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Microbial activity and metamitron degrading microbial communities differ between soil and water-sediment systems

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Abstract

The herbicide metamitron is frequently detected in the environment, and its degradation in soil differs from that in aquatic sediments. In this study, we applied $^{13}C_6$-metamitron to investigate the differences in microbial activity, metamitron mineralization and metamitron degrading microbial communities between soil and water-sediment systems. Metamitron increased soil respiration, whereas it suppressed respiration in the water-sediment system as

compared to controls. Metamitron was mineralized two-fold faster in soil than in the water-sediment. Incorporation of ^{13}C from $^{13}C_6$-metamitron into Phospholipid fatty acids (PLFAs) was higher in soil than in sediment, suggesting higher activity of metamitron-degrading microorganisms in soil. During the accelerated mineralization of metamitron, biomarkers for Gram-negative, Gram-positive bacteria and actinobacteria dominated within the ^{13}C-PLFAs in soil. Gram-negative bacteria dominated among the metamitron degraders in sediment throughout the incubation period. Actinobacteria, and actinobacteria and fungi were the main consumers of necromass of primary degraders in soil and water-sediment, respectively.

This study clearly showed that microbial groups involved in metamitron degradation depend on the system (soil vs. water-sediment) and on time. It also indicated that the turnover of organic chemicals in complex environments is driven by different groups of synthropic degraders (primary degraders and necromass degraders) rather than by a single degrader.

Key words： Microbial respiration, Mineralization; Stable isotope label, Phospholipid fatty acids; Microbial community structure

1. Introduction

Metamitron (triazinone herbicide) is used extensively to control broad-leaved weeds and grasses in sugar beet and flower bulb cultures (Janaki et al., 2013). Metamitron and its intermediates are thus frequently detected in soil (Kumar et al., 2014) and river waters (Moschet et al., 2015; Reemtsma et al., 2013) causing their pollution, and possibly, negative effects on non-target organisms (Engelen et al., 1998). Slight changes in the microbial

community and activity were noticed in soils amended with metamitron (Engelen et al., 1998). An earlier study, however, suggested that this herbicide had no negative effect on the activity of a cyanobacterial isolate as indicated by its nitrogenase activity (Gadkari, 1988). To date, it is still unclear whether metamitron has negative effects on microbial activity and microbial community composition of soil and sediment.

Microorganisms can use metamitron as a carbon source as evidenced by the incorporation of metamitron-derived carbon into amino acids in both soil (Wang et al., 2017a) and water-sediment (Wang et al., 2017b) biodegradation studies with $^{13}C_6$-metamitron. When the microorganisms die, amino acids are stabilized in organic matter (OM) of soil and sediment forming ultimately biogenic non-extractable residue (bioNER; Kästner et al., 2014). Microbial degradation of metamitron was the main pathway of its elimination from soil (Wang et al., 2017a) and water-sediment (Wang et al., 2017b). This process resulted in the formation of microbial biomass, mineralization products and harmless bioNER. The degradation of metamitron in soil was faster (Wang et al., 2017a) than in the water-sediment system (Wang et al., 2017b). Furthermore, metamitron was degraded via two pathways in soil: via the “Rhodococcus pathway” and the “desamino pathway” (Wang et al., 2017a), whereas in the water-sediment system only the “desamino pathway” could be detected (Wang et al., 2017b). Two actinobacteria, *Rhodococcus* sp. 0246b and *Arthrobacter* sp. DSM 20389, isolated from soil exposed to metamitron have been documented to degrade metamitron in pure cultures (Engelhardt et al., 1982; Parekh et al., 1994). However, only a small fraction (<10%) of the total microbial community that exists in nature can be grown under laboratory conditions (Stewart, 2012). The degradation of a pesticide in nature can be mediated by

several groups of degraders that cannot be isolated and cultured in the laboratory (Lerch et al., 2009). It is unclear why we observed differences in the biodegradation pattern of metamitron in soil and water-sediment systems (Wang et al., 2017a; 2017b) and whether this was attributed to different microbial activity or other condition. We also assume that actinobacteria are not the only metamitron-degrading microorganism in these two systems.

Phospholipid fatty acids (PLFAs) are components of cellular membranes of living cells and inform about the quantity and activity of microorganisms, and microbial community composition (Boschker and Middelburg, 2002). Total fatty acids (tFAs) include both PLFAs (in biomass) and the FAs in the necromass (Green and Scow, 2000; Kaur et al., 2005; Zelles, 1999). The kinetics in C-isotope label distribution in the PLFAs enables identification of microbial activity in the transformation of a specific compound after deliberately added tracers. The difference between the ^{13}C labeling patterns of the PLFAs and of the tFAs over time informs about the amount of necromass in OM. The combined FAs analysis with stable isotope labeling is known as FA-stable isotope probing (FA-SIP; Boschker and Middelburg, 2002). As the specific PLFAs are assigned to different bacterial groups (Gram-positive, Gram-negative, actinobacteria) or fungi (Kaur et al., 2005), PLFAs are widely used to identify which specific microbial group is metabolically active in the environment. The FA-SIP approach thus can distinguish between main groups of microbial degraders (Boschker and Middelburg, 2002), but it has a much lower taxonomic resolution than nucleic acid or proteomics-SIP based techniques (Boschker and Middelburg, 2002; Green and Scow, 2000). However, the FA-SIP requires much lower incorporation of stable isotope label into the FAs from a labeled substrate than in the DNA- or protein-SIP approaches (Boschker and

Middelburg, 2002). Therefore, FA-SIP allows performing experiments at much lower concentration of a labeled substrate than DNA- or protein-SIP.

To date, the effect of metamitron on microbial activity and microbial community composition; and the metamitron degraders in soil and water-sediment are largely unexplored. We hypothesized that (i) microbial activity and microbial community in soil differ from that in water-sediment, (ii) several groups of microorganisms are involved in the degradation of metamitron in the two systems and (iii) metamitron degrading microbial communities in soil and water-sediment are different and time-dependent. This was tested in small-scale soil and water-sediment microcosms without (as a control setting) and with $^{13}C_6$-metamitron addition over 80 days of incubation. The quantitative analyses of CO_2 (respiration), $^{13}CO_2$ ($^{13}C_6$-metamitron mineralization) and of FAs (PLFAs and tFAs) were employed to investigate the effect of metamitron addition on microbial activity and microbial community composition. The FA-SIP was conducted to quantify the time-dependent contribution of different microbial groups to metamitron degradation and it was based on the quantitation of ^{13}C-FAs in the living biomass (^{13}C-PLFAs) and in the necromass ($^{13}C\text{-}FAs_{OM}$).

2. Materials and Methods

2.1 Materials and incubation experiments

The model herbicide $^{13}C_6$-metamitron used for soil and water-sediment incubation experiments was purchased from Delta Bio Research Chemicals Ltd. (Vaughan, Ontario,

Canada). The isotopic purity of $^{13}C_6$-metamitron was 99 at % and the chemical purity was 98%. Other chemicals were obtained from VWR (Darmstadt, Germany).

The reference soil was a Haplic Chernozem sampled from the topsoil of the Static Fertilization Experiment in Bad Lauchstädt (Saxony-Anhalt, Germany), a long-term agricultural experiment. This soil received inorganic (50 kg K ha^{-1} a^{-1} and 12 kg P ha^{-1} a^{-1}) and organic fertilizers (30 t ha^{-1} farmyard manure every second year) and metamitron (applied as Goltix WG; for details see also (Wang et al., 2017b)). The characteristics of the soil are: clay, 21±0.4%; silt, 68±2.0%; sand, 11±0.5%; total nitrogen, 0.17±0.01%; total organic carbon (TOC), 2.1±0.1%; pH, 6.6±0.05 and maximum water holding capacity (WHC_{max}), 37.5±1%. The sediment and water used in this study were taken from the Getel creek in the northeastern rim of the Harz Mountains (Saxony-Anhalt, Germany, 51º45'25.02 ºN, 11º17'50.25ºE). This creek is surrounded by agricultural lowlands with continuous crop rotation and pesticide application. The sediment and water characteristics were described in detail in a previous study (Wang et al., 2016). Briefly, the sediment contained 37.7±2% of sand, 62.3±1.1% of silt + clay, 8.5±0.05% of TOC, and 1.5±0.05% of total nitrogen. The pH of the sediment was 7.1±0.1.

