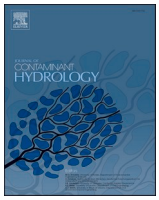
Journal of Contaminant Hydrology 247 (2022) 103979

Contents lists available at ScienceDirect

Journal of Contaminant Hydrology

journal homepage: www.elsevier.com/locate/jconhyd



Coupled steroid and phosphorus leaching from cattle slurry at lysimeter scale

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ARTICLE INFO

Keywords:
Steroids
ASE
Faecal pollution
Sub-surface pathways

ABSTRACT

Water quality degradation can be caused by excessive agricultural nutrient transfers from fertilised soils exposed to wet weather. Mitigation measures within the EU Nitrates Directive aim to reduce this pressure by including 'closed' fertiliser spreading periods during wet months. For organic fertilisers such as slurry and manure, this closed period requires sufficient on-farm winter storage and good weather conditions to relieve storage at the end of the period. Therefore, robust scientific evidence is needed to support the measure. Incidental nutrient transfers of recently applied organic fertilisers in wet weather can also be complicated by synchronous transfers from residual soil stores and tracing is required for risk assessments. The combination of nutrient monitoring and biomarker analyses may aid this and one such biomarker suite is faecal steroids. Accordingly, this study investigated the persistence of steroids and their association with phosphorus during leaching episodes. The focus was on the coupled behaviour of steroids and total phosphorus (TP) concentrations in sub-surface hydrological pathways. Cattle slurry was applied to monolith lysimeters either side of a closed period and concentrations of both steroids and TP were monitored in the leachate. The study showed no significant effect of the treatment (average $p = 0.17$), though tracer concentrations did significantly change over time (average $p = 0.001$). While the steroidal concentration ratio was validated for herbivorous faecal pollution in the leachate, there was a weak positive correlation between the steroids and TP. Further investigation at more natural scales (hillslope/catchment) is required to confirm tracer behaviours/correlations and to compliment this sub-surface pathway study.

1. Introduction

Nutrient management is essential in modern intensive agriculture and recycling of organic manures and slurries is an important way to maximise productivity (Zhen et al., 2014; Petersen et al., 2007; Haynes and Naidu, 1998). To this end, phosphorus (P) and nitrogen (N) nutrients in faecal matter applied to soils should be wholly utilised by the plant or crop (Edmeades, 2003). However, limitations to nutrient use efficiency (Dobermann, 2007), adverse weather conditions (Cassman et al., 2002) and sub-optimal management practices (Goulding et al., 2007) can result in excessive faecal matter associated nutrient loss into waters via surface runoff or sub-surface drain flow, especially when rainfall follows recent applications (Shore et al., 2016; Ramos et al., 2006). The combination of faecal matter and nutrients lost from agricultural land to water in organic fertilisers can result in microbial contamination and eutrophication (Foote et al., 2015; Chislock et al., 2013).

In the European Union (EU), the Nitrates Directive (OJEU, 1991) is the main policy instrument used to manage agricultural nutrients as a water pollution pressure (also in UK law following Brexit). In each state, regulations can include limits on organic (and inorganic) fertiliser application rates (Basso et al., 2016) and applications on sloping or frozen soils (Vadas et al., 2019; Velthof et al., 2014), restrictions to the timings of applications (Buckley, 2012), and specific yard management to ensure adequate slurry/manure storage capacity (Barnes et al., 2011). In several EU countries, both N and P are regulated within the ND due to

Abbreviations: TP, Total Phosphorus; P, Phosphorus; N, Nitrogen; EU, European Union; ND, Nitrate Directive; NVZ, Nitrate Vulnerable Zone; ASE, Accelerated Solvent Extraction; TIC, Total Ion Count; PCA, Principal Component Analysis; PC, Principal Component; K, Potassium.

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https://doi.org/10.1016/j.jconhyd.2022.103979
Received 2 August 2021; Received in revised form 14 December 2021; Accepted 17 February 2022
Available online 23 February 2022

the combined impacts of these nutrients on sensitive aquatic receptors (McDonald et al., 2019; Jordan et al., 2012).

Specific restrictions to the timing of fertiliser applications in the ND are designed to avoid 'incidental' nutrient losses during periods of heavy rainfall. These may be limited to Nitrate Vulnerable Zones (NVZs) in river catchments (Barnes et al., 2011; Lord et al., 2010; Osborn and Cook, 1997) or based on 'whole-territory' designations (Buckley et al., 2016; Velthof et al., 2014). Farmers operating under these regulations must cease fertiliser applications after a certain specified date and only resume after another specified date (Barnes et al., 2009). These 'closed period' dates vary according to climatic zone but are generally coincident with periods of high effective rainfall totals and the concomitant elevated risk of incidental pollution transfers (Fig. 1).

In agricultural livestock systems, where organic fertilisers are stored for subsequent land application, the closed period may present certain management problems related to storage capacity. For example, where slurry and manure accumulate in storage due to wetter spring and summer conditions, the pressure to spread immediately before the closed period starts increases or may run into the closed period (if derogated by government) (Shore et al., 2016). Furthermore, the manure and slurry produced from winter housed livestock may require immediate application to relieve storage capacity as soon as the closed period ends (Buckley, 2012; Barnes et al., 2009). These two scenarios may, over large catchment areas, present sudden water quality pressures and especially at times when soil moisture deficits are low and when rainfall becomes excess to soil storage and evapotranspiration.

Since residual soil nutrients are also vulnerable to runoff during periods of high effective rainfall, there is a requirement to understand how this potential pollution signal differs from incidental nutrient losses associated with organic fertiliser applications. This is necessary to enable policies such as NVZ 'closed periods' to be risk assessed or to apportion water quality pressures from multiple sources in complex catchments (Shore et al., 2016). For organic fertilisers, this should include both the faecal matter and nutrient pressure. For faecal matter, biomarker techniques have been employed, usually involving the unique properties of animal wastes, but including the use of natural steroids (Manley et al., 2020; Leeming et al., 1996). Work to isotopically label N from specific sources in upstream catchments or water bodies is well established (Collins et al., 2019; Gooddy et al., 2014; Heaton et al., 2012; Kendall, 1998) but isotope labels are more difficult to apply to P in these settings (Gooddy et al., 2016). As P is considered to be the most important limiting nutrient in many freshwater systems (Chislock et al., 2013), there is still a need to understand the coupling of faecal and P *sources*, their combined *mobilisation* from soils, and *delivery* to water bodies.

The use of steroids has the advantages of being able to target frequently detectable compounds in freshwater following faecal contamination (Fahrenfeld et al., 2016), the ability to distinguish between human and non-human sources (Shah et al., 2007; Leeming et al., 1996), and of being temporally and geographically applicable (Noblet et al., 2004; Ottoson and Stenström, 2003). However, with the definition of a biomarker being "an organic compound that maintains sufficient structural integrity between source to source to be recognised" (Leeming et al., 1996, pp. 2893), the propensity for steroids to undergo microbial based degradation can be a potential issue confounding their reliability. Therefore, it is important to investigate their persistence at different scales and especially in combination with faecal matter associated P. For example, Arnscheidt et al. (2007) used riverbed sediments as passive samplers for steroid accumulations downstream of animal (cattle/sheep) and human faecal sources. They compared these data with seasonal TP concentrations at the *delivery* end of the catchment water pollution continuum. There are fewer studies investigating steroids and P at the *mobilisation* end of the continuum (Reichwaldt et al., 2017; Nash and Halliwell, 2000), and particularly related to decay and/or dilution processes.

To address this knowledge gap, the primary aim of this study was to investigate the persistence and ratio changes to cattle slurry biomarkers (steroids) over time at plot scale in parallel to TP concentrations. A secondary aim was to investigate the presence of a steroid fingerprint in sampled soil leachates. The hypotheses were that: (i) following an application of cattle slurry, steroid and TP levels would spike and gradually decline due to being 'flushed' out by wet weather over time; (ii) that the ratios of the steroids would change as biohydrogenation would decrease the concentrations of primary steroids and increase the concentrations of secondary steroids, and; (iii) that it would be possible to identify the steroidal signals originating from herbivorous faecal matter.

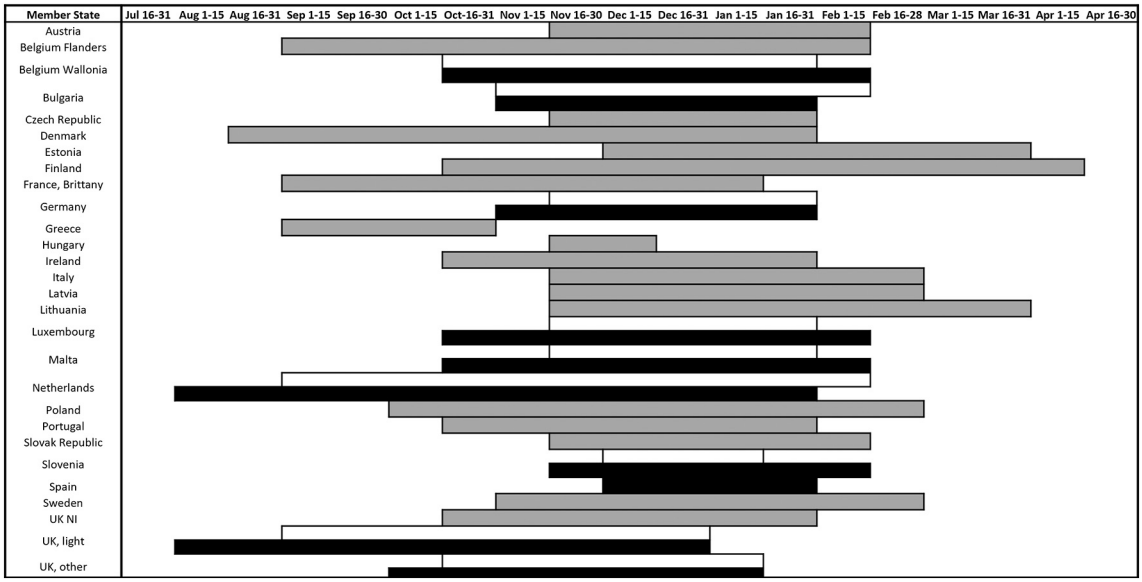

Fig. 1. A visualisation of the closed periods across the EU. White bars are grassland. Grey bars are a single closed period for both arable and grassland. Black bars are arable land. Adapted from Webb et al., 2011.

