NEW ZEALAND'S BIOLOGICAL HERITAGE

Ngā Koiora Tuku Iho

National SCIENCE Challenges

Next-generation biodiversity assessment for primary industries

Robert Holdaway, 14th July 2015

Landcare Research
Manaaki Whenua

Acknowledgements

Next Generation Biodiversity Assessment Project

- Ian Dickie, Jamie Wood, Kate Orwin, Andreas Makiola, Alexei Drummond
- Phase 1 & Phase 2 MBIE Smart Idea funding (till September 2016)
- Funding also provided by Landcare Research, Lincoln University Bio-Protection Centre, and the Department of Conservation

Biological Heritage National Science Challenge

- A national framework for bioheritage assessment across natural and productive landscapes (5 year project, started July 2015)
- Key researchers: Gavin Lear, Paul Gardner, Simon Bulman, Ian Dickie, Austen Ganley; Phil Wilcox; Kevin Collins
- Numerous other researchers and stakeholders who have contributed to the conception and development of the project

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Outline

Why assess biodiversity?

Environmental DNA 101

- What, how, why?

Primary industry applications

- Demonstrating environmental performance
- Improved management practices
- Biosecurity detection and monitoring

Future research directions

Why primary industry cares about biodiversity

- Market advantage & licence to operate
  - Biodiversity foot-printing
  - Clean green image

- Sustainable practice
  - over $500 million spent annually on fertiliser by dairy sector
  - biological farming practices can improve fertility naturally and reduce costs long-term

- Biosecurity
  - $ billions of export earnings at risk
  - early detection key to minimising potential impacts

National biodiversity assessment

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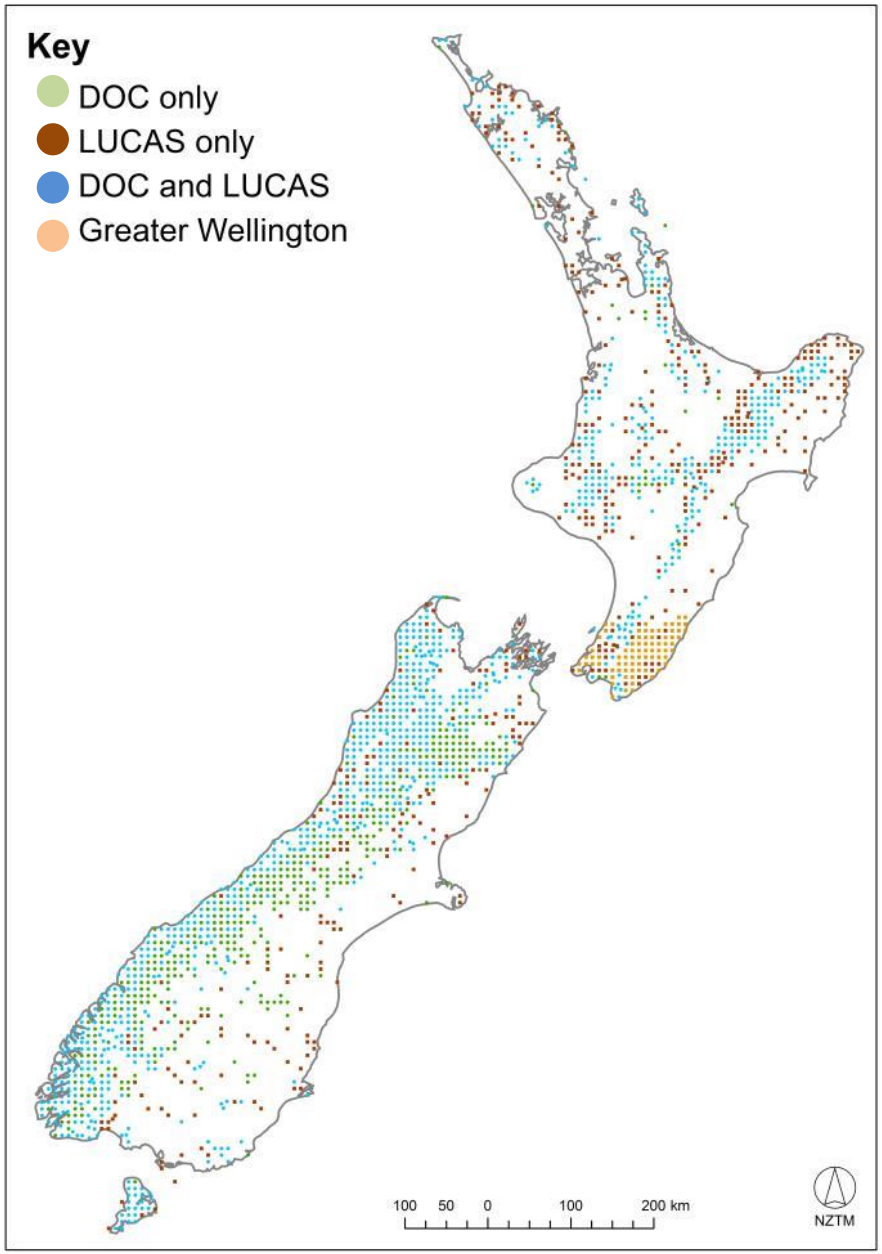

8 km grid for State of Environment reporting

- LUCAS (MfE)
- DOC Tier 1
- Regional Councils

Harmonised sample design, methods and reporting metrics

Major limitations

- Limited primary sector coverage
- the vast majority of biodiversity is not assessed

~214 birds

~2500 vascular plants

~20,000 insects

~22,000 fungi

Vertical scale = proportional to species

Cryptic biodiversity is important for primary industry

The good guys

The bad guys

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Next generation sequencing of environmental DNA provides a cost effective tool to measure all forms of biodiversity – especially cryptic biodiversity

What is environmental DNA (eDNA)?

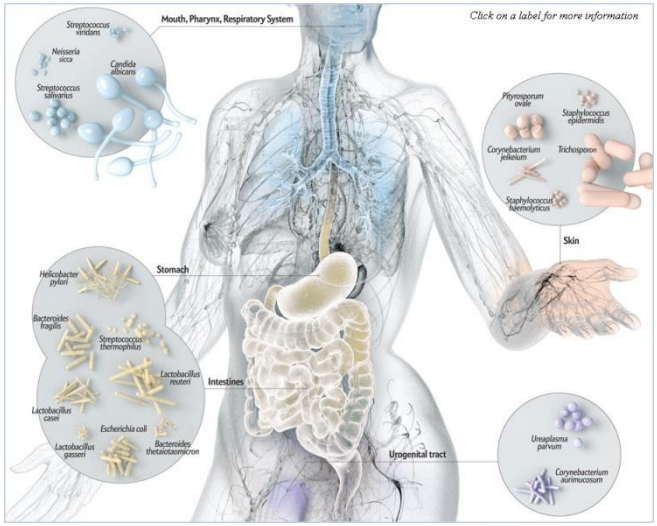
DNA extracted directly from an environmental sample

- trace DNA left behind by organisms
- undifferentiated micro-organism DNA

What is Next Generation Sequencing?

- Typically used for eDNA analysis
- High throughput, massively parallel
- Commercially available service
- Massive data outputs (millions or billions of sequences per run)

eDNA success stories



How to assess biodiversity using eDNA?

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7 fundamental steps

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How to assess biodiversity using eDNA?

7 fundamental steps

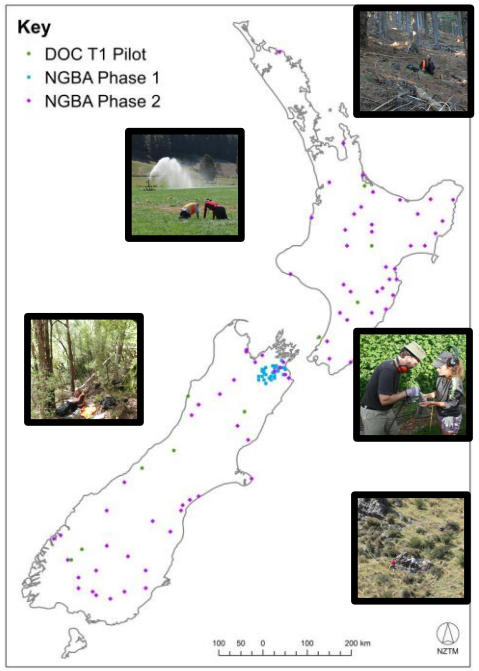
1. **Define your question**

?

How to assess biodiversity using eDNA?

7 fundamental steps

1. Define your question
2. **Design sampling**



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How to assess biodiversity using eDNA?

7 fundamental steps

1. Define your question
2. Design sampling
3. **Collect samples**

How to assess biodiversity using eDNA?

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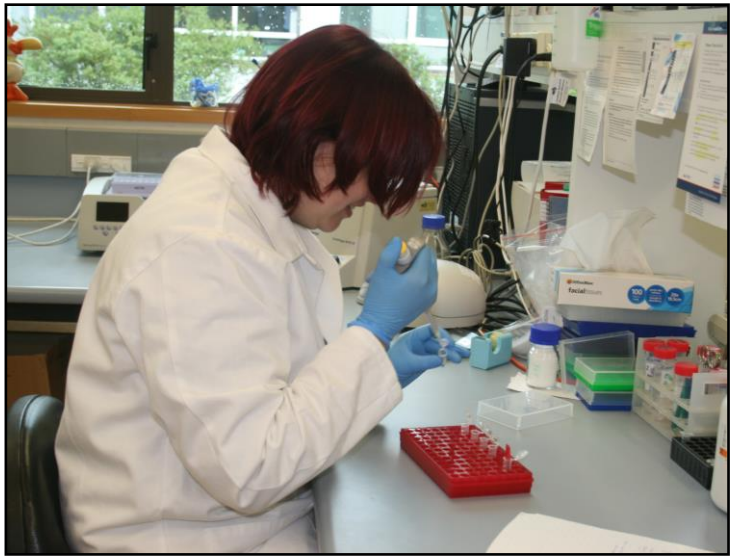
7 fundamental steps

1. Define your question
2. Design sampling
3. Collect samples
4. **Extract DNA**



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How to assess biodiversity using eDNA?

7 fundamental steps

1. Define your question
2. Design sampling
3. Collect samples
4. Extract DNA
5. **Sequence DNA**

GTGAATCATCGAATCTTTGAACG
CACATTGCGCCCCTTGGTATTCC
GAGGGGCATGCCTATTCGAGCG
TCATTATCACCCCTCAAGCCTAG
CTTGGTGTTGAGACCTGCTGTC

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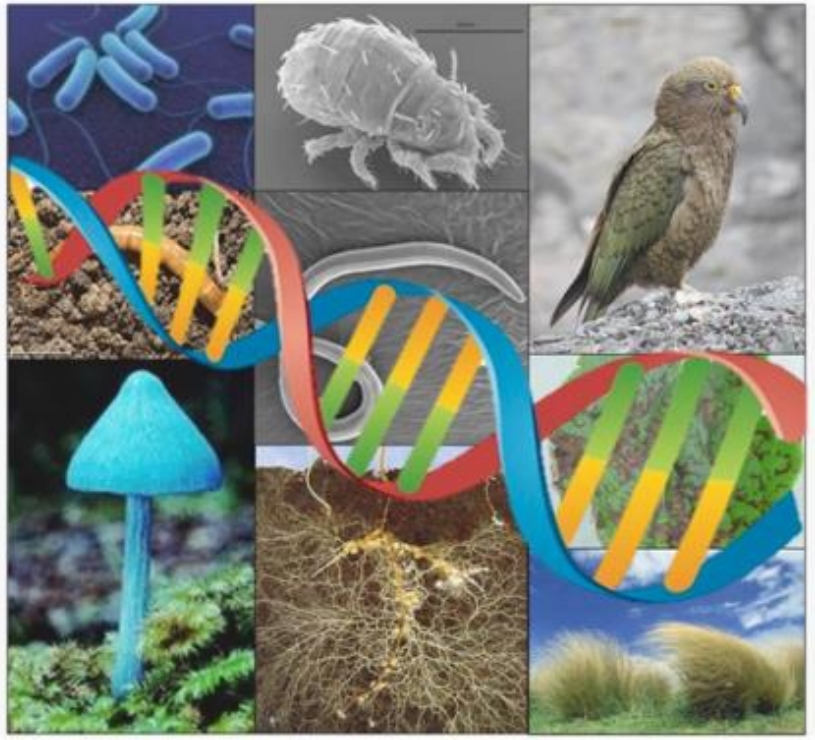
How to assess biodiversity using eDNA?

