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The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation

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ABSTRACT

Although glyphosate is a commonly used herbicide, its impacts on ecosystems are not well understood. A pot experiment, was established to explore the potential impacts of biochar, wood vinegar, and plants on the environmental fate of glyphosate. In the presence of plants (*Lolium perenne*), and irrespective of the presence of biochar or wood vinegar, leaching of glyphosate through the soil was multiple compared to the plant free systems. However, the addition of biochar to the soil decreased the leaching of glyphosate irrespective of plants. Soils treated with biochar–wood vinegar mixture showed the lowest glyphosate leaching, both with and without plants. Biochar, wood vinegar or plants, alone, had no effect on the degradation of glyphosate in soil. When the plants were present the degradation of glyphosate was highest in soils treated with biochar–wood vinegar mixture. Our results imply that biochar in particular can be applied as a soil improving agent to reduce the potential environmental risks to aquatic environments caused by glyphosate

1. Introduction

Glyphosate (*N*-(phosphonomethyl)glycine), a broad-spectrum, nonselective and postemergence herbicide is commonly-used in agricultural and non-agricultural systems [1]. Due to its rapid adsorption to soil particles and vulnerability to microbial degradation, glyphosate is supposed to be inactivated quickly after spraying. This has given rise to the common belief that glyphosate is a relatively environmentally-safe herbicide [2]. However, recent investigations have shown that the rate of degradation and sorption of glyphosate are dependent on soil properties [3,4] and the biosafety of glyphosate has been questioned. Also, plants may have a large effect on the environmental fate of glyphosate through transporting significant amounts of glyphosate from stems to roots [5]. Glyphosate in roots can enter to soil rhizosphere via root exudates or through the activities of soil microbes and fauna feeding upon decaying roots [5–7]. As a consequence, under certain environmental conditions, glyphosate and its degradation products can be prone to leaching to deeper soil layers and finally to aquatic environments [8,9].

Biochar, a solid material obtained from thermochemical conversion, such as pyrolysis, of biomass in an oxygen-limited environment [10] and added to the soil [11], has recently received much interest due to its potential for improving soil fertility and plant growth. The ability of biochar to improve soil properties, plant growth, and microbial activity has been studied extensively (see reviews [12,13]). Moreover, evidence suggests that biochar has a high capacity to adsorb to both inorganic [14] and organic [15,16] pollutants. Biochar also appears to increase the sorption potential and reduce the degradation of several pesticides to soil, such as diuron [17,18], simazine [19] and terbuthylazine [16]. However, also enhanced biodegradation of benzonitrile has also been observed in the presence of biochar [20]. To our knowledge the effects of biochar on the environmental fate of glyphosate have not been studied.

Another pyrolysis-derived 'soil interactive product' is wood vinegar, also known as pyroligneous acid, wood distillate or bio-oil. Wood vinegar is the water soluble fraction of the liquid that is produced during the pyrolysis of organic materials. Depending on the dosage, wood vinegar can act as a biocide against micro-

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organisms [21,22], weeds, insects [23,24] and mollusks [25], or when diluted sufficiently as a soil enrichment to stimulate plant rooting, shoot growth [26] and microbial activity [27]. In Japan it is common practice to apply a mixture of wood vinegar and charcoal (called Sannekka E) to improve soil fertility [28]. Despite the long history of applying wood vinegar to soils in Asia [29], only few studies concerning the toxic effects of wood vinegar to the environment are published [30–32], and knowledge about its interactions with chemical herbicides in the soil is largely unknown.

Here we examine the interactions of biochar, wood vinegar, plants, and their mixed effects on the environmental fate of glyphosate. We hypothesised that as an active sorbent, biochar reduces the leaching and decreases the degradation of glyphosate in the soil. We also hypothesised that wood vinegar, as a readily usable resource for soil microbiota, increases the degradation of glyphosate by stimulating soil microbial activity [27,31]. Furthermore, we predicted that, due to the transfer of glyphosate from plant shoots to their roots, leaching of glyphosate is enhanced in the presence of plants in comparison to plant free soil.

