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Greenhouse study and interviews indicate glyphosate residue via feed-feces-fertilizer route is a risk for horticultural producers using manure-based fertilizer

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Abstract

Background The herbicide glyphosate is the most widely used active ingredient in pesticides globally. Residues have been found in people, livestock, food and animal feed, and in the environment, but little is known about glyphosate residue in manure-based fertilizer. We describe a feed-feces-fertilizer route of glyphosate contamination with negative impacts for horticultural production. This exposure can harm sensitive plants, such as tomato, and pose a risk to effective waste disposal and nutrient cycling along principles of the circular economy.

We review the use and history of glyphosate and present a mixed methods research based on a real-world case from Finland where glyphosate residue in poultry manure fertilizer was suspected of inhibiting commercial organic tomato production. To test the fertilizer, we grew 72 'Encore' variety tomato plants for 14 weeks in a climate-controlled greenhouse according to the practices of the commercial grower. To ascertain awareness and potential contamination mitigation measures, we contacted five fertilizer companies with sales of biogenic fertilizer in Finland, two farming organizations, a feed company, and two government organizations working on nutrient cycling and agricultural circular economy.

Results The total harvest of tomatoes grown with fertilizer with the higher content of glyphosate residue was 35% smaller and the yield of first-class tomatoes 37% lower than that of the control, with lower glyphosate concentration. Two of the five fertilizer companies identified poultry manure as a source of glyphosate contamination. Companies with awareness of pesticide residues reported interest in establishing parameters for pesticide residues.

Conclusions The extent of glyphosate contamination of recycled fertilizers is unknown, but this study shows that such contamination occurs with negative impacts on crop production. Lack of testing and regulation to ensure that recycled fertilizers are free from harmful levels of glyphosate or other pesticides creates risks for agricultural producers. The issue is particularly acute for certified organic producers dependent on these products, but also for sustainable transitions away from mineral fertilizers in conventional farming. The example from Finland shows that a model of co-production between fertilizer producers and state regulatory agencies to establish safe limits can benefit both fertilizer producers and their customers.

Keywords Glyphosate-based herbicide, Agrochemical pollution, Horticulture, Sustainable agriculture, Circular economy, Organic, Organic fertilizer, Tomato, poultry manure

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Introduction

Persistence of agrichemicals is a challenge to livelihoods, health, and the environment. Glyphosate is the most widely used active ingredient in pesticides in the world [6, 70, 73], and glyphosate residues have been found in a wide range of foodstuffs, in humans and livestock, and in the environment [6, 38, 40, 43, 71, 74, 76, 79]. Organic fertilizers are fertilizers containing organic carbon (C_{org}) and nutrients and are derived from biological origin [22]. Little is known about glyphosate residues in organic fertilizers, but research and extension agencies report experience from the field that herbicide residues in organic fertilizers and mulches harm crop production [33, 36, 53]. Tomato is one of a variety of crops known to be particularly sensitive to herbicides [10, 28, 52, 75], and glyphosate-based herbicides are labeled to indicate that the products should not be used on or near tomatoes [39]. Such labelling, however, is ineffective for horticultural producers if the route of exposure is fertilizer.

Nutrient recycling is central to the agricultural circular economy, and contamination of raw materials recycled as fertilizer can limit how and where the fertilizer can be used. Failure to recycle manure and other agricultural waste results in both loss of nutrients to agriculture and increased nutrient pollution in the environment. Contamination of organic fertilizers is of particular importance for producers of organic greenhouse and field crops because mineral nitrogen fertilizer is proscribed [20] and organic fertilizers provide necessary nutrients for crops and build soil carbon.

The objectives of this study were to (1) examine the effect of a glyphosate-contaminated poultry manure fertilizer on tomato production and (2) understand the current operating environment, including industry experience and response to potential glyphosate contamination of fertilizer raw materials. For the former, we conducted an empirical study to assess the effect of glyphosate residue in manure fertilizer on the growth and total production of greenhouse tomatoes. In the latter, we contacted fertilizer companies and selected experts in Finland and asked about potential glyphosate residues in side-stream materials—particularly poultry manure—used in the production of fertilizer permitted for use in certified organic horticulture and agriculture.

Glyphosate use is complex and contentious. To adequately frame the issue, we first present glyphosate as an herbicide, how it became so ubiquitous, and the status of its regulation in Europe and the United States. Second, we describe the issue of glyphosate residue as a contaminant in fertilizers of biogenic origin (hereafter referred to as organic fertilizers or manure). Third, we present the system which was studied and the real-world case—poultry manure contamination—on which this research is based. The empirical and qualitative studies are then described sequentially in their own sub-sections in Materials and Methods and then Results. The strands of inquiry are brought together in Discussion, and future needs and directions are elaborated in Conclusion.

Glyphosate: a controversial 'miracle' herbicide

Glyphosate is a systemic, broad-spectrum herbicide used both within and outside agriculture [41, 70]. Considered a 'once in a century herbicide' [14], glyphosate was patented as an herbicide in 1971 and released into the pesticide market in 1974 under the trade name "Roundup®" [54]. Surge in use of glyphosate-based herbicides is linked to: (1) availability of generic glyphosate-based products following the expiration of the original patent in 1991 and (2) genetically modified glyphosate tolerant crops, such as soybean and maize, which became commercially available in the 1990s [6, 8, 14, 54, 73]. Monsanto Company retained exclusive rights to glyphosate in the United States until 2000 and actively maintained market share through product innovation and bundling of seeds and crop protection products [4, 54, 55].

Globally, glyphosate-based herbicides are used in agriculture for purposes, including: (1) termination of crops, including to prepare fields for sowing in minimum tillage cropping systems or to terminate perennial grasslands, such as leys or pasturage; (2) in-crop to control weeds, often in conjunction with glyphosate tolerant GM-crops; and (3) pre-harvest as a desiccant to ensure uniform ripening and drying of grain crops [1, 6, 70, 76]. Use as a desiccant is more common in northern regions in which cold and wet weather conditions are common during harvest [6].

