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Assessing mineralization rates of petroleum hydrocarbons in soils in relation to environmental factors and experimental scale

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Abstract

Mineralization rates of non-volatile petroleum hydrocarbons (HCs) in five different oil-contaminated soils with initial HC contents ranging from 0.1 to 13 g kg^{-1} are estimated as a function of environmental factors. The aim of the study is threefold, (i) to study the relevance of environmental factors that may influence the mineralization rate, (ii) to compare mineralization rates estimated in two experiments at different scales, after standardizing them to environmental reference conditions, (iii) to evaluate the CO_2 production rate as a measure for the mineralization rate of HCs. Experiments were performed at laboratory scale (30–50 cm^3 soil volume) in closed-jars under constant environmental conditions and in lysimeters (0.81 m^3 soil volume) under dynamic climatic and hydrological conditions. A biodegradation model, coupled to transport models for soil heat, water, and gas dynamics is employed for data interpretation. The transport models are used to simulate the environmental conditions that influence the mineralization rate in the non-steady lysimeter experiments. The results show that temperature, O_2 concentration and HC content have an effect on the mineralization rates. Water content could not be identified as a direct governing environmental factor. However, an indirect effect of water content is that it influences the effective gas diffusion coefficient in soils. The CO_2 production rate seems to be a good quantity to express the mineralization rate of HCs for HC contents > 1 g kg^{-1}. Measured CO_2 production rates standardized to reference conditions are similar for the two different experimental scales. This demonstrates that the usage of biodegradation rates obtained in the laboratory to predict the biodegradation rates under field conditions is sound, as long as the differences in environmental conditions have been taken into account.

Introduction

Biorestoration techniques, such as landfarming, are recognized as relatively simple and cheap methods to recover oil-contaminated soils (Wang & Bartha 1990; Harmsen 1991; Genouw et al. 1994). The success of these methods depends on the treatments that are applied to enhance aerobic biodegradation. Most treatment scenarios focus on improvement of the environmental conditions that influence the mineralization rate and the increase of biomass. Several studies (e.g. Dibble & Bartha 1979; Wang & Bartha 1990; Harmsen 1991; Freijer 1996) have shown that water content, temperature, nutrient status, availability of O_2, and the availability of the contaminant may influence the mineralization rate. Based on the response of the mineralization rate to the above mentioned environmental conditions, Dibble and Bartha (1979) clarified the feasibility and efficiency of landfarming practice under a variety of conditions, utilizing laboratory experiments. A scale up of these experiments with outdoor lysimeters was carried out by Wang and Bartha (1990). Freijer and Bouten (1993) and Li et al. (1993) applied simulation models to evaluate the effect of different bioremediation scenarios on the biodegradation rate. A major concern of model application is the fulfillment of input requirements concerning the system parameters in the biodegradation model.

Mineralization rates can easily be estimated from measurements in laboratory incubation experiments. Standard laboratory facilities are suitable to guarantee simple and time saving procedures. Field experiments and lysimeter studies on the other hand are time consuming and require expensive monitoring programs. An important issue is whether laboratory experiments represent the field situation (Dibble & Bartha 1979; Blackburn et al. 1993). If the results of laboratory experiments can be translated to the field situation, this will greatly reduce experimental effort and costs. The potential limitations of laboratory experiments are of various origin. The volumes of samples in laboratory incubation experiments are often very small and the soil properties of the samples may deviate from the bulk soil in the field. If samples are disturbed when taken from the field, transport coefficients and differential capacities of water, heat and gas may change considerably (Klute 1986). Sample preparation (drying and sieving) may induce additional irreversible changes in hydrocarbon (HC) content and microbial population. Also, most incubation studies impose steady environmental conditions, while in an outdoor climate these conditions show a dynamic behavior.

In the present study the results of experiments at two different scales to determine mineralization rates (CO_2 production rates and decrease of HC content) are compared as a function of environmental factors. Firstly, laboratory closed-jar experiments with fragmented soil contaminated with non-volatile HCs are applied under steady conditions. Secondly, greenhouse lysimeter experiments are carried out with the same contaminated soil series at dynamic hydrological and temperature regimes. The relationship between the mineralization rate and the environmental factors, given by a biodegradation model, is established from the laboratory measurements using non-linear curve fitting techniques. Simulation models for water, heat, and gas transport are employed to calculate abiotic soil conditions in the lysimeter experiments. The hypothesis that the differences in environmental conditions during the two types of experiments can explain the differences in measured mineralization rates is investigated by a comparison of standardized CO_2 production rates. The production rates measured in the two experiments are standardized to environmental reference conditions, using the biodegradation model. Finally, the CO_2 production as a measure for the mineralization rate of HCs is evaluated (Dibble & Bartha 1979; Mott et al. 1990).

Theory

Biodegradation model

The model to calculate the transformation rates in response to the abiotic environment is based on multiplicative interaction (Stroo et al. 1989; Boesten & Van der Linden 1991). Using this concept, the CO_2 production rate is given by

$$S_C = S_{r,C} f_O f_{HC} f_\theta f_T \tag{1}$$

where S_C is the actual CO_2 production rate in a given soil volume and $S_{r,C}$ the CO_2 production rate at reference conditions (for further explanation of the symbols see Notation). The environmental rate factors, *f*, are determined by response functions which give the effect of the actual abiotic conditions on the CO_2 production rate relative to the effect at the reference conditions that belong to $S_{r,C}$. Equation (1) includes rate factors to express the effect of O_2 concentration, HC content, water content and temperature. Conversion of CO_2 production rates to O_2 consumption rate can be done when the apparent respiration quotient, *r*, is known

$$S_O = -S_C/r \tag{2}$$

A similar equation for conversion of CO_2 production rates to HC-C consumption rates is given by

$$S_{HC} = -S_C/a \tag{3}$$

where *a* denotes the apparent mineralization quotient.

