Spatial uncoupling of biodegradation, soil respiration, and PAH concentration in a creosote contaminated soil

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Hotspots of PAH biodegradation in a creosote contaminated soil do not coincide with hotspots of PAH concentration, microbial biomass and respiration.

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ABSTRACT
Hotspots and coldspots of concentration and biodegradation of polycyclic aromatic hydrocarbons (PAHs) marginally overlapped at the 0.5–100 m scale in a creosote contaminated soil in southern Sweden, suggesting that concentration and biodegradation had little spatial co-variation. Biodegradation was substantial and its spatial variability considerable and highly irregular, but it had no spatial autocorrelation. The soil concentration of PAHs explained only 20–30% of the variance of their biodegradation. Soil respiration was spatially autocorrelated. The spatial uncoupling between biodegradation and soil respiration seemed to be governed by the aging of PAHs in the soil, since biodegradation of added 13C phenanthrene covaried with both soil respiration and microbial biomass. The latter two were also correlated with high concentrations of phospholipid fatty acids (PLFAs) that are common in gram-negative bacteria. However, several of the hotspots of biodegradation coincided with hotspots for the distribution of a PLFA indicative of fungal biomass.

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1. Introduction

The spatial distribution of soil microorganisms is heterogeneous and often autocorrelated at scales from millimetres to tens of metres (Robertson et al., 1997; Saetre, 1999; Grundmann and Debouzie, 2000; Amellal et al., 2001; Franklin et al., 2002), and similar scales of heterogeneity apply to geophysical and geochemical soil parameters (Röver and Kaiser, 1999; Stoyan et al., 2000; Ritz et al., 2004). Microbial activity is also variable and autocorrelated at different scales. The range of autocorrelation of soil respiration in forests is related to aboveground plant properties and belowground soil properties and may vary between 4 and 20 m (Saetre, 1999; Stoyan et al., 2000). Biodegradation of contaminants varies by a factor of 2–20 in surface and subsurface soils at the millimetre scale (Gonod et al., 2003), the metre scale (Walker et al., 2001) and the 10–400 m scale (Mills et al., 2003). The spatial resolution of sampling rarely suffices to decide whether spatial autocorrelation is present. An exception is the work by Parkin and Shelton (1992), who demonstrated that pesticide biodegradation is spatially autocorrelated at distances exceeding 20–30 m in a ploughed and tilled field.

Some few investigations have focused on the spatial heterogeneity of PAH biodegradation. Breedveld and Sparrevik (2000) found that biodegradation of 2–5 ring PAHs varied by a factor 10 between the topsoil (0–0.5 m), an organic rich layer (2–2.5 m), and a sandy aquifer underlying a creosote contaminated soil. At a smaller scale, phenanthrene biodegradation was inversely related to the distance (1–9 mm) from Lolium perenne roots (Corgié et al., 2003). The heterogeneity in PAH biodegradation may result from heterogeneous soil conditions. For instance, the fine silt and clay fractions house most PAH degraders (Amellal et al., 2001) and the greatest PAH mineralisation in a soil (Carmichael and Pfaender, 1997), and the activity of mycobacterial PAH degraders coincides with the distribution of total organic carbon (Bogan and Sullivan, 2003). Another reason for spatially variable PAH biodegradation may be specific responses of PAH degrading microbial communities to certain soil conditions, such as nutrient amendments (Durant et al., 1997). If the soil microbial community and the specific subset of PAH degraders are limited by the same soil properties, one would also expect a co-variation between soil respiration and PAH degradation, as found by Eriksson et al. (2000). The same co-variation would be observed if the PAH degraders were a quantitatively dominant group in the microbial community. In that case, the microbial community characteristics would be expected to co-vary both with microbial activity in general and with PAH degradation.
This study is an effort to analyse the within-field spatial variation of PAH biodegradation capacity and the correlation between biotic and abiotic soil properties and PAH biodegradation. It was also designed to test differences in biodegradation of added and native PAHs and their dependence on biotic and abiotic soil parameters. Soil respiration was used as a measure of the microbial activity, because we were interested in the spatial variability of PAH biodegradation across the site in relation to microbial activity in general. The study was made at a site used to impregnate railroad sleepers between 1949 and 1965. Earlier work showed that most PAHs were spatially autocorrelated and appeared with similar composition in several hotspots (Bengtsson and Törneman, 2009), and that native 2–5 ring PAHs were extensively degraded in soil samples from the site (Bengtsson and Zerhouni, 2003).

