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BOOK OF ABSTRACTS

Block C

Session C2

Soil biodiversity and ecosystem services

Table of Contents

C2 Soil biodiversity and ecosystem sesrvices

Session Description

Involved projects: AGROEcoSeqC, EnergyLink

Conveners: Alessandra Trinchera (CREA), Sebastien Fontaine (INRAE)

The concept of soil health is unavoidably connected to its multifunctionality, strongly dependent on soil biodiversity. The recently changed and still evolving environmental conditions call for management practices able to increase biodiversity and the functional redundancy of soil biological communities to ensure adequate ecosystem resilience, contemporary optimizing the synchronization of nutrients plant demand and availability in soils. This breakout session will focus on the importance of soil biodiversity and related ecosystem services: contributions describing labelling methods, molecular markers, assessment of plant diversity, geno- and phenotypic profiling of soil microbial community, enzymatic activities, soil soluble C pools, plant-microbial symbiosis, greenhouse gas emission, and indicators of ecosystem services observed in long-term experiments from crop- and grasslands, as well as application of modelling and multivariate approaches, are welcome.

Abstracts of Oral Presentations

Sculpting the soil microbiota: role of soil management and plant-diversity based farming practices

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Soil microbial community is one of the main regulators of fundamental ecosystem processes. Its composition, diversity and functional may support the long-term soil productivity and the resilience to climatic and environmental stress. However, the application of impacting agronomic practices, such as deep soil disturbance, monocropping, or use of herbicides, provoke deep changes in microbial community affecting its composition and functional diversity, generating a cascade of negative effects on C sequestration and soil-plant nutrient balance.

The adoption of agroecological practices, based on reduction of soil disturbance, increase of plant diversity by introducing service crops, or promotion of plant-microbial symbioses in field, is an effective tool able to ensure an efficient energy and nutrients complementation and exploitation by plants and soil microbiota, thus increasing ecosystems production and resilience.

For example, tillage and crop rotation change the distribution pattern of soil fungi. In ploughed soils, the fungal species are evenly distributed, while a higher spatial variability is found in no tilled ones, being fungal taxa distributed according to a small-scale pattern, corresponding to undisturbed and heterogeneously distributed micro-niches. The increase of mycorrhizal fungi abundance in no-tilled soils is due to fungi species selection, due to higher soil compaction and lower pore aeration, which increases the physical resistance to hyphal development and penetration.

With reference to plant diversity in field, the intercropping of legume with organic tomato increases soil-P availability when compared to monocropping, due to potentiation of rhizobia–mycorrhiza mycorrhiza association, especially in presence of organic fertilization.

In different EU vegetable systems, it was observed that multi-cropping increases soil microbial biomass amount and shape microbial community toward a predominance of some bacteria or fungi phyla, in the function of soil nutrient availability. The increased *Bacteroidetes* and decreased *Mortierellomycota* relative abundance in rhizosphere soil of intercropped crops are sensitive ecological indicators of an improved agro-system functionality: the *Bacterioidetes*, as evidence of low impact agricultural practice introduction, and the *Mortierellomycota* as undirect indicator of the reduced pressure made by pathogens in intercropping systems.

Finally, an overall highest plant diversity in field also improves mycorrhizal colonization of coexisting species, by developing a root-connecting external hyphal mycelial network with a positive effect on cropping system productivity in long term.

Keywords: Soil microbial diversity, mycorrhiza, no tillage, crop rotation, intercropping

Designing sustainable agrosystems by copying the biogeochemical organization of natural ecosystems

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Redesigning agrosystems by incorporating more ecological regulations has been suggested as a solution to feed a growing world population while preserving soil assets for future productivity and reducing environmental impacts such as eutrophication and greenhouse gas emissions. However, guidelines for redesigning cropping systems from natural systems remain limited. Reviewing the last knowledge of ecosystem functioning, we outlined four ecological systems synchronizing the supply of soluble nutrients by soil biota to fluctuating plant nutrient demand. This synchrony limits deficiencies and excesses of soluble nutrient, which usually penalize both production and regulating services of agrosystems such as nutrient retention and soil carbon storage. We explain why fertility should no longer be viewed as an intrinsic property of soils but as an emerging property of soil-plant interactions. We also describe how ecological systems promoting synchrony can be installed in agrosystems to improve their sustainability and reduce the use of mineral fertilizers.

