Exploring the Potential of Microbial Diversity in Dissipating Legacy Pollutants in Surface Soils

Submitted by

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Bachelor of Science (Honours) in Soil and Environmental Geosciences

Thesis submitted in total fulfilment of requirements
for the degree of Doctor of Philosophy

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September 2021

STATEMENT OF AUTHORSHIP

This work was supported by an Australian Government Research Training Program Scholarship

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Co-authors have contributed to publications presented in chapter three, four and five. Details relating to each person's contribution to each publication are outlined in the List of Publications.

This work was supported by an Australian Government Research Training Program Scholarship

Christian Krohn 19 September 2021

This thesis is dedicated to Bowie, who joined me on a journey into the unknown and remained a devoted companion - until the end.

ACKNOWLEDGEMENTS

This work was supported by an Australian Government Research Training Program Scholarship and would not have been possible without it. The PhD topic was an initiative of organochlorine officers at Agriculture Victoria, Australia. Grant Rawlin, John Ryan, Piotr Fabijański and other organochlorine officers are genuinely seeking solutions for affected farmers in their districts. Their impetus led to this PhD project, which was brought to life by my supervisor Prof. Caixian Tang and co-supervisor Ashley Franks at La Trobe University. Together with Dr. Jian Jin, who has guided me from the beginning, they have my sincere gratitude for their patience, moral support, technical guidance and knowledge. They were always available, fair, and happy to share.

I felt the same for the passionate and supportive staff and students in the Soil-Plant Interactions Group (Tang Lab) as well as for the Applied, Environmental Microbiology Group (Franks Lab) at La Trobe University. I thoroughly appreciated the limitless spirit of knowledge-sharing and desire to soak up more skills and knowledge by everyone. Special thanks to Dr. Clayton Butterly and Dr. Han Weng who were always available and have guided me with some laboratory fundamentals; Dr. Gary Clark, who inducted me into various instruments and led us to success at the national soil judging competition; Corinne Celestina who taught me molecular techniques and laboratory etiquette; Dominic Lauricella who always knows what to do, plus he helped me out with some much-needed poultry litter and biochar; James O'Sullivan and Yunyun Zheng who were my office and coffee buddies and who's positivity has kept me afloat; Tharanga Bandara who was one of the most prolific graduate researchers in the group, and who has given me important ideas and direction; Prof. Mike Stear for sharing his statistic wisdoms; Sarah Knowler who helped with some fantastic excel sheets, Gene Drendel who gave me confidence; Josh Vido who has introduced me to the world of sequencing and Qiime2 and Assoc. Prof. Steve Petrovski and Steven Batinovic for letting me use the Miseq.

Furthermore, I am extremely grateful for the contributions and support of my co-authors and collaborators, Dr. Jen Wood, Pei Zhang, Bruce Shelly, Matt Kitching, Dr. Juan Wang and Dr. Helen Hayden. Many thanks for providing resources, invaluable insights, guidance and ideas. I also am indebted to the Victorian branch of the Australian Soil Science Society, chaired by Dr. Sam Grover. The society has been an essential platform for me to connect and interact with professionals and academics, which positively impacted my PhD progress and beyond.

But most importantly, it was my wife Kim that enabled me to dedicate time to PhD research. Without her support, love and encouragement, I would not have been brave enough to enter the unknown.

THESIS SUMMARY

Agricultural topsoils, contaminated with chlorinated persistent organic pollutants (POPs), remain a significant risk for human health and the environment globally. To provide evidence-based guidance for natural attenuation strategies to policy makers and land managers, the mostly unknown biological processes inherent in natural attenuation need elucidation. This research aimed at exploring relationships of microbial diversity and soil properties with the dissipation of POP residues. It was hypothesised that soil properties, especially organic matter composition, affect a soil's metabolic potential to degrade POPs and that microbial activity can therewith be manipulated in any soil to facilitate the degradation of aged POPs. First, a literature review provides an overview of common non-microbial and microbial processes that determine the fate of POPs in the topsoils, using dieldrin as an example. This follows a systematic review of studies that examined a total of 318 POP-transformation observations in liquid media by 160 microbial isolates across 26 countries. The results of the systematic review indicated that no specific POP-degrading microbial phylotypes existed in soils. A field survey of two pasture topsoils in Victoria with contrasting dieldrin losses since 1988 then demonstrated that dieldrin dissipation was associated with decomposed carbon materials. Subsequently, a year-long soil incubation in closed chambers found that microbial growth, after the application of labile carbon, inhibited dieldrin decreases. In comparison, soil amended with biochar showed ongoing dieldrin decreases, while promoting diverse, oligotrophic growth. Hence, both the field survey and incubation indicated that efficient, slow-growing, oligotrophic communities allowed for greater participation of bacteria with capabilities to decompose recalcitrant carbon sources, including chlorinated hydrocarbons. As this was associated with decomposed carbon materials, it was proposed that 'healthy' soils are less effective for POP degradation. These conclusions may shift perspectives for future research into strategies for the natural attenuation of contaminated soils.

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ABBREVIATIONS

ASV	Amplicon sequence variant
ANOVA	Analysis of variance
Cl	Chlorine or chloride
C:N	Carbon-to-nitrogen ratio
DBP	4,4'-dichlorobenzophenone
DCP	2,4-dichlorophenol
DDA	2,2-bis(p-chlorophenyl) acetate
DDD	1,1-dichloro-2,2-bis(4-chlorophenyl) ethane
DDE	1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene
DDMU	1-chloro-2,2- bis(p-chlorophenyl) ethylene
DDNS	2,2-bis(p- chlorophenyl)ethane
DDT	Dichlorodiphenyltrichloroethane
DNRE	Department of Natural Resources and Environment
ECD	Electron capture detector
Faith's PD	Faith's phylogenetic diversity
GABBA	gamma-aminobutyric acid
GLS	Generalised least squares
HEOD	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene
H_2O_2	Hydrogen Peroxide
HOC	Humic organic carbon
IFCS	Intergovernmental forum on chemical safety
IDL	Instrument detection limit
ITS	Internal transcribed spacer
K_{oc}	Soil/water partition coefficients
K_{ow}	Octanol/water coefficient
KEGG	Kyoto encyclopedia of genes and genomes
LME	Linear mixed effect
MBC	Microbial biomass carbon
MBC:TC	Microbial-biomass-C-to-total-C ratio
MLD	Method limit of detection
MNIRS	Mid and near-infrared spectroscopy
nMDS	Non-metric dimensional scaling
NORM	National organochlorine residue management program
NSTI	Nearest sequence taxon index
PA	Pascale
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxins
PCDF	Polychlorinated dibenzofurans
PCP	Polychlorinated Phenol
PERMANOVA	Permutational multivariate analysis of variance
PFOA	Perfluorooctanoic Acids
PFOS	Perfluorooctane Sulfonic Acids
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States

POC	Particulate organic carbon
POP	Persistent organic pollutant
RDA	Redundancy analysis
rRNA	Ribosomal ribonucleic acid
ROC	Resistant organic carbon
ScaRP	Australian Soil Carbon Research Project
SEPP	SATé-enabled phylogenetic placements
Sol_{H20}	Solubility in water
sPLS	Sparse partial least squares analysis
sPLS-DA	Sparse partial least squares discriminant analysis
StARS	Stability approach to regularization selection
TE	POP transformation efficiency
TE_{bc}	TE, normalised, Box-Cox
TE_{bc_popcl}	TE_{bc} median-centered within POP type and degree of chlorination
USDA	U.S. Department of Agriculture

PUBLICATIONS

Composition of soil organic matter drives total loss of dieldrin and dichlorodiphenyltrichloroethane in high-value pastures over thirty years (Chapter 4), Science of the Total Environment, 2019, volume 691, 135-145

Christian Krohn[a]: Conceptualization, Formal analysis, Investigation, Writing - original draft; *Jian Jin*[a]: Supervision, Methodology, Investigation, Project administration, Writing - review & editing; *John Ryan*[d]: Resources. *Piotr Fabijański*[e]: Resources; *Ashley E. Franks*[b,c]: Conceptualization, Supervision, Writing, review & editing; *Caixian Tang*[a]: Conceptualization, Supervision, Fund acquisition, Writing, review & editing.

Highly decomposed organic carbon mediates the assembly of soil communities with traits for the biodegradation of chlorinated pollutants (Chapter 5), Journal Hazardous Materials, 2021, volume 404

Christian Krohn[a]: Conceptualization, Formal analysis, Investigation, Writing - original draft; *Jian Jin*[a]: Supervision, Methodology, Investigation, Project administration, Writing - review & editing; *Jennifer L. Wood*[b,c]: Writing - review & editing; *Helen L. Hayden*[g]: Writing, review & editing; *Matt Kitching*[f]: Resources, Formal analysis; *John Ryan*[d]: Resources. *Piotr Fabijanski*[e]: Resources; *Ashley E. Franks*[b,c]: Conceptualization, Supervision, Writing, review & editing; *Caixian Tang*[a]: Conceptualization, Supervision, Fund acquisition, Writing, review & editing.

Biochar reduced extractable dieldrin concentrations and promoted oligotrophic growth including microbial degraders of chlorinated pollutants (Chapter 6), Journal of Hazardous Materials, 2021, volume 423

Christian Krohn[a]: Conceptualization, Formal analysis, Investigation, Writing - original draft; *Jian Jin*[a]: Supervision, Methodology, Investigation, Project administration, Writing - review & editing; *Pei Zhang*: Resources, Training; *Jennifer L. Wood*[b,c]: Writing - review & editing; *Helen L. Hayden*[g]: Writing, review & editing; *Ashley E. Franks*[b,c]: Conceptualization, Supervision, Writing, review & editing; *Caixian Tang*[a]: Conceptualization, Supervision, Fund acquisition, Writing, review & editing.

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Chapter 1: General introduction

1.1 BACKGROUND

History of persistent organic pollutants in Australia and beyond

According to the Global Soil Partnership (http://www.fao.org/global-soil-partnership) soil pollution is a global transboundary problem and hampers the achievement of the United Nations General Assembly Sustainable Development Goals [1]. The global problem of pollution is illustrated by the presence of so-called persistent organic pollutants (POPs) in arctic snow and in the air of both hemispheres on Earth [2]. Persistent organic pollutants are some of the most persistent toxic pollutants that humans could have developed, resisting degradation while travelling large distances after volatilisation or on dust particles through the earth's wind systems [3]. They were released into the environment at a global scale until the 1980s while the increasing risks to human health and the environment gave rise to international legal action. The Stockholm Convention, a global treaty adopted by 183 countries with the aim of eliminating or restricting the production of these chemicals entered into force on 14 May 2004, after nearly 10 years of negotiations. The history and background of the Stockholm Convention are provided by Fiedler et al. [4]. Initially, twelve POPs, including dieldrin and DDT, were listed in the Stockholm convention (Table 2.3-1 in chapter 2). Since then, the list of POPs has kept growing, and today a total of 30 POPs are included. The majority are agricultural insecticides or are by-products in the production of pesticides. Others are produced for industrial uses or are released unintentionally through industrial processes. Some of these listed compounds refer to a whole class of chemicals (not a single compound), encompassing hundreds of different congeners, such as the class of polychlorinated biphenyls (PCBs), which are sold as mixtures such as Aroclor. The halogenation of these compounds largely determines their adverse behavior, and while initially, all the POPs were chlorinated hydrocarbons, more recently the prevalence of fluorinated hydrocarbon mixtures such as perfluorooctane sulfonic acids (PFOS) or perfluorooctanoic acids (PFOA) have become major concerns globally.

Figure 1.1-1. This 'WordCloud' was created in python using titles of 500 articles relating to persistent pollutants from a Web of Science search. The font size of the words is proportional to the number of times the words appeared in the titles.

This PhD project is the result of the engagement by district officers of the National Organochlorine Management Program in Victoria, Australia (NORM) [5]. Their impetus, historical data and local knowledge allowed me to investigate factors for the disappearance of extremely persistent chlorinated insecticides from valuable but contaminated agricultural surface soils. Farming families that owned these lands had struggled to use them productively since 1989, when the insecticides dieldrin and dichlorodiphenyltrichloroethane (DDT) were banned for all agricultural uses in Australia. At that time, dieldrin and DDT were two of the most commonly used organochlorine chemicals in Australia. They were used as broadacre insecticidal sprays on a range of horticultural crops, including apples and tobacco, or as a livestock ectoparasiticide. Dieldrin was also applied with fertilisers to root vegetable crops such as potatoes. Other insecticides such as Endrin and Aldrin were also used in some broadacre applications. Before they were banned, it was increasingly recognised that the extreme persistence of these toxic organochlorines, posed threats to human health and the environment globally. In Australia, organochlorines became a trade issue when the United States threatened to reject Australian veal and beef products due to the detection of residues in these products in 1987. POPs are insoluble in water but soluble in fat; hence, strongly sorb to topsoil and accumulate in the body fat of grazing cattle or any other grazing stock once ingested via contaminated soil particles. After extensive testing for POP residues in topsoils and animal carcasses in the late 1980s, some farming properties were identified that exceeded maximum residues levels. Afterwards, the NORM program was established in 1994, a joint initiative of government and industry to reduce the prevalence of POPs in beef. The NORM officers have monitored these contaminated properties until today. In Victoria, soils with dieldrin and DDT levels exceeding 0.06 $\mu g\ g^{-1}$ dry soil and 1 $\mu g\ g^{-1}$, respectively, require ongoing monitoring. After over three decades, surface soils of many of these properties still exceed the maximum residue levels in topsoil, particularly for dieldrin, which continues to affect land use options for farming families.

In Europe, DDT and its metabolites are still among the most frequently found pesticide residues in topsoils [6]. However, there are no comprehensive data on global distribution or baseline levels of POPs in the topsoil because such studies vary greatly in sampling periods, sampling strategies, different extraction methods, and the type of pesticides assessed [6,7]. This lack of global data availability for POPs in soils was perhaps one reason why the Conference of the Parties of the Stockholm Convention in 2009 recommended to use of baseline concentrations from the air and human milk or blood for ongoing monitoring [8]. This recognised that changes in global air concentrations of POPs are reflective of changes in the emissions from core sources. For example, air concentrations of PCBs at the arctic station of Storhofdi in Iceland indicated declining emissions of this POP from Western Europe [9]. In Australia, air concentrations of dieldrin were found to be highest at the urban sites of Darwin (Northern Territories) and Aspendale (Victoria), indicating that the greatest source of emission occurs in these regions. These trends of PCBs, as well as other POPs around the globe were provided in the second Global Monitoring Report by the fourth Conference of the Parties of the Stockholm Convention in 2017 [9], showing that air concentrations of some POPs are starting to be at equilibrium with environmental emission sources such as soil. Since the production of many POPs, including

dieldrin, was banned, most of the re-emission and subsequent risks for human health and the environment comes from historically contaminated soils. Consequently, given the strong sorption of chlorinated hydrocarbons to soils with half-lives of up to 20–50 years [5], it can be expected that agricultural soils will remain a major POP sink and source of risk of POP re-emission for decades to come.

1.2 CONTEXT AND PURPOSE

The quest in understanding and improving natural degradation processes of POPs

There is no common definition for the term "persistent" in the expression "persistent organic pollutant" and it is sometimes used interchangeably with the term "recalcitrant", meaning resisting environmental degradation [10,11]. This lack of a clear definition reflects the complex environmental interactions involved in chemical and biochemical transformation processes. Progress has been made in understanding the intrinsic properties of different types of semi-volatile chlorinated hydrocarbons and the various occurrent types of transformation reactions. However, it is still impossible to predict POP degradation processes in soils. Perhaps, one of its most predictable properties is the retention in soils. Retention is the principal attenuation mechanism of POPs in soils, driven by the hydrophobicity, halogenation and carbon chain length of hydrocarbons and by the amount of organic matter content of soils [12–14]. Greater soil retention of POPs reduces short-term risks for off-site transport, but at the same time it reduces the long-term bioavailability to degrading microorganisms. Hence, this low bioavailability in soils results in a lack of microbial degradation of these types of pollutants. Consequently, decreased short-term risks to humans and animals due to sorption are traded for long-term risks of chronic pollutant exposure to future generations. Such toxic effects include reduced fertility, abortion and stillbirths, eggshell thinning, immuno-suppression, contribution to cancer development, weight loss and impaired intellectual development of children [15–17].

A knowledge gap is the understanding of how biodegradation of soil-sorbed POPs by native microorganisms can be enhanced so that the long-term risks are minimised. It is generally accepted that most organochlorine chemicals can be degraded by microorganisms, either through co-metabolism, or by serving as growth or energy substrates. These processes were thoroughly reviewed in the past [10,18]. Soil metabolic diversity, especially from bacteria and archaea, is extensive, whereby bacterial cells occupy and exploit any available niche within the soil environment. In addition, it was recently found that the metabolic potential of soil microorganisms for oxidative dechlorination of POPs is ubiquitous and may have evolved with naturally occurring chlorinated organic matter [19,20]. Studies showed that some POP degradation pathways involve genes that were present before the start of the industrial synthesis of halogenated compounds [21]. However, few case studies have examined the mechanisms of natural attenuation of contaminated soils [14]. Furthermore, none seem to improve our understanding for enhancing natural attenuation processes of the most persistent pollutants in topsoil. As a result, it is impossible to predict microbial POP degradation processes in soils. Small changes in land management practices may have relatively large effects on long-

term biodegradation processes, but this is currently unknown. What advice should farmers be given, that own contaminated lands and want to practice no-till / minimal-till to sequester carbon? Perhaps, these farmers want to utilise perennial pastures or organic amendments to improve soil health in anticipation that biological activity improves pollutant degradation. Is that approach useful? Evidence-based answers to these questions will lead to appropriate advice that can maximise natural attenuation of POPs, and therefore minimise long-term risks.

Understanding soil microbial ecology is key in interpreting interactions between different taxa and their niches to exploit that knowledge for a desired function. Ecological concepts are still in their infancy and evolving with the vast genomic data generated from increasingly more accessible high-throughput sequencing platforms. Taxonomic and functional analyses rely heavily on bioinformatics because only a fraction of that diversity is culturable. Only ~10% of bacteria are culturable [22], while the definition of a bacterial species is still debated [23]. On the other hand, it is known that mechanisms of POP-sorption in soils are intrinsically linked to the composition of the soil matrix, including the soil organic matter quality [24]. Subsequently, a view is maturing that recognises the influence of organic matter quality on microbial potentials for POP degradation processes [25,26].

Interestingly, organic matter itself has been processed by microorganisms, and can reside in soils for thousands of years [27]. If POPs are sorbed into this type of 'resistant' carbon pool, then this may hint at how long POPs can reside in soils too. More importantly, it is debatable whether this resistance of native organic matter to microbial degradation is (a) due to some inherent property of the organic polymers itself or (b) whether it is due to logistical constraints, such as limited availability and organo-mineral complexation [11]. The second concept is currently challenging the concept of 'humus' as it asserts that humic substances do not exist. Instead, under the right circumstances organic polymers that make up humus are easily degraded; hence, one has to understand how environmental circumstances affect the degradation of these polymers [28]. I believe there is merit in exploring how microbial POP degradation is linked to soil organic matter dynamics, which leads to my PhD research. The research may lead to new perspectives and practical advice for land managers because any factors affecting soil organic matter quality would also impact the POP-degradation potential in a given soil. The purpose of this PhD research is to provide insights into the role of soil microbial, functional and phylogenetic diversity in the degradation of legacy POP residues in soils and how this is linked to soil organic matter quality.

1.3 SIGNIFICANCE AND SCOPE

Persistent organic pollutants will remain part of our environment and food-chain for future generations unless we learn how to enhance natural degradation processes. Health and environmental risks of these compounds are associated with their persistence, toxicity, the large scale at which they were released globally, their long-

range transport, and their tendency to accumulate in animal and human tissue. Degradation processes of POPs occur naturally in soils but are currently too slow on a human time scale.

Research in understanding microbial processes relating to POP dissipation in soils has stalled. I therefore explored associations between microbial ecology, organic matter quality and POP dissipation in soils. Due to the explorative nature of the analytical approach, it did not elucidate any new mechanisms of POP degradation in soils. Instead, conclusions provided direction for this area of research. The POP dieldrin was the focus of this research because it is the POP of concern for Victoria, Australia. We had access to historically-contaminated paddocks and their dieldrin concentrations from as far back 1987, which were kindly provided by our collaborators and organochlorine coordinators at Agriculture Victoria. It was assumed that from the conclusions made with dieldrin, inferences could be made to other chlorinated POPs as their environmental fate are largely similar, as determined by their degree of chlorination.

1.4 THESIS OUTLINE

A literature review was provided in chapter two, with relevant background information of abiotic and biotic processes that determine the fate of POPs in surface soils with emphasis on dieldrin. Chapter three provided a systematic review that assessed taxonomic associations of bacterial or fungal isolates worldwide with their efficiency of POP transformation in liquid media. This was important to understand if POP-degrading phylotypes existed and to draw inferences to a soil's potential to degrade POPs.

Experimental chapters (Chapters four to six) include two field studies and one 12-month soil incubation. These chapters provide evidence for the role of community-level diversity and function in the dissipation of dieldrin residues and how that related to other soil properties and organic amendments.

Chapter seven provides a perspective on the combined results, highlights its limitations and then gives direction for future research. The data from a 10-week plant-growth experiment are not included into this thesis but will be considered in the general discussion.

Soil diversity and estimates of the functional metagenome were assessed through sequencing of bacterial and fungal marker genes. Attempts were made to exploit multiple novel analytical methods that help to assess interaction numerically and visually. A large proportion of the work which led to the outputs of this analytical work - was learning how to handle data and code in the R programming language and understanding and applying statistical methods in microbial ecology. This was critical as it enabled the assessment of data more comprehensively. Furthermore, I was trained by the analytical chemist Pei Zhang (Agriculture Victoria) to extract and quantify dieldrin residues in soils. It took considerable time to improve the method and skills until the results were consistent.

Chapter 2: Literature review - Fate of dieldrin and related compounds in topsoil

2.1 INTRODUCTION

This literature review starts by providing a brief overview of non-microbial processes that control the fate of dieldrin in topsoil and then continues to review literature relating to microbial degradation processes of dieldrin. Some key microbial taxa are described in more detail, and degradation pathways of more soluble POPs described. Furthermore, the role of organic amendments and organic matter in these processes is reviewed to offer direction for future research that aims at improving natural attenuation processes in surface soils. As literature relating to microbial dieldrin degradation is scarce, the literature for other POPs is reviewed and put into context for dieldrin.

2.2 DIELDRIN CHEMICAL CHARACTERISTICS

Dieldrin belongs to a group of pesticides that are commonly referred to as organochlorines. This term is usually confined to ecologically problematic polychlorinated chemicals. The chemical sciences use the term organochlorine by creating the organo-element term, as in organophosphorous-, organosulfur- etc. It simply means a chemical compound in which a chlorine atom is covalently bound to a carbon atom, forming a C-Cl bond.

Dieldrin was produced by Shell Chemical and distributed worldwide as a non-systemic broad-spectrum insecticide in horticulture and agriculture, and as wood protection and termite control [29]. It was one of the initial twelve persistent organic pollutants (POPs) (at the time of writing there are thirty POPs in total) that have been recognized by the Stockholm convention (http://www.pops.int/) as causing adverse effects on human health and the environment (Table 2.3-1).

Dieldrin is a chlorinated hydrocarbon in the class of cyclodienes containing an epoxide functional group comprising six covalently-bonded chlorine atoms. Commercial dieldrin concentrates contained up to 85% of dieldrin, also known as 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene (HEOD), which was marketed as a non-systemic broad-spectrum insecticide.

It is a non-ionic, apolar, hydrophobic, lipophilic, synthetic and chlorinated organic compound in the group of cyclodiene organochlorines [30]. Pure dieldrin has a melting point of 177˚C and it is insoluble in water (Table 2.3-1). It is slightly soluble in petroleum oils, moderately soluble in acetone and soluble in aromatic solvents. Moreover, it is relatively stable to alkali, weak acids and light, and has a similar volatility to DDT [31,32]. As with all other chlorinated POPs, dieldrin is semi-volatile and has an estimated vapor pressure of 7.85×10^{-4} Pascal at 25˚C [33]. Dieldrin is chemically stable.

The toxicology of dieldrin and other organochlorines has been studied extensively [30,34]. For an assessment of the role of chlorine in biological activity and toxicity I refer to an industry dossier [35]. In animals, dieldrin acts as an antagonist for the action of gamma-aminobutyric acid (GABA) on chlorine channels of the central nervous system, which is the mode of action to control against insect pests in agriculture [30]

All chlorinated POPs have common chemical characteristics including hydrophobicity and lipophilicity as determined by the degree of their chlorination [36] (Tables 2.3-1 and 2.3-2). Therefore, it is suggested that broad inferences can be made from dieldrin to other chlorinated POPs because their environmental fate in soils is often determined by common intrinsic chemical properties. For example, a POP with a similar degree of chlorination and hydrophobicity is expected to have a similar retention and subsequent half-life in a given soil and location.

2.3 NON-MICROBIAL SOIL PROCESSES THAT AFFECT DIELDRIN CONCENTRATIONS IN TOPSOIL

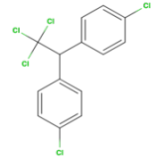
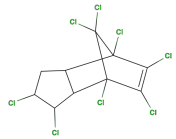
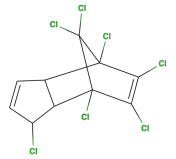
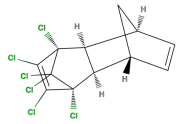
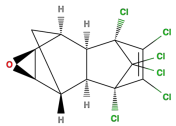
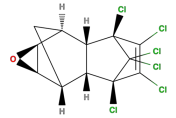
Any pesticide applied to soil undergoes transport, retention and transformation processes that are interrelated and affected by climate, vegetation and soil properties.

2.3.1 Transport

Dieldrin has the potential to volatilise into its gaseous phase and can be transported large distances through the atmosphere and redeposit in other parts of the planet [37]. Volatilisation of dieldrin from soils increases with temperature, soil water content and relative humidity, possibly because water competes with dieldrin on sorption sites [38]. Based on simulations, dieldrin volatilisation is significantly higher in silty clay soils compared to sandy soils, especially when soils were wet at the time of application, because there is a higher likelihood of the presence of dieldrin in topsoil (i.e. has not traveled down the soil profile) [39]. A perspective on risks associated with the spatial range of POPs transport is provided elsewhere [3].

Furthermore, the risk of dieldrin leaching down the soil profile and subsequent aquifer contamination was considered to be low due to insolubility of dieldrin in water [40]. However, dieldrin penetrates deeper in moist sandy soils compared to soils higher in silt and clays [41]. In coarse-textured soil profiles substantial movement of dieldrin down to 1.2 m has been observed [42,43].

Table 2.3-1. The initial twelve persistent organic pollutants (POPs) which were recognised as major threats to human and environmental health by the Intergovernmental Forum on Chemical Safety (IFCS) in 1996 (http://www.pops.int). Their chemical structure, solubilities in water (mg L^{-1}) and octanol/water partition coefficients (Log K_{OW}) are presented.

Name	Trade name examples	Structural category	Use	CAS number	MW	Chemical formula	Chemical structure	Solubility in water* (25 C, mg L^{-1})	Log K_{OW}
Dichlorodiphenyltrichloroethane (DDT)	Genitox, Anofex, Detoxan, Neocid, Gesarol, Pentachlorin, Dicophane	DDT and analogues	Pesticide	50-29-3	354.49	$C_{14}H_9Cl_5$		0.0153 [44,45] (Insoluble)	6.91 [44]
Chlordane	Octachlor, Chlordan, Velsicol 1068	Cyclodiene	Pesticide	12789-03-6 57-74-9	409.78	$C_{10}H_6Cl_8$		0.056 [46] (Insoluble)	6.16 [47]
Heptachlor	Basaklor, Gold Crest H-60, Termide, Heptagran	Cyclodiene	Pesticide	76-44-8	373.32	$C_{10}H_5Cl_7$		0.18 [48] (Insoluble)	6.10 [48]
Aldrin	Aldrec, Aldrex, Drinox, Octalene, Seedrin	Cyclodiene	Pesticide	309-00-2	364.91	$C_{12}H_8Cl_6$		0.11 (20˚C) [49] (Insoluble)	6.50 [50]
Dieldrin	Aldrec, Aldrex, Drinox, Octalene, Seedrin	Cyclodiene	Pesticide	60-57-1	380.91	$C_{12}H_8Cl_6O$		0.195 [33,51] (Insoluble)	5.40–6.20 [33,52]
Endrin	Mendrin, Hexadrin, Endrex	Cyclodiene	Pesticide	72-20-8	380.91	$C_{12}H_8Cl_6O$		0.25 [53] (Insoluble)	5.20–5.60 [53,54]

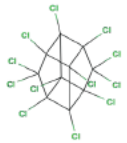
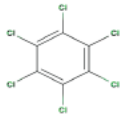
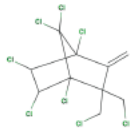
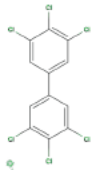
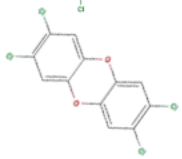
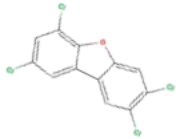
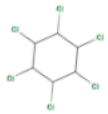
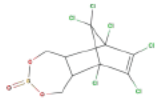
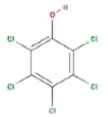
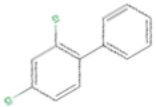
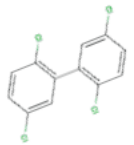
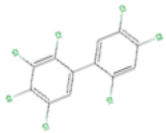
Name	Trade name examples	Structural category	Use	CAS number	MW	Chemical formula	Chemical structure	Solubility in water* (25 C, mg L^{-1})	Log Kow
Mirex	CG-1283, Dechlorane, HRS1276b	Cyclodiene	Pesticide	2385-85-5	545.54	$C_{10}Cl_{12}$		0.085 [55](20) (Insoluble)	5.28– 6.89 [55,56]
Hexachlorobenzene	Anticarie, Ceku, No Bunt	Chloro benzene	Pesticide/ Industrial use	118-74-1	284.8	C_6Cl_6		0.0047 [57] (Insoluble)	5.73 [57]
Toxaphene	Alltex, Allotox, Crestoxo,M5055, Melipax, Motox, Penphene,Cotton Tox MP82	Organic mixture of 670 chlorinated terpenes	Pesticide	8001-35-2	411.8	$C_{10}H_{6\text{-}10}Cl_{5\text{-}12}$		1.09 [45,58] (Insoluble)	5.9 [58]
Polychlorinated biphenyl (PCB)	Aroclor, Delor	Polychlorinated biphenyl (PCB)	Industrial chemical	32774-16-6	360.9	$C_{12}H_4Cl_{1\text{-}n}$		0.002 - 4 [59] (Insoluble)	4.4 - 7.1 [59]
Polychlorinated dibenzodioxins (PCDD)			Unintentional production	Various	219–425	$C_{12}H_6Cl_{2\text{-}n}O$		0.0004–1 [59] (Insoluble)	5.0–8.2 [59]
Polychlorinated dibenzofurans (PCDF)			Unintentional production	Various	202–409	$C_{12}H_6Cl_2O_2$		0.0002– 9 [59] (Insoluble)	4.6– 7.9 [59]

Table 2.3-2. Examples of other persistent organic pollutants (POPs) which were recognised as major threats to human and environmental health by the Parties of the Stockholm Convention since 2009 (http://www.pops.int). Their chemical structure, solubilities in water (mg L^{-1}) and octanol/water partition coefficients (Log K_{OW}) are presented.

Name	Trade name examples	Structural category	Use	CAS number	MW	Chemical formula	Chemical structure	Solubility in water* (25 C, mg L^{-1})	Log K_{OW}
gamma-Hexachlorocyclohexane (Lindane)	Etan 3G, Forlin, Gamaphex, Isotox, Germate Plus,	Benzene hexachlorides	Pesticide	58-89-9	290.83	$C_6H_6Cl_6$		7.3 [60] (Insoluble)	3.72 [61]
Endosulfan	Thidan, Thionex, Thionate Malix, Cyclodan, Thifor, HOE 2671	Cyclodiene	Pesticide	115-29-7	406.93	$C_9H_6Cl_6O_3S$		0.32–0.53 [62] (Insoluble)	3.62–3.83 [61]
Pentachlorophenol (PCP) and its salts and esters	PCP, Lauxtol	Chlorophenol	Pesticide	87-86-5	266.3	C_6HCl_5O		14 [62] (Insoluble)	5.12 [61]
2,4-Dichlorobiphenyl (PCB 7)[a]				33284-50-3	223.098	$C_{12}H_8Cl_2$		0.85 [59] (Insoluble)	5.11 [59]
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)[a]	Included in mixtures such as Aroclor	Polychlorinated biphenyl (PCB)	Industrial chemical	35693-99-3	292	$C_{12}H_6Cl_4$		0.050 [59] (Insoluble)	5.92 [59]
2,2′,3,4,4′,5,5′-Heptachlorobiphenyl (PCB 180)[a]				35065-29-3	395.323	$C_{12}H_3Cl_7$		0.002 [59] (Insoluble)	7.1 [59]

[a] The different congeners of PCB are shown to present increasing degrees of chlorination. PCBs were part of the initial twelve POPs and recognised prior to 2009.

2.3.2 Retention

Retention (or adsorption) of dieldrin is a reversible or irreversible physical or chemical process that is a function of climate, total organic matter content, soil texture, surface chemistry and structure of the soil matrix, as well as the chemical characteristics of the pollutant. Retention times of dieldrin in soils, expressed in half-lives from different climates, vary from > 20 years in a sandy soil in the Netherlands to 7 years on a sandy loam and a silty loam in the USA (Beltsville Maryland and Wisconsin, respectively) to 7.5 months in subtropical Taiwan and 117 days on a sandy loam in Haryana, India [43, 63–66]. In Victoria, Australia, which has a temperate oceanic climate (cfb according to the Köppen climate classification) [67], half-lives were expected to be 20 – 50 years based on the observations from the national organochlorine residue management program (NORM) [5].

A controlled long-term field trial by the Crops Research Division of the U.S. Department of Agriculture (USDA) in Beltsville, Maryland, ran from 1949 to 1966 and determined decomposition rates of various persistent organic pollutants (POPs) [63]. The mean half-life of dieldrin was 7 years in Congaree sandy loam in a humid subtropical climate (cfa according to the Köppen climate classification). In the same study and location, half-lives of DDT were determined in three different soils that showed that half-lives varied between soils, ranging from 2.5 to 35 years.

Retention in a given climate increases with soil organic matter content, hydrophobicity and chlorination [68]. Site-specific equilibrium soil-water partition coefficients (K_{oc}) are experimentally derived to define the potential of sorption of a pollutant to a given soil. For chlorinated hydrophobic chemicals such as dieldrin it is assumed that the majority of sorption occurs with organic materials, rather than with minerals, as the K_{oc} is related to the octanol-water partition coefficients (K_{ow}) [68–70]. The higher these coefficients, the greater the soil sorption potential of a POP. It was found that sorption kinetics by natural sediments are typically bimodal, with an initial rapid phase followed by a slow sorption phase that can persist for months [13]. For neutral and hydrophobic compounds such as chlorinated POPs, slow diffusion into micro and nanopores is the principal mechanism that is limiting microbial degradation [71,72]. If POPs are not available to microbial cells, they are not metabolised.

The pH-effect on half-lives in solution on non-polar organochlorines compared to polar pesticides was found to be relatively small [73]. Hence, it is expected that soil acidity only slightly increases half-lives of dieldrin in soil.

Clay minerals may also influence the sorption of pesticides through clay interlayer diffusion [71]. Bonding reactions with negatively charged mineral surfaces are less likely for the non-polar and non-ionic dieldrin unless it is transformed and dechlorinated. However, one possible mechanism was described by Green (1974) whereby non-polar pesticides could be polarised by electrostatic forces and subsequently bond to clay

surfaces through hydrogen bridges. Alternatively, van der Waals forces were proposed to be the principal force of attraction between clays and non-polar and non-ionic pesticides.

2.3.3 Photolysis

UV radiation has the potential to transform dieldrin in topsoil or leaf surfaces into a more polar molecule known as photodieldrin, which has a similar level of toxicity to dieldrin [75]. Photodieldrin molecules are chiral and exist as pairs of enantiomers and existed in soils even 40 years after dieldrin application [76]. Currently, little is known about photodieldrin, but given its toxicity and its potential effect on soil, it should be considered when assessing soil dieldrin contamination.

2.3.4 Plant uptake

Plant roots may passively adsorb dieldrin onto surface cells; however, plant uptake of dieldrin, i.e. the translocation from roots to xylem and shoots, is negligible for hydrophobic compounds with high K_{ow} such as ~5.7 for dieldrin [77,78]. However, some plants species have developed mechanisms that allow them to solubilise hydrophobic soil materials, which facilitates dieldrin translocation from roots into shoots. For example, the tropical castor bean (*Ricinus communis* L.) accumulates POPs (including dieldrin) and is a potential candidate for phytoremediation [79]. Furthermore, *Cucurbitaceae* plants such as pumpkins, zucchinis, cucumbers and melons have shown to accumulate dieldrin into shoots and fruits [80]. The mechanisms and plant physiology behind these unique processes are still being investigated. One of the hypotheses is that citric acid exudation may help to release dieldrin from the soil matrix for root uptake. Future research into these mechanisms has the potential to lead to products that assist in POP desorption from the soil matrix to enable microbial degradation/remediation strategies.

2.4 EVIDENCE OF MICROBIAL DEGRADATION AND CO-METABOLISM OF DIELDRIN

Soils comprise the most diverse ecosystem on Earth with microorganisms such as bacteria, archaea, fungi and protozoa, which mediate most soil processes, including the biodegradation or transformation of pesticides into simpler molecules [22,81]. Microorganisms can produce, or evolve to produce, the needed enzymes to biodegrade 'foreign' compounds such as POPs, while plants and animals are usually incapable of doing so [82]. For this review, microbial degradation is defined as biodegradation, a microbial-mediated process that converts an organic compound into intermediates (transformation) or into CO_2, H_2O, and/or inorganic salts (mineralisation) [18]. It describes the ability of microorganisms to use organic pollutants as sources of energy and carbon for growth, which needs to be energetically favorable for microbial cells. On the other hand, co-metabolism is defined as the transformation of organic compounds by microorganisms that grow on another substrate. In other words, enzymes produced from biodegradation/growth on a primary substrate may inadvertently react with a secondary organic compound (e.g. dieldrin); however, microorganisms neither gain

energy/carbon, nor grow through this secondary compound. Both processes are essential for POP degradation [83].

Bacteria and Archaea have greater metabolic diversity compared to fungi, and can use various electron donors (e.g. hydrogen, hydrogen sulfite, acetate, lactate, formate) and electron acceptors (e.g. oxygen, nitrate, sulfate, ferric/ferrous iron, fumarate) in aerobic or anaerobic respiration. Bacteria can further utilise vast amounts of different carbon compounds as sources of carbon and energy during respiration. This metabolic versatility leads some bacteria to develop niche enzymatic mechanisms to metabolise and grow on selected POPs. On the other hand, fungi generally only co-metabolise POPs via oxidation of other types of organic matter such as lignin because enzyme systems in fungi mainly exist to degrade lignocellulosic materials [83].

Persistent organic pollutants can either be an electron donor or acceptor depending on the redox potential in soil solution. Oxidations or reductions of various POPs such as dieldrin are both known to occur individually or sequentially and can involve dehalogenation, hydroxylation, dealkylation, or epoxidation reactions which were comprehensively reviewed by Reineke [83]. These reactions are catalyzed by dehalogenases, monooxygenases, dioxygenases, laccases and peroxidase enzymes.

2.4.1 Aerobic degradation

For highly chlorinated and water-insoluble organochlorines such as dieldrin, it is challenging to isolate microbial degraders and study their enzymatic mechanisms. Since 1967 only five studies have successfully isolated bacterial and fungal dieldrin degraders from soil (Tables 2.4-1 and 2.4-2), hence, little is known about specific phylotypes dieldrin degraders and their metabolic pathways.

Under aerobic conditions, the most limiting step in biodegradation is dechlorination, i.e. the removal of chlorine from the compound; therefore, these processes are slower for chemicals that contain a high number of chlorine substituents such as dieldrin [18]. For research on microbial degradation of polychlorinated biphenyls (PCBs), which compare the degradation efficiency of congeners with increasing degree of chlorination, it was consistently found that any congener containing more than four chlorines is difficult to biodegrade [84]. Hence, it is believed that dieldrin (consisting of 6 chlorines) and other highly chlorinated pollutants do not support microbial growth, and that co-metabolism from growth on different substrates is the primary transformation and dissipation mechanism.

In the most recent dieldrin-isolation study, a dieldrin-degrading strain of *Pseudonocardia* was isolated in 2011 using the water-soluble aldrin trans-diol as the selecting growth substrate in the enrichment medium (Table 2.4-1). However, this strain was unable to aerobically grow with dieldrin as a substrate in culture and needed pyruvic acid as a growth substrate to co-metabolise dieldrin [85]. In this example, dieldrin was only transformed (not mineralised), and the transformation product still contained five chlorine substituents.

Only one study from 2008 provided evidence of bacterial growth on dieldrin as the only carbon source (Table 2.4-1) [86]. Its authors isolated strains of *Burkholderia sp*. and *Cupriavidus sp*. (both belong to the family

Burkholderiaceae) through enrichment cultures from uncontaminated soils with a structural analogue of dieldrin (1,2-epoxycyclohexane) as carbon substrate. 1,2-epoxycyclohexane has greater water solubility (K_{ow} = 1.26) compared to dieldrin, which enabled the selection of degrading bacteria in culture. After the enrichment, strains were isolated and grown with dieldrin as the sole carbon source for the first time. Hence it appeared that this strain was able to utilise dieldrin as an electron donor.

The genera *Burkholderia* and *Cupriavidus* contain chemoorganotrophic and chemoheterotrophic species respectively, all with a strictly respiratory type of metabolism with either oxygen or nitrate as the terminal electron acceptor [87,88]. *Burkholderia* spp. also have a remarkable variety of organic compounds that can be utilised as electron donors for respiration [88]. The species *Burkholderia xenovorans* and *Burkholderia cepacia* have also shown to be potent PCB degraders with a high tolerance for chlorine toxicity [89,90]. *B. cepacia* uses the enzyme biphenyl dioxygenase, which has a broad substrate specificity, to initiate PCB degradation by inserting oxygen into the aromatic ring [91]. Hence, it is possible that similar mechanisms apply to dieldrin molecules. This is an exciting development, but further research is required to uncover the pathways involved in dieldrin degradation.

2.4.2 Anaerobic biodegradation

Organochlorines are known to be suitable electron acceptors, hence under reductive conditions, dechlorination is more likely for highly chlorinated compounds. For example, reductive dehalogenation happened more readily for highly chlorinated PCB congeners by microbial consortia under anoxic conditions [92,93]. Reductive dechlorination can be promoted by the addition of organic electron donors, such as lactate, acetate, pyruvate, ethanol, formate and glucose. For example, lactate resulted in the greatest reductive transformation of DDT in flooded microcosms using wetland soils [94]. Examples of anaerobic biodegradation of dieldrin by mixed communities, including the growth substrates, their removal rates, and incubation times, are provided in a review by Matsumoto et al. 2009. In two of these examples, the electron donor formate was successfully used for anaerobic dieldrin transformations; however, the full mechanisms are not yet understood. Reductive dechlorination reactions are usually substrate-specific and therefore do not result in complete biodegradation of pollutants; hence, may require further aerobic metabolic mechanisms [95].

2.4.3 Sequential aerobic and anaerobic biodegradation

The requirement of sequential anaerobic and aerobic conditions for complete removal of pollutants is a common observation with highly chlorinated POPs. The redox potential varies greatly within soil aggregate, whereby at near saturation of soil aggregates (-1 kPa) the redox potential decreases from the surface towards the aggregate-center [96]. Furthermore, reductive dechlorinating activity has been detected in surface soils and not only in anaerobic sediments [97]. Hence, it is possible that aerobic and anaerobic processes take place

simultaneously in the vicinity of just a few centimeters or millimeters. The potential of simultaneous aerobic/anaerobic biodegradation is well established for PCBs and DDT [98–100].

However, sequential aerobic/anaerobic processes are limited due to macro and microscale heterogeneity of both anaerobic and aerobic sites in soils [97]. Hence, this process has potential for *ex-situ* bioreactors under controlled conditions but has currently limited application in increasing natural attenuation of POPs in agricultural soils.

2.4.4 Fungal degradation of dieldrin

So far, five studies since 1967 have successfully isolated fungi from soil that show potential for dieldrin co-metabolism in culture. All of the strains belong to the genera *Trichoderma* and *Mucor* (Table 2.4-2). As soil-borne fungi were the focus for this review, wood-rotting fungi such as *Phanerochaete chrysosporium* were not considered.

Of the soil-inhabiting isolates, *Mucor racemosus* strain DDF, isolated from endosulfan contaminated soils, showed the greatest potential for dieldrin degradation. Hyphal growth was associated with increased dieldrin co-metabolism rates; hence the higher the glucose concentration in the medium, the more hyphal growth and dieldrin co-metabolism occurred. The only detected metabolite was aldrin *trans*-diol, a toxic transformation product of dieldrin, which retained all chlorine atoms while the epoxide functional group was removed. The authors speculated that the formation of aldrin *trans*-diol was catalysed by an epoxide hydrolase and noticed that it did not accumulate during the incubation, hence may have been further transformed.

This strain was also able to co-metabolise DDT, DDE, heptachlor, heptachlor epoxide and endosulfan. *Mucor racemosus* is a fast-growing facultative anaerobe. The potential of a soil-born fungi for removing POPs from soil, compared to wood rotting fungi, is that they can grow and compete in soils. Despite this promising result, no successful field trials that inoculated soils with *Mucor racemosus* spores to reduce aged POP residues have been reported.

Table 2.4-1. Studies with evidence of bacterial dieldrin co-metabolism in culture with isolates obtained from soil

Phylum	Species	Year	Medium	Metabolites	Source of Strain	TE[a] (% / day)	Ref.
Proteobacteria	*Pseudomonas sp.*	1967	YMB	trans-Aldrindiol, water and solvent-soluble metabolites	USA	0.02[b]	[101]
Proteobacteria	*Trichoderma viride*					0.006	
Firmicutes	*Bacillus sp.*					0.02	
Proteobacteria	*Klebsiella aerogenes*	1968	TSB	6,7-trans-dihydroxydihydroaldrin	USA	0.01	[102]
Actinobacteriota	*Nocardia sp.*	1972	MSM + Glucose	CO_2	Germany	< 0.00	[103]
Actinobacteriota	*Micrococcus sp.*					< 0.00	
Actinobacteriota	*Corynebacterium sp.*					< 0.00	
Proteobacteria	*Burkholderia sp.*	2008	MSM + ECH	No solvent-soluble metabolites detected, only potential water-soluble metabolites	Japan	0.04	[86][c]
Proteobacteria	*Cupriavidus sp.*					0.03	
Proteobacteria	*Burkholderia sp.*		MSM			0.01	
Proteobacteria	*Cupriavidus sp.*					0.01	
Actinobacteriota	*Pseudonocardia sp.*	2011	MSM+ pyruvic acid	Aldrindicarboxylic acid	Japan	0.09	[85]

[a] Dieldrin transformation rate (TE) in percent per day, only soil-isolates are presented with evidence of dieldrin transformations; [b] Mean of all Pseudomonas strains; [c] First study that provided evidence of bacterial growth with dieldrin as sole carbon source; YMB, yeast mannitol broth; TSB, trypticase soy broth; MSM, minimal salt medium; YPG, yeast peptone glucose; ECH, 1,2-epoxycyclohexane

Table 2.4-2. Studies with evidence of fungal dieldrin co-metabolism in culture with isolates obtained from soil

Phylum	Species	Year	Medium	Metabolite	Source of Strain	Trans. rate (% / day)[a]	Ref.
Ascomycota	*Trichoderma viride*	1967	YMB	Aldrin trans-diol, water and solvent soluble metabolites	USA	0.01	[101]
Mucoromycota	*Mucor alternans*	1970	-	none detected	USA	-	[104]
Ascomycota	*Trichoderma koningii*	1971	YMB	CO_2	USA	0.002	[105]
Ascomycota	*Trichoderma harzianum*	1993	Various C sources	-	Japan	0.02	[106]
Mucoromycota	*Mucor racemosus*	2010	CDB	Aldrin trans-diol	Japan	0.07	[107]
Ascomycota	*Trichoderma sp.*					0.01	

[a] Dieldrin transformation rate (TE) in percent per day, only soil-isolates are presented with evidence of dieldrin transformations; YMB, yeast mannitol broth; TSB, trypticase soy broth; CDB, Czapek-Dox broth.

2.5 MICROBIAL DEGRADATION OF OTHER POPS

More degradation pathways have been uncovered for increasingly water-soluble POPs such as the insecticides endosulfan and lindane and the wood preservative pentachlorophenol (PCP). These highly chlorinated POPs were included in the Stockholm Convention after 2004 (Table 2.3-2), and their water solubilities have been estimated as ~0.4, ~7.3 and ~14 mg L^{-1} respectively. Hence, these POPs are about 2, 37 and 72 times more soluble than dieldrin, although they are still in the insoluble category. For endosulfan and PCP it is now well established that the initial dechlorination occurs via a monooxygenase and was observed with *Arthrobacter*, *Flavobacterium* and *Mycobacterium* [10,108,109].

2.5.1 Biodegradation of lindane

Similarly, for lindane the complete mineralisation pathway of the *Sphingobium japonicum* strain SS86 (formerly *Sphingomonas paucimobilis*) in liquid media could be observed and seven novel enzymes were discovered. Some enzymes are constitutively expressed in this organism while others are induced by its substrates, indicating that this pathway is specific to lindane [110]. The pathway is initiated by a constitutively-expressed dehydrochlorinase (linA) for dechlorination of the first two chlorine substituents followed by hydrolysis of another two chlorines with a haloalkane dehalogenase (linB) before the removal of the final two chlorines, catalysed through a reductive dehalogenase (linD). The early steps of this pathway appear to happen in the periplasm, not in the cytoplasm, which may protect the bacterial cells from the toxic effects of chlorine [111]. This indicates that gram-negative bacteria, such as Proteobacteria may have a physiological advantage for degrading toxic substances. In fact, it is recognised that hydrophobic compounds can induce membrane toxicities in bacteria due to fluidity disruption and that gram-negative bacteria, due to their second cell-membrane physiology, may be more resistant [112]. Perhaps, the mechanism of these gram-negative bacteria to dechlorinate chlorinated compounds in the periplasm has arisen from the need for de-toxification.

Interestingly, the *S. japonicum* strain was isolated from soils in Japan, while some of these or similar genes were found in another Proteobacterium named *Rhodanobacter lindaniclasticus*, isolated from soils in France. From genomic comparisons of these different bacteria, it appears that the degradation genes were acquired relatively recently through horizontal gene transfer from other bacteria [110]. Hence, the presence of the POP lindane in soils has likely resulted in evolutionary pressure to develop biodegradation mechanisms and may become more prevalent in other bacterial taxa. Further insights into the biodegradation of lindane are provided by Zhang et al. [113].

However, evolutionary pressure from dieldrin in soils is less likely or slower due to its lower water-solubility. Both the ability to study the biochemistry of microbial degradation of POPs in culture and the bacterial evolution of biodegradation potentials depend largely on the availability of the concerned POPs to microorganisms.

2.5.2 Biodegradation of DDT

Less data regarding the evolution of DDT degradation pathways were available. So far only few bacterial strains were found that were able to grow with DDT at the sole carbon source under aerobic conditions. Typically, the investigators required up to three months of repeated enrichment of cultures before bacterial cells were isolated on agar. For example, Fang et al. [114] isolated *Sphingobacterium* sp. strain D6 from contaminated topsoil in a vegetable field in Cixi, Zhejiang Province, China. They did that by acclimatising the mixed soil community in mineral salt medium for six to eight weeks with increasing amounts of DDT. After isolating individual colonies on agar, strain D6 was capable of utilising DDT as its sole carbon and energy source. The authors discovered six metabolites (1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene; DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl) ethane; DDD, 1-chloro-2,2- bis(p-chlorophenyl) ethylene; DDMU, 2,2-bis(p- chlorophenyl)ethane; DDNS, 2,2-bis(p-chlorophenyl) acetate; DDA, 4,4'-dichlorobenzophenone; DBP) and proposed that this strain initially removed three chlorine substituents via dechlorination reactions at the trimethyl group, the central structure that links the two phenyl groups. This central structure then underwent dioxygenation and decarboxylation before the aromatic ring was cleaved on one of the phenyls. Enzymes were not identified in this study.

Similar metabolites and pathways were found with other, unrelated bacteria. For example, in 2016 a research group from the same University (Zhejiang University, Hangzhou) that discovered strain D6, proposed a strikingly similar pathway for *Stenotrophomonas sp*. DDT-1 [115]. They isolated this strain from topsoils in a vegetable field (assumably the same as above) and found it could utilise DDT as sole carbon and energy source. In this study, metagenomics was combined with metabolomics to elucidate degradation pathways. Known DDT degradation genes from KEGG pathway database (Kyoto Encyclopedia of Genes and Genomes) were compared with the genome of *Stenotrophomonas sp*. DDT-1. Again, DDT was initially converted into DDE (via *dehydrochlorinase*) and DDD (via *reductive dehalogenase*) by dechlorination at the trichloromethyl group, then into DDMU, followed by transformation into DDOH and DDA by hydroxylation and carboxylation (via *reductase* and *dehalogenase*) and finally mineralisation into CO^2.

Furthermore, Proteobacterium *Alcaligenes sp*. DG-5 as well as *Alcaligenes sp*. KK, both isolated from contaminated sites in China, have shown to transform DDT as a sole carbon and energy source in liquid culture [116,117]. The discoveries of these metabolic capabilities to mineralise DDT started to appear three decades after DDT was banned in China in 1983.

2.6 EFFECTS OF SOIL ORGANIC MATTER COMPOSITION AND AMENDMENTS ON BIODEGRADATION OF POPS

The idea to manipulate the soil microbiome to increase the potential for biodegradation of POPs in soils is an area of active research and aligns with one of the key questions for soil microbiology: "How can one modify soil populations and to what ends?" [118]. From past attempts to change soil microbial communities, it was

found that soil communities are quite resistant to change. The key driver for soil microbial diversity is the distribution of habitable niches in soils. For example, when two soils, one from the Rothamsted Research in the United Kingdom and another from the Vallombrosa Forest Reserve in Italy, were sterilised and inoculated with a subsample of the other soil, the inoculum of the 'donor' soil appeared to have no effect on the functional and taxonomic composition of the respective microbiomes [119]. The soil matrix, including its mineralogy, quantity and size of soil aggregates and pores, as well as organic matter composition, determines, to a large extent, the distribution of nutrients and water and thereby the microbial diversity and function.