2. Methods

2.1. Treatments

To investigate steroids and P mobilisation after slurry application at plot scale, monolith lysimeters of intact blocks of soil (135 cm deep and 80 cm diameter) located at Rothamsted Research North Wyke, Devon, UK (Fig. 2) were used. The lysimeters were last used more than 2 years previously; this unpublished masters experiment involved the application of nitrogen and phosphate fertilisers (triple superphosphate and calcium phosphate). Prior to this, the lysimeters had not been used for over 10 years. The lysimeters were assumed to act as analogues for hillslopes where sub-surface hydrological pathways would leach water and water-borne pollutants to deeper pathways or adjacent channels. The soils in these monolith lysimeters were collected and deployed in 1982 following a methodology described by Belford (1979). Four soil series are represented and two were used in this study: a free-draining brown earth from the Frilsham series (World Reference Base – Luvisol) and a well-drained clay loam from the Radyr series (World Reference Base – Cambisol) (Table 1). Each was replicated four times (Fig. 3). The surface of each lysimeter is situated level with the ground, with a 10 mm layer of shingle below draining leachate into pipes, silicone tubing and 25 L vessels in a collection tunnel below the lysimeters (Fig. 3).

Cattle slurry was collected from an open-air slurry lagoon on a dairy cattle farm in SW England on a grazed-grassland system with winter housing of animals. The eight lysimeters used were mown and slurry applied at a rate of 33 m^3/ha in November 2019 and March 2020 approximately either side of a typical UK winter 'closed' period (Fig. 1) (Brennan et al., 2012) (although with a slightly late first application due to operational delays). As the lysimeter plots have a surface area of 0.5 m^2, approximately 1.66 L of slurry was applied manually. Samples of the slurry were also reserved from each lysimeter plot for analysis. To act as a control, one of the plots from each soil series was selected at random to receive no slurry treatment (Radyr-4 and Frilsham-8 plots shown in Fig. 3). The slurry applications were exposed to natural rainfall periods over a period of 6 months between the 29th of November 2019 and the 4th of March 2020. Following each storm event, leachate samples were collected from the 25 L vessels in the collection tunnel. This included 9 different sampling times (Table 2), with slurry being reapplied at the same application rate after the collection of samples at sampling time 6. Samples were returned to the laboratory and analysed for steroids and TP.

Table 1
Soil characteristics of the monolith lysimeters used in this study.

Soil texture	Clay Loam	Sandy Loam
Soil series	Radyr	Frilsham
% clay (0.002 mm)	16.7	17.5
% silt (0.002–0.02 mm)	19.6	9.8
% fine sand (0.02–0.2 mm)	45.3	36.7
% coarse sand (0.2–2 mm)	16.1	32.5
% total sand (fine + coarse)	61.4	69.2
Ratio clay:total sand	0.27	0.25
Bulk density (g/cm^3)	1.39	1.37
pH	6.5	7
Total C (%)	1.92	1.92

To summarise, the experiment had been arranged as a completely randomised design for the Treated/Control/Soil Series allocations, with Sampling Time being allocated as a sub-plot treatment where the lysimeters are the plots.

2.2. Steroidal analysis

Slurry samples (taken during applications) and lysimeter leachates were passed through pre-furnaced/pre-weighed Whatman GF/F (0.7 μm) filters; these filters were then dried overnight in an oven set at 30 °C. The samples were extracted using a Dionex ASE 350 (Thermo Fisher Scientific, Hemel Hempstead, Hertfordshire, UK) by accelerated solvent extraction (ASE). The full details of the ASE methodology are described in Manley et al. (2020). Samples were inserted into ASE cells with the internal standard (20 μL of 0.2 mg/mL 5β-pregan-3α-ol) and underwent extraction. This gave the total lipid extracts (TLE) for the samples. The TLEs were then rotary evaporated, resuspended in dichloromethane (DCM):acetone (1:1) and evaporated at 40 °C under a

Fig. 2. Maps showing the location of the monolith lysimeter plots (black circle) at Rothamsted Research North Wyke, Devon, UK (grey circle) in the South-West of England, and photographs of the lysimeter plots and collection tunnel (bottom right).

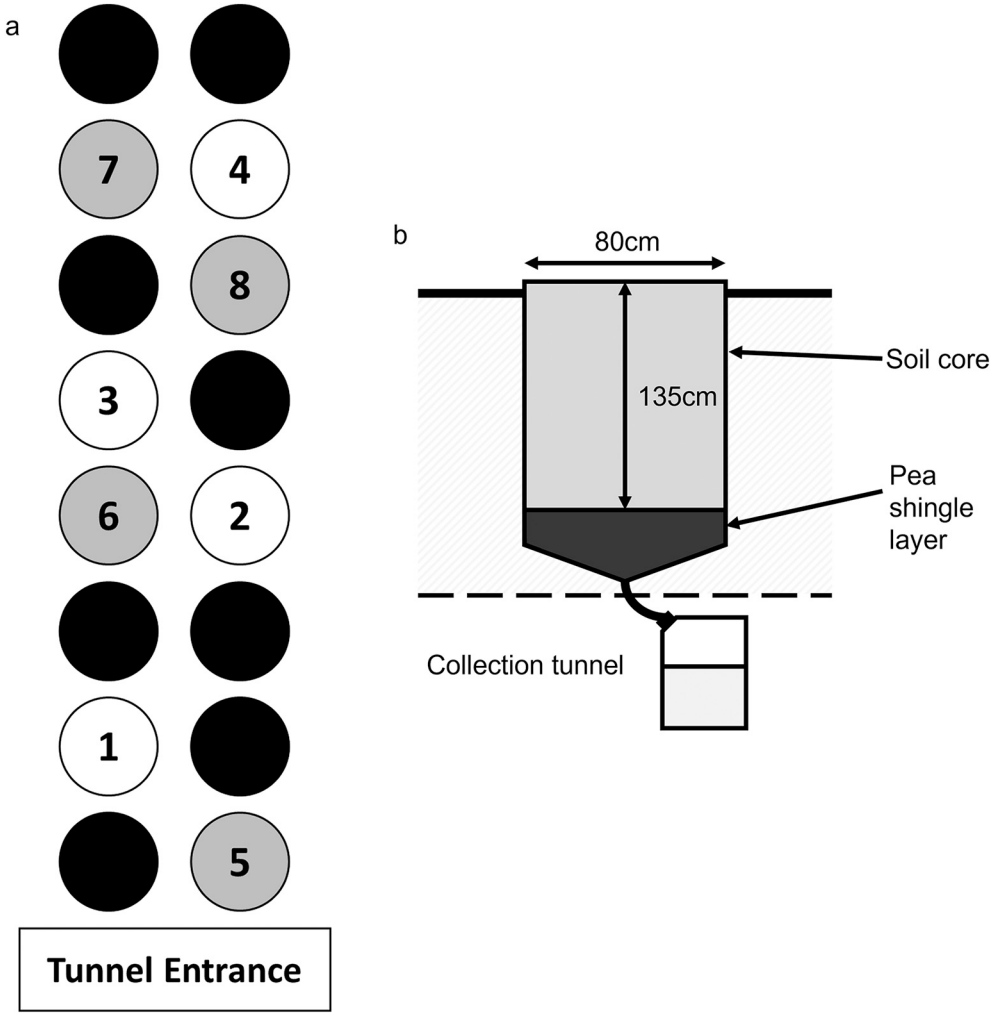

Fig. 3. A schematic showing the layout of the lysimeters. Fig. 3a shows the random placement of the two soil series used in this study. The white lysimeters are the Radyr soil series and the grey lysimeters are the Frilsham soil series. Black lysimeters were not used in this study. The numbering shown refers to the number that lysimeter had been assigned as a label. Fig. 3b shows the structure of each individual lysimeter. The lysimeters consist of 135 cm deep by 80 cm wide soil cores encased in a glass-fibre reinforced polymer casing with a pea shingle base layer. A single outlet pipe is connected at the base that allows the soils to drain naturally under gravity into the 25 L collection bottles in the underground collection tunnel.

Table 2
Overview of the dates that leachate samples were collected from the lysimeters.

Sampling Time	Collection Date
1	29/11/2019
2	12/12/2019
3	23/12/2019
4	10/01/2020
5	21/01/2020
6	03/02/2020
7	14/02/2020
8	21/02/2020
9	04/03/2020

gentle stream of N_2 (blown down).

