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Investigating semi-arid vertebrate communities from different quality habitats using eDNA derived from soils

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Index

Abstract

Since European settlement, many arid and semi-arid Australian vertebrates have suffered declines, with a disproportionally high number also going extinct. This can be attributed to introduced animals, e.g. feral cats, as well as habitat modification and loss. Native species in arid and semi-arid zones rely on refugia during dry periods, where the greenness of vegetation is linked to refugia availability. To preserve and manage native species populations, accurate biomonitoring data is crucial. Traditional techniques, e.g. camera and live trapping, are labour intensive, costly and biased towards large, common species. New techniques are being developed to improve the accuracy and reliability of biomonitoring data. One such technique is environmental DNA (eDNA). This study aimed to use soil-derived eDNA to examine the preferences of vertebrates for three different environments, as determined by a greenness index, in semi-arid zones in New South Wales, Australia. One hundred and sixty-two soil samples were collected and amplified using the 12S5V vertebrate primer. Sixteen unique taxa were detected: four reptiles, four birds and eight mammals, of which five were non-native mammals. Of these, twelve were assignable to the level of species. There was no significant difference in species richness and community composition for the different classes of greenness. However, sampling site location did have a significant effect on the community composition but not on the species richness. This suggests that greenness levels might not be the dominant driver for habitat preference, with other factors, e.g. plant community composition or water accessibility also influencing the preference of vertebrates. However, it is emphasised that this study was a pilot study and was restricted to one region over a short period. Hence, further studies are required to determine whether soil-derived eDNA can be used to determine the composition of vertebrates within arid and semi-arid regions.

Keywords: eDNA, semi-arid Australia, vertebrates, 12S5V, vegetation greenness

Introduction

Since the colonisation of Australia by the Europeans in 1788, many Australian endemic species have suffered marked declines. This includes the extinctions of three reptiles, nine birds and 34 mammals (Woinarski et al., 2019). Between 2000 and 2017, there has been on average a 35% decline in the population sizes of threatened Australian mammal species (Tulloch et al., 2023). While mammalian declines have been the most documented, there are also concerns for the long-term viability of other taxa (Walsh et al., 2013). For example, for the species of squamates (lizards and snakes) that have been assessed, the number of species considered to be threatened has nearly doubled over the last 30 years (Tingley et al., 2019). Avifauna have also suffered marked declines, with 163 species currently listed as threatened (Commonwealth of Australia, n.d.). Furthermore, the population sizes of these threatened bird species have declined by 44% between 2000 and 2016 (Bayraktarov et al., 2021). The increase in species with a threatened status and increased extinction rates are anticipated to persist (Geyle et al., 2018).

Extinctions in Australia can mainly be attributed to introduced animals. For example, the three post-European reptile extinctions were all located on tropical Indian Ocean islands (Christmas Island and Cocos Keeling Islands), and have mostly been attributed to the introduction of the common wolf snake (*Lycodon capucinus*) (Emery et al., 2021). For Australian squamates overall, in addition to invasive species such as feral cats (*Felis catus*), rats (*Rattus rattus*) and toxic cane toads (*Rhinella marina*), habitat loss and climate change are key threats (Tingley et al., 2019). For birds, the nine post-European extinctions were mostly located in Queensland and the Pacific subtropical islands (Norfolk Island and Lord Howe Island), and have been attributed to introduced rodents and hunting (Woinarski et al., 2019). For mammals, the biggest threat is predominately non-native predators, in particular, the European red fox (*Vulpes vulpes*) and feral cat (Legge et al., 2023). Extinction rates in arid and semi-arid zones are particularly high (Murphy & Van Leeuwen, 2021; Woinarski et al., 2019). The effects of predation from exotic species are compounded by the natural high mortality, especially during dry-periods, which mammals and birds face in these physiologically challenging environments (Reid & Fleming, 1992). Currently, there are large knowledge gaps regarding the status and distribution of the region's reptiles (Woinarski, Burbidge, et al., 2018).

One explanation for the disproportional decline in semi-arid mammals is the critical weight range hypothesis (Burbidge & McKenzie, 1989). This states that arid and semi-arid Australian ground-dwelling mammals with a body mass between 35g and 4200g have the highest risk of a decline in population size and going extinct (Chisholm & Taylor, 2010; Burbidge & McKenzie, 1989). There are several plausible explanations for this. Firstly, mammals within this size range are the preferred prey of introduced predators (Kinnear et al., 2002; Short & Turner, 1994). Additionally, environmental factors (e.g. rainfall and fires), introduced herbivores (McKenzie et al., 2007), and being more exposed due to the open habitat of the arid zones (Johnson & Isaac, 2009) also influence the higher extinction rate and population declines in this size class of mammals. Species outside of the critical weight range are less prone to extinction and population declines (Chisholm & Taylor, 2007). For example, smaller sized mammals often have a higher reproductive rate, and therefore, faster population growth, and this in turn helps them to recover faster after a decline due to predation (Cardillo, 2003). Furthermore, these smaller sized mammals can hide in spaces beyond the reach of the predators (Chisholm & Taylor, 2010). Mammals above the critical weight range are often too large, e.g. kangaroos and wallabies, to be the predominant prey items of invasive predators (Stobo-Wilson et al., 2021).

The critical weight range hypothesis does not include birds and reptiles. However, these two taxonomic classes are also affected by invasive predators. Feral cats eat reptiles frequently (on average 24% occurrence in cat dietary samples) and the frequency only increases in hotter, drier, mid-latitude areas (Doherty et al., 2015). It is estimated that feral cats kill about 1.8 million native reptiles per day (Woinarski, Murphy, et al., 2018). Foxes have also been implicated as a threat to some Australian lizard species (Nielsen & Bull, 2016) and as a cause for changes in Australian reptile communities (Olsson et al., 2005). For birds, the main reason for their decline in arid Australia is attributed to overgrazing by non-native herbivores with foxes and feral cats having a secondary role (Reid & Fleming, 1992). Similar to reptiles, Australian mainland birds occur more often in the diets of feral cats in drier areas (Woinarski, Murphy, et al., 2017).

Importance of biomonitoring data

Arid Australia is a unique and unusual region, it is extensive in size, has unpredictable extremities in climate, nutrient poor soils and a high level of endemism (Stafford Smith & Morton, 1990). Conservation efforts are necessary to limit declines and stop species from going extinct. Different management programmes and reintroduction efforts are being implemented. However, not all are successful, or the declines are identified too late (e.g. Woinarski, Garnett, et al., 2017). National parks and feral predator-free regions in New South Wales have been established, however, coverage is limited, especially in arid and semi-arid zones (Department of Climate Change, Energy, the Environment and Water, 2023). For species that are susceptible to predation, these feral predator-free areas offer more security for survival (Legge et al., 2018; Moseby et al., 2011; Finlayson et al., 2008). Many species that live outside of these areas are experiencing population declines as the key threats remain unmanaged (Tulloch et al., 2023). Pivotal to any conservation strategy is fundamental and reliable data on the status of species. Most notable, this includes the species' presence, distribution, and abundance. In some cases, population declines might be underestimated due to a lack of this fundamental information (Woinarski et al., 2019).

Spatial and temporal biodiversity data is especially important in arid and semi-arid zones. Most animals in these environments experience "boom and bust cycles". This can be very clearly seen in mammal species, with population sizes markedly increasing after a pronounced period of heavy rainfall (boom), with marked declines in abundances occurring during protracted dry periods (bust) (Bennison et al., 2018; Greenville et al., 2012; Dickman et al., 2011). During these bust periods, small mammals rely on refugia for their survival (Pavey et al., 2014). For most birds in arid zones, it seems like they do not experience this "boom and bust cycle" in the traditional sense. The boom response is described as driven by immigration after a period of rainfall and instead of a bust, the population slowly declines over the next couple of years (Jordan et al., 2017). Bird persistence is mostly dependent on vegetation greenness which, when it is increased, offers more resources to overcome the drier periods (Selwood et al., 2018). Reptile abundance in arid zones is also described as being determined by rainfall and temperature fluctuations in addition to vegetation conditions (Read et al., 2012). More rain increases the reptile richness, and ambient moisture is likely to increase reptile activity (Molyneux et al., 2018). During dry periods reptiles use shade microhabitat refugia to limit their water loss (Ryan et al., 2016). Vegetation level and greenness are in all cases important for the survival of animals in these arid zones. To help protect threatened vertebrates these refugia offered by vegetation should also be considered in conservation efforts.

