Centre for Ecosystem Genomics

# Getting it Together: Combining plant and mammal DNA with Lipid Biomarkers from Irish and Scottish Lakes

University of Southampton

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With

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Arts & Humanities Research Council

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LECA Laboratoire d'Écologie Alpine

## Questions & Sites:

**In a lake with pollen, diatoms – what can we add with sedaDNA and lipid biomarkers?**

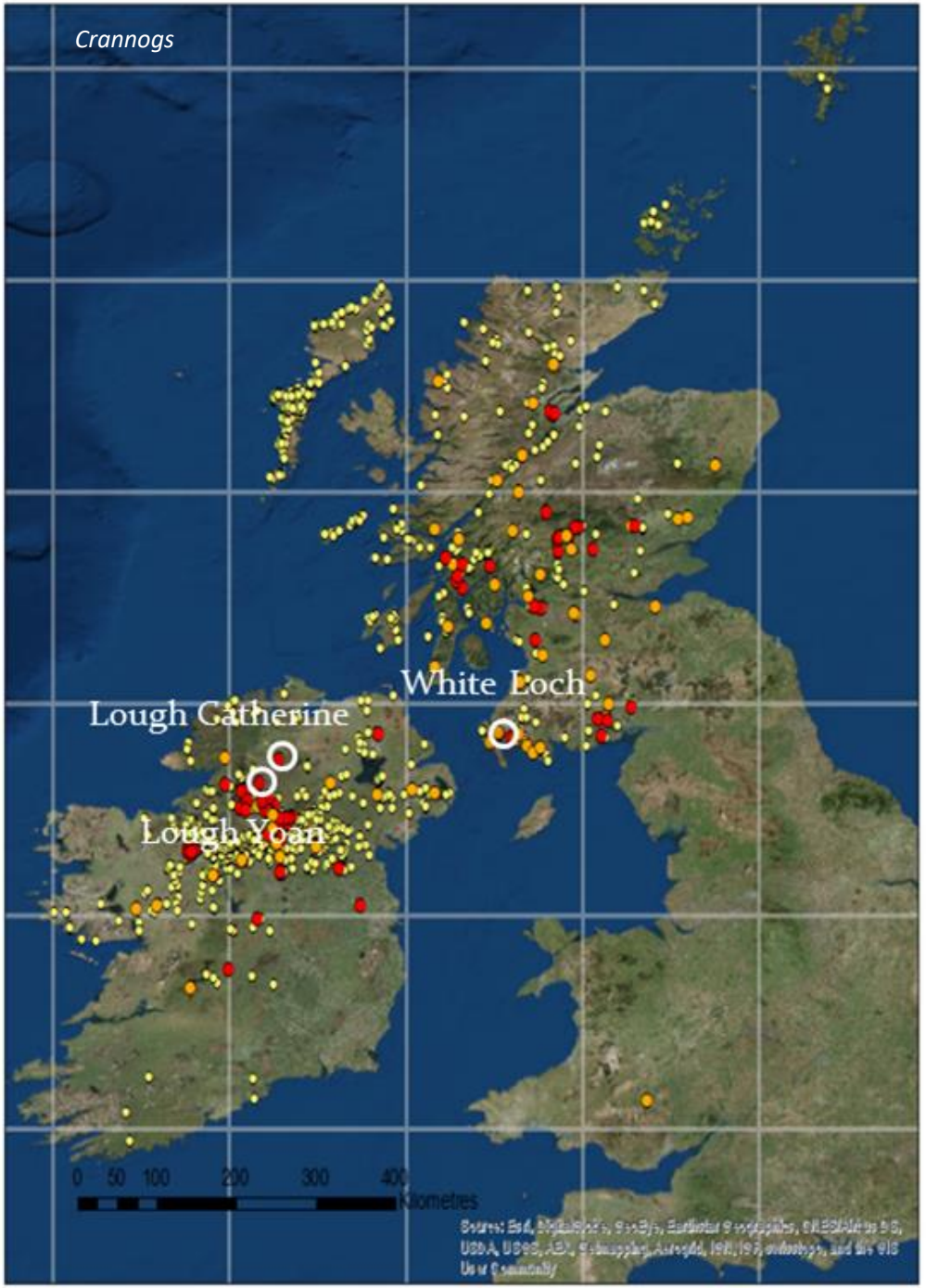
**Proximal coring to archaeological site -** can it provide: chronology, longevity of use, activities..

**Can faecal steroids indicate when the sites are occupied?**

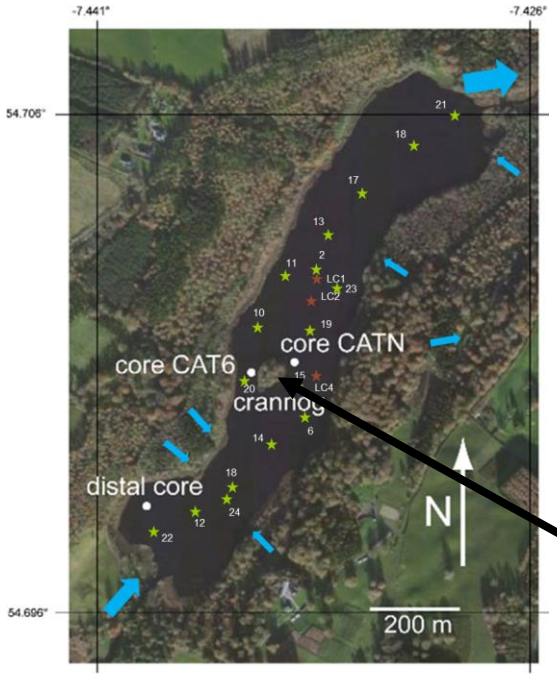
**Lough Catherine, Co Tyrone**
0-6000 cal years BP

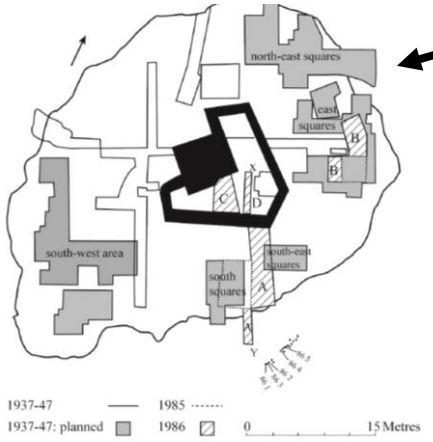
**Lough Yoan, Co Fermanagh**
800 – 2600 cal years BP

**White Loch of Murton, SW Scotland**
0-4,500 cal years BP



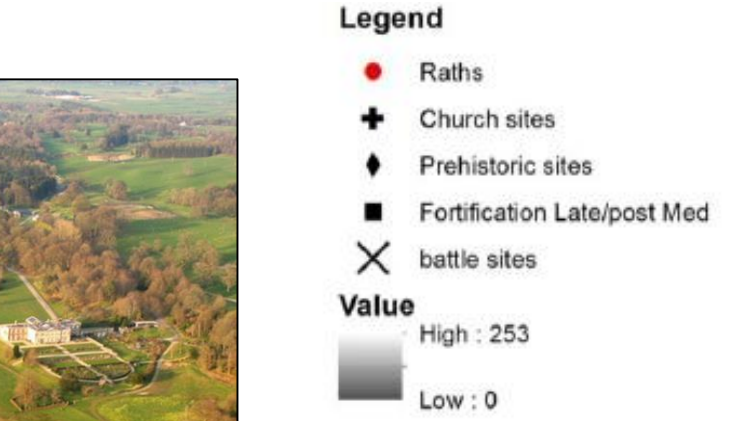
# Island McHugh – multi-period archaeological site on a natural island

