



NEW ZEALAND'S
BIOLOGICAL
HERITAGE

Ngā Koiora
Tuku Iho

National
SCIENCE
Challenges



Transforming environmental DNA data into information and knowledge about species, communities and ecosystems

Landcare Research Link Seminar

Robert Holdaway, Andrew Dopheide, Ian Dickie, Jamie Wood, Kate Orwin

EPA, Wellington, 28 February 2017

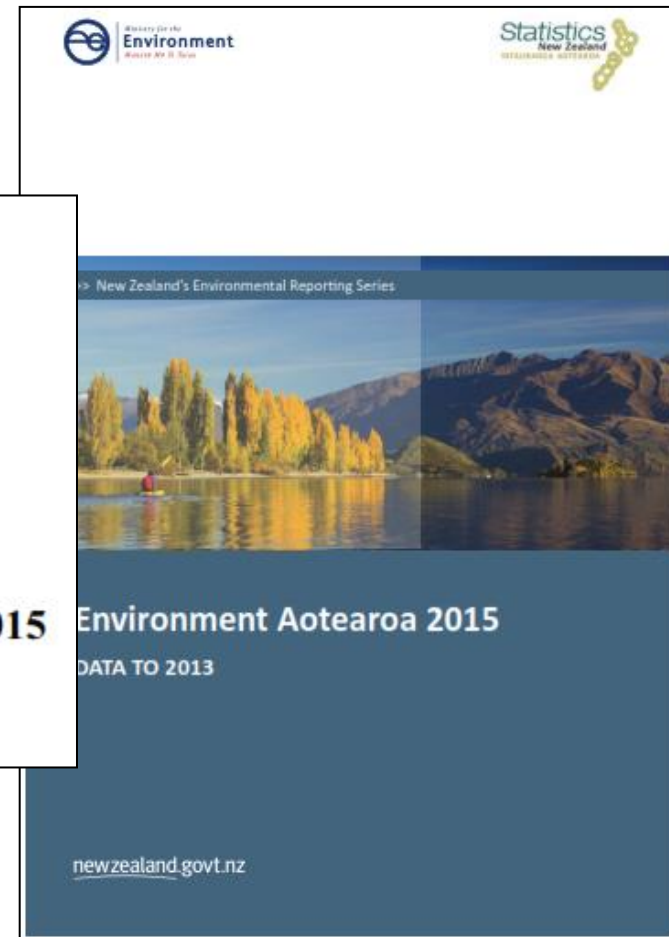
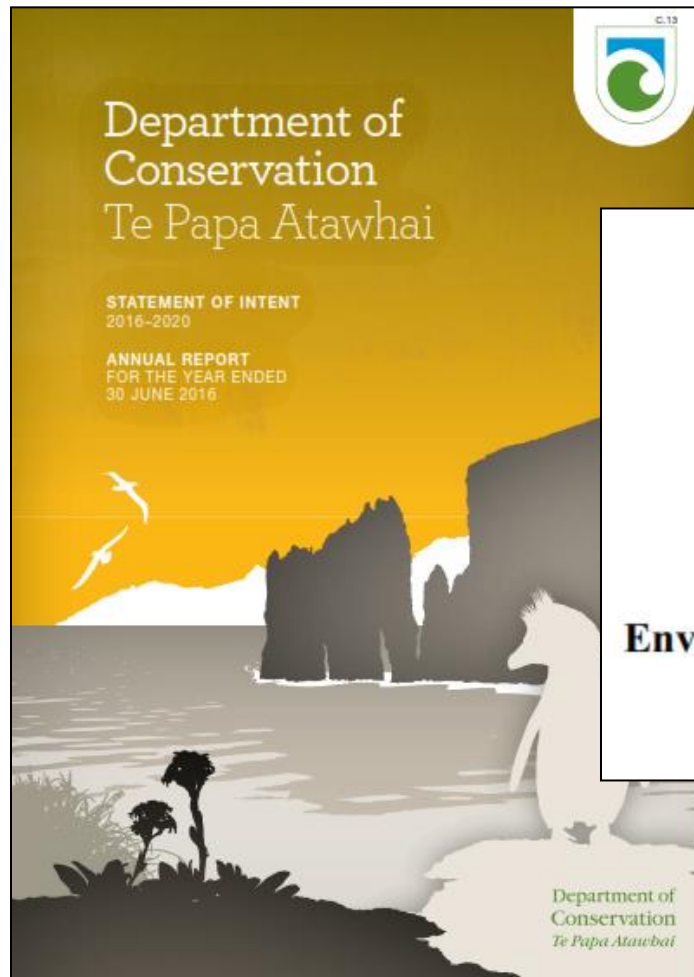


Landcare Research
Manaaki Whenua

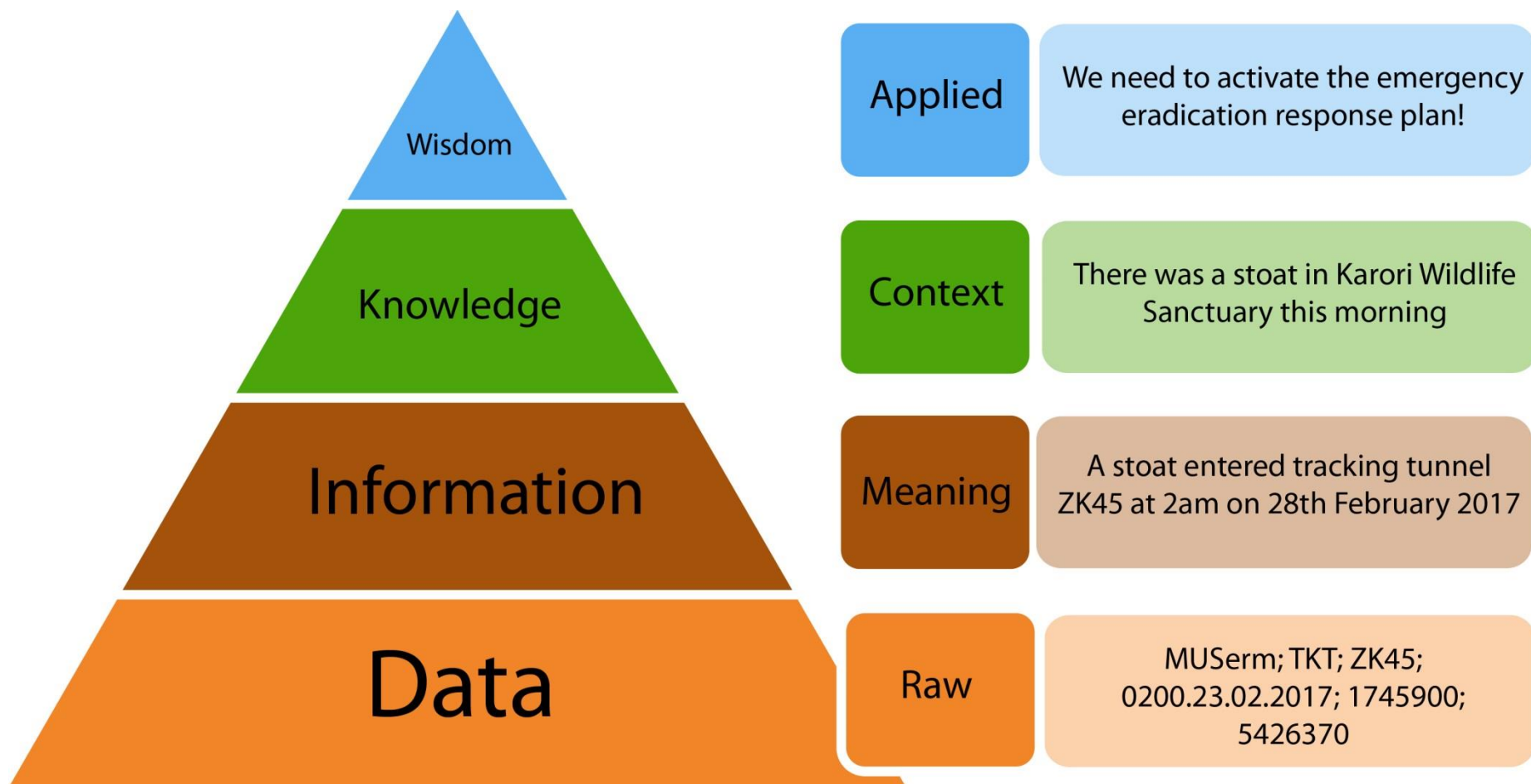
We need good data to understand the state of our environment and how it is changing, so that we can then make wise management decisions

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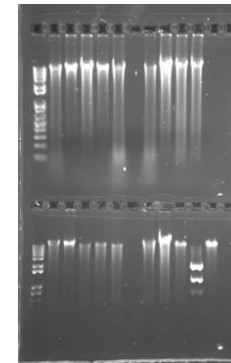


Transforming data into wisdom



Talk outline

1. eDNA basics
2. How does eDNA fit within Environmental Reporting?
3. Exploration of eDNA data
4. Field validation of eDNA data
5. Using eDNA to report on genetic diversity (example)
6. Key take home messages



1. eDNA basics

What is environmental DNA (eDNA)?

= DNA extracted directly from an environmental sample



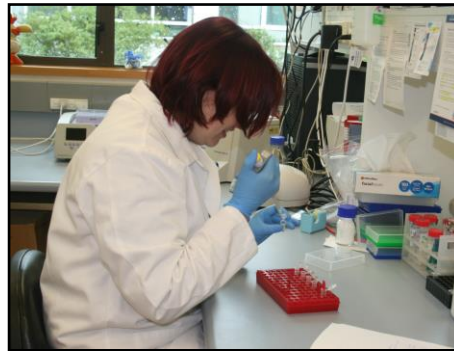
- trace DNA left behind by organisms (e.g. skin cells, faeces)
- undifferentiated micro-organism DNA (microbiomes)
- DNA samples from many pooled organisms (e.g. insects from light traps)

Biodiversity assessment using eDNA

1. Collect sample



2. Extract DNA



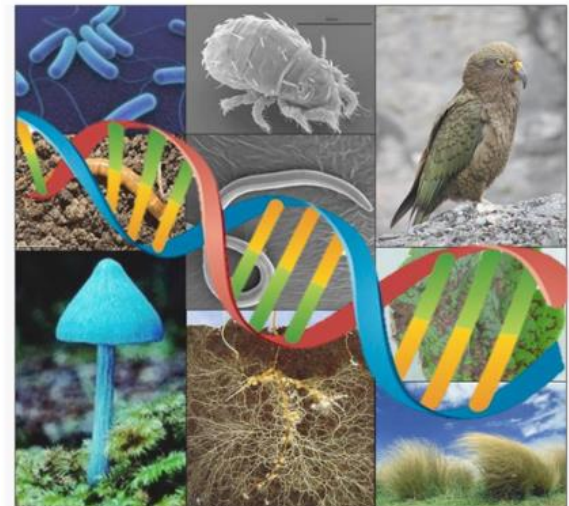
3. Amplify target DNA



4. Sequence target DNA



5. Match DNA to species



CGGCTAGCTGGATCGATCGTTG



The power of eDNA

2. How does eDNA fit within environmental reporting?

New Zealand's environment at a glance

Key findings from New Zealand's Environmental Reporting Series: Environment Aotearoa 2015



Ministry for the
Environment
Manatū Mō Te Taiao

Statistics
New Zealand
TATAUKANGA AOTEAROA

ATMOSPHERE AND CLIMATE

Our climate shapes and supports our environment, economy, and way of life, but it is slowly changing.

UV light



high rates of melanoma due to UV exposure

Carbon dioxide



CO₂ concentrations over NZ since 1972

Temperature



over the past 100 years

Global emissions



global greenhouse gas emissions since 1990

AIR

We enjoy good air quality in most places most of the time. Air quality problems can occur, usually in winter in specific locations.

Airborne particles



since 2006, leading to improved air quality

Home heating



human-made airborne particles in 2013 were from burning wood and coal

Transport emissions



carbon monoxide since 2001

FRESH WATER

It is poorer in urban and agricultural areas, and very good in national parks, native bush, and tussock.

Nitrogen on land



since 1990, from livestock and fertiliser

Nitrogen in rivers



since 1989, increasing the likelihood of slime and weeds

Water clarity



improvement since 1989

LAND

Our land has undergone dramatic change since people arrived 700–800 years ago, affecting our biodiversity and land productivity.

Land use



used for farming and forestry in 2012

Erosion



tonnes of eroded soil entering NZ waterways each year affecting water quality and productivity

Soil compaction



soils under dairy farming badly affected by compaction in 2013

Pests



area affected by possums, rats, and stoats in 2014

MARINE

Our marine environment is diverse, but changes are affecting our native seabirds and marine mammals.

Seabirds



threatened with extinction in 2012

Marine mammals



threatened with extinction in 2009

Oceans



coastal sea levels and acidity are rising

Overfishing



overfishing has declined since 2009

For more information visit www.mfe.govt.nz

Biodiversity is a cross-cutting theme

Published in October 2015
MFE 743

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Focus on land environment in this talk

Published in October 2015
MFE 737

Ecological Integrity framework for biodiversity reporting

- “ecological integrity means the full potential of indigenous biotic and abiotic features and natural processes, functioning in sustainable communities, habitats, and landscapes”
 - Environmental Reporting Act 2015

Indigenous
dominance

Species occupancy

Environmental
representation

How does eDNA data fit?

Element

Objective

Relevance of eDNA data

Indigenous
dominance



Maintaining
ecosystem
processes



Data on taxa that
underpin key
ecosystem processes

Species occupancy



Maintaining
ecosystem
composition



Composition data
for taxa not
currently measured

Environmental
representation



Improving
ecosystem
representation



Identification of
communities with
high genetic diversity

3. eDNA data

- what does it look like?



DOC eDNA pilot study



Department of
Conservation
Te Papa Atawhai



Landcare Research
Manaaki Whenua



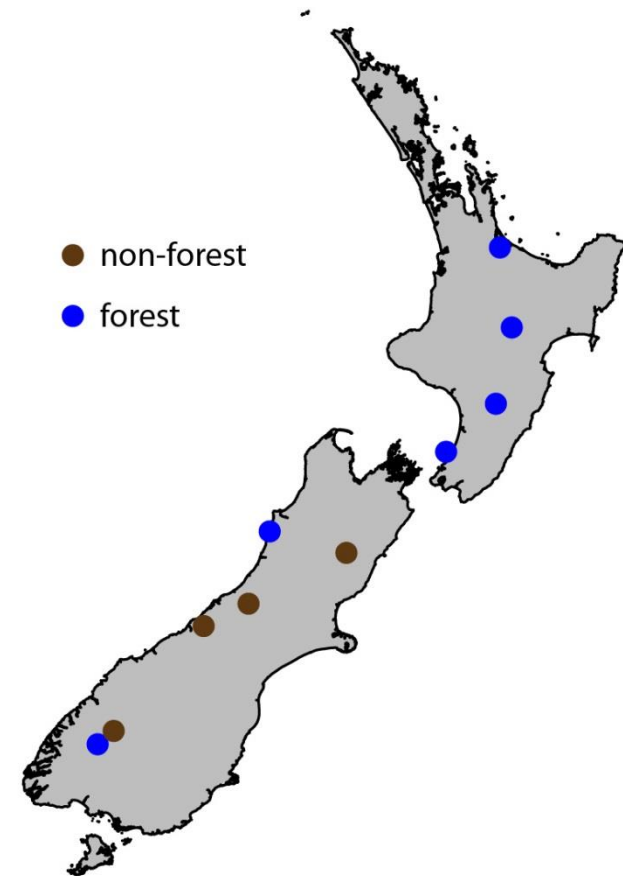
ALLAN WILSON CENTRE
FOR MOLECULAR ECOLOGY AND EVOLUTION



Bio-Protection
Bioprotection science for New Zealand

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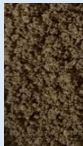
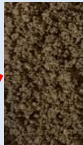
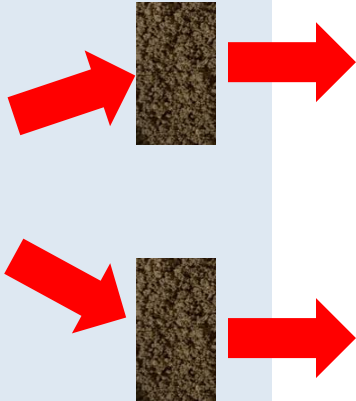


A comparison of two approaches

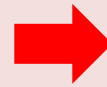
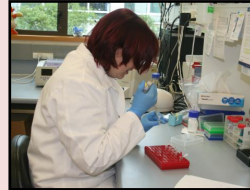
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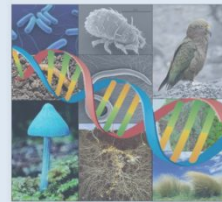
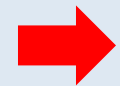
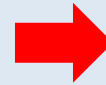
DOC & Landcare



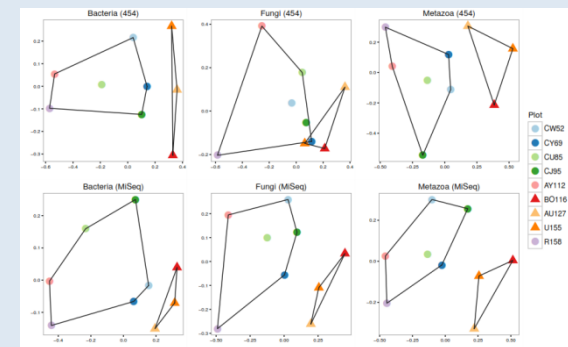
Allan Wilson Centre



Landcare



Landcare



Our questions

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Is it practical to collect eDNA data from Tier 1 plots?

What sort of data do we get?

Are there patterns between forest and non-forest plots?

Are the results from the two very different approaches consistent?



