The Behaviour of Trichloroacetic Acid in Soil and its Uptake and Effects in Sitka Spruce Trees

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I declare that the work presented in this thesis is my own unless stated otherwise.

Abstract

Trichloroacetic acid (TCA: CCl_3COOH) has been detected in all environmental compartments (rainwater, soil, vegetation, air) but is phytotoxic and has been associated with forest damage. Studies of an upland site in Scotland suggest that the catchment store of TCA is dominated by soils (90 %). A greater understanding of soil TCA cycling is required to assess the fate of chemicals since TCA has anthropogenic (chlorinated solvents) and natural sources. In this research, field and laboratory experiments were conducted using forest and agricultural soils to investigate the factors controlling TCA in soil, and estimate the relative rates of soil TCA production and elimination. To assess the effects of TCA on forest health, TCA uptake by Sitka spruce saplings (*Picea sitchensis*) via atmospheric and soil routes, and its effects on growth, enzyme activity and water retention of needles and visible damage were investigated.

The total TCA in environmental samples was determined by quantification of chloroform formed from thermal decarboxylation of TCA, using headspace gas chromatography and electron capture detection (HSGC-ECD). Other published methods determine TCA in soil aqueous extracts. To validate the HSGC-ECD method experiments were conducted involving the addition of TCA spikes to soils. It was found that TCA could not be completely extracted into water, although 100 % mass balance was obtained. Furthermore, humic acid did not contain any moieties that form chloroform on heating to 100 °C and 100 % of TCA added to humic acid solution was recovered.

Mean TCA concentrations in soils from an upland forest site and a lowland agricultural site were 48 (± 85, n = 84) ng g^{-1} and 26 (± 18, n = 5) ng g^{-1} (fresh weight). TCA concentrations varied greatly between soil types and decreased significantly with soil depth. In spruce soils TCA concentration was negatively related to water content, and positively related to soil organic matter content. A strong positive relationship ($R^2 = 0.9$, $P<0.001$) observed between soil TCA concentration and soil microbial biomass carbon, in a variety of soil types, may be evidence for formation of TCA in soils by micro-organisms.

In a 6-month field experiment, lysimeters (2.5 l) at forest and agricultural sites were dosed fortnightly with solutions containing 20 or 50 μg TCA, and soil leachates collected over 2 week periods. In all lysimeters less than 30 % of TCA applied to soil was recovered in soil leachate. The "lost" TCA was not detected in the soil, indicating that the applied TCA was degraded, or bound to organic matter within the soil. In experiments where soil was spiked with radioactive [1,2-^{14}C] TCA (≈ 380 ng g^{-1}(fwt)), $^{14}CO_2$ was produced, confirming that TCA in soil is rapidly degraded by micro-organisms, with a half-life of approximately 21 – 46 hours depending on soil type.

In a controlled greenhouse experiment 6-year old Sitka spruce saplings were exposed twice a week over two growing seasons, to 0, 10 or 100 μg l^{-1} solutions of TCA via either the foliage or soil. TCA was taken up into tree needles via both routes (with foliage route being more significant than previously thought). There was no effect of different treatments on sapling growth, but increased activity of detoxification enzymes in saplings exposed to TCA via the foliage suggested that this route of uptake causes greater stress. In a separate experiment a single dose of TCA (200 μg) was applied to Sitka spruce saplings via either the foliage or soil. Bi-weekly analyses showed rapid uptake of TCA into the needles via both routes. The half-life of TCA elimination in foliage was estimated at approximately 7 weeks during the growing season. These results suggest that trees growing in areas of frequent cloudwater episodes may be more likely to suffer long-term health effects from TCA uptake.

This research has contributed to the understanding of TCA occurrence and cycling in different soils and the effects of TCA from different sources, on tree health. Key uncertainties remaining in TCA processing in soil are TCA behaviour under environmental conditions and the specific role of micro-organism activity, especially in TCA production.

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Contents

Chapter 1 – Introduction

1.1 GENERAL BACKGROUND

Trichloroacetic acid, CCl_3COOH, (TCA, Figure 1) has in recent years, been the subject of much research due to its phytotoxic nature and purported contribution to forest dieback (Frank, 1988; Frank *et al.*, 1990, 1992, 1994; Plümacher and Schröder, 1994; Norokorpi and Frank, 1995; Sutinen *et al.*, 1997; Weiss *et al.*, 2000). Since then, TCA has been identified in most environmental media at the parts per billion range (McCulloch, 2002).

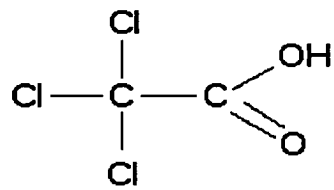


Figure 1.1. Chemical structure of trichloroacetic acid (TCA)

The main physicochemical properties of TCA are summarised in Table 1.1. TCA has a high Henry's Law constant, expressed as a liquid to gas partition ratio (Bowden *et al.*, 1998) and, with a high solubility, it exists mainly in the liquid phase and does not readily partition into the atmosphere.

Table 1.1. Physicochemical properties of TCA (WHO, 2001).

Melting point	58 °C
Boiling point	198 °C
Henry's Law constant	7.4×10^{4} mol l^{-1} atm^{-1}
pK_a	0.26
Log K_{ow}	<1 – 1.6
Water solubility	1200 g l^{-1} @ 20 °C

TCA in the form of sodium trichloroacetate was introduced as a herbicide in the 1950s, principally to control wild grasses in brassicas and similar commercial monocotyledonous crops (Lockart *et al.*, 1990). Its use has since been banned in the

European Union, except for Ireland and Italy (Hoekstra 2003), due to its indiscriminate effects on woody plant species and concern over its toxicity to humans (Juuti & Hoekstra, 1998) and the development of more effective herbicides requiring lower rates of application. During the use of TCA as a herbicide, many studies were conducted on TCA behaviour in soil which focused on the rates of degradation of large TCA applications (parts per million) in agricultural soils. However, in the 1990s there was a surge of interest in the study of TCA from a more environmental perspective as investigations by Frank *et al.* (1990, 1992, 1994), and Norokorpi and Frank (1995) reported positive correlations of TCA concentrations in needles with defoliation of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) trees in Finland and Germany. TCA has more recently been the subject of risk assessment under the OECD SIDS programme (Screening Information Data Set operated under the Organisation for Economic Cooperation and Development) which dealt with the production and use of TCA as an industrial chemical (Peters, 2000). It was concluded that TCA in soil may have adverse environmental effects and that its presence in soil could be due to the atmospheric breakdown of chlorinated solvents such as tetrachloroethene (PER). It is therefore essential that a greater understanding of TCA fluxes, particularly in the soil compartment, is acquired in order to monitor and control the potential ecotoxicological effects of TCA presence in the environment.

The presence and cycling of TCA in the atmosphere, conifer trees and soil has been widely researched since the initial suggestion by Frank (1988) that TCA may contribute to forest dieback. However, much of this research has been approached from a physical sciences perspective, with studies of TCA in environmental matrices failing to acknowledge the complexity of natural biological systems, in particular the soil compartment. As a result, many experiments have been characterised by poor experimental and analytical replication, with conclusions drawn from a few unreplicated experiments. Subsequent research has often relied on these poorly-supported assumptions, without questioning their integrity and there has been a tendency for old data to be "recycled".

Methods of TCA analysis

Concentrations of TCA in air, precipitation, surface waters and the needles of conifers have been well documented over the past decade. For most environmental media there is good consistency between reported concentrations by independent research groups using different analysis techniques. However, there are currently few and widely varying measurements of TCA in the soil environment, as discussed in Section 1.2.3. This is partly due to the lack of a standard, accurate method of analysis. The analytical method used in this study attempts to quantify the TCA present in the "whole soil" (i.e. water, organic matter and mineral components) which is achieved by decarboxylation of TCA to chloroform in a sealed vial followed by analysis of the chloroform gas by headspace gas chromatography with electron-capture detection (HSGC-ECD). Other methods require the extraction of soil TCA into water by shaking and centrifugation followed by derivatisation to, for example, the methyl ester of TCA which is then analysed by gas chromatography with mass spectrometry or electron-capture detection (Frank *et al.*, 1990; Clemens and Schöler, 1992; Hoekstra and de Leer, 1993; Scott and Alaee, 1998; Peters, 2000; Nikolaou *et al.*, 2002).

1.2 TCA IN DIFFERENT ENVIRONMENTAL COMPARTMENTS

TCA concentrations reported in selected environmental compartments by a number of researchers are summarised in Table 1.2. The literature often does not state the size of the sample, the method of TCA analysis used and whether soil and foliage TCA concentrations are expressed on a fresh weight (fwt) or dry weight (dwt) basis. Nevertheless, it is evident that TCA can be detected in most environmental matrices with the highest concentrations measured in vegetation and soil. Its relatively uniform presence in urban and rural areas, in the northern and southern hemispheres has generated much discussion about the sources of TCA and the possible risks it may pose to rural ecosystems. As TCA applied to soil as a herbicide is reported to have a half-life in the order of weeks (summarised by Foy, 1975) and its current use in rural areas is unlikely, the presence of TCA in the environment today cannot be attributed to its former use as a herbicide.

Table 1.2. Reported TCA concentrations in selected environmental compartments. (LOD = limit of detection)..

Sample		TCA concentration	Reference
Air		<LOD – 700 pg m^{-3}	7,16,19,20,22
Rainwater		<LOD – 20 μg l^{-1}	5,9,11,17,20
Snow		<LOD – 0.35 μg l^{-1}	9,11,14,
Forest throughfall	Spruce	<LOD – 2.2 μg l^{-1}	13
	Beech	<LOD – 1.03 μg l^{-1}	13
Bog water		0.33 – 1.0 μg l^{-1}	9
River water		<0.03 – 22.0 μg l^{-1}	7,9,11,12,21,22
Old ice (Alps, ca. 1900)		0.1 μg l^{-1}	9
Firn (Antarctica, 15 – 20 m deep)		0.003 – 0.04 μg l^{-1}	14
Vegetation	Conifer needles	0.7 – 180 ng g^{-1}	2,3,5,6,8,10,15,22
	Larch needles	142 – 187 ng g^{-1} (dwt)	22
	Broadleaves	<LOD – 44 ng g^{-1} (fwt)	1
	Moorland vegetation	22 – 103 ng g^{-1} (dwt)	21
Soil		<0.05 – 390 ng g^{-1}	1,4,15,18

(1) Frank (1988), (2) Frank (1991), (3) Frank *et al.* (1992), (4) Hoekstra and de Leer (1993), (5) Plümacher and Renner (1993), (6) Plümacher and Schröder (1994), (7) Frank *et al.* (1995), (8) Norokorpi and Frank (1995), (9) Haiber *et al.* (1996), (10) Juuti *et al.* (1996), (11) Müller *et al.* (1996), (12) Hashimoto *et al.* (1998), (13) Hoekstra *et al.* (1999a), (14) Von Sydow *et al.* (1999), (15) Weissflog *et al.* (1999), (16) Peters (2000), (17) Scott *et al.* (2000), (18) Reeves (2001), (19) (Bakeas *et al.* (2003), (20) Heal *et al.* (2003b), (21) Schöler *et al.* (2003), (22) Stidson *et al.* (2004a).

1.2.1 TCA in air and water

Measured TCA concentrations in air from urban and rural areas vary from <LOD (limit of detection) to 0.7 ng m^{-3} (Table 1.2) and are fairly consistent between research groups. Due to its high Henry's Law constant (and hence high solubility) TCA concentrations are not expected to be high in the gas phase and it is generally assumed that TCA in air is mainly associated with particulate matter (Bakeas *et al.*, 2003; Schöler *et al.*, 2003). Conversely, from measurements of air in Scotland and Canada, Heal *et al.* (2003a) and Martin *et al.* (2003), reported that at least half of total atmospheric TCA is associated with the gas phase rather than the particulate phase. It is possible that although TCA is initially present in the gas phase, it does not reside long before becoming associated with atmospheric condensed liquid water (Ellis *et al.*, 2001).

TCA has also been detected in most rain and snow samples ranging in concentration from <LOD to 20 $\mu g\ l^{-1}$ (Table 1.2) with no obvious pattern of variation between locations. TCA in rainwater is unlikely to be released into the gas phase due to its high Henry's Law constant and Hoekstra *et al.* (1998b) calculated that for aqueous TCA concentrations in the range 0 to1000 $\mu g\ l^{-1}$ there will be no emission from the water environment if the average air concentration is in the range of 100 to 1000 pg m^{-3} and the pH of the water ≥ 4. Although Berg *et al.* (2000) demonstrated washout of haloacetic acids in rainwater, a significant negative correlation between the depth of rainfall and TCA concentration has not been found (Clemens, 1993, cited in McCulloch, 2002; Reimann *et al.*, 1996; Heal *et al.*, 2003a). In a 2-year sampling campaign in southeast Scotland, Heal *et al.* (2003a) reported a correlation (R = 0.48) between weekly concentrations of TCA in the air (gas + particle phase) and rain, indicating that TCA behaviour in the atmosphere at this site may be controlled by an equilibrium (Henry's Law) partitioning.

Römpp *et al.* (2001) reported significantly greater TCA concentrations in cloudwater than rainwater. However, Heal *et al.* (2003a) only reported a slight enhancement of TCA in cloudwater and Stidson *et al.* (2004a) reported no significant difference between TCA concentrations of rainwater and cloudwater inputs to an upland catchment in Scotland, over one year. Heal *et al.* (2003a) observed a strong inverse relationship between TCA concentration and cloudwater depth whereas Römpp *et al.* (2001) observed no dependence of TCA concentration on fogwater volume, which further emphasises the lack of a clear and consistent picture regarding TCA presence and behaviour in the atmosphere.

Some researchers have reported higher TCA concentrations in precipitation in the summer compared to winter (Haiber *et al.*, 1996; Reimann *et al*, 1996; Schöler *et al.*, 2003) which is possibly due to higher atmospheric photochemical and biochemical activity in warmer months, assuming that atmospheric TCA is derived from these processes. Other studies have failed to detect any seasonal dependence of TCA in

precipitation (Plümacher, 1995; Berg *et al.*, 2000; Heal *et al.*, 2003a) suggesting that TCA may also be formed from other mechanisms.

As expected, due to its high solubility, TCA has also been detected in bog-water, rivers, lakes and seawater in concentrations from <0.03 to 22 $\mu g\ l^{-1}$ (Table 1.2). Due to the small amount of data available for these surface waters it is not possible to determine if there are any relationships with TCA concentrations in precipitation water, which may provide some information on the sources of TCA (Section 1.3).

1.2.2 TCA in vegetation

TCA has been detected in a variety of plant species but is generally more concentrated in conifers (McCulloch, 2002). High concentrations of TCA have been shown to affect many plants: by increasing respiration, inhibiting enzyme activity and hence reducing plant growth; and inducing leaf chlorosis and necrosis (Barrons and Hummer, 1951; Åberg, 1982). TCA concentrations reported in the needles of conifers vary extensively from 1 to 180 $ng\ g^{-1}$ in rural forests (Table 1.2) although it is not clear if these are on a fresh or dry weight basis. Investigations by Frank *et al.* (1990, 1992, 1994) and Norokorpi and Frank (1995) reported correlations of TCA concentrations in conifer needles (up to 180 $ng\ g^{-1}$) with defoliation of trees and damage of the surface wax layer, although needle loss is a non-specific phenomenon and is therefore not a reliable indicator of the effects of TCA alone. Routes of uptake of TCA into the foliage of trees have so far only been investigated in controlled environments using TCA at concentrations greater than those found in natural precipitation (Sutinen *et al.*, 1997; Forczek *et al.*, 2001, Matucha *et al.*, 2001). There are no reported studies on the effects of TCA on more mature trees exposed to environmental concentrations of TCA over more than one growing season.

Since TCA is soluble in water and the surfaces of needles are lipophilic, it has been thought unlikely that TCA is taken up directly from air or water into needles. Previous studies have indicated that the main route of uptake of TCA into conifer needles is from the soil into the root system, then to the needles via the transpiration stream (Blanchard, 1954; Sutinen *et al.*, 1995; Matucha *et al.*, 2001). However,

Stidson *et al.* (2004b) found strong indications from a year long field study that above-ground routes are important for uptake of TCA into mature forest canopies. It has also been postulated that TCA may be formed *in-situ* in foliage from chlorinated precursors such as tetrachloroethene (PER) and 1,1,1 trichloroethane (TCE) (Henschler *et al.*, 1977, cited in Hoekstra, 2003; Frank, *et al.*, 1992).

There are few measurements of TCA concentrations in deciduous trees, maybe because they are considered less vulnerable to TCA pollution, as TCA concentrations are less likely to build up to phytotoxic levels. However, the accumulation of TCA in other parts of the plant should also be considered as well as variation in physiological processes between species.

1.2.3 TCA in soil

A wide range of TCA concentrations have been reported in soil, from <0.05 to 390 ng g^{-1} (fwt), most of which have high standard deviations associated with them, as summarised in Table 1.3. Uncertainties in soil TCA concentrations most likely exist because of the heterogeneous nature of the soil, the different characteristics of sites investigated (e.g. soil type, altitude, vegetation cover, annual rainfall) and the different analysis techniques employed. Using an extraction-derivatisation method of TCA analysis, Hoekstra (2000, cited in Reeves, 2001), reported poor recoveries of TCA in his experimental procedure while concentrations of 0.2 to 4.6 ng g^{-1} (dwt) measured by Hoekstra and de Leer (1993) were not replicated. Also using an extraction-derivatisation technique, Frank (1988) and Plümacher (1995) obtained higher soil TCA concentrations with greater variability. TCA concentrations in Plümacher's soils from pine forests ranged from 1.4 to 120 ng g^{-1} (dwt) and Frank's from 20 to 380 ng g^{-1} although whether this latter figure was for fresh or dry soil is not clear. Peters (2003), in an extensive study of TCA concentrations in soils throughout Europe, found TCA concentrations to be on average a factor of 1 to 5.3 times greater in forest soils compared with soils from open land. Using the headspace gas chromatography (HSGC-ECD) method of analysis, Reeves (2001) reported TCA concentrations of up to 390 ng g^{-1} (fwt) for Sitka spruce forest soils in Scotland.

Table 1.3. Reported TCA concentrations of soils from a range of sites.

Location	Vegetation type	TCA concentration / ng g^{-1}				Reference
		Range	fwt / dwt	Mean	n	
Germany	Spruce	20 - 380	*	100 ± 200	5	Frank (1988)
Germany	Pine	1.4 – 120	*	Not stated	72	Plümacher (1995)
Holland	Douglas fir	0.2 - 1.3	dwt	0.5 ± 0.5	4	Hoekstra & de Leer (1993)
	Beech	0.2 – 0.9	dwt	0.4 ± 0.4	3	
	Peat moor	1.0 – 2.7	dwt	1.6 ± 0.9	3	
	Peat bog	2.6 - 4.6	dwt	3.4 ± 0.9	4	
Scotland	Moorland	8.0	fwt	*	*	Reeves (2001)
	Sitka spruce A	3.0 - 27	fwt	10	14	
	Sitka spruce B	6.0 – 390	fwt	200	19	
Europe	various	<0.05 – 12	dwt	0.61	48	Peters (2003)

* denotes information not provided in literature.

The characterization of soil TCA is clearly not straightforward and direct comparisons of TCA concentrations reported by different research groups should be treated with care.

1.3 SOURCES OF TCA IN THE ENVIRONMENT

1.3.1 Anthropogenic sources of TCA

1.3.1.1 Primary sources of TCA

Most manufactured TCA was historically used as a herbicide in the form of sodium trichloroacetate. Since the supposed lifetime of TCA in soil is several weeks (summarised in Foy, 1975), this herbicidal use is not likely to contribute towards contemporary concentrations of TCA in the soil environment. Combustion has also been suggested as a minor source of TCA, as all types of oxidised chlorinated organic compounds can be formed in the presence of chloride and redox-sensitive elements such as Fe and Cu (Bumb *et al.*, 1980). This may include incineration of municipal waste (Mowrer and Nordin, 1987), biomass burning and volcanic

emissions (Rasmussen *et al.*, 1980; Andreae *et al.*, 1996; cited in Keppler *et al*, 2000). However, TCA in air is readily scavenged by wet precipitation and thus, long-distance transport from anthropogenic point sources is unlikely. Therefore the presence of TCA in remote areas is more likely to be from indirect sources.

1.3.1.2 Secondary sources of TCA

TCA is a known by-product of the chlorination of drinking water (Boyce and Hornig, 1983), but this is unlikely to be a significant source in the environment as evaporation of TCA from the aqueous environment is unlikely. There is abundant evidence that TCA can be formed during the evaporation, transport and subsequent atmospheric photo-oxidation of synthetic chlorinated solvents such as 1,1,1-trichloroethane (TCE: CCl_3CH_3) and tetrachloroethene (PER: $CCl_2=CCl_2$) emitted to the atmosphere (Gay *et al.*, 1976; Franklin, 1994; Sidebottom and Franklin, 1996), via the following postulated routes;

1,1,1-Trichloroethane (TCE) → Trichloroacetaldehyde (chloral) → TCA

Tetrachloroethene (PER) → Tetrachlorooxiran → Trichloroacetylchloride → TCA

PER and TCE are highly volatile with approximate atmospheric residence times of 0.4 and 6 years respectively (Frank, 1991), and can therefore be transported over long distances and into remote areas. There are differing opinions over the relative contributions of PER and TCE to global atmospheric TCA concentrations. Müller *et al.* (1996) consider PER to be the main precursor of TCA, with other compounds having only a minor contribution and Franklin and Sidebottom (1999) estimated that approximately 5 % of PER and 1.3 % of TCE released globally into the atmosphere could be converted to TCA. According to Hoekstra (2003), the yield of TCA from TCE is unlikely to exceed 2.8 % with chloral being the principal product of the tropospheric decomposition of TCE, but on the other hand, TCE is reported to be much more abundant in the atmosphere than PER (McCulloch, 2002).

Folberth *et al.* (2003) investigated the influence of climate conditions and solar radiation intensity on gas-phase TCA formation. Atmospheric OH and HO_2

concentrations and the NO_x/HO_2 ratio were identified as the governing quantities controlling the TCA formation through TCE oxidation in the gas phase. From crude comparisons of modelled atmospheric TCA production rates (5.42×10^{-5} μg m^{-3} y^{-1} in a rural area) with measured TCA burdens in the soil (130 – 1750 μg m^{-3} (fwt)), these authors concluded that TCA formation through TCE photo-oxidation in the gas-phase is not the main atmospheric source of TCA in the area of the Caspian Sea. However, this model focused only on a gas-phase route via TCE and failed to include production pathways from other precursor species in other compartments (e.g. in the soil itself, as discussed in Section 1.3.2) or aqueous-phase processing which is thought to be of considerable importance, especially in areas with wet maritime climates such as the UK.

Uncertainties in key kinetic and photochemical data (Sidebottom and Franklin, 1996; Jordan *et al.*, 1999; McCulloch, 2002; Folberth *et al.*, 2003; Hoekstra, 2003) and various assumptions in calculations of atmospheric TCA production rates, mean that there is an ongoing debate concerning the contributions of PER and TCE to TCA concentrations in the atmosphere.

In a 2-year study of atmospheric concentrations and deposition of TCA in rural South-East Scotland, Heal *et al.* (2003a) calculated that wet and dry atmospheric TCA deposition fluxes were greater than the presumed TCA yield from emissions and atmospheric reactions of PER in the late 1990s (McCulloch, 2002). This suggests that there is an additional source of TCA, which has not been identified and included in global flux calculations. Schöler *et al.* (2003), using data from a range of literature, calculated an annual TCA input flux to soil via throughfall and litterfall of 3.3 g ha^{-1} y^{-1} and concluded that if soil has an average TCA concentration of 20 ng g^{-1}, then 20 years of TCA input to a depth of 20 cm would be necessary to obtain the same soil TCA concentration, disregarding any TCA formation or degradation processes within the soil itself. Other mass balance studies of TCA in the environment (Euro Chlor, 2001; McCulloch, 2002; Hoekstra, 2003) also reported that, while the atmospheric oxidation of PER and TCE may account for some of the

TCA detected in precipitation, large additional sources are required in order to effect a global or regional mass balance.

TCA production from chlorinated solvents in soil and vegetation

In addition to TCA formation from atmospheric conversions of chlorinated precursors there is also some evidence that TCA may be formed from these compounds in the terrestrial environment. UV light has been reported to convert TCE and PER from contaminated groundwater to TCA (Hirvonen *et al.*, 1996). This mechanism may operate in surface soil after dry deposition of these precursors but is not likely to occur at great depth as solar radiation cannot penetrate far. In aerobic conditions degradation of chlorinated solvents may occur by soil organisms such as the earthworm, *Lumbricus terrestris*, (Back and Süsser, 1992) or micro-organisms, although anaerobic conditions result in dechlorination of chlorinated solvents and TCA is unlikely to be produced (Hoekstra *et al,* 1999a).

It has been postulated that TCA may also be formed *in-situ* in plants from the degradation of chlorinated solvents, such as PER and TCE, which have been taken up by foliage directly from the atmosphere as a detoxification mechanism of the P-450 monooxygenase enzyme (Henschler *et al.,* 1977, cited in Hoekstra, 2003; Frank *et al.,* 1992; Plümacher and Schröder, 1994). This specific mechanism has so far only been directly observed in animals, although Newman *et al.* (1997) provided some evidence that poplar trees may be able to convert TCE into TCA. They applied TCE doses of 50 mg l^{-1} to the soil which resulted in TCA concentrations of 1 – 7 ng g^{-1} in the leaves, although the total mass of TCE applied is not stated. Conversely, Plümacher (1995) found no correlation of TCA concentration with PER and TCE concentrations in needles of conifer trees although this was based on "background" concentrations of TCE which were orders of magnitude lower than those used in the poplar study.

1.3.2 Natural sources of TCA

As there are many indications that the TCA presence in the terrestrial environment cannot be accounted for by atmospheric inputs alone, it has been proposed that TCA

may be formed entirely naturally in soil and vegetation. Sidebottom and Franklin (1996) found comparable concentrations of TCA in precipitation from Antarctica and the Arctic despite emissions of PER and TCE being 6 times greater in the Northern Hemisphere. If PER were the main source, much lower concentrations of TCA would be expected in the less-industrialised Southern Hemisphere. The presence of TCA in several-hundred-year-old glacier ice from the Alps and northern Sweden (Grimvall, 1995; Haiber *et al.*, 1996) and firn representing the past 100 years of snow accumulation in Antarctica (Von Sydow *et al.*, 2000) strongly indicates that chloroacetates may have occurred naturally in precipitation before there was any large-scale industrial production of reactive chlorine and chloro-organics.

Until the early 1990s it was usually assumed that organohalogens in soil and surface water were of anthropogenic origin but more recently the natural production of organohalogens has become a well-known phenomenon, particularly in marine environments (Hoekstra and de Leer, 1993) where macroalgae and microalgae have been shown to produce methyl chloride and bromoform. It has been suggested that other chlorinated compounds, including TCA can be produced naturally by similar mechanisms. Although the chemistry involved in biohalogenation is only basically understood (Urhahn & Ballschmiter, 1998), most researchers believe it occurs as a chemical defence mechanism.

Natural production of TCA in soil

In drinking water the chlorination of fulvic and humic acids produces TCA and chloroform (Rook, 1977; Reckhow *et al.*, 1990; Zhang and Minear, 2002; Sérodes *et al.*, 2003; Yoon *et al.*, 2003). Asplund *et al.* (1991) reported that humic materials in soil have high organohalogen concentrations (up to 2.5 mg Cl $(g\ C)^{-1}$) which indicates that similar chlorination processes may occur in soil. From mass balance studies Hoekstra *et al.* (1999a), reported tentative evidence of TCA production in four out of seven soils although this was not considered to be conclusive due to large uncertainties associated with the calculations.

Action of chloroperoxidase enzymes

It has been proposed that TCA may be formed naturally in soil from the reaction of inorganic chloride with humic material by a chloroperoxidase (CPO) mediated reaction (Hoekstra, 2003) (Equations 1.1 and 1.2).

$$H_2O_2 + H^+ + Cl^- \xrightarrow{CPO} HOCl + H_2O \qquad \textbf{(Equation 1.1)}$$

$$\text{Humic material} + HOCl \longrightarrow \text{Chlorinated humic material} + CHCl_3 + CCl_3COOH \qquad \textbf{(Equation 1.2)}$$

CPO enzyme activity has been observed in several soil extracts (Neidleman and Geigert, 1986; Asplund *et al.*, 1991; Laturnus *et al.*, 1995). The catalytic chlorine activity depends on the individual CPO species and soil pH, with optimum chlorination yields occurring at pH 3 - 6 (Asplund *et al.*, 1993, Haiber *et al.*, 1996; Juuti and Hoekstra, 1998; Hoekstra *et al*, 1999b). In laboratory experiments Walter and Ballschmiter (1992) reported the CPO-mediated formation of chloroform from simple organic compounds such as acetone, propionic acid and citric acid and Hoekstra *et al.* (1995) demonstrated the CPO-mediated formation of chloroform and TCA from humic acid. The source of CPO enzymes is thought to be fungal (de Jong *et al.*, 1994), and Hjelm (1996, cited in McCulloch, 2003) reported that white rot fungus has chlorination ability *in vitro*, and Wood Blewitt fungus, *Lepista nude*, *in vivo*, while Hoekstra *et al.* (1998b) also detected chloroform from five basidiomycetes and one deuteromycete. Niedan *et al.* (2000) demonstrated that CPO-mediated processes can catalyse the chlorination of phenolic building blocks, with TCA and DCA (dichloroacetic acid) also formed well above the detection limit. Haiber *et al.* (1996) reported that TCA may also be formed from acetic, malic, lactic, fumaric, malonic, citric and acetonedicarboxylic acids after incubation with the CPO enzyme in the presence of sodium chloride and hydrogen peroxide, with the most TCA formed from acetic and humic acids. TCA was also produced abiotically from the humic acid mixture in the absence of CPO enzymes, inferring that humic acid itself contains chlorinating potential. From this it was proposed that hydrogen

peroxide can oxidise chloride to some extent to elemental chlorine which may act as a chlorinating agent. Fahimi *et al.* (2003) conducted a series of experiments using soil and humic acid to test the influence of hydrogen peroxide, Fe^{2+} and Fe^{3+} on abiotic production of TCA. TCA was formed in the soil, which was attributed to the presence of humic acid. Subsequent experiments with humic acid showed that neither an oxidising agent nor a halide source was required to produce TCA and there was a positive relationship between humic acid concentration and haloacetic acid formation.

Several studies have reported the presence of chloroform in soil air (Frank, 1988; Hoekstra *et al.*, 1998a,b; Haselmann *et al,* 2000a,b, 2002; Hoekstra *et al.,* 2001). Hoekstra *et al* (1998a) confirmed that, in the field, chloroform enriched with ^{37}Cl was formed in top soil which had been spiked with $Na^{37}Cl$ solution. In separate studies Hoekstra and de Leer (1993) and Haselmann *et al.* (2000a) reported that the ratio of chloroform in top-soil air to ambient air was high compared with the ratio for other volatile chlorinated anthropogenic compounds (tetrachloromethane, TCE, trichloroethene and PER) which indicates that chloroform is formed naturally. It has been postulated that TCA and chloroform may be formed by similar mechanisms and Hoekstra *et al.* (1999b) observed a linear correlation ($R^2 = 0.88$, $P< 0.01$) between chloroform and TCA concentrations in soil air. Although correlations may also occur if TCA is either chemically decarboxylated or microbially degraded to chloroform, the authors argued that neither of these reactions is likely.

In summary, a number of studies have demonstrated that TCA may be naturally produced in the soil. Although prerequisite compounds (e.g. short-chained aliphatic acids, chloroperoxidase enzymes, Fe^{3+}, hydrogen peroxide) are thought to be naturally available in all soils (Schöler *et al.*, 2003), these experiments have all been conducted in controlled laboratory conditions using known concentrations of commercially-obtained reagents and do not provide direct evidence for similar processes occurring in field conditions. In addition, they have not attempted to compare the rates of TCA formation in different types of soil with different water and organic matter contents, pH, vegetation cover and climates. Although potential routes of natural TCA formation have been identified, any attempts to estimate the

rates of formation in soil have high uncertainties associated with them and depend on a number of assumptions and approximations regarding input and output fluxes of TCA. It is likely that TCA in the environment originates from a combination of both the anthropogenic and natural routes discussed, as well as from other, so far unidentified, sources.

1.4 REMOVAL OF TCA IN SOIL

Processes which may lead to the removal of TCA from soil include leaching, adsorption within the soil, plant uptake, chemical degradation and microbiological degradation. The relative contribution of each process is expected to depend on the properties of individual soils. Evaporation of TCA from soil is unlikely to occur due to its high Henry's Law constant. As TCA is very soluble in water it is expected to be highly mobile in the soil and easily leached out of the soil system. However, the poor recovery of TCA in the leachate of soil spiked with TCA solution (Haiber *et al.*, 1996) suggests that this is not the case and that TCA is bound or degraded within the soil matrix.

1.4.1 Chemical degradation of TCA in soil

Tests using ^{14}C-TCA have shown that TCA breaks down in water solution to form chloroform ($CHCl_3$) and CO_2 at room temperature (Leasure, 1964), especially in alkaline conditions (Kearney *et al.*, 1965). Hoekstra *et al.* (1998a) speculated that this reaction is unlikely as TCA is decarboxylated only if it is present as the trichloroacetate ion and that since the pH of forest soils is normally quite acidic (pH 3 – 5), TCA will be stable against decarboxylation. However this is a weak argument as TCA has a low pK_a (~0.26), and it is likely that the soil pH would have to be significantly lower than pH 3 to prevent decarboxylation to chloroform. Hoekstra *et al.* (1999b) later proposed that TCA degradation in soil is more likely to occur via a biological route.

1.4.2 Biological degradation of TCA in soil

Many studies on the fate of soil TCA at herbicidal concentrations (mg kg^{-1}) indicate that TCA can be decomposed in the soil by micro-organisms (Loustalot and Ferrer,

1950; Barrons and Hummer, 1951; Ogle and Warren, 1954; Martin, 1972; Foy, 1975; McGrath, 1976; Torstensson, 1976; Lignell *et al.*, 1984) to form carbon dioxide and chloride species. More recent studies have used significantly lower concentrations of TCA to investigate its degradation in soil (Haiber *et al.*, 1996; Matucha *et al.*, 2003). Haiber *et al.* (1996) found that TCA (1 $\mu g\ l^{-1}$) added to a soil lysimeter was not recovered in the leachate and no increase in chloroform in the soil was observed. Matucha *et al.* (2003) applied 1045 kBq [1,2-^{14}C] TCA to fresh soil (to a concentration of approximately 2 $\mu g\ g^{-1}$) and reported that degradation to $^{14}CO_2$ was rapid and that no other gaseous degradation products (carbon monoxide, methane or chloroform) were detected. This implies that chemical degradation of TCA in soil was negligible. In a parallel experiment using sterile soil no $^{14}CO_2$ was detected which confirmed that TCA was degraded by soil micro-organisms.

Jensen (1957, 1960) described a bacterium of undefined taxonomic position that decomposed TCA in a co-culture with Streptomyces strains or when soil extracts or Vitamin B_{12} were added to the medium. Although TCA was dechlorinated, the growth of the isolates was weak. Weightman *et al.* (1992) identified the bacteria *Pseudomonas sp, Arthrobacter sp.* and *Pseudomonas dehalogens* as well as the fungus *Trichoderma viride* as being capable of degrading TCA via a dehalogenation reaction producing CO_2 and chloride. Yu and Welander (1995) isolated, enriched and characterised aerobic bacteria which could grow with TCA as a sole source of carbon although, again, the growth of organisms was low.

Several studies have indicated that TCA added to soil may be degraded by micro-organisms, but little is known about the micro-organisms involved, the reaction kinetics and the environmental conditions needed for the reactions to occur. Whether TCA intrinsic to the soil is degraded in this way, and at what rate, remains unclear. Since soil is very heterogeneous it is difficult to determine the temporal variation in TCA at such low concentrations. In addition, the potential formation (natural or from chlorinated precursors) and degradation of TCA may occur simultaneously in the same soil thereby making it difficult to distinguish between the two processes and determine the rates of each.

1.5 REMOVAL OF TCA IN VEGETATION

Herbicidal studies of TCA indicate that TCA is not readily metabolised by plants (Barrons and Hummer, 1951; Tibbitts and Holm, 1954; Leasure, 1964). Routes and rates of TCA elimination in plants growing in natural environments are even more difficult to assess. Frank (1991) reported a half-life of 10 days in spruce needles and suggested that TCA in needles might be partly decarboxylated to the intermediate trichloromethanide which then forms chloroform. However, Forczek *et al.* (2001), Matucha *et al.* (2001) and Schröder *et al.* (2003) carried out studies on Norway spruce seedlings using labelled [1,2-^{14}C] TCA and reported that the only metabolism (or degradation) product of TCA within the plant/soil system was $^{14}CO_2$ indicating that complete mineralization of TCA takes place within the tree.

1.6 CYCLING OF TCA IN THE ENVIRONMENT

The main proposed input fluxes, output fluxes and stores of TCA in the environment, as discussed throughout this chapter, are summarised in Figure 1.2.

It is evident from the available literature that there are a number of uncertainties surrounding the origins of TCA in the environment, as its presence can no longer be attributed to its historical use as a herbicide. Anthropogenic sources of TCA in soil and vegetation from chlorinated solvents in the atmosphere cannot account for the large TCA soil burden, and hence natural formation of TCA in soil has been suggested. Since degradation of TCA is also known to occur in soil and vegetation, TCA is clearly actively and continuously cycled in the environment, although the specific routes have not all been identified and rates of production and degradation in soil have not been well quantified. In addition, the main routes of TCA uptake by vegetation (i.e. directly from the atmosphere or via soil and the roots) are not clear. Quantification of the rates of TCA uptake, and the potential risk each TCA exposure route poses to tree health, would facilitate more accurate risk assessments of TCA and its precursors.

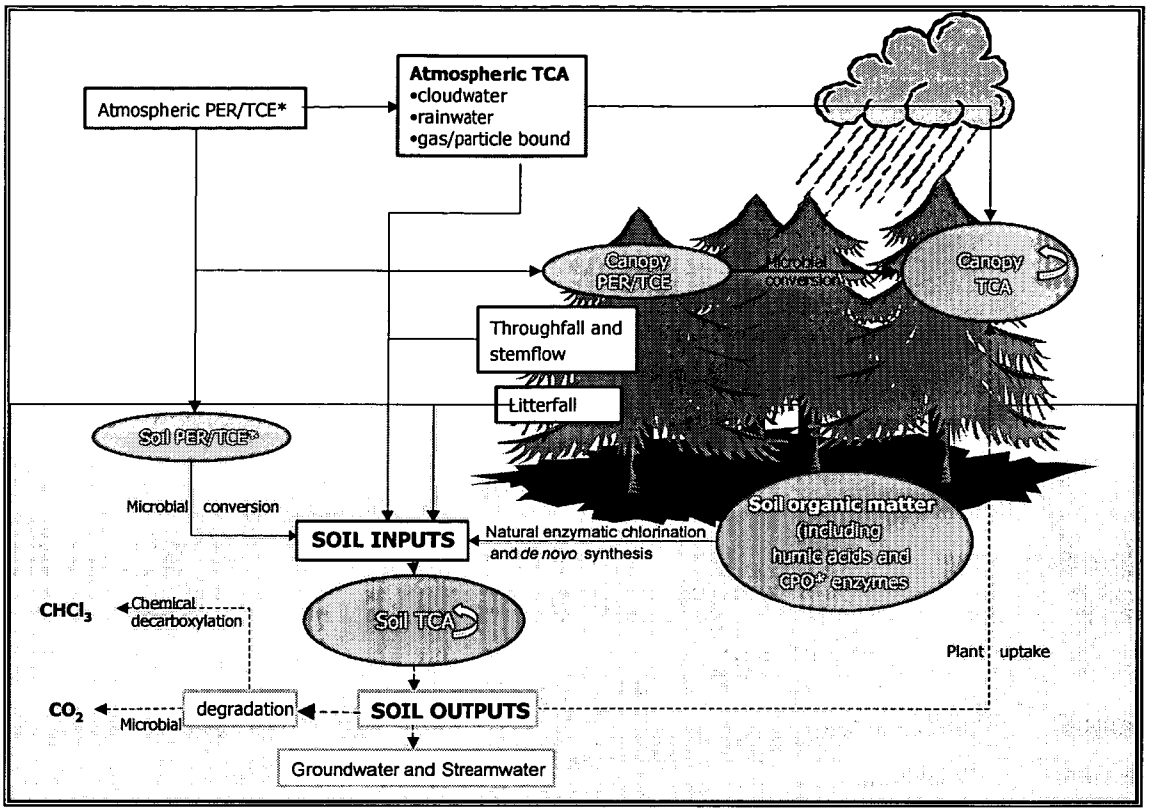


Figure 1.2. TCA fluxes and storage in different environmental compartments. Oval boxes indicate active processes, solid lines indicate TCA inputs to the terrestrial environment, dashed lines indicate TCA outputs from the terrestrial environment and arrows indicate the transfer of TCA from one compartment to another. PER = tetrachloroethene, TCE = 1,1,1-trichloroethane, CPO = chloroperoxidase.

1.7 RESEARCH AIMS

More in depth studies are required before the major sources of TCA and their relative contributions are quantified, cycling of TCA within the soil compartment is understood, and the routes of uptake by vegetation and effects on health are fully elucidated. The broad aim of this Ph.D study is therefore to address these uncertainties. The following specific objectives have been identified for investigation in this research:

1) To validate the method of TCA analysis in soil by headspace gas chromatography with electron capture detection (HSGC-ECD) via decarboxylation to chloroform. From this it may be determined if the large variation in reported TCA concentrations is due to genuine differences between regions or discrepancies between the methods of TCA analysis.
2) To characterise the presence of TCA in the soil and the influence of soil depth and specific horizon, soil pH, soil water and organic matter content, vegetation type, soil microbial biomass carbon and soil C:N ratios. It may then be possible to determine the "compartment" of the soil with which TCA is associated (e.g. water, dead organic matter, living biomass, ion exchange sites) and hence its mobility within the soil.
3) To investigate the cycling of TCA in soil in terms of the magnitudes of TCA input and output fluxes, origin of TCA and processes involved in its removal from the soil environment.
4) To identify and quantify the routes of uptake of TCA by Sitka spruce trees, its effects on tree health, and to investigate the behaviour of TCA once it has entered the tree system.

Most of this research has been carried out at Ballochbeatties in South-West Scotland (described in detail in Chapter 4), an upland site in an area of high rainfall, dominated by Sitka spruce forest and unimproved moor overlying poorly drained, highly organic soils. Comparisons were made with more mineral soils from two agricultural sites, Cowpark and Easter Howgate, in South-East Scotland which receive significantly less rainfall.

Chapter 2 – Analysis of TCA

2.1 BACKGROUND

2.1.1 Historical methods of TCA analysis

When TCA was studied during its use as a herbicide, experiments focused on understanding its movement and behaviour in the soil (including its half-life), its mode of action on target species and effects the health of both target and non-target species. The reported recommended field applications of TCA ranged from approximately 4.4 kg ha^{-1} in Western Canada (Smith, 1974) to 33 kg ha^{-1} for domestic use in Finland (Lignell *et al.* 1984) which correspond to approximately 900 to 6000 ng g^{-1} of fresh soil, assuming TCA penetrates to a depth of 0.5 m. This is several orders of magnitude greater than those TCA concentrations found naturally in the soil, of < 0.05 – 390 ng g^{-1} (Table 1.3). Due to these high concentrations it was therefore relatively simple to analyse TCA with reasonable accuracy and precision. Many of these studies involved the addition of ^{14}C radio-isotopically-labelled TCA to soil, which was then detected using liquid scintillation counting, assuming the radioactivity detected was originating from the TCA (Chow, 1970; Smith, 1974; Lignell *et al.*, 1984). Other studies used indirect methods such as colorimetric analysis of chloride released during TCA breakdown (McGrath, 1976) and the use of bioassays to follow the disappearance of TCA in soil and vegetation (Ogle and Warren, 1954; Torstensson, 1976). For example, a study by Kratochvil (1951) quantified the increase in microbial activity measured by the build-up of gas and mercury displacement in a manometer after addition of TCA to soil in a flask.

As current studies of TCA "naturally" present in the environment involve significantly lower TCA concentrations than those detected in herbicidal studies, very sensitive analysis techniques are required. In order to assess the environmental and phytotoxicological relevance of TCA, numerous samples must be analysed and so a reliable but technically undemanding method is required.

2.1.2 Trace analysis of TCA

The three main analytical techniques currently used for the determination of TCA in biological samples are colorimetry, ion chromatography and gas chromatography with electron-capture detection or mass spectrometry (Frank *et al.,* 1990). Colorimetric methods involve the determination of a pyridinium salt formed with $CHCl_3$, the decarboxylation product of TCA. Ion chromatography is more sensitive although the organic acids in plant tissue may interfere with determination of TCA at the parts per billion range (Frank *et al.*, 1990).

Gas chromatography (GC) is currently the preferred technique for TCA analysis due to its high sensitivity. However, as TCA is too involatile to be analysed directly by GC, procedures have been developed to derivatise the TCA with another reagent (Section 2.2.1), or to convert the TCA to the more volatile compound chloroform ($CHCl_3$), which can then be analysed directly by GC (Section 2.2.2).

2.1.3 Chapter aims

In this research TCA concentrations in water, soil and vegetation were determined by headspace gas chromatography (HSGC) combined with an electron-capture detector (ECD), which is particularly sensitive to halogen-containing molecules. Although the analysis of TCA derivatives by GC is also discussed, the main focus of this chapter will be the analysis of TCA by HSGC-ECD.

2.2 ANALYSIS OF TCA BY GAS CHROMATOGRAPHY

2.2.1 Derivatisation methods of TCA analysis

One of the main GC methods used is analysis of TCA as the methyl ester. TCA in aqueous samples is extracted with diethyl ether and derivatised to the methyl ester using a reagent such as saturated diazomethane (Hoekstra and de Leer, 1993; Peters, 2000). Some researchers prefer to use 2,4-difluoroaniline (Scott and Alaee, 1998) or acidic methanol (Nikolaou *et al.,* 2002) instead of diazomethane, as they are less hazardous, and analyse for the trichloroacetic acid difluoroanilide or acidic methanol derivatives respectively. These derivatives are analysed directly using GC combined

with ECD or mass spectrometry (MS). An internal standard of ^{13}C–labelled TCA (Peters, 2000) or 2,2-dichloropropionic acid (Frank *et al.*, 1990) is normally used to determine the percentage recovery of analyte during analysis. For solid samples (soil or needles) TCA must first be extracted into solution. The homogenised sample (e.g. sieved soil or ground needles) is shaken with Milli-Q water for several hours then centrifuged and filtered. The resulting aqueous extract is then extracted, derivatised and analysed by GC as for aqueous samples.

The main weakness in this method of TCA analysis is the assumption that all of the TCA in the sample matrix is extracted into aqueous solution. This is particularly questionable for soil, which is a very complex and heterogeneous material. TCA may be physically or chemically bound within the soil preventing its extraction into water, even though it is highly soluble in water.

Several steps are necessary in this analysis procedure making it not only laborious with a low sample throughput, but also increasing the chance of sample contamination at one of the many stages. Since TCA is present at low concentrations in water, it is also possible that the initial addition of water will contribute to the final measured TCA concentration. Poor recovery has been reported by some researchers (*Pers. comm.*, cited in Reeves, 2001) although others claim that TCA recovery in aqueous samples is "generally quite good" (Frank *et al.*, 1990). Despite these weaknesses, the fact that this method can uniquely define TCA and simultaneously measure the concentrations of other haloacetic acids is highly desirable.

2.2.2 Headspace gas chromatography methods of TCA analysis

2.2.2.1 Background to headspace gas chromatography

HSGC is based upon the equilibrium of volatile compounds between the liquid or solid phase of the sample and the gas phase above it. In a closed vial the ratio of concentrations changes at higher temperatures into a new equilibrium with higher concentrations in the gas phase. An aliquot of headspace is then analysed by GC. The HSGC-ECD method of TCA analysis used in this study was based on a method

developed by Plümacher and Renner (1993) and modified by Reeves (2001) using a Perkin Elmer HS40 automated headspace sampler and Autosystem GC. Reeves (2001) optimised conditions and sample preparation procedures to produce a rapid, sensitive and reproducible method of TCA analysis via thermal decarboxylation to $CHCl_3$ when heated to 100 °C (Equation 2.1), followed by equilibration at 60 °C for 1 hour.

$$CCl_3COOH \xrightarrow[1.5\ hours]{100\ °C} CHCl_3 + CO_2 \qquad \text{Equation 2.1}$$

Identification of $CHCl_3$ is based on the peak retention time and subsequent quantification of the sample TCA concentration is based on external standard solutions of known TCA concentration which are decarboxylated and analysed for $CHCl_3$ alongside the samples. Sample "blanks" heated to 60 °C only are also analysed alongside the samples heated to 100 °C to determine the background chloroform already present in the sample, which is then subtracted from the sample chloroform peak. The net chloroform concentration is that formed from TCA alone. The analysis steps are summarised in Figure 2.1.

This HSGC-ECD technique permits analysis of TCA in all matrices (including soil and needles) without the need to extract the TCA into solution. It is a straightforward and versatile method requiring no sample pre-treatment. One criticism of the HSGC-ECD method is that it does not uniquely define TCA and it is possible that other components in the sample form $CHCl_3$ between 60 °C and 100 °C. The only compound that has so far been identified in natural media that may form $CHCl_3$ under these conditions is chloral hydrate. However, Køppen *et al.* (1988) reported that the conversion of chloral hydrate to $CHCl_3$ is very pH-dependent; at pH 8 there was complete conversion to $CHCl_3$ at 60 °C after 90 minutes, but at pH 6 conversion was less than 10 %.

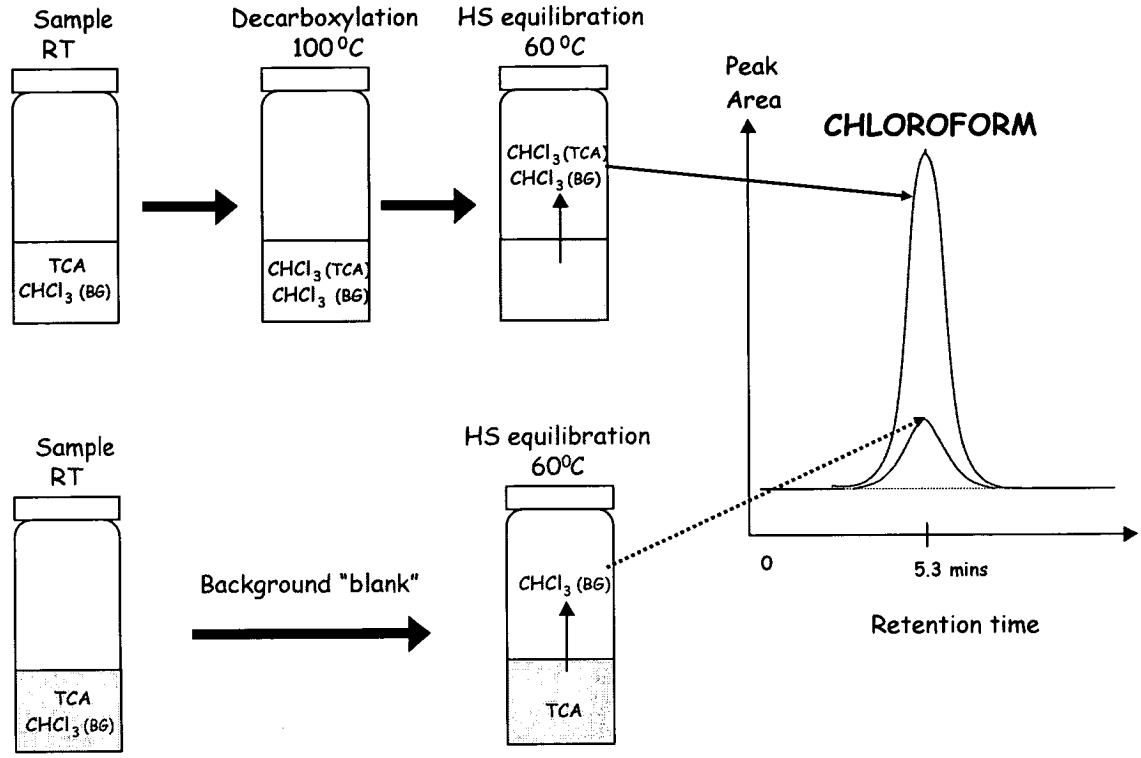


Figure 2.1 A summary of the steps involved in the analysis of TCA in environmental samples via decarboxylation to chloroform ($CHCl_3$) using headspace gas chromatography with electron-capture detection (HSGC-ECD). RT = room temperature and BG = background.

Frank *et al.* (1990) determined the TCA concentrations in spruce needles using both the extraction-derivatisation and HSGC-ECD methods of analysis. They reported that the concentrations of TCA detected in spruce needles by both methods correlated well and had similar precision (< 20 % RSD). They reported that the mean recovery of TCA in spruce and fir needles spiked with a known mass of TCA was 93 ± 9 % and 79 ± 17 % respectively, using the extraction-derivatisation technique. Recoveries for the HSGC-ECD method were not shown but the sensitivity was reported to be 40 pg which is appropriate for routine analysis of the lowest concentrations found in environmental samples.

2.2.2.2 Determination of optimum decarboxylation time

Plümacher and Renner (1993) reported that the decarboxylation of TCA to $CHCl_3$ at 65 °C is complete after 72 hours. Control experiments showed that $CHCl_3$ was stable over this period. Reeves (2001) found that heating samples to 100 °C for 90 minutes was sufficient to decarboxylate nearly 100 % of the TCA present in the sample to $CHCl_3$. He performed a recovery experiment by analysing $CHCl_3$ standard solutions alongside TCA standard solutions, for 0.1 ml of sample in the range 0 – 160 ng $CHCl_3$. The TCA solutions were decarboxylated at 100 °C for 1 hour and equilibrated at 60 °C for 2 hours whereas the $CHCl_3$ solutions were equilibrated at 60 °C for 3 hours. The calibration lines for both compounds had nearly identical gradients showing that conversion of TCA to $CHCl_3$ was 98 % complete and that the TCA was quantitatively and reproducibly converted to $CHCl_3$.

Køppen *et al.* (1988) showed that, in preliminary experiments with rat liver homogenates spiked with TCA, the rate of decarboxylation was practically independent of the pH of the sample. However, the decarboxylation rate was temperature dependent and to achieve complete conversion into $CHCl_3$ an incubation time of 90 minutes at 90 °C was necessary. Only in strongly acidic solution did loss of $CHCl_3$ occur.

2.2.2.3 Optimisation of headspace thermostatting and pressurisation times

Reeves (2001) thoroughly investigated the various parameters involved in analysis by HSGC-ECD and found that similar peak areas for standard TCA solutions were obtained for thermostatting times of 5 and 60 minutes. A time of 10 minutes was selected for speed of analysis. Pressurisation time had very little effect on the $CHCl_3$ peak area with the apparent optimum pressurisation time being 2 or 3 minutes. Therefore 2 minutes was used in future analyses. These, and other established parameters for analysis of TCA by HSGC-ECD, used in this research, are summarised in Table 2.1.

Table 2.1 Automatic parameters for analysis of TCA by headspace gas chromatography (HSGC-ECD) using the Perkin Elmer HS40 XL instrument, as determined by Reeves (2001).

Sample pre-analysis conditions	
Sample decarboxylation time	90 min
Sample decarboxylation temperature	100 °C
Sample and blank equilibration time	60 min
Sample and blank equilibration temperature	60 °C
Headspace sampler and GC temperature settings	
Thermostatting temperature	60 °C
Needle temperature	70 °C
Transfer line temperature	200 °C
Injector temperature	200 °C
Detector temperature	375 °C
Oven temperature programme	50 °C, 5 min +20 °C/min, 150 °C, 3 min
Headspace sampler and GC time programmes	
Thermostatting time	10 min
Pressurisation time	2.00min
Injection time	0.03 min
Withdrawal time	0.20 min
Vent time	0.10 min
Carrier	
Gas	Helium
Pressure	12.6 psi
Split flow rate	25 ml min^{-1}
Column	
Type	Fused silica capillary (J & W Scientific)
Length	30 m
Internal diameter	0.32 mm
Phase	DB624
Film thickness	1.80 μm

2.2.2.4 Partition ratios

The distribution of $CHCl_3$ between the sample and the gas phase is strongly dependent on sample type and is described by specific partition coefficients (Plümacher and Renner, 1993). As aqueous samples of TCA are in the same phase as the TCA standard solutions to which they are compared, the response of the HSGC-ECD should be the same. Therefore the ratio of the partition coefficients (hereafter termed the "partition ratio") should be unity. As the peak areas from solid samples (needles and soil) are also compared with aqueous TCA standard solutions, it is important to account for this difference in phase by determining the partition ratio. To determine the partition ratio experimentally, the method of standard additions may be used where increasing concentrations of TCA standard solution are added to several aliquots of the same solid samples. The ratio of the response of the spiked samples to the response of the TCA standard solutions is calculated to give the partition ratio, which is specific to each sample type. Once the partition ratios for soil and needles have been determined in this way, they can be used as a correction factor for soil and needles samples calibrated against aqueous TCA standards.

Experimental determination of partition ratios

In this study the mean partition ratio was determined for several soil types of varying water and organic matter contents. 0, 10, 20, 50 and 100 $\mu g\ l^{-1}$ solutions of TCA were prepared from one TCA stock solution and diluted with ultra-pure water (18.3 Ω). 1 ml of each TCA solution was pipetted into 20 ml headspace vials which were prepared in triplicate. Vials were sealed and heated to 100 °C for 90 minutes to decarboxylate the TCA to $CHCl_3$, then equilibrated at 60 °C for 60 minutes.

Fresh soil was homogenised by sieving through a 2-mm mesh and accurately weighed (1 ± 0.02 g) into vials. 1 ml of TCA solution was added to each soil using a pipette. Four replicate vials were prepared for each TCA standard concentration (0, 10, 20, 50 and 100 $\mu g\ l^{-1}$), three of which were heated to 100 °C for 90 minutes to decarboxylate the TCA to $CHCl_3$ then equilibrated at 60 °C for 60 minutes. The fourth replicate was heated to 60 °C only for 60 minutes, to enable correction for

background $CHCl_3$. All samples were analysed for TCA using the normal procedure described in detail in Section 2.2.3.

The $CHCl_3$ peak areas of the TCA standard solutions ("water calibration") and the spiked soils ("standard addition calibration") were plotted on a graph and the ratio of the two gradients determined. The peak areas for the water calibration were corrected for the background $CHCl_3$ by subtracting the peak areas of the water blanks. Peak areas for soil standard addition calibration were corrected for background $CHCl_3$ in both the soil itself and the added water, by subtracting the soil (+ 1 ml water) blanks. An example standard calibration graph is shown in Figure 2.2.

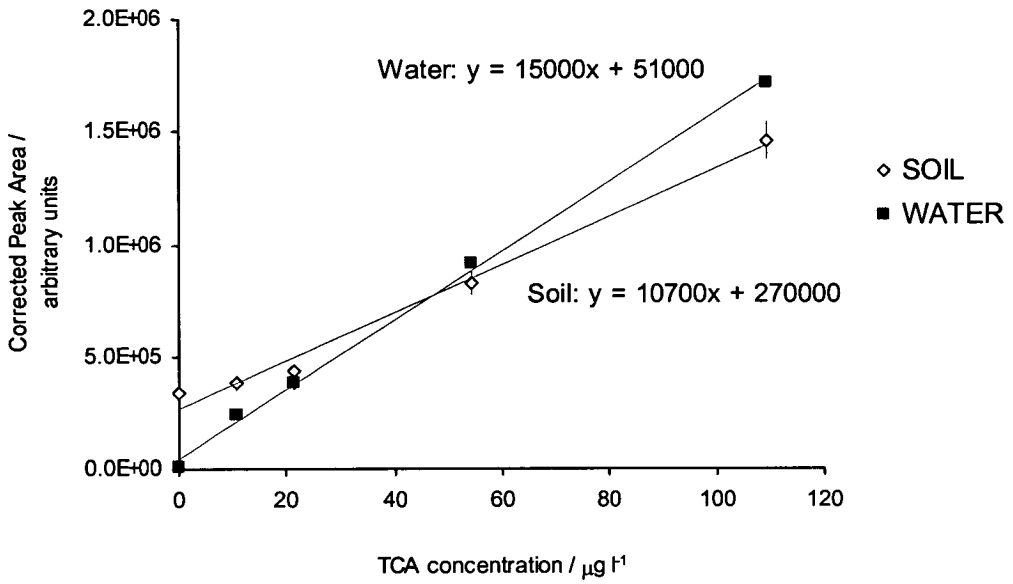


Figure 2.2. A standard addition calibration for soil (Larch B horizon) which is used to determine the partition ratio of the response factors of water and soil ($F_{w/s}$). Error bars are standard deviations of triplicate analyses.

The intercept of the soil standard addition calibration corresponds to the peak area from background $CHCl_3$ and TCA in the soil before spiking. As expected, the water calibration has zero intercept. A steeper gradient shows that a larger fraction of

$CHCl_3$ produced by decarboxylation of TCA is partitioned into the headspace by the water matrix alone than by the [soil + water] matrix. This means that lower $CHCl_3$ concentrations are detected in the headspace of the [soil + water] matrix.

The ratio of the gradients of the two plots (partition ratio) is a constant for a given set of headspace conditions such as sample mass and headspace volume. From Figure 2.2 the partition ratio can be calculated according to Equation 2.2.

$$F_{W/S} = \frac{f_w}{f_s} = \frac{15000}{10700} = 1.40 \qquad \textbf{Equation 2.2}$$

Where $F_{W/S}$ = the ratio of response factors of water to soil

A summary of the partition ratios determined for eight soils from the main study site, Ballochbeatties (described in detail in Chapter 4), is shown in Table 2.2.

Table 2.2. A summary of measured soil partition ratios (*Stidson, 2004, unpubl.)

Soil	Soil horizon	% Water	% Organic Matter	Partition Ratio
Clearfelled (deep peat)	O_1	94	6	1.11*
	O_2	75	17	1.45*
	F	84	14	1.37*
Moorland (peaty podzol)	E_a	62	11	1.18*
	O	71	14	1.21*
	B_g	46	6	1.04*
Sitka spruce forest (deep peat)	O_2	91	9	1.26*
Larch (peaty gley)	B	62	13	1.42
MEAN (± S.D.)				1.25 (± 0.15)

Partition ratios were very similar for different soils and so the mean water:soil partition ratio (1.25 ± 0.15) was used throughout this study for the determination of TCA concentrations in all soils from Ballochbeatties. A partition ratio of 2.01 was calculated for a gleyed soil from Cowpark, an agricultural site south of Edinburgh.

This soil has very different characteristics to the Ballochbeatties soils, being low in organic matter, with a high mineral content, and a very clayey texture. The value of 2.01 was therefore used for determination of TCA in this soil, rather than the mean value of 1.25 obtained for Ballochbeatties soils.

In each GC analysis run of soil samples the TCA concentration corresponding to the observed GC peak area and response factor (discussed in Section 2.2.3.2) of TCA standard solutions (prepared with water) was corrected by multiplying by this partition ratio. For determination of TCA in needles a partition ratio of 1.94 (± 0.26, n = 7) was used, as determined by Reeves (2001) and Stidson (2004, unpubl.).

Reeves (2001) found that the magnitude of the partition ratio was primarily dictated by the volume of headspace into which the $CHCl_3$ is concentrated and differences in the organic matter content density and partitioning between sample and headspace in needles and soil were relatively minor.

2.2.3 Sample preparation and analysis of TCA

2.2.3.1 Laboratory practice

As environmental samples of interest often contain only trace levels of TCA, extra care was required during sample preparation to avoid contamination. All vials were washed with de-ionised water and heated at 200 °C overnight before use to remove any traces of TCA or chloroform. The vial caps (aluminium with PTFE-coated butyl rubber septum) were heated for 20 minutes at 200 °C before use. Standard solutions of low TCA concentration were prepared by weight using flasks, which had also been heated to 200 °C overnight. Other TCA standard solutions were prepared by volume in volumetric flasks which were rinsed thoroughly with ultra-pure water before use. All samples, including solutions, were measured into vials gravimetrically using a high precision balance (Mettler AJ150) which was regularly calibrated.

When preparing vials for analysis, ultra-pure water was weighed out first, followed by the sample (aqueous, soil, needles or branchwood) and then the standard TCA solutions in order of increasing concentration to minimise any risk of cross-contamination.

2.2.3.2 Quality control

Calibration

Calibrations were regularly performed to check the linearity of the GC-ECD detector response. Direct calibrations against TCA standard solutions were carried out, rather than against $CHCl_3$ solutions. This avoided any bias that may have occurred from incomplete decarboxylation to $CHCl_3$. Calibrations were carried out at low and high TCA concentrations to check for linearity within concentration ranges likely to be experienced in a single GC analysis run of environmental samples.

A minimum of five standard TCA solutions of different TCA concentrations was prepared in triplicate from one stock solution. 5 ml of TCA solution was analysed for low TCA calibrations (up to 20 $\mu g\ l^{-1}$) and 1 ml for high TCA concentrations (up to 250 $\mu g\ l^{-1}$). Samples were analysed as for the aqueous samples described later on. The $CHCl_3$ peak areas were corrected for the $CHCl_3$ present in the ultra-pure water used to prepare the standard TCA solutions. Figure 2.3 shows an example of a high TCA calibration. Excellent linearity was achieved for both low and high calibrations with higher variability generally observed at lower concentrations. Theoretically the plots should have zero intercept on the y-axis but variability in the ultra-pure water TCA concentrations occasionally resulted in a small positive or negative intercept. Where possible, samples selected for analysis in any one GC run were compared with TCA standard solutions of similar concentrations to avoid any inaccuracies arising from poor linearity over a large concentration range.

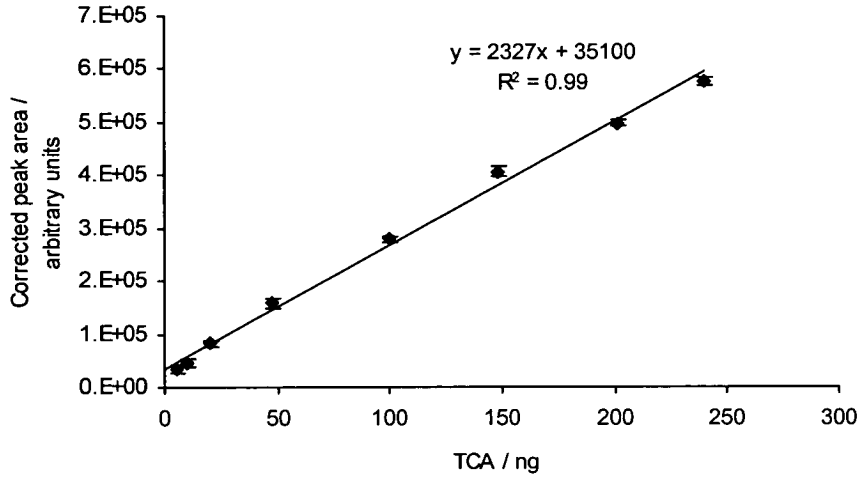


Figure 2.3. Example TCA calibration graphs for 0 – 250 μg l^{-1} TCA solution (1 ml in vial). Error bars are standard deviations of triplicate analyses.

Preparation of TCA standard solutions

Standard solutions of known TCA concentration were analysed alongside samples in every GC analysis run. Two independent stock solutions (A and B) of TCA (approximately 50 mg l^{-1}) were prepared by accurately weighing out crystalline TCA (Fisher Scientific) and dissolving it in de-ionised water. Further standards were prepared by diluting specific volumes of stock solutions (A and B) with ultra-pure water. Three standards of different TCA concentrations, including at least one each of A and B, were analysed so that the accuracy of sample preparations and linearity of the detector response could be observed. Standard TCA concentrations were selected to be within the expected range of sample TCA concentrations and were prepared and analysed in triplicate.

Response factors

The response of the HSGC-ECD instrument to $CHCl_3$ was continually monitored. "Response factors" (F_w) were calculated for every standard TCA solution by determining the peak area in relation to the TCA concentration (i.e. the unit peak area per ng of TCA). A higher response factor indicates greater detector sensitivity.

ECD detector responses are known to vary over time but changes should be gradual. Sensitivity may also be affected by routine maintenance. Therefore, although the response factor does not affect the measured TCA concentrations in individual GC analysis runs, it is a good indication of the overall performance of the instrument and variation between analysis runs. The response factor should be independent of TCA concentration for a specific volume of solution, as long as there is a linear relationship between TCA concentration and peak area, as shown in the calibration plot in Figure 2.3.

To calculate the response factors, the peak areas of the standard TCA solutions were firstly corrected for the TCA present in the ultra-pure water used to prepare the standard TCA solutions. This allowed quantification of the $CHCl_3$ formed uniquely from the known mass of TCA used to prepare the standard solutions. The corrected peak area was divided by the known TCA concentration of the standard to determine the response of the instrument (Equation 2.3).

$$F_W = \frac{(A_{STD} - A_W)}{M_{TCA}} \qquad \textbf{Equation 2.3}$$

Where:

F_W = Response factor for detection of $CHCl_3$

A_{STD} = Peak area of $CHCl_3$ from the TCA in standard solutions

A_W = Peak area of $CHCl_3$ from the TCA in the ultrapure water used to prepare TCA standard solutions

M_{TCA} = total mass of TCA in vial

A summary of response factors determined from June 2002 until May 2003 is shown in Figure 2.4. This represents a period of time over which the ECD was not subjected to any maintenance or alterations that may have significantly affected its sensitivity. Each point represents the mean of three response factors calculated from three TCA standard solutions of concentrations ranging between 10 and 53 $\mu g\ l^{-1}$.

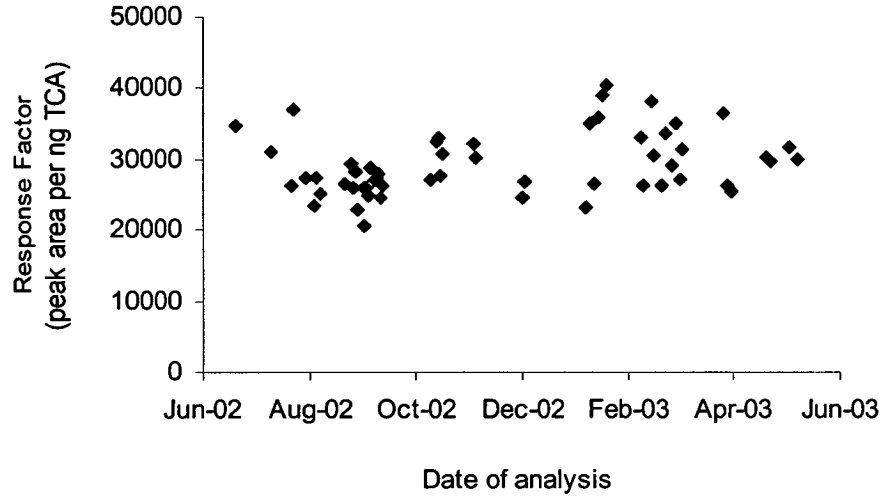


Figure 2.4. The mean response factors (F_w) from all GC analysis runs between June 2002 and May 2003. F_w was determined from standard TCA solutions of 1 ml.

The mean response factor over this period was 29200 (SD = 4400, n = 56). The magnitude and reproducibility of response factors were closely monitored within each analysis run. If standards A and B had markedly different responses or the RSD was considered too high (> 30 %), either the results of the run were discarded, or the analysis was repeated at a later date. This means that even if the response of the instrument varied from week to week, within each analysis run (approximately 13 hours) the response was fairly constant.

2.2.3.3 Sample preparation and analysis of TCA by HSGC-ECD

In order to quantify the TCA concentrations of environmental samples (needles, soil or water) after analysis by HSGC-ECD, standard solutions of TCA and ultrapure-water only were analysed alongside the samples in every GC run. To avoid confusion in terminology with ultra-pure water, environmental water samples (precipitation, river water, soil leachates and soil extracts) will be referred to as "aqueous" in this Chapter.

Ultra-pure water and blank determinations

As TCA and $CHCl_3$ are intrinsically present in ultra-pure water, five replicates of ultra-pure water samples (1 ml) were analysed in every GC run. This allowed

corrections to be made for the TCA in the ultra-pure water added to soil and needle samples (for determination of the partition ratio) and in preparation of the TCA standard solutions. These water samples were heated to 100 °C for 90 minutes to decarboxylate the TCA to $CHCl_3$, then equilibrated at 60 °C for 60 minutes. To enable quantification of the $CHCl_3$ originating uniquely from the TCA in the water, two additional replicate "blanks" of ultra-pure water were heated to 60 °C only, for 60 minutes. The $CHCl_3$ present in these blanks was then subtracted from the decarboxylated waters. Samples of ultra-pure water were also analysed for $CHCl_3$ during the analysis of aqueous solutions.

Analysis of TCA in aqueous samples

Aqueous samples were stored in the refrigerator (< 5 °C) after collection and analysed as soon as possible, often on the same day. For "natural" samples expected to have low TCA concentrations (~0 – 5 $\mu g\ l^{-1}$), 5 ml of sample was pipetted into each vial, and the exact weight recorded. For higher concentrations 1 or 2.5 ml of sample was analysed. Four replicate sample vials were prepared, three of which were heated to 100 °C for 90 minutes followed by 60 minutes of equilibration at 60 °C. The fourth replicate was heated to 60 °C for 60 minutes, to enable determination of background $CHCl_3$. A summary of the steps leading to the determination of TCA in aqueous samples is shown in Table 2.3. Shaded cells indicate the presence of $CHCl_3$ in the headspace from either TCA decarboxylation or background (BG) $CHCl_3$. The final peak area necessary to quantify the TCA concentration of the sample is that $CHCl_3$ present due to TCA alone.

Table 2.3. The steps involved in quantifying TCA in aqueous samples following the analysis of $CHCl_3$ by HSGC-ECD. Shaded cells indicate the presence of $CHCl_3$.

		$CHCl_3$ (from TCA)	$CHCl_3$ (BG)
(1) Aqueous solution (Sample)	100 °C		
(2) Aqueous solution (Blank)	60 °C		
1 - 2			

BG = background

The TCA concentration (μg l^{-1}) of an aqueous sample was determined by using Equation 2.4.

$$C_{AQ} = \frac{(A_{AQ} - A_{AQ\,BLANK})}{F_W} / M_{AQ} \qquad \textbf{Equation 2.4}$$

Where;

C_{AQ} = concentration of TCA in aqueous samples / ng g^{-1}

A_{AQ} = peak area of background (BG) $CHCl_3$ and $CHCl_3$ formed after decarboxylation of TCA in sample

$A_{AQ\,BLANK}$ = peak area of background (BG) $CHCl_3$ in sample

F_W = response factor (peak area / ng TCA, calculated according to Equation 2.3)

M_{AQ} = mass of sample / g

Analysis of TCA in soil

Fresh soil was sieved through a 2-mm mesh to remove stones and large organic matter. Homogenised soil (1 g ± 0.02) was accurately weighed into 20 ml headspace vials. 1 ml of ultra-pure water was added, as determination of partition ratios requires that 1 ml of solution is added to the soil (Section 2.2.2.4).

Four replicate sample vials were prepared, three of which were heated to 100 °C for 90 minutes followed by 60 minutes of equilibration at 60 °C. The fourth replicate was only heated to 60 °C for 60 minutes to allow determination of background (BG) $CHCl_3$ present in the soil. The ultra-pure water used to prepare the standard TCA solutions and which was also added to the samples was analysed for TCA so that the TCA from the added water could be subtracted from the final soil TCA concentrations. Water "blanks" which were heated to 60 °C only were analysed to determine the background $CHCl_3$ concentrations in the water. A summary of the analysis steps leading to the quantification of TCA in soil samples is shown in Table 2.4.

Table 2.4. Steps involved in quantifying TCA in soil (or needle) samples following the analysis of $CHCl_3$ by HSGC-ECD. Shaded cells indicate the presence of $CHCl_3$.

		SOIL		WATER	
		$CHCl_3$ (from TCA)	$CHCl_3$ (BG)	$CHCl_3$ (from TCA)	$CHCl_3$ (BG)
Soil (Sample)	100 °C				
Soil (Blank)	60 °C				
Water (Blank)	100 °C				
Water (Blank)	60 °C				
Soil 100 °C – Soil 60 °C (1)					
Water 100 °C – Water 60 °C (2)					
1 – 2					

BG = background

It is important that only $CHCl_3$ from TCA decarboxylation is used for quantification of TCA concentrations in soil. The background (BG) $CHCl_3$ already present in the soil and the ultra-pure water added to the soil before analysis must therefore be subtracted from the overall $CHCl_3$ peak area obtained for the soil samples. The final peak area required for determining the TCA concentration of soil is that of $CHCl_3$ originating from theTCA in the soil alone (1 - 2).

Equations 2.5 and 2.6 were used to calculate the soil TCA concentration after every GC analysis run. Firstly, the TCA concentration of the water added to the soil samples was calculated (Equation 2.5).

$$C_W = \frac{(A_W - A_{W\ BLANK})}{F_W} / M_W \qquad \textbf{Equation 2.5}$$

Where;

C_W = concentration of TCA (ng g^{-1}) in ultra-pure water added to soil and needles samples and used to prepare standard TCA solutions

A_W = peak area of background (BG) $CHCl_3$ and $CHCl_3$ formed after decarboxylation of TCA in ultra-pure water

$A_{W\ BLANK}$ = peak area of background (BG) $CHCl_3$ in ultrapure water

F_W = response factor (peak area / ng, calculated according to Equation 2.3)

From Equation 2.6 the TCA concentration (ng g^{-1}) of fresh soil could be calculated.

$$C_S = \frac{[(A_S - A_{SBLANK}) / F_W]}{(M_S \times F_{W/S})} - C_W \qquad \textbf{Equation 2.6}$$

Where

C_S = concentration of TCA per fresh weight of soil / ng g^{-1}

A_S = peak area of background (BG) $CHCl_3$ and $CHCl_3$ formed after decarboxylation of TCA in soil (+ 1 ml of ultra-pure water)

$A_{S\,BLANK}$ = peak area of background (BG) $CHCl_3$ in soil (+ 1 ml of ultra-pure water)

F_W = response factor of TCA in water (calculated according to Equation 2.3)

M_S = mass of soil / g

$F_{W/S}$ = partition ratio (ratio of response factors of TCA in water and TCA in soil).

Soil water and organic matter content

Soil TCA concentrations in the literature are generally expressed on a dry weight (dwt) basis. Therefore the water content for every soil sample was determined experimentally. The organic matter (OM) content of the soil was also routinely determined for scientific interest. Approximately 10 g of fresh soil was accurately weighed into ceramic crucibles and dried in an oven at 60 °C for until a constant weight was reached (about 4 days). The soil was removed from the oven, cooled in a desiccator and re-weighed. Water content was determined from Equation 2.7.

$$\%\ \text{Water} = \frac{\text{Initial soil mass (fwt)} - \text{Final soil mass (dwt)}}{\text{Initial soil mass (fwt)}} \times 100 \qquad \textbf{Equation 2.7}$$

After being re-weighed, the oven-dried soil samples were then ignited in a furnace at 550 °C for 8 hours for determination of organic matter content. The soil remaining after ignition (ash) was weighed after cooling in a desiccator. The organic matter content was calculated from Equation 2.8.

$$\%\ \text{Organic matter(OM)} = \frac{\text{Initial soil mass (fwt)} - \text{Final mass of ignited soil}}{\text{Initial soil mass (fwt)}} \times 100 \qquad \textbf{Equation 2.8}$$

The water and organic matter contents of the soil were determined from one replicate as the repeatability of the procedure was tested experimentally. The water and organic matter contents of six soils, each measured in triplicate had mean RSDs of 1 % and 2.9 %, respectively. This variation is more likely to be due to soil heterogeneity than experimental error.

After determination of the water and organic matter contents, the soil TCA concentration per g of dry weight was calculated from the from Equation 2.9, and the soil TCA concentration per g of organic matter was calculated from Equation 2.10.

$$[TCA_{SOIL}]\,ng\,g^{-1}\,dwt = \frac{[TCA]\,ng\,g^{-1}\,fresh\,weight}{1-(\%\,water\,/\,100)} \qquad \textbf{Equation 2.9}$$

$$[TCA_{SOIL}]\,ng\,g^{-1}\,OM = \frac{[TCA]\,ng\,g^{-1}\,fresh\,weight}{(\%\,OM\,/\,100)} \qquad \textbf{Equation 2.10}$$

Analysis of TCA in needles and branchwood

Needles were prepared for analysis by firstly immersing fresh spruce shoots in de-ionised water, and ultra-sonicating for 5 minutes and rinsed three times. This was to ensure that only TCA internal to the needle matrix was analysed. After drying on absorbent paper, needles were stripped from the branch with the aid of liquid nitrogen and forceps. They were homogenised to a fine powder by grinding frozen under liquid nitrogen with a pestle and mortar to ensure complete release of TCA from the needle matrix (Reeves *et al.*, 2000). Where required, branchwood was sampled from the shoot and homogenised by grinding frozen under liquid nitrogen using a pestle and mortar, as for the needles. Once homogenised the needle and branchwood samples were prepared and analysed as for soils alongside ultra-pure water samples and TCA standard solutions.

The analysis steps leading to the quantification of TCA in needle and branchwood samples were the same as for soil as summarised in Table 2.4. The calculations were

also identical to Equations 2.5 and 2.6 with the exception of the partition ratio ($F_{W/N}$) where 1.94 was used instead of the soil partition ratio ($F_{W/S}$) of 1.25.

Needle and branchwood water content

Needle TCA concentrations are generally expressed on a dry weight basis so, as for soil, the water content was determined experimentally for every sample. Approximately 5 g of fresh ground needles were accurately weighed into pyrex beakers and dried in an oven at 60 °C until a constant weight was reached (about 6 days). The needles were removed from the oven, cooled in a desiccator and re-weighed. Water content was determined in the same way as for soils (Equation 2.7) and the needle TCA concentration per g of dry weight was calculated from Equation 2.9.

The water content of the ground needles was determined from one replicate as the RSD was experimentally determined on seven replicates and found to be 1.8 %. This variation is likely to be due to both the natural variability of needles and the handling time of samples during the rinsing and grinding processes. Fresh unground needles had very similar water contents to ground needles (Stidson, 2004, unpubl.).

Precision of analysis of TCA in environmental samples using HSGC-ECD

For every sample the mean, standard deviation and relative standard deviation (RSD) of the triplicate analyses of TCA concentrations were calculated. In general, if the RSD exceeded 30 % then any obvious outlying replicates were removed. If the RSD was still 30 % after removal of replicates with outlying values, the sample was re-analysed. In the case of some soils, particularly the litter layers, high variability in TCA concentrations was observed which was assumed to be due to natural heterogeneity. Unless these concentrations were considered essential for further calculations of, for example, TCA budgets in a natural system, samples were not re-analysed. Analyses of needle samples showed good repeatability (usually less than 10 %).

Using the HSGC-ECD method of TCA analysis, Heal *et al.* (2003a) reported the mean replicate RSD values for aqueous samples to be 10 – 17 % with a limit of detection (LOD) of 0.1 $\mu g\ l^{-1}$. In another study using the same analytical method, Stidson *et al.* (2004a) reported that for aqueous samples with a TCA concentration greater than 0.8 $\mu g\ l^{-1}$, RSD of triplicate analyses was less than 30 % in 75 % of samples and less than 40 % in 94 % of samples. For aqueous samples where TCA concentrations were less than 0.8 $\mu g\ l^{-1}$, RSD values of less than 30 % and 40 % were achieved in 50 % and 62 % of cases respectively.

2.3 CHAPTER SUMMARY

Analysis of TCA via thermal decarboxylation to $CHCl_3$, followed by headspace gas chromatography with electron capture detection (HSGC-ECD) is a relatively inexpensive, simple but highly effective technique. It therefore forms the basis of TCA determinations of environmental samples throughout this Ph.D. study. A high sample throughput is possible using this method, thereby increasing the number of sample replicates that may be analysed and the confidence in the TCA concentrations determined. Regular calibrations and continual monitoring of response factors ensured confidence in the precision and accuracy of TCA concentration measured in environmental samples. The inclusion of standard solutions of three different TCA concentrations in all analysis runs and the high sample reproducibility that can be achieved for these indicates that most of the replicate variability is due to natural rather than analytical variability.

The application of this "whole soil" method avoids issues of poor recovery and contamination that may be associated with the extraction-derivatisation methods described in Section 2.2.1. Experiments to investigate the controversies surrounding the absolute accuracy of TCA measured by extraction-derivatisation methods and by headspace analysis of chloroform are presented in Chapter 3.

Chapter 3 - Evaluation of Soil TCA Analysis Methodology

3.1 BACKGROUND

In Chapter 2 the various methods of TCA analysis of environmental samples were discussed along with their inherent problems. The two most commonly used methods are: (1) the analysis of chloroform ($CHCl_3$) produced from the thermal decarboxylation of TCA in the sample using headspace gas chromatography with electron-capture detection (HSGC-ECD), and (2) the analysis of a TCA derivative using gas chromatography with mass spectrometry (GC-MS) or electron capture detection (GC-ECD). The main advantages and disadvantages of each method with specific reference to analysis of TCA in soil, are summarised in Table 3.1.

Table 3.1. A summary of the advantages and disadvantages of TCA analysis in soil by decarboxylation of TCA to $CHCl_3$ followed by analysis by HSGC-ECD; and extraction of TCA into aqueous solution followed by derivatisation and analysis by GC-MS or GC-ECD.

Method of TCA Analysis	**Decarboxylation of TCA to chloroform followed by headspace analysis**	**Extraction-derivatisation**
Advantages	• Quantification of TCA in whole soil • Ease of sample preparation • High sample throughput therefore greater replication	• Unique definition of TCA
Disadvantages	• Assumption that there are no other compounds in the soil that form $CHCl_3$ on heating to 100 °C	• Assumption that TCA is 100 % extractable from soil into solution • Multiple steps required so greater potential for contamination and/or losses • Low sample throughput therefore limited replication possible

There is considerable debate over which method of measuring TCA concentrations is more accurate, particularly in soil. As described in Chapter 2 there is no standard method used for the analysis of TCA in soils and hence a large element of uncertainty is introduced regarding absolute accuracy. It is important to determine if the variation in soil TCA concentrations of < 0.05 - 390 ng g^{-1} (Table 1.3), obtained by research groups across the world is due to geographical variation alone.

The ability to determine TCA directly from soil with the HSGC-ECD method is highly advantageous as it enables TCA in all soil compartments (air, water, organic matter and mineral) to be quantified without assuming that it is predominantly associated with one particular phase or compartment. It is assumed that all TCA present in the soil, either bound to solid particles or non-bound in the soil solution can be released on heating to 100 °C and decarboxylated to $CHCl_3$, as confirmed by Reeves (2001).

