

Metabarcoding of flying arthropods reveals patterns of biodiversity in UK plantation forests

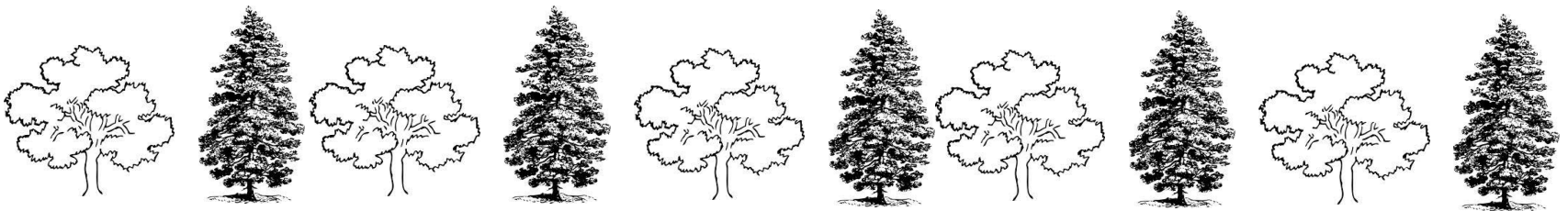
Catharine Bruce

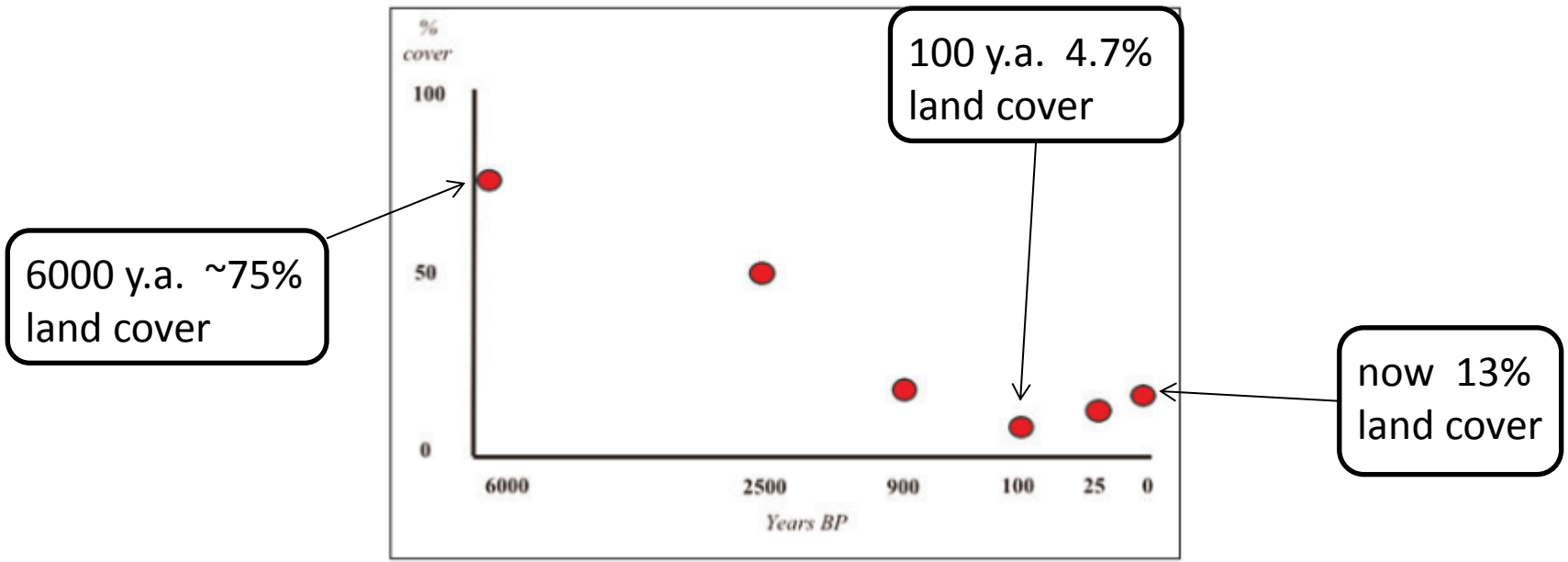
Chen-Xue Yang

Yin-Qiu Ji

Nadia Barsoum

Douglas Yu

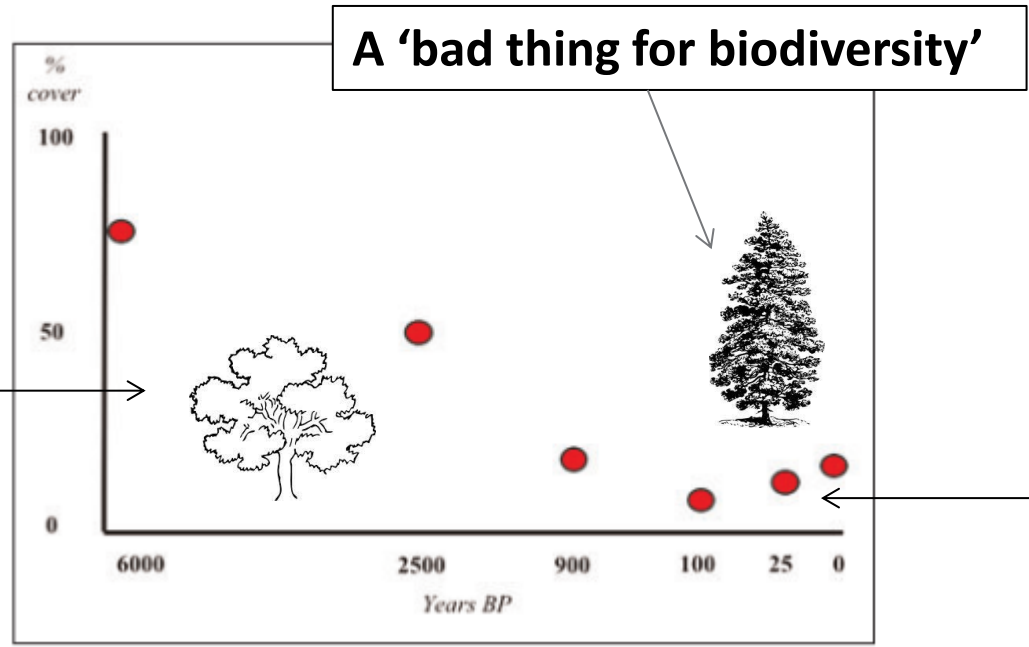




Historical forest cover in the UK (from Rackham, 1986)

Forest cover declined until the beginning of the 20th century, then reforestation

Broadleaf



Non-native conifer

Historical forest cover in the UK (from Rackham, 1986)

Forest cover declined until the beginning of the 20th century, then reforestation

Non-native conifer monocultures planted in place of broadleaf woodland

Convention for Biological Diversity (CBD), 1992 –

emphasis on management for biodiversity as well as for commercial productivity

Sustainable Forest Management

1. Maintenance and appropriate enhancement of forest resources and their contribution to **global carbon cycles**
2. Maintenance of forest ecosystems' **health and vitality**
3. Maintenance and encouragement of **productive functions** of forests (wood and non-wood)
4. Maintenance, conservation and appropriate enhancement of **biological diversity** in forest ecosystems
5. Maintenance, conservation and appropriate enhancement of **protective functions** in forest management (notably soil and water)
6. Maintenance of other **socio-economic functions** and conditions.

Key response variable – but measurement is a problem!

Sustainable Forest Management

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Key response variable – but measurement is a problem!

How can plantation forests be best managed for biodiversity?

Policy in Europe has been guided mostly by intuition

Make plantations
resemble native forest

More (native) tree
species diversity

More structural
complexity

More
deadwood

Setting the agenda

“a major scientific thrust will be needed to transform ideas on managing forests for biodiversity into practical, effective tools. The key components of this thrust will be careful natural history, controlled and replicated field experiment, and intensive monitoring”.

Simberloff (1999)

Key response variable – but measurement is a problem!

How can plantation forests be best managed for biodiversity?

