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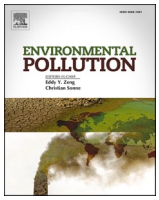

Transfer of prosulfocarb and boscalid residues from maize leaves to soil and their effects on soil microorganisms☆

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ABSTRACT

Plant materials that have been in contact with pesticides can be incorporated into the soil, posing a potential risk to non-target soil organisms and, hence, soil functions. This study investigated effects of two pesticides applied to maize leaves on the soil microbial community, activity and function. The herbicide prosulfocarb (PSC) and the fungicide boscalid (BSC) were applied alone or in combination to fresh or aged maize leaves, which were incorporated into soil. During a 56-day incubation we quantified pesticide residues in soil and maize leaves as well as maize-derived C incorporation into different microbial fractions (CO_2, extractable organic carbon, microbial biomass and main microbial groups). Prosulfocarb residues on maize and in soil decreased to below 5 % after 56 days. However, BSC residues were transferred from maize into the soil, as indicated by an increase in BSC residues in soil of around 15 %. Prosulfocarb initially inhibited the synthesis of soil bacterial phospholipids by 25–45 %, which was accompanied by a decrease in the incorporation of maize-derived C into microbial biomass by 68–70 %. Following this, microorganisms shifted their nutrient acquisition strategy towards carbon and phosphorus, which led to increased utilization of easily available maize-derived C. Boscalid transiently inhibited the growth of soil fungi, reduced soil respiration, and mineralization of maize. In the future, pesticide accumulation through transfer from plant material into soils and the mode of action dependent effects on soil microorganisms need to be considered for risk assessment.

1. Introduction

Intensively used agricultural fields are frequently treated with pesticides to control pests and to ensure both harvest quality and quantity. Their use can result in pesticide dispersal to other compartments of the environment where they may pose a hazard to non-target organisms, e.g., in soil and aquatic ecosystems (Abrantes et al., 2010; Karpouzas et al., 2016). Recent studies have found that pesticide residues are frequently detected in agricultural soils, including organic farming systems (Riedo et al., 2021; Froger et al., 2023; Silva et al., 2023). Pesticide residues are regularly found on vegetables and fruits, whereas stems, leaves and other plant parts can remain on the fields and are eventually incorporated into the soil. This can facilitate a transfer of pesticide residues from plant remnants to the soil, potentially increasing their residue levels in soils.

There, non-target soil microorganisms are also subjected to pesticide exposure. While microorganisms are capable of degrading various pesticides (Helweg et al., 1998; Jacobsen et al., 2008), thereby yielding energy or nutrients, they might simultaneously be exposed to toxic effects from pesticides (Karpouzas et al., 2016). The extent of these effects can vary with concentration, chemical characteristics and application frequency of the pesticides (Wu et al., 2014; Zhang et al., 2016; Wirsching et al., 2020), as well as with abiotic variables such as soil properties (Nguyen et al., 2016). In addition, the mode of action describes the mechanistic effects of the pesticides on the target organisms. However, they can also potentially interfere with processes present in non-target soil microorganisms. While some of these interactions have been investigated, mainly for fungicides (Yang et al., 2011; Yao et al.,

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2022), many modes of action have not been studied yet for their interference with non-target processes. By altering the abundance and composition of the soil microbial community, pesticides can also disrupt essential ecosystem functions provided by soil microorganisms, including key soil processes such as litter degradation and nutrient cycling regulation (Madsen, 2011; Hobara et al., 2014). Riah et al. (2014) reviewed the effects of pesticides on enzymes involved in carbon (C)-, nitrogen (N)- and phosphorus (P)-cycling and found both inhibition and stimulation of enzyme activities depending on the pesticide applied. Other studies found decreases in soil enzymatic activities after pesticide application (Sahu et al., 2014; Sanchez-Hernandez et al., 2017; Rouhi-Kelarlou et al., 2024).

Agricultural practices often include the application of multiple pesticides, either simultaneously or sequentially. While most studies focus on the application of one pesticide, a combination of pesticides might have interactive effects on soil microorganisms. Meidl et al. (2024) investigated the impact of up to ten pesticides in combination on soil processes and found negative effects in combination treatments, whereas single applications showed no or positive effects. They suggested potential interactive behaviors among pesticides, highlighting the importance of assessing pesticide mixtures. Similarly, we previously observed interactive, synergistic, as well as antagonistic effects of up to three pesticides on the soil microbial community and the degradation of glyphosate (Mäder et al., 2024).

Plant materials that are incorporated into agricultural fields may increase pesticide levels in soil by transferring pesticide residues into the soil during the degradation of the plant material. Yet, to our knowledge, this pathway has not been investigated yet. Additionally, this might be important when considering the bioavailability of the pesticide, which depends on adsorption/desorption properties of soils and organic substrates, and governs the contact of microorganism with the pesticide (Zabaloy et al., 2011). Also, during the degradation of the organic material, dissolved organic matter can play a minor role by competing with pesticides for binding sites, thereby increasing its bioavailability (Spark and Swift, 2002). At the same time plant materials are also able to stimulate the degradation of pesticides due to co-metabolic activities of soil microorganisms (Tran et al., 2013; Siedt et al., 2021). This effect was measured for the dissipation of MCPA and other herbicides (Saleh et al., 2016; García-Delgado et al., 2018). While the additional nutrients from the plant material might increase the degradation of the pesticides, the quality of the plant material plays an important role for the interactions. Differences in the quality of organic amendments affect the microbial community and along with these changes, both directly and indirectly, the fate of pesticides in soils (Oleszczuk et al., 2014; Safaei Khorram et al., 2016; Egamberdieva et al., 2021). As such, simple substrates generally increase microbial activity and pesticide degradation, whereas more complex substrates reduce bioavailability through adsorption, thereby reducing the toxic effects of pesticides (Mukherjee et al., 2016; Siedt et al., 2021). These studies have focused on the effects of organic amendments on pesticide degradation, but the effect of pesticides on litter degradation itself has been less frequently investigated. Recently, Meidl et al. (2024) found a decrease in litter decomposition rate in the presence of five and ten pesticides, which they attributed to synergistic effects of the pesticides on microbial function. When applied as seed dressings, pesticides had mixed results, with either no effect or a negative effect on litter degradation (Zaller et al., 2016; van Hoesel et al., 2017).

This study aimed to investigate the effects of two pesticides applied alone or in combination on the soil microbial community and their activity in the presence of maize leaves that differed in substrate quality. We aimed to improve the mechanistic understanding of these interactions using model pesticides with modes of action that could potentially affect non-target soil organisms. These pesticides were applied to maize leaves to simulate the incorporation of agricultural crop residues exposed to pesticides into the soil and to investigate the impact of a possible transfer of pesticide residues into the soil. Maize leaves were used as a proxy for residues of an agricultural crop due to its natural ^{13}C enrichment, which allowed us to track the carbon utilization by microorganisms. Two substrate qualities were used to investigate if this affects the effects of pesticides on soil microorganism due to changes to microbial functioning. Fresh litter contains a broad spectrum of organic substrates, ranging from easily available (e.g., sugars) to more recalcitrant compounds (e.g., lignin). In contrast, artificially aged litter provides a reduced fraction of easily available compounds (Jian et al., 2016). We hypothesized that (I) pesticide residues can be transferred from maize leaves into soil during degradation of the maize leaves. We further hypothesized that (II) fresh maize leaves mitigate pesticide effects on microbial activity and community structure by supplying easily available C in contrast to aged maize, which provides lower nutrient availability especially in the initial decomposition stages. Regarding pesticide specific effects, we hypothesized that (III) the mode of action of the pesticides is not limited to their target organisms, but can affect non-target soil microorganisms according to their mode of action. We selected the herbicide prosulfocarb (PSC) (further details in Supp. Table 1) due to its adsorption to organic matter (Nègre et al., 2006) and its mode of action as a lipid synthesis inhibitor (EFSA, 2007), where we hypothesized that (IV) this mode of action can inhibit the formation of soil microbial phospholipid fatty acids (PLFAs). As a second pesticide, the fungicide boscalid (BSC) (further details in Supp. Table 1) was selected due to its widespread use and frequent detection in European soils (Riedo et al., 2021; Sabzevari and Hofman, 2022; Silva et al., 2019). We hypothesized that (V) BSC would inhibit soil respiration, due to its mode of action as a succinate dehydrogenase inhibitor (Avenot and Michailides, 2010). Both pesticides represent model substances that are used for both pre- and post-emergent treatments and were tested in this study due to their characteristics.

2. Materials and methods

2.1. Soil and maize preparation

We used the standard soil 2.2 from LUFA Speyer (Speyer, Germany) classified as sandy loam (pH 5.6, SOC 1.84 %) and with no prior history of maize cultivation or pesticide application. The soil was sieved to 2 mm, stored at 4 °C and preincubated for one week prior to the start of the experiment in the dark at 20 °C. Air dried organic maize leaves were cut into 5 × 5 mm pieces. Half of the maize leaves received no further treatment and were stored in the dark, those will be referred to as fresh maize. The other half of the maize leaves was submerged in $H_2O_{deion.}$ (1 g maize leaves in 100 ml) for 24 h, drained and refilled for two additional 24 h cycles. This was done to simulate an organic substrate after early aging processes, where easily soluble compounds are leached (Poll et al., 2008). These maize leaves will be referred to as aged maize, a simplified term that describes its difference to the fresh maize. Both aged and fresh maize were dried at 40 °C to achieve similar starting conditions and stored in the dark until used. The leachates obtained after each 24 h cycle were measured on the TOC-TNb Analyzer Multi-N/C 2100S (Analytik Jena, Jena, Germany). The leachates of the aged maize contained 69, 12.5 and 3.6 μg C ml^{-1} as well as 8.5, 1.6 and 0.8 μg N ml^{-1} after 24, 48 and 72 h, respectively. The dried maize leaves were ground, weight into tin capsules and measured on an elemental analyser (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany), where C and N content as well as the $\delta^{13}C$ content of both fresh and aged maize was obtained. The C/N ratio of the maize increased from 12.5 in the fresh maize to 14.7 in the aged maize after the third washing cycle.

