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Ageing processes and soil microbial community effects on the biodegradation of soil ^{13}C-2,4-D nonextractable residues

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Ageing processes influence the NER mineralisation rate and the microbial population involved.

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ABSTRACT

The biodegradation of nonextractable residues (NER) of pesticides in soil is still poorly understood. The aim of this study was to evaluate the influence of NER ageing and fresh soil addition on the microbial communities responsible for their mineralisation. Soil containing either 15 or 90-day-old NER of ^{13}C-2,4-D (NER15 and NER90, respectively) was incubated for 90 days with or without fresh soil. The addition of fresh soil had no effect on the mineralisation of NER90 or of SOM, but increased the extent and rate of NER15 mineralisation. The analyses of ^{13}C-enriched FAME (fatty acids methyl esters) profiles showed that the fresh soil amendment only influenced the amount and structure of microbial populations responsible for the biodegradation of NER15. By coupling biological and chemical analyses, we gained some insight into the nature and the biodegradability of pesticide NER.

1. Introduction

The formation of pesticide nonextractable residues in soil is an important process and should be taken into account when assessing pollution risks. A recent review of the literature indicated that the proportion of NER formed in soil is very variable and depends very much on the nature of the pesticide (Barriuso et al., 2008). This meta-analysis of 100 pesticides showed that the presence of chemical reactive groups, such as aniline or phenol, tends to yield a larger proportion of NER. Generally, less NER originating from N-heteroatomic rings than those from phenyl-ring structures were found. The first consequence of NER formation is a decrease in the availability of pesticide residues with a concurrent increase in their persistence in soil. The extent of the reversibility between unavailable and available forms of NER is likely to play an important role in the long-term fate of pesticides. Microbial activity can have a significant effect on the formation of NER (e.g. Racke and Lichtenstein, 1985; Shapir and Mandelbaum, 1997; Liebich et al., 1999; Abdelhafid et al., 2000), through a metabolic activation that is often required to transform relatively inert parent compounds into reactive microbial metabolites which may subsequently interact with natural soil organic matter to form NER (Richnow et al., 2000). Microbial activity can also influence subsequent release of NER although this aspect has not been investigated to the same extent. Some published data suggest that only a small percentage of NER is released under the action of microorganisms. A number of studies using ^{14}C-radiolabelled soil NER have shown that, upon reincubation, NER of different xenobiotics can be mineralised (Hsu and Bartha, 1974; Roberts and Standen, 1981; Gevao et al., 2005) or rendered extractable (Khan and Ivarson, 1981; Gevao et al., 2005).

These results suggest that NER are bioavailable and can be affected by biological activity. However, the underlying mechanisms are still poorly understood. It is believed that the main interactions leading to the formation of NER imply chemical bonding (chemical stabilisation) and physical trapping (sequestration) into the organic constituents (Loiseau and Barriuso, 2002), the relative importance of which can vary with time during NER ageing (Pignatello and Xing, 1996). The existing knowledge of the mechanisms by which residues bind to soil organic matter suggests that release will be closely dependent on soil organic matter breakdown (Barraclough et al., 2005), especially in the plough layers of soils (Burauel and Bassmann, 2005). The effect of pesticide sorption onto synthetic (Dec and Bollag, 1988) or natural organic matter (Benoit and Barriuso, 1997) on the degradation rate is well documented.

Although frequently invoked to explain observed decreases of *in situ* pesticide degradation with time (e.g. Boivin et al., 2004; Amellal

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et al., 2006), the relationship between NER ageing and biodegradability by reincubation of differentially aged NER has not been investigated. A number of studies have investigated the effect of compound ageing in soils (Tang et al., 1998; Chung and Alexander, 1998). However, ageing in these studies was carried out in sterilised soils; removing microbial activity which is known to affect the ageing process (Richnow et al., 2000). The size and structure of microbial communities can affect the rate at which complex molecules are degraded (Martens, 1985). It is generally assumed that increased microbial diversity corresponds to increased catabolic potential and, hence, to better removal of metabolites and pollutants (Dejonghe et al., 2001). The interaction of these two factors (NER age and size and structure of microbial communities) is likely to determine the extent and kinetics of NER degradation. For example, should the diffusion of molecules from micropores be the rate limiting step in their degradation, then the capacity of the microbial communities to degrade will not influence the rate of degradation. On the other hand, if the chemical nature of the NER is the controlling factor then the size and structure of the microbial communities will likely play a role.

