

PhD Winter School Feeding the world: the contribution of research in agricultural chemistry to sustainable development 9-12 February 2015, Piacenza, Italy

Organizing Committee: Gian Maria Beone, Luciano Cavani, Stefano Cesco, Claudio Ciavatta, Tanja Mimmo, Roberto Terzano, Marco Trevisan

Book of Abstracts

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UNIVERSITÀ CATTOLICA del Sacro Cuore



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Beone Gian Maria - UCSC Cavani Luciano – UniBO Cesco Stefano – UniBZ Ciavatta Claudio – UniBO Mimmo Tanja - UniBZ Terzano Roberto - UniBA Trevisan Marco – UCSC

Chairmen and Coordinators of the Working Groups:

Beone Gian Maria – UCSC Laudicina Vito Armando - UniPA Renella Giancarlo – UniFI Spagnuolo Matteo - UniBA Terzano Roberto - UniBA

Speakers:

Astolfi Stefania – UniTUS Cavani Luciano – UniBO Carminati Andrea - UniGoettingen Ceccherini Maria Teresa - UniFI Cocozza Claudio – UniBA Cozzolino Vincenza – UniNA Del Buono Daniele – UniPG D'Imporzano Giuliana – UniMI Grigatti Marco – UniBO Lucini Luigi – UCSC Manoli Chiara – ILSA S.p.A. Martin Maria – UniTO Mimmo Tanja – UniBZ Monaci Elga – UnivPM Pii Youry – UniBZ Prinsi Bhakti – UniMI Said Pullicino Daniel – UniTO Schievano Andrea - UniMI Six Johan – ETHZ Sofo Adriano – UniBAS Spagnuolo Matteo - UniBA Suman Michele – Barilla S.p.A. Tomasi Nicola – UniUD Vigani Gianpiero – UniMI Zaccone Claudio - UniFG Zamboni Anita – UniVR

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The aim of the School is to bring together in a friendly environment experienced researchers and young scientists in order to share their ideas and solutions about issues related to sustainable development in agriculture. Many different aspects regarding soil, plant and environment will be covered including field applications and methodological approaches. At the end of the day, working group activities will try to provide answers to specific research and practical problems raised by the participants.

Final program

9 February 2015

afternoon: arrival and registration

15.00-15.15 Introduction of the School

15:15-16:15 Presentation of the participants (Roberto Terzano, DiSSPA, University of Bari, Italy)

16:15-16:30 Break

afternoon session: *THE SOIL-PLANT-MICROORGANISMS SYSTEM* Chair: Gian Maria Beone (UCSC, Piacenza, Italy)

16.30-17.10 Claudio Zaccone (SAFE, University of Foggia, Italy) Introduction to the Soil System

17:10-17.50 Gianpiero Vigani (DiSAA, University of Milan, Italy) Understanding plant nutrition for a sustainable agriculture

17.50-18.30 Youry Pii (FaST, University of Bolzano, Italy) An underground tale: contribution of microbial activity to plant nutrients acquisition

18:30 Get together: wine party and poster exhibition (sponsored by "Azienda Vitivinicola La Celata" and "Salumificio La Rocca")

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10 February 2015

<u>morning session</u> **SUSTAINABLE AGRICULTURAL PRODUCTIONS Chair: Vito Armando Laudicina** (DiSAF, University of Palermo, Italy)

9.00-9.45 Matteo Spagnuolo (DiSSPA, University of Bari, Italy) – **Adriano Sofo** (SSAFAA, University of Basilicata, Potenza, Italy) Sustainable farming systems: compromises and advantages in horticulture

9.45-10.30 Maria Martin – Daniel Said Pullicino (DiSAFA, University of Torino, Italy) Sustainable rice cropping systems: linking soil processes to functions

10.30-11.00 Break

11.00-11.45 Daniele Del Buono (DSA3, University of Perugia, Italy) – **Elga Monaci** (D3A, Polytechnic University of Marche, Ancona, Italy) Agrochemicals: some generalities, plant metabolism and sustainable uses

11.45-12.30 Luciano Cavani (DipSA, University of Bologna, Italy) – **Chiara Manoli** (ILSA S.p.A.) The use of biostimulants in agriculture: a collaborative overview

12.30-13.00 Open discussion (under supervision of the Chair)

13.00-14.30 Lunch break

afternoon session: Keynote lecture and Working groups

14.30-15.30 Keynote lecture: Andrea Carminati (Div. Soil Hydrology, Georg-August University of Goettingen, Germany) Root water uptake: roots and rhizosphere traits that increase plant drought tolerance

15.30-16.30 Poster Session

16.30-18.30 Working group activities (under the supervision of speakers and coordinators)

Working group 1 "Sustainable agricultural productions" - Coordinator: V.A. Laudicina

Working group 2 "Environment and waste management" – Coordinator: M. Spagnuolo

Working group 3 "New methods in agricultural research" - Coordinator: G. Renella

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11 February 2015

morning session **INNOVATIONS IN AGRICULTURE FOR A SUSTAINABLE DEVELOPMENT Chair: Matteo Spagnuolo** (DiSSPA, University of Bari, Italy)

9.00-9.30 Stefania Astolfi (DAFNE, University of Tuscia, Viterbo, Italy) Sustainable strategies for the biofortification of crops with essential micronutrients

9.30-10.00 Nicola Tomasi (DiSA, University of Udine, Italy) New solutions to improve soilless productions

10.00-10.30 Vincenza Cozzolino (CERMANU, University of Naples Federico II, Italy) Sustainable agriculture: the key role of arbuscolar mycorrhizas in agroecosystem services.

10.30-11.00 Break

11.00-11.45 Giuliana D'Imporzano – Andrea Schievano (RICICLA Group, DiSAA, University of Milan, Italy) Agroenergy, energy crops and renewable fertilizers.

11.45-12.30 Claudio Cocozza (DiSSPA, University of Bari, Italy) – **Marco Grigatti** (DiSA, University of Bologna, Italy) Compost and digestate production and their safe re-utilization in agriculture

12.30-13.00 Open discussion (under supervision of the Chair)

13.00-14.30 Lunch break

afternoon session: Keynote lecture and WORKING GROUPS

14.30-15.30 Keynote lecture: Johan Six (DESS, ETH, Zurich, Switzerland) Soil carbon sequestration: from mechanisms to predictions

15.30-16.30 Selected presentations by PhD students

16.30-18.30 Working group activities (under the supervision of speakers and coordinators)

Working group 1 "Sustainable agricultural productions" - Coordinator: V.A. Laudicina

Working group 2 "Environment and waste management" – Coordinator: M. Spagnuolo

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12 February 2015

morning session: INNOVATIVE METHODS IN AGRICULTURAL CHEMISTRY RESEARCH Chair: Giancarlo Renella (DiSPAA, University of Florence, Italy)

9.00-9.30 Luigi Lucini (UCSC, Piacenza, Italy) High resolution mass spectrometry based metabolomics: from data acquisition to mining and chemometrics

9.30-10.00 Bhakti Prinsi (DiSAA, University of Milan, Italy) Proteomic approaches to study plant biochemistry

10.00-10.30 Tanja Mimmo (FaST, University of Bolzano, Italy) Methods applied in the authentication and traceability of agricultural products

10.30-11.00 Break

11.00-11.30 Anita Zamboni (Dept. of Biotechnologies, University of Verona, Italy) Plant genomics as an essential component of green system biology

11.30-12.00 Maria Teresa Ceccherini (DiSPAA, University of Florence, Italy) Soil Metagenomics: a history of conquests

12.00-12.30 Michele Suman (Barilla S.p.A., Parma) Food safety issues from the industrial perspective: an *helicopter* view & a focus on analytical strategies devoted to mycotoxins risk prevention

12.30-13.00 Open discussion (under supervision of the Chair)

13.00-13.30 Poster prize ceremony and conclusions

lunch and departure

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Introduction to the Soil System

Claudio Zaccone

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Many natural things, including soil, lack a universally accepted definition. One of the reasons is their multifunctional role.

Land use conditioned the way soil was perceived. Historically, the prevailing concept of soil was that of agronomists (*i.e.*, a medium for plant growth) whereas for geologists soil was just a rather short phase in the long global cycle of rocks, and for engineers an unconsolidated earthy material that can be moved by machinery.

Soil is a *multiphase*, *heterogeneous*, *open*, *dynamic*, *living*, *biogeochemical* system generating and sustaining life on our planet. Soil is a *porous* media created at the land surface by weathering processes derived from biological, geological, and hydrologic phenomena. Soils differ from mere weathered rock because they show a vertical stratification (soil horizons), mainly produced by the continual influence of percolating water and living organisms. Moreover, being *ca.* three times the atmospheric pool and *ca.* four times the vegetation pool, soils represent the largest C reservoir in terrestrial ecosystems.

Consequently, soils could be defined as the "skin" of the Earth, and function as *filters*, *buffers* and *chemical* reactors.

Unfortunately, soil is probably not a renewable resource anymore, at least on "societal" time scale. Thus, the conservation of soil (quality and fertility) becomes a key link among global issues.

Understanding how physical and chemical parameters of soils affect, directly or indirectly, the cycle of nutrients and pollutants in agro-ecosystems, as well as climate changes, is of paramount importance in terms of sustainable use of soil as natural resource.

Understanding plant nutrition for a sustainable agricolture

Gianpiero Vigani

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Nowadays a worrying requirement of food haunts the scientific community: crop production must double to keep pace with growth in the global population, which will reach 9 billion people by the year 2050. Additionally, a decrease in available arable land has been estimated, therefore the plant science community should provide solutions to maintain and increase food production. Plants represent an important food source for both humans and animals. Plant production strongly depends on the availability of mineral nutrients. Macronutrients (i.e. nitrogen, phosphate, potassium, sulphate) and micronutrients (i.e. iron, copper, zinc) are essential for various aspects of plant growth and development.

