

Evaluation and Comparison of Protein Extraction Protocols from Soil Samples

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The Soil Samples:

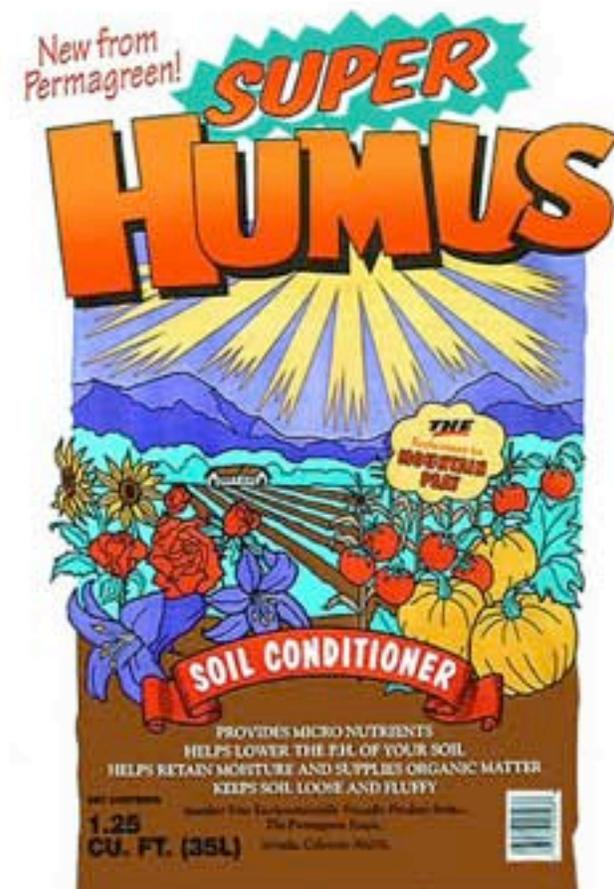
Collected and distributed by Shawna McMahon

High Organic Matter (HOM) sample

Low Organic Matter (LOM) sample



Agricultural (corn) field just outside of
Fort Collins, CO
Estimated at 3-4% OM



Commercial humus from nursery
Estimated at > 50% OM

Participating Labs

University of Warwick

Cornell University

Pacific Northwest National Lab

CNR-ISE-Pisa

Helmholtz Centre for Environmental Research

Colorado State University

Lawrence Berkeley National Lab



Soil Extraction and Purification Workflow

Extraction of Proteins
from Soil



Removal of Organic
Matter (humic acids)



Concentration and
Purification of Proteins

Soil Extraction and Purification Workflow

Extraction of Proteins from Soil

Removal of Organic Matter (humic acids)

Concentration and Purification of Proteins

- 0.1M NaOH shaken @ RT, followed by bath sonication
- 0.1M NaOH + probe sonication, followed by shaking @ RT
- K₂SO₄ (pH 6.6)+ EDTA + incubation with shaking @ RT followed by filter paper
- PBS + pulsed homogenization
- Hexafluoroisopropanol (protein denaturant)
- Lysis buffer + blender/barocycler
- Tris+0.1%SDS+3M Urea+ 2-mercaptoethanol+EDTA + probe sonication + incubation @ 100°C
- 2-step:
 - 1.) protease inhibitor+0.1%SDS+DTT+KCL+MGCl₂+ bead beater
 - 2.) protease inhibitor+ lysis soln (tris+EDTA+DTT +glycerol +pefabloc+lysosme) + bead beater

Soil Extraction and Purification Workflow

Extraction of Proteins
from Soil

- Phenol }

- PVPP

- CaCl₂ + Tween-20+ PVPP }

- Nothing

Removal of Organic
Matter (humic acids)

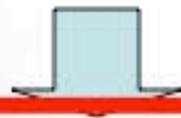
Concentration and
Purification of Proteins

Soil Extraction and Purification Workflow

Extraction of Proteins
from Soil



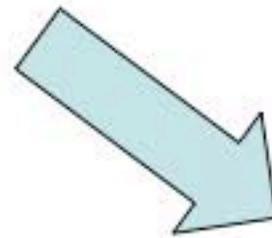
Removal of Organic
Matter (humic acids)



Concentration and
Purification of Proteins

- Precipitation with Ammonium Acetate + Methanol
- Precipitation with Acetone
- Precipitation with TCA
- 10K MWCO spin filters
- dialysis followed by 10K MWCO spin filter
- dialysis followed by 3K MWCO spin filter followed by 10K MWCO spin filter

SDS-PAGE Gels



All samples were reduced at 70°C for 10 min and boiled for additional 5 min. ALL 20 μ l of supernatant was loaded on the gel.

