Chlorine Cycling in Terrestrial Environments

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Linköping Studies in Arts and Science No. 672
Faculty of Arts and Sciences
Linköping 2016

Linköping Studies in Arts and Science • No. 672

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Distributed by:
Department of Thematic Studies – Environmental Change
Linköping University
581 83 Linköping

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Edition 1:1
ISBN 978-91-7685-813-4
ISSN 0282-9800

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Department of Thematic Studies – Environmental Change 2016

Cover image: Photo by Sivakiruthika Natchimuthu
Printed by: LiU-Tryck, Linköping 2016

Abstract

Chlorinated organic compounds (Cl_{org}) are produced naturally in soil. Formation and degradation of Cl_{org} affect the chlorine (Cl) cycling in terrestrial environments and chlorine can be retained or released from soil. Cl is known to have the same behaviour as radioactive chlorine-36 (^{36}Cl), a long-lived radioisotope with a half-life of 300,000 years. ^{36}Cl attracts interest because of its presence in radioactive waste, making ^{36}Cl a potential risk for humans and animals due to possible biological uptake. This thesis studies the distribution and cycling of chloride (Cl^-) and Cl_{org} in terrestrial environments by using laboratory controlled soil incubation studies and a forest field study. The results show higher amounts of Cl^- and Cl_{org} and higher chlorination rates in coniferous forest soils than in pasture and agricultural soils. Tree species is the most important factor regulating Cl^- and Cl_{org} levels, whereas geographical location, atmospheric deposition, and soil type are less important. The root zone was the most active site of the chlorination process. Moreover, this thesis confirms that bulk Cl_{org} dechlorination rates are similar to, or higher than, chlorination rates and that there are at least two major Cl_{org} pools, one being dechlorinated quickly and one remarkably slower. While chlorination rates were negatively influenced by nitrogen additions, dechlorination rates, seem unaffected by nitrogen. The results implicate that Cl cycling is highly active in soils and Cl^- and Cl_{org} levels result from a dynamic equilibrium between chlorination and dechlorination. Influence of tree species and the rapid and slow cycling of some Cl pools, are critical to consider in studies of Cl in terrestrial environments. This information can be used to better understand Cl in risk-assessment modelling including inorganic and organic ^{36}Cl .

Keywords: chloride, organic chlorine, chlorination, dechlorination, ^{36}Cl , risk assessment modelling

Sammanfattning

Klorerade organiska föreningar (Cl_{org}) bildas naturligt i mark och påverkar klorets kretslopp genom att de stannar kvar längre i marken. Detta stabila klor anses ha samma egenskaper som klor-36, som är en långlivad radioisotop med en halveringstid på 300 000 år. Klor-36 förekommer i olika typer av radioaktivt avfall och om klor-36 sprids i naturen finns det en potentiell risk för människor och djur genom biologiskt upptag. Syftet i denna avhandling är att öka kunskapen om fördelningen och cirkulationen av klorid (Cl^-) och Cl_{org} i terrestra miljöer med hjälp av studier i laboratoriemiljö samt en fältstudie i skogsmiljö. Resultaten visar att bildningshastigheten av Cl_{org} är högst i barrskogsjord och rotzonen tycks vara en aktiv plats. Det finns också en större mängd Cl^- och Cl_{org} i barrskogsjordar än i betesmark och jordbruksmark. Den mest betydande faktorn som styr halterna av Cl^- och Cl_{org} är trädsort, medan geografiskt läge, atmosfäriskt nedfall, och jordmån är av mindre betydelse. Bildning och nedbrytning av Cl_{org} sker med liknande hastigheter, men det tycks finnas två förråd av Cl_{org} i jorden varav ett bryts ner snabbt och ett mer långsamt. Bildningshastigheten av Cl_{org} är lägre i jordar med höga halter av kväve medan nedbrytningshastigheterna inte påverkas av kväve.

Slutsatsen från studiernas resultat är att klor i hög grad är aktivt i mark och att Cl^- och Cl_{org} halterna bestäms av en dynamisk jämvikt mellan bildning och nedbrytning av Cl_{org} . I studier av klor i terrestra miljöer bör trädsorters inverkan och nedbrytning av olika klorförråd beaktas då det kan ge varierande uppehållstider av Cl^- och Cl_{org} i mark. Denna information är viktig vid riskbedömningar av hur radioaktivt klor kan spridas och cirkulera vid en eventuell kärnkraftsolycka.

Nyckelord: klorid, organiskt klor, klorering, deklorering, klor-36, riskmodellering

List of papers

This thesis is based on the following papers, referred to in the text by their roman numerals (I-IV).

Paper I

Gustavsson, M., Karlsson, S., Öberg, G., Sandén, P., Svensson, T., Valinia, S., Thiry, Y. and Bastviken, D. (2012). **Organic matter chlorination rates in different boreal soils: the role of soil organic matter content.** *Environ. Sci. Technol.*, 46, 1504–1510.

Paper II

Montelius, M., Thiry, Y., Marang, L., Ranger, J., Cornelis, J-T., Svensson, T. and Bastviken, D. (2015). **Experimental evidence of large changes in terrestrial chlorine cycling following altered tree species composition.** *Environ. Sci. Technol.*, 49, 4921–4928.

Paper III

Montelius, M., Svensson, T., Lourino-Cabana, B., Thiry, Y. and Bastviken, D. (2016). **Chlorination and dechlorination rates – A combined modelling and experimental approach.** *Sci. Total Environ.* 554-555, 203-210.

Paper IV

Montelius, M., Svensson, T., Lourino-Cabana, B., Thiry, Y. and Bastviken, D. (2016). **Chlorine transformation and transport in an experimental soil-plant system.** (Submitted).

List of abbreviations

Cl	Chlorine
Cl ⁻	Chloride ion
Cl _{org}	Chlorinated organic compounds
VOCl	Volatile organochlorine
³⁶ Cl	Chlorine-36; a radioisotope of Cl emitting primarily beta radiation
³⁶ Cl ⁻	Chloride-36 ion
³⁶ Cl _{org}	Organically bound chlorine-36
TX	Total halogens; an operational definition based on an analysis method which strictly defined detect chlorine, bromine and iodine
TOX	Total organic halogens; an operational definition based on an analysis method which strictly defined detect chlorine, bromine and iodine
Chlorination	Transformation of Cl ⁻ to Cl _{org}
Dechlorination	Transformation of Cl _{org} to Cl ⁻

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1. Introduction

Chlorine (Cl) is one of the most abundant elements on Earth and an essential nutrient for both humans and plants (Winterton, 2000). Over the last 30 years, however, the view of Cl cycling has changed dramatically in the biogeochemical research community. The previous view was that chloride (Cl^-) is the dominant form of Cl in all environments and that Cl^- is non-reactive and simply follows the water. Therefore, Cl^- was historically used as a tracer to follow water masses in landscapes (Schlesinger, 1997; Kirschner et al., 2000). Chlorinated organic compounds (Cl_{org}) were long considered toxic and believed to be solely of anthropogenic origin. However, these views have been challenged by high concentrations of Cl_{org} measured in soil (e.g. Asplund & Grimvall, 1991) together with observed catchment-scale mass balances indicating Cl^- imbalances (Likens, 1995; Viers et al., 2001; Lovett et al., 2005; Svensson et al., 2012). Previous research has demonstrated that Cl_{org} concentrations exceed Cl^- levels in soil (Keppler & Biester, 2003; Biester et al., 2004; Svensson et al., 2007a) and that Cl_{org} is produced naturally (Öberg et al., 2002). In boreal and temperate soils, 48% to almost 100% of the total Cl has been found as Cl_{org} in the upper soil layers (Johansson et al., 2003a, 2003b; Svensson et al., 2007; Matucha et al., 2010; Redon et al., 2011). This implies that Cl is highly reactive in soil and that Cl_{org} transformation processes such as chlorination (Cl^- becoming Cl_{org}) and dechlorination (Cl_{org} becoming Cl^-) are important in the Cl cycle, as soil seems to act as both a source and a sink of Cl (Rodstedth et al., 2003).

Stable Cl is considered to have the same behaviour as radioactive chlorine-36 (^{36}Cl). In recent decades, ^{36}Cl , a long-lived radioisotope with a half-life of 300,000 years, has attracted interest because of its presence in radioactive waste (Sheppard et al., 1996). The characteristics of Cl and its high activity in soil make ^{36}Cl a potential risk for humans and animals due to possible biological uptake (Limer et

al., 2009). Knowledge of the influence of vegetation and environmental factors on chlorination and dechlorination is needed to estimate the distribution, cycling, and residence times of Cl in different environments. This would lead to both a better understanding of the natural cycling of Cl and improved long-term risk assessment models related to the handling and storage of radioactive waste (Limer et al., 2009).

