

# Differential responses of amphibians and reptiles to land-use change in the biodiversity hotspot of north-eastern Madagascar

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agroecology; agroforestry; community ecology; herpetofauna; human-dominated landscape; land-use history; slash-and-burn shifting cultivation; forest dependency.

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## Abstract

Large expanses of tropical rainforest have been converted into agricultural landscapes cultivated by smallholder farmers. This is also the case in north-eastern Madagascar; a region that retains significant proportions of forest cover despite slash-and-burn shifting hill rice cultivation and vanilla agroforestry expansion. The region is also a global hotspot for herpetofauna diversity, but how amphibians and reptiles are affected by land-use change remains largely unknown. Using a space-for-time study design, we compared species diversity and community composition across seven prevalent land uses: unburned (old-growth forest, forest fragment, and forest-derived vanilla agroforest) and burned (fallow-derived vanilla agroforest, woody fallow, and herbaceous fallow) land-use types, and rice paddy. We conducted six comprehensive, time-standardized searches across at least 10 replicates per land-use type and applied genetic barcoding to confirm species identification. We documented an exceptional diversity of herpetofauna (119 species; 91% endemic). Observed plot-level amphibian species richness was significantly higher in old-growth forest than in all other land-use types. Plot-level reptile species richness was significantly higher in unburned land-use types compared with burned land-use types. For both amphibians and reptiles, the less-disturbed land-use types showed more uneven communities and the species composition in old-growth forest differed significantly from all other land-use types. Amphibians had higher forest dependency (38% of species occurred exclusively in old-growth forest) than reptiles (26%). Our analyses thus revealed that the two groups respond differently to land-use change: we found less pronounced losses of reptile species richness especially in unburned agricultural habitats, suggesting that reptiles are less susceptible to land-use change than amphibians, possibly due to their ability to cope with hotter and drier microclimates. In conclusion, our findings emphasize existing conservation opportunities – especially for reptiles – in extensive agricultural landscapes while highlighting the precarious situation of amphibians in disappearing old-growth forests.

## Introduction

Demand for agricultural goods is on the rise due to increasing per-capita consumption, changing diets, and a growing

world population (Tilman *et al.* 2011). This situation is leading to both an expansion of croplands into natural areas and an intensification within existing production systems (Tscharntke *et al.* 2012). Most agricultural expansion in the

tropics happens at the expense of forests and leads to an increase in forest fragmentation (Hansen *et al.* 2020). This expansion affects all aspects of biodiversity: research has documented losses in species richness (Scales & Marsden, 2008), shifts in species composition (Newbold *et al.* 2016), reductions of functional diversity (Matuoka *et al.* 2020), and erosion of phylogenetic diversity (Li *et al.* 2020). Importantly, the response of biodiversity to land-use change differs among species groups (Newbold *et al.* 2014) and world regions (Williams & Newbold, 2020), but certain groups and regions are understudied compared with others. While vertebrates are comparatively well studied, we know less about the responses of amphibians and reptiles than we know about mammals and birds (Newbold *et al.* 2014). Similarly, we know less about what happens in the more biodiverse tropics compared with temperate regions (Gardner *et al.* 2009). In sum, we know that land-use change is the main driver of biodiversity decline globally (Powers & Jetz, 2019), but how the diversity of certain understudied taxa differs between land-use types remains an open question, especially in the tropics, where high land-use pressure and biodiversity coincide (Newbold *et al.* 2020).

Tropical agricultural landscapes contribute to food security but also provide opportunities for nature conservation (Perfecto & Vandermeer, 2010). The quality of the agro-ecosystem for biodiversity conservation depends on the farming systems. Agricultural landscapes dominated by large-scale industrial monocultures have lower conservation value than diverse mosaics of forest fragments, agroforestry systems, and more intensively farmed annual crop fields (Mendenhall *et al.* 2016; Murray & Nowakowski, 2021). For example, Ricciardi *et al.* (2021) reported that small-scale agricultural landscapes host more biodiversity than large-scale ones and De Palma *et al.* (2015) showed that low-intensity agriculture can maintain relatively diverse bee communities. Furthermore, forest patches within tropical small-scale agricultural landscapes increase the variety of ecological niches and benefit species with small ranges in particular (Gray *et al.* 2016). Besides the value for biodiversity, small-scale land-use mosaics can also provide essential ecosystem services and livelihoods for rural people, making such landscapes work for humans and nature (Kremen & Merenlender, 2018). Most research investigating the value of tropical agricultural landscapes for biodiversity and humans was conducted in the Neotropics (Mendenhall *et al.* 2016) while the conservation value of Afrotropical agricultural landscapes is less understood (Powers *et al.* 2011). Furthermore, it remains largely unclear how different forms of forest conversion – that is with the use of fire (slash-and-burn shifting cultivation) or without the use of fire (forest degradation and forest-derived agroforestry) – affect amphibian and reptile diversity in resulting land-use types.

Here, we investigate how herpetofauna taxonomic diversity and species composition differ across prevalent burned and unburned land-use types in Madagascar, providing insights into the vulnerability of amphibians and reptiles to various forms of land-use change. Madagascar has lost 44% of forest cover since the 1950s, mainly due to the

transformation of natural ecosystems to agricultural lands (Vieilledent *et al.* 2018). Along with unsustainable extraction rates (Whitehurst *et al.* 2009), this has resulted in more than half of evaluated Malagasy vertebrate species being at risk of extinction (IUCN, 2019). Outstanding levels of endemism (Goodman & Benstead, 2005) and ongoing threats qualify Madagascar as a global biodiversity hotspot (Myers *et al.* 2000). While the forests and protected areas of the island are increasingly well surveyed, the biodiversity in the agricultural landscapes is less known (Irwin *et al.* 2010). Focusing on amphibians and reptiles is particularly relevant since Madagascar has a diverse and highly endemic herpetofauna: amphibian species diversity is currently estimated at around 370 (AmphibiaWeb, 2020) and almost all species are endemic (Goodman & Benstead, 2005). Reptile diversity stands at around 440 species (Uetz *et al.* 2021) with 91% endemism (Goodman & Benstead, 2005). Due to cryptic taxonomic complexes, many species still await discovery or description, suggesting that total species richness numbers will increase further (Vieites *et al.* 2009). Globally, herpetofauna is sensitive to various anthropogenic threats (Vallan, 2000) including chytrid fungi, environmental pollution, collection for the pet trade, climate change, and conversion of forest habitat into agricultural lands (Hof *et al.* 2011). All of these factors are also threatening Malagasy herpetofauna, with deforestation being the top pressure (Cordier *et al.* 2021).

