

Plant Residues as Direct and Indirect Sources of Hydrocarbons in Soils: Current Issues and Legal Implications

Marco Vecchiato,^{*,†,‡} Tiziano Bonato,^{†,‡} Andrea Bertin,[‡] Elena Argiriadis,[†] Carlo Barbante,^{†,§} and Rossano Piazza^{†,§}

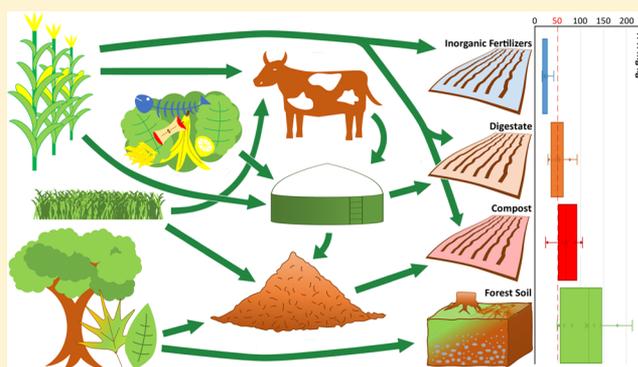
[†]Department of Environmental Sciences, Informatics and Statistics (DAIS), Ca' Foscari University of Venice, Via Torino 155, Venezia-Mestre, 30172 Venice, Italy

[‡]Società Estense Servizi Ambientali (S.E.S.A. S.p.A.), Via Comuna 5/B, 35042 Este, PD, Italy

[§]Institute for the Dynamics of Environmental Processes (IDPA-CNR), Via Torino 155, Venezia-Mestre, 30172 Venice, Italy

Supporting Information

ABSTRACT: Plant residues are the main source of organic matter in soil; this process takes place naturally in forests and with organic amendments in farmlands. Terrestrial plants also synthesize hydrocarbons. Typically, angiosperms contain hundreds to thousands of milligrams of long chain *n*-alkanes per kilogram in leaf waxes. However, petroleum pollution is a worldwide issue, and different national regulations set the guideline limit for petroleum hydrocarbons in green areas at 50 mg kg⁻¹. Focusing on the Italian legislation as a case study, we hypothesized that direct or indirect high inputs of plant residues could lead the level in the soil to exceed this limit, resulting in a false positive petroleum contamination. Therefore, we investigated the occurrence of hydrocarbons in soils with different inputs of natural or farming biomasses. The highest total petroleum hydrocarbon (TPH) concentrations were found in background soils from protected woodlands, remarkably with most samples resulting in levels above the guideline limit. Similarly, the TPH concentrations in agricultural soils amended with compost and digestate were higher than those in samples of soil in which only chemical fertilizers were used. *n*-Alkane carbon preference indices underlined the role of plant residues as a source of hydrocarbons in these samples, clearly distinguishing spiked petrogenic contamination. Possible revisions of the regulatory and analytical methods are then discussed.



INTRODUCTION

Terrestrial plants produce hydrocarbons as components of their epicuticular waxes to protect plant tissues from the external environment.¹ When plant residues are incorporated into soil, these contribute to the soil organic matter,² both naturally in woodlands and forests and artificially from crop residues or manure in agricultural lands. The addition of biomasses and fertilization with amendments, such as compost and digestate, have positive effects on the biological, physical, and chemical properties of soil, reducing the volume of waste dumped in landfill sites.^{3–5} Moreover, the organic matter in agricultural soils is considered one potential sink for the sequestration of atmospheric carbon dioxide.^{2,6}

On the other hand, petroleum hydrocarbons are some of the most widespread soil pollutants worldwide, and hydrocarbon contamination of soil is an issue that has been widely studied in the scientific literature. However, one of the main analytical and legal issues is how to distinguish actual petrogenic contamination from naturally occurring hydrocarbons and biogenic interference, because these could potentially lead to unnecessary remediation costs.⁷ However, although many parameters

(reported in the [Supporting Information](#)) have been proposed and used to identify environmental contamination caused by petroleum hydrocarbons,^{8–11} none of them is unique or effective for the different petroleum-derived products, probably except total petroleum hydrocarbons (TPH). This parameter, despite its selectivity drawbacks,⁷ is generally adopted to assess the contamination of soil by different petroleum products. The analytical determination of TPH includes a large family of different hydrocarbons, regardless of their toxicity, origin, or composition (linear, branched, cyclic, and aromatic compounds).^{12,13} Italian legislation (Legislative Decree 152/2006, Part IV, Title V, Annex 5) sets the limit for heavy hydrocarbons (with >12 carbon atoms) in public land, private land, residential green areas, and agricultural land at 50 mg (kg of dry weight)⁻¹; those sites found to exceed these limits should be remediated, or a site-specific risk analysis should prove the absence of