7 fundamental steps

1. Define your question
2. Design sampling
3. Collect samples
4. Extract DNA
5. Sequence DNA
6. **Match DNA to species**



How to assess biodiversity using eDNA?

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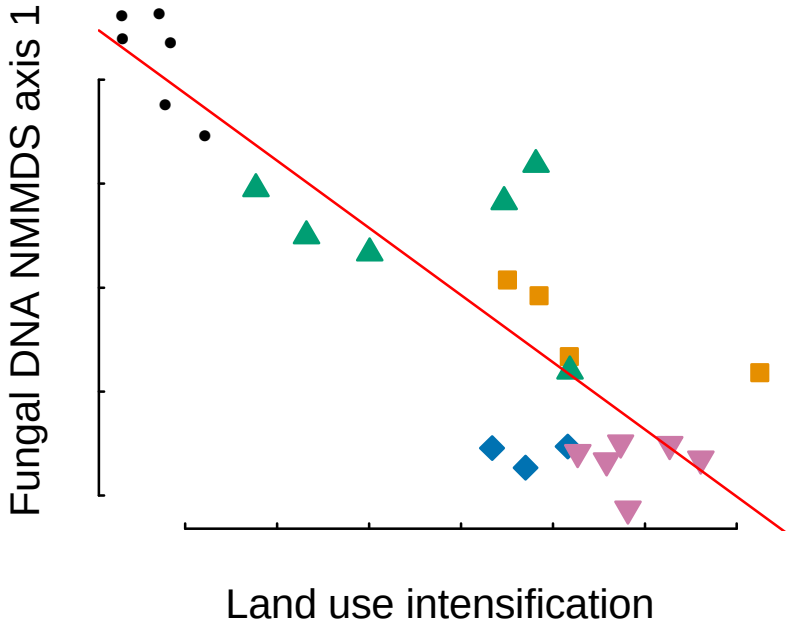
7 fundamental steps

1. Define your question
2. Design sampling
3. Collect samples
4. Extract DNA
5. Sequence DNA
6. Match DNA to species
7. **Analyse data to answer question**



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Biodiversity assessment using eDNA

7 fundamental steps

1. Define your question	HARD
2. Design sampling	MODERATE
3. Collect samples	EASY
4. Extract DNA	EASY
5. Sequence DNA	EASY
6. Match DNA to species	HARD
7. Analyse data to answer question	MODERATE

How to assess biodiversity using eDNA?

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7 fundamental steps

1. **Define your question**
2. Design sampling
3. Collect samples
4. Extract DNA
5. Sequence DNA
6. Match DNA to species
7. Analyse data to answer question

1. Define your question

Sets the spatial scale of the study

- What is the fungal community in my paddock?
- How does land use affect fungal community composition?

Determines sample media and target taxonomic groups

- What fungal endophyte species occur on kiwifruit leaves?
- How does cultivation affect the community composition of soil biota?

Main question from the Next-Generation Biodiversity Assessment Project (Phase 1)

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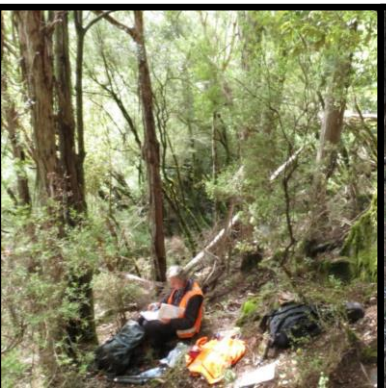
How does the richness and composition of biological communities vary within and across land uses?

Natural forest



Planted forest

Low-producing grassland

High-producing grassland

Vineyard

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Biodiversity assessment using eDNA

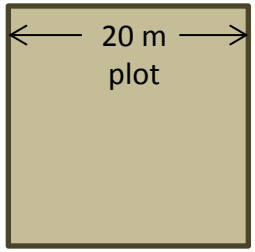
7 basic steps

1. Define your question ✓
2. **Design sampling**
3. Collect samples
4. Extract DNA
5. Sequence DNA
6. Match DNA to species
7. Analyse data to answer question

Phase 1: Proof-of-concept in a model catchment (Wairau Valley, Marlborough)

- Based on national grid of 20x20m plots used by DoC, MfE and regional councils
- Traditional plot-based measurements (plants, birds) alongside molecular methods

Field-based
Bird and plant surveys



Soil cores for molecular analysis

Biodiversity assessment using eDNA

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7 fundamental steps

1. Define your question ✓
2. Design sampling ✓
3. **Collect samples**
4. Extract DNA
5. Sequence DNA
6. Match DNA to species
7. Analyse data to answer question

3. Collect samples

- Landowner and industry permissions
- Iwi consultation
- Logistical considerations

However, eDNA sample collection is relatively straightforward and could be done by the farmers themselves

Biodiversity assessment using eDNA

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7 fundamental steps

1. Define your question ✓
2. Design sampling ✓
3. Collect samples ✓
4. **Extract DNA**
5. Sequence DNA
6. Match DNA to species
7. Analyse data to answer question

4. Extract DNA

- Commercially available kits

- Two distinct stages
  - Isolate DNA from sample
  - Use PCR to amplify target gene regions

- PCR primer selection is critical

General vs specific primers

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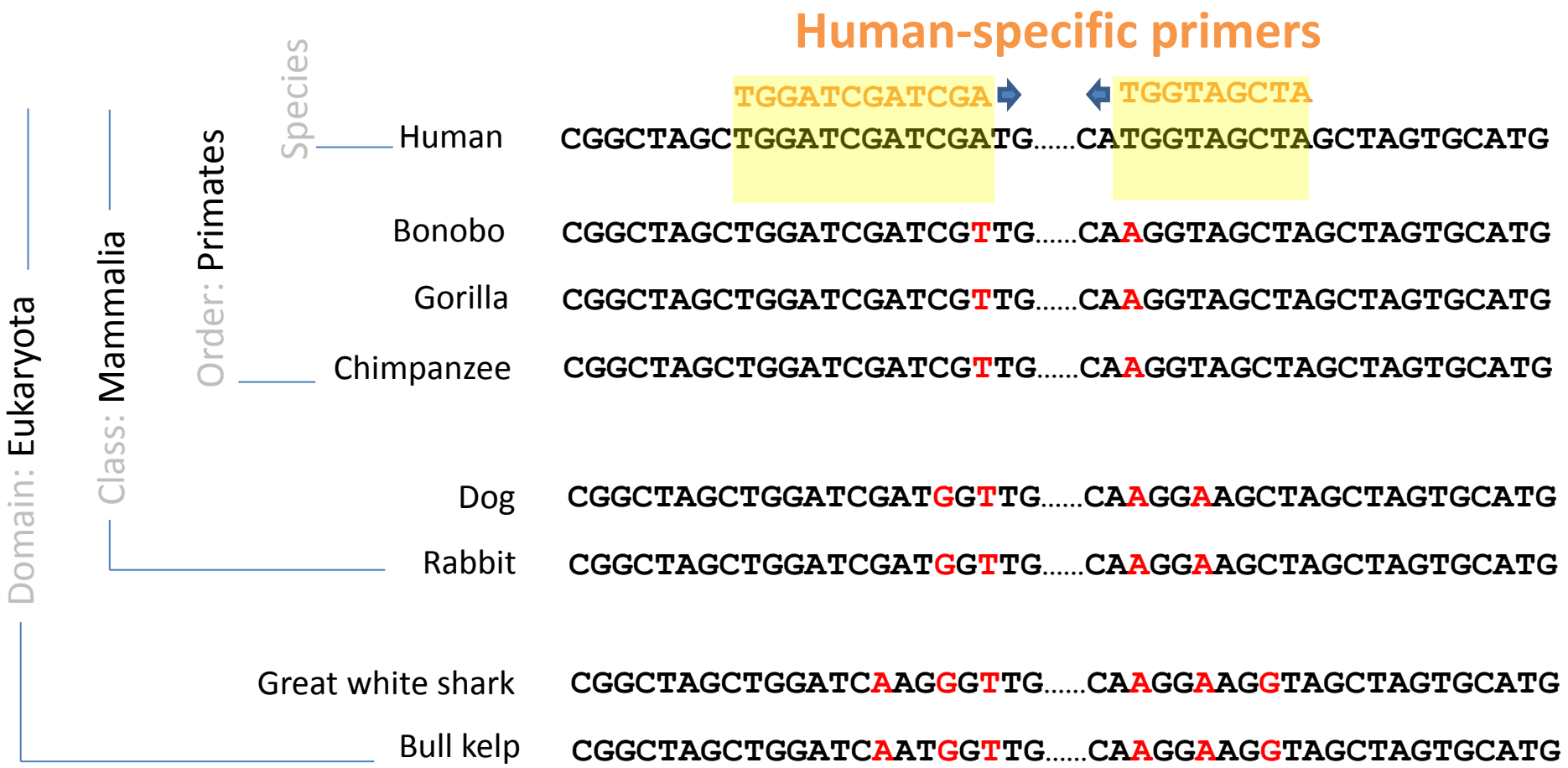

General vs specific primers

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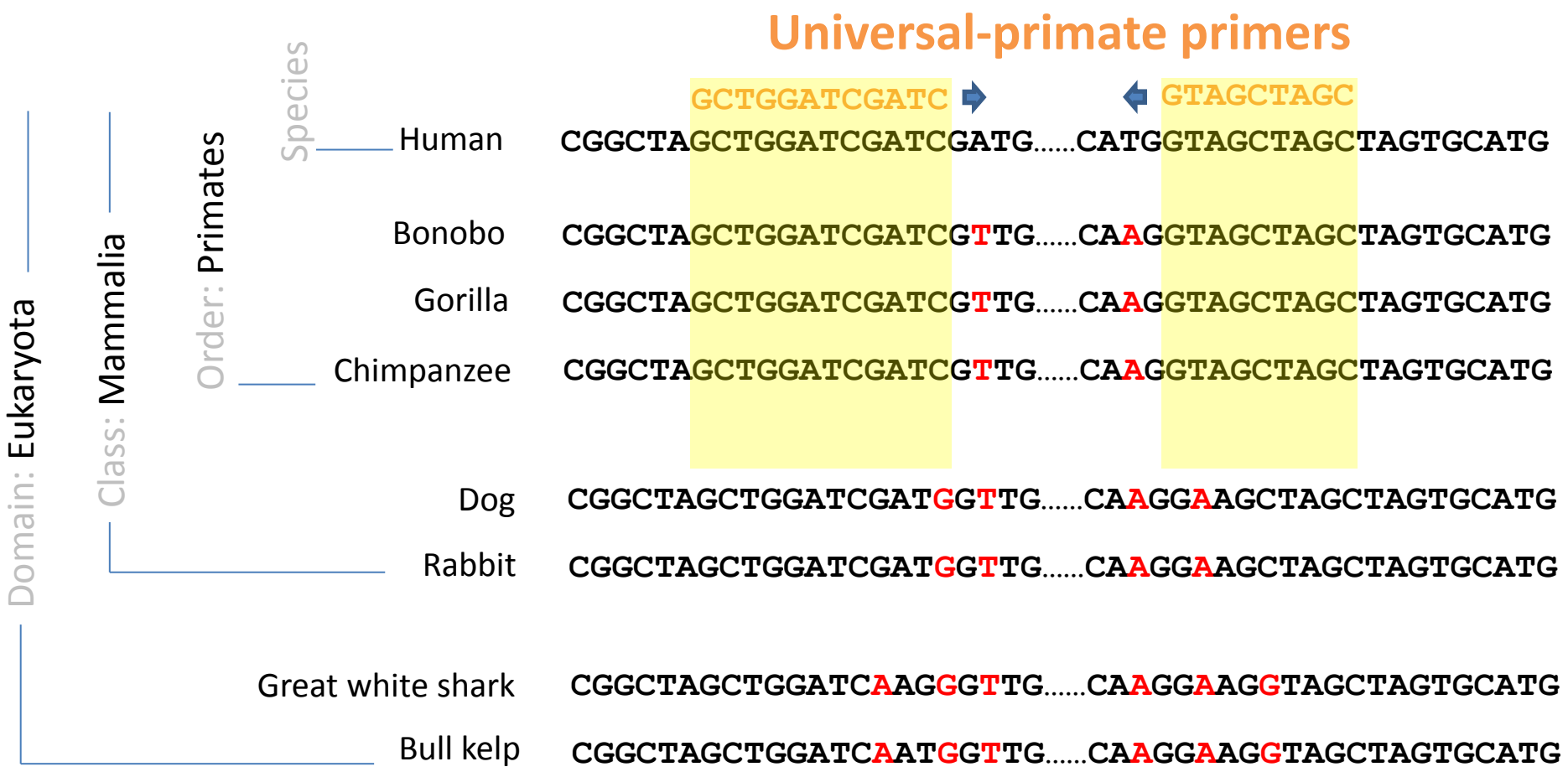