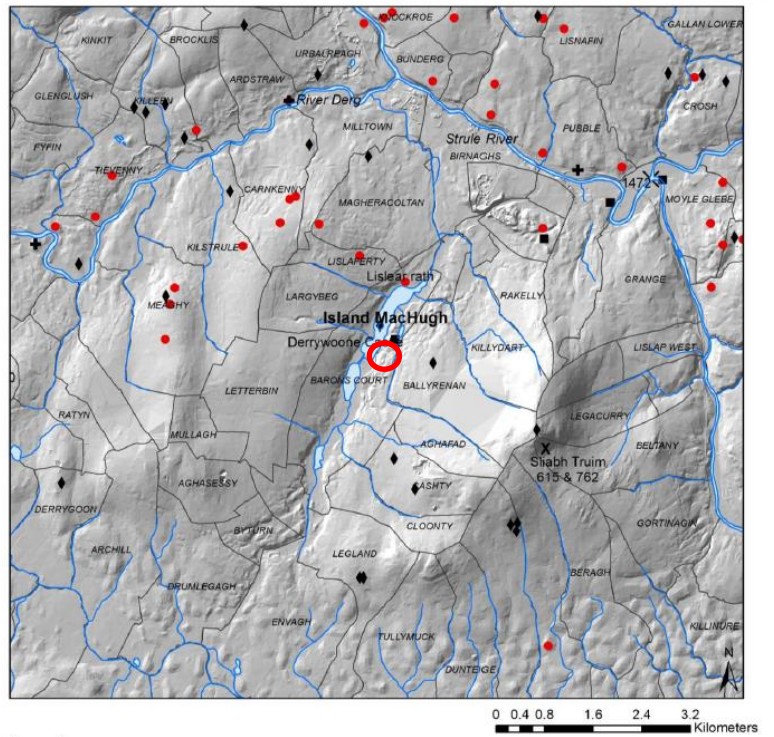


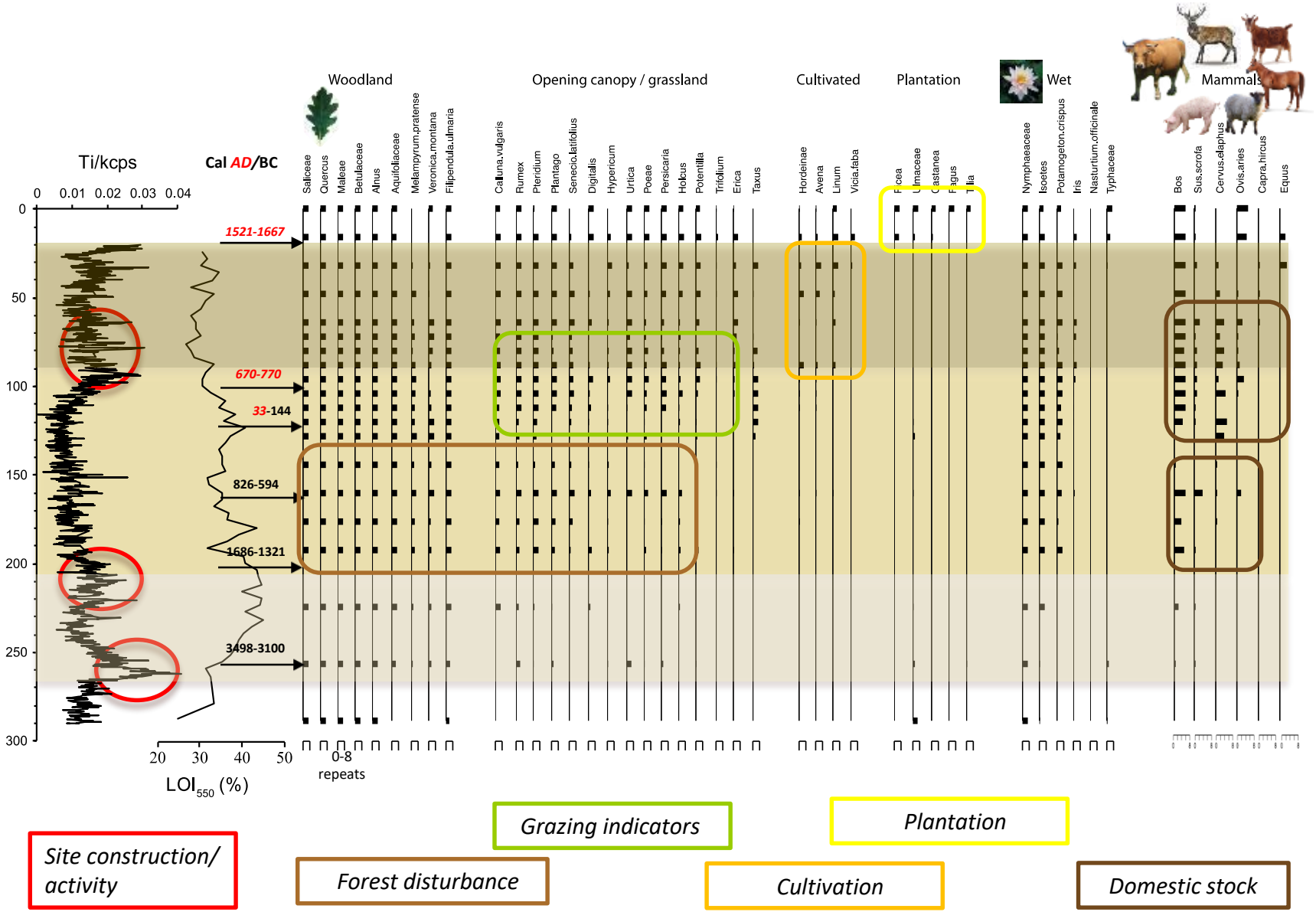

Outline plan of McHugh Island showing Davies trenches and 1980s trenches. Murray 2016

From Brown et al. Subm.