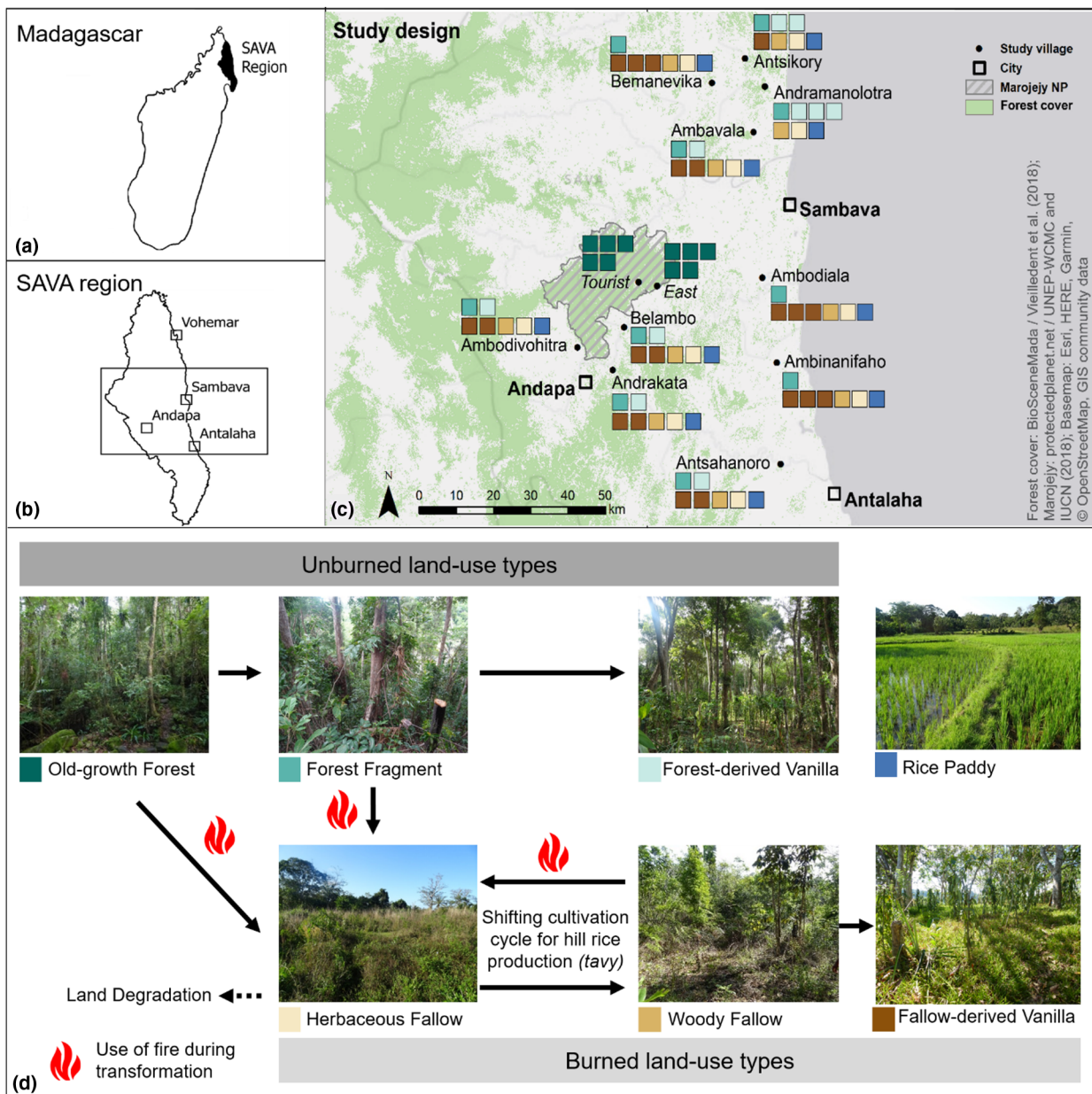
Our study focuses on north-eastern Madagascar. The area retains more forest cover than other parts of the country (Vieilledent *et al.* 2018) and is a global priority area for amphibian research (Nori *et al.* 2018). Besides being known for its remarkable biodiversity, north-eastern Madagascar is also a global center for vanilla cultivation (Hänke *et al.* 2018). The price boom of the spice between 2012 and 2019 has triggered an expansion of vanilla agroforests (Llopis *et al.* 2019), and roughly 80% of rural households in the study region farm vanilla (Hänke *et al.* 2018). How the expansion of vanilla agroforestry is impacting biodiversity is not well known, despite that the majority of global vanilla cultivation takes place across three biodiversity hotspots (Madagascar, Indonesia, and Mexico) (Myers *et al.* 2000; FAO, 2020). Investigating how vanilla agroforestry compares with other land-use types in terms of biodiversity conservation thus represents a research gap. Besides farming vanilla, the rural population in north-eastern Madagascar also practices slash-and-burn shifting cultivation for hill rice production. Valleys and plains in the study region are commonly occupied by irrigated rice paddies, forming the backbone of staple crop supply. We thus compared the taxonomic diversity and community composition of amphibians and reptiles among plots in six land-use types within the small-scale agricultural landscape and compared this with continuous old-growth forest inside Marojejy National Park. Within the small-scale agricultural landscape, we sampled vanilla agroforests of contrasting land-use history (forest- and fallow-derived vanilla agroforests), as well as forest fragment, herbaceous fallow, woody fallow, and rice paddy. Following Cordier *et al.* (2021) and Gonzalez & González-Trujillo (2021), we hypothesized that taxonomic diversity would be

highest in old-growth forest (for both amphibians and reptiles). We further expected losses in taxonomic diversity and shifts in species composition after old-growth forest conversion, particularly in those cases where land-use conversion involves the use of fire for slash-and-burn shifting cultivation. Based on previous research (Martin *et al.* 2020), we further expected that rehabilitation through agroforestry on fallow land would positively affect the taxonomic diversity of the herpetofauna.

## Materials and methods

### Study region and study design

We conducted our study in the SAVA region in north-eastern Madagascar (Fig. 1a,b) where forests outside protected areas are now highly fragmented (Vieilledent *et al.* 2018) and the landscape is dominated by smallholder agriculture.



**Figure 1** Overview of the study region and the study design. (a) SAVA region in north-eastern Madagascar; (b) study area within SAVA region; (c) study design showing the distribution of 80 plots in 10 villages and at two sites inside Marojejy National Park; and (d) overview of the studied land-use types and the typical land-use transformation trajectory from old-growth forest to forest fragments and agricultural land-uses. Panel (d) modified after Martin *et al.* (2021) and Panel (c) modified after Dröge *et al.* (2021). Rice paddy is not part of the main land-use trajectory

We collected data on circular plots with a 25 m radius (largest possible plot size in typical smallholder parcels) at low to mid-altitude (7–819 m above sea level; mean =  $192 \text{ m} \pm 207 \text{ m}$ ) surrounding 10 villages and in Marojejy National Park, a UNESCO World Heritage Site. In each village, we selected seven plots: three vanilla agroforests (forest-derived and/or fallow-derived vanilla agroforest), one forest fragment, one herbaceous fallow, one woody fallow, and one rice paddy. In the 10 villages, we first selected 30 vanilla agroforests along a canopy cover gradient. After consultation with the agroforest owner and a visual confirmation on the plot, we found that 20 vanilla agroforests were fallow-derived while 10 agroforests were forest-derived. This approach allowed us to understand the response of amphibians and reptiles to the land-use history of vanilla agroforests. Additionally, we chose 10 plots at two sites (five plots each) inside Marojejy National Park. All land-use types were replicated 10 times, except fallow-derived vanilla agroforest, which was replicated 20 times. In total, we surveyed 80 plots (Fig. 1c,d). The selection of 10 plot per land-use types was a compromise between a large number of replicates and logistical constraints. The average minimum distance between one plot and the next closest plot in the same village/site was  $719 \text{ m} \pm 438 \text{ m}$ , while the smallest minimum distance between two plots was 260 m as a compromise between independence of plots and logistical constraints.

### Sampled land-use types

We selected 10 *old-growth forest* plots at two sites with five plots each. One of the sites has experienced some selective logging in the past but is now well protected (touristic zone in Manantenina valley; site Tourist on Fig. 1), the other site suffers from ongoing occasional selective logging and trapping (Bangoabe; site East on Fig. 1), but we chose plots that did not show signs of recent disturbance. The old-growth forest plots are a minimum of 300 m from the National Park boundary. In the study region, isolated *forest fragments* occur around villages and represent remnants of the continuous forest cover that existed in the region prior to the large-scale deforestation that began in the early 20<sup>th</sup> century (Gade, 1996). The 10 forest fragments have not been burned in historic times and are all used for the extraction of timber and non-timber forest products. *Herbaceous fallows* occur after slash-and-burn shifting hill rice cultivation (locally referred to as *tavy*; Styger *et al.* 2007) and are sometimes used for grazing cows. The herbaceous fallow plots in this study had last burned at the end of 2016, 1 year before the onset of data collection in 2017. *Woody fallows* represent successional stages of herbaceous fallows, containing shrubs and small trees. Woody fallows are also occasionally grazed. The woody fallows in our study had last burnt 4–16 years before the onset of data collection in 2017 according to the reports of land-owners. The climbing vanilla orchid (*Vanilla planifolia*) is farmed in agroforestry systems with two distinct land-use histories (following Martin *et al.* 2020): *forest-derived vanilla agroforests*, where vanilla is directly planted inside

the forest after removing understory trees and shrubs and managing tall trees for shade (not involving burning). In *fallow-derived vanilla agroforests*, vanilla is planted on fallow land which resulted from slash-and-burn shifting hill rice cultivation in the past. In these fallow-derived vanilla agroforests, farmers allow natural regeneration of trees or plant trees to provide shade or as support structures for the vanilla vines. Lastly, we studied irrigated *rice paddies* that occur in valley bottoms and flood plains. Rice is planted and harvested between one to three times per year. The rice paddies chosen for our study had wider-than-average banks to facilitate movement and sampling within the plots.

