RESEARCH ARTICLE

UN Decade on Ecosystem Restoration

Impacts of herbivory by ecological replacements on an island ecosystem

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Abstract

1. The use of ecological replacements (analogue species to replace extinct taxa) to restore ecosystem functioning is a promising conservation tool. However, this approach is controversial, in part due to a paucity of data on interactions between analogue species and established taxa in the ecosystem.

2. We conducted ecological surveys, comprehensively DNA barcoded an ecosystem's flora and inferred the diet of the introduced Aldabra giant tortoise, acting as an ecological replacement, to understand how it might have modified island plant communities on a Mauritian islet. Through further dietary analyses, we investigated consequential effects on the threatened endemic Telfair's skink.

3. Dietary overlap between tortoises and skinks was greater than expected by chance. However, there was a negative correlation between tortoise and skink preferences in herbivory and minimal overlap in the plants most frequently consumed by the reptiles.

4. Changes in the plant community associated with 7 years of tortoise grazing were characterised by a decrease in the percentage cover of native herbs and creepers, and an increase in the cover of an invasive herb when compared to areas without tortoises. However, tortoise dietary preferences themselves did not directly drive changes in the plant community.

5. Tortoises successfully dispersed the seeds of an endemic palm, which in time may increase the extent of unique palm-rich habitat. We found no evidence that tortoises have increased the extent of plant species hypothesised to be part of a lost Mauritian tortoise grazed community.

6. *Synthesis and applications*. Due to a negative correlation in tortoise and skink dietary preferences and minimal overlap in the most frequently consumed taxa, the presence of tortoises is unlikely to have detrimental impacts on Telfair's skinks. Tortoise presence is likely to be beneficial to skinks in the long term by increasing the extent of palm-rich habitat. Although tortoises are likely to play a

role in controlling invasive plants, they are not a panacea for this challenge. After 7 years, tortoises have not resurrected a lost tortoise grazed community that we hypothesise might have existed in limited areas on the islet, indicating that further interventions may be required to restore this plant community.

KEYWORDS
alien species, analogue species, DNA barcoding, DNA metabarcoding, ecosystem restoration, giant tortoises, herbivory, skinks

1 | INTRODUCTION

Catastrophic declines in biodiversity and habitat degradation have led scientists to debate both a sixth mass extinction and the dawn of the Anthropocene epoch, where humans are the main driver of environmental change (Chapin et al., 2000; Dirzo et al., 2014). The loss of keystone species, including seed dispersers, grazers and apex predators, is of particular concern as they have cascading effects on ecosystems, disrupting processes, such as seed dispersal and pollination (Dirzo et al., 2014; Estes et al., 2011; Kaiser-Bunbury et al., 2010). These trophic cascades can compromise the structural and functional complexity of ecosystems leaving them less resilient to global change (Fernandez et al., 2017; Griffiths et al., 2010).

Ecological restoration has the potential to reverse ecosystem degradation by re-establishing lost species composition or ecosystem function (Corlett, 2016; Suding, 2011). In highly degraded ecosystems missing many of their original species, one restoration approach is ecological replacement; the introduction of a non-native species to functionally replace its extinct counterpart (IUCN/SSC, 2013). Ecological replacement is often associated with rewilding (Fernandez et al., 2017), which emphasises ecological function over composition, but remains controversial due to potential unresolved ecological, evolutionary, practical and societal issues (Caro, 2007). For example, there are risks of ecological replacements having unpredictable effects on biodiversity (Smith, 2005) and unforeseen interactions with native taxa (Donlan et al., 2005; Ricciardi & Simberloff, 2009; Rubenstein et al., 2006). Limited empirical studies make it difficult to support the hypothesis that ecological replacements will replicate the ecological interactions of their extinct counterparts (Donlan et al., 2005; Fernandez et al., 2017; Svenning et al., 2016). Our study addresses these issues by analysing the impacts of 7 years of ecological replacement on the flora, fauna and ecological function of the ecosystem of Round Island, Mauritius.

Two endemic species of giant tortoise, *Cylindraspis inepta* and *C. triserrata*, occurred on mainland Mauritius until the 18th century, with the last surviving individual *Cylindraspis* sp. on Round Island until the 1840s (Cheke & Hume, 2008). These tortoises likely exploited differing niches; the smaller domed *C. inepta* as a grazer and the saddle-backed *C. triserrata* as a browser (Arnold, 1979). Like extant tortoises, *Cylindraspis* spp. were probably keystone herbivores

(Gibbs et al., 2008). Tortoises would have modulated Mauritian plant community structure and composition through grazing, browsing, trampling, wallowing, seed dispersal and nutrient cycling. The extinction of tortoises resulted in the disruption of plant–herbivore interactions (Griffiths, 2014; Griffiths et al., 2011), with probable cascading effects on native fauna and flora. Reintroduction of endemic tortoises to reinstate lost species interactions was clearly impossible, but ecological replacement with a functionally similar species was feasible. The introduction of Aldabra giant tortoises *Aldabrachelys gigantea* (hereafter referred to as tortoises) to Mauritian ecosystems began in the 1990s, with the first trial releases on Ile aux Aigrettes (Tatayah et al., 2018). Eleven tortoises were introduced to enclosures on Round Island in 2007, with releases onto the island the following year (Griffiths et al., 2010). The population was supplemented from 2010, and by 2015 Round Island supported approximately 350 free-roaming individuals, the majority of which were juveniles. The suitability of *A. gigantea* as a replacement for *Cylindraspis* spp. has been discussed elsewhere (Griffiths, 2014; Griffiths et al., 2010), and the larger *A. gigantea* is capable of both grazing and browsing depending on its size.

Unintended negative impacts of species reintroductions and replacements are of particular concern on Round Island since, despite severe habitat degradation, it is a refugium for an endemic reptile community, including the IUCN Vulnerable (Cole, Goder, Roopa, et al., 2018) omnivorous Telfair's skink *Leiolopisma telfairii* (hereafter referred to as skinks) that are key seed dispersers, pollinators and predators (Zuël et al., 2012). This species was restricted to Round Island by the mid 1800s and subsequently translocated to two other Mauritian islands (Cole, Goder, Premanand, et al., 2018). In 2011, there were approximately 46,000 adult skinks on Round Island (Cole, Mootoocurpen, et al., 2018). It is a priority to evaluate any impacts on this species, which is a candidate keystone omnivore central to a web of interactions with both fauna and flora.

The detailed composition and structure of Round Island plant communities before degradation are incompletely known, but early records suggest a palm savannah (Vaughan & Wiehe, 1937) or a palm-rich forest (Cheke & Hume, 2008), which would also have been a component of the lowland areas on mainland Mauritius. Endemic tortoises, alongside the extinct Mauritius Sheldgoose *Alopochen mauritiana* (Hume, 2017) probably helped shape this vegetation community. Tortoises and geese would have maintained

open vegetation by trampling and grazing. Native endemic heliophilous grasses, sedges and herbaceous plants possessing adaptations to avoid being grazed are candidate components of this open grazing community, including the tussock grass *Chrysopogon argutus* with course silica-rich leaves (Griffiths, 2014), herbaceous plants *Aerva congesta*, *Phyllanthus revaughanii* and *P. mauritianus* (Griffiths et al., 2013). These species do not survive where they are overshadowed by other vegetation. Plants in open areas may have been similar to those in lawns maintained by tortoise grazing on Aldabra (Merton et al., 1976). In the less accessible areas, where grazing was limited, there would have been palms and screw pines, with some hardwoods. This community has been heavily disrupted and remnants of it remain only on Round Island. Our understanding of what once existed has been formed by piecing together information from early records, observations of plant and animal interactions and traits, and the ecology of other grazed ecosystems. Today, many Mauritian plant species are globally threatened, including many probable components of a grazed community. However, some species persist in specific microhabitats such as rocky zones or through non-grazing gap dynamics. We hypothesise that grazing and trampling pressures are key to their persistence within the lowland plant communities of Mauritius.

Giant tortoises have considerable impacts on island ecosystems (Blake et al., 2012; Griffiths et al., 2011; Heleno et al., 2013), and Round Island is no exception. Experimental work on Round Island indicated that tortoises reduced vegetation biomass, including flowers and seeds, and engineered areas of cropped vegetation where *C. argutus* began to recover (Griffiths et al., 2013). However, whether tortoises have triggered the recovery of a native tortoise grazed community, and the consequences for the native fauna, remain poorly known. For example, competition for limited food resources may be negative whereas facilitating dispersal of shared food resources may be positive.