Nonetheless, in a given climate and soil type, organic carbon plays a key role in moderating biological, chemical and physical properties of soil [118], and any changes to the soil organic matter composition that are driven by changes in vegetation or soil management will affect the soil microbiome and its diversity [120].

Presumably, POP degradation dynamics are related to the decomposition dynamics of soil organic carbon, wherein some forms of soil organic carbon can remain in soils for decades or even millennia. Over the years after their application, POPs such as dieldrin had time to diffuse into this 'old' pool of soil carbon in microporous regions where access to microbial cells is obstructed [72].

Lehmann et al. [121] postulated that the persistence of the undecomposed organic carbon pool is linked to microbial functional complexity. They proposed the concept of microbial functional complexity as determined by the molecular diversity of soil carbon, combined with its spatial heterogeneity and temporal variability. The concept is based on the emerging continuum model for soil organic carbon compounds that describes that it is not the material properties of compounds that control their decomposition but instead it is their environmental circumstance [28].

In relation to POP degradation, this concept implies that biodegradation is not limited by the recalcitrance of POP compounds but by their location in the soil matrix and the molecular carbon diversity surrounding them. In other words, given the chance, dieldrin could be metabolised by the microbial community. Consequently, if it was possible to manipulate the organic matter composition to promote microbial investment in energy-intensive metabolism at the dieldrin sorption sites, then in principle, natural degradation rates of POPs could increase.

2.6.1 Soluble carbon substrates

One approach to influence microbial POP degradation metabolism in soils is the application of soluble carbon substrates with the aim of increasing microbial growth and activity. However, simple stimulation of microbial growth by applying glucose to soils does not ensure increased co-metabolism of POPs. Hugenholtz and MacRae [122] showed that adding labile carbon such as glucose (water solubility 909 g L^{-1}) to soils did not increase dieldrin biodegradation. These authors incubated ~5 g of dieldrin-contaminated soil for 60 days, which were treated once with nine different carbon substrates at 100 µg g^{-1}, each with five replicates. They

found no significant dieldrin decreases in glucose-treated soils, while i-inositol (solubility in water at 25˚: ~0.2 g L^{-1}) and D-xylose (solubility in water at 25˚: ~555.0 g L^{-1}) treatments transformed 50–79% of dieldrin after 60 days. Hence, the type of sugar was important to shift the microbial community function towards the 'right' type of co-metabolic processes. Furthermore, biodegradation of dichlorodiphenyldichloroethane (DDD), a transformation product of DDT biodegradation, was either unchanged or even inhibited by acetic acid and glucose in soil-slurry microcosms [123].

Interestingly, D-xylose was also an important carbon substrate for PCB degradation in culture [124], indicating the broader utility of this substrate. D-xylose is a monosaccharide, derived from hemicellulose and is not as readily utilised as D-glucose. This is partly due to inhibition of D-xylose uptake caused by the presence of D-glucose and because different assimilation pathways are employed by a limited number of archaea, bacteria and fungi [125–127]. Hence, the growth of specific taxonomic groups may increase after the application of xylose to soils. Archaea and bacteria have similar pathways for D-xylose degradation, which are present in archaeal genera such as *Saccharolobus* and *Sulfolobus* and bacterial species such as *Azospirillum brasilense*, *Caulobacter vibrioides* CB15 and *Pseudomonas taiwanensis* [128–131]. Hence, this selection pressure of D-xylose on soil microbial community and respective functional changes may be linked to co-metabolism of POPs in soils and warrants further research.

2.6.2 Organic carbon amendments

Another approach to manipulate microbial carbon metabolism in soils is the application of common agricultural waste products such as animal manures, sewage sludge or fish hydrolysates. However, those waste products may not result in increased biodegradation potentials of dieldrin, despite increasing microbial activity overall. For example, Hemkemeyer et al. [132] showed that animal manures were ineffective for the degradation of ^{14}C-labeled 2,4-dichlorophenol (DCP), a model chlorinated pollutant (solubility in water at 20˚: ~0.45 g L^{-1}). The application of animal manure to soil increased the total abundances of bacteria, archaea and fungi but mostly in coarser particle sizes (> 2 µm). This was not surprising as larger particles of undecomposed/fresh organic matter (e.g. > 63 µm), sometimes called particulate organic carbon (POC), are typically more biologically active compared to the microbially-decomposed/processed carbon materials, which are much smaller in size [133].

However, the authors further showed that degradation of ^{14}C-labeled 2,4-dichlorophenol (DCP), happened only in the finest particle fraction (clay fraction, < 2 µm), while in the same soil, non-chlorinated ^{14}C-labeled phenol (solubility in water at 25˚: 82.8 g L^{-1}) was degraded in all particle sizes. Hence, a DCP degrading microbial community was only present in the fraction of < 2 µm and was distinct from the microbial community that degraded phenol.

The same authors showed in another experiment that DCP readily sorbed into the POC fraction in soils treated with animal manures, probably due to the increased concentrations of organic matter [25]. They proposed

that DCP degradation was slower in the soil treated with animal manure than without because the added DCP was partitioned into the POC fraction, where the 'wrong' microbial community was present. Apparently, the microbial degraders of DCP operated optimally in the fine fraction or were outcompeted in the POC fraction.

Hence, this result agreed with the carbon continuum model as the degradation of DCP was limited by spatial heterogeneity [28]. It is possible that processes for dieldrin biodegradation are affected similarly by organic amendments and warrants further investigations.

2.6.3 Role of the decomposed carbon fraction

The fine, humic carbon fraction is the largest carbon pool in soils and may play a significant role in degrading dieldrin and other POPs. Perhaps, the increased degradation potential of chlorinated pollutants in the fine fraction is linked to specific interactions of microbial cells with humic carbon. Humic carbon is the pool of microbially decomposed carbon, consisting of complexes of small carbon materials with little biological activity.

In general, soil carbon materials can be categorised into three pools that are distinct in their chemical composition, decomposability and particle size. For example, Baldock et al. [134] defined resistant organic carbon (ROC), humic organic carbon (HOC) and POC as pools of poly-aryl, alkyl and O-alkyl carbon respectively. Based on this methodology, ROC materials include all particle sizes (< 2 mm) while HOC are only fine materials (< 50 μm) and POC only coarse materials (> 50 μm). ROC is char-like and considered to be biologically inert. The HOC is dominated by decomposed, lipid-rich carbon that was microbially processed or is itself microbial biomass. In contrast, POC is dominated by less-decomposed carbohydrate-rich materials and is most biologically active. The terms HOC and decomposed carbon are used interchangeably in this review.

The decomposed soil materials have the potential to facilitate electron transport in reduction reactions of chlorinated pollutants. For example, Kappler and Haderlein [135] showed that humic acids can reduce chlorinated ethanes in solution and proposed that humic acids play a significant role as a reductant in the reductive transformation of contaminants in surface soils and that reductive transformation can be enhanced at contaminated sites. It was also postulated that this process may occur in variable oxic and anoxic microsites in soils.

In another study, humins were extracted from seven soil types and used to examine their effects on reductive dechlorination of PCP in anaerobic culture [136]. They all facilitated microbial reductive dechlorination of PCP with formate as electron donor or carbon source. From electron spin resonance spectra, quinones were considered responsible for electron transport or redox activity.

Furthermore, the electron transferring capacities of quinones from humic acids in different soils were assessed previously, and it was found that there are significant differences in a soils quinone-assisted reduction

capacity [137]. This indicated that it may be possible to apply quinone amendments to soils to increase the reductive chlorination processes of POPs. Perhaps, quinones can be designed to provide the ideal redox potential for specific POPs and applied to soils as part of a natural attenuation strategy. This is an exciting development and requires ongoing research to elucidate the potential of this technology for dieldrin biodegradation in soils.

2.7 CONCLUSIONS

Dieldrin, together with other highly chlorinated POPs, are some of the most persistent organic compounds known to man. POPs have similar intrinsic properties reflected in their high degree of chlorination and low water solubilities (Tables 2.3-1 and 2.3-2). Half-lives of residues in soils largely depend on climate and soil type. For a given soil, texture, organic matter content and type of vegetation/crop all impact abiotic and biotic removal processes. Of all potential processes that affect the fate of dieldrin residues in soils, microbially catalysed degradation of POPs is the least understood. In liquid culture, microbial isolates were able to transform various POPs via aerobic, anaerobic and sequential aerobic-anaerobic transformation reactions. However, soil retention to organic matter is limiting the availability of POP residues to microbial cells and likely slows down the evolution of aerobic degradation pathway in surface soils.

To increase a soil's potential for POP degradation, the literature indicated that the application of monosaccharides derived from hemicellulose (i.e. D-xylose) may favorably shift microbial activity and function. Applying glucose, on the other hand, generally had no effect on dieldrin degradation processes. In fact, the presence of glucose may prevent the development of this degradation potential. However, these processes are generally limited by the unavailability of POP residues, which diffuse into the small size fraction (< 2 μm) of decomposed organic materials that have low biological activity. Furthermore, amendments, such as manures, that increase the development of biologically active particulate organic matter and aggregation may prevent the assembly of the required microbial function for POP degradation. Hence, research should focus on mechanisms that drive the metabolism at the micro-scale with aim to enhance POP degradation processes. For example, some authors established that quinones, derived from humic materials, facilitated microbial reductive dechlorination of POPs. However, much more research is required to understand and exploit the mechanisms for enhancing the natural attenuation of POPs.

Chapter 3: Systematic review – Microbial transformation efficiency of POPs

3.1 ABSTRACT

Numerous studies around the globe have isolated microbial strains from soils, sediments and sludges that were capable of transforming chlorinated POPs in liquid media. However, there still is no evidence that these isolates represent phylotypes for efficient POP degradation or whether they simply are a random subset of the soil microbial community. With a focus on chlorinated POPs, this systemic review assessed whether any of the taxa among those strains that were isolated around the world were more efficient at degrading POPs in liquid media. The Web of Science database was queried, resulting in 318 POP-transformation observations in culture by 160 bacterial or fungal isolates in 78 studies across 26 countries. Transformation efficiencies were collated, normalised and median centered within POP compounds and degree of chlorination to remove POP-related biases in transformation efficiencies. In this review, the occurrences of microbial isolates across the globe could be a random subset of soil microorganisms. Furthermore, multinomial logistic regression, Kruskal-Wallis tests and Wilcoxon signed-rank tests revealed that all microbial phylotypes among the reviewed isolates had a similar efficiency in degrading POPs in liquid culture. As no specific taxon was broadly more efficient at transforming POPs, it was speculated whether a large uncultured pool of soil organisms exists that is able to degrade POPs and whether most soils are inherently able to degrade these compounds. Hence, research aiming to improve efficiencies of bioremediation of POPs in soils may need to shift its focus to the question of how this untapped genetic potential can be utilised.

3.2 BACKGROUND

Numerous studies have isolated microbial strains from soils and sludges globally that show evidence of chlorinated POP degradation or transformation in liquid media. Often the studies conclude that these isolates have the potential to enhance the biodegradation of POPs in soils through augmentation with bacterial or fungal inoculants. However, there is still no evidence of successful deployment of microbial inoculants for remediation of POPs in surface soils. Nor is it clear whether these strains, which were isolated using selective media, usually with a particular POP as carbon source, represent taxonomic groups that are in any way indicative of POP degradation in soils.

Alternatively, it is possible that the isolates are simply more resistant to POP toxicity (induced by their high degree of chlorination), which may lead to selective growth in culture supplemented with POPs. Strains that happen to show increased co-metabolism of POPs while growing on other primary carbon substrates are often chosen for further cell purification and analysis of biochemistry. However, if these isolated strains represent

phylotypes resistant to POP toxicities, it does not necessarily imply that they have an intrinsic pathway for POP degradation. They may even be less efficient in degrading POPs compared to other bacteria or fungi in soils. Or these strains are simply not competitive in a diverse soil community. This may explain the apparent gap between the strain-isolation studies and their application in contaminated lands.

Consequently, it remains unclear whether these isolated strains belong to taxonomic groups that are more efficient in degrading POPs and what role these taxonomic groups play within a diverse microbial community. If certain taxonomic groups were more efficient at degrading POPs among those isolated strains, they perhaps represent a phylotype for the degradation of persistent pollutants. Possibly, these phylotypes have the potential to become biomarkers for a soil's potential to degrade POPs. Hence, these large knowledge gaps warrant the need for future investigations into potential microbial phylotypes with an aim to develop microbial biomarkers for a soil's capacity to biodegrade POPs.

3.3 OBJECTIVE OF THE REVIEW

The objective of this review was to test if microbial phylotypes exist that show improved transformation efficiencies across all POPs in liquid media. Hence, with a focus on chlorinated POPs, the review assessed whether any taxa among those strains that were isolated around the world were more efficient at degrading POPs in liquid media. Differences in degradation efficiencies between bacteria and fungi and their different taxonomic levels were numerically analysed and visualised. Furthermore, the role of intrinsic properties of POP compounds, such as solubility in water, vapour pressure and octanol/water coefficients, in their degradability in culture was assessed.

3.4 METHODS

3.4.1 Literature search and selection criteria

The Web of Science database (Clarivate Analytics) was accessed on 15 April 2021 using the search key '#1 AND #2 AND #3 AND #4 AND #5 AND #6', whereby six sets of individual searches were combined.

- Set 1 (compounds): TS = (chlorinated hydrocarbon OR dieldrin OR ddt OR dichlorodiphenyltrichloroethane OR heptachlor OR aldrin OR endrin OR mirex OR chlorodecone OR endosulfan OR pentachlorobenzene OR hexachlorobenzene OR dicofol OR toxaphene OR pentachlorophenol OR Polychlorinated biphenyl OR PCB OR hexachlorobutadiene OR polychlorinated naphthalene OR polychlorinated dibenzodioxin OR dibenzofuran OR lindane OR hexachlorocyclohexane OR methoxychlor OR Dechloran OR PFHxS)
- Set 2 (process of microbial decomposition): TS = (transformation OR decomposition OR microb* OR biodegradation OR metabolism OR co-metabolism OR bioremediation)
- Set 3 (process of identification): TS = (identification OR isolation OR enrichment OR culture)
- Set 4 (identification of strains): TS = (strains OR species)

- Set 5 (environment): TS = (soil)
- Set 6 - (isolated or identified in title): TI = (isolat* OR identi*)

This resulted in a raw output of 365 studies which were further filtered manually. Any study that did not meet the following selection criteria was discarded, resulting in a total of 103 studies:

- Bacterial or fungal strains isolated from soil or sludge
- Study was investigating the microbial degradation or transformation of chlorinated POPs in culture.
- The POP of interest was chlorinated and listed in the Stockholm Convention.

The following considerations or adjustments to the data were made:

- If no temperature for incubation was provided, 21˚C was assumed.
- In case of PCB degradation assays that involved mixes of congeners, Cl and octanol/water coefficients of each congener was averaged.
- If the growth medium was stated to be mineral salt or minimal media, without mentioning any additional carbon source, it was assumed that POPs were the only carbon source.
- Observations relating to aldrin were not considered as aldrin readily transforms into dieldrin under environmental conditions hence is not relevant for this review
- The search was not exhaustive, and other POP degradation studies may exist, which were not included in this review. Only those search results were processes that came through from the filter key.

3.4.2 Compilation of chemical characteristics of POPs.

Some common intrinsic chemical properties of all relevant POPs were collated from different literature sources and averaged, which were then used to find associations of these properties with microbial transformation efficiencies. They included POP octanol/water coefficients (K_{ow}) and solubility in water (Sol_{H20} in mg L^{-1}) and vapour pressure in Pascale (PA) based on different methods but all at 25˚C. Chemical characteristics of multiple congeners of PCBs were averaged if they were part of a mixture that was degraded by one isolate. All relevant POPs are presented in Tables 2.3-1 and 2.3-2 accordingly (see Chapter 2).

3.4.3 Description of the data

In total 14 POPs were represented in the reviewed studies of which endosulfan, PCB, DDT, dieldrin, lindane and PCP appeared most often (Figure 3.4-1, right). Other chlorinated POPs, including alpha and beta hexachlorocyclohexane, aldrin, chlorodecone, hexachlorobutadiene, toxaphene and polychlorinated naphtalenes, were not included in this review either because they did not appear in the literature search or because the studies that appeared did not fit the selection criteria.

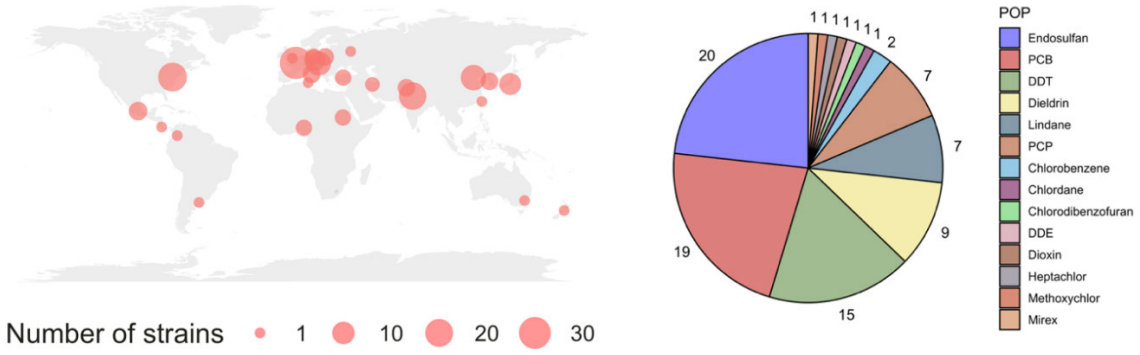


Figure 3.4-1. Left: Countries of origin of isolates and the number of different strains represented in this study. Bubbles sizes are proportional to number of isolated strains included in the review and point to capital cities of respective countries, which are not correct for source locations of isolates. Right: Pie chart showing the relative contribution and the total number of studies (outside of pie chart) per POP in this review. PCBs comprised of different congeners with varying degrees of chlorination.

Overall, 70 % of studies related to bacterial isolates and 30 % related to fungal strains, including those that were not isolated from soil or sludge. Of the 103 studies, a subset of 78 studies presented 160 strains (109 bacterial and 51 fungal strains) that were isolated from soils, sediments or sludge across 26 different countries with a total of 318 individual observations (efficiency values) of POP degradation/transformation (Figure 3.4-1, left). In addition, 25 studies were included that presented strains isolated from media other than soil/sediments with 43 individual observations of bacterial or fungal POP degradation/transformation in culture.

Taxonomic contributions of strains isolated from soils/sludge at the phylum level are presented in Table 3.4-1. These bacterial and fungal phyla were composed of 6 and 9 Classes, 16 and 12 Orders, 27 and 21 Families and finally 42 and 27 different genera respectively.

Table 3.4-1. Contributions (%) across all bacterial and fungal taxonomic groups of strains isolated from soils or sludges and were represented in 78 studies included in this review.

Bacteria	**Studies**	**%**	**Fungi**	**Studies**	**%**
Bacteria total	64	80	Fungi total	16	20
Gram stain			**Gram stain**		
negative	47	65			
positive[a]	22	35			
Phyla			**Phyla**		
Proteobacteria	45	50	Ascomycota	10	11
Actinobacteria	18	20	Mucoromycota	5	8
Firmicutes	7	8	Basidiomycota	3	3
Bacteroidota	1	1	Mortierellomycota	2	2
Class			**Class**		
Gammaproteobacteria	28	27	Sordariomycetes	9	9
Actinobacteria	18	17	Mucoromycetes	5	5
Betaproteobacteria	15	14	Eurotiomycetes	4	4
Alphaproteobacteria	10	10	Agaricomycetes	2	2
Bacilli	7	7	Mortierellomycetes	2	2
Bacteroidia	1	1	Dothideomycetes	1	1
			Leotiomycetes	1	1
			Microbotryomycetes	1	1
			Saccharomycetes	1	1

[a] Including one Gram stain variable genus; individual studies can involve multiple strains of different taxonomies

3.4.4 Statistical analysis

All data analyses were done in R (www.r-project.org). For simplicity, the term transformation efficiency (TE) was used broadly for this review although transformation products were not part of this review. Hence, transformation efficiency may also capture studies that included mineralisation of POPs.

As part of data pre-processing, 71 rows with observations of PCB (< 3 Cl substituents) transformation were removed because highly-chlorinated POPs were the focus of this review. The TE of PCB congeners with low chlorination (< 3 Cl substituents) were outliers and appeared to be part of a different population, hence it was assumed that microbial degradation processes for these compounds are different from highly-chlorinated compounds.

Raw TE in percent removal from culture per day (% day^{-1}) was non-normally distributed (Figure 3.4-2). Dividing raw TE by the number of chlorine substituents of POPS (% day^{-1} Cl^{-2}) resulted in a slight improvement towards normality of the data distribution. This transformation rate (% day^{-1} Cl^{-2}) represented the efficiency of microbial degradation in culture, independent of limitations due to chlorine toxicities. It was subsequently used for all comparisons and predictions of microbial degradation efficiencies.

$$\textbf{Transformation efficiency (TE)} = \frac{L_{total}}{d/c} \qquad \textbf{Equation 1}$$

Where L_{total} = Total percent loss of residues of the persistent organic pollutant at the end of incubation in culture, d = days of incubation, c = number of chlorine substituents of the POP. Finally, TE was standardized using Box-Cox transformations (TE_{bc}) (lambda 0.13) with the 'bestNormalize' package [138].

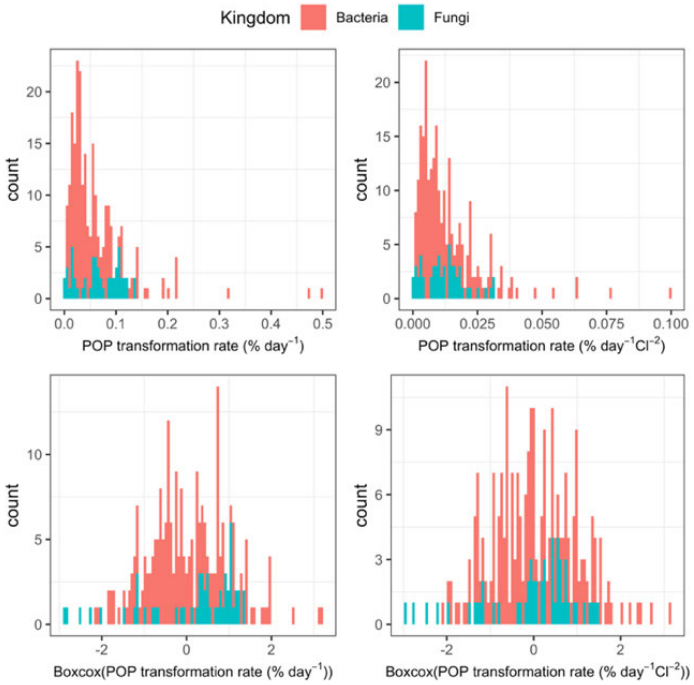


Figure 3.4-2. Distribution of dieldrin transformation efficiencies before and after Box-Cox transform (n = 247)

Principal component analysis (PCA) was chosen to represent the relationship of TE_{bc} with POP chemical characteristics such as degree of chlorination, K_{ow}, Sol_{H20} and vapour pressure. All variables were normalised with the 'bestNormalize' package and scaled.

Prior to further analysis, the bias of TE_{bc} caused by differences in intrinsic POP-degradability was removed by median centering the data within POP.

$$\textbf{TE}_{bc\_pop} = \textbf{TE}_{bc} - \textbf{median}(\textbf{TE}_{bc} \textbf{ within observations of each POP}) \qquad \textbf{Equation 2}$$

After within-POP centering, it was discovered that a declining trend of transformation rates with an increasing degree of chlorination remained. To remove the chlorination effect, TE_{bc_pop} was centered again within the degree of chlorination.

$$\textbf{TE}_{bc\_popcl} = \textbf{TE}_{bc\_pop} - \textbf{median}(\textbf{TE}_{bc\_pop} \textbf{ within observations of POPs with the same number of chlorine substituents}) \qquad \textbf{Equation 3}$$

Three different methods were chosen to assess whether any taxonomic groups were associated with improved TE_{bc_popcl}. The non-parametric Kruskal-Wallis and Wilcoxon signed-rank tests were performed to test if there was any significant variation in TE_{bc_popcl} among different bacteria or fungi at the class level. Finally, multinomial logistic regression was chosen to test if the continuous variable TE_{bc_popcl} was predictive for microorganisms on any taxonomic rank. Multinomial logistic regression was chosen because the outcome (taxonomic rank) involved more than two classes and because it tested the probability of a categorical membership based on given predictors [139]. In this case, different models were created where the outcome was set, such as Family or Genus as predicted by TE_{bc_popcl}. This was done to test if TE_{bc_popcl} was associated with a taxon at any taxonomic rank. Any taxon with < 4 observations were excluded from statistical comparisons. Wald z-tests were performed to test the significance of the regression coefficients. The output of multinomial logistic regression is presented in Tables S3.7-1 and S3.7-2.

3.5 RESULTS AND DISCUSSION

3.5.1 Role of POP characteristics in microbial transformation efficiencies

Median TE values for each POP are presented in Table 3.5-1. Just over half of all degradation studies (52 %) had another carbon source in the growth medium, showing evidence of co-metabolism of POPs. The remaining studies had analytical grade POPs as the only carbon sources for microbial growth, providing evidence of mineralisation of the respective POP. In most studies (71%, 71%, and 60% respectively) involving the degradation of PCPs, lindane and endosulfan, the bacterial strains were able to grow with these POPs as the only carbon source. However, for DDT and dieldrin only 29% and 10% of the studies (i.e. five and one study respectively) provided evidence for growth on these compounds.

As expected, the intrinsic chemical characteristics of POPs, such as Sol_{H20}, vapour pressure and the K_{ow} were closely related to each other. For example, from PCA it was apparent that a low Sol_{H20} was associated with a high K_{ow} and low vapour pressure (Figure 3.5-1). This was further shown as most of the variation was represented in the first two principal components, when these four POP characteristics (Sol_{H20}, vapour pressure, K_{ow}, and chlorination) were decomposed into components, together with bacterial/fungal degradation rates (73.1% and 94%, respectively) (Figure 3.5-1).

Table 3.5-1. Summary statistic of transformation efficiency (TE) of POPs from 69 studies with 247 transformation observations (n) with a minimum of 3 chlorine substituents (Cl). The percent of observations in which the persistent organic pollutant (POP) was the sole carbon source is shown.

TE (% day^{-1})	Cl	n	min	max	median	IQR	Sole carbon source (% of observations)
PCP	5	7	0.003	0.5	0.143	0.215	71
Methoxychlor	3	1	0.143	0.143	0.143	0	100
Heptachlor	7	1	0.067	0.067	0.067	0	0
PCB (all congeners)	3-7	142	0.004	0.216	0.053	0.055	35
Endosulfan	6	30	0.006	0.219	0.048	0.04	70
Lindane	6	12	0.005	0.158	0.041	0.097	42
DDE	4	1	0.038	0.038	0.038	0	0
Chlordane	8	1	0.036	0.036	0.036	0	0
Mirex	12	2	0.035	0.035	0.035	0	100
DDT	5	26	0.002	0.317	0.032	0.022	27
Chlorobenzene	6	5	0.007	0.09	0.022	0.027	0
Dieldrin	6	19	0.002	0.086	0.020	0.013	11

IQR, Interquartile range

When all observations, which involved various classes of POPs with > 3 Cl substituents, were included into PCA, the number of chlorine substituents was inversely related to bacterial/fungal transformation rates (Figure 3.5-1a). This indicated that highly chlorinated POPs such as mirex (Cl = 12), chlordane (Cl = 8) and dieldrin (Cl = 6) were transformed at lower rates by bacteria and fungi in culture, perhaps in part due to their high degree of chlorination (Table 3.5-1). Indeed, the median efficiency of POP transformation per chlorine substituent significantly decreased with increasing chlorine numbers in the compound (Figure 3.5-2, top left). As the transformation rates were already median centered to the number of chlorine substituents, a declining trend with increasing chlorination may be explained by another limiting property associated with chlorination. A one-way ANOVA further showed that the number of chlorine substituents (as factor) significantly affected TE_{bc} ($df = 4, 239$, $F = 4.5$, $R^2_{adj} = 0.06$, $p = 0.002$).

However, DDT and dieldrin were both transformed significantly less than the remaining POP compounds and were driving the declining trend of transformation rates (Figure 3.5-2). Hence, there appeared to be other intrinsic properties of POPs that influenced the degradation rate independently of their degree of chlorination. When only PCB congeners were included in PCA, the degree of chlorination was only slightly negatively associated with the transformation rate (Figure 3.5-1b), indicating that other factors than chlorination influenced that degradation rates. Moreover, there were significant differences in degradation rates between POPs that contained the same number of chlorine substituents (Figure 3.5-2, bottom). For example, dieldrin was degraded at significantly lower rates compared to PCB congeners and endosulfan, which were all composed of six chlorine substituents. Hence, POP chlorination alone did not sufficiently explain the different bacterial/fungal transformation rates.

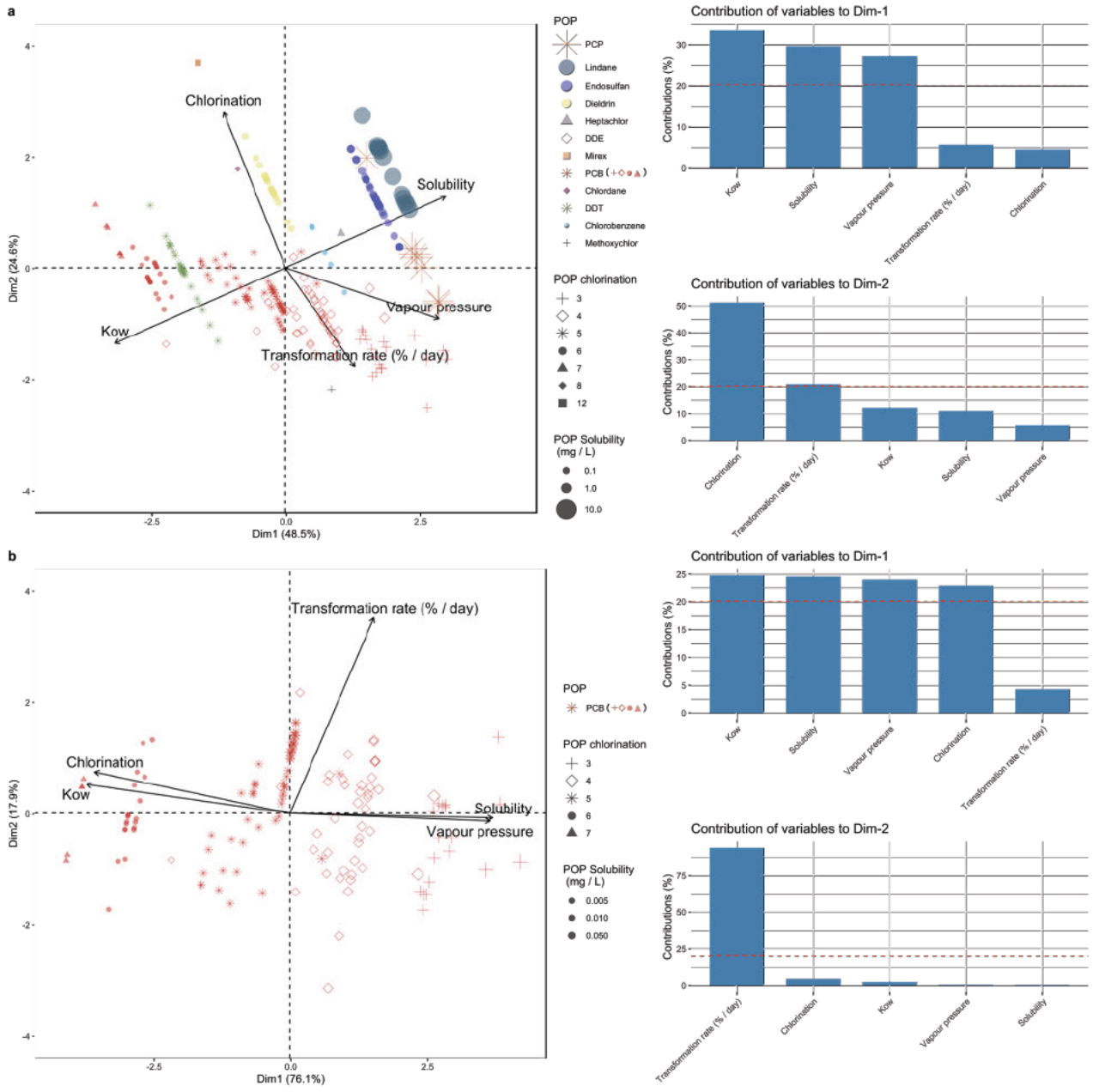


Figure 3.5-1. Variation of mean rates of POP transformation (% day^{-1}) (TE_{bc}) by bacterial/fungal soil-isolates is represented in two components of principal component analysis (PCA) together with the number of chlorine substituents in the POP compounds, POP solubility in mg L^{-1}, POP vapour pressure in Pascal (PA) and the POP octanol/water coefficient (K_{ow}). All variables are normalised (Box-Cox or Yeo–Johnson transformation) and scaled. Contributions of variables to variation is shown on the right-hand side, whereby the red dashed line indicates the expected average contribution. Any variable contributing to variation above this line is considered important. **(a)** Data includes twelve POPs with 247 observations. Their degree of chlorination and solubility is indicated by the shape and size of the symbol, respectively. Five different congeners of polychlorinated biphenyls (PCBs) with three to seven chlorine substituents are included. **(b)** A separate PCA includes a subset of 142 observations of PCB degradation efficiency only.

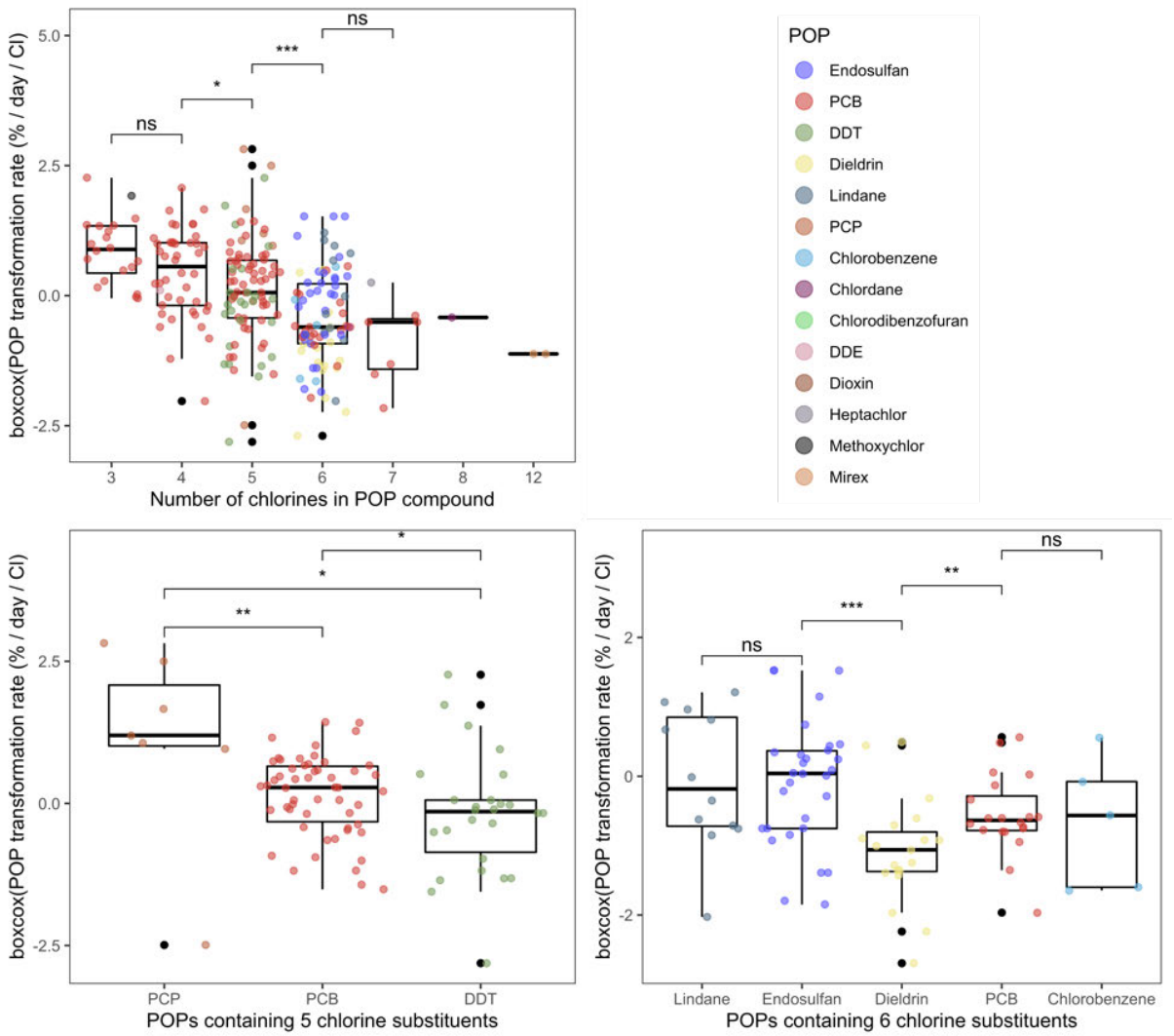


Figure 3.5-2. Comparisons of POP transformation rates (TE_{bc}) between compounds with different degrees of chlorination (top left) and compounds that contain the same number of chlorine substituents (bottom). Vertical bars of the boxplots show the maximum (top edge) and minimum (lower edge) percentiles, and boxes represent the 25th to 75th percentiles. The median is represented by the horizontal line in the boxplot. Wilcoxon signed-rank tests are shown with ns, *, ** and *** indicating not significant, significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Sol_{H20} and vapour pressure of POPs were also related to the degradation efficiency in culture. The PCA indicated that the variation of these intrinsic chemical properties was correlated with POP transformation rates (Figure 3.5-1a). To validate this, two separate ordinary linear regressions with either the POP Sol_{H20} or vapour pressure as predictor for TE_{bc} were created. The results showed that both properties significantly increased TE_{bc} (F=7.5 on 1 and 239 DF, $p = 0.007$ and F=16.5 on 1 and 239 DF, $p < 0.0001$ respectively). However, these two properties, Sol_{H20} and vapour pressure, relate to each other; hence, their effect on TE_{bc} may have been confounded.

High solubilities of POPs indicate their high availability in solution which increases the chances of their uptake by microbial cells into the cytosol, where metabolic transformations occur. It is therefore critical for

the successful isolation of strains and their growth with POPs as sole carbon source in media. The analysis showed that, PCP, the most water-soluble among the POPs in this review, was transformed at the fastest median rates in culture compared to all other POPs (Figure 3.5-2, Table 3.5-1). Furthermore, all publications used in this review that studied PCP-degradation evidenced microbial growth with PCP as a sole carbon source, indicating that it was readily available for microbial metabolism in liquid media.

Vapour pressure of PCP and other POPs were also positively associated with transformation rates. This indicated that variability in methods for degradation assays might lead to variable results, in part via unaccounted residue losses through volatilisation. For example, overestimating POP-transformation rates was likely if researchers monitored POP removal from culture in Erlenmeyer flasks on a shaker without a non-stick stopper and without evaluating non-inoculated controls. Furthermore, different authors used different shaking speeds, temperatures and periods of incubation, all of which may have affected the final TE.

3.5.2 No associations of microbial phenotypes with transformation efficiencies

Despite the apparent associations of chlorination, $Sol_{H20,}$ K_{ow} and vapour pressure with the transformation efficiencies of POPs in the studies, a part of the variation in transformation rates remained unexplained. For example, the majority of the 17.9% variation on the second principal component of PCA in Figure 3.5-1b, which represented transformation rates of PCB congeners, was mostly independent, as indicated by an almost perpendicular arrow relative to the arrows of POP characteristics. Hence, the gradient of change in POP transformation rate may have been determined in part by different microbial phenotypes and their increasing metabolic capabilities. If true, then phenotypical differences of taxonomic groups may have enabled certain taxa to be more efficient in transforming chlorinated POPs. Perhaps, they can gain more energy from chlorinated hydrocarbons and grow faster. In that case, the chance of occurrence of specific taxonomic groups/phenotypes would be higher in observations with above-median transformation efficiencies. Or particular taxa may have evolved a POP-specific enzyme; thus, the occurrence of certain taxonomic groups/phenotypes may be associated with certain POP compounds, such as endosulfan or lindane due to microbial fitness for specific compounds.

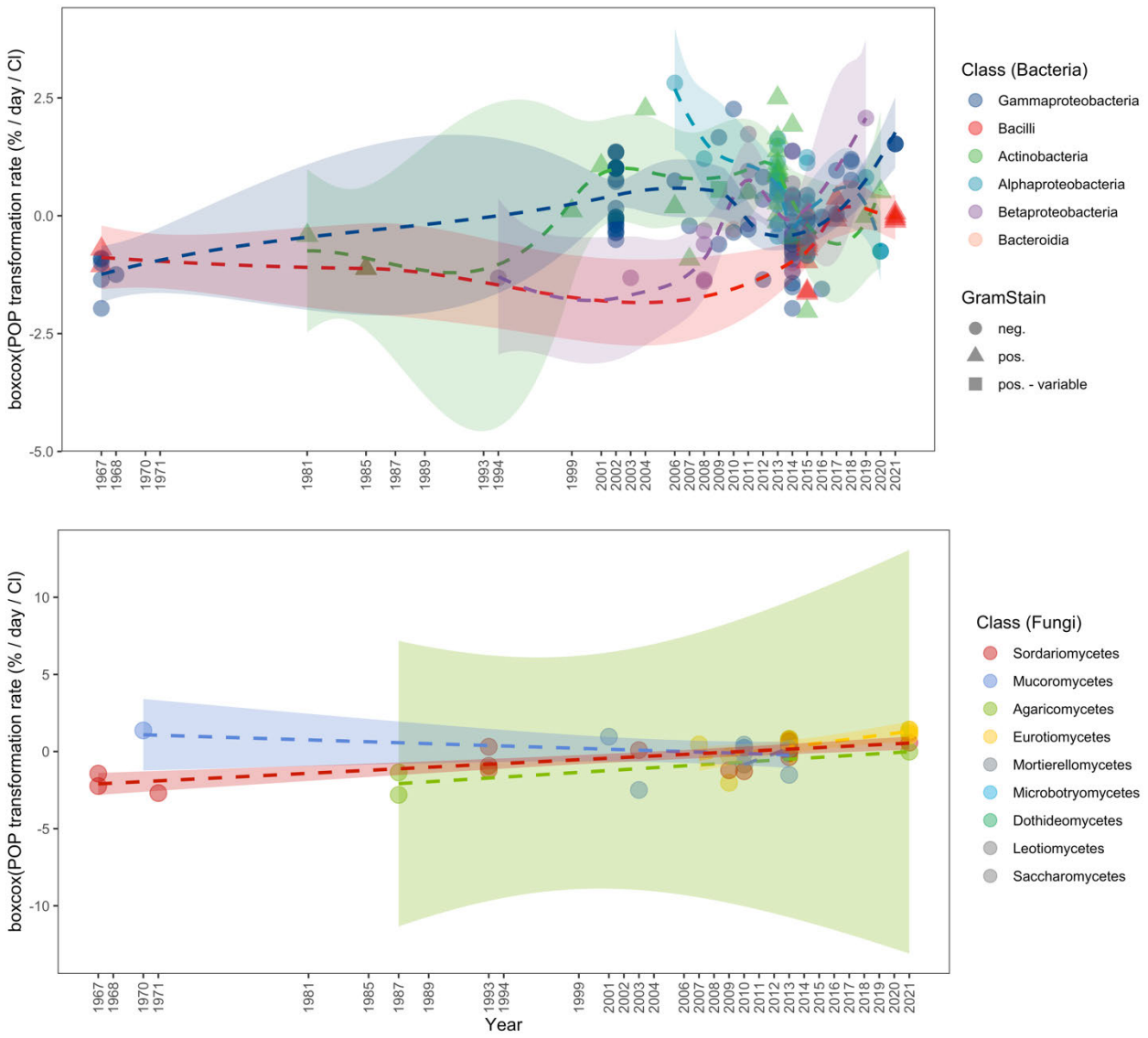


Figure 3.5-3. Overview of Box-Cox normalised POP transformation efficiencies of bacteria and fungi that were isolated from soils in 26 countries. Observations involving compounds with at least three chlorine substituents are shown. Dashed lines indicate the trend over time (polynomial and linear regression for bacteria and fungi, respectively), and the shaded area represents 95% confidence intervals.

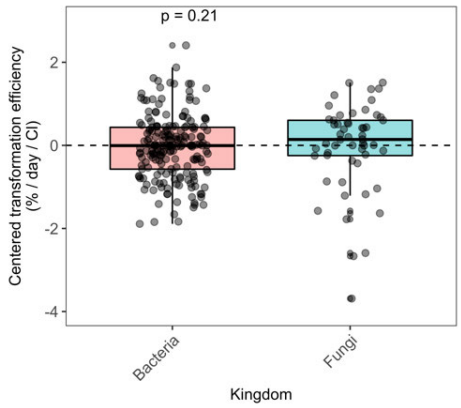


Figure 3.5-4. Centered transformation efficiencies (TE_{bc_popcl}) of bacteria and fungi across 69 studies that provided evidence of transformation of POPs with at least three chlorine substituents (247 observations). Significance of a Wilcoxon signed-rank tests between bacteria and fungi is shown.

However, after median centering the TE_{bc} to remove potential biases due to POP properties (TE_{bc_popcl}), there was no evidence that any taxonomic ranks were associated with TE_{bc_popcl}. In total, the microbial isolates belonged to four bacterial and four fungal phyla, which were isolated across all 78 studies around the globe. Since 1967, when Matsumura and Boush (1967) published results of the first successful isolation of dieldrin transforming bacteria and fungi from soil [101], no classes of bacteria or fungi have emerged that are more efficient in transforming POPs (Figure 3.5-3). The frequency of studies has increased markedly since the year 2000, likely due to an increasing interest in the development of remediation strategies for polluted soils. This increasing interest was reflected by international action of the Governing Council of the United Nations Environment Programme (UNEP), which brought the Stockholm Convention into force in 2004.

Furthermore, there was no difference in TE_{bc_popcl} between bacteria and fungi (Figure 3.5-4), nor between different classes of bacteria or fungi (Figure 3.5-5), nor between any other rank of bacteria or fungi (Multinomial regression; Tables S3.7-1 and S3.7-2). Multinomial logistic regression and Kruskal tests all confirm the null hypothesis that there is no effect of taxonomic groups on TE_{bc_popcl}.

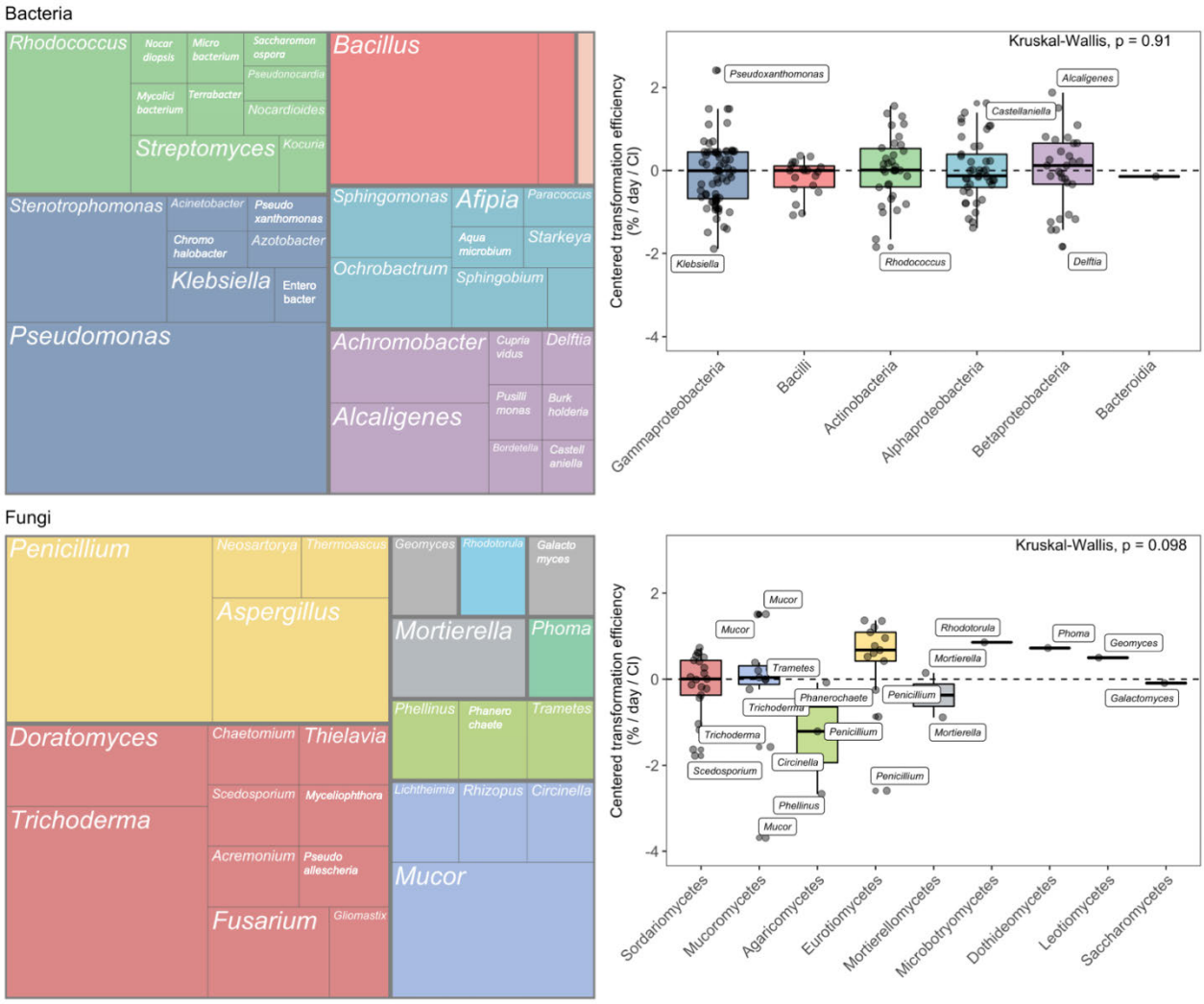


Figure 3.5-5. Treemaps showing the number of genera of soil-isolates from 69 studies that provided evidence of transformation of POPs with at least three chlorine substituents (247 observations). Colours show taxonomic classes within which genus names (white text) are shown (bacteria top and fungi bottom). Sizes of the colored rectangles are proportional to the number of different strains that were isolated. Boxplots show transformation efficiencies (TE_{bc_popcl}) between bacterial and fungal classes involving POPs with a minimum of three chlorine substituents. To remove any POP-specific effects on transformation rates, they were median-centered within POP compounds and within degree of chlorination. The dashed line shows the median transformation efficiency. Genus names of some observations are shown.

3.5.3 Potential factors for selection of phylotypes during isolation of strains from mixed communities

Only a narrow range of taxa occurred overall. All strains isolated from soils/sludges across 78 studies belonged to only four bacterial phyla and four fungal phyla (Table 3.4-1). According to the global soil biodiversity atlas, there are 30 known and recognised bacterial phyla of bacteria [22]; hence, it appeared that only specific phyla were selected when bacteria during isolation of individual strains from a mixed soil community with media that contained POPs as a carbon source. This may imply that these taxa have phylotypes that are associated with POP degradation. On the other hand, it is possible that the chance to 'catch' or isolate a bacterial phylum was related to their prevalence in soils. It is known that only few microbial phyla dominate in soils globally [140,141]. For example, as Proteobacteria are commonly the most dominant bacterium in soils, then the chances of Proteobacteria being isolated may increase.

Indeed, Proteobacteria was the most dominant phylum across all studies in this review. Among all bacterial strains in the reviewed literature, 66 % belonged to the phylum Proteobacteria showing evidence of degradation of POPs with at least three chlorine substituents. This included the classes Gammaproteobacteria (36%), Betaproteobacteria (16%) and Alphaproteobacteria (14%), which are all represented in a tree map in Figure 3.5-5. The next dominant class in this review was Actinobacteria at 20% of all bacterial strains. In that order of dominance, these two phyla were found to be the two most abundant phyla in soils across multiple continents, hence the chance of isolating Proteobacteria or Actinobacteria would be high [140].

Ascomycota was the dominant fungal phylum of strains that were isolated from soils in the reviewed studies. 71% of all fungal strains that showed evidence of degradation of POPs were Ascomycota, mainly members of the Sordariomycetes (39%) and Eurotiomycetes (27%). Again, this phylum and its classes were found to dominate soils globally [141], hence the chance to isolate fungi that belong to these taxa would be high. Broadly, these observations point to a random selection process during isolation of strains, instead of a selection of certain traits from the mixed microbial soil community.

Perhaps, within those broad taxa (i.e. phyla and classes), subsets of genera or species with such traits increase degradation efficiencies. To explore this idea, Tables 3.5-2 and 3.5-3 show the highest and lowest TEbc_popcl of individual strains including their taxonomic classifications. Most of the bacterial and fungal phyla that contained strains with the highest TEbc_popcl were also producing strains with the lowest TEbc_popcl. Additionally, a similar proportion of genera were unique with either the highest or the lowest TEbc_popcl. In other words, we found no organisms that belonged to a taxon that was associated with increased efficiencies to degrade POPs.

Table 3.5-2. Bacterial isolates that showed relatively high or low (upper or lower quartile) transformation efficiencies (TE_{bc_popcl}) in culture. Strains with TE_{bc_popcl} within the interquartile range are excluded. Bacterial orders shown in bold are unique in the groups of either low or high TE_{bc_popcl}. Genera are marked orange if they are unique in either low or high TE_{bc_popcl}.