To calculate the environmental rate factors, expressions are required to relate them to HC content, O_2. content, water content and temperature. For the HC and O_2 rate factors, first order relationships are used (Jury & Ghodrati 1989; Freijer 1996)

$$f_{HC} = C/C_r \tag{4}$$

$$f_O = X_O/X_{r,O} \tag{5}$$

The effect of water content on the mineralization rate is described by a non linear equation (Boesten & Van der Linden 1991)

$$f_\theta = (\theta/\theta_r)^n \tag{6}$$

where *n* ranges from 0 to 1. For the temperature rate factor several expressions have been described, of

Table 1. Outline of experiments

Experiment	Closed jar, laboratory	Lysimeters, greenhouse
Soil volume	30–50 cm^3	0.81 m^3
Soil profile thickness	0.5–1.0 cm	0.60 m
Dynamic conditions	X_O	X_O, T, θ, C
Steady conditions	T, θ, C	–
Macro-transport	no	yes
Sublayers number	one	multiple
Upper boundary	closed	open
Lower boundary	closed	gas: closed water: open heat: closed
Mathematical interpretation	analytical	numerical

which Q_{10} and Arrhenius relationships are frequently used. However, it was shown that optimum functions represent a more realistic description of the temperature rate factor, because microbial activity usually decreases at high temperatures (Dibble & Bartha 1979; Stroo et al. 1989). Hence, in our model an optimum function is applied following a Gaussian equation

$$f_T = \frac{exp(-\,k_T\,(T\;-\;T_{opt})^2)}{exp(-\,k_T\,(T_r\;-\;T_{opt})^2)} \tag{7}$$

The values of the four model parameters $S_{r,C}$,n, k_T and T_{opt} may vary with the type of contaminated soil and should therefore be determined empirically. The following reference conditions are used in this study, T_r = 20 °C, C_r = 4 g kg^{-1}, θ_r = 0.25 m^3 m^{-3} and $X_{r,O}$ = 9.2 mol O_2 m^{-3}.

When the environmental conditions are kept constant in time and space during an experiment, the rate factors in Equation (1) can be considered as constants. However, if the environmental conditions exhibit temporal and spatial variations, the effect of these variations on the rate factors has to be taken into account. The appropriate method for doing this is associated with the experimental setup. An outline of the two experiments used in this study is given in Table 1. The theoretical considerations for the experimental designs are discussed in the next two sections.

Design of closed-jar experiments under steady conditions

The closed-jar experiments are performed using jars containing a thin layer of moist soil contaminated with oil (Table 1). At the start of an experiment the jars are closed, and O_2 concentration and HC content both decrease due to consumption. Freijer (1996) derived an analytical solution of the governing differential equations that describe CO_2 production and O_2 consumption rates as a function of O_2 concentration in such closed-jar experiments. This solution can be used to interpret CO_2 concentration-time measurements inside the headspace of the jar to obtain the CO_2 production rate at atmospheric O_2 concentration (9.2 mol O_2 m^{-3}). For the interpretation, during intervals < 10 days, the HC content is considered to remain about constant. Temperature is kept at a constant preset value. Because the jars are closed and evaporation is thus blocked, the water content of the soil does not change. The rate factors in Equation (1) can therefore be considered as constants during the whole experiment.

Design of lysimeter experiments under dynamic conditions

In the lysimeters the environmental conditions change with time and soil depth. Soil temperature is governed by heat transport resulting from diurnal and annual temperature fluctuations at the soil surface. The water content in the lysimeter is also dynamic in space and time under alternating periods of evaporation and irrigation, imposed during the experiment. The oxygen concentration in the soil is mainly governed by dynamic gas diffusion and O_2 consumption due to the biodegradation process.

Accordingly, in the determination of the mineralization rate of the bulk soil, the variation of the environmental rate factors with time and soil height have to be taken into account. Thus, the CO_2 production rate in the bulk soil at time t is given by the integral:

$$S_C(t) \;=\; \frac{S_{r,C}}{(z_0 - z_L)} \int_{Z_L}^{Z_0} f_O(z,t) f_{HC}(z,t) f_T(z,t) f_\theta(z,t) dz \tag{8}$$

Equations (4) to (7) express the relationships of the local environmental rate factors with the environmental variables O_2 concentration, HC content, temperature, and water content. In soils, the dynamics of these variables in space and time can be modeled using one-dimensional transport models (Bouma 1991; Tiktak & Bouten 1992; Freijer & Leffelaar 1996). The basic governing partial differential equations employed in these transport models are given in Appendix 1. An important link between the water and the gas model is the effect of water content on the effective gas diffusion coefficient. The effective diffusion coefficient decreas-

Table 2. Soil properties

Property	Units	Soil				
		Eijsden	Oss	Leiden	Pernis A	Pernis B
$pH(H_2O)^a$		8.08	8.33	7.21	7.85	7.71
$pH(CaCl_2)^{a,b}$		7.49	7.60	6.90	7.42	7.43
$CaCO_3$	$g\ kg^{-1}$	58	11	9.3	90	63
CEC^c	$mmol_c\ kg^{-1}$	41	103	30	91	67
Loss on ignition	$g\ kg^{-1}$	13.6	11.1	8.6	26.0	25.3
Total nitrogen	$mg\ kg^{-1}$	315	395	300	705	630
Sand	%	25.7	96.0	96.0	72.7	83.5
Silt	%	60.4	2.6	2.3	19.0	11.5
Clay	%	14.0	1.4	1.7	8.3	5.0
Bulk density	$kg\ m^{-3}$	1450	1470	1490	1510	1450
Particle density	$kg\ m^{-3}$	2680	2680	2680	2580	2610
Porosity	$m^3\ m^{-3}$	0.459	0.451	0.444	0.415	0.444
Initial HC content	$g\ kg^{-1}$	0.30	0.40	2.7	13	6.5

^{a} 1:2.5 soil water ratio; ^{b} in 0.01 mol dm^{-3} solution; ^{c} at pH = 8.3; ^{d} 500 °C.

es with increasing water content (Equation (19)). Even at low water contents this coefficient can be reduced to near zero values. Accordingly, in moist soils the O_2 demand may exceed the O_2 diffusive flux, resulting in O_2 limitation in a major part of the soil (Borden & Bedient 1986). The decrease in HC content is modeled by numerical integration of the HC consumption rates (Equations (3) and (20)).

Materials and methods

Soils and samples

Five soils contaminated with mineral oil (Table 2) were sampled at 4 sites in the Netherlands. The contamination of the Leiden soil resulted from seepage of lubrication oil and diesel from a bus depot. An indicative microscopic study of the Leiden soil showed the presence of large numbers of *Pseudomonas* bacteria, an important HC degrading genus (Leahy & Colwell 1990). The contamination of the Eijsden and Oss soils were both caused by spills of petrol stations. The Pernis soils were sampled from an oil refinery site. Soil Pernis A mainly contained C_{10}–C_{20} alkanes, whereas in soil Pernis B C_{20}–C_{30} alkanes dominated.

