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Chloride retention in forest soil by microbial uptake and by natural chlorination of organic matter

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Abstract

Inorganic chlorine (i.e. chloride; Cl_{in}) is generally considered inert in soil and is often used as a tracer of soil and ground water movements. However, recent studies indicate that substantial retention or release of Cl_{in} can occur in soil, but the rates and processes responsible under different environmental conditions are largely unknown. We performed ^{36}Cl tracer experiments which indicated that short-term microbial uptake and release of Cl_{in}, in combination with more long-term natural formation of chlorinated organic matter (Cl_{org}), caused Cl_{in} imbalances in coniferous forest soil. Extensive microbial uptake and release of Cl_{in} occurred over short time scales, and were probably associated with changes in environmental conditions. Up to 24% of the initially available Cl_{in} within pore water was retained by microbial uptake within a week in our experiments, but most of this Cl_{in} was released to the pore water again within a month, probably associated with decreasing microbial populations. The natural formation of Cl_{org} resulted in a net immobilization of 4% of the initial pore water Cl_{in} over four months. If this rate is representative for the area where soil was collected, Cl_{org} formation would correspond to a conversion of 25% of the yearly wet deposition of Cl_{in}. The study illustrates the potential of two Cl_{in} retaining processes in addition to those previously addressed elsewhere (e.g. uptake of chloride by vegetation). Hence, several processes operating at different time scales and with different regulation mechanisms can cause Cl_{in} imbalances in soil. Altogether, the results of the present study (1) provide evidence that Cl_{in} cannot be assumed to be inert in soil, (2) show that microbial exchange can regulate pore water Cl_{in} concentrations and (3) confirm the controversial idea of substantial natural chlorination of soil organic matter.

1. INTRODUCTION

Chlorine biogeochemistry in soil is currently discussed regarding both the use of inorganic chlorine (chloride; Cl_{in}) as a tracer of soil water movements, and the possible natural chlorination of soil organic matter (SOM). The use of Cl_{in} as a tracer of soil water movements is based on the view that inorganic chlorine in soil pore water is inert (i.e. unreactive) and freely mobile (e.g. Conway, 1942; Schlesinger, 1997). This view has been questioned by recent reports of substantial Cl_{in} retention and release over different time scales (e.g. Likens, 1995; Larsson and Jarvis, 1999; Nyberg et al., 1999; Kirchner et al., 2000; Viers et al., 2001; Chen et al., 2002; Lovett et al., 2005; Bastviken et al., 2006). Proposed explanations of the Cl_{in} imbalances observed in several experiments include heterogeneous vertical transport patterns (Larsson and Jarvis, 1999), geochemical sorption including ion exchange (Hanes, 1971; Viers et al., 2001),

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Cl_{in} uptake by vegetation (Likens, 1995; Lovett et al., 2005), and microbial chlorination of soil organic matter (SOM) resulting in formation of organochlorines (e.g. Öberg, 2003).

Cl_{in} is not only retained, but can also be released from soil due to e.g. the weathering of mineral particles. Recent studies infer that decomposing chlorinated organic matter also results in the release of Cl_{in} from soil and that a substantial part of the Cl_{in} in run-off originates from decomposing organic matter (Rodstedth et al., 2003; Öberg and Sandén, 2005). Hence, it appears as if soil sometimes acts as a sink of Cl_{in} and sometimes as a source. However, it is unclear under what conditions soil acts as a Cl_{in} sink and under what conditions it acts as a source, and to what extent different processes contribute to the source-sink balance. The lack of such knowledge makes it difficult to evaluate the use of Cl_{in} as a ground water tracer.

The retention of Cl_{in} by natural chlorination of SOM represents another interesting aspect of chlorine biogeochemistry. Organochlorines in nature have been a major environmental concern for decades. Initially, all organochlorines found in soil were thought to be of anthropogenic origin, and the contribution of naturally produced organochlorines has been debated (Öberg, 2002). There is strong evidence that naturally formed organochlorines are ubiquitous in the environment, and that there are microorganisms and associated enzymes capable of chlorinating soil organic matter (e.g. Asplund et al., 1993; Öberg and Grön, 1998; Johansson et al., 2001, 2003; Gribble, 2003; van Pee and Unversucht, 2003; Öberg and Sandén, 2005). In addition, there is experimental evidence of abiotic chlorination of organic matter (Keppler et al., 2000; Fahimi et al., 2003). Previous rate estimates have focused on the consumption of chloride by microbes (Clutterbuck et al., 1940), formation of specific compounds (e.g. Keppler et al., 2003; Laturnus et al., 2005; Matucha et al., 2007), or net changes in organic chlorine during degradation of organic matter, soil or litter (e.g. Öberg et al., 1996; Hjelm et al., 1999; Myneni, 2002; Matucha et al., 2003). However, measurements of net changes in the Cl_{org} pool do not provide rate estimates of bulk SOM chlorination, since both formation (consumption of Cl_{in}) and mineralization (release of Cl_{in}) of chlorinated organic matter may take place in the soil. Hence, there are, to our knowledge, no previous direct estimates of chlorination rates in soil.

In the present study we followed the fate of added $^{36}Cl_{in}$ in different soil fractions over time (days to months) in laboratory experiments. The primary aims were to estimate (a) the extent to which Cl_{in} is retained or released under different conditions and (b) the extent to which natural chlorination of SOM contributes to Cl_{in} retention. In addition, the experimental setup allowed us to quantify microbial uptake and release of Cl_{in} which, to our knowledge, has not been considered previously in relation to the soil chlorine cycle.

2. METHODS

2.1. Soil collection

Soil was collected at the Stubbetorp catchment (58°44′N, 16°21′E) in SE Sweden in May and September 2005. The catchment is covered with coniferous forest dominated by Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) and the soil is of the spodosol type. At the site of soil collection, the uppermost centimeters of the soil was dominated by litter. Below the litter layer there was a dark brown to black organic rich layer down to approximately 15 cm depth (referred to as the A horizon). Below the A horizon, there was a leached grey layer being up to 5 cm thick (E horizon), and further below the soil was rust colored (B horizon). The catchment area is 0.87 km^2 with a broken topography and a granite dominated bedrock that is poor in chloride (Maxe, 1995). The long-term annual mean precipitation in the region is approximately 600 mm and the annual mean temperature is about 6 °C. The soil was collected with a spade and transported in plastic bags (polyethylene). In the laboratory, the soil was sieved through 2 mm mesh, transferred to a widemouth plastic bottle (2 litre, polyethylene), and stored moist in the dark at 2 °C until the start of the experiments (storage time 2–7 days).

