

Developing new environmental DNA-based ecological assessment tools for the management of coastal environment

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Stressors of different nature are affecting coastal ecosystems. Yet their combined impacts on biodiversity is poorly understood, mainly for two reasons: (i) traditional approaches for monitoring coastal biodiversity present multiple constraints that limit observations to a small number of taxa and (ii) these studies often focus on the impact of a single stressor, ignoring interacting effects. Environmental DNA (eDNA) biomonitoring is now recognized as a powerful tool for quickly obtaining comprehensive and standardized biodiversity surveys on a range of taxonomic groups. Here, we used eDNA metabarcoding to characterize several communities (eukaryote, diatom, crustacea and fish) in three estuaries subjected to different levels of anthropogenic disturbance in Northern Queensland, Australia. Diversity and community composition patterns were characterized and investigated in relation to natural and anthropogenic stressors. These data will be then combined into Bayesian Networks Relative Risk Models (BNRRM) to disentangle relative effects of stressors on estuarine communities and develop ecological risk assessment models. This process is illustrated below through several examples.

Study sites

Three estuaries in the Mackay/Whitsundays region subjected to different types/levels of anthropogenic pressures were included in this study. Sugar cane culture and grazing land in their catchments are the main local sources of environmental contaminants (nutrients, pesticides and sediments) that affect water quality (Table 1). Besides, these regions are characterized by a strong seasonality with most of land-based runoffs occurring during the wet season (November to April).

Figure 1. Study area. a) Location of the study area in Queensland, b) Location of the estuaries (Gregory, St Helens/Murray and Sandy Creek), c) Sampling sites within St Helens/Murray estuary

