S2-P1
Evaluation of *Rhizobium leguminosarum* Strains Effectiveness, Virulence and Ability of Competitiveness in Field Beans (*Vicia faba* L.)

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In agriculture systems, the bulk of the biological nitrogen fixation is derived from the cultivation of legumes. The inoculation of the legume seed material with active nitrogen fixing bacteria strains before sowing has a significant meaning for the increase of the legume yield. Inoculation can improve crop yields in cases where appropriate rhizobia are not present in the soil or the soil contains a significant proportion of nonnodulating or ineffective nitrogen-fixing strains. The successful use of rhizobial inoculants in the latter situation requires knowledge of the indigenous rhizobial population in terms of numbers, effectiveness, and competitive ability. The aim of the investigation was to detect the effectiveness, virulence and ability of competitiveness of *Rhizobium leguminosarum* strains in field beans (*Vicia faba* L.). The vegetation pot experiments were conducted at Institute of Soil and Plant Sciences of Latvia University of Agriculture and field experiment at agency Research institute of Agriculture of Latvia University of Agriculture. Before sowing field beans seeds ‘Lielplatones vietejas’ and ‘Ada’ were inoculated with *Rhizobium leguminosarum* strain: No. 110; No. 408; No. 501 and control (without inoculants) from the nodule bacteria collection of Institute of Soil and Plant Sciences of Latvia University of Agriculture. In the vegetation pot experiment was used three substratums. Plants were analyzed at six plant development stages: three leaves stage; butonisation; anthesis; pod formation; pod development; pod maturing. Analysed parameters: plant length; fresh weight; dry matter; protein amount and plant pigments. *R. leguminosarum* strains virulence and ability of concurrence were analysed by resistance on antibiotic and by molecular - genetics analyses. Taking into consideration the results of the experiment it has pointed out that all *Rhizobium leguminosarum* strains were active and inoculated plants formed nodules on the roots. It was found different *R. leguminosarum* strain effectiveness in different soils. Inoculation with *R. leguminosarum* strains doesn’t significant impact plant pigments grown in different substratum. The field beans seed inoculation with *R. leguminosarum* strains significantly increased total protein amount.

**Keywords:** *Rhizobium leguminosarum*; Competitiveness; *Vicia faba.*
S2-P2
Determination of Essential Elements in nine Barley Cultivators (*Hordeum vulgare* L.) and its Soil, Grown at Wheat Research Station Tandojam, Sindh, Pakistan

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The mineral composition of nine different cultivars of barley (*Hordeum Vulgare* L.) seeds (Jau-83, Jau-87, Haider-93, Quina, Sadabahar, Bajawar-2000, Frontier-87, Sanober-96) and soil were analysed using flame atomic absorption spectroscopy. Bajawar-2000 was found to contain the higher sodium content. The essential bulk metals in the six cultivars were found to be dominant, where K was the highest and calcium was lowest. The decreasing order of essential trace metal concentration is Fe>Zn. All barley cultivars trial grown at Wheat Research Station Tandojam, Sindh, Pakistan for two years to compare the variation of mineral uptake. In this connection, wet ashing method used to destroy the organic matrix. The concentration in mg/Kg of the soil of that specific plot was observed 4424.1 ± 291.12 (Na), 1418.0 ± 556.5 (K), 3404.4 ± 327.0 (Ca) and 3975.2 ± 3847.8 (Mg), using reference material checked the validity of digestion method.

Keywords: *Hordeum vulgare*; Fe and Zn; *Vicia faba*.

S2-P3
An Innovative Biological Growth Promoter for Corn

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Application of plant growth promoting rhizobacteria (PGPR) for boosting plant growth and development is not particularly new in agriculture biotechnology. Yet attempt to successfully utilizing PGPR to develop commercial biological growth promoter to improve yield potential of agronomically important field crops are limited. In the present investigation, we tried to develop potential biological growth promoter for corn (*Zea mays*) to enhance performance and yield of the crop. Two sulfur-oxidizing PGPR, Corn01 and Corn02 were used as possible corn inoculants in the study. Corn seeds were treated with viable cells of selected strains of *Delftia acidovorans* (RAY 209) and *Achromobacter piechaudii* (RAY 12), designated as Corn01 and Corn02, respectively. Both rhizobacterial inoculant treatments were compared with the un-inoculated controls in several field trials conducted over three years in the mid-western corn belts in the United States. Corn inoculated with these bacteria showed increased plant vigor and yield. Increased root growth & development, and dark leaves were also quite evident in some of the trials. Upon comparison between Corn01 and Corn02 treatments, more positive and consistent results were observed due to the inoculation...
S2-P4
Rapid Mass Multiplication of *Glomus mosseae* Inoculum

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Rhodes grasses (*Chloris gayana* Kunth) inoculated with *Glomus mosseae* were grown under the influence of a biotic factor - *Azospirillum*, and two abiotic factors - IAA, and Hoagland’s solution. The effectiveness of each factor was evaluated by measuring mycorrhizal root colonization and spore numbers. The pot culture experiment was carried out under polyhouse condition and observations were recorded at 45 days, 90 days and 120 days of plant growth. The harvest date finely influenced the size of mycorrhizal inoculum. But, all biotic and abiotic factors had a greater influence on root colonization and spore numbers than harvest time. The factors (biotic, abiotic) on conjunction favorably enhanced root infection and sporulation as compared to their solo treatments, with *Azospirillum* + Hoagland’s solution posing to be the best. This not only stimulated mycorrhizal development, but also accelerated the root growth. This investigation led to achieve large quantities of mycorrhizal root inocula within a relatively short time (45 days) and may help to large-scale utilization of arbuscular mycorrhizal fungi inoculant besides curtailing the production expenditure, which lacks mainly at present.

*Keywords:* *Glomus mosseae; Azospirillum; IAA.*

S2-P5
Mobilisation and Acquisition of Nutrients from Apatite and Biotite by Scots Pine Roots associated or not with a Mycorrhizosphere Bacterial Strain

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With the exception of N, soil minerals are the main source for all mineral nutrients required for tree growth in non-fertilized forest ecosystems. The major part of this nutrient reserve is, however, not directly available to trees as the nutrients are entrapped in the mineral phase. Therefore mineral weathering, a process which promotes soil nutrient bioavailability, is crucial to satisfy plant nutritional needs. A great variety of biological processes linked to plant root and micro-organism activities have been suspected to affect soil mineral weathering and to increase the nutrient supply to trees. The aims of this study are (i)
to quantify the respective effect of Scots pine roots inoculated or not with a mycorrhizosphere bacterial strain *Burkholderia glathei* PML1 (12) on the mobilization of essential nutrients such as Ca, P, K, and Mg issued from the weathering of apatite or biotite, two minerals frequently present in forest soils, and (ii) to assess the contribution of *B. glathei* PML1(12) to the nutrition of Scots pines. The strain *B. glathei* PML1 (12) was isolated in a forest soil mineral horizon from the ectomycorrhizosphere of *Scleroderma citrinum*-oak. This strain was selected within a collection of more than 500 soil bacterial isolates because of it showed highest abilities to produce weathering agents and to mobilize nutrients in different *in vitro* biotests. We carried out a column experiment in a growth chamber. Each column, containing a mixture quartz-biotite or quartz-apatite, was planted with one pine seedling except for the abiotic treatment. The half of the columns containing pine seedlings was inoculated with a calibrated suspension of *B. glathei* PML1 (12). Each column was supplied with a nutrient solution containing all the nutrients necessary to plant growth but absent from the mineral phase. The inputs and outputs of Ca, P, K, and Mg in solution as well as the immobilization of these elements by pine seedlings were measured. As regards to the biotite, the results revealed that Scots pine roots non-inoculated or inoculated with *B. glathei* PML1 (12) significantly increased K and Mg mobilization in comparison with the abiotic treatment. In addition, we observed that the inoculation of *B. glathei* PML1 (12) significantly increased K and Mg mobilization in comparison to the non-inoculated seedlings, resulting in an improvement of Scots pine nutrition and growth by a factor of 1.5. As regards to the apatite, the results also revealed that Scots pine roots non-inoculated or inoculated with *B. glathei* PML1 (12) significantly increased Ca and P mobilization in comparison with the abiotic treatment. Because the amounts of Ca and P immobilized by the pines during the experiment are not yet analyzed, we can not currently assess the effect of *B. glathei* PML1 (12) inoculation on nutrient mobilization and tree nutrition. We therefore observed the presence of oxalic acid, a strong mineral weathering agent, in the drained solutions only when Scots pine roots were inoculated with *B. glathei* PML1 (12). Our study demonstrates the capacity of a mycorrhizosphere bacterial strain to produce weathering agents and to increase the mobilization of nutrients from the soil mineral phases, thus improving tree nutrition.

**Keywords:** Apatite; Biotite; mycorrhizosphere bacterial strain.

### S2-P6

**Inoculation of Lettuce (Lactuca sativa L.) with Arbuscular Mycorrhizal Fungi (HMA) using Inorganic Matrix**

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The soil ecosystem functioning largely depends on soil microbial activity. Arbuscular mycorrhizal fungi (AMF) are one of the most important beneficial microorganism associated with most of the vegetable species which improves nutrition and growth of host plant. In Chile, the area cultivated with vegetables is around 95,000 ha where lettuce occupies an area of 7,027 ha (VII Censo Nacional Agropecuario y Forestal, 2007). The objective of this work was to study the effect of inoculation with three AMF strains using an inorganic matrix and
lettuce type Roman cv. Blonde Maraîchère as host plant. Lettuce seeding was carried out in August, transplantation at the end of September and harvest in December 2007. Treatments included four inorganic matrices: sand (S-1), perlite (S-2), vermiculite (S-3) and a mix of S-1, S-2 and S-3, (S-4); three AMF strains: *Glomus intraradices* (I-1), *Glomus claroideum* (I-2), *Glomus etunicatum* (I-3) and uninoculated control (I-4) with six replications, in a completely at random statistical design. The amount of inoculum accounted for the equivalent of 25 mL. The nursery stage covered was until 44 days after sowing (DDS) and harvest was at 90 DDS. Colonization AMF was quantified by staining the roots with tripan blue and observation under stereoscopic microscope. From 30 DDS, the number and breadth of leaves were weekly measured and chlorophyll measurements were carried out with a Minolta SPAD-502 in the first leaves avoiding aged leaves. After harvesting, dry weight of plants and leaves concentration of macronutrients Ca, Mg, K, P and Na were measured. During the cultivation, the visual appearance of inoculated plants was better than uninoculated being the former ones greater vigour, size and intensive green colour. The largest root colonization was reached with I-1 and I-2 with S-2, with a higher percentage (30%), introducing significant differences with control. However, when vermiculite (S-3) was used as matrix the highest colonization was observed in uninoculated plants due to native strains and coinciding with the highest dry weight obtained. The smallest dry weight was obtained when using S-4 as substrate and the inocula I-1, and I-3. The weight differences observed were mainly produced by the substrate rather than by inoculation, probably due to the short cycle of lettuce, lasting three months between planting and harvesting. According to the results obtained it is concluded that lettuce is a vegetable mycorrhization dependent and their performance depends on the AMF inoculum and the inorganic matrix used.

*Keywords*: Lettuce; arbuscular mycorrhizal fungi; inoculation.

