

Addressing analytical uncertainties in the determination of trichloroacetic acid in soil

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Soil is an important compartment in the environmental cycling of trichloroacetic acid (TCA), but soil TCA concentration is a methodologically defined quantity; analytical methods either quantify TCA in an aqueous extract of the soil, or thermally decarboxylate TCA to chloroform in the whole soil sample. The former may underestimate the total soil TCA, whereas the latter may overestimate TCA if other soil components (*e.g.* humic material) liberate chloroform under the decarboxylation conditions. The aim of this work was to show that extraction and decarboxylation methods yield different TCA concentrations because the decarboxylation method can also determine “bound” TCA. Experiments with commercial humic acid solutions showed there was no additional chloroform formation under decarboxylation conditions, and that all TCA in a TCA–humic acid mixture could be quantitatively determined ($108 \pm 13\%$). Anion exchange resin was used as a provider of solid-phase TCA binding; only $5 \pm 1\%$ of a TCA solution mixed with the resin was present in the aqueous extract subsequently separated from the resin, yet the decarboxylation method yielded mass balance ($123 \pm 22\%$) with TCA remaining in the resin. In aqueous extraction of a range of soil samples (with or without added TCA spike), the decarboxylation method was able to satisfactorily account for TCA in the extractant + residue post-extraction, compared with whole-soil TCA (+ spike) pre-extraction: *e.g.* mass balances for unspiked soil from Sikta spruce and larch forest were $99 \pm 8\%$ and $93 \pm 6\%$, respectively, and for TCA-spiked forest and agricultural soils were $114 \pm 13\%$ and $102 \pm 2\%$. In each case recovery of TCA in the extractant was substantially less than 100% ($<20\%$ for unspiked soils, $<55\%$ for spiked soils). Extraction efficiencies were generally lower in more organic soils. The results suggest that analytical methods which utilise aqueous extraction may underestimate whole-soil TCA concentrations. Application of both methodologies together may enhance insight into TCA behaviour in soil.

1. Introduction

Trichloroacetic acid (TCA: CCl_3COOH) has been the subject of much research since the 1990s when a possible connection between its phytotoxicity, its presence as a secondary pollutant in forest foliage and forest dieback was first suggested.^{1–4} Whilst some adverse effects of TCA on tree seedlings have been observed,^{5–8} recent debate has focused on reconciling observed concentrations of TCA in the environment with the contributions of putative sources.^{9–11} TCA is formed in the atmosphere as a product of photochemical oxidation of chlorinated hydrocarbons such as CH_3CCl_3 and C_2Cl_4 ,^{12,13} but the deposition flux of TCA in atmospheric precipitation exceeds that expected through oxidation of anthropogenic emissions of these compounds.^{14,15} Additional atmospheric flux may arise through oxidation of naturally emitted volatile chlorinated compounds¹⁶ and/or a greater rate of oxidation *via* atmospheric aqueous-phase than currently accepted.¹⁷

In addition to this lack of closure of atmospheric sources of TCA, it has been demonstrated in laboratory experiments with model systems for soil processes that TCA can be formed both through chloroperoxidase enzymes acting on aliphatic and humic acid substrates^{18–20} and through abiotic chemistry.²¹ These, and other, findings strongly implicate soil processes as an additional natural source of TCA in remote environments. In fact, attempts to close a budget for TCA in the terrestrial environment consistently point to the dominance of the soil

compartment for storage and flux of TCA.^{10,11,22,23} Despite this, measurements of TCA concentrations in soil and soil litter layer are relatively few compared with other media, and vary by orders of magnitude (<0.05 –many 100s ng g^{-1} dry weight, Table 1). This is due to the extremely heterogeneous nature of soil, the different characteristics of sites investigated (*e.g.* soil type, vegetation, altitude, climate) and, importantly, the different analytical techniques employed.

The two approaches to TCA determination in soil are: (i) extraction of TCA into aqueous solution followed by derivatisation of TCA and analysis of the derivative by gas chromatography (GC-MS or GC-ECD) or (ii) thermal decarboxylation of TCA in the whole sample to chloroform (CHCl_3) and analysis of the latter by headspace gas chromatography (HSGC-ECD). The advantages and disadvantages of both approaches are summarised in Table 2. Reagents used for derivatisation of TCA include diazomethane,²⁴ 2,4-difluoroaniline,²⁵ acidic methanol²⁶ or 1-(pentafluorophenyl) diazoethane.²⁷ The extraction-derivatisation method requires multiple preparative steps which limits sample throughput for replication and may impact on loss of analyte and contamination. More fundamentally, the requirement to extract TCA into solution may mean that not all TCA in the soil is quantified. Where extraction of soil TCA has been reported in the literature, the methodology has simply been to shake with unbuffered deionised water (*i.e.* zero ion strength) for between 1 h and 16 h followed by centrifugation and/or filtration to

Table 1 Soil TCA concentrations (dry weight (dwt)) reported from a range of sites