To cope with nutrient limitations, plants have developed a set of physiological and morphological responses to match resource availability with growth requirements. Such responses are regulated by a complex sensing and signaling mechanism that allows plants to monitor the external and internal concentration of mineral nutrients. Understanding such mechanisms represent a major issue in plant physiology and crop production, with potential impact on the design of new biofortification strategies for improving yields as well as the nutritional value of crops.

Nutrient sensing and signalling in plants involves both local and systemic pathways. When a variation in the nutrient availability is perceived by the cell, specific signal molecules induce a transcriptional reprogramming mechanism at the nucleus level. At the tissue level, roots are expected to play the initial role in sensing the local mineral nutrient status of the soil. Through a long-distance signalling pathway, nutrient stress signals reach all plant organs, inducing the plant adaptation mechanisms.

Here, the current knowledge about the nutrient sensing and signalling mechanisms related to some elements will be presented.

An underground tale: contribution of microbial activity to plant nutrients acquisition

<u>Youry Pii</u>

Faculty of Science and Technology (FaST), Free University of Bolzano, Italy, youry.pii@unibz.it

By 2050, the global population is predicted to increase by about 50% along with the demand for food. However, crops productivity is strongly limited by the scarce bioavailability in soils of several mineral nutrients, as for instance nitrogen (N), phosphorous (P) and iron (Fe). With the aim of supplying plants with readily available nutrients, the application of mineral fertilizers constantly increased in the last decades. However, crops yields have not increased proportionally, most likely because plants can take up and utilize only a small part of the nutrients supplied *via* fertilization practices.

Considering the disadvantages deriving from an extensive use of mineral fertilizers, agricultural practices are moving towards more sustainable systems, suggesting and testing alternative methods of fertilization; a very promising approach to overcome this issue might be represented by the application of Plant Growth-Promoting Rhizobacteria (PGPR) as bioinoculants. In fact, a very interesting feature of PGPR, among others, is their ability to enhance nutrients bioavailability, which has indeed a positive effect on plant nutrition. Furthermore, several observations highlight that the inoculation of plants with PGPR can have considerable effects on plants at physiological and molecular levels.

On the other hand, also plants have the ability to shape rhizosphere bacterial community in order to favour those interactions that would help overcoming stressing environments.

Nevertheless, the molecular mechanisms underlying these phenomena, the signals involved as well as the potential applications in the framework of a sustainable agriculture approach and the biotechnological aspects for possible rhizosphere engineering are still an open discussion.

Sustainable farming systems: compromises and advantages in horticulture

Matteo Spagnuolo¹ and Adriano Sofo²

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² School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Potenza, Italy, adriano.sofo@unibas.it

The key principle behind the concept of sustainability is meeting the needs of the present without compromising the needs of future generations. If natural resources such as soil, nutrients and water are used up at a rate faster than they are replenished, then the farming system becomes unsustainable. Another key concept of sustainability is to maintain a high level of biodiversity also by adopting sustainable pest management (integrated pest management) strategies in order to reduce at minimum the risk for human health and the environment (EU directive 128/2009). Vineyards and fruit trees are some of the most important and extensive crops in Mediterranean agro-ecosystems. In conventional farming systems, adopted by the majority of the farmers in Southern Italy, frequent soil tillage enhances soil erosion and loss of soil fertility, and often reduces soil microbial diversity and soil microbiota complexity, that strongly contribute to the overall soil fertility. For these reasons, these conventional agronomic practices should evolve in a more sustainable management (e.g., grass cover, pruning residues recycling, organic matter inputs) addressed to improve soil organic matter. Under semi-arid climatic conditions, the application of endogenous organic matter can be a key factor to enhance soil quality and fertility and to preserve natural resources, mainly soil and water, avoiding detrimental effects on the environment. Furthermore, agricultural practices can play an important role in carbon sequestration. The carbon stock can be viewed as measure of the relative contribution to biomass to the carbon cycle, and the capacity to store organic carbon depends to a great extent upon climate and soil properties, although the cultivation system can play a considerable part. Together with the soil management, other critical points for a sustainable horticulture are the fertilization and the irrigation system which could influence water use efficiency, yield quality, and pest and soil management. Results of experiments carried out in the last two decades will be presented. The final goal is to encourage farmers to adopt a sustainable farming system as a whole, not just as individual elements, in order to promote good-quality fruit production without negative effects on the environment.

Sustainable rice cropping systems: linking soil processes to functions

Maria Martin, and Daniel Said-Pullicino

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Rice, the most important food crop contributing to human nutrition, is generally grown in flooded paddies that results in anoxic soil conditions throughout most of the cropping period. Redox processes in these agro-ecosystems govern the biogeochemical cycling of plant nutrients, organic carbon (C) and contaminants.

Among the latter, arsenic (As) assimilation can result in relatively high As concentrations in rice grain, with important implications on food safety. As dynamics in soil are linked to the fluctuations of redox potential and pH, which are in turn affected by water and crop residue management practices. Moreover, susceptibility to As uptake seems to vary with plant growth phase and nutrient availability. The interlinking of all factors influencing As contents in rice grain are still to be fully elucidated, however, research is contributing to identify cropping systems that minimize As contents in rice.

Paddy management is also responsible for the large concentrations and fluxes of dissolved organic carbon (DOC) that characterize these ecosystems. Being the most bioavailable and mobile soil organic C fraction, DOC is the most dynamic in terms of ecosystem function. Recent studies have highlighted the role of DOC in controlling methane (CH₄) emissions, C loss and accumulation in paddy soils, intrinsically linked to the environmental sustainability of these cropping systems. Management practices may determine the source, production, mineralization and transport of DOC at field-scale. However, temporal and spatial dynamics of DOC fluxes, and the processes linking this C pool to CH₄ emissions, C export and stabilization in rice fields remain largely unknown.

Agrochemicals: some generalities, plant metabolism and sustainable uses

Daniele Del Buono¹ and Elga Monaci²

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Agrochemicals are compounds largely used in agriculture to kill or repel insects, weeds, rodents, fungi or other organisms that can affect crop productivity. They are classified on the basis of the target site in insecticides, herbicides, rodenticides and fungicides. Among them, an important sub-group is that of herbicides, weed-killers largely employed in agriculture. The plant ability to tolerate or resist to a herbicide, or more generally to toxic compounds, is based on its detoxificative metabolism. This detoxificative metabolism is represented by a three phases model, in which the xenobiotic substrate is functionalized, then conjugated to endogenous biomolecules and, at last, transferred into the vacuole to complete the degradation or re-exported and immobilized on lignin.

Many concerns have been associated to the risk that agrochemicals may enter natural ecosystems through either diffuse or point sources of contamination. Diffuse sources are originated by field treatment, during which these compounds may move from the application point. Point sources come from handling of the chemicals before and after field treatment. Both these routes can contaminate soil, superficial and ground waters and become a serious threat for natural ecosystems and human health. Following the directive 2009/128/EC, establishing the framework to achieve a sustainable use of pesticides, farmers have to apply mitigation strategies. The presence of buffer and safeguard zones may effectively reduce the exposure of water bodies to diffuse sources of contamination, whereas a number of systems, grouped under the name of biobeds, have been designed to contrast point sources of contamination at farmyard level.

The Use of Biostimulants in Agriculture: a Collaborative Overwiev

Luciano Cavani¹ and Chiara Manoli²

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The biostimulants (Bs) are not only a new kind of fertilizers, but introduce a new approach in soil fertility and plant growth.

A Bs is a substance or a microorganism or a combination of them. It stimulates biological processes in plant and/or soil to improve nutrient use efficiency (NUE), tolerance to abiotic stress and/or crop quality. It acts independently of its nutrient content, then it has not properly fertilizing effect. It does not act on biotic stress (i.e. on plant pest, weed or pathogen), then it is not a plant protection product.

du Jardin (2012)¹ has identified eight categories of plant biostimulants: (1) humic substances, (2) complex organic materials, (3) beneficial chemical elements, (4) inorganic salts (including phosphite), (5) seaweed extracts, (6) chitin and chitosan derivatives, (7) antitraspirants, and (8) free amino acids, and others N-containing substrances. In addition also some microorganisms may be considered Bs.

Many scientific studies² have demonstrated the potential of Bs to increase root growth, enhanced nutrient uptake, and stress tolerance, however several points need to be clarified:

 \rightarrow identify the active ingredient(s) (ai), and the mechanism of action of ai;

 \rightarrow develop analytical(s) methods to characterize them and to measure their biostimulant effect;

 \rightarrow define the agronomic uses of Bs (doses, time, plants, etc.);

 \rightarrow assess the environmental risk associate at the use of Bs

The European Biostimulants Industry Council (EBIC) considers that the 2012 EU market value (sales) of Bs can be estimated at \in 400-500 million, and reported that more than 6.2 million hectares in the EU are treated with Bs³. The global market for Bs is projected to reach \$2,241 million by 2018, with an annual growth rate of 12.5% from 2013 to 2018².

A collaborative partnership between public and private stakeholders is needed to achieve these ambitious objectives.

¹ du Jardin P. (2012). The science of plant biostimulants – A bibliographyc analysis. Contract 30-CE0455515/00-96.

² Calvo P., Nelson L., Kloepper J.W. (2014). Agricultural uses of plant biostimulants. *Plant Soil* 383: 3-41.

³ A Legal Framework for Plant Biostimulants and Agronomic Fertiliser Additives in the EU. Report for the European Commission Enterprise & Industry Directorate – General (2014).

