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Organic contaminants in bio-based fertilizer treated soil: Target and suspect screening approaches

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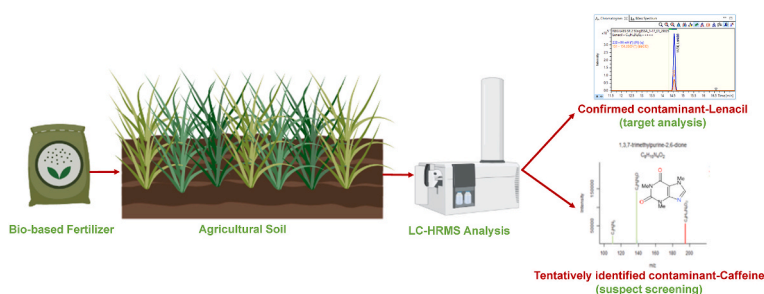
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HIGHLIGHTS

- Fifteen novel BBF-treated soil samples were analyzed for organic contaminants identification.
- Suspect screening tentatively identified 20 NORMAN-listed contaminants in soil samples.
- Most contaminants found in BBF-treated soil might come from non-BBFs sources.
- No evidence of agricultural soil contamination by BBF application.

GRAPHICAL ABSTRACT



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ABSTRACT

Using bio-based fertilizer (BBF) in agricultural soil can reduce the dependency on chemical fertilizer and increase sustainability by recycling nutrient-rich side-streams. However, organic contaminants in BBFs may lead to residues in the treated soil. This study assessed the presence of organic contaminants in BBF treated soils, which is essential for evaluating sustainability/risks of BBF use. Soil samples from two field studies amended with 15 BBFs from various sources (agricultural, poultry, veterinary, and sludge) were analyzed. A combination of QuEChERS-based extraction, liquid chromatography quadrupole time of flight mass spectrometry-based (LC-QTOF-MS) quantitative analysis, and an advanced, automated data interpretation workflow was optimized to extract and analyze organic contaminants in BBF-treated agricultural soil. The comprehensive screening of organic contaminants was performed using target analysis and suspect screening. Of the 35 target contaminants, only three contaminants were detected in the BBF-treated soil with concentrations ranging from 0.4 ng g⁻¹ to 28.7 ng g⁻¹; out of these three detected contaminants, two were also present in the control soil sample. Suspect screening using patRoom (an R-based open-source software platform) workflows and the NORMAN Priority List resulted in tentative identification of 20 compounds (at level 2 and level 3 confidence level), primarily pharmaceuticals and industrial chemicals, with only one overlapping compound in two experimental sites. The contamination profiles of the soil treated with BBFs sourced from veterinary and sludge were similar, with common pharmaceutical features identified. The suspect screening results suggest that the contaminants found in BBF-treated soil might come from alternative sources other than BBFs.

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1. Introduction

Chemical and mineral fertilizer usage revolutionized the agriculture sector and doubled global crop yields since early 1900 (Erisman et al., 2008; Roser and Ritchie, 2019). As most important plant nutrients, nitrogen (N) and phosphorus (P) form the major components of chemical fertilizers, the overuse of which has a detrimental impact on the environment worldwide (Canfield et al., 2010; Peñuelas and Sardans, 2022). While N reduction requires a lot of energy, albeit at the cost of a total of 2.1% of global greenhouse gas emissions (Canfield et al., 2010; Menegat et al., 2022; Zilio et al., 2022a), P has a finite source and with its increasing use, it is expected that the global reserve will deplete before the end of the 21st century (Jupp et al., 2021).

Bio-based fertilizers (BBFs) for agricultural soil treatment can reduce the dependency on chemical and mineral fertilizers and increase sustainability by recycling nutrient-rich side streams/organic waste (Jupp et al., 2021; Vaneekhaute et al., 2018; Ylivainio et al., 2021). Multiple studies have been conducted in recent years in this regard, and the results showed positive responses of biomass production and seed yield to direct organic fertilization, obtaining similar results with respect to mineral fertilization (Luo et al., 2021; Vaneekhaute, C. et al., 2013; Ylivainio et al., 2021; Zilio et al., 2022b). BBFs can not only fulfil the nutrient requirement for plant growth and yield, but it can also improve soil fertility and structure (Ylivainio et al., 2021; Zilio et al., 2022a). This gives the opportunity of closing the nutrient cycle and improving sustainability by reducing the demand for mineral fertilizers. However, the presence of organic contaminants in BBFs may lead to undesired chemical residues in the treated soil (Chen et al., 2021; Kacprzak et al., 2022), and a combination of repeated annual application with persistence and bioaccumulation of the chemicals in the soil may lead to concentrations building up over time (Vuaille et al., 2021). The contaminants may then either be taken up by the crop or leached to the groundwater, ultimately causing potentially severe risks to human health and the environment.