### Sampling, data collection, and field identification

To collect data in the villages, we organized two sampling campaigns during the driest period of the year (October to December 2017 and late August to December 2018) and one campaign during the wettest period (Mid-January to early April 2018). In Marojejy National Park, we also organized two sampling campaigns during the driest period (late August to early September 2018 and December 2018) and one during the wettest period (February 2019). During each campaign, we visited each plot once during the day (08:00–17:00) and once at night (18:30–23:00). Overall, we did six visits per plot, three times during the day and three times at night.

We collected data on amphibian and reptile communities during time-standardized searches (Kadlec *et al.* 2012). During the survey, we systematically searched each plot in a zig-zag pattern (Kadlec *et al.* 2012). Each search was standardized to 45 min of searching time by two observers. In sum, we thus conducted 270 min of searching time on each plot, summing up to 408 h of searching time across all plots. To detect individuals hiding under rocks, in leaf axils, tree barks, tree holes, leaf-litter, or deadwood, we actively inspected those microhabitats by lifting removable objects to check underneath.

To identify individuals to species level during fieldwork, we used morphological characteristics and referred to keys and visualizations available in “A Field Guide to Amphibians and Reptiles of Madagascar” (Glaw & Vences, 2007) and additional literature (Rakotoarison *et al.* 2017; Ratsoavina *et al.* 2019). For those individuals who we could not identify with confidence in the field, we extracted tissue samples for follow-up DNA analysis and/or collected the specimen. To denote those individuals, we used “cf.” (confer or compare with) and “sp. aff.” (affinis) in conjuncture with the genus name when we found slightly different morphological characters compared with the literature that we used or compared with a given identified species. We used sp. (sp1 or sp2) in conjuncture with the genus name when we could not find any given identified species to compare with the encountered individual. The sp. denotation was thus used to differentiate several unknown species belonging to the same genus and we consider them as morphospecies but used DNA barcodes to confirm presumed species status (see

below). Following the field identification, we resumed the searching time; thus abundance and diversity of amphibians and reptiles were independent of the time spent on the plot. Throughout this manuscript, we refer to each encountered individual as an “encounter” rather than an individual as we cannot exclude the possibility of having encountered the same individual at more than one sampling event.

### Species identification with DNA barcodes

We collected muscle or toe clips as tissue samples of individuals in case of non-reliable morphological-characteristics-based identification. We preserved tissue samples in Eppendorf tubes with 90% ethanol, stored, and analyzed at the Evolutionary Biology laboratory of Prof. Miguel Vences at the University of Braunschweig, Germany. We preserved amphibian specimens in 70% ethanol and deposited them in the collection of the Regional University Centre of the SAVA region (CURSA), Antalaha, Madagascar. We extracted genomic DNA following the standard single-tube salt extraction protocol (Bruford *et al.* 1992). To do so, we cut small pieces from the collected tissue and proceeded to protein digestion following the protocol of Cacciali *et al.* (2019). We then passed the extracted DNA to a PCR (Polymerase Chain Reaction) thermocycler, in which we amplified DNA fragments of two mitochondrial genes, “16S” (Vences *et al.* 2005) and “COI” with the primers 16SAL (5'-CGCC TGTTTATCAAAAACAT-3') and 16SBH (5'-CCGGTCT GAACCTCAGATCACGT-3') from Palumbi *et al.* (1991). Amplification was set for 45 s at 94–96°C as denaturation, for 45 s at 52°C as hybridization, and for 90 s at 72°C as elongation. The whole process was repeated for 35 cycles. We generated DNA sequences through a sequencer. We first cleaned the obtained sequences with the software Codon-Code Aligner version 8.0.2 (Codon Code Corporation, Centerville, MA, USA). Then, we processed newly generated sequences for additional quality control with a Blast search (Altschul *et al.* 1990) which queries for similar DNA sequences in the GenBank database to provide information on closest taxa or potential contamination. Finally, we built a phylogenetic tree using MEGA-X (Kumar *et al.* 2018) to reveal clusters of each newly generated sequence, allowing us to reliably cluster individuals to known or presumed species. We submitted all newly determined sequences to GenBank under accession numbers MZ019097–MZ019430. In this study, the DNA barcoding differentiated between 43 species of amphibians and 24 species of reptiles. Among them were 12 candidates for new species of which eight were documented for the first time (marked as sp. CaNEW) and four were already found by other researchers (marked as sp. Ca) but have not yet been described. The new species were based on a comparison of obtained DNA sequences with available sequences with the same extraction DNA method conducted in the same lab. In case the DNA barcoding method did not yield results due to short sequences or contaminations, we kept the field identification for those species (for 15 species, eight for amphibians, and seven for reptiles).

We attributed the red list status to evaluated species following IUCN (2019). We determined endemism according to AmphibiaWeb (2020) for amphibians and Uetz *et al.* (2021) for reptiles. Because most amphibian and reptile genera are completely endemic to Madagascar, we could attribute endemism also to morphospecies.

### Data analysis and visualization: species diversities and encounters

During the data analysis, we conducted all statistical analyses using R version 3.5.1 (R Core Team, 2019) and the R package “ggplot2” (Wickham, 2016) for visualization. To compare observed species richness and species encounters at the plot-level among land-use types, we computed a generalized linear model with observed species richness as a response, land-use type as the explanatory variable, and village (respectively old-growth forest site) as a random factor and with Poisson family for count data as distribution. We then ran the *glht* function of the R-package “multcomp” (Hothorn *et al.* 2008) applying a Tukey all-pair-comparison with Bonferroni correction. We then used the same approach to compare observed species richness between plots from unburned land-use types (old-growth forest, forest fragment, forest-derived vanilla) and plots from burned land-use types (herbaceous fallow, woody fallow, fallow-derived vanilla).

We used the encounter data to compute sample size-based extrapolation curves (Hsieh *et al.* 2016) with the “iNEXT” package to assess the diversity (species richness, Shannon, and Simpson diversity) across land-use types using the Hill number framework (Chao *et al.* 2014). We calculated species richness, Shannon diversity, and Simpson diversity (Hsieh *et al.* 2016). Chao & Jost (2012) define  $q = 0$  (0D) as species richness, that is the effective number of species in the community, giving equal weight to frequent and infrequent species;  $q = 1$  (1D) as Shannon diversity, giving more weight to more frequently observed species and  $q = 2$  (2D), as Simpson diversity interpreted as the effective number of abundant species. This approach is now well established given key advantages over traditional Shannon and Simpson diversity indices (Roswell *et al.* 2021).

To display the total species diversity in each land-use type, we sub-sampled 10 plots of the 20 fallow-derived vanilla agroforests. To do so, we randomly selected one fallow-derived vanilla agroforest from each village. As one of the villages lacks fallow-derived vanilla agroforests (village Andramanolotra, see Fig. 1), this resulted in nine agroforests. We proceeded to select one additional plot from all remaining fallow-derived vanilla agroforests, enabling a fair comparison of total species diversity across 10 plots of each land-use type.

To compare the evenness of communities between land-use types, we additionally plotted the observed and extrapolated diversity across hill numbers for each land-use type, allowing for a direct comparison of slopes between land-use types (Chao & Jost, 2015; Roswell *et al.* 2021).

