Ecological Role of Macroplastics as Habitats for Aquatic Macroinvertebrates in the Crocodile River, Mpumalanga

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Ms E Kola

School of Biology and Environmental Sciences

Mbombela Campus.

Dear **Venraate Tsuxeko Mdaka**

Protocol Reference Number: UMP/Mdaka/220432627/BIO/BScHons/2025/1

Project Title: Colonisation of macroplastics by aquatic macroinvertebrates along the Crocodile River

Approval Notification: In response to your application received on **13/05/2025** The Research Ethics Committee-Animal Sciences has considered the above mentioned application and the protocol has been granted **FULL APPROVAL**.

Any alteration/s to the approved research protocol i.e. Questionnaire/Interviews Schedule, Informed Consent form, Title of the project, Location of the study, Research Approach and methods must be reviewed and approved through the amendment/ modification prior to its implementation. In case you have further queries, please quote the above reference number.

PLEASE NOTE: Research data should be stored securely in the School/ division for a period of 5 years.

The Ethical Clearance certificate is only valid for a period of 3 years from date of issue. Thereafter, Recertification must be applied for on an annual basis.

Wishing you the best with your study.

Yours faithfully,

Ms E. Kola: SEC (Chair)

Cc: Research Office Administrator: .....

Cc: Faculty Research Committee Chair: .....

DECLARATION OF INVESTGATOR(S)

I/We fully understand the conditions under which I am/we are authorised to carry out the abovementioned research and guarantee to ensure compliance with these conditions. I agree to completion of a yearly progress report.

..... 15/05/2025

Signature Date

Introduction

- Macroplastic pollution is a major threat to freshwater ecosystems globally (Azevedo-Santos et al., 2021; Dalu et al., 2025).
- Macroplastic pollution is accelerated by human activities (Kershaw & Rochman., 2015; Oswald et al., 2015)
  - Improper disposal of waste

Problem Statement

Macroplastic pollution is a rising issue in river systems.

Studies have explored the physical and chemical impacts of plastic pollution.

However, the role of macroplastics as habitats for aquatic organisms remains underexplored (Blettler et al., 2018 ; Hoellein et al., 2024).

Aim

The study aimed to assess the ecological role of macroplastics as habitats for aquatic macroinvertebrates in the Crocodile River, Mpumalanga.

The objectives were:

To assess the colonisation patterns of macroinvertebrates on macroplastic substrates in the Crocodile River across different seasons.

To compare the diversity, abundance, and composition of macroinvertebrate communities between macroplastic and natural substrates across different seasons.

To compare physio-chemical water variables across different seasons in the Crocodile River.

Hypothesis

The study tested the following hypotheses:

There will be presence of macroinvertebrate communities on macroplastic debris within the Crocodile River across different seasons.

Macroinvertebrate communities and diversity will significantly differ between macroplastics and natural substrates.

