Science of The Total Environment
Volume 691, 15 November 2019, Pages 135-145
https://doi.org/10.1016/j.scitotenv.2019.06.497

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

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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.

Keywords: soil diversity; persistent organic pollutants; microbial bioremediation; xenobiotics; organic matter; microbial biomass

1. 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 (Chapin and Wasserstrom, 1981; Goldberg, 1975; Scheringer, 2002). 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 (Jensen, 1966; Scheringer, 2002). 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 (Department of Natural Resources and Environment (DNRE), 1996).

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 (United Nations Environment Programme, 2017). 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 (Corrigan and Seneviratna, 1990; DNRE, 1996).

Organic chemical contaminants in soils undergo transport, retention and transformation processes which are mostly mediated by soil microorganisms (Nannipieri and Badalucco, 2003). 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 (Cheng, 1990). While modifying environmental factors that control microbial growth and kinetics is a strategy for *in-situ* soil remediation of POPs (Alexander, 1999; Rittmann, 1994), further understanding of these relationships is required.

Biodegradation rates are slower when POPs are adsorbed by the soil matrix (Calderbank, 1989; Jenkinson and Rayner, 1977) with the rate of desorption from soil organic matter (SOM) limiting for degradation (Ren et al., 2018; Zhang et al., 2011). Dieldrin and DDT are fat-soluble and easily sorbed by hydrophobic organic matter (Gevao et al., 2000; Pignatello and Xing, 1995; Sharom et al., 1980). 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 (Ren et al. 2018). 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.

2. Materials and methods

2.1. 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 S1). Both locations had loamy topsoils based on texture classification of the International Society of Soil Science (1929). 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 (Isbell, 1996) which was equivalent to an Acrisol (IUSS Working Group WRB, 2014) and in the southeast as a Chromosol (Isbell, 1996) which was similar to a Luvisol (IUSS Working Group WRB, 2014) (Table S1).

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) (DNRE, 1996) and are shown in Table S2. 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.

2.2. 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 (EPA - United States Environmental Protection Agency, 2007) 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. 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).

2.3. 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 et al. (1954). Water-holding capacity (WHC) of soils was determined using the small soil core method (Cassel and Nielsen, 1986) 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 S4.

2.4. Microbial biomass C and N and inorganic N

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were estimated based on the chloroform fumigation method (Vance et al., 1987). 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 (Joergensen, 1996). To determine MBN and extractable organic nitrogen (EON), a peroxydisulfate ($K_2S_2O_8$) reagent mix was added to the K_2SO_4 extracts (1:1) and autoclaved (121 ˚C, 104 kPa) following the method of Cabrera and Beare (1993). Afterwards the NH_4^+ and NO_3^- concentrations were determined using a flow injection analyser (Lachat QuickChem 8500 Series || , USA) following the manufacturer's protocol (Harbridge 2007; Knepel, 2002). 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 (Brookes et al., 1985). 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 S5).

2.5. 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 (Kovacs et al., 2010) and the internal transcribed spacer (ITS) located between the 18S rRNA and 28S rRNA genes for fungi (Gardes and Bruns, 1993). 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 (Kovacs et al., 2010) was used for bacteria and primer pair ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA) and ITS4 (5'-TCCTCCGCTTATTGATATGC) for fungi (Gardes and Bruns, 1993).

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 (Kovacs *et al.* 2010), 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 (Lee and Taylor, 1992).

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 (Brown et al., 2005; Hewson and Fuhrman, 2006). 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. S1).

2.6. 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 (Fierer et al., 2005). 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 (Klevenhusen et al., 2011). 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 (C_t) of both plates were calculated under the same baseline fluorescence threshold. Standard deviations (STD) $\geq$ 0.3 of any quantification cycle numbers (C_t) was rejected and the corresponding C_t 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 (Ruijter et al., 2009b) 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 3.08×10^5 gene copies of amplified and purified genomic DNA of *Escherichia coli* DH5α (for bacteria) and five replicates of 1.82×10^5 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 (Ruijter et al., 2009a; Töwe et al., 2010) (Equation S3)

2.7. 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 and Cobbold (2012) (Fig. S2) 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) (Simpson, 1949) was calculated from OTU abundances for each sample, given by ARISA. The Simpson diversity index is suited for small sample sizes (Magurran, 2004) and was recommended for comparisons of microbial alpha diversity (Haegeman et al., 2013). 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).

2.8. 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 principle component analysis (PCA) (Fig. 4c and d) and boxplots by soil type (Fig. S3). 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.

2.9. Linear mixed effect modelling

We used packages lme4 (Bates et al., 2015) and nmle (Pinheiro et al., 2018) 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 Zuur et al. (2009). 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 $\log_e$-transformed (Ott, 1994; Limpert et al., 2001) 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 $\log_e$-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 Jørgensen and Pedersen (1998) and then corrected for the effect of Jensen's inequality (Equation S9) (Nakagawa et al., 2017). Thus, these estimates < 1 show negative effects and > 1 positive effects.

