SOCIETA' ITALIANA DI CHIMICA AGRARIA

PhD Winter School

Rhizosphere at work: soil-plant-microbes interactions, from plant nutrition to soil remediation

17-20 February 2014,Piacenza, Italy

Organizing committee: Tanja Mimmo, Gian Maria Beone, Luciano Cavani, Edoardo Puglisi, Nicola Tomasi, Roberto Terzano, Marco Trevisan, Stefano Cesco, Claudio Ciavatta

Book of Abstracts

University of Bari University of Bologna University of Bozen-Bolzano University of Piacenza University of Udine

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

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Organizing committee:

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Gian Maria Beone - UniPC
Luciano Cavani - UniBO
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Nicola Tomasi - UniUD
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Speakers:

Tanja Mimmo – UniBZ
Nicola Tomasi – UniUD
Gianpiero Vigani - UniMI
Luciano Cavani – UniBO
Anita Zamboni – UniVR
Stefania Astolfi – UniVT
Daniele Del Buono – UniPG
Markus Puschenreiter – UniBoku (A)
Ilenia Cattani – UniPC
Maria Chiara Fontanella - UniPC
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Chairmen and organizers of the Team work and discussion:

Edoardo Puglisi - UniPC
Matteo Spagnuolo - UniBA
Vito Armando Laudicina - UniPA
Gian Maria Beone - UniPC

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Aim of the School

The aim of the school is to give an insight into the complex processes occurring at the soil-root interface and how these processes can be exploited to improve plant nutrition and to remediate polluted soils. In particular, lectures will focus on new innovative methodologies applied in rhizosphere research.

Scientific program

17 February 2014
afternoon: arrival and registration

15.00-15.30: Introduction of the School

afternoon session: ***RHIZOSPHERE AND ROOT EXUDATION***
Chair: Edoardo Puglisi

15.30-16.15 Tanja Mimmo (FaST, Free University of Bolzano, Italy) The rhizosphere – "the underground information superhighway"

16.15-17.00 Nicola Tomasi (DiSA, University of Udine, Italy) How are exudates released from roots?

17.00-17.15 Break

17.15-18.00 Gianpiero Vigani (DiSAA, University of Milano, Italy) Where do root exudates come from? Metabolic reprogramming under nutrient deficiency: Fe nutrition as a case study

18:00-18:30 Team work and discussion*: Experimental approaches to assess roots exudation and address the complexity of tripartite soil-plant-microbiota interactions in the rhizosphere*****(*under supervision of the chair)*

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18 February 2014

morning session **SOIL MANAGEMENT AND PLANT NUTRITION**
Chair: Matteo Spagnuolo

University of Bari   University of Bologna   University of Bozen-Bolzano   University of Piacenza   University of Udine

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9.00-9.45 Luciano Cavani (DiSA, University of Bologna, Italy) Organic Fertilizers: impact on soil fertility

9.45-10.30 Anita Zamboni (DiBiotec, University of Verona, Italy) Regulation of mineral nutrition uptake in the context of root-rhizosphere relationship

10.30-11.00 Break

11.00-11.45 Stefania Astolfi (DAFNE, University of Tuscia, Italy) Sulphur in nutrient interactions

11.45-12.30 Daniele del Buono (DiSAA, University of Perugia, Italy) Agrochemicals: plant detoxification and interferences on vegetal nutrition

12.30-13.00 Team work and discussion*: Plant nutrition research: challenges to meet the need for food with a sustainable approach*****_(under supervision of the chair)_

afternoon session: **PRACTICAL SESSIONS**

14.30-17.30 Tanja Mimmo, Markus Puschenreiter, Ilenia Cattani Methods in Rhizosphere Research: Rhizoboxes and Rhizotest - limitations and implications

17.30-18.30 Nicola Tomasi How to approach a plant study in a plant-soil experimental system?

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19 February 2014

morning session **SOIL MICROBES AND THEIR INTERACTIONS WITH PLANTS**
Chair: Vito Armando Laudicina

9.00-9.45 Youri Pii (FaST, Free University of Bolzano, Italy) Interactions between plants and microorganisms: towards a better nutrient efficiency?

9.45-10.30 Edoardo Puglisi (University of Piacenza, Italy) Structure and activity of soil microbes as revealed by genomics and metagenomics approaches

10.30-11.00 Break

11.00-11.45 Davide Bulgarelli (College of Life Science, University of Dundee, UK) Underground: structure, functions and host control of the microbiota thriving at the root-soil interface

11.45-12.15 Team work and discussion*: Molecular vs. biochemical methods to study the soil microbial community*****_(under supervision of the chair)_

12.15-13.00 Selected presentations by PhD students

afternoon session: **PRACTICAL SESSIONS**

14.30-16.30 Edoardo Puglisi,Davide Bulgarelli Digging into the numbers: from Data Count Matrix to (almost) PQ pictures using R

16.30-18.30 Roberto Terzano, Maria Chiara Fontanella X-ray and mass analytical methods to study micronutrients and trace element pollutants in soil and plants

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20 February 2014

morning session: **SOIL POLLUTION AND DECONTAMINATION**
Chair: Gian Maria Beone

9.00-9.45 Ilenia Cattani (ICAA, University of Piacenza, Italy) Soil contamination and bioavailability concept

9.45-10.30 Roberto Terzano (DiSSPA, University of Bari, Italy) Soil remediation: an overview

10.30-11.00 Break

11.00-11.45 Giancarlo Renella (DiPSA, University of Firenze, Italy) Restoration of functionality of polluted soils and sediments: evidences from the GREENLAND and CLEANSED projects

11.45-12.30 Markus Puschenreiter (Institute of Soil Research, BOKU University, Wien, Austria) Rhizosphere processes involved in phytoremediation of trace element-contaminated soils

12.30-13.00 Team work and discussion*: Strategies and novel technologies for elemental speciation in rhizosphere soil solution ******(under supervision of the chair)*

13.00-13.30 Conclusions

The rhizosphere – “the underground information superhighway”

Tanja Mimmo

Faculty of Science and Technology - FaST, Free University of Bolzano, Bolzano, Italy, tanja.mimmo@unibz.it

The rhizosphere is defined as the volume of soil influenced by root activity where the chemical (pH, redox potential, nutrient concentration and root exudates), physical (temperature, water availability and soil structure) and biological characteristics (microbial associations) are shaped by plant-microbe-soil interactions. Communications between roots and between soil microorganisms and roots, i.e. rhizosphere processes, occur and change continuously influencing for instance the nutrient solubility, transport and uptake. The main driving force of these mechanisms seems to be related to root exudation processes. Root exudates comprise in fact a number of different organic (e.g. low molecular weight and high molecular weight organic compounds) and inorganic (e.g. protons and HCO_3) chemicals and can reach concentrations up to 250 mg C /g root produced of the photosyntheticaly fixed carbon. Both quality and quantity of these compounds is however influenced by soil type, plant species, plant age, nutritional status, climatic conditions, etc. Rhizodepositons or root exudates have different functions and effects and are involved in many pedogenetic and rhizospheric processes as: a) they modulate the bioavailability of essential and non-essential elements; b) they serve as protection against chemical and physical stress, c) they serve as carbon source for bacterial and fungal microbes and d) they have signaling functions to attract and/or repel microorganisms.

The high complexity inherent to rhizosphere processes and the limited size - from several millimeters in case of very soluble nutrients like nitrate and volatile compounds to < 1 mm for insoluble nutrients like phosphate – represent the main technical and methodological challenges. In addition, the difficulties in determining these processes arise on one side by the concentration of rhizodepositions, often in the nmol-pmol range, on the other by their high turnover rate, inter-conversion, adsorption and degradation processes. The selection and development of appropriate methods is therefore crucial to avoid under- or overestimations. However, most of the studies were performed in laboratories under artificial conditions, mainly hydroponic systems. As a drawback these techniques may provide data that are not representative for soil conditions, since quantity and quality of root exudates may largely differ from a soil environment (different chemical, physical and biological conditions, different root architecture, no root hairs) and roots may be damaged during the sampling procedure. In soil environment, the following approaches have been used so far: a) removal of roots from the soil and monitoring carbon loss after placing the roots in a solution; a) physical capture of exudates from roots, which are exposed in purpose-built microcosms using agar or filter-paper strips; a) localized collection of rhizosphere soil solution obtained from rhizoboxes or root windows under field conditions. All types of intervention might create artifacts in terms of both quality and quantity of exudates measured, because of the disturbance involved. In this context, sampling techniques may suffer from additional bias via adsorption of root exudates on the material used. The combination of rhizobox systems which permit the sampling of rhizosphere soil solution at a temporal and spatial resolution, with high end analytical techniques might overcome these limitations.