The objectives were to 1) reveal the spatial heterogeneity and autocorrelation of PAH biodegradation and soil respiration; 2) explore the spatial relationships between PAH biodegradation and microbial soil properties (microbial biomass, soil respiration, and microbial community characteristics); 3) analyse the spatial relationships between PAH biodegradation and soil respiration on one hand and abiotic soil properties on the other (PAH concentrations, pH, water holding capacity, fraction of organic carbon, and aggregate size distribution); 4) examine the influence of PAH aging on the relationship between PAH biodegradation and soil properties.

2. Materials and methods

2.1. Site and sampling

The Hässleholm site is situated in Southern Sweden, and the study area measures 280 by 40 m. The impregnation of railroad sleepers between 1949 and 1965 resulted in high concentrations of creosote, notably PAHs (49–5000 mg/kg soil of individual PAHs) in the soil. A regular nested sampling grid was used. One row of samples was taken along each of the short sides of the rectangular grid. Three groups of samples were then taken along the long sides of the grid, with a distance of 40 m between them. Three rows of samples were taken within each group, with a distance of 20 m between the rows. The distance between the samples along the short side of the rectangle was 10 m, which made a total of 55 samples. In addition, ten grid nodes were randomly chosen as starting positions for additional sampling points located 0.625, 1.25, 2.5, and 5 m on each side of the grid node perpendicular to the longer side of the grid rectangle, resulting in a total of 131 samples (four missing for various reasons) (the sampling strategy is shown in Fig. 1 of Törneman et al. (2008)). The samples were taken in January 2002 when the soil was covered with snow and the upper 5 cm were frozen. A stainless steel auger of 20 cm diameter was used to drill a hole to 40 cm depth. The soil was discarded, and a 10 cm diameter soil core was withdrawn from the bottom of the hole. The augers were cleaned with water in-between samplings. The soil samples were passed through a 2 mm screen and kept separately in plastic bags at 2°C for one week before the biodegradation experiment commenced. The plastic bags were vented regularly to avoid oxygen depletion.

2.2. Abiotic and biotic soil characterisation

The lower soil layers (>1 m below the surface) have sandy silty moraine, while the upper soil layer is a heterogeneous mixture of natural soil and filling material, with a sand content of 20–80%, a silt content of 5–15%, pH of 7.4–8.9, water holding capacity of 15–40% (dry wt basis), and a total organic carbon content (TOC) of 0.04–1.5%. Electric conductivity (EC), pH, water holding capacity (WHC), aggregate particle size distribution (PAD), total carbon (TC), and total organic carbon (TOC) were determined for each individual soil sample as described in Bengtsson and Törneman (2009). The phenotypic microbial community fingerprint was obtained by PLFA analysis (Törneman et al., 2008).

