lipid peroxides produced by lipid peroxidation during OS. MDA is mutagenic, tumorigenic and highly reactive which results in a variety of health disorders when in high concentration (Singh *et al.*, 2014).

8-hydroxy-2'-deoxyguanosine (8-OHdG) is produced during the oxidation of the guanine base of DNA by reactive oxygen species. It is excreted in urine by DNA repair mechanisms (Kasai *et al.*, 2008) to remove transversion mutations (Shibutani *et al.*, 1991). 8-OHdG is higher among individuals with various diseases compared to those without (Wu *et al.*, 2004, Wu *et al.*, 2017, Korkmaz *et al.*, 2018).

Neopterin is a pteridine released during the activation of the non-specific inflammatory immune response and is a product of the oxidation of 7,8-dihydroneopterin, a monocyte and macrophage synthesised antioxidant (Huber, 1984, Berdowska and Zwirska-Korczyńska, 2001, Eisenhut, 2013). As such, neopterin can be used as an estimate of OS (Wakabayashi *et al.*, 2020). It is chemically stable, even under field conditions (Danish *et al.*, 2015, Heistermann and Higham, 2015), shows no significant diurnal variation (Murr *et al.*, 2002, Behringer *et al.*, 2017) and can be used to monitor health non-invasively in urine (Higham *et al.*, 2015). Neopterin levels have been positively correlated with viral and bacterial infection (Murr *et al.*, 2002, Higham *et al.*, 2015, Behringer *et al.*, 2017, Wu *et al.*, 2018) and with disease

progression, severity and outcome (Denz *et al.*, 1990, Murr *et al.*, 2002, Prat *et al.*, 2006, Eisenhut, 2013).

Total Antioxidant Capacity (TAC) measures the cumulative antioxidant status of an organism (Buico *et al.*, 2009), defined as the moles of oxidants neutralised by one litre of antioxidant solution (Niki, 2010). Uric acid is a potent antioxidant comprising as much as 60% of antioxidant activity in human serum (Wayner *et al.* 1987). However, the antioxidant role of uric acid *in vivo* is unclear (Kang and Ha, 2014) and high levels of uric acid have been significantly linked to many diseases (Alvarez-Lario and Macarrón-Vicente, 2011). Therefore, we controlled for the potentially confounding effects of the incidental antioxidant activity of uric acid on TAC by measuring the concentrations of TAC and uric acid in each sample separately and extracting the residuals from a linear model between them (Cram *et al.*, 2015) (hereafter termed 'residual TAC'; Supplementary Material S4.2).

OS marker analysis

MDA concentration was estimated using High Performance Liquid Chromatography (HPLC). To estimate the repeatability of measurement of HPLC, we analysed 20 samples in duplicate. 8-OHdG concentration was measured using the Invitrogen DNA Damage Competitive ELISA kit (EIADNAD), TAC concentration was measured using the Cayman Chemical Antioxidant Assay kit (catalog number: 709001), UA was measured using the Cayman Chemical Uric Acid Assay Kit (catalog number: 700320) and neopterin was measured using the IBL International Neopterin ELISA (catalog number: RE59321) following manufacturer's instructions. To obtain values within the sensitivity range of the assay, we diluted samples 1:150 or 1:300 for 8-OHdG (differing between sample batches), 1:100 for TAC, 1:200 or 1:400 for UA (some samples were re-run to obtain values within the sensitivity range) and 1:25 for neopterin. We assayed each sample in duplicate for 8-OHdG, TAC, UA and neopterin within the same plate and we repeated two samples across all plates as inter-assay controls. The intra-assay coefficients of variation (CV) for 8-OHdG, TAC, UA and neopterin were 8.9%, 7.9%, 4.7% and 16.8% respectively. Mean inter-assay CVs for 8-OHdG, TAC, UA and neopterin were 24.0%, 24.7%, 14.8% and 29.9%, respectively. The 20 inter-batch replicates of MDA had a mean CV of 4.2%.

We back-calculated the original marker concentration based on the lab dilution and then adjusted these concentrations for urine dilution using specific gravity measured in the undiluted samples (Anestis *et al.*, 2009). In our first round of lab work, if a sample was above the detection range of the specific gravity meter we used (1-1.05), we excluded it (n = 80). In our second round of lab work, if a sample was above the detection range of the specific gravity

meter, we diluted the sample by 50%, remeasured specific gravity and then multiplied the value by 2 to determine the true specific gravity of the sample (Supplementary Material S4.3). We removed 4 samples because they had low specific gravity (<1.004), which was leading to inflated marker concentrations (Thompson-González *et al.*, 2020). We removed one MDA measurement, 57 8-OHdG measurements, 27 TAC measurements, 4 UA measurements and 69 neopterin measurements because they had an intra-assay CV > 15 . Finally, we excluded samples for which the ID of the individual was unknown. Not all samples were assayed for every marker due to budgetary considerations leading to differences in the size of the final analytic dataset between markers (Figure 4.2).

Statistical analysis

Effect of habitat degradation and tourist exposure on OS

To test for the effect of habitat degradation and tourist exposure on each of the OS markers, we constructed four General Linear Mixed Effects Models (GLMM) using the *lme4* package (Bates, 2015) with OS marker concentration as the response variable. Each model included 3 fixed effects: proportion of home range that is degraded, mean daily proportion of observations with tourists present and age-sex class (categorical: adult male, adult female, immature). We included age-sex class as a predictor in the model as it can affect OS values in this (Melvin, unpublished) and other species (e.g. *Homo sapiens*, Bolzán *et al.*, 1997; *Ovis aries*, Nussey *et al.*, 2009; *Macaca mulatta*, Georgiev *et al.*, 2015; *Sus scrofa*, Gassó *et al.*, 2016; *Pan troglodytes*, Thompson-González *et al.*, 2020) and we wanted to control for this. The number of freeze-thaw cycles a sample underwent prior to assaying (1-6) was included as a fixed effect for MDA, 8-OHdG and neopterin but not for residual TAC because all samples underwent exactly 3 freeze-thaw cycles prior to assaying. To account for repeated sampling of individual colobus and repeated measures from each group, we included Animal ID and Group ID as random effects in all models. For models of 8-OHdG, residual TAC and neopterin we also included Plate ID of the assay as a random factor as these assays showed high inter-plate CVs. For the residual TAC model, Group ID and Plate ID had 0 variance causing singular fit and were subsequently removed from the model. We checked for normality and homogeneity of model residuals by visual inspection of qqplots and scatterplots of fitted values vs standardised residuals respectively (Supplementary Material S4.6). We log-transformed the 8-OHdG, MDA and neopterin measurements to homogenise and normalise the residuals. Collinearity was not an issue in these models (Variance Inflation Factors < 2 ; *car* package, Fox and Weisberg, 2019) and there was no correlation between the proportion of home range classified as degraded and the mean proportion of observations with tourists present (Kendall's tau = 0.414, p-value = 0.25). We compared the optimal models with their respective

null models using a maximum likelihood (ML) fit via an ANOVA test and by comparing AIC values.

Oxidative stress and juvenile: adult female ratio

To test the prediction that groups with higher levels of oxidative damage and lower levels of antioxidant capacity will have lower recruitment, we calculated the juvenile: adult female ratio for each group. The juvenile: adult female ratio is a commonly used measure of recruitment into the adult population and therefore a proxy of population viability (Davenport *et al.*, 2019). We calculated the juvenile: adult female ratio for each group by dividing the number of juveniles by the number of adult females (Supplementary Material S4.1). We carried out a Kendall rank correlation test between the juvenile: adult female ratio and the mean concentration of each OS biomarker (8-OHdG, MDA, residual TAC and neopterin) for the identified adult females in each group. We chose to use this correlation test because there were tied ranks in the juvenile: adult female ratio between groups. The test was carried out using the *Kendall* package (McLeod, 2022).

All statistical analyses were conducted in RStudio (RStudio Team 2020) and code is available on my GitHub. The code is currently private and invitations have been sent to examiners separately.

Results

MDA values had a mean \pm standard deviation of 7.0 ± 3.8 and a range of 2.2 – 25.9 (N = 283), 8-OHdG values had a mean \pm standard deviation of 608.7 ± 323.4 and a range of 165.5 – 2040.3 (N = 196), TAC values (before transformation to residual TAC) values had a mean \pm standard deviation of 30.8 ± 10.4 and a range of 11.8 – 67.4 (N = 136), UA values had a mean \pm standard deviation of 7277.3 ± 3419.7 and a range of 2361.98 – 20564.2 (N = 164) and neopterin values had a mean \pm standard deviation of 207.3 ± 89.3 and a range of 70.0 – 472.7 (N = 91). These summary values refer to the raw concentrations adjusted for urine dilution using specific gravity.

Effect of habitat degradation and tourism on OS

The full MDA and neopterin models did not provide a better fit to the data than their respective null models (MDA: full model AIC = 277.38, null model AIC = 269.37, $\chi^2 = 1.99$, df = 5, p = 0.850; neopterin: full model AIC = 96.50, null model AIC = 95.86, $\chi^2 = 9.36$, df = 5, p = 0.095; Supplementary Material S4.4), suggesting that habitat degradation, exposure to tourism and age-sex class were not linked to MDA or neopterin concentrations.

The full 8-OHdG model and the full residual TAC model provided a better fit to the data than their respective null models (8-OHdG: full model AIC = 218.99, null model AIC = 223.05, $\chi^2=14.1$, df = 5, p = 0.015; residual TAC: full model AIC = 964.33, null model AIC = 971.09, $\chi^2=14.76$, df = 4, p = 0.005). Habitat degradation was not associated with 8-OHdG (Table 4.2) but groups living in more disturbed habitat had higher residual TAC (Table 4.3, Figure 4.3). Tourism was not associated with either 8-OHdG nor residual TAC (Tables 4.2 & 4.3). Finally, adult males had lower 8-OHdG than adult females (Table 4.2).

Oxidative stress and the juvenile: adult female ratio

The mean juvenile: adult female ratio in our study groups was 0.18 (range 0 – 0.27, N = 6). None of the OS biomarkers were significantly correlated with this ratio (8-OHdG: tau = -0.6, p-value = 0.133; MDA: tau = -0.467, p-value = 0.260; residual TAC: tau = -0.2, p-value = 0.707; neopterin: tau = -0.333, p-value = 0.452), suggesting no relationship between oxidative stress and recruitment in this population.

Table 4.2: Model output from the full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on 8-OHdG (a marker of DNA oxidative damage).

<i>Predictors</i>	Effect of anthropogenic disturbance on log(8-OHdG (ng/ml corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	6.00	0.34	<0.001	5.34
Proportion of home range that is degraded	0.88	0.42	0.112	3.55
Mean proportion of time spent with tourists per day	-0.12	0.83	0.898	2.17
Age-sex class - Adult Male	-0.22	0.10	0.026	30.07
Age-sex class - Immature	-0.23	0.15	0.127	46.48
Number of freeze-thaw cycles	-0.05	0.05	0.340	144.21

Number of observations: 196; Random effects: Number of individuals: 42, Number of groups: 6, Number of Plates: 12.

Table 4.3: Model output from the full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on residual TAC (total antioxidant capacity accounting for the effect of uric acid).

<i>Predictors</i>	Effect of anthropogenic disturbance on residual TAC (mM corr. SG)			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	-8.17	3.53	0.024	67.06
Proportion of home range that is degraded	14.00	4.13	0.001	66.61
Mean proportion of time spent with tourists per day	-5.22	4.74	0.277	37.65
Age-sex class - Adult Male	-1.22	1.70	0.480	21.22
Age-sex class - Immature	-1.41	2.59	0.588	34.07

Number of observations: 136; Random effects: Number of individuals: 41

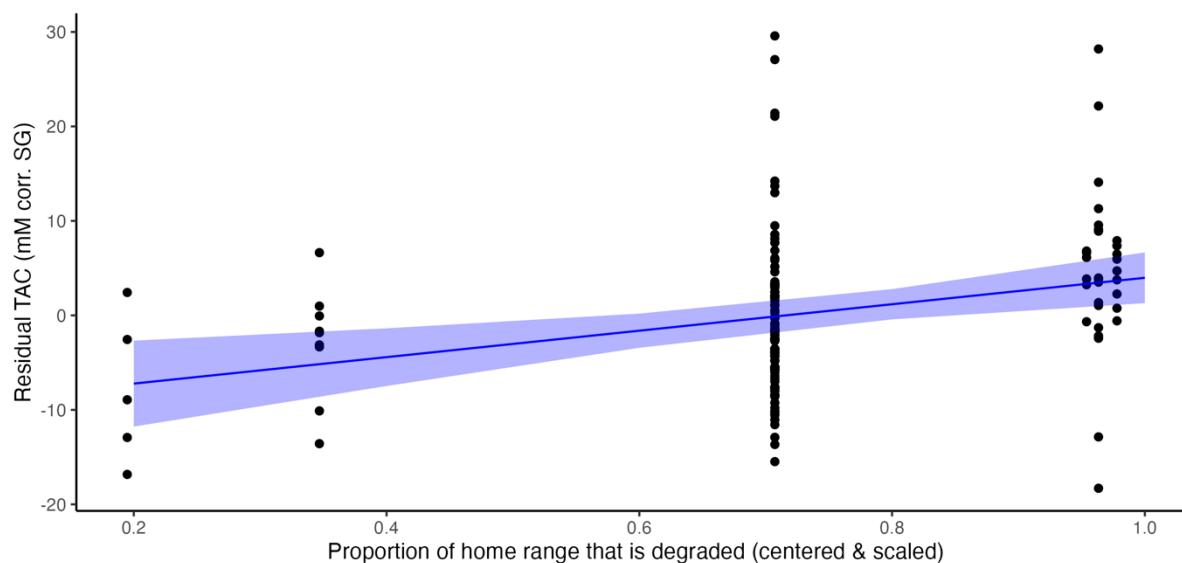


Figure 4.3: The proportion of home range classified as degraded plotted against residual TAC. The black points represent individual urine samples ($n=136$), the blue line represents the fit of the residual TAC model and the blue ribbon represents the confidence limits of the fit of the model.

Discussion

In this study we quantified, for the first time, the relationship between anthropogenic disturbance and OS in a wild primate, the Zanzibar red colobus. While our study was ultimately interested in the direct impact of anthropogenic disturbance on OS, we emphasise that our study design can only demonstrate a relationship between variables, rather than causality. We found that, across our six study groups, greater habitat degradation was associated with higher levels of antioxidant protection (residual TAC), but there was no relationship between habitat degradation and our three markers of oxidative damage (8-OHdG, MDA or neopterin). Exposure to tourists and colobus age-sex class were not associated with variation in any of the OS markers. Finally, we showed that OS levels were not associated with a demographic proxy of juvenile recruitment into the population (juvenile: adult-female ratio).

Habitat degradation and oxidative stress

Contrary to our predictions, Zanzibar red colobus living in groups whose ranges contained a greater proportion of degraded habitat did not have higher levels of oxidative damage (8-OHdG, MDA or neopterin) but instead had higher antioxidant capacity (residual TAC). This suggests that these colobus are experiencing increased production of reactive oxygen species in degraded habitats but that they are able to cope with these conditions by upregulating their antioxidant capacity (Costantini and Verhulst, 2009, Beaulieu and Costantini, 2014).

One proposed explanation for the increased production of reactive oxygen species in individuals living in more degraded habitats is an increased energy expenditure associated with altered foraging strategies. Altered foraging strategies have been linked to elevated oxidative damage/oxidant generation (but no difference in antioxidant capacity) in birds foraging in forest gaps (Gomes *et al.* 2014) and areas of low habitat quality (van de Crommenacker *et al.*, 2011). However, our results show the opposite pattern to this, namely higher antioxidant capacity in groups living in more degraded habitat and no difference in oxidative damage across levels of degradation. The discrepancy between our study and the previous studies could be because habitat degradation does not lead to increased energy expenditure on foraging behaviour in this species. In our study population, groups with high levels of habitat degradation did not spend more time feeding and had shorter daily travel distances than those with lower levels of degradation (Siex, 2003). Therefore, it seems likely that there is another mechanism responsible for elevated reactive oxygen species production in the colobus groups with higher habitat degradation in this study.

Although we do not have behavioural data or measures of glucocorticoid levels collected concurrently to the OS measures during this study, we propose that, potentially, an increased secretion of glucocorticoids in groups experiencing higher habitat degradation may explain the elevated production of reactive oxygen species. Habitat degradation exposes wildlife to a suite of psycho-social stressors which could activate the HPA axis and subsequently increase reactive oxygen species production (Costantini *et al.* 2011, Beaulieu and Costantini, 2014). In our study, groups with a higher proportion of degraded habitat in their home range also had a higher proportion of their home range that fell outside the national park (JCBNP). Groups residing outside the park generally live in and around villages where they may be hunted by humans and dogs (e.g., Georgiev *et al.*, 2019) or face persecution from farmers. Exposure to higher predation pressure in these groups may cause physiological stress, as has been observed in Ashy red colobus (*P. tephrosceles*), whereby individuals in minimally disturbed habitats had higher urinary cortisol concentrations than those in highly disturbed habitats due to predation pressure from chimpanzees (Aronsen *et al.*, 2015). Additionally, three of the four groups with relatively high proportions of habitat degradation in their home range spend large proportions of their day near to the main road transecting the national park and regularly cross the main road (ca. 5 m wide) in search of food and sleeping sites (Olgun *et al.*, 2022). Roads have the potential to increase OS through the anxiogenic effects of exposure to noise pollution (Daiber *et al.*, 2020, Ortiz-Jiménez *et al.*, 2022) or through increased exposure to oxidative chemical pollution (Lai *et al.*, 2005, Salvi and Salim, 2019). Finally, groups with a higher proportion of degraded habitat in their home range at this study site have demonstrated increased intra-specific aggression in response to food competition (Siex, 2003) which can

also lead to elevated physiological stress (Wittig *et al.* 2015). As such, it is very plausible that the physiological stress caused by higher exposure to these stressors in the groups living in degraded habitat may lead to elevated production of reactive oxygen species.

A key result from this study is that red colobus in this population were able to upregulate their antioxidant capacity to combat potential oxidative damage. Red colobus could upregulate their antioxidant capacity endogenously through increased generation of antioxidants or increased mobilisation or utilisation of existing antioxidants (Beaulieu *et al.*, 2014). Alternatively, they could increase their intake of dietary antioxidants. Two of the food items that contribute a large proportion of the red colobus diet in degraded habitats around JCBNP are *Indian almond* (*Terminalia catappa*) leaves and *mango* (*Mangifera indica*) leaves. These introduced, non-indigenous species are high in phenolic compounds (Mturi, 1991) known to have antioxidant properties in humans (Rudrapal *et al.*, 2022). As such, increasing intake of these food items could increase antioxidant capacity in these monkeys, although the antioxidant properties of these food items in red colobus monkeys requires further study. Alternatively, it could be that antioxidant capacity is elevated in groups exposed to higher levels of habitat degradation in response to infection. Parasite infection is known to trigger an antioxidant response (Cohen-Sánchez *et al.* 2023) and several studies have demonstrated that habitat degradation is associated with parasitism in primates (Gillespie and Chapman, 2006, Mhora and McPeck, 2009). As such, more research is needed to understand the mechanisms by which red colobus in more degraded habitats have higher antioxidant capacity.

Primate tourism and oxidative stress

We found no relationship between exposure to tourists in colobus groups and any of the four OS markers we assayed. We suspect this is due to habituation. Habituation occurs when animals have regular exposure to harmless stimuli and consequently perceive the risk to be low (Tablado and Jenni, 2017). Habituation to humans lead to reduced physiological stress in western lowland gorillas (*Gorilla gorilla gorilla*, Shutt *et al.*, 2014) and orangutans (*Pongo pygmaeus*; Muehlenbein *et al.*, 2012) and is thought to have been responsible for the lack of a physiological response to anthropogenic disturbance in Udzungwa red colobus (*P. gordonorum*, Barelli *et al.*, 2015). While the effect of habituation on oxidative status has not been investigated in primates, urban birds, which were thought to be more habituated to stressors than rural birds, experienced lower levels of oxidative damage than rural birds when exposed to chronic stress (Costantini *et al.*, 2014). Similarly, red colobus that are exposed to

people on an almost daily basis may no longer experience a physiological response to this stressor.

Oxidative stress and juvenile recruitment

Our results suggest that Zanzibar red colobus may be able to upregulate antioxidant capacity to prevent increases in oxidative damage due to habitat degradation. Flexibly altering antioxidant capacity to combat OS may confer fitness advantages to individuals by preventing diseases, reproductive issues and advanced aging caused by OS-induced cell damage (Keskes-Ammar *et al.*, 2003; Keaney *et al.*, 2003; Bonda *et al.*, 2010; Agarwal *et al.*, 2012, Selman *et al.*, 2012; Anderson and Maes, 2014). Previous research has shown that antioxidant capacity is highly positively correlated with annual population change (Gentoo penguins *Pygoscelis papua* and Adélie penguins *Pygoscelis adeliae*, Beaulieu *et al.*, 2013). It was suggested that the adaptive advantages conferred by higher antioxidant defences, such as increased fertility and survival (Bize *et al.*, 2008; Saino *et al.*, 2011; Losdat *et al.*, 2013), may explain this positive relationship. However, elevating antioxidant defences to mitigate the impacts of anthropogenic disturbance is likely to incur costs. Increasing antioxidant protection generally requires an energetic investment that will trade off with other important physiological mechanisms, such as somatic maintenance and immune function (Bizé *et al.*, 2008; Monaghan *et al.*, 2009; Nussey *et al.*, 2009). For example, diversion of dual-purpose compounds that act as antioxidants and immunostimulants towards mitigating oxidative stress would prevent them from being used to sustain an immune response (Beaulieu and Costantini, 2014). Diverting investment or energy and nutrients away from such mechanisms may thus carry a fitness cost. As such, the red colobus may be able to upregulate TAC to prevent damage from OS but may face reduced fitness by doing so.

Across the six colobus study groups, we found no link between OS marker concentrations and juvenile: adult female ratios, a proxy for recruitment and therefore an indicator of individual level fitness. This may be because of the opposing fitness consequences of elevating antioxidant protection described above or because the OS differences between groups was not substantial enough to affect recruitment. However, it is possible that the lack of a relationship in our study could be due to methodological limitations. The juvenile: adult female ratio is only a crude estimate of population level recruitment representing a snapshot in group composition. Additionally, we correlated this with the average OS marker concentrations for the group, removing individual variation. As such a longer-term study investigating the link between OS, individual fitness and population-level recruitment is needed to determine the long-term impacts of the upregulation of antioxidant capacity on population viability. This is

especially pertinent given that recruitment of Zanzibar red colobus is in decline across its range (Davenport *et al.*, 2019).

Age-sex class and OS

While the effect of age-sex class on OS was not a focus of this study, we did examine the relationship between the four OS markers and colobus age-sex class. We found no difference in oxidative stress markers between juveniles and either adult males or adult females. Previous research investigating the effect of age on OS has produced mixed results (*Homo sapiens*, Bolzán *et al.*, 1997; Chen *et al.*, 2007; *Ovis aries*, Nussey *et al.*, 2009; *Macaca mulatta*, Georgiev *et al.*, 2015; Speakman *et al.*, 2015; *Sus scrofa*, Gassó *et al.*, 2016; *Pan troglodytes*, Thompson-González *et al.*, 2020). We found that adult males had lower 8-OHdG than adult females but there was no difference between age-sex classes for the other OS markers tested. This result contradicts most laboratory studies investigating sex differences in oxidative stress which have found that females have lower oxidative damage (e.g., humans, Loft *et al.*, 1992, Chen *et al.*, 1999, Ide *et al.*, 2002; rats, Matarrese *et al.*, 2011, Barp *et al.*, 2002) and higher antioxidant capacity than males (rats, Bhatia *et al.*, 2012; *Drosophila melanogaster*, Niveditha *et al.*, 2017). Similarly, a study in free-ranging rhesus macaques (*M. mulatta*) showed that lactating females had lower 8-OHdG than males (Georgiev *et al.*, 2015). Some studies have shown no link between oxidative stress and sex in chimpanzees (*P. troglodytes*) in the wild (Thompson-González *et al.*, 2020) and in captivity (Costantini *et al.*, 2021). However, one study in wild chimpanzees found the same pattern we observed in this study, that 8-OHdG was higher in females than males but that there was no difference between sexes for the other markers tested (Krief *et al.*, 2023). This paper suggested that females were exposed to higher levels of pesticides than males, leading to higher oxidative stress.

One other possible explanation for females having higher 8-OHdG than males in this dataset could be the high reproductive cost females incur relative to males. In this analysis, due to restrictions in sample size and this question not being the main focus of the study, females were not sorted into reproductive phases, therefore some of the females in our sample were lactating or gestating. Reproduction is energetically costly to females with an increase in demand for resources, metabolism and potentially the production of reactive oxygen species (Metcalf and Alonso-Alvarez, 2010; Metcalf and Monaghan, 2013). This increased demand for investment in reproduction and maintenance could limit the resources available to invest in antioxidant protection also leading to elevated oxidative damage (Alonso-Alvarez *et al.*, 2017). The oxidative cost of reproduction has been identified in females of several species, for example zebra finches (*Taeniopygia guttata*) (Noguera, 2017), white-browed sparrow

weavers (*Plocepasser mahali*) (Cram *et al.* 2015) and Allen Cays rock iguanas (*Cyclura cyclura inornate*) (Webb *et al.*, 2018). However, male energetic costs may also be high if the species engages in intensive competition for mates (Emery Thompson and Georgiev, 2014; Georgiev *et al.*, 2015) and if there is high sexual dimorphism (Key and Ross, 1999). The oxidative costs of male investment in reproduction has been demonstrated in rhesus macaques, where males had higher 8-OHdG than females (Georgiev *et al.*, 2015). Rhesus macaques exhibit higher levels of aggressive competition for mates (Georgiev *et al.*, 2015) and have a higher sexual dimorphism (Turcotte *et al.*, 2022) than Zanzibar red colobus (Struhsaker, 2010). The energetic cost of male reproductive effort, relative to that of females, in Zanzibar red colobus may therefore be lower meaning that females have high oxidative stress than males in this species, at least during certain phases of the female reproductive cycle. Further research investigating the difference in oxidative stress between females in different life stages would help to understand the oxidative costs of reproduction in females and determine whether reproductive investment may be responsible for the elevated 8-OHdG in females compared to males.

Methodological considerations

In Chapter 3 of this thesis (published Melvin *et al.*, 2022), multiple methodological considerations were suggested for the measurement of oxidative stress markers in urine collected from wild animals. These were to consider the impact of storage time on OS marker concentrations and diurnal patterns of OS markers when designing sampling protocols. Due to restrictions in urine sampling due to the Covid-19 pandemic, I used a similar dataset to conduct the analysis for Chapter 3 and this chapter. As a result, I was not able to apply the two recommendations made in Chapter 3 to the sample collection for this chapter. I also was not able to remove samples or include many additional predictors in my models due to small sample sizes for some groups. As such, the results of this study should be interpreted with some caution due to the potential effects of time of day and time between sample collection and freezing on the marker values.”

Contribution to the Conservation Physiology Toolbox

This study provides novel evidence for the use of non-invasive OS markers to address conservation-relevant questions in wild animals. Our study is the first investigation into the link between anthropogenic disturbance and OS in a wild primate and, to our knowledge, the second study that considers aspects of disturbance, oxidative stress and fitness concurrently. Based on our interpretation of our results (that groups with higher habitat degradation had higher production of reactive oxygen species offset by elevated TAC production) we suggest that OS markers can provide useful insight into the nuanced impact of anthropogenic

disturbance on wild populations and their ability to respond. Further studies in other species and contexts, as well as additional tests of the link between OS and individual fitness outcomes will improve our understanding of the potential of OS markers as an early-warning system for future population declines (Beaulieu *et al.*, 2013).

Conservation implications for the Zanzibar red colobus

Even though Zanzibar red colobus appear to be coping with anthropogenic disturbance around JCBNP, habitat degradation was associated with higher levels of antioxidant capacity (which was a response to elevated reactive oxygen species levels). Given that upregulating antioxidant capacity could have fitness consequences and the long-term population-level impacts of its upregulation are unknown, we believe that protecting high quality forest habitat is important for the protection of this species. Our results suggest that tourism has a negligible impact on the OS status of Zanzibar red colobus in well-habituated groups. This is encouraging as it supports the use of tourism to generate income for conservation initiatives in JCBNP (Muehlenbein and Wallis 2014; Dunay *et al.* 2018). Tourism in JCBNP, mostly to visit the red colobus, generates over \$650,000 USD annually (JCBNP, official records 2019), which contributes to local community development. However, we recommend that tourist visits continue with the few groups that have undergone long-term habituation. We cannot determine the impact of sporadic tourist exposure on OS in unhabituated colobus from this study and therefore advise to err on the side of caution. An experimental study investigating this would be useful for informing tourism policies in this species further. We also advise that there is consideration of other risks associated with tourist exposure, such as disease transmission, that were not included in this study. It is also of note that much of the habitat degradation in Zanzibar is attributable to unmanaged development for the growing tourism industry, with tourist numbers increasing 16-fold in the last 35 years (Staehr *et al.*, 2018). As such, a holistic, environmentally-conscious approach to tourism, both within JCBNP and Unguja more widely is essential for the survival of this endangered species.

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Chapter 5

Discussion



Electra, female Zanzibar red colobus monkey (*Piliocolobus kirkii*) in Jozani-Chwaka Bay National Park, Zanzibar

Contribution to knowledge

Understanding the vulnerability of species to anthropogenic threats, and the mechanisms underlying those relationships, is an important step in designing effective and efficient conservation initiatives (Carwardine *et al.* 2012; Ofori *et al.* 2017). Different methods can be used to investigate different aspects of vulnerability (exposure, sensitivity and adaptability), each with their own strengths and weaknesses (Pacifi *et al.* 2015). Combining these methods provides a more robust and nuanced assessment of vulnerability, by allowing researchers to strengthen their confidence in their evaluation if findings converge (Hijmans & Graham 2006; Kearney *et al.* 2010; Pacifi *et al.* 2015; Martínez *et al.* 2015; Shabani *et al.* 2016) or to generate new hypotheses if they diverge (Hijmans & Graham 2006; Martínez *et al.* 2015; Rougier *et al.* 2015).

In this thesis, I took a multi-method approach to investigate vulnerability to extinction in the most threatened group of African monkeys – the red colobus monkeys (*Piliocolobus* spp.) (Linder *et al.* 2021). Red colobus monkeys are considered highly vulnerable to extinction given that they are thought to be specialised (as they are arboreally adapted and folivorous) (Harcourt 1998; Isaac and Cowlshaw 2004; Kamilar and Paciulli 2008; Korstjens 2019; Butynski and De Jong 2022; Korstjens *et al.* 2022) and are exposed to a range of anthropogenic threats and disturbances (Davenport *et al.* 2019; Linder *et al.* 2021). However, a more detailed and nuanced understanding of the differences in vulnerability among red colobus species, how anthropogenic threats may affect red colobus at different scales, and the potential mechanisms underlying vulnerability in this genus will help to target conservation efforts more effectively. This was the aim of this PhD thesis.

Below I discuss the key contributions made towards each of the four objectives of my thesis:

(A) Determining the vulnerability of red colobus (*Piliocolobus* spp.) to changes in suitable habitat due to climate change and land use change using correlative species distribution modelling on a pan-African scale

In Chapter 2, I employed correlative species distribution modelling using Bayesian Additive Regression Trees (BART) to investigate the drivers of current species distribution and vulnerability to future habitat loss in red colobus monkeys across Africa. From this research, I make three main conclusions to address this research objective.

Firstly, red colobus are vulnerable to loss of habitat under future climate change and land use change though the degree of predicted habitat loss varies by species. I found that, based on

the four species for which I conducted this analysis, red colobus were generally vulnerable to habitat loss in the future but the level of vulnerability varied between species. Three out of the four species I investigated were predicted to lose suitable habitat from their current range by the year 2070 (*P. badius*, *P. kirkii* and *P. tephrosceles*) however, the fourth species (*P. temminckii*) was predicted to experience on average a 2-4% increase in suitable habitat in its current range depending on the scenario of future climate and land use. Given their close phylogenetic relationship and their specialisation to arboreality and folivory, it may have been expected that different red colobus species respond similarly to environmental change. However, I demonstrated that this was not the case. The results of my study highlight the different responses of red colobus species to environmental change which reinforces the need for species-level conservation action and provides insights for the planning of targeted on-the-ground conservation strategies for different red colobus species. By carrying out a comparative analysis of the effect of climate change and land use change on species distributions at the genus level, my thesis has revealed more nuanced patterns of response to environmental change in red colobus that may have been masked by previous taxonomically broader analyses (Carvalho *et al.* 2019; Sweeney and Jarzyna 2022).

Secondly, I demonstrated that the extent to which red colobus will be affected by climate change and land use change is likely to be reduced through effective mitigation. Generally, the smallest losses or biggest gains in area of predicted presence (depending on the species) were found for the most optimistic scenarios of climate change and land use change, i.e., where both climate change and land use change are heavily mitigated. The biggest losses or smallest gains (depending on the species) were seen for the worst-case scenario for climate change, i.e., where the rate of land use change is somewhat mitigated and begins to decline but climate change is unmitigated. This demonstrates the importance of adhering to regulations to curb climate change and land use change for the future of red colobus species.

Thirdly, I showed that assessing the vulnerability of a species using correlative species distribution models must be done with consideration of the biology of the species and the impact that methodological decisions can have on the results. My research showed that the vulnerability of red colobus to changes in suitable habitat was mainly driven by changes in climate rather than land use. This was puzzling given that we know red colobus are generally forest dependent (Struhsaker 2010) and unable to survive in areas of human-altered land use (Decker 1994; Grubb and Powell 1999; Galat-Luong and Galat 2005; Ikemeh 2015; Mayhew *et al.* 2020; Linder *et al.* 2021; Korstjens *et al.* 2022), except for some species that seem to be more able to cope with human-altered landscapes (e.g., *P. kirkii*: Siex 2003; Nowak 2007; Nowak and Lee 2013; Davenport *et al.* 2019). Therefore, I believe this result may be an

artefact of the land use classification of the datasets used in this analysis or the methodological approach I took, rather than a true result showing that climate change is more influential than land use change. The results of Chapter 2 demonstrate the importance of 1) interpreting the results of correlative species distribution models in the context of the biology of the species, 2) understanding how decisions taken during study design can ultimately affect the conclusions of the research and 3) carrying out an open, critical examination of the datasets used.

It should be noted that the conclusions I have made about the vulnerability of red colobus are based on information from just four species for which I had sufficient data (two from East Africa and two from West Africa) and therefore may not be generalisable to the other red colobus species. Climate change and land use change are predicted to be variable across Africa. As such, these patterns of change may not reflect those that will occur in areas where the other species are found. This is particularly true for the Central African group of red colobus species, for which my dataset was particularly sparse. As such, research efforts should focus on collecting presence information and updating geographic ranges for data-deficient species (Butynski and de Jong 2022), as has been identified as the first priority for red colobus conservation more generally in the Red Colobus Action Plan (Linder *et al.* 2021). Addressing this data gap will allow us to properly assess the threats facing red colobus species and gain a more complete picture of the vulnerability of red colobus species to environmental change using the methods employed in this thesis, among others. Given that different red colobus species are likely to respond differently to environmental change, gaining an insight into the vulnerability of each red colobus species separately will be important for targeted and effective conservation of this genus.

(B) Investigating the link between niche breadth and red colobus vulnerability to extinction

Niche breadth has been identified as an important predictor of extinction risk in colobines (Kamilar and Paciulli 2008) and is one of the most robustly supported intrinsic traits determining species' vulnerability to extinction more generally (Swihart *et al.* 2003; Thuiller *et al.* 2005; Broennimann *et al.* 2006; Colles *et al.* 2009; Chichorro *et al.* 2022; Grinder and Wiens 2023). As such, in Chapter 2, I chose to carry out the first investigation into the link between niche breadth and vulnerability to extinction in red colobus monkeys specifically. As mentioned above, red colobus are assumed to be specialist (i.e., have a narrow niche) and ecologically similar to each other (Korstjens *et al.* 2022). Therefore, carrying out a trait-based analysis on such a narrow taxonomic scale (genus level) may reveal links between niche breadth and

extinction risk that have been missed in taxonomically broader comparative studies (Sweeney and Jarzyna 2022).

I found large variation in niche breadth among red colobus species (N = 16) but that this variation was not linked to differences in predicted change in suitable habitat in the four species for which sufficient data were available. This result suggests that narrow niche breadth in some red colobus species was not responsible for higher vulnerability to climate change and land use change. Instead, it may be that niche position (the position of the species' niche along each environmental axis) and level of exposure to environmental change may explain loss or gain of habitat for each red colobus species instead of niche breadth. Under this hypothesis, species specialised in niches that become more common under environmental change may gain suitable habitat even if the niche is narrow. In Chapter 2, I demonstrated that different red colobus species have significantly different niches. Furthermore, among the three species for which we had information on predicted habitat loss and niche similarity, the two with significantly similar niches (*P. badius* and *P. kirkii*) were predicted to lose habitat while the other (*P. temminckii*), with no significant niche overlap with the other two species, was predicted to gain habitat. As such, it may be that the niche occupied by *P. kirkii* and *P. badius* becomes less common in the future, despite the differences in niche breadth between the three species. However, these results should be interpreted cautiously given that they are based on a very limited number of species (N = 3). Investigating the relative contributions of niche breadth and niche position on red colobus vulnerability, as has been done for other taxa (e.g., plant species in southern Africa: Thuiller *et al.* 2005), would be an interesting and worthwhile topic for future research but would require a concerted effort to fill in missing data gaps on red colobus presence and absence.

Although the results addressing this objective need to be interpreted cautiously given the limited number of species upon which they are based, this study undoubtedly shows the ecological variability of red colobus monkeys, something that has been overlooked in the past. These results, together with those addressing objective A, demonstrate that red colobus species are not ecological surrogates for each other and are likely to respond differently to future environmental change. Again, this reinforces the need for species-level or population-level conservation strategies for red colobus monkeys, as has been proposed by the Red Colobus Action Plan (Linder *et al.* 2021).

(C) Gaining an insight into methodological considerations when measuring urinary oxidative stress in wild animals

Prior to this thesis, only one study had measured oxidative stress non-invasively in wild animals (chimpanzees, *Pan troglodytes*: Thompson González *et al.* 2020). Given the novelty of non-invasively measuring oxidative stress markers in wild animals, and the vast potential for its wider application in field studies of health and fitness (Monaghan *et al.* 2009; Beaulieu and Costantini 2014), I believed it was worthwhile to investigate how various methodological confounds associated with the field collection of urine samples may affect oxidative stress marker levels (Chapter 3).

I found that there are some methodological considerations when measuring urinary oxidative stress markers in field research. Firstly, diurnal patterns may be an important consideration for some OS markers, with MDA being higher in samples collected in the morning than in the afternoon in our study. Secondly, the length of time between sample collection and flash freezing in liquid nitrogen has variable effects on the concentration of all OS markers and therefore should be minimised where possible. Meanwhile, we found that contamination of samples from leaf surfaces and the length of time a sample is frozen at -80°C did not affect OS marker concentrations.

Given the overall high stability of urinary oxidative stress markers, I concluded that oxidative stress markers can be applied successfully in field research within some methodological constraints and that urinary oxidative stress markers are a promising new tool for field researchers. This research makes an important step towards the wider application of this method by providing practical guidelines for field researchers to measure OS robustly using non-invasive methods.

(D) Oxidative stress as a potential mechanism linking anthropogenic disturbance and recruitment (a proxy of fitness) in an island endemic, the Zanzibar red colobus (*P. kirki*)

I carried out an investigation into one potential physiological mechanism linking anthropogenic disturbance and recruitment (the addition of individuals to the population) in the Zanzibar red colobus. Oxidative stress markers are highly fitness relevant due to the link between oxidative stress and disease (Keaney *et al.* 2003; Bonda *et al.* 2010; Anderson and Maes 2014), reproductive success (Keskes-Ammar *et al.* 2003; Agarwal *et al.* 2012) and survival probability (Monaghan *et al.* 2009). Additionally, oxidative stress has been shown to be responsive to a variety of anthropogenic disturbances (Beaulieu and Costantini 2014, Chapter 4). As such,

oxidative stress is an excellent candidate for an indirect mechanism linking anthropogenic disturbance and recruitment.

I investigated the relationship between two prominent aspects of anthropogenic disturbance affecting a population of the Zanzibar red colobus at Jozani-Chwaka Bay National Park in Zanzibar (habitat degradation and exposure to tourists) and oxidative stress level markers (Chapter 4). I also then investigated the link between oxidative stress levels and the juvenile: adult female ratio (a proxy of recruitment) across six colobus groups. I found that colobus experiencing higher levels of habitat degradation, relative to colobus experiencing lower levels of habitat degradation, had elevated antioxidant capacity but similar levels of oxidative damage. Meanwhile colobus exposed to differing levels of tourism showed no difference in either aspect of oxidative stress. Additionally, there was no link between the levels of any of the oxidative stress markers and recruitment across groups.

I interpreted these results as showing that colobus monkeys exposed to habitat degradation upregulated their levels of antioxidant capacity in response to habitat degradation and successfully mitigated oxidative damage. As a result, I speculated that there was no consequences for recruitment. This result suggests that the Zanzibar red colobus I studied in and around Jozani-Chwaka Bay National Park are resilient to two aspects of anthropogenic disturbance in terms of their oxidative stress physiology. Based on these results, I made conservation recommendations for the Zanzibar red colobus, namely that protection of forest habitat from degradation should be the priority for Zanzibar red colobus conservation given that upregulation of antioxidant capacity may incur a fitness cost (with the caveat that such a cost was not possible to measure in this study). Additionally, I advised that continuing to focus tourism on a small number of habituated groups would also be beneficial given that I speculated that the lack of a relationship between oxidative stress and tourist exposure in this study is due to habituation to people.

Chapter 4 provides the first investigation into the link between anthropogenic disturbance and oxidative stress in wild primates. It is also one of the first to investigate the relationships between anthropogenic disturbance, oxidative stress and a measure of fitness in the same study (but see French *et al.* 2017 for a study of the impact of anthropogenic disturbance on oxidative stress and reproductive investment in Galápagos marine iguanas, *Amblyrhynchus cristatu*). As such, it provides useful insights into oxidative stress as a mechanism underlying the link between anthropogenic disturbance and fitness and recruitment in a wild population. In this particular case, oxidative stress does not seem to provide a mechanistic link between disturbance and recruitment but future research investigating long-term recruitment and

population changes in response to anthropogenic disturbance and oxidative stress would be useful to corroborate this finding.

Future application

The work presented in this thesis has the potential to influence policy and practice in two areas. Firstly, there are implications for the methods used by researchers to investigate and monitor species vulnerability to extinction using species distribution models and physiological methods. Secondly, there are implications for the conservation of red colobus. I present these in turn.

Implications for improving the methods used in research into species vulnerability to population declines and extinction

This thesis provides further evidence that combining methodological approaches to investigate vulnerability to extinction allows for a more comprehensive understanding than each approach in isolation would have, as has been demonstrated in previous studies (Keith *et al.* 2008; Pearson *et al.* 2014; Evans *et al.* 2015; Rougier *et al.* 2015; Visconti *et al.* 2016). By combining different methods, I was able to investigate vulnerability on various spatial, temporal and taxonomic scales to develop a more detailed understanding of extinction vulnerability of red colobus monkeys. The correlative species distribution models that I carried out in Chapter 2 highlighted that climate change and land use change are likely to reduce red colobus distributions the over the next 50 years on a scale that will lead to population declines and local extinctions. Importantly, it also demonstrated that different red colobus species are likely to respond differently to environmental change. The trait-based analysis I carried out in Chapter 2, looking into niche breadth and niche overlap between species, provided context to this result. It showed that differences in niche breadth did not explain different species responses but that differences in niche positions and exposure to environmental change may be responsible instead. These combined analyses emphasise the need for species-level conservation management and highlight the impact that level of exposure to threats can have on red colobus survival. Given that level of exposure to threats can be reduced through conservation action, this result suggests that humanity has the power to influence the future of the red colobus genus. Meanwhile the localised mechanistic investigation I carried out into oxidative stress as a potential mechanism linking anthropogenic disturbance to recruitment demonstrated that some red colobus species can cope with localised disturbances, such as tourist exposure and habitat degradation. Insights such as these allow us to determine which threats are likely to have the biggest effects on red colobus populations; in this case that localised habitat degradation and exposure to tourists may be manageable for red colobus

species but long-term, large-scale shifts in climate and land use are likely to have widespread detrimental effects. This information may be useful for determining which threats to address more urgently. As such, combining methods to assess vulnerability allows researchers and conservationists to create well-reasoned and supported conservation management plans with a more thorough understanding of the most urgent threats facing the study taxa at various scales.

This thesis also contributes information on the separate methodological considerations for each individual approach to studying vulnerability. In this thesis, I conducted an analysis into how the choice of land use dataset affects the outcomes of species distribution modelling. This methodological question arose while trying to select an appropriate land use dataset to create my SDMs for Chapter 2. I demonstrated that models created with climatic data in conjunction with each of two different land use datasets lead to different orders of variable importance and different predictions for changes in suitable habitat in the future. In some cases, these differences were large. For example, in the case of *P. kirkee*, I found that models created with climate and one of the land use datasets showed that the species would not lose any suitable habitat from their current range under any future climate/land use scenario. Meanwhile the models created with the same climatic data but the other land use dataset, predicted that they would lose 80% of suitable area under the most optimistic future climate/land use scenario and 100% under the other three future scenarios. Correlative species distribution modelling to assess how, when and where a species is likely to experience shifts in suitable habitat is now a common research practise and many conservation initiatives utilise species distribution modelling in their planning. Researchers and conservationists may be aware of discrepancies in land use datasets, but this is rarely explicitly addressed or accounted for in published studies. Understanding how the choice of environmental dataset may affect the results and conclusions of SDMs is particularly important for research that will be incorporated into on-the-ground conservation planning where the margin for error is much smaller. As such, my co-authors and I recommend that future researchers should carefully evaluate various land use products to find the best fit for their purpose, study species and study area. We also recommend that future researchers are explicit about the caveats of large spatial datasets and provide specific information on the uncertainty around predictions. Bayesian species distribution modelling methods, such as BART used in this thesis, enable researchers to model and visualise confidence around predictions in a spatially explicit way enabling transparency when utilising this information for conservation planning (Carlson 2020). The practical guidelines for species distribution modelling to assess vulnerability I provide in this thesis will hopefully make future assessments more robust and therefore more valuable for on-the-ground conservation planning.