Keywords: carbon and nutrient cycling, plant-soil interactions, agroecology, biodiversity and ecosystem functioning, synchrony, nutrient ecosystem economy

Will cover crops alter microbial carbon use efficiency? - First results from the Wageningen Clever Cover Cropping site

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The aim of EnergyLink is to unravel how crop diversity affects the microbial processing of organic carbon (C) in agricultural soils. Despite microbial respiration causing CO2 losses from soil organic matter decomposition, the microbial transformation of plant-derived C inputs is assumed to foster C stabilisation in soils: microbial-derived compounds likely associate to mineral surfaces which prevents further decomposition. The ratio of metabolised C being shared between microbial growth and respiration, i.e. microbial C use efficiency (CUE) is thought to be a key control determining the fate of organic C in soil. The microbial CUE depends amongst others on the substrate stoichiometry and chemical composition, and therefore on the C inputs entering the soil. We hypothesised that crop diversification leads to the diversification of plant-derived C inputs, which ultimately affects CUE, C processing and potentially C stabilisation.

At the project's core site, the long-term Clever Cover Cropping experiment in Wageningen, cover crops and mixtures of different cover crops are introduced into the crop rotation, representing different levels of crop diversification. The site includes eight treatments in five replicates, resulting in a total of 40 plots. Treatments are bare fallow, cover crops planted as single species (i.e. oats, radish, vetch) and full-factorial mixtures of two and three cover crops. Topsoil samples were taken in August 2022 (close to maize harvest) and December 2022 (cover crops established) to examine if potential effects of crop diversification persist during seasons without direct diversified plant C inputs. Microbial CUE was determined by the 18O-labelling method. First results on microbial CUE, growth, respiration and microbial biomass C will be presented.

Keywords: crop diversification, cover crops, microbial carbon pump, carbon stabilisation, isotopic labelling

Modelling microbial and plant diversity in multi-species agroecosystems: the DIMIVEA project

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Biogeochemical modelling is used to assess the impact of agricultural activities and climate on ecosystem carbon (C) and nutrient cycles and associated services or disservices, such as biomass production, C emissions/storage and nutrient retention. From an agroecosystem perspective, DIMIVEA is interested in the characterization and dynamic simulation of: i) the physico-chemical and biochemical properties of soils, ii) the functional diversity of plant and soil microbial communities, and iii) their role in the provision of ecosystem services. The simulation of multi-species systems (grasslands, forests, multi-species cropping systems) ensures a detailed representation of the coupled C-N cycles, but the models used remain simplified insofar as biological diversity is reduced to simplistic patterns of interactions with the environment. The creation of simulators based on the information provided by diversity attempts to overcome the paradigm of condensing biological diversity into constant parameters. This opens up new avenues of research to be explored to explain the synchronisation of nutrient demand and supply in multi-species systems by modelling some plant and microbial diversity. By focusing on the characteristics of plant and microbial communities in mixed vegetation canopies, the consortium aims to provide a conceptual framework for extending the potential of models towards a reliable estimation of the ecological processes that support the ecosystem services provided by these vegetal communities. Moving towards the creation of explicit, dynamic and integrated simulators of microbial and plant diversity, DIMIVEA represents a new paradigm that implies that related aspects of biological diversity cannot be ignored in agroecosystem modelling studies.

DIMIVEA integrates different experiences and knowledge in an attempt to model the ecological organisations that enable natural ecosystems and certain agro-systems to be productive, multifunctional (ensuring C storage, purification of drainage water, improvement of soil quality) and

low-input. The aim is also to identify the ecological organisations to be favoured according to local soil and climate contexts, and to propose agricultural practices likely to favour them in agro-systems.

For its reflections and conceptualisations, the consortium relies on the aggregate microbial modelling (i.e. stocking/de-stocking microbes) and the experimental devices of the AGROECOseqC project of the European Joint Programme Cofund on Agricultural Soil Management (EJP SOIL). The partnership is committed to organising and leading dedicated workshops within the framework of scientific events such as international conferences and study days, and to progressing towards the writing of a synthesis and positioning article, envisaged as the horizon of a collective reflection in addition to the production of model prototypes.

Keywords: dynamic modelling; plant diversity; microbial diversity; multispecies land covers

Influence of saltwater irrigation on crops and soil microorganisms under a salinity gradient

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Saltwater contamination is a major contributor to agricultural soil degradation. With rising sea levels and increasing dry periods due to climate change, ground water in temperate coastal areas is becoming increasingly brackish, forcing growers to use part-saline ground water to irrigate their crops in summer. In arid areas, saltwater contamination has been reported to decrease crop yields, but the short- and long-term effects of saltwater contamination in temperate areas have not yet been thoroughly investigated, especially its effects on the soil microbial communities. Our hypotheses are that soil microbial communities will be impacted by saltwater irrigation, and that microbial communities exposed to saltwater irrigation over a long period of time will become increasingly more tolerant to salt. To test these hypotheses, we designed a 3-year experiment using a salinity gradient to irrigate crops. During these 3 years, we will monitor the crop health and yield, as well as study the soil microbial community of each crop. This experiment started last summer, and I will be presenting the crop yield and microbial communities results we have obtained from the first year of this experiment.

