Global Ecology and Biogeography

WILEY

Global Ecology and Biogeography | A Journal of Macroecology

RESEARCH ARTICLE OPEN ACCESS

Plant Phylogenetic History, Not Diversity, Drives Insect Biodiversity Across South Africa's Biomes

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Received: 24 July 2025 | **Revised:** 30 June 2026 | **Accepted:** 8 July 2026

Handling Editor: Andrew Rominger

Keywords: beta diversity | DNA barcoding | malaise traps | phylogenetic turnover | plant-insect interactions | South Africa

ABSTRACT

Aim: Insects provide essential ecosystem services, but there is a concern that insect abundance is in rapid decline. Identifying trends in insect biodiversity is challenging because we lack baseline data for most biogeographic regions, and most insect species remain undescribed. Here, we aim to report the first nationwide inventory of insect biodiversity in South Africa.

Location: Eleven National Botanical Gardens representing four major South African biomes: Savanna, Grassland, Fynbos and Albany Thicket.

Time Period: Sampling from 1 February 2022 to 31 January 2023.

Major Taxa Studied: Insects (Arthropods) and vascular plants.

Methods: We deployed two Malaise traps per botanical garden and used DNA barcoding to identify insect specimens. We reconstructed phylogenies for insect molecular clusters (BINs) and 12,149 vascular plant species. Insect and plant community structure were compared using dissimilarity metrics and Mantel tests.

Results: We generated DNA barcodes for 337,809 specimens, representing 29,919 unique molecular clusters, as species proxies. We estimated that the true richness of above ground insect biodiversity in South Africa may be closer to 100,000 species, yet 97% of barcoded taxa could not be assigned to species level. At current rates of taxonomic description, formally cataloguing this dark diversity would take more than 200 years. Estimated insect species richness varied strongly among biomes, with higher insect diversity in the Savanna biome than the floristically rich Fynbos. Turnover in plant lineages was associated with insect community turnover, particularly insect herbivores and predators.

Main Conclusions: We show that plant species richness alone is a poor predictor of insect species richness, with biome-specific factors playing an important role in shaping insect richness gradients. However, turnover in plant lineages was a strong predictor of insect compositional differences between gardens. Our study provides both critical baseline data on insect diversity in South Africa and new insights into the ecological and evolutionary drivers of insect biodiversity gradients.

1 | Introduction

With an estimate of 1.2 million described species, insects make up much of the macroscopic diversity of life (Mora et al. 2011). They are critical to ecosystem functioning, contribute to nutrient cycling and soil formation, and support many valuable ecosystem services, including pollination of crops, seed dispersal and pest control (Jolls et al. 2019). Insects are also key ecological indicators as they are highly sensitive to environmental change (Bruce 2015). Some recent studies have suggested dramatic declines in insect abundance Hallmann et al. (2017), but the evidence and drivers remain controversial. Recent exchanges (e.g., Hallmann et al. 2025; Müller et al. 2024) highlight ongoing disagreement over the most appropriate sampling methods and response variables (e.g., biomass vs. abundance or richness) to assess insect declines. There thus remains much uncertainty in trends in insect diversity (Duffus et al. 2023; Johnson et al. 2024), and most species are undescribed (Stork 2018). Regardless of this debate, there is a broad agreement that insect losses could have cascading consequences for ecosystem integrity (Haddad et al. 2009). Here, we present the first baseline data on insect diversity for South Africa, a megadiverse country, and investigate the relationship between floristic diversity and insect richness.

The quantification of insect diversity is a daunting challenge, requiring taxonomic expertise across multiple groups and significant time and financial investment (Chimeno et al. 2022; Morinière et al. 2016). Molecular methods, such as DNA barcoding, provide a rapid and efficient method for identifying specimens through sequencing a short standardised genomic marker that can be compared to a DNA barcode reference library (Hebert et al. 2003). In animals, the mitochondrial cytochrome *c* oxidase subunit 1 (COI) is the standard DNA barcode, with sequences stored and curated on the Barcode of Life Data System (BOLD) (Ratnasingham and Hebert 2007). Because of its low cost and scalability, DNA barcoding is now a standard approach for large-scale biodiversity studies (D'Souza et al. 2021; Hebert et al. 2025; Seymour et al. 2024; Stewart et al. 2024).

DNA barcoding has been used widely in environmental assessments (Hajibabaei et al. 2012), in delineating cryptic species (Bickford et al. 2007), and in detecting invasive species (Briski et al. 2011). However, the efficacy of DNA barcoding is dependent upon the existence of comprehensive barcode libraries to which sequences can be matched and subsequently classified (Weigand et al. 2019). For many less-developed countries, the representation of insects in barcode libraries remains poor (Stewart et al. 2024). We address this gap by constructing a DNA barcode library for South African insects and examine how insect diversity varies geographically and with floristic composition.

South Africa is home to at least 21,000 species of flowering plants (Zengeya et al. 2020) and 44,000 species of insects (Myburgh et al. 2021), reflecting its varied climate, distinctive geography and complex geology. Its diversity falls within nine biomes from the Savanna to the Fynbos, a unique shrubland and the smallest of the world's six Floristic Kingdoms (Mucina and Rutherford 2006). The Fynbos is a globally recognised biodiversity hotspot, home to more than 6200 endemic plant species (Hoveka et al. 2020). South Africa also encompasses the Succulent Karoo and Maputaland-Pondoland-Albany biodiversity hotspots (Myers et al. 2000), each with many endemic plant species. However, South African biodiversity is under threat because of habitat loss due to agriculture, pollution from industrial activities, climate change and invasive species (Leisher et al. 2022). The IUCN Red List (https://www.iucnredlist.org/) has documented how these factors have increased extinction risk for mammals, birds, amphibians and plants (Atwood et al. 2020), but equivalent data for insects is effectively absent (SANBI 2010).

A Malaise trap survey within Kruger National Park by D'Souza et al. (2021) highlighted the potential of DNA barcoding to document South Africa's insect diversity. Barcoding 367,743 specimens, D'Souza et al. (2021) recorded 19,730 unique sequence clusters (Barcode Index Numbers—BINs), a species proxy. Many of these taxa were undescribed, and a recent assessment of South African sequences on BOLD revealed a large number of 'dark taxa' (Stewart et al. 2024)—species with barcode sequence data but lacking a Linnean name (Gibbs 2018). Nonetheless, by clustering sequences using the BIN system (Ratnasingham and Hebert 2013), it is possible to discriminate between samples such that broad estimates of taxonomic richness can be derived even in the absence of formal species assignments. Here, we analyse data from an extensive network of Malaise traps in botanic gardens and use DNA barcoding to describe patterns of insect diversity and turnover across South Africa.

The drivers of plant diversity and compositional turnover have been well studied and include climate, soil properties, species interactions and evolutionary history (Abraham et al. 2022; de Beer et al. 2023; Guo et al. 2017). We lack equivalent macroecological studies for insects. Seymour et al. (2024) use data from Malaise trapping to show that global arthropod beta-diversity is spatially and temporally structured by latitude, yet we do not have basic information on the underlying drivers of these gradients or if different clades (Diptera, Coleoptera, Hymenoptera etc.) and functional groups (pollinators, pests, parasites etc.) show congruent or disparate patterns. Plants and insects are intrinsically linked (Ebeling et al. 2018) because the plant community defines the niche space for insects (Schaffers et al. 2008), and insects are pests, pollinators and mutualists of plants. We might, therefore, predict as a first approximation that the distribution and compositional structure of regional insect diversity would covary tightly with plant diversity (Carroll et al. 2023); however, even this simplistic prediction has been rarely tested.

Insects provide many of Nature's contributions to people (IPBES; Díaz et al. 2015) and shifts in insect diversity may be a harbinger of more significant environmental change. Some studies have suggested dramatic declines in flying insects (Buchmann and Nabhan 1996; Hallmann et al. 2017; Wagner 2020), but it is not clear whether patterns generalise or how best to conserve insect biodiversity in the face of multiple anthropogenic pressures. A better understanding of the drivers of insect diversity would provide a guide for insect conservation and help sustain the important ecosystem services they provide. Using Malaise trapping and DNA barcoding, we present the first synthesis of flying insect diversity across South

Africa. We use these data to explore the relationship between floristic diversity and insect richness and turnover, providing a unique macroecological study of insect-plant associations.

2 | Methods

Two Townes-style Malaise traps (BT1002, Bugdorm; Townes 1972) were deployed in each of South Africa's 11 National Botanical Gardens (NBGs) situated in eight of its nine provinces. DNA barcoding was used to describe the regional composition and turnover of insect biodiversity. While the Malaise traps also capture some non-insect arthropod groups (e.g., Araneae and Collembola), most taxa in traps were insects (Insecta = 94.8%, Arachnida = 2.5%, Collembola = 1.0%, other = 1.7%), so observed biodiversity trends largely reflect variation in insect diversity. For simplicity, we thus refer to 'insect' diversity here although our data includes some taxa from other arthropod groups.