2.3. Biodegradation assay of native PAHs

The general assay strategy was to incubate soil samples in microcosms under constant moisture, light and temperature conditions. Eighty soil samples (8–12 g) from eighty positions within the site were placed in 25 ml headspace GC vials (Perkin Elmer), which had been heated for 12 h at 400 °C, and a cotton stopper was used to allow free air exchange. The vials were placed in darkness at 17 °C in two plastic covered trays. Tap water was added to the bottom of the trays to maintain a high humidity and reduce water evaporation from the samples. The vials were weighed before and after soil addition, and different amounts of water were added to each vial to maintain a water holding capacity of 50%. Once a week the vials were reweighed and an appropriate volume of water added to maintain the water holding capacity at 50%. Distilled water was added, except at every third week, when tap water was added. A mixed HgCl2 (4 mM) and NaN3 (25 mM) solution was added (50% water holding capacity) to six randomly selected abiotic control vials. The PAH concentrations at the beginning of the assay were determined from the soil samples that were kept at −20 °C. After 210 days of incubation, the assay was terminated, and duplicate or triplicate samples (2–4 g) from each vial were extracted and analysed for PAHs. The CO2 production in the vials was measured after 27, 39, 55 and 172 days (see below). Biodegradation (B) for each PAH in each vial was calculated as: B = [(C0–Ct)/C0]*100 − lbio, where C0 and Ct are the average PAH concentrations of the replicates before and after the incubation, and lbio is the average percentage loss of each PAH in the abiotic controls. The 95% confidence interval was evaluated for C0 and Ct. Unless the confidence intervals overlapped, the two values were deemed significantly different and were used to calculate biodegradation. When the abiotic losses during the 7-month incubation period were high compared with biodegradation of naphthalene, acenaphthene, acenaphthyene, and fluorene (data not shown), data were excluded from the biodegradation calculations. The anthracene peak coeluted with another chromatography peak that was not present in the soil extracts at time zero, and anthracene was excluded from the calculations. In the following, PAH biodegradation and soil respiration refers to potential PAH biodegradation and respiration of the soil, since in situ activities were not measured.

2.4. PAH analysis

The sixteen EPA priority PAHs (Keith and Telliard, 1979) were extracted using dichloromethane, saponification and ultrasonication and analysed by gas chromatography and by gas chromatography–mass spectrometry, as described in Bengtsson and Törneman (2009).

2.5. Respiration measurement during native PAH degradation

A vial was prepared for CO2 measurement by flushing it with synthetic air (containing 100 ppm CO2) and sealing with a Teflon coated headspace septum (Perkin Elmer) and an aluminium crimp cap (Perkin Elmer). An empty vial containing synthetic air was prepared for every twenty sample. After 27 h, the amount of headspace CO2 was determined in a Hewlett Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA), equipped with a thermal conductivity detector and connected to a Perkin Elmer HX 40XL headspace sampler (Perkin Elmer, Waltham, MA, USA). CO2 was separated from N2/O2 at 30 °C on a HP–PLOT Q column (15 m, ID 0.53 mm), with He as carrier gas at 6 ml min−1. The filament temperature was 300 °C and the injector temperature was 250 °C. Samples were injected at a split ratio of 1:1. After the CO2 measurement, the septum and cap was removed and replaced with a cotton stopper. Peak areas for carbon dioxide were standardized against known amounts of NaHCO3. The CO2 concentration in the assay vial was...
calculated from the standard curve, and the rate of CO2 evolution was calculated as \([\text{CO}_2(t) - \text{CO}_2(0)]/t\), where \(\text{CO}_2(t)\) is the CO2 concentration after time \(t\) and \(\text{CO}_2(0)\) is the average CO2 concentration in the abiotic control vials. The empty vials were used to monitor for leaks during the incubation. The total amount of CO2 evolved during the incubation period was obtained from a fit of a linear regression model to the CO2 evolved at each of the four sampling days.

2.6. Soil respiration and biodegradation of 13C labelled phenanthrene

Soil respiration and mineralisation of 13C-phenanthrene was determined from incubations of soil samples from ten randomly chosen positions within the site. A variety of bacterial strains can utilize phenanthrene as a sole carbon source, usually initiating the breakdown by 1,2- or 3,4-dioxygenation (See et al., 2009). The molecule undergoes several decarboxylations and hydroxylations to phthalic or salicylic acid, in which the 6-carbon position finally becomes hydroxylated. Two grams of soil (wet weight) were added to 25 mL headspace GC vials (Perkin Elmer) with Teflon lined silicone septum caps (ChromTech). Five replicates from each soil sample were prepared. 100 μL of autoclaved MilliQ water were added to each vial, which was left on a shaker at 50 rev min \(^{-1}\) for 24 h in the dark at 18 °C. Then 9.0 μL of 13C-phenanthrene (10 μg μL \(^{-1}\) in n-nonane, Cambridge Scientific), diluted in 20 μL of acetone, were added, corresponding to an increase of the in situ phenanthrene concentration by a factor of 5-10. One control without 13C-phenanthrene was also prepared for each replicate. The vials were kept on the shaker at 18 °C in the dark. Each vial was analysed for total respiration and 13CO2 every 24 h for a period of 7 days, starting one day after the 13C phenanthrene addition, using isotope ratio mass spectrometry (IRMS). To prevent leakage, the perforated membrane of each vial was covered by a thin layer of silicon after each sampling.