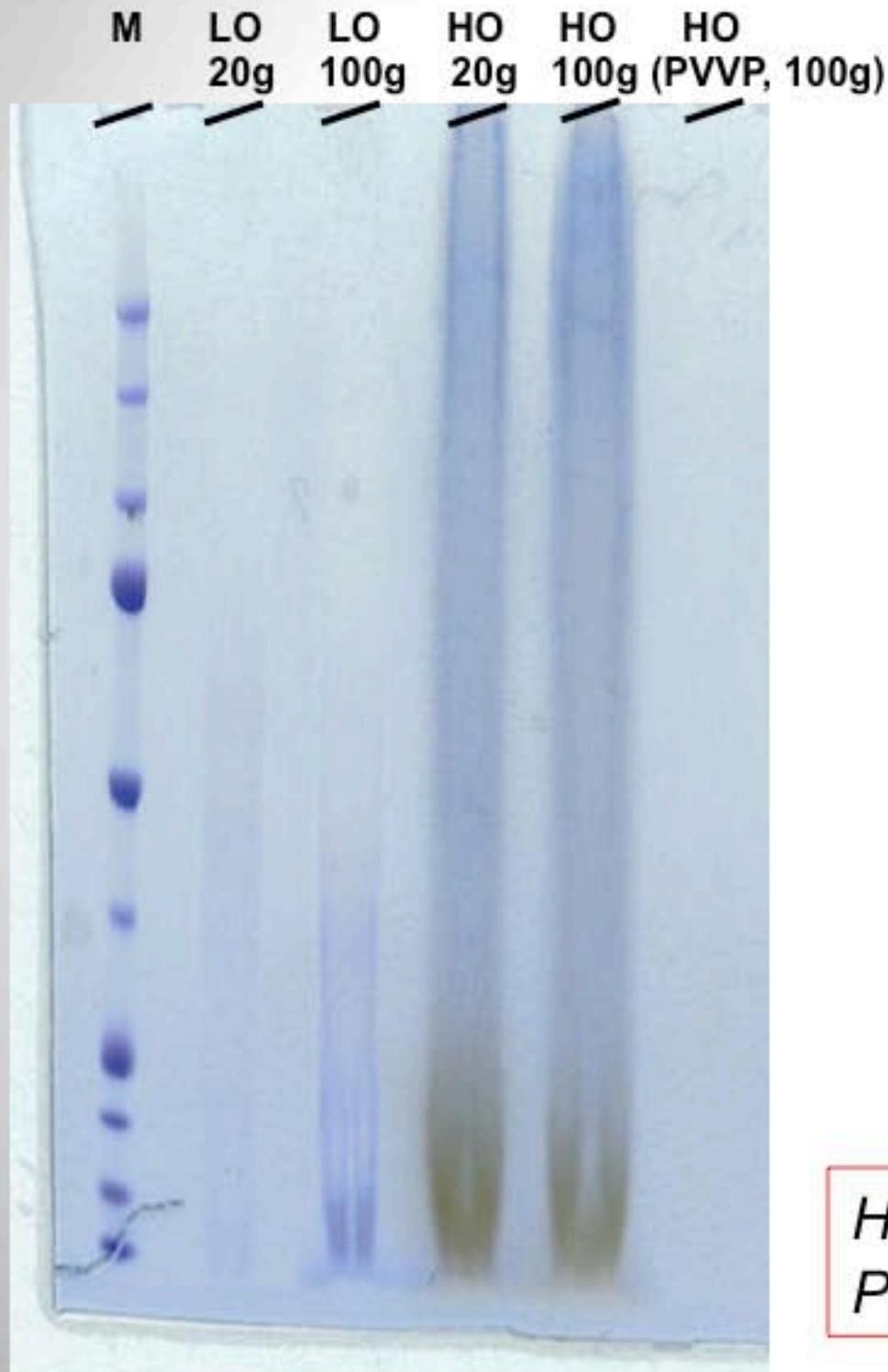
13 μ l ddH₂O
5 μ l 4X LDS sample buffer
2 μ l reducing agent

