

Heavy metal exposure alters the gut microbiota of the water hyacinth biocontrol agent *Megamelus scutellaris* (Hemiptera: Delphacidae): implications for biological control of *Pontederia crassipes* (Pontederiaceae)

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Abstract

The insect gut microbiota is critical to nutrition, digestion, and overall fitness. However, dietary exposure to heavy metal(loid)s (HMs), may result in dysbiosis including negative health implications for the host. The water hyacinth planthopper, *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), is a sap-sucking biological control agent of water hyacinth (*Pontederia crassipes* Mart., Pontederiaceae) that has been released in acid mine drainage-polluted regions across South Africa. Here, we quantified HMs in water hyacinth leaves from field sites in comparison to a mass-rearing facility (hereafter Waainek) using inductively coupled plasma optical emission spectrometry and characterised the associated gut microbiota in insects using 16S amplicon sequencing. Eleven HMs, including Fe, Mn, and Al, were elevated in plants from polluted sites relative to Waainek. A total of 75 unique amplicon sequence variants were identified in *M. scutellaris*, distributed across 7 phyla, 9 classes, 19 orders, 30 families, and 33 genera. *Rickettsia* dominated the core microbiota, co-occurring at lower abundance with *Wolbachia* and *Cardinium*. Heavy metal exposure influenced beta diversity more than alpha diversity, explaining 36% of the variation in community composition between populations. Inferred metagenome function predicted a severe reduction in the microbiome function 1 to 9-fold less in HM-exposed populations relative to mass-reared populations, including xenobiotic degradation, amino acid metabolism, and cellular processes. This study highlights the utility of the gut microbiota in understanding environmental exposure in insects. Further, it demonstrates that alterations in the gut microbiota may carry significant implications for host fitness, establishment success, and the effectiveness of biocontrol programmes against water hyacinth.

Introduction

Insect gut microbiota form associations with their hosts that range from beneficial to detrimental, and in some cases both [1]. Beneficial symbionts contribute to nutrition, immunity, detoxification, and overall fitness, for instance, by supplementing nutrients lacking in the host diet, such as essential amino acids and vitamins [2]. Others, such as *Wolbachia* and *Candidatus Cardinium* (hereafter *Cardinium*), manipulate host reproduction [3], although they can also provide fitness benefits. The gut microbiota is shaped by habitat, developmental stage, sex, and diet, and exposure to contaminants such as pesticides and heavy metals (HMs) is increasingly recognised as an additional driver of microbiota alterations [4]. Such shifts may affect insect fitness in variable environments, which is particularly relevant to biological control using insect natural enemies, where the role of the microbiota in mediating agent performance remains poorly explored.

Host-specific insect biological control agents are widely used against invasive alien plants (AIPs) as a sustainable alternative to herbicides [5] and are released across systems affected by eutrophication [6]. Because biocontrol agents may also be exposed to contaminants transferred through their host plants [7], such pollutants could favour AIP persistence where they impair agents without affecting the plants themselves. Water hyacinth (*Pontederia crassipes*) is among the world's worst aquatic AIPs and often occurs in polluted systems [8]. In South Africa, biological control has achieved substantial success, with

reduced infestations across much of the country [9, 10]. At the local scale, however, the efficacy of biological control varies among sites and can be constrained by abiotic factors such as cold temperatures [11] and by anthropogenic stressors such as pollution [12].

Heavy metal(loid)s are pollutants of particular concern in aquatic systems and are known to alter insect gut microbiota resulting in dysbiosis [13]. The most recently released water hyacinth agent in South Africa, the planthopper *Megamelus scutellaris* Berg (Hemiptera: Delphacidae), causes leaf chlorosis and subsequent plant death [14]. However, the role of HMs exposure in its performance as a biocontrol agent, and more broadly in insect-plant interactions, remains unexplored. Water hyacinth bioaccumulates HMs, underpinning its use in phytoremediation [15], which could expose agents to HMs through feeding. Anecdotal evidence from the Blesbokspruit wetland in the East Rand, South Africa, suggests that *Megamelus scutellaris* has failed to establish despite repeated releases in a catchment receiving HM-rich mining effluents [16]. The East Rand, therefore, provides an opportunity to examine whether, and how, HMs alter the gut microbiota of *M. scutellaris*, with implications for biocontrol of water hyacinth.

The oxidation of iron pyrite (FeS_2) generates acid mine drainage (AMD), which infiltrates aquatic systems, releasing sulfuric acid and HMs at high concentrations, as a documented phenomenon of the East Rand, South Africa [16]. Because water hyacinth accumulates these metals, historic evidence of mining in the East Rand increases the likelihood that *M. scutellaris* would ingest substantial concentrations of metals through feeding exposure [7]. HMs are persistent and non-biodegradable; such ingestion may lead to gut bioaccumulation and associated dysbiosis. Characterising these effects is now possible using high-throughput 16S rRNA sequencing, which captures previously unculturable taxa, and tools such as PICRUSt2 [17], which predict functional potential using curated databases such as MetaCyc [18] and KEGG [19].

Heavy metal-induced alterations in insect gut microbiota are increasingly reported across various landscapes, including agroecosystems [20]. However, similar studies on *M. scutellaris*, a widely used biological control agent, remain severely underexplored. Using the *M. scutellaris*–water hyacinth interaction as a model system, we performed a metagenomic comparison of gut microbiota from established *M. scutellaris* populations at two sites upstream of the heavily polluted Blesbokspruit Wetland that receives HMs pollution, viz. Civic Dam and Middle Lake, and from the Waainek Mass-Rearing Facility (hereafter Waainek), Makhanda, Eastern Cape, where *M. scutellaris* is mass-reared for release around South Africa. The study aimed to compare gut microbiota: (i) compositional structure, (ii) alpha and beta diversity profiles, (iii) functional potential, and (iv) heavy metal(loid) concentrations in water hyacinth leaves. Given the well-documented acid mine drainage-related pollution histories of the study region, we hypothesised that field populations would exhibit (i) reduced gut microbial richness and alpha diversity, (ii) compositional shifts in beta diversity, and (iii) altered functional potential, all relative to the mass-reared populations.