The concentration and 13C/12C ratio of the CO2 in the headspace of the vials were determined in a 20-20 IRMS (PDZ Europa Scientific Instruments) interfaced with a Hewlett Packard 6890 gas chromatograph equipped with a split/splitless injector and a CTC Combi PAL (Crelab Instruments). Headspace samples of 100 μl were injected split/splitless at 130 °C, and He was used as carrier gas at 2 mL min \(^{-1}\). CO2 was separated from other inert gases by a Poraplot Q column (l = 25 m, d = 0.32 mm) at 70 °C. The effluent from the capillary column passed through a Nafion membrane to remove water vapour, and the CO2 was then released into the 20-20 IRMS. The atom % 13C was calculated from the atom % 13C of a reference gas injected three times at the beginning and end of each chromatographic analysis. The reference CO2 was calibrated with the PeeDee Belemnite standard, and the precision of the reference gas 13C was 0.2%. Peak areas for CO2 were standardized against known amounts of NaHCO3. The CO2 atom % 13C excess in the vials that had received 13C phenanthrene was calculated by subtracting the background atom % 13C of the CO2 in the corresponding control vials and multiplying with the total concentration of CO2 in the vial.

2.7. Spatial data analysis and statistics

In classical statistics, one assumes that the mean of a survey unit is the expected value everywhere in the unit, with an estimated random error. However, this error often turns out to have spatial dependence within a given distance of the survey unit, and the basis of geostatistical methods is the recognition that parameters in an area have both random and spatial properties. The dissimilarity or experimental semi-variance (γ(e)) among observations is calculated as a function of their separation (lag, h);

\[ \gamma(e)(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} (Z(x_i) - Z(x_i + h))^2 \]

where N(h) is the number of data pairs separated by a lag distance (h) and Z(x_i) and Z(x_i + h) are measured parameter values (Z) at any two points separated by the lag h. The plot of the semi-variance (γ(e)) against the lag distance (h) is the experimental

![Fig. 2. Experimental semivariograms of observations of respiration and biodegradation of 3–6 ring PAHs in individual soil samples of known position at the Hässleholm site. The semi-variance was plotted versus the separation distances (lag, metres) between the samples and a spherical model (line), soil respiration: \(r^2 = 0.74\) fitted to the data (open circles). The y-intercept is the nugget, which represents random variance below the scale of sampling. The observations of biodegradation of 3–6 ring PAHs had no significant dissimilarity but a constant semi-variance, that is, the samples were not spatially autocorrelated but characterized by random variance. Soil respiration was spatially autocorrelated, and the range of spatial dependence (where the asymptote of the sperical model was reached) was 30–45 m. The difference between the semi-variance at this distance, the sill, and the nugget represents the spatial variance, which was 30–50% of the total variance.