I also investigated methodological considerations for measuring urinary oxidative stress markers as a mechanistic link between anthropogenic disturbance and recruitment as described above. The use of physiological markers to understand the underlying mechanisms of vulnerability is aided by the application of non-invasive methods and being able to apply them in a reliable way. Chapter 3 contributes practical guidance on the application of a novel and promising set of physiological markers to questions of species vulnerability in the future.

Implications for the conservation of red colobus

Based on the findings of this thesis, I conclude that red colobus are vulnerable to large scale anthropogenic threats such as climate change and land use change but they have some capacity to cope with smaller scale disturbances such as tourist exposure and localised habitat degradation. Therefore, tackling climate change and reducing habitat loss will be vital to safeguard the future of red colobus monkeys. Red colobus species are likely to respond to anthropogenic threats differently, which supports the use of species-specific conservation planning for red colobus (Linder *et al.* 2021). Of the four species for which we predicted habitat loss under future climate change and land use change, the results were particularly concerning for *P. tephrosceles* and *P. kirkii* because of their already restricted range (*P. tephrosceles* = 3,900 km², *P. kirkii* = 1,600 km² and restricted (naturally) to the island of Unguja in Zanzibar; Butynski and De Jong 2022). However, the four species we carried out this analysis on are the four species with the most complete datasets on presence and absence. As such, other red colobus species may experience greater habitat loss than these species. Filling in these data gaps is necessary before we can properly assess the vulnerability of the other red colobus species individually, something that is of great importance given that I demonstrate that different red colobus species are likely to respond differently to environmental change.

In light of these findings, it is important to note that this thesis investigates just a small number of a suite of threats facing red colobus today. There are other proximate threats driving red colobus population declines that I do not consider, particularly hunting (Linder *et al.* 2021). Red colobus monkeys are often the first mammal species to undergo local extirpation due to hunting (Linder and Oates 2011; Linder *et al.* 2021; Butynski and de Jong 2022). *P. preussi* and *P. pennantii* have both experienced a population decline of more than 80% in the last 30 years mainly due to hunting (Linder *et al.* 2019, 2021; Cronin 2019; Cronin *et al.* 2017). In situations such as this, immediate prevention of illegal hunting should be the first conservation priority, because if all red colobus are hunted from an area then the effects of long-term climate change and land use change would be irrelevant. Fighting the immediate threats to red

colobus survival would give them the time and space they need to cope with more long-term threats such as climate change and land use change.

However, given the likely pervasive negative impact of climate change and land use change, not only for red colobus but for the ecosystems in which they live, mitigation of these threats should not be overlooked. Furthermore, the mitigation of climate change and land use change may aid in curbing hunting of red colobus by humans given that climate change related food insecurity and habitat loss and fragmentation associated with land use change are predicted to exacerbate hunting further (Galán-Acedo *et al.* 2019; Chapman and Peres 2021). As such, a comprehensive approach to conservation, considering both the immediate and long-term threats to the survival of red colobus, would be the most beneficial.

Conclusion

This thesis demonstrates that red colobus monkeys have some capacity to cope with small-scale anthropogenic disturbance but that large-scale shifts in climate and land use are likely to have detrimental effects on their long-term survival. Mitigation of future climate change and land use change increases the chance of survival for all red colobus species. As such, while halting the most urgent threats to red colobus is the first priority, mitigation of climate change and land use change should not be overlooked. The successful long-term conservation of red colobus depends on a mixed approach of both tackling the most immediate threats and putting mitigation strategies in place now to minimise climate change and land use change in the future. As such, the survival of red colobus, as is the case for most of the tropical biosphere, will require concerted conservation management at the population, species and genus level, with cooperation across global, regional and national scales.

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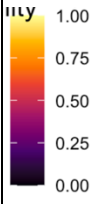
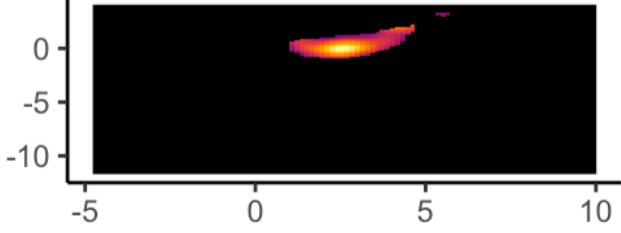
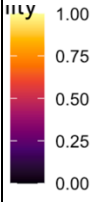
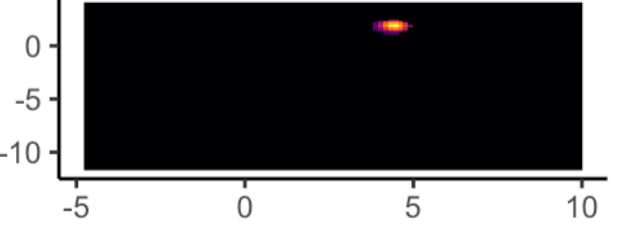
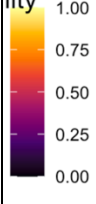
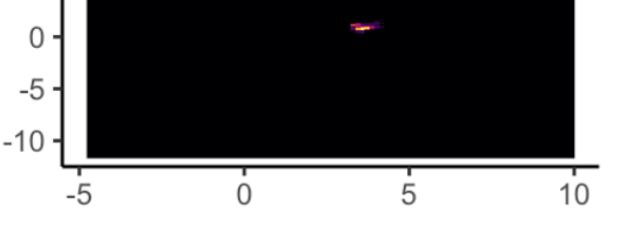
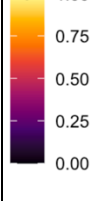
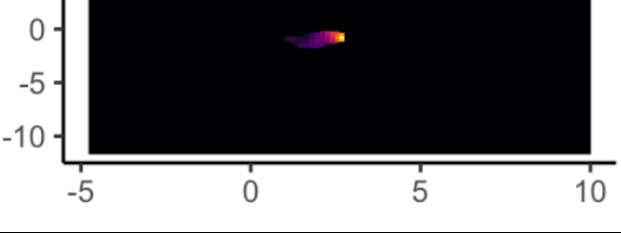
Supplementary Materials

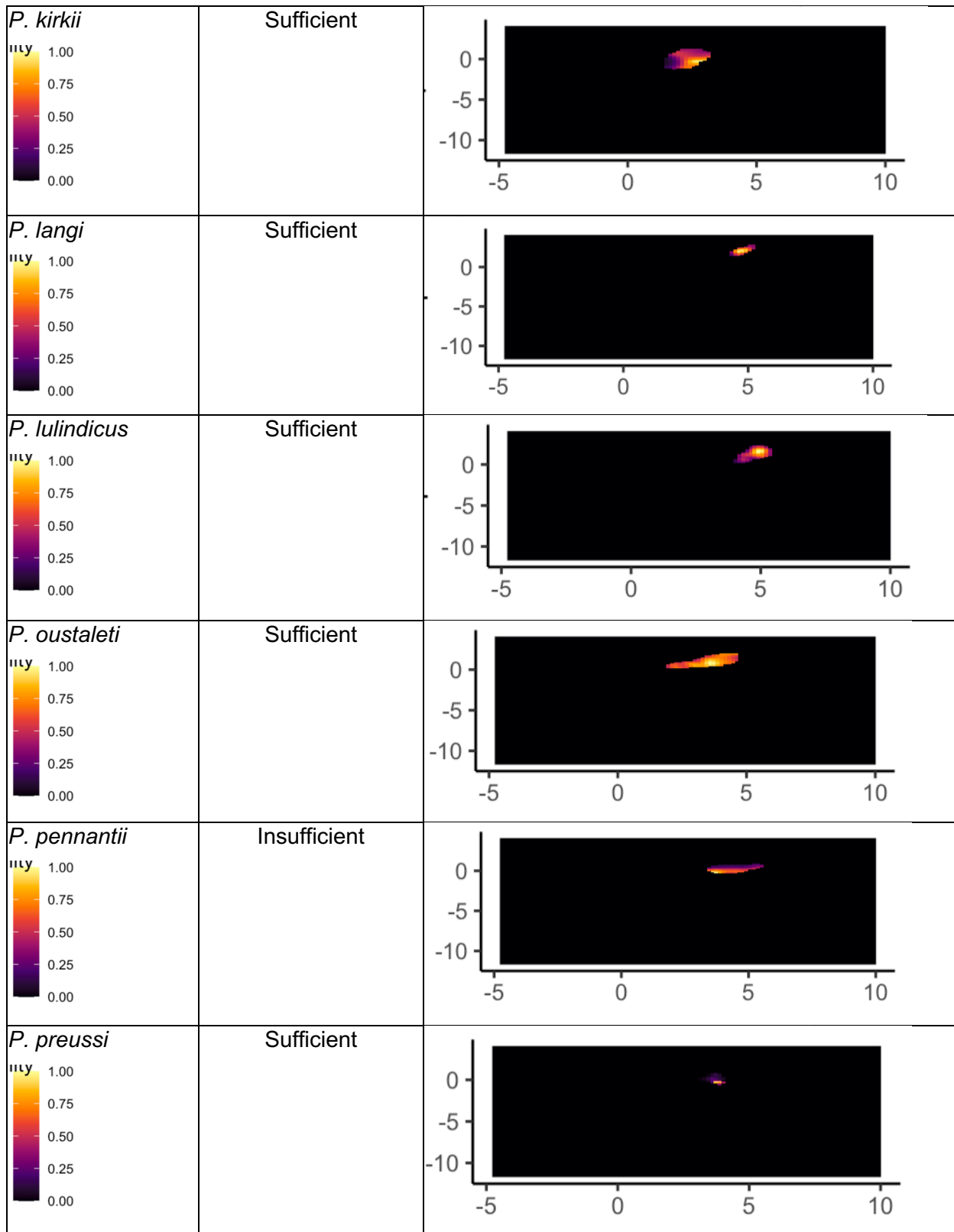
Supplementary Material for Chapter 2

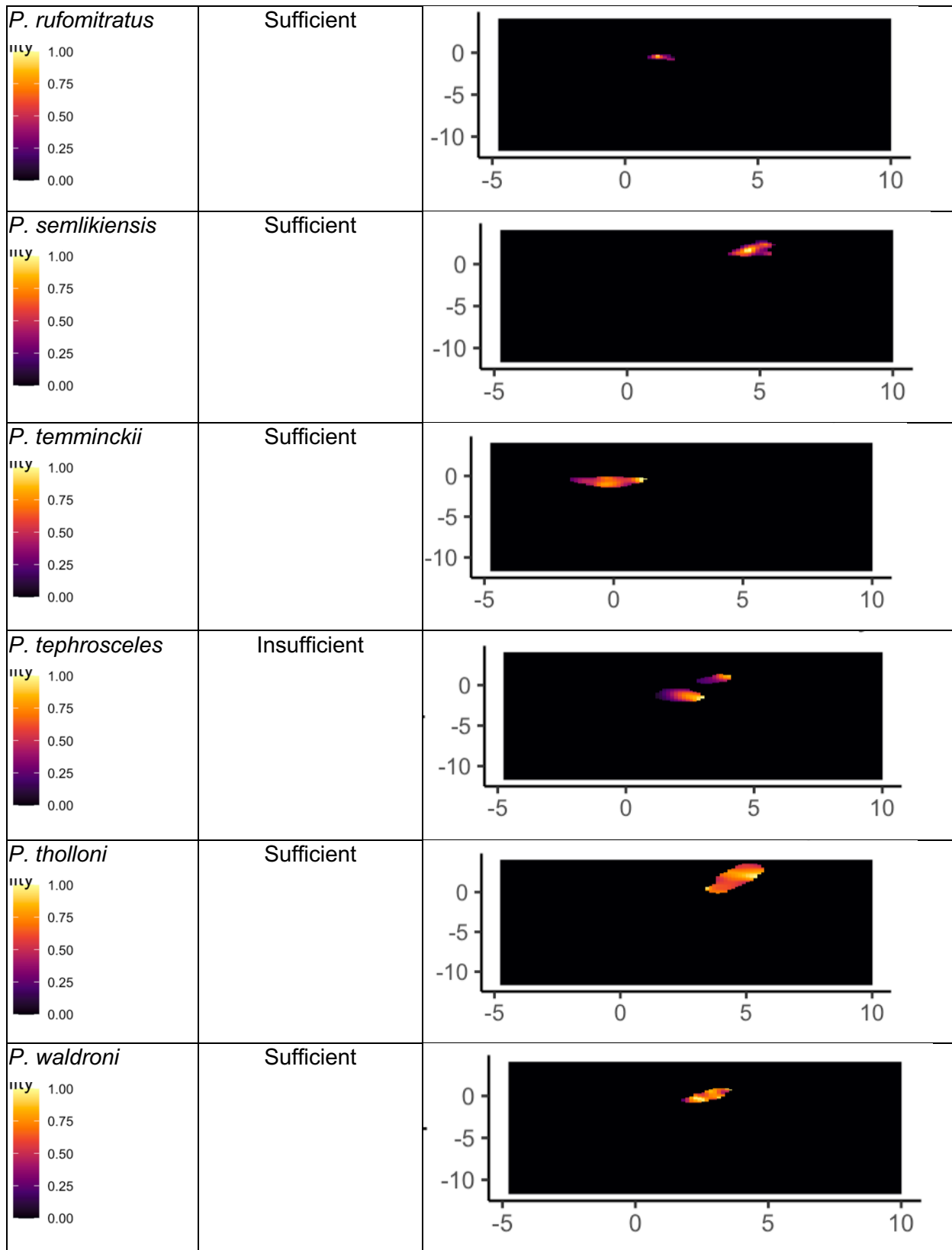
Supplementary Material S2.1: Table of kernel density estimates for the environmental niche of each species

We calculated the niche of each species in environmental space using a method developed by Broennimann *et al.* (2012) implemented in the *ENMTools* package. The kernel density estimate plots for each species are in the third column of the table below. In some cases, the kernel density estimate suffered from a methodological artefact whereby the kernel is set incorrectly and the optimal environments are seemingly along the edge of the kernel where there is little to no data. As such, three niche estimates were classified as of insufficient quality to be included in the analysis, as is specified in the Classification column of the table below.

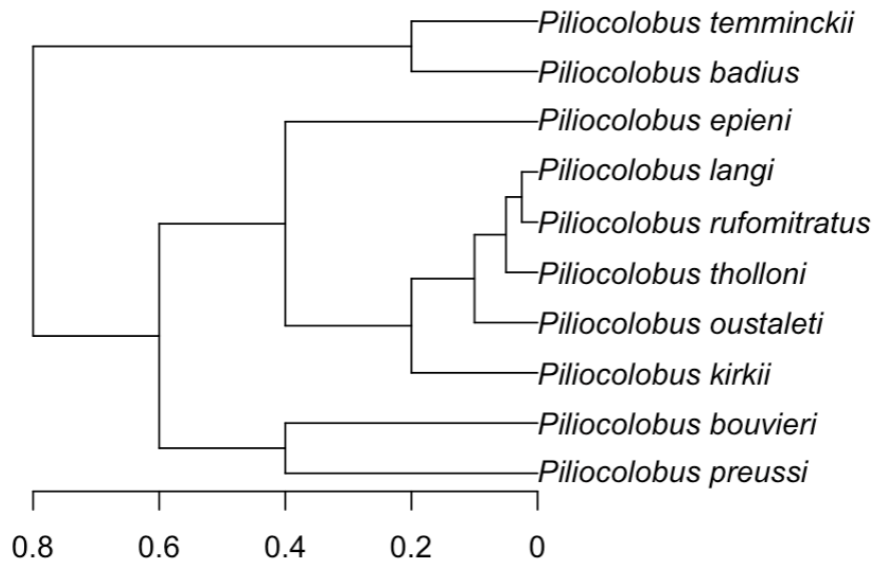
Supplementary Table S2.1: Kernel density estimates for the environmental niche of each red colobus species and the classification of whether the estimate was of sufficient quality.

Species	Classification	Kernel density plot
<i>P. badius</i> 	Sufficient	
<i>P. bouvieri</i> 	Sufficient	
<i>P. epieni</i> 	Sufficient	
<i>P. gordonorum</i> 	Insufficient	





Supplementary Material S2.2: Chronogram of red colobus species



Supplementary Figure S2.2: Chronogram of ten red colobus species used in the age-range correlation analysis. It was created using mitochondrial sequence data from Ting (2008b) aligned (with *Procolobus verus* included as an outgroup) for a 4,092 base pair dataset encompassing the regions spanning the ND3, ND4, and ND5 genes. A maximum likelihood tree was inferred using the RAxML plugin in Geneious with a GTR G+I model. Divergence times were calculated using a penalized maximum likelihood method. The 8 missing red colobus species were excluded either because we did not have sequence data or because we did not have presence data to calculate niche breadth as was required for the age-range correlation analysis.

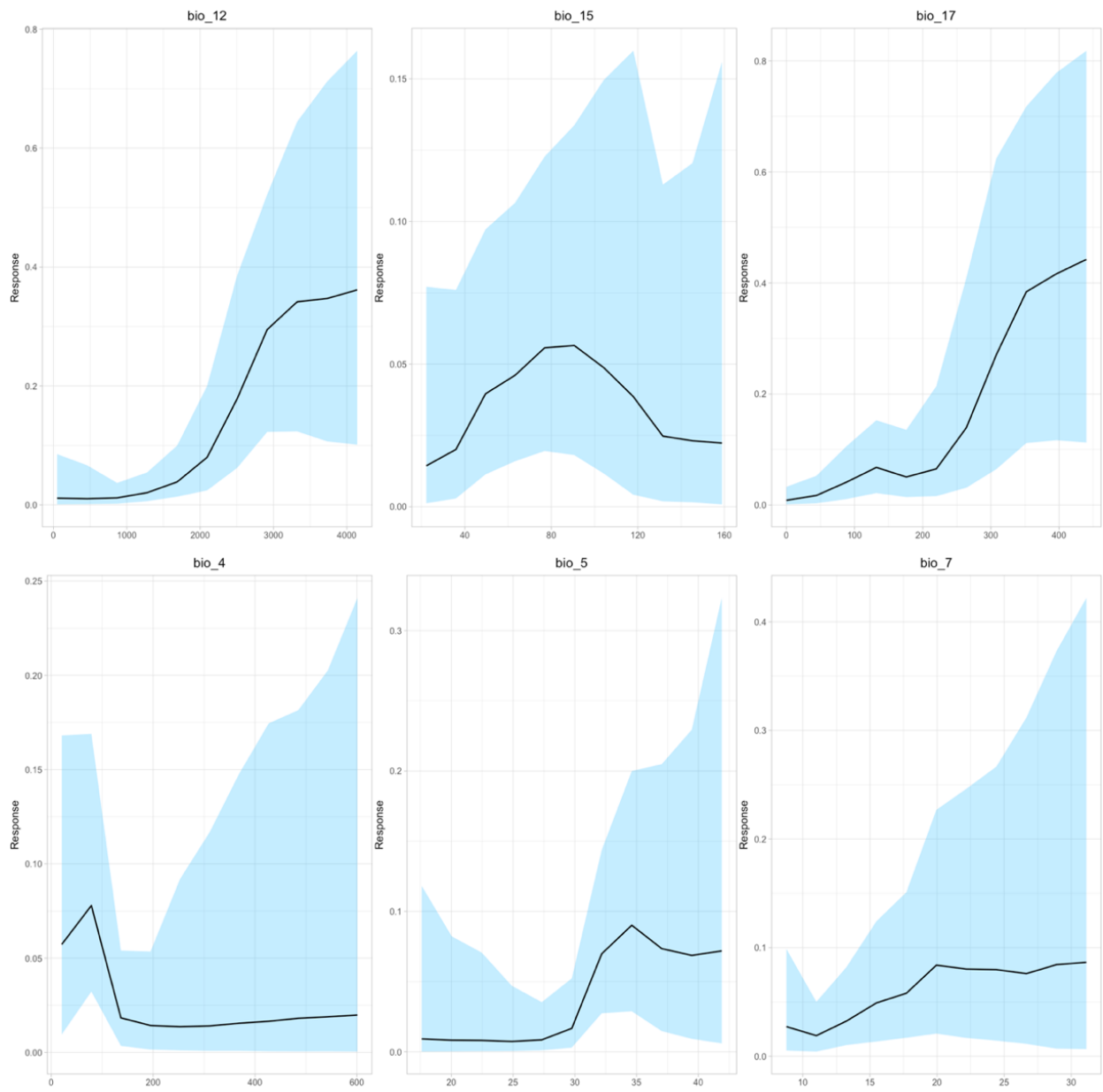
Supplementary Material S2.3: Partial dependence plots for each variable for each species

Partial dependence plots represent the relationship of a single variable to the predicted outcome, on a scale of 0 to 1, independent of the other variables fit in the model. The steepness of these curves indicates the predicted magnitude of the effect, but not necessarily the variable's importance in the model.

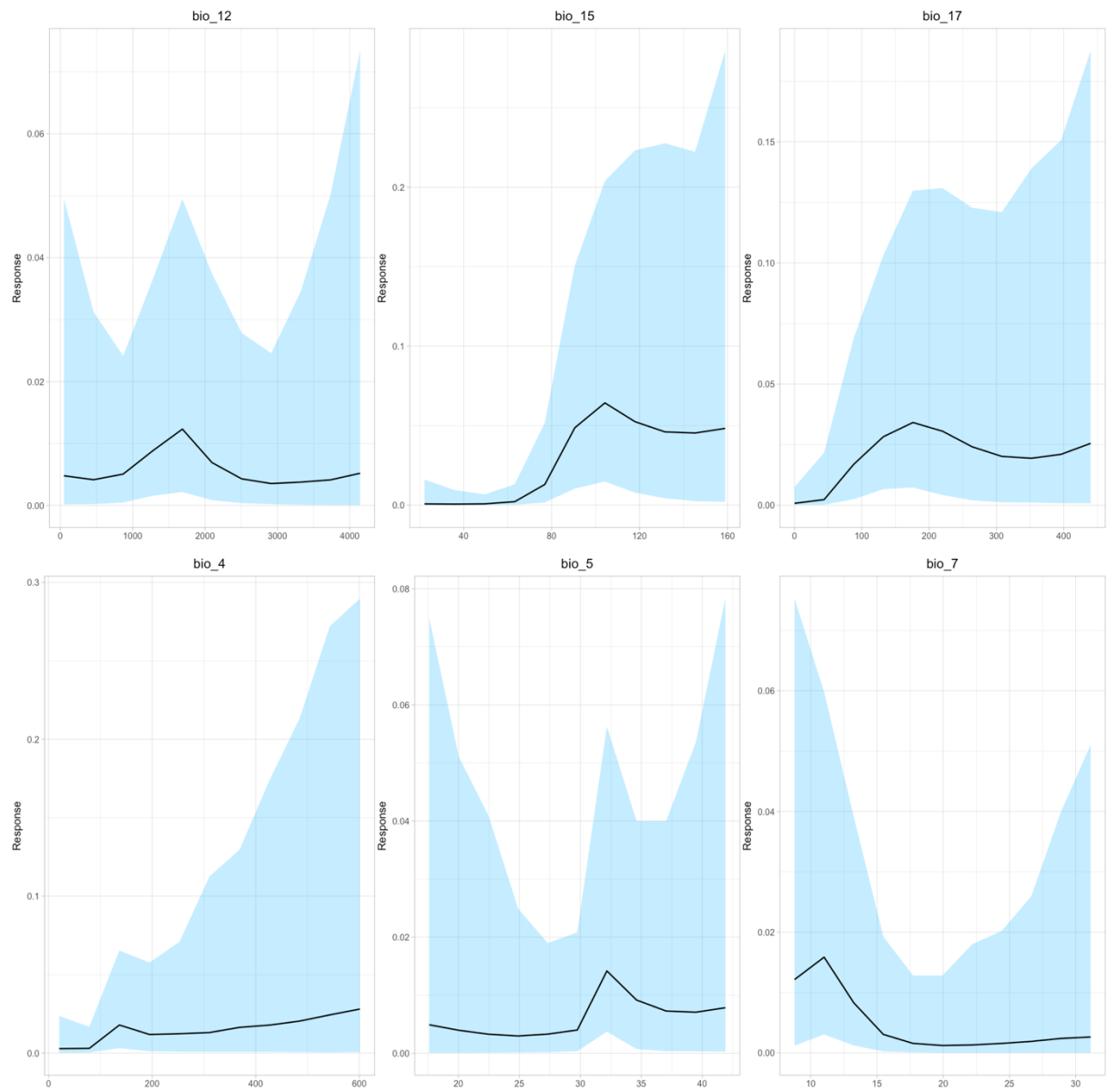
Supplementary Figure S2.3: *Partial dependence plots for each variable for each species. (BIO4 – temperature seasonality, BIO5 – maximum temperature of the warmest month, BIO7 – temperature annual range, BIO12 – annual precipitation, BIO15 – precipitation seasonality, BIO17 – precipitation of the driest quarter). The black line represents the relationship of the single variable to the predicted presence of the species, the blue shading gives a 95% credible interval from the posterior distribution of the Bayesian model.*

WorldClim only models:

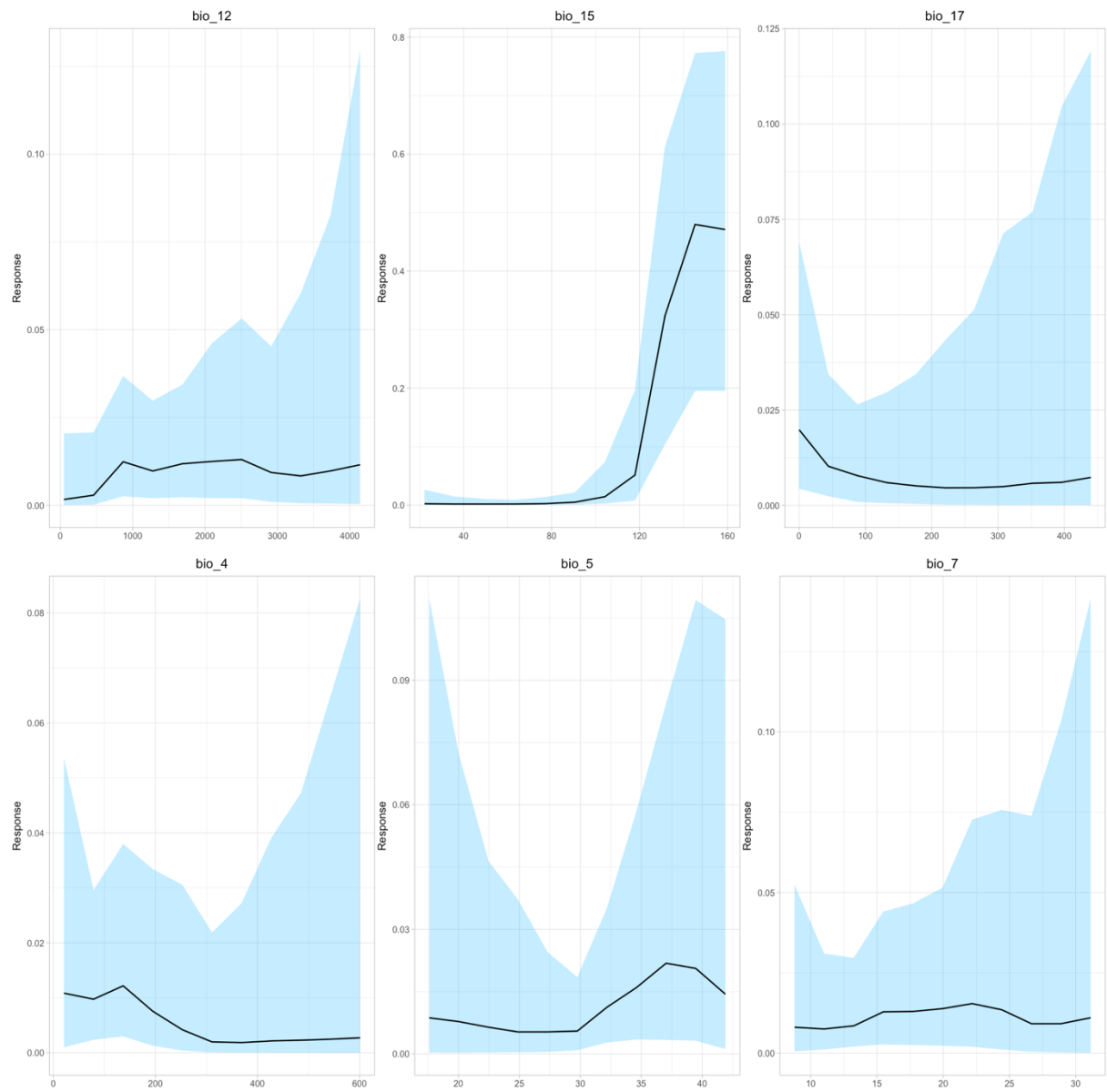
P. badius partial dependence plots:



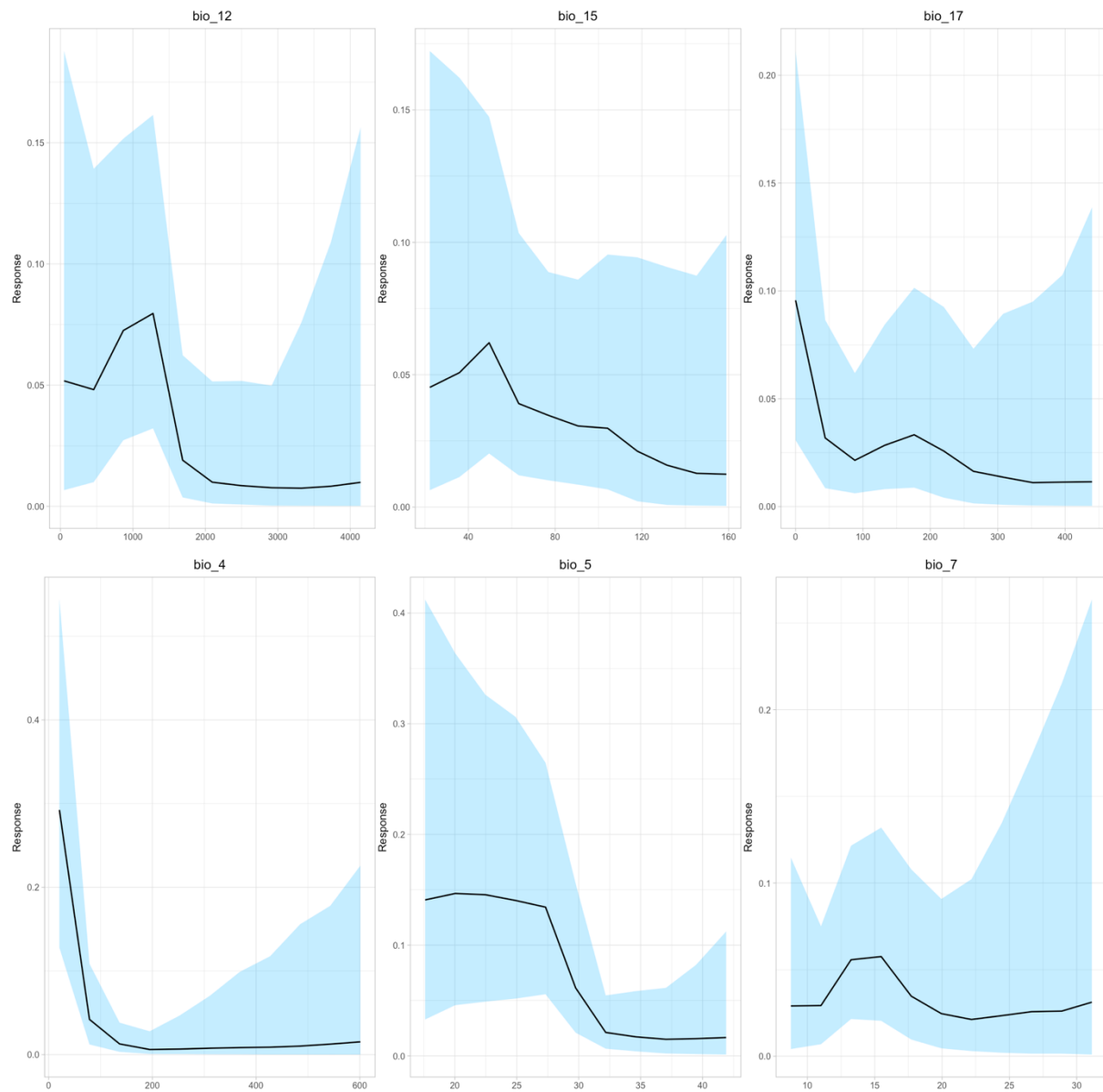
P. kirkii partial dependence plots:



P. temminckii partial dependence plots

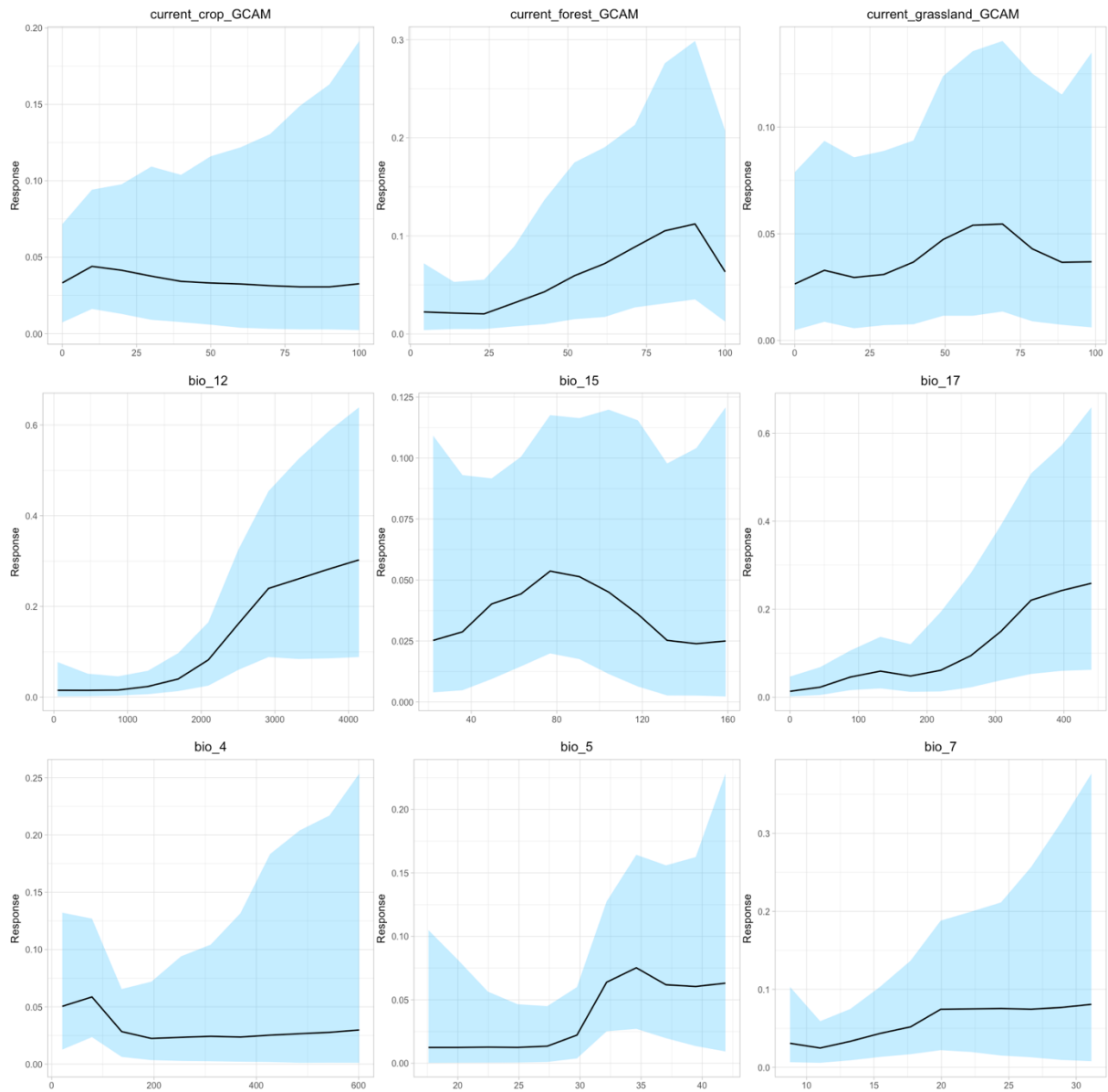


P. tephrosceles partial dependence plots:

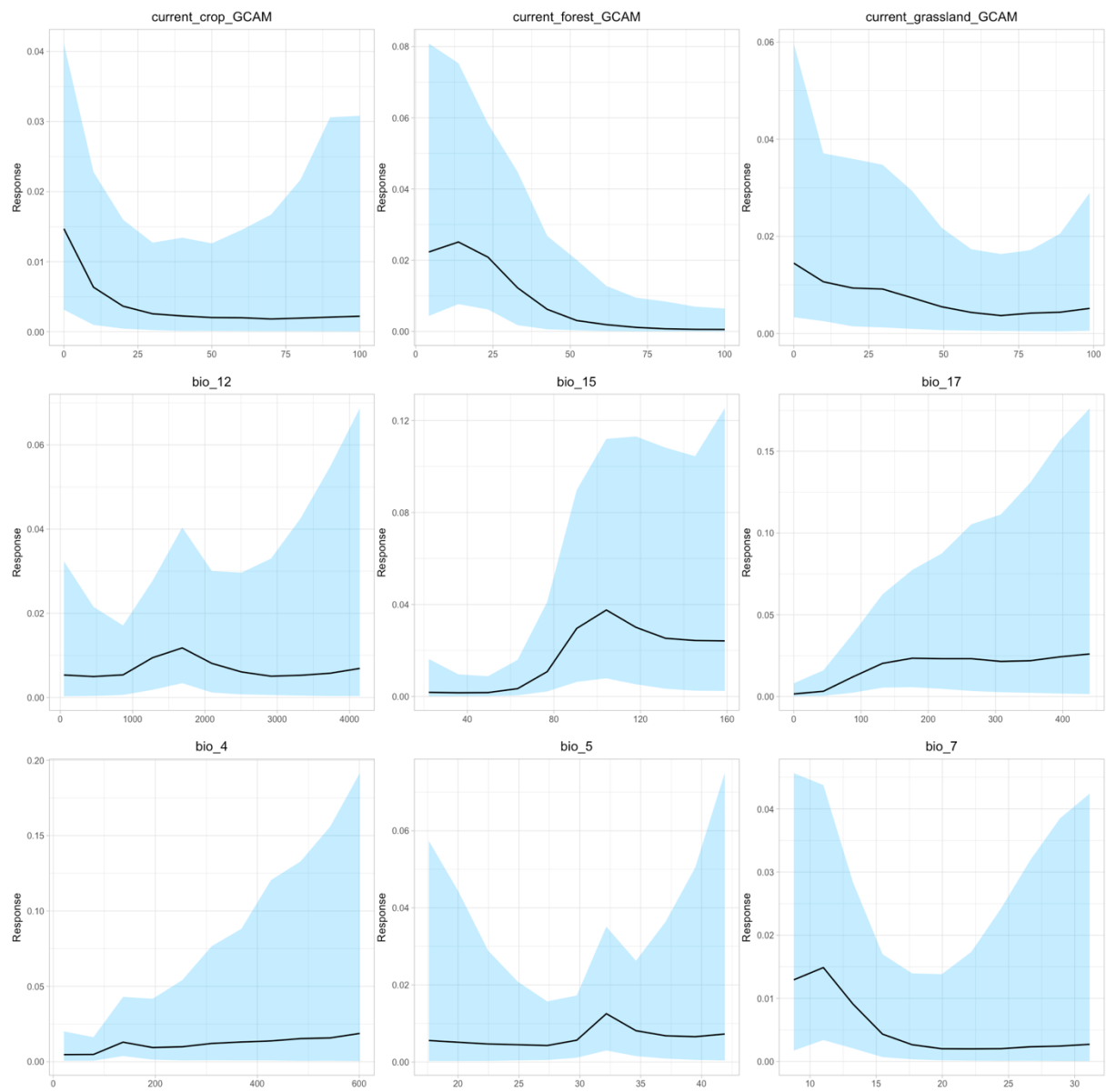


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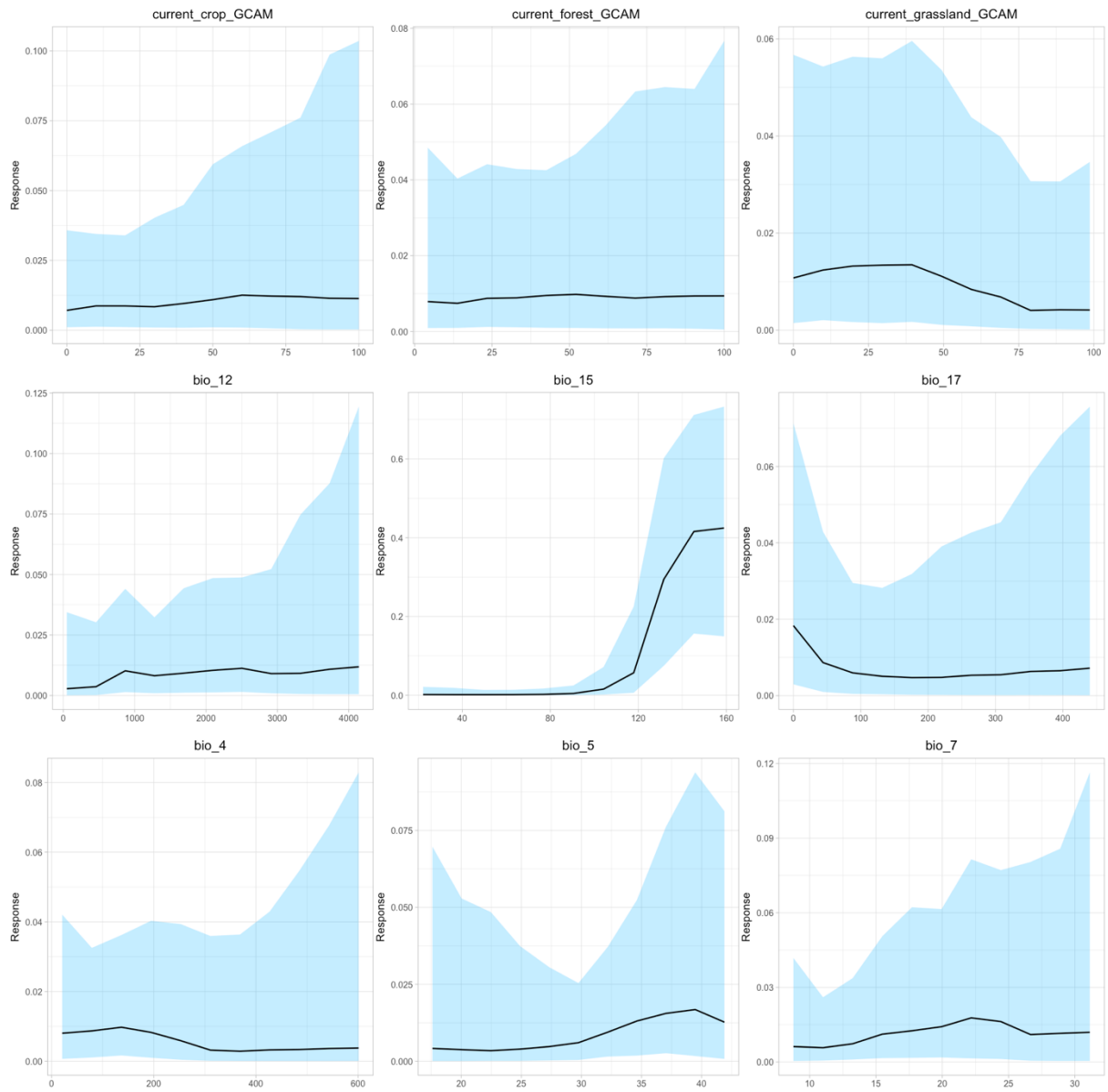
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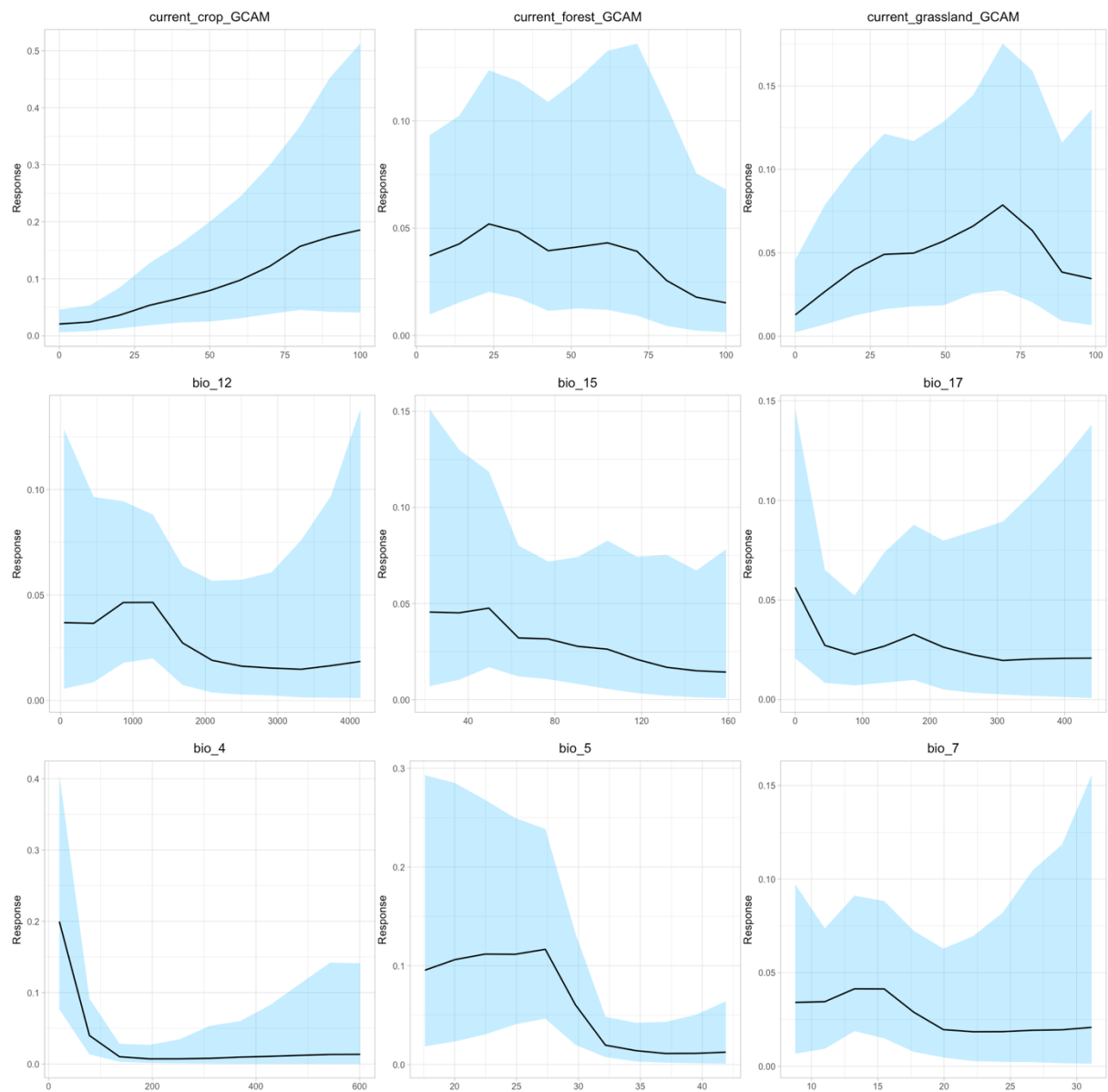
P. kirkii partial dependence plots:



P. temminckii partial dependence plots

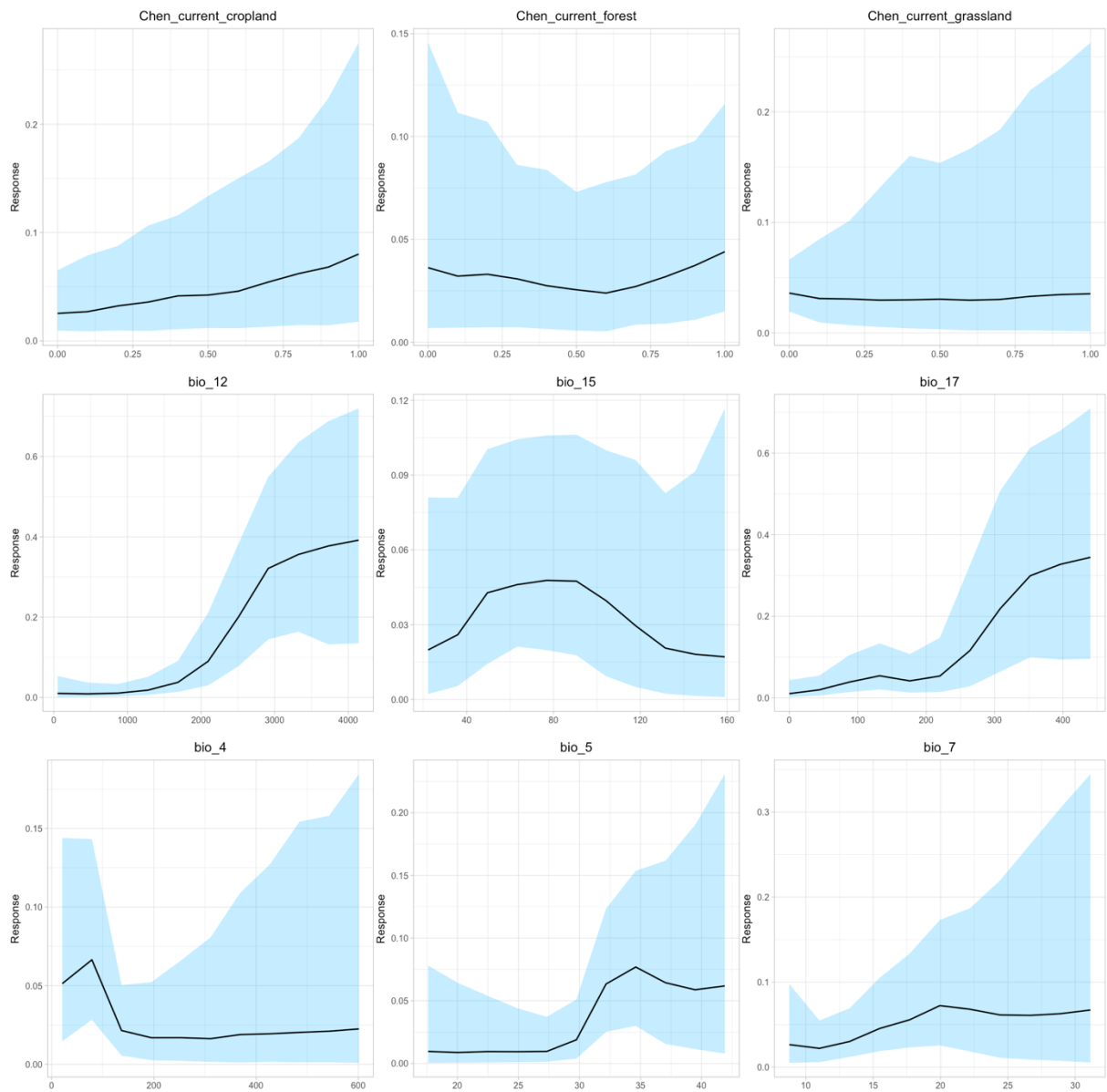


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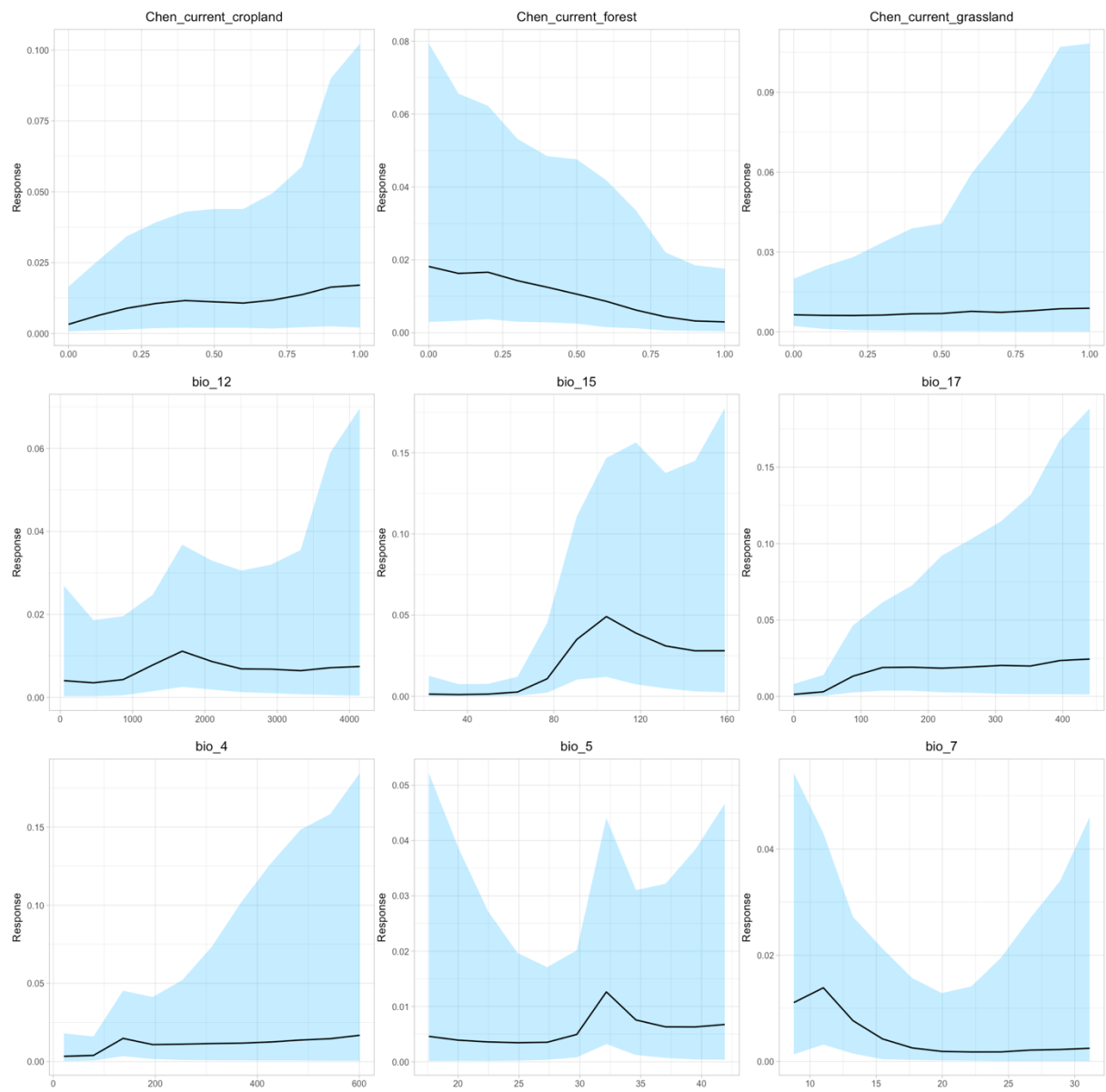


Chen and WorldClim models:

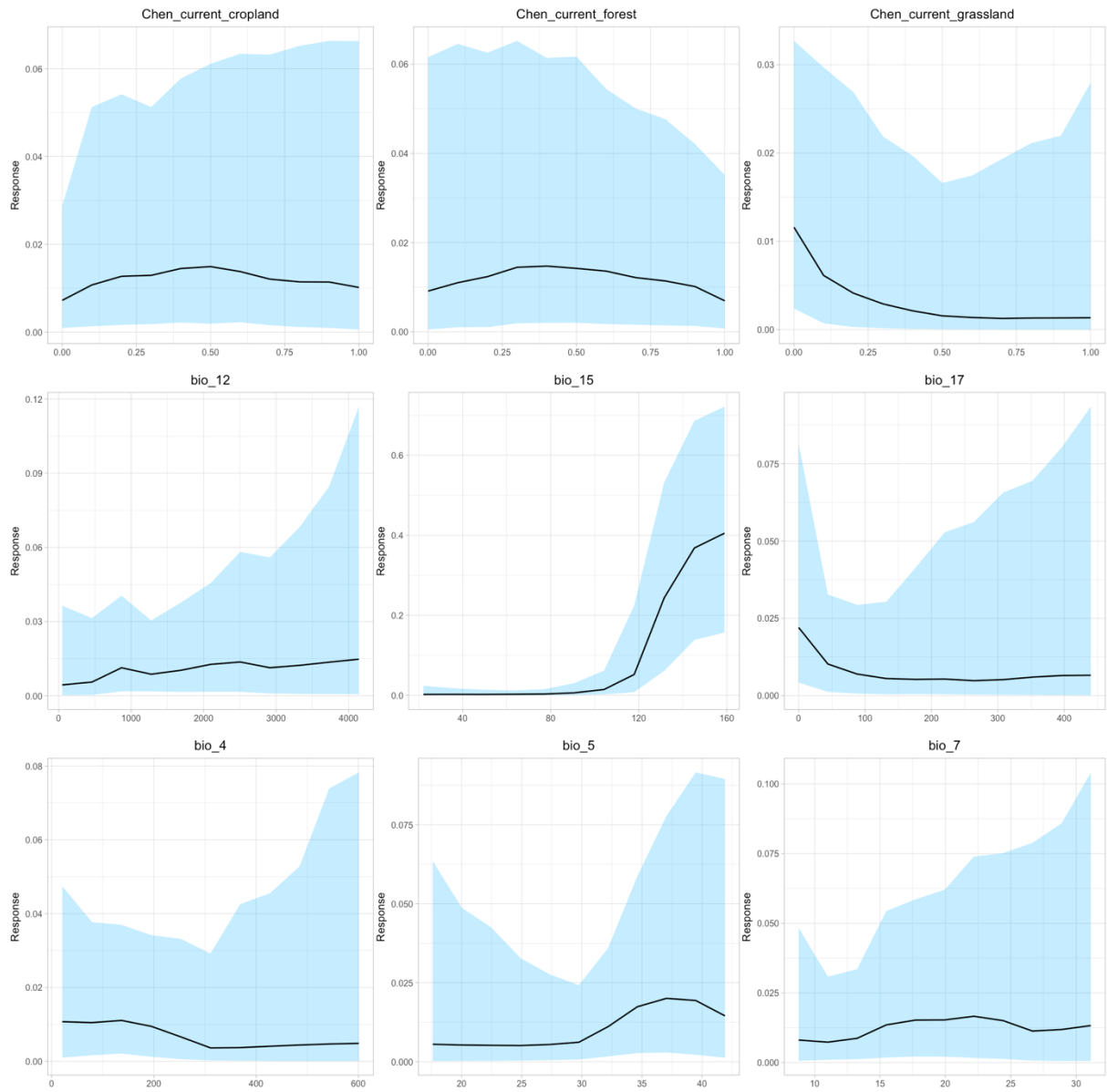
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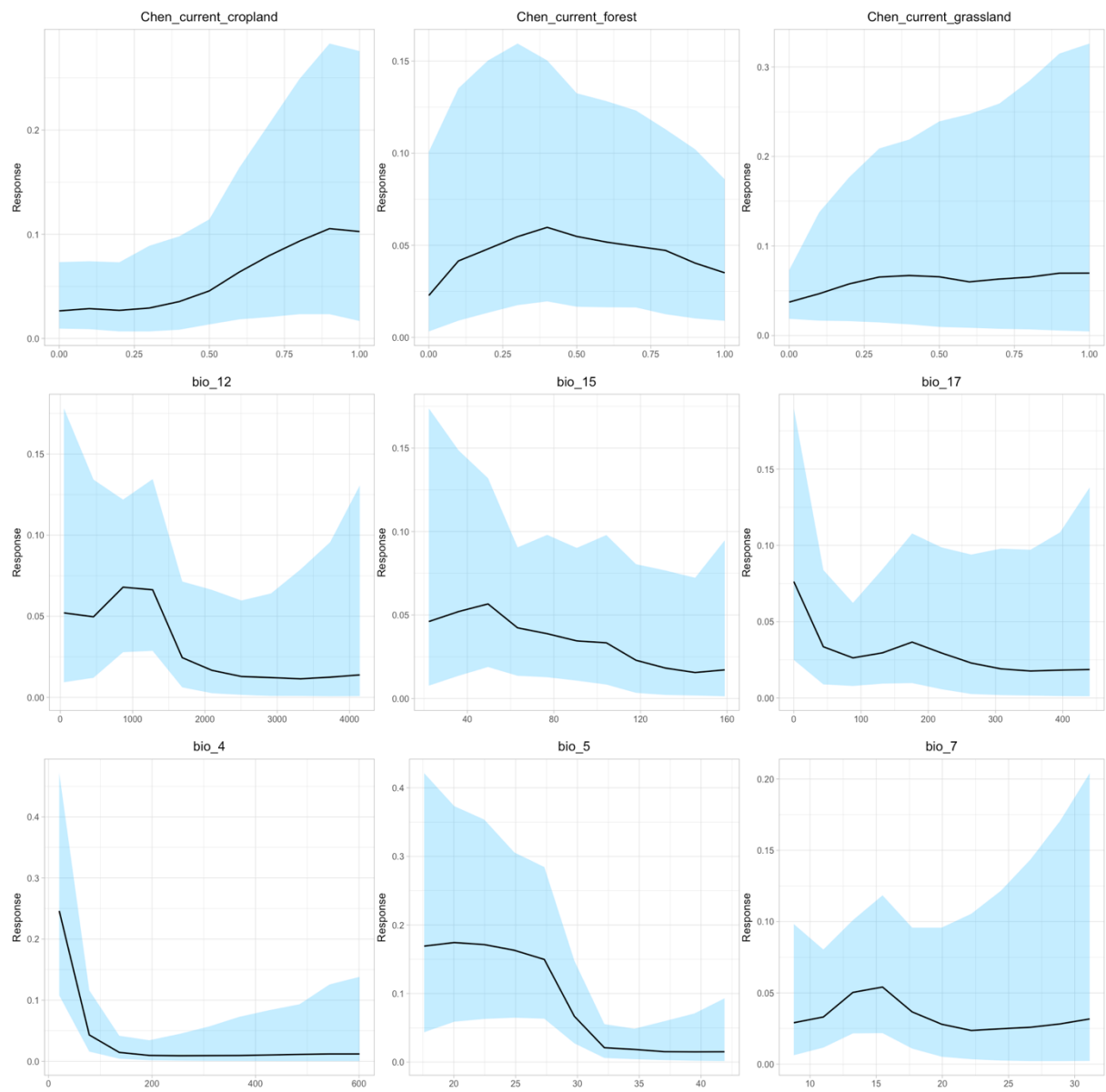
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P. temminckii partial dependence plots



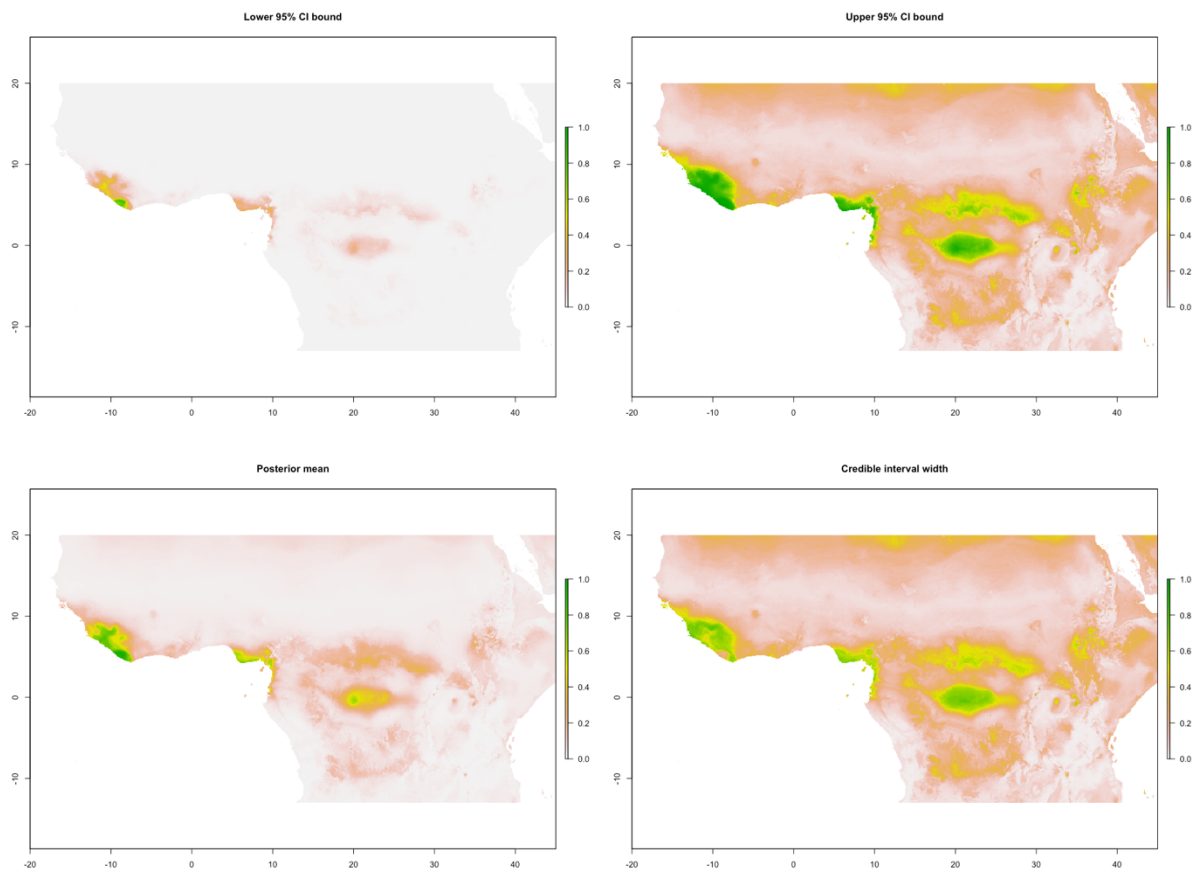
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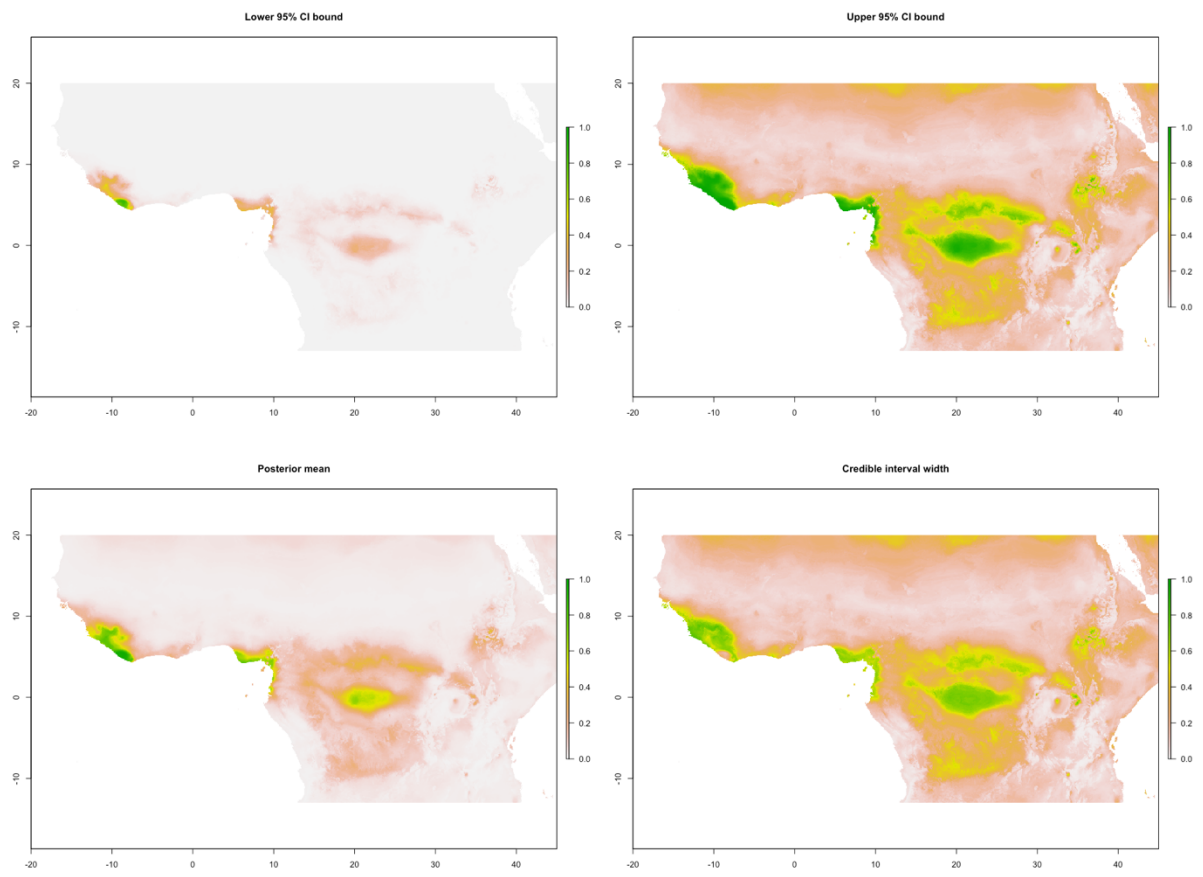
Supplementary Material S2.4: The predicted probability of presence of four red colobus species in the present and in four future scenarios (SSP1-2.6, SSP2-4.5, SSP3-7.0, SSP5-8.5)

Supplementary Figure S2.4: The posterior mean, lower 95% confidence interval (CI) bound, upper 95% confidence interval (CI) bound and the credible interval width of the predicted probability of presence of four red colobus species (*P. badius*, *P. kirkii*, *P. temminckii*, *P. tephrosceles*). The probability of presence was predicted for the current situation and for four future scenarios (SSP1-2.6, SSP2-4.5, SSP3-7.0, SSP5-8.5) using Bayesian Additive Regression Trees (BART). The BART species distribution models were created using three combinations of predictor variables: WorldClim Only (Fick and Hijmans 2017), GCAM (Chen et al. 2020) and WorldClim, Chen (Chen et al. 2022) and WorldClim. For the GCAM land use dataset, SSP3-7.0 was not available so we used the SSP3-6.0 scenario instead, meaning that the radiative forcing was set to 6.0 W/m² instead of 7.0 W/m².