The most common ways for detecting vertebrates is through cameras, live traps, and observational data. These methods are often labour-intensive, costly, invasive, and often unviable for conservation managers due to the remoteness of much of Australia's arid and semi-arid regions. Additionally, some of these traditional methods are not always reliable. Live traps work for some species but not others due to the animals' size, or because they are fossorial or arboreal (Torre et al., 2016). Camera trap performance differs considerably between camera models and even the better models have drawbacks, e.g. false triggers, drained batteries, detection probability, etc (Heiniger & Gillespie, 2018). Small animals are less likely to be detected (Jumeau et al., 2017) and misclassification is also not uncommon (Potter et al., 2019). This is especially the case for rare species, with detection probability being far lower than for more abundant species (Gu & Swihart, 2004). These characteristics of traditional methods might create biases, potentially leading to inaccurate conclusions. In turn, this may constrain the effectiveness of conservation efforts.

Environmental DNA

New techniques are being developed to improve the accuracy and reliability of biodiversity monitoring data. One such technique is the use of environmental DNA (eDNA). eDNA can be defined as a mixture of DNA (e.g. skin, hair, faeces, etc.) from different individuals found in an environmental sample such as soil, water, sediment or snow (Taberlet, 2018). The main steps of this method generally include: the collection of an environmental sample; DNA extraction from the samples; amplification of target DNA using specific primers; comparing the results to a database; and then, biological interpretation. eDNA methods are often more cost-effective and less laborious than traditional methods (Mena et al., 2021). There are indications that eDNA outperforms traditional methods, however, this result is based on only a fraction of the papers using eDNA since not all of them directly compare their results with traditional methods (Fediajevaite et al., 2021). Mena et al. (2021) compared the biomonitoring results of different traditional methods to eDNA. Their results indicated that eDNA is a good addition to the traditional methods and that a combination of methods is necessary to detect the most complete overview of the mammal community. However, this study took their eDNA samples from water sources in Neotropical forests, and thus the results might be different when taking different types of samples. Currently, eDNA methods are most commonly used in studies focussing on aquatic environments (Jiang & Yang, 2017). Consequently, sufficient studies focussing on other types of samples (e.g. soil, snow, sediment, etc.) are not as abundant (Fediajevaite et al., 2021). Leempoel et al. (2020) used soil samples as a source of eDNA to compare mammal diversity in Jasper Ridge Biological Preserve, California, USA. They suggested, similar to Mena et al. (2021), that eDNA methods are a good addition to traditional methods. Their study showed that eDNA results resembled the expected species present in the area. However, not all species that showed up on camera traps were recorded using the eDNA methods and vice versa. They specifically pointed out that eDNA works well for small mammals that were not easily detected by the camera traps (e.g. due to their size). Additionally, Mena et al. (2021) found that eDNA methods make it possible to determine the difference between species that are visually difficult to distinguish.

The quantity and persistence of eDNA in the environment are influenced by different factors. After extracellular DNA enters the environment it has three different fates. First of all, it can be broken down by microbial DNase which is present in most microbial habitats (Nielsen et al., 2007; Blum et al., 1997). Second, it can be integrated into a bacterial genome (Wackernagel, 2006; Dubnau, 1999). However, for the use of eDNA techniques, the last fate is of most significance. The DNA can adsorb to soil particles, humic substances and organomineral complexes (Crecchio & Stotzky, 1998; Lorenz & Wackernagel, 1987; Greaves & Wilson, 1969). Extracellular DNA that is adsorbed is protected against degradation by DNase (Wackernagel, 2006). Being protected against DNase in combination with the adsorption, makes it possible for extracellular DNA to persist longer in the environment (Blum et al., 1997). However, adsorption does not protect against uptake by soil bacteria (Wackernagel, 2006). Additionally, extracellular DNA is not maintained by cellular DNA repair mechanisms that are normally present within the cell. This means that it is exposed to environmental factors which in turn can lead to its fragmentation and degradation (Nielsen et al., 2007).

Multiple environmental factors influence DNA adsorption to soil and its degradation rate, including: surface area; clay content; pH; temperature; mineralogy; and moisture content (Sirois & Buckley, 2019; Levy-Booth et al., 2007; Crecchio & Stotzky, 1998; Blum et al., 1997; Widmer et al., 1996; Lorenz & Wackernagel, 1987; Greaves & Wilson, 1969). For example, a lower clay content leads to fewer binding sites which means that extracellular DNA has a lower chance of adsorbing to soil particles and a higher chance of degrading (Blum et al., 1997). The dominant binding mechanism is determined by the pH level of the soil, as it influences the charge of both the DNA and soil particles (Levy-Booth et al., 2007; Crecchio & Stotzky, 1998; Lorenz & Wackernagel, 1987; Greaves & Wilson, 1969).

DNA characteristics also influence adsorption and the degradation rate. It is thought that GC content in DNA might cause selective degradation and influence the degradation rate (Dell'Anno et al., 2002). Where a lower GC content points towards faster degradation by microbial populations (Vuillemin et al., 2017). The length of the DNA fragment influences its adsorption to the soil particles. In general, shorter fragments adsorb better to most soils (Pietramellara et al., 2009; Ogram et al., 1994). However, in sandy soils, the opposite is true, where the longer fragments adsorb better (Pietramellara et al., 2009; Ogram et al., 1988).

The quantity of extracellular DNA influences the likelihood of it being picked up during sampling. The knowledge on how the quantity is influenced by organisms and their behaviour is, as seen before with the usage of eDNA techniques and different types of samples, biased towards aquatic environments (e.g. Buxton et al., 2017; Klymus et al., 2015; Eichmiller et al., 2014). However, research on quantity pertaining to terrestrial systems is limited. However, Andersen et al. (2012) found that the biomass of individual species correlates with the amount of eDNA accumulating in the surficial soil. If this is the case it might be difficult to find elusive and rare species as their biomass will be lower compared to the more abundant species. Additionally, they determined that animal behaviour also influences the amount of DNA deposited into the environment, e.g. territorial behaviour/marking, more or less hair loss, etc. However, they also found that, if enough soil samples are taken, it can reflect the biodiversity of the sampled site.

At present, there are only a few studies using eDNA from soils to collect biomonitoring data on vertebrates, and the results are mixed. White et al. (2024) used eDNA from soils to detect spot-tailed quoll (*Dasyurus maculatus*) and determine if it was a feasible monitoring method. They collected samples from topsoil in quoll enclosures both from defecation sites and normal quoll habitat in the Otway Ranges and multiple locations in natural quoll habitat in Victoria, Australia. They concluded that eDNA from soils does not provide an improvement to spot-tailed quoll monitoring due to the sensitivity of their method substantially decreasing in natural quoll habitats compared to defecation sites. Ryan et al. (2022) compared top log hollow sediment samples (consisting of some soil and broken down heartwood material) and topsoil samples from around the hollow's entrance in two different climates, Mediterranean and semi-arid. They detected birds, mammals and reptiles, however, most mammals were common species. A wider range of species was detected using the hollow sediment compared to the soil samples. This is most likely due to the eDNA being better preserved in those conditions and the animals residing longer in hollows. Additionally, they found more species in the Mediterranean climate which is likely a representation of the overall higher species richness in that climate. Van Der Heyde et al. (2020) compared the effectiveness of different substrates for eDNA sampling in two different climates, a Mediterranean with sandy soils and an arid climate with clay soil. The topsoil was sampled randomly in different 10 x 10 m plots at both locations. The study targeted vertebrates as well as arthropods and plants. Overall the soil samples performed poorly compared to the samples from other substrates, especially in the arid climate. They suggest that more research is necessary in these types of climates to draw reliable conclusions on the usefulness of eDNA soil samples for biomonitoring.

Aim

The aim of this study was to use eDNA derived from soils to get an insight into the preferences of vertebrates for different vegetation greenness levels, and thus refuge availability, in semi-arid zones in New South Wales, Australia. Specifically, the difference in species richness and community composition between three levels of vegetation greenness was tested. Furthermore, observational data was used to compare to the results from the eDNA derived from soils. The study area was located in a large rural property, reflecting a majority of the region. Given the expansive coverage of such environments, they are critical for supporting the region’s biodiversity. I expected to detect the larger and more common mammals in the area due to their biomass. In addition, since most animals in these areas rely on refugia during dry periods, it was expected that in the treatments where the vegetation greenness levels are higher, there would be greater species richness. Furthermore, community composition was expected to differ between the different vegetation greenness levels.

Material and methods

Location, experimental design and sampling

This study was part of a broader study carried out by the New South Wales government in collaboration with Macquarie University researching mammal occurrence within semi-arid refugia. This study was performed at Nundora Station in New South Wales, Australia (30° 41' 52.8324'' S, 141° 57' 55.2492'' E), approximately 170 kilometres north of Broken Hill in the outback. Nundora Station is a working cattle farm encompassing roughly 104,000 acres. In addition to cattle, the property also farms sheep, with large numbers of wild, feral goats also present on the property (pers. obs. Vangangelt). As a working property, Nundora Station is not located within the national park system, nor does it have any feral predator-free zones. The soils on the study site are classified as rudosols by the Australian soil classification system (Department of Planning, Industry and Environment, 2021a) and silicious sands by the Great Soil Group classification system (Department of Planning, Industry and Environment, 2021b). However, the characteristics still widely vary based on the site (Isbell, 2021).

