# Forage polycultures as management tool to improve soil quality across the Mediterranean region

Presented by

**Gia Caruso Martinez**

in part fulfilment of requirements for the Master´s degree of

**Terrestrial Ecology and Biodiversity Management**

In the specialty of

**Terrestrial Ecology**

of the

**Autonomic University of Barcelona**

9 of September 2025

| Angela Ribas | Stefania Mattana | Mercedes Ibañez |
|---|---|---|
| | | |

**Project contributions by the student:**

The student started her master´s thesis in February 2024. She was not involved in data collection. She collaborated in the laboratory soil analysis. She took care of all the data processing. She wrote the entire article. She performed all the statistical analyses and produced all the figures and tables presenting the data. The scientific journal chosen to publish her work is "Agriculture, Ecosystems & Environment".

**Abstract**

The Mediterranean region is one of the most climate-vulnerable areas worldwide, facing rising temperatures, recurrent droughts, and soil degradation that threaten agricultural productivity and food security. A key concern is the decline of soil organic matter (SOM), which underpins nutrient cycling, water retention, microbial activity, and carbon storage. Enhancing biodiversity within agroecosystems has been proposed as a strategy to buffer soils against climatic stress while sustaining productivity. In particular, increasing sown species diversity may strengthen plant–soil–microbe interactions, thereby improving soil quality and resilience.

This study examined how plant diversity influences soil microbial activity and carbon dynamics across a pedoclimatic gradient of Mediterranean sites.

Results showed that species diversity enhanced microbial functional capacity, promoting greater carbon retention and decomposition of complex substrates. Species identity also played a central role: *Festuca arundinacea*, *Avena sativa*, *Dactylis glomerata*, *Cichorium intybus*, and *Plantago lanceolata* were particularly influential in shaping soil carbon dynamics. Notably, interactions among species yielded both synergistic and antagonistic effects on microbial activity and carbon accumulation, highlighting the importance of functional complementarity.

Environmental factors, especially precipitation and soil organic carbon, remained the dominant drivers of microbial responses. However, higher species diversity consistently improved microbial activity even under drought, suggesting potential for mitigating climate impacts. Overall, our findings demonstrate that targeted increases in forage diversity can enhance soil quality and carbon sequestration, offering a promising pathway for resilient and sustainable Mediterranean agriculture.

**Keywords.** Plant diversity, microbial activity, Mediterranean agriculture, drought under climate change, soil carbon accumulation, DI modeling, forage species interaction, Festuca arundinacea, Avena sativa, Dactylis glomerata, Cichorium intybus, Plantago lanceolata.

## 1. Introduction

Climate change is producing widespread impacts across the globe, with the Mediterranean region recognized as one of the most vulnerable and exposed areas. Key risk factors affecting this region include water scarcity, prolonged droughts, soil erosion, threats to agricultural productivity and food security, and sustained temperature increases (Ali et al., 2022; del Pozo et al., 2019). These climate-related challenges have had a detrimental impact on both the quality (Bezner Kerr et al., 2022; Hidalgo-Galvez et al., 2023; del Pozo et al., 2019) and quantity of crop production (Bezner Kerr et al., 2022; Dono et al., 2016; del Pozo et al., 2019).

One of the most significant effects of climate change is its impact on soil quality, particularly through the depletion of soil organic matter (SOM), a growing concern in agricultural systems (Bezner Kerr et al., 2022; Semeraro et al., 2023). Soil quality underpins the delivery of multiple ecosystem services: it supports nutrient retention, regulates physical, chemical, and biological processes, buffers pH fluctuations, enhances soil structure, increases water-holding capacity, and stimulates microbial activity. In addition, soil functions as a major carbon sink, with a storage potential estimated to be three to four times greater than that of the atmosphere. The decline of these functions undermines the availability of recalcitrant carbon in the soil (Bezner Kerr et al., 2022; Semeraro et al., 2023), thereby diminishing its carbon storage potential (Kleber, 2010). This degradation poses a direct threat to agricultural and livestock productivity (Bezner Kerr et al., 2022), particularly in climate-sensitive regions such as the Mediterranean.

In response to the urgency of the climate crisis, recent research has focused on addressing the inherent vulnerability of Mediterranean climates to climate change. New agricultural practices are being proposed to strengthen the link between soil quality, crop health, and productivity. Increasing the diversity of agroecosystems has been shown to create a buffering effect that enhances resilience to climatic events (Altieri et al., 2015), while also maintaining soil health and fertility, for example, by improving air and water availability in the soil (Semeraro et al., 2023).

Among the recommended management strategies is the adaptation of crops to the specific conditions of each region (del Pozo et al., 2019; Molénat et al., 2023), along with a focus on reinforcing plant–soil microorganism associations, which play a critical role under stress conditions (del Pozo et al., 2019). Integrated approaches that consider both aboveground and belowground

biodiversity are essential for improving the management of the plant–soil system and identifying strategies to maintain its health (Baggs, 2011).

Over the past decades, numerous studies have confirmed the positive effects of plant diversity on various ecosystem properties. These include enhanced productivity (Finn et al., 2013; Hector et al., 1999; Kirwan et al., 2007), increased soil carbon and nitrogen content (Fornara & Tilman, 2008; Steinbeiss et al., 2008), improved nitrogen use efficiency (Llovet et al., 2024; Nyfeler et al., 2011; Oelmann et al., 2007), and greater microbial activity and diversity (Eisenhauer et al., 2011; Loranger-Merciris et al., 2006). However, the specific effects of plant diversity on soil properties remain underexplored and warrant further investigation (Llovet et al., 2024).

Soil biological activity is considered a key parameter of soil quality. The interactions occurring in the rhizosphere, between plant roots and soil microbiota, enable a wide range of processes, such as soil carbon storage, nutrient cycling, and organic matter decomposition (Devi & Soni, 2020; Kleber, 2010; Orwin et al., 2006). These processes occur because heterotrophic microorganisms depend on organic matter as an energy source to sustain their metabolism. Plant roots exudates supply soluble carbon that microorganisms readily utilize, while in turn they provide essential nutrients, water availability and protection to the plant. The ability of microorganisms to decompose organic matter underpins this mutualistic exchange. Some microorganisms preferentially degrade simple carbon substrates, such as sugars or amino acids, whereas others specialize in metabolizing more complex, recalcitrant compounds. This decomposition process plays a crucial role in the carbon cycle, as microbial processing of carbon substrates not only sustains soil biological activity but also contributes to long-term carbon retention in soil (Khatoon et al., 2017).

Numerous studies have demonstrated a strong correlation between plant diversity and soil microbial biomass, suggesting that increased biodiversity stimulates microbial activity, which in turn enhances soil quality and overall ecosystem functionality (Steinauer et al., 2016). Similarly, the integration of biodiversity into agricultural systems has been shown to improve soil health, boost crop productivity, and enhance both the quantity and nutritional quality of food production (Frison et al., 2011). Despite these promising findings, the underlying mechanisms through which soil microbial activity influences key soil quality functions—particularly soil organic matter cycling—remain insufficiently understood.

In this regard, our working hypothesis is that increasing sown species diversity in polyculture systems will positively influence microbial functional diversity, thereby improving key soil quality parameters. Specifically, it is expected that higher plant diversity will lead to greater microbial biomass, enhanced microbial functional capacity, and increased carbon storage in the soil. These beneficial effects are likely to be modulated by pedoclimatic conditions, such as soil type, temperature, and moisture availability, which may influence the magnitude and direction of the microbial and soil responses.

The objectives of this study we aim to: a) characterize soil quality and microbial catabolic profiles across a pedoclimatic gradient of Mediterranean sites; b) assess whether increased plant species diversity is associated with enhanced microbial biomass and improved carbon-use efficiency, and to evaluate whether this greater sown diversity contributes to higher soil carbon (C) content and functional diversity through specific species or functional group interactions; c) determine whether the relative contributions of species identity and interaction effects on soil variables are consistent across locations and resilient to the natural drought conditions experienced during the experiment.

## 2. Materials and methods

### *2.1. Experimental sites*

The experiment was conducted across four different Mediterranean regions represented by a climatic gradient. Each location represent is situated in a different country, Spain, Slovenia, and France were considered part of the humid Mediterranean, while Lebanon was considered part of the dry Mediterranean (Table 1). However, it must be noted that the traditionally humid sites experienced a severe drought during the study years, with a decrease of 30-40% in mean annual precipitation and above-average temperatures (Table1).

### *2.2. Experimental design and selected forage species*

Our study was part of the SUSFORAGE project (https://susforage.ctfc.cat/). At each experimental site, an area of approximately 1,400 m² was established, within which a design of 40 plots arranged in two rows was implemented. Each plot measured 3 × 10 m and was separated from adjacent plots by 0.5 m corridors. For the purposes of the SUSFORAGE project, each plot was divided into two equal halves to compare grazed and ungrazed conditions. However, since the analysis of grazing treatment falls outside the scope of our study, only data from the ungrazed plots were considered.

**Table 1**. Study site location and main pedoclimatic variables. The table includes mean annual temperature (MAT) and mean annual precipitation (MAP) for the reference series, along with the temperature anomaly during the first experimental year (2022; dMAT) and the percentage change in MAP due to the 2022 anomaly (%MAP). Soil texture components (sand, silt, and clay %) and soil pH are also reported.

| Site | Longitude (º) | Latitude (º) | Altitude (m asl) | MAT (ºC) | MAP (mm) | dMAT | %MAP | Sand (%) | Silt (%) | Clay (%) | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Olius (ES) | 41.98 | 1.56 | 585 | 12.0 | 854 | +2.1 | -32.4 | 41.1 | 25.3 | 33.6 | 8.2 |
| Theix (FR) | 45.70 | 3.016 | 660 | 8.6 | 773 | +0.1 | -45.4 | 51.3 | 17.9 | 30.8 | 6.0 |
| Gorenje pri Divači (SL) | 45.69 | 13.95 | 415 | 12.0 | 1300 | +1.7 | -41.9 | 30.4 | 36.2 | 33.4 | 7.7 |
| Terbol (LB) | 33.81 | 35.99 | 900 | 15.1 | 698 | +4.4 | -6.6 | 17.4 | 23.6 | 59.0 | 8.2 |

Plant species were selected according to their functional type: grasses (G), legumes (L), and non-leguminous forbs (hereafter forbs, F). For each functional group, three species were selected, with the specific composition varying by country (Table 2). The selection prioritized native species commonly used in each respective country, ensuring better adaptation to either humid or dry Mediterranean climates, as appropriate.

Species mixtures were randomly assigned to plots. For this study, 27 plots per country were selected to ensure a balanced representation of the various mixture types and diversity levels included in the experiment (Table 2). Diversity levels were primarily defined based on the relative dominance of species within each sward mixture: centroid (all species present in similar proportions), dominance (one species predominates), co-dominance (two species dominate equally), and monoculture (only one species included). This classification enabled the assessment of how species identity and relative abundance influenced soil microbial responses under varying mixture conditions, according to the modelling proposed by Kirwan et al. (2007, 2009). For further details, see Section 2.5: Statistical Analysis.

**Table 2**. The proportion of selected species in each country, each “composition”, was used to assess soil health and quality. Each composition plot represents a specific species proportion based on the mixture, where "Mixture" refers to the species richness within each sward. Species “diversity” is treated as a categorical variable reflecting the evenness of swards. Species are grouped into three functional categories: grasses (G1, G2, G3), legumes (L1, L2, L3), and non-legume forbs (F1, F2, F3). In Spain and Slovenia, the grasses used were *Lolium perenne* (G1), *Dactylis glomerata* (G2), and *Festuca arundinacea* (G3); legumes included *Medicago sativa* (L1), *Onobrychis viciifoli*a (L2), and *Trifolium pratense* (L3); and forbs were *Cichorium intybus* (F1), *Plantago lanceolata* (F2), and *Achillea millefolium* (F3). France shares the same grasses and forbs as Spain and Slovenia, but the legumes differ: *Vicia sativa* (L1), T*rifolium repens* (L2), and *Trifolium pratense* (L3). In Lebanon, the grasses were *Lolium rigidum* (G1), *Hordeum vulgare* (G2), and *Avena sativa* (G3); legumes included *Medicago sativa* (L1), *Trifolium incarnatus* (L2), and *Vicia villosa* (L3); and the forbs were *Cichorium intybus* (F1), *Plantago lanceolata* (F2), and *Brassica napus* (F3).

| Composition | Mixture | Diversity | G1 | G2 | G3 | L1 | L2 | L3 | F1 | F2 | F3 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Monoculture | Monoculture | | 1.00 | | | | | | | |
| 2 | | | | | | | | | | 1.00 | |
| 3 | | | 1.00 | | | | | | | | |
| 4 | | | | | | | | | 1.00 | | |
| 5 | | | | | | 1.00 | | | | | |
| 6 | | | | | | | 1.00 | | | | |
| 7 | 4-sp mixture | Dominance | 0.70 | 0.10 | | | | | 0.10 | 0.10 | |
| 8 | | Co-dominance | | 0.40 | | | 0.10 | | 0.10 | 0.40 | |
| 9 | | Dominance | 0.10 | 0.70 | | 0.10 | 0.10 | | | | |
| 10 | | Co-dominance | 0.40 | 0.10 | | 0.40 | 0.10 | | | | |
| 11 | | Dominance | 0.10 | 0.10 | | 0.10 | 0.70 | | | | |
| 12 | | Co-dominance | | | | 0.40 | 0.10 | | 0.40 | 0.10 | |
| 13 | | Co-dominance | | 0.10 | | | 0.40 | | 0.10 | 0.40 | |
| 14 | | Co-dominance | 0.10 | | | 0.40 | | | 0.40 | 0.10 | |
| 15 | | Centroid | 0.25 | 0.25 | | 0.25 | 0.25 | | | | |
| 16 | | Co-dominance | 0.40 | | | 0.10 | | | 0.40 | 0.10 | |
| 17 | | Co-dominance | 0.10 | 0.40 | | 0.10 | 0.40 | | | | |
| 18 | | Dominance | 0.10 | 0.70 | | | | | 0.10 | 0.10 | |
| 19 | | | 0.10 | 0.10 | | | | | 0.70 | 0.10 | |
| 20 | | | 0.10 | 0.10 | | 0.70 | 0.10 | | | | |
| 21 | | Centroid | 0.25 | 0.25 | | | | | 0.25 | 0.25 | |
| 22 | | Co-dominance | | | | 0.10 | 0.40 | | 0.10 | 0.40 | |
| 23 | | Dominance | 0.70 | 0.10 | | 0.10 | 0.10 | | | | |
| 24 | | | 0.10 | 0.10 | | | | | 0.10 | 0.70 | |
| 25 | 6-sp mixture | Centroid | 0.17 | | 0.17 | 0.17 | | 0.17 | 0.17 | | 0.17 |
| 26 | | | 0.17 | 0.17 | | 0.17 | 0.17 | | 0.17 | 0.17 | |
| 27 | | | | 0.17 | 0.17 | | 0.17 | 0.17 | | 0.17 | 0.17 |

The experiment was established in 2021, following the agronomic calendar specific to each country, with sowing dates and densities adjusted according to the environmental conditions of each participating location. No fertilizers were applied at any of the sites, except in Slovenia, where a single application of mineral fertilizer at a rate of 300 kg $ha^{-1}$ was performed. Weeds were also not removed in any of the experimental sites, allowing natural vegetation development within the plots. All systems were maintained under rainfed conditions, except in Lebanon, where a single supplementary irrigation was applied due to extreme drought conditions.