1.1 Objective of this thesis

The overall objective of this thesis is to increase knowledge of the distribution and cycling of Cl^- and Cl_{org} in terrestrial environments. The following specific research questions were defined:

1. What are the distributions and fluxes of Cl^- and Cl_{org} in different compartments (e.g. trees and soil) in terrestrial ecosystems? (Papers I and II)
2. What is the extent of chlorination and dechlorination in terrestrial ecosystems and how do environmental factors affect these transformation processes? (Papers I, II, and III)
3. How does vegetation influence chlorination rates in soil? (Paper II and IV)

1.2 Thesis outline

This introductory chapter is followed by chapter 2, which presents an overview of the natural biogeochemical cycle of Cl. Chapter 3 briefly describes the methods and materials used in the present research; more detailed descriptions can be found in the respective Papers I–IV. Chapter 4 presents a summary of the results of Papers I–IV. Chapter 5

discusses the main results of Papers I–IV in relation to the research questions and literature on the distribution and cycling of Cl in terrestrial ecosystems. Finally, in chapter 6, I summarize the conclusions of this thesis, present practical implications, and suggest future research topics.

2. Biogeochemical cycle of chlorine

This chapter presents an overview of previous research into and concepts of the distribution and cycling of Cl in terrestrial ecosystems. First, the basic biogeochemistry of Cl is described, followed by the distribution and fluxes of Cl in terrestrial ecosystems. Finally, the transformation of Cl_{org} together with environmental factors that might influence the involved processes are presented.

2.1 Chlorine biogeochemistry

Cl is a trace element in all environmental realms except the ocean and exists predominantly as Cl⁻ in nature. The ocean is the primary sink because of the high aqueous solubility of Cl⁻, which dissolves in water as a consequence of the chemical and physical weathering of rocks (Winterton, 2000). Cl is an essential micronutrient for all living things, including humans, animals, and plants. In the human body, Cl⁻ is important for maintaining the osmotic pressure as well as for maintaining the water balance and regulating pH (White & Broadley, 2001). In plants, Cl is one of 16 elements essential for plant growth. Cl⁻ acts as a counter ion to stabilize the membrane potential and plays an important role in stomatal regulation in some plant species. It is also necessary for the water-splitting reaction in photosynthesis (Marschner, 2012).

Cl is present not only as Cl⁻ but also as Cl_{org}. Historically, synthetic Cl_{org} has been of substantial industrial and economic importance because of its diverse applications in solvents, pesticides, drugs, and plastics in various industries. The structure and number of Cl atoms in the molecule determine the physico-chemical and biological properties of Cl_{org}. The widespread use and improper handling of these anthropogenic and often toxic substances have led to the extensive distribution of, for

example, chlorinated aromatics and phenols in various environmental compartments (Gribble, 1994).

In the late 1980s and early 1990s, it was revealed that large amounts of naturally formed Cl_{org} were present ubiquitously in nature (Asplund & Grimvall, 1991; Haselman et al., 2000; Öberg et al., 2005; Svensson et al., 2007). Evidence indicates that chlorinated organic matter forms naturally and that Cl_{org} is as abundant as Cl^- in organic soils (Öberg & Sandén, 2005; Bastviken et al., 2009). At present, more than 4700 natural organic halogens are known, 2300 of which are Cl_{org} compounds such as alkenes, terpenes, steroids, fatty acids, and glycopeptides (Gribble, 2003, 2004, 2010). Naturally occurring Cl_{org} is produced by fungi, bacteria, terrestrial plants, and marine organisms (Engvild, 1986; Gribble, 2004). Forest fires are also a major source of some Cl_{org} species, such as methyl chloride (Reinhardt & Ward, 1995).

There are two stable and seven radioactive isotopes of Cl. The stable isotopes, ^{35}Cl and ^{37}Cl , constitute 76% and 24%, respectively, of all Cl, while the radioactive isotopes account for trace levels only. One of the radioactive isotopes, ^{36}Cl , has a half-life of 3.01×10^5 years, which is long enough to cause concern. The decay of ^{36}Cl corresponds to an energy level of up to 709.6 keV, and results in beta emission (98.1% of the energy) as well as electron capture producing ^{36}Ar and ^{36}S (Rodriguez et al., 2006; Peterson et al., 2007). Natural processes resulting in ^{36}Cl production include the atmospheric spallation of argon, as protons are part of the cosmic radiation, and the neutron activation of K, Ca, and Cl in soil and rocks (White & Broadley, 2001). The background radiological dose exposure is determined from the ratio of ^{36}Cl to stable chlorine ($^{36}\text{Cl}/\text{Cl}$). The natural $^{36}\text{Cl}/\text{Cl}$ ratio varies between 10^{-15} and 10^{-12} on Earth's surface depending on the geographical location (Campbell et al., 2003). In coastal areas with high levels of stable Cl, the $^{36}\text{Cl}/\text{Cl}$ ratio can be orders of magnitude lower than in inland areas with less stable Cl. Contamination with ^{36}Cl can affect the

dose, and $^{36}\text{Cl}/\text{Cl}$ ratios of up to 2×10^{-11} have been found in an extensive area (approximately 100 km^2) due to the previous operation of nuclear power reactors and a nuclear fuel reprocessing plant (Seki et al., 2007).

Nuclear weapon tests between 1952 and 1958, resulting in seawater neutron activation, led to large environmental releases of ^{36}Cl (Peterson et al., 2007). ^{36}Cl peaks from such events have been used for groundwater dating (White & Broadly, 2001; Campbell et al., 2003). In nuclear power plants, stable ^{35}Cl in several materials, including steel and concrete (construction materials), coolant water, core material, and graphite, is converted to ^{36}Cl by neutron capture (Frechou & Degros, 2005; Hou et al., 2007). ^{36}Cl formation can also be extensive in reactors with processes favouring fast neutrons and other high-energy particles (fast reactors), due to spallation involving K and Ca in concrete components (Aze et al., 2007). Levels of ^{36}Cl are often low, but high uptake rates by organisms and accumulation in biomass versus soils (White & Broadly, 2001; Kashparov et al., 2007) generate concern, calling for risk assessments based on better information on Cl transport, transformations, availability to organisms, and exposure times in various environments (Limer et al., 2009).

2.2 Chlorine in terrestrial ecosystems

In terrestrial ecosystems, Cl exists naturally as both Cl^- and Cl_{org} in soil, vegetation, and water as well as in the atmosphere (Öberg, 2002). An overview of the natural Cl cycle is presented in Figure 1. Cl from the atmosphere is deposited on land. Plants take up Cl and it is returned to the soil by leaching and litterfall. Chlorination and dechlorination processes occur in humus and in different soil layers as well as via the weathering of Cl^- from bedrock.

Figure 1. The natural chlorine cycle, showing flows and transformation processes of chloride (Cl^-) and organic chlorine (Cl_{org}) in a terrestrial ecosystem. 1. Wet and dry deposition of Cl^- and Cl_{org} 2. Cl^- and Cl_{org} from vegetation (e.g. throughfall and stemflow). 3. Uptake of Cl^- and Cl_{org} from soil by plant roots. 4. Volatilization of Cl_{org} from the soil to the atmosphere. 5. Volatilization of Cl_{org} from plants to the atmosphere. 6. Transformation of Cl^- to Cl_{org} (chlorination) in the humus layer and in the opposite direction from Cl_{org} to Cl^- (dechlorination). 7. Chlorination and dechlorination processes in the mineral soil. 8. Leaching of Cl^- and Cl_{org} from the humus layer to the mineral soil. 9. Weathering of Cl^- from the bedrock dispersed to the mineral soil and the humus layer. More detailed information about these processes is presented in the main text below.

2.2.1 Cl input and export from soil

Rock weathering from some bedrock types, such as hornblende and apatite (Peters, 1984; Lovett et al., 2005; Mullaney et al., 2009), can give an input of Cl to soil and plants. However, atmospheric deposition accounts for the largest addition of Cl to the terrestrial cycle. Atmospheric deposition consists of wet and dry deposition, which constitutes the deposition of marine salts on land. Dry deposition is the input of Cl from gases, particles, and aerosols, which can be deposited directly on the ground or adhere to tree crowns or stems, being washed out and reaching the ground when it rains. Cl^- aerosols are produced when small air bubbles break on the sea surface. The aerosols are then transported by the winds to the atmosphere where they are carried back to the sea or deposited on land via, for example, wet deposition as they are washed out by rain or snow. Cl^- deposition is higher in coastal locations than in inland areas; Cl^- is mainly deposited by wet deposition (Silva et al., 2007), which ranges from 0.5 to 220 kg ha⁻¹ y⁻¹ in Europe (Clarke et al., 2009). In addition to Cl^- , precipitation also contains Cl_{org} (Enell & Wennberg, 1991; Grimvall et al., 1991; Laniewski et al., 1995) on the order of 0.07 kg ha⁻¹ y⁻¹ (Svensson et al., 2007a).

