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Risk in the circular food economy: Glyphosate-based herbicide residues in manure fertilizers decrease crop yield

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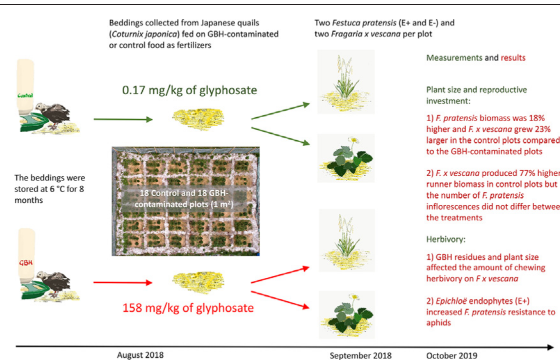
HIGHLIGHTS

- Conventional agriculture practices include glyphosate-based herbicide (GBH) use.
- The risk of GBH residues ending up in poultry feed is high.
- The manure of poultry birds given such feed contains high residues of glyphosate.
- Crop plant growth and reproduction decreases if such manure is used as fertilizer.
- Glyphosate contamination in fertilizer may affect herbivore resistance of crop plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Glyphosate-based herbicides (GBHs) are the most frequently used herbicides globally. They were launched as a safe solution for weed control, but recently, an increasing number of studies have shown the existence of GBH residues and highlighted the associated risks they pose throughout ecosystems. Conventional agricultural practices often include the use of GBHs, and the use of glyphosate-resistant genetically modified crops is largely based on the application of glyphosate, which increases the likelihood of its residues ending up in animal feed. These residues persist throughout the digestive process of production animals and accumulate in their excretion products. The poultry industry, in particular, is rapidly growing, and excreted products are used as plant fertilizers in line with circular food economy practices. We studied the potential effects of unintentional glyphosate contamination on an agronomically important forage grass, meadow fescue (*Festuca pratensis*) and a horticulturally important strawberry (*Fragaria x vescana*) using glyphosate residues containing poultry manure as a plant fertilizer in a common garden experiment. Glyphosate in the manure decreased plant growth in both species and vegetative reproduction in *F. x vescana*. Furthermore, our results indicate that glyphosate residues in organic fertilizers might have indirect effects on sexual reproduction in *F. pratensis* and herbivory in *F. x vescana* because they positively correlate with plant size. Our results highlight that glyphosate can be unintentionally spread via organic fertilizer, counteracting its ability to promote plant growth.

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1. Introduction

Risk assessment and management of substances of concern are among the central challenges in sustainable food production and transitioning toward a circular food economy (Deselnicu et al., 2018). Here, we focus on the fate of herbicides in material cycles of the poultry industry. As poultry manure is rich in essential nutrients and organic compounds, regulations for sustainable crop production suggest its use as an organic fertilizer (Bolan et al., 2010; Deselnicu et al., 2018; Kyakuwaire et al., 2019; Oenema et al., 2007). Consequently, poultry manure is increasingly used in agriculture and horticulture as well as home gardens (Hoover et al., 2019; Kyakuwaire et al., 2019). However, the possible risks associated with the accumulation of agrochemicals in poultry manure are still largely ignored (McKinnon et al., 2014) although agrochemicals used in animal feed production can accumulate in poultry manure (Ruuskanen et al., 2020a).

Glyphosate (*N*-phosphonomethyl glycine) is the most widely used contemporary biocide (Benbrook, 2016). Glyphosate-based herbicides (GBHs) are used to control weeds both in agricultural and non-agricultural environments (i.e., silviculture, urban environments, and home gardens), and during the last few decades, they have become the most commonly used herbicides globally (Baylis, 2000; Duke and Powles, 2008; Helander et al., 2012; Myers et al., 2016). The use of glyphosate-tolerant crop plants in crop production has contributed considerably to their popularity (Benbrook, 2016; Duke and Powles, 2008; Woodburn, 2000). For example, "Roundup Ready" genetically engineered (GE) soy contributed to more than 90% of the global soybean production in 2017 (ISAAA, 2017). GE soy is widely used as animal feed because soybean is an excellent protein and energy source.