2.2. Experimental setup

Either fresh or aged maize (0.52 or 0.5 g) was weighed into glass microcosms (volume: 500 ml) into which either boscalid (2 mg kg^{-1} DW soil), prosulfocarb (20 mg kg^{-1} DW soil), or their mixture (2 + 20 mg

kg^{-1} DW soil, respectively) diluted in acetone was applied directly onto the fresh or aged maize. The applied concentrations were calculated based on the active ingredient (ai) of the commercial products with their respective application recommendation for a single application event (BSC: Filan®, 50 % w/w boscalid, 0.5 kg ha^{-1} maximum individual dose and PSC: Prosulfocarb 800 EC, 800 g ai L^{-1}, 3 L ha^{-1} application rate) and the bulk density of the soil (1.21 g cm^{-3}) calculated per ha per centimeter depth of soil (see Supp. Table 2). Maize weights were chosen to cover the whole bottom of the microcosms, allowing for proper treatment with the pesticides, and are slightly higher than in the OECD 216 test for lucerne meal (5 g kg^{-1}) and adjusted to compensate for C content differences. The weights correspond to approx. 1.13 and 1.18 t ha^{-1}. An additional treatment with acetone and without pesticides was used as control. After 24 h, allowing the acetone to evaporate, 80 g dry weight (DW) soil was mixed with either aged or fresh maize in the microcosms, and $H_2O_{deion.}$ was added to reach a gravimetric soil water content of 15 %. All microcosms were closed air-tight with a rubber seal. A total of 164 microcosms were used, consisting of 4 replicas for the 8 treatments on 5 sampling days plus 4 empty microcosms used as blanks. Microcosms were placed in the dark at 20 °C for 56 days. On days 3, 7, 14, 28, and 56, four replicas per treatment were removed and sampled destructively. All microcosms were aerated once per week. The maize was separated from the soil via sieving and hand picking and both soil and maize were stored separately at −20 °C.

2.3. Pesticide residues

The extractions of BSC and PSC were performed following an adaption to the QuEChERS method as described by Silva et al. (2019). These included only the extractable parent compounds. Briefly, 1 g post-incubation soil or 0.5 g post-incubation freeze-dried maize was mixed with 10 ml acetonitrile, 5 ml $H_2O_{deion.}$ and an internal standard. Samples were shaken for 15 min at 900 rpm and QuEChERS salts (Agilent, Santa Clara, CA, USA) were added. After manual shaking for 10 s, samples were placed in an ice bath, followed by shaking (1 min. 900 rpm) and centrifugation (5 min at 3000 rpm). Supernatants were diluted with acetonitrile and measured using an Agilent 1200 chromatographic system (Agilent, Santa Clara, CA, USA) connected to an ESI/QqQ mass spectrometer Agilent Triple Quad 6410 (Agilent, Santa Clara, CA, USA). The non-detectable pesticide fraction was calculated by subtracting the extracted residues from maize and soil from the initially added amount and includes non-extractable residues, transformation and mineralization products, and analytical losses.

2.4. Microbial respiration

Titration (DIN EN ISO 16072:2011–09) was used to measure soil microbial respiration. Carbon dioxide traps containing 2 ml of 1 M NaOH were fitted to the lids on the microcosms that would be sampled on the last day. On each respiration measurement day (1–7, 9, 11, 12, 14, 16, 18, 21, 23, 25, 28, 32, 36, 42, 49 and 56), 0.5 ml of the NaOH were mixed with 0.5 ml 1 M $BaCl_2$ and titrated with 0.1 M HCl solution to neutral using phenolphthalein as indicator. The remaining NaOH was removed from the CO_2 trap and refilled with fresh 1 M NaOH before sealing the container. For determination of the $\delta^{13}C$ content in CO_2, the remaining 1.5 ml NaOH were mixed with 1.5 ml 1 M $BaCl_2$ and 40 ml of H_2O_{deion}. The solution was vortexed, centrifuged and after the supernatant was discarded, and washed with $H_2O_{deion.}$ two additional times. After drying at 60 °C, the precipitated $BaCO_3$ was weighted into tin capsules and $\delta^{13}C$ was measured on an elemental analyser (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany).

2.5. Microbial biomass

Microbial biomass C (C_{mic}) was analyzed via chloroform fumigation extraction (CFE) (Joergensen, 1996). In short, 10 g post-incubation soil was put into a chloroform atmosphere (fumigation) under vacuum for 24 h and subsequently extracted using 40 ml 0.025M K_2SO_4 solution. As background, 10 g of field fresh soil was directly extracted with the same solution. Samples were measured on the TOC-TNb Analyzer Multi-N/C 2100S (Analytik Jena, Jena, Germany). Values were converted to microbial biomass carbon using a factor of 0.45, giving the extractable fraction of total microbial biomass C (Joergensen, 1996). The measurement of the unfumigated samples was additionally used to calculate the extractable organic carbon (EOC). Extracts were concentrated in a rotary evaporator (RVC 2–25, Christ, Osterode am Harz, Germany) and the resulting salt was weighed into tin capsules. The $\delta^{13}C$ content was determined using an elemental analyzer (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany).

2.6. Phospholipid fatty acid analysis

Phospholipid fatty acid analysis was performed according to Frostegård et al. (1991). Briefly, 4 g post-incubation soil was extracted with chloroform, methanol, and citrate buffer (pH 4) (1:2:0.8 v/v/v) (Bligh and Dyer, 1959). Using silica acid SPE cartridges (Bond Elut SI, 500 mg, 3 ml, Agilent Technologies Inc, Santa Clara, CA, USA) the extract was fractionated such that only phospholipid fatty acids were collected. Using an alkaline methanolysis (Kramer et al., 2012), these were transformed into fatty acid methyl esters (FAMEs) and measured on an Agilent 8860 gas chromatograph with a 5977B mass selective detector (MSD) (Agilent, USA). The abundance of specific PLFA markers was indicative for the respective microbial groups. In accord with Ruess and Chamberlain (2010) we classified FAMEs i15:0, a15:0, i16:0, and i17:0 as gram positive bacteria (G+), cy17:0 and cy19:0 as gram negative bacteria (G-), and the sum of G+ and G- markers together with 16:1ω7 as the total bacterial FAMEs. Abundance of fungi was represented by the FAME 18:2ω6,9. A four-step fractionation was carried out to determine the $\delta^{13}C$ content in the PLFAs using n-hexane and acetone in increasing concentrations (99:1; 96:4; 90:10; 0:100, v/v) in Ag+-SPE cartridges (6 ml, Supelco, Palo Alto, USA) according to Kramer et al. (2012). This process removed monoenoic trans- and cis-FAMEs (2nd and 3rd fractions) while retaining the saturated (1st fraction) and dienoic FAMEs (4th fraction). Samples were measured on a gas chromatograph (6890 series, Agilent Technologies, Santa Clara, CA, USA) coupled to a gas chromatography incinerator III Interphase (Thermo Finnigan, Waltham, MA, USA) connected to a Delta Plus XP mass spectrometer (Thermo Finnigan MAT, Bremen, Germany).

2.7. Enzymatic activity

The activities of four enzymes involved in C-, N-, and P-cycling were determined according to Marx et al. (2001) using fluorogenic 4-methylumbelliferone (MUF) substrates. They were β-glucosidase (BG) (EC 3.2.1.21), β-xylosidase (XYL) (EC 3.2.1.37), N-acetyl-β-glucosaminidase (NAG) (EC 3.2.1.52) and acid phosphatase (AP) (EC 3.1.3.2). Substrates as well as MES buffer were prepared according to Poll et al. (2006). One gram post-incubation soil was mixed with 50 ml $H_2O_{deion.}$ and dispersed using an ultrasonic disaggregator (50 J s^{-1}) for 2 min. While stirring on a magnetic stir plate, 50 µl of the suspension was transferred into 96-well microplates (PP F black 96 well, Greiner Bio-one GmbH, Germany) to which 50 µl of 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer and 100 µl of the enzyme-specific substrate solution were added. Standards were created using 50 µl of the soil suspension with 0, 100, 200, 500, 800 or 1200 pmol MUF $well^{-1}$, yielding a final volume of 200 µl. After 30 min pre-incubation in the dark at 30 °C, the plates were exposed to a wavelength of 360 nm using a microplate fluorescence reader (Bio-Tek Instruments Inc., FLX 800, Germany) and fluorescence intensity was measured at 460 nm. For temporal resolution, the fluorescence was measured at 0, 30, 60, 120 and 180 min. Enzymatic activity (in nmol g^{-1}

h^{-1}) was calculated using the linear correlation between the measured fluorescence intensity and the enzymatic activity of the standards.

2.8. Metabolic quotient, carbon use efficiency, specific enzymatic activity and enzyme activity vector length and angles

The metabolic quotient (qCO_2), commonly used as an indicator of environmental stress (Anderson and Domsch, 2010), was calculated by dividing the amount of CO_2-C produced by the microbial biomass C following Anderson and Domsch (1985) with equation (1):

$$qCO_2 = \frac{Respiration\text{-}C}{Biomass\text{-}C} \quad (1)$$

Carbon use efficiency (CUE), used to quantify how carbon from a specific source is allocated between respiration and growth (Manzoni et al., 2012), was calculated following Geyer et al. (2016) with equation (2):

$$CUE = \frac{^{13}C_{mic}}{^{13}C_{mic} + ^{13}CO_2} \quad (2)$$

where $^{13}C_{mic}$ represents the amount of maize-derived C in the microbial biomass and $^{13}CO_2$ the amount of maize-derived C in the evolved CO_2.

