Soil Biology and Biochemistry 208 (2025) 109866

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Impact of microbial diversity and pesticide application on plant growth, litter decomposition and carbon substrate use

Ferran Romero [a,*], Shuo Jiao [b], Marcel G.A. van der Heijden [a,c,**]

[a] Plant-Soil Interactions, Research Division Agroecology and Environment, Agroscope, 8046, Zurich, Switzerland
[b] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, Shaanxi Key Laboratory of Agricultural and Environmental Microbiology, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, China
[c] Department of Plant and Microbial Biology, University of Zurich, 8008, Zurich, Switzerland

ARTICLE INFO

Keywords:
Microbial diversity
Soil function
Plant productivity
Organic matter degradation
Pesticides
Mesocosms

ABSTRACT

Microorganisms are key components of soil biodiversity and are essential for organic matter decomposition and nutrient cycling in terrestrial ecosystems. So far it is unclear whether pesticide application influences microbial communities and the contribution of microbes to soil functioning and plant growth. To address this, we manipulated soil microbial diversity and created a diversity gradient, ranging from an average of 32 fungal operational taxonomic units (OTUs) and 312 bacterial amplicon sequence variants (ASVs) to 204 OTUs and 1000 ASVs, respectively. This reduction in microbial diversity resulted in significant decreases in litter decomposition (−43.7 %), carbon-substrate usage (−56.0 %), acid phosphatase activity (−54.1 %), and plant growth (−98.6 %). Pesticide application significantly reduced fungal richness at high soil microbial diversity levels, while bacterial richness was not affected by pesticide application. Our findings also revealed that pesticides alter the role of microbial communities, especially fungi, in organic matter decomposition and plant growth. Pesticide application reduced the relative importance of fungal richness in explaining variations in plant biomass production and litter decomposition. Conversely, pesticide application increased the relative importance of bacterial richness in explaining carbon substrate utilization, particularly for carbohydrates and amino acids. Overall, this study suggests that preserving the diversity of bacterial and fungal communities within soil mesocosms is key to support essential soil functions such as organic matter degradation, and that pesticide applications could impair the beneficial effects of fungal richness on organic matter degradation.

1. Introduction

Soil is an immense habitat for diverse organisms across the tree of life. Recent research indicates that soil is likely home to 59 % of the species on Earth, including most earthworms, plants, fungi, and half of all known bacteria (Anthony et al., 2023). The multitude of life forms in soil underpin various ecological functions critical for producing food and fiber, and for maintaining both human and planetary health (van der Heijden et al., 2008; Delgado-Baquerizo et al., 2016).

One of the pivotal roles played by soil organisms involves litter decomposition. This process, crucial for recycling nutrients, involves the breakdown of plant material such as leaves, wood, and roots into simpler organic and inorganic substances (Krishna and Mohan, 2017). Litter decomposition is performed by a wide variety of soil organisms, ranging from microorganisms like bacteria and fungi to macroorganisms including earthworms and arthropods (Hättenschwiler et al., 2005). Through litter decomposition, essential nutrients such as nitrogen, phosphorus, and potassium are released, enhancing plant productivity (Freschet et al., 2013). The relationship between soil diversity and litter decomposition has been debated for the last decades, and most research points towards a positive association between the diversity of soil organisms and the rate at which litter in soil is mineralized (Petersen and Luxton, 1982; David, 2014; Frouz, 2018). For example, recent studies highlight a positive association between soil diversity and functions such as organic matter degradation and plant productivity, proving that biodiversity is an integral component of soil health (Fan et al., 2023; Romero et al., 2024). In line with this, other studies have demonstrated that both bacterial and fungal diversity positively impact carbon and

* Corresponding author.
** Corresponding author. Plant-Soil Interactions, Research Division Agroecology and Environment, Agroscope, 8046, Zurich, Switzerland.
E-mail addresses: ferran.romeroblanch@agroscope.admin.ch (F. Romero), marcel.vanderheijden@agroscope.admin.ch (M.G.A. van der Heijden).

https://doi.org/10.1016/j.soilbio.2025.109866
Received 29 January 2025; Received in revised form 24 April 2025; Accepted 23 May 2025
Available online 23 May 2025

phosphorus cycling in model and natural soils (Domeignoz-Horta et al., 2020; Wu et al., 2024). These findings are corroborated by manipulative studies under controlled conditions, which illustrate that experimentally reducing soil biodiversity can adversely affect litter decomposition and plant productivity (Wagg et al., 2014, 2019; Zhang et al., 2023). Finally, other studies have integrated field surveys with laboratory or greenhouse approaches, strengthening the evidence of a robust positive correlation between soil organic matter decomposition and the diversity of soil taxa, including invertebrates, fungi, and bacteria (Delgado-Baquerizo et al., 2017, 2020; Zhang et al., 2024).

Parallel to this, soil ecosystems are exposed to multiple environmental stressors arising from human activities and climate change (Rillig et al., 2021, 2023). For example, agricultural practices such as intense tillage, monocropping, and the application of chemical fertilizers and pesticides can lead to soil structure disruption, depletion of soil nutrients, and soil acidification (Edlinger et al., 2023). Among the many impacts derived from intensive agriculture, the application of pesticides has the potential to be particularly detrimental for soil biodiversity, with unknown consequences for ecosystem functioning (e.g., litter decomposition and plant productivity). Pesticides are prevalent in soils spanning both organic and conventionally managed fields: a recent study assessed pesticide presence across 100 agricultural fields in Switzerland, and found pesticide residues in every tested site, including 40 organic fields (Riedo et al., 2021). The study also revealed that conventionally-managed fields had double the number of pesticide residues and nine times higher concentrations than their organic counterparts. In a broader study conducted during the 2021 growing season, researchers collected 201 soil samples from fields under both conventional and organic management across 10 European countries and 8 different cropping systems and found pesticide residues in 97 % of 201 soil samples, with 88 % containing multiple substances (Knuth et al., 2024).

