NERC Open Research Archive sharing our research

Centre for Ecology & Hydrology
NATURAL ENVIRONMENT RESEARCH COUNCIL

Article (refereed)

McLoughlin, Emma; Rhodes, Angela H.; Owen, Susan M.; Semple, Kirk T.. 2009 Biogenic volatile organic compounds as a potential stimulator for organic contaminant degradation by soil microorganisms. *Environmental Pollution*, 157 (1). 86-94.
doi:10.1016/j.envpol.2008.07.029

Copyright © 2008 Elsevier Ltd All rights reserved

This version available at http://nora.nerc.ac.uk/5337/

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the authors and/or other rights owners. Users should read the terms and conditions of use of this material at http://nora.nerc.ac.uk/policies.html#access

This document is the author's final manuscript version of the journal article, incorporating any revisions agreed during the peer review process. Some differences between this and the publisher's version remain. You are advised to consult the publisher's version if you wish to cite from this article.

www.elsevier.com

Contact CEH NORA team at
nora@ceh.ac.uk

Biogenic volatile organic compounds as a potential stimulator for organic contaminant degradation by soil microorganisms.

Emma McLoughlin[a], Angela H. Rhodes[a], Susan M. Owen[a,b] and Kirk T. Semple[a*]

[a]Department of Environmental Science and the Lancaster Environment Centre, Lancaster University, Lancaster, LA1 4YQ, UK

[b]Present address: CEH Edinburgh, Bush Estate, Penicuik, EH26 0QB, Midlothian, UK

Capsule - *A amendment of soils with monoterpenes may induce organic contaminant degradation by indigenous soil microorganisms*

* Corresponding author address: Email: k.semple@lancaster.ac.uk, *Tel*: *+44 01524 594534, Fax: 0044 01524 593985*

1 **Abstract**

2 The effects of monoterpenes on the degradation of ^{14}C-2,4-dichlorophenol (DCP) were
3 investigated in soils collected from areas surrounding monoterpene and non-
4 monoterpene emitting vegetation. Indigenous microorganisms degraded ^{14}C-2,4-DCP to
5 $^{14}CO_2$, after 1 d contact time. Degradation was enhanced by prior exposure of the soils
6 to 2,4-DCP for 32 d, increasing mineralization extents up to 60%. Monoterpene
7 amendments further enhanced 2,4-DCP degradation, but only following pre-exposure to
8 both 2,4-DCP and monoterpene, with total 2,4-DCP mineralisation extents of up to
9 71%. Degradation was greatest at the higher monoterpene concentrations (≥ 1 μg kg^{-1}).
10 Total mineralisation extents were similar between concentrations, but higher than the
11 control and the 0.1 μg kg^{-1} amendment, indicating that increases in monoterpene
12 concentration has a diminishing enhancing effect. We suggest that monoterpenes can
13 stimulate the biodegradation of 2,4-DCP by indigenous soil microorganisms and that
14 monoterpene amendment in soils is an effective strategy for removing organic
15 contaminants.

16

17

18 Keywords — mineralisation, biodegradability, 2,4-dichlorophenol, monoterpenes, α-
19 pinene, limonene, ageing soils.

20

1. Introduction

Around 5000 structurally determined isoprenoids have been identified, including monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}) and higher molecular weight species (Geron *et al.*, 2000). Monoterpenes ($C_{10}H_{16}$) are the simplest family of isoprenoids (>1000 compounds), forming an array of acyclic and cyclic structures (Fall, 1999). Most studies investigating biogenic isoprenoids have focused on emissions from foliage to the atmosphere, and only limited attention has been paid to subsurface emissions (Hayward et al., 2001). However, isoprenoids have been detected in litter emissions, and are likely products of microbial activity (Isidorov and Jdanova, 2002; Isidorov et al., 2003) or derivatives of root exudates (Lin et al., 2007). The degradation of monoterpenes by indigenous soil microflora has also been demonstrated (Owen et al., 2007). In a review of secondary plant metabolites in phytoremediation, Singer et al (2003) summarised research by Focht (1995), who proposed that plant terpenes (particularly monoterpenes and sesquiterpene) might be natural analogues for PCB oxidation, and subsequent studies have investigated the efficacy of amending PCB-contaminated soil with biogenic terpenes in an effort to induce and/or enhance PCB degradation (Focht, 1995; Gilbert and Crowley, 1997; Hernandez et al., 1997; Tandlich et al., 2001). It may be that exogenous sources of terpenes may enhance the growth and reproduction of PCB-degrading microorganisms and induce the genes encoding enzymes involved in PCB degradation. Indeed, Leigh et al. (2007) demonstrated that there was a very active biphenyl-degrading capacity in indigenous microbes in the rhizosphere soil of *Pinus nigra*, using an innovative stable isotope probing technique. This suggestion that degradation of PCBs in soil might be enhanced by analogue enrichment was made almost forty years ago (Raymond and Alexander, 1971), and subsequently revived twenty years later (e.g. Brunner et al., 1985). More recently, it has been suggested that plant terpenes are likely co-metabolites for PCB degradation due to

1 their structural similarity to biphenyl (Hernandez et al., 1997; Singer et al., 2003 and
2 references therein), which can facilitate biodegradation of PCBs through co-
3 metabolism. However, biphenyl can not be applied to PCB-contaminated land because
4 it is also harmful to the environment. The ability of soil microorganisms to utilize root
5 exudates, particularly isoprenoids, as their sole carbon and energy source (Cleveland
6 and Yavitt, 1998) may justify the hypothesis that isoprenoids emitted within the soil
7 profile may also enhance the biodegradation of PCBs through co-metabolism, and they
8 have the advantage of being harmless to the environment.
9
10 The terpene isoprenoids limonene and α-pinene were selected for investigation as
11 they are plant-derived compounds that dominate woodland soil emissions (Steinbrecher
12 et al., 1999; Hayward et al., 2001; Isidorov and Jdanova, 2002; Isidorov et al., 2003; Lin
13 et al., 2007) , and are readily degraded by indigenous microorganisms (Misra et al.,
14 1996; Misra and Pavlostathis, 1997; Owen et al., 2007); both possess a cyclic structure
15 with a chemical structure closely related to 2,4-DCP. In aerated soil, 2,4-dichlorophenol
16 (DCP) is the main catabolic metabolite of 2,4-dichlorophenoxyacetic acid (2,4-D), a
17 widely used selective systemic herbicide (Tomlin, 1997). Several studies have
18 demonstrated that 2,4-DCP is biodegradable under both aerobic and anaerobic
19 conditions with several pathways of degradation identified (Haggblom, 1992). Because
20 2,4-DCP is considered representative of common soil and water contaminants (Xing and
21 Pignatello, 1998), it was selected as the test contaminant for this study. While this paper
22 focuses exclusively on terpenoid compounds as structural analogues of 2,4 DCP, it is
23 recognised that there are thousands of other secondary metabolites, such as flavonoids
24 which have a basic 10-carbon ring skeleton, which may also function as structural
25 analogues and co-metabolites of 2,4 DCP and other persistent organic pollutants in soil
26 systems.

1

2 Previous work on the influence of co-substrate concentration on contaminant

3 mineralisation are limited, although Tandlich et al. (2001) suggested that biodegradation

4 of PCBs is independent of the concentration of co-substrate used (10 mg l^{-1} and 20 mg l^{-1}

5). Aged woodland soils have also shown higher levels of [UL-^{14}C] 2,4-DCP

6 mineralisation than fresh soils, which was significantly enhanced by the addition of

7 monoterpenes (Rhodes et al., 2007).

8

9 In this study, the use of monoterpenes as a natural analogue for the stimulation of

10 2,4-DCP degradation was investigated, by quantifying (i) the ability of indigenous

11 microorganisms in soils associated with monoterpene emitting vegetation to metabolise

12 2,4-DCP; (ii) the effect of pre-exposure on degradative ability; and (iii) the effect of

13 monoterpene (co-substrate) concentration on catabolic activity.

14

1 **2. Materials and methods**

2 *2.1 Materials*

3 Non-labeled 2,4-DCP was supplied by ACROS Organics, UK, and ^{14}C-2,4-DCP in
4 toluene was obtained from Sigma Aldrich, UK. Limonene and α-pinene were obtained
5 from Fisher Scientific, UK. Goldstar, multipurpose liquid scintillation cocktail was
6 supplied by Meridan. Sodium hydroxide used for the CO_2 traps and chemicals for the
7 minimum basal salts solution were supplied by BDH Laboratory Supplies.