Class	Order	Family	Genus/Species	Strain	TE_{bc_popcl} (Quartile)	POP	POP as sole C source	Reference
Actinobacteria	**Actinomycetales**	**Micrococcaceae**	***Kocuria* sp.**	CL2	Upper	PCP	Yes	[142]
Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus sp.*	R04	Upper	PCB	No	[143]
Actinobacteria	**Propionibacteriales**	**Nocardioidaceae**	***Nocardioides* sp.**	PD653	Upper	Hexachlorobenzene	No	[144]
Actinobacteria	Pseudonocardiales	Pseudonocardiaceae	***Pseudonocardia* sp.**	KSF27	Upper	Dieldrin	No	[85]
Actinobacteria	Streptomycetales	Streptomycetaceae	*Streptomyces sp.*	885	Upper	DDT	No	[145]
Alphaproteobacteria	**Rhodothermales**	**Rhodobacteraceae**	***Paracoccus* sp.**	NITDBR1	Upper	Lindane	Yes	[146]
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingobium japonicum*	SS86	Upper	Lindane	No	[147]
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas chlorophenolica*	NA	Upper	PCP	Yes	[148]
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas sp.*	NM05	Upper	Lindane	Yes	[149]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Achromobacter sp.*	H11	Upper	PCB	Yes	[150]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes sp.*	DG-5	Upper	DDT	Yes	[116]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	***Castellaniella* sp.**	SPC4	Upper	PCB 77	Yes	[151]
Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	***Enterobacter* sp.**	EN-2	Upper	Endosulfan	Yes	[152]
Gammaproteobacteria	**Oceanospirillales**	**Halomonadaceae**	***Chromohalobacter* sp.**	LD2	Upper	Lindane	Yes	[153]
Gammaproteobacteria	Pseudomonadales	Moraxellaceae	***Acinetobacter* sp.**	ISTPCP-3	Upper	PCP	Yes	[154]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas sp.*	KS-2P	Upper	Endosulfan	Yes	[155]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas sp.*	TAH	Upper	Endosulfan	Yes	[156]
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Pseudoxanthomonas sp.*	wax	Upper	DDT	No	[157]
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas maltophilia*	EN-1	Upper	Endosulfan	Yes	[156]
Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas maltophilia*	EN1	Upper	Endosulfan	Yes	[152]

Class	Order	Family	Genus/Species	Strain	TE_{bc_popcl} (Quartile)	POP	POP as sole C source	Reference
Actinobacteria	Corynebacteriales	Mycobacteriaceae	***Mycolicibacterium*** *frederiksbergense*	IN53	Lower	PCB	No	[158]
Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus erythropolis*	E5	Lower	Endosulfan	Yes	[159]
Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus erythropolis*	H23	Lower	Lindane	No	[160]
Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	***Methylobacterium*** *rhodesianum*	NA	Lower	Endosulfan	Yes	[161]
Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	***Afipia*** *genosp*	NA	Lower	Endosulfan	Yes	[161]
Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas yanoikuyae*	Q1	Lower	Endosulfan	Yes	[161]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes eutrophus*	A5	Lower	DDT	No	[162]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes sp.*	BMD-4	Lower	DDT	No	[163]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	***Bordetella*** *petrii*	NS	Lower	Endosulfan	No	[164]
Betaproteobacteria	Burkholderiales	Alcaligenaceae	***Pusillimonas*** *sp.*	JW2	Lower	Endosulfan	No	[164]
Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	***Delftia*** *lacustris*	IITISM30	Lower	Endosulfan	No	[165]
Gammaproteobacteria	Enterobacterales	Enterobacteriaceae	*Klebsiella aerogenes*	IITISM42	Lower	Endosulfan	No	[165]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas aeruginosa*	SKL-1	Lower	Endosulfan	No	[166]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas sp.*	94	Lower	Dieldrin	No	[101]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas sp.*	NE6	Lower	Lindane	No	[160]
Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas stutzeri*	NA	Lower	PCB	Yes	[167]

Table 3.5-3. Fungal isolates that showed relatively high or low (upper or lower quartile) transformation efficiencies (TEbc_popcl) in culture. Strains with TEbc_popcl within the interquartile range are excluded. Fungal orders shown in bold are unique in the groups of either low or high TEbc_popcl. Genera are marked orange if they are unique in either low or high TEbc_popcl.

Class	Order	Family	Genus/Species	Strain	TE_{bc_popcl} (Quartile)	POP	POP as sole C source	Reference
Dothideomycetes	**Pleosporales**	**Didymellaceae**	***Phoma*** *eupyrena*	S2-26	Upper	PCB	No	[168]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium canescens*	NA	Upper	PCB	No	[169]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium citreosulfuratum*	NA	Upper	PCB	No	[169]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus fumigatus*	S2-9	Upper	PCB	No	[168]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium canescens*	NA	Upper	PCB	No	[169]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium chrysogenum*	NA	Upper	PCB	No	[169]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus jensenii*	NA	Upper	PCB	No	[169]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Aspergillus ochraceus*	NA	Upper	PCB	No	[169]
Microbotryomycetes	**Sporidiobolales**	**Sporidiobolaceae**	***Rhodotorula*** *sp.*	VITJzN03	Upper	Lindane	Yes	[170]
Mucoromycetes	Mucorales	Mucoraceae	*Mucor alternans*	NA	Upper	DDT	No	[104]
Sordariomycetes	Microascales	Microascaceae	***Doratomyces*** *purpureofuscus*	S2-32	Upper	PCB	No	[168]
Sordariomycetes	Microascales	Microascaceae	*Doratomyces verrucisporus*	S2-32	Upper	PCB	No	[168]
Sordariomycetes	Microascales	Microascaceae	*Doratomyces nanus*	S2-32	Upper	PCB	No	[168]
Sordariomycetes	Sordariales	Chaetomiaceae	***Myceliophthora*** *thermophila*	S2-1	Upper	PCB	No	[168]
Agaricomycetes	**Hymenochaetales**	**Hymenochaetaceae**	***Phellinus*** *weirii*	NA	Lower	DDT	No	[171]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium chrysogenum*	MUT 4021	Lower	PCB	No	[172]
Eurotiomycetes	Eurotiales	Aspergillaceae	*Penicillium digitatum*	MUT 4079	Lower	PCB	No	[172]
Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella sp.*	W8	Lower	Endosulfan	No	[173]
Mucoromycetes	Mucorales	Mucoraceae	*Mucor racemosus*	IM6203	Lower	PCP	No	[174]
Mucoromycetes	Mucorales	Rhizopodaceae	*Rhizopus nigricans*	NA	Lower	PCP	No	[175]
Mucoromycetes	Mucorales	Syncephalastraceae	***Circinella*** *muscae*	S2-10	Lower	PCB	No	[168]
Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma viride*	41	Lower	Dieldrin	No	[101]
Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma viride*	12	Lower	Dieldrin	No	[101]
Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma harzianum*	S2-19	Lower	PCB	No	[168]
Sordariomycetes	Hypocreales	Hypocreaceae	*Trichoderma koningii*	NA	Lower	Dieldrin	No	[105]
Sordariomycetes	Microascales	Microascaceae	***Scedosporium*** *apiospermum*	MUT 641	Lower	PCB	No	[172]

Another consideration is the bacterial cell physiology. Among the bacteria isolated in all the studies, 65% of strains were gram negative. Contaminated soils are often associated with gram-negative bacteria, which is believed to be related to their cell-membrane physiology [112]. However, the data provided no evidence that gram-negative bacteria were more efficient at transforming POPs in culture. Proteobacteria comprises the majority of all gram-negative bacteria [22] and there was no significant difference in their combined TE_{bc_popcl} in this review (Figure 3.5-5). However, it may be possible that gram-negative bacteria are more competitive in culture because they can resist overall POP toxicities better than gram-positive bacteria. Hence, that may be the reason why they are over-represented in the isolation studies. On the other hand, as Proteobacteria are commonly the dominant phyla in soils (and comprise the majority of gram-negative bacteria), the data in this review do not provide enough evidence to suggest that cell physiology is associated with POP degradation.

3.6 IMPLICATIONS AND RECOMMENDATIONS FOR RESEARCH

Most of the differences in TE was explained by intrinsic properties of POPs and likely by variation in methodologies of the degradation assays across all studies. There was no evidence that any microbial phylotypes were associated with improved degradation of POPs. Furthermore, the occurrences of microbial isolates across the globe could be a random subset of soil microorganisms. This implies that no taxonomic groups contain potential candidates for predicting a pollutant degradation potential in soils. This does not exclude the possibility that certain strains have evolved to express enzymes that catalyse the transformation of a particular POP. On the contrary, that is what happened for lindane as an example [111]. However, this evolution may be a stochastic and convergent process involving various bacterial taxa. It further indicates that taxonomic biomarkers are not suitable for assessing a soil's capability to degrade POPs.

However, the results indicate that there is potentially a larger uncultured pool of organisms that can degrade POPs. The degradation efficiency of microorganisms is not determined by a genetic pre-disposition for degradation efficiency. Given the chance, any type of bacteria may have the genetic potential to develop the needed mechanisms to degrade POPs. As discovered previously oxidative dechlorination is ubiquitous in soils and may have evolved with naturally occurring chlorinated organic matter [19,20]. Hence, phylogenetic potentials for pollutant degradation may be ubiquitous and are not the main limitation for POP degradation.

Hence, research aiming to improve efficiencies of bioremediation of POPs in soils needs to shift its focus on how this untapped genetic potential can be utilised. Instead of isolating ever more new strains from soils with the ability to degrade POPs it may be more beneficial to understand how organic matter quality, soil management and soil organic amendments alter the genetic potential for improved POP co-metabolism or mineralisation.

3.7 SUPPLEMENTARY

Multinomial logistic regression output

Table S3.7-1. Model output of multinomial logistic regression with transformation efficiency (TE) as predictor for placement into bacterial genera.

	Bacterial Genus				
Predictors	*Odds ratios*	*Confidence interval*	*p*	*df*	*Response*
(Intercept)	0.85	0.35 – 2.07	0.717	137	Alcaligenes
Transformation efficiency	2.02	0.54 – 7.52	0.294	137	Alcaligenes
(Intercept)	1.13	0.50 – 2.57	0.775	137	Aquamicrobium
Transformation efficiency	1.84	0.54 – 6.29	0.332	137	Aquamicrobium
(Intercept)	1.34	0.61 – 2.94	0.469	137	Bacillus
Transformation efficiency	0.82	0.25 – 2.69	0.746	137	Bacillus
(Intercept)	0.33	0.10 – 1.12	0.076	137	Lysinibacillus
Transformation efficiency	0.56	0.10 – 3.24	0.521	137	Lysinibacillus
(Intercept)	0.89	0.37 – 2.12	0.796	137	Ochrobactrum
Transformation efficiency	0.82	0.22 – 3.03	0.769	137	Ochrobactrum
(Intercept)	4.38	2.27 – 8.48	**<0.001**	137	Pseudomonas
Transformation efficiency	0.74	0.27 – 2.00	0.553	137	Pseudomonas
(Intercept)	1.92	0.92 – 3.98	0.081	137	Rhodococcus
Transformation efficiency	1.13	0.37 – 3.44	0.824	137	Rhodococcus
(Intercept)	0.58	0.21 – 1.58	0.288	137	Sphingobium
Transformation efficiency	0.58	0.14 – 2.48	0.465	137	Sphingobium
(Intercept)	0.45	0.15 – 1.31	0.142	137	Starkeya
Transformation efficiency	0.87	0.17 – 4.34	0.864	137	Starkeya
(Intercept)	1	0.43 – 2.31	0.999	137	Stenotrophomonas
Transformation efficiency	1.3	0.36 – 4.65	0.685	137	Stenotrophomonas
Observations	157				
R^2 Nagelkerke	0.052				
Akaike information criterion	700.401				

Table S3.7-2. Model output of multinomial logistic regression with transformation efficiency (TE) as predictor for placement into fungal genera.

	Fungal Genus				
Predictors	*Odds ratios*	*Confidence interval*	*p*	*df*	*Response*
(Intercept)	3.64	0.62 – 21.31	0.152	22	Mucor
Transformation efficiency	0.29	0.05 – 1.66	0.164	22	Mucor
(Intercept)	3.17	0.53 – 18.96	0.206	22	Penicillium
Transformation efficiency	0.32	0.05 – 1.87	0.205	22	Penicillium
(Intercept)	3.84	0.66 – 22.39	0.135	22	Trichoderma
Transformation efficiency	0.22	0.04 – 1.26	0.088	22	Trichoderma
Observations	28				
R^2 Nagelkerke	0.156				
Akaike information criterion	83.049				

Table S3. Model output of multinomial logistic regression with transformation efficiency (TE) as predictor for placement into bacterial families.

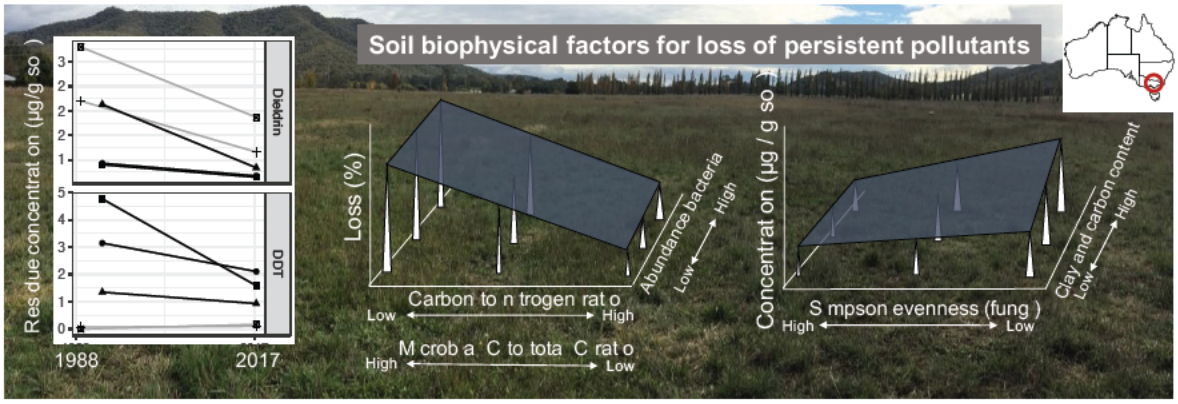
	Bacterial Family				
Predictors	*Odds ratios*	*Confidence interval*	*p*	*df*	*Response*
(Intercept)	0.61	0.32 – 1.18	0.142	153	Bacillaceae
Transformation efficiency	0.73	0.29 – 1.81	0.494	153	Bacillaceae
(Intercept)	0.41	0.19 – 0.86	0.019	153	Brucellaceae
Transformation efficiency	0.73	0.26 – 2.07	0.55	153	Brucellaceae
(Intercept)	0.16	0.06 – 0.48	0.001	153	Burkholderiaceae
Transformation efficiency	1.25	0.30 – 5.31	0.758	153	Burkholderiaceae
(Intercept)	0.92	0.51 – 1.64	0.769	153	Nocardiaceae
Transformation efficiency	0.96	0.43 – 2.14	0.916	153	Nocardiaceae
(Intercept)	0.52	0.26 – 1.04	0.064	153	Phyllobacteriaceae
Transformation efficiency	1.42	0.56 – 3.55	0.46	153	Phyllobacteriaceae
(Intercept)	0.15	0.05 – 0.48	0.001	153	Planococcaceae
Transformation efficiency	0.52	0.11 – 2.42	0.405	153	Planococcaceae
(Intercept)	2.05	1.26 – 3.35	0.004	153	Pseudomonadaceae
Transformation efficiency	0.66	0.33 – 1.31	0.233	153	Pseudomonadaceae
(Intercept)	0.42	0.20 – 0.87	0.02	153	Sphingomonadaceae
Transformation efficiency	1	0.36 – 2.77	0.993	153	Sphingomonadaceae
(Intercept)	0.74	0.40 – 1.37	0.337	153	Xanthomonadaceae
Transformation efficiency	1.22	0.53 – 2.82	0.647	153	Xanthomonadaceae
(Intercept)	1	0.32 – 1.18	0.142	153	Bacillaceae
Transformation efficiency	1.3	0.29 – 1.81	0.494	153	Bacillaceae
Observations	171				
R^2 Nagelkerke	0.036				
Akaike information criterion	732.406				

Table S4. Model output of multinomial logistic regression with transformation efficiency (TE) as predictor for placement into fungal families.

	Fungal Family				
Predictors	*Odds ratios*	*Confidence interval*	*p*	*df*	*Response*
(Intercept)	0.83	0.32 – 2.11	0.689	27	Hypocreaceae
Transformation efficiency	0.51	0.21 – 1.25	0.142	27	Hypocreaceae
(Intercept)	0.49	0.16 – 1.45	0.196	27	Microascaceae
Transformation efficiency	0.7	0.24 – 2.02	0.512	27	Microascaceae
(Intercept)	0.78	0.30 – 2.01	0.608	27	Mucoraceae
Transformation efficiency	0.68	0.27 – 1.72	0.412	27	Mucoraceae
Observations	33				
R^2 Nagelkerke	0.08				
Akaike information criterion	98.546				

Chapter 4: Composition of soil organic matter drives total loss of dieldrin and dichlorodiphenyltrichloroethane in high-value pastures over thirty years

This chapter has been published in *Science of the Total Environment*, 2019, 691, 135-145
https://doi.org/10.1016/j.scitotenv.2019.06.497



4.1 HIGHLIGHTS

- We surveyed 12 paddocks to understand degradation of insecticide residues 30 years after use.
- Loss of dieldrin and DDT in soil differed between the paddocks.
- High soil organic matter limits microbial biodegradation of dieldrin and DDT.
- Dieldrin loss correlated positively with microbial biomass but negatively with soil C/N ratio.

4.2 ABSTRACT

The residues of dieldrin and dichlorodiphenyltrichloroethane (DDT), internationally banned agricultural insecticides, continue to exceed government guidelines in some surface soils 30 years after use. Little is known regarding the soil factors and microbial community dynamics associated with the *in-situ* biodegradation of these organochlorine chemicals. We hypothesised that soil organic matter, a key factor affecting microbial biomass and diversity, affects the biodegradation and total loss of the pollutants 30 years

after use. We sampled 12 contaminated paddocks with residue concentrations monitoring data since 1988 that represent two different agricultural surface-soils. The total loss and current concentrations of the residues was correlated with soil physicochemical properties, microbial biomass carbon, microbial community diversity indices and microbial community abundance. Current dieldrin and DDT residue concentrations were positively correlated with soil organic matter and clay contents. However, key indicators for loss of residues after 23 - 30 years were low carbon-to-nitrogen ratios, high microbial-C-to-total-C ratios and high fungal community evenness. The results support the composition of soil organic matter as an important factor affecting degradation of organochlorines and that co-metabolism of dieldrin and DDT could be enhanced by manipulating the composition of soil organic matter to cater for a broad diversity of microbial function.

4.3 INTRODUCTION

Production of organochlorine insecticides such as dieldrin, dichlorodiphenyltrichloroethane (DDT), lindane, chlordane, aldrin and others peaked in the 1950s and 1960s. Hundreds of thousands of tonnes were annually produced for crop protection and the control of diseases such as malaria, typhus fever and sleeping sickness [3,176,177]. As early as the 1960s, it became clear that these organochlorine chemicals had entered and bioaccumulated within the food chains of many ecosystems with detrimental side effects [3,178]. In Victoria, Australia, dieldrin and DDT were extensively used to control a range of different crop insect pests for tobacco, potato, grains, pasture and fruits, and livestock parasites until their phasing out began in the 1960s [5].

Dieldrin and DDT remained in limited use in Victoria until being completely prohibited from all agricultural uses in 1986 (DNRE, 1996). Dieldrin and DDT were amongst the first 12 persistent organic pollutants (POPs) internationally banned through a United Nations treaty effective since 2004 [179]. Dieldrin and DDT persist in soils and continue to exceed national guidelines in rural Victoria even after 30 years since their last use. This contamination limits land use options of high-value grazing pastures due to the accumulation of POPs in cattle [180].

Organic chemical contaminants in soils undergo transport, retention and transformation processes which are mostly mediated by soil microorganisms [181]. It is notoriously difficult to predict these processes for a given site due to 'irreducible' uncertainties or 'overcomplexities' of ecosystems (Scheringer, 2002). Microorganisms are affected by factors such as climate, plant growth and soil properties which all impact degradation [24]. While modifying environmental factors that control microbial growth and kinetics is a strategy for *in-situ* soil remediation of POPs [182,183], further understanding of these relationships is required.

Biodegradation rates are slower when POPs are adsorbed by the soil matrix [184,185] with the rate of desorption from soil organic matter (SOM) limiting for degradation [70,186]. Dieldrin and DDT are fat-soluble and easily sorbed by hydrophobic organic matter [71,187,188]. Therefore, the quantity and composition of SOM may play an important role in degradation processes of POPs through adsorption,

desorption and their affected on microbial diversity dynamics [186]. This relationship needs further elucidation to understand POP degradation in agriculture soils.

We had unique access to the historical data of dieldrin and DDT residues of contaminated agriculture soils dating back to 1988. To determine if biotic and/or abiotic factors of the soil environment influenced *in-situ* degradation of dieldrin or DDT over an extended time span, we sampled surface-soils of two contaminated agricultural locations with recorded residue concentrations since 1988. The total loss of residues and current residue concentrations were modelled against soil physicochemical measurements, microbial biomass carbon, diversity indices and total gene copy numbers. We hypothesised that SOM would be a key factor affecting microbial biomass and diversity and hence affect biodegradation and total loss of the pollutants after 30 years.

4.4 MATERIALS AND METHODS

4.4.1 Site description and soil sampling

The surface soils (0-10 cm) of twelve paddocks with known contamination histories were sampled. All paddocks were converted from tobacco or potato production into pastures containing perennial and annual grasses and clovers that were grazed by cattle and sheep (for short periods at a time) or cut for hay. They were managed by five different farms and three of the sampled farms (A-C) were located in the northeast (Edi and Edi Upper in the King Valley) and two farms (D-E) southeast (Cockatoo near Gembrook) of Victoria, Australia (Table S4.8-1). Both locations had loamy topsoils based on texture classification of the International Society of Soil Science [189]. Furthermore, both locations were in high-rainfall zones with annual mean precipitation of 695 - 1060 mm. The annual maximum temperature in the northeast was 1 degree higher at 20.9 ˚C compared to the location in the southeast based on data of the closest available weather station of the Bureau of Meteorology (http://www.bom.gov.au/climate/data/, accessed 15.01.2018). Soil profiles of the soil in the northeast was classified as a Kurosol [190] which was equivalent to an Acrisol [191] and in the southeast as a Chromosol [190] which was similar to a Luvisol [191] (Table S4.8-1).

Residue concentration history from 1988 to 2015 of dieldrin, p,p'-DDT and p,p'-DDE for these paddocks were kindly provided by the National Organochlorine Residue Program (NORM) [5] and are shown in Table S4.8-2. Sampling was done using the NORM standard operating procedure which included taking 40 cores (0-10 cm) per paddock and the analysis of a mixed and quartered composite sample, without field replicates. The soil samples were sent to the National Measurement Institute (NMI) in New South Wales, Australia for extraction and analysis.

We sampled the same paddocks again in April 2017. Each paddock was divided into three separate areas (field replicates). Using a soil auger, 8 − 10 cores (0 − 10 cm) from each replicate were taken to form a composite sample. Thus, a total of 24 − 30 cores were taken resulting in three composite samples per paddock. All samples were stored at 4 ˚C and sieved (≤ 2 mm) in the following four days. Each sample was then

subdivided into three subsamples of which (1) one was used fresh for analysis of dieldrin and DDT, microbial biomass carbon/nitrogen and available nitrogen; (2) another was stored at -20 ˚C for later DNA extractions and subsequent ARISA PCR and qPCR; and (3) the third was air-dried at 24 ˚C for determination of physicochemical properties.

4.4.2 Dieldrin and DDT residues

Unless stated otherwise, the term 'DDT' in this paper denotes the sum of residues of p,p'-DDT and p,p'-DDE. Samples of field moist soils were sent to NMI for dieldrin and DDT residues analysis. The NMI followed the U.S. Environmental Protection Agency Method 8081 guidelines [192] and is the same laboratory that DNRE staff, involved in the NORM program, had used to measure residues of dieldrin and DDT. Briefly, soils (10 g in duplicate) were mixed with anhydrous sodium sulphate and extracted twice for 10 min with 1:1 hexane:acetone under sonication. To clean extracts, approximately 1.5 − 2 ml of acetone-hexane (3:2) sample extract was passed through a Pasteur pipette containing a 1.5 − 2 cm plug of alumina aluminium oxide, concentrated and then analysed using dual column (30 m of Rtc-cLPest 0.32 mm × 0.25 µm and 30 m of DB608 0.32 mm × 0.50 µm) GC/ECD (Agilent 6890) at a limit of reporting of 0.01 µg g-1 soil. The GC temperature program is supplied in Table S3-6. For quality control, soils were extracted in duplicates and laboratory control spikes and matrix spikes (Difluoro-Dichlorodiphenyldichloroethylene (DF-DDE) were added to each sample and blanks were included for each batch of 20 samples. DF-DDE recoveries were 77 − 117 %. Extracts were stored at 4˚ C in the dark.

The dieldrin concentrations measured in 2017 were used for comparison with other physicochemical soil variables. The paddock averages were found to correlate concentrations of the most previous NORM measurements in 2015 (R^2 = 0.89). For relevant analyses, the 2015 and 2017 averages were combined (Equation S1). Residue loss (%) was calculated using the first and last average concentrations (Equation S2).

4.4.3 Soil physicochemical properties

Total soil organic carbon (SOC) and total N concentrations of soil samples were determined using a dry combustion analyser (Perkin Elmer 2400 Series II, USA) after air-dried soils were ground with a ball mill (Retsch MM400, Germany) at 25 rounds per second for 30 s. Soil pH (in 1:5 w/v water and 0.01 M $CaCl_2$ solution) and electric conductivity (EC) (1:5 H_2O) were determined using a pH meter (Thermo Orion 720A+, Beverly, MA, USA) and EC meter (Hanna Instruments, Hi 1053, USA), respectively. Soil particle size distribution was determined using a laser particle size analyser (Malvern Mastersizer 2000, Worcestershire, UK) following particle dispersion in solution of 10% (w/v) sodium hexametaphosphate ($Na_6P_6O_{18}$). Available P was measured according to Olsen, Cole, Watanabe, & Dean (1954). Water-holding capacity (WHC) of soils was determined using the small soil core method [194] but disturbed soil samples were used instead of intact soil cores. Briefly, the soils are saturated overnight, then allowed to drain under capillary tension on a

1-bar ceramic plate and remaining gravimetric water content measured. The basic properties are shown in Table S3-7.

4.4.4 Microbial biomass C and N and inorganic N

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were estimated based on the chloroform fumigation method [195]. Here, two sets of 8 g fresh soil (one set fumigated in a vacuum desiccator containing chloroform, and one set without fumigation) was extracted with 32 ml of 0.5 M K_2SO_4, then shaken end-over-end for 1 h and centrifuged at 2000 rev min-1 for 2 min before filtered through a Whatman 42 filter. The concentration of organic C in the extracts was determined using a supercritical-water-oxidation analyser (GE Sievers Innovox TOC analyser, USA) that oxidises organic compounds at high temperatures in a sealed reactor to CO_2 which is then measured spectrophotometrically. Standards with known C concentration were included and used to calculate the concentrations of samples accordingly. The carbon measured in non-fumigated soils were denoted as extractable organic C (EOC) and the difference between fumigated and non-fumigated was denoted as the extractable part of MBC where total MBC was estimated using k_{EC} = 0.45 [196]. To determine MBN and extractable organic nitrogen (EON), a peroxydisulfate ($K_2S_2O_8$) reagent mix was added to the K_2SO4 extracts (1:1) and autoclaved (121 ˚C, 104 kPa) following the method of [197]. Afterwards the NH_4^+ and NO_3^- concentrations were determined using a flow injection analyser (Lachat QuickChem 8500 Series II, USA) following the manufacturer's protocol [198,199]. The nitrogen measured in non-fumigated soils were denoted as extractable organic N (EON) and the difference between fumigated and non-fumigated was denoted as the extractable part of MBN where the total MBN was estimated using k_{EN} = 0.5 [200]. Lastly, the inorganic N (NH_4, and NO_3) in soil extracts (1:1 in 2 M KCl) was determined using the same analyser. All values are converted to per unit dry soil (Table S3-8).

4.4.5 Bacterial and fungal community fingerprints

Soil community fingerprints were established with automated ribosomal intergenic spacer analysis (ARISA) targeting the highly variable intergenic spacer region between 16S rRNA and 23S rRNA genes for bacteria [201] and the internal transcribed spacer (ITS) located between the 18S rRNA and 28S rRNA genes for fungi [202]. First, DNA of 0.25 g fresh soil was extracted using Powersoil DNA isolation kit (MoBio, Calsbad, USA) and DNA concentrations measured with a nanophotometer (Implen, Munich, Germany) to normalise concentrations to 5 ng μl^{-1}. ARISA PCR was carried out with 20µl reaction mixtures in a thermocycler (TProfessional, Biometra, Goettingen, Germany). Primer pair 16S-1392f [5'-GYACACACCGCCCGT] and 23S-125r [5'-GGGTTBCCCATTCRG] with fluorescently tagged oligonucleotides [201] was used for bacteria and primer pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA) and ITS4 (5'-TCCTCCGCTTATTGATATGC) for fungi [202].

Reaction mixtures contained 0.025 U μl^{-1} of TopTaq DNA polymerase (Qiagen), 0.5 mM of dNTP mix (Qiagen), 0.5 µM each of forward and backward primers, 1.5 mM of $MgCl_2$ and 0.25 ng μl^{-1} of DNA

(bacteria) or 0.5 ng μl^{-1} (fungi). Cycle settings for bacteria were 3, 1, 1, 1.5, 6 min and 94, 94, 52, 72, 72 ˚C where Steps 2 to 4 repeated 32 times [201], and for fungi 4, 1.5, 1, 1.5, 10 min and 94, 94, 52, 72, 72 ˚C where Steps 2 to 4 repeated 35 times [203].

Ten microliters of the amplified PCR product was then sent to the Australian Genome Research Facility (AGRF) to separate fragments (AB 3730 DNA analyser) and get peak height and area using Gene Mapper 5 software to quantify abundances by proxy of relative fluorescence intensity (RFI). To minimize imprecisions of the OTU sizes, the abundance values per fragment size were binned and normalised using automatic and interactive scrips as per Ramette (2009) in R software. The optimal bin size of 4 and a shift size of 0.1 were used to bin all fragment sizes from 50 - 1200 bp resulting in 146 bacterial OTUs, and a bin size of 2 and a shift size of 0.1 used for all fragments resulting in 66 fungal OTUs [205,206]. Fungal OTUs with < 100 bp were assumed to be primer dimers and removed from the table resulting in total 53 OTUs. An abundance barchart presents the relative abundances of these OTUs (Fig. S3-5).

4.4.6 Quantitative PCR

As a proxy of their abundance, the bacterial and fungal gene copies of the same DNA were quantified using quantitative PCR (qPCR). Primer pair 1114f (5'CGGCAACGAGCGCAACCC) and 1275r (5'CCATTGTAGCACGTGTGTAGC) was used to target the bacterial 16 rRNA genes (Denman and McSweeney 2006) and ITS1f (5'-TCCGTAGGTGAACCTGCGG) and 5.8Sr (5' CGCTGCGTTCTTCATCG) to target the ITS region of fungal genes [207]. qPCR was carried out on a CFX Connect Real-Time PCR Detection System (BioRad). For bacterial qPCR each 20-µl reaction mix contained 0.2 ng community DNA, 3.3 µl Sensifast™SYBR®Fluorescein mix (Bioline), 0.14 µM of each the forward and backward primers and 14.16 µl of PCR grade water. For fungal qPCR each 10-µl reaction mix contained 0.2 ng community DNA, 5 µl Sensifast™-SYBR®-Fluorescein mix and of 0.5 µM of each primer and 2 µl of PCR grade water. Fluorescence was read after each of 40 cycles with 10 s at 94 ˚C and 30 s at 60˚C for bacterial qPCR and after each of 40 cycles with 30 s at 95 ˚C, 30 s at 55˚C and 30 s at 72 ˚C for fungal qPCR [208]. Afterwards a meltcurve was done reading fluorescence every 1 ˚C from 60 to 99 ˚C.

One batch of reaction mixture was made for two 96-well plates which each accommodated five replicates of standards and a total of 36 samples in triplicate. Quantification cycle thresholds (Ct) of both plates were calculated under the same baseline fluorescence threshold. Standard deviations (STD) ≥ 0.3 of any quantification cycle numbers (Ct) was rejected and the corresponding Ct removed from the replicate-mean. To account for differences in amplification efficiencies between samples and standards, the mean efficiency of each reaction was re-calculated using LinRegPCR software [209] based on raw fluorescence reads of all 40 cycles. Standards were made using quantified PCR products using the same primers. Five replicates were used each with 3.08 × 105 gene copies of amplified and purified genomic DNA of Escherichia coli DH5α (for bacteria) and five replicates of 1.82 × 105 gene copies of Aspergillus sp. DNA (for fungi). The fungal

standard was visualized on a 2% (w/v) agarose gel together with community soil DNA to confirm the amplicon size fits the mean amplicon profile of the domestic soil fungal DNA. Gene copies of samples were enumerated based on their efficiency fractions to the standards [210,211] (Equation S3).

4.4.7 Diversity indices

Microbial diversity profiles in soil of all paddocks were assessed across a range of sensitivities to rare OTUs using a naive similarity matrix according to Leinster & Cobbold (2012) (Figure S4.8-1) and showed that indices with lower sensitivity to rare species differentiated well between paddocks. Thus, to summarise alpha diversity, the non-parametric Simpson index (D) [213] was calculated from OTU abundances for each sample, given by ARISA. The Simpson diversity index is suited for small sample sizes [214] and was recommended for comparisons of microbial alpha diversity [215]. The Simpson index was transformed (D_2) to increase interpretability and avoid variance problems as recommended by Pielou (1975) (Equation S4). Furthermore, the Simpson evenness ($E_{1/D}$) was calculated to include a measure of OTU dominance that is not sensitive to species richness (Equation S5).

4.4.8 Calculations

The carbon-to-nitrogen ratio and microbial-C-to-total-C ratio were calculated. To visualise the relationships of selected soil measurements, we have done the principal component analysis (PCA) (Figure 4.5-4c and d) and boxplots by soil type (Figure S4.8-3). In addition, Spearman correlations were used to identify significant relationships. Paddocks 10 and 11 were excluded for any correlation involving DDT loss as these paddocks did not use DDT in previous crops.

4.4.9 Linear mixed-effect modelling

We used packages lme4 [217] and nmle [218] in R (R Core Team, 2018) to perform linear mixed-effects analyses (LME) or generalised least squares (GLS) analyses. For ordinary linear models, the R statistical packages were used. For all models, a stepwise, top-down approach was used following the protocol by [219]. A more detailed description of modelling approach, and diagnostics are referred to supplementary materials.

Dieldrin and DDT percent losses were modelled using ordinary linear regression assuming a normal distribution. Models for Simpson's diversity indices as a response variable were fitted using generalized least squares using the 'varIdent' function from the nmle package in R, allowing for different spread of residuals per farm as this improved the model fit. Dieldrin and DDT residues were loge-transformed [220,221] prior to modelling using LME models. The mixed-effect model with ln(dieldrin) and ln(p,p'-DDT+p,p'-DDE) as response variables included the factor 'Farm' (A-D) as random effect, which was a factor of five levels with uneven numbers (Equations S6 and S7). Total gene copies were loge-transformed prior to LME modelling with the factor 'Paddock ID' as random effect (Equation S8). To interpret log estimates, they were

exponentiated as explained by [222] and then corrected for the effect of Jensen's inequality (Equation S9) [223]. Thus, these estimates < 1 show negative effects and > 1 positive effects.

4.4.10 Analysis of microbial composition

To test for differences between bacterial community structure pairwise distances were calculated (Bray-Curtis) for ARISA OTU abundances and visualised on a two-dimensional ordination of non-metric dimensional scaling (nMDS). First, we used the distance-based approach with PERMANOVA [224] on Bray-Curtis distances of untransformed OTU abundances using the vegan package [225]. Secondly, we used the multivariate statistical package 'MvAbund' [226], which shows a greater power than PERMANOVA as it accounts for an increasing variance with increasing abundances. Here, the untransformed ARISA OTU abundances were modelled using generalised linear models (GLM) with negative binomial distributions, assuming independence of OTUs, with bootstrap resampling of 1000 iterations [227] and Likelihood-Ratio-Tests [228]. In addition to testing associations on all OTUs, MvAbund also provides pairwise comparisons to test which OTUs respond significantly. From this, we assessed both adjusted and unadjusted p-values. Adjusted p-values were calculated using a step-down resampling algorithm as given by [229]. Model assumptions (mean-variance relationship and independence) were checked visually using appropriate plots.

4.5 RESULTS

4.5.1 Dieldrin and DDT residues in 2017 samples and residue loss since 1988

The concentrations of dieldrin and DDT, as the sum of p,p'-DDT and p,p'-DDE, significantly differed between the five farms with concentrations ranging from 0.03 to 1.53 µg g^{-1} soil and 0.12 to 7.04 µg g^{-1} soil respectively ($p < 0.0001$) (Figure 4.5-1). In Chromosol, dieldrin residues were over four times higher and DDT residues (p,p'-DDT +p,p'-DDE) more than two times lower compared to the Kurosol.

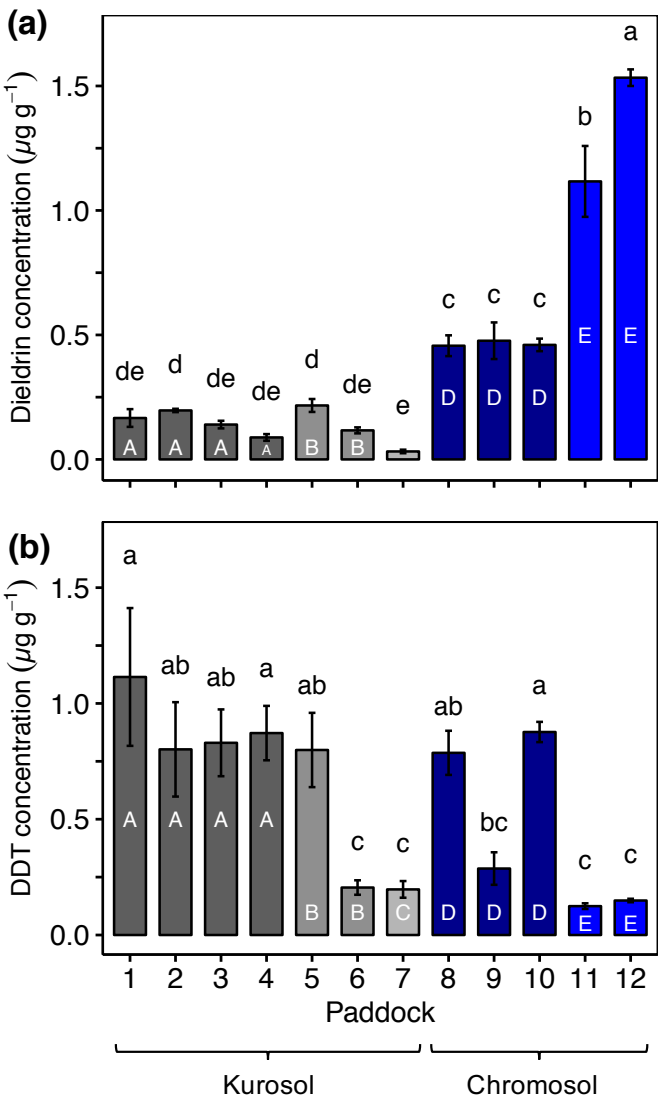


Figure 4.5-1. Mean paddock concentrations of dieldrin (a) and DDT (sum of p,p'-DDT&p,p'-DDE) (b) measured in 2017 (0–10 cm) grouped by farms as shown by capital letters A-E. Grey colour scale are farms on the Kurosol and blue colours are farms on the Chromosol. Lower case letters indicate significant differences of paddock means based on one-way ANOVA and post-hoc tests (Tukey's HSD) at the 0.05 level using log-transformed concentrations. Bars show ± standard error of the mean of three replicates.

The decline of dieldrin and DDT residues between 1988-2017 clearly differed between the two soil types (Figure 4.5-2). Dieldrin and DDT loss was 42% and 44% in the Chromosol compared to 73% and 71% in the Kurosol, respectively (Figure 4.5-2). Total loss of dieldrin residue, but not of DDT residues, was correlated negatively with carbon-to-nitrogen ratio (R_s^2 = 0.89***, Figure 4.5-3a) and positively with the microbial-C-to-total-C ratio (R_s^2 = 0.93***, Figure 4.5-3b), irrespective of soil type. The paddock-variation in the loss of DDT was inversely correlated with dieldrin concentrations (R_s^2 = 0.77***, Figure 4.5-3c), so that low dieldrin concentrations predicted increased DDT loss. Greater DDT loss occurred in paddocks with a higher bacterial abundance relative to fungi (R_s^2 = 0.61**, Figure 4.5-3d).

The linear model further showed that a unit decrease of the carbon-to-nitrogen ratio predicted an increased dieldrin loss by 11 % on average (Table 4.5-1), and that lower p,p'-DDT concentrations were associated with greater dieldrin loss (43 % for 1 µg g^{-1} of p,p'-DDT). Furthermore, an increased number of fungal OTUs increased dieldrin-loss (1% for every fungal OTU out of a total of 53 fungal OTUs based on ARISA).

The optimal linear model with average percent DDT-loss as a response variable also predicted that the lower dieldrin concentrations were associated with greater total DDT-loss (Table 4.5-1). For every decrease of 1 µg g^{-1} of dieldrin the model predicted an average 74% greater DDT-loss. Furthermore, an increase in bacterial OTU richness predicted a greater DDT-loss. With an increase of one bacterial OTU, DDT-loss was predicted to increase by 1% (out of a total of 146 bacterial OTUs based on ARISA).

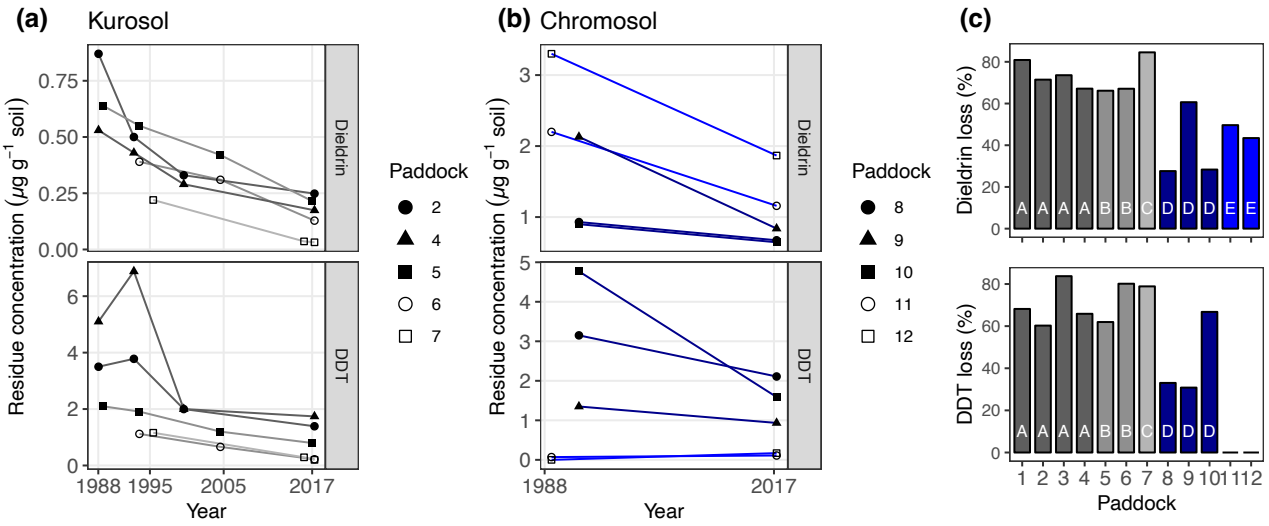


Figure 4.5-2. Mean paddock concentrations of dieldrin (a) and DDT (sum of p,p'-DDT & p,p'-DDE) (b) measured in 2017 (0–10 cm) grouped by farms as shown by capital letters A-E. Grey colour scale are farms on the Kurosol and blue colours are farms on the Chromosol. Lower case letters indicate significant differences of paddock means based on one-way ANOVA and post-hoc tests (Tukey's HSD) at the 0.05 level using log-transformed concentrations. Bars show ± standard error of the mean of three replicates.

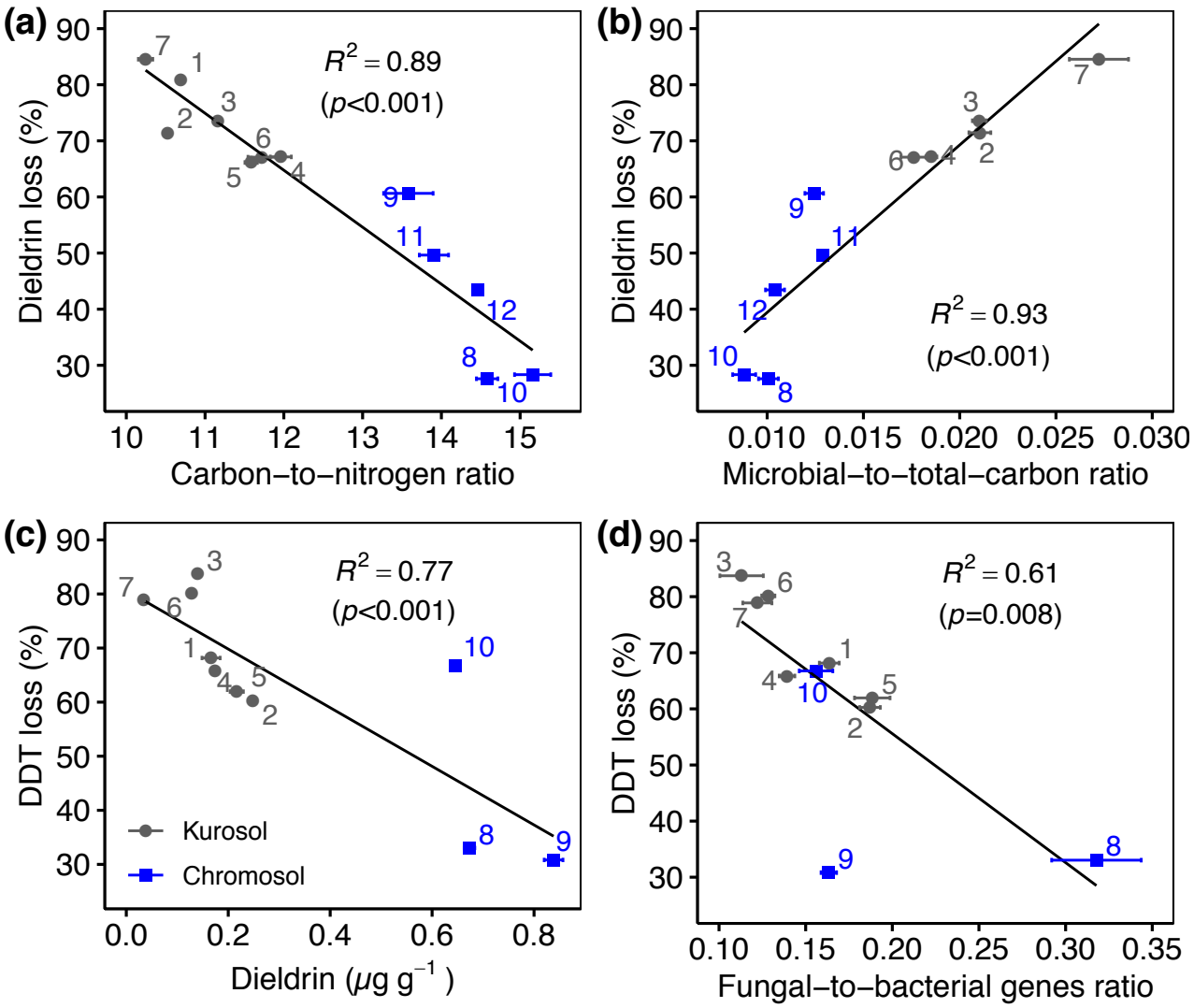


Figure 4.5-3. Scatter plots including Spearman correlation coefficients of dieldrin loss to carbon-to-nitrogen ratio (a) and microbial-C-to-total-C ratio (b). Furthermore, scatter plots with DDT loss to the combined mean dieldrin concentrations of 2015 and 2017 (c) and fungal-to-bacterial abundance ratio (d). Paddock numbers are shown next to data points. Bars show one standard error of the mean of three replicates.

Table 4.5-1. Ordinary linear models (dieldrin and DDT loss as response) and linear mixed-effect models (dieldrin and DDT concentrations as response). Samples of Farm E were excluded for all the analyses involving DDT because no DDT was used in the management of this farm.

	Dieldrin loss (%)			**DDT loss (%)**			**Dieldrin concentrations [a]**			**DDT (∑ p,p'-DDT, p,p'-DDE) concentration [a]**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	202	184 – 220	**<0.001**	7	-40 – 54	0.776	1.67	0.07 – 36.28	0.715	22633	6 – 84×10^6	**0.026**
Total organic carbon (mg g^{-1})							1.07	1.04 – 1.09	**<0.001**			
Clay (%)							1.13	1.06 – 1.20	**0.001**	1.23	1.17 – 1.37	**<0.001**
pH							0.39	0.23 – 0.67	**<0.001**			
Simpson evenness ($E_{1/D}$), fungi							0.47	0.28 – 0.78	**0.003**	0.43	0.22 – 0.82	**0.014**
log(fungal gene copies g^{-1} soil)										0.55	0.37 – 0.81	**0.004**
OTU richness (S), fungi	1	0.4 – 1.5	**0.012**									
OTU richness (S), bacteria				1.5	0.6 – 2.4	**0.017**						
Carbon-to-nitrogen ratio	-11	-13 – -10	**<0.001**									
p,p'-DDT (µg g^{-1})	-43	-66 – -20	**0.009**									
Olsen P (µg g^{-1})	-0.3	-0.4 – -0.2	**0.002**							1.06	1.05 – 1.08	**0.001**
Dieldrin (µg g^{-1})				-74	-94 – -53	**<0.001**						
Random Effects												
Residuals (σ^2)								0.05			0.08	
Intercept (σ^2)								0.42 (Farm A-E)			1.02 (Farm A-D)	
ICC								0.90 (Farm A-E)			0.93 (Farm A-D)	
Observations		12			10			36			30	
Marginal R^2 / Conditional R^2		0.99 / 0.98			0.84 / 0.80			0.52 / 0.95			0.42 / 0.96	

[a] Dieldrin and DDT estimates are shown back-transformed from log and adjusted for Jensen's inequality [e^(log(copies g^{-1} soil) + 0.5 x sigma^2)], thus estimates < 1 show negative effects and > 1 positive effects. ICC, Intraclass correlation coefficient; CI, Confidence interval.

4.5.2 Factors controlling dieldrin and DDT residues

The linear mixed-effect model (LME) indicated that dieldrin concentrations were higher in samples with higher carbon concentrations, higher clay content, lower pH and lower fungal diversity (Table 4.5-1). On average, this model estimated that for every mg g^{-1} increase in total organic C and every % increase in clay particles (< 2 μm), the concentrations of dieldrin residues increased 1.07 times and 1.13 times, respectively. A unit decrease of pH and fungal Simpson evenness ($E_{1/D}$) predicted a 2.6- and 2.1-fold increase in the concentrations of dieldrin residues, respectively.

The effect of clay content and fungal evenness ($E_{1/D}$) on DDT residue concentrations were comparable to dieldrin residues. On average, a percent increase in clay content predicted a 1.23-fold increase of DDT, and a unit decrease of Simpson's fungal evenness ($E_{1/D}$) predicted a 2.3-fold increase of DDT concentrations (Table 4.5-1). In contrast to dieldrin residues, soil carbon and pH were not associated with DDT concentrations and decreases in fungal abundance were associated with an increase in DDT concentrations.

4.5.3 Associations of microbial alpha diversity and abundance with dieldrin and DDT

The GLS models estimated that the Simpson's diversity indices (bacteria and fungi) are associated with dieldrin and DDT concentrations (Table 4.5-2). Meanwhile, the Simpson indices of both bacteria and fungi were predicted to increase with decreasing pH, and the bacterial Simpson's index was also predicted to increase with decreasing soil nitrogen and increasing bacterial abundance (Table 4.5-2).

The LME indicated that bacterial 16s rRNA gene copy number increased with fungal 18s rRNA gene copy number and vice versa (Table 4.5-2). However, only bacterial abundance increased with pH whereas fungal abundance increased with total organic C. Figure 4.5-4d further elucidated how the soil types and the farms differed in Simpson's diversity indices, bacterial and fungal abundance and in microbial-C-to-total-C ratio. It highlighted that the two soil types and 12 farms were distinctly different, with farm C (Paddock 7) on the Kurosol having the highest microbial-C-to-total-C ratio and farm D (Paddocks 8−10) on the Chromosol having the lowest ratio. The farms with higher microbial-C-to-total-C ratio had lower fungal abundances and fungal Simpson's indices.

Table 4.5-2. Generalized least squares (GLS) models with Simpson's diversity as response variable and linear mixed-effect models with gene copy numbers as response variable.

	Simpson diversity (D_2) bacteria [a]			**Simpson diversity (D_2) fungi [a]**			**Bacterial gene copies [b]**			**Fungal gene copies [b]**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	0.50	-4.35 – 5.35	0.833	5.55	3.33 – 7.77	**<0.001**	742	5.5 – 10^5	**0.014**	7778	12 – 49×10^5	**0.012**
log(dieldrin)	0.32	0.09 – 0.55	**0.007**	0.33	0.04 – 0.61	**0.026**						
log(DDT)	0.21	0.04 – 0.38	**0.019**	0.37	0.14 – 0.60	**0.003**						
Total nitrogen (mg g^{-1})	-0.34	-0.46 – -0.21	**<0.001**									
pH	-0.52	-0.78 – -0.26	**<0.001**	-0.75	-1.23 – -0.26	**0.004**	1.99	1.30 – 3.08	**0.005**			
Olsen P (µg g^{-1})	0.00	-0.01 – -0.00	**0.036**	-0.01	-0.02 – -0.00	**0.019**						
Bac. genes log(copies g^{-1} soil)	0.29	0.07 – 0.50	**0.01**							1.69	1.26 – 2.26	**0.002**
log(dieldrin):log(DDT)	0.15	0.04 – 0.26	**0.008**	0.17	0.01 – 0.32	**0.034**						
Fun. genes log(copies g^{-1} soil)							1.80	1.49 – 2.21	**<0.001**			
Bac. Simpson's diversity (D_2)							1.45	1.12 – 1.92	**0.015**			
Total organic carbon (µg g^{-1})										1.04	1.04 – 1.05	**0.006**
Random Effects												
Residuals (σ^2)		GLS (A-D)			GLS (A-D)			0.02			0.05	
Intercept (σ^2)								0.05 (Pdk 1–12)			0.01 (Pdk 1–12)	
ICC								0.72 (Pdk 1–12)			0.22 (Pdk 1–12)	
Observations		35			35			35			35	
Marginal R^2 / Conditional R^2		NA			NA			0.382 / 0.828			0.598 / 0.685	

[a] Models with Simpson diversity indices (D) were done with generalized least squares (GLS), which allowed for differing variance structures for each farm (A–D).
[b] Gene copy estimates are shown backtransformed from log and adjusted for Jensen's inequality [e^(log(copies g-1 soil) + 0.5 x sigma2)], thus estimates < 1 show negative effects and > 1 positive effects.
DDT, ∑(p,p'-DDT,p,p'-DDE); Bac., Bacteria; Fun., Fungi; CI, Confidence interval; ICC, Intraclass correlation coefficient; Pdk, Paddocks.