Location	Vegetation	TCA concentration/ng g ⁻¹ dwt			Data source
		Mean (± SD)	Min, max	n	
Germany	Spruce	100 ± 200 ^a	20, 380 ^a	5	Frank (1988) in McCulloch (2002) ³⁷
Germany	Pine	Not stated	1.4, 120 ^a	72	Plümacher (1995) in McCulloch (2002) ³⁷
Holland	Douglas fir	0.5 ± 0.5	0.2, 1.3	4	Hoekstra and de Leer (1993) ³⁸
	Beech	0.4 ± 0.4	0.2, 0.9	3	"
	Peat moor	1.6 ± 0.9	1.0, 2.7	3	"
	Peat bog	3.4 ± 0.9	2.6, 4.6	4	"
Scotland	Moorland (litter layer)	51 ^b	44, 110	5	Stidson <i>et al.</i> (2004) ²³
	Moorland (organic horizon)	42 ^b	20, 3090	8	"
	Sitka (litter layer)	559 ^b	150, 2059	6	"
	Sitka (organic horizon)	84 ^b	28, 463	7	"
	Larch (litter layer)	56 ^b	11, 231	11	"
	Larch (organic horizon)	37 ^b	26, 41	3	"
Europe	Various	0.61	<0.05, 12	48	Peters (2003) ²⁴

^a Not stated whether TCA concentrations are dry weight or fresh weight. ^b Median TCA concentrations.

obtain the aqueous fraction.^{19,24,28} More rigorous soil extraction methodology for other analytes typically uses higher ionic strength extractant buffered to an alkaline pH.

In contrast, the decarboxylation method requires no extraction, is rapid, and capable of quantifying all TCA present in the soil. It has been applied to the analysis of TCA in a variety of environmental media.^{17,29–31} The main uncertainty with the method is whether TCA concentrations are overestimated if other components of the soil matrix yield additional CHCl₃ when the sample temperature is raised to 100 °C for 1.5 h to effect decarboxylation: for example, from chloral hydrate (CCl₃CH(OH)₂), humic acid or stimulation of CHCl₃-producing microbial activity. In fact, interference from decarboxylation of chloral hydrate (if present in soils) is unlikely since laboratory experiments have already shown this to be a very pH-dependent process, with less than 10% conversion of chloral hydrate solutions of pH <6 to CHCl₃ after 90 min at 90 °C.³²

Since the two analytical methods measure different targets (water-extractable TCA *versus* whole-soil TCA), inter-comparison studies on the same soil sample cannot yield comparable data, *i.e.* soil TCA concentration is operationally defined. Instead, other experiments, as reported here, are required to address potential analytical differences between the extraction-derivatisation and decarboxylation methods for analysis of TCA in soil. The aims of this work were: (i) to examine whether CHCl₃ originates from other soil components and thus interferes with the decarboxylation method; (ii) to compare the recovery of intrinsic and extrinsic (added) TCA in soil using aqueous extraction methodology similar to that in current use with that measured by whole soil analysis. Only the decarboxylation method is able to address the latter objective of

comparing “whole soil” TCA with both “aqueous-extractable” and “residual soil” TCA; it is not possible with the extraction method to demonstrate that there is no residual TCA in the soil after extraction.

2. Experimental

2.1. Soil samples

Soil used in the experiments reported here was collected from Ballochbeatties, a remote, upland site in Ayrshire, South-West Scotland (55° 13' N, 4° 29' W) with an elevation of 300–650 m above sea level and a moist maritime climate (1824 mm mean annual rainfall 1988–1999³³). Land use consist of Sitka spruce (*Picea sitchensis*) and larch (*Larix × eurolepis*) plantation and *Molinia* moor overlying organo-mineral and organic soils. Additional clay loam soils with greater mineral content were collected from Cowpark, an agricultural site in South-East Scotland (55° 13' N, 3° 12' W), with an elevation of 200 m and lower annual rainfall (869 mm mean annual rainfall 1955–2000³⁴).

2.2. Determination of TCA in soil and aqueous samples by decarboxylation

The method of analysis for TCA in soil by decarboxylation-HSGC used here is similar to that described previously.^{23,29,35} Samples were weighed into 20 ml headspace vials and sealed with PTFE-coated butyl rubber septa and aluminium caps. All vials and caps were heated at 200 °C for a minimum of 20 min before use to remove any TCA or CHCl₃. For aqueous samples, either 5 ml or 2.5 ml of sample was analysed. For

Table 2 Advantages and disadvantages of the two methods of TCA determination in soil: decarboxylation to CHCl₃ followed by analysis by HSGC-ECD; and aqueous extraction followed by derivatisation and analysis by GC-MS or GC-ECD

Method	Advantages	Disadvantages
Decarboxylation-HSGC	Quantification of TCA in whole soil Direct and easy sample preparation High sample throughput permits greater sample replication	Assumption that no other soil compounds form CHCl ₃ on heating to between 60 °C and 100 °C for 1.5 h
Extraction-derivatisation	Unique definition of TCA Potential for parallel measurement of other haloacetic acids	Assumption that TCA is 100% extractable from soil into solution Low sample throughput limits sample replication Multiple steps are time-consuming and increase risk of loss/contamination

Table 3 HSGC-ECD conditions used in the determination of CHCl_3 formed from the thermal decarboxylation of TCA

Sample pre-analysis conditions

Sample decarboxylation	90 min at 100 °C
Sample and background equilibration	60 min at 60 °C

Headspace sampler and GC temperature and time settings

Thermostating	10 min at 60 °C
Pressurisation time	2.00 min
Needle temperature	70 °C
Injection	0.03 min at 200 °C
Withdrawal time	0.20 min
Vent time	0.10 min
Transfer line temperature	200 °C
Detector temperature	375 °C
Oven temperature programme	5 min at 50 °C, 20 °C min ⁻¹ , 3 min at 150 °C