Received: October 15, 2017

Revised: November 10, 2017

Accepted: November 10, 2017

Published: November 10, 2017

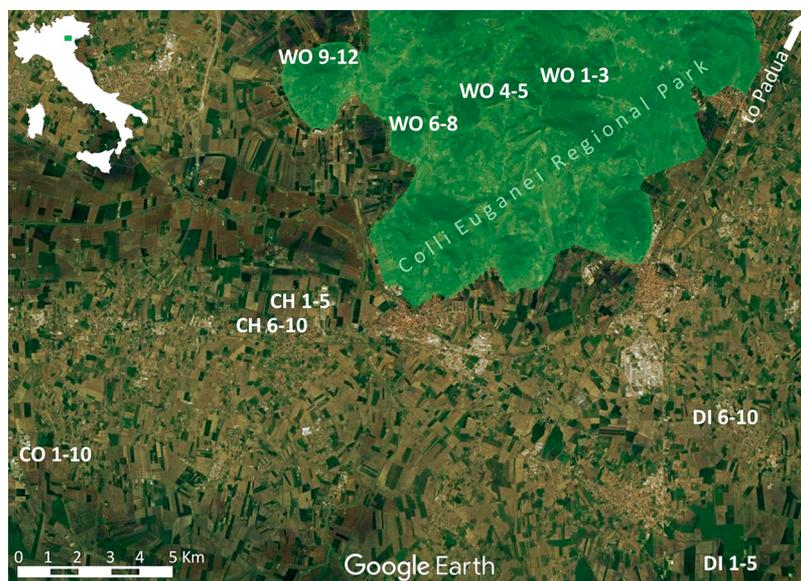


Figure 1. Locations of the sampling sites in the southern district of Padua, Italy. Agricultural soils were sampled in fields amended only with chemical fertilizers (CH), compost (CO), or digestate (DI). Woodland (WO) was sampled in different areas of the Colli Euganei Regional Park. Adapted from Google Earth, 2017.

human health risks. The parameter $C > 12$ substantially corresponds to TPH, defining a slight modification of the normal analytical method ISO 16703:2004 changing the application range from C_{10} – C_{40} to C_{12} – C_{40} .¹³

The limit of $50 \text{ mg (kg of dry weight)}^{-1}$ is not based on hydrocarbon toxicity, which is highly dependent on the speciation and the availability of the hydrocarbons, but was selected because it was considered to be high enough to avoid false positives.¹⁴ Consequently, this value was chosen as the target value for soil quality standards in different European countries.^{12,14} However, the various national regulations result in very different procedures and intervention guidelines for remediation of contamination episodes of agricultural soil. For example, there is a wide discrepancy of 2 orders of magnitude between the Italian and Dutch intervention values for remediation of hydrocarbons in soil, the limits being set at 50 and 5000 mg kg^{-1} (VROM 2012), respectively.¹² This clearly shows the need for the harmonization of national procedures for contaminated sites in the European Union.¹⁴ Outside Europe, a similar situation exists in the United States, with significant variability of methods, action, and cleanup levels for hydrocarbons in contaminated soils among the different states, in a framework of increasing complexity.¹⁵ Canadian legislation specifies different carbon ranges to analyze the total hydrocarbons in soil.⁷ Notably, the method does not include a purification step; therefore, polar biogenic compounds such as plant waxes, fatty acids, and sterols are also considered as petroleum hydrocarbon contaminants, even when their levels naturally exceed the regulatory levels.¹⁶

■ PLANT-DERIVED *n*-ALKANES

Terrestrial plants typically synthesize long chain *n*-alkanes as part of the epicuticular leaf wax, contributing to their hydrophobic properties and protecting the leaf from the external environment. Leaf *n*-alkanes are commonly produced with a strong odd-over-even predominance (OEP) and, lacking functional groups, are especially long-lived molecules.¹ For these reasons, *n*-alkanes are widely used as biomarkers to

reconstruct environmental changes in modern and paleoecological studies.^{1,17} The abundance and distribution of the *n*-alkanes in leaves depend on the plant species, the growing season, and the environmental conditions (e.g., temperature, moisture, nutrient availability, and soil properties), varying even within an individual tree.^{1,18} Typically, angiosperms produce hundreds to thousands of milligrams of *n*-alkanes per kilogram of dry leaf matter, while yields of gymnosperms are orders of magnitude lower. *n*- C_{27} , *n*- C_{29} , and *n*- C_{31} are the dominant *n*-alkanes in graminoids and woody angiosperms in variable relative abundances.¹ The carbon preference index (CPI) is a definitive measure of biologically synthesized compounds, defining the degree to which odd-carbon number *n*-alkanes dominate over even-carbon number compounds, the ratio of the sums of concentrations of odd and even *n*-alkane homologues being in a given carbon number range.¹⁹ Mean CPI values in leaves of a large majority of modern plants are around 10, although with very large variations; in a few cases, the values are $\lesssim 1$, which is the typical CPI value of petrogenic inputs.^{1,20} Another parameter is the weighted average of the various carbon chain lengths of the *n*-alkanes, usually defined as the average chain length $[ACL = \sum(C_n n) / \sum C_n]$, where C_n is the concentration of each *n*-alkane with *n* carbon atoms.¹ Leaf surface waxes can be sources of *n*-alkanes in soil in woodland and grassland,^{21,22} as well as directly from crop residues in agricultural land.²³ Other tissues of higher plants (e.g., beech and spruce roots) are characterized by short chain ($< C_{20}$) *n*-alkanes with an even-over-odd predominance, constituting a significant source of the *n*-alkanes in soil organic matter.^{22,24} In soils, other similar contributions of short chain *n*-alkanes without OEP might derive from bacterial, fungal, and algal biomass.^{18,25} These latter sources of *n*-alkanes are therefore less recognizable than inputs from epicuticular leaf waxes. Because of their stability, *n*-alkanes are also transferred to the residues of grazing animals and are used as biomarkers to reconstruct the composition of their dietary intake.²⁶