The TLE samples were processed and analysed following the methodology of Manley et al. (2020), derived from Bull et al. (2003). The TLEs were saponified to break any ester bonds and free all alcohols using 5 M potassium hydroxide in 90% methanol. Saponified extracts were then acidified and extracted in chloroform. Following this, extracts were put through drying columns using chloroform. These columns were comprised of glass Pasteur pipettes, solvent cleaned cotton wool (to plug) and 5 cm of activated sodium sulphate. Extracts were then fractionated using fractionation columns (the same structure as drying columns except activated silica instead of sodium sulphate) using DCM (fraction 1) and DCM:Methanol at a ratio of 1:1 (fraction 2). The extract fraction isolating the steroids was then blown down. Finally, fractionated extracts were derivatised via silylation using (*N,O*-bis(trimethylsilyl)trifluoroacetamide + trimethylchlorosilane) and blown down in preparation for analysis via GC–MS.

The samples were analysed using an Agilent Technologies 6890GC/5973 N GC–MS with 7683 autosampler (Agilent Technologies, Santa Clara, California, USA). The Total Ion Count (TIC) data were acquired and analysed using Agilent Chemstation software. The biomarkers were identified using known characteristic spectra and comparisons with those in the National Institute of Standards spectral library (NIST, 2012 - accessed 2019). The TIC data were quantified against internal standards and had a detection limit of 0.3 μg/g.

2.3. TP analysis

Leachate samples were agitated, and sub-samples placed into 30 mL Greiner tubes. Pre-leached slurry samples were diluted down to 1:50 by pipetting 0.5 mL of sample and 24.5 mL of milliQ water into 30 mL Greiner bottles before analyses. An Aquakem 250 discrete photometric analyser (Thermo Fisher Scientific, Hemel Hempstead, Hertfordshire, UK) was used for P analysis (Murphy and Riley, 1962). The limit of detection is 3.16 μg PO_4_P/L and the working range is 10–1500 μg PO_4_P/L. Non molybdate-reactive forms of P (organic, condensed and colloidal) are converted to orthophosphate by oxidation with acidified potassium persulphate in an autoclave at 121 °C. Total phosphate is subsequently determined colourimetrically by reaction with ammonium molybdate in acid solution to form phosphomolybdic acid and reduction to phosphomolybdenum blue which has absorbance maxima at 660 nm

and 880 nm.

2.4. Data handling and statistical analysis

Data were analysed in Genstat 19 (VSN International, 2020). Any values of 0 were due to the concentrations being below the level of detection for steroids or TP. For ANOVA, data were compared using the lysimeter number as a block, and the application of slurry as the treatment (treatment*sampling time*soil). Due to the variability in concentrations amongst steroids and the numerous values of 0, the steroid data were increased by 0.01 and then transformed (logarithm to base 10). The application of the logarithm transformation is to satisfy the ANOVA assumption of homogeneity of variance, with the addition of the small constant (0.01) to enable this transformation to be applied to the zero (below the limit of detection) observations. The TP data were not increased or transformed due to having less variability.

Principal component analysis (PCA) was then performed to assess the variation in the data using the correlation matrix. The correlation matrix was used as the scales on which different variables have been measured are different. This PCA looks for the combination of variables that explains as much of the variability in the data as possible. Correlation scale PCA treats all variables equally and looks for this combination of variables to explain variability without any variable dominating. The significance level for all statistical tests was 0.05. A final correlation test was performed on all the data (all of which were logged including TP) to investigate associations.

3. Results

The slurry samples used in this study contained on average a dry matter content of 4.63%. Table 3 shows the average steroid and TP concentrations in the slurry spread on the lysimeters per application.

The ANOVAs (Table 4) showed that the Treatment yielded no significant effects on the concentrations of any of the steroid tracers. It was a similar case for the series of Soil used (except in one instance with coprostanol $p = 0.03$). Sampling Time was the only factor that had any significant effect. This factor was extremely significant ($p <$ 0.001–0.003) across all the steroid tracers. Consequently, the two factor interactions including Sampling Time were more significant than their single factor counterparts. Whilst only coprostanol had a significant result for the interaction between Treatment and Sampling Time ($p =$ 0.015), it should be noted that the p values were still generally lower than in Treatment alone. Almost all the tracers (except cholesterol $p =$ 0.092) had significant results for the interaction between Soil and Sampling Time ($p <$ 0.001–0.026). However, only three of the tracers had significant results by the interaction between all three of the factors: coprostanol ($p = 0.007$), and 5β-stigmastanol (p = 0.015), and campestanol ($p = 0.043$).

To help illustrate the significance of the passage of time on steroid tracer concentrations (Sampling Time), Fig. 4 shows the varying concentrations of all the tracers per lysimeter over the sampling period. There is a visible difference between the tracer concentrations of the Radyr soil series (Fig. 4: Lysimeter 1–4) and the Frilsham soil series (Fig. 4: Lysimeter 5–8). The Radyr soil series appeared to begin with elevated concentrations of steroid that then decreased and remained low until the reapplication of slurry at Sampling Time 7. Here, in lysimeters 1–3, there was a spike in steroidal concentrations, particularly of cattle slurry associated steroids (5β-stigmastanol and epi-5β-stigmastanol), that decreased sharply in consequent samplings. In the control for Radyr soil (lysimeter 4), this sharp increase was not seen (except in epi-coprostanol), though there were still general though smaller increases in steroids at this time point. The Frilsham soil series appeared to start at higher concentrations of steroid than the Radyr soil series. This then sharply decreased. In the Frilsham lysimeters, there was no clear pattern (other than small variations), though there was consistently always a small peak at sampling time 7. The graph showing the levels of TP across the lysimeters over time followed the same trend; a decrease that levelled out followed by a small increase following cattle slurry reapplication.

Fig. 5 shows the different effects of the other factors in this experiment (Soil Series and Treatment) as well as how they changed over time. All steroids showed the same general trend: control mean concentrations are almost always lower than the treated concentrations (except for cholesterol at sampling time 4) and the Radyr soil series has almost always higher concentrations than the Frilsham soil series (except at the first sampling time). The TP graph has no real trend between the control/treated and Radyr/Frilsham soils, but still shows the general trend of an initially high level that decreases over time.

Table 5 shows the differing contributions of each steroid in the PCA. All the values for the steroids are quite similar in principal component (PC) 1. However, the PCA loadings of cholesterol (0.18846), β-sitosterol (0.21911), campesterol (0.22894), and 5α-cholestanol (0.24061) (Table 5) are marginally lower than the rest. This suggests that the first PC is more of a mean of all the responses of the steroids as they are all so similarly weighted. The second PC has more variation in the responses of each steroid; some of the steroids have negative loadings (epi-5β-stigmastanol, stigmasterol, 5α-cholestanol, campesterol, β-sitosterol, and cholesterol). The second principal component is therefore capturing a difference between these groups of steroids. In Fig. 6, this can be seen graphically as those steroids with higher concentrations are on the positive axis and ones with lower concentrations are on the negative axis, resulting in two groups. Fig. 6 also reveals some general groupings; the Frilsham soil series is generally on the right of the plot, as are the control lysimeter points.

The correlation plot (Fig. 7) shows the level of correlation between the variables. All the steroids have a very high positive correlation, with values ranging from 0.48 (8/ 9: epi-coprostanol/ cholesterol) to 1.00 (13/ 14: 24-ethyl-coprostenol/ 24-ethyl-epi-coprostenol). Cholesterol is generally the steroid with the smallest correlation amongst the steroids. In terms of correlations between TP and the steroids, there is a weak positive correlation between 0.14 and 0.26 and one steroid (9: cholesterol) with a negative correlation (−0.18) with TP.

Table 3
The tracer profile of the slurry used for both applications. Steroids are measured in μg/g and TP in μg/L.

Application	1	2
Coprostanol	223.79	83.06
Epi-coprostanol	59.84	20.51
Cholesterol	59.04	18.47
5α-cholestanol	39.28	14.78
5β-campestanol	192.77	80.70
Epi-5β-campestanol	142.91	64.21
24-ethyl-coprostenol	61.24	16.13
Epi-24-ethyl-coprostenol	72.83	21.01
5β-stigmastanol	646.84	288.63
Epi-5β-stigmastanol	677.81	322.20
Campesterol	18.40	26.25
Campestanol	138.30	83.34
Stigmasterol	19.18	5.39
β-sitosterol	194.77	62.97
Stigmastanol	595.91	266.73
TP	6597.69	6695.73

4. Discussion

4.1. The effect of the treatment

An application of cattle slurry at a rate of 33 m^3/ha was the treatment used in this experiment. Agricultural cattle slurry can contain high levels of nutrients such as P and N (Li et al., 2019; McGechan, 2002); slurry is also a key source of the steroids considered faecal indicators such as 5β-stigmastanol and epi-5β-stigmastanol (Prost et al., 2017;

Table 4
The results of the ANOVAs for each of the steroids. All significant values have been highlighted in italics ($p \leq 0.05$). Columns with two factors indicate any significance of interaction effects between those factors.

