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The production and degradation of trichloroacetic acid in soil: results from *in situ* soil column experiments

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Abstract

Previous work has indicated that the soil is important to understanding biogeochemical fluxes of trichloroacetic acid (TCA) in the rural environment, in forests in particular. Here, the hydrological and TCA fluxes through 22 *in situ* soil columns in a forest and moorland-covered catchment and an agricultural grassland field in Scotland were monitored every two weeks for several months either as controls or in TCA manipulation (artificial dosing) experiments. This was supplemented by laboratory experiments with radioactively-labelled TCA and with irradiated (sterilised) soil columns. Control *in situ* forest soil columns showed evidence of net export (i.e. *in situ* production) of TCA, consistent with a net soil TCA production inferred from forest-scale mass balance estimations. At the same time, there was also clear evidence of substantial *in situ* degradation within the soil (~70% on average) of applied TCA. The laboratory experiments showed that both the formation and degradation processes operate on time scales of up to a few days and appeared related more with biological rather than abiotic processes. Soil TCA activity was greater in more organic-rich soils, particularly within forests, and there was strong correlation between TCA and soil biomass carbon content. Overall it appears that TCA soil processes exemplify the substantial natural biogeochemical cycling of chlorine within soils, independent of any anthropogenic chlorine flux.

Keywords: trichloroacetic acid; TCA; soil lysimeter; microbial biomass; degradation;

Introduction

The sources, sinks and effects of trichloroacetic acid (TCA: CCl_3COOH) in terrestrial and freshwater environments continue to generate debate. The two central issues are the extent of TCA's toxicity to trees and aquatic life at the concentrations measured in the environment, and the extent to which processes involving man-made chlorinated compounds contribute to these measured concentrations. In the past, TCA and its derivatives were used as herbicides (Lockart *et al.*, 1990), but current interest in TCA dates from the 1990s when a number of studies reported an association between concentrations of TCA in conifer foliage (in the range of tens of $\mu\text{g kg}^{-1}$) and measures of tree ill-health (e.g. Frank *et al.*, 1990; Frank *et al.*, 1994; Plümacher and Schröder, 1994). At the time it was postulated that TCA in foliage derived entirely from oxidation of industrial chlorinated solvents, either within the atmosphere or after partitioning into the foliage, but it has since been argued that the flux of TCA from this source

cannot account for the observed concentrations of TCA in foliage, rainwater and soil (Jordan *et al.*, 1999; McCulloch, 2002; Hoekstra, 2003; Folberth *et al.*, 2003).

Attempts at simple mass closure calculations using field measurements imply that soil-related processes are key to understanding the biogeochemistry of TCA (Hoekstra *et al.*, 1999b; Schöler *et al.*, 2003; Stidson *et al.*, 2004a). This is supported by observations from laboratory experiments. For example, using model soil systems, TCA has been shown to be produced both by chloroperoxidase enzymes acting on aliphatic and humic acid substrates (Haiber *et al.*, 1996; Hoekstra *et al.*, 1999a; Niedan *et al.*, 2000; Matucha *et al.*, 2007) or entirely by abiotic chemistry (Fahimi *et al.*, 2003). On the other hand, there is also evidence that TCA applied to soils is readily destroyed (e.g. Heal *et al.*, 2003a; Matucha *et al.*, 2003a; Dickey *et al.*, 2004; Matucha *et al.*, 2007). The uncertainty in understanding soil-TCA relationships is exacerbated by the intrinsic heterogeneity of soil and associated difficulties in controlling soil parameters (Laternus *et al.*, 2005), and the use of different analytical methods which quantify different fractions of TCA within the soil (Dickey *et al.*, 2005). Resolving the uncertainty is important because controlled experiments confirm TCA's toxicity to trees and aquatic macrophytes at, or close to, environmental concentrations (Schröder *et al.*, 1997; Cape *et al.*, 2003; Dickey *et al.*, 2004; Hanson and Solomon, 2004; Lewis *et al.*, 2004), and consequently TCA is subject to formal risk assessment (Peters, 2003).

The work reported here included *in situ* measurements and manipulations to interpret soil TCA fluxes within a small catchment consisting predominantly of moorland and Sitka spruce plantation in the Southern Uplands of Scotland, UK. The annual external fluxes of TCA into and out of the catchment (via wet and dry deposition, and streamwater discharge, respectively) have been discussed previously (Stidson *et al.*, 2004a), as has TCA cycling through the forest canopy (Stidson *et al.*, 2004b). Two observations from this year-long monitoring programme provide strong indirect evidence for within-catchment production of TCA, associated with organic-rich forest soil in particular:

(1) About one-third of TCA deposition to the forest canopy did not appear in throughfall, stemflow or litterfall (Stidson *et al.*, 2004b), yet total TCA fluxes into and out of the catchment as a whole were at steady state, to within the estimated uncertainties (Stidson *et al.*, 2004a). The observed annual loss of TCA via the forest canopy was $\sim 800 \mu\text{g m}^{-2}$, implying that $\sim 300 \text{ g TCA}$ is generated annually *in situ* within the forested area of 0.41 km^2 to yield steady state overall (Stidson *et al.*, 2004b).

(2) The concentration of TCA in catchment streamwater was consistently higher after the stream had passed through the forest than before, as illustrated in Fig. 5 of Stidson et al. (2004a). Combining the median increase in TCA concentration in the stream ($\sim 0.35 \mu\text{g L}^{-1}$, $n = 26$) with its annual discharge ($\sim 1.4 \times 10^6 \text{ m}^3$) yields an estimate for annual net TCA production in the forest soil of $\sim 500 \text{ g}$.

Both estimation methodologies give reasonable consistency in magnitude, as well as sign, for the extent of net production of TCA within the forest soil at whole-forest scale. The study also showed that total catchment burden of TCA was dominated by the soil component and that this was approximately six times the annual external flux (Stidson *et al.*, 2004a) which further suggests that soil-TCA relationships are important to TCA biogeochemistry. Thus the aim of this work was to undertake a series of experiments with intact soil columns both *in situ* and in the lab to probe further the soil-related TCA processes inferred from the catchment-scale observations. The following hypotheses were investigated: soil TCA fluxes inferred at catchment scale are observable at the soil column scale; TCA is both produced and degraded within soil; and production and loss of TCA in soil cores is linked with biological processes associated with the soil organic matter and microbial biomass content.

