



**EJP SOIL**  
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## BOOK OF ABSTRACTS

Block C

C2 Soil biodiversity and ecosystem services

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## Block C

### C2 Soil biodiversity and ecosystem services

#### Session Description

**Involved projects:** AGROEcoSeqC, EnergyLink, ARTEMIS

**Conveners:** Julia Schroeder, Dylan Warren Raffa , Klaus Jarosch

Healthy soils can provide important ecosystem services and habitat for soil biodiversity. Crop diversification could support the provision of ecosystem services through its effects on soil fauna and microbial communities, and thereby represent a management practice to mitigate climate change in agroecosystems. However, how soil diversity relates to soil multifunctionality is not yet understood.

This breakout session will focus on the mechanisms by which above- and below-ground biodiversity drives key ecosystem functions in agroecosystems, such as biomass production, nutrient cycling, and SOC accumulation.

We invite submissions that explore the influence of plant diversity on soil fauna, microbial communities and soil organic matter quality. We particularly welcome research that investigates the relationship between biodiversity and functional diversity, microbial physiology, and carbon stabilisation, employing techniques such as isotopic labelling, molecular methods, biomarkers, greenhouse gas measurements, and modelling.

Abstracts of Oral Presentations

## Response of spontaneous flora to ecological intensification in a fruit and arable system in Mediterranean conditions: an overview of the communities' potential contribution to soil C input and storage

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Spontaneous plants in agroecosystems (i.e. weeds) are commonly considered to negatively impact provisioning services, reducing crop yield through competition. Instead, spontaneous plants can drive beneficial services such as enhanced biological control, pollination, providing supporting services (soil formation, nutrient cycling and soil stability). In the AGROECOseqC project, spontaneous flora communities are studied in agroecosystems managed in an ecological intensification perspective in comparison to local business as usual. Main focus is to assess their potential contribution to C sequestration.

Results on two project Case Studies are here reported: 1) the MAIOR Long Term Experiment (LTE, CREA) in Central Italy, an organic apricot orchard where: (i) Business As Usual (BAU—soil tillage and organic commercial fertilizer), is compared with (ii) Innovative diversified system with Cover crops, Compost and soil tillage (ICC), (iii) Innovative diversified system with Natural cover, Compost and soil tillage limited to transplanting furrow (INC); 2) La Canaleja LTE (INIA-CSIC), in Alcalá de Henares (Madrid, Spain), where (i) wheat in monoculture under minimum tillage (BAU) is compared with (ii) wheat in monoculture under no-till (No\_till\_M) and (iii) wheat in a 4-year rotation (fallow-wheat-vetch barley) under no-till (No\_till\_R).

Flora vegetational surveys were conducted during autumn 2022 (T1) and spring 2023 (T2), corresponding to minimum and maximum plant nutrient uptake.

We assumed that two main factors may drive C sequestration by the plant community: high C inputs through biomass production and low C outputs (losses) through slow decomposition (high C:

N of plant biomass). Therefore, plant functional characteristics linked to the two drivers were selected and, then, their distribution in the communities was studied through a trait analysis approach. Communities' Shannon H' diversity index was calculated and the Principal Component Analysis (PCA) on species distribution was also performed to characterize flora ecological niches.

The PCA separated the BAU communities respect to those of the other treatments in Italy at T2 and in Spain at both T1 and T2. As expected, the H' was higher in BAU at T1 in both sites, while at T2 it was lower in ICC in Italy, while a trend with lower values in BAU was recorded in Spain. No-till in both sites resulted in communities with greater ability to mycorrhize (higher values of the trait Supporting Arbuscular Mycorrhization), meaning the promotion of the rhizosphere mycorrhizal network, acting as a source or sink for C. Higher communities' Specific Leaf Area and Canopy Height (used as proxy of biomass productivity) were also recorded under no-till in both sites, suggesting potential higher C input

to the soil. In Italy, no-till resulted in higher perennials percentage, especially at T2, meaning higher soil capability to store C in plants' propagules, with ICC showing intermediate values. No clear trend was found in Spain. In INC (Italy), the communities showed also higher percentage of grass-like species (i.e. higher C:N ratio than forbs), meaning lower C outputs through slow decomposition.

In both sites, no-till seems to drive the spontaneous plant communities through a better potential for C sequestration. No effect was observed respect to the systems' diversification/diversity.

**Keywords:** Agroecology; Functional biodiversity; Conservation agriculture; Weed management; Ecosystem services

## Microbial Carbon Use Efficiency of Plant Root Exudates Depends on the Substrate and Relative Nitrogen Availability

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Microorganisms play a crucial role in soil organic matter (SOM) dynamics. While microbial activities contribute to SOM loss, microbial products are essential precursors of stable SOM. Microbial carbon use efficiency (CUE) is defined as the ratio of carbon (C) allocated for microbial growth to the total assimilated C. It describes the balance of SOM loss and stabilization, and even a slight change in the CUE may have significant consequences for C sequestration. Microorganisms are generally more efficient in utilizing labile organic compounds, such as plant primary root exudates. However, despite its implicated importance, our understanding of the microbial utilization of root exudates, and thus their contribution to SOM formation, remains limited. Furthermore, few studies on root exudates have been conducted at scales relevant to the real rhizosphere processes.