Steroid tracer	Treatment	Soil	Treatment. Soil	Sampling Time	Treatment. Sampling Time	Soil. Sampling Time	Treatment. Soil. Sampling Time
Coprostanol (μg/g)	0.082	*0.030*	0.139	*<0.001*	*0.015*	*<0.001*	*0.007*
Epi-coprostanol (μg/g)	0.256	0.093	0.179	*<0.001*	0.071	*<0.001*	0.153
Cholesterol (μg/g)	0.064	0.103	0.903	*<0.001*	0.195	0.092	0.733
5α-cholestanol (μg/g)	0.243	0.166	0.722	*<0.001*	0.190	*0.003*	0.230
5β-campestanol (μg/g)	0.091	*0.036*	0.203	*<0.001*	0.044	*<0.001*	0.200
Epi-5β-campestanol (μg/g)	0.187	0.074	0.330	*<0.001*	0.167	*<0.001*	0.073
24-ethyl-coprostenol (μg/g)	0.175	0.087	0.324	*0.001*	0.320	*0.002*	0.098
Epi-24-ethyl-coprostenol (μg/g)	0.161	0.077	0.265	*<0.001*	0.118	*<0.001*	0.051
5β-stigmastanol (μg/g)	0.132	0.098	0.307	*<0.001*	0.097	*<0.001*	*0.015*
Epi-5β-stigmastanol (μg/g)	0.222	0.293	0.646	*<0.001*	0.502	*0.002*	0.320
Campesterol (μg/g)	0.208	0.129	0.549	*<0.001*	0.781	*0.026*	0.503
Campestanol (μg/g)	0.153	0.072	0.254	*<0.001*	0.096	*<0.001*	*0.043*
Stigmasterol (μg/g)	0.230	0.209	0.646	*<0.001*	0.325	*0.007*	0.347
β-sitosterol (μg/g)	0.153	0.126	0.924	*<0.001*	0.463	*<0.001*	0.302
Stigmastanol (μg/g)	0.195	0.136	0.256	*0.003*	0.278	*0.002*	0.387
TP (μg/L)	0.798	0.392	0.926	*0.002*	1.000	*0.015*	1.000

Leeming et al., 1996). The slurry applied to the lysimeters in this experiment did indeed have these high levels of both steroids and TP (Table 4). There was however a lowering of these levels between applications that can be attributed to being stored over the study period. As such, it was unexpected to find there was no significant effect of the Treatment on any of the lysimeters in this experiment, with the ANOVA results (Table 4) ranging from 0.082 (coprostanol) to 0.798 (TP). However, it can still be seen in Fig. 5 that there is a general trend of the treated lysimeters having higher concentrations of steroid than that of the control lysimeters, just not significantly so.

Regarding TP, it would have been more expected that the Treatment would have caused a significant difference between the treated and control lysimeters, as slurry is used as a fertiliser to add P and N to soils. However, as stated, there was no significant difference between the treated and the control lysimeter regarding P in this experiment. Typically, for P to move vertically, slower and more prolonged hydrological responses are required; this allows leachate production that will penetrate deeper and may contain the P from the application of slurry. However, the autumn/winter of 2019–2020 was very wet and had many flashy rain events; for the period of sampling (November to March), the average rainfall per year was 560.7 mm for 2016/17, 1296.5 mm for 2017/18, 1156.3 mm for 2018/19, and 1787.0 mm for the sampled year (2019/20). This could explain the lack of a P signal, as the greater penetration would result in greater dilution.

For steroids, it is not as surprising that there was no effect of Treatment, though this could have been similarly affected by the heavy rainfall like P. In this study, the leachate from each of the lysimeters was collected, filtered, and analysed. The leachate was quite clear and devoid of any sediment particulates, with samples containing less than 0.01 g/L, thus requiring many litres to be collected (typically 10–18 L). This meant there was very little solid matter on the filter papers to be analysed. It is generally accepted that steroids are predominantly found in solid matrices as opposed to liquid due to the hydrophobic nature of steroids as non-polar molecules (Matić Bujagić et al., 2016; Writer et al., 1995; Brown and Wade, 1984). This coincides with the knowledge that >95% of steroids are found in the suspended sediment fraction of water (Isobe et al., 2002) as well as attached to colloidal material (Nash and Halliwell, 2000). As such, with so little solid matter being on the filter paper, and the filter papers pore size (0.7 μm) being too large to retain any colloids, it is perhaps unsurprising that a significant level of steroids had not leached from the surface through the soil and into the sampled leachate.

4.2. The effect of soil series differences

Two different soil series were used in this study: Radyr (clay loam) and Frilsham (sandy loam) (Table 1). In general, there was no significant effect caused by using the different soil series, though there were two tracers, coprostanol and 5β-campestanol (Table 4), that were significantly affected with respective p values of 0.03 and 0.036 (Table 4). However, whilst there was statistically no difference between the two soils, it can be seen in Fig. 4 that there is a visible difference in the tracer concentrations between the two-soil series, as well as amongst the Frilsham soil series where there were two distinct patterns. The differences between these soil series are further illustrated in Fig. 5 where a trend shows that the Radyr lysimeters generally have much higher steroidal concentrations than Frilsham. Whilst soil carbon content has been reported to be a factor to consider regarding steroid transport (Qi and Zhang, 2016), there is no real difference in the carbon content of the two soils used in this study (Table 1) so this can be eliminated as a factor. Another factor that is known to have an impact on the sorption of steroids to soil is particle size (Sangster et al., 2015; Qi et al., 2014; Isobe et al., 2002). Typically, sorption capacity for steroid hormones is ranked clay>silt>sand (Qi et al., 2014). As the Frilsham soil has a much higher content of coarse sands compared with clays than Radyr, it would be expected that there would be less steroid sorption (Table 1). However, this is not the case in this study where there are higher concentrations of faecal steroids within the leachate from Radyr lysimeters (Fig. 4 and Fig. 5). This is unexpected as the literature (Sangster et al., 2015; Qi et al., 2014) indicates it should be the opposite. However, the cited studies investigated steroid hormones and faecal steroids, and it has been shown that not all steroids follow this trend (Isobe et al., 2002). Further investigation would be required to fully understand sorption processes in our study soils.

Regarding TP however, after the initially high values at sampling 1 (Fig. 4) there is little fluctuation. This could indicate that an equilibrium was being maintained within the soil water due to desorption processes between the leachate water and the soil (Turner and Haygarth, 2000). These differences could be linked to the chalky nature of the soil (with an average pH of 7), as calcium fixes P by precipitation (Turner and Haygarth, 2000). Furthermore, the Frilsham soil series is known to have relatively impeded drainage due this lower layer of chalky rubble. In contrast, the Radyr soil series is freely draining.

4.3. The effect of time

The experiment spanned a period of 6 months from November 2019 to March 2020. It can be observed from Table 4 that Time was indeed the

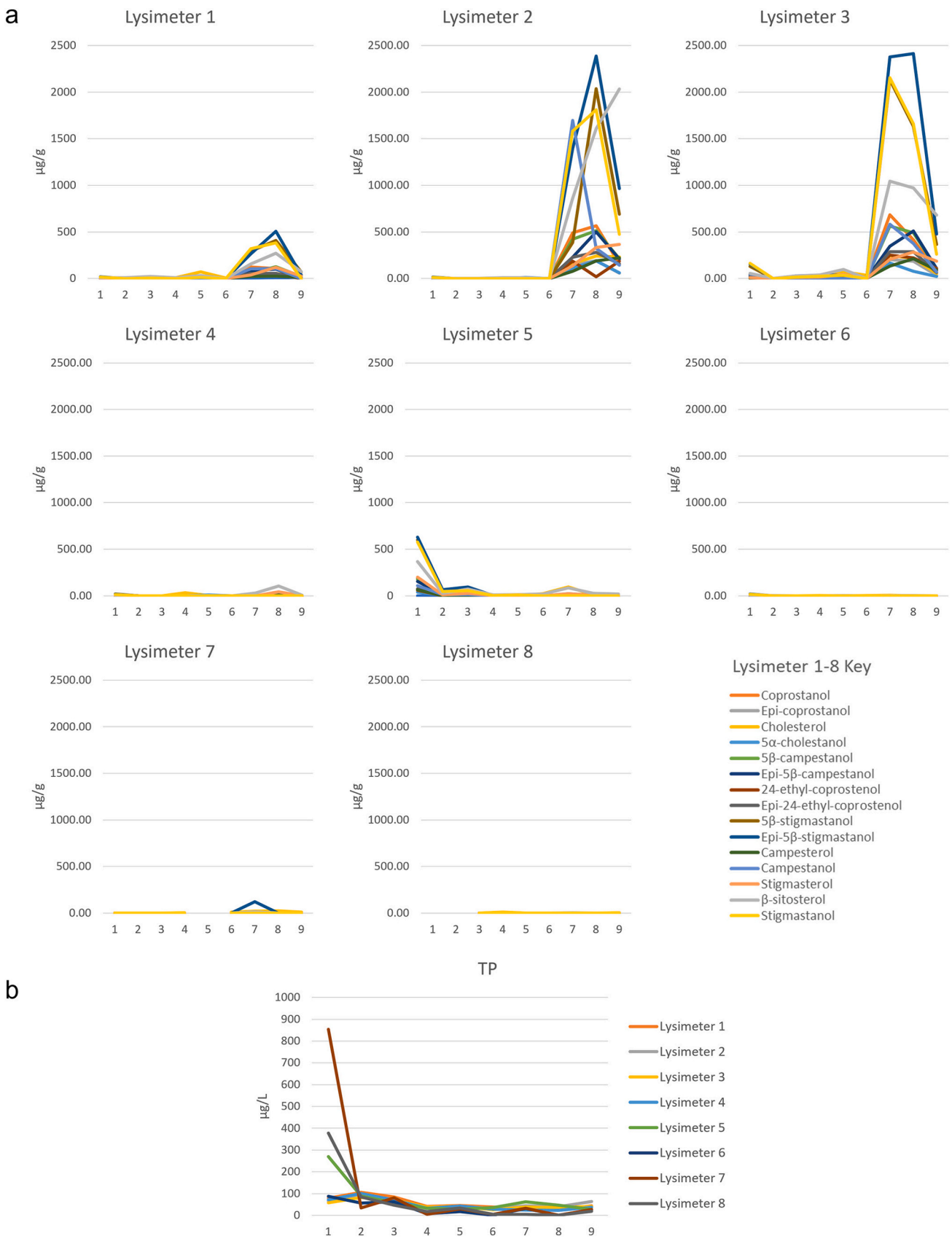

Fig. 4. Graphs showing the changing concentrations of the steroid/TP tracers over the sampling period. The top graphs (a) show the change in steroid levels over time. Lysimeters 1–8 indicate steroidal concentrations (µg/g). Lysimeters 1–4 show the results for the Radyr soil series and lysimeters 5–8 Frilsham. Lysimeters 4 and 8 are the respective controls for their soil series. The bottom graph (b) shows the change in TP levels over time (µg/L). Slurry was reapplied after Sampling Time 6. (For interpretation of the references to colour in this fig. key, the reader is referred to the web version of this article.)