![Fig. 3. Spatial patterns of PAH biodegradation (sampling points indicated by circles) and total PAH concentration (values interpolated by kriging and displayed on a contour map) at the creosote contaminated site at Hässleholm. The diameter of the circles is proportional to the PAH biodegradation (%). The circle size for 20 and 40% PAH biodegradation is shown as a reference. The variation in PAH concentrations is given by the grey-shaded gradient in the contour map (scale inserted to the right of the map): dark grey is for high concentration, light grey for low, and white for zero. The biodegradation is calculated from the difference between PAH concentrations at the beginning and end of the assay, in percentage of the initial concentration in soil samples incubated for 210 days at 17 °C in darkness. The total PAH concentration was calculated from PAH measurements of the soil sample at the beginning of the assay. Cold spots are operationally defined as sample positions with 5% or less of biodegradation, and hotspots have 40% or more. Some hotspots for biodegradation and PAH concentration overlap, for instance at 30 m longitude and 157 m latitude. Some hotspots for biodegradation have low concentrations of PAHs, for instance at 260 m latitude, and some coldspots of biodegradation appear in hotspots for PAH concentrations, for instance at 45 m and 100 m latitudes.](image-url)
The average biodegradation varied considerably between individual PAHs, and twice as much of the C20 PAHs benzo(b/k)fluoranthene and benzo(a)pyrene was degraded compared with the four ring benzo(a)anthracene and pyrene during the 210 days assay (Fig. 1). Yet, there was no significant difference in the magnitude of biodegradation for the different PAHs, mainly due to a large variation between individual samples, with a coefficient of variation (CV) ranging from 0.6 (benzo(b/k)fluoranthene) to 1.5 (fluoranthene) (Fig. 1).

The biodegradation of PAHs was not spatially autocorrelated but had a pure nugget effect, illustrated in Fig. 2 for the 3–6 ring PAHs. Hot spots and cold spots of concentration and biodegradation of PAH rarely overlapped on the contour map (Fig. 3), suggesting that they had no obvious spatial co-variation. However, the biodegradation of native 5–6 ring PAHs was significantly dependent on the soil concentration of 5–6 ring PAHs, and one unit increase of the soil concentration of 5–6 ring PAHs increased their biodegradation by 0.3–0.4 units (Table 1). The biodegradation of all PAHs was dependent on the total concentration of PAHs and pH (Table 1). The soil concentrations of PAHs explained about 20–30% of the variance of their biodegradation, suggesting that the field site variability of biodegradation and concentration of PAHs was not perfectly coinciding and that other factors than those measured may have contributed to 70–80% of the variance of biodegradation.

With one exception, the biodegradation of 3–4 ring PAHs was not correlated to any microbial soil property (microbial biomass, soil respiration, and microbial community characteristics) (Table 2). The exception was biodegradation of fluoranthene, which was correlated with soil respiration. Biodegradation of three of the 3–4 ring PAHs, phenanthrene, pyrene, and fluorene, was linked in one way or the other, but biodegradation of chrysene was not correlated with biodegradation of the other 3–4 ring PAHs or with the microbial soil properties (Table 2). The results leave out the possibility that the biodegradation of the PAHs was in general dependent on the three common microbial measures of structure and activity. The biodegradation of 13C phenanthrene was correlated to microbial biomass and soil respiration (Table 3) but independent of abiotic soil parameters (data not shown).

The soil respiration was spatially autocorrelated (range = 30–45 m, spatial variance = 30–50%, Fig. 2), as opposed to the biodegradation of PAHs, and krieded contour maps showed that the spatial distribution of soil respiration was more variable after 27 days of incubation than after 172 (Fig. 4). Soil respiration in the long term assay was independent of the PAH concentration at

### Table 2

<table>
<thead>
<tr>
<th>Phen. biodeg.</th>
<th>Pyrene biodeg.</th>
<th>Fluor. biodeg.</th>
<th>Chrysenene biodeg.</th>
<th>Microb. biomass</th>
<th>Soil respiration</th>
<th>PLFA PC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phen. biodeg.</td>
<td>Pyrene biodeg.</td>
<td>0.42/0.41</td>
<td>0.53/0.56</td>
<td>0.70/0.77</td>
<td>0.55/n.v.</td>
<td>0.55/n.v.</td>
</tr>
<tr>
<td>Fluor. biodeg.</td>
<td>0.53/0.56</td>
<td>0.70/0.77</td>
<td></td>
<td></td>
<td>0.77/0.61</td>
<td>0.74/0.34</td>
</tr>
<tr>
<td>Chrysenene biodeg.</td>
<td>0.70/0.77</td>
<td></td>
<td></td>
<td>0.77/0.61</td>
<td>0.77/0.61</td>
<td>0.51/0.28</td>
</tr>
<tr>
<td>Microb. biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.77/0.61</td>
<td>0.74/0.34</td>
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<tr>
<td>Soil respiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.77/0.61</td>
<td>0.51/0.28</td>
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<tr>
<td>PLFA PC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.55/n.v.</td>
<td>0.55/n.v.</td>
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</tbody>
</table>
concentrations below 50 mg kg\(^{-1}\) soil but positively dependent at higher concentrations (Fig. 5), so that the total PAH concentration explained 43% of the variation of soil respiration at the site (Table 4). The respiration was lower in most samples after 172 days of incubation compared with 27, except in those with total PAH concentration exceeding 300 mg kg\(^{-1}\) soil (Fig. 5). Soil respiration in both the short-term and long-term assays was dependent on the fraction of organic carbon (Table 4), the microbial community characteristics, and biomass (Tables 2 and 3). The fraction of organic carbon explained almost twice as much of the variation of soil respiration in the long term assay as in the short-term. The correlations were recalculated while controlling for the influence of the total PAH concentrations, usually resulting in a significant but lower correlation coefficient, indicating that the correlations between microbial soil properties was partly explained by their simultaneous dependence on the PAH concentration.