Carrier gas Helium, 12.6 psi, split 25 ml min⁻¹

Capillary column 30 m × 0.32 mm × 1.8 μm, DB624 (Agilent, USA)

soil analysis, 1 ± 0.02 g fresh sieved (2-mm) sample was weighed into vials and 1 ml deionised water (18.3 MΩ cm⁻¹) was added. The requirement for 1 ml of water was a consequence of the calculation of soil TCA concentration using a partition ratio (see below). Four replicate vials were prepared for each sample. Three replicates were heated to 100 °C for 90 min to decarboxylate the TCA to CHCl_3 before equilibration at 60 °C for 60 min. Experiments have previously demonstrated stoichiometric decarboxylation of TCA to CHCl_3 under these conditions.¹⁷ The fourth vial was equilibrated for 60 min at 60 °C only to quantify any background CHCl_3 in the sample. An aliquot of headspace from each vial was transferred by a PerkinElmer HS40XL headspace sampler to a PE Auto-system GC with ECD. The HSGC instrumental specifications used in this work are shown in Table 3.

The contribution of TCA to the CHCl_3 peak area in the sample was obtained by subtraction of the peak area due to CHCl_3 in the background sample not subjected to the decarboxylation conditions. For aqueous samples, the concentration of TCA corresponding to the background-corrected CHCl_3 peak area was quantified directly against external aqueous TCA standard solutions which were decarboxylated and analysed for CHCl_3 in the same run as the samples. This was

achieved *via* determination of a GC “response factor”, f_w , to TCA in aqueous solution (peak area units of CHCl_3 (ng TCA)⁻¹) calculated as shown in eqn. (1).

$$f_w = \frac{(A_{\text{STD}} - A_w)}{M_{\text{TCA}}} \quad (1)$$

where A_{STD} is the peak area of CHCl_3 from heating TCA standard solution to 100 °C, A_w is the peak area of CHCl_3 from heating water used to prepare TCA standard solution to 100 °C and M_{TCA} is the mass of TCA standard added to vial (ng).

The value of f_w was determined on every GC run from the average of 2 or 3 standards of different TCA concentrations (0.5–100 μg l⁻¹, depending on the sample type), prepared from two independent stock solutions, each analysed in triplicate. If the response factors derived from different TCA standards in the same GC run varied by > ~20% the run was re-analysed.

Given the value of f_w for a particular GC run, the concentration of TCA in any aqueous sample, C_{AQ} (ng g⁻¹), was derived straightforwardly from eqn. (2),

$$C_{\text{AQ}} = \frac{(A_{\text{AQ}} - A_{\text{AQBG}})}{f_w \times M_{\text{AQ}}} \quad (2)$$

where A_{AQ} is the peak area of CHCl_3 from heating aqueous sample to 100 °C, A_{AQBG} is the peak area of CHCl_3 from heating aqueous sample to 60 °C, f_w is the response factor (peak area (ng TCA)⁻¹), from eqn. (1) and M_{AQ} is the mass of aqueous sample (g).

The GC peak areas corresponding to CHCl_3 in the headspace above soil samples could not be quantified directly against GC peak areas of CHCl_3 in the headspace above aqueous standard solutions because of the different partitioning of CHCl_3 between headspace and aqueous solution and between headspace and soil. However, the ratio of these two partition coefficients (the “partition ratio” $F_{w/s} = f_w/f_s$) is a constant for a given set of headspace conditions such as sample mass and headspace volume. Thus, on any GC run containing soil samples, it was necessary only to include aqueous TCA standards (to quantify the run-specific value of f_w) to determine the value of f_s .

The value of the partition ratio was determined experimentally for soil using standard additions. Increasing concentrations of TCA standard solution (1 ml; 0, 10, 20, 50 and 100 μg l⁻¹) were added to aliquots of fresh homogenised soil (1 ± 0.02 g) in headspace vials. Vials of soil and standard solution were prepared and analysed for TCA using the procedure described above. The ratio of the gradients of the two standard addition plots yields $F_{w/s}$, as illustrated in Fig. 1.

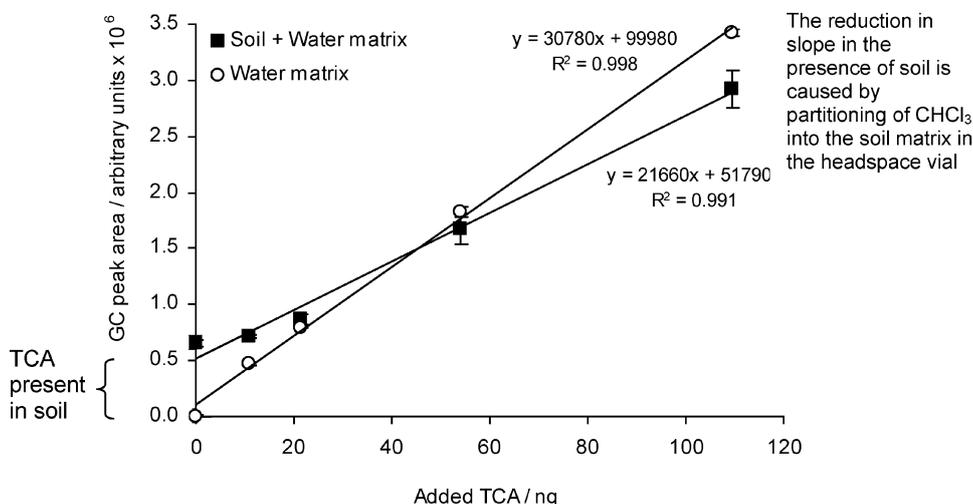


Fig. 1 Standard additions plot used to calculate the partition ratio of the response factors of water and soil; in this example, $F_{w/s} = \frac{f_w}{f_s} = \frac{30780}{21660} = 1.42$. Error bars are 1 SD of triplicate analyses.