2.10. 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 (Anderson et al., 2017) on Bray-Curtis distances of untransformed OTU abundances using the vegan package (Oksanen et al., 2018). Secondly, we used the multivariate statistical package ' MvAbund ' (Wang et al. 2012) 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 (Warton et al., 2017) and Likelihood-Ratio-Tests (Warton et al., 2012). 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 Westfall *et al.* (1993). Model assumptions (mean-variance relationship and independence) were checked visually using appropriate plots.

3. Results

3.1. 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 ($p < 0.0001$) (Fig. 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.

The decline of dieldrin and DDT residues between 1988-2017 clearly differed between the two soil types (Fig. 2). Dieldrin and DDT loss was 42% and 44% in the Chromosol compared to 73% and 71% in the Kurosol, respectively (Fig. 2). Total loss of dieldrin residue, but not of DDT residues, was correlated negatively with carbon-to-nitrogen ratio (R_s^2 = 0.89***, Fig. 3a) and positively with the microbial-C-to-total-C ratio (R_s^2 = 0.93***, Fig. 3b), irrespective of soil type. The paddock-variation in the loss of DDT was inversely correlated with dieldrin concentrations (R_s^2 = 0.77***, Fig. 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**, Fig. 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 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 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).

3.2. 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 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 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.

3.3. 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 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 2).

The LME indicated that bacterial 16s rRNA gene copy number increased with fungal 18s rRNA gene copy number and *vice versa* (Table 2). However, only bacterial abundance increased with pH whereas fungal abundance increased with total organic C. Fig. 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.

3.4. 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 3). Fig. 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 (Fig. 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 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 Fig. S4. 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 3).

4. 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.

4.1. 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 (Fig. 3a) and a high microbial-C-to-total-C ratio (Fig. 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. 3c, Table 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 (Lundquist et al., 1999; Woods and Schuman, 1986) and low carbon-to-nitrogen substrates degrade faster under these conditions (Nieder et al., 2003). For example, microbial-C-total-C ratios increase after the application of easily-digestible organic amendments such as farm-yard manure or green manures (Lundquist et al., 1999). Based on microbial-C-to-total-C ratios in crop rotation systems, Anderson and 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 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 (Quante et al., 2011; Scheringer, 2002), indicating high fat-solubility and partitioning into hydrophobic SOM (Karickhoff, 1981).

Soil organic matter consists of complexes of polymers and includes dead plant and microbial biomass (Schmidt et al., 2011). 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 (Chen and Chiu, 2003) and in soils rich in SOM (Paul, 2015; Stevenson, 1994). From previous studies, it is clear that the persistence of organic compounds (Green, 1974; Jenkinson and Rayner, 1977; Pinck and Allison, 1951), including agricultural pesticides (Calderbank, 1989; Nomura and Hilton, 1977; Riley et al., 1976; Zhang et al., 2011), is greater when they are bound to inorganic minerals or organic polymers and that strong sorption limits their degradation (Bosma et al., 1996; Rijnaarts et al., 1990).

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 (Kirkby et al., 2013; Kirkby et al., 2014). Competition for carbon might have subsequently activated a broader metabolism. Chen and 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 (Baldock et al., 1990). 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 (Paul, 2015) and consequently co-

metabolism of dieldrin or DDT. That may also explain the higher evenness of fungal communities ($E_{1/D}$, Table 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 (Fig. 4) and in paddocks with high total DDT loss (Fig. 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 (Fig. 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 (Song et al., 1986). However, due to interchangeability of functions between fungi and bacteria (Rousk and Bååth, 2007; Rousk et al., 2009; Lozupone et al., 2012), broader enzymatic capabilities of fungi (Boer et al., 2005) and potentially greater carbon-use efficiencies of fungi (Six et al., 2006), 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 (Bengtson et al., 2012; Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010), depending on plant species (Wang et al., 2016). Future research into the effects of microbial dynamics and SOM mineralisation on degradation of persistent pollutants is needed to improve understanding in this area.

4.2. 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 2 and 3, Fig. 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 3). A small part of the variation (≈ 3%) of the bacterial community composition due to DDT concentrations was further implied by PERMANOVA (Table 3). However, it was less clear if dieldrin was associated with the bacterial community. Based on the results from the statistical package MvAbund (Wang et al., 2012), which has greater statistical power than PERMANOVA, there was a significant association of microbial community composition and dieldrin concentrations (Table 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 3, MvAbund). When assessed with p values that were unadjusted for correlations between OTUs, MvAbund detected 12 OTUs that significantly responded to DDT (Table 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 (van Dorst et al., 2014; Gobet et al., 2014; Wood et al., 2016). 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.

4.3. 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 (Fig. 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 (Alexander, 1999).

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 (Alexander, 1999). 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 (Birolli et al., 2015; Bumpus and Aust, 1987; Hay and Focht, 1998; Kataoka et al., 2010; Matsumoto et al., 2009, 2008; Nadeau et al., 1994; Xiao and Kondo, 2013).

