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Research article

Water quality and pesticide contamination in agricultural streams: assessing white-rot fungi and biochar treatments as remediation strategies

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ABSTRACT

Pesticides used in agriculture, such as herbicides and fungicides, can reach water bodies through runoff and are a threat to freshwater ecosystems. Traditional methods to remove organic pesticides often lack efficiency or are too costly at scale. White-rot fungi (WRF) offer a green alternative by producing enzymes that break down persistent pollutants like pesticides. Biochar, made from organic waste, is another sustainable option due to its high adsorption capacity and carbon sequestration potential. This study evaluates water quality and pesticide contamination in five agricultural streams in Norway, and tests treatments with WRF and biochar as cost-effective, eco-friendly solutions for removing pesticides from surface waters. Eutrophication status based on total phosphorous ranged from high to bad conditions, while total nitrogen was relatively high at all locations. 35 pesticides were detected in at least one location, with concentrations ranging from 0.001 µg/l to 9.1 µg/l (for fluroxypyr). The most frequently detected compound was trifloxystrobin acid (present in all studied locations). Three water treatments were tested in the laboratory, two using WRF, in the form of spent mushroom substrate (SMS) and pellets, and one using biochar. The approaches showed promise in reducing pesticide concentrations, with biochar (average removal efficiency of 94 %) significantly outperforming WRF-based treatments (27 % average removal efficiency for WRF: SMS and 20 % for WRF: pellets). However, all treatments led to increases in total phosphorus, though the increase was lower for WRF: pellets. WRF treatments also significantly increased total organic carbon and total nitrogen, which raises concerns about the risk of eutrophication.

1. Introduction

Micropollutants pose an increasing threat to freshwater ecosystems, impacting water quality and biodiversity (Schwarzenbach et al., 2006). Micropollutants, including pesticides, industrial compounds, pharmaceuticals, and personal care products, enter water bodies through various pathways, such as agricultural runoff, wastewater discharge, and atmospheric deposition (Hörchner et al., 2024). Among micropollutants, herbicides are particularly concerning due to their widespread use, as runoff from agricultural fields may not only degrade water quality and ecological integrity but also reduce the suitability of water for downstream irrigation (Brock et al., 2000; Cessna et al., 1994; Ghosh et al., 2023).

Herbicides are largely used in agriculture to control unwanted vegetation and can reach water bodies through runoff, particularly during periods of heavy rainfall, or through leaching into groundwater. They affect freshwater species through direct and indirect mechanisms, with potential long-term impacts on populations, community structure, and ecosystems (Brock et al., 2000; Ghosh et al., 2023). Direct effects include mortality at high concentrations, while sublethal impacts, such as reduced growth, impaired reproduction, developmental abnormalities, and altered behaviour, can decrease fitness and cause population declines (Ghosh et al., 2023; Schafer et al., 2011). For example, glyphosate exposure has been shown to reduce the reproduction of *Daphnia magna*, a common zooplankton species (Ghosh et al., 2023). Indirectly, by targeting primary producers like algae and macrophytes, herbicides can affect the basis of aquatic food webs, leading to resource shortages for herbivores, altered predator-prey dynamics, and shifts in species interactions (Pesce et al., 2011; Schafer et al., 2011). For example, atrazine exposure has been shown to reduce phytoplankton biomass, impairing zooplankton reproduction (Schafer et al., 2011). Compounds that mimic the plant hormone auxin, like MCPA

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(2-methyl-4-chlorophenoxyacetic acid), mostly impact macrophytes (Brock et al., 2000; Petersen et al., 2015; Schafer et al., 2011) and have varying effects on different algae species, fish and invertebrates (Ghosh et al., 2023). Photosynthesis inhibition can lower dissolved oxygen and alter pH, and the alteration of key ecosystem processes like nutrient cycling and decomposition can further degrade water quality and overall ecosystem function, negatively affecting aquatic ecosystems (Ghosh et al., 2023; Pesce et al., 2011; Rydh Stenström et al., 2021; Schafer et al., 2011).

Herbicide persistence in the environment is influenced by the its properties, i.e. sorption to soil particles, as well as climate, soil composition, microbial activity, and water flow dynamics, all of which shape its long-term environmental impact (Rasmussen et al., 2015). Cold climates with near or below-zero winter temperatures significantly slow herbicide breakdown, leading to greater variability in degradation rates in high-latitude regions compared to temperate regions (Eklo et al., 2019). This slower degradation in colder climates can result in higher concentrations of these substances in runoff, increasing the risk of contamination in freshwater ecosystems. Increased precipitation further contributes to pesticide transport by enhancing runoff from agricultural fields, resulting in higher concentrations in streams (Oldenkamp et al., 2024). With climate change expected to lead to more precipitation and more frequent extreme weather events in Norway (Norsk klimaservicesenter, 2015), the risk of diffuse pollution into freshwater ecosystems is likely to rise, increasing environmental concerns.