In Europe, one-third of herbicide sales (volume of active ingredients) in EU28 + 3 countries are of glyphosate, but more research is needed on how and where glyphosate is applied throughout the crop rotations [2]. Although the European Union (EU) requires permits for the cultivation of genetically modified (GM) crops, including herbicide tolerant crops [1, 62], and a wide variety of GM crops are allowed into the market as food and feed [15]. The EU forbids glyphosate use 'with the intention to control the time point of harvest or to optimize the threshing', but the European Commission acknowledges that such use does occur and, therefore, advises member countries in its Implementing Regulation [19] to pay particular attention to compliance of pre-harvest uses.

In the United States, Monsanto held a patent on glyphosate products (Roundup$^{(R)}$) until 2000 when the patent expired and new products with higher glyphosate concentrations entered the market [63]. Despite packaging, including instructions for adjusting dosage based on the higher concentration, the mean application dosage of glyphosate increased, because many agricultural producers continued to apply the higher concentration products at the application rate of the prior lower concentration products (*ibid*).

As an herbicide, glyphosate acts by inhibiting plant growth via disruption of the shikimate pathway, which is a metabolic pathway found in the cells of plants and some micro-organisms but is not present in animal cells [14, 41]. This mode-of-action is why glyphosate has historically been considered a safe pesticide for humans and other vertebrates [14, 41, 68, 77]. However, there is a growing literature showing that glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), is ubiquitous in nature and the food system and has adverse consequences to human health and non-target organisms [5, 6, 8, 51, 64–66, 70]. For example, glyphosate has been shown to be lethal to amphibians [42], harms earthworms [13], and induces alterations in nervous systems of humans, rodents, fish, and invertebrates [12]. Due to glyphosate's effects on gut microbes and hormones [66], glyphosate residue in fodder can pose a risk to livestock.

In recent years, a variety of regulatory bodies have considered the safety of glyphosate with mixed outcomes. The Joint Meeting on Pesticide Residues (JMPR), which evaluates and sets maximum residue levels (MRLs) for pesticides in food on behalf of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), has declared it unnecessary to establish acute reference doses (ARfDs) or MRLs for glyphosate [78]. The decision was based on epidemiological evidence restricted to studies on cancer outcomes [30]. However, both the European Food Safety Agency (EFSA) and United States Environmental Protection Agency (EPA) have established MRL for glyphosate [16, 18, 26, 27, 72]. The MRLs set by EFSA and EPA differ substantially from each other, and controversy remains. Benbrook [6] asserts that regulatory bodies have accommodated requests by glyphosate registrants to adjust MRL upward to allow for the glyphosate residues in post-harvest foodstuffs caused by pre-harvest spraying. Scientists have presented evidence in support of lowering glyphosate MRL to a level 17 times lower than the EPA's and five times lower than EFSA's current levels [3, 6]. EFSA considers current glyphosate exposure levels via feed generally safe for livestock [24], and Sørensen et al. [71] note that EFSA proposed [25] 30% increases of MRLs for rapeseed, barley and wheat. In November 2023, the European Commission announced renewal of the approval of glyphosate for a period of 10 years. Approval is reported to come with caveats, including prohibition as a pre-harvest desiccant and unspecified restrictions to protect non-target organisms [9, 17].

Feed-feces-fertilizer: glyphosate accumulation in organic fertilizer

The route by which glyphosate may enter livestock and eventually accumulate in manure is dietary exposure. The EU's EFSA and USA's Food and Drug Administration (FDA) have monitoring programmes for commodity crops and may test crops entering the market for glyphosate and other agrochemical residues. However, the MRL are to ensure food safety for human consumption, and EU's MRLs for livestock, for example, are considered only in regard to their impact on human health [24, 25]. By virtue of diet, however, livestock exposure to glyphosate via ingestion is generally substantially higher than that of humans [71]. Feed produced and consumed on-farm or domestically via farmer-to-farmer networks is unlikely to be tested.

Both EFSA and the FDA report non-violative (below the MRL) glyphosate residues in commodities commonly used as feeds, including soybean, corn, barley, and wheat [26, 29, 73]. Pre-harvest dessication and in-crop application on glyphosate tolerant crops are likely sources of glyphosate contamination in feed [71, 76]. Glyphosate from consumed feed has been found in the urine of a range of livestock, and EFSA has previously concluded that glyphosate is rapidly excreted from the body (*cf.* [23, 48–50, 76]).

EU 2019/1009 [22] establishes limits on heavy metals and pathogens in organic fertilizers, but the only limitation for pesticides like glyphosate is that the use of the fertilizer does not result in the established residue limits in food or feed to be exceeded. To our knowledge, there are no public regulations in Europe or elsewhere establishing maximum residue levels for glyphosate in fertilizers. However, it is known that (1) some crop plants are highly sensitive to synthetic agrochemicals, including glyphosate, and (2) glyphosate from feed accumulates in the animal and may become more concentrated compared to the initial feed residues, and this concentrating effect potentially exacerbates glyphosate contamination in manure used for fertilizer [56, 66].

This study focuses on poultry manure as a fertilizer raw material. Poultry manure is one of the biological products approved as fertilizer under the European Union's organic production and implementing regulations [20, 21]. Poultry manure, a side-stream product from egg and poultry meat production, is one of the most common commercially available organic fertilizers. Its availability is facilitated by the growth of the poultry industry and the desire to recycle the manure instead of disposing of it as waste [44, 46]. Glyphosate contamination of crops via poultry manure have only been studied in a few cases. Muola et al. [56] found negative impacts of glyphosate contaminated poultry manure on both meadow fescue and strawberry in the first year of establishment. However, further study of the strawberry plants revealed a positive effect on fruit weight in the second year [37].

Finland case

The study was conducted in Finland, a European Union member state since 1995, where egg and broiler chicken production are the primary sources of poultry manure. Egg production is spread across approximately 230 farms with an average stocking rate of 20,000 hens/farm that produce a total of 77.5 million kg of chicken eggs annually [59, 60]. Broiler production takes place on approximately 200 farms with an average stocking rate of 60 000 broilers and yields about 147 million kg of meat [60].

Chicken feed in Finland commonly consists of domestic (on-farm) grains mixed with compound feeds, oil and limestone. Farms generally receive assistance in feed planning, and feed is often mixed at the farm via a mobile feed mixing service. Approximately half of the layer hens are fed with such feed mixes, while half receive commercial complete feed (Valkonen, personal communication 2024). Broilers are generally fed a mix of the farm's own wheat and complementary feed (*ibid*). Protein sources include soybean meal, domestic fava bean or peas, oil seed rape meal and fishmeal [34]. Although soy-free and GMO-free compound feeds are available, most compound feeds contain genetically modified soy (Eija Valkonen, Hankkija Oy, personal communication, 10.7.2024). Commercial poultry compound feed for the Finnish market is produced by five companies [34].