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* (Bidlan and Manonmani, 2002) 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 (Arp et al., 2001). Nonetheless, the large genetic potential of microorganisms in soils (Tate, 2000) and observed genetic adaptations to persistent pollutants over time (van der Meer, 2006) mean that future research has the potential to identify and utilise microbial strains that are effective at degrading dieldrin and DDT.

5. 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.

Acknowledgements

Historical data of dieldrin and DDT concentrations were kindly provided by The National Organochlorine Residue Management Program (NORM). Furthermore, thanks to Dr. Grant Rawlin for his help and discussions. Thanks to Dr. Clayton Butterly for help with soil chemical analysis, Dr. Gary Clark for help with soil texture analysis, Dr. Corinne Celestina and Dr. Jennifer Wood for their advice on microbial analysis. Professor Michael Stear and Dr. Xia Lee (Statistics Consultancy Platform La Trobe University, Melbourne, Victoria) provided guidance with statistical methods. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Figures and tables

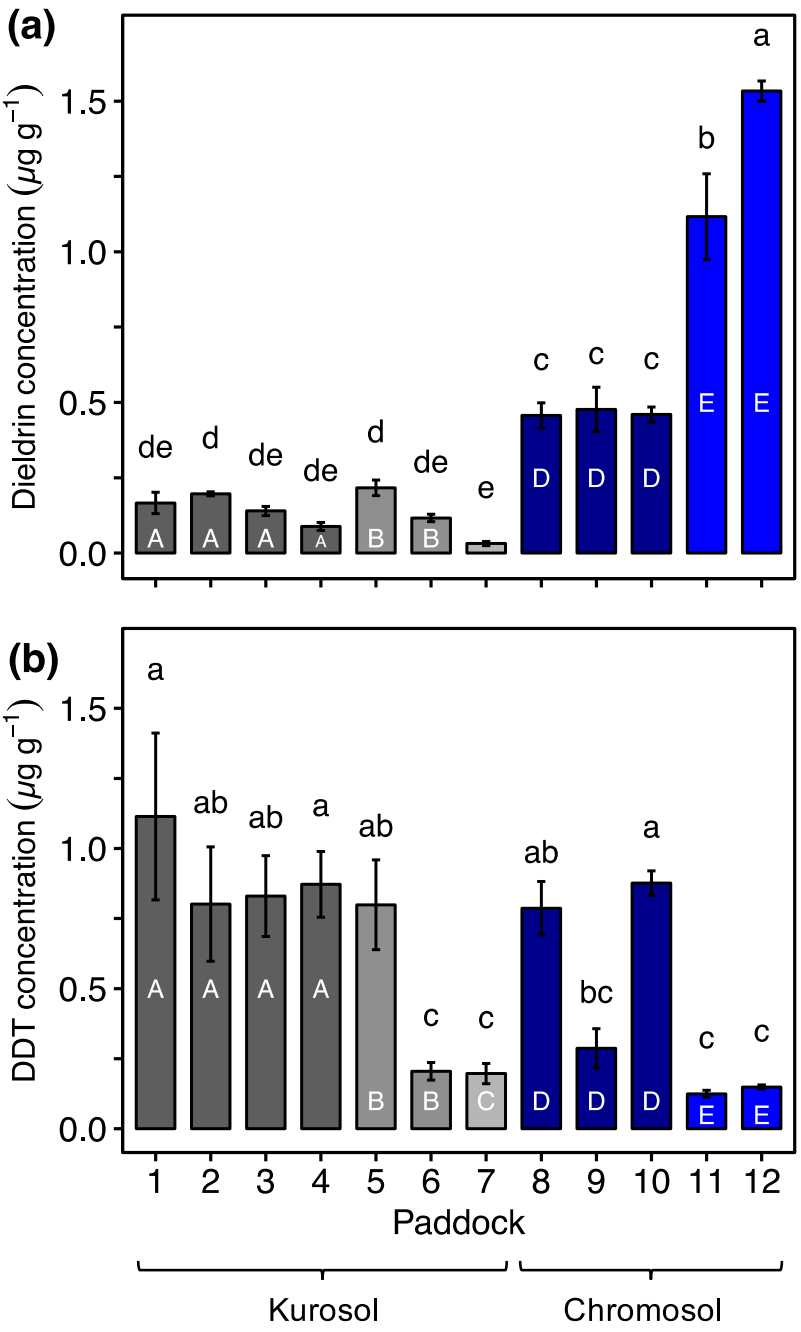

Fig. 1. Mean paddock concentrations of dieldrin (a) and DDT (sum of p,p'-DDT & p,p'-DDE) (b) measured in 2017 (0 - 10 cm) grouped by farms as shown by capital letters A-E. Grey colour scale are farms on the Kurosol and blue colours are farms on the Chromosol. Lower case letters indicate significant differences 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.