The major export of Cl from the soil pool is by the leaching of Cl^- to lakes, streams, groundwater, and deeper soil layers (Kopáček et al., 2014). The movement of Cl^- within the soil is determined by water fluxes (Tisdale et al., 1985) and is regulated mainly by factors such as precipitation and evapotranspiration (i.e. sum of water discharge from leaves and evaporation from soil). Cl could also be leached from soil as Cl_{org} , as has been demonstrated experimentally (Rodstedth et al., 2003) and is common in stream and surface waters (Asplund & Grimvall, 1991). The Cl_{org} content of runoff water from a catchment has been analysed, revealing it to be a few percent of the total Cl flux on a catchment scale (Svensson et al., 2007a).

2.2.2. Cl input and export from vegetation

Cl is readily taken up by plants and its mobility in short- and long-distance transport is high. The average concentration of Cl in plant cell walls is 2–20 mg g⁻¹ dry mass, but the minimum requirement for plant growth is 10–100 times lower (0.2–0.4 mg g⁻¹ dry mass) (Marchner, 2012). Plants take up more Cl than they need and high concentrations of Cl⁻ can be found in the upper parts of plants as a consequence of either selective uptake (active) or water uptake (passive). Cl in plants returns to soil as litter or as throughfall and stemflow when it rains, after being excreted through leaves or needles. In general, there is large variability of Cl concentration in throughfall and stemflow, depending largely on the tree species (Ashton Acton, 2011). For example, the concentration of Cl⁻ was seven times higher in throughfall from spruce than from beech (Adriaenssens et al., 2012). Some studies have measured Cl_{org} deposition from throughfall, for example, in a small spruce forest site in Denmark where the concentration was 377 g Cl_{org} ha⁻¹ (Öberg et al., 1998). The source of the Cl_{org} existing in throughfall is unknown, but aliphatic organochlorine constitutes an intrinsic component of healthy leaves (Leri & Myneni, 2010), while the most abundant forms of Cl found in humified plant material occur in high-molecular-weight aromatic structures (Myneni, 2002). A study of throughfall in a spruce forest found that Cl_{org} followed the concentration of organic carbon, which indicated a common source; that, together with the fact that the concentration of Cl_{org} in fresh leaves is very low, implies that the Cl_{org} was produced on or in leaves (Öberg et al., 1998). A later study suggests that the chlorination rates are two to three orders of magnitude higher than the rates in soil (Öberg & Bastviken, 2012).

Cl from vegetation is exported to the atmosphere by the volatilization of Cl_{org}. A variety of volatile organochlorines (VOCs), such as chloroform, is produced in soil (Hoekstra et al., 1998; Keppler et al., 2002; Svensson et al., 2007b; Rhew et al., 2008; Albers et al., 2010). VOCs are also emitted by wildfires, geothermal sources (Lobert et al.,

1999), marine sources (Laturnus et al., 1998), and plants in temperate forest ecosystems (Forczek et al., 2015). VOCl emissions are assumed to be small compared with the wet and dry deposition of Cl; for example, chloroform and chloromethane emissions correspond to 0.13 and 0.04 g Cl m⁻² y⁻¹, respectively, from a coniferous forest soil (Dimmer et al., 2001).

2.3 Transformation of organic chlorine

Processes of Cl_{org} transformation include the formation (chlorination) and degradation (dechlorination) of Cl_{org}. Present knowledge of these two processes in soil and of the mechanisms underlying them is presented below.

2.3.1 Formation of organic chlorine

Laboratory experiments have demonstrated that ³⁶Cl⁻ added to soil could be transformed to organically bound ³⁶Cl (³⁶Cl_{org}) (Silk et al., 1997; Lee et al., 2001) and that autoclaving decreased the transformation process (Silk et al., 1997). Other studies observed that microorganisms take up and release ³⁶Cl⁻ (Bastviken et al., 2007) and that chlorination in soil is temperature dependent and driven by both abiotic and biotic processes (Bastviken et al., 2009). Chlorination processes taking place in terrestrial environments are mostly attributable to enzymatic reactions, i.e. they are biotic (Reddy et al., 2002; Ortíz-Bermúdez et al., 2003; Reina et al., 2004; Wagner et al., 2009). It is suggested that enzymes secreted by plants, fungi, and bacteria play an important role in the natural chlorination of organic matter. Although the exact mechanisms are not fully understood, two types of biotic chlorination have been hypothesized in previous research: unspecific chlorination outside cells (van Pée & Unversucht, 2003) and specific chlorination inside cells (Hoekstra, 1999). Unspecific chlorination

outside cells (van Pée & Unversucht, 2003) is catalysed mainly by heme- and non-heme-containing haloperoxidases such as chloroperoxidase (CPO) (Breider & Albers, 2015). The major chlorination process outside cells appears to result from the production of reactive (free) Cl (e.g. hypochlorous acid, HOCl), which causes the unspecific chlorination of various organic compounds (Hoekstra, 1999; van Pée & Unversucht, 2003). It has been speculated that this unspecific extracellular function may play a role in microbial antagonism (Bengtson et al., 2009) or represent a way to handle oxygen stress (Bengtson et al., 2013).

The specific chlorination inside cells occurs via strictly regulated enzymatic processes and results in specific chlorinated compounds. Chlorination within cells is known to be mediated by enzymes such as FADH₂-dependent halogenases and perhydrolases (van Pée, 2001, 2012). Inside microbial cells, chlorination is speculated to have a detoxification function or to act as a chemical defence against other organisms by producing substances such as antibiotics, hormones, and pheromones (Hoekstra, 1999; Apel & Hirt, 2004).

In addition to biotic processes, there is also support for the formation of halogenated compounds by abiotic processes (Keppler et al., 2000; Fahimi et al., 2003). Most of the reported abiotic reaction schemes in terrestrial environments are linked to iron and organic matter (Schöler & Keppler, 2003). Recently, it has been demonstrated that chlorinated trichloromethane can be produced in the presence of Cl⁻, hydrogen peroxide, and Fe³⁺ by means of a Fenton-like reaction. This abiotic reaction occurs only under acidic conditions, trichloromethane not being detectable at a pH >3.7 (Huber et al., 2009).

2.3.2 Degradation of organic chlorine

Most research into Cl_{org} degradation has been conducted in relation to organochlorine pollution and bioremediation (e.g. van Pée & Unversucht, 2003) with a view to remediating soil and water contaminated with Cl_{org} from an anthropogenic source. Studies mainly report the degradation of specific environmental contaminants, such as chlorobenzenes, trichloroethylene, and dioxins (Abramowicz, 1990; Lorenz, 2000; Bunge et al., 2003; Pant & Pant, 2010). A huge number of bacterial and fungal genera possess the ability to degrade Cl_{org} compounds of various sizes under either oxic or anoxic conditions (Field & Sierra-Alvarez, 2004). For specific Cl_{org} species, microbial degradation processes are usually dominant over abiotic processes, provided that the soil habitat supports microbial growth and activity (Violante et al., 2002). Biodegradation is based on either growth or co-metabolism, i.e. the transformation of a substance without nutritional benefit in the presence of a growth substrate (Fritsche & Hofrichter, 2000). Under aerobic and anaerobic conditions, the microbial community can use the Cl_{org} compound as an electron donor and carbon source or degrade it co-metabolically while growing on another substrate. Degradation under anaerobic conditions can also occur by using Cl_{org} as the electron acceptor (halorespiration) (McCarty, 1997). Many genera of halorespiring microorganisms partially dechlorinate chlorinated ethenes (Field & Sierra-Alvarez, 2004). After 16 days, 100% of added tetrachloromethane was degraded under anoxic conditions in living soil, whereas only 20% was degraded in sterile soil (Borch et al., 2003). The degradation in sterile soil suggests abiotic processes, which include hydrolysis, mineral surface reactions, photolysis, and oxidation by molecular oxygen (Bailey et al., 2002). Hence, despite considerable research into the degradation processes of specific Cl_{org} species, as yet no studies report estimated dechlorination rates for bulk soil.

2.3.3. Environmental factors influencing transformation of organic chlorine

Knowledge of the environmental factors that might regulate chlorination and dechlorination is sparse. A few studies have indicated a decrease in the amount of Cl_{org} when nitrogen fertilizer is added to soil, though it is not known whether chlorination is hampered or dechlorination is enhanced (Öberg et al., 1996a; Johansson et al., 2001). Some studies have observed correlation between concentrations of Cl^- , Cl_{org} , organic carbon, and pH (Öberg et al., 1996b; Johansson et al., 2003a, 2003b). In these studies, the correlation with environmental variables is connected to the measured amount of Cl^- and Cl_{org} and not to the transformation rates. However, results obtained in recent decades indicate that transformation rates depend on various environmental factors, such as organic matter content, temperature, moisture, light, redox conditions, Cl^- concentration, pH, seasonal variations, and nutrient availability (Öberg & Bastviken, 2012). It is unclear how these environmental factors reported in various studies affect the transformation rates of Cl_{org} in soil.