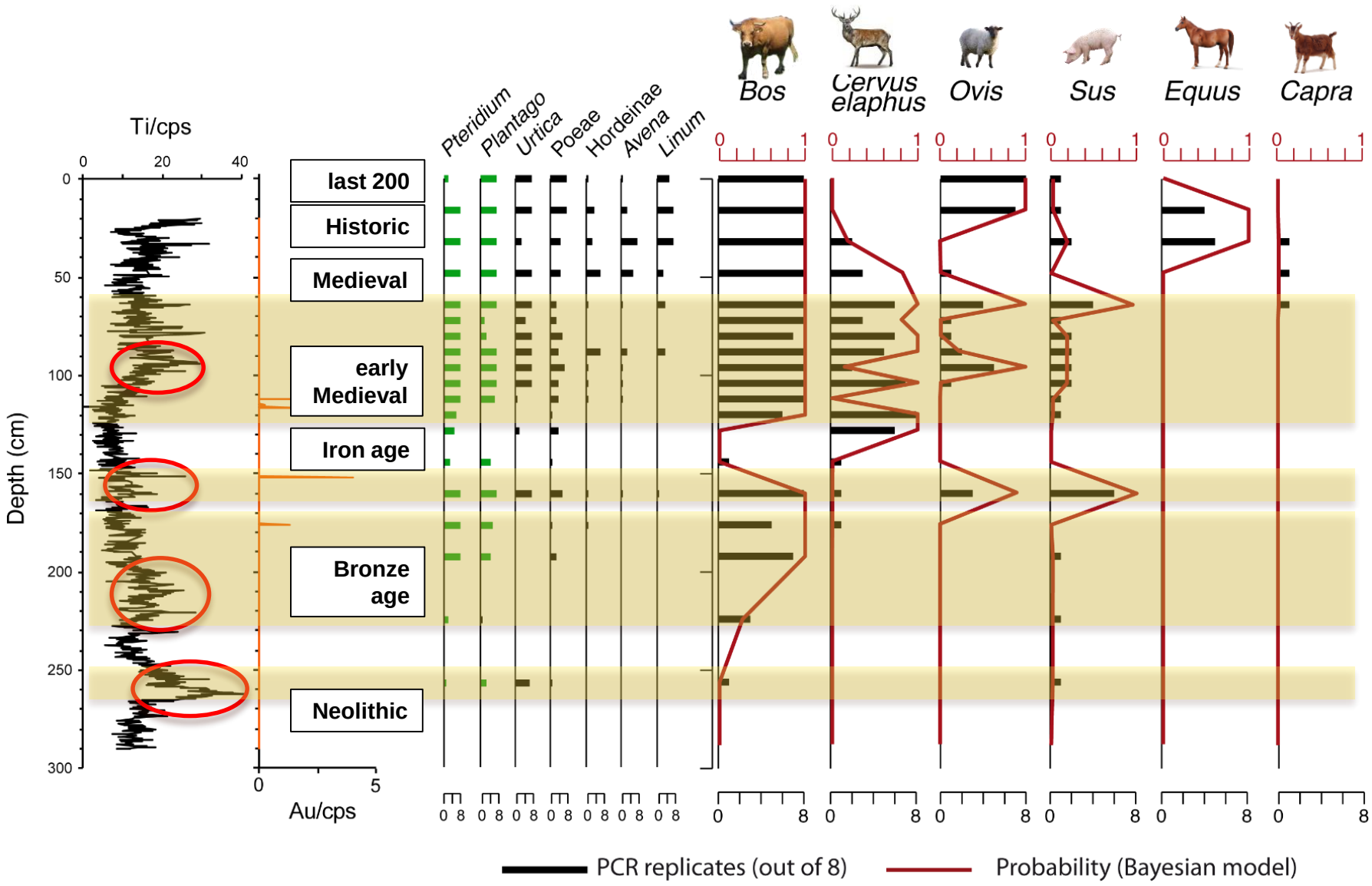



# Island McHugh, Lough Catherine



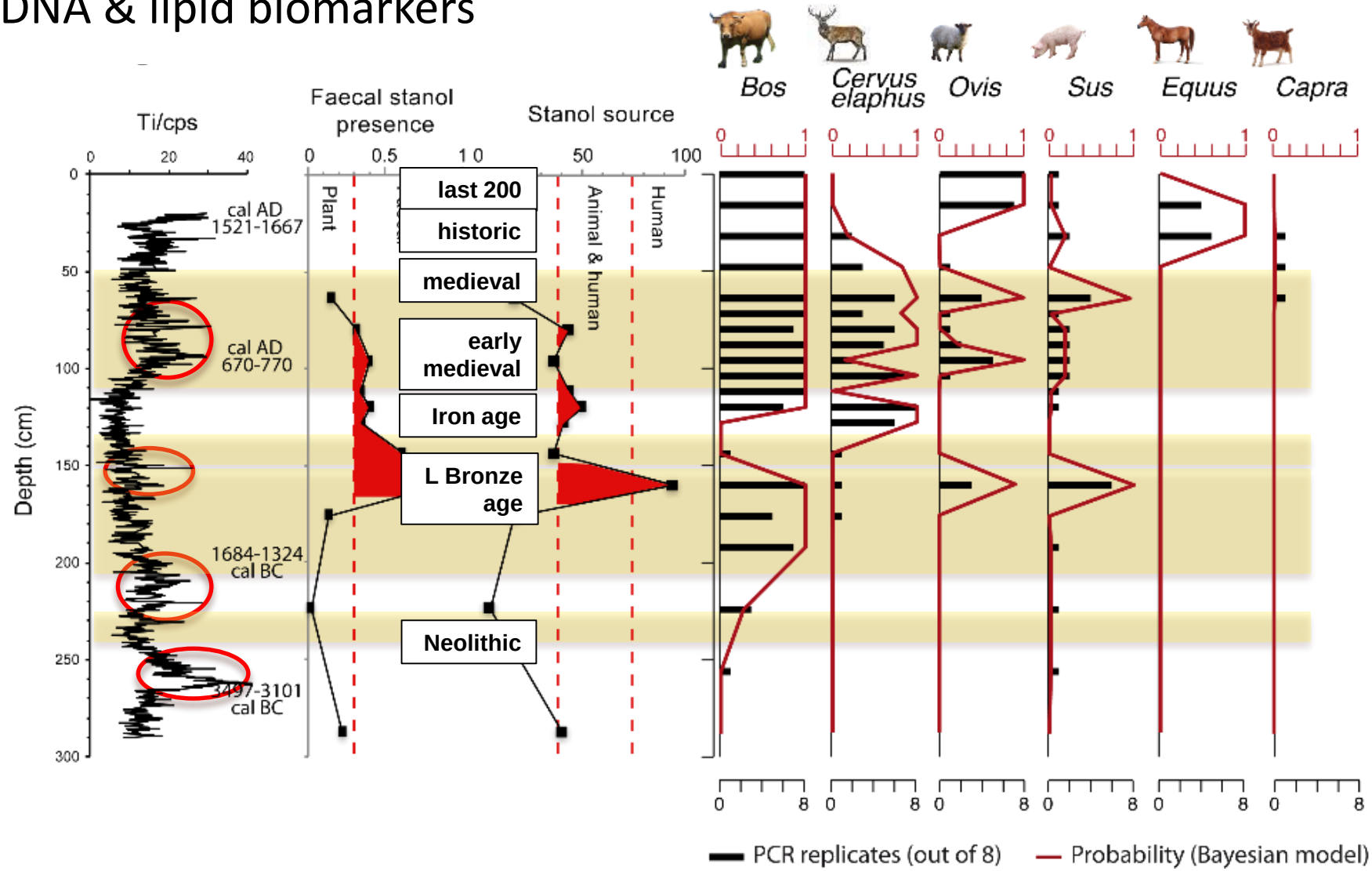

From Brown et al. Subm.

# *sedaDNA*: domesticates & related




From Brown et al. Subm.

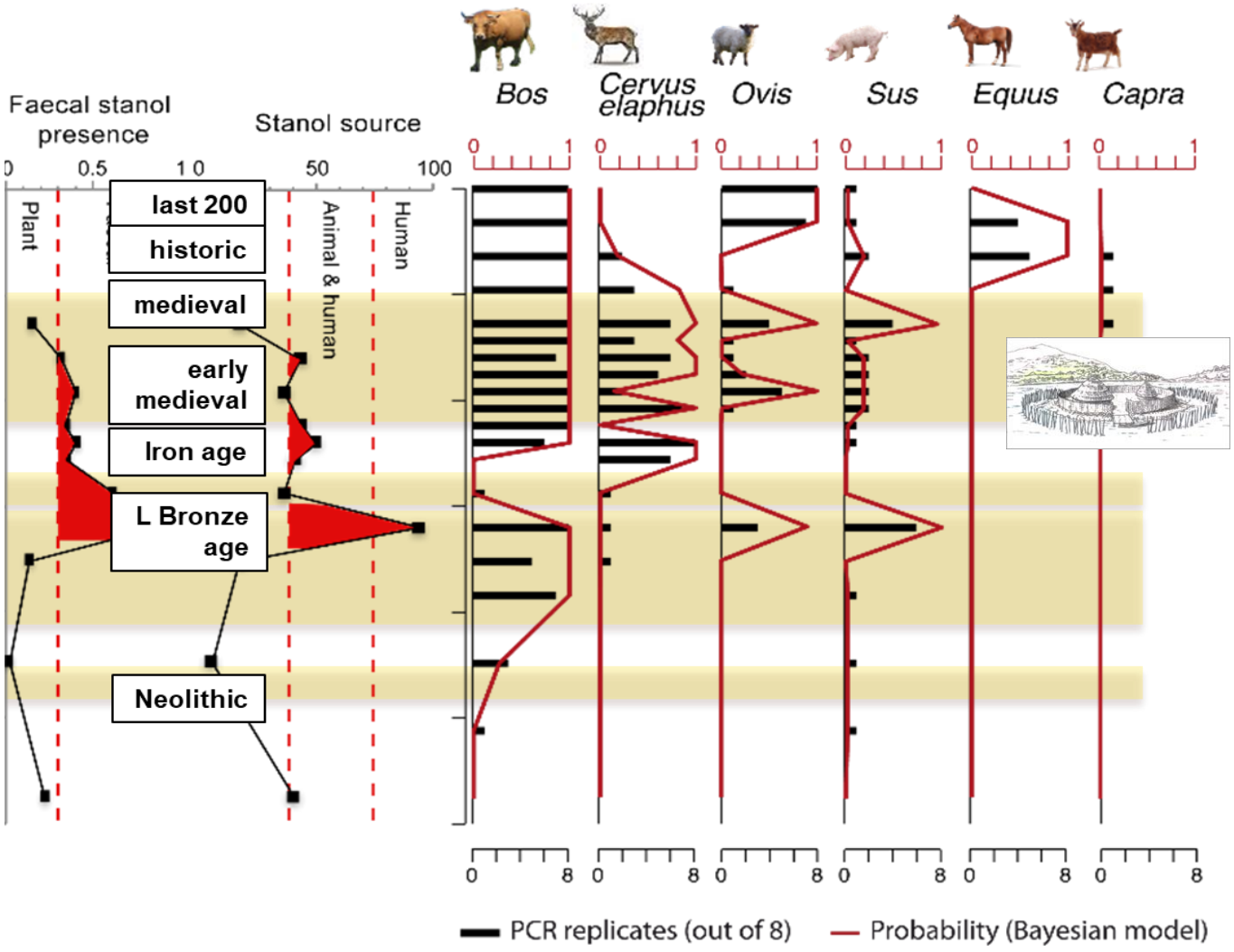
# sedaDNA & lipid biomarkers




- **Direct evidence of human activity**: human faecal matter from *ca*. 600 BC
- **Faecal signal later** than initial minerogenic input (Ti) & reduction in productivity (BSi)
  - No clear Neolithic faecal signature

From Brown et al. Subm.

# Comparison with archaeology




***Occupied in all periods - peak intensity L Bronze Age & Early Medieval***

From Brown et al. Subm.

## Post Medieval

Tower house built, attacked in AD 1602 c. 1697 AD island abandoned Derrywoone castle built c. 1622 AD on shore, Baronscourt house constructed 1745

## Late Medieval

Hearths, metalworking
Pottery, Glazed German shard
Buckle, crucibles, window glass, lead
Button, **records of stabling horses**

## Early Medieval

Timber building , pallisade (601-619 AD) with refurbishment 643 AD heightening of the island, bone comb, glass bead



**animal bone: cattle, pig, sheep (no deer)**
**barley, oats, elder**

## Iron Age

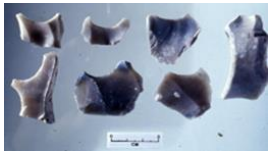
Little structural evidence (1 oak plank), pottery – cordoned urn ware
2 Roman finds (Samian ware & tile)