General vs specific primers

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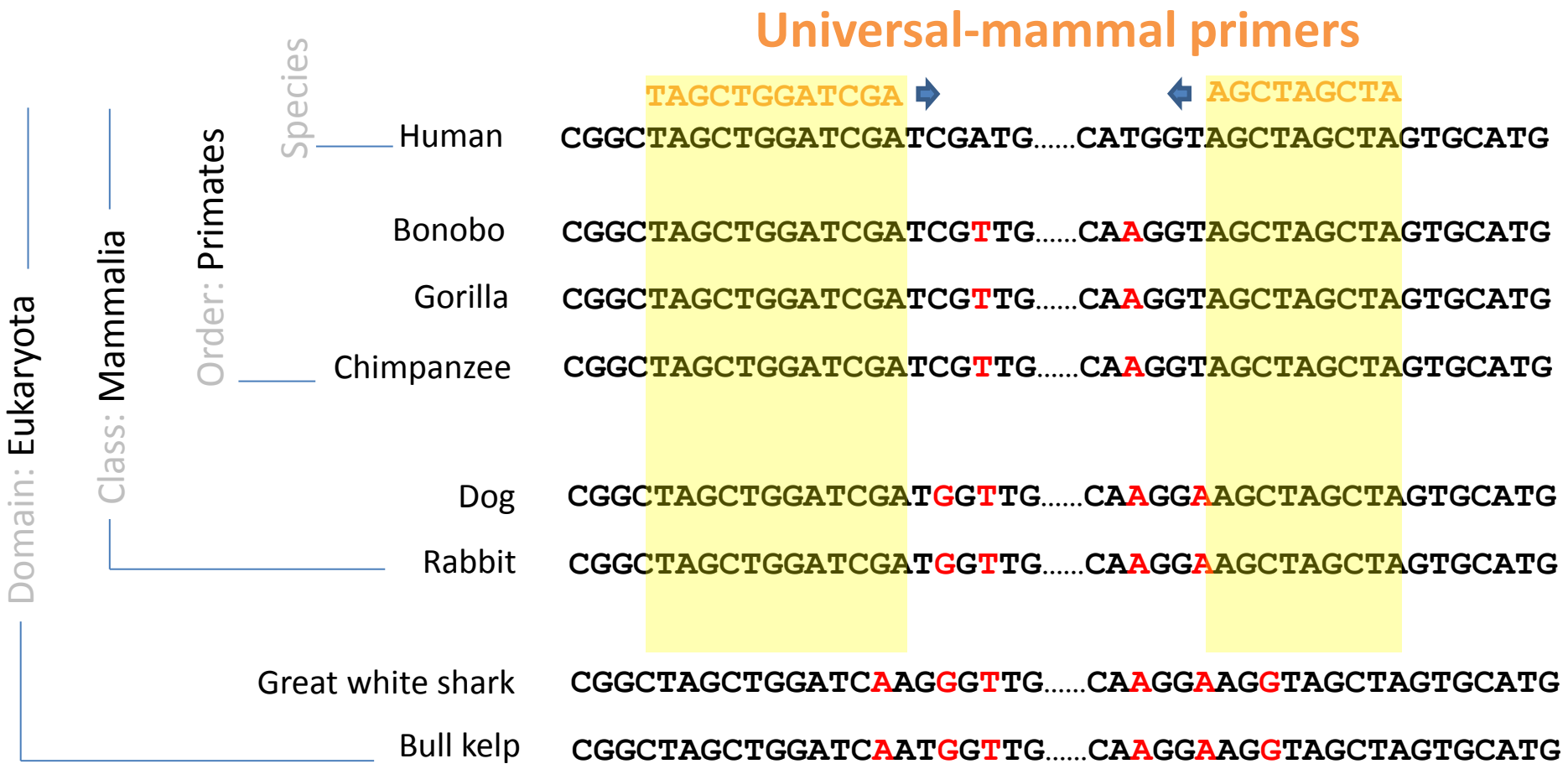

General vs specific primers

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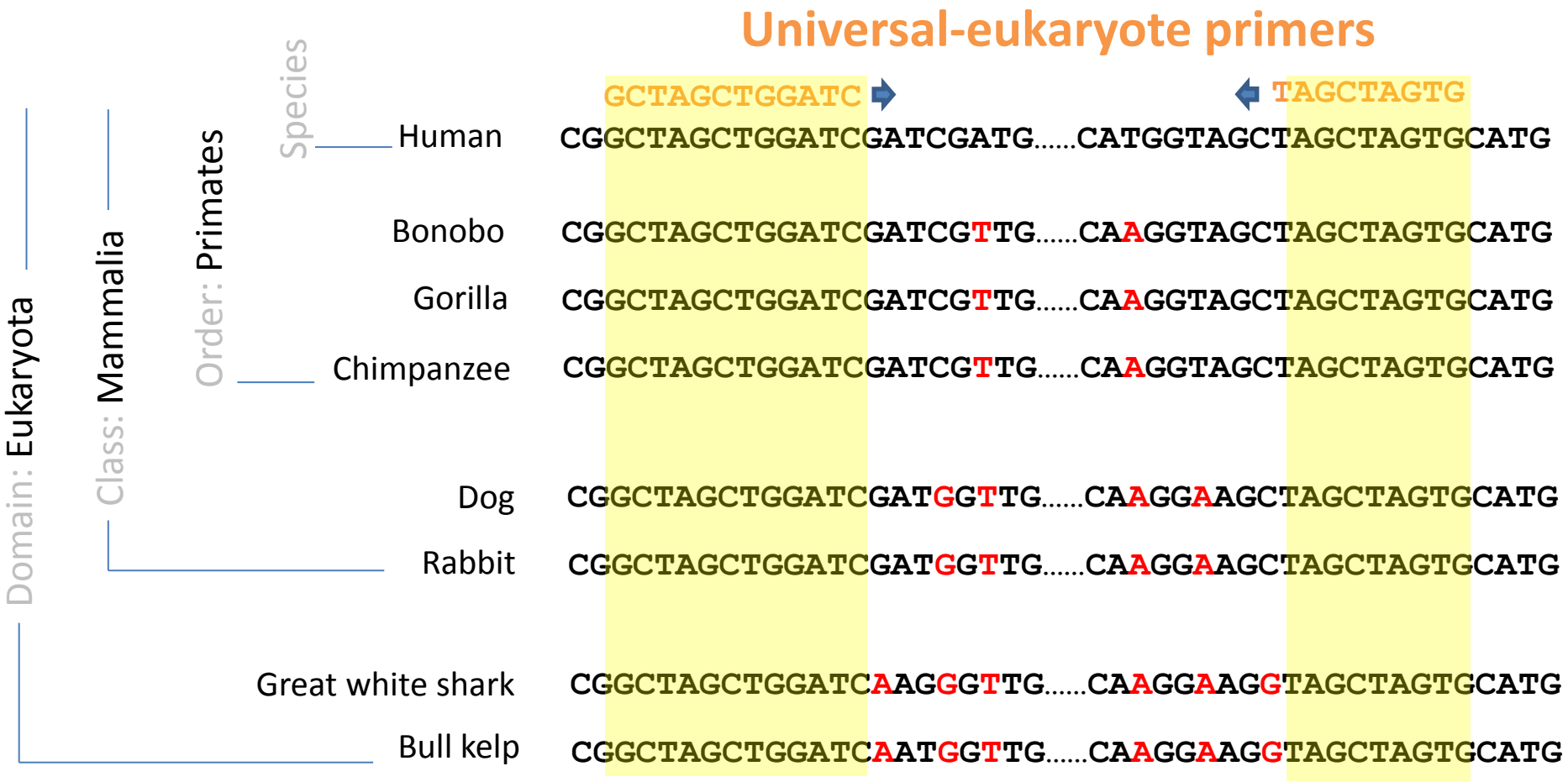

Ability of a primer to differentiate species

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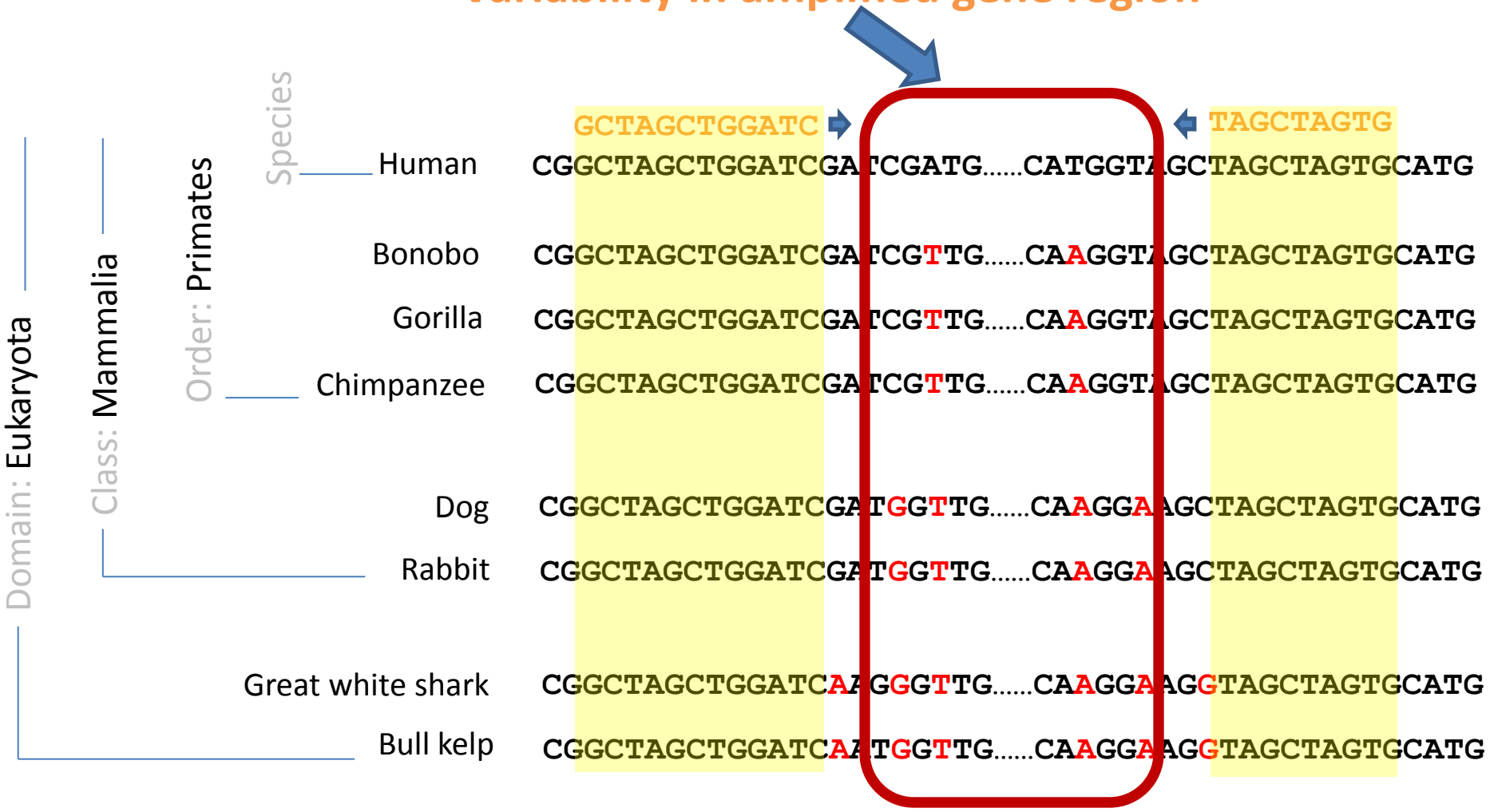