Methods

Study area

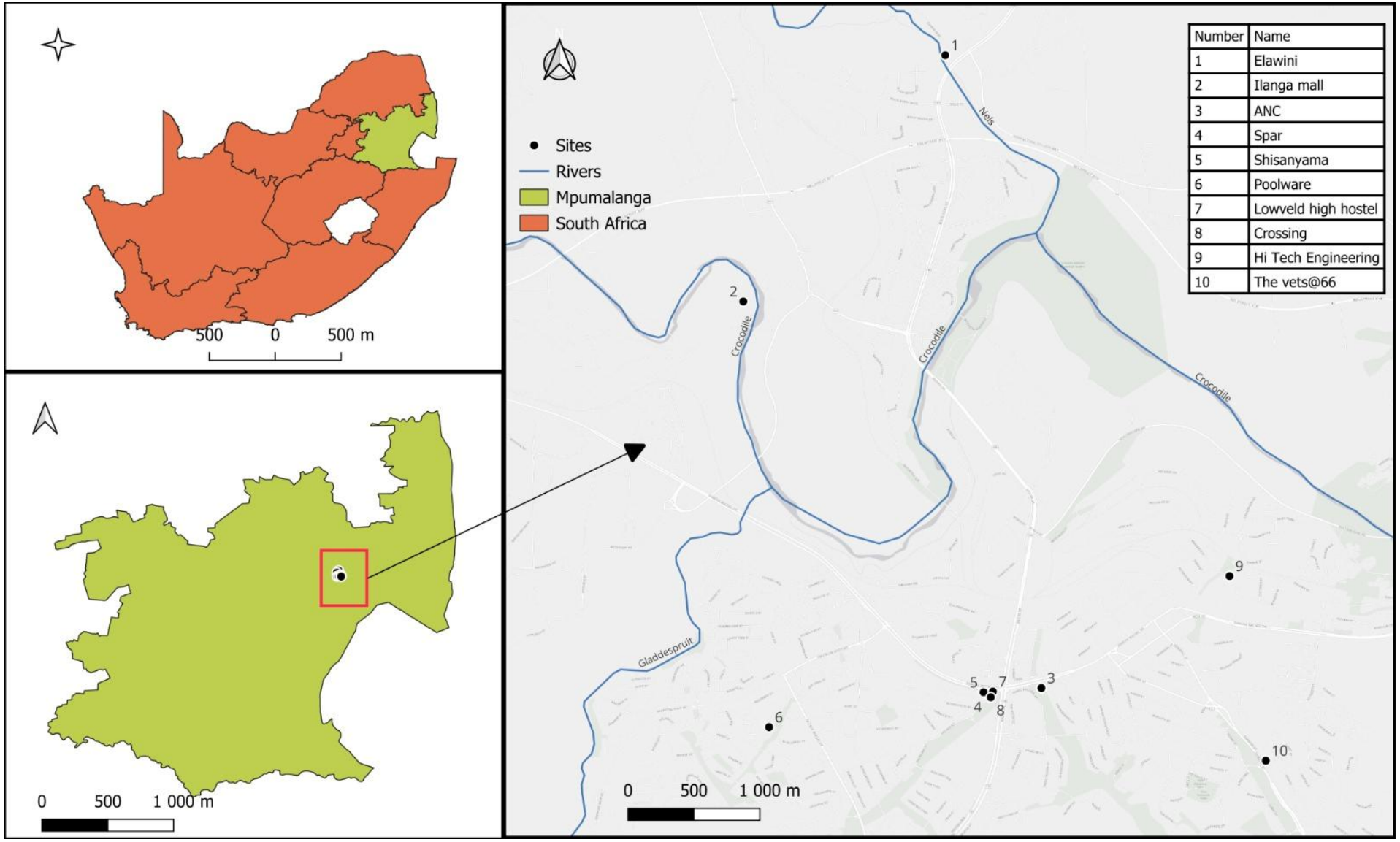


Figure 1: The Map of Crocodile River in Mpumalanga, indicating 10 study sites.

Methods

- **Environmental variables**

A multiparameter probe was used to measure water variables (i.e., temperature, pH, conductivity, DO, ORP, and dissolved solids).

- **Macroinvertebrates sampling**

A random sampling approach was employed with a 20-minute timed protocol to dislodge macroinvertebrates from substrates into a net

Samples were preserved in 70% ethanol, identified in the laboratory using a microscope, and thereafter counted.

Methods

Field Sampling:

- Sampling was conducted across two distinct seasonal periods.
  - Season 1 (Cool–Dry): Early June 2025 → Winter
  - Season 2 (Wet–Cool): Mid-September 2025 → Spring

- Substrates sampled (where present):
  - Macroplastic debris: bottles, plastic bags, plastic wrappers.
  - Natural substrates: Coarse substrates (pebbles, cobbles) and vegetation.

Methods

Data Analysis

- A Shapiro-Wilk test was conducted to assess the normality of the data.
- This is a prerequisite for using the parametric t-test to ensure the validity of its results

Environmental Variables

- To compare environmental variables between seasons, paired t-test and Wilcoxon signed-rank test were used.
- PCA biplot was used to visualise the results, making it easier to see patterns and relationships.