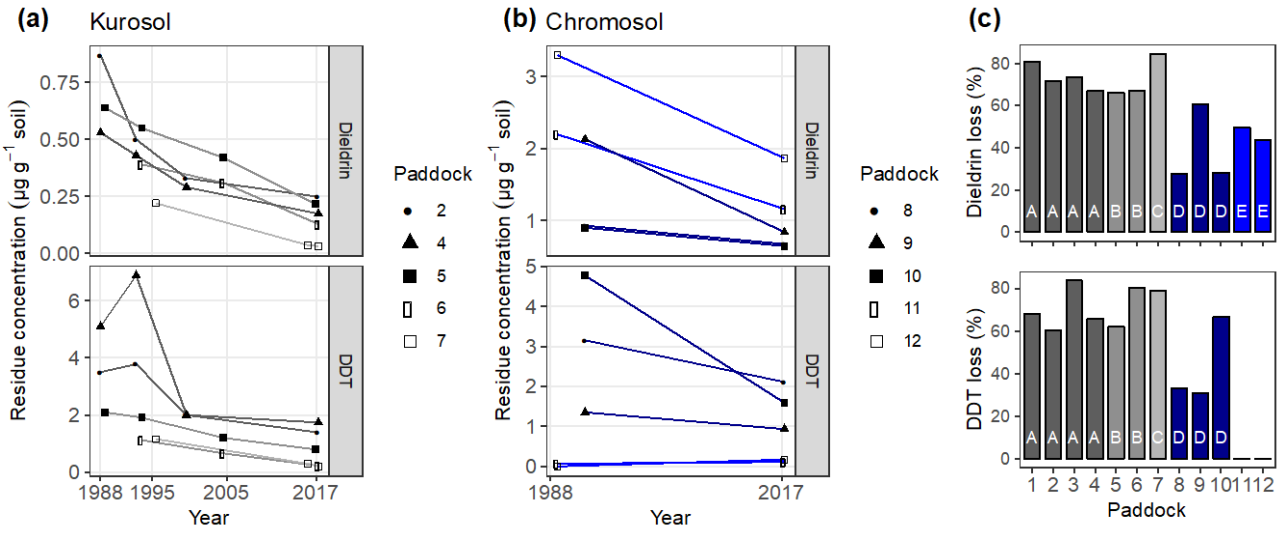

Fig. 2. Available dieldrin residue history per paddock since 1988 (a and b). Data from 1988 to 2015 was kindly provided by the National Organochlorine Residue Management program (NORM). The residue concentration shown for 2017 represent an average of measured values for 2015 and 2017. DDT values are the sum of p,p'-DDE and p,p'-DDT residues. Paddock 1 and 3 on the Kurosol were omitted from graph for clarity (a). Total residue loss in percent per paddock, grouped by farms as shown by the letters A-E (c). No analysis of variation was performed on residue loss as no field replicates were included in the NORM standard operating procedure. Grey colour scale refers to Kurosol and blue colour scale to Chromosol.