2.2. Experiment setup

The fate of Cl_{in} was studied in four radiotracer experiments using ^{36}Cl (Table 1). A four month long experiment was performed to capture both rapid and slow processes (TIME experiment). Another experiment was carried out at different temperatures (2–60 °C) for five days to examine the temperature dependence of short-term processes (TEMP

Table 1
Overview of experiments included in this study

Experiment	Start date and soil horizon[a]	Experiment time (days)	Focus
TIME	May 12, 2005 A horizon	133	Long-term Cl_{in} retention/release under oxic conditions
TEMP	September 7, 2005 A horizon	5	Cl_{in} retention at different temperatures (2-60 °C; oxic conditions)
OX-ANOX	September 14, 2005 A horizon	13	Short-term Cl_{in} retention under oxic and anoxic conditions
DEPTH	September 30, 2005 E and B horizons	12	Short-term Cl_{in} retention in deeper soil horizons

[a] For description of soil horizons see Section 2 and Table 2.

Table 2
Characteristics of the soil used in the different experiments

Experiment	Soil water content (%; $n = 6$)	LOI[a] (% of dry weight; $n = 6$)	Initial TOX[b] (μg g dry soil^{-1}; $n = 3$)	Extractable Cl^{-}[c] (μg g dry soil^{-1}; $n = 3$)
TIME	65.4 ± 0.30	51.0 ± 0.80	314 ± 12	17.4 ± 0.95
TEMP	74.1 ± 0.07	89.6 ± 0.32	476 ± 54	22.7 ± 0.11
OX-ANOX	74.2 ± 0.08	89.8 ± 1.12	476 ± 54	22.2 ± 0.44
DEPTH				
E horizon	21.2 ± 0.12	3.7 ± 0.07	48.2 ± 4.78	2.3 ± 0.33
B horizon	26.3 ± 1.24	4.8 ± 0.30	62.7 ± 24.4	3.5 ± 0.05

Values represent average ±1 SD.
[a] Loss on ignition representing the organic matter content (see text for details).
[b] Total organic halides (Asplund, 1994).
[c] Sum of chloride leached from soil during extractions with H_2O and 0.1 M KNO_3 as specified in the text. Ion chromatography was used to analyze chloride concentrations in extracts (Standardization, 1995).

experiment). To investigate if redox reactions (e.g. microbial metabolism or activity of O_2 dependent enzymes) affect Cl_{in} retention, we also performed a short-term study comparing oxic and anoxic conditions (OX-ANOX experiment; Table 1). Soil from the A horizon (high organic content) was used for all the above experiments. To study the extent of short-term Cl_{in} immobilization in deeper soil layers, we also performed an experiment comparing soil from the E and B horizons (DEPTH experiments; Table 1). The basic experimental design was identical for all experiments; 3.0 g fresh soil were added to 50 ml centrifuge tubes (Sarstedt, Germany) and amended with ^{36}Cl-chloride ($^{36}Cl_{in}$). For characteristics of the soil used in the different experiments, see Table 2.

The $^{36}Cl_{in}$ (Amersham Biotech; 0.59 MBq mg Cl^{-1}) was diluted in Milli-Q water so that $^{36}Cl_{in}$ corresponding to 205 100 disintegrations per minute (DPM; 1 Bq = 60 DPM) was added to each centrifuge tube. Given the specific activity of the source, 205 100 DPM of $^{36}Cl_{in}$ represents a mass of 5.67 μg Cl. The total DPM added was similar in all experiments, but the volume of the $^{36}Cl_{in}$ solution was adjusted for each experiment separately to make the soil semi-fluid. Thereby, homogenous distribution of the isotope could be achieved by stirring the wet soil with a syringe needle. The volume of isotope solution added to each centrifuge tube varied from 0.25 to 1.0 ml, depending on the water holding capacity of the soil which differed depending on the organic matter content in the different experiments (Table 2). After stirring, four slots crossing each other were made with the needle in the soil allowing penetration of air to the bottom of the test tube, increasing the soil area in contact with the head space gas in the tubes. Then the open tubes were placed under a fan at room temperature (20 °C) and dried until they had regained their original fresh weight. The whole procedure, from addition of $^{36}Cl_{in}$ solution to drying, took 6–12 h. Immediately after the original weight was reached, three tubes were sampled and analyzed according to the method described below to analyze the initial ^{36}Cl content in different soil fractions. In addition, the original soil and centrifuge tubes containing soil but without $^{36}Cl_{in}$ were sampled at the start of the experiment to determine soil water contents and initial concentrations of soil organic matter, total organic chlorine (TOX), and extractable Cl_{in} (Table 2). The remaining tubes were attached to a gas source (air for all oxic treatments and N_2 for the anoxic treatment in the OX-ANOX experiment). The lids of the tubes were equipped with rubber stoppers allowing gas introduction through needles, prior to entering the tubes, the gas was pumped through deionized water to increase the water content in the gas stream and maintain the water content in the soil. In all oxic treatments, air was pumped through the system with an aquarium pump. In the anoxic treatment N_2 was pushed through the system directly from the gas tube. Open ended syringe needles allowed outflow from the centrifuge tubes. In the TIME, TEMP, and DEPTH experiments, air flow was maintained for 15 min every fourth hour as regulated with a timer. In the OX-ANOX experiment the flow of air and N_2 was continuous.

Three replicate tubes were removed and sampled at different time intervals according to the design of the experiment. In the TIME experiment, tubes were sampled at 0.4, 3.6, 7.5, 13.4, 20.4, 34.5, 70.5, and 133.4 days from isotope addition. The incubation temperature was 15 °C. In the TEMP experiment triplicate tubes were incubated at 2, 10, 15, 20, 30, 37, 51, and 60 °C for 5 days. In the OX-ANOX experiment tubes were incubated at 20 °C under oxic or anoxic conditions and triplicate tubes for each treatment were sampled at 1.6, 5.6, and 12.6 days from the start of the experiment. In the DEPTH experiment tubes were sampled 5 and 12 days from experiment start.