## Data analysis and visualization: species composition

To evaluate the differences in species community composition between land-use types between burned and unburned plots, and between villages/sites, we used the *metaMDS* function of the R-package “vegan” (with 1000 permutations, Oksanen *et al.* 2020). We used non-metric dimensional scaling (NMDS) of Bray-Curtis dissimilarities to visualize the dissimilarity of species composition in two dimensions. Furthermore, to test the differences between land-use types, we used PERMANOVA (Permutational multivariate analysis of variance) implemented in the *adonis* function of the “vegan” package (Oksanen *et al.* 2020) and computed pairwise differences using the *pairwise.adonis* function with Bonferroni correction of the “*pairwise.adonis*” package (Martinez, 2020). Since PERMANOVA may confound location and dispersion, we used PERMDISP (Permutational analysis of multivariate dispersions) to see if PERMANOVA results may have been influenced by dispersion differences between groups (Anderson & Walsh, 2013). To do so, we used the function *betadisper* and *permutest* and performed pairwise comparisons using the *TukeyHSD* function. To analyze the degree of forest dependency (Rembold *et al.* 2017), we plotted the proportion of encounters for each species across seven land-use types (10 replicates per land-use type). To visualize the forest dependency, we used the *barplot* function of R graphics.

## Results

### Encounters and observed species richness

In total, we made 6215 encounters and observed 119 species of amphibians and reptiles. The 3694 amphibian encounters belong to 58 species, 15 genera, and four families (see Table S1). The most species-rich genera of amphibians are *Boophis* (11 species), *Stumpffia* (10 species), and *Gephyromantis* (eight species). We found all but one species (*Ptychadena mascareniensis*) to be endemic according to AmphibiaWeb (2020). Among the observed amphibian species, 22 (37%) could not be identified to species level in the field. Twelve of them were recognized as candidates for new species based on genetic barcoding, another eight of them were counted as morphospecies based on the criteria mentioned in the methods section, and two were conferred to previously identified species. Among the encountered amphibian species, seven are listed in the “threatened” category (IUCN 2019). Among the threatened species, we recorded six vulnerable and one endangered species.

The 2521 reptile encounters represent 61 species, 28 genera, and five families (see Table S1). The most species-rich reptile genera were *Phelsuma* (11 species) and *Uroplatus* (six species). We found 83% of reptile species to be endemic (Uetz *et al.* 2021). Amongst observed reptile species, 15 could not be identified to species level in the field; of these, seven were counted as morphospecies and eight were conferred to previously identified species. Based on the IUCN red list (IUCN 2019), seven encountered reptile species are

listed as “threatened.” Among the threatened species, we recorded four endangered, one critically endangered, and two vulnerable species.

Plot-level observed amphibian species richness differed significantly among land-use types ( $F_{6,73} = 19.59$ ,  $P < 0.001$ ) and when comparing all burned to all unburned land-use types ( $F_{1,67} = 15.89$ ,  $P < 0.001$ ; Figure S6 and Table S7). A Tukey post-hoc test revealed significant pairwise differences between pairs of land-use types (Fig. 2a and Table S2): old-growth forest had significantly higher observed amphibian species richness while rice paddy had significantly lower observed species richness compared with other land-use types (Fig. 2a). The other land-use types had similarly observed amphibian species richness with no significant differences (Table S2). Plot-level amphibian encounters also differed significantly between land-use types ( $F_{6,72} = 13.01$ ,  $P < 0.001$ ; Figure S9, Tables S10 and S12).

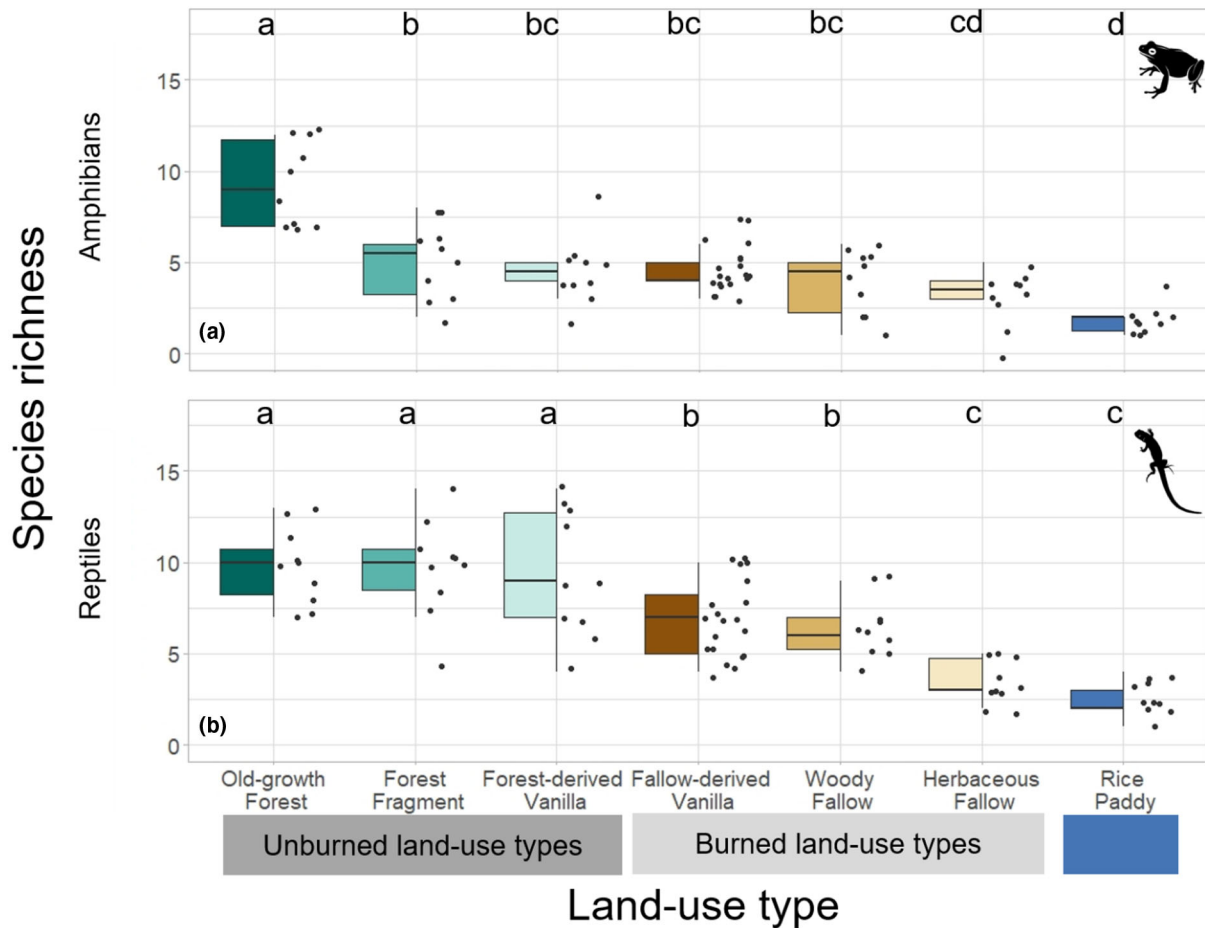
Plot-level observed reptile species richness also varied significantly among land-use types ( $F_{6,73} = 18.55$ ,  $P < 0.001$ ) and when comparing all burned to all unburned land-use types ( $F_{1,67} = 25.60$ ,  $P < 0.001$ ; Figure S6 and Table S8). Tukey post-hoc tests revealed no differences in observed species richness between old-growth forest and forest fragment ( $P = 0.88$ ) and between old-growth forest and forest-derived vanilla agroforest ( $P = 0.77$ ). These three land-use types are recorded with the highest observed species richness, with 10 reptile species on average per plot. Rice paddy had the lowest observed reptile species richness, but there were no significant differences ( $P = 0.19$ ) compared with herbaceous fallow. Observed reptile species richness was significantly higher in forest-derived vanilla agroforest than in fallow-derived vanilla agroforest ( $P = 0.01$ ; Fig. 2b and Table S2). Plot-level reptile encounters also differed significantly between land-use types ( $F_{6,73} = 14$ ,  $P < 0.001$ ; Figure S9, Tables S11 and S12).