A comprehensive understanding of the organic contaminant types and classes that might be introduced through BBF use is of importance for early identification and risk assessment. The range and types of organic contaminants present in the BBFs may be significantly influenced by their origin. However, measuring a wide range of organic contaminants, such as pesticides, pharmaceuticals, antibiotics, flame retardants, etc., using a single workflow is difficult due to the pollutants' complex nature and their potential interaction with the soil matrix. Organic contaminants are often extracted from soil samples using pressurized-liquid extraction (PLE), ultrasound-assisted extraction and microwave-assisted extraction (MAE) (Larivière et al., 2017). The QuEChERS (quick, easy, cheap, effective, rugged and safe) method is another widely and successfully used technique for extraction of broad-spectrum organic contaminants, including pesticides, pharmaceuticals, veterinary drugs and personal care products (Lehotay et al., 2010; Martínez-Carballo et al., 2007; Matamoros et al., 2012; Salvia et al., 2012). However, in most cases, the scope of the method is limited and focused on the monitoring of selected groups of compounds. After extraction, multi-residue analysis is necessarily accomplished by liquid and/or gas chromatography with mass spectrometry for target analysis. The targeted chemical screening method can detect a pre-defined list of contaminants with the help of reference standards (Paszkiwicz et al., 2022). Suspect screening, on the other hand, has been introduced in recent years, and this can be performed by finding suspect m/z values when high-resolution mass spectrometry (HRMS) based chemical analysis data are available for comparison (Gago-Ferrero et al., 2018; Paszkiwicz et al., 2022). Suspect analysis has been found to be a highly promising technique in the analysis of environmental samples mainly because reference standards are not mandatory in this approach (González-Gaya et al., 2021). However, suspect screening still represents a challenge since it can only provide semi-quantitative information about the identified compounds and lower identification confidence

levels if standard substances are not available (Chiaia-Hernández et al., 2020; Yang et al., 2020; Yang et al., 2022).

Field-scale studies are scarce, especially with respect to the potentially wide range of pollutants that might be introduced upon application of commercially available BBFs. Therefore, the objective of the present study was to apply a methodology to simultaneously check for wide range of compounds in the agricultural soil upon BBF use, and subsequently formulate advice based on the data obtained. Fifteen BBFs (seven N, and eight P BBFs) sourced from agriculture, sewage sludge and veterinary/poultry were used in this study for agricultural soil treatment as part of the LEX4BIO project, a large pan-European consortium focusing on safe BBF application (www.lex4bio.eu). A QuEChERS-based method was optimized for organic contaminant extraction from the BBF-treated soil. Based on patRoos (an R-based open-source software platform) workflows ((Helmus et al., 2021; Helmus et al., 2022) and the NORMAN Priority List (Dulio, 2017), suspect screening was used to fingerprint each treatment's chemical features. Finally, the resulting lists of confirmed compounds through target analysis and tentatively identified compounds through suspect screening were used to evaluate the possibility of organic contaminant pollution in the agricultural soils due to BBFs application and the potential for broader application of our methodology for agricultural soil screening.

2. Materials and methods

2.1. Sampling site, fertilizer application, and sampling approach

Soil samples were obtained from two experimental agricultural fields located in Seville, Spain (37°24'06.6"N 5°35'45.9"W) and Jokioinen, Finland (60°48'15.6"N 23°27'06.5"E) (Figure S1 I). Two trials, one for N and one for P, were conducted in each experimental site. Henceforth, soil samples from Spain and Finland will be referred to as Soil A and Soil B, respectively. The experimental sites were as homogeneous as possible with respect to topography, light conditions, soil characteristics, previous soil management (e.g., no application of organic fertilizers in the last five years), and pre-crops. Crop rotation was in practice in the field trials, with the first crop being either winter wheat, maize, or spring barley and the second crop a cereal or sunflower in both sites. In each trial, plot sizes were $\geq 120 \text{ m}^2$, and there was a minimum of four replicate plots (randomized complete block designed) for each BBF treatment, resulting four replicate soil samples/treatment for each trial. Altogether 15 BBFs were selected for field trial, i.e., seven N BBFs and eight P BBFs. The short name and sources of the BBFs are listed in Table 1.