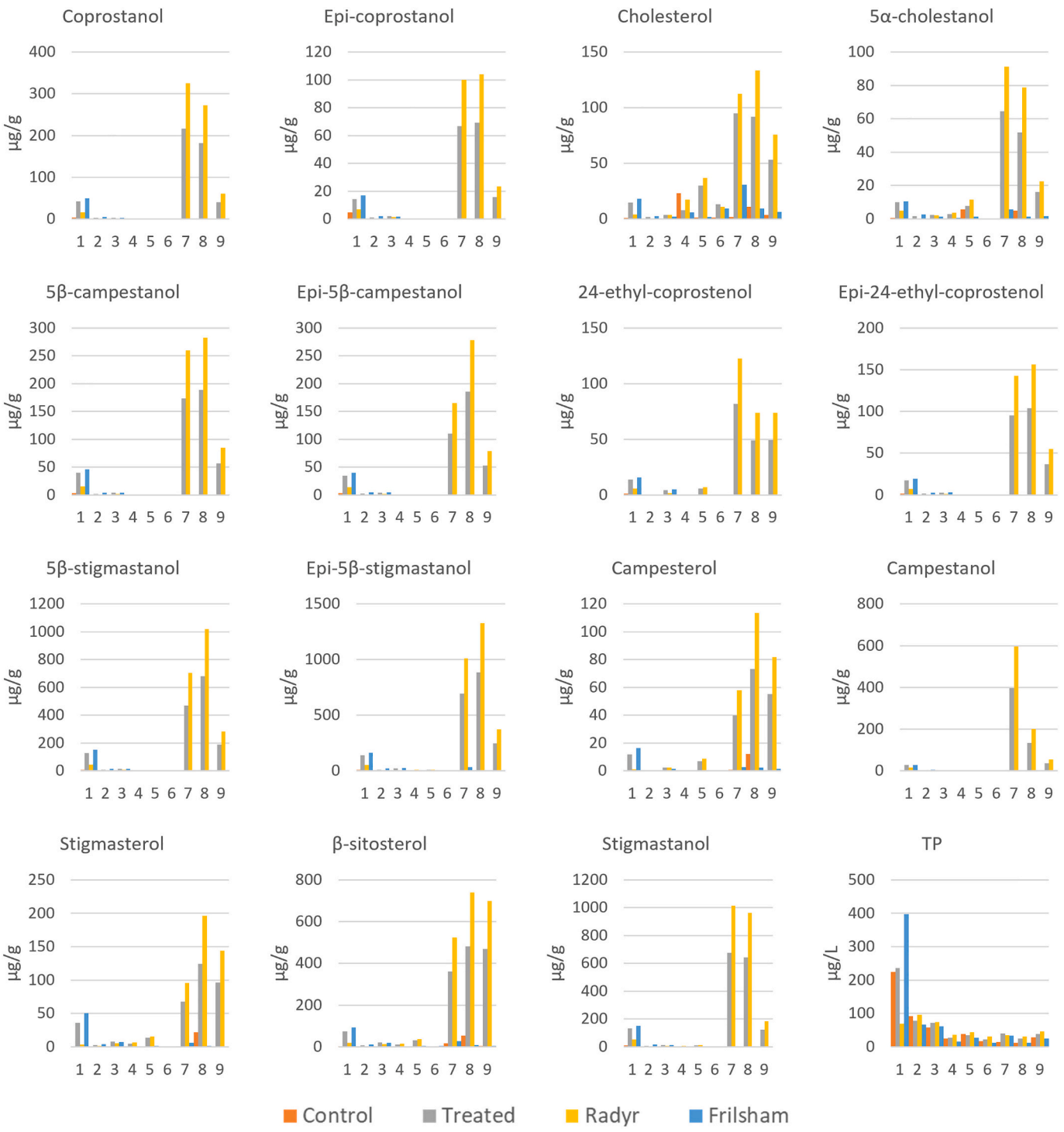

Fig. 5. Bar charts demonstrating the effects across replicate means for the steroid biomarkers and TP, illustrating the general trends. Sampling Times are on the horizontal axis with tracer concentrations (units specified on the individual graphs) on the vertical axis. The graphs show comparisons between control replicates (orange) vs treated replicates (Grey), and the Radyr replicates (yellow) vs the Frilsham replicates (blue). (For interpretation of the references to colour in this fig. legend, the reader is referred to the web version of this article.)

only factor that had any significance for all the steroid tracers in this study with ANOVA p values ranging from <0.001–0.003. However, multiple factor interactions including Sampling Time did have significant results also. This shows that whilst the differences between the Treatment application and Soil Series were not significant themselves, the significant interactions involving Sampling Time and the other factors suggests changes between the levels of the interacting factor. The significant interactions between Sampling Time and Treatment indicates that there are differences between Treated and Control plots over time; this is shown in Fig. 5 where for all steroids at all times (barring cholesterol at sampling time 4) the lysimeters treated with slurry had mean steroidal concentrations higher than that of the control lysimeters. The significant interactions between Sampling Time and Soil Series indicates that the differences between the two soils changes over time; this, again, is illustrated in Fig. 5 where the Radyr soil series is consistently always has higher mean tracer concentrations than the Frilsham

Table 5
PCA loadings (contributions) of each response variable to PC1 (79.98%) and PC2 (8.42%). The first 2 principal components explained 88.40% of the variance.

Steroid tracer	PC 1	PC 2
Coprostanol	0.27703	0.16992
Epi-24-ethyl-coprostenol	0.27607	0.2306
5β-stigmastanol	0.27589	0.07728
Campestanol	0.27536	0.23577
Epi-5β-campestanol	0.27502	0.23505
5β-campestanol	0.27468	0.18649
Epi-coprostanol	0.26924	0.26966
24-ethyl-coprostenol	0.26875	0.12309
Stigmastanol	0.2674	0.06576
Epi-5β-stigmastanol	0.26504	−0.14094
Stigmasterol	0.25242	−0.32072
5α-cholestanol	0.24061	−0.35399
Campesterol	0.22894	−0.27952
β-sitosterol	0.21911	−0.38576
Cholesterol	0.18846	−0.44631

soil series (except at sampling time 1). The significant three factor interactions between all factors indicates that the consistency of the effects of Treated vs Control in the different soils changes over time.

These interactions between factors can also be seen in Fig. 4, where the tracer concentrations in the 8 lysimeters have been separated. Over time in lysimeters 1–4 (Radyr soil series) (Fig. 4), steroidal concentrations were generally higher at sampling time 1 and then slowly declined till sampling time 6. At this point, slurry was reapplied. There was then a spike in steroidal concentrations, particularly in 24-ethyl-coprostanol, epi-24-ethyl-coprostanol, cholesterol, and epi-coprostanol. These steroids are commonly associated with cattle slurry (Leeming et al., 1996), so this spike was unsurprising. In lysimeter 4 (Radyr control lysimeter) (Fig. 4), despite not receiving treatment, the lysimeter showed a similar trend, though on a much smaller scale as the highest steroidal concentration was approximately 120 μg/g compared to 600/3000 μg/g observed in the other lysimeters. This small spike was also not from the expected cattle slurry indicator steroids mentioned previously, but epi-coprostanol.

The second soil (Frilsham) showed no obvious trends (Fig. 4). Lysimeters 5 and 6 began with a higher steroid concentration than lysimeters 7 and 8, though lysimeter 6 had a much lower general steroid

