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Mechanisms of Distinct Activated Carbon and Biochar

Amendment Effects on Petroleum Vapour Biofiltration in Soil

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Abstract

We studied effects of two percent by weight activated carbon versus biochar amendments in 93 cm long sand columns on the biofiltration of petroleum vapours released by a non-aqueous phase liquid (NAPL) source. Activated carbon greatly enhanced, whereas biochar slightly reduced the biofiltration of volatile petroleum hydrocarbons (VPHs) over 430 days. Sorbent amendment benefitted the VPH biofiltration by retarding breakthrough during the biodegradation lag phase. Subsequently, sorbent amendment briefly reduced the mineralization of petroleum hydrocarbons by limiting their bioavailability. During the last and longest study period, when conditions became less supportive of microbial growth, because of inorganic nutrient scarcity, the sorbents again improved the pollution attenuation by preventing the degrading microorganisms from being overloaded with VPHs. A 16s rRNA gene based analysis showed sorbent amendment effects on soil microbial communities. *Nocardioideae* benefitted the most from petroleum hydrocarbons in activated carbon

amended soil, whereas *Pseudomonadacea* predominated in unamended soil. Whilst the degrading microorganisms were overloaded with VPHs in the unamended soil, the reduced mobility and bioavailability of VPHs in the activated carbon amended soil led to the emergence of communities with higher specific substrate affinity, which removed bioavailable VPHs effectively at low concentrations. A numerical pollutant fate model reproduced these experimental observation by considering sorption effects on the pollutant migration and bioavailability for growth of VPH degrading biomass, which is limited by a maximum soil biomass carrying capacity. Activated carbon was a much stronger sorbent for VPHs than biochar, which explained the diverging effects of the two sorbents in this study.

Key words: Petroleum vapour, biochar, activated carbon, soil bioremediation, biofiltration

Graphical abstract

Introduction

Vast amounts of oils and fuels are used world-wide, and petroleum spillages are among the most frequently reported incidents of soil and water pollution ¹. Petroleum hydrocarbon vapours migrate from spilled liquids through the vadose zone towards indoor air, outdoor air, and groundwater, posing a significant exposure pathway in contaminated land usage scenarios ². The biofiltration of volatile petroleum hydrocarbons (VPHs) in soil provides a barrier to contaminant spreading and can considerably reduce exposure risks ³⁻⁶.

Sorption and biodegradation are key processes contributing to this natural VPH attenuation in soil ⁶, but potential interactions between sorption and biodegradation are manifold and difficult to predict.

Figures 1 a and b illustrate how an enhanced sorption capacity (expressed as linear solid-water distribution coefficient K_d) may affect the natural attenuation of a biodegradable petroleum vapour that is transported by gas diffusion over a certain distance in unsaturated soil. The predictions are based on the common assumptions that VPH degradation occurs in the soil water and can be described by first-order kinetics ^{3,7}. If these assumptions are valid, enhanced sorption (i.e., a larger K_d -value) will (i) delay VPH migration through a layer of soil and (ii) reduce the peak concentration of VPH if the source is small and finite, but (iii) the cumulative VPH mass breaking through the soil layer over the lifetime of the source will eventually reach the same level, that is, it is independent of K_d . In reality, the dynamics of soil biomass ^{8,9}, growth limitations ¹⁰, microbial ecology ^{11,12}, and metabolic regulation at the cell level ^{13,14} complicate this picture greatly, and, depending on the dominating mechanisms, enhanced sorption may potentially reduce or enhance cumulative VPH mass breakthrough. Understanding complex trade-offs between sorption and biodegradation is broadly relevant to pollutant fate predictions in the environment, and especially important for sorbent-based in-

situ soil remediation, which seeks to reduce exposure risks at contaminated sites¹⁵⁻²⁰. We performed long-term column studies, amending activated carbon or biochar to construction sand exposed to biodegradable VPHs released from a finite NAPL source to compare the sorbent amendment effects on the attenuation of typical VPHs. Our study aim was to derive the scientific understanding required for predicting coinciding sorption and biodegradation effects on the VPH fate in the environment, so that these scientific principles can then be practically applied to the design of enhanced petroleum vapour biofiltration in soil with the addition of carbonaceous sorbent materials such as activated carbon or biochar. We tested the hypothesis that the sorbent amendments would not just delay the pollutant breakthrough, but more lastingly enhance the petroleum vapour biofiltration.

Materials and Methods

Gravelly sand (Soil), biochar (BC) and activated carbon (AC). For a porous medium, we chose gravelly construction sand (89.5% by weight sand with 0.063 to 2 mm particle size). The total organic carbon content (TOC) was $1.6 \pm 0.1\%$, the total carbon content was $4.1 \pm 0.1\%$, and the pH measured in 0.01 M CaCl_2 was 7.43. A commercial biochar, produced from wood chips by fast pyrolysis at high temperature (800°C) in a fixed bed reactor, was obtained from Environmental Power International, EPI (Wiltshire, UK), and used in this study due to its large surface area. This biochar was ground to a particle size $<163 \mu\text{m}$, had a TOC of $85 \pm 2\%$, and an alkaline pH of 9.25 ± 0.16 . Type F400 activated carbon (Chemviron Carbon Ltd, Lancashire, UK) was also ground to a particle size $<163 \mu\text{m}$, had a TOC content of $73 \pm 1\%$, and pH of 8.73 ± 0.10 . Biochar and activated carbon surface areas were measured by CO_2 adsorption using an Intelligent Gravimetric Analyzer (IGA) (Hidden Isochema Ltd., Warrington, U.K) and determined to be $928 \text{ m}^2/\text{g}$ and $1012 \text{ m}^2/\text{g}$, respectively.

Chemicals. Twelve major constituents of gasoline or kerosene combined in typical ratios³ represented a well-defined non-aqueous phase liquid (NAPL) source, prepared from high purity chemicals obtained from Sigma-Aldrich (Dorset, UK)²¹. The NAPL source of each column consisted of five straight-chain alkanes (0.62 g pentane, 1.13 g hexane, 1.11 g octane, 2.02 g decane, 0.89 g dodecane), four branched and/or cyclic alkanes (1.10 g methylcyclopentane, 1.83 g methylcyclohexane, 1.24 g cyclohexane, 2.07 g isooctane), and three monoaromatic compounds (0.69 g toluene, 0.85 g m-xylene, 1.05 g 1,2,4-trimethylbenzene). These petroleum hydrocarbon compound classes vary in polarity and biodegradability²²⁻²⁴. Sulfur hexafluoride (SF₆) (Sigma-Aldrich, Dorset, UK) was used as a conservative tracer.

Column experiments. Three horizontally placed glass columns of 120 cm length and 7.8 cm internal diameter (Figure 1c) were homogeneously packed over a length of 93 cm with the gravelly sand (soil), gravelly sand with 2% dry weight (d.w.) biochar (soil & BC), and gravelly sand with 2% d.w. activated carbon (soil & AC), respectively. TOC contents were 1.6±0.1, 3.0±0.1, and 2.7±0.3 % d.w., total porosities 0.55±0.02, 0.60±0.03 and 0.59±0.02 cm³/cm³, and initial volumetric water contents 0.11±0.01, 0.12±0.02, and 0.11±0.01 cm³/cm³, for the soil, soil & BC and soil & AC columns, respectively. Thus, in agreement with previous work,²⁵ the carbonaceous amendments moderately enhanced soil macroporosity. After packing, the columns were left undisturbed for five days to monitor background soil respiration, and then, nominally on day 0, a vial containing 20 mL VPH mixture was tightly connected to a curved glass tube with 1.1 cm internal diameter at one end of the columns using a Teflon-lined rubber seal. The other column ends were purged into the fume hood with a water-saturated airflow rate of 5±1 mL min⁻¹ to strip the petroleum vapours and simulate a near-zero concentration boundary condition. Each column was equipped with seven sampling ports positioned at 15 cm distances, and the ports were sealed with GC septa (Thermogreen

LB-2, Supelco, Bellefonte, USA). Soil air samples were withdrawn through these ports with gas tight syringes for analysis. An initial SF₆ tracer test was conducted to determine the tortuosity of the gas-filled pore space ²⁶. After connecting the sources to the columns, VPH and CO₂ and O₂ concentrations in soil air were monitored weekly at each sampling port (21 in total) for the duration of the experiment. On day 430, the soil was removed from the columns in three equal sections (namely the 'VPH source side' between ports 1 and 3, the 'Middle' between ports 3 and 5, and the 'Atmospheric boundary side' between ports 5 and 7 in Figure 1c) and soil subsamples were immediately extracted with 40:60 v/v dichloromethane/pentane for residual VPH quantification. Inorganic nutrient contents, total and organic carbon contents were also determined.

Batch experiments. A series of replicated batch microcosm experiments with the three column soil types and appropriate sterile controls were performed to complement the column studies and measure VPH sorption coefficients, VPH biodegradation rates, and nutrient availability effects on VPH biodegradation and soil microbiology. Batch study details are provided as Supplementary Information, and followed protocols described by Bushnaf et al. ²¹.