In order to test this hypothesis, we set up an incubation with a 2×2 factorial design in which the NER ageing effect was tested by comparing 15 and 90-day-old NER and the role of microbial biomass and structure was tested by incubating extracted soil amended or not with fresh soil. To achieve this objective, we characterised the chemical forms and measured the mineralisation of NER and, at the end of the incubation, determined the biomass and structure of microbial degraders. Stable-isotope labelling is useful for measuring the mineralisation of molecules and the size and structure of degrading populations (Cupples and Sims, 2007; Lerch et al., 2009). Stable-isotope labelling can also be used to characterise the chemical nature of NER through the use of pyrolysis coupled to gas chromatography and isotopic ratio mass spectrometry via a combustion interface (pyrolysis-GC-c-IRMS) (Dignac et al., 2004) or nuclear magnetic resonance (NMR) spectroscopy (e.g. Thorn et al., 1992; Hatcher et al., 1993; Benoit and Preston, 2000). The aim of the study therefore, was to understand the links between the nature of NER, their degradability and the microbial communities involved using ^{13}C-labelled 2,4-D NER.

2. Materials and methods

2.1. Preparation of NER

Soil containing ^{13}C-NER originating from ^{13}C-2,4-D was obtained from an earlier experiment in which the dynamics of 2,4-D degraders were monitored (Lerch et al., 2009). Briefly, triplicate samples were amended with ring-U-labelled ^{13}C-2,4-D (10 μg g^{-1} dry soil equivalent, $\delta^{13}C_{2,4\text{-}D} = 3719‰$) and incubated at a water content equivalent to the field capacity. After 15 and 90 days incubation, sub-samples were extracted sequentially with water, methanol and dichloromethane. Soil containing NER extracted after 15 days is termed NER15 and that extracted after 90 days, NER90. The control soil (C), that had not received ^{13}C-2,4-D, was extracted at day 0. The amount of ^{13}C-NER in the extracted soil was measured by EA-IRMS on 30 replicates of 100 mg sub-samples. Based on equation (1), detailed further, the amount of ^{13}C-NER was 49 ± 2 and 43 ± 3 μg g^{-1} of dry soil for NER15 and NER90, respectively.

2.2. Molecular analysis of NER

Pyrolysis-GC-c-IRMS of soils containing NER was conducted using a microvolume Curie-point reactor. Ferromagnetic wires with a Curie-point temperature of 650 °C were subjected to a high-frequency power, inducing a rapid rise in temperature in approximately 0.15 s. The volatilized constituents were swept in splitless mode into a gas chromatograph (HP 5890) equipped with a BPX-5 capillary column (50 m × 0.25 mm × 0.32 μm) and coupled to an IRMS (Isochrom Optima, Micromass) via a combustion interface. The column temperature programme went from 30 to 350 °C at a rate of 2 °C min^{-1}. Chromatogram peaks were identified by comparing retention times with standards for 2,4-D and metabolites (2,4-dichlorophenol, 3,5-dichlorocatechol, 4-chlorocatechol, 4-chlorophenol). Five analytical replicates were used for each NER treatment. ^{13}C NMR analysis was performed as described by Benoit and Preston (2000) on one replicate of each NER treatment.