4.5.4 Associations of dieldrin and DDT concentrations with OTU composition

About 47% of the variation in bacterial composition and 27% of the variation in fungal composition could be attributed to soil type (Kurosol and Chromosol) and farm (A-E) (Table 4.5-3). Figure 4.5-4 further showed this separation in OTU dissimilarities by soil type and farm for bacteria and fungi, although it was less clear for fungal OTUs.

The PCA was used to further explore which soil properties might influence the concentrations of dieldrin and DDT residues. Axis PC1 and PC2 of the PCA explained 63% of the variation of the nine chosen soil variables (Figure 4.5-4c). The concentrations of dieldrin and DDT contributed 28% to the total variation of these nine soil variables.

The PCA further visualised that soil carbon-to-nitrogen ratio and clay content clearly differed between the two soils ($p < 0.0001$) but did not differ between farms on the same soil. However, there was no difference in water-holding capacity and pH between the two soils ($p > 0.05$) but there was a substantial difference between farms ($p < 0.0001$). The concentrations of Olsen-P and nitrate also significantly differed between the two soils and between farms A, B and C on the Kurosol.

From the PERMANOVA (Table 4.5-3), after accounting for soil type and farm, carbon-to-nitrogen ratio (5%), pH (6%) and DDT (3%) each explained a small percentage of the variation in bacterial OTU composition. Furthermore, carbon-to-nitrogen ratio (4%) and DDT (4%) explained some variation of the fungal OTU composition. However, dieldrin concentrations did not explain any more variation of the microbial composition. The variation partitioning of microbial OTU abundances was visualised in Figure S4.8-4. On the other hand, the GLMs with MvAbund detected associations of both DDT ($p = 0.01$) and dieldrin ($p = 0.008$) with the bacterial community composition (Table 4.5-3).

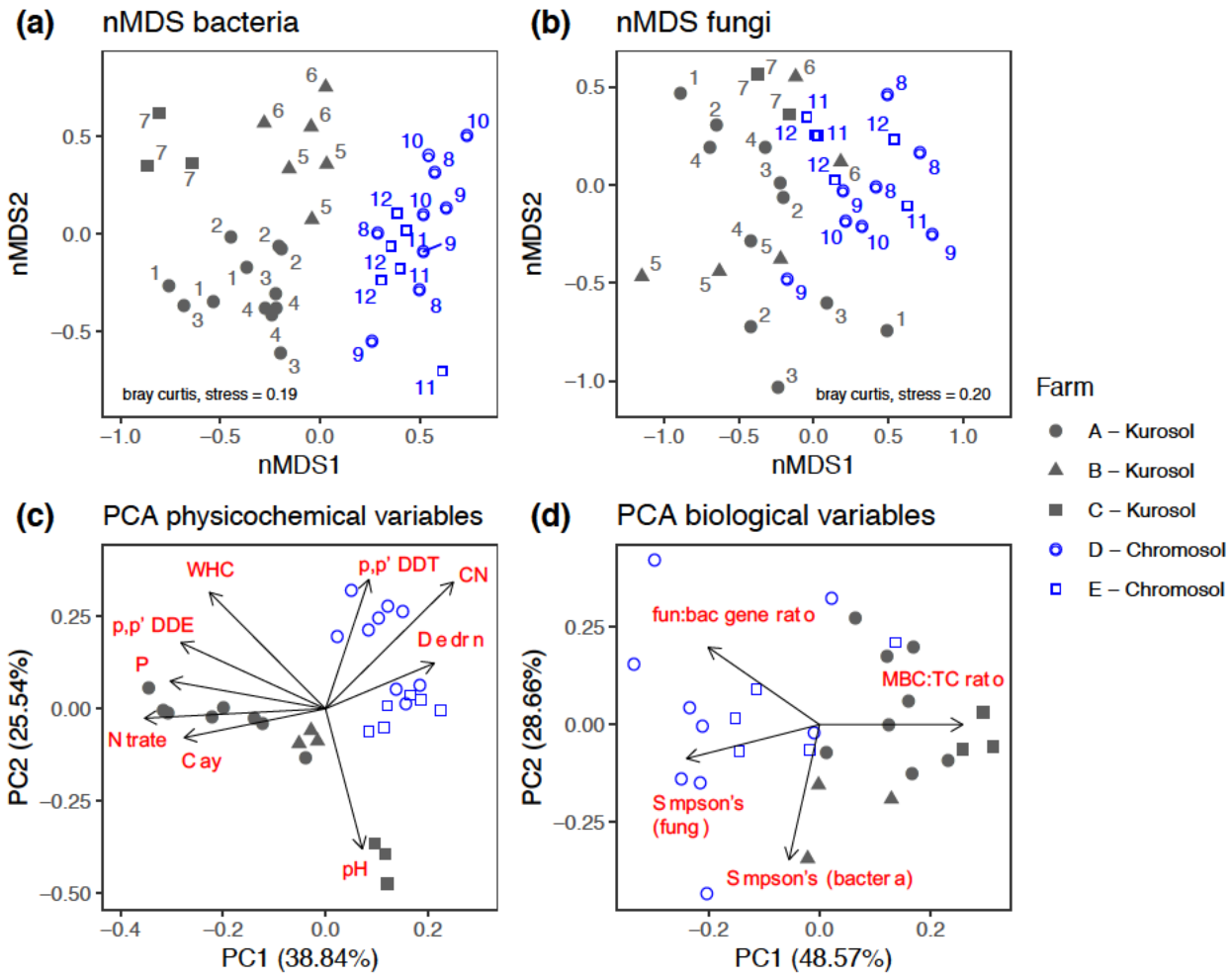


Figure 4.5-4. Plots of ordinations of non-metric dimensional scaling (nMDS) based on Bray Curtis dissimilarity matrices of the OTU abundances given by ARISA, showing 36 samples where distances between values represent similarities between samples (a and b). Principal component analysis (PCA) explaining the variation of scaled soil variables, including Olsen phosphorus (P), waterholding capacity (WHC), carbon-to-nitrogen ratio (CN), pH, proportion of clay-sized particles (clay), nitrate concentrations, p,p'-DDT, p,p'-DDE and dieldrin (c). PCA explaining the variation of scaled Simpson's index (D), total fungal-to-bacterial abundance ratio and microbial biomass carbon to total carbon ratio (MBC: TC) (d). Position of data points in PCAs show relative differences of samples in selected variables.

Table 4.5-3. PERMANOVA and GLM results, testing for the effects of soil type (factors Kurosol and Chromosol), farm (factors A - E) and selected continuous soil variables on microbial composition. Significant PERMANOVA R2 values (p b 0.05) are shown as well as MvAbund test results with the number of OTUs that significantly responded to predictors based on GLMs on OTU abundances.

	Permanova [a]					MvAbund, Generalised Linear Model with negative binomial distributions [a]									
	Bacterial OTUs			Fungal OTUs		Bacterial OTUs						Fungal OTUs			
										Responding OTUs (p<0.05)				Responding OTUs (p<0.05)	
										adjusted					
Predictor	df	F.model	R^2	F.model	R^2	Res.Df	Df.diff	Dev.	p	p	unadjusted p	Dev.	p	Adjusted p	Unadjusted p
(Intercept)						35									
Soil (factor)	1	18.42	**0.23**	4.64	**0.10**	34	1	579	**0.001**	**16**	**40**	84	**0.001**	**16**	**5**
C:N [b]	1	3.59	**0.05**	1.75	0.04	33	1	202	**0.002**	**1**	**17**	25	n.s.	1	1
Farm (factor)	3	6.33	**0.24**	2.58	**0.17**	29	4	665	**0.001**	**6**	**29**	115	**0.001**	**6**	**4**
pH	1	4.58	**0.06**	1.86	0.04	28	1	225	**0.001**	**2**	**17**	33	n.s.	2	0
Dieldrin	1	1.04	n.s.	0.29	n.s.	27	1	160	**0.008**	**0**	**8**	32	n.s.	0	1
DDT	1	2.30	**0.03**	1.89	0.04	26	1	179	**0.01**	**0**	**12**	45	n.s.	0	1
Soil×C:N	1	1.33	n.s.	0.24	n.s.	25	1	71.9	0.08	0	6	35	0.08	0	2
Farm×Dieldrin	4	1.41	**0.07**	1.23	n.s.	21	4	262	n.s.	0	10	150	**0.05**	**0**	**5**
Farm×DDT	4	1.25	n.s.	0.98	n.s.	18	4	297	n.s.	0	14	48	n.s.	0	2
Residuals	18		0.23		0.39										

[a] Values are bold when p < 0.05, not bold when p < 0.1 and n.s. when p > 0.1, [b] C:N, carbon-to-nitrogen ratio; DDT, (∑(p,p'-DDT,p,p'-DDE)

4.6 DISCUSSION

The loss, and current concentrations, of POPs in the surface soils of this study were associated with soil biology. Soil organic matter composition, including microbial biomass carbon, soluble nutrients, microbial alpha and beta diversity and abundance were interrelated and associated with dieldrin and DDT loss or concentrations. However, interpretations from these observations have to take into consideration that factors influencing DDT and dieldrin degradation may also be confounded by other variables that are unaccounted for in our measurements.

4.6.1 Factors affecting the loss of dieldrin and DDT residues

SOM and microbial growth played important roles in long-term dieldrin degradation. Paddocks with a low carbon-to-nitrogen ratio (Figure 4.5-3a) and a high microbial-C-to-total-C ratio (Figure 4.5-3b) significantly associated with increases in total dieldrin loss over 30 years. This was not observed in the loss of DDT, although average DDT loss was higher in the Kurosol than the Chromosol (73% ± 3 vs. 42% ± 14 SE), with the Kurosol having a lower average carbon-to-nitrogen ratio (11 ± 0.15 vs. 14 ± 0.21 SE). On average, paddocks with high dieldrin loss also had low DDT concentrations and vice versa (Fig. 3-3c, Table 3-2), implying that the conditions in those paddocks were conducive to the dissipation of both dieldrin and DDT.

The soil carbon-to-nitrogen ratio appeared to affect dieldrin degradation via an effect on microbial growth and function. It strongly and negatively correlated with microbial-C-to-total-C ratio (R^2 = 0.95***). Microbial-C-to-total-C ratios in soils are controlled by the quality and availability of organic carbon [230,231] and low carbon-to-nitrogen substrates degrade faster under these conditions [232]. For example, microbial-C-total-C ratios increase after the application of easily-digestible organic amendments such as farm-yard manure or green manures [230]. Based on microbial-C-to-total-C ratios in crop rotation systems, T. H. Anderson & Domsch (1989) argue that higher microbial-C-to-total-C ratios were due to a more efficient microbial metabolism that is evolved in a more complex soil environment with heterogeneous organic matter inputs.

Soil texture may also affect dieldrin and DDT degradation through sorption. It was evident that finer-textured soils, with higher SOC concentrations, stored significantly more dieldrin and DDT residues although clay appeared more important for DDT. The LME models predicted that an increase of 10 mg g-1 in SOC or a 5 - 6 % increase in clay content doubled dieldrin concentrations while the same increase in clay tripled DDT concentrations (Table 4.5-1). All pasture paddocks had high SOC contents (32 - 70 g kg-1), which led to a high sorption capacity. Dieldrin ($K_{OW} \approx 4.32$), p,p'-DDT ($K_{OW} \approx 5.98$) and its transformation product p,p'-DDE ($K_{OW} \approx 5.95$) all have high octanol-water constants [3,234], indicating high fat-solubility and partitioning into hydrophobic SOM [235].

Soil organic matter consists of complexes of polymers and includes dead plant and microbial biomass [236]. Recalcitrant, lipid-rich compounds from microbial detritus can accumulate and constitute up to 30% of the total organic C, especially in the fine fraction of SOM [237] and in soils rich in SOM [238,239]. From previous studies, it is clear that the persistence of organic compounds [74,185,240], including agricultural pesticides [70,184,241,242], is greater when they are bound to inorganic minerals or organic polymers and that strong sorption limits their degradation [243,244].

Taken together, it appeared that SOM played an important role in dieldrin and DDT degradation. This study showed that soil carbon-to-nitrogen ratios and microbial-C-to-total-C ratios were correlated with dieldrin dissipation, and the amount of SOM was less important. It indicated that residue loss was greater in paddocks where microbial growth was more efficient per unit carbon. Furthermore, N in the soils with a high carbon-to-nitrogen ratio might be limiting to microbial growth and SOC utilisation, and thus lower residue degradation. Recent studies emphasised the importance of nutrient ratios of SOM in net mineralisation activity [245,246]. Competition for carbon might have subsequently activated a broader metabolism. Chen & Chiu (2003) showed that SOM with a low carbon-to-nitrogen ratio were overall more recalcitrant than that with a high carbon-to-nitrogen ratio, especially in the fine fraction, due to the faster utilisation of the non-recalcitrant carbon sources in low carbon-to-nitrogen SOM [247]. Perhaps, the SOM of the studied pastures with high residue loss contained higher proportions of recalcitrant carbon sources such as lignin resulting in a higher degree of co-metabolism [238] and consequently co-metabolism of dieldrin or DDT. That may also explain the higher evenness of fungal communities ($E_{1/D}$, Table 4.5-1) in soils with lower dieldrin and DDT concentrations (and higher residue loss).

Interestingly, the abundance of bacteria relative to fungi was greater in paddocks with low carbon-to-nitrogen or high microbial-C-to-total-C ratios (Figure 4.5-4) and in paddocks with high total DDT loss (Figure 4.5-3d). The richness of dominant OTUs of fungi decreased in paddocks with increased microbial-C-to-total-C ratios according to the Simpson's diversity index (Figure 4.5-4d). Hence it appeared as if bacteria were more important for degradation of dieldrin and DDT, as has previously been reported for the degradation of hydrocarbons [248]. However, due to interchangeability of functions between fungi and bacteria [249–251], broader enzymatic capabilities of fungi [252] and potentially greater carbon-use efficiencies of fungi [253], an increased relative abundance and diversity of bacteria might not necessarily be representative of their increased importance for degradation of pollutants.

We hypothesize that botanical composition of pasture species of the investigated paddocks might have influenced carbon-to-nitrogen ratios and the degradation of the pollutants. Microbial priming by inputs of root exudates by grasses has shown to increase SOM mineralisation rates [254–256], depending on plant species [257]. Future research into the effects of microbial dynamics and SOM mineralisation on degradation of persistent pollutants is needed to improve understanding in this area.

4.6.2 Associations of dieldrin and DDT residues with microbial diversity

Despite the high variability in the concentration and dissipation of dieldrin and DDT residues between the paddocks, only minor associations with bacterial and fungal diversity was found. As expected, the main drivers of the bacterial and fungal alpha and beta diversity and total abundance were soil type, farm origin (i.e., management), SOM (total organic C, total N and carbon-to-nitrogen ratio) and pH (Tables 4.5-2, 4.5-3 and Figure 4.5-4). Nonetheless, DDT concentrations had small but significant associations with bacterial OTU composition ($p < 0.05$) but not with fungal OTUs ($p > 0.05$) (Table 4.5-3). A small part of the variation ($\approx$ 3%) of the bacterial community composition due to DDT concentrations was further implied by PERMANOVA (Table 4.5-3). However, it was less clear if dieldrin was associated with the bacterial community. Based on the results from the statistical package MvAbund [226], which has greater statistical power than PERMANOVA, there was a significant association of microbial community composition and dieldrin concentrations (Table 4.5-3).

Dieldrin and DDT constituted on average only about 9 × 10-4 % and 1.3 × 10-3 % of the average total SOC in this study and we assumed that most of it was inaccessible to microbial metabolism, hence it was surprising that even a small association of the residues with the bacterial community composition was detected. This association could only be detected for a family of OTUs and not for individual OTUs (Table 4.5-3, MvAbund). When assessed with p values that were unadjusted for correlations between OTUs, MvAbund detected 12 OTUs that significantly responded to DDT (Table 4.5-3, MvAbund, unadjusted $p < 0.05$). Some of these 12 OTUs also responded to soil carbon-to-nitrogen ratio and pH. The ARISA OTU tables in this study consisted of 146 bacterial and 53 fungal OTUs. However, unlike next-generation sequencing, ARISA fingerprinting only detects the most abundant community members but remains a reliable and cost-effective method to identify environmental drivers and biological relevant patterns at the community level [258–260]. Conducting 16S rRNA next generation sequencing to provide taxon data of microbial species involved in degradation of organic pollutants would be of interest in the future.

4.6.3 Decrease of residues over time and implications

Despite the clear effect of carbon-to-nitrogen ratio and microbial-C-to-total-C ratio on the total dieldrin loss, it remains unclear how the degradation processes were affected or when the majority of the residues disappeared. Because farm management would affect the pattern of residue-decline over time, we were unable to appropriately compare the rates of degradation as too few time points were available to calculate the kinetics. Nevertheless, from those paddocks where more than two time points of residue concentration were available, it was apparent that the rates of residue loss differed between the farms on the same soil type (Figure 4.5-2a). The curves of dieldrin loss over time in Paddocks 2 and 4 resemble that of first-order kinetics, whereas the curves of dieldrin loss in Paddocks 5 and 6 resemble zero-order kinetics [182].

Both the first-order and zero-order biodegradations are expected when the microorganisms are not growing selectively on the degraded compound. Zero-order kinetics has been reported frequently for organic substrates at low concentrations that do not trigger microbial growth [182]. The degradation depends mostly on limiting nutrients that become available at a constant rate. In comparison, degradation following the first-order kinetics slows down with decreasing concentrations. Based on these observations, it appears that co-metabolism but not primary metabolism (i.e. not using dieldrin or DDT as substrate) is the dominant form of metabolism involved in degradation of dieldrin and DDT in our study, similar to what has been observed in experiments where other carbon substrates were a prerequisite for microbial degradation [95,162,171,261–264].

DDT degrading microbial consortia have been enriched with DDT as sole carbon source but so far there is no evidence of their involvement in DDT degradation *in-situ* [265]. Co-metabolism requires more energy investment, the maximal rate of co-metabolic degradation of POPs is 10 - 100 times slower compared to growth-supporting degradation reactions [266]. Nonetheless, the large genetic potential of microorganisms in soils [267] and observed genetic adaptations to persistent pollutants over time [268] mean that future research has the potential to identify and utilise microbial strains that are effective at degrading dieldrin and DDT.

4.7 CONCLUSION

This study shows that a low soil carbon-to-nitrogen ratio and a high microbial-C-to-total-C ratio favoured the total loss of dieldrin over 23 − 30 year time period. A lower fungal evenness further predicted lower concentrations of both dieldrin and DDT, implying that a broader microbial community was involved in their degradation. Relative to fungi, bacterial abundance increased with increasing microbial-C-to-total-C ratio and with higher DDT loss, although it remained unclear which part of the microbial community was involved in degradation of these pollutants. Moreover, degradation was likely inhibited by N limitation and/or sorption of dieldrin and DDT residues to SOM or clay particles. Despite this, a small part of the bacterial community composition was associated with DDT concentrations, but this was less clear with dieldrin. Based on the results, co-metabolism of dieldrin and DDT could be enhanced by manipulating the quality of SOM to cater for a broad microbial diversity.

4.8 SUPPLEMENTARY MATERIALS

4.8.1 Approach and diagnostics of linear mixed-effect models for this study

Data description: Three field replicates in each of twelve paddocks across two soils. It is known that dieldrin and DDT were applied at different rates depending on management ('Farm') and soil type. Thus, values within each farm or soil type are not independent and are therefore treated as random effects.

Model approach for mixed-effect models based on the protocol by [219]:

- Assess and select appropriate distribution for the response variable. For example, concentrations of environmental pollutant commonly follow a log-normal distribution, which was confirmed for dieldrin residues in this study.
- Build beyond optimal model, meaning a model with all possible explanatory variables and as many interactions as possible. For example, with dieldrin concentrations as a response variable, the starting model included 10 physicochemical and biological variables without interactions.
- Use of Akaike Information Criterion (AIC) or Bayesian Information Criterion (BIC) with restricted maximum likelihood (REML) estimation to compare models with the same fixed effects but different random effect components to derive the best random effect structure (smallest AIC or BIC value). For example, with dieldrin as a response variable, the factor 'Farm' as random effect was the model with lowest AIC, after comparisons of nine combinations of random effects including the factors 'Soil type', 'Farm' and 'Paddock'.
- Once optimal random structure is found, de-select fixed effects (i.e. explanatory variables) one by one, based on lowest significance from estimations with REML. To obtain *p*-values of fixed effects in a model we used Wald F tests with Kenward-Roger degrees of freedom as recommended by [269][a]

Diagnostics:

- Variables with a variance inflation factor (VIF) above three were excluded unless they were inflated by interaction terms.
- Distribution of model residuals were tested for normality using the Shapiro-Wilk test and normality was assumed at the 5% significance level.
- The AIC was used to test if random effects were necessary to be included into the models, and if so, to select the most appropriate random intercept and slope construct.
- Cooks distances larger than 1 were assumed to be influential observations and removed from any model. Predictor variables were not centred prior to analysis.

Residuals of final models were plotted against fitted values to confirm homoscedasticity and then against each explanatory variable to check their homogeneity or independence.

4.8.2 Supplementary equations

Calculations for the combined mean residue concentrations of Years 2015 and 2017 (Residues1517) for each of twelve paddocks (p). Measurements per paddock from 2015 (Residues2015) without field replicates and measurements from 2017 (Residues2017) using three field replicates (n = 3).

$$\text{Residues1517} = \frac{\text{Residues2015}_{\text{p}} + \frac{\sum_{i=1}^{p_i} \text{Residues2017}}{n}}{2} \quad \text{S1}$$

(i = 1,..36 observation in p = 1,..12 paddocks, n = 3)

Residue loss (%) calculated using initial residue concentrations ($Residues_{t0p}$) and the combined mean concentrations of 2015 and 2017 (Residues1517).

$$\text{Residue loss (\%)} = \frac{\text{Residues}_{\text{t0p}} - \text{Residues1517}_{\text{p}}}{\text{Residues}_{\text{t0p}}} \times 100 \quad \text{S2}$$

(t_0 = 1988 - 1995, p = 1,..12 paddocks)

Gene copies (N0) enumerated based on their efficiency fractions to quantified DNA standards

$$\text{N0}_{\text{sample}} = \text{N0}_{\text{standard}} \times (\text{E}_{\text{standard}}^{\text{Ct}_{\text{standard}}} / \text{E}_{\text{sample}}^{\text{Ct}_{\text{samp e}}}) \quad \text{S3}$$

where N_0 is the gene copies, C_t is the mean quantification threshold and E the mean efficiency.

Simpson's index (D) and transformed Simpson's index (D_2)

$$D = 1 - \sum p_i^2 \quad \text{S4}$$

$$D_2 = -ln(D)$$

where p_i is the proportion of individuals in the i^{th} species.

Simpson's evenness ($E_{1/D}$)

$$E_{1/D} = \frac{(1/D)}{S} \quad \text{S5}$$

where S is the number of OTUs.

Mixed-effect model for $\log_e$-transformed dieldrin concentrations (*ln(dieldrin)*) for observations *i* at farm *j*. The nominal value of Farm with five levels is a random factor (*Z)* with their effect *b*. The term $X \times \beta$ represents the fixed effects included in the model. The residual ε_{ij} is assumed to be normally distributed with mean 0 and variance σ^2.

$$\ln(dieldrin_{ij}) = X_{ij} \times \beta + Z_j \times b_j + \varepsilon_{ij} \quad \text{S6}$$

$$(i = 1,\ldots 36\ observations\ in\ j = 1,..5\ farm)$$

Mixed-effect model for $\log_e$-transformed dieldrin concentrations (*ln(sum(p,p'-DDT & p,p'-DDE)*) for observations *i* at farm *j*.

$$ln(sum(pp.DDT_{ij} + pp.DDE_{ij})) = X_{ij} \times \beta + Z_j \times b_j + \varepsilon_{ij} \quad \text{S7}$$

$$(i = 1,\ldots 30\ observations\ in\ j = 1,..4\ farms)$$

Mixed-effect model for $\log_e$-transformed dieldrin concentrations (*ln(gene copies)*) for observations *i* at farm *j*.

$$\ln(gene\ copies_{ij}) = X_{ij} \times \beta + Z_j \times b_j + \varepsilon_{ij} \quad \text{S8}$$

$(i = 1,\ldots 35\ observations\ in\ j = 1,..12\ paddocks)$

where $X_{ij} \times \beta$ represents the fixed terms (predictor variables) and $Z_j \times b_j$ the random term farm, which allowed for variation between farms. ε_{ij} refers to the within-farm variation and was assumed to be independently normally distributed.

Correction of model estimates for Jensen's inequality

$$Final\ model\ estimates \qquad e^{ln(estimate + 0.5 \quad sigma\text{^}2)} \qquad \text{S9}$$

4.8.3 Supplementary tables

Table S4.8-1. Basic information of pastures investigated in this study.

Paddock	Location Victoria, Australia	Farm	Soil classification [a]	Cropping history	Area (acres)	Annual precipitation (mm) [b]	Years since first residue measurement
1	Edi, Northeast Victoria	A	Kurosol	Tobacco	18	694	29
2	Edi, Northeast Victoria	A	Kurosol	Tobacco	15	694	29
3	Edi, Northeast Victoria	A	Kurosol	Tobacco	11	694	29
4	Edi, Northeast Victoria	A	Kurosol	Tobacco	13	694	29
5	Edi upper, Northeast Victoria	B	Kurosol	Tobacco	10	1054	28
6	Edi upper, Northeast Victoria	B	Kurosol	Tobacco	3	1054	24
7	Edi upper, Northeast Victoria	C	Kurosol	Tobacco	20	1054	22
8	Cockatoo, Southeast Victoria	D	Chromosol	Potato	8	1000	25
9	Cockatoo, Southeast Victoria	D	Chromosol	Potato	4	1000	25
10	Cockatoo, Southeast Victoria	D	Chromosol	Potato	4	1000	25
11	Cockatoo, Southeast Victoria	E	Chromosol	Potato	10	1000	28.5
12	Cockatoo, Southeast Victoria	E	Chromosol	Potato	10	1000	28.5

[a] Soil classification based on the Australian soil and resource information system (ASRIS) on district level
[b] Weather data from the Bureau of Meteorology (http://www.bom.gov.au/climate/data/, accessed 15.01.2018) using closest weather station to location.

Table S4.8-2. Concentrations of dieldrin and DDT in paddock soils since 1988 as provided by the National Organochlorine Residue Program (NORM). Values represent the average per paddock based on one representative composite sample.

Paddock	Farm	Year	Dieldrin[a] ($\mu g\ g^{-1}$)	DDT[a] ($\mu g\ g^{-1}$)
1	A	1988	0.87	3.50
1	A	1992	0.50	3.78
1	A	2017	0.17	1.11
2	A	1988	0.87	3.50
2	A	1992	0.50	3.78
2	A	1999	0.33	2.00
2	A	2017	0.25	1.39
3	A	1988	0.53	5.10
3	A	1992	0.44	4.49
3	A	2017	0.14	0.83
4	A	1988	0.53	5.10
4	A	1992	0.43	6.88
4	A	1999	0.29	2.00
4	A	2017	0.17	1.74
5	B	1988	0.64	2.10
5	B	1993	0.55	1.91
5	B	2004	0.42	1.20
5	B	2017	0.22	0.80
6	B	1993	0.39	1.12
6	B	2004	0.31	0.66
6	B	2017	0.13	0.22
7	C	1995	0.22	1.16
7	C	2015	0.04	0.29
7	C	2017	0.03	0.20
8	D	1992	0.93	3.15
8	D	2017	0.67	2.11
9	D	1992	2.13	1.35
9	D	2017	0.84	0.93
10	D	1992	0.90	4.77
10	D	2017	0.65	1.59
11	E	1988	2.20	0.07
11	E	2017	1.16	0.11
12	E	1988	3.30	0.00
12	E	2017	1.87	0.17

[a]2017 values are the average of 2015 and 2017 measurements using Equation S1

Table S4.8-3. Details and conditions of gas chromatography (GC).

Column/Detector Information	
Column 1 Type:	Rtc-cL Pest (30m), 0.32mm ID x0.25 µm
Column 2 Type:	DB608 (30m), 0.32mm ID x0.50 µm
Detector type:	Micro ECD
Oven temperature program (Temp = ^{O}C, Time = min)	
Initial oven temp	110
Initial oven temp hold	0
Program 1 oven rate rise	35
Program 1 oven temp	200
Program 1 oven hold	3.5
Program 2 oven rate rise	12.5
Program 2 oven temp	260
Program 2 oven hold	0.75
Program 3 oven rate rise	17
Program 3 oven temp	280
Program 3 oven hold	5.87
Program 4 oven rate rise	10
Program 4 oven temp	300
Program 4 oven hold	2.5
Miscellaneous information	
Injection rate	Pulsed splitless
Injection volume	2 µl
Carrier gas	Hydrogen
Inlet pressure	30kpa
Inlet temp	250
Detector temp	320

Table S4.8-4. Physicochemical measurements of surface soils (0 – 10 cm). Values represent the mean ± standard error of three replicates per paddock.

Paddock	Clay, < 2 µm (%)	Silt, 2 – 20 µm (%)	WHC, Θg (%)	pH ($CaCl_2$)	EC (1:5 H_2O) (µS cm^{-1})	Total C (mg g^{-1} soil)	Total N (mg g^{-1} soil)	C:N ratio	Olsen P (µg g^{-1} soil)	EOC (mg g^{-1} soil)	EON (mg kg^{-1} soil)
1 [a]	13 ±0.6	37.6 ±1.7	41 ±0	4.51 ±0.0	115 ±34	33.6 ±1.3	3.1 ±0.1	10.7 ±0.1	45.6 ±3.8	N.A.	N.A.
2	10 ±0.4	40.1 ±5.4	40 ±0	4.35 ±0.0	129 ±31	39.4 ±0.4	3.7 ±0.0	10.5 ±0.0	58.1 ±1.1	0.15 ±0	63 ±9
3	11 ±0.4	34.9 ±1.1	40 ±1	4.63 ±0.0	99 ±9	38.4 ±0.8	3.4 ±0.1	11.2 ±0.1	44.8 ±2.5	0.12 ±0.01	27 ±4
4	10 ±0.1	33.0 ±0.1	34 ±2	4.79 ±0.0	99 ±18	33.1 ±2.0	2.8 ±0.1	12.0 ±0.3	40.6 ±1.4	0.14 ±0.01	22 ±6
5 [a]	15 ±0.2	38.8 ±0.8	35 ±0	4.39 ±0.0	42 ±2	31.8 ±0.5	2.7 ±0.1	11.6 ±0.2	62.5 ±0.8	N.A.	N.A.
6	11 ±0.1	32.7 ±2.1	31 ±0	4.31 ±0.0	69 ±4	31.5 ±1.4	2.7 ±0.1	11.7 ±0.4	49.4 ±2.6	0.19 ±0.01	18 ±1
7	10 ±0.2	35.1 ±2.5	25 ±1	5.18 ±0.1	82 ±13	25.2 ±0.6	2.5 ±0.1	10.2 ±0.2	21.1 ±1.7	0.13 ±0.01	15 ±1
8	9 ±0.8	27.4 ±1.2	36 ±0	4.34 ±0.0	58 ±4	64.8 ±2.0	4.5 ±0.2	14.6 ±0.3	40.5 ±0.8	0.31 ±0.01	20 ±1
9	6 ±0.1	31.6 ±0.9	37 ±2	4.52 ±0.1	65 ±10	70.2 ±3.2	5.2 ±0.5	13.6 ±0.6	17.1 ±5.5	0.34 ±0.04	24 ±1
10	9 ±0.5	31.7 ±1.5	35 ±0	4.42 ±0.0	41 ±5	61.1 ±0.2	4.0 ±0.1	15.2 ±0.5	29.7 ±4.1	0.33 ±0.03	18 ±1
11	10 ±0.8	27.9 ±0.0	33 ±0	4.64 ±0.1	78 ±20	54.2 ±3.1	3.9 ±0.3	13.9 ±0.4	41.6 ±4.2	0.24 ±0.02	18 ±1
12	10 ±0.4	28.9 ±0.3	33 ±1	4.54 ±0.0	75 ±12	58.6 ±2.5	4.1 ±0.2	14.5 ±0.1	25.8 ±1.5	0.28 ±0.03	19±4

[a] No fresh soil was available

WHC, water-holding capacity; EC, Electrical conductivity; EOC, Extractable organic carbon; EON, Extractable organic nitrogen; C:N, carbon-to-nitrogen ratio.

Table S4.8-5. Microbial biomass C (MBC) and N (MBN), extractable nitrates (NO_x), number of bacterial and fungal OTUs, and alpha diversity indices calculated from ARISA OTU abundances. Values are the mean of three replicates ± standard error.

Paddock	MBC ($\mu g\ g^{-1}$ soil)	MBN ($\mu g\ g^{-1}$ soil)	NOx ($\mu g\ g^{-1}$ soil)	Fungal:bacterial abundance ratio (Total gene copy ratio)	Bacterial richness (Number of OTUs)	Fungal richness (Number of OTUs)	Alpha diversity (Bacteria)		Alpha diversity (Fungi)	
							Simpson's Index (D_2)	Simpson's evenness	Simpson's Index (D_2)	Simpson's evenness
1 [a]	N.A.	N.A.	NA	0.1 6±0.01	52	14	2.6 ±0.1	0.44 ±0.05	1.1 ±0.3	0.67 ±0.1
2	0.83 ±0.06	34 ±7	49 ±3	0.19 ±0.01	45	11	2.9 ±0.0	0.57 ±0.01	0.6 ±0.1	0.57 ±0.11
3	0.81 ±0.04	66 ±3	24 ±3	0.11 ±0.03	59	15	2.6 ±0.2	0.44 ±0.02	1.1 ±0.1	0.51 ±0.07
4	0.61 ±0.03	42 ±1	18 ±5	0.14 ±0.01	44	13	2.6 ±0.1	0.43 ±0.02	1.0 ±0.3	0.60 ±0.09
5 [a]	N.A.	N.A.	NA	0.19 ±0.02	51	18	2.9 ±0.1	0.56 ±0.04	1.2 ±0.2	0.50 ±0.04
6	0.55 ±0.03	43 ±1	5 ±0	0.13 ±0.02	55	12	3.1 ±0.1	0.63 ±0.01	1.1 ±0.2	0.77 ±0.13
7	0.68 ±0.06	61 ±9	7 ±1	0.12 ±0.02	49	6	2.8 ±0.1	0.53 ±0.01	0.7 ±0.1	0.78 ±0.1
8	0.66 ±0.09	52 ±10	4 ±1	0.32 ±0.05	57	15	2.6 ±0.2	0.42 ±0.01	1.6 ±0.1	0.67 ±0.06
9	0.88 ±0.10	74 ±8	5 ±1	0.16 ±0.01	58	21	2.6 ±0.2	0.42 ±0.01	1.2 ±0.3	0.48 ±0.1
10	0.54 ±0.07	42 ±5	2 ±0	0.16 ±0.02	63	17	3.1 ±0.1	0.59 ±0.04	1.7 ±0.1	0.67 ±0.06
11	0.74 ±0.05	50 ±2	5 ±1	0.13 ±0.01	57	16	2.7 ±0.2	0.44 ±0.05	0.9 ±0.1	0.38 ±0.1
12	0.61 ±0.03	45 ±5	6 ±3	0.21 ±0.03	52	17	2.9 ±0.0	0.53 ±0.01	1.2 ±0.2	0.38 ±0.02

[a] No fresh soil was available

4.8.4 Supplementary figures

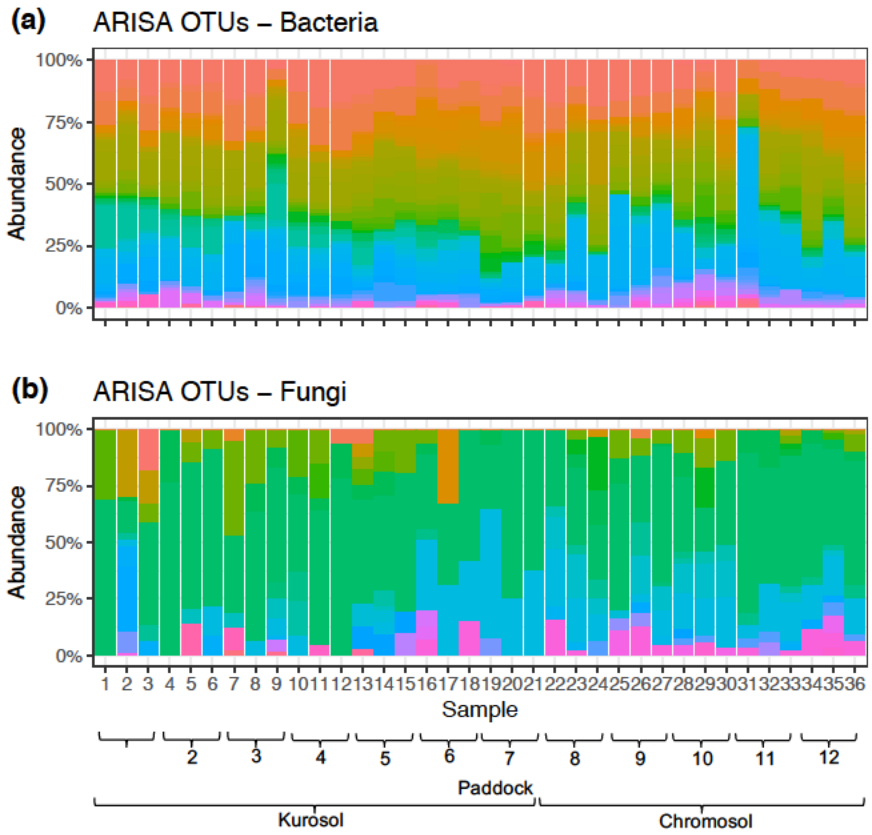


Figure S4.8-1. Stacked barcharts of ARISA relative OTU abundances for all 36 surface soils, which were samples across 12 paddocks at 3 field replicates A total of 146 bact rial OTUs and 53 fungal OTUs are represented.

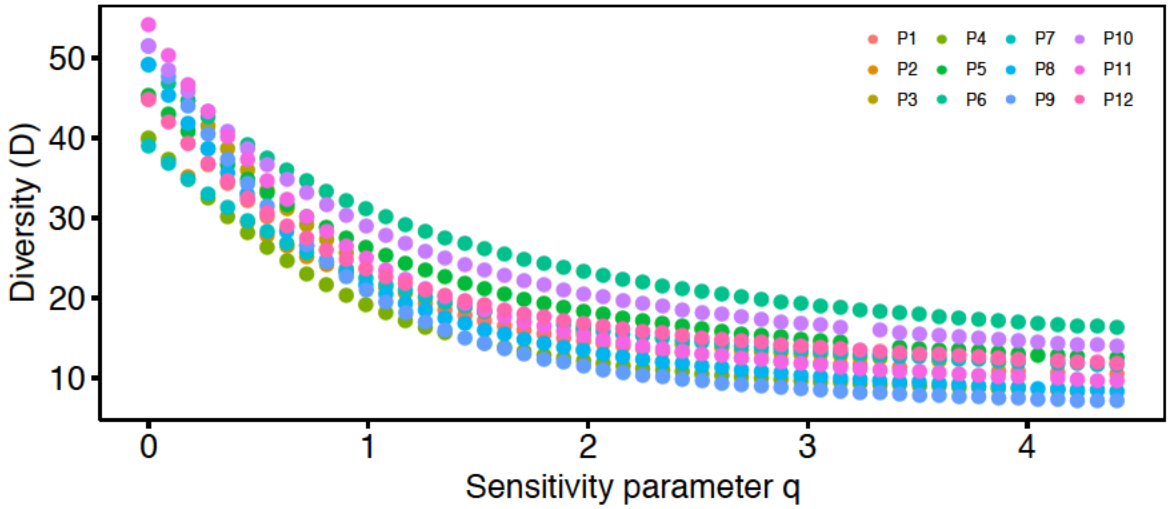


Figure S4.8-2. Diversity profiles of 12 paddocks (P1-P12) across varying levels of sensitivity to rare species assuming that all OTUs are different to each other (naive). q = zero represents individual species. As q increases, the diversity measurement becomes less sensitive to rare species and more sensitive to dominant species. q = 2 represents diversity values (D) of the Simpson's index.

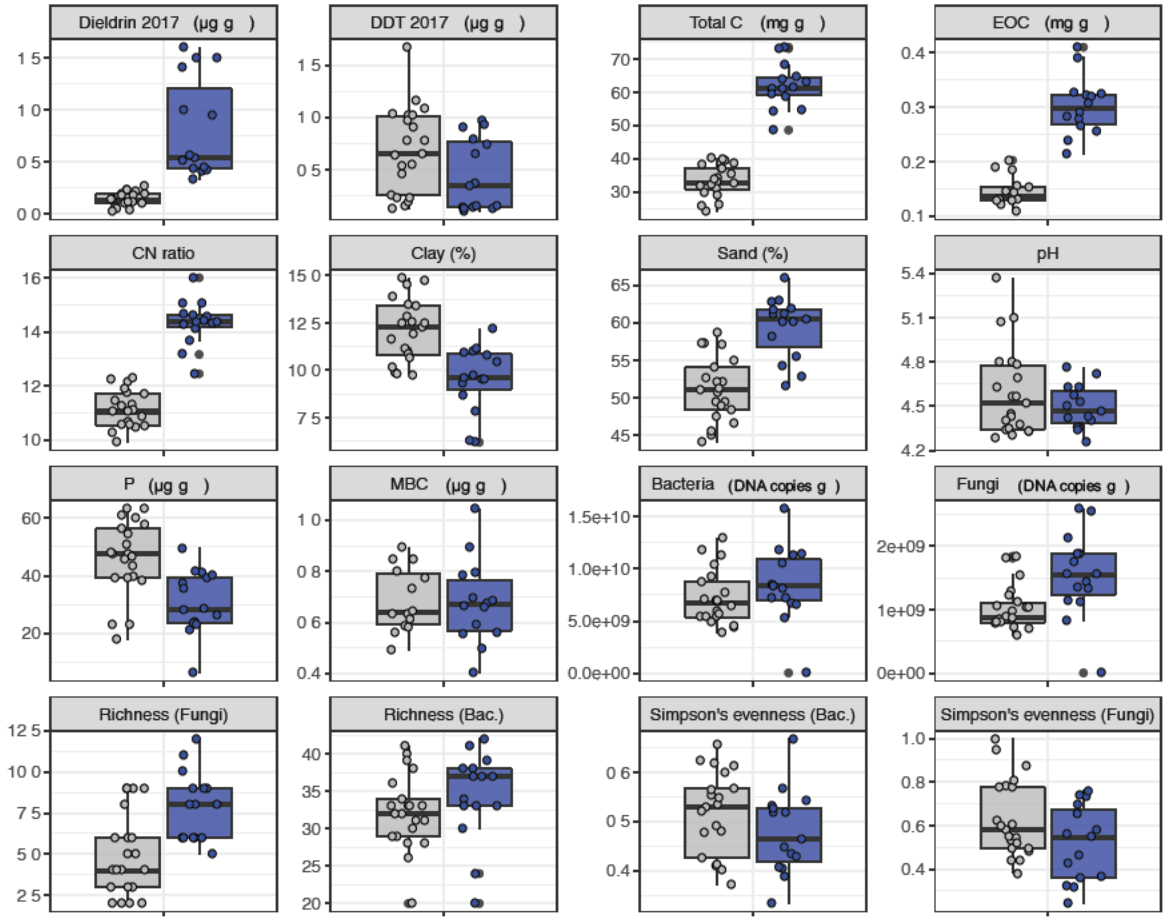


Figure S4.8-3. Boxplots of soil variables measured in this study are presented by soil type, including microbial biomass carbon (MBC), extractable organic carbon (EOC), Olsen phosphorus (P), number of OTUs (Richness) and microbial abundance (DNA copies g^{-1}). Bars show the maximum (top edge) and minimum (lower edge) percentiles, and boxes the 25% and 75% percentiles across crops, soils and years. The median (50%) percentile is represented by the horizontal line within the box.

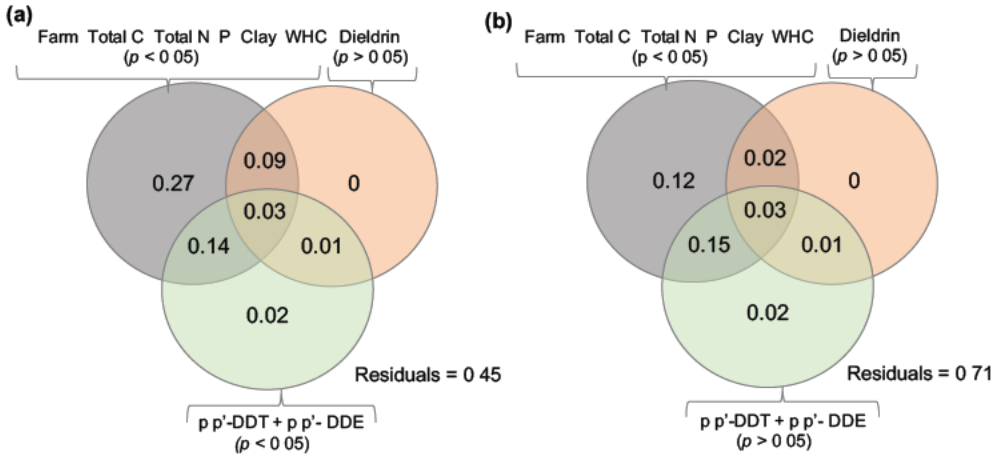
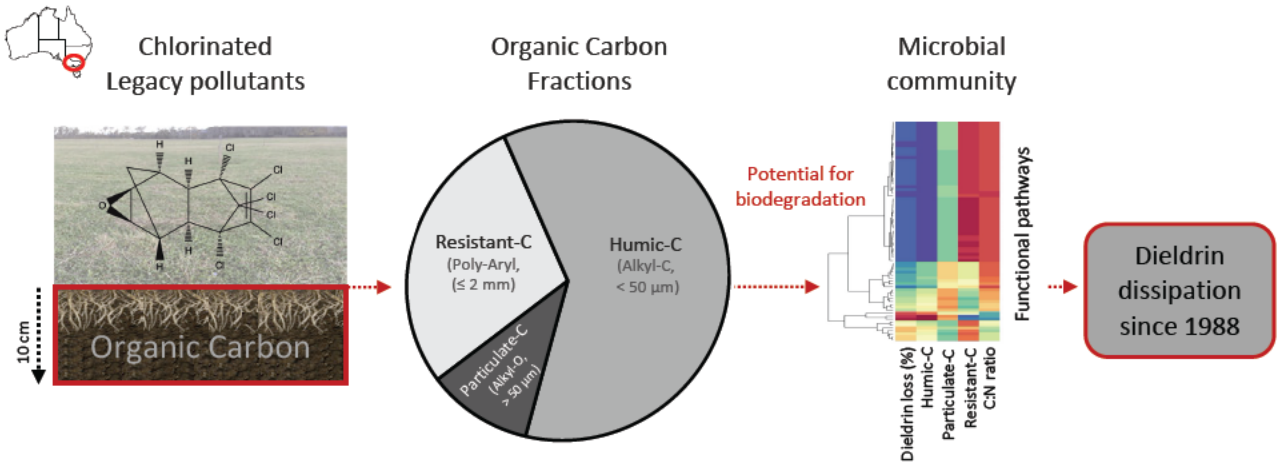


Figure S4.8-4. Variation partitioning of 146 bacterial OTUs (a) and 53 fungal OTUs (b). R^2 values are shown in circles that explain how much of the variation in microbial composition can be explained by the set of variables in each circle.

Chapter 5: Highly decomposed organic carbon mediates the assembly of soil communities with traits for the biodegradation of chlorinated pollutants

This chapter has been published in Journal Hazardous Materials, 2021, volume 404
https://doi.org/10.1016/j.jhazmat.2020.124077



5.1 HIGHLIGHTS

- A resource-limiting soil favors dieldrin dissipation.
- The persistent organic carbon pool is associated with great biodegradation potential.
- Supply of labile carbon delays biodegradation of chlorinated pollutants.

5.2 ABSTRACT

To improve biodegradation strategies for chlorinated pollutants, the roles of soil organic matter and microbial function need to be clarified. It was hypothesised that microbial degradation of specific organic fractions in soils enhance community metabolic capability to degrade chlorinated pollutants. This field study used historic records of dieldrin concentrations since 1988 and established relationships between dieldrin dissipation and

soil carbon fractions together with bacterial and fungal diversity in surface soils of Kurosol and Chromosol. Sparse partial least squares analysis linked dieldrin dissipation to metabolic activities associated with the highly decomposed carbon fraction. Dieldrin dissipation, after three decades of natural attenuation, was associated with increased bacterial species fitness for the decomposition of recalcitrant carbon substrates including synthetic chlorinated pollutants. These metabolic capabilities were linked to the decomposed carbon fraction, an important driver for the microbial community and function. Common bacterial traits among taxonomic groups enriched in samples with high dieldrin dissipation included their slow growth, large genome and complex metabolism, which supported the notion that metabolic strategies for dieldrin degradation evolved in an energy-low soil environment. The findings provide new perspectives for bioremediation strategies and suggest that soil management should aim at stimulating metabolism at the decomposed, fine carbon fraction.

5.3 INTRODUCTION

A large variety of chemistries of chlorinated pesticides were marketed globally and used extensively for crop protection with peak production around 1955 [3]. International efforts were made to eliminate the use of the most hazardous persistent organic pollutants [179] but these organic pollutants persist in agricultural soils today and continue to limit land-use options while posing long-term health risks [270,271]. Persistent organic pollutants include highly-chlorinated synthetic pesticides, such as dieldrin, and are resistant to microbial degradation leading to extremely slow biodegradation rates in soils. Biodegradation is the most important process for minimising risks of these type of soil pollutants and fundamental for bioremediation technologies and natural attenuation strategies [272,273]. However, it has been recognised that microbial biodegradation of hydrophobic and chlorinated organics is governed by retention processes and linked to the molecular composition of soil organic matter [25,70,274]. More knowledge is needed to understand how management of soil organic matter composition is associated with the *in-situ* biodegradation processes of these recalcitrant compounds.

A previous field study showed two agricultural pasture soils (0−10 cm) displayed significantly different abilities to dissipate dieldrin after three decades despite having similar soil characteristics with the exception of location and organic matter content [271]. These soils presented an opportunity to investigate factors for dieldrin dissipation revealing that low soil C/N and high microbial-C-to-total-C ratio [233] were associated with greater dissipation of aged dieldrin after three decades of natural attenuation [271]. This suggests that capabilities for the degradation of dieldrin, one of the most persistent organic pollutants, can be predicted by the presence of persistent soil organic matter and by greater microbial C assimilation efficiencies [275]. From laboratory-scale studies, it has emerged that more refractory C sources such as alkali soil extracts provide substrate for a diverse microbial community with greater metabolic capabilities for the degradation of synthetic organic pollutants [276]. It is further evident that persistent or highly decomposed C fractions

impose a stronger influence on the degradation of chlorinated pollutants compared to non-chlorinated pollutants, and that microbial community composition is intimately connected to soil C decomposability [25]. Thus, organic matter composition influences the effectiveness of microbial bioremediation strategies utilising organic matter amendments. Currently, it remains undetermined which factors may increase the effectiveness of microbial bioremediation strategies for chlorinated pollutants in surface soils aged over several decades.

This study aimed to improve understanding how *in-situ* biodegradation of dieldrin was associated with soil organic matter fractions, microbial diversity and functional profiles. We assessed the relationship of three carbon fractions with bacterial and fungal diversities as well as the metabolic profiles estimated from bacterial marker genes using PICRUSt2 [277]. It was hypothesised that microbes in soils, which comprised of more decomposed C materials had greater metabolic capabilities to degrade dieldrin.

5.4 MATERIALS AND METHODS

5.4.1 Site, soil description and dieldrin and total carbon measurements

Two agricultural surface soils (0−10 cm) of a Kurosol (n = 21) and a Chromosol (n = 15) [190], which comprised of twelve grazed pastures that were subject to the Australian National Organochlorine Residue Management Plan [5] were sampled in April/May 2017. Each sample was a composite of 8−10 soil cores representing a field replicate. Site location and details, sampling procedure and soil physicochemical characteristics were described previously [271]. The Kurosol and Chromosol consisted of seven and five fenced paddocks, respectively, for which detailed records of dieldrin concentrations since 1988 were obtained. The long-term dieldrin loss (%) per paddock was calculated using the difference of the average dieldrin concentrations from 2015/2017 (D15-17). Climate data for the paddocks in the northeast (Kurosol) and southeast (Chromosol) of Victoria was obtained from the nearest weather stations of the Bureau of Meteorology (http://www.bom.gov.au/climate/data/, accessed 22 May 2020) and are available in Table S5.8-1.

Dissipation of dieldrin since 1988 has shown to be significantly greater in the Kurosol compared to the Chromosol (Kurosol 73 ±1.5 %, Chromosol 42 ±3.4 %) [271]. It is important to note that residues of dichlorodiphenyltrichloroethane (p,p'-DDT) and its transformation product dichlorodiphenyldichloroethylene (p,p'-DDE) were also present in both soils but were not part of this study. Only dieldrin was applied to all paddocks until 1988, hence dissipation could be estimated more reliably for dieldrin. From the available data, it was found that p,p'-DDT dissipation since 1988 was also greater in the Kurosol (Kurosol 71 ±1.98%, Chromosol 43.7 ±5.84 %) [271]. Both soil types consisted of a similar texture (Clay, 12 ±0.4 and 10 ±0.4 %; Silt, 37 ±0.7 and 31 ±0.8 %; Sand, 51 ±0.9 and 59 ±1.1 % for Kurosol and Chromosol, respectively), had the similar pH (4.59 ±0.07 and 4.49 ±0.04 (Kruskal-Wallis p = 0.60), for Kurosol and Chromosol, respectively) and were both in the high-rainfall zone with similar annual maximum temperatures (20.9–21.9 ˚C) [271]. Total organic C significantly differed between the two soils (Kurosol 33.3

±1.05 mg g^{-1}, Chromosol 61.8 ±1.72 mg g^{-1}), and both the C/N and the microbial-C-to-total-C ratios strongly correlated with dieldrin dissipation (R^2 = 0.89 and 0.93, respectively) [271]. For subsequent analyses, samples were grouped into four categories based on long-term dieldrin loss (%) where the loss was either below or above the median dieldrin loss for each soil type with uneven sample sizes per group.