How are exudates released from roots?

Nicola Tomasi

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Low nutrient availability in soils limits greatly plant growth. To increase the availability of many nutrients, plants induce the release of wide array of molecules (e.g. carboxylates, carbohydrates, amino acids, phenolics, strigolactones, wax, enzymes). Only in the last years, some evidence about the mechanisms involved in the root release has been discovered.

The lecture will focus on these evidence, presenting a short general overview of root exudation and all the families of proteins that have been found to be involved in this process, i.e. Major Facilitator Superfamily (MFS), ATP-binding cassette transporters (ABC), Multidrug and toxin extrusion proteins (MATE) and Al-activated Malate Transporters (ALMT). Presenting the latest results about the transporters that have been functionally characterized to be involved directly in the release of root exudates. For examples, the membrane protein responsible of phytosiderophore release in barley/rice/maize (TOM1), the scopoletin and strigolactones “extruder” (ABCG37, PDR1), Phenolics Efflux Zero 1 and 2 (PEZ1-2), some MATEs and ALMTs involved in the release of organic acids and flavonoids. Along with the presentation of these different examples, the techniques used for the isolation of the coding sequence and those used for the characterization of the transporters will be deeply discussed.

Where do root exudates come from? Metabolic reprogramming under nutrient deficiency: Fe nutrition as a case study

Gianpiero Vigani
Silvia Donnini, Liliana Tato, Patrizia De Nisi, Marta Dell'Orto, Graziano Zocchi

Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Milano, Italy, gianpiero.vigani@unimi.it

Root exudates could be considered as communicating molecules used by plants to interact with soil. In response to particular environmental conditions, plants specifically synthesize such molecules by the induction of specific reprogramming of the whole metabolism. Therefore, to fully understand the origin of root exudates, the investigation on the metabolic adjusted mechanisms induced in plants by soil factors becomes crucial.

Iron (Fe) deficiency chlorosis is a major nutritional disorder for crops growing in calcareous soils, causing decrease in vegetative growth. Notwithstanding its abundance Fe mainly exists, in well aerated soils, as scarcely soluble compounds not freely available to plant uptake. However, Fe is an essential element for the plant growth, since it is fundamental in many metabolic reactions. Indeed, Fe is required as cofactor for a wide range of enzymes belonging to both respiratory and photosynthetic electron transport chains in mitochondria and in chloroplast, respectively. Therefore, its imbalance would affect the whole cellular metabolism, leading to a decrease in crop yield. To overcome this nutritional disorder, plants have evolved particular adapting strategies aimed at reprogramming the whole metabolism in order to take up more Fe from soil and to survive in a low Fe condition at the same time (for instance the induction of glycolysis and fermentative pathways bypassing the affected mitochondrial functionality). Under Fe deficiency several plant species extrude a huge amount of compounds/metabolites (ROC_S) into the root apoplast and rhizosphere. The main ROC_s are i) carboxylates (i.e. citrate and malate), originated by the primary metabolism and ii) a plethora of compounds such as phenolics and flavins, which are produced by the secondary metabolism.

Iron deficiency affects mitochondrial activity in root tissues leading to an accumulation of carboxylate compounds which provide, for instance, carbon skeletons to chlorotic leaves as well as reducing equivalents for Fe uptake mechanisms. Carboxylates act also inside the plant as Fe-chelators, allowing Fe translocation through the plant. As well, phenols act as Fe-chelators in plant tissues. It was demonstrated that phenolics could play an important role in facilitating the reutilization of apoplastic Fe in roots. Under Fe deficiency, carbohydrates could be diverted into secondary metabolism to produce phenols, which can be accumulated in plant tissues and/or extruded into the rhizosphere. Both oxidative pentose phosphate pathway and Calvin cycle provide carbon skeletons as erythrose-4-phosphate, which along with PEP formed from glycolysis, are used as precursors for the shikimic acid pathway. This pathway converts carbohydrates into aromatic amino acids leading to the synthesis of various phenolic compounds.

All the findings obtained so far suggest that root exudates can be synthesized both as a specific response to mobilize Fe compounds outside the cell and as unused/accumulated compounds in the cell as a result of reprogramming some metabolism pathways in response to Fe deficiency.

Team work and discussion

Experimental approaches to assess roots exudation and address the complexity of the tripartite soil-plant-microbiota interactions in the rhizosphere

Edoardo Puglisi

Istituto di Microbiologia, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy, edoardo.puglisi@unicatt.it

The rhizosphere is a tripartite system where soil components, microorganisms and plant roots interactions determine fundamental ecological processes such as the turnover of nutrients, the stimulation of microbial activities by means of root exudation processes, the promotion of plant health and productivity by the so-called plant growth promoting bacteria (pgpb). Sound experimental approaches are necessary to address these processes under conditions that should resembles as much as possible real-field conditions, reduce the disturbance on plant and microbes in order to provide meaningful and relevant results. Multidisciplinarity is a key point: experimental approaches should allow measuring altogether physical, chemical and biological variables. The team work will stimulate a discussion on the available tools for rhizosphere studies, with examples on rhizoboxes, rhizon samplers, molecular methods and chemical analyses of metabolites produced by plants and microorganisms.

Organic Fertilizers: Impact on Soil Fertility

Luciano Cavani

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The organic fertilization is an ancient practice, manure was used by Europe’s first farmers 8,000 years ago. Since the introduction of mineral fertilization its importance, especially economic, declined steadily, without being reduced to a mere marginality.

In recent decades, the spread of organic farming, the gradual decline of soil fertility, the need to reduce energy inputs to improve the sustainability of agricultural production have led resources and interest in this ancient practice. Biofertilizers, organic manures, biosolids, organo-mineral fertilizers, biostimulants, have emerged as promising component of integrating nutrient supply, promoting and enhancing agroecosystems health, biodiversity, and biological soil activities.

Nevertheless, organic fertilizers are material added to the soil in order to supply organic matter and one or more plant nutrients essential to the growth of plants. Therefore, it is not rational to use organic fertilizers without a thorough assessment of its effect on soil fertility. In fact, even the organic fertilizers are charged of different negative effects, such as the addition of organic and inorganic compounds (and pathogenic organisms) potentially toxic to plants, animals and humans; the increase of GHG and ammonia emissions; the nitrates leaching, etc... The assessment of each fertilizers requires proper physical and chemical characterization. Normally, organic fertilizers are evaluated according to their content of organic matter and nutrients for the plants, but the new type of organic fertilizers require a more structured approach. In example, an organic biomass (i.e. compost, biosolids, cow manure, etc.) to have a positive impact on soil fertility should be stable, and mature. The terms “stable” and “mature” are not generic, but identify well-defined properties, which should be accurately assessed. More, an organic nitrogen fertilizer should be assessed not only for your (organic) nitrogen content, but for the fraction of nitrogen potentially mineralizable, and for the release kinetic (in agreement with the nitrogen using efficiency approach).

Furthermore, the concept of soil fertility has been subject to an extensive revision and redefinition. Therefore, the evaluation of impact of organic fertilizers on soil fertility should adopt new methods of measurement and new assessment tools.

Regulation of mineral nutrition uptake in the context of root-rhizosphere relationship

Anita Zamboni

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Higher plants need at least 14 mineral nutrients for growth, development and reproduction. In the soil environment plants frequently cope with fluctuating and/or limited availability of these nutrients. They often deal with limited accessibility of one or more essential nutrients that can negatively affect their growth. On the other hand, high concentration of some mineral can be toxic to plant cells. Therefore, they are able to sense changes in macro and micronutrient in the environment and to respond to these variations, being sessile organisms.

Plants display a high degree of physiological and developmental plasticity to respond to different nutrient conditions using both local and systemic signalling pathway in order to control the expression of genes related to these responses (e.g. transcription factors, riboregulators, ubiquitin-related proteins).

Roots are the major uptake sites for nutrient, while shoot depends on mineral import. Nutrient deficiency needs integration of responses and communication between different parts of plants. Therefore, root system is the organ that senses nutrient availability in the rhizosphere. Root signals, under nutrient starvation, can alert shoot and, for short-time starvation plants can use compartmentalized nutrients (e.g. from vacuole or mineral-containing molecules) while in a long-period shoot signals can induce an increase in nutrient uptake in roots.