Results (eDNA)

- DNA data – Alan Wilson centre (Illumina MiSeq)
 - ~12,000 unique OTUs
 - ~ 1.2 million sequences
- DNA data – Landcare (454)
 - ~5,500 unique OTUs
 - ~ 0.4 million sequences

**What is
an OTU?**

Operational Taxonomic Unit (OTU)

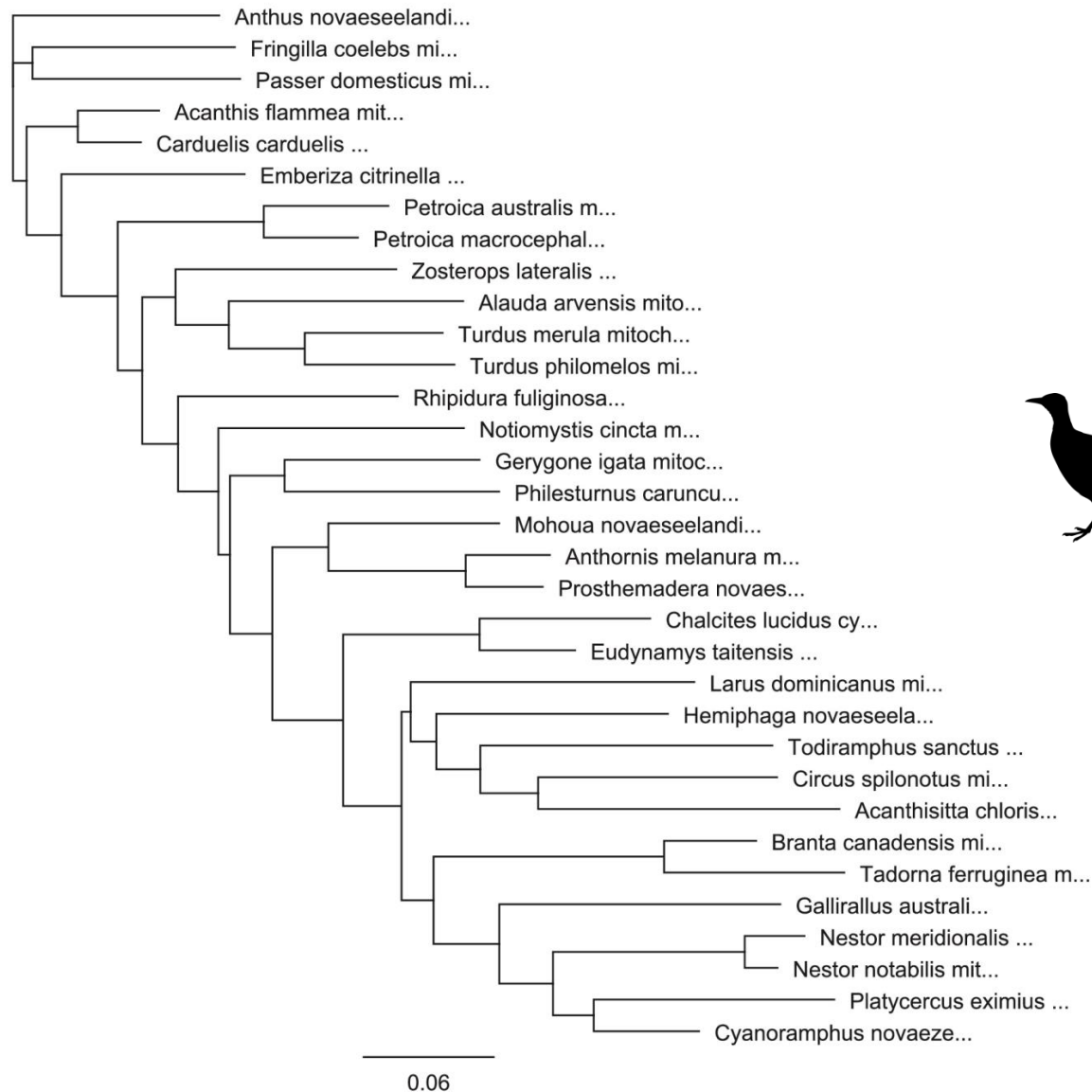
- Groups of similar sequences
- Typically clustered with 97% similarity
- ~ DNA equivalent of a species



Results (Conventional data)

- Conventional field data from same plots
 - 33 bird species
 - 352 plant species in total; 311 native, 41 exotic

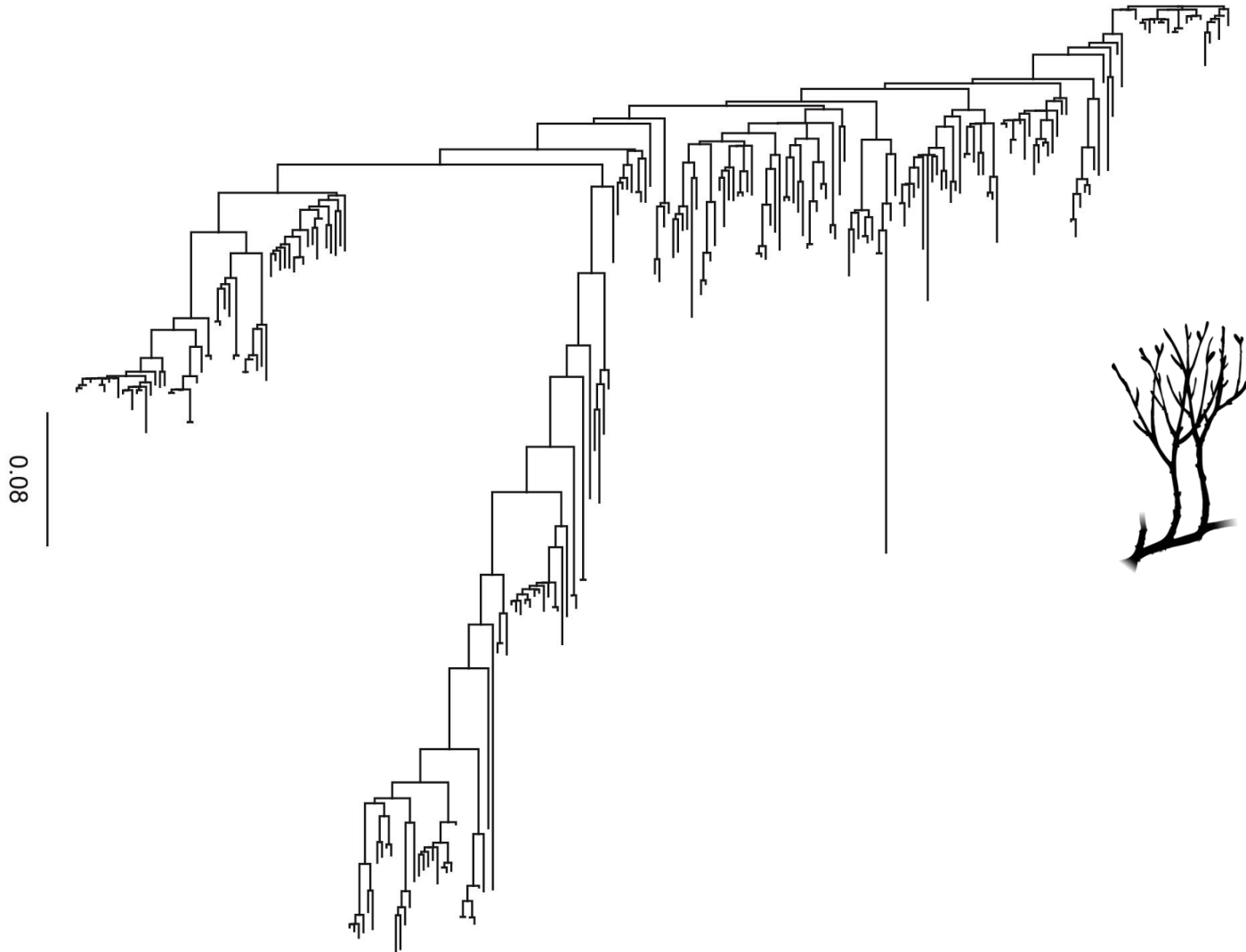
Taxonomic scope of the data - birds



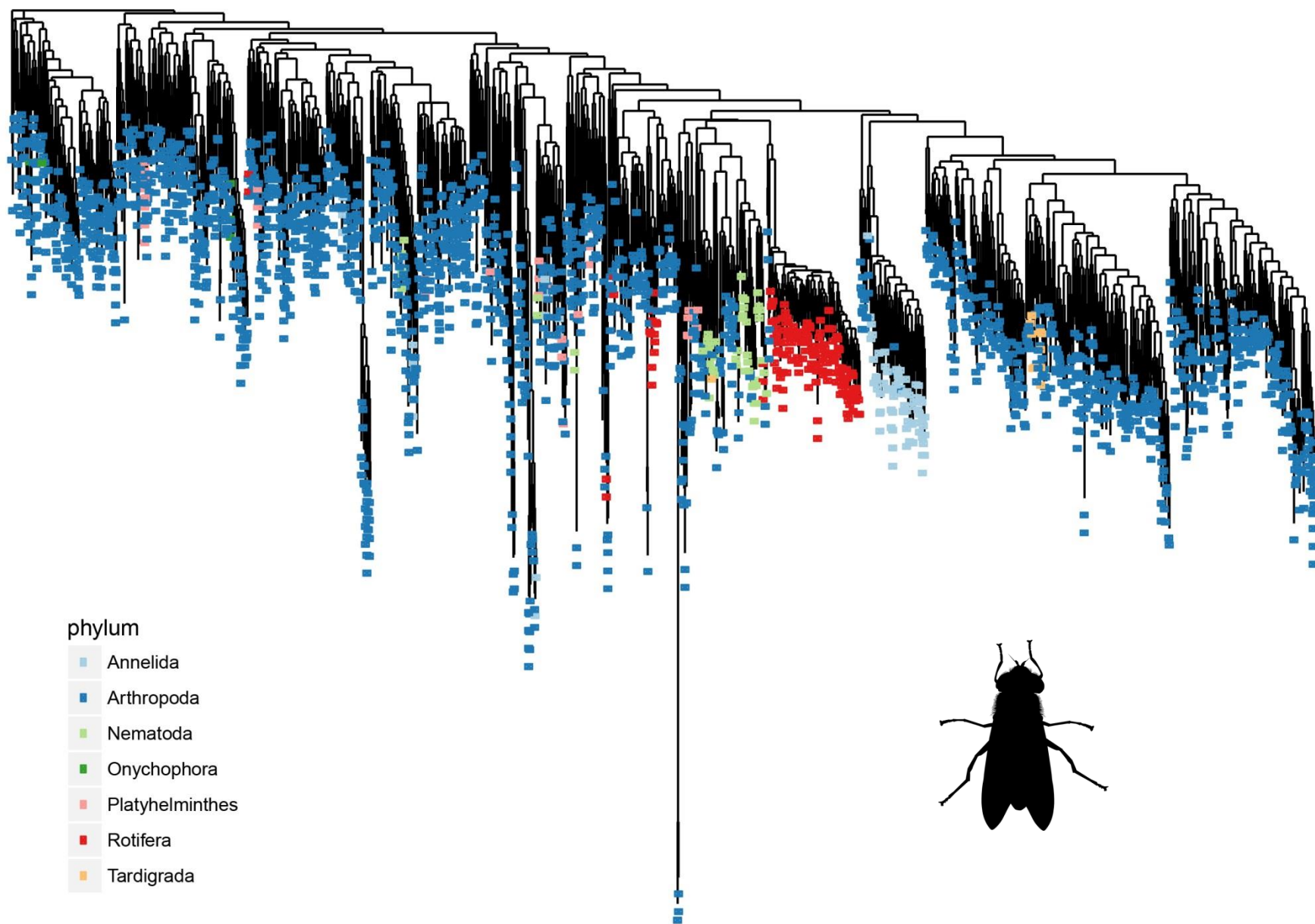
Plants (field data)

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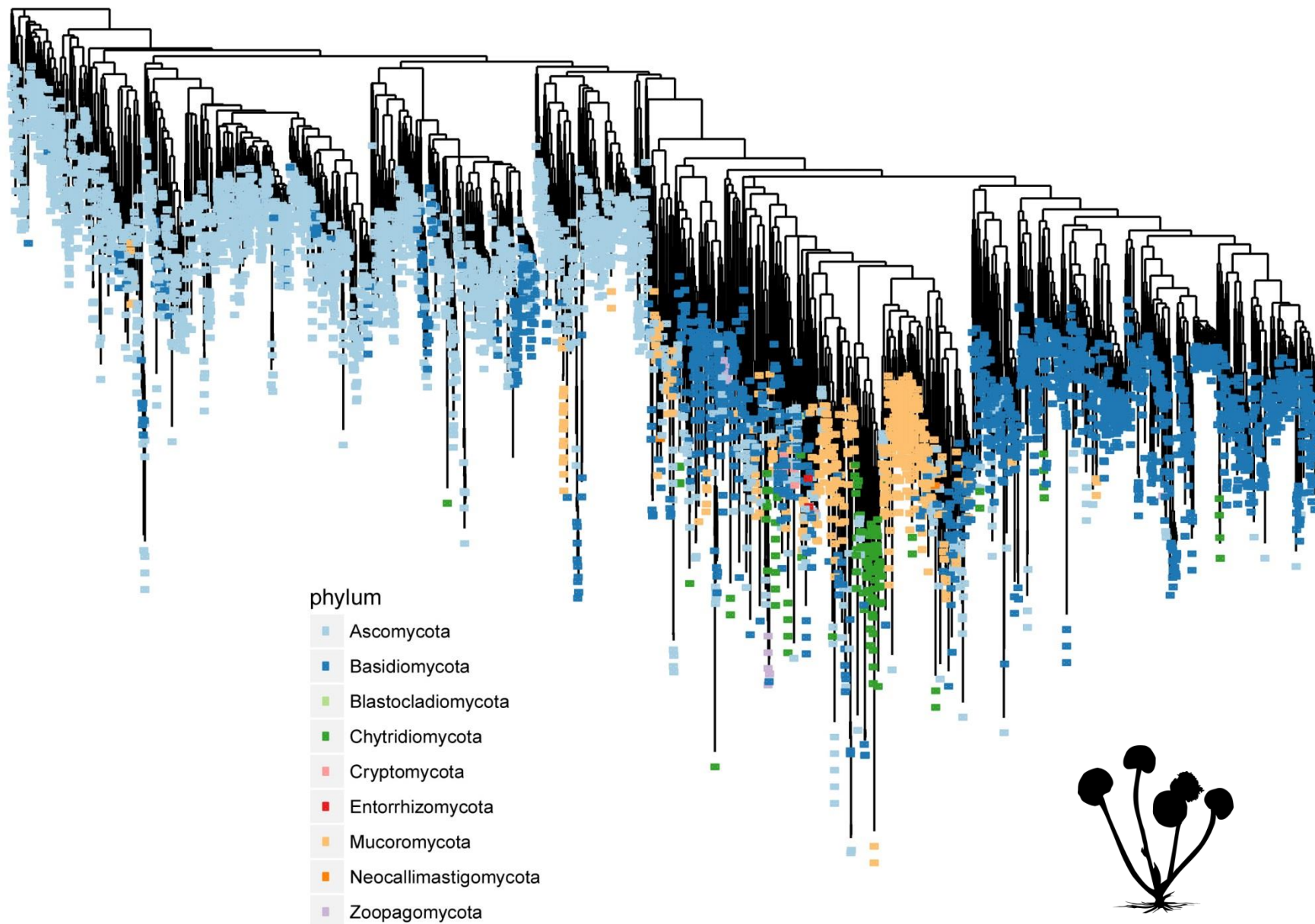
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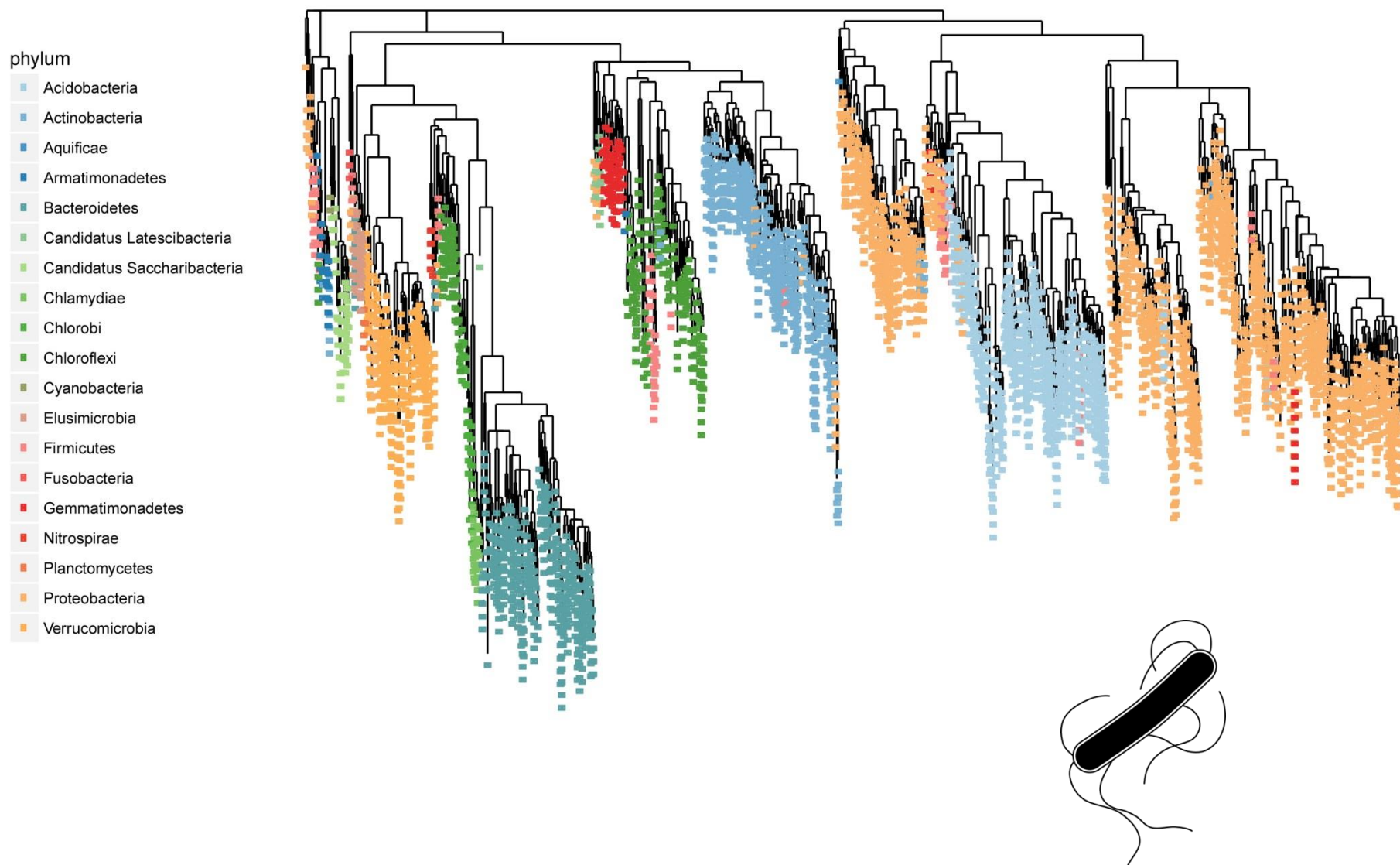
Animals (eDNA data)