5.4.2 Soil organic carbon fractions

Soil organic C fractions were predicted from diffuse reflectance spectra. Soil processing and spectra acquisition followed the method set out in Madhavan et al. (2016). Briefly, air-dried soils were sieved (≤ 2 mm) and finely ground in a ball mill (Retsch MM400, Germany). Diffuse reflectance spectra in the mid and near-infrared (MNIRS) spectra (7800−450 cm^{-1} at 8 cm^{-1} resolution) were acquired for all samples using a PerkinElmer Frontier FT-NIR-MIR Spectrometer (PerkinElmer Inc., Waltham, MA, USA) equipped with a KBr beam-splitter, a DTGS detector and AutoDiff automated diffuse reflectance accessory (Pike Technologies, Madison, WI, USA).

Spectra were pre-processed as described in Madhavan et al. (2017) and concentrations of total organic C (total-C), resistant organic C (resistant-C), humic organic C (humic-C) and particulate organic C (particulate-C) were predicted from MNIRS spectra of the Australian Soil Carbon Research Project (ScaRP) [133] using partial least squares (PLS) regression. From these spectra and methodology, resistant-C was defined as the proportion of char and lignin C (enriched in poly-aryl C) in both fine and coarse fractions, and considered to be biologically resistant. Particulate organic C was defined as the remaining coarse fraction (> 50 μm) and was considered to be relatively rich in carbohydrates (enriched in O-alkyl C) and the most biologically active and decomposable. Humic organic C was defined as the remaining fine fraction (< 50 μm) and considered to be low in carbohydrates and more decomposed (enriched with alkyl C) compared to particulate-C [133].

Estimated total-C concentrations from the PLS predictions were correlated with total-C measurements of the same samples using a dry combustion analyzer (Perkin Elmer 2400 Series II, R^2 = 0.98). Hydrogen (H) concentrations were also derived and the hydrogen-to-total-C ratio calculated as approximation for aliphatic C content in downstream analyses. Raw data are shown in Table S5.8-2. For all downstream analyses, resistant-C, humic-C and particulate-C were either transformed into percentages of total-C or into centered log-ratios.

5.4.3 Diversity measurements

DNA samples were obtained from the extraction of 0.25 g fresh soil using Powersoil DNA isolation kit (MoBio, Calsbad, USA) and stored at -20 ˚C. Marker genes were sequenced with 15% phiX control on the Illumina MiSeq platform (2 × 300) to determine bacterial and fungal diversity. For bacteria the V4 hypervariable region of the 16S-rRNA gene was targeted with primers 515F (GTGYCAGCMGCCGCGGTAA) / 806R (GGACTACNVGGGTWTCTAAT) [280]. For fungi, the Internal

Transcribed Spacer (ITS) region 2 [281] was amplified with primers FITS7 (GTGARTCATCGAATCTTTG)/ITS4 (TCCTCCGCTTATTGATATGC) [282].

The default settings of Qiime2 (v2020.2, https://qiime2.org) were used to assess the quality of paired end reads, trim primers, denoise and dereplicate sequences and filter chimeras (dada2) [283]. Sequences with number of expected errors >2 were discarded and 66% (bacteria) and 52% (fungi) of sequences were retained after filtering. A total of 19,918 and 4,712 unique amplicon sequence variants (ASVs) (bacteria and fungi, respectively) were identified. Qiime2 was then used to train primer-specific classifiers using Greengenes reference sequences (gg13.8 at 99% similarity) for bacteria (https://greengenes.secondgenome.com/) and UNITE reference sequences (v8 dynamic) for fungi (https://unite.ut.ee/) and the classifiers were then used to assign taxonomic classifications to ASVs with 'qiime feature-classifier classify-sklearn'.

For bacterial ASVs, a phylogenetic tree was created using the default options of the Qiime2 plugin q2-fragment-insertion that utilized SATé-enabled phylogenetic placements (SEPP) with the Greengenes 13.8 SEPP reference tree. The tree was subsequently used to calculate the faith phylogenetic diversity index and unifrac metric and for phylogenetic analysis of associations to dieldrin loss as outlined below.

Prior to subsequent community analyses three outliers were removed which belonged to a paddock that was not representative of the remaining paddocks in the Kurosol (final n = 33). To assess differences in diversity between samples with high and low dieldrin losses, alpha diversity indices were produced with phyloseq [284] on rarefied abundances (depth to minimum sample size; 32,417 reads) of bacteria, their predicted enzyme metagenome and fungi. The phyloseq package was used to make compositional comparisons of bacterial, enzyme and fungal abundances in principal component analysis (PCA) and redundancy analysis (RDA) after converting abundance tables into Aitchison distances [285]. ASVs were filtered to those ASVs and enzymes present in at least 25% of samples. This included 1465 and 1705 bacterial ASVs, and 398 and 403 fungal ASVs in the Kurosol and Chromosol, respectively, and further included 2093 enzymes in both soils. To assess the influence of C fractions on bacterial and fungal community composition, resistant-C, humic-C and particulate-C were used as constraining variables in RDA and significance of RDA constraints assessed using 999 permutation of a pseudoF statistic in the function `permutest` of the vegan package [225]. The functions 'betadisper' (vegan package) and 'leveneTest' (car package) were used to test for significantly different compositional dispersion between soils and variation of phylogenetic diversity between samples [286].

5.4.4 Enzyme metagenome associations to carbon fractions and dieldrin concentrations

The PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2) software [277] was used to predict the enzyme metagenome and metabolic pathways (MetaCyc, Karp, 2002) based on the bacterial ASVs. Briefly, the PICRUSt2 pipeline placed ASVs into an open-source reference tree based on prokaryotic genomes from the Integrated Microbial Genomes database [288], inferred gene

abundances per ASV and then predicted sample pathway abundances using MinPath. The full pipeline (https://github.com/picrust/picrust2/wiki/Full-pipeline-script) was run with default settings, except that the maximum Nearest Sequence Taxon Index (NSTI) was set to 0.65 in favor of greater accuracy of predictions. Prior to running the PICRUSt2 pipeline, the ASV table was filtered to those ASVs that were present in at least two samples and with a minimum frequency of 10 reads. The final table output comprised of 432 pathway abundances that represented the metabolic potentials of bacteria in each sample.

Sparse partial least squares analysis (sPLS), including sPLS discriminant analysis (sPLS-DA) of the mixOmics package [289] were chosen to explore associations between metabolic pathway potentials and soil variables of resistant-C, humic-C, particulate-C, C/N and dieldrin loss (%). The sPLS is a dimension reduction technique that integrates two high-dimensional data sets acquired from the same samples (here predicted pathway potentials and soil variables) to highlight general patterns of associations. These methods perform well when the number of samples (n) is smaller than the measured variables (p) and when variables exhibit multicollinearity. For detailed description of approach, see the supplementary section.

5.4.5 Analysis of phylogenetic associations to dieldrin losses

To test whether the presence of some bacterial clades was more likely associated with dieldrin loss, the package Phylofactor [290] was used to perform generalized phylofactorization with the mixed algorithm and a binomial distribution as described in the package tutorial (https://github.com/reptalex/phylofactor). Phylofactorization was chosen as it has the ability to detect similar shifts in microbial clades among different data (or soils) when mapped to the same phylogeny, even with minimal ASV overlap [291]. The analysis was done across both soils (n = 33) and then separately on each soil type (Kurosol, n=18; Chromosol, n=15). Any unidentified phyla and phyla with low prevalence (mean prevalence of 1 or < 100 reads) were removed. Remaining ASVs filtered to a minimum of 25 reads per ASV and converted to presence/absence, which was modelled as the response variable with dieldrin losses (%) as numeric predictors. Phylofactor partitioned the phylogeny along edges with different log-odds of presence using generalized linear models (default mixed algorithm) with binomial distributions. The identified clades were then mapped onto the phylogenetic tree using ggtree.

5.4.6 Network analysis of bacterial and fungal co-correlation

To investigate differences in community interactions of unique bacteria and fungi in the Kurosol and the Chromosol, co-correlation networks were created using sparse inverse covariance estimation for ecological association inference (Spiec-easi) [292] with glasso estimations. After filtering of ASVs to a minimum sample-presence of 25%, bacterial and fungal abundances were co-correlated using a stability approach to regularization selection (StARS) [293] with a threshold of 0.05 and 25 repetitions (other settings: nlambda = 30, lambda minimum ratio = 0.02). Network edges were processed in gephi [294] and nodes of taxa that were unique in samples with above median dieldrin losses were visualised. Furthermore, the average path lengths

were calculated and keystone taxa highlighted. Modularity was calculated [295] and strongly interacting bacteria and fungi (modules) identified. Module zero and four coincided with the presence of unique ASVs in samples with above or below median dieldrin loss (%). To investigate ecological preferences of these two groups of taxa, the standardised relative abundance (Z-score) of each module was computed and correlated to organic matter fractions (Spearman).

5.5 RESULTS

5.5.1 Carbon decomposition continuum

The two soils, Kurosol and Chromosol, differed in dieldrin dissipation, organic C composition and displayed different metabolic profiles. Samples with higher proportions of highly decomposed or lower quality C materials displayed greater capabilities for degradation of recalcitrant C materials including chlorinated hydrocarbons (Figures 5.5-1 and 5.5-2). The Kurosol contained significantly less total-C compared to the Chromosol (61.8 ±1.7 g kg^{-1} and 33.7 ±1.0 g kg^{-1}, respectively) but consisted of less charcoal and lignin-like materials (poly-aryl C) as indicated by the smaller resistant-C fraction (28 ±0.3 % and 24 ±0.6 %) (Fig. 3-1). The Kurosol had significantly greater hydrogen-to-total-C ratios but lower C/N than the Chromosol, indicating that overall the organic matter was enriched in aliphatic C sources and therefore more decomposed (Figures 5.5-1 and S5.8-1). This aliphatic C stemmed mostly from the humic-C fraction, which constituted the biggest C fraction (45 ±0.5 % and 51 ±1.0 % for Chromosol and Kurosol, respectively). The Kurosol also consisted of more fresh, decomposable material than the Chromosol, indicated by the larger particulate-C fraction (4.8 ±0.4 % and 9.5 ±0.6 % for Chromosol and Kurosol, respectively). Altogether, the organic matter in the Kurosol exhibited a higher metabolic potency for the degradation of chlorinated and aromatic carbon sources compared to the Chromosol, which coincided with increased dieldrin dissipation (Figure 5.5-2).

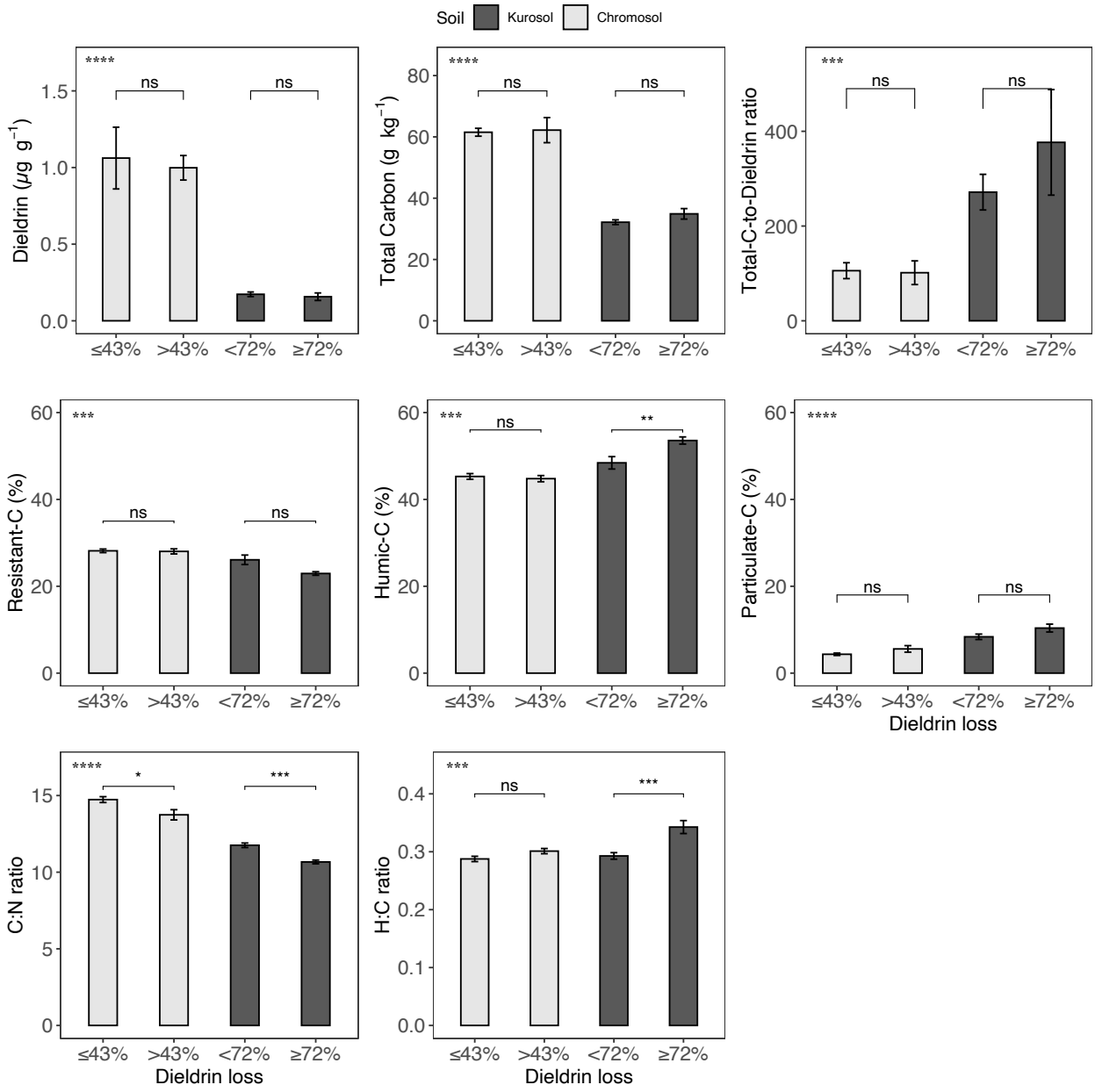


Figure 5.5-1. Comparisons of carbon quality variables between samples of low and high dieldrin losses. Samples are grouped into four categories (≤43 %, n = 9; >43 %, n = 6; <72 %, n = 9; ≥72 %, n = 12) based on long-term dieldrin loss (%) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Predictions of resistant organic carbon (resistant-C), humic carbon (Humic-C) and particulate organic carbon (particulate-C) are shown as percentages to total organic carbon. Significance levels of global Kruskal-Wallis tests for dieldrin loss categories (top-left of each panel) and Wilcoxon tests (above each group) for each soil type are shown (ns > 0.05; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001).

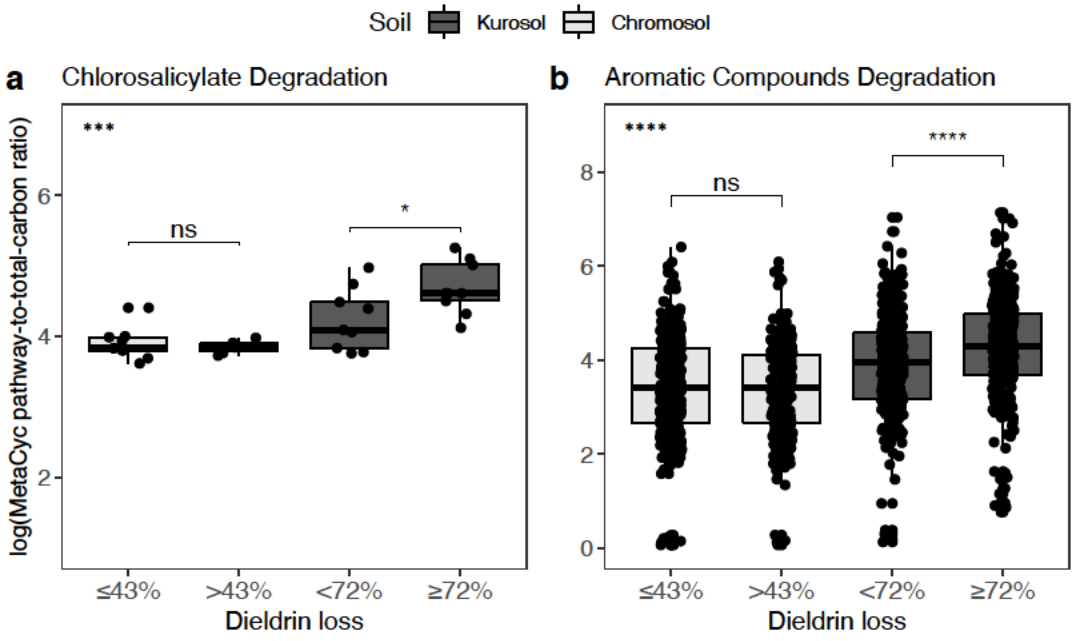


Figure 5.5-2. Comparison of degradation potentials of 1 chlorinated (a) and 39 aromatic compounds (b) predicted to be present in all samples (excluding outliers). Samples are grouped into four categories based on long-term dieldrin loss (≤43 %, n = 9; >43 %, n = 6; <72 %, n = 9; ≥72 %, n = 9) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Significances of global Kruskal-Wallis tests for dieldrin loss categories (top-left of each panel) and Wilcoxon tests (above each group) for each soil type are shown (ns > 0.05; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001).

5.5.2 Associations of MetaCyc pathway potentials with dieldrin dissipation

Abundances of 260 metabolic pathway potentials, which contributed to variations in the first three latent components in sPLS-DA, were predictive of dieldrin dissipation. Of those metabolic potentials, 10 degradation pathways (four carbohydrate, one alcohol, one carboxylate and two polyamine degradation pathways) and a vitamin biosynthesis pathway contributed most to component loadings (Figure 5.5-3). Correlations between metabolic pathways and C fractions from loadings of the first two components from sPLS further showed that the potentials for fucose, rhamnose, lactose, galactose and glycol degradation were most strongly associated with high dieldrin dissipation (Table 5.5-1, Figure S5.8-2). In contrast, oxidative glucose degradation was associated with low dieldrin dissipation since 1988 (Figure 5.5-3).

Table 5.5-1. Top correlation coefficients between dieldrin loss and soil resistant organic carbon (ROC), humic organic carbon (HOC) and particulate organic carbon (POC) and carbon-to-nitrogen ratio (C/N) obtained from component loadings of sparse-partial-least-squares analysis in regression mode.

Metacyc Pathway	Dieldrin loss[a]	ROC	HOC	POC	C/N
Sparse partial least squares model for Chromosol and Kurosol samples (n = 33)					
3-phenylpropanoate degradation	**-0.89**	0.77	-0.42	-0.78	0.90
4-aminobutanoate degradation V	**-0.87**	0.76	-0.47	-0.75	0.87
Homolactic fermentation	**-0.85**	0.77	-0.55	-0.72	0.86
Protocatechuate degradation II (ortho-cleavage pathway)	**-0.85**	0.71	-0.29	-0.76	0.86
Aromatic biogenic amine degradation (bacteria)	**-0.85**	0.73	-0.36	-0.75	0.86
Superpathway of fucose and rhamnose degradation	**0.88**	-0.74	0.33	0.78	-0.89
Superpathway of glycol metabolism and degradation	**0.88**	-0.74	0.34	0.78	-0.89
Sparse partial least squares model for Kurosol samples (n = 18)[b]					
Ubiquinol-8 biosynthesis (prokaryotic)	**-0.86**	0.47	-0.81	-0.21	0.73
tRNA processing	**-0.86**	0.50	-0.82	-0.23	0.73
Superpathway of ubiquinol-8 biosynthesis (prokaryotic)	**-0.86**	0.47	-0.81	-0.21	0.73
Inosine 5'-phosphate degradation	**-0.85**	0.56	-0.84	-0.28	0.72
Superpathway of b heme biosynthesis from glycine	**-0.85**	0.64	-0.86	-0.35	0.71
Superpathway of L-phenylalanine biosynthesis	**-0.85**	0.47	-0.81	-0.21	0.73
Superpathway of L-tyrosine biosynthesis	**-0.85**	0.47	-0.81	-0.21	0.73
Superpathway of heme-b biosynthesis from uroporphyrinogen-III	**-0.85**	0.63	-0.85	-0.34	0.71
Lactose and galactose degradation I	**0.80**	-0.70	0.84	0.41	-0.66
L-arginine degradation (Stickland reaction)	**0.83**	-0.62	0.83	0.33	-0.69

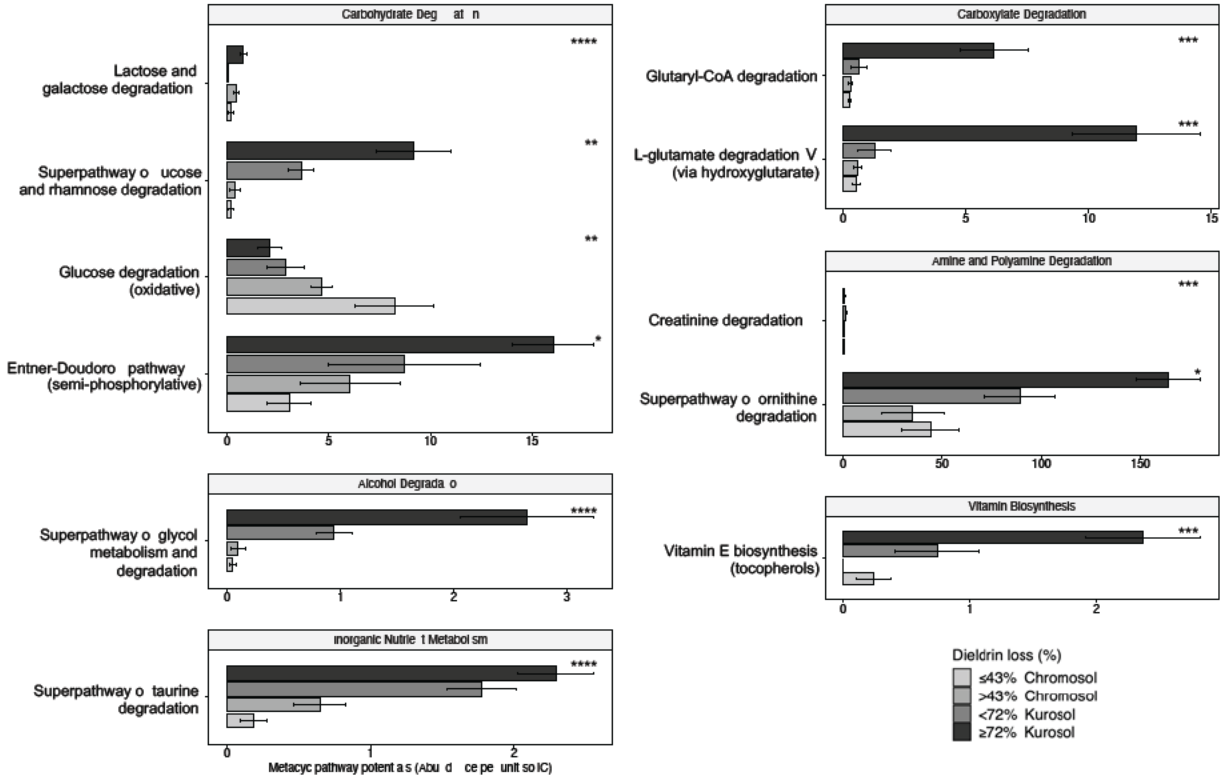


Figure 5.5-3. MetaCyc pathway potentials (Pathway abundances per g soil carbon) that were key predictors for dieldrin loss (%) in sparse-partial-least-squares analysis using the mixOmics package. Samples are grouped into four categories based on long-term dieldrin loss (≤43 %, n = 9; >43 %, n = 6; <72 %, n = 9; ≥72 %, n = 9) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Significances of global Kruskal-Wallis tests (top-left) and Wilcoxon tests (above each group) for each soil type are shown (ns > 0.05; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; **** ≤ 0.0001).

5.5.3 Compositional associations to dieldrin loss and soil C fractions

The microbial community composition differed between the Kurosol and Chromosol (Figure S5.8-3) and greater dieldrin losses coincided with significantly higher bacterial and fungal richness, as well as enzyme-encoding genes (Figure S5.8-4) and greater microbial diversity per unit of dieldrin (Figures S5.8-5). While the phylogenetic composition of bacteria was similar in the Kurosol and in the Chromosol (Figures S5.8-6 to S5.8-8), only in the Kurosol were soil C fractions associated with the composition of bacteria, enzymes and fungi (Figure 5.5-4). Permutation tests for the joint effect of resistant-C, humic-C and particulate-C were significant for the Kurosol ($p < 0.001$) but not for the Chromosol ($p > 0.05$). The Kurosol community also contained a higher number of unique ASVs with greater phylogenetic dispersion, suggesting a greater phylogenetic versatility, which coincided with increased dieldrin losses (Figures S5.8-6 and S5.8-8).

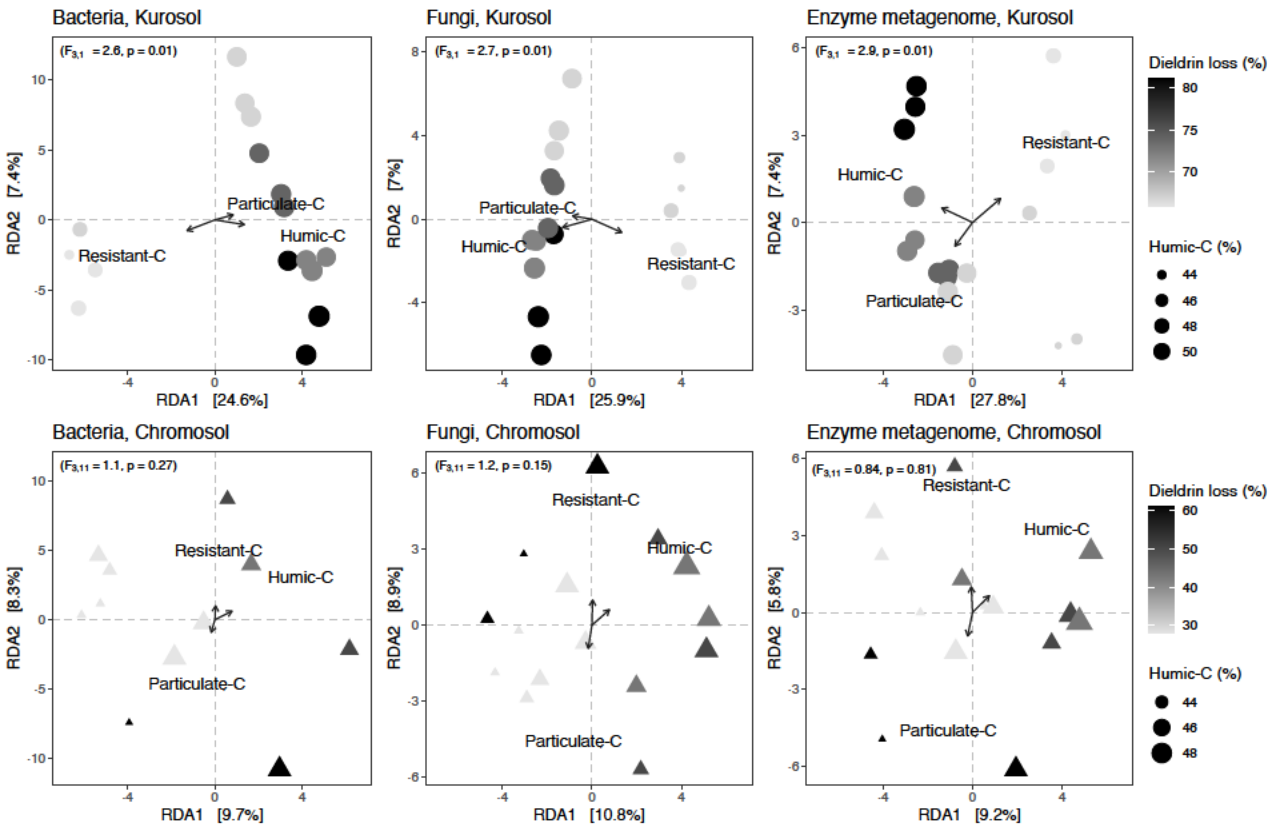


Figure 5.5-4. Redundancy analysis of bacterial and fungal abundances using the centered log-ratio transformed proportions of resistant organic carbon (Resistant-C), humic organic carbon (Humic-C) and particulate organic carbon (Particulate-C) as constraining variables. Symbols represent individual samples, and their proximity to each other indicates compositional similarity. The size and shape of symbols indicate the long-term dieldrin loss (%) of samples. Three samples from the Kurosol were removed as outliers (Kurosol, n = 18; Chromosol, n = 15).

Of the three soil C fractions, humic-C was the biggest driver of community function in the Kurosol. Individual permutation tests for humic-C ($p < 0.01$), resistant-C ($p = 0.07–0.18$) and particulate-C ($p = 0.19–0.45$) in the model indicated that humic-C was most important for bacterial, fungal and the metabolic compositions while particulate-C was least important (Table 5.5-2). The highest dieldrin dissipation (%) occurred in Kurosol samples where the microbial community and metabolic composition was associated with humic-C (Figure 5.5-4). An increase in humic-C was further associated with dieldrin loss and the metabolic potential for the degradation of lactose and galactose (Figure S5.8-2).

Table 5.5-2. Results of 999 permutations of a pseudo-F statistic on constraining variables resistant organic carbon (Resistant-C), humic organic carbon (Humic-C) and particulate organic carbon (Particulate-C).

	Kurosol				Chromosol			
Carbon Fraction	**df**	**Variance**	**pseudoF**	***p***	**df**	**Variance**	**pseudoF**	***p***
Bacteria								
Humic-C	1	980	2.65	**0.01**	1	528	1.34	0.11
Particulate-C	1	470	1.27	0.17	1	292	0.74	0.93
Resistant-C	1	576	1.56	0.09	1	310	0.79	0.85
Residual	14	5183			14	4345		
Fungi								
Humic-C	1	117	2.36	**0.01**	1	93	1.49	0.06
Particulate-C	1	60	1.21	0.19	1	45	0.72	0.96
Resistant-C	1	79	1.6	0.07	1	50	0.80	0.83
Residual	14	692			14	687		
Enzyme-encoding genes								
Humic-C	1	124	2.99	**<0.01**	1	70	1.03	0.35
Particulate-C	1	40	0.96	0.44	1	34	0.49	1.00
Resistant-C	1	54	1.3	0.18	1	46	0.69	0.92
Residual	14	582			14	745		

5.5.4 Associations of humic C with a group of bacteria and fungi and dieldrin dissipation

The combined abundance of a co-correlated group of bacteria and fungi in the Kurosol, was associated with humic-C concentrations (R^2 = 0.57***, Figures 5.5-5 and S5.8-9, Module zero). This cluster was also associated with dieldrin dissipation as it contained a group of bacteria and fungi, which were only found in samples with above median dieldrin loss (Figure 5.5-5). Bacterial interactions were generally dominant while fungal interactions increased in another cluster (Module four), which was associated with below median dieldrin loss (Figure 5.5-5; Table S5.8-3), indicating that fungal interactions were less influential on high dieldrin degradation.

A number of phylogenetic clades had a significantly higher presence in samples with high dieldrin loss as determined by generalised linear models with presence/absence as response variable (Table S5.8-4). These clades included Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes and *Geobacter sp.* (Table 5.5-3, Figure S5.8-7). Most of these were strongly representative of the humic-C-associated cluster (Module zero) of bacteria and fungi (Figure 5.5-5). For example, Actinobacteria in the order Solirubrobacterales were 12 times more frequent, while Planctomycetes in the order Gemmatales were 6 times more frequent in Module zero compared to Module four. Furthermore, Chloroflexi in the order of WCHB1-50 and Bacteroidetes in the order Bacteroidales were unique in Module zero. Of the fungal taxa, one group belonging to the order Hypocreales was the strongest representative for Module zero as it was 13

time more frequent there. The genera *Fusarium*, *Thelonectria*, *Trichoderma*, *Myxocephala* and *Monocillium* dominated in this group.

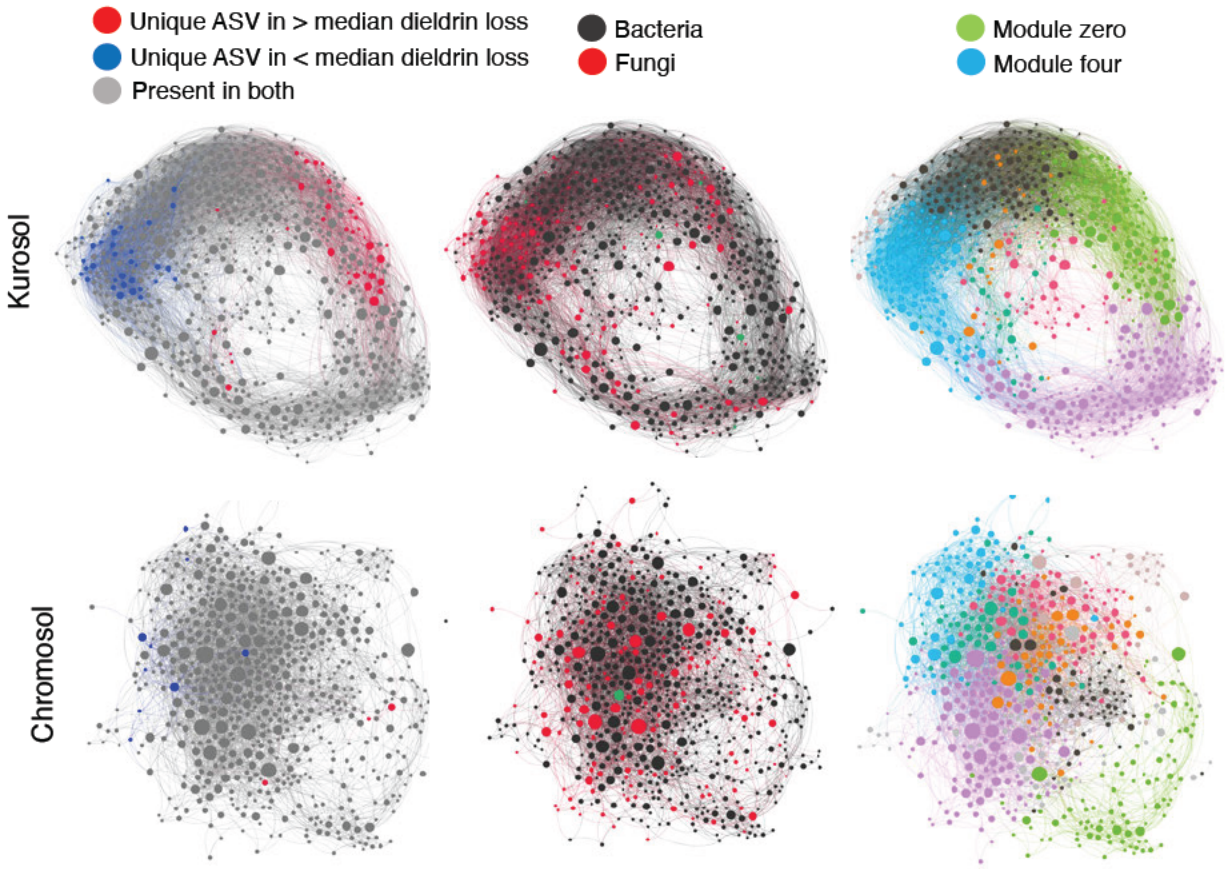


Figure 5.5-5. Co-occurrence network of fungi and bacteria in the Kurosol and the Chromosol using the SpiecEasi package (glasso) and modified in gephi. Edge lengths are proportional to distance between nodes (Force Atlas). Size of nodes indicate Betweenness Centrality. **Left**: Nodes highlight ASVs that were unique in sample groups with above or below median dieldrin loss (Median dieldrin loss was 43% and 72% in the Chromosol and Kurosol, respectively); **Middle**: Nodes are shown as either bacteria (dark grey) or fungi (Red); **Right**: Nodes are colored by modules that cluster together non-randomly (gephi modularity). The combined abundance of module zero in the Kurosol correlated with concentrations of humic carbon ($R^2 = 0.57$, $p<0.001$). Three samples from the Kurosol were removed as outliers (Kurosol, n = 18; Chromosol, n = 15).

Table 5.5-3. Richness of taxonomic groups that had a significantly higher chance of presence in samples with high dieldrin dissipation.

Phylum	Class	Order	≤43%[c]	>43%[c]	<72%[c]	≥72%[c]	Total ASVs
Acidobacteria Total (29-member Monophyletic clade)[a]			**24**	**17**	**51**	**129**	**221**
Acidobacteria	Acidobacteriia	Acidobacteriales[d]	24	17	51	129	221
Actinobacteria Total (18-member Monophyletic clade)[a]			**7**	**7**	**51**	**68**	**133**
Actinobacteria	Thermoleophilia	Solirubrobacterales	7	7	51	68	133
Bacteroidetes Total (17-member Monophyletic clade)[a]			**3**	**2**	**7**	**42**	**54**
Bacteroidetes	Unknown	Unknown	0	0	3	0	3
Bacteroidetes	Cytophagia	Cytophagales	1	0	2	3	6
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	2	2	2	4	10
Bacteroidetes	Bacteroidia	Bacteroidales	0	0	0	35	35
Chloroflexi Total (6-member Monophyletic clade)[a]			**0**	**0**	**2**	**31**	**33**
Chloroflexi	Ktedonobacteria	TK10	0	0	0	5	5
Chloroflexi	Anaerolineae	WCHB1-50	0	0	2	26	28
Planctomycetes Total (17-member Monophyletic clade)[a]			**4**	**2**	**20**	**67**	**93**
Planctomycetes	Planctomycetia	Gemmatales	4	2	20	67	93
Actinobacteria Total (589-member Monophyletic clade)[b]			**977**	**683**	**1171**	**1831**	**4662**
Actinobacteria	Actinobacteria		0	0	0	1	1
Actinobacteria	MB-A2-108	0319-7L14	0	8	9	7	24
Actinobacteria	Thermoleophilia	Gaiellales	221	168	311	385	1085
Actinobacteria	Thermoleophilia	Solirubrobacterales	213	141	255	406	1015
Actinobacteria	Actinobacteria	Actinomycetales	543	366	596	1032	2537
Bacteroidetes Total (55-member Monophyletic clade)[b]			**7**	**8**	**12**	**153**	**180**
Bacteroidetes	Unknown	Unknown	0	0	3	0	3
Bacteroidetes	Cytophagia	Cytophagales	1	0	2	3	6
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	3	3	5	9	20
Bacteroidetes	Bacteroidia	Bacteroidales	3	5	2	141	151
Chloroflexi Total (27-member Monophyletic clade)[b]			**10**	**14**	**23**	**94**	**141**
Chloroflexi	C0119	Unknown	3	2		3	8
Chloroflexi	Anaerolineae	H39	7	8	10	10	35
Chloroflexi	Ktedonobacteria	TK10	0	4	9	19	32
Chloroflexi	Anaerolineae	WCHB1-50	0	0	2	26	28
Chloroflexi	Anaerolineae	Anaerolineales	0	0	2	36	38
Firmicutes Total (44-member Monophyletic clade)[b]			**30**	**19**	**20**	**129**	**198**
Firmicutes	Clostridia	Thermoanaerobacterales	0	0	0	3	3
Firmicutes	Erysipelotrichi	Erysipelotrichales	0	0	0	6	6
Firmicutes	Clostridia	Clostridiales	30	19	20	120	189
Proteobacteria Total (24-member Monophyletic clade)[b]			**10**	**14**	**24**	**97**	**145**
Proteobacteria	Deltaproteobacteria	Desulfuromonadales[e]	10	14	24	97	145

5.6 DISCUSSION

5.6.1 Dieldrin dissipation was linked to a resource-limited soil environment

Two pasture surface soils were investigated, Kurosol and Chromosol, with differing potentials of dieldrin dissipation over the past three decades. An important finding was that metabolic capabilities of microorganisms for degradation of chlorinated pollutants were linked to adaptations to a resource-limited soil environment and driven by highly decomposed C materials. Samples that exhibited high dieldrin dissipation were enriched in aliphatic or lipid-rich C materials as indicated by the C/N and H-to-total-C ratios and humic-C proportions (Figure 5.5-1) [134,296,297]. We refer to humic-C as the pool of lipid-rich materials (< 50 μm), which were decomposed to small molecules and were energy-poor or physically-protected from microbial degradation [28]. Also known as persistent or mineral-associated organic matter, it is usually the largest fraction of soil organic matter and composed of a complex mixture of microbially-derived detritus [121,298]. Laboratory-scale studies, using synthetic alkali extracts to emulate this C fraction, showed that they provided substrate for a microbial diversity with greater metabolic capabilities for xenobiotic degradation [276]. This is consistent with this field study which showed that highly decomposed soil C (humic-C) coincided with increased potentials for degradation of chlorinated xenobiotics and aromatic compounds (Figure 5.5-2). It was concluded that dieldrin degradation processes were greater in a resource-limited soil environment containing low quality, energy-poor or more decomposed C materials.

This was further associated with increased C assimilation efficiencies, expressed as an increase in the microbial-C-to-total-C ratios [275], as our previous study showed strong correlations of total dieldrin dissipation to high microbial-C-to-total-C ratios ($R^2 = 0.93$) [271]. These increases in C-use efficiency were likely the result of more efficient maintenance processes of a microbial community that evolved in an energy-low soil environment. According to the model by Xu et al. [275], the efficiency with which microorganisms assimilate C into their biomass is determined by two factors: C assimilation efficiency and maintenance energy requirements over time. Carbon assimilation efficiencies increase with high C quality combined with low maintenance energy requirements. As the soil C quality was lower in samples with increased dieldrin losses, it was concluded that dieldrin degradation was associated with microbial strategies that increase maintenance efficiencies.

More efficient microbial activity can evolve from resource-limitations or seasonal stresses such as high temperatures and drought [275,299,300]. Seasonal stresses in the Kurosol can also explain the lower C stocks (Figure 5.5-1) as the maximum soil C content in a given location is mainly determined by maximum and minimum rainfall and temperature [301–303]. This was consistent with local climate data. In the last three decades, the King Valley region, where the Kurosol samples were taken, had greater extremes and variability in temperatures, rainfall and solar exposure (Table S5.8-1). Additionally, decadal floods occurred in seasons with heavy rainfalls. Such environmental stress has shown to increase phylogenetic dispersion of microbial communities [291], which agreed with our data showing an increase of phylogenetic variability with

increased dieldrin dissipation (Figure S5.8-8). This supported the suggestion that a stressed or resource-limited soil environment impacted on microbial degradation of dieldrin.

A resource-limited soil environment selects for microbial traits that maximise growth yield, a concept known as rate-yield tradeoff [304]. In the present study, it was observed that the potential for the Entner-Doudoroff pathway was predictive for high dieldrin dissipation (Figure 5.5-3). The Entner-Doudoroff glycolytic pathway is more common than originally thought [305] and it was found that it yielded less energy but required several-fold less enzymatic protein compared to the better known Embden–Meyerhof–Parnas pathway [306], potentially freeing up resources to invest in more complex degradation processes. It was therefore speculated that the limited access to energy and C materials selected for microbial traits, which aided in accessing energy-low materials, for example through the production of exoenzyme or extracellular glycolipids and that this increased co-metabolism of dieldrin.

5.6.2 The highly decomposed carbon C fraction was driving dieldrin dissipation

The persistent and highly decomposed C fraction played a significant role in the dissipation of dieldrin. Among the three C fractions, humic-C was the most significant driver for the microbial community and functional composition in the Kurosol (Figure 5.5-4, Table 5.5-2). There were unique associations between bacteria and fungi, which demonstrated a preference for this C pool as the combined abundance (Z-score) of a group of co-correlated bacteria and fungi (Gephi modularity, Module zero) increased with humic-C concentrations ($R^2 = 0.57$***, Figures 5.5-5 and S5.8-9). By contrast, in the Chromosol there was no effect of the C composition on the microbial community, suggesting that C quality was less important for community dynamics in this soil, which coincided with lower dieldrin dissipation (Figures 5.5-4 and 5.5-5). Moreover, functional potentials of bacteria in the Kurosol were associated to dieldrin loss and simultaneously associated to humic-C (Table 5.5-1, Figure S5.8-2), which further showed that decomposed C materials support soil functions that coincided with dieldrin degradation. Finally, the Kurosol, with greater dieldrin dissipation, contained less organic matter as well as lower proportions of charcoal or lignin materials (resistant-C), suggesting that irreversible sequestration of dieldrin was less pronounced compared to the Chromosol (Figure 5.5-1) [307]. As a result, interactions between humic-C and dieldrin molecules were likely more prevalent in the Kurosol.

Potentials of several metabolic pathways of the bacterial community gave further insights into microbial traits that were associated with highly decomposed C materials and dieldrin dissipation. The sPLS analysis showed that strongest predictors for high dieldrin losses included the metabolic potentials for degradation of microbial sugars such as fucose, rhamnose, lactose and galactose (Figure 5.5-3, Table 5.5-1). The concentration of microbial sugars in soils such as those from extracellular rhamnolipids or lipopolysaccharides in the cell membrane is typically five times lower than glucose [308–310]. Microbial sugars have been shown to be enriched in the fine C fraction and protected against microbial degradation, potentially through organo-

mineral associations [311,312]. This indicates that the higher microbial maintenance efficiencies in samples with high dieldrin dissipation were associated with metabolic adaptations to utilise microbially-derived C, which itself was associated with protected and highly decomposed materials. Microbial biomass itself becomes an important reservoir and buffer of nutrients during stress or resource-limited periods [313], hence the findings supports the idea that these conditions promote degradation of recalcitrant carbon materials including dieldrin. Taken together, dieldrin dissipation was associated with decomposed C materials, which mediated community assembly with traits that aided degradation of chlorinated organics.

These data imply that the provision of labile and decomposable C may inhibit biodegradation of chlorinated organics. This was also evident in a long-term field experiment studying the degradation of a chlorinated model compound (2,4-dichlorophenol (DCP)) [25]. Degradation of DCP was associated with the clay fraction (< 2 µm) that comprised a distinct microbial community [132], and it was found that farmyard manure reduced degradation of DCP as it reduced mass transfer of DCP to relevant microbial cells [25]. Thus, in agreement with our findings, which showed that dieldrin dissipation was associated with the finer C fraction, soil management should aim at stimulating metabolism of the decomposed fine C fraction. These findings offer new perspectives for natural attenuation strategies especially for agricultural surface soils that are contaminated with organochlorines since several decades. We speculate that disruption of the soil structure by frequent tillage is needed to stimulate biodegradation of dieldrin by indigenous microorganisms. The rational is that frequent tillage would give microorganisms access to non-labile C, including dieldrin and promote growth of relevant degraders. On the other hand, no-till practices and perennial pastures may delay dieldrin dissipation as labile C sources are constantly provided by rhizodeposites. These recommendations sit in contrast to common soil-health practices, which aim at increasing soil organic matter content. Controlled experiments are needed to verify if disturbance of soil structure and microbial communities promote the assembly of microorganisms with desired traits for degradation of chlorinated contaminants under these circumstances.

5.6.3 Microbial traits in samples with high dieldrin dissipation

Some taxonomic groups were more prevalent in samples with high dieldrin dissipation (Table 5.5-3, Figure S5.8-7). An assessment of their traits gave further insights into the potential role of highly decomposed C for the degradation of chlorinated pollutants in surface soils. To measure which phylogenetic groups related to dieldrin dissipation, probabilities of the presence of aggregated ASVs were modelled along the gradient of total dieldrin dissipation (Table S5.8-4) [314]. It was found that taxonomic groups, which were significantly associated with dieldrin dissipation (Phylofactor, $p < 0.001$), were also prevalent in the group of bacteria and fungi that were driven by humic-C concentrations (Figure 5.5-5). They included aerobic and anaerobic bacteria in the order Acidobacteriales, Solirubrobacterales, Sphingobacteriales, Bacteroidales, Gemmatales and a group of Chloroflexi and *Geobacter sp*. A similar bacterial assemblage was observed in microcosms supplemented with synthetic humic acids and harbored stronger and more diverse capabilities for degradation

of organic pollutants [19]. However, fungi were less abundant in the humic-C associated group in the Kurosol (Figure 5.5-5). Instead, fungi were more abundant in another group which was associated with below median dieldrin loss, indicating that fungi were less important for biodegradation of dieldrin (Table S5.8-3).

Common traits among these bacteria included their slow growth, large genome, complex metabolism, motility, radiation tolerance and desiccation resistance and these traits may have contributed to increased co-metabolism of dieldrin. Their complex metabolism enabled them to utilise a range of different C sources, including sugars such as fucose, rhamnose, lactose and galactose. For example, the metabolism of a clade in the order of Acidobacteriales, which comprised mostly of the genus *Candidatus Koribacter* (Table 5.5-3, Figure S5.8-7), is adapted for nutrient-poor environments and able to utilise complex C substrates at low concentrations [315]. Moreover, the Kurosol contained significantly higher frequencies of bacteria in the order Actinomycetales. Some genera in this order, such as *Pseudonocardia spp*. and *Rhodococcus spp.,* have shown to co-metabolise dieldrin and dichlorodiphenyltrichloroethane (DDT) [85,316]. Furthermore, a group of bacteria in the order Sphingobacteriales was more prevalent in the Kurosol, along with other Bacteroidetes. Some strains of *Sphingobacterium thalpophilum* are known for co-metabolic degradation of pentachlorophenol when grown on glucose [317] while other *Sphingobacterium sp.* were able to utilise DDT as a sole C source [114].

Further groups of strictly anaerobic Chloroflexi, known for their ability to dechlorinate hydrocarbons were unique in the Kurosol and most prevalent in samples with high dieldrin dissipation (Table 5.5-3, Figure S5.8-7). They belonged to the class Anaerolineae, which comprise filamentous thermophiles with diverse metabolic strategies, including reductive dechlorination of chlorinated hydrocarbons [318]. Lastly, clades of anaerobic Proteobacteria and Firmicutes, which are known to couple their growth to the reduction of humic substances, were significantly more prevalent in samples with high dieldrin dissipation (Table 5.5-3, Figure S5.8-7). Both the group of Proteobacteria in the order Desulfuromonadales, which comprised exclusively of *Geobacter spp*. and a group of bacteria in the order Clostridiales have shown to degrade chlorinated hydrocarbons in enrichment-culture and microcosms [319,320]. Humic substances acted as catalytic 'electron shuttles', which was coupled to Fe(III) reduction to enhance degradation of these pollutants [321]. Dieldrin dissipation was therefore associated with a microbial community with metabolic capabilities for degradation of complex C substrates including synthetic chlorinated pollutant.

5.7 CONCLUSION

The results from this field study add to our understanding of potential ecological drivers that affect natural attenuation or bioremediation strategies [272,273]. Two pasture soils, Kurosol and Chromosol, displayed differing potentials of dieldrin dissipation over three decades (72 % and 43 % median dieldrin loss, respectively). The study suggested that dieldrin dissipation was associated with microbial traits that evolved in an energy-low soil environment in which highly decomposed and persistent carbon materials (< 50 μm)

mediated microbial community assembly and function. This implies that the provision of labile C could slow down biodegradation of chlorinated organics and that soil management should aim at stimulating metabolism at the decomposed, fine carbon fraction. Field studies at scale are required to validate our findings and to evaluate the impacts of different soil management practices on biodegradation of chlorinated pollutants.

5.8 SUPPLEMENTARY MATERIALS

5.8.1 Sparse partial least squares analysis (sPLS) and sPLS discriminant analysis (sPLS-DA)

For sPLS, the predictor matrix (X) was composed of predicted pathway potentials, which were filtered to those present in at least 50% of samples resulting in a total of 410 pathways. This was followed by centered log-ratio transformations to account for compositionality of the data [322]. Matrix Y, the response matrix, was composed of resistant-C, humic-C, particulate-C, C:N and dieldrin loss (%) where the carbon fractions were also centered log-ratio transformed. The analysis was done in regression mode and the first two components were selected for analysis as they had sufficient predictive power based on the Q^2 cutoff of 0.0975 [323,324]. A heatmap with Ward clustering from the mixOmics package was used to display pair-wise associations between pathways and soil variables from the two components [289].

Additionally, we performed the supervised sPLS discriminant analysis (sPLS-DA) to select pathways that most predicted long-term dieldrin losses [325]. Samples were grouped into factors that were below and above the median dieldrin loss (%) of each soil, with a total of four factors representing "low" and "high" dieldrin loss in the Chromosol and Kurosol. After cross-validation based on the Balanced Error Rate [289], the first three components were selected with 70, 100 and 90 pathways, respectively. Kruskal-Wallis tests were done to test significant differences of mean abundances of metabolic pathways that contributed most to the three sPLS-DA components.

5.8.2 Supplementary Figures

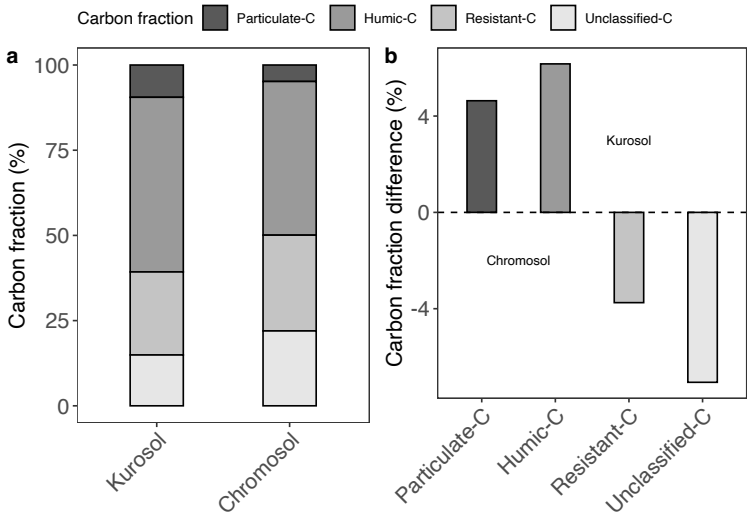


Figure S5.8-1. Comparisons of carbon fractions between Chromosol and Kurosol. Proportions of carbon (Particulate-C), humic organic carbon (Humic-C) and resistant organic carbon (Resistant-C) are shown by soil (a) and as the percent-difference of the Kurosol to the Chromosol (b). The residuals of prediction are denoted as 'Unclassified-C'.