Glyphosate has been proclaimed as safe for non-target organisms if safety period is taken into account (Williams et al., 2000) because (1) glyphosate effect is based on the inactivation of 5-enolpyruvylshikimate-3-phosphate synthase in the shikimate pathway, an essential metabolic route in plants (Duke and Powles, 2008), and (2) it should degrade fast (in two weeks) in the ecosystem. Given that animal cells lack the shikimate pathway, glyphosate is considered as non-toxic to animals (Helander et al., 2012; Williams et al., 2000). However, the shikimate pathway is present in many microbes (Bentley, 1990; Shehata et al., 2013), thus complicating the recognition of the associated risks in (agro) ecosystems. Furthermore, the prolonged persistence of glyphosate in colder climates increases the associated risks (Helander et al., 2012). Contrary to the alleged two-week safety period, glyphosate and its degradation products have been reported to persist in ecosystems even for years (Laitinen et al., 2009). Due to the extensive use of GBHs during the last few decades, an increasing number of empirical observations have revealed the residues of GBHs and their degradation products in soil, water, crop plants, and even animal tissues (Bai and Ogbourne, 2016; Bøhn et al., 2014; Helander et al., 2018; Silva et al., 2019; Ruuskanen et al., 2020a), suggesting that the residues can also cascade upward in the food chain. In tandem with these observations, numerous recent studies have reported the indirect negative effects of glyphosate via soil on non-target plants, soil microbiota, and microbes associated with plants and animals (Druille et al., 2016; Helander et al., 2018, 2019; Székács and Darvas, 2018; Van Bruggen et al., 2018). Thus, glyphosate use may harm biodiversity and ecosystem functions and services (Helander et al., 2012).

In this study, we experimentally investigated how glyphosate-contaminated poultry feed may affect crop plants via poultry manure. We focused on the agronomically important meadow fescue (*Festuca pratensis*) and the horticulturally important strawberry (*Fragaria x vescana*). As fertilizer, we used bedding material containing excrements from Japanese quails (*Coturnix japonica*) raised on feed containing glyphosate. We assumed that the bedding contains substantial amounts of glyphosate residues because our recent results demonstrated that glyphosate accumulates in high concentrations (>200 mg/kg) in the excrement of the birds (Ruuskanen et al., 2020a). We then conducted a field trial to test whether the presupposed glyphosate residues in bedding used as fertilizer affect the growth and reproduction of *F. pratensis* and *F. x vescana*. We hypothesized that in addition to the potential direct herbicidal effects of glyphosate on the growth and reproduction of the study plants, the residues might affect their ability to defend themselves against herbivores, although this would be evidenced differently in *F. pratensis* and *F. x vescana* because of their different herbivore deterrent strategies. Unlike most plants that rely on secondary metabolites, grasses primarily employ tolerance and rapid regrowth capacity to mitigate herbivory damage. Furthermore, many cool season pooid grasses are defended by alkaloid-producing symbiotic fungal *Epichloë* species. Thus, as glyphosate is known for its antifungal properties (Helander et al., 2018), it may negatively affect the *F. pratensis*-associated heritable fungal endophyte *Epichloë uncinata*, which confers defense to the host grass against herbivores by producing herbivore-deterring and toxic alkaloids (Clay, 1988; Fuchs et al., 2017; Helander et al., 2016; Saikkonen et al., 2013, 2016). In contrast, as herbivore deterrence of strawberries primarily relies on secondary metabolites, especially phenolics such as coumarins and lignin, whose production glyphosate can interrupt (Aaby et al., 2007), we expect to find a higher extent of herbivore damage in glyphosate-treated strawberry plants.