Root water uptake: roots and rhizosphere traits that increase plant drought tolerance

Andrea Carminati, Mutez A A Ahmed, Mohsen Zarebanadkouki and Eva Kroener

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The increasing demand of food production and the consequent pressure on soil and water resources are the motivation to identify mechanisms improving the ability of plants to take up water from soils, in particular when soil water is scarce. Plants can adapt to water shortage by growing deeper roots that are capable to extract water stored in the subsoil. An alternative adaptation strategy of roots consists in the modification of the soil in their vicinity, the rhizosphere.

Plant roots exude up to 10% of the carbon assimilated through photosynthesis into the soil, a process referred to as rhizodeposition. The carbon exuded into the soil helps roots to take up nutrients and promotes positive feedbacks between plants and microorganisms. Here, we show that the mucilaginous fraction of the rhizodeposits, referred to as mucilage, plays also a crucial role on soil-plant water relation and it has the potential to increase plant drought tolerance.

Mucilage is a gel that can absorb large volumes of water, altering the physical properties of the rhizosphere and maintaining the rhizosphere wet and conductive when the soil dries. Acting as a hydraulic bridge between roots and the soil, mucilage facilitates root water uptake and maintains transpiration and photosynthesis in dry soils.

In summary, by exuding mucilage into the soil, plants modify the physical soil environment, have a better access to water when water is scarce, and maintain photosynthesis for a prolonged time during drought. We propose that mucilage exudation is a plant trait conferring drought resistance.

Sustainable strategies for the biofortification of crops with essential micronutrients

Stefania Astolfi

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Mineral deficiencies affect more than half of the World's population, hindering human working ability, health and quality of life. Humans require more than 22 mineral elements, among which Fe, Zn, Cu, I and Se, are required in trace amounts. These mineral elements enter the food chain mainly through plants, so mineral deficiencies result mainly from a low content of nutrients in edible parts of crops. Therefore, improving nutrients uptake from the soil and enhancing their movement to and bioavailability in the edible parts of the plant will provide benefits for animal and human nutrition and represents a fortification solution economically worthwhile, especially for developing countries.

Fortification strategies are obviously different for each micronutrient, however can be achieved by fertilization, conventional breeding approach, to produce nutritionally improved crops relies on genetic diversity in natural populations, and/or targeted molecular genetic approaches, concerning as primary targets of biotechnological interventions the uptake from soil and the translocation to edible parts.

This talk will describe the attempts made so far to biofortify crops with Fe through conventional breeding and genetic engineering.

In addition, it will be discuss our finding that high S supply may result in the improvement of Fe use efficiency in wheat plants and the possibility to suggest an alternative approach to fortification through agricultural management.

This approach could represent an important tool to realize the improvement of the nutritional value of food crops by increasing Fe content (agronomic biofortification) without additional input of Fe fertilizers.

New solutions to improve soilless productions

Tomasi Nicola

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In the recent years, there is an increasing interest in the soilless technologies to produce food. Many motives promote the use of these cultivation methods, *i.e.* the diffusion of ready-to-eat products, the ever increasing loss of fertile soils and agglomeration of population in (mega)cities, production all over the year, reduced need of phytosanitary treatments, space travel, etc. Moreover water, for its scarcity, price and quality, is becoming an economically limiting resource, pushing for the development of cultivation systems with a higher water-use efficiency, such as floating system. Moreover this latest allow to harvest a cleaner product, with a consequent reduction of washing treatments, but also can represent an opportunity to enrich the product with beneficial elements for human health. However the quality, in particular accumulation of potentially negative elements, shelf live and organoleptic characteristics, of soilless production is often worsened in comparison of soil-grown crops.

For these reasons, researches aim at developing new soilless cultivation systems and at improving the yield, both quantitatively and qualitatively, of soilless grown crops are needed. Some examples of studies will be presented in particular about the cultivation of ready-to-eat salad in floating system. Demonstrating the opportunities to improve the yield and nutritional quality of the product acting both on the environmental conditions and the composition of the growth medium and the criticalities of this system.

Sustainable agriculture: the key role of arbuscular mycorrhizas in agroecosystems services

Vincenza Cozzolino

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Sustainable agriculture, conservation-oriented production, is the optimal target of all countries worldwide with the increased concerns on food security and resources and environmental protection. More and more biological measures and recycle technologies were attempted to apply in crop production to reduce the input of chemicals, such as fertilizer and agrochemicals. In this context, the ecosystem services rendered by soil biota in maintaining soil quality, plant health and soil resilience are extremely important. Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microorganisms developing symbiotic association with most terrestrial plants as their usually non-specific hosts. In their symbioses the fungi provide their host plant with water and nutrients in the exchange for carbon. Many studies have been carried out to determine their efficacy in promoting plant fitness, productivity, enhancing the fertilizer use efficiency and improving the soil structure and health. They also act as bioprotectants against pathogens and toxic stresses and definitely, they are seen as the key players of sustainability in agroecosystems.

In this talk, I will present the current information on the benefit of arbuscular mycorrhizal symbioses in agricultural system with data from a variety of field and pot experiments, also testing the potential benefit of commercially available bio-inoculants on crop production. The aim is to emphasize the key role that AMF symbioses can play as an ecosystem services provider to ensure plant productivity and quality in emerging systems of sustainable agriculture.

Agroenergy, energy crops and renewable fertilizers

Andrea Schievano, Giuliana D'Imporzano, Fabrizio Adani

RICICLA Group, Dipartimento di Scienze Agrarie e Agroambientali, University of Milan, Italy, andrea.schievano@unimi.it, giuliana.dimporzano@unimi.it

Is a sustainable production of renewable energy and renewable products possible from agricultural and agro-industrial resources? What bio-based fuels and other products can be thought, at what scale, and from what kind of biomass, to achieve the energetic, economic and environmental sustainability of the system? Biogas and electric energy generation from biogas are today a reality in the EU at agro-industrial scale and may play a fundamental role as base for a bio-refinery model that can combine energy-dedicated crops to waste/side-product biomass as substrates. This emerging sector has also started a new way of locally producing renewable forms of fertilizers. Bio-methane and its distribution as renewable fuel is the next step as well as market-like fertilizers based on organic and mineral renewable sources. This is a type of simple bio-refinery concept which in the next future has the potential of being improved to produce not only energy and fertilizers, but also chemicals, building blocks, active molecules, bio-materials, etc. from renewable sources.

Compost and digestate production and their safe reutilization in agriculture

Claudio Cocozza¹ and Marco Grigatti²

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Soil recycling of organic by-products can represent a winning strategy to maintain the soil fertility; they can be utilized raw or better after suitable stabilization processes.

The two established techniques available to this aim are composting and anaerobic digestion (**AD**). The former can be applied to almost all types of organic matters with the aim to increase their stability, while the latter is mainly applied to produce energy from organic residues poor of recalcitrant molecules. This can be further followed by composting.

Composting can be divided in thermophilic and curing phase. During the initial stage the microbial community operates the mass hygienization, giving a stabilized but still phytotoxic product. The curing phase operates a degradation of toxins coming from organic residues and from the microbial metabolism which are slowly decomposed, giving mature compost in the end.

Anaerobic digestion can occur in psychrophilic, mesophilic and thermophilic conditions and only the latter hygienizes the biomasses. The digestates can be liquid and/or solid according to the **AD** facility.

The quality of composts and digestates can be evaluated through several physical, chemical and biochemical methods and parameters assessment such as the respirometric tests, the germination index, beside to the determination of inerts and heavy metals content.

Those features, along with the quality of the raw materials, influence and decide the best agronomic utilization of composts and digestates.

Soil carbon sequestration: from mechanisms to predictions

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In recent years, the interest in understanding and predicting the stabilization of carbon in soils has drastically increased because of the role of soil carbon in mitigating climate change, maintaining soil fertility, preventing soil erosion, and in general upholding soil functioning. In my presentation I will outline how mechanisms for soil carbon change as one goes from the small scale of clays and aggregates to the field and landscape scale and eventually to the regional and global scale. As much as mechanisms change across spatial scales, they also change across temporal scales (i.e. from days and months, to years and decades, to centuries). The understanding of the influence of scales is important for predicting longer-term trends at bigger scales of soil carbon via modeling. However, modeling of soil carbon can also help with elucidating the scale dependency of soil carbon changes. Hence, I will argue that for a true understanding of soil carbon sequestration, we need to move across scales by a full integration of experimental and modeling studies.

High resolution mass spectrometry based metabolomics: from data acquisition to mining and chemometrics

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Untargeted screening of compounds based on high-resolution mass spectrometry, uses both accurate monoisotopic mass and isotope profile for identification of unknowns. Different extraction algorithms can be used with this purpose, having different susceptibility to false positives (that can account up to 15-20% of overall compounds). This limitation is overcome when tandem MS libraries are used, although this is rather an exception. In most cases, therefore, mass and retention time alignment and filtering are essential prior to results interpretation. This approach, when followed by a recursive identification procedure (identification, alignment and re-identification cycles) and/or by targeted approaches using inclusion lists, is effective in limiting false positive.

Besides usual statistics, covariance-based chemometrics (including principal component analysis, k-means and unsupervised clustering, PLS-DA), or the combination of both (e.g. in volcano plot analysis, that integrates ANOVA and fold-change) are essential tools to provide with meaningful data interpretations. When annotated databases are available, pathway analysis can be very informative as well.

These tools can be used in several research contexts that include descriptive studies (phytochemical profile, compounds identification, secondary metabolites across genotypes) as well as the investigation of biochemical and technological research (response to biotic and abiotic stresses, study of development processes, effect of management/processing technologies, etc.). In recent years, metabolomics have been successfully applied also to ensure food authenticity and traceability.