The data considered in this study corresponds exclusively to samples taken during year 2022 (harvest 1) and 2023 (harvest 2) in ungrazed plots. Soil sampling was carried out at the peak plant biomass, following a diagonal transect within each plot to ensure adequate representation. Three soil cores were collected from each plot using a 5 cm diameter corer, extracting soil from a depth of 0–10 cm. The cores were then homogenized to create a composite sample per plot. Samples were sieved through a 2 mm mesh and, depending on the type of analysis, either air-dried or stored at 4 °C until further processing.

*2.3 Community- level physiological profiling (MicroResp™)*

MicroResp™ assay is a microlite-plate system test that allows to perform catabolic profile of soil microbial communities by subjecting soil to a range of carbon substrate and measure the resulting respiration response over a short period. The MicroResp method provides information about the catabolic capability and functional diversity of the entire microbial community in each soil, in terms of mineralization of the chosen substrates to $CO_2$.

For the Microresp™ assay, soils were adjusted to 40% of their water holding capacity and loaded into 1.2 ml deep-well plate (ca. 0.35 g soil per well) as recommended by the manufacturer. Subsequently the samples were stored for 5 days at 25 ºC within a $CO_2$ trap (soda lime) to minimize soil disturbance induced by sampling and sieving on the microbial community. The physiological profiles were determined using different carbon sources, including five carbohydrates (D-glucose, D-fructose, sucrose, mannitol, meso-erythritol, and glycerol), three amino acids (γ-aminobutyric acid, L-proline, and L-arginine), and three carboxylic acids (α-ketoglutarate, citric acid, and L-malic acid). In addition, two polymers (cellulose and α-cyclodextrin) and one aromatic compound

(protocatechuic acid) were tested, all of which were considered recalcitrant carbon sources. Deionized water was included as a control to assess basal respiration.

After the soil incubation, 25 µl of substrate was added at each well of the deep well plate containing the soil. Subsequently, the plates were left open for 1 hour to allow the release of $CO_2$ from soil carbonates caused by addition of acid substrates. Then, the plates were sealed hermetically face to face with a second plate (detection plate) containing a colorimetric $CO_2$ gel and incubated for 6 hours at 25 ºC. The microbial activity was determined by the decrease in the absorbance measured at 570 nm with a microplate spectrophotometer (model Sunrise™, Tecan Trading AG, Switzerland).

Basal respiration was subtracted from each substrate response to obtain the net substrate-induced respiration rate. From these data, we also calculated microbial biomass carbon (MBC), using the equation by Anderson and Domsch (1978): MBC = 40.04 × glucose-induced respiration + 0.37; the microbial metabolic quotient ($qCO_2$, expressed as mg $CO_2$-C $g^{-1}$ $MBC^{-1}$); and the multiple substrate-induced respiration index (MSIR), calculated as the sum of net $CO_2$ flux across all substrates. We used MSIR as a proxy for microbial activity, microbial biomass carbon (MBC) as a proxy for the amount microbial biomass and the metabolic quotient $qCO_2$ as a proxy of microbial C use efficiency.

*2.4. Soil physical -chemical analysis*

To determine soil organic carbon (SOC) we used the Walkley & Black method (1934). This method quantifies the amount of oxidizable organic matter that is oxidized with a known amount of chromate in the presence of sulfuric acid. The remaining chromate is determined by titration with ferrous ammonium sulphate. The measured SOC was then multiplied by a correction factor of 1.72 to obtain the organic matter content.

Plant available P was determined using the Olsen protocol (Olsen & Sommers, 1982). Briefly, 1 g of soil was extracted with 10 mL of 0.5 M sodium bicarbonate (pH 8.5), and the extracted P was quantified colorimetrically by the molybdenum-blue (molybdate–ascorbic acid) method.

Total Kjeldahl nitrogen (TKN) was assessed using a modified micro-Kjeldahl method (Willis et al., 1996). Air soil samples were digested with concentrated sulfuric acid and Kjeldahl catalyst pills

at 360 °C for 3 hours. After digestion, digestates were diluted to 100 ml with distilled water, and $NH_4^+$ was measured by the salicylate method.

Water-soluble ionic concentrations were determined by ionic chromatography on a Dionex ICS-1100 ion chromatograph (Dionex, Sunnyvale, USA) using a AS4A-SC Dionex anion column for $Cl^-$, $NO_2^-$, $NO_3^-$, $HPO_4^{2-}$ and $SO_4^{2-}$ determination and a CS12A Dionex cation column for $Na^+$, $K^+$, $Mg^{2+}$, and $Ca^{2+}$ determination. Ion concentrations were estimated using linear calibration. Detection limit (LOD) estimation was stipulated as three times the standard deviation of five blank values.

*2.5. Statistical analysis*

All analyses were conducted in RStudio (R Core Team, 2024), to characterize the experimental context and support the interpretation of biodiversity effects across a geographically diverse framework, a Principal Component Analysis (PCA) was conducted to identify and describe site-level physico-chemical variation among the four study countries, Spain, France, Slovenia, and Lebanon. This analysis also aimed to capture the specific characteristics of each study site within the broader region, providing essential context for understanding how local environmental conditions may influence biodiversity outcomes.

To analyze the microbial catabolic profiles obtained with MicroResp™ we tested difference between countries using a permutational analysis of variance (PERMANOVA) using the vegan::adonis2 function (M. J. Anderson, 2001) with 999 permutations and Bray distances (Euclidean distance on Bray-transformed data). Since the PERMANOVA results were significant, we conducted pairwise comparison using the pairwise::adonis function (Martinez Arbizu, 2020), applying FDR correction to adjust p values for multiple testing. For visualization of MicroResp ™ results, we generated a heatmap to represent the microbial catabolic fingerprints of each country.

This study applied the Diversity–Interaction modelling framework developed by Kirwan et al. (2007, 2009), later generalized as General Diversity Interaction Modelling (GDIM; Connolly et al., 2013), to advance biodiversity–ecosystem functioning (BEF) analysis. The approach goes beyond species richness by integrating multiple dimensions of diversity, including species identity (composition) and interaction effects among species and functional groups. The hierarchical modelling process begins with a null model that includes only an intercept along with the structural

variables (block, density, and treatment, if specified). Species identity terms are then added to account for compositional effects. Interaction effects are subsequently modeled under different assumptions: average interaction effects (evenness model), additive species-specific contributions, and separate pairwise interactions, as outlined by Kirwan et al. (2009). Evenness (E) is given by the expression (Kirwan et al. 2007):

$$E = \frac{2S}{S - 1} \sum_{i<j} p_i \; p_j$$

Where S is the community maximum plant species number and $P_iP_j$ the species pairwise interactions.

Response variables—including MicroResp substrates (grouped by type: amino acids, carbohydrates, carboxylic acids, and polymers), functional diversity (based on the Shannon index derived from substrate activity), the multiple substrate-induced respiration index (MSRI), microbial biomass carbon (MBC), $qCO_2$, and organic carbon (CO)—were regressed against explanatory variables. Separate models were fitted for each response variable, beginning with general models based on the mean annual precipitation (MAP) from all four countries: France (FR), Lebanon (LB), Slovenia (SL), and Spain (ES). These were followed by country-level analyses using data from both harvests (2022 and 2023). Estimates were obtained using regression methods, with inference based on residual variation around the fitted models (Draper & Smith, 1998), making replication non-essential. Model selection and simplification were guided by the Akaike Information Criterion (AIC) using the DImodels package (*DI Models*, 2024), which automates the fitting and interpretation of Diversity–Interaction models. Table S4 summarizes the final models, including interaction effects, for each variable studied. And to better illustrate the influence of species evenness on soil organic carbon accumulation, pie charts were generated for all four countries,

## 3. Results

### *3.1 Soil characterization by geochemical and catabolic profile characteristics*

Figure 1 presents the results of the Principal Component Analysis (PCA). The first two principal components explained 67% of the total variance, with Dimension 1 (Dim 1, 41.5%) primarily associated with soil mineral content and precipitation, and Dimension 2 (Dim 2, 25.5%) reflecting

variation in pH and soil texture. The contribution of variables to the PCA indicates that mean annual precipitation exerts the greatest influence on the dataset (18%), followed by organic carbon (16%) and nutrients such as magnesium, nitrogen, potassium, and phosphorus (Table S1).

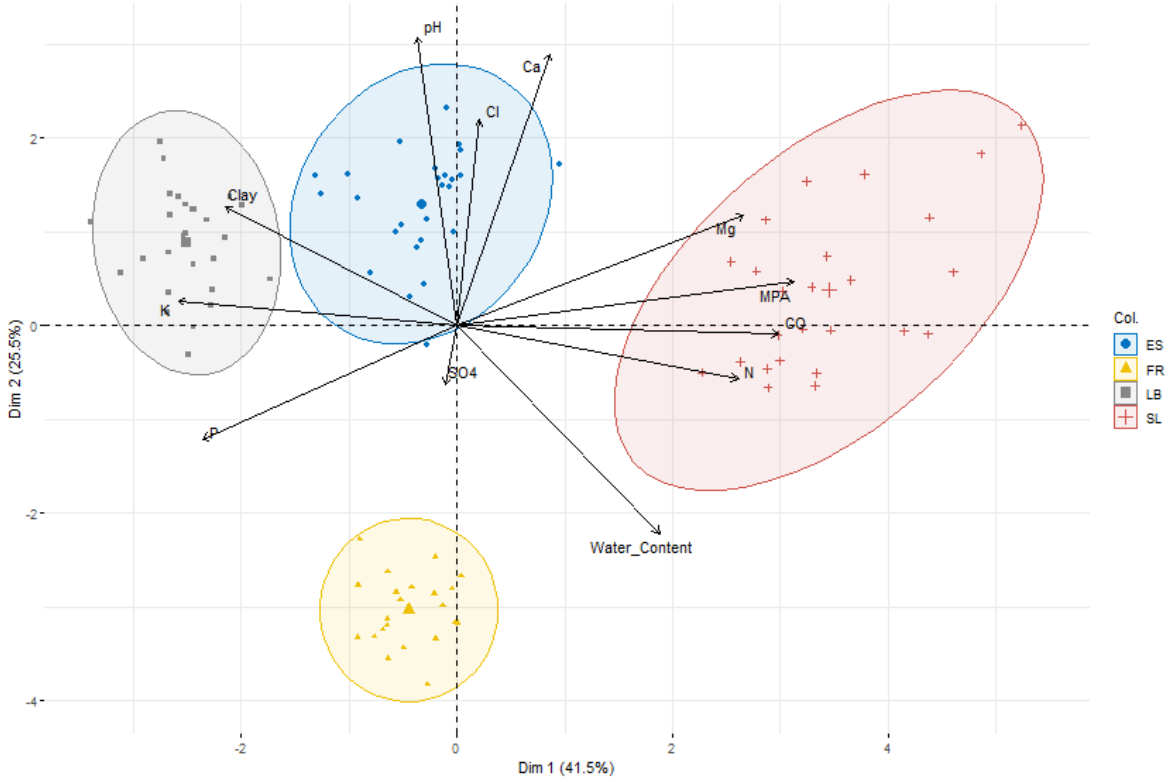


**Fig. 1**. A Principal Component Analysis (PCA) was performed to explore soil and climatic factors across the four study countries: Spain (ES), France (FR), Slovenia (SL), and Lebanon (LB). The biochemical variables analyzed included mean annual precipitation (MAP), pH, calcium (Ca, mg/kg), chloride (Cl, mg/kg), magnesium (Mg, mg/kg), sulfate ($SO_4$, mg/kg), available phosphorus (P Olsen, mg/kg), potassium (K, mg/kg), nitrogen (N, %), organic carbon (CO, %), water content, and clay content (%). Table S2 shows the correlations among variables that were statistically significant ($p \leq 0.05$).