Need biodiversity data

Direct

Fast

METABARCODING

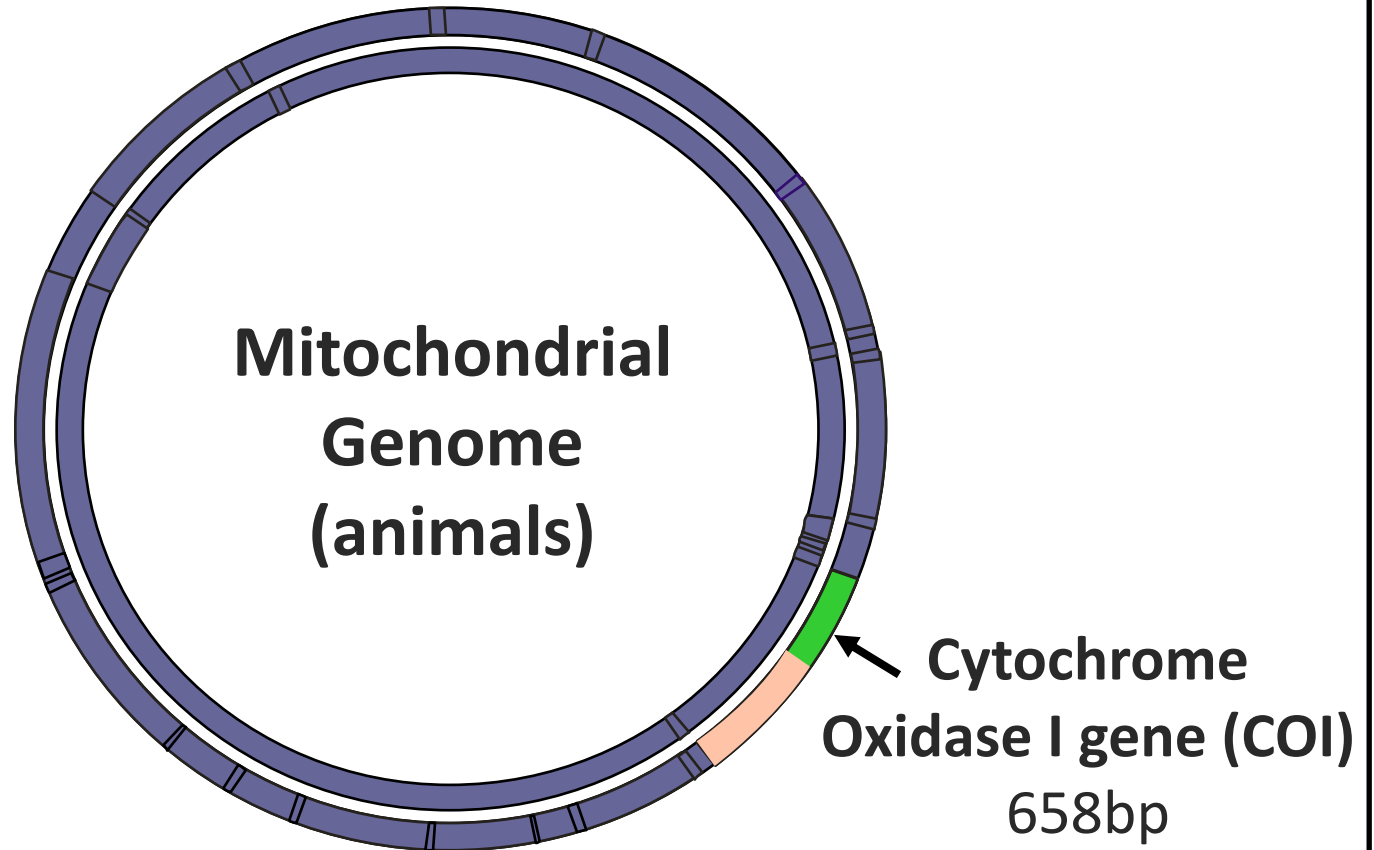
Many taxonomic
groups

No taxonomic
expertise

Powerful tool for informing management and policy decisions

DNA Barcoding

Established since 2003



Same within species but differs between species

DNA Barcoding

mosquito-COI :

CGCGACAATGATTATTTTCAACTAACCATAAGGATATTGGAACATTATATTTT
ATTTTTGGAGCTTGAGCAGGAATAGTAGGAACTTCTCTAAGTATTTTAATTTCG
AGCAGAATTAGGACACCCTGGAGCCTTTATTGGTGATGATCAAATTTATAATG
TTATTGTAACAGCTCATGCTTTTATTATAATTTTTTTTTATAGTTATACCTATT
ATAATTGGAGGATTTGGAAATTGACTAGTCCCTCTAATACTAGGGGCCCCAGA
TATGGCTTTCCTCGAATAAATAATATAAGATTTTGAATATTACCCCCCTCTT
TAACTCTTCTAATTTCTAGAAGTATAGTAGAAAATGGAGCTGGAACAGGGTGA
ACTGTATATCCTCCTCTATCCTCAGGAATTGCTCATGCAGGAGCTTCAGTAGA
TTTAGCTATTTTTTCATTACATTTAGCAGGAATTTCTTCAATTTTAGGAGCAG
TTAATTTTATTACAACAGTTATTAATATACGAGCACCAGGAATTA CTCTTGAC
CGAATACCGTTATTCGTTTGATCTGTAGTAATTACAGCAGTATTATTACT
TTCTTTACCAGTATTAGCTGGAGCTATTACTATACTTTTAAACAGATCGAAACT
TAAATACATCATTC

DNA Barcoding



PCR amplify
barcode region

GTTATACCTATTATAATTGGAGGATTTGGA

BOLDSYSTEMS Databases | Taxonomy | Identification | Workt

Advancing species identification and discovery by providing an integrated environment for the assembly and application of DNA barcodes.

