FINE-TUNING DNA APPROACH AND MULTIPLE ENZYME ASSAY TO ASSESS SOIL MICROBIAL PROPERTIES UNDER DIFFERENT FOREST FLOORS

Ascher J.[a,b], Gómez-Brandón M.[b], Fornasier F.[c], Bardelli T.[a], Arfaioli P.[a], Francioli D.[d], Egli M.[e], Sartori G.[f], Cherubini P.[g], Petrillo M.[g], Broll G.[h], Graefe U.[i], Gobat J.M.[j], Pietramellara G.[a], Insam H.[e]

[a]Department of Plant, Soil and Environmental Science, University of Florence, Italy; [b]Institute of Microbiology, University of Innsbruck, Austria; [c]Council for Research and Experimentation in Agriculture, Gorizia, Italy;
[d]Department of Soil Ecology, Helmholtz Centre for Environmental Research, Halle, Germany; [e]Department of Geography, University of Zürich, Switzerland; [f]Museo delle Scienze, Corso del Lavoro e della Scienza, 3, 38122 Trento, Italy;
[g]Swiss Federal Institute for Forest, Snow and Landscape, Birmensdorf, Switzerland; [h]Institute of Geography, University of Osnabrück, Germany; [i]IFAB Institut für Angewandte Bodenbiologie GmbH, Hamburg, Germany; [j]Institute of Biology, University of Neuchâtel, Switzerland

We monitored the small scale vertical distribution of the microbiota of acid forest topsoils under representative herbaceous vegetations and organic residues of Alpine conifer forests at South vs. North exposure by combining powerful methods: small scale sampling of the topsoil & fine-tuning DNA approach & reliable estimation of microbial biomass (dsDNA) & *multiple enzyme assay of 12 hydrolases* covering the principal biogeochemical cycles. Soil microbiological parameters were linked with principal physico-chemical parameters as a function of soil depths, forest floor representatives and exposure (*thermal signal*).

Material and Methods

The **study sites -** part of the ongoing D.A.CH-DecAlp project - are located at 1600 m asl, at North (N3) vs South (S8) exposure (Egli et al., 2006) in Trento, Italy.

- **Small-scale sampling** of the topsoil (0-15 cm) was performed in 2,5 cm intervals using a special corer (Ulfert Graefe, IFAB).
- **Principal soil properties** (pH; moisture; NH4+; NO3-; Ntot; Ctot; C/N ratio; Electrical conductivity; volatile compounds) were assessed as a function of the forest floor representatives (grass; branches; litter; moss) of the acid forest soils (paragneiss), and exposure (N vs S; *thermal signal*).
- ***Fine-tuning DNA* approach** consisting in sequential extraction of the extracellular (eDNA) and intracellular fraction (iDNA) of the soil metagenome (Ascher et al., 2009).
- **Quantitative-qualitative analyses of DNA** (eDNA, iDNA) by PicoGreen based fluorimetry (*ds*DNA; Qubit, LifeTechnologies), µl-spectrophotometry (PicoDrop) and agarose-gel electrophoresis (Ascher et al., 2012).
- **Direct extraction and Picro-Green based quantification of *crude* (not purified) double stranded DNA (*ds*DNA) of the soil metagenome (total DNA, tDNA) as reliable estimator of soil microbial biomass** (Fornasier et al., 2014), overcoming the unavoidable loss of DNA during purification when PCR compatible DNA is required for downstream analyses, and thus without the risk of underestimation.
- **Metabolic activity** was assessed by extracting and simultaneously assaying 12 hydrolytic soil enzymes involved in principal geo-biochemical cycles by a heteromolecular exchange procedure and fluorometric quantification using fluorescent, 4-methyl-umbelliferyl-(MUF) and 4-amido-7-methyl-coumarine (AMC) substrates (Fornasier and Margon, 2007).

C-cycle: Cellulase; Xylanase; alpha-glucosidase; beta-glucosidase
P-cycle: Acid phosphomonoesterase; Phosphodiesterase; Pyrophosphate-phosphodiesterase; Alkaline phosphomonoesterase
N-cycle: Leucine-Aminopeptidase; Lysine-Aminopeptidase
S-cycle: Arylsulfatase
Esterase (global hydrolytic activity index)