Country positions in PCA space revealed distinct environmental profiles: Spain and Lebanon showed negative associations with Dim 1, France with Dim 2, and Slovenia displayed positive scores on Dim 1, indicating contrasting soil and climatic conditions. Dim 1, which accounts for the largest share of variance, highlights the differentiation between Slovenia and Lebanon. Slovenia is characterized by the highest mean annual precipitation (1,300 mm) and soil water content (21% ± 0.21), along with elevated levels of organic carbon (4.8% ± 0.05), nitrogen (0.3% ± 0.03), and soluble magnesium (58 mg/kg ± 6.12) (see Table S3). In contrast, Lebanon presents nearly opposite soil conditions, with a high clay content (59%, compared to approximately 30% at the other sites), and elevated phosphorus (51 mg/kg ± 2.59) and soluble potassium (11.7 mg/kg ± 0.45). Lebanon

also experiences lower precipitation (698 mm), which is approximately 46% less than in Slovenia, as well as reduced soil water content (10% ± 0.37) and carbon levels that are approximately five times lower (1.2% ± 0.06, Table S3).

France showed a mean soil moisture of 22% ± 0.55 and is distinguished by a lower pH (6) and generally reduced nutrient concentrations. Spain, by comparison, exhibits a more alkaline pH (8.2), with elevated levels of soluble calcium (57.7 mg/kg ± 2.43) and chloride (9.2 mg/kg ± 0.45) relative to the other countries (Table S3).

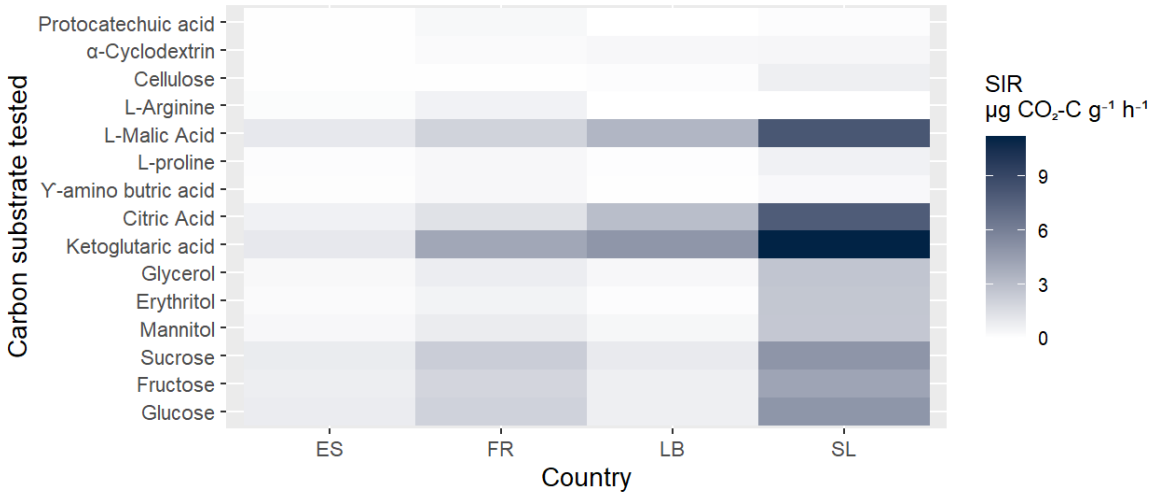


**Fig 2.** Heat map of the soil microbial catabolic profile by country, as determined by MicroResp™ assay. The 15 substrates tested are arranged in the graph according to their relative lability, from the most labile (glucose) to the most recalcitrant (protocatechuic acid). Color intensity reflects microbial activity for each substrate, with higher intensity indicating greater substrate induced respiration (SIR) (µg CO2-C g−1 h−1).

As mentioned previously, another important parameter for assessing soil quality is microbial activity. In particular, the MicroResp™ assay enabled us to study the catabolic ability of the soil microbial community. A higher catabolic capability indicates a broader capacity to metabolize diverse substrates, which in turn reflects a greater functional diversity within the microbial community.

Pedoclimatic differences played a crucial role in shaping soil microbial catabolic profiles across the studied countries (Fig. 2, $p < 0.0001$). Slovenia exhibited the highest microbial activity, as its communities decomposed larger quantities of each tested substrate and displayed broader catabolic abilities. For example, the consumption of the amino acid L-proline in Slovenian soils was approximately three times higher than the average consumption observed in the other soils. France

showed a catabolic profile similar to that of Slovenia, although overall microbial activity was lower ($p < 0.001$). Notably, French soils displayed higher catabolic activity in the decomposition of protocatechuic acid, the most recalcitrant substrate tested, characterized by its aromatic ring structure. In Lebanon, microbial activity was lower than in Slovenia ($p < 0.001$), but comparable to that in France. However, catabolic capacity remained relatively high, indicating that the microbial community was capable of metabolizing both simple and complex substrates. Spain, in contrast, displayed the lowest microbial activity. Its microbial catabolic profile was largely restricted to the use of sugars, carboxylic acids, and amino acids, with no ability to degrade more recalcitrant substrates. This indicated a reduced microbial functional diversity compared with the other countries ($p < 0.0001$).

*3.2 Assessing possible Soil Quality improvement through Diversity–Interaction Modelling*

Table 3 highlights the models that showed a statistically significant effect of sown plant diversity. In the general models, which assess effects across the entire study region, soil organic carbon (SOC) content was not significantly influenced by the diversity components of the treatment. Instead, the structural (null) model incorporating Mean Annual Precipitation (MAP) emerged as the most significant predictor (see Table S4). In contrast, site-specific models demonstrated that sown plant diversity had a meaningful impact within each country. Across all four countries, species identity consistently showed significant positive effects on soil organic carbon. Notably, G3 species identified as *Festuca arundina*cea in Slovenia, France, and Spain, and *Avena sativa* in Lebanon, were particularly influential in shaping soil organic carbon dynamics. Furthermore, in Spain, France, and Lebanon, the full model—including pairwise species interactions—had the lowest AIC, indicating that species interactions contributed uniquely to variation in organic carbon in these regions. However, these interactions had contrasting effects, sometimes offsetting one another or even resulting in negative net impacts. For example, in Spain, the interaction between *Lolium perenne* and *F. arundinacea* (G1:G3) was particularly notable, exhibiting a strongly negative effect (–8.5 ± 3.3). In contrast, G1 also showed a positive interaction with F2 (*Plantado lanceolata*), with a coefficient of 3.7 ± 1.6. In France, the combination G1:G3, produced an even stronger positive effect (11.42 ± 3.4), while the interaction between G1:F1 (*Dactylis glomerata* and *Cichorium* intybus) had a significant negative effect (–1.46 ± 0.62), as the interaction L2:F1 (*Trifolium repens* and *C. intybus)* (–7.8 ± 2.3). Conversely, the L2:F2 interaction (*T. repens* and *P.*

*lanceolata*) was both positive and significant (2.7 ± 0.8). In Lebanon, the interaction between F1:F2 (*C. intybus* and *P. lanceolata*) also showed a significant negative effect (–7.9 ± 3.2), further illustrating the divergent and site-specific nature of species interactions across the region (see Table 3 and Table S4). In Slovenia, the model that best explained the response of soil organic carbon was the functional group (FG) model, suggesting that interactions operated primarily at the functional group level. In this case, the interaction among the three forb species produced a distinct group-level effect, resulting in a significant increase in soil organic carbon (7.32 ± 2.83, $p < 0.05$; see Table S4).

On the other hand, the catabolic profile of soils also responded to the polyculture treatment. Specifically, for amino acids and polymers, the average interaction (AV) models provided the best fit. This model assumes that species interact with one another and that the strength of these interactions is uniform across all species pairs. The AV interaction coefficient reflects the mean effect of species proportions in the community, effectively capturing the influence of evenness. For amino acid degradation, a significant effect was observed only in Slovenia. In this case, most species (except G3) exhibited positive and significant identity effects, and evenness also had a significant positive effect (8.28 ± 3.4). In contrast, polymer degradation showed a significant evenness effect in the general analysis across all regions. All species contributed positively and significantly to identity effects, with grass species G3 again showing the strongest effect (13.36 ± 5.68) and a negative evenness effect marginally significant ( -2.7 ± 1.4, p=0.07) (Table S4).
Multiple substrate-induced respiration (MSIR)—defined as the sum of net substrate consumption (μg $CO_2$-C/g/hour)—reflects the overall functional capacity of the soil microbial community, i.e., its metabolic activity. The general analysis revealed an effect of forage mixtures, with the average interaction (AV) model providing the best fit. Within this framework, only the forb F2 (*P. lanceolata*) exhibited a significant positive effect across all countries (7.2 ± 3.6; see Table S4). At the country level, Slovenia was the only site where forage mixtures significantly influenced soil respiration, as indicated by the additive species (ADD) model. This model assumes that each species contributes an individual identity effect, and that interactions are additive rather than synergistic. In this context, the legumes *Medicago sativa* and *Onobrychis viciifolia*, along with the forbs *C. intybus* and *P. lanceolat*a, exhibited significant positive identity effects. However, *P. lanceolata* also showed a unique and strongly negative interaction effect (–90.56 ± 34.83) (Table S4).

**Tabla 3.** Significant models derived from the diversity–interaction modelling analysis on several dependent variables: MicroResp substrate activity (amino acids and polymers), organic carbon (CO), multiple substrate-induced respiration (MSIR), microbial biomass carbon (MBC), and the metabolic quotient ($qCO_2$). Two types of analyses were conducted: (1) a general analysis using mean annual precipitation (MAP) as the structural variable across four countries: Spain (ES), France (FR), Lebanon (LB), and Slovenia (SL); and (2) country-specific analyses using harvest time (*T1* and *T2*) as the structural variable. Species identities (*ID*) were incorporated into the models, including grasses (*G1, G2, G3*), legumes (*L1, L2, L3*), and non-legume forbs (*F1, F2, F3*), along with their interactions. The term *AV* refers to the averaged effect of evenness (E), while *wfg* and *bfg* represent within-functional group and between-functional group interactions, respectively. The term *add* denotes the additive contribution of each species when interacting with others. Statistically significant diversity-related terms are marked in bold with an asterisk (*). For detailed model coefficients, refer to Table S4.

| Dependent variable | Analysis | Fit model |
|---|---|---|
| $C_{Org}$ | ES | β + **ID*** + G1:G2 + **G1:G3*** + G1:L1 + G1:L2 + G1:F1 + **G1:F2*** + G2:L1 + G2:L2 + G2:F1 + G2:F2 + L1:L2 + L1:F1 + L1:F2 + L2:F1 + L2:F2 + F1:F2 + HarvestT1 + ε |
| | FR | β + **ID*** + G1:G2 + **G1:G3*** + G1:L1 + G1:L2 + **G1:F1*** + G1:F2 + G2:L1 + G2:L2 + G2:F1 + G2:F2 + L1:L2 + **L1:F1*** + L1:F2 + **L2:F1*** + **L2:F2*** + F1:F2 + HarvestT1 + ε |
| | LB | β +**ID*** + G1:G2 + G1:G3 + G1:L1 + G1:L2 + G1:F1 + G1:F2 + G2:L1 + G2:L2 + G2:F1 + G2:F2 + L1:L2 + L1:F1 + L1:F2 + L2:F1 + L2:F2 + **F1:F2*** + **HarvestT1*** + ε |
| | SL | β + **ID*** + **wfg_F*** + wfg_G + wfg_L + bfg_F_G + bfg_F_L + bfg_G_L + **HarvestT1*** + ε |
| AminoAcids | SL | β + **G1*** + **G2*** + G3 + **L1*** + **L2*** + **F1*** + **F2*** + **AV*** + **Harvest*** + ε |
| Polymers | General | β + **ID*** + AV + MPA+ ε |
| MSIR | General | β + G1 + G2 + G3 + L1 + L2 + F1 + **F2*** + AV + **MPA***+ ε |
| | SL | β + G1 + G2 + G3 + **L1*** + **L2*** + **F1*** + **F2*** + G1_add + G2_add + L1_add + L2_add + F1_add + **F2_add*** + **Harvest*** + ε |
| MBC | LB | β +**G1*** + G2 + G3 + L1 + L2 + **F1*** + **F2*** + G1:G2 + G1:G3 + G1:L1 + G1:L2 + G1:F1 + **G1:F2*** + G2:L1 + G2:L2 + G2:F1 + G2:F2 + L1:L2 + L1:F1 + L1:F2 + L2:F1 + L2:F2 + **F1:F2*** + **HarvestT1*** + ε |
| | SL | β + **G1*** + **G2*** + G3 + **L1*** + **L2*** + **F1*** + **F2*** + **Harvest*** + ε |
| qCO2 | ES | β + **G1*** + G2 + G3 + L1 + L2 + F1 + F2 + **G1_add*** + G2_add + L1_add + L2_add + F1_add + F2_add + HarvestT1 + ε |

The best-fitting model for microbial biomass carbon (MBC) in Lebanon was the FULL model. Within this framework, G1 (*L. rigidum*), F1 (*C. intybus*), and F2 (*P. lanceolata*) exhibited significant positive identity effects of 40.35 ± 15.1, 51.88 ± 15.53, and 47.38 ± 15.56, respectively.

Two significant interaction effects were also detected: G1:F2 showed a strong positive interaction (995.79 ± 388.6), while F1:F2 exhibited a strong negative interaction (–1132.72 ± 382.72) (see Table S4). In contrast, the best-fitting model for Slovenia was the identity (ID) model, which assumes species identity effects without interactions. In this case, all species showed significant positive effects except for G3 (see Table 3).