2. Materials and methods

2.1. Study species

We used the meadow fescue cultivar "Kasper" (*Festuca pratensis*) and the strawberry cultivar "Sara" (*Fragaria x vescana*) as the study species. *F. pratensis* is an important perennial forage grass widely used to feed cattle in Europe. The species is often found to harbor a symbiotic fungal endophyte *E. uncinata* (Saari et al., 2009). Under symbiotic conditions, a single haploid hyphae grows systemically and asymptomatically throughout the host grass. This is also true during inflorescence and seed development. As the fungus is vertically transmitted from the host grass into its offspring via seeds, the fitness of the fungus and its host grass are tightly linked (Saikkonen et al., 2004). *F. x vescana* is a hybrid cultivar between wild woodland strawberry (*Fragaria vesca*) and garden strawberry (*Fragaria x ananassa*). The hybrid was created to combine the best traits from both species for commercial use, especially berry quantity and quality as well as resistance against certain pathogens (Bauer, 1993).

2.2. Study setup

We conducted the field experiment at Ruissalo Botanical Garden (Turku, Finland, 60°26′ N, 22°10′ E) using beddings collected from Japanese quails (*Coturnix japonica*) fed on GBH-contaminated or GBH-free control food as fertilizers to study the effect of GBH residues on plant growth, reproduction, and herbivory. The beddings were collected from an aviary experiment where birds were fed with either GBH-contaminated feed ($N = 38$) or control feed ($N = 38$) from the age of 10 d to 12 months. The GBH-feed consisted of organic feed for laying chickens ("Luonnon Punaheltta," Danish Agro, Denmark) combined with Roundup Flex® equivalent to ca. 160 mg glyphosate/kg. This dose corresponds to daily intake of 12–20 mg glyphosate per kilogram body mass in full-grown Japanese quails. According the European Food Safety Authority (EFSA) estimates, the maximum glyphosate intake for poultry is 33.4 mg/kg in feed (dry matter) corresponding to maximum daily intake of 2.28 mg/kg body mass and No Adverse Effects Level (NOAEL) is 100 mg/kg body mass/day. At 12 months of exposure, the average glyphosate concentration of the excreta samples (urine and fecal matter combined) was 199 mg/kg (S.E. $= \pm 10.5$ mg/kg). The control group was fed the same organic feed with non-measurable glyphosate concentrations. The birds were reared in pairs (one female and one male) in 1 m × 1 m floor cages (N = 38) with bedding. The beddings (including wood shavings, feces, and some spilled food) were changed bi-weekly, and the used beddings were collected regularly from 8 to 12 months of exposure from each treatment. More detailed descriptions of the experimental design, GBH treatments, and rearing conditions of the birds appear in Ruuskanen et al. (2020a, 2020b). For the present experiment, the beddings were pooled (per treatment) and stored in closed containers in a dry, dark storage room (6 °C) for a maximum of 8 months. The beddings were spread on the experimental field on August 31st, 2018 and on May 7th, 2019. Altogether 25 l corresponding ca 3.8 kg beddings were spread on each 1 m^2 plot. On a field basis, this amount extrapolates ca 38 thousand kg/ha. While the application rate might be at higher side, it should be noted that approximately less than half of the weight was bird excreta and rest wood shavings. Furthermore, for small farms, home gardens and farms in organic systems, the rate used here is likely to be practical. In order to consider possible environmental gradients within the experimental field, the control (N = 18 plots) and GBH (N = 18 plots) bedding plots were arranged in a 6 × 6 chessboard grid consisting of a total of 36 plots. Experimental field has no previous history of herbicide use and it has not been used agriculturally during the last decade. The soil has been classified as Stagnosol and has sandy clay texture with relatively high proportion of organic material. Approximately one month after spreading the first beddings, in the end of September 2018, we planted two endophyte-symbiotic (E+) and two endophyte-free (E−) *F. pratensis* and two *F. x vescana* per plot (ca. 1 m^2 per plot). E+ and E− *F. pratensis* were planted crosswise in a double-dichotomy setting within the plots, and *F. x vescana* were planted on the southern side of the plots.