Proteomic approaches to study plant biochemistry

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Plant proteomics is aimed at studying the interconnections among metabolic pathways during plant biological processes and physiological responses. This goal is achieved integrating the comprehensive analysis of entire proteomes in cells and/or subcellular compartments with the detailed characterizations of enzymatic families and post-translational modifications. Hence, plant proteomics embraces several techniques, such as biochemical methods needed to manipulate complex protein samples as well as different electrophoretic and mass spectrometry approaches. Moreover, every technical choice has to be optimized according to experimental targets and plant species.

After a short overview of recent proteomic findings about abiotic stresses in crops and food production, the talk will describe the basic concepts underlying the experimental choices and the main electrophoretic and mass spectrometry approaches. To illustrate their suitability, some of our proteomic experiments, regarding relevant agricultural issues, will be described. For example, the study of the root proteome in maize (*Zea mays* L.) in response to different mineral nitrogen sources allowed the detailed characterization of isoforms and post-translational modifications of Glutamine Synthetase, a pivotal enzyme affecting the nitrogen use efficiency (NUE) in cereals. On the other hand, in order to examine the biochemical adaptation to water deficit in grapevine, we combined traditional mono-dimensional electrophoresis with novel mass spectrometry procedures for the simultaneous quantification of hundreds of proteins in complex samples. This approach allowed the comparison between two rootstock genotypes with different susceptibility to water deficit, highlighting interesting relations among abiotic stress, organ functionality, energy metabolism and hormonal balance in roots of *Vitis* spp.

Methods applied in the authentication and traceability of agricultural products

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Authentication and traceability of agricultural products has become a major issue and concern in antifraud and consumer protection in the past years and there is an increasing need by consumers for high quality food products with a clear geographical origin. For instance, the European Union released mandatory regulations to prevent frauds. Regulation no. 178/200 established the European Food Safety Authority and defined the general principles and requirements of food law. Thus, reliable analytical tools must be available along the food chain to verify the nature of food. Typically, several factors drive technique selection, including method detection limits, sample preparation, cost, and throughput. The various techniques available for testing food authenticity include ultraviolet, near infrared, mid-infrared, spectroscopy and spectrometry methods.

Isotopic ratio mass spectrometry (IRMS) has proven to be a powerful analytical technique to determine the geographic origin of agricultural products. IRMS is a technique that can distinguish chemically identical compounds based on their isotope values. In general, the isotopic composition of the constituents of agricultural products depends on various factors. Some of those factors can be expected to be indicative for the geographical origin while others are more related to the production factors including the use of fertilizers, feedings stuffs of farm animals, seasonal variations and geological factors (e.g., soil composition, altitude, etc). Especially, these latter factors that affect the stable isotope ratio can be used in assigning the regional origin of agricultural products.

Plant Genomics as an essential component of green system biology

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Genome sequencing is right at the heart of genome sciences and provides the platform for all advance studies. The excitement over genomics has not to be based on the production of sequence information but on its utility and applications. Genome sequencing teamed with generation of high-throughput data-sets, bioinformatics tools, functional and comparative studies can provide a road map for the next generation of agricultural research. The number of sequenced plant genomes and associated genomic resources is growing rapidly with the advent of both an increased focus on plant genomics from funding agencies, and the application of inexpensive next generation sequencing.

The discipline genomics has two integral elements, 'structural genomics' that deals notably with markers (landmark sequences), genome maps, cloned genome fragments, sequencing and gene discovery; and 'functional genomics' that aims to explain gene functions. Functional genomics is increasingly seen as a way towards understanding genes in isolation and within the complex networks in which genes interact.

The development of complete new technologies in biological research over the last 10 years (the foundations of green systems biology) might lead to a refinement of plant ecology (*e.g.* plant-environment interaction) and evolution as well as classical breeding and biotechnological approaches. The application of these approaches has several aims such as the improving of plant productivity and abiotic/biotic stress resistance in agriculture due to restricted land area, the increasing environmental pressures and the development of CO_2 -neutral plant resources for fiber/biomass and biofuels.

The aim of technologies such as transcriptomics, proteomics and metabolomics is the analysis of molecular data of living systems on a genome scale leading to genome-scale, dynamic molecular data in combination with a genomic template. Systems biology can be summarized as integrating experimental data, genome-scale reconstruction of metabolic networks and the derivation of mathematical models that are able to predict the molecular phenotype of the plant in its natural environment.

Soil Metagenomics: a history of conquests

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It is a small part, and a very thin one, of the terrestrial surface... (Galileo Galilei Pisa 1564 - Firenze 1642): the Soil. Less poetically, the soil is a mixture of broken rocks, minerals, organic matter, but, more than three quarters of life is below ground represented by living microorganisms. During their life they can exchange genetic information, that is the basis of the evolution, and the huge amount of genetic material represents the metagenome, a biological library where books are written in genetic words. Ever since Robert Koch established his pioneering postulates on the microbial nature of disease, the field of microbiology has centered on the process of cultivating individual microbial species. Growing an organism in pure culture has been the critical first step towards understanding the properties of a given microbe, producing the many impressive successes of microbiology in the 20th century. However, the limitations of culture-dependent studies were recognized by what has become known as the 'great plate count anomaly'. During the last 25 years microbiology has experienced a transformation that has altered microbiologists' view of microorganisms and how to study them: the metagenomics, the study of metagenomes recovered directly from environmental samples. Early environmental gene sequencing is revealing the vast majority of microbial diversity, it opens doors to a tremendous amount of scientific exploration and can lead to practical applications in many fields.

Food safety issues from the industrial perspective: a *helicopter* view & a focus on analytical strategies devoted to mycotoxins risk prevention

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In the food industry, it is necessary to check the quality of an ingredient to decide whether to use it. During production, processing, storage and transport of food and feed, a variety of potentially hazardous compounds may enter the food chain. The number of residues and contaminants (known and emerging) continue to grow, as well as increased consumers awareness and legal-toxicological implications. Frauds and adulteration risks are also growing tremendously. Globalization implies complicated import-export scenario: in this context, mold infection remains nowadays a challenge worldwide problem with more than 20% of the crops affected by mycotoxins and with economic losses estimated at billions of dollars (FAO data). Poor harvesting practices, improper drying, handling, packaging, storage, and transport conditions contribute to fungal growth and increase the risk of mycotoxins production. The incidence of mycotoxins in food crops can vary considerably from year to year depending on many factors, such as weather conditions and agricultural practices.

Analytical methods for the determination of mycotoxins in unprocessed raw materials and in finished products are highly needed in order to properly assess both the relevant exposure and the toxicological risk for humans and animals.

Liquid Chromatography hyphenated to Mass spectrometry (LC-MS) is nowadays the most flexible and effectiveness (high sensitivity and selectivity devoted to the development of multiresidual strategies) technique mainly used in order to determine mycotoxins in many different matrixes. Ultra High Pressure Liquid Chromatography (UHPLC) combined with High Resolution Mass Spectrometry (HRMS) is developing into a universal tool for a wide variety of toxicants (including mycotoxins), achieving also post acquisition interrogation of data and full scan experiments to look for unknown compounds.

Finally, there is an increasing interest for rapid screening tests for mycotoxins, to support the confirmatory\official methods (actually mainly based on HPLC or LC-MS), permitting to increase the speed at which the analysis is performed, the simplicity of the sample preparation and the low cost per analysis.

Microalgae for removal of inorganic compounds from water

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Algae are a large variety of photosynthetic species capable of growing in a variety of aquatic environments. Recent studies have demonstrated the ability of some microalgae to develop on non-conventional substrates, suggesting the possibility to treat nitrate and phosphates contaminated wastewater.

In the present work we assessed the possibility of growing the microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* in wastewater from a tomato hydroponics cultivation with the aim of obtaining the purification of the water from nitrate and phosphates.

The experimental layout was a plexiglass tank divided into eight compartments equipped with tubes to allow the air insufflation. The efficiency of the water decontamination was assessed by measuring the main water quality parameters at the beginning and at the end of experiment. Simultaneously, the growth of microalgae was measured on a standard substrate (BG11) as control.

After 56 days of microalgae inoculum application, the concentration of nitrates and phosphates in the wastewater decreased by 99% and 89%, respectively. Moreover, both species showed a slightly lower growth and an higher carbohydrate content in the wastewater respect to the control.

It has been shown that the microalgae *C. vulgaris* and *S. quadricauda* are able to grow into an agricultural wastewater leading to a significant reduction of the main eutrophic elements. Moreover, in these conditions, the capacity of the two species to produce an high percentage of carbohydrates suggests that they are promising materials as source of biomass for the production of bioethanol.

Study of the interaction between molecules and ecosystems in surfacewater photochemistry in connection with climate change

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The photochemical fate in surface waters of dissolved organic compounds, depends on the interaction between the molecules and the ecosystems. The phototransformation kinetics of a pollutant (pesticides and pharmaceuticals) depends on its reactivity toward different processes and on the ability of the water body to favor these processes. Different molecules could follow different phototransformation pathways in the same water body, and the same molecule could undergo different processes in different water bodies. To unravel the interactions between a molecule and an ecosystem, we need: to characterize the ecosystem photochemistry; to assess the compound photoreactivity through different phototransformation pathways in a water body. This work is made combining irradiation experiments and model approaches, and final testing of the model results with a field measures of the time evolution of the compound in the water body.

This work is expected to advance the knowledge about the phototransformation of organic compounds in surface waters. Photochemical processes often play a key role in the degradation of many primary pollutants and in the formation of secondary ones. However, the difficulty to generalize the results obtained in single studies prevents the prediction of the photochemical fate of a certain compound in a definite ecosystem and hampers a better understanding of the photochemical processes in surface waters.

These studies could be connected to climate changes using model since climate modification could vary the photochemistry of the water body and therefore the fate of the pollutants. Another focus of my work is to develop this *ad hoc* model that connect climate changes and water photochemistry.