Because of their widespread application, soil biodiversity is frequently exposed to pesticides in agricultural areas and adjacent soils (Jacobsen and Hjelmsø, 2014). Pesticides are predominantly tested on soil invertebrates and fauna, such as earthworms and arthropods, to assess their environmental impacts (Beaumelle et al., 2023). While these organisms are vital for soil ecosystem functioning, this approach often overlooks the effects of pesticides on microbial communities, which play crucial roles in nutrient cycling, organic matter decomposition, and greenhouse gas regulation (Cycoń et al., 2013). Some microorganisms may use pesticides as a carbon source, potentially benefiting from their presence, whereas others, particularly fungi, might be harmed by specific pesticides like fungicides (Aislabie and Lloyd-Jones, 1995; Yang et al., 2011; Edlinger et al., 2023; Riedo et al., 2021). Regarding functioning, pesticides have been shown to disrupt crucial soil functions, including nutrient cycling and litter decomposition through enzymatic activities (Riah et al., 2014; Meidl et al., 2024). However, while a range of studies assessed the direct and indirect effects of pesticides on soil communities, especially soil fauna, it is still poorly understood whether soils with different levels of soil biodiversity are affected differently by pesticide application. It is unclear whether soils with reduced soil biodiversity are more sensitive to pesticide applications and consequently it is not known whether pesticides alter the relationship between soil biodiversity and ecosystem function. Especially soil communities with reduced levels of soil biodiversity might be more sensitive to pesticide applications because such soils harbor a lower number of taxa that may compensate for functional loss following pesticide application (e.g., the insurance hypothesis of biodiversity, see Caruso et al., (2018). This underscores the need for experimental studies to directly investigate the relationship between soil microbial diversity and ecosystem function both in the presence and absence of pesticides. Understanding these interactions is crucial for sustainable agricultural practices, since it may indicate that soils with contrasting biodiversity levels might differ in terms of their capacity to buffer the negative impacts of pesticides on function.

In this study, we tested whether a mixture of pesticides applied at environmentally relevant concentrations can influence the relationship between soil microbial diversity and two soil functions (litter decomposition and plant biomass production). To that purpose, we artificially generated a soil biodiversity gradient and evaluated its impact on plant biomass production, litter decomposition, enzymatic activity, and carbon use with and without pesticide addition. We hypothesized that (I) soil functions would benefit from increased soil biodiversity in experimental mesocosms, (II) pesticide application would negatively affect soil functions, and (III) pesticide application would weaken the relationship between soil microbial diversity and function.

2. Materials & methods

2.1. Experimental approach and conditions

We conducted an experiment using self-contained mesocosms to study the effects of microbial diversity and pesticide application on organic matter decomposition and plant growth. The mesocosms were established within closed systems, each consisting of a 6.5L polyvinyl chloride (PVC) pot and a 20L Plexiglas cover (see Fig. S1). Before use, all mesocosm components were submersed in 0.5 % sodium hypochlorite for 30 min, then in 70 % ethanol with a few drops of Tween 20 for a few minutes and then dried under the laminar flow hood. To prevent contamination, the mesocosms were supplied with air and water through 0.22 μm sterile filters. The substrate for all mesocosms consisted of a 1:1 mixture of sand and grassland soil. The soil was collected from a nearby grassland (47° 25′ 38.71″ N, 8° 31′ 3.91″ E), and the sand-soil mixture had a pH of 7.6, with 9.54 mg kg^{-1} of inorganic N, 1.25 mg kg^{-1} of plant-available phosphorus, 0.61 mg kg^{-1} of K2O. The ammonium acetate-EDTA extracted amounts of Ca, P, K and Mg in mg kg^{-1} were 6.51e03, 21.8, 15.7, and 4.88 (Wagg et al., 2014). The sand-soil mixture was autoclaved twice (48-h interval) at 121 °C during 90 min before use; each mesocosm was filled with 3 kg of this mixture. The water holding capacity of the substrate was 0.348 L per kg of soil. Autoclaved substrate samples (10 g) were shaken in 100 ml of 125 mM NaCl for 10 min to extract microorganisms. Extracts were serially diluted, and 0.1 ml suspensions were spread on TSA and LB agar in duplicate. Cultures were incubated at 25 °C for 21 days, with no colony-forming units observed. The experiment consisted of 7 replicate mesocosms per treatment, resulting in a total of 56 mesocosms. The treatments included four microbial diversity levels and two pesticide levels (presence vs. absence).

Four distinct microbial diversity levels were created by sequentially sieving soil through decreasing mesh sizes: a 5-mm mesh for the most diverse community, followed by 100 μm, 11 μm, and a final autoclaved inoculum. Each mesocosm (containing 3000 g of substrate) received 250 g of the respective inoculum (7.69 % of total substrate-inoculum mixture). To correct for differences in amount of inoculum added, the inoculum not passing the sieve was autoclaved and also added to the mesocosms The total duration of the experiment was 15 weeks, including six weeks of mesocosm incubation only with autoclaved substrate and the different soil inocula (i.e., 5-mm, 100 μm, 11 μm, and autoclaved), three weeks of seedling growth, and six weeks of pesticide exposure (Fig. S2).

Specifically, mesocosms were incubated in a greenhouse for six weeks (at 25–30 °C) to allow microbial communities to establish. This duration was selected based on a previous study, where six weeks of incubation achieved microbial diversity levels comparable to those in Swiss grasslands (Romero et al., 2023b; Richter et al., 2024). After these six weeks, 15 pre-germinated seedlings of *Allium porrum* (variety Nipper) were planted per mesocosm. Leek seeds were surface-sterilized and pre-germinated on water-agar under sterile conditions for six days to improve survival. Two weeks post-planting, the number of seedlings was standardized to nine per mesocosm, and litter bags were added. Seedlings were allowed to develop for one week without pesticide addition.