Specific enzymatic activities (SEA), providing a microbial biomass independent measure of enzymatic activity, were calculated for the four enzymes by dividing the enzymatic activity by the microbial biomass C following Waldrop et al. (2000) with equation (3):

$$SEA = \frac{E}{C_{mic}} \quad (3)$$

where E represents the enzyme activity of a given enzyme (BG, XYL, NAG or AP).

Vector lengths and vector angles were calculated according to Moorhead et al. (2016), to quantify relative C versus nutrient limitation and relative P versus N limitation, respectively (Moorhead et al., 2016) using equations (4) and (5), respectively:

$$Vector\ length = \sqrt{x^2 + y^2} \quad (4)$$

$$Vector\ angle = \text{atan } 2(x, y) \quad (5)$$

where x represents the ratio of the activity of C-acquiring enzymes (BG + XYL) and P-acquiring enzymes (AP) and y represents the ratio of activity of C- and N-acquiring enzymes (NAG). Higher vector lengths indicate higher C limitation, whereas smaller vector lengths indicate less C limitation. Higher vector angles suggest comparable higher P limitation and smaller vector angles N limitation (Moorhead et al., 2016).

2.9. Statistics

All statistical analyses were performed using R 4.2.2 (R Core Team, 2022). Normality was tested by graphical inspection of QQ plots and the Shapiro-Wilk test; variance homogeneity was tested using the Levene's test. For all respiration-related data, linear mixed effect models were applied with the package *nlme* (Pinheiro et al., 2023), where the microcosms were used as random factor to account for repeated measures. For all other data, we applied a linear model with the R function *lm* from the package *stats* (R Core Team, 2022). For both, fixed factors consisted of sampling day, maize treatment and the presence of BSC and/or PSC. Using the R function *anova* from the package stats (R Core Team, 2022), F and p-values were extracted from ANOVAs applied to the models. Tukey tests were performed using the *TukeyHSD* function from the package *stats* (R Core Team, 2022) for individual days and maize treatments.

3. Results

3.1. Extractable pesticide residues

Extractable residues of PSC in soil decreased from 26 to 33 % of the initially applied amount to 1–6 % after 56 days, while residues on both aged and fresh maize decreased from 28 to 36 % to <1–2 % during the same period (Fig. 1a). An initial lag phase in PSC dissipation in soil was observed which lasted until Day 14, while PSC residues on maize decreased rapidly during the first days of the incubation. Compared to PSC, BSC residues (Fig. 1b) were more persistent. Boscalid residues decreased in both maize treatments from 52-73 % to 12–27 %, while in soil they increased from 22-27 % to 37–44 % of the initial applied concentration. In the mixed treatment of BSC with PSC, the decrease in BSC residues on fresh and aged maize exhibited a lag phase, similar to PSC, which also lasted until Day 14. The maize quality had no detectable effect on the pesticide residues.

3.2. Extractable organic carbon and maize quality

Maize treatments differed in their EOC contents (Supp. Fig. 1), with initially almost double the EOC in fresh maize as compared to aged maize. While EOC in fresh maize remained fairly constant throughout the experiment, EOC levels in aged maize increased until Day 14. The presence of the pesticides tended to increase EOC in aged maize on Day 56 but had no effect on EOC in fresh maize. The EOC of the pesticide free treatment tended to be higher than that of the pesticide treatments in fresh maize on Day 56, but was lower on Day 56 for the aged maize.

The maize-derived C in EOC (Supp. Fig. 2) was higher in fresh maize until Day 14, dropping to lower values and slowly decreasing towards the end of the experiment. In the aged maize, an initial increase of maize-derived C could be observed, reaching levels comparable to fresh maize by Day 28. The maize-derived EOC in the aged maize was initially 84–97 % lower than the maize-derived EOC in the fresh maize. Only BSC had transient effects on the maize-derived C in EOC with an increase in aged maize on Day 3 and in fresh maize on Day 7. Prosulfocarb alone and in combination with BSC, decreased the maize-derived C in EOC on Day 14–50 % of the control. For aged maize, PSC increased values on days 7 and 14. The interaction of the two pesticides tended to mainly follow the patterns of PSC, with similar changes compared to the control on various days throughout the experiment. The maize-derived EOC decreased on Day 14 in the fresh maize to the same level as the single PSC treatment. Contrary the mixture treatment did not increase the maize-derived EOC on Day 28, where the single PSC treatment increased it, therein being on the same level as the single BSC treatment and the control. In the aged maize treatment on Day 14, the maize-derived EOC increased to similar levels as the single PSC treatment.

3.3. Soil microbial community and activity

The pattern of CO_2 release differed strongly in both maize treatments: CO_2 release from fresh maize peaked early (Day 2), whereas the aged maize showed a maximum CO_2 release in a later phase of incubation (11–14 days) (Fig. 2, Supp. Fig. 3). Boscalid initially decreased the respiration rate significantly, especially in aged maize up to Day 9. In contrast, PSC initially increased the respiration rate especially in the aged maize. This led to significantly higher cumulative CO_2 production in PSC and lower cumulative CO_2 production in BSC in aged maize compared to the control. Microbial biomass remained constant in fresh maize, whereas it decreased in the first two weeks in aged maize and recovered by Day 56 (Supp. Fig. 4). The presence of PSC significantly decreased the microbial biomass in fresh maize on Day 7 by 8 %, and decreased it in the aged maize treatments on Day 56 by 14.6 %. Boscalid tended to decrease qCO_2 (Supp. Fig. 5) for days 3 and 14 in fresh, and on days 3 and 7 in aged maize. Prosulfocarb temporarily increased qCO_2 for fresh maize on Day 7 by 11 %. The abundance of G+, G- (Supp.

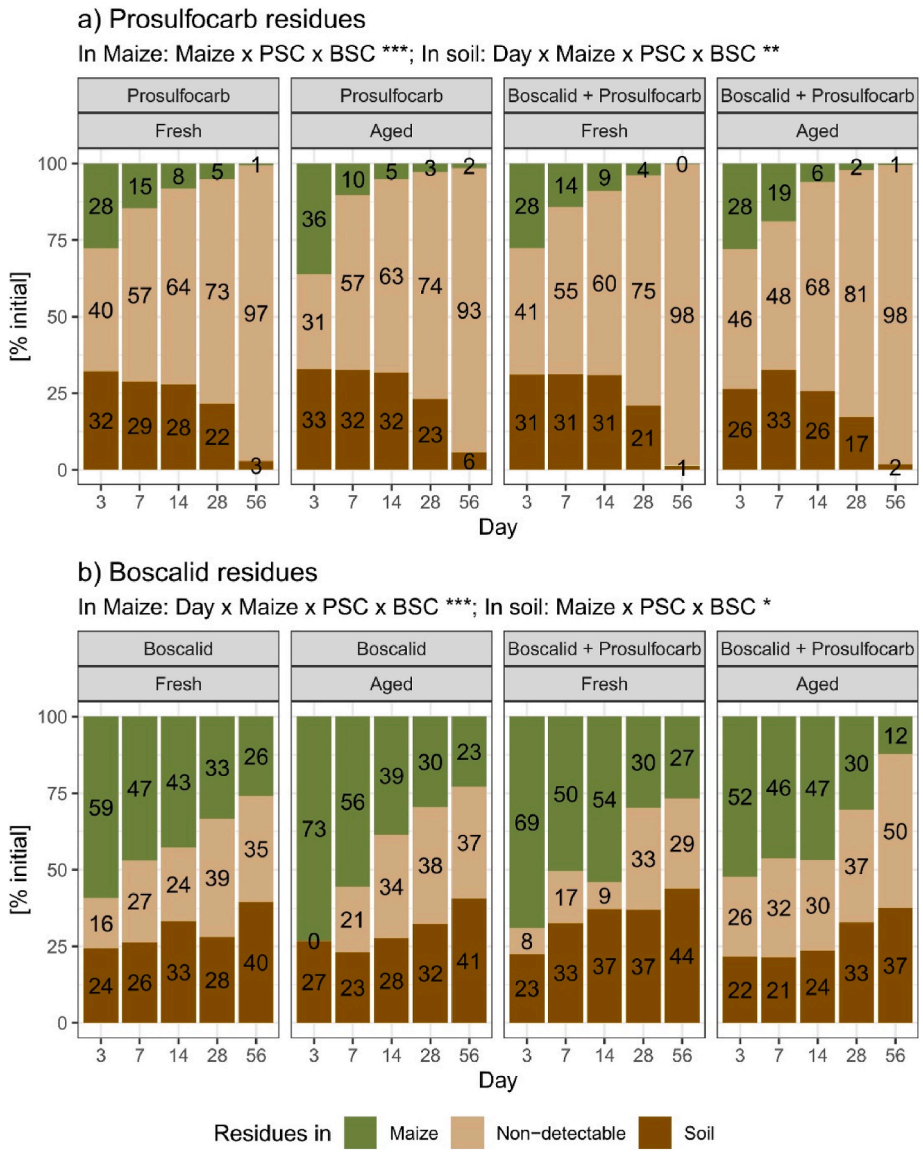

Fig. 1. Pesticide residues of a) prosulfocarb (PSC) and b) boscalid (BSC) as % of the initially applied concentration over the 56 days of incubation in/on fresh and aged maize, soil and the non-detectable fraction (n = 4).