Although complex extraction and derivatisation procedures have been developed for analysis of TCA in environmental media (Scott and Alaee, 1998; Frank *et al.*, 1990; Peters, 2000) they still cannot determine TCA from the soil directly. As these methods involve several steps, the possibility of poor recovery and the introduction of other errors is likely. More importantly, the properties of soils and their possible influences on TCA concentrations have been widely neglected. Many researchers have assumed that the TCA present in soil can be extracted with 100 % efficiency into aqueous solution. This is a large assumption considering the heterogeneity of the soil system which is a combination of solid mineral and organic material, water, air and living organisms and can be regarded as an amalgam of the lithosphere, biosphere, hydrosphere and the atmosphere. The chemical, physical and biological processes occurring within and between these compartments are highly complex and should not be ignored. There is therefore reason to suspect that the determination of "soil TCA concentrations" from water-extractable TCA substantially underestimates TCA concentrations in "whole soils". It could be argued that it is the mobile, or bioavailable TCA obtained from extraction methods that is important in terms of ecotoxicity, but more understanding of TCA binding mechanisms and conditions

which favour its subsequent release are required for any kind of accurate risk assessment.

Reeves (2001) carried out a preliminary investigation into the location and binding of TCA in the soil by a series of extractions using various reagents to extract TCA from the soil. The concentrations of TCA in the soil extracts and the soil residue were measured to determine the effectiveness of extraction. It was reported that neither water nor acid efficiently removed TCA from the soil after 2 hours of shaking, and seven out of eight extractions gave concentrations in the extractant of 6 % or less of the total bulk concentration of TCA.

The main weakness of the HSGC-ECD method of TCA analysis is that the detected $CHCl_3$ may not be produced from TCA alone and there are contributions from other compounds in the soil. The only known compound to form $CHCl_3$ between 60 °C and 100 °C is trichloroacetaldehyde (chloral; CCl_3CHO). This is a known intermediate of the photo-oxidation reaction of 1,1,1-trichloroethane in the atmosphere with an atmospheric life-time of only 3-4 hours due to rapid photolysis (Rattigan *et al.*, 1993). It has also been suggested (Hoekstra *et al.*, 1999a) that trichloroethene, a postulated precursor of TCA, may undergo oxidative conversion in the soil by methanogenic organisms to chloral, 2,2,2 trichloroethanol and dichloroacetic acid. Chloral may be oxidised further resulting in TCA formation and, possibly, chloroform. However, in contrast to tetrachloroethene (PER) and 1,1,1-trichloroethane (TCE), there is little published information about inputs of trichloroethene to the soil, and it is not possible to predict if this source of chloral is large enough to provide a significant contribution to the soil chloroform measured by HSGC-ECD. Køppen *et al.* (1988) found that at pH >8 chloral is completely decarboxylated to $CHCl_3$ after heating to 60 °C for 90 minutes. This implies that if samples were at a basic pH then any chloral present in the sample would be accounted for in the blank determination of background $CHCl_3$. Reeves (2001) confirmed this from the preparation and analysis of four different soil samples with 1 ml of NaOH instead of 1 ml of water. In addition, he found that the calculated TCA concentrations were similar for both water and base additions.

3.2 CHAPTER AIMS

This chapter reports on experiments aimed at determining whether the large variations in reported soil TCA concentrations in different environments can be explained by differences in what the two main analytical methods actually measure. This was achieved in several ways:

1) A series of soil extractions was performed in order to evaluate what proportion of TCA (or the $CHCl_3$ producing compound/s) is/are extractable:

    - "TCA-free" soil was prepared, spiked with known concentrations of TCA and then re-extracted. This enabled determination of the recovery of the added TCA without the need to distinguish between intrinsic soil TCA and TCA added to the soil.

    - Fresh soil was spiked with known concentrations of TCA and then re-extracted. The recovery of TCA in the aqueous extract was determined.

    - "Natural" TCA was extracted from fresh soil using water.

The effects of contact time between soil and extractant, and the effects of soil type were also investigated within some of the above extraction experiments.

2) The influence of humic acid on measured TCA concentrations was investigated. Different concentrations of TCA and humic acid solutions were combined and recovery of TCA determined by HSGC-ECD.

3) The extent of adsorption of TCA within the soil matrix was investigated using a commercial ion exchange resin.

3.3 EXTRACTION OF TCA FROM SOIL: GENERAL METHODS

3.3.1 Extraction procedure

The following procedure is applicable to all extraction experiments. Any deviations are described in individual sections.

Sieved (2 mm) and homogenised, field moist soil (10 g) was accurately weighed into a centrifuge tube and 10 ml of aqueous solution (ultrapure water or TCA spike solution) was added using a pipette, and the weight recorded. The tube was sealed and immediately shaken for 16 hours using an orbital mechanical shaker (Gallenkamp, 200 r.p.m.). After shaking, the soil mixture was centrifuged at 8000 rpm (relative centrifugal force of 9000g) for 15 minutes. The supernatant ("soil extract") was carefully removed to a clean vial (previously heated overnight at 200 °C to remove any chloroform and chloroform precursors) using a dropper pipette. The soil mixture was centrifuged a second time and the soil extract removed again and combined with the first extract. The weights of the residue and extract were recorded at each stage. The remaining solid ("soil residue") was stored in the centrifuge tube until analysis by HSGC-ECD. Unless stated otherwise, soil residues and extracts were analysed immediately for TCA or stored in the freezer at –30 °C until required. The stages of TCA extraction from soil are illustrated in Figure 3.1.

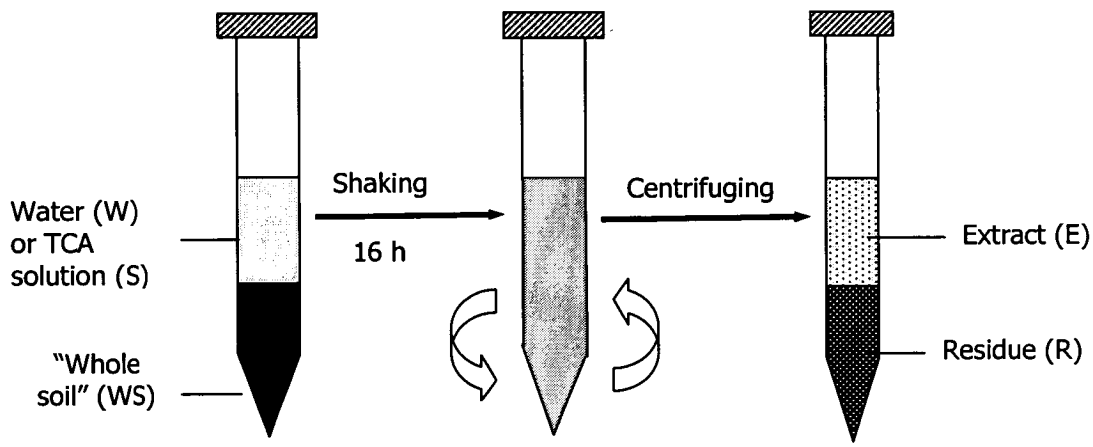


Figure 3.1. Schematic of the procedure used to re-extract TCA from soil after the addition of a TCA solution, or the water-extraction of TCA " naturally" present in soil.

3.3.2 TCA analysis of whole soil, soil residues and soil extracts

The soil residues and aqueous extracts were analysed for TCA by HSGC-ECD using the normal methods of soil and water analyses respectively, described in Chapter 2. For each of the extraction components (soil extract and soil residue), four replicate sample vials were prepared; three of which were heated to 100 °C for 90 minutes followed by 60 minutes of equilibration at 60 °C. The fourth replicate was only heated to 60 °C for 60 minutes to allow determination of background $CHCl_3$ present in the sample, as described in Chapter 2. The soil extract and soil residue, and the original "whole soil" and extractant (water or TCA solution), were all analysed for TCA in the same run.

3.3.3 Mass balance determinations

In each extraction experiment the TCA mass in each compartment (soil extract, residue, water or spike solution and whole soil) was calculated from the mass of material (g) and concentration of TCA (ng g^{-1}). The total TCA present before and after extraction was then compared. This is described as the "mass balance". If there was no loss or gain of TCA throughout the extraction process then the sum of the TCA burden (ng) of the soil and the TCA burden of the water or spike solution should equal the sum of the soil extract and the soil residue (Equations 3.1a and b). This assumes that there is no loss of analyte due transfer between containers, adsorption to containers (accounted for by controls) or degradation occurring in the soil during the experimental procedure.

If Equations 3.1a and b balance then this validates the efficiency of the analysis procedure and the method of calculating the TCA burdens. However, what is scientifically more interesting is the proportion of TCA that can be detected in the soil extract compared to the soil residue. This will clarify whether or not TCA may be extracted into solution, and therefore the accuracy of TCA analytical methods which involve extraction of TCA into water.

For **SPIKED** extractions, there is 100 % mass balance when:

$$([TCA_S] \times M_S) + ([TCA_{WS}] \times M_{WS}) = ([TCA_R] \times M_R) + ([TCA_E] \times M_E) \quad \textbf{Equation 3.1a}$$

For **WATER** extractions, there is 100 % mass balance when:

$$([TCA_W] \times M_W) + ([TCA_{WS}] \times M_{WS}) = ([TCA_R] \times M_R) + ([TCA_E] \times M_E) \quad \textbf{Equation 3.1b}$$

Where: $[TCA_X]$ = Concentration of TCA in material X
M_X = Mass of material X
S = TCA solution
W = Water
WS = "Whole Soil"
R = "Soil Residue"
E = "Soil Extract"

The percentage of TCA which is extracted into solution was calculated from Equation 3.2. This applies to both the re-extraction of TCA from spiked soils and the extraction of "natural" TCA using water. If 100 % of TCA can be extracted into water, as assumed for TCA analysis by extraction and derivatisation, then $[TCA_R]$ should be zero.

$$\text{TCA in extract } (\%) = \frac{([TCA_E] \times M_E) \times 100}{([TCA_R] \times M_R) + ([TCA_E] \times M_E)} \quad \textbf{Equation 3.2}$$

Where: $[TCA_X]$ = concentration of TCA in material X
M = Mass of material X
R = "Soil Residue"
E = "Soil Extract"

Equations 3.1a and b, and 3.2 were used to determine the mass balance and proportion of TCA extracted into water in spiked TCA-free soil extractions, spiked fresh soil extractions and extraction of "natural" soil TCA using water. The details of these experiments are described in Sections 3.4 – 3.7.

3.4 EXTRACTION OF TCA FROM SPIKED "TCA-FREE" SOIL

3.4.1. Introduction and aims

Extractions were carried out on TCA-free soil using the procedure described in Section 3.3.1. This removed the uncertainties concerning the concentration of TCA already present in the soil. The only TCA present was applied from a solution of known concentration. Therefore TCA recovery could be determined with complete certainty of the original concentration.

The main objectives of these experiments were:

1. To create a TCA-free soil by prolonged heating to decarboxylate all TCA and other $CHCl_3$-forming moieties in the soil to $CHCl_3$.
2. To determine if added TCA can be re-extracted from "TCA-free" soil, despite the possible effects of binding to organic matter or humic substances.

3.4.2 Methods

3.4.2.1 Preparation of "TCA-free" soils

Soil was collected from three different field locations; "Agricultural" (gleysol, A horizon), "Moorland" (peaty gleysol, Bg horizon) and "Larch", (peaty gleysol, Bg horizon). Samples (approximately 200 g) were sieved (2 mm), placed in Pyrex beakers and heated in an oven for 5 days at 65 °C then 1.5 hours at 100 °C. This was to remove any resident soil TCA and other compounds that may form $CHCl_3$ between 60 °C and 100 °C.

To check for complete removal of TCA, a subsample of each soil was analysed for TCA using the standard procedure (Chapter 2), but without the addition of 1 ml of water as this itself contains TCA. The soil TCA concentration could therefore not be quantified, but the $CHCl_3$ peak area of the blank was compared with the $CHCl_3$ peak area of the three sample replicates. No difference in peak area between the blank and the samples would confirm that no extra $CHCl_3$ is formed from the decarboxylation of TCA or other compounds in the soil. Ultrapure water was boiled, to remove any TCA present, for subsequent use in soil extraction experiments.

3.4.2.2 Spiking of "TCA-free" soils with TCA

A solution of approximately 50 μg l^{-1} TCA was accurately prepared using a stock solution and dilution with ultrapure water. The extractions were carried out according to the procedure described in Section 3.3.1, but dried soil was used in place of fresh soil, and 15 ml of the 50 μg l^{-1} TCA solution was added to the soil in the centrifuge tube instead of ultrapure water. Extractions were performed on three replicates of Moorland soil, two replicates of Agricultural soil and one replicate of Larch soil. Soils were extracted immediately after shaking for 16 hours with the TCA spike solution, then one replicate of each soil was immediately analysed for TCA or stored in the freezer at –30 °C before analysis at a later date (t=0). One replicate of each of Moorland and Agricultural soils was also stored for five days (t=5) in the fridge at <5 °C, after shaking with TCA spike solution, to allow more contact time with the soil. As the detector response of the GC varied slightly between runs, the TCA spike solution was analysed in each analysis run and the measured concentration used only for calculations with those samples (whole soil, soil extracts and soil residues) analysed in the same run. The TCA concentration of the whole soil was assumed to be zero.

3.4.3 Results and discussion

3.4.3.1 Creation of "TCA-free" soil

TCA was successfully removed from soil after prolonged heating. Table 3.2 shows the $CHCl_3$ peak areas obtained for the Agricultural, Moorland and Larch soils. The background $CHCl_3$ peaks were obtained from soils heated to 60 °C only for 1 hour. The peak areas of the three replicate samples were obtained from soils heated to 100 °C for 1.5 hours followed by equilibration at 60 °C for 1 hour. Although there is high variability between replicates, the values are well below the detection limit of the instrument (~0.1 μg l^{-1}) for determination of TCA concentrations. It is apparent that there is no difference between [background $CHCl_3$] and [background $CHCl_3$ + $CHCl_3$ from TCA]. It may also be assumed that any chloral in the soil has been removed as it has a boiling point of around 98 °C.

Table 3.2. $CHCl_3$ peak areas obtained from dried soil blanks (A: heated to 60 °C only for 1 hour) and samples (B: heated to 100 °C for 1.5 hours followed by 60 °C for 1 hour) and analysed by HSGC-ECD.

$CHCl_3$ Peak Area (uncorrected)	**Soil Type**		
	Agricultural	**Moorland**	**Larch**
(A) Background $CHCl_3$	5841	81	3574
Background $CHCl_3$ and $CHCl_3$ from TCA (3 analytical replicates)	301	1535	2
	1138	34	3370
	4132	1240	2622
(B) Mean (± SD, n=3)	1857 (2014)	936 (795)	1998 (1769)
$CHCl_3$ from TCA only (B – A)	-3894	855	-1576

3.4.3.2 TCA in whole soil, soil residues and soil extracts

The mass balance results for spiked TCA extractions from "TCA-free" soil are shown in Figure 3.2. The bars represent the actual TCA mass present (measured) in the centrifuge tube before ("pre-") and after ("post-"), extraction. The percentage mass balance is shown above each post-extraction TCA bar. For all soils the measured TCA burden in the centrifuge tube post-extraction was greater than the burden pre-extraction. The percentage mass balances were greatest for the Agricultural soil (156 and 139 %) and similar for both the Moorland and Larch soils (120 – 124 %).

The combined concentrations of TCA measured in the soil extract and soil residue were greater than the concentration of TCA measured in the original spike solution. As aqueous solutions were analysed alongside TCA standard solutions it is unlikely that the "pre-extraction" TCA concentration has been underestimated. It is therefore more likely that the source of the error or variation lies either with the overestimation of "post-extraction" TCA concentrations in the soil residues and extracts or a genuine trend of TCA concentrations increasing when soil is re-wetted.

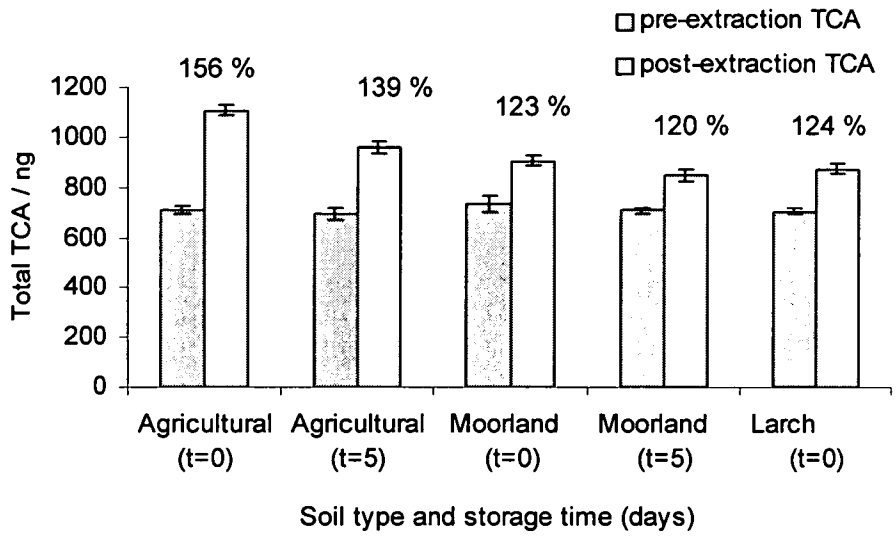


Figure 3.2. Mass balance of TCA in centrifuge tubes before extraction ("Pre-extraction" = mass of TCA in spike solution) and after extraction ("Post-extraction TCA" = mass of TCA in soil extract + mass of TCA in soil residue) for "TCA-free" soil spiked with TCA. The post-extraction TCA as a percentage of the pre-extraction TCA is shown above the bars. Error bars are standard deviations of one soil extraction (two soil extractions for Moorland (t=0)), each one analysed in triplicate.

One potential error in determining TCA concentrations in these experiments is the assumed value for the partition ratio in the analysis. Due to the small mass of sample available, it was not possible to re-determine the partition ratios of the soil residues. A soil partition ratio of 1.25 was used for Moorland and Larch soil residues, and 2.01 for Agricultural soil, as is standard for soil analyses throughout this research. The actual partition ratios of the soil residues may differ slightly from those calculated for fresh soils. It is also possible that the $CHCl_3$ in soil extracts partitions into the gas phase to a different extent from other aqueous solutions (i.e. partition ratio $\neq$ 1.00) due to its more variable consistency. Another cause of the high mass balances is possibly related to the alteration of physical, biological and chemical characteristics of soil by removal of water in the preparation of TCA-free soil. Different soils are likely to be affected to different extents by the drying process involved in creating TCA-free soil as well as responding differently to the introduction of "new" TCA.

Re-wetting of 100 % dry soil is not likely to recreate the structure of the original fresh soil, partly as the drying process induces hydrophobicity in soils. Table 3.3 illustrates how the water content of the soil residue after shaking the dry soil with TCA spike solution differs from the original moisture content, with the "new" water content being lower than the original water content for Agricultural and Moorland soils, and greater for the Larch soil.

Table 3.3. A summary of the water and organic matter contents (fwt and dwt) of the soils used for TCA-free soil extractions, before being dried.

Soil type	% Water in original soil	% Organic matter (fwt)	% Organic matter (dwt)	% Water in soil residue
Agricultural	26.7	5.3	7.0	22.9
Moorland	52.0	9.3	80.6	39.3
Larch	32.1	8.3	12.3	38.7

Another possible cause of the high mass balances is the formation of TCA in the soil which may have been stimulated during the extraction process. It is well-known that microbial activity can be stimulated when dried soil is re-wetted (Vinten and Smith, 1993). If this was the cause of the high mass balances then it is evidence of TCA production in soils. An alternative explanation is that other $CHCl_3$ precursor compounds are formed on re-wetting.

Figure 3.3a shows the post-extraction TCA apportioned between the extract and the residue. It is evident that for Moorland and Larch soils there is a greater TCA burden in the soil residue than the soil extract. Although in Agicultural soils there is a greater TCA burden in the soil extract, 39 – 43 % of TCA still remains in the soil residue. This shows that TCA cannot be 100 % extracted into solution. Even considering the mass of aqueous solution that remains within the residue, some of the TCA is still associated with the solid fraction of the soil.

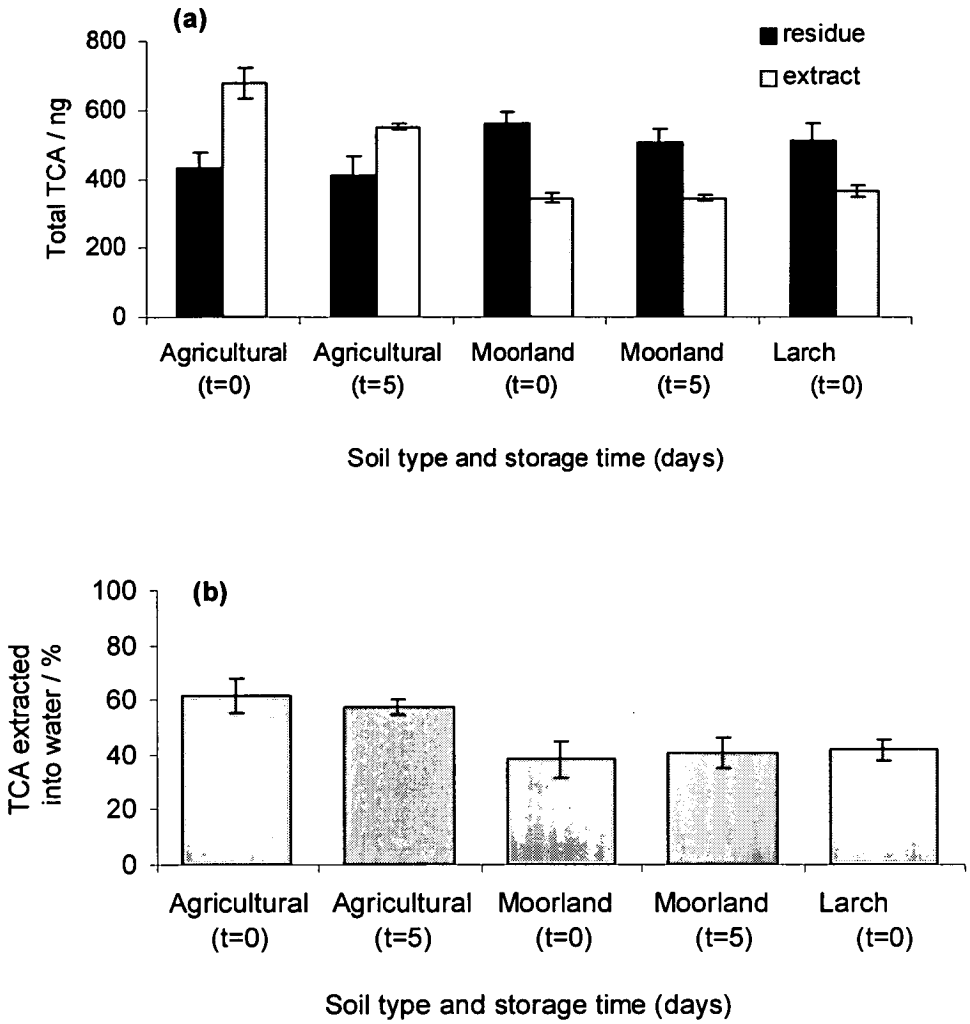


Figure 3.3. (a) The mass of TCA post-extraction in the soil residue and soil extract after addition of TCA solution to TCA-free soil and re-extraction. Error bars are standard deviations of one soil extraction (two soil extractions for Moorland (t=0)), each one analysed in triplicate; (b) The mass of TCA in the soil extract expressed as a percentage of the total TCA in the soil extract + residue. Error bars are RSDs of one soil extraction (two soil extractions for Moorland (t=0)), analysed in triplicate.

In Figure 3.3b the TCA burden in the soil extracts is expressed as a percentage of the combined TCA burden of the soil extracts and residues. Re-extraction of TCA is most efficient in Agricultural soil at both t=0 and t=5, with 61 % and 58 % of TCA respectively present in the extract. It is more difficult to re-extract TCA from the Moorland (t=0 and t=5) and Larch soils, with 38 %, 41 % and 42 % respectively,

present in the extracts. These latter soils have a higher organic matter content than the Agricultural soil and possibly have a greater capacity for TCA binding with organic compounds.

Figures 3.2 and 3.3 suggest that there is little difference between the mass balances and proportion of TCA present in the residues and extracts of soils analysed immediately (t=0) or after 5 days (t=5). Therefore any processes that occur as a result of re-wetting, or any degradation of the TCA spike solution, occur almost instantly.

These extraction experiment show that externally added TCA cannot be 100 % recovered from TCA-free, dried soil, even after shaking and centrifuging. This raises doubts about the accuracy of methods of TCA analysis which rely upon extraction of TCA from soil using water. TCA intrinsic to the soil is likely to be even more difficult to remove with water due to the lower concentrations usually involved and the fact that increased time of contact may increase the strength of TCA binding to soil components.

3.5 EXTRACTION OF TCA FROM SPIKED FRESH SOILS

3.5.1 Introduction and aims

The aim of these experiments was to determine if added TCA could be re-extracted from fresh soil, despite the possible effects of binding to organic matter or humic substances. These experiments were conducted on two different occasions with the following objectives:

(A) A preliminary study using one soil only to investigate the effect of contact time between TCA solution and soil on efficiency of TCA extraction.

(B) Further extractions using more than one soil type to investigate TCA extraction efficiencies from different soil types. A sterile soil was also used to investigate whether biological degradation affects the recovery of TCA added to soil.

3.5.2 (A) Preliminary study: Effect of contact time between TCA and soil on extraction recovery of TCA

3.5.2.1 Methods

10 ml of TCA solution (approximately 100 μg l^{-1}) was added to 10 g of Larch soil and the extractions carried out as described in Section 3.3.1. Eight replicates were prepared, four of which were centrifuged immediately after shaking and the extracts and residues either analysed for TCA on the same day or stored in the freezer until analysis. The other four replicates were stored in the fridge for 8 weeks before being centrifuged and analysed for TCA ("fridge-stored soils"). Subsamples of the original "whole" soil were stored under the same conditions (i.e. fridge or freezer) as the extraction samples and analysed alongside the extracts and residues in every analysis run.

3.5.2.2 Results and discussion

The mean TCA concentration of the whole soil at all time steps was 30.6 (± 7.7; n =13) ng g^{-1} (fwt). Concentrations ranged from 19.3 to 40.2 ng g^{-1} (fwt). This range can largely be attributed to soil heterogeneity. The soil water content was 63.7 (± 0.2) % and the organic matter content (fwt) was 25.3 (± 2.3) %. In this experiment the mean TCA concentration was used in mass balance calculations, rather than the TCA concentration of the whole soil for each individual analysis run. This was sufficient for the nature of the study since the main experimental aim was to determine if added TCA can be re-extracted into water. The analysed mean concentration of the spike solution was 109 (± 5) μg l^{-1} (n=3). The pre-extraction mass of TCA in each centrifuge tube was calculated from the mean concentration of TCA in the whole soil and the TCA concentration of the spike solution added to the soil. The mass balance was determined from Equation 3.1a and is shown in Figure 3.4.

It is immediately apparent from Figure 3.4 that the mass balance for these spiked extractions is poor. The total recovery of TCA measured in soil residues post-extraction was only 21 (± 3) % of the pre-extraction TCA concentration for freezer-stored and 18 (± 2) % for fridge-stored samples. There was no significant difference

between samples stored in the freezer and the fridge (unpaired *t*-test, P = 0.12). The magnitude of this difference in pre-extraction and post-extraction TCA masses is unlikely to be solely due to analytical error or natural variation between samples. There was negligible loss of sample throughout the extraction procedure therefore the immediate supposition is that the TCA added to the soil was rapidly degraded

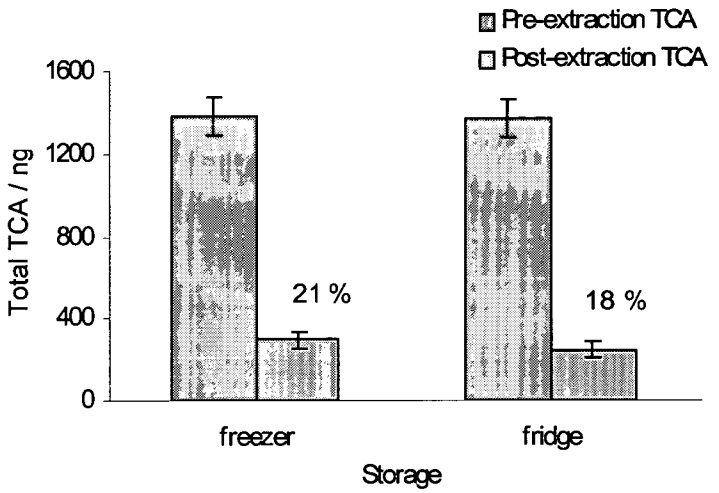


Figure 3.4. Mass balance of TCA in Larch soil in centrifuge tubes pre-extraction (mass of TCA in soil + mass of TCA in spike solution) and post-extraction (= mass of TCA in soil extract + mass of TCA in soil residue) following addition of a TCA spike. The post-extraction TCA as a percentage of the pre-extraction TCA is shown above the bars. Error bars are standard deviations of four soil extractions each one analysed in triplicate.

Figure 3.5a shows the mass of TCA present in the residue and extract after extraction. A considerably greater mass of TCA is present in the residue than the soil extract. This is shown more clearly in Figure 3.5b where the TCA in the extract is expressed as a percentage of the total TCA in the residue and extract.

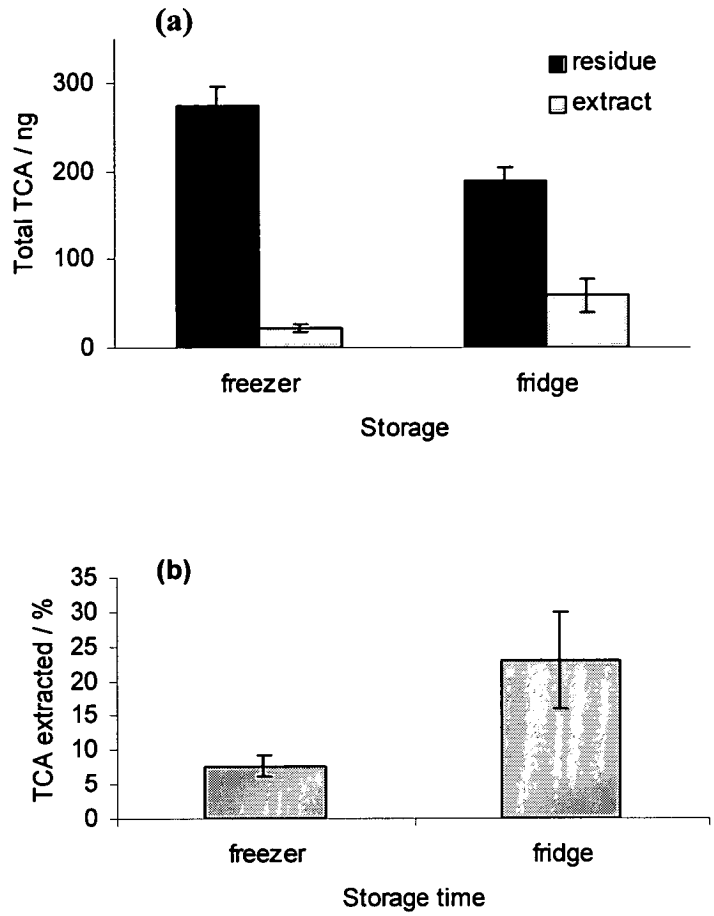


Figure 3.5. a) The mass of TCA post-extraction in the soil residue and soil extract after addition of TCA solution to Larch soil followed by re-extraction, and b) The mass of TCA in the soil extract expressed as a percentage of the total TCA in the extract + residue. Error bars are standard deviations of four soil extractions, analysed in triplicate.

For freezer-stored samples the mean recovery of TCA in the extract was 7.6 (± 1.5) %. And for fridge-stored samples the mean recovery of TCA in the extract was 22.8 (± 7.0) %. This suggests either that more TCA can be extracted with a greater contact time, or that more TCA has been degraded in the residue of fridge-stored soils resulting in a greater relative proportion of TCA in the extract. These results clearly show that added TCA cannot be easily extracted from Larch soil in aqueous solution, even considering the poor mass balances. It is also likely that added TCA is not bound to the soil as the non-extracted TCA cannot be detected in the residue.

3.5.3 (B) Spiked extractions: Effect of soil type on extraction recovery of TCA

Further extractions were carried out to confirm the observations already made in the preliminary spiked extraction and to investigate the influence of soil type, including sterile and non-sterile soils.

3.5.3.1 Methods

Three different soil types were used: "Sitka" (deep peat), "Agricultural" (gleysol), and "Larch" (peaty gleysol). To investigate the possible role of micro-organisms in the soil when TCA is artificially added, a portion of the Sitka soil was sterilised with a cobalt 60 source of gamma radiation using a dose of 27 – 35 kGy, at Ethicon Ltd. Edinburgh. 10 ml of TCA solution (approximately 90 $\mu g\ l^{-1}$) was added to 10 g of soil and extracted as described in Section 3.3.1. Four replicates of sterile Sitka soil and two replicates of non-sterile Sitka, Agricultural and Larch soil were prepared. After shaking, all samples were centrifuged on the same day and either analysed for TCA immediately ("fridge-stored") or stored in the freezer until required ("freezer-stored"). Due to time constraints some samples were frozen before being centrifuged, then defrosted, centrifuged and analysed all on the same day.

3.5.3.2 Results and discussion

Excellent mass balances were obtained for sterile Sitka (104 %), non-sterile Sitka (114 %) and Agricultural soils (102 %) although the mass balance for Larch soil (25 %) was poor (Figure 3.6). This latter result follows the trend observed in the preliminary spiked experiments (A) in which poor mass balances were obtained for all replicates of Larch, analysed either immediately or after storage in the fridge. The Larch soil used in Experiment A was collected on a different date to that of Experiment B which implies that the loss of TCA in Larch soil is a real effect, specific to Larch soil and not due to an anomalous soil sample.

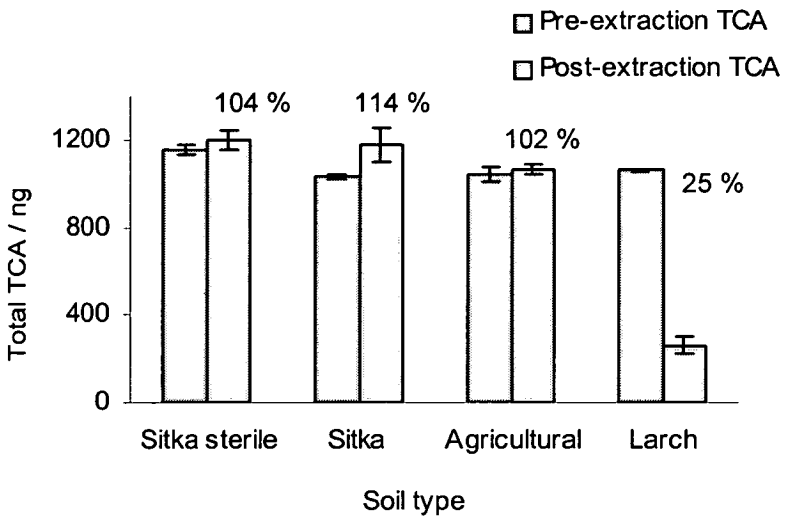


Figure 3.6. Mass balance of TCA in different soils in centrifuge tubes pre-extraction (mass of TCA in soil + mass of TCA in spike solution) and post-extraction (mass of TCA in soil extract + mass of TCA in soil residue) following addition of a TCA spike. The post-extraction TCA as a percentage of the pre-extraction TCA is shown above the bars. For Sitka sterile soil, error bars are standard deviations of four soil extractions, each one analysed in triplicate. For Sitka non-sterile, Agricultural and Larch soils, error bars are the combined standard deviations of two soil extractions, analysed in triplicate.

Similar mass balances were obtained for the sterile and non-sterile Sitka soil implying that no loss of TCA occurs after the spike solution is applied to the soil and that TCA is not degraded by micro-organisms within the time scale of this experiment. TCA loss in Larch soil may still be due to biodegradation as different soils support different microbial communities. The other possible explanation is that TCA is bound with extra strength by the Larch soil matrix such that it cannot be detected by HSGC-ECD. This would have to occur to such an extent that TCA within the soil could not be released to $CHCl_3$, even after heating to 100 °C for 1.5 hours followed by equilibration at 60 °C for 1 hour.

Figure 3.7a shows the distribution of TCA between the soil residue and soil extracts after extraction. In Figure 3.7b the mass of TCA in the extract is expressed as a percentage of the total mass of TCA in the soil residue and soil extract combined.

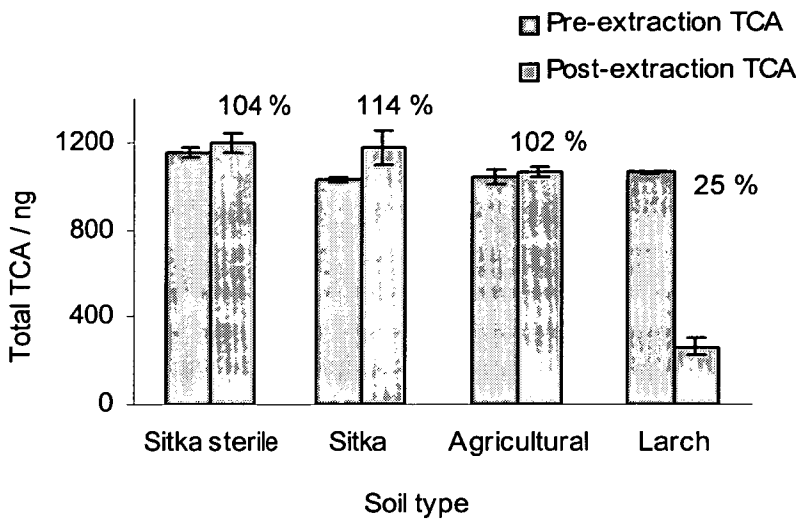


Figure 3.6. Mass balance of TCA in different soils in centrifuge tubes pre-extraction (mass of TCA in soil + mass of TCA in spike solution) and post-extraction (mass of TCA in soil extract + mass of TCA in soil residue) following addition of a TCA spike. The post-extraction TCA as a percentage of the pre-extraction TCA is shown above the bars. For Sitka sterile soil, error bars are standard deviations of four soil extractions, each one analysed in triplicate. For Sitka non-sterile, Agricultural and Larch soils, error bars are the combined standard deviations of two soil extractions, analysed in triplicate.

Similar mass balances were obtained for the sterile and non-sterile Sitka soil implying that no loss of TCA occurs after the spike solution is applied to the soil and that TCA is not degraded by micro-organisms within the time scale of this experiment. TCA loss in Larch soil may still be due to biodegradation as different soils support different microbial communities. The other possible explanation is that TCA is bound with extra strength by the Larch soil matrix such that it cannot be detected by HSGC-ECD. This would have to occur to such an extent that TCA within the soil could not be released to $CHCl_3$, even after heating to 100 °C for 1.5 hours followed by equilibration at 60 °C for 1 hour.

Figure 3.7a shows the distribution of TCA between the soil residue and soil extracts after extraction. In Figure 3.7b the mass of TCA in the extract is expressed as a percentage of the total mass of TCA in the soil residue and soil extract combined.

greater extent in the soil extract compared to the soil residue. If TCA disappeared from both soil fractions equally then the percentage recovery in the extract would not differ from the other soils. Alternatively, TCA may be bound by the soil to such an extent that the TCA cannot be released to $CHCl_3$ and analysed by HSGC-ECD. From the results of these experiments the possible explanations for poor mass balance - TCA degradation by chemical or microbial activity, or binding to the soil - cannot be distinguished from each other.

These experiments again highlight the problems of TCA analysis via extraction and derivatisation methods. Even if TCA can be extracted into solution, the efficiency varies between soils. It must be borne in mind, that although artificial application of TCA to soil gives some indication of analytical recovery, it does not provide information on whether intrinsic soil TCA may also be recovered. In addition, TCA applied to the soil as a spike solution may behave in a very different manner to TCA intrinsic to the soil and the possible effect it may have on soil micro-organisms is not known.

3.6 WATER-EXTRACTION OF INTRINSIC TCA FROM FRESH SOIL

3.6.1 Introduction and aims

The aim of these experiments was to determine if TCA intrinsic to the soil could be extracted into water, as assumed by extraction-derivatisation methods of TCA analysis. These experiments were carried out on two different occasions with the following objectives;

(A) Preliminary study using one soil only to investigate the effect of contact time between added water and soil on efficiency of TCA extraction from fresh soil.

(B) Further extractions using more than one soil type to investigate TCA extraction efficiencies from different soil types.

3.6.2 (A) Preliminary water extraction of TCA

3.6.2.1 Methods

10 ml of ultrapure water was added to 10 g of the same Larch soil used for preliminary spiked extractions in Section 3.5.2.1 and extracted as described in Section 3.3.1. Ten replicates were prepared, four of which were centrifuged immediately after shaking and the extracts and residues either analysed for TCA on the same day or stored in the freezer until required ("freezer-stored soils"). The other six replicates were stored in the fridge for 8 weeks ("fridge-stored soils") before being centrifuged and analysed for TCA. Sub-samples of the original soil were stored under the same conditions as the extraction samples and analysed alongside the extracts and residues in every analysis run.

3.6.2.2 Results and discussion

The soil TCA concentration of 30.6 (± 7.7; n = 13) ng g^{-1} (fwt), as determined for the preliminary spiked soil extractions (Section 3.5.2.1) was used for mass balance calculations and to determine the percentage of TCA recovery in the extracts.

The pre-extraction mass of TCA in each centrifuge tube was calculated from this mean soil TCA concentration and the TCA concentration of the water which was added to the soil. The mass balance was determined from Equation 3.1b and is shown in Figure 3.8. Mean mass balances were 82 (± 5.0) % for freezer-stored and 82 (± 6.3) % for fridge-stored samples. That is, the sum of the TCA in the residue and the extract was less than the burden of TCA measured in the whole soil before extraction. However given the possible partition ratio uncertainties discussed in previous sections and possible TCA degradation in the soil, these mass balances are excellent and inspire further confidence in the accuracy and precision of the HSGC-ECD method of TCA analysis.

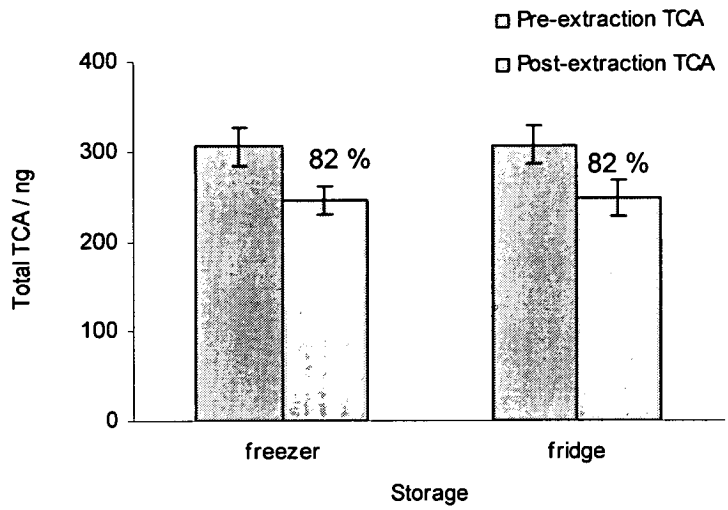


Figure 3.8. Mass balance of total TCA mass in residue and extract after aqueous extraction ("post-extraction") of "natural" TCA in Larch soil compared with the TCA mass in the whole soil and added ultrapure water ("pre-extraction"). The post-extraction TCA as a percentage of the pre-extraction TCA is also shown above the bars. Error bars are standard deviations of four (freezer), or six (fridge) extractions, analysed in triplicate.

Figure 3.9a shows the mean TCA burdens of the soil residues and soil extracts. TCA was also introduced to the soil in the ultrapure water used for extraction but it was not possible to determine how this TCA partitioned between the extract and the residue. Thus, the TCA concentration of the water was included in the calculations and shared between the residues and extracts shown in Figure 3.9a.

It is evident that the TCA burden of the extract is small compared to the residue burden. If the $CHCl_3$ measured in the whole soil and the residue is only from the decarboxylation of TCA in the soil then it is very clear that an analytical method based on extraction of soil using water yields a very small proportion of total soil TCA.

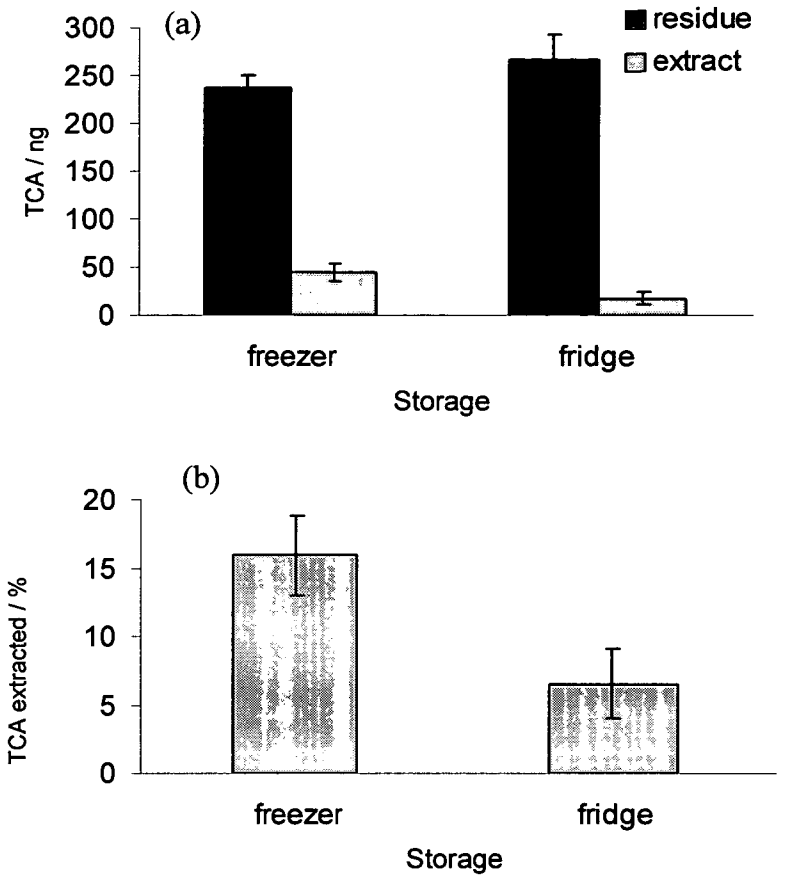


Figure 3.9. a) The mass of TCA measured in the soil residue and soil extract after aqueous extraction of fresh Larch soil, and b) The mass of TCA in the soil extract expressed as a percentage of the total TCA in the extract + residue. Error bars are standard deviations of four (freezer), or six (fridge) extractions, each one analysed in triplicate.

The percentage recoveries of TCA in the soil extract are shown in Figure 3.9b. 16 (± 2.9) % of the TCA after the extraction was found in the extract for freezer-stored samples and only 6.5 (± 2.5) % in fridge-stored samples. This firstly emphasises the poor extraction of TCA from soil into water, and secondly suggests that samples stored in the fridge have poorer extraction recoveries than those analysed immediately (unpaired *t*-test, P = 0.04). It is evident that increasing the contact time of soil and water does not increase the mass of TCA that may be extracted, which is in contrast to the results of the preliminary spiked TCA extractions where samples

stored in the fridge had better extraction recoveries. However, the concentrations of TCA in ultrapure water were approximately 100 times lower than the TCA spike solution, so the soil may respond differently to these TCA additions.

The poor extraction efficiency of TCA from soils by water may be due to binding of TCA within the soil residue, or degradation within the extract. However if TCA were degraded the mass balances of these samples (stored in the freezer and in the fridge) would differ (Figure 3.8). The good mass balances show that TCA has not been lost from the system, but the majority of TCA is not extracted by water and possibly remains bound within the soil, for example, to humic material or iron/aluminium oxides (Goring, 1967). These results provide further evidence that bound TCA can still be detected by HSGC-ECD, but not by extraction-derivatisation procedures.

3.6.3 (B) Water extraction of TCA from different soils

3.6.3.1 Method

Two different soil types were used for these extractions: "Larch" (peaty gleysol) and "Sitka" (deep peat). 10 ml of ultrapure water was added to 10 g of soil and extracted as described in Section 3.3.1. Six replicates of Sitka soil and two replicates of Larch soil were prepared. After shaking, samples were centrifuged on the same day and either analysed for TCA immediately or stored in the freezer until required. Due to time constraints some samples were frozen before being centrifuged, then defrosted, centrifuged and analysed all on the same day.

3.6.3.2 Results and discussion

The whole soil TCA concentration was 38 (± 5; n = 6) ng g^{-1} (fwt) for the Sitka soil and 21 (± 1; n = 2) ng g^{-1} (fwt) for the Larch soil. The mean TCA concentration of the water used for extractions was 5.1 (± 2.9) μg l^{-1}. Excellent mass balances of 99 (± 6) % and 91 (± 3) % were obtained for Sitka for Larch soils respectively (Figure 3.10). This is evidence of the high quality of analysis, as it means that TCA in all components has been accounted for. If biodegradation of TCA occurs more readily

in Larch soil, as suggested by the results of spiked extractions in Section 3.4, then this may account for the slightly poorer mass balance of the Larch soil. The contribution of TCA from the added water was approximately 51 ng. This TCA may be utilised by micro-organisms in the soil, resulting in the loss of TCA from the system. Sources of error contributing to a mass balance of less than 100 % include the unknown partition ratio of the soil residue (as explained in Section 3.4) and variation in water TCA concentrations as these are generally quite low and therefore more prone to analytical variation.

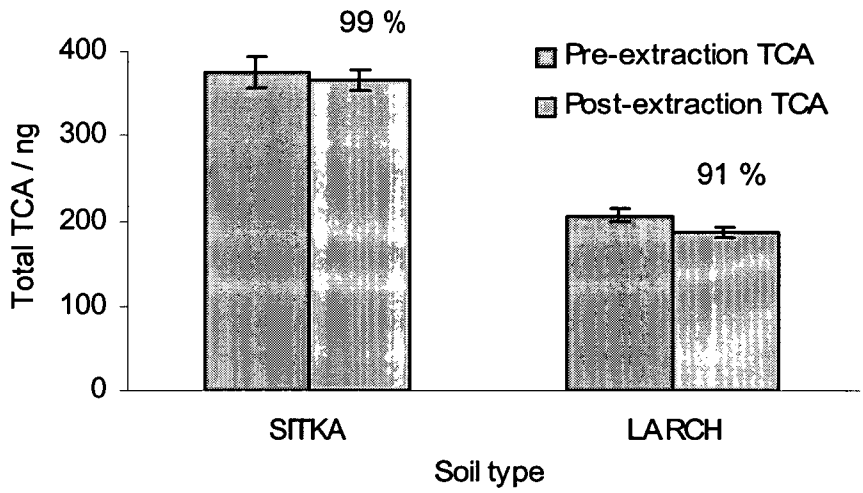


Figure 3.10. Mass balance of total TCA in the soil residue and soil extract after aqueous extraction ("post-extraction") of intrinsic TCA in fresh Sitka and Larch soils, compared with the TCA mass in the whole soil and added ultrapure water ("pre-extraction"). The post-extraction TCA as a percentage of the pre-extraction TCA has also been shown above the bars. Error bars are standard deviations of six (Sitka), or two (Larch) extractions, each one analysed in triplicate.

Figure 3.11a shows the mean TCA masses of the soil residues and soil extracts. In both Sitka and Larch soils the mass of TCA in the extract is small compared to the residue, as in the preliminary water extraction experiments using Larch soil. Figure 3.11b shows that only 17 (± 0.7) % of TCA was extracted from the Sitka soil and only 28 (± 0.7) % from the Larch soil. In the preliminary experiment an even smaller percentage of TCA was extracted in the Larch soil (Figure 3.11b), but this was probably due to soil heterogeneity, even between samples collected from the

same soil type at the same site. It is interesting that both intrinsic TCA and artificially added TCA behave differently in Larch soils compared to other soils, concerning both the mass balances obtained and the percentage of added TCA recovered in the extract after aqueous extraction.

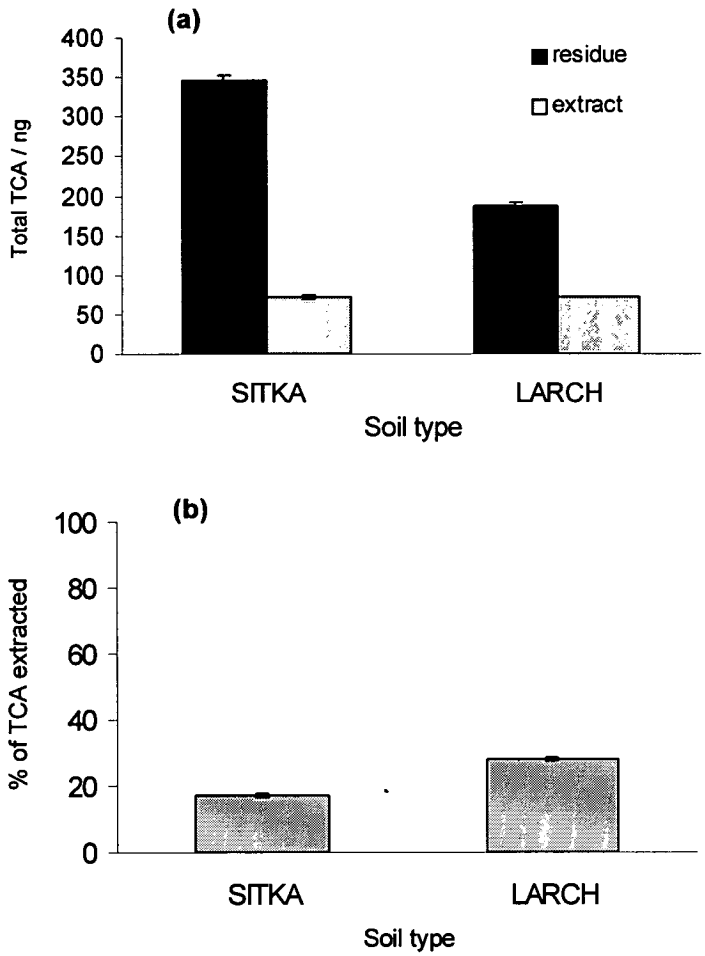


Figure 3.11. a) Mass of TCA measured in the soil residue and soil extract after extraction with water, and b) The mass of TCA in the soil extract expressed as a percentage of the total TCA in the residue + extract. Error bars are standard deviations of six (Sitka), or two (Larch) extractions, analysed in triplicate.

3.7 CONCLUSIONS OF ALL EXTRACTION EXPERIMENTS

Excellent precision was generally achieved in these experiments, both analytically and between separate soil extractions. These experiments provide good evidence that not all TCA in a range of soil types may be extracted into solution, and remained

in the soil residues. In addition to water extractions, Reeves (2001) also performed extractions using base (0.1 M NaOH, pH 13). The basic extracts were an intense orange-brown colour indicating the presence of humic acids and possible precipitation of hydrous iron oxide (Goring, 1967). They had higher TCA concentrations than water extracts (pH 7) but still only extracted up to 10 % of the TCA present in the soil. Scott *et al.* (2000) also extracted TCA from soil using base (pH 10) before analysing for TCA using a derivatisation method (Scott & Alaee, 1998). Higher TCA concentrations were obtained than for the same method using water extraction. However, using acid (0.06 M H_2SO_4, pH 1) Reeves (2001) reported that only 3 % of TCA present in the soil could be extracted which implies that if TCA is bound to anion exchange sites in the soil, it cannot be desorbed even when converted to its acid form below its pK_a of 0.26.

Asplund *et al.* (1989) proposed that the majority of organohalogens are high molecular weight products and in soils they may be derived from humic substances. Therefore attempts to extract such products with any solvent will be incomplete and quantitative analysis by conventional methods will be unsatisfactory (Nkusi & Muller (1995), cited in Grimvall (1995)).

3.8 INVESTIGATION OF THE INFLUENCE OF HUMIC ACID ON TCA CONCENTRATIONS

3.8.1 Introduction and aims

Previous studies by Haiber *et al.* (1996) have reported that TCA disappears during percolation through soils rich in dissolved organic carbon (DOC). However, the same authors also detected TCA in DOC rich waters at concentrations greater than those normally found in precipitation. They assumed from this that TCA in soil undergoes decomposition and formation processes equally. In lysimeter experiments they observed that organic-rich soils had a greater retention capacity for TCA than more mineral soils which suggests that organic matter and associated organic acids interact with TCA. The TCA recoveries of water samples spiked with humic acid (0 – 20 mg l^{-1}) were also determined. It was reported that the recovery rates of TCA

decreased substantially depending on the humic acid concentrations. This was attributed to either physical or chemical binding to the humic acid with subsequent reaction products, or decomposition of TCA via decarboxylation to $CHCl_3$.

However, it is possible that the analytical method of extraction-derivatisation used by this group failed to detect bound TCA which the headspace method can account for. It is therefore very important for the integrity of this research to verify that TCA can be completely decarboxylated from the soil matrix (i.e. both bound and non-bound TCA can be detected).

The effects of humic acid on TCA concentrations were investigated with the following objectives:

1) To determine if TCA added to humic acid can be 100 % detected by headspace analysis (HSGC-ECD).
2) To determine if humic acid itself contains chemical moieties that may produce $CHCl_3$ on heating to 100 °C.

3.8.2 Methods

Two experiments were performed to address these issues. The first experiment (I), was a preliminary experiment to gain an initial indication of the influence of the presence of humic acid on the analysis of TCA. The second experiment (II) was performed to support the findings of Experiment I and to investigate temporal effects. In both experiments TCA solutions were added to humic acid solutions and the individual TCA and humic acid solutions were analysed for TCA, alongside mixtures of the two solutions.

3.8.2.1 Experiment I

Two solutions each of TCA and commercially available humic acid (Fisher Scientific) were prepared, each of two different concentrations. This was to observe any effects of concentration that may influence measured $CHCl_3$ peak areas. Humic acid concentrations of 6.4 mg l^{-1} and 0.63 mg l^{-1} were used as humic substances are

reported to be present in the environment in concentrations of 20 μg l^{-1} in groundwater to 30 mg l^{-1} in surface water (Thurman and Malcolm, 1981, cited in Aiken, 1885), although there is very little information on humic acid concentrations in the soil. It was necessary to use TCA concentrations greater than those found in natural waters to ensure that any increase or decrease in TCA concentration could be distinguished from the natural signal, and not be confused with natural variability. Solutions of TCA solutions of 12 and 100 μg l^{-1} were therefore used in this experiment.

A stock solution of humic acid was prepared using de-ionised water. Any further dilutions were prepared with ultrapure water. The TCA solutions were prepared using the same ultrapure water source. 0.5 ml of humic acid was added to 0.5 ml of TCA solution in a headspace vial, making the total volume 1 ml. Four replicate vials were prepared for each of the four TCA + humic acid combinations (HAT12, HAT100, HBT12, HBT100), as summarised in Table 3.4. The humic acid solutions and TCA solutions were also analysed individually, using 1 ml of sample.

Table 3.4. The combinations of different concentrations of humic acid and TCA solutions which were analysed for TCA using HSGC-ECD. In Experiment I samples were analysed immediately (t = 0). In Experiment II samples were analysed immediately (t = 0) and after storage in the fridge for 2 days (t = 2) and 11 days (t = 11).

	Sample name	[Humic Acid]/ mg l^{-1}	[TCA] / μg l^{-1}
Experiment I	HA T12	0.63	12
	HA T100	0.63	100
	HB T12	6.4	12
	HB T100	6.4	100
Experiment II	HC T11	6.4	11
	HC T114	6.4	114
	HD T12	64	11
	HD T114	64	114

Three of the vials were heated to 100 °C for 90 minutes followed by 60 minutes of equilibration at 60 °C. The fourth vial was only heated to 60 °C for 60 minutes to allow determination of background $CHCl_3$ present in the sample. Samples were analysed for TCA using HSGC-ECD, as described in Chapter 2. Ultrapure water and standard solutions of TCA (1 ml) were analysed alongside the samples.

3.8.2.2 Experiment II

This was set up in a similar way to Experiment I using two different concentrations of freshly prepared humic acid and TCA solutions. TCA concentrations of 11 $\mu g\ l^{-1}$ and 114 $\mu g\ l^{-1}$ were used, which were similar in magnitude to those in Experiment I and therefore allowed direct comparisons to be made. A humic acid solution of similar concentration to HB (Table 3.4) was prepared (6.4 $mg\ l^{-1}$) as well as a humic acid solution approximately 10 times greater (64 $mg\ l^{-1}$).

1 ml of humic acid was added to 1 ml of TCA solution (instead of 0.5 ml) making the total volume in the vial 2 ml. This increase in sample volume was to ensure even better analytical precision. Four replicate vials were prepared for each of the four TCA + humic acid combinations (HCT11, HCT114, HDT11, HDT114), as summarised in Table 3.4. The humic acid solutions and TCA solutions were again analysed individually, using 2 ml of sample. This time, three sets of vials were prepared. One set of vials was analysed immediately for TCA ($t = 0$). The other two sets were stored in the fridge until analysis of TCA after 2 days ($t = 2$) and 11 days ($t = 11$), to investigate the effects of increased contact time between the humic acid and TCA solutions. Analysis of TCA using HSGC-ECD was performed as for Experiment I but using 2 ml of ultrapure water and TCA standard solutions (instead of 1 ml).

3.8.3 Results

In a separate analysis run using a volume of 5 ml instead of 1 ml, for increased accuracy and precision, the 6.4 $mg\ l^{-1}$ and 64 $mg\ l^{-1}$ humic acid solutions had TCA concentrations of 1.2 ($\pm$ 0.12; n = 3) $\mu g\ l^{-1}$ and 1.4 ($\pm$ 0.51; n = 3) $\mu g\ l^{-1}$ respectively.

There was no significant difference between the TCA concentrations of humic acid solutions and ultrapure water. This shows that humic acid itself is not a significant source of TCA or other chemical moieties that may form $CHCl_3$ on heating to 100 °C. There was also no significant difference between the background $CHCl_3$ concentrations of humic acid and water samples heated to 60 °C only.

The "post-mixing" TCA concentrations from the TCA and humic acid mixtures were corrected for the sample volumes to enable direct comparison with ultrapure water and TCA standard solutions as well as the humic acid and TCA solutions analysed separately. The "pre-mixing" TCA concentrations were determined from the sum of the TCA concentrations in the individually analysed humic acid and TCA solutions as shown by Equation 3.3.

$$[TCA]_{THEORETICAL} = [TCA]_{HUMIC\ ACID} + [TCA]_{TCA\ SOLUTION} \qquad \textbf{Equation 3.3}$$

The percentage recovery of TCA in humic acid solutions was calculated from Equation 3.4, with a recovery of 100 % indicating that all of the TCA added to humic acid can be re-detected by HSGC-ECD.

$$\%\ Recovery = \frac{[TCA]_{"post\text{-}mixing"}}{[TCA]_{"pre\text{-}mixing"}} \times 100 \qquad \textbf{Equation 3.4}$$

Figures 3.12 and 3.13 show the percentage recovery of TCA in each of the TCA and humic acid combinations in Experiments I and II respectively. Figure 3.13 also shows the percentage recovery after 0, 2 and 11 days storage in the fridge ($t = 0$, $t = 2$, $t = 11$). In Experiment I the mean percentage TCA recovery for all of the humic acid and TCA combinations was 115 (± 9.4, n = 4) % and ranged between 101 and 148 %. If the outlier of 148 % is removed then the mean is 105 (± 4.0) %.

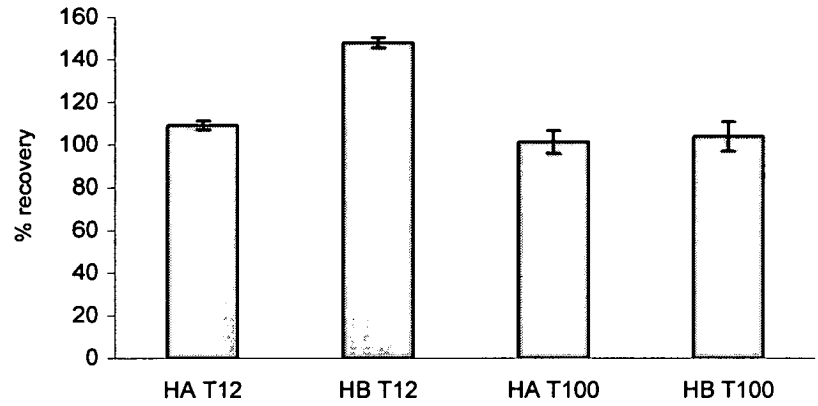


Figure 3.12. EXPERIMENT I. The percentage recovery of TCA added to solutions of humic acid. Different combinations of humic acid concentrations (6.3 mg l^{-1} (HA) and 6.4 mg l^{-1} (HB)) and TCA concentrations (12 μg l^{-1} (T12) and 100 μg l^{-1} (T100)) were analysed. Error bars are standard deviations of triplicate analyses.

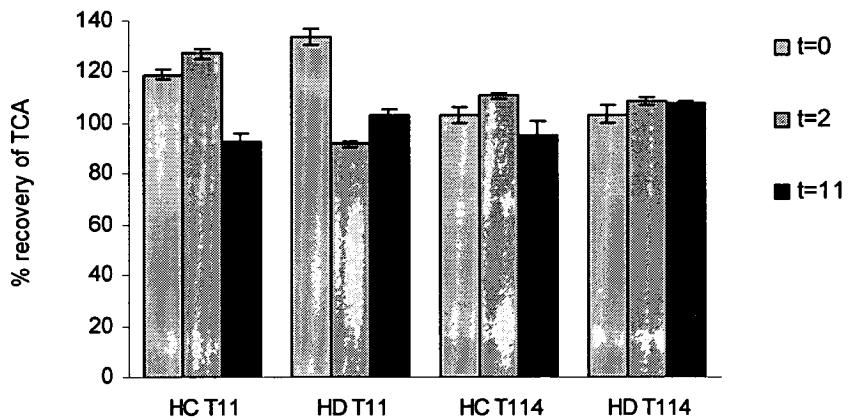


Figure 3.13. EXPERIMENT II. The percentage recovery of TCA added to solutions of humic acid. Different combinations of humic acid concentrations (6.4 mg l^{-1} (HC) and 64 mg l^{-1} (HD)) and TCA concentrations (11 μg l^{-1} (T11) and 114 μg l^{-1} (T114)) were analysed. Samples were analysed on the day of preparation (t = 0) and after 2 days (t = 2) and 11 days (t = 11). Error bars are standard deviations of triplicate analyses.

The high recovery of 148 % (HB T12) was probably due to analytical error or sample contamination as an increased recovery was not apparent in the corresponding sample in Experiment II (HC T11) which had a recovery of 119 %. In Experiment II the mean percentage TCA recovery for all of the humic acid and TCA combinations was 108 (± 3.8; n = 12) % and varied between 92 % and 134 %. There was no significant difference (2-way ANOVA, P = 0.85) in TCA recovery between the different TCA and humic acid combinations or between the storage times prior to analysis.

3.8.4 Discussion

The "post-mixing" TCA concentrations generally corresponded well with the "pre-mixing" concentrations for mixtures of TCA and humic acid solutions. This suggests that TCA is neither produced nor degraded by the humic acid used in these experiments, and its concentration is not affected by the presence of humic acid. A less likely alternative is that TCA production in the presence of humic acid is in equilibrium with TCA destruction but this would be difficult to prove. The results also show that TCA is not bound by humic acid to such an extent that it cannot be released to $CHCl_3$ by thermal decarboxylation at 100 °C.

These results contradict those of Haiber *et al.* (1996) who spiked bi-distilled water samples with TCA (1 $\mu g\ l^{-1}$) in the presence of commercial humic acid (concentration not stated) and determined the TCA concentration using the extraction and derivatisation method of Clemens and Schöler (1992). After leaving TCA in the presence of humic acid for 2 hours, only 10 % of the spiked TCA could be measured. To assess a possible decomposition pathway they measured chloroform concentrations in the lysimeter filtrates and found no significant chloroform increase implying that TCA was adsorbed or decomposed via a different route. They postulated that without continuous TCA production, the higher concentrations of TCA found in bogwater, of 1.0 $\mu g\ l^{-1}$ compared to up to 0.5 $\mu g\ l^{-1}$ in other surface waters (snow, glacier ice, bank infiltrated water, river and pond water) would be unlikely. However, as 100 % recovery of TCA was observed in TCA + humic acid solution mixtures (Section 3.8.2) using the HSGC-ECD method, this is strong

evidence that TCA in soil water can bind to humic material in the soil. The bound TCA can be released by HSGC-ECD to chloroform and quantified, but not extracted into solution for measurement using extraction and derivatisation techniques. In both the experiments described in this research, and in the studies of Haiber *et al.* (1996), commercial humic acid was used which is reported to contain more aliphatics than natural humic acid (Malcolm and MacCarthy, 1986). However, there are so many different humic acid structures in nature that it is probable that some of them would exhibit similar binding behaviour. If TCA binds to humic substances within the natural soil matrix in field conditions to the same extent as observed in laboratory and lysimeter experiments, this is evidence that reported soil TCA concentrations from analysis using extraction and derivatisation are greatly underestimated.

Although the concentrations of TCA used in these experiments were 10 or 100 times greater than those used by Haiber *et al.* (1996), the HSGC-ECD method is sensitive enough for small increases or decreases in TCA concentrations to be observed. It must be noted, however, that both these experiments observe the behaviour of externally-added TCA, and not TCA intrinsic to the soil, which is much more difficult to investigate.

The addition of TCA to humic acid is a good way of comparing the accuracy and efficiency of both analytical techniques as, in this case, chloroform production by other components of the soil is not an issue. This experiment simply tests whether the TCA added to humic acid can be detected by the two analysis procedures. As 100 % recovery was achieved by HSGC-ECD, except on one occasion where recovery was 140 %, it can be concluded that, for this particular type of commercial humic acid, chloroform is only formed from the added TCA, and not from any other functional groups which may be present within the humic acid.

3.9 DETECTION OF TCA BOUND TO ANION EXCHANGE RESIN

3.9.1 Introduction and aims

Following on from the humic acid experiments in Section 3.8, the extent of TCA binding to anion exchange sites in soil was investigated. A simple experiment was designed using a commercial anion exchange resin to find out:

1) If TCA is bound to anion exchange sites.
2) If all applied TCA can be accounted for by the headspace method, even if some of the TCA was bound to the resin and therefore unavailable for extraction.

3.9.2 Methods

DOWEX 1x8-50 strong anion exchange resin (IER) was used in this experiment. Approximately 1 g of IER was accurately weighed into a sterile centrifuge tube. 20 ml of a 20 $\mu g\ l^{-1}$ TCA solution was added using a pipette and the solution was weighed. The tube was shaken by hand, allowed to stand for 30 minutes and then centrifuged for 15 minutes at 8000 rpm (relative centrifugal force of 9000g). The supernatant was carefully removed using a pipette and stored in a sterile capped vial. The remaining solution was washed out of the tube with ultrapure water and filtered using Whatman no. 1 filter paper. Approximately 0.3 g of filtrant was weighed into a GC vial. Glass beads were added to make up 1 g of sample so that the partition ratio was assumed to be the same as for a TCA standard solution or water. The extracted TCA solution was also analysed. The IER and glass beads were analysed separately to determine the background $CHCl_3$.

3.9.3 Results and discussion

The total TCA mass in the IER residue and the supernatant after separation by centrifugation was 455 ng compared with 420 ng theoretically calculated for the resin and TCA solution separately. This mass balance of 108 % shows that all the TCA can be accounted for. Only 6 % of this TCA was detected in the extract which means that 94 % was adsorbed to the IER. This is a very favourable result for

validation of the HSGC-ECD method of TCA analysis. It demonstrates that TCA bound to an anion exchange site may still be decarboxylated to $CHCl_3$ and detected. If any complexes are present within the soil system that possess similar anion adsorption properties then methods of TCA analysis which rely on extraction of TCA into aqueous solution are undoubtedly underestimating soil TCA concentrations. Although this was a crude experiment with a number of possible errors associated with it (such as <100 % efficiency of transfer of IER to centrifuge tube and unknown mass of TCA washed off during filtration), and it only tested recovery of TCA from one specific type of anion exchange site, it is clear that TCA may be released from these sites using the headspace method of analysis. This emphasises the unique ability of this method to determine both bound and unbound TCA in the whole soil.

3.10 CHAPTER CONCLUSIONS

Aqueous extraction of TCA

- TCA intrinsic to fresh soil could not be 100 % extracted into aqueous solution after shaking and centrifugation.
- Artificially-applied TCA could not be 100 % extracted into aqueous solution from either fresh soil or dried "TCA-free" soil after shaking and centrifugation.
- Mass balances of TCA in soil residues and extracts after spiking with TCA and centrifugation were generally excellent except for Larch soil where added TCA instantly disappeared. It is possible that different soil types respond to external TCA inputs in different ways although a specific factor associated with the analytical method for TCA in Larch soil cannot be ruled out at this stage.
- Mass balances of TCA in soil residues and extracts after extraction with water and centrifugation were excellent for all soil types.
- TCA recovery in the extract from TCA-spiked Larch soil decreased with increased contact time between the TCA and the soil.
- TCA added to soil is more easily re-extracted from mineral agricultural soils than highly organic Sitka forest soils.

Effects of humic acid on TCA concentrations

- TCA is neither produced nor degraded by humic acid.
- Humic acid itself does not contain any moieties (other than TCA) that form chloroform on heating between 60 and 100 °C.
- TCA in the presence of humic acid can still yield chloroform on heating to 100 °C for 1.5 hours. Extraction-derivatisation methods have observed a decrease in TCA concentrations in the presence of humic acid. If TCA is bound to humic acid then these methods may be underestimating TCA concentrations. This is also likely to be the case for natural humic substances in soil.

Binding of TCA to Ion Exchange Resin

- TCA is bound by commercial strong anion exchange resin and cannot be extracted again into water. However the bound TCA still releases chloroform and can therefore be detected using the headspace method of analysis.

The above experiments emphasise the problems associated with the accurate determination of TCA in soil. It is possible that, although extraction-derivatisation methods clearly underestimate soil TCA concentrations, the headspace method may overestimate soil TCA concentrations if there are components in the soil that yield chloroform on heating between 60 and 100 °C. Further research is needed to investigate whether or not the suggestion is correct and to enable direct comparisons of global soil TCA concentrations, thereby leading the way for increased understanding of TCA behaviour in the soil.

An inter-comparison of HSGC –ECD and derivatisation methodologies would have to be carried out on subsamples of the same soil at the same location. TCA would be extracted from soil using water or basic solution and analysed directly using HSGC-ECD and by GC-MS after derivatisation (Scott & Alaee, 1998). This would confirm whether the "TCA" in the extract quantified by HSGC is actually TCA, and not another $CHCl_3$-forming compound present in the soil, although the problem of which compound(s) in the remaining soil residue is/are actually being converted to $CHCl_3$, and being measured by HSGC-ECD is still not really addressed. Such an

investigation may also enhance understanding of TCA extractability in relation to different soil types with physical, chemical and biological properties. Using GC-MS, other related compounds such as dichloroacetic acid (DCA) and monochloroacetic acid (MCA) could be detected which would provide a more complete picture of the concentrations and spatial variability of chloroacetic acids in the soil as a whole.

Chapter 4 – TCA in the Soil Environment

4.1 INTRODUCTION

Reported TCA concentrations in soil vary widely between different geographical regions (Table 1.3, Chapter 1). McCulloch (2002) summarised TCA concentrations measured by number of different researchers and found that 60 % of the concentrations were less than 0.05 ng g^{-1}. Hoekstra and de Leer (1993) and Hoekstra *et al.* (1995) reported TCA concentrations of 0.2 – 4.6 ng g^{-1} (dwt) in a variety of soils from four sites in the Netherlands. In a comprehensive survey of soils at 8 European locations (Germany, Netherlands, Italy, Scandinavia, U.K.) conducted by Peters (2003), mean soil TCA concentrations of 0.26 ng g^{-1} (dwt) (range <0.05 – 1.0) in open land and 0.97 ng g^{-1} (dwt) (range <0.05 - 12 ng g^{-1}) at a mountain forest site were reported. However a few studies have reported particularly high concentrations of TCA in soil, particularly in forested areas. Reeves (2001) reported TCA concentrations of Scottish soils from open moor and Sitka forest to be in the range 5 – 390 ng g^{-1} (fwt), or 15 – 3700 ng g^{-1} (dwt). Frank (1988) also reported a wide range of TCA concentrations from 20 to 380 ng g^{-1} in soils from the Black Forest in Germany, while Plümacher and Renner (1993) and Plümacher (1995) reported TCA concentrations in forest soils near Berlin, of up to 120 ng g^{-1}, although whether these concentrations are on a fresh or dry weight basis is not clear.

Soil TCA concentrations have also been reported to be variable within the same sample site as well as between different geographical regions. Reeves (2001) found TCA concentrations in soil sampled from the same depth at a Sitka spruce forest stand to range from 7 to 230 ng g^{-1} (fwt). Although considerable variability was also observed in adjacent open land, it was not as marked as in forest soils, implying that different processes occur in areas of different vegetation, which may in turn be related to a number of soil properties. Other studies have also reported TCA concentrations to be greater in soils under coniferous trees than open land, particularly Scots pine (Frank, 1988; Plümacher, 1995; Peters, 2000), and in the top soil layers (Plümacher and Renner, 1993; Plümacher 1995), although Peters (2000) failed to find a statistical correlation. These data suggest that input and output fluxes

or within-soil processes which lead to higher TCA concentrations are not evenly distributed throughout the soil.

It is difficult to detect what may be subtle differences in TCA concentrations between different regions and soil types because of high soil heterogeneity, even within the same soil horizon in the same soil profile. Soils vary greatly in physical, chemical and biological properties as well as being greatly influenced by external factors such as topography, vegetation, climate (temperature and rainfall), parent material and time taken to develop.

Chapter aims

The aims of this Chapter were to investigate why there is so much variation in soil TCA concentrations: (a) between different land-use and vegetation types, (b) between sites within a catchment and (c) between different depths within a single soil profile. More specific objectives were to:

1) Characterise TCA concentrations in soil in an upland forested site in S.W. Scotland (Ballochbeatties) and to make comparisons with an agricultural site (Cowpark) in S.E. Scotland.
2) Compare the physical, chemical and biological properties of different soils and to relate them to their TCA concentrations. The selected soil properties investigated were:
    - Composition (% water, organic matter and mineral content)
    - pH
    - Depth
    - Soil microbial biomass carbon (SMB)
    - Total carbon and nitrogen contents and C:N ratios

Soil TCA concentrations, composition, pH and depth are discussed in Section 4.2 whereas microbial biomass carbon (SMB) contents and C:N ratios are dealt with separately in Sections 4.3 and 4.4.

4.2 CATCHMENT SOIL SURVEY

This section firstly characterises the physical composition (water, organic matter and mineral content) and pH of the different soils at Ballochbeatties and Cowpark. The range and magnitudes of measured soil TCA concentrations across the Ballochbeatties study site and at different soil depths are then discussed in relation to these properties.

4.2.1 Methods

4.2.1.1 Site selection

To characterise soil TCA concentrations and properties, soil surveys were conducted on several occasions at two sites: Ballochbeatties within the Loch Bradan catchment in Ayrshire (55° 13'N, 4° 29'W) and Cowpark in the Bush Estate 11 km south south-west of Edinburgh (55° 49'N, 3° 12'W). These are shown in Figure 4.1.

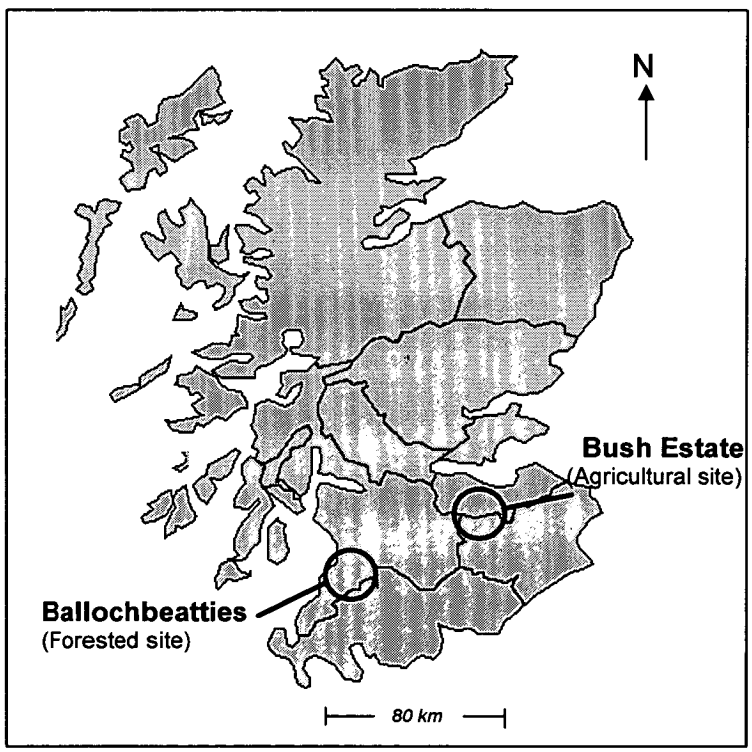


Figure 4.1. Locations of the Ballochbeatties and Bush Estate study sites in Scotland.

The Ballochbeatties catchment has an area of 0.86 km^2, ranges in elevation from 300 – 650 m and has a moist maritime climate. A mean annual precipitation of 1824 mm was measured between 1988 and 1999 (Poole, 2001) at a nearby site at approximately 200 m elevation, so the actual catchment precipitation is likely to be higher. The parent material is predominantly greywackes of the Blackcraig and Kirkcolm formations which are overlain by organo-mineral and organic soils (Hardie, 2002). The specific sampling sites within the Ballochbeatties area were selected because they represent the range of vegetation and soil types typical of the U.K. uplands. These sites were studied as part of a TCA catchment budget study (Stidson *et al.*, 2004a) and it is therefore useful to continue to study the same sites and build on current understanding. Soil was sampled on a number of occasions, from six different locations and depths at Ballochbeatties, between June 2001 and March 2003 (Table 4.1).

Table 4.1. Characteristics of soil sampling sites in the Loch Ballochbeatties catchment and at the Cowpark* agricultural site.

Site name	Elevation	Dominant vegetation	Site description	Soil types	Sampling dates
Sitka A	335 m	*Picea sitchensis*	Centre of plantation	Basin peat	Jun 01, Nov 01, Jan 02, May 02
Sitka B	345 m	*Picea sitchensis*	Centre of plantation	Basin peat	Jun 01, Jan 02, May 02, Mar 03
Sitka C	335 m	*Picea sitchensis*	Edge of plantation	Basin peat	Nov 01, Jan 02
Larch	345 m	*Larix x eurolepis*	Centre of plantation	Basin peat and peaty gleysols	Jun 01, Nov 01, Jan 02, May 02, Mar 03
Lower Moor	390 m	*Scirpus cespitosus, Erica tetralix*	Unimproved moorland	Peat, peaty gley and peaty podsols	Jun 01, Nov 01, Jan 02, May 02, Mar 03
Upper Moor	440 m	*Scirpus cespitosus, Erica tetralix*	Unimproved moorland	Peat, peaty gley and peaty podsols	Jun 01, Jan 02, May 02
*Agricultural	200 m	Perennial ryegrass	Managed for silage production	Imperfectly drained gleysol (FAO)	Nov 00, Nov 01

For comparison, soils were also sampled at Cowpark, an agricultural site south of Edinburgh, also shown in Table 4.1. This site lies at an elevation of 200 m and has a much drier climate than Ballochbeatties with a mean annual precipitation (1955 - 2000) of 869 mm (Dobbie, *pers. comm.*, 2003). The clay-loam till soils, derived from carboniferous sediment are less organic and more basic than Ballochbeatties soils. The particular field used for sampling was sown with perennial ryegrass in about 1990 and has since been managed for silage production (Dobbie and Smith, 2001). Soil was sampled on only a few occasions to provide a basic comparison of soil properties and corresponding TCA concentrations with Ballochbeatties soils. The locations of the soil sampling locations at Ballochbeatties are shown in Figure 4.2.

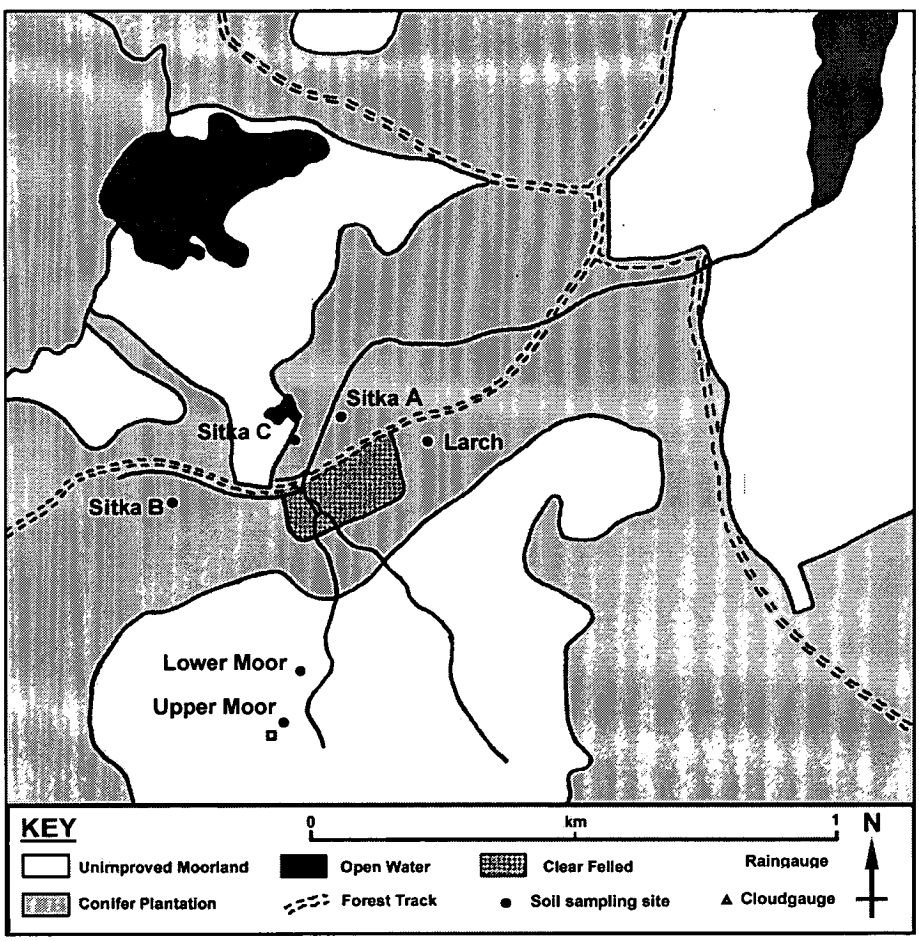


Figure 4.2. Locations of soil sampling sites (Sitka A, B, C, Larch, Lower Moor, Upper Moor), rain and cloudwater gauges in the Ballochbeatties catchment.

4.2.1.2 Soil sampling and TCA analysis

Soils were first sampled from soil pits, so that the soil profile could be assessed. Thereafter samples were taken using a screw auger, starting with the litter layer and working downwards. On each occasion a total of 20-22 soil samples were collected, at different depths, ranging from 3–6 samples at each sample site. Soil samples were placed in polythene zip-lock bags, stored in the fridge overnight and sieved wet (2 mm) the next day in preparation for TCA analysis. All soil samples were analysed for TCA by HSGC-ECD and the water and organic matter contents were determined by loss on drying and loss on ignition, using the methods described in Chapter 2.