Fungi (eDNA data)



Bacteria (eDNA data)



Results – relative richness

Birds



Plants



Bacteria



Protists



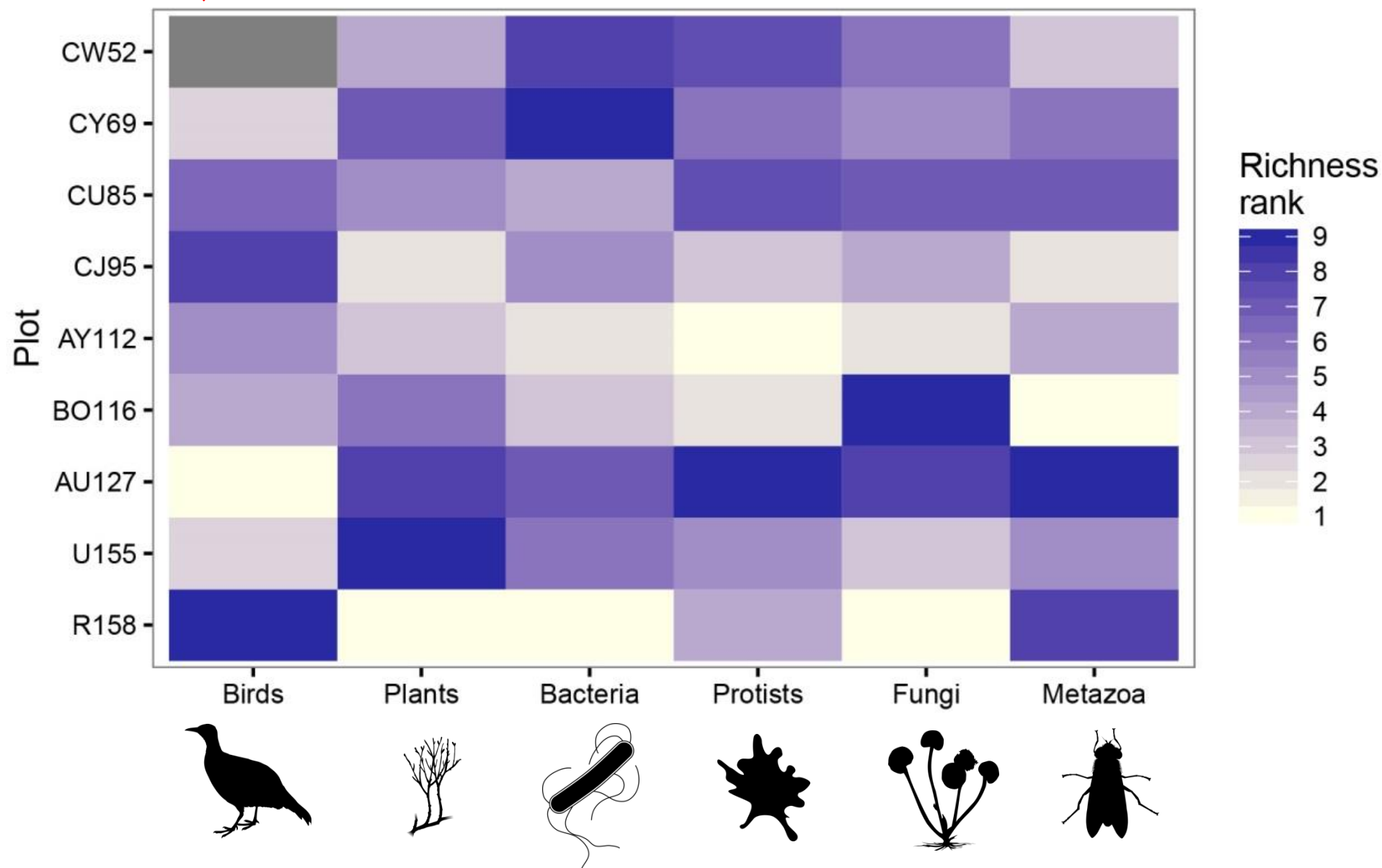
Fungi



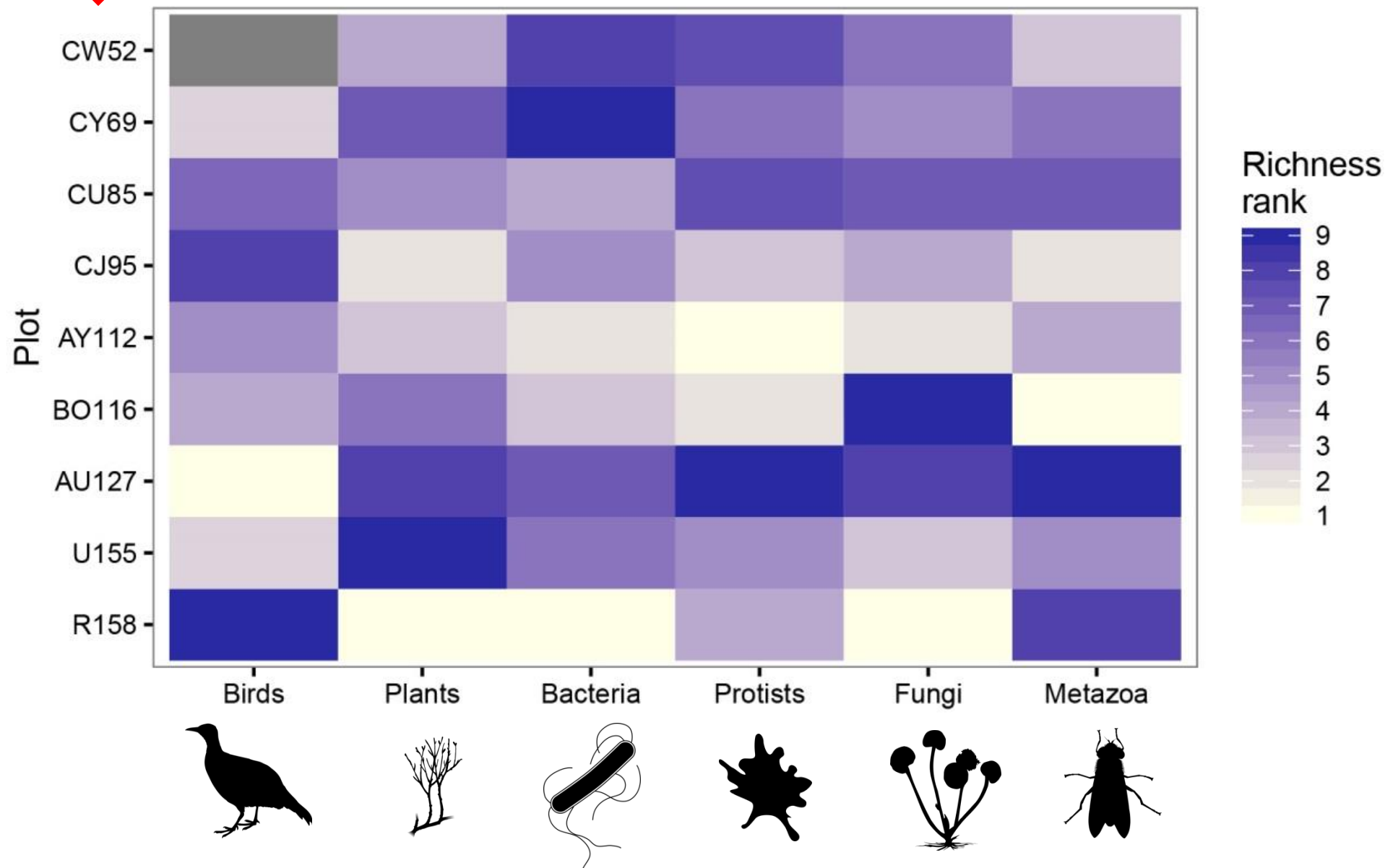
Metazoa



Results – relative richness

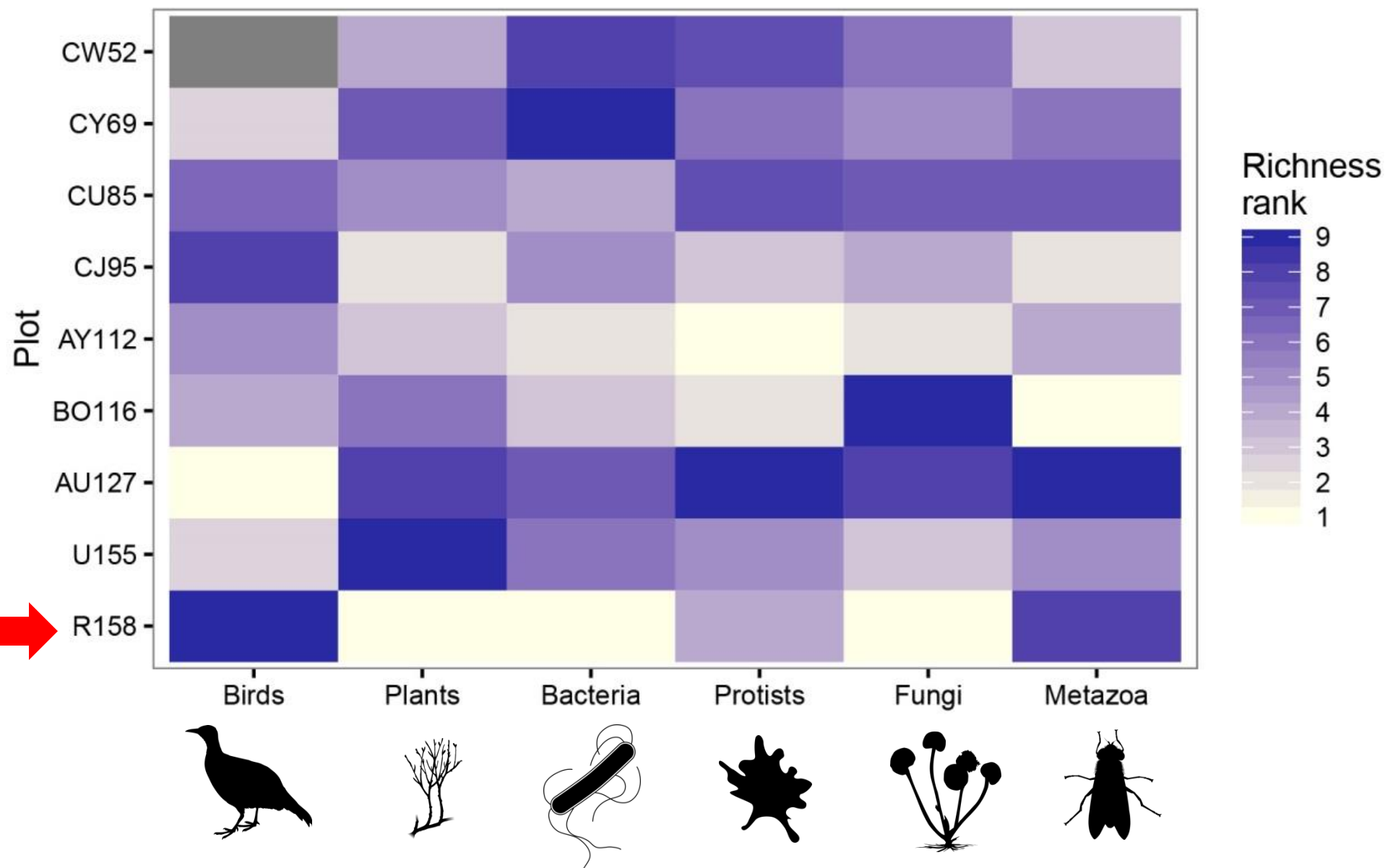


Results – relative richness





Results – relative richness

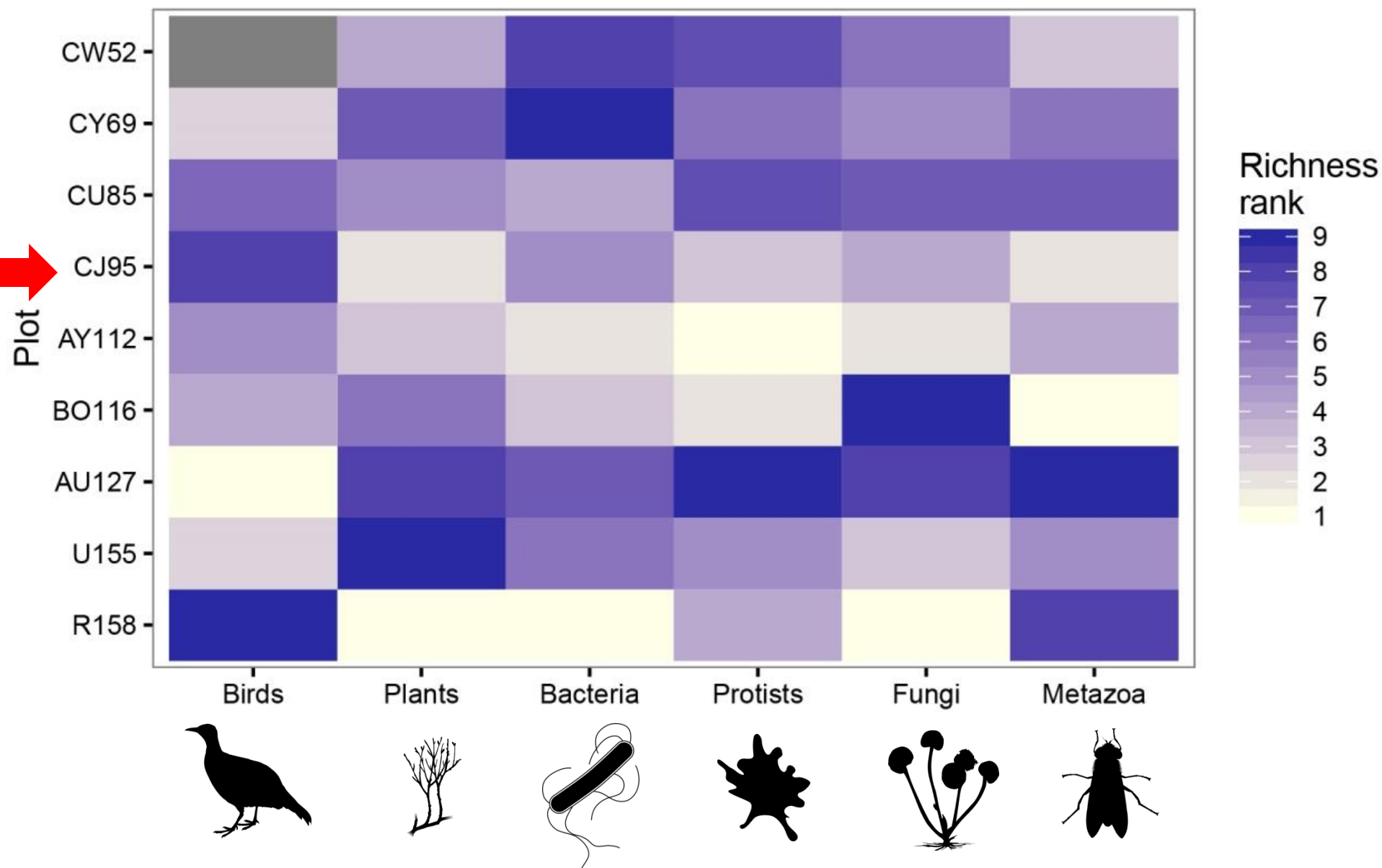






R158

Results – relative richness

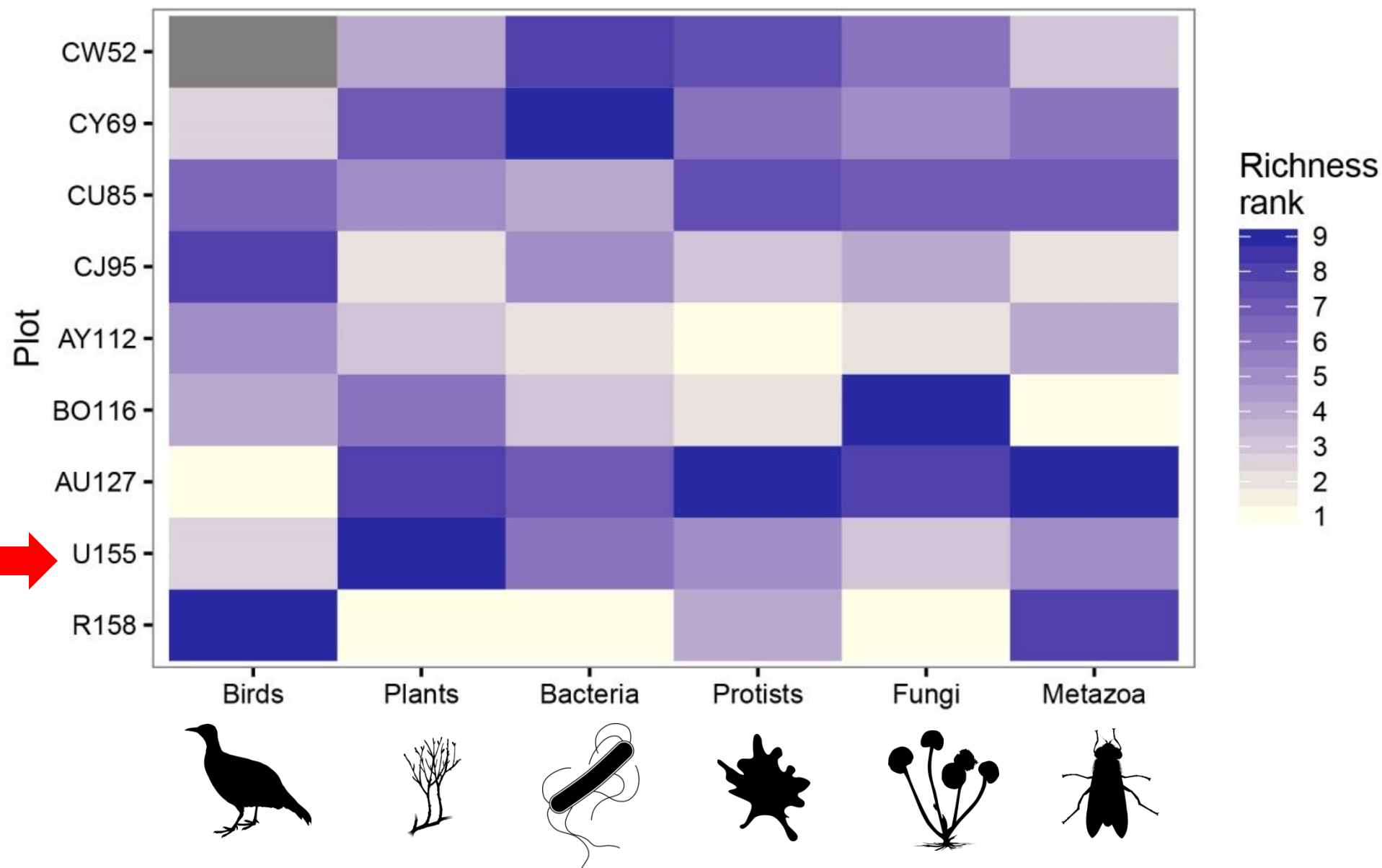