During this week, a pesticide mixture was prepared according to the recommended dosages with each pesticide diluted to the required concentration in autoclaved distilled water under sterile conditions. The pesticides included in the mixture were the fungicides Azoxystrobine, Cyproconazole, and Difenoconazole, the herbicides Pendimethalin and Diquat, and the insecticides Acetamiprid and λ-Cyhalothrin. The pesticides were combined, and 5 mL of the resulting mixture were sprayed over the soil in exposed mesocosms. Trade names, group of active ingredients, and applied dosages are available in Table S1.

2.2. Edaphic properties

We collected soil samples at the end of the experiment to perform analyses of total carbon, total organic carbon, pH, total nitrogen, and plant available phosphorus (Olsen-P) using Swiss standard protocols (FAL, 1996). Conductivity and sodium chloride (NaCl) content were measured after preparation of a soil-water extract (1:2) using an electrical conductivity meter. Microbial biomass (microbial C and N) was analyzed by the chloroform fumigation extraction method as described in Toda et al. (2023). Briefly, 15 g of the soil were fumigated with chloroform for 24 h at 25 °C. Both fumigated and non-fumigated samples were extracted with 75 mL of 0.05 M K2SO4 on an overhead shaker for 1 h. The C and N concentration of the extract was measured using a TOC/TN analyser (Elementar Analysensysteme GmbH, Langenselbold, Germany). Microbial biomass was calculated as the difference between fumigated and nonfumigated soil extractable C and N concentrations and expressed as mg C (or N) per kg of soil.

2.3. Soil biodiversity

Soil biodiversity analyses followed the procedure described in Romero et al., (2023b). Briefly, soil cores (1.7 mm diameter) were collected to the depth of the mesocosms (~25 g of fresh soil) and homogenized. A 0.25 g subsample was frozen at −20 °C until DNA extraction was performed using the Nucleospin 96 Soil DNA extraction kit (Macherey-Nagel, Düren, Germany). Microbial community composition was determined on an Illumina MiSeq platform using the Nextera XT DNA Library Preparation kit (Illumina Inc., CA, USA). Primers 515F/806R and ITS3/ITS4 targeting the 16S rRNA and ITS regions were used to characterize the bacterial and fungal communities, respectively (Caporaso et al., 2011; Yu et al., 2022). All sequences generated within this study are available at the Sequence Read Archive (SRA) database under BioProject accession number PRJNA1213498. For bacteria, demultiplexed fastq files were processed using the DADA2 (v3.19) package (Callahan et al., 2016). We first applied a quality filtering step to remove low-quality sequences based on their quality scores, then we eliminated sequences with ambiguous bases and trimmed sequences to a consistent length to reduce errors. The data was then dereplicated by collapsing identical sequences into unique sequences with their corresponding abundances. We constructed an error model specific to the sequencing run by analyzing error rates and patterns, and used this model to perform sequence inference, correcting errors and distinguishing true biological sequences from noise. Finally, we identified and removed chimeric sequences resulting from PCR amplification artifacts. This process resulted in a high-resolution set of ASVs. For fungi, sequences were clustered at 98 % operational taxonomic units (OTUs) using the VSEARCH algorithm (Tedersoo et al., 2021; Labouyrie et al., 2023). Sequences were rarefied at 30,000 for bacteria, and 5000 for fungi. Taxonomic assignment was performed using the assignTaxonomy function on the sequence table against the Silva reference database for bacteria and the UNITE reference database for fungi (Quast et al., 2012; Nilsson et al., 2019). The R package microeco V1.9.0 (Liu et al., 2021) was used to calculate microbial richness (i.e., number of bacterial ASVs and fungal OTUs per sample) and Shannon's diversity index.

2.4. Soil functions

At the end of the experiment (i.e., 15 weeks in total), leek plants were removed from each individual mesocosm, thoroughly washed to remove any soil particles, and dried at 70 °C to a constant weight (as in Romero et al., 2023a). This final weight was recorded and used to determine the plant biomass production for each mesocosm.

Litter decomposition was assessed with three 0.5-mm propyltex mesh litter-bags (6 × 6 cm) containing 1 g of sterilized *Lolium multiflorum* shoots that were added to mesocosms at the end of week eight (Fig. S2). The amount of the initial litter (1 g) that was lost at the end of the experiment (week 15, see Fig. S2) was calculated as decomposition. The mean of litter decomposition in the 3 bags employed per mesocosm was averaged and used as a result. In total, litter bags were buried for seven weeks: from week 8 and until the end of the experiment (week 15).

Carbon substrate utilization was assessed using Biolog Ecoplate™ (Biolog Inc., Hayward, CA, USA) and a modified version of the procedure described in Romero et al. (2019). Each Biolog Ecoplate™ contains three replicated wells of 31 different carbon sources and a blank with no substrate. We transferred 3 g of homogenized soil sample into 27 mL of 25 %-Ringer solution (2.25 g L^{-1} NaCl, 0.105 g L^{-1} KCl, 0.045 g L^{-1} $CaCl_2$, 0.05 g L^{-1} $NaHCO_3$) for 15 min at room temperature under soft agitation. After vortexing, the suspension was sonicated for 1 min to detach cells from soil particles, and 130 μL of the suspension were used to fill each well in the Biolog Ecoplate™. The Ecoplates were incubated at 25 °C under dark conditions and the absorbance was read every 24 h at 590 nm and 750 nm using a Spark 10 M Multimode microplate Reader (Tecan Trading AG, Switzerland). For each treatment, three plates of tryptic soy agar (TSA, 15 g L^{-1} tryptone, 5 g L^{-1} soytone, 5 g L^{-1} NaCl, 15 g L^{-1} Agar) were inoculated with 100 μl of the soil suspension and incubated for 24h at 20 °C. This ensured that the number of colony forming units (CFUs) was in the same range (~10^4 CFUs/mL of suspension) for all treatments. The data of each Biolog Ecoplate™ were evaluated following the recommendations of Sofo and Ricciuti (2019). First, the values of each well (i) were corrected for turbidity at each incubation time (h) by subtracting OD_{750} from the OD_{590} value: $i_h = OD_{590} - OD_{750}$. Then, the corrected value of the blank (b) was subtracted of the value of each well (i) at each time (h) to obtain the blank-corrected value of each well: $i_{bc-h} = i_h - b_h$. Subsequently, the color development values (c) could be calculated for each well and incubation time by subtracting the blank-corrected values at time 0 from the blank-corrected value at time h: $c_{ih} = i_{bc-h} - b_{bc-0}$. Finally, we calculated the average well color development (AWCD) of each incubation time (h) as following: $AWCD_h = \sum c_i/93$. The AWCD is a general indicator of the ability of the microbial community to use the different carbon substrates available. It is possible to combine it with c_i values to calculate the richness of substrates used (c_i equal or higher to 0.250).