4.2.1.3 Soil pH measurement

The pH of fresh soil was measured on the day of sample collection. The soil was sieved, 10 g weighed into a plastic beaker and 25 ml of distilled water was added. The mixture was stirred with a glass rod and allowed to stand for 1 hour before the pH was measured using a combination electrode (Hanna Instruments 9025), calibrated with buffer solutions of pH 4 and pH 7 (Fisher Scientific, Loughborough, U.K.).

4.2.2 Results and discussion: Soil survey

4.2.2.1 Soil water and organic matter contents

Table 4.2 summarises the mean and range of water and organic matter content of all soils samples collected from Ballochbeatties and Cowpark between November 2000 and March 2003. There was considerable variability in water, organic matter and mineral content within a single site as well as between different sites. Within-site variability can be explained by the inclusion of samples from all soil depths, as the relative composition of soil is expected to change down the soil profile. The organic matter content expressed per dry weight of soil gives a clearer indication of the organic nature of the soil, as it only shows the relative proportions of organic and mineral matter.

Table 4.2. Summary of the mean percentage water and organic matter (OM) contents in soils from Ballochbeatties and Cowpark (*). All samples collected between November 2000 and March 2003 are included. OM contents are expressed on both a fresh weight (fwt) and dry weight (dwt) basis. The range of values is shown in parentheses.

Sample site	% water	% OM (fwt)	% OM (dwt)	% mineral (dwt)	n
Sitka A	82 (47 – 85)	17 (9 – 52)	94 (77 – 99)	6 (1 – 23)	17
Sitka B	85 (79 – 89)	13 (10 – 21)	90 (2 – 98)	10 (2 – 23)	16
Sitka C	88 (82 – 92)	11 (8 – 17)	92 (64 – 99)	8 (2 – 37)	6
Larch	64 (32 – 87)	15 (8 – 26)	59 (4 – 98)	41 (2 – 97)	17
Upper Moor	82 (66 – 88)	12 (8 – 18)	75 (24 – 98)	25 (3 – 76)	11
Lower Moor	65 (15 – 87)	16 (8 – 77)	44 (11 – 93)	56 (7 – 89)	16
***Agricultural**	26 (22 – 30)	5 (3 – 6)	7 (4 – 8)	93 (92 – 96)	5

At Ballochbeatties the Sitka forest and Upper Moor peaty soils were the wettest and also had the highest organic matter contents. The mineral content of some Sitka peat soils was negligible when considered on a fresh weight basis, due to conditions favouring peat accumulation, such as low annual temperatures, poor drainage, anaerobic conditions and high soil acidity. The most mineral soils were found at the Larch and Lower Moor sites but these are still very organic compared to Cowpark agricultural soil where the mineral matter made up 93 % of the dry mass. Lower annual precipitation and litter input are likely explanations for the markedly lower water and organic matter contents in the agricultural soil. There is probably also a higher rate of organic matter decomposition at Cowpark as the soil is drier with a higher pH and temperature.

Variations in soil composition with soil depth are shown in Figure 4.3 for soils sampled from different sites at Ballochbeatties, in June 2001. In most soils the organic matter content decreased with depth. The Sitka soils were particularly wet and organic and the relative proportion of organic matter decreased with depth as the water content increased (these peats generally become anaerobic at depths of > 50 cm, and may reach depths of 2 m (Hardie, 2002)). The Larch soil is more freely draining and therefore drier and less organic than the Sitka peats. Mineral material is

present at all depths, the proportion increasing to 60 % at the greatest depth. The Lower Moor soil shows very similar trends to the Larch, but with less organic matter accumulation at the surface, which is probably due to the different vegetation types. The Upper Moor soil is a thin peat, similar to the Sitka sites. As larch is a deciduous species, a significant mass of organic matter accumulates annually on the soil surface.

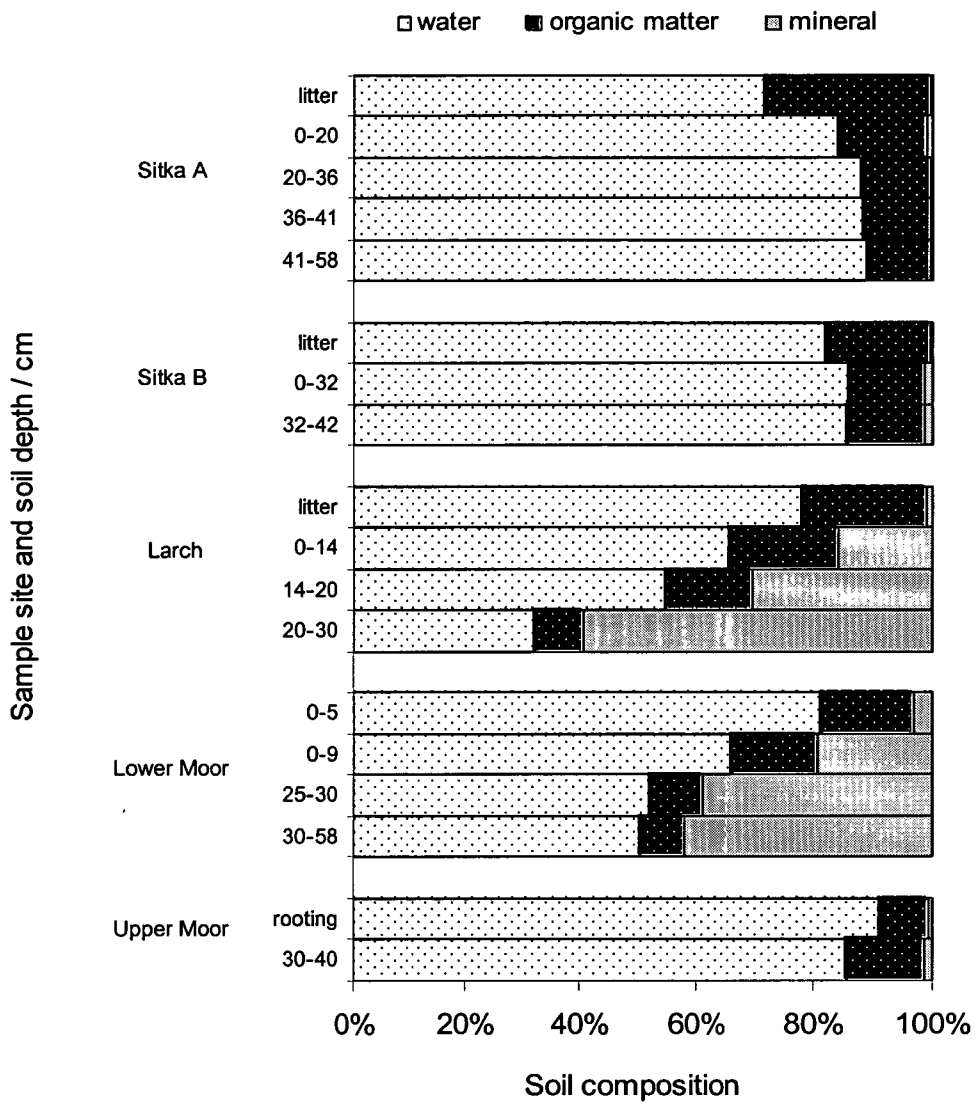


Figure 4.3. The percentage fresh weight composition of water, organic matter and mineral matter in various soils sampled at Ballochbeatties in June 2001.

The percentage composition of agricultural soil sampled from Cowpark in November 2000 is shown in Figure 4.4. Throughout the soil profile the mineral component is significant, increasing from 67 to 75 % (fwt) with depth, as the water and organic contents decrease. The soil is much more homogeneous than Ballochbeatties soils, probably due to ploughing in previous years.

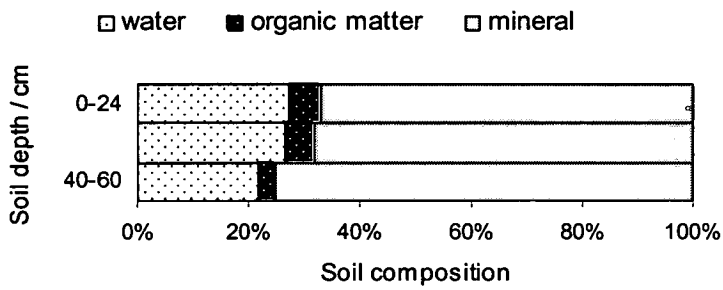


Figure 4.4. The percentage fresh weight composition of water, organic matter and mineral matter in Cowpark soil sampled in November 2000.

It is evident that there is a wide range in soil composition between Ballochbeatties forest and moorland soils as well as Cowpark agricultural soil. This will in turn influence the physical, chemical and biological processes that occur in each soil as well as other properties such as soil pH, TCA concentrations, soil microbial biomass concentrations and total C and N contents, which are discussed in the following sections.

4.2.2.2 Soil pH measurement

The mean soil pH for each soil type is summarised in Table 4.3. The relative standard deviation of soil measurements ranged from 0.9 to 2.6 % (n = 3). The Sitka forest and Larch soils are clearly the most acidic (mean pH 3.83 and 3.93 respectively). This is probably due to the acidic nature of coniferous litter as well as the efficiency with which conifer trees can intercept and scavenge acidic pollutants via the forest canopy (Puckett, 1991; Robson *et al.*, 1994; Neal *et al.*, 1997; Pierzynski *et al.*, 2000). Sitka spruce is particularly efficient due to the retention of needles all year round. In acidified soils the species diversity of flora and fauna (eg.

detritivores such as earthworms) decreases which is one reason why the rate of decomposition of organic matter beneath the Sitka forest is slow. This results in a large accumulation of litter, as observed at Ballochbeatties.

Table 4.3. The mean, range and standard deviations of soil pH measurements in soils sampled at Ballochbeatties and Cowpark between Nov 2000 and March 2003.

Soil site	Soil type	Mean pH (range)	Standard deviation	n
Sitka forest	Deep peat	3.83 (3.51 – 4.40)	0.22	26
Larch forest	Peaty gleysol	3.93 (3.90 – 4.06)	0.04	3
Lower Moor	Peaty podzol	4.42 (4.03 – 4.98)	0.37	6
Upper Moor	Peat	4.24 (4.14 – 4.39)	0.12	4
Agricultural	Gleysol	6.20 (5.40 – 6.48)	0.42	6

The Lower and Upper Moorland soils have higher pHs (4.42 and 4.24 respectively) but are still relatively acidic. The Upper Moor has a slightly lower pH than the Lower Moor, probably due to greater waterlogging and its greater accumulation of organic matter. The Cowpark agricultural soil is the least acidic with a mean pH of 6.2 which may be explained by the greater mineral content, and therefore greater concentration of base cations. The vegetation cover at Cowpark (predominantly ryegrass) is of a less acid nature than the characteristic heathland and coniferous vegetation at Ballochbeatties. Figure 4.5 (a-g) illustrates the variation of pH with soil depth at individual sites within the Ballochbeatties catchment and Cowpark.

There is some indication of an increase in pH with soil depth at some Sitka sites (b and c) but there is no observable pattern for Larch soil. There is there is a very distinct increase in the pH of the Agricultural soil (g), increasing from 5.4 at the surface to pH 6.6 at a depth of 64 cm, with the most marked increase being between 0 and 19 cm depth. With greater depth the mineral content and hence the base cation content of the soil increases and the contribution of organic acids from surface horizons will be less significant. It is evident that both the forest and moorland soils at Ballochbeatties are very acidic compared to the Cowpark agricultural soil which is

likely to have a significant influence on the types and extents of chemical and biological processes that occur.

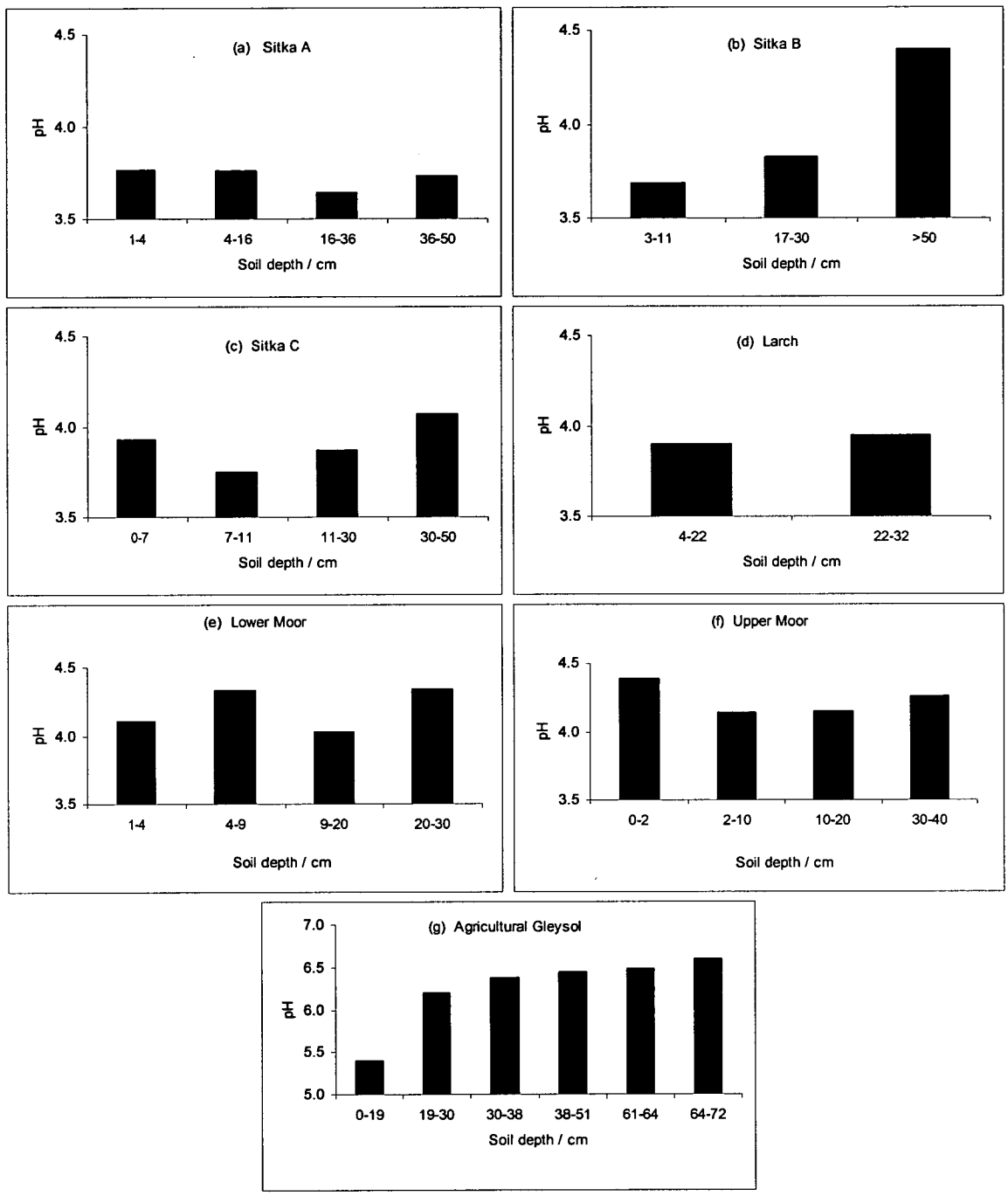


Figure 4.5. pH of soils sampled on the same day, at different sites and depths at Ballochbeatties (a – f) and Cowpark (g).

4.2.2.3 Soil TCA concentrations

The mean, standard deviation, range and median of TCA concentrations of all soils sampled and analysed at Ballochbeatties (June 2001 - March 2003) and Cowpark (November 2000 – November 2001) are summarised in Table 4.4. The standard deviations are a measure of both the natural variability in TCA concentrations between sampling depths and the analytical variability.

Table 4.4. Summary statistics for TCA concentrations (fwt) measured in soils from Ballochbeatties between June 2001 and March 2003, and Cowpark (*) between Nov 2000 and Nov 2001. The range includes all the mean values measured at each depth for all sampling dates; the overall mean, standard deviation and median values were calculated from the mean values measured at each depth for all sampling dates.

	Soil TCA concentration / ng g^{-1} (fwt)				
Sample site (Ballochbeatties)	**Range** (of means from each sampling date and soil depth)	**Overall mean** (from all sampling dates and soil depths)	**Overall standard deviation**	**Overall median** (from all sampling dates and soil depths)	**n**
Sitka A	5 – 403	97	116	51	17
Sitka B	5 – 364	103	123	33	16
Sitka C	2 - 82	26	32	10	6
Larch	2 - 43	17	11	15	17
Upper Moor	3 – 25	11	7.9	7	11
Lower Moor	4 – 16	12	5.7	11	16
***Agricultural**	4 - 47	26	28	33	5

The mean TCA concentrations of Ballochbeatties soil from all sites and depths vary from ~2 – 400 ng g^{-1} (fwt). The greatest means and ranges of soil TCA concentrations were always detected under Sitka spruce forest, where the mean soil TCA concentration was approximately seven times greater than in Moorland soil, four times greater than in Larch soil and three times greater than in Agricultural soil. TCA concentrations in Larch soils were much less variable, as well as lower than in Sitka soils. These differences may be related to the contrasting evergreen (Sitka) and

deciduous (Larch) nature of the overlying vegetation as well as the different soil types. As discussed in Section 4.2.2.2, conifers such as Sitka spruce are particularly effective at scavenging pollutants from the atmosphere and this has been suggested as a possible reason for high TCA concentrations in soil under coniferous forests. In Sitka A and B soils the median is a better approximation of TCA concentrations throughout the whole soil profile, as the mean values are dominated by a few extremely elevated TCA concentrations in the litter layers. For all other soils the mean and median values were similar in magnitude.

The Agricultural soil had TCA concentrations of up to 47 ng g^{-1} (fwt). At Ballochbeatties the soils with the lowest TCA had the highest mineral content. The Agricultural soil is more mineral-rich than all of the soils sampled at Ballochbeatties, yet has a higher TCA concentration than the Larch and Lower Moor soils, which suggests that the soil TCA concentration is not entirely dependent on its mineral content. However, there are many complex soil processes involved that are not discussed here. Although the Agricultural soil has a greater mineral content its texture is very different to soils at Ballochbeatties, with a high clay content compared to the more silty and sandy soils of Ballochbeatties which overlie greywacke and granitic parent material. The Agricultural soil is also less acidic and has a low organic matter and lower water content (Table 4.2 and Figure 4.4) therefore it is difficult to make a direct comparison on the basis of one soil characteristic.

Due to the heterogeneity of soil, it is difficult to make meaningful comparisons between TCA concentrations in soils at exactly the same depth at different sites. Soil of a particular depth is not only characteristic of the site location (in relation to factors such as parent material, water regime and vegetation cover) but also depends on the processes occurring within the whole soil profile. The variation in soil TCA concentrations with depth at different sites at Ballochbeatties and Cowpark is shown in Figures 4.6 and 4.7. Sampling in June 2001 has been used as a representative example of spatial variability of soil TCA across the Ballochbeatties catchment and within individual soil profiles. At Cowpark the soil was sampled on two occasions and the results shown are for soil sampled in November 2000.

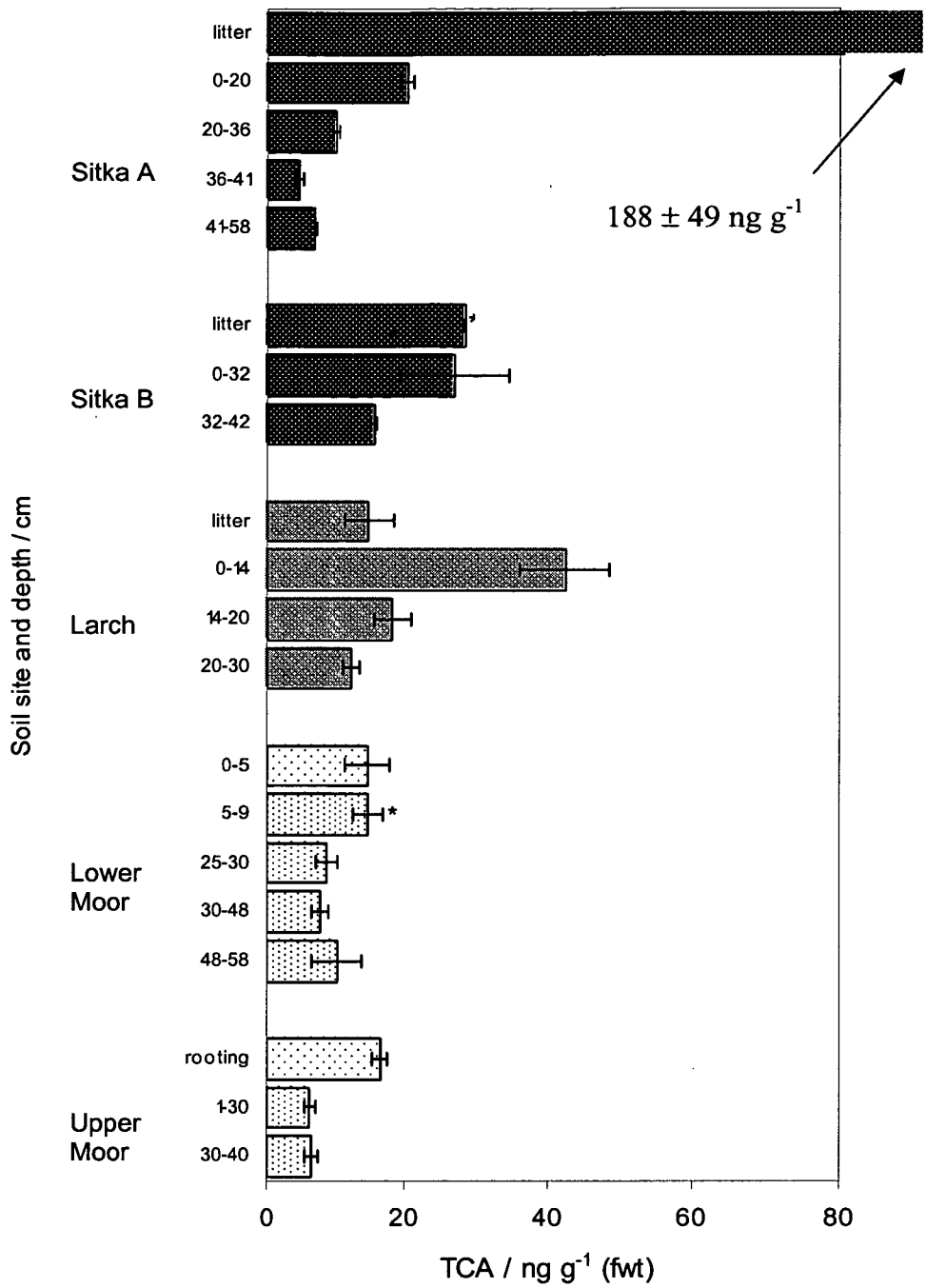


Figure 4.6. TCA concentrations of soil (fwt) from different locations and depths at Ballochbeatties, sampled in June 2001. Error bars are standard deviations of one replicate analysed in triplicate.

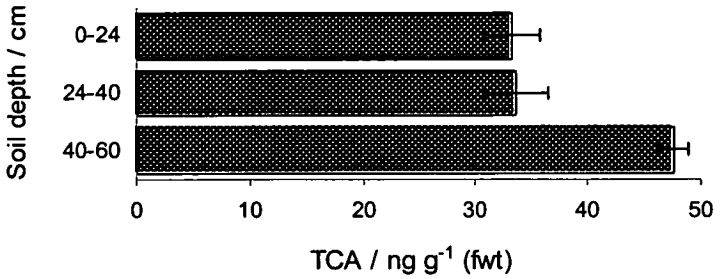


Figure 4.7. TCA concentrations of soil (fwt) from a soil profile at the Cowpark agricultural site, sampled in Nov 2000. Error bars are standard deviations of one replicate analysed in triplicate.

At all Ballochbeatties sites soil TCA concentrations generally decrease with soil depth, particularly under Sitka spruce. In contrast to the Ballochbeatties soils the TCA concentrations in the Cowpark agricultural soils were highest at the greatest depth although the range of only 15 ng g^{-1} (fwt) is small compared to soils at some Ballochbeatties sites. Due to ploughing this soil is much more homogeneous than Ballochbeatties soil and much of the organic matter has been incorporated further down the profile.

Regression curves for TCA concentrations (dwt) against soil depth are shown in Figure 4.8 for all Ballochbeatties sites between June 2001 and March 2003. The data for all Sitka forest sites has been combined, as the site characteristics were very similar. Log_e of the TCA concentration was plotted as the concentrations expressed on a dry weight basis spanned more than one order of magnitude. The data were expressed on a dry weight basis to correct for the large variation in water content between soils.

There is strong evidence of negative linear logarithmic relationships between TCA concentrations and soil depth for all sample sites at Ballochbeatties. The strongest relationship is observed for the Upper Moor soil where $R^2 = 0.55$ ($P < 0.01$). The gradients are of similar magnitudes for all sites, which indicates that the rate of decrease of log_e [TCA] with increase in soil depth is independent of the magnitude of

TCA concentration. This is true even for Sitka sites where TCA concentrations in the surface layers can be as much as 2 orders of magnitude greater than TCA concentrations in deeper or more mineral horizons. However, care must be taken when extrapolating the data in Figure 4.8 as these linear relationships can only be applied to the specific range of TCA concentrations and soil depths measured here.

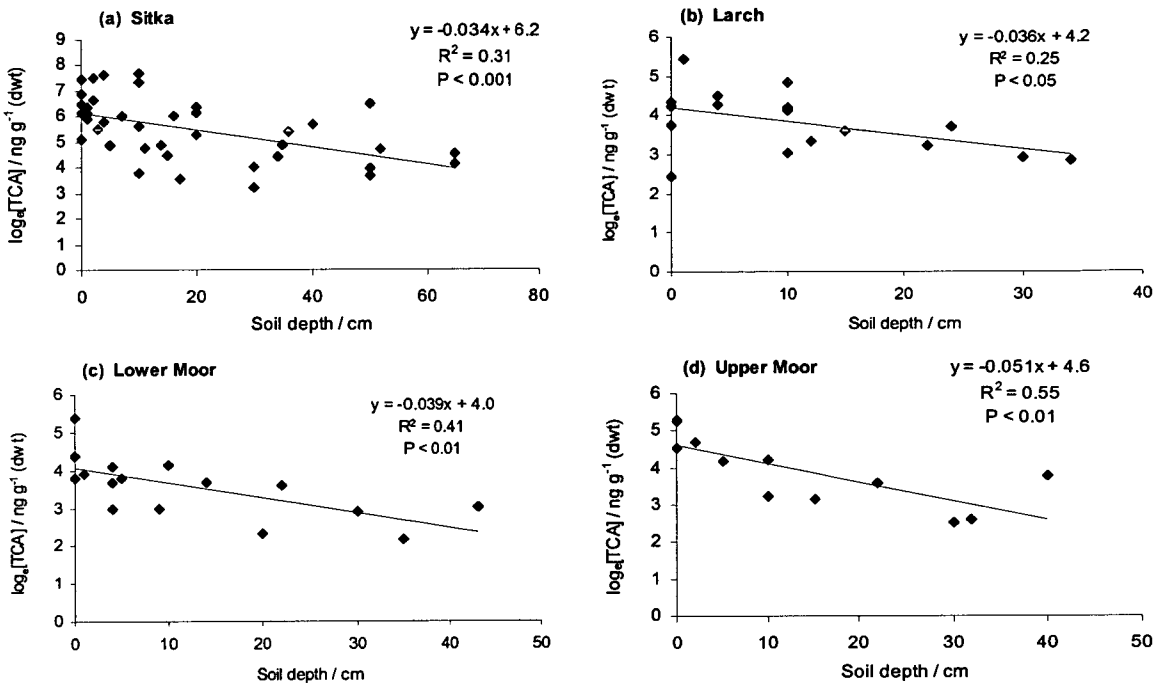


Figure 4.8. Relationship between soil TCA concentrations (log_e, dwt) and soil depth at four different sample sites at Ballochbeatties: (a) Sitka forest, (b) Larch, (c) Lower Moor and (d) Upper Moor. Data has been combined for all sampling dates between June 2001 and March 2003 and the data for Sitka A, B and C sites have been combined.

Plümacher (1995) also reported a trend of soil TCA concentrations with depth, although only two depths were sampled, compared to up to five depths in this research. They reported that the maximum soil TCA concentration was detected at the surface with 9 – 120 ng g^{-1} (dwt) in the O-horizon, and 2.4 – 14 ng g^{-1} (dwt) in the A-horizon, approximately a 9-fold difference. However, Peters (2003) reported that there was no clear correlation between soil depth and soil TCA concentration, and in a survey of European soils, at some forest sites TCA concentrations actually

increased with depth. There are several possible reasons for the observed decrease in TCA concentrations with depth as observed in this research. TCA may be degraded over time as it moves down the soil profile resulting in lower TCA concentrations. At greater depths more anaerobic conditions prevail in many of the Ballochbeatties sites, particularly in the Sitka soils, suggesting that if TCA is degraded in these horizons it must be via an anaerobic mechanism, either biotic or abiotic. Alternatively, if TCA is formed naturally in this soil, it may occur to a greater extent in the upper horizons where microbial activity is likely to be greater. These processes may depend on the type of leaf litter, which may itself be a source of TCA which becomes dispersed down the soil profile. The observed high TCA concentrations in forest soils are in agreement with Peters (2000) who reported that TCA concentrations in forest areas were significantly higher than in the open land areas by a factor of 2 – 2.5. At Ballochbeatties, neither litter from larch nor moorland vegetation was particularly enhanced in TCA suggesting that there is a particular property of Sitka litter that concentrates TCA in the soil, produces it or prevents its degradation.

The TCA concentrations for selected sites expressed on a fresh weight, dry weight and organic matter weight basis, are shown in Figure 4.9a - f. These data emphasise that care should be taken when interpreting soil TCA concentrations. To enable direct comparisons to be made between soil TCA concentrations measured in different soil types and using different methods, TCA concentrations are usually quoted on a dry weight basis. It is evident that the TCA concentration expressed on a dry weight or organic matter basis is markedly greater than the fresh weight TCA concentration and is particularly true for the Sitka B site. If TCA is predominantly associated with the dry fraction of the soil, where soil is very wet the mass of TCA present is effectively concentrated on a small mass of dry matter. Likewise, where soil is more organic the concentration of TCA per mass of organic matter is effectively lower. However, as the soils with high organic matter contents generally also have high water contents, the concentration of TCA expressed per mass of organic matter is magnified. Although the organic matter content of the soil may be

much greater than the mineral content, there is still only a small proportion onto which the TCA may be concentrated compared to the mass of water present.

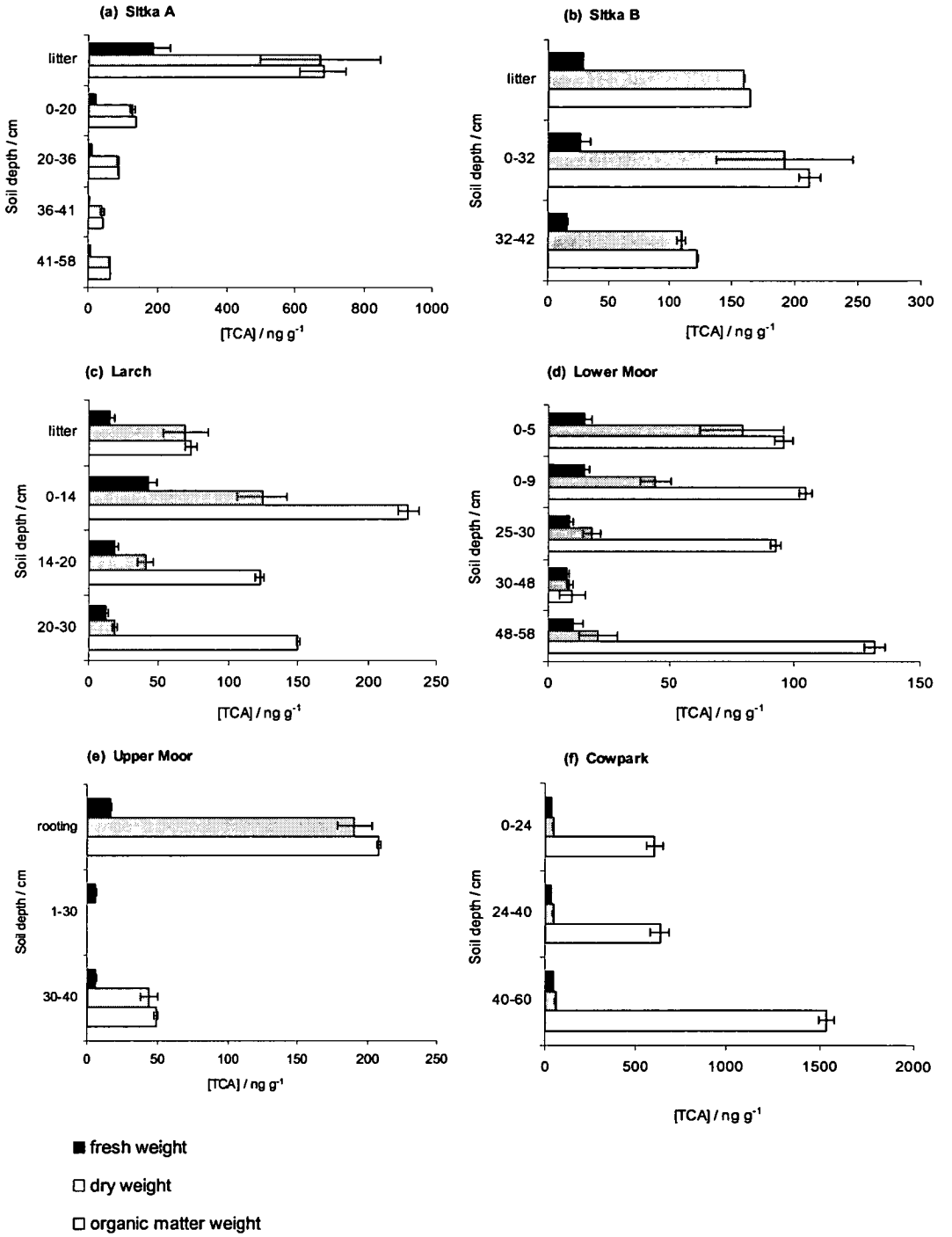


Figure 4.9. Ballochbeatties (June 2001) and Cowpark (Nov 2000) soil TCA concentrations expressed per fresh weight, dry weight and organic matter weight of soil. Error bars are standard deviation of triplicate analyses.

The trends in TCA concentrations, whether expressed on a fresh, dry or organic matter weight basis are generally similar and the peaks in all three TCA concentration expressions are usually found at the same soil depth. There are exceptions, for example the Lower Moor site (Figure 4.9 d) at a depth of 30 – 48 cm where the TCA concentration expressed per mass of organic matter decreases dramatically compared to the horizons above and below it, as there is a particularly high organic matter content and low water content. This accumulation of organic matter may be due to weak podzolic properties of the soil where the organic matter has been translocated down the soil profile. In the Cowpark agricultural soil the TCA concentrations expressed on both a fresh weight and dry weight basis are consistently low, as the water content is only about 26 %, but expressed on an organic matter basis the TCA concentrations appear to be exceptionally high due to the low organic matter content.

The component of the soil which TCA is predominantly associated with is currently not known and it is important that the TCA loading of each fraction (water, dry matter, organic matter) is taken into consideration. As the Ballochbeatties soils are very wet and organic, the percentage organic matter expressed on a fresh weight basis is driven by the percentage moisture. Moisture content is likely to exhibit much more temporal variability, even on a daily basis, than organic matter which forms over a much longer period of time. The association of TCA with soil water and organic matter is complex. A negative correlation between water content and TCA concentration is likely to be accompanied by a corresponding positive correlation between soil organic matter content (fwt) and TCA concentration. It is more meaningful to use the fresh weight TCA concentrations when considering the total soil burden of TCA and assessing the potential environmental risks associated with it.

No relationships were observed between soil water or organic matter content and soil TCA concentrations (fresh weight or dry weight) for all the soil data combined, which is in accordance with studies of Sitka spruce soils in East Scotland conducted by Reeves (2001). He proposed that TCA may be related to a specific component of

organic matter rather than the total quantity. As extremes of TCA concentrations were always detected in the litter layers of Sitka soils, the Sitka spruce soil data were considered separately. Consequently, there was a significant logarithmic linear negative correlation ($R^2 = 0.55$, $P < 0.001$, $n = 38$) between the soil TCA concentration (fwt) and soil water content (Figure 4.10a). A significant logarithmic positive correlation ($R^2 = 0.47$, $P < 0.001$, $n = 38$) was also observed between the fresh weight soil TCA concentration and fresh weight organic matter content (Figure 4.10b).

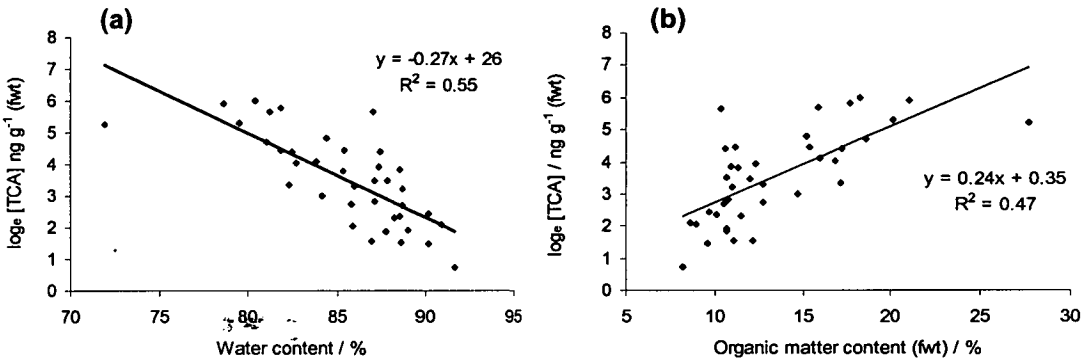


Figure 4.10. Relationship between fresh weight TCA concentrations (log_e) in all Sitka soils, and (a) water content (%) and (b) soil organic matter content (%, fwt).

These results imply that TCA occurs more in the less wet, more organic soils. Water contents of soils at Ballochbeatties generally exceed 60 % which is very wet in comparison to soils of other regions. It is likely that there is an optimum moisture content for TCA presence in soil, which may also be related to conditions which favour microbial activity, as discussed in Section 4.3. If a soil becomes too wet or too dry, TCA concentrations may decrease, although this has not been investigated further. If TCA is formed naturally in soil as suggested by several researchers (Hoekstra and de Leer, 1993; Hoekstra *et al.*, 1995, 1999a,b; Haiber *et al.*, 1996; Niedan *et al.*, 2000, Fahimi *et al.*, 2003), it is likely to occur during the degradation of organic matter, as part of the main proposed route of natural production is the chlorination of humic material (Walter and Ballschmitter, 1992). More specifically, Hjelm and Asplund (1995) and Öberg *et al.* (1996) suggested that organohalogen formation occurs during the degradation of lignin.

The pH may also influence the relative proportions of chloroform or TCA produced from reaction intermediates (Hoekstra *et al.*, 1999b). TCA yield apparently increases in basic conditions (pH < 7) whereas more chloroform is produced at pH > 8 (Juuti and Hoekstra, 1998). However these processes are not likely to be important when considering the effects of pH on Ballochbeatties or Cowpark soils, as soils at both sites were more acidic. The Ballochbeatties soils lie within the optimum pH of 3-6 for chloroperoxidase enzyme activity (Asplund *et al.*, 1993; Haiber *et al.*, 1996; Juuti and Hoekstra, 1998; Hoekstra *et al.*, 1999b) suggesting that TCA may be naturally produced in these soils by this mechanism. This possible production could contribute to the high soil TCA concentrations measured in this area compared to other parts of Europe and North America.

4.3 SOIL MICROBIAL BIOMASS-CARBON

4.3.1 Introduction

It has been shown in Section 4.2 that TCA concentrations vary widely between soils and tend to be greater in surface horizons and in Sitka spruce forest soils. The reasons for this are not understood but if TCA is formed naturally in the soil, higher production rates are likely to occur in the presence of organic matter which is a source of humic acids. To investigate further both the relationship of TCA with soil properties and a possible production route by micro-organisms, the soil microbial biomass (SMB) was measured in a variety of soils. SMB carbon is defined as the living part of the soil organic matter with the exclusion of plant roots and soil animals larger than 5 x 10^3 μm (Jenkinson and Ladd, 1981). It is measured as the mass of soil C in living and recently dead soil micro-organisms, mainly bacteria and fungi. SMB as a percentage of total soil carbon usually ranges from 2 % in arable soils to 3-4 % in grassland or woodland soils (White, 1997). The main techniques used to determine SMB in soil are:

1) Chloroform fumigation and extraction of carbon
2) Chloroform fumigation and incubation
3) Drying and re-wetting
4) Substrate-induced respiration

These methods provide no information about the species comprising the biomass but give estimates of biomass size which is valuable in modelling C turnover in soil (White, 1997). The method used in this research was chloroform fumigation followed by extraction of carbon using potassium sulphate (K_2SO_4), due to its simplicity and applicability for a wide range of soils (Vance *et al.*, 1987). The technique is based on the quantitative extraction of a particular compound, found in all components of the microbial community but in no other constituents of soil. It incorporates a chemical extraction step to remove microbial nutrients from chloroform-lysed microbial cells immediately after fumigation. This technique enables rapid analysis and avoids assumptions about the behaviour of re-colonising populations as with other methods, and has the advantage of being applicable to very acid soils and waterlogged soils (Inubushi *et al.*, 1991) such as those found at Ballochbeatties.

4.3.2 Methods

Thirteen soil samples with a range of water and organic matter contents were collected from Ballochbeatties and Cowpark in November 2001. On the day of collection, soils were sieved fresh (2 mm), divided into two subsamples for TCA analysis and SMB determination, and stored in separate bags before being frozen (-30 °C). Soils were defrosted overnight in the fridge before analysis of SMB or TCA.

Non-fumigated (NF) subsamples of each soil (20 g) were weighed into plastic beakers and extracted by shaking with 100 ml of 0.5 M K_2SO_4 (saturated) at < 100 rpm for 30 minutes. The solutions were then filtered into plastic bottles. As the solutions were in equilibrium with the soil it was not necessary to filter and retain 100 % of the liquid. A subsample of each field-wet soil (20 g) was weighed into glass beakers and fumigated (F) with chloroform for 24 h. After fumigation the remaining subsamples were extracted in the same way with K_2SO_4. Two controls (empty beakers) for both the fumigated and non-fumigated samples were subjected to the whole process to account for any adsorption of chloroform or organic carbon onto the beaker surface. Five of the thirteen soils were prepared in duplicate to obtain an indication of the variability of SMB within the same soil. Soil extracts

were analysed for organic C using a DC-80 Rosemount Dohrmann Total Organic Carbon Analyser. Extracts were shaken to precipitate out any carbonate then decanted into a glass vial. Each vial was acidified with conc. phosphoric acid (1 drop) and purged with oxygen-free nitrogen for 5 minutes. Samples were then injected into the TOC analyser and compared with carbon standard solutions prepared with potassium hydrogen phthalate.

4.3.3 Results and discussion

The SMB was determined from the difference between the organic-C concentration of the fumigated (F) and non-fumigated (NF) soils. The organic–C detected by the TOC analyser is referred to as extractable carbon (EC). EC concentrations were firstly corrected for soil water content by converting 20 g of fresh soil to 100 g of dry soil (Equations 4.1 a and b). Where there was more than one replicate the mean was calculated.

Fumigated soil **Equation 4.1a**

$$\text{Corrected EC, mg C (100 g)}^{-1}\text{ dry soil} = \frac{([EC]_F - [EC]_{BLK}) \times |(V_{K_2SO_4}) + (M_{SOIL} \times f_W)|}{(M_{SOIL} \times f_{DM}) \times 1000}$$

Non-fumigated soil **Equation 4.1b**

$$\text{Corrected EC, mg C (100 g)}^{-1}\text{ dry soil} = \frac{([EC]_{NF} - [EC]_{BLK}) \times |(V_{K_2SO_4}) + (M_{SOIL} \times f_W)|}{(M_{SOIL} \times f_{DM}) \times 1000}$$

Where
- EC_F = Extractable C from 20 g fumigated fresh soil extracts (mg l^{-1})
- EC_{NF} = Extractable C from 20 g non-fumigated fresh soil extracts (mg l^{-1})
- EC_{BLK} = Extractable C from blank samples (mg l^{-1})
- $V\ K_2SO_4$ = volume of extractant (ml)
- M_{SOIL} = mass of soil (g)
- f_W = fraction of water
- f_{DM} = fraction of dry matter

The extractable carbon (EC) is the chloroform-labile carbon pool and is proportional to the SMB. The difference between the EC from the fumigated and non-fumigated samples gives the total EC from the organic-C alone. A factor, K_{EC} is used to convert this organic-flush to SMB (Biomass-C) as shown in Equation 4.2, to account for non-extractable SMB carbon.

$$\text{Biomass-C} = \frac{[(\text{Corrected } EC_F) - (\text{Corrected } EC_{NF})]}{K_{EC}} \qquad \textbf{Equation 4.2}$$

Where Biomass-C and corrected EC (F or NF) are expressed as mg C $(100\ g)^{-1}$ (dwt)

K_{EC} is soil specific but often estimated as 0.35 (Öhlinger, 1995), the value used in this research. The assumption that this factor applies to all soils is one of the main weaknesses of the chloroform fumigation-extraction method. The values of K_{EC} used are all ultimately derived from measurements made on a small selection of organisms added to soil and fumigated. These micro-organisms are mostly grown on nutrient-rich media and therefore may not be good models for diverse populations of micro-organisms naturally present in the soil which are mostly nutrient-starved (Jenkinson *et al.*, 2004). The extent to which non-microbial components may contribute to the fumigation flush of extractable-C, and which may therefore also influence the K_{EC} factor, is unknown. Other uncertainties in the fumigation-extraction method include the variation of extraction efficiency of C with parameters such as soil pH, calcium content and moisture content and possible interference from non-biomass components in the fumigation-extraction procedure which may influence the measured carbon concentrations. The extractability of non-biomass C may be also enhanced by exposure to chloroform. Fine roots and root debris in the sample contribute non-biomass extractable-C after chloroform fumigation, resulting in an overestimation of SMB.

However these uncertainties also apply to most other methods of SMB estimation and an absolute value of K_{EC} is not necessary for making comparisons between the SMB of different soils in this research.

SMB concentrations ranged from 40 mg C (100 g) $^{-1}$ (dwt) in Agricultural soil to 967 mg C (100 g) $^{-1}$ (dwt) in the litter layers of Sitka soil (Figure 4.11). These SMB concentrations are generally higher than those reported in literature (Table 4.5) by a factor of ~ 10. This is probably a consequence of the very high water content of fresh soils from Ballochbeatties, and hence a low dry matter weight, on which the SMB carbon is effectively concentrated. To illustrate this point, if a water content of 20 % was assumed for the soils used in this research, the range in SMB carbon concentrations would be 13 – 175 mg C (100 g)$^{-1}$ (dwt) which is more within the range of the data shown in Table 4.5. The standard procedure of expressing SMB carbon concentrations on a dry weight basis makes the assumption that SMB is associated with the dry mass of soil rather than the water fraction which may not be the case and is a possible weakness in the method.

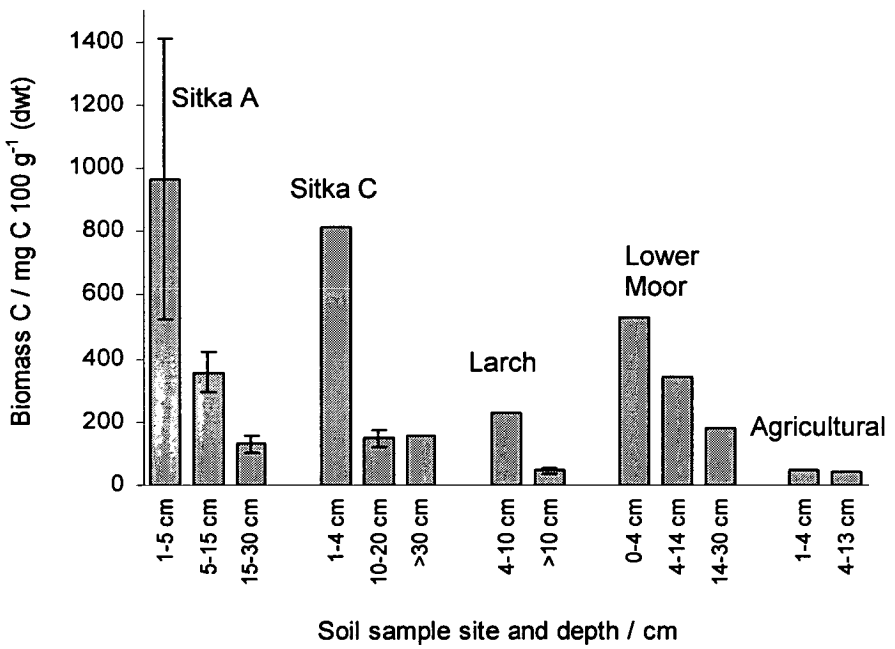


Figure 4.11. SMB concentrations (mg C (100 g) $^{-1}$ dwt) of a range of soils and depths from Ballochbeatties (Sitka A, Sitka C, Larch, Lower Moor) and Cowpark (Agricultural). Where displayed, error bars are standard deviations of two soil extractions.

The greater magnitude of the SMB concentrations in Ballochbeatties soils does not detract from the clear differences that exist between soils. The standard deviations of the SMB (where determined on two replicates) are relatively low (less than 20 % r.s.d.) except for the litter layer of Sitka A which has an r.s.d. of 46 %. Sitka litter layers are very heterogeneous, as illustrated by the variability in TCA concentrations in Table 4.4 (Section 4.2.2.3).

Table 4.5. SMB concentrations in different soil types, reported in literature.

Origin of soil sample	SMB / mg C 100 g soil^{-1} (dwt)	Reference
Coniferous woodland, U.K.	56	Webster *et al.* (2001)
Arable, U.K.	26.3 ± 2.6	Hargreaves *et al.* (2003
Mixed deciduous woodland, U.K.	56.5 ± 2.3	Hargreaves *et al.* (2003)
Arable, Germany	48.4	Wirth (1999)
Douglas fir forest, USA	66.3	Bailey *et al.* (2002)

Hargreaves *et al.* (2003) reported that SMB concentrations varied more than other soil properties such as pH, exchangeable cations and organic C, both within and between soils. They explained this by viewing SMB as a biological variable which is affected, for example, by the distribution of organic material such as root and stubble within the soil both laterally and with depth.

For all sites, the SMB concentration is greatest in the upper soil horizon. According to White (1997), most organisms are concentrated in the top 15-25 cm because C substrates are more plentiful there. The cycling of plant residues by leaf fall, root mass decay and root excretion in natural ecosystems provides the substrate for microbial activity. The concentrations of both oxygen and carbon dioxide are largely dependent on microbial activity, which in turn depends on the availability of organic carbon compounds as food. Respiration by plant roots and enhanced respiration by soil organisms near the roots are also significant processes (Brady and Weil, 1999). Subsoils are usually more deficient in oxygen than topsoils. The deeper horizons of Sitka spruce peats at Ballochbeatties were usually anaerobic which would limit the microbial activity and slow down decay of organic matter.

The relationship between SMB and TCA concentrations is shown in Figure 4.12. As it is normal practice to express SMB on a dry weight basis the dry weight TCA concentrations were also used for this comparison.

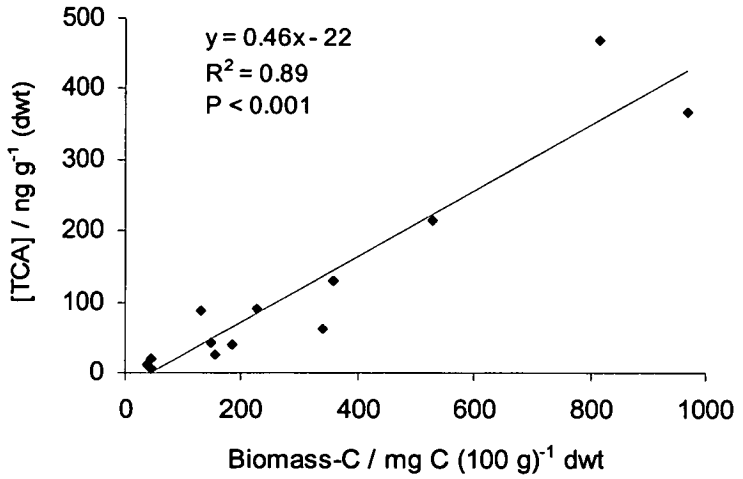


Figure 4.12. The relationship between SMB concentrations and TCA concentrations (dwt), in a range of soils from Ballochbeatties and Cowpark (n = 13).

There is a strong positive correlation between SMB and TCA concentrations in the soils investigated ($R^2 = 0.90$, $P < 0.001$, $n = 13$). This implies that where there is a high SMB concentration there is also likely to be a high TCA concentration. It does not necessarily mean one is driven by the other but if microbial activity and TCA concentrations are cause and effect it is a tentative indication of TCA production in the upper soil horizons from the breakdown of PER and/or TCE (Hoekstra *et al.*, 1999b), or entirely naturally by chlorination of humic substances (Hoekstra and de Leer, 1993; Hoekstra *et al.*, 1995, 1999a,b; Haiber *et al.*, 1996; Niedan *et al.*, 2000, Fahimi *et al.*, 2003). It is likely that a number of measured and unmeasured soil variables (e.g. pH, water and organic matter contents, temperature and soil texture) also influence both the TCA and SMB concentrations. The possibility that TCA presence in soil induces the observed increase in microbial activity (resulting in its degradation) is very unlikely as only certain micro-organism species, and only a fraction of the total soil microbial biomass, is likely to be associated with TCA in terms of its production or degradation. Therefore an increase in population of TCA-

associated micro-organisms is likely to have a negligible influence on the total biomass.

A significant positive logarithmic relationship was also observed between SMB and soil water content ($R^2 = 0.51$, $P < 0.01$) and soil organic matter content ($R^2 = 0.55$, $P < 0.01$) as a percentage of the dry mass (Figure 4.13).

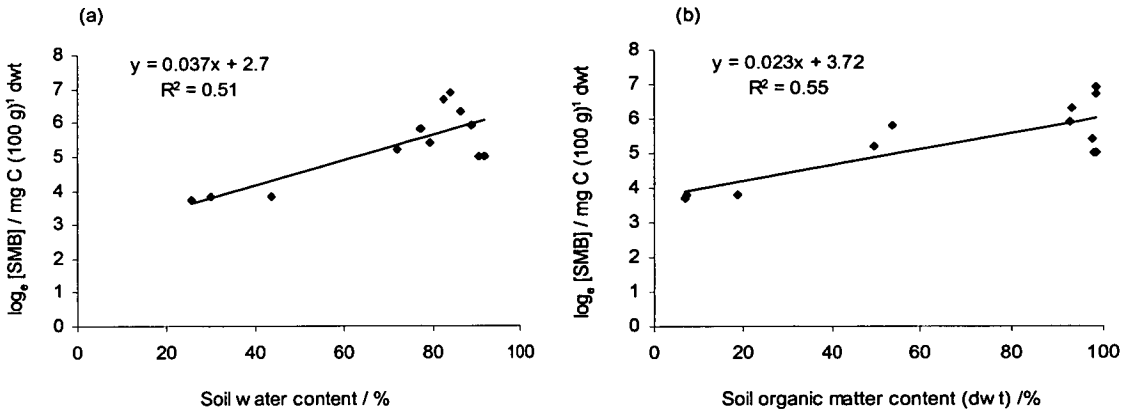


Figure 4.13. The relationship between SMB ($\log_e$) concentrations and: (a) soil water content (%), and (b) soil organic matter content (dwt, %).

The relationship with organic matter is not surprising as micro-organisms are likely to prefer habitats where is there is an abundance of N and C-rich substrate. The correlation with water content is slightly more unexpected as microbial activity can be limited by oxygen in anaerobic, waterlogged conditions. According to Singer and Munns (1996), the decay of organic matter by micro-organisms is directly influenced by the temperature and moisture content of the soil. It is interesting that a higher microbial activity was measured in organic, wet soils under Sitka forest than in the more nutrient-rich agricultural soil at Cowpark, although microbial activity in this latter soil may be limited by poor drainage and a higher dry bulk density (1.1 g cm^{-3}, Dobbie and Smith, 2001). Plant species composition, mainly through net primary productivity and litter composition, can affect SMB measurements, as well as interactions between soil organisms (Carter *et al.* 1999).

Using the chloroform fumigation extraction method the SMB can be difficult to measure in very wet compacted soils that do not completely disperse during extraction. This should not be a problem with Ballochbeatties soils as, although they contain a lot of water, they have relatively low dry bulk densities (approximately 0.1 g cm^{-3}). Although Cowpark soils are poorly draining with relatively high dry bulk densities, no problems with SMB measurement were observed in this research.

4.4 SOIL C:N RATIOS

4.4.1 Introduction

The relative proportions of carbon and nitrogen ("C:N ratio") in the soil may influence the rate of microbial activity. The total carbon and nitrogen contents and hence the C:N ratios of the same soils analysed for SMB were measured to investigate further the correlation of TCA concentration with SMB. The majority of soil organisms metabolise carbonaceous materials in order to obtain carbon for building essential organic compounds and to obtain energy for life processes. However, organisms must also obtain sufficient nitrogen to synthesise nitrogen-containing cellular components such as amino acids, enzymes and DNA. If the C:N ratio of organic material added to soil exceeds about 25:1, the soil micro-organisms will have to scavenge nitrogen from the soil solution to obtain sufficient nitrogen for growth (Brady and Weil, 1999).

4.4.2 Methods

The total carbon and nitrogen contents of the same soils as those used for SMB measurement (Section 4.3) were determined. Each soil was dried in an oven for 2 days at 80 °C then ground to a fine powder before being weighed (5 – 10 mg) into tin capsules. Samples were analysed in triplicate using a Carlo Erba Strumentazione elemental analyser. The ratio of total (inorganic + organic) carbon to nitrogen (C:N ratio) was calculated.

4.4.3 Results and discussion

4.4.3.1 Soil C:N ratios

The C:N ratios of each soil are shown in Figure 4.14. An outlying result from Sitka C soil (1-4 cm) has been omitted. The C:N ratios range from 18:1 to 38:1 in forest soils and 14:1 to 18:1 in Moorland and Agricultural soil. The C:N ratios of the Agricultural soil were considerably lower than the Sitka forest soils and similar to the Lower Moor soil, but higher than would normally be expected in arable soils.

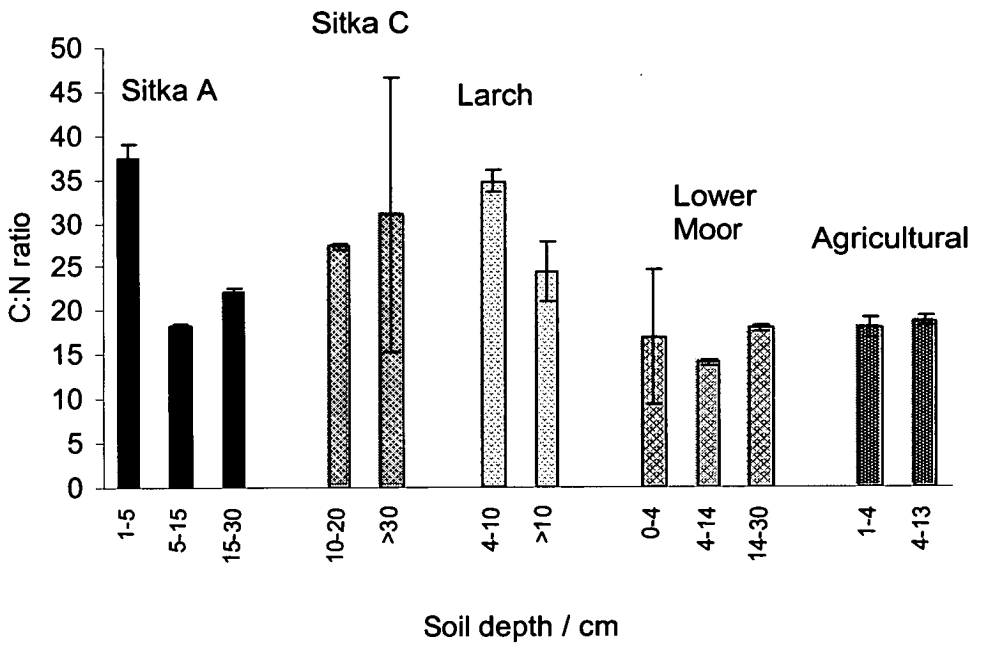


Figure 4.14. C:N ratios of a range of soils from Ballochbeatties (Sitka A, Sitka C, Larch, Lower Moor) and Cowpark (Agricultural). Error bars are standard deviations of three soil replicates.

C:N ratios are often regarded as a measure of humification (Fitzpatrick, 1986). Humic substances comprise about 60 – 80 % of the soil organic matter (Brady and Weil, 1999) and because of their complexity they are most resistant to microbial attack. Different litter types have different C:N ratios, for example, Priha and Smolander (1996) reported a C:N ratio of 30:1 for Norway spruce needles, 69:1 in Scots pine needles and 54:1 in birch leaves. Coniferous soils usually have a thick fresh litter layer due to continuous needle drop, which is often ramified by plant roots

and a tough fungal mycelium (White, 1997). Fresh litter and peat generally have high C:N ratios (sometimes greater than 100:1) as they are undecomposed or poorly decomposed As decomposition progresses the C:N ratio decreases, therefore in deeper horizons the C:N ratios are likely to be smaller. In agricultural soils the C:N ratios are most likely lower than those in forest soils due to the more favourable conditions for organic matter decomposition (e.g. good aeration, higher soil pH), rapid organic matter turnover and greater nitrogen inputs.

There was no significant relationship between dry weight soil TCA concentrations ($\log_e$) and C:N ratio, as shown in Figure 4.15, although there is some indication that soil TCA concentrations may be greater in soils with high C:N ratios.

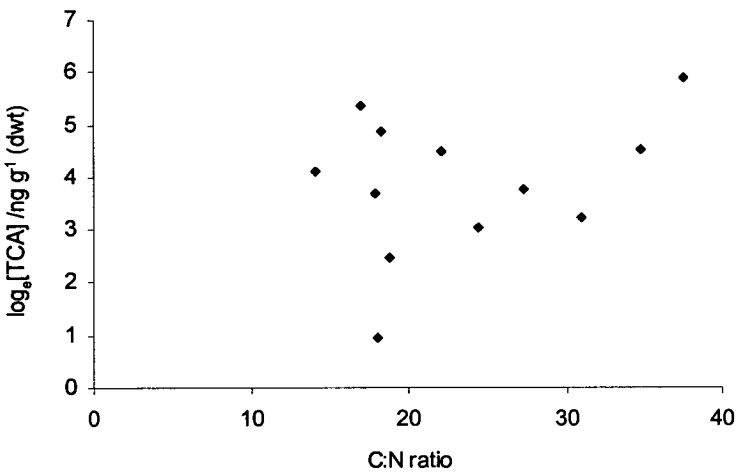


Figure 4.15. The relationship between C:N ratios and TCA concentrations of a range of soils from Ballochbeatties and Cowpark (n = 12).

When the total soil C and N contents (%) are looked at separately (Figure 4.16), there is some evidence of a significant linear logarithmic relationship between % N (R^2 = 0.37, $P<0.05$, n = 12) and soil TCA concentrations as well as % C (R^2 = 0.42, $P<0.01$, n = 12) and soil TCA concentration (dwt).

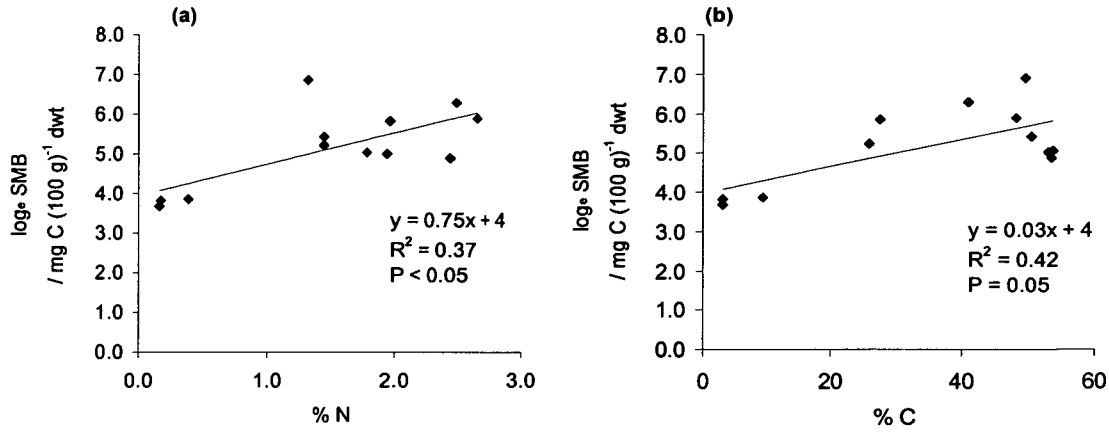


Figure 4.16. The relationship between soil TCA concentration (log_e, dwt) and (a) total soil C (%), and (b), total soil N (%) in a range of soils from Ballochbeatties and Cowpark.

In view of the previously discussed relationship between soil TCA concentration and soil organic matter content (Figure 4.10b) and the higher TCA concentrations detected in the litter layers of Sitka forest soils, indications are that TCA may be associated with higher concentrations of C and N, and possibly the less decomposable organic matter fraction in soils. This could either be due to a greater SMB concentration which may be responsible for production of TCA, or TCA may be adsorbed by the organic matter and accumulated with time.

The relationship between C:N ratios and SMB is shown in Figure 4.17. At lower (< 20:1) and higher (> 30:1) C:N ratios the SMB is greatest, but between these ratios it reaches a minimum which implies that there may be more than one optimum soil C:N ratio for microbial activity. It is also possible that the optimum activity of different species of soil micro-organism occurs at different soil C:N ratios, and Figure 4.17 may illustrate the presence of two different microbial populations.

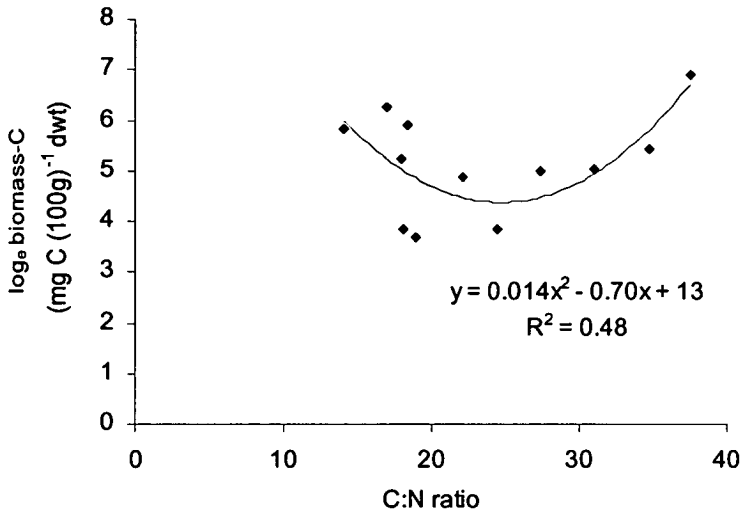


Figure 4.17. The relationship between SMB concentrations and soil C:N ratios, both expressed per dry weight of soil.

4.4.3.2 Validation of soil organic matter content determination by loss on ignition

Soil organic matter content is often estimated from the total soil carbon content by multiplying the percentage carbon by a factor of 1.72 (Fitzpatrick 1986). From the carbon content measured for determination of C:N ratios the organic matter content can be estimated and compared with the loss on ignition method which is routinely used throughout this study (described in Chapter 2). Figure 4.18 shows that there is a strong positive linear relationship ($R^2 = 0.98$, $P < 0.001$, $n = 12$) between organic matter contents determined by both methods. The gradient is close to unity (1.08) which confirms that there is little variation in absolute values obtained by both methods. This gives confidence in the total C values and resulting C:N values measured. The linear relationship implies that any error is systematic and remains constant for all soils types.

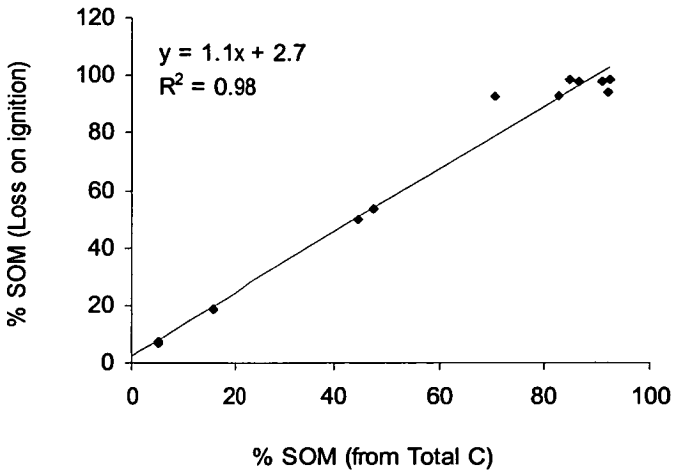


Figure 4.18. The correlation between soil organic matter (% of dry mass) measured by loss on ignition (*y*-axis) and from total C determinations (*x*-axis). Analyses were carried out on a range of soils collected from Ballochbeatties (Sitka A, Sitka C, Larch, Lower Moor) and Cowpark (Agricultural).

4.5 DISCUSSION: TCA IN SOILS

High variability of TCA was found in soils from Ballochbeatties (upland, moor and forest) and Cowpark (lowland, agricultural) both spatially between different sample sites, and vertically within a single soil profile, with the greatest variability and the highest concentrations being observed in forest soils. TCA concentrations ranged from 3 to 25 ng g^{-1} in open moorland soils whereas under Sitka spruce they ranged from 2 to 400 ng g^{-1} (fwt), which is approximately within the previously reported range of <0.05 to 390 ng g^{-1} (Table 1.3).

This large variability in measured TCA concentrations is probably due to variation in a number of different factors which are difficult to differentiate between, such as TCA input and output fluxes, soil properties which may or may not favour TCA production, degradation or anion adsorption, vegetation and climate. The complexity and interaction of the various physical, chemical and biological processes taking place in the soil need to be studied in greater detail before the sources and sinks of TCA in the soil environment are fully understood.

The greater magnitude of soil TCA concentrations in Scotland compared to other European soils is also difficult to explain, but may be related to the higher precipitation and maritime climate which could result in greater fluxes of precursor compounds such as PER and TCE, if atmospheric sources are significant. More chlorine may be introduced than in other areas and this may increase the likelihood of TCA being formed *in-situ* from reactions involving chloride ions. As there was a positive correlation between soil organic matter and TCA concentration in Sitka soils, the predominance of organic soils at Ballochbeatties may in part explain the large TCA store. The high TCA concentrations and variability observed in some Sitka litter layers may also be partly explained by differences in the overall fluxes of TCA entering the forest via throughfall and stemflow water. Puckett *et al.* (1991) reported that retention by, and leaching from, the canopy can induce spatial variability as a result of spatial heterogeneity of the biota. Robson *et al.* (1994) found that throughfall chemistry varied systematically within a forest with plot-to-plot variations and edge-to-interior effects observed, especially for marine salts.

Unlike Sitka soils at Ballochbeatties, Larch soils did not have high TCA concentrations in the litter layers which either suggests that larch is a poor scavenger of pollutants, if atmospheric deposition is an important source of soil TCA, or that Sitka litter has different chemical and/or biological properties, which more readily promote TCA production in soil. The positive relationship between soil microbial biomass (SMB) and soil TCA concentrations may be indirect evidence for TCA production within the soil. Although it does not prove that high TCA concentrations exist because of microbial activity, it shows that TCA is present in soil conditions which favour microbial activity. Laturnus *et al.* (1995) observed that CPO-like activity was present in all layers of spruce forest soils but was particularly high in organic layers where fungi and bacteria are known to be involved in the degradation of organic matter.

If TCA is produced, for example, by the chlorination of organic material in soil (Hoekstra and de Leer, 1993; Hoekstra *et al.*, 1995,1999a,b; Haiber *et al.*, 1996; Niedan *et al*, 2000; Fahimi *et al.*, 2003) following the formation of reactive chlorine

species from chloride ions and hydrogen peroxide by a peroxidase-mediated reaction (Neidleman and Geigert, 1986, Walter and Ballschmiter, 1992, Asplund *et al.,* 1993; Laturnus *et al.*, 1995) its rate of formation will depend on the abundance of the various precursor compounds involved. According to Eriksson (1959, 1960), chlorine concentrations in soil are closely related to the total mass of deposition, which is in turn related to distance to sea, precipitation and evapotranspiration rates. On a smaller scale, a number of factors influence the chlorine concentration such as wind direction, distance to forest edge and season (Potts, 1978; Beier *et al.,* 1993). Organically bound chlorine concentrations in soil have been reported to be positively related to organic carbon concentrations (Johansson *et al.*, 2003), therefore it might be expected that, assuming natural production occurs in soil, TCA concentrations are related to organic carbon.

Some researchers have postulated that TCA can be degraded in soil chemically to chloroform and carbon dioxide (Kearney *et al.*, 1965) but there is little direct evidence of this. A more widely accepted degradation route is by micro-organisms to carbon dioxide and chloride ions (Loustelot and Ferrer, 1950; Barrons and Hummer, 1951; Ogle and Warren, 1954; Martin, 1972; Foy, 1975; McGrath, 1976; Torstensson, 1976; Lignell *et al.*, 1984; Yu and Welander, 1995; Forczek *et al.*, 2001; Matucha *et al.*, 2003). It is difficult to calculate the half-life of trace amounts of TCA in soil as conditions of degradation vary spatially and temporally. Estimates based on TCA concentrations used in herbicidal applications (at the parts per million level) suggest that the half-life of TCA in soil is relatively short, in the order of weeks (summarised in Foy, 1975). Estimates based on more environmentally-relevant soil TCA concentrations (several parts-per-billion) indicate a shorter half-life of a few days (Matucha *et al.*, 2003). Therefore, if TCA is accumulating in the terrestrial environment then the rate of TCA input (via external deposition sources or within-soil natural production) must be greater than the rate of TCA output (via streamwater, within-catchment cycling or degradation).

However, the fact remains that the variation in soil TCA concentrations reported in this chapter could be due to inconsistencies in the HSGC-ECD method of analysis, if

other chloroform-producing compounds are present to varying extents in different soils within the same sample site. Differences in soil TCA concentrations between regions (i.e. Scotland and other parts of Europe and North America) may also be explained by the different methods of analysis employed. These were discussed in Chapter 3 where it was concluded that even if the headspace method of analysis (as used throughout this research) is over-estimating the true soil TCA concentrations due to the presence of other chloroform-producing moieties in the soil, other extraction-derivatisation methods are almost certainly underestimating the concentrations.

The research carried out in this chapter has provided a thorough overview of the spatially variable nature of TCA in soil. It has also generated a greater understanding of some of the soil properties that TCA may be associated with. However, this chapter has also illustrated the extent of the challenges involved in trying to understand the factors controlling TCA occurrence in the soil environment, which is a critical component of environmental TCA cycling. The main conclusions are summarised below.

4.5 CHAPTER CONCLUSIONS

1) Spatial variability of soil TCA

Between geographical locations

- The TCA concentrations at Ballochbeatties and Cowpark were higher than most concentrations reported for other European soils.
- The mean fresh weight TCA concentration of Ballochbeatties moorland and forest peaty soils was 49 ± 85 ng g^{-1} (n = 83) with a range of 2 – 400 ng g^{-1}.
- The mean fresh weight TCA concentration of Cowpark agricultural gleysol was 26 ± 28 ng g^{-1} (n = 5) with a range of 4 – 47 ng g^{-1}.

- TCA concentrations vary between different soil types with soils under Sitka forest showing the largest range in TCA concentrations. There appears to be a characteristic of forest soils that is not observed in other soils, which results in these high TCA concentrations.

Within a single soil profile

- TCA concentrations in soil generally decrease with soil depth.
- Unusually high TCA concentrations have been observed in Sitka litter layers, often higher than TCA concentrations of needles fresh from the trees, suggesting that TCA may be formed *in-situ* in the soil.

2) Factors controlling soil TCA concentrations

- No relationships between soil pH and TCA concentrations were observed at Ballochbeatties or Cowpark.
- There was a negative correlation between soil TCA concentrations and soil water content in Sitka soils
- There was a positive correlation between soil TCA concentrations and organic matter contents in Sitka soils. It is possible that TCA is associated with a particular grade of organic matter which has not been distinguished in this study.
- There was a strong positive correlation between soil TCA concentration and SMB which may be tentative evidence for natural formation of TCA in soils via the action of micro-organisms.
- There is no relationship between soil TCA concentrations and C:N ratios although there are positive linear relationships between soil TCA ($\log_e$) and the total C and N contents (%).

3) Catchment burden of TCA

- Reliable estimates of soil TCA concentrations are necessary to understand TCA cycling and stores in the environment.

Chapter 5 - TCA Cycling in the Soil Environment

5.1 INTRODUCTION

In Chapter 4 the variable nature of TCA concentrations in soil was highlighted. From this, it was concluded that the soil compartment is the key to quantifying the mass of TCA stored in the terrestrial environment, and relating this to the relative magnitudes of input and output fluxes. Although some relationships between soil TCA concentrations and selected soil properties (pH, water content, organic matter content, microbial biomass and C:N ratios) were established, they do not reveal much about the dynamics of TCA in soil. Questions remain surrounding both the identification and quantification of input fluxes to the soil (including *in-situ* production), behaviour of TCA once it has entered the soil and output fluxes of TCA from the soil. The experiments reported in this chapter deal with TCA cycling in the soil environment and attempt to elucidate if TCA is formed and / or degraded in the natural environment, and the relative time scales involved. Previous studies have investigated TCA production (Hoekstra and de Leer, 1993; Hoekstra *et al.,* 1995, 1999a,b; Haiber *et al.*, 1996; Niedan *et al.*, 2000; Fahimi *et al.,* 2003), degradation (Loustalot and Ferrer, 1950; Barrens and Hummer, 1951; Ogle and Warren, 1954; Kearney *et al.*, 1965; Martin, 1972; Foy, 1975; McGrath, 1976; Torstensson, 1976; Lignell *et al.,* 1984; Weightman *et al.*, 1992; Yu and Welander, 1995; Matucha *et al.,* 2003), and binding behaviour in soil and humic substances in the laboratory (Haiber *et al.,* 1996; Fahimi *et al.,* 2003; Schöler *et al.,* 2003), but few studies have focused on TCA behaviour in soil in the field at ambient environmental concentrations.

Chapter aims

The aims of this chapter are to build on the results of Chapter 4 which characterised soil TCA concentrations in the field and investigated the relationship of TCA with various soil properties. This chapter focuses on TCA behaviour in soil from a more dynamic perspective.

The specific objectives are to:

1) Quantify the TCA input and output fluxes in soil.
2) Determine if degradation or production of TCA occurs in soil, and by what mechanism.
3) Determine how soil TCA fluxes are influenced by soil type, particularly relating to the organic matter content and presence of a substantial litter layer.

These objectives were achieved by carrying out a series of controlled field and laboratory experiments, which are described in the following sections.

5.2 FIELD LYSIMETERS

5.2.1 Introduction

Although several studies have investigated TCA behaviour in soil in laboratory conditions (Haiber *et al.,* 1996; Fahimi *et al.*, 2003; Matucha *et al.*, 1993; Schöler *et al.*, 2003), there are few studies of the cycling of TCA in field conditions. To gain a greater understanding of the relative inputs and outputs of TCA in the environment as a whole it is important to consider the role of soil as a sink or a source of TCA. One method is to quantify the total TCA inputs and outputs to the soil using controlled experiments in the field. For this purpose lysimeters were used to study the TCA inputs to the soil via precipitation and forest throughfall, and the outputs via drainage through the soil.

5.2.2 Methods

Lysimeters were set up at Ballochbeatties (already described for soil sampling in Chapter 4) and Easter Howgate near Edinburgh, sites with contrasting land uses, climate regimes and soil types, as summarised in Table 5.1.

Table 5.1. Characteristics of field sites used for lysimeter experiments.

Site	Vegetation	Soil type	Mean annual rainfall	Elevation
Ballochbeatties	Sitka spruce	Basin peat (organic rich)	1824 mm [a]	320 m
Easter Howgate	Rough grassland	Gleysol (mineral rich)	869 mm [b]	200 m

[a] 1988 – 1999 (Poole, 2001)

[b] 1955 – 2000 (Dobbie, *pers. comm.* 2003)

Lysimeters were firstly used to study the inputs and outputs of "natural" TCA from precipitation and forest throughfall, and then later used for a long-term controlled experiment where a known mass of TCA was applied to the soil. As the main aim of the lysimeter experiments was to study TCA fluxes in a natural environment, apparatus was designed to minimise disturbance of the soil as well as to ensure free drainage of any water entering the lysimeter.

5.2.2.1 Lysimeter construction

To isolate blocks of soil for the lysimeters a plastic cylinder was pressed into the soil surface and extended roots were removed using a saw-knife. Soil-filled cores were carefully removed from the ground using a spade to cleanly slice through the soil underneath. Vegetation was retained on top of agricultural lysimeters and the needle litter layer retained on forest lysimeters. Cores had similar dimensions to the pots used to store the soil above the ground (diameter = 190 mm, depth = 140 mm). The plastic cylinder was split with a saw and the soil carefully transferred to the lysimeter pot (with holes in the base). A plastic bag (with a capacity of 3 litres) was sealed round the pot to collect any water that percolated through the soil. Pots were lined with plastic gauze and fine netting to prevent soil particulates from being washed through with the percolation water, allowing free drainage of soil water by gravity into the collection bag. Pots were supported by plastic piping, which was firmly embedded in the ground. The piping was sealed at the bottom to prevent the water table from rising up and possibly forcing the bag upwards. The experimental design of lysimeters is shown in Figure 5.1.

Soil for TCA analysis was collected from the area immediately surrounding the soil core to avoid disturbing the core itself. Samples were taken from both the litter layer and the organic layer of the Sitka soil at Ballochbeatties, and at two different depths of the Agricultural soil at Easter Howgate. These soils were analysed for TCA by HSGC-ECD as described in Chapter 2 to obtain an initial soil TCA concentration.

Seven lysimeters were established at each site in December 2001. The Ballochbeatties ("Forest") lysimeters were left *in-situ* whereas the Easter Howgate ("Agricultural") lysimeters were transported to an undisturbed area of the Edinburgh University campus (King's Buildings) for practical reasons. This site was < 10 km from the original location and was therefore exposed to a similar climate regime. At each field site, lysimeters were randomly distributed, approximately within a 5 m radius for Forest lysimeters and a 2 m radius for Agricultural lysimeters.

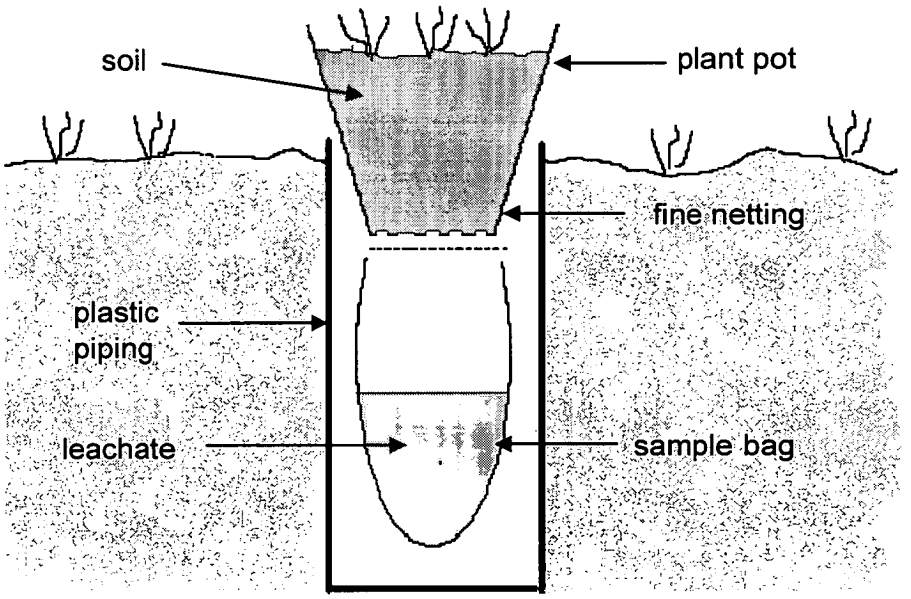


Figure 5.1. Design of soil lysimeters used to study inputs and outputs of TCA in water under field conditions.

Lysimeters were allowed to "rest" for a period of 6 (Forest) or 7 weeks (Agricultural) during which time any TCA entering the system was from wet or dry atmospheric deposition or, for Ballochbeatties, forest throughfall. During this period the percolation water ("leachate") was collected from the bags at 2-week intervals. The

volume of this leachate was measured using a 2-litre measuring cylinder and 50 ml of solution was retained for TCA analysis by HSGC-ECD as described in Chapter 2.

5.2.2.2 Lysimeter TCA dosing

After the rest period lysimeters were dosed with 0.15 μg ("Control"), 20 μg ("Low") or 50 μg ("High") of TCA. These TCA masses were considered large enough to significantly enhance the TCA concentration of the soil leachate above background TCA concentrations and thus enable determination of whether the applied TCA was detectable in the leachates or not, with a high degree of precision. The TCA was applied in a solution of 250 ml prepared from a stock solution of TCA and diluted with de-ionised water from the same source as that applied to the Control lysimeter. This is equivalent to concentrations of 0.6 μg l^{-1} (the TCA concentration of de-ionised water), 80 μg l^{-1} and 200 μg l^{-1} TCA for Control, Low and High-dosed lysimeters respectively. The dosage volume of 250 ml was considered sufficient to dilute the TCA to a concentration that was not too damaging to soil flora or fauna, and small enough that it would percolate into the soil within a few minutes without pooling on the surface. The solution was applied gradually over the soil surface taking care to distribute it evenly as well as to avoid the edge of the pot, where preferential flow was possible.

Two weeks after dosing, the volume of leachate collected was measured using a 2-litre measuring cylinder and a 50 ml sample was taken for TCA analysis. Bags were replaced, the lysimeter dosed again with the appropriate TCA solution and the leachate sampled again two weeks later. Lysimeter dosing was carried out on ten occasions at each site.

For Forest lysimeters only one control was considered necessary as other lysimeters were available in a concurrent TCA catchment budget study at Ballochbeatties (Stidson *et al.*, 2004, unpubl.) and provided background information on TCA concentrations entering and leaving the soil. Three Low and three High-dosed lysimeters were established. For Agricultural lysimeters three Controls were considered necessary as no data were available on background TCA inputs from

precipitation and outputs from soil. Two Low and two High dosed lysimeters were also set up. All the lysimeters treatments are summarised in Table 5.2.

Table 5.2. The treatments of soil lysimeters set up at Ballochbeatties (Forest) and in Edinburgh (Agricultural) and the nomenclature used throughout this chapter.

