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Natasha Balashova, Sarah Wilderspin, Chao Cai, Brian J. Reid

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Ubiquity of microbial capacity to degrade metaldehyde
in dissimilar agricultural, allotment and garden soils

Natasha Balashova¹, Sarah Wilderspin¹, Chao Cai¹,² and Brian J. Reid¹* 

¹ School of Environmental Sciences, University of East Anglia, Norwich, UK
² Institute for Urban Environment, Chinese Academy of Sciences, Xiamen, P.R. China

* Corresponding Author: b.reid@uea.ac.uk; T: +44 (0)1603 592357
Abstract

Metaldehyde is a molluscicide used to control slugs and snails. Despite its extensive use, very little is known about the capacity of soil microbial communities to degrade this chemical. This research provides a synopsis of the latent capacity of soil microbial communities, present in agricultural (n = 14), allotment (n = 4) and garden (n = 10) soils, to degrade metaldehyde. Extents of $^{14}$C-metaldehyde mineralisation across all soils ranged from 17.7 to 60.0 %. Pre-exposure (in situ, in the field) to metaldehyde was not observed to consistently increase extents of metaldehyde mineralisation. Where soils were augmented, (ex situ, in the laboratory) with metaldehyde (28 mg kg$^{-1}$), the mineralisation capacity was increased in some, but not all, soils (uplift ranged from +0.10 to +16.9 %). Results indicated that catabolic competence to degrade metaldehyde was evident in both surface (16.7 - 52.8 %) and in sub-surface (30.0 - 66.4 %) soil horizons. Collectively, the results suggest that catabolic competence to degrade metaldehyde was ubiquitous across a diverse range of soil environments; that varied in texture (from sand to silty clay loam), pH (6.15 – 8.20) and soil organic matter (SOM) content (1.2 % – 52.1 %). Lighter texture soils, in general, were observed to have higher capacity to mineralise metaldehyde. Weak correlations between catabolic competence and soil pH and soil organic matter content were observed; it was noted that above a SOM threshold of 12 % metaldehyde mineralisation was always > 34 %. It was concluded that the common occurrence of metaldehyde in EU waters is unlikely the consequence of low potential for this chemical to be degraded in soil. It is more likely that application regimes (quantities/timings) and meteorological drivers facilitate the transport of metaldehyde from point of application into water resources.

Keywords: Metaldehyde, Soil, Biodegradation, $^{14}$C-Respirometry, Land use.
1. Introduction

Slugs, snails and other gastropods are significant pests to a range of crops, including agricultural, horticultural and garden plants (Rae et al., 2009). Metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetraoxcane) is a widely used molluscicide in agriculture and domestic settings globally (including the UK, Europe, the United States and China (EPA, 2011; Gavin et al., 2012; Ma et al., 2012; Zhongguo et al., 2013; EC, 2019)).

This pesticide is normally applied to crops in autumn and winter (Environment Agency, 2009). The maximum recommended application rate of metaldehyde in the UK is currently 210 g active substance/ha (from 1st August to 31st December); 700 g active substance/ha is the maximum total dose per calendar year (Metaldehyde Stewardship Group (MSG), 2019). Similar application rates are evident across Europe; allowing a maximum of 350 g active substance/ha per single treatment, with up to two treatments per year (EFSA, 2010). In the United States the recommended single application rate should not exceed 2240 g active substance/ha with a maximum of 6 applications per year (EPA, 2011).

Bait pellets release metaldehyde, under moderately moist conditions, for approximately 10 days (Puschner, 2006). Metaldehyde is relatively water soluble (190 mg L⁻¹; PPDB, 2017) and has as low K_{OW} value (0.12; Hall, 2010). Owing to, i) its physicochemical properties (Table 1 in Supplementary Material), ii) application times that often coincide with wetter periods (when molluscs are more prevalent, compared to dry weather conditions) and, iii) the prevailing wet autumn/winter weather in the UK and northern EU countries, metaldehyde is mobile in the environment. This mobility serves to transfer metaldehyde from soil to both ground- and surface waters. Thus, metaldehyde presence in surface water and groundwater has been reported with high frequency (Busquets et al., 2014; Hillocks, 2012).

Kay and Grayson (2014) reported peak concentrations of metaldehyde in the range 0.4 – 0.6 µg L⁻¹ and highlighted that metaldehyde has been detected above the maximum
allowable concentration for drinking water of 0.1 µg L\(^{-1}\) (Council of the European Communities, 2000) during the October – December periods, when slug pellets are typically applied. These findings agree with metaldehyde concentration trends, observed by Castle et al. (2018), who reported peak concentrations of metaldehyde in the stream water of the River Thames Catchment to vary between 0.1 and 0.35 µg L\(^{-1}\) during September – January 2017. The maximum concentration of 5 µg L\(^{-1}\) was recorded in November, and no metaldehyde concentrations above 0.1 µg L\(^{-1}\) were recorded during the February – August period (Castle et al., 2018). Concentrations up to 1.5 µg L\(^{-1}\) were reported in stream water of the same catchment by Lu et al. (2017). Metaldehyde concentrations up to 2.2 µg L\(^{-1}\) were reported in a UK chalk aquifer by (Bullock, 2014), with peak concentrations observed in January and February. Metaldehyde presence in the aquatic environment has been reported in other countries. Calumpang et al. (1995) reported maximum metaldehyde concentrations of 1.57 mg L\(^{-1}\), in rice paddy water in the Philippines, following application and that concentrations fell to below the detection limit within nine days (Calumpang et al., 1995). Metaldehyde concentrations up to 6.98 µg L\(^{-1}\) were observed in run-off water from fish farming ponds in northern France within the Moselle River Basin (Lazartigues et al., 2012).