Soil microbiology analysis. Total bacterial cell numbers were determined by mixing 5 µl of sample stored in ethanol/PBS mixture with 995 µl of filtered-sterile phosphate buffer saline (PBS, Oxoid), as previously described ¹⁵. Microbial communities were investigated by high-throughput sequencing. DNA was extracted in duplicate from 500 mg of soil (wet weight), using the FastDNA Spin kit (MP Biomedicals, UK). Amplicon libraries were prepared from each extracted DNA sample by PCR amplifying the V4-V5 regions of the 16S rRNA gene ²⁷. Details are reported as Supplementary Information. The individual amplicon libraries were pooled together in equimolar amounts, and sequenced on a Roche 454 GS FLX+ System by NewGene Ltd (International Centre for Life, Newcastle upon Tyne, UK), for most samples,

and (following withdrawal of that platform by the supplier) on an Ion Torrent Personal Genome Machine (School of Engineering, Newcastle University, UK), for the samples from one of the batch studies. Further details, including about bioinformatics, are provided as Supplementary Information.

Analytical protocols. VPH analysis was performed with an Agilent HP-7890 gas chromatograph using a flame ionization detector (Agilent Technologies, Palo Alto, USA), and CO₂ and O₂ and tracer SF₆ analysis was performed on a Fisons 8060 gas chromatograph linked to a Fisons MD800 mass spectrometer ²¹. More details of analytical protocols, including for inorganic nutrient analysis, are provided as Supplementary Information.

Modelling. To interpret the column study results, an earlier petroleum vapour fate model ¹⁶ was improved by (i) considering VPH intraparticle diffusion in biochar and activated carbon, which is particularly important for simulating activated carbon amendment effects, and (ii) accounting for logistic growth with a maximum soil biomass, which provides greater flexibility as compared to the earlier model version ¹⁶, since any limiting factor, including nitrogen availability, can potentially be used to predict the soil biomass carrying capacity. Like its previous version ¹⁶, the model considers the effect of changes in source composition on VPH concentrations in soil air above the NAPL source using Raoult's law, the effect of air-water and air-solid partitioning on VPH migration from the NAPL source through the soil towards the atmospheric boundary side of the columns, the biodegradation of VPHs dissolved in soil pore water, and the growth and decay of VPH degrading biomass. Detailed modelling assumptions, equations, and boundary conditions are provided as Supplementary Information.

Results and Discussion

Sorbent amendment effects on VPH sorption. Despite their similar BET surface areas, the biochar was a weaker VPH sorbent than the activated carbon (see distribution coefficients K_d

in Table S1 in Supplementary Information). Amendment of gravelly sand with 2% biochar enhanced the sorption of straight-chained, branched, and cyclic alkanes by approximately a factor of 2 while the sorption of monoaromatic compounds was increased by up to a factor of 10, confirming preferential monoaromatic hydrocarbon sorption by this biochar ²¹. The addition of 2% activated carbon to gravelly sand much more substantially enhanced the sorption of all VPHs, by up to three orders of magnitude. Because the biochar and activated carbon had similar total surface areas, the observed differences are likely explained by different pore network structures and surface chemistries. The higher pH of the biochar for example suggests a more basic nature, which may hinder the adsorption of hydrophobic alkanes. In this context, it is important to note that sorption performance varies greatly for different biochar types ²⁰, meaning that other biochar types may be able to bind VPHs as strongly as activated carbon.

Inorganic nutrient availability and VPH sorption effects on petroleum vapour

biodegradation in batches. If added at low concentrations (0.002 mL of VPH mixture per 15 g of wet soil), all VPHs were biodegradable, as indicated by the apparent first-order biodegradation rates measured in the batch studies (Table S1 and Figure S1 in Supplementary Information). A biodegradation lag phase was noted for some VPHs such as isooctane (Figure S1b in Supplementary Information). If added at higher concentrations (0.03 mL of VPH mixture per 15 g of wet soil), VPH biodegradation was hindered by insufficient inorganic nutrient availability (Figure 2). In agreement with previous studies ²⁸, provision of inorganic nitrogen and phosphorus to achieve a VPH-C to N to P ratio of 100:10:1 significantly accelerated VPH biodegradation by intrinsic soil microorganisms (t-test, two-tailed, $p < 0.01$), while provision of only nitrogen or phosphorus had lesser benefits, which were not statistically significant (t-test, two-tailed, $p > 0.05$, blue bars in Figure 2). Inorganic nutrient amendment effects on VPH biodegradation in the soil & AC batches were not

statistically significant in any of the treatments (t-test, two-tailed, $p > 0.05$, green bars in Figure 2). Apparently, strong sorption by activated carbon became the VPH biodegradation rate controlling factor when N and P were sufficiently available. Without inorganic nutrient additions, the extent of VPH biodegradation was not significantly different in soil and soil & AC batches (t-test, two-tailed, $p > 0.05$), showing that both, sorption and inorganic nutrient scarcity, may limit VPH biodegradation in the geomeidia investigated. Observations in soil & BC batches (red bars in Figure 2) fell in between the soil and soil & AC trends, as would be expected based on the respective K_d values (Table S1 in Supplementary Information).

Activated carbon and biochar amendment effects on the petroleum vapour attenuation

in the column study. For the petroleum vapour migration exposure pathway, only those VPHs which emanate from the soil can potentially reach receptors via their presence in indoor and outdoor air. We therefore use the cumulative VPH-C emanation over the 430 day period as a measure for the incomplete VPH attenuation (Figure 3a), and by this measure, the activated carbon amended soil column most effectively, and the biochar amended soil column least effectively, attenuated the VPHs released by the NAPL source. The cumulative VPH-C emanation from the soil & AC column was at all times much less than from the soil and soil & BC columns. While the total VPH-C emanation was higher for the soil & biochar than the unamended soil column (3.18 versus 2.56 g by day 430, respectively), which could be partially due to the somewhat greater air-filled porosity of the soil & biochar column, emanation of monoaromatic VPHs, which bind strongly to the biochar used in this study, was lower for the soil & biochar as compared to the unamended soil column (0.005 versus 0.020 g monoaromatic VPH-C by day 430, respectively). The cumulative CO_2 -C emanation (Figure 3b) was initially substantially lower for the soil & AC as compared to the other two columns, but then rose more steadily to reach and surpass the flux from the other columns. Since background soil respiration measured before the connection of the NAPL source was very

low in this study, the VPH-C relative to CO₂-C ratio relates on a carbon-normalized basis the amount of VPH which was able to pass unaltered through the column (i.e. was not attenuated), to the amount of VPH which has been mineralized into CO₂. By day 430, the ratio of the cumulative VPH-C relative to CO₂-C emanation from the columns was only 1:77 for the soil & AC column, showing substantial VPH attenuation, as compared to 1:1.3 and 1:0.8 for the soil and soil & BC columns, respectively.

Notable characteristics of the petroleum vapour and CO₂ breakthrough curves

measured in the column study. VPH and CO₂ breakthrough curves measured at port 4 in the middle of the three columns are compared in Figure 4. To reduce the number of plots, the twelve VPH compounds have been aggregated into the petroleum vapour compound classes of straight-chain alkanes (Figure 4a), branched/cyclic alkanes (Figure 4b), and monoaromatic hydrocarbons (Figure 4c), while CO₂-C data provide context (Figure 4d). Characteristic features distinguished the measured VPH breakthrough curves in soil and soil & BC from those anticipated by a first-order rate model (Figure 1a) : Instead of a monotonous increase and then decline of the VPH concentrations, an initial spike in the measured VPH concentrations was followed by a temporary dip around days 5 to 10, especially notable for the straight-chain alkanes, which was followed by a secondary maximum around days 20 to 30, before the concentrations of the more volatile VPHs again declined due to the exhaustion of their finite NAPL source (Figures 4a,b,c). These features confirm the three VPH biodegradation phases tentatively identified in our previous, short-term study ¹⁶: i) a biodegradation lag phase; ii) a brief period of intensive VPH biodegradation followed by; iii) less optimal conditions for VPH biodegradation.

Mass balances, total cell counts and nutrient availability at the end of the column study.

On day 430, the remaining NAPL sources consisted to 99% of the three least volatile compounds, 1,2,4-TMB (0.2-5%), decane (11-31%) and dodecane (56-89%), showing that

the other nine VPHs had been largely exhausted from the source. The soil & AC column had the lowest NAPL source residual (Table 1), which can be explained with the enhanced sorption of VPHs by the activated carbon fostering VPH volatilization from the NAPL. VPH residuals within the columns on day 430 were highest for the soil & AC column, due to the strongest VPH retention in this column. However, the VPH residual is too small to alone explain the reduced VPH-C emanation from the soil & AC column at the atmospheric boundary side (Table 1). The VPH residual in the soil & AC column consisted to 84% of isooctane, which is one of the more recalcitrant VPH compounds ⁷. Poor extractability of VPHs from activated carbon can therefore not explain the absence of more substantial VPH residuals, since it would have affected isooctane and other alkanes similarly. Instead, an apparent increase in the soil carbon content, especially notable in the soil & AC column, but also in the two other columns, can potentially account for the unknown gaps in the VPH-C mass balances (Table 1). Increased soil carbon content can be due to VPH metabolite formation, humification of biomolecules from VPH degrading biomass, or precipitation of CO₂-C produced from VPH degradation as carbonates.

Inorganic nutrient availability in the columns was reduced on day 430 as compared to the initial condition (Table 2) and generally lowest on the NAPL source side of the columns, and especially low in the soil & AC column, where CO₂ production was highest towards the end of the experiments. Ammonium-N, which is more readily assimilated than nitrate-N, was the most depleted inorganic nutrient (>90%) as compared to the initial condition. Inorganic nutrient scarcity is thus the likely cause for suboptimal VPH biodegradation conditions in the later phase of the column study.

