## FIRST JOINT MEETING ON SOIL AND PLANT SYSTEM SCIENCES (SPSS 2019)

Natural and Human-induced Impacts on the Critical Zone and Food Production

> CIHEAM BARI, ITALY 23-26 SEPTEMBER 2019

In collaboration with











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## First Joint Meeting on Soil and Plant System Sciences (SPSS 2019)

Natural and Human-induced Impacts on the Critical Zone and Food Production

# **PROGRAMME AND ABSTRACTS**

### CIHEAM Bari, Italy 23-26 September 2019

https://SPSS2019.azuleon.org

#### **Organising Committee**

Teodoro MIANO (*Chair*) Claudio ZACCONE (*Secretary*) Gennaro BRUNETTI Claudio COCOZZA Carmine CRECCHIO Valeria D'ORAZIO Concetta Eliana GATTULLO Elisabetta LOFFREDO Donato MONDELLI Maria Pizzigallo Patrizia Ricciuti Riccardo Rolli Nicola Senesi (*Honorary President*) Matteo Spagnuolo Fabio Terribile Roberto Terzano Pandi Zdruli

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#### First Joint Meeting on Soil and Plant System Sciences







# Programme

- 9:00-13:00 Registration
- 13:00-14:00 Light lunch
- 14:00-14:30 **Opening ceremony**

#### Session I: Natural and agricultural soil systems

Chairs: Tommaso Chiti and Antonio Gelsomino

- 14:30-15:15 Invited speaker
  - **Takashi Kosaki** *President, International Union of Soil Sciences (IUSS)* Harmonization of natural and agricultural soil systems for sustaining society
- 15:15-15:30 Antonello Bonfante, A. Basile, J. Bouma Exploring the effect of varying soil organic matter contents on current and future moisture supply capacities of six Italian soils
- 15:30-15:45 **Ronen C.A. Francis, M.N. Wuddivira, G.A. Gouveia, D. Bramble** Structural and hydraulic feedbacks in humid tropical soils amended with aglime and organic residues
- 15:45-16:00 **G. Brunetti, <u>Francesco De Mastro</u>, A. Traversa, F. Nigro, C. Cocozza** Impact of different soil management on soil microbial community
- 16:00-16:30 **Coffee break**
- 16:30-16:45 <u>Andrea Ciurli</u>, D. Sega, G. Renella, L. Giagnoni, R. Pastorelli,
   A. Zamboni, Z. Varanini FePO<sub>4</sub> nanoparticles as a source of nutrients: effects on plant transcriptome

and on soil microbial communities and functions

16:45-17:00 Eleonora Grilli, M. Bastidas, M. Bijl, S. Carvalho, E. Coppola, T. La Mantia, C.A.R. Machado, M. Mastrocicco, M. Rossana, F. Pulido, M.J. Roxo, F.A. Rutigliano, P. Quatrini, A. Sarmento, S. Castaldi Drivers of soil quality in agro-ecosystems and managed forested lands of Mediterranean areas under increasing climatic risk

- 17:00-17:15 Simone Priori, R. Barbetti, L. Meini, A. Morelli, A. Zampolli, L. D'Avino
   Field scale economic land evaluation based on spatial variability of soil functions
- 17:15-18:00 **Poster presentations (Session I)**
- 18:00-19:00 General Assembly (SISS)

- 8:30-18:00 **Pedological and cultural excursion**
- 18:00-19:00 General Assembly (SIPe)

#### Session II: Soil pollution and food safety

Chairs: Valeria Ancona and Paola Castaldi

#### 9:00-9:45 Invited speaker

**Fabio Terribile** *University of Naples "Federico II", Italy* An operational tool to challenge land take at the European scale

- 9:45-10:00 Antonio G. Caporale, P. Adamo In vitro bioaccessibility of potentially toxic elements in particle-size fractions from an industrial soil of south Italy
- 10:00-10:15 Erika Di Iorio, L. Circelli, C. Colombo, A. Paltseva, M. Deeb, Z. Cheng Use of Vis-NIR Spectroscopy to predict the impact of different amendments on Pb and As bioaccessibility in urban soils
- 10:15-10:30 <u>Albert Kobina Mensah</u>, B. Marschner, S.M. Shaheen, J. Rinklebe Arsenic in an abandoned gold mine spoil in Ghana: effects of pH, geochemical fractionation and potential mobilisation
- 10:30-11:00 Coffee break
- 11:00-11:15 <u>Elio Padoan</u>, A.H. Couto, F. Ajmone Marsan Potential release of zinc and cadmium in soils contaminated by heavy metals under flooding
- 11:15-11:30 <u>Giovanni Garau</u>, A. Porceddu, B. Manunza, P. Castaldi Municipal solid wastes as gentle remediation options for the (bio)chemical recovery of As and trace metal-polluted soils
- 11:30-11:45 **Tommy Pepè Sciarria, S. Zangarini, L. Trombino, F. Adani, F. Tambone** Phosphorus recovery from liquid fraction of digestates by struvite crystallization

#### 11:45-12:45 **Poster presentations (Sessions II and III)**

12:45-14:00 **Lunch** 

#### Session III: Organic amendments and soil quality

Chairs: Paloma Campos and Claudio Cocozza

#### 14:00-14:45 Invited speaker

**José María García-Mina Freire** *Vice President, International Humic Substances Society (IHSS)* Discriminating the action of root applied- and foliar applied-humic acid on plant growth

- 14:45-15:00 <u>María Teresa Cieschi</u>, M. Caballero-Molada, I.V. Perminova, J.J. Lucena Are iron humates slow iron release fertilizers?
- 15:00-15:15 <u>Massimo Zilio</u>, F. Tambone, F. Bedussi, F. Adani Environmental impact of the use of digestates in agriculture: an open field approach

#### First Joint Meeting on Soil and Plant System Sciences

#### **PROGRAMME** • Wednesday, 25 September

### 15:15-15:30 <u>Daniela Pezzolla</u>, L. Regni, P. Proietti, E. Albertini, S. Ciancaleoni, G. Marozzi, G. Gigliotti

Agronomic reuse of olive wastewater and changes on soil chemical properties and microbial community

#### 15:30-16:00 **Coffee break**

- 16:00-16:15 A. Ioppolo, <u>Armando V. Laudicina</u>, L. Badalucco, A. Micalizzi, F. Saiano, E. Palazzolo
   Citrus hydrolates as natural biostimulants of soil microorganisms
- 16:15-16:30 **Beatrice Giannetta**, **R. Balint**, **D. Said Pullicino**, **C. Plaza**, **M. Martin**, **C. Zaccone** Redox-driven mineralogical changes in Fe (hydr)oxides across particle-size SOM fractions
- 16:30-16:45 <u>Mariia Pukalchik</u>, O. Yakimenko, K. Kydralieva, V. Terekhova Artificial intelligence approach to predict changes in biological properties of the heavy metal polluted soils after organic-based amendments addition
- 16:45-17:45 General Assembly (SICA)
- **Transfer to the social dinner venue**
- 19:30 Social dinner

#### Session IV: Plant responses to natural and human-induced drivers

Chairs: Ivana Cavoski and Andrea Ertani

#### 9:00-9:45 Invited speaker

**Philip J. White** *Programme Leader, The James Hutton Institute* Improving nutrient acquisition by plants from the soil

### 9:45-10:00 **Fabrizio Araniti, M.R. Abenavoli** Metabolic changes induced by *Cuscuta campestris* Yunck. on the host species *Artemisia campestris* subsp. *variabilis* (ten.) Greuter as a strategy to increase the parasitization success

### 10:00-10:15 A. Mannucci, L. Mariotti, <u>Antonella Castagna</u>, M.F. Quartacci, A. Trivellini, A. Mensuali-Sodi, A. Ranieri

Hormone responses to UV-B irradiation: what happens in leaves and roots of tomato plants?

10:15-10:30 <u>Emilia Dell'Aversana</u>, M. van Oosten, A. Maggio, P. Woodrow, P. Carillo, G.M. Fusco

Omeprazole enhances NUE through increased nitrogen uptake and assimilation in corn

- 10:30-11:00 **Coffee break**
- 11:00-11:15 <u>Begoña Miras-Moreno</u>, G. Colla, Y. Rouphael, M. Cardarelli, M. Trevisan, L. Lucini

Defining the active biostimulant fractions of a plant-derived protein hydrolysate using an integrated molecular fractionation and metabolomics approach

#### 11:15-11:30 <u>Bhakti Prinsi</u>, L. Brancadoro, O. Failla, L. Espen Proteomic changes in the roots of three grapevine rootstocks in response to nitrate availability

 11: 30-11: 45 L. Zanin, S. Gottardi, W. Biała, R. de Brito Francisco, S. Venuti, F. Valentinuzzi,
 T. Mimmo, S. Cesco, B. Bassin, M. Jasiński, E. Martinoia, R. Pinton, <u>Nicola Tomasi</u> Identification of an isoflavonoid transporter required for the nodule establishment of the *Rhizobium-Fabaceae* symbiotic interaction

#### 11:45-12:45 **Poster presentations (Sessions IV and V)**

12:45-14:00 **Lunch** 

#### Session V: Frontiers in plant and soil sciences

Chairs: Silvia Celletti and Daniel Said Pullicino

#### 14:00-14:45 Invited speaker

**Yiannis Deligiannakis** *President, International Humic Substances Society (IHSS)* Humic-nanomaterials hybrids as fertiliser-delivery technology and antioxidants

#### First Joint Meeting on Soil and Plant System Sciences

#### **PROGRAMME** • Thursday, 26 September

#### 14:45-15:00 **Pellegrino Conte** Applications of fast field cycling NMR relaxometry in soil science

- 15:00-15:15 Leonard Barnabas Ebinezer, G. Arrigoni, S. Trevisan, A. Manoli, P. Carletti, C. Francheschi, S. Quaggiotti, A. Masi
   Unravelling the stimulatory mechanism of APR - a novel Biostimulant: Leveraging transcriptomics and proteomics
- 15:15-15:30 Ignazio Allegretta, S. Legrand, M. Alfeld, C.E. Gattullo, M. Spagnuolo, K. Janssens, R. Terzano
   Classification of soil aggregates using SEM-EDX hyperspectral data analysis
- 15:30-16:00 Coffee break
- 16:00-16:15 L. Beneduce, C. Plaza, <u>Claudio Zaccone</u> Does physical fractionation of SOM pools preserve information about microbial taxa distribution and ecological functions?
- 16:15-16:30 <u>Maria Chiara Guerrieri</u>, M. Trevisan, E. Puglisi Isolation and phenotypic characterization of extracellular PGPR from tomato plant rhizosphere sampless
- 16:30-16:45 Simona Vingiani, P. Adamo, A.G. Caporale, S. De Pascale, L.G. Duri, M. Palladino, A. Pannico, Y. Rouphael Sustainable space agriculture using MMS1 Martian soil simulant and compost
- 16:45 Closing ceremony

#### Session I: Natural and agricultural soil systems

#### Pl.1 Filiberto Altobelli, D. Marandola, M.G. Piazza, C. Giannetti, A. Benedetti

Increasing knowledge and awareness of farmers on the role of agriculture in soil protection: LIFE4Soil

#### Pl.2 Anna Benedetti, M.G. Piazza, C. Dazzi, F. Altobelli

An Italian HUB to support the Global Soil Partnership and European Research Network Excellences on soil research: HUB SOIL

#### Pl.3 De Shorn Bramble, G. Gouveia, V. Herelle, R. Ramnarine, B. Smith, R. Farrell

The impact of aglime on soil CO<sub>2</sub> emissions is mediated by organic residue addition

### **PI.4** <u>Fatima Ezzahra Chouyia</u>, O. Pepe, M. Fagnano, I. Romano, V. Ventorino, N. Fiorentino, T. Fechtali

Isolation and characterization of phosphate solubilizing bacteria from different origins for potential use in a lake phosphate soils

#### Pl.5 Claudio Cocozza, C. Cacace, A. Traversa, F. De Mastro, G. Brunetti

Effects of Robinia pseudoacacia L. on two apulian soils

#### Pl.6 Lorenzo D'Avino, G. L'Abate, M. Sperow, E. Costantini

Assessing soil carbon trends in Italy through land use and management changes

#### Pl.7 Francesco De Mastro, E. Blagodatskaya, A. Traversa, C. Cocozza, G. Brunetti

Soil biochemical activities as affected by different tillage, fertilization and depth in a semiarid mediterranean agro-ecosystem

#### Pl.8 Daniele Del Buono, F. Luzi, P. Benincasa, J.M. Kenny, L. Torre, D. Puglia

Extraction of nanostructured starch from purified granules of waxy and nonwaxy barley cultivars

#### Pl.9 L. Circelli, Erika Di Iorio, C. Colombo

Soil organic carbon variation in organic farming in Molise (South-Central Italy) as evaluated by Diffuse Reflectance Spectroscopy

### **Pl.10** <u>Sire Diedhiou</u>, A.O.K. Goudiaby, Y. Diatta, I. Diedhiou, K.B. Assigbetsee, A.N. Badiane, M. Sène, M. Khouma, A.N.S. Samba, R.P. Dick, I. Ndoye

Do arid agroecosystem shrubs enhance soil enzyme activities during the dry season?

#### Pl.11 Chiara Ferré, R. Comolli

Land use change from vineyard to tree plantation in Piedmont hills: effects on soil organic carbon stock and other soil properties

#### Pl.12 Shadananan Nair Krishnapillai

Climate change and degradation of land and water resources: impact on agriculture production in the dry zones of India

### **Pl.13** E. Taskin, A. Fiorini, C. Misci, L. Guzzetti, D. Panzeri, N. Tommasi, F. Grassi, A. Galimberti, M. Labra, V. Tabaglio, <u>Edoardo Puglisi</u>

Assessing the effects of African Neglected and Underutilized Species and soil tillage on belowground microbial diversity and activity

#### Pl.14 E. Taskin, A. Fiorini, C. Misci, V. Tabaglio, Edoardo Puglisi

Microbial insights to sustainability of family farmers of Mt. Meru Zone in Arusha, Tanzania; Neglected and Underutilized Species vs Maize / Food vs Cash

#### Pl.15 Pere Rovira, M.T. Sauras, J. Romanyà

Soil carbon sequestration upon shifting from conventional to ecological agriculture: do we miss something?

#### Pl.16 Andreina Traversa, F. De Mastro, C. Cocozza, G. Brunetti

Use of diffuse reflectance infrared Fourier transform (Drift) to study the composition of four size fractions of soils subjected to different management

#### Pl.17 Mohammad Yaghoubi Khanghahi, G. Cucci, G. Lacolla, L. Lanzellotti, C. Crecchio

Influence of long-term tillage management on soil fertility level in a Mediterranean agro-ecosystem

#### Session II: Soil pollution and food safety

### PII.1 <u>Valeria Ancona</u>, P. Grenni, G. Aimola, G.L. Garbini, C. Campanale, L. Rolando, V.F. Uricchio, A. Barra Caracciolo

Application of a nature-based solution for recovering a multi-contaminated area in Southern Italy

#### PII.2 Paloma Campos, R. Lopez, H. Knicker, E. Fernández-Boy, J.M. De la Rosa

Application of biochar from crop residues as ameliorant of trace element polluted soils

#### PII.3 Arianna De Bernardi, C. Casucci, D. Businelli, R. D'Amato, G.M. Beone, M.C.

Fontanella, C. Vischetti

Phytoextraction ability of four plant species against nickel in carbonation lime coming from sugar industry

### PII.4 <u>Stefania Diquattro</u>, R. Manzano, G. Garau, M. Garau, C. Senette, M.V. Pinna, P.P. Roggero, P. Castaldi

Influence of biochar on trace metals mobility and leaching in contaminated mining soils

#### PII.5 V. Ancona, Ciro Galeone, N. Leone, G. Bagnuolo, V.F. Uricchio, A.P. Leone

Potential of vis-NIR reflectance spectroscopy for the prediction of soil PCBs content in a historically contaminated site of the SIN of Taranto (Southern Italy)

### PII.6 <u>Concetta Eliana Gattullo</u>, V.M. Paradiso, I. Allegretta, M. Noviello, G. Natrella, G. Gambacorta, R. Terzano

Testing synthetic zeolites obtained from recycled waste materials for oenological applications

#### PII.7 Y. Scarcia, M. Parlavecchia, Elisabetta Loffredo

Ochratoxin A removal from aqueous and aqueous ethanol solutions using low cost plant-derived adsorbents

#### Pll.8 Maria Martin, E. Barberis, G.M. Beone, M. Romani

Decreasing arsenic content in Italian rice. Progresses and constraints

#### PII.9 Giuseppe Picca, M. Parlavecchia, E. Loffredo

Potential of hemp to remove the emerging contaminants bisphenol A and metalaxyl-M from aqueous media

### **PII.10** M.S. Zangrillo, E. Taskin, G. Spini, P. Iavazzo, S. Anelli, L. Lamastra, N.A. Suciu, A. Ferrarini, S. Amaducci, M. Trevisan, <u>Edoardo Puglisi</u>

Bioaugmentation and phytoremediation: an integrated approach for the remediation of soils with low pollution levels

### PII.11 <u>Ida Rascio</u>, C.E. Gattullo, I. Allegretta, C. Porfido, R. Grisorio, G.P. Suranna, M. Spagnuolo, R. Terzano

Assessing the distribution and availability of potentially toxic metals (PTMs) in an agricultural soil after simulated fire events

#### Pll.12 Polina Tregubova, G. Koptsik

Potential for organic amendments to stabilise heavy metals in contaminated soils of technogenic barrens in Kola Peninsula

#### PII.13 Hana Voca, L. Piscitelli, A.D. Malerba, D. Mondelli, T.M. Miano, V. D'Orazio

Sorghum (Sorghum bicolor) and canola (Brassica napus) plants development on Kosovo contaminated soils

#### Session III: Organic amendments and soil quality

**PIII.1** <u>Cristina Abbate</u>, S. Fontanazza, A. Scavo, S. Lombardo, A. Restuccia, G. Mauromicale *Trifolium subterraneum* L.cover cropping as a sustainable management for mediterranean agro-ecosystem

### **PIII.2** S.R. Stazi, R. Marabottini, F. Carbone, <u>Enrica Allevato</u>, V. Vinciguerra, V. Quintarelli, S. Marinari

Land use change in Fiuggi Basin affects soil humification process leading to different fulvic acids features: implications on ground/spring water quality

#### PIII.3 Floriana Bedussi, G. D'Imporzano, F. Tambone, F. Adani

LIFE DOP: an effective example of nutrient recovery and use in the dairy sector

### PIII.4 <u>Angelica Bruno</u>, A. Velders, A. Biasone, C. Giacchetti, L. Sciulli, D. Mondelli, D. Malerba, T. Miano

Evaluation of the biostimulant capacity of biomolecules isolated from vegetable biomasses of different origin

#### PIII.5 <u>Silvia Cellett</u>i, A. Bergamo, V. Benedetti, M. Pecchi, D. Basso, M. Baratieri, T. Mimmo,

S. Cesco

Hydrothermal carbonization (HTC) of digestate and the potential of its by-products to be used in soilless culture systems

#### PIII.6 Gretha Di Donato, B. Scaglia, F. Adani

Biostimulant activity from Chlorella sp culture

#### PIII.7 S. Salati, Gretha Di Donato, V. Palumbo, M. Su, G. D'Imporzano, M. Dell'Orto, F. Adani

Cultivation of microalgae on pig manure: nutrient recovery and biomass production in *Chlorella* and *Scenedesmus* 

#### PIII.8 Giuseppe Di Rauso Simeone, G. Cesarano, M.A. Rao

Response of chemical and biochemical indicator of soil quality to OMW digestate disposal

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### PIII.9 <u>Giovanna Marta Fusco</u>, Y. Rouphael, S. De Pascale, P. Woodrow, P. Carillo, E. Dell'Aversana

A tropical plant extract biostimulant favours growth and resource use efficiency in jute under sub-optimal nutrient regimens

## **PIII.10** <u>Antonio Ganga</u>, A. Manca, M. Ribeiro da Silva, I. Amaral Guerrini, D.M. Fernandes, R. Lyra Villas Bôas, L. Cleto da Silva, A. Cássia da Fonseca, M.C. Ruggiu, C. Vilela Cruz, D.C. Lozano Sivisaca, C. de Moura D'Andréa Mateus, I. Murgia, E. Grilli, G.F. Capra

Composted sewage with bagasse as commercial substrate for Eucalyptus urophylla x E grandis seedling production

#### PIII.11 R. Sidari, M.R. Panuccio, T. Papalia, Antonio Gelsomino

A green method to treat exhausted fire-extinguishing powders for reuse as agricultural fertilizer

#### PIII.12 Amira Jouini, L.Badalucco, A.Micalizzi, E.Palazzolo, V.A.Laudicina

Metabolic responses of microbial community in soil amended with fresh leaves and leaf extracts from eucalyptus spp

### PIII.13 <u>Vito Armando Laudicina</u>, L. Badalucco, S. Muscarella, A. Alfonzo, A. Ciminata, V. Naselli, G. Moschetti, N. Francesca

Chemical and biochemical characteristics of compost obtained from dairy and wine by-products

#### PIII.14 Elisabetta Loffredo

Benefits of compost-based media for ornamental plants: growth enhancement and suppressive activity towards the phytopathogen *Fusarium oxysporum* 

### PIII.15 <u>Maria Martin</u>, E. Dinuccio, L. Rollè, D. Chabloz, C. Lerda, D. Said Pullicino, N. Pampuro, P. Balsari, L. Celi

Acid-treatment of the solid separate of pig slurries for reducing greenhouse gas emissions and improving nutrient balance

#### PIII.16 Martina Mazzon, L. Cavani, G.C. Pacini, C. Ciavatta, C. Marzadori

Management and tillage effects on soil enzyme activities in a long-term agricultural experiment

#### PIII.17 Martina Mazzon, L. Cavani, G. Campanelli, C. Ciavatta, G. Burgio, C. Marzadori

QUABIO project: the agro-ecology strategy to maintain soil quality

#### PIII.18 Donato Mondelli, A. Aly, G. Mezzapesa, L. Piscitelli, S. Dumontet, T. Miano

Short-term effects of different organic amendments on soil chemical, biochemical and biological indicators

#### PIII.19 G. Carboni, M. Dettori, Paolo Mulè, A. Porcheddu

Crop sustainable management in Mediterranean conditions focused on changes in the soil component. A preliminary study in Southern Sardinia

#### PIII.20 T. Papalia, G. Settineri, F. Romeo, C. Mallamaci, Adele Muscolo

Olive from a waste to a resource: benefits of the end products for agricultural purpose

#### PIII.21 Pasquale Napoletano, S.C. Panico, V. Memoli, G. Maisto, C. Colombo, A. De Marco

Short- and long-term effects of a single compost addition on Technosols quality

#### PIII.22 Chinyere Blessing Okebalama, B. Marschner, S. Werner, A. Buerkert

Soil fertility and aggregate associated carbon and nitrogen with biochar amendment in relation to localized soil fertilization practice after a four-year intensive vegetable production in Burkina Faso

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#### PIII.23 <u>Elio Padoan</u>, E. Montoneri, G. Fabbri, P. Quagliotto, A. Baglieri, V. Boero, M. Negre

Ozonization of fermented municipal biowaste to produce value added products

#### PIII.24 Marco Parlavecchia, E. Loffredo

Sorption-desorption of the fungicide metalaxyl-M onto a silty soil not amended and amended with biochar and vermicompost

#### PIII.25 Maria Vittoria Pinna, A. Pusino

Which is the biochar most effective in mitigating pollution due to Lumax<sup>®</sup>?

#### PIII.26 Salvatore Rapisarda, P. Gioacchini, D. Montecchio, C. Ciavatta, C. Marzadori

Effects of biodegradable plastic on soil functionality

#### PIII.27 Pere Rovira, C. Bellera, A. Sala

Commercial humic products: do they act as activators of soil microbial activity?

#### Session IV: Plant responses to natural and human-induced drivers

#### PIV.1 Francesco Bigaran, D.C. Weindorf, L. Varone, L. Gratani

Characterization of heavy metal pollution in Rome, Italy

#### PIV.2 Andrea Ertani, S. Nardi, O. Francioso, D. Pizzeghello, A. Tinti, M. Schiavon

Variation in metabolite production and physiological responses of Zea maysL. plants in response to application of commercial lignohumates

### **PIV.3** R. Lizcano Toledo, C. Lerda, <u>Maria Martin</u>, R. Gorra, I. Mania, B. Moretti, D. Sacco, E. Barberis, D. Said Pullicino, L. Celi

Effects of inorganic and organic P availability on N fixing capacity of Vicia villosa

#### PIV.4 F. Trevisan, <u>Mauro Maver</u>, D. Bulgarelli, S. Cesco, T. Mimmo

Characterization of the alkaloid hordenine and its precursors in roots of a modern barley cultivar

**PIV.5** <u>Giuseppe N. Mezzapesa</u>, A. Ghannouchi, A. Trani, D. Mondelli, F. Valerio, E.V. Perrino Ecological variability in 4 wild species of Lamiaceae in the Apulia Region: effects on chemical composition and biological activities of the essential oils

#### PIV.6 Begoña Miras-Moreno, P. Ganugi1, V. Terzi, L. Lucini, M. Trevisan

The impact of selective and non-selective herbicides on the metabolism of tomato plants

#### PIV.7 V. Cavallaro, M. Caschetto, M. Maghrebi, G.A. Sacchi, Fabio Francesco Nocito

Sulfur isotope mass balance reveals 32S/34S fractionation during sulfate uptake and translocation in rice

#### PIV.8 N. Negrini, S. Morgutti, L. Espen, Bhakti Prinsi

Variation in phenolic composition and antioxidant properties in leaves and flowers of green and red basil (Ocimum basilicum L.)

#### PIV.9 Ivana Puglisi, V. Barone, F. Fragalà, P. Stevanato, G. Concheri, A Baglieri

Effect of microalgal extracts from Chlorella vulgaris and Scenedesmus quadricauda on germination of Beta vulgaris seeds

### **PIV.10** M. Santin, A. Castagna, M.-T. Hauser, M.B. Miras Moreno, L. Lucini, <u>Annamaria</u> Ranieri

Let the sunshine in! Post-harvest UV-B radiation is able to affect the secondary metabolism in flesh of peach fruit

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### PIV.11 F. Colombo, V. Cavallaro, M. Pesenti, N. Negrini, S. Morgutti, G. Orasen, F.F. Nocito, <u>Gian Attilio Sacchi</u>

The Trehalose-6-phosphate/SnRK1 system in the response to saline conditions during germination of two rice (O. sativa L., ssp. japonica) cultivars with different salt sensitivity

#### PIV.12 M. Pesenti, A. Abruzzese, G. Orasen, F.F. Nocito, L. Espen, M. Cocucci, Gian Attilio Sacchi

Transmembrane transporters and salt tolerance in temperate japonica rice

#### PIV.13 Veronica Santoro, M. Prati, M. Martin, D. Said-Pullicino, L. Celi

Root exudates involvement in tomato plants response to low P levels

**PIV.14** <u>Adriano Sofo</u>, **C. Fausto**, **B. Dichio**, **A.N. Mininni**, **L. Lucini**, **P. Ricciuti**, **C. Crecchio** Soil quality and fertility in an olive orchard managed for 20 years with differential agronomic systems

**PIV.15** L. Zanin, S. Buoso, A. Zamboni, Z. Varanini, R. Pinton, Nicola Tomasi White lupin response to nitrogen and phosphorous deficiencies

#### PIV.16 Maria Alessandra Zicari, L. d'Aquino, A. Paradiso, S. Mastrolitti, F. Tommasi

Cerium a potential pollutant: effects on growth and metabolism in Lemna minor L.

#### Session V: Frontiers in plant and soil sciences

### PV.1 <u>Costanza Ceccanti</u>, M. Landi, G. Rocchetti, M. Begoña Miras Moreno, L. Lucini, L. Incrocci, A. Pardossi, L. Guidi

Hydroponically-grown Rumex acetosa L. and Sanguisorba minor Scop. respond differently to mowing and the storage as fresh-cut produce

### **PV.2** <u>Patrizia De Nisi</u>, G. Borlini, G. Brunoldi, M. Landoni, E. Cassani, P. Squillace, F. Adani, R. Pilu

Use of pigmented corn cob waste as a natural dye in environmental friendly textile processing (PASTEL)

#### PV.3 I. Panfili, Maria Luce Bartucca, D. Del Buono

The treatment of duckweed with a plant biostimulant or a safener improves the plant capacity to clean water polluted by terbuthylazine

#### PV.4 D. Del Buono, B. Falcinelli, Maria Luce Bertucca, M. Guiducci

Effect of light quality on gas exchange and leaf characteristics of Einkorn seedlings

#### PV.5 Antonino Ioppolo, F. Saiano, E. Palazzolo

Analysis of geochemical tracers in different systems soil-Citrus limon (L.) Osbeck

#### PV.6 Antonino Ioppolo, M. Barbera, A. Vella, F. Saiano, E. Palazzolo

New chemiometric technique applied to traceability of Sicilian honey of Sulla (Hedysarum coronarium L.)

#### PV.7 Yahya Kooch, N. Moghimian

The effect of land covers on soil microbial and enzyme activities

#### PV.8 N. Sharma, S. Dall'Acqua, S. Sut, Antonio Masi

Comprehensive multiphase NMR: a powerful technology to study the effects of PFASs on the model plant Arabidopsis thaliana

#### PV.9 S. Millan, S. Dall'Acqua, D. Jeffery, Antonio Masi

THIOLOMICS: systematic identification of low-molecular-weight thiols by HPLC-MS/MS

#### PV.10 Pasqua Murgese, P. Santamaria, B. Leoni, C. Crecchio

Use of Plant Growth Promoting Bacteria in agriculture

### **PV.11** P.F. Ambrico, M. Šimek, M. Ambrico, M. Morano, A. Minafra, G.S. Senesi, O. De Pascale, I. Allegretta, <u>Carlo Porfido</u>, R. Terzano

Treatment by atmospheric-pressure plasma improves seed germination and plant development

**PV.12** <u>Luigi Ruggiero</u>, M.C. Fontanella, C. Amalfitano, G.M. Beone, C. Di Vaio, P. Adamo Traceability of Sorrento lemon PGI by chemometric analysis of juice mineral composition: a mono- and multi-cultivar approach

#### PV.13 A. Ertani, S. Nardi, E.A.H. Pilon-Smits, C. Nicoletto, P. Paolucci, Michela Schiavon

Investigation of Se and Cr interactions in Se-hyperaccumulator Stanleya pinnata and non hyperaccumulator Brassica juncea for combining biofortification and phytoremediation technologies

#### PV.14 E. Bletsa, <u>Claudio Zaccone</u>, T. Miano, R. Terzano, Y. Deligiannakis

Testing natural Mn-todorikite as an efficient, low-cost and environmental-friendly catalyst







# Session I: Natural and agricultural soil systems

Oral presentations

#### **Invited speaker**

#### Harmonization of natural and agricultural soil systems for sustaining society

#### <u>Takashi Kosaki</u>

International Union of Soil Sciences, Vienna, Austria - Aichi University, Nagoya, Japan

Many of the global environmental problems, e.g. desertification, global warming, and the decline in biodiversity, are caused by misuse and/or mismanagement of soil resources by human beings. In order to avoid and/or mitigate the soil-related problems, appropriate strategy in soil use and management is highly required to be established. Presented here are the examples (soil organic matter deterioration under long-term mechanized farming in steppes of the temperate regions, nutrient and organic matter decline under shifting cultivation in the humid tropics, etc.) of the analysis on the type, causes, an impact of soil degradation due to the change of land use and possible countermeasures for sustainable soil management developed in different ecological conditions with taking account of the benefits given by natural soil systems.

Although some long term changes in local and/or global climate may affect the type and extent of soil degradation, human activities often contribute more directly and drastically to soil degradation. The types, causes and impact of soil degradation vary from one place to another, thus the possible countermeasures against soil degradation should be tested and evaluated with taking account of physical as well as socio-economic conditions in an individual situation. The application of a given set of countermeasures without detail analysis for natural soil system of the site in question may result in disastrous and irreversible changes in the environment and finally jeopardize human security. We need now to develop the wisdoms how to harmonize natural and agricultural soil systems.