## L Bronze Age

2 swords, piles, stone paving, pottery, spindle whorl

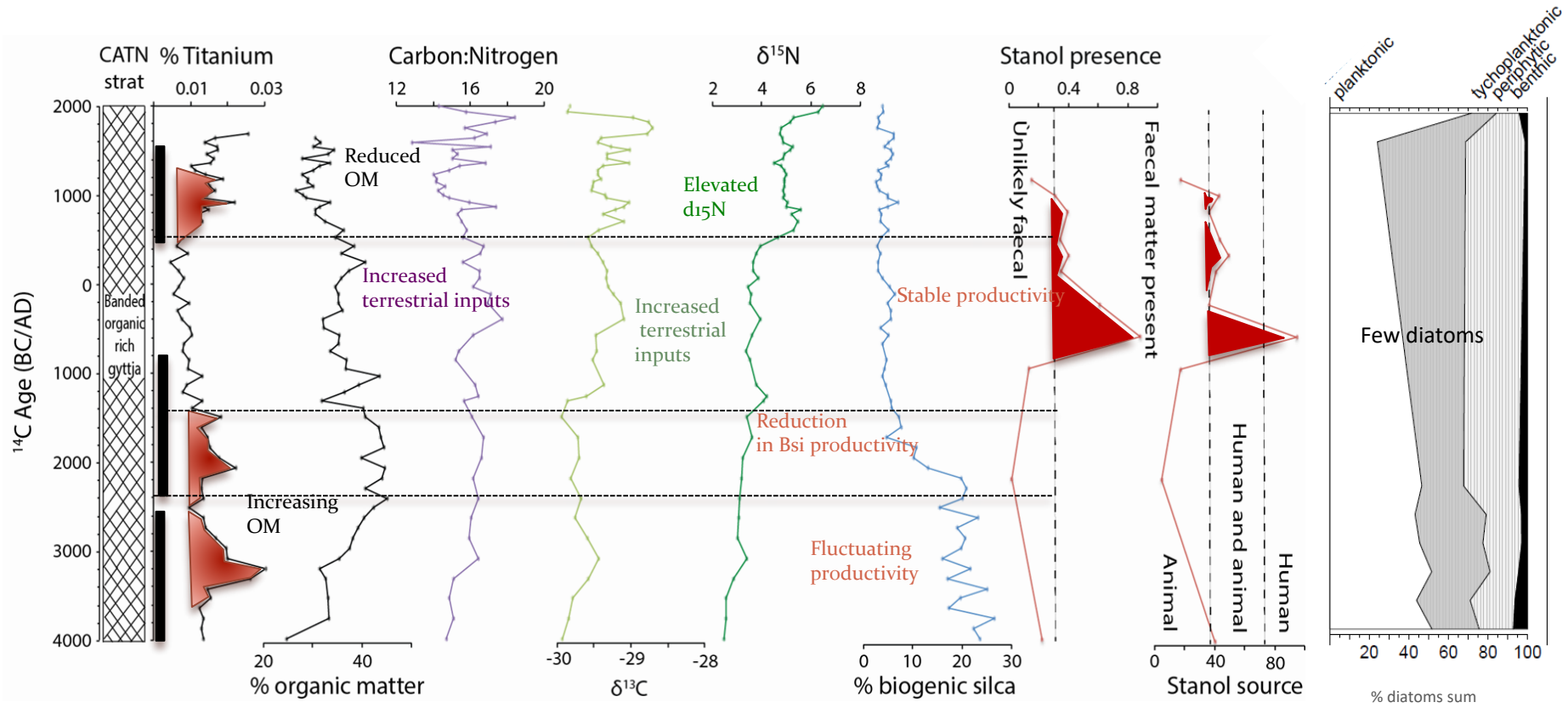
## Neolithic

Oats, hazlenuts
timber floor
pottery



# Lough Catherine, Isotopes & stanols

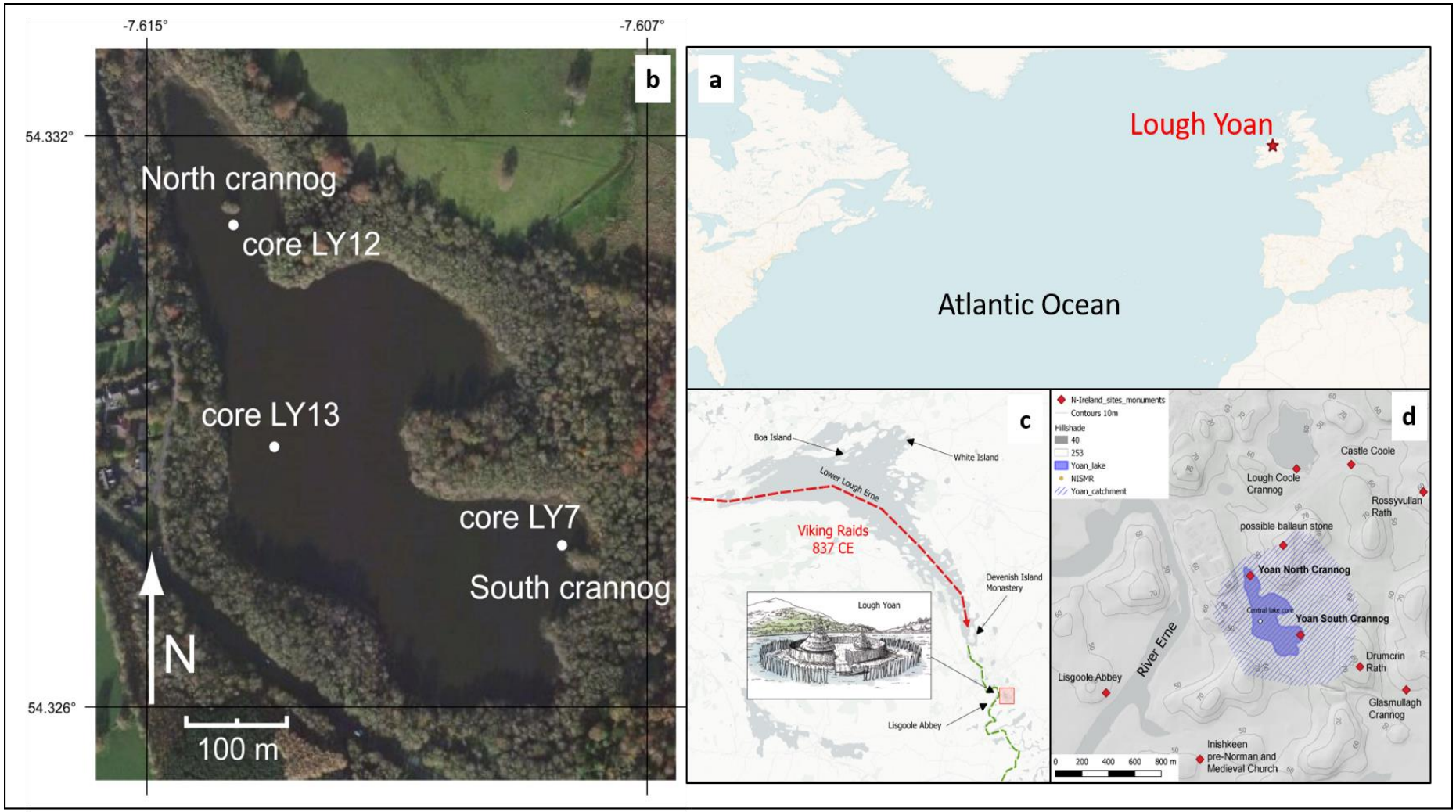
From Brown et al. Subm.




- Differences in aquatic productivity responses to construction ‘vs’ nutrients

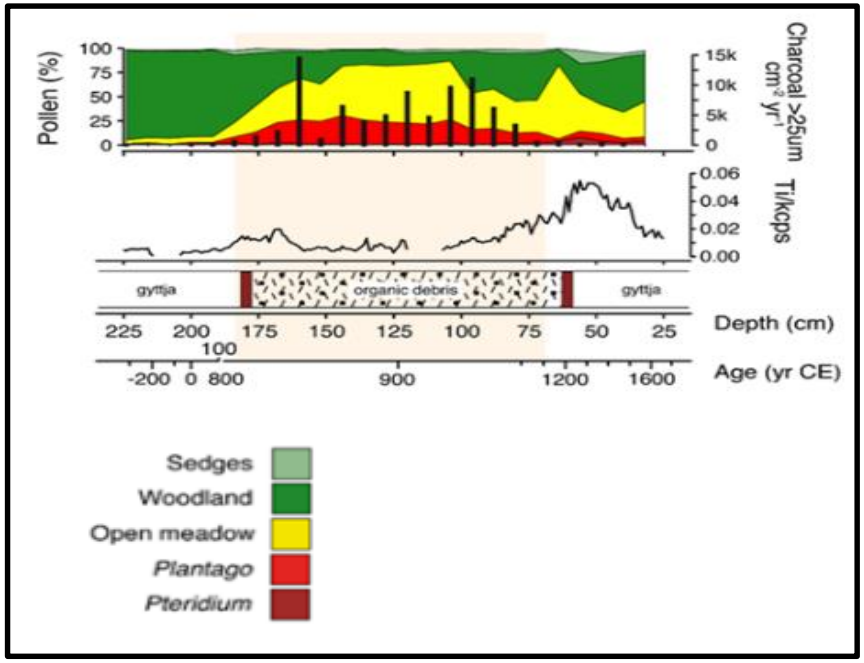
- Probably switch to non Si algal production (e.g. green algae)

# Lough Yoan: 2 Early Medieval island settlements



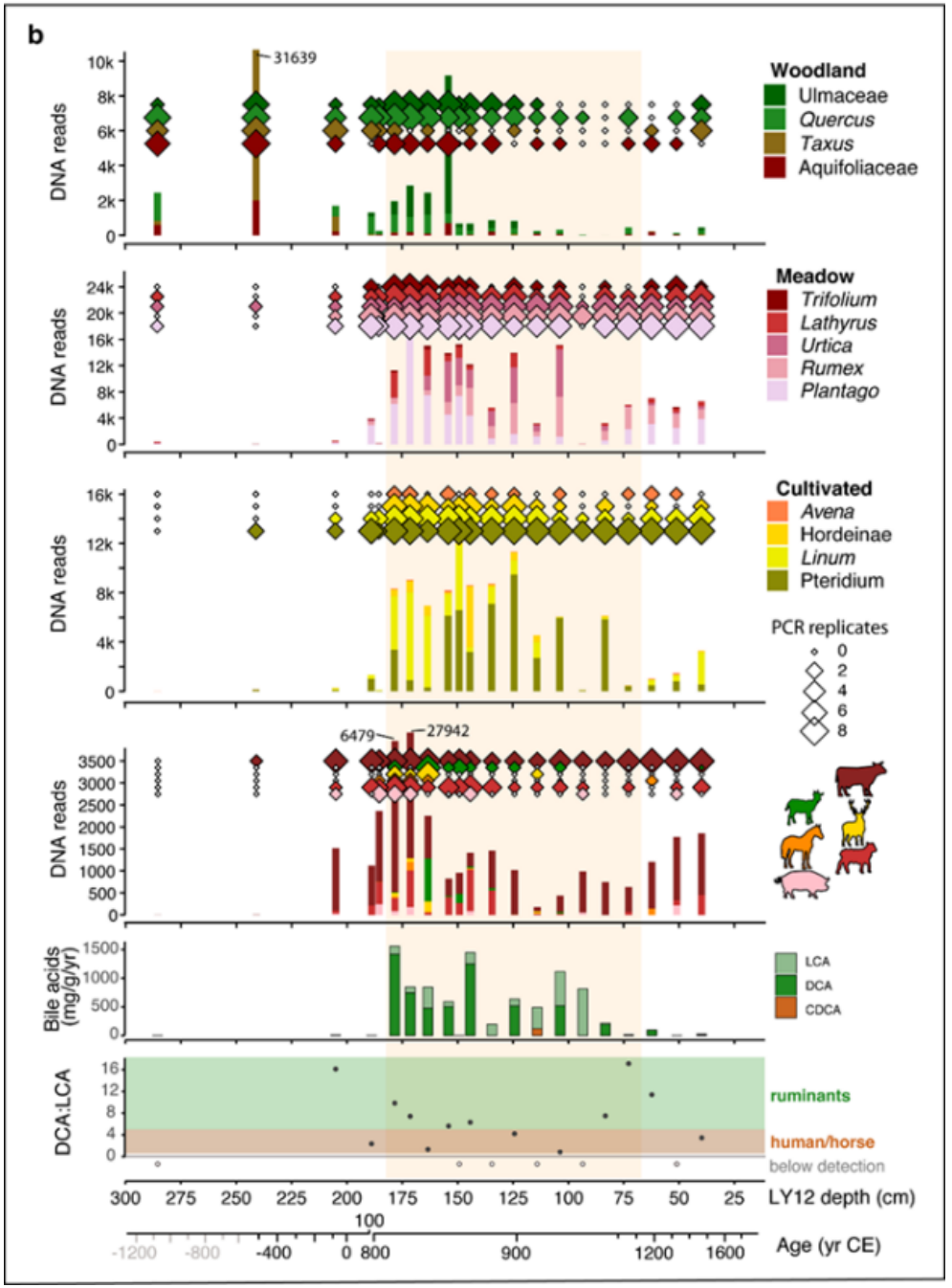

From Brown et al. Subm.