8

9 *2.2 Soil sampling and preparation*

10 Soil samples of ca. 3 kg were collected from sites located under three vegetation
11 types; pine (Pinus sylvestris), oak (Quercus robur) and mixed turf grass. Quercus robur
12 and Pinus sylvestris are isoprene- and monoterpene-emitting vegetation types (Stewart
13 et al., 2003), respectively, and grass is a non-volatile isoprenoid-emitting vegetation.
14 Woodland soils were collected from Lancaster University Campus, Lancashire, U.K.
15 and grassland soil from Mycercough College, Garstang, Lancashire, UK. Soil was
16 homogenized by sieving (2 mm) to remove stones and root material and stored at $4^{o}C$ in
17 the dark for no more than a month prior to commencement of the experimental work.

18

19 *2.3 Soil Characteristics*

20 Soil pH and moisture content were determined using standard techniques. The
21 organic matter content was determined by an acid hydrolysis followed by combustion at
22 450 ºC for 4 h and by a Carbo Erla 1108 Elemental Analyser . Phosphate and potassium
23 content were determined by acid digestion with HNO_3. Phosphate reducing agent
24 (neutralised with NaOH) was used to develop the characteristic blue colour for
25 spectrometric determination at 882 nm (Cecil Ce 1011 UV spectrometer). Potassium

1 was determined directly using a Sherwood 410 Flame Photometer calibrated against the
2 highest concentration (20 mg l^{-1}) standard solution.
3
4 *2.4 Soil Spiking*
5 Soils were spiked with non-labeled 2,4-DCP dissolved in acetone (20 ml) as the
6 carrier solvent giving a final concentration in soil of 10 mg kg^{-1} at field moisture content
7 (30-40%). A stainless steel spoon was used to blend the soils and 2,4-DCP spike to give
8 a homogenous distribution (Doick et al., 2003). Soils were subject to three different
9 preparations: (1) "Freshly spiked" soils were spiked with 2,4-DCP and amended with
10 the isoprenoid compounds at five concentrations varying in order of magnitude (0.1, 1,
11 10, 100, 1000 μg kg^{-1}) and degradation monitored immediately; (2) "2,4-DCP aged"
12 soils were spiked with 2,4-DCP and stored in amber glass jars in darkness at room
13 temperature for 32 d before amendment with isoprenoid compounds at the start of the
14 mineralisation assay; (3) "Isoprenoid and 2,4-DCP aged" soils were spiked with both
15 2,4-DCP and isoprenoid compounds and stored in amber glass jars in darkness at room
16 temperature for 32 d before starting the mineralisation assay. For each of the three
17 preparations "non-amended" soils were spiked with 2,4-DCP (dissolved in acetone)
18 without isoprenoid amendment and monitored as a control. Each treatment was
19 performed in triplicate.
20
21 *2.5 Respirometry*
22 The effects of exposure time and isoprenoid amendment on the rate and extent of
23 mineralisation of 2,4-DCP was determined by measuring $^{14}CO_2$ production using
24 respirometric assays (Reid et al., 2001). Soil samples (10 g), 30 ml minimal basal salts
25 solution, and $^{12}C/^{14}C$-2,4-DCP were added to each respirometric flask, to achieve a ^{14}C-
26 activity of approximately ~80 Bq ^{14}C-2,4-DCP g^{-1} soil and concentration/mass of 10 mg

1 kg^{-1}. A CO_2 trap, consisting of a 7 ml scintillation vial containing 1 ml NaOH (1 M)
2 solution, was suspended from the lid of each respirometer to trap $^{14}CO_2$ evolved as a
3 result of ^{14}C-2,4-DCP mineralisation. Respirometers were placed securely in an orbital
4 incubator (SANYO® Gallenkamp orbital incubator) at 20 ^oC and shaken at 100 rpm to
5 agitate and ensure adequate mixing of the slurry over the period of sampling. The ^{14}C-
6 activity in the $^{14}CO_2$ traps was assessed at regular intervals by replacing the NaOH traps
7 and adding scintillation fluid (5 ml) to each spent $^{14}CO_2$ trap. After storage in darkness
8 overnight, trapped ^{14}C activity was quantified using a Packard Canberra, Tri-Carb®
9 2300TR liquid scintillation counter. An analytical blank (containing no ^{14}C 2,4-DCP)
10 determined the level of background radioactivity. A blank, (autoclaved control) was
11 used to estimate 2,4-DCP lost through volatilization. Maximum mineralisation rates
12 were calculated from the steepest part of the $^{14}CO_2$ respirometric curve and presented as
13 % $^{14}CO_2$ d^{-1}; total extents of mineralisation are defined as cumulative mineralization
14 (%) at the end of the respirometric incubation; and lag time is defined as the time taken
15 to achieve 5% of $^{14}CO_2$ evolution.
16
17
18 *2.6 Statistical Analysis*
19 Sigma Stat® Version 2.03 and Sigma Plot® 2000 software packages were used to
20 analyze data. To compare the extents, rates and lag times of ^{14}C-2,4-DCP
21 mineralisation, two and three way analysis of variance (ANOVA) were conducted. Data
22 were ln transformed in some cases to achieve equal variance. Tukey multiple
23 comparisons test were used to identify treatments with significant differences.
24
25 **3. Results**

1 *3.1. Mineralisation of ^{14}C-2,4-DCP in freshly spiked soils*

2 The ability of indigenous soil microflora to mineralise ^{14}C-2,4-DCP was measured
3 in freshly 2,4-DCP spiked soils, sampled from sites under pine, oak and grassland
4 (Figure 1). Up to 40 % of the freshly applied ^{14}C-2,4-DCP was mineralised during the
5 17 d incubation period in the soils freshly spiked with isoprenoid compounds. Less than
6 7% of $^{14}CO_2$ release was lost by volatilization (Table 2). There were significant
7 differences in lag times between each of the soil types (>2 d in the pine and the oak
8 soils, <1 d in the grassland soils; $P<0.05$; Table 2). Overall, isoprenoid amendment did
9 not affect lag times ($P>0.05$). However, within the limonene amended soils, there was a
10 significant isoprenoid concentration effect with 100 and 1000 μg kg^{-1} amendments,
11 resulting in significantly shorter lag times than the control and the 1 μg kg^{-1} limonene
12 amendment ($P<0.05$).
13
14 Mineralisation rates varied from 2.5 % $^{14}CO_2$ d^{-1} to 13.9 $^{14}CO_2$ % d^{-1} (Table 2).
15 Mineralisation rates in the grassland soil were significantly greater than in the oak and
16 the pine woodland soils ($P< 0.001$). There were no overall significant effects of
17 isoprenoid amendment on the rates of mineralisation ($P>0.05$). However, limonene
18 concentrations of 100 and 1000 μg kg^{-1} in the grassland soil resulted in significantly
19 higher mineralisation rates than in the control soils, and in the soils amended with 0.1 or
20 1 μg kg $^{-1}$ ($P<0.05$). Mineralisation rates in the soils with limonene amendment of 1000
21 μg kg^{-1} also differed significantly from the 10 μg kg^{-1}amendment ($P<0.05$). No
22 enhancement of mineralisation rates were observed in soils with α-pinene amendments
23 ($P>0.05$).
24
25 There was an overall significant difference in the extent of mineralisation between
26 each soil type ($P<0.001$). There were no differences in extents between the limonene

1 and the α-pinene amended pine soils, nor between the limonene and the α-pinene
2 amended oak soils. However, in the grassland soil, α-pinene amendment resulted in
3 higher extents of mineralisation than in the limonene amendment ($P<0.05$). Amendment
4 with α-pinene resulted in higher extents of mineralisation in the pine soil than in the oak
5 soil (Tables 2, 3; $P<0.05$). The extents of mineralisation in the control (no amendment)
6 treatment were significantly lower than for the 100 μg kg^{-1} limonene-amended soil
7 ($P<0.05$) and lower (but not significantly) than the 1000 μg kg^{-1} amendment ($P=0.07$).
8

9 *3.2. Mineralisation of ^{14}C-2,4-DCP in aged soils*

10 The capacity of the indigenous soil microflora to mineralise ^{14}C-2,4-DCP was
11 measured in the woodland and grassland soils following a 32 d incubation in the
12 presence of 2,4-DCP (Figure 2). Up to 61% mineralisation occurred in 2,4-DCP aged
13 soils over the 17 d incubation period, with <7% of $^{14}CO_2$ release by volatilization (Table
14 3). Lag times were less than one day for all except the α-pinene amended oak and
15 grassland soils, where lag times were >1 d, and up to 3 d (Table 3). There were
16 significant differences in lag times between soil types ($P<0.05$), but there was no overall
17 significant influence of isoprenoid amendment ($P>0.05$). Lag times in the oak soil were
18 significantly greater than lag times in the pine and the grassland soils amended with
19 limonene ($P<0.05$). In the α-pinene amended soils, lag times in all of the soil types were
20 significantly different from each other ($P<0.05$), with shortest lag times in the pine soil
21 and longest in the grassland soil.
22