### Exploring the effect of varying soil organic matter contents on current and future moisture supply capacities of six Italian soils

Antonello Bonfante<sup>1</sup>, A. Basile<sup>1</sup>, J. Bouma<sup>2</sup>

<sup>1</sup>Institute for Mediterranean Agricultural and Forest Systems, CNR-ISAFOM, Ercolano, Italy <sup>2</sup>Em. Prof Soil Science, Wageningen University, the Netherlands

The static Available Soil Water Capacity (AWC) is standard data in most soil databases and expresses soil water contents in the rootzone between field capacity (FC) and permanent wilting point (WP). Literature suggests that increasing %SOM of a given soil does not significantly increase AWC and this has important implications when judging soil moisture supply to plants and potential for climate mitigation. However, real FC values vary between -0.1 and -0.5 bar in different soils and WP values between -8 and -15 bar. Standard values for AWC of -0.33bar and -15 bar do therefore not represent field conditions in many soils. When exploring AWC for six Italian soil series, ranging from clay to sand, AWC values at increasing %SOM were lower in clay soils and higher in sand as compared with actual conditions, which could be explained by considering the shape of the corresponding moisture retention curves. But rather than focus on static AWC values to define moisture supply to plants, real soil moisture supply capacities (MSC) can be obtained by dynamic modeling of the soil-water-atmosphere-plant system, including a "sinkterm" indicating a continuous relation between water uptake and negative pressure head of soil water and evaporative demand. Also, only models allow exploration of the effects of future IPCC climate scenarios. Thus, studying MSC for the six Italian soil series showed that MSC values were: (i) on average 30% higher than the corresponding AWC; (ii).distinctly different for the six soils; (iii) affected by declines of 1-9% as a result of climate change; (iv) not significantly affected by increases of %SOM when considering climate change, except for the sand. Generalizations as to the future effect of %SOM on MSC can only be realistic when more modeling is performed for soil series in different climate zones.

#### Structural and hydraulic feedbacks in humid tropical soils amended with aglime and organic residues

Ronen C.A. Francis, M.N. Wuddivira, G.A. Gouveia, D. Bramble Dept of Food Production, UWI St. Augustine, Trinidad and Tobago

Humic tropical environments are dominated by acid soils with low content of organic matter (OM). Their chemical and physical properties are largely dictated by the mineralogy of their clay fractions; hence soil management is crucial for agricultural productivity. This study aims to understand how management practices such as liming and organic residue additions impact on soil structural and hydraulic properties in three contrasting acid soils from Trinidad. An Aquic Hapludalfs (Cunupia – silty loam), Fluvaquentic Endoaquepts (Sangre Grande - clay loam) and Aquertic Eutrudepts (Talparo - clay) were incubated at field capacity (FC) with corn stover (CS) or vermicompost (VC) at 16.8 tons C/ha and aglime at their respective lime requirement plus a control treatment for 28 days in a 3x3x2 factorial replicated 5 times. Soil water repellency (SWR) was determined by water drop penetration time (WDPT) every 3 days after the first 24 hours, saturated hydraulic conductivity by the constant head (Ksat), aggregate stability by rapid wetting (WSAr) and Atterberg's limits were examined. SWR increased with increasing clay content and was most pronounced when treated with aglime-CS. Talparo was strongly repellent in aglime-CS while Cunupia and Sangre Grande were slightly repellent. No VC treatments were significantly different from the control in relation to SWR, Ksat or WSAr%. Aglime decreased WSAr% and increased Ksat; however, the effect was only statistically significant for WSAr% in Cunupia. Soil OM content was positively correlated with WSAr% and Ksat in Cunupia and Talparo (p<0.05) but had no significant effect in the Sangre Grande soil. Nonetheless, aglime-VC increased the OM in the macroaggregates (1-2mm size fraction) of the Sangre Grande soil significantly higher than the aglime-CS treatment as well as increasing its plasticity index (PI). This study indicates that less humified residues significantly increase SWR between a threshold of 20-40% FC water holding capacity. Liming was shown to exacerbate SWR for approximately 20 days. Clay mineralogy likely influenced OM mobility into the macroaggregates to impact WSAr% and Ksat. and further impacted on the expansive properties of soils.

#### Impact of different soil management on soil microbial community

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Agricultural practices greatly influence soil chemical properties and the microbial population, which plays a fundamental role in developing a good soil structure through the binding of soil aggregates by hyphae and by secretion of microbial exudates. The aim of the study was to investigate the effects of different tillage (conventional (CT), minimum (MT) and no tillage (NT)), fertilization (fertilization versus no fertilization), soil depth (0-30, 30-60, and 60-90 cm) and crops (wheat versus faba bean) on the abundance of the soil microbial bacteria, fungi and yeasts. Another objective was to understand which variables were the most responsible for the growth of the microbial population and the correlations existing between the chemical and microbiological soil parameters. Positive and significant correlations were found between total microbial population and bacterial and fungal populations. This result suggested a predominance of fungi and bacteria in the microbial community composition, compared to the yeasts which were a minority. The total microbial population was positively correlated to the content of soil organic carbon, total nitrogen and available phosphorus and negatively correlated to soil pH. With regards to tillage, MT seemed the best solution in terms of abundance of microbial population, representing a good compromise between soil aeration and available nutrients. The faba bean was the crop that favored more the microbial community due to its nitrogen fixing feature. The statistical analyses showed that depth was the parameter that mainly influenced the chemical and, consequently, microbiological properties of soils.

### FePO<sub>4</sub> nanoparticles as a source of nutrients: effects on plant transcriptome and on soil microbial communities and functions

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Nanomaterials that can provide one or more macro/micro-nutrient to the plant are commonly referred as nanofertilizers. It has been shown that iron phosphate (FePO<sub>4</sub>) nanoparticles (NPs) can provide either iron or phosphate to plants grown in hydroponic (Sega et al, *Scientific Reports* 2019, submitted), though with a different plant-based efficacy. The present study is aimed to investigate the mechanisms of action of FePO<sub>4</sub> NPs in the plant-soil system. In order to get a deeper understanding of plant early responses to FePO<sub>4</sub> NPs exposure, microarray expression analyses have been performed in maize and cucumber plants grown in hydroponic for 24 hours, and it has been shown that the responses are specific for the nano and the bulk (non-nano) FePO<sub>4</sub>. Considering the lack of knowledge concerning the impact of NPs on soil environment, two different bare soils (a silty and a clay one) were treated with FePO<sub>4</sub> NPs. Performing soil enzyme activities and respiration, together with DGGE analyses, it has been shown that there was no impact on microbial community and metabolic functions, neither toxic effects of FePO<sub>4</sub> NPs. Moreover, FePO<sub>4</sub> NPs can provide available P in a competitive way in the two bare soils, with respect to triple superphosphate (TSP). The evaluation of the FePO<sub>4</sub> NPs effects in comparison to TSP on soil-grown plants is in progress.

### Drivers of soil quality in agro-ecosystems and managed forested lands of Mediterranean areas under increasing climatic risk

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Mediterranean ecosystems are identified by the International Panel on Climate Change (IPCC) as being among the most likely ecosystems to be impacted by occurring climate changes. In particular, the most Southern Mediterranean areas are already experiencing a progressing degradation of both plant community and soils, consequence of both climatic changes and anthropic disturbance and pressure. The relationship between plant cover and soil is critical for both, being the plant inputs fundamental to maintain soil quality and vice versa being healthy soils necessary to maintain the tree cover on a long term. At present, it is not clear the extent at which the policies and management strategies applied in these areas are failing to support soil quality and functions. Within the context of the EU project Desert-Adapt "Preparing desertification areas for increased climate change", we have analyzed the baseline conditions of agroecosystems and managed forested lands of in three Mediterranean areas under strong desertification risk, Alentejo in Portugal, Extremadura in Spain and Sicily in Italy. Thirty two sites under different land use from ten areas located in the three regions were sampled and investigated, for soil chemical and physical guality and functionality, plant cover, and the soil guality was related to geomorphology, land use, land cover, disturbance. The baseline conditions of the analysed sites where used to inform the development of new sustainable management strategies focused on the maintenance and recovery of soil-based ecosystem services aimed as well as to the adaptation to the changing climate and to the productivity of the land. The overall applied strategy based on the baseline results is a framework of management we call Desert Adaptation Model (DAM), a stepwise methodological framework based on 3 pillars: market opportunities (economic benefit), sustainability criteria (environmental & climatic benefits) and stakeholder commitment (social benefits). By incorporating climate change variables and structural and functional landscape complexity, the DAM seeks to optimize resilience and adaptation to climate change, targeting the improvement of ecosystem services and sustainable socioeconomic development. Ultimately, the project proposes a conceptual revolution turning the degraded areas from low-productive systems into resourceefficient and low-carbon economies.

#### Field scale economic land evaluation based on spatial variability of soil functions

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In evaluation of agricultural parcels, estimating the economic value of the most important soil functions is related to soil features and agricultural production. Internal spatial variability of soil physical-chemical properties, and then soil functionality, may strongly affect the economic value of a field, although that is often small perceived from farmers. This communication will present an approach of economic land evaluation of about 195 ha irrigated croplands in Po river plain (Northern Italy), based on spatial variability of soil functions namely biomass production and carbon sequestration. The risk of river floods was also taken into account during economic evaluation. The soil spatial variability was mapped using proximal sensing technology (Electromagnetic Induction sensor) and few calibration points (one every five hectares, on average). Five different soil typological units were recognized and mapped. The proposed method provides for an economic evaluation of a land parcel considering the site-specific deviations of the mean land market value, published in Italy by each province and used for expropriation procedures, according to national law D.P.R. 327/2001. Biomass production of the main crops of the area, namely maize, soybean, and sorghum, was monitored and mapped for three years (2016, '17, and '18) using precision agriculture technologies. Variation of productive value of a land, was determined using the concept of capitalization rate, as ratio between the average gross saleable production of the crop yield and the mean land value. Local variation of carbon stock (CS<sub>30</sub>) value of each soil typological unit was calculated in relation to global soil organic carbon map published by FAO, considering the mean annual EU allowance price relative to the calendar year preceding the value estimation.

Flood risk of each parcel was determined according to regional map of flood risk, integrated by farmer interviews. The flood risk costs were subtracted from the mean land value, using lost annual gross sealable production and land recover costs. Following this method, the value of the land ranged in each unit from 28,019 to 38,289 €·ha<sup>-1</sup>, on the basis of soil spatial variability and flood risk. This methodology goes toward an approach of precision land evaluation and can be applied to many different arable lands.







# Session I: Natural and agricultural soil systems

Posters

### Increasing knowledge and awareness of farmers on the role of agriculture in soil protection: LIFE4Soil

<u>Filiberto Altobelli</u>, D. Marandola, M.G. Piazza, C. Giannetti, A. Benedetti Council for Agricultural Research and Economics (CREA)

Soil4Life is a European project that involves Italian, French and Croatian partners, which aims to promote sustainable use of the soil as a strategic, limited and non-renewable resource. A goal in line with the commitment signed by the European countries at the United Nations table, through adherence to the Sustainable Development Goals – SDG.

Co-financed by the European Commission through the Life programme, Soil4Life involves associations and research institutions convinced that the soil must be preserved with concrete actions and policies, supported by analyses and data that allow monitoring of the ecological status. Soil4Life pursues the application of the Voluntary Guidelines for sustainable soil management promoted by FAO, adapting them to national, regional and local contexts, and provides information and support to territorial planning involving the agricultural sector and sector professionals (agronomists, geologists, urban planners and designers). Soil4Life also aims to increase citizens' awareness of soil protection and to raise awareness among national and EU institutions of the need to adopt adequate regulations to stop soil consumption and prevent its degradation.

One of the main activities will be related to awareness campaign for technicians/professionals. This activities involves the creation of specific voluntary guidelines for the protection of the soil aimed at professionals (architects, engineers, surveyors, agronomists and foresters, geologists, etc.) and their dissemination through a series of training courses with the supply of Professional Training Credits. The Soil4Life covers the four-year period from 2018 to 2022.

### An Italian HUB to support the Global Soil Partnership and European Research Network Excellences on soil research: HUB SOIL

<u>Anna Benedetti</u><sup>1</sup>, M.G. Piazza<sup>1</sup>, C. Dazzi<sup>2</sup>, F. Altobelli<sup>1</sup> <sup>1</sup>Council for Agricultural Research and Economics (CREA) <sup>2</sup>The University of Palermo (UNIPA)

Global Soil Partnership aims to develop awareness and contribute to the development of capacities, build on best available science, and facilitate/contribute to the exchange of knowledge and technologies among stakeholders for the sustainable management and use of soil resources.

In 2018, the MIPAAFT funded the project for the establishment of a Soil HUB that started from the Italian Soil partnership with the dual purpose of allowing an easier participation in the activities of the GSP and ESP and participate to the EJP on the Soil.

The Soil Hub, intends to the establishment of a network of excellence at National level on soil research in order to overcome the fragmentation of the research itself by enhancing the knowledge to stem and mitigate the impact of climate change on agricultural systems and agricultural functions.

Furthermore, the aims of SOIL-HUB will pursue the long-term goal of promoting farmers as stewards of land and soil resources and support policy development and deployment, in particular the CAP and Climate policies.

#### The impact of aglime on soil CO<sub>2</sub> emissions is mediated by organic residue addition

<u>De Shorn Bramble</u><sup>1</sup>, G. Gouveia<sup>1</sup>, V. Herelle<sup>1</sup>, R. Ramnarine<sup>1</sup>, B. Smith<sup>1</sup>, R. Farrell<sup>2</sup> <sup>1</sup>Dept of Food Production, The University of the West Indies, St. Augustine, Trinidad and Tobago <sup>2</sup>Dept of Soil Science, University of Saskatchewan, Saskatoon, Canada

CO<sub>2</sub> may originate from agricultural lime (aglime) applied to acid soils and the lime may also drive CO<sub>2</sub> production from organic sources. The magnitude of the emissions from these sources may be regulated by organic residue addition; however, empirical evidence to support this claim is limited. This 103-day laboratory incubation study therefore assessed the impact of aglime on CO<sub>2</sub> emitted from two contrasting acid soils (Nariva clay, Mollic Fluvaquents; and Piarco loam, Typic Kanhaplaquults) that were amended with varying combinations of <sup>13</sup>C labelled aglime (0% w/w or 0.230% w/w), and organic residues (0% w/w and 1% w/w corn stover, poultry litter or glucose) in a  $2 \times 2 \times 4$  factorial with three replicates. Cumulative CO<sub>2</sub> emissions derived from organic sources was increased by 27% when aglime was applied to the noorganic residue amended Piarco soil. However, these emissions decreased by as much as 13.2% when aglime was applied to the Piarco soil treatments that received organic residues, which may be due to the carbon stabilizing effect of the Ca<sup>2+</sup> released from the aglime through physical-chemical means. However, for the Nariva soil the relative change in these emissions with aglime addition was not affected by application of organic residues. Our study also demonstrated—possibly for the first time—significant effects of organic residue on the rate of aglime-CO<sub>2</sub> release. This effect was particularly evident with the Piarco soil where the rate at which aglime-CO<sub>2</sub> was released decreased with corn stover and poultry litter but increased with glucose. As this effect was most prominent in the poultry litter treatment, a subsequent experiment was conducted to elucidate the underlying mechanism. The findings of this second experiment suggest that the carbonates in the poultry litter were responsible for this effect. These findings further suggest that there is likely to be a competing effect between the poultry litter-carbonates and the aglime-carbonates for H<sup>+</sup> ions that would likely decelerate the dissolution and subsequent release of CO<sub>2</sub> from the aglime. Overall, our findings emphasize the need to consider residue management practices when assessing the contribution of aglime to soil carbon-climate feedbacks.

**Keywords:** aglime; organic residues; acid soils; inorganic and organic derived  $CO_2$  emissions; soil carbonclimate feedbacks.

### Isolation and characterization of phosphate solubilizing bacteria from different origins for potential use in a lake phosphate soils

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The application of phosphate-solubilizing bacteria (PSB) has been reported to increase phosphate (P) uptake and plant growth. Therefore, the aim of this study was to isolate and characterize new putative PSB to use as inoculum for increasing bioavailability of P in plants. To this aim, 16 bacteria isolated from Moroccan rhizosphere soils, and 34 bacterial strains belonging to the collection of Division of Microbiology of the Department of Agricultural Sciences, were screened by semi-quantitative method for their putative P-solubilization. A total of 21 bacteria, 10 isolates and 11 strains, were selected as P solubilizers based on a clear halo formation around the colonies using modified Pikovskaya's (PVK) medium. Among them, 7 strains, showing the highest P solubilization index (from 1.63 to 4.6), were identified as Serratia marcescens A5, Pantoea ananatis AT1SB-76A, Beijerinckia fluminensis GB-G, Streptomyces albospinus MS1B15, Streptomyces noursei MS1B13, Kosakonia pseudosacchari TL8 and Kosakonia pseudosacchari TL13, and were selected for further investigations. The ability to solubilize P of the selected strains was quantitatively evaluated by Molybdenum Blue method using PVK medium. All the strains showed high P-solubilization activity (ranging from 248.57 µg/ml to 363.54 µg/ml) correlated to a pH decrease. Overall results showed that the selected bacterial strains possess a high P-solubilization activity and therefore, they could represent potential candidates as biofertilizers to increase plant growth as well as their productivity cultivated in lake P soils.

#### Effects of Robinia pseudoacacia L. on two apulian soils

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The marginal lands of Apulia region are reforested using preferentially pioneer species such as *Pinus spp.*, *Cupressus spp.* and *Robinia pseudoacacia* L. or black locust. The aim of the study was to evaluate the effects of black locust in two reforested apulian soils and compare them to the ones of pasture soils close to the robiniets. In addition, the impacts of a *Cupressus arizonica* E. Greene reforestation on soil fertility has been investigated for establishing any differences with the black locust one. Black locust enriched soils with organic matter and nitrogen with respect to the corresponding pastures. However, in comparison with the cypress soils, the C and N soil enrichment was lower. That could be the result of i) different litter deposition of cypress vs. black locust; ii) drought stress of the Mediterranean basin with respect to the black locust endemic zones; iii) absence of specific symbionts of *Robinia pseudoacacia* L. The higher carbon content observed in the cypress soils induced a statistically greater microbial community represented by the microbial biomass carbon and the dissolved organic carbon and, although not statistically significant, the soils covered by conifer showed numerically higher concentration of ß-glucosidase, phosphatase, urease and dehydrogenase. The microbial biomass, organic matter and soil enzyme activities are some of most well-known soil quality indicators (Gil-Sotres et al. 2005). Therefore, in Apulia region and in natural conditions, cypress reforestation ameliorated the soil conditions with respect to the black locust.

Gil-Sotres F, Trasar-Cepeda, C, Leirós MC, & Seoane S 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biology and Biochemistry, 37: 877–887.

#### Assessing soil carbon trends in Italy through land use and management changes

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The European Union (EU) goal of reducing greenhouse gas (GHG) emissions to 40% below the 1990 level by 2030 will contribute significantly satisfying the 2015 Paris Agreement. The amount of carbon that is prevented from returning to the atmosphere is mainly based on land use and management, soil characteristics, climate, crops, and retained biomass.

The Intergovernmental Panel on Climate Change (IPCC) has developed a 20-year-step model to assess topsoil SOC changes using climate, soil characteristics, land use, and agricultural management. Soil management is the most common change in short- and medium-term, and in EU is monitored by Corine Land Cover (CLC) inventory, that was recently updated.

The aim of this work is to describe a methodology to estimate the agricultural SOC changes over a one km raster grid, according to the Inspire EU directive requirements. This approach applies the IPCC criteria to map Italian soils and climate and to overlay these layers with 1990, 2006 and 2018 CLC maps. Since the IPCC model requires permanence of conditions for at least 20 years, CLC inventory gap considered consisted of 28 years.

The most prevalent areas in Italy: (i) have soil types assigned to the IPPC category high activity clay mineral (HACl), (ii) are considered in the warm-temperate-dry IPCC climate region and (iii) with predominant land use of forests closely followed by non-irrigated arable lands. Non-agricultural soils and soil-climate-land use combinations (triples) represented by an area less than 0.5% of total Italy area, have been excluded. Thereby, 42 triples were determined. From 1990 to 2018, semi-natural areas under warm temperate dry conditions on HACl resulted the highest increase (+1415 km<sup>2</sup>), while non-irrigate arable lands under warm temperate moist conditions on HACl showed the highest decrease (-1,214 km<sup>2</sup>). Each triple was assigned to degradation level in pastures or tillage management system and organic input in arable lands, to estimate IPCC reference carbon stock.

Further development would include a validation to compare results with other SOC surveys or legacy data, and a sensitive analysis to assess possible change in soil management, due to policies.

### Soil biochemical activities as affected by different tillage, fertilization and depth in a semiarid mediterranean agro-ecosystem

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Soil enzymes respond rapidly to changes in soil managements, and therefore are used as early and sensitive indicators of changes in soil properties induced by tillage and additions of fertilisers (Salinas-Garcia et al., 1997). The aim of this work was to compare the effects of different tillage (no tillage, minimum tillage, conventional tillage), fertilization (fertilization versus no fertilization) ad soil depth (0-30, 30-60 and 60-90 cm) on the microbial biomass and enzyme activities in a semiarid Mediterranean agro-ecosystem. This study finds differences in the microbial respiration parameters and enzymatic activities among the different soil depth. In particular, growing microbial biomass and total microbial biomass reduced with depth with the lengthening of the T-Lag and these results are related to the reduction of oxygen in the deepest soil layer and to the change of the microbial community. The activities of  $\beta$ -glucosidase and N-acetyl- $\beta$ -glucosidase reduced with depth (lower  $V_{max}$ ) presumably due to the reduced C content in the deeper layers of soils. The fertilization reduced the microbial respiration parameters, while under no tillage, all enzymes demonstrated higher potential activity in top layers of fertilized plots as compared with non-fertilized plots. Tillage smoothed the differences in microbial respiration parameters and max activity of the enzymes, while the activity of all enzymes appeared slightly higher under no tillage with respect to minimum and conventional tillage. This technology can be considered a rapid method for the study of microbial activity in semiarid Mediterranean agroecosystems.

Salinas-Garcia, J.R., Hons, F.M., Matocha, J.E., Zuberer, D.A. 1997. Soil carbon and nitrogen dynamics as affected by long-term tillage and nitrogen fertilization. *Biology and Fertility of Soils* 25, 182–188.

#### Extraction of nanostructured starch from purified granules of waxy and nonwaxy barley cultivars

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Waxy and non-waxy barley flours were selected as native material for the extraction, by acidic hydrolysis, of starch nanoparticles (NPs), with the main aim of evaluating how their different amylose/amylopectin ratio affected NPs structural characteristics. To this scope, two approaches based, respectively, on the use of NaCl/ toluene (called organic treatment) and of sodium hydroxide solutions (called alkali treatment) were adopted in order to purify the waxy flours to give starch granules. Morphological investigations of waxy flour after alkali and organic treatments, before and after acetone washing, showed that the isolation methods of the starch did not affect the general morphologic characteristics of starch granules. The method found to provide the best outcomes in terms of thermal and morphological properties was also applied to purify the non-waxy barley. The results confirmed that waxy starch characterized by granules of smaller dimensions (WS) gave amorphous nanoparticles (WNP) with a reduced mean diameter and thermal stability with respect to NPs obtained from non-waxy barley flour.

### Soil organic carbon variation in organic farming in Molise (South-Central Italy) as evaluated by Diffuse Reflectance Spectroscopy

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Assessment of soil organic carbon (SOC) spatial variability at high-detail can be very useful to comprehend the effects of management and/or erosion on carbon dynamics at farmer scale. Assessing of SOC can be improved by using Vis-NIR Reflectance Spectroscopy, which permit a quick and cheap recording of data with a high spatial density. Aims of the present work are to: 1) analyze soils with visible-near infrared (Vis-NIR) Spectroscopy; 2) compare two methods for quantifying SOC, namely loss-on-ignition (LOI) and wet-oxidation (WO); 3) monitor "carbon dynamics" in the soil and its spatial variability. This research was conducted in an organic farm specialized in production of bio-apples, located in Castel del Giudice (IS), a small village in the North-West of Molise region (Italy). In this farm, the principles of conservation agriculture have been applied for 15 years. Zero- tillage was applied to maintain the crop residues on the soil surface, in order to reduce the excessive water disturbance on top-soil, minimize water soil erosion and provide an increase of SOC. Furthermore, soil can act as carbon sink and play an important role in mitigating  $CO_2$  levels in the atmosphere. Based on these considerations, and in order to explain the link between soil and crop management, soil carbon monitoring, soil respiration rate and plants vigor measures were made. The differences in the spatial distribution of the SOC are due not only to the quantity and to quality of the SOC (the average is 23.5 g/kg with 44% of variability), but also to the variations of the temperature and humidity soil parameters. Indeed, with decreasing of soil moisture both temperature and SOC content increase, because a soil that has a less amount of moisture tends to warm up and to have a faster carbon dynamics. Maps of SOC, show a good positive correlation between both WO and LOI and soil respiration rates, expressed as ppm of CO<sub>2</sub> released by g\*h<sup>-1</sup>. The accuracy of SOC predicted maps with Vis-NIR Diffuse Reflectance Spectroscopy (DRS) allowed monitoring of the effects of agricultural management and soil carbon pool and its spatial variability.

#### Do arid agroecosystem shrubs enhance soil enzyme activities during the dry season?

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In Senegal, two Sahelian shrubs, *Guiera senegalensis* and *Piliostigma reticulatum* redistribute water from the deep soil to the surface. Shrub water redistribution influences on soil microbial community structure remain unknown. *T*his study was carried out to determine the impact of the two shrubs on soil microbial activity during both the dry season and the wet season which lasts only 3 months. The experimental site included two locations with rainfall around 300 mm to 700 mm a year. A 2 x 2 factorial design was used with three (3) soil treatments (rhizospheric soil, bulk soil and non-rhizospheric soil sampled two meters away from the shrub) and two seasons (dry and wet season). The presence of shrubs resulted in higher enzyme activities, higher MBC, and more mineral N in rhizospheric soil compared to non-rhizospheric during both the dry and wet season. Furthermore, during the dry season, mineral N was not different between the rhizospheric soil and the bulk soil. Enzyme activities were sensitive with regard to the rhizosphere and the moisture. Shrubs maintained a moister environment during the dry season which was critical in stimulating microbial activities; this has significant implications for managing Sahelian agroecosystems.

Keywords: Shrubs, Sahel, hydraulic redistribution, enzyme activities, dry season.

# Land use change from vineyard to tree plantation in Piedmont hills: effects on soil organic carbon stock and other soil properties

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Effects of conversion from vineyard to tree plantation on soil organic carbon (SOC) stock and other soil properties were investigated by sampling paired plots in a hilly area of Monferrato (Piedmont, Italy). The study area, located at Rosignano Monferrato (AL), includes a vineyard (VY) and a nearby 30-years-old tree plantation (TP) for wood production (eight poplar clones was consociated with some timber species such as wild cherry, European ash, manna ash, deodar cedar) that replaced an existing vineyard. The study area, covering a total surface of 3 ha, extends along a slighty-wavy slope with an average gradient of 15%; according to the WRB classification, soils are Calcaric Cambisols (Loamic, Aric).

The impact of land use change on soil properties was evaluated considering the spatial variability of soil characteristics, testing for autocorrelation among the model residuals. Soil sampling was performed from 3 layers (0-10 cm, 10-40 cm and 40-70 cm) at 61 and 69 points in the VY and the TP respectively, to characterize spatial distribution of soil pH in water, organic carbon content and stock, C:N ratio, soil texture and total carbonates. At TP the organic horizons were sampled and analyzed for OC determination. The common pedological origin of soils within the study area was verified and confirmed by comparability of soil texture and carbonate content of the deeper layer.

Statistical analyses showed significant (p-value < 0.05) differences between the investigated land uses with regard to pH, SOC stock and C:N ratio; the land use conversion affected all the three layers.

The VY showed in the 0-70 cm layer a weighted average pH value of  $7.9\pm0.1$ , a C:N ratio of  $7.8\pm0.7$  and a SOC stock of  $5.9\pm2.3$  kg m<sup>-2</sup>. TP was characterized by lower pH value ( $7.5\pm0.5$ ) higher C:N ratio ( $8.8\pm1.0$ ) and SOC stock ( $7.4\pm2.1$  kg m<sup>-2</sup>) than VY; the SOC stock of the organic layer was 1.0 kg m<sup>-2</sup>.

Our study provided evidence that: (1) 30 years of tree plantation strongly modified SOC stock, resulting in an increase of 25% in the first 70 cm, which became 42% if the organic layers were included; (2) soil acidification (pH difference of 0.4) and change in SOC type (C:N increase of 1) were also observed in TR compared to VY; and (3) the spatial distribution of soil properties in the VY were affected by erosive and depositional dynamics unlike the TR where vegetation contrasts erosion.

# Climate change and degradation of land and water resources: impact on agriculture production in the dry zones of India

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Maintaining food security for the large and fast rising population under a changing climate and environment is a major challenge in India. National economy and life of millions of rural population have been traditionally linked to agriculture. As a result of the change in government policy favouring globalisation and industrialisation, basis of the economy is gradually shifting from agriculture to industries, widening economic imbalance, creating food and water crisis and worsening conflict over the allocation of food and water. Farm area is shrinking very fast and the quality of soil is declining fast. Out of the total geographical area of 329 million hectares, 175 million hectares, mainly in arid and semiarid agricultural area are considered degraded. In addition to all is the change in climate leading to hydrological extremes, threatening the food security, especially in the semi-arid agricultural zones. Drylands of India are thretened by desertification. According to FAO, India could lose as much as 125 million tonnes of its rainfed cereal production due to floods and droughts under a changing climate in near future. Seasonality and intensity of rainfall are changing. Increase in rainfall is not enough to compensate for the loss in soil moisture due to rise in temperature. Shortage of water increases dependency on groundwater, but the amount is not compensated through recharge. Retreat of the Himalayan glaciers may affect the existence of major rivers in near future, leading to severe water shortage, affecting agriculture in an extensive area, mostly in the dry zones. Recent government reports show that India is heading towards a food crisis. Setbacks in agriculture leads to several socio-economic issues such as migration, conflicts over allocation, hike in food price and rural unemployment that invites unrest in society. This paper analyses the impact of climate change, environmental degradation and government policies on agricultural production in the drier zones of India and the associated socio-economic issues. Changes in water availability in an altered climate have been estimated using hydrological model and the existing policies and adaptation strategies have been critically reviewed. India urgently needs to develop an appropriate policy for agriculture, strategy for land and water management and climate change adaptation and mitigation. Guidelines for this have been provided.

# Assessing the effects of African Neglected and Underutilized Species and soil tillage on belowground microbial diversity and activity

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Jute Mallow, *Corchorus olitorius* L., is an erect woody herb that is used for both food and fiber production. Cowpea, Vigna unguiculata (L.) Walp, is an erect trailing or climbing herb that is usually cultivated for its leaves and seeds under hot and moderately wet conditions. In addition to the seeds of V. unguiculate, young fresh leaves of both species are commonly used in various African recipes. Both species can be categorized as Neglected and Underutilized Species (NUS) which are underexploited orphans of scientific interest even though they are often used/consumed in local communities without being staple crops, especially in Sub Saharan Africa(SSA) where the maize-based farming systems are the backbones of food production. In this study, both species were cultivated under various conservation agriculture management systems during one-year long field experiment in which conventional tillage (CT) and no tillage (NT) combined with normal and deficient water regimes were evaluated. Microbial diversity by PCR amplification and next generation Illumina sequencing of bacterial 16s rRNA gene and fungal ITS regions, fertility status of the soils by physicochemical analysis together with activities of  $\beta$ -Glucosidase (BGL, EC 3.2.1.21), phosphatase (PHO, E.C. 3.1.3.2), urease (URE, E.C. 3.5.1.59) enzymes were all assessed to have greater understanding of the changes caused by these plants on soils under given conditions. Significant differences in soil microbial diversity were found between CT and NT regardless of crops cultivated. Whereas the impact of the crops on soil biodiversity was highlighted in bacterial species and in fungal species, by cowpea and jute mallow, respectively. Enzyme activities on the other hand, similarly to the microbial diversity, were found to be significantly dependent on the CT and NT conditions. However, significant differences were also found also between stress and no stress conditions for both BGL and PHO only in the case of cowpea thus suggesting the plant's reaction to water stress.

#### Microbial insights to sustainability of family farmers of Mt. Meru Zone in Arusha, Tanzania; Neglected and Underutilized Species vs Maize / Food vs Cash

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Neglected and Underutilized Species (NUS) are underexploited orphans of scientific interest even though they are often consumed in local communities without being staple crops, especially in Sub Saharan Africa(SSA) where the maize-based farming systems are the backbones of food production. NUS; (i) are vital for over 60% of rural communities, (ii) have a potential to prevent malnutrition, obesity and dietrelated disorders, hidden hunger in future, together with their -often taken for granted- climate resilience. As weather extremes related to climate change have been started to take place globally, smallholder farmers of SSA are no strangers to the current challenges our world is facing. In present work, fields of small holder farmers with different farm management approaches to food production were surveyed in Mt. Meru Zone in Arusha, Tanzania to assess the functional fertility of their soils. Besides measurement of physicochemical parameters and soil enzymes activities, particular attention was paid to the contribution of biodiversity of soil microorganisms due to fact that role played by soil microbial diversity are often considered as an important contribution to resilience of the agroecosystems. Therefore, PCR amplification of bacterial 16s rRNA gene and fungal ITS regions of samples were carried out and subsequently sequenced by Next Generation Illumina Sequencing. Microbial reflection of soil fertility was highlighted by the differences between various practices applied by farmers in their field, by both bacterial and fungal biodiversity.