A key factor underpinning metaldehyde fate and mitigating its transport is the latent capacity of soil microbial communities to degrade this pesticide. Yet, literature relating to microorganisms capable of metaldehyde degradation is limited to three studies. Thomas et al. (2013, 2017) reported several metaldehyde-degrading bacterial strains that were isolated from domestic soils (liquid cultures contained 100 mg L\(^{-1}\) metaldehyde); *Acinetobacter E1* was reported to degraded metaldehyde present in solution at concentrations less than 1 nM (0.16 µg L\(^{-1}\)), other *Acinetobacter* strains were reported to be unable to degrade the pesticide. A laboratory study (EFSA, 2010), reported metaldehyde to be mineralised (50 -78 %) by soil
microbial communities under aerobic conditions; while under anaerobic conditions metaldehyde was observed to be stable. However, to date, no reports have been published that account the capacity (assessed using $^{14}$C-respirometry) of dissimilar soils from contrasting settings, to degrade metaldehyde. Thus, this current research sought to establish the level of catabolic competence of soil microbial communities to degrade metaldehyde (i.e. the competence of the microbial community to break down metaldehyde molecules into smaller units that are subsequently oxidised/mineralised to carbon dioxide). The current research considered soils obtained from three contrasting settings: agricultural fields, allotments and gardens (and both surface and sub-surface regimes). The research sought to establish intrinsic metaldehyde mineralisation potential of the microbial community within these soils and the directing influence of metaldehyde augmentation in terms of inducing metaldehyde degradation. Furthermore, it was hypothesised that soil attributes, include texture, SOM and pH would have a shaping influence upon levels of metaldehyde catabolic competence. These original lines of enquiry provide a synopsis of metaldehyde biodegradation in dissimilar soils from contrasting settings.

2. Materials and Methods

2.1. Chemicals

Metaldehyde pellets (1.5% active ingredient) were manufactured by Bayer. $^{14}$C-metaldehyde (UL-$^{14}$C; 5.1 mCi mmol$^{-1}$) was obtained from American Radiolabeled Chemicals Inc. St Louis, USA. Ultima Gold and Ultima Gold XR liquid scintillation fluids were purchased from Perkin Elmer, UK. Calcium chloride, ethanol, methanol and sodium hydroxide were supplied by Fischer Scientific, UK; and dichloromethane provided by Sigma.
Aldrich, UK. Mineral Basal Salt (MBS) components (namely: NaCl, (NH₄)₂SO₄, KNO₃, KH₂PO₄, K₂HPO₄ and MgSO₄.7H₂O) were obtained from BDH, UK.

2.2. Soils

Soil was collected from three contrasting settings: agricultural fields, allotments and gardens. Soils were collected in Norfolk and Essex, UK (Table 2). Soil samples (200 g) were collected using a Dutch auger (0-10 cm for top soil; and, 40-50 cm for sub-soil samples); four auger heads were combined to produce a single composite sample at each sampling point and a given location was sampled in triplicate (within 5 m of each other). Between sampling the auger head was thoroughly cleaned (washed with water and tissue, then sprayed with 70% ethanol solution that was allowed to evaporate). Soils were transported to the laboratory and stored (4 °C) in sealed plastic bags, for no more than 2 days, prior to assessment of catabolic competence.

Soils were characterised in terms of their: SOM content (mass loss on ignition in a muffle furnace (450 °C) for 12 h; 10 g (n = 3)) (Ghabbour et al., 2014); pH (samples (3 g (n = 3) were combined with 30 mL of distilled water in a centrifuge tube, tubes were then shaken (reciprocal shaker (IKA Labortechnik KS501) at 100 r.p.m for 14 h and the soil water pH was measured using an electrode (Jenway) and meter (Mettler Toledo FE20 Five Easy Benchtop pH Meter), and texture (samples of soil were moistened and kneaded into a ball and texture determined following the hand-texture framework of McDonald et al. (1998)). Soil characteristics are listed in Table 2, and its expanded version could be found within the Supplementary Material.