Macroinvertebrates

- For macroinvertebrate diversity analyses, diversity indices Shannon-Weiner, Simpson, and Species richness were calculated.
- To compare diversity indices between substrates, PERMANOVA and SIMPER were used.

Results

Environmental variables

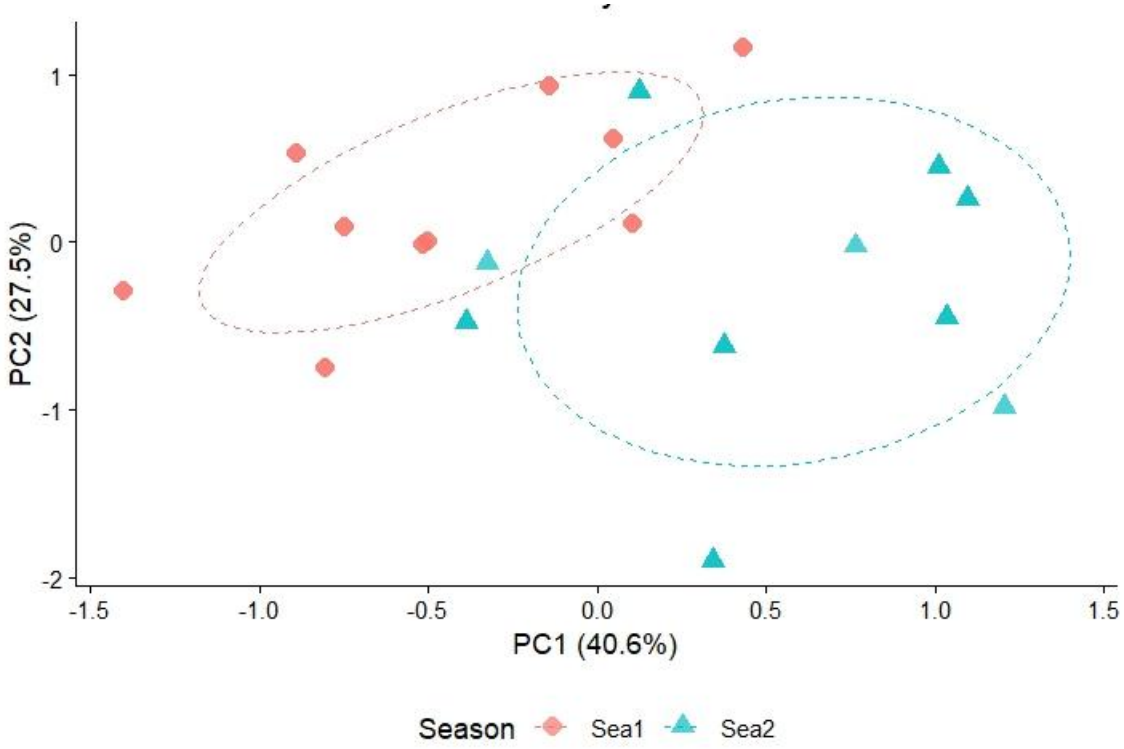


Figure 2: PCA biplot of environmental variables showing differences between S1 (cool-dry) and S2 (wet-cool) in Crocodile River, Mpumalanga.

- PCA explained **68.1%** of the variance in environmental variables.
- A clear separation between the two seasons was observed.
- S1 (winter) exhibited **greater variability** in physio-chemical conditions.
- S2 (Spring) maintained more stable conditions.

Table 1: Statistical results for seasonal water quality comparison

Variable	Test statistics	p-value
Temp	t = -9.44	0.001953 P<0.05
pH	Z = 2.6656	0.0039063 P<0.05
TDS	t = -6.044	0.0019531 P<0.05
ORP	Z = 1.78	0.083984
%DO	Z = 17838	0.083984
EC	t = -1.9245	0.0855938
Phosphorus	t =-1.1851	0.249
P Pentoxide	t = -1.356	0.19922
Phosphate	t = -1.66	0.166

Results

Species Composition

4,157 individuals were collected during S1 (cool-dry) in early June.