# Soil carbon sequestration upon shifting from conventional to ecological agriculture: do we miss something?

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The shift from conventional to organic farming is a land use change reported to result in soil carbon sequestration, but contradictory observations have been observed about this matter. These unconsistent results may be partly due to the diversity of criteria to compare the soil carbon stocks on plots; but also to the fact that relevant phenomena that occurs together with carbon sequestration are often not taken into account, namely (i) the decrease in soil bulk density, particularly in the first 20 cm of the soil, and (ii) in calcareous soils, changes in soil carbonates. With the increase in soil organic carbon stock, the stock of inorganic carbon may either increase or decrease: thus, focusing on OC changes only may drive to underestimations or overestimations of the true amounts of sequestered C.

Here we present a systematic comparison of pairs of agricultural plots, one submitted to conventional farming, other to organic farming. Some groups of plots were included, too. Plots were sampled by taking intact prismatic samples of 5 x 5 x 30 cm, thus allowing an exact volumetric reference. In addition to the classic approach (OC and IC stocks down to 30 cm), we applied the CCA (cumulative coordinates approach: Rovira et al, 2015, Catena), which allows to correct for the changes in soil compaction, which often occur in land use change situations. CCA is applied both to the organic and the inorganic carbon: thus we aim to study changes in carbon stocks in an integral way.

The shift from conventional to organic farming practices result almost always in a decrease in soil bulk density: soil expands, the amount of mineral matter down to 30 cm decreases, and this decrease distorts the changes in both OC and IC stocks, which may be over- or underestimated. The CCA method corrects for these distortions. The true C sequestration is greatly underestimated if these changes in bulk density are not properly taken into account, i.e. if only stocks down to a fixed depth are studied: underestimations of about 20 % are common, but examples of greater underestimations (up to 50 %, i.e. twice the true value) have been also found. In summary, our study stresses the need of an in-depth study of the obtained soil cores, including their changes in bulk density and carbonates, to properly assess soil C sequestration upon land use changes.

# Use of diffuse reflectance infrared Fourier transform (Drift) to study the composition of four size fractions of soils subjected to different management

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This study investigates the effects of different tillage (conventional (CT), minimum (MT) and no tillage (NT)), fertilization (fertilization vs no fertilization), and soil depth (0-30, 30-60, and 60-90 cm) on the abundance and composition of four size fractions of soils and on their organic carbon (OC) content. Fraction-size separation was conducted by ultrasonic dispersion and wet sieving according to Amelung and Zech (1999), obtaining four size fractions (A: 2000–250 μm; B: 250–53 μm; C: 53–20 μm; D: <20 μm). The OC content and the quality composition of each fraction were determined by elemental and DRIFT analyses, respectively. Results suggest that tillage and fertilization influenced the amount of each soil size fraction. In fact, CT favored the increment of the finest fraction (D) while the fertilization, promoting the microbial community, increased the amount of soil micro-aggregates (B). Clearly, soil depth influenced the amount of each soil size fraction, with higher amounts of fraction A and fraction D in the upper and deepest soil layer, respectively. The OC content was influenced mainly by the size of fraction and the soil depth since a higher OC content was found in the top soil layer (0-30 cm) and in the finest soil fraction (D). The DRIFT analysis suggested that the size of the fractions, and secondarily the depth, were the only factors responsible for the composition of soil fractions. In particular, quartz was identified in A fraction, while the signal of kaolinites appeared in the other fractions. The signals of aromatic and aliphatic organic components were present in the spectra of the finest fractions, while the nitrogen compounds were mainly related to the coarser fraction of soil (A). With depth, a reduction of the signal of polisaccharides structures was observed only in A fraction. Different managements influenced only the quantity of each soil fractions and not their composition.

Amelung W., Zech W. (1999). Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73–85.

#### Influence of long-term tillage management on soil fertility level in a Mediterranean agro-ecosystem

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Deep tillage *techniques* and rock fragmentation are common management practices to prepare farmland for planting in Southern Italy. Unlike soil physicochemical characteristics, relatively few studies have explored the effect of tillage practices on soil fertility, biochemical and microbiological parameters. The present research aimed to investigate how long-term conventional tillage (CT) and no-tillage (NT) practices applied in an agro-ecosystem of the Mediterranean area (Basilicata, Italy) affected soil fertility. Both plots have been cultivated for 10 years with a biennial rotation of durum wheat (Triticum durum Desf.) and legumes. A minimum of 5 sub-samples were taken randomly by hand auger from two different soil depths of 0–20 and 20–40 cm and combined into one composite sample per depth. Soil Biological Fertility Index (BFI) was measured to evaluate the similarities and differences between plots and soil depths. This indicator is composed by some biochemical and microbial parameters of soil including soil organic matter (SOM), microbial biomass carbon (MBC), basal soil respiration at the last day (25 days) of incubation (BSR), cumulated soil respiration during the incubation period (CSR), metabolic quotient (qCO<sub>2</sub>) and mineralization quotient (qM). According to the results, tillage techniques showed a decreasing trend in SOC, C:N ratio, MBC and nucleic acid concentration with depth. Accordingly, SOC was 1.16 and 0.99% in the top 20 cm of the NT and CT soils, respectively. The C:N ratio in the top 20 cm of the CT was significantly higher (+22.5%) than that in the NT. The highest and lowest values of microbial respiration (equal to 51.6 and 39 mg  $CO_2$ –C kg<sup>-1</sup> soil) were estimated at about the twelfth day of incubation at 0–20 cm in the NT soils and at the 13.5 days of incubation at 20–40 cm in the CT. The maximum BFI score also belonged to the 0–20 cm of NT soils (equal to 25), which was ranked in the class V (high). In conclusion, as indicated by some main biochemical and microbial parameters including C:N ratios and MBC as well as BFI, we can reasonably assume that the preservation of this agro-ecosystem under no or low disturbance, can boost soil biological fertility level in comparison with the CT, in both 0–20 and 20–40 cm soil layers. Our results can also validate the BFI to evaluate soil fertility in agricultural soils. However, more in-depth studies, including metagenomic analysis and agronomic evaluation are performing to confirm the results.







# Session II: Soil pollution and food safety

Oral presentations

## **Invited speaker**

### An operational tool to challenge land take at the European scale

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Soil Sealing by urban sprawl is one of the best-known land degradation processes proceeding at a rather high rate thus decreasing our fertile agricultural land and putting our landscape at risk. Therefore, an urgent action is required since future generations will not see a healthy soil coming back once it has been destroyed.

The interdisciplinary H2020 LANDSUPPORT project – thanks to its unique combination of knowledge and technologies – is developing a free web-based decision support tool to analyse, monitor and mitigate land take for the entire European territory.

The tool– in its prototype version – is currently in use (www.landsupport.eu) and fully active within the confines of the EU administrative boundaries. LANDSUPPORT is a Geospatial Cyber-Infrastructure strongly based on the use of free open-source geospatial libraries and programs and enables users to interact directly with the geospatial data on the map via the web.

DATA: The dataset connected to the Land Take tool includes geo-referenced data and metadata from different sources. The main types of data include: (i) Copernicus HRL, (ii) thematic maps (land use, soil, etc.) with relating databases, (iii) new data produced by simulation modelling.

MODELS: The models include (i) monitoring land take by the evaluation of soil loss or gain between different years, (ii) on-the-fly quantification of rural fragmentation to challenge urban sprawl that alters the integrity of the ecosystem functions of the rural landscape, (iii) effects of soil sealing on soil ecosystem services.

DASHBOARD: It is made up of five different sections including webgis and modelling tools.

APPLICATIONS: The system can address land take mitigation and spatial planning issues by a multitemporal accountability of land take and by performing on-the-fly calculation of critical indicators such as land fragmentation.

The system provides a support for the following use cases:

(i) National Public Authorities who require multi-temporal monitoring of land take over their territory.

(ii) Spatial Planning at Regional and Province scale where planning guide-lines/directions are to be adopted.(iii) Spatial Planning at the Municipality level which it is typically the administrative level where final land take decisions are adopted.

# *In vitro* bioaccessibility of potentially toxic elements in particle-size fractions from an industrial soil of south Italy

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Contaminants in soil can directly pose significant risks for human health through oral ingestion, particle inhalation and dermal contact, especially for humans living or working nearby the polluted areas. Currently, the evaluation of bioaccessibility of PTEs to humans is gaining interest in the environmental studies and can be profitably applied in risk assessment of potentially contaminated sites (e.g. by Risk-net 3.1 software, Reconnet). Even the recent Italian Ministerial Decree (46/2019) on soil contamination in agricultural areas imposes to investigate potentially contaminated soils by in vitro bioaccessibility tests. This study was carried out in an industrial site recycling automobile batteries. A soil geochemical characterisation highlighted a diffuse pollution by Pb (85-80152 mg kg<sup>-1</sup>), Sb (0.7-1475), Cd (0.3-235) and As (9-312), mainly distributed in the first 10 cm of soil, susceptible to wind erosion and air soil-particle dispersion. The distribution of pollutants among main soil geochemical phases was studied by BCR and Wenzel sequential extractions (SEs). In a selection of soil samples, a physical separation was performed to assess the metal distribution among five soil particle-size fractions (50-2000, 20-50, 10-20, 2-10 and  $<2 \mu m$ ). These soil sub-samples were used to study the lung, oral and dermal bioaccessibility of PTEs by analytical procedures simulating the human fluids in the upper and lower respiratory tracts (SELF and ALF methods), gastric and intestinal phases (BARGE) and skin sweat (EN 1811). Lead and Sb were found to be mainly associated with the coarsest soil particles (>50 µm, 52-58 %), while Cd and As were more concentrated in the finest ones (<10 µm, 42-49 %), i.e. the most dangerous for human health. All extractions revealed a higher lung and oral bioaccessibility of Cd (5-85 %) vs Pb (1-64 %), likewise of Sb (6-68 %) vs As (2-15 %). Overall PTE-bioaccessibility was higher in the lower than upper respiratory tracts, as well as in the gastric than intestinal digestive tracts. A considerable dermal bioaccessibility of PTEs was also found. Results from these functionally defined extractions were related to soil properties and presumed geochemical forms of PTEs in soil assessed by SEs. Our findings promoted the implementation of a phytoremediation plant onsite consisting of poplar trees and permanent grass cover, assisted by compost amendment and irrigation, as risk reduction measure minimising the soil particle resuspension.

# Use of Vis-NIR Spectroscopy to predict the impact of different amendments on Pb and As bioaccessibility in urban soils

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The aim of the research is to develop a new diagnostic screening test for Pb and As bioaccessibility in urban soils using Vis-NIR Diffuse Reflectance Spectroscopy (DRS). Soil plots at a suburban farm in New Jersey (USA) with elevated levels of Pb and As were amended with phosphates, iron oxide with elemental S, Mn sulfate monohydrate, manure compost, and raised bed soil to determine whether these treatments could change Pb and As bioaccessibility. The use of Vis-NIR DRS resulted in a good estimate of both total and bioaccessible (extracted with 0.4 M glycine) Pb and As. Total and bioaccessible elements were predicted by improving the 'cross' correlation between surface soil metal concentrations and Vis-NIR reflectance measurements via partial least-squares regression (PLSR) modelling. The two principal components (PC1 and PC2, 94% of the variation of data) allowed to identify five different clouds with different soil treatments. Both the PC1 and PC2 were correlated mainly with Vis wavelengths. These clouds depend mainly on similar adsorption in Vis range and related to several soil chemical components. The total and bioaccessible concentrations of Pb and As were qualitatively estimated (RPD>2). PC1 and PC2 likely indicate that bioaccessible Pb and As are linked to the form and distribution of the organic matter and the crystalline Fe oxides or amorphous Fe minerals. The results obtained can be summarized as follows: 1) the PLSR models for Pb and As estimation on the basis of the Vis-NIR spectra generally give good results in terms of bioaccessibility of Pb and As among the soil samples with different treatments (high R<sup>2</sup> and mean of RPD > 2); 2) the results showed a different effect of compost amendments in increasing the extractability of soil As; 3) since laboratory analyses of bioaccessible Pb and As are both costly and time consuming, the use of Vis-NIR-DRS could result in significant reductions in cost and labor. Therefore, considering the promising results we conclude that the NIR spectroscopy provides a potential diagnostic screening method for bioaccessible Pb and As in urban soils.

# Arsenic in an abandoned gold mine spoil in Ghana: effects of pH, geochemical fractionation and potential mobilisation

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Abandoned gold mine spoils pose potential threats to environmental resources and consequently raise health concerns for humans. At an abandoned gold mine spoil in Ghana, we determined arsenic contamination (As) and assessed the effects of pH on As mobilisation. We also sequentially extracted As from the bulk soil; and the fine and course fractions for the operationally defined geochemical fractions (Water-soluble arsenic (FI); Specific-sorbed arsenic/exchangeable (FII); poorly (FIII) - and well-crystalline (IV) Fe oxide; and residual (FV)). We found arsenic total contents in the abandoned mine spoil at 1807 mg kg<sup>-1</sup>. With 0.00 and 0.04 mg kg<sup>-1</sup>, As contents were lowest at pH of 5.47 and 5.87, respectively. In the alkaline range, As more or less continuously increased by a factor of > 10 from 1.25 mg kg<sup>-1</sup> at pH 7.02 till 14.08 mg kg<sup>-1</sup> at pH 10.03. This indicates higher potential environmental risks of As under alkaline conditions. The size fractionation experiment revealed higher content of As in the fine fraction consisting of clay and silt (5876 mg kg<sup>-1</sup>) than the sand fraction (521.6 mg kg<sup>-1</sup>), which indicates that arsenic might be sorbed or present as arsenopyrite in the fine size fraction. Arsenic is mainly distributed in FIII associated with amorphous iron oxides (49-65% of total As), which may indicate the presence of a large proportion of un-weathered geogenic arsenopyrite. The potential mobility ( $\Sigma$  FI – FIV) of arsenic in the coarse fraction (74.3%) was higher than in the fine fraction (59.1%). The results indicate high risks for arsenic mobilisation from the abandoned mine spoil with potential translocation into ground and surface waters.

**Keywords:** Arsenic mobilisation, geochemical fractionation, mine spoils, risk assessment, fine and coarse fractions.

#### Potential release of zinc and cadmium in soils contaminated by heavy metals under flooding

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Zinc and cadmium are two of the most common heavy metals found in contaminated sites, the main sources being anthropogenic activities such as mining and smelting operations.

When a highly contaminated soil becomes flooded, a number of chemical reactions may occur and define if the metals will be retained or released. The aims of this study were to evaluate the potential release and discuss the mechanisms that regulate Zn and Cd solubility in two mining soils under flooding conditions, using chemical extractions, mesocosm experiments and geochemical modelling.

Mine-affected soil samples were collected in the mining basin of Gorno. Following a previous characterization study, we collected two soil samples contaminated by Zn (65 - 112 g/kg) and Cd (160 - 360 mg/kg), representative of the mining area.

The waterlogging of the soils resulted in two somehow different behaviors. In Soil 1, Mn levels in the soil solution increased gradually while in Soil 2 the Mn reduction started immediately after flooding and also Fe was slowly reduced and solubilized after the 5th week of the experiment.

Zn contents in soil solution increased during the first week in both soils, decreasing almost linearly from the 2<sup>nd</sup> week and until the end of the experiment. Regarding Cd, in Soil 1 the soluble concentration decreased gradually over time while in Soil 2 a small peak of release appeared during the first days of the experiment followed by a sharp decrease after 28 days of experiment.

The geochemical modelling predicted both metals precipitating in Soil 1 as Zn and Cd carbonate. Clays and Fe oxides seem not to have a great influence on the concentrations, as both metals are preferentially complexed by SOM. In Soil 2, metals precipitate as Zn and Cd carbonate during the first days while sulphides are calculated after 60 days of submersion. In this case, Fe oxides seem to adsorb Zn while not Cd and both metals are preferentially complexed by SOM.

These results show that, during an occasional flooding, the metals could be promptly released both in the soil solution and in the aquifer. Thus, we performed a leaching experiment to evaluate the risk for the ground water due to intense rain episodes. Zn and Cd concentrations released in the soil solution were very high, 10 times the legislative limit in the waterbodies thus, in this case, a carbonatic soil seem not to protect the environment from the instantaneous release of heavy metals subsequent to a flooding.

## Municipal solid wastes as gentle remediation options for the (bio)chemical recovery of As and trace metal-polluted soils

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In the last decades several amendments have been tested as gentle remediation options (GRO) for the recovery of metal(loid)-contaminated soils. Ideal amendments should be able to reduce the concentration of labile contaminants through adsorption and/or (co)precipitation reactions as well as to improve soil fertility attributes and soil biochemical functioning. The search for such sorbent materials is nevertheless not so easy, especially when trace metals such as Pb, Cu and Zn (TM), and metalloids such as As are co-occurring in the same soil. This is due to their different speciation and chemical behaviour in the soil solution which ultimately determine their mobility and bioavailability. In this context, municipal solid wastes such as water treatment residuals (WTRs) and compost could be considered as promising amendments for gentle remediation of metal(loid)-contaminated soils.

Therefore, in this study the (bio)chemical impact of iron-based water treatment residuals (Fe-WTRs) and municipal solid waste compost (MSWC), alone and combined, has been evaluated on three different soils co-contaminated with As (up to 22,661 mg·kg<sup>-1</sup>), Pb (up to 2,162 mg·kg<sup>-1</sup>), Cu (up to 412 mg·kg<sup>-1</sup>) and Zn (up to 1,535 mg·kg<sup>-1</sup>).

Overall, Fe-WTRs and MSWC significantly increased the abundance of culturable heterotrophic bacteria, with MSWC showing the greatest impact across all soils (up to a 24% increase). Moreover, this was accompanied in most soils by a significant reduction of both the (culturable) fungal/bacterial ratio, and the proportion of culturable As(V)- and As(III)- resistant bacteria with respect to total bacterial population. Biolog carbon source utilisation profiles and 16S rRNA soil metagenome sequencing showed a clear influence of MSWC and Fe-WTRs on the structure and diversity of soil microbial community, with Proteobacteria, Actinobacteria and Firmicutes being the most affected taxa. Soil dehydrogenase activity increased approx. 20-fold in the most contaminated soil treated with MSWC, while enhanced plant growth (i.e. *Arundo donax* and *Phragmites australis*) was also recorded in amended soils. Finally, the microbial and biochemical features of treated (and untreated) contaminated soils were highly correlated with the concentrations of labile As and TM in the same soils suggesting that Fe-WTRs and MSWC could be effectively used as GRO for the (bio)chemical recovery of As- and TM-polluted soils.

#### Phosphorus recovery from liquid fraction of digestates by struvite crystallization

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The increase of worldwide population led to an increase of cereals and meat production with a connected increase of fertilizer such as livestock effluents. Although the common purpose is to limit N damages due to the livestock effluents pollution (Nitrates Directive), the attention is now focused on the P issues. Phosphorus is a non-renewable natural resource which is going to run out in the next future. The P issues are related to both its concentration in the soil non-labile pool, which is unavailable for plants, and its soluble form which may be a pollutant which can determine the water eutrophication. FAO is proposing many measures in order to improve the efficient use of fertilizers coming from phosphorus recovery. Struvite is a mineral containing P achievable from livestock effluent by adding a Mg source, with low aqueous solubility of its crystals allows a slow release of P in the soil. Recovering P from these wastewaters is considered a big challenge due to the high phosphorus concentration and solids content (TS> 4 %). This study focuses on the phosphorus recovery as struvite from different liquid fraction of digestates (TS> 4 %), i.e. from cow manure, swine manure and OFMSW, by using lab scale crystallizer operated in continuous mode (7 Ld<sup>-1</sup>). Also, in order to substitute MgCl<sub>2</sub> a by-product of salt (seawater bittern SWB) was used as Mg source. During all tests conducted a Mg/P ratio of 2:1 was used. For what concern the digestate coming from swine manure (4.5±0.2% TS), a maximum P recovery of 85% and N recovery 46 % of were obtained corresponding on an overall P recovery on the raw digestate of 70%. In the case of cow manure digestate (4.5±0.1% TS), a maximum P recovery of 60% and N recovery 30% of were obtained while using OFMSW a P recovery of 67% and N recovery 30% were obtained. Moreover, in order to evaluate the presence of struvite crystals, dried samples from the crystallizer were analyzed by X-ray diffraction (XRD), while the struvite crystals quality (purity, crystallization and precipitation dynamics) was evaluated by the scanning electron microscope (SEM-EDS). Data achieved, showed the presence of struvite in swine and cow manure digestates while it was not detected during the OFMSW test. The results obtained showed how can be possible to promote P recovery from high solids digestates by the combination of crystallizer reactor and SWB, considering that the seawater bittern, as the liquid fraction of the digestate, is a by-product too.







# Session II: Soil pollution and food safety

Posters

#### Application of a nature-based solution for recovering a multi-contaminated area in Southern Italy

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Plant assisted bioremediation is a nature-based solution for restoring contaminated soils. Numerous studies performed at laboratory scale have shown the capability of some specific plant to recover soils polluted by toxic compounds, such as polychlorinated biphenyls (PCBs) and heavy metals (HMs). However, only few field applications of phytoremediation strategies have been implemented so far. We report a field study of poplar-assisted bioremediation carried out in a multi-contaminated experimental area in Southern Italy (close to Taranto city). After a preliminary chemical and microbiological soil characterization, 750 poplar cuttings (Monviso clone) were planted in an area of about 1000m<sup>2</sup>. At 26 months after poplar planting, soils samples were collected in the four highest contaminated plots. In each plot, soils were sampled at different distance from each tree trunk (0.25m-1m) and depth (0-20 cm or 20-40 cm). Soil was characterized (e.g. pH, organic carbon, available phosphorus) and pollutant contents (PCBs and HMs with GC-MS and ICP-MS) determined. Moreover, the structure and functioning of the soil microbial autochthonous community were assessed measuring cell abundance, viability and dehydrogenase activity. At month 26, the contaminant analyses evidenced an overall reduction of total PCBs and HMs respect to their initial values. No reduction of any pollutant was found in the "control" (un-planted soil). The poplar trees were not grown homogeneously in the planted area. Microbial analyses showed that microbial activity and abundance values were quite heterogeneous among the different investigated plots, reflecting the different poplar growth. However, cell abundance were higher in the rhizosphere than in bulk soil samples. Further investigations are in progress such as soil microbial composition analyses by for identifying the bacterial communities involved in soil recovery from contamination and pollutant analyses in plant materials for evaluating its possible use for energy purposes.

#### Application of biochar from crop residues as ameliorant of trace element polluted soils

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Biochar is the solid highly aromatic carbonaceous material produced through pyrolysis of residual biomass (De la Rosa et al., 2014). It has been reported to reduce bioavailability and leaching of heavy metals in soils (Paz-Ferreiro et al., 2014). This issue is a global concern covering 37% of the degraded soils in the European Union (EEA, 2007). This study intends to determine the effects of biochar application over two Typic Xerofluvent soils from Aznalcóllar (SW Spain) on trace elements bioavailability and toxicity. The soils presented two different levels of contamination, and consequently were called MPS (moderately polluted soil) and HPS (highly polluted soil). Biochar material was produced in a 0.64 L steel reactor under  $N_2$  atmosphere at 400 and 500 °C, with reaction times of 1 and 4 h. Feedstock used was rice husk (RHB) and olive pit (OPB). The experiment was performed by mixing 0, 2, 5 and 10 % of biochar (wt/wt) in pots (triplicate) under controlled conditions (60% of water holding capacity; 25 °C; 16 h light day<sup>-1</sup>; 65 days). The bioavailability of trace elements, enzymatic activities (dehydrogenase (DHA) and  $\beta$ -glucosidase (BGA)) and basic soil properties were monitored. Subsequently, *Brassica rapa spp. Pekinensis* were sown on the pots and germination rates and biomass production were determined.

Biochar application slightly increased soil pH. RHB increased the water holding capacity in MPS and HPS. Biochar application reduced the bioavailability of specific trace elements. Specifically, 10% of RHB and OPB produced at 500 °C-4h obtained the greater reductions. DHA showed 10 times greater activity in MPS than HPS. Furthermore, biochar amended MPS showed greater DHA than control soils. BGA decreased with increasing biochar rate application. No significant differences were observed in the germination rates in MPS, whereas germination increased with biochar dose for HPS.

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# Phytoextraction ability of four plant species against nickel in carbonation lime coming from sugar industry

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A laboratory trial was performed to test the phytoextraction capacity of four plant species against watersoluble and bioavailable nickel exceeding legal limit ( $10 \ \mu g \ L^{-1}$ ) in carbonation lime disposed in a field, and sampled for a greenhouse study for autumn-winter plants (*Spinacia oleracea* and *Brassica napus*) and a field study inside boxes of 2 m<sup>3</sup>, for spring-summer plants (*Sorghum vulgare* and *Helianthus annuus*). The plants were collected and separated in epigeal and bypogean portion in the case of the greenhouse tests

The plants were collected and separated in epigeal and hypogean portion in the case of the greenhouse tests and roots, stems, leaves and infructescences in the case of sorghum and sunflower. Afetr acid digestion, Ni concentration in plants tissues was determined using ICP-MS.

Results show a noticeable reduction of Ni in lime after the plants harvest. Regarding greenhouse tests it was observed that *B.napus* plants absorbed more bioavailable Ni (from 11.9 to 21.2%) than *S.oleracea* (from 7.9 to 10.5%), probably due to the different biomass production of the two species.

In the field experiment a mass balance was difficult, due to the continue change in bioavailable and soluble portion caused by the well developed root apparatus in the boxes; however, a comparison between the Ni concentration in the control plants growed in lime without Ni and the plants in the experimetal boxes, showed an increase of Ni in both *S. vulgare* and *H. annuus*, with a higher concentration in roots respect to the aerial part and a low translocation factor. A noticeable amount of bioavailable Ni was found for both plants in the rizosphere of well developed roots, indicating a key role of root exudates in the rizodeposition. The alkaline pH (8.8-9.0) and the presence of organic matter (7.2%) could have played a role in the extraction efficiency, promoting the adsorption of Ni and the slow release in the solution.

#### Influence of biochar on trace metals mobility and leaching in contaminated mining soils

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Sardinia has been one of the most important Italian regions for mining. The principal minerals extracted were sphalerite (zinc, iron sulfide) and galena (lead sulfide). After mining stopped, and due to the absence of effective recovery, trace metals (TM) concentrations in these mining sites and the neighbouring areas often largely exceeded the legal limits. Reclamation of such TM-contaminated soils should be therefore considered as a priority objective. The use of organic amendments can represent one of the most sustainable methods to mitigate the effect of trace metals in soil, due to their potential ability to reduce the mobility and bioavailability of TM and to promote the recovery of soil functioning. The aim of this study was therefore to evaluate the influence of a biochar (added at 5% rate) on the mobility and leaching of TM in differently polluted soils. Soil samples (S1, S2 and S3) were collected at different distance from the Montevecchio dismissed mining site (Southwestern Sardinia) where argentiferous galena and sphalerite were the main ores extracted. Such soils were characterized by increasing levels of contamination (i.e. total concentrations of Pb, Cd, and Zn were 55, 318 and 1899 mg·kg<sup>-1</sup>; 0.38, 4.75 and 74.03 mg·kg<sup>-1</sup>; 115, 622 and 3803 mg·kg<sup>-1</sup> in S1, S2 and S3 respectively). Generally, biochar addition to soil (S+Bio) determined an improvement of parameters related to soil fertility (total C and N and available P in particular), and this could be of substantial help in the recovery of marginal or degraded lands. Moreover, the addition of biochar significantly decreased the TM labile fractions, i.e. those extracted using a 0.5 M Ca(NO<sub>2</sub>)<sub>2</sub> solution (e.g. < 79 and 14% for Pb; 29, and 23% for Cd; 55% and 16% for Zn in S2+Bio and S3+Bio respectively). Overall, the residual fractions of Pb, Cd and Zn, i.e. those poorly bioavailable for plants and microorganisms, increased in treated soils (>25 and 18% for Pb; >21 and 10% for Cd and >4 and 7% for Zn in S2+Bio and S3+Bio respectively). Leaching experiments highlighted a similar trend, with biochar addition determining a significant reduction of leached TM (< 80 and 60 % for Zn; < 32 and 31% for Cd; < 68 and 35% for Pb in S2+Bio and S3+Bio, respectively). Overall, the results obtained suggested that biochar addition to TM-polluted soils could be useful to reduce the labile TM pools and to increase the ability of such soils to promote plant growth.

## Potential of vis-NIR reflectance spectroscopy for the prediction of soil PCBs content in a historically contaminated site of the SIN of Taranto (Southern Italy)

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Soil contamination by polychlorinated biphenyls (PCBs) is a major environmental threat in the so-called SIN (Sites of National Interest) of Taranto (Apulia Region, Southern Italy) and the surrounding area. Soil reclamation from these compounds requires careful analysis in terms of their concentrations and spatial distribution. Conventional laboratory analyses are very expensive and time-consuming. Hence, there is a need to develop/validate alternative, rapid and low-cost techniques, to use as substitutive or integrative to conventional analytical approaches. In the last decade, visible-near infrared (vis-NIR) reflectance spectroscopy, has proved to be a useful technique to rapidly investigate various soil pollutants and other soil properties. In this study, vis-NIR reflectance spectroscopy was applied for the determination of eighteen PCBs congeners (i.e., twelve dioxin-like PCBs and six "indicators" PCBs non-dioxin-like), their sum (PCBs<sub>10</sub>) and extractable organic halogen content (EOX), which is an expression of the total content of halogen in organochlorine compounds, including the PCBs. Spectral reflectance was measured in the laboratory conditions on twenty-eight soil samples, collected within a highly-contaminated site (the ex-MATRA) of the SIN of Taranto, previously analysed through conventional laboratory analysis. Colour coordinate in different colour systems (Munsell, CIE L\*a\*b\*, CIE L\*u\*v\*, CIE L\*C\*h\*, Helmholtz) were calculated from spectroradiometric measurements. Simple linear regression analysis was carried out to predict the values of PCBs and EOX on the basis of color variables. Excellent predictive models ( $R^2 > 0.80$ ) for PCBs<sub>10</sub>, EOX, and hepta-CB, were found. Furthermore, vis-NIR spectroscopy, combined with Partial Least Square Regression (PLSR) analysis, was applied for the prediction of PCBs and EOX content. Using PLSR after pre-processing the reflectance spectra, excellent models were calibrated for the prediction of  $PCBs_{18}$  ( $R^2_{adj} = 0.91$ ; RPD = 3.47), EOX ( $R^2_{adj} = 0.909$ ; RPD = 3.40) and Hepta-CB ( $R^2_{adj} = 0.897$ ; RPD = 3.24), while a good model was calibrated for Penta-CB ( $R^2_{adj} = 0.798$ ; RPD = 2.27). The results of the present study demonstrated that i) spectroscopic determination of soil color is a promising tool for rapid screening of PCBs in contaminated soils; ii) the performance of vis-NIR improves if the whole spectra, along with PLSR statistics, are used as predictors of the investigated soil variables.

#### Testing synthetic zeolites obtained from recycled waste materials for oenological applications

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Zeolites are tectosilicates characterized by high microporosity and cation exchange capacity. Due to their adsorption properties and attitude to act as molecular sieves, these aluminosilicates are employed in a number of industrial and agricultural applications. Besides natural zeolites, several processes have been developed in the last decades to synthetize zeolites with useful chemical and structural properties. A simple procedure based on the alkaline hydrothermal treatment of glass and aluminum recovered from food and drink packaging to produce Na and K zeolites (Zeo-Na, Zeo-K) has been recently published by our research group.

The objective of this work is to evaluate the potential applications of Zeo-Na and Zeo-K for the removal of riboflavin (RF), a molecule responsible for the light-struck taste of white and rosé wines when present at concentrations higher than 100  $\mu$ g L<sup>-1</sup>. Only few studies have been published about the application of zeolites to improve the stability and shelf-life of wines, none of these adopting synthetic zeolites. Preliminary experiments were performed using a model wine solution (5 g L<sup>-1</sup> tartaric acid, 12% ethanol (v/v), pH adjusted to 3.2 with NaOH). RF was added at the concentration of 300  $\mu$ g L<sup>-1</sup> in the model solution. Each zeolite, previously characterized by X-ray powder diffraction and for physico-chemical properties, was added to the RF-enriched solution at the concentrations of 1 g L<sup>-1</sup> (Zeo-K1, Zeo-Na1) and 10 g L<sup>-1</sup> (Zeo-K10, Zeo-Na10). For comparison, two bentonites were also tested at the concentration of 1 g L<sup>-1</sup>. A control without zeolites nor bentonites was also run. The samples were stirred in the dark at 25°C up to 24 h, then filtered at 0.45  $\mu$ m and analyzed by HPLC (C18 column, DAD detector at  $\lambda$ = 440 nm). All the treatments were replicated three times.

The diffraction analysis revealed that Zeo-K contained 16% edingtonite, and Zeo-Na contained 30% zeolite-A. The remaining fraction consisted of amorphous phases. The HPLC analyses revealed that RF concentration remained unvaried in the control, whereas it decreased from 4% (Zeo-K1) to 37% (Zeo-Na10) in the treatments. Both zeolitic materials appeared less efficient than bentonites at the same concentration. Nevertheless, synthesizing zeolites with higher crystallinity is likely to lead to materials with higher efficiency, almost comparable to bentonites. Efforts are being undertaken to produce more effective zeolites from waste materials for oenological applications.