Figs. 6–7) and fungal FAMEs (Fig. 2) was higher in fresh maize compared to aged maize throughout the experiment. Prosulfocarb tended to reduce the abundance of G+ and G- FAMEs on days 7 and 28 compared to the control in aged maize and in fresh maize on Day 28. While PSC affected the bacterial groups, BSC initially decreased the fungal FAME in fresh maize until Day 14 (Fig. 2) by 18 % on Day 3 and 14.7 % on Day 7, after which its abundance increased in the BSC treatments for the remainder of the incubation compared to the control.

3.4. Utilization of maize

The rate of respired maize-derived C (Supp. Fig. 8) was higher in fresh maize until Day 14, after which the rates were higher in aged maize. Consequently, the cumulative maize-derived C (Supp. Fig. 9) respiration exhibited an initial lag phase in the aged maize while in fresh maize a sharp increase was observed. Boscalid tended to a negative effect on the cumulative maize-derived C respiration for both maize treatments.

The incorporation of maize-derived C into microbial biomass (Fig. 3 a, Supp. Fig. 10) was significantly higher in fresh maize compared to aged maize, except for Day 7 where PSC containing treatments decreased towards values of the aged maize. The presence of PSC increased the incorporation of maize-derived C in aged maize following Day 14, whereas in fresh maize transient fluctuations were observed on days 7 and 14 with a decrease by 68 % (PSC) and 70 % (PSC + BSC), and an increase by 22 % (PSC) and 32 % (PSC + BSC). In fresh maize, incorporation of maize-derived C into microbial biomass in both the single PSC treatment and the mixture treatment first decreased and then increased compared to the control. Boscalid however, showed no clear effects. Prosulfocarb decreased CUE (Supp. Fig. 11) on Day 7 in the fresh maize and increased it on Day 14, whereas in aged maize, an increase on both days 7 and 14 was observed.

Incorporation of maize-derived C into the G+ and G- FAMEs (Fig. 3 b + c, Supp. Figs. 12 + 13) was generally higher in fresh maize. After an initial increase of G+ in the presence of the pesticides, PSC treatments decreased the incorporation of maize-derived C in fresh maize (-25 % for PSC at Day 7, -25.8 % for PSC + BSC at Day 56), but had no effect in aged maize. The patterns observed for G+ in fresh maize were less pronounced in G-; however, decreases under PSC alone on Day 7 and in the mixture on Day 56 were greater, decreasing by 45 % and 40 %, respectively. In contrast to G+, the incorporation of aged maize-derived C into G-increased with the pesticide treatments following Day 14 by up

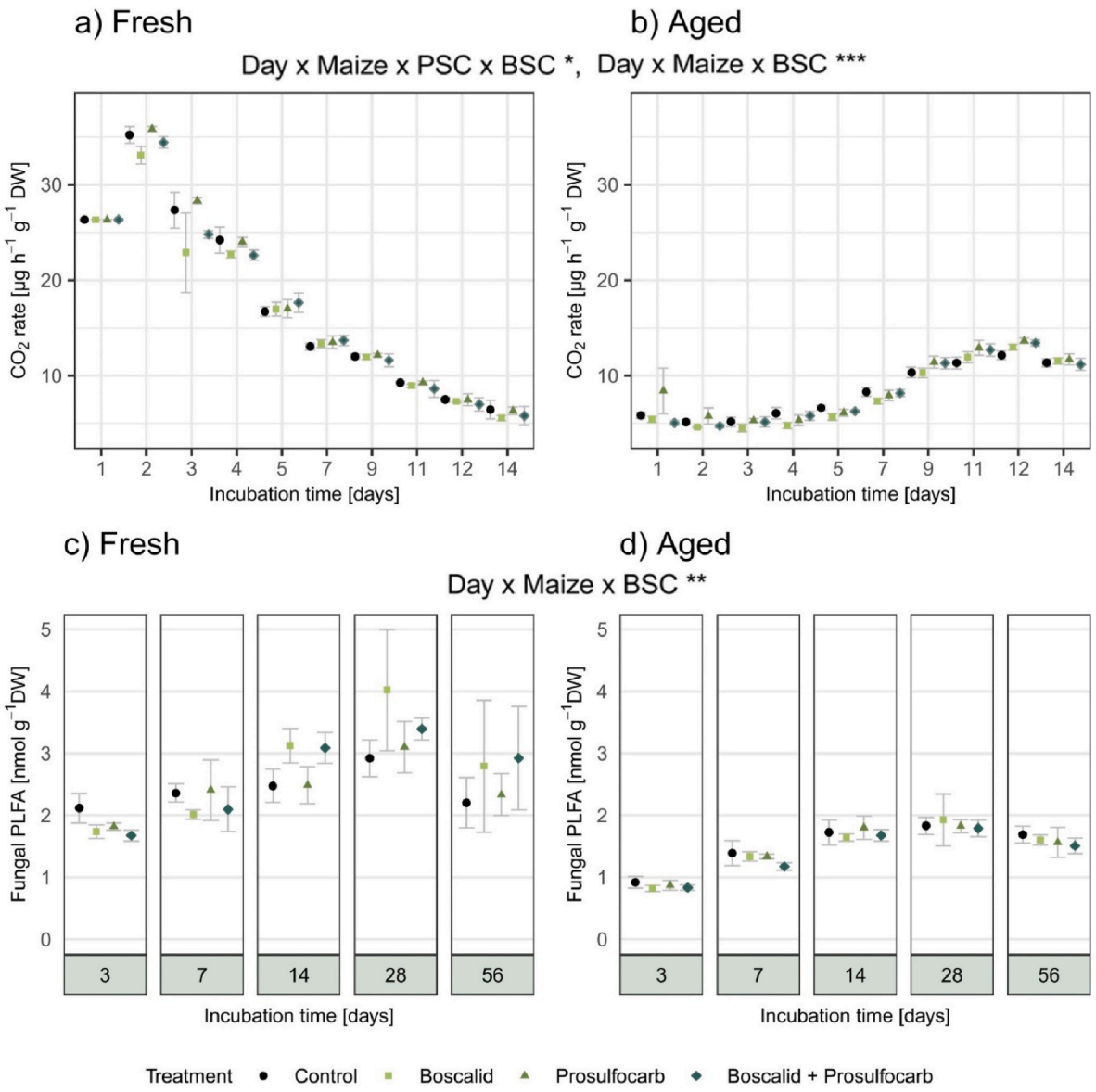

Fig. 2. Microbial parameters mainly responding to the addition of boscalid. Respiration rate during the first 14 days for a) fresh and b) aged maize (n = 4). Fungal phospholipid fatty acid abundance in c) fresh and d) aged maize (n = 4). Error bars indicate standard deviation. PSC = prosulfocarb, BSC = boscalid. Significance codes: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

to 2.3-fold. Prosulfocarb appeared to have a greater impact on aged maize incorporation by fungi compared to its effect in fresh maize (Supp. Fig. 14), with decreases on days 3 and 28. In fresh maize no clear patterns were observed concerning the studied bacterial groups.

3.5. Enzymatic activity

Enzymatic activities of BG, XYL, and NAG (Supp. Figs. 15–17) were higher in fresh maize than in aged maize. In contrast the activity of AP (Supp. Fig. 18) was slightly increased in aged maize on several days throughout the experiment.

Prosulfocarb increased the activity of BG and AP on different days throughout the experiment. For both BG and AP on Day 7 and for BG on Day 14, this increase was observed in both the single and mixed treatments. Further, these increases tended to be more pronounced in the fresh than the aged maize treatment. In line with this, the SEAs of BG, XYL and AP (Supp. Figs. 19–21) increased in the presence of PSC on Day 7. Boscalid had only minor effects on the enzymatic activity and the SEA, with transient increases in BG and transient decreases in NAG (Supp. Fig. 22).

Vector lengths (Fig. 3 d, Supp. Fig. 23), quantifying relative C to nutrient limitation increased in pesticide treatments for the fresh maize until Day 14. Vector angles (Supp. Fig. 24), quantifying relative P to N limitation were not significantly different in the presence of the pesticides. Vector angles were higher for the aged maize compared to the fresh maize.

4. Discussion

This study showed a potential transfer of BSC from maize to soil, which to our knowledge has not been reported to date. Both pesticides investigated in this study affected the soil microorganisms according to their mode of action, leading to changes in abundance, activity and function.