2. Materials and methods

2.1. Characterisation of the materials

The soil used in the experiment had no previous history of glyphosate application. It derived from an arable field used over 20 years in organic potato farming in Tammela, Finland (N 60°48.92′, E 023°42.68′). Surface soil (0–15 cm) was collected in May 2010 and stored at +4 °C for one month before being used in the experiment. To retain microbial and soil faunal functions, the soil was not dried before use. Larger roots and earthworms were removed, and the soil was sieved through a 4 mm sieve and homogenised. Particle size distribution of the original soil was determined by the Dry Sieving Method using ISO 3310 Test Sieves and a Vibratory Sieve Shaker AS 200 basic. The fine sandy soil was composed of approximately 48% sand (2–0.2 mm), 41% fine sand (0.2–0.06 mm) and 11% silt and clay ($\leq$0.06 mm) with a pH (1:2.5 H_2O) of 6.85. Bulk density of the soil was 1500 g L^{-1} (before sieving, the moisture content was 20%). The concentration of organic matter in the original soil was 4.9% (loss on ignition at 550 °C, 5.5 h) and that of total carbon was 1.7% (analysed using a Leco C/N/S 2000 analyzer, in the University of Helsinki, Lahti Campus). Total concentrations of Ca, K, Mg and P were 2034 mg kg^{-1}, 295 mg kg^{-1}, 118 mg kg^{-1} and 10.5 mg kg^{-1}, respectively, analysed by MTT Agrifood Research Finland according the protocol developed by Vuorinen & Mäkitie [33].

The biochar used here was derived from birch wood (incl. bark) and pyrolysed in a batch retort by Tisle Suomi Ltd. (Mikkeli, Finland) at 450 °C for a holding time of 23 h. A complete pyrolysis process and characteristics of the used biochar (product of the retort B) is reported in Fagernäs et al. [34]. The surface area of the biochar was 160 m g^{-2} (analysed according the PANK 2401 method in the Tampere University of Technology), the contents of carbon and nitrogen 900 mg g^{-1} and 9.1 mg g^{-1} (determined using a Leco C/N/S 2000 analyzer in the University of Helsinki, Lahti Campus), and the pH of the biochar (in water solution 1:5 v/v) was 8.75 (determined according ISO 10390). Wood vinegar, supplied by Raussi Energy Ltd. (Sippola, Finland), originated from bark-free heartwood birch (*Betula pendula*) material from a ply-wood mill. The wood vinegar used had a pH of 2.04 and it consisted of 74.7% water and 25.3% organic matter. The complete pyrolysis process (retort A), the composition and analyses of the used wood vinegar are reported in Fagernäs et al. [34].

2.2. Experimental setup

The study was performed in a glasshouse at MTT Agrifood Research Finland in Jokioinen, Finland, in the summer of 2010. The experiment was conducted in 1500 ml flowerpots (Ø 11 cm, height 19 cm) with four holes (Ø 0.5 cm) at the bottom. The four treatments, each with 20 replicates consisted of soil mixed with 1) biochar, 2) wood vinegar, 3) biochar and wood vinegar, or 4) a control system with neither biochar nor wood vinegar. Coarse gravel (Ø 0.5–1.5 cm, 100 g dry) was put at the bottom of each pot to maintain capillary action and to prevent water holes from blocking up. The application rate of biochar to the pots corresponded to 51 t ha^{-1}, assuming 10 cm incorporation depth (3.3% biochar content by dry mass). Wood vinegar concentrations applied to the pots corresponded to 2000 L ha^{-1} (0.26%). To get data on the highest possible risks and benefits of the substances, relatively high concentrations of wood vinegar and biochar were used in the experiments. Before adding 800 g of treated soil to the pots, biochar (sieved through a 2 mm sieve) and wood vinegar were homogenously mixed with the soil in a bucket, and the water content of the mixture and that of the control soil was adjusted to 20% of wet mass. The pots were randomly placed on a moist filter bed that ensured constant soil moisture during incubation. To provide optimal growth conditions for *Lolium perenne*, the pots were kept in a glasshouse with constant air temperature (23 ± 2 °C) throughout the experiment.

The experiment ran for 82 days, during which time soil and water leachate samples (see Analyses section 2.3.2) were taken three times: at 4, 46 and 80 days. After the first sampling, seeds of *L. perenne* were sown (150 per pot) in half of the pots of each treatment to determine the effects of plants on the fate of glyphosate. All the pots were covered with plastic film for 7 days to maintain soil moisture conditions. After seed germination, the plastic film was removed and all pots were fertilised (1 dl, 0.2% Yara Ferticare Kombi1, corresponding to N–P–K (14–5–21%) dose of 100 kg ha^{-1}). When the grass reached 20 cm in height (Day 36), half of the pots of each treatment (with and without plants) were treated with glyphosate (Roundup Bio; Monsanto, Copenhagen, Denmark) mixed with water (1:100) corresponding to 2000 g active ingredient ha^{-1} (ca. 2000 μg/pot). Glyphosate was sprayed according to the Good Experimental Practice GEP protocol used by the Agrifood Research Finland (MTT). The GEP standard was adopted by EEC in the Directive 93/71/EEC. Annex II in this directive specifies the requirements that are referred to as GEP. The GEP standard suites well to a variety of agricultural practices and experimentations. Grasses in glyphosate treated pots withered and died during 3–21 days after spraying the glyphosate. As part of initially added wood vinegar was obviously degraded, four days after the addition of glyphosate, a second addition of wood vinegar (500 L ha^{-1}) was made for pots that already contained wood vinegar. This was done to ensure that enough wood vinegar is present in the soil to stimulate glyphosate degradation by soil microbes. The second and third sampling events (46 and 80 d) were made 10 and 44 days after the addition of glyphosate.