Fertilizer use and quality in Finland is legislated nationally by the Fertilizers Act [32], which states that fertilizers must be safe and may not contain materials that are harmful to people, animals, plants or the environment when applied as instructed. Finland has a relatively low rate of pesticide use compared to other countries. In 2018, sale of plant protection products (pesticides) per utilized agricultural area in the EU was 3 kg/ha but only 0.6 kg/ha in Finland [45, 57]. Of this, glyphosate-based herbicides accounted for about half of all plant protection products sold for agriculture in Finland [58]. The Finnish Safety and Chemicals Agency cites EU regulation [19] in forbidding glyphosate as a pre-harvest desiccant, but glyphosate may be used under certain conditions to control weeds in feed crops prior to harvesting [35], (Kaija Kallio-Mannila, Finnish Safety and Chemicals Agency Tukes, personal communication 13,9,2023). See Discussion for conditions in detail.

This study was inspired by the experience of a commercial organic tomato producer that experienced production problems they traced to a commercial poultry manure fertilizer approved for organic use but containing glyphosate residues. The producer used a commercial chicken manure sourced from Finland and certified as suitable for organic production. The producer sent the fertilizer to an independent laboratory for testing, and the results revealed that the fertilizer contained 0.94 mg/kg of glyphosate. No other contaminants were found. With this information, the producer commissioned researchers at University of Turku, Finland to examine the effect of the fertilizer containing glyphosate residue on tomato production.

Materials and methods

Empirical study

The empirical study was undertaken in 2016 in the University of Turku research greenhouses at Ruissalo Botanical Garden, Finland. Tomato seedlings and growth substrate were provided by the commercial organic tomato producer. In the beginning of April 2016 (week 14), the producer delivered 72 "Encore" variety tomato seedlings, 36 sacks of peat (Novarbo, 70 L each) and two flexible intermediate bulk containers (FIBC) with a volume of 1000 L of organic chicken manure fertilizer. The two FIBC consisted of 'G' glyphosate residue, the original fertilizer tested by the company as containing glyphosate residue; and 'C' control, a comparable commercially available fertilizer that underwent similar testing. Prior to delivery, the seedlings were grown by the producer together with seedlings for commercial production.

The two manure-based fertilizers were produced in Europe and marketed for professional horticultural use. As manure-based fertilizers, both products were marketed as suitable for use in certified organic crop production; it does not mean the manure is from certified organic livestock. The G fertilizer was an ECOCERT-certified (https://www.ecocert.com/en/home) chicken-manure fertilizer intended for professional use. The C fertilizer was produced from manure from free-range hens certified by the Dutch inspection authority for poultry, eggs and egg products ('de Stichting Controlebureau voor Pluimvee, Eieren en Eiproducten': CPE—certified poultry farms).

Prior to commencing the study and in the end of the experiment, the fertilizers were tested for glyphosate residues. Samples were tested for AMPA, the metabolite from glyphosate, only in the end of the experiment. All tests for glyphosate and any other contaminants were conducted by Groen AgroControl-Laboratory (Delft, Netherlands; certified laboratory). The samples were analysed using liquid chromatography–tandem mass spectrometry (see details in [67]), and the detection limit was 0.01 mg/kg. Results were 0.94 mg/kg in the G fertilizer and 0.23 mg/kg in the C fertilizer. These values indicate slight variation from the 0.73 mg/kg and 0 mg/kg, respectively, previously performed by the same laboratory on samples from the same bags. Control (C) fertilizer's NPKCa nutrient composition was 4.0–2.7–2.2–10.0 kg per 100 kg and pH 7.4. Values for the glyphosate residue (G) fertilizer were 3.6–2.8–2.2–9.6 and pH 6.4.

Experimental setup and management of the tomatoes

The experiment setting consisted of 72 plants randomized in three hierarchical levels (Fig. 1). Four plants (a, b, c, d) were planted in each grow bed (replicate). The C and G treatments were alternated in every other grow bed (nine C and nine G replicates). The replicates were placed along three watering lines I–III (six replicas per line).

Each replicate was independent from the others and consisted of 140 l of peat growth medium. A small area was dug in the centre of each replicate, and 9.5 kg of either C or G fertilizer was added and lightly covered with peat before covering the whole grow bed with plastic. At a distance of approximately 15 cm from the fertilizer, holes were made at the corners of each replicate for total of four holes (a–d) per replicate. One tomato seedling per hole was planted for a total of 36 C and 36 G plants (72 plants total). Planting took place during mid-April (week 15).

The greenhouse temperature was set to 20 ℃, but outdoor heat frequently caused the temperature to rise to 24 ℃. Artificial lighting was set to 12/12 light cycle. Moisture was set to 70% with automatic mist spraying. Watering was carried out according to need so that the moisture remained optimal along the watering line. Tomatoes were managed following the conventions for commercial production, and this included weekly pruning to remove the lowest three leaves and any extraneous growth (side shoots/suckers). Plants were attached to training ties hanging from the ceiling, and the ties were let out as necessary. To ensure pollination, plants were shaken in the morning three times per week for the duration of the experiment. Predatory mites were used as biological pest control.

Measurements

Fertilizer samples were tested for glyphosate at the beginning of the experiment. Furthermore, both glyphosate and AMPA were tested at the end of the experiment when 1 L samples were taken from the centre of five of the nine C and G grow beds, respectively. In addition, ten ripe tomatoes randomly chosen from a single day's production from plants in both the C and G groups were tested for glyphosate and AMPA residues at the end of the experiment.

Plant growth and fruit production were followed for 14 weeks (Table 1). Plant length, length and width of leaves above the third fruit clutch, distance between fruit clutches, number of clutches per plant, and number of tomatoes per clutch were measured.