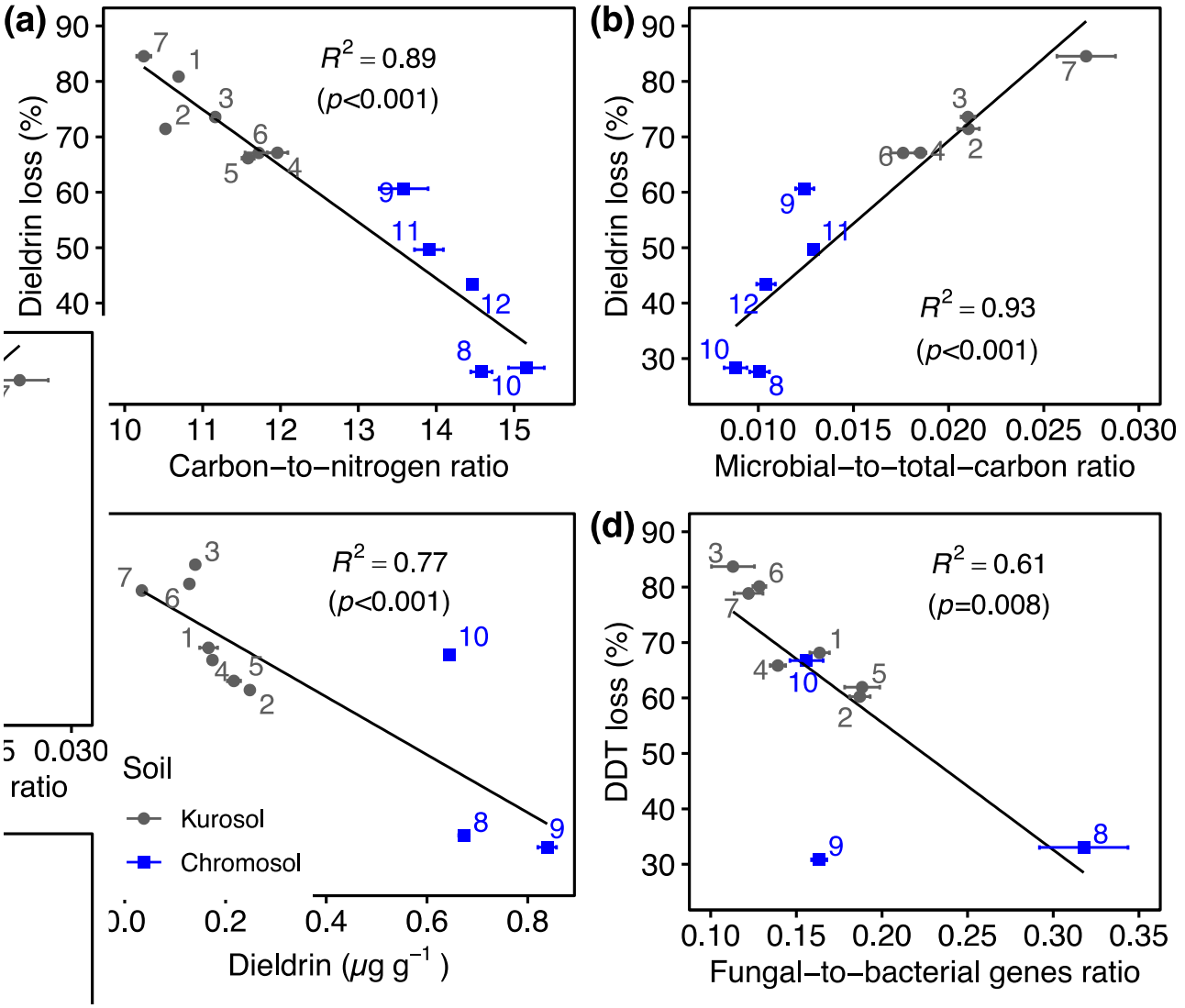

Fig. 3. Scatter plots including spearman correlation coefficients of dieldrin loss to carbon-to-nitrogen ratio (a) and microbial-C-to-total-C ratio (b). Furthermore, scatter plots with DDT loss to the combined mean dieldrin concentrations of 2015 and 2017 (c) and fungal-to-bacterial abundance ratio (d).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