Table 4 Characteristics of eight soil horizons from the Ballochbeatties site and their measured methodological TCA partition ratios ($F_{W/S}$) (see text)

Soil	Soil horizon	Water (% fwt)	Organic matter (% fwt)	pH	Fe/mg g ⁻¹ dwt	Mn/μg g ⁻¹ dwt	$F_{W/S}$
Deep peat under clearfelled area	L	84	14				1.37
	O ₁	94	6	4.4	31		1.11
	O ₂	75	17	4.7	35		1.45
Peaty podzol under moorland	O	71	14	4.1	3	24	1.21
	E _a	62	11	4.6	57	120	1.18
	B _g	46	6	5.1	120	142	1.04
Deep peat under Sitka spruce forest	O ₂	91	9	3.7	2	5	1.26
Peaty gley under larch forest	B	62	13				1.42
Mean (± 1 SD)							1.25 ± 0.15

The partition ratios determined for eight soils from the Ballochbeatties site are summarised in Table 4. The mean partition ratio ($F_{W/S}$) of 1.25 was used to calculate TCA concentrations in all soils from Ballochbeatties. There was no relationship between $F_{W/S}$ and any of the soil parameters given in Table 4. The effect of uncertainty in the value of $F_{W/S}$ is a proportional uncertainty in the derived soil TCA concentration and the effect of this uncertainty on experimental results is discussed later. A partition ratio of 2.01 was determined for soil from the Cowpark agricultural site (higher mineral and lower organic matter content).

The concentration of TCA in the soil sample, C_S (ng g⁻¹), was then calculated from an expression analogous to eqn. (2), substituting f_W with $f_W/F_{W/S}$, and correcting for any TCA present in the 1 ml of added water,

$$C_S = \left[\frac{(A_S - A_{SBG})F_{W/S}}{f_W} - (M_W \times C_W) \right] / M_S \quad (3)$$

where A_S is the peak area of CHCl_3 from heating soil (+1 ml water) sample to 100 °C, A_{SBG} is the peak area of CHCl_3 from heating soil (+1 ml water) sample to 60 °C, C_W is the concentration of TCA in added ultrapure water (ng g⁻¹), determined by application of eqn. (2) to samples of water analysed on the same GC run, M_W is the mass of water added to vial (g) and M_S is the mass of fresh soil (g).

The concentration, C_W , of TCA in the water added to the soil samples was determined using five replicates and two background samples analysed in the same GC run.

If the RSD of the triplicate analyses of a soil sample exceeded 30%, the sample was reanalysed. The mean % RSD of all soil samples was 19% ($n = 88$) compared with 10–17% for aqueous samples,¹⁷ reflecting the more heterogeneous nature of soil.

Soil moisture and organic matter contents were determined by drying to constant weight at 60 °C, and by loss on ignition at 550 °C for 8 h, respectively.

2.3. Experiments to determine whether CHCl_3 originates from other components

(1) Formation of CHCl_3 during soil sample heating. To test the hypothesis that soils heated above 60 °C in the decarboxylation step liberate CHCl_3 independently of TCA (by either abiotic or microbial processes), replicates of forest soil were heated either at 65 °C for 72 h or 100 °C for 90 min before analysis for CHCl_3 by HSGC. The former are the TCA decarboxylation conditions employed by Plümacher and Renner.²⁹

(2) Formation of CHCl_3 from humic acid. Humic acid (HA) is an important component of all soils. Experiments were conducted to investigate whether HA liberates CHCl_3 on heating between 60 °C and 100 °C, and whether TCA can be detected

by HSGC in the presence of HA. Two concentrations of TCA solution (11 μg l⁻¹ and 114 μg l⁻¹) and two concentrations of commercial HA solution (6.4 mg l⁻¹ and 64 mg l⁻¹, Fisher Scientific, UK) were used to prepare three sets of headspace vials containing the following: each HA solution only (2 ml); each TCA solution only (2 ml); each of the four combinations of TCA + HA solution (1 ml + 1 ml). One set was analysed for TCA immediately, using the method described above, and the other sets after 2 d and 11 d storage in the dark at <5 °C, to investigate any effect of contact time between HA and TCA on TCA determination. TCA concentrations equal to or greater than those found in natural waters (<0.05–20 μg l⁻¹) were used to ensure that any changes in TCA concentration resulting from the presence of HA could be clearly distinguished.

2.4. Experiments on recovery of soil TCA by aqueous extraction and whole soil analysis

Three sets of experiments were conducted using an extraction technique similar to that used in the extraction-derivatisation methods of TCA analysis to determine the recovery of intrinsic and extrinsic (added) TCA in soil between aqueous extract and soil residue. A further experiment investigated the ability of the decarboxylation-HSGC method to account for TCA bound to an anion exchange resin.