We characterise the richness (taxonomic diversity; TD) and phylogenetic diversity (PD) of insects within and between gardens. PD represents the sum of the branch lengths connecting a set of species in an evolutionary tree and captures information on the evolutionary distances separating them (Davies and Buckley 2011; Faith 1992). Phylogenetically close species typically share similar ecologies and ecosystem functions due to their shared evolutionary histories (Miller et al. 2018). PD can therefore provide insight into species ecology when taxonomic information is incomplete (Meiri and Mace 2007). PD is thus a useful diversity metric for DNA barcoding projects that generate large volumes of 'dark taxa', where thousands of unique sequence clusters (BINs) may be identified but taxonomy is poorly resolved or taxonomic expertise is lacking (for examples, see D'Souza et al. 2021 and Seymour et al. 2024). We compared the variation in insect diversity within and among NBGs to the floristic composition of the gardens.

2.1 | Study Sites and Field Sampling

Most of South Africa's 11 NBGs (Figure 1, Table S1) include both an interpretative section (cultivated) and a conservation section (uncultivated). The Hantam NBG and Kwelera NBG are exceptions as they only possessed a conservation section at the time of sampling. Importantly, the cultivated sections of South African NBGs are generally planted with indigenous plants typical of their local biome so the flora in these gardens broadly represents the regional flora. The gardens are situated in four (Grassland,

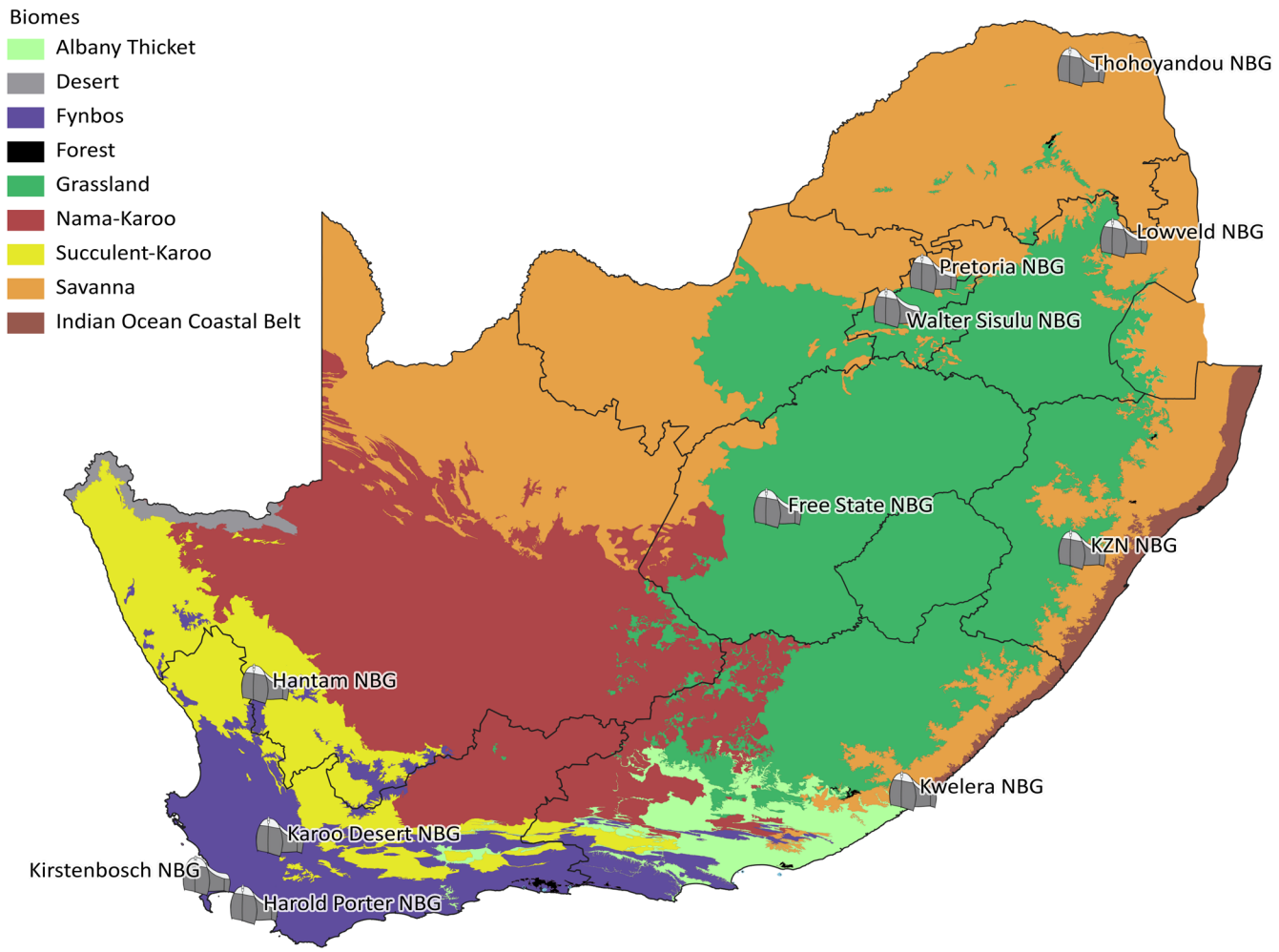


FIGURE 1 | The distribution of National Botanic Gardens (NBG) and the nine South African biomes. Colours indicate the biomes of South Africa. Lines delineate South African provincial borders.

Savanna, Fynbos, Albany Thicket) of the nine major South African biomes, and seven (Lowveld NBG, KZN NBG, Kwelera NBG, Harold Porter NBG, Karoo Desert NBG, Kirstenbosch NBG and Hantam NBG) are located within one of the three South African biodiversity hotspots (Figure 1).

We derived estimates of the insect biodiversity in each garden by pooling the results from the two Malaise traps (one from the cultivated section and the other from the conservation section; in Hantum NBG and Kwelera NBG both traps were necessarily in the conservation section; Figure 1, Table S1). The traps were overseen by staff from the respective botanical gardens for a period of 1 year (01/02/2022–31/01/2023). Samples were collected every week, decanted into a Whirl Pak bag and stored away from direct light in 95% ethanol. A team from the African Centre for DNA Barcoding (ACDB; https://www.acdb.co.za/) retrieved the samples and dispatched them to the Centre for Biodiversity Genomics (CBG; https://biodiversitygenomics.net/) every 3 months for processing.

2.2 | DNA Barcoding Protocol

A total of 1120 weekly samples (2 traps × 11 gardens × 51 weeks = 1120 weekly samples ± collection mistakes) were collected from the NBG Malaise traps. Of these, 563 samples (representing samples collected every second week) were processed through the single specimen barcoding pipeline at the CBG. Each individual insect specimen was removed from the sample bag and prepared for DNA barcoding with the exception of highly abundant morphospecies (primarily Collembola, from which only a representative subset was sampled). Specimen tissue (i.e., legs from larger specimens and full vouchers of specimens < 5 mm) was arrayed into 96-well plates for processing. To extract DNA, ethanol was evaporated from the plates and replaced with 50 μL of tissue lysis buffer (1 M KCl, 20 mg/mL Proteinase K), then plates were incubated overnight at 56°C. After lysis, DNA was purified using SPRI beads (Rohland and Reich 2012).

We followed the PCR and sequencing protocols from Hebert et al. (2025). For each specimen, we amplified the COI barcode region using the single primer cocktails C_LepFolF (LepF1 and LCO1490) and C_LepFolR (LepR1 and HCO2198). Each primer was asymmetrically indexed with a 16 bp Unique Molecular Identifier (UMI), allowing each sequence to be linked to its source specimen. PCR was performed using the following thermocycling protocol: pre-denaturation at 94°C for 2 min, five cycles of 94°C for 40 s, 45°C for 40 s and 72°C for 1 min, followed by 40 cycles of 94°C for 40 s, 51°C for 40 s and 72°C for 1 min, with a final extension at 72°C for 2 min. By employing 96 distinct forward primers and 96 distinct reverse primers, each tagged with a UMI, 9216 unique pairwise combinations were generated.

The PCR products were pooled and sequenced in batches of 9120 specimens (plus 96 negative controls for a total of 9216 samples) using single molecule, real-time (SMRT) sequencing on a Sequel or Sequel II platform with 99.9% circular consensus sequence accuracy (Hebert et al. 2025), and the sequence data were uploaded to BOLD Systems (https://boldsystems.org/; Ratnasingham and Hebert 2007). Each sequence was then identified and assigned a Barcode Index Number (BIN; Ratnasingham and Hebert 2013). The BIN system clusters DNA barcodes into operational taxonomic units (OTUs) based on a refined single linkage algorithm that initially links sequences with < 2.2% divergence, then refines the boundaries with Markov Clustering. The algorithm improves upon earlier tree-based and threshold methods by generating clusters that are more stable and reproducible across datasets; however, its single-gene focus limits resolution in cases of introgression or incomplete lineage sorting. Records with major conflicts between the voucher taxonomy and sequence-based assignment, particularly above order level, were reviewed using the voucher images. Clear voucher misidentifications were corrected, and records consistent with contamination were flagged during validation.