3. Methods

The studies reported in three of the papers, i.e. I, III, and IV, are based on laboratory soil experiments using ^{36}Cl as a radioactive tracer; the remaining paper, II, is based on an experimental forest ecosystem field study.

3.1 The laboratory soil studies (Papers I, III, and IV)

In Papers I, III, and IV, a ^{36}Cl radiotracer method (Bastviken et al., 2007) was used to study the distribution and cycling of Cl in soils. In general, $^{36}\text{Cl}^-$ solution is added to soil incubated in the laboratory and $^{36}\text{Cl}^-$ and $^{36}\text{Cl}_{\text{org}}$ levels are measured over time. The ^{36}Cl radioactive tracer method is often seen as robust and sensitive. Mass balance calculations in the experiments indicated that $98 \pm 2\%$ of the initially added $^{36}\text{Cl}^-$ was found in the analyses. Paper I focuses on chlorination rates in three soil types (i.e. forest, pasture, and agricultural) and the study examines how soil characteristics affect chlorination rates. Paper III focuses on chlorination and dechlorination rates in a coniferous forest soil influenced by different environmental conditions (i.e. addition of glucose/maltose, ammonium nitrate, and extra water). Paper IV focuses on the influence of plants (wheat) on Cl cycling (i.e. chlorination rates and Cl partitioning between Cl^- and Cl_{org} pools in the bulk soil, root zone, and aboveground parts) in agricultural soil from France. The experiments reported in Papers I, III, and IV differ in the soil type and soil sampling procedure used, but a similar experimental setup was used and the same laboratory analyses were conducted.

3.1.1 Site descriptions and sampling procedures

In Paper I, soil samples were collected from four coniferous forests, four pastures, and three agricultural fields in Sweden. The forested sites

differed in terms of, for example, tree age, canopy cover, and soil texture (i.e. proportion of various soil particle sizes). The age of the trees growing on the forest sites ranged from 25 to 50 years, while the canopy cover ranged from 50 to 80%. Pasture is here defined as land that is grazed yearly, not regularly ploughed, and may contain trees but whose main purpose is not commercial timber production. The studied pastures had been grazed for 40 to over 100 years. The canopy cover of the pastures ranged from 5 to 20%. The soil samples from the three agricultural fields were taken from the Lanna experimental farm in Västergötland, Sweden. The agricultural soils were all sampled in the same experimental area and they represent one common agricultural soil type; however, the specific fields were chosen to have different previous cropping systems or agricultural practices. All soils were sampled from the topsoil layer, 5–15 cm below the soil surface.

In Paper III, soil was sampled at Stubbetorp in south-east Sweden. The sampled area was earlier covered by coniferous forest dominated by Scots pine (*Pinus sylvestris*) and Norwegian spruce (*Picea abies*), which were felled in 2010 leaving only seed trees. Soil samples were taken from five spots within a 15-m diameter circle, from the humus layer (5–15 cm below the soil surface). The samples were pooled and mixed to form a composite sample. The soil profile was of the podsol type and the soil samples consisted of organic soil from lower parts of the humus layer, leached soil, and mineral soil.

In Paper IV, the soil used was sampled at Osne-le-Val in Eastern France. Soil samples were taken in an agricultural field with brown calcareous soil, 0–20 cm below the soil surface, from five spots and were pooled to form a composite sample.

3.1.2 Experimental setups

Experimental setup in Paper I. The soils were sieved through a 2-mm mesh, distributed in 50-mL plastic centrifugation tubes (Sarstedt, Germany), and incubated at 20°C with the addition of $^{36}\text{Cl}^-$. Briefly, 2 g of fresh soil was transferred to each tube (three replicates for each soil). For coniferous and pasture soils, diluted $^{36}\text{Cl}^-$ solutions were added to each test tube. After adding $^{36}\text{Cl}^-$ solution, the samples were dried at room temperature until they reached the original weight. The samples were then incubated in a dark room and air was pumped through the closed system. The water content and air flow were monitored weekly. On each sampling occasion, three replicate tubes per treatment were removed from the experimental setup and immediately frozen until further analysis. The coniferous forest and pasture soils were sampled on five occasions on days 0–138 and the agricultural soil was sampled on six occasions on days 0–169.

Experimental setup in Paper III. The overall strategy was to add $^{36}\text{Cl}^-$ to the experimental soil, allow some time for chlorination (e.g. the formation of $^{36}\text{Cl}_{\text{org}}$ yielding an estimate of chlorination rates), then wash away the remaining $^{36}\text{Cl}^-$, and subsequently follow the decay of the $^{36}\text{Cl}_{\text{org}}$. To accomplish this, the soil was sieved through a 4-mm mesh and then divided into two parts (i.e. experiments A and B). Experiment A was run using the original soil while the soil was washed before the start of experiment B. This prewashing was carried out by extraction with water three times to remove the Cl^- and then once with a soil extract (made from a separate portion of the same soil) to restore the ion balances. The soil in experiment B was then dried at room temperature (20°C, the temperature at which the entire experiment was performed) to the original weight before the experiment started. The experiment started with the distribution of approximately 2 g of soil into 50-mL clear plastic centrifugation tubes (Sarstedt, Germany) for both experiments A and B. A solution containing $^{36}\text{Cl}^-$ and glucose/maltose was added to the tubes to favour chlorination, because pilot studies

indicated that the addition of easily degradable sugars (glucose and maltose) increases the chlorination rates. After 15 days of incubation, the samples were exposed to four different experimental treatments: [1] a control treatment at room temperature, as in all the following treatments, but with no additions; [2] addition of 1 ml NH_4NO_3 , a concentration of 0.09 M representing levels of common forest fertilization in Sweden (150 kg ha^{-1}); [3] new addition of 1 mL of glucose/maltose solution with a concentration of 0.13 M; and [4] addition of water (water content 71% of total mass). As in Paper I, the samples were then placed in a dark room and the water content and air flow were monitored weekly. On each sampling occasion, five samples per treatment were removed from the experimental setup and immediately frozen until further analysis. The tubes with soil were sampled on nine occasions on days 0–433.

Experimental setup in Paper IV. The soil was dried at 30°C and milled (because it was clayey) before being distributed in 50-mL plastic centrifugation tubes (Sarstedt, Germany). Approximately 5 g of soil with a water content of 29% of fresh mass was placed in each tube. In half of the tubes, five wheat seeds were planted in each tube and then covered with soil from the tube; $^{36}\text{Cl}^-$ solution was added to both the soil and the soil with seeds. After adding $^{36}\text{Cl}^-$ solution, the samples were put in a climate room at a temperature of 20°C and humidity of 70%. On each sampling occasion, 20 tubes per treatment (with and without plants) were removed from the experimental setup. Bulk soil, roots (with rhizosphere soil), and the upper parts of the plant were then separated. Furthermore, four samples were pooled together as one replicate to obtain enough biomass for analysis, giving five replicates per treatment. These were weighted and immediately frozen until further analysis. The soil and plants were sampled on five occasions, i.e. days 0, 10, 25, and 50.

Analysing soil and biomass can sometimes be problematic because of the heterogeneous matrix of the samples. The current experimental design was devised to capture a wide range of variability. Composite soil samples were used and three to five replicates were prepared for each sampling occasion. Although the variation in results is sometimes large, several conclusions can be drawn.

3.1.3 Determination of soil characteristics

Soil characteristics were determined for all soils used in the experiments presented in Papers I, III, and IV. Subsamples of the original soil were collected prior to the experiment and used to determine soil water content (by drying at 105°C for 31 h), soil organic matter, pH, total Cl_{org} , and extractable Cl^- . Soil organic matter content was determined by loss of ignition (LOI) at 550°C for 8 h, assuming that the carbon content equalled 50% of LOI. pH was measured in extracts of water and 1.0 M KCl according to ISO 10390:1994. Any Cl^- present in the samples was extracted using the same procedure as used for $^{36}\text{Cl}^-$ (described below), except that the last two extractions were conducted with 0.01 M KNO_3 instead of KCl. The extracts were frozen and, after thawing, were analysed for chloride concentrations by means of ion chromatography with chemical suppression (MIC-2, Metrohm) according to ISO 10304-1:1992. The residual soil was dried and milled, and 0.02 g was incinerated and analysed to determine total organic halogen (TOX) content according to Asplund et al. (1994) using an ECS3000 analyzer (Euroglas).