DNA metabarcoding coupled with expanding DNA barcode reference libraries, has enabled the detailed and accurate reconstruction of diet and food webs (de Sousa et al., 2019). For example, a metabarcoding study of four UK colombids using the ITS2 DNA barcode showed high dietary overlap and presence of fed or cultivated anthropogenic plants in their diets, with implications for conservation of the rare species (Dunn et al., 2018). Limitations on the information gained by metabarcoding have been reviewed in detail (Lamb et al., 2019; Pompanon et al., 2012; Tercel et al., 2021) and include amplification bias, copy number variation, the effects of secondary consumption, the inability to differentiate between life stage and tissue type, and incomplete reference libraries. Combining metabarcoding with stable isotope analyses and/or morphological identification of dietary remains can help to overcome some limitations while retaining high taxonomic discrimination and dietary detail (Bonin et al., 2020). Understanding the dietary preferences of ecological replacements and other fauna within ecosystems will facilitate a better understanding of why and how plant communities are changed and allow us to predict the probable consequences for native fauna.

Here we analysed the impacts of a tortoise ecological replacement on the Round Island ecosystem. Our specific objectives were to:

(i) Determine the dietary preferences of tortoises and skinks, primarily by metabarcoding, and identify patterns in dietary overlap.
(ii) Determine if tortoises have suppressed or triggered the expansion of plant taxa, including candidate components of a tortoise grazed community, and explore possible consequences for skinks.

2 | MATERIALS AND METHODS

2.1 | Study site

Round Island is a 219 ha basaltic volcanic cone ~20 km north east of Mauritius (Figure 1). September to November are the driest months, with frequent droughts (Bean et al., 2017). The wettest months generally span January to March, when cyclones can occur (MMS, 2021). Mean annual rainfall between 2003 and 2019 was 776 mm (Mauritian Wildlife Foundation, unpubl. data). Round Island was declared a nature reserve in 1957 and is closed to the public. Introduced goats *Capra hircus* and rabbits *Oryctolagus cuniculus* were eradicated in 1979 and 1986 respectively, after more than a century of devastating plant communities through overgrazing (Cheke & Hume, 2008) including reductions in palms and increases in invasive species (Bullock & North, 1984). Subsequent erosion was severe with major soil loss (Hedding et al., 2020). Round Island is globally significant for its breeding populations of threatened reptiles and seabirds, and the last remnants of Mascarene lowland palm-rich community composed of *Hyophorbe lagenicaulis*, *Latania loddigesii*, *Dictyosperma album* var. *conjugatum* and *Pandanus vandermeeschii*. Flora restoration began in the 1980s with a focus on removing invasive non-native plants and restoring populations of native hardwoods known to have been lost from the lowland plant community. Today Round Island is a mosaic of habitat types ranging from barren rocky areas, dense areas of invasive herbaceous plants, planted hardwood areas, to closed palmoid thickets. Fieldwork on Round Island was carried out with permission from the Mauritian National Parks and Conservation Service.

2.2 | DNA barcode library

A comprehensive plant DNA barcode library was assembled, pairing Round Island with Ile aux Aigrettes, and used to design and test the ITS2 metabarcoding PCR primers used in this study (Moorhouse-Gann et al., 2018). Where possible, three individuals from each plant species known to be present were sampled. The identity of each species was confirmed morphologically and leaf tissue samples were dried over silica gel. The barcode library (Table S1) contained sequences for 91 of the 92 angiosperm species found on Round Island, of which 89 have unique ITS2 sequences at the species level.
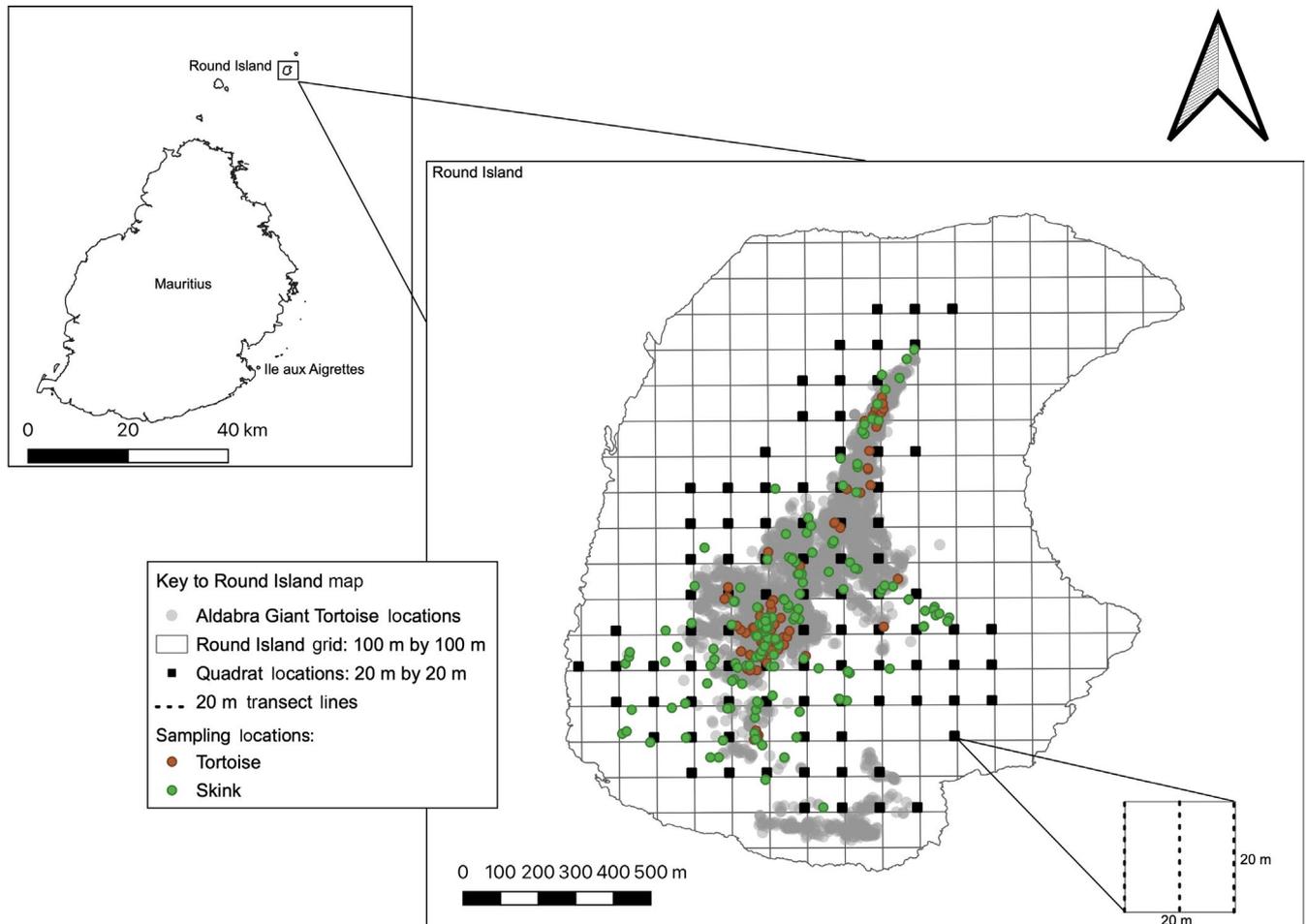
FIGURE 1 Map of Round Island in relation to the main island of Mauritius and Ile aux Aigrettes. Round Island is overlaid with a grid system composed of 1 ha cells. Approximate location of the 90 quadrats analysed in this study is shown alongside a diagram illustrating the position of the three 20 m transect lines present within each quadrat. Grey points represent a dataset of 13,127 tortoise locations collected between 2011 and 2014. Tortoise and skink sampling locations are shown

2.3 | Dietary sampling

Faecal samples from tortoises ($n = 86$) and skinks ($n = 196$) for metabarcoding were collected on Round Island during March, June and July 2015, and from skinks in December 2015. December skink samples were included as a comparison of frequently consumed plants following the driest months. To avoid over-sampling individuals, faecal samples were collected from individually recognisable animals in different areas of the island. Dung from individual tortoises was homogenised before three small (~3 cm^3) subsamples were taken from different areas of homogenised dung. All tortoises were identified from previous implanted microchips. Skinks were caught by hand or noose and individually identified by a series of measurements and distinguishing features. Photographs of the ventral and dorsal sides were taken for reference. Defecation was stimulated by abdominal massage. Faecal samples were dried over self-indicating silica gel. Ethical approval was granted by the Durrell Wildlife Conservation Trust Ethics Committee (approval no. ETH21/1510).