* Subsample of some pellets



4-12% Bis-Tris Gel
170 volt in 1X NuPAGE MOPS SDS Running buffer

The Results: Gel Separation



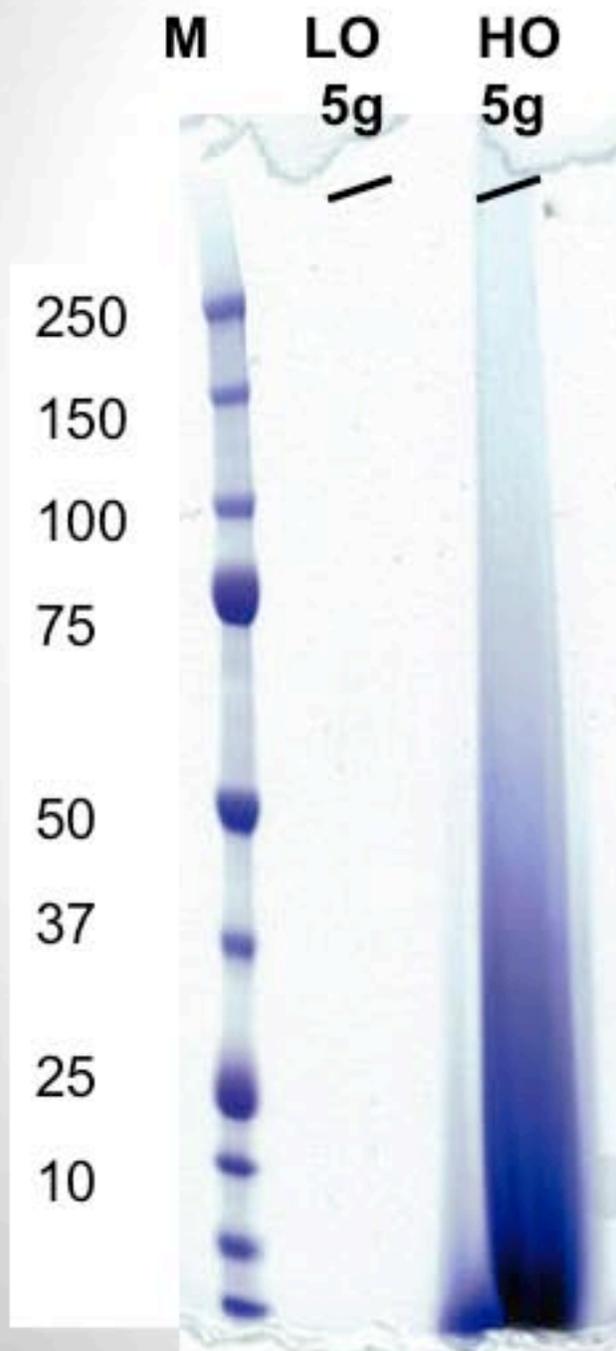
University of Warwick

Helena Wright/Ashley Johnson-Rollings

- K₂SO₄ (pH 6.6)+ EDTA + incubation with shaking @ RT followed by filter paper
- dialysis followed by 3K MWCO spin filter followed by 10K MWCO spin filter
- PVVP (100g HO sample only)
- Acetone Precipitation

*HOM and LOM, Dark brown pellets → sub-sampling
PVVP sample is light brown/white → used ALL*

The Results: Gel Separation



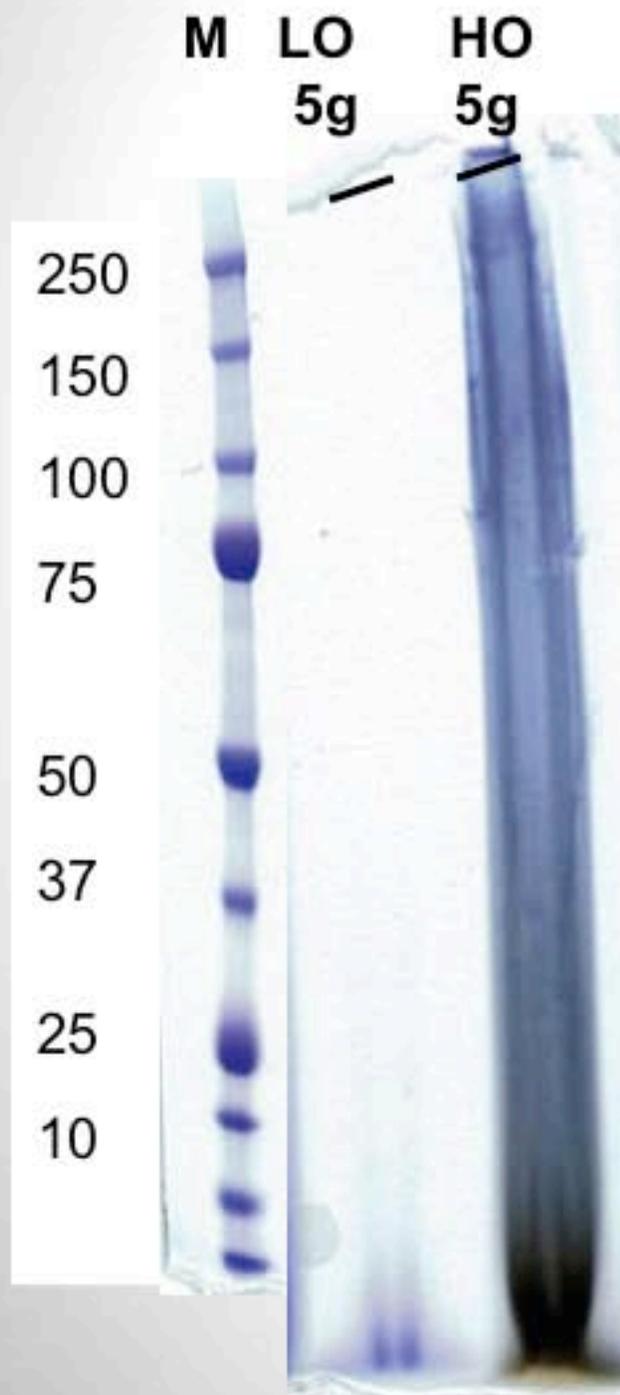
Cornell University
Ruth Richardson

- 0.1M NaOH + probe sonication, followed by shaking @ RT
- Phenol
- Acetone Precipitation