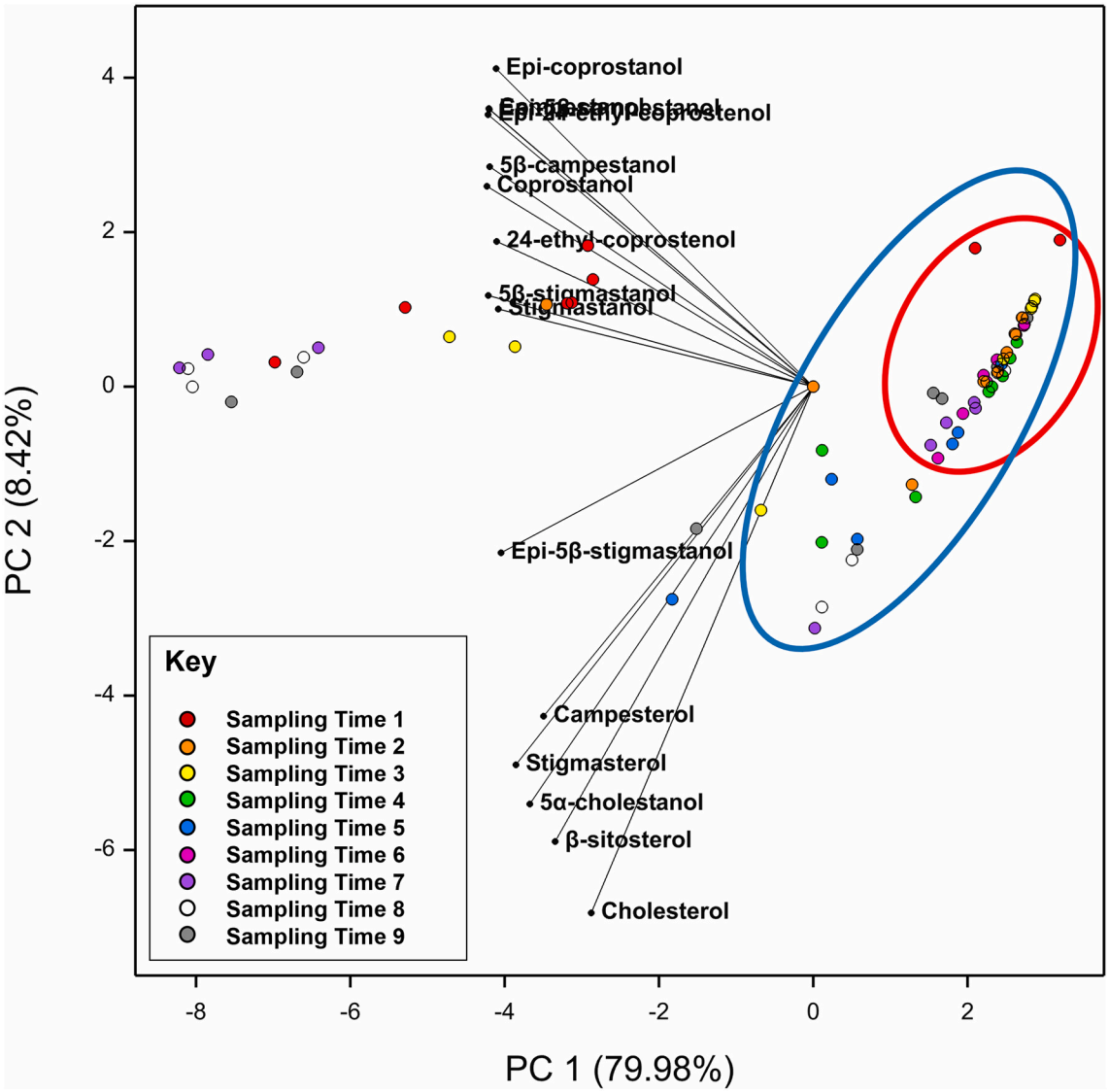

Fig. 6. A PCA biplot of the steroid tracers. Sampling Times are represented by coloured dots shown in the key. The group circled in red contains all but 2 (out of 18) of the control lysimeter points. The group circled in blue contains all but 4 (out of 36) of the Frilsham soil series points. (For interpretation of the references to colour in this fig. legend, the reader is referred to the web version of this article.)

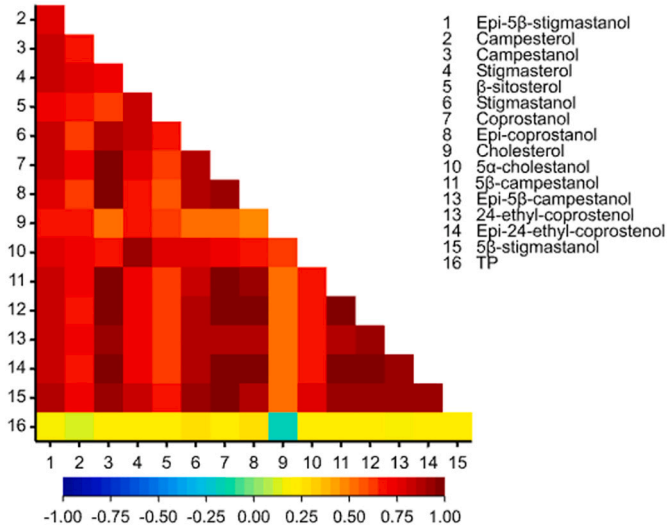

Fig. 7. Plot showing the level of correlation between all 16 variables in the study (15 steroids and TP). The scale ranges from −1 (dark blue) to 1 (dark red). (For interpretation of the references to colour in this fig. legend, the reader is referred to the web version of this article.)

concentration (approximately 30 times). This could be a consequence of innate differences amongst lysimeters of the same soil series. These lysimeters, like the lysimeters representing the Radyr soil series, had a small spike of steroid concentrations at sampling time 7.

Steroids are known to adhere to solid matrices due to their hydrophobic nature (Matić Bujagić et al., 2016), which can produce a lag in vertical transport. However, this was not the case in this study. There have been previous investigations on these lysimeters that have indicated such rapid fluxes of slurry related tracers. The lysimeters used in this study have been used previously to monitor the behaviour of nutrients from fertilisers in subsurface pathways (Alfaro et al., 2006; Turner and Haygarth, 2000). Turner and Haygarth (2000) applied fertiliser to these lysimeters between September 1993 – June 1994, and then again between October 1994 – May 1995; leachate samples were taken and measured for different forms of P. In this study, a larger than expected P concentration (in the >0.45 μm fraction) was detected in the leachate which indicated the importance of preferential flow through the soil profiles in the lysimeters. Similarly, Alfaro et al. (2006) used the lysimeters between June 2000 – June 2001 which involved applying cattle slurry and measuring potassium (K) in both leachate and the soil. Again, preferential flow and macropores were indicated as having effects within the lysimeters that caused high K) concentrations in the leachate. Whilst some work has reported that steroids move vertically quite slowly through soil profiles, the presence of preferential flow pathways and the high rainfall during the sampling period clearly resulted in rapid movement – hence a clear steroid fingerprint was detected in the leachate samples despite the relatively shorter sampling period.

As previously highlighted, the steroids themselves cannot be used individually as indicators of cattle waste pollution. Rather, they were used in ratios to do this. This includes the relative ratios of epi-coprostanol to 5β-stigmastanol [ratio 1] (Leeming et al., 1996) and coprostanol to coprostanol +5β-stigmastanol [ratio 2] (Harrault et al., 2019; Leeming et al., 1997). At approximately ratio 1, values ~2.8 indicate human faeces, whereas 0–1.2 indicates animals (Leeming et al., 1997). For Ratio 2, values <0.38 indicate herbivore faeces, and values >0.73 indicate human faeces. In this study, it was hypothesised that these ratios would be evident at both application times (1 and 7), with the ratios decreasing slightly after applications. None of the lysimeters had sufficient concentrations of steroid to use the ratios at all sampling times. Lysimeters 6–8 did not have sufficient steroid concentrations to use these ratios at all, except lysimeter 6 at sampling time one, where ratio 1 was 0.18 and ratio 2 was 0.24. Both of these values are indicative of herbivorous faecal contamination. However, within lysimeters 1–5, it was possible to use these ratios more; usually at the first 3 sampling times and the last 3 sampling times. Lysimeter 5 was the only lysimeter that had an initial ratio 1 that was <0.18 (0.10). This lysimeter then increased slightly to 0.14 at sampling time 2, and then dropped down to 0.11 at sampling time 3. This small increase could be due to a time lag. Lysimeters 1–4 had initial ratio 1 values that were higher than the value indicating herbivorous faecal pollution, but too low to indicate human faecal pollution (~2.8). For ratio 2, lysimeters 1, 2, 3, and 5 were all also indicative of herbivorous faeces (>0.38), whilst lysimeters 4, 6, 7, and 8 did not have the steroid profile to use the ratio. These differences in ratios could be because of biohydrogenation causing certain steroids to change structure into a different steroid (Leeming et al., 1996).

4.4. Limitations and next stages

This study used lysimeters as analogues to study steroid and TP transfer processes in sub-surface hydrological pathways (leaching) at the hillslope scale. The experimental set up is a useful link between laboratory scale and catchment scale experiments (Wang et al., 2012), but does not account for more surface driven, high energy hydrological pathways. The lysimeters' boundaries and consequent disruption to soil drainage (Pütz et al., 2018), and the restrictive dimensions of the lysimeters can affect the hydraulic gradient and natural flow field of the soil (Pütz et al., 2018; Corwin, 2000). Thus, even with minimal physical disturbance to the soil taken for the monolith, there would still be differences compared to natural hillslope conditions. This could result in differences in transit time for leaching (and the steroids contained) (Kim et al., 2016). As such, these differences could cause different steroidal signals to occur at larger scales. Moreover, the data here indicate that particulate matter vectors for steroids (and TP) were not high in magnitude or consistent and that any pathways may have been impacted by other soil physical properties (swelling/shrinking) or acted as chemical barriers (P adsorption). In addition to this, there was an assumption that the flux was the same for all lysimeters due to the combination of a lack of particulate matter and large leachate volumes (full 25 L bottles upon collection). Here it is noteworthy that previous work using these lysimeters also indicated flows were very similar for these lysimeters (Alfaro et al., 2006; Turner and Haygarth, 2000). In summary, the study provides a benchmark for steroid/nutrient dynamics from cattle slurry in sub-surface leaching pathways as being a low magnitude and inconsistent transport process – and likely impacted by flushing/dilution. Similar experiments (and experimental design) in surface hydrological pathways would be the next important stage.