Materials and Methods

Study area

The East Rand, east of Johannesburg on the Highveld of South Africa, lies 1200–1800 m above sea level, characterised with cool dry winters (mean minimum ~ 10°C) and hot wet summers (mean maximum ~ 25°C) [21]. The area, known for its natural wetlands, has been heavily mined since the 19th century [22]. Two lakes situated near gold mining tailings dams [23] were selected (Fig. 1): Middle Lake (26°10'53"S, 28°18'23"E; 1635 x 191 m) and Civic Dam (26°10'56"S, 28°18'58"E; 510 x 211 m), characterised by extensive water hyacinth cover and reduced *M. scutellaris* numbers. Both *M. scutellaris* field populations were established from the Centre for Biological Control's Waainek Mass-Rearing Facility, a relatively pristine site used as a control in this study. The most recent releases were made at Middle Lake in November 2023 (12,000 individuals) and Civic Dam in November 2024 (8,000 individuals). At Waainek, plants are grown using rainwater collected from the gutter systems and fertilised with CULTERRA Multisol 'N' 6.1.3 (44) water-soluble fertiliser (CULTERRA, Johannesburg, South Africa), both of which are potential HM sources, meaning baseline concentrations may not be zero. Waainek has released millions of *M. scutellaris* for the biological control of water hyacinth across South Africa since 2013.

Field sampling

Plant and insect samples were collected from Civic Dam, Middle Lake, and Waainek between 15 and 27 May 2025. For water hyacinth leaves, three sampling points (replicates) were selected within each dam (site) to ensure coverage of most of the site. At each sampling point, thirty mature leaves, five from each of six randomly selected individual plants, were cut at the base of the petiole to create a composite replicate. Between 20 and 30 adult *M. scutellaris* females were collected by electric aspirator from four sampling points per site at wading depth, spaced ~ 10 m apart. Insects were immediately transported to the laboratory for dissection.

Water hyacinth plant leaves were oven-dried at 80°C [24]. The following HMs (As, Cr, Cu, Hg, Pb, Al, B, Fe, Mn, Zn; mg kg⁻¹ dry weight *DW*) were quantified by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; Optima 2100DV, PerkinElmer) at a SANAS-accredited laboratory (Water Lab Pvt Ltd, South Africa) (Supplementary Material 1).

Gut dissection and genomic DNA extraction

Gut dissection and genomic DNA extraction

Adult *M. scutellaris* used in this study were expected to have minimal genetic differences because they originated from the same colonies maintained at Waainek. Insects (20 per replicate, four replicates per site) were immobilised at -20°C for 5–10 minutes [26] and surface-sterilised by three repeated immersions in 70% ethanol, followed by two rinses in deionised water [25]. Dissections were aseptically carried out using insect pins and forceps under a stereomicroscope in a laminar-flow hood, following

protocols adapted from Mattison et al. [27]. A detailed procedure was provided in Supplementary Material 2.

The hindgut was grasped with forceps at the base of the anus, then pulled to remove the entire gut. The forceps were flame-sterilized between successive uses on insects from different sites. Twenty guts per replicate were transferred to a 1.5 mL tube containing 0.5 mL sterile phosphate-buffered saline (PBS, pH 7.4) and stored at -80°C until DNA extraction. DNA was extracted using the DNeasy Blood and Tissue Kit (QIAGEN), with gut-free water blanks included as negative controls in both extraction and PCR, and quantity/quality assessed using the Nanodrop 1000 spectrophotometer.

Next-generation sequencing

The V3-V4 region of the 16S rRNA gene was amplified using forward and reverse primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGTATCTAAT) [28] following the Illumina 16S Metagenomic Sequencing Library Preparation guide [29], with Taq DNA Polymerase 2X Master Mix (Ampliqon) under standard cycling conditions ($95^{\circ}\text{C}/5\text{ min}$; $30 \times [95^{\circ}\text{C}/30\text{ s}, 55^{\circ}\text{C}/30\text{ s}, 72^{\circ}\text{C}/30\text{ s}]$; $72^{\circ}\text{C}/5\text{ min}$). Products were purified using Mag-Bind Total Pure NGS (Omega Bio-tek, Norcross, USA), and quality was checked using Invitrogen™ Qubit™ 4 Fluorometer (Thermo Fisher Scientific). Sequencing was performed using 600-cycle V3 chemistry on the MiSeq platform at the South African Institute for Aquatic Biodiversity (NRF-SAIAB), Aquatic Genomics Research Laboratory, Makhanda, South Africa.

Core microbiota

Taxa present in at least 80% of samples, at a mean relative abundance $\geq 0.01\%$, were considered core microbiota, following the prevalence-abundance framework of Braglia et al. [4] applied at the genus level.

Statistical analyses

Metal concentrations of Al, Mn, and Zn were analysed using one-way ANOVA, while other HMs were analysed by Kruskal-Wallis tests. Raw sequence reads were processed in QIIME2 [30]. Detailed procedures are provided in Supplementary Material 3. Alpha diversity (Chao1, ACE, Shannon, Simpson) was assessed by Kruskal-Wallis tests at $P < 0.05$. Beta diversity was evaluated using Bray-Curtis and unweighted/weighted UniFrac distances [31] using the vegan package (v2.6.4) and visualised by Non-metric Multi-Dimensional Scaling (NMDS), with significance tested by PERMANOVA (999 permutations, $P < 0.05$). Differential abundance of predicted functions was examined using LinDA [32] implemented in ggplicrust2 [33] with a Benjamini-Hochberg-adjusted $P < 0.05$ significance threshold. A detrended correspondence analysis was used to confirm the suitability of redundancy analysis (RDA) in assessing the direct link between HM exposure and gut microbiota alterations.

Results

Heavy metal(loid) concentrations in water hyacinth leaves

Mn, Fe, Al, Zn, Ni, and Cu concentrations were higher in plants from AMD-exposed sites in comparison to Waainek, while B, As, Cr, Hg, and Pb occurred at lower concentrations and were largely undetected at Waainek (Fig. 2). Al was significantly lower at Waainek than at Civic Dam (Supplementary Table 1) but not significantly different from Middle Lake ($P = 0.180$; Fig. 2A). Mn and Zn were significantly lower at Waainek than at Civic Dam, with Mn also lower than at Middle Lake (Supplementary Table 1; Fig. 2A). Pb was detected exclusively at Civic Dam (Fig. 2B), where Fe concentrations were also significantly higher than at Waainek. As Cr, Cu, Ni, and B did not differ significantly between sites (Supplementary Table 2; Fig. 2).