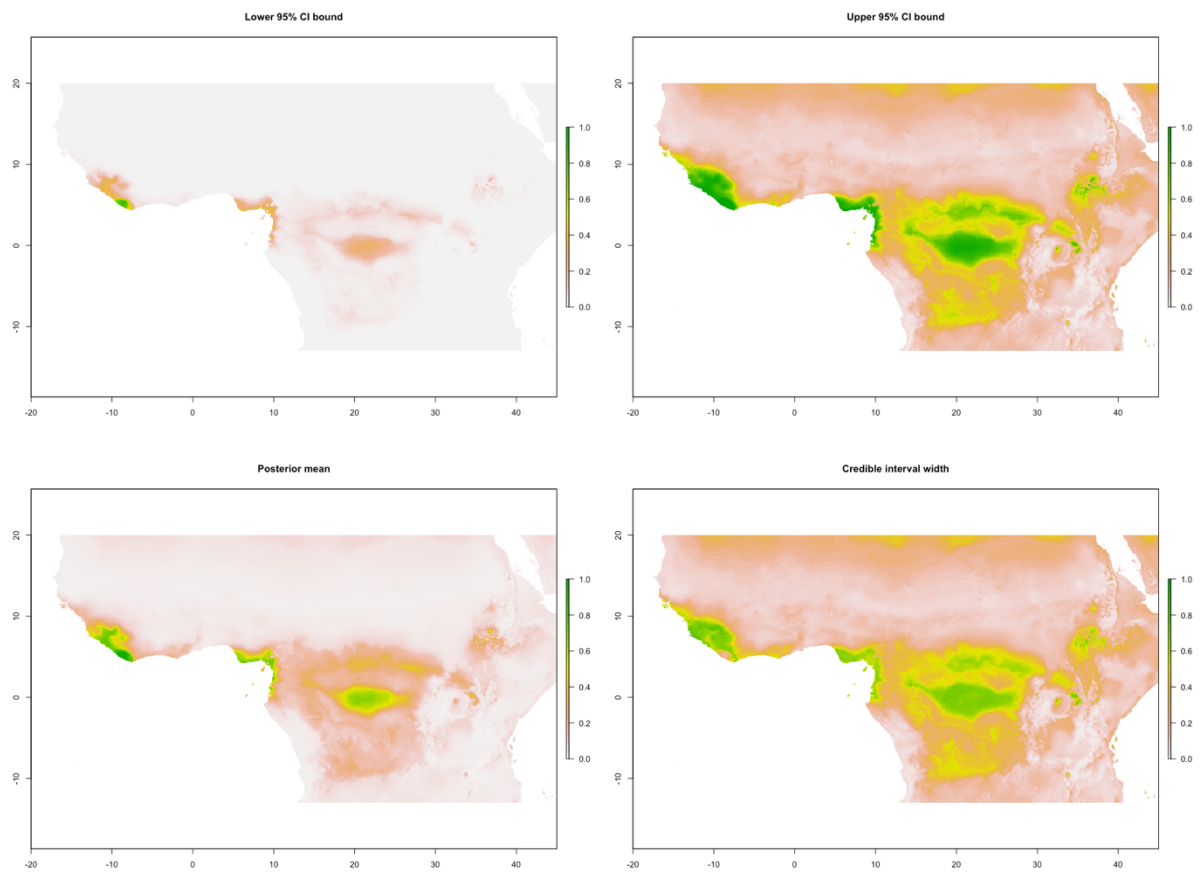
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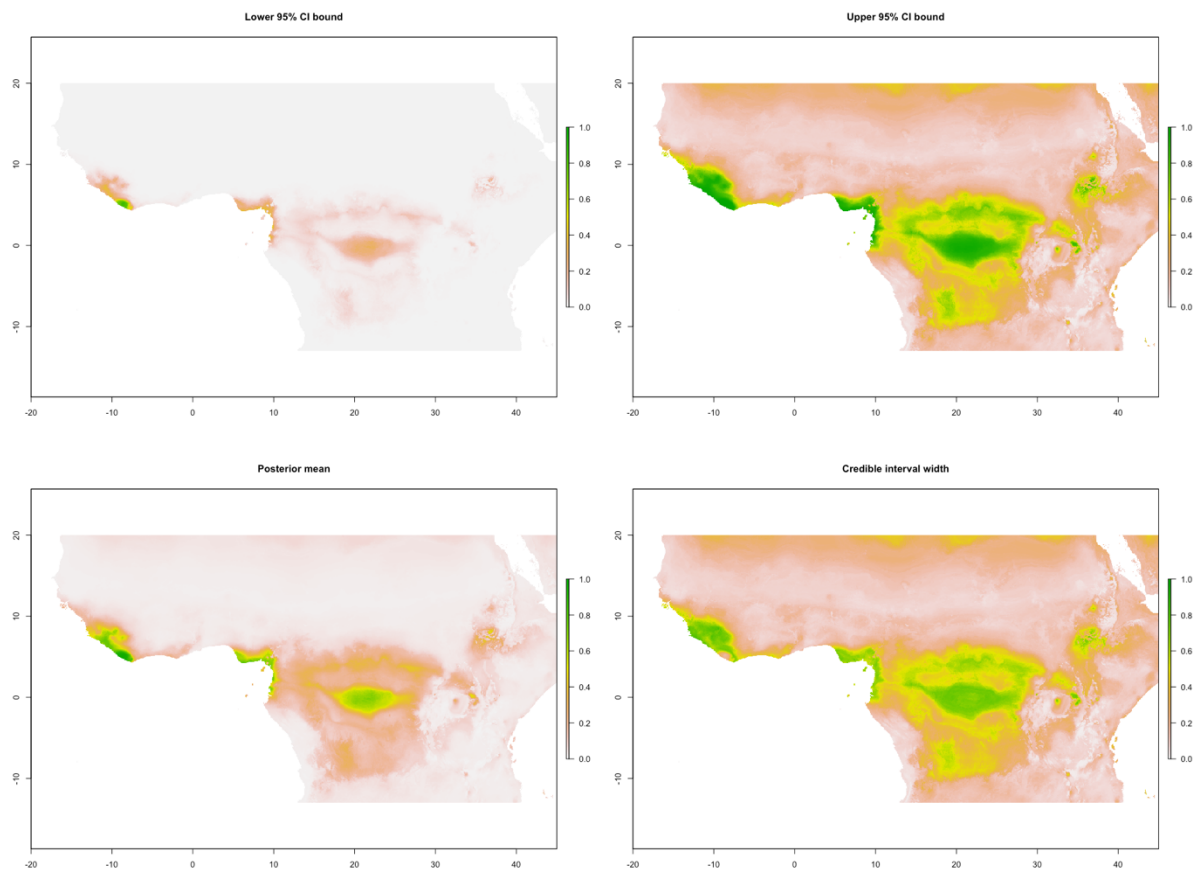
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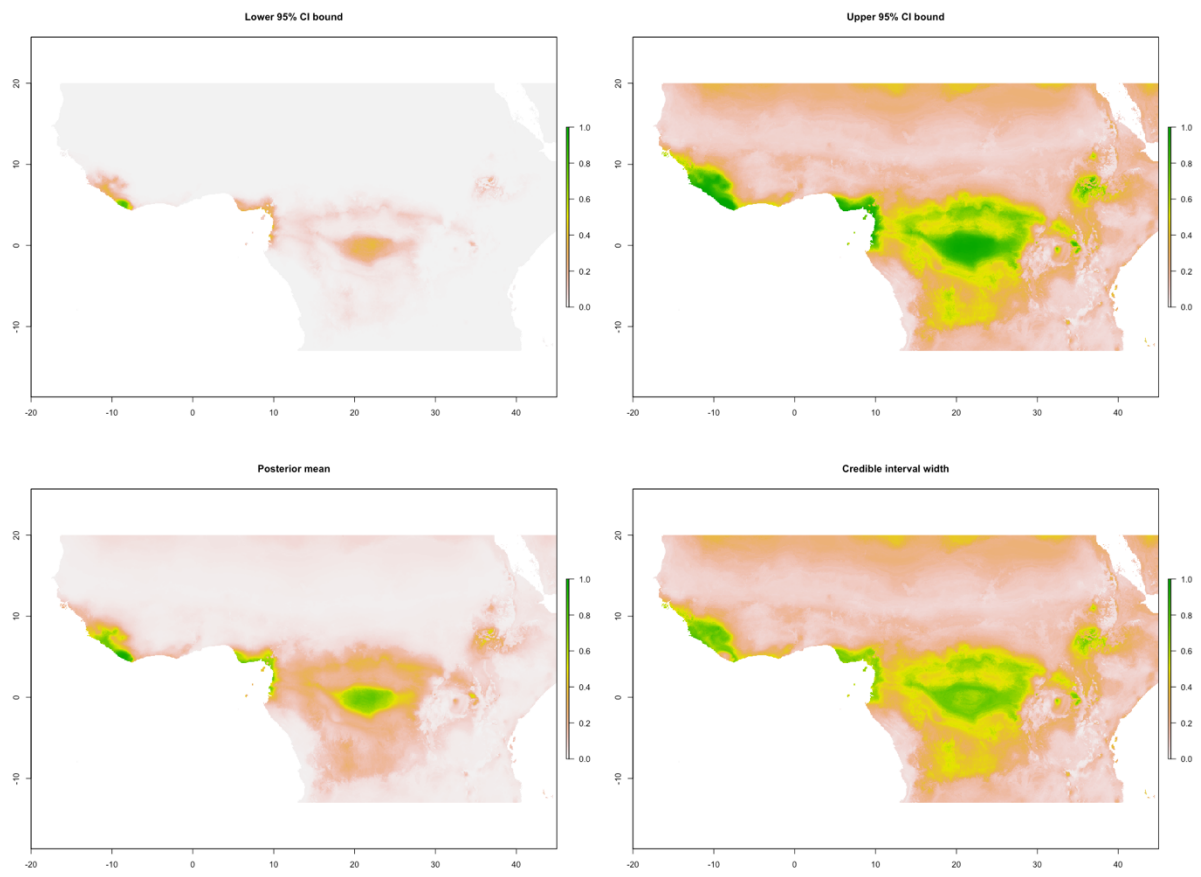
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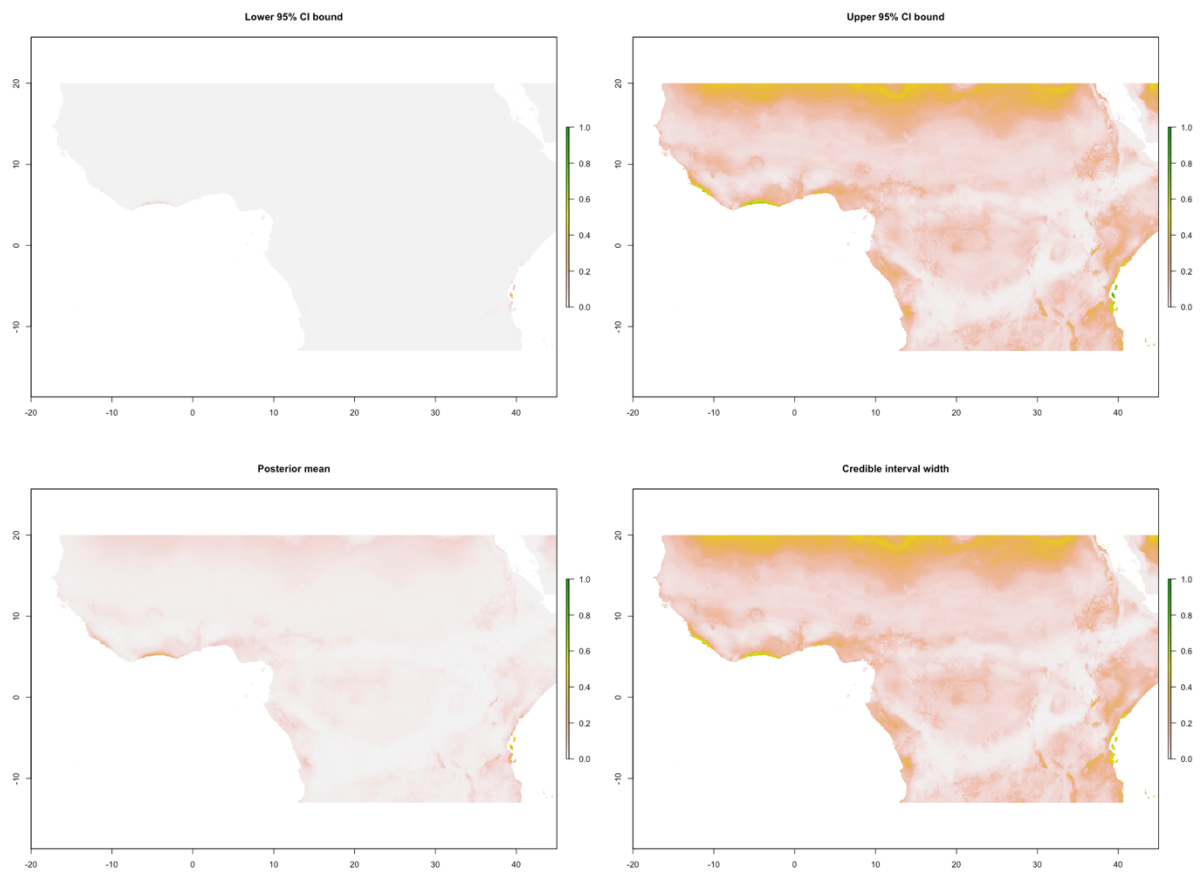
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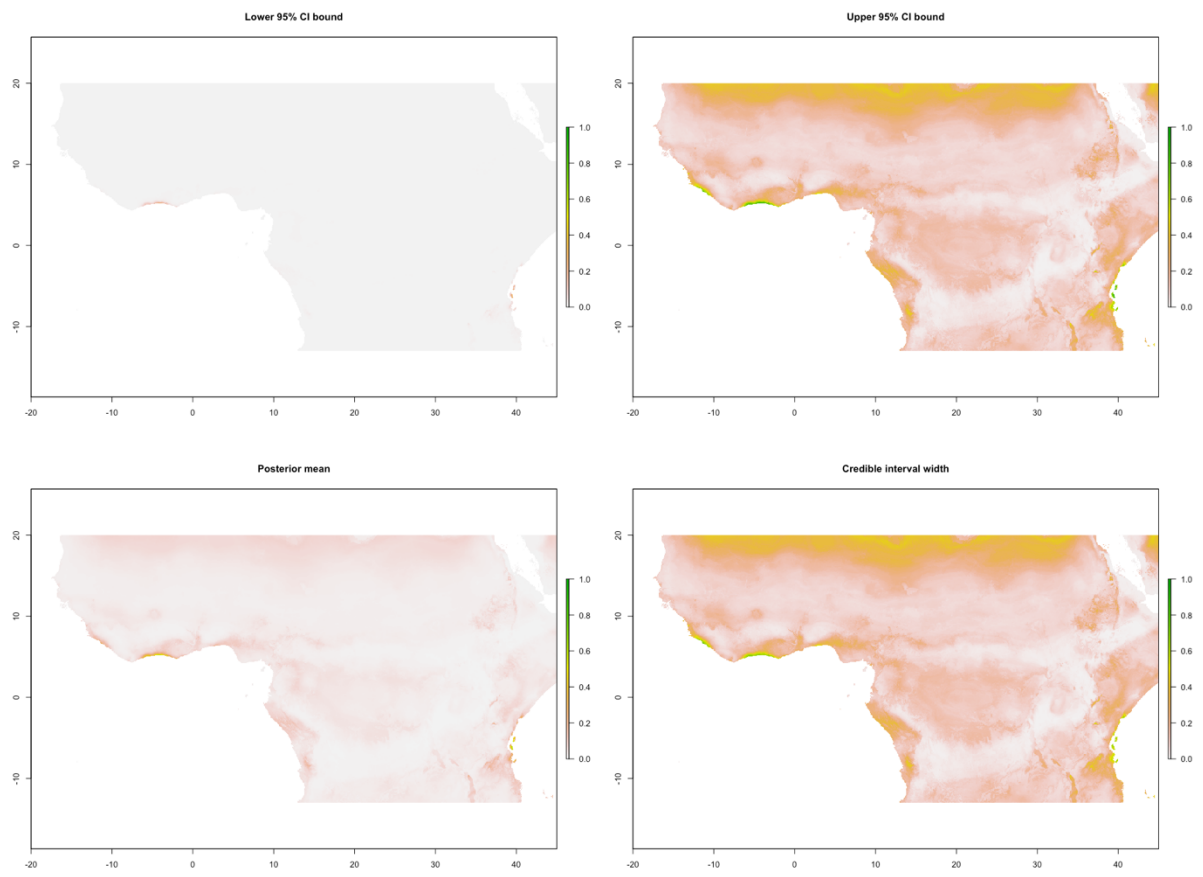
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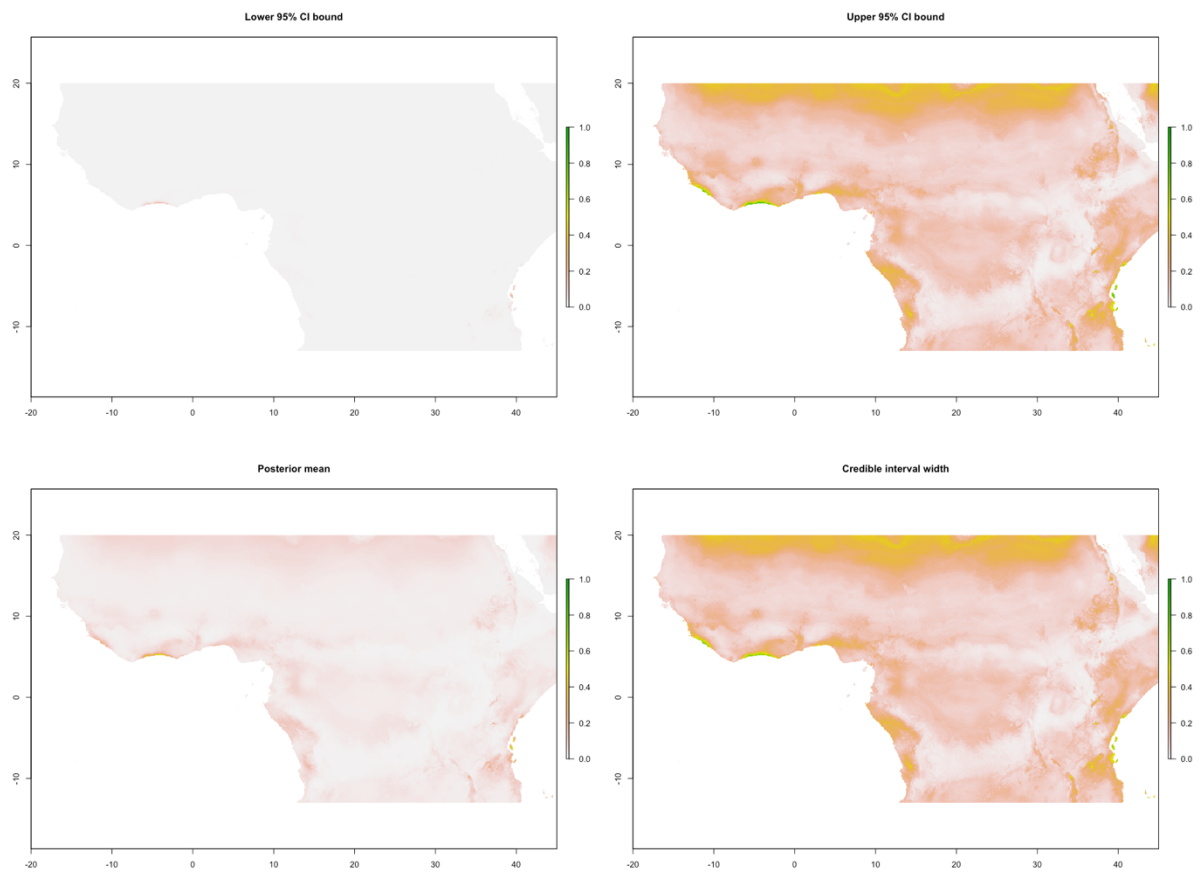
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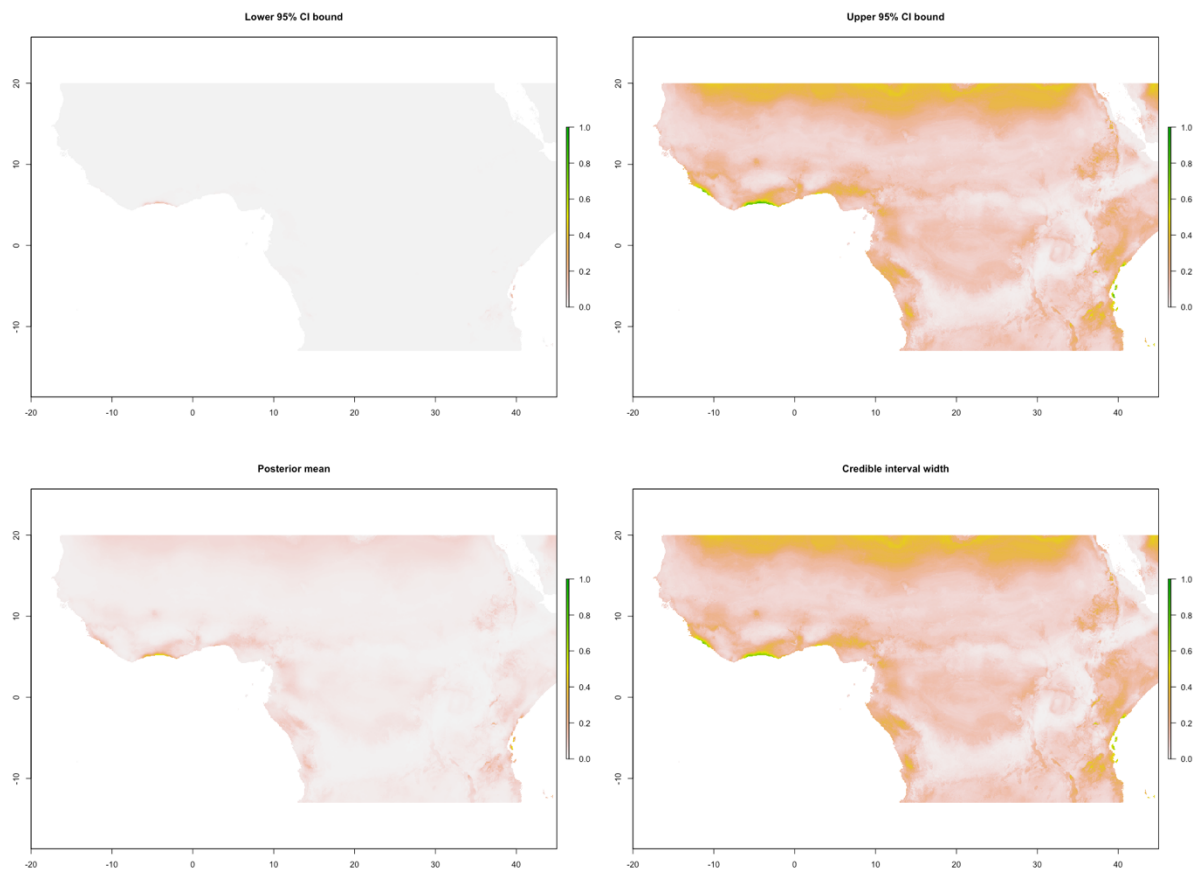
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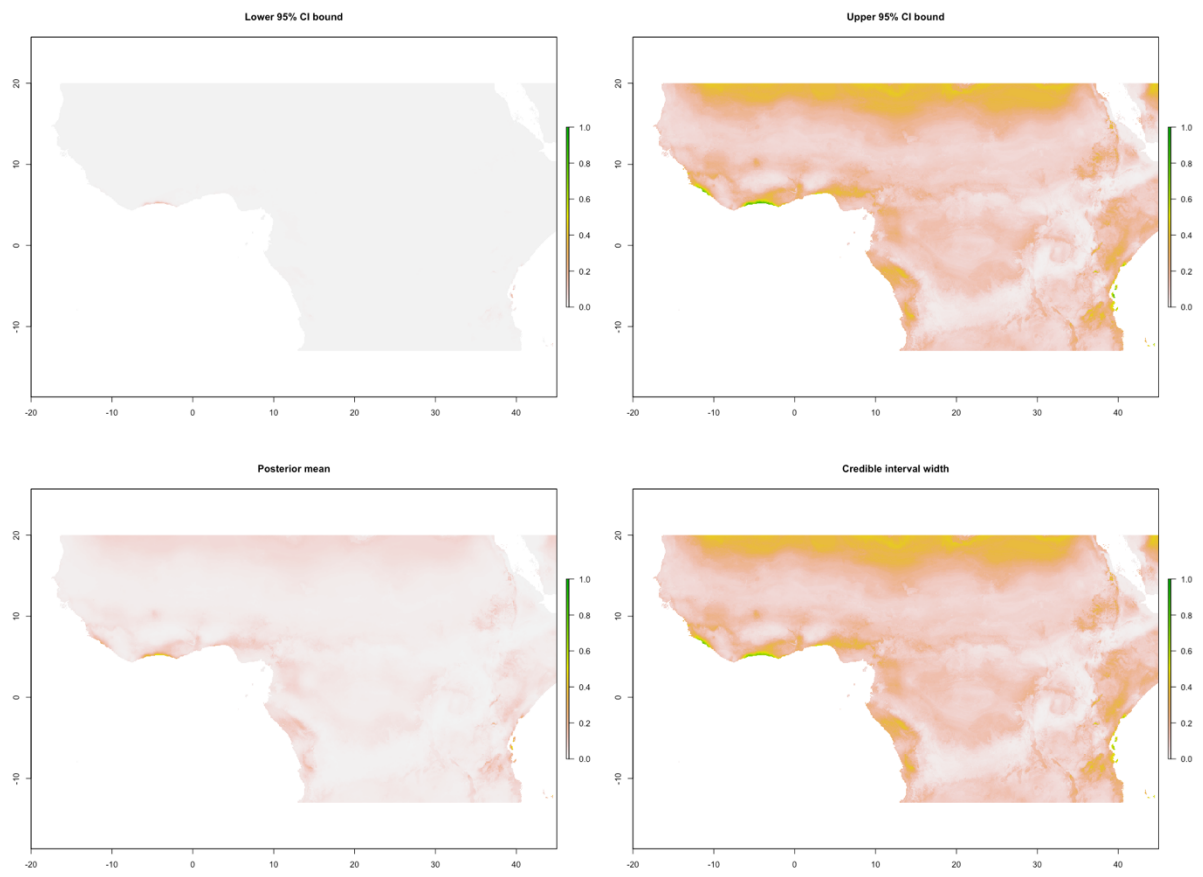
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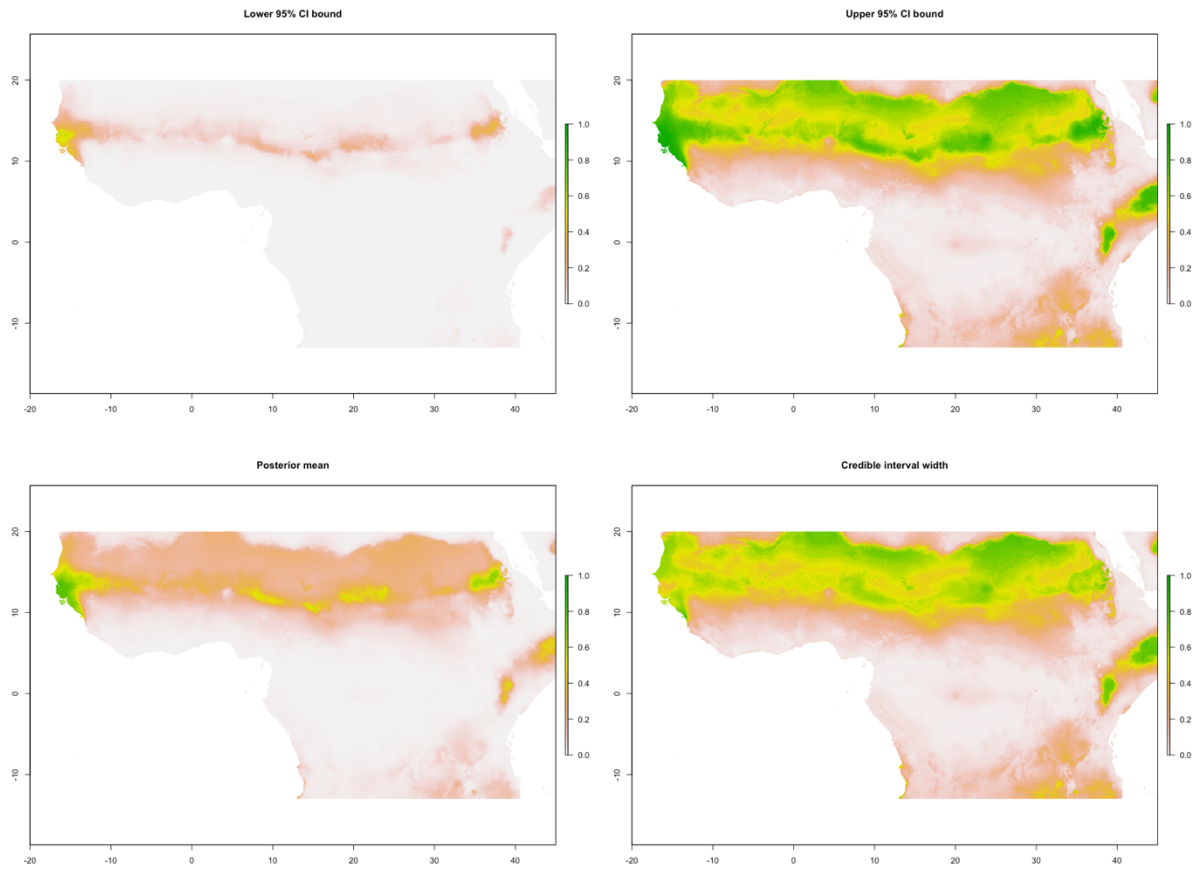
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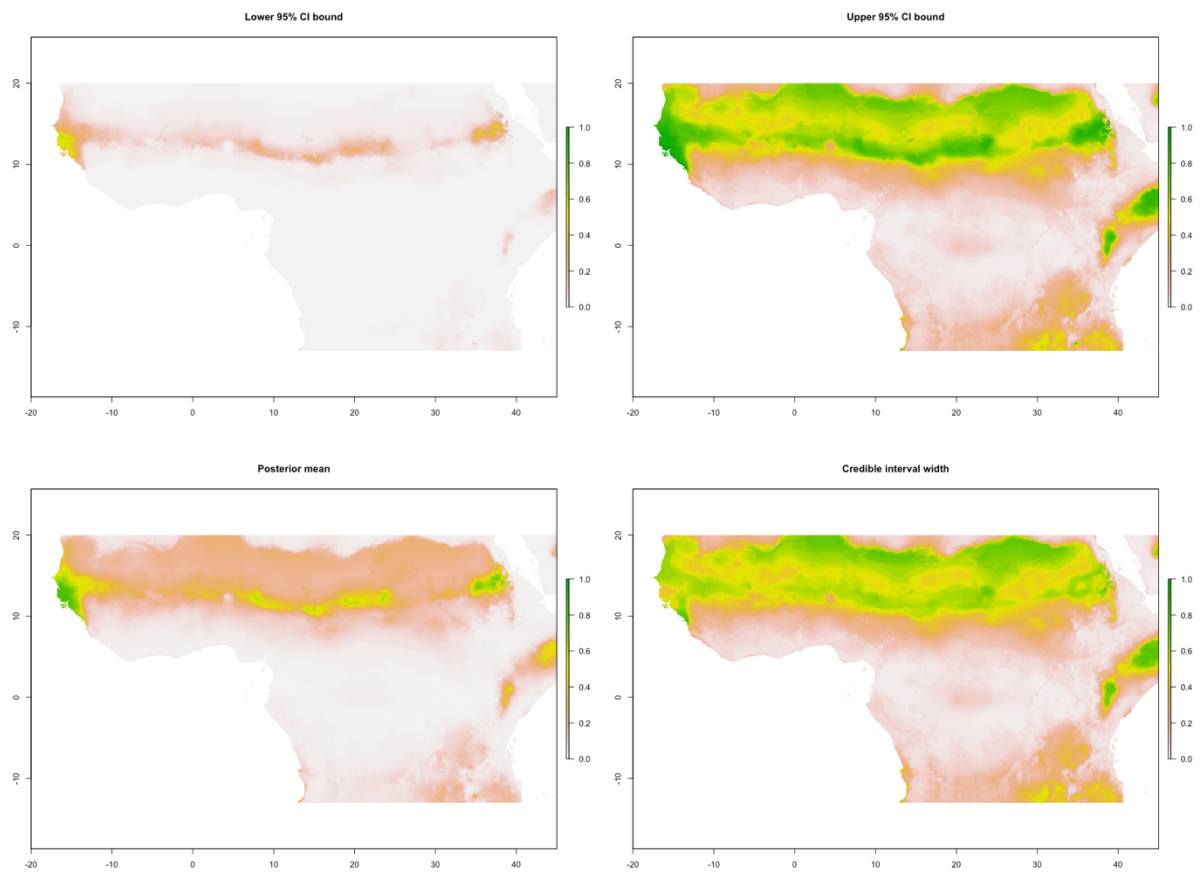
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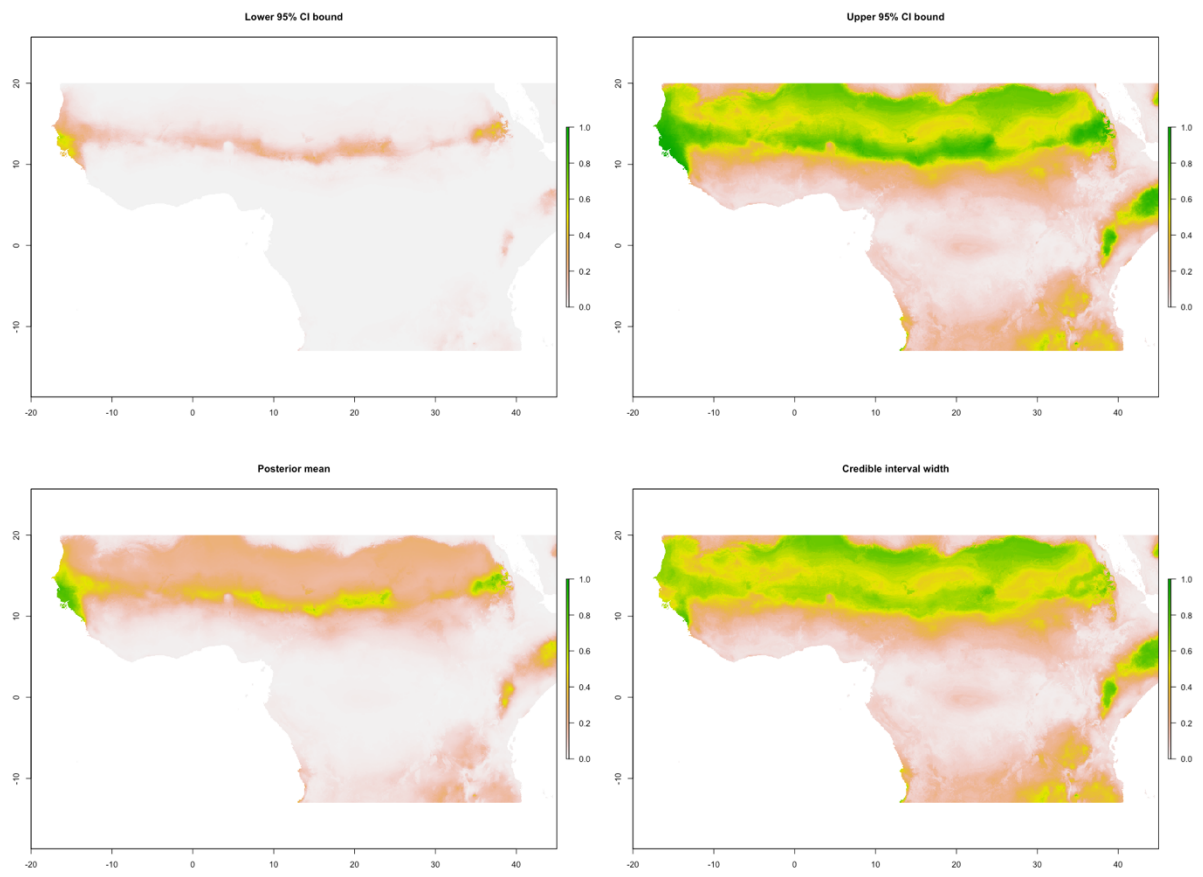
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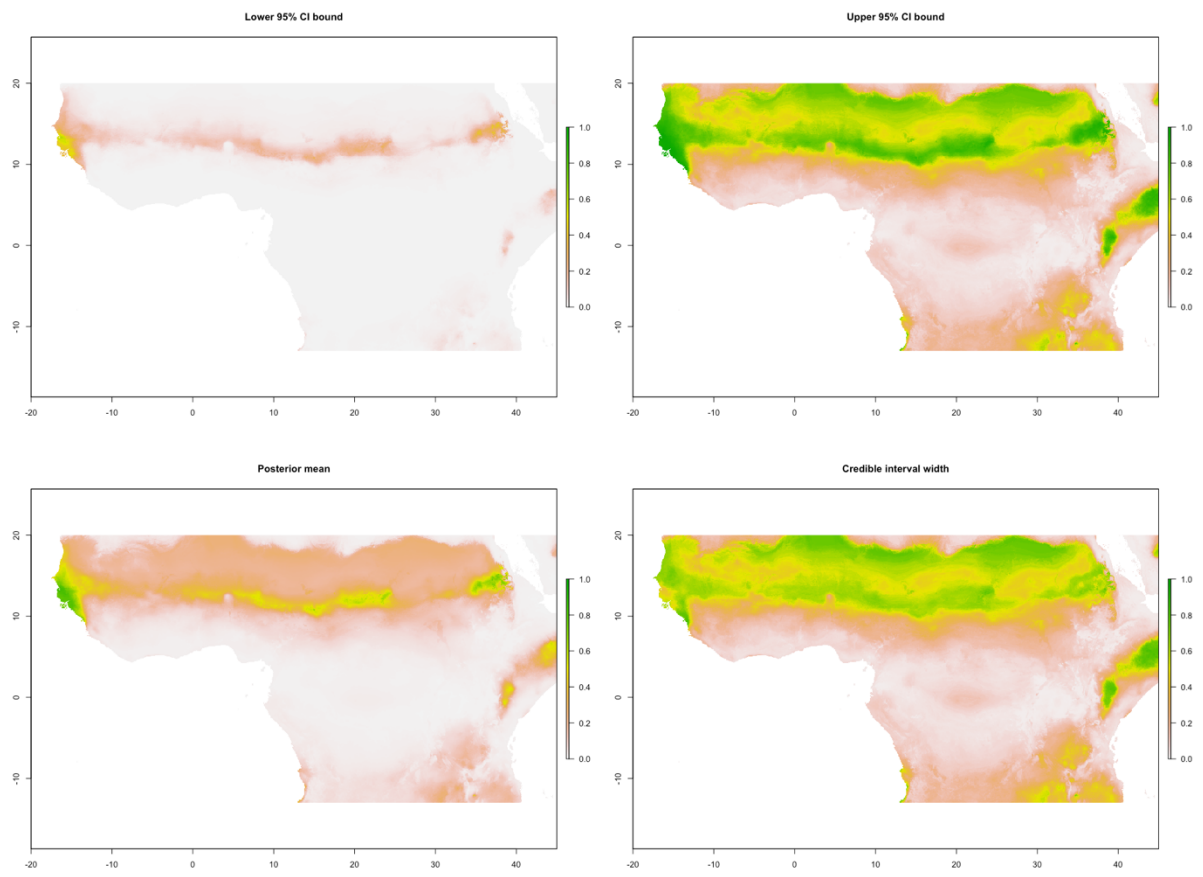
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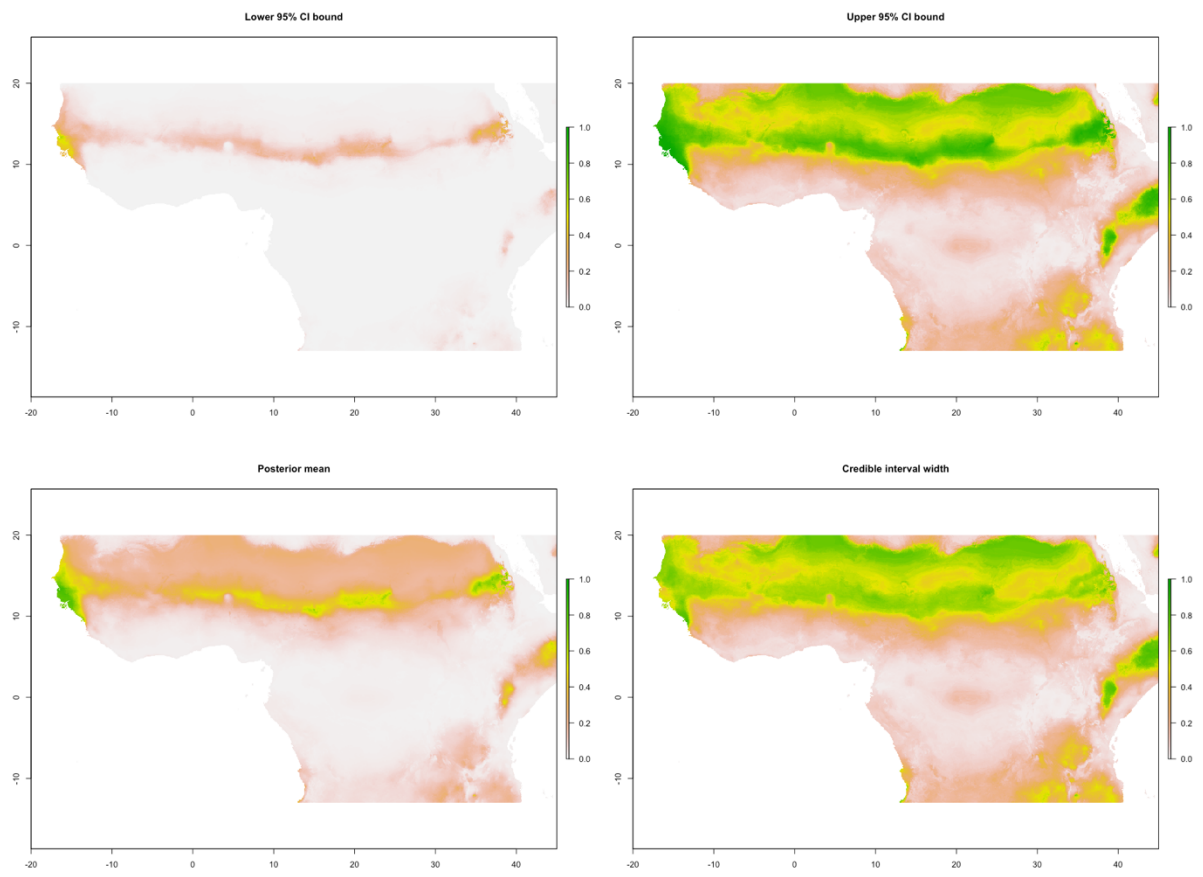
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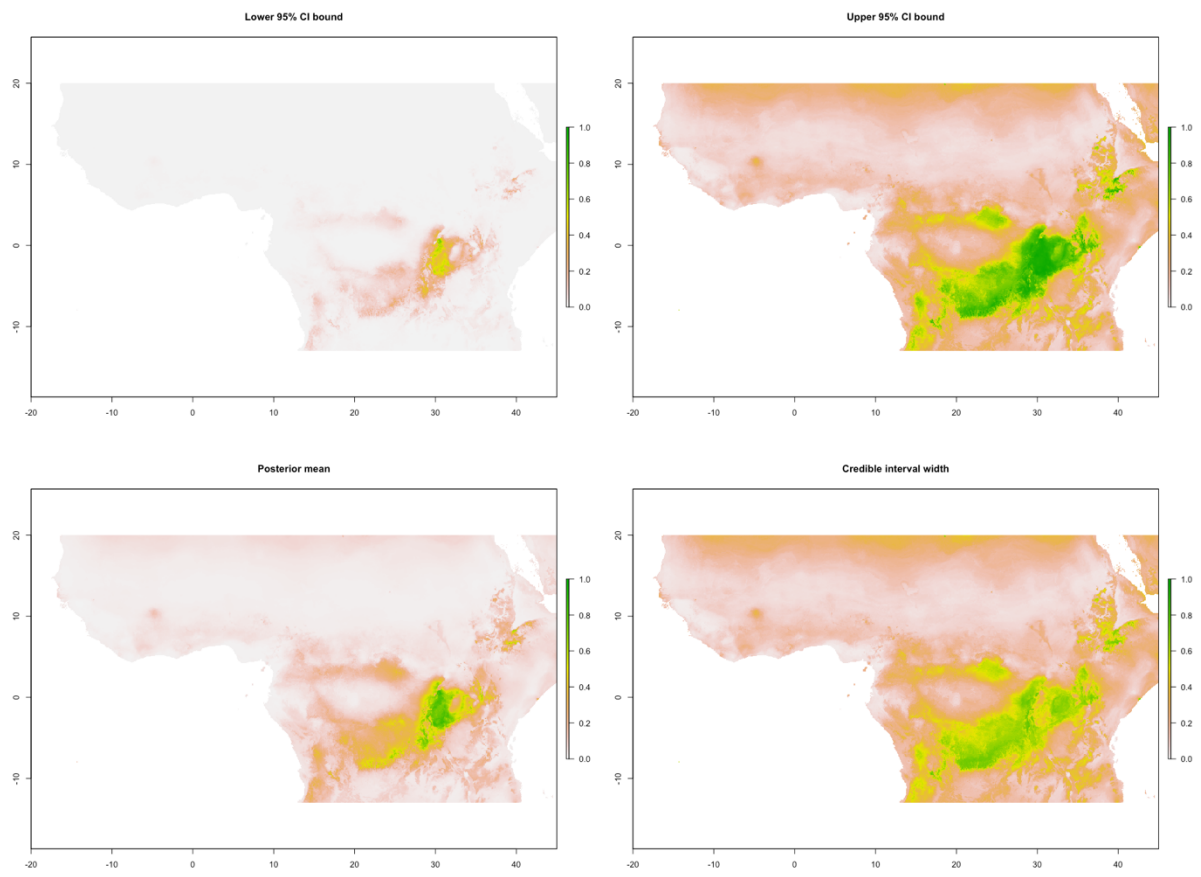
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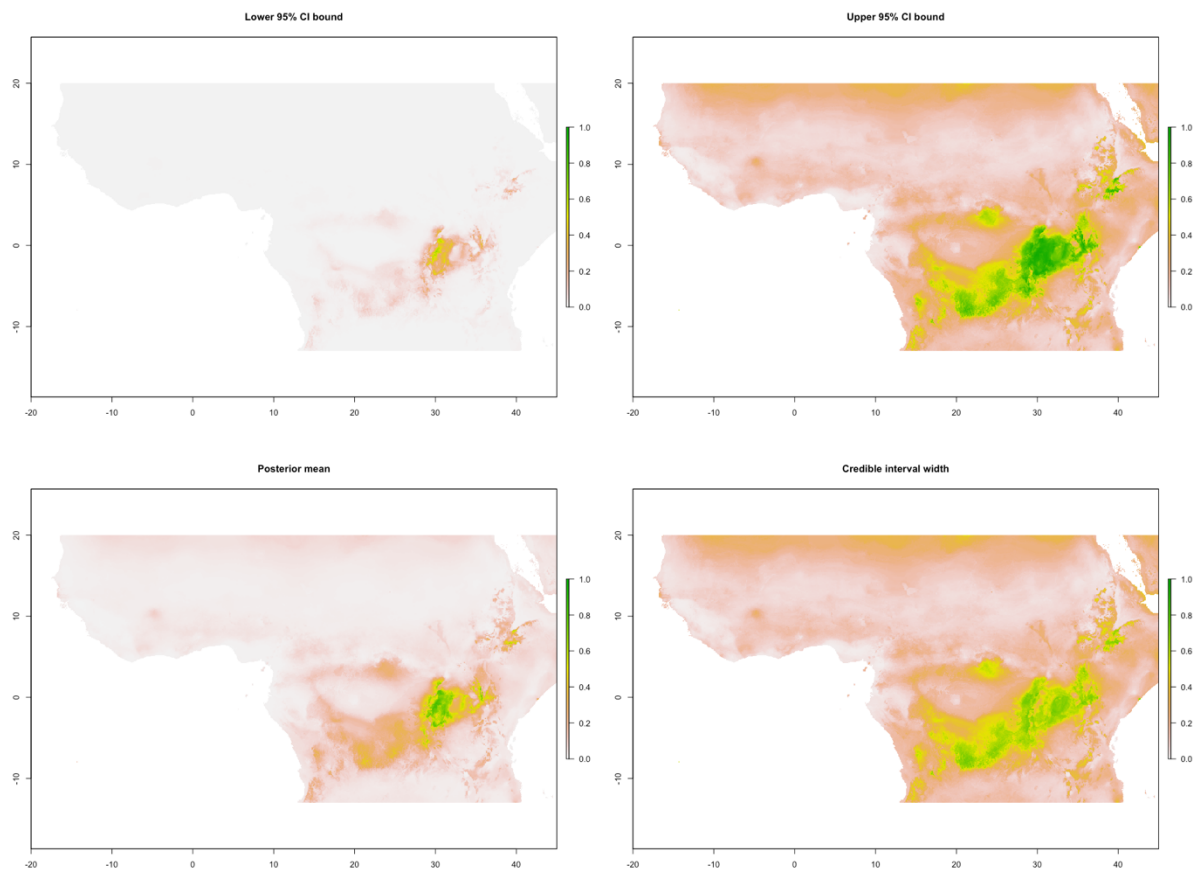
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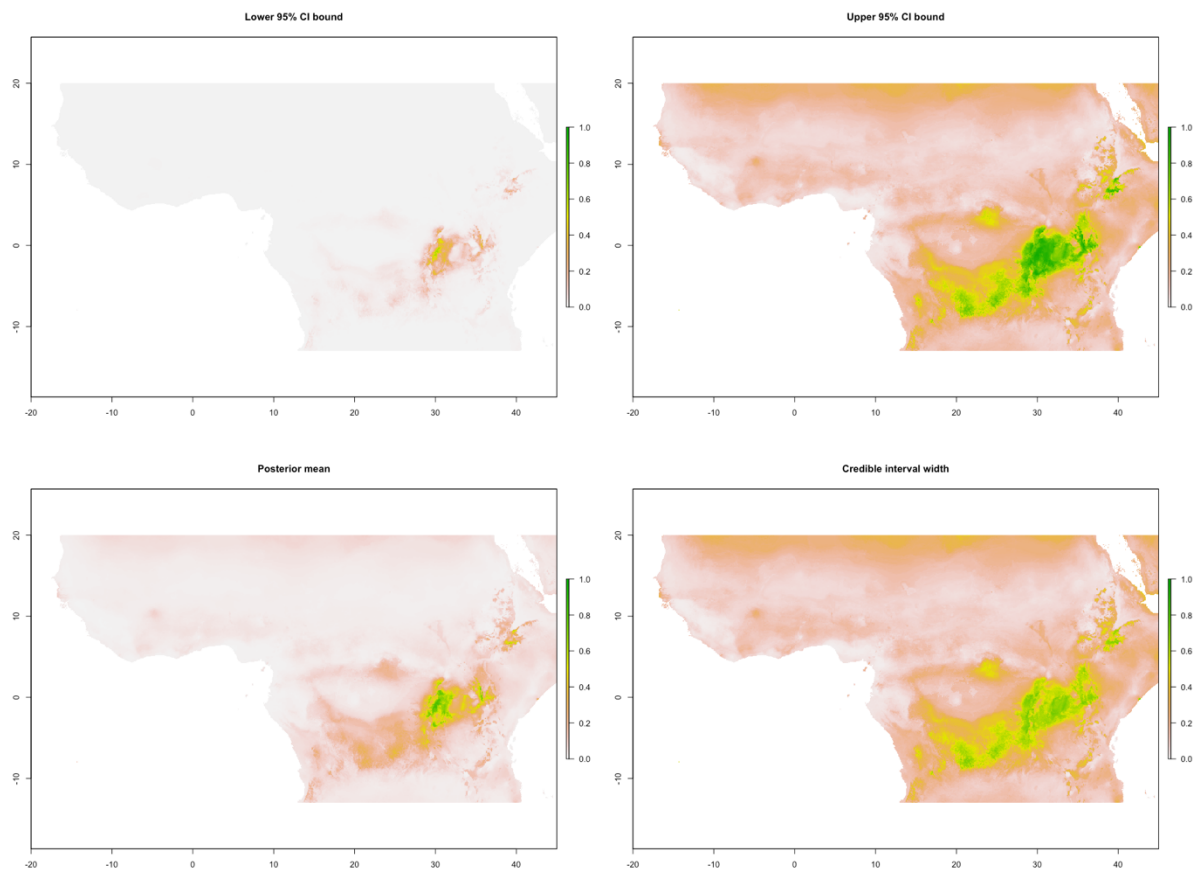
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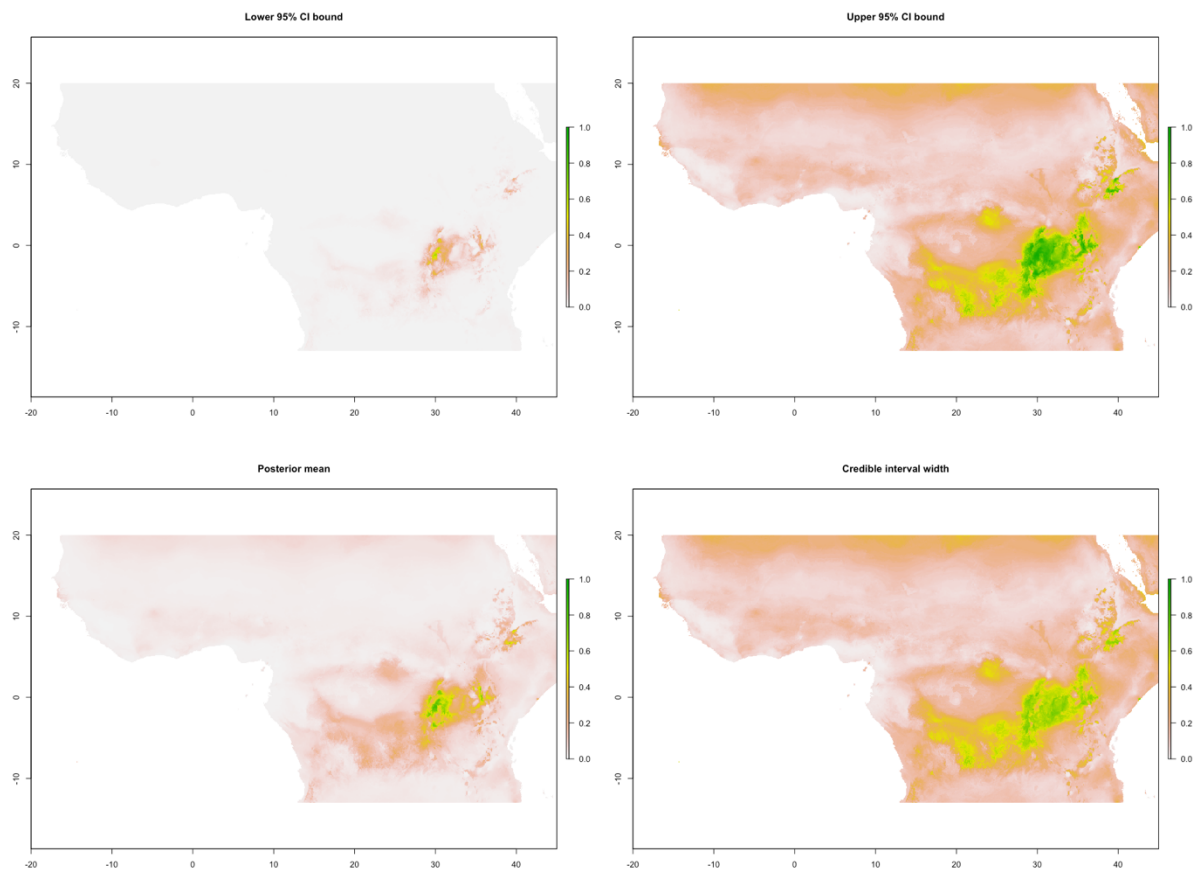
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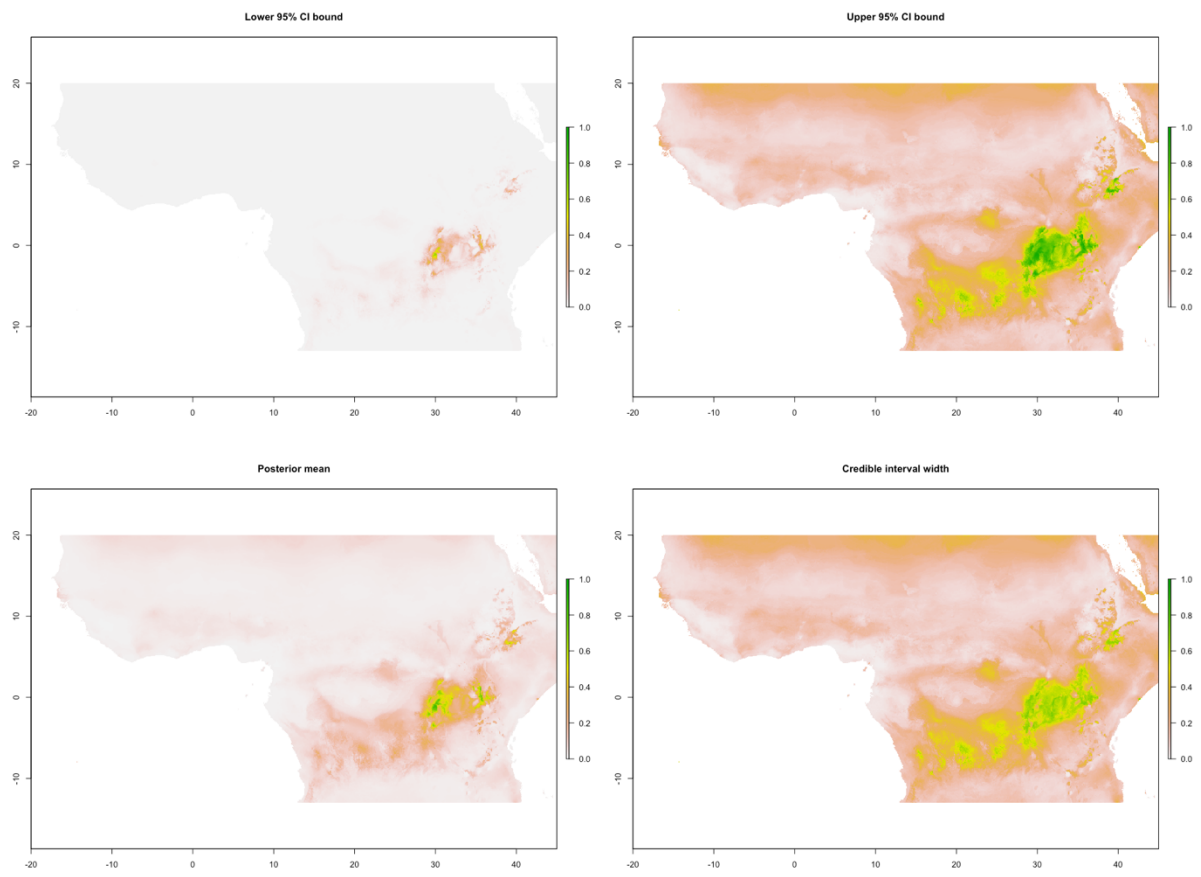
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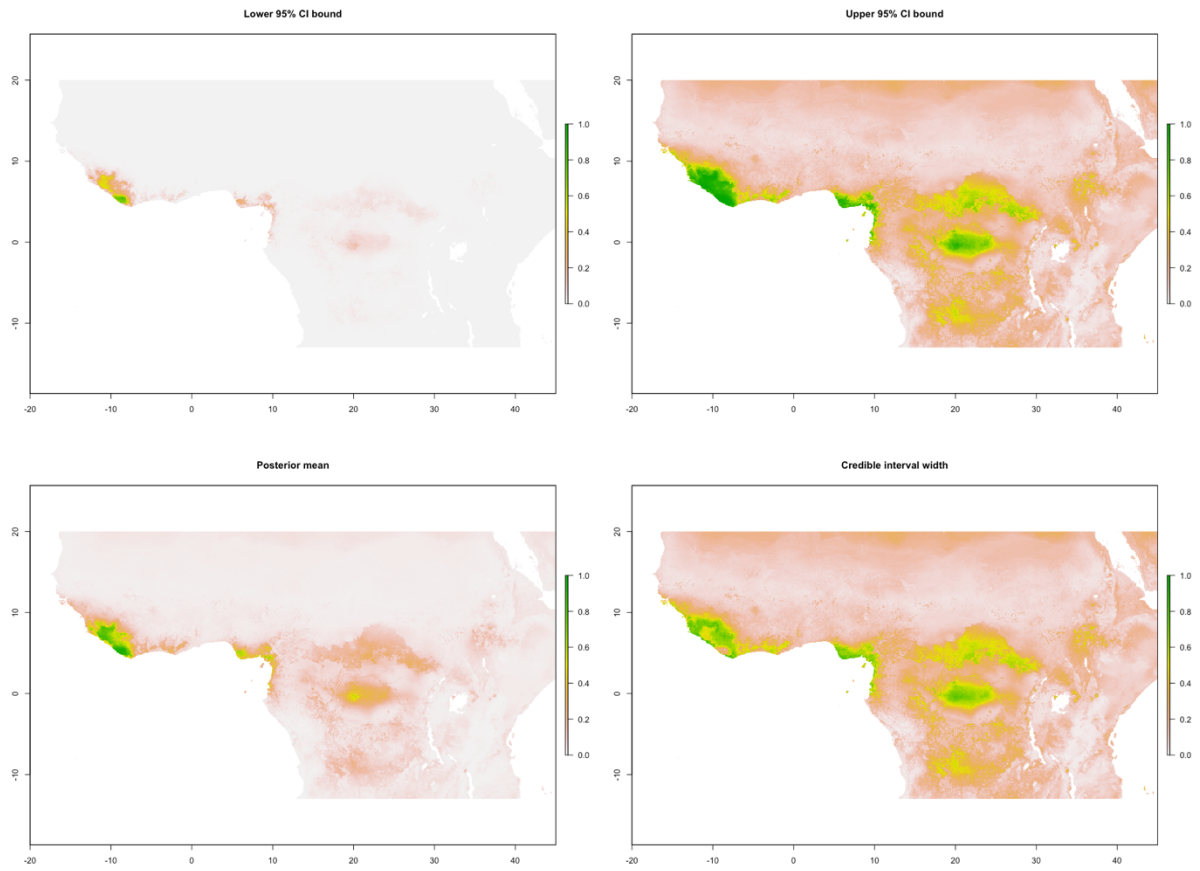


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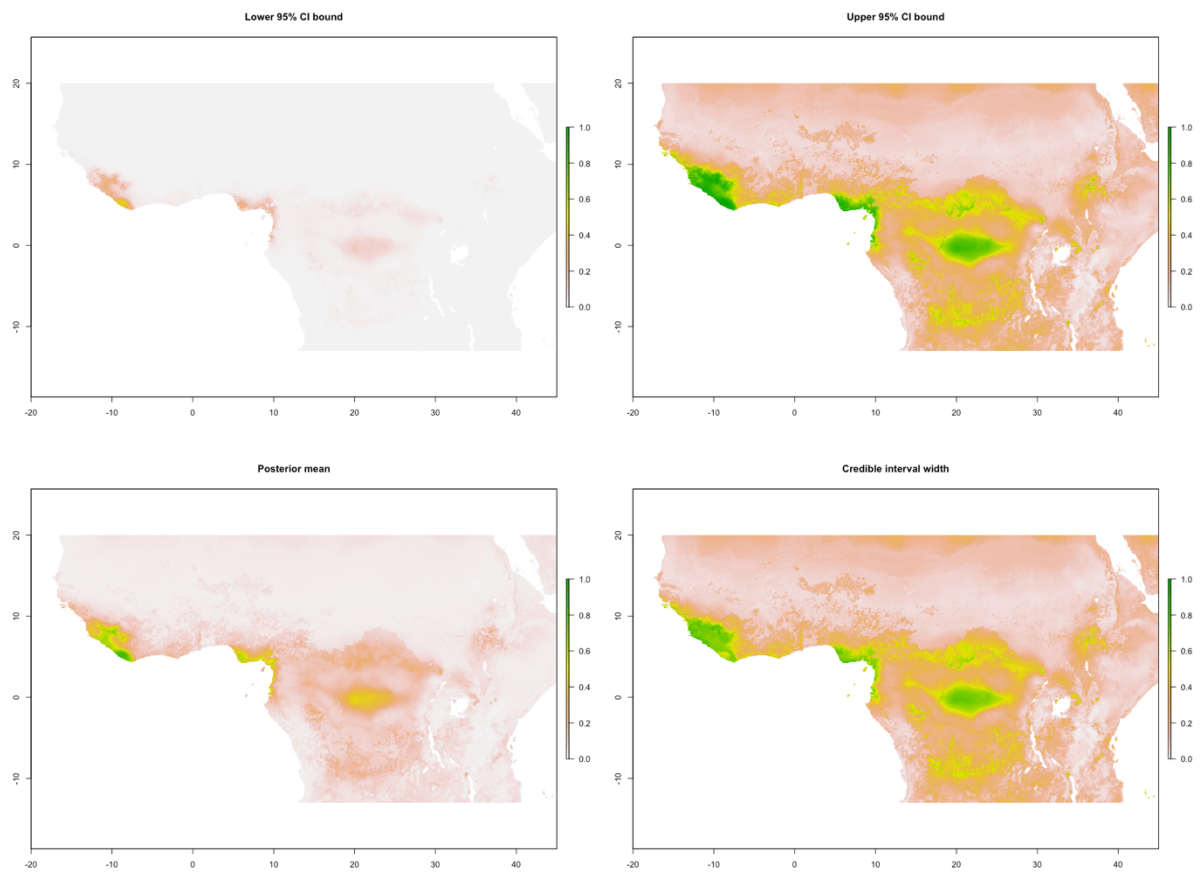