2.3. NER incubation

Unamended samples (10 g) taken from the control, NER15 or NER90 were incubated in triplicate. Three other 10 g replicates of each treatment were mixed thoroughly with 20 g of fresh soil before incubation. These treatments were named C+, NER15+ and NER90+. The fresh soil was sampled in the ploughed layer (0–30 cm) of a cultivated Luvisol at the same time as the soil used for the previous experiment. It was stored at 4 °C for 3 months and preincubated at 20 °C 2 weeks prior to use. Before mixing with soil containing NER, the amount (141 ± 4 nmol g^{-1} soil) and the composition (Fig. 1) of the fresh soil microbial biomass was estimated by FAME analyses as described below. Samples were then placed in serum bottles (120 ml) with Teflon® rubber stoppers crimped on with aluminium caps at 20 °C in the dark for 3 months. At the beginning of the incubation, the water content was adjusted to the field capacity (0.26 g water g^{-1} of oven dry soil) by adding MilliQ water (Millipore) and the bottles were flushed with CO_2 free air (19% O_2, 81% N_2).

2.4. Mineralisation measurement

Soil respiration was measured by sampling the headspace of serum bottles after 5, 10, 15, 36, 60 and 90 days of incubation. The CO_2 concentration was determined with a micro-GC (Agilent 3000A, Qplot column). The isotopic signature (δ^{13}C, ‰) of the CO_2–C was determined using a GC (Hewlett-Packard 5890) coupled with an isotopic ratio mass spectrometer (Isochrom Optima, Micromass). After each measurement the samples were flushed with CO_2 free air, sealed, and replaced in the incubation chamber. Bottles were weighed before and after the air flush and MilliQ water was added with a syringe through the Teflon® rubber if any loss of water was observed.

2.5. FAME analyses

After 3 months of incubation, soil samples were freeze-dried and the lipid fraction was extracted with a mixture of methanol and dichloromethane (Bligh and Dyer, 1959). Fatty Acid Methyl Ester (FAME) profiles of Total Lipid Fatty Acids (TLFA) were analysed rather than Phospholipid Fatty Acids (PLFA) because the former could be obtained from the same extracts used to analyse the 2,4-D and its metabolites. The lipids extracted were transesterified with BF_3/Methanol to recover the FAME and 2,4-D as methyl-2,4-D as described by Lerch et al. (2007). Lipid extracts were quantified by GC (HP 6890) coupled to a Flame Ionisation Detector (GC-FID) and identified by GC (HP 6890) coupled to an Agilent 5973 Electronic Impact (70 eV) quadripole Mass Spectrometer (GC–MS). The individual isotopic analysis was carried out using a GC (HP 5890) coupled to an Isochrom III Isotopic Mass Spectrometer (Micromass-GVI Optima) via a combustion interface (GC-c-IRMS). All GC were equipped with the same SGE BPX-5 column (50m × 0.25 mm × 0.32 μm). Column

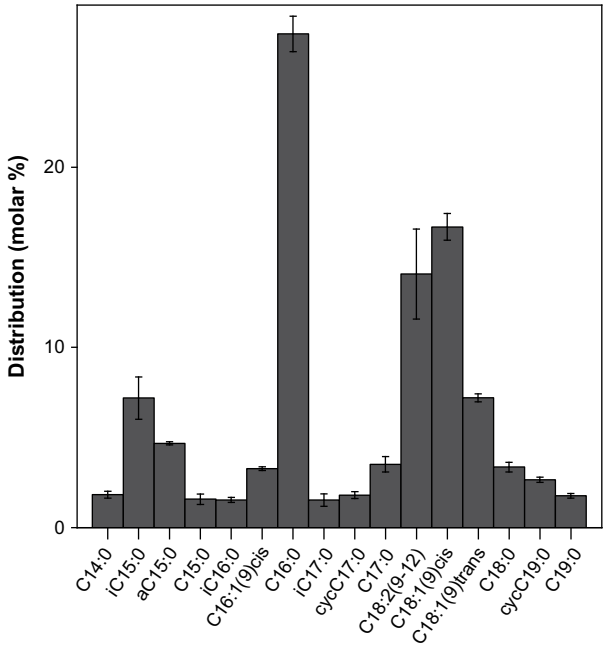

Fig. 1. Molecular composition of FAME (molar %) extracted from the fresh soil used to inoculate post-extraction soil containing NER. Error bars correspond to the standard deviation calculated for 3 replicates.