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REGULAR ARTICLE

Radiotracer evidence that the rhizosphere is a hot-spot for chlorination of soil organic matter

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Abstract

Aims The ubiquitous and extensive natural chlorination of organic matter in soils, leading to levels of chlorinated soil organic matter that often exceed the levels of chloride, remains mysterious in terms of its causes and regulation. While the composition of plant species and the availability of labile organic matter was recently shown to be important, the physical localization of chlorination in soils remains unclear but is a key for understanding regulation and patterns observed. Here we assess the relative importance of organic matter chlorination in (a) bulk soil, (b) the plant roots plus the rhizosphere zone surrounding the roots, and (c) above-ground plant biomass, in an experimental plant-soil system.

Methods A radiotracer, ^{36}Cl, was added to study translocation and transformations of Cl^- and Cl_{org} in agricultural soil with and without wheat (*Triticum vulgare*) over 50 days.

Results The specific chlorination rates (the fraction of the added $^{36}Cl^-$ converted to $^{36}Cl_{org}$ per day) in soil with plants was much higher (0.02 d^{-1}) than without plants (0.0007 d^{-1}) at peak growth (day 25). The plant root and rhizosphere showed much higher formation of $^{36}Cl_{org}$ than the bulk soil, suggesting that the rhizosphere is a hotspot for chlorination in the soil. In addition, the treatment with plants displayed a rapid and high plant uptake of Cl^-.

Conclusions Our results indicate that the rhizosphere harbour the most extensive in-situ chlorination process in soil and that root-soil interaction may be key for terrestrial chlorine cycling.

Keywords Organic chlorine · Chloride · Wheat · Chlorination · ^{36}Cl

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Introduction

During the past decades it became evident that there is ubiquitous and extensive natural chlorination of organic matter in terrestrial ecosystems. The levels of chlorinated soil organic matter (Cl_{org}) typically are as large or exceed the levels of chloride (Cl^-) in most soils (Bastviken et al. 2013, Johansson et al. 2003a, b, Redon et al. 2013, Svensson et al. 2007). Experiments with radioactive Cl (^{36}Cl) as tracer have confirmed

natural chlorination rates corresponding to as much as 50–300% of the annual wet deposition of Cl in several types of soils (Bastviken et al. 2007). Substantial chlorination of organic matter occurs in all types of soils from agricultural soils to forest soils (Gustavsson et al. 2012; Redon et al. 2013), and large spatial variability has been observed (Johansson et al. 2003a, b). A large diversity or organisms harbour enzymatic capacity for chlorination and several hypotheses regarding the reasons for and regulation of the extensive natural chlorination have been proposed, but their verification is not yet conclusive (Bengtson et al. 2009; Bengtson et al. 2013; Atashgahi et al. 2018a).

Montelius et al. (2015) observed extensive accumulation of Cl_{org} in upper soil layers over 30 years in coniferous forests, while the accumulation was lower in deciduous forests. The difference in Cl_{org} accumulation among forest types led to the conclusion that chlorination could be directly linked to the type of forest vegetation. Leri and Myneni (2010) also suggested influence of plants on soil Cl_{org} via contributions of plant litter Cl_{org}. The reasons for the plant effects observed on soil organic matter chlorination are yet unknown but plant communities can substantially influence residence times and soil pools of total Cl and Cl_{org} (Montelius et al. 2015).

The plant root-soil interface, the rhizosphere – a thin zone around the roots, is dynamic and harbours numerous biogeochemical processes that are important for terrestrial cycling of carbon and other elements. The rhizosphere is a competitive environment where various microorganisms and plant roots compete for resources such as nutrients, space, and water (Narula et al. 2009). Through root exudation of various chemical compounds, roots may regulate the nearby soil microbial community, be active in plant defence, attract beneficial or symbiotic microorganisms, change the chemical and physical properties of the soil, or inhibit the growth of competing plant species (Philippot et al. 2013). Hence, the chemical diversity around the plant roots is important for the understanding of biogeochemical processes of the soil. The majority of the studies on soil Cl cycling have hitherto focused on bulk soil in which plant roots were removed by sieving the soil, despite the knowledge that vegetation and vegetation-associated organisms have a strong influence on elemental turnover (Clemmensen et al. 2013, Pausch and Kuzyakov 2018). In a recent experimental study, increased availability of labile organic matter clearly increased the soil Cl_{org} formation rates (Svensson et al. 2017). Hence, increased chlorination rates could be expected where more labile organic matter is present.

Hence, root exudates providing labile organic matter, in combination with plant-specific microbial interactions in the rhizosphere may be important for chlorination of soil organic matter. Given the prior findings outlined above, we hypothesize that plant activity stimulates whole system formation of Cl_{org}, and that a significant part of the soil chlorine cycling can be driven by processes in narrow zones around roots, that was previously unexplored in terms chlorination activity. A laboratory radiotracer experiment was therefore performed to examine the transport and transformations of Cl^- and Cl_{org} in agricultural soil with and without wheat (*Triticum vulgare*) serving as a model plant relevant for common agricultural plots.

Methods

Soil sampling

The soil was collected at Osne-le-Val (latitude 48° 30′0′′ N, longitude 5° 11′0′′E) in eastern France in January 2014. Soil samples were collected from five points 10 m apart in field of 1–2 ha, 0–20 cm below the surface in an agricultural field with brown calcareous soil. The soil type is a Rendzic Leptosol, typical of that region, developed directly on top of the Tithonian limestone (FAO 2016). After sampling, the soil was pooled to form a composite sample, transported in polyethylene plastic bags to the laboratory, and then refrigerated at 4 °C for 3 months until used in the experiment.