### Species accumulation curves, diversity, evenness, and estimated species richness

Encounter-based accumulation curves for amphibians revealed the highest species richness, Shannon diversity, and Simpson diversity in old-growth forest and the lowest in rice paddy. In most land-use types, observed amphibian species richness curves flattened off, except for old-growth forest and forest fragment. The overlap of the 95% confidence interval of extrapolated amphibian richness for old-growth forest and forest fragment indicates no differences in species richness. Regarding the accumulated amphibian species diversity for Shannon and Simpson diversity, old-growth forest varied significantly compared with all other land-use types. Shannon and Simpson diversity also did not differ significantly between forest fragment and forest-derived vanilla agroforest, respectively, compared with burned land uses, except to rice paddy (Fig. 3 and Table 1).

Encounter-based accumulation curves for reptiles revealed the highest species richness and Shannon diversity in old-growth forest and the lowest in herbaceous fallow. Observed species richness curves flattened off in all land-use types except woody fallow and fallow-derived vanilla agroforest.





**Figure 2** Plot-level observed amphibian (a) and reptile (b) species richness across seven land-use types (replicated 10 times each except fallow-derived vanilla, which is replicated 20 times) in north-eastern Madagascar. Each dot is the observed species richness in a plot. The black horizontal line in the box shows the median. Land-use types with letters in common did not differ significantly based on pairwise comparisons that controlled for inflated false-positive errors using the Tukey HSD approach (Numeric results in SI: amphibians & reptiles Table S2). See icon reference in supplementary information (Free Icons Library)

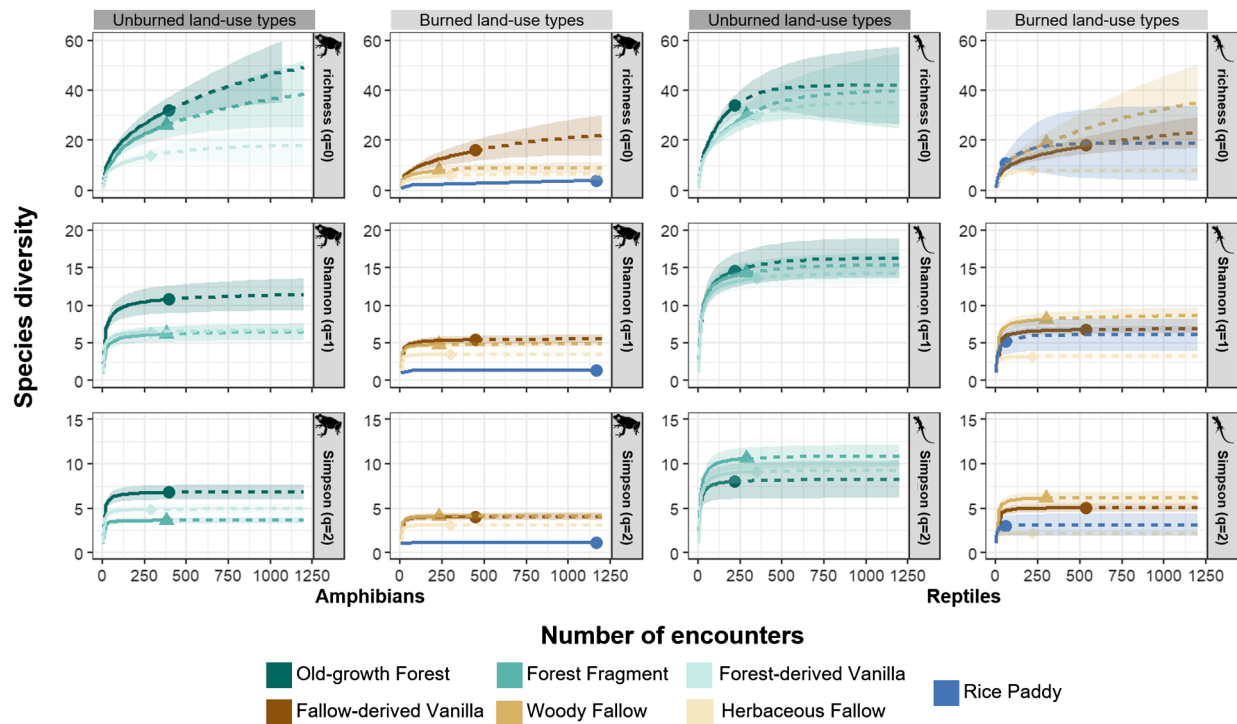
We also found an overlap of the 95% confidence intervals of extrapolated species richness as well as Shannon and Simpson diversity within unburned and burned land-use types except for Shannon and Simpson diversity in burned land-use types (Fig. 3 and Table 1).

The species diversity dropped more strongly across hill number orders ( $q = 0$  to  $q = 2$ ) in amphibians than in reptiles after old-growth forest transformation (Fig. 3, Table S3 and Figure S5), highlighting that amphibian communities were more uneven than reptile communities (Figure S5). Furthermore, the comparison shows that old-growth forest communities of both amphibians and reptiles were most uneven (Figure S5).

### Species composition and forest dependency

The composition of amphibian communities differed significantly across land-use types (PERMANOVA:  $R^2 = 0.50$ ,

$P < 0.001$ ,  $Df = 6$ ), partly driven by differences in group dispersion (PERMDISP:  $F = 4.400$ ,  $P = 0.004$ ,  $Df = 6$ , Tables S18 and S19). Pairwise comparisons showed that amphibian communities in old-growth forest and rice paddy were significantly different from those of other land-use types. Forest fragment differed significantly from fallow-derived vanilla agroforest, woody fallow, and herbaceous fallow, but no significant differences were observed between forest fragment and forest-derived vanilla agroforest. We found no significant differences between forest-derived vanilla agroforest, fallow-derived vanilla agroforest, woody fallow, and herbaceous fallow for amphibian communities (Fig. 4a, see Table S4). We compared also the amphibian communities between unburned and burned land-use types and found significant differences (PERMANOVA:  $R^2 = 0.193$ ,  $P < 0.001$ ,  $Df = 1$ , Figure S13 and Table S14), again partly driven by dispersion (PERMDISP:  $F = 34.718$ ,  $P = 0.001$ ,  $Df = 1$ , Table S16). Similarly, amphibian communities differed between villages and Marojejy National Park communities



**Figure 3** Encounter-based accumulation curves showing interpolated (solid line) and extrapolated (dotted line) diversities for amphibians (first and second columns of panel from left to right) and reptiles (third and fourth panels) in north-eastern Madagascar. Unburned (first and third panels) and burned (second and fourth panels) land-use types are separated. The species richness represented by  $q = 0$  (first row of panel from above), Shannon diversity,  $q = 1$  (second row of panel) and Simpson diversity  $q = 2$  (third row of panel) with 95% confidence intervals (shaded areas) for the amphibian and the reptile data of seven land-use types. The solid dots, triangles, and diamonds represent the reference samples, that is the observed number of encounters and species richness. See icon reference in supplementary information (Free Icons Library)

**Table 1** Estimated and observed amphibian and reptile species richness ( $q = 0$ ) for all land-use types and separated per land-use type showing the observed and extrapolated total species richness