Sequence statistics

779
072
039

Fungi & Other Life 2,328 Barcode Sequences

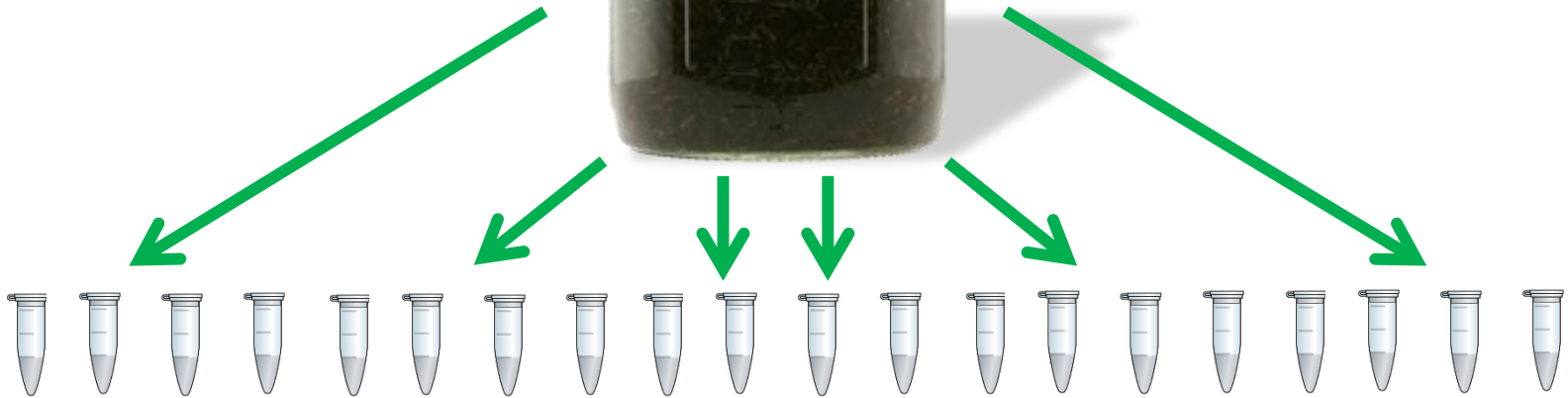
Taxonomy Search

SWALLOWTAIL BUTTERFLY

DNA Barcoding

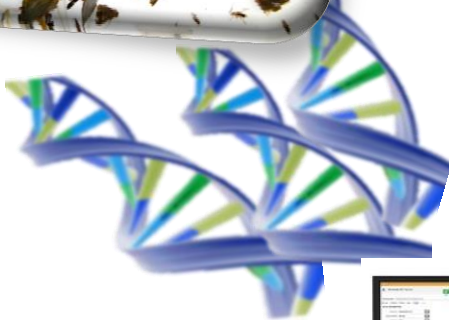
slow

expensive



Separate sequencing reaction for each specimen

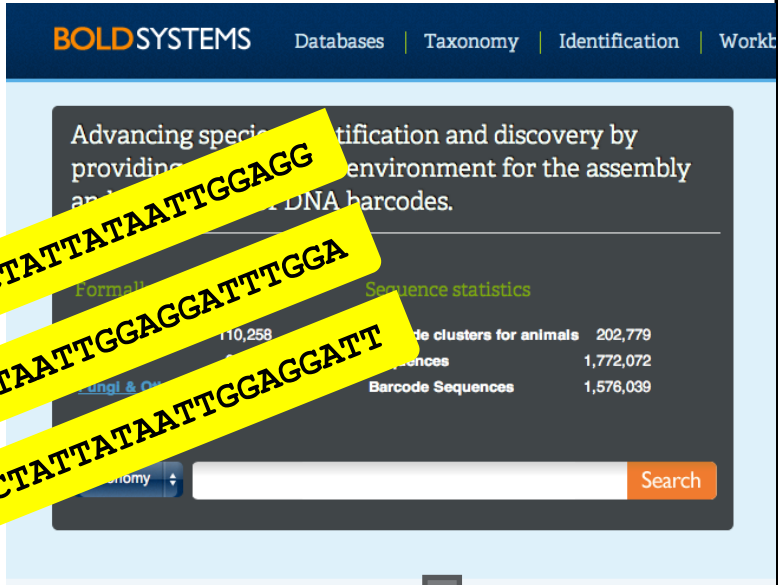
Metabarcoding



PCR amplify
barcode region



ATTTGGAGTTATACCTATTATAATTGGAGG
GTTATACCTATTATAATTGGAGGATTGGA
TGGAGTTATACCTATTATAATTGGAGGATT



SWALLOWTAIL BUTTERFLY
CARABID BEETLE 1
CARABID BEETLE 2

Next generation sequencers can sequence many taxa in parallel

Metabarcoding



You can sequence many soups in a single run

	Site 1	Site 2	Site 3	Site 4
Carabus sp.	0	0	1	15
Mosquito 345	426	33	0	2
Lasius niger	25	100	2	5

Testing using artificial communities of known composition

Metabarcoding

Methods in Ecology and Evolution



Methods in Ecology and Evolution

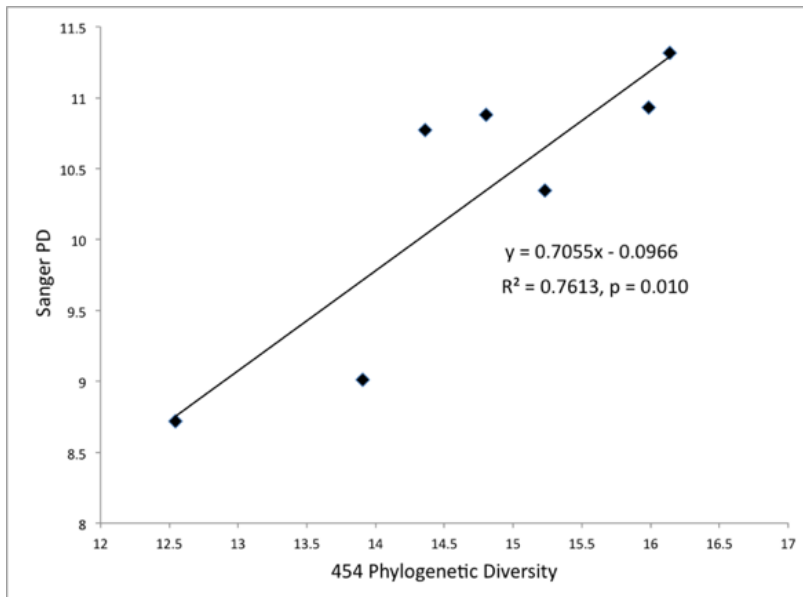
doi: 10.1111/j.2041-210X.2012.00198.x

Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring

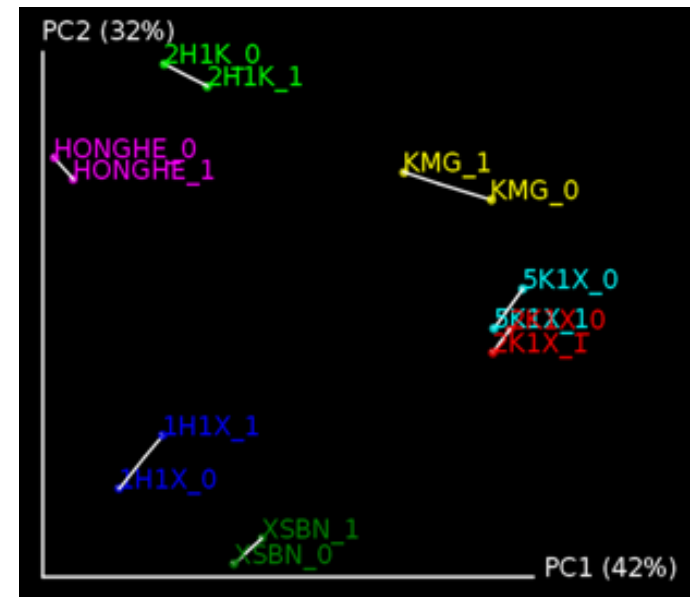
Douglas W. Yu^{1,2*†}, Yinqiu Ji^{1†}, Brent C. Emerson^{2‡}, Xiaoyang Wang¹, Chengxi Ye¹, Chunyan Yang¹ and Zhaoli Ding³

*Some drop-out of taxa
but ecological patterns
very well recovered*

Species richness



Species turnover



Testing against gold-standard conventional biodiversity assessments

Metabarcoding

Yinqiu Ji,^{1†} Louise Ashton,^{2†} Scott
M. Pedley,^{3†} David P.
Edwards,^{4,5,6†} Yong Tang,⁷
Akihiro Nakamura,^{2,8} Roger
Kitching,² Paul M. Dolman,³ Paul
Woodcock,⁹ Felicity A. Edwards,⁹
Trond H. Larsen,¹⁰ Wayne W.
Hsu,¹¹ Suzan Benedick,¹² Keith C.
Hamer,⁹ David S. Wilcove,^{4,6}
Catharine Bruce,¹³ Xiaoyang
Wang,¹ Taal Levi,^{14,15} Martin
Lott,¹⁶ Brent C. Emerson¹⁷ and
Douglas W. Yu^{1,13*}

ECOLOGY LETTERS

Ecology Letters, (2013)

doi: 10.1111/ele.12162

Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding

- *Accurately recovers ecological patterns*
- *Led to the same management decisions*
- *Fraction of the time and cost*

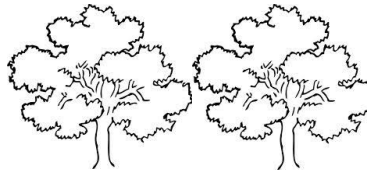
Arthropod biodiversity in UK plantation forests