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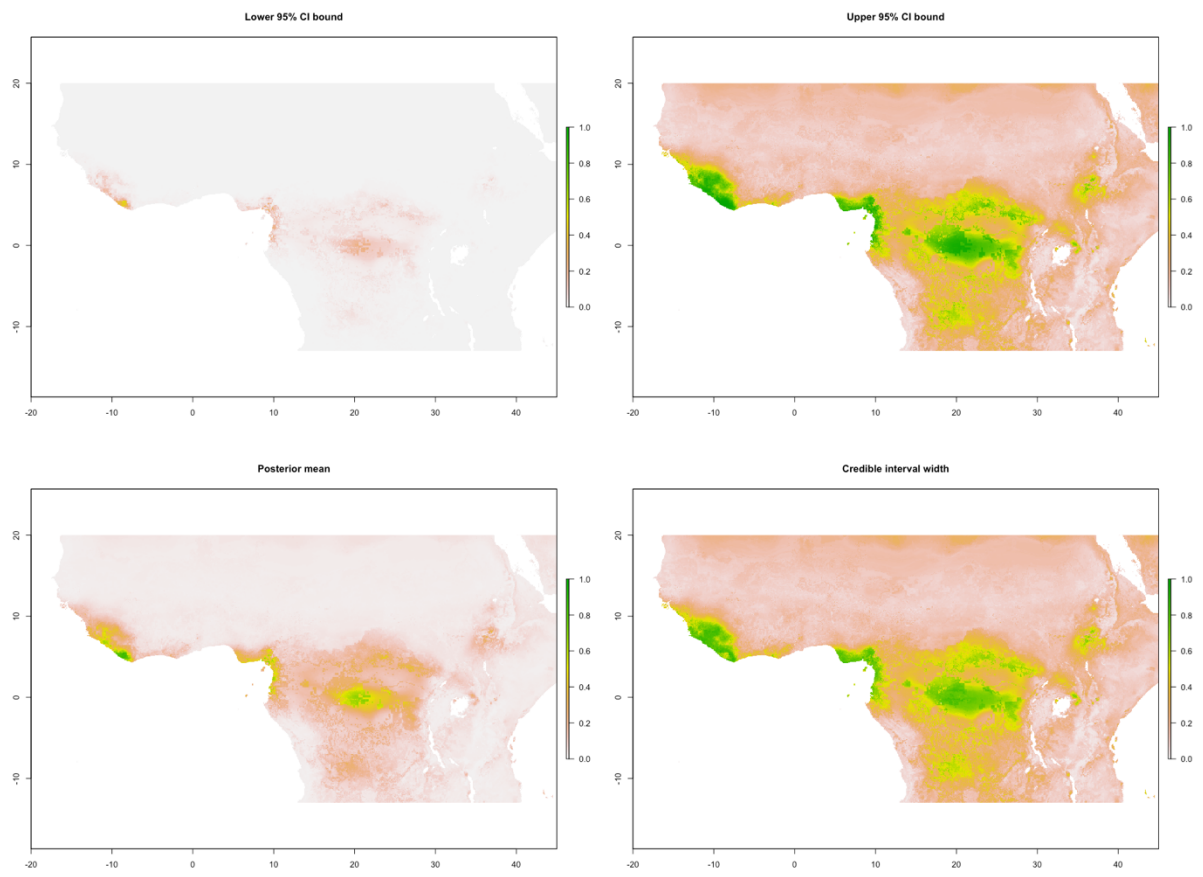
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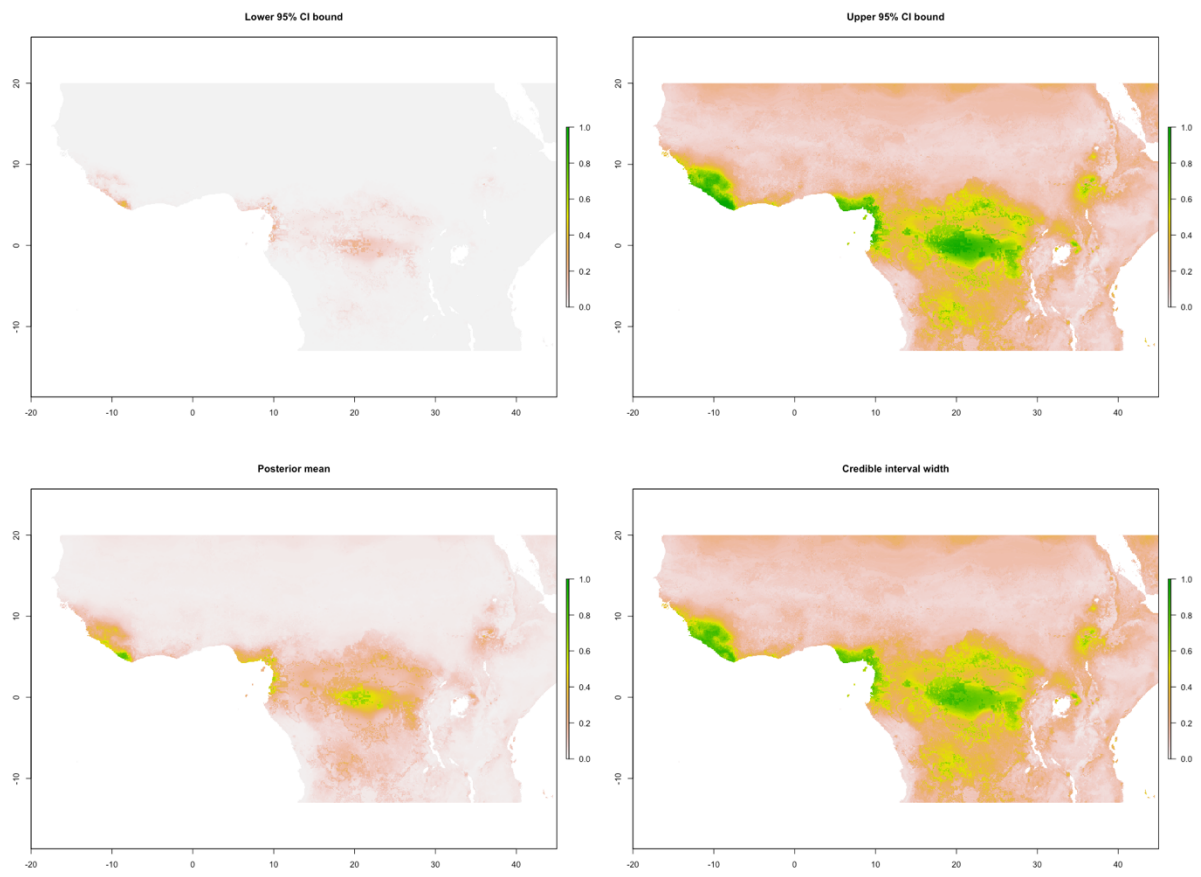
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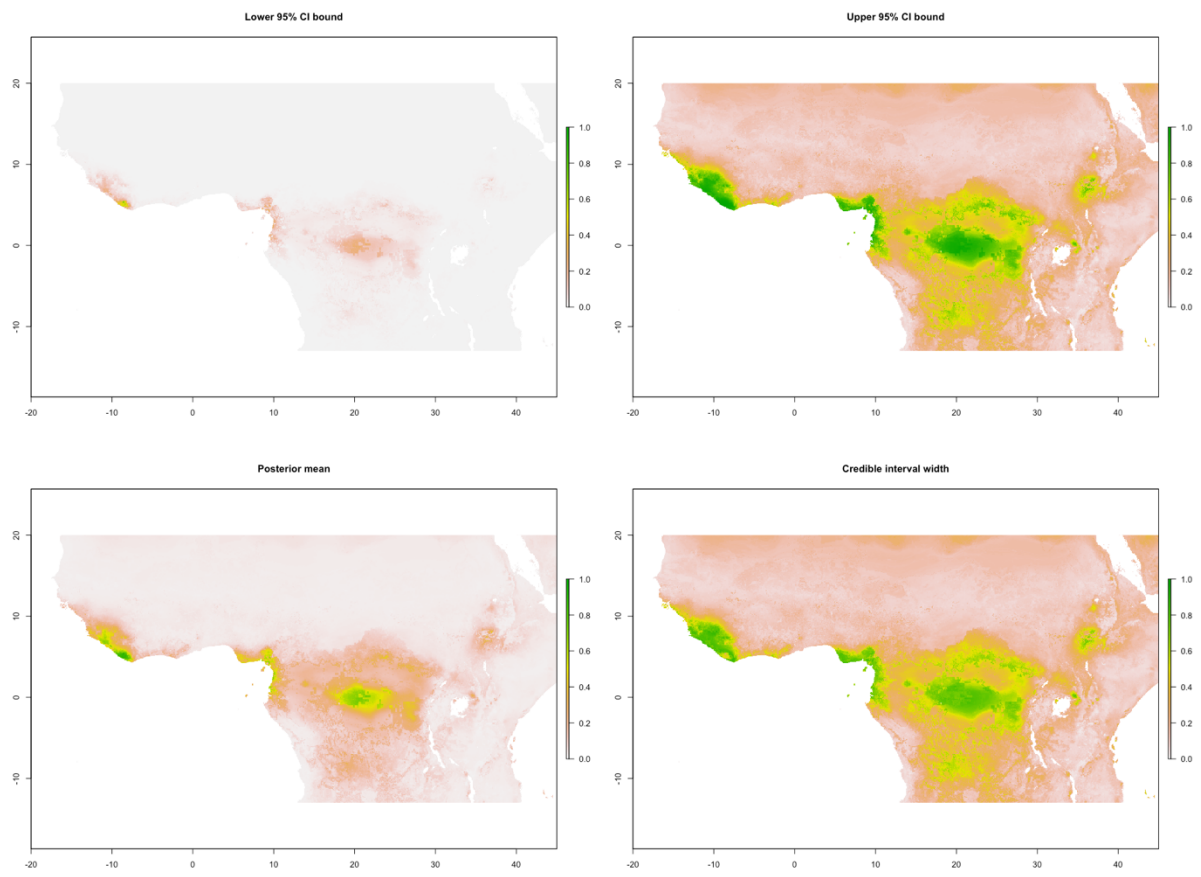
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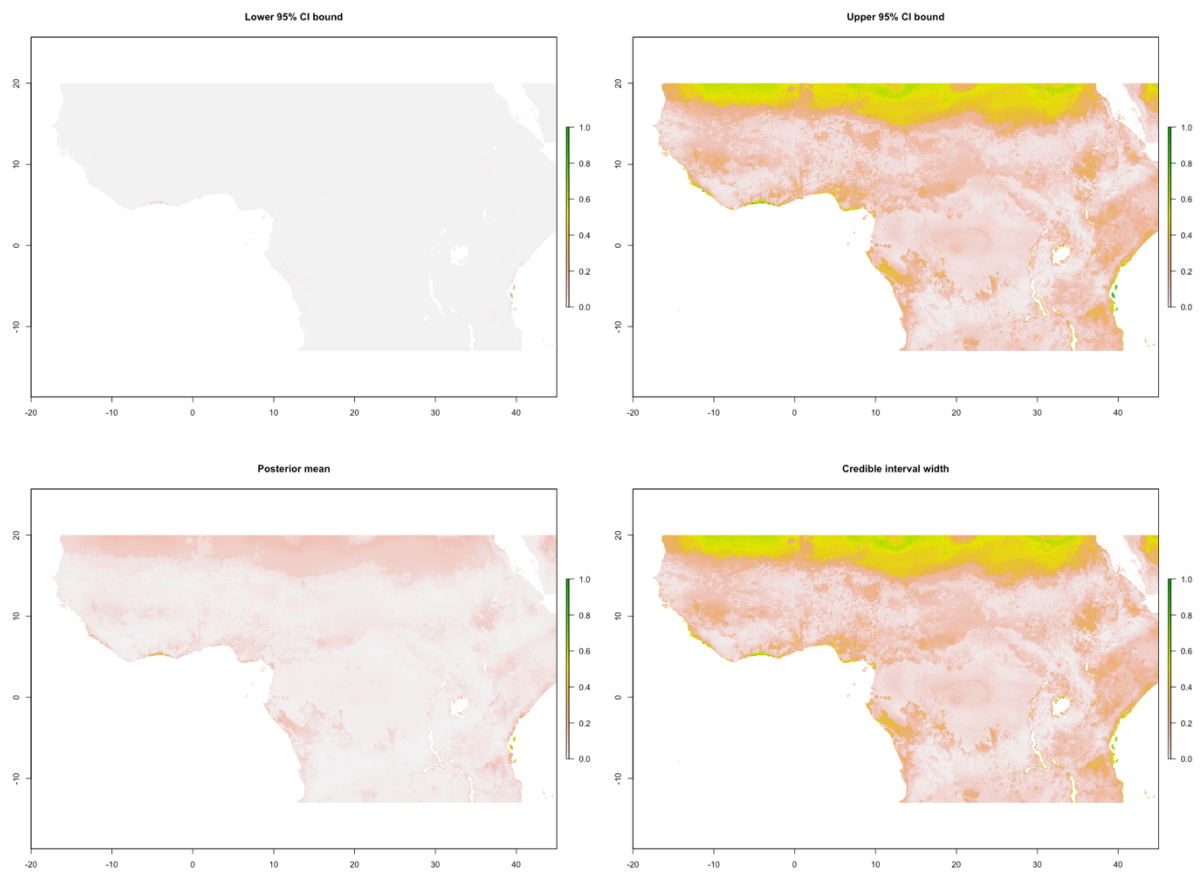
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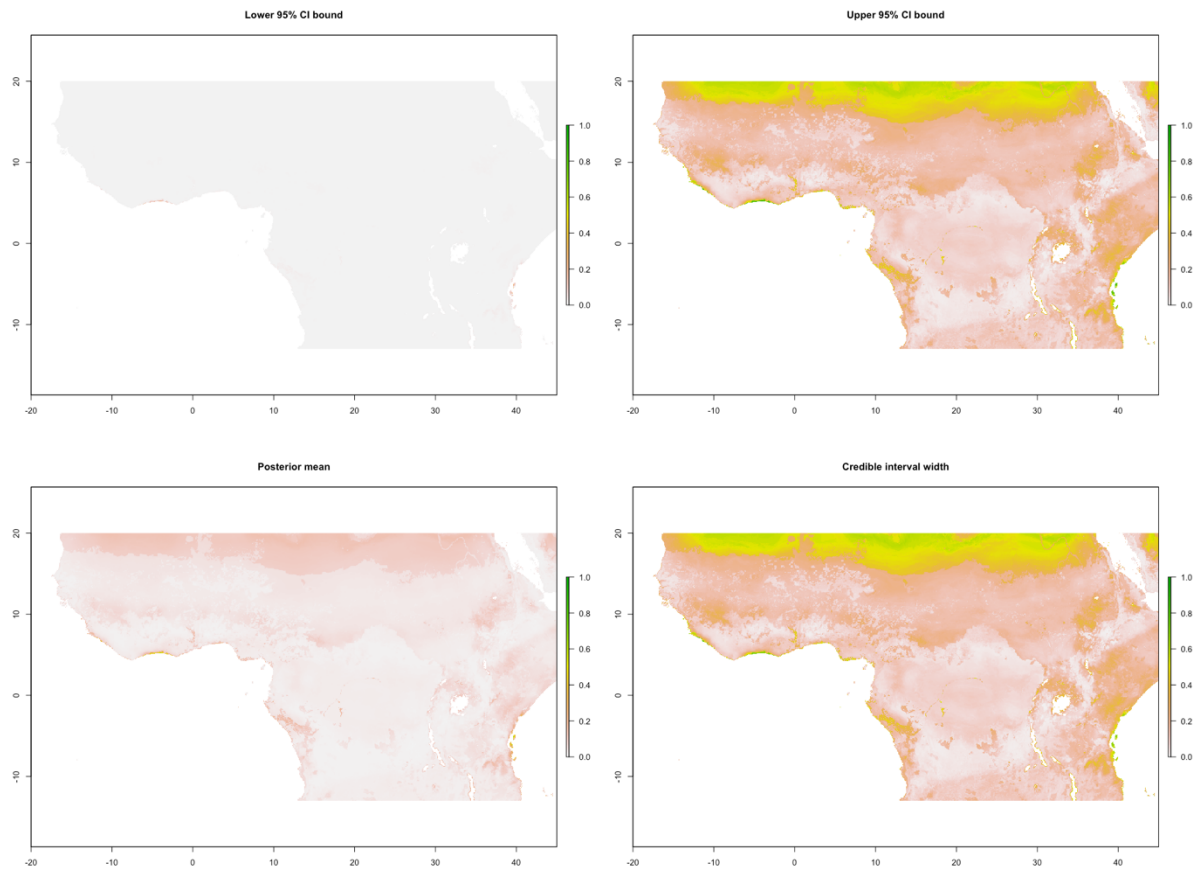
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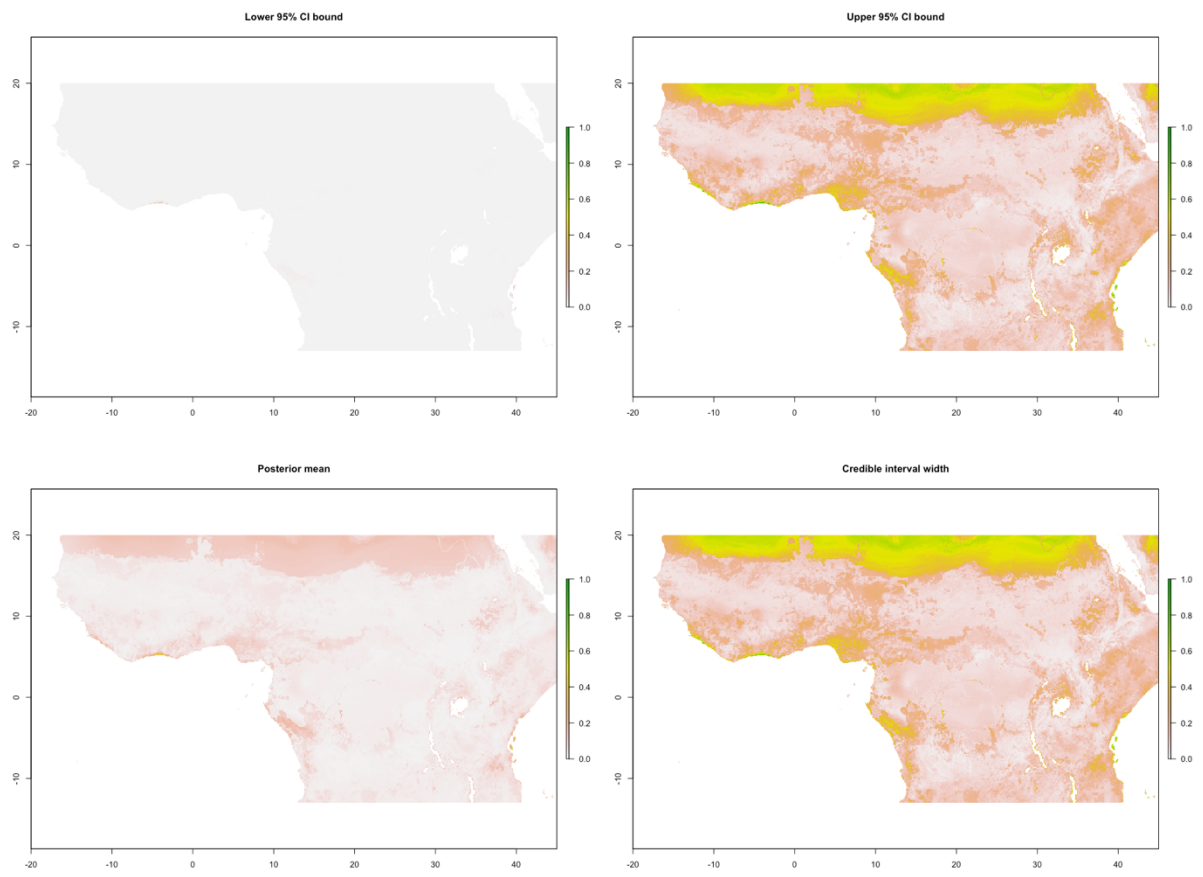
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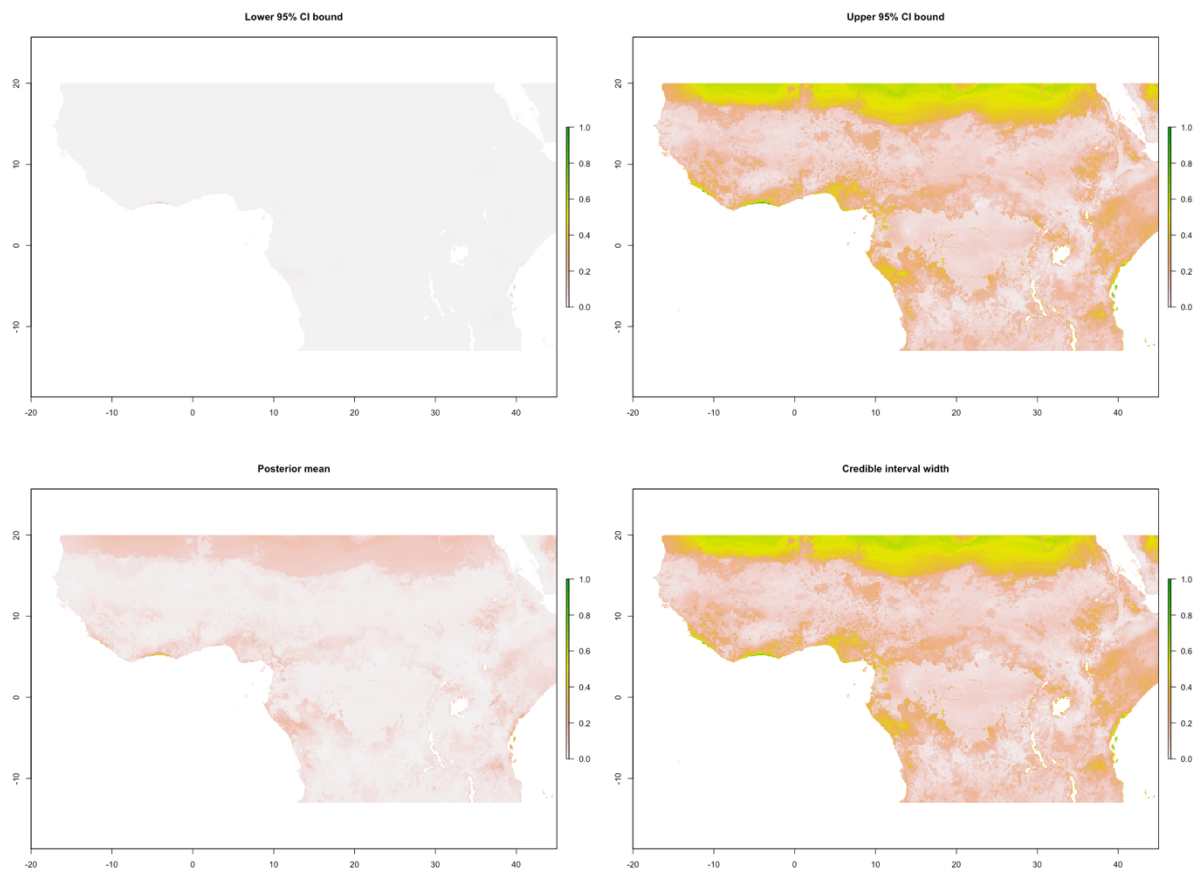
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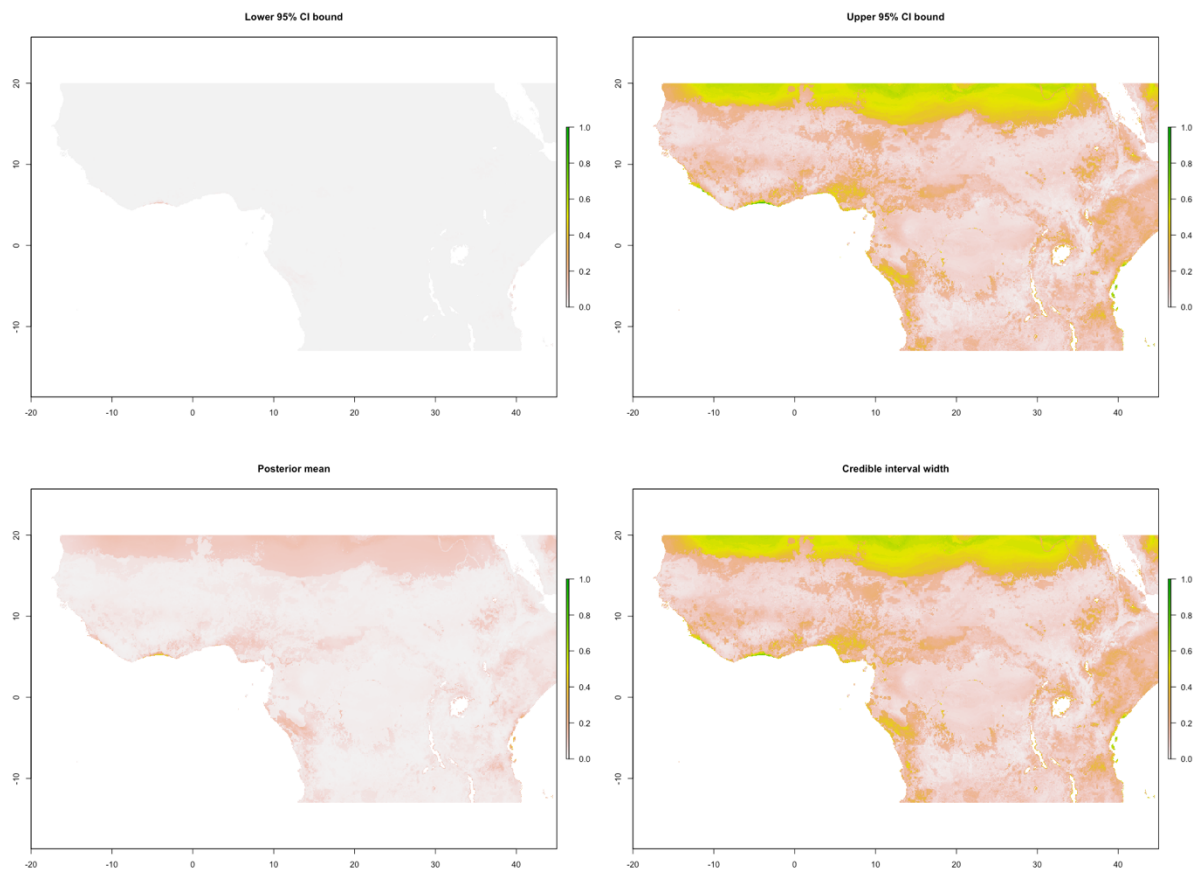
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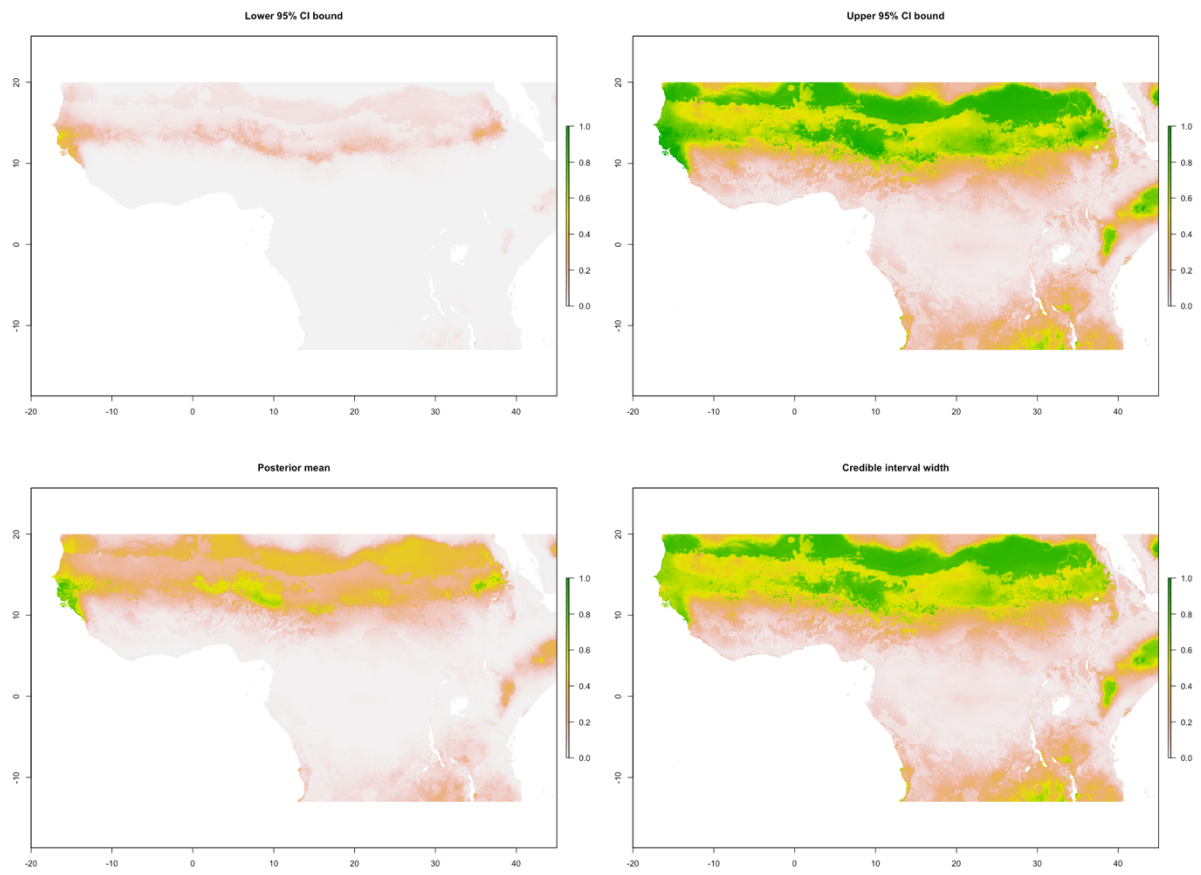
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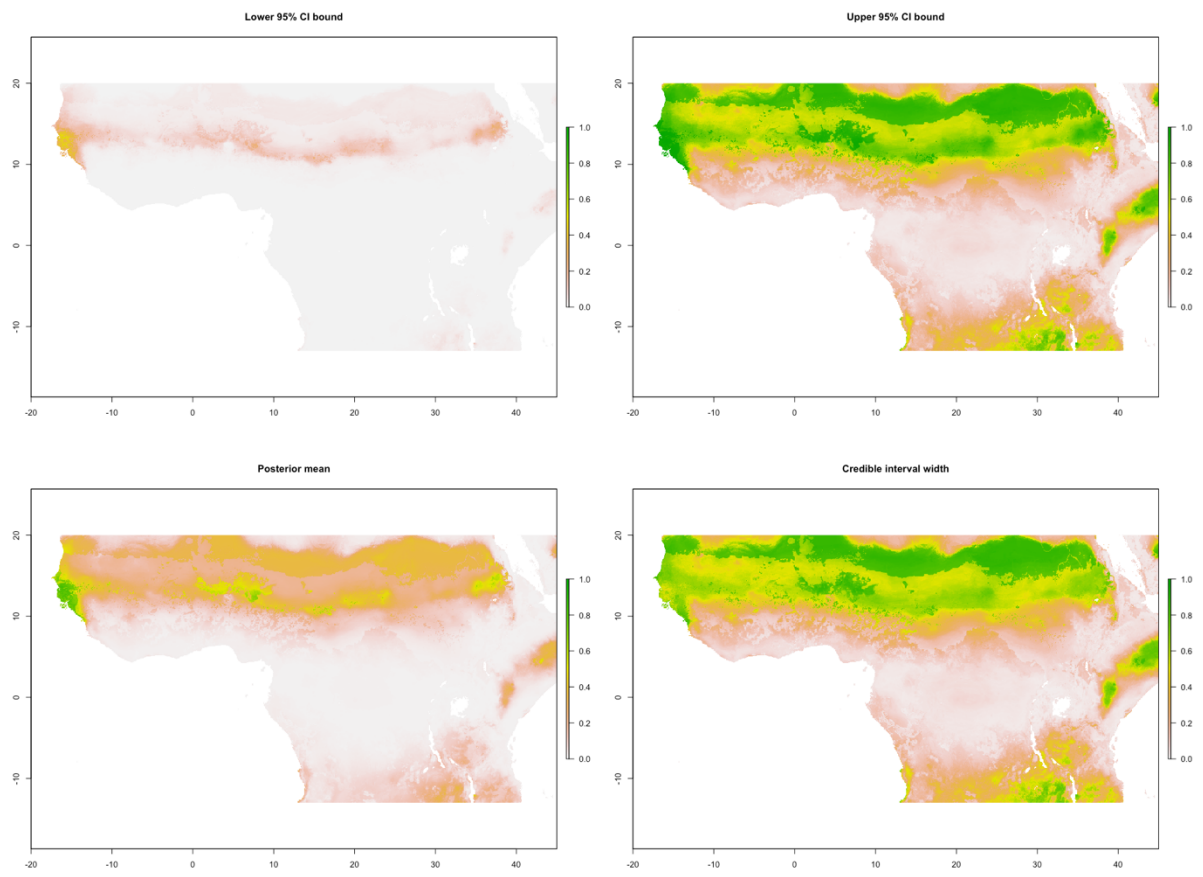
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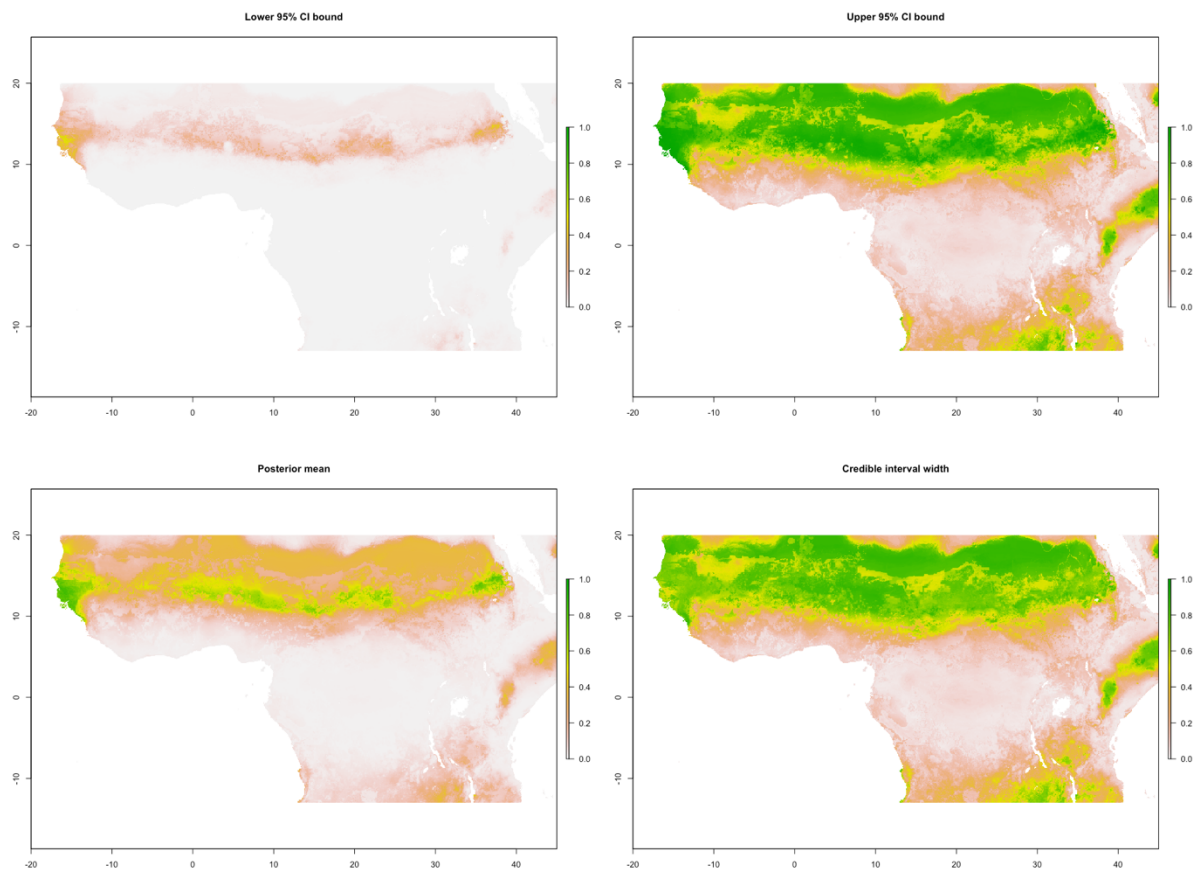
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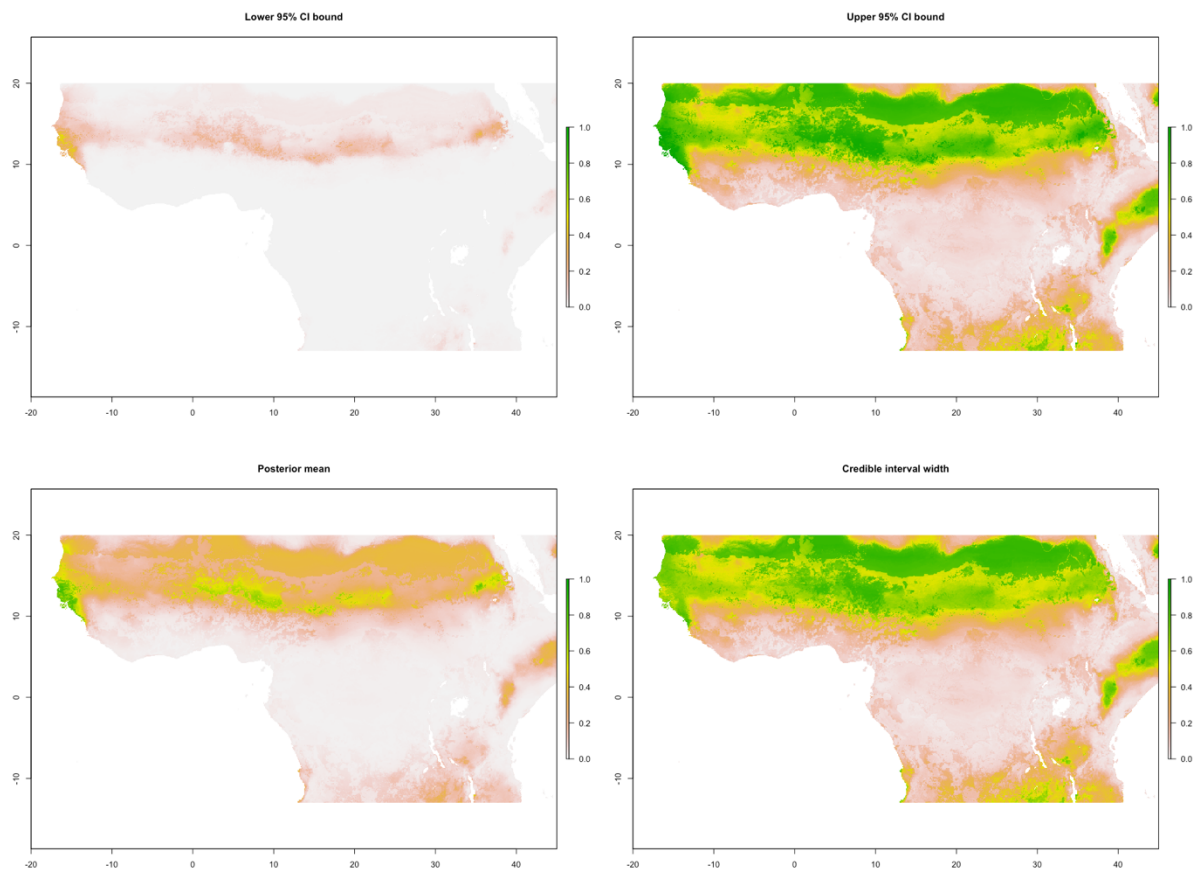
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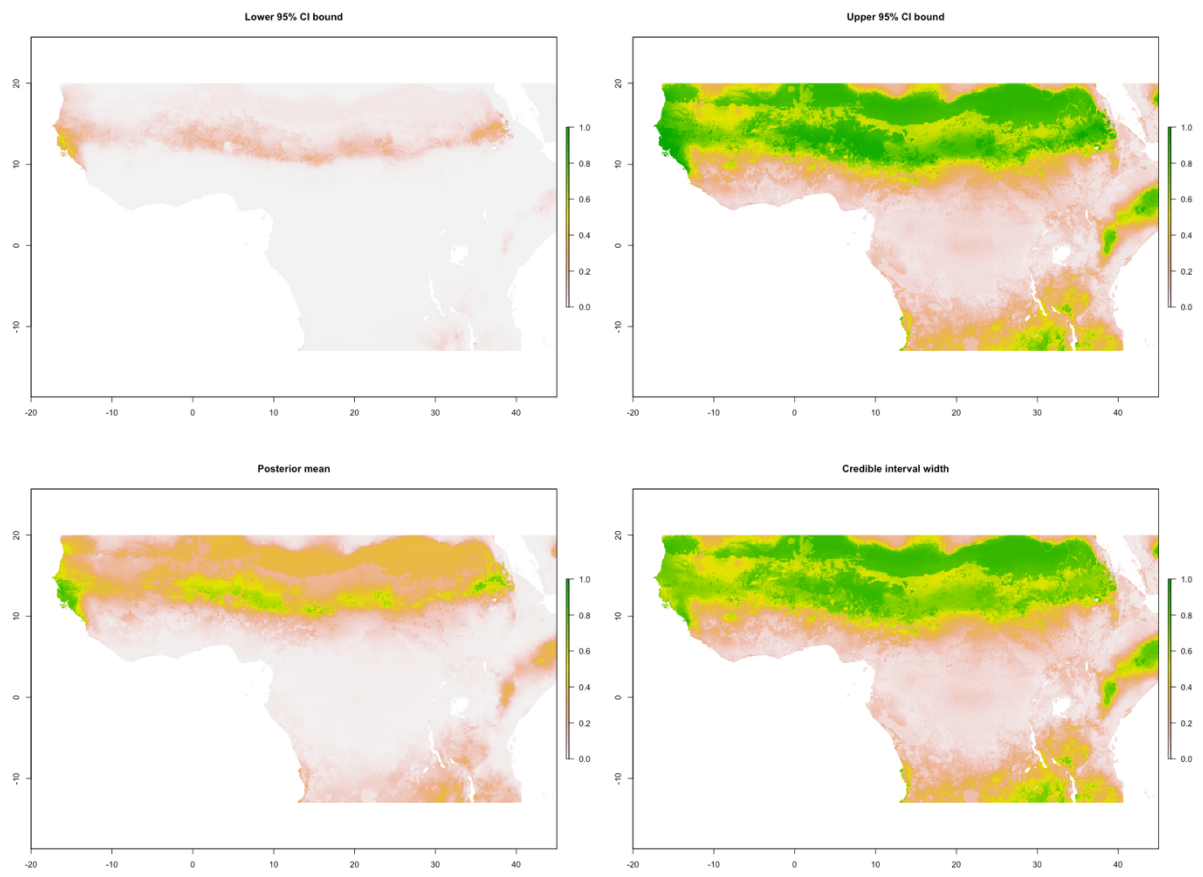
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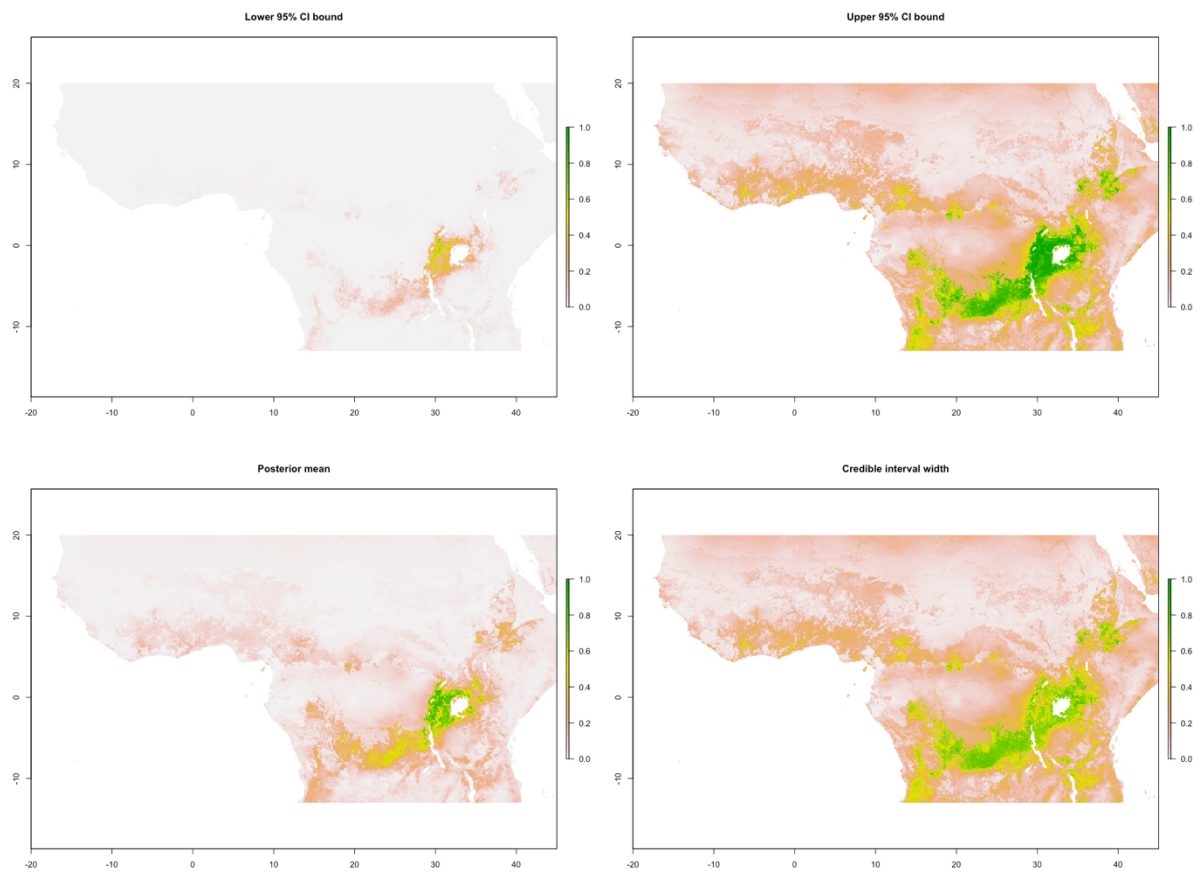
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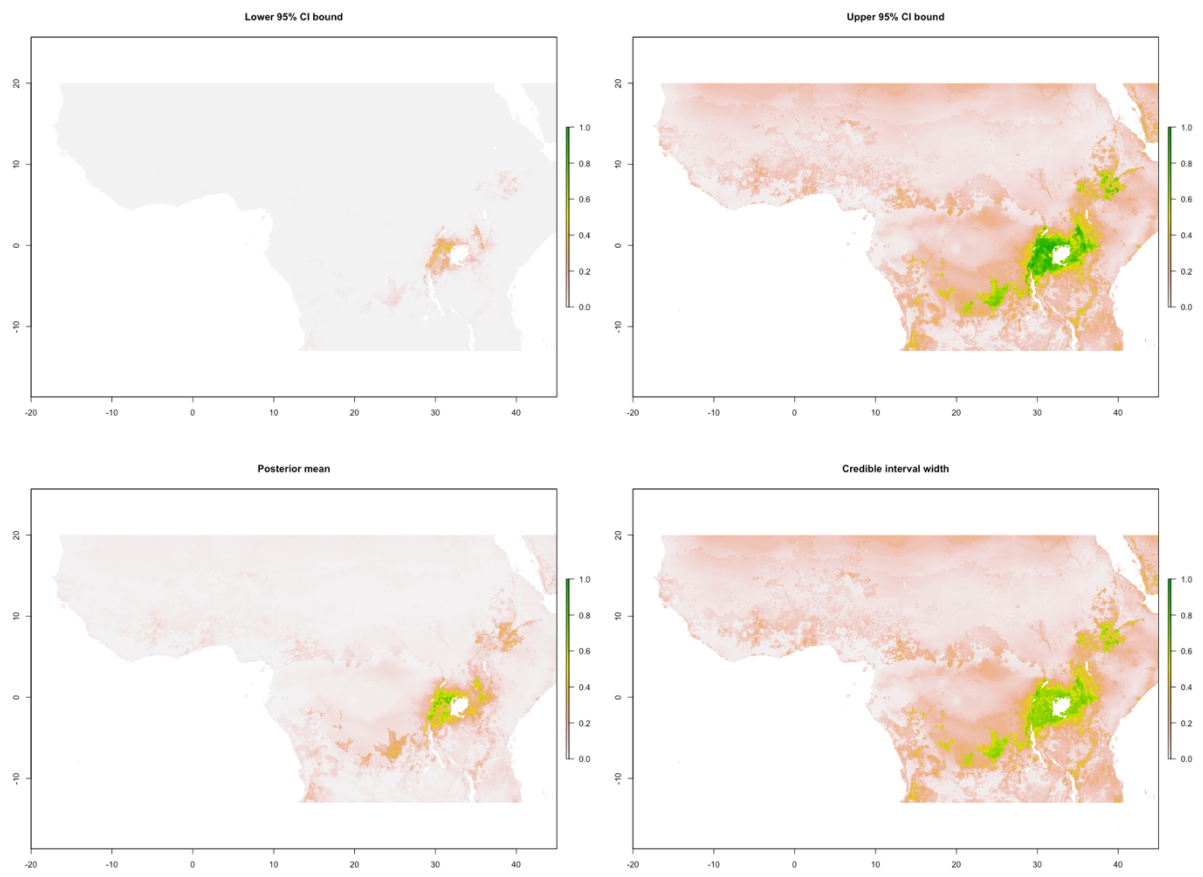
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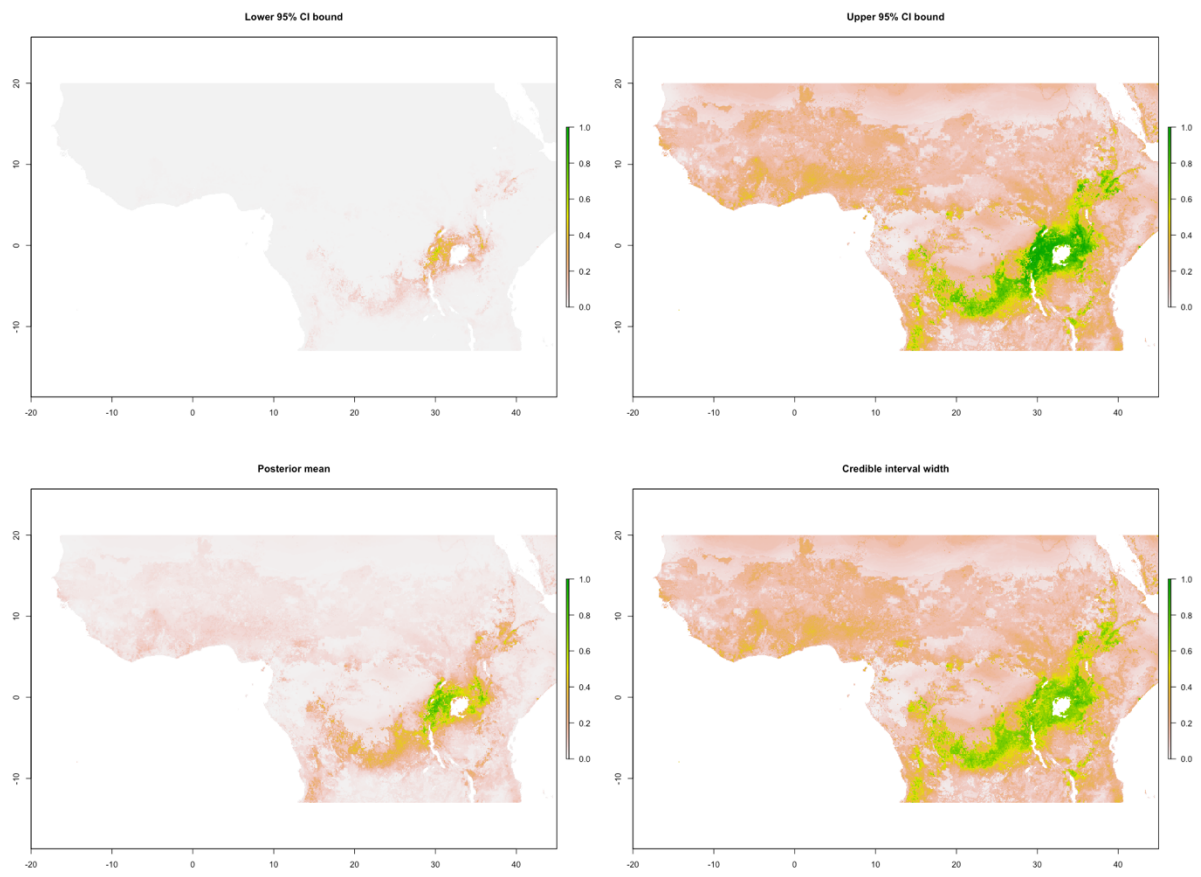
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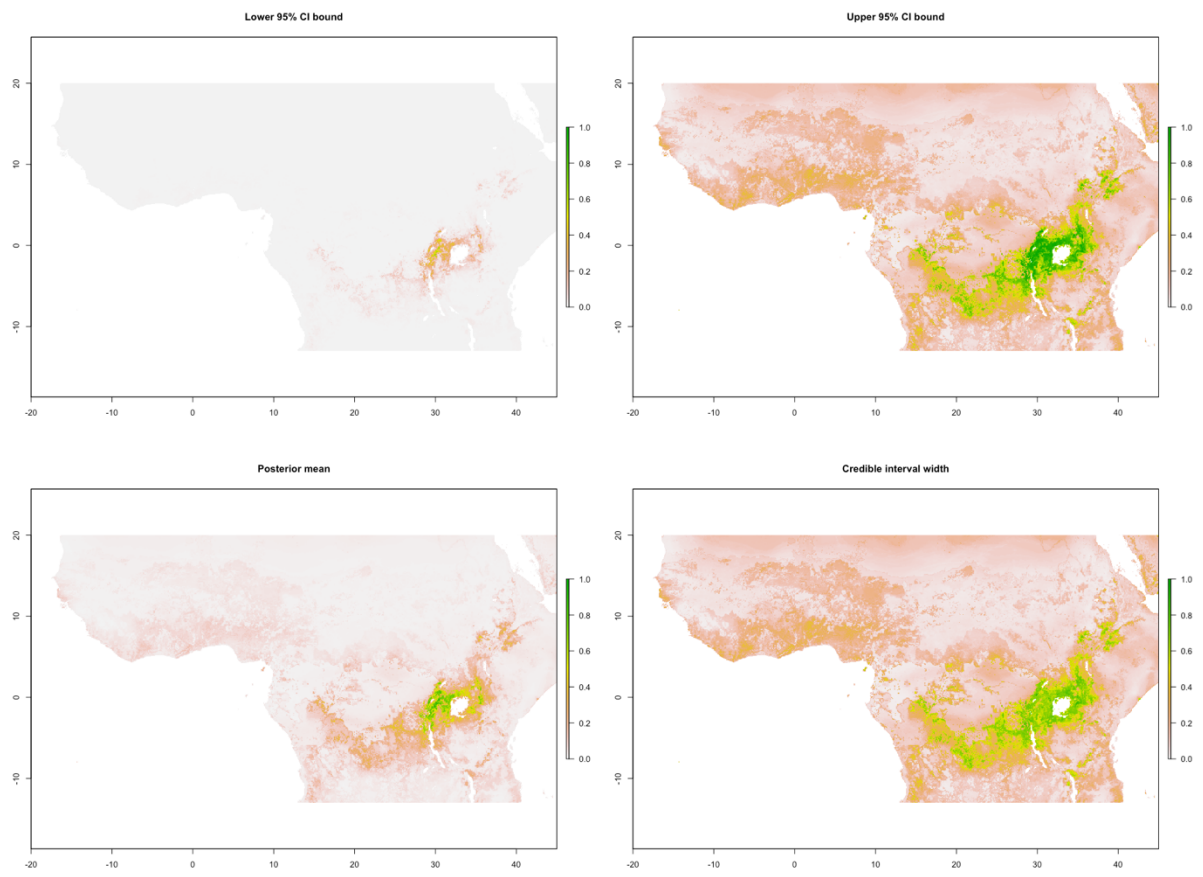
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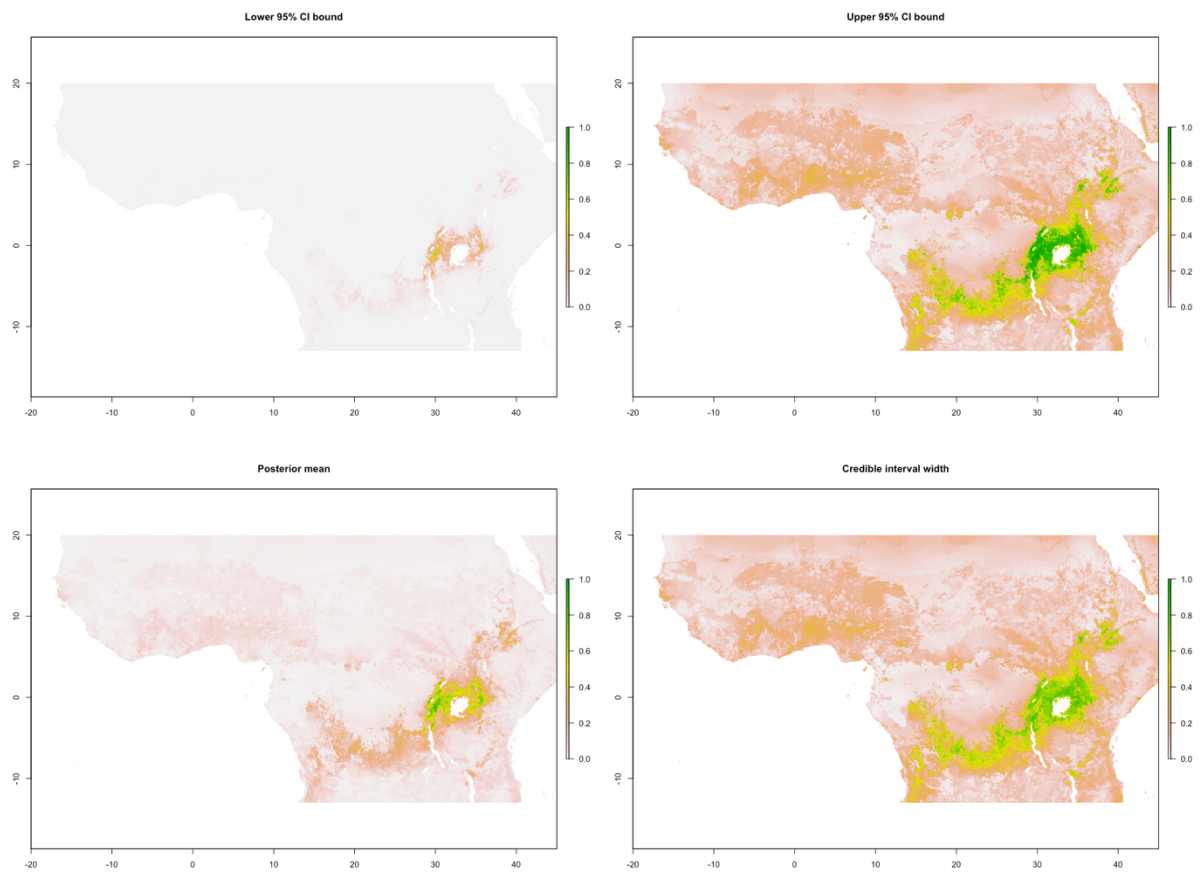
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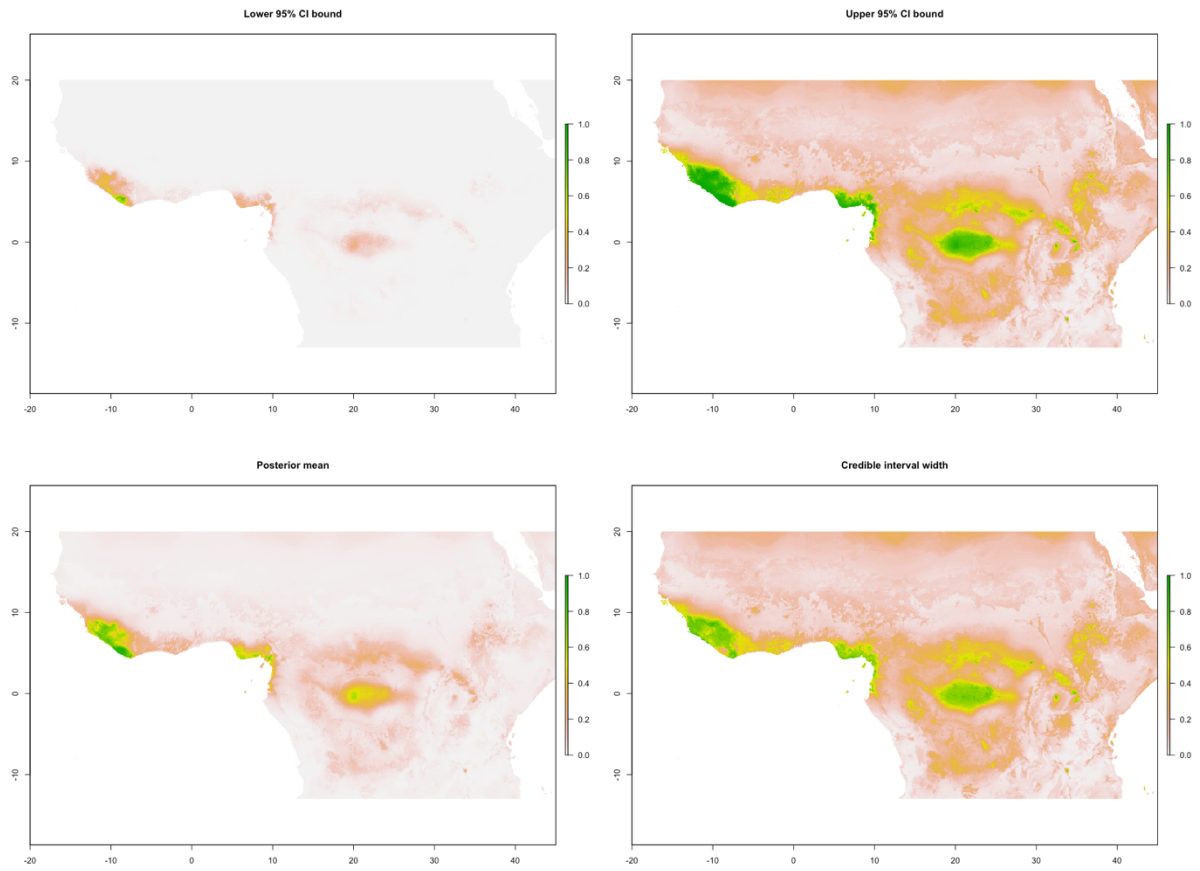