Keywords: soil, salinity, microorganism, bacteria, crop, salinity gradient, microbe, irrigation, saltwater

A guideline for appropriate estimates of carbon use efficiency with the [18]O method

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The carbon utilization efficiency (CUE) of soil microorganisms is the fraction of absorbed carbon that is allocated to microbial growth, with the remainder being released as CO_2. The CUE provides information on the carbon metabolism of soil microorganisms, but also on the carbon storage function of soils, since the microbial biomass contribute to soil organic matter building. One of the aims of the AgroecoseqC project is to estimate CUE in soils under a gradient of agroecological intensification to determine if they act as source or sink of carbon during the low and high plant nutrient demand periods. Nowadays there are multiple methods to estimate CUE. In the AgroecoseqC project, we will use the [18]O method, which measures microbial gross growth in short periods of time. Unlike the methods based on C substrate addition, with this method we avoid bias in CUE estimation due to the stimulation many new microbial populations that were previously dormant. The main assumption of this method is that all the oxygen in DNA of soil microorganisms comes from water. Hence, the method consists on the addition of [18]O labelled and to trace the incorporation of 18O in DNA in order to estimate microbial growth. Meanwhile, microbial respiration is measured by gas chromatography. Then these measures are used to feed a series of equations for estimating CUE. At the time of testing these methods for its application in AgroecoseqC, we have identified some points that are not clear in the published bibliography, being potential sources of uncertainty in the final CUE estimates. These are related with the quantity of oxygen and the impurity levels in the DNA extractions, the needed of spiking those samples with salmon DNA (to reach the minimum oxygen levels measurable by the mass spectrophotometer), and the use of different equations for estimating CUE from the measurements obtained. Here we propose a synthesis of the method, and a guideline to help future users to take the right decisions depending on the situation.

Keywords: carbon cycle; stable isotopes; soil microbials; plant/soil interactions, nutrient cycling

Early detection of microbial carbon stabilization by biomarker-SIP

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Soil organic matter (SOM) consists of two overall pools: particulate organic matter (POM) - primarily of plant origin – and mineral associated organic matter (MAOM) – primarily of microbial origin. These two pools each constitute about half the C in SOM in cultivated soil, but differ in their residence time with MAOM staying considerably longer than POM. We therefore have an interest in understanding how plant species, management and pedoclimatic conditions drive the microbial formation of this SOM pool. Studies of SOM pool changes require long-term treatments as the background SOM level often is high. In order to estimate the effect plants and management on C stabilization over shorter time spans the use of isotopes is a strong tool – especially when combined with microbial biomarkers. We here will report findings of a novel biomarker stable isotope probing (SIP) based methodology, which allow short-term evaluation of potential microbial C stabilization. The method is based on compound specific isotope analysis of PLFAs – indicating living microbial biomass – and of amino sugars – indicating living and dead microbial biomass. We applied this method in a field study with a perennial wheatgrass and lucerne and found that the legume induced a greater potential for microbial C stabilization within the upper meter of the soil profile. Using amino acid stable isotope fingerprinting and functional gene expression we identified a higher exudation of organic N compounds under lucerne to be a likely explanation for the higher microbial C use efficiency.

Keywords: Plant traits, Microbial C stabilization, Stable isotope probing, Biomarkers.

Validation of microbial community-level physiological profiles (CLPP) analysis in LAMMC and MBG Santiago-CSIC