Enzymatic activity was measured from 1 g of soil after addition of 0.5 ml of 0.115 M *p*-nitrophenyl phosphate (PNP, acid phosphatase) and 4-nitrophenyl β-glucopyranoside (PNG, beta glucosidase) and 2 ml of 0.1 M maleate buffer at pH 6.5. The mixture was then incubated for 2 h at 37 °C in darkness. After this time, the reaction product, PNP (*p*-nitrophenol), was extracted with 0.5 M NaOH and 0.5 M $CaCl_2$, and absorbance was measured with a spectrophotometer at a wavelength of 398 nm. Results are expressed as μg PNP (or PNG) g^{-1} h^{-1}.

2.5. Statistical approach

We first explored the effects of the artificially generated diversity gradient and pesticide application on soil diversity and functions. To that purpose, we built general additive models (GAMs) using the *mgcv* package in R v1.9–1 (Wood and Wood, 2015). We used a Quasi-Poisson distribution and a logarithmic link function to relate the response variable to the predictors in the model (Venables and Dichmont, 2004). Within each GAM, the factors "Diversity", "Pesticides", and their interaction term (i.e., "Diversity*Pesticides") were included as fixed factors.

The factor "Diversity" had four levels: "5 mm", "100 μm", "11 μm", and "autoclaved". The factor "Pesticides" had two levels (i.e., presence vs. absence). As response variables, we included bacterial richness (number of bacterial ASVs), fungal richness (number of fungal OTUs), decomposition (%), C-substrate utilization (number of C-substrates used), plant biomass production, and enzymatic activities. Due to very low plant biomass produced in control mesocosms with autoclaved inoculum, these data were normalized using cubic root transformation. Analysis of variance (ANOVA type III sums of squares) was performed on the GAM model to check the significance of terms. Linear and quadratic regressions were used to explore the relationships between microbial richness, including both fungal and bacterial richness (number of ASVs), and four soil functions: litter decomposition, carbon substrate use, plant biomass production, and enzymatic activity. An analysis of covariance (ANCOVA) was then applied to determine the influence of pesticide application on these relationships between microbial diversity and soil functions (Rutherford, 2012).

3. Results

3.1. Soil biodiversity manipulation – impact on microbial richness and diversity

Both bacterial and fungal richness (i.e., number of ASVs and OTUs, respectively) were significantly and successfully altered by biodiversity manipulation (Table 1, Fig. 1). Accordingly, fungal richness was highest (i.e., 171.15 ± 49.43 OTUs) in mesocosms inoculated with soil sieved through 5 mm (average of pesticide-free and pesticide-containing mesocosms, n = 13). Fungal richness decreased to 150.64 ± 32.08 OTUs in mesocosms inoculated with soil sieved through 100 μm, and to 83.43 ± 33.50 OTUs in mesocosms inoculated with 11-μm sieved soil. The lowest fungal richness was observed in mesocosms inoculated with autoclaved soil (35.93 ± 11.70 OTUs). Similarly, fungal diversity (Shannon's index) decreased from 2.29 ± 0.37 in (5 mm soils, n = 13) to 1.56 ± 0.51 in mesocosms receiving autoclaved inoculum (n = 14). Bacterial richness did not differ significantly between mesocosms inoculated with 5-mm sieved soil (760.15 ± 202.02 ASVs) and 100- μm soils (925.43 ± 262.75 ASVs) but decreased in mesocosms inoculated with 11-μm and autoclaved soils (498.00 ± 208.15 and 332.29 ± 141.23 ASVs, respectively). The same pattern was observed for bacterial diversity (Table S2).

Table 1
Summary statistics of general additive models (GAMs). First column indicates response variables. Number of mesocosms in each model was 55. "Diversity" and "Pesticides" are fixed factors, and interactive effects are indicated as "Interaction". For each model, F-value and p-value are indicated for each fixed factor and the interaction term. For each model, percentage of deviance explained is also indicated. Non-significant ($p < 0.05$) results are reported as "n.s.". Additional details on linear and quadratic regressions are available in Table S3.

Response variable	Diversity (d.f. = 3)	Pesticides (d.f. = 1)	Interaction (d.f. = 3)	Deviance explained (%)
Bacterial richness	F = 15.61 p = 3.51e-07	*n.s.*	*n.s.*	60.2
Fungal richness	F = 45.00 p = 7.34e-14	*n.s.*	F = 4.93 p = 0.00467	80.4
Litter decomposition	F = 7.81 p = 0.000249	*n.s.*	*n.s.*	51.1
C substrate use	F = 8.53 p = 0.000126	*n.s.*	*n.s.*	59.1
Acid phosphatase activity	F = 6.21 p = 0.00121	*n.s.*	*n.s.*	36.5
Plant biomass	F = 40.32 p = 4.78e-13	F = 5.00 p = 0.0302	F = 14.26 p = 9.70e-07	76.4

3.2. Soil biodiversity manipulation – impact on functions

Manipulating biodiversity had a significant impact on litter decomposition, C-substrate use, phosphatase activity, and plant biomass production (Table 1). Regarding litter decomposition, the percentage of degraded litter decreased from 77.90 ± 10.38 % (5-mm inoculum, n = 14) to 49.64 ± 16.10 % (autoclaved inoculum, n = 14). Similarly, the richness of carbon substrates degraded decreased from 25.15 ± 1.99 % to 13.36 ± 6.55 %, affecting the three major types of carbon substrates analyzed: carbohydrates, amino acids, and carboxylic acids (Fig. S3). Acid phosphatase activity decreased from 46.51 ± 15.52 to 24.73 ± 10.59 μg PNP g^{-1} h^{-1} (Fig. 1). Beta glucosidase activity was not affected by soil manipulation, with an average value between 14.30 and 20.73 μg PNG g^{-1} h^{-1} across all diversity levels (Fig. S4). Finally, we observed a decrease in plant biomass production in low soil diversity treatments (less than 1 g of dry biomass produced) (Fig. 1). Average values per treatment across all functions are available in Table S2.