Total cell counts were up to a factor of 8 higher at the end of the experiment as compared to the initial condition (Table 2), showing a lasting enhancement in the soil microbial biomass following VPH exposure. Amongst locations (source, middle and atmospheric side of the

columns) and soil types (soil, soil & BC, soil & AC) there was at most a factor of 2 variability in the total cell counts, suggesting that the extent of this long-term biomass growth was not strongly altered by the sorbent amendment or distance from the source.

Petroleum vapour addition, and biochar or activated carbon amendment effects on the soil microbial community composition. After quality filtering, 148,882 and 365,166 sequences, for batch and column samples respectively, were recovered and represented by 4,568 OTUs for the batch samples and 12,265 OTUs for the column samples at 97% similarity (i.e. distinguished at species level). The average Bray Curtis similarity matrix (log transformed relative abundance data) was mapped onto a 2D ordination space to reveal VPH and sorbent-amendment as well as spatial effects (Figure 5). Short-term effects of VPH exposure at high concentrations became apparent on day 6 in the batch studies (Figure 5a), where sorbent amendment strongly shaped bacterial and archaeal community-structure (ANOSIM on Bray Curtis similarity, Global R sorbent amendment effect = 0.655, $p < 0.001$). Long-term VPH exposure effects on microbial communities became apparent on day 430 in the column study (Figure 5b), with a significant effect of sorbent amendment on the bacterial and archaeal community structure (ANOSIM, Global R = 0.647, $p < 0.01$).

Relative OTU abundance rankings compared to the control soils at time zero were used to further evaluate the nature of the bacterial and archaeal community composition changes, induced by the VPH exposure (Figure 6 and Tables S2-4 in Supplementary Information). An improved abundance ranking of an OTU provides evidence for better ecological competitiveness of this OTU under the altered conditions. Not surprisingly, several OTUs from families known to include petroleum hydrocarbon degraders²⁹⁻³⁹ substantially enhanced their ranking following VPH exposure (Figure 6a). In batch studies with soil, members of the families *Nocardiaceae* (i.e. from the genus *Rhodococcus*) and the *Pseudomonadaceae* (i.e. *Pseudomonas umsongensis*) gained most significantly following straight-chain alkane

addition, while the *Pseudomonadaceae* benefitted more strongly than the *Nocardiaceae* from branched and cyclic alkane addition. Probably due to toxic effects at high monoaromatic hydrocarbon concentration, only small ranked abundance changes were observed following monoaromatic hydrocarbons addition.

The microbial community response to VPH addition was affected by the sorbent amendments (Figures 6b-d). While some OTUs, such as one from the genus *Acidovorax*, benefitted from the addition of the twelve VPH mixture with and without the sorbent amendments, an OTU from the genus *Achromobacter* was more successful in the soil and soil & BC than in the soil & AC batches, OTUs from the *Pseudomonadaceae* family were most successful in the unamended soil batches, an OTU from the genus *Hydrogenophaga* was most successful in the soil & BC batches, and an OTU from the *Nocardioidaceae* family most successful in the soil & AC batches (Figures 6b-d and Table S3 in Supplementary Information). Competitive interactions between groups of microorganisms with hydrocarbon degradation potential are often governed by environmental parameters: *Actinobacteria* (to which the *Nocardioidaceae* belong) tend to be most successful in soils with low carbon substrate availability, and *Proteobacteria* (to which the *Pseudomonadaceae* belong) most successful in soils with high carbon substrate availability⁴⁰. Furthermore, stable isotope labeling to identify nitrogen-incorporating bacteria in petroleum-contaminated Arctic soils showed that, within the major families of *Actinobacteria*, *Nocardioidaceae* and *Microbacteriaceae* were more effective than *Nocardiaceae* at assimilating ammonium⁴¹. Combining these insights with the current study results it can be speculated that *Nocardioidaceae* have especially high affinity for carbon substrates and inorganic nitrogen, and thus gain ecological competitiveness in the soil & AC environment, where the bioavailable VPH concentrations (Figure 4) and inorganic nitrogen concentrations (Table 2) were lower than in the unamended soil.

On day 430 in the column study, when VPH concentrations had fallen to very low levels, some of these sorbent-related short-term trends were still notable (Table S4 in Supplementary Information). An OTU from the *Nocardioidaceae* family showed the strongest relative rank abundance increase near the VPH source in the soil & AC column, while an OTU from the *Pseudomonadaceae* family had significantly increased relative abundance ranks in the soil and soil & BC column, but not in the soil & AC column. An OTU from the genus *Hydrogenophaga* again gained most consistently in the soil & BC column.

Modelling results. Figures 3-4 compare measured with simulated VPH concentrations aggregated for the different compound classes. Model parameter values are compiled in Supplementary Information (Tables S5-8). Model predictions for the soil column based on the independently measured batch study data, and some parameter values from the literature (as indicated in Supplementary Information), already captured characteristic features of the measured breakthrough curves (early peak, temporary dip, secondary peak, see Figure S2 in Supplementary Information), certainly much better than a first-order rate model (Figure 1a). Quantitatively, straight-chain alkane concentrations were well predicted, branched and cyclic alkane concentrations were under predicted, and monoaromatics concentrations over predicted, based on the independent model parameter calibration. An improved fit for the soil column data (Figures 3&4, blue lines) was obtained with the following four changes to the microbiological modelling parameters: i) increasing the lag phase for the branched and cyclic alkanes from 3 to 6 days; ii) reducing the second order growth rates for the branched and cyclic alkanes by a factor of 3.3, whilst increasing the rates for the monoaromatic hydrocarbons by a factor of 1.5; iii) reducing the soil biomass carrying capacity by a factor of 1.9; and iv) increasing the biomass decay rate, which was obtained from the literature¹⁶, by a factor of 2. Figure S3 in Supplementary Information shows the predictions for the soil & BC and soil & AC columns if only the amendment effects on the VPH sorption (K_d values in

Table S1 in Supplementary Information) were considered, i.e. assuming that the microbiological parameters fitted for the soil column study also applied in the soil & BC and soil & AC columns. These predictions already aligned well with the measured data, indicating that the main amendment effects on the petroleum vapour sorption relate to VPH sorption, and resulting effects on the VPH mobility and bioavailability. A further improved overall data fit (shown as red and green lines in Figures 3&4) could, however, be obtained, with second order growth rates adaptations for each soil type (as detailed in Table S8), in particular by increasing second-order growth rates for the soil & AC column by up to a factor of 10. Higher second-order growth rates for the activated carbon amended as compared to the unamended soil would imply that VPH degrading microorganisms in the soil & AC column had higher specific substrate affinity^{42, 43} than those predominating in the unamended soil, which aligns with the observed soil microbial community composition changes discussed above. The remaining discrepancies between measured data and model simulations generally also show a slightly overestimated VPH removal for the initial experimental period, when the VPH loading of the columns was high, and a slightly underestimated VPH removal from about day 120 onwards (Figure 3a), which also suggest that the soil microbial communities can adapt and be effective at removing VPHs when these compounds are present at low concentrations. Temporal changes in soil microbial community composition with impacts on second-order growth rates are not currently considered by the model.

Conclusions

Carefully designed sorbent amendments can stabilize the pollutant biofiltration process in soil by preventing rapid pollutant breakthrough during periods of biological inefficiency (i.e. during the lag phase), and by spreading and smoothing out pollutant concentration peaks which may otherwise overload the soil's pollutant removal capacity (i.e. when inorganic

nutrient scarcity limits further growth of pollutant degrading biomass). An activated carbon amended, near-surface soil layer could thus be a beneficial pollution control measure at VPH contaminated sites with irregular pollutant releases, for example above a polluted aquifer with a fluctuating water table.⁴⁴ Similarly, biochar or activated carbon amendments can enhance the robustness of other biofiltration designs such as stormwater biofiltration in retention ponds and soak-aways.⁴⁵ The suggestion that *Nocardioidaceae* predominate VPH degradation in the presence of sorbents like activated carbon, which strongly bind VPHs, while *Pseudomonadaceae* predominate the VPH degradation at higher bioavailable concentration levels is of broad interest to researchers studying the microbial ecology of petroleum biodegradation in soil.

Conflicts of interest.

There are no conflicts to declare.