Experimental

Field sites, soils and soil properties

The 0.86 km^2 Ballochbeatties catchment in the Southern Uplands of SW Scotland ($4^\circ 29' \text{ W}$, $55^\circ 13' \text{ N}$) which was the focus of this work has been described previously by Heal et al. (2004) in respect of hydrological closure, and by Stidson et al. (2004a; 2004b) in respect of catchment and forest scale TCA input/output measurements made two-weekly for one year. The catchment ranges in altitude from 300-480 m with the upper part consisting of Molinia moor and the lower part of forest plantation of predominantly Sitka spruce with the remainder mainly larch. Mean annual rainfall is $\sim 2000 \text{ mm}$. Soils are organic-rich throughout comprising basin peat (77-99% dwt organic matter), peaty podzol (25-50% dwt OM) and peaty gley (14-45% dwt OM). Quoted ranges reflect variation in measured OM content, primarily relating to different horizons. An agricultural grassland site in SE Scotland ($3^\circ 12' \text{ W}$, $55^\circ 13' \text{ N}$), with mineral gleysol (5-8% dwt OM) and mean annual rainfall of $\sim 900 \text{ mm}$, was also used for some comparison experiments. The soil dry and organic matter masses were determined by drying at

60 °C, and subsequent ignition in a furnace at 550 °C for 8 h, respectively. These and other soil data are given in Table S1.

A measure of the microbial biomass carbon content of the soil was determined using the methodology of chloroform fumigation and extraction with potassium sulphate (Vance *et al.*, 1987). In brief, 20 g samples of fresh soil were fumigated with chloroform for 24 h in a beaker, shaken for 30 min with 100 mL of 0.5 M K₂SO₄ and filtered. The filtrate was acidified with concentrated phosphoric acid, purged for 5 min with oxygen-free nitrogen and analysed using a total organic carbon analyser (Rosemount-Dohrmann DC-80, Santa Clara, Ca., USA). Organic carbon values were corrected using non-fumigated controls and converted to biomass carbon by dividing by 0.35 to account for non-extractable biomass carbon (Öhlinger, 1995).

Analysis of TCA

The concentration of TCA in all samples was determined using thermal decarboxylation to CHCl₃ and quantification by headspace GC-ECD. Details of the methodology as applied here to soil and aqueous samples have been extensively reported elsewhere (Heal *et al.*, 2003b; Heal *et al.*, 2003a; Dickey *et al.*, 2004; Stidson *et al.*, 2004a; Stidson *et al.*, 2004b; Dickey *et al.*, 2005). In brief, water or sieved soil samples were sealed in 20 mL headspace vials and heated at 100 °C for 1.5 h to effect decarboxylation, and re-equilibrated at 60 °C before CHCl₃ determination on a DB5 column held at 50 °C. Any background CHCl₃ was accounted for by determining CHCl₃ in a parallel vial of sample equilibrated to 60 °C only. Quantification of TCA in water samples was achieved directly against a series of standard TCA solutions taken through the same process. For soil samples, a partition ratio, the ratio of the response factor of standard additions of TCA to a soil matrix relative to the response factor of aqueous TCA standards, was determined. Each sample was analysed in triplicate. If RSD exceeded 30%, replication was repeated until this criterion was met. The limit of detection was ~0.1 µg L⁻¹.

When evaluating TCA in environmental samples it should be borne in mind that concentrations determined in aqueous samples will include the acetate form within the determination of TCA. Methods of soil analysis that are based on decarboxylation to chloroform, as is the case here, will also quantify 'bound' TCA, that is to say TCA that is not susceptible to aqueous extraction and which would not be quantified in soil analyses based on aqueous extraction. 'Bound' TCA may also include other soil material that contains a

C=O.CCl₃ functional group which undergoes decarboxylation to CHCl₃ under the analysis conditions, i.e. between 60 and 100 °C.

***In situ* soil lysimeter studies**

Twenty-two lysimeters were established at the Ballochbeathies and agricultural sites (15 and 7, respectively) by inserting lysimeter pots (diameter 190 mm, depth 140 mm) into the ground so that the upper surface of the intact soil core was level with the ground surface. Holes in the bottom of the pots enabled vertical drainage of soil water into an underlying void where it collected in large plastic bags. After a settling in period of a few weeks the volume and TCA content of collected lysimeter water was measured every two weeks for 7 months (Dec-Jul). Two broad categories of *in situ* lysimeter experiment were conducted (see Table 1):

(1) No chemical manipulation. Four lysimeters received only ambient input of wet deposition (moorland lysimeters 1&2) or forest throughfall (lysimeters 3&4 under larch and Sitka spruce canopies); a further six lysimeters received additional deionised water every two weeks to serve as “wetted” controls for the TCA-treated lysimeters (lysimeters 6&7 under Sitka spruce canopy, lysimeter 5 on the moorland, and lysimeters 8-10 in the agricultural soil).

(2) Deliberate TCA treatment. Twelve lysimeters received two-weekly doses of TCA solutions of different concentrations (lysimeter 11 on the moorland, lysimeters 12-18 under Sitka spruce canopy, and lysimeters 19-22 in the agricultural soil), as detailed in Table 1.

In all cases, ambient rainfall/throughfall hydrological and TCA inputs were taken as those measured at nearby wet deposition/throughfall gauges. The artificial TCA dose was chosen to ensure that two-weekly leachate concentration would be substantially greater than background leachate concentration if applied TCA washed through, or that total soil TCA concentration after ~7 or more TCA applications would be substantially greater than background soil concentration if the applied TCA accumulated in the soil. For the TCA-dosed lysimeters (13-22), leachate was collected for 6 or 7 two-week periods prior to the start of TCA dosing, and for a subset of these lysimeters (nos. 13, 14, 16, 17, 19, 21) leachate continued to be collected after TCA dosing ceased. Initial and final soil TCA concentrations were measured for the majority of lysimeters.

Sterile soil column experiments

Sub-samples of soil from the Sitka forest (O horizon, 10-20 cm) and larch forest (O horizon, 7-20 cm) were sterilized with 27-35 kGy of ⁶⁰Co gamma irradiation (Ethicon Ltd., Edinburgh). Six soil cores were then established for each soil type (3 sterilized and 3 non-sterilized) by

filling a pot of depth 12 cm with approximately 350 g soil. The cores were kept in an unheated greenhouse. On day zero, 4 µg TCA in 80 mL deionised water was added to each core. On day 2, when a volume of leachate sufficient for TCA analysis had accumulated in the bag beneath each core, a new bag was attached and 50 mL of ultrapure water was added to prevent drying of the soil and to flush out any further TCA. Further additions of ultrapure water were repeated on days 6, 8, 9, 14, 16 and 23.

TCA production experiments

To investigate within-soil production of TCA, soil from the O horizons (5-20 cm) of the afforested and moorland areas of the Ballochbeatties catchment were made TCA-free by drying at 60 °C for 8 day and 100 °C for 2 h to decarboxylate TCA already present (Dickey *et al.*, 2005), then rewetted with either deionised water or 7.6 mg L⁻¹ sodium chloride solution, stored in an unheated greenhouse and soil TCA concentrations re-measured after 1, 28 and 54 days.