3.3. Pesticide application – impact on microbial richness

Pesticide addition had little impact on the overall richness (i.e., number of ASVs or OTUs) of bacteria and fungi in our mesocosms (Table 1). However, we observed a decrease in fungal richness in mesocosms inoculated with 5-mm sieved soil and treated with pesticides (137.00 ± 37.51 ASVs) compared to non-treated mesocosms (203.57 ± 30.29 ASVs) (Fig. 1). This was also confirmed by a significant interaction effect between biodiversity manipulation and pesticides as indicated by general additive models (Table 1). Average values per treatment are available in Table S2.

3.4. Pesticide application – impact on functions

The decrease in fungal richness following pesticide application in mesocosms inoculated with 5-mm sieved soil was accompanied by a decrease in carbon substrate use, litter decomposition, and plant biomass production. Accordingly, the richness of carbon substrates used decreased by 7.04 %; from 26.00 ± 1.63 (pesticide-free mesocosms, inoculum 5 mm, n = 7) to 24.17 ± 2.04 in mesocosms with added pesticides (inoculum 5 mm, n = 6) (Fig. 1, Fig. S3). Similarly, litter decomposition decreased from 81.34 ± 9.54 % to 73.90 ± 10.65 %, and plant biomass production decreased by from 3.48 ± 1.55 to 0.05 ± 0.04 g (Fig. 1). However, only the decrease in plant biomass production was statistically significant following general additive models (Table 1). Average values per treatment are available in Table S2.

3.5. Relationship between microbial richness and soil function

We explored the relationships between microbial (i.e., bacterial and fungal) richness and soil functions (plant biomass production, litter decomposition, phosphatase activity, and richness of carbon substrates used). Specifically, we fitted linear and quadratic regressions and found a positive correlation between microbial richness, and soil function (Fig. 2, Table S3). The relationship between microbial richness and litter decomposition/plant biomass production was mostly linear. However, for C-substrate use, we observed a plateau at around 800 bacterial ASVs and 200 fungal OTUs, beyond which further increases in microbial richness did not enhance the richness of carbon substrates used (Fig. 2).

The presence of pesticides decreased the strength of the relationship between fungal richness and litter decomposition, from $R^2 = 0.41$ (*p-value* < 0.001) in pesticide-free mesocosms to $R^2 = 0.23$ (*p-value* < 0.001) in pesticide-containing mesocosms. This is in line with a significant interaction term in the general additive model between pesticides and diversity manipulation for fungal richness (Table 1). Similarly, pesticides decreased the strength of the relationship between fungal richness and plant biomass production from $R^2 = 0.58$ (*p-value* < 0.001) to $R^2 = 0.19$ (*p-value* < 0.050) (Fig. 2). Pesticides, however, increased