**S2-P7**

**Implications of the “Green Rust” on the Iron-Phosphorus Relationships in Flooded Soils of the Lower Orinoco River Venezuela**

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Intermittent flooding and draining of soils in floodplain result in considerable temporal variability of soil oxygen. Under saturated soil conditions, water restricts gas diffusion and limits the soil oxygen availability. Under anaerobic soil condition biogenerated Fe(II) can experience secondary chemical reactions, which lead to the formation of aqueous complexes and/or the precipitation of ferrous solids as green rusts (GR) compounds $[\text{Fe}^{\text{II}}(\text{OH})_2]^x\cdot [(\times/n)\text{A}^n(m/n)\text{H}_2\text{O}]^x$. In an earlier laboratory research with soil samples collected from a seasonal flooded forest in the lower Orinoco River, we hypothesized that anaerobic soil condition leads to the formation of the green rust compounds. In the field when the soils are exposed to the air, just at the end of the aquatic phase, we have detected dispersed spots of a blue-green colour, which we have associated with precipitation of GR compounds. In this study we examined the soil P distribution and soluble Fe$^{2+}$ content in ten samples collected from the GR spots and the adjacent soil as a control (C). Samples were taken at the end of the flood event in November 2006, following a completely random design. In both GR
and C samples, fine roots (< 2mm) were separated by manual extraction and the concentration of Fe$^{2+}$ and total P was also determined. The results showed that available (resin-Pi), moderately available (NaOH-Pi) and occluded (HCl-, and residual-Pi) inorganic phosphorus (Pi) fractions were significantly higher (P< 0.05) in GR than in C samples. A similar trend to that of the Pi fractions was obtained in the different organic P (Po) pools (NaHCO$_3$-, HCl- and NaOH- Po). Soluble Fe$^{2+}$ in the C samples was well below to that obtained in GR. Fine roots extracted from the GR showed the higher soluble Fe$^{2+}$ and total P. In this study we proposed that GR precipitation in the flooded soils of the lower Orinoco provide a small-scale spatial variability in the Fe cycle, which significantly increase the soil P bioavailability to plants growing in floodplains zones.

**Keywords:** Green rust; flooded soils; iron-phosphorus relationships.

**S2-P8**

**Arbuscular Mycorrhizal Fungi in Atriplex argentina (Chenopodiaceae) from Saline Soils of Córdoba Province (Argentina)**

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Plant root and arbuscular mycorrhizal fungi (AMF) symbiosis is one of many plant strategies in order to grow under a variety of stress conditions. The distribution of certain AMF has been related to different soil conditions (pH, P level, salinity, disturbance, etc). AMF in saline soil may improve plant salinity tolerance and growth. *Atriplex argentina* (Chenopodiaceae), a common native shrub of arid and semiarid zones of Argentina, is valued as a soil stabilizer and as palatable and nutritious forage for many herbivores. The aim of the present work was to study the relationship between seasonal changes in AMF root colonization and AMF in saline soils of Salinas Grandes (Córdoba, Argentina). Soil samples were collected from San José de Las Salinas and Árbol Blanco, Córdoba Province, in autumn and summer (1999). The AM colonization and AM fungal spores were quantified and identified respectively. Arbuscules, intercellular vesicles and inter- and intra-cellular hyphae were observed in *A. argentina* roots. The percentage of colonized root length differed between seasons, being higher in summer, but did not differ between study sites. The AMF most frequently found in the rhizosphere were species of the *Acaulospora* and *Glomus*. The data presented here provide useful information for possible reclamation of degraded areas with *A. argentina*.

**Keywords:** Arbuscular mycorrhizal, Salinity, *Atriplex*.
Remediation of contaminated soils, using plant-based systems, is known as a very perspective and rapidly developing area of biotechnology. Selective introduction of microorganisms with specific or broad-ranging biodegrading properties into a plant rhizosphere may accelerate remediation of soils polluted with organic chemicals. In this work, the results of the green-house experiments with soils artificially contaminated by diesel are presented. The seeds of peas were sown into peat and sandy soils amended with 1% and 3% of diesel, as well as inoculum of bacteria association, which was previously isolated from oil-contaminated sites. After 85 days, when peas were entering the bloom stage in the control samples, plant biomass was harvested. The presence of 1% diesel in sandy soil totally inhibited the growth of peas, although in peat soil an inhibition effect was about 50%. Addition of bacteria inoculum did not influence this effect. Harvested biomass was also tested for moist and dry weight, height, content of chlorophyll a and b. Comparison of soil physico-chemical and biochemical properties after 85 days vegetation experiment with peas showed that the noticeable changes between soils exposed by diesel and without it were occurred. Thus, dry weight of peat soil without diesel was higher almost twice, as compared to the samples with diesel, i.e. 66% and 34%, correspondingly. The pH level of peat and sandy soil was increased in the diesel-containing samples and varied in the range of 4.6 - 5.8 for peat and 6.1-7.5 for sandy soil. Redox potential varied in the range of +49 to +117 mV for peat and +6 to -39 mV for sandy soil. The total microbial count in rhizosphere was 10-100 times higher that in average soil sample. The number of colony forming units on the selective medium with diesel was higher in the samples inoculated with bacteria association. This effect was more pronounced in the samples with sandy soil. Soil was tested also for its fermentative activity, i.e. urease, FDA, and dehydrogenase activity, as well respiration, which provided more complex soil characteristics on biochemical level. Toxicity study was performed using germination test. Summarizing, these results provided the additional information on plant response to diesel contamination in the context of toxicity study and perspectives for phytoremediation. Environmental protection by using nature’s own resources would be both economically and environmentally beneficial.

**Keywords:** *Pisum sativum* L.; enzymatic activities; Sandy Soils.
S2-P10

Responsiveness To Arbuscular Mycorrhizal Fungi Of Seven Woody Species With Different Life Histories, Belonging To A Tropical Forests Cloudy In Miranda State, Venezuela

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To assess the growth and responsiveness on arbuscular mycorrhizal fungi of pioneer species Oyedaea verbesinoides, Helicocarpus americanus, the intermediate Palicurea fendleri, Vismia ferruginea and Hyeronima moritziana and late Richeria grandis and Guapira olfersiana, germinated seeds, then seedlings were transplanted to plastic pots filled with sterile soil, apply four treatments: inoculated with the fungus Glomus manihotis, inoculation and fertilization triple superphosphate (TSP), plants with TSP as a reference and untreated controls. The mycorrhizal colonization was 84 and 63 and 34% for the pioneer species, intermediate and late respectively. Also, Responsiveness resulted in 0.9, 0.57 and 0.13 for pioneers, mid and late respectively, showing a response contrasting these species. In the pioneers the effect of mycorrhizal was evident in higher values for growth, the root to shoot dry matter ratio, root length and total absorption of specific nutrients, which will be enhanced in the presence of fertilizer, in the case of intermediate and late these values were low. In conclusion, it demonstrates the highly mycorrhizal status of pioneer species and the need to take into account these fungi to recover degraded areas.

Keywords: Arbuscular mycorrhizal fungi; woody species; tropical forests

S2-P11

Seasonal Changes in the Activity of Soil Enzymes of a Flooded Tropical Forest Gradient in the Lower Orinoco River, Venezuela

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Soil enzymes play an important role in the decomposition of the organic matter and nutrient cycling. The effects of the natural hydrological fluctuations on the activity of acid phosphatase, urease and deshydrogenase were studied along a flooded tropical forest gradient in Mapire River, Venezuela. Soil samples were collected following a complete natural hydroperiod: end of the dry season (May 2004), end of the rainy season (November 2004) and end of the subsequent dry season (May 2005), and from three zones subjected to different flooding intensities: MAX, MED and MIN zones inundated for 8, 5 and 2 months per year respectively. Soil fresh of each zone and during the three collection periods, were incubated at 28°C and during 12 weeks. Throughout the collection dates, the results showed a decrease in the activity of the three enzymes from the MAX to the MIN zone, following the pattern of organic carbon of these soils. Regarding the seasonality, the three enzymes showed different patterns: Urease and deshydrogenase showed a peak in November 2004 while the acid phosphatase decreased. The increase in deshydrogenase and urease activity in November-2004 was expected considering that flooding brings with it a great contribution of labile organic matter, which with the fall of the water begins to be mineralized. At
the event of flooding are also associated processes of mineral dissolution which promotes the release of nutrients such as phosphorus (P). Therefore, a decline in the activity of acid phosphatase after the flood is logical if we consider that the P is being supplied from the geochemical pool.

**Keywords:** Soil enzymes; tropical forest; hydrological fluctuations.

**S2-P12**

**Application of Bovine Dung, Yield and Extraction of Nutriments in Three Varieties of Alfalfa**

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In the Lagoon Region approximately 39,259 are seeded has of alfalfa. This forage is an important component in the food rations of the milk bovine cattle, since it is a forage of protein high performance (19.5% PC). Reason why to look the suitable doses but of any organic product that is applied to the ground; he is determining in the agricultural production, protection and/or diminution of the contamination of the environment, etc. Logically to find the dose but suitable of dung in this case for the region it is extremely important, dice the different types from ground that exist, Reason why the objective of the work was to obtain the optimal dose of organic installment and the variety that more good interacts to increase the yield and quality of forage in alfalfa. The experiment was performed in the year of 2003-2004, in the locality of Ash of the North, Coahuila. They were evaluated, a treatment witness with chemical fertilizer (30-100-00) and five dung levels: 0, 40, 80, 120 and 160 ton ha\(^{-1}\), using an experimental design of blocks at random with three repetitions. I am carried out the measurement of the nutrimental dynamics of materials CUF 101, Sundor and Altaverde, by means of samplings of plant to determine the extraction by the culture. Also, ground samplings and bromatológico were realized. Also forage yield was evaluated by cuts.y the answer of the applied dung. The green forage production not thus showed statistical differences for the dung treatments for varieties. Occurring the highest productions in cuts 7, 6 and 5, with productions of up to 18.7 ton ha\(^{-1}\) for the Altaverde variety in cut seven, 18.26 and 18.27 ton ha\(^{-1}\) for the Altaverde varieties and CUF 101 in 16.95 cut six and ton has-1 for the Altaverde variety in cut five. With respect to dung treatments, in cut seven it excelled the B5 treatment (160 ton Ha\(^{-1}\) of dung) with 20.9 ton of forage, followed of the B3 treatment (80 ton Have of dung) with 19.8 ton of forage; in the cut the six treatments B5 and B3 behaved equal with 20.3 ton ha\(^{-1}\) of forage; in cut five it was the B4 treatment (120 ton of dung per hectare) but the excellent one with 20.5 ton ha\(^{-1}\) of forage, with a slight increase in the production in the Altaverde variety. In regard to the dung the best treatments were those of 80, 120 and 160 ton ha\(^{-1}\) with a production of 19.8, 19.1 and 20.7 ton ha\(^{-1}\) respectively of fresh forage. The nitrogen extraction by the plant was around 180 kg ha\(^{-1}\) in the dung treatments and of 130 kg ha\(^{-1}\) in the chemical fertilizer treatment and the quality of forage was better in Altaverde genotype.

**Keywords:** Bovine dung; yield; alfalfa.
S2-P13
N$_2$O Emissions from Soil Irrigated with Untreated Wastewater in Central México

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Nitrous oxide is a “greenhouse gas”, and it destroys the stratospheric ozone layer. Approximately 65% of the global N$_2$O emissions are emitted from agricultural fields (Huang, et al., 2004), and therefore this gas is considered within the Kyoto-protocol. Nitrous oxide is produced mainly during denitrification which occurs under anaerobic conditions and is the progressive reduction of nitrate (NO$_3^-$) to nitrogen (N$_2$). In the field emissions occur in so called hot spots. The most important variables that regulate N$_2$O emissions from soils are the soil moisture, the soil temperature, the pH, and the availability of nitrogen and labile organic carbon (Huang et al., 2004). Since reduction processes are more probable to occur in poorly drained and aerated soils, the redox potential is often determined to indicate the degree of aeration in the soil (Tan, 1998). This investigation was done in the Valle del Mezquital, State of Hidalgo, Central Mexico, where wastewater from the metropolitan area of Mexico-city is used to irrigate agricultural land since 1912. The objective of this study was to measure the N$_2$O emissions from a plot. This was achieved by simultaneous quantification of N$_2$O fluxes and redox potential measurements before, during and after an irrigation event. The measurements were done in an alfalfa field of 5 ha. The field is irrigated by overland flow with untreated wastewater every 28 days. We installed 4 sampling sites within the field; two of them on vertic Phaeozems, and two on the lower flat part of the field with pellic Vertisols.