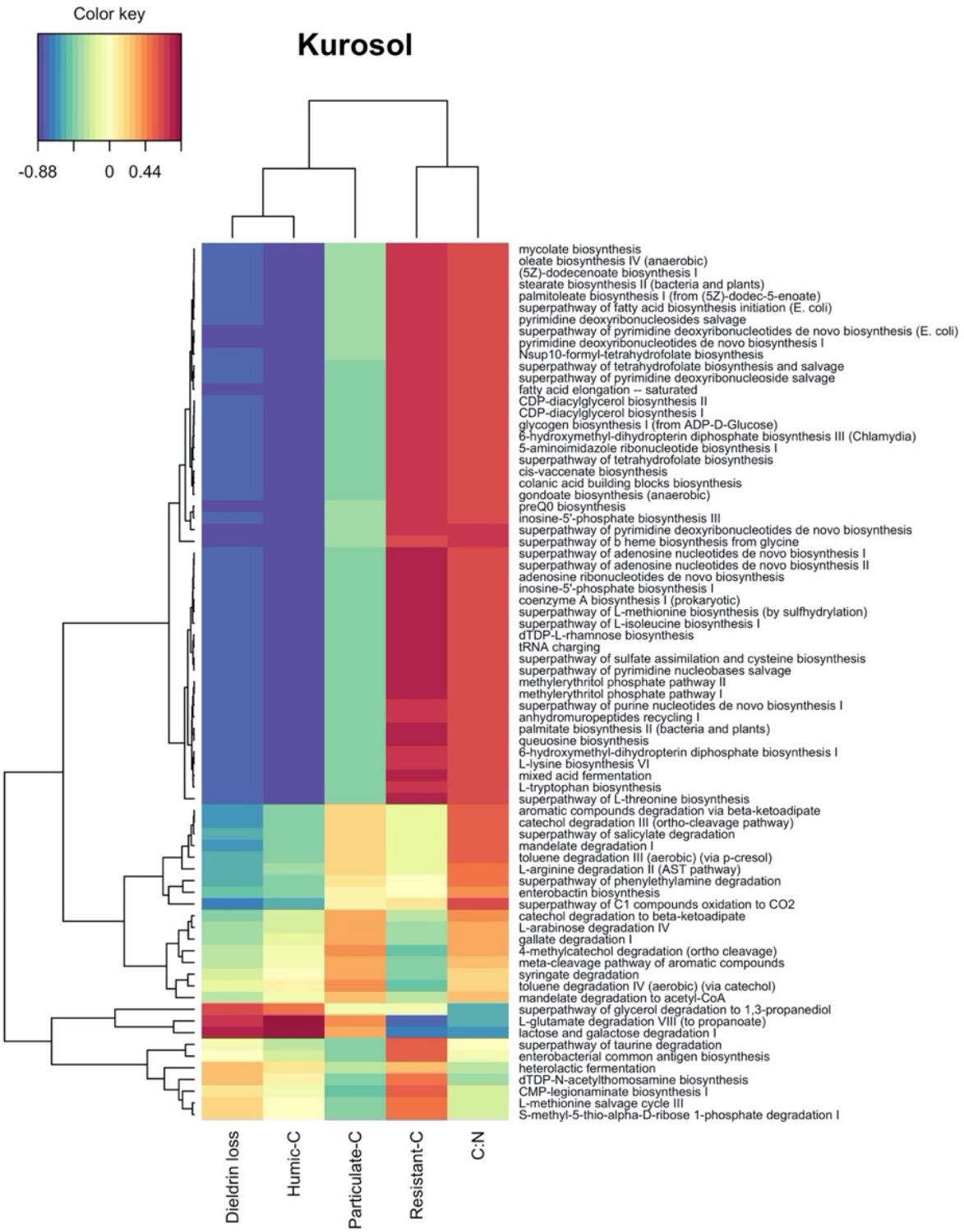


Figure S5.8-2. Ward clustered heatmap of similarity scores obtained from sparse partial least squares analysis in regression mode (R package mixOmics) for pathway potentials (rows) and soil variables (columns) for Kurosol samples. Soil variables included the long-term dieldrin loss (%), carbon-to-nitrogen ratios (C:N) and concentrations of resistant organic carbon (Resistant-C), humic carbon (Humic-C) and particulate organic carbon (Particulate-C). The pathway potentials and resistant-C, humic-C and particulate-C were centered log-ratio transformed before analysis.

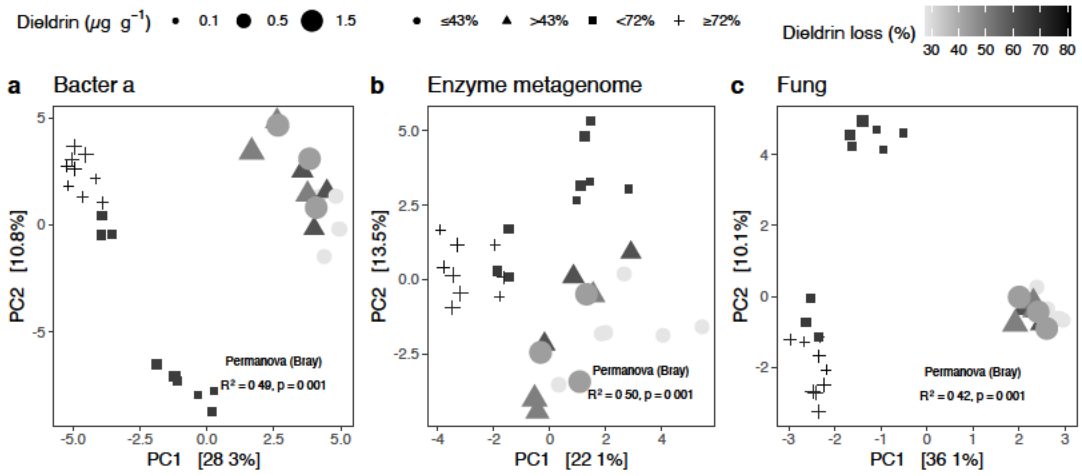


Figure S5.8-3. Ordinations of Aitchison distances were compared for compositions of bacteria and its enzyme metagenome as predicted by Picrust2 and fungi (n = 33). ASVs were filtered to those present in at least 25% of samples and included 1407 and 322 bacterial and fungal taxa, respectively, and 2091 enzyme encoding genes. Permanova results based on Bray-Curtis dissimilarities are shown. Symbols represent individual samples, and their proximity to each other indicates compositional similarity. The colour and size of symbols indicate the long-term dieldrin loss (%) and dieldrin concentrations, respectively. The shape of symbols indicates sample grouping into four factors where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively).

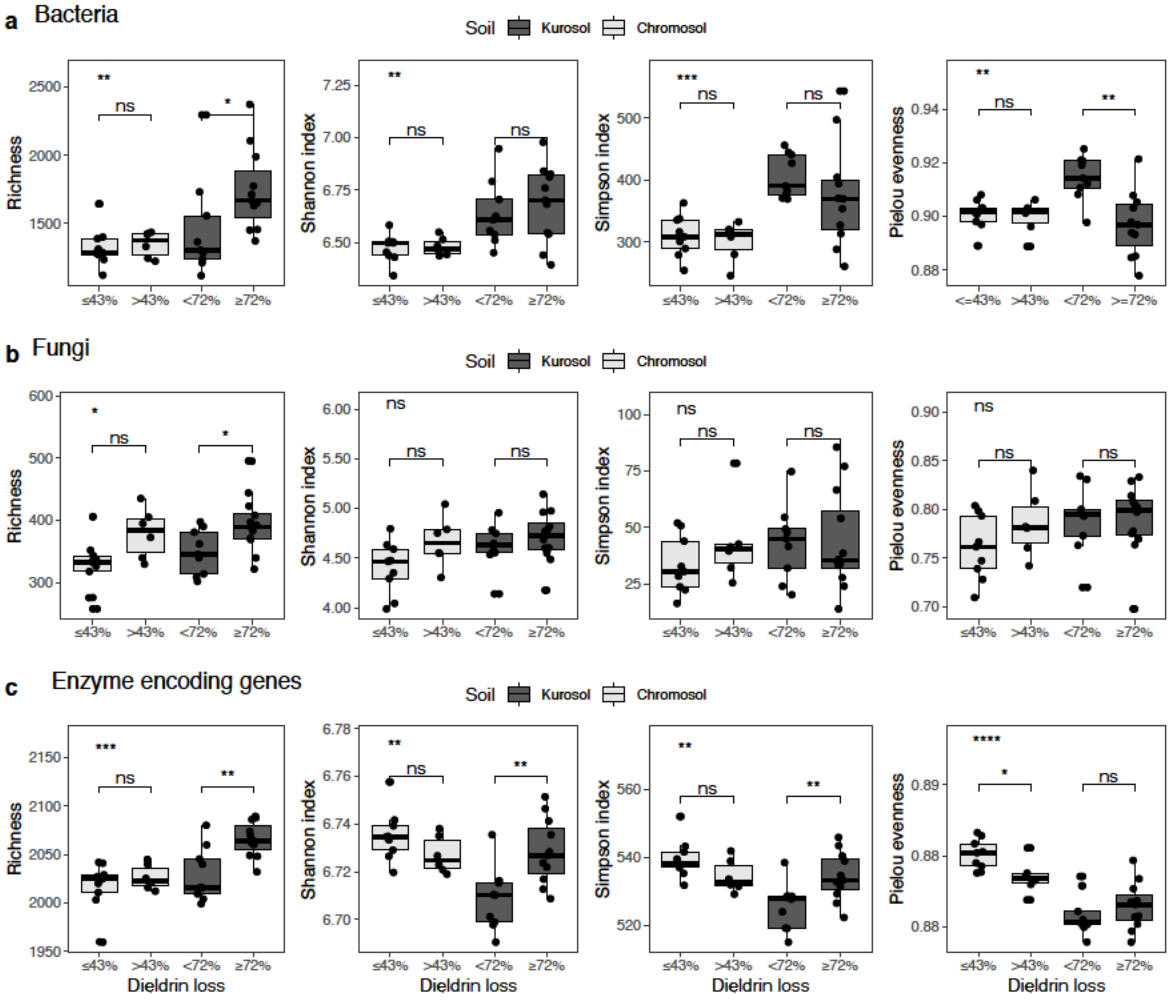


Figure S5.8-4. Alpha diversity of bacteria, fungi and the enzyme metagenome as predicted with Picrust2. Diversity indices were calculated from rarefied abundance of filtered amplicon sequence variants (ASVs) or enzyme abundances. Samples are grouped into four categories by long-term dieldrin loss (%) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Significance of global Kruskal-Wallis tests for dieldrin loss categories and Wilcoxon tests for each soil type are shown (ns, *, **, *** and **** represent p values of > 0.05, ≤0.05, ≤ 0.01, ≤ 0.001 and ≤ 0.0001, respectively).

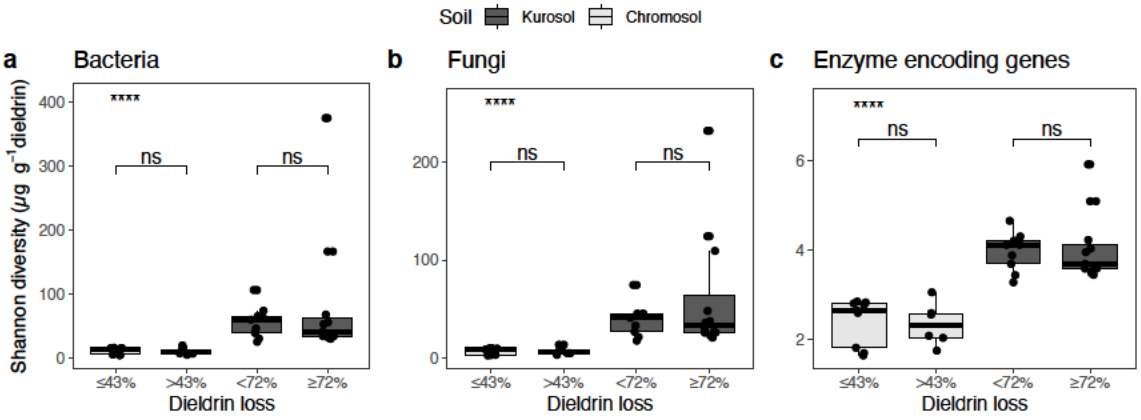


Figure S5.8-5. Shannon:dieldrin ratio of bacteria (a), fungi (b) and the enzyme metagenome as predicted from Picrust2 (c) per unit dieldrin (µg g^{-1}). Samples are grouped into four categories by long-term dieldrin loss (%) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Significance of global Kruskal-Wallis tests for dieldrin loss categories and Wilcoxon tests for each soil type are shown (ns, $p > 0.05$; **** $p \leq 0.0001$).

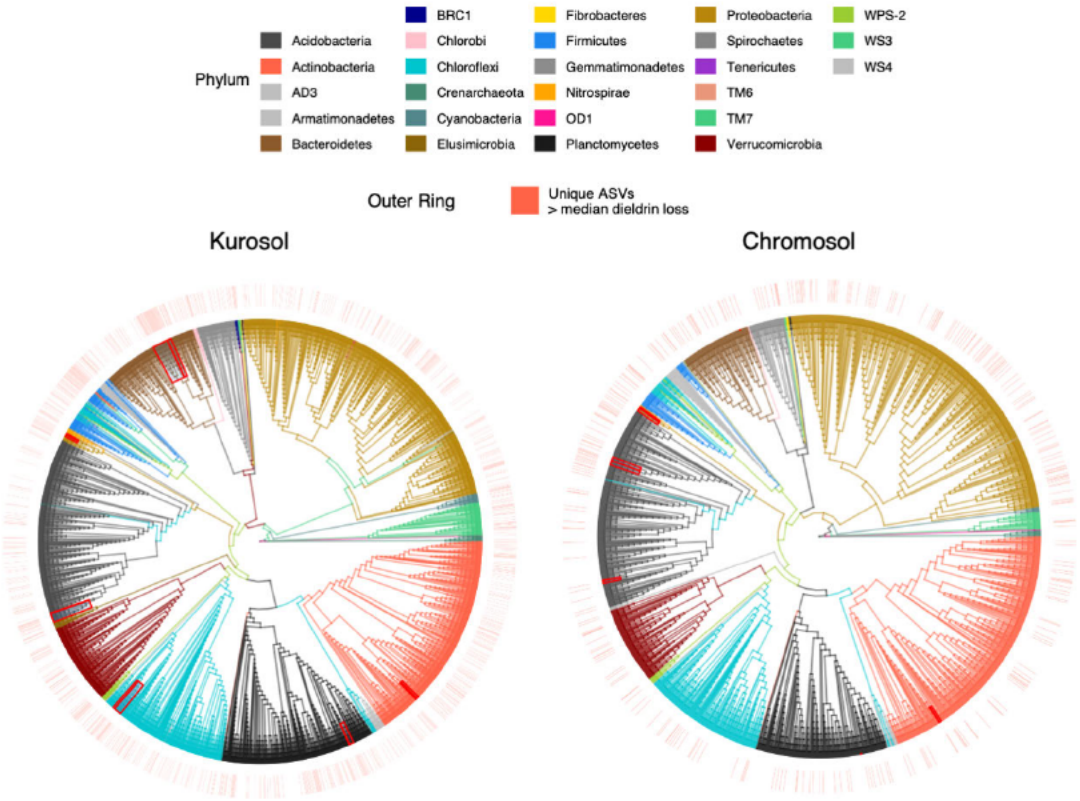


Figure S5.8-6. Soil comparison of phylogeny of all amplicon sequence variants (ASVs) present. Tips represent individual ASVs, and different colours indicate their phylum membership. Unique ASVs are shown in the outer ring where red indicates if ASVs were unique to a soil environment with above median dieldrin loss (%). On average 48 (Kurosol) or 21 (Chromosol) ASVs were unique in samples with above median dieldrin loss. Three outlier samples were removed prior to analysis (Kurosol, n = 18; Chromosol, n = 15). Polygons highlight phylogenetic clades with a higher chance of presence in 'high-dieldrin-loss' samples than could be explained by chance.

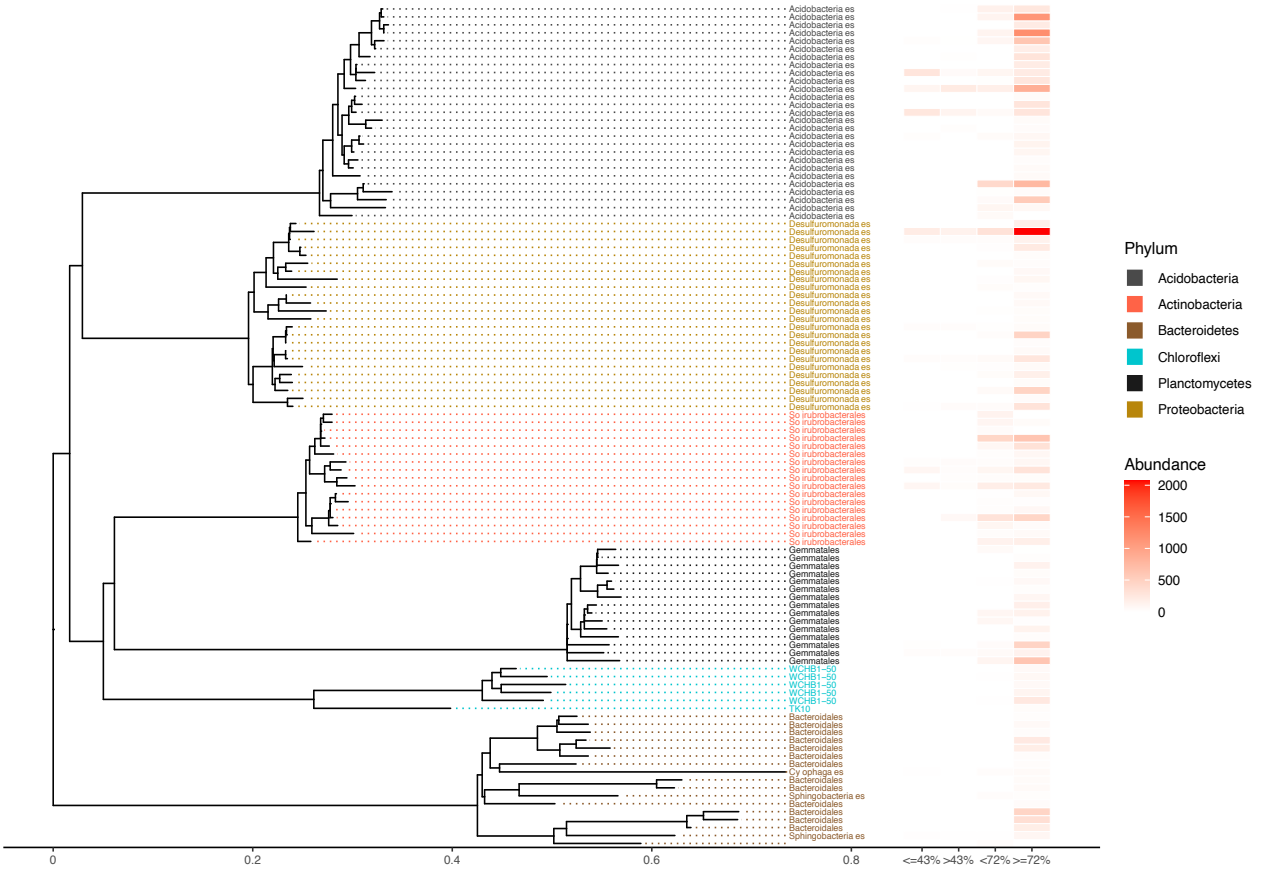


Figure S5.8-7. Phylogenetic clades with a higher chance of occurrence in 'high-dieldrin-loss' samples than could be explained by chance (Phylofactor generalised linear model with presence/absence as response variable). Samples are grouped into four categories based on long-term dieldrin loss (%) where the loss is either below or above the median dieldrin loss in each soil type (Median loss was 43% and 72% in the Chromosol and Kurosol, respectively). Prior to analysis, ASVs were filtered to those with a minimum of 10 reads. The phylofactor clade of order Desulfuromonadales comprised exclusively of the genus *Geobacter sp.*

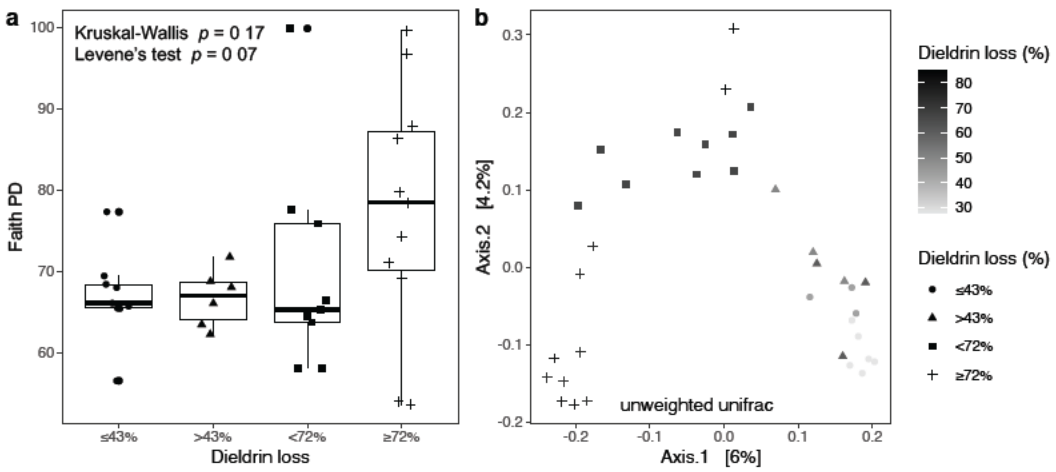


Figure S5.8-8. Faith phylogenetic diversity (Faith PD) index (a) and principal coordinate analysis (PCoA) with unweighted unifrac distances (b). Samples are grouped by long-term dieldrin loss (%) where the loss is either below or above the median loss in each soil type.

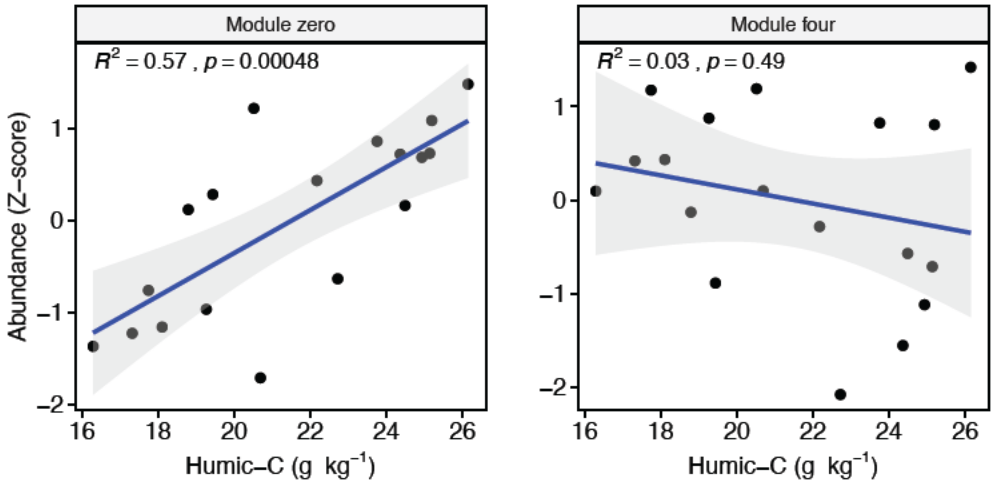


Figure S5.8-9. Scatter-plot of humic organic carbon concentration (Humic-C) and normalised abundances of the combined taxa in module zero and four from the network analysis of the Kurosol.

5.8.3 Supplementary Tables

Table S5.8-1. Climate data accessed from the Bureau of Meteorology on 22 May 2020.

Temperature (C°)

Northeast Victoria, King Valley, Edi upper, 1985 - 2020 (7 km to Kurosol paddocks)														Mean Min Max
Statistic	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	
Mean	30.4	29.8	26.6	21.4	16.5	13.3	12	13.3	16.3	20.3	24	27.	21	
Lowest	26.4	24.3	22.8	17.3	15	11.3	9.4	10.7	12.9	17.3	19.6	23	19	
Median	30.3	30.4	26.7	21.1	16.5	13.2	12.	13.4	16.4	20.1	23.8	28.	21	
Highest	35.3	33.9	30.3	25.7	18.9	15.2	14.	15.7	18.6	25.1	29.9	32.	22.4	
Min Max range	8.9	9.6	7.5	8.4	3.9	3.9	5.2	5	5.7	7.8	10.3	9.4	3.4	7.1
Southeast Victoria, Ferny Creek, 2011 - 2020 (15 km to Chromosol paddocks)														
Mean	24.5	24	21.1	17	13	10.4	9.8	10.8	13.9	17.2	19.2	22.	17	
Lowest	22.4	21.5	17.6	15	11.1	9.7	8.5	9.2	12.7	15	17.5	20.	16.3	
Median	24	24.4	22	16.8	13.2	10.3	9.7	10.5	13.7	17.2	19.5	21.	17.2	
Highest	27.8	26.2	24	19.2	14.5	10.9	11.	12.6	15.4	21.1	22.3	25.	17.4	
Min Max range	5.4	4.7	6.4	4.2	3.4	1.2	2.7	3.4	2.7	6.1	4.8	5	1.1	4.2
Southeast Victoria, Scoresby Research institute, 1948 - 2020 (23 km to Chromosol paddocks)														
Mean	26.4	26.5	24.2	20.1	16.4	13.6	13.	14.2	16.6	19.2	21.6	24.	19.7	
Lowest	21.7	22.9	20.5	16.2	13.9	11.2	11.	11.8	13.3	16.3	19.3	20.	17.7	
Median	26.5	26.5	24.1	20.1	16.4	13.6	13	14.2	16.6	19	21.4	24.	19.6	
Highest	31.6	31.2	27.8	23.6	18.8	15.7	15	16.8	19.1	24.7	26.5	29.	21.2	
Min Max range	9.9	8.3	7.3	7.4	4.9	4.5	3.6	5	5.8	8.4	7.2	8.8	3.5	6.8

Rainfall (mm)

Northeast Victoria, King Valley, Edi upper, 1985 - 2020 (7 km to Kurosol paddocks)														Mean Min Max
Statistic	**Jan**	**Feb**	**Ma**	**Ap**	**Ma**	**Ju**	**Jul**	**Au**	**Sep**	**Oct**	**No**	**De**	**Annual**	
Mean	61	57	59	57	93	114	128	119	97	77	81	79	1029	
Lowest	3	3	1	6	11	15	39	14	24	4	23	2	364	
Median	45	34	47	37	76	109	125	116	84	57	72	76	982	
Highest	204	244	224	161	208	206	247	206	201	341	173	205	1606	
Min Max range	202	241	223	155	197	190	208	192	176	337	151	203	1242	206
Southeast Victoria, Beaconsfield upper, 1968 - 2020 (8.4 km to Chromosol paddocks)														
Mean	66	62	70	81	89	88	89	96	98	99	92	81	1016	
Lowest	3	0	14	20	16	17	27	35	44	33	0	0	589	
Median	66	44	64	70	91	84	81	96	85	97	94	80	1030	
Highest	152	237	234	192	191	180	200	162	209	214	201	206	1324	
Min Max range	149	237	221	172	175	162	173	127	165	181	201	206	735	181

Solar exposure (MJ m^{-2})

Northeast Victoria, King Valley, Edi Upper (Schmidts Farm Repeater), 1990 - 2020 (3.5 km to Kurosol paddocks)														Mean Min Max
Statistic	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Annual**	
Mean	26.8	23.2	19	13.1	8.8	6.6	7.1	10.2	14.6	20.3	23.8	26.6	16.7	
Lowest	21.9	20.4	16.3	9.2	7.3	4.5	5.2	7	12.1	18.3	19.5	22.1	15.5	
Highest	31	26.6	21.8	15.8	10.5	8.8	8.7	14.1	17.8	25.9	27.4	30	18.2	
Min max dif	9.1	6.2	5.5	6.6	3.2	4.3	3.5	7.1	5.7	7.6	7.9	7.9	2.7	6.2
Southeast Victoria, Mount Burnett, 1990 - 2020 (3 km to Chromosol paddocks)														
Mean	23.6	20.5	15.8	10.7	7.3	5.9	6.6	9.3	12.6	16.9	20.5	22.8	14.4	
Lowest	20.3	17.2	11.8	8.8	6.2	4.7	5.2	7.8	9.8	14.3	17.6	19.6	13.3	
Highest	28.7	24	18.9	13.1	8.5	7	7.7	13.1	14.7	21.8	24.3	26.4	15.7	
Min max dif	8.4	6.8	7.1	4.3	2.3	2.3	2.5	5.3	4.9	7.5	6.7	6.8	2.4	5.4

Table S5.8-2. Raw sample data of dieldrin loss (%) and concentration (μg g^{-1}), resistant organic carbon (ROC), humus (HUM), particulate organic carbon (POC), carbon-to-nitrogen ratio (C:N), hydrogen-to-carbon ratio (H:C) and amplicon sequence variants (ASV). Units for carbon fractions are mg g^{-1} soil.

											Bacteria		Fungi	
ID	**Soil**	**Paddock**	**Farm**	**Dloss**	**Dieldrin**	**ROC**	**HUM**	**POC**	**C:N**	**H:C**	**Reads**	**ASVs**	**Reads**	**ASVs**
1	Kurosol	1	A	81	0.10	10.0	24.5	2.7	10.9	0.33	50,248	1,669	9,621	369
2	Kurosol	1	A	81	0.22	9.9	22.7	3.9	10.5	0.44	40,833	1,373	7,188	322
3	Kurosol	1	A	81	0.18	8.7	22.2	4.5	10.7	0.34	69,360	1,990	12,496	408
4	Kurosol	2	A	72	0.24	9.9	24.4	4.2	10.6	0.34	57,598	1,457	11,028	392
5	Kurosol	2	A	72	0.26	10.2	25.1	7.1	10.5	0.34	62,574	1,631	11,100	383
6	Kurosol	2	A	72	0.24	9.9	24.9	3.7	10.5	0.34	59,044	1,453	15,536	444
7	Kurosol	3	A	74	0.12	9.3	23.8	3.6	11.3	0.31	80,603	1,775	17,174	386
8	Kurosol	3	A	74	0.13	10.7	25.2	4.9	11.1	0.36	97,800	2,107	17,127	423
9	Kurosol	3	A	74	0.17	11.1	26.1	4.5	11.1	0.32	123,304	2,373	19,237	495
10	Kurosol	4	A	67	0.16	7.6	19.4	3.7	11.4	0.29	58,555	1,732	8,922	309
11	Kurosol	4	A	67	0.18	8.0	18.8	3.1	12.1	0.29	53,432	1,558	12,912	314
12	Kurosol	4	A	67	0.18	8.3	20.5	3.6	12.3	0.27	111,989	2,296	12,134	390
13	Kurosol	5	B	66	0.22	10.6	18.1	2.8	11.8	0.30	32,146	1,289	16,639	398
14	Kurosol	5	B	66	0.26	11.4	19.3	1.8	11.7	0.32	33,654	1,214	16,468	340
15	Kurosol	5	B	66	0.17	11.5	17.3	3.1	11.3	0.32	35,760	1,306	13,905	302
16	Kurosol	6	B	67	0.12	12.2	20.7	3.3	11.9	0.29	31,428	1,239	11,354	362
17	Kurosol	6	B	67	0.14	10.0	16.3	3.2	12.2	0.28	29,974	1,119	13,597	345
18	Kurosol	6	B	67	0.12	11.7	17.8	4.4	11.0	0.28	42,253	1,366	15,512	381
19	Kurosol	7	C	85	0.04	6.2	12.3	4.0	9.9	0.37	38,564	1,651	10,392	340
20	Kurosol	7	C	85	0.04	8.9	16.7	3.3	10.5	0.30	NA	NA	10,916	371
21	Kurosol	7	C	85	0.03	7.5	13.9	3.1	10.3	0.30	37,725	1,714	14,854	397
22	Chromosol	8	D	28	0.65	22.4	33.4	3.1	15.1	0.29	39,619	1,273	13,920	258
23	Chromosol	8	D	28	0.66	25.2	36.4	4.2	14.6	0.29	43,285	1,237	13,768	333
24	Chromosol	8	D	28	0.72	23.5	35.9	3.4	14.1	0.26	72,192	1,645	11,411	318
25	Chromosol	9	D	61	0.87	23.6	43.1	6.5	12.5	0.31	52,669	1,427	15,508	395
26	Chromosol	9	D	61	0.76	21.8	33.7	4.8	14.6	0.30	34,178	1,247	11,031	340
27	Chromosol	9	D	61	0.88	26.8	39.8	7.2	13.7	0.30	47,347	1,421	12,505	330
28	Chromosol	10	D	28	0.67	22.0	35.8	3.1	15.0	0.29	38,451	1,314	10,984	326
29	Chromosol	10	D	28	0.64	20.2	34.2	4.4	14.4	0.30	33,370	1,123	11,171	276
30	Chromosol	10	D	28	0.63	20.6	33.2	2.9	16.0	0.30	42,503	1,285	18,016	343
31	Chromosol	11	E	50	1.30	19.2	31.6	3.9	13.2	0.29	49,227	1,437	16,062	435
32	Chromosol	11	E	50	1.07	19.3	31.4	2.8	14.3	0.29	45,076	1,332	12,728	373
33	Chromosol	11	E	50	1.10	18.2	27.1	1.7	14.2	0.32	37,828	1,225	14,228	405
34	Chromosol	12	E	43	1.90	18.1	30.6	2.2	14.7	0.30	39,199	1,290	13,409	352
35	Chromosol	12	E	43	1.85	17.6	31.7	3.3	14.4	0.28	49,475	1,390	14,090	338
36	Chromosol	12	E	43	1.85	20.9	33.6	2.7	14.4	0.27	47,692	1,402	13,752	406

Table S5.8-3. Comparison of relative abundances and relative frequencies of bacteria and fungi in module zero and four of the network analysis.

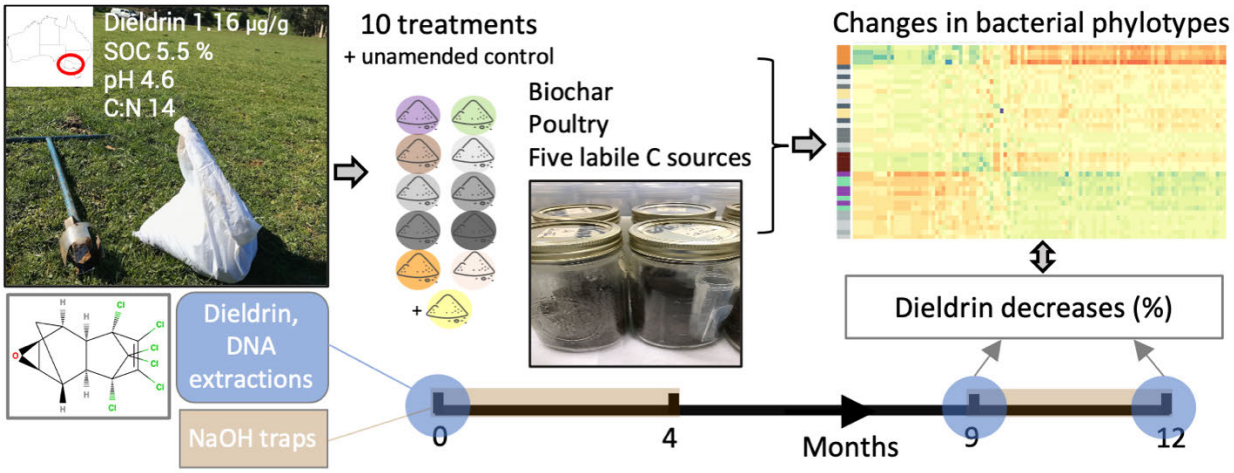
	Abundances (%)			ASV frequencies (%)		
Samples	**Bacteria**	**Fungi**	**Archaea**	**Bacteria**	**Fungi**	**Archaea**
All samples	79.5	19.8	0.7	82.2	17.4	0.4
Kurosol	81.6	17.7	0.7	83.0	16.6	0.4
Kurosol, Module 0	77.7	18.9	3.4	82.8	15.7	1.5
Kurosol, Module 4	69.5	30.5	0.0	78.6	21.4	0.0
Chromosol	76.5	22.9	0.6	81.3	18.5	0.3

Table S5.8-4. Phylofactor model coefficient and F statistic with dieldrin loss as numeric predictor for the aggregated presence of taxonomic groups. The resulting clades had a higher probability to be present in high dieldrin-loss environments (phylofactor mixed algorithm)

Phylum	n	Coefficient	df	F value	*p*	Phylofactor	Group
Chromosol & Kurosol							
Chloroflexi	33	0.34	1	394.62	< 0.001	1	6-member Monophyletic clade
Planctomycetes	33	0.1	1	386.81	< 0.001	2	17-member Monophyletic clade
Actinobacteria	33	0.07	1	371.41	< 0.001	3	18-member Monophyletic clade
Acidobacteria	33	0.05	1	353.25	< 0.001	4	29-member Monophyletic clade
Bacteroidetes	33	0.13	1	329.44	< 0.001	5	17-member Monophyletic clade
Kurosol							
Bacteroidetes	18	0.33	1	186.88	< 0.001	1	55-member Monophyletic clade
Firmicutes	18	0.19	1	142.1	< 0.001	2	44-member Monophyletic clade
Actinobacteria	18	0.05	1	116.13	< 0.001	3	589-member Monophyletic clade
Proteobacteria	18	0.16	1	40.73	< 0.001	4	24-member Monophyletic clade
Chloroflexi	18	0.14	1	32.66	< 0.001	5	27-member Monophyletic clade
Chromosol							
Acidobacteria	15	-0.21	1	0.01	0.93	1	2-member Monophyletic clade
Acidobacteria	15	0.04	1	0.00	0.97	2	19-member Monophyletic clade
Actinobacteria	15	0.06	1	0.11	0.74	3	8-member Monophyletic clade
Acidobacteria	15	0.05	1	0.31	0.58	4	15-member Monophyletic clade
Actinobacteria	15	0.18	1	0.70	0.4	5	2-member Monophyletic clade

Chapter 6: Biochar reduced extractable dieldrin concentrations and promoted oligotrophic growth including microbial degraders of chlorinated pollutants

This chapter has been published in Journal Hazardous Materials, 2021, volume 423
https://doi.org/10.1016/j.jhazmat.2021.127156



6.1 HIGHLIGHTS

- Biochar resulted in maximum decreases of aged dieldrin after 12-month incubation.
- Biochar promoted the most phylogenetically diverse, oligotrophic community
- Copiotrophic growth limited the degradation of aged dieldrin
- Labile carbon amendments may slow down the degradation of persistent pollutants

6.2 ABSTRACT

The role of organic amendments for natural degradation of aged persistent organic pollutants (POPs) in agricultural soils remains controversial. We hypothesised that organic amendments enhance bacterial activity and function at the community level, facilitating the degradation of aged POPs. An incubation study was conducted in a closed chamber over 12 months to assess the effects of selected organic amendments on

extractable residues of aged dieldrin. The role of bacterial diversity and changes in community function was explored through sequenced marker genes. Linear mixed-effect models indicated that, independent of amendment type, cumulative CO_2 respiration was negatively associated with decreases in dieldrin concentration, by up to 7% per μmol CO_2−C respired by microorganisms. The addition of poultry litter led to the highest daily carbon mineralisation, which was associated with low dieldrin dissipation after 9 months. In comparison, biochar resulted in significant decreases in extractable dieldrin residues over time, which coincided with shifts towards aerobic, oligotrophic, gram-negative bacteria, some with dehalogenation metabolism, and with increased potentials for biosynthesis of membrane components such as fatty acids and high redox quinones. The results supported an alternative theory that labile carbon promoted blooms of copiotrophic growth, which suppressed the required community-level traits and oligotrophic diversity to degrade chlorinated pollutants.

6.3 INTRODUCTION

Persistent organic pollutants (POPs) are a group of toxic legacy chemicals that resist degradation in the environment, bioaccumulate in animals and the food chain, and can be carried long distances by air as far as the arctic [2]. There are presently 29 POPs listed under the Stockholm Convention (www.pops.int), a global treaty to eliminate the production of POPs. All of them are halogenated compounds and most of them are lipophilic, chlorinated pesticides with peak production between 1950 and 1960. The Conference of the Parties of the Stockholm Convention (SC COP) reported that soils, sediments and water remain active sources for global pollution via air transport [2], hence, despite their global ban, pesticide residues in soils will continue to burden environmental and human health for decades.

In particular, agricultural soils are a major sink and source for aged POPs. For example, roughly 83 % of agricultural topsoils in Europe contain residues of at least one pesticide, with dichlorodiphenyltrichloroethane (DDT) among the most frequently found [6]. In Australia, DDT and dieldrin were the two most commonly used organochlorine pesticides for the control of insect pests in various crops until the early 1980s. Today, due to strong sorption of these chlorinated chemicals to organic matter, their residues are found at the highest concentrations in carbon-rich topsoil of former potato cropping regions with little decline of residues since 1980 [5,271]. Soil sorption is considered beneficial to prevent offsite transport. However, it also continues to limit land-use options and income of landholders. No practical solutions nor advice are available to landholders to increase natural degradation processes of aged POPs in their topsoils.

To provide solutions, agricultural practices with the potential to affect natural attenuation of aged POPs in surface soils under ambient air conditions need to be investigated as do increases or decreases in associated risks for landholders. Soils are the most diverse habitat on earth, often harboring thousands of different bacterial species, most of which remain unidentified and with functional redundancies [22]. Hence, the ability to predict the role of individual species in the fate of aged POPs upon receiving common agricultural

amendments such as lime, manures or biochars remains contradictory [326,327]. On the other hand, microbial traits that are measured at the community level may be more representative of ecosystem processes, including those that are relevant for the fate of aged POPs [291,328,329].

This study therefore explored the roles of bacterial activity, phylogenies and functional potentials in a carbon-rich Chromosol topsoil during 12-month soil incubation in a controlled closed-chamber. The aim was to better understand the roles of microbial activity and microbial phylotypes in the decreases of extractable dieldrin residues over time after the application of organic amendments. The effects of three common agricultural amendments, including lime, poultry litter and poultry-litter biochar, on changes in concentrations of extractable aged dieldrin residues were assessed.

These amendments were expected to shift the soil's organic carbon composition and biological properties in different ways. The addition of organic materials was also expected to increase adsorption processes, especially for biochar, which is known to have high sorption capacities for hydrophobic pollutants [330]. Nonetheless, the poultry-litter biochar, which was produced at low temperatures (pyrolyzed at 550˚C) has also shown to effectively improve nutrient supply and increase soil pH, and is therefore a potential stimulant for microbial activity [331]. Five soluble carbon sources were also included in this study to introduce variation in community-level responses and differentiate selection pressures of carbon and energy supply from the agricultural amendments. Bacterial taxonomies, their phylogenetic relationship and metabolic pathway potentials were predicted from bacterial marker genes to explore associations to decreases in dieldrin concentration, carbon mineralisation and the soil environment using sparse multivariate projection-based models (http://mixomics.org). We hypothesised that organic amendments enhance bacterial activity and functional traits at the community level, facilitating increased potentials for the degradation of aged POPs.

6.4 MATERIALS AND METHODS

6.4.1 Site description and soil sampling

An acidic pasture topsoil (0-10 cm) was sampled in mid-August 2018 (Winter season) on a ten-acre paddock containing known concentrations of the legacy pollutant dieldrin, on a northeast facing slope that was part of a gentle undulating landscape, which received an annual precipitation of 1015 mm. The paddock was part of a former potato operation with dieldrin as the main means of pest control until 1987. Later, it was converted to pasture and monitored by the National Organochlorine Management Program (NORM, Department of Natural Resources and Environment, 1996). The soil was a brown Chromosol [333] or Acrisol according to the international soil classification system [191] with a loamy, acidic topsoil, which overlayed a clayey subsoil and was susceptible to waterlogging. Close to 50% of dieldrin dissipated in this paddock since 1988, a decrease from 2.20 to 1.16 µg g^{-1} dry soil in 2017 [271]. Minor concentrations of dichlorodiphenyltrichloroethane (DDT) were also detected in 2017 (0.11 ± 0.01 µg g^{-1} dry soil) [271] but not in 1988. About 25 kg of soil was collected in August 2018 with an auger from 30 soil cores (0-10 cm) in a

grid pattern and homogenized into a composite on site. It was intended to incubate fresh soil and to keep disturbance to a minimum, hence it was air-dried for 24 h, then sieved (< 4 mm) before it was treated and added to incubation jars two days after sampling. The gravimetric water content (16 h at 105˚C) at time of treatment was 20%. The soil had dieldrin concentration of 1.16 ± 0.07 μg g^{-1}, pH ($CaCl_2$) 4.50 ± 0.003, electrical conductivity (1:5 water) 78 ± 20 μS cm^{-1}, total C 53.4 ±1.0 mg g^{-1}, Olsen P 41.6 ±4.2 μg g^{-1}, water-holding capacity 42%, clay 11%, silt 30% and sand 59%.

6.4.2 Experimental design

The experiment was designed to capture the dissipation of dieldrin residues that aged *in-situ* since 1988 (hence not newly applied residues) in an agricultural soil that contained high concentrations of total carbon. Two days after soil sampling, eleven treatments including ten carbon amendments and an untreated control were applied to 683 g of dieldrin-contaminated soil (dry weight basis) at 80% of water-holding capacity. Each treatment was homogenized and divided into four replicates with each replicate (171 g soil dry weight) was placed into a sealed 1-L glass fermentation jar. A total of 44 jars were randomized and incubated for 12 months in a controlled-environment room at 25˚C in the dark. A vial with 5 ml H_2O was added and CO_2 was trapped in the headspace during the first 16 weeks and the last 12 weeks of the incubation using alkali traps (40 mL 1 M NaOH), which were replaced every two weeks and stored at -20˚ until processed. To sample NaOH traps and to emulate ambient air supply while also minimising dieldrin volatilisation, oxygen was provided weekly by opening the lids for 15 min [334]. Water loss through the open headspace during oxygenation was replaced in regular intervals. Soils were subsampled (~ 25 g) from each jar at three time points, i.e. 1 day (T0), 9 months (T09) and 12 months (T12) after treatment, and soil samples stored at -20˚C until further processed. The sampling at T0 to T09 was done using a glass test tube to collect a composite from 3–4 soil cores of each jar. After T09 sampling, all soils were disturbed by re-homogenizing soils. This was done as soluble-carbon amendments were re-applied at T09 (see Treatments) and required that they were well mixed into the soil. Subsequent analyses have taken the potential effects of this disturbance on soil processes into account. A full description and justification of the experimental design is provided in supplementary materials.

6.4.3 Treatments

This study assessed eleven treatments including an untreated control, each with four replicates and sampled at three timepoints (n = 132). Of the eleven treatments, four were agricultural amendments and five were soluble-carbon amendments. These treatments intended to maximize variation in bacterial community-level responses through different types of selection pressures and to monitor dieldrin decreases over time. The agricultural amendments included poultry litter, poultry-litter biochar and lime ($CaCO_3$). Poultry litter was poultry manure together with semi-composted rice hull bedding that was collected from a from a broiler operation in Mount Wallace, Victoria, Australia [331]. The poultry-litter biochar (termed 'biochar' hereafter)

was made of the corresponding poultry litter feedstock in a slow pyrolysis batch plant (CharMaker MPP20, Earth Systems, Melbourne, Australia). The ramp rate was ~125 °C h^{-1} with a holding temperature of ~550°C, which was maintained for 45 min. Both poultry litter and its biochar materials were dried at 70 °C, finely ground (Retsch MM400, Germany) and passed through a 1-mm sieve. Nutrient and mineral composition of poultry litter and its biochar was provided by Lauricella et al. (2021) [12]. The poultry litter and biochar were applied at 20 t ha^{-1}, and lime ($CaCO_3$) at 4.55 t ha^{-1} mass equivalents. On a dry-weight basis, the biochar contained 70.1% of $CaCO_3$ equivalent to neutralise acidity. Hence, the lime treatment acted as a control for a potential pH effect of biochar on the microbial community.

The soluble carbon amendments included *myo*-inositol (CAS 87-89-8, Sigma-Aldrich, Australia), sodium formate (CAS 141-53-7, Ajax Finechem, Australia), sodium acetate hydrated (CAS 6131-90-4, Ajax Finechem, Australia), citric acid (CAS 77-92-9, Sigma-Aldrich, Australia) and fumaric acid (CAS 110-17-8, Ajax Finechem, Australia) to supply carbon and energy sources (electron donors and acceptors) for microbial growth. Citric acid has shown to affect bioavailability of organochlorines; sodium acetate and sodium formate are electron donors; *myo*-inositol is a sugar alcohol and all these compounds have shown to increase biodegradation of organochlorines [94,95,122,335]. Fumaric acid is a key metabolite in aerobic energy metabolism and can also be an electron or hydrogen acceptor in anaerobic metabolism [336]. Substrates were dissolved in milli-Q water and applied at 80 % water-holding capacity. They were re-applied once at 9 months (T09) after the first application to reinforce the potential selection pressures to the microbial community it was expected that they were completely metabolised. Concentrations (substrate C equivalents) were chosen to provide sufficient substrate-C (Table S6.8-1) to affect microbial growth in a soil that was naturally rich in organic matter. After T09 sampling and re-application of substrates, soils were mixed, and aggregates crushed using a sterile spoon to ensure a homogenous substrate distribution in soil. Soil mixing was done in all eleven treatments. As no true abiotic controls were included, the cumulative effect of biological and abiotic degradation of dieldrin was not assessed in this study.

The final two treatments were hydrogen peroxide (H_2O_2) at 3% of soil dry weight and a heat treatment where soils were autoclaved twice at 134˚C for 20 min at x 30 PSI followed by 10-min drying (Siltex, HC2D, Australia) with the aim to partially oxidise organic carbon including aged dieldrin [337] and to semi-sterilise and disturb the microbial community and organic matter composition, respectively. Heat-treatment of soils also served as a negative control for diversity indices over time. The untreated control had Milli-Q water applied.

6.4.4 Dieldrin extraction and analysis

Extraction efficiencies of dieldrin residues from soils vary among different extraction methods. To compare soil concentrations of dieldrin residues for this study, 'extractable' was defined as solvent extractable under the method described hereafter. Extractable dieldrin residues were quantified for T0, T09 and T12.

Approximately 10 g of air-dried soil was finely ground using a ball mill (Retsch MM400, Germany) at 25 rounds per minute for 1 min. Ground soil (1 ± 0.002 g) was then weighed into a 50-mL centrifuge tube, spiked with 40 µL dichlorodiphenyldichloroethane (DDD, 50 µg mL^{-1}) as internal standard and then extracted three times with 4 mL acetone:hexane (1:1) under side-by-side shaking. Extracts were cleaned and analysed as described in Method 8081 by United States Environmental Protection Agency (US EPA) [192] using Florisil (500 mg/3 mL) cartridges. Eluent was evaporated and redissolved in 1 mL hexane for analysis using a dual column GC/ECD (Varian CP-3800; DB-5MS UI 15 m × 0.32 mm, 0.25 µm; DB-1701 15 m × 0.32 mm, 0.25 µm) equipped with nickel 63 electron capture detector (ECD) and autosampler. The instrument detection limit (IDL) for dieldrin was 0.002 µg mL^{-1} and the method limits of detection (MLD) and of quantification (MLQ) were 0.025 µg g^{-1}. A detailed description of the extraction process, instrument conditions and quantification is presented in the supplementary materials.

6.4.5 Soil physicochemical properties, microbial biomass carbon and respiration measurements

The ground soil was further used to determine concentrations of total soil carbon (total C), total soil nitrogen (total N) and total soil hydrogen (H) using a dry combustion analyser (Perkin Elmer 2400 Series II, USA) at T0, T09 and T12. Soil microbial biomass C (MBC) was estimated using the chloroform fumigation method (Vance et al., 1987) as described previously [271] except that 4 g of fresh soil was extracted instead of 8 g. Soil pH was determined using a pH meter (Thermo Orion 720A+, Beverly, MA, USA) in soil extracts (1 g soil at 1:5 w/v 0.01 M $CaCl_2$) after overhead shaking for 1 hour. Raw results of these measurements are accessible under the digital object identifier (DOI): 10.17632/nbjhw7rcyj.1

Soil C mineralisation was assessed with alkali CO_2 traps, which were added to the closed incubation jars with vials of H_2O [338] and replaced bi-weekly from T0 to T04 and again from T09 to T12 (Jars were opened briefly once a week to allow for refreshing air). Traps were thawed and CO_2 was determined by back-titration of the excess alkali with HCl using a digital burette. Alkali trap solution (2 mL) was back-titrated using phenolphthalein as an indicator after removal of dissolved CO_2 and carbonates by precipitation with the addition of 8 mL of 0.25 M $BaCl_2$. The concentration of CO_2 in traps was calculated as following [338]:

$$CO_2\text{-C (mol)} = 0.5 \times (((V_{NaOH} \times C_{NaOH}) / 1000) - ((V_{HCl} \times C_{HCl})/1000) \quad (1)$$

where V and C are volume in mL and C molar concentration, respectively.

Respired CO_2 was expressed in µg CO_2-C g^{-1} dry soil after correcting for values of alkali blanks that were placed bi-weekly in empty jars together with H_2O. Mean daily CO_2 respiration (µg CO_2-C g^{-1} dry soil day^{-1}) was calculated for the first two weeks after treatment ($T0.5_{resp}$), until four months after treatment ($T04_{resp}$) and for the last 3 months of soil incubation ($T12_{resp}$). The amount of CO_2 respired per unit MBC (qCO_2) was estimated for T0, T09 and T12 by dividing mean daily respiration at $T0.5_{resp}$, $T04_{resp}$ and $T12_{resp}$ by MBC measured at T0, T09 and T12.

6.4.6 Bacterial marker gene sequencing and pathway predictions

DNA was extracted from 0.18−0.29 g soil using DNeasy Powersoil Pro extraction kit (Qiagen, Hilden, Germany) and stored at -20 ˚C for soils sampled at T0, T09 and T12, noting that the heat-treated sample at T0 yielded no DNA. The DNA was sequenced including 12% phiX on an Illumina MiSeq instrument (2 × 300 bp) after amplification of V4 regions of the 16S-rRNA gene with primer-pairs 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) (Apprill et al., 2015) and libraries preparations using Nextera XT indices. Blanks and three repeat extractions on the same soil were included to control for contamination and variation between extractions. Processing of sequences included primer trimming with cutadapt [339] followed by pairing forward and reverse reads, denoising, filtering and dereplicating of sequences using DADA2 [283] after truncating all amplicon sequences to 273 and 200 nucleotides (forward and reverse reads respectively) within the default settings of the QIIME2 (v2020.8, https://qiime2.org) pipeline. A total of 4,285,018 quality reads at a median frequency of 32,820 reads per sample and 22,271 unique amplicon sequence variants (ASVs) were obtained. This was followed by the taxonomic classification of ASVs using Silva132 at 99% similarity and the creation of a phylogenetic tree within the Qiime2 environment as described previously [340]. PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2) software (Douglas et al., 2019), together with the MetaCyc Metabolic Pathway Database (https://metacyc.org/) was used to predict a total of 412 metabolic pathway potentials from the bacterial ASVs.

6.4.7 Statistical analysis

A detailed description of statistical methods used for this study is available in supplementary materials.