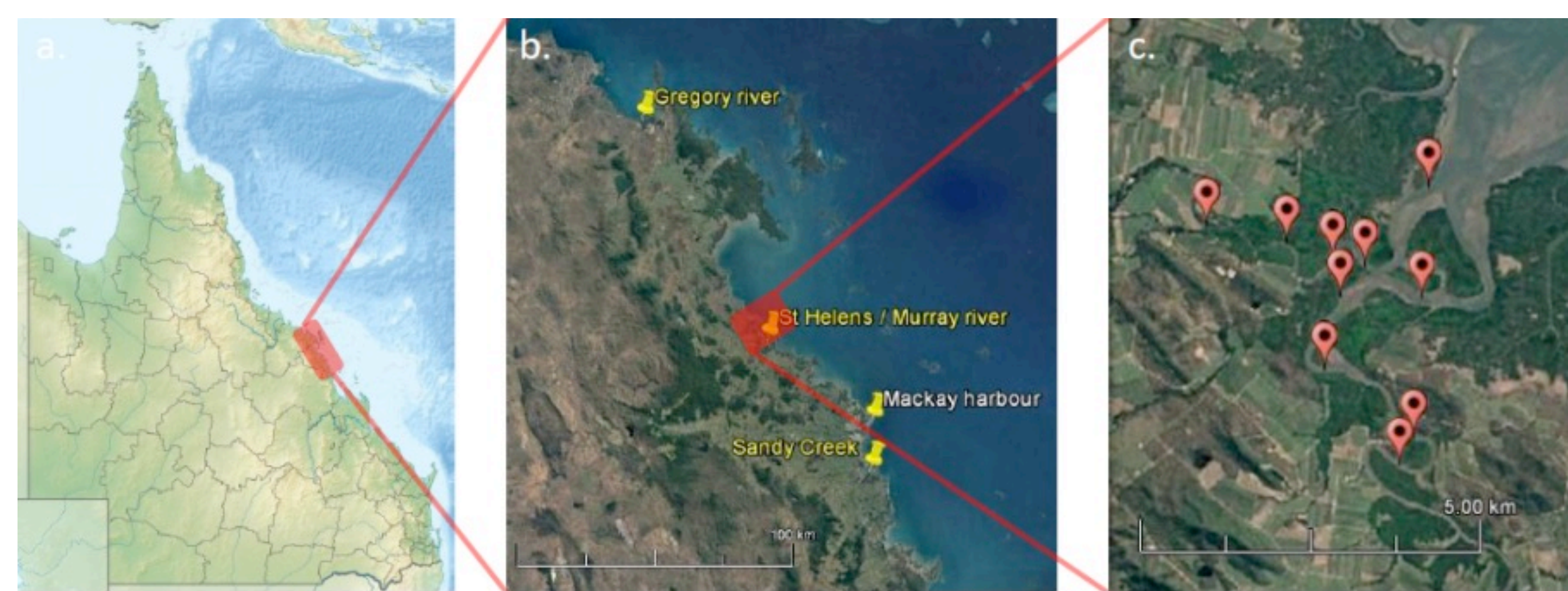


Table1. Water quality indicator scores for each estuary. Data from 2015-2019 based on the Healthy Rivers to Reef Partnership reports. Score ranges from A (very good) to E (Very poor).

	Overall water quality	Nutrients	Pesticides	Turbidity/DO
Gregory river	A/B	A/B	C	A/B
Murray / St Helens	B/C	C	B/C	C
Sandy Creek	C	C	D/E	A/B

Sampling and methods

10 sites per estuary were sampled during the dry season (October 2018). At each site, 3 water and 3 sediment samples were collected for eDNA analyses (total N = 180) to target water column and sedimentary communities, respectively. After DNA extraction, multiple DNA metabarcodes were PCR amplified to characterize groups of interests. Three PCR replicates per sample were conducted using tagged primers, and later sequenced on Illumina platform. Multiple metrics were derived to characterize local biodiversity (see part I; only results on water column communities are presented).

Sampling was replicated in wet season (January 2020) to account for seasonal variability. Wet season data are currently available only for the eukaryotic communities (Figs. 3&4). In both seasons, a range of data on local physico-chemical parameters (metals, nutrients and pesticides) were collected to first identify drivers of community changes (see II) and later to parametrize ecological risk assessment models (see part III)

I. Characterizing diversity and composition of estuarine communities from eDNA

Composition of water column communities, especially small-bodied ones (eukaryote and diatoms), exhibit a strong regional signal during the dry season (top panels) when levels of contaminants are low and salinity high (>36 ppt at all sites). This indicates a long-lasting differentiation of communities' composition between estuaries outside periods of massive run-offs. Contrarily, no consistent trend in richness was observed among taxonomic groups (bottom panels). Similar patterns were observed for sedimentary diatom and eukaryotic communities (not shown).