Several molecules, including nutrients, hormones, sugars, and small RNAs have been suggested to play a role in systemic communication that is necessary to relay information about local conditions from root to shoot and vice versa. The regulation of nutrient uptake under fluctuating availability is mainly based on the modulation of uptake systems in roots both at transcriptional and post-transcriptional/post-translational levels. In addition, the products of assimilation metabolism of nutrient can be involved in feedback regulation of uptake systems (*e.g.* glutamine on NO_3^- uptake systems).

Transcripts abundance of ion transporter often changes with the concentration of the nutrient. Some transporters are induced by a decrease in substrate concentration, other are induced by an increase in substrate from nil to low supply. In fact, genes of high affinity sulphate (SULTR1) and phosphate transporters (PT) are up-regulated upon removal of S and P from growth medium while positive modulation of member of high-affinity transporter family (NTR2) is observed after addition of small quantity of nitrate (10-50 μM) to a N-starved medium. This nutrient is also able to increase its uptake after rapid exposure of roots to the anion (“induction”). As far as micronutrient are concerned iron is most studied. Plants have developed two strategies for its acquisition to cope with Fe shortage. In *Strategy I* model plant, the transcription factors (bHLH) playing a role in Fe-deficiency responses are known. They act through the expression of genes encoding proteins belonging to Fe-uptake systems (*e.g.* IRT and FRO). In graminaceous plants (*Strategy II*) different families of transcription factors (*e.g.* IDE and bHLH) are involved in the response to Fe-shortage through the activation of Fe-deficiency induced genes.

Sulfur in nutrient interactions: the case of iron

Stefania Astolfi

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Sulfur (S) is an essential nutrient for plant growth and development, being required in many metabolites, such as amino acids and proteins. Beyond its essential role in plant metabolism, S nutrition is also important for Fe uptake and assimilation. In higher plants Fe uptake process involves complex mechanisms. Strategy I, used by all except graminaceous plants, involves the mobilization of Fe^{3+} ions from soil particles by rhizosphere acidification, likely driven by an increase in plasma membrane H^+ATPase activity, the induction of a ferric chelate reductase activity which allows higher reduction rate of Fe^{3+} to Fe^{2+}, and the uptake of the resulting Fe^{2+} via an Fe^{2+} transporter. On the other hand, in Strategy II plants (only grasses), members of the mugineic acid family of phytosiderophores (PS) are secreted by the roots into the rhizosphere, where PS are able to form stable complexes with cationic micronutrients, such as Fe^{3+}; the Fe^{3+}–PS complex is then taken up by root cells through the action of Yellow Stripe 1 (YS1) proteins.

The characterization of the metabolic responses of both Strategy I and Strategy II plants to Fe deficiency has demonstrated the key role of methionine, being required not only for the synthesis of proteins but also of S-adenosylmethionine (SAM), which is a common precursor of molecules such as nicotianamine (NA), ethylene, polyamines and PS. In particular, NA is shared by both mechanisms: in Strategy I plants, NA makes possible the transport of Fe within the plant, whereas in Strategy II plants, NA is used either for Fe homeostasis or as precursor to PS.

Our recent reports have extensively characterized the relationship between S and Fe in both Strategy I and Strategy II plants, and have proven that low S availability often limits plant capability to cope with Fe shortage, since any reduction in S availability could result in reduced methionine pool. On the other hand, our data suggest that both Strategy I and Strategy II plants have to adjust S uptake and assimilation rate to meet the higher demand for methionine in response to Fe deficiency. A further interesting output of our research was the evidence that providing S above adequate concentrations may result in the improvement of Fe use efficiency in wheat plants and this S nutritional effect seems to be especially advantageous for plants grown under severe Fe limitation. The last evidence could have important implications for the improvement of the nutritional value of food crops (agronomic biofortification) and for an alternative management practice to reduce the negative impact of Fe deficiency.

This talk will describe the recent advances made towards understanding of the various processes involved in S/Fe interactions in different crop species.

Agrochemicals: plant detoxification and interferences on vegetal nutrition

Daniele del Buono

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Agrochemicals are used to protect crops and improve the yields of cultivated fields; their utilization is increasing substantially over time. The word "agrochemicals" means all the chemicals which are produced for the use in agriculture and allied industries. They include pesticides, veterinary products, some fertilizers and other chemicals. Regarding pesticides, they are designed to kill or repel insects, weeds, rodents, fungi or other organisms that can affect crops; therefore, they are classified in insecticides, herbicides, rodenticides and fungicides.

Their mode of action is to target selectively the pests. Safety in the utilization of agrochemicals is one of the primary concern; in fact, some agrichemicals can be very hazardous to the environment and thus to human health. Among the agrochemicals, herbicides are the compounds most important and widely used. They act by killing weeds or interrupting normal plant growth and they provide an effective way to help manage weed. Herbicides can be classified in several ways: by weed control spectrum, chemical families, mode of action, etc.

A common and useful method of grouping herbicides is their mode of action. Some plants can resist or tolerate herbicides; further than alteration in the target sites, herbicide resistance has been found to be based on differential rates of metabolic herbicide detoxification in crops and weeds species. In fact, plants dispose of some enzymes which permit them to detoxify and then tolerate herbicides. However, the extensive use of herbicides has led to some concerns associated to the long persistence and toxicity of their residues in the environments.

Considering the rhizosphere, the processes occurring in this volume of soil can be directly affected by these compounds. In fact, root exudates can be significantly altered by herbicides, and, further, some treated plants may actively participate to the transformation of the chemicals. Also the root uptake of nutrients can be interfered by herbicides. In the case of Fe assimilation, some studies highlight that certain herbicides can significantly reduce the nutrient content in the treated plants. In addition, the interferences to iron nutrition is becoming prevalent in cropping systems receiving frequently applications of the same chemicals.

In conclusion, human worldwide population is increasing and this requires more efficient and productive crop systems including the use of agrochemicals. However, because of the hazardous impacts of these chemicals, more attention and detailed studies are required in order to preserve a such important and common resource as the environment.

Team work and discussion

Plant nutrition research: challenges to meet the need for food with a sustainable approach

Matteo Spagnuolo

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Crops require a supply of essential mineral elements for optimal productivity. An insufficient supply of mineral elements required in large quantities and/or mineral elements with low phytoavailability in soils often limits crop production. Hence, these elements are generally supplied as fertilizers in agricultural systems. In addition, in areas where mineral deficiencies occur in animals and/or humans, fertilizers are applied also to increase concentrations of essential mineral elements in edible portions of plants. It is estimated that up to two-thirds of the world's population might be at risk of deficiency in one or more essential mineral element, with deficiencies of Fe and Zn being most common.

However, there are both financial and mostly environmental costs to the use of mineral fertilizers. For instance, our non-renewable P resources are rapidly running out, with phosphate-fertiliser prices rapidly increasing. Even more critical is the nitrate pollution in surface and ground waters due to the non-sustainable application of nitrogen fertilizers. It is therefore important to optimize the efficiency with which fertilizers are used for a sustainable crop production. Increased fertilizer use efficiency can be achieved agronomically, through improved fertilizer-management practices, such as adopting strategies for improving the soil-plant-microbial interaction in the rhizosphere and/or genetically, by cultivating crops that acquire and/or utilize mineral elements more effectively.

Major developments in our understanding of rhizosphere processes and mineral nutrient uptake by plants are expected. Analysis of these complex interactions between soils, plants and microbes in relation with mineral elements dynamic represents a unique opportunity to progress in our knowledge of the rhizosphere ecology. These progresses are expected to provide information and tools enabling us to develop strategies to improve plant mineral nutrition and health with decreases in the application of chemical inputs. In recent years, the research has contributed to improve strategies by identifying traits and genes that can increase crop yields on soils with restricted phytoavailablity of essential mineral elements. In particular, different papers have targeted improved use efficiencies of N, P, K and Fe fertilizers.

Ultimately, sustainable crop production is achieved when stable levels of food production and quality are maintained without compromising economic profitability or the environment.

Interactions between plants and microorganisms: towards a better nutrient use efficiency?

Youry Pii

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The world's increase in both human population and environmental pollution, due to an ever greater degree of industrialization, might severely impact on food production and it is therefore desirable that agricultural productivity could increase in the next few decades. Plant growth and productivity depend on the availability of nutrients at the soil-root interface. Actually, plants take up and utilize only a small part of the nutrients available in the growing media. A wide range of factors, including the soil type and chemical-physical characteristics, plant species and genotype, and environment, determines the extent of this availability. Furthermore, the nutrient availability in the rhizosphere can be affected by the biological activities of both roots and microorganisms.

