eDNA metabarcoding to monitor biodiversity in agricultural systems

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Introduction

- Environmental (e)DNA has become established as a rapid and cost effective way to measure biodiversity
- Current applications of eDNA are appearing everywhere, but are not yet widely applied to agricultural systems
- This remains a major gap in our knowledge to assess and monitor sustainability in agricultural practices and effects on biodiversity

Methods

- We designed and implemented a sampling and sequencing protocol for cost-effective biodiversity assessment of Dutch farming systems
- Two farms were sampled, with 4 plots per farm, using air, water and soil sampling (47 environmental + 3 negative control samples)
- For each of the 50 samples per farm we selected 6 available primer sets to capture:
  - ➢ Vertebrates (12S and 16S genes)
  - ➢ Macroinvertebrates (fwh1 and fwh2 [COI mini-barcode])
  - ➢ Fungi (ITS2 region)
  - ➢ Plants (ITS2 region)

Figure 1. Schematic map of eDNA sampling at our 'pilot' farm

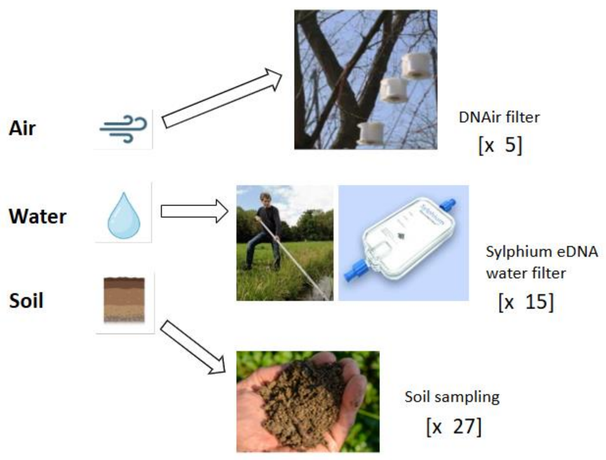

Figure 2. Overview of the types of samples taken per farm

- Samples were sequenced by an external company (Novogene), and we utilised modified bioinformatic pipelines for taxonomic assignment of reads

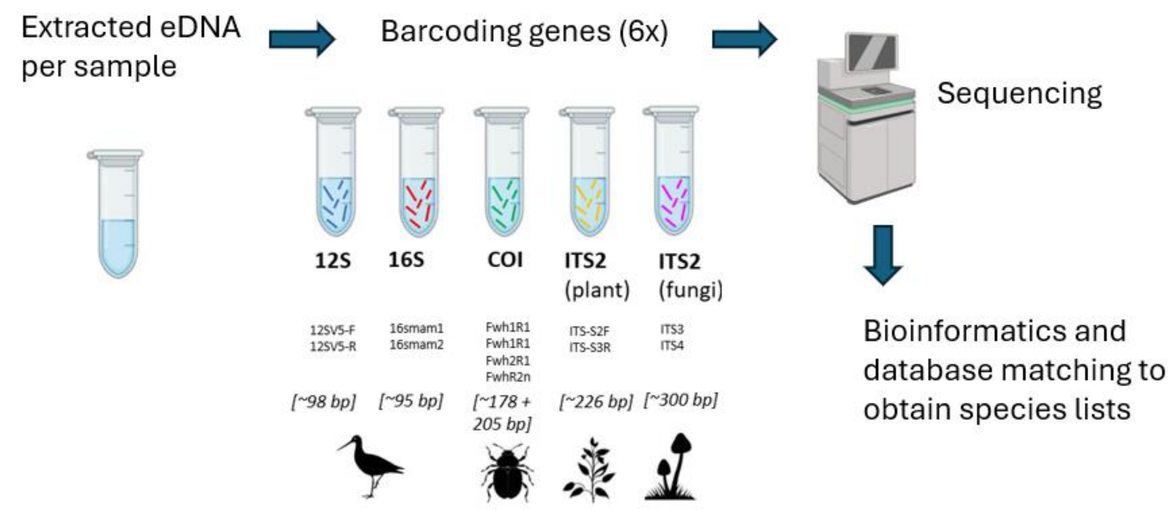

Figure 3. Workflow for eDNA extraction (left), PCR amplification (centre), and sequencing and bioinformatic processing using QIIME2, DADA2 and DNA barcoding databases (right)

Results

- All samples contained eDNA allowing taxonomic identification
- A total of 2966 Amplicon Sequence Variants (ASVs) were detected across the two farms

Table 1. Truncated example of the data output per sample, where eDNA detections are marked with an 'X'. Accumulating all air, soil and water samples per farm gives a complete eDNA-derived species list

ASV	Species	01	02	03	04	05	06	07	...
01	*Bos_taurus*	X	X	X	X	X	X	X	
02	*Arvicola_amphibius*		X	X					
03	*Anas_platyrhynchus*	X			X	X	X		
04	*Gasterosteus_aculeatus*	X	X		X		X	X	
05	*Anguilla_anguilla*		X	X					
06	*Rana_temporaria*	X	X		X	X	X		
07	*Triturus_cristatus*			X	X				
08	*Sympetrum_striolatum*	X	X				X		
09	*Rhantus_yessoensis*	X			X			X	
10	*Cricotopus_glacialis*	X	X	X	X	X	X	X	
11	*Psathyrella_sp.*								

...

- Overlap of detected species was small across air, soil and water samples, meaning that all three are important to sample for obtaining more complete biodiversity profiles per farm

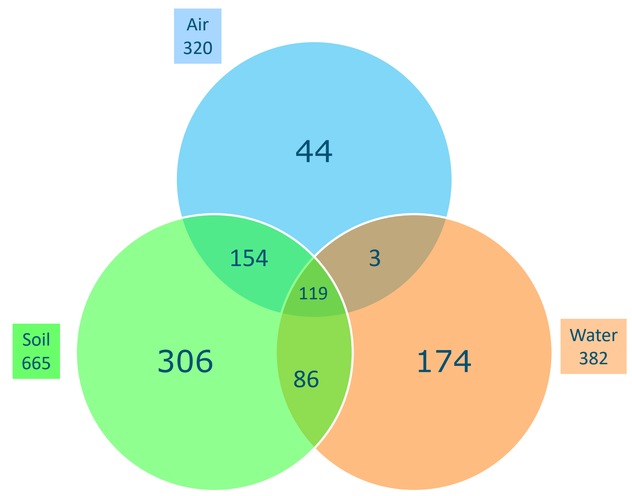

Figure 4. Numbers of eDNA based detections per sample type across farms

Conclusions

- eDNA approaches are highly promising for assessing and monitoring biodiversity of agricultural systems, and we have a working protocol
- However - these approaches do not give the complete picture of biodiversity (i.e. not all species are detected), so care must be taken and relevant indicator species/groups should be evaluated when creating biodiversity profiles based on these data

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