2.3. Analyses

2.3.1. Soil samples

At each sampling event (Days 4, 46 and 80), two soil samples were taken from each pot using a corer (Ø 1.5 cm, 5 cm deep) and stored (1–5 days) at 5 °C for the analyses of microbial activity and numbers of nematodes (see methods below). The soil *C*/*N* ratio was analysed from each sample taken at Day 80, using a LECO C/N/S analyzer (analysed in the University of Helsinki, Lahti Campus). After the last sampling, plants in the pots were uprooted, weighed, and dried (24 h, 70 °C). Shoot and root biomass of the plants were weighed separately. After completion of the experiment, replicates of each treatment were pooled and homogenised in the laboratory for the analysis of soil pH, nutritional status (Ca, K, Mg, P), glyphosate and AMPA (aminomethylphosphonic acid, the primary degradation product of glyphosate) concentrations. The samples were pooled due to the high cost of analyses. Water content of the soil samples was determined by placing the soil into an oven at 105 °C for 24 h. Soil pH was measured in a 1:2.5 (V/V) soil:distilled water suspension according to the ISO 10390 standard. Glyphosate concentration in the soil was analysed at Ramboll Finland Ltd. according to the In-house method RA4054 (GC/MSD) with the detection limit to glyphosate and AMPA at 10 $\mu g\ kg^{-1}$ (dm). Soil nutrient analyses (Ca, K, Mg, P) were conducted at the laboratory of MTT research Finland according the protocol developed by Vuorinen & Mäkitie [33].

Microbial activity of soil samples, using basal (CO_2) respiration as an estimate [35], was measured from 20 g of fresh soil using an Apollo 9000 Total Organic Carbon (TOC) analyzer. The abiotic mineralisation of biochar was measured from a 10 g biochar sample mixed with 3 ml distilled water. Before the measurements, roots were removed from the soil. The soil was placed in a 43 ml glass jar, and then allowed to stabilise for 2 h (21 °C) before the first measurement: a 0.5 ml air sample was taken from the head-space of the jar through a rubber lid using a syringe. A second air sample was taken 2 h later. Basal respiration was calculated based on the difference in the amount of CO_2 between the two measurements ($n = 5$). Nematodes were extracted from 5 g of fresh soil samples ($n = 5$) using the wet funnel method by Sohlenius [36], and the numbers of nematodes were counted under a binocular microscope.

2.3.2. *Water leachates*

One day after each soil sampling event (Days 5, 47 and 81) the pots were irrigated with 300 ml of tap water to mimic heavy rain. The water leaching through the soils was quantified and collected for analyses. After measuring conductivity, pH and TOC of each leachate sample, the leachates were pooled within a treatment to get one composite sample per treatment to analyse the concentration of glyphosate, its degradation product AMPA, and the components of wood vinegar.

The concentration of total organic carbon (TOC) in the water leachates was analysed using the APOLLO 9000 TOC Analyzer (analysed by the University of Helsinki, Lahti Campus). Leachate conductivity and pH were determined using a digital conductivity and pH meter. Concentrations of the 14 quantitatively largest compounds of wood vinegar (acetaldehyde, furan, acetone, methanol, ethanol, *n*-propanol, 1-hydroxy-2-propanone, acetic acid, furfural, *n*-propionic acid, 5-methyl-furfural, guaiacol, phenol and *m*-cresol) in the leachates were analysed by the VTT Technical Research Finland (Otaniemi) by gas chromatography–mass spectrometry (GC–MS) using Innowax-column with a detection limit of 10 $\mu g\ L^{-1}$. Glyphosate and AMPA concentrations of the leachates were analysed at Ramboll Finland Ltd. according to the FINAS-accredited method RA4012, using liquid chromatography–mass spectrometry (LC–MS) with a detection limit of 10 $\mu g\ L^{-1}$ for glyphosate and 2 $\mu g\ L^{-1}$ for AMPA.