In order to achieve an optimal fitness, the micro- and macronutrients needed by plants can be provided by means of chemical fertilization. Such agricultural practice has the advantage of quickly supplying plants with mineral nutrients, however the environment is exposed to pollution risks (e.g. leaching and eutrophication of aquatic ecosystems) deriving from fertilization excesses. Considering these facts, the agricultural practice is moving towards more sustainable systems, suggesting and testing alternative methods of fertilization, such as the combined application of chemical and organic fertilizers and the use of biological fertilizers. A very promising approach to reduce the environmental impact determined by the use of chemical fertilizers could be the inoculation of plants with plant growth-promoting rhizobacteria (PGPR).

PGPR are soil bacteria which are able to colonize plants' rhizosphere and to enhance plant growth by means of a wide variety of mechanisms like organic matter mineralization, biological control against soil borne pathogens (antibiosis, quorum sensing interference, induction of systemic resistance in host plants), biological nitrogen fixation and root growth promotion (production of phytohormones). A very interesting feature of PGPR is their ability of enhancing nutrients bioavailability. Several bacterial species have been characterized as P-solubilizing microorganisms whilst other species have been shown to increase the solubility of micronutrients, like those that produce siderophores for Fe chelation. The enhancement in the amount of soluble macro- and micronutrients in the close proximity of the soil-root interface has indeed a positive effect on plant nutrition. Furthermore, several pieces of evidence highlighted that the inoculation of plants with PGPR can have considerable effects on plant at both physiological and molecular levels (e.g. induction of rhizosphere acidification, up- and down-regulation of genes involved in ion uptake and translocation), suggesting the possibility that soil biota could stimulate plants being more efficient in retrieving nutrients from soil and coping with abiotic stresses. However, the molecular mechanisms underlying these phenomena, the signals involved as well as the potential applications in a sustainable agriculture approach and the biotechnological aspects for possible rhizosphere engineering are still matter of discussion.

Structure and activity of soil microbes as revealed by genomics and metagenomics approaches

Edoardo Puglisi

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Bacteria and Archaea play a pivotal role in maintaining and assuring the fertility of soils. Our knowledge on soil microbial structure and activity is still limited due to the immense complexity of species interacting in soil: recent measurements and estimates count up to hundreds of thousands of species thriving in a few of g of soils, most of them being not cultivable and still unknown from a taxonomical and functional point of view.

Recent advances of the post-genomic era are providing scientists with tools that have the potential for unraveling the complexity of soil microbial structure and activity, thus addressing two fundamental questions of microbial ecology: who is there and what they are doing. Next generation sequencing (NGS) technologies produce in a single run million of DNA reads of hundreds of base pairs, with read lengths and throughput being constantly increased as the technology advances.

Aim of this talk is to show the current and future applications of NGS in soil and rhizosphere microbial ecology studies. Topic will focus on the sequencing and annotation of microbial genomes, the assessment of bacterial and archaeal diversity and taxonomy by deep sequencing of 16S rRNA amplicons, the assessment and quantification of functional genes and their correlation with enzymatic activities. Insights on potential of NGS techniques for transcriptomics, metagenomics and metatranscriptomics studies will also be provided.

Structure, functions and host control of the microbiota thriving at the root-soil interface

Davide Bulgarelli

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Roots of land plants nourish rich and diverse bacterial communities in the vicinity of and within their tissues. These communities, designated rhizosphere and root microbiota, establish associations with their host that appears symptomless at first glance, possibly representing a continuum of symbiosis ranging from commensalism to mutualism. Consistently, members of the microbiota contribute, under laboratory conditions, to indirect pathogen protection and enhanced mineral acquisition for plant growth. However the structure and functions of the plant microbiota in a community context remain largely unknown.

We have grown *A. thaliana* accessions in two agricultural soils under controlled environmental conditions and we profiled the bacterial communities inhabiting the rhizosphere and the roots using a 16S rRNA gene amplicon pyrosequencing approach. Phylogenetic assignment of the generated sequencing reads revealed that the roots of *A. thaliana* are preferentially colonised by members of the phyla Actinobacteria, Bacteroidetes and Proteobacteria, while members of the phyla Acidobacteria and Firmictures are virtually excluded from these tissues. Interestingly, ribotyping of soil-incubated inert wooden matrices revealed that these substrates represent a sufficient cue for ~40% of the Arabidopsis root microbiota, with a bias for Betaproteobacteria. These results suggest that the roots of *A. thaliana* define a distinct microbial habitat whose composition mirrors the capability of members of the soil biota to respond to lignocellulosic features and other or additional cues from metabolically active host cells.

In a parallel line of research we investigated whether the structure of the microbiota correlates or is independent from plant domestication. We determined the structure of the rhizosphere and the root bacterial microbiota of wild, landrace and modern barley (*Hordeum vulgare*) accessions grown in the same soil under controlled environmental conditions. Similarly to the Arabidopsis root microbiota, the bacterial communities thriving at the barley root-soil interface appears to be gated, as indicated by a narrow phylogenetic composition, largely dominated by members of the families Flavobacteriaceae, Rhizobiaceae and Comamonadaceae. Interestingly, unlike *A. thaliana*, soil-grown barley plants were characterised by a marked “rhizosphere effect” (i.e. structural diversification of the rhizosphere microbiota from the unplanted soil). Ecological indices and linear model analysis revealed that the enrichment of specific bacterial taxa discriminates the rhizosphere from the root microbiota, possibly reflecting the functional diversification of these two microbial communities. Intriguingly, the structure of the rhizosphere communities appears to be the more divergent across barley genotypes, with the wild accession recruiting a richer and more diverse rhizosphere microbiota compared to a modern variety.

This scenario suggests a two-step selection process gradually differentiating the plant microbiota from the surrounding soil biome. In this model, rhizodeposition fuels an initial substrate-driven community shift in the rhizosphere, and host genotype–dependent factors contribute to the fine tuning of microbiota profiles. The magnitude of microbial selection in the rhizosphere compared with that of the root tissues is ultimately determined by the interactions among plant species, host genotype and soil type. The prediction of this model is that that optimum plant growth depends on specific interactions among host genotype, the microbiota and soil type.

Team work and discussion

Molecular vs. biochemical methods to study the soil microbial community

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Although our knowledge about soil-microbe-plant interactions is increased during the last decades, the complexity of interacting biological, chemical and physical factors suggests that much remains to be understood. The soil is one of the major reservoirs of biological diversity on our planet. The economic profit derived from soil biodiversity is estimated more than 1500 billion dollars, although the relative value of the associated services remains to be determined. Investigating microbial diversity is important not only for basic scientific research, but also to understand the link between community structure and function. To date both molecular and biochemical methods have been widely used to investigate genetic, functional and structural microbial diversity. Among the most widespread biochemical methods, the phospholipid fatty acids (structural diversity) and the community-level physiological profile (functional diversity) are currently used.

Molecular methods used to study microbial diversity are numerous and they mainly aim to investigate the genetic microbial diversity. Among them, DNA re-association, DNA–DNA and mRNA:DNA hybridization, DNA cloning and sequencing, and other PCR-based methods such as denaturing gradient gel electrophoresis, temperature gradient gel electrophoresis, ribosomal intergenic spacer analysis and automated ribosomal intergenic spacer analysis are to be mentioned.

However, despite the availability of such numerous methods, the study of microbial diversity is made difficult by general and methodological limitations.

General limitations are mainly due to the soil spatial heterogeneity, inability to culture all soil microorganisms and frequent taxonomical updating about several microbial groups. Methodological limitations can be even more important and concern mainly the molecular methods. In fact, although the latter has overcome the limitations of cultured-based techniques, the lack of efficient cell lysis and nucleic acid extraction techniques still show remarkable drawbacks.

In conclusion, the best way to study soil microbial community diversity does not exist yet. However, to get the largest picture possible and the most information regarding soil microbial community, a variety of methods with different endpoints and degrees of resolution should be properly selected and used, with the questions being asked and the resources available in mind.

Soil contamination and bioavailability concept

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Soil contamination is generally due to chemicals of natural or anthropogenic origin, which accumulate in the solid, liquid and/or gaseous phase of soil and change the natural soil equilibrium. This is the result of many human activities such as: leakages from sanitary sewage, acid rains, unhealthy waste management techniques, mining, commercial activities as well as transport and services, uncontrolled release of industrial waste, such as harmful gases and chemicals, agricultural pesticides, fertilizers and insecticides, unfavourable and harmful irrigation practices. Some of the most serious soil pollution effects are: decrease in soil fertility and therefore decrease in the soil yield, loss of soil and natural nutrients, soil erosion, increase in salinity, disturbance of flora and fauna residing in the soil, death of many essential organisms, biodiversity decrease, serious health problems in people consuming plant products.