4.1. Pesticide residues

Typical characteristics of pesticides that determine their fate in soils

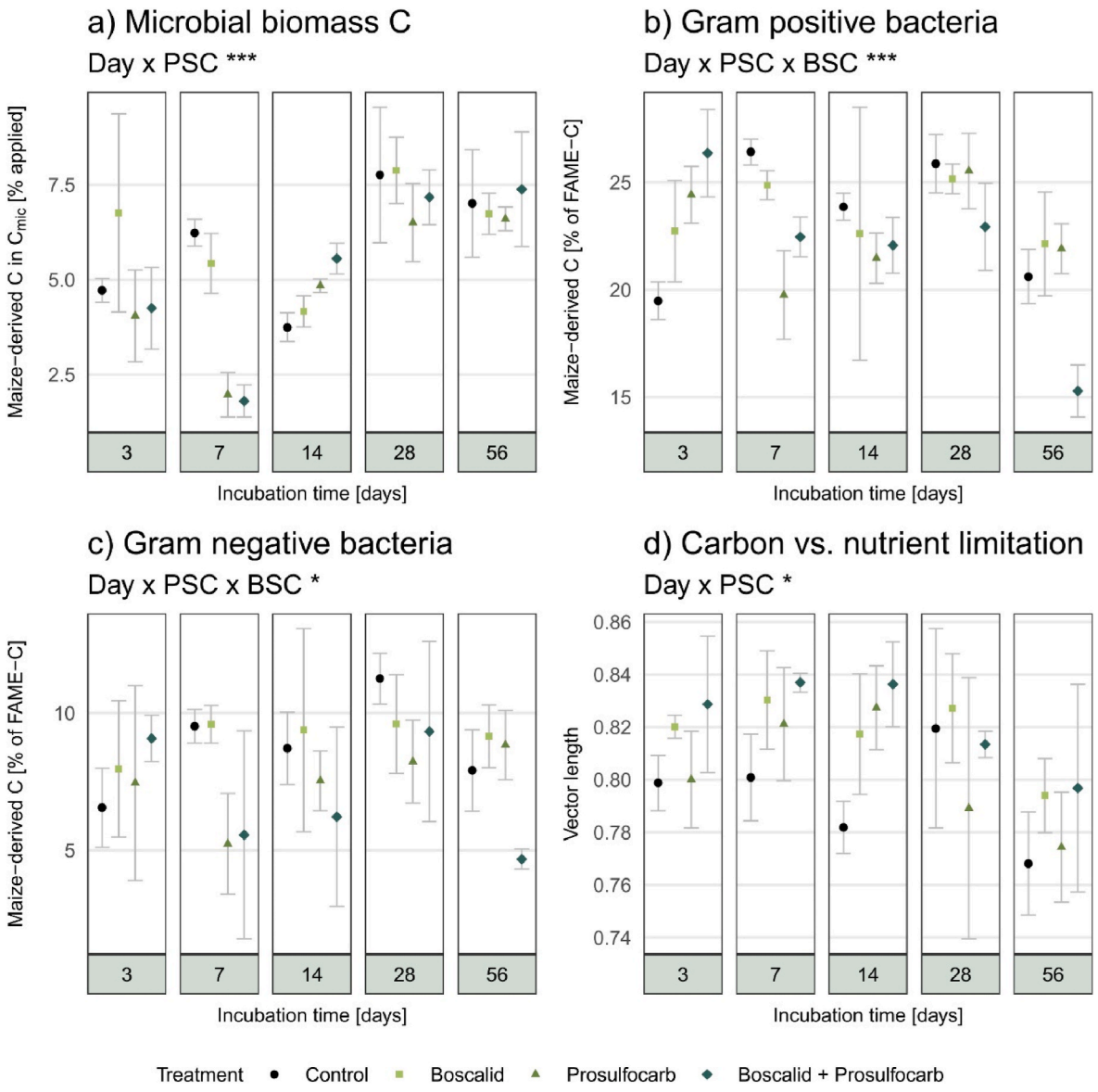

Fig. 3. Microbial parameters mainly responding to the addition of prosulfocarb. Amount of maize-derived C in the fresh maize treatment for a) the microbial biomass, b) the gram positive bacterial FAMEs and c) the gram negative bacterial FAMEs as well as d) the vector length as indicator for carbon vs. nutrient limitation over the 56 days incubation (n = 4). PSC = prosulfocarb, BSC = boscalid. Error bars indicate standard deviation. Significance codes: $p < 0.05$*, $p < 0.01$**, $p < 0.001$***.

are adsorption and desorption mechanisms, which affect their bioavailability (Gavrilescu, 2005; Barchańska et al., 2020). Here, the functional groups of a pesticide play an important role in adsorption (Sadegh-Zadeh et al., 2017). Adsorption and desorption are further influenced by soil properties such as the content of organic matter or clay content which serve as sorption sites (Li et al., 2003; Barchańska et al., 2020). These parameters, together with the chemical structure of the pesticide, can influence the bioavailability and consequently its biodegradability by microorganisms (Sánchez et al., 2004; Gavrilescu, 2005).

As such, the residues of the readily degradable pesticide prosulfocarb (PSC) decreased both in soil and on the maize. We observed a lag phase in the decrease of residues in the soil up to Day 28, regardless of maize quality or co-presence of boscalid (BSC). This was also observed by Barba et al. (2019) who reported a lag phase of 9–14 days due to initial sorption of this hydrophobic pesticide. While visible for soil, such a sorption effect was not visible on the maize, probably due to stimulated co-metabolic PSC degradation in the vicinity of maize. The presence of plant litter has been shown to enhance the degradation of pesticides (Pagel et al., 2016; Nowak et al., 2020), which is primarily caused by a general increase in microbial activity in the presence of a C source (Tran et al., 2013; Siedt et al., 2021).

In contrast to PSC, the persistent fungicide BSC showed a decrease in residues bound to maize and alongside a simultaneous increase in soil. This indicates a transfer of the pesticide from the maize to the soil, even though the total amount of BSC decreased over the duration of the experiment. A pre-requisite for this mechanism would be that BSC was desorbed from the maize and transferred onto soil particles, e.g., SOM or clay minerals (Li et al., 2003; Barchańska et al., 2020). Another possibility is that the maize was disaggregated into smaller particles during incubation, leading to their pesticide content being measured together with the soil fraction.

Interestingly, the presence of PSC with BSC led to a lag phase in the dissipation of BSC residues in both maize treatments, indicating an interactive effect between the pesticides. Similarly, a decrease in degradation rates of pendimethalin was observed in the presence of

mancozeb and thiamethoxam (Swarcewicz and Gregorczyk, 2012). While physicochemical interactions of multiple pesticides can increase the desorption and mineralization of individual components (Munira and Farenhorst, 2017; Mäder et al., 2024), we did not observe an increased dissipation of PSC. Another possible explanation might be given by microbial degradation dynamics. The presence of PSC might have exerted a toxic effect on BSC degraders and thereby inhibited the degradation of BSC. This is supported by the observation that the lag phase ended between Day 14 and Day 28, when PSCresidues already decreased, especially in maize. As there are, to our knowledge, no molecular markers for either BSC or PSC degraders, it is difficult to clarify the exact mechanisms here.

Interactions between pesticides on the potential degradation kinetics of individual pesticides pose challenges as they may complicate predictions of environmental persistence compared to single applications. This is particularly relevant given the various pesticide mixtures that are used in modern agriculture (Tang and Maggi, 2021; Knuth et al., 2024). The interactions are likely to depend on the characteristics of the pesticides present, warranting future investigations. These could investigate if similar behavior of a particular group of pesticides could enable us to make more accurate predictions within this group.

4.2. Effects on soil microorganisms

Independently of the pesticide treatments, reduced microbial activities and abundances in the aged maize treatment were due to the difference in substrate quality of the maize, and have been observed with other organic amendments (Nicolardot et al., 2007; Dungait et al., 2013). With an increased amount of both EOC and maize-derived EOC in fresh maize, microorganisms had ample supply of easily available C substrates to mineralize, which was reflected in the initial increase of respiration, indicating that they rapidly adapted to the fresh maize addition. In contrast, microorganisms in aged maize were only able to adapt to the nutrient regime (Luu et al., 2022) after about 7 days, resulting in a delayed peak in respiration.

As such, the initial increase in respiration in the presence of PSC in the aged maize suggests two possibilities: either PSC was used as a C source or the mineralization of maize was increased. Since the non-detectable fraction of PSC on Day 3 corresponded to 4.15 μg C g^{-1} DW and the increased cumulative respired CO_2-C between the PSC treatment and the control on Day 3 was 20.46 μg C g^{-1} DW, the degradation of PSC is only responsible for 20 % of the observed increase, which does not fully explain the initial increase in respiration. Instead, the observed transient increase in the maize-derived CO_2 rate on Day 3 in the aged maize leads us to the conclusion that the microorganisms mainly utilized the maize to gain energy and to compensate for potential toxic effects of PSC. Such an increase in microbial activity is indicative of a stress reaction as it has been observed for other pesticides (Kelarlou et al., 2023; Mäder et al., 2024). This effect of PSC was not observed in fresh maize, which could be explained by a general increase in respiration that masks the effect of PSC. In contrast, the decrease in maize-derived CO_2 in the presence of BSC is likely linked to the fungicide's mode of action. Boscalid acts as succinate dehydrogenase inhibitor (SDHI), targeting a broad spectrum of fungal species inhibiting respiration (Avenot and Michailides, 2010). Therefore, the initial decrease in respiration may have been due to a decrease in fungal respiration by BSC. This accords with the observed decrease in fungal PLFA abundance. Consistent with this, thifluzamide, another SDHI fungicide, has been shown to reduce fungal abundance and function (Yao et al., 2022). The similarity of effects observed in the mixed treatment further suggests that BSC dominates the effect on the microbial community even when another pesticide is present, an observation we previously reported for mixtures of the fungicide difenoconazole, the herbicide MCPA and the herbicide glyphosate (Mäder et al., 2024).