2.3. Soil extractions

Upon sampling, 20 ml Milli-Q water were added to each tube and the tubes were placed on an end-over-end shaker for 1 h. Thereafter, the tubes were centrifuged at 5000*g* for 15 min and the supernatant (i.e. water extract no. 1; Fig. 1) was transferred by pipette to new centrifuge tubes. An additional 20 ml of water were added to the residual soil and the shaking and centrifugation repeated to yield water extract no. 2. This extraction procedure was also repeated twice with 20 ml aliquots of 0.1 M KNO_3 (yielding extracts 3 and 4). Altogether, for each original tube these extractions yielded two water extracts, two KNO_3 extracts, and residual soil remaining after all four extractions (Fig. 1). The four extracts included the extractable Cl, while the non-extractable soil-bound Cl (Cl_{soil}) remained in the residual soil (Fig. 1). The extracts, as well as the residual soil, were

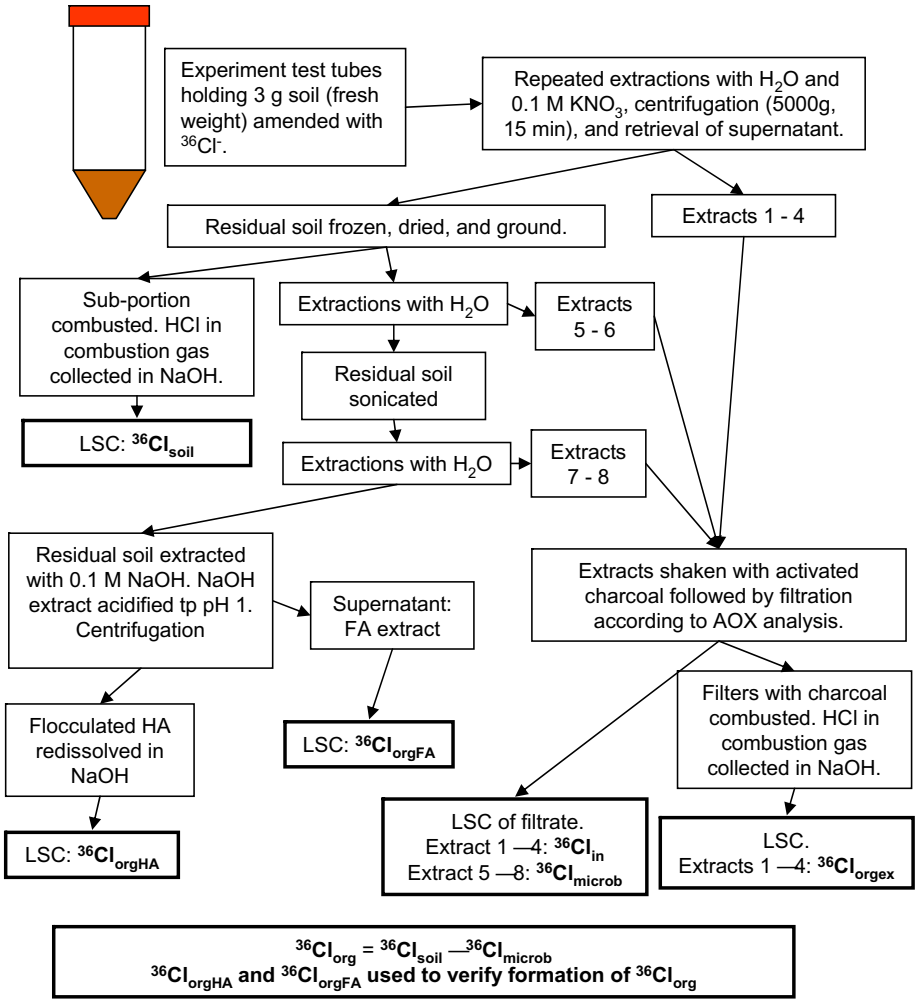

Fig. 1. Overview of extraction procedures used to obtain soil fractions for analysis of ^{36}Cl content. See Section 2 for details.

then frozen awaiting further analyses. Extracts and residual soil from 3 additional control tubes without added ^{36}Cl were also retrieved for further analyses. Aliquots of the extracts from soil without added ^{36}Cl were analyzed directly for total organic carbon (TOC) concentrations while the other samples were frozen for later analyses.

2.4. Inorganic and organic ^{36}Cl in extracts

To distinguish between $^{36}Cl_{in}$ and ^{36}Cl associated with the extracted organic matter ($^{36}Cl_{orgex}$) in extracts 1–4, a standard technique used in the analysis of adsorbable organic halogens (AOX) was applied (European Union Standard, 1996). This method has been thoroughly tested and evaluated. In brief, a volume of 1 ml acidified nitrate solution (0.22 M KNO_3, 0.02 M HNO_3) and approximately 4 drops of concentrated HNO_3 (yielding a pH <2) was added to 10 ml sub-portions of the extracts and shaken with 50 mg activated charcoal for 60 min. Thereafter the extract-charcoal mixture was filtered through a polycarbonate filter (Millipore, 0.45 μm mesh). The filter with the retained charcoal was rinsed with acidic nitrate solution (6 × 3 ml, 0.01 KNO_3, 0.001 M HNO_3), followed by acidified Milli-Q (6 × 3 ml, pH 2 by acidification with HNO_3). A 10 ml aliquot of the filtrate holding the $^{36}Cl_{in}$ was transferred to scintillation vials for subsequent liquid scintillation counting (LSC). The filter with retained $^{36}Cl_{orgex}$ associated with organic matter was combusted at 1000 °C under a stream of O_2 gas according to the procedure for analysing TOX (Asplund et al., 1994). The $H^{36}Cl$ gas formed during combustion was then trapped in 0.1 M NaOH. Tests confirmed that leading the gas stream through two scintillation vials in series, each holding 10 ml 0.1 M NaOH, yielded a recovery of >98% of the ^{36}Cl added to the soil prior to combustion. This technique to recover Cl after combustion was previously described by Laniewski et al. (1999). The filtrate was used without further treatment for LSC yielding $^{36}Cl_{in}$ (Fig. 1). The very low quantity of $^{36}Cl_{in}$ recovered in extract 4 showed that four extractions with water or KNO_3 was enough to yield all extractable $^{36}Cl_{in}$ from the fresh soil (see experiment evaluation below).

2.5. Soil-bound ^{36}Cl

To analyze total soil-bound ^{36}Cl (i.e. ^{36}Cl associated with the solid phase; $^{36}Cl_{soil}$), the frozen residual soil was

dried at 70 °C. Then, 0.1–0.3 g of soil was combusted as described for the filters with charcoal above (Fig. 1), and the ^{36}Cl liberated in gaseous form collected in scintillation vials holding 10 ml 0.1 M NaOH. The $^{36}Cl_{soil}$ potentially included both $^{36}Cl_{in}$ in microbial cells ($^{36}Cl_{microb}$) and ^{36}Cl in SOM ($^{36}Cl_{org}$), since microbial cells were alive during extractions 1–4 and may have prevented intracellular $^{36}Cl_{in}$ from being extracted. The freezing and subsequent drying at 70 °C of the residual soil after extractions 1–4 were likely to kill and to lyse a large fraction of the microbial cells. This would make $^{36}Cl_{microb}$ more readily extractable with water in contrast to the $^{36}Cl_{org}$. Hence, as a first step to separate $^{36}Cl_{microb}$ from $^{36}Cl_{org}$ we first extracted the dried soil twice with 15 ml of water each time (extractions 5 and 6). After these two extractions, another 15 ml of water were added to the residual soil and the mixture was thoroughly homogenized (Cat Homogenizer X120) and sonicated (Bandelin Sonorex RK510H) to further disrupt potentially remaining microbial cells. The homogenized and sonicated soil was then extracted another two times with water (extractions 7 and 8; Fig. 1). $^{36}Cl_{in}$ was separated from organically bound ^{36}Cl in the extracts according to above. The $^{36}Cl_{in}$ found in extracts 5–8 was assumed to represent $^{36}Cl_{in}$ in microbial cells (further discussed in Section 3).