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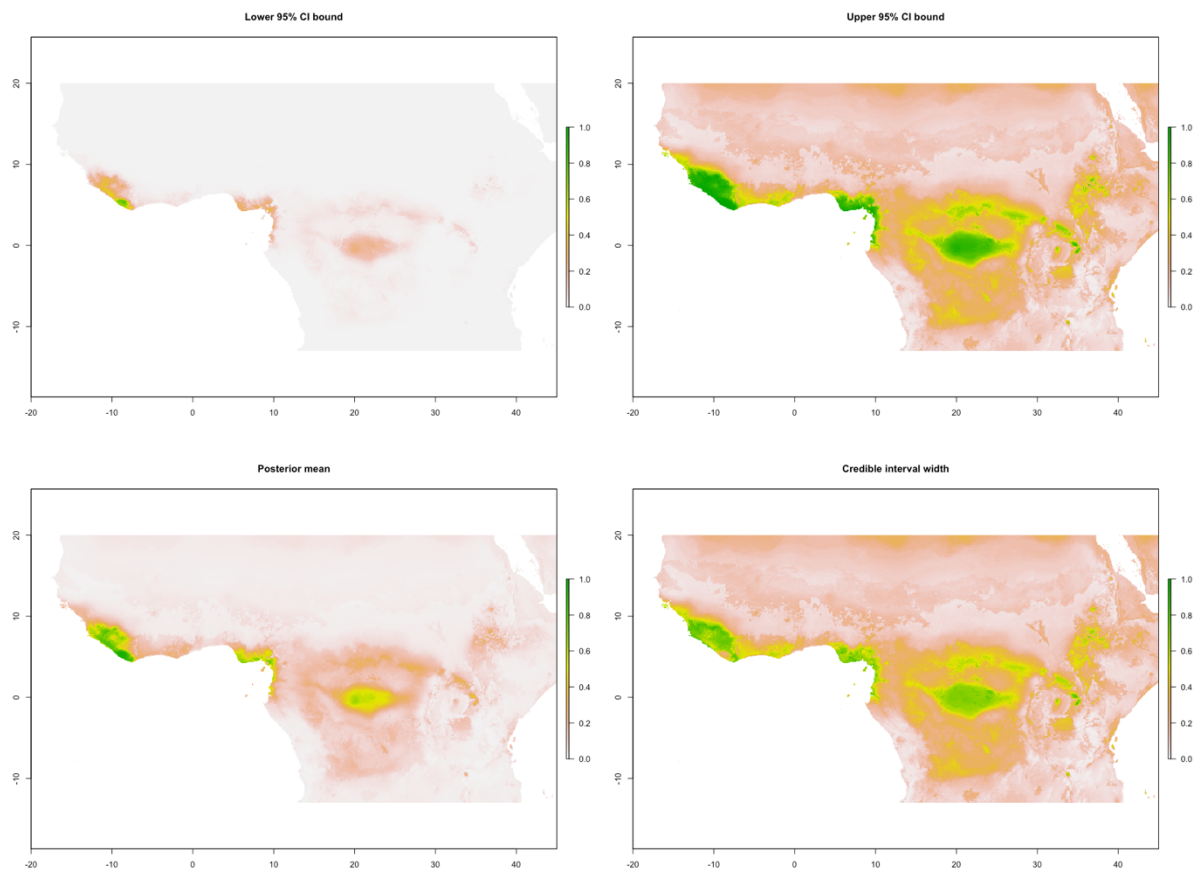


Chen and WorldClim models:

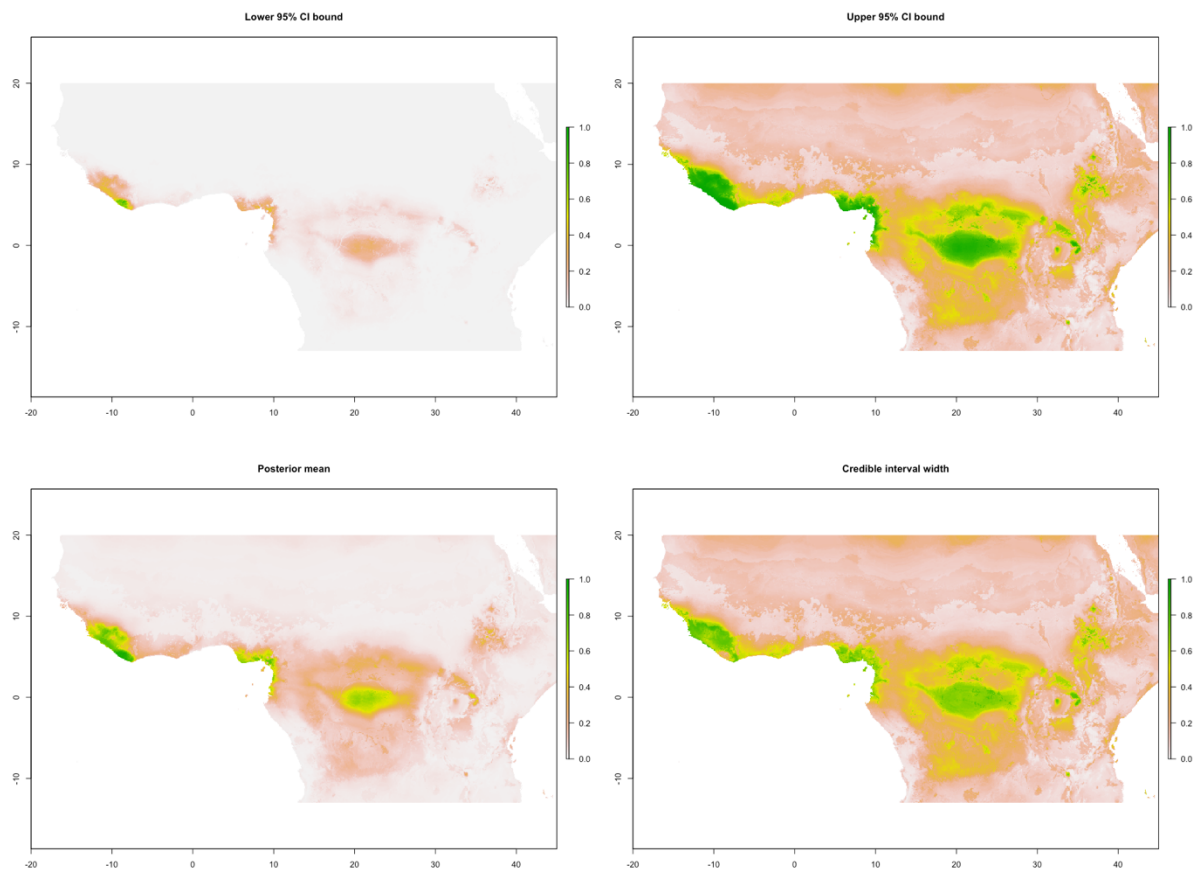
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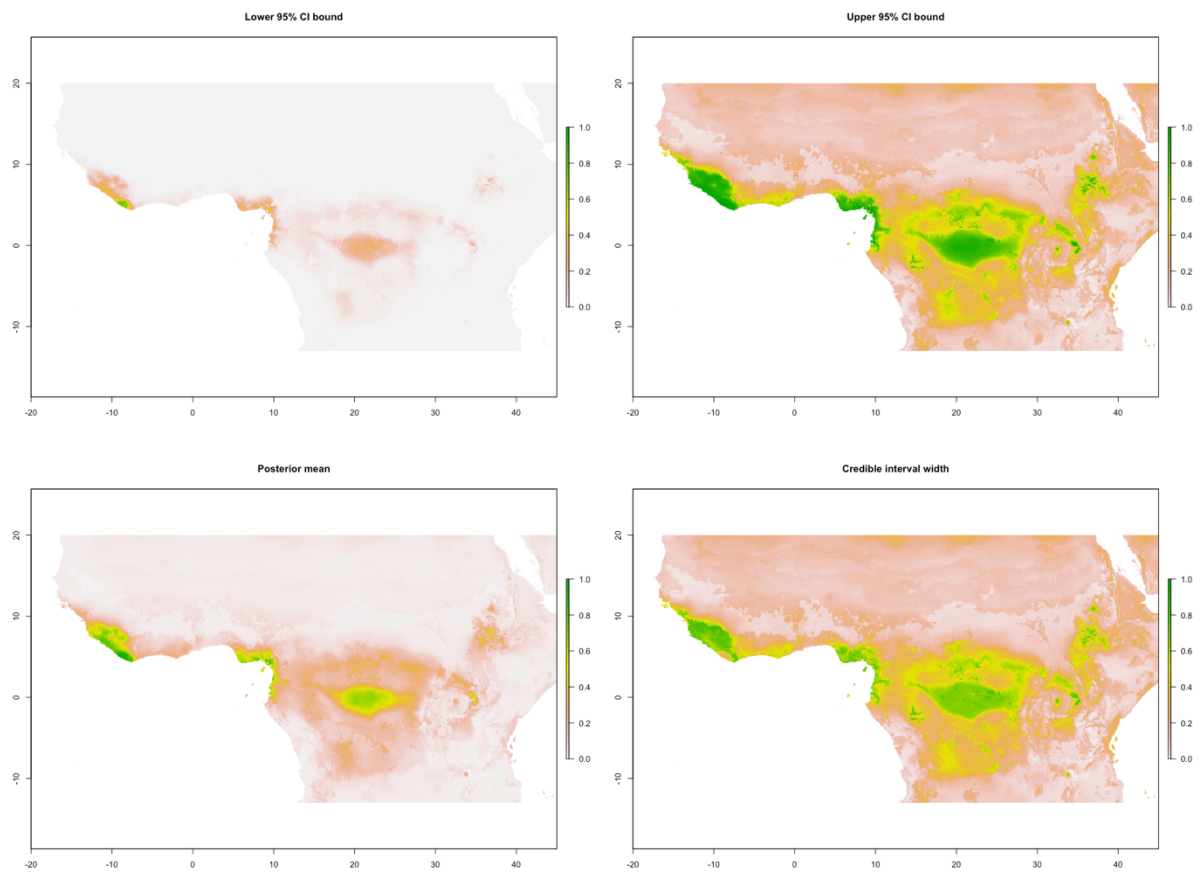
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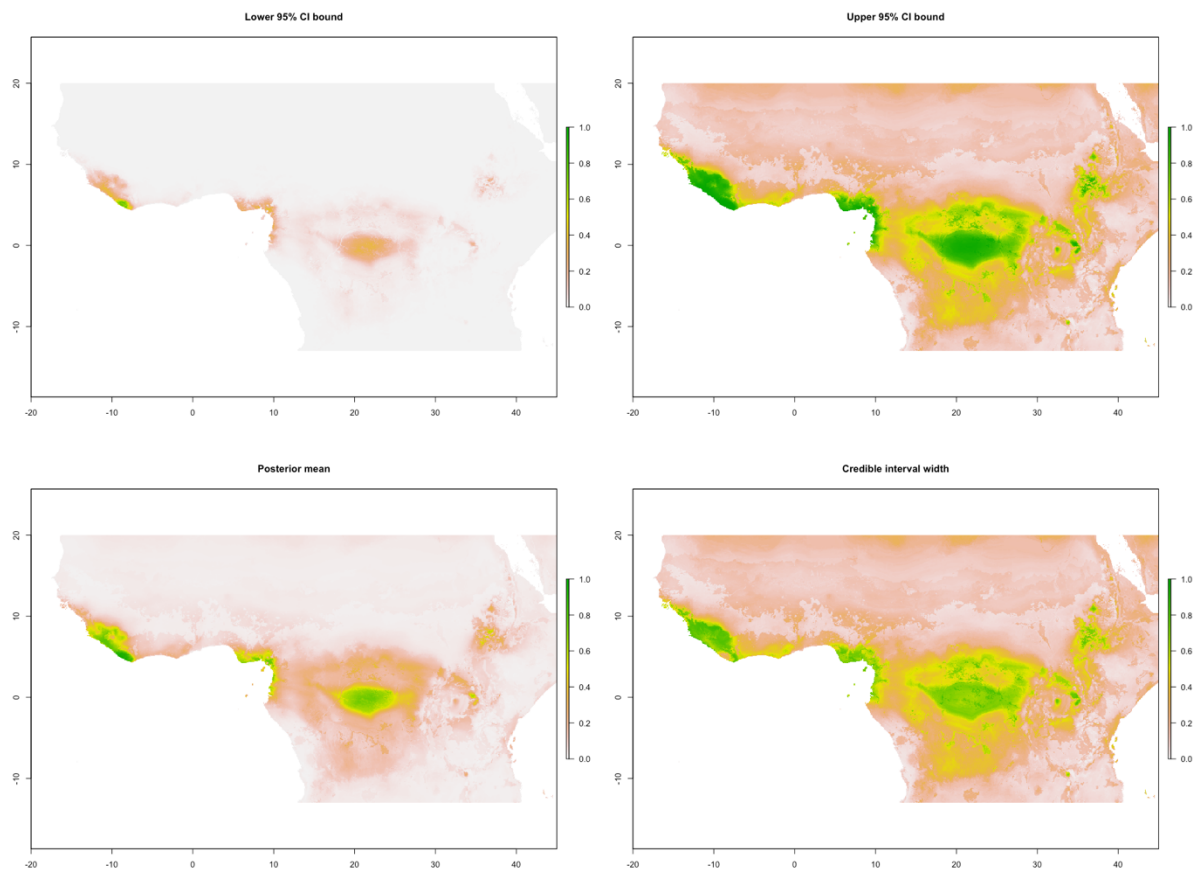
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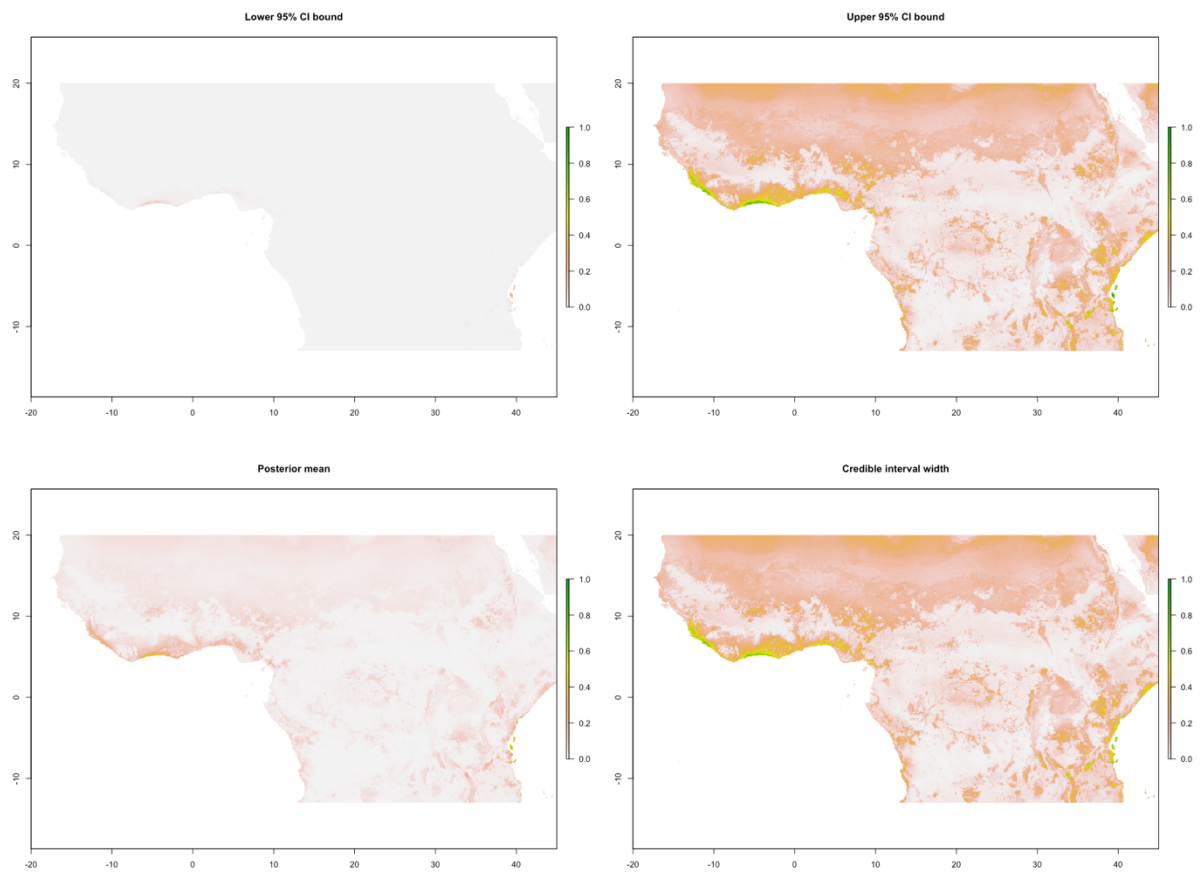
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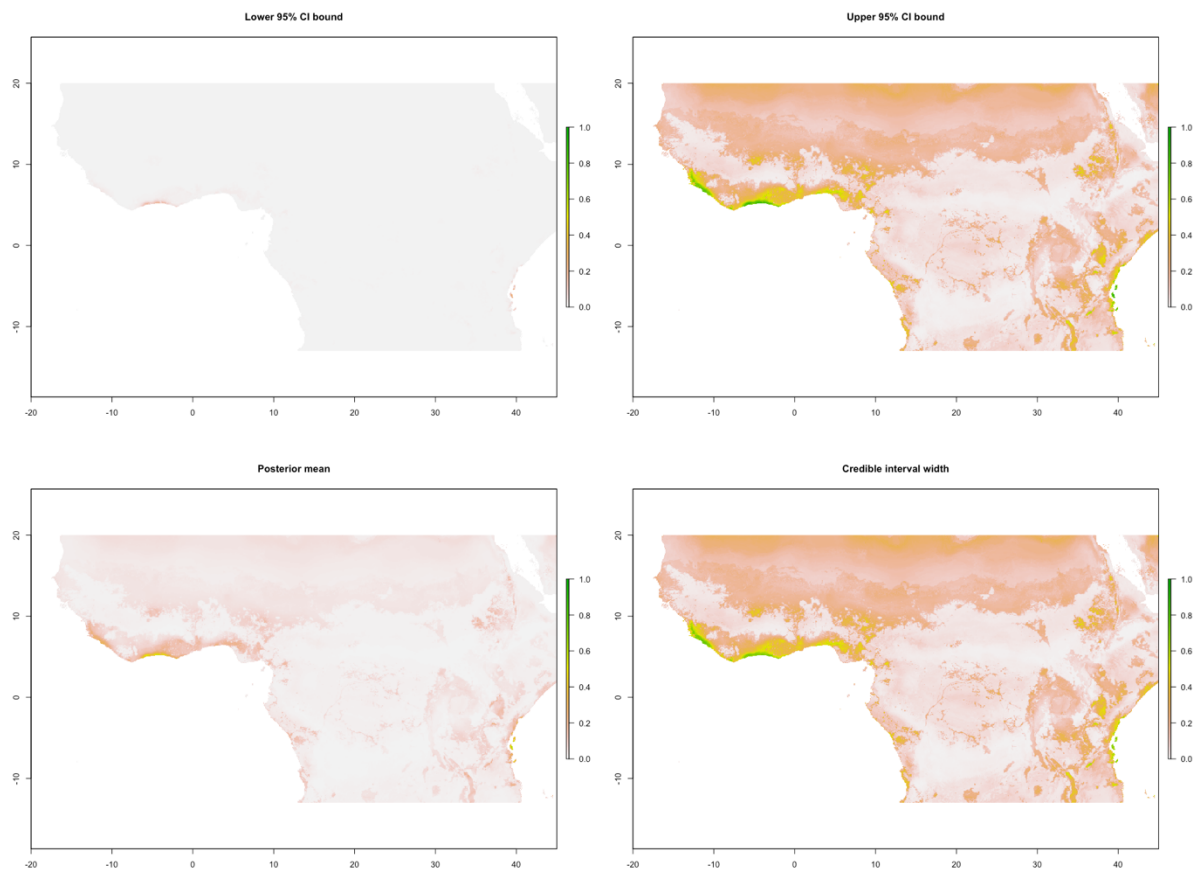
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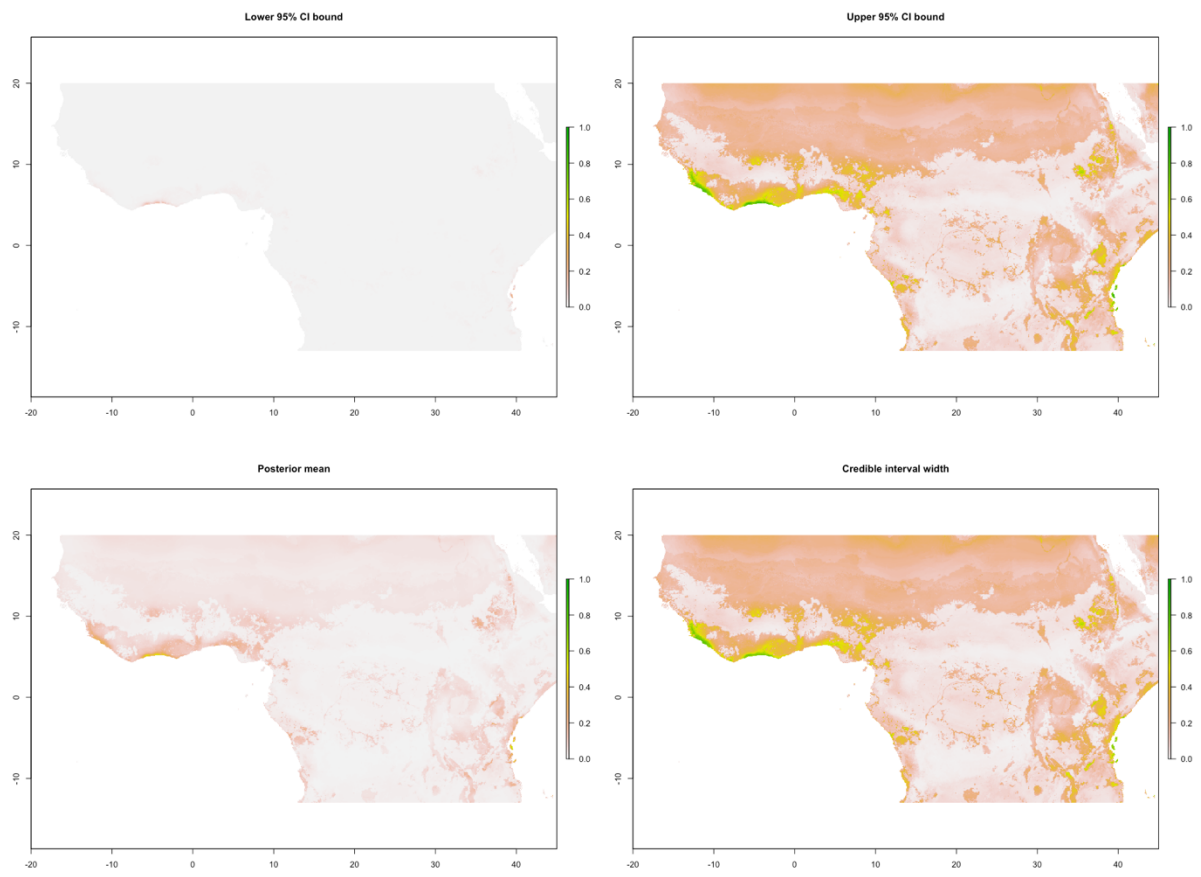
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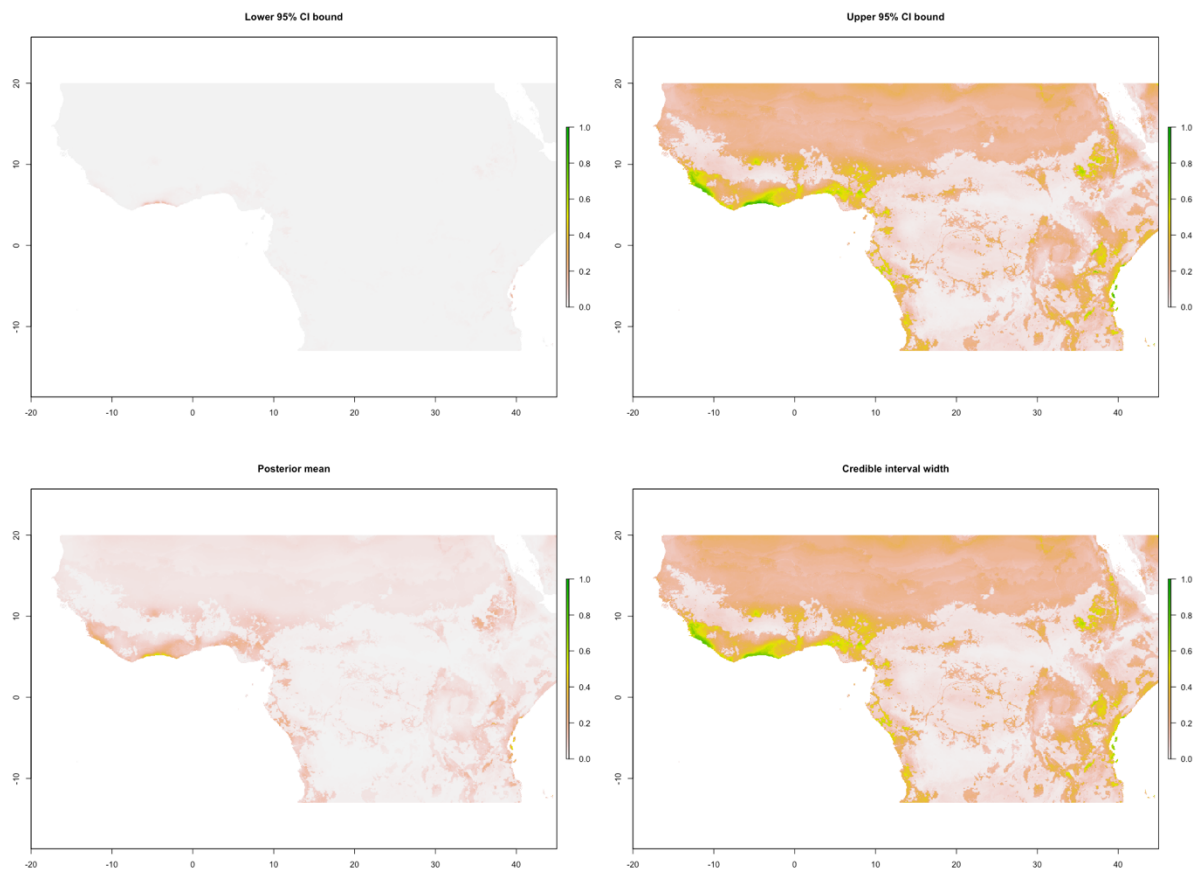
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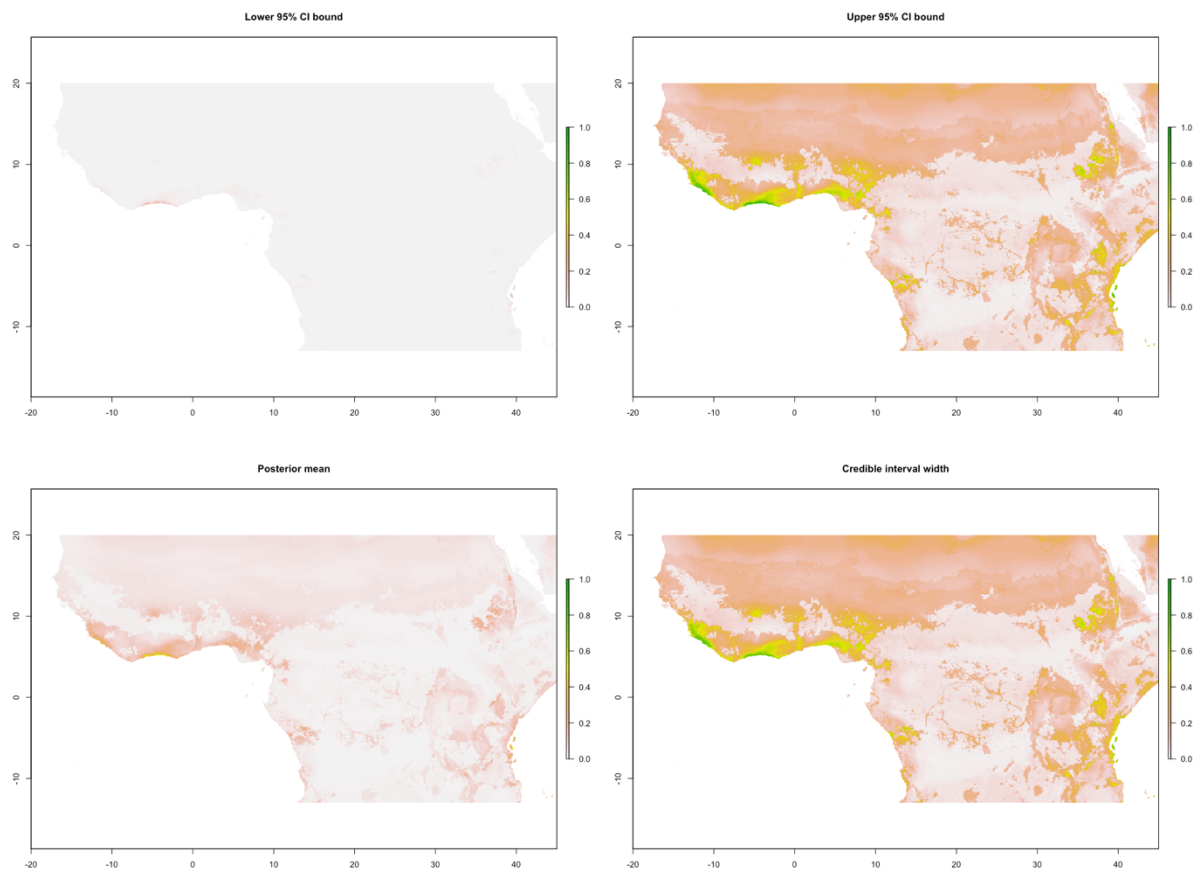
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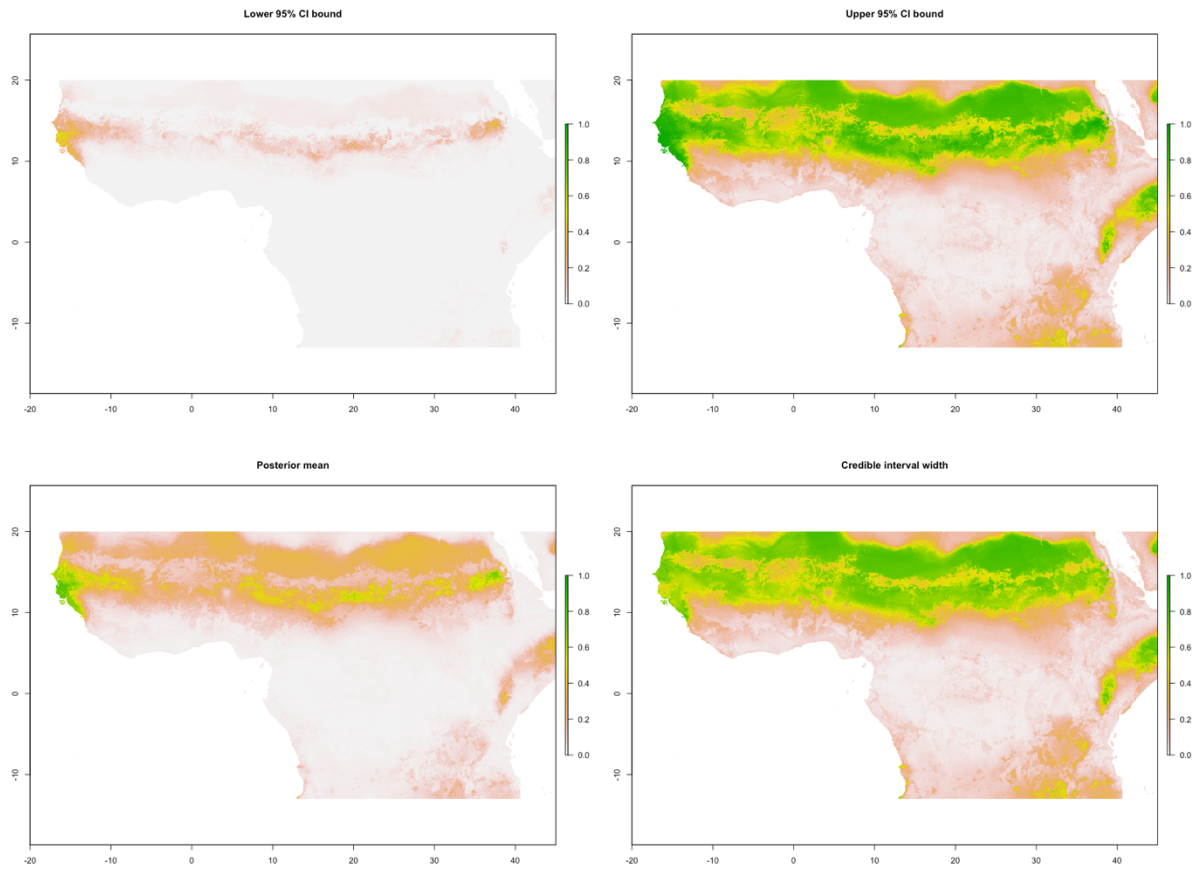
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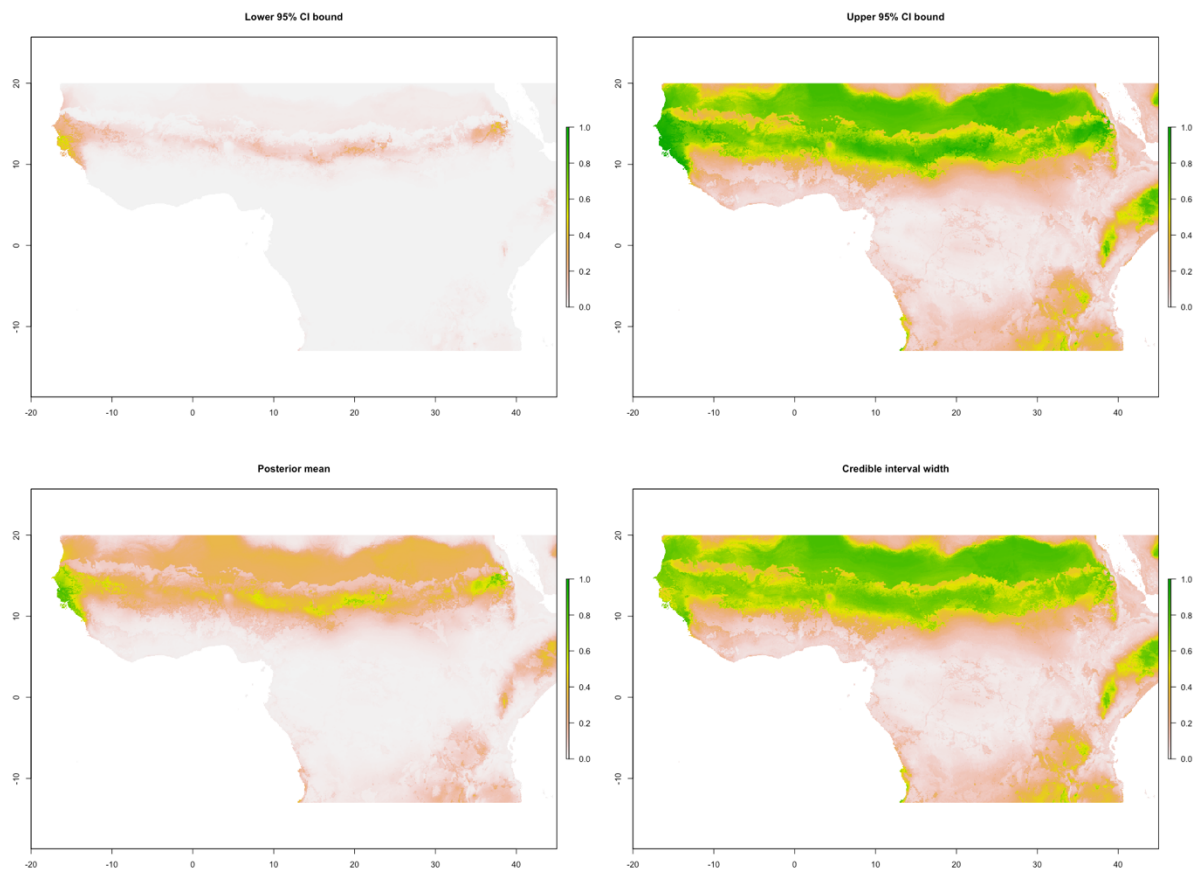
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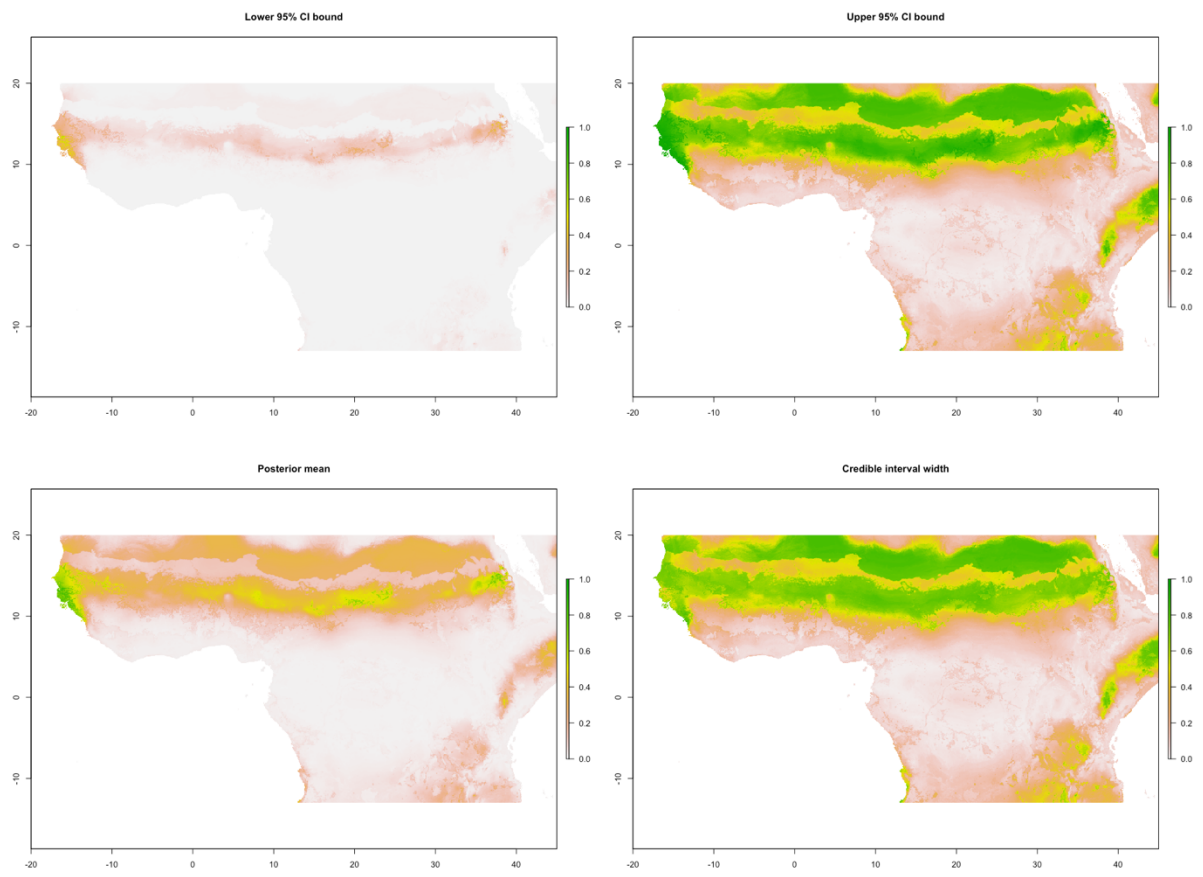
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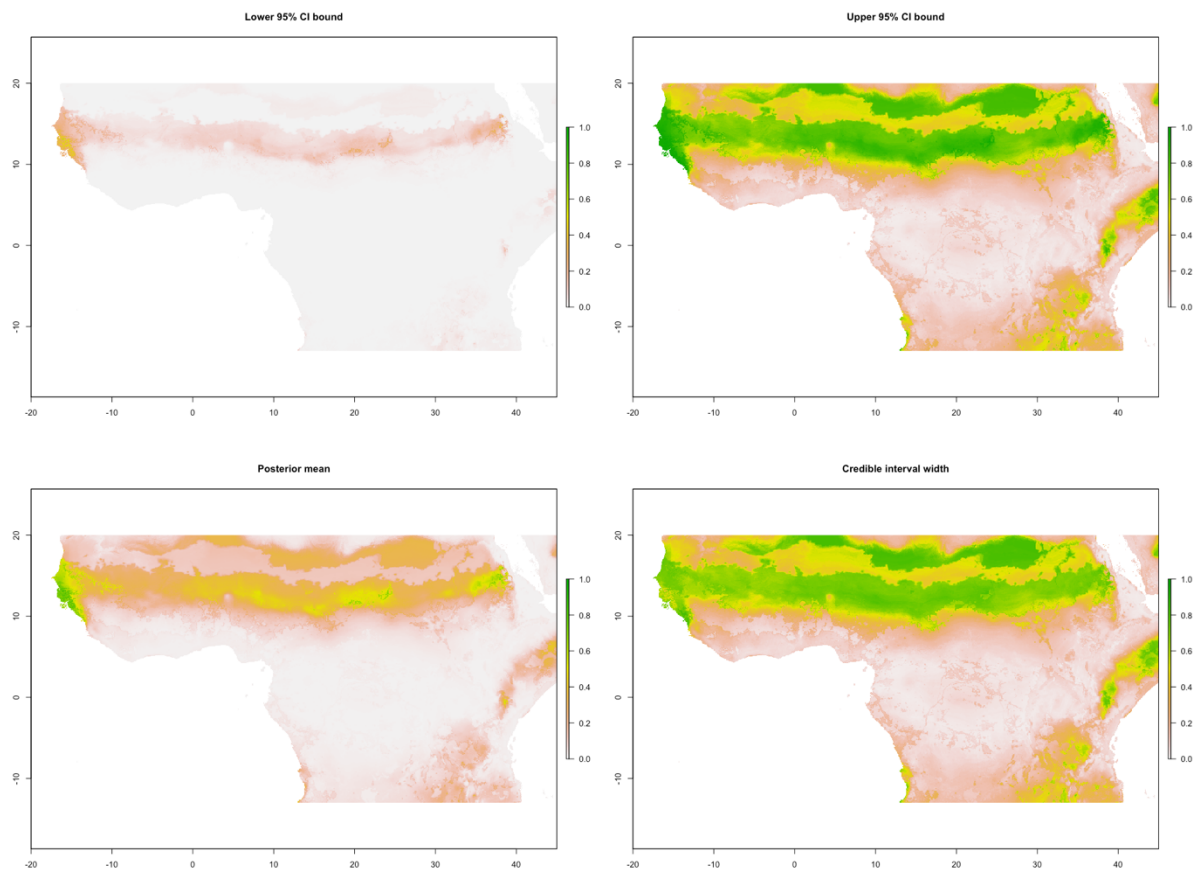
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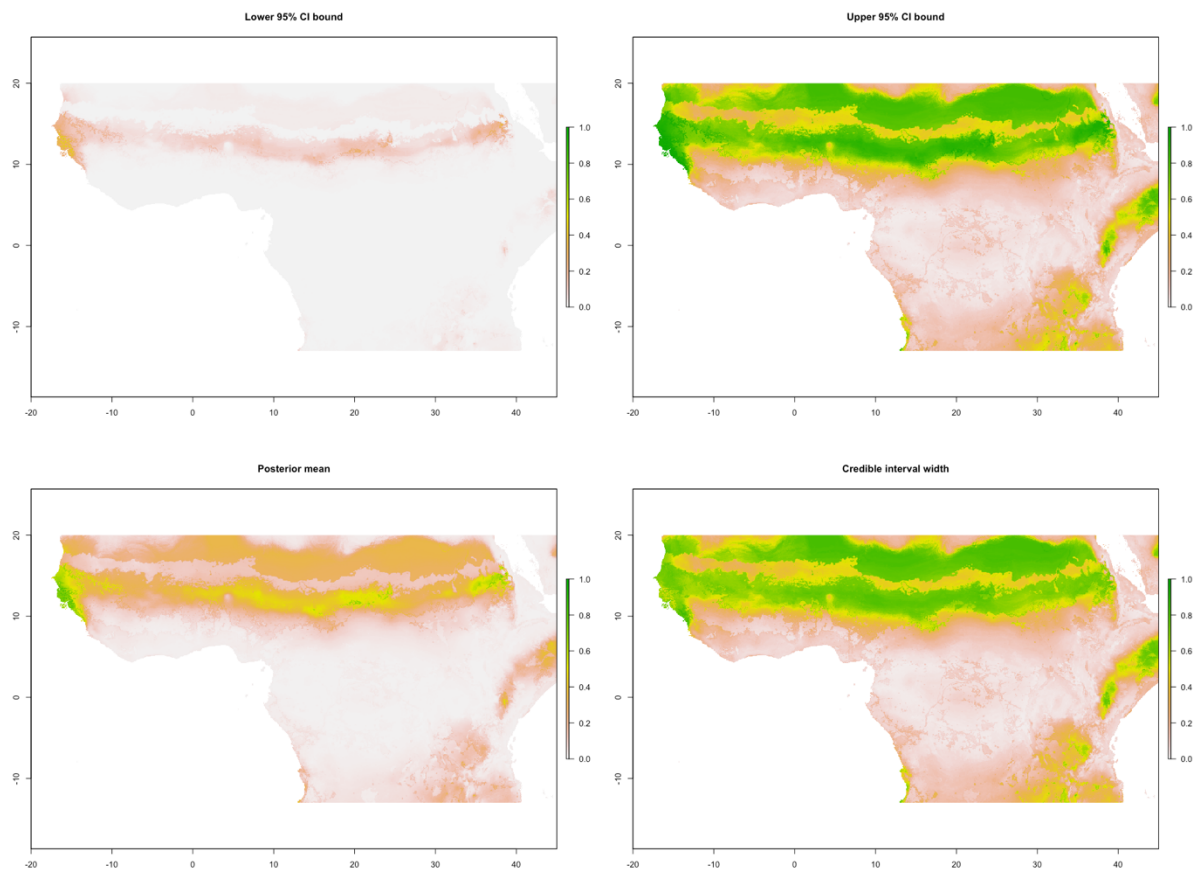
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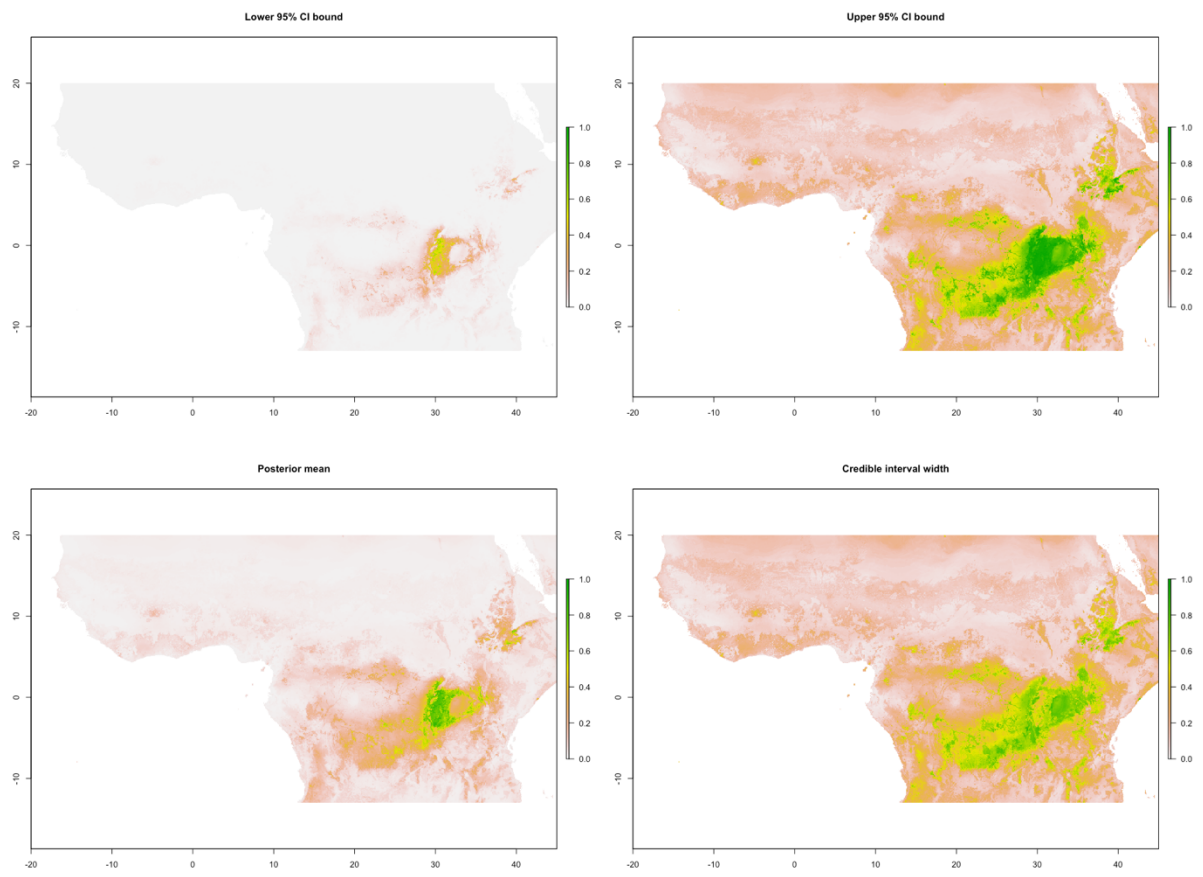
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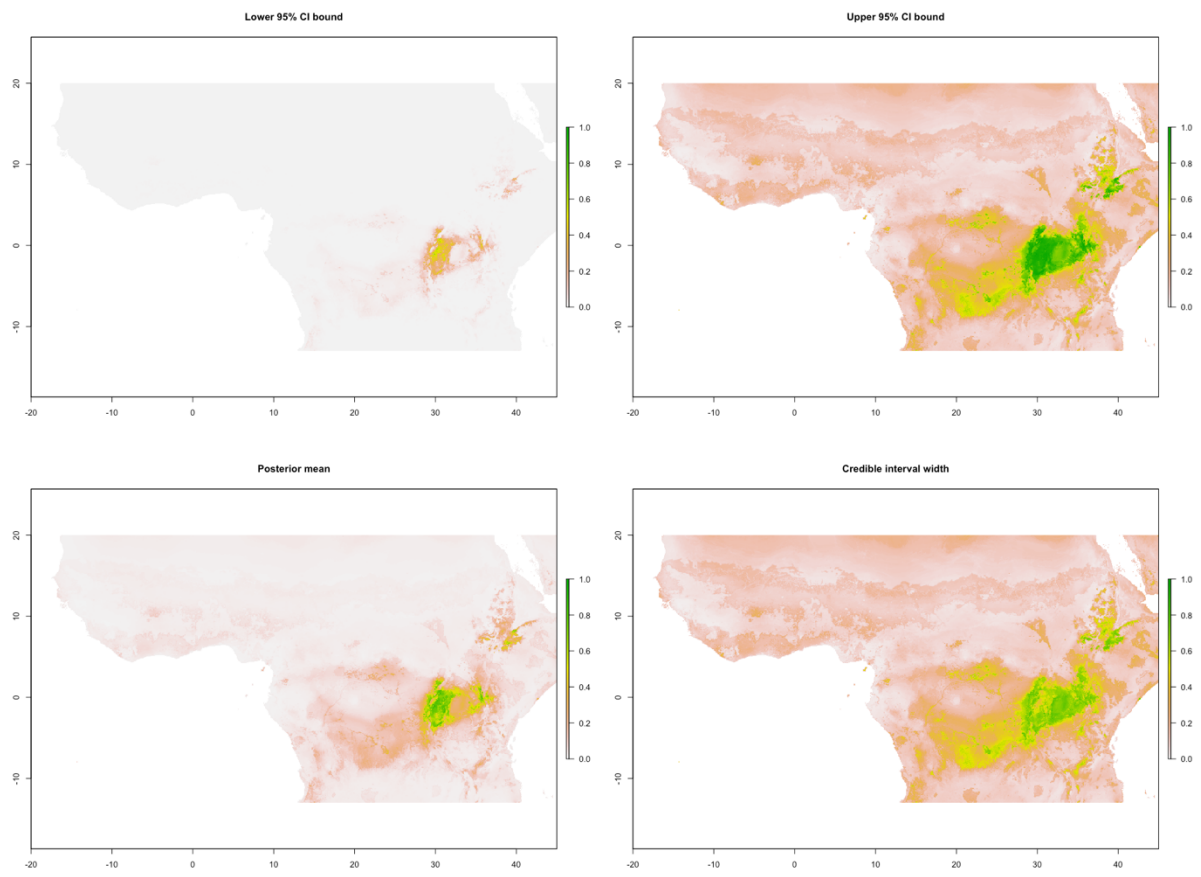
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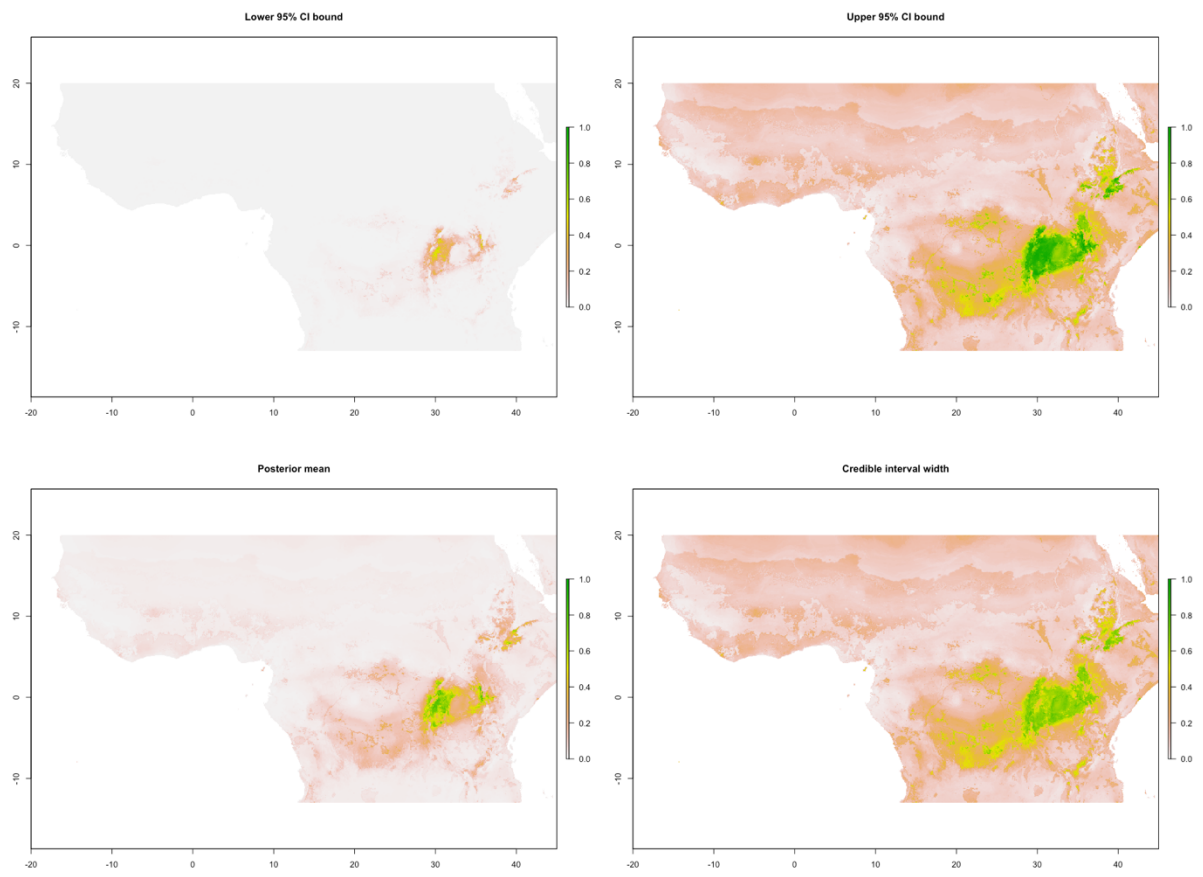
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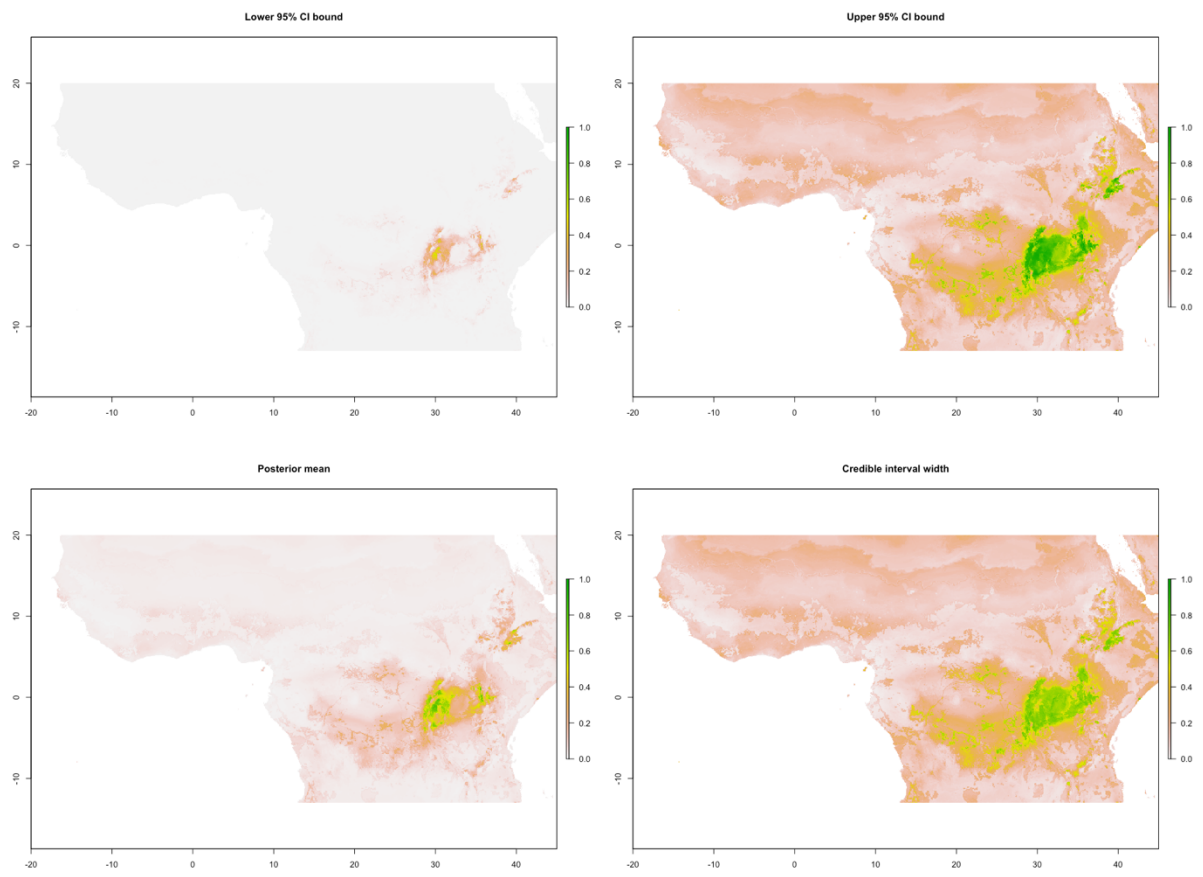
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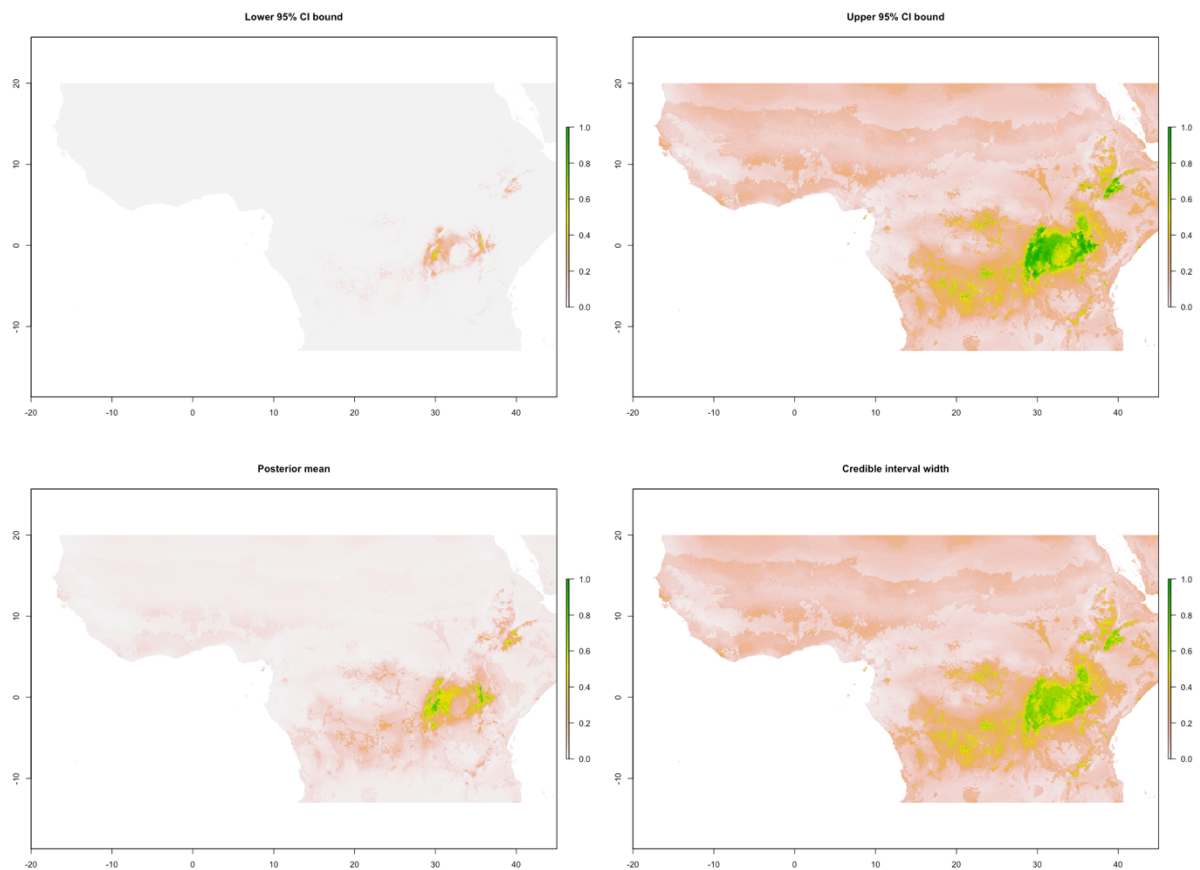
P. tephrosceles 2070 SSP2-4.5:



P. tephrosceles 2070 SSP3-7.0:



P. tephrosceles 2070 SSP5-8.5:



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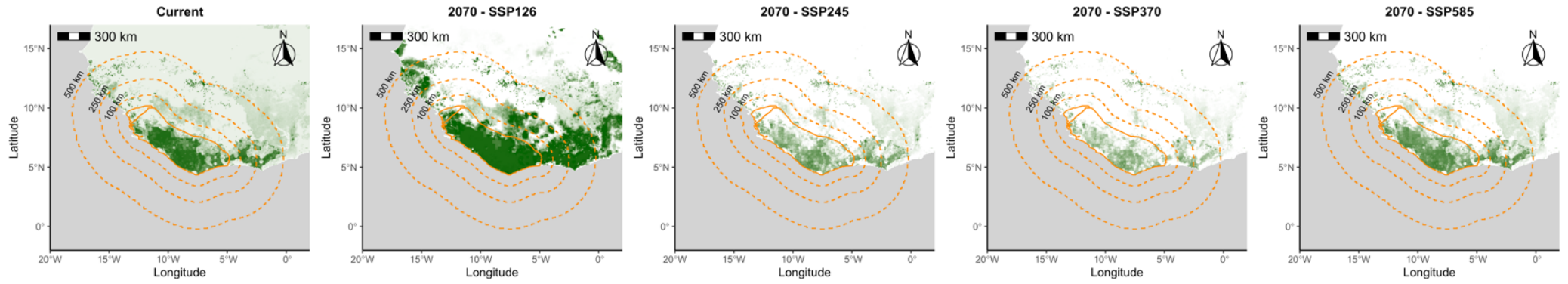
Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302-4315.

Supplementary Material S2.5: Change in forest cover by the year 2070 for four future scenarios

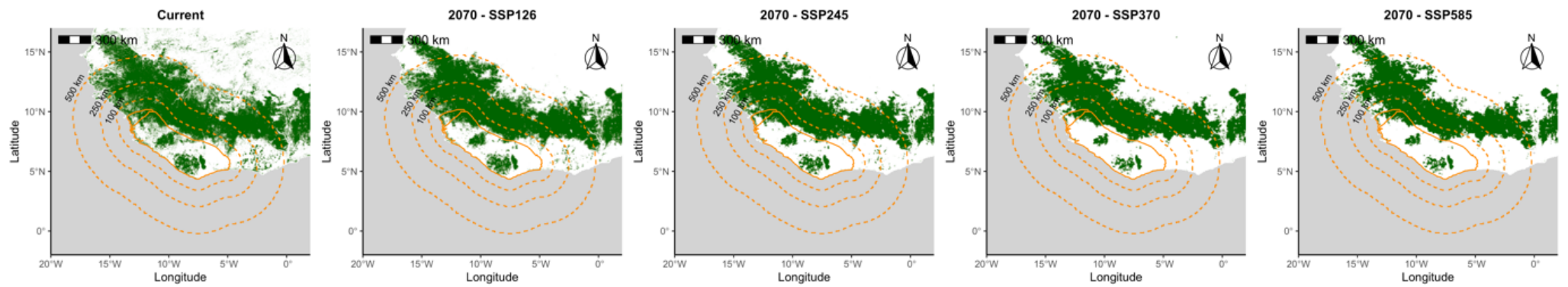
Supplementary Figure S2.5: *Proportional forest cover now and for 2070 across Shared Socio-economic Pathway – Representative Economic Pathway combinations for the GCAM dataset (Chen et al. 2020) and the Chen dataset (Chen et al. 2022). Darker green represents a higher proportion of the pixel is forest. Grey areas represent bodies of water. The area denoted by a solid orange line represents the current species range and the dashed orange lines represent 100km, 250km and 500km migration buffers. In the case of *P. kirkii*, the species is endemic to the island of Unguja and therefore is assumed to not be able to migrate beyond the coastline of that island. Therefore, this coastline is used as the migration buffer for this species.*

A) *P. badius*

GCAM dataset

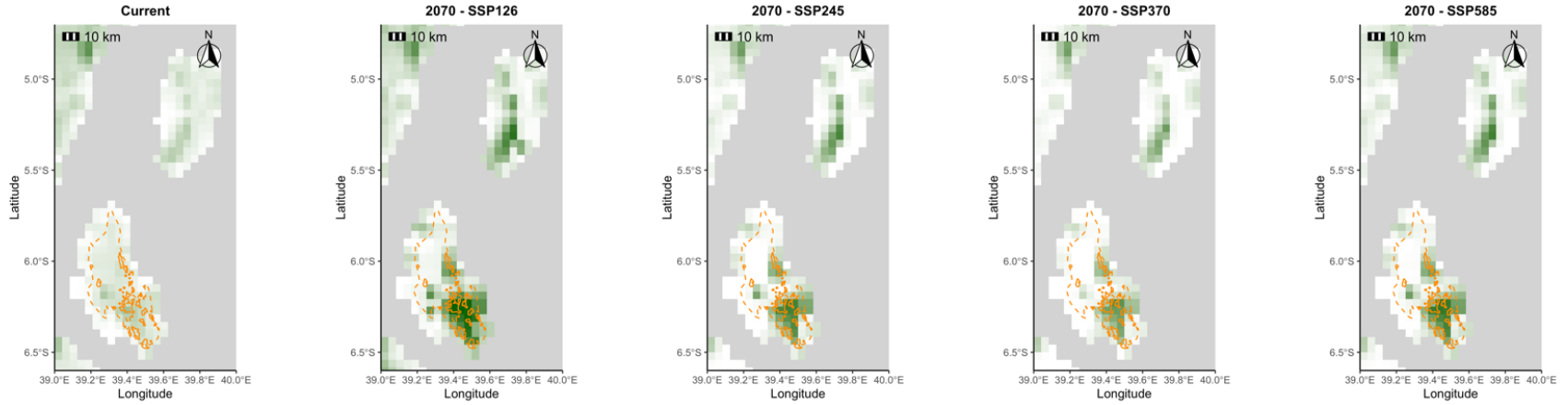


Chen dataset

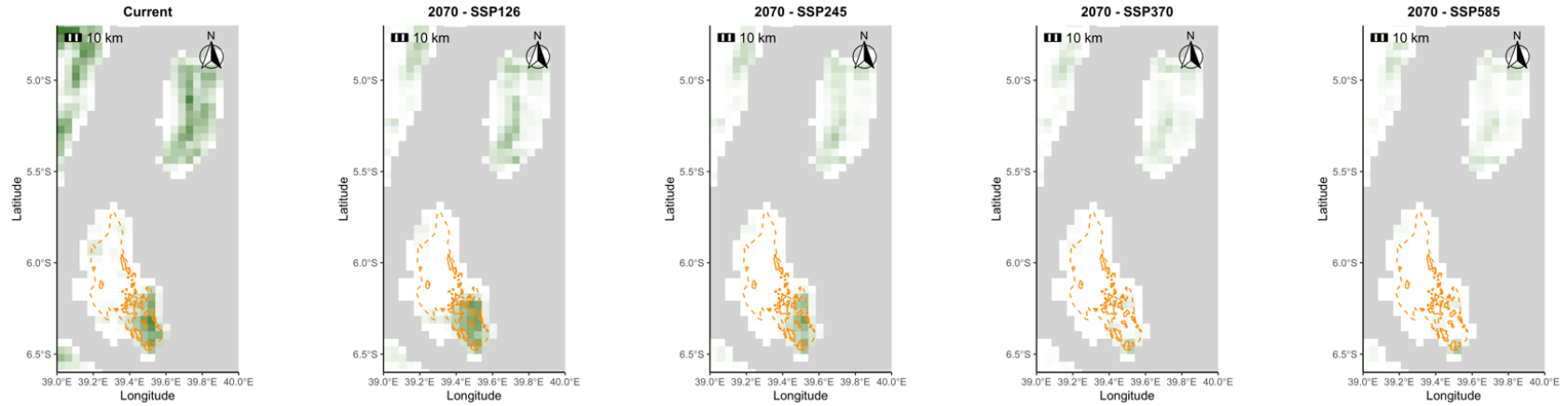


B) *P. kirkii*

GCAM dataset

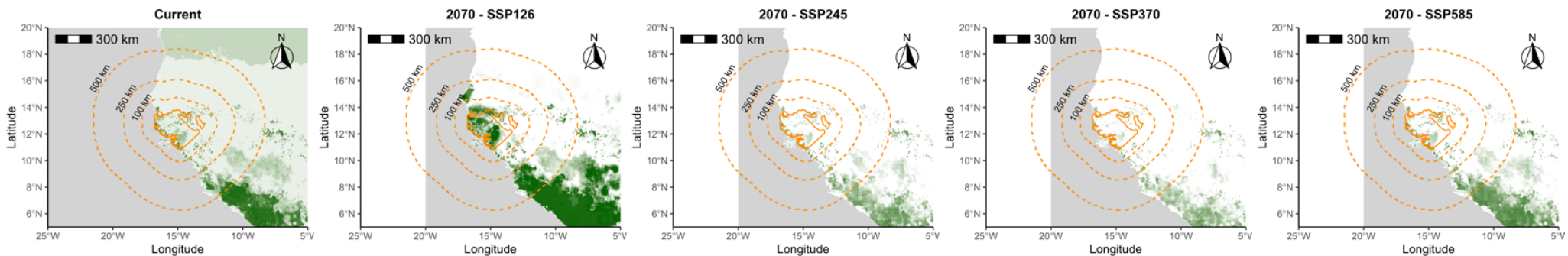


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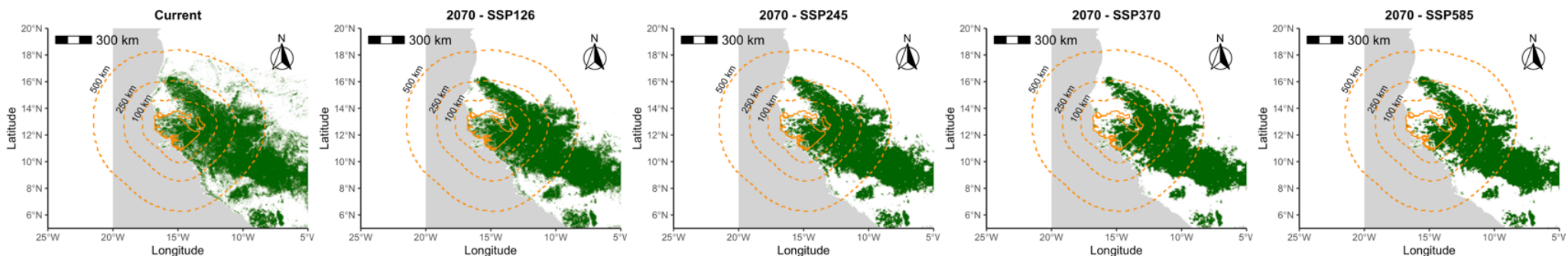