About 10 Mg of all soil types was brought in successive batches to the site where the lysimeters were installed. The lysimeters (height 1.0 m, area 0.9 × 0.9 m^2) were directly filled with the contaminated soils. For all soils except for the Leiden soil (one lysimeter), duplicate lysimeter experiments were carried out. Composite samples of 500 g loose material were periodically taken from the lysimeters during the greenhouse experiments, intended to perform closed-jar experiments at various HC contents. Totally, nine series of soils were studied in closed-jar experiments. The Leiden, Eijsden and Oss soil were once sampled; the Pernis A soil was sampled four times and the Pernis B soil was sampled twice. Before experiments started, the samples were stored at T = 2 °C; samples were not sieved or dried. Additionally, undisturbed samples were taken from the lysimeters in sample rings for laboratory measurement of the soil hydraulic parameters and the effective gas diffusion coefficient as a function of air-filled porosity.

Closed-jar experiments

For each composite sample the initial water content and the initial HC content was determined. Because field-moist soils were used, 82% of the incubated soils had water contents > 0.10 $m^3\ m^{-3}$. This indicates that only a few experiments were carried out at very low water contents. The experiments were performed in 400 cm^3 closed jars with 50 to 80 g moist contaminated sample. Experiments were conducted in at least two replicates, and at least 3 incubation temperatures in the range 2 to 45 (± 1) °C were used. After closing the jar, the CO_2 concentration in the headspace was monitored for a period of at least 6 days, with at least 4 measurements. The results were processed using Equations (18), (27), and (31) in Freijer (1996), which yield CO_2 production rates for the bulk soil at atmospheric O_2 concentration. Further details about the procedure are given in Freijer (1996). Some experiments were extended by allowing total O_2 depletion in the headspace. During these experiments both O_2 and CO_2 concentration were measured. The derivative of the linear relationship between the O_2 and the CO_2 concentration in a closed system gives an estimate of the respiration quotient (Equation (2)). Several repeated incubation runs with the same sample were also carried out to obtain a significant decrease in HC content. After adequate cumulative CO_2 production, the HC content of the sample was determined by a standard procedure (see section describing the measurement techniques). The cumulative CO_2 production and the decrease in HC content were used to calculate the mineralization quotient (Freijer 1996).

Lysimeter experiments

The lysimeters were constructed of water tight concrete, and insulated with tempex at the bottom and against the sides. At the bottom of the lysimeters a drain was installed in a 0.30 m sand layer. The oil-contaminated soils (thickness 0.60 ± 0.05 m) were placed on top of the drainage sand in the lysimeters. The lysimeters were covered with a green house and equipped with a sprinkler irrigation system. The biodegradation process was studied during periods of 198 to 268 days. The experiments thus completed at least one branch of the annual temperature cycle. Various hydrological regimes were imposed on the lysimeters by regulated irrigation, ranging from frequent (weekly) to occasional (once or twice for the whole period) applications. Drainage was kept to a minimum by opening the drains only during peak discharge, in order to prevent extensive losses of dissolved carbonate species with the drainage water. Abiotic variables were determined using an automated monitoring program, in which dataloggers were used to control the measurements and to record measured values. The following non-destructive measurements were performed: water pressure head (each day at four depths), water content (at least two times a day at five depths), soil temperature (at four depths, hourly), and air temperature (hourly). Biodegradation measurements included CO_2 surface fluxes (weekly), CO_2 concentration (weekly, 4 depths), O_2 concentration (occasionally, 4 depths), and HC content (occasionally, composite samples).

Measurement techniques

Gas concentrations were determined using a soil air sampling technique combined with gas chromatography (GC). For the closed-jar experiments the samples were taken from the head space through a septum. The lysimeters were equipped with horizontal positioned equilibration chambers (Magnusson 1989). Gases from the head space of the closed jars were sampled with a syringe and analyzed immediately (0.250 cm^3). The samples from the equilibration chambers in the lysimeter were taken using 5 cm^3 vacutainers (Magnusson 1989) and analyzed by GC after 24 hours. The GC system (Varian 3600 with a Spectra Physics integrator) was configured with a Hayesep D 80/100 8 ft 1/8″ SS column, a 13 × 45/60 6 ft 1/8″ SS molsieve, Valco valves and a hot wire detector. External standard gases were used for calibration. The following analysis conditions were applied: the Helium carrier flow was 30 $cm^3\ min^{-1}$, the injection temperature 60 °C, the oven temperature 50 °C, and the detection temperature 150 °C.

Carbon dioxide fluxes at the surface of the lysimeters were measured using the dynamic CO_2 absorption method (Freijer & Bouten 1991). For this purpose stainless steel rings were installed in the top soil of the lysimeters. Carbon dioxide flux measurements reflect the total CO_2 production rate in the soil, when changes in soil CO_2 storage are negligible. Freijer and Leffelaar (1996) showed that in an oil-contaminated soil pseudo-stationary transport occurs for long periods. Under these conditions bulk soil CO_2 production rates can be calculated from CO_2 fluxes by dividing the fluxes with the length of the soil column that provides the CO_2.

All analyses of the total HC content were carried out on duplicate or triplicate subsamples. For each subsample the following procedure was followed (De Jonge et al. 1996). Hydrocarbons were extracted by shaking 20 g moist soil during at least two hours with a mixture of 80 cm^3 pentane and 50 cm^3 acetone. This combination of aceton and pentane as a solvent is very effective in the removal of HC from soil (Genouw et al. 1994). The high volume of the solvent compared to the sample volume ensures an adequate extraction efficiency. Acetone was removed from the extracts in two steps using a separation funnel after shaking with 800 cm^3 of water. About 10 g of florisil (Merck 12518) was added to the extract, and this mixture was shaken during 30 minutes, to remove all polar substances. The residual extract was brought to 100 cm^3. Depending on the constituents of the oil a C_{36} or a C_{44} internal standard was added. Analysis was done by GC using a Carlo Erba GC 8000 system equipped with a capillary column (J & B DB-1 30 m 0.32 mm with 0.25 μm apolar film preceded by a 2 m 0.53 mm retention gap) and connected to a PC. Integration was performed with the Chromcard v. 1.30 integration program. Other analysis conditions were: the He carrier pressure was 120 kPa; initial oven temperature 60 °C, temperature increase 4 °C min^{-1} to 320 °C and 25 minutes steady at 320 °C, and FID temperature 330 °C.