The plants in the area varied from exposed soil and short dry grass to bushy with some eucalyptus trees (Appendix 2). However, most of the soil at the sampling sites was exposed. Rainfall in this area was variable over the last 20 years, ranging between 42 mm and 526 mm annually, with a mean annual rainfall of 235 mm (Bureau of Meteorology, 2024a). Temperature ranges between -3.6 °C and 47.5 °C, with an average daily temperature of 26.8 °C (Bureau of Meteorology, 2024b). The samples were taken at the end of November 2023, which was unseasonally cool. During sample collection temperatures ranged between 13 °C and 25 °C and the UV index ranged between 0 and 7 (Appendix 2).

The sampling sites were predetermined being part of a broader study conducted by the NSW government and Macquarie University. The sites were selected based on the green accumulation index, which is the sum of "green fraction over time". This index was determined based on the "green fraction over time" and the "persistent green fraction over time", reflecting a response to precipitation. The different vegetation greenness habitat quality levels (henceforth greenness levels) were determined by making a cumulative histogram. The bottom 40% was labelled as low, the next 40% of values was labelled as medium and the top 20% of classes was labelled as high. These different levels represent the refugia availability. Based on these classifications a sampling site, approximately 1 ha in size, was selected which contained three sampling plots, each a different greenness level (low, medium and high) (Fig. 1A & 2). This setup was replicated at two other sites. The sites were located ca. 500 – 1000m apart and therefore had the same management histories and climatic conditions.

In each plot, two fences were set up along which the samples were taken (Fig. 1B). The fences were made of green gauze with a small mesh size and approximately 30 cm high. In the middle of each fence, a camera with a bait station was set up. During the first four days after the fences were set up, two funnel traps were placed at each end and checked regularly. Three weeks after the fences were set up, eight soil samples were taken from each fence, four on each side. An additional sample was taken in front of the camera directly underneath the camera mat, where small animals are known to hide (pers. comm. Krystyna Jordan). There was a three week interval between setting up the fences and collecting the soil samples, subsequent to the collection of the camera trap data. Each plot was sampled in the three hours after sunrise (Appendix 2) on one occasion. In total, 162 soil samples were collected for eDNA analysis.

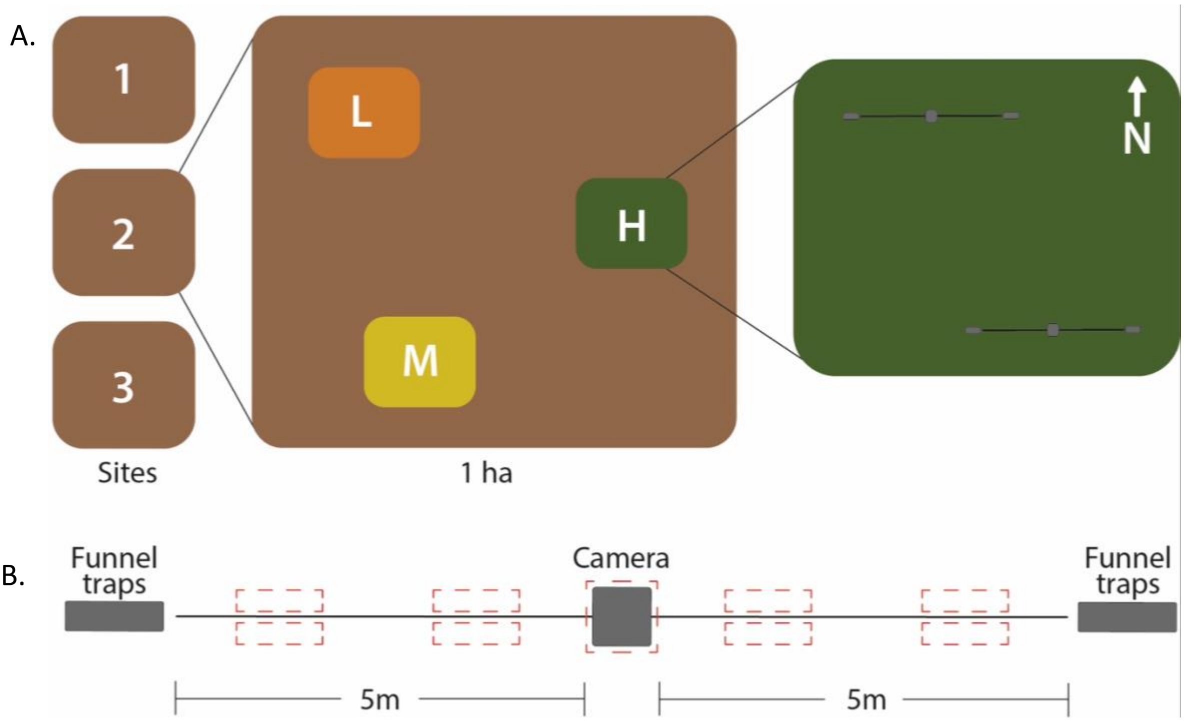

Figure 1 *Soil sampling set-up.* ***A****. Displays one of the 3 selected sites, which each contain three different greenness levels. Low = L, Medium = M and High = H.* ***B****. Overview of one of the transects. The areas within the dashed red line are where the soil samples were taken from.*

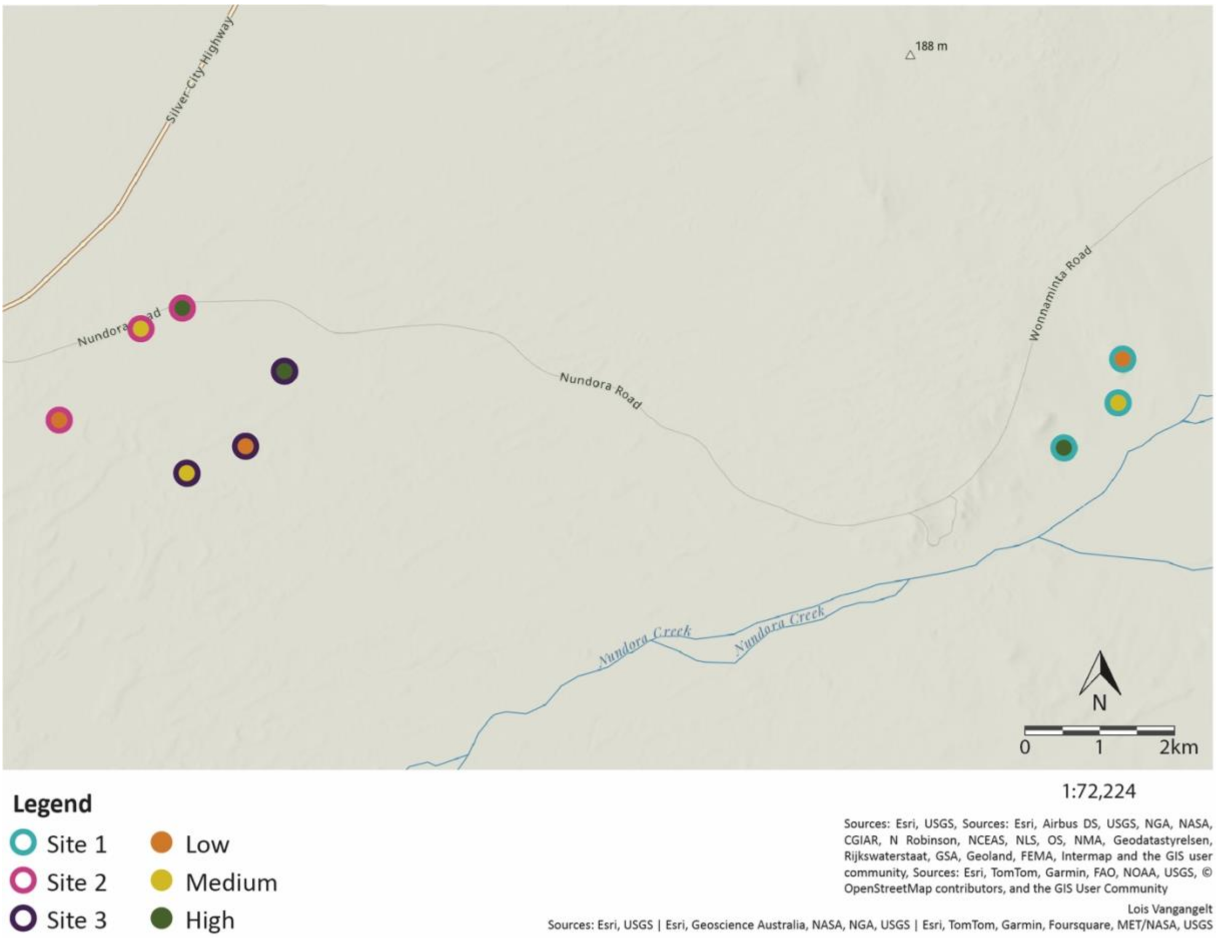

Figure 2 *Map of sampling locations specifying site and treatment for each location.*

Sample taking and soil processing

All utensils and vials were rinsed in bleach (0.4%), rinsed three times with laboratory-grade water, exposed to UV light for 15 minutes and placed in airtight bags prior to use. Silica bags, which were used to remove extra moisture from the samples, were placed under UV light for 15 minutes on each side. All vials and bags were labelled (Appendix 3). This was all clear the materials used from DNA and prevent contamination.