In this study, we conducted soil incubation experiments to investigate the CUE of low-molecular-weight compounds commonly found in root exudates. We used a microdialysis system to mimic the hotspots of microbial activity created by primary metabolite exudation into the rhizosphere. We added <sup>14</sup>C-labeled compounds (two carbohydrates, two organic acids, and two amino acids) to the soil individually, and measured the <sup>14</sup>C recovery in respiration, microbial biomass, and soil to assess the CUE. Additionally, we selected three compounds and repeated the experiment with compound mixtures (with one compound <sup>14</sup>C-labeled and the other two unlabeled) to assess how the CUE of each compound was affected by different C and N availabilities.

We observed that the microdialysis system effectively created a small volume of soil with high microbial activity, and the microbial respiration significantly differed between the microdialysis system and the commonly used single-pulse addition method. We also found significant differences in microbial respiration and biomass between compounds, as well as between individual compound additions and compound mixture additions.

Our findings suggest the CUE of root exudates depends on the substrate as well as C and N availabilities in the exudates. This implies the potential of managing plant community composition to enhance the buildup of stable SOM from root exudates.

**Keywords:** microbial carbon use efficiency (CUE), plant primary root exudation, microdialysis

## Functional and taxonomic microbial diversity profiling across soil depth in a diversified cover cropping system in the Netherlands

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Considering the important role microbial processes play in carbon cycling, it is crucial to grasp how agricultural management, in this case cover crop diversification, affect microbial physiology and its relationship with carbon dynamics. Diversifying cover crops might change the microbial community composition by increasing the chemical complexity of organic matter input from cover crop residues. This, in turn, could impact the microbial carbon use efficiency (CUE) and potentially increase carbon storage in the soil.

Furthermore, it is unclear how this effect may expand into deeper soil layers. With soil depth, the amount of soil organic carbon (SOC) and the C:N ratio of organic material decrease. While a narrower C:N ratio may positively influence CUE, nutrient scarcity may negatively affect CUE. Gradient studies within soil profiles have reported different trends in microbial CUE. Other factors, like microbial diversity, may play an important role in regulating microbial CUE.

In the EJP Soil-EngeryLink project, our main hypothesis is that greater crop diversity results in more efficient microbial use of carbon, thus enhancing soil carbon storage. To understand the link between crop diversity and the processing of organic carbon by the soil microbiome, we studied the effect of cover crop diversification on microbial CUE, microbial diversity, and carbon storage (SOC) in the top-(0-30 cm) and subsoil (30-60cm) of the Dutch long-term experiment Clever Cover Cropping.

This experiment was set up as a randomized block-design with 5 blocks of 8 plots. Each block consists of 8 cover crop treatments: three single species (radish: *Raphanus sativus*, black oats: *Avena strigosa*



and vetch: *Vicia sativa*), all possible 2- and 3- species combinations of these single cover crops and a fallow treatment. Soil samples were taken in both soil layers in December 2022 when the cover crop biomass had reached its maximum. We have analysed microbiome diversity, biomass and function and microbial CUE.

Some first results of 16S and ITS sequencing show that cover crop diversification did not significantly affect richness and Shannon diversity of the microbiome. However, the effect of soil layer was significant. Both richness and Shannon diversity were higher in the topsoil, compared to the subsoil. The correlation between microbial diversity and microbial CUE appears to have different directions in the top- and subsoil. In the topsoil microbial CUE is lower with increasing microbial diversity. In the subsoil microbial CUE is higher with increasing microbial diversity, however in the subsoil this correlation is not significant. The results and correlations for the other measurements will be presented at the EJP Soil Annual Science Days.

Preliminary results of 16S and ITS sequencing data show that cover crop diversification did not significantly affect microbial alpha diversity (richness and Shannon indices). However, the soil layer effect was significant. Both alpha diversity indices were higher in the topsoil, compared to the subsoil. The correlation between microbial alpha diversity and microbial CUE appears to have different directions in the top- and subsoil. In the topsoil microbial CUE is lower with increasing bacterial diversity, where fungal alpha diversity correlation values with CUE were small and not significant. In the subsoil microbial CUE is higher with increasing bacterial diversity, however in the subsoil this correlation is not significant. Regarding beta diversity, bacterial community also showed a big difference between top and sub soil, where the fungal community did not differ that much, though the differences between top and sub soil were significant for both, bacteria and fungi. The results and correlations for the other measurements will be presented at the EJP Soil Annual Science Days.