# Terrestrial plant and animals (domesticates) record




Sudden appearance of domesticates and red deer (*Cervus elaphus*) coincident with the start of the settlements

Also appearance of LCA, DCA & CDCA with ratio indicating both ruminants, humans and horses

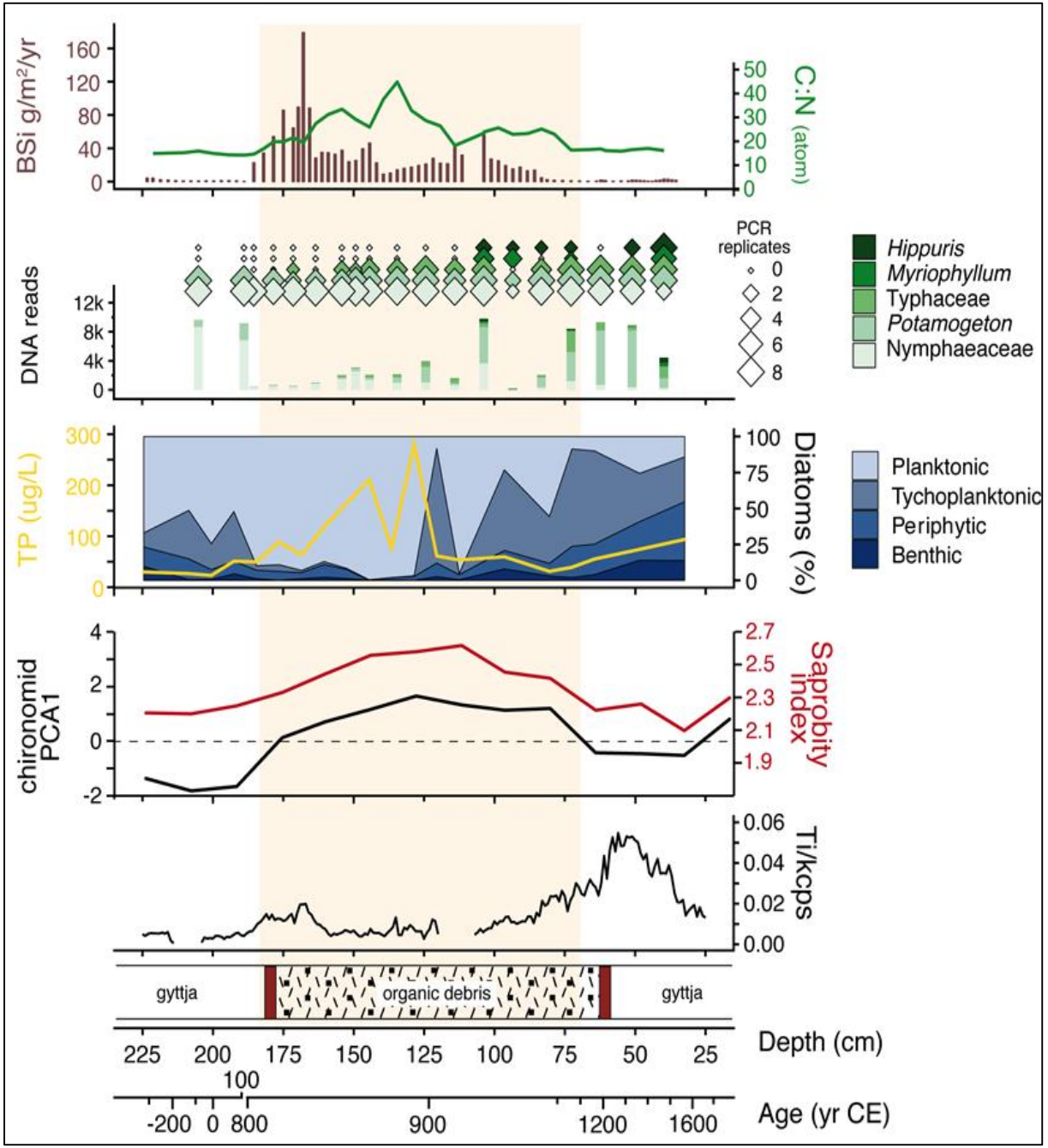



From Brown et al. Subm.

# Aquatics, diatoms and nutrients

Clear effects of settements on lake conditions:

- rise in Bsi
- rise in C: Ratio
- rise in total P
- rise in saprobity index
- delayed rise in attached diatoms
- delayed rise in emergent/marginal aquatics




From Brown et al. Subm.

# A site history from sedaDNA & lipids

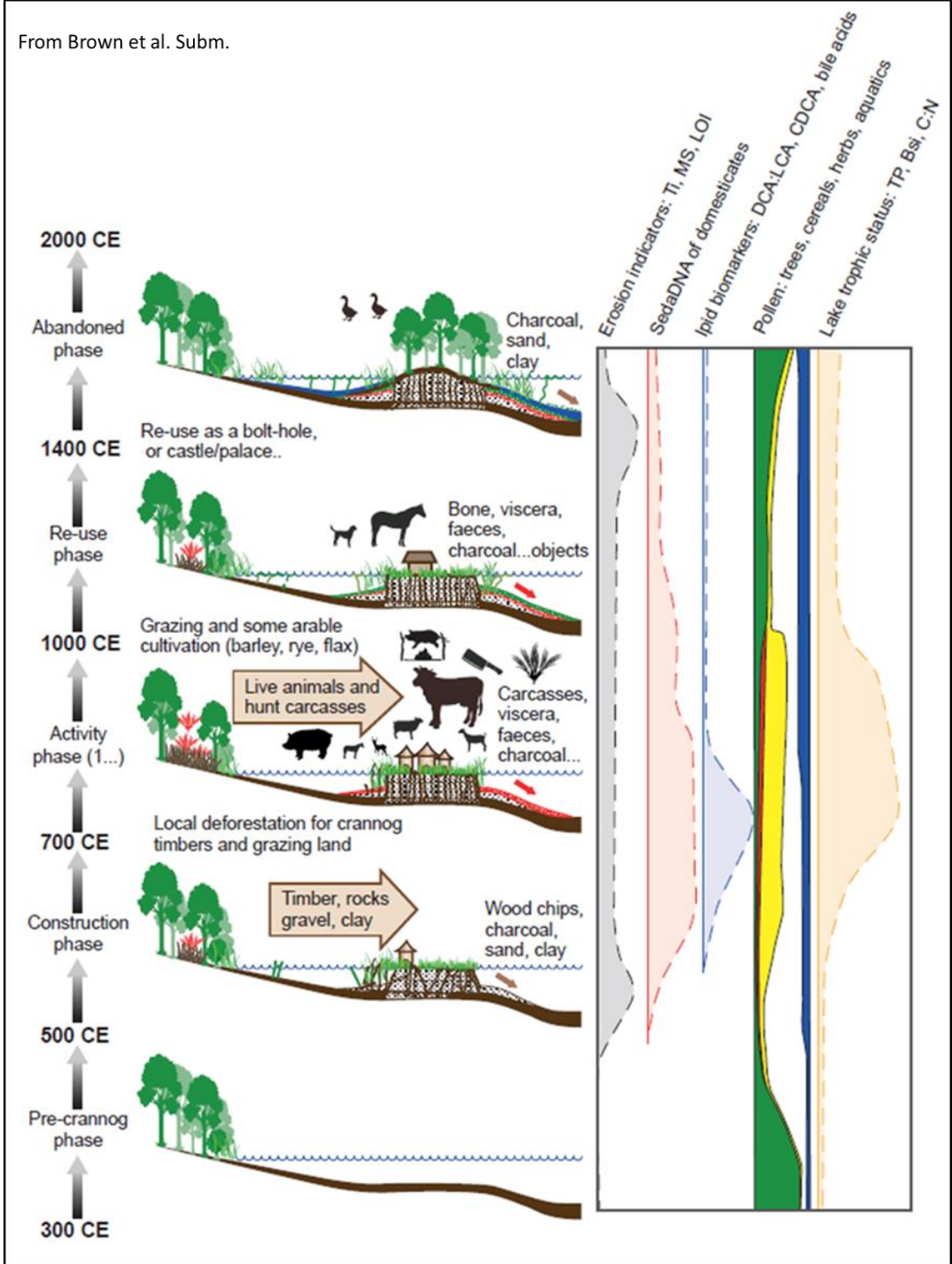
**The life-cycle of a settlement:**
construction (c. 750 AD) – use phase 1, use phase 2, abandonment of one in c. 930 AD but continued use of the other into the 1th Century AD

**Evidence of:**
construction materials (bracken)

Cereal storage/processing (barley, rye)

Slaughter of cattle, sheep/goats, pig
Red deer, horse

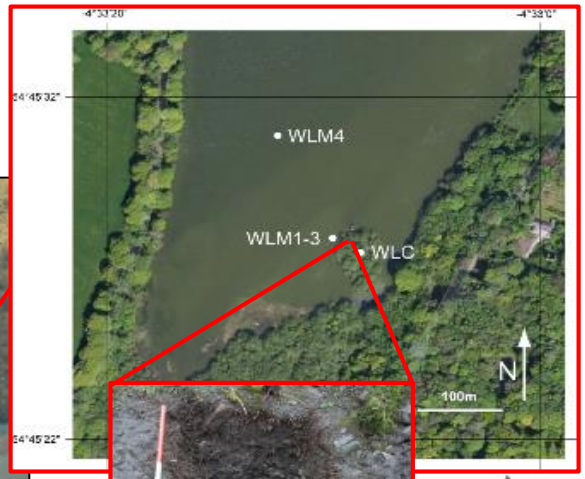
Human occupation (faeces)

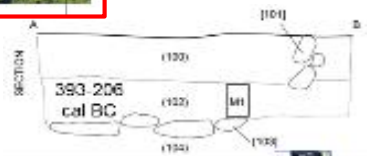


# Black and White Lochs of Myrton, SW Scotland: Iron Age Settlements

- $^{14}$C date on excavated basal timber indicates building begins *ca.* **2500 cal yr BP**







$^{14}$C date on timber base: 759-430 cal BC and 755-415 cal BC

→ **Contemporaneous with first phase of Black Loch village**.