While fungal PLFA abundance in fresh maize recovered along with the respiration rates, fungal PLFA abundance in aged maize did not recover as fast due to a longer inhibition of the respiration by BSC. This difference in effect size and duration is likely due to the substrates' quality, with both fungi and bacteria benefiting from the wider range of compounds available in the fresh maize treatment, resulting in the observed initial peak in respiration and the rapid recovery from the negative effect of BSC. We further speculate that different microbial community structures are present in the aged and fresh maize treatments, resulting from the different substrates present, and change over the duration of the experiment. Pesticide disturbance may also have caused a change in the function and community structure of the fungi, as observed by Tagele and Gachomo (2024). The community in the fresh maize treatment may have consisted of more copiotrophic fungi that responded to the higher carbon availability present (Ho et al., 2017). At the same time, the initial decline indicates these copiotrophic fungi were more sensitive to the pesticide addition. On the contrary in aged maize, oligotrophic fungi have a higher substrate utilization efficiency (Ho et al., 2017), which may have compensated for negative effects on fungal abundance. Interestingly, Zhang et al. (2014) also observed this initial decline and a subsequent recovery of the fungal community using the fungicide tetraconazole, despite not having added any substrate. In their experiment, fungi recovered to control soil levels, whereas in our study, fungi increased above control levels in the fresh maize treatment. This may indicate that the observed decline was primarily pesticide-induced and the recovery above control levels was increased by additional nutrients from the maize. Another possible explanation for the increase in fungal markers is provided by Bending et al. (2007), who argued that after the initial death of microorganisms, their biomass can be used as substrate for growth by other microorganisms. Although no decrease of bacterial PLFA was reported, we did observe a decrease in the fungal PLFA on days 3 and 7. We therefore propose that there could have been cross-feeding within the fungal community, where certain fungi benefit from the death of other fungi. Cho et al. (2017) argued that Ascomycota and Zygomycota benefited from dead cells following disturbance. This dynamic may also explain the response of the fungal community to pesticide-induced disturbance in our study.

While fungi were likely affected by the fungicide BSC, the herbicide PSC affected soil bacteria, reducing their abundance and increasing their requirement for C and P. This was evidenced by a decrease in maize-derived C in the microbial biomass as well as maize-derived C in the PLFAs of G+ and G-on Day 7, suggesting that PSC inhibited bacterial growth soon after application. As the maize-derived C in PLFAs is in newly synthesized PLFAs, our data suggest that PSC, which acts as a lipid synthesis inhibitor in plants (EFSA, 2007), may also inhibit lipid synthesis in bacteria, thereby reducing biomass formation. At the same time, the reduction in CUE in the presence of PSC indicated a microbial stress response, where less maize-derived carbon was allocated to growth and instead was respired (Manzoni et al., 2012). This is consistent with the increase in the vector length in the presence of PSC on Day 7, indicating a shift in C limitation (Moorhead et al., 2016), towards a higher requirement for C. Combined with an increase in the SEA of C and P enzymes, nutrient acquisition strategies changed, resulting in a higher requirement for C and P. We propose that microorganisms increased nutrient acquisition efforts and maintenance activities, such as enzyme regeneration and cell repair, rather than growth (Bradley et al., 2018). This response is also supported by the transient decrease in microbial biomass on Day 7. As biomass production declined, maintenance mechanisms to acquire C and P were enhanced, leading to increased utilization of readily available maize-derived EOC. Consequently, the incorporation of maize-derived C in the microbial biomass C on Day 14 indicates that the microorganisms recovered and resumed utilizing the maize-derived C source, forming a new steady state attributed to microbial resilience (Hodgson et al., 2015). At the same time the decrease in PSC residues on the maize was observed, which could indicate that the toxic effects of PSC were only transient. On the other hand, residues were still comparable to the initial concentration on Day 14 and only decreased after the microorganisms recovered. As there is no

information on the degradation pathway of PSC and genetic markers for the degrader community have, to our knowledge, not been discovered yet, it is difficult to argue what caused this. We speculate that a community more tolerant to PSC and capable to degrade PSC was formed during the first 14 days. Such a shift in the community was observed for other pesticides, e.g., MCPA (Wirsching et al., 2020; Mäder et al., 2024).

Although observed in fresh maize, the effects of PSC were not detectable in aged maize. This might be explained by different dynamics of the maize treatment and their interaction with the pesticide. We assume that PSC affects the microbial community between days 7 and 14, irrespective of maize treatment. Microbial activity in fresh maize initially increased, due to readily available nutrients, but declined rapidly after nutrients had been utilized. During this decline in activity the effect of PSC occurred, leading to the observed effects as the microbial community might have been less resistant to disturbance. In contrast, in aged maize, microbial activity increased towards Day 14, when C supply from maize increased due to an adaption to the nutrient regime and the breakdown of recalcitrant substrate (Luu et al., 2022), possibly compensating for the negative effect of PSC. This highlights the importance of substrate quality, which influences the nutrient regime at the time of a pesticide disturbance and can shape the magnitude and direction of the effect in the microbial community.

5. Conclusion

This study provides first mechanistic insights into the interactive effects of pesticides and substrate quality on soil microorganisms and their function. We have identified a transfer of pesticide residues from plant material directly to soils and an accumulation of residues in soils, which may have implications for pesticide residue prediction and modelling, where input via carriers such as plant material is currently insufficiently considered. This has to be further evaluated for different pesticides and organic inputs since the observed transfer is likely pesticide and carrier specific. Especially properties of the pesticides that increase or decrease adsorption to such carriers e.g., water solubility and the potential biodegradability by microorganisms have to be studied, as these aspects likely lead to an over- or underestimation of pesticide residues. In more detail, pesticide residues might not reach the soil directly, as they are initially on treated plants, and at the same time that these residues may be incorporated only at a later stage. Regulators and the risk assessments for pesticides need to consider such additional input pathways, as real-world exposure might not be fully covered with the existing exposure scenarios.

We could further show that this transfer was not affected by the two maize qualities used in this experiment, yet other organic substances, e.g., biochar might result in different behaviors. The microbial results highlight the individual effects that pesticides can have, depending on their mode of action, reducing microbial activity and abundance and altering their function. These effects were also dominant in the mixed treatment, where interactive effects were less pronounced than expected and single pesticide effects predominated. The absence of an interactive effect could also be linked to the individual characteristics of the pesticides. While aspects such as competitive adsorption could not be determined here, they need to be considered and investigated in future studies. The fact that PSC and BSC affected different microbial groups (bacteria and fungi, respectively) and different processes could further explain that interactive effects were less pronounced. A classification based on the modes of action could be discussed in the future, with extensive comparisons between effects of pesticides with the same mode of action. Such results could help to further group pesticide effects on non-target organisms and to predict their effects. Extending from this, the combination of different modes of action could be tested to investigate whether their combination results in similar effects even with different pesticides used.

Future studies should aim to improve the mechanistic understanding of the effects of pesticides on soil microorganisms. They could benefit from including environmental conditions that could interfere with these effects and that are typically present in agricultural scenarios. Therefore, next steps should include the investigation of such dynamics in field experiments using agricultural practices, to validate the observation in this study.

CRediT authorship contribution statement

Philipp Mäder: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Anja Listl:** Methodology, Data curation. **Zuzana Hochmanová:** Methodology, Data curation. **Wolfgang Armbruster:** Methodology, Data curation. **Paula Harkes:** Writing – original draft, Visualization. **Christian Poll:** Writing – original draft, Supervision, Conceptualization. **Ellen Kandeler:** Writing – original draft, Supervision, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2025.126862.

Data availability

Data will be made available on request.

References

Abrantes, N., Pereira, R., Gonçalves, F., 2010. Occurrence of pesticides in water, sediments, and fish tissues in a lake surrounded by agricultural lands: concerning risks to humans and ecological receptors. Water, Air, Soil Pollut. 212, 77–88. https://doi.org/10.1007/s11270-010-0323-2.

Anderson, T.-H., Domsch, K.H., 2010. Soil microbial biomass: the eco-physiological approach. Soil Biol. Biochem. 42, 2039–2043. https://doi.org/10.1016/j.soilbio.2010.06.026.

Anderson, T.-H., Domsch, K.H., 1985. Determination of ecophysiological maintenance carbon requirements of soil microorganisms in a dormant state. Biol. Fertil. Soils 1, 81–89. https://doi.org/10.1007/BF00255134.

Avenot, H.F., Michailides, T.J., 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29, 643–651. https://doi.org/10.1016/j.cropro.2010.02.019.

Barba, V., Marín-Benito, J.M., García-Delgado, C., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2019. Assessment of 14C-prosulfocarb dissipation mechanism in soil after amendment and its impact on the microbial community. Ecotoxicol. Environ. Saf. 182, 109395. https://doi.org/10.1016/j.ecoenv.2019.109395.