Natural substrates had 2,232 individuals, and plastic substrates had 1,916.

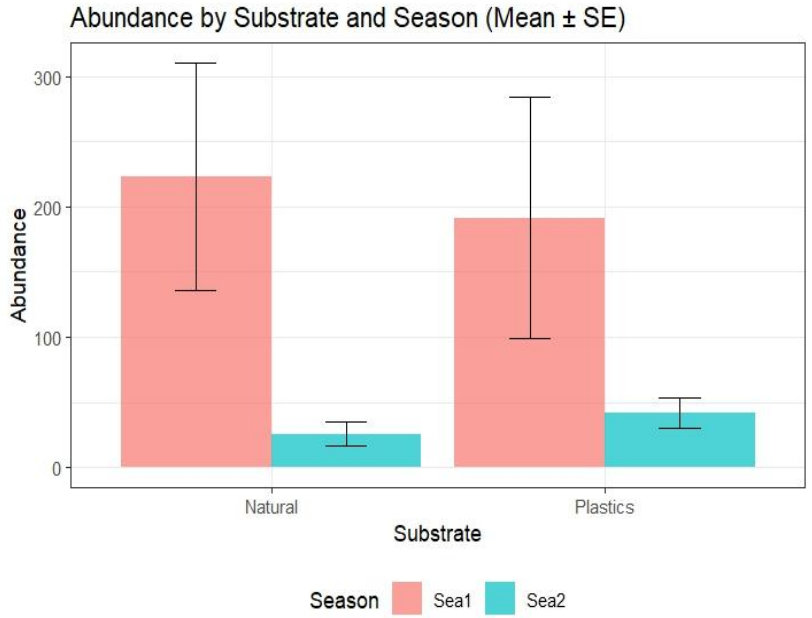
604 individuals were collected during S2 (wet-cool) in mid-September.

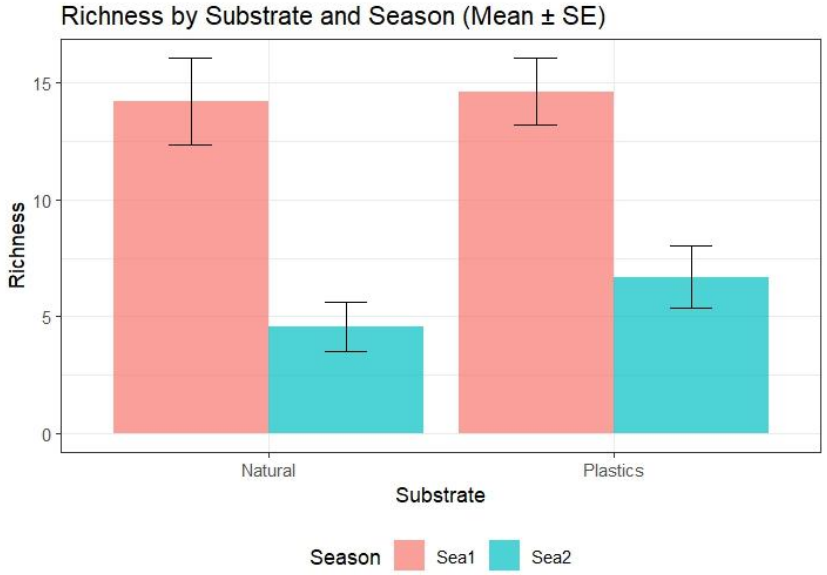
Natural substrates had 181 individuals, and plastic substrates had 423.