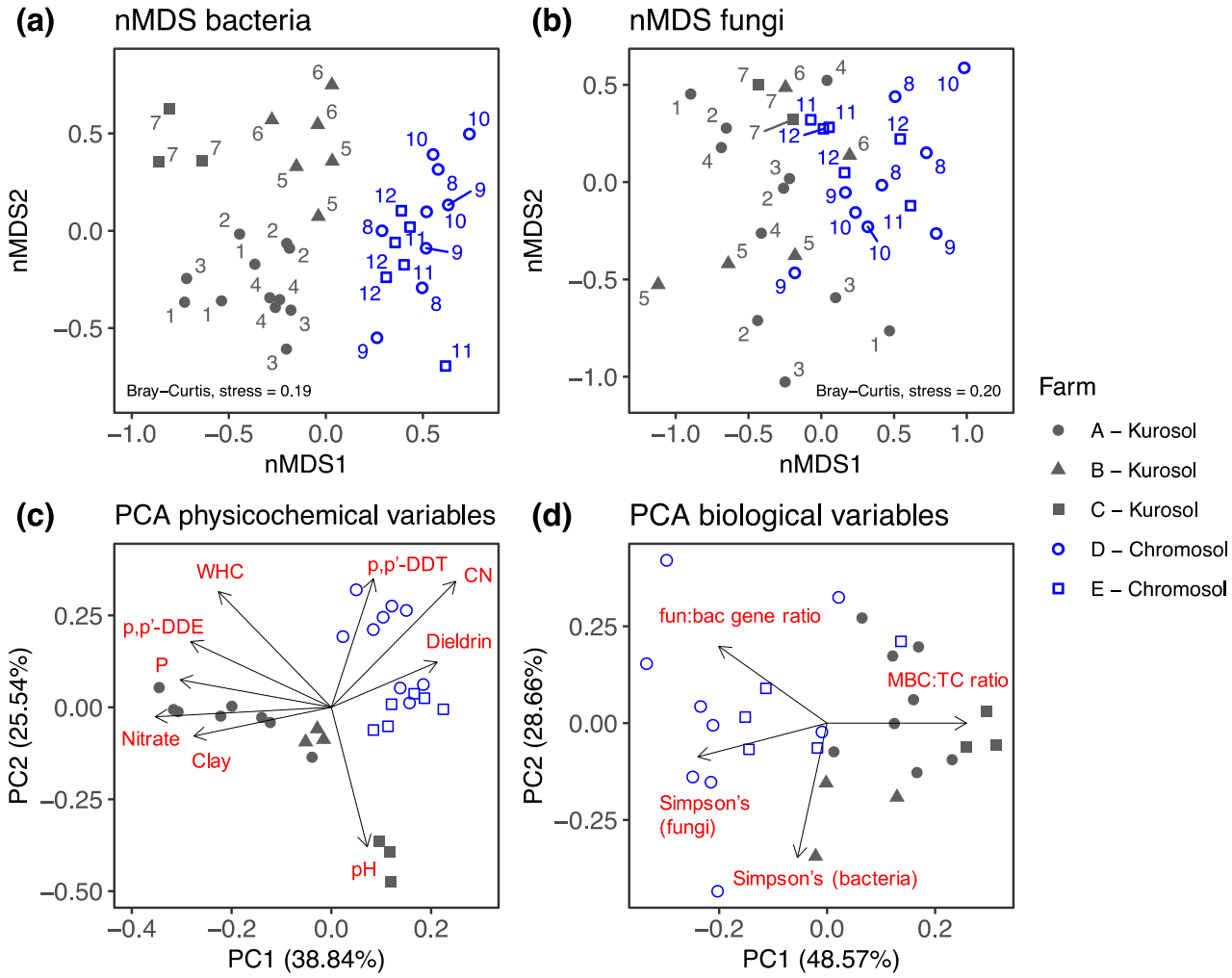

Fig. 4. Plots of ordinations of non-metric dimensional scaling (nMDS) based on Bray Curtis dissimilarity matrices of the OTU abundances given by ARISA, showing 36 samples where distances between values represents similarities between samples (a and b). Principle component analysis (PCA) explaining the 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 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	$6 – 84 \times 10^6$	**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.

Table 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	$12 – 49 \times 10^5$	**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 $sigma^2$)], 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.

Table 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 R^2 values (p<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)	
Predictor	df	F.model	R^2	F.model	R^2	Res.Df	Df.diff	Dev.	p	adjusted 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)

Supplementary Materials

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

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Supplementary description of 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 Zuur *et al.* (2009):

- 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 Halekoh and Höjsgaard (2014)[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.

[a] Halekoh, U., Højsgaard, S., 2015. A Kenward-Roger Approximation and Parametric Bootstrap Methods for Tests in Linear Mixed Models - The R Package pbkrtest. J. Stat. Softw. https://doi.org/10.18637/jss.v059.i09

Supplementary Equations S1 - S9

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).

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

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

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

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

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

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

Supplementary Table S1. 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.