Prior to the incubation experiments, soil or sediment was passed through a 2 mm sieve. Soil incubation experiments were performed according to the OECD guideline 307 (OECD 307, 2002) whereas the water-sediment incubations were based on the OECD guideline 308 (OECD 308, 2002). The detailed experimental set-up of both systems has been described previously (Wang et al., 2017a; Wang et al., 2017b). Briefly, in addition to the biotic degradation experiment ($^{13}C_6$-metamitron), we prepared three controls: I: blank (without

metamitron), II: unlabeled metamitron and III: abiotic ($^{13}C_6$-metamitron). All four set-ups were conducted as triplicate series (in total 12 incubation vessels to be sampled once per each sampling date). Soil was spiked with 55 mg kg^{-1} metamitron (either labeled or unlabeled), whereas 55 mg L^{-1} metamitron (corresponding to 90 mg kg^{-1} sediment assuming 100% partitioning into the solid phase) was added to the water of the water-sediment system. The initial concentration of metamitron in soil was eleven-fold higher than the recommended application field rate (of 5 mg kg^{-1}, Engelen et al., 1998), whereas in water-sediment was forty-fold higher than the metamitron amounts found in rivers (0.05-1.5 mg L^{-1}, Moschet et al., 2015). Those high application doses were necessary to obtain reliable isotopic enrichment results against the ^{13}C background. Furthermore, the content of TOC of sediment (8.5%) was four times higher than that of soil (2.1%); therefore, at least twice higher amount of $^{13}C_6$-metamitron as compared with soil was needed to obtain reliable results for the isotopic enrichment. Cumulative mineralization of $^{13}C_6$-metamitron in soil at 5 mg kg^{-1} was nearly similar to that at 55 mg kg^{-1} at each sampling date (Wang et al., 2017a). In contrast, in water-sediment, metamitron mineralization at lower dose (5 mg L^{-1}) was initially faster than that at 55 mg L^{-1} (Wang et al, 2017b), but the cumulative amounts of $^{13}CO_2$ on day 80 were similar. This was related to the slower partitioning of metamitron at higher concentration which delayed its mineralization.

The water content of soil was adjusted to 60% of the WHC_{max}. Two controls (blank and unlabeled metamitron) were used to correct for ^{13}C natural abundance in both incubations. Sterilized soil or water-sediment controls (sterilized by autoclaving three times for 20 min at 120°C and microwave heating for 10 min at 600 W) were installed to differentiate between

the microbial and abiotic degradation of metamitron. The incubations were carried out in the dark and at 20°C for 80 days. Soil samples (two controls and $^{13}C_6$-metamitron) were taken on day 4, 8, 16, 32, 64 and 80, whereas water-sediment systems (unlabeled metamitron and $^{13}C_6$-metamitron) were destructively sampled on day 4, 8, 16, 40 and 80; and analyzed for extractable metamitron and FAs. Due to the high number of samples yielding from all incubations and the workload associated with that, the control water-sediment system was sampled only on day 80, whereas the abiotic set-ups were sampled on day 16, 32, 64 and 80 for soil, and on day 16, 40 and 80 for water-sediment.

2.2 Daily respiration and mineralization of metamitron

The $^{13}CO_2$ evolved from the mineralized metamitron in both set-ups was trapped into 10 ml of 2M sodium hydroxide solution which was replaced regularly. The total concentration of CO_2 (respiration) was determined at each sampling date using a total organic carbon analyzer (Shimadzu TOC-5050, Duisburg, Germany). The isotopic composition ($^{12}C/^{13}C$ at%) of CO_2 was measured by gas chromatography-combustion-isotope ratio-mass spectrometry (GC-C-irMS) after separation on a BPX-5 column (50 m×0.32 m×5 µm). The temperature program for GC-C-irMS was described previously in Girardi et al. (2013).

2.3 FAs analyses: PLFAs and tFAs

The ^{13}C label incorporation from $^{13}C_6$-metamitron into FAs was determined in two fractions: (1) PLFAs indicative of living biomass and (2) tFAs comprising both PLFAs (in biomass) and the FAs in the necromass in OM (FAs_{OM}). The extraction, purification and derivatization procedures were described in detail by Nowak et al. (2011). Briefly, the PLFAs were

extracted from soil or sediment with a mixture of phosphate buffer/methanol/chloroform, purified over silica gel and then derivatized with methanol/trimethylchlorosilane (MeOH/TMCS). In contrast to PLFA, the tFAs in the dried soil or sediment sample were first derivatized with MeOH/TMCS. Thereafter, the derivatized tFAs were extracted from the soil or sediment with diethyl ether and purified over a silica gel column. The concentrations of PLFAs and tFAs were tested using GC-MS (HP 6890, Agilent) with a BPX-5 column (30 m×0.32 m×0.25 μm). The isotopic compositions of ^{13}C-PLFAs and ^{13}C-tFA were determined by GC-C-irMS. The conditions and temperature program for PLFAs and tFAs separations using GC-MS and GC-C-irMS were described in a previous study (Nowak et al., 2011).

FA biomarkers are used to estimate which groups of microorganisms are present. Each group of microorganisms is characterized by a unique FA pattern (Zelles, 1999). Iso- and anteiso-branched FAs are typical Gram-positive bacteria markers; whereas the 10-methyl branched FAs are assigned to actinomycetes (within the Gram-positive bacteria). Gram-negative bacteria are indicated by the presence of monounsaturated FAs and fungi by polyunsaturated FAs. Cyclopropyl FAs are indicators of the starvation of Gram-negative bacteria (Kaur et al., 2005). Saturated straight-chain FAs are present in the cell membranes of all microorganisms and are thus not indicative of any microbial group. However, this FA class is included into the quantitative analysis of microbial biomass.

2.4 Data analyses and data presentation

We performed all incubation experiments (biodegradation test and three controls) as triplicate series in small-scale microcosms (500 mL Schott flasks). We sampled only once from each

incubation vessel at the respective sampling date. This enabled to test the biological variability. All results in the present study are shown as average values of triplicates. The calculation of amounts of ^{13}C label in $^{13}CO_2$, ^{13}C-extractable metamitron, ^{13}C-tFAs and ^{13}C-FAs fractions were based on the quantitation of their total carbon pools (^{12}C + ^{13}C) and the determination of the ^{13}C excess over the controls as described earlier (Lerch et al., 2009; Wang et al., 2016); for details see also "*Calculation of ^{13}C excess in $^{13}CO_2$, ^{13}C-PLFAs and ^{13}C-FAs$_{OM}$*" in the Supplementary Information (SI). The amounts of ^{13}C in the respective fraction were expressed as percentages of the initially applied $^{13}C_6$-metamitron equivalents.

Respiration of soil and water-sediment ($CO_{2\ tot}\ g^{-1}\ day^{-1}$) was based on the quantitation of total CO_2 (^{12}C + ^{13}C) rate per day. Respiration was used as a proxy for microbial activity, in particular following the addition of metamitron. By contrast, the $^{13}CO_2$ daily rates refer only to CO_2 released from the mineralized $^{13}C_6$-metamitron.

FA biomarker analysis was used to detect changes in the quantity of microorganisms and microbial community composition of the soil and sediment after metamitron addition. Time-dependent changes in microbial biomass were identified by changes in the amounts of PLFAs (^{12}C+^{13}C; denoted as PLFAs$_{tot}$). The amount of FAs in necromass in OM (^{12}C+^{13}C; denoted as FAs$_{totOM}$) were calculated by difference between tFAs$_{tot}$ and PLFAs$_{tot}$.