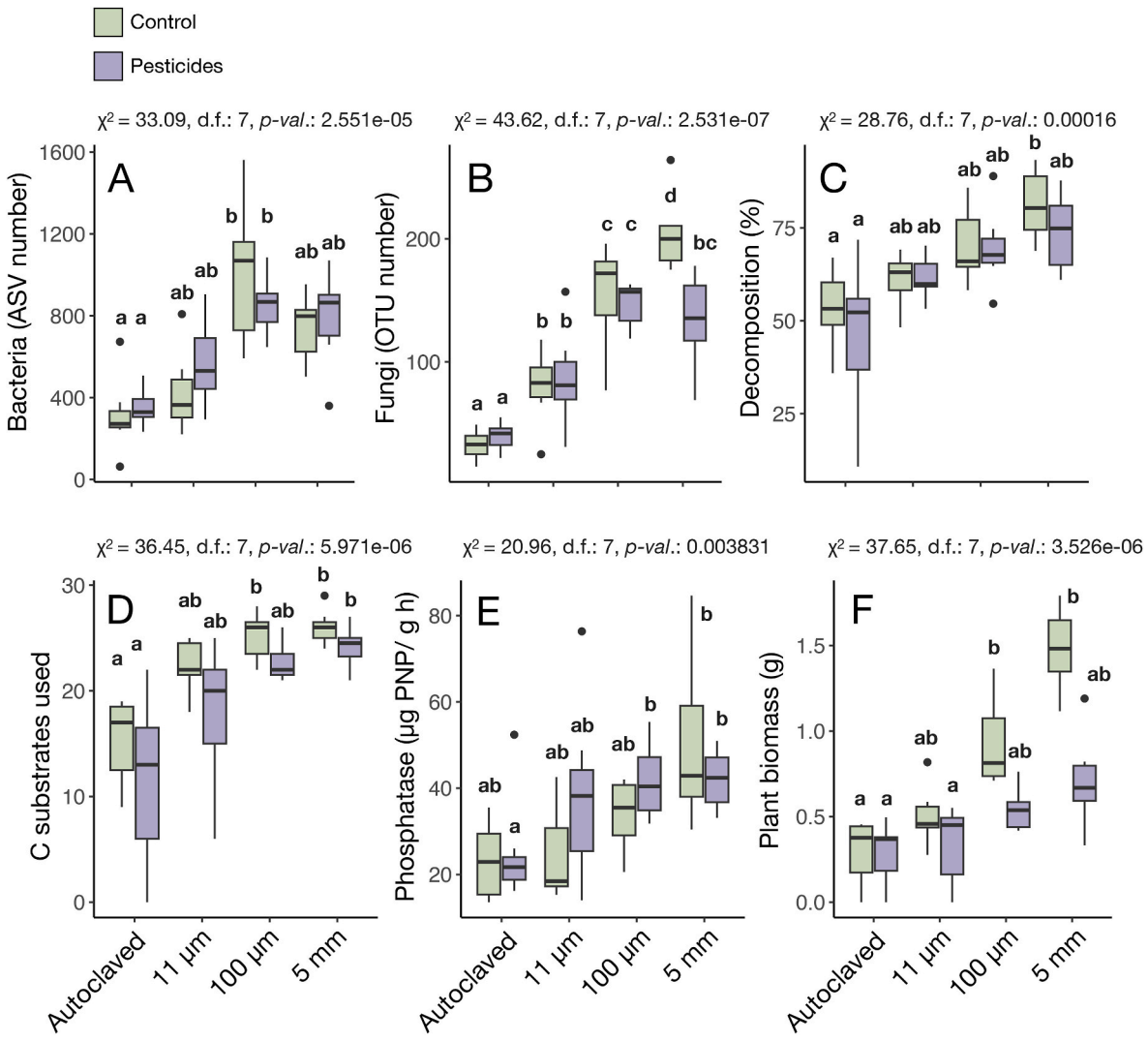

Fig. 1. Bacterial richness (A), fungal richness (B), litter decomposition (C), richness of used carbon substrates (D), acid phosphatase activity (E), and plant biomass production (F) for each treatment along the diversity gradient (mesocosms receiving autoclaved or sieved −11 µm, 100 µm, 5 mm-soil inoculum). Boxplot color indicates whether treatments received pesticides (purple) or not (green). Kruskal-Wallis test was run for each variable, and results are indicated as chi-squared (X^2) and *p-value*. Degrees of freedom are also indicated (d.f.). Different letters indicate statistically significant differences following Dunn's post hoc test with Bonferroni correction.

the strength of the association between bacterial richness and C-substrate use, from $R^2 = 0.24$ (*p-value* = 0.020) (pesticide-free mesocosms) to $R^2 = 0.39$ (*p-value* = 0.001) in pesticide-containing mesocosms. We observed weak correlations between microbial richness and acid phosphatase activity (Fig. 2). We further employed analysis of covariance (ANCOVA) to explore the interaction between pesticide application and microbial richness (i.e., to which extent the relationship between microbial richness and soil functions is shaped by pesticide application). ANCOVA results confirmed a strong influence of both fungal and bacterial richness on soil functions (Table S4). Fungal richness emerged a better predictor of plant biomass production, litter decomposition and carbon substrate than bacterial richness. ANCOVA indicated a significant interaction between fungal richness and pesticide application for plant biomass production, as well as a significant interaction between bacterial richness and pesticide application for carbon substrate use, particularly carbohydrates and amino acids (Table S4, Table S5).

3.6. Edaphic properties

Soil pH in mesocosms ranged from 7.56 ± 0.06 to 7.71 ± 0.19, and salinity ranged from 74.14 ± 17.86 to 122.71 ± 38.42 mg per 100 g of soil. Conductivity ranged from 254.71 ± 61.79 to 421.29 ± 131.83 µS cm, and phosphorus content from 9.22 ± 1.29 to 9.85 ± 1.73 mg kg. Total soil carbon ranged from 1.31 ± 0.11 % to 1.50 ± 0.11 %. Similarly, total soil organic carbon ranged from 0.35 ± 0.09 % to 0.60 ± 0.26 %. Total nitrogen ranged from 0.10 % to 0.11 %. Finally, microbial biomass ranged from 144.39 ± 48.04 to 203.91 ± 37.68 mg C kg of soil, and from 10.89 ± 13.05 to 22.28 ± 11.06 mg N kg of soil. General additive models (GAMs) were fitted on all edaphic properties and showed a significant impact of experimental treatments on NaCl content and conductivity (Table S6). Average values per treatment are available in Table S2. Information on measured variables at each individual mesocosm is available in Supplementary Dataset.

4. Discussion

In this study, we manipulated soil biodiversity in experimental mesocosms by sequentially sieving soil inoculum through decreasing mesh sizes to evaluate the effects of reduced biodiversity on organic matter degradation and plant growth. To that purpose, we employed mesocosms filled with soil, litter bags, and planted with leek (*Allium porrum*). Half of the mesocosms were exposed to a realistic mixture of