# Ochratoxin A removal from aqueous and aqueous ethanol solutions using low cost plant-derived adsorbents

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Ochratoxin A (OTA) is a secondary metabolite produced by fungi belonging to the genera *Penicillium* and *Aspergillus*. OTA contamination of agricultural commodities, including cereal grains, fruits and wine, causes serious health problems to consumers. Activated carbon, bentonite and other polymers are usually adopted to adsorb OTA from water and wine. However, the high cost of these materials and the risks of altering the nature of wine by removing valuable components induce to explore new natural adsorbents. We evaluated the capacity of widely available and low cost plant-derived materials to remove OTA from water and ethanol/water (14%, v/v) solution in batch assays.

Preliminarily, we tested dried clementine peels (CLE), coconut fiber (CON), coffee grounds (COG), ground shells of various nuts and, comparatively, strong adsorbents, such as a humic acid (HA) and wood biochar (BC) and hydrochar (HC) samples. OTA at 1 mg L<sup>-1</sup> concentration was spiked in both solutions, and a sorbent/solution ratio of 1:400 was adopted. Results indicated a significant adsorption of OTA from both media by several sorbents according to the efficiency scale in water: BC>HA>CLE>CON>HC>COG>others, and in ethanol/water mixture: CLE>CON>COG>others (HA, BC and HC excluded).

Successively, OTA sorption onto CLE, CON and COG was investigated through sorption kinetics and isotherms, using, respectively, OTA concentration of 1 mg L<sup>-1</sup> and in the range of 0.1-5 mg L<sup>-1</sup>. Desorption was also evaluated starting from the dose of 2 mg L<sup>-1</sup>. Sorption kinetics data were fitted to pseudo-first-order and pseudo-second-order models, whereas isotherms data were interpreted with the two empirical Freundlich and Langmuir equations. A rapid (about 2 h) attainment of the steady-state equilibrium of OTA onto all the three materials was obtained according to a pseudo-second-order model that indicated chemical interactions between OTA and any adsorbent. Sorption isotherm data of OTA onto CON and COG followed preferentially the Freundlich model (1/n<1, L-shaped), whereas onto CLE the Langmuir model. The K<sub>Fads</sub> values of OTA onto CON, CLE and COG were about 185, 123 and 79 (L kg<sup>-1</sup>), respectively. OTA release from CON was limited and decreasing and stopped after 4 desorption steps when about 56% of OTA was still sorbed. Overall, results obtained encourage further studies concerning the use of such recycled materials for the decontamination of agricultural commodities from this toxin and other possible contaminants.

#### Decreasing arsenic content in Italian rice. Progresses and constraints

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The greater arsenic (As) accumulation in rice grain compared with other crops is well known and in the last years a good deal of work has been done aimed to set up effective strategies to limit As uptake by rice. Some proposals are focused on a fine tuning of the redox potential in paddy fields, particularly at selected points of the rice cropping cycle; some others involve the use of amendments to immobilize As on soil solid phases, or to hamper its uptake by rice plants. Several of these strategies have shown promising performances in controlled conditions (from pot trials up to relatively small experimental plots), however a few of them have been applied and tested at farm level. When the scaling up of the experimental strategies is tried, and when the scientists have to handover the methods to the farmers, the number of variables dramatically increases; some of them can be controlled, some others cannot. Moreover, attention must be paid to the pedoclimatic conditions and to the cultivars involved in the experiments, since not always a successful strategy can be directly transferred to different environments.

Concerning Italian rice cropping, years of research have allowed to make a relatively clear portrait of the present situation, identifying the main critical points. Strategies with good performances have been set up in controlled conditions and have been tested up to farm level, identifying the main constraints emerging whit the scaling up of the methods and when setting them up in the producing context of the farm.

## Potential of hemp to remove the emerging contaminants bisphenol A and metalaxyl-M from aqueous media

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Hemp (*Cannabis sativa* L.) cultivation has been recently reintroduced in Italy for agricultural and industrial applications, and for the remediation of soil and wastewaters from mineral and organic pollutants. Bisphenol A (BPA) is an intermediate compound in the production of plastic polymers, such as PC and PVC, and epoxy resins. BPA is a xenoestrogen interfering with endocrine functions of animals and humans. Metalaxyl-M (Met) is a worldwide used fungicide that, being quite hydrophilic, can easily move and leach in soil. These compounds may reach and pollute soil and natural waters through agricultural practices and/or application and discharge of solid and liquid wastes. Decontamination is imperative to prevent the entrance of these pollutants in the food chain. Phytoremediation is a recent sustainable technology well-accepted because of its eco-compatibility and low cost. Plants suitable for this technique must not only be efficient in removing contaminants but they should also grow rapidly, be vigorous and tolerate high contamination levels. We investigated the capacity of hemp seedlings to absorb BPA and Met from aqueous media and/or to biotransform and bioaccumulate them.

Hemp seeds were germinated in water spiked with BPA or Met at concentrations ranging from 2 to 100 mg L<sup>-1</sup>. Seedlings were grown in a Phytotron for 7 days, then biometric parameters were measured to evaluate possible toxicity of the products. At the end of experiments, residual amounts of BPA and Met were measured in the germination medium and in plant tissues after extraction with methanol. BPA and Met were analysed using HPLC with fluorimeter and UV diode array detector, respectively. All experiments were replicated six times and all data were statistically analysed with ANOVA and the LSD test. In general, both BPA and, especially, Met at any dose were phytotoxic, reducing hemp seedling elongation and fresh and dry biomass averagely by more than 40 and 60%, respectively. The highest dose of Met inhibited completely germination. Despite the concurrent toxicity, seedlings were able to remove from 99 to 86% of BPA and from 94 to 67% of Met, respectively (from the lowest to the highest dose spiked in the germination medium). Plant uptake and biotransformation seemed the main mechanisms involved. Residual BPA and Met in seedlings ranged from 16.3 to 100.7  $\mu$ g g<sup>-1</sup> and from 105.6 to 3860.8  $\mu$ g g<sup>-1</sup>, respectively, and were significantly correlated with BPA (*P*=0.003) or Met (*P*=0.005) absorbed.

# Bioaugmentation and phytoremediation: an integrated approach for the remediation of soils with low pollution levels

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Worldwide soils are widely contaminated by pollutants and bioremediation can provide a viable option for the soil restoration. The aim of this work is to use indigenous microorganisms in combination with plants for the remediation of contaminated site in Mantova Municipality (Italy) where the measured levels of Polycyclic Aromatic Hydrocarbons (PAH) and mercury were slightly higher than the limits allowed in Italy for the residential areas.

Enrichment of bacterial and fungal autochthonus species were carried out using soil samples taken from the contaminated site, using the PAHs *pyrene*, *benzo(b)fluorantene* and *benzo(ghi)perylene* as sole carbon sources. The fungi and bacteria that were able to use the above-mentioned PAHs as carbon source were then isolated, identified and selected based on the best degradation efficiencies. *Stenotrophomonas maltophilia, Fusarium solani* and *Hypochnicium bombycinum* resulted the best degraders. Biosurfactant production capacity was also evaluated. Microbial consortium was then tested for its performance associated with following plant species in greenhouse conditions: *Arundo donax, Festuca arundinacea, Populus alba + Festuca arundinacea* and *Salix alba*. The same consortium was used for the field experiments with following plants: *Arundo donax, Festuca arundinacea, Populus alba + Festuca arundinacea arundinacea*.

It was observed that pyrene, benzo(b)fluorantene e benzo(ghi)perylene were best degraded by the *Stenotrophomonas maltophilia* bacterium and *Fusarium solani* and *Hypochnicium bombycinum* fungi. The degradation efficiencies of the microbial consortiums both in the greenhouse and field conditions was significant, but only in a limited number of cases total final concentrations reached level below legal limits. On the other hands, chemical measurements carried out in the greenhouse experiments showed that most pollutants were not bioavailable at the end of the study, thus pointing to the need of a risk assessment based on bioavailable rather than total concentrations.

# Assessing the distribution and availability of potentially toxic metals (PTMs) in an agricultural soil after simulated fire events

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Controlled fires are deliberately used by farmers as an agricultural practice to facilitate seeding and control weeds and pests. During fire events, temperatures at soil surface can reach values even higher than 400°C. High temperatures and long exposure times can significantly modify soil minerals and organic matter content and characteristics, thus changing soil properties and elements biogeochemistry. In addition, the distribution and speciation of potentially toxic metals (PTMs) can be altered, making them more or less available for the plant and microorganism uptake.

Even if most of the studies reported in literature aim at establishing the consequences of wildfires on soil properties, limited data are available about PTMs mobilization in soils after fire events as well as their availability for plants uptake.

In this research, the effect of fire on the availability of some PTMs (Cr, Cu, Pb and Zn) was evaluated in two agricultural polluted soils characterized by a high organic matter content.

Preliminary investigations were carried out by using thermogravimetric analysis (TGA) to evaluate the thermal behaviour of the soils under investigation. Thermal treatments were then performed in a muffle furnace on soil samples using three different thermal ramps, from room temperature up to 300°C, 400°C and 500°C, respectively, with 30 minutes of exposure time each.

Physico-chemical analysis, sequential extractions and X-ray based analyses were used to assess the changes occurred in soils after heating treatments as well as the availability of PTMs.

Preliminary data showed a significant change in some physico-chemical parameters, such as electrical conductivity (EC), pH and cation exchange capacity (CEC). Total organic carbon and nitrogen content were strongly reduced as a consequence of organic matter mineralization. These changes caused a rearrangement of the fractionation of PTMs in soils, as well as an increase in their potential bioavailability, as assessed by sequential extractions and DTPA extractions, respectively. Speciation of Cr also showed a significant increase of Cr (VI) amount in all the treated samples.

Micro X-ray analyses on soil thin sections are in progress; first results show a redistribution of Cr in soil particles of larger size at increasing heating temperature.

Further developments of the study will include the evaluation of the effect of PTMs remobilization on plants and soil microorganisms.

# Potential for organic amendments to stabilise heavy metals in contaminated soils of technogenic barrens in Kola Peninsula

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Soil contamination is one of the key factors of land degradation due to disturbance of natural soil environment functioning. These harmful changes lead to decreasing of ecosystem primary production and further problems of deforestation and desertification. Climate change, coupled with pollutant exposures, may have potentially serious adverse consequences for natural ecosystems in contaminated regions. The objectives of the current study are to compare the effects of selected organic amendments (coal humate, peat-gel, biochar, with and without mycorrhizal inoculation) with agricultural lime (CaCO<sub>3</sub>) and mineral fertilizers (NPK) on measured chemical, physical and biological properties of degraded soils in an incubation experiment.

The results demonstrate that the selected organic amendments can be recommended for restoration of acidic metal contaminated soils. Specifically, the treatments provide a measureable increase in total soil carbon, a marked decrease in acidity, a decrease in extractable metal content, together with an enhanced nutrient uptake and vegetative growth. The use of organic amendments to improve of soil physical, chemical and biological conditions and enhance plant growth could be a sustainable, environmentally friendly, and cost-effective solution to minimize contaminant metal release to environmental media. The potential for rapid risk management through containment and stabilization of the contaminant metals is complemented by a range of additional economic, social and environmental benefits, such as carbon sequestration and suppression of metal release to soil and ground waters.

# **Sorghum** (Sorghum bicolor) and canola (Brassica napus) plants development on Kosovo contaminated soils

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Modern day industrialization and extraction of natural resources in large scale is damaging the environment and causing an increasing extension of polluted areas, whose remediation is often too costly [1]. Most important heavy metal pollutants are arsenic (As), copper (Cu), lead (Pb), zinc (Zn), nickel (Ni), and their uptake through the food chain can cause serious health problems, such as cancer, cardiovascular diseases, disorders in brain, liver, kidney and many others [1]. In Kosovo, the municipalities of Mitrovicë and Zveçan were registered as highly contaminated with Pb, Zn, Cd, and As due to mining activities [2]. Two soils, A and B, have been sampled from two different sites in Mitrovicë, showing a total content of Pb and Zn, respectively, of 2153 and 3087 mg kg<sup>-1</sup>, and 3214 and 4619 mg kg<sup>-1</sup>. A pot experiment was carried out with sorghum and canola plants, reported to be co-called hyperaccumulators [3,4], aiming to understand the effect of the polluted soils on their development. Simultaneously a control has been performed (soil C).To assess the development of sorghum plant parameters were decided as length (cm), N° nodes, stem dry weight (gr), leaf dry weight (gr), ear dry weight (gr) root dry weight (gr). For canola plant measured parameters were stem dry weight (gr), leaf dry weight (gr), root dry weight (gr) and seed yield (gr). Sorghum development on soil A was significantly lower than control only in the root dry weight (gr), on the other hand plant development on soil B had significantly lower length (cm), stem dry weight (gr), leaf dry weight (gr), ear dry weight (gr). In canola plant development on soil A was significantly lower in leaf dry weight and seed yield whereas development on soil B was significantly lower in all measured parameters, dry weight (gr), leaf dry weight (gr), root dry weight (gr) and seed yield (gr). These results confirm that even though sorghum and canola plants are tolerant to heavy metals, their development is affected by pollution in soil.

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# Session III: Organic amendments and soil quality

Oral presentations

## **Invited Speaker**

### Discriminating the action of root applied- and foliar applied-humic acid on plant growth

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The humic substances (HS) capacity to enhance plant growth has promoted the use of products based on humic acids (HA) and fulvic acids (FA) as biostimulants of plant growth and enhancers of crop yields. In general, marketed HS-based products claim that they may be applied not only on the soil (root area) but also on the leaves as foliar sprays. Whereas the mechanisms of action involved in the plant growth promoting action of soil applied HS have extensively been studied, those underlying the beneficial action of HS applied on the leaves remains unknown. Indeed, it is assumed that foliar-applied HS promote plant growth by mechanisms very similar to those involved in the HS action on plant roots. However, there are many differences between the features of the interaction of HS with plant roots and with plant leaves. For example, the amount of HS that is needed to improve plant growth via foliar application is much lower than that needed via root application. Likewise, HS applied on the leaves do not interact with soil and the rhizosphere. It is therefore plausible that the mechanisms underlying the beneficial action of foliar applied-HS and root applied-HS might present relevant differences from each other.

The aim of this talk is to describe recent results obtained in our lab regarding the mechanism of action of foliar applied HA in comparison with root-applied HA. Our results indicate that although the two modes of application of HA share some common points in the mechanism that is behind to their ability to enhance plant growth, these mechanisms are diverse to each other and present great complexity.

#### Are iron humates slow iron release fertilizers?

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Leonardite iron humates (LIH) are iron fertilizers currently used in the Mediterranean basin in dripirrigated fruit tree plantations. They are cheaper than synthetic iron chelates but also less efficient in correcting iron chlorosis when they are applied to calcareous soils. In recent years, our group has studied the LIH in silico, in vivo and providing Fe to the strategy I plant in hydroponics, soil and field experiments. The LIH efficiency was evaluated using a Saccharomyces cerevisiae strain and compared to FeEDDHA<sup>1</sup>. The growth rate for the yeast culture treated with LIH was 5% less than when treated with FeEDDHA. However, LIH promoted a decrease of almost 50% of expression for the four genes (FET3, FTR1, SIT1, and TIS11) analyzed in the cells treated. Moreover, the results of Fe intracellular content in cells demonstrated that LIH was as efficient as the iron chelate providing iron to the yeast cells. LIH as a substrate for FC-R in Fe-stressed cucumber plants<sup>2</sup> presented a low reduction of Fe(III) to Fe(II) and it was attributed to a fast accumulation of LIH on the roots, which probably inactivated functional sites and/or further delayed the FC-R activity because of the nature (high-molecular-weight humic acid) and its low capability in activating Fe uptake mechanism and translocation. Besides, in a hydroponic long term experiment, crystals of jarosite deposited over soybean roots surface were detected by SEM. To evaluate the LIH efficiency in agronomical conditions, a long-term pot experiment in soil was carried out<sup>3</sup>. Three iron- humic hybrid fertilizers were synthesized from leonardite and <sup>57</sup>Fe isotope used in the form of  ${}^{57}\text{Fe}(\text{NO}_3)_3$  and  ${}^{57}\text{Fe}_2(\text{SO}_4)_3$ . Three doses of each fertilizer were applied and iron nutrition contributions to soybean plants were evaluated. Iron-humic fertilizers are less efficient than iron chelates in soil because of kinetics effects although they promote a long term effect in iron nutrition. Soybean plants exhibited continuous and long term iron uptake, the humic fertilizers remained available in the soil and were observed in fruits. The long term effect was also confirmed in a field experiment<sup>1</sup> carried out in a chlorotic orange orchard situated in Valencia, Spain.

<sup>1</sup>Cieschi et al 2017 *J. Agric. Food Chem.* 65, 6554–6563 <sup>2</sup>Cieschi et al 2018 *J. Agric. Food Chem.* 66, 13386-13396 <sup>3</sup>Cieschi et al 2019 *Front. Plant Sci.* 10, 1-17 Acknowledgments: Com. Madrid AGRISOST-CM S2018

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#### Environmental impact of the use of digestates in agriculture: an open field approach

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The exploitation of mineral fertilizers in agriculture represents a growing problem on a global scale. In fact, their production currently account for about 2-3% of the total global energy consumption. Nitrogen fertilizers are responsible for the majority of this consumption. Phosphorus moreover, another essential element for mineral fertilizers, is a finite resource that require to being extracted from mines. The intensive use of fertilizers in agriculture, which today accounts for 45% of the total nitrogen fixed on the planet, can also lead to pollution due to the emission of reactive nitrogen species both in the atmosphere (NH<sub>3</sub>, N<sub>2</sub>O) and in the underground waters (NO<sub>3</sub>).

The circular economy model can indeed represent a solution to the above-mentioned pressing environmental and economic problems, leading to recover nutrients from biowaste and reuse them in the nearby region, creating a closed and more sustainable cycle.

One of the main biowaste nutrient recovery strategies is based on anaerobic digestion. The process, from which biogas is obtained, produces a by-product called digestate rich in nutrients such as phosphorus (P), nitrogen (N) and potassium (K). The use of digestate as fertilizer in agriculture is a strategy to close the nutrient cycle, avoiding the need to synthesize new fertilizers and thus reducing the environmental impact of agriculture. As a derivative of heterogeneous biowaste matrices, digestate is a product with a complex composition, and the potential environmental effects of its use in agriculture are not yet completely clear. The objective of this work, part of the wider H2020 Systemic project, is to analyse the environmental impact on soil and atmosphere deriving from the use of digestate as fertilizer. All the experiments were carried out in open field in the year 2018, and will be repeated in the next three years using, in comparison, digestate and urea.

The results for the first year of analysis are encouraging. In fact, treatment with digestate did not entail a greater risk of soil pollution and leaching of nitrate in groundwater, compared to urea.

Over the next three years, the impact of the use of digestate on soil and atmosphere will be constantly monitored, with the aim of achieving a sustainable product that can be a valid alternative to synthetic fertilizers.

# Agronomic reuse of olive wastewater and changes on soil chemical properties and microbial community

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The olive oil extraction system produces a large amount of wastewaters, and their disposal may represent an environmental issue. The olive wastewater (OW) can be used as organic fertilizer for its chemical characteristics, but the long-term amendment can alter soil properties and microbial community structure. The aim of this study was to evaluate, through a field experiment, the effect of OW spreading on soil chemical characteristics and microbial community. Samples were collected in portion of field where the OW spreading was carried out 14 days, 120 days, one and two years before. To evaluate the risk of OW leaching, soil sampling was carried out at two different depths: 0-20 and 20-40 cm. An unamended soil was collected and used as control.

At 14 days, the decrease of pH and increase of EC suggest that the OW use could affect the availability of heavy metals and could cause phytotoxicity. The total organic C decreased in the soils treated two years before at 0-20 cm with respect to the control (1.3 and 2.0%, respectively). The water extractable organic C (WEOC) increased particularly on soil samples treated 14 days before (3574.0 and 1134.0 mg/kg at 0-20 and 20-40 cm, respectively); whereas, the soils treated two years before reached similar values to the control. The C dynamics suggest that the easy available organic C. This behaviour can explain the decrease of soluble phenols within 120 days, without any variation at 20-40 cm. The OW addition particularly increased total N and available P, even in soils treated two years before. Characterization of the microbial communities showed that *Cronobacter sakazakii*, known to be present in oil contaminated wastewaters, was one of the most abundant bacteria in OW. Moreover, it is worth noting that bacterial communities present in soils irrigated with OW two years and one year before were not statistically different from control soil. A redundancy discriminate analysis (RDA) demonstrated that the phylum *Firmicutes* is positively influenced by soil EC, P and K, WEOC and total N. Those evidences suggest that an annual OW spreading does not affect the endemic soil bacterial community of the olive grove.

#### Citrus hydrolates as natural biostimulants of soil microorganisms

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Hydrolates are waste products resulting from the industrial extraction process of essential oils (EOs) produced through the cold pressing of citrus fruit peels. During such process, drinkable water is used to remove EOs from fruit peels and carry them towards the centrifuge, where the emulsion is then separated in two phases: water solution (hydrolates) and EOs. Up to nowadays, hydrolates undergo to sewage treatment before being disposed of, with negative economic ripercussions. Since hydrolates derive from the use of drinkable water and edible fruits, it could be of interest, especially from a sustainable point of view, their possible reuse as irrigation waters. This purpose is even more desirable because hydrolates hold a high amount of dissolved organic matter. Therefore, the aim of this work was to evaluate the effects of citrus hydrolates to the soil on labile and stable C pools and on the main microbial groups when applied as they are, or diluted with water. To this purpose, the topsoil (0-10 cm) of a citrus lemon orchard was sampled, air-dried and sieved at 2 mm. Then, soil aliquots (450 g) were placed in 1L jars and moistened up to 50% of their water holding capacity (WHC) by applying hydrolates as they are or diluted with water. The tested hydrolates were obtained from Citrus sinensis (L.) Osbeck, Citrus limon (L.) Osbeck and Citrus reticulata Blanco. The hydrolates were applied at three different doses to reach 1/3 (35 mL), 2/3 (70 mL) and 3/3 (105 mL) of the 50% of soil WHC. Control soil was moistened with distilled water only. Four replicates per treatment were run. Soil samples were incubated in the dark at constant temperature (23 °C) and humidity (50% of soil WHC). Distilled water was added when necessary to complement the 50% of soil WHC. Microbial biomass C and fatty acid methyl esters were determined after 1, 4 and 8, weeks of incubation. At the same time, 20 g of soil per each treatment were similarly incubated in 200 mL jars, to monitor the emission of CO<sub>2</sub> (C mineralization) during 60 days of incubation. Results provided evidence that citrus hydrolates were an effective source of readily available carbon as they stimulated both microbial C immobilization and mineralization. The stimulation effects of hydrolates depended on both type and concentration of hydrolates. Finally, a positive priming effect can occur following the application of hydrolates.

#### Redox-driven mineralogical changes in Fe (hydr)oxides across particle-size SOM fractions

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The kinetics of iron (Fe) recrystallization and transformation processes under changing redox conditions have been mostly studied in reductionist model systems. Despite the well-known influence of redox conditions on Fe crystallinity and speciation, as well as on soil organic matter (SOM) stabilization and C turnover, the link between redox-driven changes in Fe minerals composition and crystallinity, and SOM chemical properties in the field remains elusive. Under reducing conditions, increasing concentrations of Fe(II) released in solution from the reductive dissolution of Fe (hydr)oxides may in turn accelerate ferrihydrite (Fh) transformation, although our understanding of the influence of SOM on these transformations is still lacking.

In this work we evaluated Fe(II)-catalyzed mineralogical changes in pedogenetic Fe (hydr)oxides in bulk soils and physically fractionated SOM pools (for comparison, fine silt plus clay, FSi+Cl and fine sand, FSa) of an agricultural soil unamended or amended with biochar, municipal solid waste compost, or both. Slurries containing bulk soils or size fractions were incubated for 7 days under anoxic conditions at neutral pH after addition of Fe(II). Solid phase Fe transformations were evaluated using Fe extended X-ray absorption fine structure (Fe-EXAFS) at the XAFS beamline (Elettra Sincrotrone, Trieste). The proportions of different Fe mineral phases were determined by principle component analysis and linear combination fitting (LCF) using a suite of Fe reference spectra. Statistical methods were used to determine goodness-of-fit parameters for LCF of synchrotron-based XAS data.

FSa fractions showed the most significant Fe(II)-catalyzed Fh transformations with the consequent production of well-ordered Fe oxides irrespective of soil amendment, with the only exception being the compost-amended ones. In contrast, poorly crystalline Fh still constituted *ca*.45% of the FSi+Cl fractions of amended soils only, confirming the that the higher SOM content in this fraction inhibits atom exchange between aqueous Fe(II) and the solid phase. Building on our knowledge of Fe(II)-catalyzed mineralogical changes in simple systems, our results evidence that the mechanisms of Fe mineral transformations in bulk soils may be rather complex and depend on the natural gradients in Fe mineralogy, organic C content and quality, and organo-mineral associations that exist across particle-size SOM pools.

# Artificial intelligence approach to predict changes in biological properties of the heavy metal polluted soils after organic-based amendments addition

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Soil degradation impairs the natural ecosystem functionality and eventually affects the crop system calling for a low-cost and effective solution. To prevent soil erosion and reduce soil degradation, the organic amendments like biochar (BC) or humic products (HP) seem to be the most promising. However, their unintended negative and positive effects on soil biological and physical properties remain largely understudied. This paper focuses on the influence of BC and HP, both alone and in mixtures, on biological properties of heavily multi-contaminated chernozem and cultivated phaeozems. Soil samples were analyzed after 30, 60 and 90 days using a set of variables, namely the plant-available trace element concentrations (Cu, Pd, and Zn), C/N ratio, microbial biomass carbon (Cmic), microbial quotient  $(qCO_2)$ , and soil enzyme activities involved in C-, N-, and P-cycles. Finally, we used machine learning (ML) methods to the classify high-dimensional data and predict changes in biological properties.

Both amendments, alone and in mixtures, produced a detrimental effect on biological functions of the soils. We revealed that BP and BC+HP significantly increased the C/N ratio in the soils, while HP had no impact. At the same time, the combination of BC+HP increased qCO<sub>2</sub> during incubation, while BC and HP alone slightly mitigated the negative impact from HMs to the microbial quotient. The BC and HP provided favorable soil conditions to  $\beta$ -glucosidase and urease activity in non-polluted by HMs soils; at the same time, their effects were weaker in HMs polluted soils. Parameters such as soil type, amendment, bioavailable concentration of HMs in soils, biological responses, and incubation time were used as inputs of the ML models. The relative importance of the input variables was also analyzed to understand the drivers of temporal changes in biological properties better. Overall, soil type and amendment type were identified as crucial factors to changes in biological properties of HM polluted soils, while Time was an insignificant feature. Support vector machine performed slightly better than an artificial neural network to predict the enzyme's activity ( $\beta$ -glucosidase and urease) and qCO<sub>2</sub> values.

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# Session III: Organic amendments and soil quality

Posters

# *Trifolium subterraneum* L.cover cropping as a sustainable management for mediterranean agroecosystem

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The self-seeding legumes are species fully correspond to the principles of sustainability, since they are able to exploit, add, conserve and recycle nutritive elements, control pathogens and weeds and improve soil physical characteristics. In recent decades, the need to quantify microbial populations is a pressing statement in many areas of microbial ecology and has led the scientific community to an increased interest in the ecology of nitrogen transformation.

The objective of this study was to investigate the effects of *Trifolium subterraneum* cover cropping, compared with a spontaneous flora and a conventional management, on soil nitrogen ecology in an apricot orchard. The genes *amoA* and *nifH*, involved in nitrogen cycle, and total N,  $NH_4^+$  and  $NO_3^-$  were quantified in soil samples. Moreover, the quali-quantitative composition of weed seed bank was evaluated. The experiment involved the following treatments: 1) *Trifolium subterraneum* cover cropping (TCC) leaving dead mulch on the soil surface; 2) TCC burying dead mulch in the soil; 3) spontaneous flora

cover cropping (SCC) leaving dead mulch on the soil surface; 4) SCC burying dead mulch in the soil; 5) conventional management of apricot orchard as control. *T. subterraneum* was hand-seeded at the rate of 2,000 germinable seeds m<sup>-2</sup>at 2-3 cm depth.

DNA was directly extracted from soil samples, the genes *amoA* and *nifH* were amplified by RT-PCR using the primer sets A189-amoA2R and nifH11-nifH22, respectively.

*Nitrosomonas europaea* (ATCC 19718) and *Azotobacter vinelandii* (ATCC 12518) were used for testing the primers and as standard for calibration and subsequent calculation of their amount.

Total N,  $NH_4^+$  and NO3- concentrations were determined according to Bremner (1965), Crooke and Simpson (1971). The seedbank analysis was carried out following Scavo et al. (2019).

The quantification of *amoA* and *nifH* genes showed a similar trend, in *T. subterraneum* treatments a significant higher amount of such genes has been recorded respect to spontaneous flora cover cropping and in particular to the conventional management, confirming that the presence of *T. subterraneum* stimulated the growth of microrganisms involved in N cycle.

*T. subterraneum* cover cropping with buried mulch significantly increased the quantity of the different forms (total N,  $NH_4^+$ ,  $NO_3^-$ ) of N in soil and decreased the size of weed soil seed bank in an apricot orchard, compared to a conventional management.

# Land use change in Fiuggi Basin affects soil humification process leading to different fulvic acids features: implications on ground/spring water quality

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Fiuggi chestnut forest (Anticolana Valley, Fiuggi, Lazio, Italy) provides an "unconventional" ecosystem service produced by the complex "water-chestnut forest-litter and soil". Fulvic acids in soil are water soluble in a wide range of pH (3-8): rain water flows through soil with the effect of moving fulvic acid in the ground water. The presence of chestnut groves in the Anticolana Valley has enriched Fiuggi hydrothermal water organic compounds, providing them with important properties: it prevents stones formation and/or facilitates their expulsion. Water exploitation has positive effects on local socio-economy system. The Fiuggi basin and its delicate hydrogeological balance are extremely sensitive to geological, hydrogeological features of the area. The particular sensitivity and fragility of the natural system is due the high plant cover types variability. Moreover, as observed in previous study, the different land use may affect ground/spring water quality through variation of the humic substances (fulvic acids) characteristics due to different humification process pathway.

To emphasize the ecosystem service played by the chestnut cover on the quality of spring waters, this research focuses its attention on a representatives Basin's Fiuggi area: Le Cese. This area, derived from volcanic tufa, include from the North - East to South-West direction, tree different land use soil types: golf green – chestnut forest – natural herbaceous species. The aim of this research is to study as different covers and land uses influence soil chemical, physical and biochemical properties. In particular, it was investigated the effect of different cover types on soil humification processes and on the humic substances by a qualitative and quantitative point of view.

To this end soil sampling was performed according to a randomized block design, 3 parallel transects were identify and on each transect 9 different samples were taken. All samples were analysed for physical, chemical and biochemical properties. The results that will be shown and discussed, concern: comparative analysis of humic substances using pyrolysis; biochemical and enzymatic activity of the soil microbial population involved in humification processes; analysis of ester linked fatty acids to understand the structural microbial composition of the soil from the three areas with different types of coverage.

#### LIFE DOP: an effective example of nutrient recovery and use in the dairy sector

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#### The LIFE DOP project (Demonstrative mOdel of circular economy Process in high quality dairy

**industry**) located in Mantua district, in the middle of the production chain of Grana Padano and Parmigiano Reggiano, implemented a Slurry Exchange Platform able to collect slurry, manure and slurry derived fraction from dairy stables and to valorise them in anaerobic digestion plants. The effect is that, even if the stables are small and cannot afford a large-scale biogas plant, they nonetheless benefit from the advantages of anaerobic digestion, nutrient recovery and nutrient export.

In two years of project more than 60.000 ton of slurry-manure derived fractions were sent to biogas plants, 13 million KWh of energy were produced, 350 ton of methane emission form slurry were avoided. The farmers were given reward for providing the slurry and biogas plants saved up to 50% of the cost of the feeding mix in comparisons with maize. The amount of nutrients concentrated in the solid digestate (N and P) was 3 times higher than digestate from maize and the higher value allowed to export and economically valorise the material in non-livestock areas (organic farming, horticulture, orchards). More than 9000 tons of recovered renewable fertilizers were exported. The liquid fraction of digestate was valorised locally with higher efficiency than the slurry and manure fractions before anaerobic digestion. Finally, the total carbon footprint of milk production was reduced by 8-13% in the stables involved.

The Slurry Exchange Platform demonstrated on the ground the possibility to increase the sustainability of the cheese production, to allow the export of overload of nutrients, which is a relevant problems of high intensive livestock areas, and to better manage the nutrients locally, i.e. to zeroing the use of urea for crop and valorise the use of digestate by pre sowing and coverage distribution.