Tomatoes ready for harvesting were collected, counted and weighed. The lowest tomato clutches were

Table 1 Timeline of the experiment

Experiment task	Date
Seedlings and other materials delivered	Beginning of April (week 14)
Tomatoes planted	Mid-April (week 15)
First harvesting	June 6 and 20 (weeks 23 and 25)
Ripe tomatoes harvested and classified	June 30–July 18 (weeks 26–29)
Final harvest and measuring	July 18 (week 29)

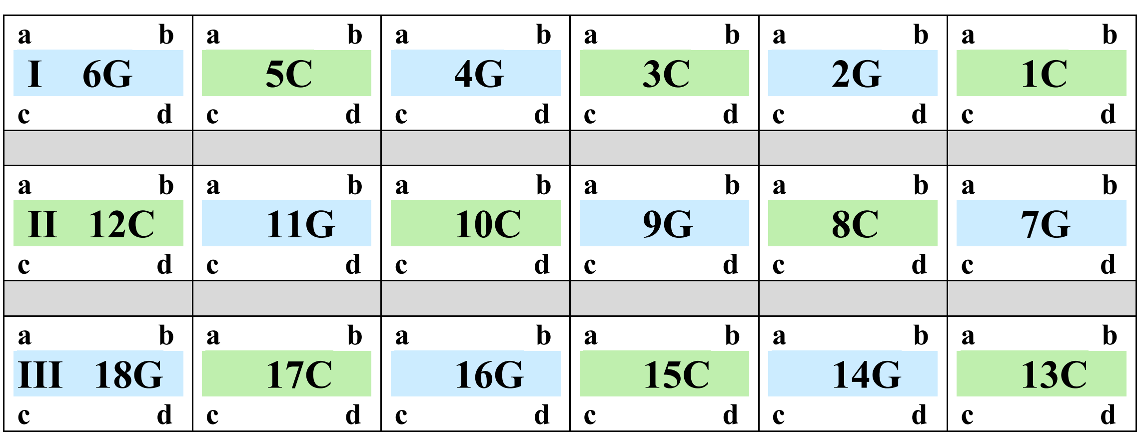

Fig. 1 Layout of the greenhouse tomato experiment. One tomato seedling was planted at each corner of the grow bed for a total of four plants (a, b, c, d) in each replicate. Number + letter indicates replicate number and treatment, where G = fertilizer containing glyphosate residue and C = control fertilizer. Roman numerals indicate the three watering lines

harvested whole when over half of the tomatoes in the clutch were ripe (harvesting dates: June 6th and 20th). During the period June 30 through July 18, ripe tomatoes were collected separately from individual clutches and were weighed and classified into either first or second class according to the criteria of the commercial producer. Quality classification was a blind test—the person classifying the tomatoes did not know whether the tomatoes were from G or C fertilized plants. The commercial tomato producer inspected the researchers' classification to assure the sorting accurately reflected professional standards of commercial tomato production. Upon conclusion of the experiment on July 18, both ripe tomatoes and tomatoes in all clutches with at least one green (raw) tomato were weighed.

Statistical analysis

Tomato production was measured in terms of (grams): (1) ripe tomatoes, (2) first class tomatoes, and (3) total yield at the end of the experiment (Table 2).

All analyses were conducted in R (version 4.3.) using linear mixed models program lme.

Treatment and time were used as fixed factors. Random factors included the four plants within replicate (rep|site) and replicates per watering line (~ 1 + line|rep). For daily yield of ripe and first-class tomatoes, replicated measurements produce a third level (time|plant). Post hoc analyses were conducted using pairwise contrasts between G and C in package emmeans. Analysis of variance of fixed effects was applied to leaf widths to study differences between C and G treatments and watering lines and their interactions due to the imbalance caused by broken leaves, which were omitted so that the analyse was based on 2–4 leaves per plant). Glyphosate analyses in the end of the experiment were tested using *t* test with degrees of freedom (df) and statistical significance (*p* value). Relationship between plant mean leaf width and tomato production variables was determined using Pearson correlations and *t* test for correlations coefficients.

Qualitative study

Based on prior knowledge and online search of fertilizer companies with sales in Finland of fertilizer permitted for use in organic production, we identified five companies to contact to gain perspective on how the industry views glyphosate contamination in organic fertilizers. We reviewed the company websites and contacted representatives of the five companies via email. We inquired about three themes:

1. Are glyphosate residues an issue in organic fertilizer production?
2. Are criteria for glyphosate residues in use?
3. Any views or experiences to share about pesticide residues in recycled fertilizers?

Interviews following initial contact aimed to elicit responses to these three themes and were tailored to the level of engagement—via email or telephone—from the respondent. Notes were taken during telephone calls, and respondents were contacted as needed for follow-up or clarification.

We also contacted five experts in Finland to gain perspectives from the poultry and organic farming associations and to understand the state of knowledge and any actions taken regarding pesticide residues in organic fertilizers. These experts represent: the Organic Growers Association, the Poultry Producers Association, Hankkija Oy (largest feed producer in Finland), the Centre for Economic Development, Transport and the Environment (SW Finland's Ely-Centre), and the national Finnish Food Authority. We also contacted Finnish Safety and Chemicals Agency (Tukes) for clarification on relevant legislation.

Table 2 Production from tomatoes grown with glyphosate-residue (G) and control (C) fertilizers was measured as harvest variables: the ripe tomatoes, the first-class tomatoes and the weight of the total yield

Measure	Description
Ripe tomatoes	Weight of all ripe tomatoes harvested during the experiment period (June 30 to July 18)
First-class	Weight of the first-class tomatoes harvested during the experiment period (June 30 to July 18)
Total yield	All ripe and unripe tomatoes (ripe tomatoes consisted of the first and second tomato clutches, ripe tomatoes from period June 30 to July 18, and any unripe tomatoes at the end of the experiment)

Fig. 2 Sample of tomato leaves. The photographed leaves are from four glyphosate (G) plants and four control (C) plants in two adjacent grow beds (bed 17 and bed 18, see Fig. 1). The parsley-like quality and lower biomass of the glyphosate-residue plants is clearly visible in the lower row

Results

Empirical study

Tests for glyphosate at the beginning of the experiment found 0.94 mg/kg glyphosate in the so-called glyphosate-contaminated (G) fertilizer and 0.23 mg/kg glyphosate in the control fertilizer (C). Thus, both fertilizers contained glyphosate, but the amount was 76% higher in the G fertilizer.

Compared to the G plants, C plants were more lush with wider leaves [G: 44.8 cm (confidence 43.2 cm–46.4 cm) and C 56.4 cm (confidence 54.7 cm–58.0 cm); $F = 99.5$, $p < 0.0001$, no effect of watering line $F = 2.1$, $p = 0.12$]. The lower leaf biomass of the G plants caused some leaves to look sparse and "parsley-like" (Fig. 2, lower row).