Considering the impact of pesticides on the aquatic ecosystem, developing efficient and cost-effective methods for their removal is of critical importance. As discussed by Dehghani et al. (2024), a range of different methods can be considered for removal of pesticide from water. However, from an applied perspective several of these methods are far too advanced, too costly or may pose ecological risks and therefore they may not be applicable in the agricultural landscape. In this study, we evaluated two low-cost approaches based on either bioremediation or biochar. The study is based on laboratory experiments that were conducted using water samples collected from an agricultural landscape in Norway, where water quality and micropollutant contamination were assessed. For the bioremediation approach, we tested white-rot fungi (WRF) in the form of both spent mushroom substrate (SMS) and fungal pellets (Hewage et al., 2025; Hultberg and Golovko, 2024). In this type of treatment, pesticide removal is expected to occur through enzymatic degradation, as ligninolytic enzymes, such as laccase produced by fungi, have been shown to be highly effective in reducing pesticide concentrations in water (Vaithyanathan et al., 2022). Additionally, contaminants are also likely to adsorb onto the added biomass, whether SMS or fungal pellets. For the biochar approach, we used a sludge-based biochar, with pesticide removal expected to be exclusively driven by adsorption (Dehghani et al., 2024). The evaluation was based on the reduction in pesticide concentrations and on the alteration of the concentration of total organic carbon, total phosphorous and total nitrogen in the treatments compared to untreated controls. White-rot fungi-based treatments and biochar filtration represent mechanistically distinct strategies for contaminant removal. In this study, we evaluated these approaches in parallel due to their shared relevance as nature-based, low-cost, and environmentally sustainable solutions, particularly suited for wastewater treatment systems. Rather than aiming to directly compare their underlying mechanisms, our objective was to assess their relative performance and practical feasibility under comparable experimental conditions. This approach is intended to inform future decisions regarding the selection, optimization, or potential integration of these strategies in real-world remediation contexts.

This study is the first to systematically evaluate and compare the performance of WRF treatments and sludge-based biochar for the removal of pesticides from surface water collected from agricultural streams in Norway, a region where cold climate and increased precipitation exacerbate pesticide runoff risks. Unlike most prior research, which typically tests these methods separately or in synthetic water matrices, this work applies them in parallel on real-world contaminated surface waters with a complex mixture of herbicides and fungicides.

2. Methods

2.1. Sampling

Water samples have been collected in Innlandet, one of the four main agricultural counties in Norway, within the municipalities of Hamar, Løten and Stange. In these municipalities, grain production dominates plant cultivation, accounting for 60 %–84 % of the agricultural area. The main cereals grown are barley, oats, and wheat, while vegetables like onion, carrot, and potato are also commonly produced. In Norway, the most commonly used plant protection products for these crops include herbicides such as glyphosate (Roundup), MCPA, 2,4-D, mecoprop, fluroxypyr-meptyl + clopyralid + MCPA (Ariane S), metsulfuron-methyl + tribenuron-methyl (Express Gold), and fluroxypyr-meptyl (Flurostar); fungicides such as prothioconazole (Proline), trifloxystrobin, and propamocarb; and insecticides including lambda-cyhalothrin (Karate), flonicamid, and acetamiprid (Norwegian Food Safety Authority, 2025).

According to the Norwegian Environmental Agency database Vann-net (vann-nett.no), 36 out of the 67 streams and rivers in these three municipalities are identified as being affected by agricultural activities, with impacts including nutrient and organic pollution as well as habitat alterations. The 40 % (n = 27) of water bodies fail to reach the target of good ecological status (or good ecological potential for the heavily modified water bodies) according to the EU Water Framework Directive (2000/60/EC). The chemical status has been assessed for only 18 of these water bodies, of which 7 are classified as having poor chemical status (vann-nett.no). We selected five sampling locations in streams or ditches adjacent to cereal crops, where agricultural fields dominate a significant portion of the drainage catchment. In June 2024, we collected water samples (3 L total per location) using 1-L polypropylene bottles that we stored −20 °C and shipped under cold conditions.

2.2. Stream classification

We sent a subsample from each location to SGS Norway for water chemistry analysis. This analysis included measurements of calcium and colour, which are used to determine the river type (Direktoratsgruppen vanndirektivet, 2018). Additionally, the concentrations of total phosphorus and total nitrogen were measured, as these parameters are used for evaluating the status of rivers, in accordance with the Norwegian implementation of the Water Framework Directive (2000/60/EC; Direktoratsguppen vanndirektivet, 2018). Specifically, total phosphorus is the main parameter for classification, while total nitrogen is only used for classification when nitrogen limitation is assumed, typically in highly eutrophic water bodies. The following analytical standards were used for water chemistry analysis: SS-EN ISO 11885:2009 for calcium; NS-EN ISO 7887 (C) for colour index; EN-ISO 15681–2:2018 for total phosphorus; and ISO 11905–1:1998 (mod.) for total nitrogen.