Our results may give indications that the relationship between microbial diversity and CUE is stronger in topsoils, but not in subsoils. Further, a higher diversity does not seem to result in increased CUE.

**Keywords:** cover crop diversification, subsoil, microbial diversity, microbial carbon use efficiency

## Assessing the Influence of Long-term Agroecological Practices on Soil Microbial Functional Diversity and Metabolic Activity

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Promoting agroecological management practices such as minimal soil disturbance and greater plant diversity significantly enhances the soil microbial community. The current focus on the composition and structure of the soil microbial community, and the changes it undergoes due to various environmental factors, is noteworthy. Furthermore, microbial activity is a reliable indicator of soil health because soil microorganisms are crucial for the breakdown of organic matter and the biogeochemical cycles that affect soil fertility. Therefore, this study aimed to assess the influence of long-term agroecological practices such as different tillage systems, along with cover crop usage, on the functional diversity and metabolic activity of the microbial community. The experimental site located in central Lithuania, at the Lithuanian Research Centre for Agriculture and Forestry (LAMMC) (55°23'50"N, 23°51'40"E). A split-plot design with three treatments, including no-tillage (NT), no-tillage + cover crop (Persian clover) (NTC), and conventional tillage (CT), was selected to evaluate the effects of agroecological practices. Microbial Community Level Physiological Profiles (CLPP) were determined using Biolog EcoPlates™ (Biolog Inc., Hayward, CA), which contain 31 different carbon sources. The average well color development (AWCD), substrate richness, and Shannon diversity index ( $H'$ ) were determined to quantify the metabolic capabilities and functional diversity. After 48 hours of incubation of the EcoPlates, it was noted that there were statistically significant differences (with  $P \leq 0.05$ ) among the treatments, in total AWCD and  $H'$  index, where NTC showed the highest AWCD and  $H'$  index values. However, the picture changed after 96 hours of incubation; there were no statistically significant differences among soils from different agroecological practices and CT, but NTC showed comparatively higher values of AWCD and  $H'$  indexes. Carbon substrate groups' utilization patterns differed among the soils. The highest carbon substrate groups, including carbohydrates, carboxylic acids, amino acids, polymers, amines, and miscellaneous utilization, were detected in the soil of NTC. The results also revealed that NTC significantly increased microbial biomass carbon (MBC) compared

to NT and CT treatments ( $p = 0.0101$ ). Pearson's correlation analysis showed moderate, statistically significant positive correlations between AWCD and MBC ( $r = 0.630$ ,  $p = 0.028$ ), as well as between H' index and MBC ( $r = 0.576$ ,  $p = 0.050$ ). These results suggest that increases in AWCD and H' index values are associated with higher microbial biomass carbon in soil. Overall, our findings demonstrate that NTC exhibited comparatively higher microbial functional diversity and enhanced metabolic activity, compared to both NT alone and CT practices. These improvements are likely due to the increased organic inputs and minimal soil disturbance, which favor a more stable and functionally diverse microbial community.

**Keywords:** soil, tillage, cover crop, community level physiological profiles, microbial biomass carbon

## Effect of agroecological intensification on root mycorrhization, soil aggregate dimension and related C content by SEM-EDS

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Fostering agroecological management practices, based on reduced soil disturbance and increased plant diversity in field, can play a pivotal role on soil fine macroaggregates and related C pools. In AGROECOseqC project, we hypothesized the no till and the crop diversification (as cover crop or rotation) can improve the physical stabilization in soil aggregates through development of mycorrhizal

root network, making SOM recalcitrant to fast degradation. To verify it, soil samples were collected from three AGROECOseqC experimental sites: S1-CREA (IT), S2-INIA-CSIC (ES), S3-LAMMC (LT). In each 4-blocks randomized experiments, following practices were tested: T1 - no tillage (NT), no crop diversification (no-Div); T2 - tillage (T), crop diversification (Div); T3 (control) – tillage (T), no crop diversification (no-Div).

At S1, Div was based on wheat+vetch cover crops sowed in apricot orchard interrow, at S3 on Persian clover cover crop after oilseed rape, while at S2 Div was the wheat-vetch-barley rotation. Twelve undisturbed soil samples in each experimental site (3 treatments x 4 blocks) were collected and sent to CREA, refrigerated. Soil samples were analysed by Scanning Electron Microscopy (SEM) and by Energy Dispersive X-ray Spectroscopy (EDS), a non-destructive technique used for morphological and chemical characterization of soil aggregates. Mycorrhizal colonization intensity (M%) was measured on undisturbed roots collected in the same 12 plots (3 plant root systems/treatment/block). Mycorrhizal colonization intensity (M%) of roots was determined using Trouvelot method (Trouvelot et al., 1986).