EXPOSURE EFFECT > FOREST FLOOR EFFECT

PHYS.- CHEM. PROPERTIES

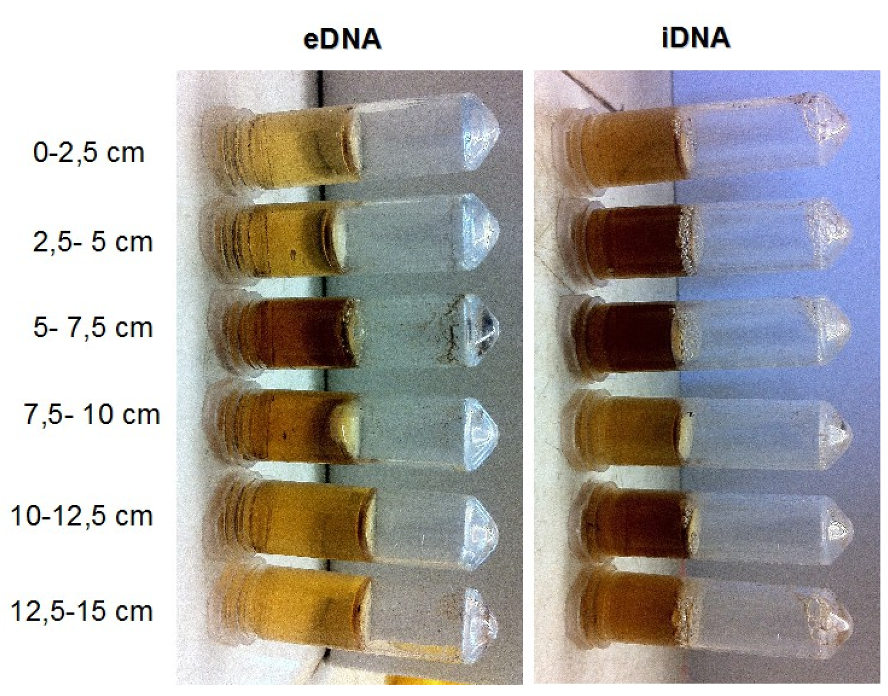
N > S — **! BRANCHES: S > N**

MICROBIOL. PROPERTIES

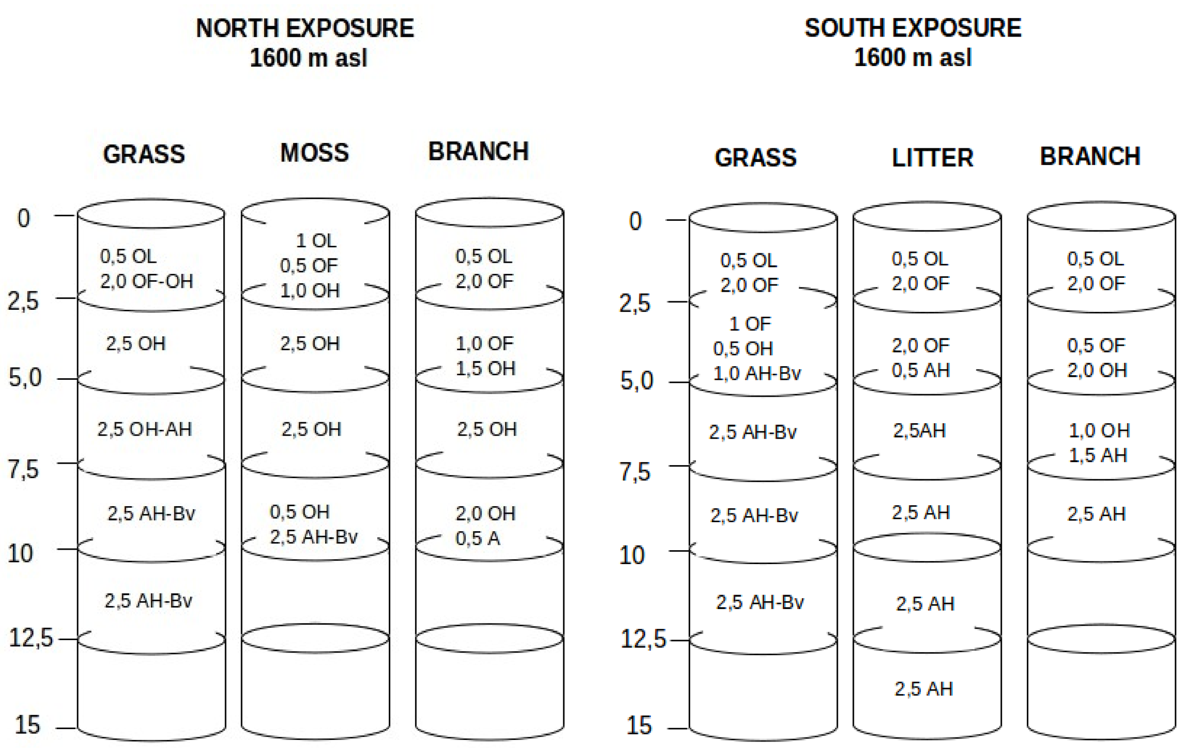
ENZYMES: N > S

! ARYLSULFATASE ~ — **! PHOSPHATASES: S > N**

tDNA: N ~ S
iDNA: N ~ S
! eDNA: N > S



Sequentially extracted extracellular (eDNA) and intracellular DNA (iDNA) from the topsoil (0-15 cm) under litter-cover (south exposure). Different colour intensities of the *crude* DNA extracts reflect the different amounts of SOM at a small scale (2,5 cm intervals).



Small-scale classification (horizons) of the topsoil layer (0-15 cm) under "active" (grass; moss) and "passive" (branch; litter) forest floors of north vs south exposed acid forests (1600 m).

Soil under BRANCHES at South exposure: special scenario with opposite trends respect to all the other study sites.

FINE-TUNING DNA APPROACH: sensitive tool to assess differences in the soil metagenome that are not evident at the level of total and/or intracellular soil DNA.

The observed bio-chemical "small scale - differences" within the topsoil layers will be correlated with the data from our mesofauna monitoring (microflora-mesofauna interaction): HUMUS FORM as PROXY for overall soil processes.

References

Ascher et al. (2009) Sequential extraction and genetic fingerprinting of a forest soil metagenome. *Applied Soil Ecology 42, 176-181.*
Ascher et al. (2012) Are humus forms, mesofauna and microflora in subalpine forest soils sensitive to thermal conditions? *Biology and Fertility of Soils 48, 709-725.*
Egli et al. (2006) Effect of north and south exposition on weathering and clay mineral formation in Alpine soils. *Catena, 67, 155-174.*
Fornasier et al. (2014) A simplified, low-cost and versatile DNA- based assessment of soil microbial biomass. *Ecological Indicators 45, 75-82.*
Fornasier and Margon (2007) Bovine serum albumin and Triton X-100 greatly increase phosphomonoesterases and arylsulphatase extraction yield from soil. *Soil Biol. & Biochem. 39, 2682-2684.*