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Monitoring mite diversity in European soils

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Background & Objectives

Within the EU FP7-project *EcoFINDERS*, various European partners collaborate to gain more insights in links between soil diversity and ecosystem services, across soils, climate types and land uses.
To allow rapid diversity screening of many soils throughout Europe, new tools are being developed for high-throughput DNA-based species identification. Alterra participates in this metabarcoding project by developing new approaches for DNA barcoding of soil mites.

Set-up

I. Soil samples were collected from various sites. Mites were extracted using Tullgren funnels, and the samples were split in two equal mixtures using a gel-based subsampling approach (Figure 1).

II. One subsample / site was subjected to morphological identification.

III. Our DNA barcoding approach involves two stadia:

● The development of a reference database with DNA barcodes of all potentially encountered species (~3 specimens / species).

● DNA-extraction from second subsample of mixed mites / site. Amplified DNA barcodes are subjected to 454 pyrosequencing and compared to the reference database to assess species composition.
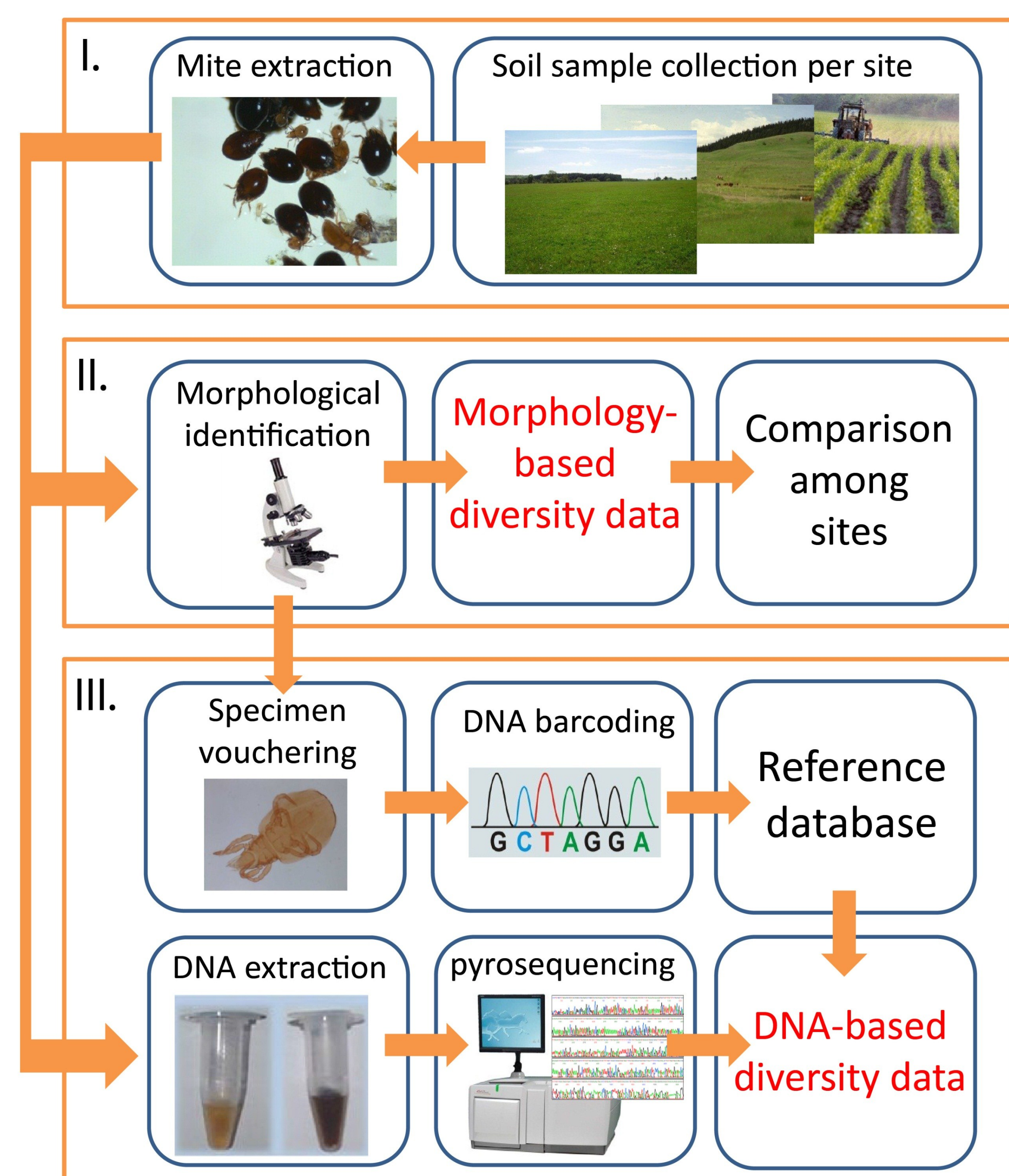

Figure 1. Schematic overview the workflow.