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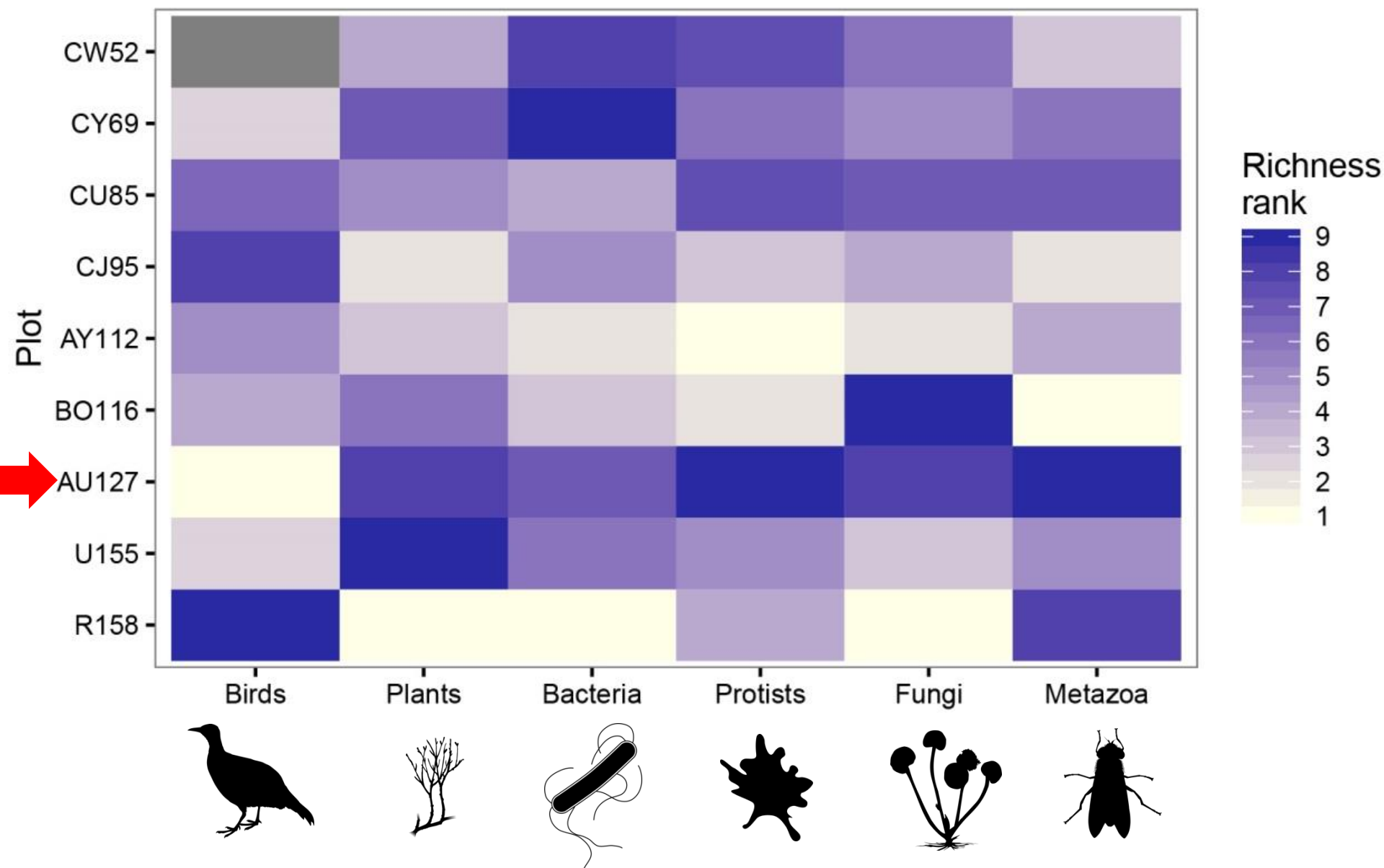


Results – relative richness



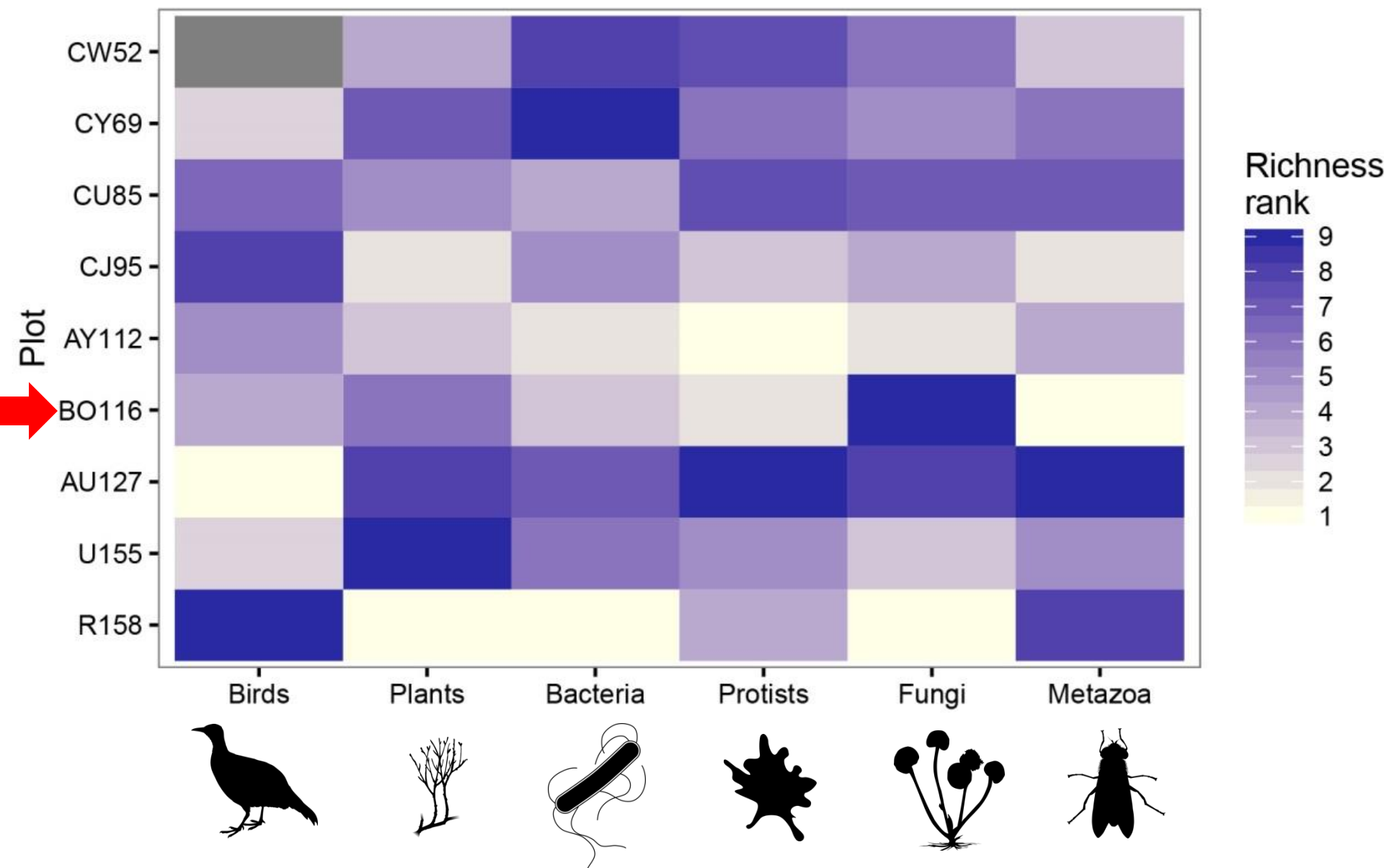


Results – relative richness



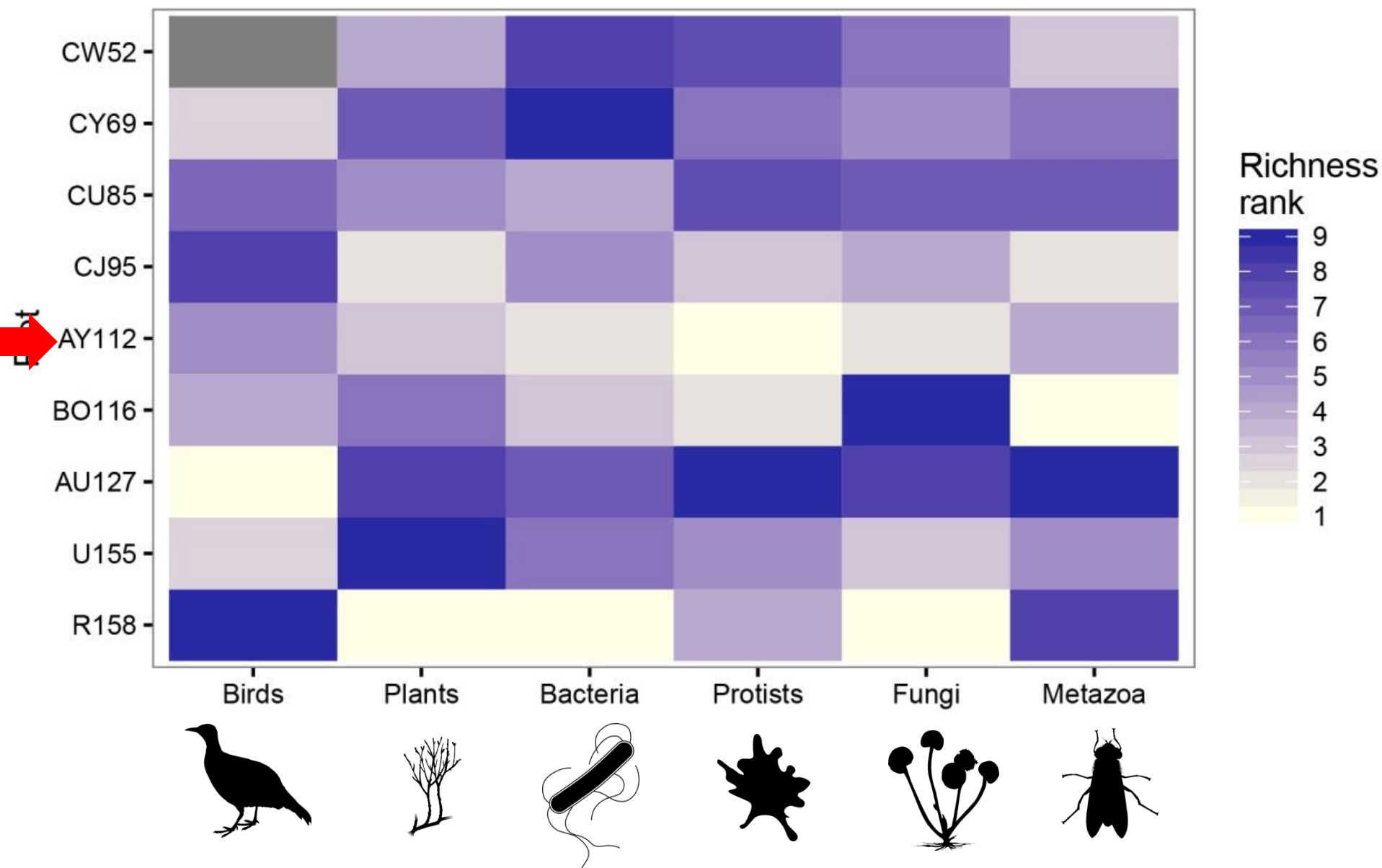


Results – relative richness



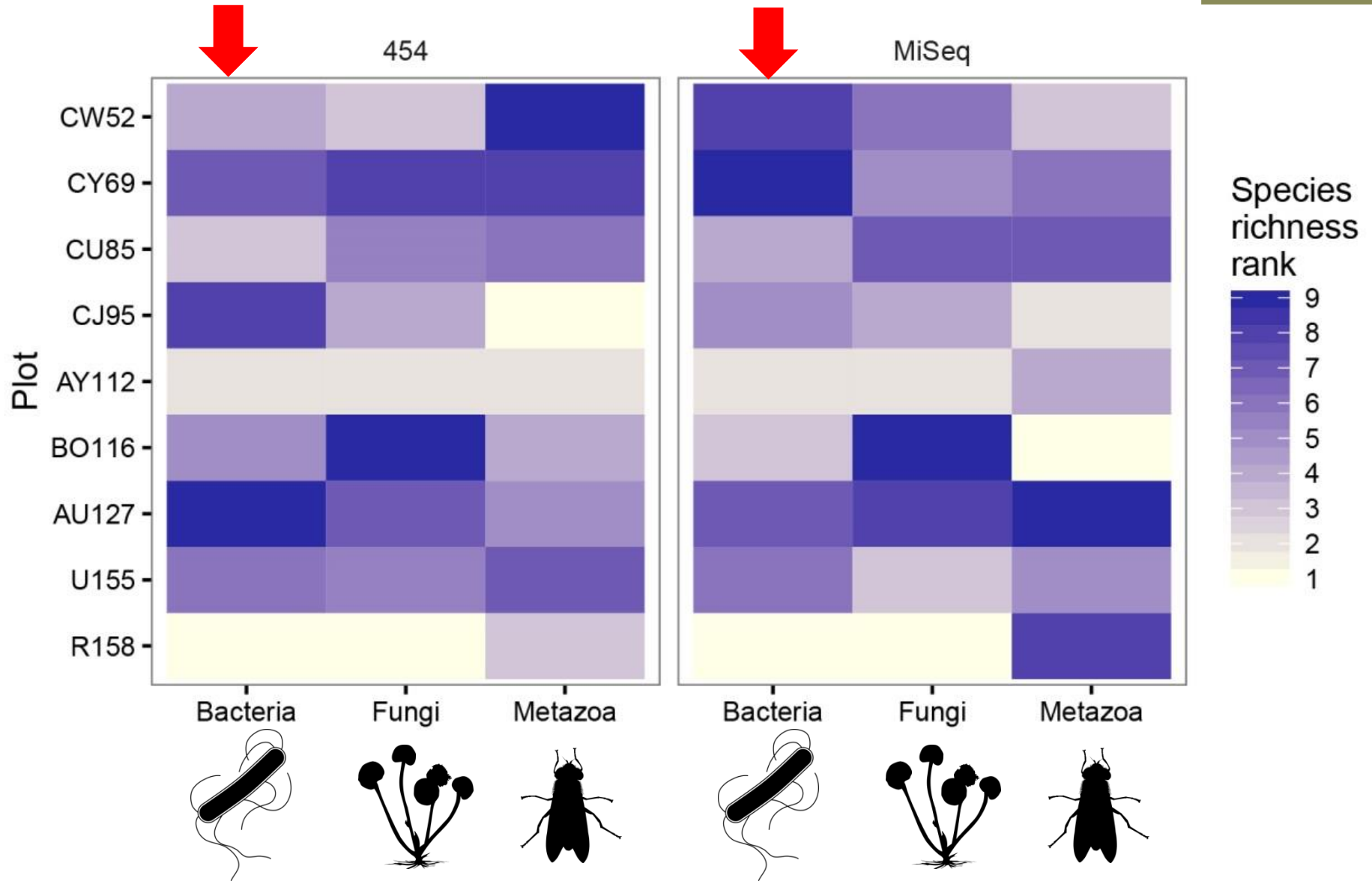


Results – relative richness

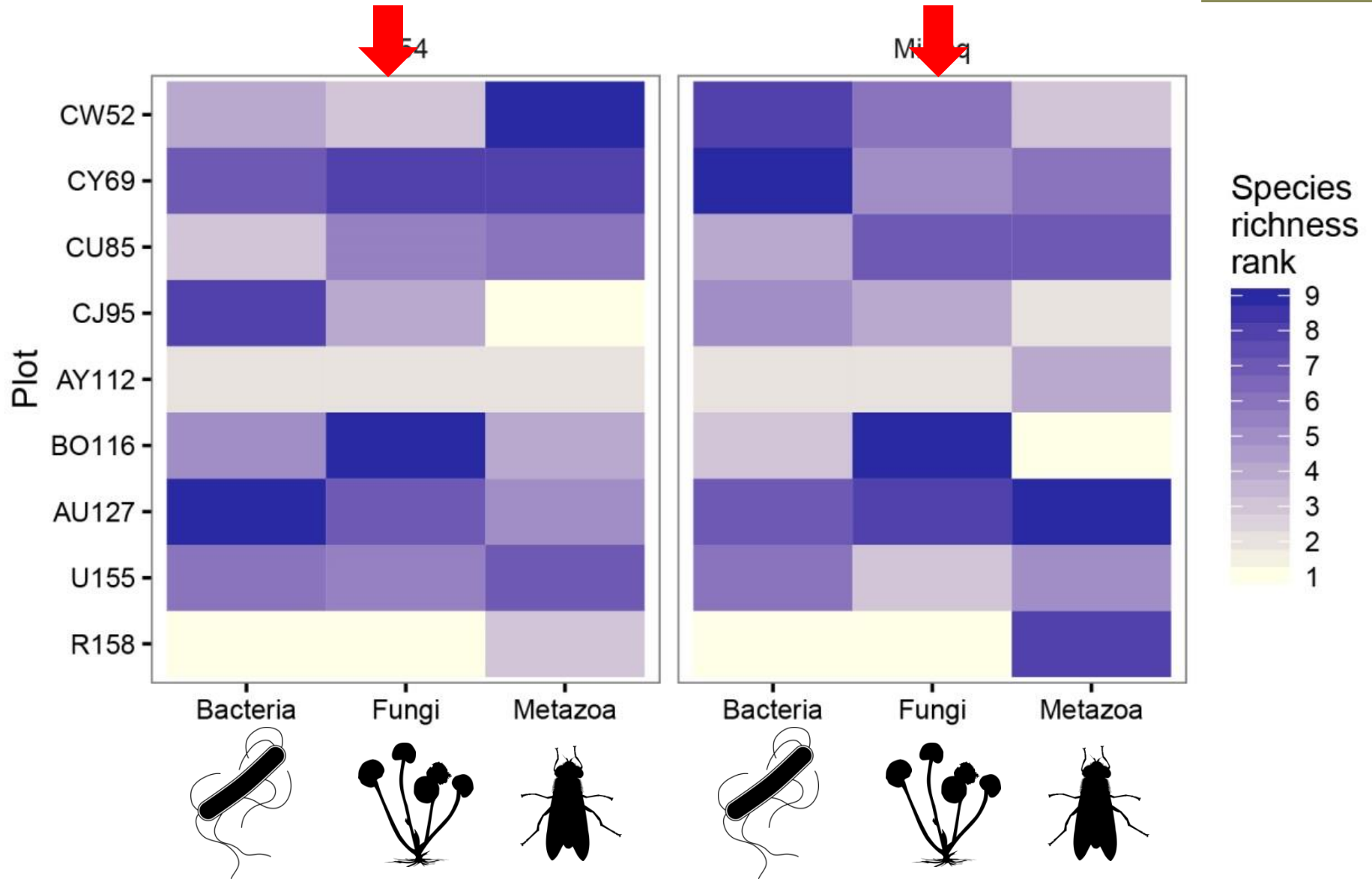




Comparison of two eDNA approaches (richness)