FA-SIP (combined analysis of PLFAs and tFAs with the ^{13}C labeling) enabled time-dependent quantification of the activity of the soil or sediment microbiome and its contribution to the transformation of $^{13}C_6$-metamitron. The ^{13}C-FAs in necromass (^{13}C-FAs$_{OM}$) were calculated by difference between ^{13}C-tFAs and ^{13}C-PLFAs. Five main groups of soil or sediment microbiome (FAs$_{tot}$) and metamitron degraders (only ^{13}C-FAs) were distinguished:

(1) Gram-positive bacteria, (2) actinobacteria (subgroup of Gram-positive bacteria), (3) Gram-negative bacteria, (4) starvation (of Gram-negative bacteria) and (5) fungi. For detailed assignment of individual FA to the groups, please refer to **Table S1** in the SI. No PLFAs ($PLFAs_{tot}$ and ^{13}C-PLFAs) and tFAs ($tFAs_{tot}$ and ^{13}C-tFAs) were detected in the water phase of the water-sediment set-up. Therefore, the results and discussion on FAs are shown only for the sediment phase of the water-sediment system.

Contribution of metamitron-derived C to microbial biomass formation in soil or sediment was calculated as the percentage of ^{13}C-PLFAs (^{13}C µmol g^{-1}) group within the respective group of $PLFAs_{tot}$ ($^{12}C+^{13}C$ µmol g^{-1}). Similarly, the contribution of ^{13}C-metamitron to necromass formation was quantified as the percentage of ^{13}C-FAs_{OM} (^{13}C µmol g^{-1}) group within the FAs_{OMtot} ($^{12}C+^{13}C$ µmol g^{-1}) group. This allows quantifying how much of ^{13}C-labeled metamitron (including ^{13}C-labeled transformation products or ^{13}C-labeled necromass in later degradation stages) is used by soil or sediment microorganisms for biomass formation and versus the other C-substrates (unlabeled ones).

The analytical uncertainty of the total carbon pool of each fraction was < 5%, whereas the uncertainty of at% ^{13}C isotope signatures was < 2% and < 4% for unlabeled and labeled samples, respectively. A repeated measures One-Way ANOVA statistics was performed to test for significant effects of metamitron on respiration and microbial biomass and community composition at the respective sampling date using IBM SPSS Statistics 19.0; effects were considered significant when $p<0.05$. A Principal Components Analysis (PCA) was conducted in order to find a correlation between the ^{13}C-PLFAs microbial groups and the ^{13}C-FAs_{OM} microbial groups in soil and sediment using the XLSTAT software, 2020.

3. Results

3.1 Respiration of soil and water-sediment

The respiration rate the in control soil without metamitron was nearly constant in the first 16 days (4.7±0.3 – 5.2±0.04 µmol g^{-1} $CO_{2\ tot}$ g^{-1} day^{-1}, $p>0.05$; **Fig. 1A**); then it decreased quickly reaching a rate of 1.9±0.2 µmol g^{-1} $CO_{2\ tot}$ g^{-1} day^{-1} after 80 days. Metamitron changed soil respiration as reflected in the increased respiration rates during 8-32 days (4.6±0.2 – 8.4±0.6 µmol $CO_{2\ tot}$ g^{-1} day^{-1}; $p<0.05$). Respiration in the abiotic soil system increased after an 8 day lag-phase. On day 16, it was two times lower (1.6±0.3 µmol $CO_{2\ tot}$ g^{-1} day^{-1}; $p<0.05$) than its biotic counterpart. On days 64 and 80, abiotic respiration rates were higher (2.5±0.1 µmol $CO_{2\ tot}$ g^{-1} day^{-1}; $p<0.05$) than in the control soil (1.9±0.2 – 2.1±0.2 µmol $CO_{2\ tot}$ g^{-1} day^{-1}) and amended with $^{13}C_6$-metamitron (1.9±0.1 – 2.1±0.1 µmol $CO_{2\ tot}$ g^{-1} day^{-1}).

In contrast to control soil, the respiration in the control water-sediment system was nearly constant throughout the incubation period (5±0.01 – 6±1.0 µmol $CO_{2\ tot}$ g^{-1} day^{-1}). Addition of metamitron to water lowered the CO_2 respiration rate on day 4 and 8 (4±0.03 µmol $CO_{2\ tot}$ g^{-1} day^{-1}, $p<0.05$; **Fig. 1B**). Thereafter, the respiration in the water-sediment system containing the herbicide was similar to the control by day 40. Respiration of the abiotic control was only 1.2-fold lower by the end than in the biotic counterpart (2±0.4 – 3.6±0.9 µmol $CO_{2\ tot}$ g^{-1} day^{-1}). Identically to the abiotic soil system, no CO_2 respiration was detected before day 16. CO_2 respiration increased until day 32 and then remained constant until day 80.

The respiration of soil treated with metamitron in the first 16 days was approximately twice higher than in the water-sediment system (5.4±0.1 – 7.4±0.1 µmol $CO_{2\ tot}$ g^{-1} day^{-1} versus 4 ±0.3 µmol $CO_{2\ tot}$ g^{-1} day^{-1}, $p<0.05$). In contrast, the respiration of abiotic water-sediment system was higher than in the soil throughout the entire incubation period (2±0.4 – 3.6±0.9 µmol $CO_{2\ tot}$ g^{-1} day^{-1} versus 1.6±0.3 – 2.5±0.1µmol $CO_{2\ tot}$ g^{-1} day^{-1}, $p<0.05$).

3.2 PLFAs in microbial biomass of soil and sediment

Similar to what was observed for respiration, the amount of microbial biomass in the soil system also differed from the one in the water-sediment system (**Fig. 2**). The $PLFAs_{tot}$ both in soil and sediment were nearly constant during the first 16 days (0.03±0.003 µmol g^{-1} and 0.10±0.002 – 0.11±0.01 µmol g^{-1}, respectively; $p>0.05$); thereafter they declined towards the end. At the end, only half of the initial $PLFAs_{tot}$ was measured in soil (0.013±0.002 µmol g^{-1}) and in sediment (0.04±0.002 µmol g^{-1}). At all sampling dates, $PLFAs_{tot}$ in soil (0.013±0.002 – 0.03±0.003 µmol g^{-1}) were lower than in the sediment (0.04±0.002 – 0.11±0.01 µmol g^{-1}; $p<0.05$).

Metamitron reduced the contents of $PLFAs_{tot}$ in soil on day 4 (0.03±0.003 µmol g^{-1}, $p<0.05$; see **Fig. S1**) as compared with the $PLFAs_{tot}$ in control soil (0.04±0.004 µmol g^{-1}). Thereafter, the $PLFAs_{tot}$ in metamitron-amended soil were nearly similar to the $PLFAs_{tot}$ in control soil ($p>0.05$; except from day 32). Analogously to soil, also the amounts of $PLFAs_{tot}$ in control sediment and in metamitron-amended sediment were comparable on day 80 (0.04±0.002 µmol g^{-1}, $p>0.05$; **Fig. S2A**).

3.3 Mineralization of metamitron in soil and water-sediment

In line with respiration, metamitron mineralization rates in soil and in water-sediment systems were also different (**Fig. 3**). The mineralization rate in soil increased rapidly reaching a maximum on day 8 (7.3±0.09% of $^{13}C_6$-metamitron equivalents per day; $p<0.05$); then it decreased strongly to 0.03±0.004% day^{-1} after 80 days (**Fig. 3**). $^{13}CO_2$ mineralization rates in water-sediment system followed a similar trend as in soil (**Fig. 3**). The highest $^{13}CO_2$ was also noticed on day 8 but it was only half of the one in soil (3.3±0.2% of $^{13}C_6$-metamitron equivalents per day; $p<0.05$). Thereafter, it dropped progressively towards the end reaching 0.2±0.02% day^{-1} after 80 days. Cumulative mineralization of $^{13}C_6$-metamitron in soil on day 80 was higher (60±0.7%; Wang et al., 2017a) than in the water-sediment system (49±4.7%; Wang et al., 2017b).