Soil samples were collected over three days, with one site being sampled per day. Samples were always collected firstly from the low greenness level site, then medium, then high. Sampling at each fence was done from west to east and the camera trap was sampled last. The samples were taken at a 106 cm interval along the fence of an area 90 cm x 22.5 cm (Fig. 1B). The top layer of soil/dust was collected with a new, sterilised spoon. To prevent contamination within and between sampling locations, gloves were replaced between each sample. Soil samples were placed into 50 mL Greiner vials (Sigma-Aldrich, St Louis, USA), with a 15 g silica bead bag added to absorb any potential excess moisture within the soils. The labelled vials were taken to the lab at Macquarie University.

Extracellular DNA extraction

To prepare the soil for DNA extraction, each sample was homogenised. Subsequently, a 15g sub-sample was taken and placed in a 50 mL falcon tube. The extracellular DNA extraction protocol was developed by Taberlet et al. (2012). Briefly, a phosphate buffer was prepared by adding 1.97 g NaH_2PO_4 and 14.7 g Na_2HPO_4 to 1L of Evian® natural mineral water. 15 mL of phosphate buffer (Na_2HPO_4; 0.12 M; pH ≈ 8) was mixed with each sample and shaken for 15 minutes. 2 mL from each sample was poured into a 2mL tube and centrifuged at 20817 rcf for 7.5 minutes. For the rest of the extraction, a NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) was used. The lysis steps were skipped and the manufacturer's protocol was followed from step 6 onwards with some minor changes. For each sample, 800 µL of supernatant was mixed with 500 µL SB buffer. The samples were then loaded into the corresponding spin column and spun down in two batches of 650 µL each. For the elution step, 100 µL of SE buffer heated to 80 °C was used. After the extractions were finished, the samples were stored in the freezer (-30 °C) until further use.

DNA amplification

A total of 162 samples, 6 extraction control samples, 6 positive controls and 6 negative controls were amplified, resulting in a total of 180 samples. The amplification for each sample was performed in triplicate. A dilution gradient was performed to determine the optimal dilution factor for a majority of the samples. The dilutions were made for all DNA extraction samples using Ultrapure DNA free water (Invitrogen, USA). The optimal dilution ratio was determined to be 1 in 10.

Vertebrate composition was performed by targeting the V5 region of the 12 rRNA gene using the primers: F 5'- ACTGGGATTAGATACCCC-3' and R 5'- TAGAACAGGCTCCTCTAG-3' primers (Riaz et al., 2011). The human blocking primer used was TACCCCACTATGCTTAGCCCTAAACCTCAACAGTTAAATC-C3spacer (Calvignac-Spencer et al., 2013).

DNA amplifications were performed resulting in a final volume of 30 µL. This included 15 µL Amplitaq Gold 360 Master Mix (Applied Biosystems, USA), 0.9 µL F 12S5V primer, 0.9 µL R 12S5V primer, 0.3 µL human blocking primer, 8.9 µL of Ultrapure DNA free water (Invitrogen, USA) and 4 µL of 1/10 diluted DNA extract. PCR conditions were based on Chivas et al. (unpublished). The mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 45 s at 59 °C and 60 s at 72 °C. Finally, an elongation step at 72 °C for 7 minutes. The final PCR products were brought to the Ramaciotti Centre for Genomics (UNSW, Sydney) for sequencing. Sequencing was performed on an Illumina Nextseq100 (2 x 150 PE) (San Diego, USA) using their standard indexing protocol.

Bioinformatics

Bioinformatics processing of the raw sequenced data (fastq files) was performed using the Greenfield Hybrid Analysis Pipeline (GHAP) (Greenfield, 2017). Operational taxonomic units (OTUs) derived from 97% similarity were matched against reference sequences downloaded from GenBank. Sequences with a <80% identity match to GenBank were discarded. OTUs were aggregated to the species level and higher. Nominal taxonomic classifications were assigned based on Blast cut-offs of: 97 % for species, 95 % for genus; 90 % for family; 85 % for order; 80% for class.

To remove potentially erroneous reads and sequences, data was further filtered based on the proportion of the positive controls in the environmental samples following the protocols of Gillmore et al. (2021). In brief, if the positive control was detected in a sample all OTUs with equal or a lesser number of reads were zeroed. Furthermore, OTUs which were only detected in one of the technical PCR replicates were also removed. Finally, any reads which contributed to <0.01 of the total reads for a sample were discarded. The final data was transformed to presence/absence prior to statistical analysis.

Statistics

All statistical analyses were performed using R studio (V4.2.2; R Core Team, 2022). The *vegan* package (Oksanen et al., 2022) was used for the bulk of the analysis. A community matrix with presence-absence data was created from the raw data using the *decostand* function. Site and treatment were used as explanatory factors. The spread and similarity of the samples index of the community matrix were visualised using non-metric multidimensional scaling (nMDS) derived from the Jaccard index using the *metaMDS* function. As the data was not normally distributed, differences in species richness based on site or treatment were tested using the non-parametric Kruskal-Wallis test. A Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations derived from the Jaccard index was conducted, using the *adonis2* function, to analyse the difference in community composition between sites, treatments and an interaction between the two. For the significant results ($P < 0,05$) a pairwise comparison was performed using the *pairwise.adonis2* function from the *pairwiseAdonis* package (Arbizu, 2017).

Results

Of the total of 486 samples (n = 162 amplified in triplicate), 221 were successfully amplified, resulting in 34 zero-radius Operational Taxonomic Units (ZOTUs). After filtering, 44 samples remained with 19 ZOTUs. eDNA detected a total of sixteen unique taxa: four reptiles, four birds and eight mammals. Twelve of those were assignable to the species level (Appendix 4).

Five non-native taxa were detected: domestic sheep (*Ovis aries*), cow (*Bos taurus*), goat (*Capra hircus*), wild boar (*Sus scrofa*) and, a taxa that could only be determined to the subfamily level, bovines (*Bovinae*). Signs of these non-native species were observed during sample taking across all sites. Using eDNA , all these species were detected at Site 1, at Site 2 only wild boar was detected and at Site 3 only sheep was detected. No non-native species were detected in the low greenness treatments across all sites (Appendix 5).

The remaining eleven unique taxa were all native species. Three mammals: red kangaroo (*Osphranter rufus*), western grey kangaroo (*Macropus fuliginosus*) and, a taxa that could only be determined to the family level, megabat family (*Pteropodidae*). Four reptiles: regal striped skink (*Ctenotus regius*), saltbush skink (*Morethia adelaidensis*), a skink of the comb eared skinks genus (*Ctenotus spp*.) and, a taxa that could only be determined to the family level, skink family (*Scincidae*). Four birds: black faced woodswallow (*Artamus cinereus*), Australian raven (*Corvus coronoides*), superb fairywren (*Malurus cyaneus*) and, a taxa that could only be determined to the family level, swallows, martins, and saw-wings family (*Hirundinidae*).

Comparison of eDNA to funnel trap data

The taxa captured by the funnel traps only belonged to the class reptiles. Twelve reptile species were caught in total (Appendix 5). Eight species were caught at Site 1, five species were caught at Site 2 and two species were caught at Site 3. The regal striped skink and the saltbush skink were the only two species that were detected using both funnel traps and eDNA and were both located at Site 1.

Species richness

The Kruskal-Wallis test did not reveal a significant difference across treatments (X^2 = 1.54, df = 2, P = 0.463) or sites (X^2 = 3.25, df = 2, P =0.197) for species richness. Across the three treatments most of the taxa overlapped and two taxa, red kangaroo and skink family, were detected in all treatments (Fig. 3A). Thirteen taxa were detected at Site 1, and thus this site had the highest species richness compared to Sites 2 and 3 which both only had five taxa. The medium and high treatments in Site 1 had a markedly higher species richness compared to the low treatment or any treatments at other sites (Fig. 4). No taxa were detected at all three sites (Fig. 3B).