After all eight extractions the remaining ^{36}Cl in the residual soil was considered to be bound to SOM (i.e. as $^{36}Cl_{org}$). To verify this, a sub-portion of the residual soil was extracted in 15 ml 0.1 M NaOH. The NaOH extract was acidified with 0.1 ml conc. HNO_3 resulting in flocculation of humic acids in the extract (Thurman, 1985). Centrifugation (5000 rpm, 15 min) and collection of the supernatant yielded ^{36}Cl in the dissolved fulvic acids as $^{36}Cl_{orgFA}$ (Fig. 1). The flocculated humic acids were washed with 15 ml water followed by centrifugation and removal of the water. Subsequently the humic acid pellet was dissolved in 0.1 M NaOH and a subportion of the solution was transferred to a scintillation vial for measurement of ^{36}Cl in humic acids ($^{36}Cl_{orgHA}$). Altogether, this procedure returned the relative proportions of ^{36}Cl in fulvic and humic acid fractions of the SOM. It should be noted that the NaOH extraction and following humus fractionation was not quantitative since the total amount of the SOM soluble in NaOH was not determined, but recovery of ^{36}Cl in the humic and fulvic acid fractions, respectively, provided firm evidence of formation of chlorinated SOM being present in the residual soil.

2.6. Analyses

Sub-portions of fresh (i.e. non-frozen) extracts from soil without added ^{36}Cl were analyzed for total organic carbon content using a Shimadzu 5000 TOC analyzer. The remaining extracts were frozen and later analysed for chloride concentrations by ion chromatography with chemical suppression (MIC-2, Metrohm) according to the standard procedure for determination of Cl_{in} of water with low contamination (European Union Standard, 1995). Briefly, the frozen extracts were thawed and filtered (0.15 μm filter, Metrohm) and separated on a column for anions (Metrosep A Supp 5—150 * 4.0 mm, Metrohm) with a carbonate eluent (0.0024 M Na_2CO_3 and 0.003 M $NaHCO_3$). A low chloride calibration standard was analysed approximately every 10th sample. The detection limit was 0.1 mg l^{-1}. The original soil was dried and milled, and then analysed for total organic halogen (TOX) content according to Asplund et al. (1994) using an ECS3000 analyser (Euroglas).

Samples from tubes with ^{36}Cl-amended soil were analyzed for ^{36}Cl by LSC in different fractions of the soil as illustrated in Fig. 1. Scintillation cocktail (Ultima Gold XR, Chemical Instruments AB) was added to all ^{36}Cl samples including untreated extracts, $^{36}Cl_{in}$, $^{36}Cl_{orgex}$, $^{36}Cl_{soil}$, $^{36}Cl_{microb}$, $^{36}Cl_{orgFA}$, and $^{36}Cl_{orgHA}$ (see Fig. 1). LSC was carried out using a Beckman LX 6300, correcting for quench using standard quench curves prepared from solutions having the same chemical composition as the samples (i.e. the various extracts and 0.1 M NaOH). Blank samples were always run together with actual samples and all ^{36}Cl measurements were corrected for background radiation.

3. RESULTS AND DISCUSSION

3.1. Experiment evaluation

The small variability in ^{36}Cl activities between replicate test tubes (e.g. Fig. 2) indicates that ^{36}Cl was distributed homogeneously within most test tubes. The distribution of both Cl_{in} and $^{36}Cl_{in}$ in extracts 1–4, with >80% retrieved in the first extract and <2% found in the fourth extract (Fig. 3), implies that Cl_{in} was efficiently extracted. Hence, the ^{36}Cl found in the residual soil after extraction cannot be explained by incomplete extraction of Cl_{in}. Water appeared to be as efficient as KNO_3 in extracting Cl_{in} from this soil (Fig. 3). The total ^{36}Cl recovery when combining all pools was close to 100% (always >94%; Fig. 4). Major increases of $^{36}Cl_{soil}$ always corresponded to a similar decrease of $^{36}Cl_{in}$ (Fig. 2a and c) suggesting that the results are robust.

The addition of $^{36}Cl_{in}$ corresponded to 5.67 μg Cl_{in} per test tube (i.e. 5.54–7.48 μg Cl g^{-1} dw depending on the experiment). This represented an increase of Cl_{in} concentrations in the investigated soil of 26–32% (Table 2). Since there are indications that chlorination of SOM may be affected by Cl_{in} concentrations (Johansson et al., 2003; Bastviken et al., 2006), it is possible that the addition of $^{36}Cl_{in}$ positively affected chlorination rates. However, the quantity added is within the range of the seasonal variations in Cl_{in} concentrations observed in the area (e.g. the difference in extractable Cl_{in} between soil retrieved in May and September, Table 2). Therefore, possible effects due to the addition of ^{36}Cl ought to be within the range of the natural variation.

3.2. Formation of soil-bound Cl (Cl_{soil})

All experiments conducted under oxic conditions showed a substantial retention of $^{36}Cl_{in}$ within a timescale of several days (Fig. 2). Even at the first sampling occasion of the TIME experiment, i.e. after 8 h, a substantial increase in $^{36}Cl_{soil}$ was observed and, after 8 days, 23% of the added ^{36}Cl had been transformed to $^{36}Cl_{soil}$ (Fig. 2a).