3.1.4 ^{36}Cl extractions and analyses

Four subsequent extractions, two with water and two with KCl (0.01 M), were used to remove $^{36}\text{Cl}^-$ from the samples. To ensure the release of intracellular $^{36}\text{Cl}^-$, the samples were frozen (24 h, -18°C), dried, and sonicated (45 seconds, 50% intensity) in a Sonorex RK510H ultrasonic

bath (Bandelin, Berlin, Germany). The amount of ^{36}Cl bound to organic matter in the extracts ($^{36}\text{Cl}_{\text{orgex}}$) was determined to ascertain its abundance relative to the $^{36}\text{Cl}_{\text{org}}$ in the residual soil after the extractions. The samples were treated according to the procedure for analysing AOX (Asplund et al., 1994), which is described in detail in Papers I, III, and IV. After that, the samples were combusted; the gas was then trapped in 0.1 M NaOH (Laniewski et al., 1999) and later analysed by liquid scintillation counting (LSC). In this procedure, the gas stream is led through two scintillation vials in series, each holding 10 mL of 0.1 M NaOH, recovering >98% of the ^{36}Cl present in the sample prior to combustion (Bastviken et al., 2007). The amount of inorganic ^{36}Cl in the extracts was determined by analysing filtrates after removing $^{36}\text{Cl}_{\text{orgex}}$, as described in Paper I. Ten-mL aliquots of filtrate were transferred to scintillation vials for LSC; after scintillation counting, the amount of $^{36}\text{Cl}^-$ was calculated taking account of all dilutions and the original soil dry mass.

The dried residual soil from the ^{36}Cl -amended treatments, remaining after soil extractions, was milled and approximately 0.2 g of soil was combusted to determine the amount of $^{36}\text{Cl}_{\text{org}}$, as was done for $^{36}\text{Cl}_{\text{orgex}}$. Previous tests have confirmed that the Cl associated with the residual soil and detected this way is organically bound and associated with humic and fulvic acids (Bastviken et al., 2007).

Finally, the radioactive samples were to be analyzed and the solutions containing trapped ^{36}Cl (NaOH solutions for $^{36}\text{Cl}_{\text{org}}$ and $^{36}\text{Cl}_{\text{orgex}}$, and water solution for $^{36}\text{Cl}^-$) were analyzed for ^{36}Cl by LSC (Beckman LX 6300). The analysis was corrected for quench using standard quench curves prepared from solutions with the same matrix composition as the samples (e.g. 0.1 M NaOH). Before analyzing the samples, a scintillation cocktail (Ultima Gold XR, Chemical Instruments AB) was added to all ^{36}Cl samples and to blank controls (Milli-Q water and scintillation cocktail). All radioactive measurements were corrected for

background radiation by subtracting the radioactivity in the blank controls.

3.1.5 Chlorination and dechlorination rates

The amount of $^{36}\text{Cl}_{\text{org}}$ in soil was plotted over time for each experiment. The specific chlorination rate determined in the experiments presented in Papers I, II, and IV is the fraction of added isotope that became organically bound every day (d^{-1}). This was determined by the slope of the regression line for the time in days (x -axis) versus the fraction of added $^{36}\text{Cl}^{-}$ recovered as $^{36}\text{Cl}_{\text{org}}$ (y -axis). The average chlorination rates expressed as $\mu\text{g Cl g}^{-1}$ dry mass soil d^{-1} were calculated by multiplying the specific rates (d^{-1}) by the total Cl^{-} content of the soil.

The chlorination rates reported in Paper IV were calculated as the fraction of $^{36}\text{Cl}^{-}$ transformed to $^{36}\text{Cl}_{\text{org}}$ between sampling days, i.e. between days 0 and 10, 10 and 25, and 25 and 50. That amount was divided by the amount of available $^{36}\text{Cl}^{-}$ in the soil, which meant that less and less $^{36}\text{Cl}^{-}$ was available in the soil-plant system as the plants grew. That was done because $^{36}\text{Cl}^{-}$ taken up by plants can no longer become $^{36}\text{Cl}_{\text{org}}$ in soil.

To distinguish between the chlorination and dechlorination rates and to estimate their specific rates, we performed modelling based on the assumption of simultaneous chlorination and dechlorination rates, both following first-order kinetics and being substrate concentration dependent. Under this assumption, a constant steady-state level of Cl_{org} would be expected when equilibrium between the chlorination and dechlorination rates is reached. The calculations were also based on the assumption that there is no $^{36}\text{Cl}_{\text{org}}$ at the beginning of the experiment (i.e. negligible background ^{36}Cl levels before adding $^{36}\text{Cl}^{-}$), meaning that the chlorination rate determined early in the experiment represents the gross chlorination rate. Given these assumptions, the model could be

used to calculate the specific dechlorination or chlorination rates by fitting the Cl_{org} over time to experimental measurements (described in more detail in Paper III).

3.1.6 Statistical analyses (Papers I, III, and IV)

In Paper I, comparisons between chlorine-to-carbon ratios and chlorination rates and among the three soil types, i.e. forest, pasture, and agricultural soils, were made using the Kruskal-Wallis test. The relationship between different environmental factors and chlorination rates were examined using Pearson's correlation coefficients.

In Papers III and IV, differences between net $^{36}\text{Cl}_{\text{org}}$ concentration, chlorination and dechlorination rates, and treatments were examined using analysis of variance (ANOVA), and post hoc testing with pairwise comparison was performed using Tukey's test. P values less than 5% were regarded as statistically significant.

3.2 The forest ecosystem study (Paper II)

The Breuil experimental forest site in France was the study object in Paper II. The site is part of the SOERE F-OreT network, which performs long-term studies of French forests in order to study forest dynamics and nutrient flows. The chosen site at Breuil-Chenue has been studied since 1976. The study reported in Paper II is an experimental field study based on sampling conducted during the years 2001–2006. The samples from the experimental site were analysed for Cl^- and Cl_{org} concentrations in different compartments of a forest ecosystem including both soil and vegetation. From these analyses, budget and residence time calculations were conducted for a better understanding of Cl cycling.

3.2.1 Site description and sampling

The experimental site is located at Breuil-Chenue (Nièvre-Morvan, in eastern France). Over the 2002–2008 period, the mean annual rainfall and temperature were 1145 mm and 9°C, respectively. The major soil type at the experimental site was an acid brown soil (pH 3.8–4.8; Bonneau et al., 1977). The native forest was dominated by European beech (*Fagus sylvatica* L.) and oak (*Quercus sessiliflora* Smith), but was cleared in 1976 and replaced with six single-tree-species plantations. Of these, we selected five forest stands: Douglas fir (*Pseudotsuga menziesii* Franco), Norway spruce (*Picea abies* Karsten), Black pine (*Pinus nigra* Arn ssp. *laricio* Poiret var. *Corsicana*), European beech (*Fagus sylvatica* L.) and oak (*Quercus sessiliflora* Smith) and their respective soil plots for the study.

Ten trees were harvested in each stand in 2001 to collect branches, stemwood, and stembark samples. Samples from standing trees were collected between 2001 and 2006 and consisted of foliage collected during the maximum growing season, litterfall collected from five litter traps per stand, and litterfall of wood. All these samples were collected to estimate the mean annual Cl^- and Cl_{org} concentrations. Under each forest stand, eight replicates of bulk humus and three soil profiles were sampled in May 2006. After sampling, the samples were dried, milled, stored in the dark, and later sent to the laboratory in Linköping. Throughfall and stemflow were collected every month. The tree biomass was evaluated according to procedures described by Ranger et al. (1995) and Saint-André et al. (2005). Briefly, the circumference of all trees was measured and branches, stemwood, and stembark were sampled from ten trees. Tree biomass (i.e. branches, stemwood, and stembark) was quantified per hectare by applying fitted equations to the stand inventory. The uncertainty of tree biomass values was 3–10% (Sicard et al., 2005).

3.2.2 Total Cl and Cl_{org} analyses

The amount of total Cl and the Cl_{org} content of the soil and tree compartments were determined by analysing total halogens (TX) and total organic halogens (TOX), respectively, by adding sieved and milled soil and tree samples to a small crucible followed by combustion using an ECS3000 analyzer (Euroglas) (Asplund et al., 1994). Cl⁻ was calculated by subtracting values of Cl_{org} from the total Cl. The mineral Cl content in mineral soil samples was analysed by TX analysis after pre-combustion of organic matter at 500°C for 4 h and washing to remove non-mineral Cl⁻. The mineral Cl was subtracted when calculating TX and TOX for mineral soil samples.

In terrestrial soil samples, the total and organic Cl concentrations are usually much higher than the bromine and iodine concentrations, and can therefore be approximated by the TX and TOX concentrations. This assumption was confirmed by comparing TOX and neutron-activation methods using forest soil samples selected from a variety of forests throughout France. The results of Cl_{org} measurements made using the TOX and neutron-activation methods differed by only 4–14% (Redon et al., unpublished).

3.2.3 Calculations of Cl ecosystem fluxes

Calculations for the forest ecosystem in Breuil were made in order to create a Cl budget for the site. Equations and explanations of the calculations are presented in detail in the Supporting Information appended to Paper II. Briefly, the annual amount of Cl incorporated into biomass, annual amount of Cl returned to the soil by litterfall, annual amount of Cl⁻ that leaches out from the tree canopy, annual amount of Cl that trees return to the soil by litterfall and leaching, annual amount of Cl that trees take up, net Cl_{org} accumulation rate in humus, possible net contribution from Cl_{org} in litter, and residence times of Cl⁻ and Cl_{org}

in humus and in trees were calculated both from measured data and from assumptions based on the literature.