Site	Treatment	Mass of TCA in dosing solution / ng	No. of times dose applied	Start of dosing	End of dosing
Forest	Control x 1	150	10	20/2/02	24/6/02
	Low A	20000	7	20/2/02	16/5/02
	Low B	20000	7	20/2/02	16/5/02
	Low C	20000	10	20/2/02	24/6/02
	High A	50000	7	20/2/02	16/5/02
	High B	50000	7	20/2/02	16/5/02
	High C	50000	10	20/2/02	24/6/02
Agricultural	Control x 3	150	10	27/2/02	13/7/02
	Low A	20000	6	27/2/02	23/5/02
	Low B	20000	10	27/2/02	13/7/02
	High A	50000	6	27/2/02	23/5/02
	High B	50000	10	27/2/02	13/7/02

To ascertain if TCA applied to soil could be completely flushed out, some of the previously dosed Low and High lysimeters were dosed only with de-ionised water for the last 3 (Forest) or 4 (Agricultural) fortnightly dosing/sampling occasions.

All background TCA inputs to lysimeters were accounted for by recording the volume and TCA concentrations of precipitation in Edinburgh and of forest throughfall at Ballochbeatties. Precipitation in Edinburgh was monitored using a bulk precipitation collector. Throughfall water was monitored using apparatus set up for a concurrent study of TCA cycling in the Ballochbeatties catchment (Stidson *et al.*, 2004b). Throughfall depth was calculated directly from the known collector surface area and the volume of water collected. Samples of throughfall and precipitation water were collected on the same day as the corresponding lysimeter leachates. All aqueous samples (lysimeter leachate, precipitation and throughfall

water) were collected in plastic vials and stored in the fridge immediately on return from the field. If analysis was not possible immediately then samples were frozen at -30 °C until a later date. Samples were prepared and analysed for TCA by HSGC-ECD, as described in Chapter 2.

The dry bulk density (DBD) of the soil in each lysimeter was determined at the end of the experiment (June 2002 and July 2002 for Forest and Agricultural lysimeters respectively) using metal corers of known volume and weight. Corers were carefully pressed into the soil, capped, then transported to the laboratory where they were dried in an oven at 80 °C until constant weight (4 days) was reached. The dried cores were weighed and the DBD was determined using Equation 5.1.

$$\text{DBD / g cm}^{-3} = \frac{\text{dry mass of soil / g}}{\text{volume of cylinder / cm}^3} \qquad \textbf{Equation 5.1}$$

It was then possible to estimate the total dry soil mass in the lysimeter pots, as the volume of the pot was known to be 2.5 l. Soil was also sampled from each lysimeter for TCA analysis by HSGC-ECD. From the estimated soil mass and known TCA concentration the TCA burden of the lysimeter was estimated and compared to the TCA input and output fluxes.

As part of a concurrent study of TCA cycling at Ballochbeatties (Stidson *et al.*, 2004, unpubl.), single lysimeters (of a similar design to those established in this study) were set up at four sites - Larch forest, Sitka forest, Upper moorland and Lower moorland. The aim of these "baseline" lysimeters was to investigate the background output of TCA in the soil leachates relative to TCA inputs and therefore determine if soil is a source or sink of TCA.

5.2.3 Results and discussion

The results of the baseline lysimeters set are discussed first, to provide information on TCA behaviour at natural ambient concentration before investigating the results of dosed lysimeter experiments.

5.2.3.1 Baseline lysimeters

Discussion of data from the baseline lysimeters focuses mainly on the single lysimeter in Sitka forest at Ballochbeatties, as this is most relevant to the controlled dosing experiment set up in the same area of forest.

Hydrological Balance

The hydrological balance of lysimeter input and output waters was monitored for one year (May 2001 – May 2002). The input water volumes to Forest lysimeters were calculated from forest throughfall water depths, as described in Section 5.2.2.2. Input volumes to Moorland lysimeters were calculated from cloudwater and bulk precipitation gauges set up nearby. The output water volume was the volume of the leachate collected in the lysimeter bag. Measurements were taken fortnightly. On occasions when the lysimeter leachate volume exceeded 2600 ml (the volume of space occupied by the lysimeter) it was assumed that overflow had occurred and the data from these dates was omitted. The remaining output volumes were plotted against the input volumes and the equation of the trendline (separate for each lysimeter) was used to estimate the output volume under high rainfall conditions. The problem of lysimeter saturation and overflow was markedly greater in the Moorland lysimeters due to the greater input volume via cloud and rainwater. The mean fortnightly hydrological input and output volumes to and from preliminary baseline lysimeters, after removal of overflow data, are summarised in Table 5.3.

Table 5.3. Summary of the mean fortnightly hydrological input and output volumes to and from baseline lysimeters at Ballochbeatties from May 2001 to May 2002. Inputs to forest lysimeters are via throughfall water and inputs to open moor lysimeters are via rain and cloudwater. Overflow data have been removed.

Lysimeter location	Throughfall or precipitation input volume /ml	Leachate output volume / ml	Output / Input volume	n
Sitka forest	1190	1040	0.88	21
Larch forest	1070	1400	1.31	21
Upper moor	1320	1040	0.79	9
Lower moor	1322	823	0.62	9

The mean input volumes were greater in the Moorland lysimeters, because of less interception loss by vegetation. In all lysimeters (except for Larch) there was less than 100 % of input volume recovered in the leachate. The greater recovery (131 %) of the Larch lysimeter leachate volume compared to the input volume may only be explained by experimental error associated with estimates of input throughfall volume, as the lysimeter leachate output can be measured accurately with a measuring cylinder and experimental error is therefore negligible. The Larch lysimeter was located several metres from the throughfall collectors thereby exposing it to slightly different conditions where, perhaps, the canopy was slightly more open allowing more rainfall to enter, or throughfall water was, by chance, preferentially deposited in the area of the lysimeter. As forest canopies are naturally heterogeneous it is likely that the volume of throughfall being deposited in one area of the forest is different to that being deposited a short distance away (as reported by Puckett *et al.,* 1991 and Robson *et al.*, 1994), although the lysimeters were assumed to received the same calculated depth per unit area. It is also assumed in this study that rain deposition in open areas is uniform over a few metres.

TCA concentrations in baseline lysimeters

The TCA concentrations and volumes of throughfall water and soil leachates for the single baseline Sitka forest lysimeter are shown in Figure 5.2. It is clear that TCA concentrations and volumes varied considerably between sampling dates. The mean TCA concentration of the leachate was 1.08 (± 0.38) $\mu g\ l^{-1}$ with a range of 0.43 – 1.76 $\mu g\ l^{-1}$ and the mean concentration of throughfall water was 1.13 (± 0.53) $\mu g\ l^{-1}$ with a range of 0.55 – 2.46 $\mu g\ l^{-1}$. There was no significant correlation between TCA concentration and volume of solution in either the throughfall input or leachate output which implies that there is no effect of precipitation volume on TCA concentrations. An unpaired *t*-test showed no significant difference between TCA concentrations in input (throughfall) and output (leachate) water. This implies that any differences observed were due to natural variability, or that the proposed processes of TCA degradation (Loustalot and Ferrer, 1950; Ogle and Warren, 1954; Jensen, 1957, 1960; Martin, 1972; Foy, 1975; Torstensson, 1976; Lignell *et al.*, 1984; Yu and Welander, 1995;) and production (Hoekstra and de Leer, 1993;

Hoekstra *et al.*, 1995, 1999a,b; Haiber *et al.*, 1996; Niedan *et al.*, 2000; Fahimi *et al.*, 2003) within the soil and the leachates balance each other. The mass of TCA reaching the terrestrial environment is very dependent on the volume of deposition. During episodes of high rainfall more TCA will be deposited which has implications for regions with high annual rainfall and may partially explain higher TCA concentrations determined in Scottish soil compared with other parts of Europe, as discussed in Chapter 4.

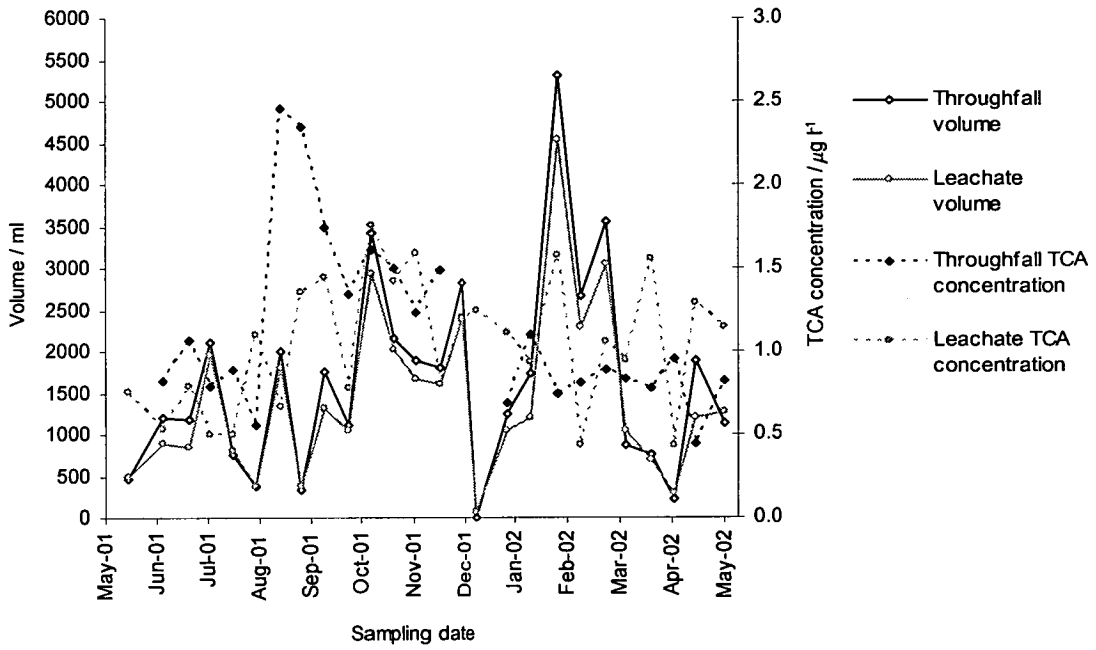


Figure 5.2. Fortnightly volume and TCA concentrations of throughfall inputs and soil leachate outputs from a single baseline soil lysimeter in a Sitka spruce forest at Ballochbeatties between May 2001 and May 2002. Some of the leachate volumes are derived from extrapolation due to overflow.

As the TCA concentrations in natural lysimeter leachates varied considerably between sampling dates, it is more informative to examine cumulative TCA masses (water volume x TCA concentrations) in lysimeter input and output waters over the whole of the experimental period (Figure 5.3).

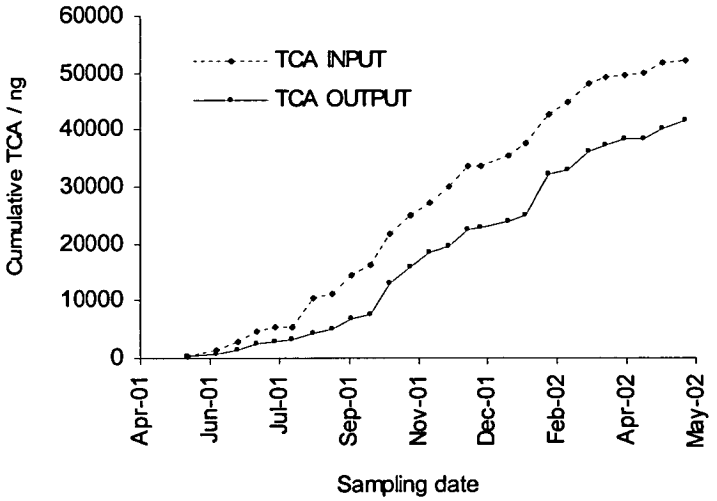


Figure 5.3. Cumulative TCA input (throughfall) to and output (leachate) from a baseline soil lysimeter in a Sitka forest at Ballochbeatties, between May 2001 and May 2002.

It is clear that for Sitka forest the total cumulative TCA input mass (52200 ng) is greater than the TCA output mass (41400 ng) and only 79 % of TCA entering the lysimeter soil was detected in the leachate. Other baseline lysimeters at Larch, Upper moor and Lower moor sites at Ballochbeatties also exhibited the same trend (Table 5.4.).

Table. 5.4 Total TCA inputs and outputs to baseline lysimeters at Ballochbeatties from May 2001 – May 2002.

Site	TCA input (ng)	TCA output (ng)	TCA output / TCA input
Sitka forest	52200	41400	0.79
Larch forest	55000	43300	0.79
Lower moor	78500	55500	0.71
Upper moor	78500	62600	0.80

However, lysimeter soils analysed at the start and end of the experiment did not show any change in TCA concentration which suggests that TCA is being lost in the soil lysimeter system. Possible reasons for this TCA loss are degradation of TCA or binding within the soil matrix or leachate. Degradation of TCA added to soil has been recorded by several authors to occur over a matter of days (Barrons and Hummer, 1951; Ogle and Warren, 1954; Martin, 1972; Foy, 1975; McGrath, 1976; Torstensson, 1976; Lignell *et al*, 1984; Forczek *et al.*, 2001; Matucha *et al.*, 2003) but few studies have reported on degradation of TCA in soil water (Haiber *et al.*, 1996) and at ambient environmental concentrations. It has been established in this research that TCA standard solutions prepared in the laboratory using distilled water last for several weeks in the refrigerator. However lysimeter leachate water is more likely to contain natural compounds and/or organisms which may degrade TCA. Binding of TCA to soil is unlikely to account for the TCA loss as soil TCA concentrations in lysimeters were not significantly different between the start and the end of the experiment. Experiments carried out in Chapter 3 showed that 100 % of TCA can be detected from TCA-spiked soil using HSGC-ECD analysis, which implies that TCA is not accumulating in the soil of the lysimeters.

5.2.3.2 Lysimeter Dosing Experiment

Hydrological Balance

For the TCA dosing experiment using Forest and Agricultural lysimeters the natural inputs and outputs (i.e. no artificial addition of water or TCA solution) were studied for the first three fortnightly sampling occasions only. The hydrological input (precipitation or throughfall, and 250 ml dosing solution), and output volumes to Forest and Agricultural lysimeters over the experimental period are shown in Figures 5.4a and b respectively. For the Forest lysimeters the hydrological data for every sampling occasion has been included but for Agricultural lysimeters the first four sampling occasions are omitted due to initial discrepancies experienced with rain gauge measurements, which were subsequently resolved. In subsequent calculations of TCA inputs and outputs, the mean input volumes of all raingauge measurements throughout the rest of the experiment are used.

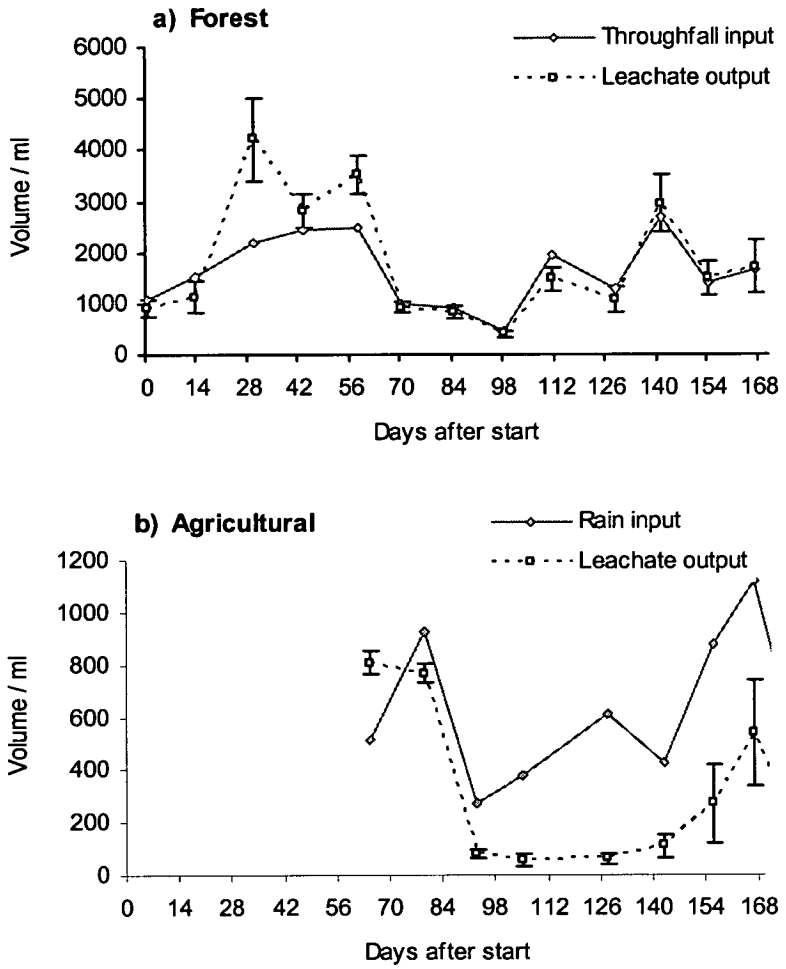


Figure 5.4. Fortnightly hydrological inputs (throughfall or rainwater) to and outputs (soil leachate) from soil lysimeters at: a) Ballochbeatties (Sitka forest), and b) Edinburgh (Agricultural). Error bars are standard deviations of seven replicate lysimeters.

The mean hydrological input (including 250 ml of dosing solution) and output volumes per fortnight at both sites are shown in Table 5.5. The total hydrological input volumes to Forest lysimeters over the whole experimental period are nearly 3 times higher than input volumes to Agricultural lysimeters which highlights a major difference in climate.

Table 5.5. Mean hydrological input and output fluxes of soil lysimeters at Forest (January – June 2002) and Agricultural (March – July 2002) sites. Standard deviations are shown in parentheses for 7 lysimeters over 13 (Forest) and 9 (Agricultural) sampling occasions.

	Mean fortnightly hydrological input / ml	**Mean fortnightly hydrological output / ml**
Forest	1624 (±191, n=13)	1803 (±328, n=13)
Agricultural	616 (±97, n=9)	312 (±103, n=9)

In Forest lysimeters there was excellent agreement between the input and output volumes. The output was, on average 110 % of the input, in contrast to the baseline lysimeter in Sitka forest where only 88 % recovery was observed over 1 year. These differences may be accounted for by inherent variation in spatial deposition of throughfall or in improved lysimeter design where overflow was not a problem.

In the Agricultural lysimeters the output volume was markedly lower than the input volume (mean of 51 % of the input volume) on all but one occasion although the relationship between input and output volumes on a fortnightly basis was similar to that of the Forest lysimeters (Figure 5.4). If the input volume was relatively large during a sample period, the corresponding output volume was also likely to be relatively large. These Agricultural lysimeters were vegetated and situated in an open area which received lower annual precipitation (Table 5.1). Evapotranspiration of water from the soil was therefore likely to occur more readily than in the forest soil, decreasing the volume of water available to percolate through the lysimeter soil into the collection bag.

TCA concentrations in dosed lysimeters

- **Control Lysimeters (dosed with water only)**

The mean "baseline" input and output TCA fluxes (TCA concentration x water volume) of all lysimeters during the first three sampling periods before dosing commenced are shown in Figures 5.5 a and b.

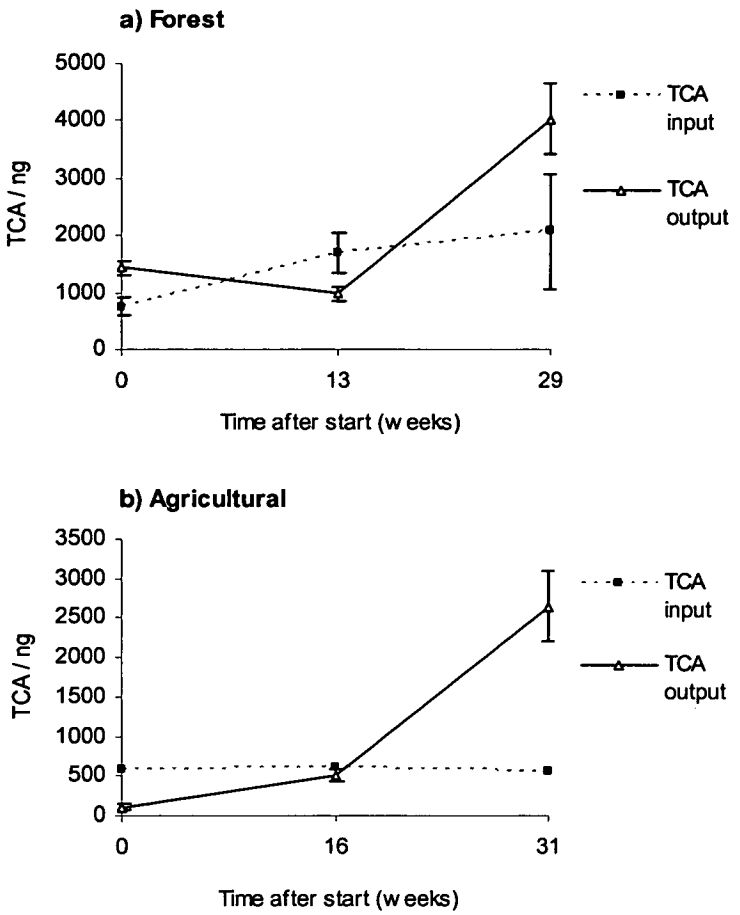


Figure 5.5. TCA masses (ng) in input (throughfall or rainfall) and mean output (leachate) water of (a) Forest and (b) Agricultural lysimeters before the start of controlled TCA dosing. Error bars are standard deviations of seven replicates analysed in triplicate.

The only aqueous TCA inputs to lysimeters were throughfall or rainwater. Forest lysimeters also received foliage litterfall from Sitka spruce trees although Stidson *et al.* (2004b) reported that only 1-2 % of the annual TCA deposition to forest sites at Ballochbeatties is in the form of foliage litterfall. For the purposes of this study the litterfall input was considered to be negligible.

Due to the small number of samples and high variability in volumes and TCA concentrations of input and output solutions it is not possible to conclude at this stage

whether natural TCA input and output fluxes are in balance for the controlled experiment. However, it is clear that TCA fluxes in Forest lysimeters were up to two times greater than those of the Agricultural lysimeters. The TCA flux is evidently driven mainly by the volume of precipitation, as observed with the baseline lysimeters.

After the "rest" period, Control lysimeters had an extra input of 250 ml of de-ionised water every fortnight for the duration of the experiment (i.e. on 10 occasions). A summary of TCA concentrations in lysimeter input and output waters is shown in Table 5.6. At both sites similar magnitudes of TCA concentration were observed in both input and output waters, although there was a significantly greater input volume (throughfall) to the Forest lysimeters compared to the Agricultural lysimeters.

Table 5.6. Summary statistics of TCA concentrations and volumes of input water (throughfall or rainwater) and output water (leachate) to and from Forest and Agricultural Control lysimeters over the whole experimental period. For all data n = 13, except for Agricultural input and output volumes, where n = 9*.

		TCA concentration / μg l^{-1}				**Volume of solution / ml**
Lysimeter site		Mean	SD	Median	Range	Mean (± SD)
Forest (n=13)	Input	0.81	0.16	0.82	0.44 – 1.10	1624 (± 191)
	Output	0.87	0.12	0.77	0.20 – 1.61	1803 (± 328)
Agricultural (n=13)	Input	0.72	0.30	0.72	0.25 – 1.03	616 (± 97)*
	Output	1.06	0.19	0.94	0.15 – 2.61	312 (± 103)*

There was no significant relationship between TCA concentrations and volumes of either input or output waters of either Forest or Agricultural lysimeters. In the case of Agricultural lysimeters, this implies that TCA in precipitation is no more concentrated during periods of low rainfall, than during storm events. However, this may not always be the case as, for example, rainfall following a long dry period may scavenge TCA from the atmosphere resulting in an episode of high TCA deposition to the terrestrial environment.

An unpaired *t*-test showed that there was no significant difference between the mean TCA concentrations of the input water and the output leachate water in any of the Forest or Agricultural Control lysimeters. There was also no significant difference between the total TCA input and output fluxes (total ng of TCA) which suggests that the soil system is in balance with regards to "natural" concentrations of TCA in throughfall or rainfall and leachate water (Figures 5.6 a and 5.7 a).

These results contradict data obtained for baseline lysimeters where it was found for all four sites (Larch, Sitka, Upper moor, Lower moor) that the TCA input mass was significantly greater than the output ($P<0.005$). The reason for this may be due to the previously described problem of lysimeter saturation which could result in under-estimation of the output volume of water.

Surprisingly, the input and output TCA concentrations of Agricultural lysimeters were not significantly different from the Forest lysimeters even although the TCA enters the Forest lysimeters via throughfall and the Agricultural lysimeters via rainwater. Over the period of this experiment the mean throughfall TCA concentration was, in fact the same (0.81 $\mu g\ l^{-1}$) as the mean rainfall reported by Stidson *et al.* (2004a) at Ballochbeatties over one year. However, in this same year-long study, the below-canopy aqueous TCA concentrations (stemflow and throughfall) at Sitka and Larch forest sites were measured by Stidson *et al.,* (2004b) to be, on average 1.4 times greater than the above-canopy aqueous TCA concentrations (rain and cloud water). This elevated TCA concentration was attributed to evaporative loss, as only 47 % of the total wet precipitation passed through the canopy as throughfall and stemflow. Therefore, although the throughfall TCA concentration was greater, the overall flux to the forest was lower than in open moorland sites. Other studies have also reported that some chemical species are elevated in throughfall water relative to rainfall (Robson *et al.,* 1994; Neal *et al.,* 1997). It is possible that over the period of the lysimeter dosing experiment described in this research, rainwater concentrations at Ballochbeatties were genuinely lower than those detected over the previous months, which would also result in lower TCA concentrations in the forest throughfall water.

The cumulative TCA inputs to, and outputs from, Control Forest and Agricultural lysimeters are shown in Figures 5.6a and 5.7a respectively. The circled data points are when dosing of lysimeters with de-ionised water commenced. There are indications that the cumulative output of TCA is greater than the cumulative input for Control Forest lysimeters although, as mentioned previously this was not statistically significant. The opposite trend was observed for Agricultural Control lysimeters but again this was not statistically significant. This demonstrates how difficult it is to draw conclusions about the natural cycling of TCA in the soil at environmental concentrations. From this study it is apparent that numerous replicate control lysimeters are necessary in order to form any definite conclusions about the relative inputs and outputs of TCA in the soil at environmental concentrations.

- **TCA-dosed lysimeters**

TCA concentrations in leachates of individual Control, Low and High-dosed Forest and Agricultural lysimeters are shown in Table 5.7. The experimental period has been divided into three stages: pre-dosing (3 sampling occasions), dosing of all lysimeters (A, B and C on 7 occasions for Forest; A and B on 6 occasions for Agricultural) and dosing of two lysimeters only at each site (C, on 3 occasions for Forest; B, on 4 occasions for Agricultural lysimeters). The quoted TCA concentrations are means of TCA concentrations (analysed in triplicate) over the duration of each experimental stage for each lysimeter.

It is clear that over the first three "pre-dosing" sampling occasions there was little difference in TCA concentrations of leachates of all lysimeters at either the Forest or Agricultural site. This is to be expected as, at this stage, they are essentially experimental replicates. During the second stage of the experimental period when all lysimeters were dosed fortnightly, with either 0.6 (Control), 80 (Low) or 200 (High) $\mu g\ l^{-1}$ TCA solution, TCA concentrations in leachates of Low or High dosed lysimeters were always higher than those of the Control. This shows that the concentration of TCA in the leachate reflects the relative magnitude of the input TCA concentration.

Table 5.7. Mean TCA concentrations and standard deviations of leachates from individual Forest and Agricultural lysimeters at three experimental stages; (a) pre-dosing, (b) during dosing of all lysimeters and (c) after termination of dosing of selected lysimeters (Forest A and B; Agricultural A). Leachates were analysed for TCA in triplicate and the overall mean of these taken over each of the three periods (no. of weeks stated below).

FOREST	**(a) Pre-dosing**		**(b) Dosing of ALL lysimeters**		**(c) Dosing of Low C and High C only**	
No. of sampling occasions	3		7		3	
	TCA concentration / µg l^{-1}					
	Mean	SD	Mean	SD	Mean	SD
Control	0.98	0.19	0.77	0.13	1.04	0.07
Low A	1.39	0.56	6.35	0.12	1.22	0.17
Low B	1.30	0.13	6.33	0.15	1.91	0.14
Low C	0.89	0.24	5.96	0.32	2.61	0.25
High A	1.07	0.14	12.6	0.17	0.51	0.09
High B	1.25	0.16	10.1	0.53	0.68	0.14
High C	1.24	0.36	12.7	0.34	10.3	1.21
AGRICULTURAL	**(a) Pre-dosing**		**(b) Dosing of ALL lysimeters**		**(c) Dosing of Low B and High B only**	
No. of sampling occasions	3		6		4	
	TCA concentration / µg l^{-1}					
	Mean	SD	Mean	SD	Mean	SD
Control A	1.35	0.11	0.91	0.11	0.65	0.09
Control B	1.92	0.34	1.13	0.41	0.75	0.12
Control C	1.38	0.57	1.10	0.21	0.62	0.07
Low A	1.30	0.22	22.1	0.21	7.41	0.64
Low B	1.08	0.19	30.9	1.08	21.5	0.17
High A	1.33	0.20	69.0	1.20	6.61	0.21
High B	1.36	0.04	70.2	1.74	90.7	0.97

During the second stage (dosing of all lysimeters), in Forest lysimeters the leachates of Low dosed lysimeters were approximately 5 times more concentrated than the Control leachates and the leachates of High dosed lysimeters were 10 times more

concentrated than the Control leachates. In Agricultural lysimeters the leachates of Low dosed lysimeters are 27 times more concentrated, and the leachates of the High dosed lysimeters are 70 times the concentration of the Control leachates. During the third stage of the experimental period, Low and high Forest lysimeters which were no longer dosed had leachate TCA concentrations comparable to those of the Control, whereas in Low and High Agricultural lysimeters which were no longer dosed, leachate TCA concentrations were up to 10 times greater than in the Control leachates. This strongly suggests that TCA is removed from the system more rapidly in Forest lysimeters than in Agricultural lysimeters. The greater TCA concentrations of Low and High dosed Agricultural leachates compared to Forest lysimeters may partially reflect differences in soil types but are mostly likely to be driven by the differences in leachate volumes, which depend on the input volumes of precipitation or throughfall.

Overall trends in TCA behaviour in lysimeters can be observed more clearly by calculating the cumulative TCA inputs to and outputs from each lysimeter over the whole experiment, as for Control lysimeters, discussed earlier. Cumulative data for Low and High Forest and Agricultural lysimeters are shown in Figures 5.6 b and c and 5.7 b and c respectively. The circled points indicate the first and last dosing occasions for Forest lysimeters A & B and Agricultural lysimeter A (the exact dates are given in Table 5.2). Forest lysimeter C and Agricultural lysimeter B were dosed until the end of the experiment.

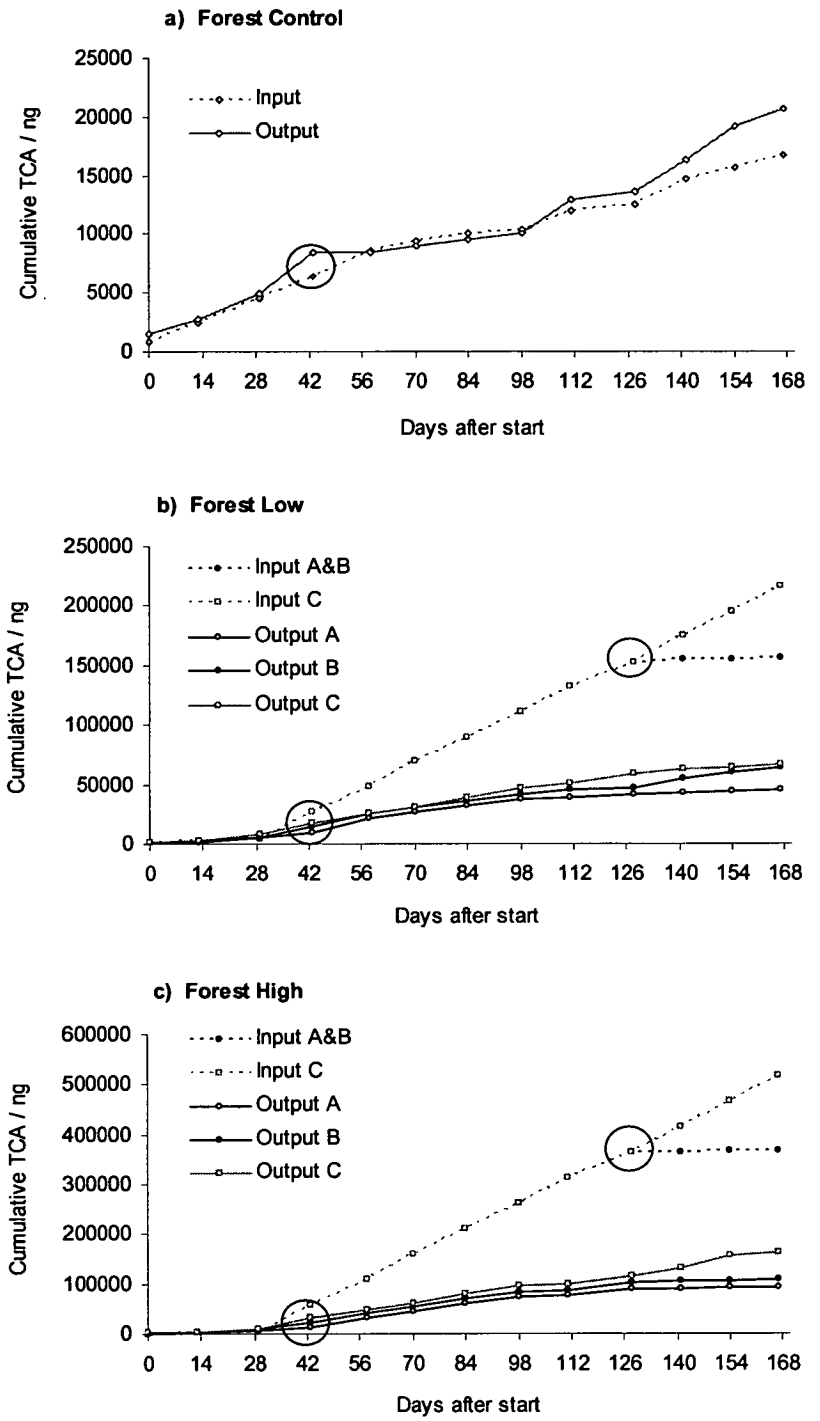


Figure 5.6 Cumulative TCA inputs and outputs to; a) Control; b) Low; and c) High dosed Forest lysimeters at Ballochbeatties over a 6-month period (Jan – June 2002). The first circled point in each graph indicates the start of dosing. The second circled point indicates the final dosing for lysimeters A and B.

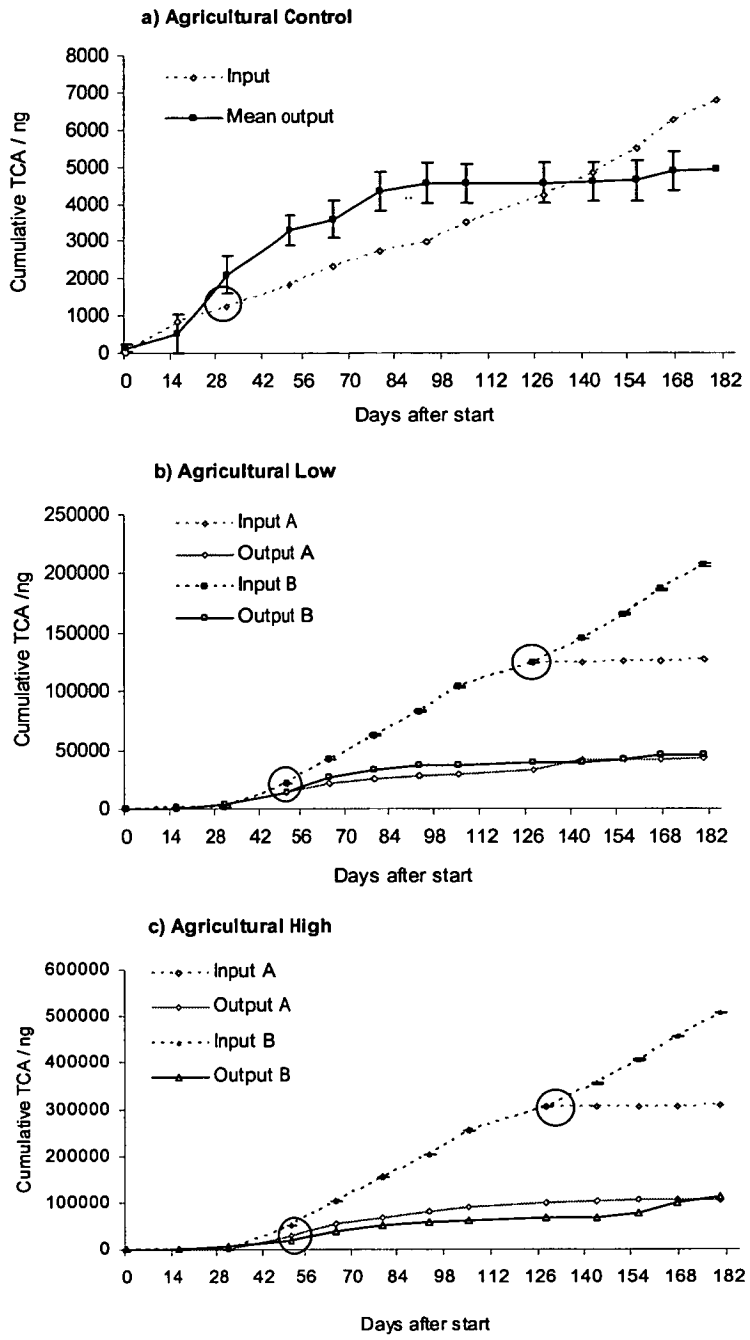


Figure 5.7. Cumulative TCA inputs and outputs to; a) Control; b) Low; and c) High dosed Agricultural lysimeters in Edinburgh, over a 6-month period (Jan 2002 – July 2002). Error bars for Control lysimeters are standard deviations of three replicates. The first circled point in each graph indicates the start of dosing. The second circled point indicates the last dosing for lysimeter A.

The Low and High-dosed Forest and Agricultural lysimeters exhibited similar overall trends in terms of TCA inputs relative to TCA outputs. The response of lysimeters to applied TCA was rapid, with leachates sampled a fortnight later having TCA concentrations significantly greater than the control and baseline lysimeters. In all Low and High-dosed lysimeters the gradient of the graph was relatively constant until dosing started, when it rose quite steeply for the first two dosing periods. It then continued to increase at a slower rate which coincided with lower rainfall from late March until early May. Although the inputs of TCA did not change significantly (the mass of TCA entering the lysimeter via rainfall was negligible in comparison to the mass present in the dosing solution) there was less water available to flush the TCA through the soil into the leachate. The TCA probably had a longer residence time in the soil before being removed, either into the leachate or via other, unidentified, removal/immobilisation mechanisms. The total cumulative TCA in the input and output water over the whole experimental period, and the final recoveries are summarised in Table 5.8 for each lysimeter. The final cumulative recoveries of TCA in the outputs of all the Low and High dosed lysimeters were of similar magnitudes of at both Forest and Agricultural sites (19 - 41 %). In Agricultural lysimeters both the Low and High lysimeters in which dosing ceased after 128 days ("A") had overall recoveries of 29 %, and in those where dosing continued until 180 days, the overall recovery was about 20 %. A similar trend was apparent for the Forest lysimeters which were dosed until the end, with a higher final recovery of 31 or 32 %. These data are remarkably consistent between the Low and High TCA dosed lysimeters. If these near-identical recoveries did not occur by chance, they may imply that the soil can only remove or immobilise a certain proportion of the input TCA, which is the same regardless of the mass of TCA applied.

Table 5.8. Total TCA inputs to and outputs from all Forest and Agricultural lysimeters over the whole experimental period of January 2002 to July 2002. The differences between the TCA input and output masses (TCA "lost") and the percentage of input TCA recovered in the output are also shown.

	Forest				**Agricultural**			
	TCA input / ng	TCA output / ng	TCA "lost" / ng	% TCA recovery in leachate	TCA input / ng	TCA output / ng	TCA "lost" / ng	% TCA recovery in leachate
Control	16700	20700	-4000	123	6890	4900	1990	71
Low A	157000	45500	111500	29	147000	42600	104400	29
Low B	157000	64400	92600	41	227000	43200	183800	19
Low C	217000	66300	150700	31				
High A	367000	92900	274100	25	357000	105000	252000	29
High B	367000	107000	260000	29	557000	114000	443000	20
High C	517000	164000	353000	32				

The percentage recoveries of TCA in the leachates relative to inputs are shown for each individual sampling date in Figures 5.8 a and b. After the first dosing (43 days for Forest and 51 days for Agricultural lysimeters) the TCA recovery in the leachate was greater in the Low lysimeters than the High lysimeters, with a mean of 37 % and 51 % of the applied TCA measured in the Low Forest and Agricultural lysimeters respectively, compared with 32 % and 39 % measured in the High lysimeters. This implies that 49 – 68 % of the applied TCA remained unaccounted for in the leachate and was either present in another compartment (e.g. soil) or degraded. After a further five (Agricultural) or six (Forest) doses, the percentage recoveries were lower with 17 % of the applied TCA being measured in the leachates of both the Low Forest and Agricultural lysimeters and 14 % and 28 % measured in the leachates of the Low and High Forest and Agricultural lysimeters respectively.

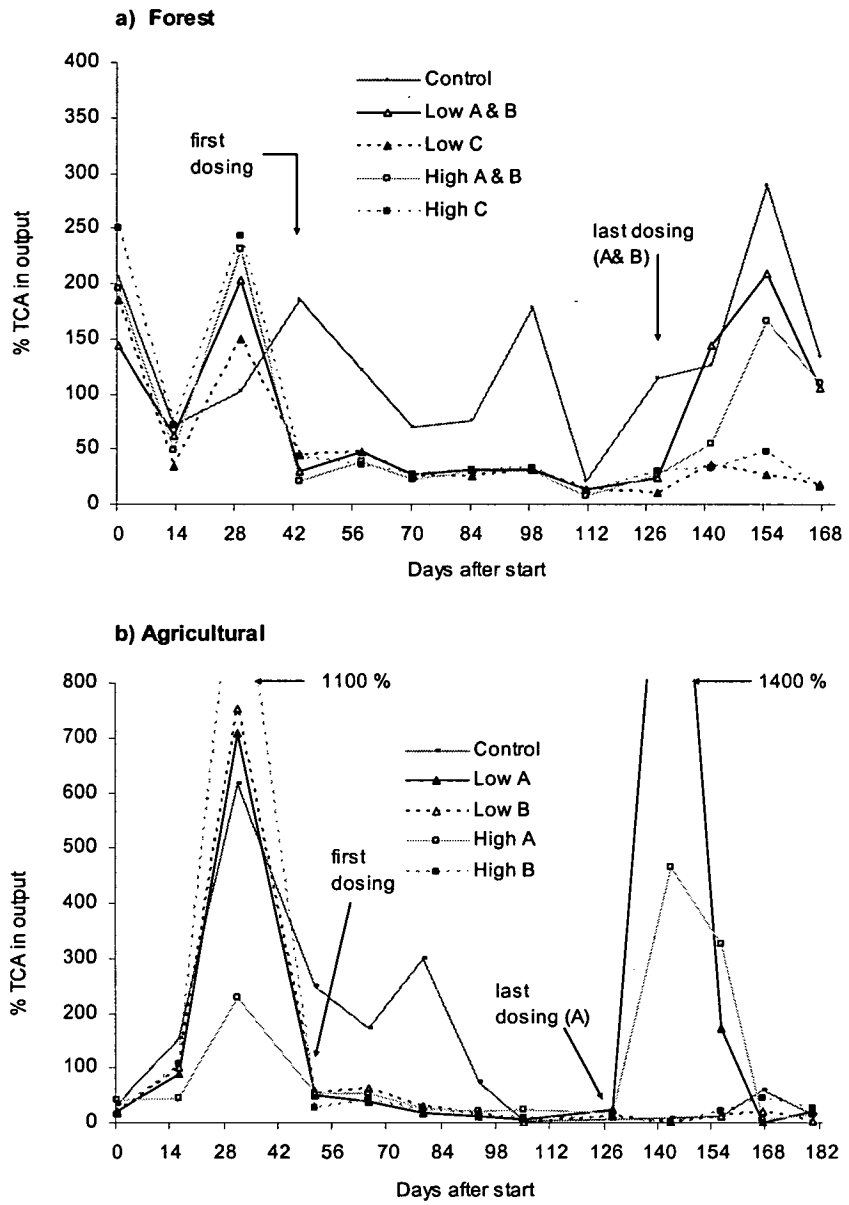


Figure 5.8. The recovery of TCA in the leachates expressed as a percentage of the input TCA for each individual sampling date, for (a) Forest and (b) Agricultural lysimeters.

It appears that, over a period of time, the soil system may adapt to external inputs of TCA and can remove it more efficiently. After dosing ended for "A & B" Forest lysimeters and "A" Agricultural lysimeter the percentage recoveries initially increased and then gradually decreased over the final weeks as the TCA present in the soil and solution was flushed through. However, although these "flushed

through" TCA concentrations were greater than the corresponding TCA input concentrations they were not large enough to account for the observed cumulative loss of TCA.

- **TCA concentration of lysimeter soil**

The percentage recovery of TCA in the leachates of both the Forest and Agricultural lysimeters were similar, despite the soils having very different characteristics and the total mass of soil in the pot differing significantly (Table 5.9). The wet mass of soil in the Agricultural lysimeters was nearly two times greater than soil in the Forest lysimeters, and the dry mass was approximately twelve times greater. As similar recoveries were obtained in the leachates of both soil types it is evident that the forest soil has a greater capacity to remove added TCA as there was effectively a greater mass of TCA applied per unit mass of soil (wet or dry).

Table 5.9. Summary of soil characteristics in Forest and Agricultural lysimeters at the start (December 2001) and termination (July 2002) of the field lysimeter TCA dosing experiment.

Variable (mean)	Date of sample collection	Forest	Agricultural
Soil fresh weight TCA concentration / ng g^{-1}	December 2001	63.6 ± 13.5	10.3 ± 0.95
	July 2002	60.9 ± 9.9	6.17 ± 0.65
Total fresh soil mass / g	Mean of Dec 01 and July 02	1894 ± 71	3514 ± 393
Total dry soil mass / g	Mean of Dec 01 and July 02	257 ± 10	3122 ± 319
Dry bulk density (DBD) / g cm^{-3}	July 2002	0.103 ± 0.004	1.25 ± 0.05
Soil water content / %	Mean of Dec 01 and July 02	86 ± 0.8	11 ± 2.3
Total TCA burden of lysimeter soil / μg	December 2001	124 ± 29	36 ± 3.1
	July 2002	117 ± 21	22 ± 2.6

The lysimeters soils were analysed for TCA in December 2001 when the lysimeters were first set up, and in June 2002 (Forest) or July 2002 (Agricultural) at the time of final leachate sampling (Figure 5.9). For Forest soils the litter layer and the soil were analysed separately and a mean TCA concentration obtained after correcting for the estimated volume occupied by each of the components in the pot.

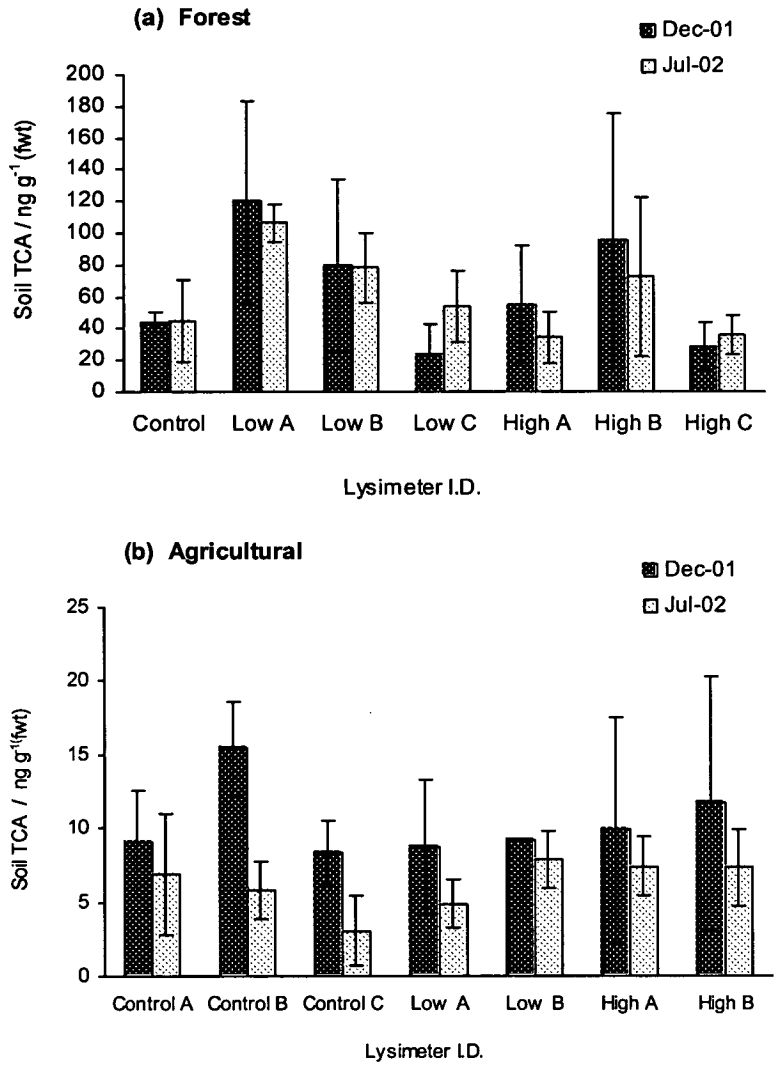


Figure 5.9. TCA concentrations of (a) Forest and (b) Agricultural lysimeter soils at the beginning (Dec 2001) and end (July 2002) of the TCA dosing experiment. Error bars are the standard deviations of 3 analytical replicates.

TCA concentrations were always greater in Forest soil than in Agricultural soil which is in agreement with soil measurements described in Chapter 4. In neither soil type did the TCA concentrations differ significantly between the start and end of the experiment for the Control, Low or High-dosed lysimeters. In fact, it appears from Figure 5.9 that they were slightly lower but this may be attributed to natural variation and soil heterogeneity, or faster degradation of TCA in warmer months. However, the presence of TCA presence in soils sampled in winter suggests that if natural production is a contributor to soil TCA, then either the production process continues throughout the colder months or TCA has accumulated from previous *in-situ* soil production and has not yet been eliminated from the soil.

These data show that the "lost TCA" is not recoverable from the soil using the HSGC-ECD method of analysis. Possible reasons are that the TCA is bound very tightly to some component of the soil, to such an extent that it is not converted to chloroform even at 100 °C, which is unlikely as shown by recovery experiments in Chapter 3. Alternatively, or in addition, the TCA may be degraded biologically by the action of micro-organisms, or chemically. These same processes may also operate in the soil solution (leachate).

The presence of TCA in the soil appears paradoxical. If TCA is so clearly removed from the soil system, then it must be questioned why TCA is present in the soil at all. This returns to the previous suggestion of TCA *in-situ* production. Alternatively, TCA that is bound to the soil is released under certain environmental conditions. As discussed in Chapter 4, Haiber *et al.* (1996) found that TCA in solution disappears during percolation through soils rich in dissolved organic carbon (DOC) but on the other hand TCA was found in DOC rich waters at concentrations of up to 1 $\mu g\ l^{-1}$. and concluded that TCA must undergo decomposition and formation processes equally. This may also be evidence that "artificial" TCA applied to a soil system behaves differently from *in-situ* soil TCA.

5.3 EFFECT OF A LITTER LAYER ON TCA CYCLING IN SOIL

5.3.1 Introduction

The field lysimeter experiments described in Section 5.2 have shown that TCA in solution added to soil and allowed to percolate through cannot be fully recovered in the soil leachate. This is particularly surprising for the Forest lysimeters given the very high TCA concentrations that have been detected in the litter layers of Sitka forest soil (up to 400 ng g^{-1} (fwt)) at Ballochbeatties (Chapter 4). It implies that high soil (or litter) TCA concentrations do not necessarily indicate high soil water concentrations (i.e. > 2.0 μg l^{-1}) and also that TCA present in the soil cannot be easily extracted into aqueous solution, supporting the results of extraction experiments presented in Chapter 3. Indications are, that Sitka litter may have unique characteristics, which influence the production or degradation of TCA in different ways to other soil horizons, resulting in greater TCA concentrations. After it has been deposited on the soil surface from throughfall or stemflow water, or produced *in-situ,* TCA may be more strongly bound in the litter layers than in deeper horizons, further contributing to the higher TCA concentrations, which can be detected by HSGC-ECD but not extraction-derivatisation methods.

Aims

To investigate the influence of a litter layer in Sitka forest soils on TCA cycling, a controlled experiment was set up in a greenhouse using soil cores with or without a litter layer.

The specific objectives were:

1) To determine if TCA from a single dose of TCA solution to soil could be recovered over time in the soil leachate.
2) To determine if the presence of a litter layer in Sitka soil influences the recovery of TCA in the soil leachate.

5.3.2 Methods

5.3.2.1 Collection of soil cores

Soil cores (I.D. = 80 mm, length = 200 mm) were collected from Sitka forest at the Ballochbeatties site on 24/1/03, in the same way as for soil lysimeters (Section 5.2.2.1). Soil immediately adjacent to the cores was sampled for determination of TCA concentration and water content. Three cores were collected from a depth of 0-20 cm (including the litter layer) and three cores were taken from the organic horizon below the litter layer (approximately 10-30 cm below the surface). These are described, respectively, as "Sitka L" (litter layer present) and "Sitka O" (no litter layer present). Cores were transported back from the field-site in an upright position and stored in the fridge overnight (<5 °C) until being set-up in an unheated greenhouse the next day to simulate field conditions as closely as possible.

5.3.2.2 Set-up of cores

The soil cores were set up in a similar manner as the field lysimeters shown in Figure 5.1. Plastic gauze and fine netting were sealed to the base of each core to prevent soil particulates from being washed through with the percolation water ("leachate") and a plastic sample bag was attached below the core to collect the soil leachate. The surface of each core was loosely covered with cling-film to minimise water loss by evaporation. The mass and volume of every core was recorded and the soil moisture content determined using the standard procedure described in Chapter 2. From this information the soil dry bulk density was calculated according to Equation 5.1.

5.3.2.3 Baseline TCA concentrations in core leachates

To obtain a baseline from which to compare the results of TCA dosing, ultrapure water was applied to each core on five separate occasions over a period of 18 days, and allowed to percolate through until a significant volume of leachate accumulated in the bag (1–3 days). The volume of leachate was recorded, and the leachate analysed for TCA by HSGC-ECD as described in Chapter 2. The original intention was to apply volumes of water proportional to the original water content of the soil in the core and ensure each core received the same relative volume of water. In theory,

this would result in total displacement of "old" soil water by "new" ultrapure water so that TCA dosing could commence after all cores had been completely flushed out. The first volume of water applied was 35 % of the initial mass of water in the core, as this was the maximum volume the cores could take without overflowing. The main problem experienced was the different drainage rates of the soil cores. In two of the cores the water remained pooled on the soil surface whereas in the other four cores the water drained within minutes through the soil to the collecting bag. This was dealt with by applying as much water as would readily drain through the core and recording the volume added so that total TCA inputs from ultrapure water could be calculated.

5.3.2.4 Dosing of cores with TCA solution

After the baseline TCA outputs in the leachates had been established, a single dose of 100 ml of 44 $\mu g\ l^{-1}$ TCA solution was prepared with ultrapure water and applied to each core on day 19 (equivalent to a total TCA dose of 4.4 μg). The leachate was collected the following day (day 20) and analysed for TCA. The cores were flushed through with ultrapure water on day 20, as well as on day 27, and the corresponding leachates collected on days 22 and 29 and analysed for TCA. Core soils and litter layers were analysed for TCA at the beginning and the end of the experiment (i.e. before and after application of a TCA dose).

5.3.3 Results and discussion

The total TCA outputs in the leachates of both Sitka L and Sitka O cores are shown in Figure 5.10. Ultrapure water itself contained up to 3 $\mu g\ l^{-1}$ TCA therefore the TCA inputs to soil cores were greater than zero even during the baseline period. The TCA outputs in leachates of both Sitka L and Sitka O followed almost identical trends before and after TCA dosing and there was no significant difference between their mean concentrations (paired *t*-test).

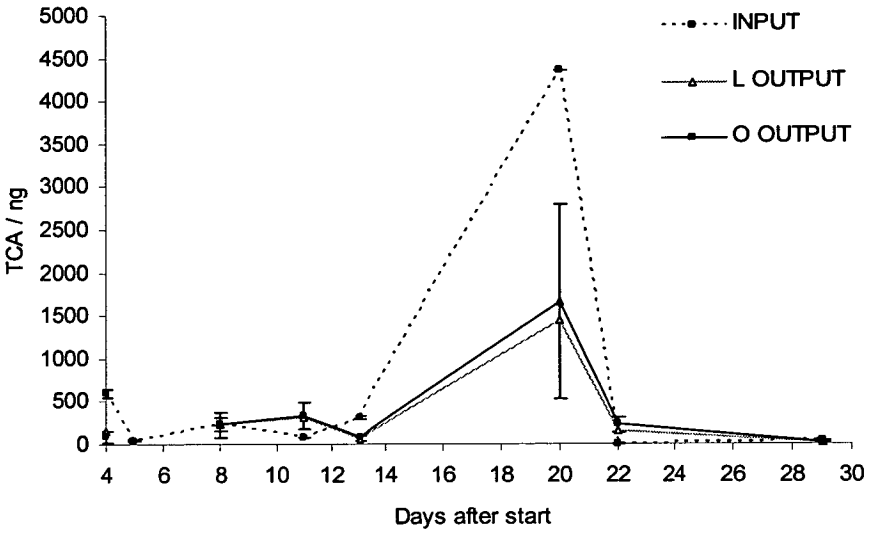


Figure 5.10. The TCA mass in leachates from soil cores from the organic horizon of a Sitka soil with (Sitka L) or without (Sitka O) a litter layer at different times (days) after the cores were set up. Error bars on outputs are standard deviations of three experimental replicates, each analysed in triplicate.

Figure 5.11 shows the total TCA inputs and outputs to cores over the whole experiment (29 days). It clearly illustrates that for all cores only a small proportion of the TCA applied to the soil cores emerges in the leachates. The mean final TCA recoveries in the leachate output were 44 (± 13) % and 47 (± 20) % for Sitka L and Sitka O cores respectively. After the addition of 4.4 μg TCA (day 19), only 33 % and 38 %, for Sitka O and Sitka L respectively of the applied TCA emerged in the leachate on day 20. Up to day 29 these recoveries increased to 57 % and 64 %, respectively (as TCA drained out of the soil in the leachate, taking into account the extra ultrapure water added to prevent the cores from drying out and to flush out any TCA remaining in the soil). However, in leachates sampled at the end of the experiment (day 29), TCA concentrations were of similar magnitudes to those detected in baseline leachates at the beginning of the experiment. This implies that either TCA was being flushed out of the soil and that it would be released very slowly at levels below analytical detection limits or that it had been "lost" in the system, as demonstrated in the field lysimeters (Section 5.2).

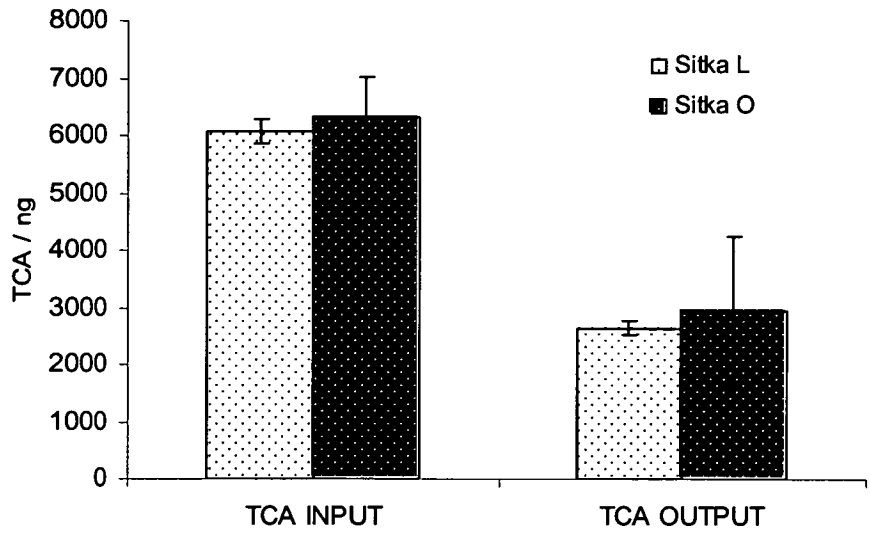


Figure 5.11. Total mass of TCA in the input (artificially applied) and output solutions (leachate) of soil cores with (Sitka L) and without (Sitka O) a litter layer. Error bars are standard deviations of three experimental replicates, each analysed in triplicate.

The problem of variable infiltration rates of applied solutions in different soil cores meant that not all soils were in contact with the applied solutions for the same length of time. There were two cores in particular where pooling on the soil surface was a problem. In Figure 5.12 the overall percentage recoveries of TCA in the leachate output are shown for individual cores Sitka L (A, B, and C) and Sitka O (A, B and C). Cores LB and OA had particularly slow infiltration rates and both of these cores had the poorest percentage TCA recovery (< 30 %) in the leachates which shows that greater contact time with soil has the effect of reducing the recovery of TCA as potential removal mechanisms such as binding or degradation have more time to take effect. However, TCA concentrations in the core soils were not significantly different between the start and end of the experiment which implies that, as with the field lysimeters, TCA is not bound to the soil, or is bound too strongly to be released to chloroform even at 100 °C. As shown by recovery experiments in Chapter 3, this latter suggestion is unlikely.

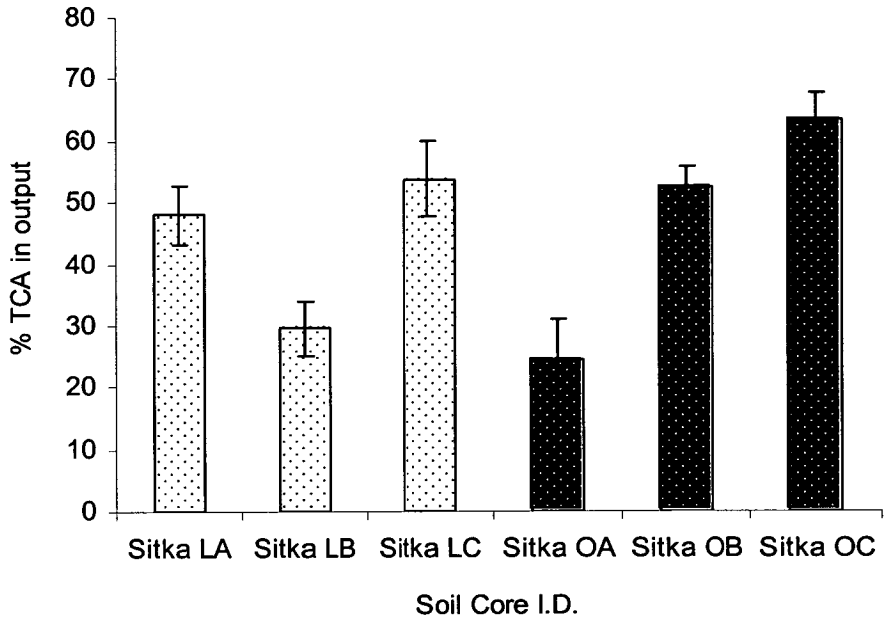


Figure 5.12. Overall recovery of TCA in the leachates of the individual cores Sitka L and Sitka O (A – C), expressed as a percentage of the total TCA inputs over the whole experimental period. Error bars are standard deviations of triplicate analyses.

5.3.4 Conclusions

It is clear that TCA from a single dose of TCA solution to soil cannot be fully recovered over time in the soil leachate. The presence of a litter layer does not appear to have any effect on the recovery of externally applied TCA at the concentration of 44 $\mu g\ l^{-1}$ used in the experiment. Although TCA appeared to be "lost" in the system, similar trends were observed for both core types. These results imply that Sitka litter does not have a greater capacity for binding and immobilising TCA than other soil horizons and does not account for the observed high TCA concentrations of Sitka spruce litter layers (up to > 400 ng g^{-1} (fwt)), which are greater than the TCA concentration of fresh needles (8 – 123 ng g^{-1} (fwt)). However, these conclusions may not apply to soil in the field which is subjected to continuous additions of low concentrations of TCA found in rain or throughfall water.

5.4 EFFECT OF MICRO-ORGANISMS ON TCA CYCLING IN SOIL

5.4.1 Introduction

It has so far been shown in controlled field and greenhouse experiments that there is less than 100 % recovery of TCA in soil leachates when TCA solution of known concentrations is added to the soil. The reasons for this are not clear. However the presence of a litter layer has been shown to have no effect on the recovery of applied TCA in the leachate, implying that the occurrence or extent of binding of TCA to organic matter or degradation in soil is not specifically associated with litter. The system was closed so that any TCA entering the core could only leave the system by percolating through the soil into the collection bag. TCA does not evaporate into the atmosphere due to its low volatility and hydrophilic nature so it appears that TCA has been degraded within the soil system. Degradation of TCA by micro-organisms was therefore investigated as a possible removal mechanism of TCA in soil.

Aims

To investigate the effect of micro-organisms on TCA cycling in the soil a controlled experiment was carried out in an unheated greenhouse using two different soil types, sterile and non-sterile.

The specific objectives were:

1) To determine if TCA can be degraded biologically in soil.
2) To compare rates of TCA degradation in two different soil types.

5.4.2 Methods

5.4.2.1 Collection and sterilisation of lysimeter soil

Approximately 3 kg (fwt) of each of Sitka forest soil (O horizon, 10 – 20 cm) and Larch forest soil (O horizon, 7 – 27 cm) was collected from the Ballochbeatties site on 24/1/03. Half of each soil was sterilised using a Cobalt-60 source of gamma radiation with a dose of 27 – 35 kGy at Ethicon Limited, Edinburgh, Scotland. This procedure was used in preference to autoclaving which involves heating soil to over 100 °C in a sealed container and therefore inevitably converts an unquantifiable mass

of intrinsic soil TCA to chloroform, and potentially alters other soil properties. It was assumed that gamma radiation killed all soil micro-organisms without changing the structure of the soil. The remaining un-sterilised soil was refrigerated at <5 °C until required. A sub-sample of each soil was also analysed for TCA by HSGC-ECD as described in Chapter 2.

5.4.2.2 Set-up of lysimeters

Lysimeters of the same design as those in the field, but at a smaller scale were set up, in an unheated greenhouse in Edinburgh to simulate field conditions as closely as possible. The sterile soil lysimeters were prepared first, using sterile gloves to reduce the risk of contamination from non-sterile soil. Each pot (depth of 12 cm) was filled with approximately 350 g of soil. As with the soil cores and field lysimeters, plastic gauze and fine netting were sealed to the base of each pot to prevent soil particulates from being washed through with the percolation water. A plastic sample bag was attached to the base of the pot to collect water which percolated through the soil ("leachate"). The surface of each pot was loosely covered with cling film to minimise water loss by evaporation as well as to reduce the risk of contamination of sterile soils by micro-organisms from non-sterile soils. However, as the greenhouse was not a sterile environment it was inevitable that some colonisation of the soil by micro-organisms would occur soon after the soil was exposed to the atmosphere. Six lysimeters were set up for each soil type (Larch or Sitka), three containing sterile soil and three containing non-sterile soil.

5.4.2.3 Dosing of lysimeters with TCA solution

To each lysimeter 80 ml of a 50 $\mu g\ l^{-1}$ solution of TCA (a total TCA mass of 4 μg) was added using a measuring cylinder (day 0). The solution was allowed to percolate through the soil into the plastic collection bag. On day 2, when a sufficient volume of leachate had accumulated, the bag was removed, the volume of leachate measured and the solution analysed for TCA by HSGC-ECD, as described in Chapter 2. A new bag was attached to each lysimeter and 50 ml of ultrapure water was added to prevent drying of the soil and to flush out any of the externally added TCA remaining in the soil. The volume of leachate was measured 4 days later (day 6) and

a sample analysed for TCA. Again, the bag was replaced and 50 ml ultrapure water added. This procedure was repeated on days 8, 9, 14, 16, and 23.

5.4.3 Results and discussion

Characteristics of the Sitka and Larch soils sampled at the start of the experiment are summarised in Table 5.10. The greater TCA concentration, water content and organic matter contents of the Sitka soil compared to the Larch soil are in agreement with measurements reported in Chapter 4 for other similar soils sampled at Ballochbeatties.

Table 5.10. Characteristics of Sitka and Larch soils used in sterile and non-sterile soil lysimeters. All values are means with standard deviations shown in parentheses for TCA concentrations (n=3, one replicate analysed in triplicate) and for soil and organic matter contents (n=2).

Soil type	"initial" soil TCA concentration / ng g^{-1} (fwt)	Soil water content / %	Fresh weight soil organic matter content / %	Dry weight soil organic matter content / %
Sitka	37.1 (± 6.0)	85.9 (±0.8)	12.7 (±0.8)	90.4 (±0.9)
Larch	10.6 (±0.5)	57.7 (±4.2)	14.4 (±1.2)	34.2 (±4.2)

The change in mass over time of TCA detected in leachates from lysimeters of sterile (S) and non-sterile (NS) Sitka and Larch soils, following TCA applications, are shown in Figure 5.13. The leachates were first analysed 2 days after TCA application. After this time only a fraction of the input TCA was recovered, with 33 % and 18 % of the input TCA detected in the leachate for Larch S and NS soil respectively, and 11 % and 8 % for Sitka S and NS soil respectively. It is clear that TCA concentrations recovered in the leachate decrease with length of time after TCA input to the soil, this trend being most obvious in the Larch soils. The greater recovery of TCA in leachate from both the Sitka and Larch sterile soils suggests that some process is occurring in the soil which is independent of individual soil characteristics and which does not occur to the same extent in non-sterile soils.

The soils were subjected to identical conditions prior to the experiment, with the exception of the sterilisation process which is assumed not to significantly alter soil properties. Therefore, the only assumed difference between the soils is the presence or absence of micro-organisms. The Sitka soil (both S and NS) showed a significantly greater loss of TCA in the leachate compared to the Larch soil (2-way ANOVA, $p < 0.001$) which implies that different soil types respond differently to external additions of TCA. Although this loss is greater in the non-sterile soil, it is still significant in the sterile soils suggesting either that micro-organisms re-colonise very rapidly and that microbial activity may increase due to the introduction of TCA to soil, or that another process in Sitka soil causes greater TCA loss, maybe binding to humic substances or chemical degradation.

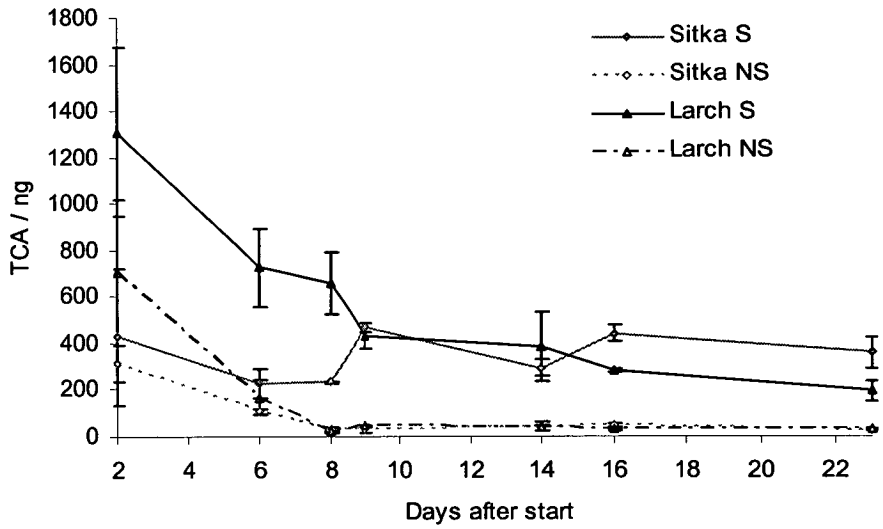


Figure 5.13. The mean TCA mass in leachates from Sterile (S) and non-sterile (NS) Sitka and Larch soil lysimeters dosed with 4 μg TCA on day 0. Error bars are standard deviation of 3 experimental replicates, each analysed in triplicate.

Over two days it is unlikely that 100 % of the input TCA could be recovered in leachate, even in sterile soils, due to the time it takes water to percolate through soil. Originally it was intended to continue the experiment until the TCA concentrations in the leachate were of similar magnitudes to the mean TCA concentration of 0.87 (±

0.7) μg l^{-1} in the ultrapure water used to water the lysimeters. The mean TCA concentrations of the final leachates collected are shown in Table 5.11.

Table 5.11. TCA concentrations in lysimeter leachates 23 days after the application of 4 μg TCA to the soil.

Soil type	Mean TCA concentration in final leachate (± S.D.) / μg l^{-1}	
	Sterile (S)	Non-sterile (NS)
Sitka	8.97 (0.42)	0.68 (0.05)
Larch	4.75 (0.50)	0.72 (0.04)

Although the baseline concentration was reached by non-sterile Sitka and Larch soils after 23 days, this was not the case with the sterile soils as the experiment was terminated due to time constraints. Figure 5.14 shows the overall lysimeter TCA outputs expressed as a percentage of the inputs over the whole experimental period (0 – 23 days).

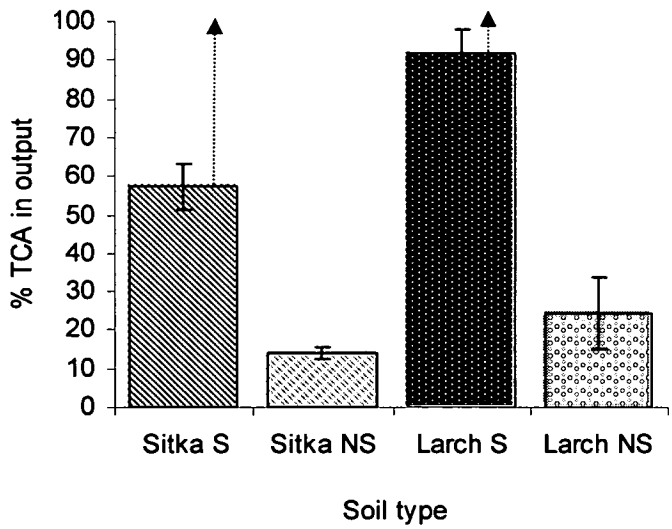


Figure 5.14. Mean recovery of TCA in the leachates of sterile (S) and non-sterile (NS) Sitka and Larch soil lysimeters, expressed as a percentage of the total TCA input over the whole experimental period. Error bars are standard deviation of 3 replicates, each analysed in triplicate.

There was a significant difference in final cumulative TCA recoveries between leachates of sterile and non-sterile soils (2-Way ANOVA, $p < 0.001$). In the sterile Larch soil lysimeters most of the applied TCA (92 %) was recovered in the cumulative outputs in the leachates, but from sterile Sitka soils only 57 % was recovered. The lower percentage recovery from the sterile Sitka lysimeters is a strong indication that TCA behaves differently in different soils. If biodegradation of TCA has occurred in sterile soils, it appears that micro-organisms re-colonised the Sitka soil more rapidly, or act at a faster rate than in the Larch soil; alternatively, chemical degradation also occurred. Different microbial species and community structures with different population dynamics are likely to exist in different soils, which may respond and adapt to the artificial introduction of TCA in different ways. From this experiment it is not possible to determine what effect the external addition of unnaturally high TCA concentrations to soil may have had on the activity of micro-organisms. Furthermore, it cannot be assumed that the micro-organisms responsible for the observed removal of TCA in this experiment act in the same way and work at the same rate as when decomposing TCA present at "natural" soil concentrations.

Since biodegradation of TCA evidently occurs at a faster rate in Sitka soils than in Larch soils, this means that *in-situ* soil production of TCA is likely to occur at a greater rate in Sitka soils in order to account for the higher TCA concentrations measured in Sitka soils compared to Larch soils. In effect, this implies that TCA cycling in Sitka soils is greater than in Larch soils and hence there is greater overall TCA formation and faster elimination.

Larch soils are expected to be more favourable for microbial activity, with a lower moisture content of 58 % (Table 5.10). The moisture content of the Sitka soils (86 %) is extremely high which is likely to generate more anaerobic conditions. This contradicts the conclusion that greater biodegradation occurs in Sitka soils. However, this experiment does not identify the nature of the micro-organisms involved in TCA degradation, whether they are operating aerobically or

anaerobically, or are using TCA as a sole source of carbon, or non-specifically utilising carbon from TCA, as one of many organic compounds present in the soil. As micro-organisms are known to degrade TCA at concentrations used for herbicidal applications (Jensen, 1957, 1960, 1963; Lode, 1967; McGrath, 1976; Torstensson, 1976) it has long been suspected that degradation by micro-organisms might also be responsible for removal of TCA at trace concentrations. Several genera of higher fungi have a capacity for biosynthesis of organohalogens but they can also cause their reductive dechlorination (Yu and Welander, 1995; Olaniran *et al.*, 2001). Microbial degradation of TCA to CO_2 and chloride was demonstrated by Weightman *et al.* (1992) and its decarboxylation to chloroform in soil has been reported in other studies (Kearney *et al.*, 1965), although this latter route is thought to be minor in natural soil conditions (Hoekstra *et al.*, 1999b). Enzymatic dechlorination of TCA to oxalic acid in pond waters has also been reported (Ellis *et al.*, 2001).

5.4.4 Conclusions

The data suggest that, had the experiment been continued for a longer period of time (several weeks), all the TCA applied to the sterile soil may have been flushed out of the soil and recovered in the leachate. This implies that no binding occurred in the soil matrix of these particular Sitka and Larch soils and that any apparent "loss" of TCA from the soil was solely via degradation by soil micro-organisms. It is clear that rates of TCA elimination by micro-organisms differ between soils. These data support the results of soil microbial biomass (SMB) measurements (Chapter 4, Section 4.3.3) which indicate that microbial activity is greater in Sitka soils than in Larch soils.

5.5 BIODEGRADATION OF TCA IN SOIL – USE OF [1,2-^{14}C] TCA

5.5.1 Introduction

Controlled soil lysimeter and core experiments conducted in the field and unheated greenhouse (Sections 5.2, 5.3 and 5.4) consistently show that not all the TCA entering the soil system can be recovered from the soil and the output water. The higher recovery of TCA in leachates from sterile soils compared with non-sterile soils is substantial evidence that micro-organisms play a role in removing TCA from the soil system (i.e. "biodegradation" of TCA occurs in soils).

In recent years, researchers in the Czech Republic and Germany (Forczek *et al.*, 2001; Matucha *et al.*, 2001; Matucha and Uhlírová, 2002; Coufal *et al.*, 2003; Matucha *et al.*, 2003; Schröder *et al.*, 2003) have used radioindicator methods to study the uptake, translocation and effects of TCA on Norway spruce saplings (*Picea abies* L. Karst.). As [1,2-^{14}C] TCA of high specific activity is not commercially available, a small-scale (< 1 mmol) one-pot synthesis of [1,2-^{14}C] TCA with > 70 % yield and specific activity of 3.7 GBq mmol^{-1} was developed by Bubner *et al.* (2001). The main advantage of the labelling technique is that the fate of added TCA is known. In previous experiments (Sections 5.2, 5.3 and 5.4) TCA losses in the soil system have been observed but the fate of TCA could not be uniquely identified. In labelled TCA experiments an independent mass balance can be calculated by radioactivity counting and all applied TCA can therefore be accounted for.

From experiments on seedlings in the laboratory using [1,2-^{14}C] TCA, Forczek *et al.* (2001) reported that uptake of TCA by the plant occurs via the soil from precipitation water followed by uptake by roots and movement into needles via the transpiration stream. Losses of [1,2-^{14}C] TCA-derived radioactivity were observed from incomplete radioactivity balances and this was thought to be due to degradation of TCA in the soil. [1,2-^{14}C] TCA was later used in controlled laboratory experiments to specifically investigate the biodegradation of TCA in soil, to $^{14}CO_2$. From a number of biodegradation experiments over several days it was concluded that the biodegradation of TCA in soil to CO_2 is fast and depends on soil TCA concentration, temperature and moisture content.

The opportunity arose to apply this technique of ^{14}C-labelling to investigate the biodegradation of TCA in Scottish soils. It is clear from data presented in Chapter 4 that most of the soils studies have TCA concentrations greater than those reported in studies of soils in other parts of Europe. Comparison of the biodegradation rates of TCA in these different soils may highlight differences that exist between the dynamics of TCA in different soils.

Aims

Experiments using [1,2-^{14}C] TCA were carried out to measure the rates of biodegradation of TCA in four different soils from the Ballochbeatties site.

The specific objectives were to:

1) Quantify the rates of degradation of [1,2-^{14}C] TCA to $^{14}CO_2$.
2) Compare the rates of [1,2-^{14}C] TCA biodegradation in four different soil types from Scotland.
3) Compare the rates of [1,2-^{14}C] TCA biodegradation of soils sampled in Scotland with soils sampled in the Czech Republic.

The experiments described in the following section were conducted in March 2003 at the Institute of Experimental Botany within the Academy of Sciences of the Czech Republic in Prague, under the supervision of Dr. M. Matucha.

5.5.2 Methods

5.5.2.1 Soil characteristics

Due to practical limitations two separate, but theoretically identical, biodegradation experiments (I and II) were performed using two different soils from the Ballochbeatties catchment in each experiment (i.e. four soils in total). Soils were sampled on 5/3/03 and sieved (2 mm) on the same day and subsamples taken for TCA analysis by HSGC-ECD, and determination of soil water and organic matter contents (Chapter 2). The remaining soil was stored in the freezer (-30 °) until transportation to the Czech Republic, and then refrigerated (<5 °C) until required.

For Experiment I, soils from a Sitka forest, (O1; 10 – 20 cm depth), and a Larch forest, (12 – 34 cm depth) were selected and for Experiment II, soils from the same Sitka forest, but from a greater depth, (O2; 20 – 50 cm), and soil from the B-horizon of a moorland site were selected. Both Sitka O1 and Sitka O2 soils had markedly greater TCA concentrations, water contents and organic matter contents (when expressed as a % of solid matter) than the more mineral Larch and Moorland soils, as summarised in Table 5.12. Two different depths of Sitka soil were used so that comparisons could be made between TCA degradation rates in different parts of the soil profile.

Table 5.12. Summary of TCA concentrations (fwt and dwt), water and organic matter contents of soils from Ballochbeatties used for biodegradation studies of [1,2-^{14}C] TCA.

Soil type	Sampling depth / cm (and horizon)	Mean TCA concentration / ng g^{-1} (± S.D, n=3)		Water content (%)	Organic matter content (fwt) (%)	Mineral content (fwt) (%)
		Fresh weight	Dry weight			
Larch *peaty gley*	12 - 34 (B)	17.4 (1.7)	28.6 (2.8)	39.2	8.3	52.6
Sitka *basin peat*	10 - 20 (O_1)	287 (6.7)	2205 (52)	87.0	10.3	2.7
Sitka *basin peat*	20 - 50 (O_2)	85.4 (1.5)	586 (29)	85.4	11.2	3.4
Moorland *peaty podzol*	25 - 30 (B)	10.2 (2.1)	23.4 (4.9)	56.6	10.8	32.6

The soils used in this experiment had very different characteristics to the soils studied by Dr. Matucha's group in the Czech Republic, being generally much wetter and more organic.

5.5.2.2 Preparation of soil and application of [1,2-^{14}C] TCA

The biodegradation rates of the above soils were measured using [1,2-^{14}C] TCA with a specific activity of 3.7 GBq mmol^{-1} and radiochemical purity higher than 98 %. To obtain the appropriate concentration of $^{14}CO_2$ and isotopic dilution, prepared [1,2-

^{14}C] TCA was diluted by bi-distilled water or a non-radioactive standard of TCA to the required concentration (Matucha *et al.*, 2003). Soil experiments were conducted in duplicate at room temperature (22 ± 2 °C). Fresh soil (stored in the freezer after sample collection) was homogenised by sieving through a 2 mm mesh. Approximately 50 g was accurately weighed into a beaker and 0.5 ml of 11.61 μCi ml^{-1} (430 kBq ml^{-1}) [1,2-^{14}C] TCA was applied to the soil drop by drop using a pipette. Care was taken to distribute the solution evenly throughout the soil. The soil was then mixed manually for several minutes using a metal spatula and transferred into a 500 ml Erlenmeyer flask. The flask was connected as soon as possible to a continuous airflow (60 cm^3 min^{-1}), provided by an air pump (Stork *et al.*, 1997), and CO_2 absorption apparatus, as illustrated in Figure 5.15.

5.5.2.3 Sampling and radioactive determination of $^{14}CO_2$

A moistened continuous air-flow was passed through a CO_2 absorber (filled with 6 cm^3 1M potassium hydroxide, KOH to remove background CO_2) followed by the Erlenmeyer flask of soil itself and finally through two more absorbers for CO_2 (and $^{14}CO_2$) released into the ambient air from the soil, as illustrated in Figure 5.15.

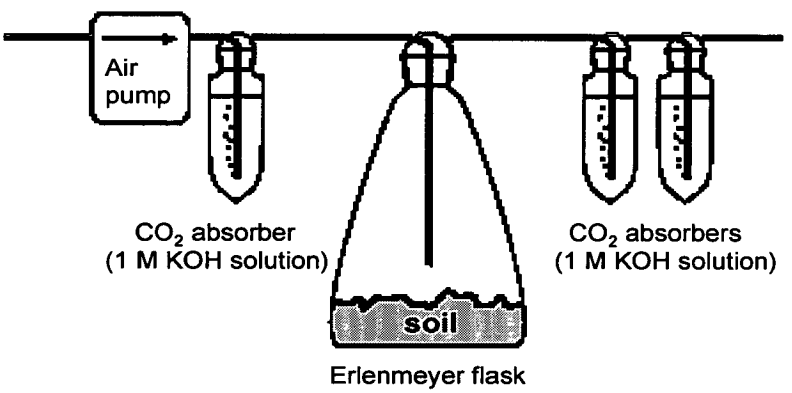


Figure 5.15. Apparatus used to study the biodegradation of [1,2-^{14}C] TCA in soil. Adapted from Schröder *et al.* (2003).

Samples of the two KOH solutions which absorbed the effluent CO_2 were taken after 1 hour, and then twice a day for 7 – 10 days, depending on the length of time required to reach a point where the rate of degradation changed very little between

sampling periods. Contents of the two absorbers were combined and 5 cm^3 of LKB (Loughborough, England) Optiphase "HiSafe" 3 scintillation cocktail was added to 1 cm^3 of KOH absorption solution. Solutions were analysed for ^{14}C using a liquid scintillation spectrometer (LSC) Beckman LS 6500 (Fullerton, Ca., USA).

At the termination of the biodegradation experiment the remaining ^{14}C in the soil was quantified so that the radioactivity balance could be determined. The soil was first oxidised using a Zinsser Analytic oxidiser, model OX-500 (Frankfurt am Main, Germany) and then analysed by LSC.

5.5.3 Results and discussion

5.5.3.1 Rates of $^{14}CO_2$ release (biodegradation) of [1,2-^{14}C] TCA

Figure 5.16 shows the rates of biodegradation (i.e. release of $^{14}CO_2$) of 215 kBq [1,2-^{14}C] TCA applied to 50 g of fresh soil, for the four soil types studied in Experiments I and II. Biodegradation rates are expressed as the radioactivity released per hour per gramme of fresh soil (Bq h^{-1} g^{-1}). For both experiments the data has been plotted for the first 7 days (168 hours) as the rates of biodegradation changed very little after this time.