Effects of herbicides on iron deficient monocots

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In order to meet the global demand for food, increases in agricultural production will be required in the coming years. Nutrients in soils are usually below the optimum level for crops. Moreover, they often attend in unavailable forms. One of the goals of the modern agriculture is to optimize the use of nutrients already present in the rhizosphere, for example by using the ability of the plants to influence the soil characteristics. In fact, they can transfer to roots up to 60% of the carbon fixed by photosynthesis; the latter can later be released in the soil as exudates (amino acids, proteins, etc.). These molecules are able to modify the availability of essential elements. Especially, monocotyledonous plants can release phytosiderophores to chelate iron and move it to the plant. In fact, iron (Fe) shortage cause strong reductions in plants productivity (iron chlorosis). As methionine (a sulphur-containing amino acid) is the precursor of phytosiderophores, Fe-acquisition is strictly related to S-metabolism. Since some herbicides act on photosynthesis, they also cause a lack of molecules available for exudation. Furthermore, they can interfere with Smetabolism, as sulphur is directly involved in plants protection-mechanism. The aim of the present study is to establish the effect of widely used herbicides on corn and barley plants grown on iron deficiency conditions, to better investigate the metabolic regulation presiding at roots exudation. The study will be carried out performing essays on roots exudation, Fecontent in plants, sulphur-metabolism, and gene-regulation mechanisms.

Plant proteomes unveil the abiotic stress effects

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The emerging technologies such as proteomics are able to investigate the adaptation of plants to environmental changes. Plant proteome dynamics under abiotic stress are investigated by a shotgun proteomic approach. In particular, environmental stresses such as salinity and heavy metals on soil, have adverse impact on crop productivity and are taking into account in our research activities. Proteomics technologies point out to deciphering mechanisms for abiotic stress resistance and tolerance in plants. The high-throughput proteomic approach should allow the implementation of new breeding strategies for improvement of crop productivity under environmental changes, including not agricultural soil or adverse condition.

Different plant tissues are analyzed taking into account proteome changes under control condition and applied stresses. Protein extracts from leaf, root and berry of some horticultural species grown under salinity or heavy metals contaminated soils are analyzed to elucidate mechanisms of tolerance. Shotgun tandem MS proteomics permits detection and identification in quali- and quantitative mode of proteins involved in response to applied stress. Proteins identified are useful to understand how environmental signals trigger plant responses and the physiological pathways being modulated.

In some cases, cytoplasmic high abundance proteins hinder the detection of lowabundance proteins. In plant leaves, RuBisCO comprises of large percentage in the total protein extracts, thus an optimized method for RuBisCO depletion is considered to deepen proteome elucidation and improving proteome coverage. Some RuBisCO depletion methods are evaluated concerning the different yield in protein detection and the applicability in gel-free button-up proteomic approaches.

The interplay between photorespiration and iron deficiency: a preliminary study in *Cucumis sativus* L.

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Iron (Fe) is an essential element for all living organisms being a cofactor of many metabolic processes such as photosynthesis, respiration, DNA synthesis. The most noticeable effect of Fe deficiency in leaves is a marked leaf chlorosis caused by a lack in chlorophyll biosynthesis which might results in a strong limitation of photosynthesis. In these conditions the generation of reactive oxygen species (ROS) can alter the redox state of the cell, inducing oxidative stress which can damage the photosystem II (PSII) and, at times, the photosystem I (PSI). Photorespiration can be considered as a cycle operating between chloroplasts, peroxisomes, mitochondria and cytosol, which helps to protect photoinhibition excessive accumulation ROS. plants from and of Metabolic characterization of *Cucumis sativus* plants grown at different Fe concentration was performed to investigate the interplay between Fe deficiency and photorespiration. In vivo analysis of photosynthetic parameters suggests limitations in photosynthesis and induction of other energy dissipation processes. Purification of the peroxisomal fraction to carry out Western Blot analysis and activity assays of enzymes belonging to photorespiration was undertaken. The activity of Fe-dependent enzymes involved in photorespiration was lower in Fe deprived plants. Our data, together with previous, suggest that the imbalance induced by Fe deficiency in the photosynthetic machinery may be balanced by increased rate of photorespiration. Future prospect will be the proteomic characterization of Fedeficient organelles involved in photorespiration.

Spectroscopic characterization of digestates obtained from sludge mixed to increasing amounts of fruit and vegetable wastes

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Anaerobic digestion (AD) represents an efficient waste-treatment technology during which microorganisms break down biodegradable material in absence of oxygen yielding a biogas containing methane.

The aim of this work was to investigate the transformations occurring in the organic matter during the co-digestion of waste mixed sludge (WMS) with an increasing amount of fruit and vegetable wastes (FVW) in a pilot scale apparatus reproducing a full-scale digester in an existing wastewater treatment plant. Samples comprised: sludge, FVW, sludge mixed with 10-20-30-40% FVW. Ingestates and digestates were analyzed by means of emission fluorescence spectroscopy and FTIR associated to Fourier self deconvolution (FSD) of spectra.

With increasing the amount of FVW from 10% to 20% at which percentage biogas production reached the maximum value, FTIR spectra and FSD traces of digestates exhibited a decrease of intensity of peaks assigned to polysaccharides and aliphatics and an increase of peak assigned to aromatics as a result of the biodegradation of rapidly degradable materials and concentration of aromatic recalcitrant compounds. Digestates with 30 and 40% FVW exhibited a relative increase of intensity of peaks assigned to aliphatics likely as a result of the increasing amount of rapidly degradable materials and the consequent reduction of the hydraulic retention time. This may cause inhibition of methanogenesis and accumulation of volatile fatty acids.

The highest emission fluorescence intensity was observed for the digestate with 20% FVW confirming the concentration of aromatic recalcitrant compounds in the substrate obtained at the highest biogas production.

Effect of decreasing levels of Fe availability on S assimilation pathway in durum wheat (*Triticum durum* L.) seedlings

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Iron is an essential element required for many crucial cellular functions. Studies in many different organisms have shown that Fe deficiency induces several responses including increased uptake of Fe from the environment. In plants, it is achieved through the activation of several components of the Strategy I and II Fe uptake system. Wheat belongs to Strategy II plants and therefore cope with Fe deficiency by increasing both the synthesis and secretion of phytosiderophores (PS), and the uptake of Fe-PS complexes. Besides the processes described for Strategy II, sulfate assimilation pathway is known to be induced upon Fe deprivation in various plant species (maize, barley and wheat), most likely because PS are derived from nicotianamine, whose precursor is methionine.

Aim of this study was to investigate if sulfate assimilation rate could be modulated in an Fe concentration-dependent manner. To this purpose, durum wheat seedlings were grown hydroponically for eight days with Fe(III)-EDTA concentrations ranging from 0 to 75 μ M.

The amount of PS released by roots gradually increased with decreasing external Fe concentrations, this result being correlated to the increase in root thiol concentration. This was supported by roots showing higher activity of both ATP sulfurylase and *O*-acetylserine(thiol)lyase, the first and the last enzyme of S metabolism, respectively.

These preliminary results suggest that the capability to induce sulfate assimilation pathway when plants sense the onset of Fe limitation seems to correlate with the general S use efficiency of a plant species as an ecological adaptation to Fe deficiency.

Assessment of carbon sequestration potential in soil and in belowground biomass of six perennial bioenergy crops

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The objective of the research was to identify the bioenergy-crop with the greatest carbon (C) sequestration potential among six perennial bioenergy-crops (woody: poplar-black locust-willow; herbaceous: giant reed-miscanthus-switchgrass) at the sixth year from plantation and in the same location.

Firstly, the variations of soil organic carbon (SOC) stock for the first 1 m soil depth and seven SOC fractions for the firsts 30 cm of the soil under the perennial bioenergy-crops compared to an adjacent arable field were measured; secondly, the root biomass allocation along the same soil profile were considered.

The results confirm that the establishment of perennial bioenergy-crops in previous arable fields can be a suitable option to sequester C belowground. However, a different C sequestration capacity was observed between woody and herbaceous crops: woody species showed the greatest SOC sequestration potential increasing the SOC stock and the physically-protected SOC fraction (iPOM) (annual rate: 105 and 25 g C m⁻² yr⁻¹ for the SOC stock and the iPOM respectively) in the first 10 cm of soil but their ability to allocate root biomass in the deeper soil layers was limited (1.2 t ha⁻¹ of root biomass under 30 cm of the soil); while, the herbaceous species allocated a high amount of root biomass in the deeper soil layers (3.7 t ha⁻¹ of root biomass under 30 cm of the soil), but only switchgrass and miscanthus sequester C in the first soil layer (annual rate: 80 and 20 g C m⁻² yr⁻¹ for the SOC stock and the iPOM respectively).

Simultaneously driven microbial N immobilization and denitrification in temperate paddy soils

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In temperate paddies nitrogen (N) dynamics are driven by different biotic and abiotic processes including soil immobilization and losses. This may significantly affect nutrient bioavailability during the rice cropping season, as well as environment quality. In this study we evaluated changes in the distribution of fertilizer ¹⁵N among available, immobilized and lost pools, with special emphasis to specific microbial communities simultaneously performing immobilization and denitrification processes.

A typical rice soil was incubated under different redox conditions, with or without rice straw, and fertilized with either ¹⁵N enriched or non-enriched ammonium sulphate. The ¹⁵N tracer was followed in different chemical and physical N pools and by means of molecular fingerprinting and stable isotope probing techniques. To evaluate the importance of abiotic factors, a parallel experiment under sterile conditions was carried out. Results suggested that abiotic processes were responsible for fast N immobilization (45-53% of applied N), independent of redox conditions. However, with time, biotic processes prevailed on abiotic ones. Addition of rice straw enhanced biotic N immobilization, particularly under flooded conditions, limiting N losses up to 25% with respect to 60% in the absence of straw. These losses were corroborated with the response of archaea *nosZ* gene, which controls the completion of denitrification. N assimilation and immobilization appeared to contribute to this response, driven by different C and N resources availability.