TCA fate experiments with [1,2-¹⁴C] TCA

Experiments were conducted at 20 ± 2 °C, in duplicate, with soils from the following locations: Sitka forest (basin peat O1 and O2 horizons, 10-20 cm and 20-50 cm, respectively), larch forest (peaty gley, B horizon, 12-34 cm) and moorland (peaty podzol, B horizon, 25-30 cm). Fresh soil was homogenised by sieving through a 2 mm mesh and 0.5 mL of 430 kBq mL⁻¹ radioactively-labelled [1,2-¹⁴C] TCA (>98% radiochemical purity, specific activity 3.7 GBq mmol⁻¹) was applied to 50 g samples, equivalent to the addition of ~380 ng TCA (g fwt soil)⁻¹. Each soil sample was mixed for several minutes and transferred to a 500 mL Erlenmeyer flask, which was connected to a moistened continuous airflow (60 cm³ min⁻¹) and upstream and downstream CO₂ absorption solutions of 1 M KOH, as described in Matucha *et al.* (2003a). The contents of the two downstream CO₂ absorbers were collected after 1 h, and then twice a day until the rate of degradation changed little between sampling periods (7–10 days). The solutions were combined and 1 cm³ mixed with 5 cm³ of Optiphase “HiSafe” 3 scintillation cocktail (LKB, Loughborough, UK) for ¹⁴C analysis using a liquid scintillation spectrometer (Beckman LS 6500, Fullerton, Ca., USA). Radioactivity balance was confirmed through quantification of ¹⁴C remaining in the soil at the end of the experiment.

Results and discussion

Hydrological and TCA output/input ratios for lysimeters and soil cores

Mean \pm 1 SD cumulative hydrological outputs from the moorland and forest lysimeters were 0.74 ± 0.07 ($n = 4$, lysimeter nos. 1, 2, 5 & 11) and 0.94 ± 0.13 ($n = 11$, lysimeter nos. 3, 4, 6, 7 & 12-18) of their respective hydrological inputs from wet deposition and throughfall. (A tabulated summary is provided in Table S2 of the supplementary material.) The lower hydrological ratio for the agricultural lysimeters of 0.33 ± 0.03 ($n = 7$, lysimeter nos. 8, 9, 10 & 19-22) is due to greater water loss by evapotranspiration in the drier climate. Although hydrological output/input ratios are well-characterised on average (Heal *et al.*, 2004), there is considerable uncertainty in the hydrological ratios for individual forest lysimeters, and hence also in the TCA mass ratios determined from the water volumes, because of the inherent spatial heterogeneity in throughfall. This is not an issue for the moorland and agricultural lysimeters since hydrological input is expected to be more spatially homogeneous.

For control lysimeters that were not artificially dosed with TCA, the mean \pm 1 SD cumulative output/input ratios of TCA over 7 months for the moorland, forest and agricultural locations were 0.96 ± 0.08 ($n = 3$, lysimeter nos. 1, 2 & 5), 1.20 ± 0.09 ($n = 4$, nos. 3, 4, 6 & 7) and 1.10 ± 0.14 ($n = 3$, nos. 8-10), respectively (Tables 1 & S2). These data provide direct evidence for net production of TCA, on average, from the forest lysimeters compared with the moorland lysimeters ($p = 0.008$, unpaired t-test). As described in the Introduction, estimated generation of TCA in the forest soil during the year of measurements was $\sim 800 \mu\text{g m}^{-2}$, which combined with average below-canopy throughfall of $\sim 1600 \mu\text{g m}^{-2}$ (Stidson *et al.*, 2004b), gives an expected average soil lysimeter TCA output/input ratio of $\sim 2400/1600 = 1.5$. Thus, although net TCA production in the forest soil has been confirmed, these experimental data values do not fully account for the total net *in situ* TCA production inferred from the catchment scale measurements. This is likely because much TCA production is associated with mycorrhizal communities at the tree root-soil interface (Laternus *et al.*, 2005) which were not present in the lysimeters.

The TCA-dosed lysimeters, on the other hand, show unequivocal evidence of *in situ* TCA degradation in all soil locations examined, with mean cumulative output/input ratios for moorland, forest and agricultural locations of 0.12 ($n = 1$, lysimeter no. 11), 0.30 ± 0.06 ($n = 7$, nos. 12-18) and 0.28 ± 0.11 ($n = 4$, nos. 19-22), respectively (Tables 1 & S2). The input TCA that does not appear in lysimeter leachate does not accumulate in the soil of the lysimeter.

There was no significant change in soil TCA concentration from start to finish for any lysimeter (Figure S1), whether control or dosed (except for one agricultural soil control lysimeter in which soil TCA concentration decreased). This is the case irrespective of the different intrinsic TCA content of the two types of soil used. Furthermore, the soil TCA concentrations determined in this work are “whole soil” rather than extractable only (Dickey *et al.*, 2005), which indicates non-recovered TCA must undergo chemical transformation rather than irretrievable binding within the soil matrix.

Evidence for the dynamics of *in situ* TCA production and degradation is obtained from evaluation of the time series of the lysimeter leachates through the pre-dosing, dosing and post-dosing regimes. The output/input TCA time series for the six TCA-dosed lysimeters in forest soil (two levels of TCA dosing) are shown in Figure 1. The equivalent time series for the four TCA-dosed lysimeters in agricultural soil are shown in Figure S2; the behaviour was similar and consistent with first-order kinetics with respect to applied TCA. The average leachate TCA concentrations for each phase of the lysimeter experiments are summarised in Table 2. During the pre-dosing period, TCA leachate concentrations were similar in all lysimeters of a given soil type, confirming the absence of intrinsic bias between lysimeters assigned as control or dosed. The TCA concentrations in the control lysimeter leachates remained constant throughout. Since hydrological fluxes were similar through all lysimeters of a given soil type (although different between soil types: mean hydrological recoveries for forest and agricultural lysimeters were 0.94 and 0.33, respectively, as noted above), the relative TCA concentrations in the leachates of lysimeters in a given soil type are a proxy for the relative TCA fluxes through the lysimeters in different phases of the experiment. For both forest and agricultural soil lysimeters, the TCA concentrations in high-dosed lysimeter leachate (during dosing) were significantly greater than in low-dosed lysimeter leachate, which in turn were significantly greater than in control lysimeter leachate (Table 2). For the agricultural soil lysimeters, the leachate concentration was approximately 2.7 times greater in those receiving the high dose compared with the low dose, which is close in value to the 2.5 ratio in applied TCA dose; for the forest lysimeters the output concentration ratio was approximately two, i.e. slightly more applied TCA was degraded in high-dosed forest lysimeters than in low-dosed forest lysimeters (average TCA output/input ratios of 0.26 ($n = 4$, lysimeter nos. 12, 16-18) and 0.34 ($n = 3$, nos. 13-15), respectively).