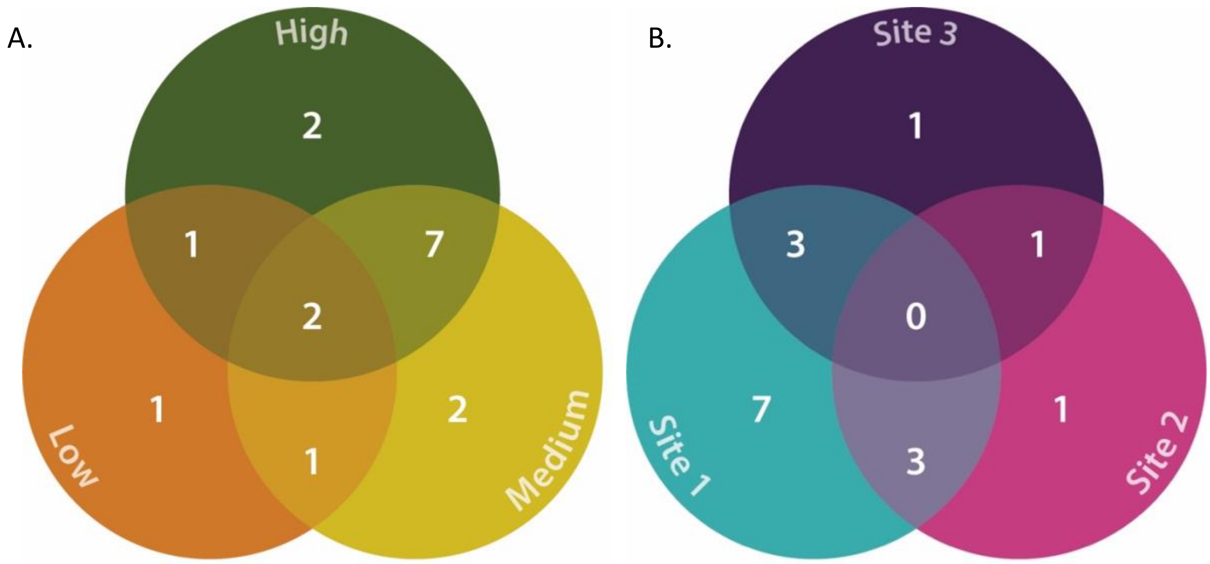

Figure 3 *The number of unique species present per treatment (**A.**) and site (**B.**). Where the overlap in circles indicates shared species between either two or all treatments or sites.*

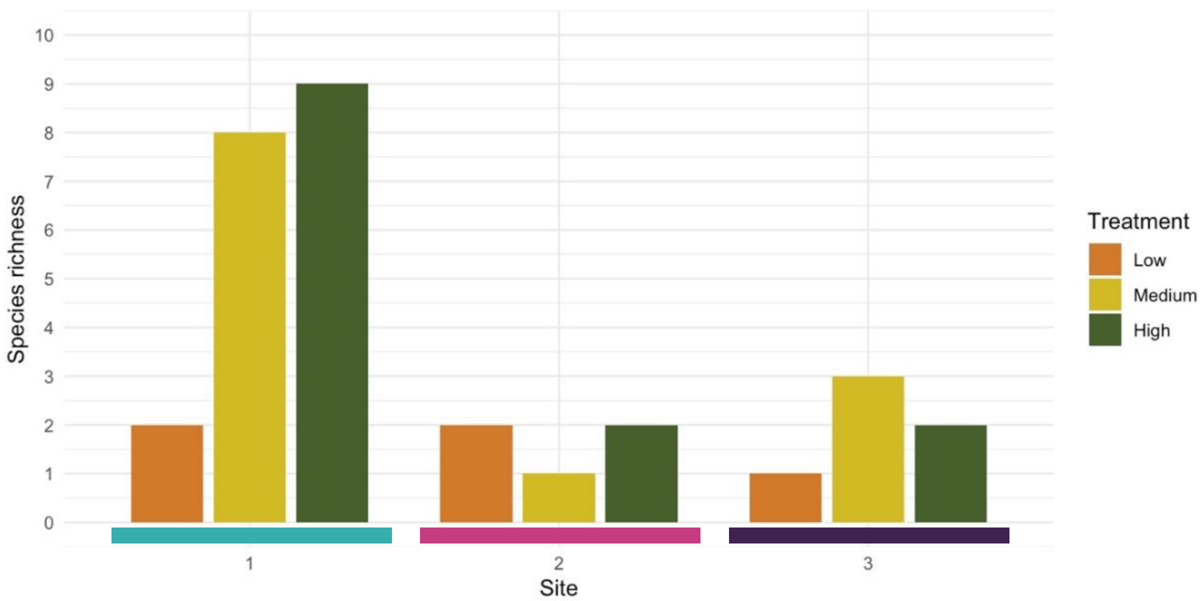

Figure 4 *Bar graph showing the species richness for each treatment within each site.*

Community composition

There was a significant interaction between treatment and site (P = 0 .001) (Table 1). The post hoc analysis shows some values missing, specifically for locations 2L and 2M, which is likely due to patchy data. Meaning the locations lack species presence data. Overall, locations 2H, 3M and 3H were significantly different from most other locations (Table 3). Another result to highlight is that samples with the same treatment but at different sites were often different from each other. The high treatments, e.g. 1H vs 2H (P = 0.001), 1H vs 3H (P = 0.081), and 2H vs 3H (P = 0.047), were markedly different from each other. The medium treatments from Site 1 and Site 3 also significantly differ from each other (P = 0.001). Additionally, with the exception of 3M vs 3H, there were no treatments within the sites that were significantly different. These results indicate that site was the dominating factor for the community composition and possibly of overriding influence on treatment.

This is confirmed by the PERMANOVA (Table 1) and the post hoc analysis of the sites (Table 2), where the sites were all significantly different (P = 0.02) from each other, and therefore, all had different vertebrate compositions. The PERMANOVA analysis failed to identify any differences in community composition between the three treatments (P = 0.539) (Table 1). This is in line with the NMDS plot (Fig. 5), there are no clear clusters and the samples of different treatments overlap.

Table 1 *Results of the PERMANOVA testing the difference in community composition. * indicates that there are significant differences in community composition for that specific parameter*

PERMANOVA					
	Df	Sum of sqs	R2	F	p-value
Treatment	2	0.83	0.04	0.95	0.539
Site	1	0.87	0.04	2.01	0.020 *
Treatment*Site	2	1.71	0.09	1.98	0.001 *
Residual	37	16.03	0.82		
Total	42	19.44	1		

Table 2 *Post hoc results from comparison of community composistion between sites*

Post hoc: Site					
	Df	Sum of sqs	R2	F	p-value
1 vs 2	1	0.97	0.06	2.17	0.005 *
1 vs 3	1	0.88	0.06	1.99	0.022 *
2 vs 3	1	1.04	0.15	2.56	0.008 *

Table 3 *Post hoc of site*treatment interaction regarding community composistion. Green cells indicate a significant difference between the two sampling locations (P < 0.05). Yellow cells indicate an almost significant difference (P < 0.1). NA indicates that there was not enough data to calculate the difference between sampling locations. The values within the rectangles show the P-values within that specific site*

Post hoc: Site*Treatment									
	1L	1M	1H	2L	2M	2H	3L	3M	3H
1L									
1M	0.213								
1H	0.114	0.129							
2L	1	0.409	0.195						
2M	1	0.392	0.196	NA					
2H	0.614	0.008	0.001	NA	NA				
3L	1	0.433	1	NA	NA	0.1667			
3M	0.14	0.001	0.267	NA	NA	0.021	1		
3H	0.4	0.073	0.081	NA	NA	0.047	0.5	0.047	

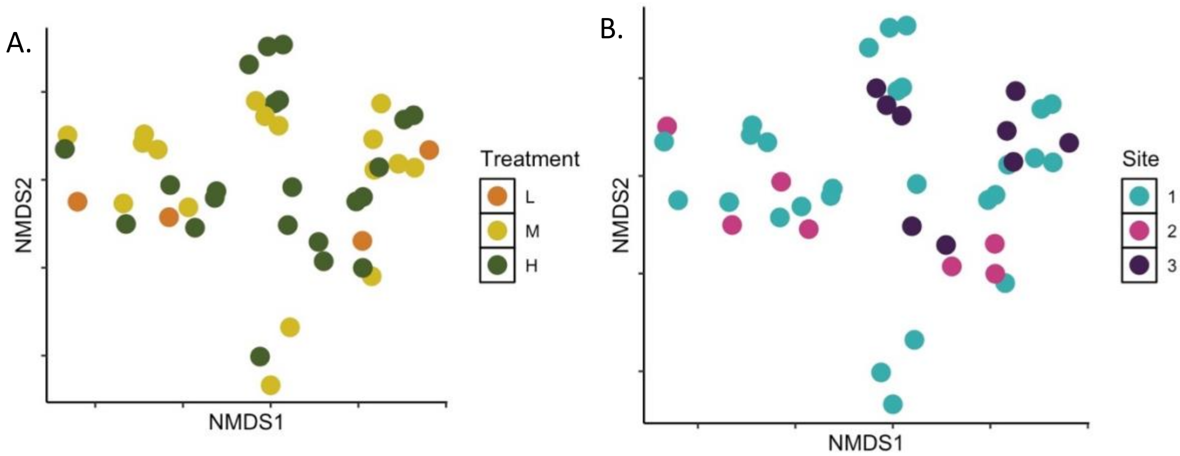

Figure 5 *Non-metric Multidimensional Scaling (NMDS) plot for (**A.**) treatment and (**B.**) site.*

Discussion

In this study I examined the preference of vertebrates for different vegetation greenness levels using eDNA derived from soils in semi-arid zones in New South Wales, Australia. As hypothesised, larger vertebrates were detected, which were predominantly mammals, e.g. two kangaroo species. Additionally, smaller reptiles and birds were also detected. Against expectation, there was no significant difference between the species richness or community composition for the different greenness levels. However, the sites all had a different community composition. This indicates that there was an overriding influence of site on treatment when it comes to community composition.