# Evaluation of the biostimulant capacity of biomolecules isolated from vegetable biomasses of different origin

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The growing increase of the world population raises the problem of the increase in food demand. New agricultural production strategies are therefore needed to guarantee high levels of production with a low environmental impact. In this sense, the aim of the research is the development of biostimulants and biofertilizers starting from extracts of two marine vegetable matrices, Posidonia oceanica (L.) Delile (PO) and Ascophyllum nodosum (AN), sources of phytostimulant substances, able to improve both production and quality of agricultural crops, in accordance with the criteria of sustainable and green agriculture. Moreover, valorisation of Posidonia residues, which represent a waste biomass, could represent an fundamental environmental and economic value as it would reduce the quantity of waste while recovering raw materials and energy. Samples of AN and PO (fresh root, fresh leaves and residues) were chemically characterized and subsequently subjected to different extraction methods (acid, alkaline and alcoholic extraction). The extracts were then subjected to qualitative characterization using mono and bi-dimensional NMR spectroscopy techniques. The chemical characterization has shown a different distribution of micro and macro elements among the different parts of the PO plant; in particular the leaves accumulate more B, Mn, Zn, Ca and Mg while the roots Fe, Mo, Cu, Na, K. The PO, except for the K and the Zn, present generally a greater content of macro and microelements than the AN. Fe and B are the most abundant in both matrices. H<sup>1</sup>-NMR spectra of acid and alkaline extracts have highlighted a region particularly rich in signals between 1.5 and 5 ppm, which refers to sugar and amino acids hydrogens, while the alcoholic extracts show peaks between 6 and 8 ppm, chemical shift region typical of aromatic compounds, and around 1-2 ppm, region of lipids. In particular, PO molecules of  $\alpha$  and  $\beta$ -D-glucose, sucrose, acrylic acid, alanine, arginine, asparagine, GABA and linoleic acid were identified or tentatively characterised. On the other hand, in AN, mannitol, alanine,  $\alpha$ -linoleic acid and phlorotannins were identified. Subsequently, the extracts will be tested on target plant species by in vitro and pot assays in order to evaluate their phytostimulant activity. On the basis of the results obtained, we will proceed with the formulation of biostimulants and experimental tests in pots and in the field, in normal and stress conditions, to test their effectiveness.

# Hydrothermal carbonization (HTC) of digestate and the potential of its by-products to be used in soilless culture systems

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The thermal post-treatment of manure-based digestate termed hydrothermal carbonization (HTC) could represent a sustainable alternative to its common treatments and could be of considerable importance to limit the problems related to land spreading. The HTC process leads to nutrient-rich solid (hydrochar) and liquid (aqueous HTC liquid, AHL) by-products. Thus, the aim of this study was to evaluate the potential of both HTC by-products to be used in soilless culture systems as either growing medium and fertigation solution. To this purpose, the HTC process was performed in a batch reactor, by varying the operating temperature and residence time, to assess the influence of these parameters on the chemical-physical characteristics of the hydrochars and AHLs. Both HTC by-products were characterized in terms of pH, electrical conductivity, and mineral concentrations by ICP-OES. Moreover, the AHLs and the hydrochar extracts were characterized by HPLC to determine the concentration of organic acids (*i.e.* lactic, formic, acetic, and fumaric acid), sugars (*i.e.* glucose), and furan-type compounds (*i.e.* HMF and furfural). Germination tests with cress seeds were also performed to assess possible phytotoxic effects of the HTC products.

The results showed that the temperature of the HTC process affected the composition of the by-products more than the process time. The chemical composition in terms of macro- and micronutrients of the AHLs was comparable to that of a conventional nutrient solution. Heavy metal concentrations were below the threshold of pollutants fixed by Italian law (D.Lgs. n. 75/2010). Germination tests revealed that the *as-is* hydrochar was phytotoxic to cress seeds while hydrochar extracts (1:10 and 1:20 m/v) allowed the seed germination, yet with a 50% reduction compared to the control.

In conclusion, the AD-HTC coupling could represent a sustainable practice in the field of biomass and waste conversion, since a management strategy, aimed at reducing and recycling the amount of waste, is needed.

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#### Biostimulant activity from Chlorella sp culture

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Biostimulant is a substance applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and or crop quality traits, regardless of its nutrients content. As last decades literature shows, biostimulant sources could be a high range of waste: agricultural, dairy, fruiting and so on or by extracts of seaweed, because these compounds have a lot of proteic fraction, necessary to improve the nutrition of plants. Biostimulant has been considered capable of enhancing plan growth and development when applied in small amounts to the soil or directly on the foliar surface. Biostimulants are extracts obtained from organic raw materials containing bioactive compounds. Biostimulants belong to four major groups: humic substances, amino acid containing products, microbial inoculants (mycorrhizal fungi and rhizobacteria), and seaweed extracts (du Jardin 2015). From a legal point of view, the bio stimulants can contain traces of natural plant hormones, but their biological action should not be ascribed to them, otherwise they should be registered as plant growth regulators. As alternative, the organic matter contains different molecules that are able to make a hormone-like effect and that are named hormone-like molecules.

The aim of this project is tested biostimulant activities from amino acids sources, obtained from *Chlorella sp* enzymatic hydrolysis, cultivated with pig manure at 6% of sterilization (Chlorella S), and pig manure not sterilized (Chlorella NS). The extraction protocol provides a mechanical treatment of the biomass, in which the cellular wall ought to break. The sonication kinetic, seen by nitrogen content, shows how can possible, after 250 minutes of handling, to obtained yield of 25.92 % and 36.02%, respectively, for Chlorella NS and Chlorella S. Following the Garcia et al. 2010 hydrolysis protocol, the degree of the reactions, is been 63.85% for Chlorella S and only 38% for the Chlorella NS. Audus test is illustrated how the solution of Chlorella S shows an auxin like profile at 89.1 mg C l-1, for the only sonicated and 16.6 mg C l-1 for the Hydrolyzed solution. Regarding Chlorella NS is needed 25,27 mg/l for the sonicated biomass to show auxin like profile and 15.65 mg/l for the hydrolyzed one.

# **Cultivation of microalgae on pig manure: nutrient recovery and biomass production in** *Chlorella* **and** *Scenedesmus*

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In the last years, strong interest has been raised by microalgae exploitation to produce a wide variety of compounds for human consumption, animal nutrition, biofertilizers, and bioenergy, as well as for wastewater treatment, nitrogen fixing and  $CO_2$  mitigation.

At the same time, global agriculture and zootechnical activities have quickly improved, driven by the increasing demand for food worldwide. These activities produce huge amounts of wastewaters rich in N and P, causing pollution and eutrophication of watercourses if not properly managed. On the other hand, they can be exploited as nutrient sources, with economic and ecological benefits. In this context, the SABANA (Sustainable Algae Biorefinery for Agriculture aNd Aquaculture) project aims to implement a microalgaebased sustainable biorefinery for the production of biostimulants, biopesticides, feed additives (high added-value) other than biofertilizers and aquafeed (low added-value). Chlorella sp, Chlorella vulgaris, Scenedesmus acutus, and Scenedesmus pannonicus were tested for their aptitude to grow using the nutrients present in pig manure (PM)-based media and for their nutrient (N-NH<sup>+</sup><sub>4</sub>, P-PO<sup>-3</sup><sub>4</sub>) recovery efficiency. The microalgae were cultivated at different PM concentrations (NH<sub>4</sub><sup>+</sup> as N source) and in a synthetic nutrient solution as a control (BG11, NO<sub>3</sub><sup>-</sup> as N source). The growth response time-course (specific growth rate,  $\mu$ ), the final biomass production and its chemical composition, the nutrient recovery ratio and the light use efficiency were detected. According to the results obtained in these trials, the most promising species to be grown in PM are *Chlorella* sp. and *S. acutus*. They did not suffer from inhibition by NH<sup>+</sup>, producing biomass amounts similar to the controls even at high PM concentrations (up to 440 mg  $L^{-1}$  NH<sub>4</sub><sup>+</sup> for Chlorella sp.). They exhibited high N and P recovery ratios and low N losses due to NH<sub>3</sub> stripping when grown in the presence of PM with a concentration up to 240 mg  $L^{-1}$  NH<sub>4</sub><sup>+</sup>. Further experiments will be run to test the response of the species to step-wise addition of PM in order to maximize N recovery and to minimize NH<sub>3</sub> stripping. Moreover, a more detailed chemical characterization will provide information to address the biomass produced to different uses.

#### Response of chemical and biochemical indicator of soil quality to OMW digestate disposal

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Anaerobic digestion (AD) of organic wastes is a promising alternative to landfilling for reducing greenhouse gas emission and it is encouraged by current regulation in Europe. AD represents a source of green energy, the strength of this process, while the by-product digestate still generates concerns for a safely disposal. The sustainability of AD plants partly depends on the management of digestion residues.

Digestate could be used in organic amendment straightaway or after composting to limit possible phytotoxicity effects on crops. This study has been focused on the environmental benefits of digested olive mill wastewater (OMW), recalcitrant agricultural wastes. OMW require a complex management due to high production volume in a limited time, fermentative processes occurring during the storage, toxicity due to phenol compounds. These latter might compromise the AD process affecting microbial metabolism. As biochar is able to adsorb and retain organic and inorganic pollutants, we used biochar as additives during AD to remove phenols and stimulate microbial activity and therefore the hydrogen or methane production. The aim of this work was to evaluate the effect of solid and liquid digestates, obtained from the AD process of OMW with biochar as additive (30 and 45%), on soil chemical and biochemical properties in order to validate its use in organic amendment in lab-scale experiment. The liquid and solid digestates were added to soil according to the maximum dose allowed by the Italian nitrates directive concerning non-vulnerable areas (91/676/EEC, DGR 209/2007). Pots containing soil differently amended with liquid and solid digestates were prepared also for the growth of *Lactuca sativa* L. seedlings.

Thirty days after treatments, positive changes in chemical and biochemical properties in soil treated with biochar-treated digestates, in particular with liquid ones, occurred. Soil organic carbon, microbial biomass carbon and some soil enzymatic activities such as dehydrogenase, phosphomonoesterase,  $\beta$ -glucosidase and fluorescein diacetate hydrolysis significantly improved after digestate-based organic amendment. The lettuce biomass enhanced too.

The assessment of the agronomic quality of liquid and solid digestates, obtained by biochar assisted AD of OMW, in organic soil amendment demonstrated that also critical biomass such as OMW, if opportunely treated, can entry in a re-use process where biogas and by-products can be part of virtuous circular economy.

#### A tropical plant extract biostimulant favours growth and resource use efficiency in jute under suboptimal nutrient regimens

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Plant biostimulants (PB) are low-dose metabolic activators able to increase plant resource use efficiency (RUE), growth, yield and quality [1]. They represent a promising and innovative tool to meet the increase food demand through a sustainable agricultural intensification while reducing chemical fertilizers and environmental impact. Among PBs, Auxym<sup>®</sup> (Italpollina, Rivoli Veronese, Italy) obtained from tropical plant biomass has been proved to enhance RUE in spinach and tomato [2,3]. In our study, we used jute (Chorcorus olitorius L.) under sub-optimal nutrient-regimes (full-, half-, and quarter-strength), to test the beneficial effects of foliar application of Auxym<sup>®</sup>. The decrease of nutrient concentrations in the culture media affected morphological and physiological parameters, in particular plant height, leaf number, dry biomass, photosynthetic rate and SPAD. PB application improved fresh yield at both half- and quarterstrength nutrient solution (15.5% and 29.5%, respectively), RUE (higher nitrate, K, and Mg contents, and lower Na content), photosynthesis and SPAD index. PB, in particular, increased chlorophyll b content probably improving plants adaptation to fluctuating light and photosynthetic efficiency. In jute plants under full nutrient strength, PB increased starch, soluble proteins, and total amino acids, while under low nutrient strength, it exerted a remobilization of nitrogen from amino acids but not from chlorophylls, preserving photosynthesis. Therefore, Auxym<sup>®</sup> was able to regulate the uptake and assimilation of mineral nutrients favoring protein synthesis and accumulation of essential amino acids under full nutritive solutions, while redistributing the nitrogen stored in some amino acids to allow plant growth and expansion even under sub-optimal nutrient conditions. Auxim<sup>®</sup> enhances jute productivity even under reduced nutrient availability.

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# **Composted sewage with bagasse as commercial substrate for** *Eucalyptus urophylla x E grandis* **seedling production**

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The commercial success of *Eucalyptus* plantations are highly dependent on the quality of seedlings, with the growth substrate playing a pivotal rule in order to achieve: i) high-quality standards in seedling production, ii) a restrain in the whole production costs. Sewage sludge and sugarcane bagasse can be used as a source of organic matter and nutrients and decompaction material, respectively, with the further possibility of creating a low-cost substrate usable in forest nurseries. This research aimed to investigate the use of composted sewage sludge with sugarcane bagasse (CSB) as a commercial substrate in nurseries to grow *Eucalyptus urophylla x E grandis* seedling (*Eucalyptus urograndis*) seedlings, representing the most used hybrid clone in roundwood production. CSB was evaluated with addition of 1.5, 3.0, and 4.5% of triple superphosphate (TP) or reactive phosphate (RP) and then compared to a control (CSB without any P addition) and a commercial substrate. After 3 months, *Eucalyptus* response in all eight substrates was assessed by measuring both plant morphological traits and chemical parameters in shoots and roots. The treatment with CSB+3% TP (TP3.0) showed statistically (p<0.05) higher performances in terms of several morphological parameters (Height, Diameter, shoots and total biomass), as well as the nutrient contents of shoots and roots. The results suggested that B and K play a fundamental role in determining improved performances in TP3.0 substrate. The multivariate statistic confirmed that B and K acts as key elements in terms of both observed variability and improved plant performances. The research demonstrated the great effectiveness of CSB as a commercial substrate for seedling production, thus encouraging the reuse of sewage sludge and bagasse in this field in order to reduce their unproductive landfill disposal.

#### A green method to treat exhausted fire-extinguishing powders for reuse as agricultural fertilizer

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The most common powdered fire-extinguishing formulations (FEF) are finely dispersed mineral salts composed of mono-ammonium phosphate and ammonium sulphate as main ingredients, together with functional additives so as to improve the rheological properties of the FEF. After its whole service life has expired (36 months, under current legislation), the presence of coating agents such as water-proofing and organosilicon-based liquids makes problematic the environmentally friendly disposal of exhausted FEF, with little chance to reuse the phosphate-ammonium salts of the particles. This work aims at developing a green biotechnology-based method alternative to the chemical extraction with highly impacting organic solvents (acetone, CH<sub>2</sub>Cl<sub>2</sub>, *n*-hexane, etc.) so as to lyse the particle external coating and recover the phosphatecontaining salts for potential use as inorganic fertilizer in agriculture. To this aim, two yeast strains were inoculated in a nutrient culture medium at 1% and 5% and grown overnight before adding the exhausted fire-extinguishing powder. After 24 and 48 h of incubation, the suspension was filtered to collect the treated particles, which were oven-dried (60 °C) and then documented under scanning electron microscopy (SEM). Untreated powder was taken as control. Whereas, the clean filtrate was analysed by ion chromatography. Results show that whatever the yeast strain, there was an increased release of ammonium, phosphate and sulphate ions in the medium. However, the two strains showed different performance according to the inoculum concentration and time of contact with the powders. Even if the treatment with bi-distilled water showed an ionic release, SEM images highlighted a more severe rupture pattern of the external coating of the particle compared to the water treatment. The external coating of the particle granule is being analysed by IR spectroscopy, TGA, and GC-MS so as to deepen the knowledge on the different mode of action of yeasts and water on the outer part of the exhausted fire-extinguishing powder. The ultimate aim is to develop an environmentally friendly strategy to recover the ammonium-phosphate content of the granules and make it suitable for use in agriculture as a chemical fertilizer directly or as an additive during the composting process.

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# Metabolic responses of microbial community in soil amended with fresh leaves and leaf extracts from eucalyptus spp

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Field observations reveal that often large areas of soil surface beneath *Eucalyptus occidentalis* Endl. are completely bare or with scant vegetation. Moreover, previous studies have showed that other species of Eucalyptus, such as *Eucalyptus camaldulensis* Dehn, may be effective in suppressing seed germination and weed growth. Such effects have been ascribed to the large number of secondary metabolites within Eucalyptus leaves. Due to their inhibition activity against weeds, leaf extracts might be used for an integrated weed management context, in accordance with the Directive 2009/128/EC. Several studies exist about the effects of Eucalyptus leaf extracts on weeds, whereas they are lacking on soil microorganisms. Therefore, the aim of this study was to assess the effects of Eucalyptus leaf extracts on soil microbial biomass and activity, as well as on the relative abundance of main microbial groups. The extracts were obtained by hydrodistillation [essential oils (EOs) and hydrolates] and by water cold extraction (aqueous extracts) from leaves of both E. camaldulensis and E. occidentalis. Also, fresh leaves were tested. The soil was spread with EOs at doses of 2.64 or 3.52 ml g<sup>-1</sup>. They were applied by adding a proper water solution containing the given amount of EOs to 350 g of dry soil, so reaching 50% of its water holding capacity. The above solution held the emulsifier Fitoil at 0.5 mL L<sup>-1</sup>. Fresh leaves, dried at 40°C for 48 hours, were chopped and applied at doses of 6.6 and 5.0 mg g<sup>-1</sup> of dry soil for E. *camaldulensis* and E. *occidentalis*, respectively. Such litter application doses were those found, at field conditions (780 and 575 g m<sup>-2</sup>, respectively). Two controls were also prepared: one with water and another with water and Fitoil. After the addition of all the treatments, soils were incubated at room temperature (20-23°C) in the dark for 35 days. At days 7 and 35, soils were analyzed for soil microbial biomass C and N. At the same days, the main microbial groups were investigated through the ester linked fatty acids (FAMEs). Moreover, 20 g of soil were incubated as above in 200 mL jars to determine the soil respiration rate (CO<sub>2</sub> emission) during 36 days of incubation. Preliminary results showed, that EOs from the two species and at both doses exerted a significant biocidal action on soil microorganisms, while hydrolates, aqueous extracts and fresh leaves stimulated both microbial biomass and activity.

#### Chemical and biochemical characteristics of compost obtained from dairy and wine by-products

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Wine and dairy chains represent two of the main agri-food leading sectors worldwide. Grape marc, a byproduct of the wine-making industries, consists of the solid parts left over following grape pressing. It is rich in P, K and Mg, as well as in potentially bioactive compounds. It also shows low pH (< 4), high C/N ratio (25–40) and potentially phytotoxic compounds (e.g. ethanol, polyphenols). Whey, a by-product of cheese production, is the liquid remaining after milk has been curdled and strained. It has a high nutrient content as holds protein N and carbohydrates. A possible way to exploit such by-products may be by composting so that phytotoxic compounds can be decomposed. Furthermore, protein N held in whey may reduce the high C/N ratio of grape marc, so favouring the composting process. Also the inoculation of compost piles with cellulolytic microorganisms can improve the composting process. This work aimed to evaluate the dynamics of nutrient (mainly total C and N) content and main microbial groups within composting grape marc piles added with whey and/or inoculated with cellulolytic microorganisms. Also, to evaluate the stability of compost and the availability of C pools to microorganisms, CO<sub>2</sub> emission rate was determined during the process. Raw vineyard materials provided potential bioactivators identified as Bacillus subtilis, Bacillus velezensis and Kocuria rhizophila showing cellulolytic activity. The bacterial strains were inoculated in the mass to be composted in order to accelerate the process. Four compost trials were performed: (i) absence of bioactivators and maintenance of humidity with deproteinized whey; (ii) presence of bioactivators and maintenance of humidity with deproteinized whey; (iii) absence of bioactivators and maintenance of humidity with water; (iv) presence of bioactivators and maintenance of humidity with water. At day 0, 15, 45, 75 and 105 an aliquot of compost from the 4 trials was sampled and analysed. Results provided evidence that i) the addition of whey stimulated microbial activity but did not increase total N in the compost, ii) the inoculation with cellulolytic microorganisms increased the CO<sub>2</sub> emission rate, so suggesting a speeding up of the composting process. This research has been supported by the grant MISE-2017-NAZ-0228 - CUP: B78I17000260008.

# Benefits of compost-based media for ornamental plants: growth enhancement and suppressive activity towards the phytopathogen *Fusarium oxysporum*

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Ornamental plants are a relevant segment of worldwide agriculture and have a great economic importance in Italy. In the last years, new substrates for container cultivation of plants have been obtained replacing partially traditional growing media, such as peat and coconut fiber, with compost, with consequent environmental and agricultural benefits. The humic fraction of compost has demonstrated suppressive properties on various soil-resident phytopathogenic fungi, thus suggesting compost application to soil to protect plants and reduce the use of fungicides. The objectives of this investigation were to test: (i) mixtures at 20%, 40% and 60% (v/v) of a green compost (CO) with peat (PE) or coconut fiber (CF) on the growth and health of China aster (*Callistephus chinensis* L. Nees) plants repeatedly infected with the fungus *Fusarium oxysporum* f. sp. *callistephi* (*FOC*); and (ii) the humic acid (HA) isolated from each medium on fungal growth *in vitro*.

Plants were grown in pots kept in a growth chamber in controlled conditions for 10, 12 and 14 weeks during which the fungus was inoculated three times in the substrate. Pathogenicity was monitored by observing the symptoms and measuring plant biometric parameters, such as height, number of leaves and the average length of the main leaf vein. In other experiments, each HA was added at concentrations of 50 and 300 mg  $L^{-1}$  to fungal medium and the radial mycelial growth was monitored for a period of 13 days. All experiments were replicated six times and data obtained were statistically analysed by ANOVA and LSD test. When infected plants were grown with PE or CF alone, they did not survive or were tremendously depressed. The use of CO only was lethal to plants even before the first fungal inoculation (6 weeks after transplanting). Conversely, almost all mixtures, especially those made with 40 and 60% of CO with PE or CF, protected China aster from *FOC* and increased noticeably, on average two or three times, the number and size of leaves per plant and plant height. Any HA significantly inhibited hyphal elongation of *FOC*, compared to control (PDA only), with maximum suppressive activity by the higher dose of HA isolated from 40% of CO with PE at 18 h after inoculation that completely impeded fungal growth. The overall findings of this study encourage the use of compost in the preparation of growing media for such plants because of favourable effects on the growth and the occurrence of disease-suppressive properties.

# Acid-treatment of the solid separate of pig slurries for reducing greenhouse gas emissions and improving nutrient balance

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Composted animal slurries can be valuable sources of soil organic matter and nutrients, however their narrow N:P ratio can cause a nutrient imbalance in the soil, leading to a progressive accumulation of P and increased potential for P losses and adverse environmental impacts. Moreover, losses of ammonia and greenhouse gases can occur both during the composting phase and after soil application of the amendment. In this experiment, we tested the effects of pig slurry acidification and of acidification with nutrient correction on ammonia volatilization, gas emissions and soil nutrient status, with respect to an untreated slurry. The solid separate was acidified with elemental sulfur prior to stabilization by composting, and subsequently pressed into pellets to favour precise mechanized distribution. In addition, a nutrient corrected pellet was produced by adding calcium and potassium nitrate to the acidified solid separate, thus obtaining a balanced nutrient source for crops. The impacts on soil nutrient balance and greenhouse gas emission were evaluated under controlled conditions. The different kinds of pellets were incubated with two soils having different initial P saturation. The gaseous emissions were measured during a time span of 92 days, and the rate of organic matter mineralization and changes in soil chemical properties were measured during 63 days of incubation. Finally, potential P losses in solution and in particulate form were evaluated. Acidification resulted in a slight improvement of the N:P ratio of the pellets, lower pH, increased nitrate concentration in soil and reduced emission of ammonia and carbon dioxide, although it did not affect organic matter dynamics. The increase in available P content and potential P environmental mobility mostly depended on the dose of pellet application rather than on the acidification treatment. The samples receiving greater amounts of compost dispersed less fine particles, due to the aggregating effect of organic matter, however, the overall potential P loss increased because of the enhanced P enrichment of the fine particles.

#### Management and tillage effects on soil enzyme activities in a long-term agricultural experiment

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Non-conservative management practices can affect soil chemical, physical and even biological properties leading, for example, to a greater risk of erosion, faster degradation of plant residues, destruction of soil aggregates and lower microbial community resilience. Nowadays, sustainable soil management practices could be a valid alternative when aiming at both agricultural production and soil preservation. However, one of the most important challenges is to evaluate the impact of management practices on soil quality. The purpose of this study is to investigate the impact of soil management and tillage on soil enzyme activities with the aim to understand which management practices promote and/or sustain soil quality. The Montepaldi Long-Term Experimental site (MoLTE) is located in San Casciano Val di Pesa (Firenze, Italy) and it is set with two management systems (conventional and organic) and three types of tillage (plowing, harrowing and ripping). The soil samples have been analyzed for C and N pools, available phosphorous, and nine extracellular soil enzyme activities.

Preliminary results indicate that the organic management promoted the increase of extractable C (+12%), organic C (+12%), microbial C (+22%) and N (+17%) while the conventional system enhanced available  $P_{Olsen}$  (+81%) and extractable N content (+49%). Plowing reduced microbial C (-37%) and N (-43%) content and negatively affected also the microbial quotient and the metabolic index. Dehydrogenase and tyrosinase activities resulted improved by the organic system (+25% and +36%) and plowing significantly downturn dehydrogenase activity (-37%). The activities of  $\beta$ -xylosidase and  $\beta$ -cellobiosidase resulted significantly enhanced with the conventional management (+15% and +24%) and the same trend has been observed also for  $\beta$ -glucosidase (+15%) and  $\alpha$ -glucosidase (+12%), while the phosphomonoesterase activity resulted significantly increased by the organic system.

Measured parameters related to microbial activity resulted negatively affected by conventional management and plowing thus indicating that these practices do not support soil biological quality. Soil enzyme activities response to management resulted to be strictly related to the effect of the different fertilization. Slight differences between harrowing and ripping have been observed indicating that both have a lower impact on soil quality. Organic management support soil C pools but slight differences in organic C content has been measured.

#### QUABIO project: the agro-ecology strategy to maintain soil quality

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Agriculture tries to include new technologies and new management strategies to meet economic, social and political changes. The agro-ecologic approach has the aim to reduce the ecological impacts of the traditional agriculture and tries to control weeds, maintain soil quality and reduce soil erosion for example using the crop rotation with cover crops, which also represent a source of nutrients, and limiting the soil tillage. Within the program "ALMAIDEA" promoted by the University of Bologna, the project "QUABIO" takes into account this thematic studying the MOnsampolo VEgetable (MOVE) organic long-term experiment which started in 2001 at the experimental fields of the Vegetable Research Unit of the Research Council for Agriculture (CRA-ORA). The site is located in Monsampolo del Tronto (AP), Marche Region, Italy and consists in a long-term comparison between conventional, organic and agro-ecologic management in vegetable farming systems.

Soil samples have been collected in June 2018 and analyzed for C and N pools, and ten extracellular enzyme activities with the aim to evaluate soil biochemical indexes response to agro-ecologic management in comparison with the organic and conventional vegetable farming systems.

The results highlight that both organic and conventional management promoted extractable C (+16%) and N (+42%). On the other hand, microbial C and N content result enhanced by the agro-ecologic management while organic C and total N content showed higher values in correspondence of the organic management. The agro-ecologic management induced an increase in the metabolic index (+60%) indicating higher metabolic efficiency. Regarding soil specific enzyme activities (SEA), the agro-ecologic system generally induced a reduction except for phosphomonoesterase and laccase activities. On the contrary to the conventional system corresponded the higher SEA except for  $\beta$ -cellobiosidase activity which was higher with the organic management.

It is possible to conclude that the three systems differ in terms of soil quality, especially the agro-ecologic one. Moreover, both organic and agro-ecologic management positively influenced microbial and organic C content indicating the favorable condition to C sequestration and soil microbial community health. Concluding, the agro-ecologic approach seems to be a valid solution to maintain and improve soil quality in vegetable farming systems, it remains to evaluate its efficiency in terms of crops productivity.

# Short-term effects of different organic amendments on soil chemical, biochemical and biological indicators

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The limited availability of animal manure and the high cost of good quality compost lead to difficult soil quality management under organic agriculture. Therefore, it is important to find out alternative organic soil amendments and more flexible strategies to sustain crop productivity and maintain and enhance soil quality. A five years study was carried out in the experimental fields of the Mediterranean Agronomic Institute of Bari located in Valenzano, Italy. The main objective of this research is to investigate the effects of different fertility management strategies on soil quality in order to estimate the role of innovative matrices for their use in organic farming.

The experiment consists of seven treatments applied to a common crop rotation and a basic crop residue mulching. The treatments include alternative organic amendments (1- olive mill wastewater OMW, 2- residues of mushroom cultivation MUS, 3- coffee silver skins COF), common soil amendments (4- compost COM, 5- *Vicia faba minor* mulching LEG, 6- cow manure - MAN) and as a reference treatment (7- mineral fertilizer COV). The soil quality was assessed before and after the application of the treatments, through biological (microbial biomass carbon and nitrogen, soil respiration and metabolic quotient), biochemical (soil enzymatic activities:  $\beta$ -glucosidase, alkaline phosphatase, urease, fluorescein diacetate (FDA) hydrolysis), and chemical (pH, soil organic carbon, soil organic matter, total nitrogen, available phosphorous, exchangeable potassium, dissolved organic carbon and total dissolved nitrogen) indicators. Based on the results obtained after five years, all treatments produced an overall decrease of soil pH, a light increase of soil organic *C*, a marked increase of available P and a limited increase of soil enzymatic activities. Data demonstrated the efficiency, the high sensitivity and a quick response of the biochemical indicators in assessing soil quality changes.

As a conclusion, it is possible to emphasize that alternative and common soil organic amendments behave similarly in enhancing the chemical, biochemical and biological properties. The alternative soil organic amendments could be appropriate candidates for substituting some commonly used one which are currently showing shortage in their supply and a lowering in their quality.

Keywords: Organic agriculture, Soil quality, Enzymatic activities, Olive mill wastewater, Residues of mushroom cultivation, Coffee chaff.

# Crop sustainable management in Mediterranean conditions focused on changes in the soil component. A preliminary study in Southern Sardinia

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The development of an innovative process for non-fermentative, fast humification of animal and vegetal by-products has led to the creation of new organic soil amendments (SOAs), currently under patent. In order to enhance the environmental sustainability of agriculture, this study aims to evaluate the effects of these new amendments, in comparison with old SOAs and ordinary N-fertilizers, in some cropping systems of Sardinia with a special focus on the changes in the main chemical-physical parameters of the soil component.

The activity has been developed on a durum wheat small-plotted yield trial and is linked with other projects related to the study of cereal and forage systems, both on a plot and field scale, aiming to study the impact on the main chemical-physical as well as microbiological characteristics of amendments deriving from animal by-products.

Starting from the USDA soil classification and from a preliminary sampling in order to ascertain the initial soil conditions of the trial area, the following chemical parameters will be monitored: organic matter, nitrogen among the main ones besides the biological soil quality index. The effects of SOAs on physiology, yield and grain quality, with the goal of determining whether SOAs can supplement or replace chemical fertilization in the ordinary agronomic management in the durum growing area will be evaluated. In this 3-year project, the following aspects will be considered:

• The use of innovative organic soil amendments from animal by-products in extensive crops in order to improve the sustainability of agricultural practices;

- the medium- and long term evolution of physical-chemical and biological soil fertility;
- the medium- and long term effects on the evolution of the soil organic matter;
- the role of different cropping systems in CO<sub>2</sub> soil sequestration (carbon sink);

• the effects of new and old SOAs in comparison with ordinary fertilizers on durum wheat yield and commercial (*i.e.* test weight, kernel weight) as well as technological quality (*i.e.* protein content, gluten strength) grain quality.

This paper will show the results of the first year.

#### Olive from a waste to a resource: benefits of the end products for agricultural purpose

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In Mediterranean countries the olive oil industry produces, yearly, a huge quantity of wastes that have negative effects on soil and groundwater quality for their phytotoxic and antimicrobial properties, due to the low pH, relatively high salinity, organic load, phenolic and lipid constituents. Biological processes represent a potential bioremediation treatment to transform these wastes into resource. In this study anaerobic digestion, aerobic digestion, vermicomposting and crude agricultural waste management system were used to produce organic fertilizers. The obtained by-products were chemically analyzed to verify if their characteristics fell into the marketability limits permitted by the current Italian regulation. Their effects on soil were subsequently assessed. Results evidenced that all the by-products were suitable as fertilizers. They were able to increase soil organic matter, microbial biomass, and nutrients with beneficial effects on soil fertility, but at different extent. The best effects were in the order: vermicompost (VC) ≈compost ©, olive pomace-sulphur-bentonite pelletized (OPSB) and digestate (DG). Considering the different amounts of olive pomace (90% in aerobic digestion, 65% vermicomposting, 12% in anaerobic digestion and 5% in crude process) that the different methodologies dispose, the processing time (4 months for compost, 2 month for vermicomposting, 1 month for anaerobic digestion and 1 day for crude process) and the processing set-up, each method can be differently competitive from an environment and/ or agricultural point of view. VC has a fertilizer effect comparable to C but with a shorter processing time. Composting is an easy process that can help to improve soil fertility in olive oil farms, reducing the cost of inorganic fertilizers or, for a commercial enterprise, can provide an additional source of revenue for the olive oil mill economy. Digestate, for its high content of nutrients can be used at different percentages, with favorable outcome, to fertilize the soils. OPSB represents a crude waste management system for producing fertilizers that contain also recovered elemental Sulphur (waste), and if the same processing time is considered it represents the method that consume the most olive pomace amount. OPSB can be considered a corrective as well as a fertilizer because it is able also to low the pH of alkaline soils.