In total, we sequenced 337,809 specimens which were identified to one of 2611 family, genus or species. Barcode sequences for each of the gardens are available on BOLD and are cross-referenced with GBIF (Table S1).

2.3 | Functional Group Classification

We assigned each of the 2611 taxa classified to family, genus or species to one of eight broad functional groups (Table 1) using information from the literature and online databases (https://bugguide.net/ and https://www.waspweb.org/). In some cases, the same taxon fell within multiple categories; in these cases, all assigned functional groups were recorded.

TABLE 1 | Descriptions of functional group types.

Functional group	Description
Pollinators	Associated with plant pollination, for example, *Apidae*, Bombyliidae
Processors	Play a role in breaking down both plant material and nutrient cycling, for example, Blattodea, Curtonotidae
Parasitoids	Parasitic relationship with other insects, for example, Chalcididae, *Chelonus*
Predators	Insects that consume other insect species, for example, Mantodea, *Theridion*
Herbivore	Consume plants and have the potential to become plant pests, for example, Aphididae, Tortricidae
Aquatics	Associated with water or aquatic environments, for example, *Kiefferulus*, Hydraenidae
Fungus associated	Feed on or are associated with fungi, for example, Ptiliidae, *Sciara*
Animal parasites	Have a parasitic relationship with non-insect organisms, for example, *Culicoides*, Mesostigmata

2.4 | Phylogenetic Reconstruction

We reconstructed the phylogenetic tree of sampled taxa from the sequenced COI barcode sequences. From the 337,809 sequences, we sampled one sequence per BIN with length closest to 658 bp returning 29,919 unique sequences (mean length = 652 bp, shortest = 452 bp, longest = 883 bp). Sequences were then grouped by taxonomic order using ape (v5.8; Paradis and Schliep 2019) and aligned using MAFFT (Katoh and Standley 2013) with default settings, alignments were visually inspected for outliers before being combined into a single matrix. The phylogenetic tree was reconstructed from the combined matrix in FastTree 2 using 10,000 bootstrap replicates and the generalised time reversible (GTR) model, selected by ModelFinder, the GTR model is one of the limited nucleotide substitution models available in FastTree 2 (Kalyaanamoorthy et al. 2017; Price et al. 2010). Because COI barcodes provide limited deep phylogenetic signal, we constrained our reconstruction with a backbone phylogeny of insect orders created using R libraries rotl (An Interface to the 'Open Tree of Life' of Life API; v3.1.0; Michonneau et al. 2016; Redelings et al. 2020), ape and TreeTools (v1.11.1; Smith 2019). We assessed the monophyly of each order using MonoPhy (v1.3.1; Schwery and O'Meara 2016) and visualised the tree using GGTree (v3.10.1; Yu et al. 2017).

2.5 | Plant Lists

A plant species list was compiled for each garden as described in (Stewart et al. 2025) but we additionally include taxa from Poales. Species lists were assembled from several sources, including species distribution models (Hoveka et al. 2020), iNaturalist observation records (downloaded from the NBGs of Southern Africa project; https://www.inaturalist.org/ on 21/03/2023), plants with historical collections in the areas (Germishuizen and Meyer 2003; Henderson 2011; Magill et al. 1983; Mucina et al. 2000; Rutherford et al. 2003, 2012), and a list provided by Botanic Gardens Conservation International (BGCI; https://www.bgci.org/). We standardised taxonomy using Kew's Plants of the World Online database and the pow_search tool within the R library taxize (v0.9.100; Chamberlain and Szocs 2013).

We assembled a phylogenetic tree for all plants recorded in the gardens using the angiosperm phylogeny from Janssens et al. (2020) as a scaffold. We then used a combination of R libraries ape, TreeTools and V.PhyloMaker (v0.1.0; Jin and Qian 2019) to place species not represented in Janssens et al. (2020) within their genus or if the genus was not represented, within their respective family. Finally, taxa not in our NBG species list were removed from the phylogeny. The final plant phylogeny consisted of 12,149 species, representing 2035 genera and 239 families, covering 17 orders.

2.6 | Taxonomic and Phylogenetic Diversity

We characterised the abundance, diversity and composition of insects and plants across NBGs using the vegan (v2.6-8; Oksanen et al. 2024) and iNEXT.3D (v1.0.5; Anne Chao et al. 2021) packages within R. Insect diversity was calculated using the Chao bias-corrected estimations with the estaccumR function in vegan using BINs as species.

We generated taxonomic and phylogenetic rarefaction curves using iNEXT3D with datatype = 'abundance' measured as the number of barcoded specimens assigned to each BIN, 100 bootstrap replicates and an endpoint of 450,000 specimens. PD was calculated as the sum of branch lengths from the phylogenetic tree (Faith 1992).

2.7 | Plant-Insect Correlations

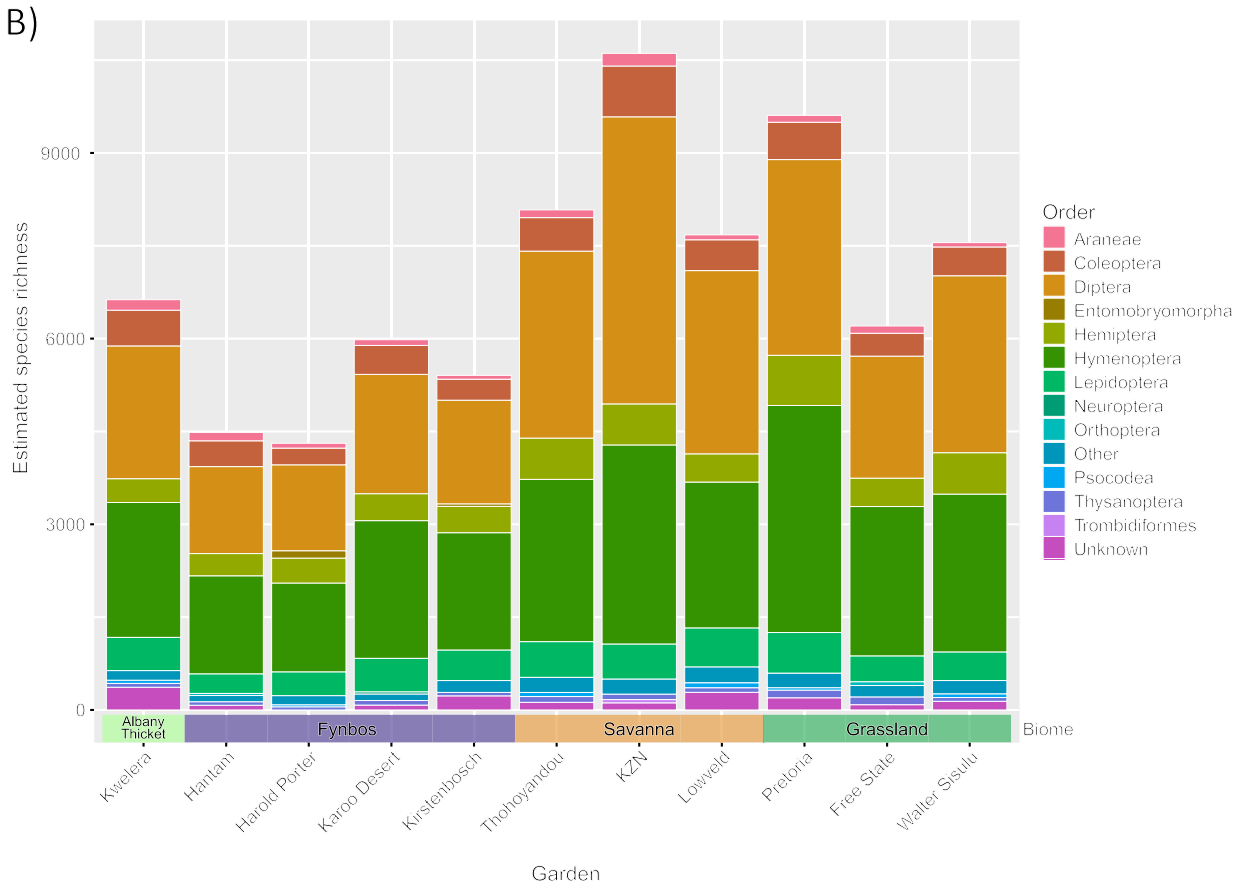
We investigated whether plant species diversity was a reliable predictor of insect diversity by examining the taxonomic and PD correlation between plants and insects across gardens and biomes.