HOM, dark brown pellet → sub-sampling
LOM, light brown → sub-sampling

The Results: Gel Separation

Cornell University
Jeff Werner

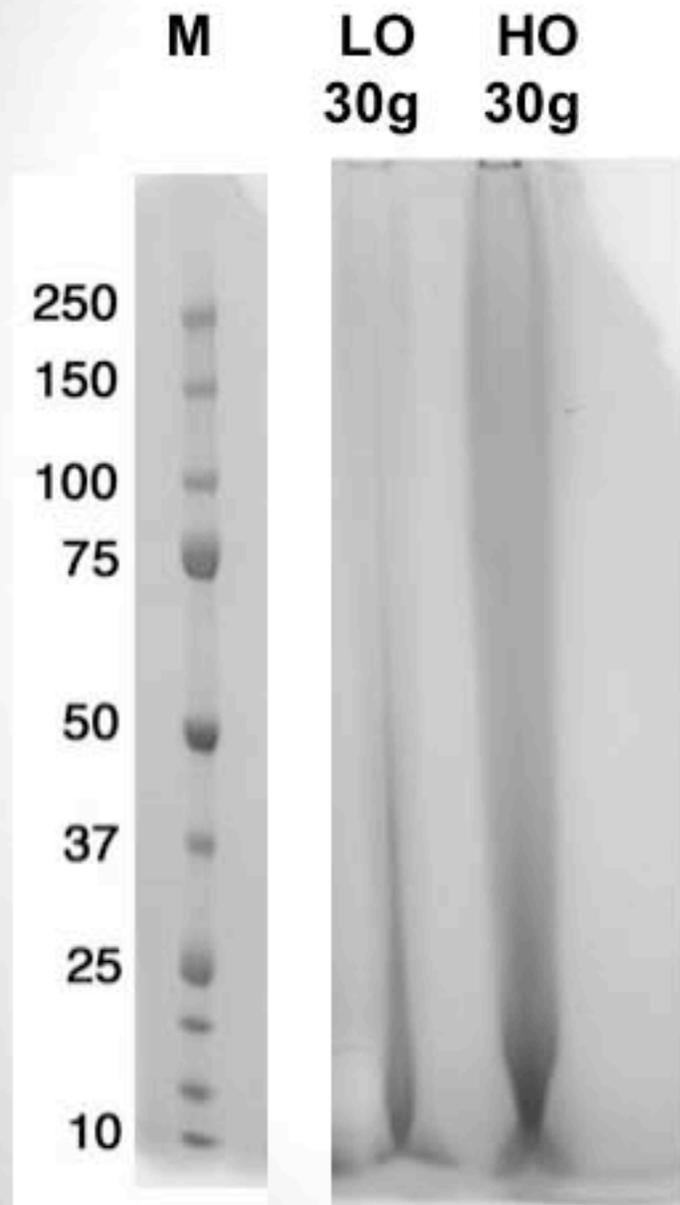


- Tris+0.1%SDS+3M Urea+ 2-mercaptoethanol +EDTA + probe sonication + incubation @ 100oC
- Precipitation with Ammonium Acetate + Methanol

*HOM, Dark brown pellet → subsampling
PVPP sample is light brown/white → used ALL*