Determination of soil characteristics

The soil was rich in limestone. All visible stones were removed before analyses and the soil homogenized. A sub-sample of soil was used to determine soil water content (by drying at 105 °C for 24 h), soil organic matter, pH, extractable Cl^-, total organic halogens (TOX), and total organic carbon (TOC) (five replicates). Soil organic matter content was determined by loss of ignition (LOI) at 550 °C for 2 h, assuming carbon content as 50% of LOI (Pribyl 2010). pH was measured in water extracts (water/soil ratio of 1:5) according to the ISO 10390 standard (1994). Cl^- in the samples was extracted by four repeated extractions according to the procedure described for $^{36}Cl^-$ (see below), except the

last two extractions that were conducted with 0.01 M KNO_3 instead of KCl. The extracts were frozen at −20 °C for approximately 3 months awaiting analysis and, after thawing, the samples were analysed for Cl^- concentrations using ion chromatography with chemical suppression (MIC-2 modular anion system; Metrohm) according to the ISO 10304-1 standard (EU 1996). The residual soil was dried and milled, and 0.02 g was incinerated and analysed to determine the total organic halogen (TOX) content according to Asplund et al. (1994) by using an ECS3000 analyser (Euroglas). TOC in soil extracts was determined using a TOC-V_{CSH} analyser (Shimadzu 5000 TOC Analyzer).

Experimental setup

The soil was dried in dark at 30 °C and milled before 4.9–5.2 g of soil (dry mass; original water content 29% of fresh mass) was distributed in each 50-mL clear plastic centrifugation tubes (Sarstedt, Germany; in total 240 tubes were prepared, exact mass in each tube was noted). In each tube for the treatment with plants, five seeds of wheat (*Triticum vulgare*; total mass of 0.1 g) were planted in the soil, and 1.8 mL of deionized water (Milli-Q) together with 0.2 mL of diluted $^{36}Cl^-$ (LEA CERA; specific activity 1.3 MBq μg Cl^{-1}) was added (Fig. 1). For the treatment without plants (control), the

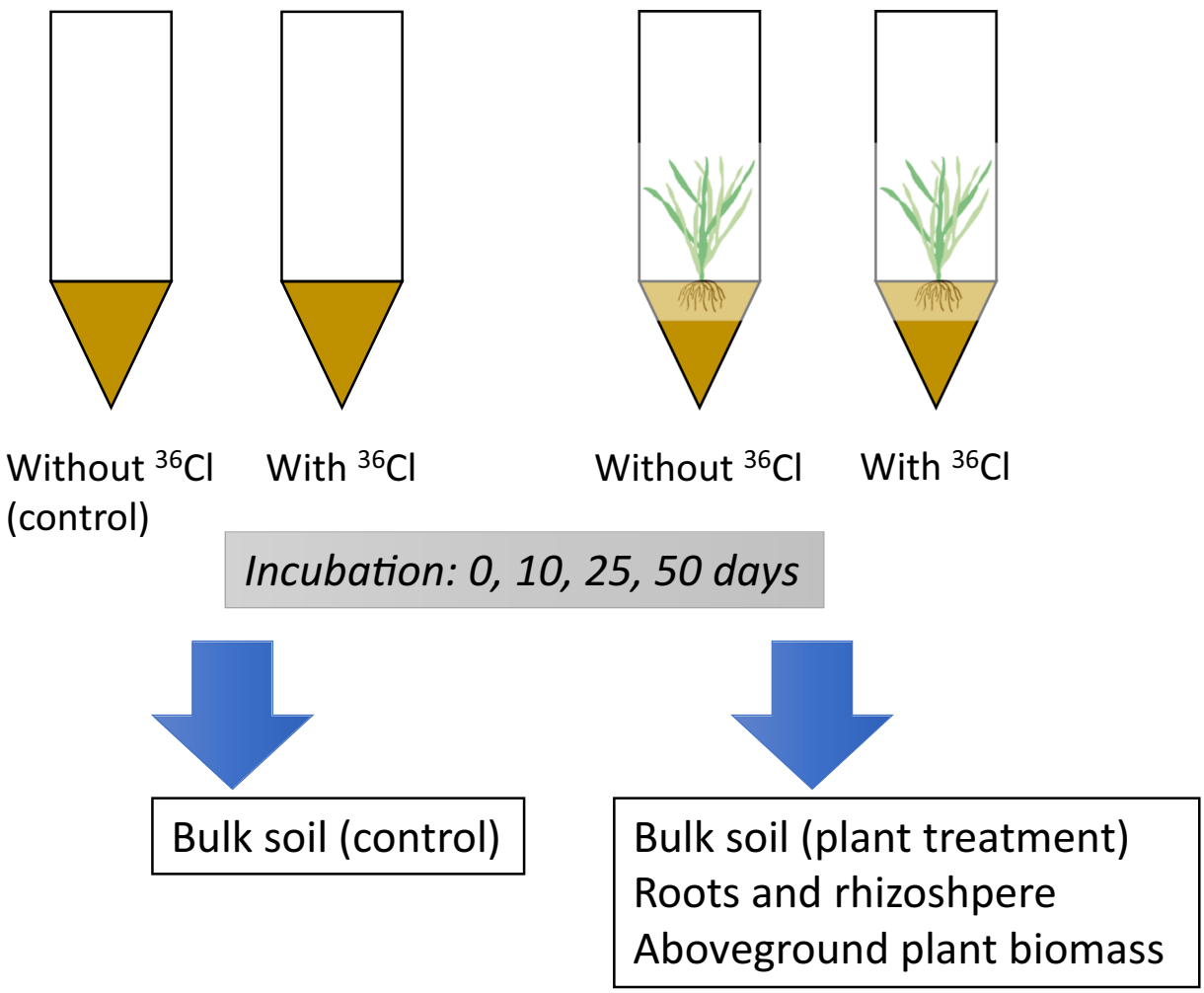


Fig. 1 Soil was distributed in tubes and in each tube for the treatment with plants, five seeds of wheat (*Triticum vulgare*) were planted in the soil and 1.8 mL of deionized water and 0.2 mL of diluted $^{36}Cl^-$ (LEA CERA, specific activity 1.3 MBq μg Cl^{-1}) was added to each tube. For the treatment without plant (control), the same amount of $^{36}Cl^-$ isotope solution was added to the soil, but without the seeds. The samples were incubated at a temperature of 20 °C and humidity of 70%. During each sampling occasion (0, 10, 25 and 50 days), 20 tubes per treatment (with and without plants) were removed from the experimental setup and four tubes were pooled to form a composite replicate, yielding five composite replicates per sample category, treatment, and sampling occasion