23 Mineralisation rates for 2,4-DCP in aged soils ranged from 4.1 % $^{14}CO_2$ d^{-1} to 45.3
24 % $^{14}CO_2$ d^{-1} (Table 3). In a given soil, there were large differences in mineralisation
25 rates between isoprenoid types, but no overall significant effect of isoprenoid
26 amendments on the mineralisation rates ($P>0.05$). Mineralisation rates were

1 significantly different between each of the soil types ($P<0.05$), with the highest rates in
2 the pine soil and the lowest in the oak soil.
3

4 Overall, isoprenoid amendment did not have a significant influence on the extents
5 of mineralisation ($P>0.05$). However, there was a significant difference in the extents of
6 mineralisation between each soil type ($P<0.05$). Within both limonene- and α-pinene-
7 amended soils, the pine soil exhibited the greatest extents of mineralisation. In limonene
8 amended soils, there was a significant influence of isoprenoid amendment ($P<0.05$) in
9 pine soil, with the 0.1 and 1 μg kg^{-1} amendment having a significantly greater extent of
10 mineralisation than all other concentrations of limonene amendments and the control
11 treatment. However, no effect of isoprenoid amendment was observed in the α-pinene
12 amended soils ($P>0.05$).
13

14 *3.3. Mineralisation of 2,4-DCP in soils aged with both limonene and 2,4-DCP*

15 The capacity of the indigenous soil microflora to mineralise ^{14}C-2,4-DCP was
16 measured in the woodland and grassland soils following a 32 d incubation in the
17 presence of limonene and 2,4-DCP (Figure 3). Over the incubation period of 9 d, up to
18 71% mineralisation was achieved in soils aged with 2,4-DCP and limonene, with <6%
19 of $^{14}CO_2$ released due to volatilization (Table 4). Lag times were <1 d for the majority
20 of treatments in the limonene and 2,4-DCP aged soils (Table 4). Isoprenoid
21 concentration had a significant effect on lag times. Lag times in incubation with no
22 limonene and the 0.1 mg kg^{-1} limonene amended soils were similar ($P>0.05$), but
23 significantly longer than lag times in the soils with 1, 10, 100 and 1000 μg kg^{-1}
24 limonene amendments ($P<0.05$). Lag times in soils with 1, 10, 100 and 1000 μg kg^{-1}
25 limonene amendments were also similar ($P>0.05$). Lag times also differed significantly

1 between soil types ($P<0.05$); the pine and the grassland soils had similar lag times,
2 which were shorter than those in the oak soil.
3
4 Overall, there was a significant isoprenoid amendment effect on mineralisation
5 rates ($P<0.05$). The mineralisation rates for the control treatment and the 0.1 μg kg^{-1}
6 amendment were not statistically different ($P>0.05$), but these rates were slower than
7 those resulting from 1, 10, 100 and 1000 μg kg^{-1} amendments ($P<0.05$). The
8 mineralisation rates in soils amended with 1, 10, 100 and 1000 μg kg^{-1} limonene were
9 similar ($P>0.05$).
10
11 Generally, increasing limonene concentration enhanced the extents of
12 mineralisation of ^{14}C-2,4-DCP ($P<0.05$; Figure 4). For the limonene amendment of 10
13 μg kg^{-1} in the pine soil and 100 μg kg^{-1} in the grassland soil, the extents of
14 mineralisation were relatively low and did not follow the general trend. The absence of
15 limonene (control) resulted in a significantly lower extent of mineralisation than all the
16 limonene amendments ($P<0.05$). The 0.1 μg kg^{-1} limonene amendment resulted in a
17 mineralisation extent similar to the no-addition of limonene treatment ($P=0.52$), but this
18 was significantly lower than extents resulting from all the other limonene amendments
19 ($P<0.01$). The 1, 10, 100 and 1000 μg kg^{-1} amendments had similarly high extents of
20 mineralisation ($P>0.05$). There was also a significant difference between soil types
21 ($P<0.05$); extents of mineralisation in the oak and the grassland soils were similar, and
22 significantly lower ($P<0.05$) than in the pine soil.
23

4. Discussion

4.1. Adaptation of soils to ^{14}C-2,4-DCP mineralisation.

In the freshly spiked soils, between 22 and 39% mineralisation occurred after 17 d incubation, which agrees with previous studies. For example, Rhodes et al. (2007) reported 19% of ^{14}C-2,4-DCP released as $^{14}CO_2$ after 14 d (in soils spiked with 10 mg kg^{-1} 2,4-DCP). Further, Boucard et al. (2005) found 23% 2,4-DCP was mineralized after 20 d (total concentration 1.4 mg kg^{-1}) and Shaw and Burns (1998) found that 25% ^{14}C-activity was mineralized after 21 d (total concentration 20 mg kg^{-1}). Mineralisation in freshly contaminated soils indicates that soils have the potential for 2,4-DCP catabolism, thus 2,4-DCP degraders were already present in the indigenous microbial community to permit immediate utilization of 2,4-DCP (Shaw and Burns, 1998; Boucard et al., 2005). It has been shown that biodegradation of 2,4-DCP is feasible in previously uncontaminated soils, due to the presence in many soil microorganisms having the tfdB gene that encodes 2, 4-DCP hydroxylase (Vallaeys et al., 1996).

In the soils, spiked and aged with either 2,4-DCP alone, or with limonene, the catabolic activity of the indigenous microflora increases with contact time. Overall, 2,4-DCP aged soils exhibited greater extents and rates of ^{14}2,4-DCP mineralisation, with degradation of up to 60% and rates of between 4.14 and 45.34 % d^{-1}. This contrasts with the maximum 39% extent of mineralisation and rates between 2.45 and 13.95 % d^{-1} for ^{14}2,4-DCP degradation in soils freshly spiked with 2,4-DCP. This has been observed for other organic contaminants in soil, particularly that of PAHs (Hwang and Cutright, 2002; Macleod and Semple, 2002; 2006; Reid et al., 2002; Lee et al., 2003). Macleod and Semple (2002) suggested that the process of increasing degradation ability (adaptation) could be via (1) induction of specific enzymes; (2) genetic changes resulting in increased metabolic capabilities; or (3) the selective enrichment for

organisms with the metabolic capability to transform the target contaminant or a combination of these mechanisms.

The properties of the sigmoidal mineralisation curves of the inoculated ^{14}2,4-DCP observed in soils freshly spiked with 2,4-DCP show that a period of microbial growth (acclimation or lag phase) is required before significant mineralisation rates occur (e.g.Grosser *et al.*, 1991). After 32 d, most soils' mineralisation curves for inoculated ^{14}2,4-DCP had shorter lag times and earlier onset of rapid rates of mineralisation compared with freshly spiked soils. This indicates that 32 d was sufficient for adaptation and enrichment in the soils of microbial populations with 2,4-DCP degradative ability.

In contrast, similar mineralisation processes lasted >24 and ≤ 76 weeks in pyrene-contaminated pasture soil, and were not completed at all in woodland soils contaminated with pyrene even after 90 weeks of pre-exposure (Macleod and Semple, 2002). This highlights the rapidity of the development of 2,4-DCP catabolic activity in the soils used in the present study. Clearly the physico-chemical properties of the molecules and their interactions with the soil will be important contributing factors, with pyrene being a more complex and less water soluble molecule than the chlorophenol.

4.2. The effect of isoprenoid and isoprenoid concentration on ^{14}C-2,4-DCP mineralisation in soil

The overall insignificant differences between isoprenoid amendments and the no-addition of isoprenoid treatment in the freshly spiked soils are comparable with the findings of Rhodes et al. (2007). In this study, Rhodes et al. (2007) found that soils freshly spiked with 2,4-DCP and amended with α-pinene, or an isoprenoid mixture

1 containing limonene, showed no enhanced 2,4-DCP mineralisation relative to the no-
2 addition of isoprenoid control. In this current study, it was found that soils aged with
3 2,4-DCP then amended with isoprenoid at the start of the mineralisation assay also
4 showed an insignificant difference in biodegradation between isoprenoid amendments
5 and the no-addition of isoprenoid control. These combined results suggest that 2,4-DCP
6 catabolic activity is not significantly influenced by the presence of isoprenoids. It
7 appears that the period of contact time with 2,4-DCP is more critical than the isoprenoid
8 concentration applied; however, although not significant, within limonene amended
9 freshly spiked soils, the 100 and 1000 μg kg^{-1} amendments resulted in a greater extent
10 of mineralisation and shorter lag times than the no-addition of limonene control in the
11 oak and the pine soils. In the grassland soil, freshly amended with limonene, greater
12 rates of mineralisation were also observed in the 100 and 1000 μg kg^{-1} amendments.
13 The greater extents, shorter lag times and faster rates observed for grass soils amended
14 with higher concentrations of limonene implied that there may have been a stimulatory
15 effect on the microbial 2,4-DCP catabolic activity above a minimum concentration.