Only minimal conversion of $^{13}C_6$-metamitron to $^{13}CO_2$ was observed in abiotic soil (**Fig. 3**) with a maximum rate of 0.2±0.04% of $^{13}C_6$-metamitron equivalents on day 32. Similarly low $^{13}CO_2$ rates were noticed in the abiotic water-sediment, with the highest rate measured on day 40 (0.5±0.06% of $^{13}C_6$-metamitron equivalents per day). In contrast to the biotic systems, the cumulative mineralization of $^{13}C_6$-metamitron in abiotic water-sediment system was twice higher (22±3.2%; Wang et al., 2017b) than that of abiotic soil (7.4±0.5%; Wang et al., 2017a).

3.4 ^{13}C labeling pattern kinetics of FAs in soil and sediment

The kinetics of ^{13}C label incorporation into the PLFAs (**Fig. 4**) in both experiments corresponded well with the kinetics of mineralization (**Fig. 3**). Along with ^{13}C mineralization, ^{13}C-PLFAs boosted rapidly in soil, reaching 0.45±0.01% of $^{13}C_6$-metamitron equivalents on

day 8 (see **Fig. 4A**; $p<0.05$). Thereafter, their amounts dropped to 0.10±0.001% after 80 days. The ^{13}C-tFAs increased until day 16 (0.96±0.04% of $^{13}C_6$-metamitron equivalents) then diminished quickly. On day 80 the ^{13}C-tFAs amounts were similar to the ^{13}C-PLFAs (0.13±0.003%, $p<0.05$).

The kinetics of ^{13}C label incorporation into the PLFAs in sediment was slower than in soil (**Fig. 4B**). The ^{13}C-PLFAs increased until day 40 peaking at 0.11±0.004% of $^{13}C_6$-metamitron equivalents ($p<0.05$). Similar to ^{13}C-PLFAs, the ^{13}C-tFAs peaked on day 40 (0.31±0.02% of $^{13}C_6$-metamitron equivalents). Thereafter, both ^{13}C-PLFAs and ^{13}C-tFAs declined towards the end (0.06±0.004% and 0.12±0.004% of $^{13}C_6$-metamitron equivalents, respectively, $p<0.05$).

Throughout the experiment, both ^{13}C-PLFAs and ^{13}C-tFAs in soil were between three- and four-fold higher than in the sediment. A maximum of 70% of the ^{13}C-tFAs was attributed to ^{13}C-FAs in necromass ($^{13}C\text{-}FAs_{OM}$) of soil on day 16. On the contrary a maximum of 92% of the ^{13}C-tFAs could be assigned to $^{13}C\text{-}FAs_{OM}$ already on day 8 in the water-sediment system. On the next sampling dates (day 16 and 40), lower amounts of the ^{13}C label from ^{13}C-tFAs were allocated to $^{13}C\text{-}FAs_{OM}$ (42% and 63%, respectively), suggesting lower stabilization of lipids in the water-sediment system than in soil.

No ^{13}C-PLFAs and ^{13}C-tFAs were found in the abiotic controls of both systems.

3.5 Microbial groups based on $PLFAs_{tot}$ and ^{13}C-PLFAs in soil and sediment

Addition of metamitron to soil did not change the microbial community composition (based on $PLFAs_{tot}$) of soil; throughout the entire incubation period (see **Fig. S3A**) it was similar to that in the control soil without metamitron amendment (see **Fig. S3B**). Both Gram-positive

(+actinobacteria) and Gram-negative bacteria (+starvation) markers prevailed equally over the other microbial markers.

Similar to soil, addition of metamitron to water-sediment system also did not induce any change on the sediment microbial community structure compared to the control on day 80 (**Fig. S2B**). In contrast to soil, the Gram-negative bacteria markers (+starvation) dominated in the sediment throughout the entire incubation period (**Fig. S4**).

In the initial phase of $^{13}C_6$-metamitron biodegradation in soil (day 4), the ^{13}C label-derived metamitron was detected mainly in the biomass of Gram-negative bacteria (0.02±0.003% of $^{13}C_6$-metamitron equivalents; $p<0.05$; see in **Fig. 5A** and **Table S2,** and $\delta^{13}C$ enrichments in **Table S3**). While an accelerated mineralization of $^{13}C_6$-metamitron with the concurrent increase of ^{13}C-PLFAs amounts occurred (8-16 days), the ^{13}C-Gram-positive bacteria (0.03±0.0006% – 0.22±0.008% of $^{13}C_6$-metamitron equivalents) and particularly ^{13}C-actinobacteria (0.09±0.002% – 0.10±0.003% of $^{13}C_6$-metamitron equivalents) constituted a major share within the ^{13}C-PLFAs (**Fig. 5A**, $p<0.05$; see also and **Fig. S5**). Markers for actinobacteria (10 Me 17:0 and 10 Me 18:0) and other Gram-positive bacteria (a 17:0) dominated over the other ^{13}C-PLFAs (see ^{13}C-amounts in **Table S2** and $\delta^{13}C$ enrichments in **Table S3**; $p<0.05$).

After $^{13}C_6$-metamitron mineralization ceased, the content of ^{13}C in the biomass of Gram-positive bacteria declined again whereas ^{13}C-Gram-negative bacteria relatively increased. The ^{13}C in the necromass (^{13}C-FAs_{OM}) of soil was dominated by Gram-negative bacteria until day 64 (0.03±0.0006 – 0.3±0.001% of $^{13}C_6$-metamitron equivalents; see **Fig.**

5B). A small contribution of the fungal biomarker to the ^{13}C-FAs_{OM} was noticed only on the last day (0.01±0.0003 % of $^{13}C_6$-metamitron equivalents).

In the sediment, the ^{13}C in the markers for Gram-negative bacteria was highest throughout the entire incubation period (0.04±0.0013 – 0.3±0.002% of $^{13}C_6$-metamitron equivalents; $p<0.05$; see **Fig. 6A**; see also **Table S3**, **Table S4** and **Fig. S5**), and in particular from day 8 to day 16 when metamitron was mineralized at maximum rates. In later degradation stages, a parallel increase in ^{13}C contents of all microbial groups was observed.

The ^{13}C label in the necromass (^{13}C-FAs_{OM}) on 4, 40 and 80 days was represented mainly by Gram-negative bacteria (0.01±0.001 – 0.04±0.001% of $^{13}C_6$-metamitron equivalents; $p<0.05$; see **Fig. 6B**), whereas on day 16 by Gram-negative bacteria and actinobacteria (0.02±0.002% of $^{13}C_6$-metamitron equivalents). On the last day, the ^{13}C label in the fungal marker prevailed over the other microbial groups in ^{13}C-FAs_{OM} (0.03±0.001% of $^{13}C_6$-metamitron equivalents).

Based on the PCA analysis, a strong correlation of ^{13}C in the necromass (^{13}C-FAs_{OM}) to the fungal marker in both soil and water-sediment systems has been found (see **Fig. S5**).

3.6 Contribution of metamitron-C to microbial biomass and necromass formation

The contribution of the metamitron-derived C to microbial biomass formation (measured as % contribution of ^{13}C-PLFAs group within the $PLFAs_{tot}$ group) in soil was bigger than in the water-sediment system (see **Fig. S6A** and **S6B**). In the very early metamitron mineralization phase (day 4), only Gram-negative bacteria and actinobacteria incorporated metamitron-derived ^{13}C, which made up 5% and 3% of their biomass C, respectively (**Fig. S6A**). On day 8, when mineralization rate of $^{13}C_6$-metamitron was highest, a large proportion

of metamitron-^{13}C was measured in the actinobacteria group (50%) and in the Gram-positive bacteria group (38%). In later degradation stages, on day 16, 32 and 80 a high percentage of C in the actinobacteria group was derived from $^{13}C_6$-metamitron (48%, 53% and 33%, respectively), and on day 32 in Gram-negative bacteria (33%). Additionally, a remarkable amount of ^{13}C-metamitron-derived C (18%) was also measured in the fungi on day 64.