To complement the metabarcoding analyses for tortoise diet, plant remains were identified morphologically from 50 faecal samples (collected April–July, September, October 2015) and 212 feeding observations (collected April–July, October, November, December 2015). These data were summarised as counts and frequency of occurrence.

2.4 | Faecal DNA extraction, metabarcoding and bioinformatics

DNA extraction from faecal samples, preparation for 250 bp paired-end Illumina MiSeq next generation sequencing, and bioinformatics followed Moorhouse-Gann et al. (2018), including the use of the UniPlant primer pair to amplify ITS2. The UniPlant primers were designed for metabarcoding on Mauritian islands to universally amplify short plant DNA fragments in degraded DNA from faecal samples, while achieving high rates of taxonomic discrimination (Chen et al., 2010; Moorhouse-Gann et al., 2018). Following initial bioinformatics steps (Moorhouse-Gann et al., 2018), the blastn algorithm (Altschul et al., 1990) was used in Blast+ (Camacho et al., 2009) to assign unique faecal DNA sequences to plant taxa in the ITS2 sequence

TABLE 1 Average conditions for quadrats with (n = 48) and without (n = 42) tortoises

Tortoises	High wind exposure (proportion)	No. shearwater burrows (mean ± SD)	Soil depth (cm, mean ± SD)	Elevation (m, mean ± SD)	Slope (°, mean ± SD)	Shaded (proportion)	Aspect (mode)	Dominant soil type (mode)	High soil compaction (proportion)
Absent	0.43	1 ± 2.26	4.94 ± 5.42	149.19 ± 67.89	20.26 ± 7.75	0.55	West-northwest	Rock	0.76
Present	0.65	1.27 ± 2.52	6.86 ± 11.96	167.46 ± 75.77	15.54 ± 6.03	0.76	South west	Rock	0.69

reference library based on BIT scores. If there was no close match to the local library, sequences were searched against the NCBI Genbank nucleotide database (Benson et al., 2013). Custom Python scripts were used to produce a final presence/absence matrix of all plant taxa detected in each sample and reduce the probability of type 1 errors across the dataset (see Appendix S1 in Supporting Information). Erroneous taxa were removed (e.g. taxa known to be consumed by field teams, fungi or known contaminants).

2.5 | Plant community surveys

A plant community dataset collected in June and July 2003 (Johansson, 2003) was used as a pre-tortoise introduction baseline. Quadrats (20 m by 20 m, n = 144) were established at the south-east corner of each 1 ha grid cell, and three 20 m transect lines running south to north were positioned within each quadrat (Figure 1). Plant taxa (or substrate: soil, leaf litter, rock) present at 50 cm intervals directly below each transect were recorded. Data collected from all three transects were combined and converted into percentage cover per species or substrate type for each quadrat. Quadrats were then searched and all plant taxa identified. Wind exposure (high, moderate, low), elevation, aspect and slope were determined per quadrat. Soil depth, type (eroding rock, loam, rock, sand, clay) and compaction (high, medium, low), and number of Shearwater burrows within 4 m² were measured at four points within each quadrat, 5 m from each corner.

A subset of this survey, using the same quadrats, was repeated in June and July 2015 using the same methodology. A dataset of 13,127 tortoise locations on Round Island (Figure 1) collected between 2011 and 2014 as part of post-release monitoring (Mauritian Wildlife Foundation, unpubl. data, Appendix S2) was used to establish which quadrats had evidence of tortoise presence. All quadrats with records of tortoises were surveyed, in addition to any quadrats with similar habitat and terrain types but with no tortoise records (n = 90 quadrats surveyed in total, Table 1). Per quadrat, the number of tortoises and tortoise dungs (complementary evidence of tortoise presence), and palmoid seedling clusters at least 3 metres away from adult trees were recorded. Tortoises are the only animals on Round Island capable of dispersing clusters of at least three palmoid seeds in their dung (Cole, N., unpubl data). Photographs of each quadrat were used to determine the availability of shade (from trees, shrubs, rocky outcrops or other features).

2.6 | Plant community analyses

The plant community between the two timepoints was compared using non-metric multidimensional scaling using Bray–Curtis dissimilarities in the VEGAN package (Oksanen et al., 2019). Bare ground (soil and rock combined) and leaf litter were included in addition to plant percentage cover estimates, and values were log+1 transformed. To test for changes through time, accounting for the repeat surveys of

each quadrat, the difference in vegetation cover per taxon within each quadrat was calculated (after rescaling the total percentage cover in each quadrat to 100%) and summarised using principal component analysis (PCA). Four axes were retained because subsequent axes showed a marked decline in the percentage of explained variance (Jolliffe, 2002). Linear models ('lm') were used to test for an association between each principal component and tortoise presence (present or absent), while controlling for confounding variables (Appendix S3). PCAs and linear models were repeated after grouping all flora into one of six life-forms (fern, herb, grasses/sedges, woody/semi-woody, vines/creepers and palmoid), and including bare ground and leaf litter. Two axes were retained in this second PCA.

The species inventory for each quadrat was examined for the presence of 14 taxa that are candidate components of a tortoise grazed community and detectable on Round Island in June/July (Table 2).

2.7 | Dietary composition: Metabarcoding

Species accumulation curves, the Chao2 index (for an estimate of the total species richness expected with unlimited sampling effort) and per-sample Shannon's diversity indices were computed in the VEGAN package (Oksanen et al., 2019). A Mann–Whitney test evaluated differences in the median per sample Shannon diversity index of tortoises and skinks. For dietary overlap, the proportion of samples testing positive for a particular plant species was calculated separately for tortoises and skinks. To test whether dietary overlap between tortoises and skinks was higher than expected by chance, null models were generated using the Pianka metric, the RA3 algorithm

and 10,000 simulations in EcoSimR (Gotelli et al., 2015) and compared to observed data.

To signify food preferences, plant species consumed in greater, lesser or equal proportions to their availability were analysed: dietary metabarcoding and food availability data (percentage cover obtained from the transects detailed above) were combined and analysed in econullnetr (Vaughan et al., 2018). The 'generate_null_net' function was implemented to run 20,000 iterations of the null model to produce frequency distributions of expected rates of herbivory based on the relative available plant food. When observed herbivory rates fell outside of the central 95% of simulated values, this indicated deviations from random herbivory. Plant species undetected by transects or metabarcoding were excluded. This analysis was repeated to account for repeated sampling of the same individual reptiles (Table S2).

A general linear model (GLM) was used to test for associations between skink dietary preferences (represented by the standardised effect size, SES, computed by econullnetr) and tortoise SES, plant life-form and status (native, non-native). A similar GLM was used to test for associations between tortoise SES and plant life-form and status. A constant of 10 was added to SES values, which were then Box–Cox transformed.

A general linear mixed effects model (GLMM) was constructed using the GLMMTMB package (Brooks et al., 2017) for zero inflated data to determine whether the differences in plant percentage cover between 2003 and 2015 were associated with tortoise SES. Tortoise SES was included as a fixed effect, with plant ID and Quadrat ID as random effects. Only Quadrats with tortoises were included in this analysis. A Gaussian error family and identity link function were specified. All analyses were conducted in R version 3.6.3 (R Core Team, 2016).