16

17 Macleod et al. (2002) suggest that higher concentrations of contaminant during
18 prior exposure result in greater catabolic potential; therefore, a greater concentration of
19 co-substrate might result in greater catabolic potential. The results presented in this
20 study support this hypothesis, but also indicate that the enhancing effect diminishes at
21 higher concentrations. There is a rapid rate of increase in the extent of mineralisation
22 with increasing limonene concentration, followed by a diminishing rate of increase in
23 mineralisation extent at high concentrations of limonene ($\geq$ 1 μg kg^{-1}). This suggests
24 that there might be both co-substrate and substrate (contaminant) concentration
25 thresholds, below which catabolic activity is not maximized (Macleod and Semple,
26 2003). However, Tandlich et al. (2003) did not report the existence of a concentration

effect, suggesting that biodegradation of PCBs was independent of the concentration of co-substrate (10 and 20 mg l^{-1}). However, it is possible that the concentrations of co-substrate applied were high enough that the enhancing effect of co-substrate concentration had stabilized and so there appeared to be no benefit in applying higher concentrations of co-substrate. Alternatively, the concentration independence might have been a function of the reduced bioavailability of PCB molecules.

^{14}C-2,4-DCP mineralisation rate was lowest in grassland soils. This may be explained by the fact that grass is a non-monoterpene emitting vegetation. If grass roots do not emit monoterpenes, then the soil microflora may not be enriched with species possessing the enzymes to degrade these compounds which are structural analogues of 2,4-DCP. Deciduous oak trees emit isoprene rather than monoterpenes (Possell et al., 2004), and mineralisation rates in the oak soil were statistically similar to those in the grassland soils. In contrast, pine trees are monoterpene emitters (Janson, 1993), and mineralisation rates in the pine soils were higher. Hence, natural exposure of the pine woodland microbial communities to monoterpenes, which are structurally similar (natural analogue) to 2,4-DCP, appears to confer a greater inherent ability to adapt to the presence of 2,4-DCP. Natural exposure of pine soils to monoterpenes from roots and litter may also result in a larger and/or more active community of 2,4-DCP degrading microorganisms, and is an example of how different plant communities could influence the microflora in the rhizosphere.

4.3. Influence of soil characteristics on ^{14}C-2,4-DCP mineralisation

Soil organic matter (SOM) content differed significantly between the different soil types, with the greatest SOM content in woodland soils (48% in pine and 17% in oak). Sorption of 2,4-DCP is strongly correlated to SOM content, with increased sorption at

1 greater SOM contents (Benoit *et al.*, 1999; Yang *et al.*, 2004). It has recently been
2 proposed that in soils with high SOM content, the rate of transfer of contaminants from
3 solid to aqueous soil phases and hence to microorganism limits mineralisation (Macleod
4 and Semple, 2002). However, in this present study, mineralisation was greatest in soils
5 that contained the highest SOM content; this supported the findings Benoit *et al* (1999).
6 A possible explanation for the greater mineralisation in soils with higher SOM content
7 may be that SOM provides a source of energy and a reservoir of nutrients for the
8 indigenous soil microflora. Low SOM frequently limits the growth of microbes, thus the
9 stimulatory effects of SOM content on microbial biomass might override the possible
10 reduced biodegradation rates as a consequence of high SOM to give a greater overall
11 extent of 2,4-DCP mineralisation in woodland soils. Further, it has been reported that
12 the presence of organic matter does not significantly affect the biodegradation of
13 monoterpenes (Misra *et al.*, 1996).
14
15 All soils were acidic, reflecting their high organic matter content. The woodland
16 soils had significantly lower pH than the grassland soil (pH 3.7 ± 0.02 and pH 3.9 ±
17 0.03, for pine and oak, respectively; pH 5.1 ± 0.06 for grass). Acidity affects the
18 efficacy of some chlorinated pesticides (e.g. chlorophenols) which become less mobile
19 in acidic soils (Jensen, 1996). This may also have contributed to mineralisation rates of
20 ^{14}C-2,4-DCP in the different soils.
21

22 **4. Conclusions**

23 Soil-contaminant contact time is a critical factor affecting the development of catabolic
24 activity within indigenous soil microflora. Isoprenoid amendment has an enhancing
25 effect only when sufficient time has elapsed for microbial adaptation. Following
26 adaptation, increasing isoprenoid concentrations enhanced biodegradation, with the

1 enhancing effect diminishing at higher concentrations. Degradation was greatest in 2,4-
2 DCP and limonene aged soils. Overall, this study supports the suggestion that
3 amendment of soils with monoterpenes induces organic contaminant degradation by
4 indigenous soil microflora. Therefore, it is feasible to suggest that there is potential for
5 *in-situ* remediation of contaminated soils through the stimulation of indigenous
6 microorganisms through applications of exogenous isoprenoid such as terpene rich plant
7 residues or the planting of isoprenoid emitting vegetation.
8

9 **Acknowledgements**

10 The authors acknowledge the advice and assistance in the laboratory of Kieron Diock,
11 Nadia Dew and Jackie Stroud. We thank the Natural Environment Research Council,
12 UK for financial support of post-graduate research.
13

14 **References**

15 Atlas, R.M., Bartha, R., 1998. Microbial Ecology: Fundamentals and Applications,
16 4th ed. Benjamin/Cummings Publishing Company, California.

17 Benoit, P., Barriuso, E., Soulas, G., 1999. Degradation of 2,4-D, 2,4-dichlorophenol,
18 and 4-chlorophenol in soil after sorption on humified and nonhumified organic
19 matter. Journal of Environmental Quality 28, 1127-1135.

20 Boucard, T.K., Bardgett, R.D., Jones, K.C., Semple, K.T., 2005. Influence of plants
21 on the chemical extractability and biodegradability of 2,4-dichlorophenol in soil.
22 Environmental Pollution 133, 53-62.

23 Brunner, W., Sutherland, F.H., and Focht, D.D.,1985. Enhanced biodegradation of
24 polychlorinated biphenyls in soil by analogue enrichment and bacterial
25 inoculation. Journal of Environmental Quality 14, 324-328.

1 Cleveland, C.C., Yavitt, J.B., 1998. Microbial consumption of atmospheric isoprene in
2 a temperate forest soil. Applied and Environmental Microbiology 64, 172-177.

3 Doick, K.J., Lee, P.H., Semple, K.T., 2003. Assessment of spiking procedures for the
4 introduction of a phenanthrene-LNAPL mixture into field-wet soil.
5 Environmental Pollution 126, 399-406.

6 Fall, R., 1999. Biogenic Emissions of Volatile Organic Compounds from Higher
7 Plants, in: Hewitt, C.N. (Ed.), Reactive Hydrocarbons in the Atmosphere.
8 Academic Press, London, pp. 43-97.

9 Focht, D.D., 1995. Strategies for the improvement of aerobic metaolism of
10 polychlorinated-biphenyls. Current Opinion in Biotechnology 6, 341-346.

11 Geron, C., Rasmussen, R., Arnts, R.R., Guenther, A., 2000. A review and synthesis of
12 monoterpene speciation from forests in the United States. Atmospheric
13 Environment 34, 1761-1781.

14 Gilbert, E.S., Crowley, D.E., 1997. Plant compounds that induce polychlorinated
15 biphenyl biodegradation by Arthobacter sp. Strain B1B. Applied and
16 Environmental Microbiology 63, 1933-1938.

17 Gonod, L.V., Chenu, C., Soulas, G., 2003. Spatial variability of 2,4-
18 dichlorophenoxyacetic acid (2,4-D) mineralisation potential at a millimetre scale
19 in soil. Soil Biology and Biochemistry 35, 373 - 382.

20 Grosser, R.J., Warshawsky, D., Vestral, J.R., 1991. Indigenous and enhanced
21 mineralisation of Pyrene, Benzo[a]pyrene and Carbazole in soils. Applied and
22 Environmental Microbiology 57, 3462 - 3469.

23 Haggblom, M.M., 1992. Microbial breakdown of halogenated aromatic pesticides and
24 related-compounds. FEMS Microbiology Reviews 103, 29-72.

1 Hayward, S., Muncey, R.J., James, A.E., Halsall, C.J., Hewitt, C.N., 2001.
2 Monoterpene emissions from soil in a Sitka spruce forest. Atmospheric
3 Environment 35, 4081-4087.

4 Hernandez, B.S., Koh, S.C., Chial, M., Focht, D.D., 1997. Terpene-utilizing isolates
5 and their relevance to enhanced biotransformation of polychlhlorinated
6 biphenyls in soil. Biodegradation 8, 153-158.

7 Hwang, S., Cutright, T.J., 2002. Biodegradability of Aged Pyrene and Phenanthrene in
8 a natural Soil. Chemosphere 47, 891 - 899.