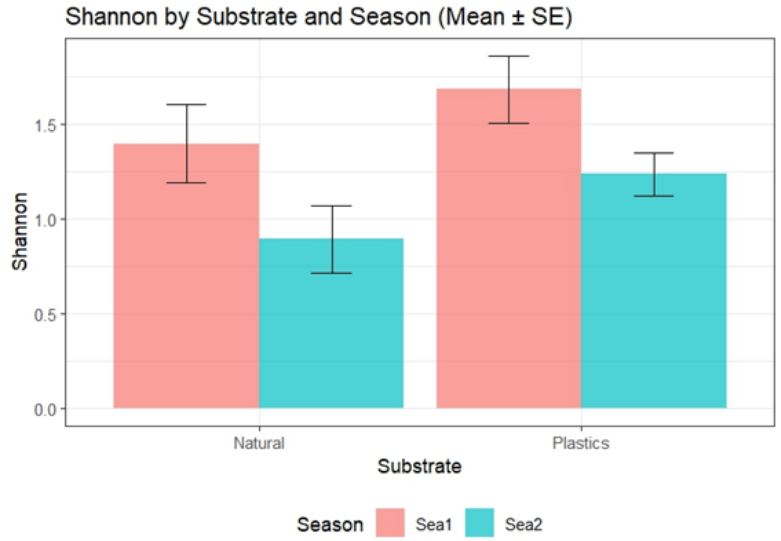
Macroinvertebrate community composition was higher in S1 compared to S2.