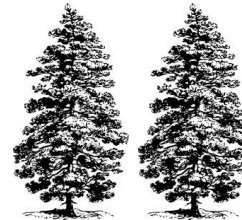


Sampled flying arthropods from 15 forest stands

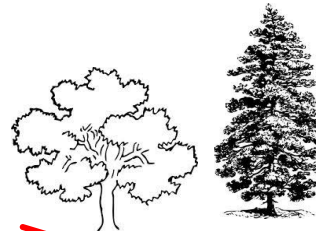
5 x *pure oak* crops



4 x *pure Scots pine* crops



6 x *mixed oak and Scots pine* crops



Stands
approximately
1-4 ha

Understory
allowed to
regenerate

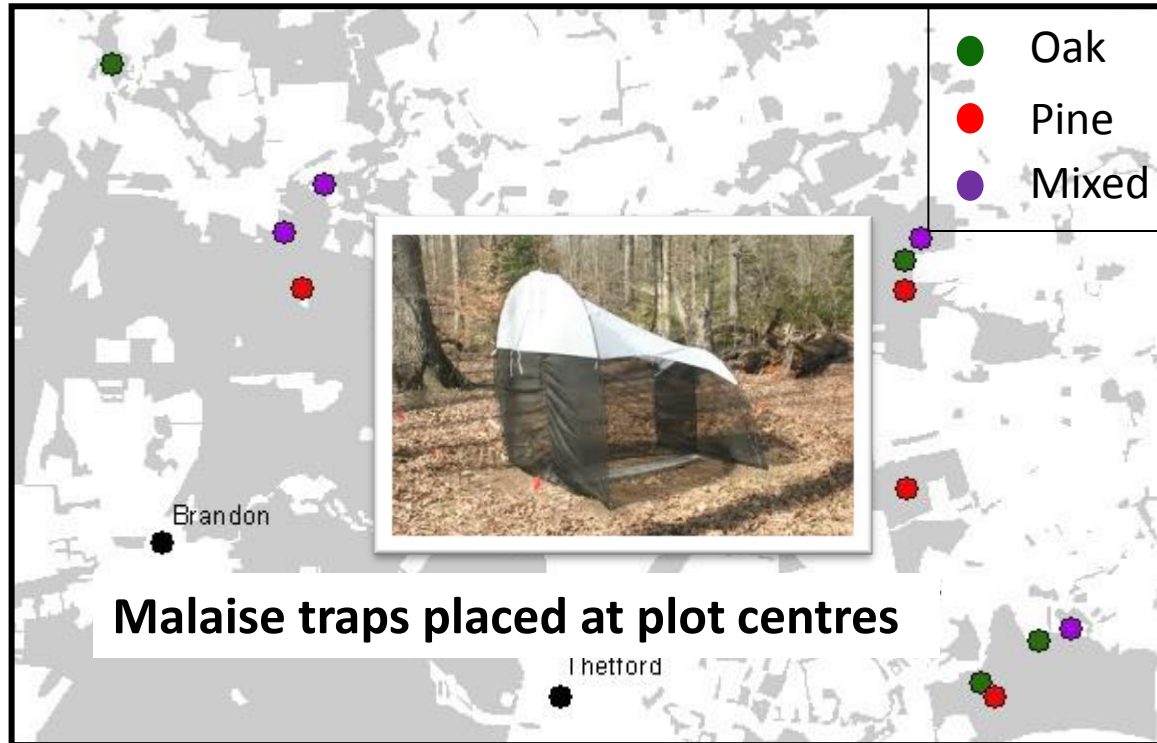
**Suggested by
INTUITION!!**



Thetford Forest, UK



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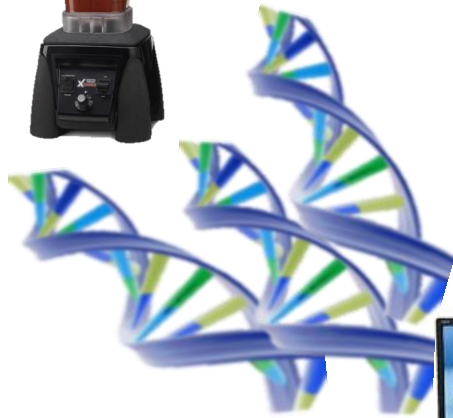
*8 weeks of trapping – samples collected
each week*



x 120



x 120



PCR amplify
barcode region



x 1

Metabarcoding

BOLD SYSTEMS Databases | Taxonomy | Identification | Workflows

Advancing species identification and discovery by providing a user-friendly environment for the assembly and analysis of DNA barcodes.

Formal name: *Swallowtail butterfly*

Sequence statistics

Number of clusters for animals	202,779
Number of sequences	1,772,072
Barcode Sequences	1,576,039

Search

ATTTGGAGTTATACCTATTATAATTGGAGG

GTTATACCTATTATAATTGGAGGATTGGA

TGGAGTTATACCTATTATAATTGGAGGATT



SWALLOWTAIL BUTTERFLY
CARABID BEETLE 1
CARABID BEETLE 2

Next generation sequencers can sequence many taxa in parallel



- > Initial quality control (QIIME, PyNAST)
- > Homopolymer errors corrected (MACSE)
- > *De novo* & reference based chimera detection (UCHIME)
- > OTU-picking at 97% similarity (CROP)
- > Taxonomic assignment (SAP)

Bioinformatics
pipeline from Yu *et al.*, 2012 in QIIME
environment

< 2 months after sampling

1128 arthropod species detected

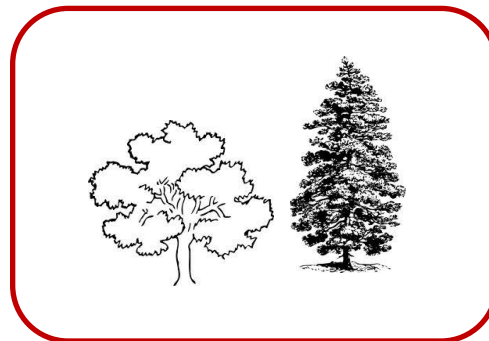
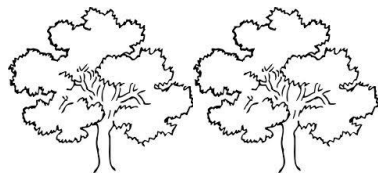
> 60% are Diptera

Lower prevalence of Hymenoptera, Hemiptera, Lepidoptera,
Coleoptera, Arachnida, and others



Questions

Are mixed crop stands better for biodiversity than single crop stands?

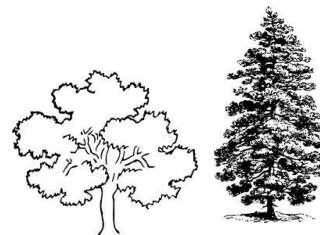
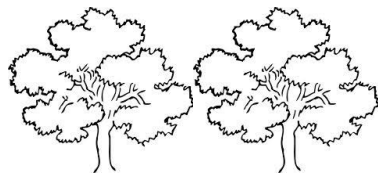




Questions

Are mixed crop stands better for biodiversity than single crop stands?

Do structural indicators predict diversity?



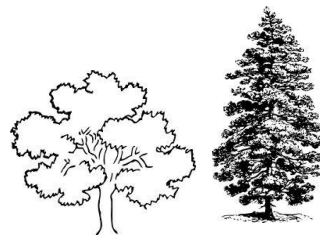
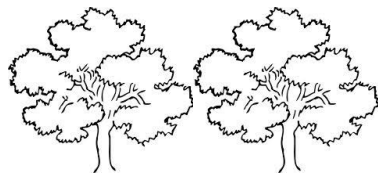


Questions

Are mixed crop stands better for biodiversity than single crop stands?

Do structural indicators predict diversity?