same amount of $^{36}Cl^-$ isotope solution was added to the soil, but without the seeds. The isotope addition resulted in a final $^{36}Cl^-$ concentration corresponding to 225,800 disintegrations per minute (DPM; 1 Bq = 60 DPM). The solution added a mass of 3 ng $^{36}Cl^-$ per test tube in all treatments. The total volume of added $^{36}Cl^-$ solution and water (2.0 mL) was determined by testing how much water was needed to wet all the soil for easy mixing of the isotope, while also producing a suitable water content for seed growth. After addition of the $^{36}Cl^-$ solution, the samples were put in a climate room at a temperature of 20 °C and humidity of 70%. During each sampling occasion, 20 tubes per treatment (plant treatment and control, respectively) were removed from the experimental setup. To obtain enough plant biomass material for analysis, four tubes were pooled to form a composite replicate. The content of tubes with plants was divided into three categories: (a) bulk soil, (b) roots including tightly attached rhizosphere soil, and (c) above-ground biomass. This yielded five composite replicates (each representing material from four tubes) per sample category, treatment, and sampling occasion. The above-ground plant biomass, roots and rhizosphere, and bulk soil in the pant treatment, and bulk soil in control tubes without plants, were sampled on four occasions, i.e., days 0, 10, 25, and 50 after experiment start. The samples were weighted and immediately frozen until further analysis right after the experiment.

Extraction of $^{36}Cl^-$ in the soil

The $^{36}Cl^-$ in the soil samples was recovered by a series of four extractions, two with water and two with KCl (0.01 M; Bastviken et al. 2009). To facilitate the release of intracellular $^{36}Cl^-$, the samples were frozen (24 h, −18 °C), dried, and after rewetting sonicated (45 s, 50% intensity; Sonorex Super RK 510 H ultrasonic rod; Bandelin, Germany) (Bastviken et al. 2007). The extraction procedure was performed as follows: After freezing, the samples were thawed at room temperature for 2 h, after which 20 mL of deionized water (Milli-Q) was added to each tube. The tubes were agitated on an end-over-end shaker for 30 min and then centrifuged at 6000 g for 10 min (Biofuge Primo centrifuge; Thermo Scientific, USA). The supernatant (extract no. 1) was transferred by pipette to new centrifuge tubes. The soil was then dried at 60 °C for 24 h, milled, rewetted with 5 mL of water, and sonicated. Subsequently, 15 mL of water was added followed by shaking, centrifugation, and supernatant removal as above, yielding extract no. 2. With the addition of 20 mL of 0.01 M KCl, the shaking, centrifugation, and supernatant removal procedure was repeated twice more, producing extracts no. 3 and 4. The extracts were finally frozen, and the residual soil was dried at 60 °C for 24 h and stored until further analysis.

Analysis of ^{36}Cl in soil extracts

The amounts of ^{36}Cl bound to organic matter in the soil extracts ($^{36}Cl_{org\text{-}ex}$) were determined in selected samples according to procedures described by Bastviken et al. (2007) for comparison with that of $^{36}Cl_{org}$ in the residual soil solid phase ($^{36}Cl_{org\text{-}s}$) after the extractions. Before the analyses, 1 mL of acidified nitrate solution (0.2 M KNO_3, 0.02 M HNO_3) and 0.2 mL concentrated HNO_3 (68% *w*/w; yielding a pH <2) were added to 10 mL of each extract. The mixture was shaken with 50 mg of activated carbon for 60 min and filtered through a 0.45-μm polycarbonate filter (Millipore, USA). The filter with the activated carbon and adsorbed $^{36}Cl_{org\text{-}ex}$ was rinsed with acidic nitrate solution (6 × 3 mL, 0.01 KNO_3, 0.001 M HNO_3) and then with acidified deionized water (6 × 3 mL, pH 2 by acidification with HNO_3) to remove any remaining Cl^-. The filter with the adsorbed $^{36}Cl_{orgex}$ was combusted at 1000 °C under a stream of O_2 gas according to the procedure for analysing adsorbable organic halogens (AOX; e.g. Asplund et al. 1994). The $H^{36}Cl$ gas formed during combustion was then trapped in 0.1 M NaOH (Laniewski et al. 1999). This procedure, leading the gas stream through two scintillation vials in series, each holding 10 mL of 0.1 M NaOH, yielded a recovery of >98% of the ^{36}Cl present in the sample before combustion (Bastviken et al. 2007). The trapped ^{36}Cl, corresponding to $^{36}Cl_{org\text{-}ex}$, was determined by liquid scintillation counting (LSC; see below). The amount of $^{36}Cl^-$ in each extract was determined by analysing filtrates after removal of $^{36}Cl_{org\text{-}ex}$, as described above. Aliquots of 10 mL were transferred to scintillation vials for LSC.

Organic ^{36}Cl in the soil

The dried residual soil (after the four extractions described above) from the ^{36}Cl-amended treatments was milled and approximately 0.02 g of soil was combusted to determine the amount of organically soil solid-phase bound ^{36}Cl ($^{36}Cl_{org\text{-}s}$), as described above for the determination of $^{36}Cl_{org\text{-}ex}$ and in e.g. Bastviken et al. (2007)

Previous tests have confirmed that ^{36}Cl associated with the residual soil and detected this way was organically bound and associated with humic and fulvic acid organic matter fractions (Bastviken et al. 2007). The total organically bound ^{36}Cl ($^{36}Cl_{org}$) hereafter equals the solid-phase $^{36}Cl_{org\text{-}s}$) plus the water extractable $^{36}Cl_{org\text{-}ex}$.