2.3. Pesticide analysis

In total 138 pesticides were included in this study. All standards used were of high analytical purity (>97 %) and compared versus reference mix of known concentration before first use. Native standards and isotopically labelled standards were acquired from Teknolab AB (Kungsbacka, Sweden) or from LGC Standards GmbH (Wesel, Germany). A list of the pesticides included in the study is given in Supplementary Table S1.

Procedures for preparation of water samples for instrument analysis were done as described previously (Jansson and Kreuger, 2010). Briefly, water samples (5 ml) were pH-adjusted, spiked with internal standards solution and filtered with 0.2 μm RC-filters. Samples were

preconcentrated online using an Agilent 1200SL HPLC system equipped with isocratic and binary pumps, a thermostatted autosampler with 15 × 6 mL vial capacity, a column compartment, and a micro-degasser. Detection was performed on an Agilent G6410A triple-quadrupole mass spectrometer with ESI operated in both positive and negative modes. Online SPE used Strata C18–E and Strata X columns (20 × 2 mm, 20–25 μm, Phenomenex). Separation was done on a Zorbax Eclipse Plus C18 column (100 × 3 mm, 3.5 μm) without a guard column. System control was via MassHunter software. Reported concentrations reflect the total concentrations since the internal standards are added before the filtration and continuous validation samples, to determine relative recoveries and precisions, are prepared by adding native pesticide solution to whole water samples.

2.4. Evaluated treatments

Based on the results of the pesticide analysis, the water sample from the location with the highest number of pesticides was selected for the bioremediation experiments.

Treatment with spent mushroom substrate (WRF: SMS). Grain spawn of the white-rot fungus *Pleurotus ostreatus* M2191 was obtained from Mycelia BVBA, Belgium. The spawn was mixed into a substrate composed of 75 % of sawdust (Birch, 2–4 mm) and 25 % of wheat bran in a concentration of 10 % of dry weight (dwt). The substrate had a moisture content of 65 %, was subjected to pasteurization at 70 °C for 5 h and allowed to cool down before the spawn was added. The inoculated substrate was incubated at 22 °C for 18 days. After this time, the substrate was completely colonized by the fungi and fructification was performed as described by Östbring et al. (2023). After harvest of the fruiting bodies, the substrate was crumbled to pieces and used for treatment of the water.

Treatment with white-rot fungal pellet (WRF: pellets). The grain spawn described above was inoculated in sterile distilled water (20 g/l) and lignin (Sigma-Aldrich 370,959) was added (4 g/l). This suspension was cultivated on horizontal orbital shaker (VWR, Advanced 5000 Shaker, Radnor, PA, USA) operated at 100 rpm at room temperature (20–22 °C). After 24 h, the grain was harvested on nylon filters (mesh size 2 mm) and transferred to the contaminated water.

Treatment with biochar. The biochar was produced from sludge from a municipal wastewater plant for the producer Skånefrö AB, Sweden. Before use, the biochar was filtered through a metal sieve (2 mm) to obtain a uniform size of the granules. The biochar is not yet available commercially and detailed information about it is available as supplementary material (Supplementary Table S2).

2.5. Experimental set-up

Experiments were performed in 300-ml Erlenmeyer glass flasks on a horizontal orbital shaker (VWR, Advanced 5000 Shaker, Radnor, PA, USA) operated at 100 rpm at room temperature (20–22 °C). Each replicate consisted of 50 ml of water from sampling location 3. All treatments were applied in a concentration of 20 g/l (dwt). After 24 h of treatment, the solid phase was removed by centrifugation at 3000*g* for 15 min. Untreated control samples underwent the same procedures as the treated samples. The liquid samples were immediately stored in a freezer at −20 °C before analysis, which was performed within one week.

2.6. Analysis

The Hach (https://se.hach.com/) spectrophotometric system and kits were used to determine the impact of the treatments on nutrients (total nitrogen (TN) and total phosphorous (TP) and total organic carbon (TOC). The analyses were performed before and after treatment as described by the manufacturer. Laccase activity was determined in the supernatant colorimetrically by detecting the oxidation product 2,6-dimethoxyphenol (DMP), as described by Parenti et al. (2013). The reaction mixture contained 0.45 ml of sample, with appropriate dilution, and 0.5 ml of 10 mM DMP in 100 mM acetate buffer (pH 5). Absorbance was measured at 468 nm and one unit (U) of enzyme activity was defined as formation of 1 μmol of product per min.