In association with excavations

$^{14}$C date on charcoal (*Corylus avellana*) from a context in the occupation phase: 393-206 cal BC

→ Contemporaneous with 2nd phase of Black Loch village.

From Van Hardenbroek et al. Subm.

Bile acids, stanols, animals (domesticates) Terrestrial and plant record

**Almost first appearance** of domesticates: cattle, sheep and pig

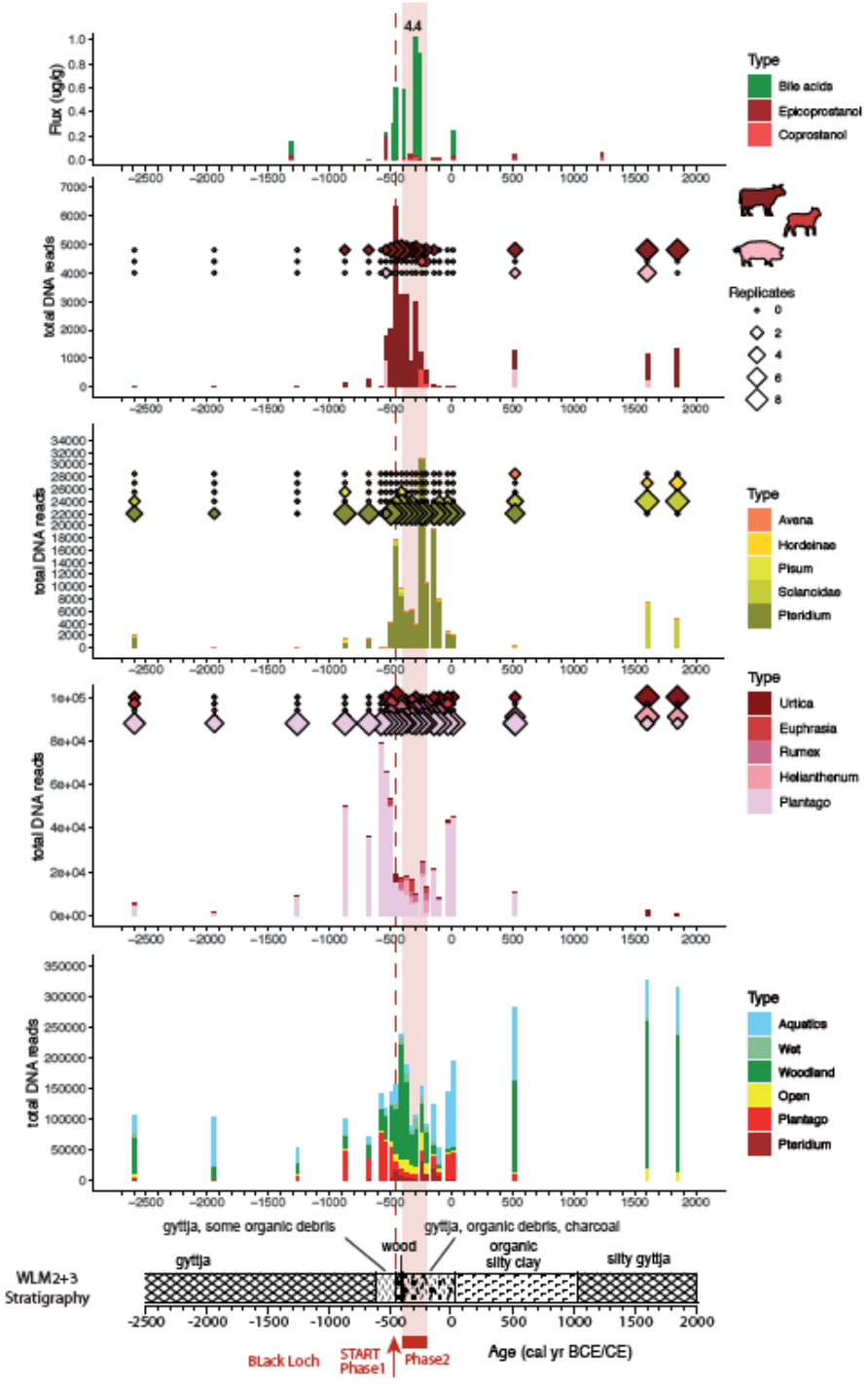
**First appearance** of crops: oats, barley, peas

**Increase in** bracken and very large increase in plantain (Plantago lanceolata)

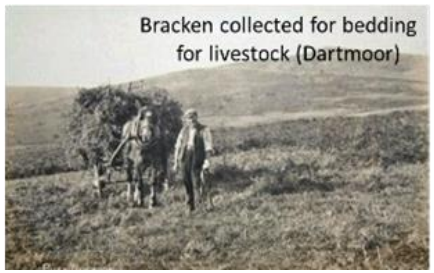
**Large increase** in bile acids and spike in coprostanols

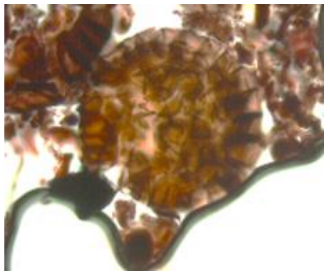
**The Plantago Problem**
Also seen in pollen diagrams from L Yoan and other island sites.

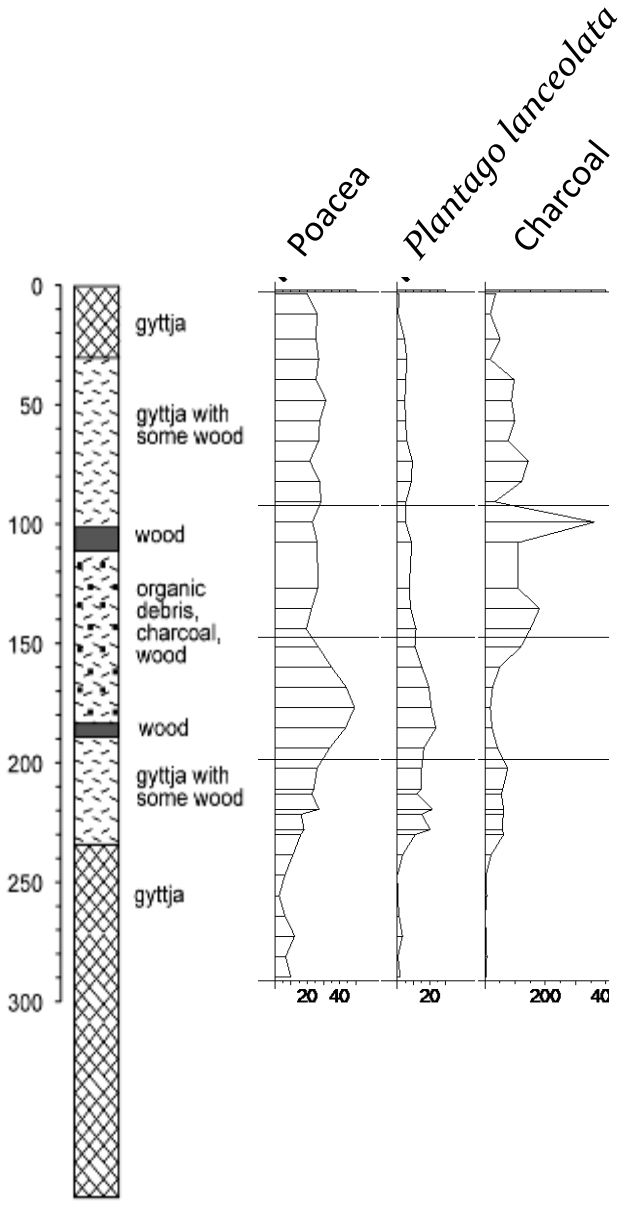
Plantain rich meadows created by sheep grazing