What management strategy will maximise biodiversity across the whole landscape?
(gamma diversity)



Results



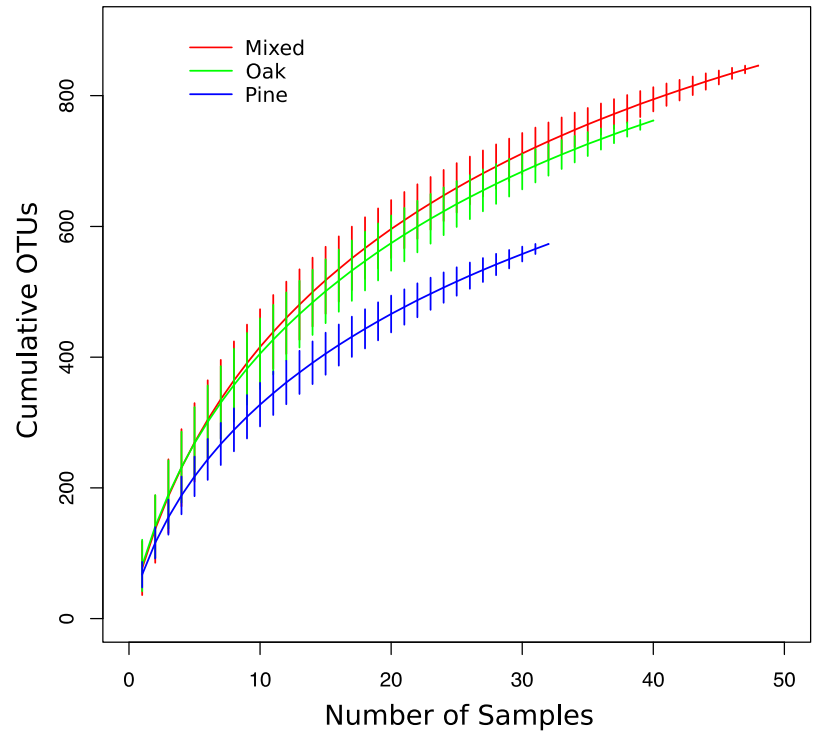
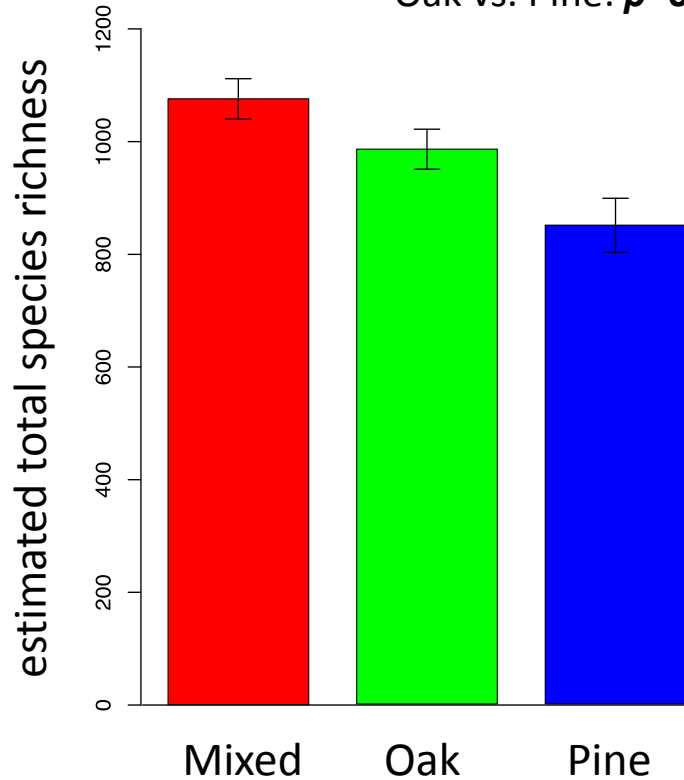
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Result 1: *Mixed stands are not more species rich than Oak stands*