The results highlighted the predominant microbial regulation of N cycling, with particular attention to the ecological implications of archaea on controlling N immobilization and losses in temperate paddy soils.

Biochar to remediate water and soil contaminated by organic pollutants

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Among the most dangerous pollutants released by human activities into the environment, polycyclic aromatic hydrocarbons (PAHs) and chlorophenols (CPs) are also listed because they can cause serious effects, such as disease and death in humans and animals.

Recently, among various materials, biochar arises particular interest due to high porosity and remarkable capacity to absorb pollutants.

Therefore the aim of the present study was to evaluate the efficiency of biochars from poplar and conifer in water and soil remediation. The attention was focused on two different organic contaminants, phenanthrene (Phe) and pentachlorophenol (PCP), as representative of polycyclic aromatic hydrocarbons and chlorophenols, respectively, having different structure and probably interaction with biochar. A combination of biochar and compost was also tested in the treatment of contaminated soil, to verify a possible synergistic effect of organic amendment able to achieve also pollutant degradation.

The remediation process was influenced by both the nature of contaminants and biochar. Hydrophobic contaminants or however less hydrophilic, like Phe and PCP, found higher affinity for biochar having more hydrophobic surface properties such as biochar from conifer. In addition the amount of biochar added in remediation test affected the treatment efficiency, especially for PCP.

All these factors as well as remediation time was an important factor in favouring the immobilization of contaminants on carbonaceous matrices thus limiting their bioavailability. The use of biochar can be considered a valid *in situ* remediation technique, although further investigations need to be carried out to confirm these interesting results.

Soil Ecosystem Services Provided by Bioenergy Buffer Strips: the HEDGE-BIOMASS project

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The HEDGE-BIOMASS project addresses the promotion of a new perennial linear landscape element: bioenergy buffers. Bioenergy buffer strips are narrow arable field margins converted to warm-season grasses and short-rotation woody crops for bioenergy applications. Within the HEDGE-BIOMASS project a 2 year integrated field and laboratory study was set up to monitor the following soil-based ecosytem services: biogeochemical cycling of C and nutrients (N,P), climate change mitigation (GHG emissions) and water quality regulation. With a litter-bag approach, the biochemical mechanisms driving the interactions among organic matter stabilization, litter chemistry, microbial biomass and extracellular enzyme activities during the decomposition of bioenergy leaf litter are studied. Furthermore, using a 3-source-partitioning approach ¹⁴CO₂-labelled switchgrass plants are grown on a soil after C3-C4 vegetation change to reveal mechanisms and drivers of priming effects induced by C rhizodeposition. To elucidate the mechanisms of soil organic C storage the dynamics of C within aggregate-size fractions are being tracked from the establishment of bioenergy buffers. Automated closed chambers are used on the field trials to estimate GHG emissions and particularly to study soil CO₂ pathways and N₂O hotspot emissions. Linking ecoenzymatic stoichiometry to litter/root/soil resources, GHG emissions, soil aggregates dynamics and microbial biomass dynamics, new insights into key mechanicistic processes for nutrient cycling and carbon sequestration in bioenergy cropping systems will be gained. With regard to water quality regulation, a network of piezometers on shallow groundwater has been installed to monitor monthly the bioenergy buffers removal efficiency of N-NO₃. This research has been set up to contextualize bionergy buffers into the EU renewable energy and CAP 2014-2020 policies. Our main goal is to prove that if bioenergy crops are strategically planted along the water bodies and unproductive field margins, they can provide a large set of key ecosystem services for climate change mitigation and agroecosystem health.

Cadmium increases the critical concentration for external sulfate in *Arabidopsis thaliana*

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Sulfur (S) is an essential element for plants. The main S source is sulfate (SO₄²⁻), which is taken up by roots through specific plasma-membrane transporters. Once inside the plant, $SO_4^{2^2}$ is allocated to different sinks where it is first assimilated into cysteine (Cys), a key intermediate from which most of S containing compounds are synthesized. Considering the central role of Cys, it appears evident that both $SO_4^{2^2}$ uptake and assimilation have to be modulated to meet the demand for S arising from Cys consuming activities.

Several stresses increase the demand for Cys derived compounds, causing an increase in the activity of the SO_4^{2-} assimilatory pathway. An example of this has been largely described in plants exposed to cadmium (Cd) in which the activation of S-consuming adaptive responses increases the demand for SO_4^{2-} and S metabolites.

To better understand the relationship between Cd accumulation and S metabolism, plants of *Arabidopsis thaliana* were grown under a wide range of SO_4^{2-} concentrations, in the presence or absence of steady amounts of Cd, to evaluate if Cd changed the critical concentration for $SO_4^{2^-}$, defined as the external concentration of the anion which produces 95% of the maximum shoot fresh weight. Results indicated that Cd significantly changed the total demand for S and the critical value for $SO_4^{2^-}$. Moreover, the negative effects exerted by the lowest Cd concentration analyzed (0.1 μ M) on shoot growth decreased as $SO_4^{2^-}$ concentration in the medium increased, until to become negligible for $SO_4^{2^-}$ external concentrations higher than the critical one.

A proteomic approach to study microalgae metabolism in different growth conditions to optimize the synthesis of biomolecules of nutraceutical interest

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Algal ecology, physiology and biochemistry have been reviewed extensively over the decades. Microalgae of different origins have a tendency to resemble each other in terms of cell composition, particularly in the relative amounts of crude proteins, lipids and carbohydrates that they contain when grown under more or less optimal growth conditions. For a single species, on the other hand, the variation in cell composition may different many fold, according to the culture conditions under which it is grown; for example, numerous studies show that the biosynthesis and accumulation of lipids is enhanced in nitrogen-limited conditions. Furthermore, an increasing interest is addressed to nutraceutical properties. The optimization of the growth conditions as well as the recognition of the factors influencing the synthesis of these biomolecules are crucial aspects from the technological point of view. "-Omic" approaches represent an innovative tool for in-depth analysis of the metabolic responses to environmental growth conditions in several organisms. The aim of the project is to improve information about metabolism of Arthrospira platensis, Phaeodactylum tricornutum and Pavlova lutheri, microalgae known to synthesize high levels of fatty acids and antioxidant compounds like a ω -3 and ω -6. Classical biochemical approaches and 2-DE technique will be used to show whether the effects of the changed growth conditions (physical and/or chemical factors) could have a positive influences on the synthesis of the biomolecules. Particular attention will be turn to the effects of nutrient availability in the growth medium, such as nitrogen. This research started from November 2014 and will be performed all along the Ph.D. (2014-2017).

The contribution of the soil biophysical properties to the management of temperate agrosystems

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Several studies point to the influence of soil aggregation on microbial communities and their activities. The soil aggregates, or more in detail their pores are habitats where microorganisms live and biogeochemical processes occur.

The most widely used traditional approaches for the aggregates separation are based on the wet sieving or on density fractionation. On the obtained aggregate fractions it is then possible to determine the microbial biomass functionality. In contrast with the standard methods, in this project we will study fractions of macro and microaggregates separated on the base of the different breakdown mechanisms induced by water.

The goal is to identify and to test biophysical, innovative and effective indexes for the assessment of the maintenance or improvement of soil quality induced by different management procedures, in forest and agricultural ecosystems.

More in detail, for the forest ecosystem the study concerns an oak wood and an alfalfa grassland located in the Apennine mountain belt, while for the agricultural ecosystem agrarian sites differently managed for their fertility will be considered in Bologna plain.

For both ecosystems the main pools of organic matter and microbial activity will be investigated both in soils and in aggregate fractions. The microbial activity evaluation concern the quantification of extracellular enzyme activity using fluorimetric assays in microplate scale.

The obtained data will be related to the data of the aggregates stability and soil porosity in order to identify the representative parameters of soil quality and, therefore, the useful indicators for the assessment of the soil quality changes caused by the different management.

Selection, application and metal recovery from hyperaccumulating plants grown on waste incineration residues

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Sewage sludges as well as ashes and slags from waste treatment and incineration plants are known accumulation sinks of many elements that are either important nutrients for biological organisms (phosphorus, potassium, magnesium, etc.) or valuable metals in pure form (nickel, chrome, zinc, etc.); they are also serious pollutants when they occur at anthropogenic end- of-stream points. Often more than 90% of these same metals have to be imported from abroad for technological use.

The project BIO-ORE aimed to explore new pathways to concentrate metals from diluted sources such as sewage sludge and wastewater by using highly efficient biological absorption and transport mechanisms. These enzymatic systems from plants work with very little energy input. The process is called bioaccumulation and can be most effectively observed in hyperaccumulating metalophytes, which are studied for its suitability to be incorporated in metal recovery processes.

In a systematic series of tests under laboratory conditions the accumulation behaviour for a variety of metals of a selection of candidate plants growing on different waste streams was assessed (quantitavely and qualitatively). The results provide the groundwork for further research that may bring to practical implementation a technological option with potentially huge benefits:

- The recovery of valuable metal resources from waste by environmentally friendly and low energy means
- Simultaneous decontamination of the input substrates from heavy metals
- Simultaneous generation of biomass, which can yield usable energy surplus

A national follow-up research project started in 2014, where two years of field trials with hyperaccumulative plants and further phytomining strategies will be developed.

Physiological and molecular characterization of magnesium deficiency in grapevine rootstocks with different tolerance

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Magnesium (Mg) is an essential mineral macro-nutrient for plants. Surprisingly, a relative limited scientific literature is available on its impact on plant physiology and crop production.