Acute toxicity of the leachates (5, 47, 80 d) was investigated using the *Daphnia magna* Acute Immobilisation Test [37]. The test was performed with minor modifications. Dormant eggs (Daphtoxkit™ F magna, MicroBioTests Inc.) of *D. magna* were hatched in reconstituted water (Elendt M7), prepared in accordance with OECD guidelines [38]. Three replicates of each leachate were prepared and five juveniles up to 24 h old were added to sealed glass vessels (vol. 20 mL), containing 10 mL of experimental water. The test vessels were kept in darkness at 20 °C. Immobilisation was observed after 24 and 48 h.

2.4. *Statistical analyses*

Repeated measures MANOVA was used to examine treatment effects of biochar, wood vinegar, vegetation and glyphosate on microbial respiration, numbers of nematodes, the soil C/N ratio and leachate properties (pH, conductivity, TOC) at the second and third sampling events. Soil and leachate samples were analysed separately. In case of leachates, a significant interaction effect was observed between time and biochar and the effects of biochar on pH, conductivity, TOC of leachates in different sampling events were studied separately using Simple-effects model [39]. Transformations (log, ln) were used to normalised the data.

Samples taken 5 days after the study started differed from those taken 40 and 80 d after the start due to the addition of grasses (Day 6) and glyphosate (Day 36) to some of the pots. Therefore the effects of biochar and wood vinegar on nematodes, microbial respiration, TOC, conductivity and pH of the samples taken during day 5 were performed separately using MANOVA. Soil and leachate samples were analysed separately. All analyses were carried out using SPSS c.15 for Windows [40]. As glyphosate concentrations in the leachates and soils were calculated from pooled samples (representing average of five replicates), statistical analyses were not performed.

3. Results

3.1. *Effects of biochar and wood vinegar on soil nutrients and carbon*

Biochar increased soil total nitrogen concentrations being 1.51 $mg\ kg^{-1}$ in biochar treated pots and 1.22 $mg\ kg^{-1}$ in control pots at the beginning of the study. During the experiment, total nitrogen concentrations decreased by 12% (mean 0.14 $mg\ kg^{-1}$) in the control soils, while loss in the biochar-treated systems was significantly lesser being only 3% (mean 0.04 $mg\ kg^{-1}$) irrespective of plants ($F_{1,\ 64} = 17.23$, $p < 0.001$; $n = 40$) (Table 1). At the end of the study (Day 80), the pH of control and biochar-treated soils was 6.83 and 6.95, respectively.

Biochar increased soil total C significantly ($F_{1,\ 64} = 692{,}95$, $p < 0.001$ $n = 40$) at the beginning of the study being 17 $mg\ g^{-1}$ in control pots and 47 $mg\ g^{-1}$ in biochar treated pots, but biochar had no effect on soil Ca, K, Mg and P concentrations. Wood vinegar had no effects on the concentrations of soil total N, Ca, K, Mg, P at the end of the experiment.

3.2. *Effects of plants, biochar and wood vinegar on the environmental fate of glyphosate*

The volume of water leached out of the pots varied between 165 and 230 ml with no differences between the treatments except the pots with plants treated with wood vinegar lost 25% more water

Table 1
The effects of biochar on i) soil total nitrogen content at the beginning (Day 1) and at the end (Day 80) of the experiment and on ii) soil microbial respiration (μg C/g dm soil/h) at Day 46 and 80. Data include both vegetated and plant-free pots. Means ($n = 40$) and S.E (±) are presented.

	Day	N mg/g dm soil	μg C/g dm soil/h
Biochar	1	1.507 (±0.021)	n.d.[a]
	44	n.d.[a]	0.634 (±0.043)
	80	1.466 (±0.027)	0.316 (±0.027)
Control	1	1.218 (±0.089)	n.d.[a]
	44	n.d.[a]	0.554 (±0.041)
	80	1.075 (±0.117)	0.229 (±0.079)

[a] Not determined.

than the control pots with plants. Plants had a substantial effect on the leaching of glyphosate: the concentration of glyphosate in water leachates was up to six times higher than in plant-free control pots (Fig. 1). This effect was evident in soils with and without biochar or wood vinegar addition.