In contrast to the soil, much lower contribution of the metamitron-^{13}C to sediment biomass formation was observed during the accelerated degradation of $^{13}C_6$-metamitron (4-16 days; see **Fig. S6B**). The ^{13}C label was detected only in the Gram-negative bacteria group on day 4 (0.3%). Similarly, also low amounts of the metamitron-^{13}C were noticed in the Gram-negative bacterial markers (0.7-8%), actinobacteria (0.09-5%) and Gram-positive bacteria (1.3%) during 8-16 days. When the metamitron ceased (40-80 days), the metamitron-^{13}C was measured mainly in the actinobacteria group (11-22%). Similarly to what was observed in soil, also remarkable contents of metamitron-^{13}C were determined in the fungi (8.7-10%) in later degradation stages (day 40 and 80).

The contribution of the metamitron-derived C to necromass formation (measured as % contribution of ^{13}C-FAs_{OM} group within the FAs_{totOM} group) was lower than the metamitron-C contribution to microbial biomass formation (see **Fig. S6** and **S7**). Furthermore, the proportional contributions of metamitron-^{13}C to necromass formation in soil and in sediment were comparable (see **Fig. S6A** and **S6B**; see also a positive correlation of ^{13}C-FAs_{OM} to the fungal marker in both systems in **Fig. S5**). During the first 16 days, the metamitron-^{13}C in soil contributed mainly to the markers for necromass of Gram-negative bacteria (incl. starvation; 6-10%; see **Fig. 7A**). In later degradation stages, metamitron-^{13}C contributed to the necromass

of actinobacteria on day 64 (32%) and in the fungal necromass (28%). In contrast to soil, in the very early metamitron degradation phase (day 4), equal contributions of the metamitron-^{13}C (2.9%) were found in the necromass of Gram-negative and Gram-positive bacteria (incl. actinobacteria) in water-sediment (**Fig. 7B**). In later metamitron degradation stages, the metamitron-^{13}C contribution was particularly high in the necromass of actinobacteria (13-31%). Similar to what was observed in soil, a significant contribution of metamitron-^{13}C was detected in the necromass of fungi (8%) on the last day.

4. Discussion

4.1 Metamitron changed microbial activity of both soil and water-sediment system

The addition of metamitron at 55 mg kg^{-1} to soil promoted respiration. A similar effect was observed in soil amended with an eleven-fold higher amount of metamitron than the recommended application field rate of 5 mg kg^{-1} (Engelen et al., 1998). However, the soil respiration in the study by Engelen et al. (1998) increased between two and eight weeks of incubation, but not at the beginning as in our study (8-32 days). The enhanced soil respiration in both studies could be related to either of either stress response or adaptation to the high dose of metamitron. It is difficult to explain the divergence in time-dependent soil respiration kinetics in these two studies. Potential reasons for this include different experimental set-up, soil type, exposure history to metamitron, availability of metamitron to microorganisms and different sensitivity of the microbial community to metamitron. However, enzymatic activities (dehydrogenase, urease and nitrification) in soil treated with metamitron were comparable

with the control soil without this herbicide (Engelen et al., 1998). This suggests no detrimental effect of metamitron on soil microbial community. By contrast, we observed reduced amounts of $PLFAs_{tot}$ in biomass of metamitron-amended soil as compared with the control on day 4. Afterwards, the $PLFAs_{tot}$ in our study were similar to the ones in control and the microbial community composition in soil remained unchanged following metamitron addition. This finding disagrees with the study by Engelen et al. (1998), who noticed slight changes in the abundance of several unidentified microbial clones (16S rRNA) in metamitron-amended soil. Nonetheless, the nucleic acid approach is more sensitive with regard to the taxonomic resolution than FA analysis. Thus, it is likely that metamitron could have induced negative effects on soil microbial community composition that remained undetectable using the FA approach.

In contrast to soil, metamitron suppressed the respiration of water-sediment systems initially (day 4 and day 8). Similar to what was observed for soil, the changed respiration in water-sediment system also could be a result of either stress response or adaptation to a high dose of metamitron. The initially applied amount of metamitron to water-sediment was higher than in soil (90 mg kg^{-1} versus 55 mg kg^{-1}). However, the enhanced metamitron concentration in water-sediment system is presumably not the reason for the suppressed respiration. Metamitron was added to the water phase of water-sediment system; therefore, this herbicide will partition into the solid phase of water-sediment system, which requires an equilibration time (Katagi, 2013; Wang et al., 2017b). Microorganisms inhabit preferentially solid surfaces in aquatic environments rather than swimming planctonically (Warren et al., 2003). Therefore, metamitron can exert toxicity on microorganisms in aquatic ecosystems only when it adsorbs

onto solid surfaces. Approximately 55% of the initial amounts of metamitron were partitioned into the sediment on day 8 (Wang et al., 2017b). Thus, a similar amount of metamitron was absorbed onto sediment (ca. 50 mg kg^{-1}) than the initially applied amount of metamitron to soil (55 mg kg^{-1}); therefore, the larger inhibition of respiration cannot be explained by a higher exposure of microbes in the sediment. The microbial biomass (based on $PLFAs_{tot}$) and the microbial community composition of sediment amended with metamitron were similar to that in the control set-up on the last day suggesting no detrimental long-term effect of metamitron. As we measured the $PLFAs_{tot}$ in the biomass of control sediment only on day 80, we may have overlooked temporal toxic effects caused by metamitron application. The combined analysis of $PLFAs_{tot}$ kinetics and community fingerprinting (e.g. 16S rRNA gene sequencing) in control and metamitron-amended sediment is still necessary to validate our results on transitory toxic effects of metamitron to the microbiome.

The respiration in both abiotic settings was lower than in the biotic ones and it was delayed. The delay was caused by re-growth of heat-resistant bacteria after the sterilization process. However, the increase in respiration was more pronounced in the abiotic water-sediment than in the abiotic soil. This could be related to either higher number of heat-resistant microorganisms or better availability of nutrients or carbon-substrates for re-growing microorganisms (Cranwell, 1982; van Oevelen et al., 2006; Zhang et al., 2019).

4.2 Activity and composition of the microbiome differed between soil and sediment

The $PLFAs_{tot}$ concentration was lower in soil than in sediment, indicating a lower microbial biomass in soil. However, the TOC concentrations were also different, and if referred to TOC

concentrations, the size of microbial biomass (per unit OM) was similar. The ratios of soil-$PLFAs_{tot}$ to soil-TOC were in the range 1.2-2.7×10^{-4}, whereas the ratios of sediment-$PLFAs_{tot}$ to sediment-TOC ranged between 1.1×10^{-4} and 2.5×10^{-4}. However, soil respiration normalized to microbial biomass (as indicated by the ratio of soil respiration to soil-$PLFAs_{tot}$ [91-300]) was higher than water-sediment respiration (water-sediment respiration / sediment-$PLFAs_{tot}$ = 34-141). The discrepancy in the microbial activity in these two systems is difficult to explain. Several factors might have contributed to that, for instance: different water and oxygen contents, different amounts of total organic carbon or different C/N ratios in soil and water-sediment systems (Cranwell, 1982; van Oevelen et al., 2006). The content of oxygen and the C/N ratio in aquatic sediments are usually lower than in soils (Cranwell, 1982).

Gram-positive and Gram-negative bacteria equally dominated within the soil microbiome, whereas the Gram-negative bacteria dominated in the sediment microbiome during the entire incubation period. The fast-growing Gram-negative bacteria are known to utilize more labile substrates, whereas the slowly-growing Gram-positive bacteria degrade more recalcitrant compounds of OM (Billings and Ziegler, 2008; Breulmann et al., 2014; Fanin et al., 2019; Kramer and Gleixner, 2008; Moore-Kucera and Dick, 2008). The upper water phase in the water-sediment might have increased the availability of labile nutrients and carbon substrates to microorganisms as well as their quality and quantity (Cranwell, 1982; van Oevelen et al., 2006; Zhang et al., 2019). Therefore, Gram-negative bacteria in sediment microbiome had presumably better conditions for growth than the ones in soil.