2.3. ¹⁴C-radiorepirometry assessment of intrinsic and induced catabolic competence
Prior to undertaking the respirometry, soils were transferred to the laboratory incubator for 24 h to bring them back to a temperature of 18 °C. Soil samples (10 g) were added to sterile Duran Schott bottles (250 mL) containing sterile MBS (30 mL) (0.3 g NaCl, 0.6 g (NH₄)₂SO₄, 0.6 g KNO₃, 0.25 g KH₂PO₄, 0.75 g K₂HPO₄ and 0.15 g MgSO₄·7H₂O dissolved in 1 L of deionised water) (Hickman et al. (2008). To each bottle ¹⁴C-metaldehyde was added (100 Bq in 100 µL of ethanol). To capture ¹⁴CO₂ generated from the mineralisation of ¹⁴C-metaldehyde, a glass scintillation vial (7 mL) containing 1M NaOH (1 mL) was suspended (using a stainless-steel clip) from the top of a Teflon™ lined respirometer lid. Bottles were continuously shaken on an orbital shaker (IKA Labortechnik KS501) at 100 r.p.m and the vials were removed and replaced periodically over the 120 h (5 d) assay time. Removed vials were wiped with a tissue, and Ultima Gold scintillation fluid (6 mL) added. Vials were sealed, shaken and stored in the dark (for a minimum of 24 h) and then analysed by liquid scintillation counting (Perkin-Elmer TriCarb 2900TR liquid scintillation analyser; count time 10 mins). Results were corrected for background radiation using un-spiked respirometers (Reid et al., 2001). The respirometer system was previously validated by Reid et al. (2001), who reported that up to 400 µmol CO₂ could be accommodated in a single trap and a ¹⁴C activity balance of 101±8.9 %. In order to assess the inducible capacity of soil microbial communities in response to metaldehyde augmentation the above procedure was repeated with the addition of a metaldehyde pellet to each respirometer bottle. Each pellet had a mass of 0.028 g and a metaldehyde content of 1.5 %. Thus, each respirometer was dosed with the equivalent of 28 mg metaldehyde kg⁻¹ soil. Sterile respirometers, containing MBS (30ml), were spiked with ¹⁴C-metaldehyde to evaluate abiotic degradation and volatilisation of ¹⁴C-metaldehyde. All respirometer assays were run in triplicate.
2.4. Sample codes

Samples have been coded to indicate: land use regime, Field (F), Allotment (A) and Garden (G); the location qualifier (1-10; see Table 2); if samples were top soil (T) or subsoil (S); if the in situ regime had metaldehyde application (p) or no metaldehyde application for at least the last 4 years (n), and; if the ex situ laboratory assay was conducted in the presence of a slug pellet (+) or its absence (-). For example, F2Tp+ corresponds to Field 2, a topsoil sample that was exposed to metaldehyde in situ and was screened for catabolic competence in the presence of a metaldehyde pellet. In presenting the data, soils have been organised with lighter (sandier) textures presented first and heavier (clay) textures presented last.

2.5. Statistics

Significant differences between intrinsic and induced mineralisation levels were established using ANOVA post hoc Tukey Tests (SPSS Statistics 22); a significance level of 0.05 (95 % level of confidence). Pearson’s correlation test was applied to determine linear correlation between mineralisation and pH/SOM values, a significance level of 0.05.

3. Results

3.1. Control flasks and blanks

Abiotic degradation/volatilisation of $^{14}$C-metaldehyde was evident at a modest level ($7.8 \pm 3.9 \%$). This value was commensurate with a fugacity (Mackay, 2001) driven pseudo-equilibrium (theoretical value = 9.5 %), where: the respirometer MBS media volume was 30ml, the trap volume was 1 ml and the trap was changed three times over the assay period. Background $^{14}$C-radiation was negligible (0.06 % of the activity delivered in the respirometer spike).
3.2. Agricultural Field Soils (FT, FS)

Intrinsic catabolic competence (i.e. in assays with no metaldehyde pellet added (-)) was ubiquitous across all agricultural field soils; mineralisation varied between 17.6 % (FT(p)7) and 31.0 % (FT(n)1) (Figure 1).

In most instances soils with light texture (FT(n)1, FS(n)2, FS(n)4 – sand, FT(n)2 – loamy sand, FT(n)3 – FT(p)6 – sandy loam, FT(p)7 – silty loam), were observed to have higher intrinsic capacities to mineralise $^{14}$C-metaldehyde. Soils with heavier texture (FT(n)8, FT(p)9 and FT(p)10 – loam, FT(p)11 – sandy loam clay, FT(p)12 – silty clay loam) were observed to have lower intrinsic catabolic competence (Figure 1).

Similarly, induced (with pellet present (+)) catabolic competence was observed to be higher in lighter textured soils (FT(n)1 – FT(p)7) than in heavier textured soils (FT(n)8 – FT(p)12). This was also the case for the Field Subsoil samples (FS(n)2, FS(n)4 – sandy texture), where an uplift in induced mineralisation was observed (+8.9 % and +0.1 %) (Figure 1). The extent of induced mineralisation in FT (where a pellet was added to the respirometer) varied from 16.5 % (FT(n)8) to 30.3 % (FT(n)3) (Figure 1); this range was almost identical to the intrinsic catabolic competence range, suggesting that catabolism of metaldehyde was operating at its maximum capacity before the pellet was added.