The observed release of $^{14}CO_2$ shows that the applied [1,2-^{14}C] TCA was degraded by micro-organisms (i.e. biodegradation), where CO_2 is a waste product. Similar experiments by Forczek *et al.* (2001) did not detect any chloroform, carbon monoxide or methane during the experiment which implies that the only loss of radioactivity into the atmosphere was in the form of CO_2. The biological nature of this degradation was confirmed by an experiment in which the speed of degradation in sterilised (autoclaved) soil was less than 1 % of that observed in non-sterile soils (Matucha *et al.*, 2003).

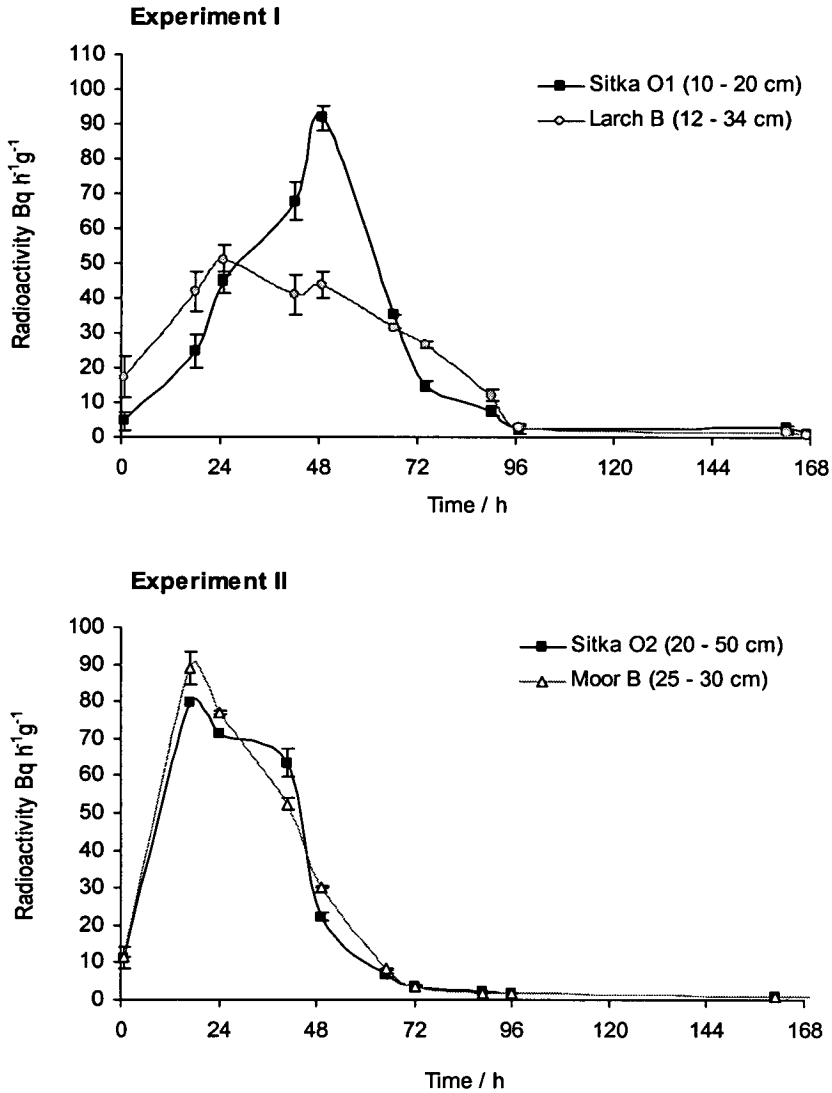


Figure 5.16. Rate of biodegradation of 215 kBq [1,2-^{14}C] TCA applied to 50 g of (I) Sitka O1 and Larch B soils, and (II) Sitka O2 and Moor B soils. Rates of biodegradation were measured as the release of $^{14}CO_2$ in Bq h^{-1} g^{-1} fresh soil. Error bars are standard deviations of two experimental replicates analysed in duplicate.

In these experiments the rates of radioactivity ($^{14}CO_2$) release varied with time and soil type, as shown in Table 5.13 which summarises the initial, maximum and final rates of biodegradation in the four different soil types.

Table 5.13. Comparison of the initial, maximum and final rates of [1,2-^{14}C] TCA degradation to $^{14}CO_2$ and the corresponding time after the start of the experiment (hours), for four different soil types.

	Rate of biodegradation ($^{14}CO_2$ release) of [1,2-^{14}C] TCA in fresh soil / Bq h^{-1} g^{-1}					
Soil type	Initial rate	Time (days)	Maximum rate	Time (hours)	Final rate	Time (hours)
Sitka O1 (10 – 20 cm)	4.6	0	91.7	49	0.9	167
Larch B (12 – 34 cm)	17.3	0	50.9	25	0.7	167
Sitka O2 (20 – 50 cm)	11.1	0	79.7	17	1.1	161
Moor B (25 – 30 cm)	11.6	0	88.8	17	0.9	161

For each soil in both Experiments I and II the rate of release in radioactivity (Bq $h^{-1}g^{-1}$ of fresh soil) increased to a peak, then decreased fairly rapidly to a low constant value. The soils in Experiment I took the longest to reach a maximum rate of radioactivity release, with the Larch soil reaching a maximum of 51 Bq $h^{-1}g^{-1}$ after 25 hours and the Sitka O1 soil reaching a greater maximum of 92 Bq $h^{-1}g^{-1}$ after 49 hours. In Experiment II both the Sitka O2 and Moor B soils reached a maximum after only 17 hours, with peak radioactivities of 80 Bq $h^{-1}g^{-1}$ and 89 Bq $h^{-1}g^{-1}$ respectively. As the mean radioactivity release was calculated over a number of hours, the exact time and magnitude of maximum radioactivity could not be quantified. The greatest rate of increase of biodegradation rate was shown by Sitka O2 and Moor B soils, as the radioactivity increased by 77 and 69 Bq $h^{-1}g^{-1}$ respectively in 16 hours. This implies that there was a rapid increase in population of micro-organisms, which reached a maximum then started to decline, possibly as the introduced source of carbon (TCA) became limited. Poor aeration is unlikely to have been an influential factor as there was a continuous moistened air flow passing into the flasks throughout the experiment. Also, during sampling the soils were inevitably disturbed, and therefore more aerated than they might naturally be. The initial rates of increase in radioactivity from both the Sitka O1 and Larch B soils were slower than the Sitka O2 and Moor B but so were the subsequent rates of decrease, with a more constant plateau being reached after 96 hours instead of 72

hours. This suggests that the overall process of biodegradation is slower in Sitka O1 and Larch B soils, possibly due to less favourable conditions for micro-organisms.

The difference in the shapes of the degradation curves of the Sitka O1 and O2 soils is surprising as both had very similar water and organic matter contents and therefore would be expected to support similar populations of micro-organisms. However, the quality of the organic matter may differ between the O1 and O2 horizons and it may be this, rather than the total mass of organic matter, which is important in determining the size and type of microbial community and rates of biodegradation of TCA. According to the soil characteristics shown in Table 5.12 the Moor B soil may be expected to be most favourable for microbial activity as the relatively low water content would induce more aerobic conditions and the higher mineral content would provide a more nutrient-rich environment. Matucha *et al.* (2003) reported that the water content of soil influences the rate of TCA degradation. In their experiments using soils of different water contents, the lowest TCA degradation rates were observed in soils with highest (79 %) and lowest (22 %) water contents. In this experiment the Sitka soils were the wettest (> 85 % water) with the highest dry organic matter and lowest mineral contents. Another possible explanation for the difference in biodegradation between the two Sitka horizons is that the Sitka O2 soil was more anaerobic in field conditions but once it had been handled and prepared for the experiment it became aerobic. This may have resulted in a rapid increase in population of colonising aerobic micro-organisms. The differences between soils from different sites (Sitka, Larch, Moor) may be explained by different mineral contents and chemical composition of organic matter which are more or less available for micro-organisms to utilise.

It cannot be determined from these experiments whether a number of microbial populations of different species are involved in TCA breakdown, or if one specific species is stimulated which utilises TCA as its sole source of carbon. It is debatable whether or not the absolute mass of TCA applied to soil in these experiments can cause the accumulation of a specific soil microflora responsible for the

biodegradation process, as it does when higher masses of energy rich organic compounds are available (Siciliano *et al.*, 2001).

5.5.3.2 Radioactivity remaining in soil

The percentage radioactivity remaining in the soil was calculated by subtraction of radioactivity released as $^{14}CO_2$ from total initial radioactivity added in the form of [1,2 -^{14}C] TCA, as shown in Figure 5.17 for Experiments I and II.

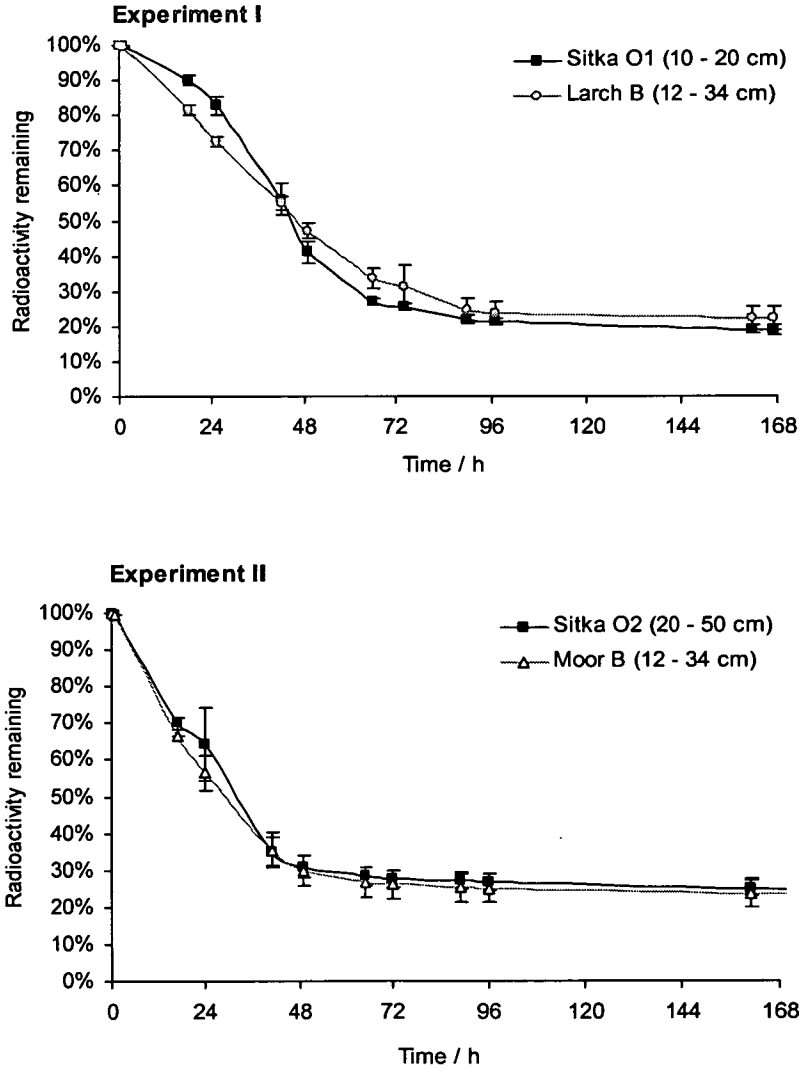


Figure 5.17. Calculated percentage of radioactivity remaining in Sitka O1 and Larch B soils (Experiment I) and Sitka O1 and Moor B soils (Experiment II) over 7 days following the application of 215 Bq [1,2-^{14}C] TCA to 50 g fresh soil. Error bars are standard deviation of two experimental replicates analysed in duplicate.

The most important observation is that not all of the radioactivity applied to the soil as [1,2-^{14}C] TCA was accounted for by the released $^{14}CO_2$. After 97 hours in Experiment I, and 72 hours in Experiment II, the decrease in rate of $^{14}CO_2$ release between sampling occasions was very small (< 1 %). After 167 and 161 hours for Experiments I and II respectively there was still significant radioactivity remaining in the soil (19 – 23 %) which suggests that some of the applied [1,2-^{14}C] TCA was not degraded to $^{14}CO_2$. In a similar experiment, Forczek *et al.* (2001) concluded that radioactivity "loss" could be accounted for by [1,2-^{14}C] TCA remaining in the soil which was unavailable to micro-organisms.

If it is assumed that all the remaining radioactivity in the flask is associated with the soil and is present as [1,2-^{14}C] TCA, and not another conversion product of TCA, then estimations of the half-life of TCA in soil can be made from Figure 5.17, which are summarised in Table 5.14.

Table 5.14. Estimations of the half-life of [1,2 ^{14}C] TCA in four different soil stored in flasks at room temperature (approximately 22 °C).

Soil type	Half-life / hours
Sitka O1	45
Sitka O2	45
Larch B	46
Moor B	21

These half-lives of TCA in soil are shorted than those reported from past herbicidal studies of several weeks (summarised in Foy, 1975). The TCA concentrations applied to the soils used in this study (~ 380 ng g^{-1} (fwt)) are up to 3 orders of magnitude lower than those used in herbicidal applications and are therefore likely to be removed more quickly. Furthermore, it is possible that herbicidal application concentrations of TCA were more likely to have toxic effects on soil micro-organisms resulting in a decrease in microbial activity and therefore a decrease in the capacity of the soil to remove TCA.

5.5.3.3 Radioactivity balance

To ensure that all of the applied radioactivity could be accounted for at the termination of the experiment, the radioactivity of soil residues was measured, and compared in Figure 5.18 with the calculated percentages of radioactivity remaining shown in Figure 5.17.

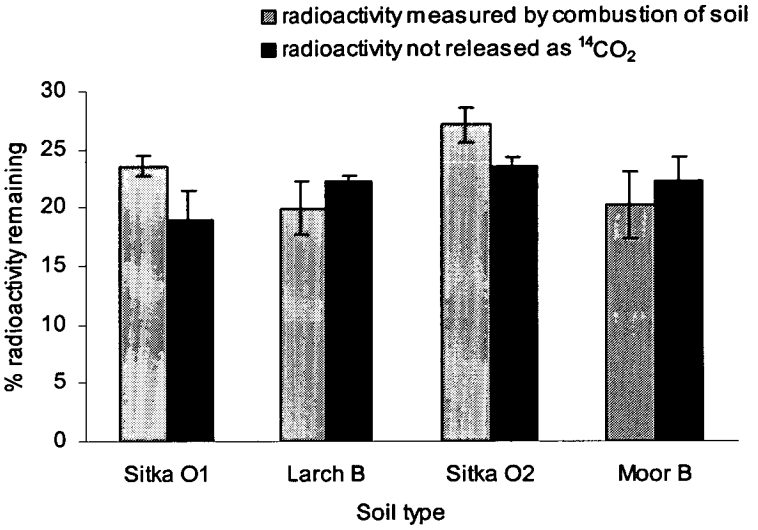


Figure 5.18. Radioactivity remaining in the soil at the end of the experiment, determined using two methods: (a) direct analysis of final radioactivity in soil by combustion followed by LSC, and (b) subtraction of radioactivity released as $^{14}CO_2$ from the total radioactivity present in the soil at the start of the experiment. Error bars are standard deviations of two replicate experiments, each analysed in duplicate.

There was excellent agreement between the results of direct measurement of remaining ^{14}C radioactivity and the calculated values which confirms that CO_2 was the only volatile degradation product of TCA and that all labelled TCA was satisfactorily accounted for. The greatest discrepancy was observed for Sitka O1 soil and, considering the individual the error associated with each method (shown as standard deviation of 2 replicates in Figure 5.17) over a time scale of over 7 days this gives sufficient confidence in the method to assume that all of the applied radioactivity has been accounted for.

It is possible that the radioactivity remaining in the soil was not present as [1,2-^{14}C] TCA and was in fact converted to another non-gaseous compound in which the ^{14}C

was still incorporated. To confirm this, the soil could be analysed for TCA using a standard method of analysis. However the presence of "natural" TCA intrinsic to the soil would have to be accounted for in the results, especially if the soil has high TCA concentrations. The variability in soil TCA concentrations described in Chapter 4, as well as the debate over which method should be used for TCA analysis mean that it is difficult to draw conclusions on the form of ^{14}C present with any level of confidence.

The differences in biodegradation rate observed between Experiments I and II suggest that the behaviour of the soils may differ between experiments as well as between soil types. The reason for this is not clear as the soils for both experiments were prepared in exactly the same way and the experiments were run under identical conditions. None of the soils were exposed to TCA prior to the experiment which eliminates the possibility of micro-organisms being conditioned to degrade TCA. Although there is clear evidence that TCA applied to soil is degraded by micro-organisms, to $^{14}CO_2$, this was for one particular dose of TCA. Application of 215 kBq of [1,2-^{14}C] TCA to 50 g of soil is approximately equivalent to a soil concentration of 380 ng g^{-1} (fwt), in addition to the TCA intrinsic to the soil. This is at the upper range of TCA concentrations detected in Ballochbeatties soil and two orders of magnitude greater than the TCA concentrations detected in natural precipitation or throughfall. Matucha *et al.* (2003) reported that degradation of TCA in soil is dependent on the concentration added. They observed the highest relative degradation rate (%) in soil with the lowest application of TCA (42 μg TCA to 100 g soil). and the highest absolute degradation rate (μg kg^{-1} h^{-1}) in soil with the highest application of TCA (883 μg TCA to 100 g soil). They also found that degradation rates did not increase in a linear manner with increase in TCA concentrations which may be due to the limited biodegradation capability or to inhibition of microbial activity at high concentrations. These experiments have demonstrated that TCA degradation in soil by micro-organisms is a significant process and may be important for quantifying TCA input and output fluxes in the environment as a whole. Nevertheless, degradation of TCA intrinsic to soil at trace concentrations has still not been proved.

5.6 LABORATORY STUDIES OF TCA BEHAVIOUR IN SOIL

5.6.1 Effect of contact time with soil on TCA concentrations of aqueous solutions

5.6.1.1 Introduction

Previous soil lysimeter and core experiments in this chapter have shown that TCA applied to soil is removed from the soil system to varying extents and that the removal process is dominated by degradation to CO_2 by soil micro-organisms. There is evidence that TCA behaves differently in different soils and, although it was proposed that the presence of a litter layer may have affected rates of TCA degradation in the soil, when water or TCA solution was allowed to freely drain through the soil, no differences in recovery of TCA in the leachates were observed between Sitka soils with or without a litter layer. Results from soil core experiments indicated a possible effect of contact time of TCA with soil on the recovery in the soil leachate. An experiment was therefore conducted to investigate the effect of contact time with soil on TCA removal from solution.

Aims

A simple laboratory storage experiment was set up to investigate further, at a basic level, the differences in behaviour of TCA in solution between sterile and non-sterile soils of different types.

The specific objectives were:

1. To determine if TCA intrinsic to the soil can be leached out by prolonged contact with ultrapure water and if there is a difference between soil types.
2. To determine if the TCA concentration of a solution of known TCA concentration changes after prolonged contact with soil and to see if there is a difference between soil types
3. To determine if TCA concentrations in either ultrapure or TCA solutions differ between sterile and non-sterile soils after prolonged contact, and to determine from this if TCA still "disappears" in the absence of micro-organisms.

5.6.1.2 Methods

Three different soils from Ballochbeatties were sampled on 24/1/03 for this investigation: Larch B horizon, Sitka litter layer ("Sitka L") and Sitka O1 horizon ("Sitka O"). This allowed the differences between soil types, as well as the presence or absence of a litter layer in the Sitka soils, to be investigated. Soils were sieved through 2 mm mesh on the day of collection and stored in the freezer at -30 °C until required. A portion of each soil was sterilised by gamma radiation, as described in Section 5.4.2. Approximately 5 g of sieved fresh soil was accurately weighed into a plastic sampling vial and either 25 ml of ultrapure water (Experiment I), or 25 ml of approximately 20 $\mu g\ l^{-1}$ TCA solution (Experiment II) were added using a pipette. The mixtures were shaken by hand and then left to settle. Three replicates were prepared for both sterile and non-sterile samples of each soil type. The supernatant was removed from the soil-water mixture with a 5 ml pipette and deposited directly in a headspace vial for analysis of TCA using HSGC-ECD as described in Chapter 2. Water solutions were analysed for TCA after 13, 20 and 31 days and TCA solutions were analysed after 2, 8 and 17 days. Samples were stored in the dark in the refrigerator until analysis for TCA.

5.6.1.3 Results and discussion

The results for the ultrapure water solutions (Experiment I) and TCA solutions (Experiment II) will be discussed separately. In both cases the initial "applied" TCA concentration of the solution was calculated taking into account the total water content of the fresh soil as well as the volume of liquid added. It was assumed that any water intrinsic to the soil would mix with the added water. This is not likely to have significantly altered the TCA concentrations of the water solutions in Experiment I as the magnitudes of TCA concentrations were expected to be similar. However, the applied TCA solutions in Experiment II were assumed to be diluted by the water already present in the soil, resulting in slightly lower net TCA concentrations. The "measured" TCA concentration refers to the actual concentration measured after different storage times with ultrapure water or TCA solution.

Experiment I: Soil immersed in ultrapure water

Figure 5.19 shows the TCA concentrations measured in ultrapure water stored with Larch, Sitka L and Sitka O, sampled over time. Due to the large error associated with all of these measurements there is no statistical evidence for any differences between the soil types, sterile or non-sterile treatments or the time of analysis. Even so, there are still some trends that may be observed.

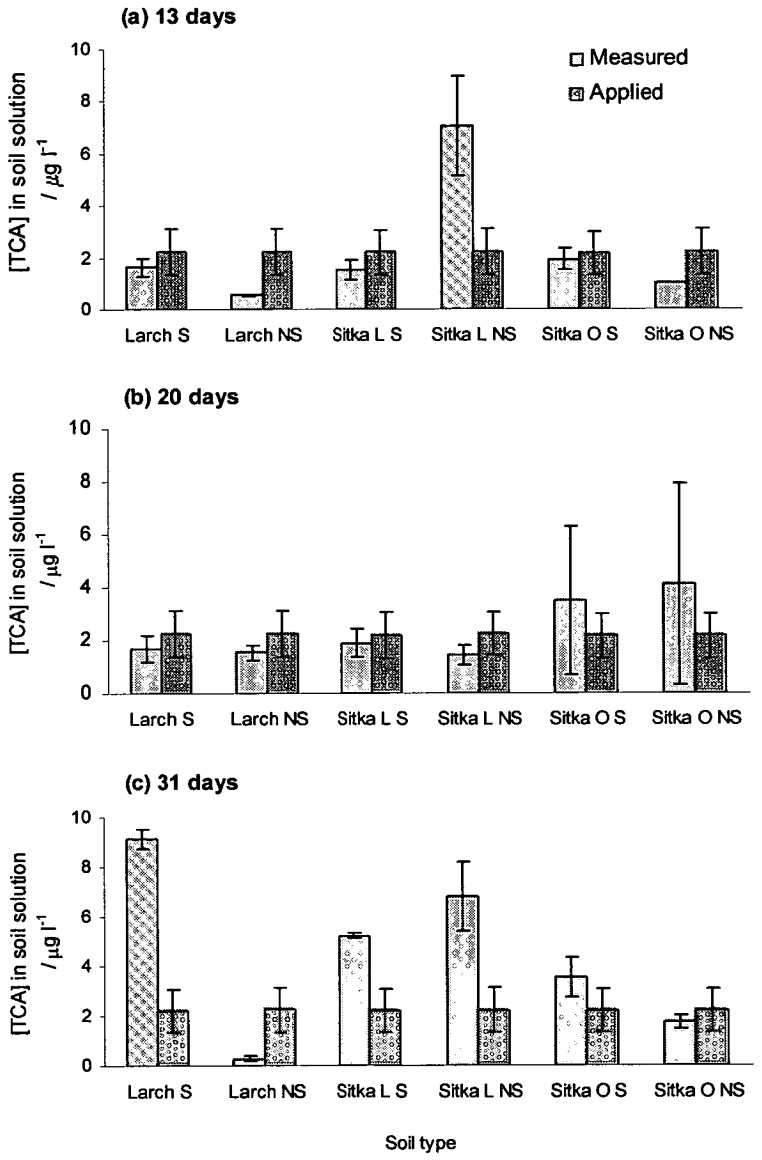


Figure 5.19. TCA concentrations in ultrapure water stored with sterile (S) and non-sterile (NS) Larch, Sitka litter (L) and Sitka O soils for (a) 13 days, (b) 20 days and (c) 31 days. Error bars are standard deviations of one sample analysed in triplicate.

After 13 days the measured TCA concentration was lower than the applied TCA concentration (2.2 $\mu g\ l^{-1}$) for all samples except for the non-sterile Sitka litter layer. This suggests that the TCA in the applied solution was degraded or bound to the soil and indicates that, at this point, intrinsic soil TCA had not been leached out. After 20 days the same trend is apparent but this time both the sterile and non-sterile Sitka O soils show a greater TCA concentration in the measured solution than the applied solution although these have particularly high standard deviations associated with them. After 31 days the trend seems to reverse slightly and in the sterile Larch, Sitka L and Sitka O soils and the non-sterile Sitka L soil, the measured TCA concentration was up to 4 times greater than the applied TCA solution. If this observation is genuine, it suggests that either more TCA has been leached out of the soil, or that natural production has become more prominent, perhaps as micro-organisms involved in the production process have adapted to the artificial introduction of TCA resulting in a population increase.

Experiment II: Soil immersed in TCA solution

The initial solution applied to the soils ($t = 0$ days) had a TCA concentration of 25.2 ($\pm$ 1.1) $\mu g\ l^{-1}$, which was corrected to approximately 22 $\mu g\ l^{-1}$ after the contribution from intrinsic soil solution was taken into account. The TCA concentrations of solutions analysed at subsequent time intervals are shown in Figure 5.20.

Solutions sampled and analysed ("measured") after 2 days had greater TCA concentrations than the initial solution added to the soil ("applied"), except for non-sterile Sitka O, where the measured TCA concentration was lower than that of the applied solution. After 8 days the results differed between soils, with solutions from sterile Larch soil and sterile and non-sterile Sitka L soil still having greater TCA concentrations than the applied solution. It is clear that in the non-sterile Larch and Sitka O soils the solutions become significantly less concentrated in TCA over time compared to the applied solution. This suggests that TCA has been microbially degraded over time. After 17 days all the solutions in non-sterile soils had significantly lower TCA concentrations. This is shown more clearly in Figure 5.21

where the "measured" TCA at 2, 8 and 17 days, is expressed as a percentage of the applied TCA concentration.

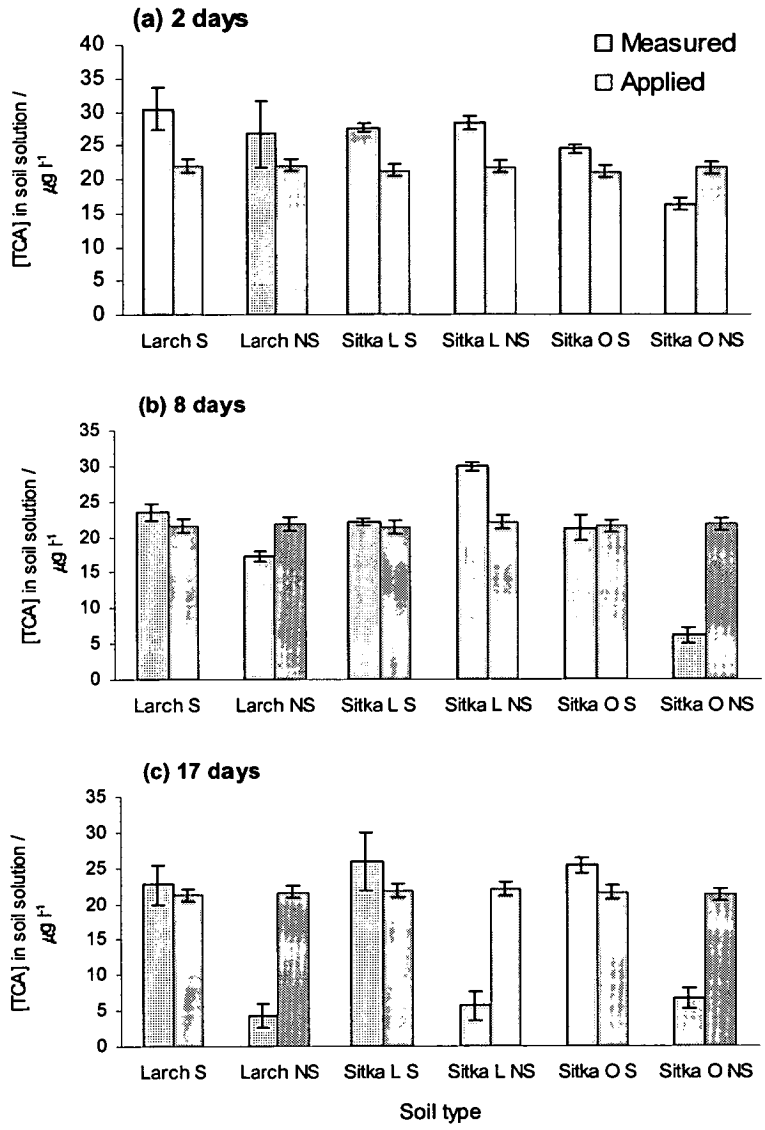


Figure 5.20. Measured TCA concentrations in TCA solution stored with sterile (S) and non-sterile (NS) Larch, Sitka litter (L) and Sitka O soils for (a) 2 days, (b) 8 days and (c) 17 days. Error bars are standard deviations of one sample analysed in triplicate.

After 17 days the percentage of applied TCA measured in solutions varied between 20 and 31% in the non-sterile soils and between 107 and 119 % in the sterile soils. The mean TCA concentrations of measured solutions after 2 days were significantly

lower than those measured at 17 days (General Linear Model, $P<0.05$) in both sterile and non-sterile soils but there was no significant difference between soil type on either occasion.

Between 2 and 8 days, measured TCA concentrations decreased the most in the non-sterile Sitka O soil, from 16 to 6 μg l^{-1}, but did not decrease over the next 9 days implying that in Sitka O soil, biodegradation occurs immediately and rapidly. In contrast, in the Larch and Sitka L soils biodegradation occurs more gradually and is not stimulated as quickly in the first instance. The solution in the Larch soil had the lowest final TCA concentration at 17 days, of 4 μg l^{-1} which was a decrease of 22 μg l^{-1} over 15 days. The TCA concentrations of solutions in sterile soils remained in the range 21 – 30 μg l^{-1} throughout the whole experiment, providing further evidence that the poor recovery of TCA in the measured solutions of non-sterile soils must be associated with microbial activity. Statistical analysis of all using the General Linear Model showed that TCA concentrations of solution stored with sterile soils were significantly greater ($P<0.001$) than with non-sterile soils.

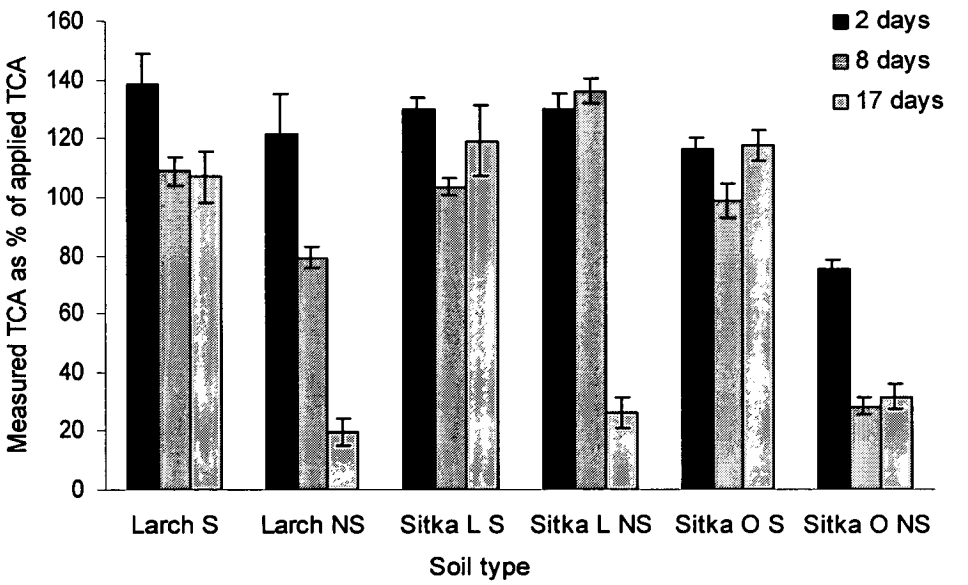


Figure 5.21. TCA concentration of TCA solution (= "Measured") after storage with soil for 2, 8 and 17 days, expressed as a percentage of the initial TCA concentration. Error bars are relative standard deviations of one sample analysed in triplicate.

One factor which could not be monitored throughout this experiment was the availability of oxygen within the storage vials. It may be assumed that up to 2 days any biodegradation was aerobic, but after 8 and 17 days it is possible that the soils and the solutions became anaerobic. Therefore different species of micro-organisms may be responsible for TCA degradation at the end (17 days) compared to those at the start of the experiment (2 days).

It is not clear why the initial concentrations of nearly all the measured solutions were markedly greater that those of the applied solutions. As the same trend was observed in sterile and non-sterile soils it is not likely to be due to TCA production by micro-organisms. It is possible that the introduction of "new" water to a natural soil stimulates the chemical production of TCA and/ or chloroform, or another compound that produces chloroform on heating between 60 and 100 °C.

5.6.2 Addition of ultrapure water or sodium chloride solution to "TCA-free" soils

5.6.2.1 Introduction

This chapter has so far focused on the degradation of TCA in soil, particularly by micro-organisms. Although earlier experiments (Section 5.5) showed indications of an increase in detected TCA which was stimulated by the addition of aqueous solution to fresh soils, no study has conclusively shown that TCA may be produced in soil. It is possible that within experiments where the degradation of TCA in soil by micro-organisms has been unquestionable, TCA has also been produced, but at a slower rate than degradation and has therefore not been identified. If so, this would mean that degradation rates of TCA in soils in the field are faster than estimated in the experiments reported in this chapter, as the observed degradation of TCA may actually be the net rate of loss after accounting for TCA produced in the soil. However, all the evidence of biodegradation of TCA in soil has been based on adding solutions of known TCA concentration to soil and monitoring the recovery of TCA either in leachate, or as radioactive $^{14}CO_2$. The degradation of TCA has not been observed in experiments in which ultrapure water is added to soil (Section

5.6.1) nor in field lysimeters fed by precipitation or forest throughfall (Section 5.2), and it is still not clear how TCA already "naturally" present in the soil behaves.

Several researchers have postulated that TCA may be naturally produced *in-situ* in the soil (Hoekstra *et al.*, 1995, 1999a; Niedan *et al.*, 2000), although this has not been demonstrated in the field. It has also been suggested that the concentration of chloride ions in the soil may influence the rate of TCA production (Haiber *et al.*, 1996; Fahimi *et al.*, 2003). This may be one explanation for the higher soil TCA concentrations reported in Scotland, which has a maritime climate with significant NaCl deposited to the terrestrial environment from the atmosphere.

An experiment was conducted to test the hypothesis that TCA may be produced in "TCA-free" soils in the presence of ultrapure water or water enriched with NaCl.

Aims

The main aim of this experiment was to see if TCA can be produced in artificially created "TCA-free" soils. Assuming natural production of TCA is observed, the specific additional objectives were:

1) To determine how the rates of TCA production differ between soil types.
2) To determine if TCA production is more evident in soil re-wetted with NaCl solution than soil re-wetted with ultrapure water.
3) To determine if TCA production in soil is influenced by length of contact time of applied ultrapure water or NaCl solutions.

5.6.2.2 Methods

Soil was collected from a Moorland B horizon, Sitka organic horizon (10 – 30 cm) and Larch organic horizon at Ballochbeatties on different dates, sieved (2 mm mesh) and stored in the freezer until use in this experiment. Each soil was placed in a Pyrex beaker and dried at 60 °C for 8 days followed by 100 °C for 2 hours to remove any intrinsic TCA or chloroform. The moisture contents of each soil were also

determined. Ultrapure water used in the analyses and experiments was boiled before use to remove any traces of TCA or chloroform.

NaCl solution was prepared with ultrapure water to a concentration of 7.6 mg l^{-1} which lies within the range of NaCl concentrations reported in natural rain and cloudwater in the UK (Reynolds and Pomeroy, 1988; Robson and Neal, 1996; Neal *et al.*, 1997). Dried soil (4 – 5 g) was accurately weighed into plastic sample vials and either ultrapure water or NaCl solution was added and the vial sealed. The volume of liquid added to each soil (5.8 ml to Moor soil, and 12.6 ml to Larch) was calculated from the mass lost during drying. However, the dried Sitka soil was quite hydrophobic and did not easily absorb the applied solution, so only 10 ml was applied to each replicate (12.8 ml less than the volume lost through drying). Six replicates for each treatment (water and NaCl) and soil (Moor, Larch, Sitka) combination were prepared; three replicates were stored in the refrigerator in the dark and three in the laboratory in the light at room temperature. Soils stored in the laboratory were analysed for TCA after 1, 28 and 54 days and those stored in the fridge were analysed after 30 and 54 days. It was assumed that the initial (after 1 day) TCA concentrations of soil stored in the fridge would be similar to those of soil stored in the laboratory.

5.6.2.3 Results and discussion

Although the soils were re-hydrated with volumes of solution calculated to re-create the original water content of the fresh soil (except for Sitka), it is inevitable that natural properties of soil were irreversibly altered during the drying and re-hydration processes. The TCA concentrations of re-hydrated soils after 1, 28 and 54 days' storage in the laboratory and after 30 and 54 days' storage in the fridge are shown in Figures 5.22 a and b respectively.

In all re-hydrated soils it is evident that TCA concentrations were greater than zero and hence, in these experiments, TCA was formed during the process of re-wetting with water or NaCl solution. The stimulation of soil microbial activity by re-wetting is a well-known phenomenon (Vinten and Smith, 1993). This strongly implies that

the presence of TCA is associated with microbial activity and may therefore be evidence for the natural production of TCA in soils by micro-organisms. Alternatively, the chloroform being detected by HSGC-ECD may be formed from a compound other than TCA, as discussed in Chapter 3.

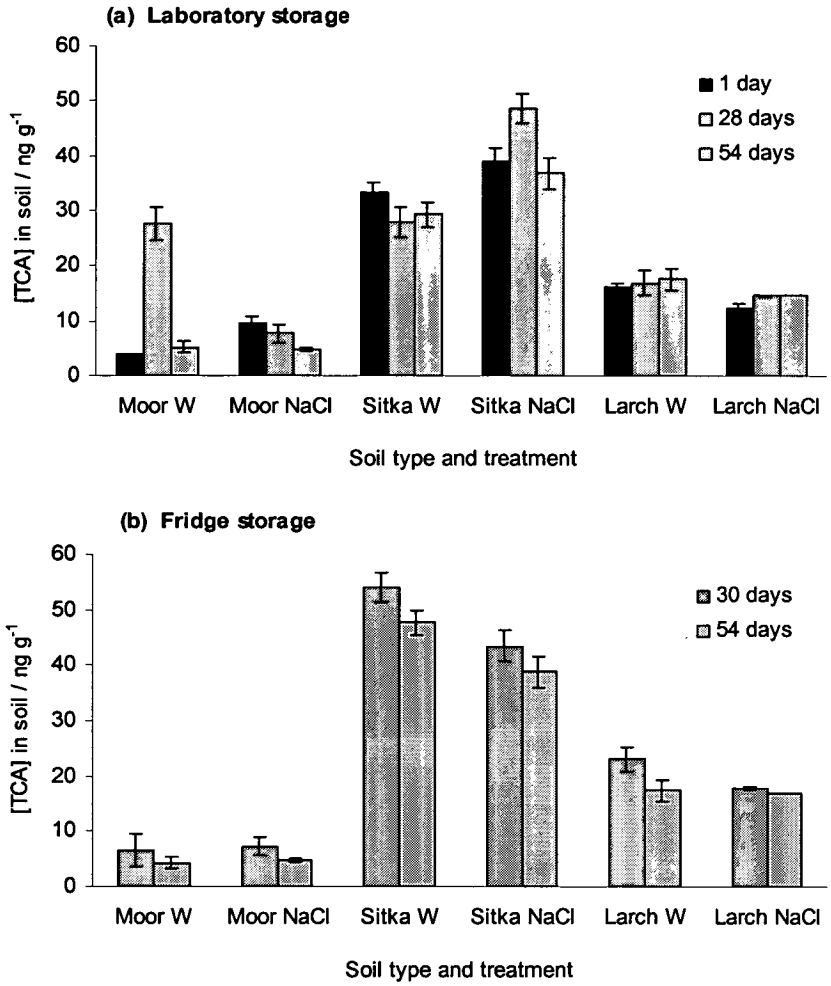


Figure 5.22. TCA concentrations of dried "TCA-free" soils re-hydrated with water (W) or sodium chloride (NaCl) and stored in (a) the laboratory or (b) the refrigerator for different lengths of time. Error bars are standard deviations of one sample analysed in triplicate.

On every occasion, for both water and NaCl additions, TCA concentrations of soils stored in the laboratory (Figure 5.22a) were greatest in the Sitka soil where up to 37 ng g^{-1} (fwt) of TCA was detected after 1 day of storage in the laboratory. This is

approximately a total of 980 ng of TCA. In Larch soil up to 16 ng g^{-1} (fwt) TCA (280 ng TCA in total) and in Moor soil up to 9.5 ng g^{-1} (fwt) TCA (100 ng in total) was detected after 1 day. These soil TCA concentrations are comparable to the mean TCA concentrations detected in fresh soils from the same sites and depths at Ballochbeatties, reported in Chapter 4, with 60 (± 80) ng g^{-1} (fwt) TCA detected in Sitka soils (excluding the litter layer), 16 (± 7) ng g^{-1} (fwt) in Larch soils and 13 (± 4) ng g^{-1} (fwt) in lower moorland soils. This suggests that each soil reaches a different steady state between production and degradation processes.

Similar rates of TCA production and similar maximum TCA concentrations were reached in soils stored in the fridge in the dark, re-wetted with either water or NaCl solution, as those stored in the laboratory. In addition, the same general trends were observed between soil types. From this it is apparent that TCA production in these soils occurs to a similar extent at <5 °C in the dark (fridge) or 22 °C in the light (laboratory). This may because, despite the different storage conditions, all replicates of each soil type initially had the same physical, chemical and biological characteristics and therefore most likely supported the same microbial communities. In the field, differences in environment (including temperature and available light) are likely to have an influence on soil formation over a much longer period of time, resulting in the colonisation of different species of micro-organisms which may produce TCA at different rates.

If the TCA detected in the soils in these experiments was chloroform produced chemically from intermediates in these dried soils on re-wetting, it can only have formed from compounds which were introduced in the liquid at trace concentrations (unlikely in ultrapure water), and not from the dried soil which should have been free from any such compounds.

In general, TCA concentrations in re-wetted soils were similar over the whole experiment which implies that any production of TCA (or chloroform) occurs almost instantaneously, or that it is being continually produced and degraded at similar rates. In Figure 5.22 (b) there appears to be a trend of lower TCA concentrations after 54

days compared to 30 days' storage, but this was not statistically significant (2-way ANOVA). There were no significant differences in TCA concentration between soils re-wetted with water or NaCl which implies that at the concentrations used in this experiment (7.6 mg l^{-1}), chloride ions do not have any effect on TCA production in soil. The Ballochbeatties catchment has a moist maritime climate and therefore it is likely to have a significant annual atmospheric input of chloride. In this experiment, it may have been more informative to compare Ballochbeatties soils with soils from areas with a more continental climate where soils are known to have low chloride concentrations. It would also be interesting to compare the chloride concentrations of different soil types within the Ballochbeatties catchment to see if there is a relationship between soil chloride and TCA concentrations.

Although the method used to create "TCA-free" soil by heating and drying the soil to remove TCA, followed by re-wetting, is artificial, there is no doubt that there was a significant increase in detected chloroform in the soil, which was most probably produced from decarboxylation of TCA naturally formed in soil. The extent of this "TCA" formation was not influenced by the concentration of chloride ions.

5.7 CHAPTER CONCLUSIONS

The experiments reported in this chapter have increased the understanding of TCA behaviour and rates of production and degradation processes in different soil types. It has been confirmed that TCA added to soil is degraded by micro-organisms, and that this degradation is immediate and rapid. There is also some evidence of TCA production which shows that TCA may be actively cycled within the soil. The main findings are summarised below.

TCA degradation in soil

- Field lysimeter experiments demonstrated that over a 6-month experimental period soil TCA input and output fluxes were in balance and there was neither net accumulation nor net loss of TCA in the soil environment. This raises the question of why the soil store of TCA is so large, if TCA is not accumulating from external inputs.
- Less than 30 % recovery of TCA was generally observed in leachates from soil lysimeters dosed fortnightly with 80 or 200 $\mu g\ l^{-1}$ TCA solution (total of 20 μg or 50 μg of TCA). Clearly, TCA applied to soil artificially and at higher concentrations than found in natural precipitation or throughfall waters, behaves differently to TCA already intrinsic to the environment.

- Experiments involving addition of TCA to soil from a Sitka spruce forest with or without a litter layer also confirmed TCA loss in the soil system. Since no difference in the proportion of TCA loss was observed between soil with or without a litter layer, it can be concluded that, in this particular soil, litter does not have an increased retention capacity of TCA. It therefore does not explain either the observed "loss" of TCA or the high TCA concentrations detected in Sitka litter layers. However, in experimental conditions soil and litter horizons were treated the same whereas in the field the litter horizon is dried and rewetted more often than lower horizons which may have an effect on TCA cycling.

- The role of micro-organisms in removing TCA from the soil system (i.e. "biodegradation") was confirmed in a controlled experiment in which 4 μg TCA

was applied once to sterile and non-sterile soil lysimeters. After 23 days nearly 60 % and over 90 % of added TCA was recovered from sterile Sitka and Larch soils, respectively, compared to only approximately 10 % and 20 % in non-sterile Sitka and Larch soils respectively.

- Biodegradation rates of TCA in soil were quantified in experiments where [1,2-^{14}C] TCA ($\approx$ 380 ng g^{-1} (fwt)) was added to soil in a sealed experimental system. Degradation to $^{14}CO_2$ was immediate and rapid, and after 2 – 4 days degradation had almost completely ceased. Approximate half-lives of the applied [1,2-^{14}C] TCA ranged from 21 to 46 hours in four different soils, but are likely to be dependent on the initial TCA concentrations as well as soil properties (pH, water content, organic matter content, initial soil microbial biomass concentration, C:N ratio) and experimental conditions such as humidity of the airflow and temperature. The initial TCA concentration of the soil and whether or not it has previously been exposed to TCA (soil "conditioning") may also affect the numbers and species of micro-organisms present and hence, the absolute rate of TCA degradation.

TCA production in soil

- Laboratory experiments have provided tentative evidence for TCA production in soil. TCA concentrations of ultrapure water increased after being stored with soil in a sealed vial for 2 days, although this may have been due to the stimulation of micro-organisms by re-wetting of the soil.

- There was evidence of TCA production in "TCA free", dried soils which were re-hydrated with either ultrapure water or NaCl solution. Sitka soils showed the greatest initial increase in TCA concentration (37ng (g^{-1} fresh weight soil)) followed by Larch (16 ng (g^{-1} fresh weight soil)) and Moorland soil (9.5 ng (g^{-1} fresh weight soil)), after one day. These TCA concentrations are of a similar magnitude to corresponding soils analysed fresh from the field. This suggests that, in stable conditions, TCA inputs and outputs in the soil will reach a steady state which, if perturbed, will eventually return to the same steady state. There

was no difference in TCA concentrations between soils re-hydrated with ultrapure water or NaCl solution suggesting that, under these experimental conditions, higher concentrations of chloride ions do not result in greater production of TCA.

Although the soil TCA input fluxes via precipitation and forest throughfall and the output fluxes by percolation through the soil have been closely investigated in field and laboratory conditions, most of the experiments have involved the addition of known masses of TCA. Cycling of TCA "naturally" present in the environment is much more difficult to quantify and it is likely that both soil and TCA interact with each other differently at lower TCA concentrations. The heterogeneity of soil and the observed spatial variability of intrinsic soil TCA, as well as the trace concentrations present in input waters (precipitation, cloudwater and forest throughfall) and output waters (soil leachate) make it difficult to distinguish "real" changes in TCA concentration from natural variability.

Results from experiments in this chapter suggest that the most important controls on TCA concentrations in soil are internal processes of production and degradation, the magnitudes of which are driven by the suitability of different soil type for supporting microbial biomass. The aqueous inputs and outputs of TCA to and from soil are fairly insignificant for soil TCA concentrations.

The role of vegetation in the TCA cycle as a potential source and, in particular as a removal route, has not yet been discussed in this research. In Chapter 6 the routes of uptake of TCA (including the role of soil TCA) by Sitka spruce saplings, its behaviour in the sapling and effects on sapling health are investigated in detail.

Chapter 6 – TCA Cycling in Sitka Spruce Saplings

6.1 INTRODUCTION

Since TCA has herbicidal effects against woody plant species (Barrons and Hummer, 1951), reports of correlations between the TCA content of needles and the extent of defoliation have led to the suggestion of a cause-effect link (Frank, 1991; Norokopi and Frank, 1995). Many studies, both field based and controlled experiments, suggest that exposure to TCA has a negative influence on tree health (Frank *et al.*, 1990; Plümacher & Schröder, 1994; Norokorpi & Frank, 1995; Sutinen *et al.*, 1997; Weiss *et al.*, 2000). Effects are complex and may range from direct physical effects on tree growth to more subtle effects on tree physiological functioning. There is particular concern regarding the effects of TCA exposure on coniferous trees because TCA may accumulate in foliage to phytotoxic levels over several years.

TCA concentrations in conifer needles are reported to range from <1 to 180 ng g^{-1} in rural forests (Frank, 1991; Frank *et.al.*, 1992; Plümacher and Renner, 1993; Plümacher and Schröder, 1994; Norokorpi and Frank, 1995; Juuti *et al.*, 1996; Weissflog *et al.*, 1999; Stidson *et al.*, 2004a). The origin of TCA in needle foliage is not clear, and postulated routes of uptake and relative rates have not been well-quantified. The presence of TCA in conifer needles in rural areas implies that potential TCA precursor compounds may be transported long distances. Fluxes of TCA in atmospheric precipitation significantly exceed estimates of its production via photo-oxidation reactions of chlorinated solvents such as 1,1,1- trichlorethane (TCE) and tetrachloroethene and (PER) emitted to the troposphere (McCulloch, 2002).

TCA is extremely soluble, with a Henry's law coefficient of 7.4 x 10^4 mol l^{-1} atm^{-1} (Bowden *et al.*, 1998), so will partition almost exclusively into the aqueous phase. As the surfaces of the needles are lipophilic, it has previously been thought unlikely that TCA is taken up through needle surfaces directly from air or water, but rather from the soil pore water via the roots, (Blanchard,1954; Sutinen *et al.*,1995; Forczek *et al.* 2001)

If a direct atmospheric input of TCA to foliage exists, then this may have implications for the health of trees on the edge of forest stands, which are regularly exposed to cloud water which can form a significant proportion of wet deposition in upland areas. Trees may also be subjected to TCA concentration effect as water droplets on the canopy evaporate.

Another possible source of TCA in needles is from *in-situ* formation within the plant from C_2-chlorocarbon precursors, such as tetrachloroethene, which have been taken up directly into the needles (Frank, 1991). Transformation of precursors to TCA may occur either by photolysis or by enzymatic detoxification by P-450 monoxygenase (Frank *et al.* 1992).

Although there have been several studies on the effects of TCA used in high concentrations as a herbicide, there are few reports focusing on the chronic effects of TCA on tree health. Most controlled experiments to investigate the effects of TCA on tree health have used TCA doses greatly in excess of those that have been reported in the environment. Quantification of the rates of uptake and elimination under controlled conditions would improve understanding of TCA cycling in the forest system, and is important for the risk assessments of TCA and its precursors. It must be questioned whether TCA present in needles is related to the TCA in the atmosphere or whether it reflects the TCA concentrations of the soil with only minor atmospheric contributions.

The high TCA concentrations detected in soil, at two Scottish field sites relative to other sites in the rest of Europe and North America have been highlighted in Chapter 4, and in Chapter 5 the cycling of TCA in the soil environment in terms of relative inputs (atmospheric deposition and forest throughfall) and outputs (soil leachate) was investigated. However, the relationship between soil TCA concentrations and the presence of TCA in the foliage of trees has not yet been discussed.

In the U.K. Sitka spruce (*Picea sitchensis*) is one of the most widely planted trees, and in 1995 accounted for 49 % of the land area covered by coniferous trees

(Forestry Commission, 2002). It is therefore important for both environmental and economic reasons to improve understanding of the cycling of TCA in Sitka spruce trees and the possible effects it may have on tree health.

Chapter aims

In this study two controlled experiments were carried out to investigate the cycling of TCA in Sitka spruce saplings, its effects on sapling health and routes and kinetics of TCA uptake and elimination. The specific objectives of each experiment are described below.

(I) Long-term exposure of saplings to TCA

Sitka spruce saplings were exposed to environmentally-relevant doses of TCA over two growing seasons to investigate:

- The routes of uptake of TCA solution by Sitka spruce saplings via the atmosphere (TCA solution sprayed directly onto the foliage) and the roots (TCA solution applied to the soil);
- TCA stores and fluxes within the sapling-soil system;
- The effects of TCA exposure over 2 years on sapling health (growth, visual damage, needle enzyme activity and needle protein content).

(II) Kinetics of uptake and elimination of TCA in saplings

A single pulse of TCA solution was applied to either the foliage or the soil of Sitka spruce saplings to investigate the kinetics of uptake and elimination in needle foliage.

6.2 EXPERIMENT I: LONG TERM EXPOSURE OF SITKA SPRUCE SAPLINGS TO TCA

6.2.1 Methods

6.2.1.1 Sapling material

This experiment was initially set up by Reeves (2001) and conducted for one year (May 1999 – May 2000) for which results are reported in Cape *et al.* (2003). These Sitka spruce saplings were later used in the research presented here for a similar 2-year study (May 2000 – October 2002) in which TCA uptake and storage by saplings, and effects on tree health were investigated over a longer period of time. The experiment was conducted on saplings of Sitka spruce [*Picea sitchensis* (Bong.) Carr] of Queen Charlotte Island provenance, which were planted in 1997, and grown at the Centre for Ecology and Hydrology, Edinburgh. The saplings were grown in an unheated greenhouse and treated according to normal nursery practice, including fertilisation and regular watering. Pots were placed in plastic saucers and watered by filling the saucers with de-ionised water, which contained low concentrations of TCA (< 5 $\mu g\ l^{-1}$). All saplings were watered with the same volume and frequency.

6.2.1.2 Experimental design and exposure technique

120 saplings were divided into 6 blocks of 20 saplings. One of the six tallest saplings was randomly assigned to each block, one of the second six tallest and so on until each block had one of the six smallest saplings. These blocks of 20 were split by height into 4 batches (H1, H2, H3, H4) of 5 trees with H1 in all blocks containing the tallest saplings and H4 containing the shortest saplings.

The saplings were exposed to TCA over two growing seasons. A volume of solution equivalent to 2 mm of rain was applied, as this was the estimated canopy retention depth (i.e. before throughfall and dripping occurs). In the first season (May 2001 to October 2001) saplings were dosed with 106 ml of solution on 44 occasions and in the second season (May 2002 – October 2002) they were dosed with 200 ml on 46 occasions. The treatments were performed on average every 3.5 days. Each block was subjected to one of 2 x 3 treatment-level combinations consisting of two treatment techniques; directly to the soil (Ts) and by spraying a fine mist onto the

foliage (Tf); and three concentrations (levels): TCA 1.5 μg l^{-1} (de-ionised water, L0), 10 μg l^{-1} (L10), and 100 μg l^{-1} (L100). The order of spraying was always L0, L10 then L100 to remove risk of contamination of the control (L0) or L10 saplings. The blocks were arranged randomly in an unheated greenhouse. Paper plates were placed around the stems of all saplings to prevent any drips from the spray applications from contaminating the soil, and also to help retain soil moisture. Due to the fading of some of the H3 and H4 labels for the L10Ts block, the saplings could not be distinguished from each other and therefore samples from both batches were combined. The L10 treatments were not applied in the 2002 growing season after analysis of results from the 2001 season showed no marked deviation from those of the controls.

6.2.1.3 Needle and soil sampling

Needles from the previous year's growth (C+1) and soil samples were collected at the start of the growing season before dosing commenced. At the end of the growing season, about a week after dosing ceased, "new" needles (year C) and further soil samples were taken. In October 2002 needles from the previous year class (C+1) were also sampled. Needles were collected by cutting a whole shoot from the 2nd or 3rd whorl of every tree. Samples were batched by height class within each block, stored in sealed polythene bags and either analysed immediately or stored at –30° C until analysis for TCA.

Soil was sampled from the sapling pots at 4 cm from the base of the sapling trunk using a 10-cm by 2-cm soil corer. On the day of collection the soil was sieved through 2-mm mesh to remove stones and to homogenise it, then stored in polythene bags at –30° C until analysis for TCA.

6.2.1.4 TCA analysis

Shoots for the sapling experiment were immersed in de-ionised water, rinsed, and the excess water allowed to dry, to ensure that only TCA internal to the needle matrix was analysed. Needles were stripped from the branch and homogenised by grinding frozen under liquid nitrogen with a pestle and mortar to ensure complete release of

TCA from the needle matrix. Homogenised needles (1 g) were weighed into vials for analysis of TCA by HSGC-ECD, as described in Chapter 2. Soil samples were also prepared and analysed as described in Chapter 2.

6.2.1.5 Sapling health

Sapling growth

The heights and stem diameters of each sapling were measured at the start and end of each growing season. The sapling heights were measured from the rim of the pot to the tip of the lead shoot. The stem diameter was measured at two perpendicular points at rim height using vernier callipers, and the mean taken. The growth rates were assessed by comparing the percentage increase of stem diameter, height and trunk volume for each sapling over the growing season.

Visible damage

This was carried out by Mr Rolf Koren (Honours project student, University of Edinburgh, 2002). Saplings were visually assessed for signs of damage, such as loss of needles and foliage yellowing, at the end of the growing season in October 2001 on four separate occasions. Saplings were inspected in a random order and the extent of damage estimated using a scale of 1 (least damage) – 4 (most damage).

Glutathione and peroxidase enzyme activities

In this study the activities of both peroxidase (POX) and glutathione-S-transferase (GST) enzymes were used as an indicator of sapling health as both enzymes have been found to be involved in the detoxification of xenobiotics in plants (Schröder *et al.*, 1997). Year C needles were collected from each sapling in October 2001 and samples from each height class H1 (tallest) – H4 (shortest) were bulked. Shoots were rinsed with de-ionised water, dried carefully with tissue paper and stored at –80°C before being sent in dry ice to Forschungszentrum für Umwelt und Gesundheit (GSF), Neuherberg, Germany where they were analysed for GST and POX activity by Dr. P. Schröder.

GST activity was determined using the procedure described in Schröder *et al.* (1997). Needle samples were ground with liquid nitrogen to a powder, and 10 volumes (w/v) of 100 mM Tris/HCl buffer added at pH 7-8 containing 1 % PVP K30, 5 mM EDTA and 0.25 % Nonidet™ P40. The slurry was homogenised, allowed to stand, centrifuged and the supernatant filtered. GST activity was determined spectrophotometrically in triplicate in the purified extract using 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) and dichloromethane (DCM) as substrates. POX activity was determined from the change in absorbance measured at 420 nm for 5 minutes of 1 ml assay (910 µl 0.05 M potassium phosphate buffer (pH 6.0), 20 µl 3.4 M guaiacol as substrate, 20 µl 0.9 mM H_2O_2, 50 µl enzyme). Blanks were subtracted and activity was calculated from a molar extinction coefficient of 26.6 mM^{-1} cm^{-1}. Protein content was determined in duplicate in the same needles by the method of Bradford (1976) using bovine serum albumin as a standard.

Needle physical properties

Koren (2002) investigated the rates of water loss from needles sampled in September 2001 from saplings used in this research, to assess the effects of TCA application on the physical properties of needles. Two excised needles per leader shoot of each sapling were pooled by height block, fully hydrated overnight, weighed (at time t = 0) and then placed in an atmosphere of constant humidity > 50 % for 72 hours. The needles were re-weighed 18 times throughout this period and then placed in an oven at 70 °C for three days to obtain the dry needle masses. The data were analysed as described by Cape and Percy (1996) using Equation 6.1, followed by Equation 6.2;

$$R(t) = \frac{m(t) - m_d}{m_f - m_d} \qquad \textbf{Equation 6.1}$$

Where $R(t)$ = relative needle water content at time t

$m(t)$ = mass of needles at time t

m_f = fresh needle mass

m_d = dry needle mass

$$R(t) = R_{\infty} + (R'_0 - R_{\infty})e^{-kt}$$ **Equation 6.2**

Where R'_0 = extrapolated relative needle water content at $t = 0$

R_{∞} = extrapolated relative needle water content at infinity

k = first-order rate coefficient corresponding to a measure of needle surface integrity for a given specific needle surface area

Using least squares non-linear regression, Equation 6.1 was fitted to the rate of weight loss with time for each sample, for times >3 hours, to obtain estimates for R'_0, R_{∞} and k for each of the 24 group-block combinations.

Statistical analysis

Throughout this chapter statistical analysis was performed using the General Linear Model followed by Tukey's pairwise comparisons (Minitab 12), unless stated otherwise. All statistical analysis was carried out by the author, with the exception of the needle water loss data which was carried out by Dr. K. Heal (School of GeoSciences, University of Edinburgh).

6.2.2 Results and discussion

6.2.2.1 Routes of TCA uptake into foliage

TCA in current (C) needles

All needle TCA concentrations are expressed per dry weight (dwt) of material. The mean TCA concentrations in current needles for all treatments are shown in Figure 6.1 for October 2001, April 2002 and October 2002. The mean (± 1 SD) TCA concentration in needles of all control (L0) saplings was 51 (± 22) ng g^{-1} with a range of 12 - 98 ng g^{-1} which is within the usual range of concentrations reported in literature of up to 180 ng g^{-1} (fwt) for trees in natural conditions.

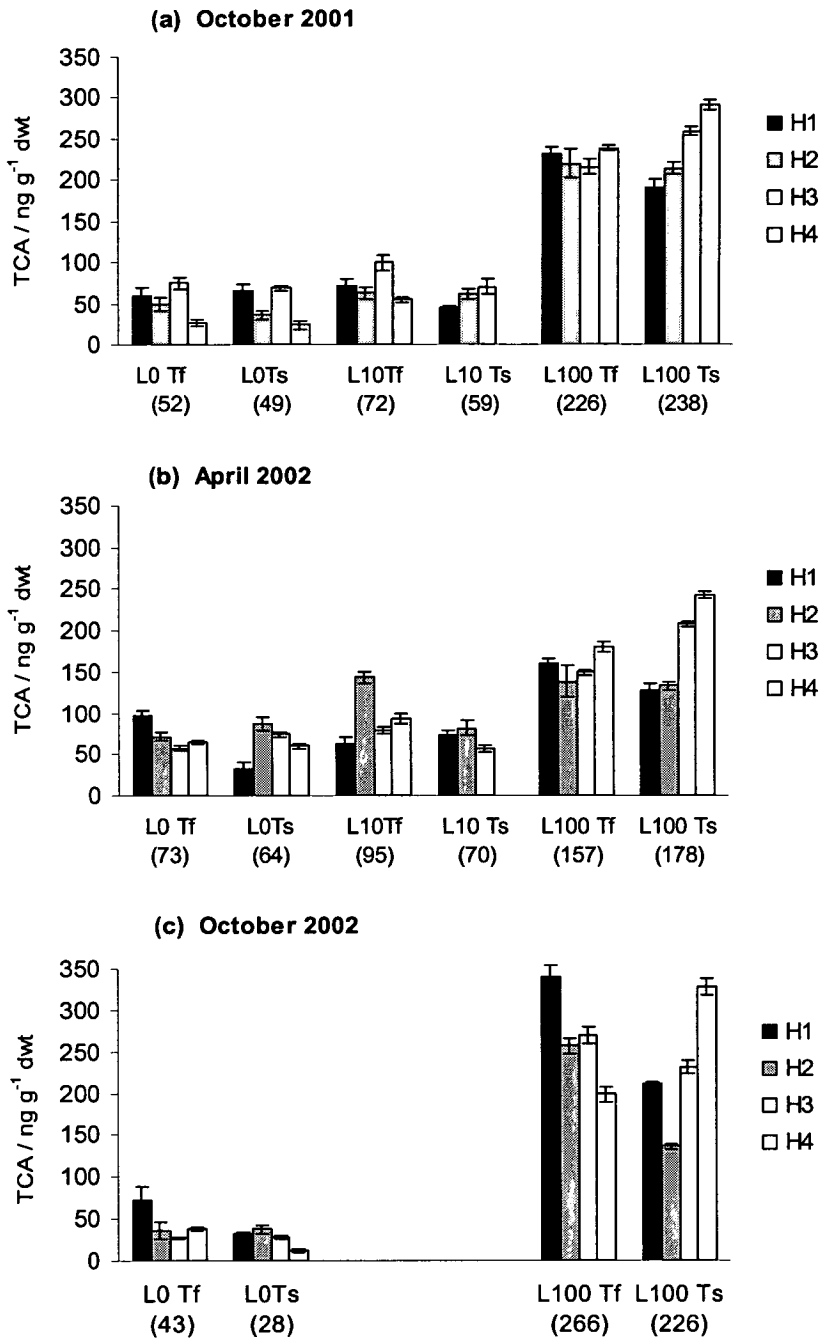


Figure 6.1. TCA concentrations (dwt) in the most recent needle class (year C) sampled in (a) October 2001, (b) April 2002 and (c) October 2002. Needles sampled in April 2002 are the same cohort as those sampled in October 2001. Error bars are 1 SD of five samples pooled by height block (H1 – H4) and analysed in triplicate. The mean values for each level-treatment group are shown in parentheses below the *x*-axis.

TCA concentrations in needles of L10 were not significantly different to TCA concentrations in needles of L0 saplings. This is possibly due to the variability of measured TCA concentrations within each treatment, although it may also result from background TCA present in the de-ionised water that was used to water the saplings and make up treatment solutions (measured on 5 occasions, mean TCA 1.5 $\mu g\ l^{-1}$). TCA concentrations in needles of L100 saplings were significantly greater than both the L0 and L10 saplings in October 2001 ($P < 0.001$) and April 2002 ($P < 0.001$), and significantly greater than the L0 saplings in October 2002 ($P < 0.01$) for both foliage (Tf) and soil (Ts) applications.

There was no significant difference in needle TCA concentrations between L100Tf and L100Ts saplings. This is evidence that TCA can be taken up via both foliage and soil routes and that an above-ground route is particularly efficient since a greater proportion of TCA dosing solution is likely to be lost due to incomplete interception by the canopy.

It was not possible to directly compare the net accumulation of TCA in the needles of the Tf and Ts treatments because the exact proportion of TCA solution intercepted by foliage in the spray-treated batch is not known. Despite the protection by paper plates, the possibility of spray drift from the Tf treatment to the soil cannot be excluded but can be assumed to be minimal.

TCA concentrations in the needles of L100Ts saplings decreased significantly with height ($P<0.05$), a trend that was not apparent in needles of L100Tf saplings. Either a dilution effect is observed in Ts saplings, or smaller saplings exposed to TCA via the soil are not as efficient at metabolising TCA. This suggests that there may be differences in TCA uptake and metabolism mechanisms in saplings between TCA applied to foliage or to soil. The observed foliage uptake was not due to TCA measured on the needle surface since the needles were washed prior to analysis to eliminate this possibility.

Temporal changes in needle TCA concentrations

TCA concentrations (dwt) in the same cohort of needles sampled in October 2001, April 2002 and October 2002 are shown in Figure 6.2. Needles sampled at the end of the growing seasons in October 2001 and October 2002 had significantly greater TCA concentrations ($P < 0.01$ and $P < 0.005$ respectively) than needles sampled in April 2002 for both foliage (Tf) and soil (Ts) application methods. It is evident that, once TCA has accumulated in needles it is subsequently eliminated even over winter when saplings are less metabolically active.

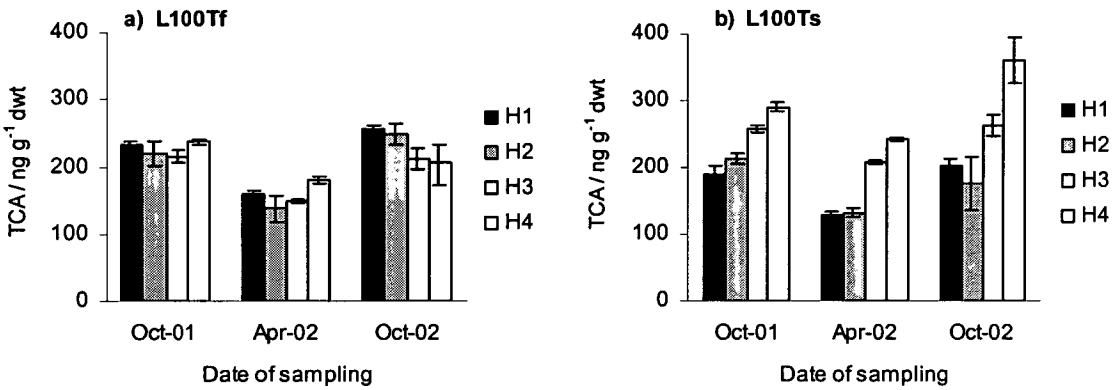


Figure 6.2. TCA concentrations (dwt) in the same cohort of needles established in the 2001 growing season, sampled in October 2001, April 2002 and October 2002, for saplings dosed with 100 μg l^{-1} TCA solution via: (a) the foliage (L100Tf) and (b) the soil (L100Ts). Error bars are 1 SD of samples pooled by height block and analysed in triplicate.

On average, the needle TCA concentrations in L100 dosed saplings in April 2002 were 69 % and 75 % of the needle concentrations at the end of the 2001 growing season for the Tf and Ts treatments respectively. Mean needle concentrations decreased from 226 to 157 ng g^{-1} for L100Tf and 238 to 178 ng g^{-1} for L100Ts. The TCA may have been broken down within the system to, for example, carbon dioxide, or it may have been relocated within the system. Although the saplings did not increase in height over the winter, the stem diameters increased over the winter period resulting in a mean trunk volume increase of 17 (± 9) %. According to estimates of stemwood mass (discussed in more detail in Section 6.2.2.2), and assuming that the same foliage mass ratios apply throughout the year, the mean

percentage increase in needle mass was 14 % (approximately 6 g). These foliage mass increases may result in dilution of the TCA present in the system and account for some of the apparent "loss" of TCA, in addition to elimination.

In October 2002 TCA concentrations in the needles of both Tf and Ts saplings were not significantly different to concentrations detected in the same needles in October 2001. The net accumulation in TCA concentration in the second season was only 74 (± 37) ng g^{-1} for L100Tf and 73 (± 33) ng g^{-1} for L100Ts saplings treatment compared with net accumulations of 266 (± 58) ng g^{-1} and 226 (± 79) ng g^{-1} respectively, in the C needles over the same period. Either "new" TCA initially accumulates more slowly in these C+1 needles compared with the C needles, or it is eliminated at a faster rate. It is possible that needles only have a certain capacity for TCA uptake, after which a saturation level is reached. Similar trends of uptake and elimination were observed by Cape *et al.* (2003).

It is evident that TCA does not accumulate linearly in needles over a prolonged period of TCA exposure. However, no information was obtained about the rate of TCA accumulation in the saplings at a single point in time during the growing season, only the increase in needle TCA concentrations between the start and end of the season. Likewise, the rates of elimination of TCA from the saplings during the growing season could not be easily quantified. Based on the effective loss of about 25 - 30 % of TCA over the 6-month winter period, a rough estimate of 1 year can be made for the half-life of TCA in Sitka spruce needles during winter. The rates of uptake and elimination of TCA by saplings are investigated in more detail in Experiment II (Section 6.3).

TCA concentrations in needles of different ages

TCA concentrations in two cohorts of needles of different age classes (years C and C+1) from L100 saplings sampled in October 2002 are shown in Figure 6.3. There was no significant difference in TCA concentrations between needles classes for either the L100Tf or L100Ts applications which is evidence that TCA may be taken up by needles of different ages and not only by the youngest needles.

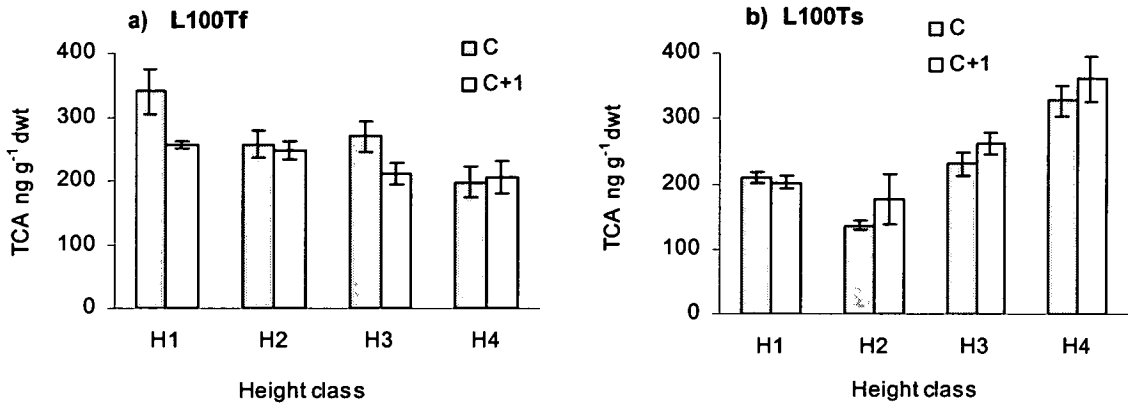


Figure 6.3. TCA concentrations (dwt) in year C and C+1 needles sampled in October 2002 for saplings dosed with 100 μg l^{-1} TCA solution via (a) foliage (L100Tf) and (b) soil (L100Ts). Error bars are 1 SD of samples pooled by height block and analysed in triplicate.

Most field and laboratory studies show that TCA concentrations are greater in older needles, probably due to lower metabolic activity, (Frank *et al.*, 1990; Plümacher and Schröder, 1994; Juuti *et al.*, 1996; Sutinen *et al.*, 1997; Hafner *et al.* 2002 and Stidson *et al.*, 2004a). However, Forczek *et al.* (2001), on applying ^{14}C-labelled TCA to soil in a controlled experiment detected greater radioactivity in current needles than in older needles, and suggested that this is because of a faster transpiration stream in young needles. This implies that "new" TCA initially enters the C needles first but can then be eliminated more rapidly. Results in Figure 6.3 suggest that initially, TCA is taken up equally by both C and C+1 needles. Over time this TCA may be eliminated more efficiently in the C needles, and ultimately lead to lower TCA concentrations than in C+1 needles.

Results from needle analyses described in this section have shown that TCA may be taken up by foliage as well as via the soil and roots of Sitka spruce saplings which provides evidence for an atmospheric route. However, uptake into the foliage may not necessarily be through the needles themselves but via branchwood and

stemwood. TCA concentrations (dwt) in branchwood sampled in October 2002 are shown in Figure 6.4.

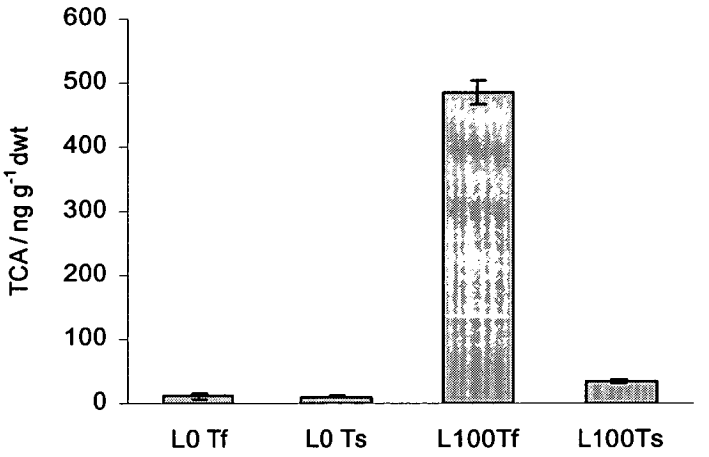


Figure 6.4. TCA concentrations (dwt) in branchwood of saplings dosed with 1.5 μg l^{-1} (L0) or 100 μg l^{-1} (L100) TCA solution via the foliage (Tf) or the soil (Ts), sampled in October 2002. Error bars are 1 SD of samples pooled by treatment and analysed in triplicate.

There was strong evidence ($P<0.001$) that TCA concentrations in branchwood of saplings treated with 100 μg l^{-1} TCA solution via the foliage (Tf) were significantly greater (by over an order of magnitude) than those treated via the soil (Ts). This suggests that TCA initially enters the sapling via branchwood and is subsequently translocated into the needles via the transpiration stream, possibly because branchwood is more hydrophilic than coniferous needles. Other ions have also been reported to enter coniferous trees through branchwood rather than needles (Percy and Baker, 1989).

6.2.2.2 TCA distribution in the sapling/soil system

To gain a better understanding of the routes of uptake of TCA by Sitka spruce trees and its cycling within the forest system, TCA distribution within the sapling system was investigated by determining the proportion of TCA stored in needles, branchwood, stemwood and soil compartments.

TCA in soil of the sapling/soil system

Soil in the pots of saplings was analysed for TCA to determine the proportion of applied TCA that could be accounted for in the soil. Soil TCA concentrations (dwt) are shown in Figure 6.5 and ranged from 8 to 53 ng g^{-1} (mean = 19 ng g^{-1}, SD = 18 ng g^{-1}) in April 2001, and 10 to 51 ng g^{-1} (mean = 18 ng g^{-1}, SD = 8 ng g^{-1}) in October 2001.

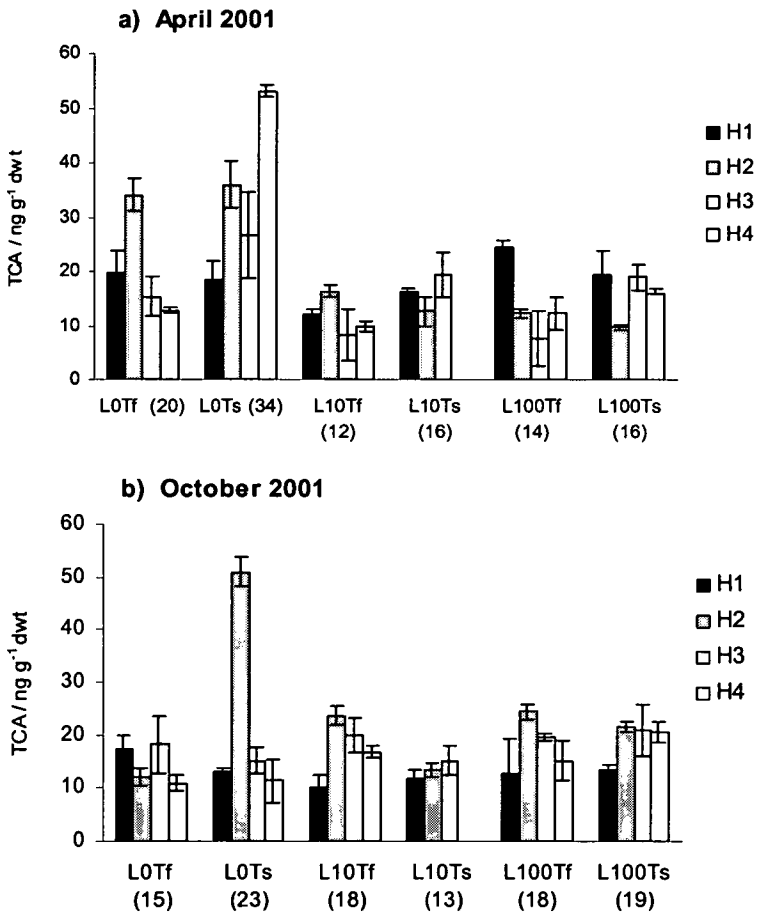


Figure 6.5. TCA concentrations (dwt) in soil collected from sapling pots in (a), April 2001 and (b), October 2001. Error bars are 1 SD of samples pooled by height block and analysed in triplicate. The mean values for each level-treatment group are shown in parentheses below the *x*-axis.

Soil TCA concentrations were not significantly different either between treatments or between the start and end of dosing. This implies that the range in soil TCA concentrations measured is due to natural variability. Although Ts saplings had TCA

applied directly to the soil twice a week for 5 months, this TCA evidently did not accumulate and was most likely broken down within the soil (as discussed in detail in Chapter 5) or taken up into the saplings via the root system and transpiration stream.

Mass of material in each compartment within the sapling / soil system

The masses of needles, branchwood and stemwood per sapling were calculated for each year using the methods of Cannell *et al.* (1983), as described below.

1) The stemwood volumes of all saplings were estimated from direct measurements of stem height and diameter by assuming the stem is a cone (Equation 6.3).

$$v = \frac{\pi r^2 h}{3} \qquad \textbf{Equation 6.3}$$

Where v = volume of stem (cm^3)
r = radius of stem at root collar of sapling (cm)
h = height of sapling (cm)

2) Stemwood mass was determined from the stemwood specific gravity (reported for Sitka spruce to be 0.40 g cm^{-3} for 5-year old saplings and 0.36 g cm^{-3} for 6-year old saplings) and the fresh stemwood volume (υ), as shown in Equation 6.4.

Mass of stemwood = Stemwood specific gravity x v **Equation 6.4**

3) Needles and branchwood masses were estimated from the dry matter mass ratio of stemwood:branchwood:needles = 37:29:34.

A sapling was harvested in February 2003 and the dry masses of each compartment were measured directly to check that estimations from Cannell *et al.* (1983) were sufficiently accurate to be applied throughout the study. Good agreement was found

between the measured dry mass (232 g) of branchwood and needles and calculated estimate (261 g).

The soil mass was calculated from the volume of the pot and six measurements of dry bulk density. It was assumed to be the same for each sapling and to remain constant throughout the experiment. Table 6.1 shows the mean distributions by mass of needles, stemwood, branchwood and soil in the sapling system in October 2001 and 2002.

Table 6.1. The mean dry masses of needles, stemwood, branchwood and soil per sapling for each level-treatment group in October 2001 and 2002. Standard deviations (parentheses) for needles, stemwood and branchwood are of the 4 height blocks within each treatment. Standard deviation (parentheses) for soil is of the soil density determinations.

Treatment	Mean (± SD) dry mass of material / g per sapling			
OCTOBER 2001	**Needles**	**Stemwood**	**Branchwood**	**Soil**
L0Tf	43.4 (6.0)	47.2 (6.5)	37.0 (5.1)	3580 (691)
L0Ts	46.3 (1.7)	50.4 (1.8)	39.5 (1.4)	3580 (691)
L10Tf	42.6 (3.3)	46.4 (3.6)	36.4 (2.8)	3580 (691)
L10Ts	41.2 (3.8)	44.9 (4.1)	35.2 (3.2)	3580 (691)
L100Tf	43.4 (2.9)	47.2 (3.2)	37.0 (2.5)	3580 (691)
L100Ts	41.1 (9.5)	44.7 (10.3)	35.0 (8.1)	3580 (691)
OCTOBER 2002				
L0Tf	73.7 (10.6)	80.3 (11.5)	62.9 (9.0)	3580 (691)
L0Ts	75.0 (3.9)	81.6 (4.2)	63.9 (3.3)	3580 (691)
L100Tf	66.6 (4.1)	72.5 (4.4)	56.8 (3.5)	3580 (691)
L100Ts	66.3 (10.7)	72.1 (11.6)	56.5 (9.1)	3580 (691)

In both years the foliage compartments all accounted for similar proportions of the total sapling mass but soil mass was approximately 2 orders of magnitude greater. One factor which was not accounted for due to the impracticalities of measurement was the volume taken up by the sapling roots. Neither the mass nor the TCA concentrations were measured. In the 2002 season the roots were likely to occupy a

greater volume than in 2001 due to sapling growth, and therefore reduce the volume occupied by the soil. The mass of soil in turn is likely to change in response to microbial breakdown of organic matter and use of resources by the saplings. However, it is still likely to be the case that soil dominates the mass and TCA storage within the sapling-soil system.

Mass of TCA stored in each compartment

The TCA stored in branchwood, stemwood, needles and soil was calculated by multiplying the dry mass of material by the TCA concentration of each compartment. The TCA concentration of stemwood was assumed to be negligible. Table 6.2 shows the mass of TCA stored in each compartment of the sapling-soil system, in October 2001 and 2002 determined from measured TCA concentrations in soil, needles and branchwood.

Table 6.2. The mean mass of TCA in needles, stemwood, branchwood and soil per sapling for each level-treatment group in October 2001 and 2002, calculated from the TCA concentrations and masses of sapling material. Standard deviations (parentheses) are for the 4 height blocks within each treatment. Stemwood TCA concentration was assumed to be zero. Soil TCA concentrations in October 2002 were assumed to be the same as those measured in October 2001.

Treatment	Mean (± SD) TCA mass / µg per sapling			
OCTOBER 2001	**Needles**	**Stemwood**	**Branchwood**	**Soil**
L0Tf	2.30 (0.97)	0	0.45 (0.06)	52.2 (13.0)
L0Ts	2.26 (1.04)	0	0.39 (0.01)	81.1 (68.0)
L10Tf	3.03 (0.57)	0	0.44 (0.03)	62.8 (20.6)
L10Ts	2.50 (0.29)	0	0.35 (0.03)	49.6 (5.78)
L100Tf	9.81 (0.75)	0	17.9 (1.20)	64.0 (18.3)
L100Ts	9.52 (1.21)	0	1.20 (0.28)	68.4 (13.4)
OCTOBER 2002				
L0Tf	6.85 (2.57)	0	0.76 (0.11)	52.2 (13.0)
L0Ts	8.11 (6.13)	0	0.63 (0.03)	81.1 (67.9)
L100Tf	12.8 (1.67)	0	27.5 (1.67)	64.2 (18.3)
L100Ts	12.3 (3.31)	0	1.93 (0.31)	68.4 (13.4)

The soil TCA burden was markedly greater than that of the needles, stem and branchwood in each of the treatments. This is not surprising given the large mass of soil. Before dosing commenced in May 2001 the mean soil TCA burden in all treatments was 17 times greater than that of the foliage. In October 2001 after a season's dosing the soil TCA burdens in Ts treatments were 17 and 6 times greater than the foliage TCA burden for L10 and L100 saplings, respectively and in October 2002 after a season's dosing, the soil TCA burdens in Ts treatments were 5 times greater than the foliage TCA burden in L100Ts saplings. Although needle TCA concentrations increased over the growing season when dosed with TCA solution, and soil concentrations remained the same, the relative burden of the soil was still considerably greater. This supports previous studies which have found the TCA burden of soil in the natural environment to be approximately 6 times the annual input flux (Stidson *et al.*, 2004a)

The inputs of TCA to the sapling system and the change in storage of TCA within the sapling system over the 2001 and 2002 growing seasons are shown in Table 6.3. The changes in TCA storage between the start and end of each growing season were determined by the difference in storage at the start and end of the growing season. The percentage recovery of TCA is the proportion of input TCA (by dosing) accounted for by change in storage. Thus, if the mass of TCA applied over the season is equal to the measured increase in TCA stored within the sapling system in October, the recovery will be 100%.