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References

1. Environment Agency, Oil pollution, <http://www.environment-agency.gov.uk/research/library/position/41233.aspx>, (accessed 06.05, 2010).
2. R. De Búrca, H. Morgan, J. Jeffries and N. Earl, *Soil Guideline Values for toluene in soil* Report Science Report SC050021 / toluene SGV, 2009.
3. G. Pasteris, D. Werner, K. Kaufmann and P. Höhener, *Environmental Science & Technology*, 2002, **36**, 30-39.
4. N. Dakhel, G. Pasteris, D. Werner and P. Höhener, *Environmental Science and Technology*, 2003, **37**, 2127-2133.
5. H. Luo, P. R. Dahlen, P. C. Johnson and T. Peargin, *Environmental Science & Technology*, 2013, **47**, 1977-1984.
6. T. H. Wiedemeier, H. S. Rifai, C. J. Newell and J. T. Wilson, *Natural attenuation of fuels and chlorinated solvents in the subsurface*, Wiley, New York, 1999.
7. P. Höhener, C. Duwig, G. Pasteris, K. Kaufmann, N. Dakhel and H. Harms, *Journal of Contaminant Hydrology*, 2003, **66**, 93-115.
8. D. W. Ostendorf, T. H. Schoenberg, E. S. Hinlein and S. C. Long, *Environmental Science & Technology*, 2007, **41**, 2343-2349.
9. N. C. Choi, J. W. Choi, S. B. Kim and D. J. Kim, *Applied Microbiology and Biotechnology*, 2008, **81**, 135-141.
10. P. Höhener, N. Dakhel, M. Christophersen, M. Broholm and P. Kjeldsen, *Journal of Contaminant Hydrology*, 2006, **88**, 337-358.
11. A. Elazhari-Ali, A. K. Singh, R. J. Davenport, I. M. Head and D. Werner, *Environmental Pollution*, 2013, **173**, 125-132.
12. N. L. Capiro, M. L. B. Da Silva, B. P. Stafford, W. G. Rixey and P. J. J. Alvarez, *Environmental Microbiology*, 2008, **10**, 2236-2244.
13. M. K. Chang, T. C. Voice and C. S. Criddle, *Biotechnol. Bioeng.*, 1993, **41**, 1057-1065.
14. M. A. Dinamarca, A. Ruiz-Manzano and F. Rojo, *Journal of Bacteriology*, 2002, **184**, 3785-3793.
15. P. Meynet, S. E. Hale, R. J. Davenport, G. Cornelissen, G. D. Breedveld and D. Werner, *Environmental Science & Technology*, 2012, **46**, 5057-5066.
16. P. Meynet, E. Moliterni, R. J. Davenport, W. T. Sloan, J. V. Camacho and D. Werner, *Soil Biology and Biochemistry*, 2014, **68**, 20-30.
17. L. Jakob, T. Hartnik, T. Henriksen, M. Elmquist, R. C. Brändli, S. E. Hale and G. Cornelissen, *Chemosphere*, 2012, **88**, 699-705.
18. U. Ogbonnaya and K. Semple, *Agronomy*, 2013, **3**, 349.
19. J. Tang, W. Zhu, R. Kookana and A. Katayama, *J Biosci Bioeng*, 2013, **116**, 653-659.
20. S. E. Hale and D. Werner, in *Biochar for Environmental Management: Science, Technology and Implementation*, eds. J. Lehmann and S. Joseph, Routledge, Milton Park, UK, 2nd edn., 2015, pp. 625-654.
21. K. M. Bushnaf, S. Puricelli, S. Saponaro and D. Werner, *Journal of Contaminant Hydrology*, 2011, **126**, 208-215.
22. C. J. Englert, E. J. Kenzie and J. Dragun, *Bioremediation of petroleum products in soil*, Lewis publisher, Chelsea, MI 48118, 1993.
23. F. Solano-Serena, R. Marchal, M. Ropars, J. M. Lebeault and J. P. Vandecasteele, *Journal of Applied Microbiology*, 1999, **86**, 1008-1016.
24. K. H. Baker and D. S. Herson, *Bioremediation*, McGraw-Hill, New York, 1994.

25. H. M. S. K. Herath, M. Camps-Arbestain and M. Hedley, *Geoderma*, 2013, **209–210**, 188-197.
26. D. Werner and P. Höhener, *Environmental Science and Technology*, 2002, **36**, 1592-1599.
27. L. Wilhelm, G. A. Singer, F. Christina, T. J. Battin and K. Besemer, *International Society for Microbial Ecology*, 2013, **7**, 1651-1660.
28. R. M. Atlas and R. Bartha, *Adv. Microb. Ecol.*, 1992, **12**, 287-338.
29. S. J. Assinder and P. A. Williams, *Advanced Microbial Physiology*, 1990, **31**, 1-69.
30. F. Chaillan, A. Le Fleche, E. Bury, Y. Phantavong, P. Grimont, A. Saliot and J. Oudot, *Research in Microbiology*, 2004, **45**, 629-634.
31. M. Deng, J. Li, F. Liang, M. Yi, X. Xu, J. Yuan, J. Peng, C. Wu and J. Wang, *Marine Pollution Bulletin*, 2014, **83**, 79-86.
32. A. Fahy, T. J. McGenity, K. N. Timmis and A. S. Ball, *FEMS Microbiology Ecology*, 2006, **58**, 260-270.
33. G. D. Gojic-Cvijovic, J. S. Milic, T. M. Solevic, V. P. Beskoski, M. V. Ilic, L. S. Diokic, T. M. Narancic and M. M. Vrvic, *Environmental Pollution*, 2011, **159**, 616-622.
34. N. Hamamura and D. J. Arp, *Microbiology Letters*, 2000, **186**, 21-26.
35. N. Hamamura, S. H. Olson, D. M. Ward and W. P. Inskeep, *Applied and Environmental Microbiology*, 2006, **72**, 6316-6324.
36. S. Meyer, R. Moser, A. Neef, U. Stahl and P. Kampfer, *Microbiology*, 1999, **145**, 1731-1741.
37. S. Mukherjee, N. K. Bardolui, S. Karim, V. V. Patnaik, R. K. Nandy and P. K. Bag, *Environmental Science and Health Part A*, 2010, **45**, 1048-1053.
38. N. J. Palleroni, D. H. Peiper and E. R. B. Moore, in *Handbook of Hydrocarbon and Lipid Microbiology.*, ed. K. N. Timmis, Springer-Verlag, Berlin, Heidelberg, 2010, pp. 1787-1798.
39. R. E. Parales, J. L. Ditty and C. S. Harwood, *Applied and Environmental Microbiology*, 2000, **66**, 4098-4104.
40. T. H. Bell, E. Yergeau, C. Maynard, D. Juck, L. G. Whyte and C. W. Greer, *The ISME Journal*, 2013, **7**, 1200-1210.
41. T. H. Bell, E. Yergeau, C. Martineau, D. Juck, L. G. Whyte and C. W. Greer, *Applied and Environmental Microbiology*, 2011, **77**, 4163-4171.
42. F. P. Healey, *Microbial Ecology*, 1980, **5**, 281-286.
43. K. Kovárová-Kovar and T. Egli, *Microbiology and Molecular Biology Reviews*, 1998, **62**, 646-666.
44. D. Werner and P. Höhener, Sheffield, UK, 2002.
45. B. A. Ulrich, E. A. Im, D. Werner and C. P. Higgins, *Environmental Science & Technology*, 2015, **49**, 6222-6230.

Table 1: Volatile Petroleum Hydrocarbon (VPH) carbon mass balance on day 430. Initially, 12.5 g VPH-C was present in each source.

Column	Source (g)	Retained as VPH in soil (g)	Emanated as VPH (g)	Emanated as CO ₂ (g)	Change in Biomass C ^a (g)	Total accounted (g)	Change in total soil C (g)
Soil	1.15±0.09	0.0	2.56	3.32	0.080±0.026	7.11	+10±15
Soil & 2% BC	1.36±0.10	0.01±0.01	3.18	2.63	0.037±0.023	7.22	+16±20
Soil & 2% AC	0.65±0.03	0.40±0.05	0.045	3.45	0.063±0.019	4.61	+51±28

^aCalculated assuming a carbon content of 10⁻¹³ g per cell.

Table 2: Water extractable inorganic soil nutrients and total soil cell count data

Sample ID	Ammonium ($\mu\text{g NH}_4^+\text{-N g}^{-1}$)	Nitrate ($\mu\text{g NO}_3^-\text{-N g}^{-1}$)	Phosphate ($\mu\text{g H}_2\text{PO}_4^-\text{-P g}^{-1}$)	Total cell count (cells g^{-1})
Before the column experiment				
Soil	2.9 $\pm$ 0.2	8.2 $\pm$ 2.0	0.8 $\pm$ 0.07	2.7 $\pm$ 0.2 $\times 10^7$
Soil & 2% biochar	5.8 $\pm$ 0.5	5.5 $\pm$ 0.57	0.2 $\pm$ 0.02	7.8 $\pm$ 0.2 $\times 10^7$
Soil & 2% activated carbon	6.1 $\pm$ 1.2	6.5 $\pm$ 1.0	0.3 $\pm$ 0.03	3.9 $\pm$ 0.7 $\times 10^7$
At the end of the column experiment				
Soil, VPH source side	0.18 $\pm$ 0.04	4.0 $\pm$ 0.4	0.2 $\pm$ 0.02	21.4 $\pm$ 1.3 $\times 10^7$
Soil, middle	0.18 $\pm$ 0.05	6.8 $\pm$ 0.3	0.4 $\pm$ 0.03	11.5 $\pm$ 6.6 $\times 10^7$
Soil, zero conc. boundary side	0.24 $\pm$ 0.03	6.5 $\pm$ 0.5	0.7 $\pm$ 0.03	18.6 $\pm$ 4.1 $\times 10^7$
Soil & 2% biochar, VPH source side	0.06 $\pm$ 0.003	1.1 $\pm$ 0.5	0.07 $\pm$ 0.01	20.2 $\pm$ 1.6 $\times 10^7$
Soil & 2% biochar, middle	0.05 $\pm$ 0.01	2.1 $\pm$ 0.1	0.12 $\pm$ 0.02	13.9 $\pm$ 1.1 $\times 10^7$
Soil & 2% biochar, zero conc. boundary side	0.04 $\pm$ 0.01	2.2 $\pm$ 0.1	0.13 $\pm$ 0.03	11.2 $\pm$ 4.6 $\times 10^7$
Soil & 2% activated carbon, VPH source side	0.04 $\pm$ 0.01	0.0 $\pm$ 0.0	0.17 $\pm$ 0.02	18.0 $\pm$ 4.6 $\times 10^7$
Soil & 2% activated carbon, middle	0.05 $\pm$ 0.01	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	12.5 $\pm$ 1.1 $\times 10^7$
Soil & 2% activated carbon, zero conc. boundary side	0.05 $\pm$ 0.01	2.4 $\pm$ 0.02	0.12 $\pm$ 0.02	17.9 $\pm$ 9.1 $\times 10^7$

Figure 1: Schematic illustration of the predicted sorbent amendment effects on (a) the VPH concentration in soil; (b) the VPH attenuation in soil; and (c) an illustration of the column study set-up. The qualitative illustrations of the K_d effects on VPH concentrations and attenuation in soil are based on the assumption of a finite source of one biodegradable compound passing through the column by gas-phase diffusion with first-order rate biodegradation occurring only in the soil pore water phase.