With the exception of FT(n)1 (light sandy texture) and FT(n)8 (medium loamy texture), all agricultural soils that were not exposed to metaldehyde in situ (n) were observed to show an uplift of catabolic competence following the addition of a metaldehyde pellet (+) (FS(n)2 – FT(n)5). Lighter FT soil textures included sand (FS(n)2, FS(n)4), loamy sand (FT(n)2), sandy loam (FT(n)3 – FT(n)5). The same outcome was observed for light soils where metaldehyde was used in situ (p) (FT(p)6 – sandy loam, FT(p)7 – silty loam).

FS(n)2 was the only sample among all agricultural Field soils in which a significant difference between intrinsic and induced mineralisation was observed (P < 0.05) (+8.9 %)
The maximum level of observed catabolic activity did not exceed 38.9 % (induced mineralisation in FS(n)2 sample) in the Agricultural Field soils (Figure 1).

### 3.3. Allotment Soils (AT, AS)

Intinsic (-) catabolic competence was ubiquitous across all Allotment soils; mineralisation varied between 34.3 % (AT(p)2) and 60.0 % (AS(n)1) (Figure 2). Similar to the Field soils, Allotment soils with lighter texture (sand) exhibited higher intrinsic mineralisation capacities when compared to soils with slightly heavier texture (loamy sand) (Figure 2).

Relative difference between intrinsic (-) and induced (+) mineralisation in lighter textured subsoils were also higher, particularly in soil with previous *in situ* metaldehyde application history (p) (AS(p)2) (Figure 2). Sandy Subsoil sample (AS(n)1) with no previous metaldehyde application had the highest metaldehyde mineralisation (both induced and intrinsic).

Like Field soils, Allotment soils exhibited elevated mineralisation levels in the presence the of metaldehyde (Figure 2). In the presence of metaldehyde, the extent of mineralisation varied from 35.7 % (AT(p)2) to 66.4 % (AS(n)1) (Figure 2).

Only in the case of AS(p)2, intrinsic and induced levels of $^{14}$C-metaldehyde mineralisation were significantly different (P < 0.05) (a +9.9 % uplift in mineralisation was observed). The maximum level of observed catabolic activity did not exceed 66.4 % (induced mineralisation in AS(n)1 sample) (Figure 2).

### 3.4. Garden soils (GT)

As observed for Field and Allotment soils, competence to degrade metaldehyde in garden soils was ubiquitous across soil types (Figure 3). In the absence of a metaldehyde
pellet, the extent of intrinsic metaldehyde mineralisation varied from 28.9 % (GT(n)7) to 52.8 % (GT(n)6) (Figure 3).

In general, as was the case with Field soils (Figure 3), lighter textures (sandy loam and loamy sand); GT(n)1 through GT(n)6) indicated higher levels of catabolic competence to mineralise metaldehyde when compared to heavier textures (sandy clay loam and sandy clay) (Figure 3).

In the presence of metaldehyde all soils showed elevated levels of mineralisation (Figure 3); the extent of mineralisation varied from 39.9 % (GT(n)2) to 53.0 % (GT(n)6). Uplift in mineralisation, in the presence of a metaldehyde pellet (+), was greatest for soils observed to have lower intrinsic catabolic competence; conversely, where soils were observed to already have high catabolic competence only small increases (a few %) in mineralisation were observed following metaldehyde augmentation (e.g. GT(n)1 and GT(n)6; Figure 3).

In several instances the augmentation resulted in significant (P < 0.05) increases in mineralisation GT(n)3, GT(n)7, GT(n)9 and GT(n)10; +14.4 %, +15.3 %, +12.8 % and +16.9 %, respectively. Again, as observed for Field and Allotment soils, the maximum catabolic capacity of 50-55 % appeared to be a ceiling beyond, which catabolic capacity was not exceeded.

4. Discussion

The degradation of any pesticide depends upon its physical and chemical characteristics, e.g. aqueous solubility and inherent recalcitrance (Semple et al., 2003) and the physical, chemical and biological properties of the soil (Rao et al., 1983), such as pH, redox conditions, matrix attributes, carbon:nitrogen:phosphorus (C:N:P) elemental ratio, temperature, moisture content (Arias-Estévez et al., 2007). Presence/absence/activity of
catabolic enzymes in soils affect pesticide degradation directly (Deng et al., 2016) while pesticide bioavailability/bioaccessibility indirectly influence pesticide degradation (Arias-Estévez et al., 2007). Additionally, pesticide transport, biological degradation and chemical transformation processes are affected by application regime (rates/methods and timing), as well as hydrological and weather conditions (Borgesen et al., 2015). Thus, site-specific physical, chemical and biological properties control the fate and transport of pesticides in the environment and determine the variation in spatial distribution of pollutants.