Biochar amendment decreased the leaching of glyphosate: when plants were present, the reduction was 18% 10 days and 35% 44 days after glyphosate treatment. Throughout the study, the leaching of glyphosate from the biochar treated pots (with plants) was 24% less than from the control pots. In the plant-free pots, biochar decreased the leaching of glyphosate by 40% 10 days after glyphosate treatment as compared to control (no biochar) pots. However, 44 days after glyphosate addition, glyphosate concentrations in the leachates were low and there were no differences between treatments. Overall, in the absence of plants biochar decreased the leaching of glyphosate by 27% as compared to control pots during the study (Fig. 1).

The effects of wood vinegar on glyphosate leaching were inconsistent, in the presence of plants wood vinegar increased glyphosate leaching whereas in plant free pots the effect was the opposite. Soils treated with biochar–wood vinegar mixture showed the highest decrease in glyphosate leaching, both with and without plants (Fig. 1).

At the end of the study (Day 80, 44 days after glyphosate addition), 17–27% of glyphosate added to the pots were still present in the soils. The degradation of glyphosate in soil was not affected by biochar, wood vinegar (Fig. 2) or plants alone. In the presence of plants the degradation of glyphosate was highest in soils treated with the biochar–wood vinegar mixture (Fig. 2). Concentrations of AMPA in the soils varied between 160 and 210 µg/kg.

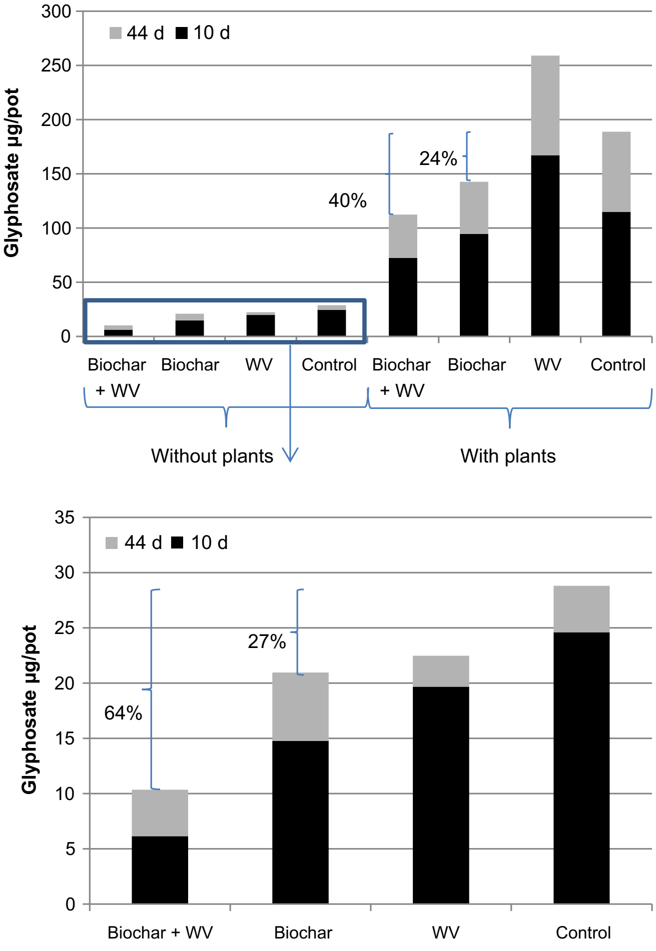

Fig. 1. The effects of plants, biochar and wood vinegar on glyphosate dose leached out of the pots at 10 (black column) and 44 (grey column) days after glyphosate addition. Each column represents results of a composite sample pool of 5 replicates. Percentage values denote the difference to the control.

3.3. *Effects of biochar and wood vinegar on soil organisms and plants*

Biochar had no effect on soil microbial activity at the first sampling event (Day 4) ($F_{1,\ 20} = 2.31$, $p = 0.14$), but a significant increase in microbial activity in the biochar-treated soils was observed at Days 46 and 80 ($F_{1,\ 64} = 12.39$, $p = 0.001$) (Table 1). Biochar affected neither the number of nematodes ($F_{1,\ 64} = 1.19$, $p = 0.28$) nor the above ground and root biomass ($F_{1,\ 16} = 1.38$, $p = 0.25$, $F_{1,\ 16} = 2.40$, $p = 0.14$, respectively) of *L. perenne*. Biochar mixed with distilled water produced low amounts of CO_2, but this explained only a minor part (<5%) of the observed increase in microbial activity in the biochar-treated soils.

Wood vinegar, glyphosate and vegetation had no effect on the rate of soil respiration or on the number of nematodes ($p > 0.05$) at any of the sampling events. Neither *L. perenne* shoot nor root biomass was influenced by wood vinegar ($F_{1,\ 16} = 2.45$, $F_{1,\ 16} = 0.16$, $p > 0.05$).

