

Conceptual Boxes

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Here we create three text boxes for the adjacent manuscript. These serve to illustrate three main ideas which we found difficult to incorporate into the written text. We also produce one diagram, which serves to outline the entirety of the process we engaged in.

1) The Applications of Meta-barcoding with Plants

Table 1: Applications of Plant Metabarcoding

Application	Example	Citations
Plant-Animal Interactions	Dietary Preferences of herbivores	Soininen et al. 2009
Noxious Weed Detection	Presence of species by hydrologic Basin	Coghlan et al. 2021
Detection of Rare Species	Presence of Rare Aquatic Species	Tsukamoto et al. 2021
Forensic Science	Identifying the provenance of materials found at crime scene	Allwood et al. 2020
Pharmaceutical	Identifying adulterants in wholesale products	Bell et al. 2022

2) Major Issues and bottlenecks currently related to plant Meta-barcoding.

Table 2: Current Issues Facing Plant Metagenomics

Issue	Our Approach	Issue Citation
Taxonomic Resolution	A353	Coissoc et al. 2016, Kress 2017
Reference Library - Phylogenetic	Kew PAFTOL, no phylogenetic biases	Kress 2017, Bell et al. 2022
Reference Library - Spatial	Some bias persists towards Europe	Cheng et al. 2018, Darwin Tree of Life 2022, Lewin et al. 2020, Bell et al. 2021
Reference Library Generation	Spatial Modelling; Code within	Bell et al. 2022
Uncertainty with Matches	Temporal Filter System	Bell et al. 2022
Species Surrogates	Temporal Filter System	?

Issue	Our Approach	Issue Citation
False Positives	Spatial & Temporal Modelling, Jaccard Index, high quality reference loci	Bell et al. 2021

3) The Summary of Limitations which each method we utilized contains

Table 3: Limitations Faced and Possible Solutions

Method Component	Limitations	Paths Forward
Stage 1 Species Filter	Test Data	Flash Plant Species Surveys on Plot
Species Distribution Modelling	Number of Records; Taxonomically Difficult Groups	Develop and Disseminate Education Materials; Herbaria Collections
Phenological Modelling	Post-Initiation of Climate Change Records	Advocate Herbarium Collections
Database Generation	Adequate Phylogenetic/Spatial Representation	Plant and Fungal Tree of Life; 10kP
Read Re-assignment	Discrete Frequentist Data	Posterior-Probabilities; Floral Abundance, Nectar/Pollen Nutrition
False Positives	Which True Species?	Jaccard Index, Plot Abundance
Semi-Quantitative Inference	Genome Size, Pollen Grain Size?	Spike Samples with Reference Materials; Several Genome Sizes

4) Diagram of Major conceptual ‘filtering steps’