Results

Taxonomic diversity





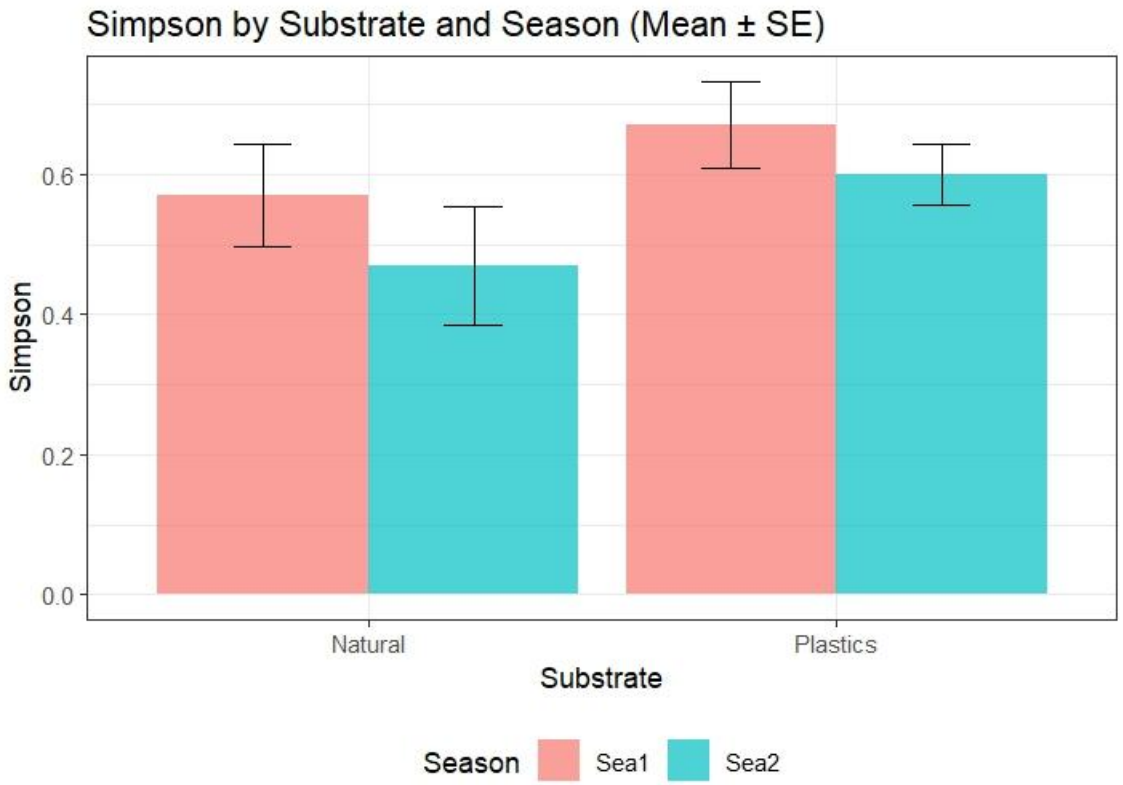


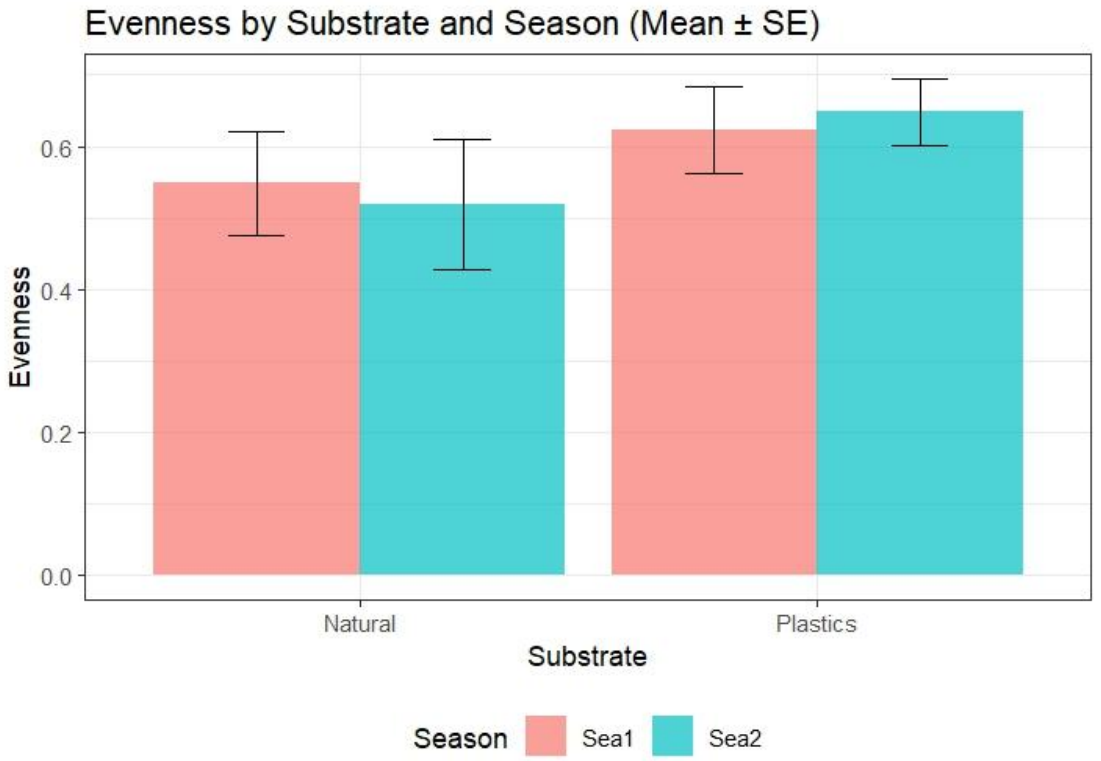
Sea1 (cool-dry), Sea2 (wet-cool)

Table 2: Significance of Macroinvertebrate Diversity Indices Between Substrates and Seasons

Diversity indices	Substrates	Seasons
Abundance	0.38318 (P>0.05)	0.0016899 (P<0.05)
Species richness	0.17626 (P>0.05)	0.00011253 (P<0.05)
Evenness	0.30899 (P>0.05)	0.14237 (P>0.05)
Simpson diversity	0.0136 (P<0.05)	0.064322 (P>0.05)
Shannon	0.0106 (P<0.05)	0.0068626 (P<0.05)

Results





Sea1 (cool-dry), Sea2 (wet-cool)

- **Evenness and Simpson diversity did not differ significantly between the two seasons.**
- **Simpson diversity showed significant variation in substrates. P<0.05**