The microbial biomass metabolic quotient ($qCO_2$), which represents the amount of $CO_2$ released by microbial activity relative to the microbial biomass carbon, serves as an indicator of microbial metabolic efficiency—lower values suggest greater efficiency. The only analysis showing a significant identity effect was conducted in Spain, where the additive species (ADD) model identified G1 (*L. perenne*) as the only species with a significant positive identity effect (175.04 ± 27.31), alongside a very strong negative interaction effect (–315.05 ± 108.16) (see Table S4).

For the remaining variables analyzed, the null model provided the best fit, suggesting that diversity components did not significantly influence their variation. Instead, environmental variables were the primary drivers of the observed variation.

*3.3 Components of Sward diversity that enhance soil organic Carbon*

In Figure 3A, carbon accumulation is shown as a function of evenness in Slovenia, with the highest predicted value occurring at an evenness level of 0.54. This corresponds to a diversity–dominance scenario involving four species proportions: *L. perenne* (10%), D. *glomerata* (10%), *C. intybus* (10%), and *P. lanceolata* (70%). This polyculture configuration outperformed all monoculture scenarios in terms of carbon accumulation. In Spain (Figure 3B), the highest carbon accumulation was associated with centroid diversity, where six species contributed equally (16.7% each): *D. glomerata*, *F. arundinacea*, *O. viciifolia*, *Trifolium pratense*, *P. lanceolata*, and *Achillea millefolium*. Similarly, in France, the highest predicted carbon accumulation also occurred under co-dominan diversity, with four species and an evenness of 0.74, including *D. glomerata* (10%), *T. repens* (40%), *C. intybus* (10%), and *P. lanceolata* (40%) (Figure S3A). In Lebanon, the highest carbon accumulation was linked to a dominant diversity scenario at an evenness level of 0.54, involving six species: *L.rigidum* (70%), *Hordeum vulgare* (10%), *C. intybus* (10%), and *P. lanceolata* (10%) (Figure S3B). Overall, polycultures with moderate species evenness (0.54–0.74) were optimal for enhancing soil carbon, consistently outperforming monocultures, especially when combining grasses, legumes, and forbs. Dominant species such as *P. lanceolata* and *L. rigidum*

played a crucial role in specific scenarios, while centroid diversity proved particularly effective in Spain and France. The species most frequently associated with high carbon accumulation included grasses like *D. glomerata*, *Lolium perenne*, *F. arundinacea*, *L. rigidum*, and *H. vulgare*; legumes such as *T. pratense*, *T. repens*, and *O. viciifolia*; and forbs including *P. lanceolata*, *C. intybus*, and *A. millefolium*.

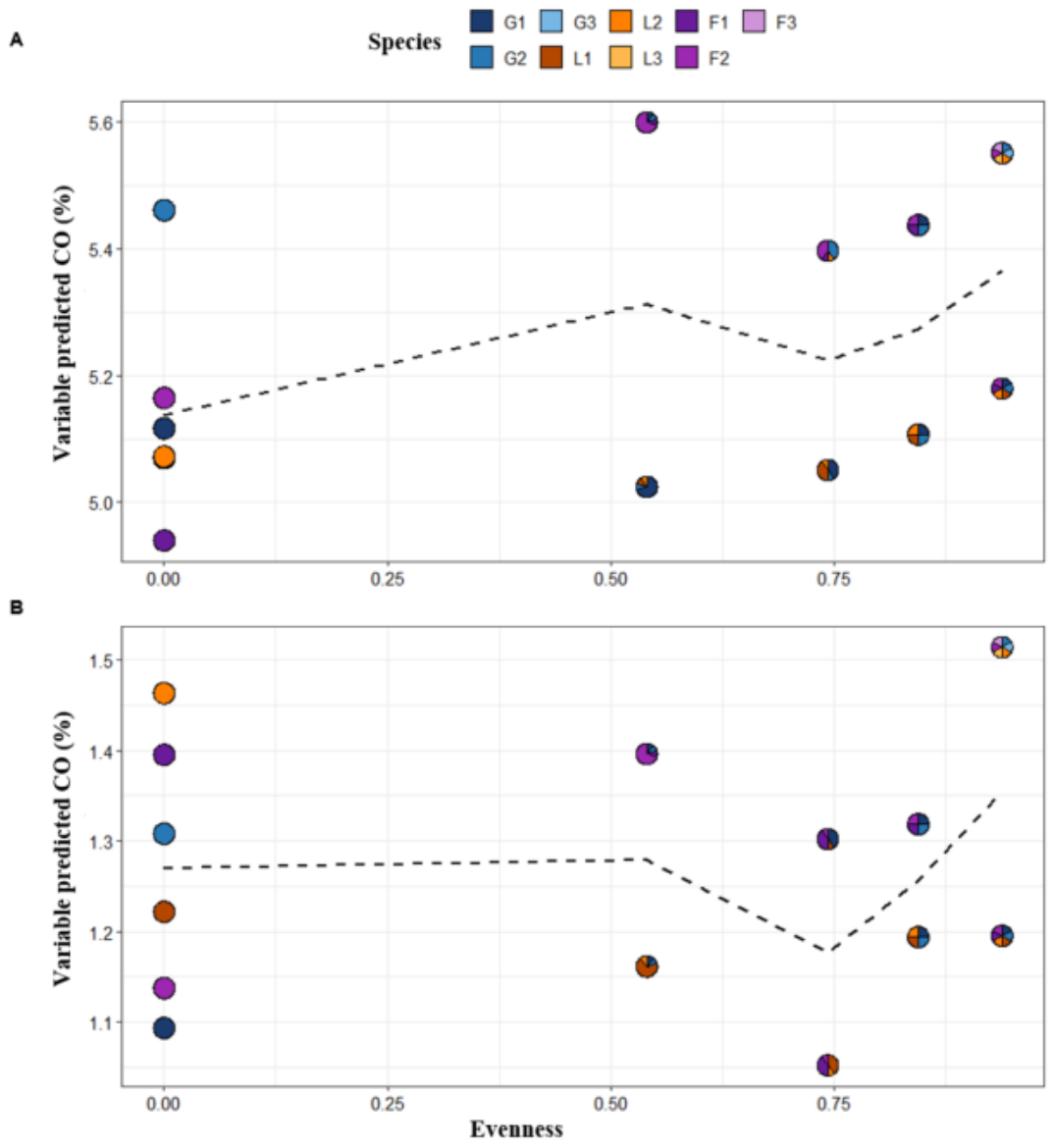


**Fig 3.** Predicted values of organic carbon in relation to species evenness in Slovenia (A) and Spain (B), based on Diversity–Interaction modelling. Each point represents a specific species mixture or functional group (e.g., G1–G3 for grasses, F1–F3 for forbs, L1–L3 for legumes). The x-axis indicates species evenness, ranging from low to high, while the y-axis shows the predicted organic carbon response. The charts illustrate the maximum and minimum predicted values across the evenness gradient evenness, the value of 0, represent predicted carbon values for monocultures of each studied species.

## 4. Discussion

### *4.1 Characterizing Environmental Gradients in Soil Chemical Properties and Functional Diversity Profiles Across the Studied Region*

Our results revealed distinct differences in soil chemical properties and microbial catabolic profiles among the four study countries. Mean Annual Precipitation (MAP) and soil organic carbon emerged as the main drivers of this variation. Among sites, Slovenia demonstrated the most favorable conditions for supporting complex soil dynamics.

Microbial catabolic activity further reinforced this pattern, revealing a marked contrast between Slovenia and the other countries and highlighting its microbial functional potential—even during the severe drought affecting some of the regions studied. This heightened activity was associated with greater microbial biomass and broad carbon source utilization, indicative of a more diverse soil microbiome (Campbell et al., 2003; Creamer et al., 2009) (Figure 2).

Previous studies support this relationship, showing that increased soil organic matter enhances microbial biomass and diversity, thereby promoting the degradation of complex substrates such as lignin and aromatic compounds (Bongiorno et al., 2020; Creamer et al., 2016). These findings align with the broader understanding that decomposition of diverse organic substrates is mediated by distinct microbial communities, particularly those capable of producing specialized enzymes (Khatoon et al., 2017). Importantly, the assembly and functional complexity of such communities depend on environmental stability, which shapes both their resistance to disturbance and resilience of function (Bongiorno et al., 2019; Graham & Knelman, 2023).

France also experienced climatic anomalies in 2022, with an average precipitation decline of –45.4%, however, changes in mean annual temperature were minimal (0.1 °C; Table 1). This suggests that, possibly due to the limited temperature change, soil moisture remained relatively stable during the experiment, likely because of reduced evapotranspiration. Consistently with this, microbial activity in France was higher than in Spain and Lebanon (Fig. 2). Ren et al. (2017) similarly observed that microbial biomass and activity can be sustained when soil moisture is preserved, even under altered precipitation regimes. In contrast, Spain showed strong negative correlations between microbial activity, soil carbon, and soil water content, reflecting the detrimental effects of prolonged drought.

Lebanon presented a distinct case, with negative correlations between microbial activity and key environmental parameters. According to Ren et al. (2017), strict conditions—such as limited water availability—typically lead to declines in both microbial biomass and activity. Despite having the lowest MAP among the study countries, Lebanon was less affected by precipitation anomalies, suggesting that its microbial communities did not experience widespread mortality but instead persisted under relatively stable conditions. This aligns with Ren et al. (2017), who reported that microbial activity can be maintained under stress when environmental stability is preserved.

Overall, our findings emphasize that soil moisture, pH, and organic matter—rather than vegetation or climate alone—are key determinants of microbial functional potential in Mediterranean agroecosystems. They also challenge the assumption that drought-adapted communities are uniformly resilient, underscoring the reciprocal links between soil properties and microbial activity in shaping rhizosphere dynamics (Creamer et al., 2016; Nielsen et al., 2010).

*4.2 Sown species diversity as drivers of soil fertility.*

Diversity–Interaction (DI) models showed that soil variables were primarily shaped by environmental conditions. Nonetheless, across all four countries, sown species diversity consistently influenced soil carbon content. Regarding species identity effects, *Festuca arundinacea* emerged as the most influential species in Spain, Slovenia, and France, while *Avena sativa* played a central role in Lebanon. These findings are consistent with previous research, which identifies *F. arundinacea* for its high carbon capture potential through photosynthesis and its robust root system that promotes soil aggregation and stabilizes organic matter (Handayani et al., 2011). Certain varieties of *F. arundinacea* can further enhance microbial biomass carbon and micro-aggregate formation, contributing to long-term carbon sequestration. Similarly, *A. sativa* improves soil structure and nutrient retention through its fibrous root system and high biomass production, which supports microbial activity and organic matter input (Kumar et al., 2023). Furthermore, our results indicate that both species had strong positive effects on polymer degradation, highlighting the underlying mechanisms through which they contribute to soil carbon accumulation.

Other grasses also exhibited notable species identity effects in various models. *Lolium perenne* and *Lolium rigidum* (in Lebanon) showed consistent contributions across most carbon dynamics models, except for MSIR. Both species are recognized for their adaptability to diverse soil conditions and their role in erosion control and organic matter input, facilitated by their dense root

systems and high turnover rates (Esmaeili et al., 2025). Similarly, *Dactylis glomerata*, characterized by its deep-rooted morphology (Xu et al., 2025), contributed to a more active catabolic profile. Our results align with those of Béraud et al. (2023), who found that *Dactylis glomerata* supports a diverse and functionally active microbial community. Additionally, identity effects from forbs—primarily *Plantago lanceolata*—also contributed to enhanced microbial activity. These findings are consistent with previous research indicating that *P. lanceolata* is known for its high rapid organic matter cycling, due to its ability to generate biomass that quickly returns to the soil, enhancing nutrient turnover under moderate drought conditions, particularly under intensive grazing regimes (Cranston, 2014).

On the other hand, the inclusion of pairwise interaction terms and the averaged interaction effect (AV) further supported the influence of species composition—particularly evenness and functional group balance—on soil carbon dynamics. Notably, in Slovenia, within-functional group (WFG) interactions among forbs were especially relevant. Moreover, specific species interactions revealed additional context-dependent effects. For example, *Lolium perenne* exhibited a significant negative interaction in Spain's $qCO_2$ analysis when combined with other species, suggesting improved microbial carbon use efficiency and potential for increased carbon storage. Similarly, *Plantago lanceolata* in Slovenia exhibited additive negative effects when interacting with other species across multiple induced respiration responses. This suggests that, in general, *Plantago lanceolata*—and forbs more broadly, in contrast to grasses—exerts a facilitative influence in mixed plant communities, although primarily under non-extreme environmental conditions. Its traits appear to modulate microbial dynamics in ways that slow decomposition and potentially promote carbon stabilization, highlighting its key role in shaping belowground microbial processes. However, this role may be site-dependent, as evidenced by the negative interactions observed between *P. lanceolata* and *Cichorium intybus* in Lebanon, where carbon accumulation was reduced.

Consistent with previous studies (e.g., Skinner & Dell, 2016), our results revealed that grasses tended to behave more competitively, exerting strong negative effects both within their own functional group and across others. Notably, Skinner & Dell (2016) found that *Festuca arundinacea* was dominant in both two-species and five-species mixtures, with higher soil carbon levels observed in the more diverse assemblages. This outcome may reflect shifts in species'

performance depending on environmental conditions, suggesting that competitive or facilitative behaviors can be site-dependent. This pattern is further supported by McElroy et al. (2017) , who observed a similar trend: under moderate environmental conditions, species interactions remained stable and generally positive. However, when nitrogen levels increased excessively—shifting conditions toward environmental extremes—these interactions became negative. These findings highlight the site-dependence of plant–soil feedback, where microbial interactions can shift from mutualistic to antagonistic along environmental gradients (Bever et al., 2010; van der Putten et al., 2013).