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A rapid community-level approach to assess sole carbon source utilization in mixed microbial samples may be used to study metabolic potential of microbial community. The method involves direct inoculation of environmental samples into a Biolog Ecoplates and uses color formation by reduction of a tetrazole dye to assess the utilization of 31 carbon sources over a few days of incubation period. Current studies were carried out to validate the microbial community-level physiological profiles (CLPP) analysis in LAMMC and MBG Santiago-CSIC laboratories and subsequently evaluate the influence of the sustainable agricultural practices on the metabolic potential of the soil microbial communities at the moment of maximum nutrient plant uptake by cover plants (i.e., when the soil functions as a C source). Soil samples (total 12) were collected from LAMMC long-term soil tillage experiment. Selected treatments included: ploughing without cover crops, no-tillage without cover crops, and no-tillage with cover crops. A harmonized and unified assay protocol was used in both laboratories. The absorbance data every 24 hours (until 144 hours) obtained in both labs were compared. The values at 72 h or 96 h were considered as the most representative, because degradation of some substrates (e.g., cellobiose, lactose, phenylalanine, glycyl glutamic) could be detected only after incubation of 3 or 4 days. In general, there was an agreement between the results of the two laboratories, although for some substrates the differences were more pronounced. The degradation of these substrates might be affected by the storage during the trip to MBG Santiago-CSIC. The analysis of the data suggests that the substrates more affected are those with lower degradation or with degradation patterns with higher variability. Among the tested treatments, highest metabolic activity was detected in no-tillage treatment with cover crops following by no-tillage without cover crops. The study suggests that the usage of sustainable agricultural practices, such us no-tillage and cover cropping, may positively influence the metabolic potential of soil microbial community.

Variation in soil bacterial community structure under different tillage intensity

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Soil bacterial community structure is the key player identifying the strengthening of ecosystem stability. The biodiversity of soil bacteria and other functional groups can affect a row of soil properties, as well as chemical processes, physical parameters, and indirectly, through previously mentioned aspects - crop yield. Because of methodological difficulties of microbial researched there is still lack of information about variation and functional responses of bacterial communities to different tillage. The aim of the present study was to identify bacterial community composition and diversity under different soil tillage. *Dystric Glossic Retisol* was analysed, geographical coordinates 55⁰43'38"N, 21⁰27'43"E. We assessed soil bacterial communities in two different management regimes - deep ploughed and more environmentally friendly - shallow ploughless tillage. Composition and diversity of soil bacterial communities were assessed by sequencing of 16S rRNA genes. All sequences were classified below phylum level. Results show that, two types of bacteria are dominant: *Actinobacteria* and *Proteobacteria.* Their relative abundance ranges about 33% and 28% respectively. The most widespread family was *Micrococcaceae*, and *Hyphomicrobiaceae,* which accounts for 6% and 3% respectively. The number of taxonomic units in different agroecosystems shows the abundance of organisms but does not allow for the estimation of biodiversity; therefore, five different biodiversity indices were calculated. Shannon and Simpson biodiversity indices found that 10 most abundant species were the same in tested soils, just relative abundance various. The ACE, Chao1, and JackKnife biodiversity indices varied in the analysed soils – the highest indices calculated for shallow ploughless soil. This leads us to the assumption that mentioned type of soil management contribute to the protection of soil functions.

Keywords: stakeholders, criterion, framework and model, engagement, values and practices.

REHABILITATION OF SOILS CONTAINING HIGH SALT LEVELS WITH BENEFICIAL FUNGI

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Soil salinity not only reduces the quality of the soil, but also causes ionic imbalance in the plant, competition in the nutrient uptake and toxic effects at high concentration. Combating salinity, which has become a greater stress factor due to impact of drought and high temperatures, is of great importance. In this study, 3 kg soil in pots, adjusted to 12 mS/m EC, under four different treatments (3 different fungi species and control) were compared in a laboratory environment. The experiment was designed with three replications for each subject and one plant in each pot. Fungi species (*Clononotachys rosea*, *Trichoderma* sp., *Taloromyces funiculosus*) were used as soil inoculant to reduce soil salinity. The soil electrical conductivity (EC) was between 9.25 and 9.95 mS/m within 1 month in tomato cultivated soils, while the EC of the plant-free medium was between 10.4 and 11.4 mS/m: thus, the salt content did not differ statistically. EC values of the saline soils was decreased by 21-, 17-, 29 %, respectively, when *Trichoderma* sp., *Taloromyces funiculosus*, *Clononotachys rosea* were applied to tomato plant growing soils. These fungi also decreased the soil EC values by 15-, 16-, 25 %, respectively, in the plant-free environment. The chlorophyll SPAD value of tomato plants grown in saline soils decreased from 22.65 to 0, however, the SPAD values of tomato plants grown in soils with Clononotachys rosea, Trichoderma sp., Taloromyces funiculosus were increased from 22.4-, 25.6-, 24.1 to 29.3-, 50.2-, and 24.5. Similar findings were also observed for the increase in the chlorophyll content of the tomato plant. The fungi mentioned above also contributed to the increase of soil macro (Ca, Mg, K, P) and micro (Fe, Cu, Zn, Mn) element contents, and to the improvement of soil available phosphorus amount (P_2O_5) with the increase in soil phosphorus content. Fungi inocula induced a statistically significant increase of soil micronutrient concentrations ($p > 0.05$). Sodium (Na) concentration in saline soils did not change in inoculated-non-inoculated soils. These findings were interpreted as fungi inocula were not able to assimilate the salt from the soil, but they instead contributed to the release of macro and micro elements that were unavailable under saline conditions, possibly due to their metabolic activities. According to the results of soil analysis at the end of the experiment, it was revealed that the pH value was 7.72 in the control pots and 3.28 in the pots treated with Taloromyces funiculosus, and the EC value was 12.34 and 9.74 mS/m, respectively. Among the