Soil pollutants may be divided into two main groups, according to their chemical structures and properties: i) inorganic and inorganic-organically bound (nitrates, phosphates, salts, trace elements, radionuclides), ii) organic toxic compounds (pesticides, hydrocarbons).

Even if soil contamination has long been recognized as an environmental issue of public concern, remediation is prescribed by considering both the risk that the land represents for human health and the potential commercial value of the site. It is know that the metal content in the solid phase often does not well predict toxic effects in soil dwelling organisms and plants, hence a risk-based clean-up of a contaminant can be determined - with minimum uncertainty- by taking into account its bioavailability. Bioavailability can be considered to consist of three processes: a physicochemically driven desorption by which the compound equilibrates between the solid phase and the pore water, a physiologically driven uptake from the solid or liquid phase by the organism that is studied, and the internal distribution in the organism. So, the bioavailable fraction may be the fraction that is mobile and available for uptake by plants, animals and humans. This fraction may vary with time, depending on soil properties (texture, pH, redox potential, CEC, organic matter, inorganic constituents, enzyme content, biomass content, rhizosphere processes), speciation and chemical transformations (oxidation, reduction, hydrolysis, substitution, elimination, photolysis, biodegradation) of the considered substance: e.g. ageing of contaminated soils results in sequestering compounds within the soil particles or in its degradation and consequently decreasing the bioavailability.

The methodologies currently preferred for assessing the bioavailability of chemicals in the environment are:

- extraction procedures: the amount of bioaccesible compound is obtained as a function of that released by soil extraction by simple aqueous washes, complexing agents, acids, bases, salts or organic solvents. In this case, the final assay is always chemical rather than biological.

- bioassays: a test organism (e.g. microbe, plant) is exposed to the sample, and afterwards either the negative effects on the tested population or the bioaccumulation are quantified. Some of these methodologies are based on challenging the test organisms with soil extracts, whereas others attempt to bring the organism in direct contact with the non-extracted soil sample.

Soil remediation: an overview

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Because of human activities, every year we are losing soil at a rate estimated at approximately 11.6 ton ha^{-1} y^{-1}, equivalent to a reduction in soil thickness of about 0.38 mm y^{-1}. For this reason, not only we should protect the soil we have but also we have to repair the damage caused by polluting the soil and bring it back into beneficial use.

Soil remediation is a very complex issue since it has to deal with a number of different aspects which only marginally pertain to technology and science. In fact, very often legislative as well as economic, social and engineering constraints dictate the rules toward the choice of the remediation technology to be employed for a specific polluted site.

From a technological point of view, there are several available well-established methods for soil remediation, including biological, thermal and chemical-physical treatments. All of them can be applied *in situ* (i.e. without excavation and movement of the polluted soil), or *ex situ* (i.e. with soil displacement) with soil treatment performed *on site* or *off site* (i.e. in treatment plants located outside the site under remediation). All these methods fulfill the general purpose to remove the source of pollution from the source-pathway-receptor linkage.

Despite the large number of available technologies, only few of them are sustainable and therefore further research on new remediation methods is needed. Applied to remediation, sustainability means that any remedial treatment should achieve a balance between protecting the environment now and not limiting the use of the environment in the future, being accepted by the general population, and not being too expensive.

The objective of this talk is to present an overview of all the available technologies for soil remediation and of the main advantages and drawbacks of the different methods. Also, the scientific principles at the basis of the primary classes of technologies will be discussed.

At last, some general guidelines concerning how to choose the best remedial technology will be provided, taking cues from real case studies.

Restoration of functionality of polluted soils and sediments: evidences from the GREENLAND and CLEANSED projects

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Several phytoremediation options can be used for the sustainable management of contaminated soils and sediments. In particular, the phytoremediation using tolerant plants or short rotation coppice of with tolerant clones of woody plants can lead degradation of the organic pollutants in the rhizosphere and phytoextraction or phytostabilization of trace elements in polluted soils and sediments, with and without the use of (in)organic amendments. Phytoremediation allows the preservation and restore the quality of the polluted soils which are non renewable natural resources.

The phytoremediation can reduce the chemical mobility of metals and metalloids and the total concentrations of organic contaminants, thus reducing soil ecotoxicity particularly in the rhizosphere. This leads to soil revegetation and restore or promote functional activity and nutrient fluxes in the remediated soils and sediments.

While it is well established that phytoremediation increases soil functionality and mineralization capacity, studies on soil bacterial and fungal species richness at community level have shown that soil microbial diversity in various aided phytostabilization trials can be increased at different levels even after several years of implementation. However, to date, there is not a clear common trend between microbial diversity and the apparent restoration of soil ecosystem services in the phytoremediated soils. Probably, this is mainly due to the fact that most of the microbial diversity studies have been carried out using a 16S- and 18S rDNA PCR-fingerprinting techniques. The low resolution level of the molecular PCR-DGGE techniques applied during previous studies makes it uncertain whether the lack of increased microbial diversity in the phytostabilized soils was due to a low sensitivity, or ecological factors such as slow microbial colonization after metal stress relief, the absence of complex ecological interactions between microbial species, and microbial selection induced by the incorporation of amendments (e.g. compost, iron grit, and beringite) into the soils.

Higher levels of resolution can be achieved by using open metagenomic approaches, mainly based on soil metagenome sequencing, or closed metagenomic approaches such as microarray techniques. Among the microarray techniques, the GeoChip technology allows the detection of microbial functional groups involved in the nutrient biocycles, metal resistance, and degradation of organic contaminants. The GeoChip technology has been successfully used to unravel the dynamics of microbial communities in remediated soils.

The results of the functional activity and functional diversity of soils and sediments from the EU – FP7 GREENLAND and LIFE12 CLEANSED projects showing the effects of phytoremediation on the soil and sediment ecological functions will be discussed and compared to the heavy metal solubility and organic contaminant concentrations.

Rhizosphere processes involved in phytoremediation of trace element-contaminated soils

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The few millimeters of soil surrounding the plant roots are called ”rhizosphere”. While roots represent the hidden half of entire plants, the microenvironment around roots constituting the rhizosphere has been referred to as the “hidden half of the hidden half”, bearing numerous secrets yet not completely revealed. In spite of its limited volume the rhizosphere plays a key role in controlling the soil-plant relationship. Rhizosphere soil differs largely from the bulk soil and represents the microenvironment in which root activities and soil properties closely interact. Plant-microbe relationships ranging from symbiosis to parasitism as well as microbial population dynamics are also influenced by these particular conditions.

The rhizosphere is an extremely dynamic environment characterized by numerous feedback processes linking phenomena in the biosphere with processes in the pedosphere. The high complexity of multiple plant-induced microbial and soil physical / chemical processes linked to each other and occurring simultaneously at a micro-scale makes rhizosphere research an extremely difficult task. In particular, access to study these processes and phenomena in situ is limited as they occur below-ground and separation of the components involved, such as roots, root hairs, soil solid phase, soil solution, gaseous phase and microbes, is very challenging in respect of experimental designs and technology.

The solubility and the bioavailability of trace elements in the rhizosphere is largely influenced by the activities of roots and associated microbes, leading to a decrease or an increase of concentrations in comparison to the bulk soil. The main processes include exudation of chelating compounds (e.g., carboxylates, siderophores), changes of pH and/or redox potential, uptake by living cells of roots or microbes, and others. Key factors influencing these processes are the general soil characteristics, plant genotype and the physiological status of the plant (e.g. nutrient deficiency).

Due to methodological challenges, the information about the fate of trace elements in the rhizosphere is still limited. However, due to recent developments, novel insight into the rhizosphere dynamics was gained. Novel rhizobox techniques allowed the assessment of rhizosphere gradients at high spatial resolution and 2D imaging techniques were the basis for obtaining new insight into the differentiation of rhizosphere processes along the root axis.

Many rhizosphere processes involved in phytoremdiation of trace element-contaminated soils are still unknown or only partially clarified. Trace element accumulating plants serve as model organisms to unravel the root-soil interactions involved in trace element biogeochemistry. Improving our understanding of trace element dynamics in the rhizosphere is a prerequisite for the optimization of phytoremediation efficiency.

Team work and discussion

Strategies and novel technologies for elemental speciation in rhizosphere soil solution

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Rhizosphere is spatially defined as the soil portion under the influence of plant roots. This microenvironment usually consists of a layer of few mm close to the roots. Chemical and biological reactions occurring in the rhizosphere modify soil properties and contribute to define the element chemical forms, so as to determine their behaviour in terms of toxicity, mobility and bioavailability. The main factors which are effective on element mobility and bioavailability in soil are: pH, Eh, cation and anion exchange capacity, organic matter content, both content and type of clay and inorganic soil constituents. The total concentration of an element in the rhizosphere does not allow to obtain a realistic information on its dangerousness for the environment. A correct assessment of the real risk and of the suitable decontamination techniques for a contaminated soil cannot leave speciation studies out of consideration.