5. Conclusion

This study used monolith lysimeters that had cattle slurry applied both at the beginning and end of a slurry spreading closed period. The leachate from this was then analysed using ASE and the Aquakem to monitor faecal steroids and TP respectively. During the length of this experiment there were two small steroid concentration spikes that occurred following slurry applications that then decreased over time. This was particularly true for 5β-stigmastanol and epi-5β-stigmastanol (typical ruminant faecal indicator steroids). From this, it could be seen that whilst the treatment of cattle slurry itself had no significant effect ($p \geq 0.05$), sampling time did ($p \leq 0.05$). This could have been due in part to the treatment potentially being understated by the particularly wet year (causing heavy dilution), or perhaps due to the near negligible amount of sediment found in the sampled leachate since steroids typically adhere to particulate matter. Regardless, the study showed that (indeed), tracer concentrations do spike following slurry application and decrease over time (hypothesis (i)).

Regarding the actual concentrations of the steroids, these too

changed over time. The ratios used in this study work by making comparisons between primary and secondary steroids. In other words, comparing precursor (primary) steroids to their resultant (secondary) steroids that form following biohydrogenation by microbes. However, this is not always possible due to either insufficient or non-existent concentrations of steroids (potentially due to the aforementioned issues). In the cases within this study where steroid profiles had enough data to use these ratios (such as in lysimeter 5), it was possible to identify correctly the steroids as having come from ruminant faecal contamination. Thus, the study showed that there were differences in primary/secondary steroid concentrations over the course of the experiment, and that it was also possible to use these concentrations to identify the origin of the contamination (hypotheses (ii) and (iii)).

The next logical step to extend this study would be to take it to hillslope plot scale, completing experimental work examining the persistence of steroid tracers in both surface and subsurface pathways, or even further to catchment scale. This would (tackle the limitations addressed here and) further confirm the utility of the approach for disentangling incidental and residual nutrient sources.

CRediT authorship contribution statement

Amber Manley: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Project administration. **Adrian L. Collins:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. **Adrian Joynes:** Methodology, Formal analysis, Writing – review & editing. **Per-Erik Mellander:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Phil Jordan:** Conceptualization, Methodology, Writing – review & editing, Supervision, 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.

Acknowledgements

We acknowledge the Walsh Fellowship (Ref: 2016115) provided by Teagasc to Ulster University, the Teagasc Agricultural Catchments Programme (funded by the Irish Department of Agriculture, Food and the Marine) and Rothamsted Research for part funding this research. We thank the farmer for providing slurry, as well as Andrew Mead, Robert Dunn, and Neil Donovan at Rothamsted Research for their invaluable help. Rothamsted Research receives strategic funding from UKRI-BBSRC (UK Research and Innovation-Biotechnology and Biological Sciences Research Council) and the contribution to this work by ALC was supported by the Soil to Nutrition strategic programme under Project 3 (grant award BBS/E/C/000I0330).

References

Alfaro, M.A., Jarvis, S.C., Gregory, P.J., 2006. Factors affecting potassium leaching in different soils. Soil Use Manag. 20, 182–189. https://doi.org/10.1111/J.1475-2743.2004.TB00355.X.

Arnscheidt, J., Jordan, P., Li, S., McCormick, S., McFaul, R., McGrogan, H.J., Neal, M., Sims, J.T., 2007. Defining the sources of low-flow phosphorus transfers in complex catchments. Sci. Total Environ. 382, 1–13. https://doi.org/10.1016/J.SCITOTENV.2007.03.036.

Barnes, A.P., Willock, J., Hall, C., Toma, L., 2009. Farmer perspectives and practices regarding water pollution control programmes in Scotland. Agric. Water Manag. 96, 1715–1722. https://doi.org/10.1016/J.AGWAT.2009.07.002.

Barnes, A.P., Willock, J., Toma, L., Hall, C., 2011. Utilising a farmer typology to understand farmer behaviour towards water quality management: nitrate vulnerable zones in Scotland. J. Environ. Plan. Manag. 54, 477–494. https://doi.org/10.1080/09640568.2010.515880.

Basso, B., Dumont, B., Cammarano, D., Pezzuolo, A., Marinello, F., Sartori, L., 2016. Environmental and economic benefits of variable rate nitrogen fertilization in a nitrate vulnerable zone. Sci. Total Environ. 545–546, 227–235. https://doi.org/10.1016/J.SCITOTENV.2015.12.104.

Belford, R.K., 1979. Collection and evaluation of large soil monoliths for soil and crop studies. J. Soil Sci. 30, 363–373. https://doi.org/10.1111/J.1365-2389.1979.TB00993.X.

Brennan, R.B., Healy, M.G., Grant, J., Ibrahim, T.G., Fenton, O., 2012. Incidental phosphorus and nitrogen loss from grassland plots receiving chemically amended dairy cattle slurry. Sci. Total Environ. 441, 132–140. https://doi.org/10.1016/J.SCITOTENV.2012.09.078.

Brown, R.C., Wade, T.L., 1984. Sedimentary coprostanol and hydrocarbon distribution adjacent to a sewage outfall. Water Res. 18, 621–632. https://doi.org/10.1016/0043-1354(84)90213-6.

Buckley, C., 2012. Implementation of the EU nitrates directive in the Republic of Ireland — a view from the farm. Ecol. Econ. 78, 29–36. https://doi.org/10.1016/J.ECOLECON.2012.02.031.

Buckley, C., Wall, D.P., Moran, B., O'Neill, S., Murphy, P.N.C., 2016. Farm gate level nitrogen balance and use efficiency changes post implementation of the EU nitrates directive. Nutr. Cycl. Agroecosyst. 104, 1–13. https://doi.org/10.1007/S10705-015-9753-Y.

Bull, I.D., Elhmmali, M.M., Roberts, D.J., Evershed, R.P., 2003. The application of steroidal biomarkers to track the abandonment of a Roman wastewater course at the agora (Athens, Greece)*. Archaeometry 45, 149–161. https://doi.org/10.1111/1475-4754.00101.

Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. AMBIO: A J. Hum. Environ. 31, 132–140. https://doi.org/10.1579/0044-7447-31.2.132.

Chislock, M.F., Doster, E., Zitomer, R.A., Wilson, A.E., 2013. Eutrophication: causes, consequences, and controls in aquatic ecosystems. Nat. Educat. Knowl. 4, 10.

Collins, A.L., Burak, E., Harris, P., Pulley, S., Cardenas, L., Tang, Q., 2019. Field scale temporal and spatial variability of δ13C, δ15N, TC and TN soil properties: implications for sediment source tracing. Geoderma 333, 108–122. https://doi.org/10.1016/J.GEODERMA.2018.07.019.

Corwin, D.L., 2000. Evaluation of a simple lysimeter-design modification to minimize sidewall flow. J. Contam. Hydrol. 42, 35–49. https://doi.org/10.1016/S0169-7722(99)00088-1.

Dobermann, A., 2007. Nutrient Use Efficiency – Measurement and Management. Agronomy & Horticulture – Faculty Publications.

Edmeades, D.C., 2003. The long-term effects of manures and fertilisers on soil productivity and quality: a review. Nutr. Cycl. Agroecosyst. 66, 165–180. https://doi.org/10.1023/A:1023999816690.

Fahrenfeld, N.L., Del Monaco, N., Coates, J.T., Elzerman, A.W., 2016. Fecal sterol and runoff analysis for nonpoint source tracking. J. Environ. Qual. 45, 315–322. https://doi.org/10.2134/JEQ2015.06.0312.

Foote, K.J., Joy, M.K., Death, R.G., 2015. New Zealand dairy farming: milking our environment for all its worth. Environ. Manag. 56, 709–720. https://doi.org/10.1007/S00267-015-0517-X.

Gooddy, D.C., Macdonald, D.M.J., Lapworth, D.J., Bennett, S.A., Griffiths, K.J., 2014. Nitrogen sources, transport and processing in peri-urban floodplains. Sci. Total Environ. 494–495, 28–38. https://doi.org/10.1016/J.SCITOTENV.2014.06.123.

Gooddy, D.C., Lapworth, D.J., Bennett, S.A., Heaton, T.H.E., Williams, P.J., Surridge, B.W.J., 2016. A multi-stable isotope framework to understand eutrophication in aquatic ecosystems. Water Res. 88, 623–633. https://doi.org/10.1016/J.WATRES.2015.10.046.

Goulding, K., Jarvis, S., Whitmore, A., 2007. Optimizing nutrient management for farm systems. Philos. Transact. Royal Soc. B: Biol. Sci. 363, 667–680. https://doi.org/10.1098/RSTB.2007.2177.

Harrault, L., Milek, K., Jardé, E., Jeanneau, L., Derrien, M., Anderson, D.G., 2019. Faecal biomarkers can distinguish specific mammalian species in modern and past environments. PLoS One 14, e0211119. https://doi.org/10.1371/JOURNAL.PONE.0211119.

Haynes, R.J., Naidu, R., 1998. Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr. Cycl. Agroecosyst. 51, 123–137. https://doi.org/10.1023/A:1009738307837.

Heaton, T.H.E., Stuart, M.E., Sapiano, M., Micallef Sultana, M., 2012. An isotope study of the sources of nitrate in Malta's groundwater. J. Hydrol. 414–415, 244–254. https://doi.org/10.1016/J.JHYDROL.2011.10.037.

Isobe, K.O., Tarao, M., Zakaria, M.P., Chiem, N.H., Minh, L.Y., Takada, H., 2002. Quantitative application of fecal sterols using gas chromatography - mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam. Environ. Sci. Technol. 36, 4497–4507. https://doi.org/10.1021/es020556h.