4.3 Mineralization of metamitron in soil was faster than in water-sediment

Mineralization of metamitron in soil was faster than in the water-sediment system. This is consistent with the observed higher respiration rates of soil during 8-16 days. The activity of soil is generally highest at the beginning of incubation upon the addition and mixing of a substrate (Jarvis et al., 2007; Lado-Montserrat et al., 2014). The smaller content of oxygen in aquatic sediments than in soils slows down aerobic microbial turnover of organic compounds (Cranwell, 1982; Katagi, 2013). After oxygen in the water-sediment system is depleted, anaerobic degradation processes may prevail over the aerobic degradation (Katagi, 2013). Anaerobic degradation processes are generally known to be slower than aerobic degradation processes (Katagi, 2013; Wang et al., 2016). We therefore consider this the major reason why metamitron mineralization in water-sediment was slower.

Although the sediment biomass (based on $PLFAs_{tot}$) was higher than in soil, the contribution of sediment microbiome to metamitron degradation was smaller than in soil as evidenced by ^{13}C-PLFAs. However, the soil used for the experiment had received the Goltix WG formulation, which contains metamitron, for at least 10 years. Therefore, the enhanced microbial degradation of metamitron in soil could be a result of presence of high number of metamitron degraders upon repeated metamitron application (Arbeli and Fuentes, 2007). Furthermore, different physico-chemical properties of soil and sediment may also have affected the extent of metamitron sorption and thereby its mineralization (Gavrilescu, 2005). The amount of necromass ($^{13}C\text{-}FAs_{OM}$) in the water-sediment system was high only for a short period of time (maximum 92% of ^{13}C-tFAs on day 8 with the rapid decrease to 42% on

day 16) as compared with the soil. This finding agrees well with the generally low preservation of microbial biomass in sedimentary OM (Cranwell, 1982; Katagi, 2013).

4.4 Metamitron degraders depend on the system and are time-dependent

Initially, only Gram-negative bacteria degraded metamitron in soil. Gram-positive bacteria and in particular actinobacteria dominated on day 8 and 16. A previous study on microbial turnover of ^{13}C-labeled metamitron in soil evidenced the biodegradation of this herbicide via two pathways: the "Rhodococcus pathway" and the "desamino pathway" (Wang et al., 2017a). Two bacterial strains capable to degrade metamitron via the "Rhodococcus pathway" (*Rhodococcus* sp. 0246b) and the "desamino pathway" (*Arthrobacter* sp. DSM 20389) were isolated from soil exposed to metamitron (Engelhardt et al., 1982; Parekh et al., 1994; see also Metamitron Pathway Map). *Rhodococcus* sp. (Parekh et al., 1994) and *Arthrobacter* sp. (Engelhardt et al., 1982) are aerobic actinobacteria (Gram-positive bacteria subgroup). *Rhodococcus* sp. inhabits a broad range of environments like soil and water (Martinkova et. al., 2009), whereas *Arthrobacter* sp. is commonly found in soil (Mongodin et al, 2006). As the typical PLFA biomarkers for actinobacteria (10 Me 17:0 and 10 Me 18:0) were highly abundant in soil, it is possible that *Rhodococcus* sp. or *Arthrobacter* sp. degraded metamitron in soil. However, the label in Gram-negative bacteria markers prevailed both in the biomass at the very early (day 4) and at later phase of metamitron degradation (32-80 days) as well as in the necromass of soil (8-64 days). Gram-negative bacteria were also the main degraders of the herbicide 2,4-D in soil during the accelerated 2,4-D degradation phase (Lerch at al., 2009). Gram-negative bacteria preferentially utilized available metamitron, and thus dominated

immediately upon substrate addition (Jarvis et al., 2007; Lado-Montserrat et al., 2014). This suggests that Gram-negative bacteria are the metamitron degraders in soil. As the Gram-positive bacteria and actinobacteria markers were highly enriched in ^{13}C on day 8 and 16, we cannot exclude that these microbial groups also degraded metamitron in soil from the start of the incubation. Therefore based on that, we can conclude that both Gram-negative and positive bacteria and actinobacteria were metamitron degraders. However, actinobacteria presumably degraded metamitron slower than Gram-negative bacteria.

After metamitron was depleted, the predominant Gram-negative bacteria could not continue label uptake as they cannot degrade the necromass of primary metamitron degraders. The necromass-degrading actinobacteria could have supported the growth of Gram-negative bacteria via excretion of ^{13}C-exudates from their biomass or through the mobilization of ^{13}C-compounds of other decaying microorganisms (Fanin et al., 2019; Moore-Kucera and Dick, 2008; Rinnan and Bååth. 2009). Actinobacteria thus consumed presumably the necromass of primary metamitron degraders at both early and later stages of the experiment. Similar results were also noticed in the FA-SIP study with $^{13}C_6$-2,4-D by Lerch at al. (2009) where Gram-positive bacteria assimilated ^{13}C from the principal 2,4-D degraders.

In contrast to soil, the Gram-negative bacteria dominated within the microbial community of sediment during the entire period. The degradation of metamitron in water-sediment occurred presumably only via the “desamino pathway” (Wang et al., 2017b) which has been documented in the actinobacterial soil isolate *Arthrobacter* sp. (Parekh et al., 1994; Metamitron Pathway Map). As the Gram-negative bacteria markers predominated during the initial degradation of metamitron, this microbial group could have been the metamitron

degraders also in the water-sediment system. Similar to what observed in soil, highly abundant in ^{13}C-actinobacteria in the later degradation stage also presumably degraded the necromass of metamitron degraders in water-sediment system. In addition to actinobacteria, fungi could also have contributed to the degradation of necromass of metamitron degraders in water-sediment system. Fungi have a unique ability to decompose complex substrates (Fabian et al, 2016) and thus could also have enhanced the availability of the decomposing labeled biomass compounds (Fabian et al, 2016; Li et al., 2015).

4.5 More metamitron-C was used for biomass formation in soil than in sediment

In the very early phase of metamitron degradation, only low amounts of metamitron-C was used for biomass and necromass formation in both soil and water-sediment systems. At that time, the C derived from metamitron was used by Gram-negative and Gram-positive bacteria, as well as actinobacteria. However, all three groups of bacteria utilized preferentially other (unlabeled) substrates over metamitron (<10%) as a C source until day 4. Multiple use of different C-substrates in nature is well-known (Rinnan and Bååth, 2009). In contrast to the sediment, the contribution of metamitron-C to microbial biomass formation in soil increased from day 4 onwards and it was between two- and five-fold higher than in the sediment. This remarkable difference in the relative contribution of metamitron-C to biomass formation could be attributed to different activities of the two microbiomes. The microbial activity in both experiments can be affected by different conditions, e.g. oxygen and nutrient availabilities, microbial ability to degrade metamitron or exposure history to metamitron (Billings and Ziegler, 2008; Cranwell, 1982; Fanin et al., 2019). The large contribution of

metamitron-C to biomass formation and in particular to Gram-positive bacteria and actinobacteria biomass formation soil is a result of the enhanced capability of these microbial groups to degrade metamitron upon repeated metamitron application (Arbeli and Fuentes, 2007).

The striking contribution of metamitron-C in fungal markers to necromass in both systems is related to higher stabilization potential of fungal residues in the OM. Fungi generally contribute more than bacteria to OM formation through necromass accumulation, as their necromass is characterized by a slower turnover rate than bacterial necromass (Li et al., 2015; Schweigert et al., 2015).

5. Conclusions

This is the first study which compared the effect of metamitron addition on microbial activity and the extent of metamitron degradation in two different model environments – terrestrial and aquatic. Metamitron initially increased soil respiration whereas it suppressed respiration in the water-sediment system. Metamitron mineralization in soil was faster than in the water-sediment system. The contribution of metamitron-derived C to the formation soil microbial biomass and necromass was higher than in water-sediment. Gram-negative bacteria, Gram-positive bacteria and actinobacteria were the metamitron degraders in soil, whereas Gram-negative bacteria were the only metamitron degraders in water-sediment system. Actinobacteria acted as the necromass degraders of primary degraders in soil, while both actinobacteria and fungi were necromass consumers in water-sediment.