Microbial community structure

Illumina sequencing of 12 samples comprising four replicate pools (20 individuals each) per site across three sites yielded 647,480 raw paired-end reads, from which 75 amplicon sequence variants (ASVs) were identified using DADA2. Proteobacteria dominated at the phylum level (93.4%), followed by Bacteroidota (6.6%) (Fig. 3A). At the class level, Alphaproteobacteria (78.1%), Betaproteobacteria (15.3%), and Bacteroidia (6.6%) predominated (Fig. 3B). At the genus level, *Rickettsia* was most abundant (71.7%), followed by *Arsenophonus* (7.8%), *Wolbachia* (7.1%), *Cardinium* (6.7%), *Pseudomonas* (4.9%), and *Stenotrophomonas* (1.1%) (Fig. 3C). Notably, *Arsenophonus* occurred only in HM-exposed populations, while *Pseudomonas* was restricted to Waainek. Several low-abundance genera (< 1%), including *Herbaspirillum*, *Variovorax*, *Bosea*, and *Methylobacterium-Methylobacterium*, were also detected. In total, 7 phyla, 9 classes, 19 orders, 30 families, and 33 genera were identified, of which *Rickettsia*, *Wolbachia*, *Cardinium*, *Variovorax*, and *Stenotrophomonas* comprised the core microbiota, present in $\geq 80\%$ of samples (Fig. 3D).

Microbial diversity patterns

Diversity was assessed on data rarefied to 40,000 reads per sample, the depth at which rarefaction curves plateaued (Supplementary Fig. 1). The Waainek population showed higher mean richness (Chao1, ACE) and diversity (Shannon, Simpson) than HM-exposed populations (Fig. 4A). Richness differed significantly across sites (Chao1: $\chi^2 = 6.9$, $df = 2$, $P = 0.032$; ACE: $\chi^2 = 6.5$, $df = 2$, $P = 0.039$), although post-hoc pairwise comparisons were not significant after BH correction ($P > 0.05$; Supplementary Table 3). Diversity indices did not differ significantly (Shannon: $\chi^2 = 1.5$, $df = 2$, $P = 0.472$; Simpson: $\chi^2 = 2.0$, $df = 2$, $P = 0.368$), though richness measures showed greater variability among HM-exposed populations than at Waainek (Fig. 4A).

Beta diversity differed significantly across sites based on Bray-Curtis dissimilarity ($F = 2.357$, $P = 0.030$), weighted UniFrac ($F = 2.975$, $P < 0.001$), and unweighted UniFrac ($F = 2.975$, $P < 0.001$) distances (999 Permutations) (Fig. 4B).

Relationship between heavy metals in plant leaves and the gut microbiota

Detrended correspondence analysis (DCA) gradient length of 2.23 after Hellinger-transformation confirmed Redundancy analysis RDA as the appropriate ordination method [34]. Because HMs were strongly collinear (Hg–Pb, $r = 1.00$; Zn with Al, Cr, Fe, and Mn, $r = 0.83–0.92$), concentrations were summarised by principal component analysis (PCA). PC1 explained 74.5% of the total variation, with Al, Cr, Fe, and Zn showing the strongest loadings (Supplementary Table 4). This 'HM gradient' predictor in the RDA (Supplementary Table 5) accounted for 36% of the variation in gut microbiota composition between AMD-exposed sites and Waainek ($R^2 = 0.36$, adjusted $R^2 = 0.30$, $F = 5.62$, $P = 0.010$; Fig. 5; Table 3).

Table 3
Permutation test of the RDA model. Significance was assessed at $\alpha < 0.05$.

Model (HM gradient)	Df	Variance	F	Pr(> F)
	1	0.0435	5.62	0.010
Residual	10	0.0774		

Inferred metagenome function in Megamelus scuterallis gut microbiota

A total of 440 MetaCyc pathways were inferred in *M. scutellaris*, of which 186 differed significantly across sites (LinDA, $P < 0.05$). However, only the 70 most significant are displayed in Fig. 6. Hierarchical clustering grouped pathways into three clusters: amino acid metabolism, nucleotide/nitrogen recycling, and carbohydrate degradation; aromatic/xenobiotic degradation (catechol, protocatechuate, salicylate, phenylacetate, beta-ketoadipate pathways); and fatty acid metabolism with cofactor biosynthesis.

A total of 415 KEGG pathways were inferred in *M. scutellaris*, of which 213 differed significantly across sites ($P < 0.05$). The top 30 differentially abundant pathways for each comparison (Civic Dam vs. Waainek; Middle Lake vs. Waainek) are highlighted in Fig. 7. Pathways including cellular processes, transcription, amino acid and lipid metabolism, energy metabolism, secondary metabolite biosynthesis, and xenobiotic degradation were reduced 1- to 9-fold in AMD-exposed populations relative to Waainek.

Discussion

This study provides the first characterisation of the gut microbiota of *Megamelus scutellaris* and infers its functional potential in populations exposed to heavy metals, relative to mass-reared populations maintained under unpolluted conditions at Waainek. Microbial community structure was similar at the phylum and class levels, with differences apparent at the genus level. Microbial richness (Chao1, ACE) was lower, though not significantly, in exposed populations, partly contradicting our first hypothesis. Beta diversity differed significantly between exposed and mass-reared populations, supporting our second

hypothesis. Inferred functional potential, including amino acid and carbohydrate metabolism, cellular processes, and xenobiotic degradation, was suppressed in exposed populations, confirming our third prediction.

Microbial community structure

Proteobacteria dominated the gut microbiota of *M. scutellaris*, consistent with reports for other delphacid planthoppers [35]. At the genus level, *Arsenophonus* was detected only among AMD-exposed populations. This genus is a recognised endosymbiont of planthoppers [36], and in aphids it has been associated with improved host performance under nutritional stress through interaction with the obligate symbiont *Buchnera aphidicola* [37]. Whether *Arsenophonus* performs a comparable role in *M. scutellaris* under metal stress remains to be examined. Additionally, *Rickettsia*, *Wolbachia*, *Cardinium*, *Variovorax*, and *Stenotrophomonas* were identified as members of *M. scutellaris* core microbiota. Core taxa are consistently shared across host individuals and are often hypothesised to underpin essential host functions [38]. Specifically, *Rickettsia* dominance mirrors patterns in other phloem-feeding Hemiptera, including the whitefly *Bemisia tabaci* [3], suggesting it may perform a similarly beneficial role in *M. scutellaris*.

