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Background

The protection of soil biodiversity and ecosystem services are increasingly being addressed throughout Europe and implies a diagnosis of soil health. Soil biodiversity research and monitoring studies are challenged by the lack of taxonomic expertise and the impossibility to identify cryptic species and juvenile stages. So here we present a soil invertebrate metabarcoding study of agricultural soil comparing the performance of metabarcoding with conventional species level biomass estimation.

There is a demand for validating eDNA techniques for soil biodiversity monitoring. However, a number of questions regarding optimization of each step from sampling, DNA extraction, library building, bioinformatics and data analysis need to be investigated before large-scale monitoring can go ahead and employ standard procedures. These include:

- Sample number, spatial distribution and soil amount for DNA extraction
- DNA extraction reagents
- Choice of metabarcoding approach: PCR based or PCR-free methods (probe capturing, shotgun and mitogenomics)
- Populate reference databases with more barcodes
- Performance of metabarcoding compared to conventional diversity inventories (covered here)

Method

Earthworms and soil microarthropods were sampled in Oct. 2017 and June 2018 in two long-term field experiments comparing tillage and no-tillage. Metabarcoding were done on phosphate buffer DNA extracts of soil using the 70 bp earthworm 16S minibarcode primer set (Bienert *et al.* 2010. Mol. Ecol.).

Metabarcoding main result

In spite of the commonly known PCR bias obscuring the abundance estimation and creating a risk to overlook species, OTUs and species list generated by metabarcoding can still capture changes in community structure of disturbed communities (Fig. 2) similar to conventional species biomass compositions.



Fig. 1. A 20 year old long-term field study of no-tillage farming in Jerslev, Zealand (DK) CT: Conventional Tillage; CA: Conservation Agriculture

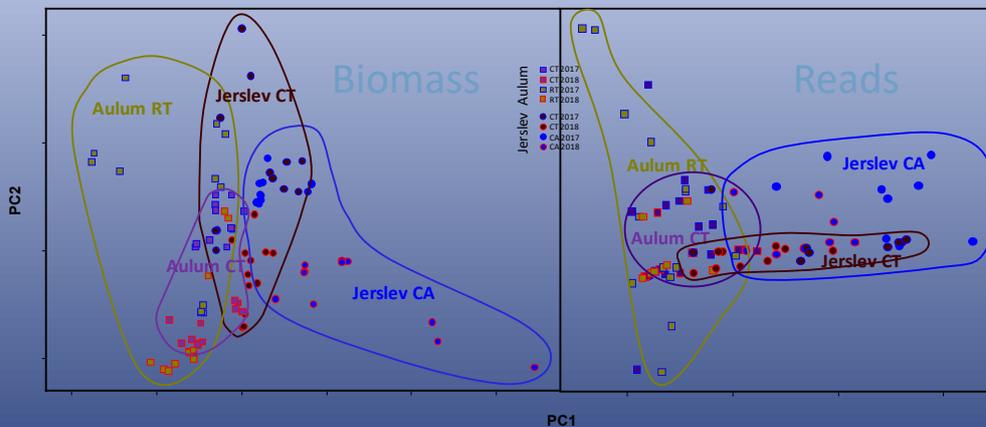


Fig. 2. A multivariate analysis (PCA) of earthworm conventionally estimated biomass and metabarcoding DNA reads from Illumina NGS. Samples came from two locations, Aulum and Jerslev, with contrasting farming tillage practices. CT: Conventional Tillage; RT: Reduced Tillage; CA: Conservation Agriculture

Conclusions

- ☐ Metabarcoding reveal earthworm community structure changes comparable to conventional methodology
- ☐ Cryptic species such as *L. herculeus* and difficult species are detected by metabarcoding
- ☐ Metabarcoding of earthworm communities reveal effects of farming systems as well as location specific communities.



Fig. 3. Extracting the deep-burrowing earthworms with a mustard powder solution after hand-sorting the top soil to find all earthworms.