Agricultural practices differently affected the distribution of soil fine ( $250\mu\text{m} < \phi < 1.0\text{mm}$ ) and coarse ( $1.0\text{mm} < \phi < 2.0\text{mm}$ ) macroaggregates, depending on experimental sites. In apricot orchard interrow (S1), the highest percentage of soil fine macroaggregates was recorded in tilled system with cover crops (T2), as such as in S2 wheat cropping system under no-tillage (T1): as expected, in S2 the soil microaggregates ( $< 250\mu\text{m}$ ) were predominant in tilled plots (T3). Contrastingly, in S3 oilseed rape cropping system the highest percentage of soil fine macroaggregate were found in tilled plots

(T3), while no-tillage gave the highest percentage of soil microaggregates. By evaluating together all the tested sites, a decrease of soil microaggregates in favour of 250µm÷500µm fine macroaggregates was observed under no-till and crop diversification. M% was reduced by tillage compared to no-tillage in the tested experimental sites, while no effect of crop diversification on root mycorrhization was observed, except in S3, where Persian clover was introduced as cover crop. SEM analysis gave also evidence of no-till to maintain the root mycorrhizal extra-hyphal mycelium intact. M% was also positively correlated to soil aggregates diameter (linear regression:  $R^2 = 0.6106$ ). Contrastingly, semi quantitative analysis of C% in soil aggregates did show neither a significant correlation with average diameter of soil aggregates, nor with M%.

In conclusion, no-till practice corresponds to an increased root mycorrhization in field and to a predominance of soil fine macroaggregates, probably due to the mycorrhizal extra-hyphal mycelium increasing the soil particles adhesion. In no-tilled systems, a trend to C% increase in studied soil aggregates was recorded also.

**Keywords:** no tillage, crop diversification, SEM-EDS, soil aggregates, mycorrhization.

## Soil nitrogen pool dynamics in an agroecological gradient

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One of the main targets of agroecology is the reduction of fertilizing application while maintaining crop productivity, resulting in less fertilization costs for the farmer and a decrease in nitrogen leaching and its derivate ecological impacts (e.g. water eutrophication, biodiversity loss or toxic algae growth). This could be achieved by increasing the synchrony between the plant nutrient demand and the nitrogen cycle. In soil, the two main processes involving nitrogen are mineralization (N transformed from organic N to mineral N forms, available for plants but also leached) and immobilization (N transformed from mineral to organic forms), whose balance determine nitrogen leaching or accumulation in soil. In this frame, the AgroecoseqC project aims to study the soil nitrogen supply resulting from soil organic nitrogen dynamics at low and high nutrient demand under different agroecological intensification conditions. We collected soil samples on 9 European experimental sites with 3 equivalent treatments (1 control, and 2 levels of agroecological improvements) at two dates corresponding to low and high plant nutrient demand. Besides, the practices applied on the different sites constituted a gradient of agroecological intensification, from low to high intensity management practices. We estimated soil nitrogen mineralization and immobilization by the <sup>15</sup>N isotope dilution method of Davidson et al. (1991). After an addition of <sup>15</sup>NH<sub>4</sub><sup>+</sup> to soils, this method allows to quantify 1) the gross N mineralization using the dilution of <sup>15</sup>NH<sub>4</sub><sup>+</sup> pool by the <sup>14</sup>NH<sub>4</sub><sup>+</sup> released from soil organic nitrogen and 2) the gross N immobilization using the incorporation of <sup>15</sup>N in soil organic nitrogen. Here we present some preliminary results for the already available data, showing differences between demand periods, sites and agroecological practices.

**Keywords:** nitrogen cycle; stable isotopes; soil microbials; plant/soil interactions, nutrient cycling, nitrogen mineralization and immobilization balance

Abstracts of Poster Presentations

## Cover crop diversification can alter microbial life-death cycle and enhance carbon sequestration in agricultural soil

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Sequestering atmospheric CO<sub>2</sub> within soil organic matter via shifts in agricultural practices represents a compelling strategy for enhancing soil ecosystem services and mitigating global change. Traditionally, the perception of soil carbon (C) stability is focused on intrinsic characteristics of organic matter inputs, such as lignin content. Recent studies, though, challenge this perspective, proposing a more effective approach centered on managing how the soil microbiome processes C inputs (Sokol et al., 2019; Poepflau et al., 2019).

This change of perspective prompts the exploration into the intricate connection between aboveground plant communities and belowground diversity of the microbiome, as well as the associated metabolic processes governing C sequestration. Building on this, Lehmann et al. (2020) presented a theoretical framework, interpreting the persistence of C in soil as a consequence of interactions between the molecular variability of organic matter input and the spatio-temporal microbial heterogeneities within the soil system. This perspective emphasizes the need of a comprehensive understanding of the dynamic interplay shaping C sequestration, moving beyond static views of organic matter stability.

