Additional File 25. Workflow schematic of the DNA-SIP with methanol, the DNA SIP with carbon dioxide and the sequencing and quantification of the methanol dehydrogenase genes and 16S rRNA gene from the soil habitats

Collection, drying and homogenisation of Church Farm (CF) soil

DNA stable isotope probing with $^{13}CO_2$

Growth of plants

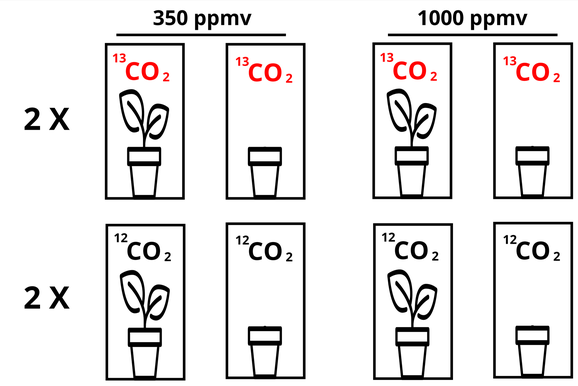
16 days

8 X Unplanted soil

8 X pea rhizosphere soil

Supply of growing pea plant and unplanted controls with $^{13}/^{12}CO_2$ at 350/1000 ppmv

12 days



Processing of samples

DNA extraction from the rhizosphere soil of all test groups → **Ultracentrifugation and fractionation of all DNA samples** → **16S rRNA gene sequencing of heavy and light fractions**

DNA stable isotope probing with ^{13}C-methanol

Growth of plants

28 days

6 X Unplanted soil

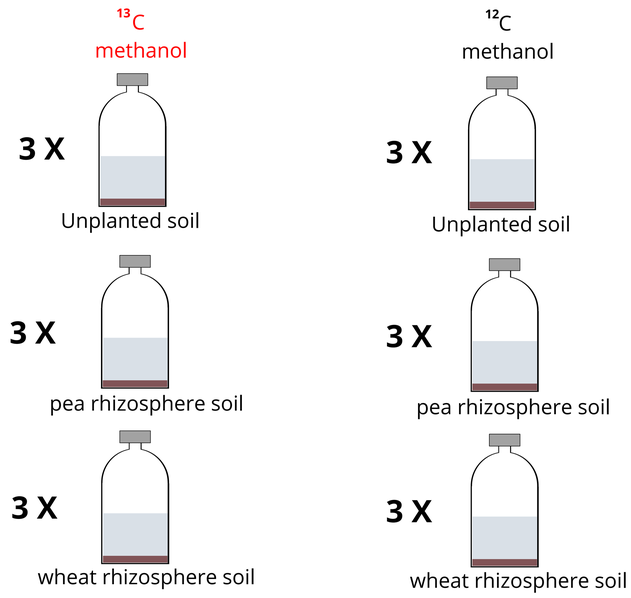
6 X pea rhizosphere soil

6 X wheat rhizosphere soil

Enrichment of soil methylotrophs with $^{13}C/^{12}C$ methanol

T1 7 days

T2 20 days



Processing of samples

DNA extraction from all T1 samples → **Ultracentrifugation and fractionation of all DNA samples** → **16S rRNA gene sequencing of heavy and light fractions**

DNA extraction from all T2 samples → **Ultracentrifugation and fractionation of all DNA samples** → **16S rRNA gene sequencing of heavy and light fractions**

DNA extraction from all T2 samples → **Ultracentrifugation and fractionation of all DNA samples** → **Shotgun sequencing of the heavy fractions of all C enriched test groups**

Quantification and characterisation of the diversity of mxaF, xoxF, mdh2 and 16S rRNA genes in the pea and wheat rhizosphere soils and unplanted soil

Processing of samples

DNA extraction from CF soil, unplanted soil, pea rhizosphere soil and wheat rhizosphere soil

- **16S rRNA gene amplification by PCR** → **16S rRNA gene sequencing**
- ***xoxF, mxaF*** **and** ***mdh2*** **amplification by PCR** → ***xoxF, mxaF*** **and** ***mdh2*** **sequencing**
- **Quantification of 16S rRNA genes, *xoxF5* and *mxaF* by qPCR**