AARHUS UNIVERSITY

Niels Bohse Hendriksen and Anne Winding
Department of Environmental Science, Aarhus University, Denmark


nbh@dmu.dk aw@dmu.dk

Extracellular enzyme activity assay as indicator of soil microbial functional diversity and activity

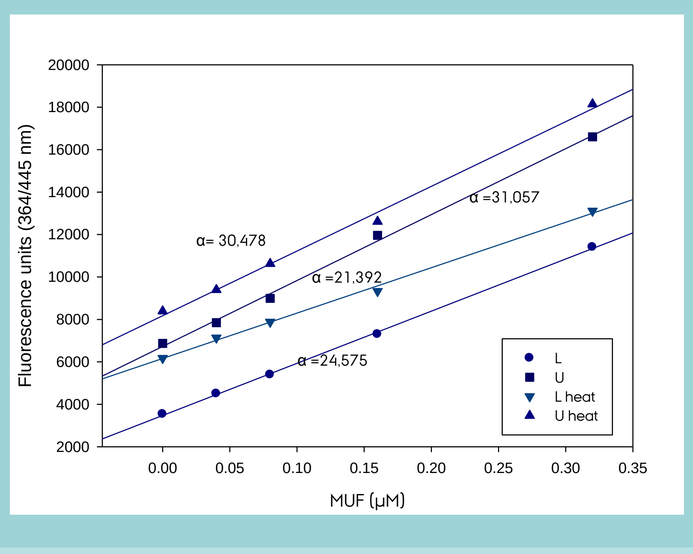
Aim

Standard Operating Procedure for extracellular enzyme activity (EEA) assay optimized for soil enzymes

Results

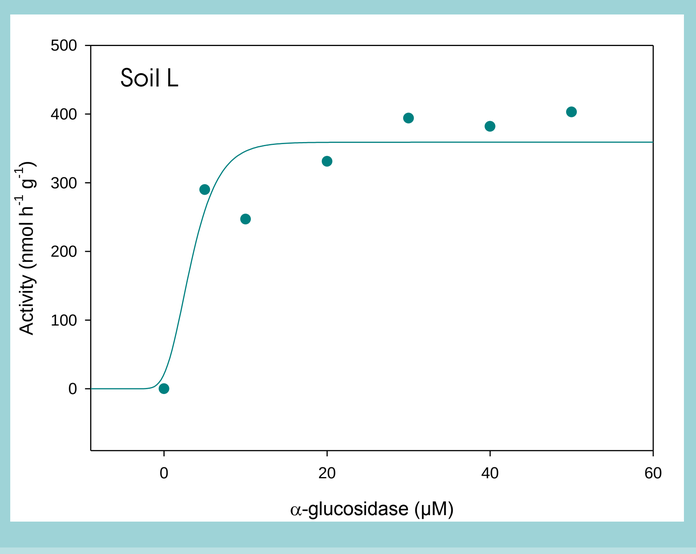
Standard Curves for each soil

Two soils +/- inactivation (90°C 30 min). R^2>0.995. Standard curves for individual soil extractions necessary