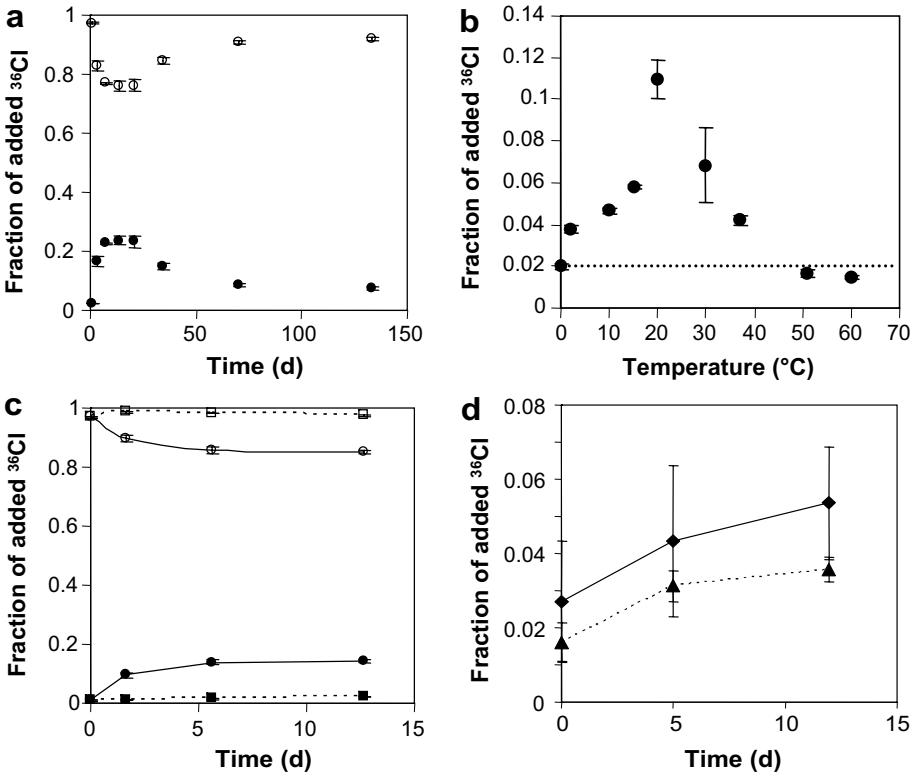

Fig. 2. Fractions of the added ^{36}Cl recovered as extractable inorganic chlorine ($^{36}Cl_{in}$; open symbols) and soil-bound non-extractable chlorine ($^{36}Cl_{soil}$; solid symbols) in the experiments. (a) The TIME experiment (see Table 1). (b) The fraction of ^{36}Cl added recovered as $^{36}Cl_{soil}$ after 5 days at different temperatures (the TEMP experiment). The dotted line represents initial levels of $^{36}Cl_{soil}$ (12 h after isotope addition; see Section 2 for details). (c) The decline in $^{36}Cl_{in}$ and the simultaneous increase in $^{36}Cl_{soil}$ with time under oxic (circles) and anoxic (squares and dotted lines) conditions in the OX-ANOX experiment. (d) Formation of $^{36}Cl_{soil}$ in (a) and (b) horizons (diamonds and triangles, respectively; DEPTH experiment). Averages ± 1SD are shown ($n = 3$).

This rapid increase in $^{36}Cl_{soil}$ was also seen in the TEMP experiment, in which $^{36}Cl_{soil}$ reached 11% of the added ^{36}Cl in 5 days at 20 °C (Fig. 2b). Similarly, the oxic treatment of the OX-ANOX experiment resulted in a $^{36}Cl_{soil}$ fraction of 14% after 6 days (Fig. 2c). The formation of $^{36}Cl_{soil}$ in soil from the deeper horizons was lower as compared with the soil from the top-layer and corresponded to approximately a two-fold increase within 12 days (Fig. 2d). These results show that a substantial amount of the added $^{36}Cl_{in}$ was rapidly transformed to soil-bound chlorine which was non-extractable with water or KNO_3 (Cl_{soil}).

The results of the TEMP experiment strongly suggest that the initial formation of $^{36}Cl_{soil}$ was due to microbial activity, since the temperature response corresponded to the expected temperature response of microbial activity (Fig. 2b). The temperature response, showing an initial increase in $^{36}Cl_{soil}$ formation from 2 to 20 °C followed by a systematic decrease as the temperature exceeded 20 °C, can best be explained by an enzyme mediated process and explanations based on abiotic associations between $^{36}Cl_{in}$ and soil particles (e.g. adsorption) can most likely be excluded.

Formation of $^{36}Cl_{soil}$ was detectable under anoxic conditions, but at much lower rates than in the presence of O_2 (Fig. 2c). The large difference in $^{36}Cl_{soil}$ formation between oxic and anoxic conditions adds further evidence against adsorption. Instead, the reduced initial formation of $^{36}Cl_{soil}$ under anoxic conditions supports the idea of rapid microbial $^{36}Cl_{soil}$ formation since microbial activity should be lower under anoxia due to the lower metabolic energy yield in anaerobic metabolism. Furthermore, if the rapid formation of $^{36}Cl_{soil}$ is caused by microbial activity, lower rates of formation would be expected in deeper soil horizons with lower microbial populations and less favorable conditions for microbial activity (less SOM and nutrients). Hence, the lower formation rate of $^{36}Cl_{soil}$ in the deeper horizons as compared with experiments conducted with A horizon soil at 20 °C (DEPTH experiment; Fig. 2d versus Fig. 2a–c) also suggest $^{36}Cl_{soil}$ formation due to microbial activity.

3.3. Retention of Cl_{in} due to microbial exchange

Based on the reasoning above, the retention of Cl_{in} during a time frame of up to three weeks was a result of microbial formation of Cl_{soil}. It is well known that soil microorganisms, like all other organisms, contain and are able to take up Cl_{in}. The intracellular Cl_{in} concentration is vital for cellular functions and for maintaining the intracellular ion balance and osmotic potential. Therefore, Cl_{in} exchange with the surrounding environment is likely to be actively regulated as long as the cells are alive. Our approach to estimate the amount of intracellular $^{36}Cl_{in}$ in

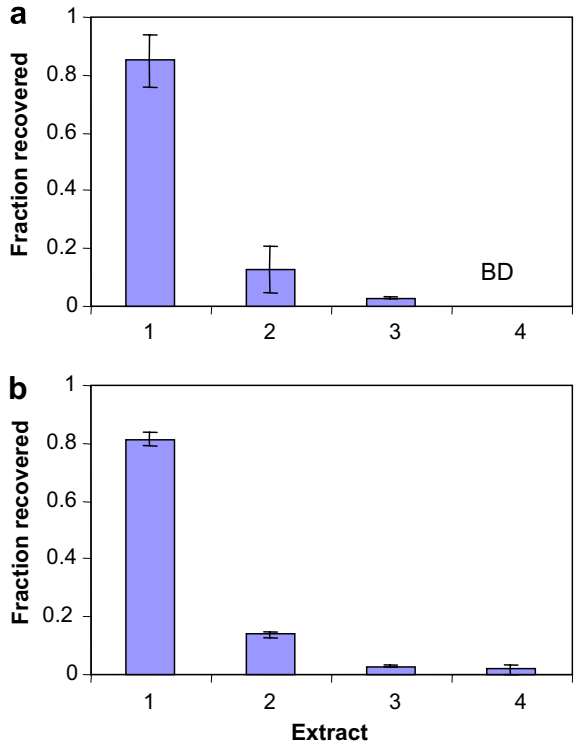

Fig. 3. Relative recoveries of the extractable inorganic chlorine (Cl_{in}; average ± 1SD) in extracts 1–4 (see text for details). (a) Cl_{in} concentrations in extracts of the original soil based on ion chromatographic analysis ($n = 9$; BD denotes that concentrations were below detection limit). (b) The relative $^{36}Cl_{in}$ content in extracts from the TIME, TEMP, and OX-ANOX experiments ($n = 69$). Extraction 1–2 was performed with Milli-Q water and extraction 3–4 with 0.1 M KNO_3.