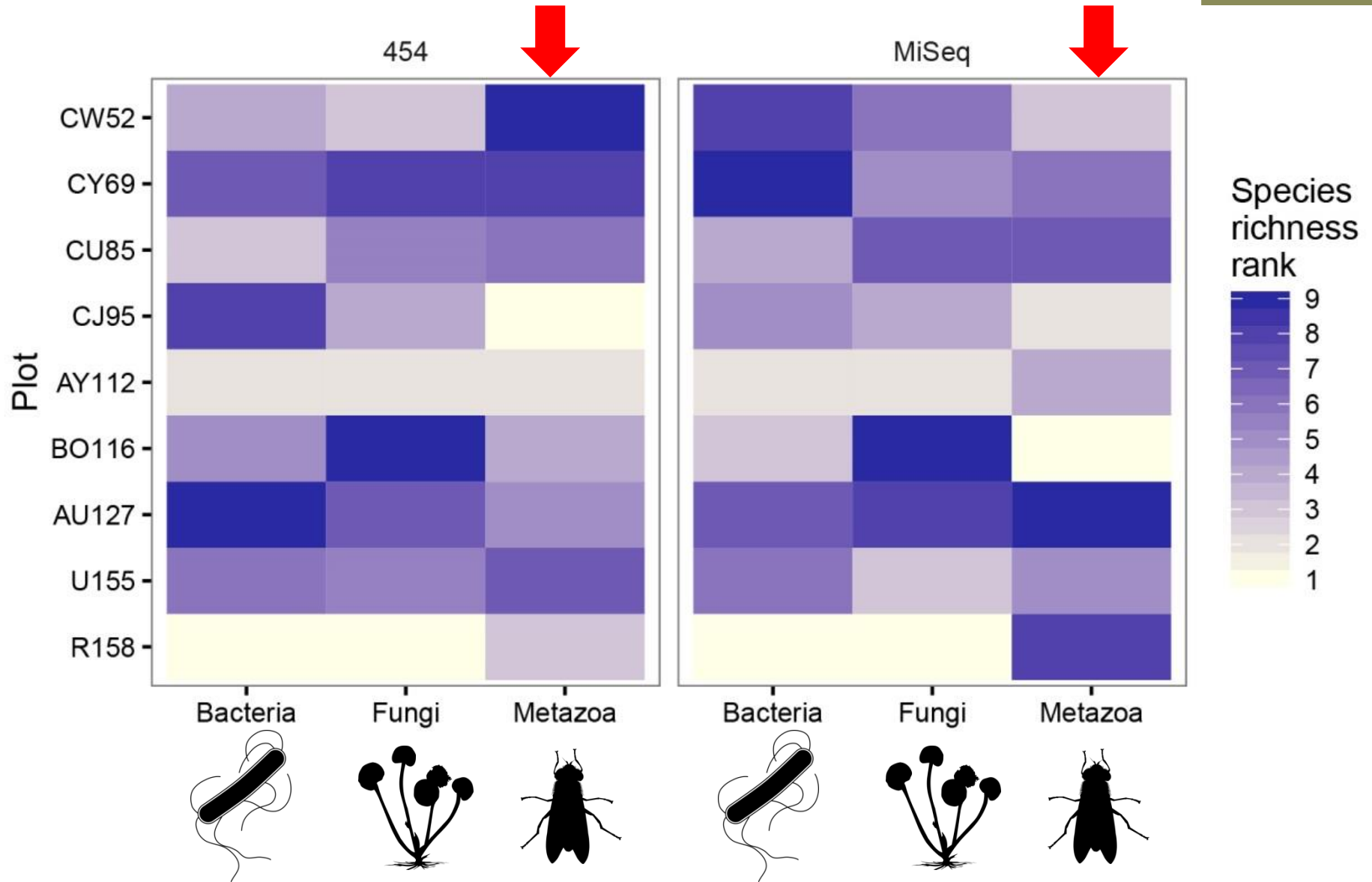
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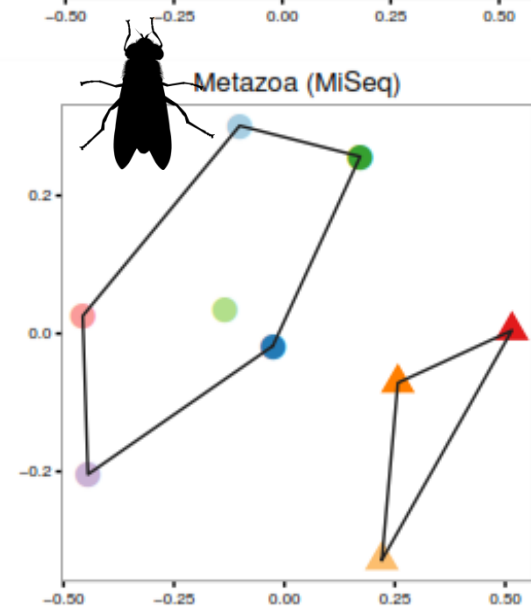
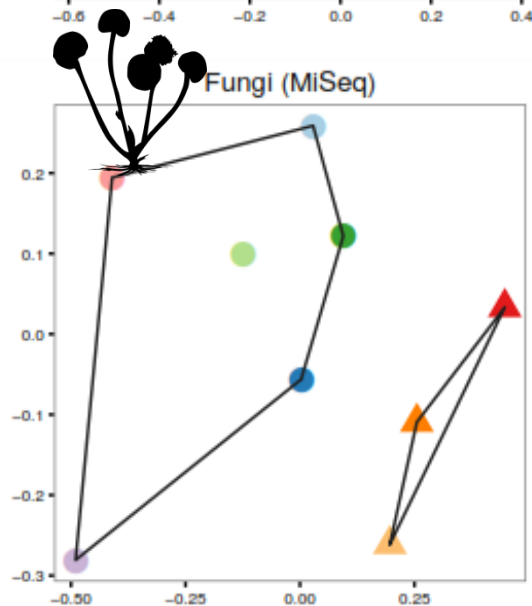
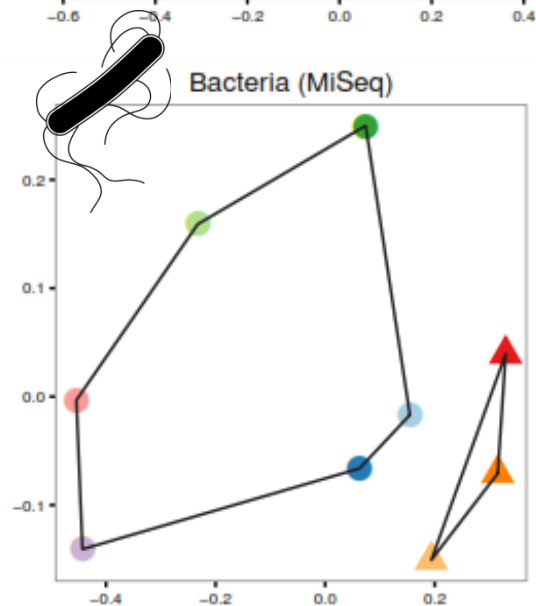
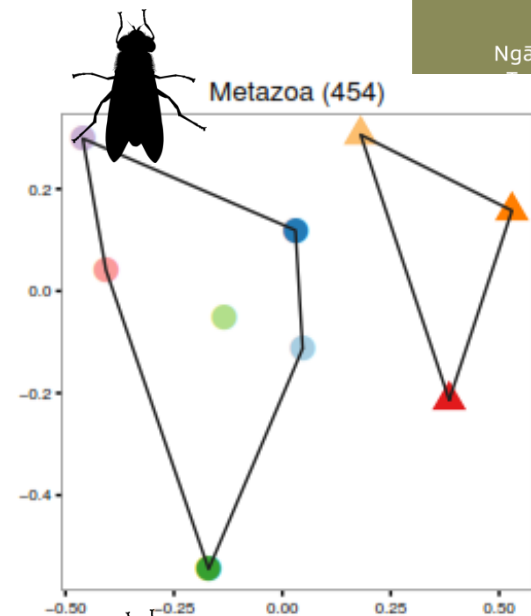
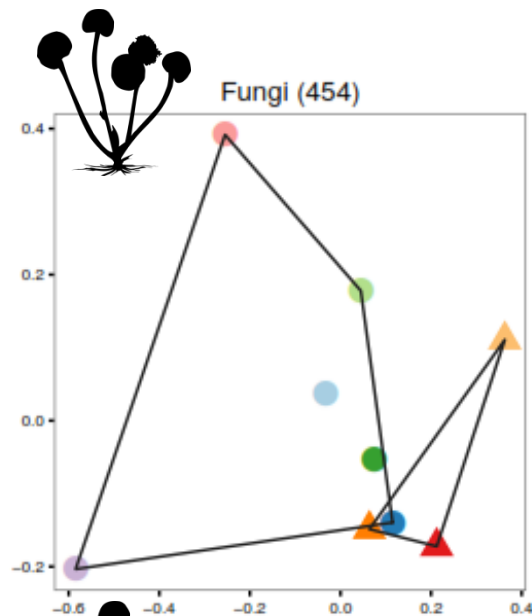
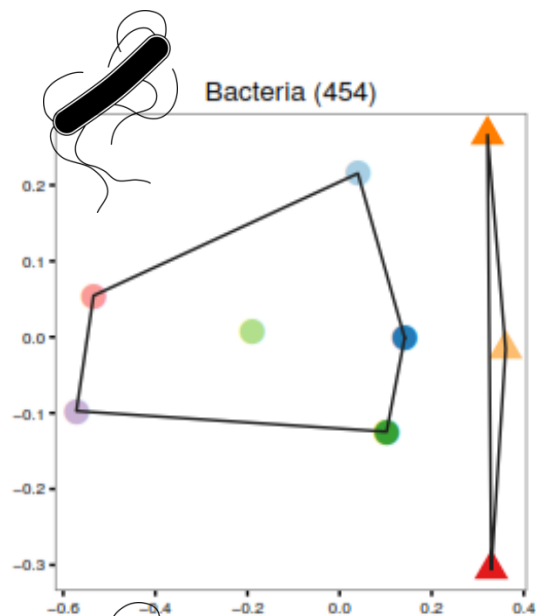
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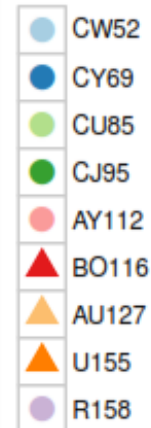
Composition comparison

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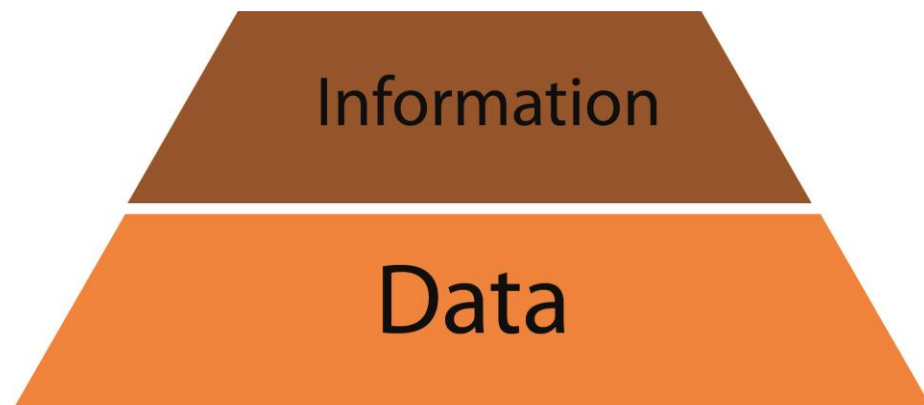
Plot



Conclusions from T1 eDNA pilot

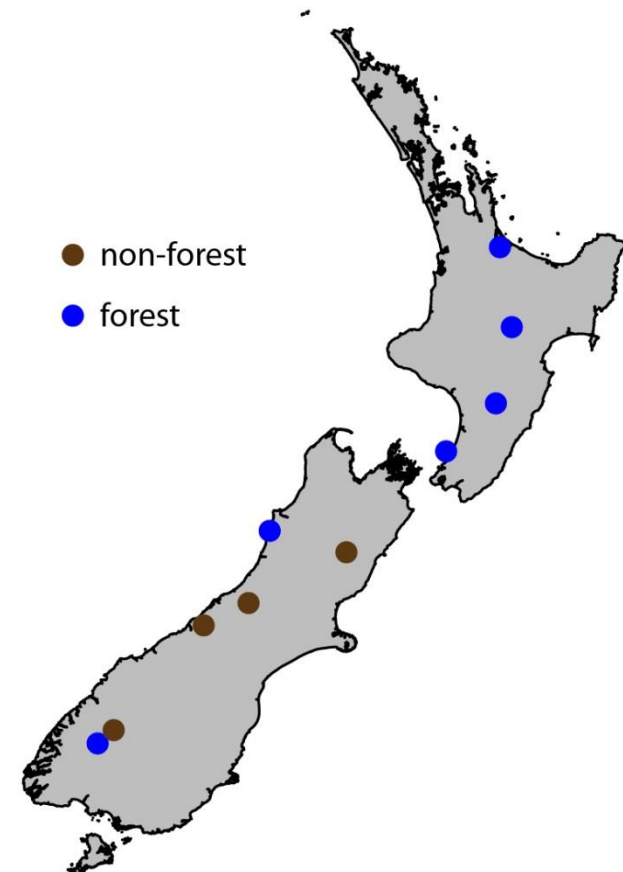
- Is it practical to collect eDNA data from Tier 1 plots? **YES**
- What sort of data does the method generate? **LOTS**
- Are there patterns between forest and non-forest plots? **YES**
- Are the results from the two very different approaches consistent? **Somewhat**

4. Field validation of eDNA data



Field data vs DNA data

- Plants as test-case
- Initially trialed sampling from soil cores but results were poor
 - Sampling not optimal for plants
 - Low sequence count
 - Poor reference data

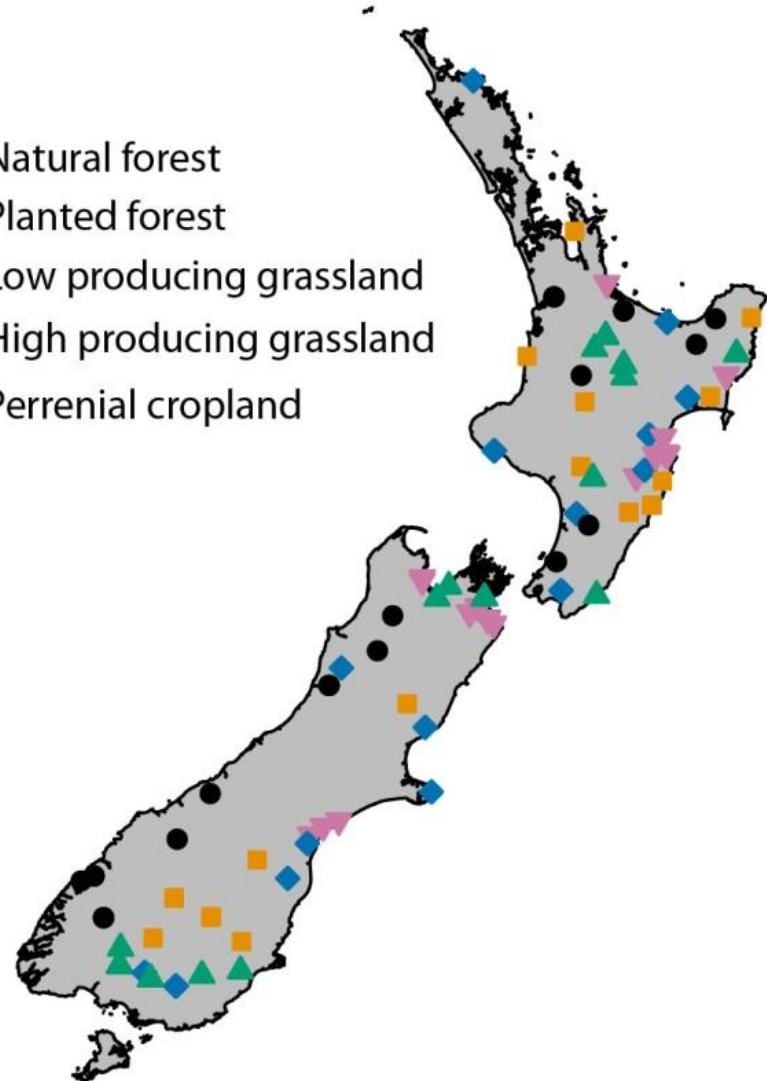


Plant field data vs DNA data

Phase 2 NGBA project

- MBIE Smart Idea
- 125 locations sampled 2015/2016
- Uses 8km grid
- 5 different land uses
- Updated primers and sampling method for plants