Soil from all four replicates of each trial plot was treated with only one BBF. The local advisory recommendation for the specific crop was followed for both experimental sites, with all BBFs being applied only once at the start of the trial and at same total N and P rates of 200 kg N/ha and 20 kg P/ha. The mode of application was as close to practical farming conditions as possible. BBFs were applied to the surface and were incorporated into the soil (at approximately 10 cm depth) within 24 h of application. Soils from the field experiment sites were collected from the top 20–30 cm before and after applying BBFs, henceforth referred to as 'before soil' and 'after soil'. All after soils were collected within 72 h of BBF application, to avoid the possibility of the pollutant migrating away from the application site and/or being degraded before analysis and detection. A composite sample was created from 20 soil cores taken across each plot of the field experiment. Refrigerated composite soil samples from the field sites were transported to IBED, UvA, for analysis. A representative subsample of the after soil was used for soil characterization (SI Table S1), and before soil samples were used for extraction method validation.

2.2. Chemicals and reagents

The target list in this study contained 35 compounds of interest,

including NORMAN priority pollutants in soil, current and legacy agricultural herbicides, fungicides and insecticides, frequently detected pharmaceuticals, their transformation products and one veterinary drug (Chiaia-Hernandez et al., 2017; Degrendele et al., 2022; Gworek et al., 2021; Kalyva, 2017; Narain-Ford et al., 2022; Rose et al., 2022; Silva et al., 2019). All selected contaminants are amenable to analysis by liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). Reference standards and isotope labelled standards (listed in SI Table S2 & S3) were purchased from Sigma Aldrich (Switzerland and The Netherlands) at purities $\geq 95\%$ (analytical grade). Methanol ($\geq 99.9\%$) was purchased from Fisher Scientific (Wohlen, Switzerland). Ultrapure water was obtained from ELGA water purification system (Veolia Water Technologies Netherlands B.V., Ede, the Netherlands). Sodium acetate ($\geq 99\%$) used as the mobile phase modifier was purchased from Merck (Darmstadt, Germany).

2.3. Sample extraction

A QuEChERS method from Acosta-Dacal, A. et al. (2021) was adapted to extract pesticides and pharmaceuticals from Soil A and Soil B (Acosta-Dacal et al., 2021). Briefly, 10 g (± 0.1 g) of composite (made by mixing four replicates), freeze-dried and homogenized soil samples were weighed into 50-mL centrifuge tubes, and 10 mL acetonitrile with 2.5% formic acid was added. The solution was then vortexed for 30 s. After equilibrating the samples for 20 min, 6 g of $MgSO_4$ and 1.5 g of CH_3COONa were added, energetically shaken for 30 s, and sonicated for 20 min in an ultrasonic bath (VWR® USC300TH) operating at 50 Hz and 80 W. After that, samples were placed in a mechanical shaker for 30 min. Finally, the samples were centrifuged for 10 min at 4200 rpm at ambient temperature. An aliquot of the clear supernatant extract was filtered through 0.20 μm Chromafil® PET filters (0.2 μm , 13 mm, Macherey-Nagel, Düren, Germany), and diluted five times with H_2O before analysis by LC-QTOF-MS/MS. One laboratory blank was prepared following the same protocol described above but without any added soil. To test the efficiency of the extraction method, recovery and matrix effects were determined. 'Before soil' was used for the recovery

experiment and spiked with two different volumes of reference standard solution to achieve 10 ng g^{-1} and 50 ng g^{-1} target concentrations. Once the method was adequately validated, a single replicate per BBF-treated Soil A and Soil B was extracted following the above-described procedure along with laboratory blank and before soil samples.

2.4. Instrumental analysis

The instrumental analysis method was based on a recently published work by Narain-Ford, D. et al. (2022) (Narain-Ford et al., 2022). The analyses were conducted using a UHPLC system (Nexera, Shimadzu, Den Bosch, The Netherlands) coupled to a Bruker Daltonics maXis 4G high-resolution QTOF-MS upgraded with HD collision cell and equipped with an ESI source (Wormer, The Netherlands). A reversed-phase Phenomenex Kinetex® 1.7 15 μm Biphenyl 100 Å, LC Column 150 \times 2.1 mm (Phenomenex, Utrecht, The Netherlands) column was used to achieve separation. Ultrapure water with 0.05% acetic acid (mobile phase A) and MeOH (mobile phase B) were used as the mobile phase. Sample separation was attained by a gradient starting from 0% mobile phase B at 0–2 min, increased linearly to 100% at 17 min, and kept equal until 25 min. The total flow rate was 0.3 mL min^{-1} . The autosampler was maintained at 15 °C, and 20 μL of the sample was injected for positive and negative ESI mode analysis. The column oven was 40 °C. The MS detector was internally calibrated before starting an analysis batch by infusing 2 mM sodium acetate solution in H_2O and MeOH (1:1, v:v) in both positive and negative ESI mode. The initial conditions were restored over 7 min, and a 50 μM sodium acetate solution in H_2O :MeOH (1:1, v:v) was introduced automatically for m/z recalibration of the system between each sample injection. Data-independent MS/MS scans were used for target screening and data-dependent MS/MS acquisition was used for suspect screening as previously described by Narain-Ford, D. et al. (2022) (Narain-Ford et al., 2022). Details of the acquisition method can be found elsewhere (Narain-Ford et al., 2022)