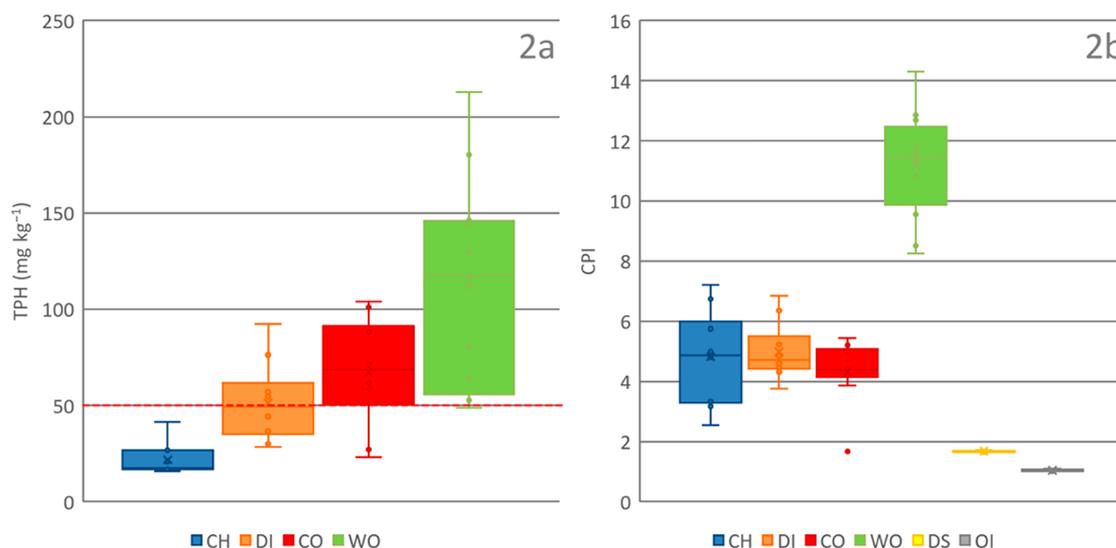


Figure 2. Distribution of the values of TPH (2a) and distribution of the values of the carbon preference index [$CPI = (\sum_{odd} C_{21-33} + \sum_{odd} C_{23-35}) / 2 \sum_{even} C_{22-34}$] (2b) in the different soil types. Box plots show the median, upper and lower quartiles, and maximum and minimum values, and crosses are at the arithmetic average, with outliers noted. The dashed line indicates the corresponding law limit for the concentration of hydrocarbons in green and agricultural areas.

OBJECTIVES

The main aim of this work is to investigate the occurrence of hydrocarbons in soil derived from plant residues of natural or farming origin, in relation to the corresponding legislative limits for green areas. This goal translates into a series of open questions. Can the parameter TPH naturally exceed the legal limits in cases of high organic matter inputs, as in background woodlands? Can certain agricultural practices, such as soil fertilization with plant residues and related products, such as compost and digestate, lead to increased hydrocarbon values in the soil? How can we distinguish the natural occurrence of hydrocarbons in soil from cases of real contamination by petroleum-derived products? To answer these questions, we analyzed TPH and *n*-alkanes in samples of soils subject to very different inputs of biomass, including protected woodlands and agricultural fields with different fertilization management.

MATERIALS AND METHODS

The study area was set in the southern district of Padua, Italy. To compare soils with different types of inputs of plant residues, we collected the samples shown in Figure 1. The sampling sites for agricultural soils were chosen because of their fertilization treatments, where during at least the past 10 years only chemical fertilizers (CH samples), only compost from green and organic urban wastes (CO), or only digestate from biogas reactors fed with cattle and pig manure and corn silage as cosubstrates (DI) were used. Two fields were selected for each of the three types of fertilizers, and five soil samples were collected in each field. The top 40 cm of soil was excavated, homogenized, and finally subsampled (April and May 2016). In the more recent years, these fields were used mainly for corn (*Zea mays* L.) production, occasionally in rotation with soybean (*Glycine max* L.) and rapeseed (*Brassica napus* L.) crops.

The non-anthropogenic vegetal inputs of hydrocarbons in soils were investigated in wooded areas of the nearby protected Colli Euganei Regional Park. Twelve topsoil samples (WO) were collected, avoiding surface litter of dry leaves and digging down to the skeleton horizon (approximately 15 cm depth). Details of samplings and of the tree species are listed in Table

S1. According to decree 152/2006, woodland and cultivated soils belong to the same category. To simulate the petrogenic contamination of farmland, e.g., leakages from agricultural machinery, parts of the CH samples were pooled and homogenized, and aliquots of ~20 g of soil were spiked with 10 μ L of diesel (DS; $n = 5$) or mineral oil (OI; $n = 5$). Also, one of the CO samples (CO7) was blind spiked with diesel prior to *n*-alkane analyses to evaluate the ability of the method to distinguish different and mixed sources. Details of TPH and *n*-alkane analytical determinations are reported in the Supporting Information.