Interestingly, some of our species' combinations demonstrated resilience under contrasting conditions. For example, *Lolium perenne* maintained positive interactions with *Onobrychis viciifolia* both in Lebanon (under non-stressful conditions) and in Spain (under stressful conditions), suggesting that certain plant pairings can sustain beneficial relationships despite environmental constraints.

Overall, these results highlight the identity effects of grasses and forbs over those of legumes. However, when interacting under certain limitations or extreme conditions, competition increases, and—as previously demonstrated by other authors—interactions among these species can become highly negative. Therefore, the nuanced effects of species interactions—both positive and negative—demonstrate that functional traits and ecological compatibility are essential considerations in designing plant mixtures for soil carbon enhancement. For example, Rodriguez et al, (2022) have showed that moderate legume presence can increase SOC, whereas excessive legume abundance may reduce it, partly due to the balance between the recalcitrance and the lability of the organic matter they contribute.

Our findings underscore that species combinations—not merely species richness—play a critical role in shaping microbial responses and soil carbon outcomes. The interactions between species, whether within the same functional group or across different ones, highlight the complexity of biodiversity effects. These effects are not uniformly positive but depend on the nature of species interactions and the surrounding environmental context. This aligns with results from long-term grassland experiments, which have shown that plant diversity enhances soil carbon storage primarily through increased biomass production and nutrient retention. However, the influence of functional group composition and species interactions can vary significantly depending on

environmental conditions (Cong et al., 2014; Lange et al., 2015). Our findings support these conclusions and further emphasize the importance of soil microbial mechanisms as key drivers of ecosystem functioning.

## 5. Conclusions

Our study demonstrated that environmental conditions modulate microbial catabolic capacity and can amplify the positive effects of forage mixture diversity. This site-dependence reflects broader ecological patterns, where plant–soil feedback and microbial interactions shift in response to environmental gradients (Bever et al., 2010; van der Putten et al., 2013). For example, certain species combinations yielded synergistic effects on microbial biomass and carbon accumulation, while others exhibited antagonistic interactions, underscoring the importance of functional compatibility and trait complementarity.

Therefore, it is suggested that future studies should focus on the effects of species interactions, which demonstrate strong performance under adverse environments or potential climate change scenarios. The aim would be to identify an appropriate species proportion that allows them to express their functional attributes while, at the same time, minimizing negative effects on the response variable under competitive conditions. This approach could ultimately contribute to improving long-term soil quality and resilience.

## References


Ali, E., Cramer, W., Carnicer, J., Georgopoulou, E., Hilmi, N. J. M., Le Cozannet, G., & Lionello, P. (2022). Mediterranean Region. In *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 2233–2272). Cambridge University Press.

Altieri, M. A., Nicholls, C. I., Henao, A., & Lana, M. A. (2015). Agroecology and the design of climate change-resilient farming systems. *Agronomy for Sustainable Development*, *35*(3), 869–890. https://doi.org/10.1007/s13593-015-0285-2

Anderson, J. P. E., & Domsch, K. H. (1978). A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, *10*(3), 215–221. https://doi.org/10.1016/0038-0717(78)90099-8

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, *26*(1), 32–46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Baggs, E. M. (2011). Soil microbial sources of nitrous oxide: Recent advances in knowledge, emerging challenges and future direction. *Current Opinion in Environmental Sustainability*, *3*(5), 321–327. https://doi.org/10.1016/j.cosust.2011.08.011

Béraud, C., Cantarel, A., Gervaix, J., Creuzé des Châtelliers, C., Delort, A., Boisselet, C., Poussineau, S., Lacroix, E., & Piola, F. (2023). *Impact of Two Acquisitive Plants on N-Cycle on a Large Range of Soils: The Invasive Knotweed Does it and so Does the Native Cocksfoot!* (SSRN Scholarly Paper No. 4673023). Social Science Research Network. https://doi.org/10.2139/ssrn.4673023

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., Rillig, M. C., Stock, W. D., Tibbett, M., & Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, *25*(8), 468–478. https://doi.org/10.1016/j.tree.2010.05.004

Bezner Kerr, R., Hasegawa, T., Lasco, R., Bhatt, I., Deryng, D., Farrell, A., Gurney-Smith, H., Ju, H., Lluch-Cota, S., Meza, F., Nelson, G., Neufeldt, H., & Thornton, P. (2022). Food, fibre, and other ecosystem products. In H.-O. Pörtner, D. C. Roberts, M. M. B. Tignor, E. S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, & B. Rama (Eds), *Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Bongiorno, G., Bünemann, E. K., Brussaard, L., Mäder, P., Oguejiofor, C. U., & de Goede, R. G. M. (2020, October). Soil management intensiity shifts microbial catabolic profiles across a range of European long-term field experiments. *Elsevier B.V*, *154*, 13.

Bongiorno, G., Bünemann, E. K., Oguejiofor, C. U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., & de Goede, R. (2019). Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. *Ecological Indicators*, *99*, 38–50. https://doi.org/10.1016/j.ecolind.2018.12.008

Campbell, C. D., Chapman, S. J., Cameron, C. M., Davidson, M. S., & Potts, J. M. (2003). A Rapid Microtiter Plate Method To Measure Carbon Dioxide Evolved from Carbon Substrate

Amendments so as To Determine the Physiological Profiles of Soil Microbial Communities by Using Whole Soil. *Applied and Environmental Microbiology*, *69*(6), 3593–3599. https://doi.org/10.1128/AEM.69.6.3593-3599.2003

Cong, W.-F., van Ruijven, J., Mommer, L., De Deyn, G. B., Berendse, F., & Hoffland, E. (2014). Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *Journal of Ecology*, *102*(5), 1163–1170. https://doi.org/10.1111/1365-2745.12280

Connolly, J., Bell, T., Bolger, T., Brophy, C., Carnus, T., Finn, J. A., Kirwan, L., Isbell, F., Levine, J., Lüscher, A., Picasso, V., Roscher, C., Sebastia, M. T., Suter, M., & Weigelt, A. (2013). An improved model to predict the effects of changing biodiversity levels on ecosystem function. *Journal of Ecology*, *101*(2), 344–355. https://doi.org/10.1111/1365-2745.12052

Cranston, L. M. (2014). *Chicory (Cichorium intybus) and plantain (Plantago lanceolata); physiological and morphological responses to water stress, defoliation, and grazing preference with implications for the management of the Herb and Legume Mix: A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science, Massey University, Te Kunenga ki Pūrehuroa, New Zealand* [Massey University]. http://hdl.handle.net/10179/6002

Creamer, R. E., Bellamy, P., Black, H. I. J., Cameron, C. M., Campbell, C. D., Chamberlain, P., Harris, J., Parekh, N., Pawlett, M., Poskitt, J., Stone, D., & Ritz, K. (2009). An inter-laboratory comparison of multi-enzyme and multiple substrate-induced respiration assays to assess method consistency in soil monitoring. *Biology and Fertility of Soils*, *45*(6), 623–633. https://doi.org/10.1007/s00374-009-0374-y

Creamer, R. E., Stone, D., Berry, P., & Kuiper, I. (2016). Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. *Applied Soil Ecology*, *97*, 36–43. https://doi.org/10.1016/j.apsoil.2015.08.004

del Pozo, A., Brunel-Saldias, N., Engler, A., Ortega-Farias, S., Acevedo-Opazo, C., Lobos, G. A., Jara-Rojas, R., & Molina-Montenegro, M. A. (2019). Climate Change Impacts and Adaptation Strategies of Agriculture in Mediterranean-Climate Regions (MCRs). *Sustainability*, *11*(10), Article 10. https://doi.org/10.3390/su11102769

Devi, S., & Soni, R. (2020). Relevance of Microbial Diversity in Implicating Soil Restoration and Health Management. In R. S. Meena (Ed.), *Soil Health Restoration and Management* (pp. 161–202). Springer. https://doi.org/10.1007/978-981-13-8570-4_5

*DI Models*. (2024). https://dimodels.com/index.php

Dono, G., Cortignani, R., Dell'Unto, D., Deligios, P., Doro, L., Lacetera, N., Mula, L., Pasqui, M., Quaresima, S., Vitali, A., & Roggero, P. P. (2016). Winners and losers from climate change in agriculture: Insights from a case study in the Mediterranean basin. *Agricultural Systems*, *147*, 65–75. https://doi.org/10.1016/j.agsy.2016.05.013

Draper, N. R., & Smith, H. (1998). *Applied Regression Analysis*. John Wiley & Sons.

Eisenhauer, N., Milcu, A., Sabais, A. C. W., Bessler, H., Brenner, J., Engels, C., Klarner, B., Maraun, M., Partsch, S., Roscher, C., Schonert, F., Temperton, V. M., Thomisch, K., Weigelt, A., Weisser, W. W., & Scheu, S. (2011). Plant Diversity Surpasses Plant Functional Groups and Plant Productivity as Driver of Soil Biota in the Long Term. *PLOS ONE*, *6*(1), e16055. https://doi.org/10.1371/journal.pone.0016055

Esmaeili, S., Salehi, H., Khosh-Khui, M., Fallah Shamsi, S. R., Mikaeili Tabriz, A. R., & Xiang, M. (2025). Habitat Suitability Mapping on Significant Differences Between Lolium perenne and L. rigidum. *International Journal of Horticultural Science and Technology*, *12*(2), 439–452. https://doi.org/10.22059/ijhst.2024.379984.893

Finn, J. A., Kirwan, L., Connolly, J., Sebastià, M. T., Helgadottir, A., Baadshaug, O. H., Bélanger, G., Black, A., Brophy, C., Collins, R. P., Čop, J., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Ghesquiere, A., Golinska, B., Golinski, P., … Lüscher, A. (2013). Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: A 3-year continental-scale field experiment. *Journal of Applied Ecology*, *50*(2), 365–375. https://doi.org/10.1111/1365-2664.12041

Fornara, D. A., & Tilman, D. (2008). Plant functional composition influences rates of soil carbon and nitrogen accumulation. *Journal of Ecology*, *96*(2), 314–322. https://doi.org/10.1111/j.1365-2745.2007.01345.x

Frison, E. A., Cherfas, J., & Hodgkin, T. (2011). Agricultural Biodiversity Is Essential for a Sustainable Improvement in Food and Nutrition Security. *Sustainability*, *3*(1), Article 1. https://doi.org/10.3390/su3010238

Graham, E. B., & Knelman, J. E. (2023). Implications of Soil Microbial Community Assembly for Ecosystem Restoration: Patterns, Process, and Potential. *Microbial Ecology*, *85*(3), 809–819. https://doi.org/10.1007/s00248-022-02155-w

Handayani, I. P., Coyne, M. S., & Phillips, T. D. (2011). Soil organic carbon fractions differ in two contrasting tall fescue systems. *Plant and Soil*, *338*(1), 43–50. https://doi.org/10.1007/s11104-010-0352-z

Hector, A., Schmid, B., Beierkuhnlein, C., Caldeira, M. C., Diemer, M., Dimitrakopoulos, P. G., Finn, J. A., Freitas, H., Giller, P. S., Good, J., Harris, R., Högberg, P., Huss-Danell, K., Joshi, J., Jumpponen, A., Körner, C., Leadley, P. W., Loreau, M., Minns, A., … Lawton, J. H. (1999). Plant Diversity and Productivity Experiments in European Grasslands. *Science*, *286*(5442), 1123–1127. https://doi.org/10.1126/science.286.5442.1123

Hidalgo-Galvez, M. D., Matías, L., Cambrollé, J., Gutiérrez, E., & Pérez-Ramos, I. M. (2023). Impact of climate change on pasture quality in Mediterranean dehesas subjected to different grazing histories. *Plant and Soil*, *488*(1), 465–483. https://doi.org/10.1007/s11104-023-05986-9

Khatoon, H., Solanki, P., Narayan, M., Tewari, L., & Rai, J. (2017). Role of microbes in organic carbon decomposition and maintenance of soil ecosystem. *International Journal of Chemical Studies*, *5*(6), 1648–1656.