This study clearly showed that different microbial taxa contributed to metamitron turnover in soil and water-sediment and that their contributions shifted over degradation stages. The synthropic interactions between different microbial groups during turnover of pesticides in terrestrial and aquatic ecosystems should be investigated in detail. New studies should distinguish between the direct carbon fluxes (pesticide → primary consumers) and the indirect carbon fluxes (primary consumers → secondary consumers) from a pesticide. Furthermore, still little is known on whether interactions between different microbial taxa upon pesticide application can induce unwanted effects on microbial community composition.

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Figure captions

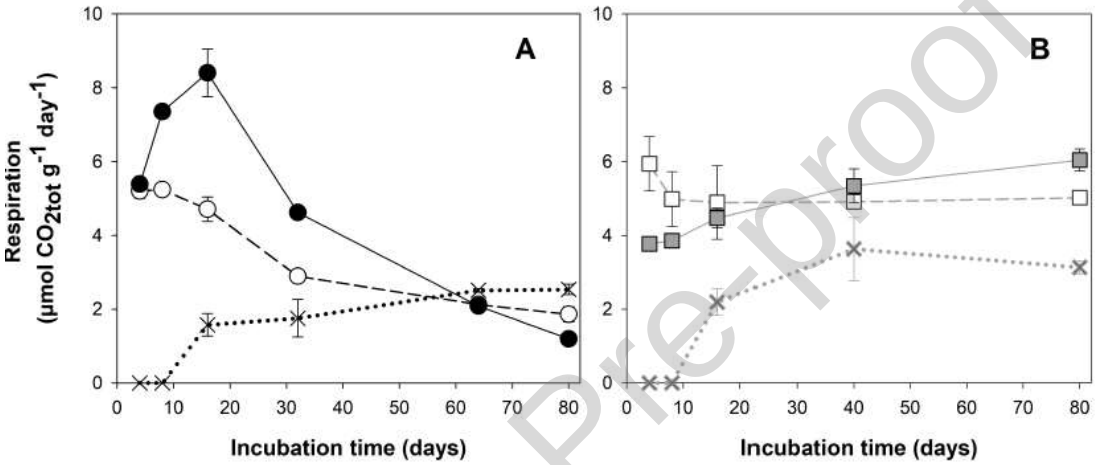


Fig. 1. Respiration (µmol CO_{2tot} g^{-1} day^{-1}) of soil (**A**) and water-sediment (**B**) system over 80 days of incubation.

Soil: Control without metamitron (○), Labeled metamitron (●), Abiotic control (×).

Water-sediment: Control without metamitron (□), Labeled metamitron (■), Abiotic control (⊠).

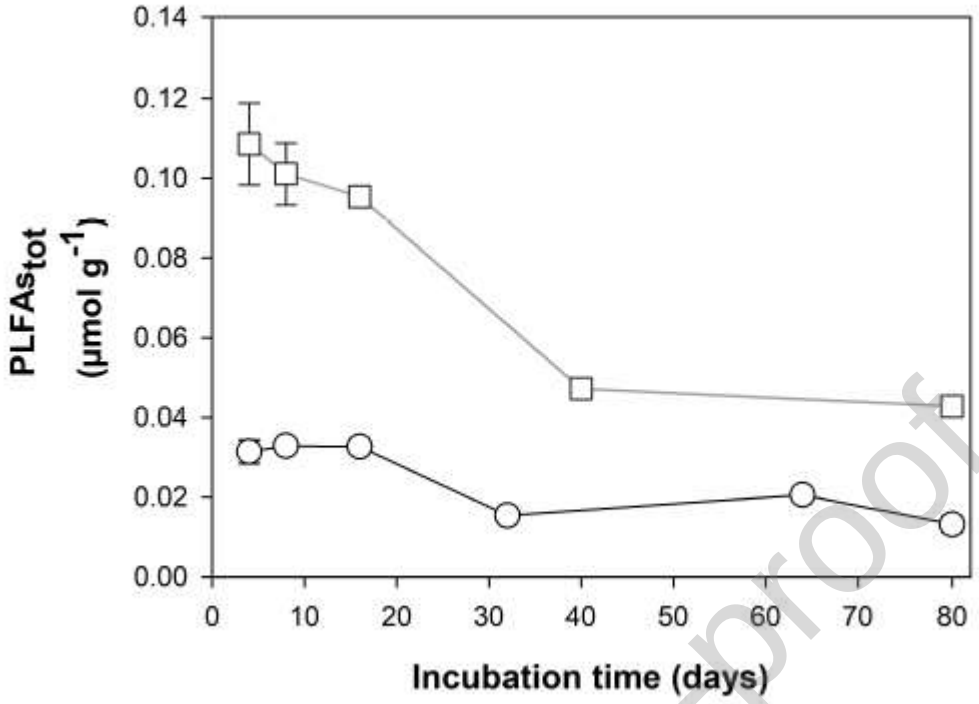


Fig. 2. The $PLFAs_{tot}$ (µmol g^{-1}) in soil (○) and sediment (□) amended with $^{13}C_6$-metamitron over 80 days of incubation.

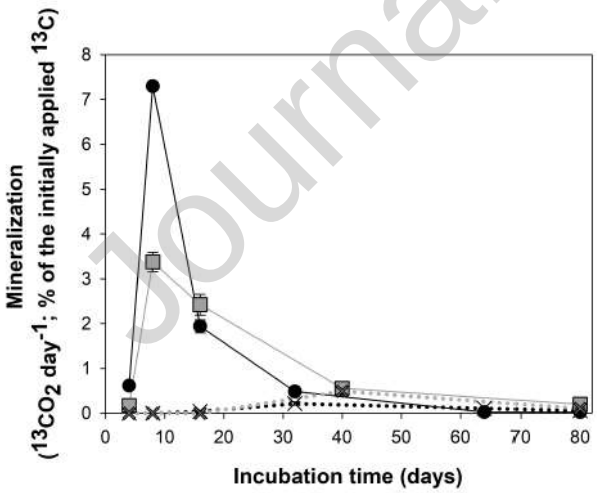


Fig. 3. Mineralization of $^{13}C_6$-metamitron (day^{-1}, % of the initially applied ^{13}C) over 80 days of incubation in soil and water-sediment.

Soil: Biotic (●), Abiotic soil (×). *Water-sediment*: Biotic (■), Abiotic water-sediment (⊠).

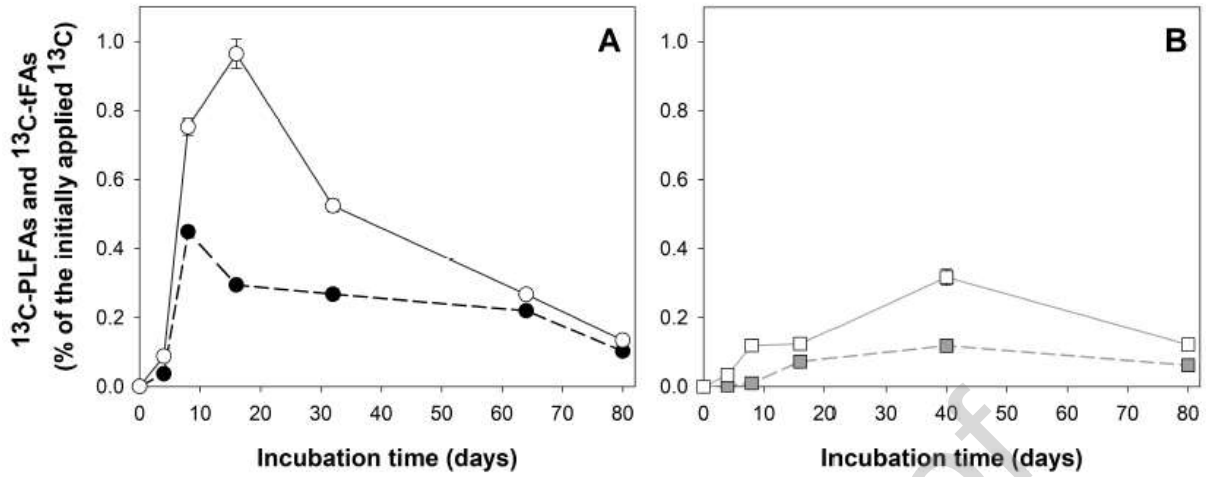


Fig. 4. Incorporation of ^{13}C from $^{13}C_6$-metamitron into PLFAs and tFAs over 80 days of incubation in soil (**A**) and sediment (**B**) as % of the initially applied ^{13}C. *Soil*: PLFAs (●), tFAs (○). *Water-sediment*: PLFAs (■), tFAs (□).