We described pair-wise differences in species presences (Jaccard's dissimilarity), barcoded specimen abundance (Bray–Curtis's dissimilarity) and evolutionary history (Jaccard's phylogenetic dissimilarity) using the vegan and betapart R libraries (v1.6; Baselga et al. 2023). We further partitioned beta diversity into nestedness (richness differences) and turnover (species identity differences) following Baselga (2010), using the vegdist, beta.pair and phylo.beta.pair functions from the vegan and betapart packages. Last, we separately analysed beta diversity for the eight insect functional groups (Table 1) to explore how trends varied by ecological role. We assessed the correlation between beta diversity metrics of insects and plants using Mantel tests in vegan (Figure S4), with the number of permutations = 50,000, and method set to 'spearman' as it is less sensitive to outliers. To assess whether singleton BINs (those represented by one specimen) influenced the beta-diversity analyses, we repeated the Mantel analyses excluding BINs represented by only one specimen, this filtering did not qualitatively change our main results (Table S9 and Figure S7). For simplicity, we report the Mantel test statistics for Jaccards's phylogenetic turnover in the main text; equivalent results for abundance weighted Bray-Curtis and phylogenetic metrics are provided in Table S2.

3 | Results

We sequenced 337,809 specimens representing 29,919 unique BINs from 563 collection sample bottles and 22 Malaise traps spread across eight of the nine South African provinces. Samples were collected weekly over 12 months. Due to logistical constraints related to the very large number of specimens, we followed D'Souza et al. (2021) in DNA barcoding samples from collections made every other week. Of the assigned BINs, more than 12,000 were represented by a single specimen (Figures S5 and S6). Both observed and estimated (from rarefaction curves) taxonomic richness varied across gardens and biomes (Figure 2; Tables S5 and S6). Because the true richness in gardens is likely better captured by estimated species richness, we focus on comparisons using this estimate.

The Savanna (Thohoyandou, Lowveld and KZN) biome was estimated to have the highest insect diversity (both TD and PD) followed by the Grassland (Free State, Pretoria and Walter



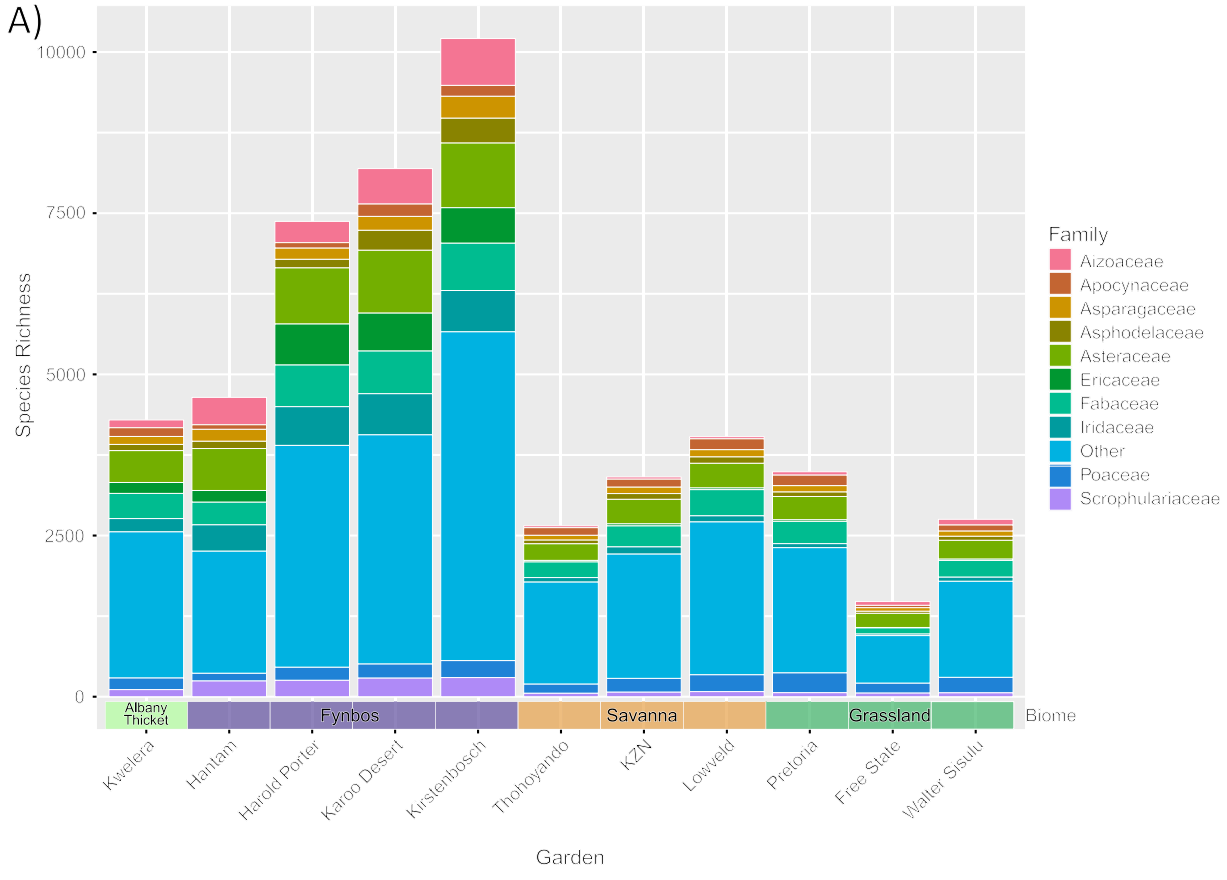


FIGURE 2 | Legend on next page.

FIGURE 2 | (A) Top 10 most species-rich plant families in each of the South African National Botanic Gardens. (B) Top 10 insect orders represented in each of the gardens. Colours on the *X*-axis labels represent the different biomes: Orange = Savanna; purple = Fynbos; dark green = Grassland; light green = Albany Thicket. Plant families and insect orders are ranked alphabetically, for a more detailed breakdown of orders see Tables S3 and S8.

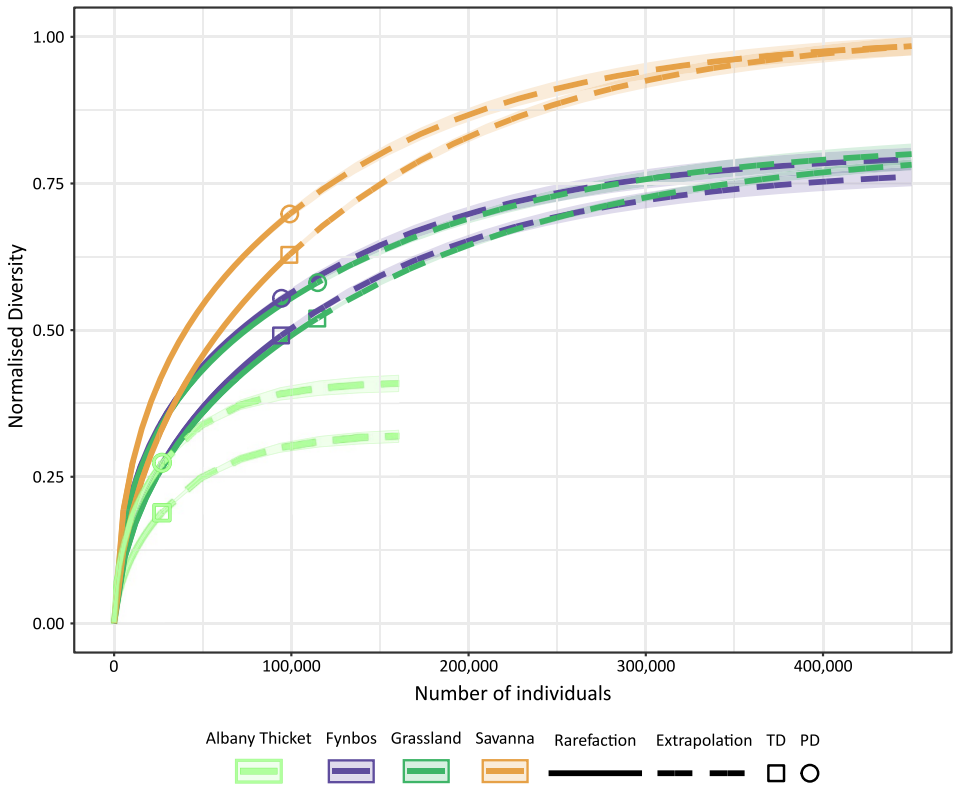


FIGURE 3 | Normalised rarefaction and extrapolation curves of taxonomic diversity (TD) and phylogenetic diversity (PD) for each biome, scaled by their respective maximum observed values (TD_{max} = 19,760; PD_{max} = 2216). Colours indicate biomes: Orange = Savanna, purple = Fynbos, dark green = Grassland, light green = Albany Thicket. Solid lines show rarefied diversity; dashed lines represent extrapolated diversity. Squares denote TD and circles denote PD. Shaded regions show 95% confidence intervals. See Figures S1 and S2 for unscaled diversity curves.