Plant height did not differ significantly between the C and G plants. Control plants did, however, produce more tomato clutches (mean: G 10.2 clutches vs. C 11.2 clutches; $t = 3.4$, $df = 54$, $p = 0.002$) (Fig. 3), and clutches were produced more densely on the plant (mean distance of clutches: C 25.2 cm vs. G 26.6 cm; $t = 2.0$, $df = 57$, $p = 0.049$).

Growth variables were correlated with each other. In addition, some growth variables, particularly the mean width of leaves (Table 3), length of leaves and clutch distance were clearly correlated to production variables.

Initially, ripe tomatoes developed at the same pace in both treatments and all watering lines. However, C plants

Fig. 3 Control plant (C) on the left has more tomato clutches than the glyphosate-residue plant (G) on the right

Table 3 Relationship of leaf width (cm/plant) to production variables (kg/plant)

Measure	Leaf width
All tomatoes, total	0.31**
Ripe tomatoes, total	0.22 ns
July 18 harvested ripe	0.21 °
July 18 harvested raw	0.64 ***

Results are presented with *t* test (df = 70) of correlation coefficients, with * indicating the strength of the correlation (**$p < 0.01$, ***$p < 0.0001$, ° $p = 0.09$, *ns* not significant)

Table 4 Mixed model analyses of production of total tomatoes in glyphosate and control treatments between June 30 and July 18. Statistical significance is indicated as *$p<0.05$, **$p<0.01$, ***$p<0.0001$

	NumDF	DenDF	*F* value	*p* value
Time	1	575	421.2	< 0.0001***
Treatment	2	70	33.3	< 0.0001***
Time*treatment	1	575	4.7	0.0293*

Table 5 Mixed model analyses of production of first-class tomatoes in glyphosate and control treatments between June 30 and July 18. Statistical significance is indicated as *$p<0.05$, **$p<0.01$, ***$p<0.0001$

	NumDF	DenDF	*F* value	*p* value
Time	1	575	145.0	< 0.0001***
Treatment	2	70	47.8	< 0.0001***
Time*treatment	1	575	10.5	0.0012**

began to overtake G plants in harvest variables of total production of tomatoes (Table 4) and production of first-class tomatoes (Table 5) during the period July 6–13 (weeks 27–28). In the middle of the experiment, on July 9th, C plants produced, on the average, 27% more total tomatoes compared to G plants [C plants: 0.62 kg/plant (confidence 0.52 kg–0.72 kg), G plants: 0.48 kg (confidence 0.38 kg–0.58 kg)]. Results for first class tomatoes are similar (Table 5). On July 9th control plants produced 45% more, i.e., 0.41 kg (confidence 0.33 kg–0.49 kg) and G plants 0.28 kg (confidence 0.20 kg–0.36 kg).

At the end of the experiment, the C plants had produced a total of 121.4 kg, while the G plants produced a total of 78.9 kg of tomatoes, which is a 35% difference. Difference in total production was particularly pronounced for the raw tomatoes. The C tomatoes increased production rate around the time when the production rate in the G tomatoes began to level off for both ripe (Fig. 4) and first-class fruits (Fig. 5).

The G plants underperformed by all measures of production compared to the C plants (Table 6). For the ripe tomatoes, C plants produced 49.2 kg and G plants produced 37.9 kg, which is a 23% decline in ripe tomatoes. For the first-class tomatoes, C plants produced 34.3 kg and the G plants 21.5 kg, a difference of 37%.

Mean glyphosate residues at the end of the experiment were 0.37 mg/kg (AMPA 0.99 mg/kg) for G grow beds and 0.23 mg/kg (AMPA 0.59 mg/kg) for C grow beds ($t = 1.9$, $df = 8$, $p = 0.10$). Correlation coefficient between glyphosate level of the bed at the end of the experiment and mean yield of the four plants in the bed was significant ($r = 0.61$) ($t = 2.2$, $df = 8$, $p = 0.06$). No glyphosate or AMPA residues were found in the ripe tomatoes from plants in either treatment.

Qualitative study

Industry representatives

The five fertilizer companies interviewed represent the majority of fertilizer sales in Finland. The representatives interviewed held researcher, operations director or other positions in which they were well placed to know about company policy and experience regarding quality control and potential contaminants. All the companies produce fertilizer marketed for use in organic farming in Finland. The company profiles varied (Table 7), as did their knowledge and experience of glyphosate or other pesticide contamination in side-stream raw materials or potential raw materials considered for upcycling as fertilizer. Companies A, B, and E were the most knowledgeable and forthcoming on experiences, procedures, and expressing views about glyphosate and other pesticide residues.

Representatives from the Finnish Organic Association and the Poultry Producers Association emphasized the importance of feed consistency in production and confirmed that feed is normally mixed in large batches on-farm using the farm's own grains and additional protein, such as soybean, oilseed, and fish by-products as well as oils and minerals. While the Association approves of the use of manures from conventional agriculture in organic farming and finds the term "suitable for organic farming" sufficient for labelling purposes, the Finnish Organic Association expressed specific interest in discussions about the legislative definition of 'industrial/ factory farming' and the effect the definition has on the quality of fertilizer products used in organic farming. Manure from factory farming is not allowed in certified organic production [20] and [21].