Median dieldrin concentrations and the interquartile range (IQR) as well as mean dieldrin decreases (Decreases (%) = (T0 - T_{month} / T0) × 100) were calculated. Dieldrin concentrations (μg g^{-1} dry soil) or dieldrin decreases (%) were predicted from treatments as well as from soil variables and CO_2 respiration using four linear mixed-effect (LME) models. The terms decreases and dissipation were used interchangeably. To explore relations between dieldrin decreases since T0 and decreases in, total C and extractable organic C (EOC) as well as to microbial diversity indices, principal component analysis (PCA) was performed at T09 (n = 40) and T12 (n = 40).

Alpha diversity indices such as richness and Pielou's evenness were calculated from rarefied abundances. Effects on bacterial community composition were analysed using principal component analysis (PCA) and redundance analysis (RDA), after transforming abundances into centered log-ratios. To test the effect of treatments (excluding heat treatment, n = 120) on the presence/absence of bacterial taxonomic groups, the package Phylofactor [314] was used to perform generalized phylofactorisation. To elucidate which taxonomic groups were predicted by low/high dieldrin dissipations, differential abundances of bacterial taxonomic groups at T09 and T12 (n = 40) were regressed with a Gaussian distribution using function 'PhyloFactor' in

the package Phylofactor. Lastly, sparse partial least squares (sPLS) regression was performed with the package mixOmics [289] with dieldrin decreases (%), decreases in MBC (%), decreases EOC (%) and mean respiration (μmol CO_2-C g^{-1} soil day^{-1}) as response variables predicted from potentials of bacterial metabolic (Metacyc) pathways at T09 and T12 (n = 40).

6.5 RESULTS

6.5.1 Associations of treatments with decreases in dieldrin concentrations over time

Dieldrin concentrations and subsequent decreases are summarised for each sampling time (Table 6.5-1). During the first nine months dieldrin decreases were minimal. Furthermore, soil disturbance was minimal, which resulted in increased soil aggregation as would be expected after periods of microbial activity. At T09 soil aggregates were crushed and the soil in the incubation jars was mixed, which coincided with a dieldrin decrease of 9.6 % in the following 3 months from T09 to T12 (Table 6.5-1).

Concentrations of extractable dieldrin significantly decreased in all treatments compared to the untreated control (Figure 6.5-1a, Table S6.8-2). These treatment effects on extractability of dieldrin were observed from the start of incubation including T0 soils, which were sampled one day after treatments were applied (Figure 6.5 1a).

However, repeated measures analysis showed that the decrease rate of dieldrin concentrations was significantly slower after 9 and 12 months for soils treated with poultry litter and H_2O_2 relative to the unamended control (Table S6.8-2). Furthermore, soils treated with fumaric acid and sodium acetate also exhibited significantly slower decreases in dieldrin concentrations after 12 months (Table S6.8-2).

Biochar treatments resulted in the greatest decreases in extractable dieldrin (8.3 % ±1.17 SE and 17.3 % ±1.75 for T09 and T12, respectively). This was significantly improved compared to poultry litter, H_2O_2 and fumaric acid, based on multiple comparison test (Fisher's least significant difference) with Holm corrected significance at the 5 % level (Figure 6.5-2). However, it was not significantly different from total decreases of the untreated control (Figure 6.5-2). In addition, poultry litter and H_2O_2 resulted in a small increase in extractable dieldrin concentrations at T09 compared to T0 although this was not significant compared to the untreated control based on multiple comparison tests (Figure 6.5-2).

Table 6.5-1. Summary of dieldrin concentrations and decreases across all treatments for each month after treatment (MAT).

MAT	n	Median concentration (µg g^{-1})	IQR	Mean concentration (µg g^{-1})	SE	Mean decrease (%)[a]	95% CI
T0	44	1.072	±0.066	1.076	±0.008	0	0
T09	44	1.067	±0.061	1.053	±0.010	2.1	0.7–3.5
T12	44	0.953	±0.056	0.950	±0.008	9.6	8.2–11.0
T0–T12	44					11.6	10.2–13.0

[a] Calculated as the mean of percent decreases of each incubation jar (n); IQR, interquartile range; SE, standard error; CI, confidence interval.

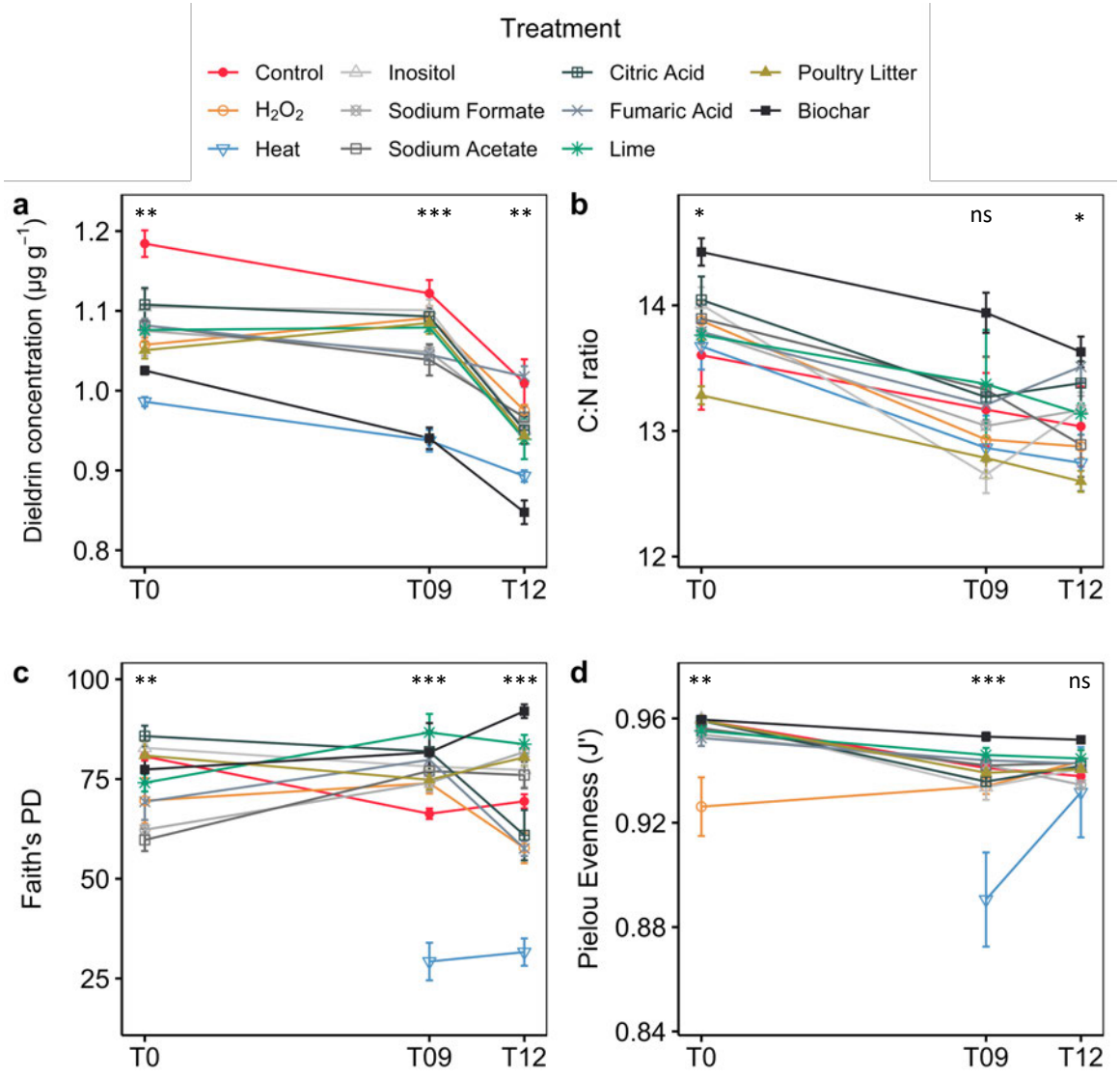


Figure 6.5-1. Repeated measurements at 1 day (T0), 9 months (T09) and 12 months (T12) after treatment. At T09, soils were re-homogenised and soluble-carbon amendments (inositol, sodium formate, sodium acetate, citric acid and fumaric acid) re-applied. Extractable dieldrin concentrations, carbon-to-nitrogen (C:N) ratio and the diversity indices Faith's PD and Pilou's evenness are presented (**a–d**). No DNA was present in heat-treated soils at T0 hence no diversity was assessed for these samples. ns, *, **, *** and **** indicate no significance at $p = 0.05$, significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and $p < 0.0001$, respectively, of Kruskal-Wallis tests of treatments at each sampling event. Bars represent ± standard error of the mean of four replicates.

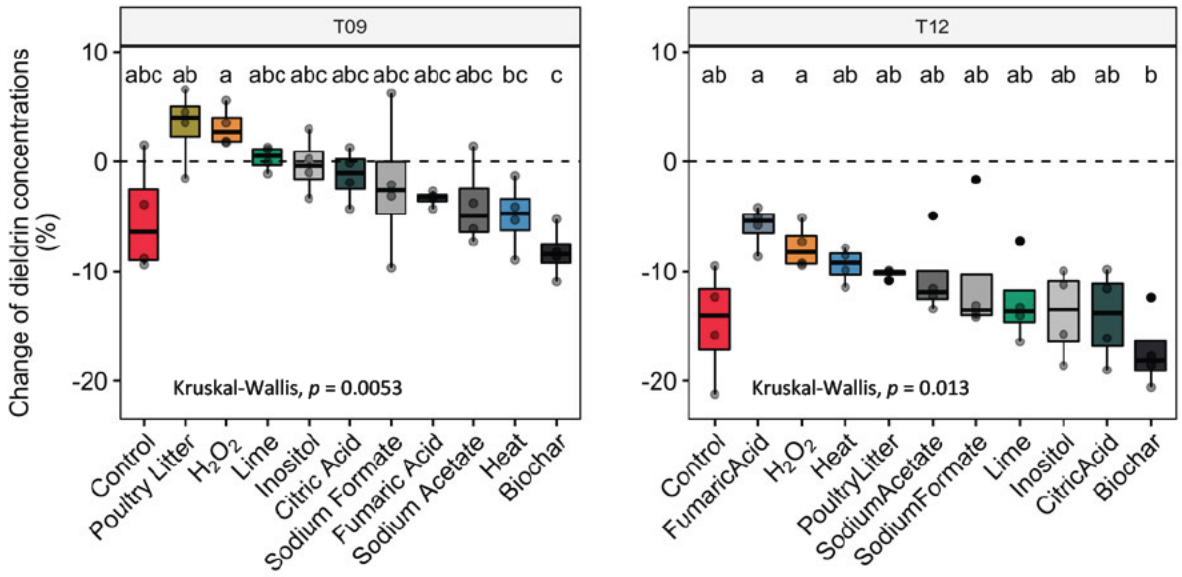


Figure 6.5-2. Changes in dieldrin concentrations after 9 months (T09) and 12 months (T12) since application of soil treatments. The means followed by a common letter are not significantly different after multiple comparison tests at the 5 % level (Fisher's least significant difference, Holm corrected *p* value).

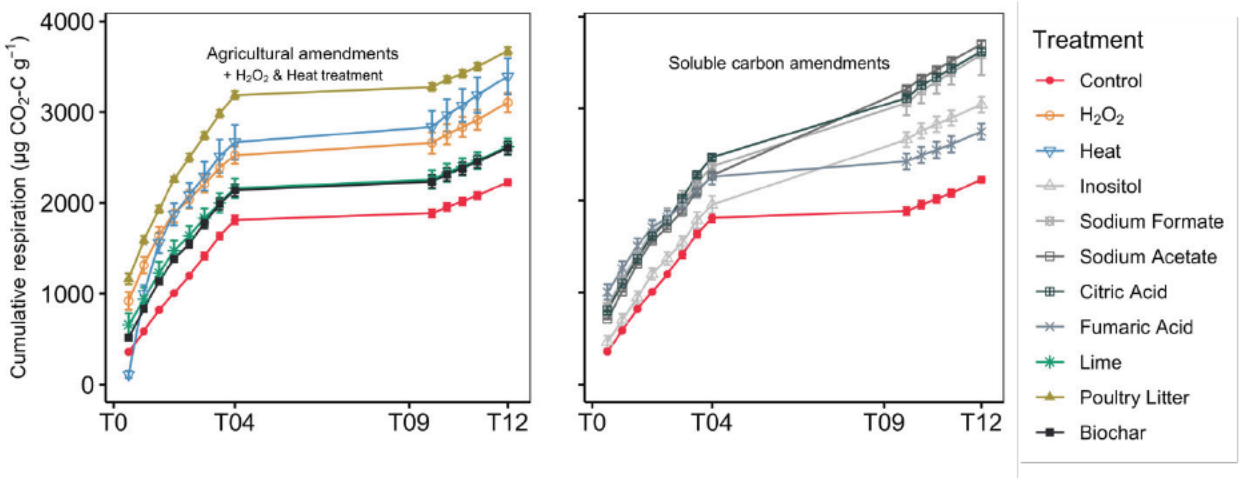


Figure 6.5-3. Cumulative CO_2 respiration from bi-weekly measurements during the first four and the last three months of soil incubation. Treatments are divided into agricultural amendments (left) and soluble-carbon amendments (right). At T09, the incubated soil was re-homogonized and soluble-carbon amendments (inositol, sodium formate, sodium acetate, citric acid and fumaric acid) re-applied.

Table 6.5-2. Linear mixed-effect model with decreases (%) in dieldrin concentrations at 9 (T09) and 12 months (T12) of treatment as response and soil variables as predictors (LME Model S3 and S4 respectively).

	Dieldrin decrease (T09)				**Dieldrin decrease (T12)**			
Predictors	*Estimates*	*CI*	*p*	*df*	*Estimates*	*CI*	*p*	*df*
(Intercept)	-0.11	-2.2 – 2.1	0.92	34	10.95	6.5 – 15.4	**<0.001**	34
Soil hydrogen decrease (%)	1.47	0.1 – 2.9	**0.039**	34				
Mean respiration[a] (μmol CO_2-C g^{-1} soil day^{-1})	-7.05	-11.3 – -2.8	**0.001**	34	-5.12	-11.2 – 1.0	0.101	34
Pilou Evenness	3.37	0.6 – 6.2	**0.018**	34				
Microbial biomass C decreases (%)					-2.83	-6.0 – 0.3	0.078	34
Faith's PD					2.4	0.7 – 4.1	**0.007**	34
Random Effects								
σ^2	9.29				11.98			
τ_{00} Treatment	2.44				3.39			
ICC	0.21				0.22			
N Treatment	10				10			
Observations	40				40			
Marginal and Conditional R^2	0.47 / 0.58				0.36 / 0.50			

[a] Respiration in the first 4 months and the last 3 months was measured. Hence, the mean respiration that was included for the model at T09 is the daily mean respiration from T0–T04; the mean respiration for T12 is the daily mean from T0–T04 + T09–T12.

6.5.2 Association of dieldrin dissipation with low microbial activity

Linear mixed-effect models, which controlled for treatment effects, provided evidence that respiration was associated negatively with decreases in dieldrin concentration (Table 6.5-2, n = 40, $p = 0.001$) and positively with dieldrin concentrations (Table S6.8-3, n = 120, $p = 0.007$). This inhibitory effect of respiration on dieldrin decreases was independent of treatments and relatively large, limiting dieldrin decreases 7 % for each µmol CO_2−C respired after 9 months of incubation (Table 6.5-2, n = 40, $p = 0.001$). Overall, samples that produced the greatest mass of cumulative CO_2−C in the first two months correlated with poor decreases of dieldrin (0.32, $p < 0.001$) (Figures 6.5-3a and 6.5-5a). This was observed again from cumulated CO_2 respiration after re-applying soluble C at T09 (0.49, $p < 0.001$) (Figure 6.5 4b).

Samples treated with poultry litter, which released the most CO_2 (total of 3672 µg C g^{-1} dry soil) (Figure 6.5 3) and maintained the highest daily CO_2 release for the first 4 months of incubation, were associated with low dissipations of dieldrin concentrations (Figure 6.5-5). The PCA further highlighted that this increased microbial activity in treatments such as poultry litter was associated with declines in total C and EOC and together was associated with low dissipation of dieldrin concentrations in soil (Figure 6.5-5). Moreover, PCA showed that total declines in MBC were negatively associated with dieldrin dissipation (Figure 6.5-5). For example, initial microbial growth from poultry litter at T0 was followed by a steep decline of MBC after 9

and 12 months of incubation, which contrasted to treatments such as biochar, where the microbial biomass was relatively stable over time and showed improved dieldrin dissipation (Figure 6.5-5, Table S6.8-4).

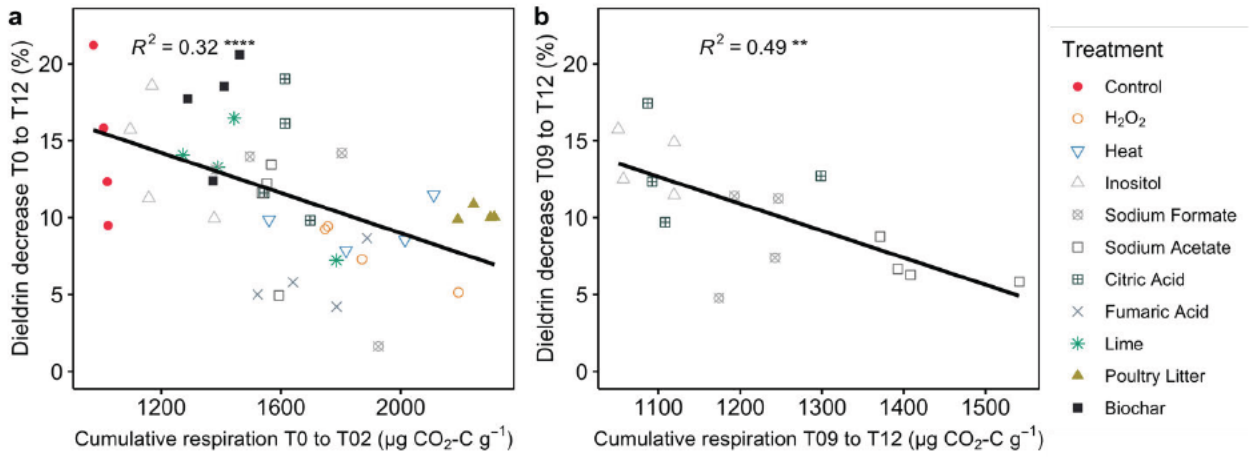


Figure 6.5-4. Correlations between cumulative soil respiration of the first 2 months after treatments (T02) and decreases in dieldrin concentration after 12 months (T12) (**a**), and between cumulative soil respiration of soluble-carbon amendments and decreases in dieldrin concentration from 9 months (T09) to 12 months (T12), excluding samples treated with fumaric acid (**b**).

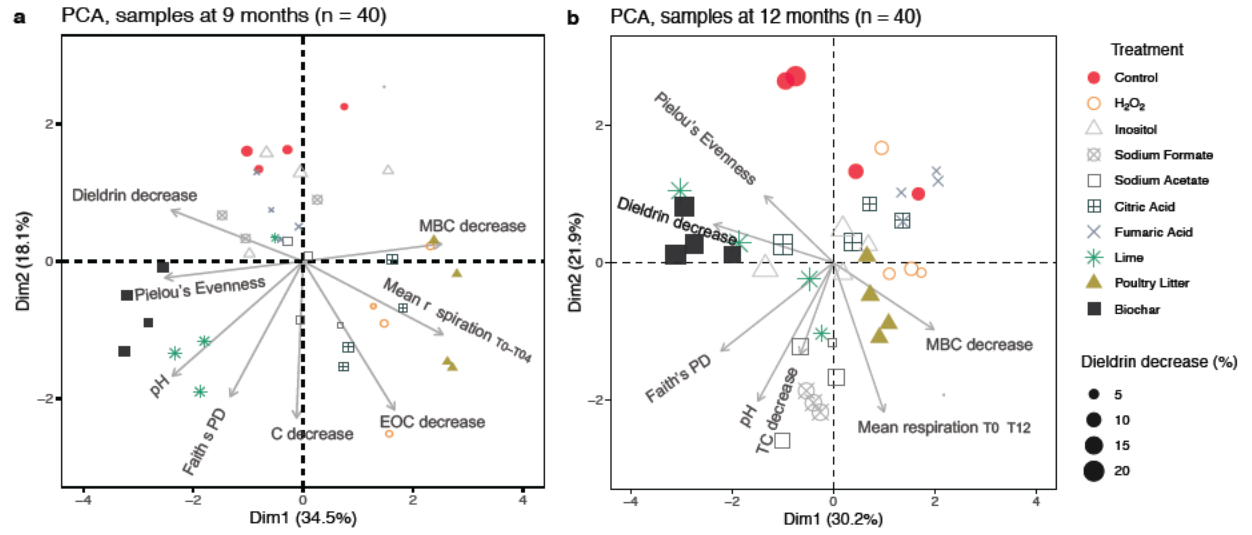


Figure 6.5-5. Principal component analysis (PCA) of soil samples collected after 9 (T09) (**a**) and 12 (T12) (**b**) months of treatment. Variables include the percent-decreases of total carbon concentrations (TC), extractable organic carbon (EOC), microbial biomass carbon (MBC) and extractable dieldrin concentrations since time T0. Furthermore, pH, mean daily respiration (µg CO_2-C day^{-1}) and the diversity indices Faith's phylogenetic diversity and Pielou's evenness are included. All variables were scaled and centered. Size of symbols indicates total dieldrin decreases (%) of samples.

6.5.3 Relationship of bacterial diversity with dieldrin dissipation and organic amendments

Bacterial phylogenetic diversity (Faith's PD) increased in the biochar treatment but decreased with poultry litter in the first 9 month of incubation (Figure 6.5-1c). After 12 months, biochar-treated soils contained the greatest phylogenetic diversity of all treatments. Furthermore, Pilou's evenness was greatest for biochar-treated samples (Fig. 1d). In addition, PCA and linear mixed models, controlling for treatment effects, provided evidence that evenness of bacterial abundances and Faith's phylogenetic diversity both coincided with improved dieldrin dissipations (Figure 6.5-5, Table 6.5-2). The soil treatments further affected bacterial community composition with distinct changes over time that differed between biochar and poultry litter treatments. Redundancy analysis revealed that around 10.8 % of the captured variation in the bacterial communities changed over time although the treatments themselves had a relatively small effect (~ 2.1 %) (Figure 6.5-6). It further showed that bacterial communities in soils treated with biochar and lime developed into a different community composition compared to poultry litter and the untreated control (Figure 6.5-6).

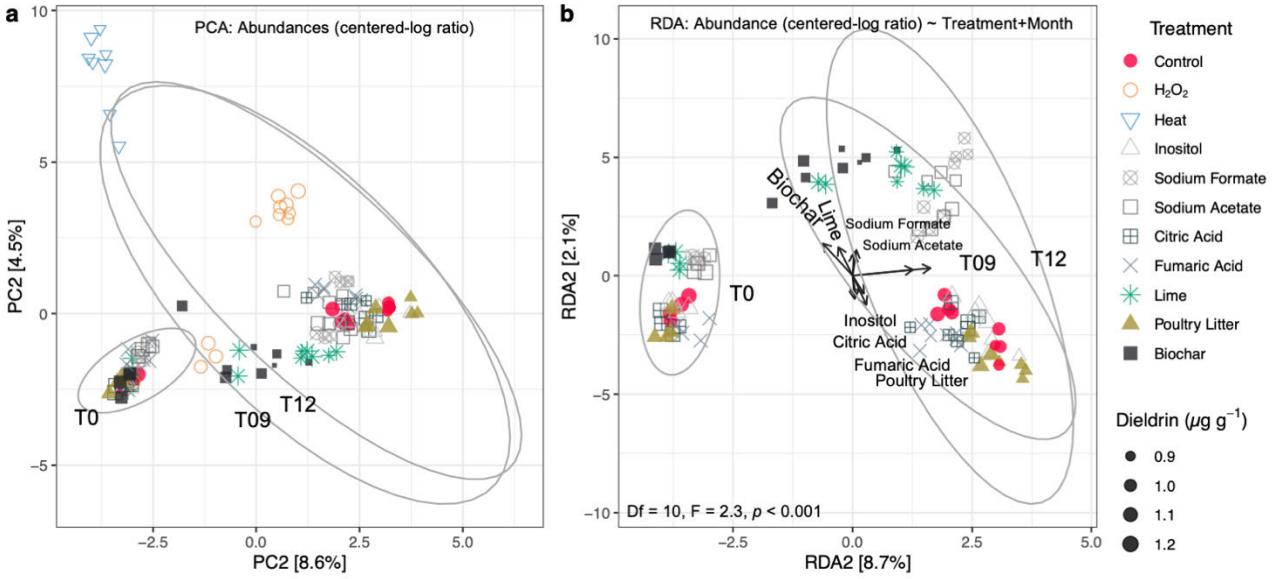


Figure 6.5-6. Principal component analysis (PCA) and redundancy analysis (RDA) of transformed bacterial abundances (centered log-ratio). Each datum point represents distances of the first two principal components (PC) of a sample. Date ellipses represent the 95% probability contours, i.e. the change of bacterial compositions, from month T0, T09 to T12. The PCA excludes heat-treated samples at start of incubation (T0) (n = 128) as no DNA was recoverable (**a**). The RDA with treatment and time (months after treatment) as predictors for bacterial abundances excluding heat-treated soils for all three sampling events (n = 120) (**b**). Arrows indicate strength and direction of associations between bacterial compositions and treatments and time. Size of symbols indicate dieldrin concentrations (µg g^{-1} soil) of samples.

Soil treatments resulted in shifts in the presence and relative abundance of phylogenetic groups and their associated gene pools with different metabolic potentials. The taxonomic profile of bacteria in biochar and limed soils developed similarly over time but differed from the poultry litter treatment or the untreated control (Figure 6.5-6). For example, in the poultry litter treatment, Firmicutes and Chloroflexi became the most abundant bacterial groups while the contribution of most other taxa declined over time (Figure 6.5-7). With

biochar, most phyla remained relatively evenly abundant until the end of incubation at T12, including Actinobacteria, Proteobacteria, Planctomycetota, Verrumicrobiota, Gemmatimonadota and Bacteroidota. The treatments of biochar and lime increased the diversity within these mentioned phyla. Biochar and lime led to the largest combined increase of richness in Proteobacteria, Gemmatimonadota, Myxococcota, Bacteroidota, the class Thermoleophilia of phylum Actinobacteria and for the smaller phyla Nitrospirota, Desulfobacterota and RCP2-54 compared to the control (Figure S6.8-1, phylofactor 1 remainder group 2).

Furthermore, the combined richness of Acidobacteriota, Verrucomicrobiota and Planctomycetota was the greatest in the biochar treatment, followed by the lime treatment (Figure S6.8-1, phylofactor 3). It was further observed that the Thermoleophilia '*Solirubrobacterales* bacterium 67-17' were most prevalent (highest number of ASVs) in the biochar and lime-treated soils (Figure S6.8-1, phylofactor 10) and these ASVs were associated with improved dieldrin dissipation (Figure S6.8-3, phylofactor 39).

Similar phylogenetic shifts were observed from phylofactor models of bacterial abundances with dieldrin decreases as the explanatory variable. The combined relative abundance of Proteobacteria, Gemmatimonadota, Myxococcota, Bacteroidota and Thermoleophilia Actinobacteriota was associated with decreases in dieldrin concentrations, together with the relative abundances of Planctomycetota, Verrumicrobiota and some classes of the Chloroflexi including Dehalococcoidia (Figure S6.8-2, group 2 of phylofactor 3 and 19; Figure S6.8-3 group 2 of phylofactor 4). Lastly, increases in the relative abundances of uncultured *Xanthobacteraceae* (α-Proteobacteria) were associated with improved dieldrin dissipation (Figure S6.8-2, phylofactor 8).

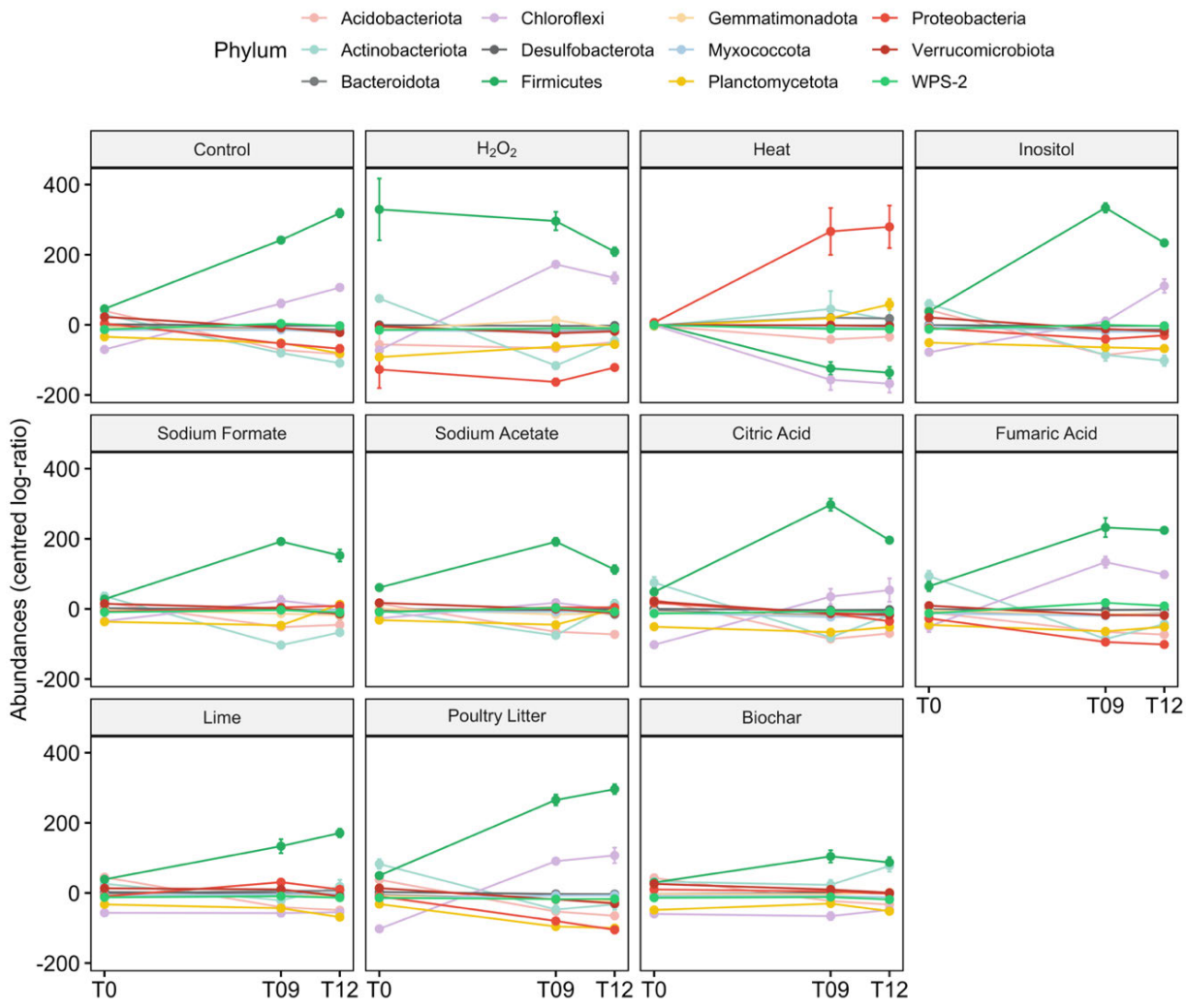


Figure 6.5-7. Relative abundances (centered log-ratio) of major bacterial phyla after incubation for 0 (T0), 9 (T09) and 12 months (T12). Bars represent ± standard error of the mean of four replicates.

Figure 6.5-8. Clustered heatmap of association scores of important pathways from sparse partial least square (sPLS) regression using T09 (9 months) samples excluding heat-treated soils (n = 40) with decreases in dieldrin concentration, microbial biomass C (MBC), extractable organic carbon (EOC) in percent and mean respiration as response variables and pathway abundances as predictors for pathway potentials. Red rectangles highlight pathways of interest

sPLS analysis of pathway abundances further highlighted that the bacterial gene-pool in soils treated with biochar and lime contained functional pathways that were associated with lower dieldrin concentrations (Figure 6.5-8). These pathways were lacking in the bacterial gene-pool in most treatments treated with soluble C, poultry litter and H_2O_2, and the untreated control (Figures S6.8-4 to S6.8-6). The majority of these pathways were related to the biosynthesis of components of the cytoplasmic membrane including unsaturated fatty acids, electron carriers and cofactors. For example, at both T09 and T12, bacterial potentials for the metabolic pathway of heme b biosynthesis from uroporphyrinogen-III and from glycine were predictive of improved dieldrin dissipations and were associated with biochar and lime treatments (Figure 6.5-8 and S6.8-4 to S6.8-6). Furthermore, the potentials for anaerobic biosynthesis of unsaturated fatty acids such as 5-dodecenoate, palmitoleate and oleate were predictive of improved dieldrin decreases as well as the potentials for the biosynthesis of other fatty acids including palmitate, mycolate and stearate (Figure 6.5-8 and S6.8-4 to S6.8-6). Only α- and γ-proteobacteria have the genes for the anaerobic synthesis of 5-dodecenoate [341].

Moreover, bacterial metabolic potentials for biosynthesis of electron carriers contrasted between the type of electron carrier that were associated with low or high dieldrin concentrations. The potential for the biosynthesis of ubiquinol, the quinone with greatest redox potential, was predictive of improved dieldrin dissipation, while the potential for the biosynthesis of menaquinol, the quinone with lowest redox potential, was predictive of low dieldrin dissipation (Figure 6.5-8) [342]. Most aerobic Proteobacteria contain ubiquinone as the sole quinone, which is present only in strictly aerobic microorganisms [342].

6.6 DISCUSSION

This incubation study evaluated the effects of ten soil treatments on changes in concentrations of extractable dieldrin in a carbon-rich topsoil that remained contaminated with dieldrin residues since the 1980s. At the end of 12-month incubation, the concentrations of extractable dieldrin decreased by an average of 11.6 % across all treatments although the effect of the treatments on total dieldrin dissipations relative to the untreated control was not significant (Figure 6.5-2, Table 6.5-1). Nonetheless, there was significant variation in total dieldrin decreases among the treatments, mainly between biochar, H_2O_2 and poultry litter (Figure 6.5-2).

It was further noted that all treatments, including biochar and poultry litter significantly lowered the concentrations of extractable dieldrin one day after their application (T0) (Figure 6.5-1a, Table S6.8-2). Extraction efficiency of organochlorine residues from soils can be affected by various factors [343]. While the heat-treated soil samples may have lost dieldrin residues through volatilisation, biochar and poultry litter may have increased sorption processes of dieldrin. The addition of salts such as sodium acetate or sodium formate may have further have affected extractability. However, it was assumed that dieldrin extraction efficiencies were constant over time and that therefore comparisons of dieldrin decreases between treatments were valid. Dieldrin decreases during the incubation may have been the result of various abiotic and biotic

processes such as volatilisation, adsorption, enzymatic transformation or mineralisation. Nevertheless, it was assumed that decreases in extractable dieldrin concentrations over time would be partly due to microbially catalysed processes and were subsequently termed 'potential degradation' in the context of microbial community associations. This potential for degradation was expected to stem from community-level changes in enzyme expression and co-metabolism, rather than dieldrin specific reactions, because the highly chlorinated dieldrin compound is a poor growth substrate on its own.

6.6.1 Copiotrophic growth inhibited dieldrin decreases

As expected, the addition of carbon sources resulted in microbial growth and carbon mineralisation as evident from an increase in microbial biomass and respiration at T0, which was followed by the decomposition of soil carbon as observed from decreases of extractable organic C and the C:N ratios over time (Figures 6.5-1b, 6.5-3 and 6.5-5, Table S6.8-4). Increasing carbon supply enhanced the microbial response. For example, the H_2O_2 treatment resulted in a release of extractable organic C from the native organic C and subsequently increased microbial respiration. Poultry litter, which was applied at a maximum rate of 20 t ha^{-1} mass equivalent, provided the richest source of carbon, nitrogen and other nutrients [331] and subsequently resulted in the greatest response of microbial growth and respiration (Figure 6.5-3).

However, this microbial growth or activity was evidently not associated with a reduction in extractable dieldrin concentrations. In fact, the opposite was observed. Across all treatments, increased cumulative respiration was associated with low dissipation of dieldrin over time (Figure 6.5-4, Table 6.5-2). Furthermore, treatments that had the greatest effect on microbial respiration, such as poultry litter and H_2O_2, significantly slowed down the rate of dieldrin decreases after 9 and 12 months relative to the control (Table S6.8-2). The data further showed that dieldrin concentrations in these treatments might even have increased after 9 months of incubation, pointing to soil processes that increased extractability or bioavailability of aged dieldrin (Figure 6.5-2). Hence, despite active microbial mineralisation of soil carbon, dieldrin concentrations did not decrease. The hypothesis that increased microbial activity from carbon-amendments improved the potential for degradation of aged dieldrin within 12 months is therefore rejected. However, the data supported the alternative view that, instead of promoting the potential for dieldrin degradation, increased microbial activity inhibited processes required for dieldrin degradation.

Increased microbial growth and respiration were associated with low dieldrin dissipation (Figure 6.5-4, Table 6.5-2). This supported the idea that the bloom of copiotrophic growth after the addition of labile carbon suppressed required community-level traits of an oligotrophic diversity to degrade dieldrin. For example, Firmicutes bacteria such as Bacilli are commonly associated with copiotrophic growth [344,345] and in this study Bacilli were associated with poultry litter and poor dieldrin decreases (Figure S6.8-2, phylofactor 2). Copiotrophs, also known as r-strategists, prefer labile carbon and nutrient-rich sources, and have high growth rates, which means that they quickly outcompete the slow-growing oligotrophs as soon as fresh, energy-rich

carbon sources are available [346]. In contrast, oligotrophs (also called k-strategists) exhibit slower growth and outcompete copiotrophs in environments where carbon quality and nutrient availability are low [347]. Oligotrophs are suspected to be most important for degradation of aged, non-labile and recalcitrant carbon including organic pollutants [348] while their suppression under high levels of labile substrates is well documented [346].

6.6.2 Associations of oligotrophic growth with improved dieldrin dissipation

Oligotrophs are defined by their ability to grow under low-nutrient conditions and are known for their efficient and diverse metabolic capabilities to slowly degrade complex carbon compounds at the expense of growth rate. The idea that oligotrophic growth was important for dieldrin decreases agreed with relevant changes in bacterial taxonomy and diversity in this study. Acidobacteria and Verrumicrobia are chemoorganotrophic phyla that are consistently associated with oligotrophic environments [347,349]. Both of these phyla were part of a group of bacteria, which together were associated with improved dieldrin dissipation (Figures S6.8-2 and S6.8-3). Other bacterial taxa in this group associated with dieldrin dissipation were Planctomycetota, Gemmatimonadota, Bacteroidota and 7 ASVs of an uncultured species in family *Xanthobacteracea* of order Rhizobiales (α-Proteobacteria). All of these taxa have been associated with oligotrophic growth in previous studies [349]. Moreover, this was a similar taxonomic assemblage that was associated with increased dieldrin dissipation since 1988 in our recent field survey [340]. Here, the metabolic capabilities for degradation of dieldrin in two historically-contaminated soils were assessed and from the results we speculated that labile and decomposable carbon might inhibit biodegradation of chlorinated pollutants.

In further support of oligotrophs being important for dieldrin dissipation, a higher community-level phylogenetic diversity was evident in soils treated with biochar (Figure S6.8-1c), the treatment that resulted in maximum dieldrin dissipation throughout this study. Oligotrophic community traits are postulated to exhibit a high affinity for and indiscriminate uptake of a variety of carbon substrates [350]. Hence, a greater phylogenetic diversity would be expected in oligotrophic soils to metabolise a more diverse set of carbon sources. Moreover, as expected for oligotrophic environments, relatively evenly distributed, microbial abundances in this study enabled bacteria to access a variety of carbon materials, without dominant groups that outcompete others. Pilou's evenness, a measure of the distribution of species-counts per sample, was consistently highest with biochar (Figure 6.5-1d) and the abundances of major phyla remained relatively even compared to other treatments (Figure 6.5-7). Improved dieldrin dissipation in the biochar treatment might be associated with the ability of biochar to moderate oligotrophic growth, thus increasing the diversity in metabolic mechanisms and the chances of (co-)metabolism of chlorinated compounds.

The potential for metabolism or co-metabolism of chlorinated hydrocarbons exists in some of the taxa identified in this study as known oligotrophs. In particular, the seven uncultured *Xanthobacteracea* ASVs detected in phylofactor 8 (Figure S6.8-2) were noteworthy. They were associated with improved dieldrin

dissipation and were highly abundant, comprising 15.4 % of total *Xanthobacteracea* abundances (7,362 of 47,804 reads) across all treatments at 9 months. All members of the gram-negative family *Xanthobacteraceae* grow as aerobic chemoheterotrophs but often also as facultative chemolithoautotrophy with hydrogen and/or sulfur as electron donors and often with nitrogen-fixing metabolism. To identify the potential genera/species of these seven *Xanthobacteracea* ASVs, a Basic Local Alignment Search Tool (BLAST) search was undertaken using their 16s rRNA genes (https://blast.ncbi.nlm.nih.gov/). It showed that among the bacteria with highest sequence alignment were strains of *Xanthobacter, Ancylobacter* and *Starkeya sp.* The genera *Xanthobacter* and *Ancylobacter* can metabolise and co-metabolise chlorinated and brominated alkanes, alkenes, and aromatic compounds. They were isolated from various chlorinated hydrocarbons as the sole carbon and energy source, and possess hydrolytic haloalkane dehydrogenases with a broad substrate specificity [351]. The data from this incubation therefore suggest that oligotrophic conditions allowed these organisms to increase their participation in potential dieldrin degradation. Biochar-treated soil samples were among the greatest relative abundances of these organisms while poultry litter and H_2O_2 samples were among the lowest (Figure S6.8-2).

It is further noteworthy that for *Xanthobacteracea* the major isoprenoid quinone was ubiquinol-10 (coenzyme-10), which is indicative of aerobic respiration [351]. Overall, this study found that the bacterial gene-pool associated with improved dieldrin dissipation was consistently associated with the biosynthesis of a variety of high-redox ubiquinones while low dieldrin dissipation was associated with low-redox menaquinones, indicating that aerobic respiration was important for dieldrin degradation potentials (Figures 6.5-8 and S6.8-4 to S6.8-6).

6.6.3 Biochar a potential promoter for a dieldrin-degrading community

Biochar increased the soil pH but pH *per se* was not associated with dieldrin dissipation. After 9 months, the soil pH in the biochar (70.1% calcium carbonate equivalent) and lime treatments increased by approximately 0.64 units to 5.14 ± 0.06 SE, which represented a four-fold reduction in acidity. However, only biochar, but not lime, showed maximum decreases in dieldrin concentrations after 9 months of incubation (Figure 6.5-2). Meanwhile, the pH in the control and poultry-litter treatments remained at 4.52–4.55 (Table S4). Soil acidity affects a wide range of soil chemical and biological properties and limits the activity of pH-sensitive organisms. However, it may only weakly affect dieldrin retention processes within the soil matrix. For non-polar organochlorines such as dieldrin, it was observed that pH had little effect on their half-lives in soils [73].

Nonetheless, pH was associated with an increase in phylogenetic diversity over time (Figure 6.5-5). Furthermore, both biochar and lime drove metabolic shifts of gram-negative bacteria (Figures 6.5-6 and S6.8-1 to S6.8-3). Gram-negative bacteria, due to their cell-membrane physiology, are known to be more competitive under the stress induced by chlorinated hydrophobic compounds [352], indicating that pH promoted the formation of a microbial community that is resilient to toxicities. However, biochar might

further promote the potential for dieldrin degradation via other mechanisms. For example, previous studies showed that biochar materials increased electron exchange capacities and could facilitate reductive dechlorination of pentachlorophenol by bacteria [353,354]. On the other hand, biochar might have increased the adsorption potential of available dieldrin molecules onto porous and aromatic biochar surfaces. This was indicated by the presence of different types of hydrophobic materials in biochar samples (Figure S6.8-7) and by the observation that less dieldrin was extractable (under the given extraction method) a day after biochar application (Figure 6.5-1a). This in turn might have reduced the effects of toxic elements such as dieldrin on microbial community dynamics.

It is well established that lipophilic hydrocarbons, such as dieldrin, are toxic to bacterial and eukaryotic cells because they disrupt cytoplasmic membranes and thereby inhibit respiration and energy transduction [352]. The type of biochar used in this study might therefore simultaneously act as a protector and promotor of dieldrin degraders by providing habitat with lower toxicity for microorganisms and maintaining proximity to sorbed dieldrin [355,356]. It was found that the gene-pool of bacteria that were associated with increased dieldrin dissipation shifted towards increased potentials for the biosynthesis of membrane components such as various fatty acids and heme b from glycine (Figures 6.5-8 and S6.8-4 to S6.8-6). This indicated that bacteria able to modify the fatty acid composition of their lipid bilayer and resist dieldrin toxicities, might be associated with capabilities to (co-)metabolise dieldrin. Taken together, the significantly improved dieldrin decreases in the biochar treatment compared to the poultry litter or H_2O_2 treatments may be linked to the growth of gram-negative bacteria, immobilisation of toxic elements and mechanisms related to biochar surface chemistry.

6.6.4 Potential role of soil aggregation and tillage

The number of soil macroaggregates visibly increased during the first 9 months of incubation. This was expected after the addition of labile C and subsequent microbial processing of labile C into polysaccharide materials and mineral-associated C [357]. Soil aggregation increases with microbial activity, whereby more microbial polysaccharide materials are secreted, which increases affinity to soil particles [358]. However, after soil sampling at 9 months, soil aggregates were crushed and homogenised across all treatments. This destruction of soil aggregates coincided with faster decreases in extractable dieldrin concentrations across all treatments during the remaining three months of incubation (Figure 6.5-1a). These results implied that the destruction of aggregates, such as tillage, may have promoted microbial degradation of dieldrin.

This result was not expected because anaerobic conditions within aggregates would favor reductive dechlorination of dieldrin. The redox potential towards the aggregate-center can be anoxic under saturated aggregates (-1 kPa) [96,359], which would have favored the activity of obligate anaerobes. However, as the moisture of incubated soils was maintained at 80 % of field capacity (~ -25 kPa) oxygen may have diffused deeper into aggregates. Under field conditions, conventional tillage had resulted in superior pollutant

degradation compared to minimal tillage, mainly due to decreased adsorption and higher pollutant bioavailability under the conventional tillage [360,361].

The results from our study suggested that the supply of oxygen after crushing the aggregates may have accelerated oxidation of aromatic compounds. After 12 months of incubation in this study, the gene pool of bacteria in treatments with maximum dieldrin decreases was associated with aerobic degradation of aromatic compounds (Figure S6.8-6), which was not apparent at 9 months. Interestingly, this pathway of aromatic compound degradation via beta-ketodiate is always present in bacterial strains that degrade chlorinated aromatic compounds [362]. Hence, the acceleration of dieldrin dissipation after aggregates were crushed, may be linked to microbial oxidation of recalcitrant C and subsequent co-metabolism of dieldrin.

6.7 IMPLICATIONS AND CONCLUSIONS

The study showed that microbial growth after addition of labile carbon, even if only applied once or twice, may affect a soil's potential to degrade persistent pollutants. The supply of labile carbon to soils promoted the 'wrong' type of microbial growth for the potential degradation of aged, chlorinated pollutants as it inhibited dieldrin decreases within 12 months of application. Hence, land managers may need to be aware that the application of any organic soil amendment, including manure, vermicompost, seaweed sludges or similar [363] that directly supply labile carbon or indirectly via increased plant/pasture growth and rhizosphere deposits, may inhibit microbial degradation of POPs such as dieldrin in soil. Microbial communities can be highly specific to soil type and thus it remains unclear if this phenomenon was soil or dieldrin specific, with these results requiring validation with other soil types and different POPs.

On the other hand, the benefits of biochar for landholders seeking remediation strategies for aged POPs in topsoil may be two-fold. Firstly, the biochar used in this study increased retention of dieldrin residues and secondly, dieldrin dissipation continued over the course of 12 months. Biochar promoted oligotrophic growth and a phylogenetically diverse microbial community that taxonomically showed potential to metabolise or co-metabolise recalcitrant carbon, including chlorinated hydrocarbons. The results from this incubation study did not reflect natural conditions and require thorough validation with long-term field trials. Future research is needed to determine how different types of biochar materials can be utilised in soil to promote microbial reactions to biodegrade chlorinated pollutants. Lastly, the crushing of aggregates might have promoted the oxidation of aromatic and chlorinated hydrocarbons, hence more research is required to elucidate the role of surface-soil tillage in the microbial decomposition of chlorinated pollutants.

6.8 SUPPLEMENTARY MATERIALS

6.8.1 Experimental design

The experiment was designed to capture the dissipation of dieldrin residues that aged *in-situ* since 1988 (hence not newly applied residues) in an agricultural soil that contained high concentrations of total carbon. It

required a relatively long incubation time as dieldrin dissipation was expected to be slow due to residue sorption and occlusion by carbon materials. The freshly sampled soil mass for the microcosms contained a microbial community that was relatively close to field conditions at the start of the incubation and changes of this community were observed after treatments. Two days after soil sampling, eleven treatments including ten carbon amendments and an untreated control were applied to 683 g of dieldrin-contaminated soil (dry weight basis) at 80% of water-holding capacity. No additional dieldrin was applied to ensure that only aged dieldrin residues were assessed throughout the incubation. Each treatment was homogenized and divided into four replicates with each replicate (171 g soil dry weight) was placed into a sealed 1-L glass fermentation jar. A total of 44 jars were randomized and incubated for 12 months in a controlled-environment room at 25˚C in the dark. A vial with 5 ml H_2O was added and CO_2 was trapped in the headspace during the first 16 weeks and the last 12 weeks of the incubation using alkali traps (40 mL 1 M NaOH), which were replaced every two weeks and stored at -20˚ until processed. Days after the application of the organic substrates CO_2 respiration/carbon mineralisation drastically reduced, which indicated that substrates were fully utilised. Hence, sampling of NaOH traps stopped after 16 weeks, as no major differences in CO_2 respiration between treatments were expected. To sample NaOH traps and to emulate ambient air supply while also minimising dieldrin volatilisation, oxygen was provided weekly by opening the lids for 15 min [334]. Water loss through the open headspace during oxygenation was replaced in regular intervals. Soils were subsampled (~ 25 g) from each jar at three time points, i.e. 1 day (T0), 9 months (T09) and 12 months (T12) after treatment, and soil samples stored at -20˚C until further processed. The sampling at T0 to T09 was done using a glass test tube to collect a composite from 3–4 soil cores of each jar. After T09 sampling, all soils were disturbed by re-homogenizing soils. This was done as soluble-carbon amendments were re-applied at T09 (see Treatments) and required that they were well mixed into the soil. Subsequent analyses have taken the potential effects of this disturbance on soil processes into account.

6.8.2 Dieldrin extraction and analysis

The concentrations of total extractable dieldrin were measured at 0 (T0), 9 (T9) and 12 months (T12) as following: approximately 10 g of air-dried soil was finely ground using a ball mill (Retsch MM400, Germany) at 25 rounds per minute for 1 min. Ground soil (1 g ±0.002) was then weighed into a 50-ml centrifuge tube, spiked with 40 µL dichlorodiphenyldichloroethane (DDD, 50 µg/ml) as internal standard and then extracted three times each with 4 ml acetone:hexane (1:1) by vortexing for 10 sec followed by side-to-side shaking at 225 rpm for 30 min. Extracts were centrifuged at 2500 rpm for 2 min, supernatants were decanted into amber glass bottles and stored at 4˚C in the dark for later analysis. One mL of the soil extract was blown down to just dryness under nitrogen then dissolved in 1 mL hexane before Florisil clean up. The Florisil (500 mg/3 mL) cartridge was first conditioned with 3 mL hexane, then loaded with 1 mL soil extract in hexane, followed by eluting with 5mL of acetone:hexane (1:9). Eluent was evaporated to just dryness and redissolved in 1 mL hexane for analysis using a dual column GC/ECD (Varian CP-3800; DB-5MS UI 15 m × 0.32 mm, 0.25 µm;

DB-1701 15 m × 0.32 mm, 0.25 μm) equipped with nickel 63 electron capture detector (ECD) and autosampler. A total of 2 μL was injected and separated into two columns. The temperature of injector and detector was held at 280 ˚C and 320 ˚C, respectively. Analytes were measured at a column temperature of 210 ˚C, a flow rate of 2.3 mL/min and a run time 20 min. The known concentration of spiked DDD was used as internal standard for a calibration curve. The calibration curve was created by injecting three levels of dieldrin standards (0.47, 0.094 and 0.0094 μg mL^{-1}) and one DDD standard (0.17 μg mL^{-1}). The ratio of the peak areas of dieldrin and DDD was used for quantification. The instrument detection limit (IDL) for dieldrin was 0.002 μg mL^{-1} and the method limit of detection (MLD) and limit of quantification (MLQ) were 0.025 μg g^{-1}. No background levels of DDD were detected previously in this soil. Dieldrin recovery was 89 - 119 % based on previous trials with spiked dieldrin. Six extractions were included of untreated reference soil (airdried, ground and stored in the dark) at different extraction batches. The standard deviation of extracted dieldrin concentrations of this reference soil was of 0.028 μg g^{-1} dry soil.

6.8.3 Detailed description of statistical analysis used in this study

All analyses were done in R software (https://www.r-project.org/). Heat-treated samples were excluded from all formal analyses as it yielded no DNA at T0. Dieldrin concentrations (μg g^{-1} dry soil) or dieldrin decreases (%) were predicted from treatments as well as from soil variables and mean CO_2 respiration using four linear mixed-effect (LME) models with the nlme and lme4 packages. Dieldrin concentrations were normally distributed across all treatments and timepoints. Mean daily CO_2 respiration (μmol CO_2 C g^{-1} soil day^{-1}) for the models was calculated for T0, T04 and T12 by averaging mean daily respiration for the first two weeks (T0 T0.5), the first 4 months (T0 T04) and the first 4 months plus the last 3 months (T0 T12), respectively. All models are defined in supplementary materials (LME model S1 to LME model S4). Model approach, selection of fixed effect, random effects and diagnostics were based on [219] and supplementary-described previously [271]. To explore how variation of dieldrin decreases since T0 related to decreases in microbial biomass C, total C and extractable organic C as well as to microbial diversity indices, two principal component analysis (PCA) were performed at T09 (n = 40) and T12 (n = 40). All variables were scaled and centered. The first two components explained 49 % and 57 % of the variation at T09 and T12, respectively.