Supplementary Table S2. 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

Supplementary Table S3. 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

Supplementary Table S4. 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

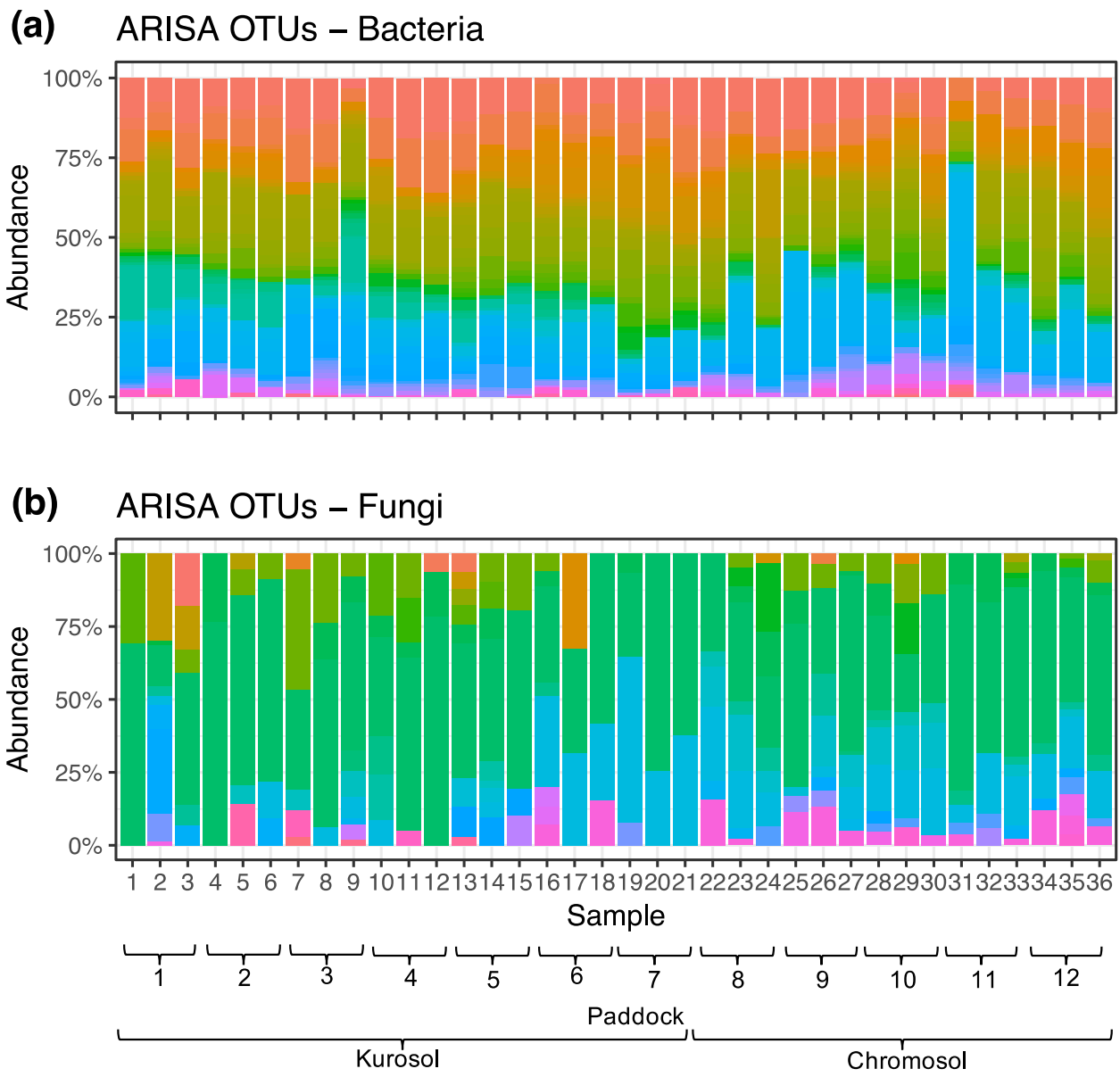
[a] No fresh soil was available

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

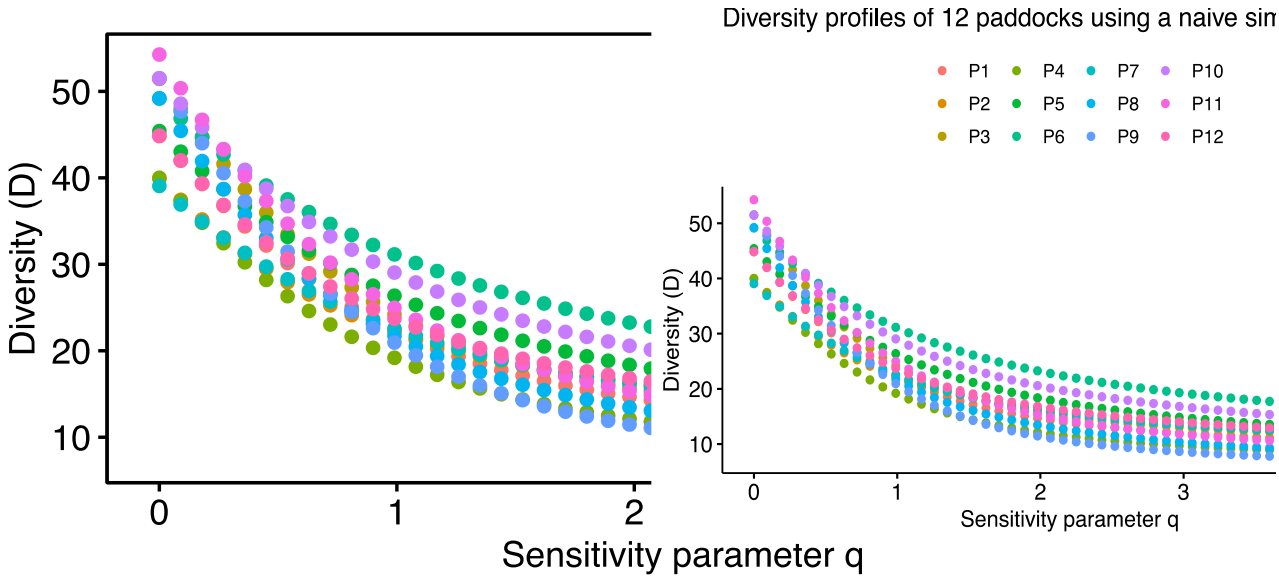
Supplementary Table S5. Microbial biomass C (MBC) and N (MBN), extractable nitrates (NO_x), number of bacterial and fungal OTUs, and alpha diversity indices calculated from ARISA OTU abundances. Values are the mean of three replicates ± standard error.