2.3. Plant measurements

To study whether the glyphosate-contaminated and control beddings affected the performance of experimental plants differently, we used the following growth and reproduction attributes: (1) To estimate the growth of *F. pratensis*, the aboveground parts of the plants were harvested, oven-dried (at 65 °C for 48 h), and weighed at the end of the growing season in late October 2019. (2) Prior to that, in mid-August, we counted the number of *F. pratensis* inflorescences as a measure of reproduction. (3) To estimate the growth of *F. x vescana*, we measured plant size as the diameter and height of the rosette of each plant individual in late August 2019. Diameter and height were then used to calculate the plant size index (diameter × height). *F. x vescana* did not produce flowers, probably because of the high amounts of nitrogen leaching from the quail manure in the beddings, which prevents flowering in many strawberry species (Sharma et al., 2019). Furthermore, *F. x vescana* cultivars typically produce a high number of runners during the first year (Bauer, 1993). (4) Thus, to estimate *F. x vescana* reproductive output, we measured their vegetative reproduction. We collected runners at the end of July 2019, dried them at room temperature (ca. 22 °C) for two weeks, and determined their weight. In addition, (5) as an estimate of herbivory, we monitored the presence of aphids on each individual *F. pratensis* and *F. x vescana* plant in early July, and calculated the number of chewing insect-damaged *F. x vescana* leaves (as indications of hymenoptera, lepidoptera, and/or coleoptera damage) in late August. Leaves of *F. x vescana* were considered to be damaged if at least 2% of the leaf area (by visual inspection) indicated chewing herbivore damage.

2.4. Glyphosate residue determination in the beddings

To determine the residues of glyphosate and its degradation products, gluphosinate and aminomethylphosphonic acid (AMPA) in the beddings, we sampled the bedding material directly after it was spread in May 2019. On average, 25 g of bedding from all treatment and control plots was collected, mixed, and pooled (per treatment) for further analysis. Air-dried samples were stored in 4 °C and analyzed using liquid chromatography-tandem mass spectrometry (see Ruuskanen et al., 2020a) at Groen Agro Control (Delft, Netherlands; certified laboratory).

2.5. Statistical analyses

We used general linear models to analyze how the use of GBH-contaminated and control beddings as fertilizer affected the growth and reproduction of the study plants. We used *F. pratensis* biomass and *F. x vescana* plant size index as measures of growth, and the number of inflorescences and runner biomass as measures of reproduction for *F. pratensis* and *F. x vescana*, respectively. The endophyte status (E+ or E−) and its interaction with the GBH treatment were included as fixed factors for analyses with *F. pratensis* and GBH treatment for analyses with *F. x vescana*. To control the effect of plant size on reproduction, we used the measures of growth as covariates when analyzing how the use of GBH-contaminated and control beddings as fertilizer affected the reproduction of the study plants. We used regression analysis to confirm the association between plant size and reproduction.

In addition, we analyzed how the use of GBH-containing and control beddings as fertilizer affected the chewing herbivore damage in *F. x vescana* by conducting a general linear model, wherein the number of herbivore-damaged leaves was used as a response variable and GBH treatment as fixed factor. Plant size index was included as a covariate in the model to control the potential effect of plant size on herbivory. Additionally, we analyzed the absence or presence of aphids in both *F. x vescana* and *F. pratensis* using generalized linear models (binomial error distribution and logit link function) with the GBH treatment (GBH-contaminated vs. control beddings) as an explanatory fixed factor. In addition, for *F. pratensis*, we included the endophyte status of each plant (E+ or E−) and its interaction with the GBH treatment as explanatory fixed factors in the analyses. In all the models with plant size as a covariate, interactions with covariate and explanatory variables were tested and nonsignificant interactions were removed from the final models. The normality and homoscedasticity of the residuals were checked by visual examination and Levene's test, respectively. All statistical analyses were performed with Proc GLIMMIX of the SAS/STAT® Software, Version 9.4 (SAS Institute Inc., 2013).