Results

PERMANOVA

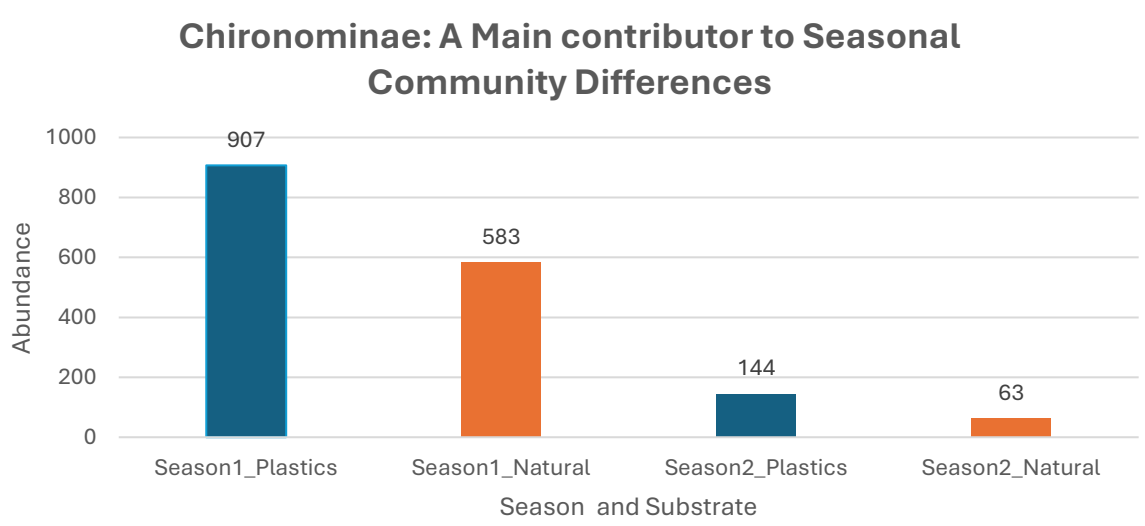
- **Substrate:** Not significant (p= 0.711, -F= 0.820)
- **Season:** Highly significant (p= 0.001, -F= 2.369)
- **Substrate × Season:** Significant (p= 0.017, -F= 1.443)