2.7. Statistical analysis

All experiments had three replicates per treatment. Statistical analyses were carried out using Minitab version 2020 and data were tested for significant differences ($p < 0.05$) using ANOVA and Tukey's post-hoc test and *t*-test. Values presented are mean ± standard deviation (std).

3. Results

3.1. Stream classification

Two of the locations (S1-2) can be classified as river type "calcium rich and humic" and three (S3-5) as "calcium rich and clear" (Table 1). Based on the stream type and total phosphorus values, S1 is classified as being in high status, two locations (S4, and S5) are classified as being in good status, one location (S2) in poor status, and one (S3) in moderate status. Total nitrogen levels were relatively high at all locations (Table 1).

Table 1
Water chemistry at the five locations. The classification follows the Water Framework Directive, as implemented in Norway (2000/60/EC; Direktoratsguppen vanndirektivet, 2018), see Norwegian Environment Agency (2025) for the threshold values for the classification.

Location	Parameter	Value	Uncertainty	Unit	Classification
S1	Calcium	79	±12	mg/l	Calcium rich
	Colour index (after filtration)	51	±5.1	mg Pt/l	Humic
	Total phosphorus	16		μg P/l	High
	Total nitrogen	2000		μg N/l	Poor
S2	Calcium	130	±20	mg/l	Calcium rich
	Colour index (after filtration)	38	±3.8	mg Pt/l	Humic
	Total phosphorus	83		μg P/l	Poor
	Total nitrogen	6400		μg N/l	Bad
S3	Calcium	120	±18	mg/l	Calcium rich
	Colour index (after filtration)	23	±2.3	mg Pt/l	Clear
	Total phosphorus	30		μg P/l	Moderate
	Total nitrogen	2400		μg N/l	Bad
S4	Calcium	98	±15	mg/l	Calcium rich
	Colour index (after filtration)	15	±1.5	mg Pt/l	Clear
	Total phosphorus	21		μg P/l	Good
	Total nitrogen	4200		μg N/l	Bad
S5	Calcium	110	±17	mg/l	Calcium rich
	Colour index (after filtration)	22	±2.2	mg Pt/l	Clear
	Total phosphorus	21		μg P/l	Good
	Total nitrogen	3200		μg N/l	Bad

3.2. Analysis of surface water for pesticides content

A total of 138 pesticides were analyzed across the five sampling locations, with 35 pesticides detected in at least one location. The concentrations of these pesticides ranged from 0.001 μg/l (for atrazine-desethyl and azoxystrobin) to 9.1 μg/l (for fluroxypyr) (Fig. 1, Supplementary Table S3). The composition of detected compounds and their concentration ranges varied significantly between the sampling locations. The most frequently detected compound was trifloxystrobin acid, which was present in all studied locations, with concentrations ranging from 0.005 to 0.077 μg/l. The highest pesticide detection frequency was observed at locations S3 and S5 (Fig. 1, Supplementary Table S3). Water from location S3 was selected for further treatment analysis.

3.3. Pesticides removal under WRF and biochar treatment

In the water from location S3, a total of 21 of the analyzed pesticides were detected and the total sum was 19.4 ± 0.7 μg/l. Of the 21 pesticides, 8 were herbicides while 13 fungicides were detected (Table 2). The herbicides were dominating, fluroxypyr comprised approximately half of the total sum and MCPA was detected in a high concentration. The fungicides detected in highest concentration were fludioxonil and the metabolite of trifloxystrobin, trifloxystrobin -acid, which both comprised approximately 0.2 % of total sum of pesticides in the water before treatment. The metabolite of trifloxystrobin, trifloxystrobin -acid, and fluopyram were detected in higher concentrations in the treatment with WRF compared to the initial values, indicating that these fungicides might have been present in the SMS and the plant material used for production of pellets.

A significant reduction in pesticide concentrations was observed in all treatment groups relative to the untreated control. However, the treatments differed from each other and the biochar treatment performed considerably better compared to the treatments with the WRF (Table 2). The biochar treatment removed 94 ± 0.8 % of the pesticides, using WRF: SMS caused a reduction of 27 ± 2 % while WRF: pellets reduced 20 ± 1 % of total amount detected initially in surface water from location 3. When comparing the impact of treatment for the three herbicides which dominated, fluroxypyr, MCPA and clopyralid, it was evident that biochar was more efficient in removing these compounds compared to the treatments based on WRF (Fig. 2). The WRF treatments differed in the removal of fluroxypyr with SMS being more efficient compared to fungal pellets while clopyralid was removed to a larger extent by fungal pellets. For MCPA, the WRF treatments performed similarly. The laccase activity differed in the fungal treatments with WRF: SMS having a considerably lower laccase activity of 12 ± 4 U/l after treatment (24 h) compared to the WRF: pellets which reached an activity of 135 ± 26 U/l.