The non-destructive measurement of water content was carried out with Time Domain Reflectometry (TDR). Sensor design, measurement system and signal analysis were in accordance with Heimovaara and Bouten (1990). The equation proposed by Topp et al. (1980) was used to calculate water content from dielectric permittivity. Pressure heads were measured by tensiometry. The tensiometers were connected to a pressure transducer via a scanivalve. Both the control over the scanivalve and registration of measured values was arranged by a Campbell CR10 datalogger. Soil temperature was recorded using calibrated thermistors connected via multiplexers to the CR10 datalogger to establish data storage and unit control. Air temperature was measured by a PT100 sensor, which was directly connected to the CR10 datalogger.

Data analysis and standardization of CO_2 production rates

The comparison of the mineralization rates measured at the two experimental scales concentrated on deriving standardized CO_2 production rates, mineralization quotients, and respiration quotients from the experimental results. The following procedure was followed.

First, we determined the relevant environmental rate factors that influence the mineralization rate. For this purpose various model configurations constructed from Equations (1), (4), (5), (6), and (7) were fitted to the results obtained with the closed-jar experiments. Non-linear parameter optimization using the simplex method (Nelder & Mead 1965) was performed to find $S_{r,C}$ and the parameters in the selected rate factor functions from series of measured CO_2 production rates at $X_{r,O}$, and actual temperature, water content, and HC content. The numbers of free parameters and independent variables were varied in order to find the simplest model with the best performance. The product moment correlation coefficient (R^2) between measured and predicted actual CO_2 production rates served as a performance index (Whitmore 1991).

Next, in the closed jar experiments, we obtained the standardized CO_2 production rates as follows. Each actually measured CO_2 production rate was standardized to reference conditions by dividing the actual measured CO_2 production rate with the environmental rate factors:

$$S_{s,C} = S_{m,C}/(f_O f_{HC} f_\theta f_T) \tag{9}$$

The environmental rate factors are given as in Equations (4) to (7). In Equation (9) the measured actual biodegradation rate is represented by $S_{m,C}$. The standardized biodegradation rate is an adequate quantity to compare the results of different experiments, because it rules out the effect of differences in environmental conditions between different observations. In an analogous way, rate factors can be processed using measured CO_2 production rates, the remaining rate factors, and optimized $S_{r,C}$ values. Thus, residual rate factors for temperature and HC content were obtained by:

$$f_{s,T} = S_{m,C}/(S_{r,C} f_O f_{HC} f_\theta) \tag{10}$$

$$f_{s,HC} = S_{m,C}/(S_{r,C} f_O f_T f_\theta) \tag{11}$$

Additionally, the apparent mineralization quotient, *a*, and the apparent respiration quotient, *r* are important parameters for evaluating to what extent the CO_2 production is related to consumption of the contaminant (El-Din Sharabi & Bartha 1993; Hickey 1995). Values of *a* were calculated from simultaneous measurements of cumulative CO_2 production and decrease in HC content. Simultaneously measured O_2 and CO_2 concentrations in the closed jars and the lysimeters were used to estimate *r* (Freijer 1996).

Finally, the data obtained in the lysimeter experiments were processed. The biodegradation model was coupled to the numerical transport models. The transport models were used to simulate the rate factors as a function of time and depth in each different lysimeter. All initial input requirements regarding system parameters of the simulation models (e.g. effective diffusion coefficient and soil hydraulic properties) were measured for each soil type using standard laboratory methods. For interpretation of the lysimeter experiments, the simulation models of water and heat transport were applied to interpolate between the measurements obtained during field monitoring. Water dynamics was simulated first, and water contents as a function of time and depth were subsequently input to the soil temperature model. Then, simulated soil temperatures and water contents served as boundary conditions to the gas transport and the biodegradation model.

For the parameters in the biodegradation model the optimized values obtained from the closed-jar experiments were used as initial estimates. A calibration period was carried out by trial and error to improve the agreement between simulated and measured data. The calibration procedure allowed fine-tuning of a limited number of system parameters in their range of uncertainty. The values of $S_{r,C}$, a, and r were thus optimized by minimizing the difference between simulated and measured HC content, CO_2 production, O_2 concentration and CO_2 concentration. The parameters T_{opt} and k_T were not altered. Simulated rate factors at different depths obtained with the simulation models were then used to calculate standardized CO_2 production rates, $S_{s,C}$, from the measured CO_2 fluxes at different moments in time, employing the following equation:

$$S_{s,C}(t) = S_{m,C}(t)(z_0 - z_L) / \int_{z_L}^{z_0} f_O(z,t) f_{HC}(z,t) f_T(z,t) f_\theta(z,t) dz \tag{12}$$

with the expressions for the rate factors given in Equations (4), (5), (6), and (7).

Results and discussion

Rate factors under steady conditions

The closed-jar experiments were performed for nine soil series. The performances of the biodegradation model configurations, with different response functions and different numbers of free parameters, are given in Table 3. The effect of O_2 is not included in this table, because data processing of the results obtained with the closed jar experiment yields CO_2 production rates at atmospheric O_2 concentrations. The experimental results and the theoretical considerations in Freijer (1996) demonstrate the effect of O_2 concentration on the mineralization rate for soil Pernis A and Pernis B.

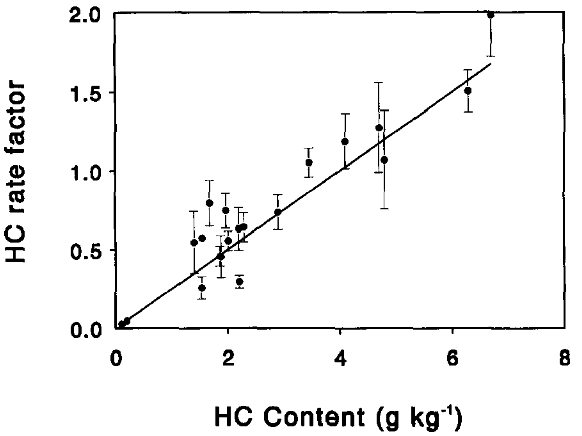

Figure 1. Residual (solid circles) and fitted (solid line) HC rate factor as a function of HC content. Vertical bars indicate standard deviations.