#### Short- and long-term effects of a single compost addition on Technosols quality

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Technosols are made up of 50 % in anthropogenic materials in the first 10 cm depth whose features are strongly influenced by own derivation. The chemical-physical and biological properties of Technosols, and then quality, mostly depend on the soil origins and the anthropogenic derivatives. Particularly, total and available element contents can influence the soil capability to sustain the functioning of the ecosystems. The aim of this work was to evaluate the temporal variations of Technosols quality after a single addition of green compost in a mesocosm trial. The compost (20 t ha<sup>-1</sup>) was added to quicken the soil evolution and to stimulate the edaphic community. In 2006, 5 vessels (each with area:  $16m^2$ , depth: 2 m) were filled with Andosols. Successively, they were mixed with cracked building rubble and, as result of this, defined as Ekranik Technosols. In 2010, two weeks before the single addition of green compost, surface soil (0-10 cm depth) sampling was performed (pre-treatment); in addition, in 2011 and 2018 (one and eight years after the compost addition, respectively) soils were also sampled. The soil quality was assessed through the evaluation of a synthetic index (SQI) taking into account: pH, water content, organic matter, total and available contents of Cr, Cu, Ni, Pb, Zn, Fe, K, Mg, Mn, N and Na, C/N ratio, microbial biomass ( $C_{mic}$ ), total and active fungal biomass, soil respiration (Resp), metabolic quotient (qCO<sub>2</sub>) and endogenous mineralization coefficient (CEM).

The SQI showed values that reflected a good starting Technosols quality at the pre-treatment (0.65  $\pm$  0.02 SE). The soil quality improved one year after the compost addition (0.72  $\pm$  0.03 SE) and decreased eight years later (0.54  $\pm$  0.02 SE). The temporal trend of the SQI would seem to be due to the increases of pH, C/N, Cmic, Resp, qCO<sub>2</sub> and CEM at short term and to the decreases of water and organic matter contents, total and active fungal biomass at long term. The compost addition induced a variation of composition and activity in edaphic community: in fact, at long term the treatment induced the decrease of fungi than bacteria and a fast rate of organic matter mineralization. In addition, total and available contents of the investigated elements increased at long term. However, a single application of green compost would seem to be not enough to maintain or increase Technosols quality.

# Soil fertility and aggregate associated carbon and nitrogen with biochar amendment in relation to localized soil fertilization practice after a four-year intensive vegetable production in Burkina Faso

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Biochar has been identified to enhance soil fertility and soil carbon sequestration but soil aggregate C and N dynamics with biochar addition in relation to traditional soil fertility management practice has not yet been thoroughly assessed. Four replicates of four treatments; control (CTL), Urea and cattle fertilizer (UCF), corn cob biochar (CCB), and UCF+CCB, were arranged in randomized complete block design, following a four-year rotation of eleven vegetable crops on actual producing farmers field. We tested the hypotheses that the effect of combined application of UCF+CCB treatments on aggregate C and N storage would be additive of the corresponding effects of their sole applications. The weakly alkaline Haplic Lixisol, experienced acidification due to applied treatments. Soil C and N stocks were reduced by individual additions of UCF and CCB but increased maximally by UCF+CCB, indicating a relatively additive effect. However, C/N was maximal with CCB than UCF+CCB treatment. Corn cob biochar drastically reduced soil available P by 314%, and also contributed to 27% P reduction by UCF+CCB treatment. The treatments increased Ca<sup>2+</sup> and Na<sup>+</sup>, but significant increase in Mg<sup>2+</sup> concentration was only obtained with UCF+CCB treatment. Aggregate N content amongst the treatments followed similar distribution trend, increasing from 1.00-2.00 mm to <0.25 mm aggregates with maximum and minimum N content by UCF+CCB and CCB treatments, respectively. Accordingly, the micro aggregates (> 0.25 and <0.25mm) stored more N than the macro aggregates (> 2.00 and 1.00-2.00mm) amongst the treatments. The effect of UCF+CCB treatment on aggregate N content was rather synergistic since N content amongst the aggregate fractions of CTL and UCF were statistically similar. Decrease in C and C/N distributions with decreased aggregate size fractions by CCB indicates more C in macro aggregates due to high aggregate C/N and associated reduced decomposition. So, while there are more C in micro aggregates with UCF, UCF+CCB and CTL treatments, the UCF+CCB combine moderated aggregate C/N and enhanced release and retention of the highest C and N nutrients across aggregate fractions compared to their sole applications. The soil characterization results reveal maximum improvement and retention of C, N, Ca, Mg and Na with UCF+CCB treatment. Although, UCF+CCB effect on C and N storage of the whole soil was additive, a rather synergistic effect exists with hierarchical aggregate N but not aggregate C.

#### Ozonization of fermented municipal biowaste to produce value added products

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Soluble biobased lignin-like polymeric substances (SBO) isolated from the alkaline hydrolysates of compost, digestate and municipal biowaste (www.biochemenergy.it) have been reported as a promising and competitive source of biopolymers and biobased chemical auxiliaries. They have been tested in several diverse processes, e. g. as emulsifiers and surfactants in detergents, textile dyeing baths, flocculants, auxiliaries for soil and water remediation, nanostructured materials for chemical and biochemical catalysis, biopolymers, soil fertilizers and plant biostimulants for agriculture. These results offer the perspective to convert a municipal biowaste treatment plant into a biorefinery, integrating biochemical and chemical technology to produce biomethane and valued added bio-based chemicals.

To upgrade processes, properties and uses for the above SBO, a low temperature oxidation through ozonisation has been developed.

The anaerobic digestate and compost of a municipal biowaste treatment plant have been hydrolysed to yield biopolymers. These products are a mix of molecules, most of which have molecular weight ranging from 100 to over 750 kDa. They are multipurpose products for use in the chemical industry by virtue of their molecular weight and surfactants properties. Ozonization of the biopolymers changes significantly the molecular weight distribution and properties of the pristine biopolymers, yelding a product with improved surfactant properties and a higher ratio of small molecules. They are new products with no commercial counterparts, while the small molecules are the biobased counterpart of commercial chemicals obtained from fossil sources.

These results prospect the development of a biobased chemical industry built on the integration of biochemical and chemical technologies to convert municipal biowaste to value added products.

# Sorption-desorption of the fungicide metalaxyl-M onto a silty soil not amended and amended with biochar and vermicompost

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Agro-industrial waste biomasses can be recycled using traditional and modern technologies. The carbonrich materials obtained, used as soil amendments, enable carbon sequestration, reducing greenhouse gases emission. Among these products, there are biochar (BC) and vermicompost (VC) which in soil interact with organic compounds, including pesticides. We investigated quantitative aspects of sorption-desorption of the fungicide metalaxyl-M onto an unamended silty soil, two BCs from grapevine pruning residues (BC-G) and red spruce wood (BC-S), two VCs produced vermicomposting digestates from a mixed feedstock (VC-M) and buffalo manure (VC-B), and the soil amended with each material at a dose of 2% (w/w). Adsorption kinetics and sorption-desorption isotherms were performed with the batch equilibration method using a metalaxyl concentration of 2 mg L<sup>-1</sup> for kinetic studies and a concentration range of 1-20 mg L<sup>-1</sup> for isotherms. Analysis of metalaxyl-M in solution was performed using HPLC and UV diode array detector. Kinetics data were fitted to pseudo-first order and pseudo-second order models. On all the adsorbents, the steady-state equilibrium was rapidly reached (few hours) following preferentially a pseudo-secondorder model, that indicates the formation of chemical bonds between the product and any adsorbent. Sorption isotherms data were interpreted with the empirical Freundlich and Langmuir equations. The adsorption of metalaxyl-M on BCs and VCs fitted better the Freundlich model (1/n < 1, L shaped), whereas on all soil samples it occurred according to a linear model. The Freundlich sorption constants obtained for BCs (890 L Kg<sup>-1</sup>, averagely) and VCs (54 L Kg<sup>-1</sup>, averagely) indicate that both these materials have a high capacity to retain metalaxyl-M. The value of the distribution coefficient, Kd, of the unamended soil (1.3 L Kg<sup>-1</sup>) increased significantly when the soil was treated with BCs (3.5 L Kg<sup>-1</sup>, averagely) or VCs (2.4 L Kg<sup>-1</sup>, averagely). Freundlich desorption constants of all soil samples (unamended and amended with each material) were all higher than the corresponding sorption constants, demonstrating that the release of the product from soil occurred to lesser extent and rate than adsorption. Overall, our findings suggest that the addition of BC or VC in soil increases its capacity to retain pesticides even at low hydrophobicity, like metalaxyl-M, thus avoiding or reducing the risk of leaching in soil and contaminate natural water bodies.

#### Which is the biochar most effective in mitigating pollution due to Lumax°?

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Pesticide residues are frequently detected in soil, water, and air, and there is a growing concern for their toxic side effects to environment and non-target organisms. Lumax, composed of S-metolachlor, terbuthylazine, and mesotrione, is a pre-emergence selective herbicide used for maize. Terbuthylazine and metolachlor have been widely found in groundwater at concentrations that often exceed the limit imposed by legislation. Mesotrione belongs to triketone herbicides designed to be eco-friendly although the mechanisms controlling their environmental fate as well as their toxicity and impact on ecosystems are still under investigation. New strategies are continuously put in act to restore the agricultural environment from adverse effects of agrochemicals, including the biochar soil amendment. Biochar is a carbon rich by-product obtained by pyrolysis of biomass at high temperature in absence of oxygen. It is able to adsorb the organic contaminants, therefore useful in the soil and water remediation by removing or reducing the pesticide contamination. The biochar adsorption efficiency is due to its physico-chemical properties, which in turn depend on raw materials and pyrolysis process variables such as temperature and heating time. Therefore, the sorption capacity of biochars, obtained from coppiced hardwoods at two different pyrolysis temperatures, for the Lumax<sup>®</sup> active ingredients was investigated to identify the pyrolysis conditions to obtain the fitting biochar in the case of pollution due to the Lumax<sup>®</sup>. In addition, the same study was repeated on each Lumax<sup>®</sup> active component to check both the sorption features of each individual component on two biochars and if the co-presence of all three active ingredients of Lumax<sup>®</sup> could influence the single component sorption behaviour. Our results showed that the biochar performances depend on both physico-chemicals properties of biochar and pollutants, in every case the biochar obtained at high temperature can be considered the most effective, likely due to its high aromaticity, in mitigating pollution due to Lumax<sup>®</sup>.

#### Effects of biodegradable plastic on soil functionality

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Biodegradable mulch films represent an eco-friendly alternative to the conventional poly-ethylene (PE) films. They do not need to be removed from soil and disposed at the end of the crop cycle, but they can be mineralized in soil thanks to their biodegradability (in accordance with the main standards), leading to reduced environmental impact and management costs.

This study aims to assess the effects of biodegradable plastic on soil functionality through the measurement of some of the most important soil quality indicators (basal respiration, microbial biomass carbon and nitrogen, water extractable organic carbon, mineral nitrogen, alkaline phosphomonoesterase activity and potential nitrification).

Soil incubation, carried out in controlled conditions of temperature and humidity, compared different doses of biodegradable plastic and pure cellulose; the soil without any plastic or cellulose addition was included in the experiment as a control. Mater-Bi<sup>®</sup> material for producing mulch films, supplied by Novamont S.p.A., is the biodegradable plastic used in this experiment, while cellulose represents the common reference in this type of laboratory test. The doses chosen for the biodegradable plastic were 10, 100, 1,000 and 10,000 mg/kg of dry soil for the cellulose. Three replicates for each treatment were prepared. The incubation is planned to last for one year and the analysis are performed at pre-established times (0, 2, 4, 8, 16, 24, 32, 48 weeks).

Outcomes collected in the early eight months showed that both the highest doses of biodegradable plastic and cellulose (10,000 mg/kg of dry soil) exhibited an increase of basal respiration. These treatments showed a remarkable increase of microbial biomass N and C after the early two months of incubation, and a parallel increase in N immobilization was observed during the same period. Alkaline phosphomonoesterase activity and potential nitrification were higher in these treatments with respect to the control. Mater-Bi<sup>°</sup> bioplastic demonstrated to have a slower biodegradation than cellulose and not significant changes on soil functionality were recorded.

#### Commercial humic products: do they act as activators of soil microbial activity?

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Commercial humates, widely used in agriculture in many countries, are often presented as enhancers of crop production. It is usually assumed that they are also enhancers of the soil microbial activity: such an enhancement should be partly responsible for the improved crop production, for it implies enhanced N and P mineralization, and in an overall sense an enhanced functioning of soil as an ecosystem. Nevertheless, whereas the hormone-like activities of these humates has been extensively verified (direct effect on roots and plant cells), their enhancing effects on soil microbial activity have been less verified. Our study investigates how the soil microbial activity is enhanced or inhibited by the addition of humates, taking into account two main factors: (i) the origin of this humate (uncomposted sheep manure, leonardite, farm compost, and a pine forest OH horizon), and (ii) how this humate has been obtained, either at room temperature (as usual in scientific studies), or at high temperature (as usual at industrial scale). These humates were applied to soil samples, in an incubation experiment under optimal temperature and soil humidity (25°C, 60% WHC). As a reference, an additional C source was applied (glucose), and, of course, control soils without any addition of humates. Soil respiration was monitored throughout. Not all added products had a stimulatory effect on soil microbial activity. Glucose had the highest positive effect. On the contrary, the humates derived from the forest OH horizon had an inhibitory effect. In some cases, an initial stimulation but a further inhibition is observed (e.g. manure-derived humates obtained at low temperature). In humates derived from manure, compost or a forest OH horizon, the humate obtained by boiling with alkali had a better effect (enhancer of soil microbial activity) than that obtained at room temperature. For leonardite-derived humates, the opposite was found: the humate obtained at room temperature had a positive, stimulatory effect, whereas that obtained by boiling with alkali had an inhibitory effect.

Even though more studies are needed to explore the effects of a wide variety of humates, our results show that as a general rule humates can not be assumed to enhance soil microbial activity. The effects depend on the origin of the humate, and its obtention method.







# Session IV: Plant responses to natural and human-induced drivers

Oral presentations

# **Invited Speaker**

#### Improving nutrient acquisition by plants from the soil

#### Philip J. White

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For crop production to keep pace with human demand, there is an incessant need to increase yields per hectare of land. Thus, the gap between the actual and potential yields of crops must be reduced continuously. The greatest contribution to yield gaps is often inadequate irrigation or crop nutrition. In low-input agricultural systems, the availability of N, P, K and S often limits crop production. In addition, restricted phytoavailability of Fe, Zn, Mn and Cu can limit crop production on alkaline and calcareous soils, whilst P, K, Ca, Mg and Mo deficiencies, together with proton, Al and Mn toxicities, limit crop production on acid soils. Such limitations can be addressed either by management strategies that improve soils, optimise soil pH and increase the phytoavailability of nutrients, or by the cultivation of crop genotypes with greater tolerance of imperfect conditions. There is also an imperative to develop crops that use inputs, such as water and fertilisers, with greater efficiency to reduce economic and environmental costs. Since mineral nutrients are generally acquired from the soil by the root system, the development of crop genotypes with traits that improve nutrient acquisition by roots should not only increase yields on infertile soils but also lead to greater resource use efficiency in high-input agricultural systems. This presentation will examine plant traits that are likely to improve the acquisition of mineral nutrients and the search for a genetic understanding of these traits that might be utilised in developing molecular markers for crop breeding programmes. It will illustrate that, although the efficient acquisition of a particular nutrient might require a specific set of plant traits, suites of traits can be identified that benefit the acquisition of a group of mineral nutrients. One group of mineral nutrients that share beneficial plant traits are macronutrients subject to leaching, such as N and S; another group are macronutrients with limited movement in the soil, such as P and K; and, a third group are the micronutrients Fe, Zn, Cu, Mn and Ni, which are present at low concentrations in the soil solution, respond similarly to soil chemistry, and share common uptake pathways in plants. It is argued that breeding crops for a limited number of distinct ideotypes, addressing particular combinations of nutritional constraints, could be pursued advantageously.

# Metabolic changes induced by *Cuscuta campestris* Yunck. on the host species *Artemisia campestris* subsp. *variabilis* (ten.) Greuter as a strategy to increase the parasitization success

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*Cuscuta campestris* is a holoparasitic species, which parasitizes wild and crop species causing yield and economic losses. Among them, *Artemisia campestris subsp. variabilis*, a species of a great pharmaceutical and nutraceutical interest, is one of the most affected.

Although some information is available concerning host recognition and anatomical alterations induced to the host plants, no data are reported on Cuscuta-*A. campestris* interaction mechanisms and on the effects induced on primary and secondary metabolism of the host plants after the parasitization.

In the present study, the composition of volatile organic compounds (VOCs), involved in the host-parasite interaction, and the changes induced on primary metabolism, as well as on several physiological parameters of the host species, have been deeply studied.

The VOCs analysis highlighted a reduction of molecules involved in plant defense and cuscuta parasitization. In particular, a reduction in sequiterpenes content, important for plant defense against cuscuta, was observed. On the contrary, the monoterpenes profile, molecules involved in the host recognition, was not affected by the parasitization. Finally, cuscuta inhibited the 3-hexen-1-ol acetate production, a secondary metabolite with a repellent activity against cuscuta.

At physiological level, cuscuta parasitization altered the photosynthetic machinery of the host inducing alterations of several parameters connected to photosystem II (PSII).

Moreover, significant changes in leaf osmotic potential, in hormonal and protein content as well as alterations in fresh and dry biomass were observed.

In addition, metabolomic analysis highlighted that parasitization significantly altered the amino acidic and glycogen metabolism strongly increasing the osmoprotectants production, which generally accumulate in plants as strategy defense from oxidative stress.

Finally, a significant accumulation of two sugars trehalose and 2-deoxygucose in the parasitized plants was observed. These sugars are known to interfere with the cell wall polysaccharides of cuscuta vines tip causing necrosis and reducing the parasitization process.

Concluding, we hypothesize that cuscuta parasitization induces an increase of internal plant defenses (primary metabolites fundamental for plant survival) at the expense of external ones (secondary metabolites) in the host species *Artemisia*, limiting plant defense against further parasitization.

#### Hormone responses to UV-B irradiation: what happens in leaves and roots of tomato plants?

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UV-B radiation influences many aspects of plant physiology and biochemistry through a signalling route triggered by UV-B perception by the specific photoreceptor UVR8. Within the complex network of signals interacting with the UVR8 pathway, hormones are involved in both photomorphogenic and stress responses to UV-B<sup>[1]</sup>. Most studies, however, investigated only the behaviour of the above-ground organs directly reached by UV-B, despite this radiation influences root morphology as well.

Based on these premises, the present research aimed to understand whether, and how, low doses of UV-B radiation influenced the hormonal balance not only at leaf level, but also in the roots. To this aim, the level of stress-related hormones (ethylene, ET; abscisic acid, ABA; jasmonate, JA; salicylic acid, SA), and of the growth-promoting hormone indoleacetic acid (IAA), were quantified in leaves and roots of Micro-Tom tomato (Solanum lycopersicum L.) plants subjected to UV-B irradiation (1.19 KJ/m<sup>2</sup> per day, 15 min/day) and harvested after 8 (UVB<sub>8</sub>) and 11 (UVB<sub>11</sub>) days of treatment and after 3 days of recovery (UVB<sub>11+3</sub>). Hydrogen peroxide accumulation, phenolic and flavonoid content of leaves and roots and the profile of leaf photosynthetic pigments were checked to ensure that UV-B dose did not induce oxidative stress, potentially overlapping with the specific UVR8-mediated response.

ET emission decreased in UV-B-treated leaves, confirming that its production is repressed by photomorphogenic UV-B levels<sup>[1]</sup>. Such an effect was not evident in the roots, suggesting that low UV-B doses probably influence ET biosynthesis only in the organs directly exposed to the radiation. Consistent with the ET influence on auxin synthesis and distribution<sup>[2]</sup>, a marked reduction in IAA levels was detected in UVB<sub>11</sub> and UVB<sub>11+3</sub> leaves. Moreover, IAA decreased in UVB<sub>11+3</sub> roots, probably due to a reduced basipetal transport consequent to the lower leaf production, though inhibition of root biosynthesis could not be excluded.

SA markedly increased in leaves and roots of  $UVB_8$  samples, followed by a significant decrease below (leaf) or to the control levels (root) thereafter, while ABA showed only slight fluctuations and JA was below the detection limit, indicating that tomato plants acclimated to low UV-B doses, probably activating the UVR8-mediated responses rather than the stress signalling pathway.

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#### Omeprazole enhances NUE through increased nitrogen uptake and assimilation in corn

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In the last decades, the remarkable growth in the world's population has increased agricultural demand leading farmers to depend more on the use of synthetic nitrogen (N) fertilizers. The improvement of nitrogen use efficiency (NUE) could represent a sustainable method in agriculture to limit the need for chemical fertilizers, thus reducing the consequent environmental degradation and pollution [1]. Omeprazole (OP) is a member of a family of substituted benzimidazoles that act as proton pump inhibitors (PPIs) in mammals. It has previously been shown to act at micromolar concentrations in tomato as an enhancer of growth [2] and salinity tolerance [3,4]. In our study, we used maize plants (p1619 line) grown for four weeks in hydroponics with two modified Hoagland's solutions containing 1mM NO<sub>3</sub><sup>-</sup> (Low N) or 10mM NO<sub>3</sub> (High N) to test the effect of OP on NUE. At 14 days after germination, when seed reserves became insufficient to meet demand for nitrogen (N), OP 1µM was added. Under Low N, maize plants treated with OP showed lower growth limitations than control plantsin terms of fresh and dry weight both in leaves and roots as well as leaf area. OP was able to promote the mobilization of the N resources already present in the shoot tissues to synthesize amides to transport to root, by promoting protein catabolism and the reorganization of amino acids. OP treatment considerably decreased nitrate content in the roots compared to the controls, by increasing the rate of assimilation in these tissues. Starch and sucrose increased both in leaves and in roots of treated plants. Our results indicate that OP enhances NUE by improving nitrogen assimilation, but its targets are still unclear. Understanding how to regulate these processes is important to enhance NUE and afterwards developing sustainable crops with lower environmental impacts.

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# Defining the active biostimulant fractions of a plant-derived protein hydrolysate using an integrated molecular fractionation and metabolomics approach

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Plant biostimulants are compounds having a positive effect on different parameters related to crop yield, nutrients use efficiency and tolerance to environmental stress. Among them, foliar protein hydrolysates (PH) have been reported to increase growth, yield and fruit quality of agricultural crops, as well as to act as plant growth regulators likely due to the presence of specific peptides. Interestingly, some bioactive plant peptides have been reported to have phytohormone-like activities. In fact, previous studies from our group highlighted that the plant-derived protein hydrolysate "Trainer" regulates nitrogen uptake and elicits an auxin- and gibberellin-like activity (Colla et al., 2014).

In order to better elucidate the mode of action of this PH, a fractionation was performed by using a 0.5-1 kDa dialysis membrane, and then fractions were tested on tomato plants for *in vitro* hormone-like activities. Thereafter, an untargeted metabolomic approach using a hybrid quadrupole-time-of-flight mass spectrometer coupled to an UHPLC chromatographic system (UHPLC-ESI/QTOF-MS) was carried out. Statistical analysis allowed to discern the pathways and processes elicitated by the PH and a selected PH fraction. Over 3000 compounds were putatively annotated, of which 361 were identified as discriminants and upload to a pathway tool software from Plantcyc. Secondary metabolism was the most involved process, since over 160 related metabolites were affected. However, the pathways involved depended on the treatment. Hormones, alkaloids, glucosinolates and some phenylpropanoids were some of the discriminant compounds between treatments. Interestingly, our results showed a similar effect on plant metabolic reprogramming between the PH fraction and the synthetic auxin positive control (IBA).

#### Proteomic changes in the roots of three grapevine rootstocks in response to nitrate availability

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Although the biochemical mechanisms involved in nitrate assimilation are overall known, especially in herbaceous species, specific information on perennial plants, such as grapevine, remain yet incomplete. Few studies have investigated the root responses to nitrate availability in this species. Considering that grafting practice is widely used in viticulture, the role of rootstock in nitrogen metabolism as well as its effects on scion must be better characterized. In this view, the responses of roots to nitrogen availability, as well as to its fluctuations in the soil, are among the main factors that influence plant growth and productivity from both quantitative and qualitative point of view.

The aim of this study was to analyze the metabolic events occurring in the roots of three grapevine rootstocks, M3, M4 and 1103P, in responses to the addition of 10 mM nitrate, after a period in which the plants were grown in absence of nitrogen.

Firstly, the changes of some biochemical parameters (such as nitrate, sugar and amino acid contents) as well as the evaluation of abundances of nitrate reductase and glutamine synthetases by Western blot analyses were used to define the time course of the induction of nitrogen metabolism in roots. Taken together, the results indicated that in all genotypes the nitrogen metabolism significantly increased after 30 h from the addition of nitrate. Moreover, at this time the comparison among the three genotypes revealed a greater assimilative capacity in 1103P.

To gain a better characterization of the biochemical responses in roots, a proteomic comparison among the three genotypes, in control condition and after 30 h of induction, was conducted. Proteomic analyses were performed by GeLC-MS/MS, a technique by which proteins are purified by SDS-PAGE, in-gel digested, and then identified and quantified by mass spectrometry. Functional classification was made according to the bin hierarchical tree developed by MapMan ontology. Only proteins showing at least a fold change of 40% in abundance (Student's t-test, p < 0.05) were considered significantly affected by the treatment. This approach allowed the identification of some hundreds of proteins, with high reliability and good reproducibility, revealing in all genotypes changes of proteins/enzymes involved in nitrogen assimilation as well as in metabolic pathways useful to sustain the requests of carbon skeletons and of energy.

# Identification of an isoflavonoid transporter required for the nodule establishment of the *Rhizobium-Fabaceae* symbiotic interaction

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Nitrogen (N) is a key nutrient determining crop productivity, but its soil availability is frequently inadequate for plant growth. Legumes have developed a strategy to overcome this limitation by forming a symbiotic relationship with N-fixing soil bacteria called rhizobia and are thus able to grow in soils with low N contents and without any supply of artificial fertilizers. The legume-rhizobial symbiosis starts with a signal exchange between the host plant and its microsymbiont. Root released isoflavonoids are signaling molecules perceived by compatible bacteria. Subsequently bacteria release nod factors, which induce signaling cascades that allow the bacteria to enter the plant root without inducing a defense reaction, finally leading to the formation of functional N-fixing nodules.

Here we report the identification and functional characterization of a plasma membrane localized MATEtype transporter (LaMATE2) involved in the release of genistein from white lupin roots. The gene encoding this transporter is overexpressed in the root in condition of low N as well as low phosphate availability, two nutritional deficiencies that induce the release of this isoflavonoid. Transport assays in yeast vesicles demonstrated that LaMATE2 acts as a proton-driven isoflavonoid exporter. Silencing of *LaMATE2* drastically reduced genistein efflux and even more the formation of symbiotic nodules in silenced plants, supporting the crucial role of LaMATE2 in isoflavonoid root release and nodulation.

In conclusion, we present the identification of the long sought-after isoflavonoid transporter needed to establish a N-fixing symbiotic nodule.







# Session IV: Plant responses to natural and human-induced drivers

Posters

#### Characterization of heavy metal pollution in Rome, Italy

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Urban vegetation contributes to human health and well-being through a multitude of services, and atmospheric pollution is one of the most important public health problems in cities, especially in densely populated urban areas. Particles in the atmosphere are usually associated with metals in airborne dust generated by traffic or industrial activity. The city of Rome is a highly urbanized area where extensive public and private transportation are utilized. Urban soils are affected by atmospheric deposition of heavy metals, found in PM10 and PM2.5. Those PM have the same size of silt and clay and have a strict relationships with the cation exchanging complex; as charged particles, clay bind the heavy metals. Further, they are a key marker since they are not degradable by natural processes. Portable X-ray fluorescence (pXRF) is a fast, accurate, non-destructive method to investigate the metal concentration in different matrices with implications for environmental quality assessment. Previous studies have just begun to investigate the use of pXRF for heavy metal characterization; certain elements have shown strong correlation with traditional laboratory characterization. The aim of this research is to assess the heavy metal pollution in soil and vegetation, specifically the metal accumulation capability of plants. *Platanus acerifolia* (Aiton) Willd was introduced in Rome in the late 1800s and is widely distributed in parks, gardens and avenues. Measurements of soil and leaf metal concentration were conducted June 2019 at different sites in Rome: high traffic density sites (P sites) and parks (C sites). This type of investigation is a fast and accurate way to help city planners, researchers, and other stakeholders assess the bioremediation potential of natural or semi natural plants in urban settings.

# Variation in metabolite production and physiological responses of *Zea maysL*. plants in response to application of commercial lignohumates

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Conventional agronomical techniques that employ excess of mineral fertilizers to meet high crop yield demandshave long adversely impacted on soil properties and pose environment-related concerns. The decline of soil quality is normally accompanied by the decrease of soil fertility, reduced content of soil organic carbon and impoverishment of microbial biodiversity. Therefore, new strategies are envisioned in support of sustainable crop productions. Among them is the use of biostimulant products, including humic substances, to reduce nutritional inputs from fertilizers in agriculture. Humic substances or humates represent the most stable and recalcitrant component of soil organic matter and originate from the chemical and microbial degradation of vegetal and animal deposits. They are pivotal components of soils because they markedly improve soil quality. Also, they positively influence several aspects of plant metabolism and root morphological traits, by interacting either directly or indirectly with an array of biochemical and physical processes occurring at the plant-soil interface. In this study, seven humates (Hs) including five lignosulfonates (LS1-5), one commercial leonardite-humate (PH) and one commercial lignosulfonate (LH), were analyzed for their carbon, nitrogen and sulfur contents, and the distribution of functional groups using Fourier Transform Infrared (FTIR) and Raman spectroscopies. Hs were further supplied for 2 days to Zea mays L. in hydroponics at 1 mg C L-1 to test their capacityto triggerchanges in physiological target-responses. LS1, LS2, LS3 and LS5 determined the most pronounced effects on plant growth and accumulation of nitrogen metabolites (proteins and phenolics), perhaps because of their chemical and spectroscopic features. Root growth was more increased than leaf growth. This effect was ascribed to higher stimulation of N metabolism in roots according to the increased activity of N-assimilation enzymes. Hs enhanced photosynthesis, as suggested by increased values of RuBisCOactivity, SPAD index and leaf sugar accumulation. In conclusion, the current study provides clear evidence that lignohumates produced through industrial processes could be used as biostimulants during crop cultivation, being really effective in promoting the performance of plants with respect to growth, carbon and nitrogen metabolic pathways.

### Effects of inorganic and organic P availability on N fixing capacity of Vicia villosa

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Cover crops offer various ecosystem services that contribute to the sustainability of intensive cropping systems. In particular, leguminous cover crops may represent an important source of nitrogen (N) for the following income crop. Through the symbiotic association between plant roots and N<sub>2</sub> fixing bacteria, biological N fixation (BNF) may contribute as much as 65-95% of the total plant N. However, BNF requires considerable amounts of energy in the form of ATP, and its efficiency could therefore depend on soil phosphorus (P) availability. Apart from the uptake of readily available inorganic P (Pi), leguminous plants can favour the hydrolysis and P availability from organic P sources (Po), through the production of phosphatase enzymes in order to overcome P deficiencies. However, there is scant information on the possible limiting effect of low P availability on BNF, and it is still unclear if, and to what extent, the dominant form of P in soil (Po vs Pi) can affect BNF. The aim of this work was to understand how the availability and forms of soil P can affect the production of biomass and BNF efficiency of a typical cover crop (*Vicia villosa*). Vetch plants were cultivated in mesocosms utilizing a P-poor agricultural soil at three P addition levels (no P: control; low P: +50 mg P kg<sup>-1</sup>; high P: +200 mg P kg<sup>-1</sup>) applied in form of inorganic (KH<sub>2</sub>PO<sub>4</sub>) or organic P (inositol hexaphosphate). Soils and plants were destructively sampled after 30, 50 and 70 days. Above and below ground biomass production, the total amount of nodules, as well as C, N and P contents were quantified. BNF efficiency was evaluated by isotope dilution after application of <sup>15</sup>NO<sub>3</sub><sup>-</sup>. Soil inorganic N forms and available P were also determined and the microbial activity in the rhizosphere has been studied.

Results evidenced that above ground biomass was proportional to the amounts of Pi applied and, hence, to Pi availability. With adequate Pi supply, the shoot/root ratio is the highest, suggesting that the plant allocated less C and other nutritional resources below ground for soil exploration for nutrient acquisition by developing an extended rooting system. Plants receiving organic P, conversely, needed a longer time to increase P availability with the progressive hydrolysis of organic P sources, requiring a greater investment of plant resources.