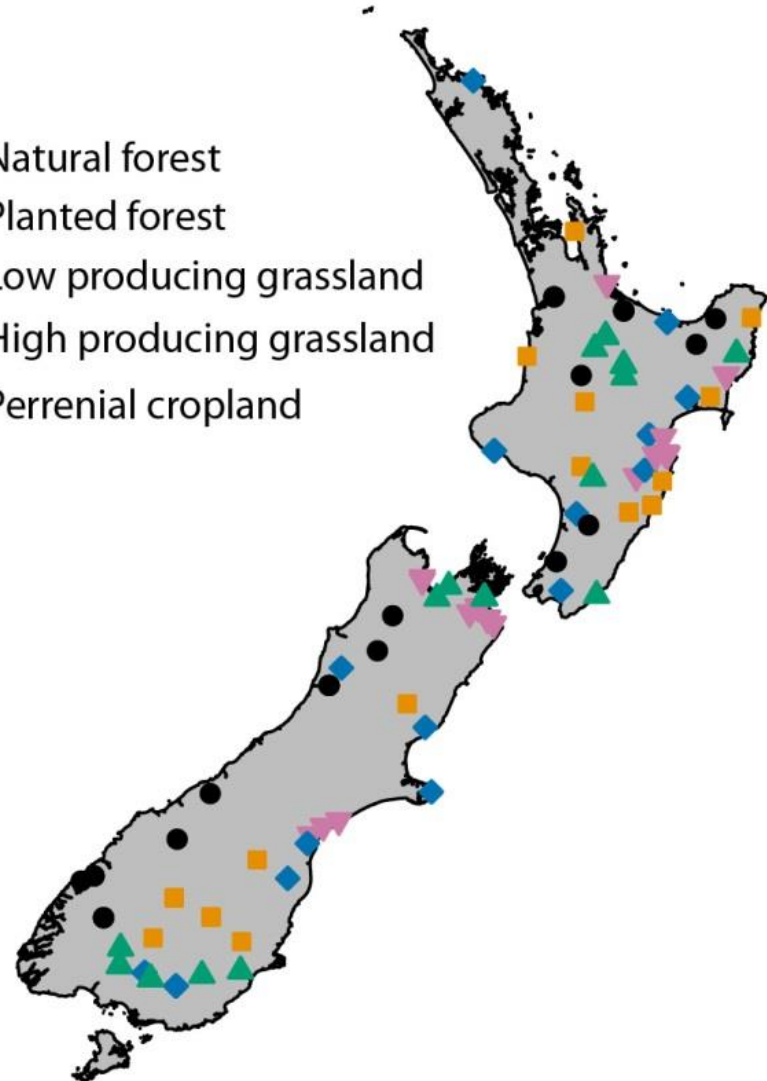
- Natural forest
- ▲ Planted forest
- Low producing grassland
- ◆ High producing grassland
- ▼ Perennial cropland



Plant field data vs DNA data RESULTS

- Field data
 - 671 species in total;
406 native, 265 exotic
- DNA data
 - 787 unique OTUs
 - ~ 1.8 million sequences

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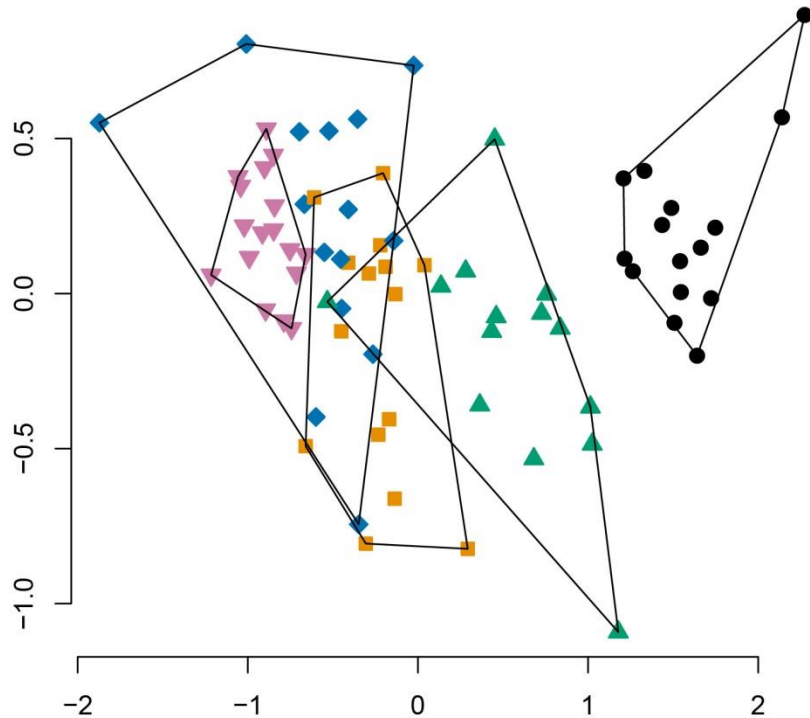


Community composition comparison

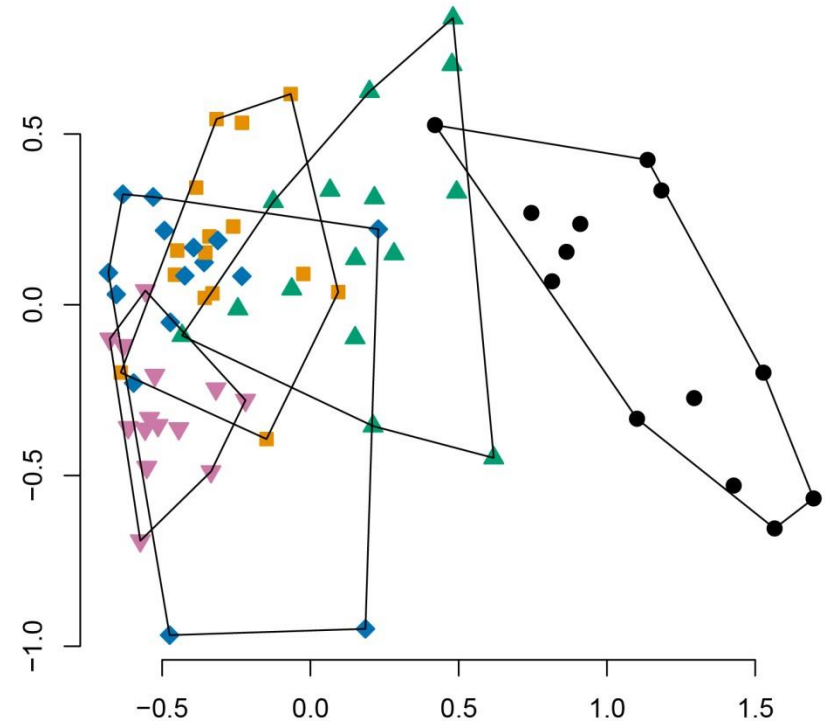
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Field data



DNA data



● Natural forest

■ Low producing grassland

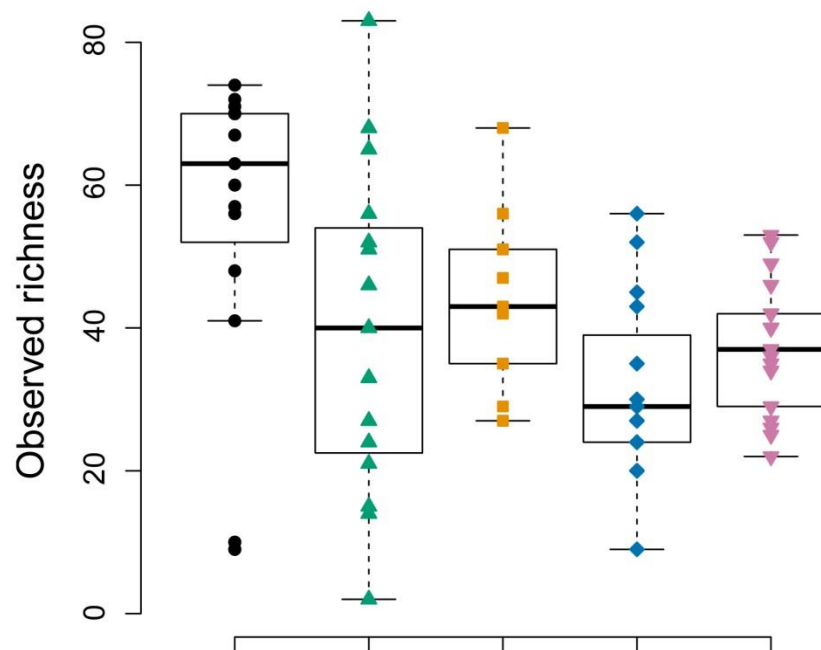
▼ Perennial cropland

▲ Planted forest

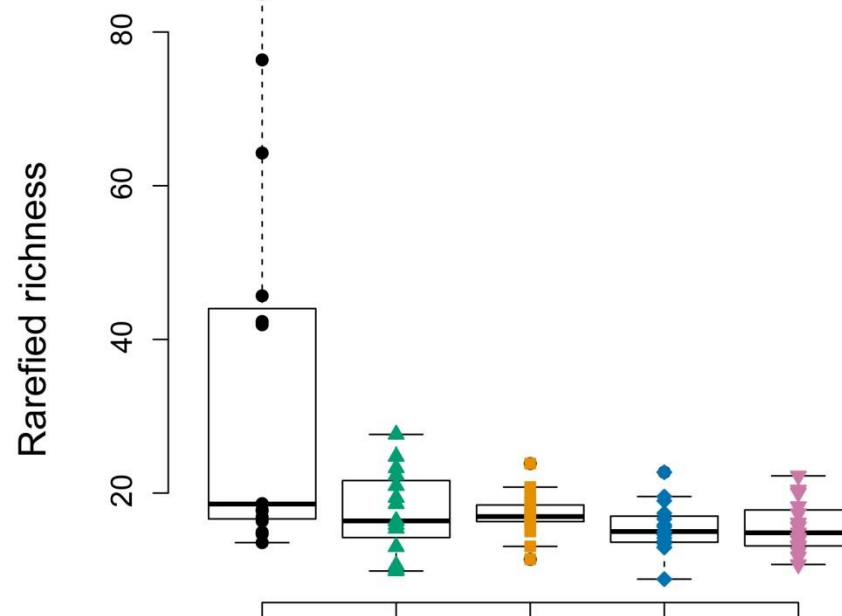
◆ High producing grassland

Relative richness comparison

Field data – Observed richness



DNA data – rarefied richness



● Natural forest

▲ Planted forest

■ Low producing grassland

◆ High producing grassland

▼ Perennial cropland

Species identification

How many of the field-identified species do we detect on the same plot using eDNA?

	Species	Genus	Family
Raw match	26%	53%	62%

But how good is our reference data?

Species level

- 412 species (64%) have 1 or more sequences
- 200 species (36%) have none.

Genus level

- 279 (92%) have genus matches
- 25 genera do not

Species identification

How many of the field-identified species do we detect on the same plot using eDNA?

	Species	Genus	Family
Raw match	26%	53%	62%
Species with reference data	37%	64%	77%

Detection issues? Long tail of rare species



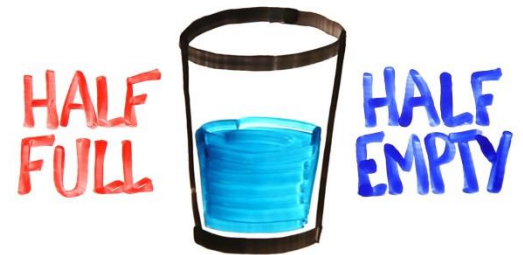
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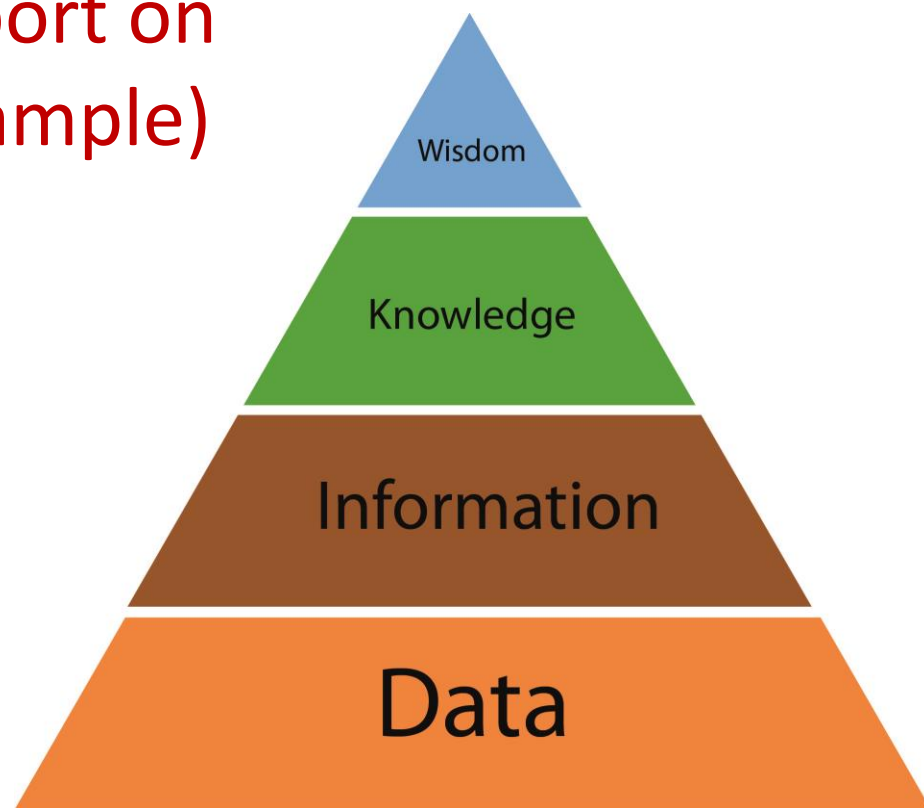
	Species	Genus	Family
Raw match	26%	53%	62%
Species with reference data	37%	64%	77%
With reference data and abundant	47%	78%	91%

What does this tell us about the data?

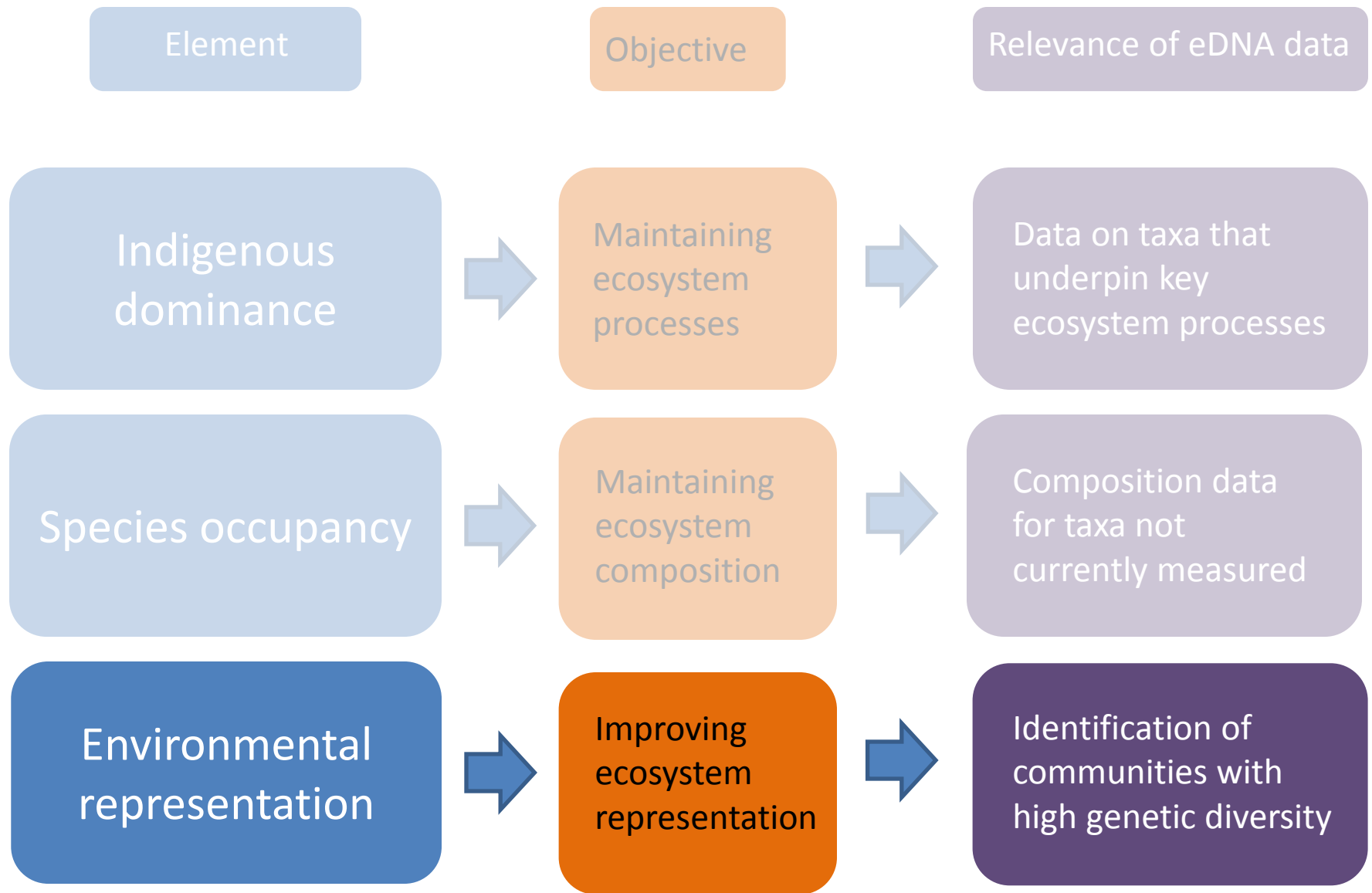
- eDNA identifies similar compositional patterns but richness not so well
- Species level matches can occur but are not the norm
 - Treat name assignments with caution!
- Plant data are a difficult test-case
 - High field data standards
 - Funky genomes
- More accurate matches at higher taxonomic levels
 - these are more meaningful for other diverse taxa