Consequently, within the EnergyLink framework a range of microbial markers were investigated to illuminate potential physiological changes at a microbial level across several European agricultural field sites with different cover crop management types. Specifically, to discern shifts in microbial necromass composition and quantity, we focused on amino sugars (galactosamin, glucosamine, mannosamine and muramic acid). To assess effects on potential growth rates, we quantified <sup>14</sup>C incorporation into ergosterol for fungi and <sup>3</sup>H-leucine incorporation for bacteria. To investigate shifts in nutrient acquisition strategies, we also examined extracellular enzyme activities for different nutrient classes. Additionally, we determined C:N:P ratio for bulk soil and microbial biomass. Here we present first results and discuss implications of diversified cover crops on soil carbon properties.

**Keywords:** Cover cropping; microbial necromass, microbial growth, amino sugars, microbial turn over



# The effect of crop diversification and season on microbial carbon use efficiency across a European gradient

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Given the critical role of microbial carbon (C) transformations in C cycling, it is important to understand the influence of land use and management practices on microbial physiology and its connection to C dynamics. Crop diversification may alter microbial physiology by altering the chemical complexity of plant-derived organic matter inputs to the soil and eventually microbial community composition. This in turn could influence the mean residence time of plant-derived C in soil. The microbial carbon use efficiency (CUE) describes how much of the metabolised C is directed to microbial biomass or lost as CO<sub>2</sub> to the atmosphere.

Within the EJP SOIL project EnergyLink, we investigated the effects of crop diversification on microbial CUE across a pan-European pedo-climatic gradient. In total, topsoil from eight long-term experimental sites representative for different crop diversification measures (i.e. cover crops, ley farming, vegetation stripes) across Europe were sampled and analysed for microbial CUE, growth, respiration and biomass C using the <sup>18</sup>O-labelling method. On a subset of five sites, a second sampling was performed to test whether the effect of crop diversification was influenced by the growing season.

The general response of these microbial parameters and SOC stocks to overall crop diversification and individual measures was calculated extracting weighed effect sizes for each sampling (i.e. site and season), accounting for site clusters. We tested for differences in CUE between sampling time points using a site-wise ANOVA and TukeyHSD.

Crop diversification did not cause a distinct alteration in microbial physiology. The establishment of vegetation stripes between rows of olives or vines increased overall microbial abundance and activity (i.e. microbial biomass C, respiration and growth), without changes in CUE. Higher abundance and activity were likely related to higher C inputs in vegetation stripes as compared to bare soil. Most noticeable, CUE was significantly different for samples taken at different time points, potentially implying a seasonality effect on microbial physiology. This should be considered when comparing CUE values across sites sampled at different time points.

Furthermore, a numerical approach to extract seasonality information from weather data (i.e. temperature and precipitation) to account for different climatic conditions across the pan-European gradient is presented. This information can help to further investigate the drivers of the observed seasonality effect.

**Keywords:** crop diversification, microbial carbon pump, carbon stabilisation, isotopic labelling, microbial physiology

## A guideline for estimating carbon use efficiency with the $^{18}\text{O}$ method

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The carbon use efficiency (CUE) of soil microorganisms is the fraction of absorbed carbon that is allocated to microbial growth, with the remainder being released as  $\text{CO}_2$ . The CUE provides information on the carbon metabolism of soil microorganisms, and 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 disentangle if they act as source or sink of carbon during the low and high plant nutrient demand periods. Among all the multiple methods available to estimate CUE, In the AgroecoseqC project we will use the  $^{18}\text{O}$  method, which aims the estimation of microbial gross growth in short periods of time. The greatest difference with the methods based on C substrate addition, is that with this method we avoid bias in CUE estimation due to the stimulation of 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  $^{18}\text{O}$  labelled and to trace the incorporation of  $^{18}\text{O}$  in DNA in order to estimate microbial growth. Simultaneously, microbial respiration is measured by gas chromatography. Then these measures are used in a series of equations for estimating CUE. At the time of testing these methods for their application in AgroecoseqC, we have identified some unclear points in the published literature, introducing potential uncertainty in the final CUE estimates. These unclear points involve 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), the variability of the replicates performed with the same soil sample 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

## Integrated modelling of microbial-plant interactions in multi-species agroecosystems: the project MODIMIV

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The MODIMIV (“Modeling relationships between microbial and plant diversity in multi-species agroecosystems”) exploratory project (2024-2025) within the INRAE Biosefair meta-programme (<https://biosefair.hub.inrae.fr>) sets out to refine our understanding of biotic interactions within multi-species vegetation stands (<https://biosefair.hub.inrae.fr/rubriques-verticales/nos-actions/projets-exploratoires/modimiv-projet-exploratoire-2024-2026>). Recognising the pivotal role played by soil microbial diversity and plant diversity in governing carbon and nutrient cycles, MODIMIV is committed to unravelling these complex interactions, with grasslands serving as revealing case studies for broader multi-species ecosystems.