TABLE 2 Plant taxa identified as candidate components of a tortoise grazed community. The count and percentage of quadrats within which taxa were detected in June/July 2003 and 2015 is shown. *Distribution incompletely known

Plant taxa	Life-form	Mauritius status	2003 (*n* = 144)		2015 (*n* = 90)	
			Count	Percentage	Count	Percentage
Aerva congesta	Herb	Endemic	1	0.69	0	0.00
Dichondra repens	Herb	Native	4	2.78	1	1.11
Sida pusilla	Herb	Native	6	4.17	1	1.11
Phyllanthus mauritianus	Herb	Endemic	5	3.47	0	0.00
Phyllanthus revaughanii	Herb	Native	0	0.00	0	0.00
Brachiaria serpens	Grass	Endemic*	0	0.00	0	0.00
Chloris filiformis	Grass	Endemic	3	2.08	0	0.00
Chrysopogon argutus	Grass	Endemic	45	31.25	15	16.67
Cymbopogon caesius	Grass	Native	57	39.58	37	41.11
Lepturus repens	Grass	Native	5	3.47	0	0.00
Stenotaphrum spp.	Grass	Native	2	1.39	6	6.67
Cyperus exilis	Sedge	Native	46	31.94	18	20.00
Fimbristylis cymosa	Sedge	Native	11	7.64	7	7.78
Aloe tormentorii	Succulent	Endemic	2	1.39	1	1.11

3 | RESULTS

3.1 | DNA metabarcoding

ITS2 DNA sequences derived from plants growing on Round Island were retrieved from 80 tortoise samples from 63 individuals and 91 skink samples collected between March and July, and 75 skink samples from December. There was no known repeated sampling of the same individual skink, but 13 tortoises were sampled between two and four times each.

Bioinformatics summaries are reported in Appendix S1. After applying thresholds to reduce biases caused by tag-jumping and other errors, unique sequences were assigned to 88 taxa. Following the removal of erroneous taxa (taxa known not to grow wild on Round Island, originating from human contamination, fungi or UK laboratory contaminants), 53 were retained for further analysis. Of these, 52 (98%) could be assigned to species. *Boerhavia* spp. was assigned to genus.

3.2 | Plant community analyses

There was evidence of tortoise activity in 48 of 90 quadrats. Eleven quadrats contained palmoid seedling clusters (35 seedling clusters overall). All seedlings were identified as *Latania loddigesii*, the dominant endemic palm species on the island, and only in quadrats with tortoise activity. The majority of taxa identified as candidate components of a tortoise grazed community were absent or rare in both 2003 and 2015 (Table 2).

There was an overall change in plant communities across the island between the two surveys, and the magnitude and direction of this change was similar in quadrats with and without tortoises (Figure 2). In both quadrat types, there was a relative increase in species such as *Adiantum rhizophorum*, *Hibiscus tiliaceus* and *Phyllanthus amarus* at the expense of species such as *Amaranthus viridis*, *Chloris filiformis* and *Lepturus repens* (Figure S1). Superimposed on this overall change, there was an increase in *Desmodium incanum*, and a reduction in *Boerhavia* spp. and *Ipomoea pes-caprae* in tortoise positive quadrats in comparison to negative quadrats (Figure 3a). Tortoise presence was associated with significantly lower PC1 scores (lm: −5.502 ± 2.744, $t = -2.005$, $p = 0.0496$; Figure 3c; Table S3). A change in the cover of three taxa in particular had a strong influence on PC1: *Boerhavia* spp., *I. pes-caprae* and *D. incanum*. Both *Boerhavia* spp. and *I. pes-caprae* increase along PC1, indicating that plots where tortoises were absent were typically characterised by an increase in the
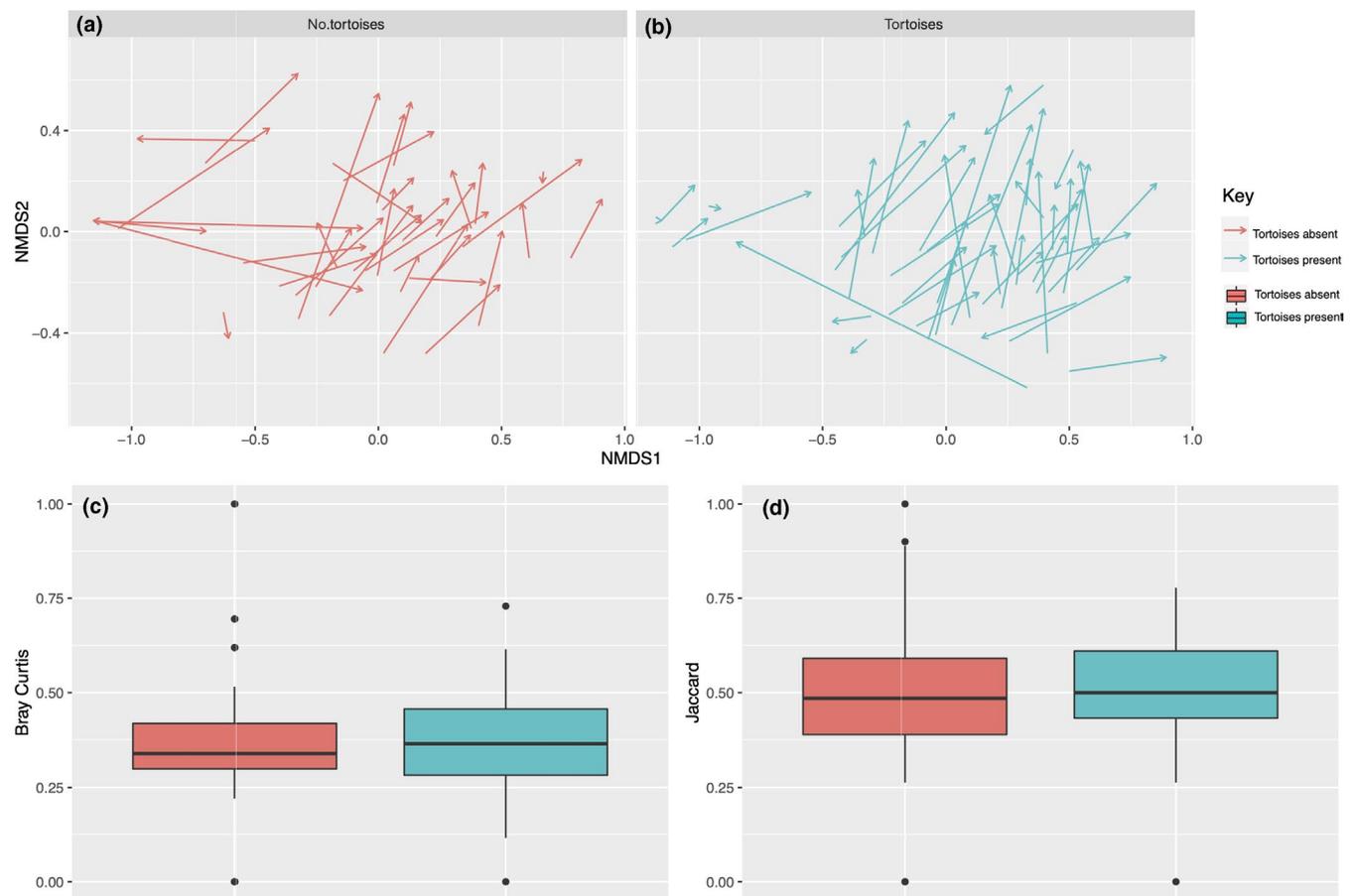
FIGURE 2 Magnitude and trajectory of change in vegetation, leaf litter and bare ground percentage cover between 2003 and 2015. (a) Ordination (NMDS) plot illustrating the trajectory (arrow direction) and magnitude (arrow length) of change in each quadrat between 2003 and 2015. Bray–Curtis (b) and Jaccard (c) dissimilarities from 2003 to 2015 in quadrats with and without tortoises
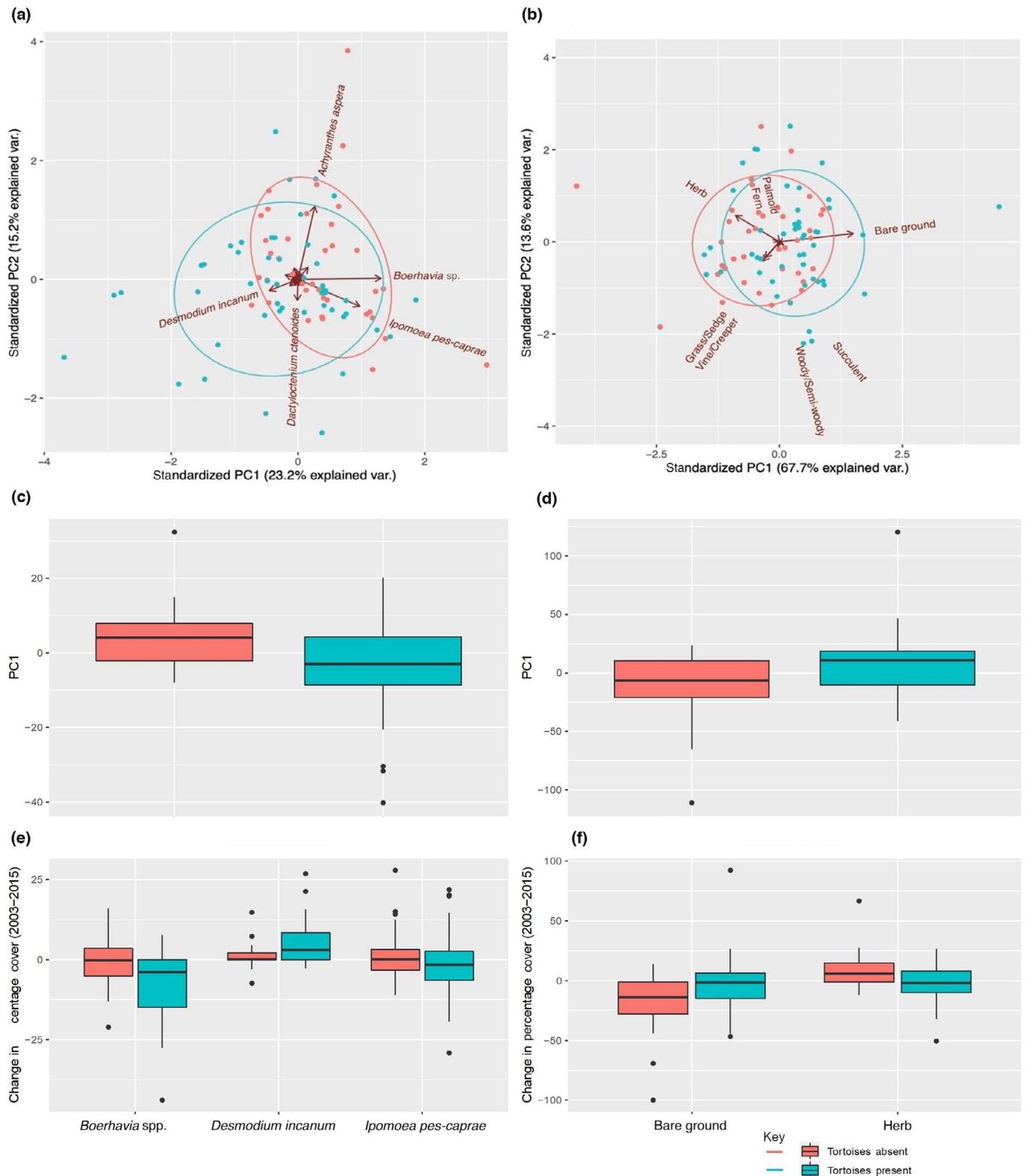
FIGURE 3 Differences in percentage cover at the taxon and plant life-form level. PCA biplots illustrating the analysis of the difference in (a) taxon (note reduced labelling of variables for clarity; *D. incanum* and *A. aspera* are non-native) and (b) life-form percentage cover in 2015 in comparison to 2003. Arrows represent magnitude and direction of change. The relationship between PC1 and tortoise presence for the taxon (c) and trait (d) analyses. Change in percentage cover between 2003 and 2015 for variables identified to be of influence by PCA for taxa (e) and life-forms (f)