At each sampling point a soil profile was dug in which we installed in total 10 Pt electrodes at different depths. N$_2$O emissions were measured by the static chamber method (Teira, 1998). Statistical analyses were performed with the STATISTICA 6.0 software. Before irrigation (figure below), all soils had oxic conditions with mean Eh values of 400 to 650 mV and very small N$_2$O emissions (0.01-0.04 mg N-N$_2$O m$^{-2}$ h$^{-1}$) occurred. During irrigation redox potentials (RP) decrease (-100 to 500 mV) and anoxic and suboxic conditions prevail (≈60 to 400 mV) for the following two days; in this period N2O is emitted reaching up to 3.42 mg N-N$_2$O m$^{-2}$ h$^{-1}$ in one of the Phaeozem subplots. As wastewater infiltrates and the top soil starts to dry out, a transition from suboxic to oxic conditions (300 to 600 mV) is observed and the N$_2$O emissions decrease until the initial flux values are reached again. The N$_2$O emissions measured in this plot are comparable with the N$_2$O fluxes published for fertilized arable soils. Teira (1998), reported fluxes ranging between 0.166 to 1.620 mg N-N$_2$O m$^{-2}$ h$^{-1}$ for a clay rich agricultural soil.

**Keywords:** Nitrogen emissions; wastewater; soil irrigated.

**References**


S2-P14

Carbon and Nitrogen Pools in Soil Physical Fractions Following Land Use Changes from Native Forest to Hoop Pine Plantation in Subtropical Australia

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Soil physical structure causes differential accessibility of soil organic matter (SOM), including carbon (C) and nitrogen (N) pools, to decomposer organisms and is an important determinant of SOM storage. Physical fractionation method of SOM in conjunction with elemental as well as isotopic analyses (C, N, $\delta^{13}$C, $\delta^{15}$N) of those soil fractions are used in this study to determine the land use and management induced changes of SOM and associated processes in three adjacent sites of native forest (NF), first (1R) and second rotation (2R, including tree planting row (2R-T) and windrow of harvest residues (2R-W)) of hoop pine plantations in southeast Queensland, Australia. The results suggest that there is a greater accumulation of C and N in the light fraction (LF) of NF site than that of plantation sites (1R and 2R), and the C and N losses following conversion from mixed species NF to the single-species plantation are attributed to the reduction in C and N stocks for all physical fractions separated by wet sieving. In contrast, the C and N losses induced by the rotation practices (e.g., between 1R and 2R-T) are attributed to the C and N decreases in the LF and macroaggregates (250-2000 µm) only. The C and N increases upon the residue management (e.g., between 2R-W and 2R-T) are primarily attributed to the C and N increases in the LF and macroaggregates as well. Coupled with 30 soil chemical and biological parameters obtained in our previous studies, further principal component and multivariable regression analyses were conducted and the results showed that both the LF and macroaggregates were highly related to the status of C and N pools, the processes of N transformation and soil respiration, and the diversity of microbial communities, and thus could serve as diagnostic SOM fractions responsible for the changes in soil properties and processes induced by the land uses and management practices. Knowledge of the interactive relationships between aggregate classes within SOM and soil chemical and biological processes in this study represents a further step towards diagnostically measuring and understanding important soil C and N processes in response to the land use and management changes in the soil ecosystems such as forests in subtropical Australia.

Keywords: Carbon and nitrogen pool; native forest; pine plantation.

S2-P15

Current Information on Phytase-Producing Bacteria

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During last two decades there has been an increase in the biotechnological applications of microbial phytases in various fields, such as animal feed, processing and manufacturing of
food for human consumption, environmental protection, aquaculture and agriculture. Phytases are group of enzymes capable of releasing phosphate from phytate and they can be found in many organisms (plants, animals and microorganisms). Phytate is by far the most prevalent form of organic P in soils. Thus, phytase-producing bacteria (PPB) have attracted the attention of microbiologists and agriculturists as inoculants to improve the phosphorus-uptake by plants. The present study summarizes the current information on phytase-producing bacteria contained in public databases, such as National Center of Biotechnology Information (NCBI) and Swiss-Prot. The analyses of literature showed that phytases have been detected in a wide variety of bacteria, such as bacilli, enterobacteria, anaerobic rumen bacteria and pseudomonas. The analysis of protein sequences revealed that phytase-like proteins are found in a great diversity of bacterial groups, particularly in γ-proteobacteria. Based on amino acids sequences, phylogenetic analyses of representative phytase-like proteins into γ-proteobacteria shows clustering of the three classes of phytases (cysteine phytases, histidine acid phosphatases and β-propeller phytases) reported. The importance of bacterial phytases and PPB as potential biotechnological tools has been recognized in various fields. However, this study shows that only a limited number of bacterial phytases have been reported and studied, and our knowledge on mechanisms and factor regulating phytase activity is limited. The use of PPB for improving plant nutrition in the field also represents a promising potential for agriculture, although major efforts will be required to refine and implement this technology prior to its becoming economically viable.

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Keywords: Bacterial phytase, soil, bioinformatic.

S2-P16

Influence of the Temperature on Urease Activity in Andisols Fertilized with Urea

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Nitrogen (N) is an essential element for plant nutrition and is one of the most important factors that limits crop and pasture yields. In the soil-plant system, N availability mainly depends on the mineralization of organic N into ammonium (NH4+-N) by urease enzymes from soil organic matter, organic residues and urea-based fertilizers. Thus, the changes in the urease activity can be used as an indicator indirect of the variation in the pool of potentially available N in a soil. The aim of this study was evaluate the effect of time, temperature and urea supply level on the urease activity in two Andisols. In order to evaluate the effect of soil temperature, duplicate 100 g soil samples of each soil [(Piedras Negras soil (PNS) and Freire soil (FS)] were treated with urea at rates equivalent to field applications of 0, 100, 200, 400 and 800 kg N ha⁻¹. The samples were incubated in plastic bags at 5, 10 and 21°C under aerobic conditions, and subsamples were taken at 24 and 48 h to determine the urease activity. To assay the urease activity a modified method of Nannipieri et al. (1980) was used. Soil sub-samples of 1 g were incubated with 4 mL of 0.1 M phosphate buffer (pH 7.1) and 1 mL of 1.067 M urea at 5, 10 and 21°C by 2 h, and after
incubation 5 mL of 2 M KCl were added to terminate the reaction. The N-NH$_4^+$ was determined by ion selective electrode. In both Andisols, the urease activity were fitted to a simple Michaelis-Menten kinetics and Michaelis-Menten constant (K$_m$) and the maximum enzyme reaction velocity (V$_{max}$) were determined. Comparatively, PNS registered a higher urease activity than FS at 24 and 48 h of soil incubation. Although FS showed pH values slightly superior than those of PNS, the highest organic matter content of PNS (220 g OM kg$^{-1}$) may explain the greatest activation of the urease enzyme in this soil with respect to FS (170 g OM kg$^{-1}$) at all the temperatures. A differential activation of the urease was also observed by effect of the incubation time in each soil. Thus, the urease activity was raised in PNS as the incubation time increased from 24 to 48 h. In FS, an inhibition of urease occurred after 48h at 10 and 21°C, whereas at 5°C the enzyme activity increased. Likewise, urease enzyme was activated when the temperature increased from 5 to 21°C in both soils, but at 48 h of soil incubation the opposite behavior was observed in FS. In fact, the K$_m$ decreased and V$_{max}$ increased with the increase of temperature in PNS and FS at 24 h and in PNS at 48 h of incubation. Furthermore, at 24 and 48 h of PNS incubation, the catalytic efficiency of the enzyme reaction (V$_{max}$/K$_m$) increased 56.1 and 29.4%, respectively, with the increase of temperature from 5 to 21°C. In FS, the V$_{max}$/K$_m$ ratio increased 3.2 times at the highest temperature after 24 h of incubation, whereas it was reduced in 44.1% later. The decrease of both the urease activity and the V$_{max}$/K$_m$ ratio at 48 h of FS incubation suggest that most of applied urea was hydrolyzed before 24 h at 10 and 21°C. In contrast, the activation of urease and the slight changes in catalytic efficiency in PNS by effect of the temperature and incubation time indicates that the urea concentration was not a limiting factor for the enzyme activity, and denotes a slower urea hydrolysis in this soil compared with FS.

Acknowledgements: FONDECYT Grant 1061262 and PSD 26 CONICYT-UFRO

Keywords: Nitrogen, urease activity, Andisols.

References
of wheat and sunflower stems was more intensive. The amounts of available P and K nutrients were higher, while the amount of N remained around at the same level in the soil, near the stems. The root fresh weight and the sugar contents were higher in the field experiment, measured in June. The PHYLAZONIT MC® treatment equalized the effect of 80 kg/ha nitrogen fertilization in the field experiments. The contents of „harmful” nitrogen, and other compounds in the sugar-beet were smaller than in the non-treated plants, which is advantageous for industrial processes. The extra yield, calculated in sugar, was: 2.6 t/ha. The application of PHYLAZONIT MC® increased the yields in the laboratory and in the field experiments. The physiological basis is: an increased chlorophyll synthesis, a more intensive uptake of nutrients causes a more intensive growth of leaves. Due to the more active bacterial soil life the level of several organic compounds, as malic acid, citric acid, and syderophores are higher, that makes the solubility and availability of nutrients easier in the rhizosphere. We observed a moderate effect of Al-toxicity when PHYLAZONIT MC® was applied. Supposedly the complex formation of released organic acids by the bacteria prevents the uptake of Al, even under Al-stress. We came to the conclusion, that the PHYLAZONIT MC® is an alternative for replacing chemical fertilizers with a biologically active, environmentally protective agent.

**Keywords:** bio-fertilizer; ion-uptake; Al-tolerance.

**References**

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**S2-P18**

**Impact of the Biodegradation of Bovine Manure in the Alfalfa Production**

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This study had as general objective to determine the nutritamental necessities of alfalfa forage under several environmental conditions. The main agricultural problem in the Comarca Lagunera Region is that farmers do not have information about nutrient uptake for alfalfa forage and their effect on forage production. The alfalfa is the most important forage for cow milk production, and income for farmers. The experiments were established in three locations of Ejido Fresno del Norte Coahuila, Venecia and Horizonte Durango, respectively. The two factors were studied alfalfa varieties as factor one in three levels, CUF 1001, Sundor and Alta Verde and the factor B had six levels: 0, 40, 80, 120, 160 ton ha\(^{-1}\) of cow manure with one additional level of chemical fertilizer (30-100-00). The combination among factors gave 12 treatments which were established in a complete randomized block design with split block arrangement replicated three times. Plant and soil parameter were measured each harvest period to compare treatments such as dry and soil temperature and moist, respectively. Results indicated a low increment in forage dry and wet production in Sundor variety in tree harvesting times. With respect to cow manure with a wet forage yields of 19.8, 19.1, and 20.7 ton ha\(^{-1}\) at seven yield of forage respectively. The nitrogen uptake by plant was in average in chemical fertilizer and the control treatment. The soil temperature varies in average from 25 to 32°C in spring and from 15 to 19°C in winter this condition in part
explains the low yield in winter. As a main conclusion according to the results, a cow manure application at the beginning of alfalfa forage varies from 80-160 ton ha$^{-1}$ with only one application of cow manure.