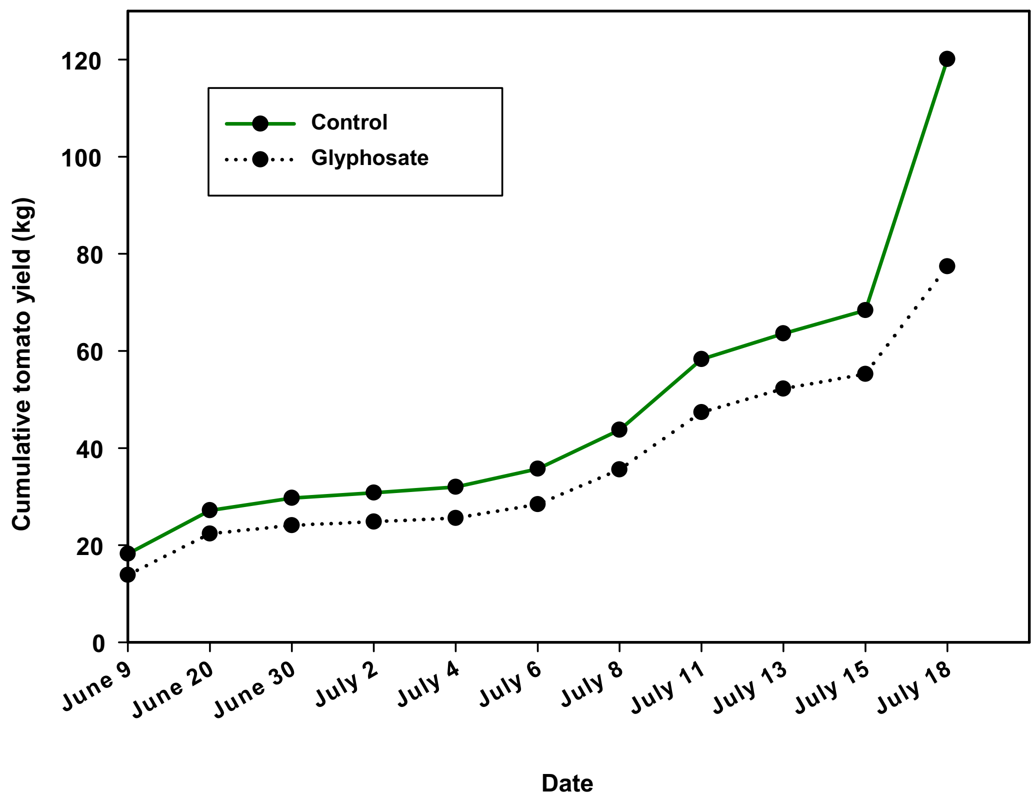

Fig. 4 Total tomato harvest ($n = 36$ plants per treatment) during the experiment period June 9–July 18. Figure is drawn from the raw data

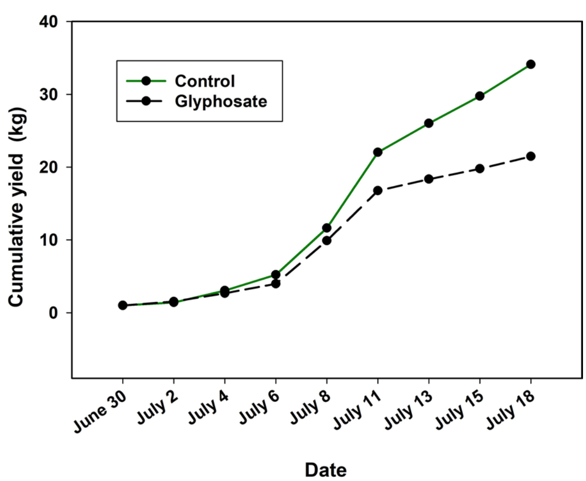

Fig. 5 Production of first-class tomatoes ($n = 36$ plants per treatment) during the experiment period June 9–July 18. Difference in production is statistically significant starting from July 13. Figure is drawn from the raw data

Glyphosate residues in fertilizer production

Two companies, A and B, stated that glyphosate contamination of poultry manure is an issue of concern or past concern and is explicitly addressed in their quality control. Company A identified domestic grains as a prior source of contamination, and Company B identified imported feed, specifically soybean, as the major source of contamination for poultry manure (Table 8). Company A traced contamination to feed grain on individual farms and described a case where they worked together within their network to educate the farmers about the problem and monitor manure from specific farms to eliminate contamination. To reduce glyphosate contamination, Company B's manure providers are under contract that manure comes from livestock fed GMO-free feed. Company C reported no knowledge of glyphosate residues in their poultry manure, but also indicated that their manure is sourced from GMO-free poultry and expected that this should limit potential contamination. Company D stated that they are unaware of glyphosate residues in the manure they source and emphasized that pre-harvest desiccation is not allowed in Finland. Company E indicated that they have not encountered glyphosate in the side-stream products they use, which do not include animal manure.

Companies A, B and E have internal testing and criteria to monitor for pesticide residues, including glyphosate (Table 8). According to its representative, Company C does not test for glyphosate or other

Table 6 Linear mixed models analyses of final harvest results (ripe tomatoes, first class tomatoes, total harvest) of the 14-week greenhouse experiment and green tomatoes in July 18th and weight of first two tomato clutches for the tomatoes with either glyphosate (G) or control (C) fertilizer. Statistical significance is indicated as *p <0.05, **p<0.01, ***p<0.0001

	G	C	Difference %	*F* value	*p* value
Ripe tomatoes	37.44	48.60	22.96	21.5	0.0001***
First-class tomatoes	19.84	23.69	16.26	250.4	< 0.0001***
Total harvest (excluding first two clutches)	56.88	94.32	39.69	61.0	< 0.0001***
Green tomatoes, July 18th	19.01	45.47	58.19	126.8	< 0.0001***
Weight of first two tomato clutches	22.07	26.60	17.05	245.0	< 0.0001***

Table 7 Overview of the companies contacted in this research

Stakeholder	Types of manures used
Company A	Domestic chicken manure
Company B	GMO-free domestic chicken manure
Company C	GMO-free domestic chicken manure
Company D	Variety of organic fertilizers from livestock manure, including cattle, pigs and poultry
Company E	No manure used. Organic-certified (private label) fertilizer from non-manure raw material

The list is not exhaustive of all raw materials used by the companies

pesticide contaminants. Company D reported only that they follow all applicable regulations if such exist. Representatives for A, B and E reported testing for pesticide residues, primarily using external laboratories, and one company informed that they also have a wide range of crop experiments, because 'not everything can be analyzed and the plants know more'.

Identifying risks, knowledge gaps, and residue limits

Concerns can be illustrated by questions and comments, especially from companies A, B and E. Two asked whether glyphosate breaks down during processing for biogas or through heat treatment used in sugar beet production, and one asked if the researchers know of or could suggest recommended limits for glyphosate in manure or fertilizers. One representative commented in depth:

> *'If some risk is known, it is better to respond to that risk than just ignore it. Glyphosate and pyralids should be measured the same way as other contaminants such as heavy metals. We need to know how quickly they break down in the conditions we*

Table 8 Summarized responses for thematic question 'Is glyphosate residue an issue in organic fertilizer production?'