cover of these plants. *Desmodium incanum* cover on the other hand decreased along PC1, indicating that plots where tortoises were present were typically characterised by an increase in this species

(Figure 3a,e). Tortoise presence was associated with changes in coverage of plant life-forms, which were captured in PC1 (Figure 3b). Tortoise presence was associated with significantly

higher PC1 scores (lm: 26.010 ± 7.111, $t = 3.658$, $p = 0.0005$; Figure 3d; Table S4). Tortoise plots were typically characterised by a reduction in herbaceous cover and an increase in bare ground in comparison to areas without tortoises. On average, tortoise plots had a 5% reduction in herbaceous cover and a 13% increase in bare ground in comparison to plots without tortoises (Figure 3b,f).

3.3 | Dietary composition

Analysis of species accumulation (metabarcoding data) indicated that sampling for both tortoises and skinks was approaching completeness (Figure S2). Approximately 77% and 82% of the total diet was sampled for tortoises and skinks respectively (Chao2 index). Dietary overlap between tortoises and skinks was significantly higher than expected by chance ($p < 0.001$). The per sample Shannon's diversity index was significantly higher in tortoises in comparison to skinks ($W = 1846$, $p < 0.001$, Figure S2). The metabarcoding, morphology and observation methods identified 47, 21 and 24 taxa in tortoise diets respectively (Table 3). Thirty-eight and 36 taxa were identified by metabarcoding in skink faeces in wet and dry months respectively (Table 3; Table S5).

Skink SES was significantly negatively correlated with tortoise SES (glm: −0.004 ± 0.002, $t = −2.153$, $p = 0.021$). There was no significant association between skink SES and plant life-form or status ($p = 0.070$ and 0.787 respectively) and the model explained approximately 32% (multiple R^2) of the variation in the data. Tortoise SES was significantly associated with plant life-form ($p = 0.002$), but not status ($p = 0.88$). Specifically, the vine/creeper category was significantly less preferred by tortoises than the grass/sedge category (glm: −0.780 ± 0.246, $t = −3.173$, $p = 0.003$; multiple $R^2 = 34\%$).

The Round Island flora list contained 99 taxa (92 angiosperms, 5 pteridophytes, 1 bryophyte and 1 lycopodiophyte; Table S1) at the study outset. Ninety-three taxa were present in the DNA barcode library (94%), the only angiosperm missing was the Round Island Hurricane Palm *Dictyosperma album* var. *conjugatum*. Dietary analyses detected an additional three taxa, increasing the flora list to 102 taxa: *Asparagus* sp. (differing from *A. umbellulatus*, also present), *Morinda citrifolia* and *Stenotaphrum dimidiatum*. A mean of 5.95 and 3.69 (range = 1–14; 1–10) taxa per faecal sample, and approximately 46% and 37% of the island's flora were detected for tortoises and skinks respectively (December samples excluded, Figure 4). Frequency of occurrence of plant taxa in faecal samples differed between tortoises and skinks; both reptiles consumed plants with a range of life-forms and statuses (Figure 5; Table 3). In general, tortoises consumed herbs and grasses most frequently. The morphology and observation data indicate that tortoises frequently consume the seeds and leaves of non-native plants. Both reptiles had preferences for a variety of species exhibiting a range of life-forms, with no clear preferences for particular plant life-forms or statuses aside from avoidance of vines and creepers by tortoises (Figure 6; Table 3).

There was no significant association between the difference in percentage cover of plant taxa between the survey years and tortoise SES (glmTMB: −0.055 ± 0.092, $z = −0.602$, $p = 0.547$).

4 | DISCUSSION

We evaluated the effect of an introduced tortoise, acting as an ecological replacement, on an ecosystem's plant community. Through analysis of tortoise and skink dietary preferences, we explored consequential effects of an ecological replacement on a sympatric endemic skink.

4.1 | Impacts of tortoises on plant communities

A comparison of vegetation data from before and after tortoises was introduced revealed that plant community changes were broadly similar in areas with and without tortoises. Through feeding and trampling large herbivores engineer the structure of landscapes, for example by maintaining vegetation heterogeneity, and dispersing seeds (Bakker et al., 2016; Hunter et al., 2013; Owen-Smith, 1987). On Round Island, the effect of tortoises was difficult to detect against the much larger effects of the earlier eradication of goats and rabbits, and the subsequent regeneration of native and invasive non-native plants (North et al., 1994). It is worth noting here that the majority of tortoises were juveniles which may have less of an impact than larger adults, and impacts may be density dependent.