Figure 2: VPH mass residuals after fifteen days in live soils with and without 2% biochar (BC) or activated carbon (AC) amendment relative to sterile controls. Nitrogen (N) and/or phosphorus (P) amendments were added based on a mass ratio of 100:10:1 for VPH-C to $\text{NH}_4^+\text{-N}$ to $\text{H}_2\text{PO}_4^-\text{-P}$.

Figure 3: Measured, cumulative (a) VPH carbon and (b) CO₂ carbon emanation from the soil columns, shown as diamond symbols. Solid lines show the model predictions for the soil (blue) soil & BC (red) and soil & AC (green) columns, with optimized microbiological parameter values.

Figure 4: Carbon-content normalized VPH and CO₂ concentrations measured in the middle of the columns for the sum of the a) straight-chain alkanes, b) branched/cyclic alkanes, c) monoaromatic hydrocarbons, and d) CO₂. Inserts show the early data for the first two months. Solid lines show the model predictions for the soil (blue), soil & BC (red) and soil & AC (green) columns, with fitted microbiological parameter values.

Figure 5: Non metric multi-dimensional scaling (NMDS) analysis on Bray-Curtis similarities at species level (log transformed) for the sorbent amended and unamended soil samples (duplicates), in the a) batch study on day 6, with nutrients (WN) and without nutrients (WON) and b) column study on day 430.

Figure 6: Illustration of relative abundance rank changes following the addition of 0.03 mL VPHs as a) compound class or b)-d) complete mixture of the twelve VPHs in the batch study for a)-b) soil, c) soil and biochar and d) soil and activated carbon.

Supplementary Information:

Mechanisms of Distinct Activated Carbon and Biochar

Amendment Effects on Petroleum Vapor Biofiltration in Soil

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Additional method descriptions:

Batches experiments. Amber vials (65 mL, Jencons, a VWR Division, Leicestershire, UK) closed with Teflon Mininert valves (Supelco, Bellefonte, USA) were prepared in triplicates, and contained wet construction sand without sorbent amendment (soil), and with 2% d.w. biochar amendment (soil & BC) or 2% d.w. activated carbon amendment (soil & AC). Similarly, sterilized controls were prepared by autoclaving the same soil types at 121 °C for 30 min. Water contents of the vials were determined by the weight loss of control vials upon drying in the oven at 105 °C. Three kinds of batch experiments were performed. Firstly, VPH headspace concentrations were monitored after injecting 0.002 mL of VPH mixture as liquid into the batches containing 40, 25 and 13 g of wet construction sand for soil, soil & BC, and soil & AC, respectively, to determine VPH vapor biodegradation rates (live soils) and sorption coefficients (sterile soils). Secondly, to study the effects of nutrients on the biodegradation of VPHs, VPH residuals were extracted 30 days after the addition of 0.03 mL of VPH mixture to 15 g wet construction sand, equivalent to 0.018 g VPH-carbon, from the various soil types (soil, soil & BC, soil & AC) with and without the addition of 0.0018 g of nitrogen in the form of NH_4Cl and/or 0.00018 g of phosphorus in the form of KH_2PO_4 (i.e., nitrogen and phosphorus were added together or separately). Autoclaved controls with and without VPH mixture addition were also set up in parallel. On day 4 and day 6, 10 mL of the headspace air in the nutrient amended batches containing soil with or without biochar were replaced with pure air using gas-tight syringes to keep the batch aerobic, and the amount of VPHs removed with the syringe was measured and considered as non-degraded VPHs in the mass balance. Thirdly, batch experiments were performed to investigate soil microbiology. CO_2 was monitored in the headspace of batches with and without 0.03 mL of VPH mixture addition, with and without nutrient addition, or with

the addition of 0.03 mL of equal weight mixtures of either the straight chain alkanes, the branched and cyclic alkanes, or the monoaromatic hydrocarbons. After between 8 and 15 days, soil DNA was extracted for 16s rRNA gene sequencing. Batch study data interpretation followed protocols described by Bushnaf et al. ¹. The apparent first-order biodegradation rates in the gas phase ^{2,3} was estimated by linear regression of the natural logarithm of the VPH concentration in soil air versus time in the part of the experimental data that showed a clear decrease, e.g. after an eventual lag phase, and the rate had to be greater than the one observed in the sterile control. Exemplary data is shown in Figure S1 for hexane (no lag phase) and isooctane (lag phase). First-order biodegradation rates in the water were derived from these measurements as explained in Bushnaf et al. ¹.

Ammonium quantification. Readily available nutrients were extracted using 1:1 w:w ratios of soil:CaCl₂ 0.01 M (CaCl₂ from VWR, Leicestershire, UK) in centrifuge tubes which were shaken for 20 h. The slurries were centrifuged at 4,000 rpm for 10 min and the supernatant was immediately filtered through syringe filters (pore size 0.2µm, VWR, Leicestershire, UK). Ammonium determination was carried out using an ammonium reagent kit (Merck no. 1.4752.002, Merck, Darmstadt, Germany) and a Spectroquant Nova 60 photometer (Merck, Germany). The sample of supernatant solution (5 ml) was put into test tube, and 0.6 ml of sodium hydroxide buffer solution and 0.5 g of EDTA was added. After 5 min of shaking 3 drops of sodium nitroprusside indicator were added.

Nitrate, sulphate and phosphate quantification. Nitrate, sulphate and phosphate analysis was performed on a Dionex ICS 1000 Ion Chromatography system provided with a conductivity detector (Dionex, Sunnyvale, CA, USA). An aliquot of the sample (5 mL) was injected by a Dionex AS40 automated sampler. The separation was performed on an Ionic pac AS14A

analytical column (250 mm × 4 mm i.d., Dionex, Sunnyvale, CA, USA). The Ion Chromatograph was held isothermally at 20°C with 8.0 mM Na₂CO₃/1.0 mM NaHCO₃ solution as the mobile phase (flow rate of 1 ml min⁻¹, initial pressure 1800 psi).

Total carbon and total organic carbon. Total carbon (TC) and total organic carbon (TOC) contents of soil, biochar, activated carbon and soil with 2% biochar or activated carbon were measured using a Leco CS230 carbon-sulphur analyser (LECO Corporation, Michigan, USA). Samples for TOC were treated with 50% hydrochloric acid to remove carbonate before measuring the carbon content.

CO₂, O₂ and SF₆ quantification. GC-MS analysis of CO₂, O₂ and SF₆ was performed on a Fisons 8060 Gas Chromatograph linked to a Fisons MD800 MS (electron voltage 70 eV, filament current 4A, source current 800uA, source temperature 200°C, multiplier voltage 500V, interface temperature 150°C). The sample (60 µL) was injected in split mode with a 100 µL Hamilton gastight syringe. The separation was performed on a HP-PLOT-Q capillary column (30 m x 0.32 mm i.d) packed with 20 µm Q phase (Agilent Technologies, Palo Alto, USA). The GC was held isothermally at 35°C with helium as the carrier gas (flow rate of 30 mL min⁻¹, initial pressure 65 kPa, split at 100 mL min⁻¹). The instrument was calibrated using standard CO₂, O₂, and N₂O (Scientific and Technical Gases, Staffordshire, UK) and SF₆ (Sigma –Aldrich, Dorset, UK) gases for a five point calibration.

VPH quantification: GC-FID analysis was performed on an Agilent HP-7890 Gas Chromatograph (Agilent Technologies, Palo Alto, USA). The gas sample (60 µL) of headspace gas was injected with a 100 µL Hamilton gastight syringe. The separation was performed on a HP-5 capillary column (30 m × 0.25 mm i.d.) with 0.25 µm film thickness (Agilent Technologies, Palo Alto, USA). The injection port used a split ratio of 10 and was heated to

200°C. The gas chromatography column temperature was held at 30°C for 5 minutes, increased to 120°C at a rate of 10°C min⁻¹, and then held constant for 6 min with hydrogen as carrier gas. The instrument was calibrated using dilutions of headspace gas concentrations sampled from Mininert-valve (Supelco, Bellefonte, USA) closed batches containing the VPH mixture liquid for a five point calibration.