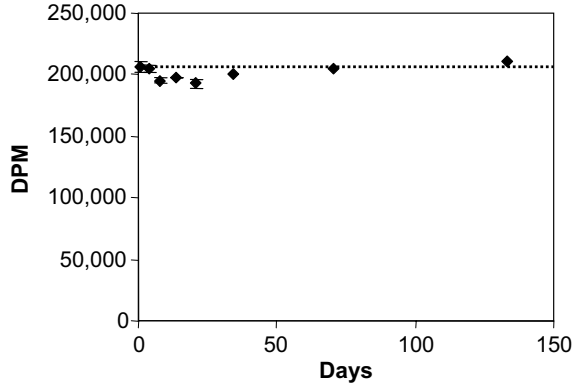

Fig. 4. Total recovery of ^{36}Cl combining all analyzed ^{36}Cl pools in the TIME experiment (average ± 1SD, $n = 3$). 205100 DPM ^{36}Cl was added as indicated by the dotted line.

the $^{36}Cl_{soil}$ pool (i.e. $^{36}Cl_{microb}$) confirms the idea of a substantial microbial uptake of $^{36}Cl_{in}$. Essentially all of the rapid formation of $^{36}Cl_{soil}$ during the first three weeks in the TIME experiment corresponds to formation of $^{36}Cl_{microb}$ (Fig. 5). Within this short time frame almost all $^{36}Cl_{soil}$ was returned to the $^{36}Cl_{in}$ pool by the measures taken to kill and lyse microbial cells and by extractions 5–8. In addition, no or very little ^{36}Cl associated with humic or fulvic acids could be found in the early samples when the $^{36}Cl_{microb}$ pool peaked.

In the TIME experiment, the proportion of ^{36}Cl as $^{36}Cl_{soil}$ remained high (23%) until day 20. Thereafter the fraction of $^{36}Cl_{soil}$ started to decrease and reached a lower plateau at 8% of the added ^{36}Cl by day 133 of the experiment (Fig. 2a). The decrease of the $^{36}Cl_{soil}$ pool in the TIME experiment occurred synchronously with a decrease of $^{36}Cl_{microb}$ (Fig. 5) in correspondence with the expected trend of microbial growth and subsequent decline in a closed system. The initial disturbance of the soil microcosms at the start of the experiment was likely to stimulate microbial activity until resource depletion resulted in a decline in microbial activity and stabilization at lower levels during the remainder of the experiment. Hence, the decline in $^{36}Cl_{soil}$ and $^{36}Cl_{microb}$ further support the conclusion that porewater Cl_{in} concentrations were regulated by microbial exchange of Cl_{in} during the first weeks of the TIME experiment.

We are not aware of any previous comparable estimates of Cl_{in} uptake by soil microorganisms. However, the extent of the $^{36}Cl_{microb}$ formation appears reasonable given current knowledge about microbial biomass and intracellular Cl_{in} concentrations. Soil microbial biomass is typically in the order of tens of mg (g dry soil)$^{-1}$ (Insam, 1990; Friedel et al., 2006). Assuming a cellular Cl content of approximately 0.5% dw (i.e. 5 mg g^{-1}; Stanier et al., 1976), the Cl present in microbial cells may correspond to 50 μg Cl g^{-1} dw or more, which is a substantial amount given the porewater Cl_{in} concentration of approximately 20 μg Cl g^{-1} dw (Table 2). Given this, a microbial biomass increase in the order of 10% could decrease the porewater

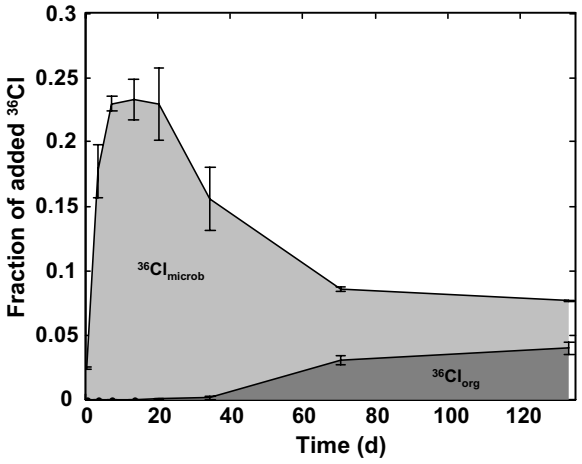

Fig. 5. Area graph showing fractions of the added ^{36}Cl recovered as chloride associated with microbial cells ($^{36}Cl_{microb}$), and as organically bound chlorine ($^{36}Cl_{org}$) over time in the TIME experiment (average ± 1SD, $n = 3$). Initially, $^{36}Cl_{microb}$ totally dominated the soil solid phase associated ^{36}Cl ($^{36}Cl_{soil}$), but in the end of the experiment $^{36}Cl_{soil}$ was composed of approximately equal proportions $^{36}Cl_{org}$ and $^{36}Cl_{microb}$.

Cl_{in} concentrations by 25% by formation of Cl_{microb} in the studied soil.

The evidence that $^{36}Cl_{microb}$ represents intracellular $^{36}Cl_{in}$ appears consistent and we conclude that Cl_{in} retention in the short-term (up to one month) under the conditions of our experiments was dominated by microbial uptake. Apparently, microbial exchange of Cl_{in} has the potential to cause substantial retention or release of soil porewater Cl_{in} over short periods of time. Microbial exchange presumably has the greatest effect on porewater Cl_{in} concentrations following any kind of environmental change affecting the microbial activity. Such changes could range from relatively trivial and frequent events (e.g. rewetting of dry soil following precipitation) to very dramatic though much less frequent events (e.g. clear-cutting of forest). In more stable periods between such changes the microbial exchange may very well be in steady-state with no net changes in porewater Cl_{in} concentrations due to microbial uptake. The microbial exchange of chlorine deserves more attention since it has the potential to cause significant uncertainties in estimates of Cl budgets and when using Cl_{in} as a tracer of soil water movements. In addition, as discussed above, the amount of Cl_{in} present in microbial cells may be greater than the porewater Cl_{in} concentration. This is important to consider when choosing methods to assess the Cl_{in} content in soil, since the typical room temperature extraction of Cl_{in} with water or nitrate solution may not account for intracellular Cl_{in} and thereby substantially underestimate the total Cl_{in} present in the soil.