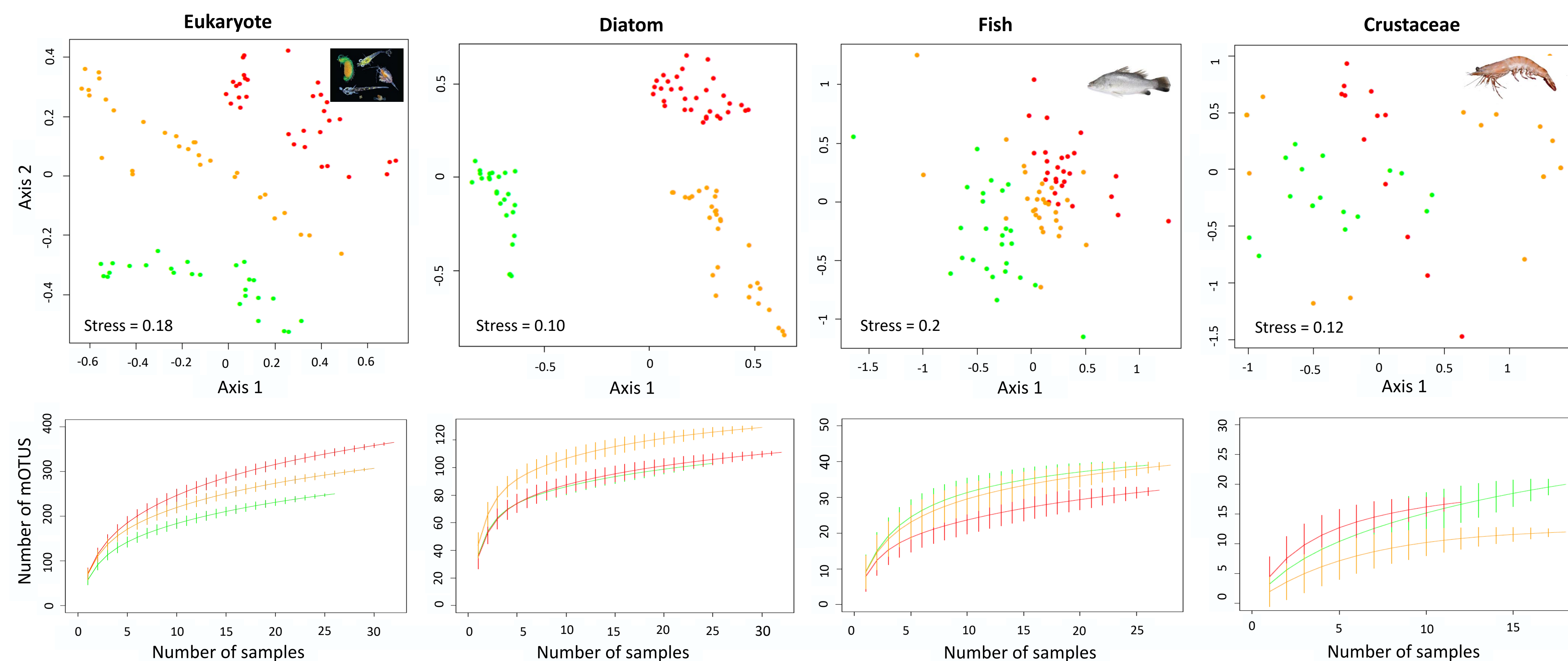


Figure 2. Dissimilarity in composition and richness of water column communities (eukaryote, diatom, fish and crustacea) in the three estuaries. Top panels: NMDS ordinations reflecting (dis-)similarity in community composition between water samples. Bottom panels: rarefaction curves illustrating richness of community in each estuary. Colors reflects the estuary (green: Gregory river, orange: Murray/St Helens, red: Sandy Creek).

II. Identifying natural and human-induced drivers of community composition

Eukaryotic communities composition in the water column changes dramatically between seasons (Fig. 3). However, the inter-estuary differentiation is observed all year round, although it is less marked during the wet season (Fig. 4). At this period, estuaries are characterized by a strong salinity gradient (from freshwater to saltwater) and high concentration of contaminants, especially in Sandy Creek. Community composition is correlated to several water physico-chemical parameters in both seasons ($P < 0.01$): salinity, dissolved oxygen (DO), water acidity (pH) and chlorophyll concentration. Pesticide (red arrows) and nutrients (green arrows) concentrations explain partially the differentiation between estuaries, although they are very low in dry season.

Figure 3. Seasonal composition of eukaryotic communities (at the phylum level) in each estuary.