Mix vs. Oak: $p=0.076$

Mix vs. Pine: $p=0.001$

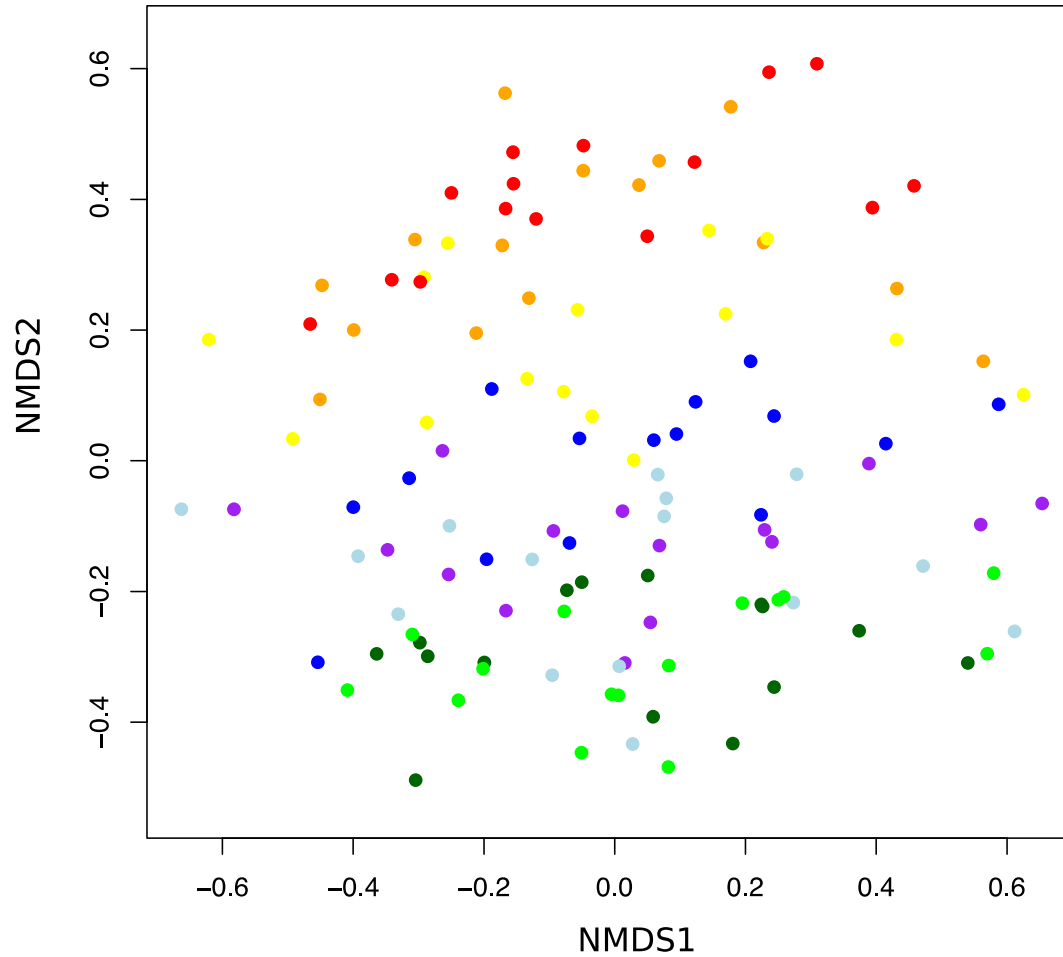
Oak vs. Pine: $p=0.041$



Results – Beta diversity



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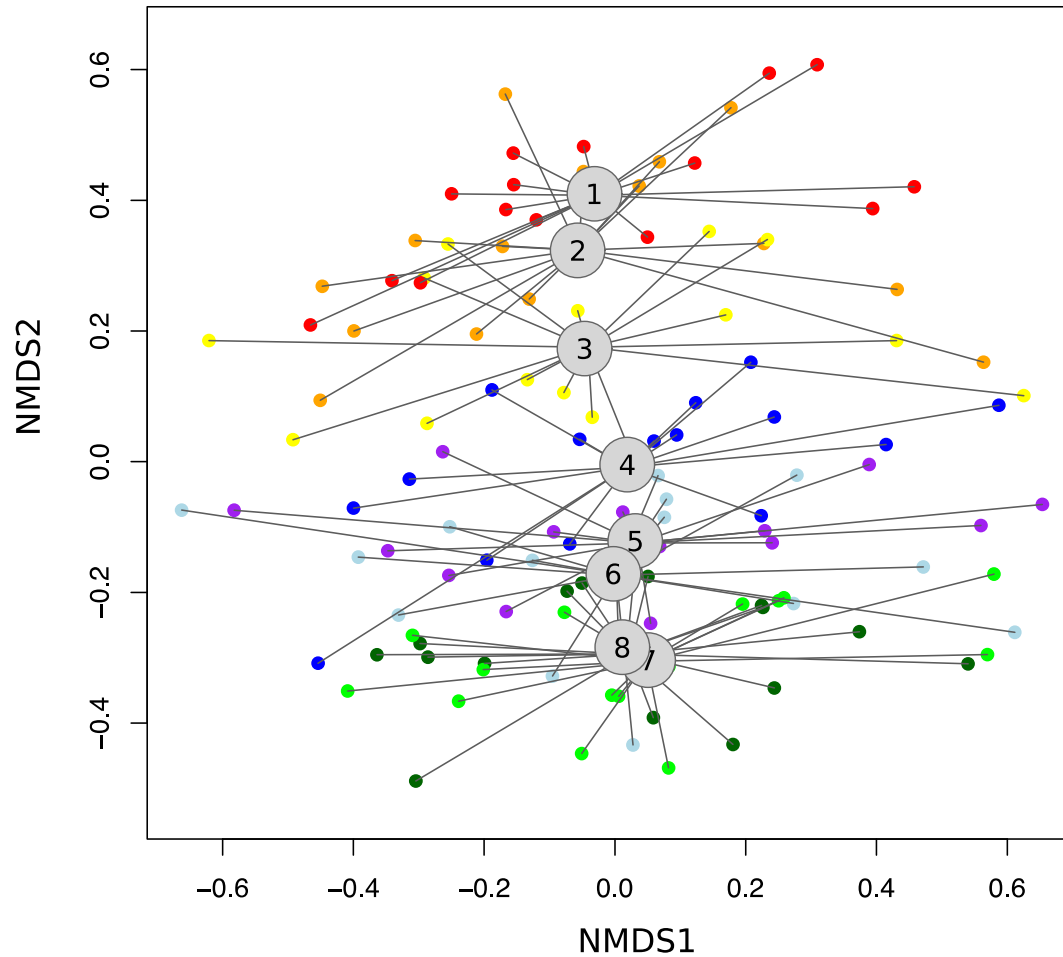
- | | | | |
|----------|----------|----------|----------|
| ● Week 1 | ● Week 2 | ● Week 3 | ● Week 4 |
| ● Week 5 | ● Week 6 | ● Week 7 | ● Week 8 |

Results – Beta diversity



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Result 2: *Communities change over time*

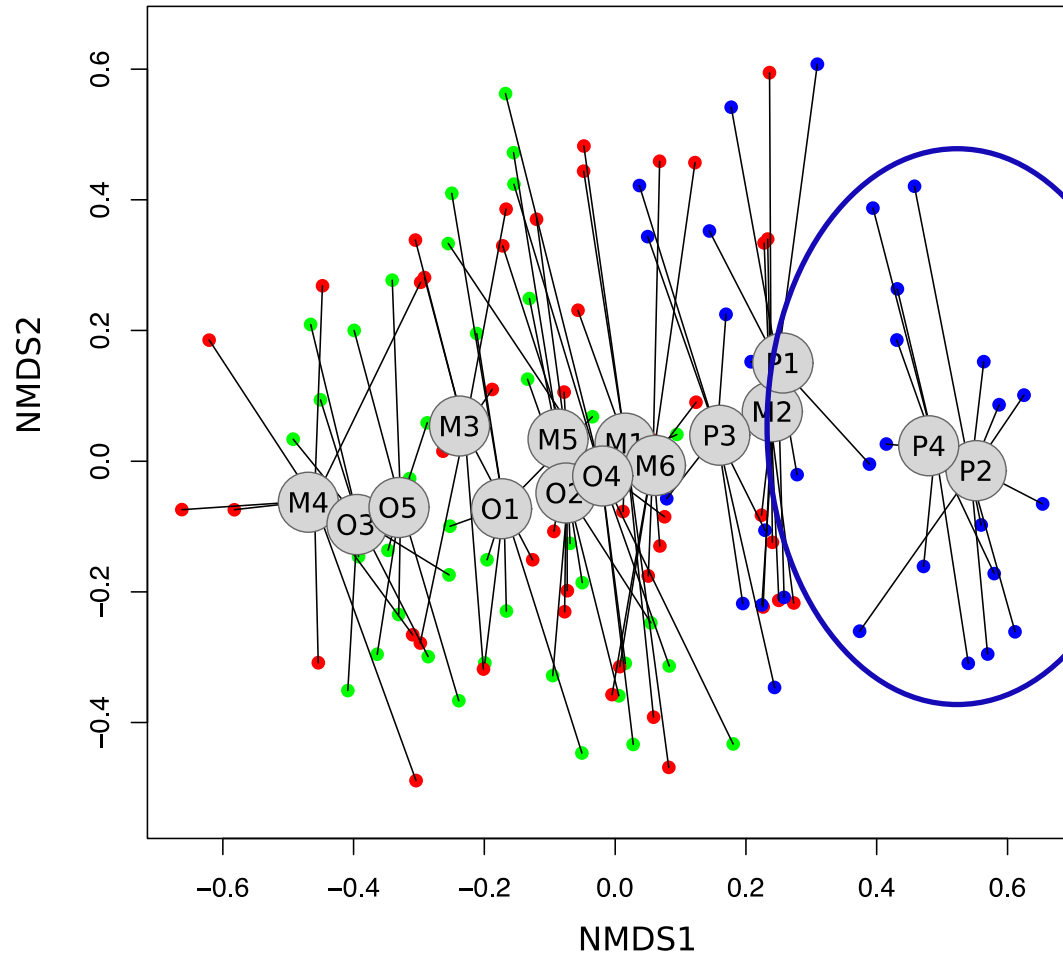


- | | | | |
|----------|----------|----------|----------|
| ● Week 1 | ● Week 2 | ● Week 3 | ● Week 4 |
| ● Week 5 | ● Week 6 | ● Week 7 | ● Week 8 |



Results – Beta diversity

The horizontal axis sorts samples by site



**Important for
regional
biodiversity**

'Bad-for-biodiversity' forest?



'Bad-for-biodiversity' forest?