3. Results

3.1. Glyphosate residues in beddings

Glyphosate and its degradation products were detected in the beddings used as fertilizer in the experiment. In the beddings originally collected from the cages of the birds fed with GBH-contaminated feed and kept 8 months in the storage, 158 mg/kg of glyphosate, <0.01 mg/kg of gluphosinate, and 1.3 mg/kg of AMPA were detected. In contrast, the corresponding concentrations detected in the control beddings were 0.17 mg/kg, <0.01 mg/kg, and <0.05 mg/kg. The limits of detection (l.od.) for all tested compounds are 0.01 mg/kg (Groen Agro Control).

3.2. Effects of glyphosate residues on plants

GBH residues affected the growth *F. pratensis* and *F. x vescana* and the reproduction of *F. x vescana* negatively. The biomass of *F. pratensis* was 18% higher and the plant size index of *F. x vescana* was 23% higher in the control plots compared to the GBH-contaminated plots (Fig. 1 and Table 1). Endophyte status did not affect *F. pratensis* growth, either alone or in interaction with GBH contamination (Table 1). *F. x vescana* runner biomass was 77% higher in control plots than in the GBH-treated plots (Fig. 2 and Table 1). The number of *F. pratensis* inflorescences did not differ between the treatments (Fig. 2 and Table 1). Likewise, endophyte status did not affect the number of *F. pratensis* inflorescences (Table 1). Positive correlations between the growth, number of inflorescences, and runner biomass in *F. pratensis* and *F. x vescana*, respectively suggest that the larger plants had higher reproductive output independent of the treatment (Table 1 and Fig. 3).

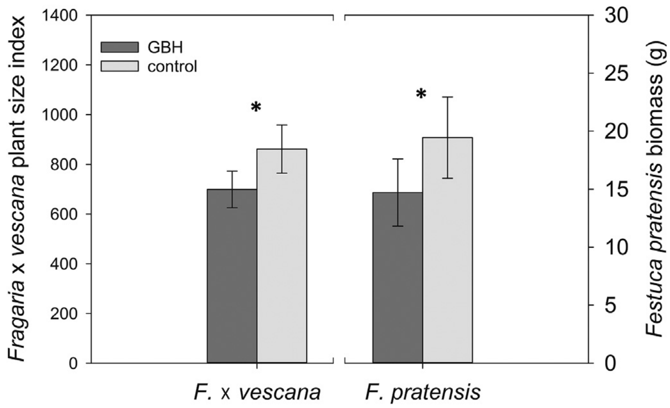

Fig. 1. Effect of manure fertilizer containing glyphosate based herbicide (GBH) residues on growth of *Fragaria x vescana* and *Festuca pratensis*. Growth of *F. x vescana* was measured as plant size (plant size index calculated as diameter (cm) × height (cm)) and that of *F. pratensis* growth was measured as biomass (g, dry weight). Means ±95% confidence intervals. *F. x vescana*: *N* (GBH) = 35, *N* (control) = 36; *F. pratensis*: *N* (GBH) = 69, *N* (control) = 70. The asterisk indicates a statistically significant difference ($P < .05$).

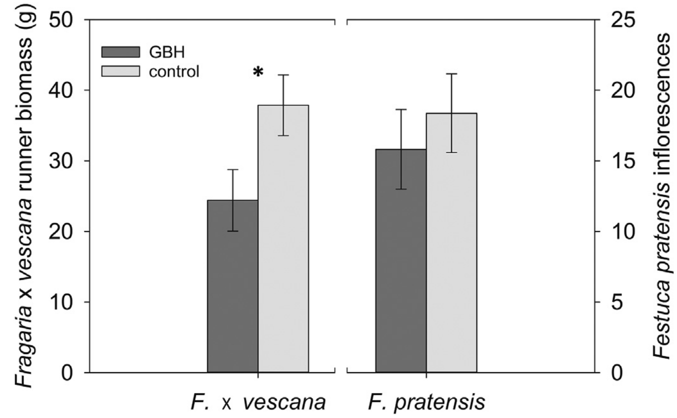

Fig. 2. Effect of manure fertilizer containing glyphosate based herbicide (GBH) residues on reproduction of *Fragaria x vescana* and *Festuca pratensis*. Reproduction of *F. x vescana* was measured as runner biomass (g) and that of *F. pratensis* was measured as number of inflorescences. Marginal means ±95% confidence intervals. *F. x vescana*: *N* (GBH) = 35, *N* (control) = 36; *F. pratensis*: *N* (GBH) = 69, *N* (control) = 70. The asterisk indicates a statistically significant difference ($p < .05$).