3.2.4 Statistical analyses (Paper II)

In Paper II, Cl^- and Cl_{org} concentrations in different soil layers were compared between tree species, with Cl_{org} normalized to carbon in soil, using ANOVA and Tukey's test as a post hoc test. P values less than 5% were regarded as statistically significant.

4. Results

This chapter presents the results of Papers I–IV.

4.1 Chlorination in different soil types (Paper I)

The objective of the study presented in Paper I was to study organic matter chlorination rates in soils from eleven locations distributed among coniferous forests, pastures, and agricultural fields and to investigate whether environmental factors such as soil organic matter, pH, total Cl_{org} , and extractable Cl^- affect chlorination rates. The results indicate that chlorination occurred in all studied soil types. The highest mean specific chlorination rate was found in the coniferous forest soils (0.001 d^{-1}), which was two to three times higher than in pasture soils (0.0005 d^{-1}) and agricultural soils (0.0004 d^{-1}). The same pattern was observed in absolute chlorination rates. The highest chlorination rate was detected in one of the forest soils ($90 \text{ ng Cl g}^{-1} \text{ dry mass d}^{-1}$). The average chlorination rate in forest soil was $50 \text{ ng Cl g}^{-1} \text{ dry mass d}^{-1}$, which was significantly higher than the average rate in pasture soil ($4 \text{ ng Cl g}^{-1} \text{ dry mass d}^{-1}$) or agricultural soil ($3 \text{ ng Cl g}^{-1} \text{ dry mass d}^{-1}$). The amount of initially added $^{36}\text{Cl}^-$ transformed to $^{36}\text{Cl}_{\text{org}}$ by the end of the experiment was 14–25% in the forest soil, considerably higher than in pasture and agricultural soils where 3–7% of $^{36}\text{Cl}^-$ was transformed.

The results further indicated that the specific chlorination rates were significantly correlated with different environmental variables, i.e. Cl^- concentration, LOI, TOX, pH, and water content. The Cl^- concentrations were 3–10 times higher in the forest soils than in the agricultural and pasture soils. That difference could not be explained by Cl^- deposition or by weathering. Cl^- deposition differed only two-fold between the eleven sites (and therefore cannot explain a 3–10-fold difference) and the bedrock at the forest and pasture sites is dominated by acidic slow-weathering minerals, which contribute to low

concentrations of Cl^- from bedrock. This indicates that the Cl^- concentrations of the forest soils were not directly influenced by Cl^- deposition. As such, higher Cl^- concentrations in forest soils result from high rates of turnover between Cl^- and Cl_{org} . Not only the chlorination rate but also the mineralization rate (release of Cl^-) is higher in forest soils than in the other soil types. A large pool of Cl_{org} in combination with a high mineralization rate results in an increased concentration of Cl^- in the soil-water. Much of the Cl^- then becomes Cl_{org} and is not leached from the system. This process could explain the relationship between organic matter, Cl^- , and Cl_{org} .

4.2 Tree species affect Cl cycling in soil (Paper II)

The objective of Paper II was to investigate how tree species influence overall terrestrial Cl cycling by studying the balance between Cl^- and Cl_{org} in the soil and in different tree species planted on the same soil. The results indicated that almost 30 years after the reforestation was initiated, Cl concentrations in both tree tissue and humus layer were significantly higher in the Norway spruce plots than in the other tree plots. Average Cl^- and Cl_{org} concentrations in the humus layer were 1–7 and 1–9 times higher, respectively, in experimental tree plots with coniferous trees than in plots with deciduous trees. Therefore, dominant tree species did influence ecosystem levels of both Cl^- and Cl_{org} in the humus layer. However, levels in the mineral soil were similar across all plots.

Concentrations of Cl^- and Cl_{org} varied greatly among plant parts (e.g. leaves, branches, and stems) and tree species in the forest ecosystem. In general, fresh leaves and needles contained higher concentrations of Cl than did the other parts of the tree (e.g. bark, branches, and wood). For example, fresh leaves contained 15–20 times more Cl than did wood in all tree species. There is also large variation between the humus layer

and the mineral soil layers. The humus layer contained more Cl per dry mass than did the mineral soil. Most of the Cl in the humus layer comprised Cl_{org} irrespective of the tree species. In general, Norway spruce plots had the highest levels of Cl⁻ and Cl_{org} of all the plots, followed by Douglas fir, which had higher levels than the pine, beech, and oak plots. This was true for most of the studied ecosystem components in the plots. For example, when comparing the mass of Cl per hectare in the biomass and in the humus layer, the Norway spruce plots exhibited 10-fold and four-fold higher Cl⁻ and Cl_{org} storage in the biomass, respectively, and seven-fold and nine-fold higher storage of Cl⁻ and Cl_{org} in the humus layer, respectively, than did oak plots. Ecosystem Cl fluxes and residence times were estimated to illustrate the overall Cl cycling (Table 1).

Table 1. Mean residence time in experimental plots with different tree species; Cl⁻ and Cl_{org} denote chloride and organic chlorine, respectively.

	Oak	European beech	Black pine	Douglas fir	Norway spruce
Residence time of Cl⁻ (tree) (yr)	6.2 (3.6–8.7)	1.0 (0.58–5.9)	0.8 (0.5–17)	0.6 (0.3–0.9)	3.0 (1.3–8.6)
Residence time of Cl_{org} (tree) (yr)	3.3 (2.2–5.8)	15 (8.8–41)	13 (8.9–26)	32 (22–54)	19 (11–35)
Residence time of Cl⁻ (humus) (yr)	0.1 (0.1–0.2)	0.2 (0.1–0.2)	0.2 (0.1–0.3)	0.3 (0.2–0.5)	0.9 (0.5–1.3)
Residence time of Cl_{org} (humus) (yr)	11 (8.2–15)	30 (22–45)	65 (46–111)	52 (44–63)	45 (34–68)

The results indicate that there was generally more extensive Cl⁻ uptake by trees and higher storage of Cl_{org} in the humus layer. Longer Cl residence times were found in coniferous trees than in deciduous trees.

4.3 Chlorination and dechlorination in forest soil (Paper III)

The objective of the study presented in Paper III was to estimate chlorination and dechlorination rates and to elucidate the potential

effects of environmental factors such as water, nitrogen, and labile organic matter. The results indicate that chlorination occurred at the beginning of the experiment, 15% of the added $^{36}\text{Cl}^-$ being transformed to $^{36}\text{Cl}_{\text{org}}$ during the first 15 days. After addition of glucose/maltose, ammonium nitrate, and water, approximately 20–30% of the added $^{36}\text{Cl}^-$ was turned to $^{36}\text{Cl}_{\text{org}}$ within 35 days, depending on the treatment. In general, the net change in $^{36}\text{Cl}_{\text{org}}$ was lower in the NH_4NO_3 treatment than in the other treatments, suggesting that addition of ammonium nitrate had a hampering effect on chlorination rates. No clear differences were observed in the net amount of $^{36}\text{Cl}_{\text{org}}$ between the control, water, and glucose/maltose treatments.

The significant decrease in the amount of $^{36}\text{Cl}_{\text{org}}$ between days 365 and 433 indicated that dechlorination occurs in this forest soil. Addition of glucose/maltose, water, and ammonium nitrate had no strong direct effects on modelled specific dechlorination rates. The observed specific chlorination rates were lower than the estimated specific dechlorination rates. This implies that the amount of Cl^- should exceed the levels of Cl_{org} if all Cl_{org} was easily dechlorinated.

4.4 Influence of vegetation on chlorination rates in soil (Paper IV)

The objective of the study presented in Paper IV was to investigate how Cl^- and Cl_{org} are distributed and cycle in a soil–plant system. The results indicate that the treatment with plants showed a rapid and high plant uptake of Cl^- compared to the soil without plants. The results from the ^{36}Cl radiotracer analyses show that most of the $^{36}\text{Cl}^-$ initially added to the soil with plants were taken up by the roots as soon as the seeds started to germinate. With time, as the plants grew, increasing amounts of $^{36}\text{Cl}^-$ were found in the green plant biomass. After 50–days of incubation, $75 \pm 12\%$ of the initially added amount of $^{36}\text{Cl}^-$ could be

detected in the green parts of the plant. The amount of $^{36}\text{Cl}_{\text{org}}$ in the plant was generally very low. The amount of $^{36}\text{Cl}_{\text{org}}$ in the roots was 2% on day 10, increasing to 11% by day 25 and decreasing to 2% by day 50, the pattern being the same for $^{36}\text{Cl}_{\text{orgex}}$. The distribution of $^{36}\text{Cl}^-$ and $^{36}\text{Cl}_{\text{org}}$ in the soil and plant is illustrated in (Figure 2).