RESULTS AND DISCUSSION

The samples are characterized by very different concentrations of TPH in soil (Figure 2a). The lowest mean levels [22 ± 8 mg (kg of dry weight)⁻¹] were found in the CH samples, ranging from 16 to 41 mg kg⁻¹. This probably accounts for the relatively low inputs of biomasses in these agricultural soils where only chemical fertilizers were used. On the contrary, the highest levels were detected in the WO samples, where oak woods [*Quercus pubescens* Wild. (Table S1)] generally dominate, with TPH concentrations of ≤ 213 mg (kg of dry weight)⁻¹, while the soils from areas in which locust trees (*Robinia pseudoacacia* L.) dominate exhibited relatively lower levels (Table S2). Remarkably, 11 of 12 samples collected in woodland exceed the guideline limit for the concentration of hydrocarbons in green areas (50 mg kg⁻¹), and the twelfth was very close (49 mg kg⁻¹) to it. The other agricultural soils are at a halfway point, the mean concentrations of TPH in DI and CO samples being 51 ± 20 and 67 ± 27 mg (kg of dry weight)⁻¹, respectively. Examples of the GC-FID chromatograms are shown in Figure S1.

The concentration of TPH is in accordance with the content of organic carbon (adjusted $R^2 = 0.660$; $N = 42$; $p = 0.0364$) (Figure S2), with the highest OC values being found in the WO samples (average $OC_{WO} = 18\%$), at levels similar to those of other forest soils.²⁷ On the other hand, the lowest OC concentrations were found in the CH samples (0.09–0.18%), reflecting the low inputs of biomasses in these soils. The

relatively higher average OC levels in DI and CO samples (0.34 and 0.29%, respectively) indicate that, compared to that of inorganic fertilizers, the application of digestate and compost is effective in increasing the content of organic matter in soil. This is well-known to enhance soil health, plant growth, and production yields.^{2,6} The WO samples were coarser than plain agricultural soil likely because of the stronger influence of the shallow parent stone in the hill forest soils (Table S2).

The results for the *n*-alkanes are similar to those for TPH, with the highest concentrations in the woodland soils and the lowest in CH samples (Table S2), although with different proportions. The sum of the main odd *n*-alkanes ($C_{27} + C_{29} + C_{31} + C_{33}$) represents 0.9–11% of the TPH values in agricultural soils, while it constitutes $\leq 58\%$ of the TPH in WO samples. Notably, in two samples (WO6 and WO7) the guideline limit is exceeded considering the sum of these four *n*-alkanes only. The different soil types can be distinguished considering the CPI [calculated as $CPI = (\sum \text{odd}C_{21-33} + \sum \text{odd}C_{23-35}) / 2\sum \text{even}C_{22-34}$]¹ (Figure 2b). The highest CPI values were found in the WO soils (mean CPI = 11), reflecting the relevant inputs of leaf waxes and resulting in a lower relative level of dispersion in comparison to total *n*-alkanes and TPH values (relative standard deviations of 16, 48, and 97%, respectively). Lower CPIs, with an average of ~ 5 , were detected in the agricultural samples, ranging from 3.8 to 6.9 in DI soil and from 3.9 to 5.4 in nonspiked CO samples. The lowest CPI values were found in the soils artificially contaminated with diesel and mineral oil and reflect such petrogenic sources, the CPI values being in the ranges of 1.6–1.7 (DS) and 1.0–1.1 (OI). Despite large relative variations in the total *n*-alkane concentrations (RSD values of 27 and 43%, respectively), the corresponding CPIs in DS and OI are much less dispersed, showing RSD values of 2 and 4%, respectively. Moreover, blind spiked sample CO7 clearly falls in this petrogenic CPI range, resulting as an outlier compared to the other CO soils (Figure 2b) and confirming the ability of the CPI approach to distinguish different sources.

Compared to WO soils, the lower CPI values in the agricultural samples are mainly caused by degradation processes of the original vegetal material and the contribution of bacteria, fungi, and biomasses with low CPIs.^{1,18,23,28,29} Moreover, the relative abundance of short chain *n*-alkanes ($\leq C_{20}$) with even-over-odd predominance in the CH, DI, and CO samples (Table S2) also confirms the role of grasses and other plant tissues (roots and spruce needles) as sources of hydrocarbons in cultivated soils.²² The same considerations are confirmed considering, in the C_{10} – C_{35} range, the parameter ACL,²⁴ which is higher in the WO samples (ACL = 29) than in CH, DI, and CO soils, with ACLs of 22, 25, and 25, respectively, as a consequence of relatively higher inputs of microbial biomass.²⁸ With a focus on the heavier *n*-alkanes, the most common carbon number maximum (C_{\max}) of the WO set is C_{29} , which is typical of the input from higher plants, while C_{\max} generally is C_{31} in agricultural soils, accounting for the influence of grasses.^{19,22} Similar concentrations and distribution patterns of the *n*-alkanes were found in other cultivated soils.^{28,30}