Analyses of plants and roots/rhizosphere

The amounts of $^{36}Cl_{org\text{-}s}$, $^{36}Cl_{org\text{-}ex}$, and $^{36}Cl^-$ in dried and milled above-ground plant parts and root/rhizosphere samples were determined by the same principles as described above for the bulk soil samples. $^{36}Cl_{org\text{-}s}$ was measured after washing $^{36}Cl^-$ from the material according to the procedure used for $^{36}Cl_{org\text{-}ex}$, without the addition of activated carbon (also described for total organic halogens (TOX) in Asplund et al. 1994). $^{36}Cl_{org\text{-}s}$ was measured by combustion, after washing away $^{36}Cl^-$ and $^{36}Cl_{org\text{-}ex}$ from the material by filtration as the activated carbon was washed in the procedure used for $^{36}Cl_{org\text{-}ex}$ (see also Asplund et al. 1994 for details). $^{36}Cl_{org\text{-}ex}$ was analysed according to above. $^{36}Cl_{org\text{-}ex}$ and $^{36}Cl_{org\text{-}s}$ was summed to estimated solid-phase bound ^{36}Cl.

Levels of total stable (i.e., non-radioactive) Cl^- and Cl_{org} were analysed in plant, root and rhizosphere, and extracted soil samples at day 0 and day 50 by determining total halogens (TX) and total organic halogens (TOX) according to Asplund et al. (1994), but using the extraction procedure described above for the samples with added ^{36}Cl.

Liquid scintillation counting (LSC)

The solutions containing trapped ^{36}Cl (NaOH solutions for $^{36}Cl_{org}$ and $^{36}Cl_{orgex}$, and water solution for the $^{36}Cl^-$) were analysed for ^{36}Cl by means of LSC (LX 6300 analyser; Beckman Coulter, USA). The analysis was corrected for quenching using standard quench curves prepared from solutions with the same matrix composition as the samples (e.g., 0.1 M NaOH or water). Before analysing the samples, scintillation cocktail (Ultima Gold XR; Chemical Instruments AB, Sweden) was added to all ^{36}Cl samples and also to blank controls (deionized water and scintillation cocktail). All radioactive measurements were corrected for background radiation by subtracting the radioactivity in the blank controls.

Chlorination rates

The amount of $^{36}Cl_{org}$ was plotted over time, and the specific chlorination rate is expressed as the fraction of the standing stock $^{36}Cl^-$ that became organically bound per day (d^{-1}). This rate was determined by the slope of the least squares regression line for the time in days (*x*-axis) versus the fraction of added $^{36}Cl^-$ (adjusted for the remaining $^{36}Cl^-$ after above-ground plant uptake) recovered as $^{36}Cl_{org}$ (*y*-axis) between days 0–10, 10–25, and 25–50, respectively. The average soil chlorination rates expressed as μg Cl g dry mass $soil^{-1}$ d^{-1} were calculated by multiplying the specific rates (d^{-1}) by the total content of Cl^- in the soil. The rates for days 0–10 largely reflect gross chlorination rates, as there was no $^{36}Cl_{org}$ before the experiment started, while the rate during the later period, day 10–50, reflects net chlorination in a situation in which both chlorination and dechlorination may occur simultaneously (Montelius et al. 2016).

To enable comparison between chlorination in soil and in plants (which have large density differences making units per mass less relevant) we compared total accumulation of $^{36}Cl_{org}$ as well as the formation of $^{36}Cl_{org}$ per carbon (C) content in the three pools. Plant carbon content was assumed to be 50% of the dry mass (Houghton et al. 2009; Pribyl 2010). Root and rhizosphere carbon content was estimated by assuming the root mass was similar to above-ground biomass (Houghton et al. 2009) and the remaining mass represented rhizosphere soil.

Results

The soil characteristics (water content, organic matter content, pH, C:N ratio, and TOC in soil extracts) are shown in Table 1. The mean germination of the seeds in tubes was 92 ± 12%. The green biomass in the soil–plant system was visible above-ground on day 5 and biomass increased throughout the experiment (Table 2); the plants reached a height of approximately 14 cm on day 50. The root biomass increased until day 25 and then decreased until the experiment terminated on day 50 (Table 2). The decrease in root biomass probably reflects the experimental setup, as the space in the tubes for roots to expand was limited after day 25, and we hereafter regard the results from days 0–25 as more reliable than those from days 25–50 when space limitation in the experiment tubes may

Table 1 Average chloride (Cl^-) and chlorinated organic compound (Cl_{org}) concentrations in soil with and without plants, root and rhizosphere soil, and above-ground plant parts on different days, and initial soil characteristics; standard deviation ($n = 4 \times 5$) (see Methods section for description)

	Cl^- (μg g^{-1} dry mass)	Cl_{org} (μg g^{-1} dry mass)	LOI (% of dry mass)	Water content (fraction of fresh mass)	pH	TOC (mg L^{-1})	C:N (ratio)
Bulk soil (day 0)	8 ± 1	15 ± 2	7.7	0.29	8.2	27	14
Bulk soil (control) (day 50)	7 ± 1	15 ± 1					
Bulk soil (plant treatment) (day 50)	*9 ± 1*	*16 ± 2*					
Root and rhizosphere soil (day 50)	*ND*	*43 ± 22* [a]					
Above-ground biomass (day 50)	*1143 ± 155*[b]	*150 ± 82* [b]					

ND means "not detected". The italicized rows denote treatments with plants. Note that the seed Cl content corresponded to approximately 61% of the total Cl after addition of seeds to the tubes

[a] The dry mass represents a mixture of root biomass and rhizosphere soil

[b] The high numbers depend on the low dry mass of organic matter compared to dry soil. Compare with the total mass given in Table 2

have severely inhibited plant development which probably influenced all processes.

In the plant treatment, the original Cl content in soil was the same as in the controls, but the seeds added substantial amounts (seed Cl content corresponded to approximately 61% of the total Cl after addition of seeds to the tubes). In the plant treatment at the end of the experiment (day 50), 39% of the total Cl was found as Cl^- in green biomass and 5% as Cl_{org} in the green biomass. The root and rhizosphere contained 29% of the total Cl as Cl_{org}, while 27% of the total Cl remained in the bulk soil (two thirds of this was Cl_{org}).