If all four parameters in the model are considered to be unique for each soil type (9 × 4 = 36 parameters) the model response is highest (Table 3). Decreasing the number of parameters by allowing only one value of the parameters T_{opt}, k_T, and n for the whole data set (9 + 3 = 12 parameters) also yields a high R^2 value, and the applicability of the model becomes more generic. Leaving out the response function for the water content rate factor, which is an additional simplification, gives the same R^2 as in the 12 parameter model. Apparently, the effect of water content on mineralization rate is not very distinct within the water content range examined in this study. These results confirm those obtained by Dibble and Bartha (1979), who found that water contents in the range of 0.30 to 0.90 of water holding capacity did not inhibit nor favor aerobic biodegradation. The lack of inhibition at low water contents is probably related to the presence of the HCs. The HCs render some surfaces hydrophobic, which reduces the water holding capacity of the soil and thus increases the availability of the water that is present (Dibble & Bartha 1979). If the response functions for the other rate factors (f_T and f_{HC}) are omitted the performance of the model becomes unacceptably low (Table 3). Therefore, the 11 parameter model with $S_{r,C}$ unique for each soil, single values of k_T and T_{opt}, and without f_θ was adopted. Optimized values for the parameters in the expression for the temperature rate factor are $k_T = 1.902\ 10^{-3}\ °C^{-2}$ and $T_{opt} = 46.2\ °C$.

Figures 1 and 2 visualize the modeled and the residual rate factors. In Figure 1 the linear relationship between f_{HC} and HC content assumed in the model is confirmed by the residual rate factors. The bars (standard deviations) and the points (averages), however, show a considerable scatter. The modeled and residual temperature rate factors, f_T, as a function of temperature do agree much better (Figure 2), and the left branch of the optimum curve distinctly fits the residual values. This effect of temperature on the min-

Table 3. Model performance (R^2 values) of various response function configurations and numbers of free parameters for the 9 soil series studied; models were fitted to 163 observations; for explanation see text

Functions in model				Number of free parameters				R^2
$S_{r,C}$	f_r	f_{HC}	f_θ	$S_{r,C}$	T_{opt}	k_T	n	
+	+	+	+	9	9	9	9	0.951
+	+	+	+	9	1	1	1	0.948
+	+	+	-	9	1	1	-	0.948
+	+	-	+	9	1	1	1	0.831
+	+	-	-	9	1	1	-	0.760
+	-	+	-	9	-	-	-	0.465
+	-	+	+	9	-	-	1	0.449
+	-	-	+	9	-	-	1	0.448
+	-	-	-	9	-	-	-	0.351

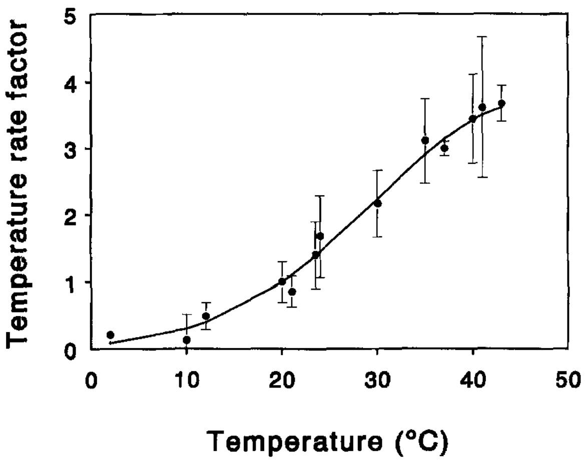

Figure 2. Residual (solid circles) and fitted (solid line) temperature rate factor as a function of temperature. Vertical bars indicate standard deviations.

eralization rates was also demonstrated in many other studies (e.g. Dibble & Bartha 1979).

Rate factors under dynamic conditions

An example of the employment of simulation models to determine the environmental rate factors in a dynamic lysimeter system is given in Figures 3 and 4. The treatment that is used in this case is limited to two irrigation applications. The water content as a function of time (Figure 3a) shows an increase at day 140 (due to irrigation), followed by a decrease towards a rather steady dry situation. Soil temperature (data not shown) was approximately 14 °C in early spring (day 100), increasing to an average of 30 °C in summer (day 193) and decreasing to 8.5 °C in winter (day 350). After calibration, the simulation models were used to calculate the total rate factors at different heights and the total CO_2 flux at the surface. The total rate factor is defined as the product of the local rate factors f_T (z, t), f_{HC} (z, t) and f_O (z, t).

The calculated CO_2 fluxes at the soil surface (Figure 3b) resemble the measured values. Using the biodegradation model and the measured CO_2 fluxes, fluxes standardized to reference conditions were determined, that scatter around an average value of 1.31 mol m^{-2} d^{-1} (Figure 3b). It can therefore be concluded that the environmental rate factors included in the model explain most variation of the CO_2 flux in the experimental period. The total rate factor displayed in Figure 3c at several heights in the soil shows that in the beginning most CO_2 is generated in the surface layer. In the second part of the experimental period the rate factors at all heights decrease, but the contribution of the different soil layers inverts.

The individual rate factors f_T, f_O and f_{HC} are given in Figure 4 at several heights. Figure 4a reflects the temperature wave during the year, which can also be recognized in Figure 3c. It is highly dynamic at the soil surface and dampened in the deeper layers. The rate factor f_O (Figure 4b) decreases with decreasing height, and reaches very low levels in the deeper soil, thus dominating the total rate factor at this height (Figure 3c). This is due to the O_2 concentration gradient that develops from O_2 consumption. An indirect effect of water content on biodegradation appears when irrigation is applied (Figure 3a). Because the water content increases, the effective gas diffusion coefficient and air-filled porosity decrease (Equation (19)), and O_2 con-

Table 4. Averages of standardized mineralization rates ($S_{s,C}$) at reference conditions (T = 20 °C, C = 4 g kg^{-1} and X_O = 9.2 mol m^{-3}), mineralization quotients, a, respiration quotients, r, determined using results of closed-jar experiments. Numbers in parentheses are standard deviations

Soil	$S_{s,C}$	Observations	a	r
	mol CO$_2$ m^{-3} day^{-1}	#	mol mol^{-1}	mol mol^{-1}
Eijsden	2.70 (0.53)	16	nd*	0.65
Oss	7.31 (2.28)	18	nd	nd
Leiden	2.53 (0.91)	20	1.21	0.60
Pernis A	2.04 (0.27)	5	0.86	0.58
	1.94 (0.27)	9	0.61 to 1.03	nd
	3.15 (0.93)	48	nd	nd
	1.88 (0.72)	21	nd	nd
Pernis B	2.40 (0.53)	5	0.71	0.58
	2.24 (0.78)	21	nd	nd

* nd is not determined.