3.3. Effects of glyphosate residues on herbivory

The number of chewing herbivore-damaged leaves did not differ between the *F. x vescana* growing in the GBH-contaminated and control plots (Table 2). However, plant size affected the amount of chewing herbivory, as larger *F. x vescana* plants experienced more damage than smaller ones (Table 2). Likewise, the aphid occurrence was higher for larger *F. x vescana* plants, while glyphosate residues did not affect the occurrence of aphids in *F. pratensis* or *F. x vescana* (Table 2). *Epichloë* endophytes increased *F. pratensis* resistance to aphids, and we found aphids only on endophyte-free plants (Table 2).

4. Discussion

Our results demonstrate the risk associated with glyphosate residues in poultry manure in a circular food economy. We found that poultry manure can accumulate high residues of GBHs, decrease plant growth and reproduction, and thus inhibit the growth-promoting effects of manure when applied as fertilizer. These results demonstrate that the residues pass through the digestive process of birds, and more importantly, they persist in the manure fertilizer over long periods. In our case, we recorded residues first in the birds' excreta and then in the beddings 8 months later, showing that the glyphosate contamination remained relatively stable during the storage period. GBH-contaminated bedding used as fertilizer in the field decreased the growth of both plant species and the vegetative reproduction of *F. x vescana*. These results were most likely caused by glyphosate partially blocking 5-enolpyruvylshikimate-3-phosphate synthase (Duke and Powles, 2008). If GBHs are repeatedly used in agricultural practice, the negative effects of their residues in the field may result in GBH accumulation in human and animal food, and cascade in the food chain, leading to GBH residues in surrounding ecosystems.

Contrary to our expectations of more herbivory on GBH-treated plants, we did not find any differences in the chewing herbivore-damage or aphid presence between *F. x vescana* growing on plots fertilized with GBH-contaminated manure and the control plants. However, our results suggest that GBHs may indirectly affect herbivory, as the residues decreased plant growth, and larger strawberry plants suffered more damage from chewing herbivores and had higher aphid presence than smaller ones. On average, *F. x vescana* plants grown on the control plots were 28% larger than those growing on plots fertilized with GBH-contaminated manure. These results support the "plant vigor hypothesis", namely that larger plants are more attractive to herbivores (Price, 1991).

By blocking 5-enolpyruvylshikimate-3-phosphate synthase (Duke and Powles, 2008), glyphosate also blocks the biosynthesis of chorismate, the key precursor for the three aromatic amino acids (tryptophan, phenylalanine, and tyrosine) that are essential for plant survival, growth,

Table 1
Results of general linear models testing the effects of manure fertilizer containing glyphosate based herbicide (GBH) residues and the endophyte status (E+/E−) on growth and reproduction of *Festuca pratensis*, and of the manure fertilizer containing GBH residues on growth and reproduction of *Fragaria x vescana*. *F. pratensis* growth and reproduction were measured as dry biomass (g) and the number of inflorescences, respectively. *F. x vescana* growth and reproduction were measured as plant size index calculated as diameter × height (cm) and the dry biomass (g) of runners, respectively. Plant size was used as a covariate in the models testing the effect of GBH residues on plant reproduction.