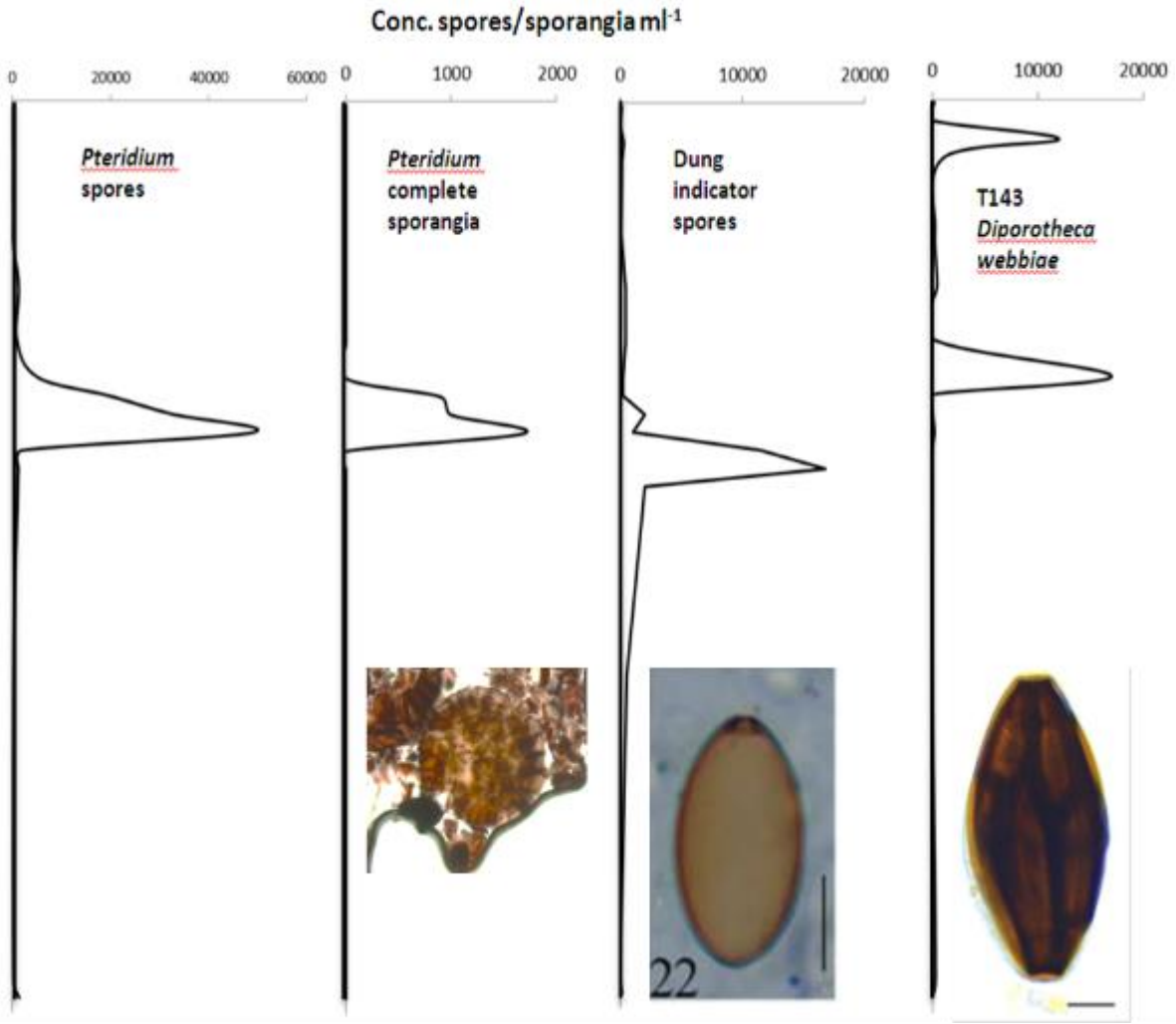


# Other evidence of animals – dung and bedding/roofing

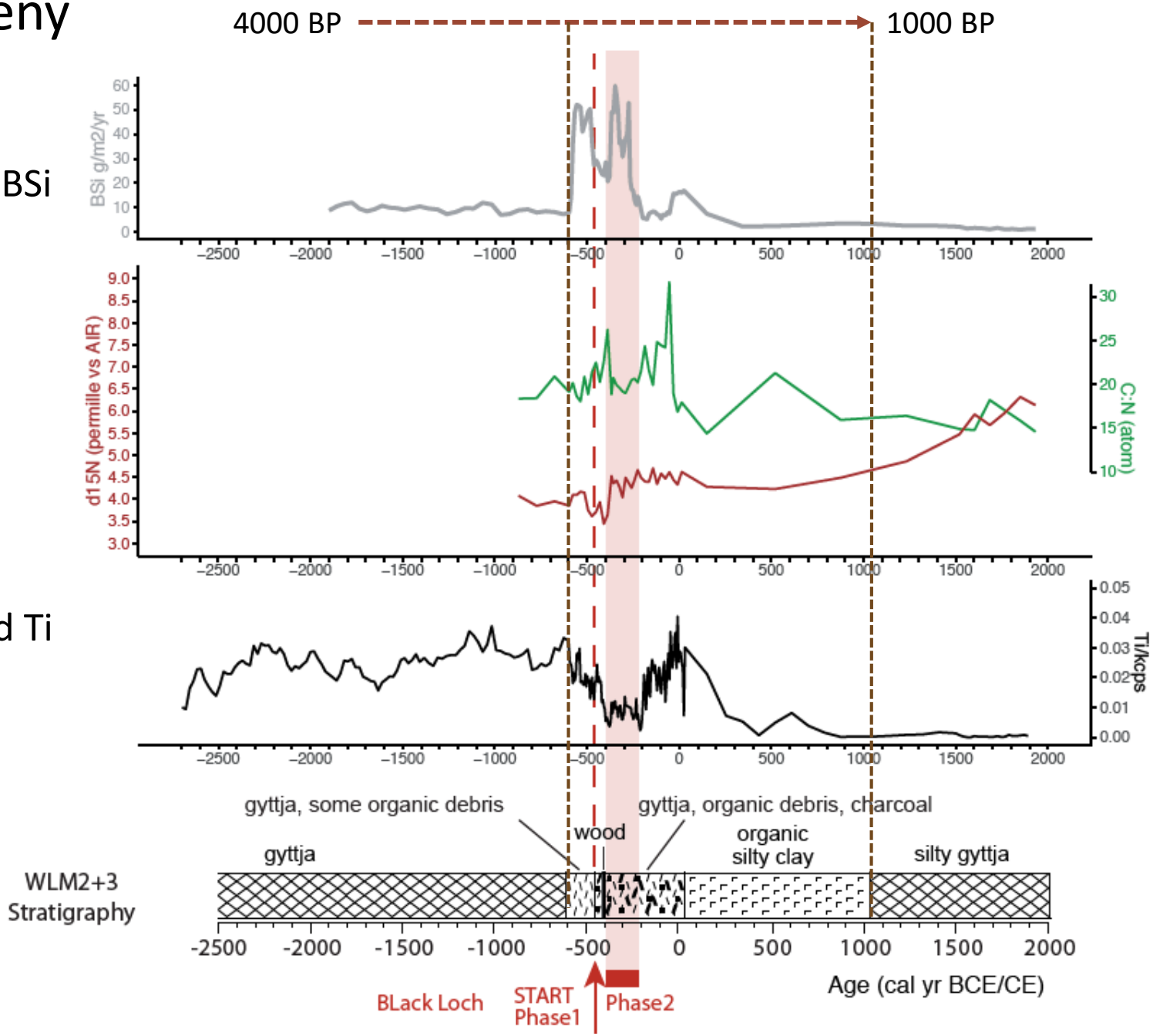


# Loch Ontogeny

Sudden rise in BSi

More complex changes in C:N ratio, d15N and Ti




From Van Hardenbroek et al. Subm.

## Conclusions

1. Lake cores from the edge of archaeological sites can give a wealth of site-related information from both sedaDNA and lipid biomarkers

2. The closeness of match to the site activity suggest both reflect local and not catchment conditions

3. Faecal stanols can indicate periods of peak activity on sites and support sedaDNA

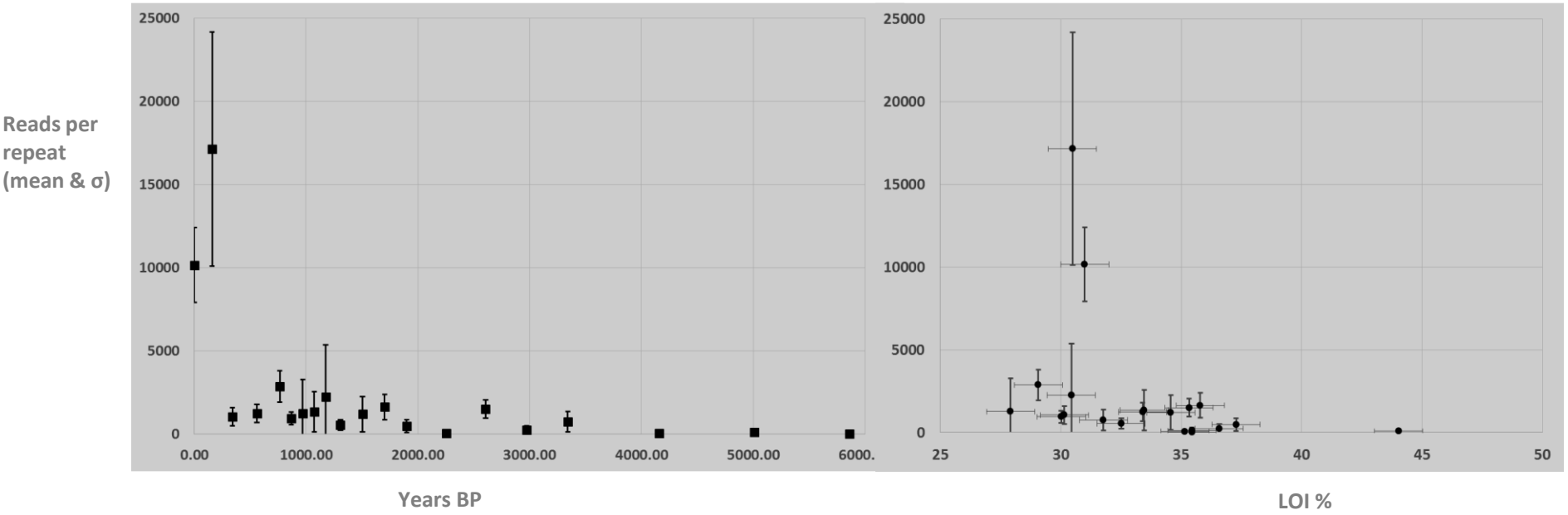
4. The strength of the record of domesticates suggests slaughter of animals on the islands – this supports bone evidence

5. These sites are best explained as ‘protected elite residences’ from the Bronze Age to the Post Medieval period

6. Island settlements are the most suitable but ‘moated settlements’are also working…

# Supplementary Information Slides

Taphonomic/PCR  Bias? (samples all in a single batch for PCR & sequencing)