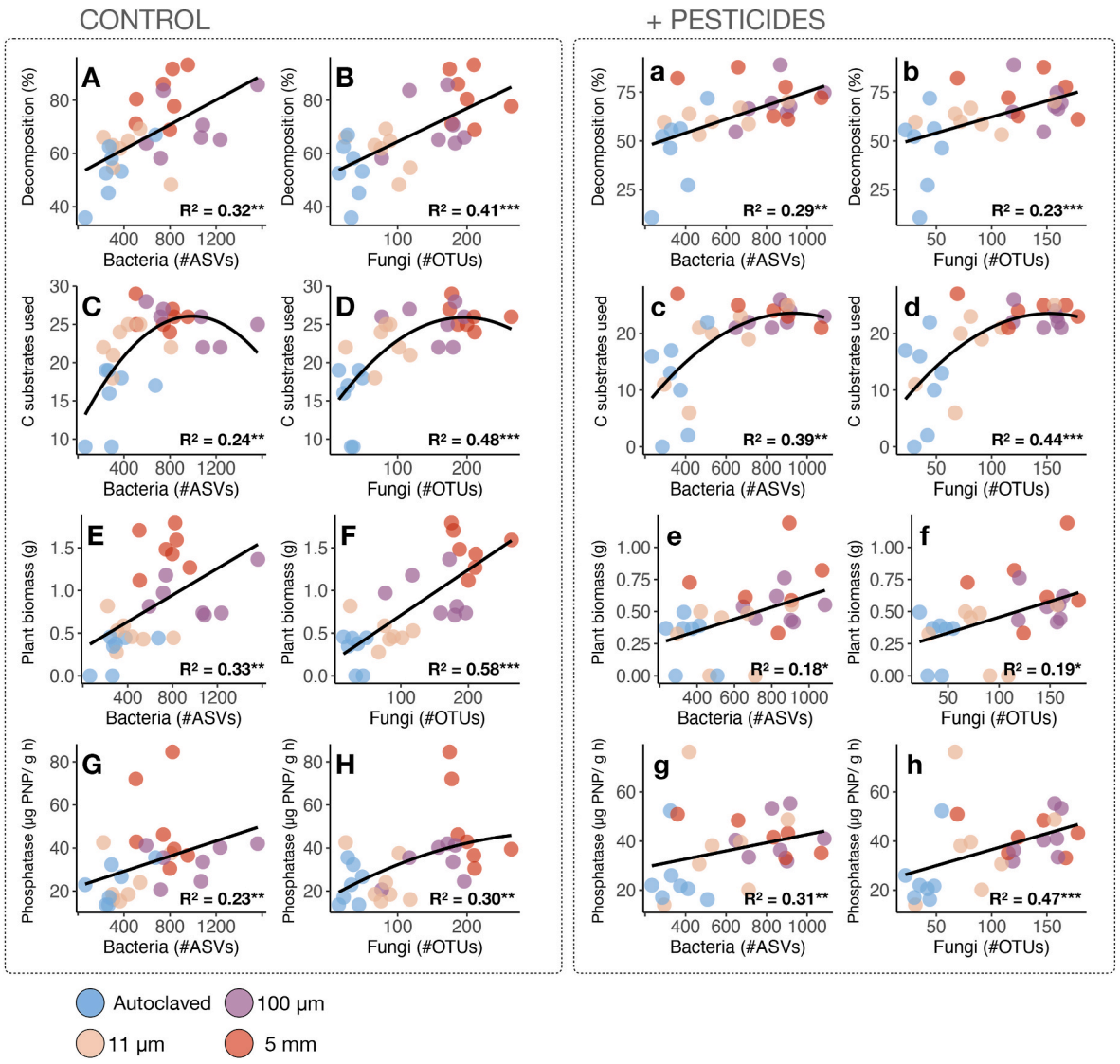

Fig. 2. Relationship between microbial richness (bacterial ASVs and fungal OTUs) and litter decomposition, C-substrate use, plant biomass production, and phosphatase activity. Left plots (A–H) indicate relationships for control mesocosms (no pesticides), right plots (a–h) indicate relationships for mesocosms receiving pesticides. Different colors indicate diversity treatments. Fit statistics are indicated as R^2 (0–1) and *p-values* are indicated with asterisks (***; *p-value* < 0.001, **; *p-value* < 0.010, *; *p-value* < 0.050). Non-significant (*p-value* > 0.05) regressions are denoted with "n.s.". Additional information on fitted linear and quadratic regressions is available in Table S3.

pesticides to explore the interaction between biodiversity and pesticide exposure. The main finding in this study is that differences in microbial diversity are more important than pesticide applications in determining plant growth and organic matter use in soil mesocosms. Furthermore, we found that pesticide presence in soil alters the contribution of microbial richness to soil function. Particularly, we observed that pesticides decreased the relative contribution of fungal richness to plant biomass production production and litter decomposition.

Our study shows that sequential sieving of an initial soil inoculum leads to established soil communities with decreasing diversity levels, in line with earlier work (Wagg et al., 2014, 2019). The highest levels of microbial diversity that we achieved in our mesocosms were ≈200 fungal OTUs and ≈1000 bacterial ASVs, while the lowest diversity values (≈32 fungal OTUs and ≈312 bacterial ASVs) often corresponded with mesocosms inoculated with autoclaved soil. These diversity values are in line with previous research (Pierre-Alain et al., 2018; Romero et al., 2023b), but are lower than other similar studies: for example, Wagg et al. (2021) employed a similar approach and retrieved up to 300 fungal and 3500 bacterial OTUs. In line with this, Chen et al. (2020) manipulated soil microbial diversity using the dilution-to-extinction approach and retrieved between 2000 and 4000 bacterial OTUs. The observed differences in microbial diversity across mesocosm studies arise from variations in experimental systems, soil inoculum origin, potential external contamination, duration of the experiment and the methods used for sequence processing and taxonomy assignment, among other factors (Delgado-Baquerizo et al., 2017; Pierre-Alain et al., 2018; Romero et al., 2023b). These variations underscore the need for more studies using diverse experimental systems and conditions. While main findings are consistent, these differences complicate cross-comparisons between studies. We suggest that future mesocosm studies should collect and mix soil from multiple sources to achieve broader biodiversity gradients.

In our study, plant biomass production, litter decomposition, and carbon substrate degradation decreased in mesocosms receiving autoclaved inoculum compared to mesocosms receiving soil inoculum sieved through 5 mm (i.e., highest diversity treatment), confirming our first hypothesis. Most studies exploring the relationship between soil diversity and organic matter degradation have relied on enzymatic

activities due to their standardized use and applicability (Pu and Liu, 2023). While this approach offers valuable insights, it typically targets specific molecules like hemicellulose or lignin, leaving out the complex array of compounds in plant litter (Kögel-Knabner, 2002). To address this limitation, we combined the use of enzymatic activities with litter bags and BIOLOG EcoPlates™ to assess microbial community metabolic diversity through the utilization of 31 different carbon sources (Gomez et al., 2006). Among the different substrates in the microplate (carbohydrates, carboxylic acids, and amino acids), we found that bacterial richness was positively associated with all substrate types, suggesting a broad metabolic versatility of bacteria. In contrast, fungal richness was only marginally associated with carboxylic acid degradation, reflecting their specialized roles in complex organic matter breakdown. This difference likely reflects the complementary ecological roles of bacteria and fungi in driving multiple soil functions (Wagg et al., 2014, 2019; Delgado-Baquerizo et al., 2017; Zhang et al., 2024). In our study, a one order of magnitude reduction in microbial alpha diversity was required to detect observable effects on soil functioning, and such a decline is unlikely to occur under typical field conditions (but see Muñoz-Arenas et al., 2020). Note however, that reduced abundance or disappearance of specific groups of soil microorganisms, such as arbuscular mycorrhizal fungi (Lutz et al., 2023) can have a strong impact on plant biomass production, and further studies are necessary to assess this. Likewise, we only assessed microbial diversity at one time point. In order to obtain a better overview of community dynamics, future studies should regularly monitor soil microbial diversity over time, to better assess the extent and ecological relevance of diversity losses in response to specific environmental perturbations.