5. Using eDNA to report on genetic diversity (example)



Genetic diversity in a reporting context



(Phylo)genetic diversity as a potential indicator

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A measure of evolutionary relatedness

Community A



Community (B) has higher genetic diversity compared with community (A)

Community B



Community (B) has greater evolutionary potential



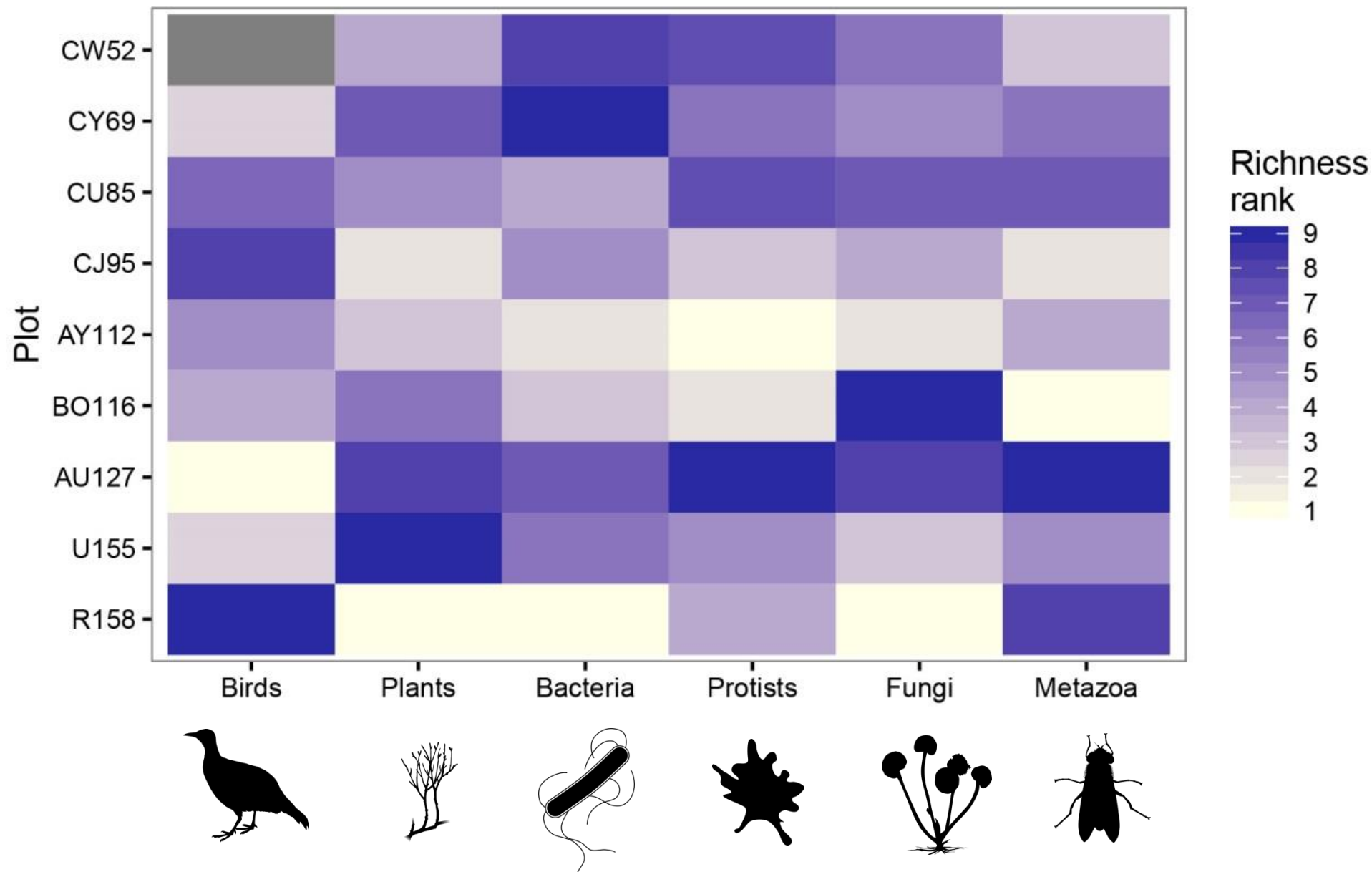
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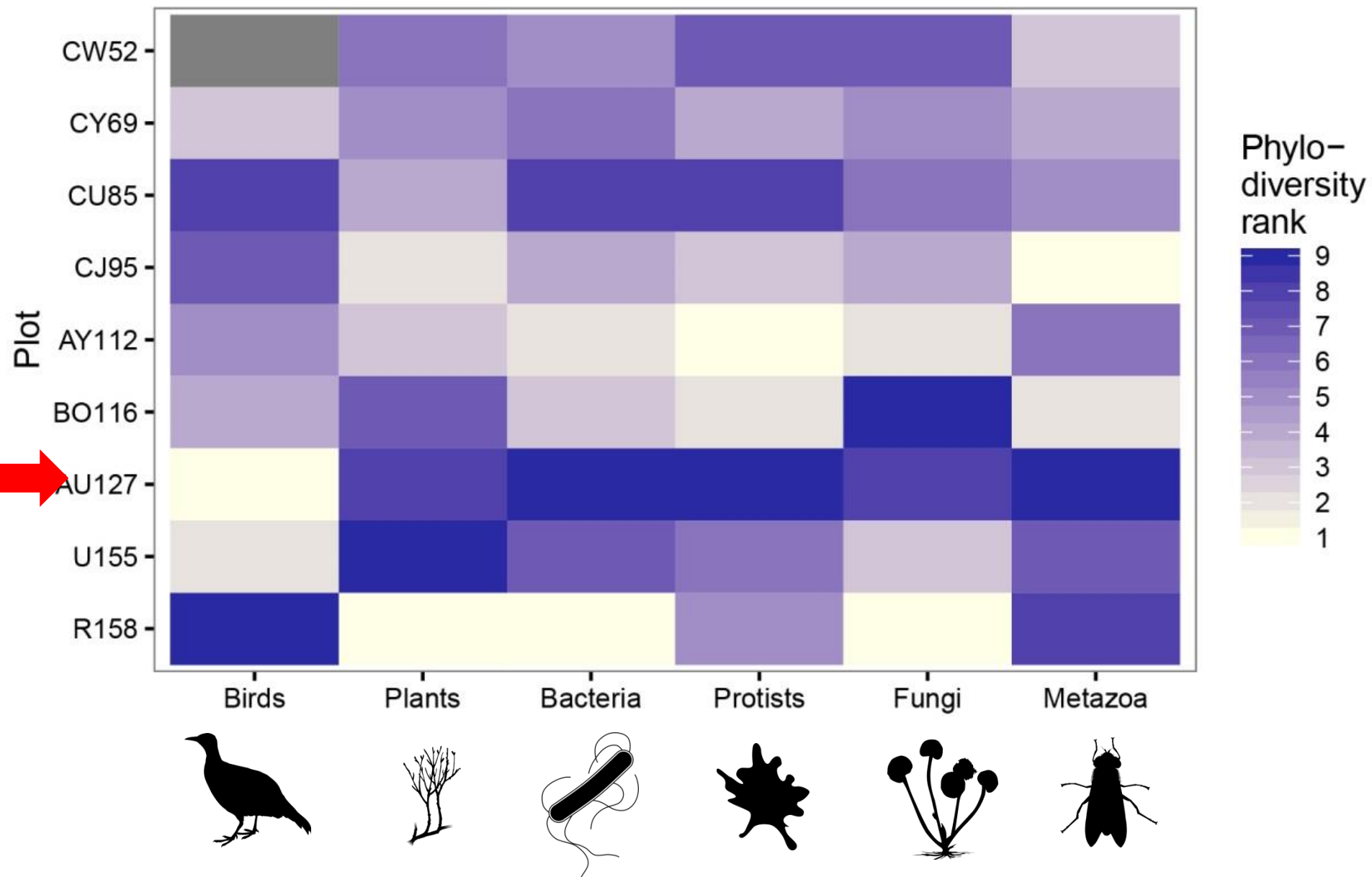
Strengths of phylogenetic indicators

- Robust to taxonomic (name) uncertainty and change
- Capture information about the entire community
- Can be calculated directly from eDNA data

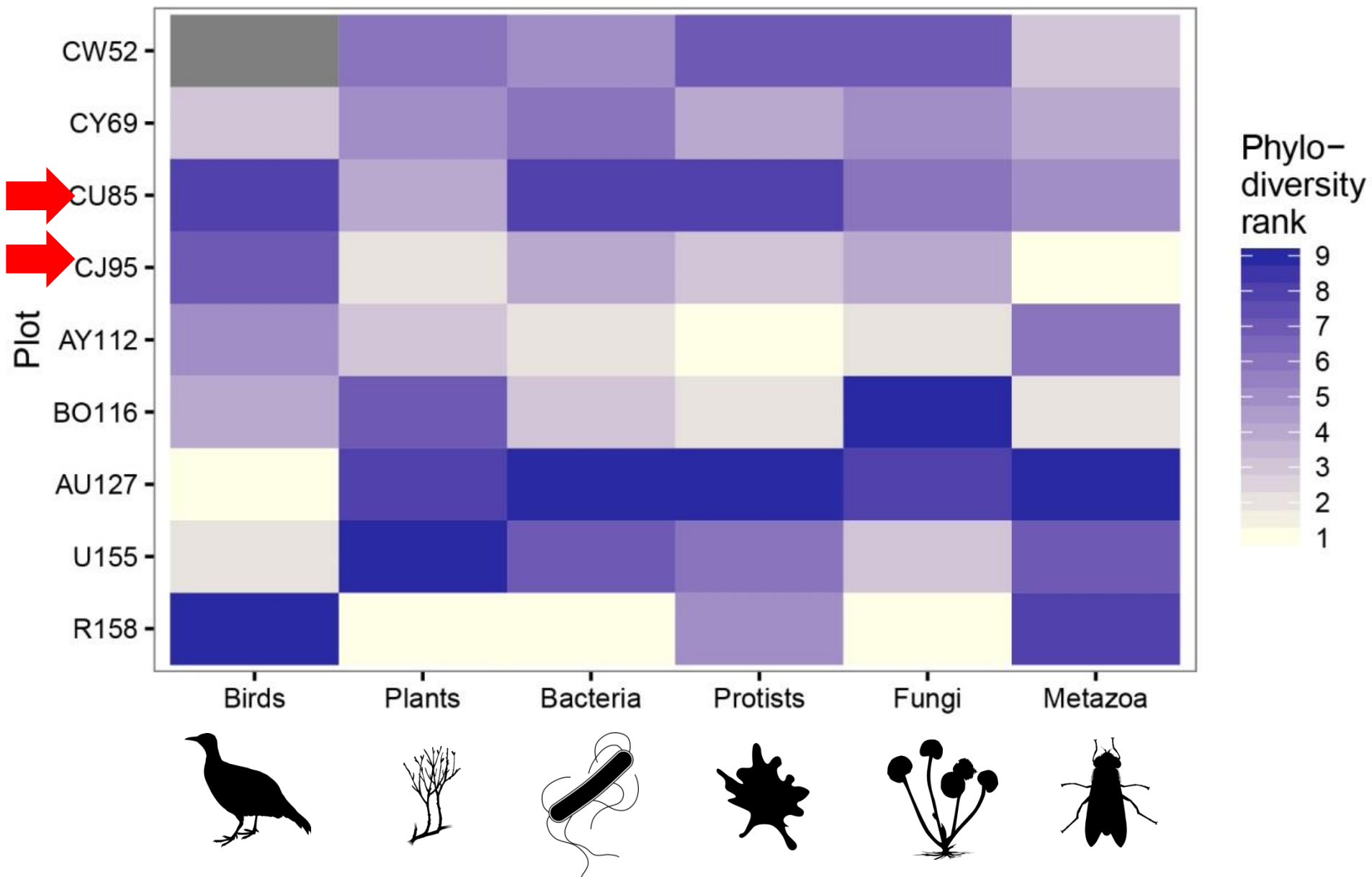
Results – relative richness



Results – Phylogenetic diversity



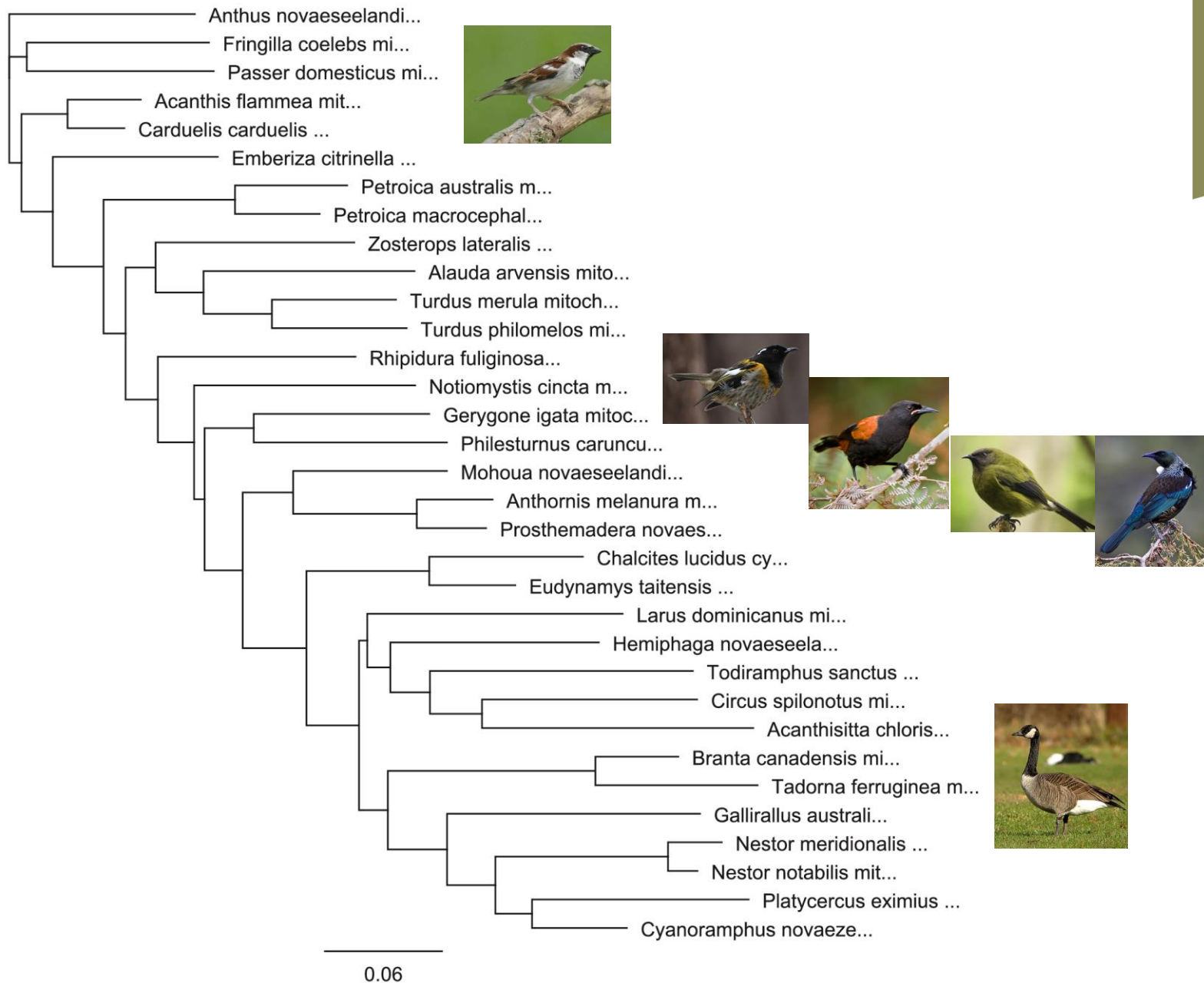
Results – Phylogenetic diversity





Which has the highest Phylogenetic diversity?





But what about endemism?

- Species found in a small number of locations are a greater conservation priority than widespread species
- Areas of endemism are where components of biodiversity with restricted ranges are concentrated
- Can quantify this within our dataset using **phylogenetic endemism** - Rosauer et al. 2009
- Calculation combines presence/absence (range size) data with data on phylogenetic relatedness
- Can be calculated using OTUs – you do not need to have a name or know if a species is native or exotic



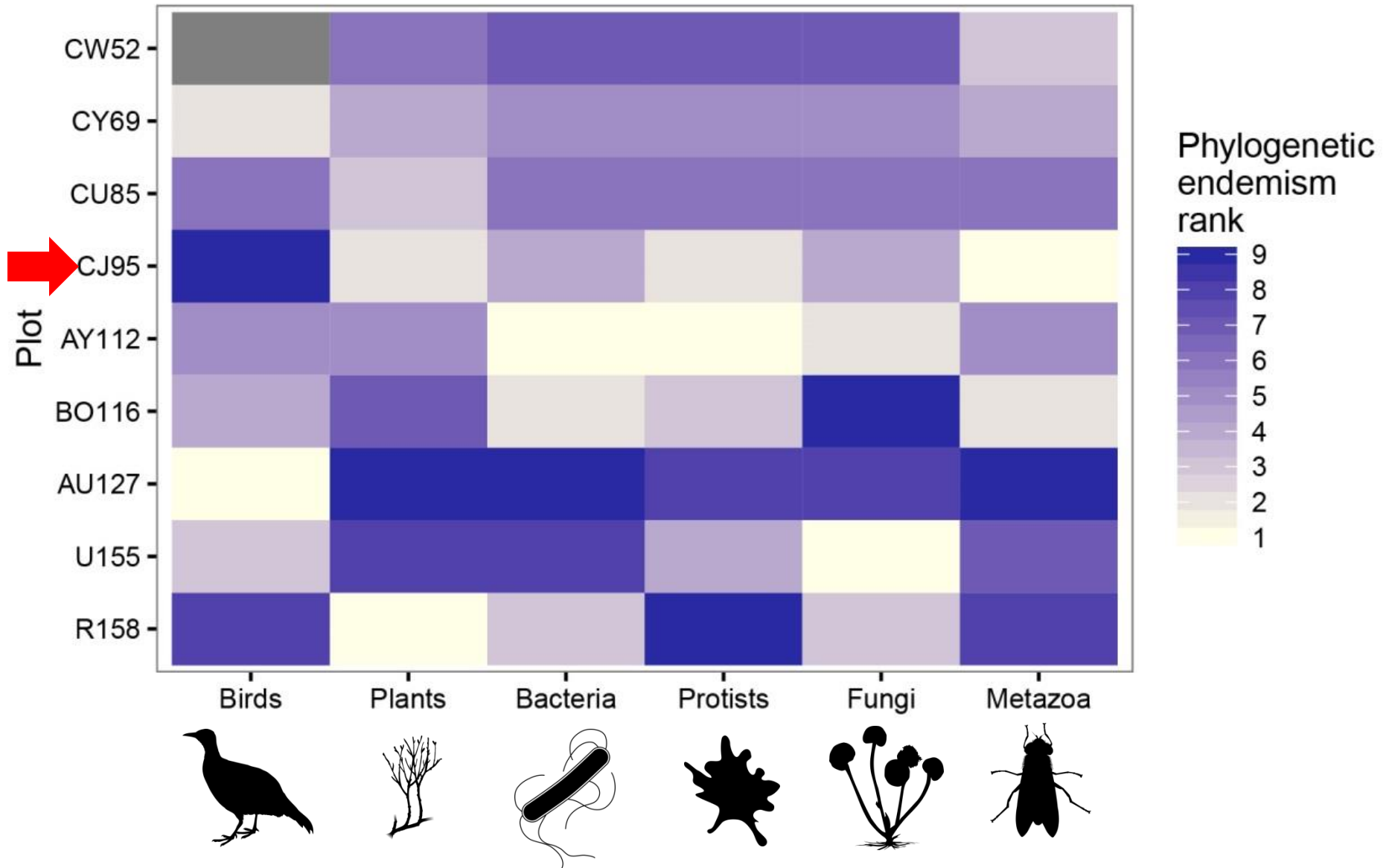
Which has the highest phylogenetic endemism?