Table 1

Sources, technologies and manufacturers used in production of the N and P Fertilizers included in this study. Total N and P are reported as % of fresh weight (FW).

| Short Name | N Fertilizer: Source | Technology | Manufacturer's Name | N (% of FW) | Short Name | P Fertilizer: Source | Technology | Manufacturer's Name | P (% of FW) |
|------------|--|---|--|-------------|------------|--|-------------------------------|--------------------------|-------------|
| BA6 | Purely plant-based, bioethanol residue amended with residual molasses, potato fruit water and maize steeping water | Fermentation and distillation | Agrana, A | 5.57 | BA1 | Vegetable origin | Fermentation and distillation | Agrana, AT | 1.2 |
| PAL | Fermented biochar and vegetable by-products and other soil-forming additives such as clay and stone powder | Anaerobic digestion, pelletizing/granulating (1–4 mm) | Palaterra Betriebs-und Beteiliungsgesellschaft mbH, DE | 4.89 | MO14 | Natural origin (45% vegetable by-products) | ^a NA | MeMon, NL | 6.1 |
| FEK | Chicken manure | Drying and processing (extrusion process) | Fertikal, BE | 3.94 | MB1 | Meat & bone meal | Pyrolysis | Hauert, CH | 6.2 |
| MO13 | 100% feather meal | Pelletizing | MeMon, NL | 14.2 | CGO | Struvite | ^a NA | Ostara, NL | 12.5 |
| ECO | Blood meal, animal by-products | Pelletizing | Ecolan Oy | 11.6 | ADC | Calcined phosphate from sewage sludge | ^a NA | Outotec, DE | 8.1 |
| OG2 | Hornmeal (pig bristles) | Hydrolysis | Daka | 13.9 | OPU | Chicken manure pellets | Pyrolysis, Pelletizing | Optisol, CH | 1.7 |
| BIO | Meat bone meal, apatite, vinasse, chicken manure and potassium sulphate | Pelletizing | YARA, FI | 7.39 | EPH | Sunflower husk ash | ^a NA | Orij Group Ltd., Ukraine | 1.7 |
| – | – | – | – | – | PLA | Poultry litter ash | ^a NA | BMC Moerdijk | 3.6 |

^a NA- Not available, technology information was not shared by the manufacturer.

2.5. Post-acquisition data treatment

2.5.1. Target screening and quantification method

Quantitative data analysis was performed using TASQ version 2021.0316 (Bruker Daltonics, Wormer, the Netherlands). Target screening and quantification method set-up were also based on [Narain-Ford, D. et al. \(2022\)](#). In short, TASQ software requires analytes formulas, retention times and qualifier ions. All this information was generated by analyzing a mixture of standards in autoMS/MS mode. The most intense fragments were manually inspected and checked in the bbCID MS/MS mode. The fragments were added to the database as qualifiers of their respective precursors after successful confirmation. The screening parameters for the target analytes are provided in [SI Table S3](#).

2.5.2. Suspect screening

Post-acquisition data treatment was performed using the open-source software platform patRoom, based on the four steps generic workflow published by [Helmus et al. \(2021\)](#) ([Helmus et al., 2021](#)). A schematic overview of the workflow is given in [SI Figure S2](#). All the raw HRMS data were manually m/z calibrated using Bruker DataAnalysis 4.4 software (Bruker Daltonics, Bremen, Germany). The m/z calibration method was set to the search range 0.15 m/z and the calibration error was verified to be < 0.5 ppm. Next, centroiding (vendor algorithm) and conversion to mzML format were performed using ProteoWizard v. 3.0.22119 ([Kessner et al., 2008](#); [Martens et al., 2011](#)).