Conventional modelling approaches often compartmentalise plant and microbial components, overlooking their dynamic interplays. MODIMIV challenges this view by integrating the dynamics of microbial and plant diversity into simulation tools, thereby offering key insights for sustainable agricultural management practices. Microbial diversity significantly shapes processes such as nitrification, denitrification, nitrogen fixation and carbon sequestration, while plant diversity holds promise for optimising microbial carbon and nitrogen use by harmonising nutrient supply and demand.

At the core of MODIMIV lies the ambition to develop integrated simulators capable of capturing the material flows both within and between microorganisms and plants. By aligning the availability of nutrients with the needs of plants and microbes, this approach seeks to shed light on the nuanced interplay of biological compartments within ecosystems.

The main objectives of MODIMIV include:

- Investigating how biodiversity shapes carbon and nutrient cycling across diverse, multi-species vegetation cover to enhance our understanding of ecosystem dynamics.
- Developing advanced simulation tools that seamlessly integrate plant and microbial diversity to enrich the modelling of ecological processes associated with herbaceous covers.
- Assessing the effects of plant and microbial diversity on nutrient cycling and carbon sequestration, highlighting their central role in supporting ecosystem sustainability.
- Providing long-term prediction models: Equipping stakeholders with reliable and easy-to-use predictive models to strengthen sustainable agricultural practices by offering foresight for sustainable management strategies.

To enhance the refinement of modelling prototypes, MODIMIV will leverage data from multi-year mesocosm studies and replicated field experiments conducted on grassland and agro-grassland treatments in Clermont-Ferrand (France). Moreover, the project actively welcomes collaboration with other initiatives, including the EJP Soil AGROECOSeqC project. With grasslands as its primary focus, MODIMIV aims to show the effectiveness of its simulators, particularly in low-input agroecosystems. These ecosystems serve as compelling examples of how diverse plant communities modulate nutrient availability to soil microbiota, with benefits for plant growth. Through these collaborative efforts, MODIMIV seeks to transcend conventional modelling paradigms and advocates for the adoption of sustainable agricultural practices across a diverse array of multi-species vegetation covers.

**Keywords:** grasslands, integrated modelling, microbial-plant dynamics, multi-species agroecosystems

## Exploring the interplay of plant and soil biodiversity in mediating soil organic carbon dynamics in European agroecosystems

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Plant and soil biodiversity are critical for soil health and ecosystem stability. The interactions between soil and plant biodiversity mediate essential processes that underpin multiple ecosystem services. In the last decades the study of functional biodiversity emerged as a new lens to analyse, monitor and promote the role of the soil life and plant assemblages on ecosystem services. However, the mechanisms underlying these interactions and how farmers can manage biodiversity to balance environmental and productive goals remain unclear.

In the AGROECOseqC project we have been collecting plant and microbial biodiversity data in order to investigate their effects on soil organic carbon dynamics from several locations across Europe. Specifically, we conducted phytosociological survey of plant communities and applied Next Generation Sequencing analysis to characterize the soil microbial (bacteria and fungi) communities. Based on these data we have studied the functional diversity of those communities by selecting functional traits linked to soil carbon dynamics. The statistical analysis employed alternative strategies, including partial least squares structural equation modelling.

Our preliminary results indicate that specific plant traits, such as Specific Leaf Area and taproot presence, significantly affected organic carbon content in soil. These plant traits also influenced microbial functional groups, particularly bacterial groups involved in carbon degradation and mycorrhizal fungi. Some selected bacterial and fungal groups exhibited significant negative effects on soil carbon, including soil organic carbon and water-extractable carbon. Additionally, farm management practices were found to influence both plant and microbial functional groups.

This functional approach offers a valuable tool for exploring the impacts of plant and soil microbial biodiversity on soil health and ecosystem services. Understanding these interactions can help in developing strategies for managing biodiversity to achieve both environmental sustainability and agricultural productivity.

## Regional Assessment of Soil Organic Matter Stability under No-till and Diversified Agricultural Management Practices

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The relationship between soil structure and the ability of soil microbiome to stabilize soil organic matter is a key element in soil carbon (C) dynamics. Water extractable organic carbon (WEOC) pool can be used as prompt indicator of soil organic matter stability, associated to soil microbial C (SMBC). Therefore, this study aims to validate the established correlation between the water stable aggregates (WSA, %), soil organic carbon (SOC, %), WEOC ( $\text{g kg}^{-1}$ ), and SMBC ( $\mu\text{g g}^{-1}$ ) in bulk soil and different soil aggregates (fine: 0.25-1 mm and coarse: >1mm) under no-till and diversified practices across different environmental conditions at experimental sites in Italy (S1-CREA), Spain (S2-INIA-CSIC), and Lithuania (S3-LAMMC). Soil samples were collected from 3-blocks with three sub-replications in two no tillage treatments: T1 - no crop diversification (no-Div) and T2 - crop diversification (Div). At S1, Div was based on wheat + vetch cover crops in-between apricot tree rows, at S2 - on the wheat-vetch-barley rotation, and at S3 - on Persian clover cover crop grown after oilseed rape. Undisturbed soil samples (0-20 cm in depth) were taken for the aggregate stability analysis. Dry sieving was performed by Retch sieve shaker: mesh sizes 8.0, 5.6, 4.0, 2.0, 1.0, 0.5, and 0.25-mm and soil aggregates of 0.25-1 mm and >1mm were subjected to SOC and WEOC analysis. Aggregates from 1 mm sieve were wet sieved by Ejkelkamp apparatus (Velykis, Satkus, 2018). Bulk soil samples for the SOC, WEOC, and SMBC analysis were sieved through a 2 mm sieve. The content of SOC was determined according to the Nikitin-modified Tyurin method (Nikitin, 1999), WEOC - by the IR detection method after UV-catalyzed persulphate oxidation, SMBC - by the chloroform fumigation extraction method (Vance et al., 1987).