Table 5. Averages of standardized mineralization rates ($S_{s,C}$) at reference conditions (T = 20 °C, C = 4 g kg^{-1} and X_O = 9.2 mol m^{-3}), mineralization quotients, a, respiration quotients, r, determined using results of lysimeter experiments. Numbers in parentheses are standard deviations

Soil	$S_{s,C}$	Observations	a	r
	mol CO$_2$ m^{-3} day^{-1}	#	mol mol^{-1}	mol mol^{-1}
Eijsden	6.60 (2.93)	20	3.0	0.65
	7.63 (2.01)	15	3.0	0.65
Oss	5.24 (1.77)	21	2.5	0.65
	4.01 (1.86)	20	3.0	0.65
Leiden	3.18 (0.62)	30	1.2	0.60
Pernis A	2.42 (0.49)	37	0.45	0.58
	2.27 (0.90)	38	0.45	0.58
Pernis B	2.15 (0.51)	36	0.80	0.58
	2.37 (0.27)	36	0.80	0.58

centration drops rapidly as biodegradation proceeds, resulting in low values of f_O (Figure 4b). In Figure 4c f_{HC} is shown. In the beginning, no height gradient is observed, because it is assumed that the HCs are homogeneously distributed through the soil. As biodegradation proceeds, HC content decreases mainly at the surface due to high total rate factors (Figure 3c). However, as the HC content decreases in the top soil it affects the total rate factor in the top soil, which is responsible for the inversion of the gradient in the total rate factor (Figures 3c and 4c).

Summarizing, it can be concluded that one direct effect (temperature), one indirect effect (water content) and two negative feed-back mechanisms (O$_2$ concentration and HC content) govern the process of biodegradation. High mineralization rates demand high O$_2$ and HC contents. Due to continuous biodegradation the pools of O$_2$ and HCs exhaust and the mineralization rate decreases. The O$_2$ pool is partly restored by O$_2$ diffusion from the surface into the soil. For the HC pool developments are paradoxical. As the HC content decreases, approximating the goal of biorestoration, the HC rate factor also decreases, resulting in an extremely long treatment time to obtain a completely recovered soil.

The CO$_2$ production rate and experimental scale

The results in Table 4 to 5 show the effect of experimental scale on CO$_2$ production rates, after standardizing these rates to reference conditions for temperature, O$_2$ concentration and HC content. When comparing the experimental scales (Table 4 to 5), differences in $S_{s,C}$ are small for the Pernis soils and the Leiden soil. They

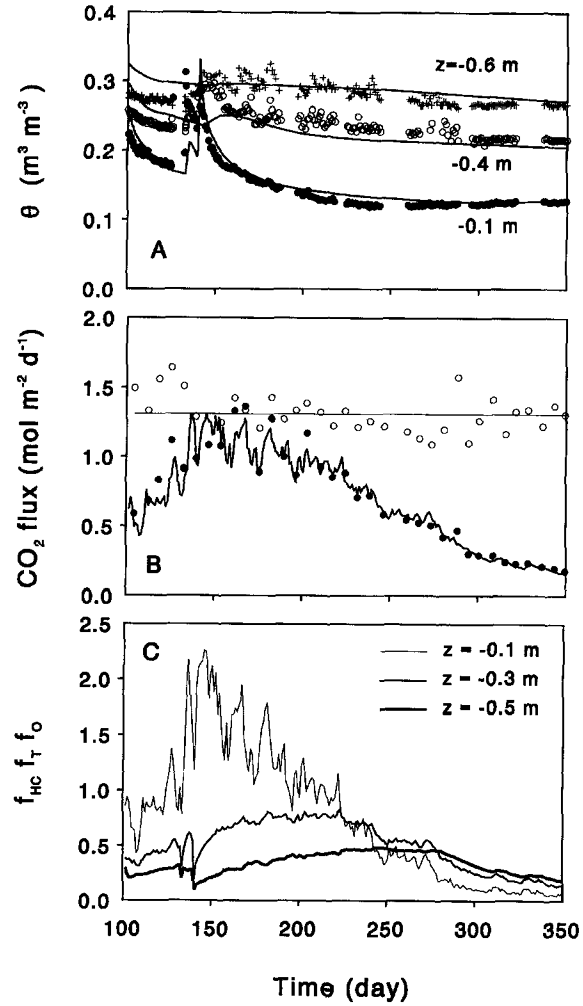

Figure 3. Biodegradation in a lysimeter filled with soil Pernis B and using a dry hydrological scenario, as a function of time. (*A*) simulated (lines) and measured (symbols) water content at various soil heights; (*B*) measured (solid circles) and simulated (thick line) actual CO_2 surface flux, and residual surface CO_2 flux standardized to reference conditions (open circles) with average of all standardized fluxes (thin line); (*C*) simulated total rate factors at various soil heights.

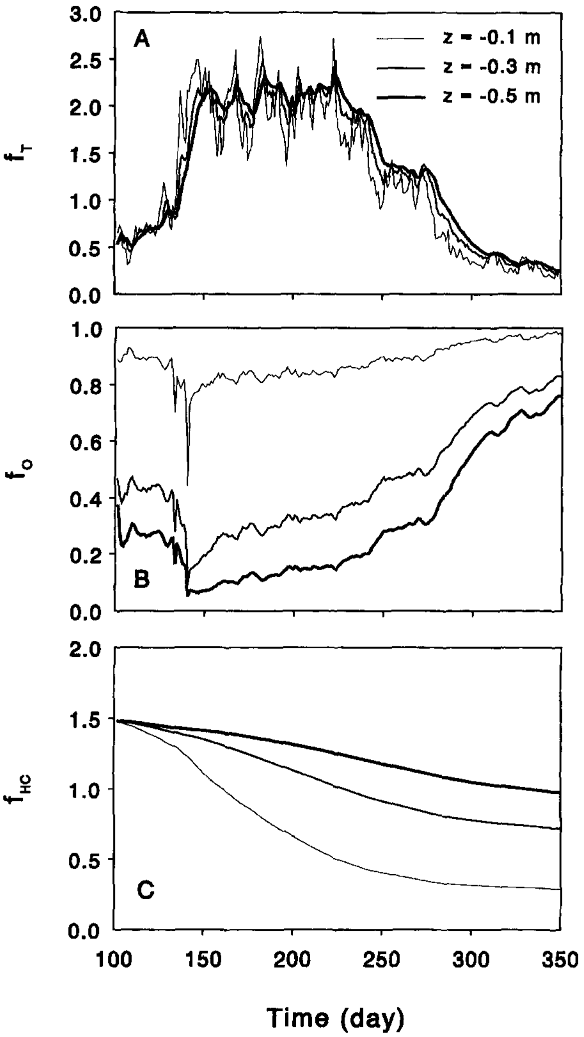

Figure 4. Biodegradation in a lysimeter filled with soil Pernis B and using a dry hydrological scenario, as a function of time. Simulated rate factors at various soil heights (*A*) temperature rate factor; (*B*) O_2 concentration rate factor; (*C*) HC content rate factor.

are comparable to the values of the standard deviations of the standardized CO_2 production rates, $S_{s,C}$. Results for the Oss and Eijsden soils show larger discrepancies. For these soils, with a low initial HC content (Table 2), values of $S_{s,C}$ obtained at the two experimental scales differ up to a factor three. The relatively high standard deviations, however, indicate that systematic differences are unlikely.