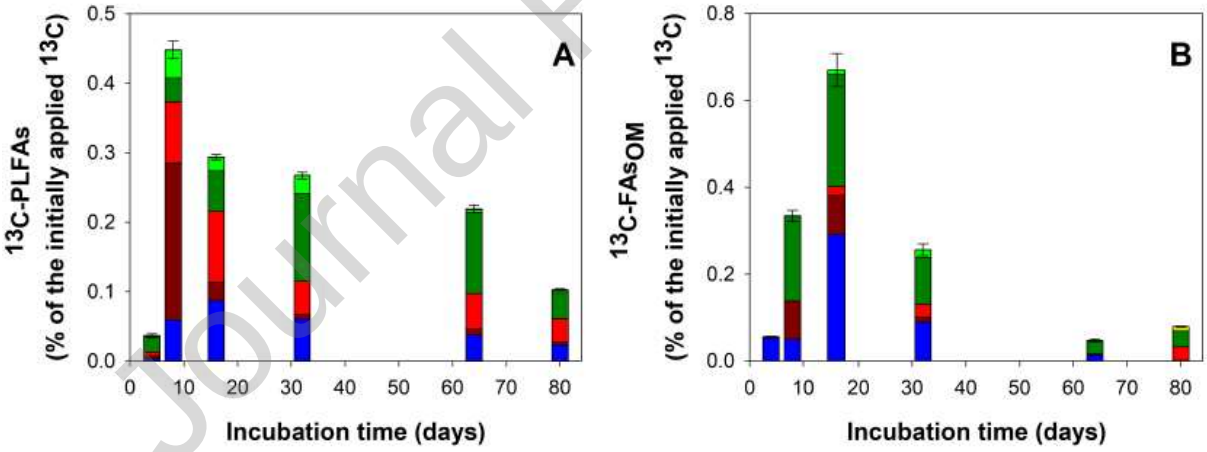


Fig. 5. Groups of ^{13}C-PLFAs (**A**) and ^{13}C-FAs$_{OM}$ (**B**) in soil over 80 days of incubation in % of the initially applied ^{13}C. General (■), Gram-positive bacteria (■), actinobacteria (subgroup of Gram-positive bacteria) (■), Gram-negative bacteria (■), starvation (Gram-negative bacteria) (■), fungi (■). Unspecified ^{13}C-PLFAs or ^{13}C-FAs$_{OM}$ are included into the General FAs group.

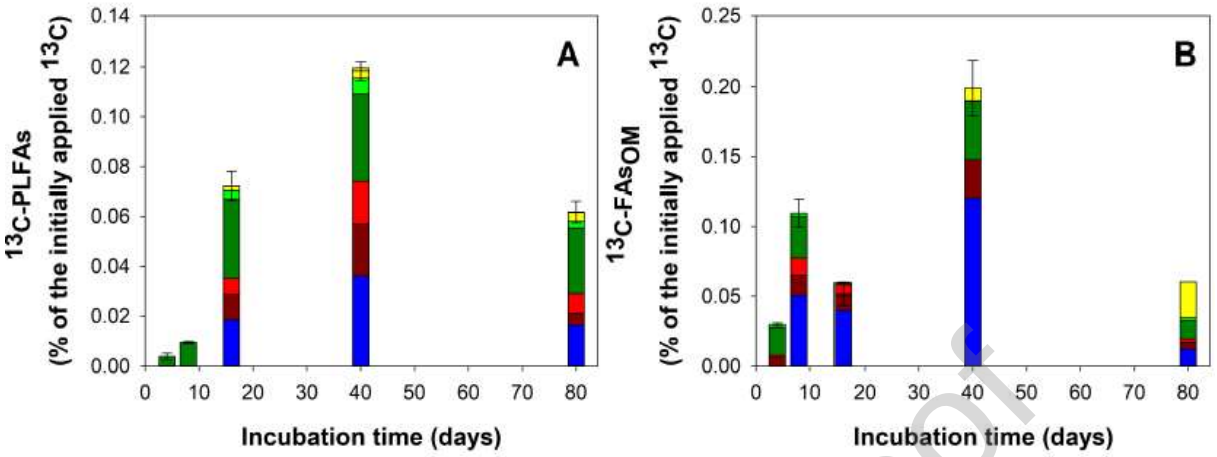
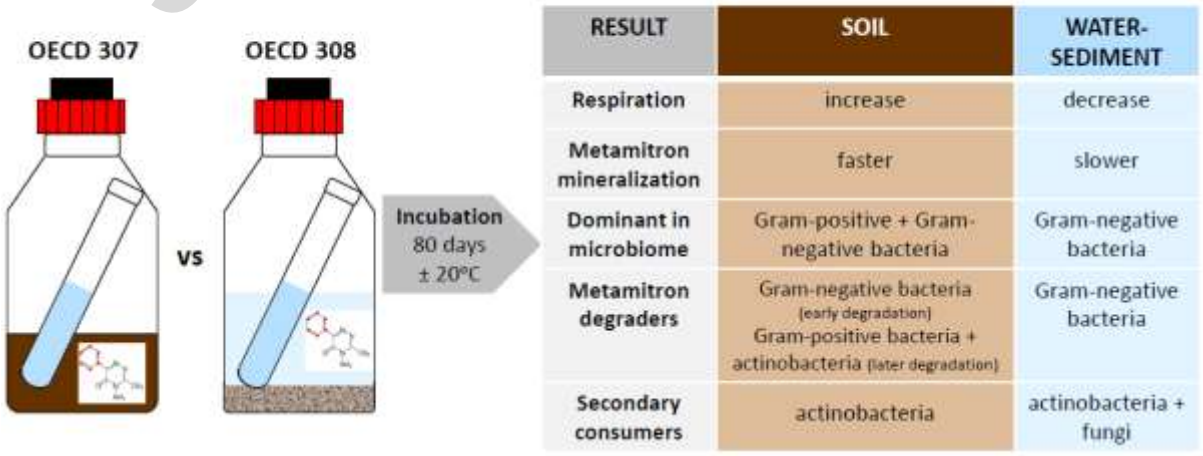


Fig. 6. Groups of ^{13}C-PLFAs (**A**) and ^{13}C-FAs$_{OM}$ (**B**) in sediment over 80 days of incubation in % of the initially applied ^{13}C. General (■), Gram-positive bacteria (■), actinobacteria (subgroup of Gram-positive bacteria) (■), Gram-negative bacteria (■), starvation (Gram-negative bacteria) (■), fungi (■).Unspecified ^{13}C-PLFAs or ^{13}C-FAs$_{OM}$ are included into the General FAs group.

Graphical abstract



AUTHOR CONTRIBUTIONS FORM

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"Microbial activity and metamitron degrading microbial communities differ between soil and water-sediment systems"

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On behalf of all authors,

Karolina Nowak

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Metamitron increased respiration in soil but suppressed it in water-sediment
- Mineralization of metamitron in soil was faster than in water-sediment
- Gram-negative, positive bacteria and actinobacteria were metamitron degraders in soil
- Gram-negative bacteria were metamitron degraders in water-sediment
- Actinobacteria and fungi were secondary consumers of primary degraders