fungi studied, *Taloromyces funiculosus* was found to be more effective than other two fungi on decreasing soil salinity and increasing micronutrient availability to plants.

On-farm regeneration of microbiology for healthy agricultural soil

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Agriculture is bearing the full brunt of pressures, related to rising demands of food and fiber higher energy costs and climate change. Promoting soil organic carbon (SOC) sequestration has gained immediate concern for maintaining and restoring soil health in order to ensure continuous soil fertility and functioning. Despite extensive research effort for farming practices that promote soil health applicable measures from farms are still scarce.

In the present study we followed an on-farm approach comprising 21 sites in North-Eastern Austria to compare two farming systems (an innovative 'pioneer' and a standard system) and semi-natural field margins as a reference. Soils from pioneer farms have been managed according to soil health-oriented principles combining multiple measures such as: conservational tillage, diverse crop rotations, intensive cover cropping and organic amendments to improve soil biology. While the `standard" system represents the current state-of-the-art conventional practice on neighbouring fields with the same soil type. The study focused on available nutrients, microbial biomass C, nitrogen (N) and phosphorus (P), ergosterol, potential activities of C-, N- and P-acquiring enzymes as proxies for microbial functioning, and amino sugar concentrations as proxies for microbial necromass. Beside management effects, we also investigated whether differences in soil texture and soil depth (0-5, 5-20, 20-35 cm) affect microbial biomarkers.

Our results indicate that microbial parameters, especially microbial biomass and necromass C, are significantly enhanced in soils of pioneer farming systems. Yet, pioneer cultivation did not reach the levels prevailing in the semi-natural reference system. Likewise, differences between systems were most pronounced in the topsoil and declined with soil depth. Soil texture had a serious leverage on management effects. Significant management predictors were observed for extractable organic C contents, which is an important pathway for microbial-mediated SOC sequestration. Our on-farm approach provides significant knowledge on how farming systems can be changed towards more sustainability and higher C sequestration.

Keywords: conservation agriculture, microbial indicators, SOC sequestration, microbial carbon pump

Abstracts of Poster Presentations

Influence of agricultural management on soil biodiversity of Mediterranean soils. SOILBIO

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Soil biodiversity, including edaphic fauna and microorganisms, are key to the functioning and health of agroecosystems. These organisms interact with each other and with plants and biota of the ecosystem, forming a complex system of biological activity. Soil organisms provide a series of essential services for the sustainability of all ecosystems. These services are a fundamental resource for a sustainable management of agricultural systems. These organisms are primary agents for driving the nutrient cycle, regulating the dynamics of matter soil organic matter, carbon sequestration in the soil and greenhouse gas emissions, modifying the physical structure of the soil and water storage, increasing the amount and availability of nutrients for vegetation and increasing plant health. Soil biodiversity and agricultural production is endangered by challenges as important as climate change and intensive agricultural management, which is leading to soil degradation. Approximately 88% of agricultural soils in Spain have a high risk of degradation.

However, considering that the soil is one of the main C reservoirs, it is necessary to promote management strategies that allow enhancing carbon sequestration in agricultural soils and maintain soil biodiversity (EU Biodiversity Strategy 2030; Farm to Fork Strategy-Green Deal).

The lack of standardized experimental designs in Spain to evaluate the loss of biodiversity and organic carbon within the context of existing climate change, as well as the influence of existing agricultural practices, has led to carrying out the project that is presented.

C2 Soil biodiversity and ecosystem sesrvices

The Goal of the project that will be develop during 2022-2025 is to evaluate the effect of agricultural practices (conventional, ecological and conservation) on extensive herbaceous crops (wheat, Triticum), through different indicators of biodiversity (microorganisms, meso- and macro-fauna), together with chemical parameters and microbial activity and functionality, allow to evaluate the areas of greatest biodiversity and soil health and C stocks within a context of global change.