Biodiversity assessment using eDNA

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7 fundamental steps

1. Define your question ✓
2. Design sampling ✓
3. Collect samples ✓
4. Extract DNA ✓
5. **Sequence DNA**
6. Match DNA to species
7. Analyse data to answer question

5. Sequence DNA

- Next-generation sequencing platforms
- Commercially available service
- Can be done on a pooled DNA sample

- Technology rapidly evolving
- Big data outputs

Biodiversity assessment using eDNA

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7 fundamental steps

1. Define your question ✓
2. Design sampling ✓
3. Collect samples ✓
4. Extract DNA ✓
5. Sequence DNA ✓
6. **Match DNA to species**
7. Analyse data to answer question

6. Match DNA to species (or OTUs)

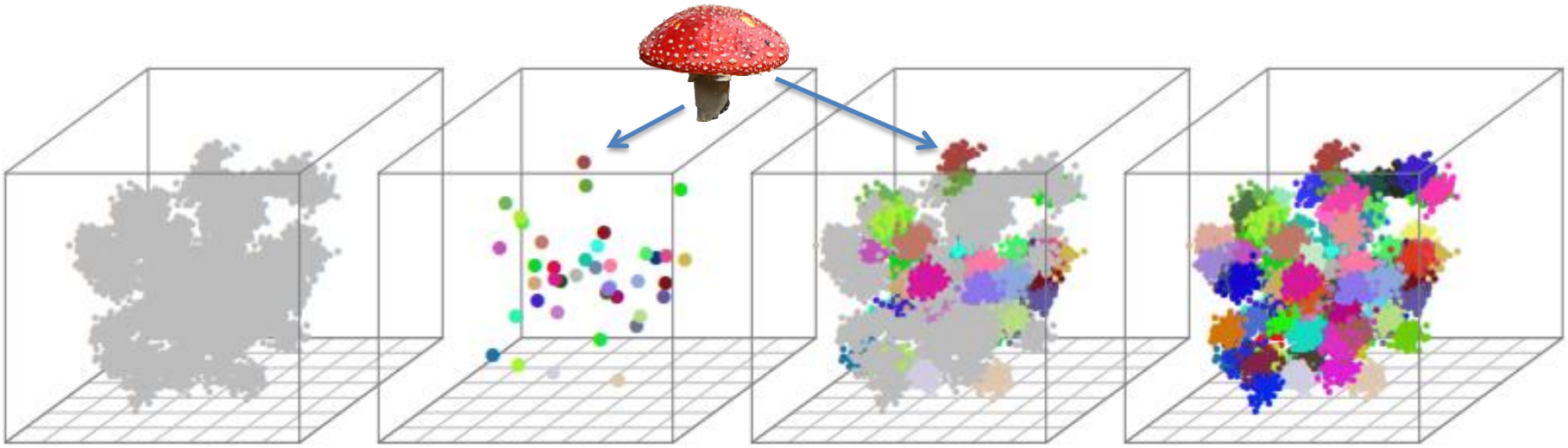
Operational taxonomic units (OTUs) allow identification of 'species' without taxonomic matches

Assigning species names to OTU's is reliant on reference databases

- Genbank, GreenGenes

Going from DNA to OTU's &/or species is **challenging**

- Incomplete reference data
- Which bioinformatics method to use?
- What taxonomic resolution is most appropriate?

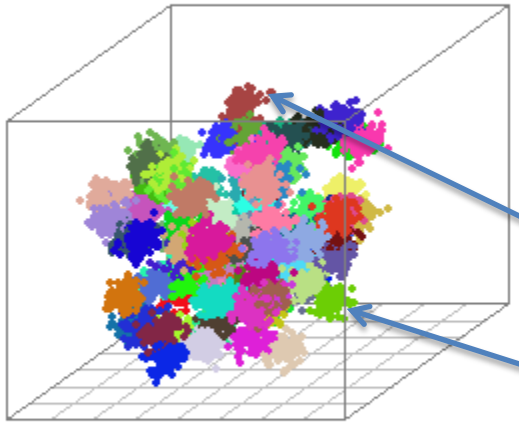


1: start with DNA data in N-dimensional similarity cloud

2: "Seed" space with known species from reference library (e.g., from a specimen)

3: Match unknown data to these known references

4: Form new "unknown" clusters where no known species match



5: Assign names & identity based on:

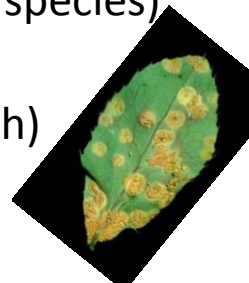
- known "seed" that formed cluster

OR

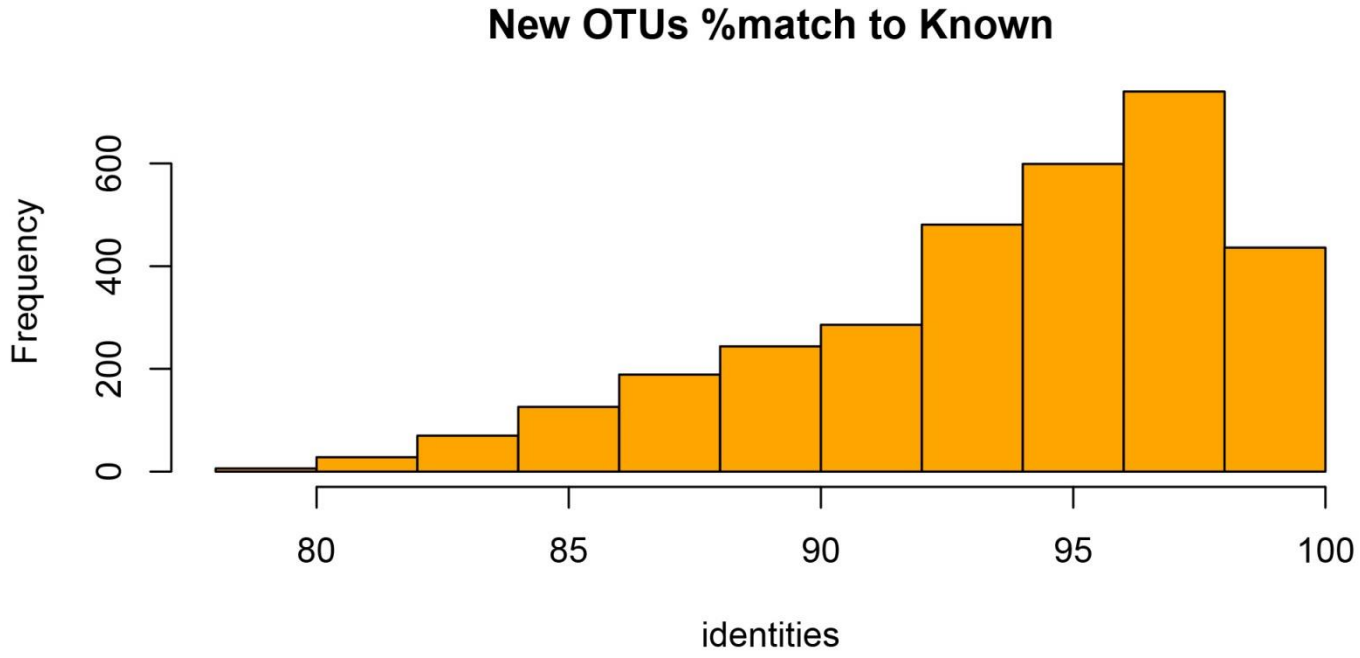
- nearest known species in database (to some level of confidence)

Amanita muscaria (OTU matched to known species)

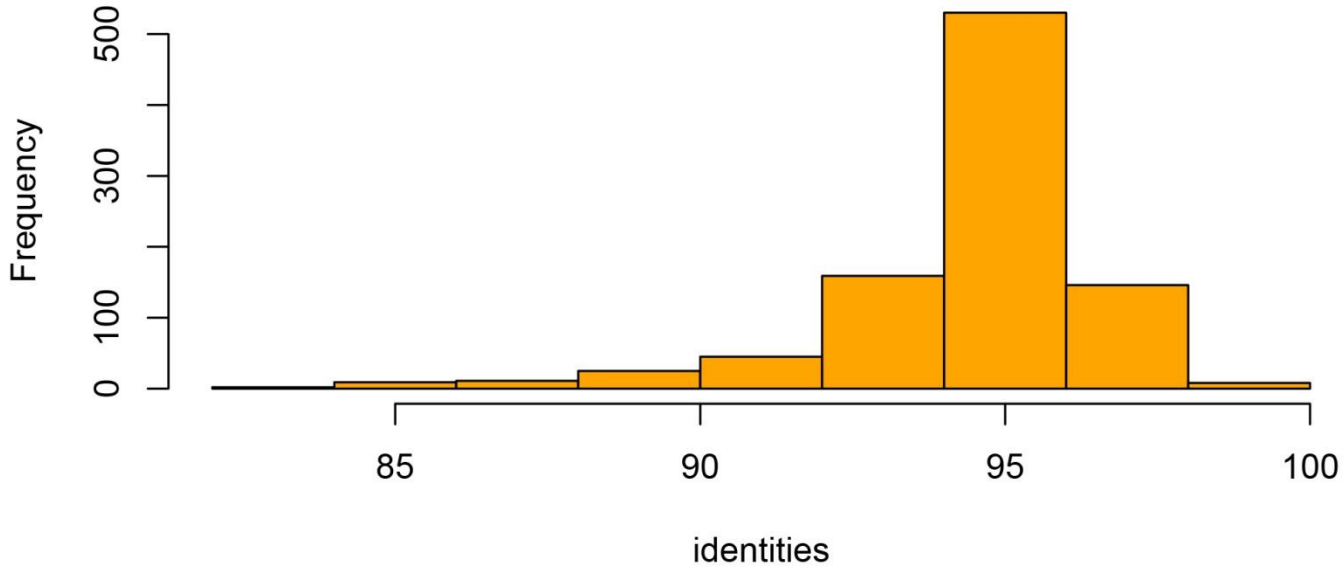
Unknown rust fungus (OTU family level match)



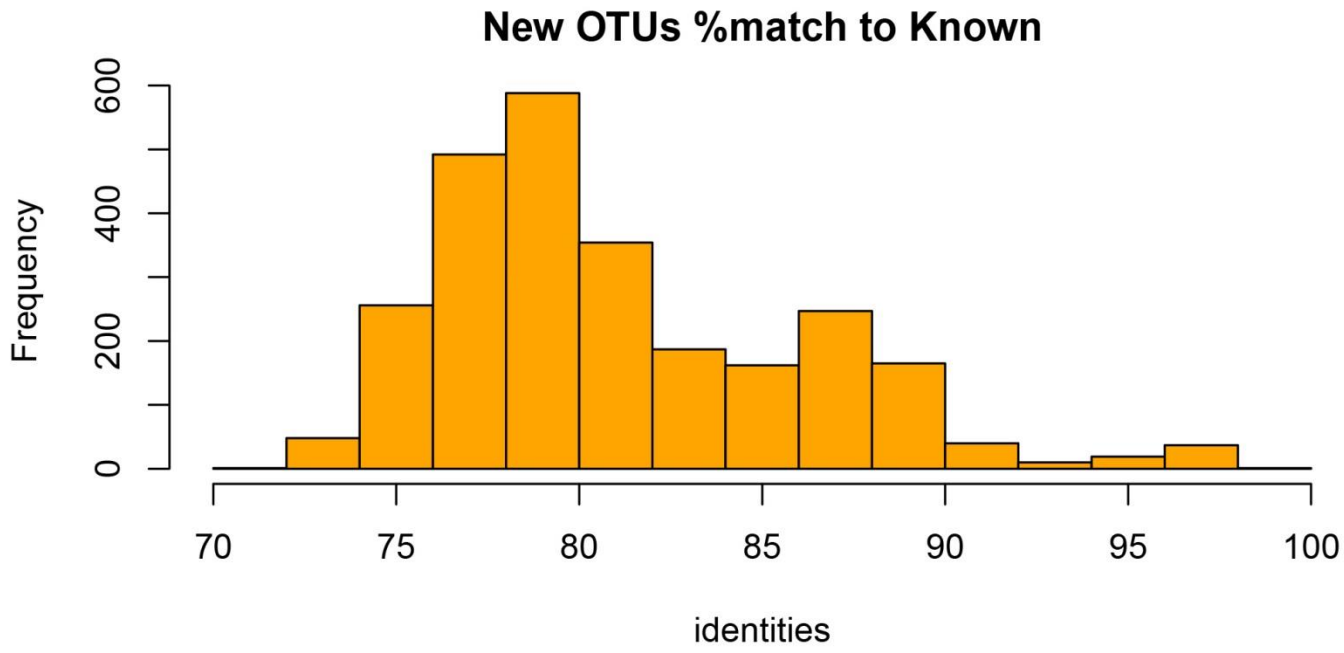
Fungi ITS1F OTU matching
0
1000
2000
3000
Unmatched
WrongTaxa
TooShort
NameErorr
NewOTU
KnownOTU


New OTUs %match to Known
Frequency
0
200
400
600
80
85
90
95
100
identities

Plants OTU matching
0
200
600
Unmatched
WrongTaxa
TooShort
NameErorr
NewOTU
KnownOTU


New OTUs %match to Known
Frequency
0
100
300
500
85
90
95
100
identities

Invertebrates OTU matching
0
500
1500
2500
Unmatched
WrongTaxa
TooShort
NameErorr
NewOTU
KnownOTU


New OTUs %match to Known
Frequency
0
200
400
600
70
75
80
85
90
95
100
identities

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Biodiversity assessment using eDNA

7 fundamental steps

1. Define your question ✓
2. Design sampling ✓
3. Collect samples ✓
4. Extract DNA ✓
5. Sequence DNA ✓
6. Match DNA to species ✓
7. **Analyse data to answer question**

How does the **composition** of biological communities vary with land use?