Soil aggregate stability differed among sites, but not among treatments, with highest WSA at S1 (93.9%) followed by S3 (77.0%), and S2 (47.1%). Highest content of SOC and WEOC were obtained in bulk soil at site S1 (2.2% and  $0.30 \text{ g kg}^{-1}$  respectively), and lowest - at site S2 (1.1% and  $0.16 \text{ g kg}^{-1}$  respectively) and S3 (1.6% and  $0.17 \text{ g kg}^{-1}$  respectively). No significant differences among different soil



aggregate fractions and treatments for SOC and WEOC were found at any site. SMBC differed among the S1, S2 and S3 (367.6  $\mu\text{g g}^{-1}$ , 267.3  $\mu\text{g g}^{-1}$  and 92.6  $\mu\text{g g}^{-1}$  respectively) and Div' - at S1 and S3. Higher SMBC was found in no-Div (433.1  $\mu\text{g g}^{-1}$ ) compared to Div (313.4  $\mu\text{g g}^{-1}$ ) at S1, and opposite, less SMBC - in no-Div (238.9  $\mu\text{g g}^{-1}$ ) compared to Div (291.4  $\mu\text{g g}^{-1}$ ) at S3. Positive and statistically significant correlations were detected among all variables tested. Most of the very strong correlations ( $r > 0.8^{**}$ ) were found among SOC and WEOC and its amounts in different soil aggregates. SMBC strongly correlated with WSA ( $r = 0.87^{**}$ ), SOC and WEOC in bulk soil ( $r = 0.67^{**}$  and  $r = 0.62^{**}$  respectively). Strongest WSA correlations obtained with SOC in fine aggregates ( $r = 0.73^{**}$ ) and WEOC in bulk soil ( $r = 0.69^{**}$ ).

This study emphasizes the complex relationship between soil structure, microbial activity, and carbon cycling across diverse environmental conditions and agricultural practices. The strong correlations observed among SMBC, WEOC, SOC, and WSA highlight the pivotal role of soil organic matter stability in regulating soil carbon processes. Integrated agricultural management strategies are essential for improving soil carbon dynamics in response to these findings.

**Keywords:** soil aggregate stability, soil microbial carbon, soil organic carbon, water extractable organic carbon

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## Soil-, management-, and climate-related drivers of yield stability in organic and conventional farming systems

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Soils provide multiple ecosystem services that enable a continuous production of crops. This ability is largely affected by the actual land management system in place. Farming systems that promote ecosystem services are also expected to be more resilient against external stressors, enabling a more stable crop production. To better understand the soil-, management- and climatic- related drivers of yield stability in different farming systems, the EJP SOIL ARTEMIS project is analysing different long-term field experiments across Europe on these parameters. Here we present the results of the Swiss DOK-experiment, that continuously compares organic and conventional farming systems since more than 45 years at two fertilisation levels (standard and halved).

We calculated different yield stability indicators including the coefficient of variance for the main crops of winter wheat, maize, soy bean, potatoes as well as grass-clover leys that are part of the 7-year crop rotation. Yield stability showed no general treatment trend, i.e. in some years organically managed crops performed better and vice versa. To better identify treatment differences, we then decoupled climate-related drivers from soil-related drivers (such as soil organic carbon, pH and nutrient available N) using linear mixed effect models. We further included different management practices for pest management that are strongly differing between these systems into the statistical modelling.

Preliminary results indicate a strong relationship between yield productivity and agroclimatic data, especially for estimates for the whole growing season of each specific crop. We further show that the positive effects of standard fertilisation levels on yield levels are for both systems significantly stronger during adverse climatic stress events.

**Keywords:** yield stability; organic farming; long-term field experiment

## Effects of different agriculture practices on ecosystem services in cereal fields worldwide

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Cereals are critical to support food production and energy across the globe, occupying the largest crop acreage in the world. Advancing our knowledge about the effects of the different agricultural practices on the ecosystem services provided by these cereal crops is critical to support food production and ecosystems sustainability. Here, we put together a global database to investigate the effects of conventional agricultural, organic and sustainable management in driving multiple ecosystem services from nutrient cycling to carbon stocks. Our meta-analysis reveals that different agricultural practices affect soil microbial communities and nutrients, leading to variations in their activities and composition.