The next steps were performed in RStudio. Pre-treated data were loaded as a data.frame with the file information of the sample analyses and blank assignments. The pre-treated data were screened using the NORMAN priority contaminants list, which includes 967 compounds of environmental concern including pesticides, pharmaceuticals, preservatives, colorants, surfactants, and a long list of industrial chemicals ([Dulio, 2017](#)). The lists of suspects included information about the compound's name, molecular formula, CAS number, smiles and other information, all extracted from the NORMAN database. The suspect list can be found in an Excel file provided as supporting information. The OpenMS algorithm was used to generate unique chromatographic and mass spectral properties or feature lists ([Röst et al., 2016](#)). To further refine the quality of the dataset, basic rule-based filtering was applied, and further data reduction was achieved by removing all detected features in the before soil sample and remove any features that do not match suspect assignments. Next, components were automatically generated by using the RAMClustR algorithm and features were grouped based on their adducts i.e., "[M - H]⁻" or "[M+H]⁺" ([Broeckling et al., 2014](#)). Finally, features were annotated assigning the candidate formula (GenForm algorithm) and structure (MetFrag) from the PubChem database ([Meringer et al., 2011](#); [Ruttkies et al., 2016](#)). The confidence levels of the tentatively identified compounds were assigned to the final candidates following a set scoring level ([SI Figure S2](#)) and the scheme proposed by Schymanski et al. ([Schymanski et al., 2014](#)), considering the characteristic fragments match with the experimentally found fragments that are available in the spectral libraries such as the European MassBank, the MassBank of North America and PubChem ([Horai et al., 2010](#); [MassBank-consortium and its contributors, 2021](#)).

2.6. Quality control and quality assurance

Method recovery experiments were conducted in triplicate in Soil A and Soil B according to the methods described in Section 2.3. Before extraction, samples were spiked with a solution containing known concentrations of mixed analytes. The percent recovery was determined by comparing the response of the analyte spiked pre-extraction (A) with the response in a pure standard solution (B), where % Recovery = $[(A/B) - 1] \times 100$. The precision was determined by calculating the relative standard deviation for the spike and recovery tests. The matrix effect was calculated by comparing the analytes' response in the presence of

the soil matrix spiked post-extraction (C) with that in the absence of the matrix (D), where %ME = $[(C/D)-1] \times 100$. A positive %ME indicates signal enhancement, while negative %ME suggests signal suppression due to the matrix effect and zero refers to no matrix effect. The method performance for target screening was evaluated by determining the linearity of the calibration curve, method recovery rate, precision, limit of detection (LoD) and limit of quantitation (LoQ), and the matrix effect (ME) ([SI Table S4](#) and [SI Table S5](#)). The linearity of the method was evaluated by injecting a set of eight-point calibration standards at a concentration range from 0.5 ng mL⁻¹ to 30 ng mL⁻¹ for the targeted compounds. For quality assurance, procedural blanks, and 'before soil' samples were run at the start of the sequence. Calibration standards were measured at the beginning and the end of each sequence, and the QC standard was measured repeatedly every 15–20 injections, to check for signal stability.

Descriptive statistics for all measurements was performed Microsoft® Excel® for Microsoft 365 MSO (Version 2205 Build 16.0.15225.20394) to summarize and describe the main characteristics (i.e., mean, median) and variability (i.e., relative standard deviation) of the measurements.

3. Results

3.1. Validation of the analytical method

Recoveries for Soil A and Soil B were within 60–130% for 74% of targeted compounds ([SI Figure S3](#)). The repeatability was $\leq 20\%$ for >85% of the compounds and <30% for 100% of compounds, which indicates good precision of the analytical method. Correlation coefficients were ≥ 0.99 for all compounds except trietazine (0.98). The LOQ values for the targeted compounds ranged from 0.1 to 6.0 ng g⁻¹, and LODs from 0.01 to 2.0 ng g⁻¹. The matrix effect results indicated signal suppression for all compounds ([SI Table S4](#) and [Table S5](#)). The results showed that for 83% of the targeted compounds matrix effect ranged between 0% and -50%. Higher signal suppression was observed in the target pharmaceutical compounds. Detailed information regarding method validation parameters is summarized in [Tables S4](#) and [S5](#).

3.2. Occurrence and composition of contaminants in the BBFs-treated soil samples

[Table S6](#) and [Table S7](#) summarize the results found for the before-, N- and P-treated soil samples. Surprisingly, none of the target compounds was detected in any of the samples except for ibuprofen, one of its primary metabolites 1-hydroxyibuprofen and lenacil. The highest ibuprofen and 1-hydroxyibuprofen concentrations measured in Soil A samples were 0.6 ng g⁻¹ and 1.5 ng g⁻¹ dry soil weight, respectively ([Table S6](#) and [Table S7](#)). 1-hydroxyibuprofen was also found in Soil B (highest concentration 1.5 ng g⁻¹ dry soil weight); however, ibuprofen was not detected in Soil B samples ([Table S6](#) and [Table S7](#)). The highest lenacil concentration (28.7 ng g⁻¹) was detected in the Soil B sample that received BA1 as P treatment. Lenacil was also present in two N-treated Soil B samples, which received BA6 and PAL as N treatment.