In grapevine, one of the most economically important fruit crop in the world, Mg deficiency often occurs in acidic and sandy soil with negative effects on quantity and quality of the final products.

In this work we assessed the responses to Mg starvation of two rootstocks known to differ in the field in their tolerance: SO4 (*Vitis berlandieri x Vitis riparia*) susceptible to Mg shortage and 1103 Paulsen (*Vitis berlandieri x Vitis rupestris*) with a good adaptability to Mg starvation.

The analysis of growth and physiological parameters performed after 4 and 14 days of starvation in microcuttings hydroponically cultivated, confirmed field observation that the two rootstocks differ in their tolerance. The increase of soluble sugars accumulation found in leaves of both rootstocks, shows, as occurs in other species previously characterized, that one of the first symptoms of Mg deficiency in grapevine is accumulation of sugars in leaves.

Microarray analysis performed comparing transcriptomic response in roots of 1103 Paulsen and SO4 to Mg deprivation, revealed several noticeable differences. After 4 days 219 genes are differentially expressed between the two genotype, most of them encoding proteins involved in metabolic process, transport and oxido-reduction processes. After 14 days the number of genes differentially expressed increases to 361 and increase the number of transcripts involved in abiotic-stress (e.g. heat shock proteins), transports and oxidative stress. These results suggest that the different adaptation to macronutrient absence is genetically based.

Ammonium transport in *Zea mays* roots: a physiological and molecular characterization

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In most soils, NH_4^+ and NO_3^- are the predominant sources of N that are available for plant nutrition. Although the average NH₄⁺ concentrations of soils are often 10-1000 times lower than those of NO₃, the difference in soil concentrations does not necessarily reflect the uptake ratio of each N source. Many plant species show a preference towards the absorption of NH₄⁺, as the process of assimilation does not require a reduction reaction, like the case of NO₃. Optimal plant growth is usually achieved when N is supplied in both forms. Previously works performed in rice and Arabidopsis have revealed the existence of two transport systems for NH4⁺ with high (HATS) and low (LATS) affinity. Members of ammonium transporter/methylamine permease/rhesus (AMT/MEP/Rh) family are involved in NH₄⁺ transport at concentration below 1 mM. In Arabidopsis thaliana 5 members of the AMT1 subfamily were identified. The genes AtAMT1:1, AtAMT1:3 and AtAMT1:5 are expressed in the root of N-deficient plants and their activity contributes mainly predominantly to the high-affinity transport of NH4⁺. Information regarding molecular aspects of NH₄⁺ uptake in maize is very limited. The purpose of this work is the biochemical and molecular characterization of NH4⁺ transport in seedlings of two maize inbred lines (Lo5 and T250) identified in field experiments as a high (Lo5) and low (T250) NUE lines.

Nutritional stress and epigenetic variations in plants

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Organisms are constantly exposed to environmental stresses, which have significant negative impacts on organism growth, development, and reproduction. They often determine the environmental and geographical distribution of plant species, and provides a selective evolutionary pressure on a given population.

Different strategies can be applied by living beings to minimize stress influence. Obviously, because of their sessile nature, it can be expected that plants have evolved sophisticated mechanisms to cope with unavoidable adverse environmental conditions. One of these mechanisms can be epigenetics, *i.e.* changes in gene expression and/or phenotype caused by mechanisms other than alterations in the primary DNA sequence.

DNA methylation is one of the best characterized epigenetic modification. The interest in this particular epigenetic mechanism derives from the possibility that this type of epigenetic changes induced by stresses could be transmitted to the progenies thereby stabilizing stress-dependent gene expression changes.

The aim of my PhD is to evaluate the potential role of DNA methylation in the plant response to stresses, using an experimental setup based on nutritional stress in the model plant *Arabidopsis*. Specifically, nitrogen deficiency stress has been chosen among many nutrients as it is the most prominent mineral nutrient required by plants. Moreover, it is known that nitrate, the source of N that will be used, is a signal eliciting the rapid gene expression of transporters and metabolism enzymes as well as changes in root morphology and physiology. Moreover I will investigate if epigenetic modifications influence the nitrogen efficiency, in particular nitrate acquisition, and if these changes are transmitted to the progeny of N-stressed plants.

Remediation of dredged sediments for its use as growing substrate

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Rivers and ports are periodically dredged to provide more efficient traffic and to prevent environmental pollution (Bianchi et al, 2010). In Europe, dredging produce about 200 million $m^3 y^{-1}$ of sediments (Bortone et al., 2004), resulting in serious management problems. This issue gets worse as large part of sediments turn out to be polluted by organic and inorganic contaminants. Although sediments are generally landfilled, several decontamination technologies, originally tested for contaminated soils, are potentially applicable to polluted sediments remediation; therefore clean sediments can be employed for many "beneficial use".

The principal aims of my research are (1) trialling co-composting process for sediment's conversion into a fertile artificial soil and (2) testing treated sediments as growing medium for plants.

In co-composting experiment slightly polluted sediments from Navicelli canal (Pisa, Italy) have been mixed with pruning residues from Florence urban green (1:1, 3:1 rates), and compared with two controls, only sediment and only pruning wastes. Composters have a volume of about 200L, with no gas or energy input. Chemical, eco-toxicological and microbiological analyses are periodically done. Co-composting products will be tested for plants growth.

Regarding sediments re-use as growing substrate, I am following an experimentation involved in CLEANSED project (LIFE-12-ENV-IT-000652). This trial employed Navicelli's sediments treated for three years by phytoremediation. Three ornamental species (Photinia x fraseri, Viburnum tinus, Eleagnus macrophylla) were transplanted on two sediment:soil mixes (1:1, 1:3 rates) and on soil-controls. Chemical, microbiological and eco-toxicological analyses are performed. It is expected that treated sediments have good performance as outdoor substrates.

Effects of plant essential oils used for weed control on soil bioindicators

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Herbicides could bio-accumulate in crops, affect soil ecosystem and pollute groundwater. Moreover, they could also affect directly human health by contact. To avoid some of the disadvantages arising from the utilization of traditional chemical herbicides, organic herbicides whose activity is based on allelopathic substances obtained from other plants are already available on the market.

Aim of this study was to evaluate the effects of essential oils extracted from different plants (*Eucalyptus camaldulensis*, Dehnh; *Eriocephalus africanus*, L.; *Thymus capitatus*, L.; Tangerine 'Clemenules' and Lemon 'Eureka') on soil microbial biomass and community structure, and on its activity. The experiment was carried out in a greenhouse using an orchard soil placed in pots of about 0.5 L. Three doses of each essential oil have been tested. The pots were maintained at 50% of the water holding capacity for 120 days after the essential oils were applied to soil. Samples were collected on the day 0, 15, 30, 90 and 120. Soil samples were used to determine microbial biomass carbon, microbial community structure and carbon mineralization.

In the poster, the results of this study will be reported and discussed.

Partitioning and mobility of heavy metals in particle size fractions of road-deposited sediments

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Road-deposited sediments (RDS) are a complex environmental media originating from a plethora of sources, both natural and anthropogenic, including soil dust, traffic, industrial emissions, pesticides etc. RDS are one of the main sinks of pollutants, including heavy metals, and are predominantly constituted of fine particles that can be easily re-suspended and transported by wind or traffic where they can enter the respiratory system and adversely affect human health. Many studies on solid environmental media, including RDS, have concentrated on total concentrations, but few studies have examined the geochemical fractionation of metals and assessed the mobility, availability and, consequently, the toxicity of trace metals. Furthermore, to our knowledge, no one work were done on the metal availability and bioaccessibility of RDS in the fine fractions (in particular, particles less than $10 \ \mu$ m), considered to be the most important in relation to human health.

Dust, sediment and soil samples will be collected from various urban and rural sites and chemical and physical properties will be determined as well as the partitioning of metals in the size fractions (<2 to 2000 μ m).

The main objectives of this work are: (1) to study the fractionation patterns of inorganic elements in RDS with grain size, (2) to quantify the partitioning of heavy metals in RDS with different techniques (e.g. EDTA, BCR, SBET, DGT) to assess the mobility and availability of contaminants.

Study of an innovative method to preserve stored grain in developing countries

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Surveys carried out in some Congolese villages in the surroundings of Kabinda showed that the preservation of grains after harvest is a serious problem. Insect infestations are the main cause of damage. Among coleopteran pests, the genus Sitophilus is widely distributed and it was often encountered during the inspections of our operating units in Congo. Many approaches are currently adopted to solve this problem, but they are often inadequate or insufficient, as the manual removal of visible insects. Chemicals are almost unused, both for their unavailability and their high costs. At the Faculty of Agriculture, Food and Environmental Sciences of Piacenza we are currently testing a new method to protect jute bags and other containers in which the grains are stored. The protection system consists in a fine-mesh polyester net coated with a synthetic pyrethroid that gives repellent and insecticide effect. Preliminary bioassays with adults of Sitophilus oryzae showed that a contact time shorter than 5 minutes is sufficient to knock-down 50% of insects, and 9 minutes to knock-down 95%. Further laboratory bioassays (in Italy) and field studies (in Congo) will be carried out to test the efficacy of the treated net. The coating of the structures for grain preservation could be a viable sustainable strategy of intervention for many populations in developing countries.