Stakeholder	Glypho-sate residue an issue? (Yes/No)	Summary of responses
Company A	Yes	Glyphosate residue in raw material was first encountered in poultry manure about 10 years ago and was traced to on-farm feed. Residue in poultry manure was resolved through communication with poultry producers and monitoring. Glyphosate and AMPA are occasionally found in other side-stream products. In 2018, the company rejected domestic oilseed rape meal as unsuitable due to contamination levels of 16 mg/kg glyphosate and 0.33 mg/kg AMPA. Bakery waste has high levels of glyphosates. Other pesticides are also found, particularly pyralids and diuron
Company B	Yes	Aware of potential glyphosate contamination of poultry manure and have set a condition with poultry manure providers for a maximum permitted level of 0.2 mg/kg but are continuing to revise this limit. Strategy to limit glyphosate risk is to purchase manure from GMO-free poultry producers who do not use glyphosate-tolerant soybean feed
Company C	No	The company does not test for glyphosate residue in manure. According to the company, the producer supplying the poultry manure has an agreement with the feed provider for GMO-free feed, and the company thinks this should be sufficient
Company D	No	No knowledge of glyphosate residue in company's production. They are aware that KRAV (Swedish organic label) would like information about possible pesticide-residues for some pesticides
Company E	No	Does not use manure but does use plant waste. No glyphosate or AMPA residues were found from the side-stream products (e.g., sugar beet processing leftovers) tested (Galab 500PLUS). However, clopyralid is a challenge because it causes problems, e.g., for tomatoes, at the level allowed for feed and is not suitable for field use. Diuron has also been found

have here. In the same way as for sludge, we need to research and control these other side-streams.'

Four of the five companies have experience cooperating with research or governmental organizations on the safety of recycled fertilizers. One of these projects is *'Kiertokas: Towards a sustainable circular economy: Residue of plant protection agents in recycled fertilizers and growing media, and their management'* [33]. Kiertokas is the first project in Finland to focus exclusively on pesticide residues in organic fertilizers. The project is funded for the period 2023–2026 with Finnish Food Authority, Finnish Ministry of Agriculture and Forestry, and several fertilizer companies as partners. A key aim of the project is to establish upper thresholds for pesticides, including glyphosate, in organic fertilizers.

The need for common standards for glyphosate and other potential contaminants was described by the contacted stakeholders as having multiple benefits, including product quality, providing a legal foundation for the fertilizer companies if they are sued for crop damage attributed to pesticide residues in fertilizer, and in providing clear guidelines for the providers of raw materials. Some of the companies noted their legal obligation to provide a clean product and expressed concern about attempts to valorize side-stream products as fertilizer raw materials without understanding and examining potential risks:

'We want cleaner production, and independent research is needed to take it forward... Some do not understand the risks, and some just ignore them.'

Standards for pesticide residues in fertilizers were also considered important because feed, raw materials and fertilizers travel across borders. Based on their own internal testing, the companies identified some products of concern, including sugar beet, oilseed rape, side-stream materials from insect production, and bakery waste. Pesticide residues of concern included especially diuron and pyralids (specifically clopyralid). Glyphosate was considered as mainly under control through testing. Some of the companies noted that the allowable residue limits (MRL) for glyphosate and pyralids in crops resulted in pesticide residue levels that are problematic for sensitive crops including tomatoes and peas.

Interviews with two researchers in the Kiertokas project further elucidated the challenges in measuring and monitoring for pesticide residues and establishing MRL for fertilizers. The project is partially influenced by prior efforts to include chemical testing in legislation, as well as knowledge that Sweden and Norway already have some recommendations for pesticide residues in fertilizers. The researchers reported that key challenges of the project include what pesticides to test for and on which products. They indicated that although all raw materials should be tested, this is not possible due to resource limitations. Based on their own discussions with fertilizer producers, the Kiertokas researchers confirmed that the producers are particularly interested in the decomposition processes of the pesticide residues, including the possible effects of light, temperature, composting, and biogas production in accelerating decomposition. As a starting point, the Kiertokas project researchers indicated that the project intends to use, where available, the fertilizer producers' internal residue limits as reference values in testing. Project results will be available online as they become available [33].

Discussion

Negative impacts of glyphosate residues

Except for the glyphosate content, the two fertilizers were substantively similar. Hence, the difference in tomato production between the two treatments can reasonably be attributed to the glyphosate residue present in a commercial fertilizer marketed (at the time) as suitable in certified organic horticultural production including for tomatoes.

Tomatoes are known to be highly sensitive to many herbicides [39, 47], and tomato has been described as the "canary in the coalmine" of the vegetable world for herbicide drift, including glyphosate [39]. The difference in harvest between the glyphosate residue and control tomato was particularly pronounced toward the end of the experiment, which suggests that that the difference in tomato harvest would have been even greater if the study were continued longer.

Because both the C and G fertilizers contained some amount of glyphosate but the C plants performed better according to the production metrics, it is likely that glyphosate becomes significantly harmful to tomatoes at a level somewhere between these two measures.

The correlation between the growth variables measured early in the experiment and the production variables measured at the end suggest that plant production can be predicted based on growth behaviour at the beginning of the growing season.

How widespread is glyphosate residue in fertilizer?

Currently, no-one knows how widespread glyphosate residue is in fertilizers, and it probably varies globally. Our empirical study, however, shows that glyphosate residue in fertilizer at the level found on a batch on the market can be a risk for herbicide-sensitive crops like tomato, and even environmental certification is not necessarily a guarantee that the products are free from harmful levels of pesticide residue. In this case, the Ecocert certified manure contained more glyphosate than the Dutch CPE certified product. Quality certification programs should consider testing for pesticide residues in their fertilizers.

Interviews with the fertilizer producers revealed that glyphosate can enter the fertilizer production chain through contaminated raw materials such as poultry manure and that vigilance is needed to prevent such contamination. The potential for glyphosate contamination entering via feed depends on a variety of factors including how glyphosate is used and what types of monitoring are in place to minimize the risk of contaminated product reaching the market.

The finding by one company that herbicide residue in bakery waste is too high for fertilizer production illustrates that foodstuff MRLs alone do not assure that products can be safely recycled into the food system as fertilizer. Finland's major grain purchasers require farmers to guarantee their grain has not been desiccated with glyphosate or other herbicides, but bakery waste includes both imported and domestic grains.