On the other hand, *Wolbachia*, a maternally transmitted symbiont, can induce reproductive manipulations such as cytoplasmic incompatibility, feminisation, male killing, and parthenogenesis [39]. In our case, its functional role in *M. scutellaris* warrants investigation, as reproductive manipulations could affect population densities and sex ratios of released agents. More so, Pb exposure reduced fitness more severely in *Wolbachia*-infected than uninfected *Drosophila melanogaster* [40], suggesting HM exposure may similarly alter *Wolbachia*-host dynamics, with implications for host fitness. Furthermore, *Cardinium*, an obligate intracellular endosymbiont infecting 6–13% of arthropod species [41], was consistently detected across populations, suggesting stable maternal transmission. Like *Wolbachia*, *Cardinium* influences host biology [41], including improving thermotolerance in other insects [42], a benefit that may also apply to *M. scutellaris*.

Microbial diversity under acid mine drainage exposure

Plants at Civic Dam and Middle Lake, in the East Rand, demonstrated a high level of Mn, Fe, Al, Zn, Ni, and Cu, consistent with reports on AMD pollution in the East Rand [43]. Although richness was lower at Middle Lake than at Civic Dam, the difference was not significant. This observation is incongruent with the pygmy grasshopper *Eucriotettix oculatus*, where HM-contaminated sites showed significantly reduced gut richness and a shift towards pathogenic *Aeromonas* [44]. Furthermore, in this study, beta diversity differed significantly among sites, mirroring findings for *Anopheles arabiensis* under metal exposure [13]. This suggests that metal exposure restructured the gut microbial community of *M. scutellaris* between populations without significantly altering richness (alpha diversity). Heavy metals explained 36% in the observed microbial community variation, a meaningful effect size for a field study in which microbiota composition is shaped by multiple co-occurring factors [45]. Overall, the effects of metal exposure on the gut microbiota observed here are consistent with reports for other insect orders,

namely Diptera (*A. arabiensis*) [13] and Lepidoptera (*Chilo suppressalis*) [46], suggesting HM exposure is a common driver of gut microbiota alteration across insect taxa.

Inferred metagenome function

Gut endosymbionts mediate several functional pathways in planthoppers [47]. In this study, pathways related to amino acid, energy, and lipid metabolism, secondary metabolite biosynthesis, and xenobiotic degradation were predicted to be 1- to 9-fold lower in HM-exposed populations than at Waainek. This agrees with earlier results for ground beetles exposed to agrochemicals [48] and for silkworms (*Bombyx mori*) exposed to heavy metals [49]. As a phloem feeder with a diet rich in sucrose but poor in essential amino acids [50], *M. scutellaris* likely relies on endosymbionts to supply missing amino acids, as in the pea aphid, where *Buchnera aphidicola* synthesises threonine, isoleucine, and lysine [51]. The predicted suppression of lysine metabolism in exposed populations suggests reduced capacity to supplement amino acids, with implications for host fitness.

Populations exposed to heavy metals also showed predicted suppression of arginine and proline metabolism, a pathway associated with cold tolerance in *Bactrocera dorsalis* (Hendel, 1912) (Diptera: Tephritidae) [52]. Because Middle Lake and Civic Dam lie in the South African Highveld, where winters are extremely cold, *M. scutellaris* at these sites may be more vulnerable to cold stress. Similarly, predicted suppression in aromatic/xenobiotic degradation, including protocatechuate degradation, may indicate a reduced capacity to degrade plant secondary metabolites such as phenolics and terpenoids found in water hyacinth [53]. Gut microbiota capable of degrading such compounds have been reported across diverse insects, including the aphid *Sitobion avenae* (Fabricius, 1775) (Hemiptera: Aphididae) [54], the diamondback moth *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae) [55], and fungus-farming leaf-cutter ants [56]. Compromised detoxification capacity may therefore further undermine host fitness.

Our study demonstrated that *Megamelus scutellaris* core microbiota was dominated by *Rickettsia*, with *Wolbachia* and *Cardinium* present at lower abundance. Heavy metal exposure altered gut community composition (beta diversity) and predicted a severe reduction in the microbiome function, including amino acid metabolism and xenobiotic degradation. These results indicate that HM exposure may compromise *Megamelus scutellaris* fitness and its efficacy as a water hyacinth biological control agent. This study provides a strong baseline for future studies on shotgun metagenomics, which can provide direct evidence of the functional changes inferred here. We recommend that water management authorities prioritise the remediation of heavy metal-polluted systems to safeguard biological control, which remains the most effective and sustainable management option for water hyacinth.

Declarations

Ethical Clearance

All procedures involving live insects were conducted in accordance with institutional and national guidelines for animal research. Ethical approval was granted by the Rhodes University Animal Research Ethics Committee (RU-AREC) (Approval no. 2025-8120-10221).

Consent to Participate

Not applicable.

Consent to Publish

No individual personal data was used in this study.

Competing interests

The authors declare no competing interests.

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Author Contribution

T.B. (Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing—original draft, Writing—review and editing), R.J.W. (Conceptualization, Methodology, Supervision, Writing—original draft, review and editing), J.A.C (Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—original draft, Writing—review and editing), and G.M (Writing—review and editing).