TOC was increased by both WRF treatment, especially the use of SMS was observed to cause a considerable increase in organic carbon (Table 3). For TN a similar pattern was observed with a significant increase after treatment with WRF, especially for the SMS treatment, while no impact was observed after biochar treatment. The biochar treatment caused an increase in TP, slightly more than 1 mg/l was added in this treatment. Still, this increase was considerable smaller than the use of SMS which caused an increase of almost 7 mg/l. Both the biochar treatment and WRF: SMS increased pH significantly compared to the initial value.

4. Discussion

This study evaluated two cost-effective and environmentally sustainable methods, using WRF and sludge biochar, for the removal of

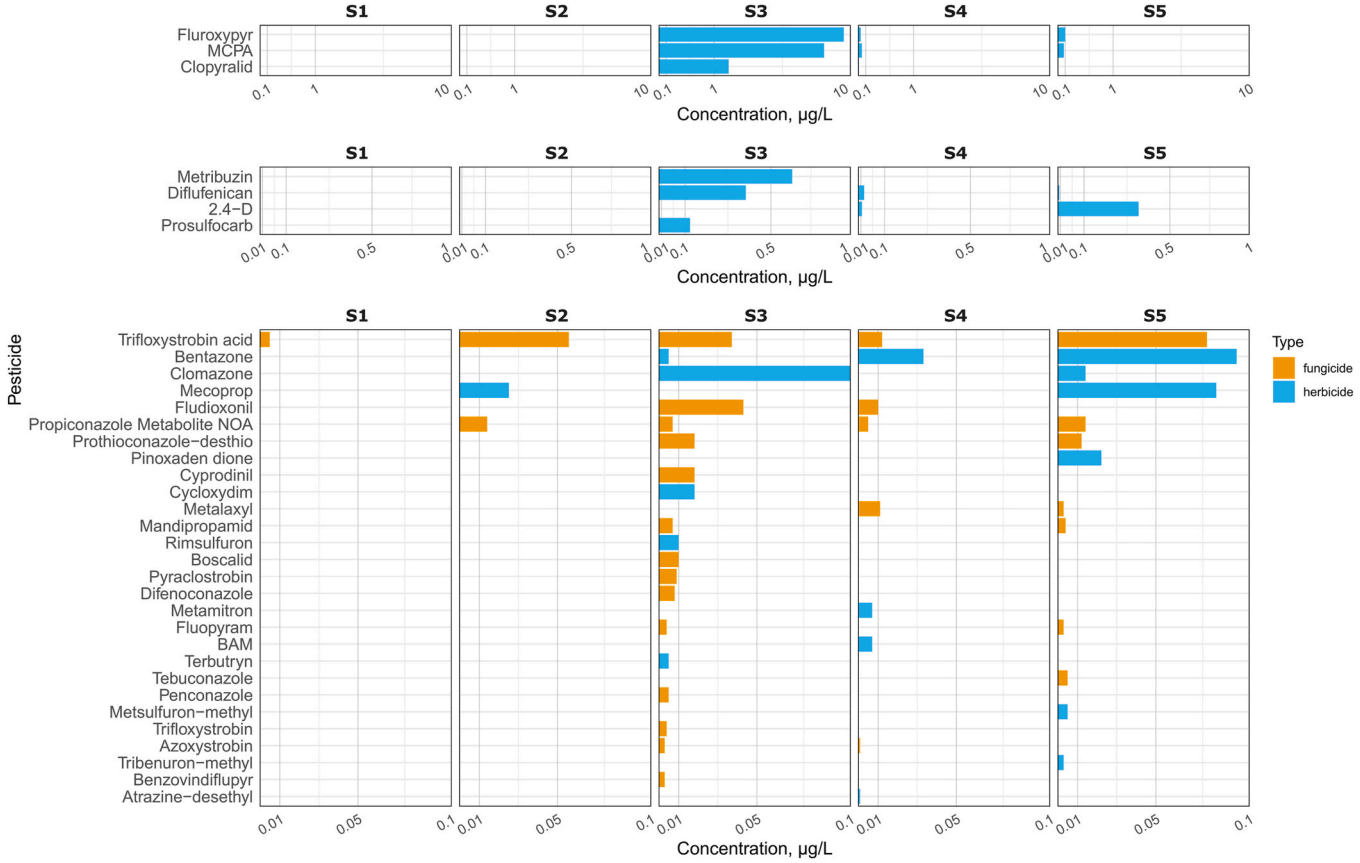

Fig. 1. Positive pesticides in the five studied locations (S1-5). Pesticides are grouped into three categories (displayed in separate horizontal panels) based on their maximum concentrations. Note that each panel uses a different logarithmic scale.