As expected, leaf waxes of trees predominate as the sources in *n*-alkanes in woodland, while the contribution of grasses, also containing hundreds to thousands of milligrams of *n*-alkanes per kilogram of dry weight,³¹ becomes more relevant in the agricultural soils. The sources of this latter “grasslike” pattern are likely only the crop residues in the CH samples, while the higher hydrocarbon concentrations in DI and CO soils deriving

from the additional inputs from the amendments. For example, corn, which is the main crop cultivated in the sampled fields, is a C_4 grass, and its contribution of *n*-alkanes from shoots and roots can be relevant in a cultivated soil;³² however, leaves can be major sources.²³ The digestate and compost can also contain *n*-alkanes from grass sources. Biogas reactors receive *n*-alkanes directly from the corn silage, where the whole plant is used,^{23,33} and indirectly from the livestock manure. In fact, the *n*-alkane signal of the vegetal feed can be maintained through the digestion of the grazing animals.²⁶ Also, the soils fertilized with the compost, produced with organic household wastes mixed with 20–30% grass cuttings and the remains of pruning, reflect these sources of leaf waxes. The concentrations of *n*-alkanes in compost can be similar to those of the starting vegetal materials, with the contribution of bacterial and fungal biomasses.³⁴ Moreover, under certain conditions, *n*-alkanes can be preserved during the composting processes because of their recalcitrant character,³⁵ and compared to other plant components, in the environment *n*-alkanes are more resistant to degradation and are prone to accumulating in soils.²⁷ Although the compost of urban wastes can contain considerable amounts of organic pollutants,³⁶ TPH is biodegraded during the composting process, and the application of compost enhances the bioremediation of diesel-contaminated soils.^{37,38}

From an analytical point of view, in addition to *n*-alkanes, alkenes and a variety of other plant-derived hydrocarbons⁸ can contribute to the TPH concentrations in soil as determined according to ISO 16703:2004 and similar protocols. These methods aim to analyze mineral oil and petrogenic products but cannot distinguish similar hydrocarbons of a biological origin, because only polar bioderived lipids, e.g., *n*-alkanols,¹⁷ are adsorbed and not adsorbed during the purification step. This issue underlines the importance of the polar biogenic interference as potential false positive contamination in the case of Canada, where also a biogenic interference calculation (BIC) index was proposed to determine levels of organic compounds in peat soils.^{39,40}

These findings clearly show that the different soil types are characterized by various TPH concentrations. However, using a simple CPI approach, it is possible to distinguish actual petrogenic contamination from natural hydrocarbon occurrence and between the inputs of different biomasses. In other heavily polluted Italian sites, TPH contamination can reach concentrations that are orders of magnitude higher than those of samples presented above, constituting up to 1% of the soil.⁴¹ Similar levels were found in different contaminated environments.^{16,42,43} On the other hand, total concentrations at levels comparable to those of WO samples were found at other Italian sites in contaminated industrial areas,⁴⁴ but in these cases, the petrogenic sources were revealed by typical CPI values of ~ 1 . With a focus on the concentrations in the WO soils and the corresponding CPIs, the detected levels are comparable to those found in other background and forest areas worldwide,^{7,16,21,27,45,46} demonstrating that the input of hydrocarbons from leaf waxes can naturally exceed the 50 mg kg⁻¹ threshold in soil. This answers one of our initial questions, and the results of the DI and CO samples highlight that the fertilization of soils with digestate and compost leads to an increase in the level of hydrocarbons in comparison to those of the soils for which only inorganic fertilizers were used. However, this increase, along with the OC increase, is likely due to the inputs of plant residues that constitute the amendments, as indicated by the CPI values. These ratios can in turn be a useful tool for

distinguishing the cases of real contamination by petroleum-derived products, as shown by the DS and OI samples.

■ LEGISLATIVE REVISION

The results described above indicate that the Italian legislation needs to be revised. We can likely suppose that large parts of the woodland and protected areas of Italy are actually exceeding the intervention limit at 50 mg (kg of dry weight)⁻¹, leading to the paradox that these soils should be remediated because of the presence of leaf wax *n*-alkanes. These restrictive limits are very conservative and protective for human health, but this excessive caution leads to a number of false positives in those cases in which the natural occurrence of *n*-alkanes in soil is considered petrogenic pollution. A possible legislative modification should also be considered by other nations that have adopted similar guidelines. Moreover, the Italian legislation should specify the petrogenic origin in the soil contamination limits. In our opinion, this regulatory revision of the forensic and analytical methods can follow three different approaches, each with specific advantages and disadvantages. (I) The first and readily applicable approach is to increase the intervention limit to newly defined background values, to exclude those soils with high inputs of leaves and vegetal organic matter. This has the drawback of also excluding possible false negative cases of low levels of real petrogenic contamination, although the change would make the Italian legislation more similar to other national regulations.¹⁴ Lawmakers should also take into account the effects of fertilization of fields with digestate and compost, considering the importance of these farming practices for organic waste management and carbon dioxide sequestration.^{3,6} (II) Another easily applicable approach is to include in the analytical protocols the determination of *n*-alkanes, using the resulting CPI values to discriminate between the petrogenic and leaf wax hydrocarbons. The major drawback of this approach is that it could not work in the likely very frequent cases of mixed sources.^{7,47} Natural or intentional high inputs of vegetal biomasses with strong OEP in soils *de facto* would allow higher levels of petrogenic contamination to cover the relatively low CPI signal. Nevertheless, this approach would be applicable in those cases in which it is the mere presence of the heavy *n*-alkanes typical of leaf waxes that exceeds the legal limits. (III) The third and more complex approach is to determine and compare a variety of source-specific biomarkers and ratios (see a paragraph in the [Supporting Information](#)), each diagnostic of distinct sources for fingerprinting different types of petrogenic pollution.^{12,43,48} This integrated approach requires greater analytical efforts but would be applicable in specific contamination episodes to identify the sources of pollution.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.estlett.7b00464](https://doi.org/10.1021/acs.estlett.7b00464).