(1) Extraction of intrinsic soil TCA. Six experimental replicates of fresh Sitka soil and two experimental replicates of fresh larch soil were extracted with ultrapure water. In each extraction, 10 ml of ultrapure water was added to 10 g of sieved (2 mm), homogenised, fresh soil in a centrifuge tube. The tube was sealed, shaken for 16 h using an orbital mechanical shaker (Gallenkamp, 200 rpm), centrifuged at 9000 g for 15 min and the supernatant (“soil extract”) removed to a clean vial using a pipette. The soil mixture was centrifuged again and any additional supernatant removed and combined with the first extract. The soil extract and remaining solid (“soil residue”) were analysed for TCA by HSGC in the same GC run as a sample of unextracted soil (“whole soil”) and ultrapure water. The masses of the soil, added water, residue and extract were recorded prior to analysis. The TCA mass in each of the soil extract and soil residue was calculated from the product of their respective measured masses and TCA concentrations, and the total TCA in both these fractions was compared to the total mass of TCA determined similarly in the whole soil and in the water extractant to calculate the extent of mass balance at the end of the experiment.

(2) Extraction of extrinsic soil TCA. “TCA-free” soils were obtained by heating soil samples for 5 d at 65 °C and 1.5 h at 100 °C to remove intrinsic soil TCA and CHCl_3 . This was done on three experimental replicates of moorland soil (52% water, 9% organic matter), two experimental replicates of agricultural

soil (27% water, 5% organic matter) and one experimental replicate of larch soil (32% water, 8% organic matter). Triplicate analyses by HSGC of a subsample of each soil showed that the TCA concentrations were below the limit of detection ($\sim 0.1 \mu\text{g g}^{-1}$). Subsamples ($\sim 10 \text{ g}$) of the TCA-free soils were rehydrated with 15 ml of $50 \mu\text{g l}^{-1}$ TCA solution and extracted and analysed as described above.

(3) Extraction of intrinsic and extrinsic soil TCA. The efficiency of recovering both intrinsic and extrinsic soil TCA by aqueous extraction was investigated by spiking samples of fresh soil (10 g) with 10 ml of $\sim 90 \mu\text{g l}^{-1}$ TCA solution which were then extracted and analysed as described above. Two replicate experiments on each of the following soil types were performed: Sitka (deep peat: 84% water, 15% organic matter), agricultural (gleysol: 25% water, 5% organic matter) and larch (peaty gleysol: 67% water, 12% organic matter).

(4) Recovery of TCA added to anion exchange resin. 20 ml of a $20 \mu\text{g l}^{-1}$ TCA solution was added to approximately 1 g of Dowex $1 \times 8-50$ anion exchange resin (AER) in a sterile centrifuge tube. The tube was shaken by hand, allowed to stand for 30 min and then centrifuged for 15 min at 9000 g. The supernatant was removed using a pipette and analysed for TCA. The AER was washed out of the tube with ultrapure water, recovered on a Whatman no. 1 filter paper and three sample replicates and one blank, each of approximately 0.25 g, were weighed into headspace vials for TCA analysis. The masses of the AER, TCA solution, AER residue and supernatant were recorded before analysis. TCA concentration in the AER and AER residue samples were calculated assuming a range of partition ratios (1, 1.25 and 1.5) since partition ratios were not determined for these materials.

3. Results and discussion

Quoted uncertainties are ± 1 SD of experimental replicates unless otherwise stated.

3.1 Experiments to determine whether CHCl_3 originates from other soil components

TCA concentrations measured in replicates of forest soil decarboxylated at 65°C over 72 h or 100°C over 90 min were not significantly different: 337 ± 28 ($n = 3$) and 267 ± 15 ($n = 3$) ng TCA g^{-1} fwt, respectively. This shows that heating soil above the temperature of 60°C used for background CHCl_3 correction does not result in the detection of enhanced CHCl_3 concentrations.

In the experiments conducted with HA, there was no significant difference in TCA concentration between ultrapure water and the samples of either 6.4 mg l^{-1} or 64 mg l^{-1} HA solution ($P > 0.05$, unpaired t test). The HA used was therefore neither a source of TCA nor of interfering CHCl_3 when heated to decarboxylation conditions. The TCA concentrations determined in the mixed HA + TCA solutions ("post-mixing TCA") were compared with the concentrations determined in the parallel single component TCA and HA solutions ("pre-mixing" TCA). The recoveries of TCA "post-mixing", expressed as a percentage of TCA "pre-mixing", are shown in Fig. 2 for each of the HA + TCA combinations after 0, 2 and 11 d storage. The mean percentage TCA recovery for all of the HA + TCA combinations was $108 \pm 13\%$ ($n = 12$, range 92–134%). There was no significant difference (2-way ANOVA, $P = 0.85$) in TCA recovery between the different TCA and HA combinations or between mixtures stored for different times prior to analysis.

The following is concluded from these data: (a) TCA is neither produced nor degraded by HA in solution; (b) TCA

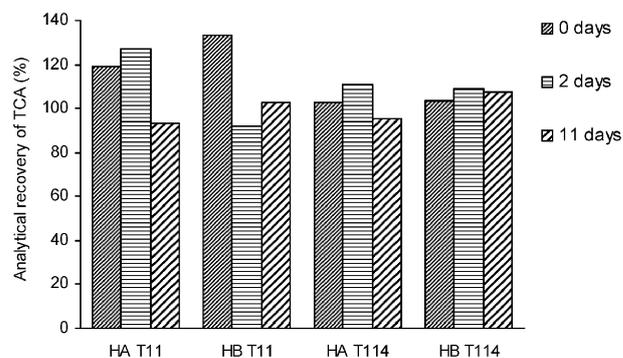


Fig. 2 The percentage analytical recovery of TCA added to solutions of humic acid. Different combinations of humic acid concentrations (6.4 mg l^{-1} (HA) and 64 mg l^{-1} (HB)) and TCA concentrations ($11 \mu\text{g l}^{-1}$ (T11) and $114 \mu\text{g l}^{-1}$ (T114)) were analysed. Samples were analysed on the day of preparation (day 0) and after 2 d and 11 d.

remains quantitatively detectable by decarboxylation to CHCl_3 at 100°C in the presence of HA (regardless of whether or not a proportion of TCA binds to HA); (c) HA does not liberate additional CHCl_3 when heated between 60°C and 100°C and (d) the above conclusions apply for contact of at least 11 d between TCA and HA.