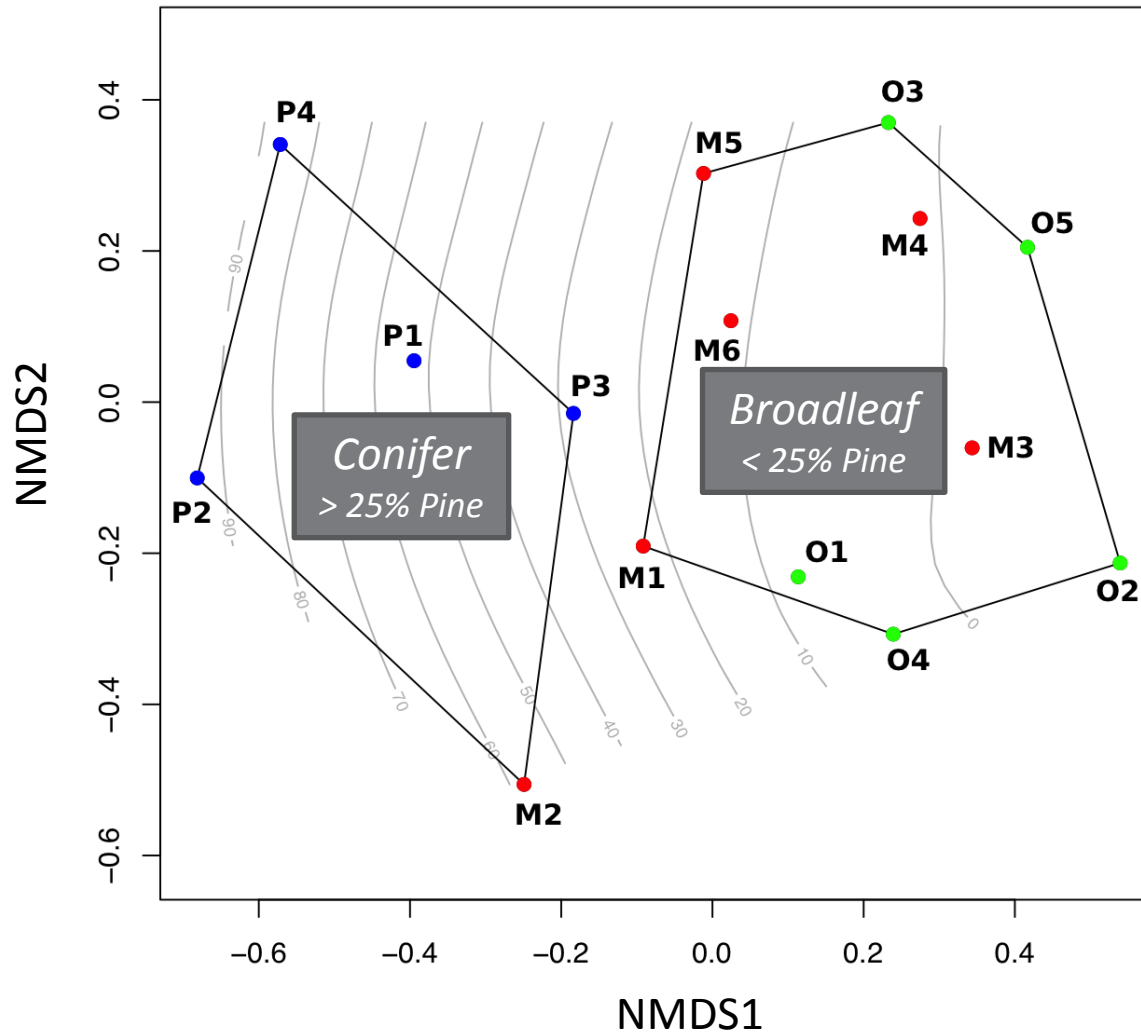


May have biodiversity benefits



Results

Species turnover strongly affected by dominance of conifers

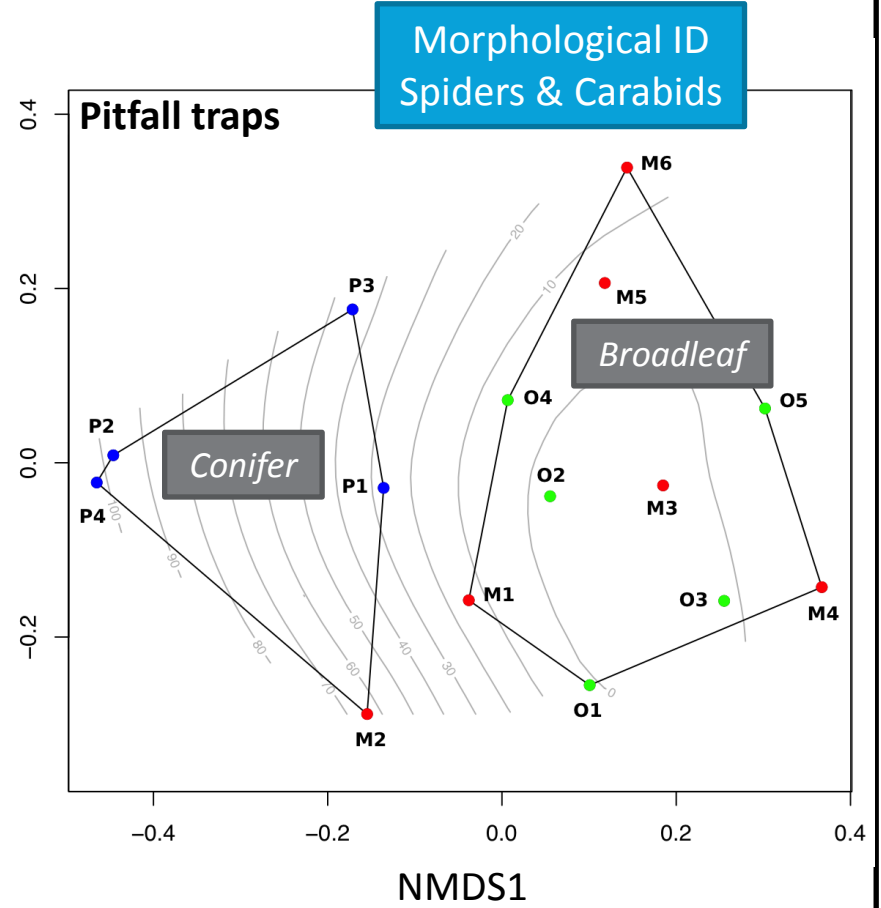
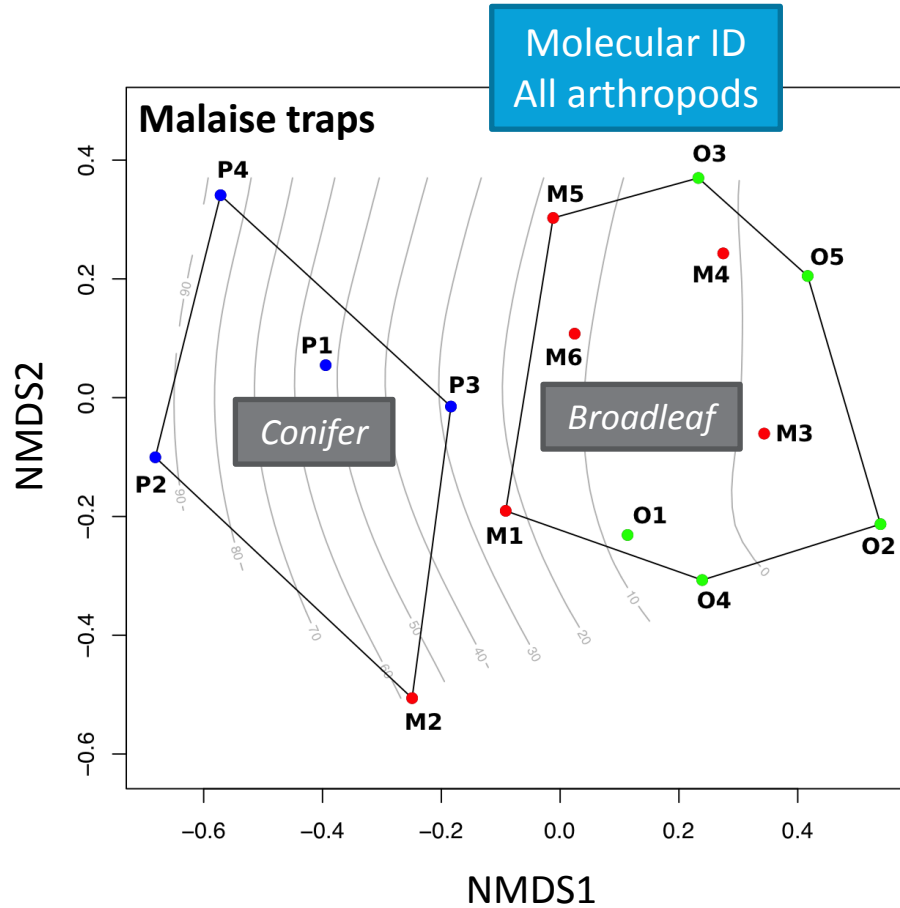