According the definition by IUPAC, chemical speciation is a specific form of an element defined as to isotopic composition (e.g. for C, N, O, S, Pb, and Sr), electronic or oxidation state (e.g. CrIII/CrVI, AsIII/AsV and HgII/HgI/Hg0), organometallic compounds (e.g. Hg, As, Pb and Sn compounds) and inorganic and organic compounds of various origin. On the other hand, by 'speciation of an element' we mean the distribution of an element amongst defined chemical species in a system.

During the discussion, some aspects involving both the strategies and the techniques to investigate the speciation in rhizosphere soil solution, with particular emphasis on their principles, strong points, limitations and applicability, will be considered.

Some basic concepts calling attention to the different steps of chemical speciation procedures will be mentioned: sampling, storage, pretreatment, analysis and result evaluation. Finally, some recommendations will be given about the strategies that researchers should have to adopt, if possible, to improve the quality of their analytical data, including the use of isotopic techniques.

Among the different analytical techniques, the atomic spectrometry (AAS, ICP-AES, and the various types of ICP-MS), hyphenated or not with other equipments (e.g. chromatographic separations, Field Flow Fractionation, and Laser Ablation), will be dealt with in details.

Methods in Rhizosphere Research: Rhizoboxes and Rhizotest - limitations and implications

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The rhizosphere is the volume of soil around plant roots that is influenced by their activities. The high complexity of a multitude of plant-induced, microbial and soil physical-chemical processes linked to each other and occurring simultaneously at a micro-scale makes rhizosphere research an extremely challenging task. In particular, access to study these processes and phenomena *in situ* is limited as they occur below-ground and separation of the components involved, such as roots, root hairs, soil solid phase, soil solution, gaseous phase and microbes, is very challenging in respect of experimental designs and methodology.

In the past, several methods have been developed that allow the separation of rhizosphere from bulk soil. The main approach includes the use of rhizoboxes, where either porous membranes or microtomes where used to obtain rhizosphere soil at defined distance from roots. The development of rhizobox-based methods facilitated major developments in rhizosphere research, however, the systems used in the past still had a number of limitations that may have caused significant artifacts. Recently a novel rhizobox design has been developed that overcomes these limitations by (i) employing a visible root only compartment containing a well-defined root monolayer of known biomass and age, (ii) use of a soil compartment on top of the rhizobox where roots grow in direct contact to the soil and thus limiting growth constraints during the experiment. To avoid artifacts caused by separation of soil layers in the rhizosphere compartment, slicing of rhizosphere soil was recommended to be done on non-frozen rhizosphere soil. Microelectrodes or micro suction cups may be fitted into the rhizosphere compartment to obtain information on either soil pH or redox potential or on the soil solution composition at defined distance from the planar root mat during the experiment.

Another experimental approach is the so-called rhizotest. The soil-based biotest procedure includes two successive steps: (i) pre-growth of the plants in hydroponics and (ii) growth of the plants in contact with soil samples. Compared to the rhizobox approach, the rhizotest provides more standardized growth condition and the possibility to calculate net element fluxes from soil to the whole plant. The rhizotest is a powerful tool for screening for rhizosphere effects in a large number of soils, treatments or replicates, however it does not provide the same high spatial resolution as it can be obtained with rhizoboxes.

How to approach a plant study in a plant-soil experimental system?

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After the practical session focusing on the soil aspect, the scope of this one is to shed light on the problematic of the study of the plant-soil system from a molecular physiologist's point of view. This session will not be based on some practical works but on a discussion between all participants on how to setup an experimental setup to access (part of) the complexity of how plants respond to the interactions with soil, recognizing advantages and disadvantages of different systems and giving some tools to choose the most adapted depending the scope of the research.

There are major difficulties in studying the plant in a plant-soil system. First is that the study generally focused on the response at the root level, where there is actually the problem to access the root in a soil and to take into account the heterogeneity of the soil-plant interactions and the activities of microorganisms. Moreover the plant species used is often critical, as most of the knowledge for the molecular side is still about *Arabidopsis.* However this plant is not adapted for this kind of study as it has a small and fragile root system; therefore it is needed to find/choose a plant that has strong roots, well known genome and if possible with a well characterized root physiology.

Once found a model plant you still need to go to find a system to grow it in a way to simulate as closing as possible what is occurring in the field, e.g. field experiments, pots, rhizobox, but you will need *in situ* techniques, e.g. biosensors, to measure the root physiology or to be able to collect the roots cleanly and without damage in order to do the measurements *in vitro*. In alternative, most of the researchers will go to a simplified setup, e.g. aeroponic or hydroponic cultivation, without any heterogeneity or with some, e.g. split-root, compartmentalization. Moreover these last setups are normally in (semi-)sterility or at least without any soil microorganism community.

For the molecular part, you might have the possibility to use some mutants (knock out, overexpressing or transient silencing), where you might just need to check the physiological part. If not you have to collect generally the roots from the ground, remove the adherent soil and extract RNA from these tissues. Then you might need to find a method to isolate your gene(s) of interest if you do not know it(them) yet and to follow its/their expression level. Thereafter you might want to go up to characterize functionally this(ese) gene(s).

Digging into numbers: from Data Count Matrix to (almost) PQ pictures using R

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Next Generation Sequencing (NGS) technologies provide several gigabytes of data made up of millions of sequenced reads with length up to a few hundreds bp. Handling and analyses of these high-throughput data is a crucial step, which has become more time- and cost-consuming than the work being carried out in the lab. A number of bioinformatics methods should be wisely applied in order to correctly test scientific hypotheses and extract meaningful conclusions from experiments relying on NGS data.

The practical session will provide examples of a number of available bioinformatics tools for the analyses of NGS data. Topics will cover the analyses, quality screening and conversion of FASTQ reads, the assembly, functional annotation of microbial genomes, the application of pipelines for the analyses of 16S rRNA amplicons. The second part of the session will focus on multivariate data reduction and hypothesis testing. We will discuss basic approaches in microbial ecology, such as alpha and beta diversity indices, the features of the most common ordination methods and how these can be applied to the analysis of NGS dataset, with emphasis for rRNA gene amplicon data. In addition, we will provide a brief overview of the statistical tools deployable to correlate NGS data with experimental variables. The final part of the session will be dedicated to the analysis of NGS data provided by the instructors using open source tools such as R.

X-ray and mass analytical methods to study micronutrients and trace element pollutants in soil and plants

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Heavy metals (Z>20) and metalloids are frequently present in soils both deriving from natural geologic sources or from anthropogenic inputs in the environment. Some of these elements are important micronutrients for plants (Fe, Mn, Zn, Cu, Ni, Mo) while others (Cr, V, Co, As, Pb, Hg, Cd) can exert toxic effects, especially when their chemical forms in soil are available for plant uptake and translocation.

In most of the cases, determining only the total metal content in soil is not sufficient to estimate how much available for nutrition or dangerous that element can be for plants. A better approach is to assess metal speciation and/or potential/actual bioavailability of trace elements in soil. In addition, following the fate of that element inside the plant is extremely useful to evaluate if it can be profitably exploited by plants (in the case of nutrients) or dangerously translocated in the plant edible parts (in the case of toxic elements).

For this type of studies, two groups of analytical techniques are particularly advantageous: those based on X-rays and those detecting the exact mass of the analytes.

As for X-rays, they have the capacity to penetrate inside matter and to give rise to a number of physical phenomena which can be profitably exploited by scientists to extract chemical as well as structural information from the sample under investigation. Physical processes such as diffraction, fluorescence and absorption can be used to study trace element speciation in soil and plants. In addition, microanalytical X-ray-based techniques can be adopted to elucidate chemical and structural properties of the sample down to the micrometer and nanometer scale.

In this practical session, an overview of these X-ray based techniques will be presented and the use of X-ray fluorescence to analyse soil and plant materials will be shown.

Another powerful method for speciation analysis is HPLC-ICP-MS (High Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry). Such a technique combines the separation capabilities of HPLC with the high sensitivity of ICP-MS detection to identify and quantify the different forms in which trace elements are present in plant and soil samples.

The advantages associated with the HPLC-ICP-MS technique include high elemental specificity, the possibility to record real time chromatograms, the ability to separate the signals of interfering species from the peaks of interest, a high linear range, a multi-element capability, and the possibility to obtain isotopic information.