In contrast, Haiber *et al.*,¹⁸ using mixtures of commercial HA and spring water spiked with $1 \mu\text{g l}^{-1}$ TCA solution and analysis of TCA by an extraction-derivatisation method,³⁶ reported that TCA recoveries decreased with increased HA concentration (in the range $0-20 \text{ mg l}^{-1}$) and with time elapsed between TCA addition and analysis (0–7 d). Both sets of experiments are compatible with the hypothesis that TCA binds to HA so that only a proportion remains aqueous-extractable. Care must be taken in applying these conclusions to TCA in soil in field conditions because of potential differences in the characteristics of HA in solution and humic material in soil.

The experimental results described in this section show no evidence for CHCl_3 formation of non-TCA origin under the decarboxylation conditions employed in this work.

3.2 Recovery of soil TCA by aqueous extraction and whole soil analysis

In the set of experiments investigating extraction of intrinsic soil TCA, the Sitka and larch whole soil TCA concentrations were 38 ± 5 ($n = 6$) and 21 ± 1 ($n = 2$) ng g^{-1} fwt, respectively. Fig. 3 shows that excellent analytical mass balance was

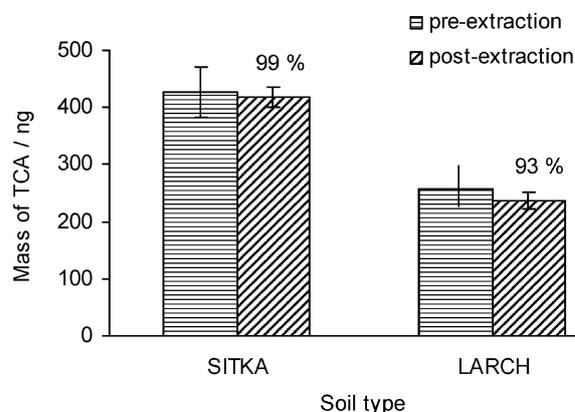


Fig. 3 The mass of TCA present in the whole soil + extractant water pre-extraction, and in the soil extract + soil residue post-extraction. The mass balance (TCA measured post-extraction as a percentage of TCA measured pre-extraction) is displayed above the post-extraction bars. Error bars are standard deviations of six (Sitka), or two (larch) experiments, with each TCA analysis in triplicate.

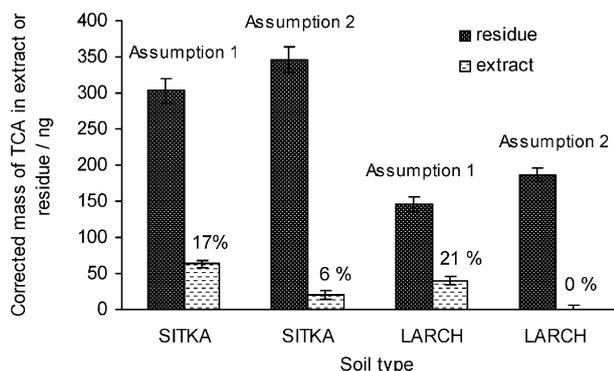


Fig. 4 The mass of TCA measured in both soil residue and soil extract after extraction of different soil samples with 10 ml ultrapure water. Recovery of TCA in the soil extract (as a percentage of the total TCA in the extract + residue) is stated above the extract bars, where correction for TCA already present in the water (~ 50 ng) has been performed in two ways: Assumption 1 – complete mixing; or Assumption 2 – all added TCA remains in the extractant phase. Error bars are standard deviations of six (Sitka), or two (larch) soil experiments, with each TCA analysis in triplicate.

achieved between the sum of TCA measured in the whole soil plus extractant water prior to extraction, and the sum of TCA measured in the soil extract plus residue after extraction. Post-extraction TCA was $99 \pm 8\%$ and $93 \pm 6\%$ of pre-extraction TCA, respectively, for the Sitka and larch soils, showing that mass balance was provided by the decarboxylation method.

Note on uncertainties: the uncertainties in the above figures are ± 1 SD of experimental replication. Uncertainty in the value of the analytical partition ratio, $F_{W/S}$, (± 1 SD of the mean is 12%, Table 4) contributes additional uncertainty to calculation of whole-soil and soil-residue TCA concentrations, but not to aqueous TCA concentrations which do not require a partition ratio. Including the partition ratio uncertainty does not change the values of the mass balances quoted above, but increases their uncertainties from $\pm 8\%$ and $\pm 6\%$ to $\pm 14\%$ and $\pm 13\%$ for the Sitka and larch soils, respectively. In the remainder of the discussion of soil extraction experiments the quoted uncertainties are for experimental replication only. Allowance for uncertainty in the analytical partition ratio would increase the quoted uncertainties by a few percentage points but not change the quoted mean value.