The concentration of TCA in leachate from dosed agricultural soil lysimeters was 4-5 times greater than that from dosed forest soil lysimeters (Table 2). This concentration disparity is consistent with the 4-5 times smaller hydrological flux through the agricultural soil lysimeters due to both the lower rainfall input and the greater evapotranspiration for these lysimeters. The much shorter flush-through time for the forest lysimeters must be balanced by a greater rate of TCA destruction activity in these organic-rich soil matrices in order to give the observed situation overall that for both soil types approximately 30% of applied TCA passed through in leachate and 70% was degraded.

Table 2 and Figures 1 and S2 show that the response of the lysimeters to changes in applied TCA occurred within the two week timescale between dosing and leachate collection. Once TCA dosing ceased (in lysimeters A & B), there was return of leachate TCA concentration to control lysimeter leachate concentration levels. The ratio of cumulative TCA output/input for individual lysimeters also remained fairly constant with time (gradients of lines in Figures 1 and S2) both during and after dosing. This confirms that TCA drainage and degradation processes act on timescales of just a few days and is shorter for the forest soil than for the agricultural soil.

Processes of TCA production and degradation

Is this chemical transformation of TCA biotic or abiotic? In the experiments with γ -irradiated (“sterile”) forest soil cores, a highly significantly greater proportion of applied TCA was recovered in the leachate than from untreated control soil cores (Figure 2). Recovery exceeded 90% for sterilized larch soil cores. This strongly implicates biotic processes mediating the permanent loss of TCA. Similarly, observation that $^{14}\text{CO}_2$ is released from the soils dosed with 1,2- ^{14}C labelled TCA (Figure 3) implicates biological processes causing mineralisation of TCA. The areas under the curves in Figure 3 equate to 73-80% of the applied labelled TCA being lost by mineralisation to CO_2 . These values are entirely consistent with the average proportion of ~70% for non-retained (i.e. “lost”) TCA inferred from the TCA-dosed *in situ* field lysimeters (Table 1, and discussion above). The time profiles of radioactive CO_2 release in Figure 3 also confirm that soil degradation of TCA operates on a time scale of tens of hours to day.

The permanent mineralisation of TCA applied in these radioactivity experiments and to the *in situ* soil lysimeters is likewise consistent with observations of permanent loss of TCA applied

to the soil surrounding Sitka spruce seedlings (Heal *et al.*, 2003a; Dickey *et al.*, 2004). Biological dehalogenation of TCA (and other haloacetic acids) at environmental concentrations has been reported previously (Ellis *et al.*, 2001; McRae *et al.*, 2004), and the ubiquity of microorganisms containing dehalogenation enzymes has been demonstrated using gene sequencing on samples taken throughout water treatment processes (Leach *et al.*, 2009).

What about *in situ* production of TCA in soil? The soil cores made TCA-free showed production of TCA from only 24 h after rewetting at room temperature (Figure 4). The amount of TCA generated was not dependent on rewetting with water or with NaCl solution, but was significantly different between soils from different locations, with soil from within the Sitka spruce forest yielding significantly greater TCA than soil from within the larch forest or moorland soil. It is not possible to conclude from this experiment the extent to which the production of the TCA is biotic, through stimulation of soil biomass, or abiotic, via processes such as those proposed by Fahimi *et al.* (2003). (The latter involves Fenton-type redox cycling with Fe^{2+} /peroxide coupled to oxidation of chloride and chlorination of methoxy-type organic functional groups.) However, the strong correlation between soil TCA and measured soil microbial biomass C concentration (Figure 5) indirectly suggests that biologically-mediated processes dominate. Figure 5 shows that the organic-rich peaty horizons of the forest and moorland soils are associated with both greater microbial biomass and TCA content, consistent with the greater TCA-related activity for these soils inferred from the other experiments. These observations are also consistent with recent process-based studies using ^{36}Cl radioactively-labelled NaCl which have directly demonstrated TCA formation from laboratory experiments with mixtures of humic or acetic acids and chloroperoxidase enzyme and with forest soils (Matucha *et al.*, 2003b; Matucha *et al.*, 2007). Figure 4 also shows that although TCA production is rapid, the soil cores move to a steady state in TCA concentration, such that TCA degradation and TCA production must operate simultaneously. This is also the conclusion from the radioactive tracer experiments (Matucha *et al.*, 2003b; Matucha *et al.*, 2007).

Implications for TCA cycling in the environment

The overall picture that emerges from our *in situ* catchment and lysimeter studies, and work from other groups, is that the soil and forest environment is not passive with respect to TCA. We have previously shown that a coniferous forest moderates TCA fluxes via the canopy (Stidson *et al.*, 2004b) and hydrological pathways (Stidson *et al.*, 2004a). Soil TCA activity

inferred from catchment scale measurements has been supported by the detailed soil-focused studies presented here. We have shown that the soil has the facility both to generate and degrade TCA on the timescale of a few days and that this is particularly the case for highly organic and biomass-C-rich forest soils. The available evidence is consistent with *in situ* biologically-mediated processes being predominant for both the production and destruction processes operating simultaneously, although contribution from geochemical (abiotic) processes is not excluded. The soil processes relating to TCA as one example of a chlorinated compound are likely only one component in the larger picture emerging of microbially-mediated chlorination and subsequent degradation of soil organic matter (Laternus *et al.*, 2005; Clarke *et al.*, 2009; Rohlenova *et al.*, 2009).

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Figure captions

Figure 1. Cumulative TCA inputs and outputs to soil lysimeters in Sitka spruce forest at Ballochbeatties, dosed two-weekly with 20 µg TCA (Low) or 50 µg TCA (High) over a 6-month period (Jan – June 2002). On each occasion the leachate present was collected before the lysimeter was dosed. The input data are the sum of throughfall plus dosing input up to that point. The first arrow in each graph indicates the first TCA dosing and the second arrow indicates the final dosing for lysimeters A and B. The final % recoveries of input TCA in the leachates are shown in the legend.

Figure 2. Mean recovery after 23 days of TCA in the leachates of control (untreated) and sterile Sitka spruce and larch soil columns, expressed as a % of the total TCA input on day zero. Error bars are standard deviation of 3 replicate columns, each analysed in triplicate.

Figure 3. Rate of production of $^{14}\text{CO}_2$ from 215 kBq [1,2- ^{14}C] TCA applied to 50 g of Sitka O1, Sitka O2, Larch B, and Moor B soils. Error bars are ± 1 SD of two experimental replicates each analysed in duplicate.

Figure 4. TCA concentrations of dried organic-rich “TCA-free” soils re-hydrated with water (W) or sodium chloride solution (NaCl) and stored at room temperature for different lengths of time. Error bars are ± 1 SD of triplicate analyses.

Figure 5. The relationship between soil microbial biomass-C concentrations and TCA concentrations, in a range of soils and horizons from Ballochbeatties forest and moorland, and agricultural grassland.