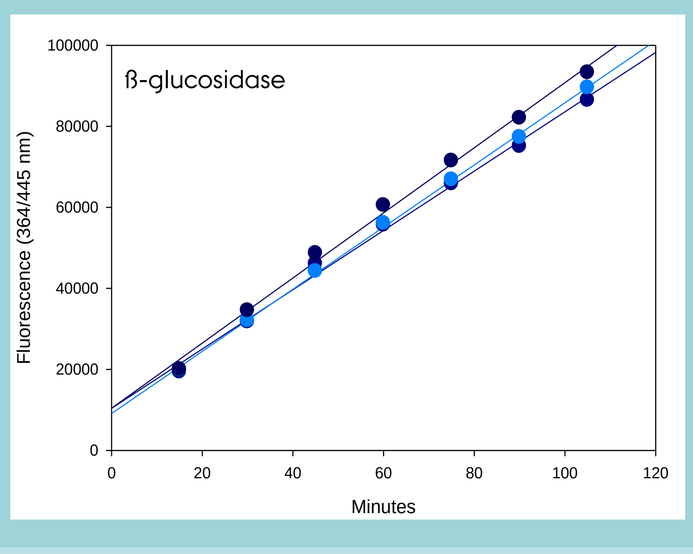
Substrate saturation

V_{max} approx. 15 µM.
All following experiments were performed at 50 µM



Replication within extractions

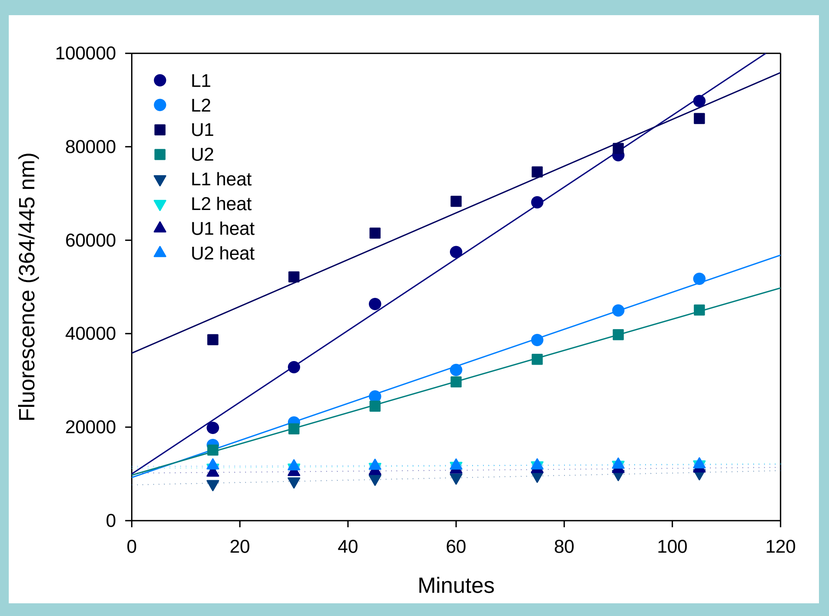
Triplicates of identical soil extractions (2 g dw + 20 ml, 5 min sonication, 5 min wrist hand shaking. Activity rates 732-803 fluor. units min^{-1}. 200 µl microtiter well $^{-1}$)



α-gluocosidase

Soil	Activity rate (fluorescence units min-1)	r2
L1	78.9	0.996
L2	45.2	0.991
U1	70.6	0.987
U2	52.2	0.995
L1 heat	15.4	0.896
L2 heat	5.0	0.558
U1 heat	12.9	0.940
U2 heat	8.4	0.748

β-gluocosidase



β-gluocosidase

Soil	Activity rate (fluorescence units min-1)	r2
L1	767.5	0.997
L2	396.7	0.997
U1	500.1	0.970
U2	333.9	0.999
L1 heat	25.5	0.975
L2 heat	8.8	0.972
U1 heat	10.5	0.926
U2 heat	3.5	0.628

- Replicate variation between extractions of identical soils is significant, while variation within each soil extraction is insignificant.
- Heat inactivation significantly reduces activity rate, confirming enzymatic origin of activity and excluding chemical activity.

Background

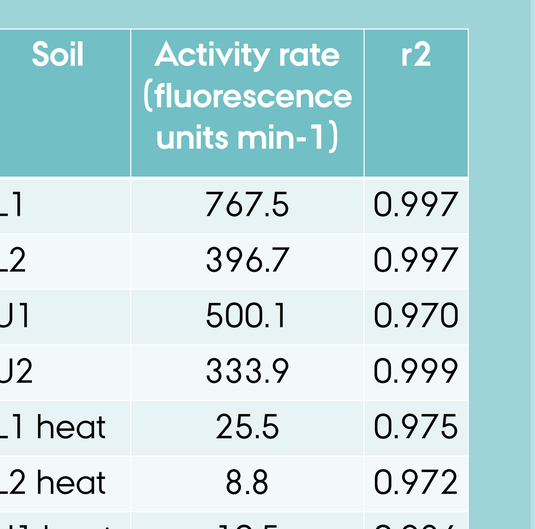
Loss of biodiversity and threats to the ecosystem services provided by soil have fostered the need for indicators of microbial biodiversity of soils. Such indicators need to be standardized and validated across time, land use, and climate. Extracellular Enzyme Activity (EEA) assay has been suggested as an indicator of both potential activity and functional diversity of soil microbial communities (Ritz et al. 2009).

EEA assay has been developed and used over decades to study natural and anthropogenic effects on microbial soil communities and an ISO/TC 22939:2010 standard has been developed. The EEA assay is microtiter based and uses fluorogenic substrates indicating enzymatic degradation of either C, N or P based substrates (Marx et al. 2001).

Depending on the design, EEA assess **potential activity** in a specific soil or **diversity** of soil microbial communities across different soils.

Future

1. Replicate variation will be minimized by e.g. increasing sample size
2. EEA assay will be tested at 6 LTOs in EU representing 4 climate zones with arable land-use at two different seasons: fall and spring. Within two of the LTOs the contrasting land-use grassland will also be assessed. By this we will test sensitivity and robustness of the EEA assay and compare with other biodiversity assays of microbes and fauna
3. EEA assay will be employed at a transect across EU with 80 sites from north to south and east to west during fall 2012 to:
   - Establishing Normal Operating Range maps for soil biodiversity
   - Identify functional diversity across EU



Acknowledgment: Technical assistance: Tina Thane. Funding: EU FP7 EcoFINDERS project no. FP7-264465
References: Marx et al. 2001 Soil Biol. Biochem. 33:1633-1640. Ritz et al. 2009 Ecol. Ind. 9:1212-1221.