Alpha diversity indices were calculated after rarefying abundances to a depth of minimum sample size (9,214 reads) after removing heat-treated samples at T0, which yielded no DNA. Common diversity indices including species richness (N0) and Shannon index (H') were calculated with function 'estimate_richness' from the Phyloseq package [364] while Pielou evenness (J') was calculated as follows [365]:

$$J' \quad \frac{H'}{\ln(N0)}$$

The effects of incubation time on bacterial community abundances were analysed using principal component analysis (PCA) and redundance analysis (RDA), performed after abundances were transformed into centered

log-ratios. Heat-treated samples at T0 were removed prior to PCA (n = 132) and samples treated with heat and H_2O_2 were removed prior to RDA (n = 108) as these treatments had too much influence on the overall variance of bacterial community composition (i.e. were outliers). The RDA modeled clr ratios as response with Months ×Treatment as categorical predictors. The model explained 10.9 % of the clr variance (R^2 adjusted) and was significant based on a global permutation test for RDA (999 permutations, $p < 0.001$).

To test the effect of treatments (excluding heat treatment, n = 120) on the presence/absence of bacterial taxonomic groups, the package Phylofactor [314] was used to perform generalized phylofactorization with function 'gpf' using the mixed algorithm, a binomial distribution and the choice parameter 'var' (Phylofactor model S1) as described in the package tutorial (https://github.com/reptalex/phylofactor) and described previously [340]. The first 10 phylofactors were processed. In addition, aggregated and transformed abundances (isometric log-ratios, ILRs) of bacterial taxonomic groups (phylofactors) at T09 (n = 40, Phylofactor model S2) and T12 (n = 40, Phylofactor model S3) were regressed with a Gaussian distribution using function 'PhyloFactor' where a number of factors were produced based on the Kolmogorov–Smirnov threshold of $p = 0.0001$ to elucidate which taxonomic groups were predicted by low/high dieldrin decreases. Each phylofactor represents balances or abundance ratios (ILRs) of the combined abundances of the phylofactor group (group 1) and the remainder group (group 2). ILRs reflect the response of group 1 relative to group 2 and vice versa.

Sparse partial least squares (sPLS) regression was performed with the package mixOmics [289] with dieldrin decreases (%), decreases in microbial biomass C (%), decreases extractable organic C (%) and mean respiration (μmol CO_2-C g^{-1} soil day^{-1}) as response variables predicted from bacterial metabolic (Metacyc) pathways potentials at T09 (n = 40) and T12 (n = 40). Pathway potentials were pruned to those with at least 1 % of total abundances, which retained 319 and 318 pathways for T09 and T12, respectively, and were centered log-ratio (clr) transformed as guided by the mixOmics project (http://mixomics.org). For pathways and principal component selection 'Mfold' validation with 10 folds, 100 repeats and a Q2 cutoff of > 0.098 was used. A heatmap with Ward clustering from the mixOmics package was used to display pair-wise associations between pathways and soil variables from two principal components, which explained most variation.

Linear mixed-effect (LME) models used in this study:

(LME model S1)

$$dieldrin_{tij} = \beta_0 + \beta_1 X_{tij}^{(1)} + ... + \beta_p X_{tij}^{(p)} + \mu_{0injection\_date} + \mu_{0sample\_id} + \varepsilon_{tij}$$

(i = 1,..120 samples; j = 1,...11 treatments x 1,..3 months; t = T0, T09 or T12; GC injection_dates = 1,..12; sample_id = 1,..44))

where $\varepsilon_{tij} \sim N(0,\sigma^2)$ and $X_{tij}^{(1)} + ... + X_{tij}^{(p)}$ represents p predictors (treatment × month), $\mu_{0injection\_date}$ represents the random intercept specific to GC injection dates and $\mu_{sampleid}$ represents the random intercept specific to each sample.

LME Model S1 syntax with the lme4 package in R (Output as per table S1):

Model <− lmer(log1p(Dieldrin) ~ Treatment×Month + (1|Injectiondate) + (1|SampleNo),

data = lme.df, REML = TRUE)

(LME Model S2)

$$dieldrin_i = \beta_0 + \beta_1 MicrobialQuotient + \beta_2 MeanRespiration + \beta_3 TotalC + \beta_4 Hydrogen{:}TotalC\ ratio +$$

$$\beta_5(Microbial\ biomass\ C) + \mu_{0treatments} + \mu_1 MonthT0_{treatment} + \mu_2 MonthT09_{treatment}$$

$$+ \mu_3 MonthT12_{\ treatment} + \varepsilon_i$$

(i = 1,..120 samples, treatments = 1,..11)

where $\varepsilon_i \sim N(0,\sigma^2)$ μ_0 represents the random intercept specific to each treatment and μ_1,.. μ_{3Month} represents the random effect of each sampling month.

LME Model S2 syntax with the lme4 package in R:

Model <− lmer(log1p(Dieldrin) ~ MicrobialQuotient×MeanRespiration + TOC + HC

+ (1 + Month | Treatment), data = lme.df, REML = TRUE)

(LME Model S3)

$$DieldrinDecreaseT09_i = \beta_0 + \beta_1\ HydrogenDecreaseT09 + \beta_2 MeanRespirationT0\_T04 + \beta_3 PilouEvennessT09 +$$

$$\mu_{0treatments} + \varepsilon_i$$

(i = 1,..44 samples, treatments = 1,..11)

LME Model S3 syntax with the lme4 package in R:

Model <− lmer(DieldrinDecrease ~ HydrogenDecrease + MeanRespiration + PilouEvenness + (1 | Treatment), data = lme.df_T09, REML = TRUE)

(LME Model S4)

$$DieldrinDecreaseT12_i = \beta_0 + \beta_1 MicrobialBiomassDecreaseT12 + \beta_2 MeanRespirationT0\_T12 + \beta_3 FaithpdT12 +$$

$$\mu_{0treatments} + \varepsilon_i$$

(i = 1,..44 samples, treatments = 1,..11)

LME Model S4 syntax with the lme4 package in R:

```
Model <- lmer(DieldrinDecrease ~ MicrobialBiomassDecrease + MeanRespiration +FaithPD + (1 | Treatment),
data = lme.df_T12, REML = TRUE)
```

Outputs of Phylofactor models used in this study:

Phylofactor model S1

Phylofactor object from function gpf (Presence and absence as response, treatment as explanatory, Samples across all three sampling events, excluding heat-treated samples, n = 120)

Method : gpf
Algorithm : mix
Formula : cbind(Successes, Failures) ~ phylo * Treatment
Partitioning Variable : Treatment
Number of species : 4879
Number of factors : 10
Largest non-remainder bin : 3425
Number of singletons : 0
Paraphyletic Remainder : 1318 species

Factor Table:

	Group1	Group2
Factor 1	3425 member Monophyletic clade	1454 member Monophyletic clade
Factor 2	15 member Monophyletic clade	3410 member Paraphyletic clade
Factor 3	959 member Monophyletic clade	2451 member Paraphyletic clade
Factor 4	19 member Monophyletic clade	2432 member Paraphyletic clade
Factor 5	31 member Monophyletic clade	1423 member Paraphyletic clade
Factor 6	26 member Monophyletic clade	1397 member Paraphyletic clade
Factor 7	5 member Monophyletic clade	2427 member Paraphyletic clade
Factor 8	11 member Monophyletic clade	948 member Paraphyletic clade
Factor 9	23 member Monophyletic clade	2404 member Paraphyletic clade
Factor 10	79 member Monophyletic clade	1318 member Paraphyletic clade

Phylofactor model S2

Phylofactor object from function PhyloFactor (T09 samples only, excluding heat-treated samples, n = 40)

Method : glm
Choice : var
Formula : PhylogeneticTree ~ Dieldrin_decrease
Number of species : 3743
Number of factors : 115
Frac Explained Variance : 0.0136
Largest non-remainder bin : 803
Number of singletons : 67
Paraphyletic Remainder : 1864 species

Factor Table:

	Group1	Group2	ExpVar	F	Pr(>F)
Factor 1	24 member Monophyletic clade	3719 member Monophyletic clade	0.00104860	6.3224	0.01627500
Factor 2	342 member Monophyletic clade	3377 member Paraphyletic clade	0.00087304	14.8860	0.00042899
Factor 3	478 member Paraphyletic clade	2899 member Paraphyletic clade	0.00056453	7.3689	0.00992450
Factor 4	15 member Monophyletic clade	2884 member Paraphyletic clade	0.00032593	5.8867	0.02011100
Factor 5	7 member Monophyletic clade	2877 member Paraphyletic clade	0.00031913	14.0690	0.00058697
Factor 6	4 member Monophyletic clade	2873 member Paraphyletic clade	0.00030982	6.3336	0.01618800
Factor 7	tip	2872 member Paraphyletic clade	0.00022571	11.1000	0.00193100
Factor 8	7 member Monophyletic clade	2865 member Paraphyletic clade	0.00021497	13.5200	0.00072687
Factor 9	13 member Monophyletic clade	2852 member Paraphyletic clade	0.00019277	5.5575	0.02365400
Factor 10	11 member Monophyletic clade	2841 member Paraphyletic clade	0.00019070	3.9031	0.05549200
	...	...	...	...	...
	Group1	Group2	ExpVar	F	Pr(>F)
Factor 19	803 member Paraphyletic clade	2007 member Paraphyletic clade	1.4635e-04	4.9060	0.032832
Factor 115	tip	786 member Paraphyletic clade	4.9612e-05	7.0716	0.011401

Phylofactor model S3

Phylofactor object from function PhyloFactor (T12 samples only, excluding heat-treated samples, n = 40)

Method : glm
Choice : var
Formula : PhylogeneticTree ~ Dieldrin_decrease
Number of species : 3523
Number of factors : 58
Frac Explained Variance : 0.00949
Largest non-remainder bin : 2511
Number of singletons : 33
Paraphyletic Remainder : 818 species

Factor Table:

	Group1	Group2	ExpVar	F	Pr(>F)
Factor 1	15 member Monophyletic clade	3508 member Monophyletic clade	0.00063574	4.7144	0.03622200
Factor 2	6 member Monophyletic clade	3502 member Paraphyletic clade	0.00049920	7.2506	0.01048600
Factor 3	2 member Monophyletic clade	3500 member Paraphyletic clade	0.00035296	6.8365	0.01273500
Factor 4	2511 member Paraphyletic clade	989 member Paraphyletic clade	0.00036168	4.5507	0.03942300
Factor 5	8 member Monophyletic clade	2503 member Paraphyletic clade	0.00036687	9.3068	0.00414820
Factor 6	5 member Monophyletic clade	2498 member Paraphyletic clade	0.00035833	3.8421	0.05734500
Factor 7	tip	2497 member Paraphyletic clade	0.00033013	17.6360	0.00015554
Factor 8	7 member Monophyletic clade	2490 member Paraphyletic clade	0.00029885	10.4280	0.00256100
Factor 9	tip	2489 member Paraphyletic clade	0.00028711	14.7260	0.00045600
Factor 10	10 member Monophyletic clade	979 member Paraphyletic clade	0.00025637	4.9842	0.03154900
	...	...	...	...	...
	Group1	Group2	ExpVar	F	Pr(>F)
Factor 58	tip	818 member Paraphyletic clade	1.4635e-04	4.9060	0.032832

6.8.4 Supplementary tables

Table S6.8-1. Overview of treatments and substrate carbon added to each treatment at day 1 (T0) and 9 months (T09) after incubation. Soil C concentrations are means ± s.e. (n=4).

Treatment	Molecular formula	MW	Carbon MW	Elemental C ratio	Substrate C (g kg^{-1})	Substrate C added T0 (% Soil C)[a]	Soil C concentration T0 (g kg^{-1})[b]	Substrate C added T09 (% Soil C)	Soil C concentration T09 (g kg^{-1})
Control						0.00	5.34 ±0.09	0.00	5.15 ±0.05
Hydrogen peroxide	H_2O_2	34.01				0.00	5.37 ±0.04	0.00	5.13 ±0.05
Autoclave (135˚C)						0.00	5.30 ±0.05	0.00	5.10 ±0.01
Inositol	$C_6H_{12}O_6$	180.16	72.06	0.40	0.06	0.11	5.48 ±0.01	2.47	5.25 ±0.04
Sodium formate	HCOONa	68.00	12.01	0.18	0.2	0.37	5.36 ±0.13	1.72	5.24 ±0.06
Sodium acetate	$C_2H_3NaO_2$	136.08	24.02	0.18	0.40	0.75	5.52 ±0.03	2.66	5.26 ±0.04
Citric acid	$C_6H_8O_7$	192.12	72.06	0.38	0.63	1.19	5.46 ±0.05	2.55	5.11 ±0.03
Fumaric acid	$C_4H_4O_4$	116.07	48.04	0.41	0.64	1.20	5.55 ±0.04	0.51	5.36 ±0.1
Lime	$CaCO_3$	100.09	12.01	0.12	0.42	0.29	5.37 ±0.02	0.00	5.16 ±0.02
Poultry litter					5.08[c]	9.50	5.83 ±0.08	0.00	5.51 ±0.06
Biochar					5.59[c]	10.37	5.91 ±0.08	0.00	5.58 ±0.05

[a] Equivalent mass of substrate was added to 683 g soil dry weight each; [b] Measured one day after treatments were applied; [c] Calculated from C content provided in Table S1. Poultry litter and biochar were applied at a rate of 15.4 g kg^{-1} dry soil or 20 t ha^{-1} equivalent.

Table S6.8-2. Linear mixed-effect model for the analysis of repeated measurements with dieldrin concentrations as response and treatment × time as explanatory factor (LME Model S1). The confidence interval (CI), probability (*p*) and degree of freedom (df) are also shown.

	Dieldrin (µg g^{-1})			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	1.17	1.14 – 1.21	**<0.001**	96
Treatment [H_2O_2]	-0.11	-0.17 – -0.06	**<0.001**	96
Treatment [Heat]	-0.19	-0.24 – -0.14	**<0.001**	96
Treatment [Inositol]	-0.06	-0.10 – -0.02	**0.006**	96
Treatment [SodiumFormate]	-0.09	-0.13 – -0.05	**<0.001**	96
Treatment [SodiumAcetate]	-0.09	-0.13 – -0.05	**<0.001**	96
Treatment [CitricAcid]	-0.05	-0.10 – -0.01	**0.017**	96
Treatment [FumaricAcid]	-0.08	-0.12 – -0.04	**<0.001**	96
Treatment [Lime]	-0.09	-0.15 – -0.04	**<0.001**	96
Treatment [PoultryLitter]	-0.12	-0.17 – -0.07	**<0.001**	96
Treatment [Biochar]	-0.15	-0.20 – -0.09	**<0.001**	96
Month [T09]	-0.05	-0.10 – -0.01	**0.016**	96
Month [T12]	-0.17	-0.21 – -0.12	**<0.001**	96
Treatment [H_2O_2] * Week [T39]	0.08	0.02 – 0.14	**0.009**	96
Treatment [Heat] * Week [T39]	0	-0.05 – 0.06	0.925	96
Treatment [Inositol] * Week [T39]	0.03	-0.02 – 0.08	0.183	96
Treatment [SodiumFormate] * Week [T39]	0.02	-0.03 – 0.07	0.479	96
Treatment [SodiumAcetate] * Week [T39]	0.01	-0.04 – 0.06	0.722	96
Treatment [CitricAcid] * Week [T39]	0.02	-0.03 – 0.07	0.378	96
Treatment [FumaricAcid] * Week [T39]	0	-0.05 – 0.05	0.992	96
Treatment [Lime] * Week [T39]	0.05	-0.01 – 0.11	0.088	96
Treatment [PoultryLitter] * Week [T39]	0.08	0.02 – 0.14	**0.008**	96
Treatment [Biochar] * Week [T39]	-0.04	-0.10 – 0.02	0.212	96
Treatment [H_2O_2] * Week [T52]	0.08	0.02 – 0.14	**0.007**	96
Treatment [Heat] * Week [T52]	0.07	0.01 – 0.13	**0.017**	96
Treatment [Inositol] * Week [T52]	0	-0.05 – 0.05	0.895	96
Treatment [SodiumFormate] * Week [T52]	0.04	-0.01 – 0.09	0.103	96
Treatment [SodiumAcetate] * Week [T52]	0.05	0.00 – 0.10	**0.043**	96
Treatment [CitricAcid] * Week [T52]	0	-0.05 – 0.05	0.96	96
Treatment [FumaricAcid] * Week [T52]	0.1	0.05 – 0.15	**<0.001**	96
Treatment [Lime] * Week [T52]	0.02	-0.04 – 0.08	0.56	96
Treatment [PoultryLitter] * Week [T52]	0.05	-0.01 – 0.11	0.092	96
Treatment [Biochar] * Week [T52]	-0.02	-0.08 – 0.04	0.548	96
Random Effects				
σ^2	5.80E-04			
$\tau_{00\ SampleNo}$	1.50E-04			
$\tau_{00\ Injectiondate}$	3.40E-04			
ICC	0.46			
$N_{\ Injectiondate}$	12			
$N_{\ SampleNo}$	44			
Observations	132			
Marginal R^2 / Conditional R^2	0.846 / 0.916			

Table S6.8-3. Linear mixed-effect model with log-transformed dieldrin concentrations as response and microbial quotient, total C, microbial biomass C as % total C and the hydrogen-to-carbon ratio as predictors (LME Model S2). Heat-treated samples were excluded, and all variables were scaled prior to analysis. The confidence interval (CI), probability (p) and degree of freedom (df) are also shown.

	Dieldrin (µg g^{-1})			
Predictors	*Estimates*	*CI*	*p*	*df*
(Intercept)	1.03	1.00 – 1.06	**<0.001**	107
qCO_2 (μg CO_2-C g day^{-1} C_{mic}^{-1})	0.01	-0.00 – 0.03	0.099	107
Mean respiration (μmol CO_2-C g soil day^{-2})	0.03	0.01 – 0.04	**<0.001**	107
Microbial biomass C (% of Total C)	0.02	0.00 – 0.04	**0.012**	107
Total C (g kg^{-1})	0.02	0.01 – 0.04	**<0.001**	107
Soil hydrogen-to-total C ratio	0.01	0.00 – 0.02	**0.023**	107
Random Effects				
σ^2	0.001			
τ_{00} Treatment	0.004			
τ_{11} Treatment[Month T09]	0.001			
τ_{11} Treatment[Month T12]	0.003			
ICC	0.78			
N Treatment	10			
Observations	120			
Marginal R^2 / Conditional R^2	0.32 / 0.85			

Table S6.8-4. Summary of repeated measurements of pH, extractable organic C (EOC) and microbial biomass C (MBC) of incubated soils after 0 (T0), 9 (T09) and 12 (T12) months after treatments (MAT) (se = standard error).

Treatment	MAT	n	pH	±se	n	EOC (mgC g^{-1})	±se	n	MBC (µgC g^{-1})	±se
Control	T0	3	4.50	0.007	4	0.256	0.003	4	237	6
Control	T09	4	4.52	0.033	4	0.263	0.003	4	75	6
Control	T12	4	4.47	0.021	4	0.282	0.009	4	96	22
H_2O_2	T0		NA	NA	4	0.428	0.003	4	283	14
H_2O_2	T09	4	4.72	0.004	4	0.324	0.018	4	73	26
H_2O_2	T12	4	4.66	0.003	4	0.323	0.005	4	40	5
Heat	T0		NA	NA	4	1.257	0.008	4	790	28
Heat	T09	4	5.08	0.019	4	0.517	0.068	4	144	6
Heat	T12	4	5.00	0.03	4	0.333	0.021	4	20	10
Lime	T0		NA	NA	4	0.24	0.031	4	290	8
Lime	T09	4	5.14	0.057	4	0.232	0.004	4	105	16
Lime	T12	4	5.17	0.057	4	0.238	0.005	4	90	33
Poultry Litter	T0		NA	NA	4	0.314	0.007	4	344	21
Poultry Litter	T09	4	4.55	0.013	4	0.266	0.006	4	61	16
Poultry Litter	T12	4	4.49	0.018	4	0.274	0.008	4	41	23
Biochar	T0		NA	NA	4	0.188	0.004	4	280	12
Biochar	T09	4	5.14	0.03	4	0.195	0.002	4	114	7
Biochar	T12	4	5.06	0.019	4	0.196	0.002	4	96	13
Inositol	T0		NA	NA	4	0.218	0.008	4	289	6
Inositol	T09	4	4.61	0.018	4	0.255	0.005	4	93	7
Inositol	T12	4	4.63	0.013	4	0.274	0.004	4	67	9
Sodium Formate	T0		NA	NA	4	0.216	0.001	4	292	7
Sodium Formate	T09	4	4.67	0.003	4	0.244	0.004	4	94	9
Sodium Formate	T12	4	5.37	0.023	4	0.227	0.002	4	36	18
Sodium Acetate	T0		NA	NA	4	0.305	0.005	4	300	19
Sodium Acetate	T09	4	4.71	0.021	4	0.246	0.002	4	83	8
Sodium Acetate	T12	4	5.24	0.016	4	0.212	0.004	4	81	22
Citric Acid	T0		NA	NA	4	0.334	0.02	4	299	22
Citric Acid	T09	4	4.62	0.018	4	0.27	0.003	4	63	11
Citric Acid	T12	4	4.60	0.009	4	0.256	0.005	4	107	27
Fumaric Acid	T0		NA	NA	4	0.237	0.005	4	270	9
Fumaric Acid	T09	4	4.57	0.007	4	0.257	0.003	4	93	6
Fumaric Acid	T12	4	4.53	0.015	4	0.253	0.004	4	42	9

6.8.5 Supplementary figures

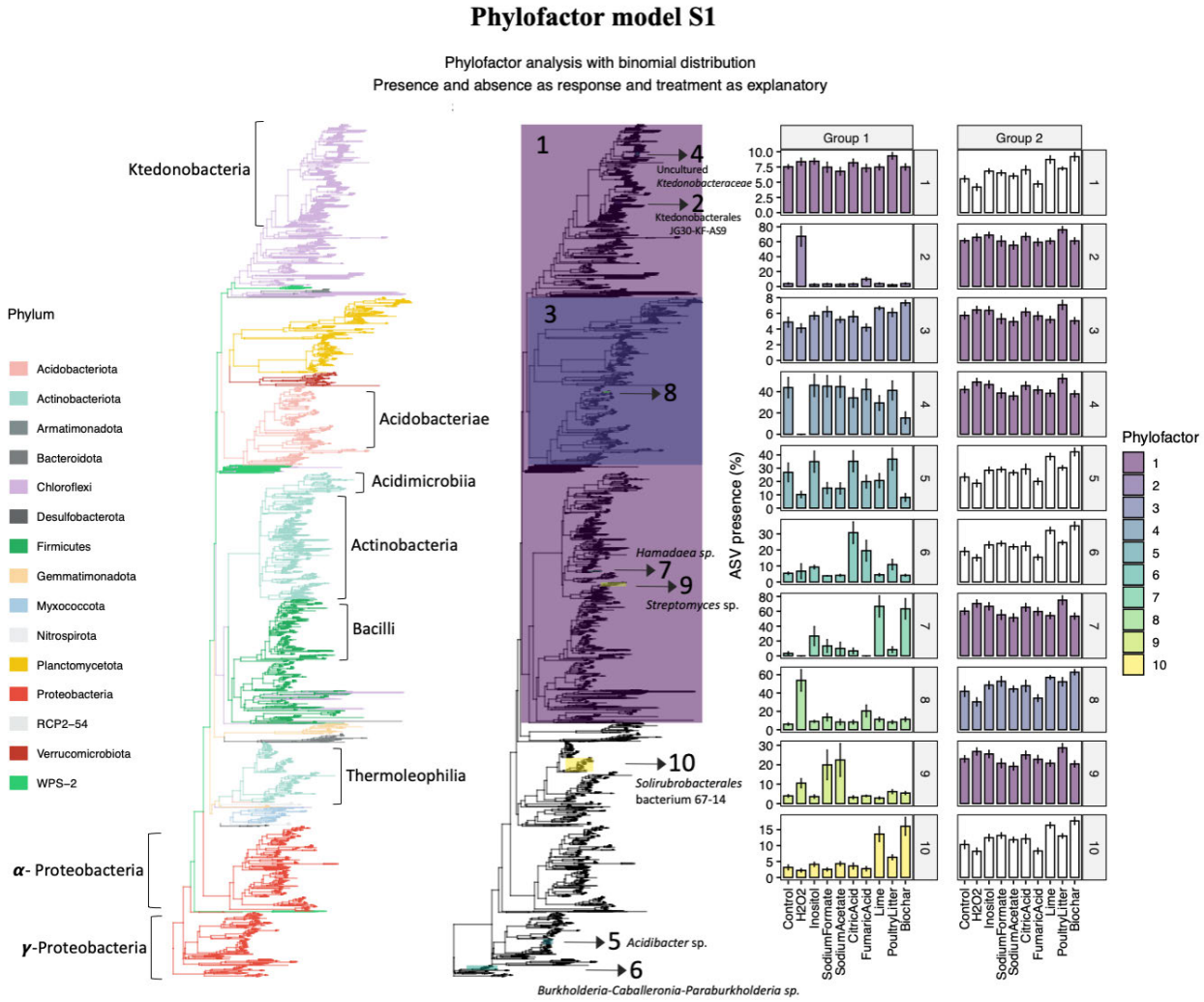


Figure S6.8-1. Visual representation of Phylofactor model S1. [**Left**] Phylogenetic tree across samples from 0 (T0), 9 (T09), and 12 months (T12) (excluding heat-treated samples, n = 120) with edges colored by phylum membership. Each tip represents one amplicon sequence variant (ASV). [**Right**] The same phylogenetic tree is shown but phylofactors (group 1) are highlighted, which indicate a taxonomic group that was significantly more or less likely to be present in a treatment compared to the remainder group (group 2). Barplots show the percent presence of phylofactors (group 1) and their remainder groups (group 2).

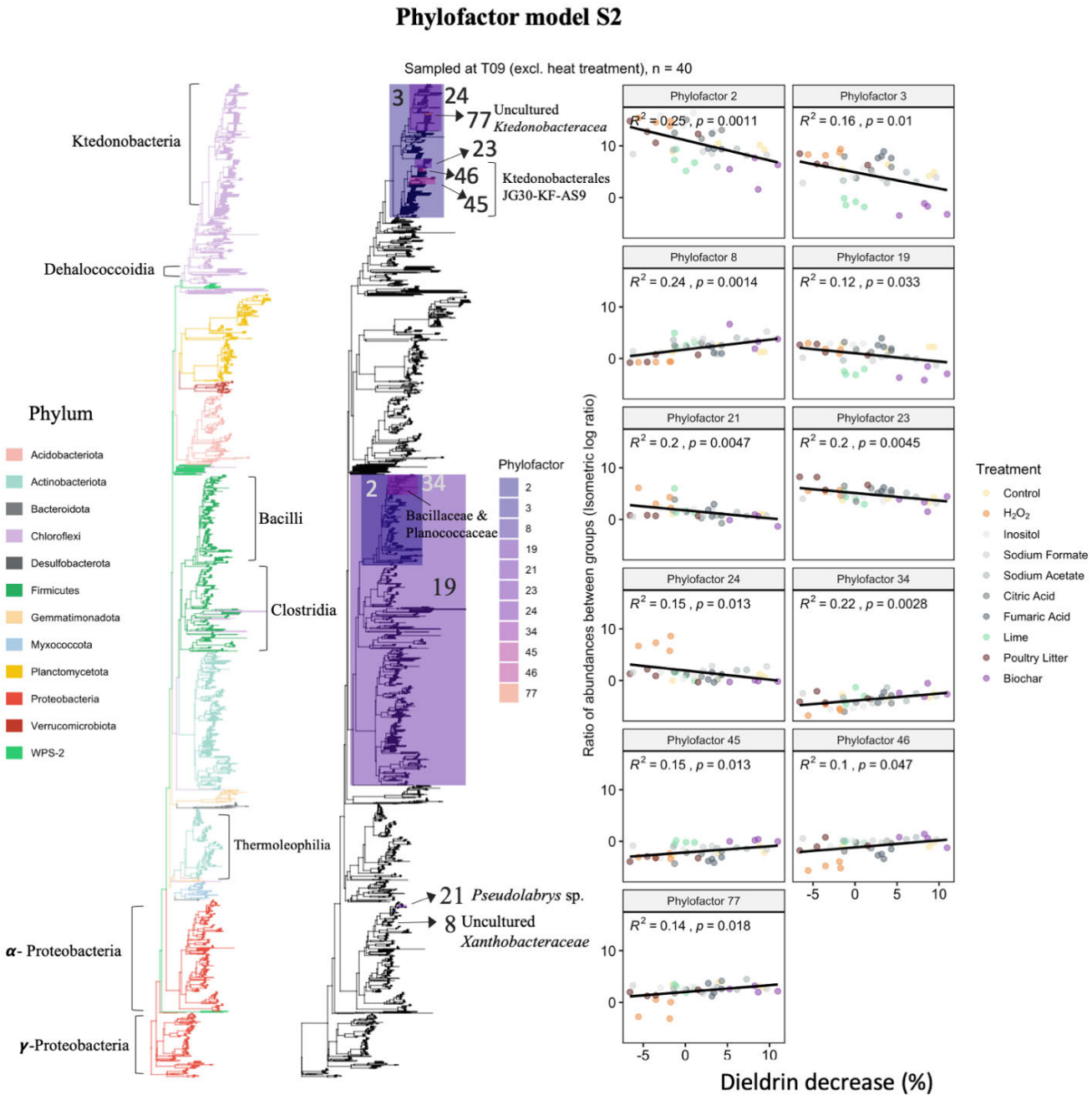


Figure S6.8-2. Models of isometric log-ratios (ILR) across edges of phylogenetic trees at 9 months (T09) (Phylofactor model S2). [**Left**] Phylogenetic tree of T09 samples (excluding heat-treated samples, n = 40) with edges colored by phylum membership. Each tip represents one amplicon sequence variant (ASV). [**Right**] The same phylogenetic tree but phylofactors (group 1) are highlighted, for which the gradient of ILRs was associated with dieldrin decreases. Scatterplots show the relationship between ILRs and dieldrin decreases (%), where the ILR is derived from the aggregated abundances of each phylofactor (group 1) and its remainder group (group 2) in the phylogenetic tree. ILRs are relative; hence, a decrease of group 1 is an increase of group 2 and vice versa.

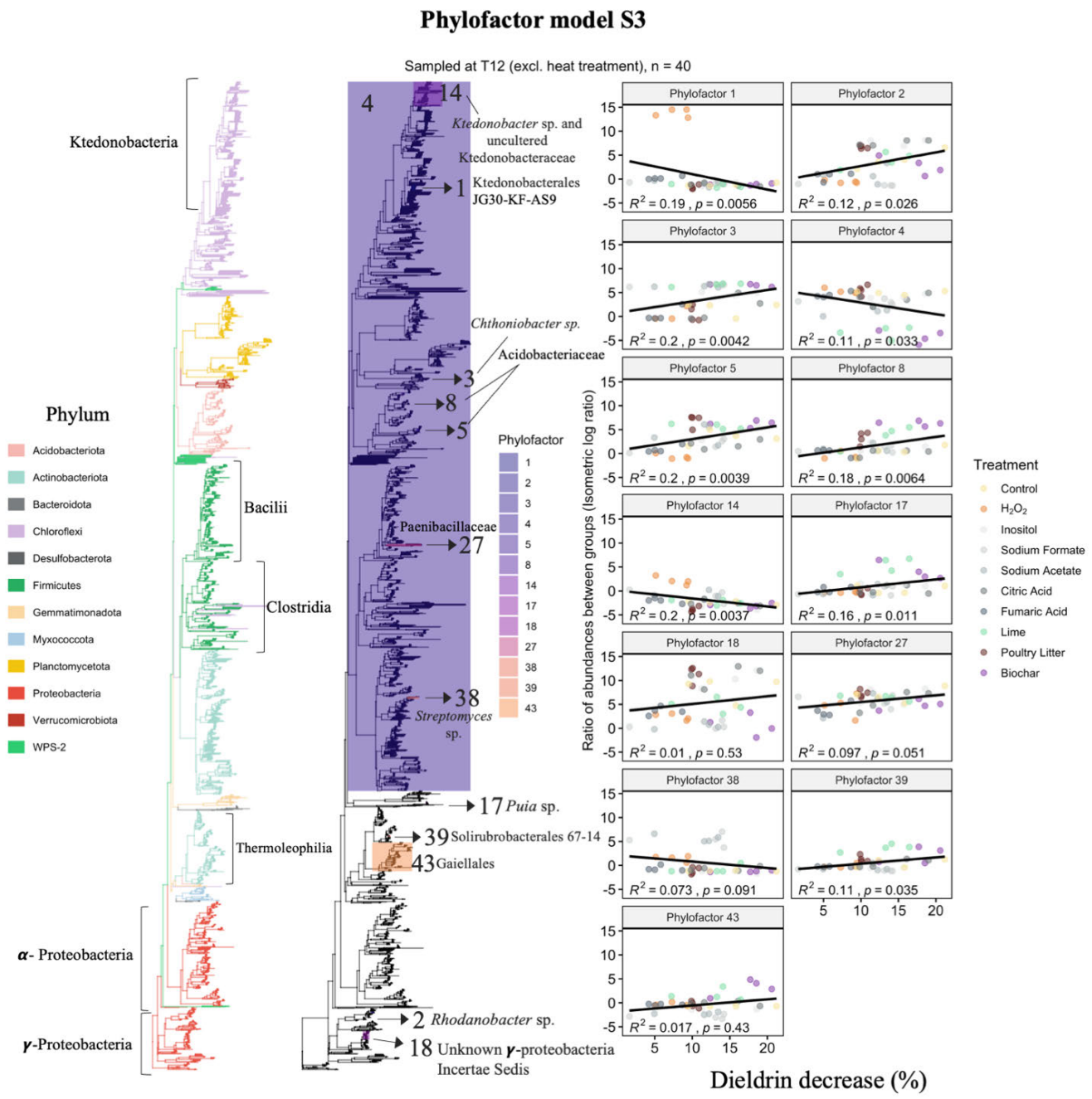


Figure S6.8-3. Models of isometric log-ratios (ILR) across edges of phylogenetic trees at 12 months (T12) (Phylofactor model S3). [**Left**] Phylogenetic tree of T12 samples (excluding heat-treated samples, n = 40) with edges colored by phylum membership. Each tip represents one amplicon sequence variant (ASV). [**Right**] The same phylogenetic tree but phylofactors (group 1) are highlighted, for which the gradient of ILRs was associated with dieldrin decreases. Scatterplots show the relationship between ILRs and dieldrin decreases (%), where the ILR is derived from the aggregated abundances of each phylofactor (group 1) and its remainder group (group 2) in the phylogenetic tree. ILRs are relative hence a decrease of group 1 is an increase of group 2 and vice versa.

Figure S6.8-4. Clustered heatmap of association scores of metabolic pathway abundances for each sample (T09 excluding heat-treated soils, n = 40) from sparse partial least squares (sPLS) regression with the mixOmics package. Pathway abundances were modelled as predictors for dieldrin decreases, mean respiration, decreases in microbial biomass and decreases in extractable organic carbon. Red colors indicate positive associations, blue colors negative associations.

Figure S6.8-5. Clustered heatmap of association scores of important pathways from sparse partial least square (sPLS) regression using **T12** (12 months) samples excluding heat-treated soils (n = 40) with dieldrin decrease, decreases in microbial biomass C (MBC), decreases of extractable organic C (EOC) and mean respiration as response variables and pathway abundances as predictors.

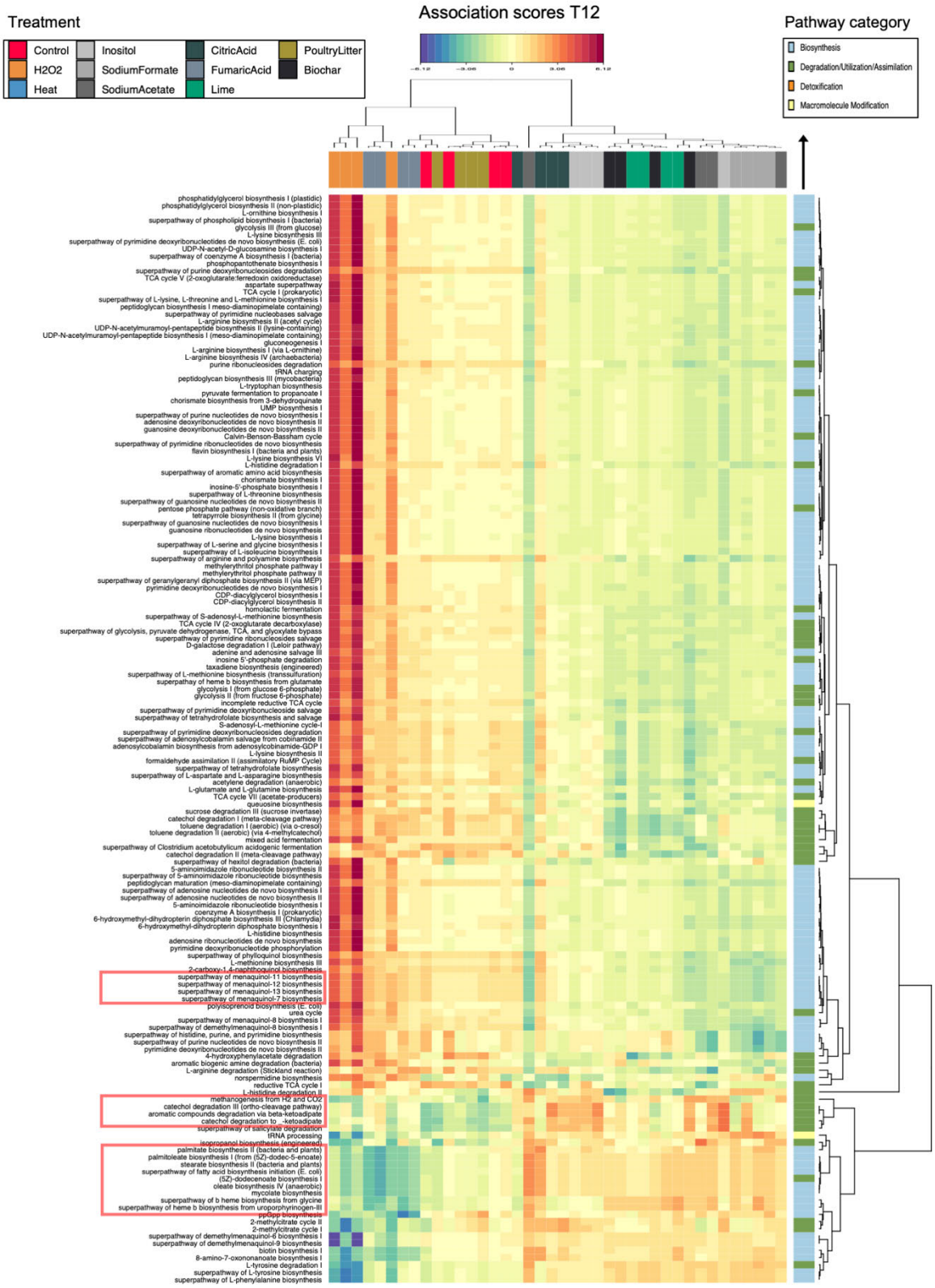


Figure S6.8-6. Clustered heatmap of association scores of important pathways from sparse partial least square (sPLS) regression using T12 (12 months) samples excluding heat-treated soils (n = 40).

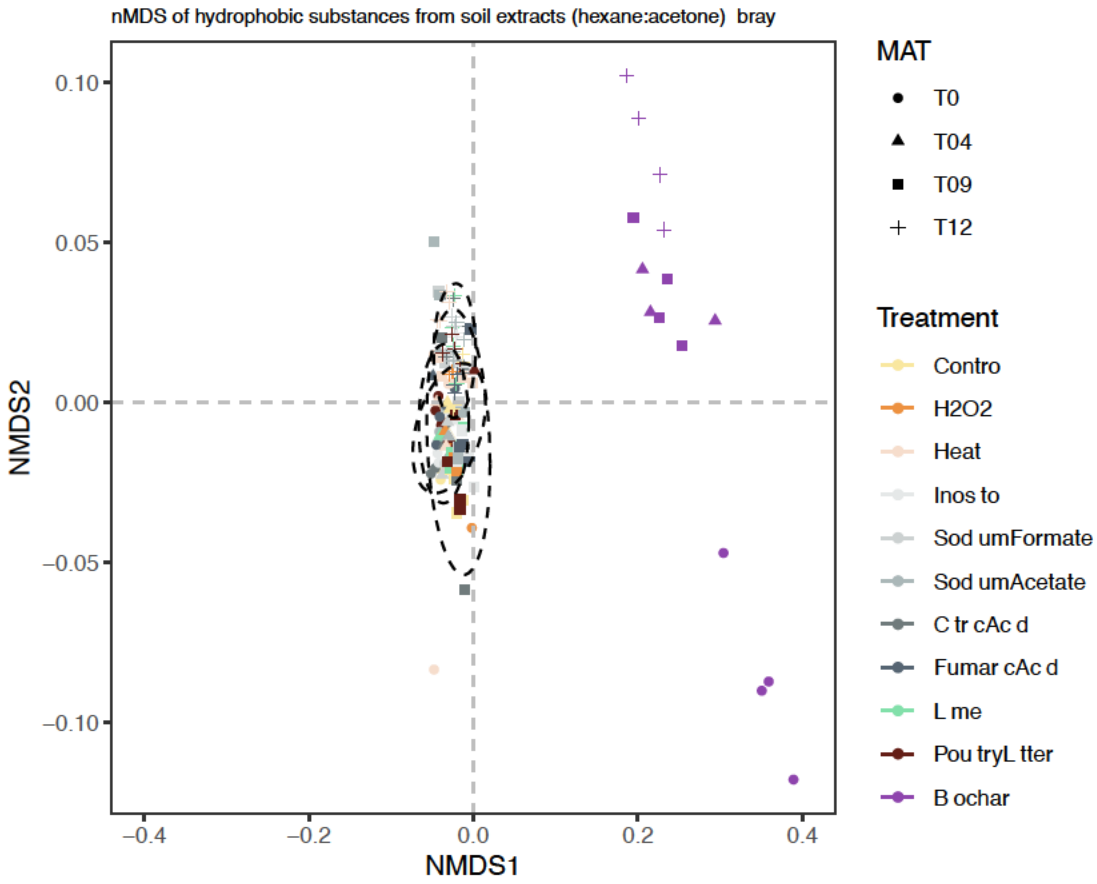
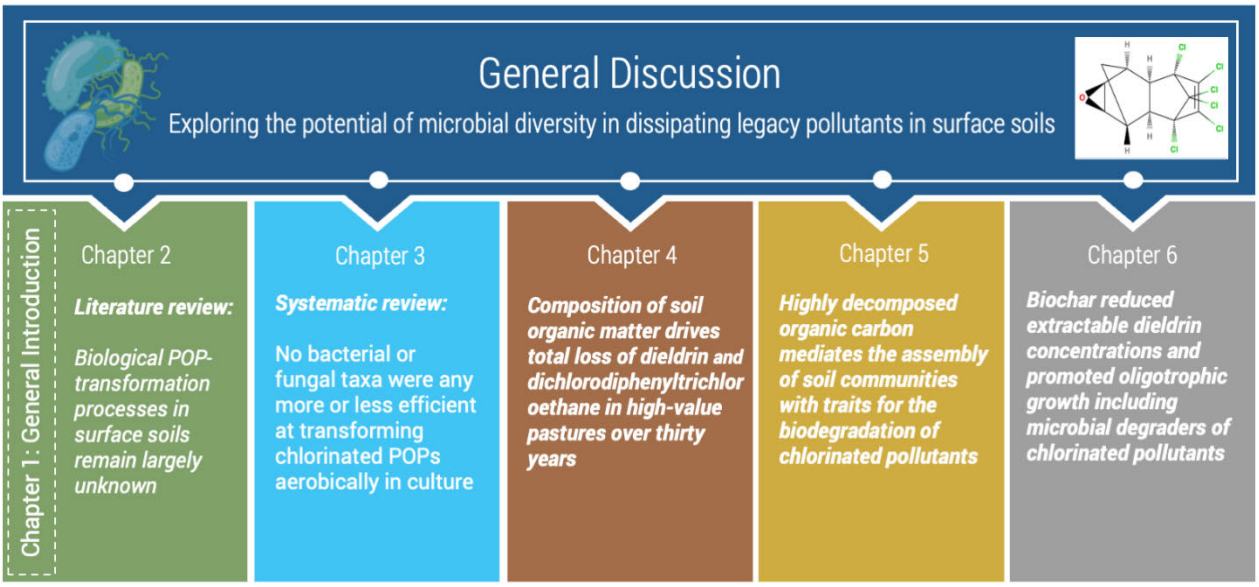


Figure S6.8-7. Non-metric multidimensional scaling (nMDS) of ratios of peak areas derived from gas chromatography with electron capture detector (GC ECD) between all extracted substances from soils and the internal standard dichlorodiphenyldichloroethane (DDD). Shapes indicate dieldrin extractions at 0 (T0), 4 (T04), 9 (T09) and 12 (T12) months after treatments (MAT).

Chapter 7: General discussion



7.1 FOUNDATIONS FOR THIS RESEARCH

The majority of POP residues were released into the environment at a global scale until the 1980s. Today, topsoils remain a major sink and source for POP residues worldwide. It is believed that natural attenuation or biodegradation is the most effective, long-term risk-mitigation strategy for soil pollutants [14]. However, aerobic natural attenuation processes of these extremely persistent and highly chlorinated POPs are too slow on a human timescale. Hence, research is required to elucidate these processes with aim to speed up their natural dissipation. Once applied to topsoils, POP residues undergo various abiotic processes that determine the fate of POPs in the environment, as outlined in chapter two, using dieldrin as an example.

Based on the reviewed literature, soil retention is the most limiting process for microbial availability to POP residues and therefore slows down the evolution of degradation pathways. Hence, although many studies globally have provided evidence of POP transformation reactions by bacterial and fungal isolates in liquid media (as described and analysed in Chapters 2 and 3), it remained unclear how to exploit this degradation potential in topsoils. Biological POP-transformation processes in surface soils remain a black box and are largely unknown. However, identifying potential drivers of microbial POP dissipation within the soil matrix is critical for policy makers and land managers to implement strategies that speed up natural attenuation in soils.

In Chapter 3, a systemic review of 78 bench-scale studies in liquid media since 1967 showed that no bacterial or fungal taxa were any more or less efficient at transforming chlorinated POPs aerobically in culture (Chapter 3). Furthermore, the type of bacterial and fungal taxa that were isolated from soils in 26 different countries, with POPs in selective media, resembled the most common soil phyla and classes. Hence, there does not appear to be a taxonomic pre-disposition for the aerobic enzymatic transformation of chlorinated pollutants. Consequently, any bacteria could likely evolve the mechanisms to catalyse transformation reactions in surface soils if conditions are suitable. In other words, any surface soil may have the genetic potential to evolve the required function for POP degradation.

This phenomenon was observed previously. For example, converged evolution of pesticide-degrading genes was established for atrazine, a herbicide with a similar water solubility to lindane. Within a period of three decades, entirely new pathways for atrazine mineralisation among genetically different bacteria appeared around the world [366]. A similar phenomenon was observed with lindane, as outlined in Chapter 2.5. Hence, if any bacteria in any soil can evolve a metabolic pathway for POP degradation, then it is crucial to understand how this evolution can be enhanced. To progress this area of research it may be necessary to focus on community-level interactions with soil properties and ultimately with the toxic residues. Once we understand how functional pathways are related to POP degradation in terrestrial environments, it will be possible to advise on best land management practices.

7.2 SOIL ORGANIC MATTER COMPOSITION A POTENTIAL DRIVER FOR POP DEGRADATION

As a result, the remaining chapters explored links of soil properties with a soil's potential to degrade POPs in surface soils. In particular, the role of soil organic matter composition was of interest due to the intrinsic relationship of soil organic matter and POP degradation as outlined in Chapter 2.6 and reviewed by Ren et al. previously [186]. This thesis focused on the POP dieldrin, as dieldrin residues remained the greatest concern for farmers in Victoria, Australia. We had unique access to topsoil (10 cm) of two contaminated agricultural sites and their dieldrin residue history since 1988. This was kindly facilitated by organochlorine coordinators in Australia, who had been monitoring these properties and were collaborators in this project. A field survey of these soils was conducted as detailed in Chapters 4 and 5, which explored the links of soil properties, including carbon quality, to POPs degradation processes.

From the field survey results in Chapters 4 and 5, it was apparent that the prevalence of degraded organic carbon, i.e. carbon of low energy value for the microbial community, was associated with improved dieldrin dissipation. This increased potential for dieldrin dissipation was linked to a metabolically efficient microbial taxonomy. In particular, a broad diversity of oligotrophs was noted to be involved with evenly distributed abundances. These microbial traits likely evolved in an energy-low soil environment where highly decomposed and persistent carbon materials mediated microbial community assembly and function.

Furthermore, the results indicated that, compared to fungi, bacteria played a much greater role for POP dissipation in terms of total and relative abundance.

7.3 CHANGES TO CARBON DEGRADATION DYNAMICS INFLUENCED POP TRANSFORMATION POTENTIALS

In a closed-chamber incubation experiment for Chapter 6, it was further discovered that the supply of labile carbon sources to a contaminated and highly POP-retentive soil promoted the growth of the 'wrong' type of microbes and inhibited dieldrin decreases within 12 months. After applying various soluble carbon sources, the sudden increase in the activity of copiotrophic microbes was associated with smaller decreases of dieldrin residues. By contrast, slow-growing, gram-negative, oligotrophic bacteria were associated with improved dieldrin decreases. In further agreement with the results from the field survey, an evenly distributed community and function were related to enhanced dieldrin decreases in this experiment. These results showed that equitable access to soil carbon resources allowed for greater participation of bacteria that are more resistant to chlorine toxicity and have metabolic capabilities to decompose recalcitrant carbon sources, including chlorinated hydrocarbons. However, these oligotrophic bacteria are easily outgrown; as soon as other bacteria grow on high-energy carbon substrates, the required oligotrophic participation may reduce. Hence, any soil amendments that include soluble carbon sources may reduce as soil's potential to degrade POPs, even after only one or two applications. This may include root exudates of pastures that constantly supply glucose among other substrates and, therefore, may prevent the development of POP-degradation potentials altogether.

This assertion was in line with observations from a 10-week plant-growth experiment, which was conducted from 08 May to 17 July 2019 with help of Honours student Matt Brewer at La Trobe University. The results of this experiment were not presented in the thesis as the growth of a key plant species failed. Briefly, eleven agricultural plant species were grown in a dieldrin-contaminated and highly POP-retentive soil. These included eight pasture species and three cropping species. After 10 weeks of plant growth, there was no difference in dieldrin-residue concentrations in the rhizosphere of any plant species to the reference concentration at the start of the experiment. Hence, root exudation had no short-term effects on the degradation potential of POPs such as dieldrin.

Instead, the effects of biochar made from poultry litter looked promising. As shown in Chapter 6, the addition of biochar to soils immediately decreased the concentrations of extractable dieldrin (likely through strong sorption onto porous and aromatic biochar surfaces). Furthermore, dieldrin decreases continued for the remaining 12 months after application. Biochar promoted oligotrophic growth and a phylogenetically diverse microbial community that displayed the potential to metabolise or co-metabolise recalcitrant carbon including chlorinated hydrocarbons. Hence, these results warrant further research into the use of biochars as a soil amendment to reduce long-term POP risks. Furthermore, an unintended but nonetheless important side-

observation from the experiment in Chapter 6, was that soil disturbance enhanced the potential of aerobic degradation of recalcitrant compounds, and therefore, may enhance POP degradation. Combined, these results provided direction for future research.

7.4 LIMITATIONS FUTURE PERSPECTIVES

All of the results from this PhD project show relationships or associations only. No mechanisms were uncovered, that prove causalities. As a consequence, the results of the field survey, which assessed the topsoil of two historically dieldrin-contaminated sites, requires validation through further field surveys of other contaminated sites. Furthermore, mechanistic studies are required to document the biochemical processes leading to co-metabolism of dieldrin. Future PhD projects could be created for students to access these records and sites to repeat the field survey on more soils in Victoria and other states in Australia. Therefore, it is crucial that records of POP residues collected by organochlorine officers across Australia from the late 1980s are maintained and made available where possible. Furthermore, my studies had focused on dieldrin as dieldrin was the primary POP of concern in the investigated region. It was assumed that other chlorinated POPs undergo similar fates in soils, as determined by their intrinsic chemical properties. However, this still requires rigorous validation.

Currently, no full dieldrin degradation pathways are known for microbial isolates, due to the difficulties in growing microorganisms with dieldrin as a sole carbon source (Chapter 2). Hence, there are no known dieldrin-degrading genes that could be quantified. Consequently, no microbial degradation mechanisms, such as enzymatically catalysed transformation reactions were uncovered. Instead, this research focused on exploring relationships between decreases in the concentration of extractable dieldrin residues over time, soil properties and community-level diversity and function.

Nonetheless, the results may provoke a fundamental shift in the approach to natural attenuation strategies of POPs and provide a new direction for future research that aims to facilitate natural attenuation of POPs through land management practices. The message is that 'good' soils, which are rich in organic matter, well-structured and covered by a perennial pasture that is microbially active, are probably 'not good' in degrading POPs. As a result, the following questions require answering through future research:

1. Do all soils have an intrinsic potential for microbial POP degradation?
2. Do all chlorinated POPs associate with decomposed organic carbon materials, and how can oligotrophic growth be 'switched on' in those materials?
3. Is microbial resistance to chlorine toxicity associated with improved POP degradation potentials, and what are the relevant mechanisms?
4. What are the physicochemical interactions between biochar and POPs, and how do they relate to microbial potentials for POP degradation?

5. Does soil tillage increase the potential for aerobic POP degradation, and what are the relevant mechanisms?
6. Where are POP residues located within soil aggregates, and in what size of aggregates? How is this associated with microbial degradation potentials?

To answer these questions it may be necessary to develop a method that combines DNA stable-isotope probing (DNA-SIP) of ^{13}C-labeled POPs with metagenomics as done for other pollutants, for instance, by Thomas et al. [367] and Uhlik et al. [368]. This would allow for tracing dieldrin residues to microbial functions in soils. The challenge for this method is to overcome ^{13}C-DNA yield constraints as microbial metabolism involving dieldrin may be extremely slow.

It remains critical to find solutions for policy makers and land managers around the globe to minimise long-term risks of POP exposure. Long-term funding, plus an inter-regional and inter-disciplinary approach would be required to fully investigate these complex interactions and may include research in agronomy, soil organic matter dynamics, hydrocarbon biochemistry, soil microbial ecology and metagenomics. My aspiration is that authorities and land-care groups around the world are able to recommend land management practices to farmers that assure maximum biodegradation of POPs in a given topsoil. All in all, my findings provided promising perspectives and should encourage future research.

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