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Native forest

Pine forest

Low producing grassland

High producing grassland

Vineyard

How does the **composition** of biological communities vary with land use?

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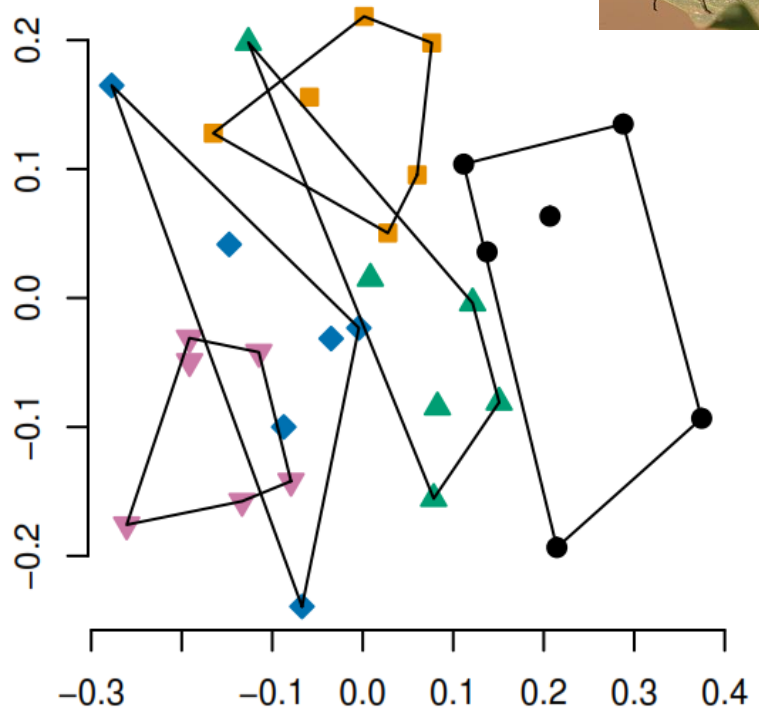

Native forest

Pine forest

Low producing grassland

High producing grassland

Vineyard

Plant community
(based on non-DNA field measurements)

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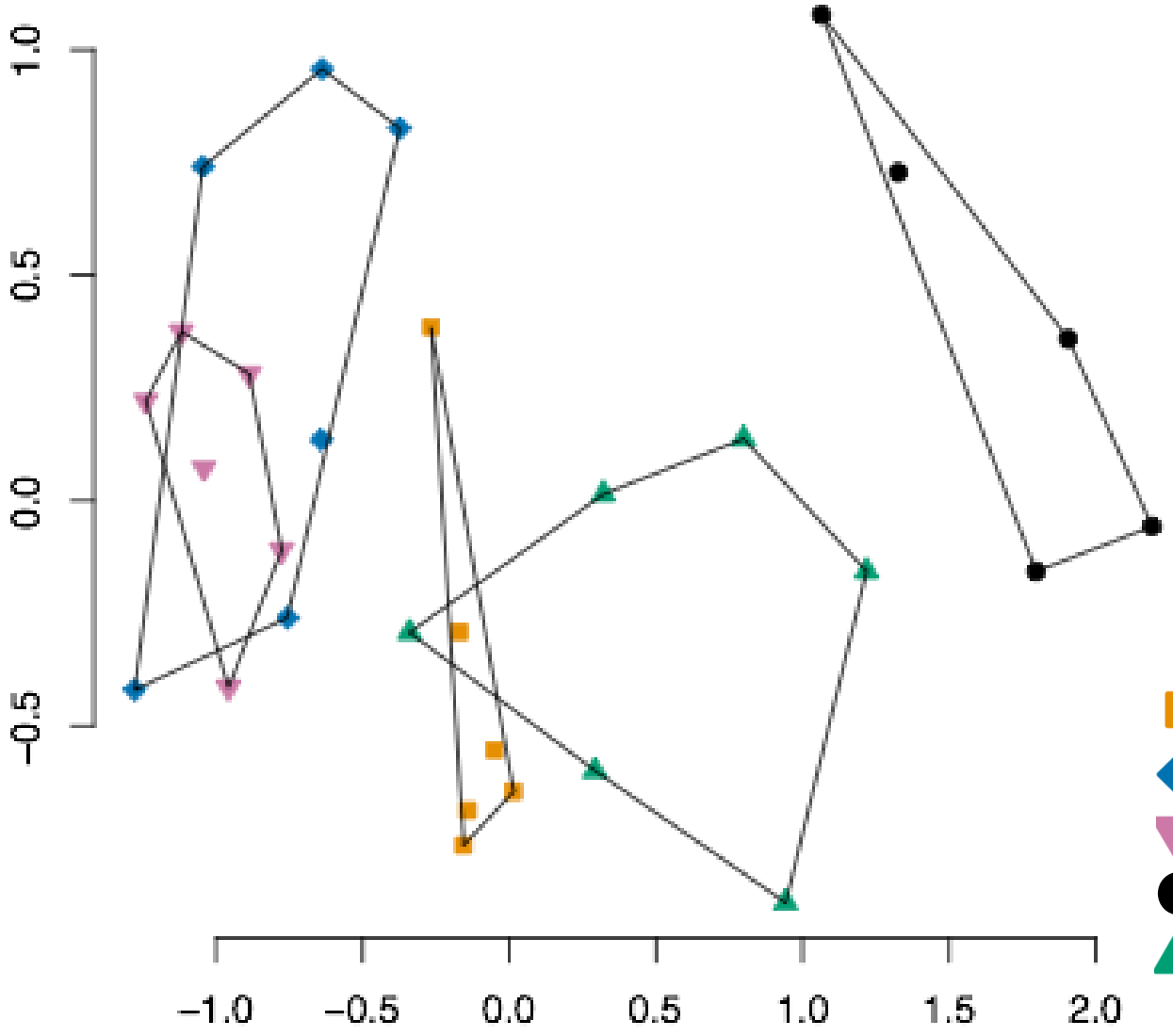

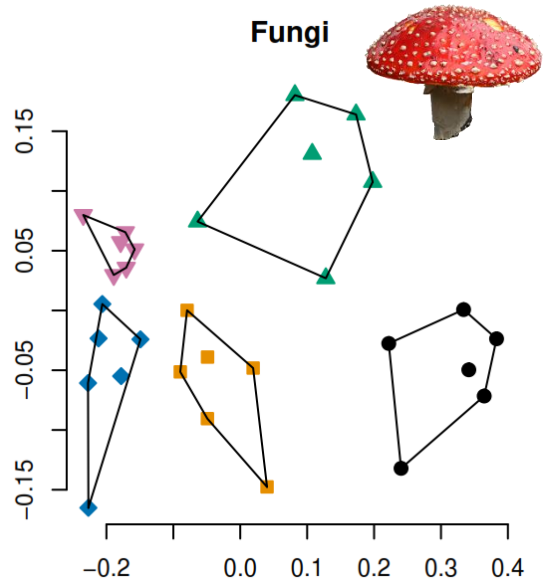

- Low producing grassland
- High producing grassland
- Vineyard
- Native forest
- Pine forest

How does the **richness** of biological communities vary with land use?

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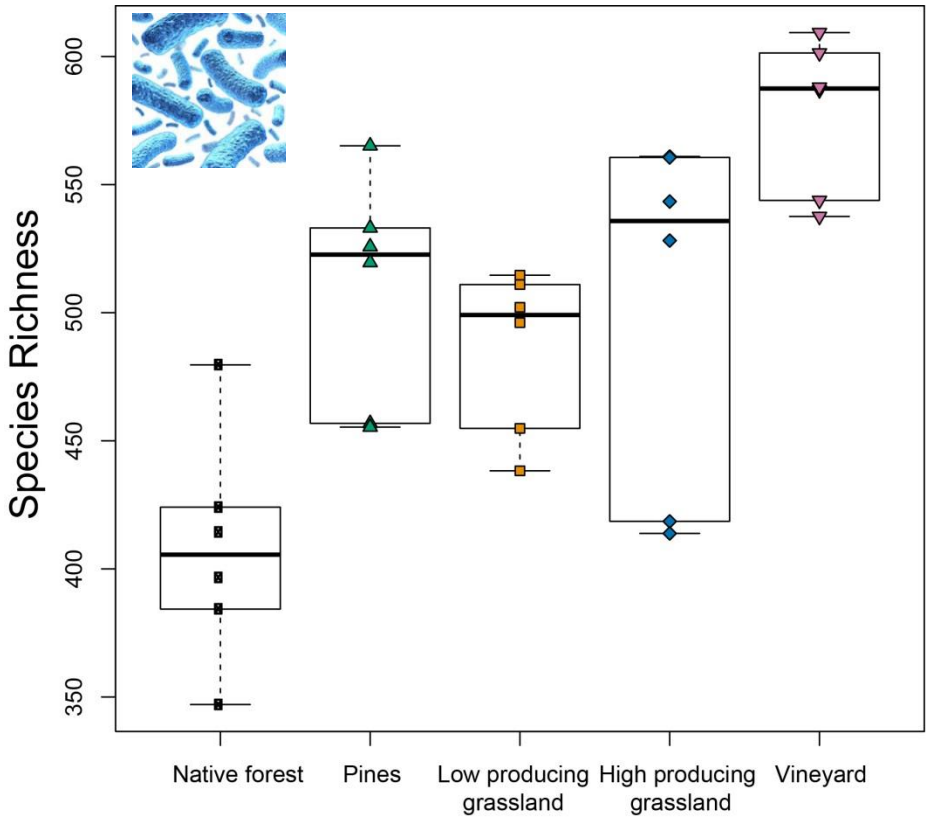
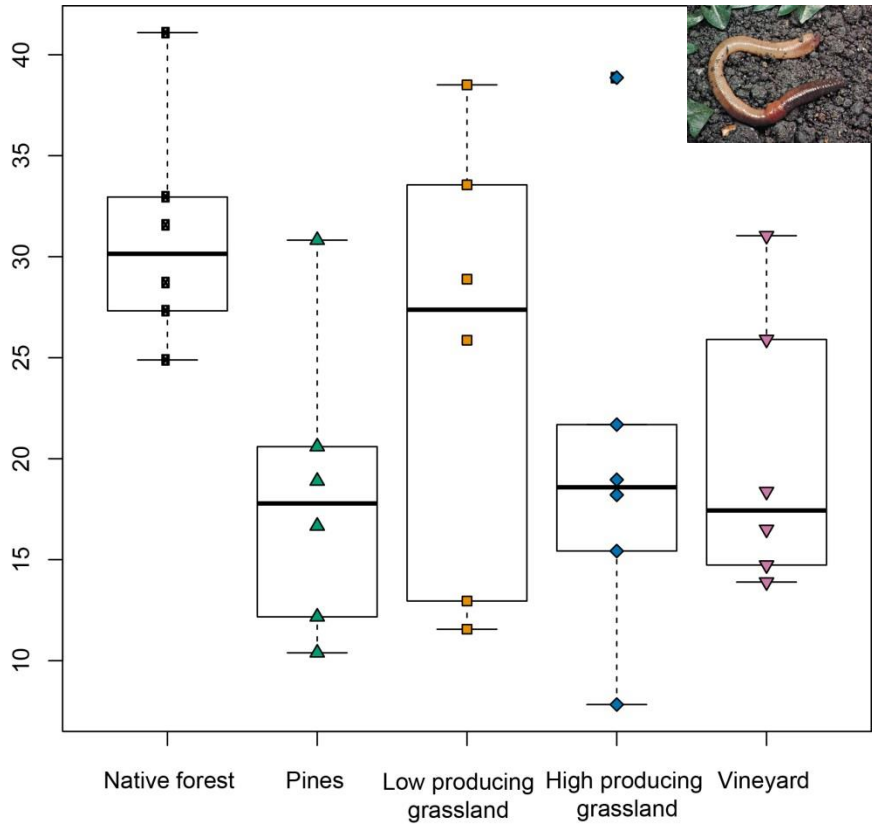
Bacteria



Invertebrates



Land use type

How does the **richness** of biological communities vary with land use?