Figure 1.

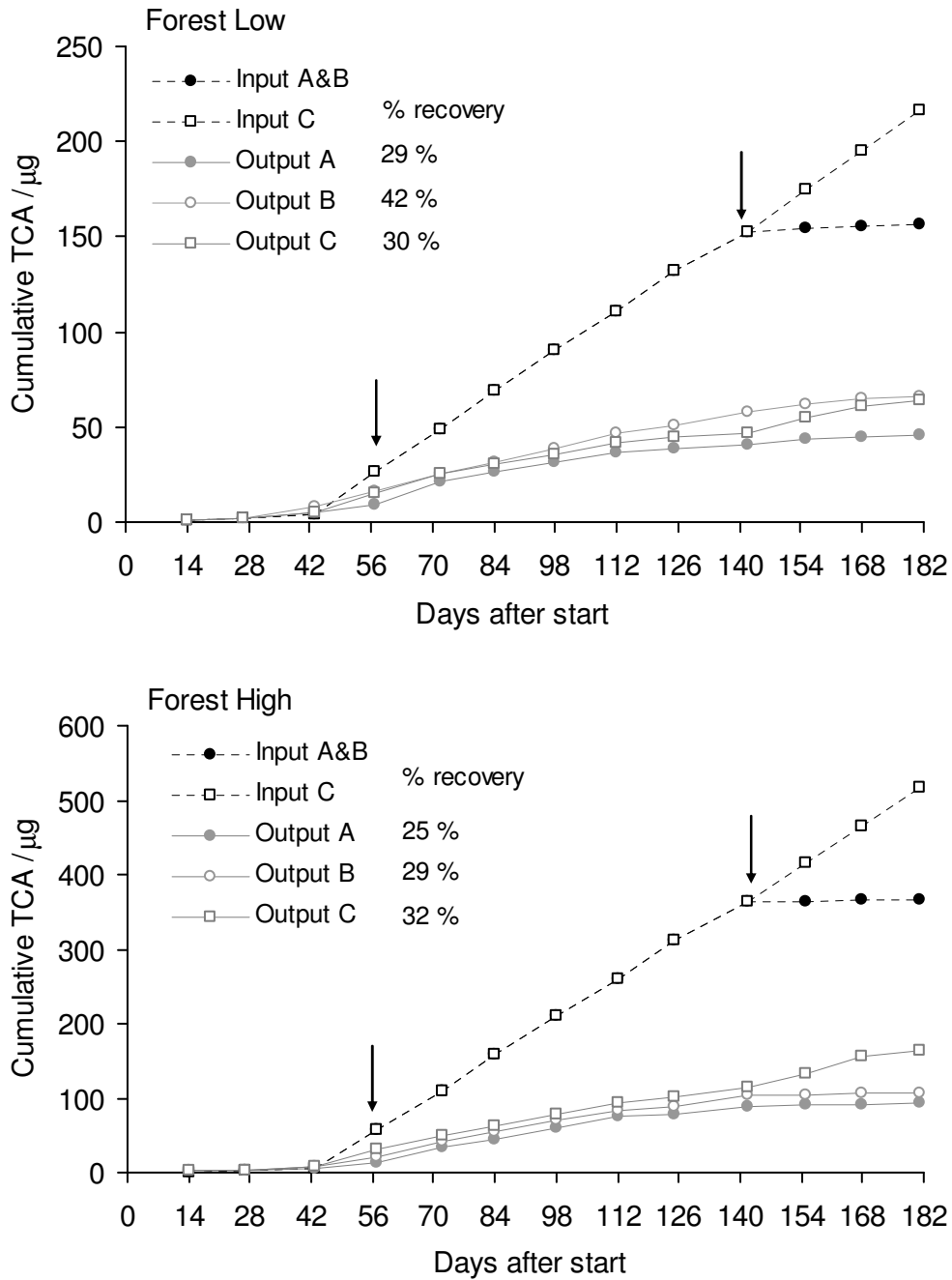


Figure 2.

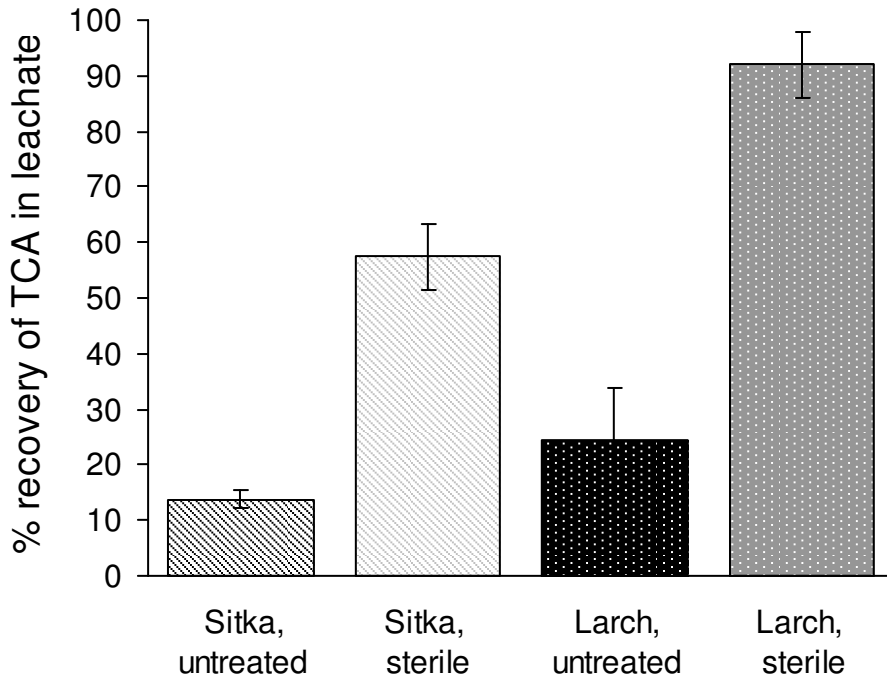


Figure 3.