A variety of vertebrates were detected, including the non-native domestic sheep, cow, goat and boar. The area in which the samples were taken belonged to Nundora station, which is a working cattle station, which also keeps sheep and goats, with all the detected non-native mammals being observed during sampling. And hence, the high rate of detections for these taxa was expected.

Although eleven native species were detected, none of these were small terrestrial mammals, possibly reflecting their biomass, both a reflection of their size and low abundance. It has been suggested that DNA deposition, and therefore detectability, is related to biomass and behaviour (Andersen et al., 2012). Since these small mammals have a lower biomass they might have deposited less DNA in the environment. Behaviour may have also played a role. Small mammals in semi-arid zones in Australia use torpor as a way to cope with the extreme environment, where they go into torpor at night or early morning and arise during midday or the afternoon (Geiser, 2004). They use this mechanism in a range of different ambient temperatures (-2.4 to 35.5 °C) (Körtner & Geiser, 2009; Warnecke et al., 2008). In some small arid zone marsupials it has been shown that the time spent in torpor increases with colder nights (Körtner & Geiser, 2009). Samples were taken just after sunrise with temperatures ranging between 13 and 25°C, and temperatures during the night were even lower. These unseasonally low temperatures most likely increased the torpor duration of the small mammals if they were present at the study site, leading to lower quantities of small mammal DNA present in the soil due to inactivity during their torpor. In addition, the DNA that was deposited into the environment during their active period might have been partially degraded during the day due to exposure to the elements (Sirois & Buckley, 2019). However, this was unlikely, as samples were collected early in the day limiting the amount of time the DNA was exposed the UV radiation and high temperatures. Importantly, the small native terrestrial mammals were also not captured in the funnel traps, suggesting that they were either absent or inactive during the sampling period. During sustained periods of low rainfall the density of small mammals is markedly lower (Dickman et al., 2011). Furthermore, small mammals have limited mobility compared to bigger mammals who can move across different sites, and hence increase their likelihood of detection. Long range movements of small mammals do occur but are driven by rainfall, where they will move towards the area with rainfall, and even then their movement only ranges up to multiple kilometres depending on the species (Dickman et al., 1995). Collectively, this suggests that the chances of detecting (eDNA) or capturing (traps) small terrestrial mammals is site dependent, and it is unclear whether they still persist within the study region.

Interestingly, eDNA did capture native birds and reptiles, which in comparison to the mammals captured, also have a lower biomass. A reason for this is that their abundance likely was high enough to deposit enough DNA into the environment to be captured by eDNA. This was also prevalent in the field, where multiple skinks and flocks of birds were seen during soil sample collection (pers. obs. Vangangelt). However, the observational data from the live trapping showed that there were a lot more reptile species present at the sample sites that were not captured using eDNA. This might point to eDNA not having captured the full range of vertebrates present at the sampling site. Since only one primer set was used during this study there might have been some vertebrates that have been missed due to the specificity of this primer. To capture a more complete representation of the vertebrates present in the sampling area it might be beneficial to use multiple primers that specifically select reptile, bird or mammal DNA in addition to a primer that selects all vertebrates (Taberlet, 2018). Additionally, using multiple primer sets in parallel has been shown to reduce taxonomic biases and increase taxonomic coverage improving the overall performance of eDNA (Jeunen et al., 2020; Alberdi et al., 2018)

Surprisingly, no feral cats were detected in any of the samples. Cat density is driven by prey availability and is on average higher after a period of rainfall since their preferred prey, small mammals, become more abundant at that time (Letnic & Dickman, 2010; Pavey et al., 2008). However, both reptiles and birds are part of the feral cat diet (Woinarski, Murphy, et al., 2018, 2017) and especially reptiles are consumed by feral cats when they become more active during the summer months (Paltridge, 2002). However, I did not capture any small native terrestrial mammals. Additionally, the samples were taken during an unseasonally cold period which might have affected the activity of reptiles even though I did capture multiple different reptiles. Both the lack of small terrestrial mammals and low reptile activity could have contributed to the low amount of prey available with the absence of feral cats as a consequence.

A factor that might have influenced the range of captured vertebrates is the soil itself. At the study site, the soil is classified as rudosols (Department of Planning, Industry and Environment, 2021a) and silicious sands (Department of Planning, Industry and Environment, 2021b), which means the texture ranges from sandy to clayey sand (Sleeman & Walker, 1979). This overlaps with our observations in the field, it was very dry and sandy. The soil characteristics vary based on the site (Isbell, 2021). However, arid zone soils are known for low humus content (Stott & Martin, 1989) which contains key substances that extracellular DNA binds to which increases its persistence in the soil (Levy-Booth et al., 2007). Additionally, sand is suggested to have poor binding capacity for DNA due to its lower surface area (Blum et al., 1997; Lorenz & Wackernagel, 1987). This suggests that the soils at the study site were not optimal for the persistence of extracellular DNA leading to less DNA present in the samples.

The soil samples were taken along fences that were set up three weeks in advance of sampling. They were set up so that the area directly alongside it was (mostly) free of vegetation. This means that the sampling areas were more exposed to the elements in comparison to shaded areas with more vegetation coverage, for example underneath bushes or trees. Sampling more exposed areas might not have been the optimal sampling strategy since DNA exposed to the elements degrades faster (Sirois & Buckley, 2019). Ryan et al. (2022) showed that samples taken from tree hollow sediments led to a wider range of species being detected compared to the more exposed soil samples taken from the surrounding of the tree hollow. They expected this was partially due to animal behaviour as they spend an extensive amount of time inside these hollows and as a consequence, more DNA is deposited there. The link between time spent and DNA deposition has also been confirmed by previous studies (e.g. Kucherenko et al., 2018; Andersen et al., 2012). However, they also expected this was due to the tree hollow being shielded from the outside elements which improved eDNA preservation (Ryan et al., 2022). This suggests that the samples taken alongside the fence might have caused a limited range of species to be detected due to low DNA persistence.

To reiterate, there was an overriding influence of site on treatment as well as sites being significantly different from each other regarding community composition. In addition, even though it was not statistically proven, the species richness at Site 1 was notably higher than at Sites 2 and 3. Which might indicate a preference for Site 1. This might be explained by Site 1 being located further away from Sites 2 and 3, which were located closer together, and it being markedly different. From what could be observed, the surroundings of Site 1 had a higher plant biodiversity and water present at the site (pers. obs. Vangangelt). However, since vegetation greenness had no significant effect on either species richness or community composition it is likely that other factors, that were outside of the scope of this study, were driving the seeming preference for Site 1. Factors that affect the occurrence of vertebrates in arid and semi-arid zones vary per class and can range from vegetation structure, water availability, wildfire to introduced predator abundance. Repeated large fires have been shown to negatively affect small mammal populations (Lawes et al., 2015). In reptiles, the response to wildfire depends on the species with some increasing in population size and others decreasing in population size after a wildfire (Pianka & Goodyear, 2012). Rainfall positively influences small mammal abundance and species richness (Letnic & Dickman, 2010). Similarly, in reptiles rainfall is also positively associated with reptile species richness (Molyneux et al., 2018). Vegetation structure has been shown to be important in small mammal distribution but the response is species specific (Kelly et al., 2013). Further research would be necessary to determine the driving factors for habitat preference at this specific study site.