mvabund:
 $P = 0.006$
LR = 3163

Results - Indicators



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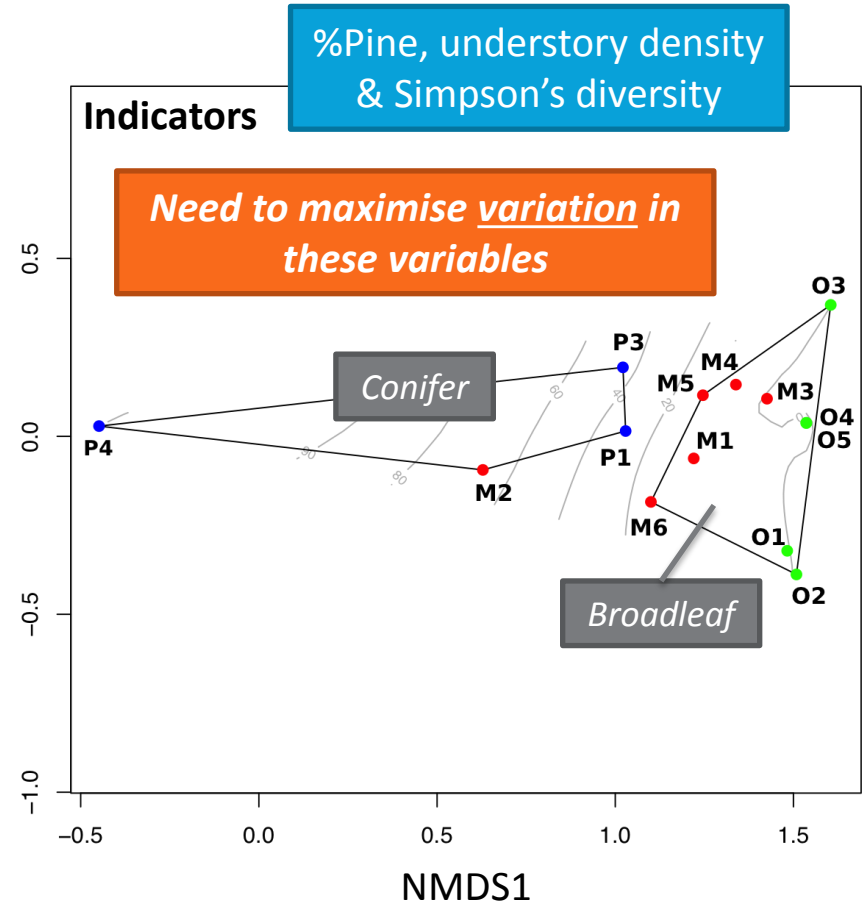
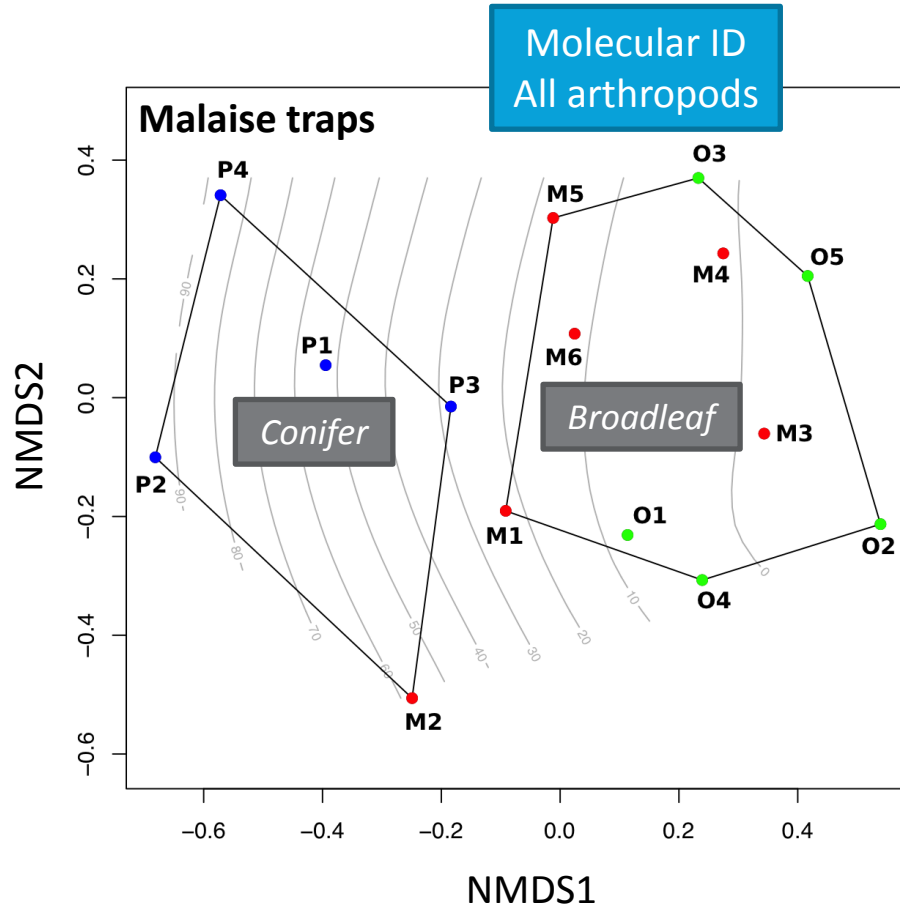


Procrustes $R^2 = 0.71$
 $p=0.001$

Results - Indicators



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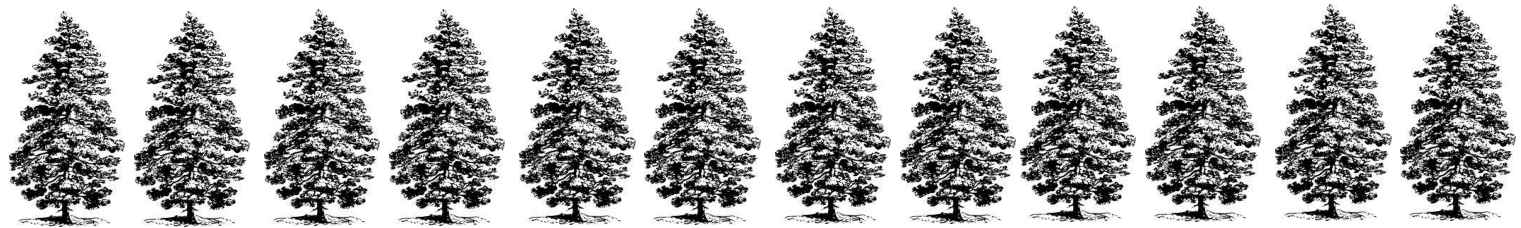


Procrustes $R^2 = 0.61$
 $p=0.003$

“Management for diversity calls for diversity of management”

Lindenmayer, Franklin, and Fischer, 2006

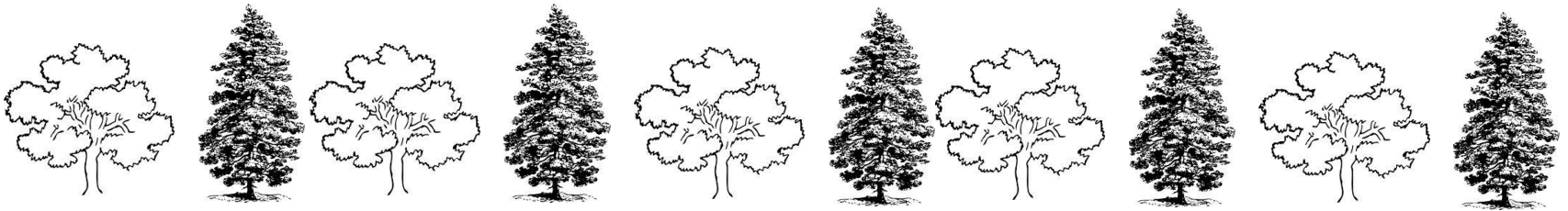
i.e. Don't do the same thing everywhere



“Management for diversity calls for diversity of management”

Lindenmayer, Franklin, and Fischer, 2006

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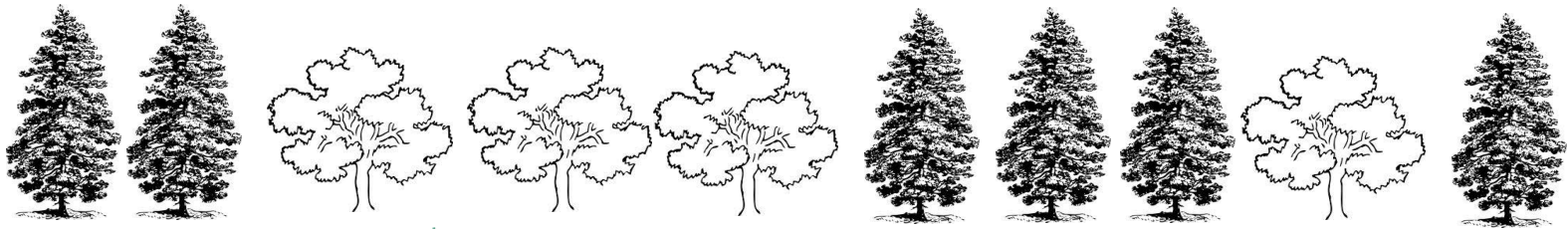


“Management for diversity calls for diversity of management”

Lindenmayer, Franklin, and Fischer, 2006

i.e. Don't do the same thing everywhere

Thank you



Results – Beta diversity



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