3.3. Identification of suspects in the BBF-treated soil

The suspect screening in the BBF-treated soil yielded 39 hits for the N treatment and 25 hits for the P treatment for confidence level 4 and above ([Fig. 1](#)). Note that confidence levels were automatically assigned by patRoom. Additional investigations of the MS/MS spectra were performed to reach tentative identification for all suspects for confidence level 3 and above. No further manual interpretation was conducted for level 4 compounds. This workflow further reduced the number of hits resulting in 14 hits for N treatment and 7 hits for P treatment. [SI 2 Tables S1](#) and [S2](#) summarizes the tentatively identified compounds

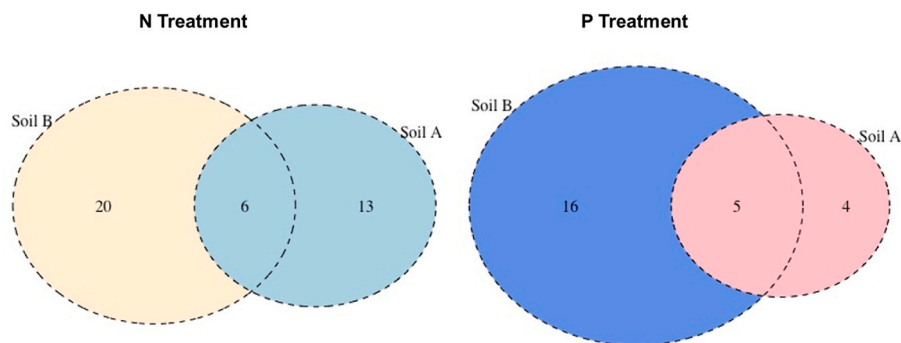


Fig. 1. Distribution of the detected suspect compounds in the Soil A and Soil B samples.

showing their structure, molecular formulas, neutral mass, detected adducts, MS/MS fragments, confidence level and information related to their use and/or area of application.

The heatmaps in Figs. 2 and 3 provide an overview of tentatively identified compounds in representative samples from Soil A and Soil B treated with N and P BBFs concerning their peak area counts. In the N-treated soil, most tentatively identified compounds were industrial chemicals (i.e., six out of fourteen identified compounds) and were mainly detected in Soil B (SI 2 Table S1). At least one of the two experimental sites detected five pharmaceuticals, namely acetylsalicylic acid, caffeine, gemfibrozil, MDMA, and phenobarbital, along with two herbicides, bentazone and benzothiazole-2-sulfonic acid, and one flame retardant, tri-isobutyl phosphate. Out of the seven tentatively identified compounds with P treatment, one compound was identified at level 2, and six compounds at level 3 (SI2 Table S2). The concentrations of the compounds identified at level 3b were below the set intensity threshold and, therefore, might not be present or at least not very relevant regarding peak area counts (Figs. 2 and 3).

4. Discussion

In total, 23 organic contaminants were identified using target and

suspect screening (tentative), of which 3 contaminants were detected by target and 20 by suspect screening analytical approaches. Ibuprofen is one of the three detected contaminants through targeted analysis, which is the world's third most-used anti-inflammatory drug. Many studies reported ibuprofen as a soil contaminant, which predominantly comes from wastewater irrigation (González-Naranjo et al., 2013; Gworek et al., 2021; Hiller and Sebesta, 2017). Vazquez-Roig et al. conducted a study in Valencia, eastern Spain and reported 74% of the irrigation water samples were contaminated with ibuprofen (Vazquez-Roig et al., 2012). As ibuprofen and 1-hydroxyibuprofen were detected in one of the before-treatment soil samples, it is not conclusive if BBFs are the contamination source or if other land management practices have introduced it. Lenacil is the third detected contaminant and a common current-use herbicide in agricultural land. Lenacil concentrations measured in this study were 14.1 ng g^{-1} for BA6 and 1.7 ng g^{-1} for PAL, which might be attributed to the use of BBF treatment. This is likely because both BA6 and PAL for N and BA1 and MO14 for P treatments are sourced from agricultural by-products, where many studies reported this agrichemical contaminant in sugar beet and fodder crops (Kucharski et al., 2011; European Food Safety Authority, 2009). However, lenacil was found to be moderately persistent in field dissipation trials ($DT_{50} = 18\text{--}88$ days) and a low-risk compound for soil organisms like