9 Isidorov, V., Jdanova, M., 2002. Volatile organic compounds from leaves litter.
10 Chemosphere 48, 975-979.

11 Isidorov, V.A., Vinogorova, V.T., Rafalowski, K., 2003. HS-SPME analysis of
12 volatile organic compounds of coniferous needle litter. Atmospheric
13 Environment 37, 4645-4650.

14 Janson, R.W., 1993. Monoterpene Emissions from Scots Pine and Norwegian Spruce.
15 Journal of Geophysical Research-Atmospheres 98, 2839-2850.

16 Jensen, J., 1996. Chlorophenols in the terrestrial environment, Reviews of
17 Environmental Contamination and Toxicology 146, 25-51.

18 Kleinheinz, G.T., Bagley, S.T., St.John, W.P., Rughani, J.R., McGinnis, G.D., 1999.
19 Characterization of alpha-pinene degrading microorganisms and application to a
20 bench-scale biofiltration system for VOC degradation. Archives of
21 Environmental Contamination and Toxicology 37, 151-157.

22 Leigh, M,B., Pellizari, V.H., Uhlik, O., Sutka, R., Rodrigues, J., Ostrom, N.E., Zhou, J.,
23 Tiedje, J.M., 2007. Biphenyl-utilizing bacteria and their functional genes in a pine
24 root zone contaminated with polychlorinated biphenyls (PCBs). The International
25 Society for Microbial Ecology Journal 1, 134-148.

1 Lin, C., Owen, S.M., Penuelas, J., 2007. Volatile organic compounds in the roots and
2 rhizosphere of Pinus spp. Soil Biology & Biochemistry 39, 951-960.

3 Macleod, C.J.A., Semple, K.T., 2002. The adaptation of two similar soils to pyrene
4 catabolism. Environmental Pollution 119, 357 - 364.

5 Macleod, C.J.A., Semple, K.T., 2003. Sequential extraction of low concentrations of
6 pyrene and formation of non-extractable residues in sterile and non-sterile soils.
7 Soil Biology and Biochemistry 35, 1443-1450.

8 Misra, G., Pavlostathis, S.G., 1997. Biodegradation kinetics of monoterpenes in liquid
9 and soil-slurry systems. Applied Microbiology and Biotechnology 47, 572-577.

10 Misra, G., Pavlostathis, S.G., Perdue, E.M., Araujo, R., 1996. Aerobic biodegradation
11 of selected monoterpenes. Applied Microbiology and Biotechnology 45, 831-
12 838.

13 Owen, S.M., Boissard, C., Hewitt, C.N., 2001. Volatile organic compounds (VOCs)
14 emitted from 40 Mediterranean plant species: VOC speciation and extrapolation
15 to habitat scale. Atmospheric Environment 35, 5393-5409.

16 Owen, S.M., Clark, S., Pompe, M., Semple, K.T., 2007. Biogenic volatile organic
17 compounds as potential carbon sources for microbial communities in soil from
18 the rhizosphere of Populus tremula. FEMS Microbiology Letters 268, 34-39.

19 Possell, M., Heath, J., Nicholas Hewitt, C., Ayres, E., Kerstiens, G., 2004. Interactive
20 effects of elevated CO2 and soil fertility on isoprene emissions from Quercus
21 robur. Global Change Biology 10, 1835-1843.

22 Raymond, D.G.M., and Alexander, M., 1971. Microbial metabolism and cometabolism
23 of nitrophenols. Pesticide Biochemistry and Physiology 1, 123-130.

24

Reid, B.J., MacLeod, C.J.A., Lee, P.H., Morriss, A.W.J., Stokes, J.D., Semple, K.T., 2001. A Simple ^{14}C-Respirometric Method for Assessing Microbial Catabolic Potential and Contaminant Bioavailability. FEMS Microbiology Letters 196, 141 - 146.

Rhodes, A.H., Owen, S.M., Semple, K.T., 2007. Biodegradation of 2,4-dichlorophenol in the presence of volatile organic compounds in soils under different vegetation types. FEMS Microbiology Letters 269, 323-330.

Shaw, L.J., Burns, R.G., 1998. Biodegradation of 2,4-D in a noncontaminated grassland soil profile. Journal of Environmental Quality 27, 1464-1471.

Singer, C.S., Crowley, D.E. and Thompson, I.P. 2003. Secondary plant metabolites in phytoremediation and biotransformation. Trends in Biotechnology 21, 123-130.

Steinbrecher, R., Hauff, K., Rossler, J., Durr, M., Seufert, G., 1999. Monoterpene emission from soils in orange plantations of the Valencian Citrus belt, Spain. Physics and Chemistry of the Earth Part B-Hydrology Oceans and Atmosphere 24, 695-698.

Stewart HE, Hewitt CN, Bunce RGH, Steinbrecher R, Smiatek G, Schoenemeyer T (2003) A highly spatially and temporally resolved inventory for biogenic isoprene and monoterpene emissions: Model description and application to Great Britain. Journal of Geophysical Research-Atmospheres 108, Art. No. 4644.

Tandlich, R., Brezna, B., Dercova, K., 2001. The effect of terpenes on the biodegradation of polychlorinated biphenyls by Pseudomonas stutzeri. Chemosphere 44, 1547-1555.

Tomlin, C.D.S., 1997. The Pesticide Manual, 11th ed. British Crop Protection Council, Farnham.

1 Vallaeys, T., Fulthorpe, R.R., Wright, A.M., Soulas, G., 1996. The metabolic pathway
2 of 2,4-dichlorophenoxyacetic acid degradation involves different families of
3 tfdA and tfdB genes according to PCR-RFLP analysis. FEMS Microbiology
4 Ecology 20, 163-172.

5 Xing, B.S., Pignatello, J.J., 1998. Competitive sorption between 1,3-dichlorobenzene
6 or 2,4-dichlorophenol and natural aromatic acids in soil organic matter.
7 Environmental Science & Technology 32, 614-619.

8 Yang, K., Zhu, L.Z., Lou, B.F., Chen, B.L. 2004. Significance of natural organic
9 matter in nonlinear sorption of 2,4-dichlorophenol onto soils/sediments. Water
10 Resources Research 40, (1) Article Number: W01603

11

12

13

List of Figures

Figure 1. Mineralisation (%) of ^{14}C-2,4-DCP by indigenous soil microorganisms in 3 soil types; (pine, oak and grass), freshly spiked with 2,4-DCP and amended with limonene (A, B, C) or α-pinene (D, E, F) at 5 different concentrations; 0.1 (○), 1.0 (□), 10.0 (Δ), 100.0 (◊) and 1000.0 μg kg^{-1}(∇). A no-addition of limonene/α-pinene control (■) is plotted, and an autoclaved control (●), represents loss of 2,4-DCP through volatilisation. Error bars are the S.E.M of the triplicates.

Figure 2. Mineralisation (%) of ^{14}C-2,4-DCP by indigenous soil microorganisms in 3 soil types (pine, oak and grass), aged with 2,4-DCP for 32 d and amended with limonene (A, B, C) or α-pinene (D, E, F) at the start of the mineralisation assay, at 5 different concentrations; 0.1 (○), 1.0 (□), 10.0 (Δ), 100.0 (◊) and 1000.0 μg kg^{-1}(∇). A no-addition of limonene/α-pinene control (■) is plotted, and an autoclaved control (●), represents loss of 2,4-DCP through volatilisation. Error bars are the S.E.M of the triplicates.

Figure 3. Mineralisation (%) of ^{14}C-2,4-DCP by indigenous soil microorganisms in 3 soil types (pine, oak and grass), aged with 2,4-DCP and limonene for 32 d, at 5 different concentrations; 0.1 (○), 1.0 (□), 10.0 (Δ), 100.0 (◊) and 1000.0 μg kg^{-1}(∇). A no-addition of limonene control (■) is plotted, and an autoclaved control (●), represents loss of 2,4-DCP through volatilisation. Error bars are the S.E.M of the triplicates.

Figure 4. Dependency of mineralisation extent (%) of ^{14}C-2,4-DCP by indigenous soil microorganisms in 3 soil types (pine, oak and grass) aged with 2,4-DCP and limonene,

1 on concentration of applied limonene. Note: the limonene concentration = 0 μg kg^{-1} was

2 allocated a value of 10^{-10} μg kg^{-1} to facilitate plotting the logarithmic axis

Table Captions

Table 1. The physico-chemical properties of each soil type (mean ± S.E.M).

Table 2. Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, freshly spiked with 2,4-DCP and limonene or α-pinene at 5 different concentrations; 0.1, 1, 10, 100 and 1000 μg kg^{-1}. A no-addition of isoprenoid treatment shows the effect of isoprenoid addition and an autoclaved control represents loss of 2,4-DCP through volatilisation.