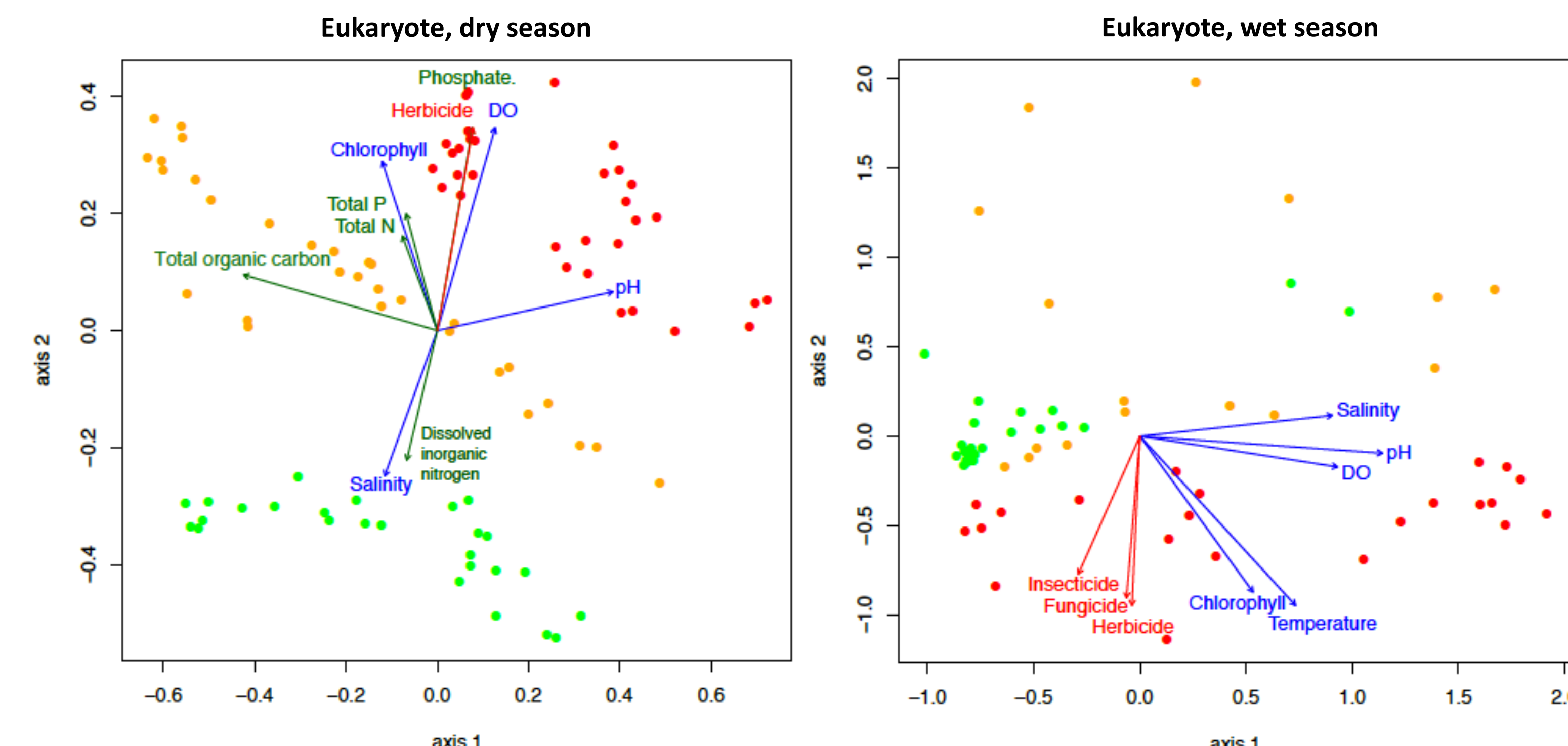
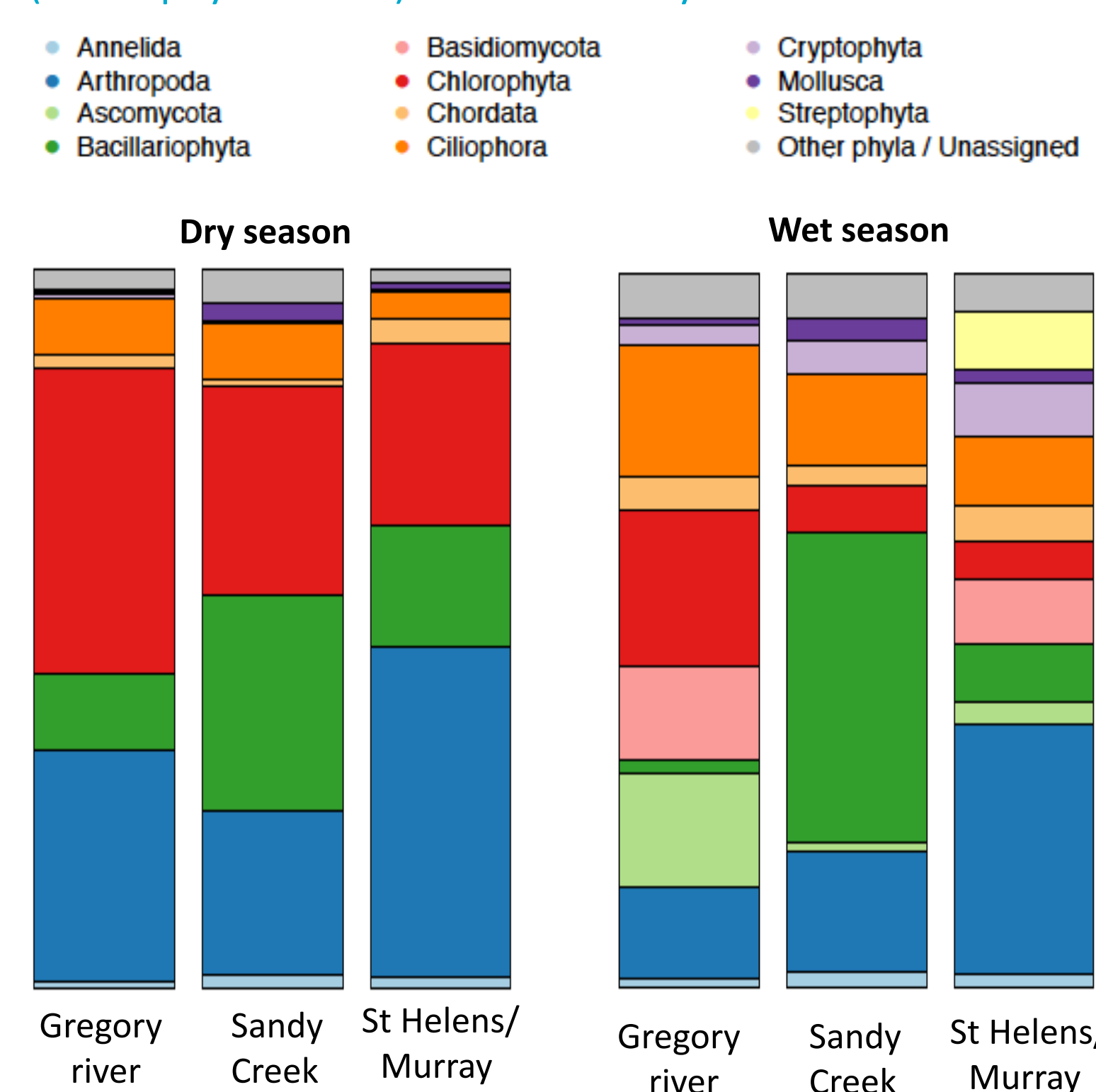