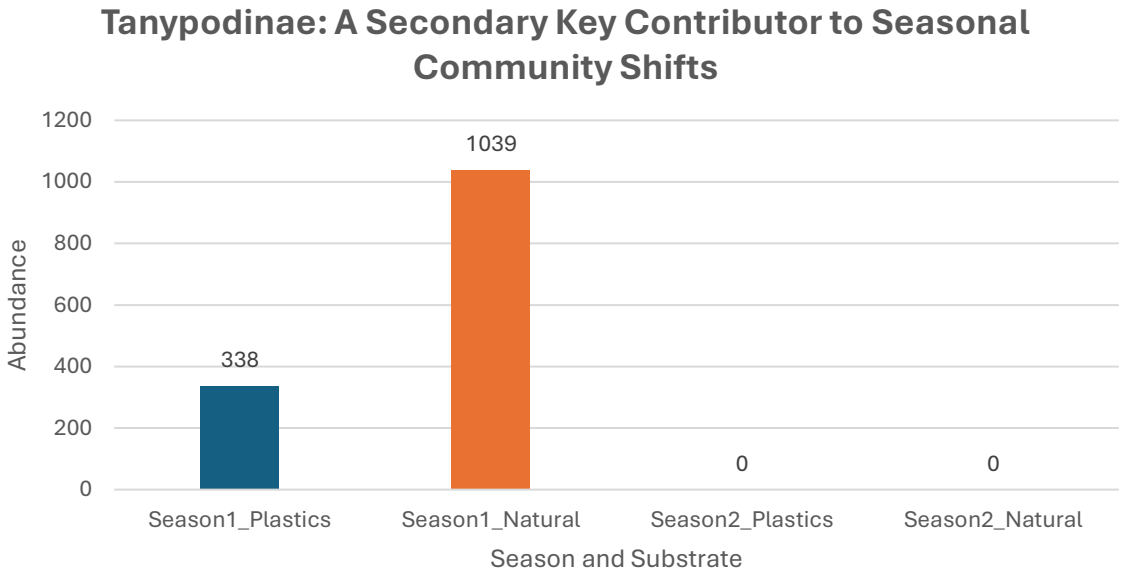
SIMPER

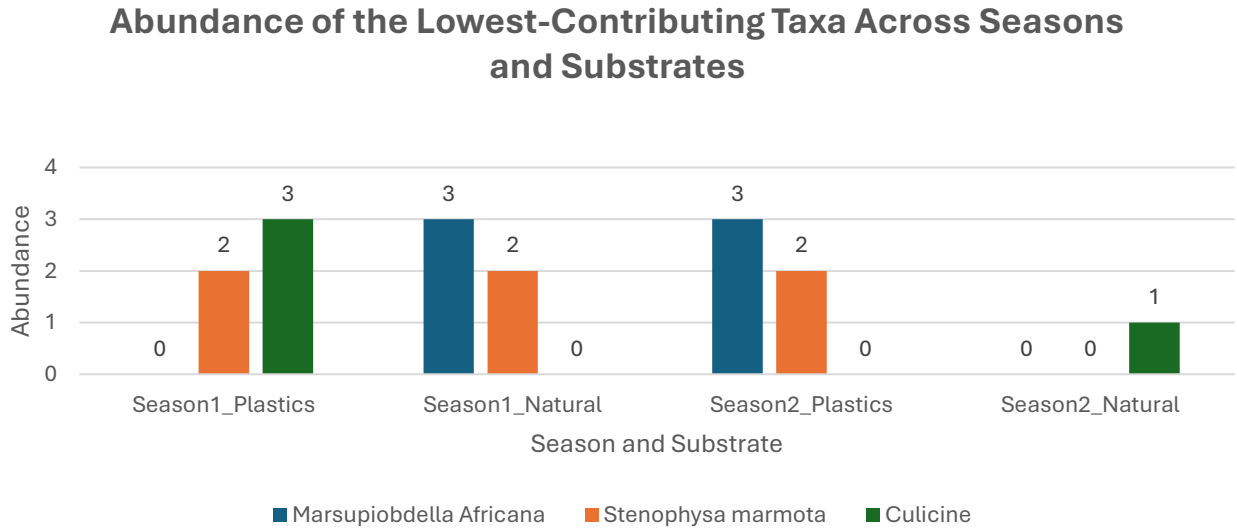
- ***Chironominae*: 21.4%** of dissimilarity, plastics (**52.6%**) & natural substrates (**32.3%**).
- ***Tanypodinae*:** Dissimilarity **(15.4%),** natural substrates **(52%) &** plastics (**16.9%**).
- ***Marsupiobdella Africana, Stenophysa marmota, and Culicine*** Dissimilarity (**1<%** each).

Results

SIMPER







Discussion

Cause of dramatic seasonal decline in macroinvertebrates

Life Cycle Patterns:

- Many aquatic insects have synchronized life cycles.
- The high abundance in S1 (cool-dry) likely reflects mature larvae before emerging as flying adults.
- The low abundance in S2 (wet-cool) most larvae have emerged, leaving only a few ones.

Physical Disturbance:

- Rainfall a week prior likely increased flow and displaced organisms.
- Higher flows during the wet season can sweep invertebrates off from natural substrates.

Causes of plastics supporting more diversity in Season 2

Habitat Stability & Complexity:

- During high-flow events, natural substrates like leaves and small rocks are easily displaced.
- Macroplastics provide stable refuges during disturbances because of their size and ability to snag.

Conclusion

The study confirms that aquatic macroinvertebrates actively colonize macroplastic debris in the Crocodile River, with macroplastics acting as functional habitats like natural substrates.

Additionally, macroplastics may alter community structures and provide stable refuges under changing environmental conditions.

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Thank you

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