4.1. Soil Microbe Response to Chemicals Inputs

The ability of microbial communities to respond to organic compounds (e.g. pesticides) presence/augmentation is well documented for a range of compound classes, including: several semi-volatile hydrocarbon pollutants (Kelsey and Alexander 1997; Reid et al., 2002; Springael and Top, 2004; Hickman et al., 2008), pesticides (Duah-Yentumi and Johnson, 1986; Reid et al., 2005; Bending et al., 2006; Posen et al., 2006; Trinh et al., 2012; Reid et al., 2013) and antibiotics (Islas-spinosa et al., 2012; Bennet et al., 2017). These studies confirm the capacity of microbial communities to respond to organic compound input by becoming more catabolically competent (Reid et al., 2005; Bending et al., 2006; Posen et al., 2006; Reid et al., 2013). For example, Reid et al. (2005) reported soil microbial communities, of initially low catabolic competence, to degrade the herbicide isoproturon, (mineralisation C. 5 %) to increase in their competence following the incubation of soil with a low (0.05 μg kg\(^{-1}\)) application of the herbicide (mineralisation increased to C. 40 %). In column studies, Trinh et al. (2012) reported three phases of attenuation/degradation of these herbicides isoproturon and MCPA: an initial sorption phase, followed by an acclimatisation/adaptation phase and a final rapid degradation phase (resulting in complete removal of the herbicides).
Several studies on biodegradation of metaldehyde have been reported, for example, Thomas et al. (2013, 2017) isolated and characterised metaldehyde-degrading bacteria in domestic soils. They reported Acinobacter E1 strain to be able to degraded metaldehyde to a concentration below 1 nM. However, to date, the response of soil microbial communities, present in agricultural, allotment and garden soils, to metaldehyde augmentation has not been reported. Thus, our results confirm the potential for soil microbial communities to increase in their competence to degrade metaldehyde following exposure. In keeping with observations for other compounds, metaldehyde catabolic competence was observed to increase significantly, following slug pellet addition (in some cases increasing by a factor of 2). Largest increases in catabolic competence following metaldehyde augmentation were observed for FS(n)2 (+8.9 %), AS(p)2 (+9.9 %), GT(n)3, GT(n)7, GT(n)9 and GT(n)10 (+14.4 %, +15.3 %, +12.8 % and +16.9 %, respectively).

In contrast to other pesticides, where low catabolic competence is exhibited in unexposed soils, high levels of intrinsic catabolic competence to degrade metaldehyde were observed (up to 66.0 %). Metaldehyde is a cyclic tetramer of sub-units that can depolymerise, through microbial activity, into acetaldehyde (Castle et al., 2017; Tomlin, 2003). High levels of metaldehyde degradation in the soil environment have been reported in the literature. For example, Bieri (2003) reported fast degradation rates of metaldehyde in agricultural soils in Germany; with, DT$_{50}$ values ranging from 5.3 to 9.9 days. Coloso et al. (1998) reported metaldehyde concentration in pond sediment to rapidly decrease from an initial concentration of 80 mg kg$^{-1}$ to 1 mg kg$^{-1}$ after 15 days. Ma et al. (2012) studied metaldehyde residues in agricultural soils in China and reported metaldehyde residue of up 9 mg kg$^{-1}$ to decrease below 0.3 mg kg$^{-1}$ over 7 days. While Calumpang et al. (1995) reported metaldehyde concentrations in paddy soil to fall from 0.13 mg kg$^{-1}$ to below the analytical detection level within 22 days.
We suggest the ubiquity of high levels of catabolic competence observed in our research are likely due to the degradation of the simple metaldehyde molecule to acetaldehyde (the primary degradation product), and the subsequent degradation of acetaldehyde to acetate; this being assimilated into Krebs tricarboxylic acid (TCA) Cycle (Tomlin, 2003) and respired as carbon dioxide.

4.2. Catabolic competence and its relationship with soil properties

All soil types, drawn from all settings (Field, Allotment and Garden), were observed to exhibit significant levels of catabolic competence. As already highlighted, soil texture had a shaping influence on the extent of $^{14}$C-metaldehyde mineralisation; with sandy soils supporting, in general, higher level of catabolic competence. It is widely recognised that soil texture has a substantial influence on the soil environment. It controls soil porosity, and thus, has a directing influence on soil hydrology (Luna et al., 2017) and soil atmosphere (Pagliai et al., 2004). In turn, these drivers exert a shaping influence on soil microbial community structure (Fierer, 2017). Schroll et al. (2006) reported optimum pesticide mineralisation at a soil water potential of $-0.015$ MPa; pesticide mineralisation was markedly reduced when soil moisture approached soil water holding capacity.

Acknowledging the considerable influence soil texture has on soil moisture conditions, it is unsurprising that levels of catabolic competence observed have been influenced by soil texture. We suggest that the higher levels of catabolic competence for metaldehyde, observed in the lighter soil textures, could be linked to a higher redox potential in these more freely drained soils (Voroney and Heck, 2015). These conditions would, putatively, support a more active microbial community with greater capacity to degrade organic substrates (including metaldehyde). In general, pesticide degradation (Fenner et al.,
2013), and metaldehyde degradation specifically (EFSA, 2010), have been reported to be faster under aerobic conditions.