Petrogenic fingerprinting, analytical methods, WO dominant species (Table S1), chromatograms (Figure S1), OC vs TPH (Figure S2), and TPH, *n*-alkanes, CPIs, ACLs, OCs, and soil texture (Table S2) ([PDF](#))

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: vecchiato@unive.it. Phone: +39 0412348545.

ORCID

Marco Vecchiato: [0000-0002-5112-8472](https://orcid.org/0000-0002-5112-8472)

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was financially supported by the public utility company Società Estense Servizi Ambientali (S.E.S.A.), Este, PD, Italy.

■ REFERENCES

- (1) Bush, R. T.; McInerney, F. A. Leaf wax *n*-alkane distributions in and across modern plants: Implications for paleoecology and chemotaxonomy. *Geochim. Cosmochim. Acta* **2013**, *117*, 161–179.
- (2) Lal, R. Soil carbon sequestration to mitigate climate change. *Geoderma* **2004**, *123*, 1–22.
- (3) Tambone, F.; Scaglia, B.; D'Imporzano, G.; Schievano, A.; Orzi, V.; Salati, S.; Adani, F. Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost. *Chemosphere* **2010**, *81*, 577–583.
- (4) Hargreaves, J. C.; Adl, M. S.; Warman, P. R. A review of the use of composted municipal solid waste in agriculture. *Agric., Ecosyst. Environ.* **2008**, *123*, 1–14.
- (5) Weiland, P. Biogas production: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 849–860.
- (6) Stockmann, U.; Adams, M. A.; Crawford, J. W.; Field, D. J.; Henakaarchchi, N.; Jenkins, M.; Minasny, B.; McBratney, A. B.; Courcelles, V. de R. de; Singh, K.; et al. The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agric., Ecosyst. Environ.* **2013**, *164*, 80–99.
- (7) Wang, Z.; Yang, C.; Kelly-Hooper, F.; Hollebhone, B.; Peng, X.; Brown, C. E.; Landriault, M.; Sun, J.; Yang, Z. Forensic differentiation of biogenic organic compounds from petroleum hydrocarbons in biogenic and petrogenic compounds cross-contaminated soils and sediments. *J. Chromatogr. A* **2009**, *1216*, 1174–1191.
- (8) Volkman, J. K.; Holdsworth, D. G.; Neill, G. P.; Bavor, H. J., Jr. Identification of natural, anthropogenic and petrogenic hydrocarbons in aquatic sediments. *Sci. Total Environ.* **1992**, *112*, 203–219.
- (9) Wang, Z.; Fingas, M. Differentiation of the source of spilled oil and monitoring of the oil weathering process using gas chromatography-mass spectrometry. *J. Chromatogr. A* **1995**, *712*, 321–343.
- (10) Xiong, W.; Bernesky, R.; Bechard, R.; Michaud, G.; Lang, J. A tiered approach to distinguish sources of gasoline and diesel spills. *Sci. Total Environ.* **2014**, *487*, 452–462.
- (11) Vecchiato, M.; Argiriadis, E.; Zambon, S.; Barbante, C.; Toscano, G.; Gambaro, A.; Piazza, R. Persistent Organic Pollutants (POPs) in Antarctica: Occurrence in continental and coastal surface snow. *Microchem. J.* **2015**, *119*, 75–82.
- (12) Pinedo, J.; Ibáñez, R.; Lijzen, J. P. A.; Irabien, Á. Assessment of soil pollution based on total petroleum hydrocarbons and individual oil substances. *J. Environ. Manage.* **2013**, *130*, 72–79.
- (13) Istituto Superiore per la protezione e la ricerca ambientale. Procedura per l'analisi degli idrocarburi > C12 in suoli contaminati. n. 75/2011; ISPRA-Manuali e Linee Guida: Rome, 2011.
- (14) Carlon, C.; D'Alessandro, M.; Swartjes, F. Derivation methods of soil screening values in Europe. A review and evaluation of national procedures towards harmonisation. EUR 22805-EN; Carlon, C., Ed.; European Commission, Joint Research Centre: Ispra, Italy, 2007.
- (15) Tomlinson, P.; Ruby, M. V. State and federal cleanup levels for petroleum hydrocarbons in soil: State of the states and implications for the future. *Hum. Ecol. Risk Assess.* **2016**, *22*, 911–926.
- (16) Wang, Z.; Yang, C.; Yang, Z.; Hollebhone, B.; Brown, C. E.; Landriault, M.; Sun, J.; Mudge, S. M.; Kelly-Hooper, F.; Dixon, D. G. Fingerprinting of petroleum hydrocarbons (PHC) and other biogenic organic compounds (BOC) in oil-contaminated and background soil samples. *J. Environ. Monit.* **2012**, *14*, 2367–2381.