Another speciation method which will be shown during the practical session is the coupling of separation techniques with the measurement of different isotopes of a given element by ICP-MS. Isotope dilution analysis (IDA) is a well-known analytical technique based on the measurement of isotope ratios in a sample where its isotopic composition has been altered by the addition of a known amount of an isotopically enriched element. The first advantage of isotope dilution analysis is that any variation of this parameter due to instrumental instabilities such as signal drift or matrix effects will have no influence on the final value for the element concentration in the sample.

Metabolic reprogramming of rice mutant plants knocking-down in the Mitochondrial Iron Transporter (MIT) expression

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Iron (Fe) is an essential element for plant life being a fundamental constituent in many metabolic processes, including cellular respiration which is located in mitochondria. Reduced bioavailability of this element strongly impairs this important cellular compartment. Plants, however, have different regulatory mechanisms that allows flexible metabolic adjustments in response to nutritional disorders related to Fe deficiency. Under these conditions there is a general metabolic reprogramming at the root level (activation of glycolysis and secondary metabolism). This re-modulation of mitochondrial activity plays a key role in iron deficiency response. It has recently been suggested that mitochondria might be involved in a Fe sensing and signalling mechanism in the cell. The understanding of the mechanism is necessary to optimize the acquisition of Fe by plants.

In this work, the metabolic adjustment of rice *mit-2* mutant plants, grown at different concentrations of Fe, knocking-down in the expression of the Mitochondrial Iron Transporter (MIT), has been investigated.

Ionomic and transcriptomic analysis of leaf and root tissues have been performed on Fe-sufficient (+Fe) and Fe-deficient (-Fe) wild type and *mit-2* plants. The latter analysis of the *mit-2* plants showed a strong alteration of expression (up/down regulation) of genes encoding enzymes of both primary and secondary metabolisms. In addition to this, more mitochondrial alterations in gene expression were observed in leaf than in root. Western blot analysis carried out on the mitochondrial fraction and organic acids quantification by HPLC of root tissues, showed that under control condition (+Fe) the *mit-2* plants reprograms the mitochondrial metabolism similarly to that induced by a Fe deficiency. These findings support the hypothesis that mitochondria might represent the cellular compartment involved in the determination of metabolic responses induced by Fe deficiency.

Iron deprivation results in a rapid but not sustained increase of the expression of genes involved in iron metabolism and sulphate uptake in tomato (*Solanum lycopersicum* L.) seedlings

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Characterization of the relationship between sulphur and iron in both Strategy I and Strategy II plants, has proven that low sulfur availability often limits plant capability to cope with iron shortage. Here it was investigated whether the adaptation to iron deficiency in tomato (*Solanum lycopersicum* L.) plants was associated with an increased root sulphate uptake and translocation capacity, and modified dynamics of total sulphur and thiols accumulation between roots and shoots.

Most of the tomato sulphate transporter genes belonging to Groups 1, 2 and 4 were significantly up-regulated in iron-deficient roots, as it commonly occurs under S-deficient conditions. The up-regulation of the two high affinity sulphate transporter genes, *SlST1.1* and *SlST1.2*, by iron deprivation clearly suggests an increased root capability to take up sulphate. Furthermore, the up-regulation of the two low affinity sulphate transporter genes *SlST2.1* and *SlST4.1* in iron-deficient roots, accompanied by a substantial accumulation of total sulphur and thiols in shoots of iron-starved plants, likely supports an increased root-to-shoot translocation of sulphate. Results suggest that tomato plants exposed to iron-deficiency are able to change sulphur metabolic balance mimicking sulphur starvation responses to meet the increased demand for methionine and its derivatives, allowing them to cope with this stress.

An example of agro-ecosystem sustainability

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The goal is to delineate the main chemical-physical characteristics of the agricultural soils of a sustainable farming in Northern Italy at 403 m a.s.l. elevation. We focused the study of the soil farm and its vegetables production. We have studied 4 different vegetables from 4 Families: Chenopodiaceae, Cucurbitaceae, Solanaceae, Composite. We have taken samples during the different growth cycle of the vegetables. In order to achieve the objectives of the project we provide extensive investigation of the link between a) general study of the physical-chemical characteristics and physics properties of natural and agricultural agro-ecosystem soil b) knowledge on farming practices and quality-quantity characteristics of the products, quantitative and qualitative analysis on soil, humus, plant production, agric management carried out and confronted with experience and opinions of the farmer c) using the results of the soil research we have suggested for a new farming sustainable management. The results of the project bring us an understanding of the nutrient cycle in the soil farm during all the growing period of the plants. We recognize this agriculture as an example of environmentally friendly agriculture in Europe, translating the scientific nutrients dynamic and soil fertility knowledge into management practices that can be applied at the farm and landscape levels in order to preserve the natural fertility of the soil.

Studio delle interazioni pianta-microrganismi, in un suolo contaminato da PCB, in presenza della specie foraggiera *Medicago sativa*

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I Policlorobifenili (PCB) sono una classe di sostanze organiche xenobiotiche, prodotti industrialmente fino agli anni ‘70 ed attualmente presenti nell’ambiente come contaminanti persistenti in miscela di più congeneri (es. l’Apirolio, prodotto in Italia ed usato per i trasformatori elettrici). La loro degradazione avviene principalmente attraverso l’attività microbica. Importante è il ruolo svolto dalle piante, le quali, attraverso la secrezione di essudati radicali, possono stimolare o reprimere, in modo specifico, la presenza e l’attività dei diversi taxa microbici nella rizosfera. La degradazione dei contaminanti dipende fortemente sia dalla capacità delle specie vegetali che delle comunità microbiche di resistere al loro effetto tossico, ma soprattutto dall’interazione sinergica tra pianta e microrganismi nella rizosfera. Inoltre in suoli particolarmente degradati, non solo dalla presenza di contaminanti ma anche per scarso contenuto di sostanza organica, la scelta della specie vegetale è fondamentale per la selezione della comunità microbica, per la formazione di una fitta rizosfera e per l’arricchimento nutritivo del suolo, che può essere ulteriormente incrementato attraverso l’aggiunta di compost.

Al fine di indagare le interazioni tra pianta e microrganismi autoctoni della rizosfera, campioni di suolo, provenienti da un sito contaminato da PCB, sono stati utilizzati per allestire un esperimento di degradazione in presenza della specie foraggera *Medicago sativa*, nonché in presenza/assenza di Apirolio e di compost di derivazione da rifiuti solidi urbani.

Le analisi microbiologiche e chimiche, effettuate in diversi momenti dell’esperimento, hanno permesso di valutare le variazioni nella struttura e funzione delle popolazioni microbiche, in relazione alle diverse condizioni sperimentali.

Cadmium translocation in rice: a possible zinc independent pathway

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Zinc is a micronutrient required for plant growth and development. Interestingly, the movement of Zn through the plant has been seen to be often coupled with that of cadmium, both at the uptake and translocation level.

Although divergent results have been obtained, they generally suggest that Zn independent pathways for Cd translocation in plant could be possible.

Here we present a study aimed at better understanding the Zn and Cd translocation pathways in rice plants.

Rice plants were hydroponically grown and differentially exposed to increasing Cd or Zn external concentrations, maintaining a steady amount of Zn and Cd, respectively.

Cd and Zn concentrations were measured by ICP-MS in xylem sap, roots and shoots. Results were related to total Cd and Zn contents in root fractions obtained by an exhaustive extraction procedure which enabled us to estimate the amount of Zn and Cd ions of the roots potentially available for translocation.

Interestingly, almost no competition of Zn over Cd has been proved at the translocation level, whereas a strong one has been observed in the opposite direction, i.e. of Cd over Zn. Such an outcome reasonably suggests that the xylem loading of Cd in rice occurs via different transport pathways, just partially shared with Zn.

In order to identify the possible Zn-insensitive pathway involved in Cd translocation we are focusing our attention on members of the P_{1B}-type ATPase family - OsHMA2, OsHMA4 and OsHMA5 - whose selectivity is being studied by expressing the relative cDNAs in yeast.

The Role of Flavonoids in Promoting the Mobilization of Fe and Mn in soil

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Flavonoids are polyphenolic compounds that can be released by plants into the rhizosphere, where they exert multifunctional roles. Little is known about their interactions with soil constituents and their effect on nutrient mobilization.

In this research, the interactions between three flavonoids, quercetin, rutin, and genistein, and two soils (one acid and one alkaline) were studied. Synergistic and competitive effects with three organic acids usually present in root exudates (citric, oxalic, and malic acid) were also assessed for the mobilization of different major and trace elements.