Suggestions for future soil-derived eDNA studies

It is important to highlight that this was a pilot study, and constrained to one property and a single time point. The study aimed to provide an insight into what the eDNA data may look like, if the semi-arid soils were suitable as environmental samples and whether detections from domestic animals would hinder the detection of native species. The broader study by the NSW government and MQ University will build upon the findings of this pilot study. As such, several points are worth considering or reflecting upon as the broader study evolves, Firstly, this present study utilised only a single primer set, 12S5V, and hence, it has been proposed that the broader study will include multiple primer sets used in conjunction with the 12S5V primer used in this study. This will increase the taxonomic coverage and provide a more complete picture of the biodiversity at the study site. Potential additional primer sets include the 16SMam1/2 for mammals (Boessenkool et al., 2012) or Aves01 for birds (Epp et al., 2012). Given the ubiquitous presence of some exotic mammals, e.g. cattle and goats, it might also be worth considering the addition of a blocking primer for these species as a means of increasing the chance of detecting native species. Secondly, as highlighted by the statistical challenges associated with patchy detection data, a more extensive sample collection is necessary to enhance the robustness of the study. It has been suggested that increasing sampling spatially and quantitatively results in more meaningful estimates of biodiversity (Andersen et al., 2012). Additionally, a combination of several different substrates increases the range of biodiversity detected (Van Der Heyde et al., 2020). Incorporating these suggestions, in combination with sampling from more protected surface soils (e.g. inside burrows, underneath vegetation, etc.), should yield better eDNA results that are more representative of the actual biodiversity at the study site. Previous studies also suggested combining eDNA with other traditional biomonitoring methods, for example, live and camera trapping, to increase the chance of capturing the full range of biodiversity at the study site (Mena et al., 2021). During our time in the field, additional soil samples were collected from burrows and underneath vegetation at the same sites as this study. Additionally, camera trap data was collected. These soil samples and camera trap data will both be included in the broader study. In addition to the funnel trap data, the camera trap data will also be compared to what was captured using eDNA. This will give a better insight into the taxonomic coverage of eDNA at the study site and might result in a different preference for either treatment or site.

Conclusion

Our pilot study provided insight into the use of eDNA to analyse vertebrate communities in semi-arid Australia. Vegetation greenness did not affect the species richness or community composition. However, some significant differences were found between the community compositions, where not vegetation greenness but site location was the driving factor. This suggests that habitat preference might be determined by other factors outside of the scope of this study. Additional research is necessary to identify what these other potential driving factors are. Once these factors are identified they could give more insight into how species management outside of feral predator-free zones could be improved to limit declines that are currently prevalent in population sizes of native species (Tulloch et al., 2023). Since this study was limited in region and time it is difficult to definitively say if eDNA can be used to determine the vertebrate composition within arid and semi-arid regions in Australia. Hence, further studies expanded temporally and spatially, are required to gain more insight into the use of eDNA in these regions.

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Appendix 1 Popular science summary

Animal communities in semi-arid Australia: how much green do they prefer?

Animals that live in semi-arid Australia are exposed to extreme environments and long dry periods affect their population sizes. During these periods they rely on refugia for their survival. Refugia are microclimates created by plants that offer resources like food and shelter. The amount of refugia available is related to the amount of plant coverage (greenness) in the area. It is important to study the diversity of animals in habitats with different greenness levels to see if there is a preference. If we know what they prefer we can incorporate this into management programmes to better protect animals in these semi-arid areas.

How do we measure diversity?

To measure diversity in habitats with different greenness levels we need to collect biomonitoring data. This data tells you something about the presence, abundance and distribution of different animal species. There are different ways to collect this type of data. During our study, we chose to use a method called environmental DNA or eDNA.

What is eDNA?

When an animal moves through its environment it leaves behind little traces of hair, skin, faeces, etc. These traces remain in the soil, water, snow or sediment. If you take an environmental sample you can use it to trace the animals that have been in that specific area. For our study, we chose to collect soil samples.

Our study site

Our soil samples were collected at Nundora station in New South Wales, Australia (Fig. 1). In total we had three sites and each site had three sampling locations corresponding with different vegetation greenness. These were divided into three levels: low, medium and high.

What did we find?

In total, we could trace back 8 mammals, 4 birds and 4 reptiles. There seemed to be no preference for any of the different vegetation greenness levels since the different species were found spread across all greenness levels. However, most of the species were found at one of the sites. This indicates that there might be a preference for this specific site which is not related to the vegetation greenness levels. There might have been other factors causing this preference that were not included in our study. Plant communities or water availability might have been the driving factors. More research needs to be done on this topic to say for certain what drives the preference for vegetation greenness in semi-arid zones.

Figure 1 Map of Australia. The red dot indicates where our study site was located, Nudora station.

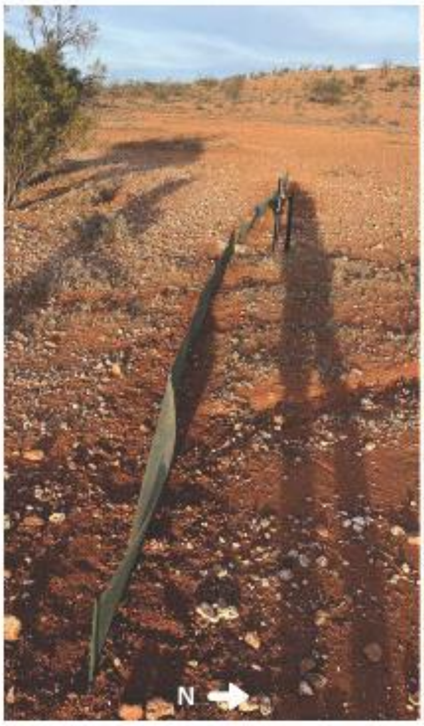
Appendix 2 Sampling site information

Nundora site 1

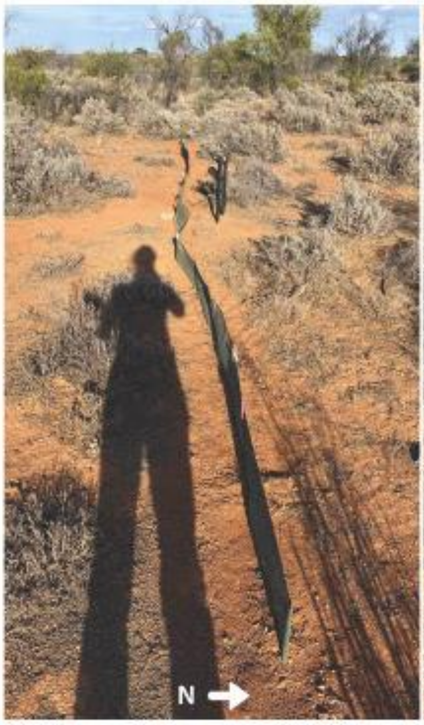
Sampled on	18-11-2023	
	Start	Finish
Time	06:27	10:06
Temperature (°C)	13	25
UV - index	0	4
First light	05:50	
Notes	1LN - very rocky, 1HN - lots of goat/ cow poo	

Nundora site 1 Low South (LV 1LS)		
Coordinates	Lat: -30.702760	Long: 142.061603
Time	Start: 06:30	Finish: 07:00

Nundora site 1 Low North (LV 1LN)		
Coordinates	Lat: -30.702223	Long: 142.062255
Time	Start: 07:03	Finish: 07:37



Nundora site 1 Medium South (LV 1MS)		
Coordinates	Lat: -30.707806	Long: 142.061398
Time	Start: 07:56	Finish: 08:23



Nundora site 1 Medium North (LV 1MN)		
Coordinates	Lat: -30.707222	Long: 142.061965
Time	Start: 08:26	Finish: 08:56

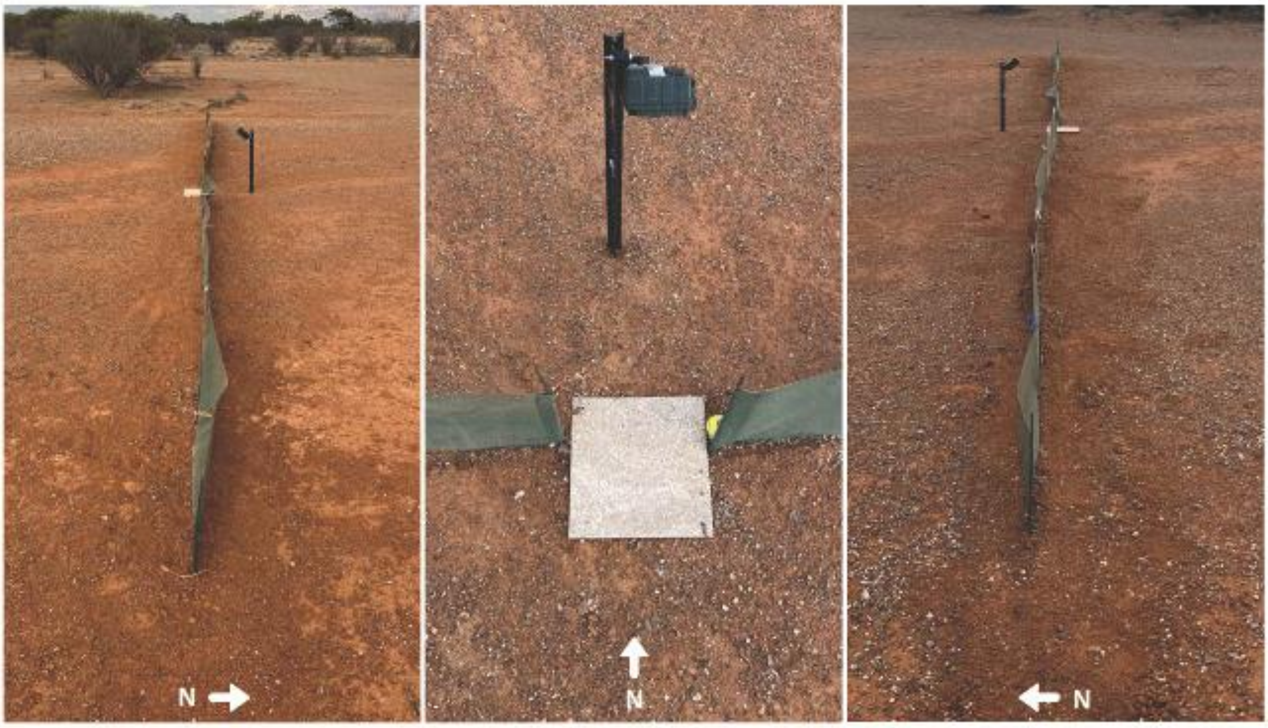
Nundora site 1 High North (LV 1HN)		
Coordinates	Lat: -30.712355	Long: 142.053948
Time	Start: 09:08	Finish: 09:35