Sisulu), Fynbos (Kirstenbosch, Karoo Desert, Harold Porter, Hantam) and Albany Thicket (Kwelera) biomes (Figure 3; Table 3; Tables S3 and S8). Insect orders with the highest estimated species richness included Diptera and Hymenoptera with 27,183 (SE = 1054) and 26,168 (SE = 1351) species, respectively (Table S3). Notably, we estimated a higher species richness of Diptera in KZN (4642; SE = 128) than the total estimated insect species richness in Hantam (4485, SE = 443) or Harold Porter (4309, SE = 453).

The proportion of unsampled TD relative to the total estimated diversity within most biomes is consistently higher than the relative proportion of unsampled PD (Table 3). We estimate that more than 450,000 individuals would need to be sampled in each of the Fynbos, Grassland and Savanna biomes to capture their total species richness, while this number for the Albany Thicket is ca. 150,000 individuals. Thus, we would need to sample an additional ~1.5 million individuals to capture the complete species richness of these biomes, four times more than sampled in this study.

3.1 | Compositional Similarities Within and Between Biomes

The taxonomic composition of plant communities generally clusters by biome, for example, Thohoyandou, Lowveld and KZN in the Savanna biome and Kirstenbosch, Karoo Desert, Harold Porter and Hantam within the Fynbos biome (Figure 4A), but this clustering is less evident for insect communities (Figure 4B).

Despite apparent similarities in vegetation structure, gardens in the Grassland biome (Pretoria, Free State and Walter Sisulu) do not cluster as closely as gardens in other biomes (Mean plant Jaccard dissimilarity within biomes: Fynbos = 0.54 ± 0.01, Savanna = 0.55 ± 0.01, Grassland = 0.64 ± 0.02; Figure S2; Table S4). Contrasting with observations for plants, insect compositional differences are greatest among gardens within the Fynbos and least for gardens within the Grassland biome (Mean insect Jaccard dissimilarity within biomes: Fynbos = 0.91 ± 0.05, Savanna = 0.87 ± 0.05, Grassland = 0.81 ± 0.04; Table S4).

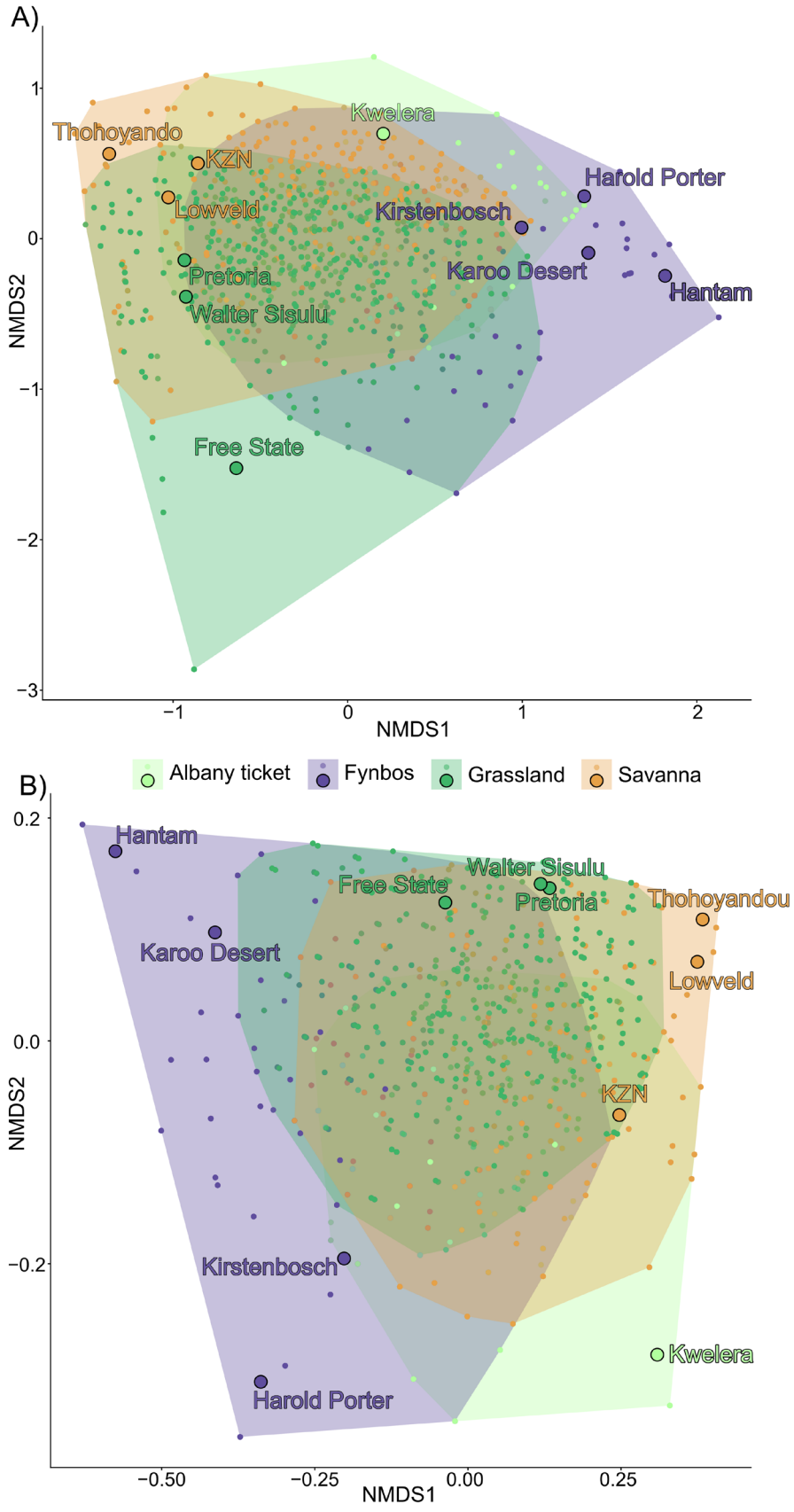


FIGURE 4 | Compositional similarity of plant (A) and insect (B) communities from the different gardens and their respective biomes, visualised using Non-Metric Multidimensional Scaling (NMDS) with the metaMDS function in vegan, $k = 2$, and distance = 'jaccard'. The colours of the polygons represent the different biomes: Orange = Savanna; purple = Fynbos; dark green = Grassland; light green = Albany Thicket.