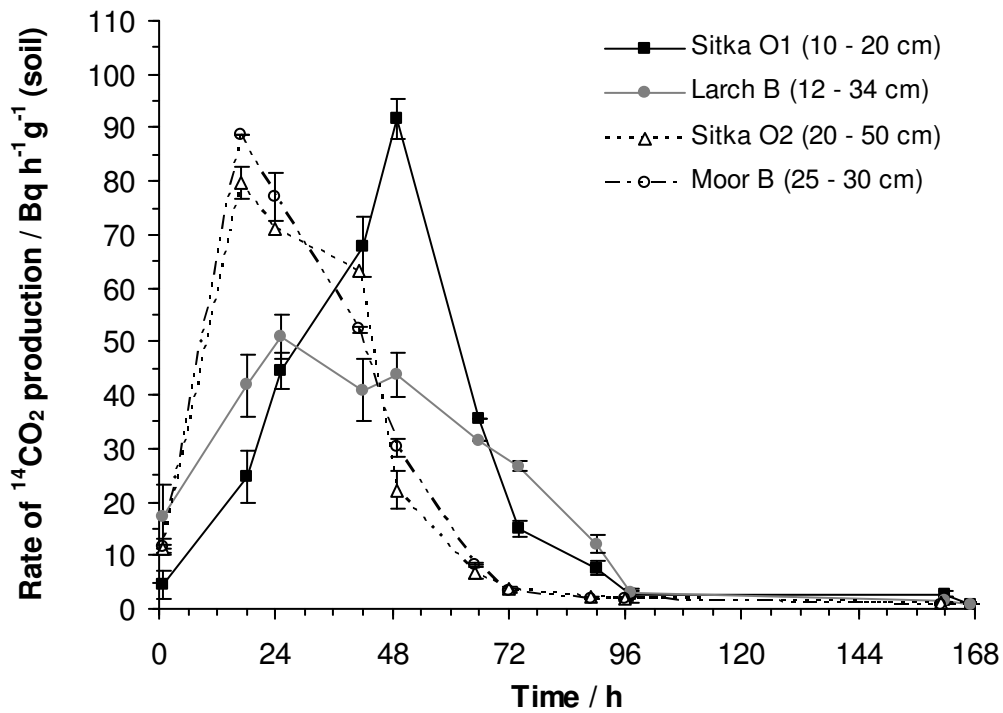


Figure 4.

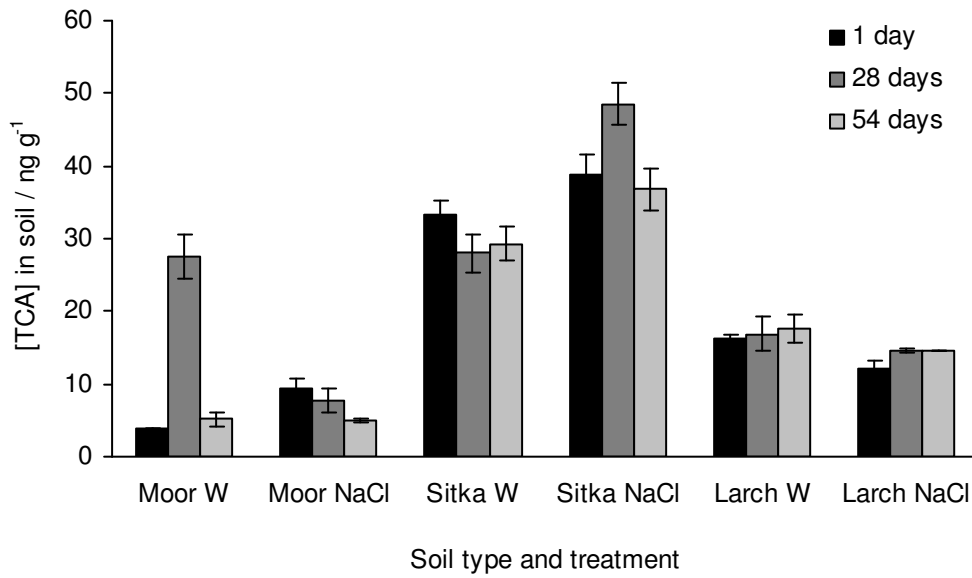


Figure 5.

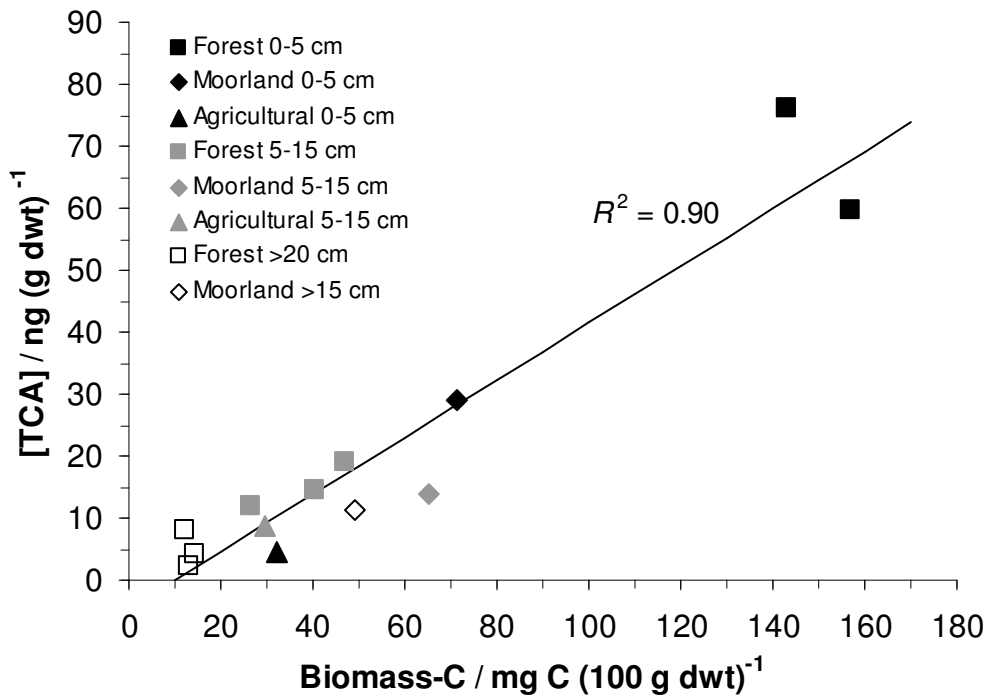


Table 1: Summary of individual lysimeter experiments.

Location	Land use	Soil classification ^a	Application	Dose	No. of doses ^b	Cumulative out/in ratio for water	Cumulative out/in ratio for TCA
1	Ballochbeaties	Wet heath moorland	Peaty podzol, O, B	None	-	0.69	0.94
2	Ballochbeaties	Wet heath moorland	Peaty podzol, O, B	None	-	0.82	1.05
3	Ballochbeaties	Larch plantation	Peaty gley, O	None	-	1.14	1.27
4	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	None	-	0.87	1.06
5	Ballochbeaties	Wet heath moorland	Peaty podzol, O, B	250 mL	18	0.78	0.90
6	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL	18	0.91	1.23
7	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL	13	1.05	1.23
8	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL	13	0.34	0.96
9	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL	13	0.38	1.24
10	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL	13	0.29	1.13
11	Ballochbeaties	Wet heath moorland	Peaty podzol, O, B	250 mL, 200 µg L ⁻¹	18	0.68	0.12
12	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 200 µg L ⁻¹	18	0.84	0.24
13	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 80 µg L ⁻¹	7	0.67	0.29
14	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 80 µg L ⁻¹	7	0.92	0.42
15	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 80 µg L ⁻¹	10	0.94	0.30
16	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 200 µg L ⁻¹	7	0.92	0.25
17	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 200 µg L ⁻¹	7	1.08	0.29
18	Ballochbeaties	Sitka spruce plantation	Basin peat, OI	250 mL, 200 µg L ⁻¹	10	1.01	0.32
19	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL, 80 µg L ⁻¹	6	0.35	0.34
20	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL, 80 µg L ⁻¹	10	0.34	0.22
21	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL, 200 µg L ⁻¹	6	0.32	0.34
22	Nr. Edinburgh	Agricultural grassland	Gleysol, O	250 mL, 200 µg L ⁻¹	10	0.30	0.22

^a Soil Survey of Scotland (MLURI, 2002)^b All lysimeters were left to settle for several weeks after installation before commencement of dosing. Where water or solution was applied, frequency was in all cases two-weekly.