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Data Availability

Raw 16S rRNA V3-V4 amplicon sequence data were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1454826 and are publicly available at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1454826>

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57. Statements & Declarations

Figures

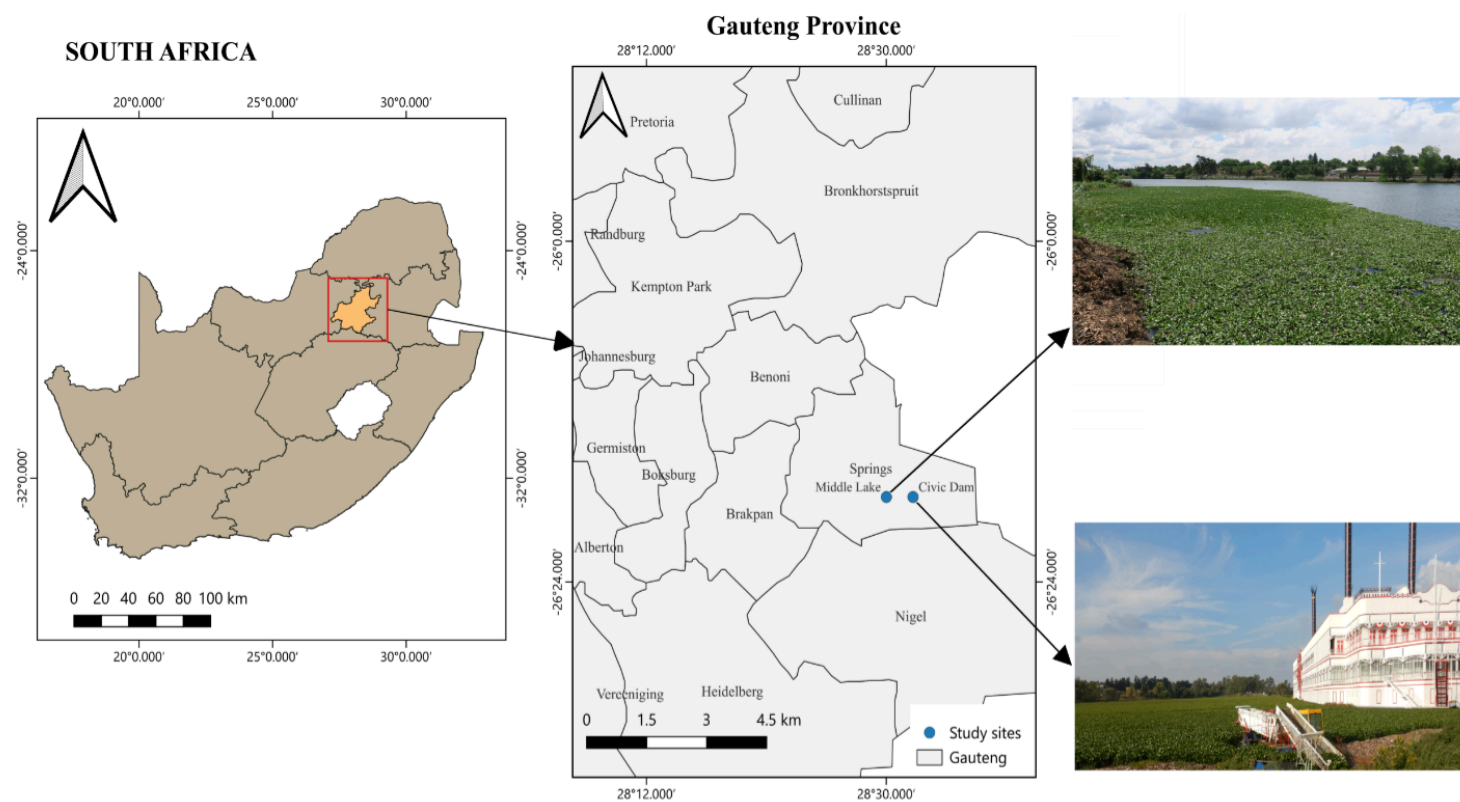


Figure 1

Locations of the water hyacinth-invaded sites, Civic Dam and Middle Lake, in the East Rand, South Africa, where *Megamelus scutellaris* and plant samples were collected.