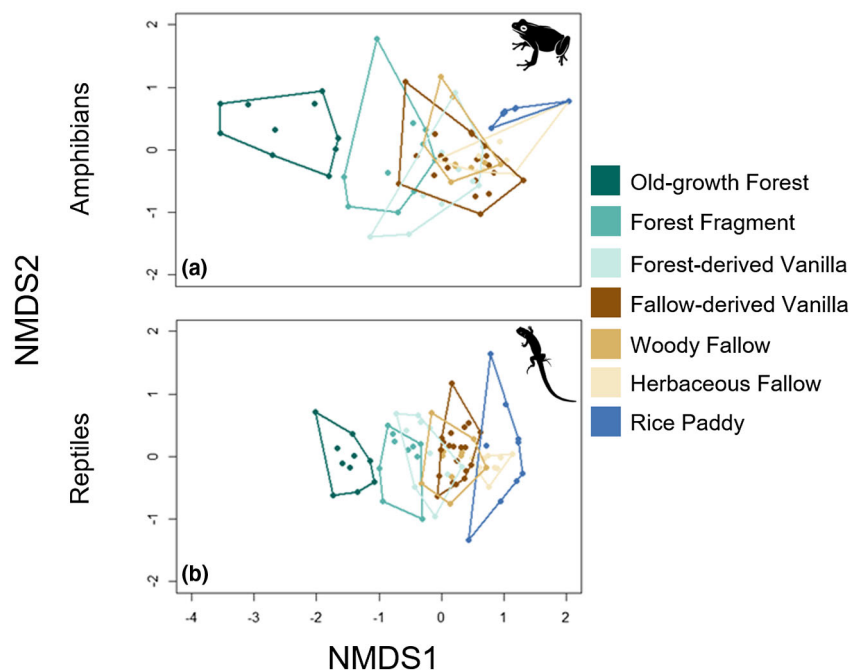
Species Group (measure)	Land-use type							
	All 80 plots	Old-growth forest	Forest fragment	Forest-derived vanilla agroforest	Fallow-derived vanilla agroforest	Woody fallow	Herbaceous fallow	Rice paddy
Amphibian (observed)	58	32	26	14	16	8	6	4
Amphibian (extrapolated)	NA	60.0 (20.4–99.6)	46.1 (12.4–79.8)	18.0 (9.1–26.9)	25.0 (7.0–42.9)	9.0 (7.4–10.6)	6.5 (3.9–9.1)	5.0 (4.3–5.6)
Reptile (observed)	61	34	30	30	18	19	8	11
Reptile (extrapolated)	NA	42.0 (20.4–63.5)	40.1 (10.2–69.9)	35.1 (18.2–52.4)	26.9 (8.2–45.7)	46.6 (11.5–81.7)	8.0 (6.8–9.2)	18.9 (4.3–33.4)
Total (observed)	119	66	56	44	34	27	14	15

Each land-use type is represented by 10 plots; for fallow-derived vanilla, the 10 plots are down-sampled from 20 plots. Extrapolated species richness is based on 5000 encounters and includes the lower and upper 95% confidence interval in italics in the brackets. See SI for results of  $q = 1$  and  $q = 2$  (amphibians and reptiles: Table S3).

(PERMANOVA:  $R^2 = 0.416$ ,  $P < 0.001$ ,  $Df = 10$ , Figure S21, Tables S22 and S24), in this case not driven by differences in group dispersion (PERMDISP:  $F = 1.254$ ,  $P = 0.283$ ,  $Df = 11$ , Table S25). Here, communities were very similar between villages (Figure S21).

Reptile species composition showed significant differences among land-use types (PERMANOVA:  $R^2 = 0.40$ ,  $P < 0.001$ ,  $Df = 6$ ) not driven by differences in group dispersion (PERMDISP:  $F = 1.870$ ,  $P = 0.088$ ,  $Df = 6$ , Table S20) indicating the homogeneity of group dispersion.





**Figure 4** Species composition across seven land-use types in north-eastern Madagascar. Non-metric dimensional scaling (NMDS) showing community dissimilarity of amphibian (a) and reptile (b) communities. Old-growth forest shows unique species composition for both amphibians and reptiles. See icon reference in supplementary information (Free Icons Library)

Pairwise comparisons showed that the reptile communities in old-growth forest and forest fragment were significantly different from all other land-use types. The communities in other land-use types showed no significant differences (Fig. 4b and Table S4). Old-growth forest showed unique communities of reptiles and amphibians. The reptile species composition between unburned and burned land-use types showed significant differences (PERMANOVA:  $R^2 = 0.210$ ,  $P = .001$ ,  $Df = 1$ , Table S15) not driven by differences in group dispersion (PERMDISP:  $F = 3.918$ ,  $P = 0.057$ ,  $Df = 1$ , Table S17). The reptile communities between villages and Marojejy National Park showed significant differences (PERMANOVA:  $R^2 = 0.389$ ,  $P < 0.001$ ,  $Df = 10$ , Table S23 and S24) not driven by differences in group dispersion (PERMDISP:  $F = 0.835$ ,  $P = 0.626$ ,  $Df = 11$ , Table S26).

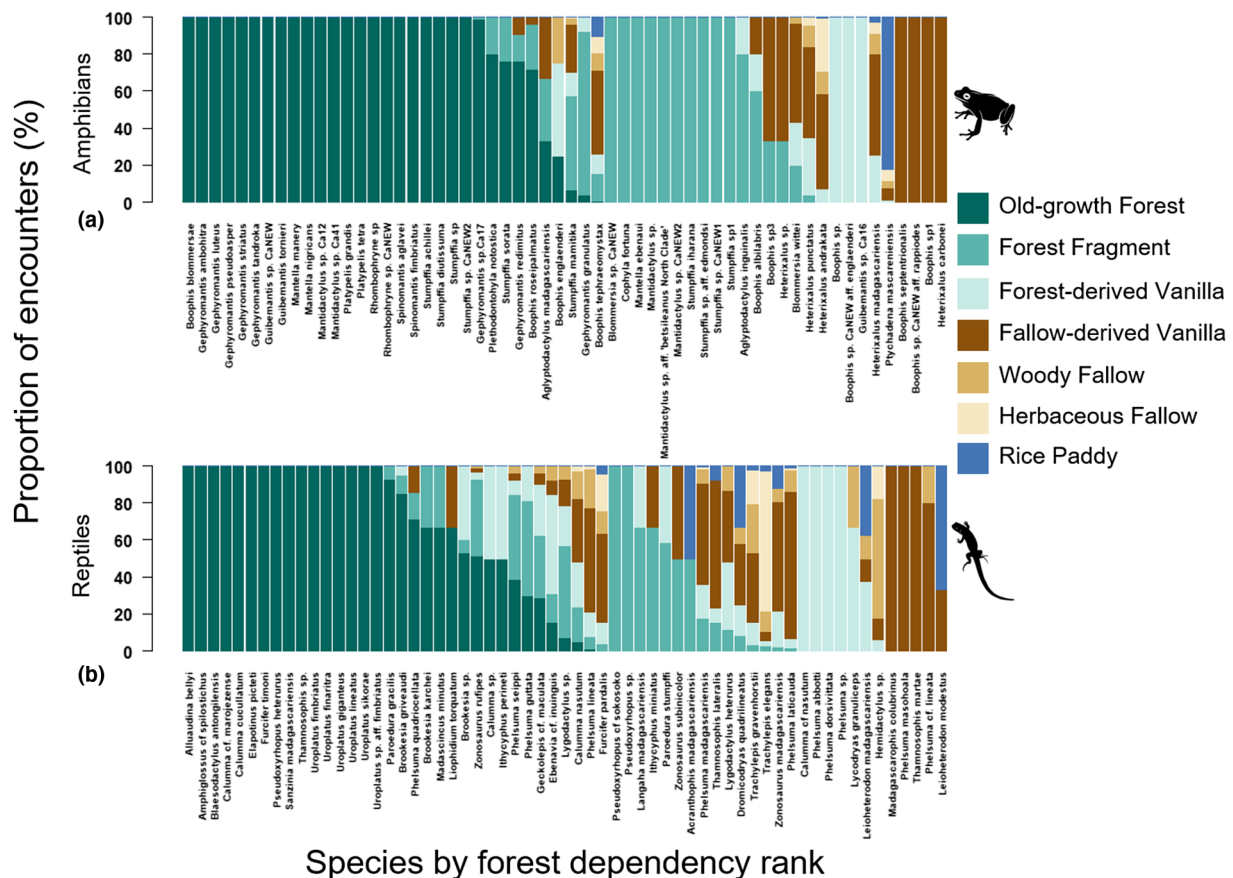
Finally, the analyses of forest dependency revealed that 22 amphibian species (38%) were found exclusively in old-growth forest, 11 amphibian species (19%) exclusively in forest fragment, three amphibian species (5%) exclusively in forest-derived vanilla agroforest, and four amphibian species (7%) exclusively in fallow-derived vanilla agroforest (Fig. 5a).