### 4. Discussion

A number of characteristics appeared at first to qualify the biodegradation potential to be spatially correlated with the PAH concentration and the microbial biomass/respiration. First, biodegradation was substantial, arriving at 60% for some PAHs and average values of 15–40% in 210 days (Fig. 2). A similar capacity was observed by Bengtsson and Zerhouni (2003), who added groundwater and DOC (dissolved organic carbon) to soil from the same site to get 20–50%, 10–45% and 10–20% biodegradation of 3, 4, and 5–6 ring PAHs, respectively, after 60 days of incubation. Similar observations were also made by Haeseler et al. (1999) (89, 67, and 23% of 3, 4, and 5–6 ring PAHs, respectively, after 3 months incubation of soil with similar TOC as in Hässleholm), Breedveld and Sparrevik (2000) (70, 0, 20% after 6 months of incubation of 4% TOC surface soil), and Brauner et al. (2002) (10–40% of 4–5 ring PAHs after 8 months of soil incubation). High rates of PAH degradation seems to be a characteristic of PAH contaminated soils (Carmichael and Pfender, 1997).

Second, the spatial variability of PAH biodegradation was considerable (Figs. 2 and 3). This is a common phenomenon for contaminant biodegradation in soil environments. For instance, 2,4-D mineralisation varied by a factor ten at the mm scale in a cultivated field soil (Gonod et al., 2003) and phenanthrene biodegradation by a factor 2–3 in a ryegrass rhizosphere (Corgié et al., 2003). The variability of the 2,4-D mineralisation was attributed to an uneven distribution of degrading microorganisms rather than to a variability of soil characteristics, and the uneven distribution of root exudates was suggested to create a variable number of phenanthrene degraders in the rhizosphere. At a larger scale, isoproturon biodegradation varied by a factor of seven between transects spaced 50 m apart and by a factor of 3–4 within transects in an agricultural field (Walker et al., 2001), in accord with small pH variations.

Yet, the variogram analysis demonstrated a large nugget effect for PAH biodegradation (Fig. 2), meaning that the variable was irregular, discontinuous and not spatially autocorrelated. This may be found in situations where extreme values are surrounded by smaller values (Juang et al., 2001), as shown in Fig. 3, where samples with little or no PAH biodegradation are close to samples with high. These irregularities may represent a more complicated dependence pattern than the geostatistical analysis primarily can reveal. Since 1) the soil concentration of PAHs explains 59% of the variance of the microbial biomass (the total amount of PLFAs) (Törneman et al., 2008), 2) the microbial biomass explains 37% of the variance of the soil respiration (Table 2), and 3) the soil concentration of PAHs explains only 27% of the variance of the biodegradation (Table 1), other characteristics than the spatial distribution of the PAHs, the total microbial biomass, and its respiratory activity must make a substantial contribution to the spatial variability of biodegradation.