Beyond its influence on soil, physical, hydrological and biological attributes soil texture also controls pesticide bioavailability (Gavrilescu, 2005). Numerous studies have sustained the general trend that lighter sandy soil textures assist biodegradation by maintaining high pesticide bioavailability and, in contrast, heavier clay textures tend to facilitate greater sorption and entrapment of pesticide (e.g. Reid et al., 2000; Gavrilescu, 2005). Thus, heavier clay textures tend to decrease the potential for degradation though stronger sorption. These strong interactions have been reported to preclude the opportunity for pesticides to induce catabolic competence (Reid et al., 2013).

In addition to their texture, the dissimilar soils also varied in their SOM content. SOM has been reported to influence the fate, behaviour and biodegradation of pesticides (Hatzinger and Alexander, 1995). However, to date, there have been no reports accounting how soil properties (specifically, SOM and pH) influence the biodegradation of metaldehyde by soil microbial communities. To elucidate any such relationships, SOM and soil pH were correlated with $^{14}$C-metaldehyde mineralisation under intrinsic and induced regimes and across all settings (Figure 4).

SOM varied (from 1.17 % to 52.14 %) across the dissimilar soil types obtained from contrasting settings (Table 2) and extent of mineralisation in these soils also varied greatly (from 16.51 % to 66.44 %). Considering all soils, $^{14}$C-metaldehyde mineralised was observed to increase with increasing SOM for both intrinsic and induced assessment (Figure 4A). While the correlations between mineralisation extent and SOM were not significant ($r = 0.34$, $p = 0.08$; intrinsic and induced mineralisation vs. SOM); the data supports the conclusions that i) beyond a SOM content of 12% metaldehyde mineralisation was consistently $> 34\%$, and, ii) where SOM content was less than 12% metaldehyde mineralisation was observed.
across a very broad range (from 16.5 to 60 %) (Figure 4A). These results suggest that efforts to sustain SOM levels in soil could assist in promoting higher levels of metaldehyde degradation, and thus, reduce the opportunity for metaldehyde to transfer to water resources.

In one hand, SOM controls sorption of pesticides in soil (Chiou et al., 1983). Sorption is responsible for retention of pesticides in soil, preventing leaching and decreases pesticide bioavailability (Singh, 2008). While, on the other hand, SOM is the cornerstone of soil food webs, and its amount and quality underpins microbial diversity and its capacity to utilise a broad range of substrates (Neumann et al., 2014). With regards to metaldehyde, as a relatively water soluble compound (190 mg L\(^{-1}\)) and as a labile carbon source (Bieri, 2003; EFSA, 2010), we suggest sorption onto SOM is unlikely to be a significant influence on biodegradation. It more likely that SOM has a synergistic influence on metaldehyde biodegradation as it acts as a primer for microbial activity. The higher levels of catabolic competence observed to be synonymous with SOM content of >12% (Figure 4A) support this linkage.

Where pH was correlated with mineralisation across all soil types and regimes, no relationship was observed for intrinsic mineralisation (r = 0.19, p = 0.34) (Figure 4B). A slightly positive correlation was observed between increasing pH and induced mineralisation (r = 0.44, p = 0.02) (Figure 4B). More useful, perhaps, is the observable distinction between soils of pH lower than 6.9, where mineralisation never exceed 30 %, and soils where pH was greater than 6.9, and mineralisation was more often observed to be greater than 35 % (Figure 4B). Thus, while pH influence on pesticide degradation has been reported for other compounds (e.g. atrazine (Houot et al., 2000) and pirimicarb and metsulfuron-methyl (Kah et al., 2007)), it influence upon metaldehyde mineralisation was inconsistent.
The results reported herein highlight soil microbial communities, in dissimilar soils under Agriculture, Allotment and Garden regimes, to all have a considerable latent capacity to degrade metaldehyde (Figures 1-3). Our results suggest that soil microbial communities across these regimes, and present in both top-soil and sub-soil, are well predisposed to degrade metaldehyde. We suggest that it is unlikely that the, at times, high levels of metaldehyde detected in water (Castle et al., 2017; Kay and Grayson, 2014) are due to low degradation capacity in the soil system. It is more likely that runoff and fast leaching of metaldehyde is the main driver underpinning the high incidence and high concentrations of metaldehyde sometimes reported in water resources (Calampung et al., 1995; Coloso et al., 1998; Council of the European Communities, 2000; Bieri, 2003; Hillocks, 2012; Ma et al., 2012; Busquets et al., 2014; Lu et al., 2017).

With metaldehyde being applied in autumn and winter, when slug populations are higher due to wet weather (and when young crops are most vulnerable), the opportunity for metaldehyde transport is increased. The situation is further antagonised by metaldehyde having a relatively high aqueous solubility (190 mg L\(^{-1}\)). In support of this view there is considerable evidence that pesticides applied to the soil surface can be transported rapidly, bypassing the unsaturated soil zone, to groundwater (Arias-Estévez et al., 2007; Johnson et al., 1995; Lopez-Perez et al., 2006). Indeed, metaldehyde has frequently been detected in groundwater at levels higher than the EU Drinking Water Framework Directive limit (0.1 µg L\(^{-1}\)) (EC, 1998); in some cases, concentrations of metaldehyde of up to ten times this limit have been reported (UKWIR, 2013).