Kirwan, L., Connolly, J., Finn, J. A., Brophy, C., Lüscher, A., Nyfeler, D., & Sebastià, M.-T. (2009). Diversity–interaction modeling: Estimating contributions of species identities and interactions to ecosystem function. *Ecology*, *90*(8), 2032–2038. https://doi.org/10.1890/08-1684.1

Kirwan, L., Lüscher, A., Sebastià, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., Helgadottir, A., Baadshaug, O. H., Brophy, C., Coran, C., Dalmannsdóttir, S., Delgado, I., Elgersma, A., Fothergill, M., Frankow-Lindberg, B. E., Golinski, P., Grieu, P., Gustavsson, A. M., Höglind, M., … Connolly, J. (2007). Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology*, *95*(3), 530–539. https://doi.org/10.1111/j.1365-2745.2007.01225.x

Kleber, M. (2010). What is recalcitrant soil organic matter? *Environmental Chemistry*, *7*(4), 320–332. https://doi.org/10.1071/EN10006

Kumar, P. R., Meenakshi, S., Shalini, S., Devi, S. R., & Boopathi, S. (2023). Soil Quality Prediction in Context Learning Approaches Using Deep Learning and Blockchain for Smart Agriculture. In *Effective AI, Blockchain, and E-Governance Applications for Knowledge*

*Discovery and Management* (pp. 1–26). IGI Global Scientific Publishing. https://doi.org/10.4018/978-1-6684-9151-5.ch001

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., Mellado-Vázquez, P. G., Malik, A. A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B. C., Trumbore, S. E., & Gleixner, G. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications*, *6*(1), 6707. https://doi.org/10.1038/ncomms7707

Llovet, A., Llurba, R., Aljazairi, S., Mattana, S., Plaixats, J., Nogués, S., Sebastià, M. T., & Ribas, A. (2024). Nitrogen facilitation was maintained in sown Mediterranean forage mixtures despite drought stress conditions with concurrent general benefits upon plant aboveground water status and yield. *Agriculture, Ecosystems & Environment*, *375*, 109187. https://doi.org/10.1016/j.agee.2024.109187

Loranger-Merciris, G., Barthes, L., Gastine, A., & Leadley, P. (2006). Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. *Soil Biology and Biochemistry*, *38*(8), 2336–2343. https://doi.org/10.1016/j.soilbio.2006.02.009

Martinez Arbizu, P. (2020). *pairwiseAdonis: Pairwise multilevel comparison using adonis* (Version 0.4,1) [R]. https://github.com/pmartinezarbizu/pairwiseAdonis

McElroy, M. S., Papadopoulos, Y. A., Glover, K. E., Dong, Z., Fillmore, S. A. E., & Johnston, M. O. (2017). Interactions between cultivars of legume species (Trifolium pratense L., Medicago sativa L.) and grasses (Phleum pratense L., Lolium perenne L.) under different nitrogen levels. *Canadian Journal of Plant Science*, *97*(2), 214–225. https://doi.org/10.1139/cjps-2016-0130

Molénat, J., Barkaoui, K., Benyoussef, S., Mekki, I., Zitouna, R., & Jacob, F. (2023). Diversification from field to landscape to adapt Mediterranean rainfed agriculture to water scarcity in climate change context. *Current Opinion in Environmental Sustainability*, *65*, 101336. https://doi.org/10.1016/j.cosust.2023.101336

Nielsen, U. N., Osler, G. H. R., Campbell, C. D., Burslem, D. F. R. P., & van der Wal, R. (2010). The influence of vegetation type, soil properties and precipitation on the composition of soil mite and microbial communities at the landscape scale. *Journal of Biogeography*, *37*(7), 1317–1328. https://doi.org/10.1111/j.1365-2699.2010.02281.x

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., & Lüscher, A. (2011). Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems & Environment*, *140*(1), 155–163. https://doi.org/10.1016/j.agee.2010.11.022

Oelmann, Y., Kreutziger, Y., Bol, R., & Wilcke, W. (2007). Nitrate leaching in soil: Tracing the NO3− sources with the help of stable N and O isotopes. *Soil Biology and Biochemistry*, *39*(12), 3024–3033. https://doi.org/10.1016/j.soilbio.2007.05.036

Olsen, S., & Sommers, L. (1982). Methods of Soil Analysis; Chemical and Microbiological Properties. Part 2. In *Methods of Soil Analysis; Chemical and Microbiological Properties. Part 2* (Page, A.L. | Miller, R.H. | Keeney, D.R., pp. 403–430). American Society of Agronomy / Soil Science Society of America.

Orwin, K. H., Wardle, D. A., & Greenfield, L. G. (2006). Ecological Consequences of Carbon Substrate Identity and Diversity in a Laboratory Study. *Ecology*, *87*(3), 580–593. https://doi.org/10.1890/05-0383

R Core Team. (2024). *RStudio (version 4.4.1)* (Version 2024.04.2 Build 764) [R]. Posit Software, PBC. https://posit.co/download/rstudio/

Rodríguez, A., Canals, R. M., & Sebastià, M.-T. (2022). Positive Effects of Legumes on Soil Organic Carbon Stocks Disappear at High Legume Proportions Across Natural Grasslands in the Pyrenees. *Ecosystems*, *25*(4), 960–975. https://doi.org/10.1007/s10021-021-00695-9

Semeraro, T., Scarano, A., Leggieri, A., Calisi, A., & De Caroli, M. (2023). Impact of Climate Change on Agroecosystems and Potential Adaptation Strategies. *Land*, *12*(6), Article 6. https://doi.org/10.3390/land12061117

Skinner, R. H., & Dell, C. J. (2016). Yield and Soil Carbon Sequestration in Grazed Pastures Sown with Two or Five Forage Species. *Crop Science*, *56*(4), 2035–2044. https://doi.org/10.2135/cropsci2015.11.0711

Steinauer, K., Chatzinotas, A., & Eisenhauer, N. (2016). Root exudate cocktails: The link between plant diversity and soil microorganisms? *Ecology and Evolution*, *6*(20), 7387–7396. https://doi.org/10.1002/ece3.2454

Steinbeiss, S., BEßLER, H., Engels, C., Temperton, V. M., Buchmann, N., Roscher, C., Kreutziger, Y., Baade, J., Habekost, M., & Gleixner, G. (2008). Plant diversity positively affects short-

term soil carbon storage in experimental grasslands. *Global Change Biology*, *14*(12), 2937–2949. https://doi.org/10.1111/j.1365-2486.2008.01697.x

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van de Voorde, T. F. J., & Wardle, D. A. (2013). Plant–soil feedbacks: The past, the present and future challenges. *Journal of Ecology*, *101*(2), 265–276. https://doi.org/10.1111/1365-2745.12054

Walkley, A., & Black, I. A. (1934). AN EXAMINATION OF THE DEGTJAREFF METHOD FOR DETERMINING SOIL ORGANIC MATTER, AND A PROPOSED MODIFICATION OF THE CHROMIC ACID TITRATION METHOD. *Soil Science*, *37*(1), 29.

Willis, D., Moore, A. R., Frederick, R., & Willoughby, D. A. (1996). Heme oxygenase: A novel target for the modulation of inflammatory response. *Nature Medicine*, *2*(1), 87–90. https://doi.org/10.1038/nm0196-87

Xu, Q., Zhao, Y., Xu, M., Dong, J., Yin, W., Ma, J., & Wang, X. (2025). Grass-legume mixture optimizes soil new carbon sequestration by leveraging microbial necromass dynamics in a pear orchard. *Agriculture, Ecosystems & Environment*, *394*, 109898. https://doi.org/10.1016/j.agee.2025.109898

## Supplementary material

**Table S1.** presents the contributions of each variable to the first two principal components (Dim.1 and Dim.2) derived from the PCA. Variables with higher percentage values contribute more significantly to the variation explained by each component. Abbreviations used are as follows: MAP refers to Mean Annual Precipitation; MBC denotes microbial biomass carbon; $qCO_2$ represents the microbial metabolic quotient; and MSIR stands for the multiple substrate-induced respiration index.

| Variable | **Dim.1** | **Dim.2** |
|---|---|---|
| **MAP** | 18.12 | 0.66 |
| $C_{Org}$ (%) | 16.56 | 0.03 |
| **Mg** | 13.11 | 4.16 |
| **N** | 12.65 | 0.99 |
| **K** | 12.37 | 0.21 |
| **P** | 10.35 | 4.49 |
| **Clay** | 8.55 | 4.85 |
| **Water Content (%)** | 6.57 | 14.92 |
| **Ca** | 1.37 | 25.32 |
| **pH** | 0.25 | 28.63 |
| **Cl** | 0.08 | 14.54 |
| **$SO_4$** | 0.02 | 1.21 |

Table S2. Correlations among variables with significant values. Correlations indicate the strength of association between the original variables and the principal components, highlighting the variables driving the separation along each axis.

| Variable | Variable | Correlation | Pvalue |
|---|---|---|---|
| MPA | Water_Content | 0.49 | <2.2e-16 |
| MPA | CO | 0.95 | <2.2e-16 |
| Water_Content | CO | 0.66 | <2.2e-16 |
| MPA | Clay | -0.49 | <2.2e-16 |
| Water_Content | Clay | -0.45 | <2.2e-16 |
| CO | Clay | -0.41 | <2.2e-16 |
| Water_Content | pH | -0.74 | <2.2e-16 |
| Clay | pH | 0.5 | <2.2e-16 |
| MPA | N | 0.68 | <2.2e-16 |
| Water_Content | N | 0.56 | <2.2e-16 |
| CO | N | 0.69 | <2.2e-16 |
| Clay | N | -0.51 | <2.2e-16 |
| pH | N | -0.23 | 2.22E-02 |
| MPA | P | -0.69 | <2.2e-16 |
| CO | P | -0.5 | <2.2e-16 |
| Clay | P | 0.58 | <2.2e-16 |
| pH | P | -0.29 | 3.90E-03 |
| N | P | -0.43 | <2.2e-16 |
| MPA | K | -0.7 | <2.2e-16 |
| Water_Content | K | -0.37 | 2.00E-04 |
| CO | K | -0.65 | <2.2e-16 |
| Clay | K | 0.55 | <2.2e-16 |
| N | K | -0.52 | <2.2e-16 |
| P | K | 0.62 | <2.2e-16 |
| MPA | Mg | 0.78 | <2.2e-16 |
| Water_Content | Mg | 0.31 | 1.60E-03 |
| CO | Mg | 0.72 | <2.2e-16 |
| Clay | Mg | -0.33 | 8.00E-04 |
| N | Mg | 0.59 | <2.2e-16 |
| P | Mg | -0.58 | <2.2e-16 |
| K | Mg | -0.51 | <2.2e-16 |
| MPA | Ca | 0.38 | 1.00E-04 |
| Water_Content | Ca | -0.38 | 1.00E-04 |
| CO | Ca | 0.26 | 9.70E-03 |
| Clay | Ca | 0.29 | 3.80E-03 |
| pH | Ca | 0.8 | <2.2e-16 |
| P | Ca | -0.41 | <2.2e-16 |
| Mg | Ca | 0.51 | <2.2e-16 |
| Water_Content | Cl | -0.31 | 1.90E-03 |
| pH | Cl | 0.42 | <2.2e-16 |
| P | Cl | -0.24 | 1.44E-02 |
| Mg | Cl | 0.37 | 2.00E-04 |
| Ca | Cl | 0.51 | <2.2e-16 |
| Mg | SO4 | -0.3 | 2.10E-03 |
| Cl | SO4 | -0.2 | 4.21E-02 |
| Water_Content | SiteES | -0.67 | <2.2e-16 |
| CO | SiteES | -0.39 | 1.00E-04 |

| | | | |
|---|---|---|---|
| Clay | SiteES | -0.31 | 1.50E-03 |
| pH | SiteES | 0.42 | <2.2e-16 |
| P | SiteES | -0.5 | <2.2e-16 |
| Ca | SiteES | 0.21 | 3.65E-02 |
| Cl | SiteES | 0.35 | 4.00E-04 |
| MPA | SiteFR | -0.3 | 2.40E-03 |
| Water_Content | SiteFR | 0.58 | <2.2e-16 |
| Clay | SiteFR | -0.41 | <2.2e-16 |
| pH | SiteFR | -0.97 | <2.2e-16 |
| P | SiteFR | 0.4 | <2.2e-16 |
| Mg | SiteFR | -0.34 | 5.00E-04 |
| Ca | SiteFR | -0.86 | <2.2e-16 |
| Cl | SiteFR | -0.42 | <2.2e-16 |
| SiteES | SiteFR | -0.31 | 1.40E-03 |
| MPA | SiteLB | -0.54 | <2.2e-16 |
| Water_Content | SiteLB | -0.41 | <2.2e-16 |
| CO | SiteLB | -0.43 | <2.2e-16 |
| Clay | SiteLB | 1 | <2.2e-16 |
| pH | SiteLB | 0.43 | <2.2e-16 |
| N | SiteLB | -0.51 | <2.2e-16 |
| P | SiteLB | 0.64 | <2.2e-16 |
| K | SiteLB | 0.58 | <2.2e-16 |
| Mg | SiteLB | -0.37 | 1.00E-04 |
| Ca | SiteLB | 0.21 | 3.28E-02 |
| SiteES | SiteLB | -0.36 | 2.00E-04 |
| SiteFR | SiteLB | -0.32 | 1.00E-03 |
| MPA | SiteSL | 0.97 | <2.2e-16 |
| Water_Content | SiteSL | 0.54 | <2.2e-16 |
| CO | SiteSL | 0.98 | <2.2e-16 |
| Clay | SiteSL | -0.31 | 1.40E-03 |
| N | SiteSL | 0.65 | <2.2e-16 |
| P | SiteSL | -0.54 | <2.2e-16 |
| K | SiteSL | -0.64 | <2.2e-16 |
| Mg | SiteSL | 0.76 | <2.2e-16 |
| Ca | SiteSL | 0.39 | 1.00E-04 |
| SiteES | SiteSL | -0.34 | 5.00E-04 |
| SiteFR | SiteSL | -0.31 | 1.90E-03 |
| SiteLB | SiteSL | -0.35 | 3.00E-04 |