Recovery of ^{36}Cl

The recovery of ^{36}Cl (average $^{36}Cl^- + {}^{36}Cl_{org}$) were 100% + 2%, 98% + 4%, 97% + 4% of the initial added amounts of $^{36}Cl^-$ in the control treatment, and 99% + 5%, 92% + 11%, and 88% + 6% in the plant treatment, on days 10, 25, and 50, respectively. Clearly, the high recovery in the plant treatment on Day 25 represents an outlier that can be explained by the combined uncertainties in both extraction, sample handling, analyses, and mass determinations of all samples. To ensure that we do not overestimate the impact of the root/rhizosphere zone processes when testing our hypothesis, we assigned all this uncertainty to the Cl_{org} results in this zone. We therefore reduced the measured Cl_{org} levels in the root and rhizosphere samples by 50% in all calculations and results reported (i.e. the root and rhizosphere Cl_{org} dpm values of each of the five replicates at day 25 was multiplied by 0.5), which if true would lead to the same total ^{36}Cl recovery levels as on Day 10 (this target level lead to the selecting of 50% reduction). Hence, our below

Table 2 Total mass of four pooled tube replicates (average ± SD, $n = 5$) mass (g dry mass) in bulk soil with and without plants, root and rhizosphere soil, and above-ground plant parts on different days (see Methods section for description)

	Day 0 (g dry mass)	Day 10 (g dry mass)	Day 25 (g dry mass)	Day 50 (g dry mass)
Control treatment (no plants)				
Bulk soil	20.0 ± 0.2	20.2 ± 0.2	20.1 ± 0.1	20.0 ± 0.1
Plant treatment				
Above-ground plant biomass		0.2 ± 0.02	0.3 ± 0.04	0.4 ± 0.05
Root and rhizosphere		5.8 ± 0.9	8.1 ± 1.9	7.9 ± 1.4
Bulk soil		15.2 ± 0.8	12.4 ± 1.9	12.8 ± 1.2

assessment of the relative importance of the root and rhizosphere zone $^{36}Cl_{org}$ formation is conservative and real rhizosphere chlorination may be up to twice our reported values. The recovery of less than 100% on day 50 can be due to the combined uncertainties but it is also consistent with the loss previously observed over long incubation times in similar tracer studies (Bastviken et al. 2009). It has been speculated that this could be due to the formation and evasion of volatile chlorinated compounds (Bastviken et al. 2009; Jiao et al. 2018; Forczek et al. 2015; Svenssson 2019).

Translocation of Cl

There was a rapid and high plant uptake of $^{36}Cl^-$. Most of the $^{36}Cl^-$ initially added to plant treatment was taken up by the roots as soon as the seeds started to germinate (Figs. 2 and 3a). With time, as the plants grew, increasing amounts of $^{36}Cl^-$ were found in the above-ground plant parts as a result of translocation from roots to the green biomass. After 50 days of incubation, 75 ± 12% of the initially added amount of $^{36}Cl^-$ could be detected in the green parts of the plant. The relative amount of $^{36}Cl_{org}$ in plant green biomass was low corresponding to ≤1% of the total ^{36}Cl over the whole treatment period.

Cl_{org} formation

The highest chlorination activity was occurring in the rhizosphere regardless of how the chlorination was expressed (e.g. per the whole tube, or per g C; Figs. 2, 3; Table 3), also after reducing the rhizosphere numbers by 50% (see above). The amount of $^{36}Cl_{org}$ in root and rhizosphere was 3.7% of the added ^{36}Cl on day 10, which increased to 9% by day 25 and was five times greater than in the bulk soil (Figs. 2 and 3b). The proportion of extractable Cl_{org} in root and rhizosphere (i.e. $^{36}Cl_{org\text{-}ex}$) were approximately 40% of all $^{36}Cl_{org}$, compared to a few percents in the bulk soil and above-ground plant biomass.

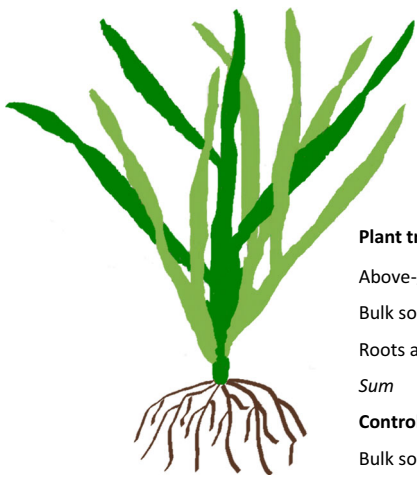
Chlorination rates

The specific chlorination rates below-ground (including bulk soil, roots and rhizosphere) were the highest in the plant treatment (Table 4). Between day 0 and day 10, the plant treatment had 3-fold higher specific chlorination rates, while on days 10–25 the plant treatment had 14-fold higher specific chlorination rate (average 0.01 d^{-1}) than the control soil (average 0.0007 d^{-1}) (Table 4). The absolute below-ground chlorination rates, at day 10–25, was 0.006 μg Cl g^{-1} soil dry mass d^{-1} and 0.09 μg Cl g^{-1} soil dry mass d^{-1} for the control and plant treatment, respectively.