Hence, it seems that the impact of environmental factors on the mineralization rate is fairly distinct and explains the differences in measured actual CO_2 production rates at different experimental scales. Accurate documentation of the conditions during measurements is therefore always necessary to enable a comparison of biodegradation rates obtained in different types of experiments.

The CO_2 production rate as a quantity for the hydrocarbon mineralization rate

Aerobic mineralization of HCs is characterized by the simultaneous production of CO_2, water, metabolites and biomass, and the consumption of O_2 and HCs.

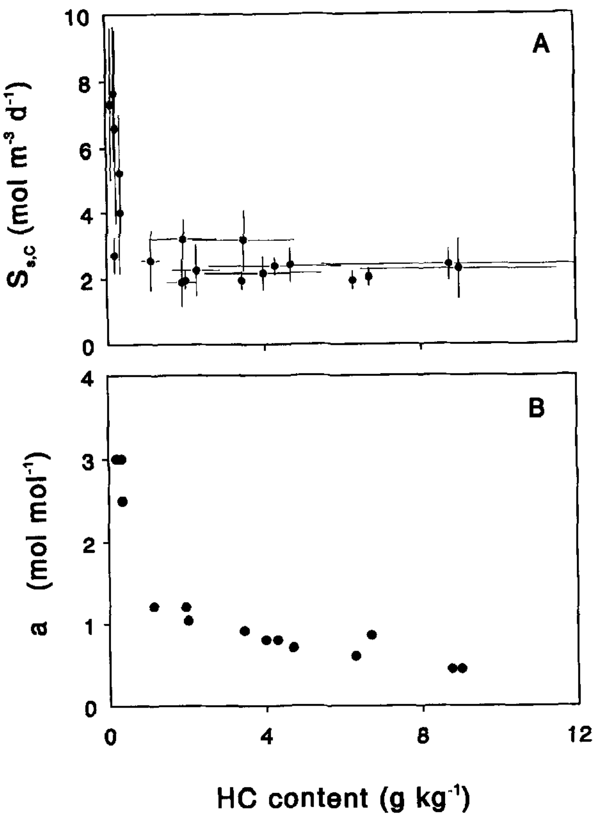

Figure 5. (*A*) Standardized CO_2 production rates, $S_{s,C}$, and (*B*) apparent mineralization quotients, a, as a function of HC content. Vertical bars indicate standard deviations and horizontal bars HC content range.

When the HCs are completely mineralized, and the mineralization quotient, a, equals one, the value of the respiration quotient, r, is determined by the C-H-O ratio of the carbon source. Theoretically, r is equal to 0.66 for HCs with a C-H ratio of 0.5, whereas for most of the constituents in soil organic matter values range from 0.80 to 1. If part of the HCs are transformed into biomass and metabolites, then a is much lower than one. From the simultaneously measured total CO_2 production and HC content decrease in oil sludge, presented by Dibble and Bartha (1979) values of a ranging from 0.66 to 0.91 can be calculated. If other carbon sources are mineralized additionally to the HCs, values of a derived from measurements of total CO_2 production and measured decrease of HC content can be much higher than one. Under microbial growth conditions, the respiration quotient, r, decreases compared to the value that is expected from the C-H-O ratio of the compound (Hickey 1995; Freijer 1996).

Consequently, the values of a and r indicate to which extent CO_2 production is related to aerobic biodegradation of HCs. In the present study, values of r estimated from the closed-jar experiments (Table 4) vary between 0.65 and 0.58, which comes very close to the theoretical value of HCs at a = 1.0, which is 0.66. Values of a vary from 0.61 to 1.03 in the Pernis soils suggesting that most HCs are mineralized to CO_2. For the Leiden soil a = 1.21, which indicates that other carbon sources are possibly also mineralized. In the lysimeter experiments the results obtained with the closed-jar experiments are confirmed for the Pernis and Leiden soils. The Oss and Eijsden soils show values of $a > 2.5$. It thus seems that in these soils the CO_2 production is only weakly related to HC consumption.

If all values of a and $S_{s,C}$ obtained from the experiments are plotted against HC content, some interesting similarities can be observed (Figure 5). In Figure 5a the standardized CO_2 production rates, $S_{s,C}$ are plotted against HC content. Results indicate that the relationship between $S_{s,C}$ and HC content can be approximated by a hyperbole. The same trend can be observed for a as a function of HC content (Figure 5b). High values of a for HC content < 1 g kg^{-1} demonstrate that CO_2 production no longer coincides with an ample HC consumption in this range. A possible explanation for the sudden steep increase of a and $S_{s,C}$ with decreasing HC content can be given if biomass production is considered. Song and Bartha (1990) studied the microbial population in soil contaminated by jet fuel. They found that the disappearance of the jet fuel within the first two weeks of incubation coincided with a fast increase in microbial numbers (2 to 2.5 orders of magnitude). After reaching peak levels in microbial numbers, and disappearance of 80% of the original applied jet fuel, microbial numbers declined and decrease in HC content decelerated. Microbial activity on the other hand remained at high levels and sometimes increased. Projecting these results on those presented in Figure 5 thus suggest that the increase of a and $S_{s,C}$ with decreasing HC content may be caused by a relative increase in mineralization of dead biomass. Because the availability of HCs diminishes during biodegradation, the share of dead biomass mineralization in total CO_2 production increases rapidly.

Conclusions

- The mineralization rate of HCs in soils is influenced by temperature, HC content, and O_2 concentration. Water content could not be identified as a direct governing environmental factor for water contents > 0.10 $m^3\ m^{-3}$. An indirect effect of water content is, however, that it influences the effective gas diffusion coefficient in soils.