C) *P. temminckii*

GCAM dataset

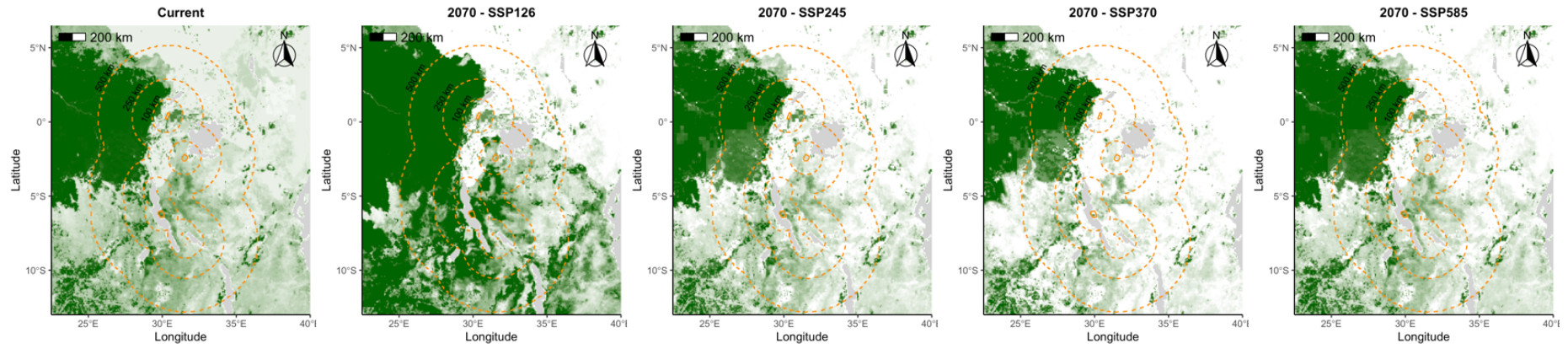


Chen dataset

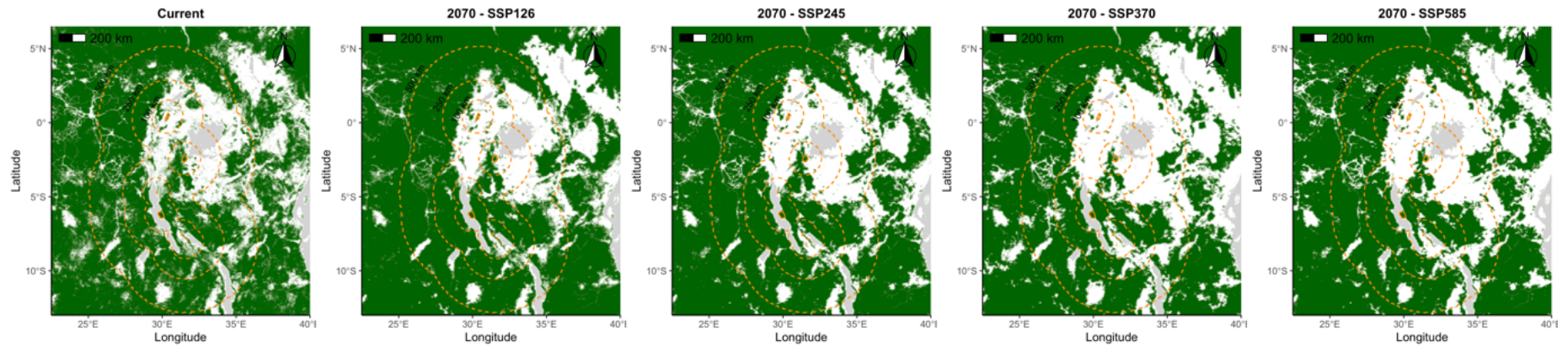


D) *P. tephrosceles*

GCAM dataset



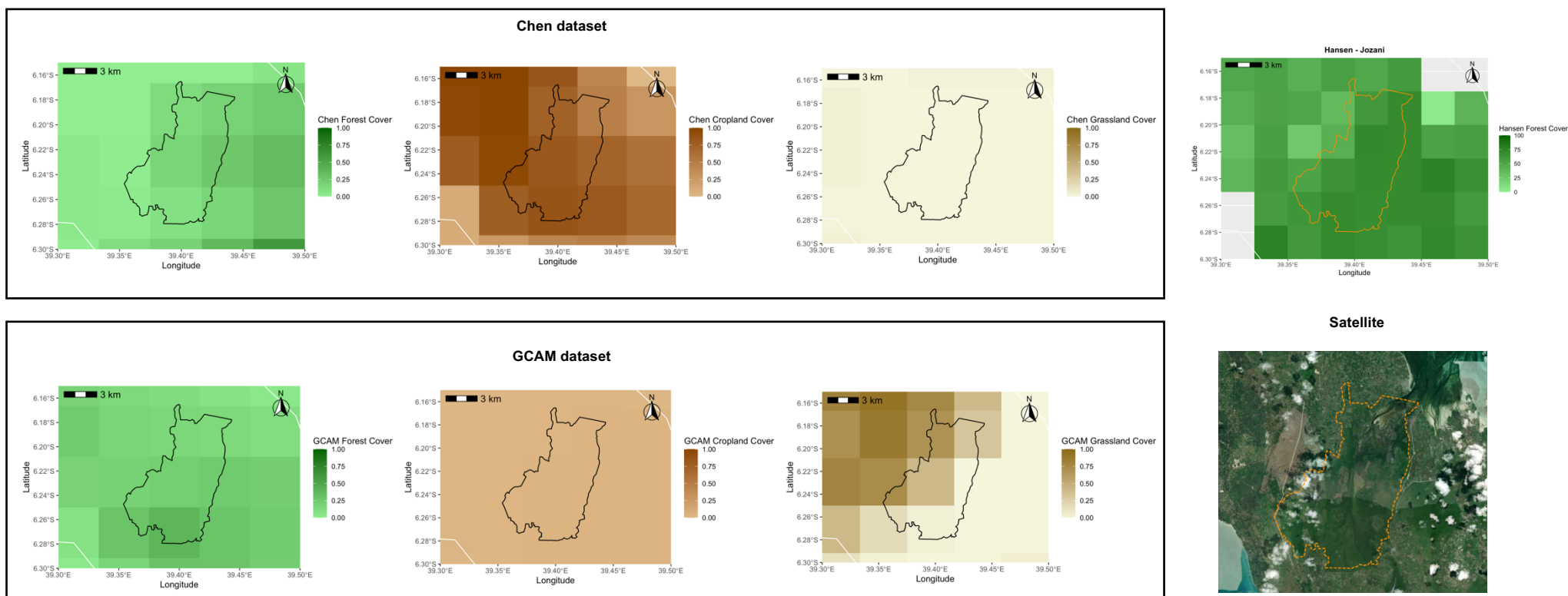
Chen dataset



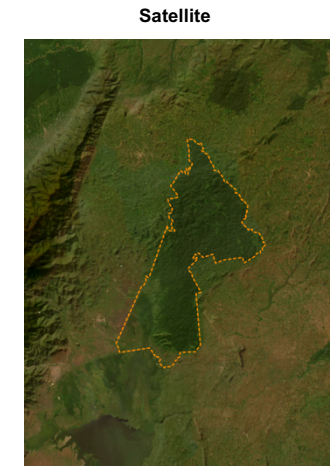
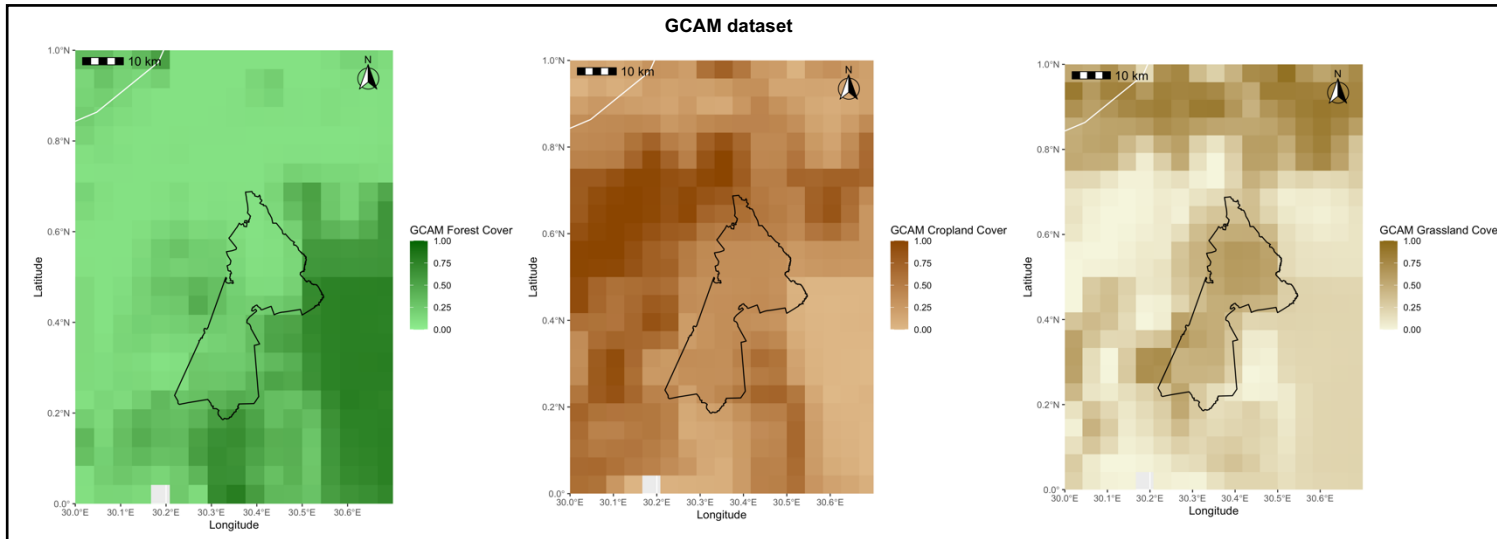
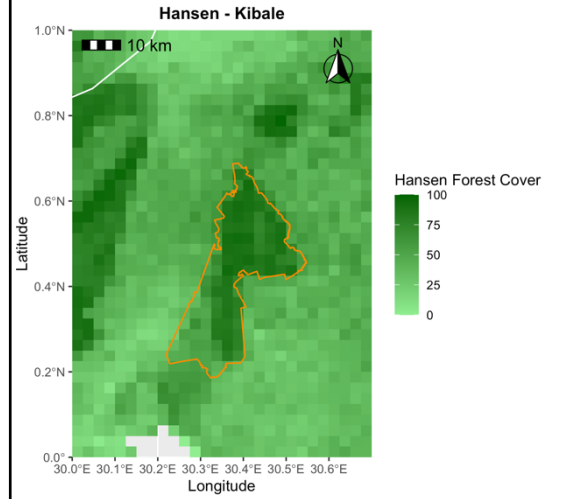
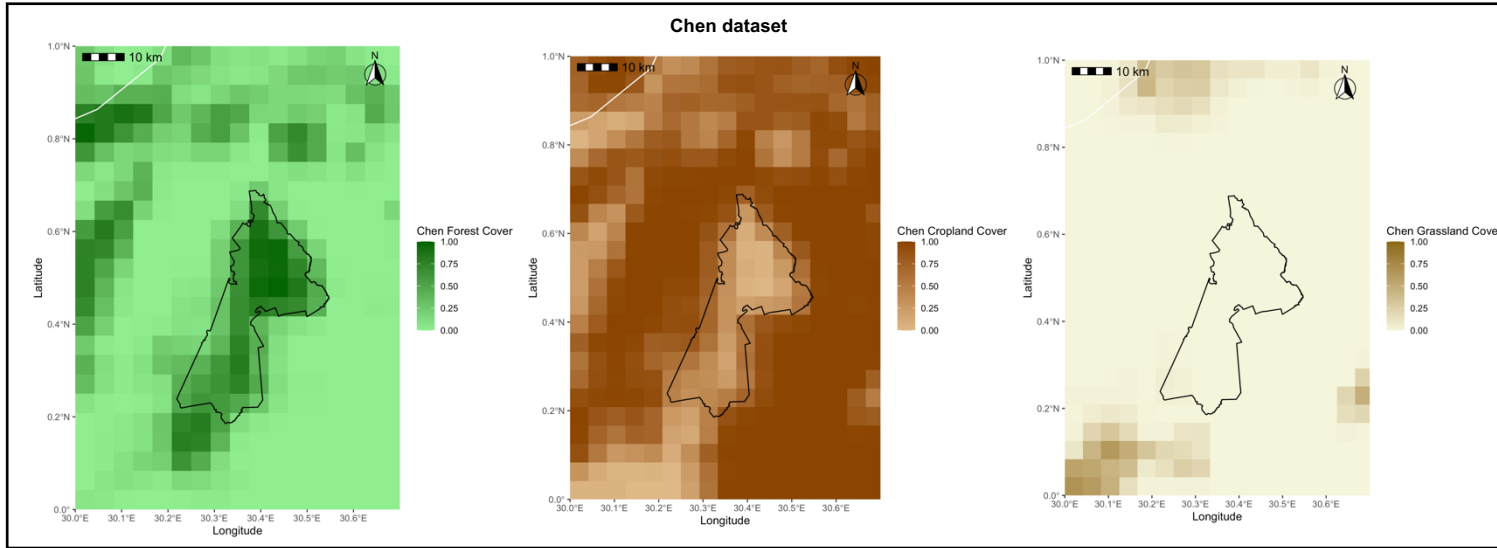
Supplementary Material S2.6: Land use classification for Jozani-Chwaka Bay National Park and Kibale National Park

Supplementary Figure S2.6: Current proportional forest, grassland and cropland for the GCAM dataset (Chen et al. 2020) and the Chen dataset (Chen et al. 2022) as well as a satellite image and forest cover measured in the Hansen dataset (Hansen et al. 2013) for Jozani-Chwaka Bay National Park, Zanzibar (6.233°S , 39.404°E) and Kibale National Park, Uganda (0.4364°N , 30.3667°E).

A) Jozani-Chwaka Bay National Park



B) Kibale National Park



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Supplementary Material for Chapter 3

Supplementary Material S3.1: Model selection using AICc

Supplementary Table S3.1: AICc tables used for model selection and multi-model inference for the candidate set of models for each OS marker and the subsequent estimates and standard errors for each model parameter. Method = collection method (plastic or leaves), Freezing D = freezing delay (decimal hours), Time = Time of sample collection (decimal hours past midnight) and Frozen Storage = length of time in frozen storage (decimal weeks). The null model included just the random effects. K = number of parameters, AIC_c = the second-order Akaike's Information Criterion, ΔAIC_c = the difference between the AICc of each model and the top-ranked model, AIC_cWt is the AIC weight of each model. Estimates and SE are set to 0 for models that do not contain the variable of interest so as not to inflate estimate values. The models in bold represent the 95% confidence set of models.

A

log(8-OHdG (ng/ml corr. SG)) Model structure	K	AICc	ΔAICc	AICcWt	Method		Frozen Storage		Time		Freezing D	
					Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Method	4	96.22	0	0.19	-0.21	0.1	0	0	0	0	0	0
Method + Time	5	96.48	0.26	0.16	-0.2	0.1	0	0	-0.02	0.01	0	0
Method + Freezing D	5	96.85	0.63	0.14	-0.22	0.1	0	0	0	0	0.03	0.02
Method + Time + Freezing D	6	97.64	1.42	0.09	-0.21	0.1	0	0	-0.02	0.01	0.03	0.02
Method + Frozen Storage	5	98.42	2.2	0.06	-0.21	0.1	0	0	0	0	0	0
Time	4	98.69	2.47	0.05	0	0	0	0	-0.02	0.01	0	0
Null	3	98.7	2.49	0.05	0	0	0	0	0	0	0	0
Method + Frozen Storage + Time	6	98.72	2.5	0.05	-0.2	0.1	0	0	-0.02	0.01	0	0
Method + Frozen Storage + Freezing D	6	99.07	2.85	0.05	-0.22	0.1	0	0	0	0	0.03	0.02
Method + Frozen Storage + Time + Freezing D	7	99.89	3.68	0.03	-0.21	0.1	0	0	-0.02	0.01	0.03	0.02
Freezing D	4	99.93	3.71	0.03	0	0	0	0	0	0	0.02	0.02
Time + Freezing D	5	100.34	4.12	0.02	0	0	0	0	-0.02	0.01	0.02	0.02
Frozen Storage + Time	5	100.57	4.35	0.02	0	0	0	0	-0.02	0.01	0	0
Frozen Storage	4	100.59	4.38	0.02	0	0	0	0	0	0	0	0
Frozen storage + Freezing D	5	101.65	5.43	0.01	0	0	0	0	0	0	0.03	0.02
Frozen Storage + Time + Freezing D	6	102.08	5.87	0.01	0	0	0	0	-0.02	0.01	0.02	0.02

B

log(MDA (μM corr. SG))					Method		Frozen Storage		Time		Freezing D	
	Model structure	K	AICc	ΔAICc	AICcWt	Estimate	SE	Estimate	SE	Estimate	SE	Estimate
Frozen Storage + Time	6	162.52	0	0.27	0	0	0.01	0	-0.02	0.01	0	0
Method + Frozen Storage + Time	7	163.53	1.01	0.16	-0.08	0.07	0	0	-0.02	0.01	0	0
Frozen Storage + Time + Freezing D	7	164.6	2.08	0.1	0	0	0.01	0	-0.02	0.01	-0.01	0.02
Frozen Storage	5	165.33	2.82	0.07	0	0	0.01	0	0	0	0	0
Method + Frozen Storage + Time + Freezing D	8	165.7	3.18	0.06	-0.08	0.07	0	0	-0.02	0.01	0	0.02
Method + Frozen Storage	6	166.22	3.7	0.04	-0.08	0.08	0	0	0	0	0	0
Method + Time	6	166.81	4.29	0.03	-0.11	0.07	0	0	-0.02	0.01	0	0
Time	5	166.99	4.47	0.03	0	0	0	0	-0.02	0.01	0	0
Null	3	167.43	4.91	0.02	0	0	0	0	0	0	0	0
Frozen Storage + Freezing D	6	167.49	4.97	0.02	0	0	0.01	0	0	0	0	0.02
Method + Frozen Storage + Freezing D	7	168.37	5.85	0.01	-0.09	0.08	0	0	0	0	0	0.02
Time + Freezing D	6	168.88	6.36	0.01	0	0	0	0	-0.02	0.01	-0.01	0.02
Method + Time + Freezing D	7	168.91	6.39	0.01	-0.11	0.07	0	0	-0.02	0.01	-0.01	0.02
Method	5	169.16	6.64	0.01	-0.12	0.07	0	0	0	0	0	0
Method + Freezing D	6	171.31	8.79	0	-0.12	0.08	0	0	0	0	0	0.02
Freezing D	5	171.54	9.02	0	0	0	0	0	0	0	0	0.02

C

TAC (mM corr. SG) Model structure	K	AICc	Δ AICc	AICcWt	Frozen Storage		Time		Freezing D	
					Estimate	SE	Estimate	SE	Estimate	SE
Frozen Storage + Time	6	697.25	0	0.31	0.12	0.06	-0.43	0.25	0	0
Frozen Storage	5	697.84	0.59	0.23	0.12	0.06	0	0	0	0
Frozen Storage + Time + Freezing D	7	699.24	1.98	0.12	0.12	0.06	-0.4	0.26	0.3	0.52
Frozen Storage + Freezing D	6	699.31	2.06	0.11	0.12	0.06	0	0	0.46	0.52
Time	5	699.94	2.68	0.08	0	0	-0.4	0.26	0	0
Null	4	700.09	2.84	0.08	0	0	0	0	0	0
Freezing D	5	701.61	4.36	0.04	0	0	0	0	0.44	0.53
Time + Freezing D	6	701.9	4.65	0.03	0	0	-0.38	0.27	0.29	0.53

D

log(UA (μM corr. SG))	Model structure	K	AICc	ΔAICc	AICcWt	Frozen Storage		Time		Freezing D	
						Estimate	SE	Estimate	SE	Estimate	SE
Frozen Storage + Time		5	110.19	0	0.28	0.01	0	-0.02	0.01	0	0
Frozen Storage		4	110.45	0.26	0.24	0.01	0	0	0	0	0
Frozen Storage + Freezing D		5	111.72	1.53	0.13	0.01	0	0	0	0.03	0.03
Frozen Storage + Time + Freezing D		6	111.85	1.66	0.12	0.01	0	-0.02	0.01	0.02	0.03
Time		4	112.46	2.27	0.09	0	0	-0.02	0.01	0	0
Null		3	112.87	2.68	0.07	0	0	0	0	0	0
Time + Freezing D		5	114.25	4.06	0.04	0	0	-0.02	0.01	0.02	0.03
Freezing D		4	114.31	4.12	0.04	0	0	0	0	0.02	0.03

Supplementary Material for Chapter 4

Supplementary Material S4.1: Zanzibar red colobus study group descriptions

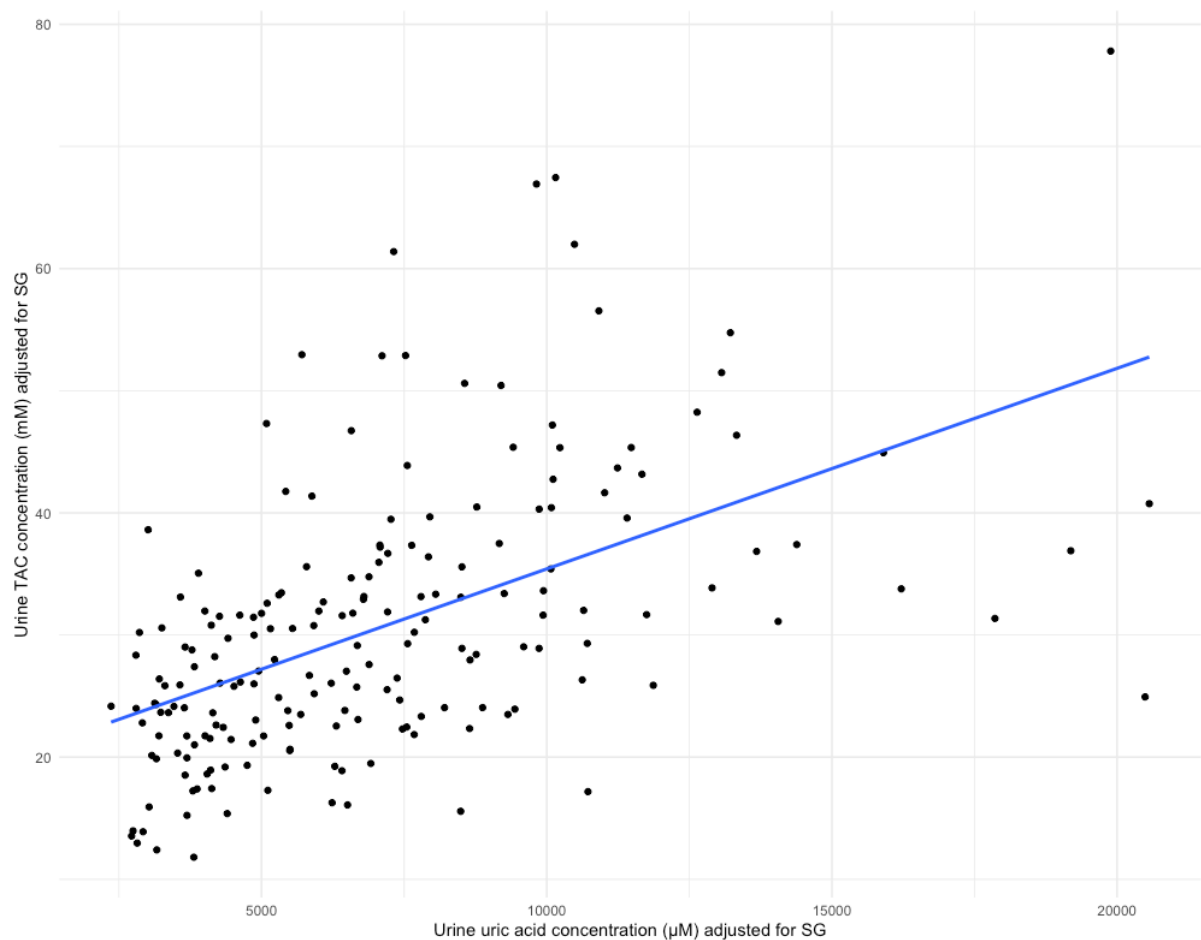
Supplementary Table S4.1: Description of study groups, AM = Adult Male, AF = Adult Female, SAM = Sub-adult Male, SAF = Sub-adult female, J = Juvenile, I = Infant

Group	Code	Group composition	Juvenile: Adult Female ratio	No. of urine samples	Sample collection period	Proportion of home range that is degraded	Mean proportion of observations with tourists present
Camp Group	CG	4 AM, 11 AF, 2 SAM, 1 SAF, 3 J, 10 I	3:11	222	09/2018 – 03/2020	0.707	0.367
Half-tail Group	HTG	2 AM, 8 AF, 2 J, 4 I	2:8	32	02/2019 – 03/2020	0.963	0.003
Apple Tree Grove Group	ATG	3AM, 9 AF, 4 I, 1 J	1:9	22	09/2018 – 04/2019	0.954	0.021
Baobab Group	BG	3 AM, 7 AF, 1 SAF, 6 I	0:7	13	02/2020 – 02/2020	0.978	0.185
Joshi Group	JG	AM, 7 AF, 1 SAF, 1 J, 3 I	1:7	24	03/2020 – 03/2020	0.347	0
Deep Forest Group	DFG	3 AM, 5 AF, 1 SAF, 1 J, 3 I	1:5	26	02/2020 – 03/2020	0.195	0

Supplementary Material S4.2: Residual TAC background and methods

Uric acid is a weak acid that represents the final breakdown product of purine nucleotide metabolism (Chen *et al.*, 2016). In primates, over 90% of uric acid is re-absorbed by the URAT1 and GLUT9 specific anion transporters in the kidneys but is not further oxidised by uricase to form allantoin as it is in other animals. Allantoin is 100 times more soluble than uric acid and is therefore more efficiently excreted in urine. Humans and higher primates lack a functional gene to produce uricase and therefore they have a plasma uric acid concentration 10 times higher than other mammals (Alvarez-Lario and Macarrón-Vicente, 2010). The loss of uricase activity 15 million years ago provided important adaptive advantages for survival, one of which was an increased antioxidant capacity (Totan *et al.*, 2019). Uric acid is a powerful antioxidant, contributing as much as 60% of free-radical scavenging in human serum (Wayner *et al.*, 1987). As such, in primates, there is a strong correlation between the concentration of uric acid and total antioxidant activity. However, the role of uric acid in antioxidant protection *in vivo* is unclear, with some studies showing that it sometimes acts as a pro-oxidant depending on its chemical environment (Kang and Ha, 2014), and high levels of uric acid have been significantly linked to many diseases including obesity, insulin resistance, gout, type II diabetes, kidney disease and hypertension (Alvarez-Lario and Macarrón-Vicente, 2011). Therefore, it can be beneficial to extricate the remaining TAC excluding uric acid.

We found a strong linear relationship between uric acid concentration and TAC concentration (see Supplementary Figure 4.2: linear mixed effects model with monkey identity and TAC plate number as random factors: $df = 189$, estimate = 0.002, Std. Error = 0.0002, $t = 9.266$, $p\text{-value} < 0.0001$). This model was checked for normality and heteroskedasticity of the residuals using diagnostic plots. Therefore, we calculated residuals from a linear model with TAC as the response term, uric acid as the sole predictor, and monkey ID and TAC plate as random effects to yield a measure of antioxidant capacity devoid of that coming from the activity of uric acid (Cram *et al.* 2015).



Supplementary Figure S4.2: The linear relationship between urinary uric acid and urinary TAC. The values for both markers have been adjusted for urine concentration using specific gravity. The line indicates a linear regression between TAC and uric acid with no random effects and points indicate the raw data.

Calculating TAC in this way removed the confounding effects of uric acid on total antioxidant activity. However, the residual TAC only reflects a portion of the total antioxidant protection the animal is experiencing and therefore should be interpreted with some caution. Having said that, previous ecological research demonstrates that residual TAC is a meaningful measure of circulating antioxidant activity that is associated with other antioxidants in serum (Cohen *et al.*, 2007; Cohen *et al.*, 2009a) and health and fitness measures (Cohen *et al.*, 2008; Cohen *et al.*, 2009 a,b; Kilgas *et al.*, 2010; López-Arrabé *et al.*, 2014).

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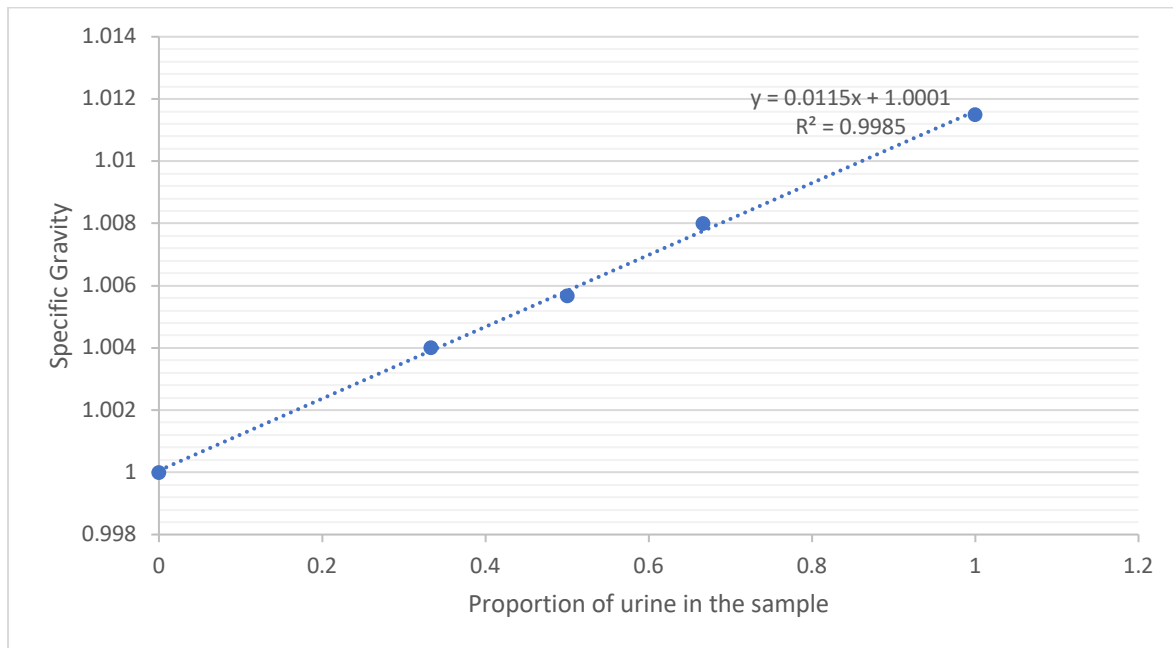
López-Arrabé J, Cantarero A, Pérez-Rodríguez L, Palma A, Moreno J (2014) Plumage ornaments and reproductive investment in relation to oxidative status in the Iberian Pied Flycatcher (*Ficedula hypoleuca iberiae*). *Can J Zool* 92: 1019-1027.

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Supplementary Material S4.3: Dilution factors and specific gravity

When measuring specific gravity in the Zanzibar red colobus (*Ptilocolobus kirkii*) urine samples, we found that 29% of samples had specific gravity measures outside the measurement limit of the specific gravity meter we were using (1 - 1.05), most of which were above this range. As such we conducted a mini-experiment to determine the relationship between dilution factor of the urine samples and specific gravity. We took a pure urine sample and diluted it with distilled water to create aliquots at 1/3, 1/2 and 2/3 dilutions. We measured the specific gravity of these aliquots, as well as the pure urine sample and pure distilled water. We found that specific gravity varies with dilution in a linear fashion (Supplementary Figure 4.3). As such, we can see that by doubling the measured specific gravity of a sample diluted by 50%, we can accurately determine the true specific gravity of the original sample.



Supplementary Figure S4.3: The relationship between the specific gravity measurement of an aliquot and the proportion of urine to distilled water in the sample.

Supplementary Material S4.4: Model output for the MDA and neopterin models

These models were not better than their respective null models.

Supplementary Table S4.4a: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on MDA.

<i>Predictors</i>	Effect of anthropogenic disturbance on log(MDA (μM corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	1.68	0.38	0.009	4.34
Proportion of home range that is degraded	0.44	0.49	0.419	3.61
Mean proportion of time spent with tourists per day	-0.27	1.01	0.811	2.60
Age-sex class - Adult Male	0.00	0.10	0.998	25.71
Age-sex class - Immature	-0.07	0.14	0.638	45.37
Number of freeze-thaw cycles	0.01	0.05	0.888	243.39
Random Effects				
σ^2	0.12			
T00 Animal_ID	0.04			
T00 Group_ID	0.10			
ICC	0.53			
N Group_ID	6			
N Animal_ID	50			
Observations	283			
Marginal R ² / Conditional R ²	0.028 / 0.546			

Supplementary Table S4.4b: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on neopterin.

<i>Predictors</i>	Effect of anthropogenic disturbance on log(neopterin (nmol/L corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	4.91	0.64	<0.001	15.34
Proportion of home range that is degraded	-0.26	0.57	0.679	3.06
Mean proportion of time spent with tourists per day	-1.15	1.18	0.425	2.20
Age-sex class - Adult Male	0.04	0.16	0.827	21.99
Age-sex class - Immature	-0.47	0.21	0.035	18.55
Number of freeze-thaw cycles	0.20	0.11	0.073	64.00
Random Effects				
σ^2	0.08			
T00 Animal_ID	0.09			
T00 Neopterin_Plate_ID	0.01			
T00 Group_ID	0.13			
ICC	0.76			
N Group_ID	6			
N Neopterin_Plate_ID	7			
N Animal_ID	37			
Observations	91			
Marginal R ² / Conditional R ²	0.1590.795			

Supplementary Material S4.5: Model results using Joshi Group’s home range calculated based on 2 days of GPS data collection

The results below are from the models carried out using the proportion of home range that is degraded calculated using the “true” home range of Joshi Group (as opposed to the buffer calculated to match the mean home range size of the other five groups). The results of the analysis do not change.

Supplementary Table S4.5a: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on 8-OHdG.

<i>Predictors</i>	Effect of anthropogenic disturbance on log(8-OHdG (ng/ml corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	5.89	0.35	<0.001	6.17
Proportion of home range that is degraded	0.99	0.41	0.073	4.02
Mean proportion of time spent with tourists per day	-0.07	0.74	0.931	2.10
Age-sex class - Adult Male	-0.22	0.10	0.026	30.32
Age-sex class - Immature	-0.22	0.15	0.144	46.50
Number of freeze-thaw cycles	-0.05	0.05	0.329	147.29
Random Effects				
σ^2	0.13			
T00 Animal_ID	0.02			
T00 8OHdG_Plate_ID	0.03			
T00 Group_ID	0.05			
ICC	0.43			
N Group_ID	6			
N Animal_ID	42			
N 8OHdG_Plate_ID	12			
Observations	196			
Marginal R ² / Conditional R ²	0.148 / 0.517			

Supplementary Table S4.5b: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on MDA.

<i>Predictors</i>	Effect of anthropogenic disturbance on log(MDA (μM corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	1.64	0.41	0.012	4.58
Proportion of home range that is degraded	0.48	0.52	0.406	3.83
Mean proportion of time spent with tourists per day	-0.24	1.00	0.830	2.61
Age-sex class - Adult Male	0.00	0.10	1.000	25.71
Age-sex class - Immature	-0.07	0.14	0.650	45.33
Number of freeze-thaw cycles	0.01	0.05	0.889	243.39
Random Effects				
σ^2	0.12			
T00 Animal_ID	0.04			
T00 Group_ID	0.10			
ICC	0.53			
N Group_ID	6			
N Animal_ID	50			
Observations	283			
Marginal R^2 / Conditional R^2	0.028 / 0.543			

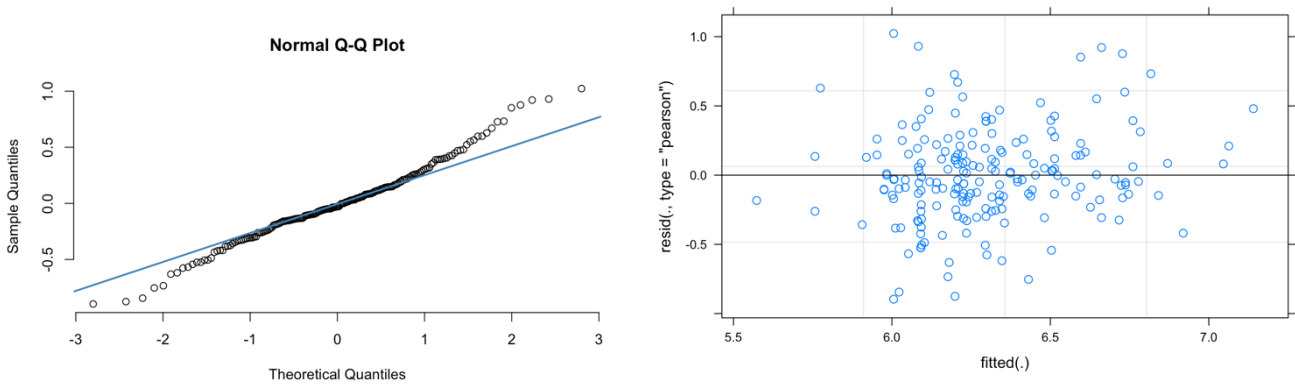
Supplementary Table S4.5c: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on residual TAC.

<i>Predictors</i>	Effect of anthropogenic disturbance on residual TAC (mM corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	-9.87	3.94	0.015	64.30
Proportion of home range that is degraded	15.64	4.55	0.001	63.98
Mean proportion of time spent with tourists per day	-3.79	4.78	0.432	39.01
Age-sex class - Adult Male	-1.26	1.70	0.465	21.38
Age-sex class - Immature	-1.11	2.60	0.673	34.60
Random Effects				
σ^2	63.86			
T00 Animal_ID	2.11			
ICC	0.03			
N Animal_ID	41			
Observations	136			
Marginal R ² / Conditional R ²	0.110 / 0.138			

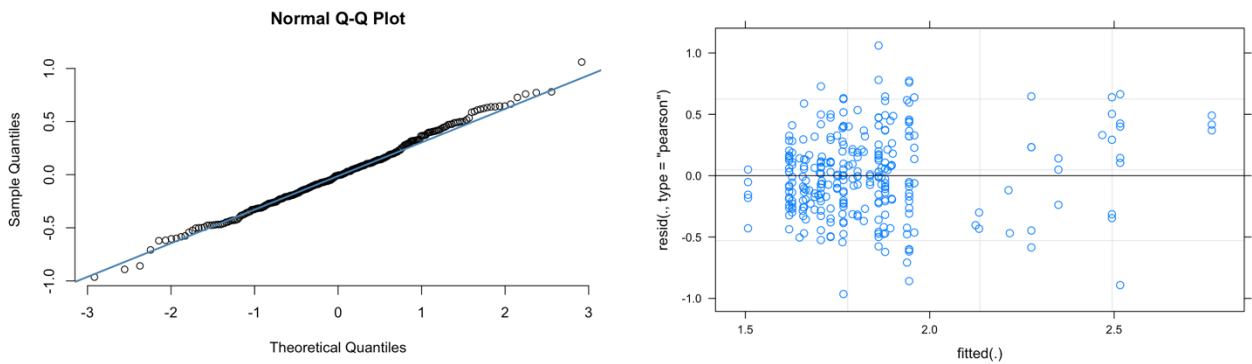
Supplementary Table S4.5d: Model output from full general linear mixed models testing for the effect of habitat degradation, tourist exposure and age-sex class (categorical: adult male, adult female, immature) on neopterin.

<i>Predictors</i>	Effect of anthropogenic disturbance on log(neopterin (nmol/L corr. SG))			
	<i>Estimates</i>	<i>std. Error</i>	<i>p</i>	<i>df</i>
(Intercept)	5.00	0.66	<0.001	15.00
Proportion of home range that is degraded	-0.39	0.59	0.555	3.40
Mean proportion of time spent with tourists per day	-1.13	1.14	0.413	2.27
Age-sex class - Adult Male	0.04	0.16	0.820	22.06
Age-sex class - Immature	-0.48	0.21	0.033	18.55
Number of freeze-thaw cycles	0.20	0.11	0.073	64.10
Random Effects				
σ^2	0.08			
T00 Animal_ID	0.09			
T00 Neopterin_Plate_ID	0.01			
T00 Group_ID	0.12			
ICC	0.75			
N Group_ID	6			
N Neopterin_Plate_ID	7			
N Animal_ID	37			
Observations	91			
Marginal R ² / Conditional R ²	0.159	0.789		

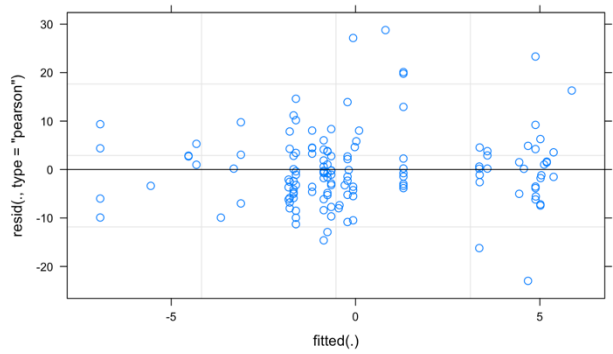
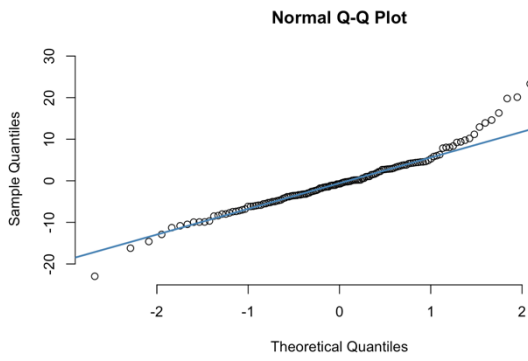
Supplementary Material S4.6: Model validation



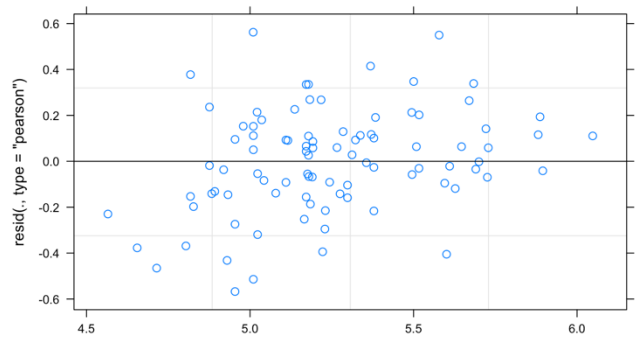
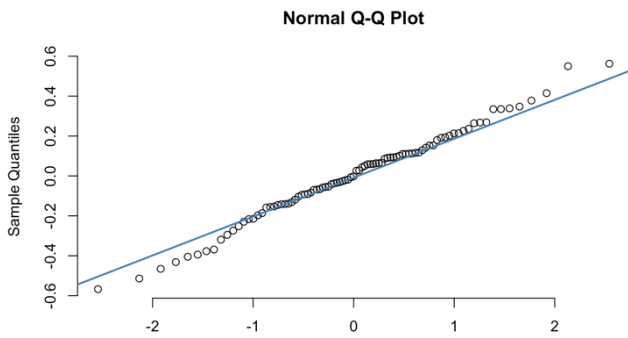
Supplementary Figure S4.6a: qqplots and scatterplots of fitted values vs standardised residuals used to check model residuals for normality and homogeneity of residuals for the 8-OHdG model.



Supplementary Figure S4.6b: qqplots and scatterplots of fitted values vs standardised residuals used to check model residuals for normality and homogeneity of residuals for the MDA model.



Supplementary Figure S4.6c: qqplots and scatterplots of fitted values vs standardised residuals used to check model residuals for normality and homogeneity of residuals for the residual TAC model.



Supplementary Figure S4.6d: qqplots and scatterplots of fitted values vs standardised residuals used to check model residuals for normality and homogeneity of residuals for the neopterin model.