#### Characterization of the alkaloid hordenine and its precursors in roots of a modern barley cultivar

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Barley (*Hordeum vulgare* L.) is one of the most important crops in the world and it has been well known along the centuries as being able to contrast both crops and weeds. Its allelopathic ability consists in the synthesis and release of allelochemicals. In particular, the alkaloids gramine and hordenine have been identified as one of the main responsible of this ability in barley. Hordenine is synthetized starting from the amino acids phenylalanine and tyrosine. The metabolic pathway passes through the synthesis of tyramine, which is then methylated twice obtaining N-methyltyramine and finally hordenine. Although the complete metabolic pathway of hordenine is known, its synthesis within different plant tissues, its possible translocation between leaves and roots and time and/or stress dependent synthesis are still not well characterized in barley.

Thus, this study focuses on the biosynthetic, metabolomics and allelopathic aspects of hordenine in roots in modern the barley cultivar Solist. Hordenine and its precursors tyramine and N-methyltyramine content has been monitored in roots of barley seedlings up to 8 days after germination. In addition, barley plants were subjected to different nutrient deficiencies to evaluate the effect of abiotic stress on the biosynthesis pathway. Germination tests with cress (*Lepidium sativum* L.) were also carried out.

This work has shown that the root content of hordenine and its two precursors decrease with seedling age, in particular within the first days after germination. Metabolites synthesis influenced by different nutrient starvation showed that nitrogen deficiency led to the significantly highest concentration of hordenine and N-methyltyramine, followed by iron and sulfur deficiency, while phosphorus deficient barley plants showed similar concentration as the control plants after four days. In addition, no day light dependent diurnal pattern was observed for any of the three metabolites studied. Germination tests with cress revealed a low efficacy of hordenine as allelochemical towards cress seeds.

All the performed analysis has helped improving the knowledge about the biosynthetic patterns of tyramine, N-methyltyramine and hordenine in barley. Further studies are needed to study the influence of other biotic and abiotic stresses on the synthesis of the allelochemicals and the allelopathic effects.

# Ecological variability in 4 wild species of *Lamiaceae* in the Apulia Region: effects on chemical composition and biological activities of the essential oils

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A study was carried out on four taxa of Lamiaceae family (Clinopodium suaveolens, Satureja montana subsp. montana, Thymbra capitata, Salvia fruticosa subsp. thomasii) growth in different areas of the Apulia region. Two different sites were sampled for each species in order to evaluate the effect of the habitat on the composition and bioactivity of their essential oils extracted (EOs). A total of 8 different were sampled, two for each species. The effect of the different ecology on the composition and bioactivity of the essential oils extracted from the studied species was evaluated. The extraction was carried out for 6h by hydrodistillation using Clavenger apparatus. The collected EOs were analyzed using GC-MS for qualitative determination and tested for antimicrobial activity. A total of 57 compounds were identified in the analyzed samples, mostly belonging to the terpenes group. The ecosystem affects the EOs composition in two out of four of the studied species. The main constituents of EOs of S. fruticosa subsp. thomasii, referable to the Oleo sylvestris-Ceratonion siliquae alliance, were Eucalyptolo, Camphor and Camphene; the relative abundance of the two last compounds showed a significant difference (p < 0.05) between the two area of growth. A significant effect of the growth area on the abundance  $\alpha$ -Pinene, Ocimene, Limonene and Thymol was observed in S. montana subsp. montana. This species was detected in two different ecologic contexts related to Cisto cretici-Ericion manipuliflorae alliance. No significant differences were observed on the compounds abundance and pattern for *T. capitata* and *C. suaveolens*, although *T. capitata* has been surveyed in different ecological contexts always related to Cisto cretici-Ericion manipuliflorae syntaxon. The lowest compositional complexity of EOs was observed in C. suaveolens (10 compounds represented more than 99.1% of total chromatogram area). The results of the antimicrobial activity confirmed the differences observed in composition analysis. The EOs of *C. suaveolens* resulted the more effective on molds whereas that of *T. capitata* showed the best antibacterial activity.

#### The impact of selective and non-selective herbicides on the metabolism of tomato plants

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Herbicide are agrochemicals commonly used in agriculture. Although the main purpose of herbicides is the control of weeds, recent evidence highlighted possible effects on plant metabolism affecting therefore fruit quality, following its application. However, most of the studies about herbicides have focused on their behavior in relation to absorption/desorption, conduct in the environ or risk to human health rather than the plant physiological effect. Due to the fact that herbicides might represent a stress toward crop plants and therefore towards industrial final products, there is an interest in understanding the effect of these chemical products on plant metabolism. Taken into account all above mentioned, the goal of this work was to test the impact of four different herbicides on metabolic processes in industrial tomatoes. According to this aim, tomato plants were grown and subsequently treated either with selective herbicides (metribuzin and rimsulfuron) and non-selective herbicides (glyphosate and pelargonic acid). Thereafter, leaves were collected and an Untargeted screening of metabolites were carried out by using a hybrid quadrupole-timeof-flight (Q-TOF) mass spectrometer coupled to an UHPLC chromatographic system (UHPLC/Q-TOF). The untargeted metabolomics annotated over 2500 metabolites. 186 significant compounds were selected by multivariate statistics and used to discuss the changes at molecular level. Data interpretation using the Plantyc Pathway Tool Software leaded to a better understanding of the physiological processes involved in the responses to herbicides application. Primary metabolism, mainly amino acid related compounds, was affected. Furthermore, nitrogen-containing secondary metabolites showed significant changes after the treatment. Concerning the effects on berries, our findings showed that the treatments had significant impact on their weight, antioxidant activity, total polyphenol content and percentage of carbon.

#### Sulfur isotope mass balance reveals <sup>32</sup>S/<sup>34</sup>S fractionation during sulfate uptake and translocation in rice

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Four stable isotopes of sulfur exist ( ${}^{32}$ S,  ${}^{33}$ S,  ${}^{34}$ S,  ${}^{36}$ S) whose natural isotopic percentage abundances are 0.94499, 0.0075, 0.0425 and 0.0001 atom fraction, respectively. The most abundant isotopes –  ${}^{32}$ S and  ${}^{34}$ S – are now commonly measured using elemental analyzers coupled with isotope ratio mass spectrometers (EA-IRMS). Such an approach is based on the complete transformation of total S to SO<sub>2</sub>, which is subsequently analyzed by the mass spectrometer with regards to masses 64 ( ${}^{32}$ S<sup>16</sup>O<sub>2</sub>) and 66 ( ${}^{35}$ S<sup>16</sup>O<sub>2</sub>or  ${}^{32}$ S<sup>16</sup>O<sup>18</sup>O) atomic mass units. S stable isotopes have been used to trace the movements of the related compounds in plants, in testing S flux models, and in identifying and determining the impact of natural and anthropogenic S sources on the environment. However, the isotope technique applied for S metabolism investigations, as well as for sulfate transport and allocation within the plants, is limited by our current knowledge of the potential  ${}^{32}$ S/ ${}^{34}$ S isotope discrimination that may occur during both S metabolism and sulfate transport.

The relative <sup>34</sup>S abundance is traditionally quantified using the  $\delta$  value:  $\delta^{34}S = (R_{sample} - R_{standard})/R_{standard}$ , were R is <sup>34</sup>S/<sup>32</sup>S isotope ratio.

The  $\delta^{34}$ S signature of the total biomass produced by a plant generally reflects that of the available sulfate in the soil solution, thus suggesting the fractionation against <sup>34</sup>S during sulfate acquisition negligible. However, a careful analysis of rice plant organs revealed that the  $\delta^{34}$ S-SO<sub>4</sub><sup>2</sup>-was higher in the shoot than in the root, indicating that fractionation occurs during sulfate uptake, allocation and metabolism.

Now we are approaching the topic of  ${}^{32}S/{}^{34}S$  fractionation in rice by investigating the hypothesis that the  $\delta {}^{34}S-SO_4^{-2}$  of the leaves could be determined by the activities of the sulfate transporters involved in sulfate uptake, as well as in root-to-shoot sulfate translocation. The experimental approach will be mainly based on the comparison of the effects of different growing conditions – known to modulate sulfate uptake and/or translocation – on the  $\delta {}^{34}S$  signature of the sulfate ions in the leaves. Results will be related to the relative transcript levels of the sulfate transporter genes involved in sulfate uptake and translocation, in order to obtain a comprehensive picture of the  ${}^{32}S/{}^{34}S$  isotope effects occurring during sulfate distribution within the plant.

# **Variation in phenolic composition and antioxidant properties in leaves and flowers of green and red basil** (*Ocimum basilicum* L.)

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Sweet basil (Ocimum basilicum L., Lamiaceae) is a culinary and medicinal herb, cultivated worldwide and highly appreciated for its organoleptic and antioxidant properties. These properties are partly attributed to a high content of phenolic compounds, such as rosmarinic acid and chicoric acid. Besides the green cultivar 'Italiano Classico' (IC), widely cultivated in the Mediterranean area, sweet basil includes varieties with red leaves and flowers, known as *purpurascens*, that might represent rich sources of highly glycosylated anthocyanins. Among the red varieties, 'Red Rubin' (RR) was proposed as a novel culinary herb, while 'Dark Opal' (DO) is mainly appreciated as ornamental plant. Several studies indicate a high variability in the leaf phenolic composition in Ocimum spp., while to date any investigation has not been conducted in flowers. The purpose of this work was to characterize the antioxidant properties and composition in phenolic acids and flavonoids in leaves and flowers of IC, RR and DO, by colorimetric assays and reverse phase chromatography - tandem mass spectrometry (LC-ESI-MS/MS). This study provided novel molecular information about anthocyanins in leaves, highlighting differences in total contents and composition between the two red varieties. Moreover, the analyses of polyphenolic acids in leaves and flowers revealed qualitative and quantitative differences among all the three varieties. In particular, flowers showed higher antioxidant properties than leaves and a very specific composition. Indeed, this first chemical profiling of basil flowers revealed the presence of interesting bioactive compounds, such as several members of the salvianolic acid family.

Overall, the study provides the basis for future investigations about the physiological roles of phenolic compounds in basil, and for improvements in the use of this important nutraceutical resource.

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# **Effect of microalgal extracts from** *Chlorella vulgaris* **and** *Scenedesmus quadricauda* **on germination of** *Beta vulgaris* **seeds**

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Sugar beet (Beta vulgaris subsp. vulgaris) is a commercially important biennial root crop providing about 20% of the world annual sugar production. Seed quality is crucial for an adequate plant growth and it is strictly associated with the quanti-qualitative production of the crop. Unfortunately, the productivity of sugar beet is often limited by low or irregular germination in the field also due to the presence of inhibitory substances in the pericarp of the seed. In order to improve sugar beet germination process, the effect of different concentration of microalgal extracts from C. vulgaris and S. quadricauda on several indices (i.e. germination percentage GP, mean daily germination MDG, germination index GI, etc.), useful to evaluate the germination performance, was investigated. Moreover, root morphological analysis was performed by using WinRHIZO software and seedling length (SL), root surface area, total number of root tips, root diameter, root volume and lateral root were determined. The two microalgae extracts were firstly characterized by CNS, Fourier transform infrared spectroscopic analysis (FT-IR), and carbon-13 nuclear magnetic resonance (13C NMR). Beta vulgaris seeds were soaked with six different concentrations of the microalgal extracts, considering the quantity of organic carbon (C<sub>ore</sub>) in each extract: 1) Untreated  $= 0 \text{ mg } C_{\text{org}}/\text{L}; 2) \text{ C1} = 0.1 \text{ mg } C_{\text{org}}/\text{L}; 3) \text{ C2} = 1 \text{ mg } C_{\text{org}}/\text{L}; 4) \text{ C3} = 2 \text{ mg } C_{\text{org}}/\text{L}; 5) \text{ C4} = 5 \text{ mg } C_{\text{org}}/\text{L}; 6)$  $C5 = 10 \text{ mg } C_{org}/L$ . Our results show that these microalgal extracts exert a positive effect on sugar beet germination, making more efficient as well as regular this critical process for Beta vulgaris seeds. Among all the treatments the best results, in term of germination indices as well as root morphological traits, were reached by using *C. vulgaris* extract at the concentrations C2 (1 mg  $C_{org}/L$ ) and C3 (2 mg  $C_{org}/L$ ).

# Let the sunshine in! Post-harvest UV-B radiation is able to affect the secondary metabolism in flesh of peach fruit

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The potentiality of UV-B radiation in promoting phenolics accumulation has been elucidated in many fruit and vegetables<sup>1</sup>. Previous studies reported that 10 min and 60 min of UV-B irradiation were effective in stimulating a strong accumulation of phenolics, especially dihydroflavonols, anthocyanins and flavones, in peach skin<sup>2,3</sup>. However, almost the entire relevant literature has considered the UV-B-driven phenolics changes only in the fruit skin, since it represents the outermost tissue and therefore directly exposed to the UV-B radiation. It is also important to point out that most people use to peel the fruit due to the possible presence of harmful chemicals, e.g. pesticides and fungicides, thus they would not benefit from the phenolics enrichment in the skin. In the light of above, this work aimed to figure out whether the UV-B exposure might influence the secondary metabolism within the peach flesh, focusing particularly on phenolic compounds. Based on these considerations, melting flesh yellow peaches (Prunus persica L., cv. Fairtime) were exposed to UV-B radiation (2.31 W m<sup>-2</sup>) for 10 and 60 min, and the flesh was sampled at two different recovering times, 24 and 36 h. Through an -omics approach using a UHPLC-ESI/QTOF-MS technique followed by a fold-change-based multivariate analysis, we were able to find which metabolites were mostly affected by UV-B radiation in the flesh. Phenolics compounds were highly affected by UV-B radiation, showing a slight decrease after 24 h from the irradiation, and an accumulation after 36 h. Since this behaviour reflects what was already observed in the skin, a possible transduction mechanism of the UV-B signal from the skin to the flesh below is likely to occur. Indeed, nowadays, no studies have measured the UV-B transmittance within the peach skin, although it has been previously found that UV transmittance across tomato peel is only about 0.5%. Besides phenolics terpenoids were also highly affected by UV-B radiation, showing a great increase of most terpenoid subclasses, 36 h after the irradiation. In detail carotenoids showed the highest increase among terpenoids after both 24 and 36 h recovery timepoints. These findings highlight the effectiveness of UV-B radiation to increase most of secondary metabolites, not only in the skin, but also in the flesh.

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# The Trehalose-6-phosphate/SnRK1 system in the response to saline conditions during germination of two rice (*O. sativa* L., ssp. *japonica*) cultivars with different salt sensitivity

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Soil salinity is extremely harmful for crops, and, among cereals, for rice (*Oryza sativa* L.), with particular regard to the ssp. *japonica*. Seed germination and seedling emergence are among the phenological stages particularly sensitive to this stress condition.

A Genome Wide Association Study (GWAS) with a total of 31.421 SNPs was conducted on a collection of 277 *japonica* rice accessions phenotyped under mid-salinity considering germination kinetic parameters and seedling emergence rate. A few Marker-Trait Associations were identified on the basis of significant genotype-phenotype association analysis. Among the genes putatively involved in the salt response, two were particularly interesting: *OsTPP7* (chromosome 9) and *OsTPP10* (chromosome 7), both belonging to the family encoding Trehalose-6-Phosphate Phosphatase (TPP) catalyzing the dephosphorylation of Trehalose-6-Phosphate (T6P) to Trehalose.

Salt stress affects carbohydrate production and the mobilization/use of C storage compounds altering the sink-source relationships, sugar allocation and energy metabolism. In this framework, the ratios T6P/Tre, regulated by TPP activity, acts as a signal in the cascade of events that regulate, through sugar metabolism, plant development with particular regard to seed germination and seedling growth. In turn, T6P regulates the activity of Snf1-Related protein Kinase-1 (SnRK1), a metabolic sensor able to regulate  $\alpha$ -amylase and fundamental in maintaining C homeostasis under stress.

Aim of the work was to establish the role of the SnRK1/Tre6P/Tre/Sucrose system and in particular of the *OsTPP7* and *OsTPP10* genes in the tolerance of *japonica* rice to salt stress during seed germination and early seedling development. In two model rice accessions (Olcenengo, tolerant, and SR113, sensitive) with opposite behavior in salt stress, biochemical and molecular analyses were conducted. In particular, have been considered: in the growing embryos, T6P, Tre, and sucrose levels, and *OsTPP7-OsTPP10* gene expression; in the endosperm, the time-course of  $\alpha$ -amylase activity. The results define a picture coherent with the different effects of salt stress in Olcenengo and SR113. Functional characterization of the *OsTPP10* gene and its allele mining analysis within the 277 rice accessions are in progress.

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#### Transmembrane transporters and salt tolerance in temperate *japonica* rice

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Several investigations aimed at identifying molecular tools useful for the selection and/or the constitution of high-yield salt tolerant rice have been successfully carried out, concerning in particular *indica* and/ or tropical rice genotypes. The global warming process is nowadays determining the intrusion of saline wedge into coastal fresh-water streams, and the soil salt concentration of many European rice areas, where temperate rice cultivars are mainly grown, is more and more increasing.

In order to identify molecular markers and/or new *loci* related to salt tolerance, a Genome Wide Association Study (GWAS) has been carried out using a panel of 277 *japonica* rice accessions. The panel has been subjected to Genotyping By Sequencing and phenotyping concerning tolerance to a mild-salt stress soil condition (5 dS m<sup>-1</sup>) expressed at the 4<sup>th</sup>-5<sup>th</sup> leaf developmental stage using the Standard Evaluation Score (SES) proposed by IRRI. On the basis of GWAS, a QTL including a few genes that in the *indica* rice genome are localized within the major salinity tolerance-related QTL 'SalTol' have been identified. Among them, the *Os01g0337500* gene encoding the vacuolar H<sup>+</sup>-pyrophosphatase 6 (*OsOVP6*) is present. Since the role of the OsOVP6 activity is considered central in regulating the cellular Na<sup>+</sup> homeostasis in both roots and leaves, investigations comparing some elements of the complex mechanisms involved in this process have been carried out. A physiological approach evaluating this possibility has been conducted in two *japonica* rice varieties (Galileo and Virgo) that resulted salt-tolerant, in one *japonica* rice variety (PL12) known to be quite salt-susceptible, and in the salt-tolerant *indica* inbred genotype FL478 (containing the 'SalTol' QTL) as reference genotype.

The root and shoot Na<sup>+</sup>/K<sup>+</sup> ratio, Na<sup>+</sup> influx and K<sup>+</sup> efflux, H<sup>+</sup> extrusion activity, cytosolic and vacuolar pH by *in vivo* <sup>31</sup>P-NMR techniques were evaluated in roots of the four rice genotypes. The results obtained, together with the electrophysiological evaluation of the whole root Na<sup>+</sup> conductance, allow to define a picture that may explain the different salt tolerance observed among the rice genotypes analyzed. As a whole, these results confirm the interest towards a deep allele mining analysis, concerning *OsOVP6*, within the most significant members of the *japonica* rice accession panel under investigation.

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#### Root exudates involvement in tomato plants response to low P levels

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Phosphorus (P) is an essential macronutrient required by plants but, in most soils, its concentration can be very low because of abiotic processes (adsorption on mineral surfaces and precipitation as insoluble salts) that immobilize P reducing its mobility and limiting the fertilizers diffusion. Plants have evolved several strategies to exploit localized sources of P to cope with P deficiency, as a higher production of exudates including electron-rich species that can reduce iron oxides and cause the release of P and enzymes like phytases and phosphatases able to hydrolyse organic P-compounds. Also strigolactones (SL), a class of signalling molecules involved in the regulation of plant architecture, response to nutrient availability and establishment of symbiosis with AM-fungi, have been suggested to be involved in plants response to P availability. In the context of the Horizon 2020 TOMRES project, aiming at enhancing tomato plants resilience to combined water and nutrient stress, we are studying the root exudates involvement in plants response to low P conditions. We grew up wild-type (WT) tomato plants and plants with gene for SL synthesis silenced (SL-). After a period of normal nutrient conditions, plants were kept in P stress regimen. Root exudates were then collected and analysed for inorganic P, total C, N and P, organic acids and polyphenols. Plants biomass parameters were measured and then roots and shoots were analysed for total C, N and P content as well. Root architecture was also investigated via X-ray computed tomography (CT). Results highlighted differences in WT and SL- plants biomass, in P distribution between roots and shoots and in exudate composition. In particular, the main diversities were found for organic acids and polyphenols amount and composition. As in soils P is often adsorbed onto iron (Fe) (hydr)oxides surfaces or precipitated with Fe as insoluble salts, the ability of some of the organic acids and polyphenols identified in the exudates to dissolve P-Fe systems and release P either by complexation or reductive dissolution was texted.

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#### Soil quality and fertility in an olive orchard managed for 20 years with differential agronomic systems

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Climate change, in terms of increased temperature and extreme precipitation regimes, will have agricultural consequences due to the interrelations between climate, land and water use, soil degradation and landscape changes. Conservation agriculture offers new chances to mitigate the effects of climate change. In sustainable agro-forestry systems, management practices are able to increase carbon (C) inputs into the soil and possibly reduce GHGs emissions due to some revised field operations (e.g., irrigation techniques, use of recycled water, pest and disease management, fertilization, soil and plant farming systems). Carbon enrichment increases biological activities by improving soil structure, as well as the soil moisture and nutrient contents, that are beneficial to plant growth and production. This study reports results on the effects of changed soil practices of an experimental olive orchard from a conventional management ( $C_{mng}$ : soil tillage, mineral fertilizers, burning of pruning residues) to a sustainable management ( $S_{mng}$ : no-tillage, pruning residues, cover crop retention, and compost application) on soil quality/fertility and soil/plant microbiota. Results show that a 20-year period of  $S_{mng}$  (including C inputs at a mean rate of 8-9 t C ha<sup>-1</sup> year-1) caused increases in soil organic carbon (SOC) from 1.0-1.3% w/w up to 1.7-2.0% in the topsoil, and in soil water retention (up to 40% more) and permeability (from 13 to 160 mm H<sub>2</sub>O day<sup>-1</sup>). The adoption of a correct irrigation management had a key role in the potential role of orchards in C sequestration (soils become from C sources to C sinks) and in the consequent greenhouse effect mitigation. Indeed, compared to dry areas, wetted soils generally had a higher microbial respiration and SOC mineralization, and a faster bacterial C and N turnover. Finally, the  $S_{mng}$  brought beneficial effects on plant yield, that was improved by 30-50%, compared to  $C_{mng}$ , and on the levels of a wide range of plant protective secundary metabolites in the xylem sap. The endogenous C additions and the different irrigation systems also affected the reserves of soil nutrients (N, P, K, Ca, Mg) and CO<sub>2</sub> soil emission. Promoting cost-effective sustainable land use strategies can avoid SOC decline, soil erosion and soil degradation, with consequent benefits in terms of soil quality parameters. This is essential for sustaining and improving yield and quality of olive plants.

#### White lupin response to nitrogen and phosphorous deficiencies

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White lupin (*Lupinus albus* L.) is considered a model plant for studying plant adaptation to P deficiency; in this condition, white lupin plants strongly modifies the root architecture with the formation of bottlebrush like structures, the so-called proteoid or cluster roots. Being a leguminous species, lupin plants are able to establish association with *Bradyrhizobium* sp. (*Lupinus*), but white lupin responses to low N availability has been still hardly investigated. Therefore, we undertake a research to study the response of white lupin plants to N-deficiency and to the double deficiencies (-N-P).

Under P and N deficiencies (-N-P), lupin roots developed pseudo cluster roots with peculiar morphological, physiological and molecular responses. The nutritional stress induced changes in the nodulation of lupin roots, leading -N-P plants to develop several groups of closely spaced nodules along the primary root. Moreover changes in availability of N and P in nutrient solution influenced the elemental composition in lupin tissues of nutrients: N, P, Zn, K, Mn and B.

The real time RT-PCR analyses allowed to monitor the expression values during plant growth under the different nutritional conditions. Sixteen genes involved in N, P and Fe acquisition and regulation were analysed. After 28 days, the expression of genes encoding malate synthase, urea transporter, acid phosphatase was overexpressed in +N-P in comparison to -N-P which expression was higher than in -N+P. Moreover some genes known to be involved in N and Fe acquisition were found to be responsive to P-deficiency (+N-P) and showed a lower expression values when both nutrients were lacking (-N-P), as those coding for PEP carboxylase, asparagine synthetase, proton pump, glutamate synthase and ferric reduction oxidase (FRO).

The better comprehension of physiological and molecular mechanisms undertaken to N and P nutritional pathways in plants will help to find out new strategies to improve nutrients' use efficiency in plants and optimizing the use of fertilizers.

#### Cerium a potential pollutant: effects on growth and metabolism in Lemna minor L.

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Rare earth elements (REEs) are a group of 17 elements, from lanthanum to lutetium, commonly named "lanthanides", plus scandium and yttrium. They share similar chemical properties and accumulate in the same ore deposits. They are widely used for high-technology applications, as well as in agriculture and husbandry. Due to the intense rate of REEs extraction form their ores, increasing levels of such elements have been detected in terrestrial and aqueous environment. REEs effects on ecosystems and human health are underevaluated. Although many data in the literature reported REEs toxicity in plants and animals, these elements are not officially considered as dangerous pollutants and no specific regulations about their environmental levels have so far been establishred. The purpose of this work was to investigate the effects of Cerium (Ce), that is one of the most abundant REEs, in plants commonly used for ecotoxicological studies in order to obtain data about Ce toxicity thresholds. Lemna minor plants, grown under controlled conditions, were treated with millimolar Ce concentrations at pH 5.5 to investigate the effects of such element on plant growth, photosynthetic pigments content and lipid peroxidation levels. Ce ions affected L. minor growth following a biphasic trend, with stimulatory effects at lower concentrations and inhibitory effects at higher concentrations. In plants treated with higher Ce concentrations chlorotic symptoms and lower concentrations of photosynthetic pigments were detected. We also investigated the effect of pH 4.4 on Ce plant responses. An inhibition growth from 7 days of incubation at all tested concentrations was accompanied both by an increase in lipid peroxidation levels at all tested times and concentrations and by an decrease of photosynthetic pigments thus indicating that pH value modulates the plant-Ce interaction and that the toxicity is greater at low pH values. Ce concentration in plant tissues was also determined and detectable Ce levels were found only in plants grown on Ce-supplemented media. No effects on all tested parameters were recorded in plants treated with the same Ca nitrate concentration compared to the control, therefore was excluded nitrate effects. Thus, effects of Ce supply on L. minor are related to its ability to enter in cells and to be accumulated in plant tissues. L. minor is proposed as a tool for biomonitoring of Ce-polluted freshwater.







# Session V: Frontiers in plant and soil sciences

Oral presentations

# **Invited Speaker**

## Humic-nanomaterials hybrids as fertilizer-delivery technology and antioxidants

#### Yiannis Deligiannakis

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Humic Substances (HS) and solid oxides constitute active components of soils, controlling the soil's physical chemistry (redox, pH, water retention) as well as the biovailability of nutrients (metals, electrolytes, organics). HS themselves bear antioxidant capacity which originates from their polyphenolic character. Composites of [HS] with [engineered nanooxides] allow the production of innovative materials which –in principle- can incorporate the properties of the HS and the nanooxide. This can anticipated as a technology which mimics desired aspects of the soils in real environments. The techno economic feasibility of such technology will be analyzed based on case-studies of production of Fe-, P-, Ca, Zn-based materials by industrial scale Flame Spray Pyrolysis (FSP) technology [see, nanomaterials.physics.uoi.gr]. The controlled release of nutrients [Fe, P,] at aquatic environments will be discussed. The rates and amounts of nutrients release can be determined by targeted engineering of [i] the type of HS, [ii] the composition and phase of the nanooxide, [iii] the interface of HS-with the-nanooxide. The effect of [HS] on the dynamics of the [oxide], and the effect of the [oxide]-on-the-antioxidant properties of [HS] will be exemplified.

## Applications of fast field cycling NMR relaxometry in soil science

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Fast field cycling (FFC) NMR relaxometry allows the understanding of the molecular dynamics at the solidliquid interfaces. Therefore, it is a very important technique to reveal the behavior of water (and solutes therein) in soils. Here, it is reported the importance of FFC NMR relaxometry for the understanding of soil erosion and nutrient dynamics. Different case studies are illustrated and a model for the molecular dynamics of soil nutrients is provided.

# Unravelling the stimulatory mechanism of APR - a novel Biostimulant: leveraging transcriptomics and proteomics

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Plant productivity largely depends on the regulation of growth and on its ability to manage abiotic and biotic stresses. Over the last decade, novel approaches to reorient conventional agriculture towards sustainability have been implemented, like the use of biostimulants to modify plant physiology optimizing productivity. Biostimulants promote plant growth, generally at low quantities, without direct nutrients or fertilizer effects. Major classes of biostimulants include microorganisms, humic substances, seaweed/ plant extracts, protein hydrolysates and amino acids. The biostimulant in the current study is a collagen derived protein thermobaric hydrolysate, known as APR developed by ILSA S.p.A. The effects of APR at two dosages (A1/2 & A1) on the physiology of maize seedlings, its root transcriptome and proteome were investigated. Consistent with the characteristic feature of biostimulants, lower dose of APR (A1/2) induced significantly higher root biomass (+20 %) than A1 (12 %).

Transcriptome analysis indicated that 262 and 608 transcripts were differentially expressed in response to APR at concentrations A1/2 and A1, respectively. Quantitative proteomics resulted in identification of 128 and 242 differentially abundant proteins in response to A1/2 and A1, respectively. Comprehensively, transcriptomic analysis suggests the involvement of plant hormones and induction of flavonoid and phenylpropanoid biosynthesis while proteomic analysis reconfirmed the upregulation of phenylpropanoid biosynthesis and indicated that the central metabolic pathways including glycolysis, citrate cycle, pentose phosphate pathway and oxidative phosphorylation were responsive to APR treatment.

Concatenating the inferences, the stimulatory effect observed with APR treatment could involve hormone (especially brassinosteroid) - mediated regulation of several central pathways related to carbohydrate, amino acid metabolism, energy production and biosynthesis of metabolites including phenylpropanoids (coumarin) and flavonoids with direct or indirect impact on plant growth. In addition, induction of glutathione metabolism with phenylpropanoids and flavonoids could also potentially aid the plants in coping with various biotic and abiotic stresses. It was also exemplified in our study that transcriptome and proteome analysis are complementary approaches: both were indispensable to gain a comprehensive understanding of the molecular mechanisms responsive to APR in maize seedlings.

## Classification of soil aggregates using SEM-EDX hyperspectral data analysis

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Soil is a complex system characterized by peculiar chemical, physical and biological properties. Soil characteristics are the results of the attributes of micrometric and submicrometric domains. For this reason, the use of sensitive techniques with high spatial resolution is mandatory. Scanning electron microscopy (SEM) is a very powerful analytical technique for the analysis of morphology and microstructure of the soil and its components. When SEM is coupled with energy dispersive (EDX) or wavelength dispersive (WDX) x-ray detectors, the chemical analysis of the sample can also be performed. The combination of microstructural and chemical data can give information about elemental associations within minerals or soil aggregates, allowing their detailed characterization. Chemical databases are not fully used and scientists often superimpose different chemical maps in order to find correlations among element on the base of different colour scales. However, by elaborating data this way, most of the objective hyperspectral database information is lost and the obtained results rely on the scientist's subjective choice. To overcome this problem, in the present work "Datamuncher gamma", a software recently developed for the analysis of SEM-EDX hyperspectral data, is presented and applied to study soil samples. Datamuncher gamma allows to obtain elemental maps from hyperspectral datasets and to compare the characteristic fluorescence lines of all the elements found in the sample. The visual recognition of particular correlations then allows to identify particular mineral phases and soil features.

Specifically, datamuncher\_gamma was applied for the study of chromium polluted soil aggregates. SEM-EDX analysis were conducted on soil thin sections with a Zeiss  $\Sigma$ igma 300 VP FEG-SEM working at 15 kV and equipped with an Oxford EDX C-Max<sup>N</sup> SDD.

Different element correlations with characteristic ratios were recognised. For example, Si vs Al scatterplots were useful for the identification of different aluminosilicates and a more precise characterization was obtained by comparing the Si signal with the signal of K, Ca and Mg. Chromium was found mostly associated with aggregates having high C/O ratio. Among them, four different types of Cr-aggregates were recognised on the base of Fe/Cr scatterplot.

Such hyperspectral approach using SEM-EDX data could be used to investigate many soil processes regarding metal(loid) pollutants or micronutrients at the microscale.

# Does physical fractionation of SOM pools preserve information about microbial taxa distribution and ecological functions?

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In a previous study (Zaccone et al., Appl. Soil Ecol., 2018), a preliminary evaluation of potential ecological partition of total, bacterial and plant DNA across soil organic matter (SOM) fractions linked to conceptual stabilization mechanisms was provided. Here, we investigated if different ecologically meaningful SOM fractions share the same microbial communities. To test this hypothesis, DNA was recovered from SOM pools that differ in quality and level of physical and chemical protection from decomposition: free SOM located between aggregates (FR), SOM occluded within macro (MA) and microagreggates (MI), and mineral-associated SOM (MIN) (Plaza et al., Soil Biol. Biochem., 2013). Bacterial communities were then assessed by amplification of V3-V4 region of 16S rDNA while fungal communities by amplification of ITS region. NGS was performed by Illumina Miseq platform and the sequences analysis carried out using QIIME2 v2018.6.0.