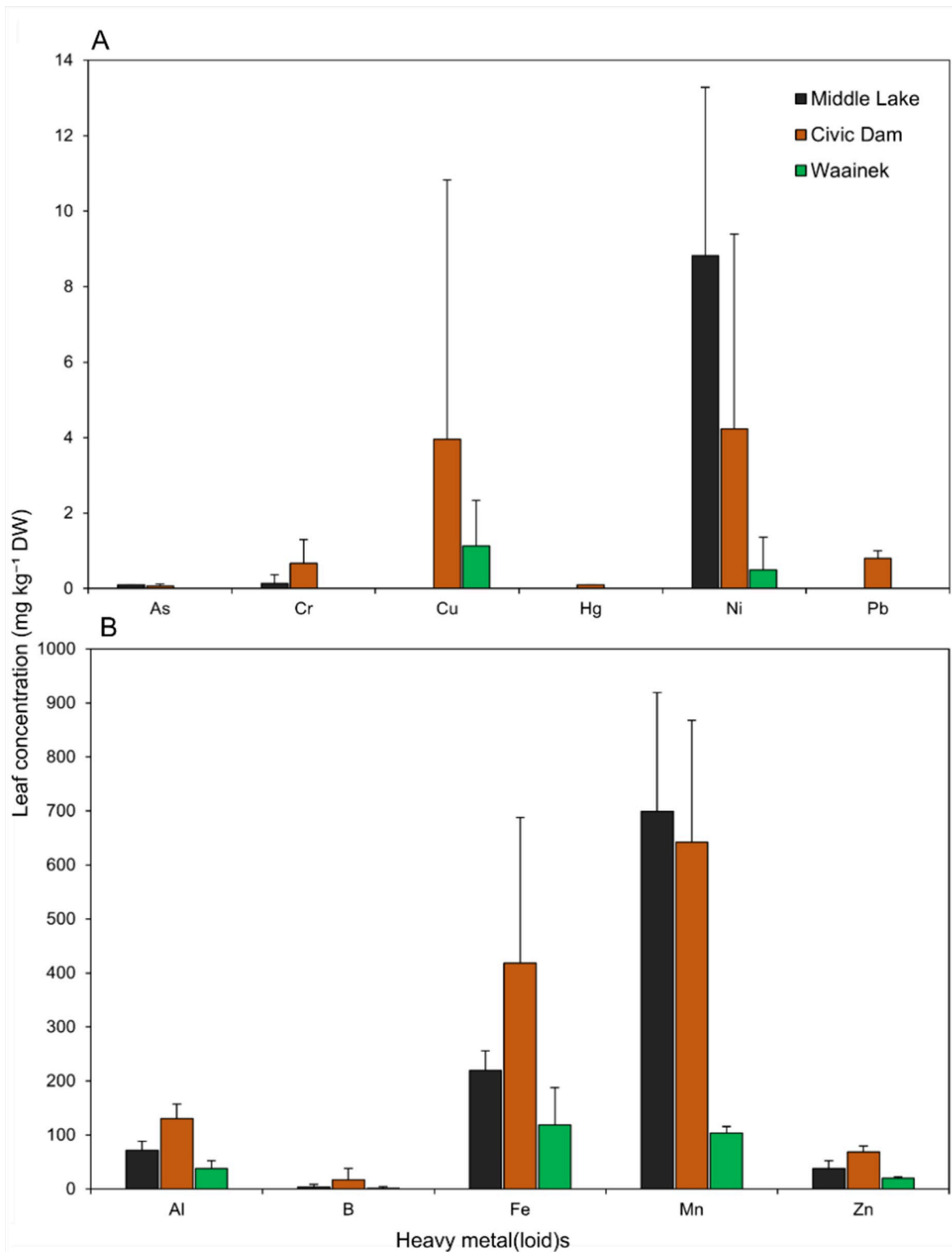


Figure 2

Heavy metal(loid) concentrations in water hyacinth leaves between acid mine drainage-exposed sites and a relatively pristine mass-rearing facility, Waainek. Concentrations are expressed as mg kg⁻¹ dry weight (DW) (A) 0–900 mg kg⁻¹ DW range. (B) 0–14 mg kg⁻¹ DW range. Error bars indicate standard error of the mean (n = 3).

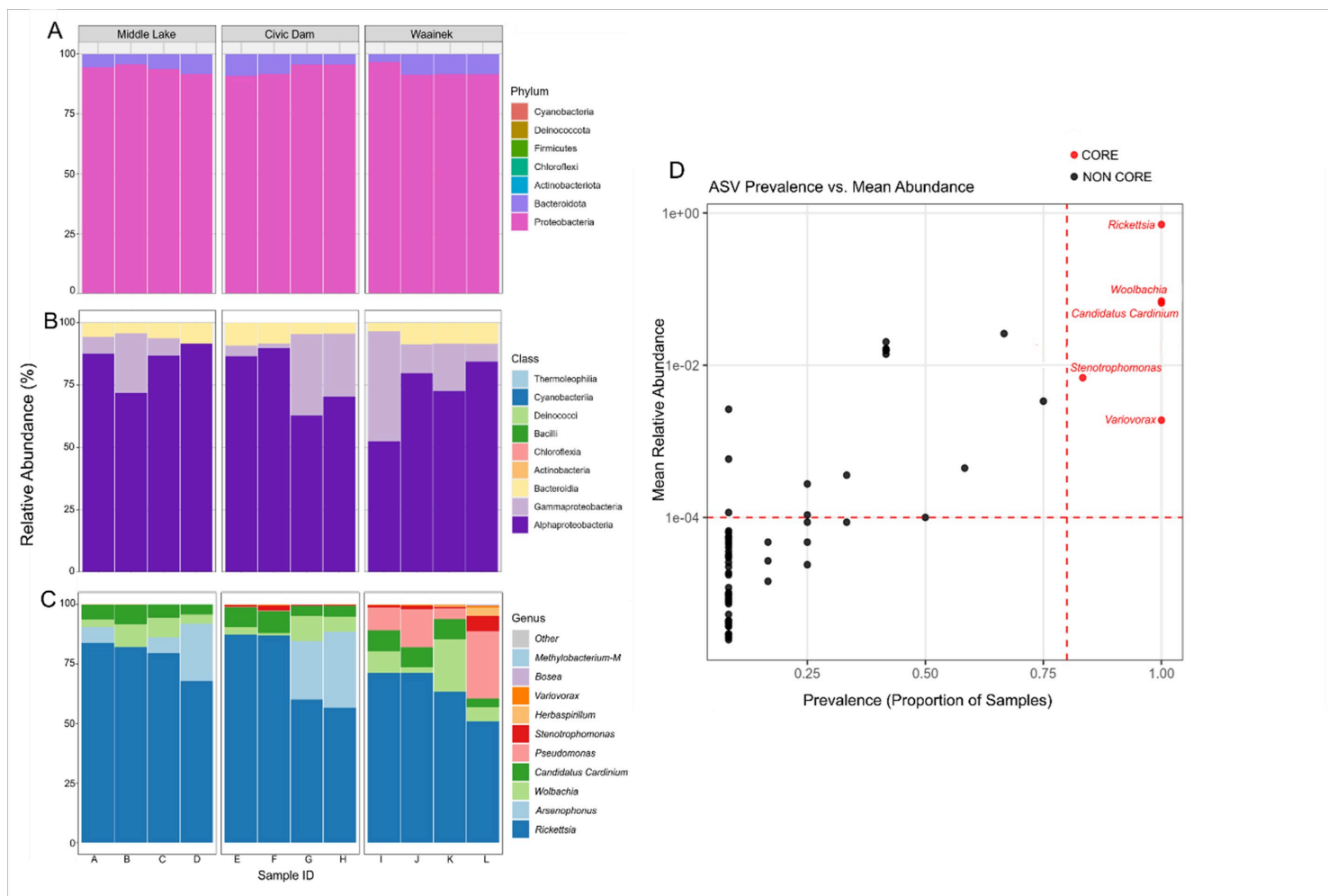


Figure 3

Compositional biplot of *Megamelus scutellaris* gut microbiota highlighting the top 10 taxa at the A) phylum, B) class, and C) genus levels across Civic Lake, Middle Lake, and Waainek. Letters A to L represent replicates per site, with 20 insects in each replicate. D) Prevalence-abundance distribution scatter plot of *M. scutellaris* gut microbiota. Core taxa (red) were present in at least 80 % of samples with a mean abundance of $\geq 0.01\%$, as marked by the dashed red thresholds.