Keywords: Biodiversity, Ecosystem, Global change, Indicators, agricultural soils

Soil proteins as biochemical indicators of the plant-soil-microorganism system as a whole

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Soil macromolecules have been considered with a view to developing indicators of the functionality of the plant–soil-microorganism system, considered as a whole (Mesplou Sylvain, 2022).

Soil proteins, including glomalin thought to be produced (or partially) by mycorrhizal fungi, are attractive candidates to consider as products of the overall soil biological activity, involving both below- and above-ground organisms, as well as inputs of organic matter from dead material. In addition to reflecting biological quality, soil macromolecules have a role in aggregation and carbon storage properties.

Although extensively studied for years, the diversity of extraction protocols and methods for designating the glomalin protein family, the complexity of specific assays and the use of non-specific methods such as Bradford for quantification, have led to some ambiguities and confusions. This study proposes a biochemical indicator of soil quality and fertility based on the non-specific dosage of the total compartment of extractable proteins. After improving the extraction and assay protocols, this model was compared to the one based on glomalin according to the most commonly used published protocol.

Total proteins, in addition to being able to discriminate culture modalities and systems, showed excellent correlations with the amount of microbial DNA, carbon, nitrogen, phosphorus and structural stability. In this comparative study, it was found that total proteins better discriminated cultural practices than glomalins alone. This indicator could potentially be useful in routine analyses.

Keywords: *Proteins, Glomalin, Soil, Indicator*

Linking soil microbial carbon sequestration to cover crop diversification in agricultural soil systems across Europe

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Sequestering atmospheric CO_2 into soil organic matter through changes in agricultural practices is an appealing idea to improve soil ecosystem services and to mitigate global change. The old view of carbon (C) stability in soil, based on the intrinsic properties of the organic matter inputs (e.g. lignin content), would lead policy towards greater percentages of recalcitrant organic matter content in crops. Recent research suggests otherwise and that managing how the soil microbiome process C inputs is a more fruitful approach (Sokol et al., 2019, Poeplau et al., 2019). It is therefore to decipher and evaluate the link between the aboveground plant community and the complex belowground diversity of the microbiome and their metabolic processes that mediate C sequestration. Lehmann et al. (2020) proposed a theoretical framework in which the persistence of C in soil can be understood as the outcome of interactions between the molecular variability of organic matter input and spatio-temporal microbial heterogeneities of the soil system.

Within the EnergyLink framework we therefore investigate various microbial markers to illuminate possible physiological changes across several European agricultural field sites with different cover crop management types. Specifically, for detecting shifts in microbial necromass composition and quantity we target amino-sugars (galactosamin, gluctosamine, mannosamine and muramic acid), for evaluating effects on growth rates we measure ^{14}C incorporation into ergosterol for fungi and ^{14}C-leucine incorporation for bacteria and to grasp changes in uptake strategies we test extra cellular enzyme activities for different nutrient classes. Additionally, we determine C:N:P ratio for bulk soil, microbial biomass and above ground plant biomass to estimate stoichiometric imbalances. Here we present preliminary results from our first sampling campaign and discuss implications of diversified cover crops on soil carbon properties on a European scale.

Keywords: cover cropping, soil microbiome, necromass, exoenzymes, microbial growth

On-farm evaluation of microbial physiology and carbon-use efficiency on innovative pioneer farming systems

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Increasing pressure on arable land related to climate change as well as recent policy frameworks have generated widespread interest on the effect of sustainable management practices on soil organic carbon (SOC) sequestration into arable soils. As soil microorganisms and their functioning are the key drivers of long-term SOC accrual, we require a better understanding of the underlying microbial physiological mechanisms governing SOC sequestration into arable soils. In this regard, the efficiency of soil microorganisms to utilize carbon (CUE) has recently been suggested as a key leverage factor for the sequestration of SOC; however, on-farm studies investigating the effect of conservation management practices are scarce.

Here, we present results from a 21-site on-farm study in North-Eastern Austria, where we evaluate soil bacterial and fungal growth rates, respiration and CUE at innovative 'pioneer' farming systems, adjacent conventional farming systems and undisturbed reference soils (i.e., field margins) across a wide range of arable soil types at three soil depths (0-5, 5-20 and 20-35 cm). These pioneer farming systems have the operational target of increasing SOC and biological activity by differently combining measures such as high rotation diversity, multi-species cover crop mixtures, minimum tillage and organic fertilization.