Barchańska, H., Czaplicka, M., Kyzioł-Komosińska, J., 2020. Interaction of selected pesticides with mineral and organic soil components. Arch. Environ. Protect. 46, 80–91. https://doi.org/10.24425/aep.2020.134538.
Bending, G.D., Rodríguez-Cruz, M.S., Lincoln, S.D., 2007. Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere 69, 82–88. https://doi.org/10.1016/j.chemosphere.2007.04.042.
Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. https://doi.org/10.1139/o59-099.
Bradley, J.A., Amend, J.P., LaRowe, D.E., 2018. Bioenergetic controls on microbial ecophysiology in marine sediments. Front. Microbiol. 9. https://doi.org/10.3389/fmicb.2018.00180.
Cho, H., Kim, M., Tripathi, B., Adams, J., 2017. Changes in soil fungal community structure with increasing disturbance frequency. Microb. Ecol. 74, 62–77. https://doi.org/10.1007/s00248-016-0919-1.
Dungait, J.A.J., Kemmitt, S.J., Michallon, L., Guo, S., Wen, Q., Brookes, P.C., Evershed, R.P., 2013. The variable response of soil microorganisms to trace concentrations of low molecular weight organic substrates of increasing complexity. Soil Biol. Biochem. 64, 57–64. https://doi.org/10.1016/j.soilbio.2013.03.036.
EFSA, 2007. Conclusion regarding the peer review of the pesticide risk assessment of the active substance prosulfocarb. EFSA Scientific Report 111, 1–81. https://doi.org/10.2903/j.efsa.2007.111r.
Egamberdieva, D., Jabbarov, Z., Arora, N.K., Wirth, S., Bellingrath-Kimura, S.D., 2021. Biochar mitigates effects of pesticides on soil biological activities. Environmental Sustainability 4, 335–342. https://doi.org/10.1007/s42398-021-00190-w.
Froger, C., Jolivet, C., Budzinski, H., Pierdet, M., Caria, G., Saby, N.P.A., Arrouays, D., Bispo, A., 2023. Pesticide residues in french soils: occurrence, risks, and persistence. Environ. Sci. Technol. 57, 7818–7827. https://doi.org/10.1021/acs.est.2c09591.
Frostegård, Å., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163. https://doi.org/10.1016/0167-7012(91)90018-L.
García-Delgado, C., Barba, V., Marín-Benito, J.M., Igual, J.M., Sánchez-Martín, M.J., Rodríguez-Cruz, M.S., 2018. Simultaneous application of two herbicides and green compost in a field experiment: implications on soil microbial community. Appl. Soil Ecol. 127, 30–40. https://doi.org/10.1016/j.apsoil.2018.03.004.
Gavrilescu, M., 2005. Fate of pesticides in the environment and its bioremediation. Eng. Life Sci. 5, 497–526. https://doi.org/10.1002/elsc.200520098.
Geyer, K.M., Kyker-Snowman, E., Grandy, A.S., Frey, S.D., 2016. Microbial carbon use efficiency: accounting for population, community, and ecosystem-scale controls over the fate of metabolized organic matter. Biogeochemistry 127, 173–188. https://doi.org/10.1007/s10533-016-0191-y.
Helweg, A., Fomsgaard, I.S., Reffstrup, T.K., Srensen, H., 1998. Degradation of Mecoprop and Isoproturon in soil influence of initial concentration. Int. J. Environ. Anal. Chem. 70, 133–148. https://doi.org/10.1080/03067319808032610.
Ho, A., Di Lonardo, D.P., Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 93. https://doi.org/10.1093/femsec/fix006.
Hobara, S., Osono, T., Hirose, D., Noro, K., Hirota, M., Benner, R., 2014. The roles of microorganisms in litter decomposition and soil formation. Biogeochemistry 118, 471–486. https://doi.org/10.1007/s10533-013-9912-7.
Hodgson, D., McDonald, J.L., Hosken, D.J., 2015. What do you mean, 'resilient'? Trends Ecol. Evol. 30, 503–506. https://doi.org/10.1016/j.tree.2015.06.010.
Jacobsen, C.S., van der Keur, P., Iversen, B.V., Rosenberg, P., Barlebo, H.C., Torp, S., Vosgerau, H., Juhler, R.K., Ernstsen, V., Rasmussen, J., Brinch, U.C., Jacobsen, O.H., 2008. Variation of MCPA, metribuzine, methyltriazine-amine and glyphosate degradation, sorption, mineralization and leaching in different soil horizons. Environ. Pollut. 156, 794–802. https://doi.org/10.1016/j.envpol.2008.06.002.
Jian, Q., Boyer, T.H., Yang, Xiuhong, Xia, B., Yang, Xin, 2016. Characteristics and DBP formation of dissolved organic matter from leachates of fresh and aged leaf litter. Chemosphere 152, 335–344. https://doi.org/10.1016/j.chemosphere.2016.02.107.
Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: calibration of the k_{EC} value. Soil Biol. Biochem. 28, 25–31. https://doi.org/10.1016/0038-0717(95)00102-6.
Karpouzas, D.G., Tsiamis, G., Trevisan, M., Ferrari, F., Malandain, C., Sibourg, O., Martin-Laurent, F., 2016. LOVE TO HATE" pesticides: felicity or curse for the soil microbial community? An FP7 IAPP Marie Curie project aiming to establish tools for the assessment of the mechanisms controlling the interactions of pesticides with soil microorganisms. Environ. Sci. Pollut. Control Ser. 23 (18), 18947–18951. https://doi.org/10.1007/s11356-016-7319-4.
Kelarlou, T.R., Golchin, A., Toolarood, A.-A.S., 2023. Evaluation of the impacts of clopyralid and butisanstar herbicides on selected soil microbial indicators. Water, Air, Soil Pollut. 234, 572. https://doi.org/10.1007/s11270-023-06597-x.
Knuth, D., Gai, L., Silva, V., Harkes, P., Hofman, J., Šudoma, M., Bílková, Z., Alaoui, A., Mandrioli, D., Pasković, I., Polić Pasković, M., Baldi, I., Bureau, M., Alcon, F., Contreras, J., Glavan, M., Abrantes, N., Campos, I., Norgaard, T., Huerta Lwanga, E., Scheepers, P.T.J., Ritsema, C.J., Geissen, V., 2024. Pesticide residues in organic and conventional agricultural soils across Europe: measured and predicted concentrations. Environ. Sci. Technol. 58, 6744–6752. https://doi.org/10.1021/acs.est.3c09059.
Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K.U., Walker, F., Scheu, S., Kandeler, E., 2012. Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. Pedobiologia 55, 111–119. https://doi.org/10.1016/j.pedobi.2011.12.001.
Li, H., Sheng, G., Teppen, B.J., Johnston, C.T., Boyd, S.A., 2003. Sorption and desorption of pesticides by clay minerals and humic acid-clay complexes. Soil Sci. Soc. Am. J. 67, 122–131. https://doi.org/10.2136/sssaj2003.1220a.
Luu, A.T., Hoang, N.T., Dinh, V.M., Bui, M.H., Grandy, S., Hoang, D.T.T., 2022. Effects of carbon input quality and timing on soil microbe mediated processes. Geoderma 409, 115605. https://doi.org/10.1016/j.geoderma.2021.115605.
Mäder, P., Stache, F., Engelbart, L., Huhn, C., Hochmanová, Z., Hofman, J., Poll, C., Kandeler, E., 2024. Effects of MCPA and difenoconazole on glyphosate degradation and soil microorganisms. Environ. Pollut. 362, 124926. https://doi.org/10.1016/j.envpol.2024.124926.
Madsen, E.L., 2011. Microorganisms and their roles in fundamental biogeochemical cycles. Curr. Opin. Biotechnol. 22, 456–464. https://doi.org/10.1016/j.copbio.2011.01.008.
Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol. 196, 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x.
Marx, M.-C., Wood, M., Jarvis, S.C., 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biol. Biochem. 33, 1633–1640. https://doi.org/10.1016/S0038-0717(01)00079-7.
Meidl, P., Lehmann, A., Bi, M., Breitenreiter, C., Benkrama, J., Li, E., Riedo, J., Rillig, M. C., 2024. Combined application of up to ten pesticides decreases key soil processes. Environ. Sci. Pollut. Control Ser. 31, 11995–12004. https://doi.org/10.1007/s11356-024-31836-x.
Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol. Biochem. 93, 1–7. https://doi.org/10.1016/j.soilbio.2015.10.019.
Mukherjee, S., Tappe, W., Weihermueller, L., Hofmann, D., Köppchen, S., Laabs, V., Schroeder, T., Vereecken, H., Burauel, P., 2016. Dissipation of bentazone, pyrimethanil and boscalid in biochar and digestate based soil mixtures for biopurification systems. Sci. Total Environ. 544, 192–202. https://doi.org/10.1016/j.scitotenv.2015.11.111.
Munira, S., Farenhorst, A., 2017. Sorption and desorption of glyphosate, MCPA and tetracycline and their mixtures in soil as influenced by phosphate. Journal of Environmental Science and Health, Part B 52, 887–895. https://doi.org/10.1080/03601234.2017.1361773.
Nègre, M., Passarella, I., Boursier, C., Mozzetti, C., Gennari, M., 2006. Evaluation of the bioavailability of the herbicide prosulfocarb through adsorption on soils and model soil colloids, and through a simple bioassay. Pest Manag. Sci. 62, 957–964. https://doi.org/10.1002/ps.1264.
Nguyen, D.B., Rose, M.T., Rose, T.J., Morris, S.G., van Zwieten, L., 2016. Impact of glyphosate on soil microbial biomass and respiration: a meta-analysis. Soil Biol. Biochem. 92, 50–57. https://doi.org/10.1016/j.soilbio.2015.09.014.
Nicolardot, B., Bouziri, L., Bastian, F., Ranjard, L., 2007. A microcosm experiment to evaluate the influence of location and quality of plant residues on residue decomposition and genetic structure of soil microbial communities. Soil Biol. Biochem. 39, 1631–1644. https://doi.org/10.1016/j.soilbio.2007.01.012.
Nowak, K.M., Miltner, A., Poll, C., Kandeler, E., Streck, T., Pagel, H., 2020. Plant litter enhances degradation of the herbicide MCPA and increases formation of biogenic non-extractable residues in soil. Environ. Int. 142, 105867. https://doi.org/10.1016/j.envint.2020.105867.
Oleszczuk, P., Jośko, I., Futa, B., Pasieczna-Patkowska, S., Pałys, E., Kraska, P., 2014. Effect of pesticides on microorganisms, enzymatic activity and plant in biochar-amended soil. Geoderma 214–215, 10–18. https://doi.org/10.1016/j.geoderma.2013.10.010.
Pagel, H., Poll, C., Ingwersen, J., Kandeler, E., Streck, T., 2016. Modeling coupled pesticide degradation and organic matter turnover: from gene abundance to process rates. Soil Biol. Biochem. 103, 349–364. https://doi.org/10.1016/j.soilbio.2016.09.014.
Pinheiro, J., Bates, D., R Core Team, 2023. Nlme: Linear and nonlinear mixed effects models. R package version 3.1-163. URL: https://cran.r-project.org/web/packages/nlme (Acessed 24.10.23).
Poll, C., Marhan, S., Ingwersen, J., Kandeler, E., 2008. Dynamics of litter carbon turnover and microbial abundance in a rye detritusphere. Soil Biol. Biochem. 40, 1306–1321. https://doi.org/10.1016/j.soilbio.2007.04.002.
Poll, C., Ingwersen, J., Stemmer, M., Gerzabek, M.H., Kandeler, E., 2006. Mechanisms of solute transport affect small–scale abundance and function of soil microorganisms in the detritusphere. Eur. J. Soil Sci. 57, 583–595. https://doi.org/10.1111/j.1365-2389.2006.00835.x.
R Core Team, 2022. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://r-project.org/ (Acessed 24.10.23).
Riah, W., Laval, K., Laroche-Ajzenberg, E., Mougin, C., Latour, X., Trinsoutrot-Gattin, I., 2014. Effects of pesticides on soil enzymes: a review. Environ. Chem. Lett. 12, 257–273. https://doi.org/10.1007/s10311-014-0458-2.
Riedo, J., Wettstein, F.E., Rösch, A., Herzog, C., Banerjee, S., Büchi, L., Charles, R., Wächter, D., Martin-Laurent, F., Bucheli, T.D., Walder, F., van der Heijden, M.G.A., 2021. Widespread occurrence of pesticides in organically managed agricultural soils—the ghost of a conventional agricultural past? Environ. Sci. Technol. 55, 2919–2928. https://doi.org/10.1021/acs.est.0c06405.
Rouhi-Kelarlou, R., Golchin, A., Toularoud, A.A.S., 2024. Ecotoxicological impact of butisanstar and clopyralid herbicides on soil microbial respiration and the enzymatic activities. Chemosphere 357, 142029. https://doi.org/10.1016/j.chemosphere.2024.142029.
Ruess, L., Chamberlain, P.M., 2010. The fat that matters: soil food web analysis using fatty acids and their carbon stable isotope signature. Soil Biol. Biochem. 42, 1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020.
Sabzevari, S., Hofman, J., 2022. A worldwide review of currently used pesticides' monitoring in agricultural soils. Sci. Total Environ. 812, 152344. https://doi.org/10.1016/j.scitotenv.2021.152344.