Citrate was the most effective organic acid in acid soil, whereas oxalate was more efficient for Cu, Zn and Ni extraction from alkaline soil. Synergistic effects among organic acids were observed for Mn in acid soil, and for Si in alkaline soil. Competitive effects occurred for Cu, Zn and Ni in alkaline soil.

Rutin was 25 times more effective than citrate for Fe mobilization in alkaline soil, and quercetin was 50 times more efficient than citrate in extracting Mn from acid soil. Quercetin (35 µM) combined to citrate (1 mM) increased five times the amount of Mn extracted by citrate alone.

Citrate may promote the element desorption from soil minerals through complexation. Differently, rutin mobilized Fe from soil by reduction to Fe(II) (reductive dissolution). Quercetin reduced soil-Mn to Mn(II) but, when combined with citrate, Mn was also partly reduced to Mn(III) which was immediately complexed and stabilized by citrate.

These results are extremely important to understand the biogeochemical cycle of trace elements in rhizosphere soil.

Application of the Isotopic Dilution spectrometry for the determination of the exavalent Chromium in soil samples

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The oxidation number of Chromium in nature can vary from 0 to VI, but Cr(III) and Cr(VI) are the more stable forms in the environment. Even if the first one is essential for human nutrition, Cr(VI) is carcinogenic.

The proposed procedure consist of an extraction, a chromatographic separation and an ICP-MS quantification. It is howhever necessary to verify the possible interconversion of the two forms that during the extraction may occur. At this purpose different studies suggest the utilization of Speciated Isotope-Diluition Mass Spectrometry (SIDMS). This method requires the addition of a known amount of a solution that contains a chemical form of the element, enriched in one of its stable isotopes. This allows to follow the behavior of Chromium during the analytical steps.

In our case we have analyzed some samples of soil with a different content of organic substance and Iron (that tend to reduce Cr(VI)). Even adopting a so promising technique ("Fast and accurate procedure for the determination of Cr(VI) in solid samples by Isotope Dilution Mass Spectrometry", Fabregat-Cabello N. at al.2013), in some of these samples the Cr(VI) added before the extraction as a spike, was completely reduced, preventing the quantification of hexavalent Chromium.

Our attention was focalized on the improvement of the conditions of extraction (temperature, cycles of warming, EDTA molarity, pH values, addition of ammonium nitrate and fenantroline as Fe(II) complexant).

The optimization of the procedure allowed the determination of the Cr(VI) in the majority of the investigated soils.

The complex world of ectomycorrhizal fungi: soil fauna biodiversity in *Tuber aestivum* brulé

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Some species of ectomycorrhizal fungi, such as *Tuber aestivum* Vitt, create a zone (called burn or brulé) around the mycorrhized plant where the growth of other plants is inhibited. The aim of this study still in progress is to characterize soil fauna diversity inside the brûlé and highlight eventually differences compared to outside. The study, started on June 2013, is carried out in 9 spontaneous brûlé of *T. aestivum* Vitt., located in the Piacenza province. Soil samples were collected inside and outside the burn for: chemical-physical analysis (pH, TOC, organic matter content and humidity) microarthropods extraction (10x10x10 cm), nematode extraction (soil cores of 500 ml in volume). Sampling was repeated every first week of the subsequent months for 8 months. Microarthropods extraction was performed using Berlese-Tüllgren funnel. Soil biological quality indices (QBS-arthropods, QBS-collembola and Acari/Collembola ratio -A/C-); and biodiversity indices (Shannon-Weiner diversity index -H'- and Pielou's index -J-) were applied. Nematodes were extracted with modified Baermann funnel, fixed with hot formalin (5%) and identified to genus level. Maturity Index (M.I.) was applied.
Preliminary results show some differences between inside and outside the burn. Soils showed a moderately higher pH inside the burn while organic matter content and TOC were higher outside the burn. Taxa densities and soil quality and biodiversity indices were higher outside the burn. Regarding nematode community, although the determination phase is still in progress, the preliminary results seem to highlight differences in terms of relative abundance, species composition and M.I. values between the two "environments".

Effects of water-saving cultivation of rice on the ion and metabolite contents in the grain

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For the modern rice varieties the average value of water productivity (WP) is about 1.1 g grain kg^{-1} evapotranspired water, but in irrigated lowland condition it drops off to 0.4 g grain kg^{-1} water. Global warming challenge and industrial or civic requirements for water might cause water shortages for irrigated lowland rice systems. Thus, the cultivation of aerobic rice become an important technique in several countries.
Soil water status, but in irrigated affects some commercial qualitative traits of grains, whereas scarce information exist for what concern its effect on the grain contents in dietary trace elements, undeliverable elements (Cd and As), nutraceutical or anti-nutritional phytochemicals. In order to acquire information on this aspect the levels of ions and metabolites were evaluated on brown rice samples obtained from four cultivars of temperate japonica rice genotypes grown in field under flooded or aerobic condition. Ionomics investigation by ICP-MS technique showed that, in all the varieties, in the grain of aerobically-grown plants the levels of Cd, Ni, Pb and Cu in the grains resulted higher (about 8, 5, 2, 1.5 fold, respectively), whereas those of As resulted lower (-30%). No differences were observed comparing the levels of the other elements. The water save technique did not influence the levels of tocopherols, tocotrienols and γ-oryzanol, detected by HPLC techniques, with respect to soil flooding. The genetic and physiological basis of some observed differences among the rice genotypes are under investigation.

Mobility and bioavailability of pollutants in soils from 'Terra dei Fuochi' (Campania region)

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This work was carried out within the LIFE project ECOREMED *Implementation of eco-compatible protocols for agricultural soil remediation in Litorale Domizio-Agro Aversano RIPS* (ex-NIPS L. 426/98), Campania region (Southern Italy). The area, commonly known as 'Terra dei Fuochi', is characterized by a diffuse land abuse mainly through legal and illegal activities of wastes dumping and incineration. The LIFE project intends to define an operative protocol for agriculture-based bioremediation of polluted soils, including the use of biomasses for renewable energy production.

This study was aimed to characterize the potentially toxic elements (PTE) pollution in terms of fractionation of total content among main soil geochemical forms and main pools or fractions available to plants.

Four abandoned agricultural sites were chosen in the district of Giugliano (NA), Teverola, Trentola Ducenta and Castel Volturno (CE). From each site, soil samples, according to a grid of 10x10m cells, was collected. Pseudototal content of 16 PTE defined by Italian legislation (DL 152/2006) was measured by *acqua regia.* EU-MTP sequential extractions, EDTA 0.05 mol l^{-1} pH7 and leaching test UNI EN ISO 12457-2/2004 were applied to define forms, phytoavailability and mobility of elements in soil.

Copper, Pb, Zn and Cr were the main pollutants exceeding the Italian legal limits (respectively 150, 100, 150, 150 mg/kg). Beryllium, Sn and V, also above the limits (in order 2, 1, 90 mg/kg), were naturally enriched in soils according to Ispra 2001 (baselines for Be 6, Sn 4, V 150 mg/kg).

A very low mobility characterizes all pollutants, likely due to the general alkaline pH and fine texture of the studied soils. Nevertheless, the potentially phytoavailable quantity (as assessed by EU-MTP and EDTA) was for some pollutants a consistent fraction of the total (for Cu 48-65%), indicating a potential effectiveness of the phytoextraction remediation techniques.

Bioavailability studies for food safety, agroecosystem qualification and phytoremediation

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Specifically concerning toxic elements in soil, the contamination degree can’t be expressed only by their total content, and the contaminant chemical form is one of the crucial factors to be considered in order to assess the environmental impact, as from that depend element toxicity and mobility. The contamination degree is better represented by the elemental bioavailable fraction, which in turn depends - other than on the elemental total content and chemical form - on soil physical-chemical characteristics and biological properties, and on environmental factors. The elemental bioavailability and the mechanisms of translocation from soil to plant may affect many different aspects related to land use, from food safety (in terms of elemental content of crops), up to the efficiency and effectiveness of phytoremediation processes.

ENEA-UTAGRI is engaged in studies and researches aimed to assess the contamination degree of agricultural soils and to study the influence parameters on element diffusivity, mobility and bioavailability. In this work we report about ENEA-UTAGRI activities on the development and application of analytical methods for evaluating elemental bioavailability, for studying the influence parameters on plant uptake and for establishing specific provisional *soil-plant translocation* functions. These studies are aimed either to the development of strategies for the reduction/prevention of food product contamination, the qualification/valorization of primary production areas with evaluation of "territorial attitudes", other than to the optimization of processes for phytoremediation of polluted sites.

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