Table 3. Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, aged with 2,4-DCP (32 d) and amended with limonene or α-pinene, at the start of the mineralisation assay, at 5 different concentrations; 0.1, 1, 10, 100 and 1000 μg kg^{-1}. A no-addition of isoprenoid treatment shows the effect of isoprenoid addition and an autoclaved control represents loss of 2,4-DCP through volatilisation.

Table 4. Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, aged with 2,4-DCP and limonene for 32 d, at 5 different concentrations; 0.1, 1, 10, 100 and 1000 μg kg^{-1}. A no-addition of limonene treatment shows the effect of limonene addition and an autoclaved control represents loss of 2,4-DCP through volatilisation.

1 **Table 1**. The physico-chemical properties of each soil type (mean ± S.E.M).

	Soil		
	Pine	**Oak**	**Grass**
Texture	sandy loam	sandy loam	sandy clay loam
Moisture content (%)	30.6 - 81.5	16.0 - 40.3	11.3 - 39.4
pH	3.72 ± 0.023	3.92 ± 0.027	5.10 ± 0.062
ΔpH	0.75 ± 0.024	0.70 ± 0.049	0.32 ± 0.072
Loss on ignition (%)	48.42 ± 1.40	17.24 ± 0.49	4.24 ± 0.37
PO_4 (ppb)	342.81 ± 1.25	249.00 ± 8.12	579.47 ± 1.26
K (ppm)	8.07 ± 0.39	7.69 ± 0.37	13.23 ± 0.79

2

3

1 **Table 2**. Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, freshly
2 spiked with 2,4-DCP and limonene or α-pinene at 5 different concentrations; 0.1, 1, 10, 100 and 1000 μg kg^{-1}. A no-addition of isoprenoid
3 treatment shows the effect of isoprenoid addition and an autoclaved control represents loss of 2,4-DCP through volatilisation.

Soil Type	Treatment	limonene			α-pinene		
		Extent (%)	Rate (% d^{-1})	Lag time (d)	Extent (%)	Rate (% d-1)	Lag time (d)
Pine	Autoclaved	2.51 ± 1.79	0.49 ± 0.41	n/a	5.61 ± 2.17	0.72 ± 0.45	n/a
	0	29.25 ± 1.57	3.82 ± 0.33	5.00 ± 0.35	28.59 ± 1.12	3.45 ± 0.71	2.97 ± 0.09
	0.1	31.59 ± 0.58	4.47 ± 0.15	4.93 ± 0.11	27.06 ± 0.27	3.75 ± 0.32	3.45 ± 0.11
	1.0	28.25 ± 2.90	3.81 ± 0.33	5.36 ± 0.27	27.63 ± 0.65	4.18 ± 0.42	3.23 ± 0.09
	10.0	30.61 ± 0.73	3.69 ± 0.08	4.90 ± 0.04	27.74 ± 0.18	3.92 ± 0.29	3.10 ± 0.04
	100.0	32.50 ± 0.35	3.88 ± 0.07	4.62 ± 0.09	27.89 ± 0.90	3.49 ± 0.18	3.03 ± 0.06
	1000.0	30.96 ± 0.18	3.49 ± 0.14	4.75 ± 0.06	29.54 ± 2.99	3.85 ± 0.17	2.77 ± 0.63
Oak	Autoclaved	5.71 ± 2.52	1.02 ± 0.47	n/a	3.40 ± 0.29	0.42 ± 0.05	n/a
	0	22.20 ± 0.34	2.97 ± 0.25	4.11 ± 0.14	24.28 ± 1.47	3.94 ± 0.35	5.91 ± 0.23
	0.1	23.83 ± 0.14	2.58 ± 0.22	3.72 ± 0.07	24.57 ± 1.20	4.39 ± 0.21	6.06 ± 0.48
	1.0	25.04 ± 0.57	2.55 ± 0.08	3.90 ± 0.20	35.70 ± 4.05	6.35 ± 0.94	4.53 ± 0.78
	10.0	24.80 ± 0.67	3.26 ± 0.07	3.63 ± 0.18	23.67 ± 1.23	4.61 ± 0.35	6.13 ± 0.62
	100.0	25.17 ± 0.74	2.99 ± 0.33	3.54 ± 0.22	25.24 ± 1.66	5.02 ± 0.48	5.97 ± 0.02
	1000.0	25.20 ± 0.13	2.45 ± 0.25	3.42 ± 0.11	26.27 ± 0.75	4.48 ± 0.52	5.88 ± 0.28
Grass	Autoclaved	6.42 ± 1.61	1.32 ± 0.73	n/a	6.34 ± 0.78	3.60 ± 2.07	n/a
	0	25.24 ± 2.15	7.31 ± 0.48	0.75 ± 0.08	38.66 ± 2.45	13.95 ± 3.24	1.13 ± 0.11
	0.1	24.24 ± 1.99	7.12 ± 0.83	0.96 ± 0.10	36.52 ± 1.94	11.58 ± 2.22	1.22 ± 0.01
	1.0	25.52 ± 0.31	7.31 ± 0.07	0.85 ± 0.003	34.07 ± 1.60	9.76 ± 1.73	1.26 ± 0.13
	10.0	26.72 ± 0.85	8.11 ± 0.33	0.68 ± 0.04	35.47 ± 1.34	10.52 ± 1.79	1.13 ± 0.02
	100.0	28.13 ± 0.41	8.61 ± 0.09	0.65 ± 0.02	36.05 ± 2.31	11.20 ± 2.33	1.28 ± 0.08
	1000.0	28.55 ± 0.43	9.89 ± 0.30	0.51 ± 0.02	37.92 ± 2.26	12.25 ± 2.26	1.22 ± 0.05

4 * n/a indicates lag times was greater than the 17 d period of the experiment.

1 **Table 3**. Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, aged with
2 2,4-DCP (32 d) and amended with limonene or α-pinene, at the start of the mineralisation assay, at 5 different concentrations; 0.1, 1, 10, 100 and 1000
3 μg kg^{-1}. A no-addition of isoprenoid treatment shows the effect of isoprenoid addition and an autoclaved control represents loss of 2,4-DCP through
4 volatilisation.

Soil Type	**Treatment**	**limonene**			**α-pinene**		
		Extent (%)	**Rate (% d^{-1})**	**Lag time (d)**	**Extent (%)**	**Rate (% d^{-1})**	**Lag time (d)**
Pine	Autoclaved	0.64 ± 0.06	0.05 ± 0.01	n/a*	2.72 ± 0.13	0.30 ± 0.05	n/a
	0	44.33 ± 0.45	7.67 ± 0.05	0.66 ± 0.01	60.22 ± 2.64	45.34 ± 2.39	0.11 ± 0.007
	0.1	45.78 ± 1.82	13.48 ± 1.40	0.38 ± 0.04	57.55 ± 0.98	43.52 ± 0.58	0.11 ± 0.003
	1.0	51.26 ± 0.62	14.32 ± 0.40	0.35 ± 0.01	55.51 ± 2.91	41.96 ± 3.01	0.12 ± 0.009
	10.0	45.59 ± 5.96	8.24 ± 1.26	0.75 ± 0.15	57.24 ± 1.78	40.67 ± 2.99	0.12 ± 0.006
	100.0	48.81 ± 1.14	7.46 ± 0.10	0.69 ± 0.02	50.83 ± 4.27	38.43 ± 3.59	0.13 ± 0.012
	1000.0	48.81 ± 1.11	7.63 ± 0.08	0.69 ± 0.01	57.09 ± 2.55	43.42 ± 2.81	0.12 ± 0.007
Oak	Autoclaved	1.22 ± 0.55	0.15 ± 0.08	n/a*	4.07 ± 0.33	0.42 ± 0.05	n/a
	0	38.95 ± 0.66	7.47 ± 0.09	0.78 ± 0.03	33.26 ± 2.31	4.73 ± 0.59	1.80 ± 0.30
	0.1	41.45 ± 0.65	6.94 ± 0.60	0.93 ± 0.07	34.30 ± 1.81	5.62 ± 1.04	1.34 ± 0.28
	1.0	39.63 ± 1.20	7.36 ± 0.38	0.88 ± 0.04	35.84 ± 2.47	5.55 ± 0.61	1.38 ± 0.16
	10.0	42.83 ± 0.89	8.21 ± 0.41	0.80 ± 0.02	36.44 ± 2.45	5.24 ± 0.65	1.45 ± 0.30
	100.0	41.23 ± 2.50	7.23 ± 0.57	0.92 ± 0.06	32.79 ± 4.13	4.47 ± 1.00	2.23 ± 0.91
	1000.0	39.57 ± 1.00	7.44 ± 0.70	0.87 ± 0.11	29.86 ± 0.32	4.14 ± 0.14	2.24 ± 0.30
Grass	Autoclaved	3.89 ± 0.16	0.39 ± 0.05	n/a*	6.28 ± 0.87	0.89 ± 0.18	n/a
	0	26.66 ± 0.11	9.03 ± 0.09	0.55 ± 0.01	52.16 ± 0.35	34.51 ± 0.84	2.15 ± 0.01
	0.1	25.84 ± 0.94	8.46 ± 0.17	0.59 ± 0.01	36.72 ± 1.71	16.30 ± 1.59	2.36 ± 0.03
	1.0	25.70 ± 0.55	7.58 ± 0.50	0.67 ± 0.05	47.48 ± 2.54	26.31 ± 3.81	2.18 ± 0.03
	10.0	26.70 ± 0.19	8.97 ± 0.34	0.56 ± 0.02	44.93 ± 1.84	25.28 ± 3.01	2.22 ± 0.03
	100.0	28.17 ± 0.34	8.99 ± 0.56	0.56 ± 0.03	48.20 ± 4.33	27.83 ± 6.24	2.10 ± 0.04
	1000.0	26.08 ± 2.37	7.96 ± 0.96	0.65 ± 0.09	48.37 ± 2.61	26.81 ± 3.37	2.17 ± 0.03

5 n/a indicates lag times was greater than the 17 d period of the experiment.