Given its ubiquity in water resources, metaldehyde has been subject to scrutiny, voluntary initiatives and evolving regulation. Specifically, in the UK the Get Pelletwise campaign of the Metaldehyde Stewardship Group (MSG, formed in 2008), aimed to promote
sustainable use of metaldehyde by applying principles of Integrated Pest Management and introducing guidelines for metaldehyde application (MSG, 2019). This guidance recommended, the use of the minimum amount of active compound per hectare; that soil conditions, topography and fields proximity to watercourses are factors to be considered in assessing the risk of metaldehyde loss to streams, and; that metaldehyde application is discouraged during heavy rain events and if field drains are flowing (MSG, 2019). However, metaldehyde is still regularly detected at the concentrations above the DWD limit of 0.1 µg L⁻¹ (Castle et al., 2017; Lu et al., 2017). Thus, in order to mitigate metaldehyde transfer still further a reduction in the nominal loading of metaldehyde in pellets (e.g. from 3 % to 1.5 % active ingredient) and the development of pellets that afford stronger metaldehyde attenuation might offer further opportunity for improvements.

We highlight that soil itself is likely to be a significant reservoir of metaldehyde. With respect to this soil burden, the results reported herein suggest that, there is good prospect that, given time, the indigenous soil microbial communities will degrade this reservoir of metaldehyde. However, further research regarding the levels of microbial catabolic activity, specifically under lower substrate concentrations, should be undertaken.

5. Conclusions

Results indicate substantial catabolic competence to degrade metaldehyde in soils with various texture (from sand to silty clay loam), pH (6.15 – 8.20) and organic matter content (1.2 – 52.1 %). Ubiquitous catabolic competence was observed in both topsoil (16.7 – 52.8 %) and subsoil horizons (30.0 – 66.4 %). In general, soils with lighter texture (sand, sandy loam and loamy sand; average mineralisation 37.3 %) had higher levels of ¹⁴C-metaldehyde mineralisation when compared to soils with heavier texture (sandy clay, sandy clay loam and silty clay loam; average mineralisation 33.3 %). When soils were augmented
with metaldehyde (in the laboratory) an increase in mineralisation was observed in some, but not all soils (up to 16.9 % increase in the Garden Soil GT(n)10 (sandy clay)). Overall, pH and organic matter content were weakly correlated with $^{14}$C-metaldehyde mineralisation. However, soils with higher SOM (>12%) were, in general, observed to support higher levels of metaldehyde mineralisation. It is suggested that the higher SOM status of these soils exerted a beneficial shaping influence upon soil microbial communities and their capacity to degrade metaldehyde. Collectively, results suggest that the concentrations of metaldehyde (that are at times high), detected in water, are unlikely due to insufficient microbial capacity to degrade this pesticide. It is suggested that application regime (rate and timing), the high mobility of metaldehyde and its loss to the watercourses via runoff and leaching are the driving factors underpinning the ubiquity of metaldehyde in surface and ground water resources. To reduce metaldehyde runoff to watercourses, the application timing should not coincide with wet weather conditions. The use of pellets with reduced concentrations of metaldehyde and development of the pellet products with stronger attenuation capacity could further assist in the effort to reduce metaldehyde transfer to the aquatic environment.

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Figures
**Figure 1.** Catabolic competence ($^{14}$C-metaldehyde mineralisation (%) after 5 days assay time) in Field topsoil (FT(n)1-FT(p)12) and Field subsoil (FS(n)2, FS(n)4): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each texture class. Error bars are standard error of the mean (n = 3). A star indicates significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

**Figure 2.** Catabolic activity ($^{14}$C-metaldehyde mineralisation (%) after 5 days assay time) in Allotment soils (AT – Allotment topsoil, AS – Allotment subsoil): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each class. Error bars are standard error of the mean (n = 3). A star indicates significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

**Figure 3.** Catabolic activity ($^{14}$C-metaldehyde mineralisation (%) after 5 days assay time) in Garden soils (GT(n)1-GT(n)10): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each class. Error bars are standard error of the mean (n = 3). Stars indicate significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

**Figure 4.** Correlation of intrinsic (black), and induced (white), catabolic activity (% mineralisation) with: OM (A) and pH (B); for, Field soils (○), Allotment soils (□) and Garden soils (△). Errors bars are ± 1 standard deviation (n = 3). Lines of best fit indicates correlations between intrinsic (solid) and induced (dashed) mineralisation capacity and SOM (A) and pH (B).
Figure 1. Catabolic competence (\(^{14}\)C-metaldehyde mineralisation (%) after 5 days assay time) in Field topsoil (FT(n)1-FT(p)12) and Field subsoil (FS(n)2, FS(n)4): soil only treatments (white bars) and soil with metaldehyde addition (black bars). Soil types are ordered by texture and then by mineralisation (%) for each texture class. Error bars are standard error of the mean (n = 3). A star indicates significant difference (p < 0.05) between soil only (-) and soil with metaldehyde (+) couplets.