Figure 2. Overview of the distribution of radioactive chloride ($^{36}\text{Cl}^-$), radioactive chlorinated organic chlorine ($^{36}\text{Cl}_{\text{org}}$), and dissolved radioactive organic chlorine from extracts ($^{36}\text{Cl}_{\text{orgex}}$) in the plants on days 10, 25, and 50 of the experiment; shown as per cent of initially added amount of $^{36}\text{Cl}^-$. The presented values originate from four samples pooled to form one replicate; five such replicates are then used to calculate the means and standard deviations ($n = 4 \times 5$).

Furthermore, the results indicate that chlorination occurred in the agricultural soil without plants. After 50 days of incubation a net change

in the amount of $^{36}\text{Cl}_{\text{org}}$ was observed and 6% of the initially added $^{36}\text{Cl}^-$ had been transformed to Cl_{org} . The rest of the added $^{36}\text{Cl}^-$ was found in the soil solution. The rapid plant uptake of the added $^{36}\text{Cl}^-$ led to lower amount of $^{36}\text{Cl}^-$ being available for chlorination in the soil. The soil–plant system had a 10-fold higher specific chlorination rate by day 10–25 compared to soils without plants, and the rates increased after day 10. The highest chlorination activity was found in the root zone. The increased $^{36}\text{Cl}_{\text{org}}$ formation after day 10 in the treatment also indicates that roots may influence specific chlorination rates in soil. In conclusion, the root zone seems to be the most active site for formation of Cl_{org} in soils and Cl^- is rapidly taken up by the plants at higher concentrations than those needed for growth.

5. Discussion

The overall objective of this thesis is to increase knowledge of the distribution and cycling of Cl^- and Cl_{org} in terrestrial environments. The results of Papers I–IV are discussed below.

5.1 Distribution and fluxes of Cl^- and Cl_{org} in trees and soil

The results indicate large variations in the distribution of Cl^- and Cl_{org} among different soil types, with higher concentrations of both Cl^- and Cl_{org} in forest soils than in soils from pasture and agricultural fields (Paper I). This is in line with previous results of a study that measured 51 soil samples from forest, pasture, and agricultural fields in France (Redon et al., 2013). As demonstrated in Paper II, concentrations differ greatly between soil depths, with the highest concentrations of Cl^- and Cl_{org} found in the humus layer (Paper II; see also Redon et al., 2011). The composition and properties of plant litter are essential for formation and degradation of soil organic matter in terrestrial ecosystems (Kögel-Knabner, 2002). The cycling of Cl^- and Cl_{org} in the humus layer was clearly affected by the tree species planted in different plots (Paper II). Higher Cl^- and Cl_{org} concentrations were found in humus layers under tree species with the highest concentrations of Cl in their litterfall (i.e. Norway spruce and Douglas fir). Cl concentrations in both tree tissue and the humus layer were higher in Norway spruce stands than in the other investigated tree plots. In general, fresh leaves and needles contain mainly Cl^- , though the ratio between Cl^- and Cl_{org} decreases in litterfall leaves and even more in litterfall branches.

The higher amount of total Cl and Cl_{org} and faster chlorination rate in coniferous forest reported in Paper I are consistent with the results in Paper II, which indicate that forest ecosystems with coniferous trees are likely to accumulate higher amounts of both Cl^- and Cl_{org} than are

deciduous forest ecosystems. These results explain the patterns observed in several previous studies finding high Cl_{org} concentrations in forest soils (Johansson et al., 2003a; Redon et al., 2013). Furthermore, the results in Papers I and II indicate that Cl_{org} levels are not directly affected by climate or deposition. The mechanisms underlying the observed higher total Cl and Cl_{org} levels are not clear, but it is speculated that trees themselves may contribute to cycling and retention through the internal production of Cl_{org} in biomass or by differential Cl uptake. Another possible explanation is that trees can have a more indirect effect on Cl cycling by means of tree-related soil microbial communities that can chlorinate organic matter or influence the soil organic matter content. The rhizosphere microbial community structure has been demonstrated to vary depending on tree species (Lohmus et al., 2006). The conclusion from the results of Papers I and II is that tree species and their associated microbial communities seem to be the most important factors determining Cl^- and Cl_{org} levels in soils.

5.2 Chlorination and dechlorination: the influence of environmental factors

Research conducted in recent decades has found that the transformation rates of Cl depend on various environmental factors, such as organic matter content, temperature, moisture, light, redox conditions, Cl^- concentration, pH, seasonal variations, and nutrient availability (Öberg & Bastviken, 2012). The results of Paper I indicate that chlorination occurs in various soil types but that the rates differ among forest, agricultural, and pasture soils. The highest specific chlorination rates were found in forest soil, while rates in pasture and agricultural soils were significantly lower. The rates in forest soil range from 0.0004 to 0.004 d^{-1} (Bastviken et al., 2009; Rohlénova et al., 2009; Paper I). In comparison, the specific chlorination rates reported in Paper III varied between 0.0005 and 0.01 d^{-1} and were higher than those reported in

Paper I. In this case, the faster rate and larger net change of $^{36}\text{Cl}_{\text{org}}$ reported in Paper III could be explained by the addition of easily degradable carbon at the beginning of the experiment. Results of a pilot study indicated an increase in the chlorination rate when glucose and maltose were added to the soil. Considerable variability in Cl_{org} transformation rates is expected because the chlorination of organic material seems to be mainly biotic (Bastviken et al., 2009; Rohlénova et al., 2009) and the rate of CPO-driven chlorination is dependent on intertwined environmental factors (Manoj, 2006). The difference in rates could also be explained by different soil characteristics or diverse soil microbial communities in the forest soils.

The chlorination rates reported in Paper I were significantly correlated with all of the studied environmental variables, i.e. organic matter content, moisture, pH, and Cl^- concentration. This is in line with previous findings, which indicate that organic matter (Johansson et al., 2003; Redon et al., 2011) and Cl^- are related to the formation of Cl_{org} (Johansson et al., 2003; Matucha et al., 2007). A hypothesis is that a sufficient amount of organic material is a key requirement for chlorination (Paper I). Bacteria and fungi use extracellular enzymes to break down organic matter and these play a central role in the degradation of litter and soil organic matter. If Cl_{org} is produced when microbes break down organic matter using extracellular enzymes, then factors that support microbial decomposition and growth contribute to faster chlorination rates. The quality of carbon is particularly important for the decomposition of organic matter because it constrains the supply of energy for microbial growth and enzyme production (Fontaine et al., 2003). Even though microbes can break down ancient carbon, fresh carbon is essential to sustain the long-term activity of decomposer populations (Fontaine et al., 2007). As demonstrated by Veres et al. (2015), extracellular enzyme activities are significantly related to easily degradable carbon but not to total soil organic carbon, suggesting that enzymes respond to pools that are more immediately degradable and

unprocessed. The production of enzymes has also been demonstrated to be sensitive to pH and other soil properties (Sinsabaugh et al., 2008). However, a source of fresh carbon input to soil seems to be an important requirement for enzyme activities. These factors create possibilities for the chlorination of organic material.

The observed correlation between chlorination rates and organic matter in Paper I might be an indirect effect of tree species and their associated microbial communities. The results of Paper II indicated that the major differences in humus-layer Cl_{org} levels between the different tree species plots remained after normalizing the data against organic carbon. In addition, the results of Paper III indicate that there are at least two pools of Cl_{org} in soil. Dechlorination rates seem to be higher than chlorination rates, but more than 50% of the total Cl in forest soils is still Cl_{org} . The experiments therefore indicate that all Cl_{org} prone to dechlorination is rapidly dechlorinated, but that some Cl_{org} must also be resistant to dechlorination and gradually accumulate to explain the observed high levels in soils. Accordingly, the steady-state partitioning between $^{36}\text{Cl}^-$ and $^{36}\text{Cl}_{\text{org}}$ reported in Paper III was approximately 60% ($^{36}\text{Cl}^-$) versus 40% ($^{36}\text{Cl}_{\text{org}}$) in the experiment in which the $^{36}\text{Cl}_{\text{org}}$ was recently produced. In the sampled soil, this partitioning was 14% versus 86% for the Cl^- and Cl_{org} . Furthermore, unpublished data from a pilot study support the indication that some Cl_{org} is resistant to degradation and gradually accumulates. It was found that the organic matter Cl:C ratio increased 100-fold from fresh plant tissue to deep soils, indicating that some Cl_{org} is more refractory than average organic matter. This is supported by observations of increased concentrations of Cl, bromine, and iodine with depth in humic acids (Pereira et al., 2011).