1 **Table 4.** Mineralisation extent, initial rates, and lag times (mean ± S.E.M) for ^{14}C-2,4-DCP degradation in 3 soil types; pine, oak and grass, aged with 2 2,4-DCP and limonene for 32 d, at 5 different concentrations; 0.1, 1, 10, 100 and 1000 μg kg^{-1}. A no-addition of limonene treatment shows the effect of 3 limonene addition and an autoclaved control represents loss of 2,4-DCP through volatilisation.

Soil type	**Treatment**	**Extent (%)**	**Rate (% d^{-1})**	**Lag time (d)**
Pine	Autoclaved	1.54 ± 0.12	0.20 ± 0.02	n/a
	0	40.65 ± 1.37	7.94 ± 0.17	0.77 ± 0.01
	0.1	51.60 ± 0.64	19.14 ± 0.42	0.26 ± 0.001
	1.0	70.91 ± 1.91	54.81 ± 1.76	0.09 ± 0.003
	10.0	37.58 ± 0.67	5.58 ± 0.13	1.01 ± 0.01
	100.0	64.48 ± 4.46	48.34 ± 3.55	0.10 ± 0.01
	1000.0	70.52 ± 4.38	55.03 ± 4.61	0.09 ± 0.01
Oak	Autoclaved	1.48 ± 0.04	0.23 ± 0.03	n/a
	0	19.45 ± 0.99	3.49 ± 0.55	1.95 ± 0.14
	0.1	15.74 ± 1.04	2.30 ± 0.15	2.85 ± 0.27
	1.0	54.46 ± 1.28	26.11 ± 0.51	0.19 ± 0.003
	10.0	48.84 ± 2.37	17.18 ± 0.85	0.29 ± 0.01
	100.0	57.24 ± 4.12	42.68 ± 1.19	0.45 ± 0.34
	1000.0	60.57 ± 4.33	43.81 ± 2.99	0.11 ± 0.01
Grass	Autoclaved	5.89 ± 1.14	0.90 ± 0.27	n/a
	0	24.84 ± 0.13	10.10 ± 0.43	0.50 ± 0.02
	0.1	37.48 ± 1.14	17.02 ± 0.73	0.30 ± 0.01
	1.0	38.49 ± 0.44	10.97 ± 0.76	0.47 ± 0.31
	10.0	43.55 ± 1.79	25.58 ± 1.47	0.20 ± 0.01
	100.0	31.35 ± 2.06	17.55 ± 1.31	0.29 ± 0.02
	1000.0	41.18 ± 3.83	23.04 ± 2.15	0.22 ± 0.02

4 * n/a indicates lag times was greater than the 9 d period of the experiment.

5

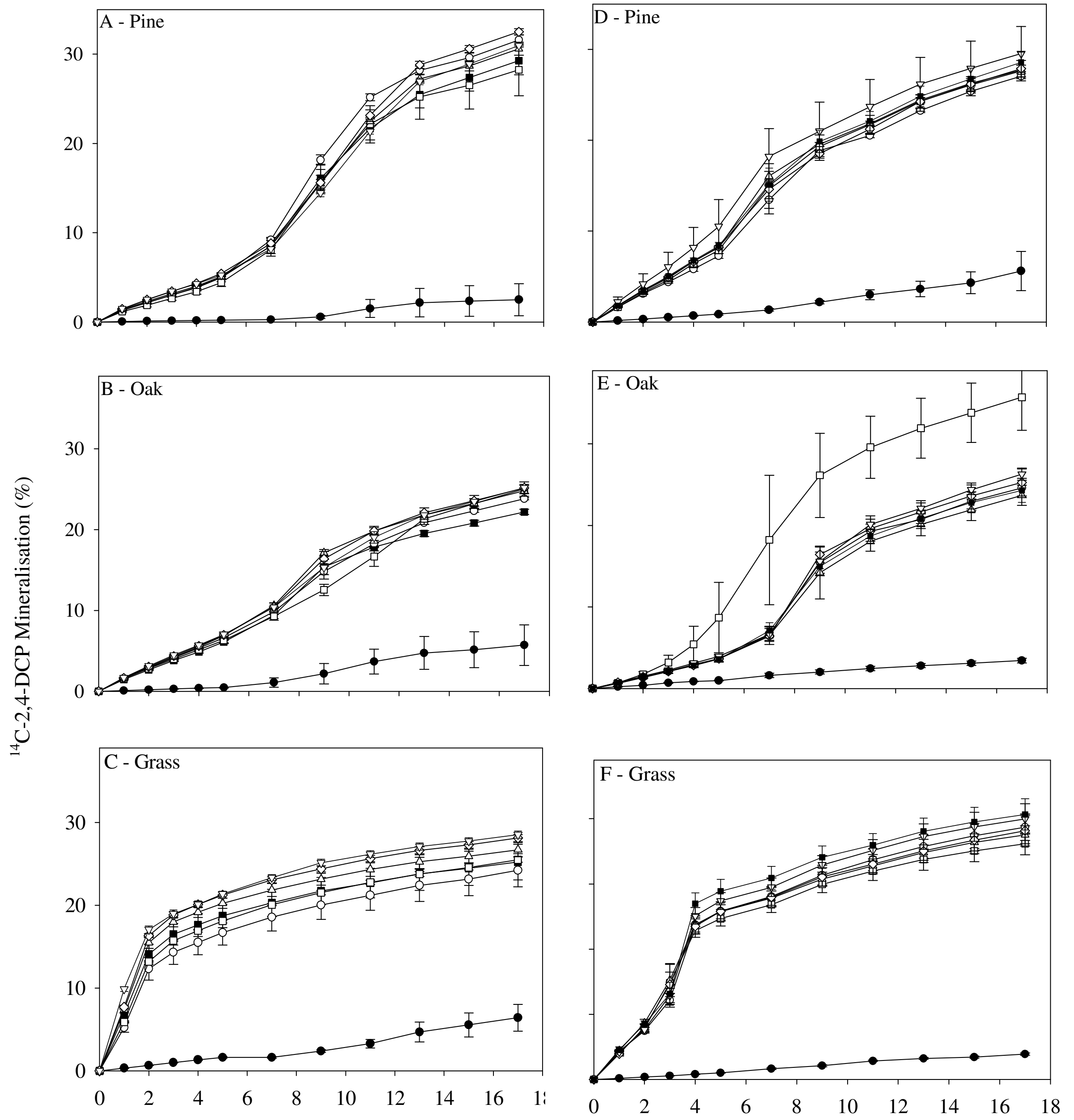
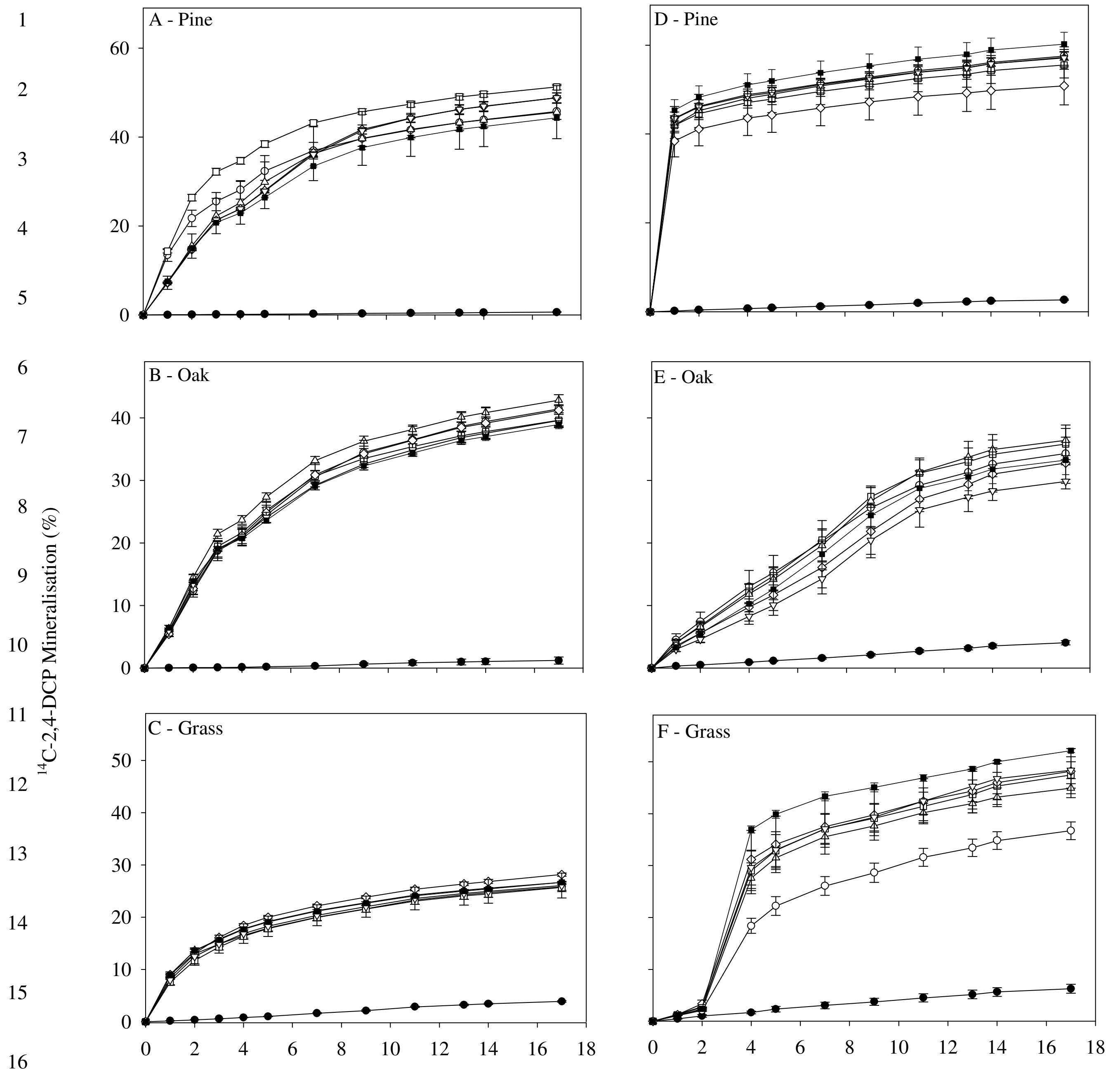