The PLFA PC1 was mainly determined by high concentrations of PLFAs that are common in gram-negative bacteria, and since the soil concentration of PAHs explained 61% of their variance (Törneman et al., 2008) and their distribution was not significantly correlated with the distribution of the PAH biodegradation (Table 2), other groups of microorganisms may have an impact on

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**Table 3**

Pearson product moment correlation coefficients for the relationships between biodegradation of \(^{13}\)C phenanthrene and measures of microbial structure and function in the 7 days assay with \(^{13}\)C phenanthrene added to samples of PAH contaminated soil from the Hässleholm site. The number following slash is the partial correlation after controlling for (e.g., partialling out) the PAH concentration from the correlation. Data for soil respiration were obtained from the total amount of CO\(_2\) produced during the incubation period, and data for biomass and PLFA PC1 were obtained from Törneman et al. (2008). The correlations were only calculated for data at lag > spatial range, so that the data fulfilled the assumption of independence between measurements. Correlations with \(p < 0.05\) are shown. The analysis shows that soil respiration explains 55% (squared partial correlation) of the variability of biodegradation of \(^{13}\)C phenanthrene and that microbial biomass explains 17% of the variability of soil respiration.

<table>
<thead>
<tr>
<th>Phen. biodeg.</th>
<th>Microb. biomass</th>
<th>Soil respiration</th>
<th>PLFA PC1</th>
</tr>
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<tbody>
<tr>
<td>0.32/0.34</td>
<td>0.71/0.74</td>
<td>0.35/0.37</td>
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**Fig. 4.** Spatial pattern of soil respiration at the Hässleholm site shown on contour maps derived from values interpolated by kriging of 131 samples. The contour maps show the respiration pattern after 27 and 172 days of soil incubation at 17 °C in darkness. A quantitative scaling guide is inserted to the right of the maps: the variation in respiration is given by the grey-shaded gradient, with dark grey for high rates and light grey for low.
the biodegradation pattern. One candidate group would be fungi. The PLFA 18:2o6:9 is an indicator of fungal biomass, which is the most common PLFA in the cold spots of PAH concentrations at the Hässleholm site and is negatively correlated with the PAH concentration (Törneman et al., 2008). However, several of the hotspots of biodegradation (Fig. 3) coincide with hotspots for the distribution of the 18:2o6:9 (Fig. 1 in Törneman et al. (2008)), and since different groups of fungi are proficient PAH degraders (Sack et al., 1997), they may add some explanation to the biodegradation pattern in the Hässleholm soil. Biodegradation may also be controlled by spatially variable soil properties that were not quantified (bioavailability, nutrients, electron acceptors, other toxic elements, etc).

In contrast to PAH biodegradation, soil respiration was clearly spatially autocorrelated (Fig. 1), and its spatial pattern followed that of PAH concentrations (compare Figs. 3 and 4). Soil respiration has often a strong spatial autocorrelation (Robertson et al., 1997; Saetre, 1999; Stoyan et al., 2000), and its spatial variability can be controlled by tree canopy (Saetre, 1999), urine patches (Ritz et al., 2004), and soil moisture (Bengtsson et al., 2005).

The dependence of soil respiration on the PAH concentration (Table 4, Fig. 5) and the uncoupling of native PAH biodegradation from soil respiration and microbial biomass indicates that other aromatic compounds in the creosote were at least equally important carbon sources for the bacteria as the quantified PAHs. In addition to the 16 EPA priority PAHs measured here, there are hundreds of other substituted and unsubstituted PAHs in creosote, all of which may biodegrade (Lundstedt et al., 2003). Their influence on respiration is evident in Fig. 5, where the base line respiration becomes lower after 172 days of incubation in soil samples with low PAH concentrations but remain high for high PAH concentrations.