- A response model based on multiplicative interaction of environmental rate factors to calculate the CO_2 production rate in soils as a function of temperature, HC content, and O_2 concentration, was successfully fitted to measured CO_2 production rates.
- Using modeled rate factors, all measured actual CO_2 production rates were standardized to environmental reference conditions. The standardized CO_2 production rates are comparable for the different experiments. Apparently, the differences in environmental conditions between the experiments are responsible for the variation in actual CO_2 production rates. Consequently, simple laboratory experiments under different environmental conditions are adequate to parameterize the biodegradation model to predict mineralization rates under field conditions.
- The CO_2 production rate seems to be a good quantity to express the mineralization rate of HCs in mineral soils. Carbon dioxide production rates are strongly related to HC consumption rates for HC contents > 1.0 g kg^{-1}. This can be concluded from the values of measured mineralization quotients in both experiments. For HC contents < 1 g kg^{-1} the mineralization quotient increases steeply with decreasing HC content. This shows that the share of HC mineralization in total CO_2 production decreases, probably due to depletion of the available HC pool.

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Appendix

The models for determining the environmental conditions as a function of time and soil depth are based on the numerical solution of transport equations in a 1-dimensional soil system. The fundamentals of these partial differential equations are described in detail in e.g. Richter (1987). The following equations are employed (for a definition of the symbols see Notation):

Heat transport

$$\frac{\partial T}{\partial t} = \frac{\lambda}{C_h}\frac{\partial^2 T}{\partial z^2} \tag{13}$$

The heat conductivity, λ, and the heat capacity, C_h, are both a function of water content. The upper boundary condition is soil surface temperature, and the lower boundary condition is a zero flux. Specific information on the heat transport model is given in Bouma (1991).

Water transport

$$C_w\frac{\partial h}{\partial t} = \frac{\partial}{\partial z}\left(K\frac{\partial(h+z)}{\partial_z}\right) \tag{14}$$

The differential capacity C_w, $d\theta/dh$, is a function of h, and K is a function of θ. The upper boundary conditions are precipitation and evaporation, and the lower boundary condition is a known pressure head. Specific information on the water transport model is given in Tiktak and Bouten (1992).

Gas transport

$$\frac{\partial(\varepsilon X_i)}{\partial t} = -\frac{\partial F_i}{\partial z} - \frac{\partial F_{p,i}}{\partial z} + S_i \tag{15}$$

with

$$\frac{\partial F_i}{\partial z} = -D_{e,F_i}\frac{\partial^2 X_i}{\partial z^2} \tag{16}$$

The second term is calculated using the isobaric equilibrium condition:

$$\sum_{j=1}^{v}\left(\frac{\partial F_{p,j}}{\partial z}\right) = \sum_{j=1}^{v}\left(-\frac{\partial F_j}{\partial z} + S_j\right) - \frac{\partial(p\varepsilon/(RT))}{\partial t} \tag{17}$$

$$\frac{\partial F_{p,i}}{\partial z} = Y_i\sum_{j=1}^{v}\left(\frac{\partial F_{p,j}}{\partial z}\right) + \frac{\partial Y_i}{\partial z}\sum_{j=1}^{v}F_{p,j} \tag{18}$$

with temperature, T, in K.

Air-filled porosity, ε, is calculated by subtracting water content from total porosity. The effective diffusion coefficient is a function of air-filled porosity

$$D_{e,F_i} = D_{0,F_i}e(\varepsilon - g) \tag{19}$$

The upper boundary condition is the atmospheric gas concentration and the lower boundary condition a zero flux at an impermeable layer. Specific information on the gas transport model is given in Freijer and Leffelaar (1996).

HC consumption

$$dC*/dt = S_{HC} \tag{20}$$

The initial situation is the estimated HC-C content as a function of height. Notice that the units of $C*$ are mol m^{-3} and can be converted to g kg^{-1} through division by the dry bulk density and the molar C content of one gram of HC. The dry bulk density is a soil property that is specific for each soil type, the molar C content of one gram of HC is approximately 0.071 mol g^{-1}.

Notation

a	apparent mineralization quotient (mol mol^{-1})
e	empirical parameter in the effective diffusion coefficient function
f_r	temperature rate factor
$f_{s,T}$	residual temperature rate factor
f_O	oxygen concentration rate factor
f_{HC}	HC content rate factor
$f_{s,HC}$	residual HC content rate factor
f_θ	water content rate factor
g	empirical parameter in the effective gas diffusion coefficient function (m^3 m^{-3})
h	pressure head (m)
k_T	system parameter in expression for f_T ($^\circ C^{-2}$)
n	system parameter in expression for f_θ
p	pressure (Pa)
r	apparent respiration quotient (mol mol^{-1})
t	time (day)
v	number of gas components
z	height or gravitational head (m)
z_0	height at the soil surface (m)
z_L	height at the bottom of the soil profile (m)
C	total HC content (g kg^{-1})
$C*$	total HC-C content (mol m^{-3})
C_h	heat capacity (J m^{-3} K^{-1})
C_w	Differential water capacity (m^{-1})
C_r	reference HC content (g kg^{-1})
$D_{0,Fi}$	Fickian diffusion coefficient of gas i in air (m^2 day^{-1})
$D_{e,Fi}$	effective Fickian diffusion coefficient of gas i in soil (m^2 day^{-1})
Fi	Fickian diffusive gas flux density of gas i (mol m^{-2} day^{-1})
$F_{p,i}$	pressure adjustment flux density of gas i (mol m^{-2} day^{-1})
K	hydraulic conductivity (m day^{-1})
R	gas constant (J mol^{-1} K^{-1})
S_C	CO_2 production rate (mol m^{-3} day^{-1})
S_{HC}	HC-C consumption rate, a negative value denotes consumption (mol m^{-3} day^{-1})
S_i	Production rate of gas species i (mol m^{-3} day^{-1})
$S_{m,C}$	measured CO_2 production rate (mol m^{-3} day^{-1})
S_O	O_2 consumption rate, a negative value denotes consumption (mol m^{-3} day^{-1})
$S_{r,C}$	CO_2 production rate at reference conditions $X_{r,O}$, C_r, θ_r and T_r (mol m^{-3} day^{-1})
$S_{s,C}$	measured CO_2 production rate standardized to reference conditions $X_{r,O}$, C_r, θ_r, and T_r (mol m^{-3} day^{-1})
T	temperature (°C)
T_{opt}	optimum temperature in f_r (°C)
T_r	reference temperature (°C)
X_i	gas concentration of species i (mol m^{-3})
X_O	oxygen concentration (mol O_2 m^{-3})
$X_{r,O}$	reference oxygen concentration (mol O_2 m^{-3})
Y_i	mole fraction of species i in the gas phase (mol mol^{-1})
ε	air-filled porosity (m^3 m^{-3})
θ	water content (m^3 m^{-3})
θ_r	reference water content (m^3 m^{-3})
λ	heat conductivity (Jm $^{-1}$ day^{-1} K^{-1})

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