**Table S3**. Mean values (±SE) of soil variables by country. Abbreviations: MAP stands for Mean Annual Precipitation.

| Variable (units) | Site | Mean | SE |
|---|---|---|---|
| MAP ( mm) | Spain | 854 | - |
| | France | 773 | - |
| | Lebanon | 698 | - |
| | Slovenia | 1300 | - |
| Water Content (%) | Spain | 7.17 | 0.27 |
| | France | 22.09 | 0.55 |
| | Lebanon | 10.21 | 0.37 |
| | Slovenia | 21.11 | 0.21 |
| CO (%) | Spain | 1.29 | 0.03 |
| | France | 1.87 | 0.03 |
| | Lebanon | 1.21 | 0.06 |
| | Slovenia | 4.88 | 0.05 |
| Clay (%) | Spain | 33.6 | - |
| | France | 30.8 | - |
| | Lebanon | 59 | - |
| | Slovenia | 33.4 | - |
| pH | Spain | 8.2 | - |
| | France | 6 | - |
| | Lebanon | 8.2 | - |
| | Slovenia | 7.7 | - |
| N (%) | Spain | 0.16 | 0 |
| | France | 0.21 | 0.01 |
| | Lebanon | 0.1 | 0.01 |
| | Slovenia | 0.33 | 0.03 |
| P Olsen (mg/kg) | Spain | 9.16 | 0.68 |
| | France | 44.71 | 2.47 |
| | Lebanon | 51.45 | 2.59 |
| | Slovenia | 6.93 | 1.08 |
| K (mg/kg) | Spain | 7.15 | 1 |
| | France | 7.72 | 0.6 |
| | Lebanon | 11.67 | 0.45 |
| | Slovenia | 2.02 | 0.24 |
| Mg (mg/kg) | Spain | 20.2 | 2.84 |
| | France | 4.92 | 0.62 |
| | Lebanon | 5.71 | 0.69 |
| | Slovenia | 58.19 | 6.12 |
| Ca (mg/kg) | Spain | 57.68 | 2.43 |
| | France | 17.33 | 1.2 |
| | Lebanon | 57.63 | 1.71 |
| | Slovenia | 64.23 | 2.54 |
| Cl (mg/kg) | Spain | 9.24 | 0.45 |
| | France | 5.7 | 0.34 |
| | Lebanon | 7.7 | 0.48 |
| | Slovenia | 8 | 0.52 |
| $SO_4$ (mg/kg) | Spain | 5.16 | 0.6 |
| | France | 6.41 | 1.02 |
| | Lebanon | 6 | 0.77 |
| | Slovenia | 5.86 | 0.78 |

**Table S4.** The table presents the results of the diversity–interaction modeling, including estimates, standard errors (SE), and p-values, for several dependent variables: MicroResp substrate activities (amino acids, carbohydrates, carboxylic acids, and polymers), organic carbon ($C_{Org}$), the multiple substrate-induced respiration index (MSIR), microbial biomass carbon (MBC), and microbial metabolic quotient ($qCO_2$). A separate model was fitted for each variable. Initially, a general model was applied to data from all four countries—France (FR), Lebanon (LB), Slovenia (SL), and Spain (ES)—using harvest data from the second year of the experiment. These general models included Mean Annual Precipitation (MAP) as a structural factor. Subsequently, country-specific models were developed using data from both the first and second harvests (years 1 and 2). When statistically significant, models also incorporated species identity effects—*G1, G2,* and *G3* (grasses); *L1, L2*, and *L3* (legumes); and *F1, F2*, and *F3* (non-legume forbs)—as well as pairwise species interactions and/or a unique mean interaction effect (*AV*). The *AV* term represents the average effect of Evenness (E), while *wfg* and *bfg* denote within-functional group and between-functional group interactions, respectively. Model selection was based on the Akaike Information Criterion (AIC). Statistically significant p-values, $p \leq 0.05$, are indicated with an asterisk (*).

| Variables | Analysis | Model | Factor effect | Estimate | SE | Pr(>\|t\|) | |
|---|---|---|---|---|---|---|---|
| $C_{Org}$ | | | (Intercept) | 1.21 | 0.04 | 0.00 | * |
| | **General** | Null | MPA773 | 0.66 | 0.06 | 0.00 | * |
| | | | MPA854 | 0.07 | 0.06 | 0.23 | |
| | | | MPA1300 | 3.68 | 0.06 | 0.00 | * |
| | **ES** | Full | G1_ID | 1.13 | 0.06 | 0.00 | * |
| | | | G2_ID | 1.34 | 0.06 | 0.00 | * |
| | | | G3_ID | 5.17 | 0.41 | 0.00 | * |
| | | | L1_ID | 1.26 | 0.06 | 0.00 | * |
| | | | L2_ID | 1.50 | 0.06 | 0.00 | * |
| | | | F1_ID | 1.43 | 0.06 | 0.00 | * |
| | | | F2_ID | 1.17 | 0.06 | 0.00 | * |
| | | | `G1:G2` | -0.02 | 1.35 | 0.99 | |
| | | | `G1:G3` | -8.48 | 3.33 | 0.01 | * |
| | | | `G1:L1` | 0.47 | 0.64 | 0.47 | |
| | | | `G1:L2` | -0.23 | 1.52 | 0.88 | |
| | | | `G1:F1` | -0.36 | 0.60 | 0.55 | |
| | | | `G1:F2` | 3.77 | 1.62 | 0.02 | * |
| | | | `G2:L1` | 0.27 | 1.52 | 0.86 | |
| | | | `G2:L2` | 0.13 | 0.64 | 0.84 | |
| | | | `G2:F1` | -1.73 | 1.61 | 0.29 | |
| | | | `G2:F2` | 0.52 | 0.67 | 0.44 | |
| | | | `L1:L2` | -1.87 | 1.31 | 0.16 | |
| | | | `L1:F1` | -0.28 | 0.84 | 0.74 | |
| | | | `L1:F2` | 0.33 | 2.35 | 0.89 | |
| | | | `L2:F1` | -2.93 | 2.34 | 0.22 | |
| | | | `L2:F2` | 0.06 | 0.84 | 0.94 | |
| | | | `F1:F2` | -0.82 | 1.57 | 0.61 | |
| | | | HarvestT1 | -0.04 | 0.03 | 0.16 | |
| | **FR** | Full | G1_ID | 1.90 | 0.06 | 0.00 | * |
| | | | G2_ID | 1.85 | 0.06 | 0.00 | * |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | G3_ID | 3.74 | 0.42 | 0.00 | * |
| | | | L1_ID | 1.80 | 0.07 | 0.00 | * |
| | | | L2_ID | 1.97 | 0.07 | 0.00 | * |
| | | | F1_ID | 1.83 | 0.07 | 0.00 | * |
| | | | F2_ID | 1.88 | 0.07 | 0.00 | * |
| | | | `G1:G2` | 0.02 | 1.30 | 0.99 | |
| | | | `G1:G3` | 11.42 | 3.44 | 0.00 | * |
| | | | `G1:L1` | -0.74 | 0.66 | 0.27 | |
| | | | `G1:L2` | -2.85 | 1.49 | 0.06 | |
| | | | `G1:F1` | -1.46 | 0.62 | 0.02 | * |
| | | | `G1:F2` | 2.43 | 1.58 | 0.13 | |
| | | | `G2:L1` | 1.15 | 1.49 | 0.44 | |
| | | | `G2:L2` | 0.44 | 0.66 | 0.51 | |
| | | | `G2:F1` | 1.94 | 1.59 | 0.23 | |
| | | | `G2:F2` | -1.32 | 0.69 | 0.06 | |
| | | | `L1:L2` | 1.96 | 1.26 | 0.13 | |
| | | | `L1:F1` | 2.21 | 0.87 | 0.01 | * |
| | | | `L1:F2` | -3.34 | 2.35 | 0.16 | |
| | | | `L2:F1` | -7.85 | 2.34 | 0.00 | * |
| | | | `L2:F2` | 2.71 | 0.87 | 0.00 | * |
| | | | `F1:F2` | -0.04 | 1.56 | 0.98 | |
| | | | HarvestT1 | -0.01 | 0.03 | 0.80 | |
| | | | G1_ID | 4.78 | 0.14 | 0.00 | * |
| | **SL** | FG | G2_ID | 5.12 | 0.14 | 0.00 | * |
| | | | G3_ID | 16.38 | 0.84 | 0.00 | * |
| | | | L1_ID | 4.74 | 0.15 | 0.00 | * |
| | | | L2_ID | 4.74 | 0.15 | 0.00 | * |
| | | | F1_ID | 4.60 | 0.15 | 0.00 | * |
| | | | F2_ID | 4.84 | 0.15 | 0.00 | * |
| | | | FG_bfg_F_G | -0.61 | 0.77 | 0.44 | |
| | | | FG_bfg_F_L | -0.61 | 0.55 | 0.27 | |
| | | | FG_bfg_G_L | -0.39 | 0.65 | 0.55 | |
| | | | FG_wfg_F | 7.32 | 2.83 | 0.01 | * |
| | | | FG_wfg_G | -0.86 | 1.71 | 0.62 | |
| | | | FG_wfg_L | 1.33 | 2.23 | 0.55 | |
| | | | HarvestT1 | 0.34 | 0.07 | 0.00 | * |
| | | | G1_ID | 1.13 | 0.13 | 0.00 | * |
| | **LB** | Full | G2_ID | 1.17 | 0.20 | 0.00 | * |
| | | | G3_ID | 3.05 | 0.85 | 0.00 | * |
| | | | L1_ID | 1.22 | 0.16 | 0.00 | * |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | L2_ID | 1.02 | 0.13 | 0.00 | * |
| | | | F1_ID | 1.37 | 0.13 | 0.00 | * |
| | | | F2_ID | 1.40 | 0.13 | 0.00 | * |
| | | | `G1:G2` | 3.21 | 2.76 | 0.25 | |
| | | | `G1:G3` | -0.05 | 7.59 | 0.99 | |
| | | | `G1:L1` | 0.01 | 1.56 | 0.99 | |
| | | | `G1:L2` | -5.95 | 3.15 | 0.07 | |
| | | | `G1:F1` | 1.90 | 1.25 | 0.13 | |
| | | | `G1:F2` | 4.76 | 3.25 | 0.15 | |
| | | | `G2:L1` | -3.04 | 3.22 | 0.35 | |
| | | | `G2:L2` | 0.99 | 1.55 | 0.53 | |
| | | | `G2:F1` | -1.31 | 3.22 | 0.69 | |
| | | | `G2:F2` | 0.61 | 1.32 | 0.65 | |
| | | | `L1:L2` | 4.43 | 2.58 | 0.09 | |
| | | | `L1:F1` | 0.99 | 1.86 | 0.60 | |
| | | | `L1:F2` | -4.79 | 4.70 | 0.31 | |
| | | | `L2:F1` | 1.38 | 5.08 | 0.79 | |
| | | | `L2:F2` | 1.72 | 1.84 | 0.36 | |
| | | | `F1:F2` | -7.89 | 3.20 | 0.02 | * |
| | | | HarvestT1 | -0.21 | 0.06 | 0.00 | * |
| **Amino Acids** | | | (Intercept) | 20.10 | 1.01 | 3.7E-37 | * |
| | **General** | Null | MPA773 | 2.84 | 1.43 | 4.9E-02 | * |
| | | | MPA854 | -3.22 | 1.43 | 2.6E-02 | * |
| | | | MPA1300 | -4.40 | 1.44 | 2.9E-03 | * |
| | **ES** | Null | (Intercept) | 21.87 | 0.66 | 3.6E-47 | * |
| | | | HarvestT2 | -5.00 | 1.13 | 3.4E-05 | * |
| | **FR** | Null | (Intercept) | 13.75 | 0.58 | 2.0E-36 | * |
| | | | HarvestT2 | 9.19 | 0.98 | 3.5E-14 | * |
| | **SL** | AV | G1_ID | 13.30 | 1.86 | 6.6E-10 | * |
| | | | G2_ID | 16.31 | 1.85 | 7.0E-13 | * |
| | | | G3_ID | 19.61 | 11.92 | 1.0E-01 | |
| | | | L1_ID | 14.57 | 1.96 | 2.2E-10 | * |
| | | | L2_ID | 15.49 | 1.96 | 3.1E-11 | * |
| | | | F1_ID | 11.17 | 2.05 | 7.4E-07 | * |
| | | | F2_ID | 12.21 | 1.96 | 3.4E-08 | * |
| | | | AV | 8.28 | 3.40 | 1.7E-02 | * |
| | | | HarvestT1 | 3.23 | 0.97 | 1.4E-03 | * |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **LB** | Null | (Intercept) | 27.41 | 0.80 | 3.1E-45 | * |
| | | | HarvestT2 | -7.32 | 1.29 | 3.0E-07 | * |
| **Carbohydrates** | **General** | Null | (Intercept) | 22.79 | 6.18 | 0.000 | * |
| | | | MPA | 0.02 | 0.01 | 0.001 | * |
| | **ES** | Null | (Intercept) | 46.33 | 1.09 | 0.000 | * |
| | | | HarvestT2 | 13.02 | 1.86 | 0.000 | * |
| | **FR** | Null | (Intercept) | 49.74 | 1.63 | 0.000 | * |
| | | | HarvestT2 | -6.63 | 2.78 | 0.020 | * |
| | **SL** | Null | (Intercept) | 39.82 | 1.29 | 0.000 | * |
| | | | HarvestT2 | 9.28 | 2.24 | 0.000 | * |
| | **LB** | Null | (Intercept) | 16.25 | 1.24 | 0.000 | * |
| | | | HarvestT2 | 9.43 | 2.00 | 0.000 | * |
| **Carboxylix Acid** | **General** | Null | (Intercept) | 48.19 | 5.43 | 0.000 | * |
| | | | MPA | -0.01 | 0.01 | 0.019 | * |
| | **ES** | Null | (Intercept) | 28.99 | 0.67 | 0.000 | * |
| | | | HarvestT2 | -4.89 | 1.18 | 0.000 | * |
| | **FR** | Null | (Intercept) | 37.07 | 1.42 | 0.000 | * |
| | | | HarvestT2 | -4.92 | 2.38 | 0.042 | * |
| | **SL** | Null | (Intercept) | 38.82 | 1.19 | 0.000 | * |
| | | | HarvestT2 | -4.91 | 2.07 | 0.020 | * |
| | **LB** | Null | (Intercept) | 52.80 | 1.25 | 0.000 | * |
| | | | HarvestT2 | -1.22 | 2.03 | 0.551 | |
| **Polymers** | **General** | AV | G1_ID | 3.51 | 1.09 | 0.002 | * |
| | | | G2_ID | 5.77 | 1.09 | 0.000 | * |
| | | | G3_ID | 13.36 | 5.68 | 0.021 | * |
| | | | L1_ID | 3.26 | 1.13 | 0.005 | * |
| | | | L2_ID | 4.80 | 1.13 | 0.000 | * |
| | | | F1_ID | 3.21 | 1.12 | 0.005 | * |
| | | | F2_ID | 2.98 | 1.13 | 0.010 | * |
| | | | AV | -2.75 | 1.49 | 0.068 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | MPA | 0.00 | 0.00 | 0.053 | |
| | **ES** | Null | (Intercept) | 2.80 | 0.46 | 0.000 | * |
| | | | HarvestT2 | -1.37 | 0.79 | 0.087 | |
| | **FR** | Null | (Intercept) | 2.12 | 0.51 | 0.000 | * |
| | | | HarvestT2 | -0.31 | 0.88 | 0.727 | |
| | **SL** | Null | (Intercept) | 2.53 | 0.32 | 0.000 | * |
| | | | HarvestT2 | -1.24 | 0.55 | 0.026 | * |
| | **LB** | Null | (Intercept) | 3.54 | 0.97 | 0.001 | * |
| | | | HarvestT2 | -0.90 | 1.57 | 0.571 | |
| **MSIR** | **General** | AV | G1_ID | 0.32 | 3.47 | 0.927 | |
| | | | G2_ID | 4.63 | 3.46 | 0.185 | |
| | | | G3_ID | -1.91 | 18.07 | 0.916 | |
| | | | L1_ID | 1.32 | 3.60 | 0.715 | |
| | | | L2_ID | 5.07 | 3.59 | 0.161 | |
| | | | F1_ID | 5.63 | 3.57 | 0.118 | |
| | | | F2_ID | 7.20 | 3.60 | 0.048 | * |
| | | | AV | -7.93 | 4.75 | 0.098 | |
| | | | MPA | 0.01 | 0.00 | 0.000 | * |
| | **ES** | Null | (Intercept) | 4.18 | 0.37 | 0.000 | * |
| | | | HarvestT2 | 6.50 | 0.64 | 0.000 | * |
| | **FR** | Null | (Intercept) | 18.46 | 0.93 | 0.000 | * |
| | | | HarvestT2 | -3.48 | 1.58 | 0.031 | * |
| | **SL** | ADD | G1_ID | 4.20 | 7.24 | 0.564 | |
| | | | G2_ID | 13.69 | 7.26 | 0.064 | |
| | | | G3_ID | 36.60 | 33.71 | 0.282 | |
| | | | L1_ID | 18.07 | 7.52 | 0.019 | * |
| | | | L2_ID | 31.46 | 7.49 | 0.000 | * |
| | | | F1_ID | 33.90 | 7.63 | 0.000 | * |
| | | | F2_ID | 35.49 | 7.51 | 0.000 | * |
| | | | G1_add | 34.46 | 27.99 | 0.223 | |
| | | | G2_add | 45.09 | 29.23 | 0.128 | |
| | | | L1_add | -20.41 | 30.71 | 0.509 | |
| | | | L2_add | -27.34 | 30.83 | 0.378 | |
| | | | F1_add | 49.07 | 34.09 | 0.155 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | F2_add | -90.56 | 34.83 | 0.012 | * |
| | | | HarvestT1 | 45.11 | 3.16 | 0.000 | * |
| | **LB** | Null | (Intercept) | 32.49 | 1.50 | 0.000 | * |
| | | | HarvestT2 | -19.63 | 2.42 | 0.000 | * |
| **MBC** | | | (Intercept) | 30.58 | 6.23 | 0.000 | * |
| | **General** | NUll | MPA773 | 27.73 | 8.80 | 0.002 | * |
| | | | MPA854 | 32.96 | 8.80 | 0.000 | * |
| | | | MPA1300 | 66.59 | 8.89 | 0.000 | * |
| | **ES** | Null | (Intercept) | 19.91 | 2.64 | 0.000 | * |
| | | | HarvestT2 | 43.63 | 4.52 | 0.000 | * |
| | **FR** | Null | (Intercept) | 84.22 | 4.68 | 0.000 | * |
| | | | HarvestT2 | -25.91 | 8.01 | 0.002 | * |
| | **SL** | ID | G1_ID | 66.10 | 23.80 | 0.007 | * |
| | | | G2_ID | 81.31 | 23.91 | 0.001 | * |
| | | | G3_ID | 203.30 | 144.23 | 0.163 | |
| | | | L1_ID | 79.12 | 25.47 | 0.003 | * |
| | | | L2_ID | 107.28 | 25.62 | 0.000 | * |
| | | | F1_ID | 172.60 | 26.88 | 0.000 | * |
| | | | F2_ID | 104.38 | 25.77 | 0.000 | * |
| | | | HarvestT1 | 146.94 | 13.76 | 0.000 | * |
| | **LB** | Full | G1_ID | 40.35 | 15.10 | 0.010 | * |
| | | | G2_ID | -3.58 | 23.36 | 0.879 | |
| | | | G3_ID | 79.74 | 100.05 | 0.429 | |
| | | | L1_ID | 31.88 | 18.34 | 0.089 | |
| | | | L2_ID | 15.43 | 15.52 | 0.325 | |
| | | | F1_ID | 51.88 | 15.53 | 0.002 | * |
| | | | F2_ID | 47.38 | 15.59 | 0.004 | * |
| | | | `G1:G2` | 97.52 | 331.34 | 0.770 | |
| | | | `G1:G3` | -7.59 | 896.06 | 0.993 | |
| | | | `G1:L1` | -99.73 | 183.63 | 0.590 | |
| | | | `G1:L2` | -365.13 | 377.10 | 0.338 | |
| | | | `G1:F1` | -59.81 | 147.01 | 0.686 | |
| | | | `G1:F2` | 995.79 | 388.60 | 0.014 | * |
| | | | `G2:L1` | 197.87 | 384.77 | 0.609 | |
| | | | `G2:L2` | 127.07 | 182.95 | 0.491 | |
| | | | `G2:F1` | 483.95 | 385.17 | 0.215 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | `G2:F2` | -44.53 | 155.38 | 0.776 | |
| | | | `L1:L2` | 153.10 | 311.02 | 0.625 | |
| | | | `L1:F1` | 155.34 | 218.87 | 0.481 | |
| | | | `L1:F2` | -442.41 | 558.82 | 0.433 | |
| | | | `L2:F1` | 332.28 | 632.18 | 0.602 | |
| | | | `L2:F2` | 139.40 | 226.37 | 0.541 | |
| | | | `F1:F2` | -1132.72 | 382.72 | 0.005 | * |
| | | | HarvestT1 | 22.41 | 6.52 | 0.001 | * |
| **Shannon Index** | **General** | Null | (Intercept) | 1.87 | 0.11 | 0.000 | * |
| | | | MPA | 0.00 | 0.00 | 0.080 | |
| | **ES** | Null | (Intercept) | 2.19 | 0.02 | 0.000 | * |
| | | | HarvestT2 | 0.02 | 0.03 | 0.526 | |
| | **FR** | Null | (Intercept) | 2.10 | 0.04 | 0.000 | * |
| | | | HarvestT2 | 0.04 | 0.07 | 0.523 | |
| | **SL** | Null | (Intercept) | 2.11 | 0.03 | 0.000 | * |
| | | | HarvestT2 | -0.02 | 0.05 | 0.653 | |
| | **LB** | Null | (Intercept) | 1.66 | 0.03 | 0.000 | * |
| | | | HarvestT2 | 0.15 | 0.04 | 0.001 | * |
| **$qCO_2$** | **General** | | (Intercept) | 184.19 | 32.98 | 0.000 | * |
| | | | MPA | -0.10 | 0.04 | 0.004 | * |
| | **ES** | ADD | G1_ID | 175.04 | 27.31 | 0.000 | * |
| | | | G2_ID | 53.26 | 27.90 | 0.061 | |
| | | | G3_ID | 143.13 | 127.18 | 0.265 | |
| | | | L1_ID | 36.70 | 28.32 | 0.200 | |
| | | | L2_ID | 34.36 | 28.81 | 0.238 | |
| | | | F1_ID | 42.75 | 28.30 | 0.136 | |
| | | | F2_ID | 50.89 | 28.42 | 0.078 | |
| | | | G1_add | -315.05 | 108.16 | 0.005 | * |
| | | | G2_add | -17.86 | 110.87 | 0.873 | |
| | | | L1_add | 102.83 | 115.92 | 0.378 | |
| | | | L2_add | 7.21 | 118.09 | 0.952 | |
| | | | F1_add | 38.85 | 126.75 | 0.760 | |
| | | | F2_add | 4.89 | 130.78 | 0.970 | |
| | | | HarvestT1 | 5.45 | 12.11 | 0.654 | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **FR** | Null | (Intercept) | 53.57 | 5.63 | 0.000 | * |
| | | | HarvestT2 | 62.48 | 9.63 | 0.000 | * |
| | **SL** | Null | (Intercept) | 26.00 | 2.93 | 0.000 | * |
| | | | HarvestT2 | 33.52 | 5.07 | 0.000 | * |
| | **LB** | Null | (Intercept) | 81.78 | 11.80 | 0.000 | * |
| | | | HarvestT2 | 52.13 | 19.61 | 0.010 | * |