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**Figure 4.** Correlation of intrinsic (black), and induced (white), catabolic activity (mineralisation, %) with: OM (A) and pH (B); for, Field soils (○), Allotment soils (□) and Garden soils (△). Errors bars are ± 1 standard deviation (n = 3). Lines of best fit indicates correlations between intrinsic (solid) and induced (dashed) mineralisation capacity and SOM (A) and pH (B).
Table 2. Field, Allotment and Garden soil properties.

| Soil Code | Setting   | Texture            | Metaldehyde application            | OM (%)      | pH          
|-----------|-----------|--------------------|------------------------------------|-------------|-------------
| FT(n)1    | Field 1   | Sand               | >4 years ago                       | 3.49 ± 0.1  | 6.68 ± 0.23 |
| FT(n)2    | Field 2   | Loamy Sand         | >4 years ago                       | 3.08 ± 0.2  | 7.55 ± 0.5  |
| FS(n)3    | Field 3   | Sand               | >4 years ago                       | 1.82 ± 0.4  | 7.55 ± 0.2  |
| FT(n)4    | Field 4   | Loamy Sand         | >4 years ago                       | 2.53 ± 0.1  | 6.57 ± 0.1  |
| FS(n)5    | Field 5   | Loamy Sand         | >4 years ago                       | 3.85 ± 0.03 | 6.35 ± 0.1  |
| FT(n)6    | Field 6   | Sandy Loam         | >4 years ago                       | 2.38 ± 0.1  | 7.21 ± 0.4  |
| FS(n)7    | Field 7   | Sandy Loam         | >4 years ago                       | 4.52 ± 0.3  | 7.19 ± 0.3  |
| FT(p)8    | Field 8   | Sand               | >4 years ago                       | 2.79 ± 0.2  | 8.2 ± 0.1   |
| FT(p)9    | Field 9   | Sandy Loam         | Seasonal (ongoing)                | 3.89 ± 0.1  | 7.24 ± 0.1  |
| FT(p)10   | Field 10  | Loam               | Seasonal (ongoing)                | 4.02 ± 0.3  | 6.15 ± 0.1  |
| FT(p)11   | Field 11  | Sand               | Seasonal (ongoing)                | 3.4 ± 0.1   | 7.11 ± 0.2  |
| FT(p)12   | Field 12  | Silty Clay Loam    | Seasonal (ongoing)                | 2.67 ± 0.1  | 7.73 ± 0.2  |
| AT(n)1    | Allotment 1 | Loamy Sand     | No previous application           | 7.91 ± 0.4  | 7.58 ± 0.01 |
| AS(n)1    | Allotment 1 | Sand            | No previous application           | 1.36 ± 0.3  | 7.05 ± 0.2  |
| AT(p)2    | Allotment 2 | Loamy Sand     | Seasonal (ongoing)                | 5.24 ± 0.1  | 7.44 ± 0.5  |
| AS(p)2    | Allotment 2 | Sand            | Seasonal (ongoing)                | 1.17 ± 0.1  | 7.18 ± 0.1  |
| GT(n)3    | Garden 1  | Sandy Loam        | >6 years ago                       | 52.1 ± 1.0  | 7.1 ± 0.03  |
| GT(n)4    | Garden 2  | Loamy Sand        | >6 years ago                       | 7.2 ± 0.2   | 7.54 ± 0.03 |
| GT(n)5    | Garden 3  | Loamy Sand        | >6 years ago                       | 25.3 ± 0.2  | 6.92 ± 0.02 |
| GT(n)6    | Garden 4  | Loamy Sand        | >6 years ago                       | 16.2 ± 0.3  | 7.49 ± 0.04 |
| GT(n)7    | Garden 5  | Loamy Sand        | >6 years ago                       | 11.8 ± 0.5  | 8.02 ± 0.02 |
| GT(n)8    | Garden 6  | Loamy Sand        | >6 years ago                       | 11.4 ± 0.4  | 8.01 ± 0.01 |
| GT(n)9    | Garden 7  | Sandy Clay Loam   | >6 years ago                       | 10.2 ± 0.1  | 7.65 ± 0.01 |
| GT(n)10   | Garden 8  | Sandy Clay Loam   | >6 years ago                       | 12.4 ± 0.4  | 7.52 ± 0.01 |
| GT(n)11   | Garden 9  | Sandy Clay Loam   | >6 years ago                       | 5.5 ± 0.1   | 8.15 ± 0.01 |
| GT(n)12   | Garden 10 | Sandy Clay       | >6 years ago                       | 8.6 ± 0.3   | 7.7 ± 0.02  |

Highlights

- Results indicated ubiquitous catabolic competence to degrade metaldehyde in dissimilar soils
- Metaldehyde catabolic competence was evident in garden, allotment and field soils
- Metaldehyde mineralisation ranged from 17.7 to 60.0 %
• Higher levels of catabolic competence were observed in the lighter soil textures

• Pre-exposure to metaldehyde sometime, but not always, resulted in higher catabolic competence