Results – Phylogenetic endemism

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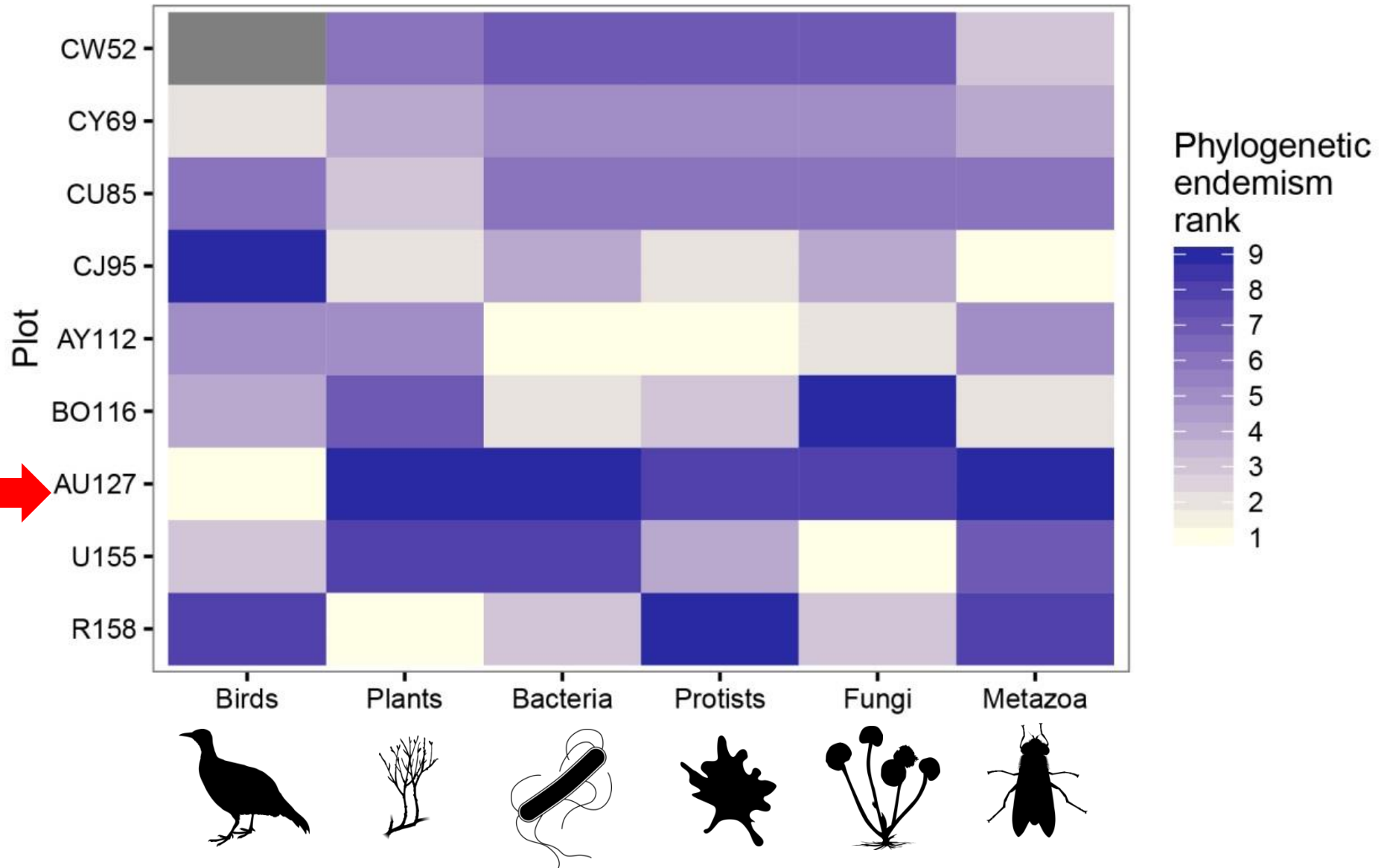
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Results – Phylogenetic endemism

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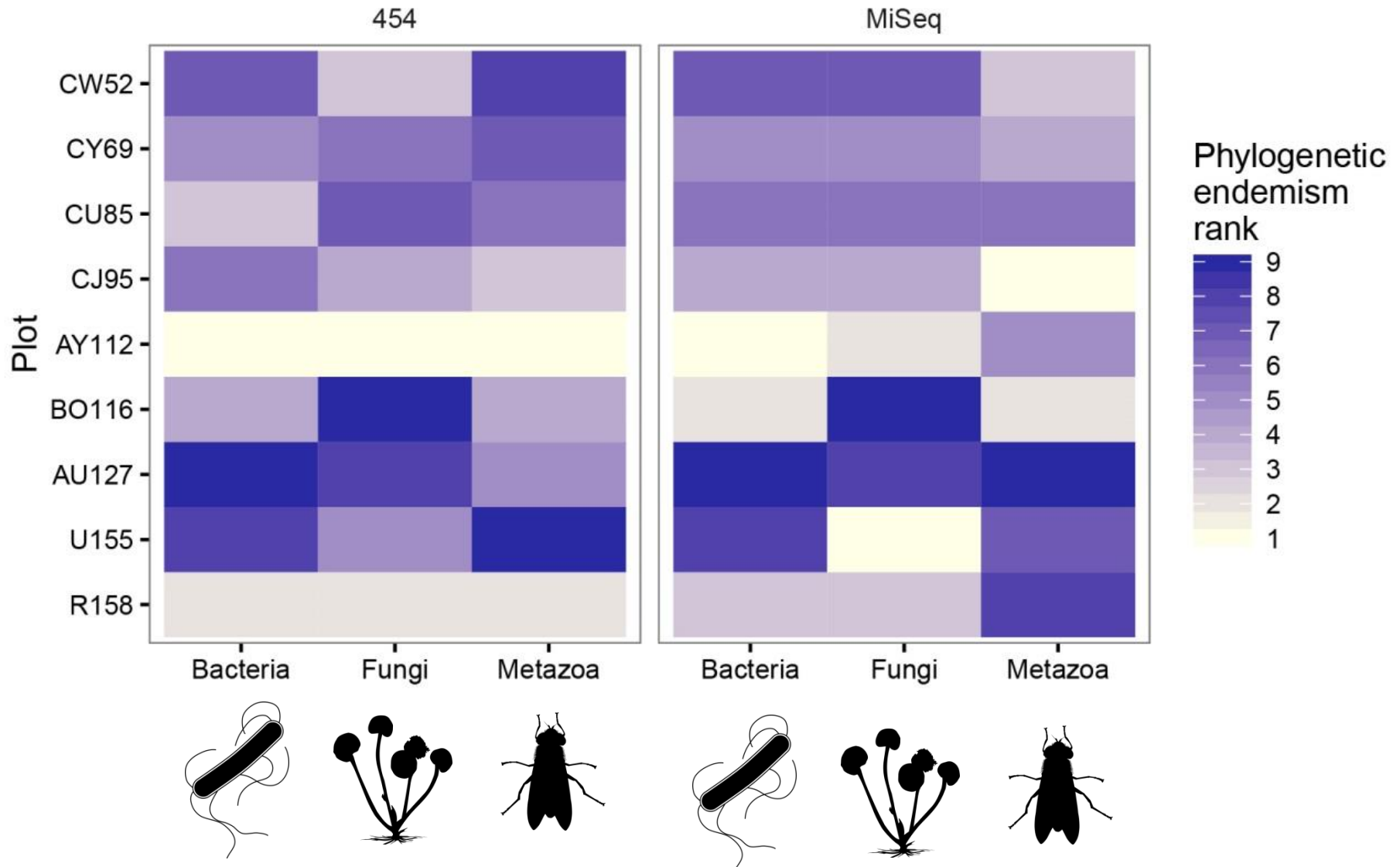
Ngā Koiora
Tuku Iho



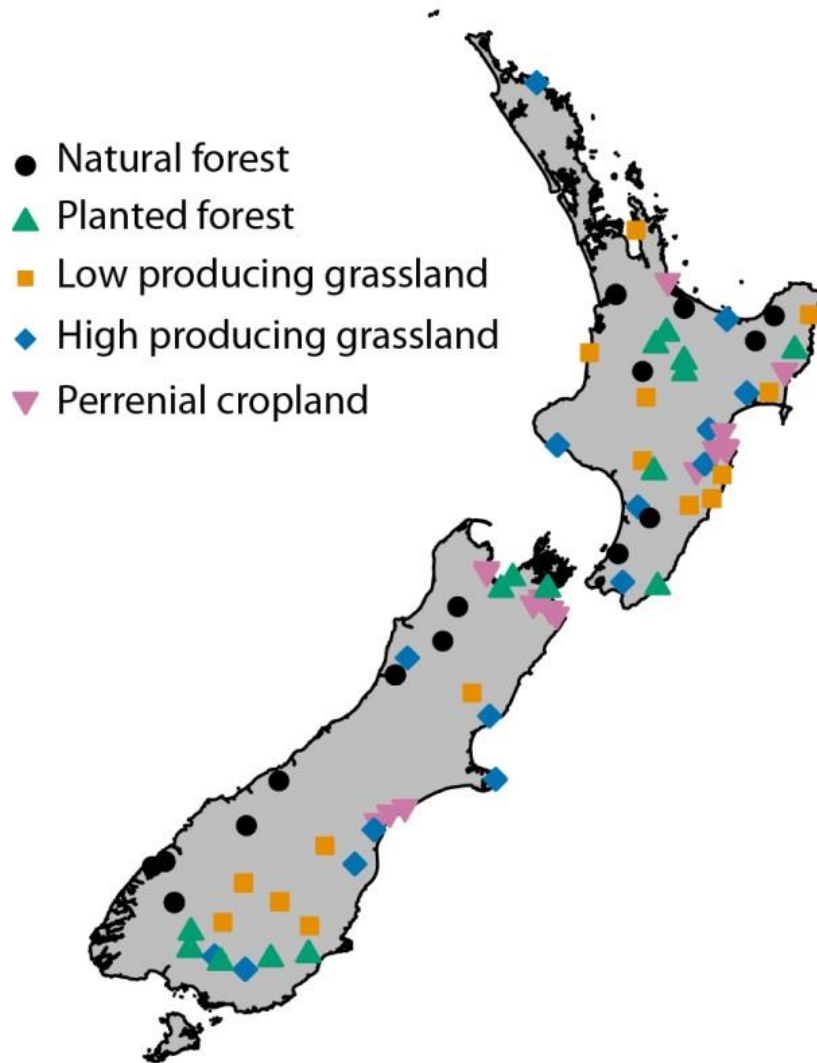
How reliable is phylogenetic endemism as an indicator?

NEW ZEALAND'S
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How is land use impacting genetic diversity?

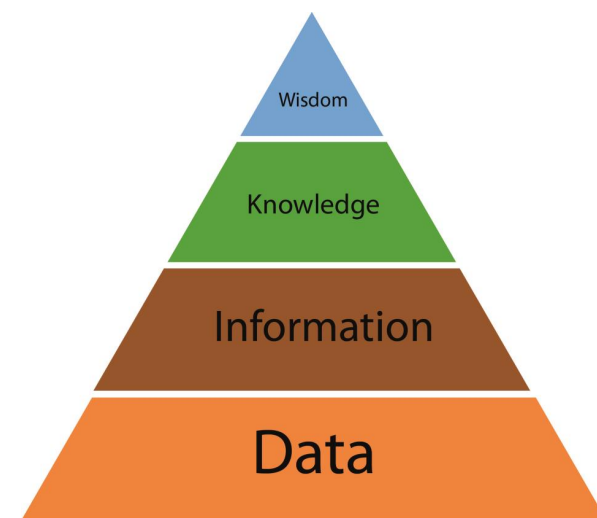


We have the
data to tell a
national story

..... But that's
for another day



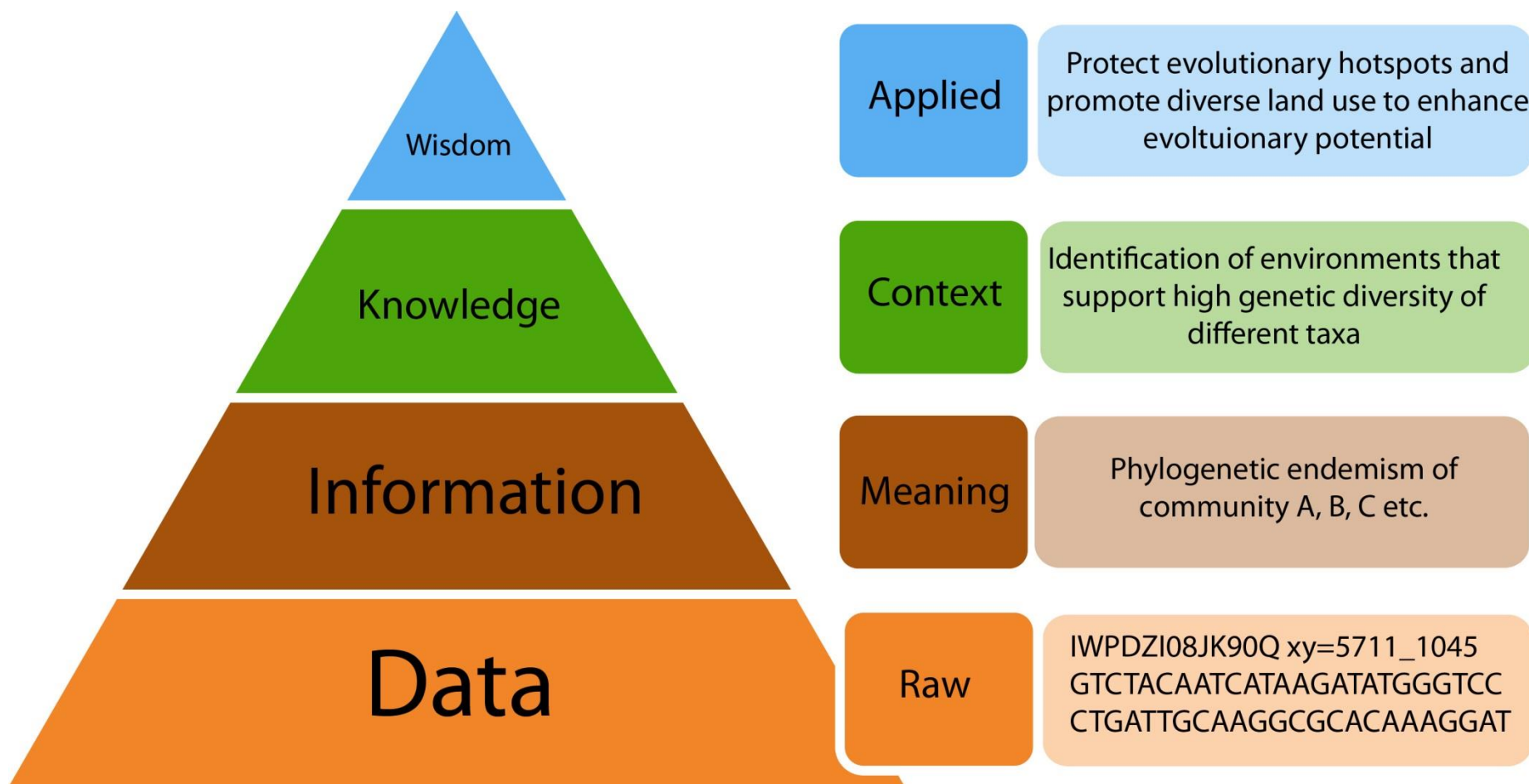
6. Key take home messages



Key take home messages

1. eDNA studies generate deep, complex datasets with many potential uses but they also have limitations
2. Analysis and interpretation is very much a work in progress – and is by no means straightforward or well developed, yet.
3. We need to properly understand the information content of eDNA data so that we are confident in its application
4. Genetic diversity indicators calculated using eDNA data show promise for environmental reporting

From Data to Wisdom (or policy)?



Acknowledgements

Projects

- Next Generation Biodiversity Assessment Project (MBIE Smart Idea)
- Department of Conservation Tier 1 eDNA pilot study (DOC, LCR, BioProtection, AWC)



Landcare Research
Manaaki Whenua



Bio-Protection
Bioprotection science for New Zealand

People

- Numerous researchers have contributed their knowledge and ideas to the above projects and the ideas communicated in this presentation.
- Special thanks to all those who are contributing to the National Science Challenge eDNA Project & associated workshops.



**Department of
Conservation**
Te Papa Atawhai



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