- (17) Diefendorf, A. F.; Freeman, K. H.; Wing, S. L.; Graham, H. V. Production of n-alkyl lipids in living plants and implications for the geologic past. *Geochim. Cosmochim. Acta* **2011**, *75*, 7472–7485.
- (18) Kirkels, F. M. S. A.; Jansen, B.; Kalbitz, K. Consistency of plant-specific n-alkane patterns in plaggén ecosystems: A review. *Holocène* **2013**, *23*, 1355–1368.
- (19) Simoneit, B. R. T. Organic matter of the troposphere - V: Application of molecular marker analysis to biogenic emissions into the troposphere for source reconciliations. *J. Atmos. Chem.* **1989**, *8*, 251–275.
- (20) Yang, C.; Yang, Z.; Zhang, G.; Hollebone, B.; Landriault, M.; Wang, Z.; Lambert, P.; Brown, C. E. Characterization and differentiation of chemical fingerprints of virgin and used lubricating oils for identification of contamination or adulteration sources. *Fuel* **2016**, *163*, 271–281.
- (21) Jansen, B.; Nierop, K. G. J.; Kotte, M. C.; de Voogt, P.; Verstraten, J. M. The applicability of accelerated solvent extraction (ASE) to extract lipid biomarkers from soils. *Appl. Geochem.* **2006**, *21*, 1006–1015.
- (22) Kuhn, T. K.; Krull, E. S.; Bowater, A.; Grice, K.; Gleixner, G. The occurrence of short chain n-alkanes with an even over odd predominance in higher plants and soils. *Org. Geochem.* **2010**, *41*, 88–95.
- (23) Lichtfouse, E.; Elbisser, B.; Balesdent, J.; Mariotti, A.; Bardoux, G. Isotope and molecular evidence for direct input of maize leaf wax n-alkanes into crop soils. *Org. Geochem.* **1994**, *22*, 349–351.
- (24) Griepentrog, M.; Bodé, S.; Boeckx, P.; Wiesenberg, G. L. B. The fate of plant wax lipids in a model forest ecosystem under elevated CO₂ concentration and increased nitrogen deposition. *Org. Geochem.* **2016**, *98*, 131–140.
- (25) Rao, Z.; Zhu, Z.; Wang, S.; Jia, G.; Qiang, M.; Wu, Y. CPI values of terrestrial higher plant-derived long-chain n-alkanes: A potential paleoclimatic proxy. *Front. Earth Sci. China* **2009**, *3*, 266–272.
- (26) Sun, H. X.; Zhou, D. W. Prediction of diet composition and intake of sheep grazing soybean and maize residues. *J. Appl. Anim. Res.* **2009**, *35*, 125–129.
- (27) Marseille, F.; Disnar, J. R.; Guillet, B.; Noack, Y. n-Alkanes and free fatty acids in humus and A1 horizons of soils under beech, spruce and grass in the Massif-Central (Mont-Lozère), France. *Eur. J. Soil Sci.* **1999**, *50*, 433–441.
- (28) Wiesenberg, G. L. B.; Dorodnikov, M.; Kuzyakov, Y. Source determination of lipids in bulk soil and soil density fractions after four years of wheat cropping. *Geoderma* **2010**, *156*, 267–277.
- (29) Pietrogrande, M. C.; Mercuriali, M.; Pasti, L.; Dondi, F. Data handling of complex GC-MS chromatograms: characterization of n-alkane distribution as chemical marker in organic input source identification. *Analyst* **2009**, *134*, 671–680.
- (30) Wiesenberg, G. L. B.; Schwarzbauer, J.; Schmidt, M. W. I.; Schwark, L. Source and turnover of organic matter in agricultural soils derived from n-alkane/n-carboxylic acid compositions and C-isotope signatures. *Org. Geochem.* **2004**, *35*, 1371–1393.
- (31) Rommerskirchen, F.; Plader, A.; Eglinton, G.; Chikaraishi, Y.; Rullkötter, J. Chemotaxonomic significance of distribution and stable carbon isotopic composition of long-chain alkanes and alkan-1-ols in C₄ grass waxes. *Org. Geochem.* **2006**, *37*, 1303–1332.
- (32) Quéneá, K.; Largeau, C.; Derenne, S.; Spaccini, R.; Bardoux, G.; Mariotti, A. Molecular and isotopic study of lipids in particle size fractions of a sandy cultivated soil (Cestas cultivation sequence, southwest France): Sources, degradation, and comparison with Cestas forest soil. *Org. Geochem.* **2006**, *37*, 20–44.
- (33) Miller, S. S.; Reid, L. M.; Butler, G.; Winter, S. P.; McGoldrick, N. J. Long Chain Alkanes in Silk Extracts of Maize Genotypes with Varying Resistance to *Fusarium graminearum*. *J. Agric. Food Chem.* **2003**, *51*, 6702–6708.