VPH residual quantification. To examine VPHs residuals, 15 g soil samples were extracted twice for 24 hours in 10 mL dichloromethane/pentane mixture (40:60 by volume). The combined extracts were passed through a glass chromatographic column (45 cm×10 mm i.d.) pre-packed with 3 g activated silica gel topped by 1.5 g sodium sulphate (Sigma–Aldrich, Dorset, UK). Columns were rinsed with an additional 20 mL of the dichloromethane/pentane solvent and all solvent was collected in 40 mL amber vials from which 1 mL was transferred into a 1.0 mL GC-vial for analysis. Pentane could not be quantified by this method because of incomplete separation from the solvent peak. Compounds in cleaned-up solvent extracts were identified and quantified by GC-FID analysis on a Hewlett Packard 5890 series II in split less mode, injector temperature at 280°C. The separation was performed on an Agilent fused silica capillary column (30 m × 0.25 mm i.d.) coated with 0.25 µm diethyl poly-siloxane (HP-5) phase (Agilent Technologies, Palo Alto, USA). The GC temperature was programmed from 50-310°C at 5°C/min and held at the final temperature for 20 min with hydrogen as the carrier gas.

Amplicons library preparation for next-generation sequencing assays. For both pyrosequencing and Ion Torrent assays, individual sequencing libraries were prepared from each DNA sample by amplifying the V4 and most of the V5 regions of the 16S rRNA gene, using primer set 515f (5'-GTGNCAGCMGCCGCGGTAA-3') and 926r (5'-CCGYCAATTYMTTTRAGTTT-3'). The primers contained the specific adapters for

pyrosequencing (GS FLX Titanium adapter A, 5' - CGTATCGCCTCCCTCGCGCCATCAG – 3', and adapter B, 5' – CTATGCGCCTTGCCAGCCCGCTCAG – 3') or for the Ion Torrent (Ion adapter A, 5'- CCATCTCATCCCTGCGTGTCTCCGACTCAG-3', and Ion adapter trP1, 5'- CCTCTCTATGGGCAGTCGGTGAT-3'). Samples were differentiated by a unique 8 bp (pyrosequencing) or 12bp (Ion Torrent) barcode, added to the 5' –end of both the forward and reverse primers through a GA linker (pyrosequencing) or to the 5'-end of the forward primers through a GAT spacer (Ion Torrent). Each PCR reaction was composed of 0.5 µl DNA template, 0.4 µmol l⁻¹ primers, 0.2 mmol l⁻¹ dNTPs (PCR grade Nucleotide Mix, Roche), FastStart High Fidelity Enzyme Blend (2.5U/reaction), 1.8 mM MgCl₂ in the FastStart High Fidelity Reaction Buffer (Roche Diagnostics GmbH, Mannheim, Germany). The following PCR thermal cycling programme was used: an initial denaturation cycle at 95 °C for 4 minutes; 25 cycles of denaturation at 95 °C for 1 minute, annealing of primers at 55 °C for 45 seconds, and elongation at 72 °C for 1 minute; one final elongation step at 72 °C for 7 minutes. For samples in which the product was not sufficiently concentrated, multiplex PCR were prepared, pooled together and cleaned using the QIAquick PCR purification kit (QUIAGEN, Crawley, UK). For the Ion Torrent, single libraries were further purified and size selected using double-sided solid-phase reversible immobilisation (SPRI) beads (Agencourt AMPure XP system, Beckman Coulter), following the manufacturer's instructions. The single libraries were then individually quantified using a Qubit dsDNA HS Assay on a Qubit 2.0 Fluorometer (Invitrogen, UK), following the manufacturer's instruction, pooled together in equimolar amounts and sequenced on a Roche 454 GS FLX+ System by NewGene Ltd (International Centre for Life, Newcastle upon Tyne, UK) or (for the batch study in which the biodegradation of the straight chain alkanes, the branched and cyclic alkanes, and the monoaromatic hydrocarbons in soil was investigated separately) on a

126 Personal Genome Machine (PGM) at the School of Civil Engineering and Geosciences,
127 Newcastle University, using a 316 ion chip. The raw sequences were processed using the QIIME
128 (v 1.7.0) bioinformatics pipeline. Briefly, the reads were filtered for quality (filtering criteria:
129 perfect match to sequence barcode/primer, 200bp minimum sequencing length). For
130 pyrosequencing data, QIIME Denoiser ⁴ was used to detect and correct sequencing errors, and
131 the data were reintegrated into the QIIME pipeline by inflation. Sequences were then clustered
132 into Operational Taxonomic Unit (OTU) at 97% sequence similarity level by the uclust
133 algorithm. A representative sequence from each OTU was selected and taxonomically identified
134 using the Greengenes database ^{5, 6}. Representative sequences and correspondent taxonomic
135 assignment were used to build a table of OTU abundances at different levels of taxonomy.
136 Microbial community diversity within and across the samples was determined by using the
137 QIIME (v.1.7.0) pipeline ⁷ and Primer v6 software ⁸. The Bray Curtis similarity metric was
138 generated and represented by a 2-dimensional non-metric multidimensional scaling (nMDS) plot.

139 **Tracer test.** To examine the tortuosity factor of the packed columns, 10 mL of SF₆ gas were
140 injected into the column center at port 4. The soil air was sampled through the injection point and
141 at a sampling port at 15 and 30 cm distance on either side (ports 3 and 5), after 20 min, 40 min,
142 60 min, 80 min, 100 min, 130 min and 160 min. Headspace purging was stopped for the duration
143 of the tracer experiment. To avoid artifacts due to the spatial limitation of the experimental
144 system, the duration of the experiments was no longer than 160 min. Samples of 60 µL of soil
145 gas were taken with a gas-tight syringe and injected directly into the gas chromatograph for SF₆
146 analysis by mass spectrometry. The measured concentration of SF₆ was compared with the
147 modelled concentration which was obtained by interpreting the diffusive transport with an
148 equation for an instantaneous plane source of sulphur hexafluoride (SF₆) in a porous medium as

149 described by Werner and Hohener ⁹. Simulated SF₆ concentration when using $\tau=0.4$, 0.6, and 0.8
150 were compared with measured concentrations, and $\tau=0.8$ provided the best fit. Therefore, $\tau=0.8$
151 was used as the tortuosity value to simulate the volatilization and migration of VPHs or CO₂.
152 Such a high value of the tortuosity factor may be due to the fact that the columns were relatively
153 loosely packed in comparison with natural soils.

Modelling

Underpinning assumptions:

The VPH concentrations in soil air above the NAPL source are described by Raoult's law. The VPH concentration on the atmospheric boundary side of the columns is zero, and this results in a net diffusive VPH flux from the NAPL source towards the atmospheric boundary. The VPHs migrate through the column by Fickian diffusion in soil air. Advective gas transport is neglected. The distribution of VPHs between soil air and water is described by Henry's law. VPH sorption to the soil and biochar or activated carbon solid matrix is linear and reversible. The kinetics of the VPH uptake or release by biochar or activated carbon is described by sorption-retarded intraparticle diffusion of VPHs in the biochar or activated carbon pores, which are water-filled. The VPH degrading biomass is present in the water in between, but not within particles, and can simultaneously grow on all the VPH compounds. This biomass can only degrade VPHs which are present in the water in between soil particles. Sorbed VPHs and VPHs in soil air are not directly bioaccessible. The growth rate of VPH degrading biomass is dependent on the VPH concentration in the water in between particles, and limited by a maximum biomass concentration.

Coordinates, time:

z (cm)	Distance from the pollution source zone
t (s)	Time

Soil and carbonaceous sorbent parameters:

$\theta_a, \theta_w, \theta_s, \theta_c$ (-)	Air, water, soil solid and carbonaceous sorbent-filled soil volume fractions
ρ_s (gcm ⁻³)	Density of the soil solids
τ_a (-)	Tortuosity factor for the air-filled soil pore space

180	ρ_c (gcm ⁻³)	Skeletal, solid density of the carbonaceous sorbent
181	$p_{w,c}$ (-)	Water-filled porosity of the carbonaceous sorbent
182	R_c (cm)	Particle radius of the carbonaceous sorbent
183		
184	<u>Chemical parameters for compound k:</u>	
185	$C_{a,k}$ (gcm ⁻³)	Concentration of compound k in soil air
186	$S_{c,k}$ (gcm ⁻³)	Volumetric concentration of compound k in the carbonaceous sorbent
187	H_k (-)	Dimensionless air-to-water partitioning coefficient (Henry constant)
188	$K_{d,k}$ (cm ³ g ⁻¹)	Soil solid-to-water partitioning coefficient
189	$K_{c,k}$ (cm ³ g ⁻¹)	Carbonaceous sorbent solid-to-water partitioning coefficient
190	$D_{a,k}$ (cm ² s ⁻¹)	Molecular diffusion coefficient of compound k in soil air
191	$D_{w,k}$ (cm ² s ⁻¹)	Molecular diffusion coefficient of compound k in water
192	$D_{c,k}$ (cm ² s ⁻¹)	Apparent diffusion coefficient of compound k in the carbonaceous sorbent
193	x_k (-)	Mole fraction of compound k in the VPH source
194	$C_{pl,k}$ (gcm ⁻³)	Pure liquid vapor concentration of compound k
195		
196	<u>Biological parameters:</u>	
197	C_b (gcm ⁻³)	Biomass concentration in soil water
198	$C_{b,max}$ (gcm ⁻³)	Maximum biomass concentration in soil water
199	dec (s ⁻¹)	Biomass decay rate
200	g_k (s ⁻¹)	Biomass growth rate on compound k
201	Y_k (gg ⁻¹)	Biomass yield coefficient for compound k
202	sg_k (cm ³ g ⁻¹ s ⁻¹)	Second order growth rate on compound k
203		
204	<u>Column parameters:</u>	
205		
206	r_{column} (cm)	Column radius
207	l_{column} (cm)	Column length
208	r_{neck} (cm)	Source neck radius
209	l_{neck} (cm)	Source neck length
210		