The total TCA applied to the sapling system over a whole growing season could not be fully accounted for in the increase in needle, stemwood, branchwood or soil TCA concentrations over either the 2001 or 2002 growing seasons. In all control (L0) sapling systems in 2001 and 2002, and the L10 sapling systems in 2002, the estimated change in TCA storage over the growing season was negative. This implies that either: (1) TCA which was intrinsic to the sapling system before dosing with TCA solution (1.5 $\mu g\ l^{-1}$ or 10 $\mu g\ l^{-1}$) was partially eliminated in addition to the externally applied TCA, maybe because the detoxification capacity of trees increases with age, or (2) approximations involved in calculations of the material mass (foliage

and soil) and therefore TCA storage resulted in systematic errors and random uncertainty in all of the calculations involved. However, as the TCA storage was calculated using the same method for every sapling, the final recoveries of TCA are directly comparable between treatments, even if the magnitudes are not entirely accurate. It is unquestionable that the percentage of TCA recovered in the sapling/soil in either October 2001 or 2002 was a tiny fraction of the total mass of TCA applied over the growing season.

Table 6.3. Mean changes in TCA storage of the sapling-soil system from May - October 2001 and May - October 2002, compared with the mass of TCA applied (input). The standard deviations shown in parentheses are for the 4 height blocks within each treatment.

Treatment	Change in sapling system storage	Input	Input – Change in sapling storage	Recovery (± SD)
OCTOBER 2001	TCA / ng	TCA / ng	TCA / ng	%
L0Tf	-976	7000	7970	-14.0 (20)
L0Ts	-28	7000	7020	-0.4 (17)
L10Tf	-1260	53600	54900	-2.4 (7.0)
L10Ts	-573	53600	54200	-1.1 (3.6)
L100Tf	24000	475000	451000	5.1 (0.5)
L100Ts	7480	475000	468000	1.6 (0.2)
OCTOBER 2002				
L0Tf	-1290	13800	15100	-9.4 (4.9)
L0Ts	-1020	13800	14800	-7.4 (9.4)
L100Tf	17300	933800	917000	1.9 (0.4)
L100Ts	7360	933800	926440	0.8 (0.4)

The percentage recovery was < 6 % for L10 and L100 saplings in 2001 and < 2 % for L100 saplings in 2002. The percentage recovery was always greater in Tf treated saplings which can be attributed to the greater branchwood TCA concentrations. In Ts treated saplings there was either rapid degradation of TCA in soil or rapid uptake into the sapling whereas in Tf treated saplings it is possible that TCA remained

adsorbed onto the needle surface until being washed off during preparation for analysis, or degraded on the needle surface. Alternatively, TCA was accumulating in a compartment not identified in this experiment. Forczek *et al.* (2001) also reported poor recovery (~ 22 %) of [1,2-^{14}C] TCA applied to a Norway spruce sapling experimental system and attributed this to degradation in the soil and adsorption by both roots and litter. The pulse experiment, described in Section 6.3, also indicates loss of TCA in the total sapling/soil system and attempts to estimate the proportion lost in the various "compartments".

6.2.2.3 Sapling health

Growth of saplings

Percentage changes in sapling stem diameter, height, and trunk volumes of saplings between May 2001 and October 2002 are presented in Figure 6.6 a, b and c. The trunk volume is the combination of stem diameter and height, given in Equation 6.3.

There was no significant difference in stem diameter or height measurements either between concentration (L0 or L100) or application method (Tf or Ts) which implies that sapling growth was not influenced by application of TCA over two growing seasons. There was also no significant difference in the trunk volumes, indicating that there is no observable combined effect of stem diameter and height. This does not exclude possible long-term effects of TCA exposure. In addition, saplings in nature are exposed to much harsher environments in general (e.g. low temperatures, poor soils, disease) so that a low-level effect of TCA may then give rise to an observable detrimental effect on growth.

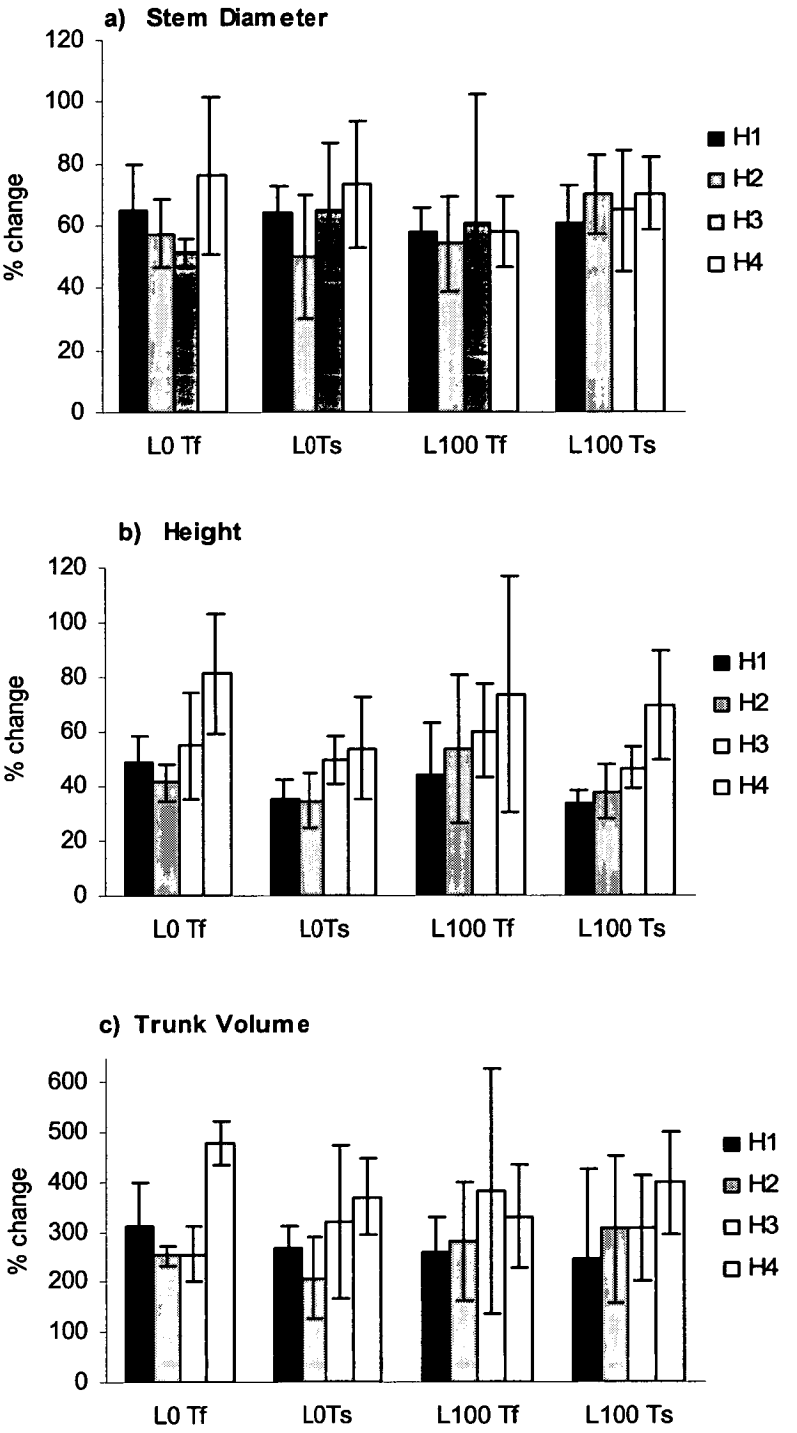


Figure 6.6. Percentage changes in (a) stem diameter, (b) height and (c) trunk volume of L0 and L100 saplings between May 2001 and October 2002. Error bars are 1 SD for the 5 replicate saplings in each height block.

Damage survey

The results of visible damage surveys carried out on saplings are shown in Figure 6.7 for October 2001. Although there was no significant effect of TCA concentration, foliage-dosed (Tf) saplings showed significantly more signs of damage ($P < 0.05$) than soil-dosed saplings (Ts). There was also a clear effect of height where H4 showed significantly less damage than H1 ($P < 0.05$) and H2 saplings ($P < 0.01$). These data suggest that damage to Sitka spruce saplings is more dependent on exposure route than concentration of applied TCA solution.

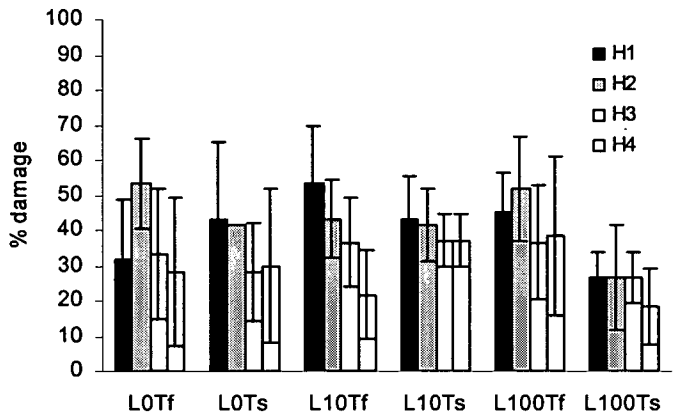


Figure 6.7. Visible damage (%) of saplings in October 2001 where 0 % represents no visible damage, 25 % represents light patches of needles discoloration, 50 % represents needles browning throughout most of the sapling and 100 % represents heavy browning of needles and needle loss. Error bars are standard deviation of 5 saplings within each height class.

Enzyme analysis

The activity of certain enzymes has been used as an indicator of pollution stress in plants. Peroxidase enzymes (POX) are ubiquitous in plants and are known to oxidise xenobiotics in plants with a wide substrate specificity (Lamoureux and Frear, 1979, cited in Schröder *et al.*, 1997). Gluthathione-S-transferase enzymes (GST) have been found to catalyse the conjugation of glutathione in plants with a number of pesticides and herbicides (Lamoureux and Rusness, 1989, 1993a; Sandermann 1992, 1994). Schröder *et al. (1997)* found that in a long-term exposure experiment of pine

saplings to TCA via root uptake or acid mist treatments, exposure to TCA influenced the activity of xenobiotic detoxification enzymes (peroxidase and GST) in the needles, indicating that the trees were under environmental stress.

Peroxidase (POX) enzyme activities in needles from October 2001 are shown in Figure 6.8a. No significant effect of TCA concentration, application method or sapling height was found. In a study carried out by Schröder *et al.* (1997) where 2-year old Scots pine seedlings were exposed to 0.1 mg l^{-1} TCA solution, needles exhibited greater POX activity. This may be explained by the greater overall TCA dose applied per mass of seedling than in this study.

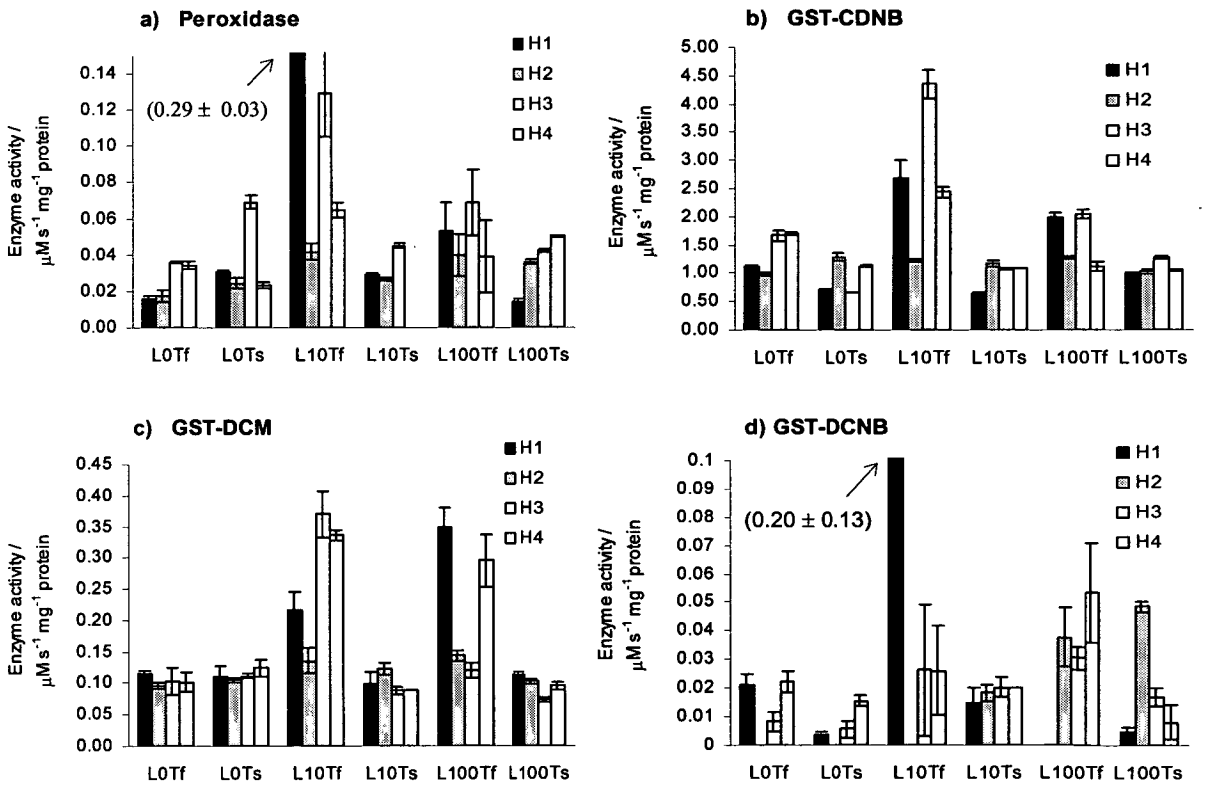


Figure 6.8. Enzyme activities in needles of Sitka spruce saplings at the end of the dosing season in October 2001. Peroxidase enzyme activities are shown in (a) and GST enzyme activities in (b) - (d), with (b) CDNB and (c) DCM, and (d) DCNB as the xenobiotic substrates. Error bars are standard deviations of five samples pooled by height class and analysed in triplicate.

Glutathione-S-transferase (GST) enzyme activities in response to various xenobiotics from needles in October 2001 are shown in Figure 6.8 (b - d) for DCM, CDNB and DCNB as the xenobiotic substrates. GST activities were significantly greater in the needles of foliage-dosed (Tf) saplings for DCM ($P < 0.05$) and CDNB ($P < 0.01$) but not DCNB which suggests that cells were under stress, causing them to express more enzyme to deal with the xenobiotic. GST activities were significantly greater ($P < 0.05$) in the L10 than L100 saplings for CDNB, which implies that GST enzyme activity may be induced by lower TCA concentrations and inhibited by higher concentrations.

The protein content (Figure 6.9) was significantly lower in L100 needles ($P<0.001$) and L10 needles ($P<0.01$), than control needles (L0) and there was a highly significant difference between application methods Tf and Ts ($P < 0.001$) where the protein content of Tf needles was lower than Ts needles. This inverse relationship between needle TCA concentrations and protein content was also observed by Plümacher and Schröder (1994) in Norway spruce needles and may be explained by the protein precipitating properties of TCA resulting in a reduction of the capacity of protein to conjugate and detoxify xenobiotics.

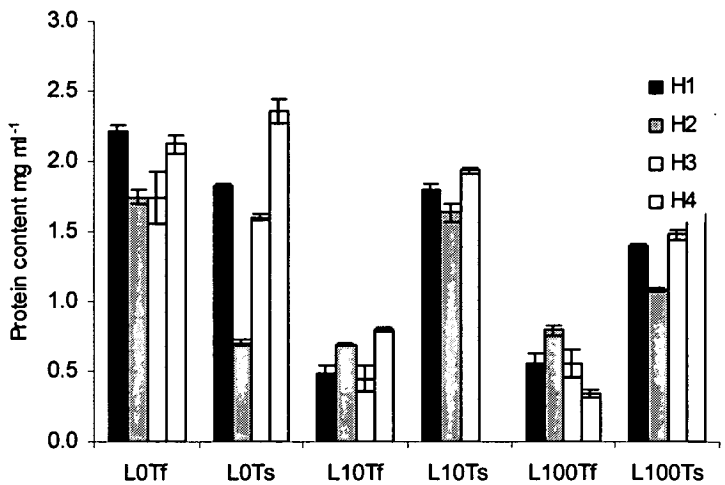


Figure 6.9. Protein content (mg (ml extract)$^{-1}$) of sapling needles in October 2001. Error bars are standard deviations of five samples pooled by height class and analysed in triplicate.

These data support the findings of the visible damage survey where Tf saplings were also found to be more damaged by TCA input at the L100 level. Enzyme activity was not induced by wetting of needle surfaces, as needles of control saplings did not exhibit greater enzyme activities in Tf treatments compared with Ts.

Needle physical properties

An example fit of Equation 6.1 (Dr. K. Heal, School of GeoSciences, University of Edinburgh) to the relative water loss for one of the needle samples is shown in Figure 6.10. Three variables, R'_0, k and Ω, were derived from the curve fitted to each needle sample. k and R'_0 are as defined earlier and $\Omega = R_\infty (m_f - m_d) / m_d$, where m_f is the fresh needle mass at $t = 0$ and m_d is the oven-dry needle mass.

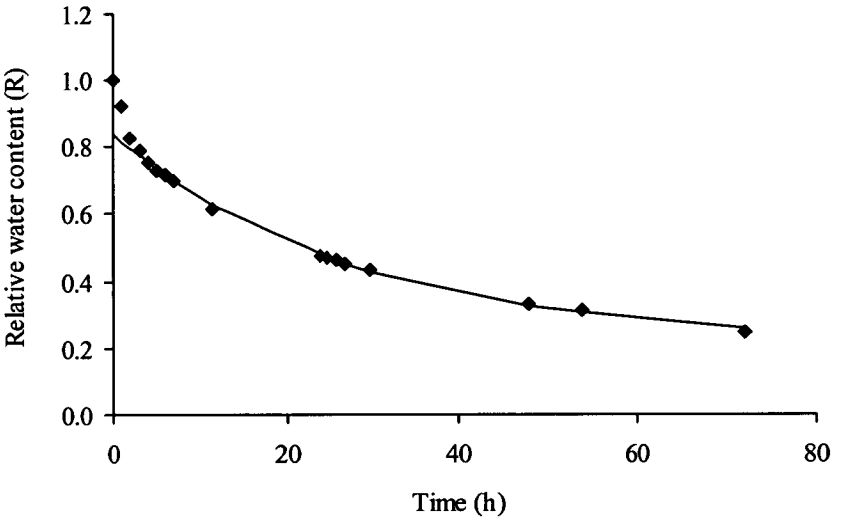


Figure 6.10. Rate of water loss from pooled needles (sampled in September 2001) from saplings of height block 1 dosed with 10 µg l^{-1} TCA to foliage (L10TfH1). Equation 1 is fitted to the data for t > 3 hours.

There was no significant effect of TCA concentration, application method or sapling height on values of k and Ω, nor on fresh mass/dry mass (m_f/m_d) ratios. However, there was a significant interaction between TCA concentration and application method ($P<0.05$) for values of R'_0 (relative water content at $t = 0$) (Table 6.4).

Table 6.4. Mean R'_0 values (%) for sapling needles sampled in September 2001, derived from fitting Equation 6.1 to measurements of needle weight loss against time at constant humidity. Values with the same superscript in a column or row are not significantly different. Interaction between level and treatment was significant at $P < 0.05$.

TCA dose level	Treatment method		Level mean
	Foliage (Tf)	Soil (Ts)	
Control	80.9	80.0	80.4^{ab}
10 μg l^{-1}	81.9	85.6	83.7^{a}
100 μg l^{-1}	64.7	82.7	73.7^{b}
Treatment mean	75.8^{a}	82.7^{b}	

For most treatments, R'_0 exceeded 80 %, but it was significantly lower (65 %) for the 100 μg l^{-1} TCA foliage treatment. Main effect differences in R'_0 were significant for TCA concentration ($P<0.05$) and application method ($P<0.05$). Since an estimate of the proportion of initial needle water lost through stomata is given by the quantity ($1-R'_0$), saplings with lower R'_0 values may be more susceptible to water loss through the stomata in dry conditions, resulting in yellowing of needles and risk of drought. These results are consistent with the damage survey, enzyme analysis and protein content results, providing further evidence that the application of TCA solution to tree foliage has an adverse effect on visual sapling health and functioning, in this case through water control in the needles.

Sutinen *et al.* (1995) reported that adsorption of TCA on needle surfaces caused the disintegration of epicuticular waxes and stomatal cells, an effect not seen after root uptake. This has implications for trees that are regularly exposed to wet cloud. Cloud water often has a greater pollutant loading than rain and can represent a significant proportion of total wet deposition (Crossley *et al.*, 1992). Percy *et al.* (1990) reported that fog can concentrate and deposit acidic particles, pesticides and other pollutants onto plant surfaces, which in turn, can initiate physiological and morphological responses. In a study on the biophysical aspects of fog deposition on the needles of three conifers Jagels (1991) reported that unless fog episodes are of prolonged duration or are followed directly by rain, droplets deposited onto the leaf surfaces are not immediately washed off. After fog dissipation, droplets on leaf

surfaces evaporate and successive fogs can lead to increasing dry deposition accumulation. Sitka spruce saplings retain their needles for a relatively long period of time (6-8 years compared with 2-3 years for Scots pine) therefore TCA may accumulate to toxic levels over this period (Cannell, 1987).

6.2.3 Conclusions: Experiment I

The exposure of Sitka spruce saplings to TCA over two growing seasons, has increased understanding of the routes of TCA uptake into the sapling, its behaviour within the sapling-soil system and effects on sapling health, as summarised below.

Routes of TCA uptake into saplings

TCA may be taken up into the needles via both the soil and foliage in Sitka spruce saplings. Similar TCA concentrations (up to 340 ng g^{-1} dwt) are detected in the current (C) needles for 100 $\mu g\ l^{-1}$ of TCA applied by both methods. These results imply that TCA input via foliage is a greater input pathway than previously thought. There is also evidence that TCA may enter needles indirectly via branchwood.

TCA behaviour in the sapling system

Not all artificially-applied TCA that enters the system can be accounted for by storage in needles, stemwood, branchwood or soil. Although there are increases in TCA concentrations in plant material, the TCA concentration of the soil in the pots does not change. This means that TCA applied via the soil is either taken up rapidly from the soil and eliminated once in the sapling system, broken down in the soil before it can be taken up, or it is accumulating in a compartment that has not been accounted for.

Sapling health

The effects of TCA on Sitka spruce saplings are more dependent on route of TCA uptake than TCA concentration. GST enzyme activities were greater, and protein contents were lower, in needles of foliage-dosed saplings than soil-dosed saplings (Ts). Foliage-dosed (Tf) saplings also showed more visual damage. Neither the

height nor stem diameter of saplings were influenced by application method or concentration of TCA applied to the saplings, nor did the percentage change in height and diameter differ between these treatments.

6.3 EXPERIMENT II: PULSE EXPERIMENT TO INVESTIGATE THE ROUTES AND KINETICS OF TCA UPTAKE BY SITKA SPRUCE SAPLINGS

6.3.1 Methods

6.3.1.1 Sapling material

A total of 40 Sitka spruce saplings (*Picea sitchensis* (Bong.) Carr), planted in 1997 and grown in an unheated greenhouse according to normal nursery practice, as for Experiment I (Section 6.2.1.1), were used in this experiment.

6.3.1.2 Experimental design and exposure technique

The saplings were systematically divided into four blocks such that each block contained one of the four tallest saplings, one of the second four tallest, and so on. Blocks were arranged randomly in the greenhouse.

Each sapling in two of the blocks was dosed with 200 ml of 1000 $\mu g\ l^{-1}$ TCA solution (i.e. a total of 200 μg TCA) applied once only on 30th July 2002, to the soil surface (block TCA-S), or sprayed as a fine mist to the foliage (block TCA-F). The application volume was chosen to correspond to a 2 mm precipitation depth over the projected canopy surface area (the estimated canopy retention depth before throughfall). Although this TCA concentration is not typical of those found in natural precipitation, it is only a few times the estimated annual ambient wet deposition flux. Control blocks (CON-S and CON-F) were dosed with de-ionised water instead of TCA solution. To avoid spray drift, the TCA-F saplings were removed to another greenhouse for spraying and returned when dry. A paper plate was placed around the base of the stem of every sapling to prevent drips from the

spray applications from contaminating the soil, and to maximise soil moisture retention. Saplings were irrigated on average twice a week via the plastic saucers beneath their pots.

6.3.1.3 Needle sampling

Samples of the current needles (year class C) were collected from all saplings immediately prior to treatment ($t = 0$ days), then approximately twice a week for 6 weeks and weekly until $t = 85$ days on 23rd October 2002 (a total of 17 sampling occasions). Needles were always sampled from branches on the second whorl of the sapling, and batched within each block. On two occasions ($t = 34$, $t = 85$) samples of the previous year's needles (C+1) were also collected. When needles were not analysed immediately the shoots were stored in sealed bags at –30° C.

Prior to analysis, shoots were immersed in de-ionised water, ultra-sonicated for 5 minutes, rinsed, and the excess water removed by patting with a tissue to ensure that measured TCA was internal to the needle matrix and not adsorbed to the surface. Needles were stripped from the branch and homogenised by grinding frozen under liquid nitrogen with a pestle and mortar, to ensure complete release of TCA from the needle matrix. The homogenised needles (1 g) were heated to 100 °C in sealed 20 ml vials for 90 min to decarboxylate TCA to chloroform and analysed by HSGC-ECD, as described in Chapter 2.

6.3.2 Results

The mean (± SD) height and stem diameter of the saplings at the start of the growing season (April 2002) were 105 (± 12) cm and 2.3 (± 0.2) cm, respectively. TCA concentrations were expressed per fresh weight (fwt) of needle, as analysed, instead of on a dry weight basis as in Section 6.2. The mean water content of the needles varied very little throughout the experiment (58.1 ± 1.9 % SD), so this parameter did not introduce any temporal bias to the data when expressing the concentration data as fresh weight.

6.3.2.1 TCA concentrations of current (C) needles

Mean TCA concentrations (fwt), in the year C needles of the control saplings are shown in Figure 6.11 for each sampling occasion. Mean needle TCA concentrations for the whole experimental period were 22 (± 6) ng g^{-1} (n = 9) and 14 (± 5) ng g^{-1} (n = 9) for the soil-dosed (CON-S) and foliage-dosed (CON-F) control saplings, respectively.

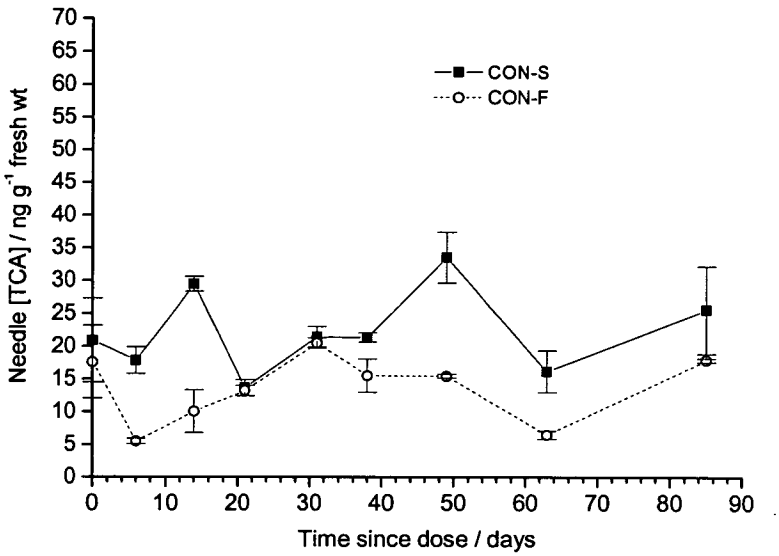


Figure 6.11. TCA concentrations (fwt) in the current year (C) needles of control saplings following application at *t* = 0 of de-ionised water to the soil only (CON-S) or to the foliage only (CON-F). Error bars are standard deviations of pooled needle samples taken from each of the 10 saplings within a group on each sampling occasion, and analysed in triplicate.

There was a significant difference between the needle TCA concentrations of CON-S and CON-F saplings which may only be explained by differences arising by chance when the 40 saplings were divided into groups of 10 at the start of the experiment. The presence of TCA in the needles of control saplings was most probably carry-over from previous background exposure to de-ionised water used for irrigation which has been shown to contain TCA (Section 6.2.1.2). Figure 6.11 shows that despite the intrinsic variability associated with the biological system, concentrations in control saplings did not show a trend with time. The variability in each data set

gives an estimate of the variability associated with sampling different needles of different saplings at different times.

The 95 % confidence interval for measurement of needle TCA concentration at a single point in time is 1.96 times the standard deviation for all corresponding control values. This yields 95 % confidence interval factors for within-group variation, relative to the corresponding mean, of 0.4 – 1.6 and 0.3 – 1.7 for soil and foliage treatments. The needle TCA concentrations (fwt) of soil-dosed (TCA-S) and foliage-dosed (TCA-F) saplings with time after dose are shown in Figures 6.12 a and b respectively.

There was a clear initial accumulation of TCA in needles and subsequent elimination for both TCA-S and TCA-F saplings. For TCA-S saplings, the needle TCA concentrations increased from 18 (± 3) ng g^{-1} at $t = 0$, to a maximum of 60 (± 2) ng g^{-1} after 31 days, decreasing to 33 (± 2) ng g^{-1} after 85 days. The accumulation and elimination of TCA in needles of the TCA-F saplings were less marked, but still significant, increasing from 14 (± 3) ng g^{-1} at $t = 0$, to a maximum of 40 (± 2) ng g^{-1} after 28 days, then decreasing to 15 (± 1) ng g^{-1} after 85 days. It is not possible to compare directly the accumulation of TCA in needles of the TCA-S and TCA-F treatments because the exact proportion of TCA solution intercepted by foliage in the spray-treated batch was not known. However, the existence of independent below-ground and above-ground pathways of TCA uptake cannot be disputed, as well as uptake just a few days after application.

The TCA-S needle TCA concentrations remained significantly higher at $t = 85$ than at $t = 0$ suggesting that either TCA was eliminated more slowly than in TCA-F saplings, or that they were still taking up TCA from the soil which partly masks the process of elimination. The kinetics of TCA uptake and elimination in needles are discussed in Section 6.2.3.3.

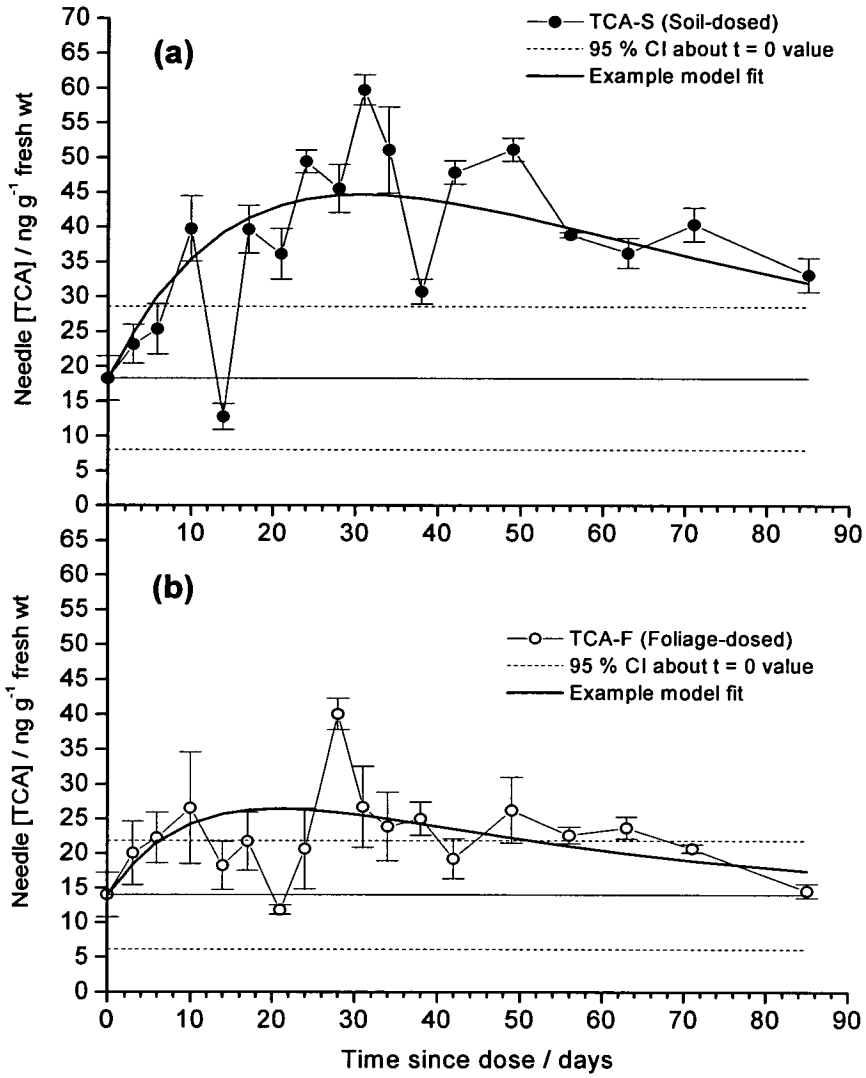


Figure 6.12. TCA concentrations in current year (C) needles following the application at t = 0 of a single dose of 200 μg TCA per sapling to (a), the soil only (TCA-S) or (b), the foliage only (TCA-F). Error bars are the standard deviation of pooled needle material taken from each of the 10 saplings within a group on each sampling occasion, and analysed in triplicate. The horizontal lines in each figure are the concentration at t = 0, and the associated 95 % confidence interval for within-group variability of measurement at a single time point, as determined using the control group data in Figure 6.11. The solid curves are model fits to the data of the kinetic scheme shown in Figure 6.14.

Soil from the CON-S and TCA-S pots was analysed for TCA at the end of the experiment. Samples were pooled from the 10 sapling pots for each treatment and analysed in triplicate for TCA. TCA concentrations of 16.5 (± 3.5) ng g^{-1} and 4.7 (± 0.4) ng g^{-1} (dwt) were detected for CON-S and TCA-S respectively which showed that the applied TCA at t = 0 could not be detected after 85 days. The difference between the treatments can be attributed to the natural variability of soil TCA concentrations.

6.3.2.2 TCA concentrations of C+1 needles

On the two occasions sampled (t = 34 and t = 85 days), the previous year's needle class (C+1) of TCA-S saplings had higher TCA concentrations than the corresponding controls (CON-S), showing that TCA was also taken up into older needles following a single application via both above and below ground routes. Figure 6.13 shows that for both sampling occasions, concentrations in C and C+1 needles of TCA-S saplings were comparable, whereas concentrations were clearly greater in C+1 needles than C needles for TCA-F saplings.

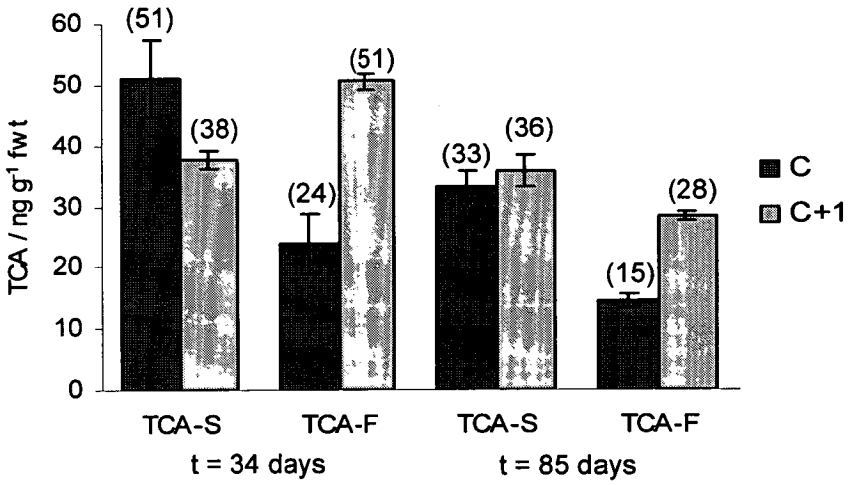


Figure 6.13. TCA concentrations of current year (C) and previous year (C+1) needles sampled at 34 and 85 days after the application at t = 0 of a single dose of 200 µg TCA per sapling to the soil only (TCA-S) or the foliage only (TCA-F). Error bars are standard deviations of pooled needle material taken from each of the 10 saplings within a group on each sampling occasion and analysed in triplicate. Mean values are shown in parentheses above the bars.

As samples of C+1 needles were collected and analysed on only two occasions, natural variability cannot be excluded as an explanation for observed differences between TCA concentrations of C and C+1 needles.

These results contrast with those of Experiment I (Section 6.2) where no significant difference was found between TCA concentrations of C and C+1 needles at the end of the growing season of saplings dosed twice a week for 5 months with 200 $\mu g\ l^{-1}$ TCA solution. It is likely that saplings exposed to TCA twice a week over 5 months respond differently to saplings exposed to a single large dose of TCA in terms of rates of uptake and detoxification capacity. There were no visible short-term adverse effects of saplings following this large pulse dose of TCA, although the physiological and biochemical changes damage that may have been incurred were not specifically investigated in this study. C+1 needles may be more susceptible to damage by pollution than C needles, a trend that would be more apparent when large doses of TCA are applied. It is also likely that a solution with a TCA concentration of 1000 $\mu g\ l^{-1}$ applied directly to the foliage may damage detoxification enzymes, resulting in slower elimination of TCA from needles.

6.3.2.3 Kinetics of TCA uptake and elimination

The kinetic modelling discussed in this section was carried out by Dr. M. Heal (School of Chemistry, University of Edinburgh). The observed time-dependence in needle concentration (Figure 6.12a and b) is typical of a two-step sequential kinetic system involving first-order uptake into the sapling from an initial "reservoir" (the TCA-dose) and first-order elimination from the sapling (by metabolism/detoxification). In addition, the existence of a competing parallel loss from the initial reservoir due to the loss of TCA in the soil (microbial degradation), or on the foliage surface (chemical and/or biological degradation) before uptake into the sapling, must also be included.

The data were modelled according to the simple kinetic scheme illustrated in Figure 6.14 using the total mass of TCA, rather than concentration as the time-dependent

variable. Mass transfer from each compartment was assumed to follow first-order kinetics, and the first-order rate constants were the fitting parameters.

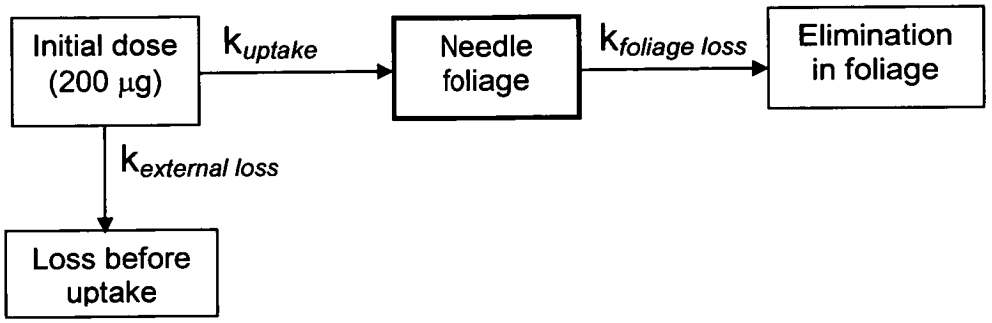
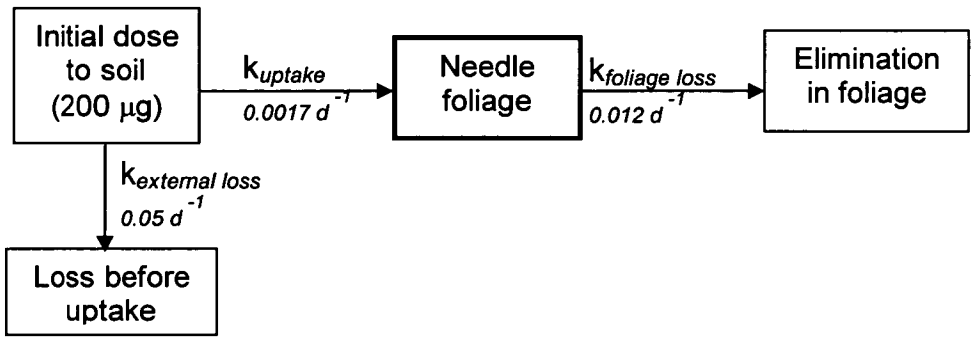


Figure 6.14. Kinetic scheme used to fit observed needle TCA concentration data.

Example fits to needle TCA concentrations with time are shown in Figure 6.12, and the corresponding fitted rate constant values for the TCA-S and TCA-F saplings are shown in the kinetic schemes in Figure 6.15. The inverse of each rate constant is the lifetime for the associated transfer process.

A fresh mass of 130 g needles for a 6 year Sitka spruce sapling was estimated from the data of Cannell *et al.* (1983) as described in Section 6.2.2.2. It was assumed that there was negligible extra contribution to total sapling TCA burden from branchwood and stemwood. Since, in reality, not all TCA dosed to the soil or the foliage is likely to be "available" TCA for uptake, the initial dose is not actually a well-characterised fixed parameter. In practice, the kinetic fitting was fairly insensitive to assumed values of available TCA between 150 and 200 μg per sapling, although, overall, the model was not very well constrained. Despite this, the following quantitative conclusions emerged from trials of model fits.

(a) Soil-dosed (TCA-S) saplings



(b) Foliage-dosed (TCA-F) saplings

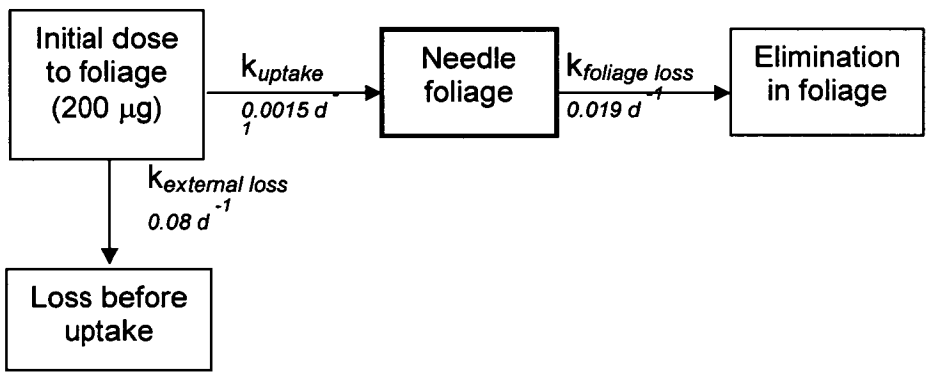


Figure 6.15. Kinetic scheme used to fit observed needles TCA concentration data for (a) TCA-S and (b) TCA-F saplings.

First, the data were best fit when there was provision for net loss of initial TCA in parallel to uptake into the needles. In the case of the soil-dosed experiment, this is taken to indicate chemical/biological degradation of TCA in soil. This is consistent with evidence from the long-term exposure experiment described in Section 6.2 and reported by Cape *et al.* (2003), as well as results of lysimeter experiments discussed in Chapter 5 which show that TCA added to soil cannot be fully recovered in leachate water. It is assumed that similar degradation occurs on the foliage surface.

Secondly, the values obtained from the fits both for the kinetic parameters, and the proportions of TCA passing through each compartment, are broadly consistent between both application regimes. Uptake of TCA into the needles has a first-order lifetime, in this experiment, of several hundred days (±100). This parameter is not of relevance to environmental situations since trees are continuously exposed to "fresh" TCA directly to the canopy or from the soil via TCA in precipitation and, possibly, soil sources. The model estimates of first-order lifetimes corresponding to within-foliage elimination rate, and the loss rate from the initial reservoir in parallel to uptake, are ~70 ± 40 days and ~ 20 ± 10 days, respectively. (Quoted ranges are approximate 95 % confidence intervals obtained in the non-linear fitting model and are large because of difficulties in constraining the model).

Thirdly, very approximately, after the 85 days of observation, the kinetic fits suggest that ~ 1 % of initial TCA dose applied per sapling was present in the needle foliage, ~2 - 7 % remained in the soil or externally on the foliage, ~89 - 95 % was lost externally before uptake, and ~2 - 3 % was eliminated within the needles. Regardless of undertaking kinetic modelling, it is evident from Figure 6.12 that only a small proportion (<1 %) of the initial dose of TCA applied was present in the needles after 85 days. This is in accordance with results from Experiment I (Section 6.2) where only a small proportion of the total mass of TCA applied to the system remained in the needles. Loss is presumed to occur via rapid degradation, for example within the soil, as discussed in Chapter 5 and reported by Forczek *et al.* (2001) from experiments where radioactively labelled [1,2-^{14}C] TCA was applied to Norway spruce saplings.

The needle TCA elimination half-life of ~50 days applies to an actively growing sapling. The elimination rate reported here is slower than that observed in Scots pine saplings by Sutinen *et al.* (1997) during an active growing season. They noted that during the four weeks after exposure of saplings to 500 ng TCA for 2 weeks, 5 times a week, needle concentrations decreased from a peak of 250 ng g^{-1} to 36 ng g^{-1}fwt, even although TCA was still being applied at an average of 1.3 times per week.

The observation of a direct canopy route of uptake of TCA from solution supports the findings of Experiment I. Thorough rinsing of the needles prior to analysis for TCA ensured that all TCA measured was intrinsic to the needle matrix and not adsorbed on the surface. A laboratory study by Reeves (2001) showed that there is no direct partitioning of TCA from solution through the needle cuticle. However, in Section 6.2.2.1, elevated TCA concentrations in branchwood were reported for saplings dosed with TCA directly to the foliage (L100Tf) which is further indication that TCA may be taken up initially through the branchwood and hen transferred to other parts of the sapling including the needles.

Benesch and Gustin (2002) obtained similar results for trifluoroacetic acid (TFA) after exposing the foliage of *Pinus ponderosa* saplings to TFA as a mist 5 times a week for 4 months. They found that needles accumulated TFA as a function of concentration and time, which demonstrates that uptake of TFA from rain and fog is a pathway by which TFA may concentrate in leaf tissue as well as via soil and roots.

6.3.3 Conclusions: Experiment II

After a single dose of TCA solution (1000 $\mu g\ l^{-1}$) to Sitka spruce saplings there was a clear accumulation of TCA in needles with time followed by subsequent elimination. This was evident in saplings dosed via either the foliage or the soil. The uptake of TCA into Sitka spruce saplings was almost comparable via above-ground or below-ground routes. Kinetic modelling of the data indicated that the half-life for within-needles elimination (during the growing season) was approximately 7 weeks.

6.4 CHAPTER CONCLUSIONS

Experiments described in this chapter have shown that TCA may be taken up into needles of Sitka spruce saplings via both the soil and foliage. Uptake by the soil has been previously well-documented but direct foliar uptake has been assumed to be minimal due to the lipophilic nature of coniferous needles. However, this research shows that TCA may first enter the foliage via the branchwood and stemwood before being relocated into the needles, rather than being taken up directly through the needle cuticle.

Changes in enzyme activity and protein content and greater visible damage were observed in saplings exposed to TCA via direct spraying to the foliage but saplings exposed to TCA via the soil were unaffected. This direct uptake by foliage is of particular concern regarding the health of forest stands in the natural conditions, as a considerable proportion of wet precipitation is intercepted by the forest canopy. Forests in the UK are frequently exposed to cloudwater for prolonged periods, which may have implications for tree health as cloudwater has often been reported to have a higher pollutant loading than rainwater, and it is more likely to remain adsorbed to the foliage for longer periods of time. TCA in saplings was eliminated slowly between two growing seasons which implies that over many years Sitka spruce trees may accumulate high levels of TCA.

There may be other effects of TCA on sapling health that appear over a longer period of time which have not been observed in this experiment. These may include a sapling's ability to adapt to environmental stresses such as frost, drought, nutrient deficiencies and disease. In the natural environment, trees are almost certainly exposed to several different pollutants in addition to TCA, therefore any effects on tree health are likely to be more complex and may not be attributable to any one pollutant species.

Chapter 7 – Research Conclusions

The research reported in this Ph.D study is a valuable contribution to current understanding of TCA cycling in the environment, particularly in the soil compartment. Detailed investigations of TCA in both laboratory and field conditions have built on previous knowledge of TCA analytical methodology, spatial variability of TCA in soil, magnitudes and rates of TCA degradation and production in soil and the routes of TCA uptake by trees and the effects on tree health. In the following sections the issues associated with different methods of TCA analysis are first of all discussed followed by the various findings reported from each chapter which are pulled together in an attempt to obtain a logical overview of how TCA is behaving in the environment as a whole.

7.1 METHODS OF TCA ANALYSIS

Throughout this research, the method of TCA analysis by headspace chromatography with electron capture detection (HSGC-ECD) has proved to be capable of determining TCA concentrations in a wide range of sample matrices (including soil, forest litter, needles, branchwood, soil and rainwater). Pre-analysis preparation time of samples is minimal and therefore the only limitation on the speed of sample throughput is the number of vials that can be analysed in one day (~65). This permitted all samples to be analysed in triplicate, as reported throughout this research. In contrast, extraction-derivatisation methods of TCA analysis are more limited by the availability of equipment and laboratory space, making it difficult to analyse more than two samples (in duplicate) per day (Matucha, 2003, *pers. comm.*). However, despite the comparable TCA concentrations of air, water and needles reported using both extraction-derivatisation and headspace techniques there still remains some uncertainty surrounding the reported concentrations of TCA in soil which can vary between geographical regions by up to 2 orders of magnitude, on a fresh weight basis, and up to 3 orders of magnitude on a dry weight basis.

Analysis of soil using HSGC-ECD avoids the issues of poor recovery often associated with extraction-derivatisation methods, although it has been suggested

that other compounds in the soil may form chloroform on heating between 60 and 100 °C for 1.5 hours, the experimental conditions used to convert TCA to chloroform for analysis by HSGC-ECD (Chapter 2). Throughout this research, the origin of chloroform analysed by HSGC-ECD is presumed to be solely from TCA in the soil (after accounting for background chloroform) although there has been not conclusive evidence to show this. There is therefore a possibility that reported "TCA concentrations" of soil and soil solutions in this research may actually be over-estimated, arising from more than one compound that may produce chloroform between 60 and 100 °C. However, the only other postulated chloroform precursor in soil is chloral hydrate ($Cl_3CCH(OH)_2$), which is known to be formed when trichloroacetaldehyde (Cl_3CCHO) enters water bodies from industrial discharges or as a by-product during the chlorination of water containing organic precursor molecules (Hoekstra *et al.*, 1999b). Trichloroacetyl-type precursors have been observed alongside the biogenesis of chloroform in the soil top layer, although their conversion to chloral has not been confirmed (de Leer, *et al.*, 1985; Hoekstra *et al.*, 1998a). Chloral hydrate is highly soluble in water and is therefore likely to be mobile in the soil. If chloral hydrate contributes to the high "TCA" concentrations detected in Ballochbeatties soils, via its conversion to chloroform, then the soil leachates from field lysimeters and other controlled greenhouse experiments (Chapter 5) could be expected to have high "TCA" concentrations. Since there was no significant difference between the input (rainwater or forest throughfall) and output TCA concentrations (soil leachates) of undosed lysimeters, this strongly suggests that chloral is not present at significant concentrations, if at all, in soil. Nevertheless, it is still possible that chloral is bound within soil to such an extent that it is only desorbed when the temperature is increased (i.e. during decarboxylation of "TCA"). It has also been suggested (Keppler, *pers. comm.*, 2003) that chloroform may be formed from compounds in the soil other than TCA due to the activation of microbial activity at temperatures greater than 90 °C. Reeves (2001) disproved this theory by decarboxylating subsamples of the same soil at either 65 ° C for 72 hours, or 100 °C for 90 minutes, and analysing both samples for chloroform. The same chloroform concentrations were obtained by either method. In this research (Chapter 5), humic acid, a fundamental component of soil which has been postulated to be

present in some of the proposed TCA production processes in soil, (Hoekstra *et al.*, 1995; Haiber *et al.*, 1996; Fahimi *et al.*, 2003) was found to contain similar concentrations of chloroform and TCA to distilled water when analysed using HSGC-ECD. This indicates that humic acid itself does not contain moieties which may form chloroform on heating to 100°C. However, it must also be noted that humic acid can exist in numerous complex forms and may differ in nature from the commercial humic acid used in this experiment.

Results of laboratory experiments (Chapter 3) to extract TCA from soil with water have provided good evidence that neither TCA intrinsic to the soil nor TCA externally applied to soil can be completely extracted with water. A 100 % mass balance was obtained (with the exception of Larch soil) when the sum of the TCA content of the extracted soil residue and aqueous extract was calculated and compared with the sum of the TCA added and the TCA already present in the soil at the start of the experiment. This strongly suggests that TCA concentrations detected by extraction-derivatisation methods underestimate whole soil TCA concentrations.

The relatively high TCA concentrations determined by HSGC-ECD analysis may, in fact, be evidence that soils from the Ballochbeatties catchment genuinely have greater TCA concentrations than those in the rest of Europe. In addition, most of the Ballochbeatties soils have particularly high water contents (up to 92 %) and organic matter contents (up to 52 % fresh weight and 99 % dry weight) compared to other soils analysed, therefore expressing soil TCA concentrations on a dry weight instead of a fresh weight basis results in apparently exaggerated TCA concentrations for very wet soils. Use of dry weight soil TCA concentrations may therefore be misleading as the overall catchment store of TCA is the same regardless of whether the soil is wet or dry since TCA is involatile.

Peters (2003) carried out a sampling survey of soils from eight European sites under open and forested land. Soil TCA concentrations determined using an extraction-derivatisation method of analysis are summarised in Table 7.1, and compared with soil TCA concentrations determined in this research by HSGC-ECD. Although

measured TCA concentrations of Ballochbeatties soils in this work are generally greater by at least one order of magnitude, the same trend was observed, of Sitka and Larch forest soils having higher TCA concentrations compared to open land, even excluding the high Sitka litter concentrations.

Table 7.1. TCA concentrations (dwt) of Ballochbeatties soils analysed using the HSGC-ECD method, compared to TCA concentrations of soil from 8 European sites reported by Peters (2003) and analysed using an extraction-derivatisation method. Soil TCA concentrations are shown for soils from both forest and open land.

		Soil TCA concentration / ng g^{-1} (dwt)				
Analyst	**Soil type**	**Mean**	**Standard deviation**	**Median**	**Range**	**n**
Ballochbeatties	Sitka forest	488	589	268	25 - 2000	39
	Larch forest	61	53	43	11 - 231	17
Peters (2003)	*All forest*	*0.97*	*2.3*	*0.22*	*<0.05 - 12*	*24*
Ballochbeatties	Open land	60	58	42	9 - 196	26
Peters (2003)	*Open land*	*0.26*	*0.40*	*0.16*	*<0.05 -1.0*	*24*
Ballochbeatties	All soils	264	459	78	9 - 2000	82
Peters (2003)	*All soils*	*0.61*	*1.7*	*0.20*	*<0.05 - 12*	*48*

In addition to the clear differences in measured TCA concentrations of soil by the HSGC-ECD and extraction-derivatisation techniques, variations in the specific methodology of each basic technique also exist between research groups. Examples of these include, for extraction-derivatisation methods, the pH of the aqueous solution used to extract soil TCA and the specific organic compound used for derivatisation, and, for HSGC-ECD methods, the decarboxylation time and temperature programmes used. It could be argued that the fraction of TCA in the environment that is most cause for concern is the water-extractable fraction measured by extraction-derivatisation methods. TCA bound to soil is less mobile and therefore less available for plant uptake. However binding may be temporary, and, with variations in climate and soil conditions (pH in particular), this TCA may eventually be mobilised becoming available for plant uptake, or leaching into water courses or degradation.

Due to the presence of TCA in soil at trace concentrations, it is difficult to assess the efficiency of measurements of soil TCA using any available method, without externally adding TCA to determine its recovery. It is highly probable that externally applied TCA behaves differently to TCA already present "naturally" in the soil, as has frequently been discussed throughout this research.

In summary there is, so far, insufficient scientific evidence to draw valid conclusions about the overall accuracy of one particular method of TCA analysis in soil. Although the TCA concentrations of other environmental matrices may continue to be determined with confidence, using either HSGC-ECD or extraction-derivatisation, further work needs to be carried out to investigate the analysis of TCA in soil. Ideally, a standard method will be developed where TCA can be accurately and directly determined in the whole soil and uniquely identified, enabling direct comparisons to be made between research groups.

7.2 Storage of TCA in soil

If it is assumed that TCA in soil originates solely from atmospheric inputs, the "expected" TCA concentrations in soil from the Ballochbeatties catchment can be estimated from the known TCA inputs via rainwater and cloudwater. Stidson *et al.* (2004a) reported that the total inputs of TCA to the catchment via rainwater and cloudwater were 1804 g and 275 g TCA, respectively, from May 2001 to May 2002. Over the catchment area of 0.84 km^2, this is equivalent to a total combined TCA input of 2480 $\mu g\ m^{-2}$ of soil. Assuming that, over a year, this water penetrated to a depth of 30 cm in soil of density 1 $g\ cm^{-3}$ (fwt) then the soil TCA concentration would be approximately 8 $ng\ g^{-1}$ (fwt). In comparison, the mean TCA concentration of all soils sampled at Ballochbeatties between 2001 and 2003 was 48 (± 85) $ng\ g^{-1}$ (fwt) which is six times greater than the expected concentration. The median TCA concentration of 15 $ng\ g^{-1}$ (fwt), which excludes the high concentrations of up to 400 $ng\ g^{-1}$ (fwt) in Sitka litter layers is much closer to the estimated value. However, Stidson *et al.* (2004b) measured significant depths of litter, from 4 to 14 cm (median = 11 cm, n = 5) under Sitka spruce, which account for approximately 40 % of the Ballochbeatties catchment area. Excluding the TCA concentrations of Sitka litter

from the calculation may lead to considerable underestimation of the true soil TCA store. This calculation implies that more TCA is present in the soil than can be accounted for by atmospheric inputs alone.

The soil data for the Ballochbeatties catchment presented in Chapter 4 were used in a study by Stidson *et al.* (2004a) to quantify the total external TCA fluxes and stores of all key compartments of the Ballochbeatties catchment from May 2001 to May 2002 (Figure 7.1). This was achieved by measuring TCA concentrations of all the possible TCA inputs (wet and dry deposition) and TCA outputs (streamwater) to and from the catchment every two weeks for one year. Since the Ballochbeatties catchment overlies relatively impermeable geology it was assumed that there was no loss to groundwater. TCA concentrations were also determined in tree foliage, branchwood and stemwood, forest litter layers, moorland foliage and soil.

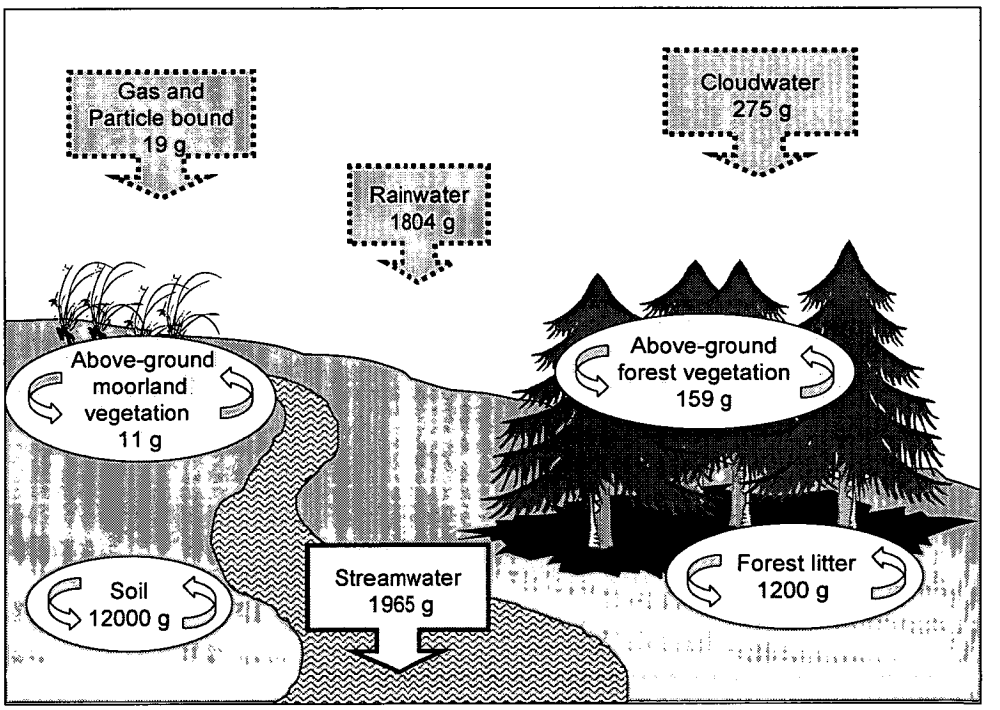


Figure 7.1. The input (dashed boxes) and output (solid box) fluxes of TCA and the mass of TCA stored (oval boxes) within the Ballochbeatties catchment during the period May 2001 – May 2002 (Stidson *et al.*, 2004a). Within-catchment cycling is indicated by curved arrows.

An annual TCA input flux of 2098 g (wet and dry atmospheric deposition) and output flux of 1965 g of TCA (in streamwater) were estimated. After examination of the uncertainties associated with each measurement, the TCA inputs and outputs were considered to be equal. The TCA store in the catchment was estimated to be 13 kg which is approximately six times the annual external fluxes. This store was dominated by soils (12 kg) followed by forest litter, forest branchwood and stemwood, forest needles and moorland vegetation (Table 7.2). Any uncertainties in soil TCA concentrations will therefore have a large impact on the catchment store calculations.

Table 7.2. Summary of TCA masses present in each measured environmental compartment in the Ballochbeatties catchment (Stidson *et al.*, 2004a).

Compartment		**Catchment TCA store / g**
Sitka spruce *(Picea sitchensis)*	Needles (years C, C+1 and C+2)	61
	Branchwood (years C, C+1 and C+2)	18
	Stemwood	55
Larch *(Larix x eurolepis)*	Needles	7
	Branchwood	8
	Stemwood	10
Moorland vegetation *(Calluna vulgaris, Vaccinium myrtillus, Nardus Stricta, Molinia caerulea, Scirpus cespitosis*		11
Sitka spruce litter		1180
Larch litter		47
Soils (Sitka, Larch, Moorland)		12000
TOTAL		**13397**

This estimated Ballochbeatties catchment soil TCA store assumes that analysis of chloroform from thermal decarboxylation of TCA is an accurate measurement of the TCA concentrations in soil. If soils from the Ballochbeatties catchment had the same mean TCA concentration of 0.61 (± 1.7) ng g^{-1} (dwt) as determined by Peters (2003) using the extraction-derivatisation technique (Table 7.1), the total catchment TCA store would be approximately 200 g, nearly 60 times lower than that determined from TCA concentrations using the HSGC-ECD method. This would mean that the annual catchment TCA flux is approximately 1.3 times *greater* than the catchment

TCA store (TCA in vegetation, litter and soil). However, using the highest concentration measured by Peters (2003) of 12 ng g^{-1} (dwt), the annual catchment TCA flux is 2.7 times *lower* than the catchment TCA store of 5000 g. Therefore, even using the results from the same analysis method, conflicting conclusions may be drawn showing that the uncertainty in the mass of soil in the catchment is small compared with uncertainty in TCA concentrations within the soil. In the analysis of Stidson *et al.* (2004a), soil TCA comprises approximately 96 % of the total TCA mass in the Ballochbeatties catchment and is therefore crucial to the understanding of TCA fluxes and stores in the environment. Over- or under-estimating the soil TCA concentrations in the catchment may lead to different interpretations of within-catchment TCA processes.

Other researchers (McCulloch, 2002; Folberth *et al.*, 2003; Hoekstra, 2003; Schöler *et al.*, 2003) have carried out approximate catchment-budget calculations from smaller data sets which also indicate that the soil store of TCA is large in relation to reported inputs and outputs of TCA to the terrestrial environment.

7.3 TCA PRODUCTION AND DEGRADATION IN SOIL

The large store of TCA in the soil exists in spite of the abundant evidence (Chapter 5) that TCA added to soil is degraded. The experiments reported here show that TCA degradation occurs mostly by the action of micro-organisms, is observed immediately after TCA application, and occurs fairly rapidly. The estimated half life of [1,2-^{14}C] TCA (~380 ng g^{-1} (fwt)) externally applied to different soils were 21, 45 and 46 hours for Moorland B, Sitka O1 and O2 and Larch B-horizons respectively, at room temperature. Other studies carried out on the herbicidal properties of TCA (summarised in Foy, 1975) reported half-lives of several weeks which may reflect the higher masses of applied TCA (up to 6 μg g^{-1} of fresh soil), different soil types and different experimental conditions. Assuming that either of these timescales is realistic, then, in order to maintain the observed soil TCA concentrations there must be a steady input of TCA to the soil, over and above TCA deposited via atmospheric deposition alone. In addition, "control" field lysimeters indicated that TCA inputs to

soil via atmospheric deposition and/or forest throughfall, and TCA outputs in soil leachate water were in balance. Thus, if degradation occurs, as has been previously established, there must be a process occurring in the soil or soil water that compensates for this "loss" of TCA.

The proposed additional input of TCA to the soil may be from natural formation of TCA within the soil, as indicated by laboratory experiments where "TCA-free" soil was re-wetted with ultrapure water or NaCl solution. In one day, TCA production of 10, 16 and 37 ng g^{-1} (fwt) in re-wetted soil was observed in Moor, Larch and Sitka soils respectively. This suggests that either microbial activity or TCA-forming chemical reactions are stimulated by the re-wetting of soil. The greater TCA production in Sitka soils may correspond to typical field conditions where the upper Sitka layers are generally more freely draining, have low dry bulk densities (<0.1 g cm^{-3}), are exposed to the atmosphere and therefore likely to dry out more quickly than deeper soil horizons and in other soil types. Cycles of drying and re-wetting of the Sitka litter layer may stimulate TCA production and partly explain the high TCA concentrations detected.

TCA production is hypothesised to occur via the action of CPO enzymes on chloride ions and organic matter substrates in soil (Hoekstra and de Leer, 1993; Hoekstra *et al.*, 1995; Haiber *et al.,* 1996; Hoekstra *et al.,* 1998b, 1999a,b) or a natural abiotic route (Hoekstra *et al.*, 1995, Haiber *et al.*, 1996) involving the oxidation of chloride ions by hydrogen peroxide (H_2O_2), which may be enhanced by the addition of Fe (III) (Fahimi *et al.*, 2003).

Fahimi *et al.* (2003) also demonstrated that TCA may be formed from humic acid prepared with bidistilled water, without the addition of hydrogen peroxide or chloride ions. This opposes the findings of this research (Section 3.8) where there was no observed TCA or chloroform formation from humic acid prepared in de-ionised water after 0, 2 or 11 days storage. If the increase in TCA observed by Fahimi *et al.* (2003) was due to natural TCA formation then the different conclusions generated by the two studies must arise from the different compositions of the water

used to prepare the solutions. Fahimi *et al.* (2003) suggested that the observed TCA formation was from impurities of up to 0.9 % Fe and 1.5 % chloride present in the humic acid from the manufacturing process which reacted with organic matter. They also used humic acid concentrations up to 300 times greater than those normally found in nature (20 μg l^{-1} in groundwater to 30 mg l^{-1} in surface water) by Thurman and Malcolm (1981, cited in Aiken, 1985) compared to 64 mg l^{-1} in this research. Higher concentrations of humic acid will inevitably mean higher concentrations of impurities and potential precursors to TCA formation.

Although there are many indications of TCA formation directly from controlled laboratory experiments, or indirectly from field measurements of potential TCA precursors or by-products, there is no direct evidence of TCA formation in the field under natural conditions.

In the Ballochbeatties catchment, Sitka soil (below the litter layer) had a mean TCA concentration of 47 (± 76, n = 26) ng g^{-1} (fwt), and Sitka litter layers had a mean TCA concentration of 173 (± 128, n = 13) ng g^{-1}(fwt), with a combined mean of 89 (± 112, n = 39) ng g^{-1} (fwt). This is about twice the mean TCA concentration of needles on the tree of 40 ng g^{-1} (fwt), (range = 14 – 123 ng g^{-1} n = 30) reported by Stidson *et al.* (2004a). If TCA concentrations are expressed on a dry weight basis, the TCA concentrations in Sitka soil increase even more, relative to actively growing needles, by up to two orders of magnitude as actively growing needles had a mean moisture content of 56 (± 6, n = 30) %, compared to Sitka soils in which the moisture content can be as high as 90 %. In Chapter 6 it was reported that there is continuous elimination of TCA in the needles of Sitka spruce saplings. If TCA is still entering the forest soil via atmospheric deposition, forest throughfall and needle fall, but is not being eliminated in dead litter as the needles detoxification mechanism are no longer active, then TCA may be accumulating at a greater rate than it is being eliminated. It is also possible that there is an additional process occurring in the needles when they start to decompose on the forest floor, which results in an increase in TCA concentration over and above the rate of TCA degradation. Laturnus *et al.* (1995) observed high chlorination activity in the soil organic layer and about 100-

fold lower chlorination activity in deeper soil layers (15 – 180 cm) which suggests that TCA may be produced in the litter layers via proposed routes of natural TCA formation from the reaction of inorganic chloride with humic material by a chloroperoxidase mediated reaction (Hoekstra *et al.*, 1995; Haiber *et al.*, 1996; Hoekstra *et al.*, 1999a,b; Niedan *et al.*, 2000). However in this research, laboratory experiments with "TCA-free" soil (Chapter 5) found no increase in TCA production when NaCl was present instead of ultrapure water.

The observation of a highly significant positive linear relationship between soil microbial biomass (SMB) and soil TCA concentrations for a range of different soil types is interesting, as initially it suggests that microbial activity may be responsible for TCA production in soils, in spite of the substantial evidence for the presence of micro-organisms in soil which degrade TCA. The observed SMB concentrations include all species of bacteria and fungi present in the soil of which only a few may be actively involved in TCA cycling. It is not possible to determine what proportion, if any, of this activity is associated with TCA production or degradation, but there is no doubt that it is an indication of the dynamic nature of TCA in soil. It is evident that Sitka litter layers are more active, in terms of TCA turnover, than deeper horizons and in other soil types investigated. However, although micro-organisms may be involved in the "production" of TCA, TCA is also a by-product of other compounds present in the soil, i.e. TCA is a metabolite. It is therefore possible that high TCA concentrations are observed in Sitka litter layers because high concentrations of natural or anthropogenic TCA precursors in this litter occur here. The known anthropogenic precursors, 1,1,1-trichloroethane (TCE) and tetrachloroethene (PER), are relatively insoluble and are unlikely to partition into rain or cloudwater and their routes to foliage or soil will therefore be via gas-phase transfer. Other, soluble precursors (so far unidentified) may be more concentrated in litter layers due to their accumulation on needle surfaces from atmospheric deposition, and subsequent wash-off and deposition on the soil surface from forest throughfall water.

When the biodegradation of [1,2-^{14}C]TCA added to soil (Section 5.5.1) was quantified by measuring the evolution of $^{14}CO_2$, the initial rate of degradation was lower in the organic layers (O1 and O2) of Sitka soils than in Larch and Moor soils. Additionally, as the half-life was almost twice as long as in the Moor soil it is tempting to suggest that TCA production processes are more dominant in Sitka soils than degradation processes resulting in higher soil TCA concentrations. However, other experiments presented in this research where TCA was added to sterile and non-sterile soils suggested that TCA degradation was more complete in Sitka soil than in Larch soil at the end of the experiment (23 days). Also, micro-organisms re-colonised sterile Sitka soils more quickly than sterile Larch soils when they were exposed to the atmosphere. From this it is apparent that cycling of TCA in soil is very complex and probably depends on the species of micro-organisms and other chemical compounds present in the soil which may encourage TCA production or degradation and govern the relative rates of each process.

Reasons for differences in TCA concentrations between different soil types are not clear, but there are some indications that soils with higher organic matter contents also have greater TCA concentrations. Öberg *et al.* (1997) hypothesised that micro-organisms produce reactive chlorine which can chemically degrade relatively resistant organic matter such as lignin with organic chlorine being produced as a by-product. This suggests that the composition of soil organic matter may be an important factor which influences the production or degradation of TCA and, hence, its net concentration in soil. Soil pH may also affect the rate of suggested natural TCA production in soil. At Ballochbeatties the soil pHs were lowest in the forest soils (pH 3.5 – 4.0), and greatest in moorland soils (4.0 – 5.0). These lie within the reported pH range for optimum activity of chloroperoxidase enzymes, believed to be involved in TCA production, of pH 3 – 4 (Asplund *et al.*, 1993; Haiber *et al.*, 1996), although Juuti and Hoekstra (1998) and Hoekstra *et al.* (1999b) claim this range extends to pH 6. It is clear that the typical organic matter contents and pHs of Ballochbeatties soils favour the natural production of TCA in soil which may partly explain the high TCA concentrations measured in these soils.

The lower TCA concentration detected in soil water (e.g. lysimeter leachates) compared to the associated soil is further evidence of the complexity of TCA cycling in the soil system. This observation supports the results of laboratory experiments in Section 3.3 where only a fraction of applied TCA could be extracted from the soil with ultrapure water. From this it was concluded that TCA is bound to soil to such an extent that it can only be desorbed when heated to 100 °C. Alternatively, the lower TCA concentration of soil water may be due to rapid degradation of TCA as it percolates through the soil.

7.4 ENVIRONMENTAL IMPLICATIONS OF TCA

It was demonstrated in this research that the direct foliar uptake of TCA by trees is a more significant route than previously thought. Changes in protein content and enzyme activities of Sitka spruce needles exposed to regular foliar inputs of TCA at 10 $\mu g\ l^{-1}$ and 100 $\mu g\ l^{-1}$ suggest that even at TCA concentrations similar to those of natural precipitation, TCA may adversely affect tree health. Although only saplings exposed to 100 $\mu g\ l^{-1}$ TCA via the foliage showed visible signs of damage, this was over a relatively short time period of two 5-month growing seasons whereas in the natural environment trees are subjected to TCA all year round from atmospheric deposition. Sitka spruce trees in U.K. plantations are usually harvested after 40 – 60 years, over which time significant concentrations of TCA may build up in the foliage, potentially to phytotoxic levels. In addition, Sitka spruce trees retain their needles for 6 – 8 years (Cannell, 1987) which implies that these trees are potentially a large store of TCA. Coniferous trees are particularly efficient collectors of cloud droplets relative to moorland vegetation (Fowler *et al.*, 1989) and can therefore greatly increase the deposition of pollutants at high altitude by direct interception of cloudwater (Crossley *et al.*, 1992). Since Sitka spruce trees thrive in moist soils, they are typically found in areas of high rain and cloudwater deposition. Cloudwater can be a particularly damaging source of pollutants as, unless the cloud episode is of prolonged duration or is followed directly by rain, droplets deposited onto leaf surfaces evaporate and successive fogs can lead to increasing dry deposition accumulation (Jagels, 1991). As TCA is non-volatile its concentration on the surface of Sitka spruce needles is likely to increase until it is washed off by rain. It is

difficult to quantify the true extent of TCA damage to trees in the natural environment where trees are exposed to all kinds of environmental stresses including variable weather conditions (e.g. extremes of temperature, periods of drought or waterlogging), exposure to other pollutants, disease and pests. It is likely that observed forest damage (Frank *et al.*, 1990, 1992; Norokorpi and Frank, 1995) is from the cumulative effects of several pollutants acting individually, or the formation of other, more phytotoxic compounds resulting from chemical reactions of these pollutants in the vegetation. The high soil TCA concentrations (up to 400 ng g^{-1}, (dwt)) detected in the litter layers of Sitka spruce forests are also cause for concern as the Sitka spruce sapling experiments show that TCA applied to the soil may also be taken up via the roots. Although there were no visible or observed physiological effects in saplings dosed with TCA via the soil over the experimental period, effects of continual soil uptake of TCA over a number of decades are not known.

Ahlers *et al.* (2003) assessed the potential environmental risk of TCA, particularly in relation to plant uptake from soils reported to have high TCA concentrations. Belkov and Semenova (1973, cited in Ahlers *et al.*, 2003)) conducted a study with pine and spruce seedlings from which they determined a 60-day EC_{10} (the concentration of TCA required to produce 10 % of the possible effect of TCA) of 120 μg kg^{-1} (dwt) TCA for reduction of root weight, although neither the method of TCA exposure, nor how the value was derived are stated. From this, Ahlers *et al.* (2003) derived a $PNEC_{soil}$ (predicted no effect concentration) for seedlings of 2.4 ng g^{-1} (dwt) soil TCA which is significantly lower than TCA concentrations determined in soil at Ballochbeatties. However, 60 days cannot be considered sufficient to determine the true risk that TCA may pose to Sitka spruce or pine trees in the natural environment.

There is abundant evidence that TCA in the atmosphere may be formed from photo-oxidation of 1,1,1-trichloroethane (TCE) and tetrachloroethene (PER) and it has also been proposed that TCA can be formed from these precursors *in-situ* in the soil or vegetation as a detoxification mechanism of the P-450 monooxygenase enzyme (Frank *et al.*, 1992; Plümacher and Schröder, 1994; Plümacher, 1995).

Environmental concern should therefore not be restricted to the phytotoxic effects of TCA alone, but also to its potentially environmental damaging precursors, of which TCA is an indicator. A good understanding of TCA behaviour in soil and breakdown products is therefore important for accurately assessing the environmental risks posed by TCE and PER.

One of the weaknesses in most TCA research is the short period of study relative to the life-span of trees. Controlled studies of TCA uptake by conifer trees and associated health effects have been conducted for a maximum of two years (Schröder *et al.*, 1997; Sutinen *et al.*, 1997; Forczek *et al.*, 2001; Matucha *et al.*, 2001; Cape *et al.*, 2003; Heal *et al.*, 2003b; Matucha *et al.*, 2003; Dickey *et al.*, 2004) and mass balance studies in the field have generally been estimated from data collected from variety of sources over one or two years (McCulloch, 2002; Folberth *et al.*, 2003; Hoekstra, 2003; Schöler *et al.*, 2003; Stidson *et al*, 2004a,b). These studies only provide a snapshot of TCA concentrations over a particular period of time, from which it is difficult to determine how TCA concentrations alter, if at all, in response to changing environmental conditions. If a long-term study was carried out which monitored TCA concentrations over several decades, it may be possible to determine if changes in TCA concentrations of soil and conifer needles reflect changes in atmospheric concentrations of TCA and the precursors TCE and PER since the total global emission fluxes of these compounds have been continually declining since the early 1990s (Sidebottom and Franklin, 1996). It may also be possible to see if changes in TCA concentrations in conifer needles reflect changes in soil TCA concentrations. As the manufacture of TCE was banned in most countries in the late 1990s, it should be possible to elucidate over the next few years the main anthropogenic source of TCA. However, environmental processes are complex and constantly evolving so any explanations for changes in soil or vegetation TCA concentrations over time must be treated with care, as they may not be related to concentrations of any one particular precursor. In addition, subtle temporal changes in soil properties such as moisture, temperature, depth of litter layer and nutrient status may affect the rate of TCA production and degradation processes.

7.5 OVERVIEW AND KEY UNCERTAINTIES

It is evident from this research that TCA is actively cycled within the environment although it is difficult to observe temporal changes in soil TCA concentrations because of the highly heterogeneous nature of soil and relatively short study period (~2 years). Despite this, there is evidence from field lysimeter experiments that TCA concentrations in the environment are in equilibrium and if perturbed (e.g. by artificial addition or removal of TCA) they return to a steady state concentration. In other words, soil may act as a buffer towards TCA.

Many consistent trends in TCA behaviour in the soil have become apparent throughout this research, such as "loss" of applied TCA in soil, low concentrations of TCA in soil water compared to the soil itself and the high TCA concentrations detected in Sitka litter layers. However, there are also confounding results such as the high degradation rates of TCA in Sitka soils despite the strong positive relationship observed between soil microbial biomass (SMB) and TCA concentration, and the greater production rates of TCA in Sitka soils (Section 5.6.2) compared to Larch and Moor soils.

This research has contributed significantly to the understanding of TCA cycling in the soil compartment and its uptake and effects on Sitka spruce trees. However, it has also highlighted the differences in soil TCA concentrations reported by different research groups, the difficulties of estimating the soil TCA store due to soil spatial heterogeneity and the challenges involved in quantifying of the rates of TCA production and degradation in soil at environmental concentrations. Key uncertainties which still need to be addressed are behaviour TCA intrinsic to the soil in environmental conditions and the specific role of micro-organism activity, especially in TCA production.

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APPENDICES

APPENDIX A

Calculation of TCA concentrations in aqueous samples

As described in Chapter 2, the TCA concentration of an aqueous sample is quantified from the analysis of chloroform produced in a sealed vial from the thermal decarboxylation of TCA in the sample. An aliquot of chloroform in the headspace is transferred by an HS40-XL headspace autosampler to a Perkin-Elmer Autosystem GC with an electron-capture detector (HSGC-ECD).

Order of analysis for aqueous samples
The maximum number of vials that can be analysed by HSGC-ECD in one carousel is 40. All vials are heated to 100 ° before analysis, except for sample "blanks" which are heated to 60 ° to enable quantification of background chloroform. A typical analysis run includes:

- 6 samples of ultrapure water (the first one is always discarded) as standard calibration solutions are prepared using this water.
- Typically, 6 calibration standard solutions of two known TCA concentrations (3 replicates of each). Periodically, 9 calibration standards of three known concentrations (and therefore only 6 samples) are used to check for linearity of the detector response.
- 7 samples of interest of unknown TCA concentration: 4 vials for each unknown sample; one for determination of background chloroform ("blanks" heated to 60 °C only) and 3 sample replicates for determination of chloroform produced from TCA in the sample (heated to 100 °C).

Calculation of TCA concentrations in an aqueous sample from chloroform peak areas
An example analysis run for the determination of TCA concentrations of aqueous samples is shown on page 263. From this data, the calculations used to determine the final TCA concentrations of a Control lysimeter leachate sample (Cell B14) are shown below:

1) Mean chloroform peak area of ultrapure water

= CellF11 + F12 + F22 + F32 + F47
= 164751

2) Response factor for each calibration standard solution
e.g. for CalA1, Cell A9

Response factor
= Attenuation x [(Cal A1 peak area - mean ultrapure water peak area) / concentration of CalA1] x mass of CalA1 in vial
= 1 x (3056444 – 164751) / 15.3 x 5.0215

= 37638 (arbitrary units)

3) Mean response factor for all standard calibration solutions

= 36166

4) Corrected peak area of each aqueous sample

= Attenuation x [(sample peak area (100°C) x standard mass/actual mass) – (blank sample peak area (60°C) x standard mass / actual mass)]
= 1 x [(126546 x 5 / 5.0249) – (14303 x 5 / 5.0249)]
= 111687

5) Final TCA concentration of the aqueous sample

= Corrected peak area of sample / mean response factor
= 111687 / 37638
= ***3.08 ng g^{-1}*** (or μg l^{-1})

This is the final TCA concentration of an aqueous sample. The same calculations are repeated for the other two sample replicates and the mean taken.

For aqueous solutions the partition ratio of chloroform is expected to be the same as for standard solutions and therefore no correction factor is necessary.

Appendix A: Example calculations of TCA concentrations in aqueous samples

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
2	Sample type	Bradan lysimeter water				Sample collection:		07-Mar-02		Attenuation	1		St. mass / g	5
5														
6	Sample ID		Code		Mass / g	Chloroform peak area	Corrected peak area	Response Factor	TCA (ng)	TCA (ng g^{-1})				
7							Attenuation * ((sample area/sample mass)-(sample blank area/sample blank mass)))	Attenuation * ((Cal area - water$_{100}$ area) / (mass of TCA in vial))	(Peak area / average response factor) * partition ratio	Mean	sd	CI	n	rsd
8	W0		02 C9	001		182500								
9	CAL A1			002	5.0215	3058444		37638						
10	CAL B1			003	5.0132	542350		37660						
11	W1			004	5.0249	101339								
12	W2			005	5.0249	198853								
13		BLK		006	5.0249	14303								
14	Control	1		007	5.0249	126546	111687		3.08					
15		2		008	5.0249	150582	135604		3.74					
16		3		009	5.0249	126558	111798		3.08	3.30	0.38	0.94	3	12
17		BLK		010	5.0249	28754								
18	Low 1	1		011	5.0249	727395	695179		19.17					
19		2		012	5.0249	745571	713265		19.67					
20		3		013	5.0249	774387	741938		20.46	19.77	0.65	1.61	3	3
21	CAL A2			014	5.0388	3068799		37668						
22	W3			015	5.0249	169017								
23		BLK		016	5.0249	16786								
24	Low 2	1		017	5.0249	554484	535034		14.76					
25		2		018	5.0249	467868	448845		12.38					
26		3		019	5.0249	507472	488254		13.47	13.53	1.19	2.95	3	9
27		BLK		020	5.0249	7786								
28	Low 3	1		021	5.0249	425075	415219		11.45					
29		2		022	5.0249	465857	455799		12.57					
30		3		023	5.0249	390541	380856		10.50	11.51	1.03	2.57	3	9
31	CAL B2			024	4.9936	495639		33130						
32	W4			025	5.0249	207520								
33		BLK		026	5.0249	15316								
34	High 1	1		027	5.0249	947249	927313		25.57					
35		2		028	5.0249	968001	947962		26.14					
36		3		029	5.0249	962935	962822		26.55	26.09	0.49	1.22	3	2
37				030	5.0249	15201								
38	High 2			031	5.0249	907468	887846		24.49					
39				032	5.0249	904741	885132		24.41					
40				033	5.0249	864614	845204		23.31	24.07	0.66	1.63	3	3
41				034	5.0249	19055								
42	High 3			035	5.0249	812888	789899		21.78					
43				036	5.0249	860964	857041		23.64					
44				037	5.0249	829904	806831		22.25	22.56	0.96	2.39	3	4
45	CAL A3			038	5.0249	2988871		36734						
46	CAL B3			039	5.0032	512288		34731						
47	W4			040		148927								
48														
49			Mean ultrapure water			164751	Mean Response Factor	36166						
50							sd	1908						
51							% rsd	5.3						

APPENDIX B

Calculation of TCA concentrations in soil

As described in Chapter 2, the TCA concentration of a soil sample is quantified from the analysis of chloroform produced in a sealed vial from the thermal decarboxylation of TCA in the sample. An aliquot of chloroform in the headspace is transferred by an HS40-XL headspace autosampler to a Perkin-Elmer Autosystem GC with an electron-capture detector (HSGC-ECD).

This procedure is the same for analysis of vegetation with the exception of the specific partition ratio used (1.94 for needles; 1.25 for soil).