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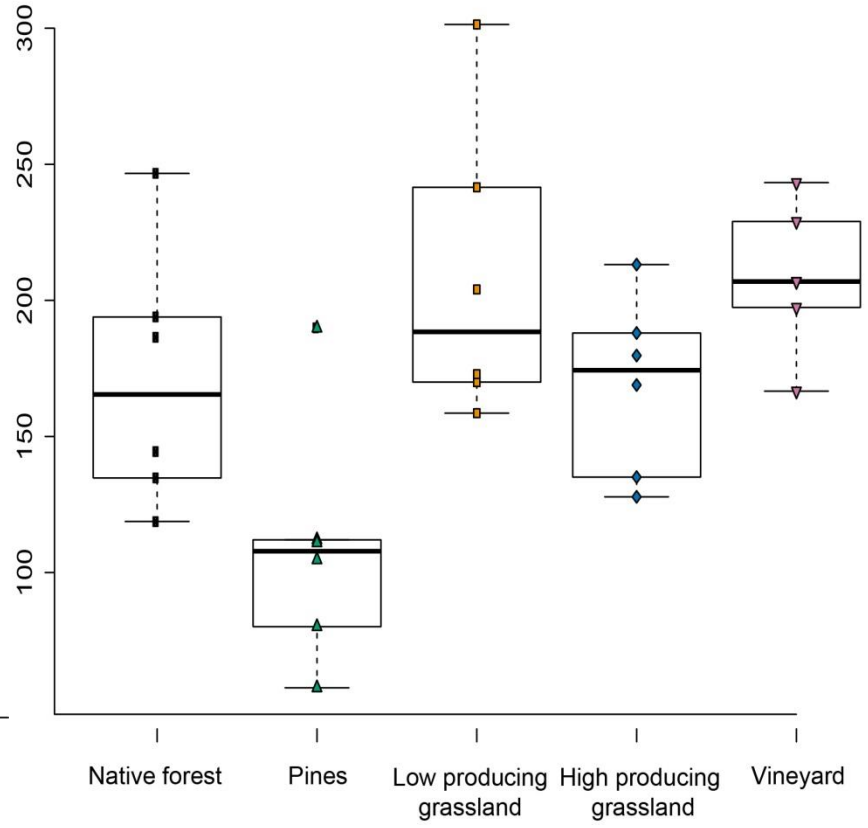
Plants

Fungi



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Primary sector eDNA applications

- Demonstrating environmental performance
- Improving farm management
- Biosecurity detection and monitoring

Application 1
Demonstrating environmental performance

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- Demonstrating positive effects of industry on biodiversity is an increasing market requirement
- Benchmarking within sector groups provides a framework to improve management practices
- eDNA derived indicators could be used to demonstrate maintenance (or enhancement) of whole-ecosystem biodiversity

Application 1
Demonstrating environmental performance

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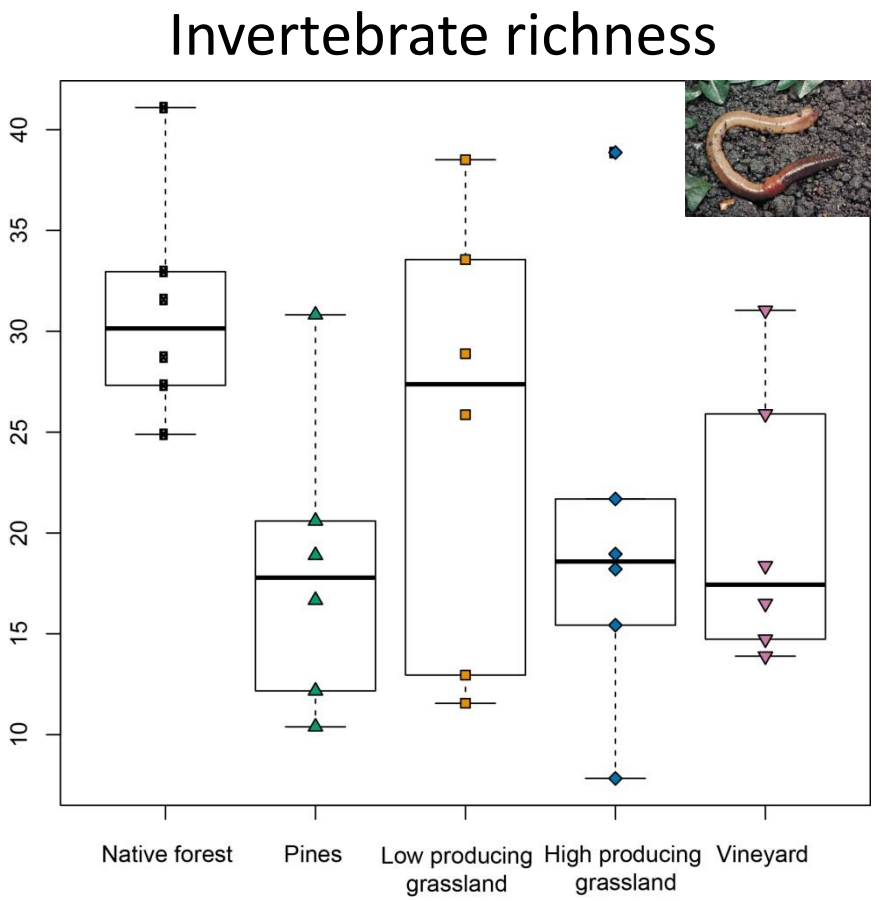
- (e.g.)Maintenance of invertebrate species richness
- Large variability within land use types
  - Environmental or management driven?



Application 1
Demonstrating environmental performance

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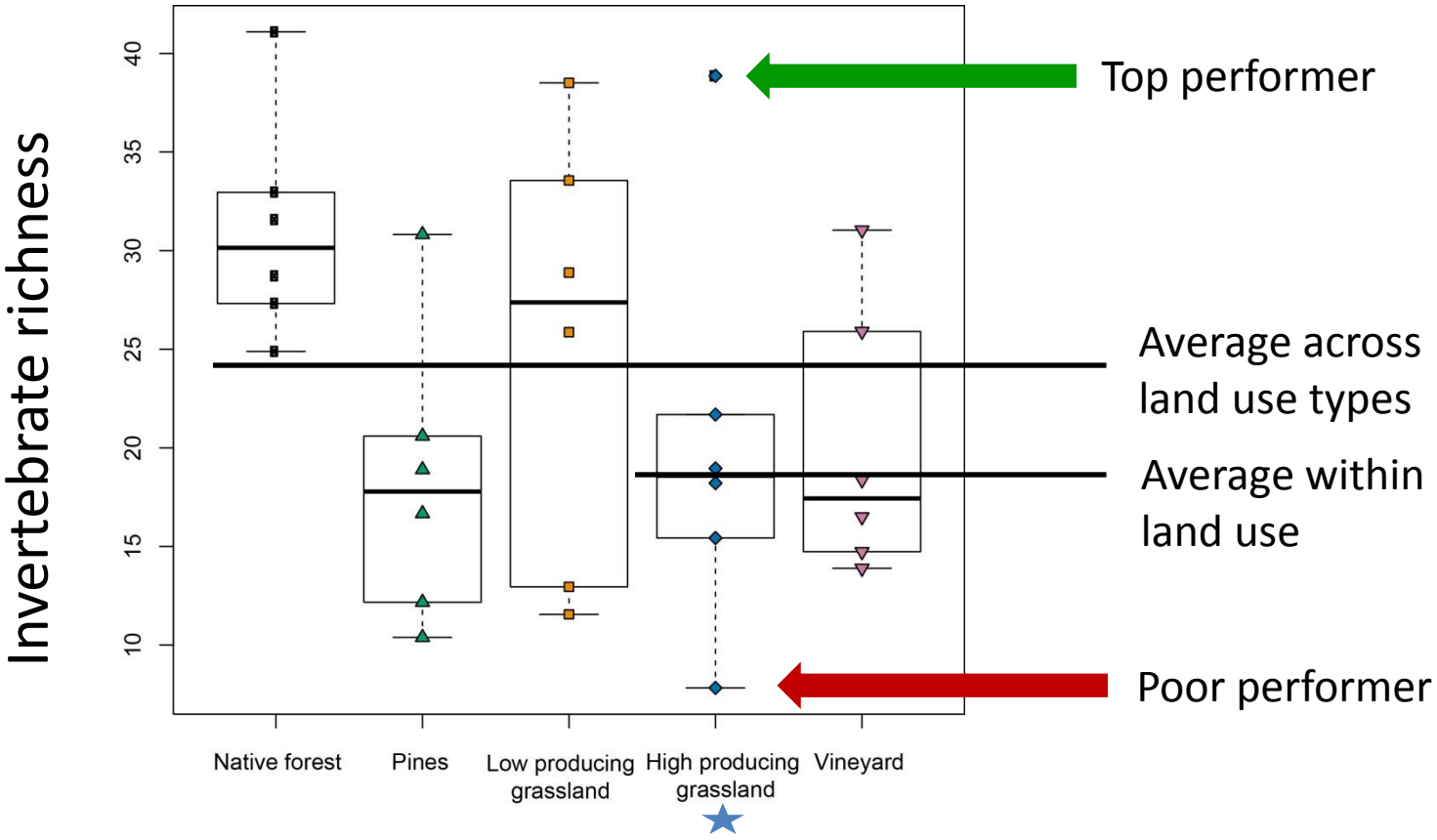
- Benchmarking within and across land use



Application 2
Improving farm management

Future farming systems depend on healthy living soil

- Some biota in particular are known to be essential for productive systems
  - nitrogen fixing bacteria, mycorrhizal fungi, predatory mites
- eDNA can be used as a tool to understand the effect of soil biota (or the soil community) on farm productivity