Another environmental factor possibly influencing the transformation of Cl is nitrogen. Previous studies have found that nitrogen fertilization either hampers chlorination or enhances dechlorination (Öberg et al., 1996a; Johansson et al., 2001). In Paper III, specific chlorination rates

were found to be hampered by the addition of ammonium nitrate but were otherwise similar among the treatments. Similar indications have been observed in field studies in which the net formation of Cl_{org} during the decomposition of spruce needles was hampered by nitrogen fertilization (Öberg et al., 1996a). In previous studies, soil organic matter had a lower degree of chlorination in soil from fertilized plots than in soil from control plots (Svensson et al., 2013), and ammonium nitrate caused a decrease in the concentration of soil Cl_{org} (Johansson et al., 2001). A possible explanation for the small amount of incorporated $^{36}\text{Cl}_{\text{org}}$ found in nitrogen treatments is that the fungal community is inhibited by the addition of ammonium nitrate. Nitrogen fertilization has been found to reduce the taxon richness of decomposer fungi in soils (Allison et al., 2007; Treseder, 2008). Several microcosm and field studies have demonstrated that excess nitrogen has a negative influence on the ectomycorrhizal fungal biomass (e.g. Högberg et al., 2011; Wallander and Nylund, 1992).

Interestingly, tree species seems to be the most important factor explaining C:N ratios in European forest soils (Cools et al., 2014). It is suggested that microbial decomposition of organic matter is influenced by tree species primarily via differences in litter lignin and nitrogen content (Vesterdal et al., 2008, 2012). If so, this might explain the observed high amount of Cl_{org} in coniferous soil (Papers I and II). Generally, evergreen tree species have higher C:N ratios than do deciduous trees (Cools et al., 2014). In humus under coniferous tree species, there is less nitrogen per carbon where the highest amount of Cl_{org} is found. This is also consistent with results of Paper III indicating decreased chlorination rates in the presence of ammonium nitrate.

The results of Paper III indicate that chlorination and dechlorination occurred simultaneously under all tested environmental conditions, i.e. additional water, labile organic matter, and ammonium nitrate. Dechlorination is well known for specific chemicals (e.g. Wiegert et al.,

2013) but not for bulk soil. Soil processes including dechlorination have been suggested to explain Cl imbalances observed in catchment studies (Kopáček et al., 2014; Svensson et al., 2012). The results of Paper III further indicate that additional sugars, water, and ammonium nitrate had no strong direct effects on specific dechlorination rates. If this is valid, absolute dechlorination rates may depend primarily on chlorination and Cl_{org} levels. This would be compatible with previous discussions of the possibility that the net mineralization of Cl_{org} was greater in plots fertilized with nitrogen than in control plots in a forest (Öberg et al., 1996a), as inhibition of chlorination by nitrogen fertilization would lead to a net Cl_{org} loss until a new equilibrium between chlorination and dechlorination is established.

5.3 Influence of vegetation on chlorination in soil

The uptake of Cl^- by plants has frequently been assumed to be passive (with Cl^- following the water flow), resulting in toxic effects in leaves under high or moderate salt stress (Moya et al., 2003; Storey & Walker, 1999). However, citrus plants reportedly actively take up Cl^- and accumulate Cl in leaf tissues to levels that exceed the critical content (Brumós et al., 2010). Another study demonstrates that Cl^- concentrations 500 times higher than those needed for growth regulate leaf size and water relationships in tobacco plants (Franco-Navarro et al., 2015). An extensive “luxury” uptake of Cl^- was also observed in Douglas fir in Paper II. If Cl^- accumulation to macronutrient concentrations is driven by active uptake, it is feasible to think that Cl^- plays a broad and poorly understood biological role in plants, as active uptake requires considerable energy (Brumós et al., 2010). A previous study hypothesizes that biological functions regulated by Cl^- availability probably influence adaptive mechanisms that regulate water homeostasis and the ability of plants to withstand water deficit (Franco-

Navarro et al., 2015). All of this indicates that Cl^- might have a more active role than previously thought.

The results of Paper IV showed that the soil–plant systems had a 10-fold higher specific chlorination rate by day 10–25 than in soils without plants. Increased Cl_{org} formation as well as high chlorination activity was found in the root zone and this indicate that roots may influence specific chlorination rates in soil. Plant roots can stimulate microorganisms in the rhizosphere by supporting a favourable microenvironment and by root exudates (Dundek et al., 2011) which supplies soil microorganisms with labile organic carbon (Cheng et al., 2014). The root zone seems to be an active site for the formation of Cl_{org} in soils. Cl^- is rapidly taken up by the plants at higher concentrations than those needed for growth and the reason for this additional uptake is unknown. The results of Papers II and IV indicate that tree species and one common agricultural crop, wheat, have a high uptake of Cl^- . This affects the chlorination rates, as less Cl^- is available for possible chlorination of the soil. An extensive Cl^- uptake by vegetation might induce Cl^- deficiency for the microbial communities when plant growth is high. A way for the microorganisms to solve this problem could be to use extracellular enzymes to release Cl^- stored in Cl_{org} molecules. A similar hypothesis has been discussed by Johansson et al. (2003a). They found that plant and microbe induced dechlorination to access Cl^- . This might explain decreased Cl_{org} levels in soil during the most extensive vegetation growth period in the summer. As plant uptake of Cl^- affect the turnover rates of both Cl^- and Cl_{org} in soil, plants can therefore affect the residence times of ^{36}Cl in a soil–plant system.

6. Conclusions and implications

This thesis has increased knowledge of the distribution and cycling of Cl^- and Cl_{org} in terrestrial environments. Papers I–IV demonstrate highly active Cl cycling in soils, where Cl^- and Cl_{org} levels result from a dynamic equilibrium between chlorination and dechlorination.

The first conclusion is that there are higher amounts of Cl^- and Cl_{org} in coniferous forest soils than in pasture and agricultural soils. Tree species is the most important factor regulating Cl^- and Cl_{org} levels, whereas geographical location, atmospheric deposition, and soil type are less important (Papers I and II). The correlation between chlorination rates and soil organic matter (Paper I) seems to be an indirect effect of tree species (Paper II).

The second conclusion is that chlorination rates are higher in coniferous forest soil than in pasture and agricultural soils (Papers I and II). Moreover, dechlorination occurs in forest soil within the same range of rates as chlorination (Paper III), with the root zone apparently being an active site of chlorination (Paper IV). In addition, there seem to be at least two major Cl_{org} pools, one being dechlorinated easily and one being dechlorinated much more slowly (Paper III).

The third conclusion is that lower chlorination rates are expected in soils higher in nitrogen. Dechlorination rates, on the other hand, seem not to be affected by nitrogen (Paper III).

The implications of these conclusions are that the influence of tree species and the rapid cycling of some Cl pools, while other pools have very long residence times, are clearly critical variables to consider in all studies of Cl in terrestrial environments. This information can be used to understand Cl in risk-assessment modelling when developing a model including both $^{36}\text{Cl}^-$ and $^{36}\text{Cl}_{\text{org}}$, to investigate the transport and

distribution of ^{36}Cl from a possible accident. $^{36}\text{Cl}_{\text{org}}$ residence times will be six times longer in the humus layer of a coniferous forest soil than in a deciduous forest soil. Plants in agricultural fields will probably take up a large part of $^{36}\text{Cl}^-$ depending on the plant type and soil conditions. Plant uptake of Cl^- may reduce the total amount of $^{36}\text{Cl}^-$ turned to $^{36}\text{Cl}_{\text{org}}$, which otherwise could be retained in the soil. This means that most of the $^{36}\text{Cl}^-$ will follow the plant as it is harvested and enter the food chain or return to the soil if the plants are left on the field.

Future research should continue to investigate the influence of environmental factors, such as various nutrients and temperatures, on chlorination and dechlorination rates. The effects of vegetation could be further studied through lysimetric experiments adding isotopes to large pots of plants. Active microbial communities could be studied and extracellular enzymes measured to obtain a better understanding of how and when chlorination and dechlorination occur and under what conditions.

Acknowledgements

First, I would like to thank my supervisors David Bastviken and Teresia Svensson: David, for your good ideas, comments on the text, and the opportunity to conduct this thesis research; Teresia, for your generous support in not just research but also in everything associated with it.

I also wish to thank Andra and EDF for financing this project and my colleagues in France, Yves Thiry, Laura Marang, Beatrice Lourino-Cabana, and Francoise Siclet, for useful research input and for fun times when you showed me French sights and especially French food.

The research engineers at Tema M deserve my gratitude: the wise Lena Lundman, who taught me a lot about chemistry and life; “the Brain” Susanne Karlsson, who is always nice to talk to; “the Muscles” Mårten Dario, who is really good with details; as well as Ingrid Sundgren and Henrik Reyier, who were always helpful. Thank you Karin Hjerpe for being my mentor and for helping me with future plans.

I wish to thank all of my colleagues at Tema M. Thank you to all the past and present Ph.D. students whom I had the opportunity and privilege to know. I would especially like to thank Eva-Maria, Magali, Siva, Luka, Roberta, Fausto, Lotten, and Francesco: You, my friends, have made my days so much more fun – you are always welcome at my home anytime!

I would like to thank my family and friends, especially my mother Maria, father Magnus, brother Mattias, and the whole Montelius clan. A special thanks to my mother, who has listened to all my research related problems, and to Hanna who always been there. And finally thanks to the most important people in my life:

♡♡♡ Otto, Olle, and Clara ♡♡♡

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