Order of analysis for soil samples
The maximum number of vials that can be analysed by HSGC-ECD in one carousel is 40. All vials are heated to 100 °C for 1.5h before analysis of headspace, except for water and soil "blanks" which are heated to 60 °C only to enable quantification of background chloroform. A typical analysis run includes:

- 5 samples of ultrapure water (the first one is always discarded) since standard calibration solutions are prepared using this water.
- 2 samples of ultrapure water ("blanks"), heated to 60 °C only in order to determine the background chloroform. From these the TCA concentration of the ultrapure water is determined since 1 ml of this water is added to soil samples.
- 5 calibration standard solutions of two known TCA concentrations (3 replicates of concentration A and two replicates of concentration B). Periodically, a third calibration standard of a different TCA concentration (and therefore only 6 samples analysed) is also used to check for linearity of the detector response.
- 7 soil samples of interest of unknown TCA concentration: 4 vials for each unknown sample; one for determination of background chloroform ("blanks", heated to 60 °C only) and 3 sample replicates for determination of chloroform produced from TCA in the soil (heated to 100 °C only).

Calculation of TCA concentrations in a soil sample from chloroform peak areas
An example analysis run for the determination of TCA concentrations of soil samples is shown on page 267. From this data, the calculations used to determine the final TCA concentrations of Ballochbeatties soil sample (Upper Hill 10- 20 cm, Cell B14) are shown below:

1) **Response factor for each calibration standard solution**
e.g. for CalA1, Cell A10

Response factor
= Attenuation x [(Cal A1 peak area - mean ultrapure water peak area) / concentration of CalA1] x mass of CalA1 in vial

= 2 x [(292226 – 51251) / (10.2 x 1.0055)]
= 46992 (arbitrary units)

2) Mean response factor for all standard calibration solutions
= 42339

3) TCA concentration of ultrapure water

a) Mean chloroform peak area of ultrapure water
= Cell F12 + F22 + F32 + F48
= 51251

b) Mean peak area of ultrapure water "blank"
= Mean of Cell F45 and F46
= 18816

c) Corrected peak area of ultrapure water (subtracting blank)
= Attenuation x (mean ultrapure water peak area – mean ultrapure water blank peak area)
= 2 x (51251 – 18816)
= 64871

d) TCA concentration of ultrapure water
= Corrected peak area of ultrapure water / Response factor
= 64871 / 42339
= 1.5 ng g^{-1} (μg l^{-1})

4) Corrected peak area of each soil sample

= Attenuation x [(sample peak area (100°C) x standard mass/actual mass) – (blank sample peak area (60°C) x standard mass / actual mass)]
= 2 x [(307875 x 1 / 1.0197) – (17297 x 1 / 1.0073)]
= 569511

5) Final TCA concentration of the soil sample

= [(Corrected peak area of sample / mean response factor) x soil partition ratio] – TCA concentration of 1 ml ultrapure water in sample
= [(569511 / 42339) x 1.25] – 1.5
= 16.8 – 1.5 ng g^{-1} (fwt)
= ***15.3 ng g^{-1} (fwt)***

This is the final fresh weight TCA concentration of the soil sample. These calculations are repeated for the other two replicates and the mean taken.

Appendix B: Example calculations of TCA concentrations in soil samples

2	Sample type	BRADAN SOILS - Winter survey				Sample collection:		21-Jan-01		Attenuation	2		Partition ratio	1.25	
3						Preparation:		01-Feb-02		Conc Cal A	10.2	ppb	St. mass	1	
5															
6															
7	Sample ID		Code		Mass / g	Chloroform peak area	Corrected peak area	Response Factor	TCA (ng)	TCA (ng g^{-1})	TCA (ng g^{-1})			rsd	
8							Attenuation * ((sample area/sample mass)-(sample blank area/sample blank mass)))	Attenuation * ((Cal area - water$_{100}$ area) / (mass of TCA in vial))	(Peak area / average response factor) * partition ratio	(TCA ng in sample-averageTCA in water) / standardised mass sample	Mean	sd	CI	%	
9	W0		02 B1	001		70652									
10	CAL A1			002	1.0055	292226		46992							
11	CAL B1			003	1.0007	1021429		38320							
12	W1			004		52638									
13		BLK		005	1.0073	17297									
14	Upper Hill 10-20 cm	1		006	1.0197	307875	569511		16.81	15.28					
15		2		007	0.9871	300254	574012		16.95	15.41					
16		3		008	1.0091	256999	475020		14.02	12.49	13.95	2.07	18.56	15	
17		BLK		009	1.0007	32373									
18	Upper Hill 2-10 cm	1		010	1.0128	426525	777568		22.96	21.42					
19		2		011	0.9870	433948	814627		24.05	22.52					
20		3		012	0.9811	434319	820671		24.23	22.70	22.61	0.13	0.31	1	
21	CAL A2			013	1.0059	293000		47124							
22	W2			014		60002									
23		BLK		015	1.0035	24570									
24	Sitka Road 3-11 cm	1		016	1.0132	577332	1090652		32.20	30.67					
25		2		017	1.0048	530484	1006931		29.73	28.20					
26		3		018	1.0043	556421	1059109		31.27	29.74	29.53	1.25	3.10	4	
27		BLK		019	1.0165	7063									
28	Upper Hill 30-40 cm	1		020	1.0249	118409	217168		6.41	4.88					
29		2		021	1.0114	120178	223750		6.61	5.07					
30		3		022	1.0089	135173	254064		7.50	5.97	4.98	0.14	0.34	3	
31	CAL B2			023	0.9959	1004874		37848							
32	W3			024		42365									
33		BLK		025	1.0068	7296									
34	Sitka Road -50 cm	1		026	1.0166	157292	294954		8.71	7.18					
35		2		027	0.9876	176859	343261		10.13	8.60					
36		3		028	1.0085	120484	305689		9.03	7.49	8.05	0.78	1.95	10	
37				029	1.0144	4798									
38	Sitka Road 17-30 cm			030	1.0007	88907	199682		5.90	4.36					
39				031	1.0001	134622	259757		7.67	6.14					
40				032	1.0163	145338	276554		8.16	6.63	5.71	1.19	2.96	21	
41				033	1.0259	60278									
42	Upper Hill - rooting			034	0.9981	434896	753939		22.26	20.73					
43				035	1.0246	528247	913620		26.97	25.44					
44				036	1.0117	485361	802450		23.69	22.16	22.78	2.42	6.00	11	
45	W BLK 1			037		22795									
46	W BLK 2			038		14836									
47	CAL A3			039	0.9956	267634		41409							
48	W4			040		49999									
49															
50		Mean ultrapure water				51251	Mean response factor	42338.5							
51							sd	4520.1							
52							% rsd	10.7							
53															
54		Corrected ultrapure water peak area				64871	Mean ultrapure water TCA	1.5							

APPENDIX C

List of publications

Heal, M.R., Dickey, C.A., Cape, J.N. and Heal, K.V. (2003) The routes and kinetics of trichloroacetic acid uptake and elimination in Sitka spruce (*Picea sitchensis*) saplings via atmospheric deposition pathways. *Atmospheric Environment* **37 (31),** 4447 - 4452.

Dickey, C.A., Heal, K.V., Stidson, R.T., Koren, R., Schröder, P., Cape, J.N. and Heal, M.R. (2004) Trichloroacetic acid cycling in Sitka spruce saplings and effects on sapling health following long term exposure. *Environmental Pollution* **130**, 165-176.

Stidson, R.T., Dickey, C.A., Cape, J.N., Heal, K.V. and Heal, M.R. (2004) Fluxes and reservoirs of trichloroacetic acid at a forest and moorland catchment. *Environmental Science and Technology* **38 (6),** 1639-1647.

Heal, K.V., Stidson, R.T., Dickey, C.A., Cape, J.N. and Heal, M.R. (2004) New data for water losses from mature Sitka spruce plantations in temperate upland catchments. *Hydrological Sciences Journal*, **49 (3),** 477-493.

Stidson, R.T., Heal, K.V., Dickey, C.A., Cape, J.N. and Heal, M.R. (2004) Trichloroacetic acid fluxes through a conifer forest canopy: results from a year of detailed measurements. *Environmental Pollution.* In Press.

Dickey, C.A., Heal, K.V., Cape, J.N., Stidson, R.T., Reeves, N.M. and Heal, M.R. (2004) Addressing analytical uncertainties in the determination of trichloroacetic acid in soil. Submitted to *Journal of Environmental Monitoring.*

Dickey, C.A., Heal, K.V., Matucha, M., Cape, J.N. and Heal, M.R. (2004) Processes and rates of trichloroacetic acid elimination from soil. Submitted to *Chemosphere.*

Dickey, C.A., Heal, K.V., Matucha, M., Cape, J.N. and Heal, M.R. (2004) Further evidence for, and rates of TCA production in soil. Submitted to *Chemosphere.*

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The routes and kinetics of trichloroacetic acid uptake and elimination in Sitka spruce (*Picea sitchensis*) saplings via atmospheric deposition pathways

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Abstract

A major flux of trichloroacetic acid (TCA) to forests is via wet deposition, but the transfer of TCA into tree foliage may occur by an above- or below-ground pathway. To investigate the routes and kinetics of TCA uptake, two groups of 10 Sitka spruce saplings (with an equivalent number of controls) were exposed to a single application of 200 μg TCA in solution, either to the soil only, or sprayed as a mist to the foliage only. The needle foliage was subsequently analysed regularly for TCA for 3 months during the growing season. Significant uptake into current year (C) needles was observed from both routes just a few days after application, providing direct evidence of an above-ground uptake route. Uptake of TCA was also observed in the previous year needle class ($C+1$). Kinetic modelling of the data indicated that the half-life for within-needle elimination (during the growing season) was $\sim 50 \pm 30$ days. Most of the applied TCA appeared to be degraded before uptake, either in the soil, or externally on the sapling foliage.

Keywords: Wet deposition; Needles; Degradation

1. Introduction

Elevated concentrations of trichloroacetic acid (TCA) up to 180 ng g^{-1} fwt have been measured in the foliage of forests in many countries (see recent reviews by McCulloch, 2002; Schöler et al., 2003). Since TCA has a herbicidal effect against woody plant species (Barrons and Hummer, 1951), reports of correlations between the TCA content of needles and the extent of defoliation have led to the suggestion of a cause-effect link (Frank, 1991; Norokorpi and Frank, 1995).

The origin of TCA in needle foliage is not entirely obvious. Since TCA is extremely soluble, with a Henry's law coefficient of the order of 10^5 M atm^{-1} (Bowden et al., 1998), it will partition almost exclusively into the aqueous phase. Fluxes of TCA from the atmosphere in precipitation significantly exceed estimates of its production via currently understood photooxidation reactions of chlorinated solvents emitted to the troposphere (McCulloch, 2002). Furthermore, an issue to address is whether TCA in needles arises by direct "above-ground" uptake from the atmospheric aqueous phase (via needles or branchwood), or indirect uptake from the soil via the vascular system. The former may be important since many coniferous forests are in zones of prolonged contact with convective cloud even if actual wet precipitation is low. Quantification of the rates of uptake and elimination under controlled conditions will improve understanding of the cycling of TCA through the forest system, and is important for the risk

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assessments of TCA and its precursors imposed on manufacturers of relevant chlorinated compounds.

In this work, Sitka spruce saplings were pulsed with a single dose of TCA solution to soil or foliage only, during a growing season, to follow the kinetics of uptake into needle foliage and subsequent elimination. Previous similar studies have not investigated uptake routes in Sitka spruce and have generally used multiple doses of TCA applied to considerably younger and smaller seedlings. This study used six-year old saplings which are likely to have up to 100 times the volume of plant material giving 100 times lower dose per plant. Sitka spruce is the major commercial forest species in the UK (Mason and Sharpe, 1992).

2. Materials and methods

The 40 six-year old Sitka spruce saplings (*Picea sitchensis* (Bong.) Carr.) of Queen Charlotte Island provenance, used in this experiment were grown in pots containing a 3:1:1 peat:loam:grit mixture in an unheated greenhouse. The mean ($\pm$s.d.) height and root collar diameter of the saplings at the start of the growing season (April 2002) were 105 ± 12 and 2.3 ± 0.2 cm, respectively. The saplings were systematically divided into four groups each containing one of the four tallest saplings, one of the second four tallest, etc. The groups were arranged randomly in the greenhouse.

Each sapling in two of the groups was dosed once only on 30th July 2002 with 200 ml of 1000 $\mu g l^{-1}$ TCA solution (i.e. a total of 200 μg TCA) applied either to the soil surface (group TCA-S), or sprayed as a fine mist to the foliage (group TCA-F). The application volume was chosen to correspond to $\sim$2 mm precipitation depth over the projected canopy surface area (the estimated canopy retention depth before throughfall). The dose rate was therefore equivalent to $\sim$2000 μg TCA m^{-2}. Although this TCA concentration is not typical of those found in natural precipitation, it is comparable to the annual ambient wet deposition flux of $\sim$1000 μg TCA m^{-2} measured at an upland Sitka spruce site in Scotland (Heal et al., 2003).

Two control groups of saplings (CON-S and CON-F) were dosed in the same manner with deionised water.

To avoid contamination by spray drift, the TCA-F saplings were removed to another greenhouse for spraying and returned when dry. All plants, including those in soil-dosed groups, had a paper plate with water repellent coating placed around the base of the stem to prevent drips from the spray applications from contaminating the soil, and to ensure parity in soil moisture retention for all saplings. The possibility that some of the spray solution applied to the foliage may have found its way into the soil via stem flow cannot be absolutely excluded but any contribution will have been small. In addition, it is inevitable that a proportion of spray solution will not have landed on the foliage but have been lost by drift.

Plants were irrigated with water twice a week on average via plastic saucers beneath their pots. This water was shown to contain low levels of background TCA.

Samples of the current year needles (year class C) were collected from all plants immediately prior to treatment ($t = 0$ days), then approximately twice a week for 6 weeks and weekly until $t = 85$ days on 23 October 2002 (a total of 17 sampling occasions). Needles were always collected from branches on the second whorl of each sapling, and pooled within each group, i.e., for every sampling occasion, the needle material always included needles from each sapling in that group. Sampling is obviously destructive so it was not possible to resample the same needles at each time point. It was also necessary to pool needle material to avoid excessive physical damage to the saplings in an experiment requiring removal of foliage on 17 occasions. Therefore reported TCA values reflect both analytical variability and within-group sapling variability. Material was deliberately sampled from the same whorl on each occasion in order to control for one possible source of sampling variability. This precaution has not been reported in previous studies.

Samples of the previous year's needles ($C+1$) were similarly collected on days 34 and 85.

Prior to analysis, shoots were immersed in deionised water, ultra-sonicated for 5 min, rinsed, and the excess water removed by tissue to ensure that measured TCA was internal to the needle matrix and not adsorbed to the surface. Needles were stripped from the branch and homogenised by grinding frozen under liquid nitrogen with a pestle and mortar, to completely release TCA from the needle matrix.

TCA was quantified by the method of thermal decarboxylation to chloroform developed by Plumacher and Renner (1993). The homogenised needles (1 g) were heated to 100°C in sealed 20 ml headspace vials for 90 min to convert TCA to chloroform. The vials were re-equilibrated at 60°C and the chloroform quantified against aqueous TCA standard solutions, undergoing the same process, using headspace sampling and gas chromatography with ECD detection. Parallel samples equilibrated at 60°C only provided the background chloroform present in the needles. Further details are provided in Heal et al. (2003) and Cape et al. (2003). It was necessary to correct the measured TCA concentration in needle samples for the different degree of partitioning of chloroform between headspace and needle matrix or water matrix. The ratio of these partition factors was derived from standard addition experiments in which a series of concentrations of TCA solution were added either to water or to needle. Example results are shown in Fig. 1. The ratio of the

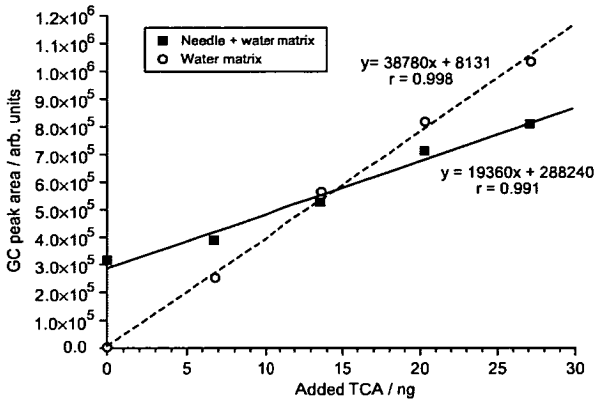

Fig. 1. Example standard additions of TCA to Sitka spruce needle and deionised water matrices. A larger fraction of chloroform produced by decarboxylation of TCA is retained by the (needle + water) matrix than by water alone, leading to smaller concentrations in the headspace. The ratio of the two gradients (2.00 in this example) yields the partition ratio which is a constant for a given set of headspace conditions.

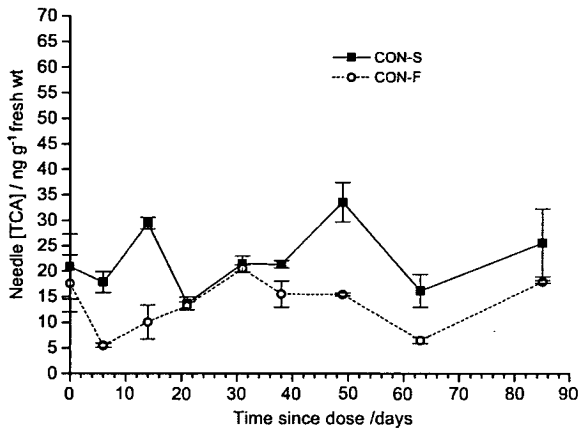

Fig. 2. Concentrations of TCA in the current year needle class of control saplings following application at $t = 0$ of deionised water either to the soil only (group CON-S) or to the foliage only (group CON-F). Error bars are standard deviation of analytical triplicates of pooled needle material taken from each of the 10 saplings within a group on each sampling occasion.

gradients in Fig. 1, termed the partition ratio, is a constant for a given set of headspace conditions (volume of vial, mass of matrix, headspace aliquot analysed, etc.), regardless of any run-to-run variability in ECD response sensitivity. Standard addition plots as shown in Fig. 1 were repeated on a number of occasions yielding a mean partition ratio of 1.94 (s.d. = 0.26, $n = 8$). This partition ratio was used to correct needle sample TCA values quantified on subsequent GC runs against just a water calibration plot.

The decarboxylation method has the advantage of being a whole-sample technique whereas extraction-derivatisation methods must assume that all intrinsic matrix-bound TCA is extracted into solution. However, the decarboxylation method does not directly quantify TCA, although samples are always blank-corrected for chloroform quantified at 60°C so that only chloroform produced by the sample after 1.5 h at 100°C is quantified as TCA. The linearity of the two plots in Fig. 1 demonstrate the applicability of the standard addition methodology, including the correction for any background chloroform prior to decarboxylation.

All samples were analysed in triplicate. All TCA concentrations were expressed per fresh wt needle. The water content of the needles (mean 58.1 ± 1.8% s.d., $n = 25$) did not vary significantly with sampling date or treatment type, so this parameter did not introduce any bias to the analyses.

3. Results and discussion

The concentration of TCA in the needles of the control group saplings, CON-S and CON-F, are shown in Fig. 2. The variation in concentrations provide an estimate of the within-group experimental and analytical variability in measurement of TCA needle concentration in the absence of TCA dosing. Fig. 2 shows there was no significant trend with time in needle TCA concentrations for either CON group, although mean CON-S and CON-F concentrations did differ significantly. The presence of TCA in the needles of the CON saplings is the result of previous background exposure over the entire lifetime of all saplings from irrigation tapwater shown to contain TCA (Cape et al., 2003). The variability in the CON-F and CON-S needle concentrations with time (and presumably similar variability in the TCA treated saplings) reflects intrinsic variability in TCA content of shoots both within-sapling and between-sapling. Within- and between-tree variability of TCA concentration in environmental foliage is well-documented. The origin of the mean difference between CON-S and CON-F concentrations presumably reflects chance between-group differences when the 40 saplings were divided into groups of 10 at the start of the experiment.

The TCA concentrations in the needles of the TCA-S and TCA-F groups following the single application of TCA at $t = 0$ to soil or foliage only are shown in Figs. 3a and b, respectively. These values are analysed with respect to the change from $t = 0$ using the variation in the CON sapling values as the estimate of the likely variation in the treated saplings. As indicated above, this latter variation includes both experimental (i.e. sapling/sampling) variability as well as analytical variability. The mean ± 1 s.d. concentration of TCA in the year C needles of the CON-S and CON-F saplings were 22 ± 6 ng g^{-1} fwt ($n = 9$) and 14 ± 5 ng g^{-1} fwt ($n = 9$),

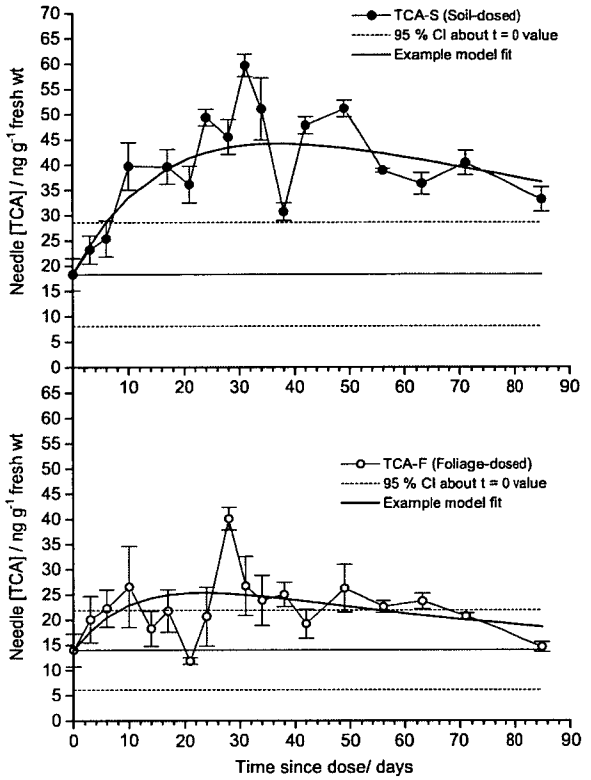

Fig. 3. Concentrations of TCA in the current year needle class following application at $t = 0$ of a single dose of 200 μg TCA per sapling either to the soil only (group TCA-S, (a)) or to the foliage only (group TCA-F, (b)). The error bars are standard deviation of analytical triplicates of pooled needle material taken from each of the 10 saplings within a group on each sampling occasion. The horizontal lines in each figure are the $t = 0$ concentration, and associated 95% confidence interval for within-group variability of measurement at a single time-point, as determined using the control group data in Fig. 2. The solid curves are model fits to the data of the kinetic scheme shown in Fig 4. For TCA-S data, $k_{\text{uptake}} = 0.0017\,\text{d}^{-1}$, $k_{\text{foliage loss}} = 0.012\ \text{d}^{-1}$, $k_{\text{external loss}} = 0.05\,\text{d}^{-1}$. For TCA-F data, $k_{\text{uptake}} = 0.0015\,\text{d}^{-1}$, $k_{\text{foliage loss}} = 0.019\,\text{d}^{-1}$, $k_{\text{external loss}} = 0.08\,\text{d}^{-1}$.

respectively. The 95% confidence interval for measurement of needle TCA concentration at a single time point is 1.96 times the s.d. concentration. This yields 95% confidence interval factors for within-group variation, relative to the corresponding mean, of 0.4–1.6 and 0.3–1.7 for soil and foliage treatments, respectively. These 95% confidence values about the $t = 0$ values, are also shown in Figs. 3a and b.

Figs. 3a and b clearly show there was significant uptake and subsequent elimination of TCA in needles of both soil-dosed (TCA-S) and foliage-dosed (TCA-F) saplings. In particular, concentrations in needles of the TCA-S group remain significantly higher than $t = 0$ even after 85 days. For the TCA-S saplings, needle TCA concentrations increased from $18 \pm 3\,\text{ng g}^{-1}$ fwt at $t = 0$, to a maximum of $60 \pm 2\,\text{ng g}^{-1}$ fwt after 31 days, decreasing to $33 \pm 2\,\text{ng g}^{-1}$ fwt after 85 days. The change in TCA in needles of TCA-F saplings was lower, but still significant, increasing from $14 \pm 3\,\text{ng g}^{-1}\,\text{fwt}^{-1}$ at $t = 0$, to a maximum of $40 \pm 2\,\text{ng g}^{-1}\,\text{fwt}^{-1}$ after 28 days, then decreasing to $15 \pm 1\,\text{ng g}^{-1}\,\text{fwt}^{-1}$ after 85 days. (The errors quoted are 1 s.d. of analytical triplicates of a pooled sample from all 10 saplings in the group.) The additional variability with time reflects the sampling variability discussed above for the CON groups.

It is not possible to compare directly the net accumulation of TCA in needles of the TCA-S and TCA-F treatments because the exact proportion of TCA solution intercepted by foliage in the spray-treated batch is not known. However, the existence of independent below-ground and above-ground pathways of TCA uptake is unequivocal, as is observation of uptake from a few days after application.

The observed time-dependence in needle concentration in Fig. 3 is typical of a sequential kinetic system involving TCA uptake into the needles from an initial "reservoir" (the TCA dose) and elimination from the needles, presumably by metabolism/detoxification. In addition, the existence of a competing parallel loss from the initial reservoir due to loss of TCA in the soil, or on the foliage surface, before uptake into the sapling, must also be included. In this context, loss means not available for uptake into the needles. This could include chemical and/or biological degradation and/or immobilisation and, for the soil application route, the possibility of permanent leaching from the soil into the irrigation saucer (although this cannot be the case for the foliage application route).

The data were modelled according to the simple kinetic scheme illustrated in Fig. 4, in which mass transfer from each compartment was assumed to follow first-order kinetics, and the first-order rate constants were the fitting parameters. Example fits to needle TCA concentration with time are shown in Fig. 3; fitted rate constant values are quoted in the caption. The inverse of each rate constant is the lifetime for the associated transfer process. A nominal fresh mass of 130 g needles

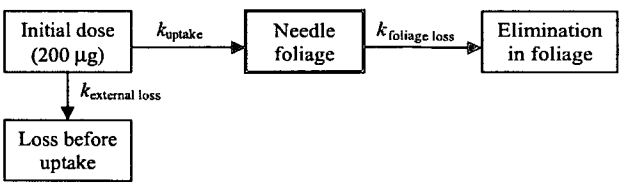

Fig. 4. Kinetic scheme used to fit observed needle TCA concentration data.

for a six-year Sitka spruce sapling was derived from the data of Cannell et al. (1983), and validated by destruction of one sapling at the end of the experiment.

Not all TCA dosed to the soil or the foliage is likely to be "available" for uptake, so the initial dose is not actually a well-characterised fixed parameter. (Some of the foliage dose will have been lost by drift or canopy drip, whist some of the soil dose may have leached out of the pot.) In practice, the kinetic fitting was fairly insensitive to assumed values of initial available TCA between 150 and 200 μg per plant although, overall, the model was not very tightly constrained. Despite this, the following conclusions emerge from model fits. First, the data are best fitted when there is provision for substantial net loss of initial TCA in parallel to uptake into the needles. In the case of the soil-dosed experiment, this is taken to indicate chemical/biological degradation of TCA in soil and is consistent with evidence from chronic sapling exposure studies (Cape et al., 2003; Dickey et al., 2003) and unpublished lysimeter measurements from this group, which all show that TCA added to soil is rapidly degraded on a timescale of a few days to weeks. For the foliage-dosed experiment, this loss is presumed to be similar degradation on the foliage surface.

Secondly, the values obtained from the fits, both for the kinetic parameters and the proportions of TCA passing through each compartment, are broadly consistent between both application regimes. Uptake of TCA into the needles has a first-order lifetime of several 100 days ($\pm \sim 100$ days). This parameter is not of relevance to environmental situations since trees are continuously exposed to "fresh" TCA directly to the canopy or from the soil via TCA in precipitation and, possibly, soil sources. The model estimates of first-order lifetimes corresponding to within-foliage elimination rate, and the loss rate from the initial reservoir in parallel to uptake, are $\sim 70 \pm 40$ days and $\sim 20 \pm 10$ days, respectively. (Quoted ranges are approximate 95% confidence intervals obtained in the non-linear fitting model and are large because of difficulties in constraining the model.)

Thirdly, very approximately, after the 85 days of observation, the kinetic fits suggest $\sim 1\%$ of initial TCA dose applied per sapling was present in the needle foliage, ~ 2–7% remained in the soil or externally on the foliage, ~ 89–95% was lost externally before uptake, and ~ 2–3% was eliminated within the needles. Regardless of kinetic modelling, it is evident from Fig. 3 that only a small proportion ($< 1\%$) of the initial dose of TCA applied is present in the needles after 85 days.

The needle TCA elimination half-life of ~ 50 days applies to an actively growing plant. In a separate study, elevated TCA concentrations in Sitka spruce seedlings at the end of the growing season in October were reduced by only about one-third by April the following year (Dickey et al., 2003) indicating very little detoxification metabolic activity over winter, as expected. The elimination rate reported here is slower than that observed in Scots pine seedlings by Sutinen et al. (1997) who noted that during the four weeks after repeated exposure to TCA, needle concentrations decreased from a peak of 250 ng g^{-1} to 36 ng g^{-1} fwt.

The analysis of older needles (year class $C+1$) sampled after 34 and 85 days clearly showed the presence of TCA in older needles via both application routes. On both sampling occasions, and for both treatments, concentrations in C and $C+1$ needles were not significantly different (taking into account the natural sampling/analytical variability discussed for Figs. 2 and 3), although there was a (non-significant) trend for concentrations greater in $C+1$ needles than in C needles for foliage application route. (N.B. $n = 2$ data only.)

The observation of a direct canopy route of uptake of TCA from solution initially appears surprising given the highly hydrophobic nature of the needle cuticle. The measurements are not simply residual TCA on the needle surface because needles were thoroughly rinsed in water before analysis. Further field evidence of canopy uptake from measurements in a mature Sitka spruce forest has been shown by this group (unpublished). A laboratory study has shown no direct partitioning of TCA from solution through the needle cuticle (Cape et al., 2003), as is also the case for other anions, e.g. SO_4^{2-} (Percy and Baker, 1989). However, elevated TCA concentrations have been observed in the branchwood of foliage-treated saplings indicating that transfer through the branchwood is the likely uptake route (Cape et al., 2003). Recently, Benesch and Gustin (2002) have also demonstrated an above ground route for accumulation of trifluoroacetic acid (TFA) in needles of *Pinus ponderosa* saplings following repeated exposure to mists of TFA solution.

An observational assessment of external sapling health (categorisation according to foliage density and colour) revealed no observable short-term adverse health effects arising from application of this single dose of TCA. However, absence of visual damage does not imply absence of toxicity; physiological and biochemical changes, if causal, are likely to result from chronic exposure to environmental levels of TCA (Cape et al., 2003; Dickey et al., 2003).

In conclusion, this work has demonstrated uptake of TCA into Sitka spruce saplings via above-ground only and below-ground only routes. The former is important given the considerable proportion of wet precipitation intercepted by the forest canopy. The retention of TCA in foliage is relatively long-lived after exposure by either route, with a half-life of approximately 7 weeks during the growing season.

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Trichloroacetic acid cycling in Sitka spruce saplings and effects on sapling health following long term exposure

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"Capsule": *TCA stored in Sitka spruce needles may affect the health of saplings.*

Abstract

Trichloroacetic acid (TCA, CCl_3COOH) has been associated with forest damage but the source of TCA to trees is poorly characterised. To investigate the routes and effects of TCA uptake in conifers, 120 Sitka spruce (*Picea sitchensis* (Bong.) Carr) saplings were exposed to control, 10 or 100 $\mu g\, l^{-1}$ solutions of TCA applied twice weekly to foliage only or soil only over two consecutive 5-month growing seasons. At the end of each growing season similar elevated TCA concentrations (approximate range 200–300 $ng\, g^{-1}$ dwt) were detected in both foliage and soil-dosed saplings exposed to 100 $\mu g\, l^{-1}$ TCA solutions showing that TCA uptake can occur from both exposure routes. Higher TCA concentrations in branchwood of foliage-dosed saplings suggest that atmospheric TCA in solution is taken up indirectly into conifer needles via branch and stemwood. TCA concentrations in needles declined slowly by only 25–30% over 6 months of winter without dosing. No effect of TCA exposure on sapling growth was measured during the experiment. However at the end of the first growing season needles of saplings exposed to 10 or 100 $\mu g\, l^{-1}$ foliage-applied TCA showed significantly more visible damage, higher activities of some detoxifying enzymes, lower protein contents and poorer water control than needles of saplings dosed with the same TCA concentrations to the soil. At the end of each growing season the combined TCA storage in needles, stemwood, branchwood and soil of each sapling was <6% of TCA applied. Even with an estimated half-life of tens of days for within-sapling elimination of TCA during the growing season, this indicates that TCA is eliminated rapidly before uptake or accumulates in another compartment. Although TCA stored in sapling needles accounted for only a small proportion of TCA stored in the sapling/soil system it appears to significantly affect some measures of sapling health.

Keywords: Enzyme activity; Exposure; Forest damage; Needles; Protein, soil

1. Introduction

Trichloroacetic acid (TCA, CCl_3COOH) is a phytotoxic chemical that has been detected in all environmental compartments (McCulloch, 2002). Although banned as a herbicide in the late 20th century (partly due to its indiscriminate effects on non-target plant species), TCA is still actively forming in the environment today and there is considerable debate about its present-day sources. The major source of TCA in the environment is postulated to be the atmospheric photooxidation of anthropogenically-produced chlorinated C_2-hydrocarbons, but natural formation in soils has also been reported (deJong and Field, 1997; Haiber et al., 1996; Hoekstra et al., 1998, 1999a,b; Keppler et al., 2000).

TCA has been widely detected in rural forests, particularly in conifer needles at concentrations of <1–180 $ng\, g^{-1}$ (Frank, 1991; Frank et al., 1992; Juuti et al., 1996; Norokorpi and Frank, 1995; Plümacher and Renner, 1993; Plümacher and Schröder, 1994; Stidson et al., 2004)

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but the routes of uptake of TCA into tree foliage are poorly quantified. It is not clear if the TCA measured in needles is taken up as TCA (from the atmosphere or soil) or is formed in the plant from C_2-chlorocarbon precursors. Frank et al. (1992) suggested that TCA in trees is formed from the needle uptake of chlorinated solvents, such as tetrachloroethene, which are subsequently transformed to TCA in the plant either by photolysis or by detoxification by the P-450 monoxygenase enzyme. According to Blanchard (1954) and Sutinen et al. (1995), TCA can enter plants in soil pore water taken up by the roots and transported to foliage via the transpiration stream. Direct uptake of TCA on needle surfaces from air or water has been thought unlikely since TCA is highly soluble in water and the surfaces of needles are lipophilic. However, if a direct atmospheric route of TCA input to foliage exists there may be significant implications for trees in forests which are regularly exposed to cloudwater.

Many field-based and controlled experiments suggest that exposure to TCA has adverse effects on tree health (Frank et al., 1990; Norokorpi and Frank, 1995; Plümacher and Schröder, 1994; Sutinen et al., 1997; Cape et al., 2003). Reported impacts of TCA exposure range from impaired growth to more subtle alterations of tree physiological functioning, such as changes in the structure of chloroplasts (important for photosynthesis) and needle surface waxes (Sutinen et al., 1995). There is particular concern regarding the effects of TCA exposure on coniferous trees because TCA may accumulate in foliage to phytotoxic levels over a number of years. Most experiments to investigate the routes of uptake and effects of TCA on trees have applied TCA in short-term studies that may overlook any chronic effects of TCA on tree health. Here, results are reported from an experiment in which 6–7 year-old saplings of Sitka spruce were exposed to multiple doses of TCA over two full growing seasons at near-realistic long-term application rates. Sitka spruce is an economically-important conifer species in Great Britain, accounting for 49% of the area under conifers in 1995 (Forestry Commission, 2002). The experimental aims were to identify for the saplings: (1) the routes of TCA uptake (atmospheric vs. soil); (2) TCA stores and fluxes within the experimental system; (3) the effects of chronic TCA exposure at near-realistic loading on health (growth, visual damage, needle enzyme activity, needle physical properties).

2. Materials and methods

2.1. Experimental design

The experiment was conducted on 120 potted saplings of Sitka spruce (*Picea sitchensis* (Bong.) Carr) of Queen Charlotte Island provenance in an unheated greenhouse at the Bush Estate, Scotland (55° 52′ N, 3° 12′ W). The saplings had been grown according to normal nursery practice and were 6 years old at the start of the 2001 growing season. The potted saplings were placed in plastic saucers and were irrigated identically during the experiment by adding de-ionised water to the saucers. The saplings had, as younger plants, been used for a single-season TCA exposure experiment during 1999 (Cape et al., 2003). This experiment used the same plants in the same experimental design as in 1999 to examine the long-term effects of TCA exposure. No TCA treatments were given in 2000, but plants were maintained in the greenhouse throughout the year, and re-potted in the intervening period.

The saplings were divided into six groups of 20 saplings. One of the six tallest saplings was randomly assigned to each group, one of the second six tallest saplings to each group and so on, until each group contained one of the six smallest saplings. The groups were then divided by height into four blocks (H1, H2, H3, H4) of five plants with H1 containing the tallest saplings and H4 containing the shortest saplings.

During both the 2001 and 2002 growing seasons each group of saplings received the same one of six treatment-level combinations consisting of three TCA concentrations (levels): de-ionised water control (L0), 10 $\mu g\ l^{-1}$ (L10), or 100 $\mu g\ l^{-1}$ (L100) TCA solution; and two treatment techniques: pouring directly onto the soil (Ts), or spraying a fine mist onto the foliage (Tf). The six groups are subsequently referred to as L0Ts, L0Tf, L10Ts, L10Tf, L100Ts and L100Tf (with height blocks H1 to H4 in each group). The treatments were applied on average every 3.5 days on 44 occasions during the first growing season (3 May–8 October 2001) and on 46 occasions during the second growing season (30 April–4 October 2002). The volume of solution applied per sapling on each occasion was 106 ml in 2001 and 200 ml in 2002, calculated to be equivalent to an estimated canopy interception storage capacity of ~2 mm (before throughfall and stemflow occur) from measurements of the average projected sapling canopy surface area at the start of each season. These data yield a total application rate of TCA per growing season of ~900 and ~9000 $\mu g\ m^{-2}$ for the two TCA dose levels, respectively. An annual wet deposition flux of ~1000 μg TCA m^{-2} has been measured at an upland Sitka spruce site in Scotland (Heal et al., 2003b). So although the concentrations of individual TCA doses in this experiment are higher than ambient, the chronic exposure rate (particularly at the lower level) is broadly realistic.

The blocks were arranged randomly in the unheated greenhouse and were moved around once during each growing season. Cardboard disks were placed on the soil surface around the trunks in all pots to prevent the spray applications from contaminating the soil and to ensure parity of soil moisture retention for all saplings. A

couple of the saplings in the H3 and H4 blocks of the L10Ts group could not be distinguished from each other due to fading of labels during the 2001 growing season so samples from both batches were amalgamated for analysis. The L10 treatments were discontinued in the 2002 growing season after analysis of the 2001 results showed no marked difference from the controls.

2.2. Sampling of sapling material and soil

Needle and soil samples were collected at the start of each growing season before dosing commenced and at the end of each growing season about one week after dosing ceased. The most recent needles (year C) were sampled by cutting a whole shoot from the 2nd or 3rd whorl of every tree. In October 2002 needles from the previous year class (C+1) and branchwood were also sampled from the same shoot. Samples were pooled by height block within each group and either analysed immediately or stored in sealed polythene bags at −30 °C until analysis. Soil was sampled by taking a core (2 cm in diameter, 10 cm in length) from every pot at 4 cm from the base of the sapling stem. On the day of collection soil samples were homogenised and grit removed by passing through a 2 mm sieve and then stored in polythene bags at −30 °C until analysis.

2.3. TCA analysis

TCA was determined in triplicate analyses of all soil and sapling shoot samples following the method of Plümacher and Renner (1993) in which TCA is thermally decarboxylated to chloroform. Full details of the analytical methodology as applied here are given in Cape et al. (2003) and Heal et al. (2003a) and only summarised here.

Before analysis the sampled shoots were immersed in de-ionised water, rinsed, and blotted dry to remove any surface TCA. Needles were stripped from the shoot and ground to a powder under liquid nitrogen with a pestle and mortar to ensure complete release of TCA from the needle matrix. Branchwood was prepared in the same manner. Homogenised needles or branchwood (1 g) were weighed into vials, 1 ml of water added, and the capped vials heated to 100 C for 1.5 h to effect decarboxylation of TCA to chloroform. An aliquot of the headspace was transferred by Perkin Elmer HS40 automated headspace sampler and the chloroform detected by GC-ECD. The TCA was quantified against chloroform produced by 1 ml TCA aqueous calibration solutions in vials undergoing the same process. Replicate needle samples were heated and analysed at 60 °C only to determine the background chloroform present in the needles. The TCA concentration equivalent to the background-corrected chloroform was corrected using a previously-determined partition ratio of 1.94 to allow for the different partitioning of TCA between headspace and water or needle+water matrices. The sieved fresh soil samples were analysed in the same way as the needles, but using a separate partition ratio.

To correct measured TCA concentrations to dry weight, the moisture content of needle, soil and branchwood samples were determined from weight loss of fresh samples dried to constant weight at 60 °C.

2.4. Sapling health

Sapling health following chronic exposure to TCA was assessed in four ways:

1. The height and stem diameter of each sapling was measured at the start and end of each growing season. Sapling height was measured from the rim of the pot to the tip of the lead shoot. Stem diameter along two perpendicular axes was measured at pot rim height using vernier callipers, and the mean taken.
2. Saplings were visually assessed for signs of needle damage on three separate occasions in September 2001 towards the end of the growing season. On each occasion saplings were assessed in a random order and the extent of damage assigned to a four-value scale of 0 (descriptor: no visible damage), 25, 50 or 75 (descriptor: heavy browning of needles on all branches including youngest shoots, evidence of loss of needles).
3. The activities of peroxidase (POX) and glutathione-S-transferase (GST) enzymes were determined in year C needles at the end of the 2001 growing season as an indicator of sapling stress. Both enzymes have been found to be involved in the detoxification of xenobiotics in plants (Schröder et al., 1997). A shoot was cut from each sapling at the end of the growing season in October 2001 and samples were pooled by height block within each group. Shoots were rinsed with de-ionised water, blotted dry and stored at −80 °C before analysis in triplicate for GST and POX activity. GST activity was determined using the procedure described in Schröder et al. (1997). Needle samples were ground with liquid nitrogen to a powder, and 10 volumes (w/v) of 100 mM Tris/HCl buffer added at pH 7–8 containing 1% PVP K30, 5 mM EDTA and 0.25% Nonidet™ P40. The slurry was homogenised, allowed to stand, centrifuged and the supernatant filtered. GST activity was determined spectrophotometrically in triplicate in the purified extract using substrates of 1-chloro-2, 4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) (Habig et al., 1974), and dichloromethane (DCM) (Schröder and Belford, 1996). POX activity was determined from the change in absorbance measured at 420 nm for 5 min of 1 ml assay (910 μl 0.05 M potassium phosphate buffer

(pH 6.0), 20 μl 3.4 M guaiacol as substrate, 20 μl 0.9 mM H_2O_2, 50 μl enzyme). Blanks were subtracted and activity was calculated from a molar extinction coefficient of 26.6 mM^{-1} cm^{-1}. Protein content was determined in duplicate in the same needles by the method of Bradford (1976) using bovine serum albumin as a standard.

4. Rates of water loss from needles collected on 17 September 2001, near the end of the first growing season, were measured to assess the effects of TCA application on physical needle properties. Two excised needles per leader shoot of each sapling were pooled by height block, fully hydrated overnight, weighed (at time $t=0$) and then placed in an atmosphere of constant humidity >50% for 72 h. The needles were reweighed on 18 occasions during this period and then placed in an oven at 70 °C for three days to obtain the dry needle mass. The data were analysed as described by Cape and Percy (1996) using Eq. (1),

$$R(t) = R_\infty + (R'_0 - R_\infty)e^{-kt} \quad (1)$$

where,

$$R(t) = \frac{m(t) - m_d}{m_f - m_d} \quad (2)$$

is the change in relative needle water content with time ($m(t)$ is mass of needles at time t, and m_f and m_d are the fresh and dry masses, respectively), R'_0 is the extrapolated relative needle water content at $t = 0$, R_∞ is the relative needle water content at infinity, and k is a first-order rate coefficient corresponding to a measure of needle surface integrity for a given specific needle surface area. Using least squares non-linear regression, Eq. (1) was fitted to the rate of weight loss with time for each sample, for times >3 h, to obtain estimates for R'_0, R_∞ and k for each group-block combination.

All data were analysed using ANOVA or General Linear Model parametric statistics.

3. Results and discussion

3.1. Routes of TCA uptake into foliage

3.1.1. TCA in current needles

TCA concentrations in current needles in October 2001, April 2002 and October 2002 are shown in Fig. 1. (Sampling in April 2002 was prior to fresh needle growth so needles sampled at this time belong to the same cohort sampled in October 2001; needles sampled in October 2002 are the new growth). Needle concentrations are within the range measured in forest foliage (up to 180 ng TCA g^{-1} fresh weight). The mean (± 1 S.D.)

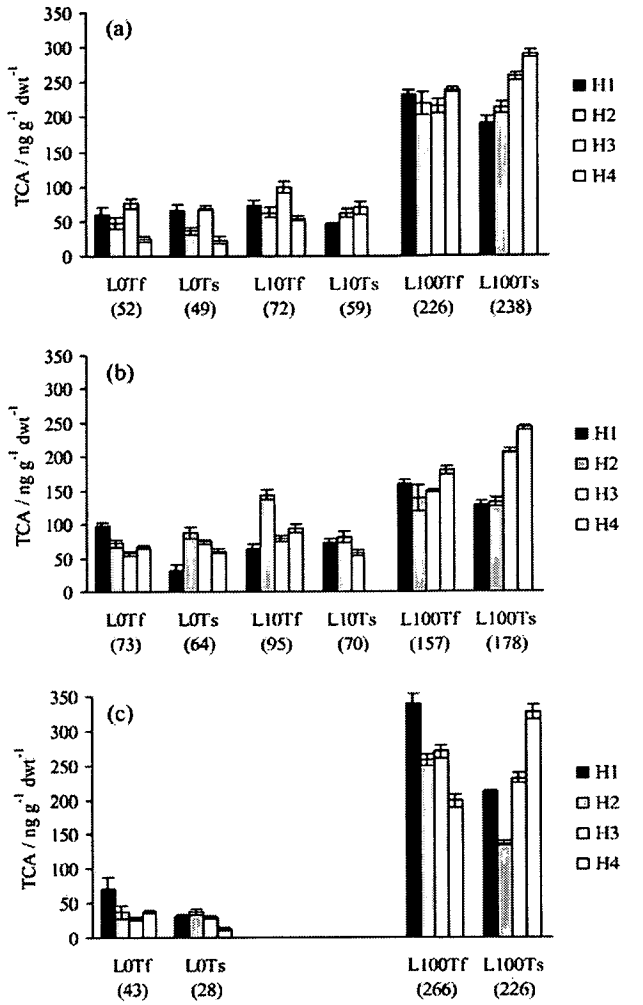

Fig. 1. TCA concentrations in most recent needle class sampled in (a) October 2001, (b) April 2002 and (c) October 2002. Needles sampled in April 2002 are the same cohort as sampled in October 2001. Error bars are 1 S.D. of analytical triplicates of samples pooled by height block. The mean values for each level-treatment group are shown in parentheses below the x-axis.

TCA concentration in needles of all control (L0) saplings in October 2001, April 2002 and October 2002 was 51 (± 22) ng g^{-1} dwt with a range of 12–98 ng g^{-1} dwt arising from variability between saplings. There was no significant difference in needle TCA concentrations between L0 and L10 saplings in October 2001 and April 2002. The control saplings were also subject to background TCA in the de-ionised water (measured on five occasions, mean TCA concentration 1.5 μg l^{-1}) that was used to water the saplings and make up treatment solutions.

Concentrations of TCA were very significantly greater in needles of L100 saplings than in L0 and L10 saplings on each sampling occasion ($P<0.001$ in October 2001 and $P<0.01$ in April 2002 and October

2002) for both foliage (Tf) and soil (Ts) applications (Fig. 1). There was no significant difference in needle TCA concentration between L100Tf and L100Ts saplings. These results demonstrate that TCA applied to either foliage or soil is taken up into the sapling needles and that the saplings do not discriminate between TCA from the two sources. TCA uptake from the foliage treatment appears to be particularly efficient compared with direct application to the soil since not all of the TCA solution sprayed onto the foliage will have been intercepted by the canopy. One mechanism that could explain this is that canopy evaporation may increase the effective TCA concentration at the canopy surface, creating a concentration gradient that accelerates TCA movement through the plant cuticle. The evidence of a canopy only uptake route is not due to TCA measured on the external needle surface since the needles were washed prior to analysis to eliminate this possibility.

The saplings were deliberately dosed at approximately the same specific rate (TCA dose per mass of sapling) in each growing season. The resultant similar concentrations of TCA in the fresh (year C) needles of the L100 dosed saplings at the end of both growing seasons (Fig. 1a and c) suggests that uptake and elimination rates remained fairly uniform. The absence of enhanced concentrations in needles of L10 dosed saplings suggests that saplings were able to degrade low level chronic TCA application.

The pathway of TCA uptake into sapling needles when TCA is applied to soil is assumed to be uptake through roots and then movement to the needles in the transpiration stream. The uptake into needles from foliar application of TCA is likely to occur through initial uptake into branchwood; TCA concentrations measured in branchwood sampled in October 2002 were very significantly greater in L100Tf saplings than in L100Ts saplings (Fig. 2). In addition, no uptake into needles was observed in an experiment in which excised Sitka spruce needles were immersed in de-ionised water, 10 μg l^{-1} or 100 μg l^{-1} TCA solutions at pH 7 or 4 for

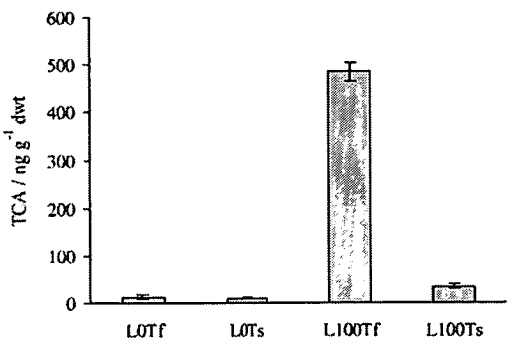

Fig. 2. TCA concentrations in sapling branchwood sampled in October 2002. Error bars are 1 S.D. of analytical triplicates of samples pooled by height block.

up to 24 h (data not shown). Other ions, e.g. SO_4^{2-}, have also been shown preferentially to transfer through branchwood rather than needles in conifer saplings (Percy and Baker, 1989), corroborating the suggestion that TCA from atmospheric sources initially enters saplings via branchwood and subsequently translocates to needles via the transpiration stream.

There was no significant effect of sapling height on needle TCA concentration across treatment method or dose level. However, in the L100 treatments, needle TCA concentrations decreased significantly with height in the Ts saplings ($P<0.05$) but not in the Tf saplings. This suggests that there may be differences in TCA uptake and metabolism mechanisms in saplings between TCA applied to foliage or to soil. TCA applied to the soil may be taken up readily into the needles of all saplings via the transpiration stream, but slower growing saplings could be less efficient at metabolising TCA in the needles. In the saplings exposed to foliar TCA applications, TCA movement into plant tissue may be controlled by the properties of the plant surfaces which are independent of sapling height.

3.1.2. Temporal changes in needle TCA concentrations

Fig. 3 shows the change in time of TCA concentrations in needles of the same age cohort in the L100 saplings during the experiment (i.e. year C in October 2001 and year C+1 in April and October 2002). In both foliage and soil-treated saplings, needle TCA concentrations were significantly lower at the start of the 2002 growing season in April 2002 than at the end of the growing seasons in October 2001 and October 2002 ($P<0.01$ and $P<0.005$ for October 2001 and October 2002 comparisons, respectively). On average, the needle TCA concentrations in L100 dosed saplings in April 2002 were 69 and 75% of the needle concentrations at the end of the 2001 growing season for the Tf and Ts treatments, respectively. (Mean needle concentrations decreased from 226 to 157 ng g^{-1} dwt for the L100Tf group and from 238 to 178 ng g^{-1} dwt for the L100Ts group). However, needle TCA concentrations in the L100 saplings in April 2002 remained significantly greater than in needles of the control saplings (by a mean of 84 ng g^{-1} dwt for Tf saplings and 114 ng g^{-1} dwt for Ts saplings). A similar pattern of TCA uptake and elimination in needles was observed in Sitka spruce seedlings by Cape et al. (2003).

The loss of TCA from needles during the winter between treatment periods could be due to elimination within the needles or relocation within the sapling. Alternatively the "loss" may arise from dilution of TCA by the plant growth observed over this period from sapling height and stem diameter measurements. Using relationships for sapling biomass (see Section 3.2), the needle mass of the L100 saplings was estimated to have increased by a median value of 7 g between October

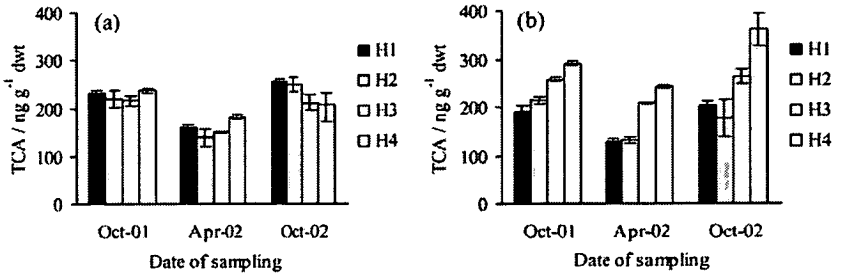

Fig. 3. TCA concentrations in the same cohort of needles established in the 2001 growing season, sampled in October 2001, April 2002 and October 2002, for 100 μg TCA l^{-1} (a) foliage-dosed saplings (L100Tf) and (b) soil-dosed saplings (L100Ts). Error bars are 1 S.D. of analytical triplicates of samples pooled by height block.

2001 and April 2002 which could theoretically account for ~45% of the observed decrease in needle TCA concentration over this period, assuming no TCA elimination or relocation in the saplings or addition of TCA through plant watering.

The above data show that TCA accumulated in the needles in the high TCA treatments was eliminated only slowly over winter when saplings are less metabolically active. A net effective loss of 25–30% of TCA in the 6 months from October 2001 to April 2002 corresponds to a half-life of ~350 days for needle TCA elimination during winter. As expected, this is considerably longer than a half-life of ~50 days for needle TCA elimination in actively growing Sitka spruce saplings derived from an experiment applying a single dose of TCA near the start of the growing season (Heal et al., 2003a).

Fig. 3 also shows that needle TCA concentrations in both L100 Tf and Ts saplings in October 2002 were not significantly different from concentrations detected in the same cohort of needles at the end of the previous growing season in October 2001. The net TCA additional concentration that accumulated in C+1 needles in the 2002 growing season was 74 (± 37) ng g^{-1} dwt in L100Tf and 73 (± 33) ng g^{-1} dwt in L100Ts, compared to 266 (± 58) ng g^{-1} dwt and 226 (± 79) ng g^{-1} dwt, respectively, accumulated in the C needles of the same treatments over the same period (Fig. 1c). These results show that TCA in sapling needles is not linearly cumulative with dose during prolonged exposure to atmospheric or soil TCA.

3.1.3. TCA concentrations in needles of different ages

There was no significant difference in TCA concentrations of needles of different age classes (C and C+1) from L100 saplings sampled in October 2002 (Fig. 4). In contrast, field measurements and some other laboratory experiments have found that needle TCA concentration increases with needle age in conifers (Frank et al., 1990; Plümacher and Schröder, 1994; Juuti et al., 1996; Sutinen et al., 1997; Hafner et al., 2002; Stidson et al., 2004). These apparently contradictory results can be reconciled if it is assumed that TCA is taken up rapidly into current needles [as has been indirectly demonstrated through the application of [1,2-^{14}C]TCA to Norway spruce seedlings by Forczek et al. (2001)], perhaps due to a faster transpiration stream. After needle uptake, TCA may be eliminated at a faster rate in current needles than in older needles, resulting in the measurement of greater net TCA concentrations in older needles. At the higher TCA treatment level applied in this experiment (relative to environmental concentrations) the rate of TCA application and uptake may have exceeded the rate of metabolic elimination of TCA within the needles, masking the effect of needle age on TCA concentration.

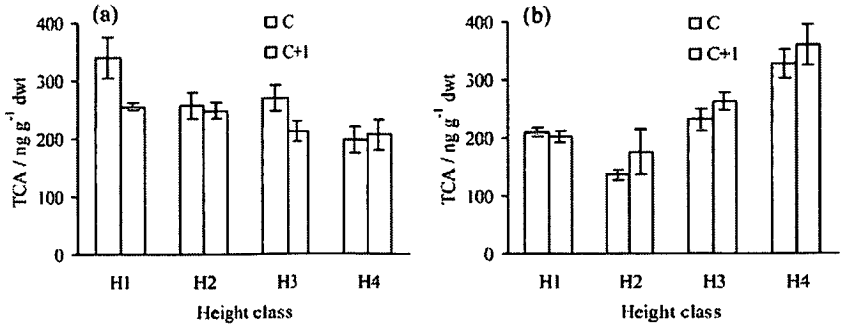

Fig. 4. TCA concentrations in C and C+1 needles sampled in October 2002 for 100 μg TCA l^{-1} (a) foliage-dosed saplings (L100Tf) and (b) soil-dosed saplings (L100Ts). Error bars are 1 S.D. of analytical triplicates of samples pooled by height block.

3.2. TCA behaviour in the sapling/soil system

Soil from the sapling pots was sampled before and after treatment in the 2001 growing season and analysed for TCA to determine the proportion of applied TCA that could be accounted for in the soil. Soil TCA concentrations (Fig. 5) ranged from 8 to 53 ng g^{-1} dwt (mean = 19 ng g^{-1}, S.D. = 18 ng g^{-1}) in April 2001, and 10–51 ng g^{-1} dwt (mean = 18 ng g^{-1}, S.D. = 8 ng g^{-1}) in October 2001. Soil TCA concentrations were not significantly different either between level-treatment groups at the end of the 2001 growing season or between the start and end of TCA applications. These data show that TCA applied directly to the soil for six months in the Ts treatments did not accumulate in the sapling soil, indicating that the applied TCA was broken down within the soil, and/or was irreversibly leached from the soil into the saucer, and/or was taken up into the sapling by the roots.

The masses of TCA stored within the different compartments of the experimental system were estimated to improve understanding of TCA uptake and storage in the sapling-soil system. The stemwood mass for each sapling was estimated by multiplying the stemwood specific gravity of Sitka spruce saplings [from Cannell et al. (1983)] by the fresh stemwood volume (derived from the sapling height and diameter measurements and assuming that the stem is a cone). Needle and branchwood masses were then estimated from the dry matter mass ratio for stem:branches:needles of 37:29:34 measured in Sitka spruce saplings of the same age by Cannell et al. (1983). Good agreement was found between the measured dry mass (232 g) of branches and needles of a sapling harvested in February 2003 and the estimated dry mass (261 g), showing that this method provided reasonable estimates of sapling dry mass. The soil mass in each pot was estimated by multiplying the pot volume by the mean of six measurements of soil bulk density and is therefore the same for all treatments. Soil dry mass was assumed to remain constant throughout the experiment whilst foliage masses were recalculated for every occasion on which sapling height and stem diameter were measured. Table 1 shows the mean estimated mass of needles, branchwood, stemwood and soil per sapling in the different treatments in October 2002. The soil accounts for up to 95% of the soil-sapling system mass.

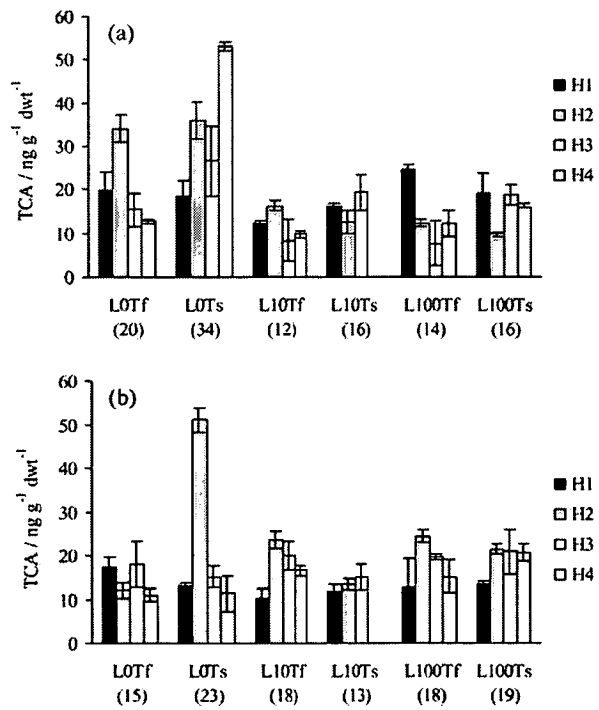

Fig. 5. TCA concentrations in soil samples collected from sapling pots at the (a) start, and (b) end, of the 2001 growing season. Error bars are 1 S.D. of analytical triplicates of samples pooled by height block. The mean values for each level-treatment group are shown in parentheses below the x-axis.

The mass of TCA stored in each compartment was calculated by multiplying the dry mass by the measured TCA concentrations in soil, needles and branchwood. Soil TCA concentrations measured in 2001 were used for the 2002 calculations. The stemwood TCA concentration was assumed to be zero since Sitka spruce stemwood has been found to contain negligible TCA (Stidson et al., 2004). Table 2 shows the mean TCA mass per sapling in each compartment at the end of the experiment in October 2002 for each level-treatment group. The soil TCA store is markedly greater than the foliage TCA store, accounting for 93–97% of the total TCA in the sapling-soil system in control groups, and 59 and 80%, respectively, of total TCA store in L100Tf and L100Ts groups. The difference for the L100Tf group is due to the extra TCA in the branchwood of saplings in this group (Fig. 2).

TCA inputs to, and changes in TCA storage within, the sapling-soil system for each treatment over the 2001 and 2002 growing seasons are shown in Tables 3 and 4, respectively. The data take into account measured

Table 1
Mean dry masses of needles, stemwood, branchwood and soil per sapling for each level-treatment group in October 2002, derived as described in the text. Standard deviations shown in parentheses for needles, stemwood and branchwood are of the four height blocks within each group. Standard deviation shown in parentheses for soil is of the soil density determinations

Treatment	Mean dry mass of material/g per sapling			
	Needles	Stemwood	Branchwood	Soil
L0Tf	74 (11)	80 (11)	63 (9)	3500 (700)
L0Ts	75 (4)	82 (4)	64 (3)	3500 (700)
L100Tf	67 (4)	72 (4)	57 (4)	3500 (700)
L100Ts	66 (11)	72 (12)	57 (9)	3500 (700)

Table 2
Mean TCA mass in needles, stemwood, branchwood and soil per sapling for each level-treatment group in October 2002, calculated from the TCA concentrations and masses of sapling material. Standard deviations shown in parentheses are for the four height blocks within each group. Stemwood TCA concentration was assumed to be zero. Soil TCA concentrations were assumed to be the same as those measured in October 2001

Treatment	Mean TCA mass/μg per sapling			
	Needles	Stemwood	Branchwood	Soil
L0Tf	3.3 (±2.0)	0	0.8 (±0.1)	52 (±13)
L0Ts	2.1 (±0.9)	0	0.6 (±0.03)	81 (±67)
L100Tf	17.8 (±4.5)	0	27.5 (±1.7)	64 (±18)
L100Ts	14.7 (±4.7)	0	1.9 (±0.3)	68 (±13)

changes in sapling biomass during the growing season. TCA inputs include the background TCA in the deionised water used for watering and in the treatment solutions. Allowing for within-group variability, there was no difference in TCA present in the saplings between the start and end of the growing seasons for L0 saplings and (for the 2001 growing season) L10 saplings. This indicates that the saplings were able to degrade whatever proportion of the L0 and L10 TCA dose was taken up into the saplings. From these observations a half-life of a few 10s days for within-sapling degradation during the growing season can be very approximately estimated, in general agreement with the half-life of ~50 days determined separately by Heal et al. (2003a).

For the L100 dosed groups, change in TCA stored in the saplings was <6 and <2% of total mass of TCA applied for the 2001 and 2002 growing seasons, respectively (Tables 3 and 4). Combined with estimated within-sapling degradation rates, this suggests that only a small proportion (<~15%) of the TCA applied to these high dose groups is taken up into the saplings. In the soil treatment the TCA unaccounted for could result from rapid degradation of TCA in the soil and/or rapid uptake into the sapling, followed by elimination in the

Table 3
Mean changes in TCA storage of the sapling system between May and October 2001 compared with the mass of TCA applied (input). The standard deviations shown in parentheses are for the 4 height blocks within each group

Treatment	Change in sapling TCA storage during season/μg	Input of TCA during season/μg	Proportion of input TCA present at end of season/%	Mass of input TCA unaccounted for at end of season/μg
L0Tf	−0.9 (±1.4)	6.9	0	
L0Ts	0.0 (±1.2)	6.9	0	
L10Tf	−1.2 (±3.7)	54	0	
L10Ts	−0.6 (±1.9)	54	0	
L100Tf	24.0 (±2.4)	475	5.1 (±0.4)	451 (±2.4)
L100Ts	7.5 (±0.7)	475	1.6 (±0.2)	468 (±0.7)

Table 4
Mean changes in TCA storage of the sapling system between April and October 2002 compared with the mass of TCA applied (input). The standard deviations shown in parentheses are for the 4 height blocks within each group

Treatment	Change in sapling TCA storage during season/μg	Input of TCA during season/μg	Proportion of input TCA present at end of season/%	Mass of input TCA unaccounted for at end of season/μg
L0Tf	−1.3 (±0.7)	13	0	
L0Ts	−1.0 (±1.3)	13	0	
L100Tf	17.3 (±4.1)	934	1.9 (±0.4)	917 (±4.1)
L100Ts	7.4 (±3.6)	934	0.8 (±0.4)	926 (±3.6)

plant tissue. In the foliage treatment, possible pathways for TCA loss include degradation of TCA on the needle surface or adsorbed TCA which was washed off during sample preparation for analysis. Alternatively, TCA could be accumulating in a compartment not accounted for, such as the sapling roots. Forczek et al. (2001) indirectly also recovered only 22% of [1,2-^{14}C]TCA applied to the soil in a Norway spruce seedling experiment and suggested that the TCA loss resulted from degradation in the soil and absorption by bark, roots and litter. However, although the proportion of TCA processed with the sapling may be comparatively small, it remains the case that TCA is efficiently biomagnified in the sapling which may have important consequences on sapling health.

3.3. Sapling health

3.3.1. Sapling growth

The cumulative growth over two seasons was assessed in order to test for association between long-term, or chronic, exposure to TCA and sapling growth. The mean percentage increases in sapling stem diameter, height and stem volume (approximated as a cone) during the experiment were 51.2, 63.2 and 311%, respectively. No significant differences were found for absolute or relative changes in sapling growth either between treatment level (L0 or L100) or application method (Tf or Ts), apart from for the affect of application method on relative change in sapling height ($P < 0.01$, Tf > Ts) (Fig. 6). Therefore in this experiment sapling growth was not affected by application of TCA over two growing seasons, although spraying solution on sapling foliage appeared to increase sapling height compared with applying solution to the soil.

3.3.2. Observation of foliage damage

There was no significant effect of TCA concentration on apparent damage. However sapling height did have a significant effect, with the blocks of shortest saplings (H4) showing significantly less damage (mean 29%)

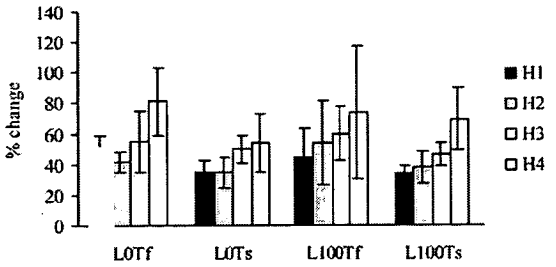

Fig. 6. Percentage changes in stem height of L0 and L100 saplings between April 2001 and October 2002. Error bars are 1 S.D. for the five replicate saplings in each height block.

than the two blocks of tallest saplings (H1: mean 41%, $P<0.05$; and H2: mean 43%, $P<0.005$; Tukey test). Significantly more damage was observed in the foliage-dosed (Tf) saplings than the soil-dosed saplings (Ts) (means of 39 and 33%, respectively, $P<0.05$), suggesting that spraying solution on foliage, regardless of TCA content, caused the visible damage. Interestingly, the foliage-dosed saplings also had significantly greater height growth rates compared with the soil-dosed saplings (Fig. 6), implying that the stimulation of sapling growth may be linked to needle browning and loss.

A highly significant interaction existed between TCA concentration and application method ($P<0.005$) in that there was no difference in visible sapling damage between the foliage and soil applications in the control and 10 μg l^{-1} TCA treatments, but in the 100 μg l^{-1} TCA treatment considerably more damage was observed in the foliage-dosed saplings than the soil-dosed saplings. This result suggests that the exposure route of conifers to TCA is an important control on the extent of visible damage as well as the solution concentration, with trees whose foliage is regularly exposed to TCA solution (e.g. via cloudwater) being particularly vulnerable. Recent measurements of TCA in rainwater and cloudwater for an upland forest in south east Scotland showed that TCA concentrations were slightly enriched in cloudwater by a mean factor of 1.2, compared to rainwater, and that cloudwater deposition accounted for 13% of total TCA deposition to the forest (Heal et al., 2003b). Greater enhancement of TCA has been reported in fogwater (Römpp et al., 2001). Furthermore, needles of Sitka spruce saplings are retained for a relatively long period of time [6–8 years compared with 2–3 years for Scots pine (Cannell, 1987)] so TCA may accumulate in the needles of trees exposed to TCA in cloudwater over this time.

3.3.3. Enzyme activity in needles

No significant effect of TCA concentration, application method or sapling height was found for peroxidase (POX) activity (data not shown), in contrast to experiments on 2-year old Scots pine seedlings in which greater POX activity was measured in 0.1 mg l^{-1} TCA treatments, a considerably greater specific dose than in these experiments (Schröder et al., 1997). The GST enzyme activity results differed for the three xenobiotic substrates used (DCNB, CDNB and DCM). For DCNB (data not shown) no significant effects were observed of TCA concentration, application method or sapling height on GST activity.

When CDNB was used as a substrate (Fig. 7a), there was no significant difference in GST enzyme activity in needles with TCA concentration in soil-treated (Ts) plants, but in foliage-treated plants, activity was significantly greater in plants that had received the 10 μg l^{-1} doses than either the control or the 100 μg l^{-1} doses ($P<0.005$). With DCM as GST substrate (Fig. 7b), enzyme activity again was only enhanced in plants where foliage was treated, and in this case the effect was statistically significant for both 10 μg l^{-1} ($P<0.005$) and 100 μg l^{-1} ($P<0.05$) treatments. An explanation for these observations is that GST activity may be induced at the 10 μg l^{-1} TCA dose to detoxify the added TCA, but become inhibited at higher TCA doses. These results are consistent with the observation of greater visible

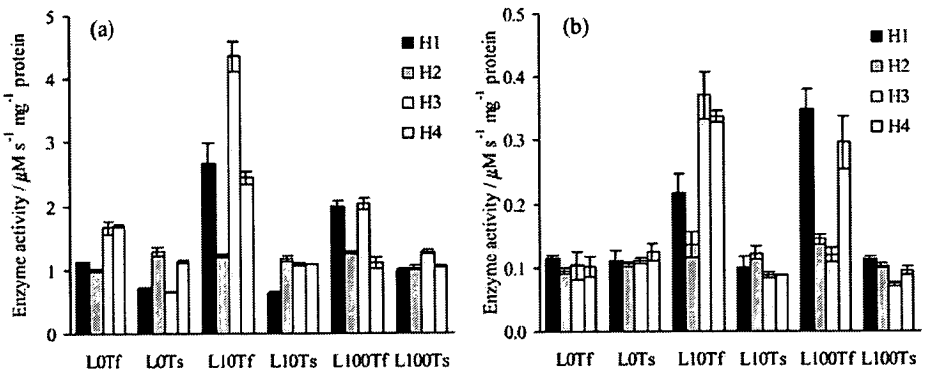

Fig. 7. GST enzyme activities in sapling needles in October 2001 with substrates of (a) CDNB and (b) DCM. Error bars are 1 S.D. of analytical triplicates of samples pooled by height block.

Table 5
Protein content (mg (ml extract)$^{-1}$) of sapling needles sampled in October 2001. Values with the same superscript in a column or row are not significantly different. Interaction between level and treatment was significant at $P<0.001$

Group	Height 1 (tallest)	Height 2	Height 3	Height 4 (shortest)	Group mean	Level mean	Treatment mean
L0Tf	2.21	1.75	1.74	2.13	1.96	1.79^{a}	
L0Ts	1.83	0.71	1.61	2.36	1.62		
							Tf: 1.04^{a}
L10Tf	0.49	0.69	0.45	0.80	0.61	1.22^{b}	
L10Ts	1.79	1.64	1.94	1.94	1.83		
							Ts: 1.62^{b}
L100Tf	0.55	0.80	0.56	0.34	0.56	0.98^{b}	
L100Ts	1.40	1.08	1.48	1.63	1.40		
Height mean	1.38^{ab}	1.11^{a}	1.29^{ab}	1.53^{b}		Level-treatment interaction $P<0.001$	

damage in the foliage-dosed saplings compared with the soil-dosed saplings in October 2001. Cape et al. (2003) report a similar result for enhanced needle GST activity in Sitka spruce seedlings exposed to TCA by foliage routes, but with DCNB as a substrate. In that work, a foliage-wetting effect was apparent since needle GST activity was also enhanced by application of the control dose to the foliage. Although there are broadly consistent trends in all these data, the observed significant differences in needle GST activity for some substrates between trees exposed to TCA by different routes suggests that enzyme activity measurements should be interpreted with care as indicators of tree stress in experiments of this nature.

As with GST activities, TCA exposure route had a significant effect on needle protein content (Table 5) ($P<0.001$) with lower protein contents measured in foliage-dosed saplings than soil-dosed saplings. Of particular note in the needle protein content results was the significant interaction between TCA concentration and application method ($P<0.001$). In the L10 and L100 treatments considerably less needle protein occurred in the foliage-dosed saplings than in the soil-dosed saplings suggesting that TCA solution applied to the foliage is more potent to sapling needles than TCA applied to the soil. The observation of significantly lower needle protein content in TCA dosed plants compared with control plants (Table 5, $P<0.001$) is consistent with previous measurements in Norway spruce (Plümacher and Schröder, 1994) and Sitka spruce (Cape et al., 2003). Lower protein contents are probably due to the protein precipitating property of TCA, which results in an associated reduction of the capacity of protein to conjugate and detoxify xenobiotics. Sapling height also had a significant effect on needle protein content ($P<0.05$) with higher protein contents generally occurring in the shorter saplings. Again this result is consistent with the observation of less visible damage in the shorter saplings.

In summary, although measured needle enzyme activities differ between tree TCA exposure experiments and with substrate used and experimental artefacts may also exist (the stimulation of enzyme activity by spraying solution on foliage), in this experiment the needle enzyme activity and protein content results indicate that stress occurs in conifers exposed to TCA and that the degree of stress is influenced by the route of TCA exposure. Greater stress was apparent in the needle biochemistry of foliage-dosed Sitka spruce saplings than in soil-dosed saplings, in agreement with the visible damage observations. However, it should be cautioned that observations of associations do not necessarily imply a cause-effect relationship.

3.3.4. Needle physical properties

An example fit of Eq. (1) to the relative needle water loss for one of the needle samples is shown in Fig. 8. Three variables, R'_0, k and Ω, were derived from the curve fitted to each needle sample. k and R'_0 are as defined earlier and $\Omega = R_\infty\ (m_f - m_d)/m_d$, where m_f is the fresh needle mass at $t=0$ and m_d is the oven-dry needle mass. There was no significant effect of TCA concentration, application method or sapling height on values of k and Ω (data not shown), nor on fresh mass/dry

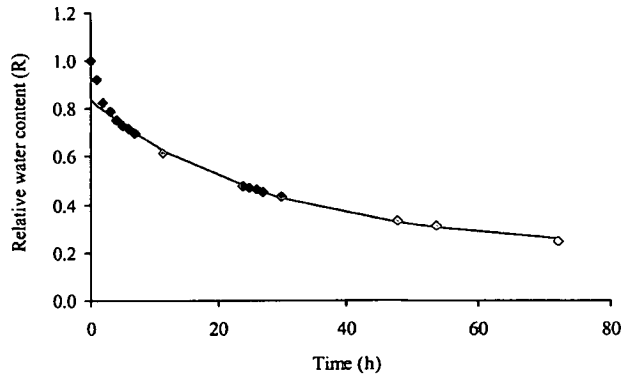

Fig. 8. Rate of water loss from pooled needles (sampled in September 2001) from saplings of height block 1 dosed with 10 μg l^{-1} TCA to foliage (L10TfH1). Eq. (1) is fitted to the data for t > 3 h.

Table 6
Mean R'_0 values (%) for sapling needles sampled in September 2001, derived from fitting Eq. 1 to measurements of needle weight loss against time at constant humidity. Values with the same superscript in a column or row are not significantly different. Interaction between level and treatment was significant at $P<0.05$

TCA dose level	Treatment method		Level mean
	Foliage (Tf)	Soil (Ts)	
Control	80.9	80.0	80.4^{ab}
10 μg l^{-1}	81.9	85.6	83.7^{a}
100 μg l^{-1}	64.7	82.7	73.7^{b}
Treatment mean	75.8^{a}	82.7^{b}	Level-treatment interaction $P<0.05$

mass (m_f/m_d) ratios. However, there was a significant interaction between TCA concentration and application method ($P<0.05$) for values of R'_0 (relative water content at $t=0$) (Table 6). For most treatments, R'_0 exceeded 80%, but it was significantly lower (65%) for the 100 μg l^{-1} TCA foliage treatment. Main effect differences in R'_0 were significant for TCA concentration ($P<0.05$) and application method ($P<0.05$). Since an estimate of the proportion of initial needle water lost through stomata is given by the quantity $(1-R'_0)$, saplings with lower R'_0 values may be more susceptible to water loss through the stomata in dry conditions, resulting in yellowing and droughting of needles. This is again consistent with both the damage survey and protein content results, providing further evidence that the application of TCA solution to tree foliage has an adverse effect on sapling functioning, in this case through water control in the needles.

4. Conclusions

Exposure of 6–7 year old Sitka spruce saplings to 100 μg l^{-1} TCA over two 5-month growing seasons showed that TCA applied either to the soil or foliage was detected in current year needles at similar concentrations in both growing seasons (up to 340 ng g^{-1} dwt), providing further evidence for both above- and below-ground routes of TCA uptake in conifers. The main pathway of needle uptake of foliage-applied TCA is most probably through branchwood and stemwood rather than through the needle cuticle since high TCA concentrations were measured in branchwood of foliage-treated saplings. TCA in sapling needles was eliminated slowly between the two growing seasons suggesting that TCA in conifer needles persists after exposure to TCA from above or below-ground routes. Estimated TCA budgets for the experimental sapling-soil system showed that the vast majority of the applied TCA was unaccounted for, probably due to degradation in the soil or on foliage surface, or metabolism within the sapling. TCA dosing had no measurable effect on sapling growth during the experiment. However the survey of visible damage and assays for needle enzyme activities and protein content at the end of the first growing season showed that there is a significant interaction between TCA concentration and exposure route. Repeated exposure of foliage to TCA in solution causes measurable changes in needle enzyme activity, protein content and physical properties at concentrations as low as 10 μg TCA l^{-1}, whereas no health effects were detected in saplings exposed to the same TCA concentrations via the soil. There may be other effects of TCA on sapling health that only become apparent over a longer time period than in this experiment, e.g. the adaptability of saplings to environmental stresses such as frost, drought, nutrient deficiencies and disease. Overall, TCA in needles only accounted for <16% of TCA stored in the sapling-soil system, but the evidence indicates this TCA may have significant effects on sapling health. Although the results from these controlled experiments may not be directly applied to forest ecosystems, tree saplings (taller than 1 m) are good analogues for investigating routes of TCA uptake and cycling in trees.

Acknowledgements

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Fluxes and Reservoirs of Trichloroacetic Acid at a Forest and Moorland Catchment

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The concentrations and input/output fluxes of trichloroacetic acid (TCA) were measured in all relevant media for one year at a 0.86 km^2 upland conifer plantation and moorland catchment in SW Scotland (n > 380 separate samples analyzed). Annual wet precipitation to the catchment was 2.5 and 0.4 m for rain and cloud, respectively. TCA input to the catchment for the year was 2100 g, predominantly in rainwater (86%), with additional input via cloudwater (13%) and gas plus particle dry deposition (1%). There were no seasonal trends in TCA deposition, and cloudwater concentration was not enhanced over rainwater. TCA in precipitation exceeded concentrations estimated using currently accepted routes of gas-phase oxidation from anthropogenic chlorinated hydrocarbon precursors, in agreement with previous studies. Export of TCA from the catchment in streamwater totalled 1970 g for the year of study. The TCA concentration in streamwater at outflow (median 1.2 μg L^{-1}) was significantly greater than that before the stream had passed through the conifer plantation. To well-within measurement uncertainties, the catchment is currently at steady-state with respect to TCA input/output. The catchment reservoir of TCA was dominated by soils (~90%), with the remainder distributed in forest litter (~9%), forest branchwood and stemwood (~0.7%), forest foliage (~0.5%), and moorland foliage (~0.1%). Although TCA is clearly taken up into foliage, which consequently may be important for the vegetation, this was a relatively minor process for TCA at the catchment scale. If it is assumed, on the basis of laboratory extraction experiments, that only ~20% of "whole soil" TCA measured in this work was water extractable, then total mass of TCA in the catchment is reduced from ~13 to ~3.5 kg. Comparing the latter value with the annual flux yields an average steady-state residence time for TCA in the catchment of ~1–2 y, if all TCA is involved in catchment turnover. Considering that other evidence indicates the lifetime of TCA in soil and biota is considerably shorter than this (weeks rather than years), the magnitude of the TCA reservoir is suggested to be strong evidence for net natural TCA production in soils and/or that the majority of TCA in the reservoir is not involved with external fluxes.

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1. Introduction

Trichloroacetic acid (TCA: CCl_3COOH) is a known phytotoxic chemical. Some years ago concern was raised over proposed causal links between observation of TCA in remote forest foliage, stress on forest productivity, and an atmospheric route to production of TCA from certain anthropogenic chlorinated solvents (*1*). Since then, TCA has been identified ubiquitously in most types of environmental media (air, rain, snow, rivers, foliage, soil, etc.) at concentrations in the parts per billion range (*2*–*4*).

However, controversy remains surrounding the modern-day sources of TCA in rural ecosystems (*5*). Although TCA salts and derivatives were formerly used as herbicides, and TCA is known to be produced during oxidative water treatment from paper manufacture and water treatment, TCA is very soluble and fully dissociated in water (1200 g L^{-1} at 20 °C (*6*); pK_a = 0.3 (*7*)) and is not expected to volatilize. A potential anthropogenic source of TCA in the remote environment is as a photooxidation product of chlorinated hydrocarbon solvents such as 1,1,1-trichloroethane (CH_3CCl_3) and tetrachloroethene (C_2Cl_4) emitted to the atmosphere (*1*). TCA may also be formed in-situ in foliage after uptake of the chlorocarbons. Postulated natural sources include natural chloroperoxidase enzymes acting on chloride ions and organic matter substrates in soils. TCA and chloroform have been formed in laboratory experiments from humic material, hydrogen peroxide, chloride, and the chlorinating enzyme chloroperoxidase (*8*, *9*). However, although this latter source might contribute, either wholly or in part, to observed TCA in soil, foliage, and river water, it cannot contribute to TCA in atmospheric precipitation.

Attempts to quantify and rationalize known or postulated sources and fluxes of TCA in the environment have been generally limited to sub-compartments of the anticipated full cycling of TCA in the environment. For example, Franklin and Sidebottom (*10*) calculated the mean concentrations of TCA expected in precipitation in Europe, Antarctica, and the Arctic, based on estimated releases of chlorinated solvent precursors into the atmosphere and atmospheric oxidation reaction pathways. (Assumed upper limits of conversion to TCA from C_2Cl_4 and CH_3Cl_3 were 5% and 1.3%, respectively). The concentrations anticipated from atmospheric precursor measurements were at the low end of the range of actual observations, from which the authors concluded that large additional sources of TCA (in the atmosphere) were required in order to effect a global or regional mass balance. More recent modeling similarly concluded that the rate of formation of TCA from gas-phase photooxidation of CH_3Cl_3 was insufficient to account for TCA burdens in the environment (*11*).

Berg et al. (*12*) estimated the aquatic fluxes of TCA in the precipitation, wastewater, and river water of Switzerland and concluded that, of the nationally averaged TCA input of 490 g km^{-2} y^{-1}, 73% was from wet deposition and the remaining 27% was from communal wastewater. Of this, 120 g km^{-2} y^{-1} (23%) could be accounted for by exports in river water, but the remaining 77% was assumed to reflect either *loss* by degradation or accumulation in the soil and groundwater. On the other hand, other studies have used mass balance calculations for the soil top layer as evidence for the *formation* of TCA in soil (*13*). The enormous range and uncertainties

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TABLE 1. Location and Frequency of Sampling for Hydrological Parameters and TCA Concentration at Ballochbeatties Catchment, May 2001 to May 2002

	measurement	frequency of sampling
rain (×2 sites)	depth	15 min
	integrated sample for TCA analysis	2 weeks
cloud	depth	2 weeks
	integrated sample for TCA analysis	2 weeks
air	integrated sample for TCA analysis	2 weeks
streamwater	depth, velocity & flow	15 min
	flow-proportional sample for TCA analysis	2 weeks
	grab sample at gauging station	weekly
	grab sample as stream left moorland	2 weeks
forest foliage	3 years of needle classes at 4 sites	Sept 2001, Jan 2002, May 2002
stemwood	cores from 8 trees	May 2002
moorland vegetation	5 most abundant species	Aug 2001, Jan 2002, May 2002
soils	2 moor and 4 forest locations (to 85 cm depth)	June 2001, Jan 2002, May 2002

in the derived estimates for soil production rate (ranging from 0 ± 0.4 mg m^{-2} y^{-1} for Douglas fir forest to 60 ± 90 mg m^{-2} y^{-1} for spruce forest) reflect both the undoubted heterogeneity of the environmental matrixes and the limited amount of TCA data for such a calculation.

None of the above studies have attempted to quantify TCA flux and budgets through and within all key compartments of the same catchment simultaneously. The aim of the work presented here was to undertake such a study by measuring concentrations of TCA in all relevant media for a year in a forest and moorland upland catchment in SW Scotland. TCA input to the catchment consisted of wet (rain and cloud) and dry (gas and particle) deposition, while TCA output consisted of streamwater. These were each measured at two-week intervals to enable flux balance comparison. Extensive measurements were made of the TCA burden in the catchment (tree foliage, branchwood and stemwood, forest litter layer, moorland foliage, and soil) to compare TCA flux with the catchment reservoir and to give insight into the biogeochemical cycling of TCA.