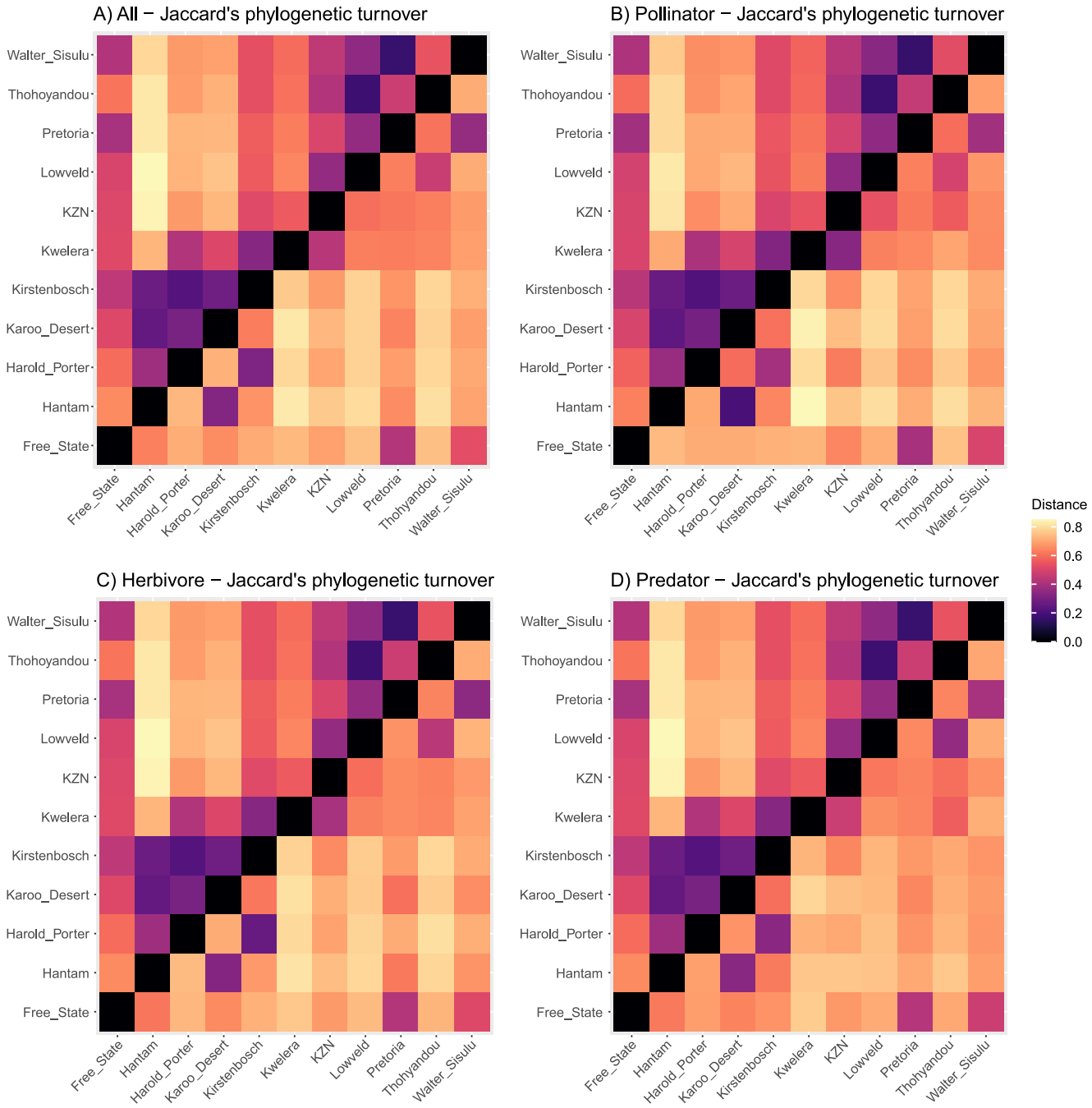


FIGURE 5 | Jaccard's phylogenetic turnover between gardens for plants (upper triangle) and insects (lower triangle). (A) Jaccard's phylogenetic turnover of all insects. (B) Jaccard's phylogenetic turnover of pollinators. (C) Jaccard's phylogenetic turnover of herbivores. (D) Jaccard's phylogenetic turnover of predators. Equivalent plots for the less well represented functional groups and the additional beta diversity components (Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness, and Jaccard's phylogenetic turnover) can be found in Figure S4. Lighter cells indicate higher dissimilarity, and darker cells indicate higher similarity between the respective NBG.

Broadly mirroring patterns of insect diversity, gardens within the same biome exhibit lower Jaccard's phylogenetic turnover in insects than gardens in different biomes (Figure 5A; Table S4). High phylogenetic turnover (Jaccard's phylogenetic turnover: Fynbos = 0.80 ± 0.05, Savanna = 0.78 ± 0.06, Grassland = 0.77 ± 0.05, Albany Thicket = 0.80 ± 0.05) and low nestedness (Jaccard's phylogenetic turnover: Fynbos = 0.041 ± 0.02, Savanna = 0.033 ± 0.02, Grassland = 0.036 ± 0.02, Albany Thicket = 0.025 ± 0.2) indicate that biomes are associated with distinct insect lineages rather than being nested subsets. Notably, insect phylogenetic composition varies more strongly between gardens within the same biome than does plant phylogenetic composition. For example, the mean Jaccard's phylogenetic turnover within the Fynbos gardens is 0.28 ± 0.05 for plants, but 0.74 ± 0.09 for insects.

3.2 | Correlation Between Floristic Composition and Insect Composition

Jaccard's phylogenetic turnover in insect community composition is significantly correlated with turnover in floristic composition between gardens (Mantel $R = 0.62$; $p < 0.001$), although, on average, the turnover in plant composition is lower than that for insects (mean plant turnover $= 0.56 \pm 0.2$ vs. mean insect turnover $= 0.79 \pm 0.05$; Table S4). However, the strength of the correlation in compositional turnover varies strikingly by insect functional group (Table 2). Floristic turnover is strongly correlated with the turnover in pollinators (Mantel $R = 0.67$; $p < 0.001$), predators (Mantel $R = 0.68$; $p < 0.001$) and fungus-associated (Mantel $R = 0.68$; $p < 0.001$) insects, but only weakly correlated with turnover in animal parasites (Mantel R-value $= 0.29$; $p = 0.08$). Excluding BIN singletons did not qualitatively alter correlation trends, but correlation strengths tended to be marginally weaker on average. We provide a detailed breakdown of functional groups by garden and order in Table S7.

3.3 | Dark Taxa

Dark taxa (BINs with no species level identification) dominate specimens in all biomes (Grassland 92.05%, Savanna 97.54%, Fynbos 96.06% and Albany Thicket 98.05%). Diptera, Hymenoptera and Coleoptera have the highest proportion of dark taxa (Figure 6), while Araneae have the highest proportion of specimens and BINs identified to species (Figure 6), with 17.78% of the specimens within the Fynbos classified at the species level. The estimated number of dark taxa for each biome was as follows: Grassland 14,330, Savanna 18,282, Fynbos 13,994 and Albany Thicket 6042. These values were derived by multiplying the proportion of BINs lacking species-level identifications in each biome by the asymptotic species richness estimates (Table 3).

4 | Discussion

We sequenced 337,809 individual specimens, representing 29,919 unique barcode taxa (BINs) spread across 522 families. These data, sampling from NBGs over the course of 1 year, provide the first baseline data on insect diversity for South Africa. We show that compositional differences in insect diversity are well predicted by plant phylogenetic turnover. Generally, gardens within the same biome have more similar insect communities than gardens in different biomes. However, contrary to expectations—that insect diversity would be highest where plant species richness is highest—we document that insect diversity peaks in the Savanna and not the plant-rich Fynbos. Nonetheless, we demonstrate a strong relationship between plant phylogenetic turnover and insect phylogenetic turnover, suggesting a shared ecological and evolutionary mechanism links these dynamics.

4.1 | Species Richness

We found a large variation in insect species richness among gardens. Kirstenbosch NBG is one of the region's best-known

TABLE 2 | Description of insect functional groups and their top three contributing orders, number of specimens and Mantel statistics testing for the correlation between Jaccard's phylogenetic turnover of plants and insects. For a more detailed breakdown of the functional groups per garden and order, see Figure S4 and Table S7.

	No. order	1st order (Observed specimens)	2nd order (Observed specimens)	3rd order (Observed specimens)	No. specimens	Mantel *R*	*p*
Total	37	Diptera (187,574)	Hymenoptera (58,841)	Coleoptera (15,100)	337,809	0.62	< 0.001
Pollinator	11	Diptera (25,879)	Coleoptera (4348)	Hymenoptera (3233)	36,458	0.67	< 0.001
Processor	18	Diptera (85,880)	Entomobryomorpha (5893)	Coleoptera (2732)	101,807	0.53	< 0.001
Parasitoid	12	Diptera (65,518)	Hymenoptera (49,246)	Sarcoptiformes (1102)	117,804	0.59	< 0.001
Predator	18	Diptera (53,950)	Hymenoptera (4360)	Coleoptera (3299)	70,683	0.68	< 0.001
Herbivore	16	Diptera (101,721)	Hemiptera (30,053)	Lepidoptera (12,922)	161,243	0.62	< 0.001
Aquatic	10	Diptera (41,083)	Trombidiformes (335)	Amphipoda (145)	42,144	0.39	< 0.001
Fungus associated	6	Diptera (70,720)	Coleoptera (1432)	Thysanoptera (516)	72,929	0.68	< 0.01
Animal parasite	4	Diptera (23,332)	Mesostigmata (92)	Hemiptera (47)	23,493	0.29	< 0.05