**Keywords:** Manure; alfalfa; biodegradation.

**References**


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**S2-P19**

**Management Strategies and Practices for Preventing Nutrient Deficiencies in Organic Crop Production**

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In organic farming, synthetic fertilizers/chemicals are not applied to increase crop production. In the Canadian Prairie Provinces, most soils under organically farmed systems are deficient in available N for optimum yield. There are many organically farmed soils low in available P, and some soils contain insufficient amounts of S and K for high crop yields. The N deficiency in soil on organic farms can be corrected by growing N-fixing legume crops in the rotations, but if soils are deficient in available P, K, S or other essential nutrients, the only alternative is to use external sources to prevent their deficiencies. Field experiments are underway in Canada to determine the influence of management practices (crop diversification with deep taproot and shallow fibrous root crops, crop residue, green manure, legumes for seed/forage, cereal-legume intercropping) and amendments (*Penicillium bilaiae*, rock phosphate, elemental S, gypsum, compost manure, wood ash, alfalfa pellets) on crop yield. In the cropping systems study established in 1995, crop yields for organic system without any chemical input were 30-40% lower than the conventional system with high inputs. But, lower input costs plus price premiums for organic produce normally more than offset lower yields, resulting in favorable economic performance and energy efficiency. Legume, green manure, compost manure helped to replace nutrients lacking in the soil and improved crop yields. In the organic system, amount of P removed in crop exceeded that of P replaced and this can be a major yield limiting factor. In amendments experiments, there was small effect of granular rock phosphate fertilizer and/or *Penicillium bilaiae* in increasing soil P level and crop yield in the application year. Other findings suggested the use of elemental S fertilizer, gypsum, compost manure, wood ash or alfalfa pellets to improve nutrient availability, and yield and quality of produce. In conclusion, the findings suggest that integrated use of management practices and amendments has the potential to increase sustainability of crop production as well as improve soil quality plus minimize environmental damage.

**Keywords:** Organic crop; compost; *Penicillium bilaiae*. 
S2-P20

The Enzymatic and Abiotic Hydrolysis of Glucose-1-Phosphate Adsorbed on Goethite: An ATR Infrared Spectroscopy in situ Study

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In order to make phosphorus in organophosphates bioavailable, hydrolysis is usually a required step. This process may be catalyzed by exoenzymes present in soil. However, organophosphates have due to their phosphate moiety a strong affinity for environmental particles, which may affect their biodegradation. In this study the hydrolytic reaction is investigated with focus on a water-mineral interface at which adsorption of glucose-1-phosphate occurs. Glucose-1-phosphate has been used in bioassays as a model substrate and is found to be equally available as orthophosphate. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR FTIR) is a technique which allows in situ measurements of surface reactions occurring on e.g. metal oxide particles in aqueous solutions. In the present work ATR FTIR is used to monitor the enzymatic hydrolysis by a phosphatase from sweet potato of glucose-1-phosphate adsorbed at the water-goethite interface. Substrate desorption is investigated using the same molecular-level technique. The kinetics of hydrolysis and desorption, and the stability of the adsorbed glucose-1-phosphate in absence of enzyme will be discussed. The spectroscopic data will be presented together with results from quantitative measurements of levels of substrate and hydrolytic products, showing that abiotic hydrolysis due to surface sorption on goethite is not likely the reason to the bioavailability of glucose-1-phosphate.

Keywords: organophosphates; exoenzymes; ATR Infrared Spectroscopy.

S2-P21

Biological Fertility of an Intensive Farming Soil Affected by Various Management Practices

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In areas with intensive agricultural productions soil fertility can be depressed by practices addressed to optimize the crop and not attentive to store physical, chemical and biological fertility in long term. The preferential use of mineral fertilizers to the detriment of organic amendments leads to a decrease in soil productivity and plant growth. The depletion of the organic carbon content is a common response in these soils and a very strictly relation ship with biological properties can be observed. Soil enzymes and microbial biomass are sensitive to the presence of organic matter and they are largely known as valid indicators of soil biological fertility. The aim of this work was to study the effect of organic fertilizations on some enzymatic activities (dehydrogenase, arylsulphatase, β-glucosidase, phosphatase, urease) and microbial biomass of an agricultural soil under crop rotation, sited in Campania
Region (Italy). At the beginning of the experimental plan, compost from vegetable residues, ricin seed exhaust and straw were supplied singly or in association on plots treated or not with a preventive sterilization by solarization and calcium cyanamide to control soilborne pathogens and weeds. The physical, chemical and biological analysis of the agricultural soil were carried out after the initial treatment (September 2005), after the first crop, lettuce (December 2005), and after pepper and lettuce rotation (January 2007). All plots amended with compost, and especially those treated with all three organic materials, showed higher contents of organic carbon and total nitrogen, correspondingly higher values of enzymatic activities and microbial biomass were detected. Conversely, solarization and calcium cyanamide depressed biochemical and biological parameters. At the last sampling amended plots showed a decrease of organic carbon content to the initial level registered in the control plot to indicate that the organic matter supplied by this compost or ricin seed exhaust was not stable and did not ensure a durable nutrient source.

**Keywords:** Biological fertility; soil enzymes; microbial biomass.

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**S2-P22**

**Microbial Mobilization of Mineral Phosphates Co-inoculated with Diazotrophic Bacteria on the Yield of the Pasture *Brachiaria decumbens* Stapf.**

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The bioavailability of N and P in soils plays a fundamental role in the sustainable management of a pasture yield. The present research evaluates two microbiological processes: the mineral phosphate bioconversion by phosphate solubilizing microorganisms and the increase of nitrogen uptake by nitrogen fixing bacteria. A low P acidic soil was amended with the Venezuelan mineral phosphate (MP) of Monte Fresco; this mineral was used as an insoluble inorganic phosphate fertilizer. The phosphate solubilizer fungus *Penicillium rugulosum*, strain IR-94MF1, was evaluated in a randomized greenhouse experiment in conjunction with different diazotrophic rhizobacteria. These rhizobacteria include the following: *Enterobacter cloacae*, *Azospirillum amazonensis*, *Rhizobium* sp., and two unknown diazotrophic Gram-negative strains. The effect of these consortia was evaluated on the growth of the tropical pasture *Brachiaria decumbens* Stapf. The diazotrophic strain, which showed higher potential based on the dry matter yield of shoots and their P and N contents, was selected for the use of a field experiment. The microbial treatments were as follows: an uninoculated control, *P. rugulosum* strain IR-94MF1 alone and *P. rugulosum* strain IR-94MF1 co-inoculated with the rizobacteria *E. cloacae* strain 17. The mineral phosphate was applied at a rate of 0 (control) and 750 kg ha⁻¹ using a bifactorial analysis of variance with repeated measurements over time. The study was performed during dry and rainy seasons in an established pasture of *B. decumbens*. The results for dry matter yield, %N and %P were evaluated for five cuts during seven months. If compared to the uninoculated controls, the inoculated treatments with and without the application of the MP, showed significant increases (*P<0.05*) of the accumulated dry matter yield, and N and P contents based on a measurement of Kg/ha/7months. The application of the fungus with the nitrogen-fixing bacteria did not show significant statistical differences with respect to the productivity of *B. decumbens*. However, without the application of the MP, *E. cloacae* showed a positive tendency of 15% for the
accumulation of N in Kg ha\(^{-1}\). It seems that both microorganisms, isolated from the Monte Fresco phosphate mine may function in a synergistic way when they are inoculated in a less rich nutrient rhizosphere. However, after the application of the MP, this positive tendency was reversed; the fungus MP solubilization capacity might activate native nitrogen fixing microflore allowing for better assimilation of the nutrients by the pasture. Moreover, the simple inoculation of the fungus \textit{P. rugulosum} strain IR-94MF1 fulfilled the bioconversion of the MP resulting in an increase of 48% P in the shoots and 36% for the P accumulated relative agronomic efficiencies. Indeed, these bioinoculants could be considered as a sustainable biotechnological option, which could increase the nutritional value of \textit{B. decumbens} pasture.

**Keywords:** \textit{Penicillium rugulosum}; \textit{Enterobacter cloacae}; mineral phosphate.

**S2-P23**

**Use of Indigenous N\(_2\)-Fixing Soil Cyano-Bacteria for Low-Input, Sustainable Improvement of Overall Fertility of Degraded Lands in Semiarid Tropics**

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For a sustainable improvement of both, biogeochemical performance and physical fertility of degraded soils in marginal lands of three Southern Africa Countries (Tanzania, Zimbabwe and South Africa), an holistic approach was adopted to asses the possibility of locally growing and applying to soil, of biomasses of selected strains of indigenous cyanobacteria. Some strains of these photosynthetic, autotrophic micro-organisms are able to fix atmospheric Nitrogen, and to produce exo-poly saccharides (EPS), capable to bind soil mineral particles, thus increasing aggregates stability. The main results of the investigations undertaken can be summarized as follows:

a) **Cyanobacteria identification and selection.** A large number of cyanobacteria strains (about 200) have been identified. Some of these strains were found able to fix consider- able amounts of atmospheric Nitrogen and to produce abundant EPS. The most productive indigenous strains for each Country were successfully selected, purified and grown with suitable procedures and techniques specifically adapted to obtain large amounts of biomass, even under stress. These biomasses were used for laboratory and field studies and experiments aimed to increase permanently the overall fertility and productivity of degraded soils.

b) **Effects on chemical fertility.** Laboratory, greenhouse and field experiments demonstrated that cyanobacteria were capable to grow even under limiting conditions (low nutrients availability, low pH). In spite the metabolic cyanobacteria activity decreases the NO\(_3\)- and NH\(_4\)+ concentration, as they use these forms of N to grow, the overall soil C and N content increased. In soils with low nutrients content, cyanobacteria increased the amount of total and available phosphate, potassium, calcium and magnesium and also the concentration of micronutrients. In soils with higher inherent fertility in which cyanobacteria developed to larger extent, a decreased concentration of available macro- and micronutrients was found, due to sorption on the negative charged surface of the EPS produced by these micro-organisms. Moreover, cyanobacteria modify the SOM characteristics and increase the more stable humic fraction.
c) Effects on physical fertility. The results of laboratory, greenhouse, and preliminary field experiments showed an increase of aggregates stability due to cyanobacteria inoculation on poorly structured soils. This improvement appeared shortly after inoculation and increased with time and growth of cyanobacteria biomass. The increased stability is accompanied by changes of soils surface microstructure and formation of organo-mineral aggregates.

d) Effects on crop growth. Greenhouse experiments with rice and maize showed that inoculation with cyanobacteria had limited effect on the first crop, but the yield of the subsequent crop increased significantly. The effect of cyanobacteria on crop growth varied with soil types and this depended on the different extents of cyanobacteria establishment. Preliminary, short-term field experiments seem to confirm the beneficial effect of cyanobacteria on improving crops yield.

Keywords: Cyano-Bacteria; exo-polysacharides; nitrogen.