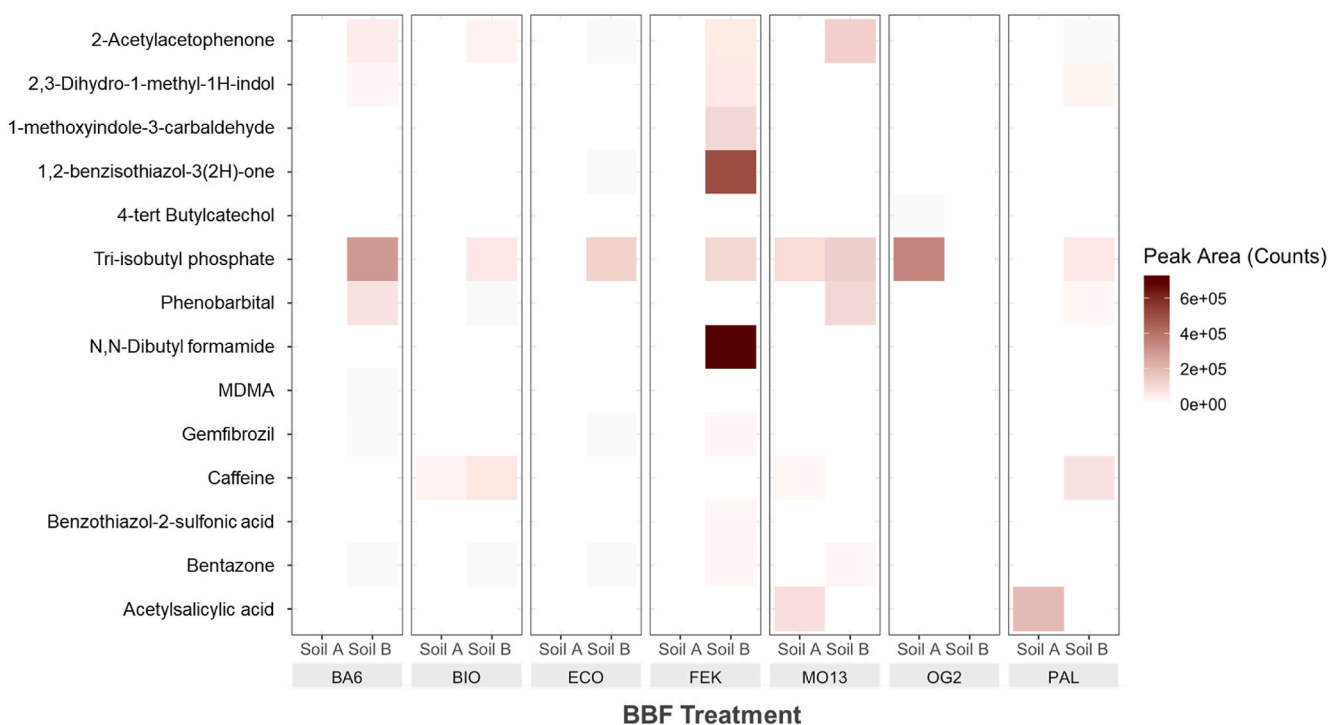


Fig. 2. Tentatively identified compounds in N-treated soil samples.

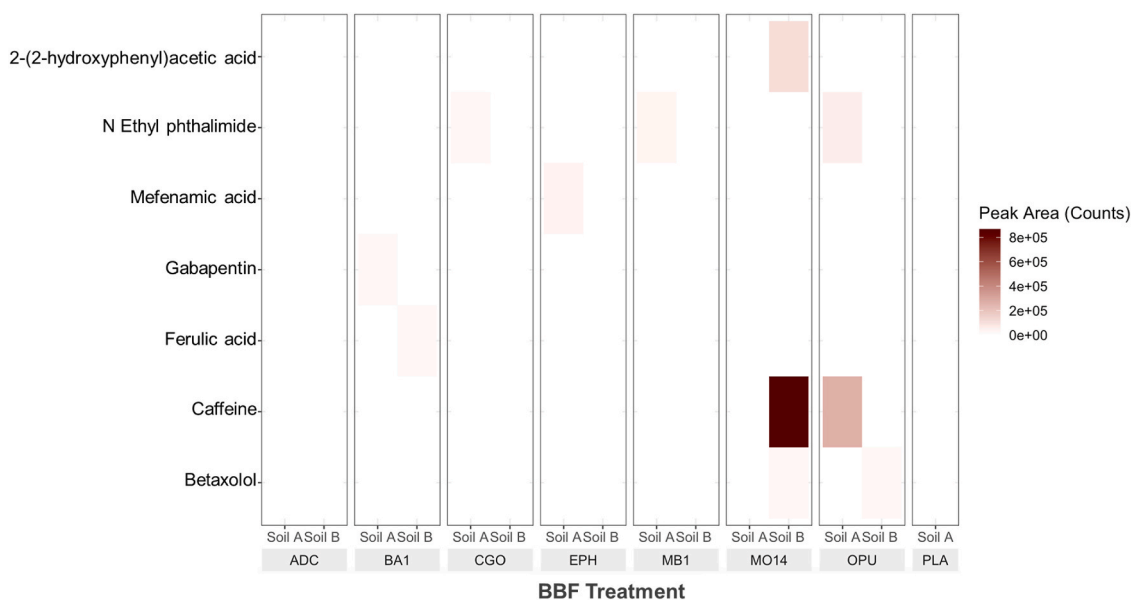


Fig. 3. Tentatively identified compounds in P-treated soil samples.

earthworms (European Food Safety Authority, 2013).