We show that bacterial growth rates were lowest in the conventional farming systems and highest in the reference systems; fungal growth rates on the contrary showed the opposite trend. Moreover, we observed significant effects of site and soil depth on the physiology of soil microorganisms: growth rates tended to be higher in light-textured soils and decrease with soil depth. Against our expectations, CUE was highest in the conventional farming systems and lowest in the reference soil systems. Moreover, CUE increased with soil depth. These results suggest a complex interplay between site and microbial physiology characteristics and a potential link between the relative C limitation of soil microorganisms and their CUE: with higher labile C inputs (through e.g. cover cropping or organic

fertilization in the pioneer farming systems or through the permanent plant cover in the undisturbed reference systems), C limitation might be released relative to other nutrients, thus reducing CUE. This implies that other nutrients such as phosphorus or nitrogen might become more important drivers of microbial metabolism and physiology.

Our results challenge the current perception of the role of microbial CUE for the sequestration of SOC through conservation agriculture practices, and we suggest that other parameters such as bacterial and fungal biomass turnover might be more promising predictors of SOC accrual. Clearly, more studies are required to decipher the key aspects of microbial-driven SOC sequestration in agroecosystems.

Benchmarking soil biodiversity through eDNA metabarcoding

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Critical knowledge gaps about soil health in relation to specific land-use practices hinder the design and implementation of policies that could protect soil functions and ecosystem services. A current specific area of weakness is characterising, quantifying, and monitoring soil biodiversity. In light of new policy priorities (e.g. EU Biodiversity Strategy for 2030) on biodiversity conservation and bio-resource management, there is a need for reliable indicators to facilitate the impact assessment of policy decisions. Due to the enormous diversity of life in soil, the development of indicators with high relevance for impact assessment studies, policy design and biodiversity monitoring, necessitates a broad coverage of the soil biome, from microorganisms to meso- and macrofauna. To acquire such coverage within a reasonable amount of time, the emerging technique of eDNA metabarcoding might be a viable option to recover as much as possible of the biodiversity present. Genetics can be used to develop indicators for each land use type (e.g. grassland, cropland), specific soil functions (e.g. organic matter and nutrient turnovers, water infiltration) or soil threats (e.g. soil contamination, salinisation, compaction). Additionally, the presence of organisms of interest from a functional point of view (e.g. plant symbionts and pathogens, decomposers, bioremediators) could be evaluated. Sufficient coverage is however dependent on the specific biodiversity recovered, which is in turn highly dependent on the sampling protocols and protocols for DNA analysis used. Therefore, the aim of this research is to evaluate the sampling and molecular protocols used for the 2018 and 2022 LUCAS Soil biodiversity component. As part of EJP-SOIL WP6, we performed double sampling of 11 biopoints in Belgium from the 2022 LUCAS Soil survey to analyse the taxonomical and functional diversity recovered when comparing LUCAS and newly developed national protocols by means of eDNA metabarcoding. For the 2018 LUCAS Soil biodiversity campaign, one DNA-extraction method and four primer sets were chosen to cover the broadest possible spectrum of soil organisms. We evaluate whether the extraction of DNA from a larger amount of soil, mixing more subsamples, analysing an additional deeper soil layer, and the amplification of DNA with an additional three primer sets is beneficial to increase the biodiversity detected. This might especially be important for soil organisms other than prokaryotes and microbial eukaryotes, for which the methodology is less well established. The inclusion of sampling protocols, DNA-extraction methods and primer sets that target functional groups (e.g. Arthropoda and Annelida) which might have been partially overlooked by the

methodology used for the 2018 LUCAS Soil biodiversity component, could contribute towards capturing a better picture of the status of the European soil biomes.

Keywords: Soil biodiversity; eDNA metabarcoding, LUCAS, monitoring, indicators

Wheat roots can modulate soil microbiome to increase sustainability and efficiency of nitrogen fertilizer inputs.

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Wheat is the most widely grown crop in agricultural system and is the staple crop for 35% of the world population, providing 20% of daily protein and food calories. To meet the sustainable development goal of 'food security' it is necessary to maintain healthy soils and to restore degraded agricultural land. Effective strategies require identifying, and targeting, the parameters that define a healthy soil but also the effectors. The WISH-ROOTS project (Wheat Improving Soil Health through Root traits) addresses key marker of soil health: the capacity to provide essential nutrients to plants and soil organisms while maintaining an optimum balance, focusing on nitrogen (N) cycling and impact of root architecture in soil structure.