S2-P24
Mineralization of Cow Manure and Their Impact in Production and Quality of Corn Forage under Subsurface Irrigation System

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At the present time the excessive chemical fertilizer use and the little use of organic amendments have brought with the deterioration of the physical, chemical and biological properties of the ground. On the other hand little technology has been generated to use this valuable remainder of the cattle activity. Combined to the previous thing the change of the system of traditional irrigation is urgency in the Lagunera Region. The work was made in the Agriculture research center of the Agriculture and Zootecnic Collage in the Ejido Venecia, County of Gómez Palacio, Durango, México. The variety of corn “San Lorenzo” (generated in this College) was planted in 1982 and the variety of Soya “Cajeme”. The exploration spaces were the following: Factor (cow manure): with the levels; B1 = 0 ton ha⁻¹ (control); B2 = 40 ton ha⁻¹; B3 = 80 ton ha⁻¹; B4 = 120 ton ha⁻¹; B5 = 160 ton ha⁻¹; B6 = 100-150-00 (chemical fertilization). Treatments distributions were made with a randomized block design using a split block arrangement replicated three times. The physical characteristics texture, capacity of field, witting point, usable humidity and soil temperature. Chemical variables also were measured such as: electrical Conductivity, organic Matter, pH, Ammonium and Nitrates. The main variables measured in the plant were: yield of green and dry forage. The physical-chemistries characteristics of the ground indicate that the soil is a clay until the depth of 90 cm and salty clay from 90-120 cm, which shows that this type of ground retains nutrients and water in significant form for the culture compared with any other type of mineral ground, since its water available (CC and PMP) is high in comparison with the other 10 textural classes that exist (Cadahia, 1998). pH in general is alkaline, the value of organic matter is poor (less than 1,1%) in the layer of 0-30 cm, and decreases up to 0.51% at 90-120 cm of depth. The value of EC (1.5 dSm⁻¹) is normal for the establishment of any agricultural crop. With respect to N-NO₃ a poor ground is considered since the maxima concentration found was 17.5 mg kg⁻¹ to 0-15 cm and 3 mg kg⁻¹ in the layer 90-120 cm, indicating that the ground requires chemical or organic fertilization for a good development of the crop. The phosphorus content is considered low; the value of the potassium (k) 119.37 mg
kg·L⁻¹ is high, which is a characteristic of the regions with calcareous soils. For the value of N-NH₄⁺, like for nitrate poor soil is considered with less than 14.52 mg kg⁻¹, in general terms these results are considered like normal for soil of the region. The forage production during years 2001 and 2002 had very similar values, not thus in 2003 that corn forage production decreases; nevertheless, the tendency was the same with respect to the manure treatments, where the 80 and 120 ton ha⁻¹ of cow manure had the highest production with 86 and 84 ton ha⁻¹ respectively of green forage for 2001, respectively; 84 and 86 t ha⁻¹ of green forage in 2002 and 73 and 72 ton ha⁻¹ of green forage for 2003.

**Keywords:** Cow manure; corn forage; irrigation.

**References**

**S2-P25**

**The Fate of Organic and Inorganic Phosphorus in Soils - Analysing a Single Root Model**

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A mathematical single root model describing the effect of root exudates on the bioavailability of both organic and inorganic phosphorus is analysed using the method of nondimensionalisation and perturbation methods. Phosphorus (P) is mainly taken up by plants in the form of ortho-phosphate (Pi). The amount of total P bound to organic forms varies largely between soils and may contribute significantly to the P nutrition of plants. In order to be utilized by plants, organic P must be hydrolyzed to inorganic P by phosphatases which may be of plant or microbial origin. The proposed model includes solute transport in the rhizosphere as well as the effect of roots exuding both low molecular weight organic acids and phosphatases on the P uptake of a single root. Each of these processes has a characteristic time and spatial scale. When compared to each other, some processes may be dominant while other processes may appear negligible. Information about the importance of the processes compared to each other is useful for management decisions concerning the P nutrition of agricultural crops. The method of nondimensionalisation provides a mathematical tool to analyse this. It is a process of changing variables by scaling so that the new variables have no units. This procedure leads to a simpler form of equations with fewer parameters that are all dimensionless. These parameters describe the relative importance of different processes included in the model compared with each other and determine the behaviour of the solution. The proposed dimensionless model shall help to interpret the complex chemical, physical and biological interactions in the rhizosphere with regard to P and exudate control mechanisms, and lead to a better understanding of the relevant processes involved.

**Keywords:** Mathematical model; inorganic and organic phosphorus; nondimensionalisation.
S2-P26

Effects of “Helper” Microorganisms on Rhizobial and Actinorhizal Nitrogen-Fixing-Symbioses

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The aim of the present study was to examine the effect of saprophytic actinomycetes as “helper” microorganisms on two different nitrogen-fixing-symbioses: rhizobial and actinorhizal. Experimental assays were carried out under laboratory conditions, employing the strains Streptomyces MM40, Actinoplanes ME3 and Micromonospora MM18, which were previously isolated from the rhizosphere of Ochetophila (Discaria) trinervis and characterized as rhizoactinomycetic microorganisms. Two symbiotic systems, Medicago sativa-Sinorhizobium meliloti and Ochetophila trinervis-Frankia were used. The corresponding plants were inoculated with the symbiotic strains Sinorhizobium meliloti 2011 and Frankia BCU110501, and coinoculated with the above mentioned rhizoactinomycetes strains. Double inoculations were performed either simultaneously or asynchronously, and the plants were fertilized with different N concentrations. In both nitrogen-fixing-systems, the nodulation and, consequently the plant growth were significantly stimulated by simultaneously or asynchronously coinoculations with rhizoactinomycetes strains, compared to those plants, which were inoculated only with the symbiotic strains. In asynchronous coinoculations, this effect was more evident when the plants were first inoculated with Sinorhizobium or Frankia, and later coinoculated with rhizoactinomycetes strains. However, the nitrogen-fixation rate, estimated as acetylene reduction activity (ARA/mg⁻¹) was not significantly modified by rhizoactinomycetes strains. The analysis of the nodulation kinetics with simultaneous or asynchronous coinoculations of rhizoactinomycetes strains with S. meliloti or Frankia suggests, that the effect of saprophytic strains operates in early stage of infection and nodule development, counterresting the autoregulation of nodulation by the plant. The inoculation of rhizoactinomycetes alone did not show any effect on plant growth. In spite of the different infection types -Alfalfa is infected via root hairs and O. trinervis is infected via intercellular invasion-, apparently the rhizoactinomycetes “helper” effect on nodulation may operate by a common step in both systems. As it was expected, a high N-concentration inhibited the nodulation by S. meliloti in single inoculations. On the contrary, in all coinoculations with rhizoactinomycetes strains, the development of nodules could be observed, even under high N-fertilization. These results on the effect of rhizoactinomycetes strains on the nitrogen fixing symbioses, open new perspectives on the applications of the nitrogen fixing plants as the agronomical-uses of legumes and as the restoration of degenerated forest soils (actinorhizal plants).

Keywords: Ochetophila trinervis; Medicago sativa-Sinorhizobium meliloti; nitrogen-fixing-symbioses.
Organic Fertilizer S in Hot-Water Extract from Calcareous Arable, Fallow and Forest Soils as Affected by Different Sources of Organic Carbon Additions

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Hot-water extractable organic carbohydrates are reported to be mostly microbially-derived compounds that constitute a reservoir of readily available nutrients for plants. However, little is known about the impact of more or less labile carbon sources on the availability of organic sulphur (S) in hot-water extract. This study examined the effects of salicylate, glutamate and glucose on fertilizer S immobilization and the subsequent releases of fertilizer S in hot-water extract (HW-S) after incubation of three calcareous soils differing in organic C contents: an arable soil (2.3% organic C), a fallow soil (3.9% organic C) and a forest soil (7.3% organic C). The amounts of carbon of the three substrates were added at two rates (0 and 1000 mg kg\(^{-1}\) soil) to the three soils after a 1-week soil pre-incubation at 25°C to regenerate microbial biomass and then incubated for 1, 2, 6 and 12 weeks more with a \(\text{Na}_2^{35}\text{SO}_4\) solution (674 kBq kg\(^{-1}\) dry soil and 20 mg S kg\(^{-1}\) soil) prior to analysis. All soils received the same amount of 80 mg N kg\(^{-1}\) soil as NaNO\(_3\). HW-S was obtained after sequential extraction of SO\(_4^{2-}\)-S with 0.009 M Ca(H\(_2\)PO\(_4\))\(_2\). The use of \(^{35}\text{S}\) makes it possible to calculate the amount of fertilizer S immobilized and that of fertilizer S in hot-water extract. Fertilizer S immobilization gradually increased over time, indicating a progressive integration of fertilizer S in the structure of humified compounds via microbial biomass turnover. Across all soils, the average amounts of fertilizer S immobilized were in increasing order: control < salicylate < glutamate < glucose. For all substrates, the quantities of fertilizer S immobilized were on average in the order: arable soil < fallow soil < forest soil, indicating a largest fertilizer S immobilization in the forest soil mostly rich in organic C. In contrast, the average values of HW-S expressed as percentage of immobilized fertilizer S across all soils were in increasing order: glucose = glutamate < salicylate < control, suggesting a greater yield of recalcitrant microbial S products from labile organic carbon source additions such as glutamate and glucose, basically present in root exudates. The addition of salicylate, a breakdown product of lignin in the detritusphere, resulted in more percentage labile HW-S production compared with glucose and glutamate. Based on the largest S immobilization with glucose, the non difference in HW-S observed between glutamate and glucose suggests that glucose proportionally produced more labile HW-S than glutamate. This is reflected in the sulphate specific activity average values that were in the increasing order: glutamate < glucose < salicylate < control. This indicates a lowest turnover of fertilizer S in the presence of glutamate. Overall, our results showed that the efficient S immobilization induced by labile C sources such as glutamate and glucose was associated with a greater yield of recalcitrant S products as determined in hot-water extract. Freshly immobilized fertilizer S was more labile than the older one. Apparently, fertilizer S immobilization with glutamate engendered more recalcitrant S compounds than with glucose.

Keywords: Organic fertilizer S; organic carbon; forest soil.
Effects of Glucose on Sulphur Dynamics in Calcareous Arable and Fallow Soils

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The magnitude of microbial sulphur (S) immobilization as well as the synthesis of inducible enzymes such as arylsulphatase (ARS) are basically controlled by carbon (C) additions. In order to assess soil fertility after a period of fallow a better knowledge of S dynamics including S immobilization and ARS activities of a natural fallow soil (3.9% organic C) in comparison with an arable soil (2.3% organic C) is required. Thus, the objectives of this work were to (i) examine the effects of glucose-C additions, a representative labile organic C in the rhizosphere, on the intensities of S immobilization in relation with ARS activities and (ii) distinguish extra- and intra-cellular ARS activities induced by glucose-C applied to the two soils. The amounts of glucose-C were added at six rates (0, 125, 250, 500, 750 and 1000 mg kg\(^{-1}\) soil) to the studied soils after a 1-week soil pre-incubation at 25°C and then incubated one week more (to promote microbial S immobilization over S remineralization) with a Na\(^{35}\)SO\(_4\) solution (518 kBq kg\(^{-1}\) dry soil and 20 mg S kg\(^{-1}\) soil) prior to analysis. All soils received the same amount of 80 mg N kg\(^{-1}\) soil as NaNO\(_3\). Soil SO\(_4^{2-}\)-S content was extracted with 0.009 M Ca(H\(_2\)PO\(_4\))\(_2\). The use of \(^{35}\)S makes it possible to calculate the amount of fertilizer S immobilized. The ARS method used determines the total ARS which corresponds to the ARS located either outside the cells (extra-cellular ARS) or inside the cells (intra-cellular ARS). Recovery tests using 0.009 M Ca(H\(_2\)PO\(_4\))\(_2\) showed a 100% recovery of SO\(_4^{2-}\)\(^{35}\)S introduced in the sample and extracted immediately after addition. Therefore, physical immobilization (adsorption of SO\(_4^{2-}\)\(^{35}\)S onto soil particles) was excluded and the \(^{35}\)S immobilized corresponded exclusively to microbial immobilization. Glucose-C additions increased both microbial S immobilization and ARS activities. Related to the rate 0 (control), largest increase in immobilized-S was observed in the arable soil (300.7%) compared with the fallow soil (153.1%). In contrast, the ARS activity increased by 16.4% in the arable soil versus 32.1% in the fallow soil. These results indicate that glucose proportionately affected more the intensities of immobilized-S than those of ARS. We found positive and significant correlation coefficients of ARS activities with immobilized-S in the arable soil (r = 0.86, P < 0.05) and in the fallow soil (r = 0.83, P < 0.05). Accordingly, by extrapolating to zero immobilized-S (or microbial biomass-S), the results showed a presence of extra-cellular ARS activity of 38.7 mg p-nitrophenol kg\(^{-1}\) soil h\(^{-1}\) in the arable soil and of 63.5 mg p-nitrophenol kg\(^{-1}\) soil h\(^{-1}\) in the fallow soil. Without glucose-C (rate 0), values of 3.7 and 20.0 mg p-nitrophenol kg\(^{-1}\) soil h\(^{-1}\) of intra-cellular ARS activity (difference between total ARS and extra-cellular ARS activity) were found in the arable and fallow soil, respectively. It was concluded that fallowing generated greater ARS activities. Fallow regime re-established soil fertility through increasing soil organic matter content and thereby enhancing the activities of extra- and intra-cellular ARS enzyme compared with the arable soil that is regularly disturbed by tillage, pesticide treatment and fertilization.