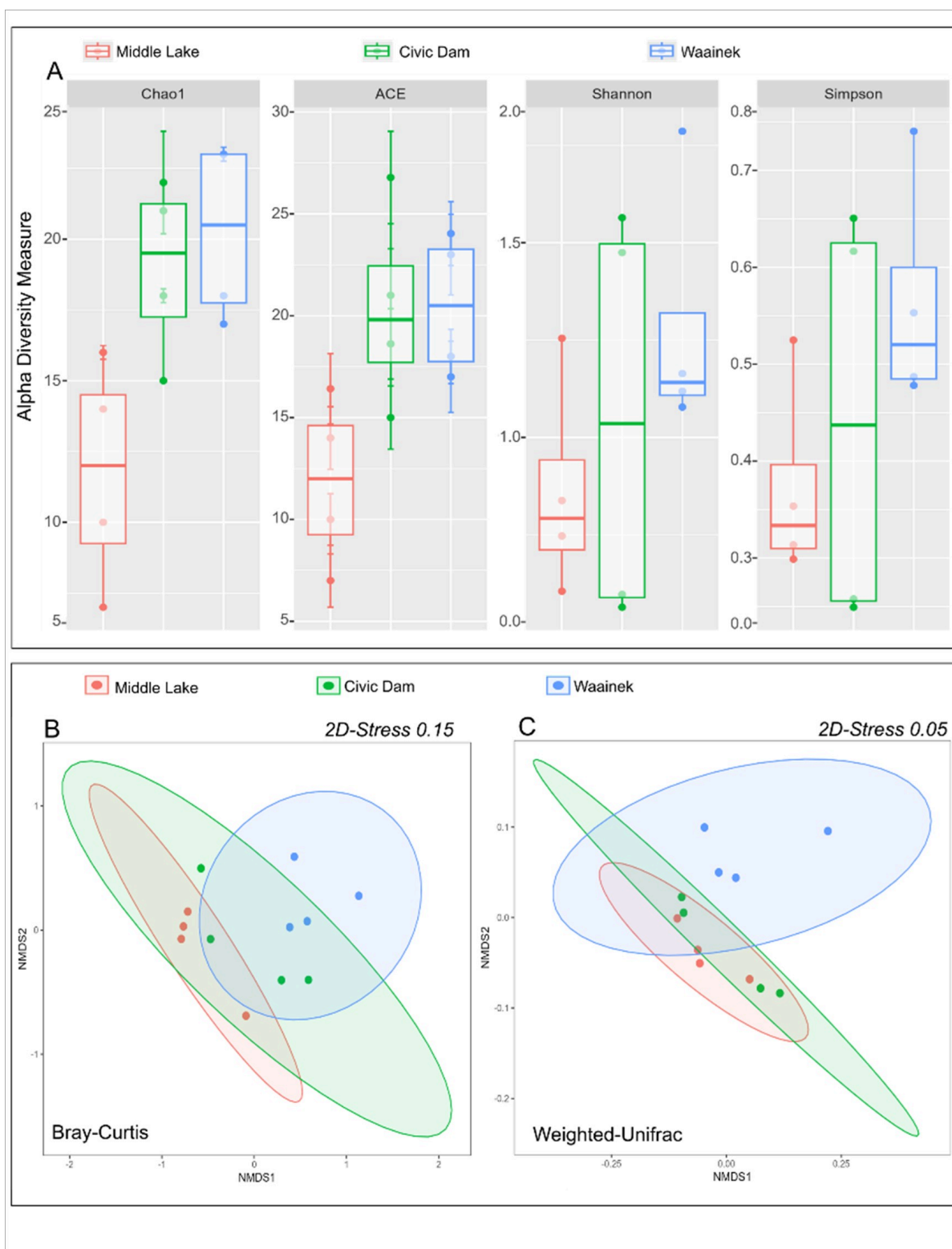


Figure 4

Diversity of *Megamelus scutellaris* gut microbiome across Middle Lake, Civic Lake, and Waainek populations. (A) Alpha diversity was estimated using Chao1, ACE, Shannon, and Simpson indices. (B, C) Beta diversity visualised by NMDS ordination based on Bray–Curtis dissimilarity and Weighted UniFrac distances, respectively. Ellipses denote 95 % confidence intervals.