The modest effect of tortoises can be summarised as a reduction in *Boerhavia* spp. and *I. pes-caprae* and an increase in *D. incanum*. *Boerhavia* spp., was preferentially grazed, explaining its reduction. *Ipomoea pes-caprae* was avoided overall, probably a consequence of tortoises consuming only leaves, fruits and flowers in preference to extensive creeping tough stems. For 15 years, *I. pes-caprae* has increasingly encroached on critically endangered *Aerva congesta* (Pynee et al., 2018) sites, likely contributing to local extinction. Expansion of tortoise distribution to these sites may assist the recovery of *A. congesta* in the long term. Increased *D. incanum* cover indicates tortoise grazing (consumed as expected) is unlikely to control this hyperabundant invasive non-native species, which is known to withstand heavy grazing (CABI, 2021). Complementary morphology data indicated that tortoises frequently disperse *D. incanum* seeds, but tortoises may also create favourable conditions for expansion, for example, through grazing competitors and fertilisation of areas through their dung.

Historic overgrazing by introduced mammals has caused poor plant regeneration, the loss of native plant species and severe soil erosion on Round Island (Hedding et al., 2020; North et al., 1994). Reduced erosion and soil retention are key objectives of the ecological restoration of Round Island. Bare ground decreased overall between 2003 and 2015, but to a lesser extent

TABLE 3 Tortoise and skink diet expressed by Count, FOO (frequency of occurrence = (no. occurrences/sample size)*100), and SES (standardised effect size representing dietary preferences computed in econullnetr). March–July data shown for metabarcoding, data from all months surveyed by morphology or observation. *Taxa absent from plant DNA barcode library. **Latania loddigesii was not fruiting during this sampling period but see Table S5

| Life-form | Plant taxa | DNA metabarcoding | | | | | | | | Morphology | | Observation | |
| | | Tortoise (n = 80) | | | | Skink (n = 91) | | | | Tortoise (n = 50) | | Tortoise (n = 212) | |
		Count	FOO	SES	Interaction strength	Count	FOO	SES	Interaction strength	Count	FOO	Count	FOO
Fern	Microsorum scolopendria	3	3.750			1	1.099						
Grass/Sedge	Cenchrus echinatus	64	80.000	5.4181	Stronger	32	35.165	0.1903	As expected	37	74	35	16.509
	Chloris barbata	7	8.750			7	7.692			2	4		
	Cymbopogon caesius	2	2.500	−3.0434	Weaker	2	2.198	−2.0621	Weaker			1	0.472
	Cyperus dubius	3	3.750	12.1143	Stronger			−0.1863	As expected	1	2	2	0.943
	Cyperus exilis	1	1.250	0.6304	As expected			−0.5737	As expected				
	Dactyloctenium ctenoides	13	16.250	−7.9734	Weaker	7	7.692	−6.3627	Weaker	45	90	46	21.698
	Digitaria horizontalis	42	52.500	11.0650	Stronger	13	14.286	2.8461	Stronger			14	6.604
	Eragrostis amabilis	1	1.250			2	2.198						
	Stenotaphrum dimidiatum	4	5.000	4.5148	Stronger	1	1.099	1.0414	As expected				
Herb	Abutilon indicum	11	13.750	3.3902	Stronger	34	37.363	19.5524	Stronger			1	0.472
	Achyranthes aspera	30	37.500	−3.8442	Weaker	31	34.066	−0.8253	As expected	5	10	7	3.302
	Amaranthus viridis	9	11.250	5.9986	Stronger	4	4.396	3.0908	Stronger			4	1.887
	Bidens pilosa	30	37.500	7.8905	Stronger	5	5.495	−0.1735	As expected			9	4.245
	Boerhavia spp.	58	72.500	3.3448	Stronger	20	21.978	−3.2028	Weaker			34	16.038
	Commelina benghalensis									3	6	4	1.887
	Desmodium incanum	33	41.250	0.1229	As expected	11	12.088	−3.0511	Weaker	44	88	59	27.830
	Erigeron bonariensis	2	2.500	2.0186	As expected	1	1.099	1.2010	As expected				
	Euphorbia prostrata	1	1.250										
	Euphorbia thymifolia	8	10.000			2	2.198						
	Oxalis corniculata	3	3.750	2.1538	As expected			−0.7642	As expected			1	0.472
	Phyllanthus amarus	8	10.000	23.2037	Stronger	1	1.099	3.3965	As expected				
	Portulaca oleracea	8	10.000	11.9103	Stronger			−0.5024	As expected			1	0.472
	Sida pusilla	1	1.250	2.5444	As expected	4	4.396	14.5128	Stronger				
	Solanum americanum	1	1.250	−0.3905	As expected	19	20.879	19.0845	Stronger				
	Solanum lycopersicum	3	3.750			4	4.396						
	Sonchus asper	1	1.250	1.5969	As expected			−0.3843	As expected				
	Tridax procumbens	38	47.500	3.0904	Stronger	10	10.989	−2.0945	Weaker			46	21.698

TABLE 3 (Continued)

Life-form	Plant taxa	DNA metabarcoding Tortoise (n = 80) Count	FOO	SES	Interaction strength	Skink (n = 91) Count	FOO	SES	Interaction strength	Morphology Tortoise (n = 50) Count	FOO	Observation Tortoise (n = 212) Count	FOO
Palmoid	Dracaena concinna									1	2	1	0.472
	Hyophorbe lagenicaulis									3	6		
	Latania loddigesii**	0	0.000	−7.2951	Weaker	1	1.099	−5.1188	Weaker	1	2	0	
	Pandanus vandermeeschii									1	2		
Unknown	Asparagus sp.*	1	1.250										
Vine/Creeper	Ipomoea pes-caprae	23	28.750	−6.2000	Weaker	23	25.275	−3.1252	Weaker	2	4	16	7.547
	Passiflora suberosa	1	1.250	−7.9639	Weaker	39	42.857	3.8638	Stronger	18	36	8	3.774
	Vincetoxicum confusum	2	2.500	−4.6617	Weaker	10	10.989	−0.7778	As expected				
Woody/Semi-woody	Asparagus umbellulatus	1	1.250	0.4416	As expected			−0.6353	As expected			2	0.943
	Desmanthus virgatus	8	10.000	0.1185	As expected	2	2.198	−1.3378	As expected	2	4	3	1.415
	Dodonaea viscosa	1	1.250	0.6389	As expected	1	1.099	1.1899	As expected	1	2		
	Elaeodendron orientale	0	0.000	−1.0946	As expected	2	2.198	1.5255	As expected	1	2	4	
	Eugenia lucida	2	2.500	2.7377	As expected			−0.4693	As expected	1	2		
	Ficus reflexa	1	1.250	0.6523	As expected			−0.5714	As expected				
	Gagnebina pterocarpa	13	16.250	5.7956	Stronger	8	8.791	4.5067	Stronger	4	8	2	0.943
	Hibiscus tiliaceus	5	6.250	8.7296	Stronger	5	5.495	11.1739	Stronger				
	Hilsenbergia petiolaris	4	5.000	3.3404	Stronger	8	8.791	10.1142	Stronger				
	Leucaena leucocephala	3	3.750										
	Margaritaria anomala	8	10.000			8	8.791			1	2		
	Maytenus pyria	1	1.250	0.0603	As expected	1	1.099	0.5530	As expected	2	4	1	0.472
	Morinda citrifolia	1	1.250			1	1.099						
	Premna serratifolia	9	11.250	1.1764	As expected	2	2.198	−0.9881	As expected	2	4	1	0.472
	Psiadia arguta	0	0.000	−0.9455	As expected	1	1.099	0.6489	As expected				
	Scaevola taccada	2	2.500	−1.7724	As expected	12	13.187	4.2936	Stronger	11	22	1	0.472
	Thespesia populnea	3	3.750										
	Volkameria heterophylla	2	2.500			1	1.099			1	2		
	Total taxa detected	47				38				21		24	