Overall, soil respiration was slightly higher in the experiment with 13C phenanthrene than in the long-term PAH bioassay (Figs. 5 and 6). Due to aging processes (Johnsen et al., 2005) and low water solubility of native aromatics and natural carbon, the small amount of added 13C phenanthrene (and acetone) may have stimulated the microbial community, possibly indicating that the soil bacteria were carbon limited, as commonly found (Griffiths et al., 1999). Solvents and other sources of dissolved organic carbon have been added to contaminated soils to stimulate biodegradation, and PAH degradation in coal tar-contaminated soils doubled by acetone addition (Lee et al., 2001). That work used 50 times higher concentrations of acetone (0.5 mL g-1 soil) than in our assay, so the effect was probably more to increase the bioavailability by desorption of aged PAHs than to stimulate microbial growth. The addition of acetone in our assay corresponded to 0.4 g kg-1 soil of DOC, which was close to 10% of the average TOC at the site and may have represented some initial growth stimulus. In this situation, the respiration of the microbial community may have become

![Image](image-url)

**Fig. 5.** The relationship between soil respiration (µg CO2 d-1 g soil-1) and the sum of the native concentrations of the 16 PAHs determined (sum PAH, mg kg soil-1) in individual spatially separated soil samples at the Hässleholm site. The respiration was measured after 27 (left) and 172 (right) days of incubation at 17 °C. Altogether 80 soil samples were assayed, and each circle represents an individual sample.

![Image](image-url)

**Fig. 6.** The relationship between biodegradation of 13C labelled phenanthrene (ng g soil-1 d-1) and soil respiration (µg CO2 d-1 g soil-1) of ten individual, spatially separated soil samples from the Hässleholm site. The samples were incubated for a total of 7 days at 17 °C and soil respiration and mineralized 13C phenanthrene were measured daily, starting on the second incubation day.

**Table 4**

Stepwise multiple regression analysis, forward procedure (F to enter = 1.5, F to remove = 0) of soil respiration on abiotic soil properties (PAH concentration, PAD (aggregate particle size distribution), WHC (water holding capacity), pH, EC (electric conductivity), fOC (fraction of organic carbon), TC (total carbon)) in the long term (172 days incubation) and short term (7 days incubation) assay of individual soil samples from the Hässleholm site. The calculations were limited to data for lags that were longer than the spatial range, so that the data fulfilled the assumption of independence between measurements. Only significant regressions (p < 0.05) are shown. The electric conductivity explained more (53%) of the variability of respiration in the long term incubation than the concentration of PAHs. A comparison of the linear regression coefficients with the fraction of organic carbon shows that respiration was 5.5 times faster per fraction of organic carbon in the long term incubation than in the short term.

<table>
<thead>
<tr>
<th>Dependent Factor</th>
<th>Factors in model</th>
<th>Regression Coefficient</th>
<th>Model R²</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO2 respiration (long term incubation)</td>
<td>sum PAH</td>
<td>0.18</td>
<td>0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>fOC</td>
<td>0.99</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.89</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>CO2 respiration (short term incubation)</td>
<td>fOC</td>
<td>0.18</td>
<td>0.27</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
uncoupled from the PAH concentrations (Table 4) and covaried with the phenanthrene biodegradation (Fig. 6, Table 3). Enrichment of soils with other sources of dissolved organic carbon has been 1) without effect on native PAH degradation (phthalate; Singleton et al. (2008), humic substances; Seibeld et al. (1996)), 2) depending on the DOC concentration and the size of the PAH molecule (extracted soil DOC; Bengtsson and Zerhouni (2003)), or 3) limiting it (complex forming macromolecules; Allan et al. (2007)).

In conclusion, whereas soil respiration, microbial biomass, and concentration of PAHs are spatially autocorrelated and coinciding at the creosote contaminated site, the spatial pattern of biodegradation is highly irregular, and the distribution of its hotspots and coldspots only marginally overlapping with those of the PAH concentration. The spatial uncoupling between biodegradation and soil respiration seems to be governed by the aging of PAHs in the soil, since biodegradation of recently added phenanthrene covered with soil respiration. The issue of predicting biodegradation of PAHs from the spatial heterogeneity of their concentration and soil respiration at a contaminated site may be resolved by describing the spatial heterogeneity of the fungal biomass. Its hotspots coincide with those of biodegradation and with the coldspots of PAH concentration. Thus, attempts to predict the potential in situ biodegradation of PAHs at abandoned hazardous waste sites from hotspots of contaminants run the risk of being largely erroneous.

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