The Finnish Safety and Chemical Agency (Tukes) has settled on a strict interpretation of the EU legislation on glyphosate [19] and forbidden pre-harvest application of glyphosate in grains destined for human consumption. However, it does allow pre-harvest application of glyphosate for control of couch grass (Elytrigia repens) in feed crops of barley and oat and in rapeseed under conditions of maximum 30% moisture content and with a 10-day holding period (Kaija Kallio-Mannila, Finnish Safety and Chemicals Agency Tukes, personal communication). It is likely that the legislative loophole that allows pre-harvest spraying for weed control in livestock feed crops is the source of the domestic glyphosate residue identified by Company A. This supposition is supported by the fact that the company was able to eliminate the glyphosate by raising awareness with the manure providers and conducting farm-level testing of the manure.

At first glance, Company B and C's strategy of avoiding genetically modified feed is a prudent choice in line with research that glyphosate residue in livestock and feed is primarily from genetically modified crops, such as soybean and pre-harvest dessication [7, 68, 76]. It does not, however, eliminate the risk from pre-harvest dessication or pre-harvest weed control. Furthermore, there appears to be a discrepancy about the presence of genetically modified organisms (GMO) in the feed, because most producers use at least some commercial compound feed, and the soy in the compound feed is GMO soy. As the interviewed experts considered GMO soy a risk for glyphosate residue, this potential misunderstanding on the part of the fertilizer producers could create a false sense that glyphosate residue is unlikely to be present in the manure.

Mechanisms of glyphosate breakdown is a knowledge gap

The companies' questions about the effect of biogas production and whether other heat treatment may possibly break down pesticide residue components indicates an extremely relevant knowledge gap for recycled fertilizers that could be addressed through cooperative, multi-actor research with processers and fertilizer companies. Prior efforts to understand glyphosate degradation systems have revealed both abiotic and biotic mechanisms [11]. The abiotic mechanisms, photolysis (radiation) and especially adsorption (for example, using biochar) are worth studying in more detail. Presently, microbial degradation pathways and the relevant enzymes are better known. Microbes have two main ways to break down glyphosate, i.e., consume glyphosate components [11, 69], where one degradation pathway produces AMPA—which is more stable and toxic for most organisms—and the other produces sarcosine that readily mineralizes to NH_3 and CO_2. The sarcosine pathway has been found in, e.g., in some *Pseudomonas* and *Agrobacterium* lines, but their use in field conditions has turned out to be difficult. Furthermore, their relevant enzymes are poorly active in field conditions in Finland characterized by cold climate and acidic soils.

Addressing pesticide contamination

Although the focus of the interviews with the companies was glyphosate, three of the companies brought up clopyralid and diuron residues as issues of even greater concern. The finding that companies are interested in collaboration with officials to establish maximum limits for pesticide residues in fertilizers is in line with the finding by consulting services organization Visia Cooperative consulting services [61], which states that fertilizer producers have a positive attitude toward efforts to update legislation governing organic fertilizers.

Overall, the results of this empirical study, the stakeholder interviews and the background to glyphosate presented in the introduction show that glyphosate contamination is a complex issue with far-reaching implications. This study supports the finding that although use of poultry manure as fertilizer is encouraged by EU policy, glyphosate residues originating in feed can inadvertently affect other agricultural production [37, 56]. It also supports the assertion by Ferrante et al. [31] that glyphosate can reach non-target destinations and that a One Health perspective accepting that human, animal, plant and microbial health are interwoven is essential to understanding the complete impacts of glyphosate.

Conclusion

Uptake of organic fertilizers is important for fostering nutrient cycling as part of circular economy, meeting organic production aims, and reducing dependency on mineral fertilizers. However, glyphosate residues and other contentious compounds can cause unforeseen production problems that may be difficult and costly to identify. For these reasons, it is particularly important to establish parameters for herbicide residues in fertilizers.

Persistence of glyphosate and other agrichemicals in the agricultural production chain are a challenge for just transitions toward sustainable agriculture. This research highlights that certified organic producers of herbicide sensitive crops like tomato are particularly vulnerable as long as fertilizers remain untested for pesticide residues. Contamination through a feed-feces-fertilizer chain has received insufficient attention in research and is mainly unregulated. Science-based limits on pesticide residues in organic fertilizers are urgently needed. Multi-actor networking that engages stakeholders throughout the production chain can be effective in identifying and addressing contaminants in fertilizer raw materials, but effective legislation that reduces the source of contamination, i.e., reduces pesticide loads, is also needed. A 'One Health' or a similar holistic approach is essential to understanding and effectively resolving this risk to organic producers specifically and agricultural circular economy broadly.

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Author contributions
All authors contributed substantially to the manuscript. M.H. and I.S. conceptualized the empirical study, which was carried out by M.H. and K.S. Statistical analysis was done by I.S. and visualization by M.H. and K.S. The manuscript was written by T.B., who also conducted the qualitative research. All authors participated in revision of the manuscript.

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Availability of data and materials
The data sets generated and/or analysed during the current study are available in the Dryad Digital Repository.

Declarations

Ethics approval and consent to participate
The research contains results of communications, either emails or telephone interviews, with stakeholders from fertilizer companies operating in Finland. Respondents were made aware when contacted that they are being contacted as a part of research conducted by University of Turku on the issue of glyphosate/ herbicide residues in fertilizer raw materials. Participation in the study, including level of engagement with the researchers, was voluntary. No personal information was collected beyond name and job title of the respondent. In accordance with EU GDPR and applicable national legislation, respondent privacy has been protected. Results of the fertilizer company inquiry are presented in an anonymized fashion with identifying details removed. Professional organizations and expert individuals who provided knowledge and expertise have been acknowledged in Acknowledgements. All attributed personal communications have been verified and approved for publication by the subject.

Competing interests
The empirical research of this project was paid for by Ikaalisten Luomu Oy, an organic horticultural producer. The producer approached the researchers about investigating the effect of glyphosate residue in fertilizers on tomato production after experiencing unexplained problems in production and an independent laboratory identified glyphosate residue in a batch of manure fertilizer used in the production. The horticulture producer provided the fertilizers and seedlings as well as advice on growth method. The research itself was conducted independently by the university researchers in the university's facilities. The authors declare that they do not have any conflicts of interest.

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