Table 2
The pesticides detected in surface water sampled from location 3 and impact of treatments on their concentration (μg/l). The control represents initial values before treatment. Mean ± std is shown, n = 3.

Pesticides		Initial concentration	Concentration after treatment		
(f = fungicide, h = herbicide)			Biochar	WRF: SMS	WRF: pellets
Azoxystrobin	f	0.002 ± 0.000	BDL**	BDL	BDL
Boscalid	f	0.009 ± 0.001	BDL	BDL	BDL
Clomazone	h	0.085 ± 0.006a*	BDL	0.020 ± 0.001 b	0.024 ± 0.001 b
Clopyralid	h	1.467 ± 0.121a	0.497 ± 0.042c	1.467 ± 0.058a	1.2 ± 0.031 b
Cyprodinil	f	0.013 ± 0.001	BDL	BDL	BDL
Diflufenican	h	0.242 ± 0.021a	0.003 ± 0.001 b	0.018 ± 0.001 b	0.020 ± 0.002 b
Dimethomorph	f	0.002 ± 0.000	BDL	BDL	BDL
Fludioxonil	f	0.038 ± 0.002	BDL	BDL	BDL
Fluopyram	f	0.004 ± 0.000 b	BDL	0.011 ± 0.001a	BDL
Fluroxypyr	h	9.567 ± 0.855a	0.377 ± 0.050c	5.600 ± 0.100 b	6.967 ± 0.058 b
Mandipropamid	f	0.006 ± 0.001	BDL	BDL	BDL
Metribuzin	h	0.588 ± 0.028a	0.016 ± 0.001 d	0.110 ± 0.020c	0.223 ± 0.006 b
Penconazole	f	0.004 ± 0.000	BDL	BDL	BDL
Propiconazole Metabolite NOA	f	0.005 ± 0.001a	BDL	BDL	0.007 ± 0.001 b
Prosulfocarb	h	0.088 ± 0.008	BDL	BDL	BDL
Prothioconazole-desthio	f	0.015 ± 0.001	BDL	BDL	BDL
Pyraclostrobin	f	0.005 ± 0.001	BDL	BDL	BDL
Rimsulfuron	h	0.009 ± 0.001a	BDL	0.010 ± 0.001a	0.007 ± 0.001 b
Trifloxystrobin	f	0.003 ± 0.000	BDL	BDL	BDL
Trifloxystrobin acid	f	0.038 ± 0.002c	BDL	0.067 ± 0.004a	0.048 ± 0.001 b
MCPA	h	7.250 ± 0.302a	0.293 ± 0.031c	6.600 ± 0.100 b	6.833 ± 0.058 ab
Sum		19.436 ± 0.732a	1.185 ± 0.120 d	13.903 ± 0.104c	15.330 ± 0.104 b

* Values within rows followed by different letters are significantly different ($p \leq 0.05$) **BDL, Below detection limit.

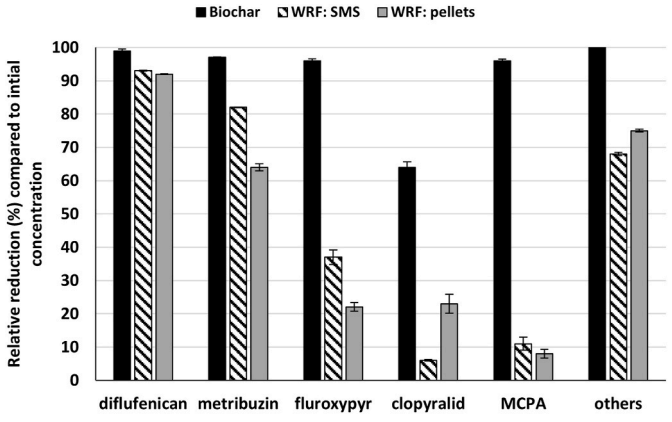

Fig. 2. Relative reduction (mean ± std, n = 3) by treatments of the pesticides detected in the highest concentration in the surface water sampled from location 3. The group "others" refer to pesticides present in a concentration below 1 % of total amount and include 16 compounds.

pesticides from surface water in agricultural landscapes. Our findings demonstrate that while both approaches showed promise in reducing pesticide concentrations, biochar significantly outperformed WRF-based treatments, achieving an average removal efficiency of 94 % compared to 27 % and 20 % for WRF: SMS and WRF: pellets, respectively.

Table 3
The impact of the treatments on total organic carbon (TOC, mg/l), total nitrogen (TN, mg/l), total phosphorous (TP, mg/l) and pH. Mean ± std is shown, n = 3.