3.4. *Effects of wood vinegar and biochar on the chemical composition of leachates*

The effects of wood vinegar and biochar on pH, conductivity and TOC of leachates were slight and mostly transient (Table 2). Plants decreased TOC and conductivity, and increased the pH of leachates at Day 44 but these effects did not last to the end of the study (results not shown).

None of the 14 quantitatively most abundant compounds of wood vinegar (see Materials and methods; Water leachates) were detected in the leachates. Consequently, survival of *D. magna* in the leachates was not affected by the treatments.

4. Discussion

4.1. *Effects of plants on the retention of glyphosate*

The main objective of this study was to explore whether biochar, wood vinegar and plants affect the environmental fate of glyphosate in arable sandy soil. Understanding of the consequences of the widespread glyphosate is important, especially in the northern climates where soil types and often extreme climatic conditions enable a long persistence of glyphosate [6]. Of the three factors, plants had the largest effect on the fate of glyphosate in the soil. We hypothesised that, due to the transfer of glyphosate from plant shoots to roots, and finally the soil by root exudates and decaying roots, leaching of glyphosate is enhanced in the presence of plants. As expected, leachates from the plant systems contained up to six times more glyphosate than leachates from the plant-free systems, indicating that the mobility of this pesticide was enhanced by rye grass. As reported by Ruiz et al. [41], dead roots form channels in the soil, enabling water and the soil macrofauna to reach deeper soil layers. Kjær et al. [42] and Stone and Wilson [43] reported a considerable proportion of glyphosate transport can occur together with colloidal soil particles via macropores and cracks in the soil, as well as through root release and via root channels [5–7]. Results of our study lend support to these findings, suggesting that the root of weeds may, at least to some extent, control the fate of glyphosate and its degradation products.

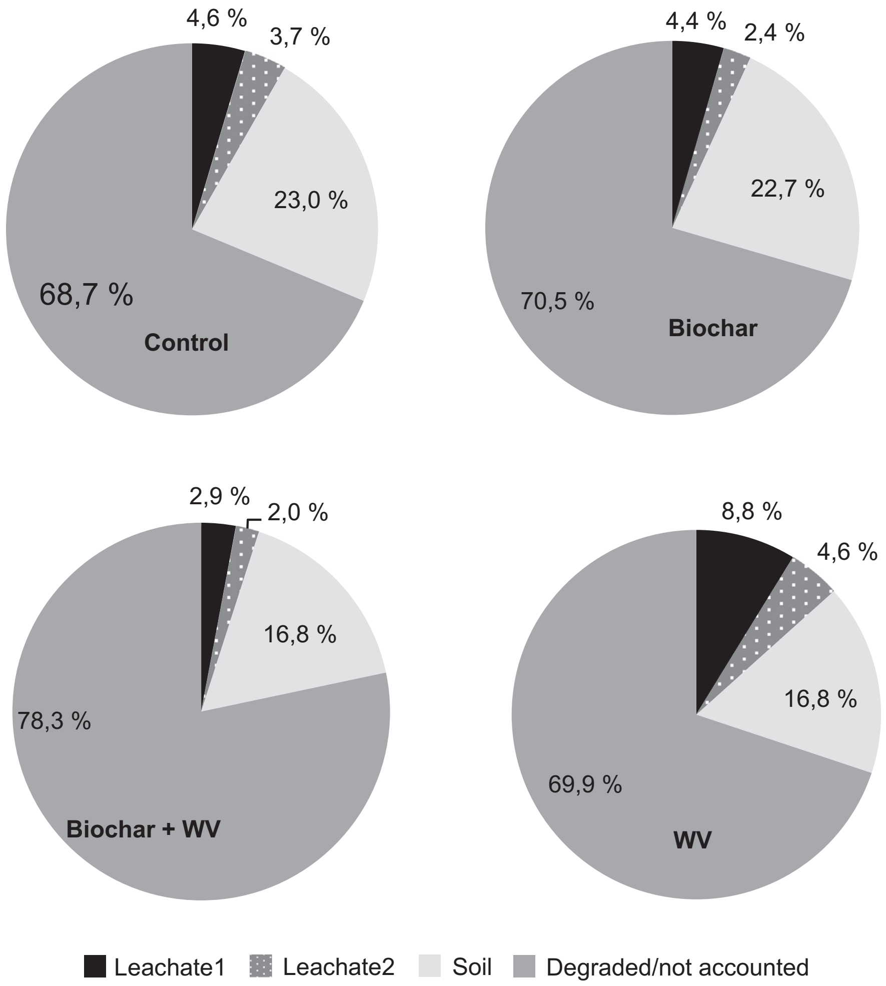

Fig. 2. The proportions (%) of glyphosate leached out of the pots, still present in soils or degraded (not accounted) at the end of the study in the pots with plants.