Application of N fertilisers to agricultural soils supports half of the world's food production. However, approximately 50% of this N is lost worldwide to the environment by leaching/gaseous emissions due to rapid transformation by soil bacterial nitrifying communities shortly after fertilizer application. Biological nitrification inhibitors (BNIs) exuded from the roots of certain varieties of plants can reduce loss of N-fertilizer by delaying N transformation. Particularly, some historic landraces of bread wheat (*Triticum aestivum L.)* have shown evidence of BNI activity in their root exudates. Introducing this agronomic trait into modern cultivars could improve the efficiency of use of N-fertilizer by crops while reducing N loses to the environment.

Performing 16S sequencing analysis for rhizosphere soil from modern wheat varieties and historic landraces with BNI capacity has revealed a significant segregation in rhizosphere microbiome composition between elite and historic cultivars, with significant differences in the abundances of guilds involved in the transformation of N in soil (*Nitrospiraceae, Nitrosomonadaceae* and *Nitrosococcaceae*). The prediction of the functions of these communities using PICRUSt2 and shotgun sequencing has confirmed differences for several associated ecological functions related to N transformation between moder and historic cultivars, including aerobic ammonia oxidation and nitrification.

Wheat cultivars with contrasting BNI capacity were grown in the field under three regimes of N-fertilizer application (none, one and two applications of 48 kg N/ha). Six plots were established for each cultivar and N dose. Roots and rhizosphere soil were sampled at tillering, flowering and immediately after harvest. Root architecture and rhizosphere nitrifying communities were examined.

Root image analysis revealed a significant increase in number of root tips, root length and depth with increasing N-fertilizer for both cultivars. Rhizosphere soil DNA extraction and amplification of the 16S rRNA hypervariable V3-V4 region (Illumina) revealed significant differences in rhizosphere microbiome composition for both cultivars and for the N dose, particularly at flowering. Significant differences in *Nitrososphaerales* abundance were consistent with the contrasting BNI capacity of the wheat cultivars. Increasing N dose resulted in a significantly greater *Nitrososphaerales* abundance in the rhizosphere of the elite wheat cultivar, while *Nitrososphaerales* was significantly reduced in the rhizosphere of the wheat landrace.

Control of N-cycling using the BNI trait identified in historic wheat cultivars provides a sustainable strategy to optimize N-fertilization in wheat crop systems.

Keywords: Rhizosphere microbiome; Nitrogen cycling, Wheat rhizosphere, Wheat root architecture

Influence of long-term application of different tillage system with cover crop and glyphosate management practices on greenhouse gas emissions

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Tillage is an important agricultural practice used to prepare land for crop production by ploughing, cultivating, and levelling the soil. However, these activities can significantly impact soil organic matter content, nutrient availability, microbial activity, and associated greenhouse gas (GHG) emissions. Arable lands are one of the sources of GHG that are influenced by the chemical and physical properties of the soil and are an important contributor to climate change. Accordingly, different tillage practices can impact GHG emissions in different ways. Therefore, this study aimed to evaluate the long-term management of agricultural practices such as different tillage systems along with cover crops and glyphosate on GHG emissions. Field trials were a long-term study consisting of three tillage systems (no-till, conventional and reduced), cover crops (white clover and no cover crops), and glyphosate with the cultivation of soil seed rape. Field trials was consisting of three tillage systems (conventional tillage, reduced tillage, and no-tillage), cover crops (with and without cover crops), and glyphosate (with and without glyphosate) with the cultivation of oil seed rape in 2022 in the crop rotation of 5 sequences. The emissions of greenhouse gasses (carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) were captured directly by a closed static chamber system. By studying these management techniques, the soil physicochemical properties being a critical factor in GHG emissions were also monitored for the period under consideration. The study results showed that N2O emissions were significantly higher in reduced and conventional tillage treatments in combination with glyphosate and cover crops treatment respectively. For CH_4, reduced tillage and cover crop treatments had higher emissions while CO_2 emissions varied across all treatments under study. Interestingly, GHG emissions flattened out in all the treatments after the harvest (end of the cultivation period) signifying that the maximum uptake of nutrients by the main plants during the cultivation period impacted on the GHG fluxes. Additionally, GHG emissions were weakly correlated to water filled pore spaces (WFPS), soil moisture and soil temperature. These initial results suggested that reduced and conventional tillage could negatively impact on plants function in association with their nutrient release/uptake especially as it related with the temperate climatic conditions in Lithuania.

Keywords: soil, greenhouse gas emission, tillage, glyphosate, cover crops