It was not possible to know how the TCA present in the water used for extraction ($5.1 \pm 2.9 \mu\text{g l}^{-1}$) became apportioned between the extract and residue. Therefore, for this set of experiments, the masses of TCA measured in the soil residue and extract were corrected for the TCA added in the extractant water using either: (1) the assumption of complete mixing of added TCA, or (2) the assumption that all TCA in the extractant remained in the extract phase. Fig. 4 shows that if all TCA in the water extractant is assumed to remain in the extract phase, the proportion of intrinsic TCA extracted from the soil was negligible for both the Sitka and larch soils. The figure shows that even if the TCA in the water extractant is assumed to be proportionally mixed in the residue and extract, the proportion of intrinsic TCA extracted from the soil was still small, with only $17 \pm 2\%$ of TCA in the Sitka soil and $21 \pm 1\%$ of TCA in the larch soil being extracted.

Fig. 5 shows the results of the experiments in which extrinsic TCA was extracted from “TCA-free” soils. The poorer analytical mass balances ($148 \pm 12\%$, $122 \pm 3\%$ and 124% , for agricultural, moorland and larch soils, respectively, Fig. 5b) reflect the fact that the same partition ratio was used to calculate TCA concentrations in the whole “TCA-free” soil as for fresh soil even though soil characteristics were probably altered during preparation of the “TCA-free” samples by heating and drying. Nevertheless, it is clear from Fig. 5a that

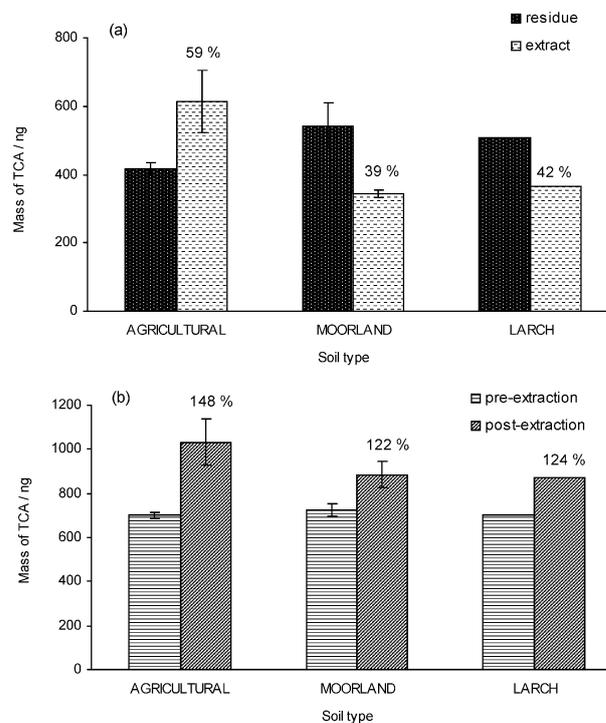


Fig. 5 (a) The mass of TCA measured in the soil residue and soil extract after addition of 15 ml of $50 \mu\text{g l}^{-1}$ TCA solution to “TCA-free” soil, and re-extraction. Recovery of TCA in the soil extract (as a percentage of the total TCA in the extract + residue) is stated above the extract bars. (b) The mass of TCA present in the whole soil + spike solution pre-extraction, and in the soil extract + residue post-extraction. The analytical mass balance (TCA present post-extraction as a percentage of TCA pre-extraction) is displayed above the post-extraction bars. Error bars are standard deviations of three experiments for moorland soil and two experiments for agricultural soil, with each TCA analysis in triplicate.

the applied TCA still cannot be fully recovered by aqueous extraction. Less TCA was recovered in the extracts from larch (42%) and moorland ($39 \pm 3\%$) soils compared to the agricultural soil ($59 \pm 3\%$), which could be due to the higher organic matter contents of the former soils and possibly a greater capacity for TCA binding with humic materials. (Note that in this and the other extraction experiment involving addition of a TCA spike, the measured TCA masses in the residue and extract were used directly to calculate the recovery of TCA in the extract since TCA added in the extractant water was small compared with TCA added in the spike).

Fig. 6 shows the results of the experiments in which spiked fresh soils were extracted with water. For the Sitka and agricultural soils, excellent analytical mass balances of $114 \pm 13\%$ and $102 \pm 2\%$, respectively, were again obtained (Fig. 6b), but again the extraction was far from complete, with only $50 \pm 3\%$ and $55 \pm 3\%$ of the sum of intrinsic soil TCA plus spike solution TCA recovered in the extracted component (Fig. 6a). In contrast, analytical mass balance for the spiked larch soil was poor ($25 \pm 5\%$, Fig. 6b), although, as for all the other extraction experiments, the proportion of TCA measured post-extraction that was in the extracted component was minor ($23 \pm 2\%$). The reason for the poor analytical recovery of TCA spiked to fresh larch soil is not known but, interestingly, it was also observed in a separate experiment in which $20 \pm 3\%$ mass balance and $15 \pm 12\%$ TCA proportion in the extract were obtained for eight replicate extractions of larch soil spiked with $100 \mu\text{g l}^{-1}$ TCA. Since analytical mass balance was achieved for larch soils in the other extraction experiments described above, the discrepancy cannot be due to larch soil specific TCA degradation processes or other processes in the soil causing inability to detect TCA by decarboxylation.