Precipitation can also influence glyphosate leaching; when the application of glyphosate is followed by heavy rain fall, large amounts of glyphosate can be transported to deeper soil layers via soil macropores [44]. Furthermore, in our study, fine sandy soils in the absence of plant (root) activities leached very low amounts of glyphosate, suggesting that the risk of glyphosate leaching in typical Northern agricultural soils, devoid of plant cover and significant root biomass, is low, even during heavy rain fall. It is worth noting that, due to the pooling of the glyphosate samples, the glyphosate data was not submitted to statistical analysis. However, as there was the same remarkably clear trend in glyphosate leaching among the sampling times we conclude that the plants can have a great influence on the transfer of glyphosate in soils.

Table 2
The effects of biochar, wood vinegar and the mixture of biochar and wood vinegar on leachate properties 5, 47 and 81 days after their amendment on soil. Treatment effects that are statistically significant (repeated-measures ANOVA $p \leq 0.05$, $n = 20$) are marked with asterisk (*). Means ($n = 40$) and S.E (±) are presented.

	Day	pH	Conductivity (mS/m)	TOC (mg C/L)
Control	5	7.49 (±0.02)	0.23 (±0.00)	16.2 (±0.86)
	47	7.80 (±0.02)	0.34 (±0.02)	19.5 (±0.75)
	81	7.51 (±0.02)	0.35 (±0.02)	22.1 (±0.83)
Biochar	5	7.43 (±0.01)	0.23 (±0.00)	18.0 (±0.45)
	47	7.84 (±0.02)	0.34 (±0.02)	21.8* (±0.90)
	81	7.74* (±0.03)	0.33 (±0.01)	23.3 (±1.03)
Wood vinegar	5	7.52 (±0.02)	0.18* (±0.00)	27.6* (±0.06)
	47	7.87* (±0.01)	0.29* (±0.01)	19.7 (±0.83)
	81	7.65* (±0.04)	0.34 (±0.01)	22.2 (±0.97)
Biochar + wood vinegar	5	7.52 (±0.02)	0.18* (±0.00)	26.2* (±0.86)
	47	7.87* (±0.02)	0.33 (±0.01)	24.3* (±0.96)
	81	7.74* (±0.03)	0.33 (±0.01)	21.6 (±0.63)

4.2. *Effect of biochar and WV on the environmental fate of glyphosate*

As it was hypothesised the biochar reduced the leaching of glyphosate from the soil. Compared to other pesticides, glyphosate has unique sorption characteristics in the soil. It has a high soil adsorption coefficient ($K_d = 61$ g cm^{-3}) and a very low octanol/water coefficient ($K_{ow} = 0.00033$) suggesting that, in spite of its high water-solubility (12 g L^{-1}, 25 °C), glyphosate is rather immobile and is thus unlikely to leach through the soil [45]. The adsorption of glyphosate is not, or is sometimes negatively, correlated with soil organic matter content [3]. Instead, Albers et al. [46] reported rather high glyphosate sorption values in purified humus samples and Shen et al. [47] showed that activated carbon has glyphosate absorption capacity. Many studies have reported decreased leaching of herbicides [16,19] after the addition of biochar. In line with these studies, we showed that birch wood derived biochar can influence the fate of glyphosate by reducing the likelihood of glyphosate leaching out of the soils. This effect was evident irrespective of the presence or absence of plants and there was the same remarkably clear trend in glyphosate leaching among the sampling times. We conclude that the biochar can have a great influence on the leaching of glyphosate in soils. However, as biochar is produced from different parent materials and by varying pyrolysis technologies, the interactions of different kinds of biochar with soil constituents and applied agrochemical inputs are expected to be highly variable [12].

Contrary to our hypothesis, the presence of biochar had no clear effect on the glyphosate degradation in the soil. The role of biochar in the degradation of chemical pesticides is not clear. Several studies [18,19] have demonstrated a greater persistence and limited degradation of pesticides, such as simazine and diuron in biochar-amended soils. In contrast, Zhang et al. [20] observed that nutrients from biochar enhanced biodegradation of benzonitrile. These authors concluded that biochar can stimulate soil microbial communities by increasing the organic matter and nutrient content of soils. The degradation of glyphosate in soils is mainly a microbiological process, and microbial respiration in the soil can be used to estimate the degradation of glyphosate [48]. Here we showed that biochar also stimulated soil microbial activity when compared to control soils. However, in our study the larger and more active microbial population in these biochar systems had no effect on glyphosate degradation reflecting importance of understanding complex chemical and/or soil specific sorption processes that evidently decreased availability of the strongly sorbing glyphosate to microbes [49].