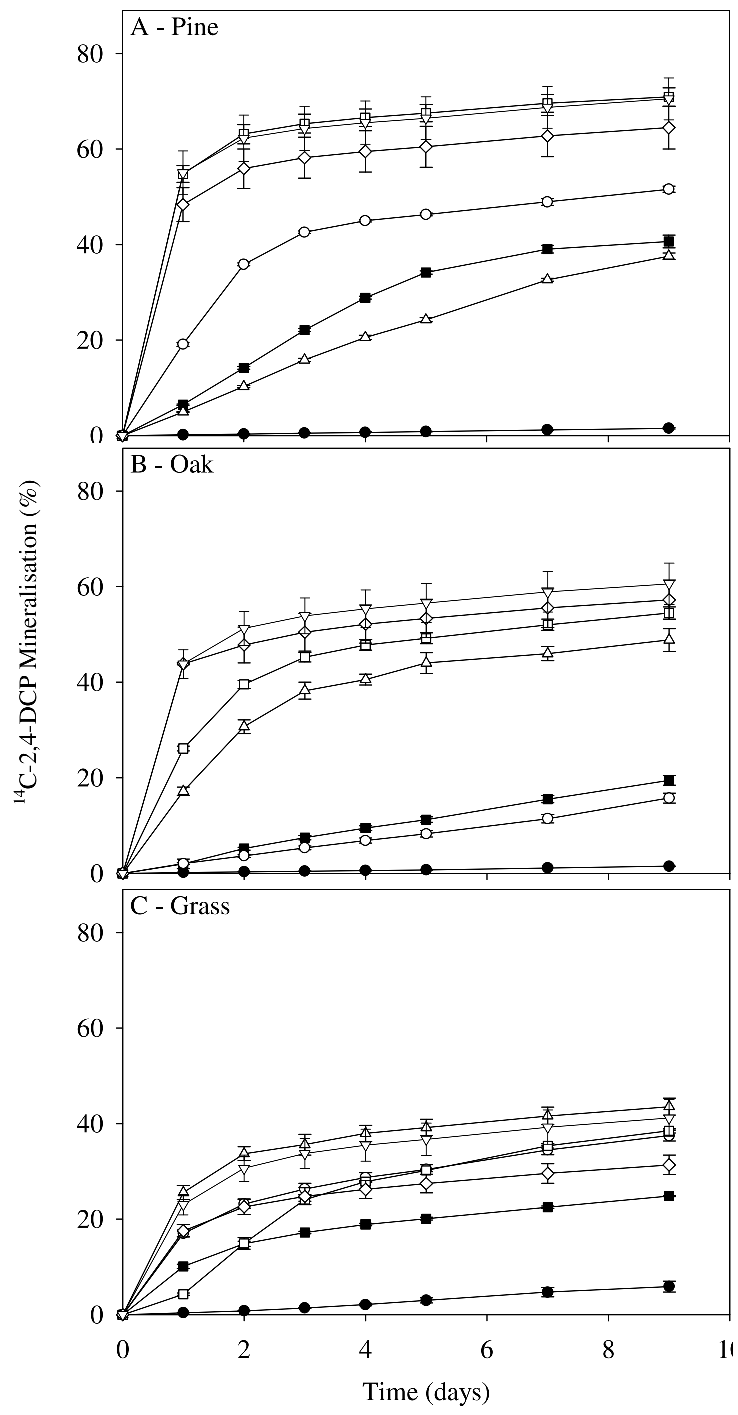
Figure 1

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16



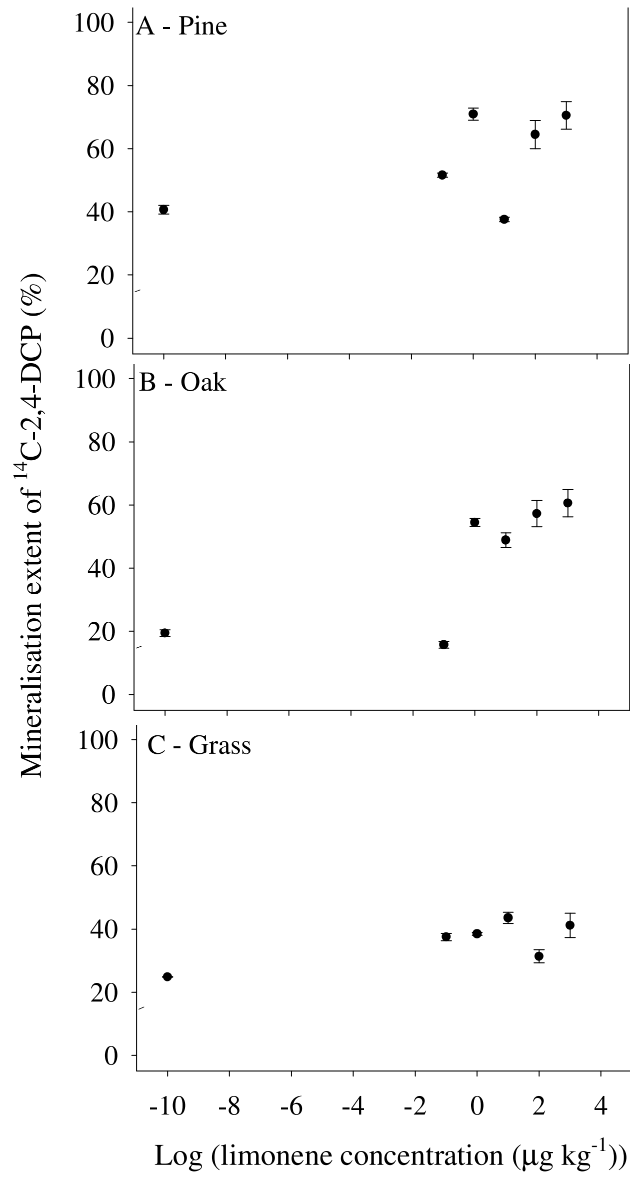
17 Figure 2.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22



23 Figure 3.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18



19 Figure 4.