The tentatively identified pharmaceuticals in the suspect screening corresponded to the most common medical substances, which are used as either anti-inflammatory drugs, beta-blockers or stimulants (Biel-Maeso et al., 2018; Martínez-Piernas et al., 2018). Only one (tentatively) identified pharmaceutical (i.e., caffeine) overlapped between two experimental sites and treatments. Caffeine, along with all other tentatively identified pharmaceuticals, was previously detected in soil and water bodies (Biel-Maeso et al., 2018; Martínez-Piernas et al., 2018). Gravert et al. (2021) conducted a non-target analysis to profile organic contaminants in agricultural soil amended with organic wastes and reported pharmaceuticals as the main group of tentatively identified contaminants, which is consistent with our results (Gravert et al., 2021). Vuaille et al. (2021) also identified pharmaceuticals and personal care products after long-term soil fertilization with urban and animal wastes (Vuaille et al., 2021). Although our results represent snapshots that are comparable to other similar studies, tentatively identified compound lists are different in the two experimental sites. This is probably because the tentatively identified compounds were not introduced through BBFs, but through other previous land management practices like wastewater irrigation, plant protection products use, and agricultural machine uses (Chiaia-Hernández et al., 2020; Gravert et al., 2021; Vuaille et al., 2021).

None of the three contaminants that were detected using target analysis were identified as part of the suspect screening, in spite of being present in the NORMAN suspect list. The most likely explanation for the non-identification of two of these, ibuprofen and 1-hydroxyibuprofen, in the suspect screening is the fact that the 'before soil sample' already contained these chemicals. Since the 'before soil sample' was used for a blank subtraction during suspect screening, the signal of the mentioned compounds was removed during this process. The non-detection of the third chemical in the suspect screening as compared to the target analysis, was that the compound height was below the set intensity threshold. This also means that there might be more suspects present that were not detected with current set intensity threshold.

Overall, it is well established that suspect screening allows the detection of additional contaminants, which could be missed by target screening. However, suspect screening may generate false positives, which can occur due to sample contamination or interferences from other compounds present in the sample. Indeed, target analysis is required for the confident identification and quantification of

environmental contaminants. Nevertheless, suspect screening is a significant addition to target screening and can be used for qualitative screening purposes. However, challenges still need to be addressed regarding the quantification of tentatively identified compounds and analytical gaps resulting from the selected extraction and instrumental methods.

5. Conclusion

In our study we applied a novel, broad screening method to simultaneously detect a wide range of pesticides and pharmaceuticals in soils via retrospective analysis of 35 target contaminants and suspect screening of 976 priority contaminants in 2 agricultural fields upon application of 15 bio-based fertilizers (BBFs) of agricultural, sludge and veterinary/poultry origin. The distribution of contaminants in soil before and after treatment with BBFs was compared to draw basic conclusions on the possibility of organic contaminant contamination in agricultural soil due to BBF application. Overall, only a very limited number of contaminants was found at low intensities. The annotation of detected features highlighted pharmaceuticals and industrial chemicals as the main groups of organic pollutants. Soil treated with veterinary and sludge sourced BBFs displayed similar pollution profiles with features annotated as common pharmaceuticals.

A full target analysis to quantify the suspects using standards will be required to draw a full conclusion. Nevertheless, the applied suspect screening approach searched the samples against the priorities suspect list (database) that is feasible with reference materials. Further BBF treated soil samples need to be collected and analyzed over several years to compare their chemical profiles and highlight individual suspect pollutants before, just after fertilization and after several years to understand the fate of the chemicals in the natural environment. Overall, soil treatment with BBFs provides a promising way to recirculate essential nutrients for crop growth, otherwise placed in landfills or incinerated, but appropriate application thresholds need to be considered to contain unintended secondary effects.

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Author contributions statement

S. Das: Conceptualization, sample extraction and analysis, data interpretation and writing the first draft of the manuscript. **R. Helmus:** Input on suspect analysis, instrumental analysis, scientific contribution, and manuscript correction. **Y. Dong & S. Beijer:** Soil analyses. **A. Praetorius & J. Parsons:** Conceptualization & reviewing the manuscript, **B. Jansen:** Conceptualization, writing, reviewing and editing the manuscript.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2023.139261>.

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