To our knowledge, the impacts of wood vinegar on the degradation of herbicides have not yet been studied. We hypothesised that wood vinegar would increase the degradation of glyphosate by stimulating the activity of microbes that can use glyphosate as a resource. We based this hypothesis on the observation that wood vinegar stimulates soil microbial activity soon (ca. 1 day) after application [27,31]. However, wood vinegar had no measurable effect on soil microbial activity in our study. It is probable that soil microbes had already consumed this energy-rich resource before the respiration analysis took place. Previously noted increase in microbial activity has been short-term (see Refs. [27,31]) and probably thus had no influence on glyphosate degradation. However, in the presence of plants the degradation of glyphosate was highest in soils treated with the biochar–wood vinegar mixture. This result was unexpected as neither wood vinegar nor biochar, when applied alone, had an effect on glyphosate degradation. A mechanistic understanding for the observed results is not clear warranting further examination.

None of the compounds that were most abundant in the wood vinegar were found in the leachates. Thus the increased TOC-value in the leachates at Day 5 (Table 2) is, at least partly, an indirect effect of wood vinegar and may be caused for example by a rapid, temporal increase of microbial activity and mineralisation processes after wood vinegar application [27,31].

4.3. *Effects of biochar on soil fertility and plant growth*

Biochar contains various plant nutrients, such as N, P, K, Ca and S [50]. In our study, total nitrogen of the soil increased by almost 20% as a result of biochar amendment. However, despite the increase in N, the loss of total nitrogen from biochar amended soils was only 3% compared to 12% in control soils irrespective of plants. Nitrogen loss from control pots can be explained by the substantial amounts of water that leached through soils after irrigations. Biochar can affect N cycling by influencing nitrification rates, the adsorption of ammonia, and increase NH_4-storage by enhancing the cation exchange capacity in soils [51]. The decreased loss of total nitrogen from biochar-treated soils in our study may result from the strong sorption of N to the surface of biochar [51]. Furthermore, as carbon input often increases N immobilisation in the soil [52], it is also possible that biochar carbon that exists in labile form was readily available for the soil microbial uptake which lead to the immobilisation of N into soil microbial biomass.

Given the likely changes in N dynamics in biochar-treated soils, it is not surprising that biochar had no impact on the growth of *L. perenne*. For example, Spokas et al. [53] showed that changes in the soil N cycle can increase crop production in biochar treated soils. Several studies have reported that biochar increases crop yields, but virtually all of these were conducted in the tropics with large variation in effect sizes. In their review, Verheijen et al. [13] explained that this variation is likely due to divergent properties of the biochar applied, soil and crop types. They suggest that the liming effect induced by biochar is one of the main mechanisms explaining increased plant productivity. In our study, pH of the parent soil (6.83) was rather high and the addition of biochar increased it only slightly (6.95), which may explain the unresponsive growth of *L. perenne* to biochar. However, the increased total N in the soil as well as the decreased loss of total N had no effect on the growth of *L. perenne* in our study. Obviously, due to the lack of a significant interaction between treatments “plant” and “biochar”, the observed treatment effects on (i) microbial respiration, (ii) N dynamics, and (iii) glyphosate leaching and degradation were not indirect effects via plants, but actual effects of biochar on these variables.

5. Conclusions

Our study shows for the first time that birch wood biochar has potential to influence the fate of glyphosate in the soil by decreasing its leaching. Since the transfer of glyphosate into deeper soil layers seems to be strongly dependent on plant root release and translocation via root channels, mixing or ploughing biochar deep into the soil is likely to minimise the translocation of glyphosate from the above ground milieu to the below ground system. This would reduce ground and surface water contamination risks by glyphosate. Our study also corroborates previous findings that mixing of biochar to agricultural soils can decrease soil nitrogen losses and thereby reduce the fertiliser inputs on aquatic environments.

Our findings that none of the most abundant compounds of wood vinegar were found from the leachate, and that there were no differences in the survival of the *D. magna* between control waters or waters leached through differentially treated soils, support our earlier claims that wood vinegar is of low environmental risk and is rapidly decayed through microbial activity.

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