We observed 16 reptile species (26%) exclusively in old-growth forest, three reptile species (5%) exclusively in forest fragment, four reptile species (7%) exclusively in forest-derived vanilla agroforest, and one reptile species (2%) exclusively in fallow-derived vanilla agroforest (Fig. 5b). No species were exclusively found in woody fallow, herbaceous fallow, and rice paddy for both groups.

## Discussion

In this study, we provide a comprehensive assessment of the response of amphibian and reptile species diversity to land-use change in the biodiversity hotspot of north-eastern Madagascar. Overall, we document a highly diverse herpetofauna with 58 amphibian and 61 reptile species and presumed species, and a high proportion of endemic species (98% for amphibians and 83% for reptiles).

At a plot-level, observed species richness for both amphibians and reptiles was very high, with up to 12 and 14 species, respectively. For both groups, old-growth forest was significantly different from all other land-use types in terms of total estimated species richness and community composition. Rice paddy and herbaceous fallow harbored the lowest observed species richness for amphibians and reptiles, respectively. Reptile species diversity varied significantly between forest-derived and fallow-derived vanilla agroforests whereas amphibian species richness showed no differences. We found no unique species in woody fallow, herbaceous fallow, or rice paddy plots for either taxon. Importantly, we found that amphibians and reptiles responded differently to land-use history. After any kind of old-growth forest conversion, whether through burning or not, amphibian species communities both declined in richness and shifted in structure away from old-growth forest. Deforestation for shifting cultivation through slash-and-burn led to species losses in reptiles as well, but changes were less pronounced if old-growth forest was transformed to forest fragment or forest-



**Figure 5** Proportion of species encounters across seven land-use types (with 10 replicates each) in north-eastern Madagascar. All 58 amphibian species with 3694 encounters (a) and all 61 reptile species (b) with 2521 encounters are displayed by forest dependency rank: 38% of amphibian species and 26% of reptile species exclusively occurred in old-growth forest, despite that old-growth forest is only accounting for 12.5% of the total plots. See icon reference in supplementary information (Free Icons Library)

derived vanilla agroforest since these transformations refrain from using fire.

## Outstanding diversity of amphibians and reptiles in north-eastern Madagascar

The diversity of amphibians and reptiles documented here was exceptional, both within Madagascar and compared with other tropical biodiversity hotspots. The few available studies on the response of amphibian communities to land-use change in Madagascar reported lower overall values, with 32 and 28 species, respectively (Vallan, 2000; Ndriantsoa *et al.* 2017). Andreone *et al.* (2000) found 42 amphibian and 23 reptile species in north-eastern Malagasy rainforest. Despite differences in methodology and study sites, our study provides additional evidence that the north-east of Madagascar is one of the most species-rich regions for herpetofauna in the country, as previously suggested (Brown *et al.* 2016). The documented diversity also exceeds values found in other tropical biodiversity hotspots (Cordier *et al.* 2021; Murray & Nowakowski, 2021) – for example, Urbina-Cardona *et al.*

(2006) found 21 amphibian and 33 reptile species in the region of Veracruz, Mexico, and Kurz *et al.* (2014) found 25 amphibian and 20 reptile species in a region of north-eastern Costa Rica (versus 58 amphibian and 61 reptile species in this study). The high species diversity may partly be driven by our extensive sampling effort, in terms of number of plots (total  $n = 80$ ), diversity of different land-use types (seven), and search effort (total of 270 min searching time by two observers per plot), and is likely also influenced by the use of genetic samples for species identification.

## Response of amphibian diversity to land-use change

We found a strong negative response of amphibian species richness to any anthropogenic land use. Old-growth forest had a significantly higher observed amphibian species richness with 38% species exclusively occurring there. The amphibian species composition is unique compared with all other land-use types, driven by a high share belonging to the Microhylidae family and the *Spinomantis*, *Guibemantis*, and

*Mantella* genera (Fig. 5a). Only forest fragments harbored comparable accumulated amphibian species richness. Subsequently, we infer that Malagasy amphibians are very sensitive to habitat change. This result is in line with findings from Ndrantsoa *et al.* (2017) in disturbed habitats of eastern Madagascar which documented strongly impoverished frog communities in secondary vegetation and rice fields compared with forests. Forest fragments are thus valuable for Malagasy amphibians to maintain diversity within the agricultural landscape but cannot substitute large and continuous old-growth forest (Vallan, 2000).

The other land-use types (fallow that form part of the slash-and-burn cycle, vanilla agroforests, and rice paddies) are of minor importance for amphibian conservation, given the low species diversity that consists mainly of common and widely ranging species. Nonetheless, amphibians could play an important functional role in these habitats: the abundance of amphibians is high throughout, reflected by the high number of encounters, particularly in rice paddies (see Fig. 3b and Figure S9). As such, they may be an important food source for other taxa or could provide pest control services in crops (Hocking *et al.* 2014).

The strong negative response of amphibians to deforestation could be explained by the fact that numerous species rely on moist environments to avoid dehydration (Watling & Braga, 2015), especially species living in evergreen forest habitat (Hof *et al.* 2011). Given that selective logging, forest fragmentation, and deforestation severely change microclimate conditions (Ewers & Banks-Leite, 2013), many species may struggle to cope. Furthermore, amphibians have different habitat requirements throughout their life cycle stages (Hof *et al.* 2011; Hocking *et al.* 2014). Such heterogeneity is often lost in concert with the simplification in forest structure (Wanger *et al.* 2009), making new habitats unsuitable for many species.

### Response of reptile diversity to land-use change

We found a strong effect of land-use history on reptile diversity. Unburned land-use types had a significantly higher average species richness, more uneven communities, more unique species, and a distinct species composition compared with burned land-use types and rice paddy. The distinct species composition in unburned land-use types was caused by an increased number of species belonging to the Chamaeleonidae family and the *Uroplatus* genus (Fig. 5b) compared with other land-use types. This is, to our knowledge, the first study shedding light on the response of reptiles to land-use change in Madagascar's humid eastern escarpment. Studies from the drier southern region (Scott *et al.* 2006; Gardner *et al.* 2016; Nopper *et al.* 2018) show that Malagasy reptile species react less strongly to habitat change than other taxa, especially if diurnal (Nopper *et al.* 2018).

Interestingly, the less pronounced effects of land-use change on reptiles compared with amphibians in tropical landscapes has also been demonstrated by others (Wanger *et al.* 2009; Kurz *et al.* 2014) and was found in a review by

Palacios *et al.* (2013). This may be due to the high thermotolerance in reptiles (Thompson & Donnelly, 2018), thanks to a fairly impermeable skin covered with scales. This structure prevents excessive evaporative water loss and adapts reptiles to various microclimates (Watling & Braga, 2015). Furthermore, egg development is not heavily impacted by temperature rise, which reduces the size of hatchlings but accelerates incubation (Packard *et al.* 1982). Reptile traits associated with more open, hotter, and drier environments (Morin, 2005) may make them less vulnerable to land-use change.

### Land-use history of vanilla agroforests affects reptiles but not amphibians

In our study, we have separated forest-derived vanilla from fallow-derived vanilla agroforests, thereby explicitly accounting for land-use history (Martin *et al.* 2020). For amphibians, we found no differences between the two agroforestry systems across metrics. Reptile communities, on the other hand, were significantly more diverse in forest-derived vanilla agroforests on plot-level, more species-rich overall, more uneven, and compositionally different compared with fallow-derived vanilla agroforest. Reptile communities observed in forest-derived vanilla agroforests are thus more similar to old-growth forest and forest fragment. The reptile communities recorded in fallow-derived vanilla agroforests were comparable with fallow land. Both kinds of agroforest thus resembled the land-use types they were derived from. However, we observed an increase in accumulated amphibian species richness from fallow land to fallow-derived vanilla agroforest, which suggests rehabilitation through agroforestry (Osen *et al.* 2021). These findings are in line with the prediction from a recent study by Martin *et al.* (2020) that found that the land-use history of agroforestry systems matters for biodiversity.