Discussion

Rapid Cl^- uptake by plants

Our results show that $^{36}Cl^-$ levels were the highest in the root and rhizosphere on day 10 and in the above-ground biomass on day 50 (Fig. 2 and 3a), which is consistent



	Day 10		Day 25		Day 50	
	Cl^-	Cl_{org}	Cl^-	Cl_{org}	Cl^-	Cl_{org}
Plant treatment						
Above-ground plant biomass	33 ± 4.2	0.3 ± 0.1	62 ± 4.2	0.3 ± 0.2	75 ± 11.9	1.0 ± 0.3
Bulk soil (plant treatment)	16 ± 2.1	0.6 ± 0.2	7.5 ± 1.4	1.7 ± 1.5	8.2 ± 1.5	1.1 ± 0.8
Roots and Rhizosphere	50 ± 13.5	3.7 ± 1.8	23 ± 6.0	9.2 ± 2.5	4.3 ± 2.6	3.7 ± 2.1
Sum	*99 ± 11.2*	*5 ± 1.9*	*92 ± 3.7*	*11 ± 2.6*	*88 ± 10.1*	*5.8 ± 1.7*
Control without plants						
Bulk soil (control)	100 ± 2.0	2.4 ± 1.3	98 ± 0.4	3.5 ± 1.3	97 ± 2.3	5.9 ± 3.9

Fig. 2 Overview of the distribution of ^{36}Cl in different experimental compartments expressed as a percentage of the initial added amounts of $^{36}Cl^-$. Five composite replicates (each based on material from four test tubes) were used to calculate the mean and standard deviation ($n = 4 \times 5$); see text for details

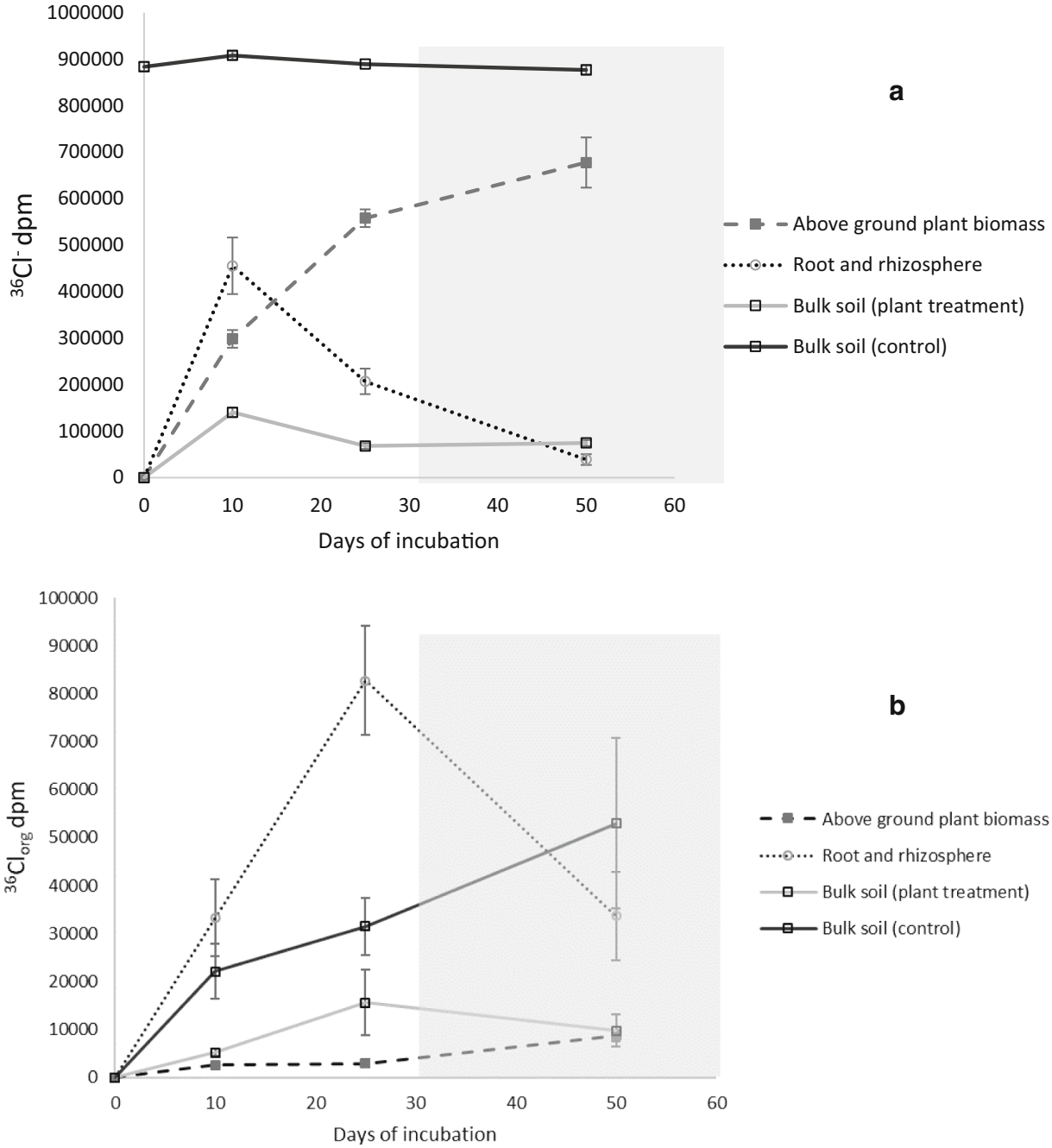


Fig. 3 The amount of $^{36}Cl^{-}$ (Panel **a**) and $^{36}Cl_{org}$ (Panel **b**) expressed as disintegrations per minutes (dpm) in each composite sample for four tubes in soil without plants (bulk soil), in bulk soil with plants, in the roots and rhizosphere, and in above-ground plant biomass and roots (n = 5 composite samples; mean ± 1SD). The shaded area denotes the time when space limitation in experiment tubes hampered development in plant

with previous observations that Cl^{-} moves through the root and into the xylem and further to the shoot, where it accumulates or is redistributed throughout the plant via the phloem (Atwell et al. 1999; MacAdam 2009).