Jordan, P., Melland, A.R., Mellander, P.E., Shortle, G., Wall, D., 2012. The seasonality of phosphorus transfers from land to water: implications for trophic impacts and policy evaluation. Sci. Total Environ. 434, 101–109. https://doi.org/10.1016/J.SCITOTENV.2011.12.070.

Kendall, C., 1998. Tracing nitrogen sources and cycling in catchments. In: Isotope Tracers in Catchment Hydrology, 519–576. https://doi.org/10.1016/B978-0-444-81546-0.50023-9.

Kim, M., Pangle, L.A., Cardoso, C., Lora, M., Volkmann, T.H.M., Wang, Y., Harman, C.J., Troch, P.A., 2016. Transit time distributions and StorAge selection functions in a sloping soil lysimeter with time-varying flow paths: direct observation of internal and external transport variability. Water Resour. Res. 52, 7105–7129. https://doi.org/10.1002/2016WR018620.

Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. Water Res. 30, 2893–2900. https://doi.org/10.1016/S0043-1354(96)00011-5.

Leeming, R., Latham, V., Rayner, M., Nichols, P., 1997. Detecting and distinguishing sources of sewage pollution in Australian inland and coastal waters and sediments. ACS Symp. Ser. 671, 306–319. https://doi.org/10.1021/BK-1997-0671.CH020.

Li, Y., Jones, D.L., Chen, Q., Chadwick, D.R., 2019. Slurry acidification and anaerobic digestion affects the speciation and vertical movement of particulate and nanoparticulate phosphorus in soil after cattle slurry application. Soil Tillage Res. 189, 199–206. https://doi.org/10.1016/J.STILL.2019.01.009.

Lord, E.I., Anthony, S.G., Gooday, R.D., 2010. Assessing the impact of nitrate vulnerable zones in England on nitrate loss from agricultural land. Int. J. River Basin Manag. 7, 233–243. https://doi.org/10.1080/15715124.2009.9635386.

Manley, A., Collins, A.L., Joynes, A., Mellander, P.E., Jordan, P., 2020. Comparing extraction methods for biomarker steroid characterisation from soil and slurry. Water Air Soil Pollut. 231, 1–15. https://doi.org/10.1007/S11270-020-04871-W.

Matić Bujagić, I., Grujić, S., Jauković, Z., Laušević, M., 2016. Sterol ratios as a tool for sewage pollution assessment of river sediments in Serbia. Environ. Pollut. 213, 76–83. https://doi.org/10.1016/J.ENVPOL.2015.12.036.

McDonald, N.T., Wall, D.P., Mellander, P.E., Buckley, C., Shore, M., Shortle, G., Leach, S., Burgess, E., O'Connell, T., Jordan, P., 2019. Field scale phosphorus balances and legacy soil pressures in mixed-land use catchments. Agric. Ecosyst. Environ. 274, 14–23. https://doi.org/10.1016/J.AGEE.2018.12.014.

McGechan, M.B., 2002. SW—soil and water: transport of particulate and colloid-sorbed contaminants through soil, part 2: trapping processes and soil pore geometry. Biosyst. Eng. 83, 387–395. https://doi.org/10.1006/BIOE.2002.0136.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Nash, D.M., Halliwell, D.J., 2000. Tracing phosphorous transferred from grazing land to water. Water Res. 34, 1975–1985. https://doi.org/10.1016/S0043-1354(99)00359-0.

NIST, 2012. National Institute of Standards and Technology Spectral Library Search Program. US Department of Commerce, Gaithersburg, Maryland, US.

Noblet, J.A., Young, D.L., Zeng, E.Y., Ensari, S., 2004. Use of fecal steroids to infer the sources of fecal indicator bacteria in the lower Santa Ana River watershed, California: sewage is unlikely a significant source. Environ. Sci. Technol. 38, 6002–6008. https://doi.org/10.1021/ES049799V.

OJEU, 1991. Council directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. Off. J. Eur. Union 1–8.

Osborn, S., Cook, H.F., 1997. Nitrate vulnerable zones and nitrate sensitive areas: a policy and technical analysis of groundwater source protection in England and Wales. J. Environ. Plan. Manag. 40, 217–234. https://doi.org/10.1080/09640569712191.

Ottoson, J., Stenström, T.A., 2003. Faecal contamination of greywater and associated microbial risks. Water Res. 37, 645–655. https://doi.org/10.1016/S0043-1354(02)00352-4.

Petersen, S.O., Sommer, S.G., Béline, F., Burton, C., Dach, J., Dourmad, J.Y., Leip, A., Misselbrook, T., Nicholson, F., Poulsen, H.D., Provolo, G., Sørensen, P., Vinnerås, B., Weiske, A., Bernal, M.P., Böhm, R., Juhász, C., Mihelic, R., 2007. Recycling of livestock manure in a whole-farm perspective. Livest. Sci. 112, 180–191. https://doi.org/10.1016/J.LIVSCI.2007.09.001.

Prost, K., Birk, J.J., Lehndorff, E., Gerlach, R., Amelung, W., 2017. Steroid biomarkers revisited – improved source identification of Faecal remains in archaeological soil material. PLoS One 12, e0164882. https://doi.org/10.1371/JOURNAL.PONE.0164882.

Pütz, T., Fank, J., Flury, M., 2018. Lysimeters in vadose zone research. Vadose Zone J. 17, 1–4. https://doi.org/10.2136/VZJ2018.02.0035.

Qi, Y., Zhang, T.C., 2016. Transport of manure-borne testosterone in soils affected by artificial rainfall events. Water Res. 93, 265–275. https://doi.org/10.1016/J.WATRES.2016.01.052.

Qi, Y., Zhang, T.C., Ren, Y., 2014. Testosterone sorption and desorption: effects of soil particle size. J. Hazard. Mater. 279, 493–501. https://doi.org/10.1016/j.jhazmat.2014.06.077.

Ramos, M.C., Quinton, J.N., Tyrrel, S.F., 2006. Effects of cattle manure on erosion rates and runoff water pollution by faecal coliforms. J. Environ. Manag. 78, 97–101. https://doi.org/10.1016/J.JENVMAN.2005.04.010.

Reichwaldt, E.S., Ho, W.Y., Zhou, W., Ghadouani, A., 2017. Sterols indicate water quality and wastewater treatment efficiency. Water Res. 108, 401–411. https://doi.org/10.1016/J.WATRES.2016.11.029.

Sangster, J.L., Oke, H., Zhang, Y., Bartelt-Hunt, S.L., 2015. The effect of particle size on sorption of estrogens, androgens and progestagens in aquatic sediment. J. Hazard. Mater. 299, 112–121. https://doi.org/10.1016/j.jhazmat.2015.05.046.

Shah, V.G., Hugh Dunstan, R., Geary, P.M., Coombes, P., Roberts, T.K., von Nagy-Felsobuki, E., 2007. Evaluating potential applications of faecal sterols in distinguishing sources of faecal contamination from mixed faecal samples. Water Res. 41, 3691–3700. https://doi.org/10.1016/J.WATRES.2007.04.006.

Shore, M., Jordan, P., Melland, A.R., Mellander, P.E., McDonald, N., Shortle, G., 2016. Incidental nutrient transfers: assessing critical times in agricultural catchments using high-resolution data. Sci. Total Environ. 553, 404–415. https://doi.org/10.1016/J.SCITOTENV.2016.02.085.

Turner, B.L., Haygarth, P.M., 2000. Phosphorus forms and concentrations in leachate under four grassland soil types. Soil Sci. Soc. Am. J. 64, 1090–1099. https://doi.org/10.2136/SSSAJ2000.6431090X.

Vadas, P.A., Stock, M.N., Arriaga, F.J., Good, L.W., Karthikeyan, K.G., Zopp, Z.P., 2019. Dynamics of measured and simulated dissolved phosphorus in runoff from winter-applied dairy manure. J. Environ. Qual. 48, 899–906. https://doi.org/10.2134/JEQ2018.11.0416.

Velthof, G.L., Lesschen, J.P., Webb, J., Pietrzak, S., Miatkowski, Z., Pinto, M., Kros, J., Oenema, O., 2014. The impact of the nitrates directive on nitrogen emissions from agriculture in the EU-27 during 2000–2008. Sci. Total Environ. 468–469, 1225–1233. https://doi.org/10.1016/J.SCITOTENV.2013.04.058.

VSN International, 2020. Genstat for Windows, 19th edition. VSN International, Hemel Hempstead, UK.

Wang, Q., Cameron, K., Buchan, G., Zhao, L., Zhang, E.H., Smith, N., Carrick, S., 2012. Comparison of lysimeters and porous ceramic cups for measuring nitrate leaching in different soil types. N. Z. J. Agric. Res. 55, 333–345. https://doi.org/10.1080/00288233.2012.706224.

Webb, J., Sørensen, P., Velthof, G.L., Amon, B., Pinto, M., Rodhe, L., Salomon, E., Hutchings, N., Burczyk, J., Reid, J.E., 2011. Study on Variation of Manure N Efficiency throughout Europe. AEA Technology plc.

Writer, J.H., Leenheer, J.A., Barber, L.B., Amy, G.L., Chapra, S.C., 1995. Sewage contamination in the upper Mississippi River as measured by the fecal sterol, coprostanol. Water Res. 29, 1427–1436. https://doi.org/10.1016/0043-1354(94)00304-P.

Zhen, Z., Liu, H., Wang, N., Guo, L., Meng, J., Ding, N., Wu, G., Jiang, G., 2014. Effects of manure compost application on soil microbial community diversity and soil microenvironments in a temperate cropland in China. PLoS One 9, e108555. https://doi.org/10.1371/JOURNAL.PONE.0108555.