Combined total taxa 31
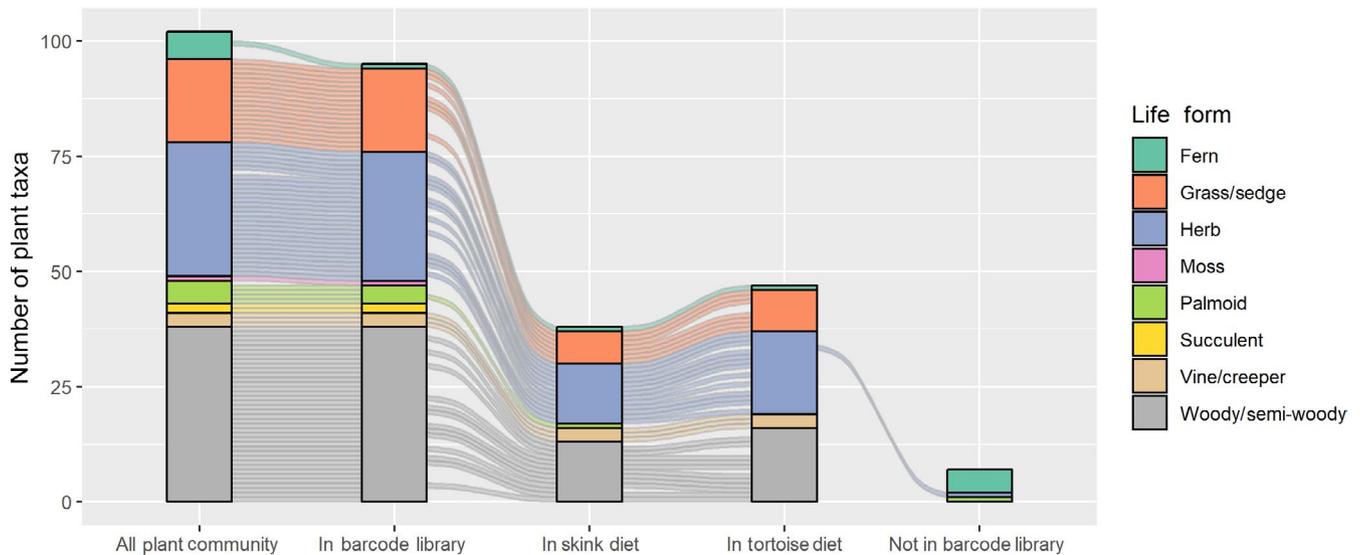
FIGURE 4 Breadth of reptile diets as determined by metabarcoding in the context of the Round Island plant community. The number of taxa in the Round Island plant community is shown, alongside the fraction of the community present or absent in the DNA barcode library, and present in skink and tortoise March–July faecal samples. December samples exclude
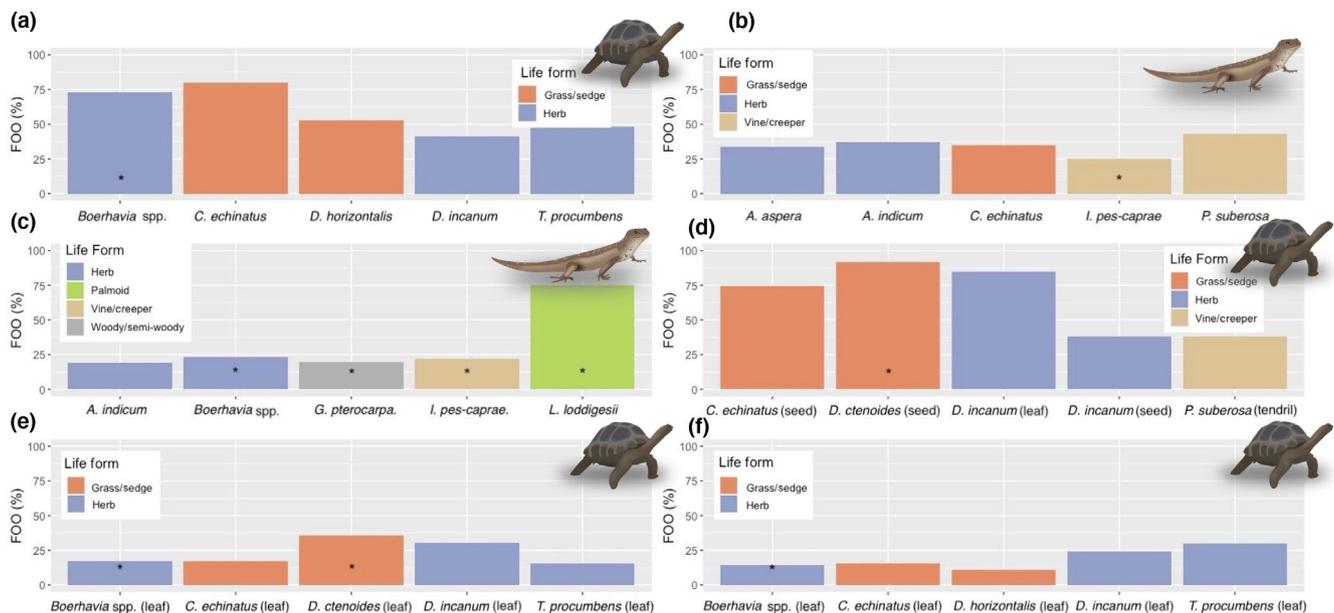
FIGURE 5 Five most frequently occurring plant taxa detected in reptile diets using a range of methodologies. Frequency of occurrence (FOO = (no. occurrences/sample size)*100) displayed on the y-axis. (a) March–July tortoise metabarcoding data (n = 80); (b) March–July skink metabarcoding data (n = 91); (c) December skink metabarcoding data (n = 75); (d) April–July tortoise morphology data (n = 47); (e) April–July tortoise observational data (n = 129); (f) November–December tortoise observational data (n = 83). September–October tortoise morphology data not shown due to a small sample size (n = 3). Plant parts are shown where applicable. *Taxa native/endemic to Mauritius. See Table 3 and Table S5 for details of all detected species and full binomial names

in tortoise areas. In the pre-degraded ecosystem this could provide opportunities for slower growing plant taxa with anti-herbivore adaptations to persist in a tortoise grazed community, reminiscent of the impacts of *A. gigantea* in their native range

(Gibson & Hamilton, 1983). On Round Island, in some areas heavily grazed by tortoises, the native tussock grass *Chrysopogon argutus* rapidly colonised, benefiting from the reduced competition from the fast-growing exotic species (Griffiths et al., 2013).

FIGURE 6 Dietary preferences of tortoises and skinks as computed in econullnetr using metabarcoding data. (a) Bipartite food web, upper and lower boxes scaled to represent sample size and availability of plants; plant taxa ordered by life-form. (b, c) Dot plots displaying dietary preferences of tortoises (b) and skinks (c); horizontal lines represent the selection predicted by the null model (the number of samples taxa would be present in if taxa were consumed in proportion to their availability) ±95% confidence intervals. December skink samples excluded
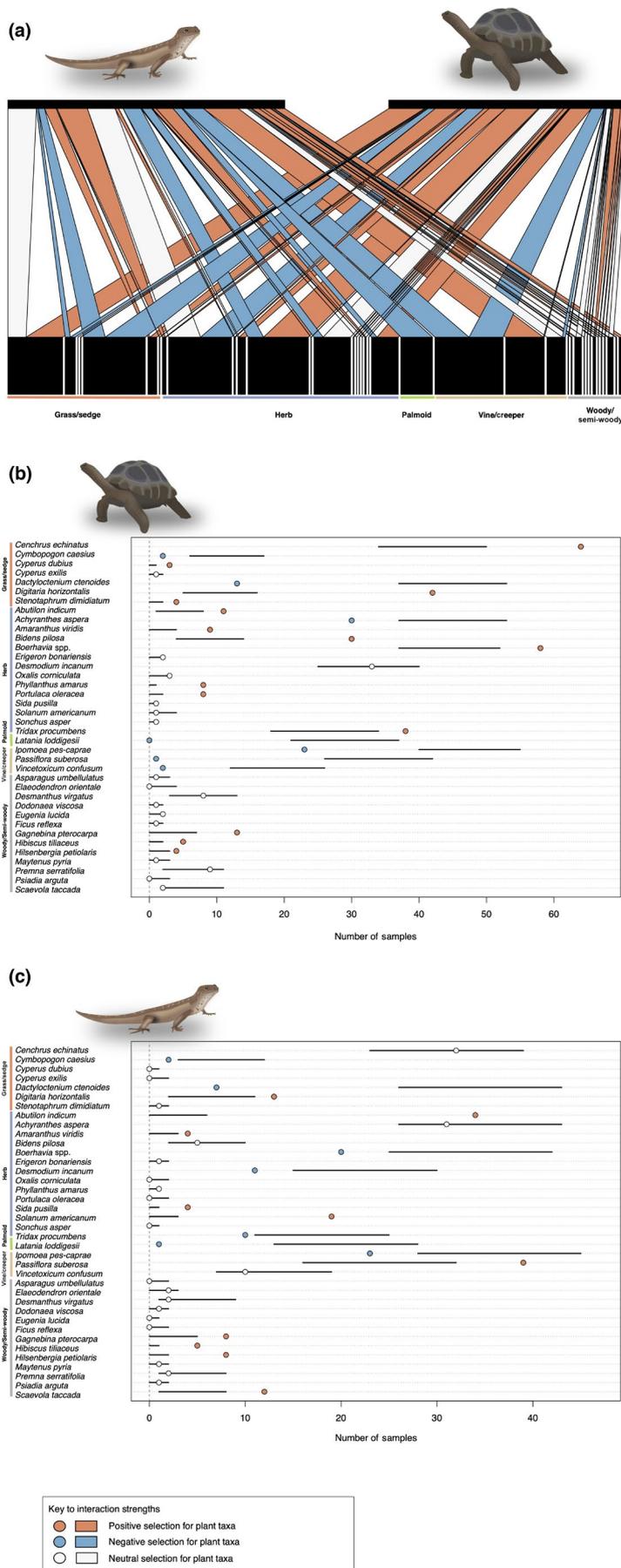
Anecdotal observations suggest that tortoise adapted plants thrive on frequently trampled paths.