The Results: Gel Separation

Pacific Northwest National Lab
Stephen Callister/Carrie Nicora

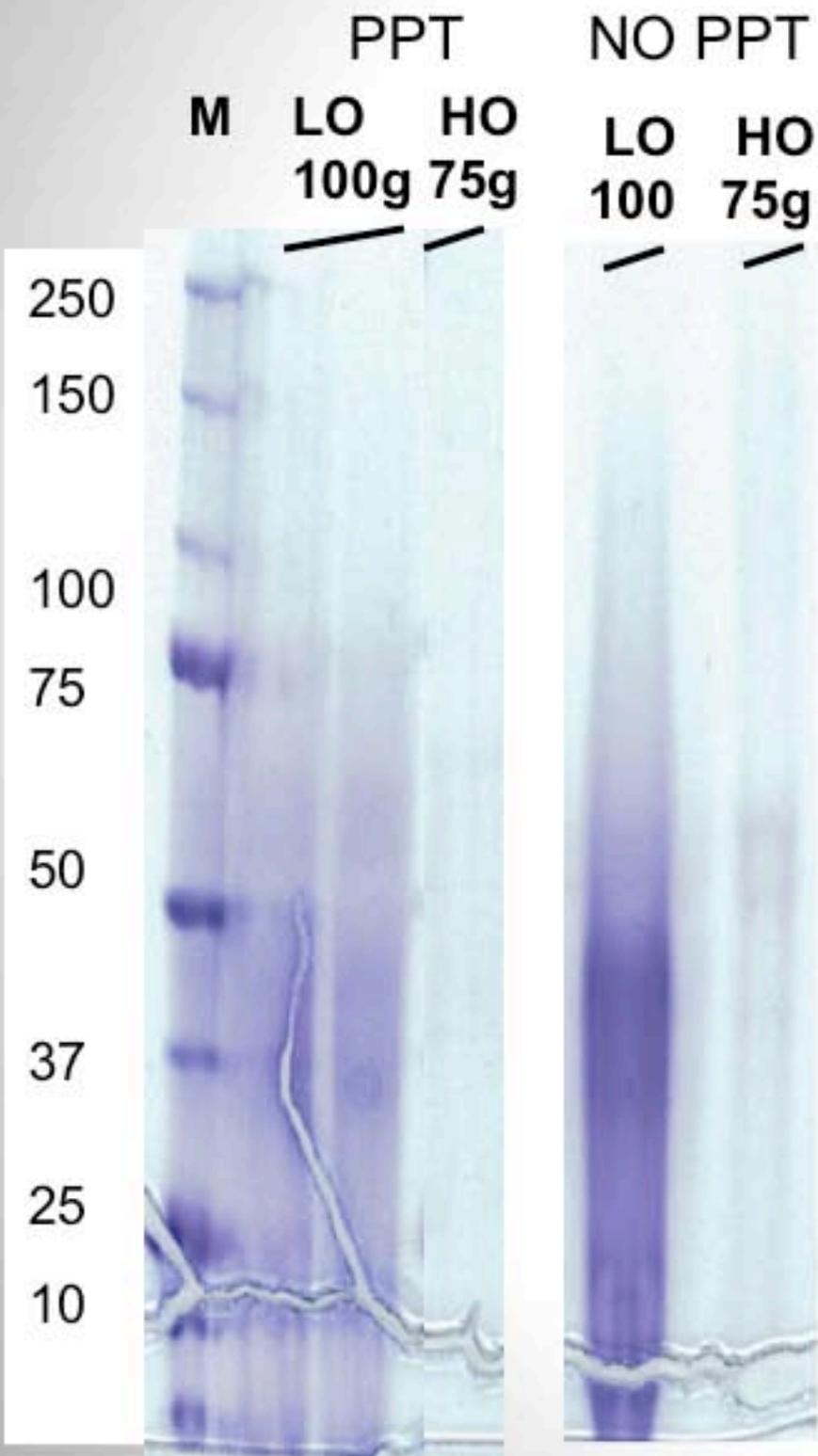


- Lysis buffer + blender/barocycler
- PVPP (multiple treatments both samples)
- 10K MWCO spin filters
- acetone precipitation