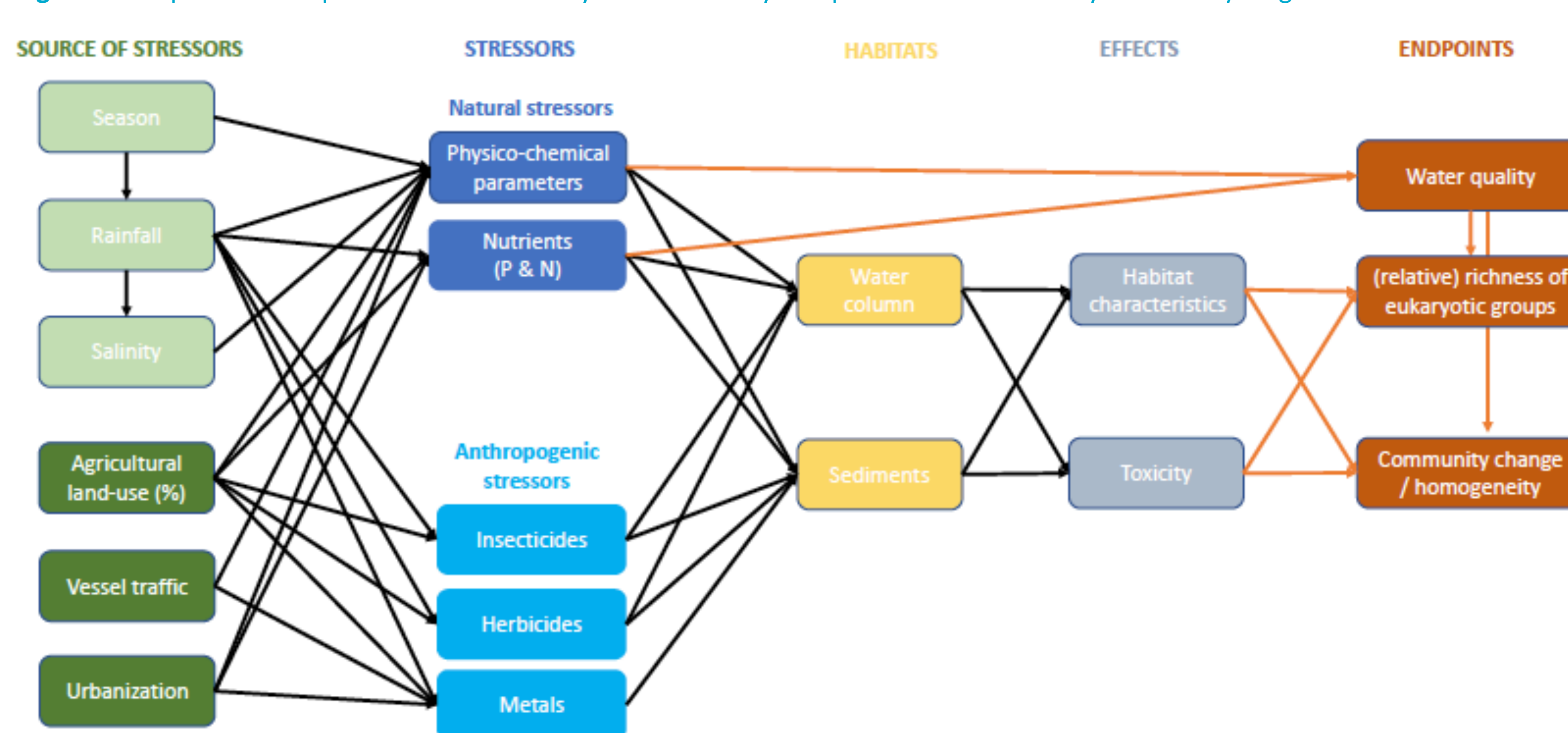