Sadegh-Zadeh, F., Wahid, S.A., Jalili, B., 2017. Sorption, degradation and leaching of pesticides in soils amended with organic matter: a review. Advances in Environmental Technology 3, 119–132. https://doi.org/10.22104/AET.2017.1740.1100.

Safaei Khorram, M., Zhang, Q., Lin, D., Zheng, Y., Fang, H., Yu, Y., 2016. Biochar: a review of its impact on pesticide behavior in soil environments and its potential applications. J. Environ. Sci. (China) 44, 269–279. https://doi.org/10.1016/j.jes.2015.12.027.

Sahu, N., Raha, P., Sahu, A., Singh, U.B., 2014. Effect of organophosphorus pesticides on enzyme activities in alluvial soil (typic ustochrepts). Nat. Environ. Pollut. Technol. 13, 775–780.

Saleh, O., Pagel, H., Enowashu, E., Devers, M., Martin-Laurent, F., Streck, T., Kandeler, E., Poll, C., 2016. Evidence for the importance of litter as a co-substrate for MCPA dissipation in an agricultural soil. Environ. Sci. Pollut. Control Ser. 23, 4164–4175. https://doi.org/10.1007/s11356-015-4633-1.

Sánchez, M.E., Estrada, I.B., Martínez, O., Martín-Villacorta, J., Aller, A., Morán, A., 2004. Influence of the application of sewage sludge on the degradation of pesticides in the soil. Chemosphere 57, 673–679. https://doi.org/10.1016/j.chemosphere.2004.07.023.

Sanchez-Hernandez, J.C., Sandoval, M., Pierart, A., 2017. Short-term response of soil enzyme activities in a chlorpyrifos-treated mesocosm: use of enzyme-based indexes. Ecol. Indic. 73, 525–535. https://doi.org/10.1016/j.ecolind.2016.10.022.

Siedt, M., Schäffer, A., Smith, K.E.C., Nabel, M., Roß-Nickoll, M., van Dongen, J.T., 2021. Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. Sci. Total Environ. 751, 141607. https://doi.org/10.1016/j.scitotenv.2020.141607.

Silva, V., Gai, L., Harkes, P., Tan, G., Ritsema, C.J., Alcon, F., Contreras, J., Abrantes, N., Campos, I., Baldi, I., Bureau, M., Christ, F., Mandrioli, D., Sgargi, D., Pasković, I., Polić Pasković, M., Glavan, M., Hofman, J., Huerta Lwanga, E., Norgaard, T., Bílková, Z., Osman, R., Khurshid, C., Navarro, I., de la Torre, A., Sanz, P., Ángeles Martínez, M., Dias, J., Mol, H., Gort, G., Martins Figueiredo, D., Scheepers, P.T.J., Schlünssen, V., Vested, A., Alaoui, A., Geissen, V., 2023. Pesticide residues with hazard classifications relevant to non-target species including humans are omnipresent in the environment and farmer residences. Environ. Int. 181, 108280. https://doi.org/10.1016/j.envint.2023.108280.

Silva, V., Mol, H.G.J., Zomer, P., Tienstra, M., Ritsema, C.J., Geissen, V., 2019. Pesticide residues in European agricultural soils – a hidden reality unfolded. Sci. Total Environ. 653, 1532–1545. https://doi.org/10.1016/j.scitotenv.2018.10.441.

Spark, K., Swift, R., 2002. Effect of soil composition and dissolved organic matter on pesticide sorption. Sci. Total Environ. 298, 147–161. https://doi.org/10.1016/S0048-9697(02)00213-9.

Swarcewicz, M.K., Gregorczyk, A., 2012. The effects of pesticide mixtures on degradation of pendimethalin in soils. Environ. Monit. Assess. 184, 3077–3084. https://doi.org/10.1007/s10661-011-2172-x.

Tagele, S.B., Gachomo, E.W., 2024. Evaluating the effects of mefenoxam on taxonomic and functional dynamics of nontarget fungal communities during carrot cultivation. Sci. Rep. 14, 9867. https://doi.org/10.1038/s41598-024-59587-2.

Tang, F.H.M., Maggi, F., 2021. Pesticide mixtures in soil: a global outlook. Environ. Res. Lett. 16, 044051. https://doi.org/10.1088/1748-9326/abe5d6.

Tran, N.H., Urase, T., Ngo, H.H., Hu, J., Ong, S.L., 2013. Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Bioresour. Technol. 146, 721–731. https://doi.org/10.1016/j.biortech.2013.07.083.

van Hoesel, W., Tiefenbacher, A., König, N., Dorn, V.M., Hagenguth, J.F., Prah, U., Widhalm, T., Wiklicky, V., Koller, R., Bonkowski, M., Lagerlöf, J., Ratzenböck, A., Zaller, J.G., 2017. Single and combined effects of pesticide seed dressings and herbicides on earthworms, soil microorganisms, and litter decomposition. Front. Plant Sci. 8, 215. https://doi.org/10.3389/fpls.2017.00215.

Waldrop, M.P., Balser, T.C., Firestone, M.K., 2000. Linking microbial community composition to function in a tropical soil. Soil Biol. Biochem. 32, 1837–1846. https://doi.org/10.1016/S0038-0717(00)00157-7.

Wirsching, J., Pagel, H., Ditterich, F., Uksa, M., Werneburg, M., Zwiener, C., Berner, D., Kandeler, E., Poll, C., 2020. Biodegradation of pesticides at the limit: kinetics and microbial substrate use at low concentrations. Front. Microbiol. 11, 2107. https://doi.org/10.3389/fmicb.2020.02107.

Wu, X., Xu, J., Dong, F., Liu, X., Zheng, Y., 2014. Responses of soil microbial community to different concentration of fomesafen. J. Hazard Mater. 273, 155–164. https://doi.org/10.1016/j.jhazmat.2014.03.041.

Yang, C., Hamel, C., Vujanovic, V., Gan, Y., 2011. Fungicide: modes of action and possible impact on nontarget microorganisms. Int. Sch. Res. Not. 2011, 130289. https://doi.org/10.5402/2011/130289.

Yao, X., Liu, Y., Liu, X., Qiao, Z., Sun, S., Li, X., Wang, J., Zhang, F., Jiang, X., 2022. Effects of thifluzamide on soil fungal microbial ecology. J. Hazard Mater. 431, 128626. https://doi.org/10.1016/j.jhazmat.2022.128626.

Zabaloy, M.C., Zanini, G.P., Bianchinotti, V., Gomez, M.A., Garland, J.L., 2011. Herbicides in the soil environment: linkage between bioavailability and microbial ecology. In: Soloneski, S., Larramendy, M.L. (Eds.), Herbicides. InTech. https://doi.org/10.5772/12880.

Zaller, J.G., König, N., Tiefenbacher, A., Muraoka, Y., Querner, P., Ratzenböck, A., Bonkowski, M., Koller, R., 2016. Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. BMC Ecol. 16, 37. https://doi.org/10.1186/s12898-016-0092-x.

Zhang, M., Xu, Z., Teng, Y., Christie, P., Wang, J., Ren, W., Luo, Y., Li, Z., 2016. Non-target effects of repeated chlorothalonil application on soil nitrogen cycling: the key functional gene study. Sci. Total Environ. 543, 636–643. https://doi.org/10.1016/j.scitotenv.2015.11.053.

Zhang, W., Xu, J., Dong, F., Liu, X., Zhang, Y., Wu, X., Zheng, Y., 2014. Effect of tetraconazole application on the soil microbial community. Environ. Sci. Pollut. Control Ser. 21, 8323–8332. https://doi.org/10.1007/s11356-014-2844-5.