Nundora site 1 High South (LV 1HS)		
Coordinates	Lat: -30.713140	Long: 142.054086
Time	Start: 09:38	Finish: 10:06

Nundora site 2

Sampled on	19-11-2023	
	Start	Finish
Time	07:00	09:40
Temperature (°C)	21	25
UV - index	0	4
First light	05:50	
Notes	2LS - very rocky, S2HS S2HN many flies	

Nundora site 2 Low South (LV 2LS)		
Coordinates	Lat: -30.709756	Long: 141.913923
Time	Start: 07:04	Finish: 07:23



Nundora site 2 Low North (LV 2LN)		
Coordinates	Lat: -30.708974	Long: 141.913914
Time	Start: 07:27	Finish: 07:52

Nundora site 2 Medium South (LV 2MS)		
Coordinates	Lat: -30.699927	Long: 141.924460
Time	Start: 08:01	Finish: 08:21

Nundora site 2 Medium North (LV 2MN)		
Coordinates	Lat: -30.699123	Long: 141.924685
Time	Start: 08:24	Finish: 08:48

Nundora site 2 High South (LV 2HS)		
Coordinates	Lat: -30.698550	Long: 141.930547
Time	Start: 08:56	Finish: 09:15

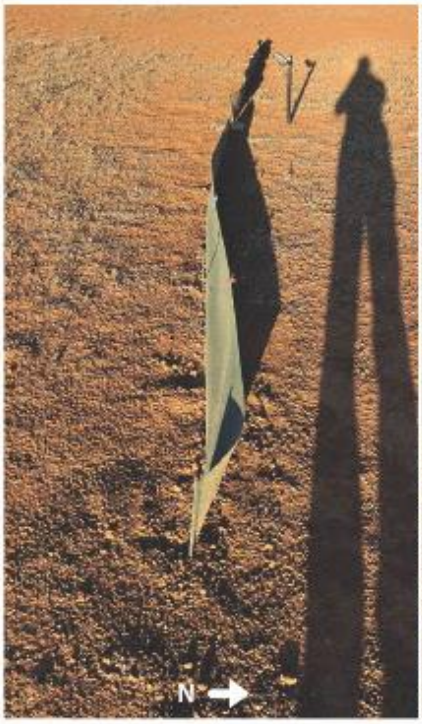
Nundora site 2 High North (LV 2HN)		
Coordinates	Lat: -30.697781	Long: 141.930522
Time	Start: 09:20	Finish: 09:40

Nundora site 3

Sampled on	17-11-2023	
	Start	Finish
Time	06:00	10:22
Temperature (°C)	14	24
UV - index	0	7
First light	05:50	
Notes	3LS - west side of fence down, mouldy boards, 3MSS2 silica bag fell on soil, 3HN 3HS lables swapped around	

Nundora site 3 Low South (LV 3LS)		
Coordinates	Lat: -30.713071	Long: 141.938266
Time	Start: 06:32	Finish: 07:09

Nundora site 3 Low North (LV 3LN)		
Coordinates	Lat: -30.712548	Long: 141.938951
Time	Start: 07:12	Finish: 07:49



Nundora site 3 Medium South (LV 3MS)		
Coordinates	Lat: -30.716508	Long: 141.930085
Time	Start: 07:59	Finish: 08:31

Nundora site 3 Medium North (LV 3MN)		
Coordinates	Lat: -30.715848	Long: 141.930426
Time	Start: 08:36	Finish: 09:10

Nundora site 3 High South (LV 3HS)		
Coordinates	Lat: -30.703537	Long: 141.944781
Time	Start: 09:22	Finish: 09:51

Nundora site 3 High North (LV 3HN)		
Coordinates	Lat: -30.703118	Long: 141.943890
Time	Start: 09:58	Finish: 10:22

Appendix 3 Labelling key

Initials	Site	Greenness	Fence	Side	# sample
LV	1	L (Low)	N (North)	N (North)	1 - 4
LV	2	M (Medium)	S (South)	S (South)	C (camera)
	3	H (High)			

Appendix 4 Taxa detected in soil-derived eDNA

Order	Suborder	Family	Subfamily	Genus	Species	Common name
Domestic and non-native mammals						
Artiodactyla	Ruminantia	Bovidae	Bovinae	Bos	Bos taurus	Domestic cow
Artiodactyla	Ruminantia	Bovidae	Caprinae	Capra	Capra hircus	Goat
Artiodactyla	Ruminantia	Bovidae	Caprinae	Ovis	Ovis aries	Domestic sheep
Artiodactyla	Suina	Suidae		Sus	Sus scrofa	Wild boar
Artiodactyla	Ruminantia	Bovidae	Bovinae		*	*
Native mammals						
Diprotodontia		Macropodidae		Macropus	Macropus fuliginosus	Western grey kangaroo
Diprotodontia		Macropodidae		Osphranter	Osphranter rufus	Red kangaroo
Chiroptera	Megachiroptera	Pteropodidae			*	*
Reptiles						
Squamata		Scincidae	Sphenomorphinae	Ctenotus	Ctenotus regius	Regal striped skink
Squamata		Scincidae	Eugongylinae	Morethia	Morethia adelaidensis	Saltbush skink
Squamata		Scincidae	Sphenomorphinae	Ctenotus	Ctenotus spp.	*
Squamata		Scincidae			*	
Birds						
Passeriformes		Corvidae		Artamus	Artamus cinereus	Black faced woodswallow
Passeriformes		Corvidae		Corvus	Corvus coronoides	Australian raven
Passeriformes		Maluridae		Malurus	Malurus cyaneus	Superb fairywren
Passeriformes		Hirundinidae			*	*

Appendix 5 eDNA and observational data combined

Order	Family	Genus	Species	1L	1M	1H	2L	2M	2H	3L	3M	3H
Domestic and non-native mammals												
Artiodactyla	Bovidae	Bos	Bos taurus									
Artiodactyla	Bovidae	Capra	Capra hircus									
Artiodactyla	Bovidae	Ovis	Ovis aries									
Artiodactyla	Suidae	Sus	Sus scrofa									
Artiodactyla	Bovidae											
Native mammals												
Diprotodontia	Macropodidae	Macropus	Macropus fuliginosus									
Diprotodontia	Macropodidae	Osphranter	Osphranter rufus									
Chiroptera	Pteropodidae											
Reptiles												
Squamata	Agamidae	Ctenophorus	Ctenophorus nuchalis									
Squamata	Agamidae	Ctenophorus	Ctenophorus pictus									
Squamata	Agamidae	Pogona	Pogona vitticeps									
Squamata	Diplodactylidae	Lucasium	Lucasium damaeum									
Squamata	Diplodactylidae	Lucasium	Lucasium microplax									
Squamata	Elapidae	Suta	Suta suta									
Squamata	Gekkonidae	Gehyra	Gehyra versicolor									
Squamata	Scincidae	Ctenotus	Ctenotus regius									
Squamata	Scincidae	Ctenotus	Ctenotus schomburgkii									
Squamata	Scincidae	Ctenotus	Ctenotus spp.									
Squamata	Scincidae	Menetia	Menetia greyii									
Squamata	Scincidae	Morethia	Morethia adelaidensis									
Squamata	Scincidae											
Birds												
Passeriformes	Corvidae	Artamus	Artamus cinereus									
Passeriformes	Corvidae	Corvus	Corvus coronoides									
Passeriformes	Maluridae	Malurus	Malurus cyaneus									
Passeriformes	Hirundinidae											

Family	Species	
		only eDNA
		only Obs
		Obs & eDNA
		Not detected