Model equations:

Compound k mass balance equation:

$$\left(\theta_a + \frac{\theta_w}{H_k} + \frac{\rho_s \theta_s K_{d,k}}{H_k} \right) \cdot \partial / \partial t C_{a,k}(z, t) = \theta_a \tau_a D_{a,k} \cdot \partial^2 / \partial z^2 C_{a,k}(z, t) \\ - \theta_c \cdot \partial / \partial t \left[\frac{3}{R_c^3} \int_0^{R_c} r^2 S_{c,k}(z, r) dr \right] - \theta_w \cdot \frac{g_k}{Y_k} \cdot C_b(z, t)$$

if time > lag phase

$$g_k = s g_k \cdot \frac{C_{a,k}(z, t)}{H_k} \cdot \frac{C_{b,max} - C_b(z, t)}{C_{b,max}}$$

else

$$g_k = 0$$

Carbonaceous sorbent intraparticle diffusion equation ¹⁰:

$$\partial / \partial t S_{c,k}(z, r, t) = \frac{D_{c,k}}{r^2} \cdot \partial / \partial r \left(r^2 \partial / \partial r S_{c,k}(z, r, t) \right)$$

Estimated intraparticle diffusion coefficient ¹⁰:

$$D_{c,k} = \frac{p_{w,c}^2}{p_{w,c} + (1 - p_{w,c}) \cdot \rho_c \cdot K_{c,k}} \cdot D_{w,k}$$

Biomass equation:

$$\partial / \partial t C_b(z, t) = \left(\sum_k g_k - dec \right) \cdot C_b(z, t)$$

237 Zero-concentration boundary condition at the column outlet:

238
$$C_{a,k}(z = l_{column}, t) = 0$$

239

240 Boundary condition at the NAPL source:

241
$$C_{a,k}(NAPL\ source, t) = x_k(t) \cdot C_{pl,k}$$

242

243 Mass conservation at the NAPL source – soil interface:

244

245
$$r_{column}^2 \cdot \theta_a \cdot \tau_a \cdot \partial / \partial z C_{a,k}(z = 0, t) \approx r_{neck}^2 \frac{C_{a,k}(z = 0, t) - C_{a,k}(NAPL\ source, t)}{l_{neck}}$$

246

247 Boundary condition at the soil water – carbonaceous sorbent interface:

248

249
$$S_{c,k}(z, R_c, t) = (p_{w,c} + (1 - p_{w,c}) \cdot \rho_c \cdot K_{c,k}) \cdot \frac{C_{a,k}(z, t)}{H_k}$$

250 Boundary condition in the carbonaceous sorbent particle core:

251

252
$$\partial / \partial r S_{c,k}(z, 0, t) = 0$$

253

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255 **Table S1:** Compound-specific parameter values determined in batch experiments.

	Apparent first-order biodegradation rate in water, k_w (s^{-1})	Distribution coefficient K_d (cm^3g^{-1})		
	Soil	Soil	Soil & 2% BC	Soil & 2% AC
n-Pentane	$6.5 \pm 0.9 \times 10^{-3}$	53 ± 16	86 ± 19	23200 ± 3000
n-Hexane	$5.3 \pm 0.2 \times 10^{-3}$	68 ± 6	112 ± 24	133000 ± 27000
n-Octane	$8.1 \pm 1.9 \times 10^{-3}$	167 ± 77	334 ± 190	53300 ± 19900
n-Decane	$8.9 \pm 2.9 \times 10^{-3}$	805 ± 355	1091 ± 437	39800 ± 6900
n-Dodecane	$2.5 \pm 0.6 \times 10^{-2}$	1714 ± 490	2151 ± 295	206900 ± 5800
Methylcyclopentane	$1.2 \pm 0.07 \times 10^{-3}$	13 ± 2	18 ± 1	10600 ± 700
Methylcyclohexane	$5.5 \pm 0.6 \times 10^{-4}$	15 ± 2	26 ± 9	28800 ± 8800
Cyclohexane	$6.0 \pm 0.3 \times 10^{-4}$	6 ± 0.8	8 ± 1	3600 ± 260
Isooctane	$1.1 \pm 0.06 \times 10^{-2}$	83 ± 14	124 ± 32	249000 ± 76000
Toluene	$6.9 \pm 0.9 \times 10^{-5}$	1.3 ± 0.2	12 ± 5	170 ± 59
m-Xylene	$4.8 \pm 1.2 \times 10^{-5}$	1.8 ± 0.6	6 ± 2	59 ± 20
1,2,4 TBM	$1.6 \pm 0.7 \times 10^{-4}$	4.4 ± 2.0	10 ± 3	59 ± 10

257 **Table S2:** Summary of OTUs distinguished at 97% similarity that showed a minimum 16-fold increase in their relative abundance
258 rank fifteen days after the addition of 0.3 mL of different VPH compound classes to soil batches.

Taxon (highest level classification)	Relative abundance rank increase for different treatments compared to the soil only control + 2-fold, ++ 4-fold, +++ 8-fold, ++++ 16-fold, +++++ 32-fold, ++++++ 64-fold, ++++++ 128-fold, , ++++++ 320-fold or greater, equivalent decrease for minus signs			
	Soil Rank	Soil+monoaromatic hydrocarbons	Soil+straight chain alkanes	Soil+branched and cyclic alkanes
g__Rhodococcus	665	+	++++	
g__Rhodococcus	249	+	+++++	+
f__Nocardiodaceae	107	+	++++	
s__Desulfosporosinus_meridiei	153	+	++++	
g__Tepidibacter	532		+++++	
g__Phenylobacterium	782		+++++	
g__Polaromonas	185	+	+++++	+
g__Perlucidibaca	749		++++	+
f__Pseudomonadaceae	593	+	+++++	+++++
f__Pseudomonadaceae	230		+++++	++++
g__Pseudomonas	428	+	+++++	+++++
g__Pseudomonas	232	++	+++++	+++++
s__Pseudomonas_umsongensis	264	+	++++	+++++
g__Pseudoxanthomonas	600		++++	

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262 **Table S3:** Summary of OTUs distinguished at 97% similarity which showed a minimum 8-fold increase in their relative abundance
 263 rank eight days after the addition of 0.3 mL of the VPH mixture to batches with soil, soil + biochar, or soil + activated carbon.

Taxon (highest level classification)	Relative abundance rank increase for different treatments compared to the soil only control + 2-fold, ++ 4-fold, +++ 8-fold, ++++ 16-fold, +++++ 32-fold, ++++++ 64-fold or greater, equivalent decrease for minus signs						
	Soil d0 Rank	Soil+VPHs	Soil+VPHs +nutrients	Soil+BC +VPHs	Soil+BC +VPHs+ nutrients	Soil+AC +VPHs	Soil+AC +VPHs+ nutrients
f__Nocardiodaceae	65			-		++++++	++++++
g__Paenisporsarcina	615	+++++	+++++	++++	++++	+++	
g__Sphingopyxis	615	+	+++	+	+		
o__Burkholderiales	615	++	++	+++	+++	+++	+
g__Achromobacter	93	++++++	++++++	++++++	++		--
g__Acidovorax	615	++	+++++	++++	++++	++++	+
g__Hydrogenophaga	222			++++	+++	++	+
g__Cupriavidus	562	+	+	+		+	+++
f__Pseudomonadaceae	615	+	++++	+++	+	+	
g__Pseudomonas	24	+++++	+++++	+	++	+	-
s__Pseudomonas__umsongensis	139	+++++	++++	++	++	+	-
g__Arenimonas	615	++	++	+++	+++	+	

270 **Table S4:** Summary of OTUs distinguished at 97% similarity which showed a minimum 8-fold increase in their relative abundance
271 rank 430 days after the connection of a VPH NAPL source to columns filled with soil, soil & biochar, or soil & activated carbon.