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

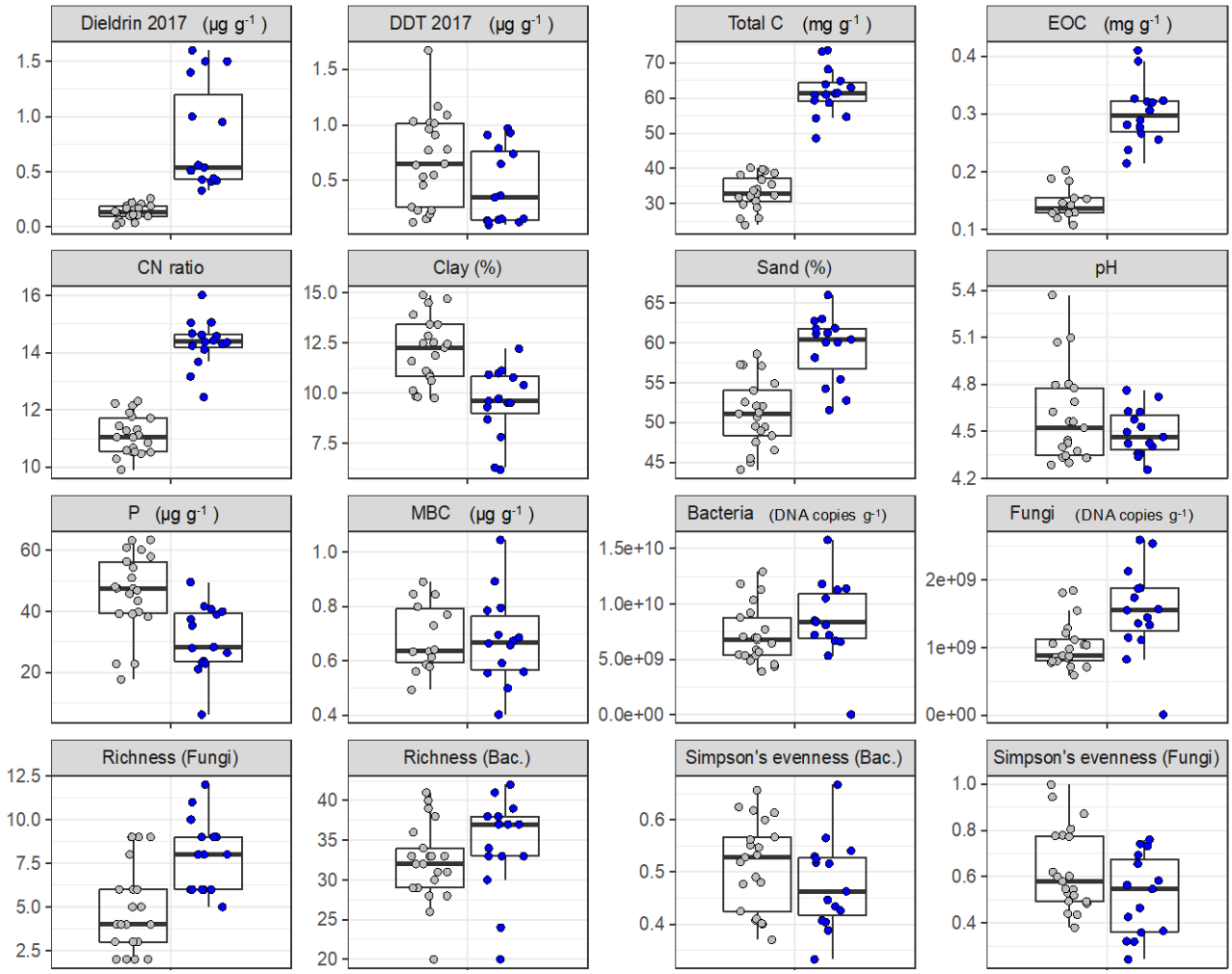
[a] No fresh soil was available



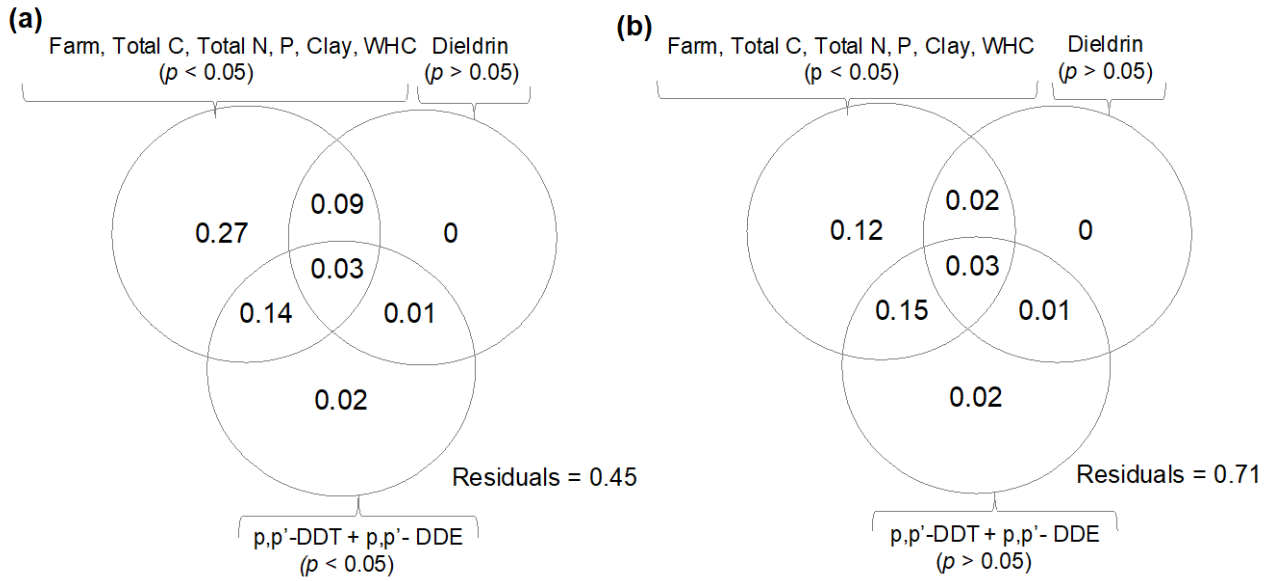
Supplementary Fig. S1. Stacked barcharts of ARISA relative OTU abundances for all 36 surface soils which were samples across 12 paddocks at 3 field replicates. A total of 146 bacterial OTUs and 53 fungal OTUs are represented.



Supplementary Fig. S2. 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.



Supplementary Fig. S3. 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.



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