HOM, light brown pellet → sub-sampling
LOM, light brown pellet → used ALL

The Results: Gel Separation

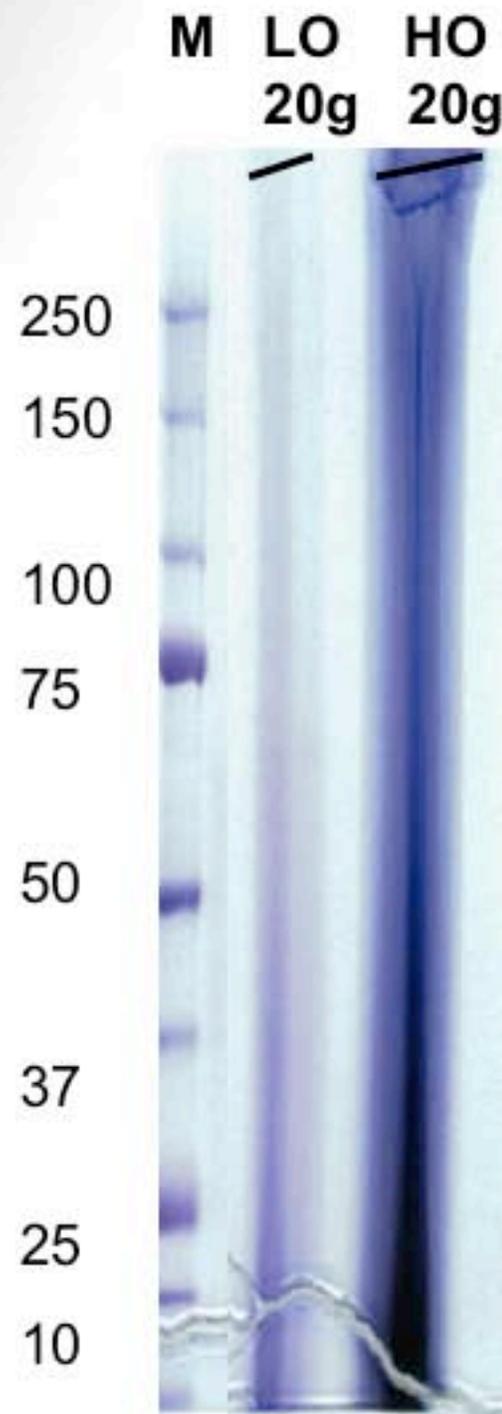
CNR-ISE-Pisa
Cristina Macci



- K₂SO₄ (pH 6.6)+ EDTA + incubation with shaking @ RT followed by filter paper
- PVPP (HO samples only)
- dialysis followed by 10K MWCO spin filter
- acetone precipitation (PPT lanes only)

NO PPT, LOM, light brown → used ALL
NO PPT, HOM, white → used ALL
PPT, LOM, light brown → used ALL
PPT, HOM, white → used ALL

The Results: Gel Separation



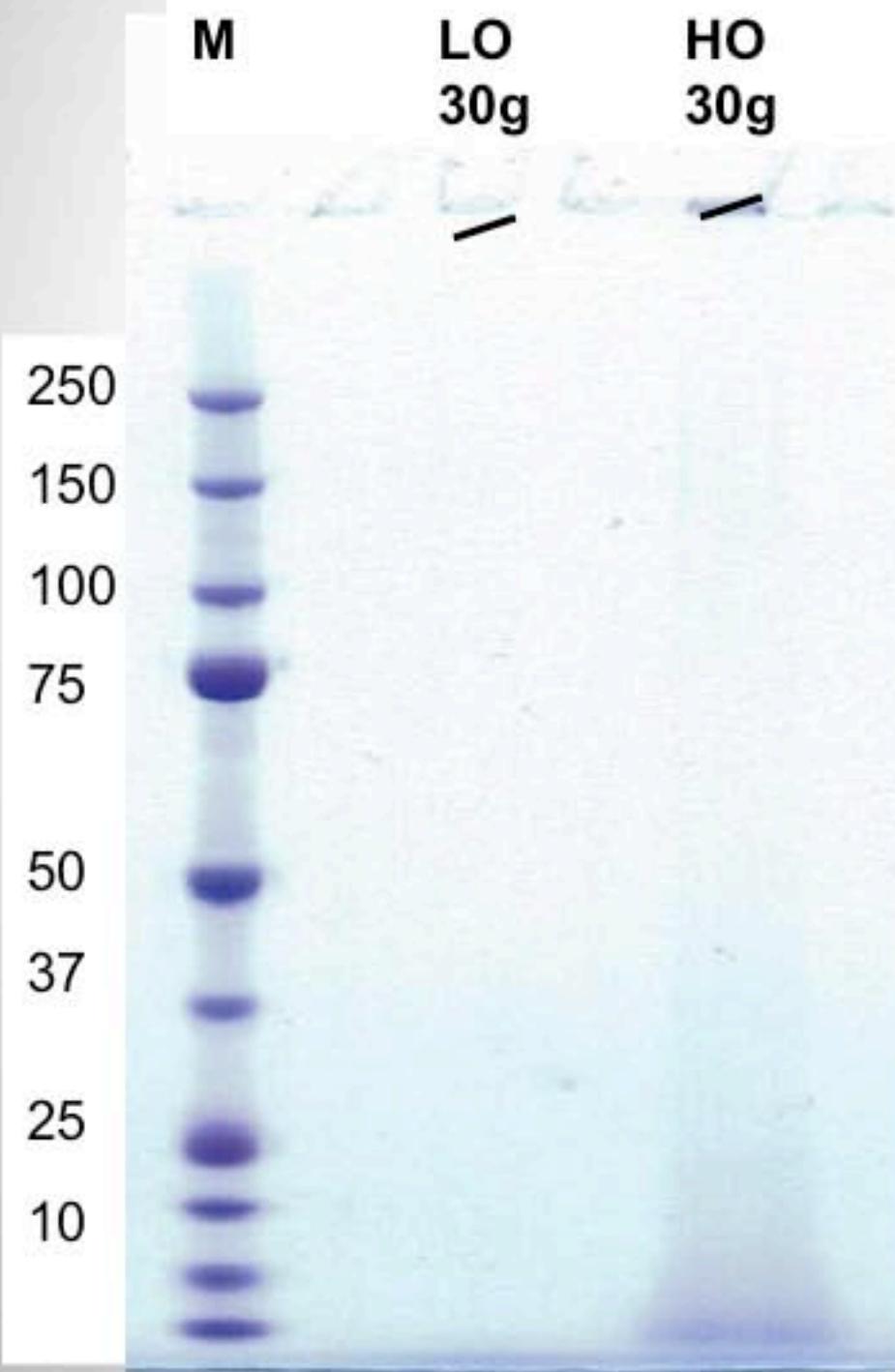
Helmholtz Centre for Environmental Research
(Jana Seifert/Martin von Bergen)