Effects of conventional and organic agronomic practices on soil features in different sites in Northern Italy

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The replant disease and yield decline affecting continuous crops are common effects of land degradation of agricultural soils. Two of the main causes of land degradation are intensive exploitation of crops and climatic characteristics. Soil sickness can be challenged by enhancing microbial biomass and diversity in agricultural soils. The aim of this PhD research is to evaluate the effect of different agricultural techniques on soil systems and to assess soil biological fertility in intensive cropping systems integrating different approaches. Two trials are being investigated: one study site belongs to a Natura 2000 Network site (Podere Pantaleone, Bagnacavallo, Ravenna) which had been a conventionally managed vineyard until the 1970's (now undergoing a renaturalization process); and the other study is an open-field trial conducted in Laimburg (BZ), Northern Italy (apple orchard set up during a 5 year period) and aims to test and compare the effects of different fertilizers, application timing and rate, tillage techniques and different locations on soil features and fertility. Chemical and biochemical parameters, as well as the molecular analyses of microbial communities (DGGE, high throughput sequencing) of soil microbiological communities were conducted. Data obtained are elaborated in order to describe the relationships between diverse parameters and to identify particular microbiota communities involved in soil supressiveness and in soil fertility.

Speciation, bioavailability and ecotoxicity of As in contaminated soils

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Arsenic (As) is a metalloid element, naturally associated with gold, sulphur, iron and heavy metals; it is often found at high concentration in soils and waste around former mines and industrial plants treating As-bare minerals. The risks associated with the uptake of this element, both for environment and human's health, make the As contamination an important issue of research. For this reason, my Ph.D. research deals with the speciation of As in contaminated soils, in order to evaluate its ecotoxicity and the its actual risks. In fact, bioavailability and mobility depend on the chemical forms and the oxidation states of the As, thus the most accurate speciation is required. This research will involve chemical, mineralogical and biochemical analyses. A multi-methodological approach based on X-ray techniques will be performed in our new μX -ray Lab. The mineralogy of the soils will be investigated via X-ray Powder Diffraction (XRPD), while wavelength dispersive X-ray fluorescence (WD-XRF) and also Total-reflection X-ray Fluorescence (TXRF) will be used for chemical analyses. Thin sections will be mapped with µ-XRF to evaluate the As distribution in soil. Earthworms (Eisenia andrei) exposed to the contaminated soil will be used for biological tests (e.g. Comet Assay and Neutral Red Retention Time on coelomocytes) to evaluate the ecotoxicity of As, while sections of the same exposed earthworms will be mapped with µ-XRF to evaluate the As bioaccumulation and distribution in their tissues. At present, soil samples coming from Valle Anzasca (Northern Piedmont) and Scarlino (Grosseto, Tuscany) are going to be analysed. Portable XRF performed in situ, revealed an As concentration ranging from about 300 to 21000 ppm for the area of the old plant of treating gold ore in Valle Anzasca, and from 150 to 800 ppm for two samples from Scarlino. Two soils with low Arsenic concentration sampled in the same area were chosen as control samples. Part of the soils sampled is being used for earthworms exposure experiments.

Genome-Wide association studies for the characterisation of 297 rice cultivars

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Rice is adapted to grow both in anaerobic (flooded) and aerobic soils. Changes in agroenvironmental and socio-cultural conditions, as well as the reduction in the availability of irrigation water in some temperate rice-growing areas, have led to the introduction and spreading of different cultivation practices with respect to traditional continuous flooding. It is well known that the soil water status affects plant development and qualitative traits of the rice grain, such as headness, calkness, and amylose content. On the contrary, poor information exists concerning the grain contents of dietary essential trace elements (Fe, Zn, Mn, Mg and Cu) or undesirable inorganic toxic elements (i.e. Cd and As). For better understand the effect of water management on the ions content in different rice cultivars, grain samples of 297 cultivars, grown in field trial, both in submerged and irrigated soil, as well as shoot samples of a subset of 134 rice cultivars grown for 40 days in a greenhouse under flooded soil condition, were carried out. The content of thirteen mineral elements in grain and shoot was determined with ICP-MS techniques. The shoot ions content was compared with the grain content of the same cultivars. Very slight correlations seem to exist, excluding the possibility to predict the grain ions content by analyzing their shoot levels in the early growth stages of the plant. The phenotyping result was correlated with the genotype diversity through genome-wide association (GWA) mapping using the diversity panel of the 297 cultivars and 53k single nucleotide polymorphism (SPNs). Some loci significantly associate with ion content variation in shoot and grain. The locus identification and characterization are in progress.

Phosphate solubilization, indole acetic acid and siderophores production of Plant Growth Promoting Rhizobacteria

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Plant Growth Promoting Rhizobacteria (PGPR) are a heterogeneous group of bacteria which influence plant growth by biofertilization, biostimulation and bioprotection against plant pathogens.

The aim of this study is the characterization of some plant growth promoting traits of rhizobacteria, such as phosphate solubilization, indole acetic acid and siderophores production.

PGPR were previously isolated on Nutrient Agar from soil of barley and tomato plants grown in the RHIZOtest system, in condition of Fe deficiency as well as of Fe sufficiency.

Two hundred colonies for each soil sample were screened for their ability to produce siderophores using CAS Agar plates and measuring the diameter of the orange zone.

Eighty isolates, showing positivity to siderophores production, were tested for their capability to solubilize phosphates and to produce indole acetic acid (IAA). Firstly, qualitative IAA production was detected by colorimetric method using Salkowski reagent; the presence of IAA will be further confirmed and quantified by HPLC.

Qualitative estimation of P-solubilization was carried out on Pikovskaya agar; quantitative estimation of P-content in NBRPI broth using the spectrophotometric method of ascorbic acid is in progress.

Eighty isolates were also characterized by phylogenetic analysis, by amplifying a region of the16S rRNA gene of about 1 kb and sequencing both strands of the amplicons. The resultant sequences were aligned to the NCBI database using BLASTn. Phylogenetic trees were carried out using the Seaview 4 software according the maximum likelihood method. The isolates belong to the genus of Pseudomonas sp., Azotobacter sp., Rhizobium sp., Chryseobacterium lathyri, Agrobacterium tumefaciens.

Magnesium transport in grapevine: molecular aspects

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Magnesium (Mg) is an essential macronutrient for plants, with an ubiquitous importance in plants physiology and biochemistry, being involved in photosynthesis, protein synthesis, and in cell energetic balance. Mg deficiency causes an impairment of carbohydrates partitioning between source and sink organs, leading in several plant species to a modification of the root/shoot ratio, and an accumulation of starch and soluble carbohydrates in source leaves. This can cause a reduced CO₂ fixation in source leaves, with photooxidative damage under high light conditions.

Despite the undisputable importance of Mg²⁺ in plant physiology, scientific literature regarding Mg²⁺ uptake and distribution within the plant is quite limited, in particular if woody plants like grapevine are concerned. At molecular level, the best known Mg²⁺ transport system in eukaryotes is MRS2 family, a family of transporters that belongs to CorA/Mrs2/Alr superfamily. The MRS2 transport protein was firstly identified and characterized in yeast and subsequently homologues were identified and characterized in *Arabidopsis thaliana* and *Oryza sativa*.

An attempt was made to characterize the molecular mechanisms underlying Mg²⁺ transport in grapevine; we firstly identified through bioinformatic analyses seven putative members of MRS2 family in grapevine genome. We started to clone one (VIT_05s0020g04720) of these genes in two different rootstocks, 1103P and SO4. Sequence alignment identified one conservative and two semi-conservative substitutions between 1103P and SO4. In order to prove the Mg²⁺transport activity of this protein, a chimeric gene for heterologous complementation of *Saccharomyces cerevisiae* strain DBY747 *mrs2* was constructed. Yeast strain transformation and phenotype complementation assays are now in progress.

Transcriptional and physiological aspects of Fe deficiency response in roots of *Zea mays*

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Plants react to iron (Fe) deficiency using different adaptive strategies. Under limited Fe availability maize, an economically important crop and a model species for *Strategy II* plants, improves Fe acquisition via the release of phytosiderophores (PS) into the rhizosphere and the subsequent uptake of the Fe-PS complex into root cells. In this work, microarray analysis identified 376 genes differentially modulated by Fe-deficiency in roots (289 up- and 87 down-regulated). Of particular interest, genes coding for many transcription factors and for the synthesis and release of PS were found induced by the nutritional stress. The capacity of maize plants to respond to the Fe-deficiency was further evaluated exposing roots to soluble or poorly soluble Fe-sources for up to 24 hours. Beside real time RT-PCR analyses, ⁵⁹Fe uptake experiments showed that the mechanisms involved in Fe acquisition were induced by the nutritional stress; however the downstream pathway involved in the translocation and distribution of the micronutrient within the plant were not yet activated in Fe-deficient plants.

Selenium biofortification of *Valerianella locusta* grown in a soil-less system

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The use of cultivation systems for the production of fresh-cut vegetables with a higher water-use efficiency, like soil-less cultivation, is particularly desirable in those regions where water, for its scarcity, price and quality, is becoming an economically limiting resource. Moreover, soil-less cultivation practices, such as floating system, may allow to harvest a cleaner product, with a consequent reduction of washing treatments, but also can represent an opportunity to enrich the product with beneficial elements for human health.

In order to improve, both qualitatively and quantitatively, the production of *Valerianella locusta*, the possibility to biofortificate the edible part with selenium (Se), an essential micronutrient for human, but not for plants, has been evaluated.

Se has been supplemented to the nutrient solution at a concentration of 0, 10 and 40 μ M. The results show that the presence of Se does not determine a significant variation of biomass yield, but increases the chlorophyll content; furthermore, a decrease of nitrate content in edible part, characteristic associated with the healthiness of this product, is observed. Selenium content in plant tissues rises with the increasing of Se concentration in growth medium. In addition, part of Se accumulated in these tissues is incorporated in the amino acids Se-cysteine and Se-methionine, the most available forms of Se for human nutrition.

Overall the results indicate that the soil-less cultivation of *Valerianella locusta* in the presence of Se may lead to a biofortification of the product similarly to what has been observed in other horticultural products, determining a qualitatively improvement of the vegetables with a consequent potential competitive advantage on the market of this improve corn salad product.

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