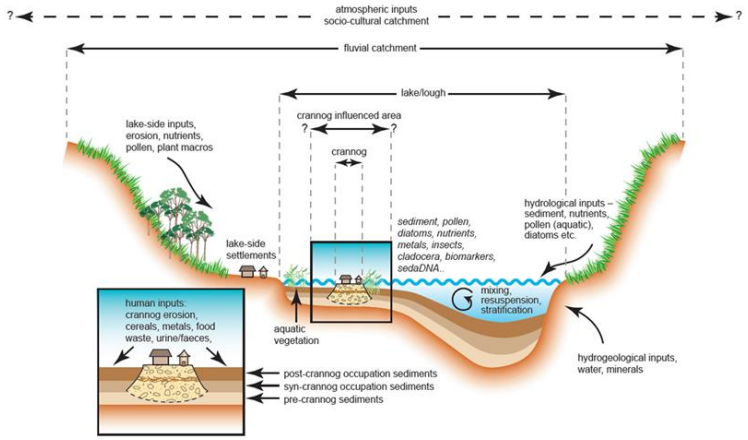

After upper two samples no significant variation in total reads over time

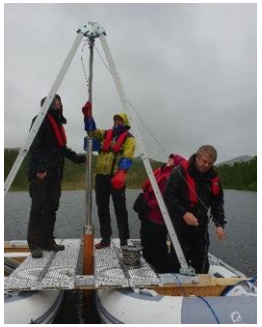
No relationship between animal or plant reads & LOI (%) (or BSi %)

No relationship between plant read total and animal read total

*This is **not** template DNA, but does show that results are not the result of just a few repeats with very high values, or associated with high or low LOI*

# From sampling to data



**Sampling**

**DNA extraction**

**PCR**

**Identifying**

**Bioinformatic**

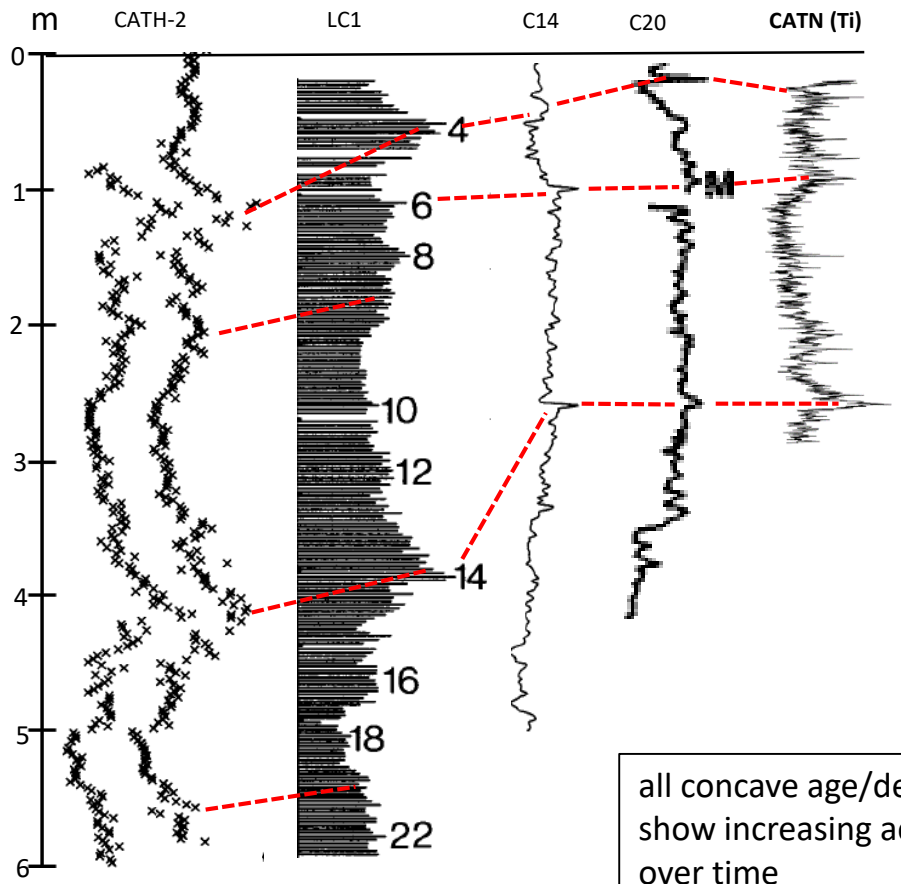
AGTGGGCCTAA
TCTGGGCCTAA
ATTGGGCCTAA
T

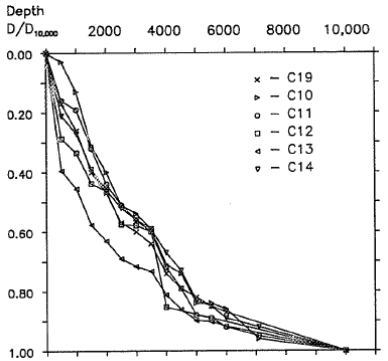
**Sequencing**

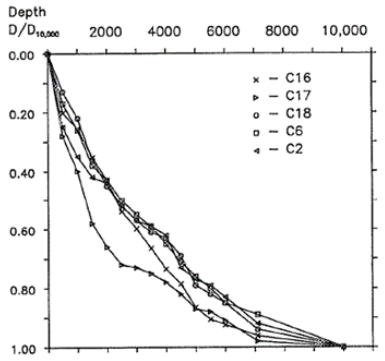
**METHODs**

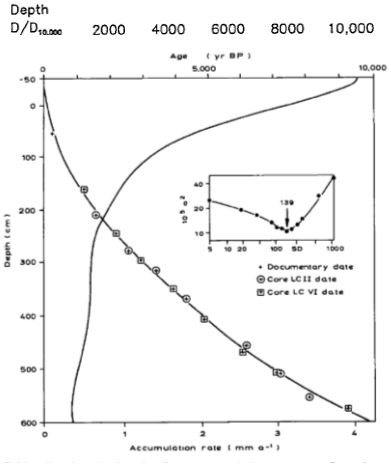
- Extraction with PowerMax Soil Kit in clean lab.
- Mammal primer P007:
  MamP007F, 5'-CGAGAAGACCCTATGGAGCT-3'
  MamP007R, 5'-CCGAGGTCRCCCCAACC-3'
- Plant primer gh (p6 loop of chloroplast *trn*L (UAA) intron (Taberlet et al. 2007)
  g, 5'-GGGCAATCCTGAGCCAA-3'
  h, 5'-CCATTGAGTCTCTGCACCTATC-3'
- 45-50 PCR cycles
- Illumina HiSeq 2500
- OBITools (Boyer et al. 2015 Mol. Ecol. Res.)

Multiple cores (c. 30) have shown consistent lake sedimentology

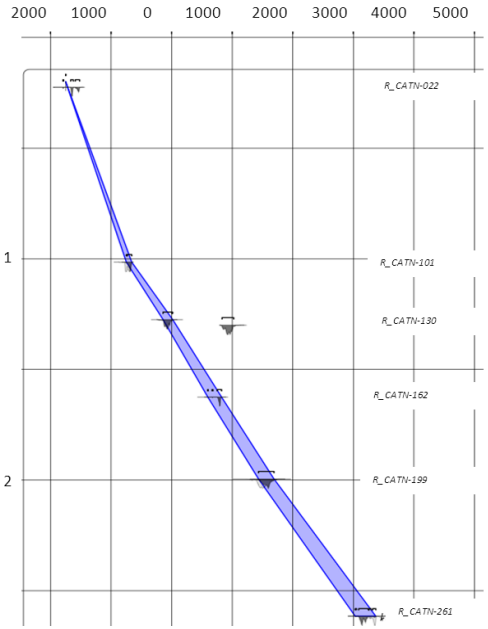



Modelled date BC/AD




all concave age/depth curves all show increasing accumulation rate over time

From Thompson & Edwards 1982; Edwards & Thompson 1984; Hirons & Edwards 1986; Snowball & Thompson 1990; this study

# Getting it Together: Combining plant and mammal DNA with Lipid Biomarkers from Irish and Scottish Lakes

Tony Brown[1,2], Thierry Fonville[2], Helen Mackay[3], Maarten van Hardenbroek[3,] Francesco Ficetola[4] and Inger Alsos[1]

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## Abstract

Lowland lakes in Scotland and Ireland have been heavily impacted by human activity since the Neolithic due to forest clearance, agriculture and lakeside settlement. Whilst plant macrofossils, pollen and other microfossils, especially diatoms, have been able to outline these changes many uncertainties remain about the origin and exact nature of these impacts. Obtaining independent measures of both vascular plants and mammals (and other animals) allows for more taxonomically precise reconstructions and the study of long-term biotic interactions. Lipid biomarkers, such as faecal steroids and bile acids, can both validate the mammal DNA, and also indicate the magnitude of agricultural and human lake inputs into the lake ecosystem. Our initial work focused on small artificial islands (crannogs) common in the Celtic parts of the UK. Unusually strong sedaDNA and lipid biomarker results from both plants and animals are believed to result from the creation of a biogeochemical halo around the crannogs due to the direct input of bone and viscera, rapid organic and clastic sedimentation, and a lack of disturbance. The human activities on the artificial islands, such as slaughter, butchery and feasting, caused severe eutrophication of the smaller lakes, which only partially recovered after the abandonment of the sites. Similar but less pronounced effects can be seen at lake-side settlement sites in larger lakes and away from archaeological sites which reflect catchment-wide influences. This paper will present data from crannogs, lake-side sites and from a new study of lakes on small islands on the Celtic Seaboard. These island sites are being studied to test the narrative of 'marginality' and a perceived lack of resilience in small islands during the last two thousand years. Overall our sedaDNA and steroid results complement data from both archaeological excavation, survey and traditional palaeoenvironmental proxies to provide a more detailed and comprehensive image of the environment in which our ancestors were operating, the changes they had on their ecosystems and our inheritance of this today.