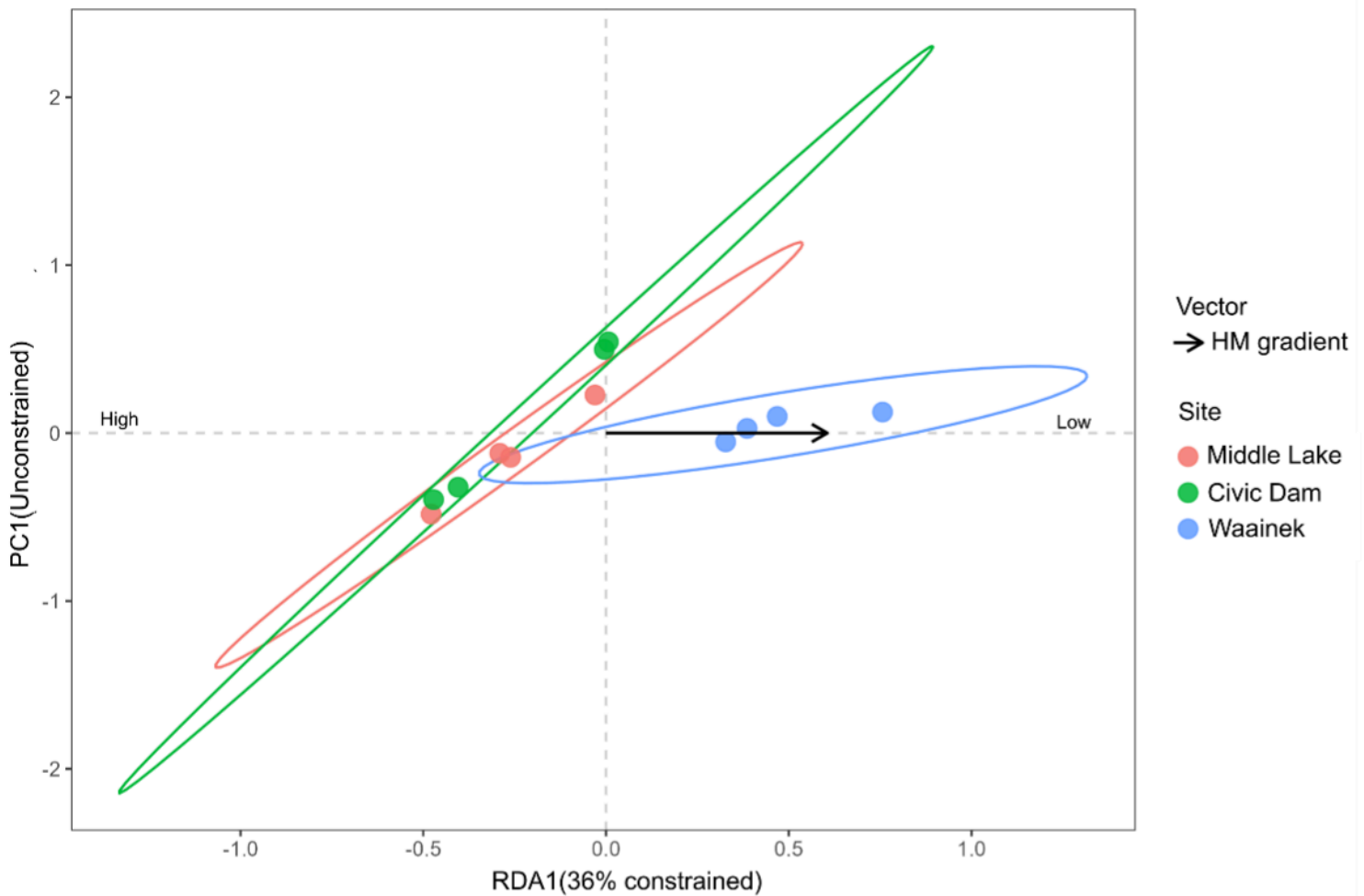


Figure 5

Redundancy analysis (RDA) biplot of *Megamelus scutellaris* gut microbiome composition (Hellinger-transformed ASV data) constrained by heavy metals. RDA1 (x-axis) captures 36 % of the total variation across sites, while PC1 (y-axis) represents unconstrained residual variation. The HM gradient vector indicates decreasing metal concentrations. Points represent samples (n = 4 per site); ellipses denote 95 % confidence regions.



Figure 6

Redundancy analysis (RDA) biplot of *Megamelus scutellaris* gut microbiome composition (Hellinger-transformed ASV data) constrained by heavy metals. RDA1 (x-axis) captures 36 % of the total variation across sites, while PC1 (y-axis) represents unconstrained residual variation. The HM gradient vector indicates decreasing metal concentrations. Points represent samples (n = 4 per site); ellipses denote 95 % confidence regions.

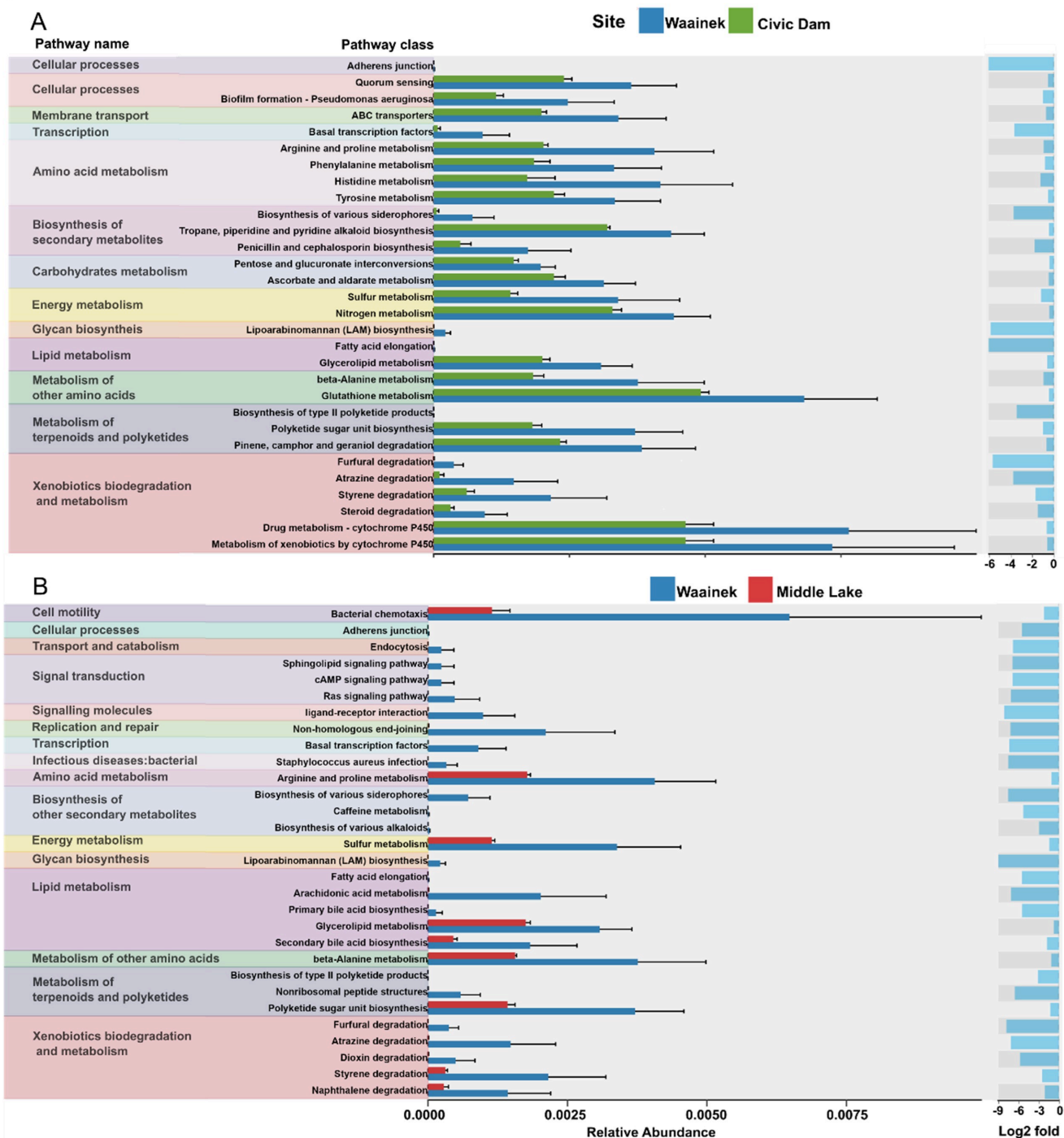


Figure 7

Redundancy analysis (RDA) biplot of *Megamelus scutellaris* gut microbiome composition (Hellinger-transformed ASV data) constrained by heavy metals. RDA1 (x-axis) captures 36 % of the total variation across sites, while PC1 (y-axis) represents unconstrained residual variation. The HM gradient vector indicates decreasing metal concentrations. Points represent samples (n = 4 per site); ellipses denote 95 % confidence regions.

Supplementary Files

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