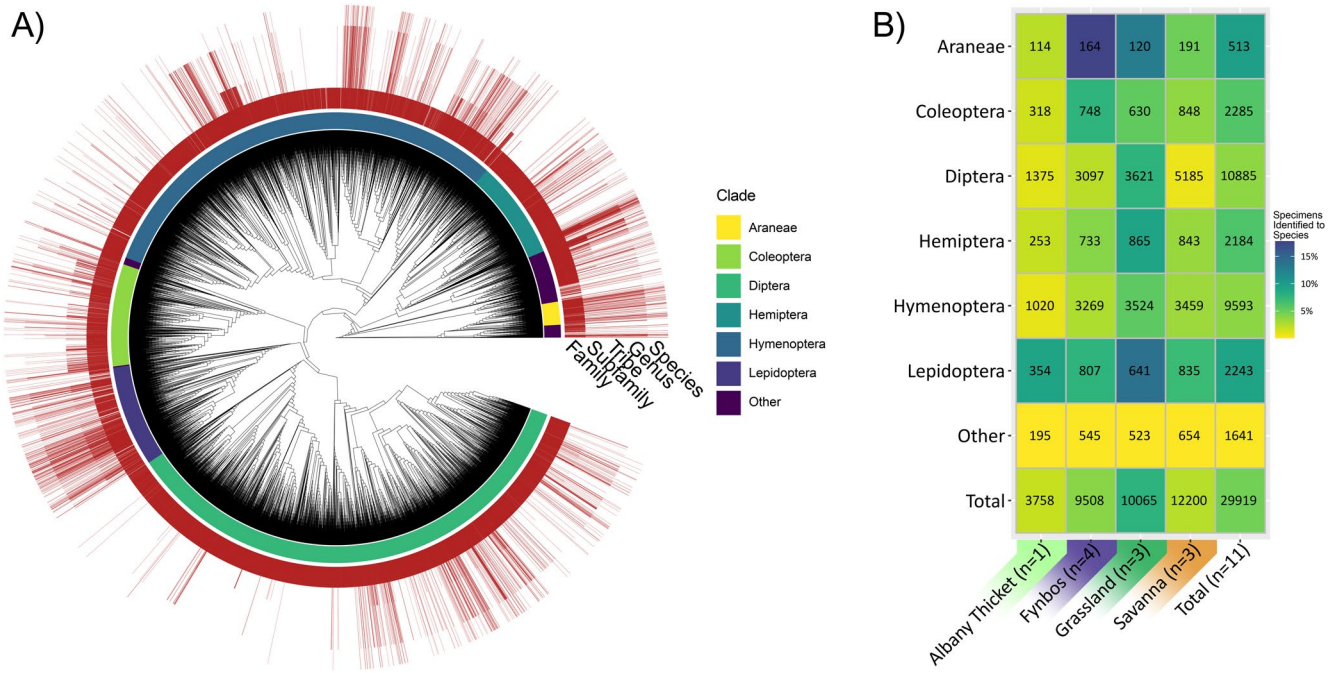


FIGURE 6 | (A) Maximum likelihood phylogeny of all 29,919 unique BINs and the taxonomic level to which they have been identified. Orange bars highlight the taxonomic resolution of classified BINs, moving from family (innermost circle) to species (outermost circle). The tree is displayed as a cladogram. (B) Number of BINs and their taxonomic resolution within biomes of the top 6 orders. Cell colours indicate the percentage of specimens that have been identified to the species level; the darker the cell the more specimens identified, all taxonomic orders per biome are shown in Figure S3.

botanical gardens and the most floristically rich, including many plants from the species-rich Fynbos (Arnolds and Guo 2024; Botha 1976; Hitchcock 2006). However, it does not support the greatest insect diversity; this rank goes to the KZN NBG, with almost double the number of taxa recorded in Kirstenbosch. KZN NBG is situated within the Savanna biome, the most geographically extensive vegetation type in South Africa. This garden is also South Africa's oldest, established in 1974 and originally managed by the Natal Botanic Society in 1874 before being incorporated into the South African National Biodiversity Institute (SANBI) in 1969. The lowest insect diversity was documented in Harold Porter NBG, also within the Fynbos, capturing ca. 40% of the estimated species richness of KZN NBG. Notably, the estimated species richness for gardens within the Fynbos (Harold Porter = 4309, Kirstenbosch = 5406, Karoo Desert = 5982, Hantam = 4485) was all below the estimated richness for gardens within the Savanna biome (Lowveld = 7676, Thohoyandou = 8080, KZN = 10,609). Gardens within the Albany Thicket (Kwelera NBG = 6629) and Grassland biomes (Free State NBG = 6204 and Pretoria NBG = 9607) had intermediate richness.

Plants form the niche space for insects, and thus we might have a priori assumed that the floristically rich Fynbos would support the greatest insect diversity. However, there are many possible explanations for gradients in species richness, including climate (Wang et al. 2019), resource availability (Rabosky and Hurlbert 2015), geography (Igea and Tanentzap 2020; Seymour et al. 2024) and evolutionary timescales (Tietje et al. 2022). The Fynbos occurs on nutrient-poor soils, and while floristically rich, it may be relatively resource-poor (Braschler et al. 2012). In contrast, the Savanna biome, where insect diversity is highest, is relatively resource-rich (Marchant 2010) and geographically more extensive. Our observations are consistent with experimental evidence indicating that insect richness responds more strongly to plant productivity than to plant species richness (Siemann 1998). In addition, the Savanna includes a mosaic of habitats, and the vegetation within it encompasses diverse plant types (as defined by Higgins et al. 2023), including C3 grasses, C4 grasses, perennial forbs, annual forbs, geophytes, restiods, climbers, succulents, shrubs and trees. Trees are notably absent from the Fynbos. We suggest the greater diversity of plant types, larger geographical area and higher overall density of vegetation in the Savanna may explain why it supports higher insect diversity than the number of plant species per se (see also Morpurgo et al. 2024; Tscharntke and Kruess 2002).

4.2 | Dominant Insect Communities

Across gardens, a few key taxa dominate insect communities, with Diptera (true flies) and Hymenoptera (bees and wasps) each contributing approximately one-third of total insect diversity. The dominance of these two taxa broadly matches global observations (Seymour et al. 2024), and we detect a much lower proportion of Diptera and a much higher proportion of Hymenoptera and Coleoptera (beetles and weevils). We also find that dominant taxa differ between gardens (compare also with reported taxonomic sampling in the Kruger National Park by D'Souza et al. 2021). For example, Hymenoptera comprise the highest proportion of the BINs in the Fynbos, while Diptera dominates in the Savanna. It is likely that the greater diversity of flowers in the Fynbos supports higher pollinator diversity (Procheş and Cowling 2006), whereas greater vegetative biomass

TABLE 3 | Observed and asymptotic species diversity estimates from Figure 3. The asymptotic species estimates are accompanied by standard error values in parentheses (SE). All values are presented with their respective normalised values. Phylogenetic diversity is measured in substitutions per site.

	Taxonomic diversity		Phylogenetic diversity		Unsampled diversity	
	Observed TD	Asymptotic species estimate (SE)	Observed PD	Asymptotic estimate (SE)		
	NV = normalised value	NV = normalised value (SE)	NV = normalised value	NV = normalised value (SE)	Taxonomic diversity	Phylogenetic diversity
Savanna	11,996	19,338 (215)	1510	2168.17 (20)	7342	658
	NV = 0.62	NV = 1.00 (0.01)	NV = 0.68	NV = 0.98 (0.01)	NV = 0.38	NV = 0.3
Grassland	9911	15,568 (101)	1256	1780.48 (21)	5657	524
	NV = 0.51	NV = 0.81 (0.01)	NV = 0.57	NV = 0.80 (0.01)	NV = 0.3	NV = 0.23
Fynbos	9354	14,878 (125)	1195	1743.22 (22)	5524	548
	NV = 0.48	NV = 0.77 (0.01)	NV = 0.54	NV = 0.79 (0.01)	NV = 0.29	NV = 0.25
Albany Thicket	3223	6162 (126)	591	892.30 (25)	2939	301
	NV = 0.19	NV = 0.31 (0.01)	NV = 0.27	NV = 0.40 (0.01)	NV = 0.12	NV = 0.13

in Savanna might support a higher diversity of insect herbivores such as Leaf-miner flies and Aphids (Maraia et al. 2024).

Our results help illustrate how vegetation type shapes insect communities, with some habitats potentially favouring beneficial insects, including natural predators of pests and plant pollinators, while others may favour insect herbivores and plant pests. We find that insect predators have a particularly strong relationship with plant community composition, highlighting the tight connectivity between plant diversity, herbivorous insects and their predators. Previous work has demonstrated that community composition can affect arthropods across trophic levels (Scherber et al. 2010; Schuldt et al. 2019), and can strengthen predator-mediated control of insect herbivores (Barnes et al. 2020). Our results indicate that predator turnover may reflect not only plant diversity, but also the identity and evolutionary history of the plant communities that structure herbivore assemblages (Pellissier et al. 2013; Volf et al. 2017). Importantly, these higher trophic links may contribute to natural pest control, as insect predators and parasitoids can regulate pest outbreaks (Tscharntke et al. 2016).

4.3 | Estimated and Undescribed Diversity in South Africa

Hymenoptera and Diptera contribute the most taxa in our samples, but we estimate that more than half of their species remain unsampled. This knowledge gap is a concern given their importance as pollinators and primary consumers, respectively. Failing to capture the full diversity of these groups may mislead inferences on the resilience or vulnerability of ecosystems. In general, Malaise traps are inefficient at capturing ground-dwelling insects and taxa with limited flight capabilities, generating a bias in taxonomic representation (Bertoia et al. 2023). In addition, we speculate that the fine mesh size of the Townes Malaise trap may have been easier for dragonflies and large Aculeata to see and avoid (Uhler et al. 2022), which could help explain why Odonata numbers are low relative to some previous estimates (Samways and Niba 2010; Suh and Samways 2005). It is also possible that we may have missed pollinators in sites where the blooming period was short, such as Hantam NBG. This garden is known as the 'bulb capital of the world' (with ca. 2200 bulb species in the area Willis 2018) and flowering is limited to 2 weeks at the end of August and the beginning of September. To improve the representation of ground-dwelling insects, we suggest pairing Malaise traps with pitfall traps and/or using metabarcoding on the soil below the traps (Kirse et al. 2021). Environmental DNA (eDNA) metabarcoding may provide an efficient approach to further expand sampling intensity (Buchner et al. 2025), but like metabarcoding, it is limited by the completeness of the barcode reference library (Rattray et al. 2024).