Cl^{-} is essential for plants and has a direct role in photosynthesis, and important in osmotic adjustment of the plant and plays an essential role in stomatal regulation (White and Broadley 2001). Considering the relative high

Table 3 The amount of total $^{36}Cl_{org}$ expressed as disintegrations per minutes (dpm) per g carbon (C) in bulk soil (control and plant treatment, respectively), root and rhizosphere, and in above-ground plant biomass of four pooled tube replicates ($n = 5$; mean ± 1SD) (see Methods section for description)

	Day 10 (dpm per g C)	Day 25 (dpm per g C)	Day 50 (dpm per g C)
Control treatment (without plants)			
Bulk soil	22,276 ± 11,260	31,880 ± 11,868	53,363 ± 35,786
Treatment with plants			
Above-ground plant biomass	32,750 ± 7138	22,805 ± 12,869	13,202 ± 13,202
Root and rhizosphere	89,863 ± 32,360	160,052 ± 17,737	56,426 ± 23,193
Bulk soil	5166 ± 1612	15,820 ± 14,065	9869 ± 7004

observed stable Cl concentrations, the wheat plants did not seem to suffer from Cl deficiency. The minimum requirement for plant growth is 0.2–0.4 mg g^{-1} dry mass, which is on average 10–100 times the concentration of Cl in plant cell walls (Marschner 2012), and the above-ground biomass had 1.1 mg Cl^- g^{-1} dry mass at day 50 in the experiment (Table 1). The observed large uptake appears to be common among plants. Earlier studies indicate that Cl^- is rapidly taken up by plants at higher concentrations than those needed for growth (Hurtevent et al. 2013, White et al. 2001). Cl^- therefore tend to accumulate in plant tissue and the concentrations in fresh plant biomass is 1.5 to 305-fold higher than soil water concentrations for common agricultural plants (Kashparov et al. 2007a, b; Marschner 2012). Another study demonstrates that Cl^- concentrations 500 times higher than those needed for growth influence leaf size and water regulation in tobacco plants (Franco-Navarro et al. 2016). Montelius et al. (2016) found similar excess uptake in coniferous trees leading to extensive crown-leaching and throughfall of Cl^-. The reasons and mechanisms for this excess Cl^- uptake in plants are still unclear. It may be an indirect consequence of the water uptake or could indicate that Cl^- may play yet unknown roles in plant physiology.

Cl_{org} levels in plant biomass

The analyses of stable (i.e. non-radioactive) Cl, indicated that approximately 10% of the total Cl in green biomass was Cl_{org} (Table 1). The incorporation of $^{36}Cl_{org}$ in the green plant biomass in the experiment were generally low (≤1% of the total ^{36}Cl over the whole treatment period). Hence, the Cl_{org} levels of 10% of the stable Cl are rather high and indicate that transfer of stable Cl_{org} present in the original soil to the plant cannot be excluded. Although Cl^- dominates in plants, over 130 chlorinated organic compounds have been isolated from higher plants (Engvild 1986; Gribble 2010), but the information in literature on the function and relative abundance of Cl_{org} in plants are scattered (Bastviken et al. 2013). A Cl_{org}-containing plant growth hormone is produced in plants such as peas, lentils, vetch, and fava beans (Gribble 1998). The Japanese lily produces several Cl_{org} fungicides to protect itself from pathogenic fungi (Monde et al. 1999). In addition, it is well-known

Table 4 Average specific chlorination rates (d^{-1}) and chlorination rates (μg Cl g^{-1} dry mass d^{-1}) in Bulk soil and Rhizosphere combined, for control and plant treatment. The range presented correspond to ±1 SD for the data used in calculations. "Chl." denote chlorination

	Specific chl. rate (d^{-1}); of control	Specific chl. rate (d^{-1}); of plant treatment	Chl. rate (μg Cl g^{-1} dry mass d^{-1}) of control	Chl. rate (μg Cl g^{-1} dry mass d^{-1}) of plant treatment
Days 0–10	0.002 (0.001–0.004)	0.006 (0.004–0.009)	0.02 (0.01–0.03)	0.05 (0.03–0.07)
Days 10–25	0.0007 (0.00066–0.00075)	0.01 (0.089–0.014)	0.0056 (0.005–0.006)	0.09 (0.07–0.11)

that plants can emit volatile Cl_{org}, but the underlying mechanisms are unclear and both biotic and abiotic pathways have been suggested (Svenssson 2019). Flodin et al. (1997) observed Cl_{org} concentrations in meadow grass as 0.3% of total Cl. The percentage of Cl_{org} in the foliage of oak, European beech, black pine, Douglas fir, and Norway spruce was much higher (7–15%; Montelius et al. 2015), which is comparable to observations from the present work.

Higher Cl_{org} formation in soil with plants

After 25 days of incubation, 1.7% of the initially added $^{36}Cl^-$ had been transformed to $^{36}Cl_{org}$ in bulk soil of the plant treatment, which is slightly lower than the 3.5% in the control bulk soil (Figs. 2, 3b; Table 3). The large plant uptake of $^{36}Cl^-$ (see above) made less $^{36}Cl^-$ available for the soil microbial communities and for soil chlorination, which could explain the lower amount of $^{36}Cl_{org}$ in plant treatment bulk soil starting on day 10. The transformation to $^{36}Cl_{org}$ was higher for the rhizosphere, reaching 9.2% on day 25; five times higher than in the bulk soil. Considering all soil pools studied in each experiment tube, 11% of the added $^{36}Cl^-$ had been converted to $^{36}Cl_{org}$ in the plant treatment tubes (combining soil, root/rhizosphere, and plant biomass) on day 25, which is three times higher than in the control bulk soil. The observed chlorination of soil organic matter in the control treatment is in line with previous studies of agricultural and pasture soils observing that 3–7% of the total added $^{36}Cl^-$ had become $^{36}Cl_{org}$ after 50–80 days (also without plants) (Gustavsson et al. 2012; Lee et al. 2001).

Soil with plants clearly exhibited higher chlorination capacity than bulk soil without plants. The observed high below-ground $^{36}Cl_{org}$ formation in the plant treatment indicates that root and rhizosphere may influence specific chlorination rates in soil. Plant roots can stimulate microorganisms in the rhizosphere by creating a favourable microenvironment and by means of root exudates that supply labile organic carbon (Cheng et al. 2014; Dundek et al. 2011). The information regarding Cl in roots and rhizosphere around the roots are scarce. Van den Hoof and Thiry (2012) estimated the Cl_{org} pool in roots to be almost equal to the above-ground biomass pool in Scots pine (*Pinus sylvestris L.*).