Keywords: Sulfur dynamics; calcareous soil; aryl sulphatase.
Eucalyptus sp. Litter Deposition and its Colonization by Ectomycorrhizal Fungi

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Eucalypt forests deposit high litter quantities on the soil surface, promoting an effective cycling of nutrients. This work aimed at evaluating the content and concentration of nutrients in the litter layer of a Eucalyptus sp. forest, grown for 2.5 years, in a mountainous area in the region of Viçosa, MG. Litter samplings were done in three topographical positions, namely Top, Slope, Lowland. The litter was separated in four fractions according to the degree of decomposition and analyzed for the contents and concentrations of P, Ca, Mg, and K. Litter colonization by ectomycorrhizal fungi and the activity of acid phosphatases of ectomycorrhizas present in the litter layer were also evaluated. Litter deposition, in kg ha⁻¹, decreased in the following order (p<0.05): Top, 2,460 > Lowland, 1,730 > Slope, 1,239. The larger fraction of the litter layer corresponded to that showing the most advanced level of decomposition, being composed of dark fragments of leaves and branches in direct contact with the soil. Generally, nutrient content in the forest litter, in Kg ha⁻¹, decreased as follows: Ca (33.4) > K, (4.9) > Mg (1.7) = P, (1.7). Ca content in the litter and its low concentration in the soil evidence the importance of the litter layer as a Ca reservoir for eucalypts. The highest concentrations and contents of P in the litter were verified in the Top position, however, the activity of acid phosphatases of ectomycorrhizas did not differ among the topographical positions evaluated. Fruit bodies of Laccaria, Pisolithus, Scleroderma, and of an unidentified fungus were observed in the area under study. The examination of the most decomposed litter layer and of the interface soil-litter showed an intense colonization by eucalypt roots, with the presence of eight distinct ectomycorrhizal morphotypes, hyphae, rhizomorphs, and basidiomes, suggesting an important role for ectomycorrhizal fungi in the cycling and storage of nutrients in the eucalypt forests.

Keywords: Eucalyptus sp.; litter deposition; ectomycorrhizal fungi.

Soil Microbial Biomass and Urease Activity in a Permanent Pasture under Different Grazing Systems

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In grassland systems, the pasture productivity can be influenced by the grazing management, due to its impact on soil microorganisms and, therefore, on biological processes that affects the nutrient cycling. The aim was to evaluate the soil microbial biomass carbon, microbial biomass nitrogen and the urease activity in a permanent pasture of Southern Chile under different grazing managements. During spring 2005 to winter 2006, four grazing systems were evaluated: frequent-heavy (FH), frequent-light (FL), infrequent-heavy (IH) and
infrequent-light (IL) and a no-grazing control (C), with three replicates, in a randomized block design. According to the results, the values of microbial biomass carbon fluctuated between 28 and 697 mg C kg\(^{-1}\), whereas the microbial biomass nitrogen values ranged between 3 and 70 µg N g\(^{-1}\) during the four seasons. Comparatively, the FH grazing system showed the highest average of microbial biomass carbon (297 mg C kg\(^{-1}\)) and microbial biomass nitrogen (21.6 µg N g\(^{-1}\)). On the other hand, the urease activity oscillated between 84 and 1137 µg N-NH\(_4^+\) g\(^{-1}\) 2h\(^{-1}\), and the highest enzyme activities occurred in the grazed pastures with annual averages between 319 and 339 µg N-NH\(_4^+\) g\(^{-1}\) 2h\(^{-1}\). Additionally, the pasture yield was improved by increasing the grazing intensity, and the greatest production was observed in FH (9.8 ton DM ha\(^{-1}\)) and IH (10.3 ton DM ha\(^{-1}\)). The results showed that, after grazing, a higher flux of organic residues in the soil stimulated the microbial biomass and, therefore, an increase of the urease activity and the microbial biomass -carbon and -nitrogen was observed. Thus, in grazing systems, the increase of the amount of residues in soil from both the plant and the cattle excretes (dung and urine) improves the biological fertility, the nutrient availability and the pasture production.

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**Keywords:** Microbial biomass; urease activity; grazing systems.

**S2-P31**

**Dynamics of Nitrogen and Phosphorus Losses in a Volcanic Soil of Southern Chile**

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The use of nitrogen (N) and phosphorus (P) in fertilizers in cattle production systems of Southern Chile has increased over the last ten years, so that it is expected that the environmental risk in the area will increase. Most of the production is based on grazing systems on volcanic soils, which are characterised by high organic matter content (>15%) and high P fixation capacity. The objective of this work was to establish the dynamic and main forms of N and P losses in runoff and leaching in a volcanic soil. The experiment was carried out on an Andisol of the Osorno soil series (Typic Hapludands). Nitrogen and P dynamics in surface runoff were determined using three surface lysimeters (5 x 5 m). Runoff samples were analysed for nitrate and ammonium. Total N was also determined and organic N (DON) was calculated as the difference between total N and the available N forms. Leaching losses were determined using the ceramic suction cups technique and samples were analysed as indicated previously for runoff samples. In runoff samples, reactive P (RP) and total P (TP) were determined and organic P (OP) was estimated as the difference between TP and RP for each sample. Of the total N lost in runoff, available forms represented on average c. 24% each, as the result of urine lost in runoff immediately after excretion and N lost after nitrate fertilizer application. Dissolved organic N represented c. 50% of total. This could be related to the high soil organic matter content in the topsoil and the expected high soil biomass activity registered at the site. A high proportion of the N leached (c. 70%) was lost as nitrate with ammonium averaging less than 10% of the total inorganic losses. Dissolved organic N in leachates samples represented

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25% of total. Total P losses were mainly as RP (70% on average). Organic P losses represented only 30% of the total P lost in runoff. High soil organic matter played a major role on N and P dynamic losses. Thus, DON was the second most important N form in leachates and the main form in runoff losses. Fertilizer management played a fundamental role on P dynamic losses.

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Keywords: Nitrogen and phosphorus losses; volcanic soils; grazing systems.

References

S2-P32
Effect of Acid Phosphatase Immobilized in Allophanic Clay on P Availability in Soil

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Organic-P represents 20–80% of the total P in most soils and consequently represents a significant reserve of potentially available P. Low phosphorus availability in soil, however, remains one of the largest constraints to agricultural production worldwide. Organic P contributes to the P nutrition of plants primarily after being mineralized into organic P. The rate of P mineralization depends on the activity of hydrolytic enzyme such as phosphatases. In soil, enzymes are naturally immobilized in inorganic, organic and organo-mineral soil constituents. The activity of immobilized enzyme and its catalytic behaviour strongly depends of the nature of the immobilizing support. In a previous work, we are demonstrated that the immobilization of acid phosphatase in allophanic clay increase their catalytic efficiency. Therefore, the immobilization of enzyme in this support could be increase the hydrolysis of soil organic substrates. However, the role of immobilized phosphatase as catalyst in hydrolylising soil organic P have not been well investigated. In order to estimate the role of immobilized phosphatase in P bioavailability in soils we are investigate the effect of addition of acid phosphatase immobilized on allophanic clay on P availability in soil under laboratory conditions. The Andisol soil used in this study has a organic matter of 9 % and 6 mg kg⁻¹ of Olsen P. For the immobilization of acid phosphatase, complexes were formed by the interaction between acid phosphatase and the clay fraction extracted from a Chilean Andisol. Two levels of enzyme (0.0029 and 0.0143 mg protein g⁻¹ of clay) were used for immobilization. Then, the soil was incubated with these complexes at 20°C by 8 days. For comparison, also was added a free enzyme to the soil and the control treatments was the soil without enzyme. At 0, 1, 4 and 8 days, samples of these soils were taken and the amount of available P, determined by the phosphomolybdenum blue method, was measured. By applying immobilized acid phosphatase to the soil, the amounts of soluble inorganic phosphate increased. The highest P content, 15 mg kg⁻¹, was obtained the fourth day for the treatments corresponding to complex with 0.0143 mg of enzyme. At this time, the available P increased by up to 150 % relative to soils without immobilized enzyme. Nevertheless, to the eighth day the available P concentration decreased to 12 mg kg⁻¹. It has been frequently reported that the activity of acid phosphatase can be controlled by the solution P concentration decreasing by a raise of inorganic P concentration. When complex with 0.0029 mg of enzyme was applied alone, the Olsen P increased from 6 to 10 mg kg⁻¹ at the fourth day. While, no significant differences in Olsen P content was observed by addition of free enzyme to the soil. This result indicate that the bioavailability of organic-P is likely to be largely dependent on the amount of
active phosphatases in soil solution. This result showed that acid phosphatase immobilized in allophanic clay can release available inorganic phosphates from soil.

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**Keywords:** Acid phosphatase; enzyme immobilization; allophanic clay.

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**S2-P33**

**Screening and Isolation of Phosphate Solubilizing Fungi from Soils of Southern Chile**

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Chile has excellent conditions to become a major food producer and exporter, as it has done with fruit. In this way, free-living phosphate solubilizers fungi (phosphofungi) can play an important role in plant nutrition by allowing use part of accumulated P in the soil, which has low availability for plants, especially in volcanic soils. However, it is necessary to isolate and select native phosphofungi adapted to the climate-soil conditions in each region and well evaluated on their ability to solubilise phosphate. For this, a lot of liquid media have been suggested but all of them with different sensitivity. Hence, the aim of the study was: 1) To make a screening of native phosphofungi present in volcanic soils from southern Chile. 2) To assess the P solubilization capacity of a selected phosphofungus in four liquid media. The screening of phosphofungi was carried out from several soils of our region under different crops and managements using Martin medium agar-rose bengal-streptomycin, with Ca$_3$(PO$_4$)$_2$ or phytate-Ca as insoluble inorganic or organic phosphate source, respectively. A halo around the colony indicated solubilization. After 5 d incubation at 28ºC an index of solubilization was calculated as the total ratio diameter / diameter of the colony. The colonies with high phytate-Ca solubilization capacity were analyzed for phosphatase (P-ase) activity secretion picking up on Martin medium supplemented with phenolphthalein phosphate; a red halo around these colonies revealed with ammonia vapours indicated secretion of P-ase. A phosphofungus strain with high index of solubilization (*Penicillium* sp.) was used as a model for starting the stage of election of a liquid media to assess phosphate solubilization capacity of the others strains. Erlenmeyer flasks with 50 mL of sterilized liquid medium were inoculated with three 0.5-cm-diameter of fungal mycelium grown on solid agar. The liquids media were as: Agnihotri, Asea-Wakelin, Pikovskaya and Nahas *et al.* Ca$_3$(PO$_4$)$_2$ was used as the P source which was previously autoclaved and then added to the sterile solutions. The growing conditions were: darkness, 20ºC +/- 1, initial pH 6.5 +/- 0.2, intermittent orbital agitation 9 min. every 1 h. After 7 days the solid was separated from the liquid phase by Whatman Nº1 filter paper. In the filterings soluble P and pH were determined. The solid phase was dried at 60ºC for 24 h, and then calcined in a muffle furnace for 6 h at 500ºC; the difference in weight between the two procedures accounted for the fungal biomass. At the controls it was applied the same treatment but without inoculum. All treatments were performed with four replicates. Phosphofungi were detected in the 90% of the soils analyzed, a few showed a promising solubilization capacity of Ca$_3$(PO$_4$)$_2$ or phytate-Ca on Martin medium. The highest solubilization indexes ranged 1.2-1.4 mainly found in medium with
phytate. There was P-ase secretion in 80% of phytate solubilizer colonies analyzed. The *Penicillium* sp. strain showed the highest solubilization capacity in the liquid medium of Asea-Wakelin. In conclusion, it is possible to isolate phosphofungi with high potential P solubilizing capacity to be used for mobilizing accumulated P in volcanic soils.