Specifically, we found that sustainable agriculture has a significant positive effect on fertility, soil habitat, and carbon sequestration, while organic agriculture only shows a significant positive effect on soil habitat compared to sustainable agriculture. Despite this, no significant effect of these practices on crop production is observed, indicating that both soil management practices maintain agricultural production while promoting key ecosystem services.

Our study demonstrates the critical role of farm management in supporting food production in a world facing such major changes.

**Keywords:** soil fertility, soil biodiversity, crop yield, ecosystem services

## ANALYSIS OF SOIL MICROBIAL COMMUNITY ASSOCIATED WITH CEREAL CROP UNDER SUSTAINABLE MANAGERMENTS IN DIFFERENT EUROPEAN COUNTRIES

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### Abstract

Climate change poses an escalating threat to food security and overall life, with significant effects on crops and ecosystems. Adaptation and mitigation strategies are imperative to safeguard agricultural production and ensure the sustainability of food systems and human well-being. This comprehensive European study compares biodiversity and functionality across different regions of Europe in cereal crops, applying different ecological scales in terms of eco-efficiency. Furthermore, some studies recently revealed that even rare bacterial and fungal species may be highly important for soil function, which should be identified and monitored. The experiments consisted in testing three different treatments: sustainable farming (T1), consisting in the use organic fertilizers, crop rotation, and reducing the chemical inputs; agroecological farming (T2), adopting organic and environmental-friendly practices; conventional farming (T3), taken as control. We observed that both the structure and predicted functionality of fungal and bacterial communities were differently affected by T1 and T2 treatments, compared to conventional farming (T3) across the different regions. These results indicate that both sustainable and agroecological agricultural practices may have a similar, great

impact on soil microbial composition and functionality in different European regions, thus benefiting the agroecosystems through increasing soil health and fertility.

Keywords: biodiversity, functionality, sustainable managements, soil

## Exploring Endophytic Bacteria from *Artemisia* spp.: Antagonistic Potential Against Pathogens and Contributions to plant growth promotion

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Endophytic microorganisms might present sustainable alternatives to environmental concerns associated with conventional agricultural practices. Therefore, our study focused on the isolation, identification, and characterization of endophytic bacteria from the root, stem, and leaf tissues of four *Artemisia* plant species. A total of eighty-four endophytic bacterial isolates were selected based on morphological characteristics, and their molecular identification was performed using a 16S rDNA gene sequence-based method. The isolated bacteria belonged to diverse genera, including *Bacillus*, *Pseudomonas*, *Enterobacter*, and *Lysinibacillus*. Inhibition growth tests revealed that 61 bacterial isolates inhibited the growth of two pea root rot pathogens. AR11, and VR24 strains, isolated from *A. absinthium* and *A. vulgaris* roots, respectively, exhibited significant inhibition growth activity, against *Fusarium* sp. Root rot agent of pea. Furthermore, twenty-two strains demonstrated phosphate solubilization ability, with AR11 displaying the highest Phosphate Solubilization Index (2.93) after 10 days. Additionally, thirteen isolated strains exhibited positive reactions for indole production. The majority of effective strains belonged to the *Bacillus* genus, particularly from the root parts of *Artemisia* spp. The study underscores the multifaceted benefits of endophytic bacteria in sustainable agriculture, providing valuable insights into their role in pathogen suppression and plant growth promotion.

**Keywords:** Endophytic bacteria, *Artemisia* plants, *Fusarium* sp. Plant growth promotion

## INCREASES of ORGANIC CONTENTS OF SOILS THROUGH *Taloromyces funiculosus* APPLICATION

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### ABSTRACT

Soil nutrient status tend to decrease gradually over the years even if fertilization is made. Inorganic and organic compounds tend to accumulate and lead to pollution if they are not decomposed. These compounds also increase the soil electrical conductivity (EC) level and pose a threat to crop plants especially in the seedling stages. In this study, over a 3-year period between 2021-2023, we applied *Taloromyces funiculosus* to the soils cultivated with barley or soybean as cover plants in winter seasons. We observed that total organic carbon (TOC) contents and soil organic matters (SOM) increased significantly over a 3-year period. The soils cultivated with barley or soybean without the fungus application exhibited a gradual increase in TOC and SOM contents. We evaluate that *T. funiculosus* has a significant role for improving and maintaining the soil chemical and physical properties. We are in the process of elucidating if the fungus acts as a biofertilizer due to possible synthesis of soil enzymes. But much more proof is needed in this area.

Keywords: *Taloromyces funiculosus*, total organic carbon, soil organic matters, barley, soybean.