3.4. Retention of Cl_{in} due to formation of organically bound chlorine (Cl_{org})

As $^{36}Cl_{soil}$ and $^{36}Cl_{microb}$ decreased in the TIME experiment, there was a simultaneous increase in $^{36}Cl_{org}$ (Fig. 5). The formation of $^{36}Cl_{org}$ could be confirmed since almost all the ^{36}Cl found in the NaOH extract (Fig. 1) was associated with humic and fulvic acids. By the end of the TIME experiment approximately 4% of the added $^{36}Cl_{in}$ had been transformed to $^{36}Cl_{org}$ (Fig. 5) which was shared equally between humic and fulvic acid fractions. The rate of $^{36}Cl_{org}$ formation was highest between day 35 and day 70 of the experiment (Fig. 5). We also found ^{36}Cl associated with the extracted organic matter ($^{36}Cl_{orgex}$; Fig. 1), but in much lower amounts. The formation of $^{36}Cl_{orgex}$ in the TIME experiment accounted for 1–2‰ of the added ^{36}Cl corresponding to the fraction of the organic matter being extracted (Fig. 6).

While rate estimates regarding chlorine transformation in soil are rare, there are several previous studies reporting natural formation of Cl_{org} in soil environments. Lee et al. (2001) observed that a substantial proportion of the $^{36}Cl_{in}$ added to soil columns became associated with low molecular weight humic acids in experiments run for 78 days or 4 years. Silk et al. (1997) performed ^{36}Cl labeling experiments in peat and recovered substantial fractions of the added ^{36}Cl in NaOH extracts or residual peat after 8 weeks of incubation (up to 6% and 2%, respectively). Hence, they report rates similar to ours, but it is unclear to what extent their NaOH extract includes Cl_{microb} since Cl_{org} and Cl_{microb} were not separated. There are also studies indicating natural Cl_{org} formation without the use of ^{36}Cl. Myneni (2002) reported formation of non-volatile Cl_{org} during degradation of plant material starting within six months after the plants became senescent. Formation of Cl_{org} in soil in association with reduction of Fe(III) has been reported (Keppler et al., 2000; Fahimi et al., 2003). Rapid natural formation and degradation of chloroacetic acids in soil has also been observed (Matucha et al., 2003, 2007; Laturnus et al., 2005). Furthermore, several experiments and catchment studies focusing on chlorine biogeochemistry have suggested natural formation of Cl_{org} (e.g. Öberg, 2002; Öberg et al., 2005; Öberg and Sandén, 2005). Hence, our results of formation of Cl_{org} in a forest soil reaching substantial levels after approximately one month of incubation (Fig. 5) are in line with, and supplement previous work by indicating natural chlorination of water extractable, fulvic acid, and humic acid fractions of SOM, and by providing information about potential rates of SOM chlorination.

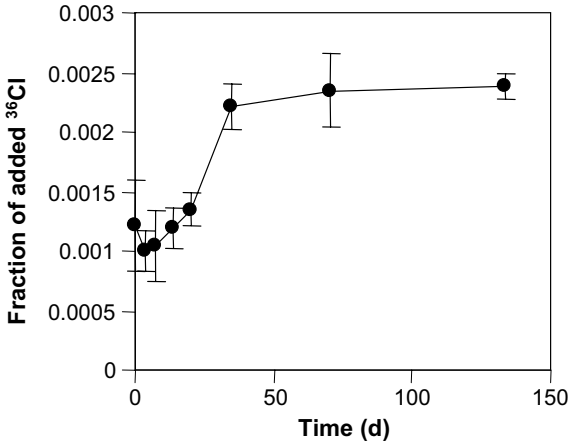

Fig. 6. Fraction of the added ^{36}Cl recovered as extractable organic ^{36}Cl ($^{36}Cl_{orgex}$) with time in the TIME experiment (average ± 1SD, $n = 3$).

The net-formation of 4% Cl_{org} relative to Cl_{in} in the TIME experiment corresponds to a Cl_{org} formation of 0.8 μg g^{-1} dw over 133 days if the Cl_{in} concentration is 20 μg g^{-1} dw (Table 2). Assuming that this rate applies to field conditions during 260 days per year (i.e. not when the ground is frozen during winter), the Cl_{org} formation corresponds to approximately 1.6 μg g^{-1} dw yr^{-1}, or 0.1 g m^{-2} yr^{-1} in the area where the soil was collected. As rough as it may appear, our estimate is supported by a previous chlorine budget for this area, and based on a catchment mass-balance approach, the net-formation of chlorinated organic matter in soil was estimated to 0.2 g m^{-2} yr^{-1} (Öberg et al., 2005). Given a Cl_{org} formation of 0.1 g m^{-2} yr^{-1}, the Cl_{org} formation may constitute 25% of the total wet deposition of Cl_{in} in this catchment (0.4 g m^{-2} yr^{-1}; Maxe, 1995). Hence, from a catchment budget perspective, the formation of Cl_{org} represents a substantial retention of Cl_{in} in the studied soil and a quantity of Cl_{in} corresponding to the whole porewater Cl_{in} pool could be immobilized by formation of Cl_{org} over 15 years.

Given an annual Cl_{org} formation of 1.6 μg g^{-1} dw it would take 200–300 years to build up the Cl_{org} content found in the soil (TOX of 314–476 μg g^{-1} dw; Table 2). Thereby, the observed formation of Cl_{org} means a minor contribution to the standing stock of Cl_{org}. However, since the studied soil is substantially older than 200–300 years, our rate estimate infers that there must be processes degrading Cl_{org}, resulting in turnover of Cl_{org} within the soil.