Species	Dependent variable	Effect	*F*	*Ndf, ddf*	*p*
F. pratensis	Growth	GBH	4.22	1, 136	0.0419
		E+/E−	0.01	1, 136	0.9130
	Reproduction	GBH	1.61	1, 134	0.2063
		E+/E−	1.93	1, 134	0.1669
		plant size	44.01	1, 134	<0.0001
F. x *vescana*	Growth	GBH	7.32	1, 69	0.0086
	Reproduction	GBH	18.39	1, 68	<0.0001
		plant size	18.62	1, 68	<0.0001

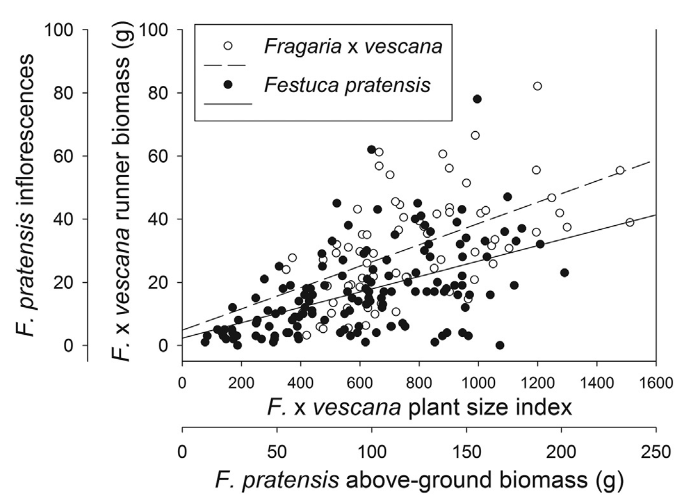

Fig. 3. Positive association of plant size and reproduction in *Fragaria x vescana* and *Festuca pratensis*. Larger plants invested more in reproduction in both plant species. *N* (*F. x vescana*) = 71, *N* (*F. pratensis*) = 139.

Table 2
Results of generalized linear model testing the effects of (a) manure fertilizer containing glyphosate based herbicide (GBH) residues, endophyte status (E+/E−), and plant size (covariate) on the probability of aphid infection in *Festuca pratensis*, and (b) manure fertilizer containing GBH residues and plant size (covariate) on the probability of aphid infection in *Fragaria x vescana*. Results of linear model testing the effects of manure fertilizer containing GBH residues and plant size (covariate) on damage by leaf-chewing herbivores on *F. x vescana*. Marginal means and 95% CIs are also presented.

Species	Dependent variable	Effect	Marginal mean	95% CI		*F*	*Ndf, ddf*	*P*
				Lower mean	Upper mean			
F. pratensis	Aphid presence	GBH	0.29	0.19	0.41	0.01	1, 135	0.9346
		Control	0.29	0.19	0.42			
		E+	0.19	0.11	0.30	8.09	1, 135	0.0051
		E−	0.41	0.30	0.53			
		Plant size[a]	0.001 ± 0.004			0.07	1, 135	0.7963
F. x vescana	Leaf damage	GBH	7.59	5.94	9.25	2.84	1, 68	0.0965
		Control	9.61	7.98	11.25			
		Plant size[a]	0.10 ± 0.05			4.75	1, 68	0.0328
	Aphid presence	GBH	0.03	0.00	0.19	2.64	1, 68	0.1088
		Control	0.15	0.07	0.34			
		Plant size[a]	0.002 ± 0.001			4.10	1, 68	0.0469

[a] For plant size, β ± SE are given.

defense, and secondary metabolite production (Helander et al., 2012). Thus, prevention of chorismate biosynthesis can also decrease plant nutritive value and attractiveness to herbivores (Hernandez et al., 1999; Santos-Sánchez et al., 2019). These findings point to the importance of evaluating how GBH residues in organic fertilizers affect plant chemistry, and thereby plant attractiveness and quality, for herbivores. For example, wild barnacle geese chose to forage on plants growing on GBH-treated plots over control plants in our recent field study (Helander et al., 2019). Thus, future studies should elucidate the mechanisms of how GBH affects the attraction of plants to vertebrate and invertebrate herbivores.

Based on recent studies revealing the antifungal properties of glyphosate (Helander et al., 2018), we predicted that GBH residues should negatively affect *Epichloë* endophytes that are well known for their ability to promote plant performance and resistance to herbivores (Clay, 1988; Helander et al., 2016; Lehtonen et al., 2005; Sabzalian et al., 2004; Saikkonen et al., 2013, 2016). However, *F. pratensis* symbiotic with *Epichloë* endophyte had lower aphid presence, suggesting that *Epichloë* endophytes may not be susceptible to GBH residues in the organic fertilizers.