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**Keywords:** Screening; phosphate solubilizing fungi; Andisols.

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**S2-P34**

**Effect of the Co-inoculation with Two PGPRs Strains (C26 and C139) on Spring Wheat (cv. Pandora) in Volcanic Soils of Southern Chile**

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Spring wheat (Pandora) was inoculated using two strains of PGPR (Plant Growth Promoting Rhizobacteria) C139 (*Pseudomonas* sp) and C26 (*Bacillus* sp.) both isolated from volcanic ash soils of Southern Chile. The taxonomy is being done. The effect was study on plants maintained at Santa Rosa Experimental field, Valdivia, South Chile (39°45’ S and 73°14’ O). Low levels of P Olsen (8 ppm) and mineral N (9.8 mg kg$^{-1}$) were determinated. Both strains showed at laboratory assays: Indols acids production and fixer Nitrogen (strain C26) and of solubilization P (C139). Seven treatments were established at field as follows: Control with full NP, C26 without N, C26 without N and P, C139 without P, C139 without N and P, C139 plus C26 without N and P and control without N and P. Plants were established at 400 plants m$^{-2}$ density in November 2007 and maintained until harvesting time. The Measured traits was total aerial biomass (g m$^{-2}$), leaf area index (g m$^{-2}$), spikes biomass (g m$^{-2}$), radiation interception, leaf protein content, leaf pigments content (chlorophyll *a*, *b*, and carotenoids) and fluorescence of PSII, using Fv/Fm [Fv as variable fluorescence and Fm as maximal fluorescence]. These parameter was using of plant vitality indicator. Samples were taken at tillering initiation, flowering and harvest. Plants with C26 without N presented higher biomass (55%) and higher grain yield (57%) than control without NP and lower than Control NPK treatment (only 76.4% in biomass and 78.5% in grain yield). The treatment C139-P increased biomass and grain yield by 29% and 27% above the control –NP, respectively. At harvest co-inoculation treatments (C139 plus C26 without N and P) showed low yield and biomass than separated treatments (C26 without N). Non significant differences were observed in Fv/Fm parameter, chlorophyll and leaf between treatments at tillering initiation and flowering. All the inoculated treatments with C130 or C26 were significant different than control treatment (without NP) in grain yield and biomass. No positive interaction has been found between strains C26 and C139.

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**Keywords:** PSII; PGPRs strains; volcanic soils.
S2-P35

Interactions between Fe and N Cycles: Role of Fougerite as a Competitor of Nitrate in Anaerobic Respiration and as a Direct Abiotic Reductor of Nitrate

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In moderately reducing conditions, anaerobic respirations rely on different electron acceptors, such as nitrate N(V) and ferric Fe(III) compounds, such as goethite or Fougerite. Fougerite is the natural green rust mineral responsible for the blue-green color of gley. It is a mixed Fe(III)-Fe(II) hydroxide with a brucitic-type layer and a well crystallized mineral with definite crystal structure: the excess positive charge of the layer is compensated in the interlayer by intercalated anions (Trolard et al., 2007). As it contains Fe(III), Fougerite can act as an electron acceptor. Depending on the nature of Fe(III) compounds considered and on pH, nitrate can be reduced before or after Fe(III), so that nitrate and Fe$^{2+}$ can coexist in solution. Thermodynamic calculations show that when Fougerite is not present, nitrate is reduced before goethite, while when Fougerite is present, it is reduced either before nitrate in acidic conditions (pH=6, Fig.1), but is reduced after nitrate in alkaline conditions (pH = 8.5). This implies that Fougerite can compete with nitrate as an electron acceptor in anaerobic respiration. Due to its labile character, Fougerite reduction proceeds easily and poises Eh so that nitrate does not undergo denitrification in anoxic conditions when Fougerite is present. Nitrate cannot be directly reduced by Fe$^{2+}$, but Hansen et al. (1996) obtained the abiotic reduction of nitrate into ammonium by synthetic green rusts, e.g. GR-SO$_4$ in several hours. This is not denitrification, but abiotic reduction, and this is not a depolluting process, as ammonium is more toxic to fish than nitrate. The following mechanism can be proposed: the first step is an anion exchange between sulphate and nitrate in the interlayer. When the extent of the exchange is large enough, the GR structure changes from GR2 (2 layers of water molecule) to GR1 (1 layer), and the nitrate anion is located in between two Fe$^{3+}$ cations, and surrounded by 6 divalent cations Fe$^{2+}$ or Mg$^{2+}$ in the upper layer and 6 others in the lower layer (Fig.2). In this situation, 8 Fe$^{2+}$ can simultaneously donate an electron to N(V), while Fe – OH – Fe bonds transform into Fe – O – Fe bond (oxolation). The GR oxidizes into hematite. Thus N cycle cannot be fully understood without reference to biotic and abiotic interactions with Fe cycle.

**Figure 1:** Scales of reduction with and without Fougerite.

**Figure 2:** Mechanism for direct reduction of nitrate into ammonium by Fougerite.

**Keywords:** Fe and N cycles; Fougerite; anaerobic respiration.
S2-P36
Effect of Nitrogen Supply on Phosphate Dynamic in Perennial Ryegrass (*Lolium perenne* L.) Rhizosphere

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A rhizotron experiment was conducted to evaluate the ryegrass rhizosphere behavior in response to nitrogen fertilizers (N-NH\(_4^+\) y el N-NO\(_3^-\)) supply. In the present work, the effect of N applications on pH values, phosphorous (P) availability, and oxalate concentration in both, bulk soil and ryegrass rizosphere solution, were studied. The assay was carried out under environmental controlled conditions using soil samples (Chilean Andisol) previously incubated with P (300 mg P kg\(^{-1}\) of soil, using KH\(_2\)PO\(_4\)). Ryegrass plants (5 plants per rhizotron) were grown for a period of 12 days. Nitrogen doses of 0, 100 and 300 mg N kg\(^{-1}\) of soil were applied immediately after the rhizotron installation. The rhizospheric solution was collected with the micro-suction cup/rhizobox technique. The micro-suction cups were placed 0-2 mm, 3-5 mm and 6-8 mm in front of growing ryegrass fine roots. Results showed N-NH\(_4^+\) supply diminished the rhizospheric pH. Thus, soil pH values of 5.55 and 5.21 were reached with N-NH\(_4^+\) doses of 100 and 300 mg N kg\(^{-1}\), respectively (average pH of 6.49 in control soil solution). P availability was increased when N-NH\(_4^+\) was applied, nevertheless, for all N treatments rhizospheric solution P concentrations were lower than 1 mg P L\(^{-1}\). A maximum P concentration of 0.7 mg L\(^{-1}\) was recorded with N treatment of 300 mg N kg\(^{-1}\). On the other hand, oxalic acid concentration in the rizosphere solution was increased in response to N-NH\(_4^+\) applications. The highest oxalate concentrations were detected at the distances of 3-5 and 6-8 mm from the ryegrass root surfaces. The highest rhizospheric solution NO\(_3^-\) levels were obtained with N-NO\(_3^-\) doses of 300 mg N kg\(^{-1}\), reaching a maximum of 562 mg N L\(^{-1}\) at 6-incubation day. For all treatments, results indicate that there is a difference of P concentrations in the bulk soil respect to the values detected in ryegrass rhizospheric solution. A decreasing gradient on P availability in the nearest distances to the roots was observed.  

**Acknowledgements:** This work was supported by the FONDECYT project 1061262.  
**Keywords:** Ryegrass; rhizosphere; nitrogen.

S2-P37
The Role of Antioxidative Enzymes and Carboxylates Exudation on the Tolerance to Manganese Toxicity in Perennial Ryegrass (*Lolium perenne* L.)

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Manganese (Mn) is an essential micronutrient in plants, but may become toxic when present in excess in the rizosphere. In Chile, most forage species are cultivated in soils derived from volcanic ashes, like Andisols. About 50% of Chilean Andisols have high soil acidity levels and hence, Mn amounts that may be highly toxic to plants. The biochemical mechanisms underlying differential tolerance to Mn toxicity in cultivars of perennial ryegrass, one of the dominant forage in southern Chile, are poorly understood. In this study, activity of antioxidative enzymes and root

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exudation of organic acid anions were evaluated in four ryegrass cultivars grown in nutrient solutions with increasing supply of Mn. A growth reduction caused by Mn toxicity was smaller in Jumbo and Kingston than Nui and Aries cultivars. Shoot Mn accumulation varied among the ryegrass genotypes in the order Nui > Aries > Kingston > Jumbo. In general, ascorbate peroxidase (APX) and guaiacol (GPX) peroxidase activities increased proportionally to Mn excess. These enzymes were particularly strongly induced in shoot tissues of Mn-tolerant cultivars Jumbo and Kingston; correspondingly, lipid peroxidation was relatively low in these cultivars. Kingston was the cultivar best tolerating high tissue Mn concentrations; it also had the highest superoxide dismutase (SOD) activity. Thus, increased activity of antioxidative enzymes (such as peroxidases and especially SOD) in Mn-tolerant cultivars could protect their tissues against oxidative stress triggered by excess Mn. Mn toxicity induced root exudation of organic acid anions in all cultivars. In the case of Mn-tolerant cultivars, Kingston showed high exudation of oxalate and Jumbo high exudation of citrate. The findings obtained of root organic acid exudation measurements suggest that citrate was the most efficient organic acid anion in decreasing availability of external Mn. On the other hand, oxalate exudation may be linked to increased intracellular concentrations of this ligand that detoxify Mn by complexation.

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Keywords: Antioxidative enzymes; carboxylates exudation; manganese toxicity.