We estimated that Hantam and Harold Porter, both within the Fynbos biome, had marginally higher proportions of missing species (0.45 and 0.46, respectively) than other NBGs. We suggest that additional sampling in Hantam NBG could be particularly valuable, as several recently described species have been found in the garden, including the long-tongued fly (*Prosoeca marinusi* Barraclough), a pollinator of plants endemic to Hantam

(*Lapeirousia oreogena* Schltr. ex Goldblatt, *Babiana vanzyliae* L. Bolus, and *Babiana framesii* L. Bolus) that was noted in early pollination studies but only formally described 20 years later (Barraclough et al. 2018). We suspect additional sampling in Harold Porter is also likely to reveal new species. Several species have been discovered there, including *Elpiscladius capicola* Harrison & Cranston, found in the Davidskraal River, and *Trioza bullatae* Burckhardt, Drohojowska & Giliomee, a gall-inducing species on black stinkwood (*Ocotea bullata*; Burckhardt et al. 2012; Harrison and Cranston 2007).

Current estimates of global biodiversity range between 8 and 100 million (Mora et al. 2011). Insects make up the bulk of these estimates. In sub-Saharan Africa, there are ca. 100,000 described insects (Miller and Rogo 2001), with perhaps ca. 44,000 described species in southern Africa (Scholtz 2016; Scholtz and Holm 1985; Scholtz and Chown 1995). However, the true diversity of the region is almost certainly much greater (Hamer 2013). We identified almost 30,000 barcode taxa (equivalent to 65% of the 44,000 described specimens), of which only 3% were identified to species level, while many of the remainder likely represent undescribed or cryptic species. If these data are representative, it might indicate that the total above ground insect diversity in South Africa could be close to 100,000 species. In some biomes, such as the Albany Thicket, we are closer to sampling the total diversity, although even here we estimate that ca. 2900 species are missing from our samples. Based on the current description rates from Hamer (2013)—355 years to describe around 80,000 species—it would take more than 200 years to describe the estimated > 52,000 dark taxa suggested by our study. Nonetheless, we captured a greater proportion of the insect tree of life, and much of the missing diversity likely falls within the sampled clades, as indicated by the proportionately lower fraction of missing PD.

DNA barcoding is a powerful tool for accelerating species identification, biomonitoring and the generation of important biodiversity baseline data. Globally, South Africa ranks fourth in the number of insect records on BOLD systems (Stewart et al. 2024). However, many of the barcoded species are 'dark taxa' which lack formal taxonomic descriptions. Some taxonomic groups typically attract more research attention than others, such as Diptera and Lepidoptera (Salis et al. 2024), although most major clades are still lacking species-level identification. While larger-bodied groups and those with charismatic traits, including Araneae and Lepidoptera, tend to be better studied (Salis et al. 2024), 85% of these groups remain dark. Even within otherwise well-studied clades, some highly speciose groups have endured taxonomic neglect, such as parasitic wasps within Hymenoptera (Forbes et al. 2018).

5 | Conclusion

We provide the first comprehensive baseline dataset of insect diversity across South Africa, contributing over 29,919 unique BINs from 337,809 specimens. A congruence between plant and insect diversity would allow one group to provide a proxy for diversity in the other and indicate that insect diversity might be a useful general indicator of biodiversity (Landmann et al. 2023). We show that insect species richness is not well predicted by plant species richness, with biome-specific factors playing a significant role in shaping insect richness gradients. However, turnover in plant lineages is a good predictor of insect compositional differences between gardens. Our results parallel observations by Novotny et al. (2006) and Ødegaard et al. (2005) who showed that insect communities co-vary more closely with the diversity and turnover of host-plant lineages rather than species composition. We highlight this distinction in contrasts between the floristically rich Fynbos and the more insect-rich Savanna, where biome area, vegetation structure and resource availability may additionally contribute to shaping insect diversity.

Our data, in conjunction with future barcoding efforts, have a wide range of potential ecological and environmental applications, including biomonitoring, documenting species interactions, tracking biological invasions, assessing the effectiveness of conservation actions and identifying potentially pathogenic vectors. To realise this potential, we must expand sampling in (1) space to other biomes, (2) time intervals, sampling more frequently during key seasonal events, such as flowering and (3) temporal extent, allowing longer-term records of biodiversity trends.

Funding

This work was supported by New Frontiers in Research Fund, NFRFT-2020-00073; Canada Foundation for Innovation, MSI-42450, LEF-20984, LEF-460488; Genome Canada, OGI-208, OGI-233; Ontario Research Foundation, #18406; Canada First Research Excellence Fund, #000054; Gordon and Betty Moore Foundation, #2966, #8493, #12262; Natural Sciences and Engineering Research Council of Canada, RTI-401629.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All DNA barcode data and specimen metadata are available through the Barcode of Life Data System (BOLD) under project codes (DS-PTIN, DS-WSIN, DS-FSIN, DS-LVIN, DS-TDIN, DS-KZIN, DS-KWIN, DS-KBIN, DS-KBIN, DS-HPIN, DS-HPIN, DS-KDIN, DS-HTIN). Respective BOLD URL's: http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-PTIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-WSIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-FSIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-LVIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-TDIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-KZIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-KWIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-KBIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-HPIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-KDIN, http://www.boldsystems.org/index.php/Public_SearchTerms?query=DS-HTIN. The respective data is also available on Global Biodiversity Information Facility (GBIF) URL's: https://doi.org/10.15468/dl.9vw3gn, https://doi.org/10.15468/dl.2mjy3r, https://doi.org/10.15468/dl.gvuqq5, https://doi.org/10.15468/dl.cvhzus, https://doi.org/10.15468/dl.7j6e7p, https://doi.org/10.15468/dl.6qsckq, https://doi.org/10.15468/dl.gvrbrh, https://doi.org/10.15468/dl.2ep7ze, https://doi.org/10.15468/dl.wbxwnv, https://doi.org/10.15468/dl.x6jj4p, https://doi.org/10.15468/dl.3u49kz. The complete working dataset, phylogenetic trees, plant community matrix and associated metadata are archived in Dryad at https://doi.org/10.

5061/dryad.47d7wm3w5. The *R* scripts used to reproduce the analyses, figures and Supporting Information tables are available on GitHub at https://github.com/rossdstewart/Plant-phylogenetic-history.git.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Rarefaction curves for phylogenetic diversity (PD) in four South African biomes. **Figure S2:** Rarefaction curves for taxonomic diversity (TD) in four South African biomes. **Figure S3:** Number of BINs and their taxonomic resolution within biomes for all insect orders. Cell colours indicate the percentage of specimens that have been identified to the species level; the darker the cell, the more specimens are identified. **Figure S4:** Heat map of the correlations among six beta diversity metrics (Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness, and Jaccard's phylogenetic turnover) of all iterations from eight functional groups (all insects, Pollinators, Processors, Parasitoids, Predators, Herbivores, Aquatics, Fungi, Animal Parasite) against plants diversity. **Figure S5:** Distribution of Barcode Index Numbers (BINs) among specimen-abundance classes across the complete dataset. **Figure S6:** Mean number of specimens per BIN (±SD) for each field sample, shown separately for the 22 Malaise-trap locations and ordered by collection date. **Figure S7:** Heat map of the correlations from the no singleton run of the six beta diversity metrics (Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness and Jaccard's phylogenetic turnover) of all iterations from eight functional groups (all insects, Pollinators, Processors, Parasitoids, Predators, Herbivores, Aquatics, Fungi, Animal Parasite) against plants diversity. **Table S1:** Deployment locations for the 22 Malaise traps with GPS coordinates and site descriptions. **Table S2:** The Mantel statistics for seven beta diversity metrics (Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness, Jaccard's phylogenetic turnover) and all iterations from the various functional groups (all insects, Pollinators, Processors, Parasitoids, Predators, Herbivores, Aquatics, Fungi and Animal parasites). **Table S3:** The observed richness, estimated richness and proportion of unsampled taxa in each National Botanic Garden separated by insect order. **Table S4:** A breakdown of the mean beta diversity statistics for Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness, and Jaccard's phylogenetic turnover of both the insects and plants for each biome. **Table S5:** The number of specimens analysed, the number of BINs, estimated species, number of families and phylogenetic distance for each site. **Table S6:** Descriptive diversity metrics of all biomes, including the number of specimens analysed, number of BINs, estimated species, number of families and phylogenetic distance. **Table S7:** A breakdown of the functional groups per garden and insect order. **Table S8:** The observed richness and estimated richness of each site are separated by insect order. **Table S9:** The Mantel statistics with no signletons for seven beta diversity metrics (Bray–Curtis's dissimilarity, Jaccard's dissimilarity, Jaccard's nestedness, Jaccard's turnover, Jaccard's phylogenetic dissimilarity; Jaccard's phylogenetic nestedness, Jaccard's phylogenetic turnover) and all iterations from the various functional groups (all insects, pollinators, processors, parasitoids, predators, herbivores, aquatics, fungi and animal parasites).