Table 2. Average TCA concentration in leachate for the pre-dosing, dosing and post-dosing periods in experiments in which lysimeters of forest or agricultural soil were dosed two-weekly with 20 μg (Low) or 50 μg (High) of TCA. Control lysimeters received 0.14 μg TCA via the control volumes of water applied to them. In the period referred to as “post-dosing,” dosing ceased in lysimeters labelled A (and B) but continued in lysimeters labelled C for comparison.

Mean (\pm 1 SD) two-weekly TCA concentration of lysimeter leachate / $\mu\text{g l}^{-1}$			
<u>Forest soil lysimeters</u>			
<u>Forest</u>	Pre-dosing period (3 occasions)	Dosing period (7 occasions)	Post-dosing period (3 occasions)
Control ($n = 1$) (#7) ^a	0.98 (\pm 0.60)	0.74 (\pm 0.41)	1.04 (\pm 0.40)
Low dosed ($n = 3$) (#13-15)	1.19 (\pm 0.28)	6.3 (\pm 5.2)	1.25 (\pm 0.45) (A,B) 3.24 (\pm 0.96) (C)
High dosed ($n = 3$) (#16-18)	1.19 (\pm 0.41)	12.8 (\pm 11.2)	0.68 (\pm 0.32) (A,B) 10.3 (\pm 4.8) (C)
<u>Agricultural soil lysimeters</u>			
	Pre-dosing period (3 occasions)	Dosing period (6 occasions)	Post-dosing period (4 occasions)
Control ($n = 3$) (#8-10)	1.55 (\pm 0.32)	1.12 (\pm 0.85)	0.67 (\pm 0.33)
Low dosed ($n = 2$) (#19,20)	1.19 (\pm 0.29)	24.7 (\pm 20.5)	5.56 (\pm 3.9) (A) 21.5 (\pm 33.9) (C)
High dosed ($n = 2$) (#21,22)	1.35 (\pm 0.16)	67.6 (\pm 56.8)	6.6 (\pm 9.5) (A) 68.1 (\pm 59.3) (C)

^a Lysimeter identifiers refer to the assignments in Table 1.

Supplementary Material

Figure S1. TCA concentrations of *in situ* (a) forest and (b) agricultural lysimeter soils at the start (Dec 2001) and end (July 2002) of the TCA dosing experiment. Error bars are ± 1 SD of triplicate analyses.

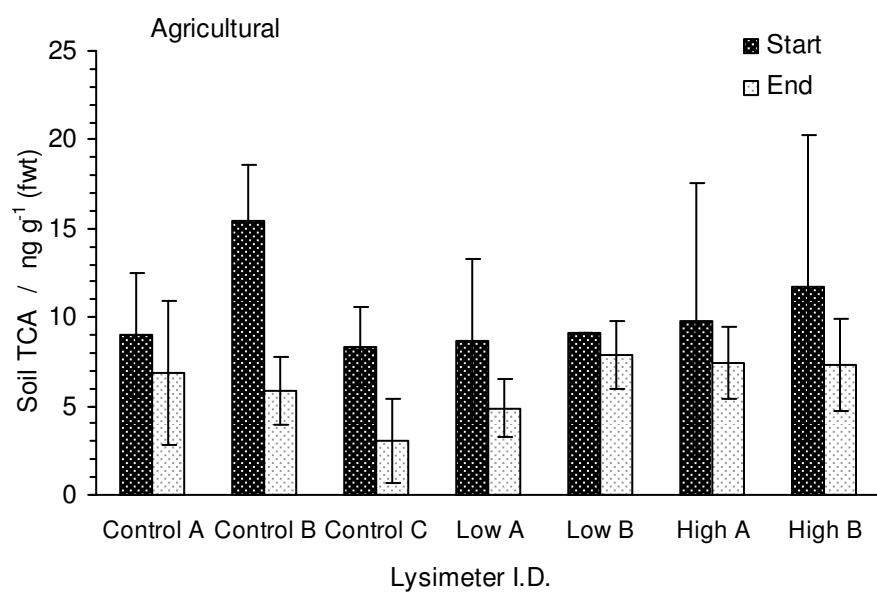
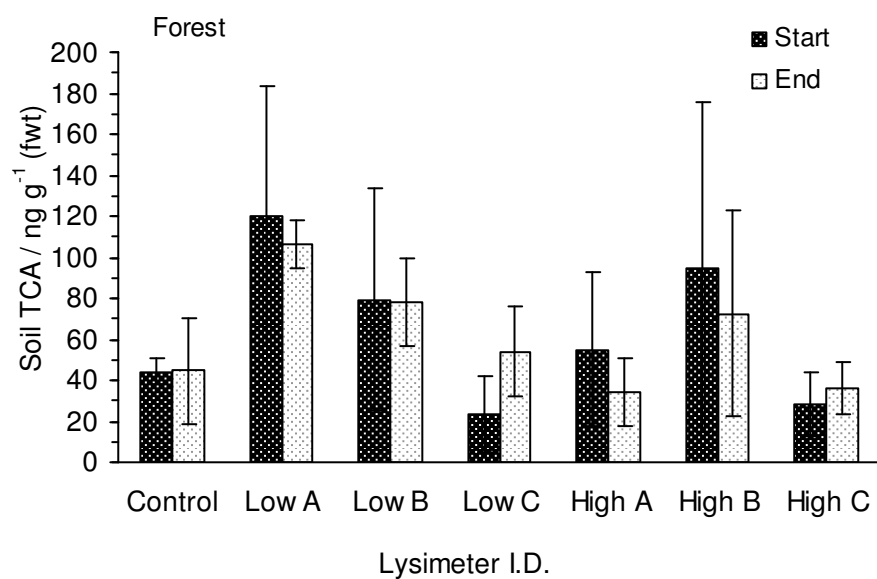


Figure S2. Cumulative TCA inputs and outputs to lysimeters from an agricultural site near Edinburgh, dosed two-weekly with 20 μg TCA (Low) or 50 μg TCA (High) over a 6-month period (Jan – June 2002). On each site visit the leachate present was collected before the lysimeter was dosed. The input data is the sum of rainwater plus dosing input up to that point. The first arrow in each graph indicates the first TCA dosing and the second arrow indicates the final dosing for lysimeter A. The final % recoveries of input TCA in the leachate are shown in the legend.