Figure 4. NMDS ordinations reflecting (dis-)similarity in community composition between water samples in dry and wet seasons, with vector fitting of environmental parameters. Only significant variables are represented. Nutrients data were not yet available for the wet season and are thus not represented on the right panel.

III. Combining eDNA biomonitoring data with Bayesian Network Relative Risks Models (BNRRM) for ecological risk assessment

BNRRM have been widely used to assess ecological risks as they can account for various stressors and response types over large spatial scales. These models allow to weight the relative importance of each stressor on a set of ecological endpoints in different locations. Here, eDNA-derived biodiversity metrics are used as ecological endpoints. A conceptual model linking sources of stressors to habitats where they have an effect and finally to ecological endpoints is first built (Fig. 5). This model is based on causal relationships between the variables in the system, which are derived from analyses of field data (see part II) and literature. In this series of causal networks, interactions between the nodes are described by conditional probability tables. Using prior knowledge and field data, Bayesian Networks allows to calculate the probability of a specific response (e.g. high diatom diversity) occurring, and the associated uncertainty, according to the state of stressors influencing it. Collectively, the approach can then be used to test different management scenarios.

Figure 5. Simplified conceptual model for eukaryote community composition in the Mackay-Witsundays region



Perspectives

The notion of studying a single stressor on a small number of taxa is now outdated. eDNA biomonitoring can provide comprehensive information on coastal biodiversity while BNRRM offers an appropriate framework to disentangle the relative effect of multiple stressors on these communities. Combining both approaches to develop ecological risk assessment models at a scale relevant for managers will help developing effective biodiversity management strategies.

FOR FURTHER INFORMATION

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