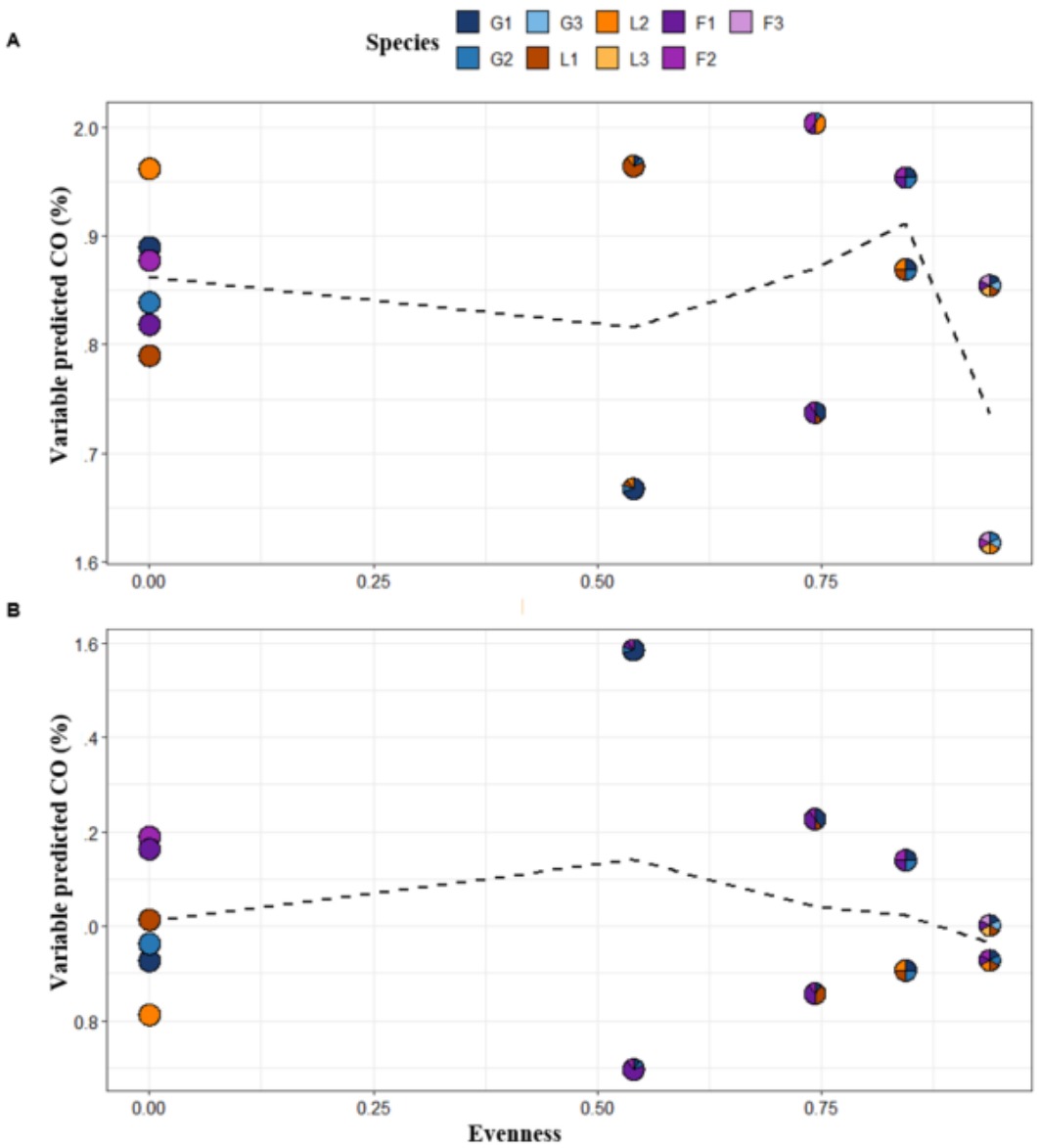


**Figure S1.** Predicted values of organic carbon in relation to species evenness in France (A) and Lebanon (B), based on Diversity–Interaction modelling. Each point represents a specific species mixture or functional group (e.g., G1–G3 for grasses, F1–F3 for forbs, L1–L3 for legumes). The x-axis indicates species evenness, ranging from low to high, while the y-axis shows the predicted organic carbon response. The charts illustrate the maximum and minimum predicted values across the evenness gradient evenness, the value of 0, represent predicted carbon values for monocultures of each studied species. The highlight the influence of species composition and relative abundance on soil organic carbon content.