Application 2
Improving farm management

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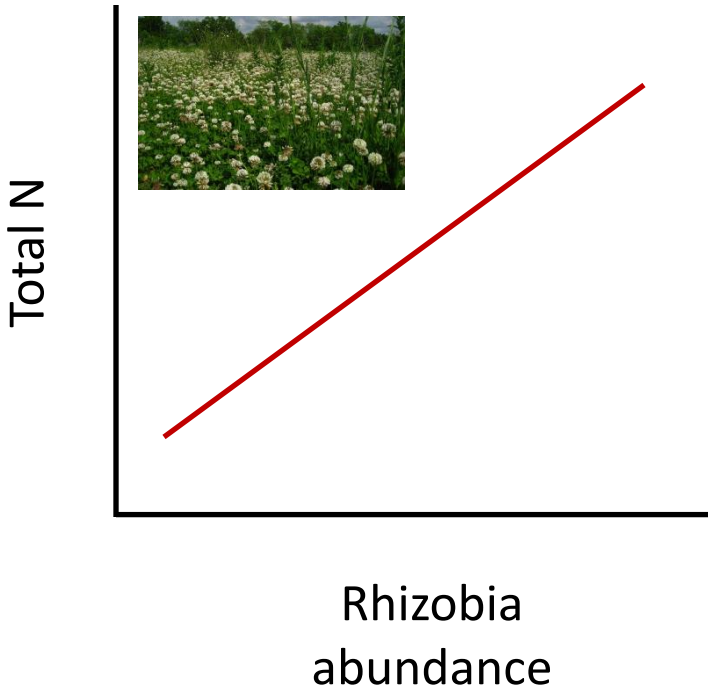
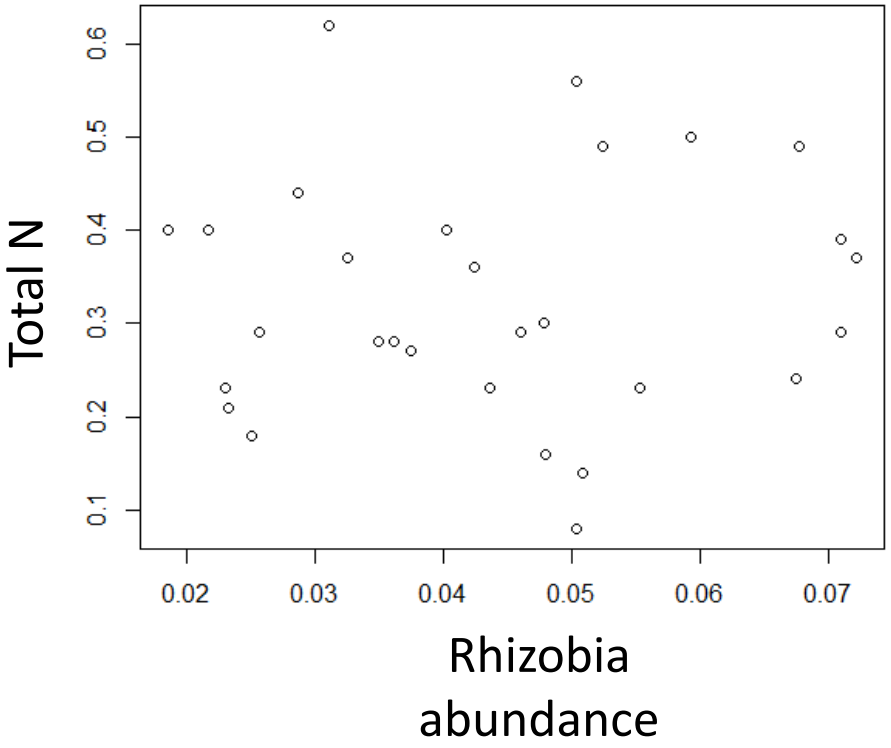
Nitrogen fixing Rhizobia bacteria

Theory

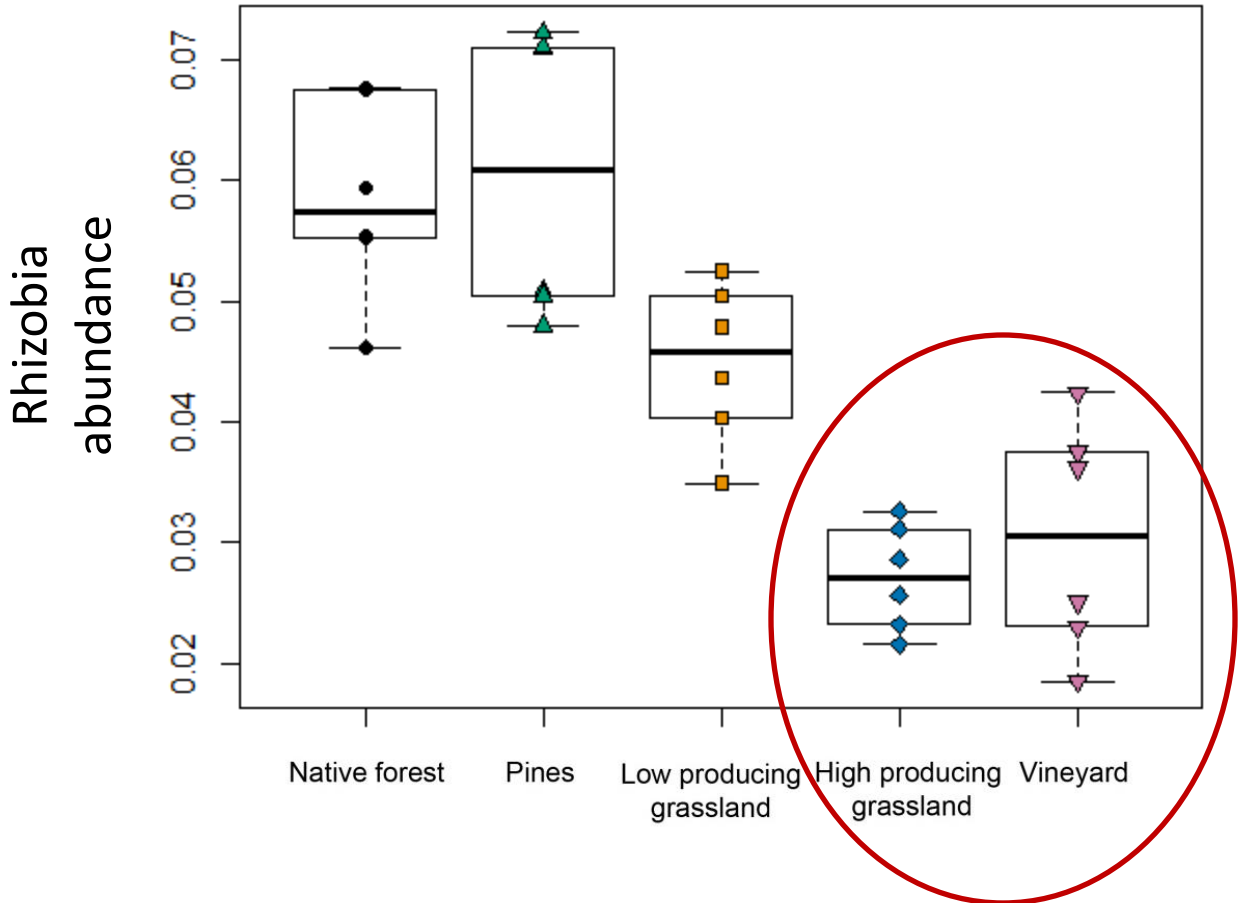


Phase 1 data



Application 2
Improving farm management

Nitrogen fixing Rhizobia bacteria



High N inputs from fertiliser

Application 2
Improving farm management

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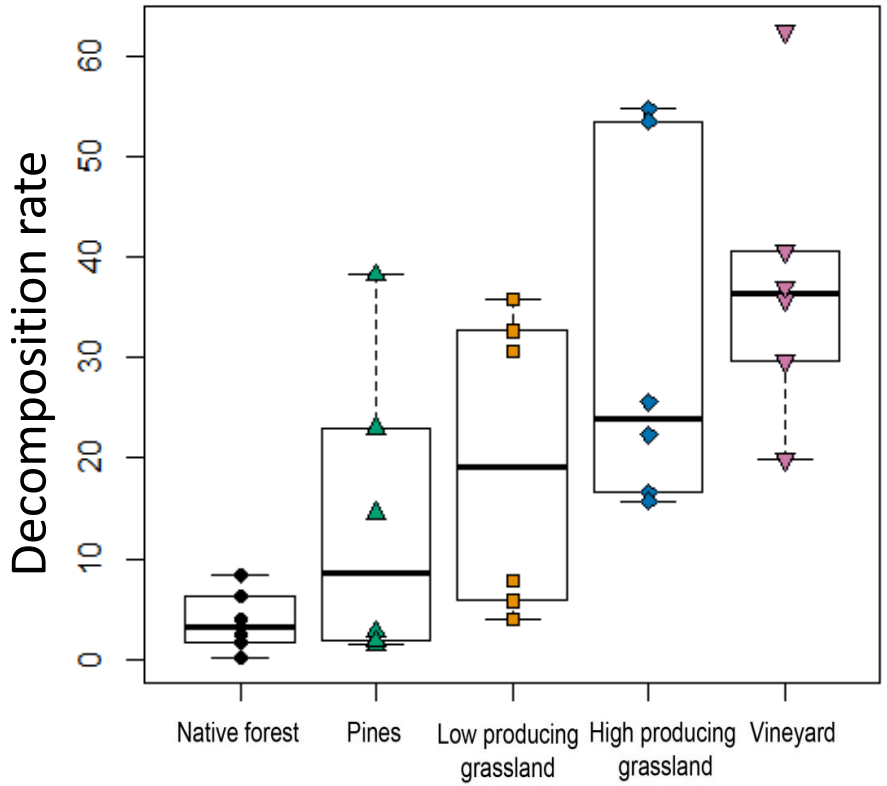
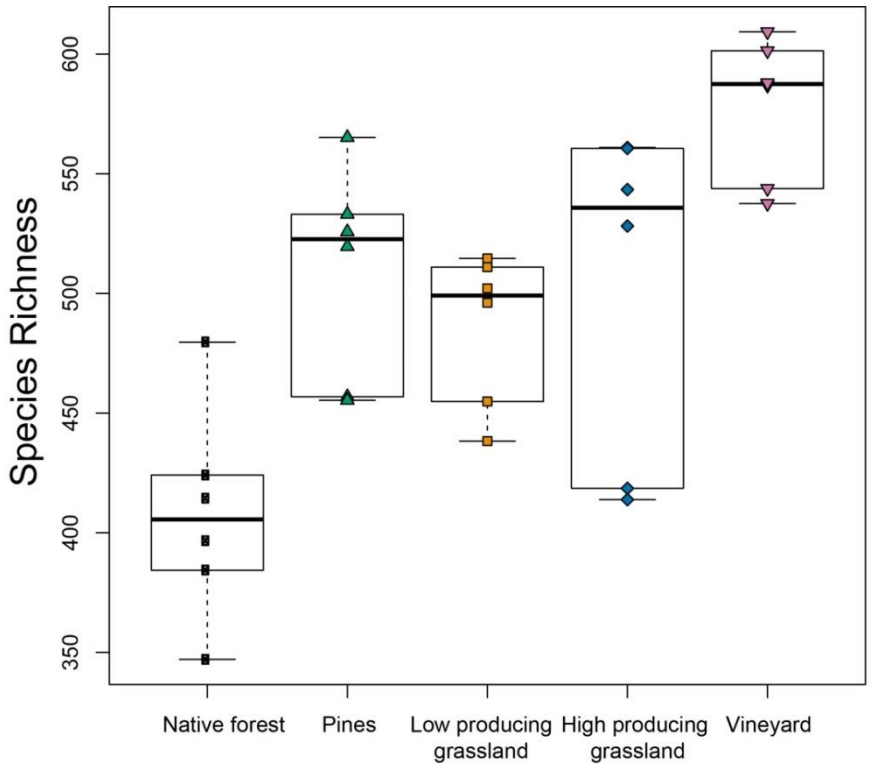
Biotic controls of decomposition

Phase 1 (bacteria)





Application 3
Biosecurity detection and monitoring

- Detecting presence of a threat before the symptoms appear
  - e.g. detecting kauri dieback before trees die