Taxon (highest level classification)	Relative abundance rank increase for different treatments compared to the soil only control + 2-fold, ++ 4-fold, +++ 8-fold, ++++ 16-fold, +++++ 32-fold, ++++++ 64-fold or greater, equivalent decrease for minus signs									
	Soil d0 Rank	Soil+VPHs d430 source	Soil+VPHs d430 middle	Soil+VPHs d430 Atmos	Soil+BC +VPHs d430 source	Soil+BC +VPHs d430 middle	Soil+BC +VPHs d430 atmos	Soil+AC+ VPHs d430 source	Soil+AC +VPHs d430 middle	Soil+AC+ VPHs d430 atmos
g__Nitrosopumilus	72	++++		---			-	-	--	--
g__Microbacterium	458			+++	+		+			
f__Nocardiodaceae	94			+			+	+++		+
g__Aeromicrobium	495			+	+	+	+		+	+++
g__Pseudonocardia	204	+	+	++	++	++	+++	+++++	+++++	+++++
o__Bacteroidales	629	+++++	+++	+++	+++	+++++	+++++	+	+	
f__Chitinophagaceae	86			++++		+				+
f__Flammeovirgaceae	9			+++	+	++	+	+		+
c__Chloroflexi	458	++	++	++	++	++	++	+++	+++	+++
f__Phyllobacteriaceae	131			+++			+		+	++
g__Oleomonas	629			+++++						+
g__Sphingobium	80	---	--	++++	---	---	+	---	---	+
f__Comamonadaceae	79	++	++	+++	+	++	++	++	+++	+++
g__Hydrogenophaga	116	+	++	++++	++++	++++	+++++	++	+++	+++++
o__Myxococcales	17			+++	-	-			++	++++
f__Haliangiaceae	36				+	+	++	++	++	+++
g__Nannocystis	420	+	+		-	-	-	++++	+++	++
f__Alteromonadaceae	629	+	+		+	++	+++		++	+
g__HB2-32-21	272	++	++++	++	+++	++++	++++	+	++++	+++++
f__Chromatiaceae	629	++++	+++	++	++	++++	++++	+		
o__PYR10d3	181			+++		+	+++++			
f__Pseudomonadaceae	166	+	+++	+	+++	+++++	++++	-	-	
f__Sinobacteraceae	105			++++	+	+		+	++	+
g__Pseudoxanthomonas	235			+++	+	+	++	+	++	++

Table S5: Measured column parameters, and *parameters obtained from the literature*¹¹.

	Volume fraction air (-)	Volume fraction water (-)	Tortuosity (-)	Weight fraction sorbent amendment (gg ⁻¹)
Soil	0.44	0.11	0.8	0
Soil & BC	0.48	0.12	0.8	0.02
Soil & AC	0.48	0.11	0.8	0.02

Soil-filled column length 93 (cm); soil-filled column radius 3.9 (cm); source neck length 10 (cm); source neck radius 0.55 (cm); soil solids density 2.5 (gcm⁻³); *BC solid matrix density* 1.2 (gcm⁻³)¹¹; *AC solid matrix density* 1.96 (gcm⁻³)¹¹; BC and AC intraparticle porosity 0.5 (cm³cm⁻³); BC and AC particle radius 0.005 cm.

Table S6: Physicochemical *model parameter values from the literature*^a

Compound	Molecular weight (g/mol)	Pure liquid vapour conc. (g/cm ³)	Henry's constant (-)	Diffusion coefficient in water (gcm ⁻²)	Diffusion coefficient in air (gcm ⁻²)	Carbon mass fraction (-)	Initial moles in source (-)
Pentane	72	0.002	50	0.000013	0.082	0.83	0.0086
Hexane	86	0.00065	70	0.000012	0.074	0.84	0.0131
Octane	114	0.000089	120	0.000009	0.064	0.84	0.0097
Decane	142	0.000014	293	0.000008	0.057	0.84	0.0142
Dodecane	170	0.000002	293	0.000007	0.051	0.85	0.0052
Methylcyclopentane	84	0.00060	15	0.000012	0.079	0.86	0.0131
Methylcyclohexane	98	0.00020	4.2	0.000011	0.073	0.86	0.0186
Cyclohexane	84	0.00042	7.3	0.000012	0.080	0.86	0.0147
Isooctane	114	0.00027	132	0.000009	0.064	0.83	0.0181
Toluene	92	0.00014	0.26	0.000011	0.078	0.91	0.0075
m-Xylene	106	0.000047	0.26	0.000010	0.072	0.91	0.0080
1,2,4-TMB	120	0.000015	0.27	0.000009	0.066	0.90	0.0087

^aBased on ¹, pure liquid vapor pressures at 25°C reported in ³.

Table S7: Biogeochemical model parameter values measured in batch experiments

Compound	Soil-water partitioning coefficient K _d (cm ³ g ⁻¹)	Biochar-water partitioning coefficient K _{bc} (cm ³ g ⁻¹)	AC-water partitioning coefficient K _{ac} (cm ³ g ⁻¹)	Yield coefficient (g biomass-C g ⁻¹ VPH)
Pentane	53	1596	1157000	0.2183
Hexane	68	2131	6647000	0.2209
Octane	167	8180	2656000	0.2209
Decane	805	13479	1949000	0.2209
Dodecane	1714	20152	10258000	0.2236
Methylcyclopentane	13	237	529000	0.2262
Methylcyclohexane	15	535	1439000	0.2262
Cyclohexane	6	94	180000	0.2262
Isooctane	83	1965	12446000	0.2209
Toluene	1.3	533	8433	0.2393
m-Xylene	1.8	208	2858	0.2393
1,2,4-TMB	4.4	276	2726	0.2367

Table S8: Biogeochemical model parameter values measured in batch experiments or *obtained from the literature*¹², and fitted from the column study data.

Compound	Second-order growth rate, soil batch experiments (cm ³ g ⁻¹ s ⁻¹)	Second-order growth rate, soil column, fitted (cm ³ g ⁻¹ s ⁻¹)	Second-order growth rate, soil & BC column, fitted (cm ³ g ⁻¹ s ⁻¹)	Second-order growth rate, soil & AC column, fitted (cm ³ g ⁻¹ s ⁻¹)
Pentane	97	97	97	292
Hexane	80	80	80	240
Octane	122	122	122	367
Decane	134	134	134	403
Dodecane	382	382	382	1146
Methylcyclopentane	18.5	5.6	3.7	55.6
Methylcyclohexane	8.5	2.6	1.7	25.5
Cyclohexane	9.3	2.8	1.9	27.8
Isooctane	166	50	33	498
Toluene	1.1	1.7	2.3	3.4
m-Xylene	0.79	1.2	1.6	2.4
1,2,4-TMB	2.6	3.9	5.2	7.8

Initial VPH degrading biomass = 0.0000146 (g biomass C/cm³ water), based on the initial cell count and¹². Soil biomass carrying capacity derived from available nitrogen contents = 0.0011 (g biomass C/cm³ water); Soil biomass carrying capacity fitted from the soil column study = 0.00058 (g biomass C/cm³ water); *biomass decay rate* from¹² = 0.00000025 (s⁻¹); biomass decay rate fitted from the soil column study = 0.00000050 (s⁻¹); lag phase all compounds from batch experiments = 259200 (s); lag phase branched and cyclic alkanes fitted from the soil column study = 518400 (s).

296 **Figure S1:** Exemplary data from the batch experiments with soil showing fitted time trends for
 297 a) hexane and b) isooctane.

Figure S2: Carbon-content normalized VPH concentrations measured in the middle of the soil column for the sum of the a) straight-chain alkanes, b) branched/cyclic alkanes, c) monoaromatic hydrocarbons, and d) total VPH-carbon emanation from the soil column. Inserts show the early data for the first two months. Measured data is shown as diamond symbols. Solid lines show the model predictions for the unamended soil column (solid blue line) based on parameter values which were independently measured or obtained from the literature (see Tables S5-8).

Figure S3: Carbon-content normalized VPH concentrations measured in the middle of the soil column for the sum of the a) straight-chain alkanes, b) branched/cyclic alkanes, c) monoaromatic hydrocarbons, and d) total VPH-carbon emanation from the soil column. Inserts show the early data for the first two months. Measured data is shown as diamond symbols. Solid lines show the model predictions for the unamended soil column (blue), and the soil & biochar column (red) and soil & AC column (green) based on microbiological parameter values optimized for the soil column.

320 **References**

- 321 1. K. M. Bushnaf, S. Puricelli, S. Saponaro and D. Werner, *Journal of Contaminant*
322 *Hydrology*, 2011, **126**, 208-215.
- 323 2. P. Höhener, C. Duwig, G. Pasteris, K. Kaufmann, N. Dakhel and H. Harms, *Journal of*
324 *Contaminant Hydrology*, 2003, **66**, 93-115.
- 325 3. G. Pasteris, D. Werner, K. Kaufmann and P. Hohener, *Environmental Science &*
326 *Technology*, 2002, **36**, 30-39.
- 327 4. J. Reeder and R. Knight, *Nature Methods*, 2010, **7**, 668-669.
- 328 5. D. McDonald, M. N. Price, J. K. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G.
329 L. Andersen, R. Knight and P. Hugenholtz, *ISME*, 2012, **6**, 610-618.
- 330 6. J. J. Werner, O. Koren, P. Hugenholtz, T. Z. DeSantis, W. A. Walters, J. G. Caporaso, L.
331 T. Angenent, R. Knight and R. E. Ley, *ISME*, 2012, **6**, 94-102.
- 332 7. J. G. Caporaso, J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello,
333 N. Fierer, P. A. Gonzalez, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D.
334 Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M.
335 Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T.
336 Yatsunenko, J. Zaneveld and R. Knight, *Nature Methods*, 2010, **7**, 335-336.
- 337 8. K. Clarke Robert, P. J. Somerfield and M. G. Chapman, *Experimental Marine Biology*
338 *and Ecology*, 2006, **330**, 55-79.
- 339 9. D. Werner and P. Höhener, *Environmental Science and Technology*, 2002, **36**, 1592-
340 1599.
- 341 10. S. C. Wu and P. M. Gschwend, *Water Resour. Res.*, 1988, **24**, 1373-1383.
- 342 11. D. Werner, U. Ghosh and R. G. Luthy, *Environ Sci Technol*, 2006, **40**, 4211-4218.
- 343 12. P. Meynet, E. Moliterni, R. J. Davenport, W. T. Sloan, J. V. Camacho and D. Werner,
344 *Soil Biology and Biochemistry*, 2014, **68**, 20-30.
- 345