2. Methodology

2.1 Site Description. The study catchment was the Ballochbeatties burn in Ayrshire, SW Scotland (55°13′N, 4°29′W), 70 km south of Glasgow. The location and sampling sites are shown in Figure 1. The area is remote from major population or industrial centers. The catchment has an area of 0.86 km^2 and ranges in elevation from 320 to 480 m. The upper part (0.45 km^2) is Joint Nature Conservation Committee National Vegetation Classification community type M15 (*Scirpus cespitosus – Erica tetralix* Wet Heath) (*14*) underlain by peat, peaty gley, and peaty podsols. The lower section is 1964-planted commercial conifer forest, largely on basin peat, dominated by Sitka spruce (*Picea sitchensis*) but additionally containing hybrid larch (*Larix x eurolepis*, 16% by area), lodgepole pine (*Pinus contorta*, 6%), and small proportions of additional species. Glacial drift and relatively impervious graywackes underlie the catchment. This, in combination with the observation that hydrology is dominated by overland and near-surface through-flow, indicate that groundwater influences are likely to be minimal. Hydrological inputs to the catchment are therefore composed of rain and cloud deposition (2500 mm and 400 mm, respectively, for the year under study); while streamwater is the only output (mean discharge 47 L s^{-1} during the year).

The catchment was visited every two weeks for one year commencing in May 2001. The sampling frequency for each environmental medium is given in Table 1.

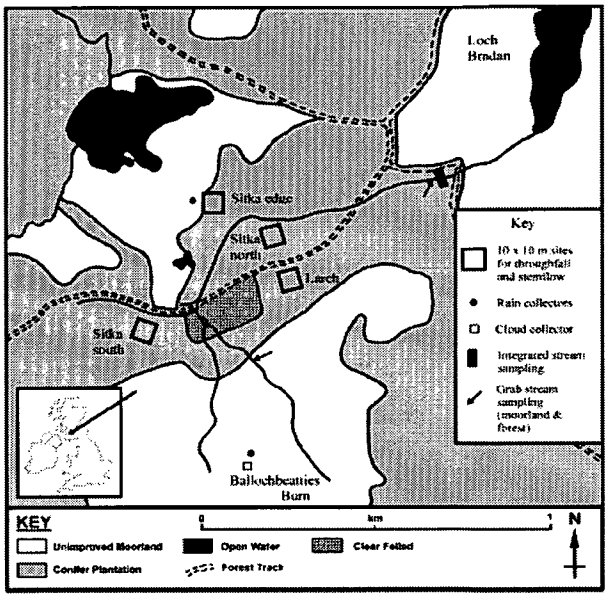

FIGURE 1. Plan of Ballochbeatties catchment indicating location of sampling sites.

2.2 Sampling of Catchment Inputs and Outputs. *Air.* Air was sampled for total (gaseous and particle-bound) TCA, as described by Heal et al. (*15*), by drawing air through two 47-mm-diameter open-face Na_2CO_3-impregnated filters (Gelman A/C glass microfiber) in parallel to provide duplicate samples. Analysis blanks consisted of unexposed Na_2CO_3-impregnated filters. The total flow rate through both filters was around 20 L min^{-1}, measured cumulatively with an in-line gas meter. Experiments with Na_2CO_3-impregnated filters in sequence established there was no breakthrough from a single filter.

The filters and pump were enclosed in a plastic container to provide shelter from rain. Because the air sampler required main power it was located 4 km NE of the catchment at 260 m asl. Atmospheric TCA was assumed to be homogeneous over this short distance.

Rainwater and Cloudwater. Rainfall depth was recorded at both an upper (430 m asl) and lower (330 m asl) site in the catchment (Figure 1) using two ARG100 tipping-bucket raingauges, each with a collection area of 0.51 m^2 and rim ~0.4 m above the ground. Bulk precipitation gauges situated near each tipping-bucket gauge collected samples for TCA

analysis via a 150-mm-diameter Pyrex glass funnel, set 1.5 m above ground level, draining into a polypropylene bottle.

Cloudwater was collected at the upper site using a passive harp wire device, as described by Crossley et al. (*16*). The collector had the form of an open inverted cone with closely spaced polypropylene filaments (0.6-mm diameter) strung around the sides. Cloud collection occurred by droplet impaction on the filaments. The apex of the cone drained into a funnel and into a polypropylene bottle. The cloud precipitation rate was calculated by first correcting the recorded cloudwater volume for the rainwater also collected by the device, and then by the average capture efficiencies of the cloud detector and vegetation (0.29 and 0.05, respectively).

Sub-samples of rain and cloudwater for chemical analysis were collected in 500-mL HDPE bottles (Nalgene) and stored at 4 °C until analysis (generally within 1−4 days).

Streamwater. Streamwater discharge was quantified within a rectangular cross-section 1.15 m wide installed in the stream at the catchment outlet. Vertical panels of marine plywood formed the sides of the cross-section and the natural streambed formed the base. Additional panels set into the banks prevented streamflow around the sides. An area-velocity flow logger (Isco 4150) in the center of the section recorded stream depth (via pressure transducer) and velocity (via Doppler ultrasound method) every 15 min. These were combined in real-time with the cross-sectional area data to yield discharge. The cross-section base was periodically remeasured throughout the year and the flow-logger was adjusted appropriately for any (small) changes. The automatic flow logger measurements agreed well with manual flow measurements made on three occasions using a Price type "mini" current meter (Scientific Instruments, Inc.).

Streamwater samples for TCA analysis were collected using a flow-proportional sampler (Isco 6700) controlled by the flow logger in order to obtain an unbiased measure of the integrated streamwater flux of TCA. The sampler was additionally programmed to take a grab sample between each visit. Grab samples of streamwater were also collected manually both at the catchment outlet and upstream as the stream left the moorland before entering the forest (Figure 1).

2.3 Sampling Within the Catchment. *Forest Sites.* Prior to sampling, the diameter at breast height (DBH) was measured of each tree within 10 10 m × 10 m quadrants distributed throughout the forest. DBH values ranged from 2.5 to 35.7 cm with a median of 10.5 cm. The median stem density of the sites was 4850 stems ha^{-1}. Four of the quadrants were selected as sites for the monitoring program (Figure 1): three of Sitka spruce and one of larch, in proportion to the overall distribution of species in the catchment. The Sitka North and Sitka South sites were situated well-within the forest, while the Sitka Edge site was used to assess edge effects. Although TCA concentrations in throughfall and stemflow were also measured during this study, they are not relevant to the assessment here of whole-catchment external fluxes and are reported in a separate discussion of the influence of the forest canopy on TCA cycling (*17*).

Tree foliage was collected three times during the year (Table 1) using squirrel pruners, at a height of ~4 m, from branches below the third whorl. On each sampling occasion needles and branchwood from three year classes were collected from three randomly selected trees near each site. In the laboratory the needles were stripped and pooled for each site, according to age class, within 0−2 days of sampling, and the needles and branchwood were frozen (−18 °C) until analysis.

Stemwood cores of 4-mm diameter were collected in May 2002 using a borer at 1.3 m height from eight trees (two per site) with DBH values representative of the distribution determined in the DBH survey. The cores were frozen until analysis.

Moorland Foliage. The five most abundant species of moorland vegetation, in descending order, were *Calluna vulgaris* (heather), *Molinia caerulea* (purple moor grass), *Vaccinium myrtillus* (bilberry), *Scirpus cespitosus* (deer grass), and *Nardus stricta* (mat grass). Each of these species was sampled at several randomly selected locations throughout the moorland area on three occasions (Table 1). On each occasion samples were pooled by species. Samples were either analyzed the following day or frozen on the day of sampling.

Soils. Detailed information on soil types, horizons, and depths for the catchment was obtained from the Soil Survey of Scotland (*18*). Soil TCA concentrations were determined in samples collected from small soil pits or by screw auger from the four forest sites and two sites of differing elevation in the moorland. Soil sampling was conducted in June 2001, January 2002, and May 2002 (Table 1). On each occasion 20−22 soil samples were collected, randomly at each site, eight from the moorland part of the catchment and the remainder from beneath the forest, and at different depths between 0 and 85 cm within the same core.

2.4 Analytical Methodology. The TCA concentration in all samples was determined using thermal decarboxylation of TCA to chloroform ($CHCl_3$) (*19*). Full details of the method as applied here are given in Heal et al. (*15*, *20*). Samples were sealed in 20-mL headspace vials and heated at 100 °C for 1.5 h to convert TCA to $CHCl_3$. The vials were reequilibrated at 60 °C for 1 h, and an aliquot of the headspace was transferred to a Perkin-Elmer Autosystem GC by an HS-40 headspace autosampler. Chromatography for $CHCl_3$ was performed isothermally at 50 °C on a DB-5 column (injector temperature 200 °C, detector temperature 375 °C). The presence of any background $CHCl_3$ in the sample, which would interfere with $CHCl_3$ from TCA decarboxylation, was accounted for by subtracting the amount of $CHCl_3$ determined in a parallel vial of sample equilibrated to 60 °C only.

Water samples were analyzed by sealing 5 mL of sample into the headspace vial and calibrated directly against 5 mL of standard TCA solution, ranging in concentration from 0.2 to 4 $\mu g\ L^{-1}$ processed in exactly the same way. Every sample was analyzed in triplicate. For samples with a TCA concentration greater than 0.8 $\mu g\ L^{-1}$, triplicate RSD was better than 30% in 75% of samples and better than 40% in 94% of samples. For samples where TCA concentration was less than 0.8 $\mu g\ L^{-1}$, RSD values of less than 30% and 40% were achieved in 50 and 62% of cases, respectively. The limit of detection of this method was determined to be around 0.1 $\mu g\ L^{-1}$.

Soil samples were sieved before analysis. Needle, branchwood, stemwood, and vegetation samples were ground to a powder in a pestle and mortar under liquid nitrogen to enhance TCA extraction and replicate precision. A 1-g aliquot of sample (±0.02 g) was sealed into a headspace vial. For calibration, a partition ratio (the ratio of the response factor of standard additions of TCA to a particular environmental matrix (needles, soil, etc.) relative to the response factor of aqueous TCA standards) was determined (*19*, *20*). The partition ratio is invariant to changes in absolute detection sensitivity so it was only necessary to determine the response factor to aqueous standards on each analysis run. The dry mass of soil and vegetation samples was determined from mass loss on drying for 6 days at 60 °C. The organic mass of soil samples was calculated from the additional mass loss of organic matter after heating at 550 °C for 8 h.

Each air sample filter (sample or blank) was heated in an open vial for 45 min at 60 °C to remove any background $CHCl_3$. The vial was then capped, heated at 100 °C to decarboxylate TCA, reequilibrated at 60 °C, and analyzed as

above. Subtraction of the blank filter peak accounted for any TCA intrinsic to the filter or the Na_2CO_3 solution. External calibration was performed against peaks from water standards prepared as described above. Analysis of filters was destructive, although sampling yielded duplicate sample filters every two weeks. The LOD for a two-week sample was ~10 pg m^{-3}.

The headspace decarboxylation method has the advantage of being a whole-sample technique whereas extraction-derivatization techniques, while specific for TCA, must assume that all intrinsic matrix-bound TCA is extracted into solution. The decarboxylation method does not directly quantify TCA so it is possible that some other compound(s) in the sample matrix might yield $CHCl_3$ under the sample decarboxylation conditions and be erroneously quantified as TCA. However, all samples were always blank-corrected for $CHCl_3$ quantified at 60 °C so that only $CHCl_3$ produced by the sample during 1.5 h above 60 °C is quantified as TCA. This potential bias should be recognized when interpreting TCA data presented here. For soils in particular, the decarboxylation method gives consistently larger TCA concentrations for whole soil than those obtained from aqueous extraction of soil, whether the extracts are analyzed by derivatization (*21*) or decarboxylation. It is not yet clear whether the decarboxylation method reflects total TCA, or the sum of water-soluble TCA and some interfering moiety. Our experiments on soils from this catchment indicate that only ~20% of "whole soil" TCA is water-extractable TCA. In this paper, TCA measured in soil is referred to as TCA* to emphasize that not all may be mobile in the soil.

3. Results

3.1 Hydrological Balance. The extent of catchment hydrological balance in the relationship

Wet (rain + cloud) deposition = stream discharge + loss by evapotranspiration in forest + loss by evapotranspiration in moorland

was fully evaluated to ensure that subsequent calculations of aqueous TCA fluxes were based on full characterization of the hydrology (*22*). The hydrological evaluation also included data from throughfall and stemflow collectors and in-situ soil lysimeters under the canopy at each forest site and in the moorland (not described here) to derive estimates of evapotranspiration losses. In summary, the hydrological dataset for the year of study in the Ballochbeatties catchment was comprehensive with almost no missing data. The only parameter not measured directly was loss by forest transpiration which was taken as the mean value of estimates of annual transpiration water depth reported for other U.K. upland coniferous forests. Applying all data, the two sides of the hydrological equality given above agreed to within 7%, well within likely measurement uncertainty. Overall, about 40% of hydrological input to this catchment was lost by evapotranspiration.

3.2 Catchment TCA Input and Output: Concentrations and Fluxes. *Air.* The concentration of total (gas and particle-bound) TCA in air was low, ranging from 5 to 131 pg m^{-3} (as two-week averages) with a median of 26 pg m^{-3} (Table 2 and Figure 2). There have been few measurements of TCA in air, but values measured here are consistent with concentrations ranging from <LOD to 600 pg m^{-3}, and from <LOD to 110 pg m^{-3}, for Edinburgh, and another rural site in Scotland, respectively (*15*), 6–700 pg m^{-3} in Germany (*23*), and mean concentrations of ~140 pg m^{-3} and ~200 pg m^{-3}, respectively, in Guelph and Toronto, Canada (*24*).

There was no obvious seasonal trend, in agreement with recent measurements in Canada (*24*); the notable peak in

TABLE 2. Summary of TCA Concentrations (and Accumulated Annual Total TCA) for Input and Output Fluxes to Ballochbeatties Catchment (area 0.84 km²), May 2001 to May 2002

	median TCA concn ($\mu g\ L^{-1}$)[a]	min, max TCA concn ($\mu g\ L^{-1}$)[a]	*n*	annual catchment volume (m^3)	annual catchment flux of TCA (g)
air (pg m^{-3})	26[a]	5, 131[a]	24		19
rainfall	0.81	0.32, 2.30	51	2.15×10^6	1804
cloudwater	0.81	<0.1, 3.45	24	3.54×10^5	275
streamwater	1.23	0.26, 2.02	26	1.48×10^6	1965

[a] Concentration units for air: pg m^{-3}.

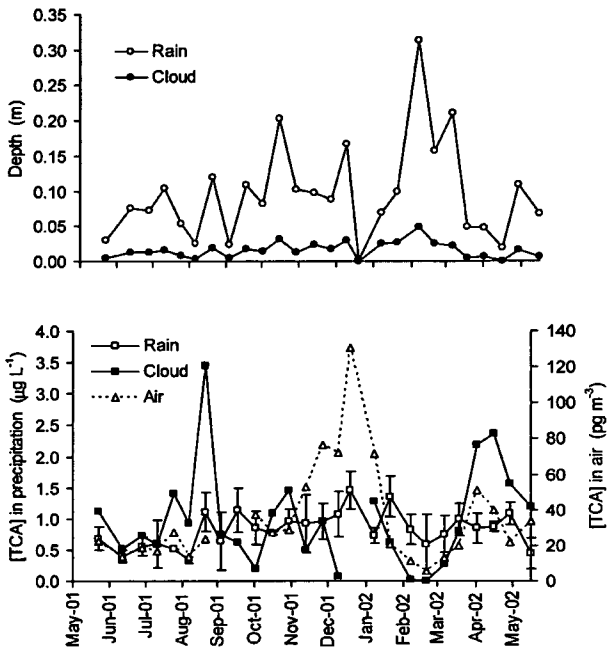

FIGURE 2. Top: rain and cloud precipitation depth. (Rain depth is the mean of two tipping-bucket rain gauges). Bottom: corresponding average concentrations of TCA in rain, cloud, and air. All data measured approximately every two weeks, May 2001 to May 2002. For clarity, error bars (±1 SD of triplicate TCA analyses) are shown for rainwater concentration only, but are typical of other measurements.

TCA concentration in mid-December 2001 in Figure 2 coincided with a period of stable high-pressure weather and a documented air pollution episode for the region. For example, the mean concentrations of PM_{10} particulate matter and NO_x gases recorded for this measurement period at an urban center location in Glasgow (70 km north of the catchment) were ~2.5 and ~6.5 times their annual averages, respectively.

To calculate the dry deposition flux of TCA, a single dry deposition velocity of 2×10^{-2} m s^{-1} was assumed as a maximum for both gas and particle deposition. Two-week dry deposition fluxes were summed to yield an estimated total annual dry input of 19 g for the whole catchment (Table 2). The uncertainty in dry deposition velocity has negligible consequence in comparison with wet deposition fluxes (see below).

Rainwater. Two-week averaged TCA concentrations from the upper and lower site are presented in Figure 2 and summarized in Table 2. The two-week rain precipitation depth, calculated as a mean of both tipping-bucket rain

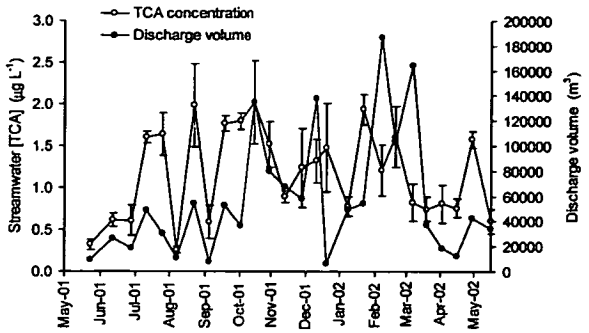

FIGURE 3. Cumulative streamwater volumetric discharge and discharge-weighted streamwater TCA concentration, determined approximately every two weeks at catchment outlet, May 2001 to May 2002. Streamwater samples were obtained by flow-proportional sampling during each two-week period. Error bars are ±1 SD of triplicate TCA analyses.

gauges, is also plotted in Figure 2. Rainwater TCA concentrations ranged from 0.32 to 2.3 $\mu g\ L^{-1}$ (median 0.81 $\mu g\ L^{-1}$). No significant difference in TCA concentrations was observed between the upper and lower sites, nor any evidence of seasonality. However, as expected for a soluble species, concentrations of TCA in air and rainwater correlated significantly, as observed previously elsewhere in Scotland (*15*).

The rainwater TCA concentrations are comparable to measurements from SE Scotland (*15*), but slightly higher than mean rainwater concentrations reported elsewhere, although data are highly variable. Rainwater and snow concentrations measured in Switzerland in 1996 and 1997 ranged from <0.002 to 2.064 $\mu g\ L^{-1}$ (mean 0.238 $\mu g\ L^{-1}$) (*12*). In recent reviews, McCulloch (*2*) reports a mean TCA concentration of 0.26 $\mu g\ L^{-1}$ and concentrations of up to 1.58 $\mu g\ L^{-1}$ for various rainwater samples in Canada, while Schöler et al. (*4*) report rainwater TCA concentrations in Europe in the range <0.01−20 $\mu g\ L^{-1}$.

The two-week flux of TCA in rain was calculated as the product of TCA concentration, precipitation depth, and catchment area, and summed to yield an annual rainwater input of 1804 g TCA (Table 2).

Cloudwater. The harp-wire cloud gauge collected both rainwater and cloudwater, so cloudwater-only TCA concentration was calculated from

$$[TCA]_{cloud-only} = (V_{cloud+rain}[TCA]_{cloud+rain} - V_{rain}[TCA]_{rain})/V_{cloud}$$

where V_x = volume of precipitation collected for sample type x.

The two-week cloudwater-only TCA concentrations and deposition depths are plotted in Figure 2. Concentrations ranged from <0.1 to 3.45 $\mu g\ L^{-1}$ with a median of 0.81 $\mu g\ L^{-1}$ (Table 2), and are comparable to cloudwater concentrations measured elsewhere in Scotland (*15*). There was no evidence of seasonality in cloudwater TCA concentration, nor of significant difference from rainwater TCA concentration, consistent with the review by McCulloch (*2*). At another Scottish site of higher elevation (602 m asl), cloudwater TCA concentration was moderately enhanced (~20%) over that of rainwater (*15*).

The annual flux of TCA in cloudwater to the catchment was calculated as for rainwater and yielded a value of 275 g.

Streamwater. Two-week integrated streamwater TCA concentrations and discharge volumes are plotted in Figure 3. Integrated streamwater TCA concentrations ranged from 0.26 to 2.02 $\mu g\ L^{-1}$, with a median of 1.23 $\mu g\ L^{-1}$. Average TCA

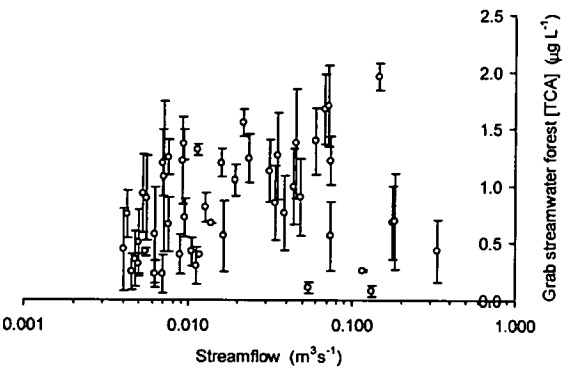

FIGURE 4. Relationship between streamwater TCA concentration in a "grab" sample at the catchment outlet and the mean streamwater volumetric flow rate for the preceding 2 h. A grab sample was taken manually at each two-week site visit, and automatically by the stream sampler at each intervening week. Error bars are ±1 SD of triplicate TCA analyses.

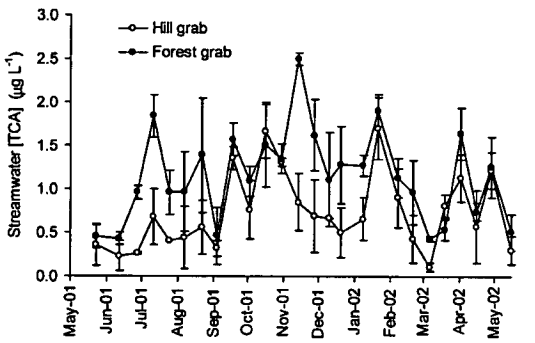

FIGURE 5. Concentrations of TCA in streamwater samples taken manually upstream before it entered the forest ("hill grab") and at the catchment outlet ("forest grab") after it had passed through the forest, May 2001 to May 2002. Error bars are ±1 SD of triplicate measurements.

concentration in streamwater exceeded that in rainwater, but such a comparison does not take into account concentration changes arising from loss of water by evapotranspiration in the catchment.

Figure 3 suggests that integrated streamwater TCA concentrations increased as the discharge volume increased. This is investigated further in Figure 4 which plots the TCA concentration of grab streamwater samples collected weekly at the catchment output against the mean discharge for the preceding 2 h. For discharges <~0.08 $m^3\ s^{-1}$ there was a trend for higher TCA concentration with higher discharge, although the relationship does not hold for the extreme flood events. At higher hydrological flux a greater proportion of discharge is likely to derive from flow through near-surface soil horizons which have greater TCA concentrations than lower soil horizons. Extreme discharge periods include contributions from overland flow where rainwater does not pass through soil at all. Also, during extreme rainfall events, the proportion lost by evaporation is low so there is no concentrating effect on discharge.

The TCA concentrations in the streamwater grab samples collected concurrently at the forest outlet and upstream before the stream enters the forest (see Figure 1) are plotted in Figure 5. On 25 out of the 26 sampling occasions the stream had enhanced TCA concentrations after passing through the forest. The concentrations in the grab samples after the stream had passed through the forest ranged from 0.08 to 2.50 $\mu g\ L^{-1}$ (median 1.09 $\mu g\ L^{-1}$); those taken before the stream

TABLE 3. Summary of TCA Concentrations in Each Environmental Compartment in the Ballocbeatties Catchment, and the Resultant Estimates of Total TCA Present in Each Compartment in the Whole Catchment

compartment (*C* = current year class)		TCA concn (ng g^{-1} dry weight) median	TCA concn (ng g^{-1} dry weight) min, max	*n*	dry mass in catchment (kg)	catchment TCA reservoir (g)
Sitka needles	C	78	35, 161	8	1.3×10^5	10
	C+1	99	64, 197	9	1.3×10^5	13
	C+2	145	78, 226	9	2.6×10^5	38
Larch needles		165	142, 187	2	4.1×10^4	7
Sitka branchwood	C	14	9, 58	7		
	C+1	15	9, 69	9	1.2×10^6	18
	C+2	19	13, 172	8		
Larch branchwood		29	28, 30	2	2.6×10^5	8
Sitka stemwood		11	4, 22	6	5.2×10^6	55
Larch stemwood		6	2, 11	2	1.5×10^6	10
moorland vegetation	heather (*Calluna vulgaris*)	26	16, 40	3		
	bilberry (*Vaccinium myrtillus*)	22	15, 25	3		
	mat grass (*Nardus stricta*)	59	51, 736	3	3.02×10^5	11
	purple moor grass (*Molinia caerulea*)	44	14, 161	4		
	deer grass (*Scirpus cespitosis*)	103	67, 139	2		
forest litter	Sitka	351	78, 979	8	3.38×10^6	1180
	Larch	56	11, 231	4	8.45×10^5	47
Moorland soils[a]	vegetation layer	190 (17)	79, 195	3		
	litter layer	51 (12)	44, 110	5		
	organic horizon	42 (8)	20, 3090	8		
	mineral horizon	16 (7)	9, 37	8		
Sitka soils[a]	litter layer	559 (194)	150, 2059	6		
	organic horizon 1	192 (46)	77, 1549	7	3.34×10^8	12000
	organic horizon 2	84 (16)	28, 463	7		
	organic horizon 4	57 (9)	12, 131	8		
Larch soils[a]	litter layer	56 (11)	11, 231	4		
	organic horizon 1	71 (26)	61, 124	3		
	organic horizon 2	37 (15)	26, 41	3		
	mineral horizon	18 (12)		1		
					total	13400

[a] All data for soils represent TCA*, as discussed in the text. Data in parentheses for soils indicate concentration of TCA* as ng g^{-1} fresh weight.

entered the forest ranged from 0.11 to 1.70 μg L^{-1} (median 0.67 μg L^{-1}).

Two-week stream fluxes of TCA were calculated by multiplying measured discharge volume and integrated streamwater TCA concentration, and summed to obtain a total annual output of TCA from the catchment of 1965 g.

3.3 Within-Catchment TCA: Concentrations and Catchment Reservoirs. For the reasons outlined in Section 2.4, caution must be exercised in making direct comparisons of TCA concentrations measured by different analytical methods.

Needles, Branchwood, and Stemwood. The TCA concentrations in Sitka spruce (3 year classes) and larch (current year) from three sampling occasions are summarized in Table 3. There was little evidence of a significant difference in TCA concentrations between trees sampled at sites within or along the edge of the forest. Concentrations in spruce needles ranged from 35 to 226 (median 100) ng g^{-1} dry weight, or 14 to 123 ng g^{-1} fresh weight. Median concentration in larch foliage was 165 ng g^{-1} dry weight. These data are within the range of approximately 4−180 ng g^{-1} for TCA in coniferous foliage summarized in recent reviews of the literature (*2*, *4*).

The concentration of TCA in branchwood was generally lower than that in foliage (Table 3), ranging from 9 to 172 ng g^{-1} (median 15 ng g^{-1}) dry weight for spruce and from 28 to 30 ng g^{-1} dry weight for larch. The needle TCA concentration always increased with year class (needle age). Branchwood concentrations tended to increase with year class but were more variable. TCA concentrations in stemwood were lower again and ranged from 2 to 22 ng g^{-1} dry weight, with median values of 11 and 6 ng g^{-1} for Sitka spruce and larch respectively (Table 3). Data for TCA in branch and stemwood of trees have not been reported previously.

By combining the data on measured tree sizes and distribution, planting maps, and a wide range of literature sources for relationships between biomass and tree DBH or age, best-judgment estimates were derived of forest biomass in different compartments, as dry mass per m^2 and total biomass in catchment. The median TCA concentration per dry weight measured for each compartment was multiplied by the calculated total dry mass (kg) of that compartment in the catchment to obtain total mass of TCA in the catchment in each of: Sitka spruce years C, C+1, and ≥C+2 needles, Sitka branchwood, Sitka stemwood, larch needles, larch branchwood, and larch stemwood (Table 3). Summation yielded a total mass of 159 g TCA in all forest vegetation in the catchment.

Forest Litter. The total mass of TCA stored in the soil litter horizon under Sitka and larch forest was calculated from the soil survey results by multiplying the total dry litter mass (area × median litter depth × dry bulk density of litter) by the median litter TCA dry weight concentration. The concentrations used in these calculations are shown in Table 4. The calculated mass of TCA stored in forest litter was 1.2 kg (Table 3).

Moorland Foliage. TCA concentrations in the five most abundant vegetation species are summarized in Table 3. These are the first reported concentrations of TCA in these vegetation types. Median TCA concentration for each species varied between 22 and 103 ng g^{-1} dry weight with the grasses

TABLE 4. Data Used to Calculate Catchment Reservoir of TCA in the Litter Layer Beneath Sitka Spruce and Larch Plantation in the Ballochbeatties Catchment

	area in catchment (m^2)	dry bulk density of litter ($kg\ m^{-3}$)	median (min, max) litter depth (m)	median (min, max) TCA concentration ($ng\ g^{-1}$ dry weight)	total TCA catchment reservoir (g)
Sitka spruce	3.28×10^5	103	0.10 (0.04, 0.14) $n = 7$	351 (78, 979) $n = 8$	1180
Larch	8.20×10^4	assumed as spruce	0.10 (0.04, 0.10) $n = 3$	56 (11, 231) $n = 4$	47

generally having higher concentrations than heather or bilberry. The concentrations were generally lower than those in tree foliage yet still indicate that moorland vegetation biomagnifies TCA compared with aqueous input.

Estimates of moorland vegetation dry biomass for each species present were derived by combining data from the vegetation survey with literature biomass data. The dry biomass of each vegetation species was multiplied by the median TCA concentration per dry weight for that species, and summation yielded a total TCA mass in all moorland vegetation in the catchment of 11 g (Table 3). This is less than 10% of TCA contained in the forest biomass which covers about the same area in the catchment as the moorland.

Soils. As discussed in Section 2.4, concentrations of TCA in soil measured here are referred to as TCA*. A summary of the TCA* concentrations measured in soils across the catchment is given in Table 3. TCA* concentrations ranged from 4 to 404 ng g^{-1} (median 15 ng g^{-1}) fresh weight. Even allowing for the question of proportion-extractable TCA (see Section 2.4) these values are high compared with previous data recently summarized in the literature (*2*, *4*). The concentrations decreased with depth and were generally greatest in the deep peat soil under Sitka spruce forest, both of which are in agreement with previous observations. Particularly high concentrations of TCA* were measured in surface litter layers of spruce forest. It was not possible to discern any seasonal patterns due to the heterogeneous nature of the soil data.

Interestingly, TCA concentrations measured in soil lysimeter leachate (data to be considered in a later publication), were generally low (<2 $\mu g\ L^{-1}$) and not obviously different from concentrations in rainwater or throughfall.

Areas of soil associations within the catchment were calculated from a 1:25 000 soil map (*18*) and comprised (in units of m^2) the following: Blanket peat (122 500), Dalbeattie Garrary 38 750), Ettrick Finlas (172 500), Ettrick Brochloch (43 750), and Ettrick Darnaw (482 500). Each soil association contained one to six component soils, data on which were supplied by the Macaulay Land Use Research Institute. Soil dry mass for each horizon was calculated as follows: (area of soil association) × (fraction cover of soil component) × (horizon depth) × (horizon dry bulk density). Measured concentrations of soil TCA* were assigned to an appropriate soil survey association and horizon, and the median TCA* for each horizon was used to calculate a total soil TCA* burden in the catchment. The total was 12.0 kg (Table 3).

4. Discussion

This discussion is based on data from ~230 separate samples of catchment input and output TCA flux and ~150 separate samples for catchment TCA burden.

The total output of TCA via streamwater from the Ballochbeatties catchment for the year under study was 1965 g, or ~94% of the total annual input of 2098 g via dry (19 g) and wet deposition (2079 g) (Table 2). These input and output fluxes are equal to well-within likely uncertainties in the values. There was no obvious seasonal hysteresis in two-week input and output TCA fluxes to this catchment, which is expected given that wet precipitation is distributed evenly throughout the year in the UK. The principal nonanalytical sources of uncertainty in quantifying inputs and outputs are the measurements of cloudwater depth and stream discharge. Sources of cloudwater uncertainty include the subtraction of cloudwater depth from rainwater plus cloudwater depth, and the values of the capture efficiencies of the cloudwater gauge and forest canopy required to convert measured cloudwater depth to actual precipitation depth. Total cloudwater depth uncertainty is estimated to be ~±25%. Considering cloudwater accounts only for ~13% of annual catchment TCA deposition, the effect of even large uncertainties in its value on calculated TCA input is small. In contrast, measurement of stream discharge, which directly affects calculated total output TCA flux, may, realistically, have an uncertainty of the order ±15%. The mean difference of three manual/automated discharge comparisons was 10%. However, flow-proportional sampling was used to derive the streamwater flux value which explicitly takes into account the variation of streamwater TCA concentration with discharge volume (Figure 4). Random analytical uncertainties in measurement of TCA in aqueous samples averaged around 20–30% (Section 2.4). Therefore, total uncertainty in input and output TCA fluxes is likely to be in the range ±30%. The measured input and output TCA fluxes differ by less than 7%.

Wet deposition dominates (99%) the input of TCA. The TCA input flux at Ballochbeatteies is equivalent to 2.4 kg $km^{-2}\ y^{-1}$. This is higher than the wet TCA deposition fluxes of ~1.0 kg $km^{-2}\ y^{-1}$ reported for a separate upland site in Scotland (*15*) or 0.36 kg $km^{-2}\ y^{-1}$ TCA estimated for Switzerland (*12*). The higher TCA flux mainly reflects the considerably higher annual precipitation (rain and cloud) in the Ballochbeatties catchment (2.91 m, compared with 1.29 m for Dunslair Heights in SE Scotland, and 1.50 m for Switzerland), but also the higher TCA concentration in Scottish precipitation (Scottish median TCA concentrations ~0.7–0.8 $\mu g\ L^{-1}$, Switzerland mean ~0.24 $\mu g\ L^{-1}$).

Atmospheric deposition of TCA must represent new TCA to the catchment because the high solubility and Henry's Law coefficient of TCA prevent its re-volatilization to the atmosphere. The data from this study confirm that the atmospheric flux of TCA is greater than that predicted from currently accepted oxidation reactions of 1,1,1-trichloroethane and tetrachloroethene in the atmosphere (*2*). If these reactions are correct, the observations suggest an additional source of TCA, which may arise from greater rates of aqueous-phase reactions within the maritime climate of Scotland (*15*).

Summing the individual compartment TCA burdens yields an estimated total mass of TCA in the Ballochbeatties catchment of ~13 kg (Table 3), which is approximately six times the annual through-flux. The catchment reservoir of TCA (or TCA*) is dominated by soils (~90%), with the remainder distributed in forest litter (~9%), forest branchwood and stemwood (~0.7%), forest needles (~0.5%), and moorland vegetation (~0.1%) (Table 3). Clearly, the uncertainty in total TCA burden is dominated by the uncertainty

for TCA in soil. Soil TCA burden is the most uncertain quantity anyway because of the inherent difficulty of accurately scaling up the spatial heterogeneity associated with soil properties and TCA concentration. In this work, detailed information on soil horizons, depths, and bulk densities was available and utilized. However, even a crude approximation applying a single value for mean fresh weight soil TCA* concentration to a single soil depth over the catchment yields a similar estimate of many kg TCA in catchment soil, in line with the value of 6.4 kg km^{-2} estimated for soil by Schöler et al. (*4*). As discussed in Section 2.4, soil TCA* may be an over-estimate due to analytical interference or because only a proportion TCA of TCA* is extractable. Our laboratory aqueous extraction experiments suggest that only ~20% of "whole-soil" TCA is water extractable. If the nonextractable fraction of TCA* is not included as TCA then the TCA burden in the Ballochbeatties catchment would be ~3.5 kg km^{-2}, giving a ratio of reservoir to annual flux of around 2, much smaller than the ratio 20 estimated by Schöler et al. (*4*). However, the latter work applied averaged literature values from a range of studies to a generic forest catchment, whereas the values reported here are based on a complete and consistent set of measured data.

Because the input/output fluxes suggest no current net TCA accumulation from atmospheric deposition, possible explanations for this observed within-catchment TCA are (1) past enhanced deposition, (2) a marginal excess input accumulated over decades, (3) in-situ net production within soils. Both the first two explanations require that externally added TCA has a lifetime in soil and biota of many years or decades, whereas experiments indicate that TCA is degraded comparatively quickly in soils. For example, Foy (*25*) suggested that phytotoxic residues of TCA "usually disappear" from soil within 30–90 days, with fastest degradation occurring on wet soil with a high organic content. Experiments by this group have shown rapid loss of TCA spiked to the pots of Sitka spruce seedlings (*20, 26*) or to soil lysimeters in the field or laboratory, with an estimated half-life of 1–2 weeks. Experiments using [1,2-^{14}C]-labeled TCA applied to soil of potted Norway spruce seedlings also implied degradation of TCA in soil on a time scale of days to weeks (*27*).

TCA is also degraded by coniferous foliage. Comparison of the below-canopy throughfall to the above-canopy deposition in this Sitka spruce plantation (*17*) has shown that TCA is degraded within or on the canopy at an annual rate approximately twice the forest reservoir of TCA. Other experiments with controlled application of TCA to tree seedlings have reported half-lives for TCA loss in needles (during the growing season) of ~50 days or ~10–15 days for Sitka spruce (*20*) or Scots pine (*28*), respectively. However, TCA uptake and elimination in the forest biomass, although potentially important for the tree, has a minor role in TCA cycling in a catchment because the mass of TCA within vegetation is only ~1.5% of total catchment TCA burden.

If the catchment is at steady-state, the flux and reservoir values imply an average catchment residence time for TCA of ~6 years if all reservoir TCA is involved in catchment through-flux, or 1–2 years if only the extractable fraction of TCA* in soil is considered. In contrast, the average hydrological residence time for the catchment is estimated to be 4–6 months. However, as stated above, TCA is also presumed to be subject to within-catchment degradation. Therefore, in conclusion, (1) there must be production of TCA within the soil of the Ballochbeatties catchment, and/or (2) only a proportion of measured TCA in the soil is actively involved in catchment TCA cycling, even taking into account the water-soluble fraction. The first conclusion is supported by reports of TCA formation in laboratory experiments of soil (*9, 29*) and other attempts at TCA mass balance calculations (*3, 4, 13*). Additional catchment evidence is provided by observation of enhancement of TCA concentration in the stream as it passes through the organic-rich soil of the forest (Figure 5). The second conclusion is supported by our own experiments which have shown that when TCA is extracted from catchment soil using water, only a minor proportion of "whole-soil" TCA*, as determined by the headspace method, is extracted into solution. This suggests that only a proportion of intrinsic soil TCA may be mobile in hydrological pathways. It also suggests that whole-soil TCA concentrations may be underestimated by methods that require the assumption of 100% extraction into water.

Acknowledgments

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New data for water losses from mature Sitka spruce plantations in temperate upland catchments

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Abstract Accurate estimates of water losses from mature Sitka spruce (*Picea sitchensis*) plantations in the UK uplands are required to assess the sustainability of water supply in the event of land-use change. Many investigations have demonstrated that afforestation increases water losses from temperate upland catchments, to up to 40% of annual site rainfall. In a 0.86 km^2 upland water supply catchment in southwest Scotland, interception loss in a Sitka spruce-dominated 37-year old plantation, was 52% of annual precipitation (2912 mm), considerably higher than reported in previous studies of similar catchments. From direct measurements of rainfall, cloudwater, discharge and soil evaporation, the catchment water balance was 96–117% complete, within the limits of measurement error. The most probable explanation for the higher forest interception loss reported here is the inclusion of cloudwater measurements.

Key words catchment water balance; cloudwater; conifer forest; evapotranspiration; interception; stemflow; throughfall; UK; upland catchment; water loss

Nouvelles données pour les pertes d'eau de plantations adultes de sapins de Sitka en bassins versants tempérés d'altitude

Résumé Des estimations précises de l'eau perdue par des plantations adultes de sapins de Sitka (*Picea sitchensis*), dans les zones d'altitude du Royaume Uni, sont nécessaires pour évaluer la durabilité de l'alimentation en eau en cas de changement d'occupation du sol. De nombreuses recherches ont démontré que le reboisement augmente les pertes d'eau subies par des bassins versants d'altitude tempérés, pouvant atteindre 40% des précipitations annuelles. Dans un bassin versant du sud-ouest de l'Ecosse, de 0.86 km^2, situé en altitude et dédié à l'approvisionnement en eau, la perte par interception au niveau d'une plantation dominée par des sapins de Sitka âgés de 37 ans, a atteint 52% de la précipitation annuelle (2912 mm), ce qui est considérablement plus important que ce qui est montré dans les études précédentes menées sur des bassins similaires. Des mesures directes de la pluie, de l'eau des nuages, du débit et de l'évaporation au sol, ont permis de boucler le bilan du bassin versant avec une précision de 96–117%, ce qui correspond aux erreurs de mesure. L'explication la plus probable de cette perte par interception forestière plus importante est la prise en compte des mesures de l'eau des nuages.

Mot clefs bilan hydrologique de bassin versant; eau de nuage; forêt de conifères; évapotranspiration; interception; ruissellement le long des troncs; pluie au sol; Royaume-Uni; bassin versant d'altitude; perte d'eau

INTRODUCTION

Assessments of the effect of land-use changes on runoff from temperate upland catchments are important for water supply, flood prediction and maintaining ecologically acceptable flows. The UK uplands have experienced a substantial change in land use since the 1930s with the planting of conifer forests, dominated by Sitka spruce (*Picea sitchensis*), to reduce reliance on imported softwood timber and pulp.

Planting has been concentrated in the UK uplands on land of marginal agricultural value, replacing the existing vegetation cover of heather (*Calluna vulgaris*) and grass-dominated moorland. In Great Britain the total area of Sitka spruce forest now stands at 6920 km^2 (Forestry Commission, 2002) and 85% of conifer plantation is in upland regions (Rowan, 1986). Initially water authorities favoured the afforestation of upland catchments that supply water to the major UK urban conurbations outside of London and southwest England. The dense forest cover reduced soil erosion and sedimentation in reservoirs and also restricted public access to reservoirs, thereby minimising the risk of water contamination from human activity. It was also believed (incorrectly) that the forest cover enhanced catchment rainfall, resulting in increased water resource availability (McCulloch & Robinson, 1993). However, lysimeter studies in the 1950s of the hydrology of reservoir catchments in northwest England demonstrated that conversion of the land cover of temperate upland catchments from heather moorland or grass to conifer plantation caused an increase in water loss and a decrease in runoff (Law, 1956, 1958). This finding was of considerable concern to water authorities as the change in land cover of water supply catchments from moorland to forest reduced water resource availability. Law's results have been confirmed by subsequent detailed investigations in the UK, particularly by the former Institute of Hydrology in paired catchment experiments in the 1970s and 1980s at Plynlimon (mid Wales), Coalburn (northern England) and Balquhidder (southern Highlands, Scotland) (Institute of Hydrology, 1991a, 1991b, 1998). From these studies it is now widely-accepted that conifer afforestation of upland catchments with annual precipitation in excess of 1000 mm causes increased water losses of approximately 35-40% of incident precipitation compared with heather moorland or grass (Calder & Newson, 1979). Departures from this figure may occur in catchments where snow forms a large proportion of the annual water input and/or shrubs with a high canopy storage capacity grow in exposed areas (e.g. Balquhidder—Institute of Hydrology, 1991b). The cause of increased water losses when upland catchments are afforested is increased interception losses arising from differences in canopy properties between coniferous trees, such as Sitka spruce, and heather and grasses. A higher proportion of incident precipitation is intercepted within the canopy of coniferous trees and is available for loss by evaporation. Evaporation rates from wetted forest canopies have been shown to be higher than those from heather and grass due to the lower aerodynamic resistance of the wetted forest surface and the greater availability of energy within the forest as the result of advection and/or radiation balance modifications (Ward & Robinson, 1999).

The majority of field studies estimate water losses from forested catchments in one of two forms—evapotranspiration or interception. Interception (I) is incident precipitation stored in the canopy and lost to the atmosphere by evaporation. Evapotranspiration (E_T) is the sum of I plus forest transpiration (t) and evaporation from the forest soil and understorey vegetation (often assumed to be negligible in humid temperate forests). Interception is normally estimated in plot or lysimeter studies as the difference between incident wet precipitation to the forest canopy (P) and the sum of throughfall (T) and stemflow (S) below the canopy. Evapotranspiration has been estimated for whole catchments in numerous studies (e.g. Institute of Hydrology, 1991a, 1991b) from application of the catchment water balance equation to measurements of precipitation inputs and discharge outputs (Q) over a minimum period of a year. Over this time it is widely assumed that the net change in

groundwater and soil water storage is zero in upland catchments with steep gradients, thin soils and relatively impervious geologies. The two approaches of estimating catchment forest water losses are summarised in equations (1) and (2):

Whole catchment studies:

$$E_T = P - Q \quad (1)$$

Plot/lysimeter studies:

$$E_T = I + t = [P - (T + S)] + t \quad (2)$$

The majority of field studies reported have utilized only one approach of estimating forest water loss. In this paper forest water losses, derived from both approaches, are reported from a study of an upland water supply catchment in southwest Scotland, UK, that is dominated by mature Sitka spruce forest (37 years of age). Explanations are discussed as to why the measured water loss in this study from mature Sitka spruce forest is considerably higher than reported in other studies, expressed as percentage of incident precipitation.

STUDY SITE

The study site was the Ballochbeatties catchment, located in Ayrshire, southwest Scotland, UK (55°13′N, 4°29′W) (Fig. 1), and constituting part of the catchment of Loch Bradan, the seventh largest water supply source (by yield) in Scotland (Jowitt & Hay-Smith, 2002). The climate is humid, temperate, cool and windy. Summary climate data for the nearest weather station (4 km northeast of the catchment) at 250 m elevation at Loch Bradan Treatment Works are shown in Table 1. The catchment area (measured from a 1:5000 scale topographic map) is 0.86 km^2 and elevation ranges from 320 to 480 m. Slope gradients vary from 2° in the lower part of the catchment to in excess of 25° in the upper part and the underlying geology is hard metamorphosed greywackes overlain with glacial drift. For these reasons the catchment is expected to be largely hydrologically self-contained, with a flashy runoff regime dominated by overland and near-surface throughflow and with minimal contribution from groundwater. A recent reservoir yield assessment estimated groundwater recharge in the wider Loch Bradan catchment to be <100 mm $year^{-1}$ (Jowitt & Hay-Smith, 2002) and hydrological studies of nearby Loch Fleet showed that groundwater contributed only 5% of the mean loch outflow (Cook *et al.*, 1991).

Catchment land use and soil types are typical of the UK uplands. The upper catchment consists of unimproved moorland (NVC community type M15, *Scirpus cespitosis–Erica tetralix* wet heath) (0.45 km^2) underlain by peat, peaty gley and peaty podzols of the Dalbeattie and Ettrick soil associations (0.2–1.0 m depth), with occasional rock outcrops. The lower part of the catchment comprises forestry plantation (0.41 km^2), planted largely on basin peat (>2 m depth in places) and dominated by Sitka spruce (*Picea sitchensis*) and hybrid larch (*Larix eurolepsis*) (67 and 16% of forested area, respectively). The forestry plantation has been managed in the standard manner with preparation of ground by ploughing and construction of drainage ditches prior to planting in 1964. In 2000, the mean tree density was 1 stem per 2.2 m^2 and the mean forest basal area was 53 m^2 ha^{-1} (from diameter at breast height (DBH) measurements of 471 trees in ten plots of 10 m × 10 m, selected to represent the forest tree species and site conditions).

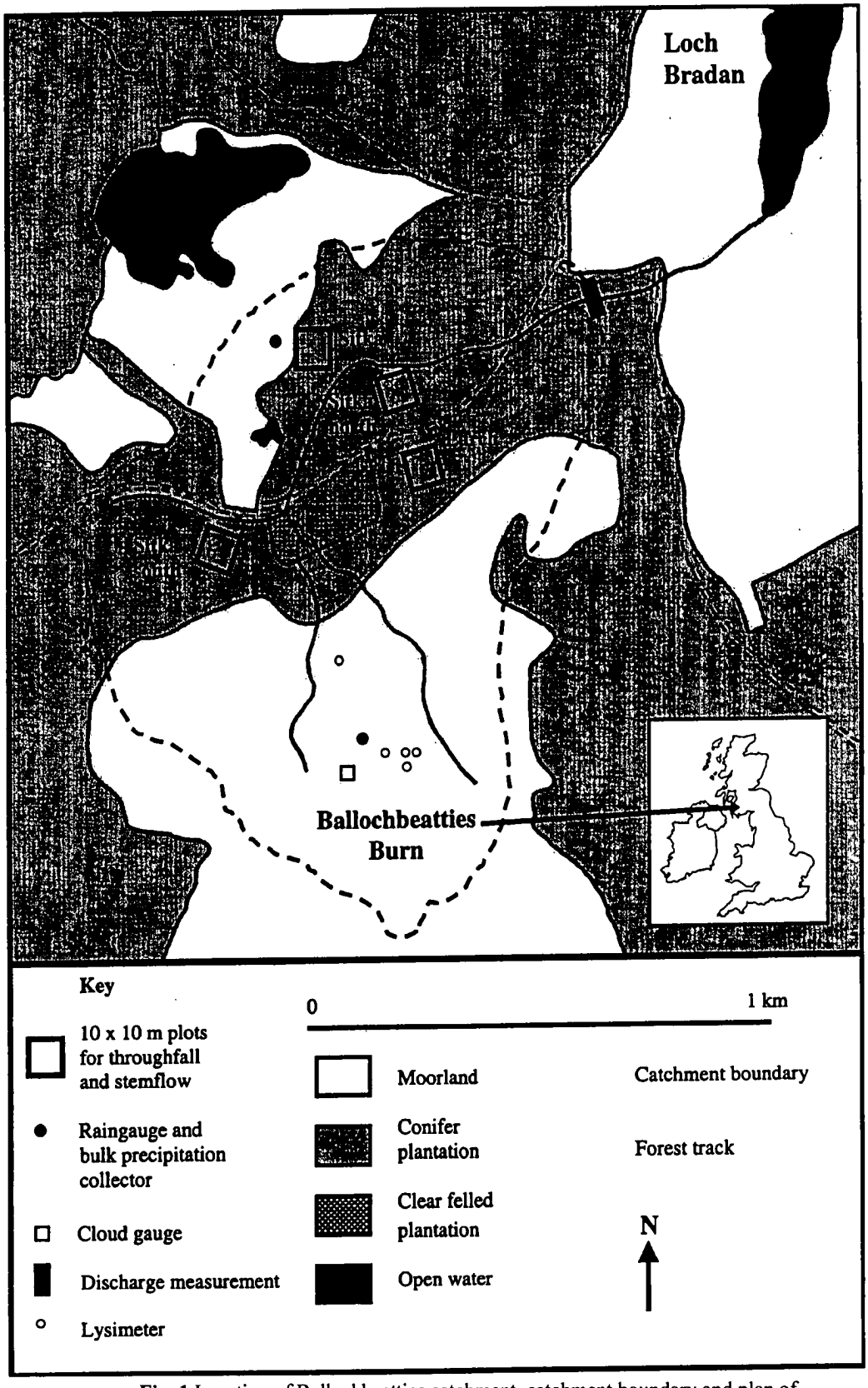

Fig. 1 Location of Ballochbeatties catchment, catchment boundary and plan of catchment hydrological measurement network.

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Table 1 Summary climate data from Loch Bradan Treatment Works weather station located at 250 m elevation.

Mean annual rainfall (mm)	1824 *	1988–1999
Mean daily minimum air temperature (°C)	4.7	1993–1998
Mean daily maximum air temperature (°C)	11.4	1993–1998
Mean daily wind speed (m s^{-1})	3.7	1993–1998
Mean daily maximum wind speed (m s^{-1})	16.7	1993–1998

* Missing daily values interpolated from six weather stations within a 20 km radius (Poole, 2001).

METHODS

In 2001 the Ballochbeatties catchment was instrumented for a one year study of trichloroacetic acid (TCA) cycling, necessitating the measurement of rainfall, cloudwater, throughfall, stemflow, soil water behaviour in lysimeters and discharge at the catchment outlet (Fig. 1). Rainfall and discharge were recorded at 15-min intervals and all other measurements were made as fortnightly totals on 26 occasions from 9 May 2001 to 15 May 2002. Rainfall was monitored by two ARG100 tipping bucket raingauges (rim 0.4 m above ground surface) in the moorland (430 m elevation) and forested (in a clearing at 330 m elevation) parts of the catchment, ensuring that the gauges were located the minimum distance of two times the height from the nearest tallest object (Met Office, 1997). Although aboveground raingauges are expected to systematically underestimate actual rainfall to the ground surface due to the effect of wind drift, rainfall data were not corrected since the loss of catch has been shown to vary seasonally, from storm to storm and also between sites (Rodda & Smith, 1986). Furthermore, the underestimate of rainfall catch by the ARG100 raingauges is expected to be less than documented in other studies due to the aerodynamic design of the gauges which minimizes drag by presenting a reduced side area to the wind compared to standard cylindrical aboveground raingauges. Cloudwater was measured at 440 m in the upper reaches of the catchment using a passive harp-wire device strung with closely-spaced 0.6 mm diameter polypropylene filaments (as described by Crossley *et al.*, 1992) and mounted, at 1.5 m above the ground, on a funnel draining to a 2.5-l container. This container overflowed on four occasions and was replaced with a 10-l container which subsequently only overflowed on one occasion when 314 mm rain fell in a fortnight. The linear relationship between rainfall and water collected in the 10-l container ($r^2 = 0.875$, $n = 20$) was used to correct the cloud gauge data for the occasions when the 2.5-l container overflowed. Cloudwater volume was calculated as the difference in water volume collected by the cloud gauge and a nearby bulk precipitation collector (also mounted at 1.5 m above the ground) since the cloud gauge was exposed to driving rain. Cloud deposition was calculated as the cloudwater volume divided by the capture efficiency of the harp-wire collector (0.29) and multiplied by the average capture efficiency of Sitka spruce (~0.05). The estimates of the capture efficiencies of the gauge and vegetation are from field measurements in a Scottish upland Sitka spruce forest (Beswick *et al.*, 1991; Crossley *et al.*, 1992) and have been used to estimate cloudwater deposition in other UK upland catchments (Crossley *et al.*, 1992; Heal *et al.*, 2003). The effect of errors in rainfall and cloudwater measurement on catchment water balance are assessed in the Results.

In situ lysimeters (volume 3800 cm^3, top diameter 20.2 cm) were constructed by placing intact soil cores (and attached vegetation for the moorland only) into plastic

containers with a perforated base. The soil-filled containers were then replaced in the soil core holes in the field and soil drainage was collected in a polyethylene bag (volume *c.* 3 l) suspended below the container. Five lysimeters were located in the moorland part of the Ballochbeatties catchment and five in the forest (four under Sitka spruce and one under hybrid larch) (see Fig. 1) to assess TCA behaviour in soil and soil water. Fortnightly measurements ($n = 26$) of the water volume inputs and outputs from the forest and moorland lysimeters from May 2001 to May 2002 were used to estimate evaporation from forest soil and evapotranspiration from the moorland, respectively. The inputs were rainfall and cloudwater to the moorland lysimeters and throughfall only to the forest lysimeters, which were located away from tree trunks. Overflow of the output collection bag occurred on five occasions for the moorland lysimeters and on one occasion for the forest lysimeters. The output volumes on these occasions were estimated by applying linear relationships, fitted between output and input volumes for each lysimeter for the measurement periods when overflow did not occur, to the measured input volumes.

Forest stemflow and throughfall were measured in four of the 10 m × 10 m forest plots surveyed in 2000 (Table 2, Fig. 1), adhering to recommended methods (Puckett, 1991; Thimonier, 1998). The plots were selected to represent the relative proportions of tree species in the forest and included a forest edge site (Sitka Edge in Table 2) to take account of any edge effects on forest water loss. Three stemflow and three throughfall collectors were installed within each plot. The trees for stemflow measurements were selected to be representative of the distribution of measured DBHs in the forest, by choosing trees with DBHs corresponding to the median value of nine evenly spaced noniles for all ranked Sitka spruce DBH measurements (for Sitka spruce) and three evenly spaced terciles for all ranked larch DBH measurements (for larch). Stemflow collectors consisted of flexible plastic tubing (2 cm diameter), with a narrow slit opening, attached in a spiral around the tree trunk at approximately 1.5 m above the ground. Any gaps between the tubing and the tree trunk were filled with sealant. Stemflow collected in the tubing was directed in a closed tube to a 25-l lidded tank at the base of the tree.

Throughfall was collected in each plot in three below canopy inclined guttering systems, with a total area per plot of 0.92–0.95 m^2, that drained into a 25-l lidded tank. Every fortnight, water depths in the throughfall and stemflow tanks were measured in the field and converted to volumes from the calibration of an identical tank in the laboratory. Water loss from the tanks by evaporation between site visits is likely to be very small as the tanks are lidded and located beneath the forest canopy in a cool, temperate climate. Since throughfall is highly spatially variable beneath forest canopies, additional throughfall tanks were deployed in each forest plot as a check on the validity of the throughfall measurements. The fortnightly throughfall depths measured in these tanks was always within the range of depths from the main throughfall collectors. The throughfall tanks overflowed on five occasions for which throughfall

Table 2 Characteristics of the forest throughfall and stemflow measurement plots, Ballochbeatties catchment. DBH = diameter at breast height.

	Sitka Edge	Sitka North	Sitka South	Larch
No. of stems in 100 m^2	42	64	45	40
Mean DBH (cm)	9.9	9.5	13.0	13.5

depths were calculated from the good linear relationship between fortnightly throughfall depth and mean catchment rainfall for all the throughfall collectors in the four forest plots ($r^2 = 0.941$, $n = 21$). The use of a linear function based on lower rainfall periods to estimate throughfall in high rainfall periods normally underestimates throughfall because, in low rainfall periods, repeated wetting and drying of the canopy will occur, enhancing interception loss and reducing throughfall. During higher rainfall the canopy storage will be full frequently, and the opposite effect will occur: interception losses will be limited and throughfall will be enhanced relative to rainfall. However, since the study catchment has a very high annual rainfall (2500 mm), this effect is expected to be negligible as the forest canopy is wet most of the time. Furthermore, even if throughfall was underestimated by 20% during the wettest fortnight (in which 13% of the annual rainfall occurred) the annual throughfall total only increases by 2.6%.

Discharge was measured at 15-min intervals, in a stabilized stream section at the catchment outlet, using a Doppler ultrasonic velocity gauge and pressure transducer (Isco 4150). The stream cross-sectional area was remeasured throughout the year and adjustments for any (small) changes in area made. On three occasions discharge was measured manually in the same section, using a Price type "mini" current meter (Scientific Instruments, Inc.), and demonstrated reasonable agreement (±17%) with the automated discharge measurements, considering the uncertainties of both methods.

RESULTS

Field measurements

The depths of cloudwater, rainfall, discharge, throughfall and stemflow are shown for each measurement period in Fig. 2. Table 3 contains a summary of the hydrological data for the Ballochbeatties catchment for May 2001–May 2002. Rainfall was calculated as the mean depth measured by the two automatic raingauges to represent the range of elevations in the catchment. Fortnightly rainfall depths at the two gauges were strongly correlated ($r^2 = 0.979$) and 7% more annual rainfall was measured at the upper gauge, as expected. Estimated cloudwater inputs account for 14% of the total annual precipitation input to the Ballochbeatties catchment. Other measurements of cloudwater deposition as a proportion of total precipitation input at upland sites in southern Scotland support this figure (24%—Crossley *et al.*, 1992; 11%—Heal *et al.*, 2003). Fortnightly discharge depth was calculated by dividing the total fortnightly discharge volume by the catchment area (860 000 m^2). The fortnightly discharge depths were then summed to obtain the annual discharge depth. Fortnightly river discharge depths closely track rainfall depths (Fig. 2(a)), showing that discharge responds rapidly to rainfall inputs and that the effect of subsurface storage is negligible in the Ballochbeatties catchment.

There were no apparent differences in measured throughfall depths between the Sitka spruce and hybrid larch forest plots nor between Sitka Edge and the other Sitka plots (Fig. 2(b)). The fortnightly mean throughfall depth was calculated from the three throughfall samplers at each plot and then summed to obtain the annual throughfall depth in each plot. Annual forest throughfall in the catchment was calculated as the mean of the four annual plot values, since the averaging of contributions from three

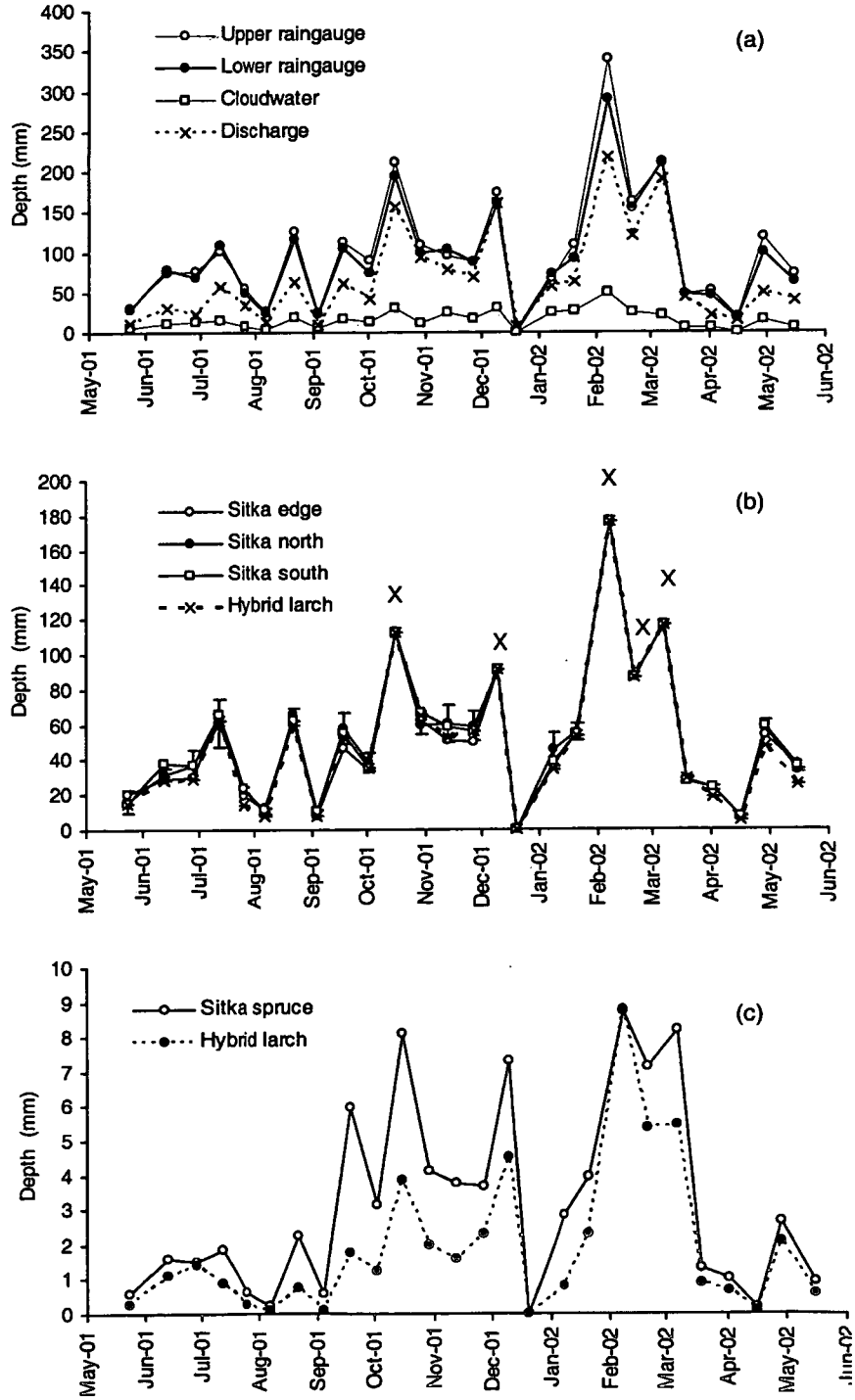

Fig. 2 Water depths measured in the Ballochbeatties catchment, 2001–2002: (a) rainfall, cloudwater, discharge, (b) mean throughfall of three collectors at each forest plot (±1 SD at the Sitka north plot to illustrate variability between collectors), (c) mean stemflow for Sitka spruce and hybrid larch. The crosses in (b) indicate the five occasions on which the throughfall collectors overflowed and throughfall was estimated from the relationship between throughfall and rainfall on all other occasions.

Table 3 Summary of hydrological data measured in the Ballochbeatties catchment, 9 May 2001–15 May 2002.

Mean rainfall depth	2500 mm
Cloudwater depth	412 mm
Total precipitation depth	2912 mm
Cloudwater as % of total precipitation	14%
Mean forest throughfall depth	1331 mm
Mean forest stemflow depth	76 mm
Discharge depth	1726 mm
Forest interception loss (of total precipitation) [Total precipitation – (throughfall + stemflow)]	1505 mm
Forest interception loss (of mean rainfall) [Mean rainfall – (throughfall + stemflow)]	1093 mm
Forest interception loss as % of total precipitation	52%
Forest interception loss as % of mean rainfall	44%
Mean forest soil evaporation loss (±1 SD of 5 forest soil lysimeters)	10 ± 26% of throughfall
Mean moorland evapotranspiration loss (±1 SD of 5 moorland soil lysimeters)	29 ± 7% of total precipitation

Sitka spruce plots and one hybrid larch plot reflects very closely the overall areal weighting in the forested part of the catchment of 67% Sitka spruce to 16% hybrid larch.

Fortnightly stemflow depths below Sitka spruce and hybrid larch in the catchment were calculated separately (Fig. 2(c)), to take account of any relationship between stemflow volume and tree basal area for individual tree species. For Sitka spruce, linear relationships were fitted for every fortnight between stemflow volume measured in each Sitka spruce collector and the $(DBH)^2$ of the collecting tree. There was generally a very good linear relationship between stemflow volume and $(DBH)^2$ and an example for one fortnight is shown in Fig. 3. Next, the relationship for each fortnight was applied to the root mean square DBH of deciles of the distribution of Sitka spruce DBHs, from the 2000 DBH survey, to calculate mean stemflow volume for each decile. This volume was multiplied by the number of trees in each decile (16 000) to calculate stemflow volume per decile. The stemflow volumes of all the deciles were then summed and divided by the area under Sitka spruce (328 000 m^2) to arrive at the fortnightly stemflow depth under Sitka spruce in the Ballochbeatties catchment. A

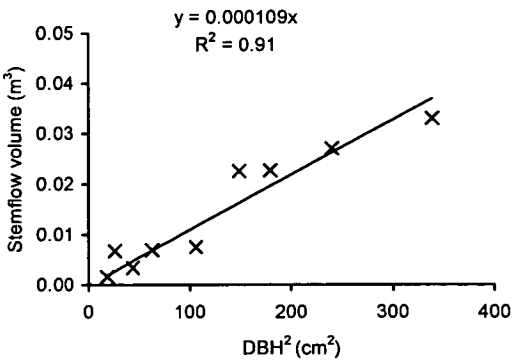

Fig. 3 Relationship between stemflow volume and $(DBH)^2$ for collectors in the Sitka spruce plots, Ballochbeatties catchment, for the period 7–20 January 2002.

similar approach, using basal area, was reported by Aboal *et al.* (1999) to be the most accurate method for scaling up stemflow values from individual trees to the whole stand. There was no apparent relationship between stemflow volume and $(DBH)^2$ for the three stemflow collectors in the hybrid larch plot in the Ballochbeatties catchment. The fortnightly larch stemflow depth was therefore calculated as the mean of these three stemflow collectors multiplied by the number of hybrid larch trees in the catchment (29 000) and divided by the area under larch (82 000 m^2).

Fortnightly stemflow depth under Sitka spruce was always greater than or equal to stemflow under hybrid larch in the Ballochbeatties catchment (Fig. 2(c)). This difference is probably caused by the differences in bark roughness between tree species, rather than tree spacing (Teklehaimanot *et al.*, 1991), as stem densities are similar in the Sitka spruce and hybrid larch plots (Table 2). The greater bark roughness of hybrid larch causes more absorption of water on the tree trunk before stemflow starts. Similar differences in stemflow production between tree species have also been observed by Aboal *et al.* (1999).

The mean fortnightly forest stemflow depth was calculated by summing the stemflow volumes under Sitka spruce and hybrid larch and dividing by the total area of forest (410 000 m^2). The mean annual stemflow depth was then obtained by summing the mean fortnightly forest stemflow depths.

The total annual water input and output volumes measured in the forest and moorland lysimeters provide annual estimates of evaporation from the forest floor and moorland evapotranspiration, respectively, in the Ballochbeatties catchment. Mean annual evaporation from the forest floor was 10% of throughfall. This figure is for soil evaporation only since there was no understorey vegetation below the dense forest canopy and the lysimeters did not contain any active tree roots. Mean annual evapotranspiration from the moorland was 29% of total annual precipitation, very similar to the evapotranspiration loss of 22% of annual precipitation from grass and heather measured in the Balquhidder upland catchment, Scotland (Institute of Hydrology, 1991b). It is probable that moorland evapotranspiration has been overestimated in this study because of the assumption, in the calculation of cloudwater deposition, that the vegetation capture efficiency of cloudwater is the same for moorland vegetation as for conifer trees. In practice it is expected that moorland grasses and shrubs will be less efficient at capturing cloudwater than conifer trees.

Catchment water losses

Annual catchment water loss (evapotranspiration) was calculated, using the field hydrological data (Table 3) and literature values for transpiration as:

$$E_{T,C} = A_F(E_{Tr,F} + E_{S,F} + I_F) + (1 - A_F)E_{T,M} \quad (3)$$

where $E_{T,C}$ is the catchment-average water loss (mm), A_F is the fraction of catchment covered by forest, $E_{Tr,F}$ is the estimated forest transpiration (mm), $E_{S,F}$ is the measured soil evaporation below the forest (mm), I_F is the measured interception loss for the forest (mm) and $E_{T,M}$ is the measured total water loss for the moorland (mm).

Forest interception loss was calculated as the difference between total precipitation (2912 mm) and throughfall (1331 mm) plus stemflow (76 mm) penetrating the canopy, giving a fraction of total precipitation lost by interception of 0.52, higher than the

Table 4 Measurements of annual forest transpiration in forested UK upland catchments (from Roberts, 1983).

Reference	Location	Study years	Tree species	Tree age (years)	Transpiration (mm year^{-1})	Method
Law (1956, 1958)	Stocks Reservoir, Lancashire	1954–55	Sitka spruce	25–26	340	Large lysimeter
Calder *et al.* (1982)	Severn, Plynlimon	1974–76	Norway spruce	29–31	290–340 (Same stand, 3 separate years)	Large lysimeter + soil moisture measurements

typical figure of 0.35–0.40 measured in many other upland UK Sitka spruce forests. From very consistent measurements of transpiration in similar UK upland forests (Table 4), annual forest transpiration was taken to be 320 mm. From the soil lysimeter measurements, soil evaporation below the forest was 133 mm and the total water loss from the moorland was 844 mm. Inserting these values in equation (3), and assuming that total precipitation is uniform across the catchment, the calculated total annual water loss from the Ballochbeatties catchment is 1375 mm, or 47% of the annual precipitation input. This figure is of similar magnitude to the total annual reference evapotranspiration (the potential evapotranspiration from a well-watered short green grass surface) of 1191 mm estimated for the Ballochbeatties catchment for 1995 in a separate study (Hardie, 2002) from daily evapotranspiration calculated using the Penman-Monteith method within the Reference Evapotranspiration Program (Hess, 1998).

Catchment water balance

As a check on the accuracy of the field hydrological measurements, and assuming that there is no long-term change in soil water and groundwater storage, the annual water balance of the Ballochbeatties catchment was calculated as:

$$P = Q + E_{T,C} \tag{4}$$

For the Ballochbeatties catchment, P = 2912 mm year^{-1} and Q = 1726 mm year^{-1}, both obtained from direct field measurement, and $E_{T,C}$ = 1375 mm year^{-1}, derived entirely from direct field measurement apart from a literature value for transpiration as described above. Since the estimated annual water outputs only exceeded the inputs by 6%, the water balance for the Ballochbeatties catchment for May 2001–May 2002 can be considered complete, demonstrating that all water fluxes in the catchment have been accounted for satisfactorily. A sensitivity analysis was conducted of the effect on the catchment water balance of varying the estimates of forest transpiration, moorland evapotranspiration, rainfall, cloudwater and discharge within the limits of measurement error (Table 5). The analysis showed that the catchment water balance is 96–117% complete and that the accuracy of discharge measurement is the major source of uncertainty. Indeed the catchment water balance is probably closer to 100% complete since the cloudwater inputs to the moorland area of the catchment have probably been overestimated, because of the assumption that the moorland vegetation capture efficiency is the same as conifer trees. Therefore all water fluxes in the Ballochbeatties catchment have been accounted for satisfactorily, almost entirely from the field measurements, validating the assumption that changes in soil water and

Table 5 Sensitivity analysis of annual Ballochbeatties catchment water balance to hydrological parameters.

Scenario	Rainfall (mm)	Cloud water (mm)	Forest transpiration (mm)	Discharge (mm)	Moorland evapotrans-piration (mm)	Catchment water out / water in (%)
Mean [a]	2500	412	320	1726	844	106
Max forest transpiration value [b]	2500	412	340	1726	844	107
Min forest transpiration value [b]	2500	412	290	1726	844	106
Rainfall + 10% [c]	2750	412	320	1726	844	101
Rainfall - 10%	2250	412	320	1726	844	113
Cloudwater + 10 %	2500	453	320	1726	844	106
Cloudwater - 10 %	2500	371	320	1726	844	107
Discharge + 17 %[d]	2500	412	320	2020	844	117
Discharge - 17 %[d]	2500	412	320	1433	844	96
Moorland evapotranspiration mean literature value[e]	2500	412	320	1726	582	102

[a] Calculated using mean field measurements and mean transpiration from literature.

[b] From literature cited in Table 4.

[c] Estimated loss of catch for a standard aboveground raingauge compared to a ground level raingauge in this part of the UK (Rodda & Smith, 1986).

[d] Mean % difference between logger and manual measurements of river discharge.

[e] Mean literature value for moorland evapotranspiration = 0.2 of annual precipitation from whole catchment studies of UK upland catchments with grass and heather vegetation cover (Calder & Newson, 1979; Institute of Hydrology, 1976, 1991b; Shuttleworth & Calder, 1979).

groundwater storage during the study period are negligible and also demonstrating that the large interception losses observed from the forest canopy are real and not a measurement artefact.

DISCUSSION

Although the calculated annual water balance for the Ballochbeatties catchment is complete, within reasonable uncertainty, the measured annual forest interception loss (0.52), expressed as a fraction of the annual precipitation input, is considerably higher than interception losses reported in other studies of UK upland catchments dominated by Sitka spruce plantation (Table 6). In these studies, reported annual forest fractional interception losses were typically 0.35–0.40 of annual rainfall input (although it is often not clear whether annual precipitation includes cloudwater or not). Excluding cloudwater, the forest interception loss from the Ballochbeatties catchment, expressed as a fraction of annual rainfall, reduces slightly to 0.44 (Table 3). Although forest interception loss varies spatially and temporally due to the interaction between local climate and forest canopy factors, it is important to use realistic values in assessments of the effects of upland land-use change on water resource availability.

Despite the higher observed forest interception loss, the fractions of stemflow and throughfall of net precipitation below the forest canopy in the Ballochbeatties catchment are similar to measurements conducted in Sitka spruce stands in other UK upland catchments. Indeed the mean stemflow and throughfall results for Sitka spruce

Table 6 Mean interception losses measured in Sitka spruce stands in other UK upland catchments. All measurements were made using standard raingauges, throughfall and stemflow collectors, except where stated.

Reference	Location	Altitude (m a.s.l.)	Tree age (years)	Study years	Mean annual rainfall (mm)	Mean annual interception/ mean annual rainfall
This study	*Ballochbeatties, SW Scotland*	*350*	*37–38*	*2001–2002*	*2912*[a]	*0.52*[a]
Law (1956, 1958)	Stocks Reservoir, NW England	~200	25–26	1954–1955	984	0.38
Calder (1990)	Stocks Reservoir, NW England	~200	26–40	1956–1970	1496	0.38
Ford & Deans (1978)	Greskine Forest, S Scotland	355	14–16	1975–1977	1639	0.30
Johnson (1990)	Kirkton, Balquhidder	~300	50–53	1983–1986	2130	0.28
Anderson & Pyatt (1986)	Kielder, NE England	223	25–28	1977–1980	1037	0.29
Anderson & Pyatt (1986)	Kielder, NE England	230	63–65	1979–1981	997	0.50
Gash *et al.* (1980)	Kielder, NE England	220	27–28	1977–1978	969	0.32
Gash *et al.* (1980)	Hafren Forest, Plynlimon	410	29–32	1975–1978	1867	0.27
Calder (1990)	Dolydd, Plynlimon	~290	31–33	1981–1983	2004	0.38[b]
Calder (1990)	Crinan, SW Scotland	~50	~8–10	1978–1980	2000	0.36[b]

[a] Including cloudwater.
[b] Net precipitation below canopy measured with plastic-sheet net-rainfall gauges.

in the Ballochbeatties catchment, expressed as a fraction of net precipitation, provide further support for the relationship proposed by Johnson (1990) between Sitka spruce tree age and the fractions of net precipitation occurring as stemflow and throughfall (Fig. 4). From measurements in a number of UK upland forests, the maximum fraction of net precipitation occurs as stemflow when trees are 10–15 years old and decreases in older stands, probably due to an increase in leaf area index causing a higher proportion of precipitation to be intercepted by the canopy foliage.

A number of explanations may account for the higher forest interception loss measured in the Ballochbeatties catchment compared with other UK upland catchments, including: measurement uncertainty, tree age, stem density, differences in methodology, and climate change. The main sources of measurement uncertainty in this study are for discharge and cloudwater. Because cloudwater only accounts for 14% of the annual precipitation input the overall effect of even large uncertainties in its value on the overall catchment water balance are small, as has been already demonstrated. Other sources of error in the cloudwater measurement have already been discussed. Rainfall, throughfall and stemflow were measured in the Ballochbeatties catchment using standard methods and measurements at replicate sites showed close agreement. Evaporation loss from the inclined troughs of the throughfall samplers prior to storage in lidded tanks is likely to be negligible as the trough angles followed the recommendations of Thimonier (1998). Furthermore the cool, humid microclimate below the forest canopy is not conducive to evaporation. The strong relationship

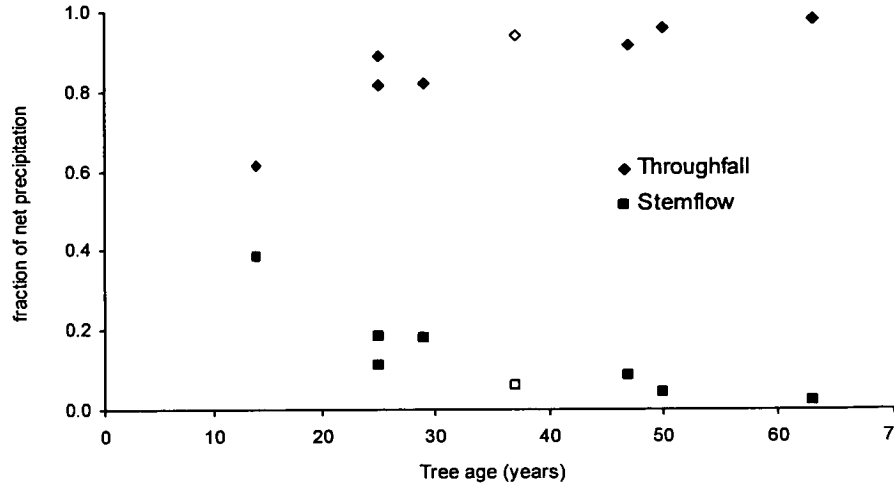

Fig. 4 Fractions of stemflow and throughfall of net precipitation as a function of tree age in Sitka spruce stands in UK upland catchments (after Johnson, 1990). The unfilled points are the Sitka spruce stand in the Ballochbeatties catchment.

observed in the Ballochbeatties catchment between stemflow volume and $(DBH)^2$ for the Sitka spruce stemflow collectors indicate that systematic errors in these measurements are small. The greatest source of uncertainty in this study is the measurement of discharge which has measurement errors of ±17%.

Differences in tree age and stem density between the Ballochbeatties catchment and other UK upland catchments could account for the observed differences in forest interception loss. Tree leaf area index, and hence canopy interception characteristics, change with tree age. However, tree age in the Ballochbeatties catchment at the time of this study (37 years) lies within the range of tree ages in other studies (14–63 years). Stem density in the Ballochbeatties catchment (4775 stems ha^{-1}) is at the high end of reported stem densities in other Sitka spruce stands studied (Table 7), although this may be due partly to the widespread occurrence of forked stems in the Ballochbeatties plantation. Ideally, basal area data should be compared between Sitka spruce stands but these figures are not available for other studies.

Table 7 Stem density of UK upland Sitka spruce stands in which interception studies have been conducted.

Reference	Location	Altitude (m a.s.l.)	Tree age (years)	Thinned	Stem density (ha^{-1})
This study	*Ballochbeatties, SW Scotland*	*350*	*37*	*No*	*4775*
Anderson & Pyatt (1986)	Kielder Forest, NE England	223	25	No	3450
Ford & Deans (1978)	Greskine Forest, SW Scotland	355	14	No	3594
Gash *et al.* (1980)	Kielder Forest, NE England	220	27	No	3600
Gash *et al.* (1980)	Hafren Forest, Powys	410	29	No	4250
Johnson (1990)	Kirkton, Balquhidder	~300	50	Yes	2500

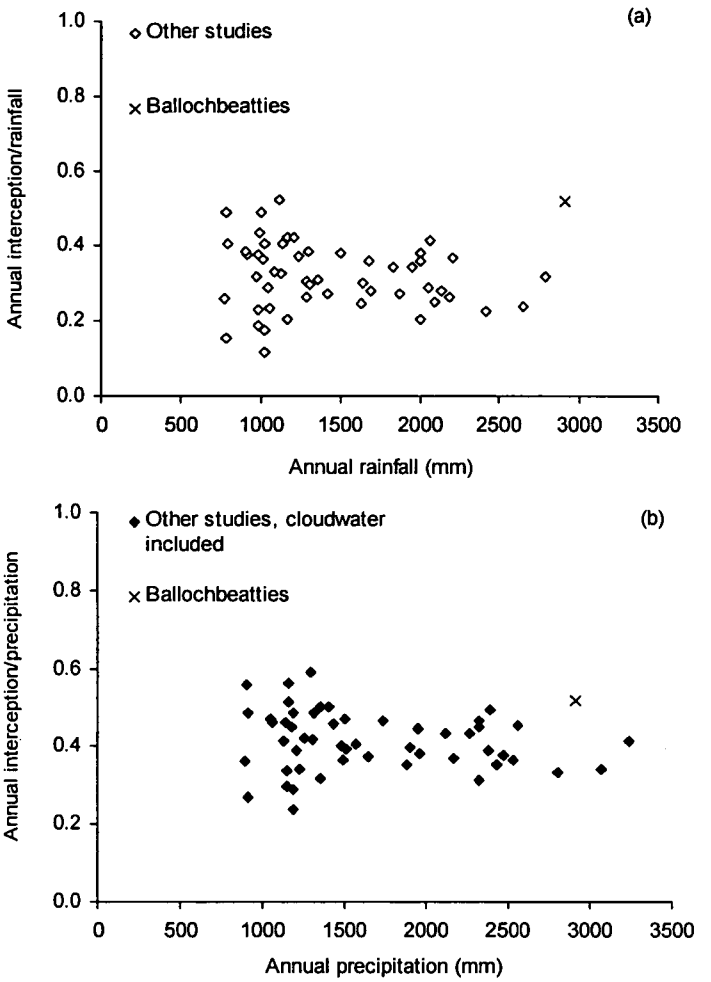

Fig. 5 Annual fractional stand interception loss plotted against: (a) annual rainfall (excluding cloudwater), (b) annual precipitation (including estimated cloudwater contribution). (Data from Table 6 and Cape & Lightowlers (1988)). In (a) and (b) the Ballochbeatties value is expressed as the fraction of precipitation (including cloudwater).

Differences in methodology, in particular the inclusion of cloudwater in precipitation measurements in this study, could also account for the higher forest interception loss measured in the Ballochbeatties catchment compared to other UK upland catchments. Because interception loss is calculated as the difference between precipitation onto the forest canopy and throughfall and stemflow measured below the canopy, if cloudwater is not taken into account then interception loss may be underestimated. When data from other interception studies in Sitka spruce and European larch stands in the UK uplands at similar elevations are plotted as annual interception expressed as a fraction of annual rainfall (not including cloudwater), the Ballochbeatties result (which includes cloudwater) appears as an outlier (Fig. 5(a)). However if the data from other studies are recalculated, assuming the same proportion of cloudwater to annual rainfall as in the Ballochbeatties catchment (although in reality this proportion will vary between sites), the Ballochbeatties result is less of an anomaly (Fig. 5(b)) and the mean annual forest interception loss of other studies increases from

0.32 to 0.41 of total annual precipitation. The importance of including cloudwater for calculating catchment water budgets, particularly in temperate upland forested catchments, has been reported elsewhere. For example, for a small headwater catchment in southern Germany, the underestimate of annual evapotranspiration calculated by the catchment water budget method, compared with the eddy covariance energy budget method, was attributed to fog deposition (Zimmermann & Zimmermann, 2002). Consideration of cloudwater is also essential for catchment planning and management in assessing the effect of land-use and vegetation changes on interception losses and, hence, water resource availability.

A final possible explanation for the higher forest interception loss measured in the Ballochbeatties catchment compared to other studies in UK upland forests is recent climate change. Decadal climate change, particularly increased cloudiness and decreased atmospheric specific humidity deficit, was suggested as a partial explanation for declining water loss observed in the forested Severn catchment, mid-Wales, from 1969 to 1988 (Hudson & Gilman, 1993). The majority of other studies of interception losses in UK upland forests were conducted in the 1970s and 1980s. The higher forest interception loss measured in the Ballochbeatties catchment in 2001–2002 could be due to higher air temperatures, particularly in winter, causing an increased rate of evaporation of water intercepted in the forest canopy. This hypothesis cannot be tested from the data currently available and would require a further modelling study to hindcast forest interception losses from the Ballochbeatties catchment from 1970s and 1980s weather records.

CONCLUSIONS

The annual water budget of the Ballochbeatties catchment, a small UK headwater catchment with 48% forest cover, has been well-characterized and completed, within the limits of measurement uncertainty. The proportions of throughfall and stemflow of net precipitation through the forest canopy are similar to other forested UK upland catchments and follow the trend of increasing significance of throughfall in net precipitation as tree age increases. However forest interception loss in the Ballochbeatties catchment, 52% of total annual precipitation, was considerably higher than interception losses of 35-40% measured in similar catchments. The most probable explanation for this difference is that cloudwater inputs have not been included in calculations of interception loss in other studies, resulting in an underestimate of catchment water loss as the result of vegetation change in upland catchments. It is important that the effects of vegetation change on catchment water losses are fully understood and quantified in order to inform the sustainable management of catchment water resources.

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