Bacterial communities showed a clear separation among the different SOM fractions, independently from the kind of the amendment applied to the soil (i.e., biochar, municipal compost). In particular, FR and MIN fractions were dominated by *Firmicutes* (65-75%), followed by *Actinobacteria* (~13%). On the opposite, MA and MI had a lower level of *Firmicutes* (~50%) and higher level of *Actinobacteria* (20-30%). Interestingly, among Bacillales, *Bacillus* and *Brevibacillus* spp. (aerobic) were largely dominant in FR and MIN, whereas the facultative anaerobic Limnochordaceae were more represented in MA and MI, and the anaerobic sporeforming *Clostridium* spp. Mainly in MI. These data clearly depose for a 'selection' of the microbial taxa according to the level of physical and chemical protection of SOM, with O<sub>2</sub> availability as one of the main drivers. Although at a less extent, also fungal population was related to the SOM pools rather than to the amendment, with MIN and FR differing from MA and MI. In particular, *Sordariomycetes* are a fungal class that can represent a proxy of SOM evolution.

In conclusion, (i) the physical fractionation method caused no mixing over of bacterial DNA through all SOM pools, and (ii) different microbial taxa seem to be specifically associated to SOM fractions linked to conceptual stabilization mechanisms.

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# Isolation and phenotypic characterization of extracellular PGPR from tomato plant rhizosphere samples

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Plant Growth Promoting Rhizobacteria (PGPR) can enhance the plant growth and development in various ways. The activity of PGPR is carried out through direct mechanisms, aimed at stimulating the growth and nutrition of the host (e.g. nitrogen fixation, phosphorus solubilization and IAA production), and indirect mechanisms, aimed at the biocontrol of pathogens (e.g. production of HCN and other secondary metabolites). In the pursuit of efficient PGPR strains with multiple activities, a total of 112 bacteria have been isolated from rhizospheric soil of tomato plants. Considering the several activities of PGPR, we have decided to structure the screening with a hierarchic approach, starting from testing the capability of fixing nitrogen. The isolation of nitrogen-fixing bacteria has been occurred through the use of a semi-selective semi-solid nitrogen-free growth medium (NFb). Afterwards, the obtained bacteria have been processed through the molecular typing technique REP-PCR (Repetitive Extragenic Palindromic) in order to discriminate same microbial strain profiles. In order to measure the effective ability to fix nitrogen, the acetylene reduction assay (ARA assay) has been carried out. Finally, the evaluation of tricalcium phosphate solubilization of isolated strains has been assessed using GY/Trycalcium phosphate solid medium, while the 3-indoleacetic acid (IAA) production was detected using the Salkowski's reagent. The bacterial isolates have been also tested *in vitro* for their antifungal activity against the common pests of tomato plant.

#### Sustainable space agriculture using MMS1 Martian soil simulant and compost

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The sustainability of long duration manned missions in space and planet exploration depend on plant cultivation for food production and waste recycling. Being able to use resources from explored planets to grow plants can provide a huge reduction of energy and transport cost. Regolith, as natural element of Moon and Mars, has a potential to be used as growing substrate for human's gardens in space. Nevertheless, lack of organic matter in this mineral substrate can be filled with inedible produced biomass from human's plantation converted into compost. Regolith/compost fraction can influence the agronomic and qualitative aspects of plants, which is the core of the present experiment. In this work we mainly focused on the assessment of the main physical, chemical, mineralogical and hydrological properties of a Martian soil simulant and to which extent they are modified by compost addition in view of its potential use as plant growing medium. In terms of chemical and physical properties, the Mojave Mars Simulant (MMS-1) shows a sandy texture (sand 810 and clay 25 g kg<sup>-1</sup>), strongly alkaline reaction (pH 8.9), low carbonates (2.7 %) and very low organic matter content  $(0.42 \text{ g kg}^{-1})$ , as well as low CEC  $(7.94 \text{ cmol}(+)\text{kg}^{-1})$  with a medium content of exchangeable Ca and Mg (1030 and 106 mg kg<sup>-1</sup>) and high K (248 mg kg<sup>-1</sup>), carrying to an unbalanced ratio Mg/K (1.39) due to K excess. The mineralogy of the sand fraction is dominated by Ca-plagioclase (anorthite) and amorphous material (glass), even low content (around 3%) of iron oxides (hematite) is also detected; zeolite is found only in the fine sand fraction (250-20 µm) while the clay fraction is mainly made by smectite and hematite, even anorthite is still found. In terms of chemical classification, the Martian simulant is an intermediate (SiO<sub>2</sub> = 57.3%) peraluminous ( $K_2O + Na_2O$ )/ $Al_2O_3 = 0.5$ ) igneous trachy-andesitic rock, having Ba, Zr, Sr and Ni as most abundant trace elements. A pot experiment was arranged by growing Green and Red Salanova lettuces for 19 days in different substrates obtained by mixing MMS-1 regolith simulant and a green compost at varying volume percentage (0:100,30:70,70:30;100:0). To fulfill plant requirements, modified Hoagland solution was also provided throughout the experiment. Crop performance in terms of yield, mineral and bioactive-compound contents basically revealed that the substrates 30:70 and 70:30 MMS-1/compost allowed the lettuce plants to produce a larger and healthier biomass.







# Session V: Frontiers in plant and soil sciences

Posters

# Hydroponically-grown *Rumex acetosa* L. and *Sanguisorba minor* Scop. respond differently to mowing and the storage as fresh-cut produce

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Wild edible plants have been used in cooking since ancient times. Recently, their value is improving thanks to scientific evidence of their nutraceutical properties [1, 2].

In this experiment, *Rumex acetosa* L. and *Sanguisorba minor* Scop., which were selected among other wild edible species for their nutraceutical properties and their agronomic traits [2], where hydroponically-grown and stored as fresh cut products for 15 days. This process was carried out for two consecutive mowing (first mowing, FM; second mowing, SM). Leaves were analysed during the storage (days 0-15) for their nutraceutical properties and antioxidant capacity. An untargeted metabolomics approach was utilized to fingerprint phenolics and other health-related compounds in both the species, as well as to unveil differences between the two mowing.

Severe variations were observed between the two mowing in both the species, whereas less marked variations were observed upon the storage as fresh-cut product. Leaves from the SM accounted for lower values of total phenol, ascorbic acid, and flavonoid concentration than the leaves from the FM. Metabolomics by UHPLC-QTOF semiquantitative analysis allowed to putatively annotate 458 metabolites when considering the two mowing of *R. acetosa*, while 467 compounds were found in those of *S. minor*. Among phenolic sub-classes, flavones, flavanols/flavonols, phenolic acids, tyrosols, lignans, stilbenes and alkylphenols showed an average down-accumulation in the SM, as observed for fold change values < 1 in *R. acetosa*. Conversely, an increment in flavonoids (principally flavones) was observed in *S. minor* (with no variations observed for other phenolic sub-classes) between FM and SM. Considering toxic compounds, oxalate concentration was considerable in *R. acetosa* leaves from both the two mowing; on the other hand, nitrite content was not negligible in *S. minor*.

This experiment provides evidence of the nutraceutical value of the selected wild species, even when hydroponically grown, thus promoting their use as fresh-cut products. Further research is still essential to: i) maintain the nutraceutical value of these species in multiple mowing and, ii) minimize the level of oxalate and nitrite, to effectively support their use as *nutrafood*.

[1] Romojaro A., Botella M.Á., Obón C., Pretel M.T. (2013). *International Journal of Food Sciences and Nutrition*, 1465-3478

[2] Ceccanti C., Landi M., Benvenuti S., Pardossi A., Guidi L. (2018). Molecules, 23, 2299, 1-15

# Use of pigmented corn cob waste as a natural dye in environmental friendly textile processing (PASTEL)

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The increased demand for textile products and the related increase in their production, as well as the use of synthetic dyes, contributed to make dye wastewater one of the main responsible of the severe pollution problems in current times. For these reasons and taking into consideration the importance of this sector in the Lombardy Region, the aim of the PASTEL project (Granted by Cariplo Foundation) is to develop a new natural dyeing process of natural fibers based on the use of anthocyanins (dyes) and tannins (mordanting) extracted from maize cobs, i.e. the residual waste coming from the cultivation of red corn. At present, anthocyanins are mainly extracted from red grape skin and other red berries, but since the cultivation of pigmented corn is growing in Lombardy, because of beneficial properties of contained anthocyanins, cobswaste could become a cheaper source of natural dye.

With this purpose we studied new materials and traditional varieties to find the best candidates for anthocyanins production. Anthocyanins belong to the class of flavonoids, secondary metabolites synthesized by a complex metabolic pathway consisting of about 20 biosynthetic genes and regulated by two types of transcription factors encoded by the bHLH and MYB gene families. The varieties selected for anthocyanins production carry the two strong regulatory genes involved in flavonoids synthesis Pl (purple plant 1) and P1 (pericarp color 1), leading respectively anthocyanins and phlobaphene synthesis in pericarp. We set up a quick and cheap extraction method starting from dried cobs to obtain the flavonoid pigments used to stain different natural fibers (wool, silk, cotton and flax).

HPLC-MS analyses have been performed on the different repartitions water/ethanol extracts to individuate the best composition for the staining process.

In this poster we'll present preliminary data regarding the entire process, from the waste colored cobs to the pigment extraction and natural fiber staining.

# The treatment of duckweed with a plant biostimulant or a safener improves the plant capacity to clean water polluted by terbuthylazine

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Water pollution is becoming alarming since thousands of contaminants are dispersed in the aquatic environments, and agricultural practices, for the massive use of pesticides, are contributing to exacerbating this problem.

In this context, a research aimed at investigating the ability of duckweed (Lemna minor), a free-floating aquatic species widespread throughout the world, to remediate water polluted with five different concentrations of a herbicide - terbuthylazine (TBA) - was carried out. In addition, duckweed was treated with a plant biostimulant and a safener with the aim of increasing the plant's capacity to tolerate and remove the TBA from the water. The results evidenced that the herbicide affected the duckweed already at the lower concentrations, reducing its capacity to proliferate and the area of its fronds. On the contrary, when the TBA treatments were performed in combination with the biostimulant or the safener the average area of the fronds was affected of lesser extents, compared to the plants treated with the herbicide only. Antioxidant enzymes, namely ascorbate peroxidases (APX) and catalases (CAT), were investigated and it was found that the bio-stimulated and safened duckweed showed increased activities of these enzymes, compared to the plants treated with TBA only. At last, some

phytofiltration experiments were planned. The biostimulated and safened duckweed removed more TBA from polluted water than the plants treated with the herbicide alone. In conclusion, this research showed that duckweed is suitable for cleaning water polluted with TBA and this potential can be successfully improved by treating the species with a biostimulant or a safener.

## Effect of light quality on gas exchange and leaf characteristics of Einkorn seedlings

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LED lamps produce monochromatic light making very efficient the study of its effect on plant physiology and growth. In general, the effects of the light spectrum have been studied for several species, but never for einkorn. Therefore, the aim of this work was to investigate the effects of red and blue light on gas exchange, plant morphology and growth of einkorn seedlings.

Einkorn (*Triticum monococcum* L. ssp. *monococcum*, cv. Monlis) was sown in plastic trays filled with sand of quartz and positioned in a growth chamber under controlled conditions. Different proportions of blue, red and intermediate wavelengths in the PAR region were adopted: B100R0 (100% blue photons), B0R100 (100% red), B25R75 (25% blue+75% red), R50B50 (50% blue+50% red) and B75R25 (75% blue+25% red) and B0R0 (only intermediate wavelengths).

Light treatments strongly affected einkorn seedlings. The highest  $CO_2$  assimilation rate was recorded in B25R75 and the lowest ones either under monochromatic light (B100R0 and B0R100) or under intermediate wavelengths (B0R0). Within dichromatic spectra, the assimilation rate proportionally decreased with increasing the blue/red ratio. The same behavior was observed in term of water consumption. The light spectrum greatly affected either the leaf shape or the pigments and nitrogen concentrations in leaves. In absence of blue light plants developed thin leaves, very poor in pigments and N. For the other treatments, leaf length decreased and leaf width increased with the proportion of blue light, while the highest pigments and N concentrations were recorded in B50R50 and B75R25.

Results showed that the quality of the light can influence the assimilation rate of einkorn seedlings through changes in the morphology of the plant or the chemical composition of the photosynthetic apparatus. The differences in water consumptions clearly indicate that the effect of light quality on the stomatal conductance was predominant. In fact, the assimilation rate was highly correlated to the plant evapotranspiration. In addition, non-stomatal effects should have also occurred, as low assimilation rates have been linked with low concentrations of pigment and nitrogen in leaves.

In conclusion, our data confirm that blue light is necessary to regulate many metabolic mechanisms involving the synthesis of pigments, nitrogen assimilation and photoreceptors activity and that plants cannot optimally develop with monochromatic red light alone.

#### Analysis of geochemical tracers in different systems soil-Citrus limon (L.) Osbeck

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Today is increasing the attention of consumers for the origin of food and high reputation of products with a distinct geographical identity. Food traceability is an important issue in food safety and quality control, with impacts on food security, its quantity and overall availability. The knowledge of a chemistry relationship between the soil and the agricultural products is an important tool for the quality assessment of food. Citrus Limon is the most important fruit tree crop in the world and the detection of potential fraud could improve by using tools linking the chemistry composition of this production to its typical growing area. This study use rare earth elements (REEs) as geochemical tracers. The REEs are a set of 14 elements, from lanthanum to lutetium that can be divided in light rare earth elements (LREEs), from La to Gd and heavy rare earth elements (HREEs), from Tb to Lu. The REEs have recognized as very useful tracers due to their generally coherent and predictable behavior. The aim of the research is to observe whether the fruits of various cultivars of citrus cultivated on the same soil and their products (fruit and juice) reproduce the same distribution of REEs. Taking into account of our previous works carried out on grapevine – soil system, we applied the same technique to evaluate and trace the REEs distribution in soil- Citrus Limon fruits system. Ten different varieties of Lemon fruits (Citrus limon Osbeck (L.)) were sampled. Lemon plant were grafted onto an unique rootstock (Citrus × aurantium L.), grown on volcanic soil in two experimental farms, located at the "Acireale" region (latitude 37 ° 37'23 N, longitude 15° 09'51.00 E and 205 m a.s.l., in Sicily, Italy) and "Portici" region (latitude 40 ° 81' 55 N, longitude 14° 34'75.00 E and 75 m a.s.l., in Campania, Italy). A sample of 2 kg of fruit was collected at technological ripeness from each plant of three for every cultivar. As well as, three soil samples (about 2 kg) in the field corresponding to lemon sampling were collected and to reduce any surface contamination from a depth of 10–30 cm. The REEs amount, the HREEs/LREEs relations and their distribution in the fruit and citrus juice with respect to the own soil were determined and calculated. Results obtained were treated with statistical methods. The intriguing results obtained with a geochemical approach are the first on the soil-Citrus Limon fruits system.

#### New chemiometric technique applied to traceability of Sicilian honey of Sulla (Hedysarum coronarium L.)

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The consumers have an increasing interest about food traceability with respect to safety, quality and typicality issues. Furthermore, the possibility of tracing the origin of foodstuff is assuming an increasingly important role at the legislative level, as a tool that may allow to prove on product authenticity and to control adulteration. In the last years, several of analytical techniques have been tested to found ways to establish the geographical origin of different kinds of food and many works reported that the combination of different analytical methods associated a multifactorial analysis of the data seems to be the most promising system to establish univocal traceability systems.

The honey is a natural food well appreciated in the world and the detection of potential fraud could be favored through tools linking the chemistry composition of this production to producing area. Recent works have been demonstrated the potentiality of the study of the distribution patterns of rare earth as a promising analytical method for traceability of food products due coherent and predictable chemical behavior of these compounds.

Also, have been demonstrated that their distribution in soil keeps unaltered in plants growing on that soil and eventually in agricultural products obtained from those plants and through the normalization of the REEs distribution, it is possible to appreciate their relative enrichments in soil-plant- agro food products. The aim of the research was to observe if the REEs normalizated pattern of honey of Sulla (*Hedysarum coronarium L.*) was kept unaltered respect the soils of production to establish a correlation between geographic area and honey product.

For this study, different production areas of Sulla in Sicily were considered. The production fields explored by the bees within 2 km of the hives were taken into consideration, the soil and the flowering plants were sampled. Flowers and leaves were analyzed from the plant. In the hive, pollen and honey were taken in three different days during the flowering period of the Sulla.

Of same areas were sampled to the same methods in different periods where other monofloral honeys were product, these samples were used for a control. In this study the REE approach, give very intriguing results in the geographical traceability of honey samples and are the first. The results have been supported by statistical treatment.

#### The effect of land covers on soil microbial and enzyme activities

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Degradation of forest habitats and land use change are among the factors affecting the variability of soil characteristics. With the aim of studying and evaluating the effect of forest, rangeland and crop cover on microbial characteristics and soil enzymes activities, the mountainous habitat of Kodir was considered from the Kojur region in the south-east of the Noshahr city. In the present study, four types of vegetation including forest (Carpinus orientalis - Quercus macrocarpa), Rangeland dominated by Astragalus balearicus -Teucrium subspinosum, rangeland dominated by Stachys byzantina and agriculture (Triticum aestivum) were selected. Following field trip, in each of the studied land uses, three transects (50 meters apart from each other) with 200 meters in length were considered. Soil samples ( $25 \times 25$  cm area) to a depth of 15 cm were taken at the first, middle and at the end of each transect. A total of 9 soil samples from land uses were transferred to the laboratory for analysis of physico-chemical, biological, microbial and enzymatic activity. The highest values of basal respiration, substrate induced respiration, microbial biomass (carbon and nitrogen, phosphorus), urease, acid phosphatase, arylsulfatase and invertase were observed in the forest land use, while the studied microbial indices (i.e. qCO2, microbial ratio and carbon capability index) did not show significant statistical differences among the different land uses. Principal component analysis (PCA) also shows higher values of soil microbial, enzymatic, biological and fertility in the forest site and shows a completely different location. In general, the results of this study showed that different soil characteristics under forest ecosystem have better condition than the other studied sites, while forest degradation and land use change is due to decreasing organic matter quality, the activity of soil microbial activity and biochemistry.

# **Comprehensive multiphase NMR: a powerful technology to study the effects of PFASs on the model plant** *Arabidopsis thaliana*

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Nuclear magnetic resonance (NMR) spectroscopy, being a powerful technique has become a key method for high throughput comparative analysis in plant metabolomics. There has been a novel NMR approach named Comprehensive Multiphase-Nuclear Magnetic Resonance (CMP-NMR) which possesses a great potential for the *in-situ* study of natural samples in their native state without sample destruction (Courtier-Murias *et al.*, 2012). This new technology helps to understand the stress biology and acclimation response in plants by identifying different compounds, toxicity, its mode of action, contaminate fate and the remediation (Simpson *et al.*, 2012; Jenne et al., 2019).

Here, in our study, in collaboration with Dr. Andre Simpson (University of Toronto, Canada) the sterilized wild-type Columbia (Col-0) *Arabidopsis thaliana* seeds were grown in sterilized Murashige and Skoog (MS) growth medium containing 1% (w/v) glucose. For the NMR experiments, uniformly labelled <sup>13</sup>C<sub>6</sub>-glucose at 99% <sup>13</sup>C enrichment (Silantes, GmbH) was used (Jenne *et al.*, 2019). The three experimental setups were established; PFOS and PFOA at 10 ppm each separately, 11 different PFASs at 11ppm and the other one without any treatment (Control). The plates with sterilized seeds were transferred to the dark for fourteen days at 21 °C and were collected for NMR analysis.

The overall aim of our study was to assess which metabolites can be influenced in *A. thaliana* by exposure to PFOA, PFOS and a mixture of eleven different PFASs. Soluble compounds as well as metabolites in cell wall and in non-soluble part of the tissues were monitored and data were compared in control versus different treatment groups. The specific condition of plant growth in the dark and <sup>13</sup>C carbon labelled glucose in the nutrition solution allow the enrichment of <sup>13</sup>C of the whole plant metabolites and give the opportunity to have an experimental model that is feasible for the study of changes in metabolic pathways of the plant when exposed to PFAS.

The overall results indicated that the annotated metabolites levels are not significantly modified when plant seedlings are treated with PFOA, PFOS at the selected concentration and in the proposed experimental conditions. On the other hand, the mixture showed modification of Alanine and Glutamine levels with an increase of 30 and 10-fold respectively, compared with the control. Support from the University of Padova for the acquisition of the LC-MS-QQQ is gratefully acknowledged.

#### THIOLOMICS: systematic identification of low-molecular-weight thiols by HPLC-MS/MS

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Low Molecular Weight (LMW) thiols are a class of highly reactive organic compounds which play a primary role in the maintenance of redox homeostasis in cells, thanks to the reactivity of their nucleophilic -SH group. In plants, they can make complexes with xenobiotics and toxic compounds and deactivate them; they are also implicated in the plant response to stress factors and they can post-translationally modify regulatory enzymes, participating in the modification of the redox state of sensitive molecules. Researches show a huge amount of LMW existing in plants, but most of them remain to be identified. Their identifications represent one major challenge, due to their low concentration in plant tissues. In order to discover new unidentified thiols, in this work a new high-performance liquid chromatographytandem mass spectrometry (HPLC-MS/MS) method has been developed and applied to seven leaves samples of Brassicaceae family from the Botanical Garden of University of Padova. The samples were previously extracted in acidic conditions and derivatized with the label 4,4'-Dithiodipyridine (DTDP). Thiols-DTDP complexes are characterized by the formation of three thiol-specific fragments in mass spectrometry using an electrospray ionization source (ESI) in positive ionization mode. Through the formation of these three fragments, a list of mass/charge (m/z) ratio was obtained for each matrix using the precursor ion scan mode, with a Kinetex C18 column coupled to an HPLC-QqQ triple quadrupole mass spectrometer. The subsequent analysis with high-resolution mass spectrometer (HRMS) Quadrupole-Time of flight (QTOF) have provided the exact molecular mass and molecular formula required for the identification of unknown thiols.

Results show that extracts from different Brassicaceae species had distinctive thiol composition, as confirmed by chromatographic analysis, indicating that several thiols are species-specific compounds. Given their role in plant metabolism, the identification and definition of these novel LMW thiols could open new prospective studies, which aim to understand how they could influence plant metabolism. Support from the University of Padova – Progetto Grandi Attrezzature Scientifiche is gratefully acknowledged for the acquisition of the LC-MS instrumentation.

#### Use of Plant Growth Promoting Bacteria in agriculture

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The intensive application of chemical fertilizers to overcome nutrients deficiency and achieve maximum agricultural productivity has led to environmental impacts. An important strategy to reduce the application of agro-chemicals and to prevent the lost of environmental biodiversity can consist in the technology based on suitable levels of fertilization in association with Plant Growth-Promoting Bacteria (PGPB) inoculation. The aim of this work is to test in vivo some PGPB strains (BFD160 *E. asburiae*,TFD26 *P. koreensis* and BFS112 *P. lini*,), previously isolated and characterized in vitro, on plants of *Cucumis melo* L. The effects of these bacteria have been evaluated on plants in a soilless system to understand the concrete possibility of using these PGPB as biofertilizers. The experiments have been performed in a greenhouse using four different thesis: fertigation consisting of full or half dose of Hoagland solution, 100% FERT and 50% FERT, respectively and fertigation plus mixed bacteria inoculation (100% FERT + I and 50% FERT + I). At the end of vegetative cycle both molecular and phenotypic parameters have been evaluated.

The molecular analyses consisted of the study of transcriptional expression of genes, amplified by Real Time-PCR, involved in ammonium (*AMT2*), iron (*FRO*, *IRT1*) and phosphorus (*PHT1*) transport and uptake by plant. Our results reveal that PGPB inoculum transcriptionally up-regulates *AMT2* gene in plants with full dose of fertigation; *FRO*, *PHT1* genes in plants with half dose and *IRT1* in both treatments. The phenotypic analyses included roots scanning, fruit analysis, measurements of chlorophyll content and morphological parameters. The data showed that the impact of PGPB on plant nutrition may result in effects on plant nutrient uptake and plant growth rate: the bacterial treatments anticipate the production of fruits and yields, influenced significantly morphological parameters, increased the total soluble solids and chlorophyll content. At the opposite, the decrease of volume and radical cumulative length in inoculated plants probably due to a higher availability of nutrients, may bring to metabolic savings that, in turn, influence positively fruit productivity.

#### Treatment by atmospheric-pressure plasma improves seed germination and plant development

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Recent agriculture 2.0 development is increasing the collaboration between scientists with different expertise with the aim of innovating agricultural technologies and guarantee in the future sufficient and high-quality food for an always-increasing number of people around the world. In this respect, the use of non-thermal plasma technology in agriculture has recently started a new research trend aiming, for example, at improving seed germination, surface decontamination and resistance to pathogens. In this research, basil (*Ocimum basilicum*) seeds were treated for different exposure times (from 10 s to 3 min) with low-temperature non-equilibrium plasma produced by volume dielectric barrier discharge (VDBD) in humid air at atmospheric pressure. Plasma treatment did not change the seed structure and morphology, as visualized by high resolution computed X-ray microtomography (μ-XCT). Seed germination rate was evaluated as well as plantlet development after 2 and 3 weeks of age. A faster and higher germination rate was observed, especially for the seeds treated with plasma for 1 and 3 minutes. Also the plantlet, after both 2 and 3 weeks, showed a more developed root apparatus, compared to non-treated plants.

Basil seeds were also investigated for their electrical impedance (EI) and for the internal distribution of macro and micronutrients, by using micro X-ray fluorescence spectroscopy ( $\mu$ -XRF).

Significant variations in EI were observed after plasma treatment as well as a redistribution of mineral elements such as P, Mg, K and Zn. In particular, P, K and Mg concentrated in the radicle, moving from the endosperm, while Zn, initially concentrated in the cotyledon, appeared more homogeneously distributed inside the whole seed after the plasma treatment.

It is likely that this element redistribution in the seed was triggered by the electrical field associated with the VDBD plasma, which caused a movement of important micro and macro nutrients from the storage regions of the seed towards the radicle tissues. This ion movement could explain the observed faster germination of the plasma-treated seeds. In fact, such movement is similar to what is generally observed in seed tissues during germination. The plasma treatment is therefore somehow boosting the mobilization of key-nutrients towards the radicle, thus resulting in a faster and higher germination of the seeds as well as improved characteristics of the basil plantlet, especially at the root level.

#### Traceability of Sorrento lemon PGI by chemometric analysis of juice mineral composition: a monoand multi-cultivar approach

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In the last decades the growth of high value agri-food trade and origin-based marketing strategies has generated in EU a strong need to protect the product provenience with the "Geographical Indication" (GIs) labelling. The Sorrento lemon (Citrus limon (L.) Burm. f. cv. Ovale di Sorrento), is known for its characteristic cultivation on terraces in Sorrento peninsula in Campania region. On 2000 it has been awarded with the Protect Geographical Indication (PGI).

Main goal of the work is to protect the PGI Sorrento lemons by frauds with lemons produced in not PGI areas. Thus, we explored the feasibility of multi-elemental fingerprinting of lemon juice to discriminate the geographical origin of lemon fruits. Lemons were collected in three mono- (Ovale di Sorrento) and multi-cultivar (Ovale di Sorrento, Sfusato Amalfitano, Femminello Cerza and Femminello Adamo) lemon groves from different areas of Campania region. In this work we report the total content of 12 trace elements in lemon juices measured by acid digestion and ICP-MS. The efficacy and robustness of a chemometric approach of geographic traceability to discriminate the elemental profiles of Sorrento lemon PGI is discussed. According to the one-way ANOVA, 10 out of 2 lemon juice elements (Ti, Fe, Co, Cu, Zn, Se, Rb, Sr, Mo, Ba) were significant and so normalised and scaled. Exploratory analysis by PCA was satisfactorily carried out with the first two PC explaining 58% of the cumulate variance for Ovale di Sorrento dataset (39 samples) and 55% for all cultivars dataset (63 samples). The scatter plots (PC1 vs PC2) showed a clear grouping of juices from the three different production areas in both cases. Then Linear Discriminant Analysis (LDA) was applied. The best model for mono-cultivar dataset, Ovale di Sorrento, achieving 100% of correct classification and showing an optimal discrimination between the 3 groups with different provenience, was based on the same 10 elements. The model was cross-validated with very good results. LDA applied to multi-cultivar dataset showed a satisfactory discrimination between lemon groves of different provenience. An external validation with 30% of samples randomly chosen produced optimal results underlining the ability of multi-elemental fingerprinting of lemon juice to trace provenience also at small geographical scale levels. Future investigations including a more significant number of cases are needed to support these preliminary results.

# **Investigation of Se and Cr interactions in Se-hyperaccumulator** *Stanleya pinnata* **and non hyperaccumulator** *Brassica juncea* **for combining biofortification and phytoremediation technologies**

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Selenium (Se) is not essential to plants, which however represent the main portal of Se entry into the food web. Plant enrichment with Se could be a valid tool to counteract increasing Se deficiency in the world population. Se biofortification can be coupled with Se phytoremediation, which exploits the propensity of plants to accumulate Se to remove excess Se from naturally seleniferous soils or Se-polluted soils. The Se-enriched plant material may be used to alleviate Se deficiency in low-Se areas. One issue is that Se-polluted soils could contain other pollutants, and thus the plant material must be carefully checked before use. In this respect, Se-hyperaccumulators could be preferred to other species, because they seem to possess specific mechanisms for Se uptake and tolerance. In this study, we evaluated the efficiency of Se-hyperaccumulator Stanleya pinnata and non hyperaccumulator Brassica junceato accumulate Se (supplied as selenate) in presence of chromium (Cr, supplied as chromate), and the capacity of Se to reduce Cr accumulation by plants. Six week-old plants were grown in hydroponics for 7 days without Se and Cr (control), Se 50mM, Cr 50 mM, or Se 50mM+Cr 50mM. The fresh weight of S. pinnatawas stimulated by Se, but impaired by Cr. However, it was restored by Se in Cr treated plants. The dry biomass was also improved by Se alone. In *B. juncea*, Se and Cr treatments caused a reduction of biomass, more evident in plants treated with Cr alone. Cr did not affect Se accumulation in roots of Se-treated S. pinnata, but reduced it by 2-fold in leaves, thus suggesting an inhibitory effect of Cr on Se root to shoot translocation. In B. juncea, Cr reduced leaf and root Se accumulation. Cr in both species was mainly accumulated in roots, but its concentration was reduced in leaves and roots by Se, to mean that Se inhibited Cr uptake by plants. S. pinnataaccumulated 2-fold more Se than Cr, perhaps because the shared uptake systems were more specific for Se or the existence of selective transporters for Se. Conversely, B. juncea accumulated more Cr than Se. Analysis of oxidative stress in the two species evidenced that Se positively influenced the antioxidant systems of *B. juncea*, while *S. pinnata* displayed constitutive high activity of antioxidant enzymes, regardless of Se and Cr treatments. Based on these results, we propose that plant material derived from *S. pinnata* grown in a Se/Cr polluted substrate is to prefer for further biofortification programs.

#### Testing natural Mn-todorikite as an efficient, low-cost and environmental-friendly catalyst

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Manganese (Mn) oxide minerals occur in a wide variety of geological settings. Besides being major components of Mn nodules that pave huge areas of the ocean floor, Mn oxide minerals are ubiquitous in soils and sediments and participate in a variety of chemical reactions affecting groundwater and bulk soil composition.

Todorokite  $[(Ca,Na,K)_{X}(Mn^{4+},Mn^{3+})_{6}O_{12}\cdot 3.5H_{2}O]$  is one of the major Mn minerals identified in ocean Mn nodules, but it is also a major mineral in the oxidized zones of many terrestrial Mn deposits. As todorokite shows large zeolite-like tunnels, there has been considerable interest in producing synthetic analogues for possible utilization as catalysts or molecular sieves.

A natural Mn-todorokite mineral collected in the Apulia region (Italy) has been evaluated as oxidation catalyst for degradation of methyl-orange (MO) dye. This mineral has been firstly characterised by X-ray diffraction, wavelength dispersive X-ray fluorescence, BET, scanning electron microscopy, attenuated total reflectance Fourier transform infrared spectroscopy and thermogravimetry.

The catalytic dye-degradation data show that this Mn-todorokite can operate under strongly oxidizing potentials >+400 mV *vs.* standard hydrogen electrode performing fast MO degradation ( $t_{1/2}$  <5 minutes). A detailed study using Electron Paramagnetic Resonance spectroscopy revealed that, at Eh >+450 mV, the active Mn-centers of Mn-todorikite evolve rapidly through Mn<sup>3+</sup>/Mn<sup>4+</sup> states and this is correlated with the rapid catalytic degradation of MO.

These results suggest Mn-todorikite mineral as an efficient, low-cost and environmental-friendly catalyst which can be used for industrial purposes.







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