The chlorination of soil organic matter is primarily believed to be driven by biotic processes, but also include abiotic processes (Atashgahi et al. 2018a, 2018b; Bastviken et al. 2009). The capability of chlorination among various groups of organisms are widespread including bacteria, fungi, and vascular plants is widespread (Clutterbuck et al. 1940; Hunter et al. 1987; de Jong and Field 1997; Öberg 2002; Bengtson et al. 2009; Bengtson et al. 2013). There are several proposed mechanisms behind the chlorination in soil, such as intracellular chlorination regulated by enzymatic processes and alternatively extracellular chlorination. The intracellular chlorination processes may be associated with metabolic by-products and act as detoxification agents or are believed to represent production of compounds serving as chemical defence (van Pée and Unversucht 2003). The extracellular chlorination is thought to be driven by formation of reactive Cl (e.g. hypochlorous acid, HOCl), from reactions between hydrogen peroxide and Cl^-, where the reactive Cl reacts with surrounding organic matter leading to an unspecific chlorination of various organic compounds in the large and complex pool of soil organic matter. The extracellular chlorination could benefit microbes in multiple ways, including cutting complex organic molecules to smaller pieces being more available as substrates (van Pée and Unversucht 2003), serving as a chemical defence (Bengtson et al. 2009) and to detoxify reactive oxygen species (Bengtson et al. 2013). In this study, we cannot elucidate whether the observed chlorination was due to biotic or abiotic processes, but the presence of plants significantly enhanced soil organic matter chlorination. We hypothesize this was because root exudates of labile organic matter stimulated microbial activity in ways the promoted chlorination, in accordance with Svensson et al. (2017).

The space limitation for roots was reached by day 25. As a result, there was a decrease in growth and signs of root deterioration was clearly visible. This coincided with the decrease in root and rhizosphere $^{36}Cl_{org}$ from day 25 to day 50. The amount of $^{36}Cl^-$ per carbon in roots and rhizosphere of the plants (Fig. 3) also decreased and the growth curves for roots showed a stagnation from days 25 to 50 (Table 2). The decrease in Cl^- between day 25 and 50 indicates that plant biomass Cl^- stocks can be rapidly exchanged with the environment.

Chlorination rates

The observed specific chlorination rate in the control treatment was higher than agriculture soils (sieved soil without plants) in Sweden of 0.0003–0.0006 d^{-1}, but in the range of previous estimates from other soils without plants by Gustavsson et al. (2012). Hence, there was a

large influence of growing plants on soil organic matter chlorination, leading to a dramatic increase in overall ecosystem specific chlorination, and a 3-fold increase in amounts Cl_{org} formed under the experimental conditions. Clearly, for assessing in-situ natural chlorination levels, the plant influence should not be ignored.

On days 25–50, the net chlorination rates in plant treatment bulk soil became negative, indicating that dechlorination processes were dominant, which was consistent with reduced plant activity (as described above). A situation in which dechlorination dominates over chlorination as detected from ^{36}Cl tracer, could have been caused by a combination of high uptake of $^{36}Cl^-$ by plants limiting the amount available for $^{36}Cl_{org}$ formation in the soil or root zone, while the Cl_{org} pool became large enough to sustain dechlorination. If so, the effect of emerging Cl^- limitation on chlorination would have been severe in the small experimental test tubes without continuous Cl^- input, but it is unclear how frequently this could happen in situ. Hence, the results from day 25–50 should not be extrapolated beyond this experiment. However, a recent study demonstrated that dechlorination rates can exceed chlorination rates in soil (Montelius et al. 2016), supporting the finding that net dechlorination periods may occur in natural environments.

Net changes of Cl^- and Cl_{org} in bulk soil

The stable Cl^- and Cl_{org} concentrations remained constant throughout the experiment (Table 1) in the bulk soil. This means that in spite of extensive and rapid Cl cycling revealed by the ^{36}Cl tracer, the net change of Cl^- and Cl_{org} in the bulk soil was small, which indicates simultaneous and largely balanced chlorination and dechlorination processes in line with previous investigations (Montelius et al. 2016). However, the portion of Cl_{org} in the rhizosphere of the plant treatment at day 50, was approximately twice as high as the Cl_{org} in the bulk soil, which coincide with the ^{36}Cl results.

In summary, the results of the study suggest that the root zone is the most active site for Cl_{org} formation in soils. Despite the fact that the current study is laboratory based and the results cannot directly be extrapolated or upscaled to field conditions, it is clear that extensive natural chlorination and dechlorination of organic matter in soil and Cl turnover is likely linked to common ecosystem processes and that plants and plant/root-associated organisms can have a major influence on these processes. Indeed, different Cl accumulation rates have been linked to forest types and chlorination rates were recently associated with the microbial activity (Montelius et al. 2015; Svensson et al. 2017). The results indicate that Cl^- is rapidly taken up by plants at higher concentrations than those needed for growth, though the reason for this additional uptake is unknown. These results are not only relevant to stable Cl dynamics but are also relevant to the behaviour of the long-lived radionuclide ^{36}Cl ($t_{1/2}$ = 3.01×10^5 years) present in the radioactive waste. ^{36}Cl has been identified as a radionuclide of interest that may enter the food chain (Kashparov et al. 2005; Sheppard et al. 1996). A better understanding of how Cl circulates in the terrestrial environment would be useful when making environmental risk assessment models, for example, when calculating residence times and human intake doses from crops when simulating the potential ^{36}Cl contamination in soils (Le Dizès and Gonze 2019). Our results imply that chlorination and dechlorination processes, as well as the presence and activity of microorganisms contributing to these processes, in the root zone need further attention. The spatial distribution of Cl transformation processes also needs to be considered in risk assessments and in other models in which Cl cycling is relevant.

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