I: Sample collection

• Grasslands in the Veluwe area (Netherlands) differ in time after last disturbance: 7-13 yrs (recent), 17-24 yrs (middle), 27-30 yrs (old)

• Sites near Lusignan (France) differ in land use: permanent arable land, arable / grassland rotation, permanent grassland. This corresponds to gradient in disturbance frequency (high→low).

II: Variation in abundance and taxonomic diversity

• In total, we found 37 different mite taxa near Lusignan and 69 taxa in the Veluwe area. Species composition differs strongly among sites.

• Both total abundance and number of taxa increases with increasing age after last disturbance (Veluwe; Figure 2)

• The total abundance of mites is lowest at intermediate levels of disturbance. In frequently disturbed plots some species become highly dominant. Taxonomic diversity is low, but the total number of individuals slightly rises (Lusignan; Figure 2).
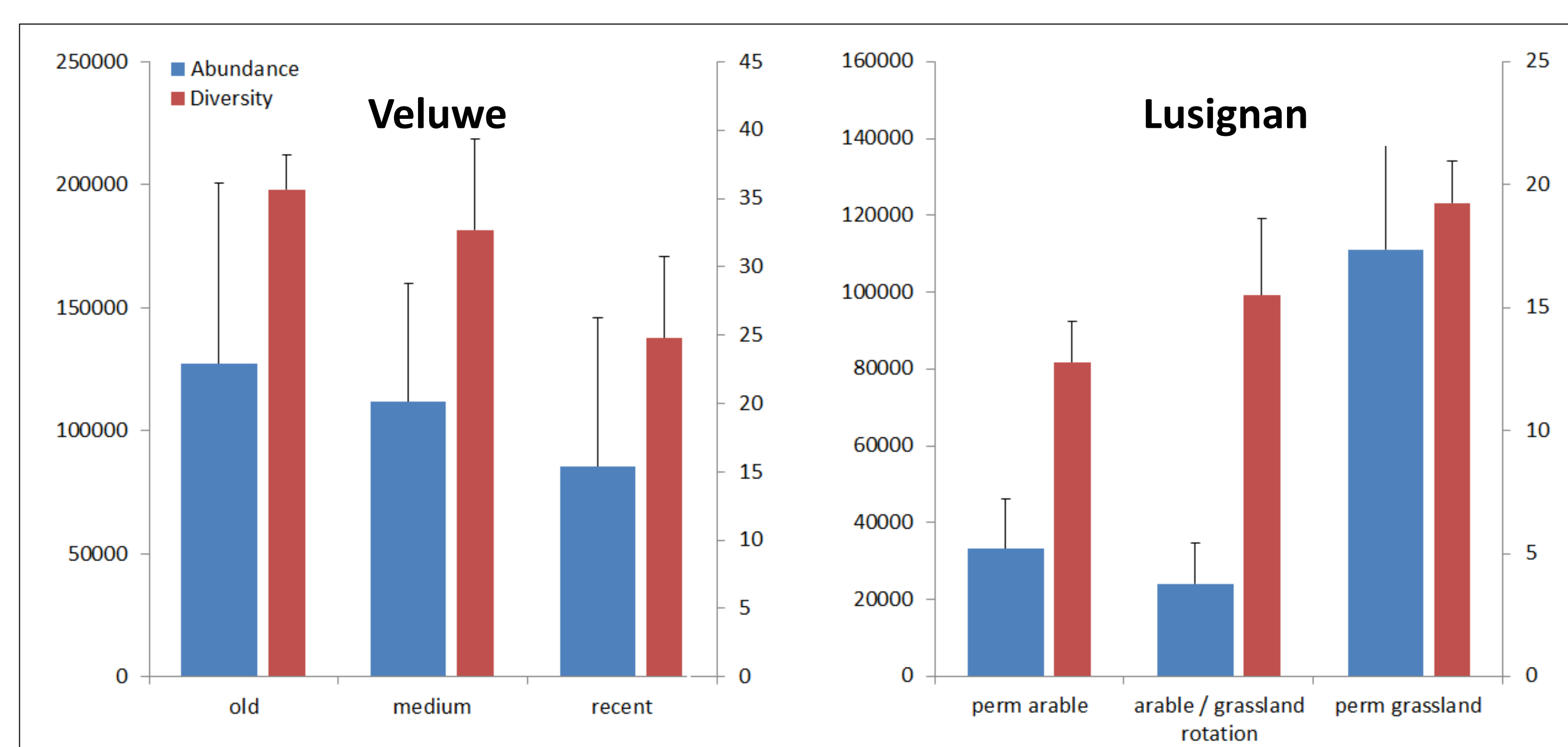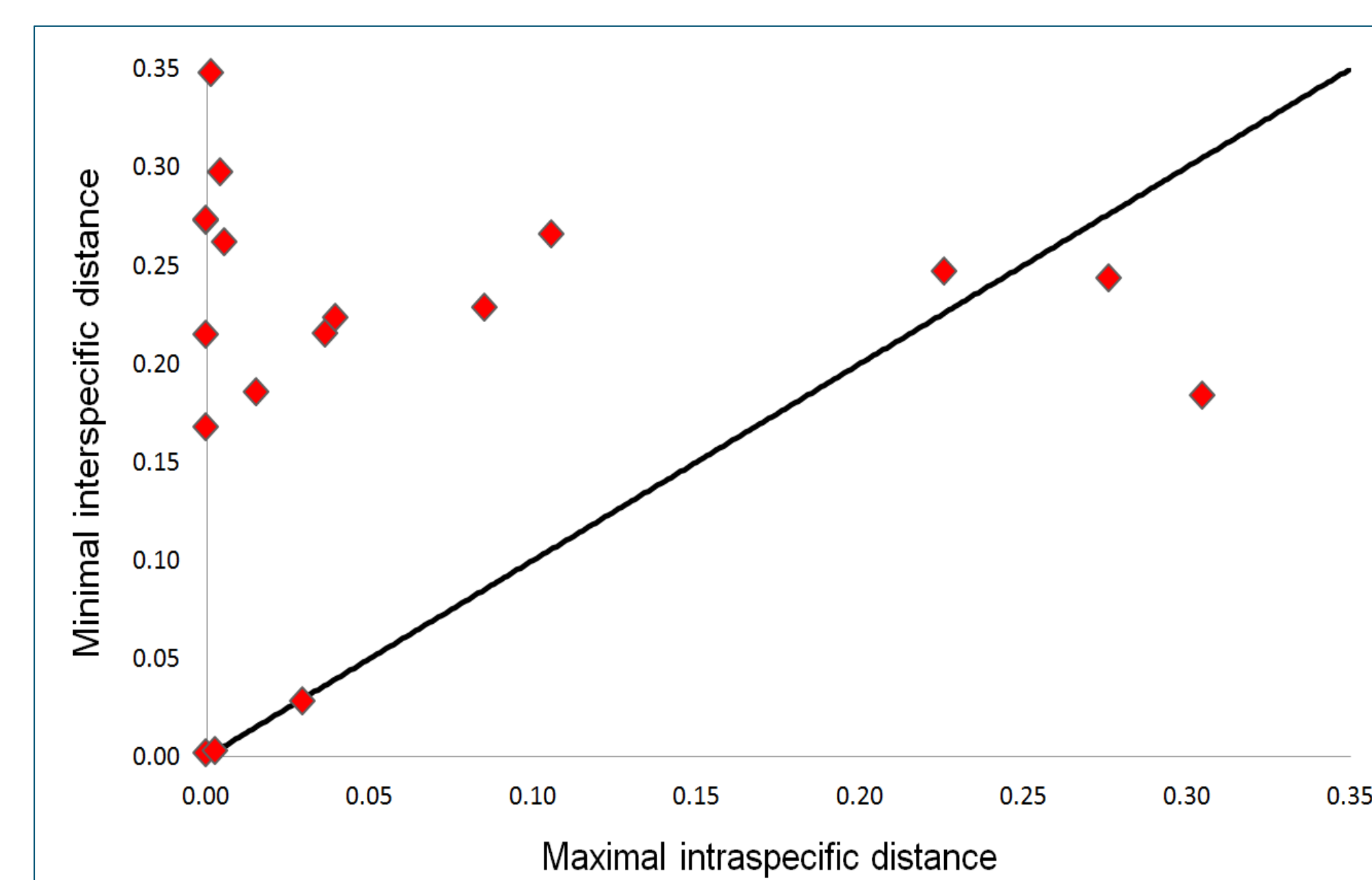

Figure 2. Variation in abundance (# individuals / m^2) and diversity (# taxa) among sites in time after last disturbance (Veluwe) or frequency of disturbance (Lusignan).

III: High-throughput DNA barcoding

● Reference database with CO1-barcodes for Dutch agricultural and (semi-)natural grasslands is approaching completion.

● 89 % of the species and 84% of the genera in the database formed its own well-supported monophyletic clade. 87% of the species showed a proper "barcoding gap" (Figure 3).

Figure 3. Sequence divergence between species, given as the relation between the maximal intraspecific and minimal interspecific K2P genetic distances. Only species with multiple samples were included (each red dot = 1 species). A proper barcoding gap is present in case the interspecific variation is larger than the intra specific variation (above the black line (X=Y)).

• We used sequencing data, augmented with data from BOLD, to develop a 209bp minibarcode located within the CO1-fragment.

• Results of 454 pyrosequencing (currently conducted) will allow validation of the barcoding approach by direct comparison of relative species abundances between the molecular and morphological datasets.

• Extraction of animals from soil is time consuming. Currently, we develop procedures based on direct extraction of DNA (including *environmental DNA* or *eDNA*) from soil samples.