S2-P38
Effect of Urea Supply on Phosphate Fertilization and Nitrogen Forms in Perennial Ryegrass (Lolium perenne L.) Rhizosphere

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Nitrogen (N) fertilizers of acid reaction can affect phosphorus (P) availability in the rhizosphere and hence, reduce the growth and shoot P concentration in plants. A greenhouse experiment was conducted to evaluate the effect of nitrogen and phosphate fertilizers applications on nutrient distribution and forms in perennial ryegrass rhizosphere. The assay was carried out in pots using soil samples (Chilean Andisol) treated with 3 doses of N (0, 100 y 300 mg N kg⁻¹ of soil, using urea) and P (100, 200, 400 mg P kg⁻¹ of soil, using triple superphosphate). Ryegrass plants were grown for a period of 34 days. To study rhizospheric P depletion, 2 rhizobox were inserted in each pot. Results indicated that ryegrass dry weight (DW) and shoot P concentration significantly increased with increasing doses of P. In addition, shoot P concentration in ryegrass decreased in a doses dependent manner with N application. Shoot P concentrations ranged from 1540 to 2534 mg kg⁻¹. As expected, P availability in the rhizosphere increased in response to P supply. Moreover, the rhizospheric concentrations of N-NO₃⁻ and exchangeable aluminium were incremented at increasing N doses. Under all treatments, N- NH₄⁺ soil levels were low. The pH values diminished and varied from 4.1 to 5.4 in response to urea supply during the assay period. Thus, the highest pH variations were detected in samples treated with the higher urea doses. A decreasing gradient on P availability in the nearest distances to the roots was observed. P depletion conditions were provoked by N applications at the distance range of 0-2 mm from the root surfaces. These results allow concluding that the N fertilizers of acid reaction could reduce P uptake and availability in the ryegrass rhizosphere.

Acknowledgements: This work was supported by the FONDECYT project 1061262.

Keywords: Urea; rhizosphere; ryegrass.
S2-P39
Assessment of Interspecific Lines of *Oryza* spp. Inoculated with *Azotobacter chroococcum* and *Azospirillum amazonense* in Soil *Typic haplustalf* of Ibague, Colombia

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In rice crops, it is necessary to generate alternatives that reduce costs through the use of fewer nitrogen fertilizers, e.g. by using biological fixation of nitrogen (BFN) and breeding programs to obtain genotypes that are efficient in their nitrogen use. The genus *Oryza* has 20 wild species and two cultivated: *O. sativa* L. and *O. glaberrima* Steud. Wild species represent an additional source of genes for improving rice potential characteristics, then, the objective of this research was to evaluate the response of interspecific lines of rice (*Oryza* spp.) to inoculation with *Azotobacter chroococcum* and *Azospirillum amazonense* on the efficiency in the use of N, in a *Typic haplustalf* soil of the plateau of Ibague, Colombia. Each material was evaluated with and without inoculation, with a biofertilizer with a concentration of 10¹⁰ CFU/mL of *A. chroococcum* (IBUN Nº 142111) and *A. amazonense* (IBUN Nº 121111). The seeds were inoculated with 1 cc of biofertilizer 250 g⁻¹ per cultivar and were cultivated immediately after. Each cultivar, with or without inoculation, was studied with three doses of N: 0, 50 and 100%, 100% corresponded to 250 kg N ha⁻¹, the source used was granular urea (46% N). ANOVA was conducted in accordance with a subdivided plot design where the N rates corresponded to the main plot; with and without the inoculum in each subplot; and genotypes (10 in total) to each subplot with three repetitions. Tests were conducted by comparing the average DMRT (Duncan Multiple Range Test) and by a multivariate analysis. Inoculation with *A. chroococcum* and *A. amazonense* produced more vigorous rice plants, and more radical and shoot biomass, especially in the interspecific lines CT13941 and CT16049 when they received 50% of N. The use of N by interspecific rice lines produced a higher agronomic efficiency, when the plants received average doses of nitrogen fertilizer plus inoculation with *A. chroococcum* and *A. amazonense*, with the highest values in the lines CT13941, CT14524 and CT16049-1. The highest N recovery rate was obtained with the wild phenotype *O. rufipogon* Griff., with 50% N plus inoculation. In addition, there were differences between cultivars in this variable, where interspecific line CT16049-1 with 50% of N plus inoculation surpassed the commercial variety Fedearroz 50. The physiological efficiency was high on the interspecific lines CT16049-2 and CT1394-1 surpassing the other cultivars, with 100% N and without inoculation. The yield of interspecific lines CT13941 and CT13943 increased with the inoculation of the NFB and the implementation of 50% N, surpassing all other cultivars. It is considered a good alternative for reducing costs, by reducing the use of nitrogenous fertilizers and generating higher yields.

**Keywords:** biological nitrogen fixation, rice, interspecific lines.
S2-P40
Soluble Organic Matter and Humic Acids in Agricultural Soil Amended with Sewage Sludge and Sewage Sludge Compost

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Biosolids are a valuable source of organic matter, N and P at low cost in agricultural soils. However, one of the main concerns regarding their application to the soil is the presence of potentially toxic elements (PTE). There are still some uncertainties about the final destination of PTE in the soil where sewage sludge is applied. For instance, metals introduced with sewage sludge can be released and become available to organism, but the organic matter can also immobilise them. The behaviour of DOM and HA in the soil and its effect on pollutant chemistry is of a particular interest to researchers, since de DOM and HA play a fundamental role in immobilisation and transport of pollutants. The effect of the sewage sludge or sewage sludge compost to the soil on the quality of the dissolved organic matter (DOM) and humic acids (HA) in the soil, was investigated under field conditions. The study site is located in an agricultural area of the municipality of Toluca, in Mexico’s central region, where 9 land plots of 6 x 8 m each were defined and distributed in a Latin square. Three plots were used as controls (S) utilizing fertilizer (120-90-60 kg ha⁻¹ de N-P-K), another three were amended with 18 Mg ha⁻¹ of biosolid on a dry base (Sw-S) and the remaining three plots were treated with equal doses of sewage sludge compost (SwC-S). From the control and amended soil, were taken monthly samples throughout the agricultural cycle (June-November 2003), in order to carry out the pH analysis, organic matter content (OM), DOM and HA quality through the infrared spectroscopy (IR). The evaluation of the cationic exchange capacity (CEC), interchangeable cations (Ca, Mg, Na and K), organic carbon total, total nitrogen, as well as the total and available heavy metals content (Cd, Cu, Pb and Zn) were determined in an initial, intermediate and final period (June 2003, August 2003 and November 2003). The SwC-S showed the highest OM (4.2%), Nt (0.13%), CEC (16 Cmol kg⁻¹) and Cu (9.20 mg kg⁻¹). The Sw-S showed the highest C/N ratio (18.0), Ca²⁺ (7.2 Cmol kg⁻¹), Mg²⁺ (3.5Cmol kg⁻¹), K⁺ (0.28 Cmol kg⁻¹) and total Zn (31.7 mg kh⁻¹) content. The DOM of Sw-S indicated the presence of carboxyl groups, aliphatic compounds, lignin and protein content, while SwC-S showed an increased of carbohydrates. The behaviour of HA was different with respect to the DOM. The HA of SwC-S application showed an increased of proteins, lignins, carbohydrates and aliphatic compounds respect to S and Sw-S. No significant differences were found on total and available heavy metals content between S, Sw-S and SwC-S.

Keywords: Soluble organic matter; humic acids; sludge compost.
S2-P41
Molybdate and Phosphate Kinetic Sorption in Chilean Andisols

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Sorption of molybdate and phosphate by soil constituents affect the mobility and bioavailability of Mo and P in soil-solution-plant systems (Barrow, 1999). Both anions are strongly and specifically sorbed on the soil (Goldberg and Forster, 1998), mainly on variable charge minerals (Al-oxides, Fe-oxides, allophane, imogolite). Several studies have described the kinetics of phosphate reaction in heterogeneous soil and clay minerals by various kinetic models. Nevertheless, reported information about the kinetic of molybdate sorption on soil constituents is very limited. The aim of this work was to compare the effect of time and soil properties on molybdate and phosphate sorption in Andisols with different properties. The sorption behavior was evaluated as a function of time (0-72 h) at ionic strength 0.1 mol L\(^{-1}\) KCl (25ºC) in four Chilean Andisols. Data from sorption experiments were fitted by the Elovich equation modified (Chien and Clayton, 1980). Results showed a fast sorption in the first 0.5 h, being 55% for molybdate and 62% for phosphate. For both anions the reaction continued slowly until 72 h reaching 90% for molybdate and 97% for phosphate. The release of OH\(^-\) ions at the solution was increased with the time, raising the pH in 0.85 units for molybdate sorption reactions and 0.65 units for phosphate reactions. The Elovich parameter \(\alpha\) (sorption rate) value for both anions was relative to the organic matter content (O.M.) of soils, and also can be associated to humus–Fe and humus–Al complexes. The \(\alpha\) values for molybdate were 2.24 x 10\(^{15}\) mmol kg\(^{-1}\) h\(^{-1}\) for Vilcún soil (15 % O.M), 2.49 x 10\(^{12}\) mmol kg\(^{-1}\) h\(^{-1}\) for Pemehue soil (16 % O.M), 8.76 x 10\(^{10}\) mmol kg\(^{-1}\) h\(^{-1}\) for Osorno soil (20 % O.M.) and 3.11 x 10\(^7\) mmol kg\(^{-1}\) h\(^{-1}\) for Piedras Negras soil (24 % O.M.), whereas for phosphate the \(\alpha\) values were 3.89 x 10\(^7\) mmol kg\(^{-1}\) h\(^{-1}\) for Vilcún soil, 5.21 x 10\(^{10}\) mmol kg\(^{-1}\) h\(^{-1}\) for Pemehue soil, 3.11 x 10\(^{12}\) mmol kg\(^{-1}\) h\(^{-1}\) for Osorno soil and 1.08 x 10\(^{16}\) mmol kg\(^{-1}\) h\(^{-1}\) for Piedras Negras soil. Desorption constant values (\(\beta\) ) ranging from 0.47 to 0.28 for molybdate and 0.22 to 0.94 for phosphate from Vilcún to Piedras Negras soils. The \(K_{eq}\) values determined by the relationship between \(\alpha\) and \(\beta\) constants for Vilcún and Pemehue soils were higher for molybdate than phosphate, in contrast, \(K_{eq}\) values for Piedras Negras and Osorno soils were higher phosphate than molybdate. Results showed that mineralogical composition and the O.M. content of the soils regulate the kinetic behavior of both anions. Thus, the sorption of molybdate was associated to higher affinity of the Fe-oxide and Al-oxide and the phosphate sorption was regulated by the humus-Fe and humus-Al complexes.

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Keywords: Molybdate; phosphate; kinetic sorption.

References
Plant growth promoting rhizobacterias (PGPR) are those exert beneficial effects on plant growth and development. Field and pot experiments were carried out, using volcanic soil, to evaluate wheat growth promotion produced by *Raoultella terrigena* and *Pseudomonas fluorescens* wheat. Both experiments had four treatments: (i) optimal phosphorus (P) fertilization; (ii) no P fertilization; (iii) no P fertilization plus *Raoultella terrigena* inoculation and (iv) no P fertilization plus *Pseudomonas fluorescens* inoculation. Treatments were arranged in a complete randomized blocks design with three replicates. Wheat seeds (cv. Pandora) in the field experiment were inoculated with $1.5 \times 10^5$ cfu seed$^{-1}$. In the field experiment, at grain harvest evaluation inoculated treatment with both PGPR increased significantly ($P<0.05$) total aboveground biomass (34%), grain yield (33%), grain number (36%) and P uptake (25%) over the control without P fertilization. The pot experiment was planned growing ten inoculated plants ($3.75 \times 10^5$ cfu plant$^{-1}$) in boxes of 375 cm$^2$ and 100 cm depth. Each box was put in 60° respect to ground and had a glass wall, in order to draw roots length at different times during growing period. At wheat heading stage evaluation *Raoultella terrigena* increased significantly ($p<0.05$) aboveground biomass (248%) and root length (30%) over control without P fertilization. These results suggest a potential use of *Raoultella terrigena* and *Pseudomonas fluorescens* as wheat growth promoting rhizobacteria in volcanic soils.

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**Keywords:** *Raoultella terrigena*; *Pseudomonas fluorescens*; wheat.