- Requires better reference databases for many taxa

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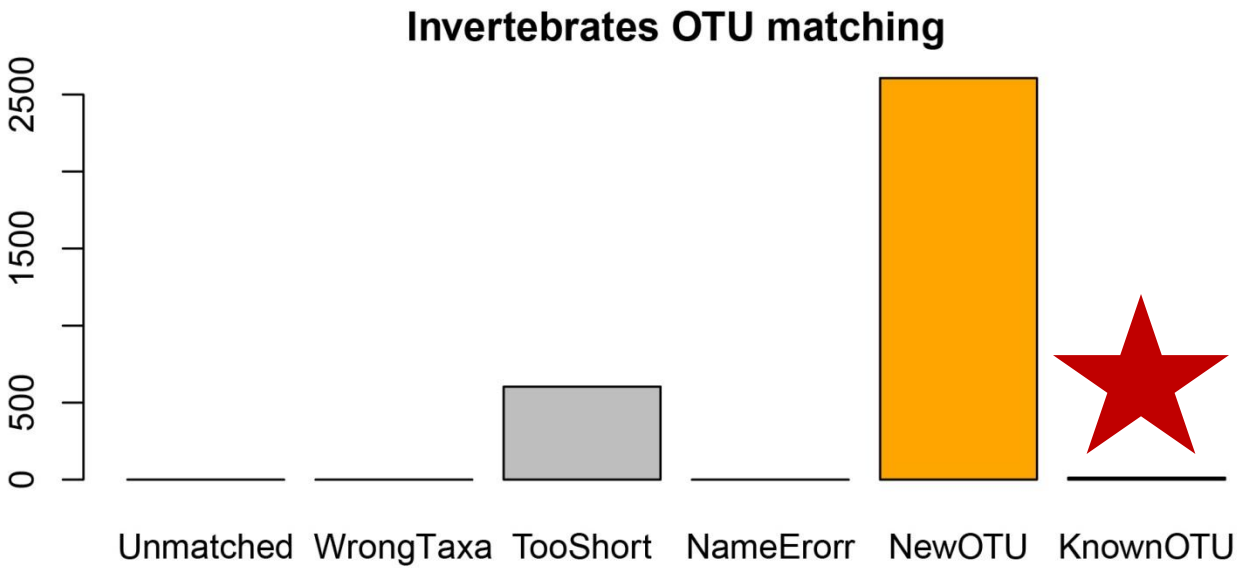
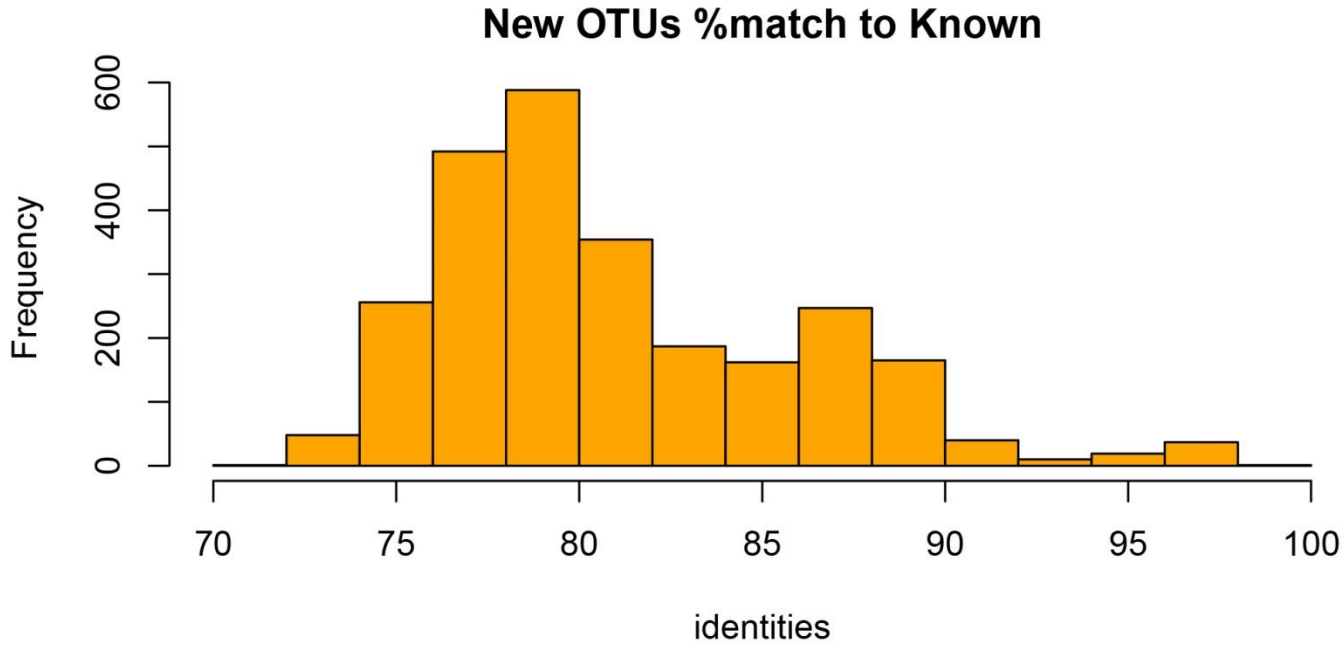




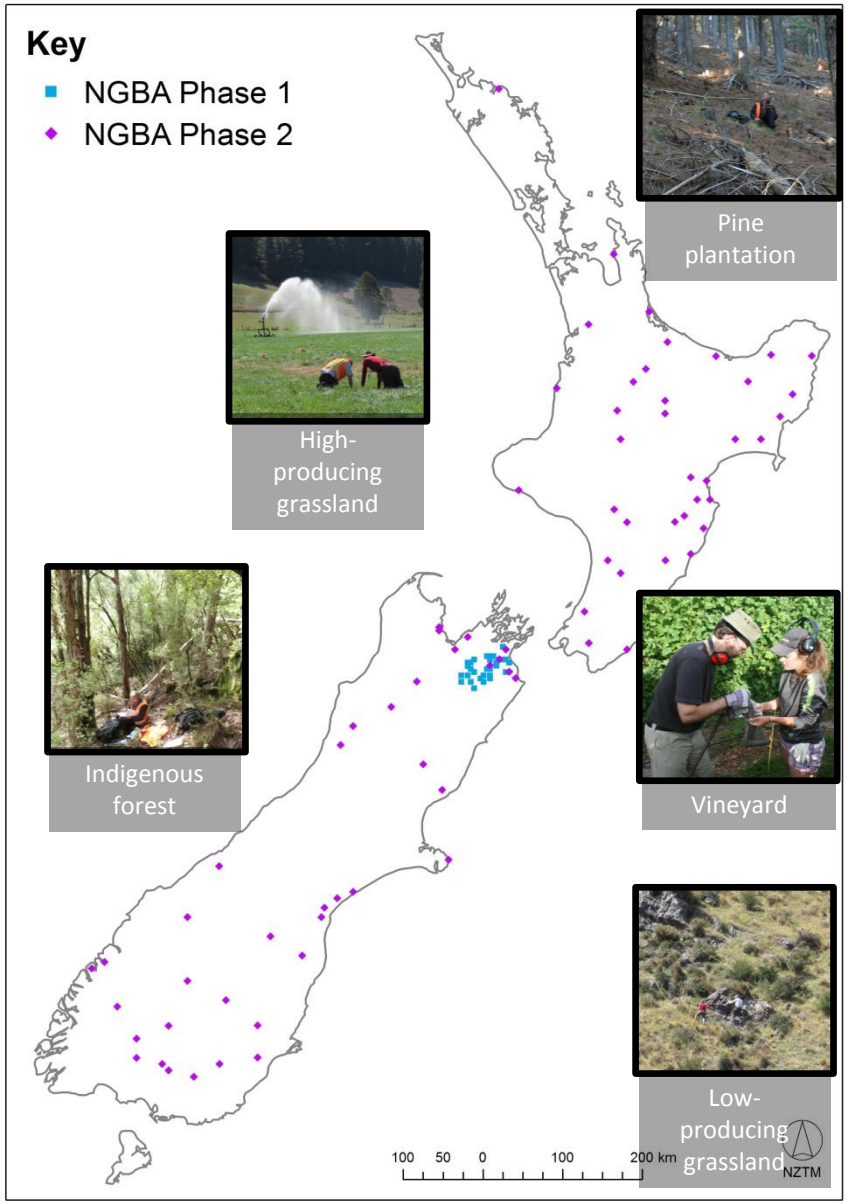
NEW ZEALAND'S BIOLOGICAL HERITAGE

Ngā Koiora Tuku Iho

Future directions

- Phase 2 of MBIE Smart Idea
- Biological Heritage National Science Challenge eDNA project

Phase 2 – National & local variability




14/01/2015

National
SCIENCE
Challenges

NEW ZEALAND'S BIOLOGICAL HERITAGE

Ngā Koiora
Tuku Iho

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NEW ZEALAND'S BIOLOGICAL HERITAGE

Ngā Koiora Tuku Iho

National SCIENCE Challenges

MISSION

Reverse the decline of New Zealand's biological heritage, through a national partnership to deliver step change in research innovation, globally-leading technologies, and community and sector action.

A national framework for bioheritage assessment across natural and productive landscapes (Project 1.3)

NEW ZEALAND'S BIOLOGICAL HERITAGE

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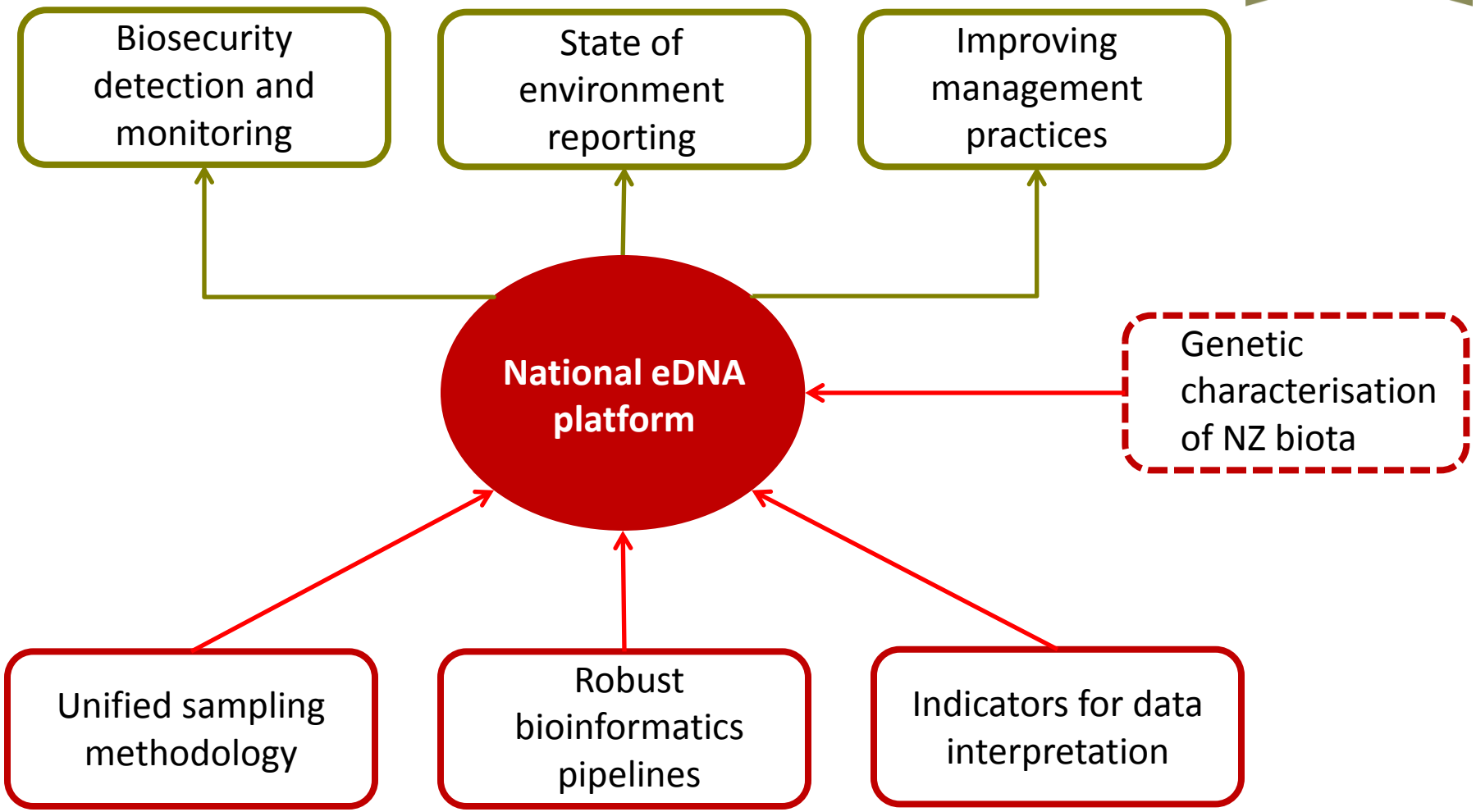
- Aims to develop a New Zealand-wide framework for biodiversity assessment using environmental DNA data

Two key focus areas

- Develop methods and infrastructure to realise the full potential of eDNA technology
- Build partnerships to consistently apply eDNA technology across the whole New Zealand landscape

5 year project outcome



NEW ZEALAND'S BIOLOGICAL HERITAGE

Ngā Koiora Tuku Iho

eDNA end-user workshop

Feeling inspired & thinking of potential eDNA applications?

We want you to help shape the future of environmental DNA research in New Zealand

When: September 28th-29th 2015

Where: Wellington

Register your interest now

Robert Holdaway - holdawayr@landcareresearch.co.nz

Kevin Collins - kcollinsconsult@gmail.com

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