- 0.1M NaOH shaken @ RT, followed by bath sonication
- Phenol
- Precipitation with Ammonium Acetate + Methanol

HOM, dark brown pellet → used ALL
LOM, light brown pellet → used ALL

The Results: Gel Separation

Lawrence Berkeley National Lab
 Janet Jansson/Olivia Mason



- PBS + pulsed homogenization + Centrifuge followed by Hexafluoroisopropanol
- Aqueous Precipitation followed by resuspension in tris/glycine/SDS
- Acetone Precipitation

HOM, white pellet → sub-sampling
LOM, white pellet → sub-sampling

The Results: Gel Separation

M LOa LOb LOc HOa HOb HOc



LOMa K₂HPO₄/EDTA
 LOMb CaCl₂/Tween
 LOMc OM 2 step
 HOMa K₂HPO₄/EDTA (brown when added)
 HOMb CaCl₂/Tween
 HOMc 2 step (brown when added)

Colorado State University
 Matt Wallenstein/Jessica Ernakovich

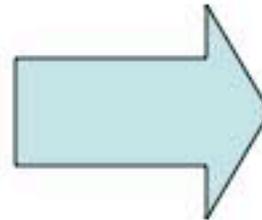
- 2-step:
 - 1.) protease inhibitor
 +0.1%SDS+DTT+KCL
 +MgCl₂+
 bead beater
 - 2.) protease inhibitor+ lysis
 soln (tris+EDTA+DTT
 +glycerol +pefabloc
 +lysosme) + bead beater
- CaCl₂ + Tween-20+ PVPP
- Precipitation with TCA

The Results: Mass Spectrometry

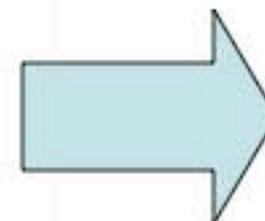
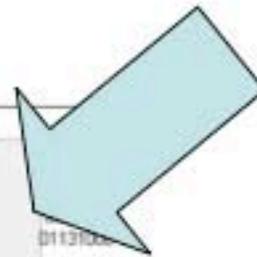
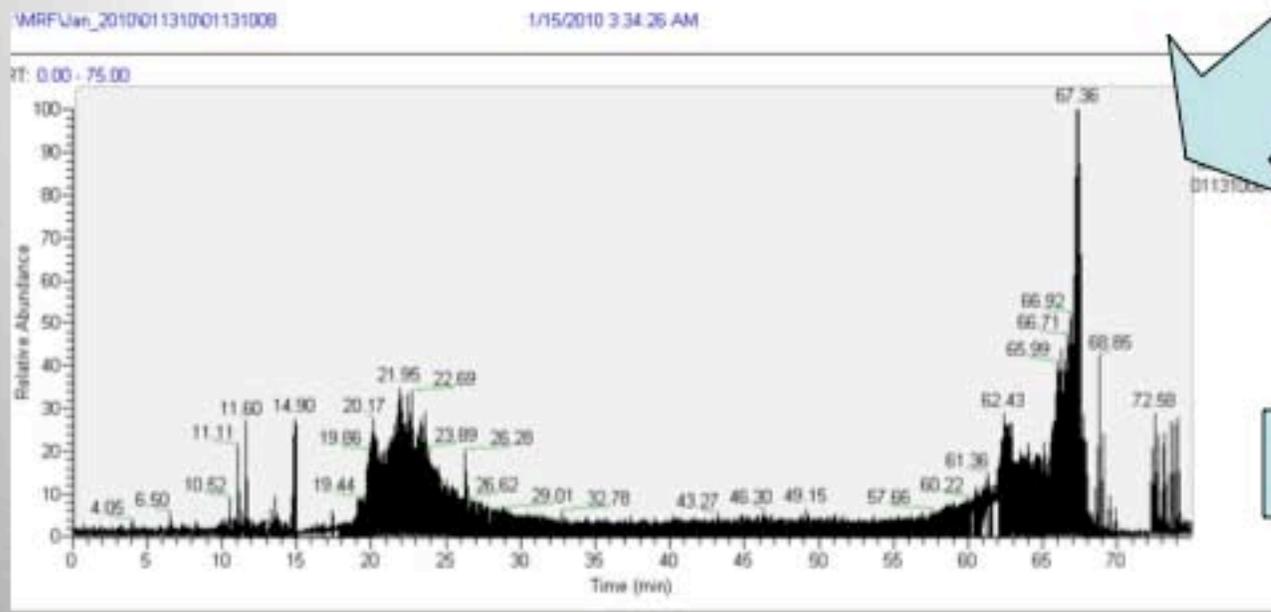