- (34) Spaccini, R.; Baiano, S.; Gigliotti, G.; Piccolo, A. Molecular Characterization of a Compost and Its Water-Soluble Fractions. *J. Agric. Food Chem.* **2008**, *56*, 1017–1024.
- (35) El Fels, L.; Lemee, L.; Ambles, A.; Hafidi, M. Identification and biotransformation of aliphatic hydrocarbons during co-composting of sewage sludge-Date Palm waste using Pyrolysis-GC/MS technique. *Environ. Sci. Pollut. Res.* **2016**, *23*, 16857–16864.
- (36) Brändli, R. C.; Bucheli, T. D.; Kupper, T.; Furrer, R.; Stahel, W. a.; Stadelmann, F. X.; Tarradellas, J. Organic pollutants in compost and digestate. Part 1. Polychlorinated biphenyls, polycyclic aromatic hydrocarbons and molecular markers. *J. Environ. Monit.* **2007**, *9*, 456–464.
- (37) Semple, K. T.; Reid, B. J.; Fermor, T. R. Impact of composting strategies on the treatment of soil contaminated with diesel fuel.pdf. *Environ. Pollut.* **2001**, *112*, 269–283.
- (38) Namkoong, W.; Hwang, E. Y.; Park, J. S.; Choi, J. Y. Bioremediation of diesel-contaminated soil with composting. *Environ. Pollut.* **2002**, *119*, 23–31.
- (39) Kelly-Hooper, F.; Farwell, A. J.; Pike, G.; Kennedy, J.; Wang, Z.; Grunsky, E. C.; Dixon, D. G. Field survey of Canadian background soils: Implications for a new mathematical gas chromatography-flame ionization detection approach for resolving false detections of petroleum hydrocarbons in clean soils. *Environ. Toxicol. Chem.* **2014**, *33*, 1754–1760.
- (40) Kelly-Hooper, F.; Farwell, A. J.; Pike, G.; Kennedy, J.; Wang, Z.; Grunsky, E. C.; Dixon, D. G. Is it clean or contaminated soil? Using petrogenic versus biogenic GC-FID chromatogram patterns to mathematically resolve false petroleum hydrocarbon detections in clean organic soils: A crude oil-spiked peat microcosm experiment. *Environ. Toxicol. Chem.* **2013**, *32*, 2197–2206.
- (41) Guarino, C.; Spada, V.; Sciarillo, R. Assessment of three approaches of bioremediation (Natural Attenuation, Landfarming and Bioaugmentation e Assisted Landfarming) for a petroleum hydrocarbons contaminated soil. *Chemosphere* **2017**, *170*, 10–16.
- (42) Teng, Y.; Zhou, Q.; Miao, X.; Chen, Y. Assessment of soil organic contamination in a typical petrochemical industry park in China. *Environ. Sci. Pollut. Res.* **2015**, *22*, 10227–10234.
- (43) Wang, Z.; Yang, C.; Parrott, J. L.; Frank, R. A.; Yang, Z.; Brown, C. E.; Hollebone, B.; Landriault, M.; Fieldhouse, B.; Liu, Y.; et al. Forensic source differentiation of petrogenic, pyrogenic, and biogenic hydrocarbons in Canadian oil sands environmental samples. *J. Hazard. Mater.* **2014**, *271*, 166–177.
- (44) Riccardi, C.; Di Filippo, P.; Pomata, D.; Di Basilio, M.; Spicaglia, S.; Buiarelli, F. Identification of hydrocarbon sources in contaminated soils of three industrial areas. *Sci. Total Environ.* **2013**, *450–451*, 13–21.
- (45) Rao, Z.; Wu, Y.; Zhu, Z.; Jia, G.; Henderson, A. Is the maximum carbon number of long-chain n-alkanes an indicator of grassland or forest? Evidence from surface soils and modern plants. *Chin. Sci. Bull.* **2011**, *56*, 1714–1720.
- (46) Jansen, B.; Nierop, K. G. J.; Hageman, J. A.; Cleef, A. M.; Verstraten, J. M. The straight-chain lipid biomarker composition of plant species responsible for the dominant biomass production along two altitudinal transects in the Ecuadorian Andes. *Org. Geochem.* **2006**, *37*, 1514–1536.
- (47) Scholz-Böttcher, B. M.; Ahlf, S.; Vázquez-Gutiérrez, F.; Rullkötter, J. Natural vs. anthropogenic sources of hydrocarbons as revealed through biomarker analysis: A case study in the southern Gulf of Mexico. *Bol. Soc. Geol. Mex.* **2009**, *61*, 47–56.
- (48) Kaplan, I. R.; Galperin, Y.; Lu, S. T.; Lee, R. P. Forensic environmental geochemistry: Differentiation of fuel-types, their sources and release time. *Org. Geochem.* **1997**, *27*, 289–317.