The risk of glyphosate residues in poultry manure used as fertilizer have largely been ignored. Poultry manure is readily available globally, and since the poultry industry is growing fast, the availability of the manure is increasing (Hoover et al., 2019). At the same time, regulations call for sustainable use of excretion products, including their use as manure on crop fields (Deselnicu et al., 2018). For instance, the European Union posits a circular economy achieving zero waste as one of the key goals in its sustainable growth strategies (Deselnicu et al., 2018). However, given that glyphosate residues are not controlled in animal feed and glyphosate is not broken down during the digestive process of birds (Ruuskanen et al., 2020a), the accidental introduction of herbicides with the application of manure is likely to occur. This not only counteracts the growth-promoting features of poultry manure, but might also cause unnoticed and uncontrolled herbicide contamination of soil and other non-target organisms. These aspects can have long-lasting effects, especially in northern habitats, where GBH residues persist longer in the soil due to the cold climate (Helander et al., 2012).

The risks associated with exposure to GBHs are thus particularly high in agro-ecosystems. Today, genetic engineering is commonly used to increase crop resistance to GBHs in maize, soybean, oilseed, and alfalfa crops, which comprise the most commonly used crop species in livestock feeds (Aumaitre et al., 2002; Faust, 2002). The risks are further increased by the accepted agricultural practices in animal feed production. The use of glyphosate-tolerant crops also facilitates weed control in fields after the germination of the plants, which may lead to even more intensive use of GBHs. In addition to their use as a herbicide, GBHs are used as “harvest aids” in conventional crop production (Goffnett et al., 2016; Nelson et al., 2011; Zhang et al., 2017). These practices are likely to lead to increased concentrations and associated risks of glyphosate residues on crops used in human or animal food production, or consumed by wild birds feeding on them (Cuhra, 2015; Székács and Darvas, 2018). Furthermore, given the high stability of glyphosate in the digestive process of birds, GBH introduction into vulnerable ecosystems via the excretion of migratory birds could happen.

5. Conclusions

Our study demonstrated how GBH residues accumulated in poultry excrements can have negative effects on crop plants when poultry manure is used as a fertilizer. Regulations to promote sustainable food production/circular economy, for instance, encourage the use of poultry manure as fertilizer, which increases the risk of indirect und unnoticed introduction of GBHs. Simultaneously, the current agricultural practices, especially for “glyphosate-ready” GM crops used for animal feed, are heavily dependent on GBHs. Both agricultural practices (the increased use of manure and the increased use of GBHs) promote unintended and indirect introduction of GBH residues to the environment and especially to agricultural systems. These residues can then persist in ecological systems, affecting several non-target organisms over years. The consequences include (1) decreased efficiency of manure as fertilizer, (2) long-lasting GBH contamination of agricultural cycles, (3) uncontrolled GBH contamination of non-target areas, (4) increased threat to vulnerable non-target organisms, and (5) increased risk of emerging GBH resistances. Thus, we suggest that more holistic analyses of herbicide applications, beyond the “direct” and “targeted” ones and including “indirect” effects, be conducted. Furthermore, we emphasize that more studies are needed to reveal the extent of the GBH residues in organic fertilizers and how they may impact sustainability in different cropping systems.

CRediT authorship contribution statement

Anne Muola: Conceptualization, Methodology, Writing - original draft, Funding acquisition. **Benjamin Fuchs:** Conceptualization, Writing - original draft. **Miika Laihonen:** Investigation, Formal analysis. **Kalle Rainio:** Investigation, Data curation, Formal analysis. **Lauri Heikkonen:** Investigation, Data curation. **Suvi Ruuskanen:** Writing - review & editing. **Kari Saikkonen:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **Marjo Helander:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

Declaration of competing interest

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

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Data accessibility

Data is stored at the Dryad Digital Repository (https://doi.org/10.5061/dryad.cjsxksn40).

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