The mechanisms of Cl_{org} formation have been debated and both abiotic and biotic processes have been suggested. For example, Keppler et al. (2000) and Fahimi et al. (2003) report abiotic Cl_{org} formation of volatile halogenated organic carbon and chloroacetic acids, respectively. It has been hypothesized that the abiotic formation of volatile organochlorines is associated with redox reactions involving humic matter and Fe^{3+}/Fe^{2+}. Abiotic conversion from Cl_{in} to chloromethane (CH_3Cl) in plant material with pectin as the methyl donor has also been reported (Hamilton et al., 2003). On the other hand, biotic synthesis of a numerous organochlorine compounds is well known to be performed by many organisms (Gribble, 2003). Most previous knowledge about biotic chlorination concern intracellular chlorination. However, the quantitatively dominating biotic chlorination of soil organic matter is believed to occur primarily through the action of extracellular chloroperoxidase-like enzymes (Asplund et al., 1991, 1993; Hoekstra, 1999; van Pee and Unversucht, 2003). Such enzymes produce reactive chlorine that can break C–C bonds in complex organic molecules and at the same time result in chlorination of the organic matter. It has been shown that chloroperoxidase activity enhances the biodegradability of lignin compounds (Ortiz-Bermúdez et al., 2003). Our results indicate that the formation of $^{36}Cl_{org}$ was non-linear and delayed until the decrease of the $^{36}Cl_{microb}$ (Fig. 5). We hypothesize that the Cl_{org} formation was a result of microbially regulated activity of choroperoxidase-like enzymes to degrade refractory organic matter, and that the delayed $^{36}Cl_{org}$ formation could be explained by a shift in conditions and possibly in microbial communities. During the early phase of the experiment, with an abundance of

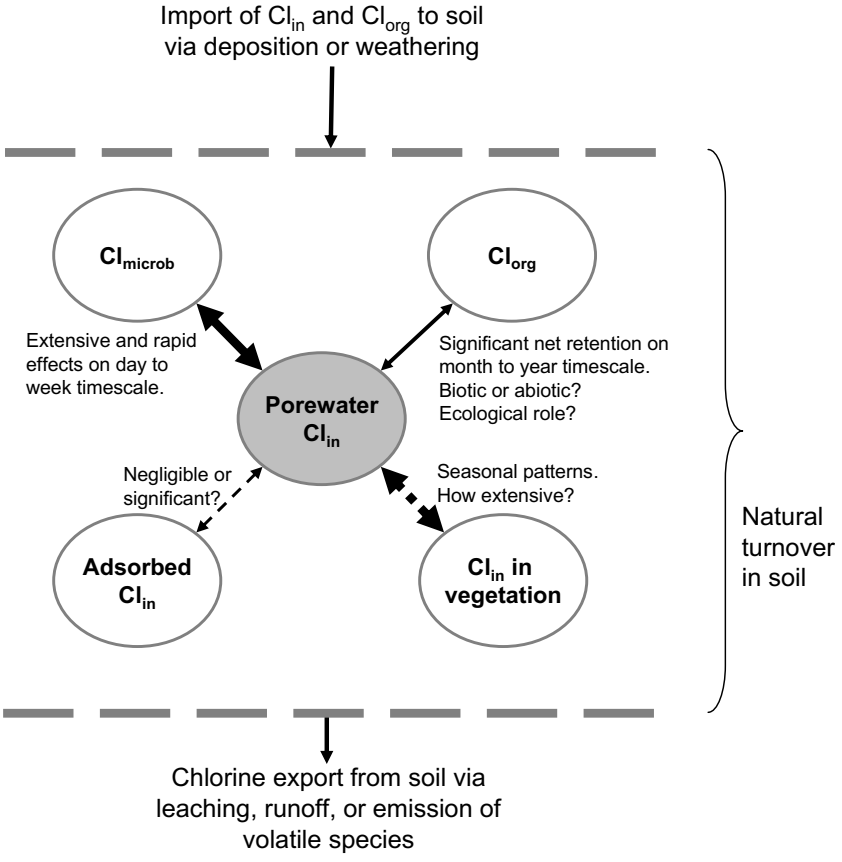

Fig. 7. Conceptual illustration of the exchange between chloride (Cl_{in}) in porewater and other soil chlorine pools. Extensive cycling between porewater Cl_{in} and Cl_{in} in microbial cells (Cl_{microb}), as well as significant Cl_{in} retention by chlorination of soil organic matter was indicated by the present study. The dotted arrows indicate exchange processes proposed by other studies (see Section 1 for references). Flux rates will vary among ecosystems, but believed relative flux magnitudes are indicated by arrow sizes. Some comments and questions regarding the different exchange processes are included. This picture indicates that porewater Cl_{in} concentrations are regulated by many processes acting at different timescales, which should be considered when using Cl_{in} as a tracer of soil water flow. Note that this is not a complete picture of soil chlorine cycling and several processes are missing. For example, Cl_{org} may not only be produced from porewater Cl_{in}, but may also be derived from Cl_{microb} or Cl_{in} in vegetation.

easily available organic substrates, microorganisms grew rapidly resulting in the rapid increase in $^{36}Cl_{microb}$ with no requirements for chloroperoxidase-like activity to access substrates. However, upon depletion of the easily available substrates the microbial community crashed as indicated by the decrease in $^{36}Cl_{microb}$, and chloroperoxidase-like activity became more important to access organic substrates, which resulted in the formation of Cl_{org}. The formation of $^{36}Cl_{orgex}$ occurred just ahead of the $^{36}Cl_{org}$ formation and simultaneously as the $^{36}Cl_{microb}$ decreased (Figs. 6 and 7). In line with our hypotheses above, we speculate that the increase in $^{36}Cl_{orgex}$, representing easily extractable chlorinated SOM, may have been associated with the onset of chlorination activity. The detected $^{36}Cl_{orgex}$ may include small chlorinated and water extractable organic compounds such as chloroacetic acids. It has been shown that both DCA and TCA represent important products of SOM chlorination although present at very low concentrations since microbial degradation is rapid (Matucha et al., 2007).

Obviously, the formation and degradation of chlorinated SOM need further attention. While this study illuminates the effect of natural SOM chlorination on Cl_{in} retention, in general very little seems to be known about chlorinated SOM in spite of the fact that Cl_{org} is very abundant and dominates the chlorine pool in many soils. Important unanswered questions include to what extent formation of Cl_{org} is biotic or abiotic, and what biogeochemical, ecological, and potentially toxicological roles naturally chlorinated SOM have.

3.5. Conclusions

The present study provides evidence that Cl_{in} can be retained in forest soil by at least two different processes acting over different time scales. First, 11–24% of the added $^{36}Cl_{in}$ was retained within one week in soil from the A horizon by microbial uptake, and similar amounts were released after about three weeks in the long-term experiment. This indicates a rapid and extensive cycling of porewater Cl_{in} due to microbial uptake and release. This cycling is probably regulated by changes in environmental conditions affecting microbial activity. Second, there was a less extensive but still substantial net Cl_{in} retention due to natural formation of chlorination of organic compounds associated to the fulvic and humic acid fractions of the SOM. These results have several important implications. For example, the use of chloride as a conservative tracer of soil and groundwater movements should be reconsidered. Apparently, porewater chloride is not conservative on a short-term basis and, instead, the whole chloride pool appears to be rapidly and continuously turned over in soil due to microbial uptake and release. The results also indicate long-term net natural chlorination of SOM potentially representing a substantial Cl_{in} sink over time periods of years to decades. Hence, the apparently conservative behavior of porewater Cl_{in} on a long-term or large-scale basis seems, instead, to be a delicate balance between many processes acting at different time scales (Fig. 7).

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