8M Urea
Reduce/Alkylate (DTT and IAA)
Dilute to 2M Urea
Digest with Trypsin

Zip-tip purify



nanoLC-MS/MS (LTQ linear ion trap)



Mascot (ALL NCBI nr)
Trypsin & Keratin
(Bacteria only → nothing)

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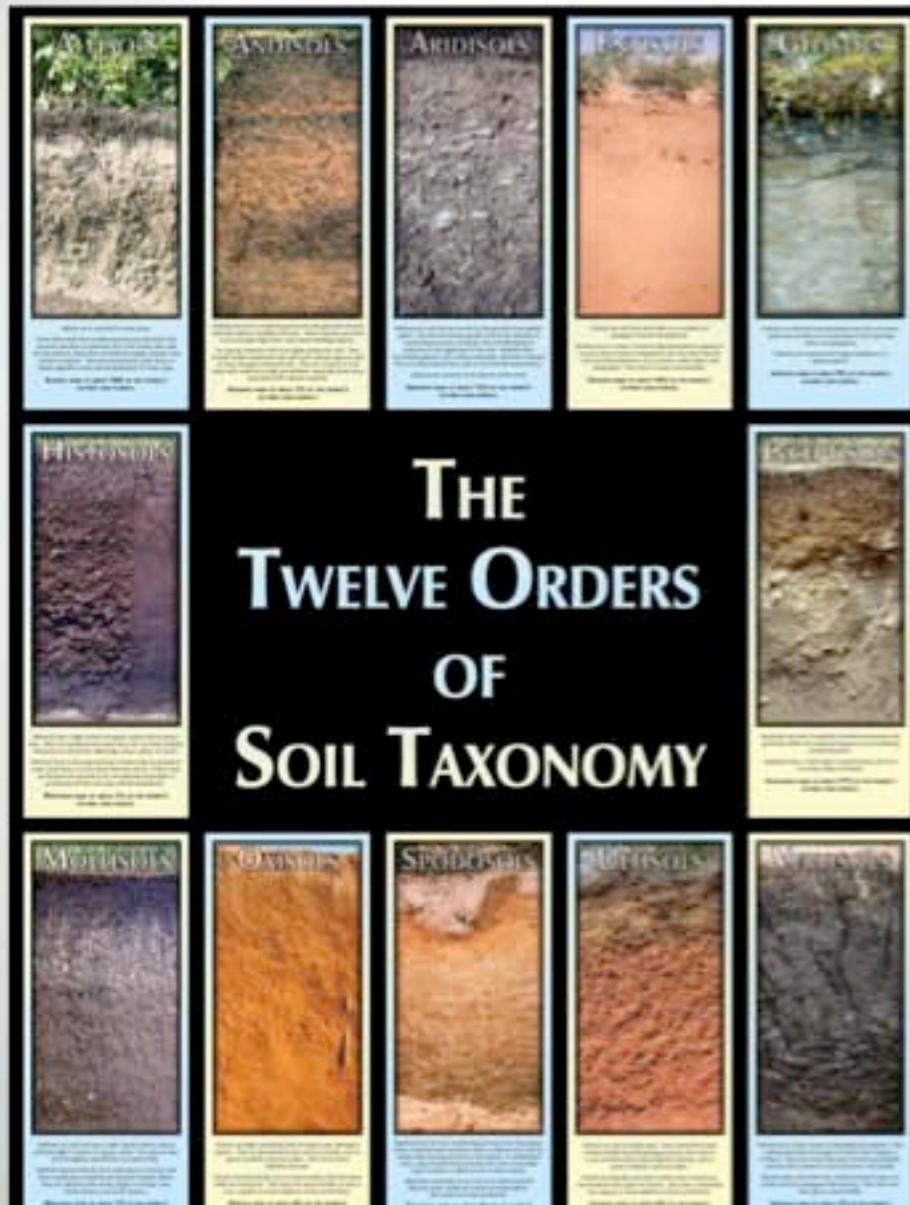
Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and perspectives

F. BASTIDA, J. L. MORENO, C. NICOLÁS, T. HERNÁNDEZ & C. GARCÍA

Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), Department of Soil and Water Conservation and Waste Management, Campus Universitario de Espinardo, Espinardo, Murcia 30100, Spain

“Unfortunately, there are no published studies that compare the efficiency, in terms of yield and quality, of different extraction protocols....”

Workshop Goal? Designing a Comparative Experiment



1. What types of soil are represented?
2. How much soil is used?
3. Which protocols are compared?
 1. Buffers/reagents?
 2. pH?
 3. Temperature?
4. What metrics are used?
 1. Visual (1D gels)
 2. Protein assay? Which one?
 3. Mass Spectrometry