	TOC	TN	TP	pH
Control	8.4 ± 0.9c	2.6 ± 0.5c	<0.05	7.1 ± 0.2c
Biochar	5.3 ± 0.4c	2.7 ± 0.1c	1.4 ± 0.2 b	8.0 ± 0.2a
WRF: SMS	250.7 ± 3.2a	35.4 ± 0.9a	6.7 ± 1.6a	7.6 ± 0.02 b
WRF: pellets	55.4 ± 2.4 b	6.9 ± 0.3 b	0.1 ± 0.03 b	7.4 ± 0.1bc

*Values within columns followed by different letters are significantly different ($p \leq 0.05$).

The good performance of biochar in this study is consistent with previous findings that highlight the high adsorption capacity of biochar for a wide range of organic micropollutants, including herbicides and fungicides (Dehghani et al., 2024). The porous structure, high surface area, and the presence of functional groups capable of interacting with hydrophobic or ionic pesticide molecules likely contributed to the observed removal efficiencies (Sarkar et al., 2024). As detailed in Supplementary Table S2, the biochar used in this study showed a porous structure and a high surface area, along with the presence of mineral components originating from the wastewater sludge feedstock. These characteristics enhance its ability to adsorb a wide range of organic micropollutants through mechanisms including hydrophobic partitioning, electrostatic interactions, hydrogen bonding, and surface complexation. Among the compounds tested, herbicides such as fluroxypyr, MCPA, and diflufenican showed the highest removal efficiencies (>90 %), likely due to their moderate hydrophobicity (log Kow: fluroxypyr = 2.20, MCPA = 3.25, diflufenican = 4.9), which favors adsorption onto carbon-rich surfaces. In contrast, clopyralid—a more polar (log Kow = 1.06) and weakly sorbing compound—was less efficiently removed, which aligns with previous observations that highly polar pesticides exhibit lower affinity for biochar. While our study employed a 24-h treatment period under batch conditions, detailed kinetic modelling was not performed. Future studies should evaluate sorption kinetics, including time-to-equilibrium and adsorption rate constants, to better understand and optimize biochar performance in dynamic flow systems.

Compared with biochar, the performance of WRF treatments was less efficient, likely due to several interacting factors, including limited contact time, variability in laccase activity, and potential competition for enzyme binding sites (Bilal et al., 2019). Interestingly, the WRF: pellet treatment showed higher laccase activity than the WRF: SMS treatment (135 vs. 12 U/l), yet it showed slightly lower pesticide removal. This suggests that enzymatic degradation alone may not fully explain the observed outcomes and that additional factors, such as adsorption to the treatment matrix or compound-specific degradation kinetics, may have played a role (Asgher et al., 2008).

Of the dominating pesticides detected in the most contaminated location, both fluroxypyr and MCPA showed a relative reduction of more than 90 % compared to initial concentrations after exposure to biochar while the treatment of clopyralid was less successful (Fig. 2). Fluroxypyr and MCPA are used for the control of broadleaf weeds by acting as synthetic auxins, disrupting plant growth processes. They both exhibit toxicity toward aquatic organisms. At high concentrations, fluroxypyr was found to inhibit the growth of the green alga *Chlamydomonas reinhardtii* and induce oxidative stress, peroxide accumulation, and DNA degradation (Zhang et al., 2011). MCPA can cause inflammatory and immunosuppressive effects in fish, such as altered blood parameters in common carp (Lutnicka et al., 2018), and damage aquatic plants like *Hydrilla verticillata* by reducing growth, impairing chlorophyll production, and triggering oxidative stress (Weerakoon et al., 2018). The marked reduction of these herbicides in our treatments therefore represents a decrease in potential ecotoxicological pressure on aquatic ecosystems. Clopyralid belongs to the group of pyridine carboxylic acids with a relatively low acute toxicity to aquatic organisms (Fairchild et al.,

2009). However, clopyralid is a subject of concern due to side-effects observed in non-target crops such as tomatoes, peas and peppers (Namiki et al., 2019). Risk for contamination by the compound, followed by considerable plant damage and economical loss for growers, have been observed to occur in relation to use of certain organic plant fertilizers (Fuchs and Fomsgaard, 2025). This is a known problem and within EU there are recommended measure which should be taken to avoid transfer of exposed plant residues into the production chain of organic plant fertilizers (European Commission, 2021). In the present study, clopyralid was detected in an approximate concentration of 1.5 μg/l in the surface water. For sensitive crops, damage has been observed in very low concentrations of these compounds, even below 1 ppb (Fuchs and Fomsgaard, 2025). Thus, this surface water is not suitable for irrigation of sensitive crops. Additionally, use of this water for irrigation of non-sensitive crops may result in plant uptake and assist in uncontrolled spreading. This finding highlights the need for extended control of these contaminates in surface water in agricultural areas.