We rarely detected candidate taxa for Round Island's tortoise grazed community both before and after the tortoise introduction. These taxa probably no longer persist in the seed bank and populations of wind-pollinated species are too small to recolonise, amplified by severe soil erosion and competition from dominant plant species. We conclude that tortoises alone are unlikely to restore lost Mauritian tortoise grazed communities, echoing Griffiths et al. (2013). After centuries of degradation, additional restoration approaches, working alongside tortoises, are required to assist regeneration of a plant communities.

Tortoises play a role in regenerating a palm-rich community, evidenced by *Latania loddigesii* seedling clusters exclusively in tortoise areas. *Latania loddigesii* seeds were previously identified in tortoise dung from Round Island (Griffiths et al., 2013), but undetected here due to the misalignment of fruiting and sampling periods. Tortoises are dispersers of two other large-fruiting Mauritian endemics, *Diospyros egrettarum* (Griffiths et al., 2011) on Ile aux Aigrettes, and *Pandanus vandermeeschii* on Round Island (Griffiths, 2014).

4.2 | Consequential effects on Telfair's skinks

Dietary overlap between tortoises and skinks was greater than expected by chance but dietary preferences between the two species were negatively correlated. Tortoises may reduce the coverage of plants frequently consumed by skinks, such as *Boerhavia* spp. and *I. pes-caprae*. Despite frequent consumption by skinks, these taxa were not preferred dietary items so reduced availability of these very common foods is unlikely to be detrimental for skinks. Analyses of interaction strength (preferences) allowed us to differentiate between food frequently consumed because it is widely available and those rarer preferred foods. We were unable to analyse mechanisms underpinning preferences, such as selection for fruits (Vaughan et al., 2018), or the scale at which reptiles of differing sizes make foraging decisions. Tortoises disperse the seeds of *L. loddigesii*, previously shown to be a seasonally important food resource for skinks (Zuël, 2009) and the plant taxon most frequently consumed by skinks following the driest months (this study). The benefits of increased palm-rich habitat will extend to the wider reptile community. Palms provide food and habitat for ornate day geckos *Phelsuma ornata*, Günther's geckos *Phelsuma guentheri*, Durrell's night geckos *Nactus durrellorum*, Bojer's skinks *Gongylomorphus bojerii* and keel-scaled boas *Casarea dussumieri* (Bullock, 1986; Cole, Mootoocurpen, et al., 2018; Vinson, 1975). By 2011, populations of seven reptile species had increased substantially on Round Island following the eradication of mammals and subsequent vegetation regeneration (Cole, Mootoocurpen, et al., 2018), highlighting the importance of plant community restoration.

4.3 | DNA metabarcoding to inform ecological replacement

The current study corroborates the importance of key species identified by Griffiths et al. (2013) and Zuël (2009) in the diet of tortoises and skinks respectively, and uncovered greater dietary breadth. Metabarcoding generally delivers more detailed dietary information with greater taxonomic discrimination in comparison to morphological methodologies (e.g. da Silva et al., 2019). DNA metabarcoding identified approximately twice the taxa of either morphology or observation, with herbaceous taxa in particular going undetected more often using the latter methods. All methods identified almost all taxa to species, assisted by considerable knowledge of the plant communities (observation and morphology) and a comprehensive DNA barcode library (metabarcoding). Our approach of comprehensively barcoding the plant community prior to metabarcoding has furthered our understanding of ecological replacement by allowing for the detailed analysis of trophic interactions at the species level. Drawbacks of metabarcoding include the inability to differentiate between plant parts (seeds, flowers, leaves etc.) and secondary consumption of plant material via the ingestion of herbivorous invertebrates by omnivores (da Silva et al., 2019). By combining DNA barcoding with traditional methodologies, we were able to identify some of the plant parts that were recognisable after digestion or identified from grazing observations. These complementary techniques have deepened our knowledge of ecosystem processes. Although a portion of the taxa in skink diets may have originated from secondary consumption (but see Guenay et al., 2021), these taxa may still contribute to skink dietary needs and the secondarily consumed plants are important for invertebrates consumed by skinks. We employed a presence/absence approach over relative read number. Both approaches suffer biases, but further calibration studies are required to accurately relate relative read number to plant biomass consumed (Deagle et al., 2019). A broader temporal span of sampling, particularly for the metabarcoding data, would undoubtedly enhance our understanding of trophic interactions on Round Island (Alberdi et al., 2019).

5 | CONCLUSIONS

Our data indicate that in severely degraded and isolated ecosystems with depleted soil and seed banks, ecological replacement of grazing and trampling functions alone is likely to be insufficient to restore lost plant communities composed of species with small populations. During the 7 years of the study, tortoises maintained areas of bare ground. In the absence of further restoration interventions a native tortoise grazed plant community, which we hypothesised might have existed and been maintained by extinct *Cylindraspis* spp., is unlikely to regenerate in this space. We also showed that additional interventions to control invasive plant species are required since tortoise grazing does not appear to be a panacea for this conservation challenge on Round Island. Ongoing monitoring of tortoise impacts on the plant community is required, since the numbers, distribution and

density of the tortoises on Round Island will increase and because many of the impacts will likely take more time to arise. By the end of 2020, the tortoise population was estimated to be approximately 680 individuals (Mauritian Wildlife Foundation, unpubl. data), almost twice the 2015 estimate. Over time, the age structure will move towards having a greater proportion of larger adult tortoises, which are likely to have a greater impact on the ecosystem.

This study represents the first attempt to use metabarcoding to understand the impacts of an ecological replacement grazer on an ecosystem via the analysis of trophic interactions. As ecological replacements are likely to be increasingly used as part of rewilding interventions, there will be a greater need for practical techniques and empirical data to evaluate the efficacy of this approach. We advocate the use of metabarcoding as a useful complementary tool to investigate and monitor resulting changes to food webs.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

W.O.C.S., N.C.C. and R.J.M.-G. conceived the ideas and designed methodology; fieldwork by R.J.M.-G., M.G., M.C.R. and N.C.C.; laboratory work by R.J.M.-G., I.-R.M.R. and D.M.; bioinformatics by R.J.M.-G., H.H. and D.W.G.S.; data analysis by R.J.M.-G. and I.P.V.; R.J.M.-G. led the writing of the manuscript. All authors contributed critically to the drafts and gave approval for publication.

DATA AVAILABILITY STATEMENT

Plant DNA barcodes are archived on NCBI Genbank (Table S1 includes accession numbers). Raw MiSeq data are archived in the NCBI Sequence Read Archive (BioProject accession: PRJNA781763). Plant community and processed DNA metabarcoding data are available from the Dryad Digital Repository https://doi.org/10.5061/dryad.gtht76hnp (Moorhouse-Gann et al., 2021).

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