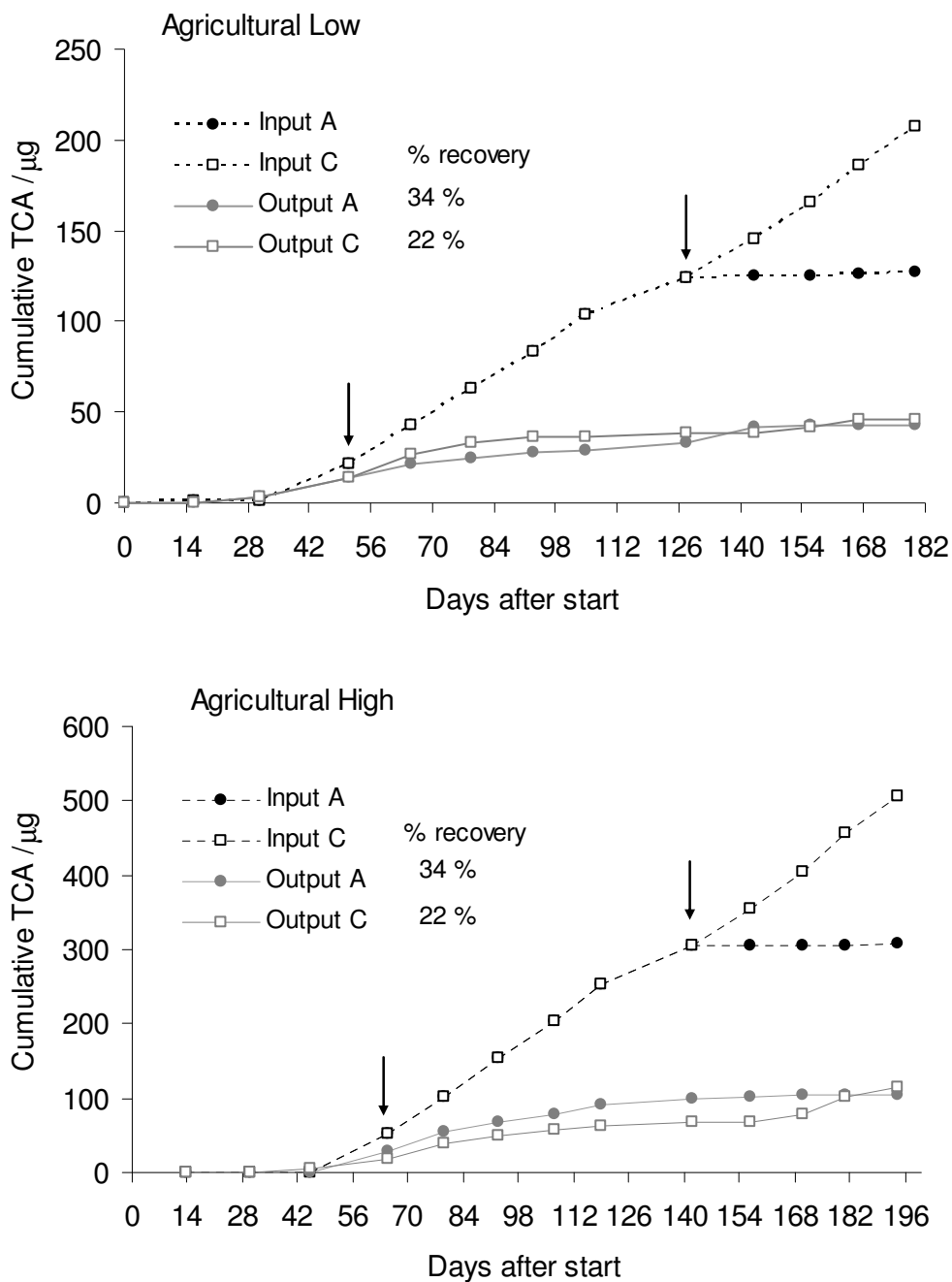


Table S1: Physical and chemical properties of the soil types sampled in the proximity of the lysimeter experiments. Analyses were conducted using standard methods detailed in Dickey (2004)¹. Values are means (number of samples in parentheses).

Land use (soil horizon)	Lysimeter identifiers ^a	% water ^b	% organic matter (dwt) ^b	pH in deionised water ^b	% C ^c	% N ^c	C:N ratio ^c
Sitka spruce forest (basin peat O1)	4, 6, 7, 12-18	85 (16)	90 (16)	3.83 (26)	52	2.1	25
Larch forest (peaty gley O)	3	64 (17)	59 (17)	3.93 (3)	27	1.0	27
Moorland (peaty podzol B)	1, 2, 5, 11	65 (16)	56 (16)	4.42 (6)	31	2.0	16
Agricultural grassland (gleysol O)	8, 9, 10, 19-22	26 (5)	7 (5)	6.20 (6)	3.0	0.17	18

^aAs defined in Table 1.

^bSoil sampled on 2-5 occasions from November 2000 to May 2003.

^cSoil sampled in November 2001.

For comparison the lysimeter experiments were conducted between December 2001 and July 2002.

¹ Dickey, C.A. (2004) The behaviour of trichloroacetic acid in soil and its uptake and effects in Sitka spruce trees, PhD thesis, The University of Edinburgh.

Table S2: Means of individual ratios of cumulative output/input for water volume and for TCA mass for different combinations of soil lysimeters.

Lysimeter grouping	Lysimeter identifiers^a	n	Mean ratio \pm 1 SD
<u>Water volume out/in ratio</u>			
Forest	3, 4, 6, 7, 12-18	11	0.94 \pm 0.13
Moorland	1, 2, 5, 11	4	0.74 \pm 0.07
Agricultural grassland	8, 9, 10, 19-22	7	0.33 \pm 0.03
<u>TCA mass out/in ratio</u>			
No TCA dosing (forest)	3, 4, 6, 7	4	1.20 \pm 0.09
No TCA dosing (moorland)	1, 2, 5	3	0.96 \pm 0.08
No TCA dosing (agricultural grassland)	8, 9, 10	3	1.10 \pm 0.14
Dosed with TCA (forest)	12-18	7	0.30 \pm 0.06
Dosed with TCA (moorland)	11	1	0.12
Dosed with TCA (agricultural grassland)	19-22	4	0.28 \pm 0.07
Dosed with TCA (all locations combined)	11-22	12	0.28 \pm 0.08

^aAs defined in Table 1.