Our study also found that organic matter decomposition increased with the richness of bacteria and fungi in the soil, as evidenced by reduced litter biomass remaining in the litter bags at the end of the experiment in soils with higher diversity of microbial taxa. Soil properties including microbial biomass were only slightly affected by our diversity treatments, suggesting that differences in organic matter degradation and plant growth were primarily driven by changes in soil diversity. We argue that bacteria and fungi in our systems degraded plant litter, and nutrients were either used by these microorganisms or absorbed by plants, as no significant changes in soil organic carbon or total nitrogen were observed. We observed, however, that pH was slightly higher in mesocosms with autoclaved soil compared to those with living communities, though high variation within treatments rendered the results non-significant. We suggest that higher pH in low-diversity mesocosms may result from a lack of respiration and decomposition, which would otherwise produce carbon dioxide and organic acids, thereby lowering soil pH (Aciego Pietri and Brookes, 2008).

Among the functions assessed, only plant biomass production responded to pesticide applications, partially confirming our second hypothesis. In line with our third hypothesis, we also found that the impact of pesticide exposure on the relationship between microbial richness and soil function varied by organism. Bacterial contribution to litter decomposition remained relatively stable under pesticide exposure, while fungal richness became decoupled from organic matter use. Similarly, fungal richness better predicted plant biomass production in control (pesticide-free) mesocosms compared to contaminated mesocosms. This suggests that in the presence of pesticides, increased fungal richness does not correspond to higher decomposition and plant biomass production, as it does in pesticide-free conditions. Several mechanisms could explain these differences: the pesticide mixture, which includes fungicides, may have disrupted fungal metabolism, leading the community to rely more on easy-to-degrade substrates. This shift likely occurs as the fungi allocate more resources to defense mechanisms rather than organic matter decomposition (Fernández et al., 2015). Additionally, bacteria's ability to use pesticides as a carbon source could enhance their tolerance and even benefit from pesticide presence (Aislabie and Lloyd-Jones, 1995). In line with this, we observed that bacterial richness was a better predictor of carbohydrate and amino acids degradation in mesocosms exposed to pesticides compared to control mesocosms. This might be due to the limited number of carbon sources (31) in the microplates, where a plateau in carbon source utilization is reached at around 1000 ASVs. In pesticide-exposed mesocosms, the maximum bacterial richness observed was around 1000 ASVs, resulting in a stronger association, while control mesocosms reached higher richness (up to 1600 ASVs) without further increase in carbon source utilization. Finally, although we used pesticides that are authorized for leek cultivation and applied them at recommended dosages, we cannot fully exclude the possibility of direct phytotoxic effects on plant growth. To minimize this risk, we applied the pesticide mixture three weeks after seedling emergence, following standardization of plant number per pot. We chose to apply all pesticides as a single mixture incorporated into the soil to ensure consistency across treatments, recognizing that applying each substance according to its specific agricultural guidelines would have introduced inconsistencies in mode (e.g., foliar vs. soil application) and timing of exposure (e.g., conditional application based on pathogen presence). Our primary objective was to target soil microbial communities, and we successfully induced a reduction in fungal diversity, with more limited effects on bacteria. Notably, this disturbance weakened the predictive link between diversity and soil functions such as plant biomass and litter decomposition. We therefore suggest that future studies should complement this approach with preliminary toxicity assessments to better disentangle direct chemical effects on plants from indirect effects mediated through soil biota.

Due to the size of our experimental systems, we excluded larger organisms such as invertebrates. We argue that future experiments should take into consideration the inclusion of soil fauna, as they represent a considerable proportion of soil biomass, play an indispensable role in organic matter degradation, and are known to be impacted by pesticides. Moreover, their presence can influence microbial diversity through top-down control, potentially affecting the outcomes of diversity–function relationships. (Petersen and Luxton, 1982; Anthony et al., 2023; Beaumelle et al., 2023). Likewise, future studies should build upon our results to test if generating the soil inoculum with methods not based on size organisms (e.g., dilution-to-extinction technique) lead to the same conclusions.

Finally, future studies should explore the interaction between soil microbial diversity and pesticides across soils from various land uses (e.g., arable lands, wetlands, forests) and climatic regions, as they host differing levels of microbial richness, and therefore contrasting responses to pesticides could be expected (Labouyrie et al., 2023, 2024). Overall, this study suggests that preserving the diversity of bacterial and fungal communities within the soil ecosystem is key to support essential soil functions such as organic matter degradation, and that pesticide applications could impair the beneficial effects of fungal richness on organic matter degradation.

CRediT authorship contribution statement

Ferran Romero: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shuo Jiao:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Marcel G.A. van der Heijden:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability

All sequences generated within this study are available at the Sequence Read Archive (SRA) database under BioProject accession number PRJNA1213498. Data on soil properties, measured functions, and microbial richness per mesocosm is available as a Supplementary Dataset. The R Code used to analyze data and generate all figures and

tables included in this study is available at https://github.com/fromerob/Romero-et-al-2025-SBB.git.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

M.G.A.v.d.H and F.R. acknowledge funding from the Swiss National Science Foundation (SWITZERLAND) through grant no. 310030–188799 and from the European Union Horizon 2020 research and innovation programme under grant agreement no. 862695 EJP SOIL-MINOTAUR. Data produced and analyzed in this paper were generated in collaboration with the Genetic Diversity Centre (GDC), ETH Zurich.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109866.

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