We further hypothesize that the strong importance of forest-derived vanilla agroforests for reptiles may be in part driven by leaf-litter depth, which is known to positively influence reptile diversity and abundance (Urbina-Cardona *et al.* 2006), and which is typically deeper in forest-derived vanilla agroforests. According to the same study, these microenvironmental variables also showed positive effects on amphibians and thus cannot explain why there are no differences between the two kinds of agroforests for amphibians. Elsewhere, the loss of canopy cover had negative impacts on amphibian and reptile species richness (Scott *et al.* 2006). Since canopy cover differed significantly between forest-derived vanilla agroforest (higher) and fallow-derived vanilla agroforest (lower) in the same study site (Osen *et al.* 2021), this calls for further investigation combining habitat characteristics with species traits (Oliveira *et al.* 2017) to elucidate the drivers of change in both species groups.

### Conservation implications

The strong negative response of amphibians to old-growth forest modification and the high old-growth forest dependency of

amphibians and reptiles calls for the effective protection of the last remaining old-growth forests. Additionally, conserving forest fragments within the agricultural landscape will be important to many reptile and amphibian species that are absent from other land-use types. This is particularly important given that numerous species are micro-endemics (Brown *et al.* 2014), meaning that they only occur very locally (Vieites *et al.* 2009), possibly in only a handful of forest fragments. Management strategies are thus needed to safeguard the long-term existence of forest fragments and to improve the connectivity between fragments. The protection of large continuous forests throughout the region is also important under a changing climate (Hof *et al.* 2011) and in light of emerging threats, such as the chytrid fungi (Kolby & Skerratt, 2015) and the recent spread of the invasive Asian common toad (*Duttaphrynus melanostictus*, Soorae *et al.* 2020). These conservation needs are underscored by the exceptional diversity of reptiles and amphibians in north-eastern Madagascar as well as by the high proportion of endemic species.

Our findings from north-eastern Madagascar also have broader relevance to herpetofauna conservation in other tropical biodiversity hotspots. The differential response of amphibians and reptiles to land-use change shows that conservationists should not treat herpetofauna as a homogenous group when devising conservation programs. Furthermore, the distinct differences in reptile communities between plots of contrasting land-use history shows that past land-use must also be considered, and suggests that the maintenance of remnant forest fragments and forest-derived agroforests is important. Lastly, we highlight the importance of unburned compared with burned land-use types in the agricultural matrix for the conservation of reptiles.

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samples to Germany under CITES permits DE-E-03377/18, DE-E-03378/18, DE-E-02422/19, DE-E-02423/19, DE-E-02424/19, and DE-E-02425/19 granted by the German Federal Agency for Nature Conservation, Bonn. This study was financially supported by the Niedersächsisches Vorab of Volkswagen Foundation as part of the research project “Diversity Turn in Land Use Science” (Grant number 11-76251-99-35/13 (ZN3119)) and by the German Academic Exchange Service (DAAD) within the “Partnerships for Supporting Biodiversity in Developing Countries” initiative (Project Nr. 57449386). Open Access funding enabled and organized by Projekt DEAL. WOA Institution: GEORG-AUGUST-UNIVERSITÄT GÖTTINGEN. Blended DEAL: Projekt DEAL

## Conflict of interest

No conflict of interest to declare.

## Author's contributions

TRF, DAM, KO, AW, HK, AA, and FMR designed the study. TRF, EB, RB, and RR collected amphibian and reptile data under the lead of TRF. TRF and DAM analyzed the data. TRF and DAM wrote the first manuscript draft. All authors revised the manuscript.

## Data Availability Statement

Data are available from Zenodo: <http://doi.org/10.5281/zenodo.4548955>

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## Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Table S1.** List of encountered amphibian and reptile species per land-use types with IUCN status and endemism to Madagascar, ‘*cf.*’ and ‘*sp. aff.*’: confer to identified species, ‘*sp.*’: not identified species and shows dissimilarity of morphological characters to identified species, ‘*sp. CaNEW*’ (sometimes followed by number, e.g. *sp. CaNEW1*): Candidate for new species according to DNA barcoding recorded during the current study, ‘*sp. Ca*’ (sometimes followed by a number, e.g. *sp. Ca17*): Candidate for new species according to DNA barcoding which has already been recorded by other researchers, LC: Least Concern, NA: Not Applicable, VU: Vulnerable, NE: Not Evaluated, EN: Endangered, NT: Near Threatened, CR: Critically Endangered, OGF (Old-growth

forest), FF (forest fragment), VFST (Forest-derived vanilla agroforest), VFLW (Fallow-derived vanilla agroforest), WF (woody fallow), HF (herbaceous fallow), RP (rice paddy).

**Table S2.** Tukey post-hoc pairwise comparisons for observed amphibian and reptile species richness with plot as sample between land-use types.

**Table S3.** Extrapolated species diversity (species richness, Shannon diversity and Simpson diversity) based on 5000 encounters and includes the lower and upper 95% confidence interval for amphibians and reptiles.

**Table S4.** Pairwise-adonis test for amphibian and reptile species composition between land-use types.

**Figure S5.** Curves showing the evenness of (A) amphibian and (B) reptile communities across seven land-use types in north-eastern Madagascar using order of  $q$  between 0 and 3 with 95% confidence intervals.

**Figure S6.** Plot-level observed amphibian (A) and reptile (B) species richness of unburned land-use types (old-growth forest, forest fragment, and forest-derived vanilla agroforest) and burned land-use types (herbaceous fallow, woody fallow, and fallow-derived vanilla agroforest) in north-eastern Madagascar.

**Table S7.** Comparison of observed amphibian species richness between unburned and burned land-use types.

**Table S8.** Comparison of observed reptile species richness between unburned and burned land-use types.

**Figure S9.** Plot-level amphibian (A) and reptile (B) species encounters across seven land-use types in north-eastern Madagascar.

**Table S10.** Comparison of amphibian species encounters across seven land-use types.

**Table S11.** Comparison of reptile species encounters across seven land-use types.

**Table S12.** Tukey post-hoc pairwise comparisons for amphibian and reptile species encountered between land-use types.

**Figure S13.** Species composition in unburned and burned land-use in north-eastern Madagascar.

**Table S14.** Adonis test of community dissimilarity for observed amphibian species between unburned and burned land-use types.

**Table S15.** Adonis test of community dissimilarity for observed reptile species between unburned and burned land-use types.

**Table S16.** Permutation test for homogeneity of multivariate dispersions for amphibian species composition within unburned and burned land-use types.

**Table S17.** Permutation test for homogeneity of multivariate dispersions for reptile species composition within unburned and burned land-use types.

**Table S18.** Permutation test for homogeneity of multivariate dispersions for amphibian species composition within seven land-use types.

**Table S19.** Tukey multiple comparisons of means for homogeneity of multivariate dispersions for amphibian species composition within seven land-use types.

**Table S20.** Permutation test for homogeneity of multivariate dispersions for reptile species composition within seven land-use types.

**Figure S21.** Species composition of amphibian and reptile per village and in Marojejy National Park in north-eastern Madagascar.

**Table S22.** Adonis test of community dissimilarity for amphibian species between villages and Marojejy National Park (MJ).

**Table S23.** Adonis test of community dissimilarity for reptile species between villages and Marojejy National Park (MJ).

**Table S24.** Pairwise-adonis test for amphibian and reptile species composition between villages and Marojejy National Park.

**Table S25.** Permutation test for homogeneity of multivariate dispersions for amphibian species composition within villages and Marojejy National Park (MJ).

**Table S26.** Permutation test for homogeneity of multivariate dispersions for reptile species composition within villages and Marojejy National Park (MJ).