While biochar demonstrated high efficiency in pesticide removal, its application has some limitations. Notably, the release of total phosphorus (TP) from the sludge-based biochar raised concerns about secondary pollution risks, which may offset the benefits of pesticide removal. TP leakage was also found in a previous study where this specific biochar was evaluated for inclusion in mushroom substrate and a significant increase in phosphorous content in the fruiting bodies were observed (Karlsson et al., 2025). Although this release was significantly lower than that observed with the WRF: SMS treatment, it underscores the need to carefully evaluate the leaching potential of biochar, particularly when applied to nutrient-sensitive environments (Yao et al., 2012).

The WRF-based treatments also posed environmental challenges resulting in significant increases in TOC, TN, and TP, especially following SMS application. This increase reflects the release of organic and nutrient-rich components from the partly degraded substrate. For the pellet treatment, the increase can be explained by exudates from the partially decomposed grain spawn and residues of lignin, which was used to stimulate laccase production. While these additions may not pose direct toxicity risks, they could contribute to eutrophication or disrupt ecological balances in nutrient-limited systems. In agriculturally influenced streams already subject to elevated nutrient pressures, such as those examined in this study, such these inputs may further intensify eutrophic conditions and compromise ecosystem functioning. Additionally, following WRF application the metabolite of trifloxystrobin (trifloxystrobin acid) and fluopyram were detected at higher concentrations compared to initial levels, indicating a potential release of fungicide residues, likely introduced through the spent mushroom substrate (SMS) or the plant material used in pellet production. Although these compounds were present in low concentrations, their detection raises concerns about chemical contaminants in WRF-based materials (Anagnostopoulou et al., 2022).

Both biochar and WRF have received attention in recent years considering removal of organic pollutants from water. Still, most of the published studies have been performed in laboratory in a bench scale (Dasgupta et al., 2024; Fang et al., 2025). Considering real world applications, it is important to mention that biochar offers rapid and efficient removal primarily through adsorption, but the potential for desorption and contaminant release under changing environmental conditions (e.g., pH, ionic strength) is a well-recognized limitation (Ao et al., 2025). In contrast, WRF-based treatments aim to degrade pollutants enzymatically or incorporate them into biomass, which may reduce long-term re-release risks—but also introduces concerns related to metabolite formation. It is well established that WRF have a key role in biotransformation of organic environmental contaminates, including herbicides (Koroleva et al., 2015; Torres-Farradá et al., 2024). Despite that most studies report decreased toxicity after treatment with WRF (Torres-Farradá et al., 2024) more knowledge needs to be developed regarding specific contaminates, including for the dominating pesticides detected in this study. Thus, it is important for future studies to incorporate non-target screening and toxicity assays to monitor both parent and degradation products. Ecotoxicological assays, e.g., using Daphnia magna, algae, or fish embryo tests, should be included to comprehensively assess the biological safety of treated effluents and to better inform the trade-offs between contaminant removal and nutrient loading.

5. Conclusion

Effective removal of pesticides from water remains a critical challenge for protecting environmental and human health. When comparing biochar and WRF-based treatments, our results indicate that although both methods effectively reduced pesticide levels, biochar performed considerably better. From an applied perspective, biochar emerges as a strong candidate for in-situ treatment of agricultural runoff or implementation in buffer zones adjacent to croplands. Its simplicity, low operational cost, and high efficiency make it suitable for decentralized water treatment strategies. However, managing “exhausted” biochar, once its adsorption capacity is saturated, is crucial. Potential solutions include regeneration, thermal reactivation, or safe disposal, each with its own environmental and economic considerations. WRF-based treatments, while less efficient in this study, offer other advantages such as the potential for continuous enzymatic activity and the biotransformation of pollutants. Their use could be enhanced by optimizing conditions such as contact time, oxygen availability, and nutrient supplementation, or by integrating fungal systems into constructed wetlands or bioreactors (see e.g. Mustafa et al., 2024). The use of WRF may also be preferable in settings where the reuse of agricultural or forestry residues is prioritized, aligning with circular economy principles.

Future research should explore the combined use of WRF and biochar to harness the benefits of both biological degradation and adsorption. Synergistic effects could enhance removal efficiency, while biochar might help mitigate the nutrient release associated with fungal treatments. Additionally, long-term studies under field conditions are needed to assess treatment longevity and ecosystem-level impacts. The findings of this study provide a foundation for the development of low-cost, modular treatment systems that can be deployed in rural and agricultural landscapes.

CRediT authorship contribution statement

Francesca Pilotto: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Oksana Golovko:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Malin Hultberg:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization.

Data statement

All data supporting the findings of this study are provided in the tables and supplementary materials.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Francesca Pilotto reports financial support was provided by Nordforsk. If there are other authors, they 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.jenvman.2025.127282.

Data availability

All data supporting the findings of this study are provided in the tables and supplementary materials.

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