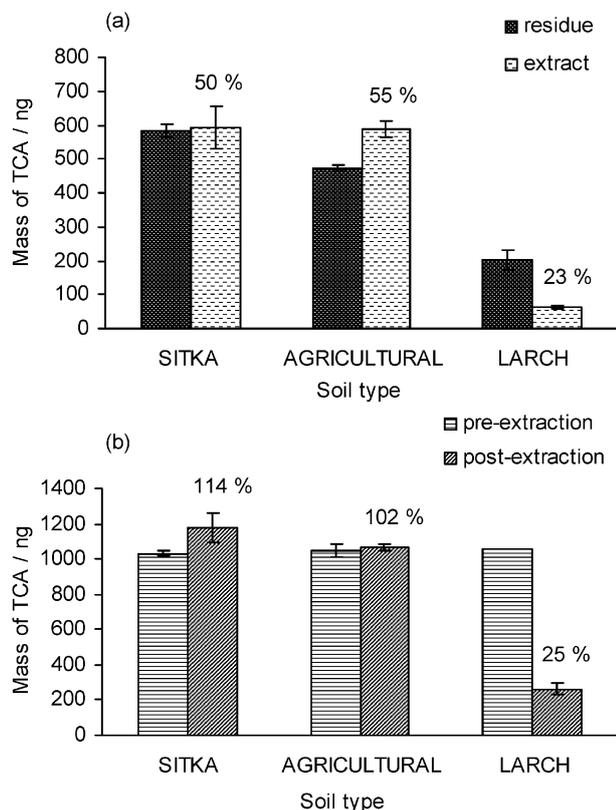


Fig. 6 (a) The mass of TCA measured in the soil residue and soil extract after addition of 10 ml of $90 \mu\text{g l}^{-1}$ TCA solution to different soil types and re-extraction. Recovery of TCA in the soil extract (as a percentage of the total TCA in the extract + residue) is stated above the extract bars. (b) The mass of TCA present in the whole soil + spike solution pre-extraction, and in the soil extract and soil residue post-extraction. The analytical mass balance (TCA measured post-extraction as a percentage of TCA pre-extraction) is displayed above the post-extraction bars. Error bars are standard deviations of two experiments for each soil type, with each TCA analysis in triplicate.

The results of the extraction experiments show that TCA, either intrinsic or added to the soil, cannot be fully recovered by aqueous extraction across a range of different soil types. This is very likely due to binding of TCA to ion exchange sites in the soil, such that bound TCA cannot be determined by deionised water extraction but can be determined by decarboxylation. The TCA-binding suggestion is supported by the results of the experiment in which TCA solution was shaken with anion exchange resin (AER) before separation into supernatant and AER residue. In this experiment, the mass of TCA in the TCA solution and resin prior to separation was determined to be 418 ng, whilst the sum of the masses of TCA present in the AER residue and aqueous supernatant after separation by centrifugation was 419 ng, an analytical mass balance of 100%. Of this latter total, 94% was in the AER residue and only 6% was in the supernatant. These data were determined using a partition ratio of 1 for quantifying CHCl_3 in the presence of AER in the vial. However, as indicated in Section 2.4, the partition ratio was not experimentally determined, so the data were also calculated taking values for the partition ratio of 1.25 or 1.5. Applying these partition ratios caused deterioration in the analytical mass balance values to 123% or 145%, respectively. This uncertainty does not alter the fundamental observation that the proportion of TCA observed in the supernatant is small relative to that remaining in the AER. (The proportion TCA in the supernatant was amended to 5% or 4% for calculations using the two alternative partition ratios).

That only a small proportion of TCA is found in the aqueous phase in the presence of an anion exchange resin is

expected since TCA in solution exists predominantly as the trichloroacetate anion. The point of the experiment is to illustrate two key conclusions: (1) the decarboxylation HSGC method can determine TCA bound within a solid phase; (2) TCA in the presence of anion exchange sites is not readily water extractable. It seems reasonable to assume that similar conclusions apply to TCA in the soil, namely, that the binding of a proportion of TCA to particular sites within the soil matrix prevent its determination by extraction, but not by decarboxylation.

4. Conclusions

Mass balance experiments provide good evidence that the decarboxylation-HSGC method of analysis for TCA in a range of soil types can account for all TCA within the matrix and that neither the intrinsic soil TCA nor TCA added to fresh or dried soil, can be efficiently extracted into solution. Extraction efficiencies were poorer in more organic soils suggesting that "bound" TCA may be associated with the organic fraction of the soil. This is supported by experiments which show that TCA in humic acid solution can be quantified by the decarboxylation technique, in contrast to results reported from a similar study which showed TCA could not be recovered from humic acid solution using an extraction method of analysis.¹⁸ Furthermore, TCA cannot be extracted from anion binding sites but remains detectable by decarboxylation-HSGC. There is little evidence that other entities in the soil aside from TCA yield CHCl_3 under the decarboxylation condition of raising the temperature of the soil from 60°C to 100°C for 1.5 h. However, even if soil TCA concentrations are being over-estimated by the decarboxylation-HSGC method, a range of evidence has been presented which suggests that extraction-derivatisation methods of TCA analysis may significantly underestimate whole soil TCA concentrations. Water-extractable TCA may be a useful surrogate for biologically-available TCA, but this is not yet known. Neither is the extent of variability of aqueous extraction efficiency between soil types. Overall, the two prevailing methods for soil TCA analysis, if used together, may enhance insight into TCA behaviour in soil.

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