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Compositional Changes in the Hydrophobic acids fraction of Drainage Water from Different Land Management Practices.

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Abstract

Dissolved organic matter (DOM) can play a key role in many environmental processes, including carbon cycling, nutrient transport and the fates of contaminants and of agrochemicals. Hydrophobic acids (Ho) , the major components of the DOM, were recovered from the drainage waters from a well drained (WDS) and a poorly drained (PDS) Irish grassland soils in lysimeters and amended with N fertiliser (F) and with bovine urine (U) and were studied using 1D and 2D solution state Nuclear Magnetic Resonance (NMR) spectroscopy. The Diffusion Edited (DE) ^{1}H NMR spectra indicated that the Ho consisted largely of larger molecules, or of molecules that formed rigid aggregates, and the 1D and the 2D (Heteronuclear Multiple Quantum Coherence – HMQC, the Total Correlation Spectroscopy –TOCSY, and the Nuclear Overhauser Effect –NOESY) spectra indicated that the samples were composed of lignin residues, carbohydrates, protein/peptides, and aliphatic components derived from plant waxes/cuticular materials and from microbial lipids. The F amendments increased the concentrations of Ho in the waters by 1.5 and 2.5 times those in the controls in the cases of WDS and PDS, respectively. The lignin-derived components were increased by 50% and by 300% in the cases of the Ho from the WDS and PDS, respectively. Applications of F + U decreased the losses of Ho, (compared to the F amendments alone) and very significantly decreased those of the lignin derived materials, indicating that enhanced microbial activity from U gave rise to enhanced metabolism of the Ho components, and especially of lignin. In contrast the less biodegradable aliphatic components containing cuticular materials increased as the result of applications of F + U. This study helps our understanding of how management practices influence the movement of C between terrestrial and aquatic environments.

Keywords

Grassland; Dissolved organic matter; Hydrophobic acids; Drainage water; Fertiliser; Urine; Solution state NMR

1. Introduction

Dissolved organic matter (DOM) is a complex, heterogeneous mixture found in all natural waters, and it represents the largest fraction of mobile carbon (C) on earth. It provides an intimate link between the terrestrial and aquatic environments (Lam et al., 2007). Soil derived DOM can play a key role in many environmental processes, including carbon cycling, nutrient transport and the fates of contaminants and of agrochemicals (Qualls and Haines, 1991; Royer et al., 2007; Zsolnay, 2003). Despite its obvious importance, the structural components of soil DOM and the variations of these components with different land management practices, have not been well resolved (Royer et al., 2007).

Temperate grassland ecosystems, which comprise 32% of the earth's natural vegetation (Frank and Dugas, 2001), can be considered to have a significant role in the uptake of atmospheric CO_2 and in balancing the global C budget (Batjes, 1998). Grassland, the dominant ecosystem in Ireland, represents 90% of agricultural land and 56% of the total land area (Jaksic et al., 2006). Article 3.4 of the Kyoto protocol (UNFCCC, 1998) makes provision for the use of soil C stock changes in grazing lands to offset greenhouse gas (GHG) emissions and to facilitate the achievement of emissions reduction targets (Byrne et al., 2007). On that basis, there is a need to better understand the organic components leached from these carbon stocks under different management practices.

Soils in long-term pasture are in a steady-state with regard to soil organic matter (SOM) content. Carbon accumulation in grassland ecosystems occurs mostly below ground and changes in soil organic C (SOC) stocks may result from changes in land uses management (Soussana et al., 2004). Grassland C stocks represent at least 10% of the global total, and some sources suggest up to 30% of that total (Scurlock and Hall, 1998). The stocks of SOM result from the balance between inputs and outputs of C. Inputs are primarily from leaf and root detritus. Outputs are dominated by the efflux of carbon dioxide (CO_2) and of methane (CH_4) from the soil surface and by the hydrologic leaching of dissolved and particulate C (Davidson and Janssens, 2006). The pool of SOM is of particular interest because even small changes in flux rates into or out of such a large pool could lead to the accumulation of significant quantities of greenhouse gases (Billings and Ziegler, 2008).

Although land use and related management practices are known to affect the amounts and compositions of SOM and soil properties, their influences on the amounts and compositions of DOM have not been extensively studied (Chantigny, 2003). Various aspects of the effects of elevated nitrogen (N) deposition and of N fertilization have been studied, yet little is known about their effects on DOM turnover (Kalbitz et al., 2000). The same is true for organic amendments such as urine. The OM in amendments is biodegradable and is generally readily transformed by soil microbes. That may result in transient increases in the soil DOM (Chantigny, 2003). Amendment with slurry has been found to increase nitrogen (N) immobilisation through increased microbial activity (Hoekstra, 2009). This may lead to an increase in carbon mineralization and a decrease in DOM export. However, to our knowledge, detailed studies have not been carried out on changes in DOM compositions following mineral fertilization and organic amendments. Soil hydrology is also likely to affect DOM dynamics. Differences have been found between DOM fractions isolated from different drainage regimes (Hayes et al.,1997), and research has shown that DOC exports were 33 Kg ha $^{-1}$ lower from drained than from undrained plots (McTiernan et al., 2001).

In this study we characterise, in detail, the Ho from the DOM formed from two soils, one well drained (WDS) and the second poorly drained (PDS), each amended with fertiliser, and with fertiliser and urine. The emphasis is on the characterisation of the components of the Ho released in the drainage water from these soils using advanced Multidimensional nuclear magnetic resonance spectroscopy (NMR) techniques that are widely used to study structures and interactions in environmental chemistry (Simpson and Brown, 2005; Thrippleton and Keeler, 2003).

2. Materials and Methods

2.1 Source of samples

Intact soil monoliths lysimeters (0.8 m diameter by 1 m deep) were sampled from a well drained (WDS) Brown Podzolic soil (Haplic Podzol (Anthric)) (FAO, 2007) and from a poorly drained (PDS) Gley (Luvic Stagnosol (Eutric, Siltic)) (FAO, 2007) were installed in 2004 in lysimeters in a pasture field at the Teagasc Environmental Research Centre (ERC), Johnstown Castle, Wexford, Ireland. The sand, silt, and clay contents of the soils are given in Table 1. The soils were collected as undisturbed monoliths and installed according to an established protocol (Cameron et al., 1992). Briefly this involved isolating a 1 by 1m soil column and then carefully, reciprocally, pushing a 0.8 m HDPE pipe through the soil column. When the pipe reached 1 m a cutting plate was hydraulically pushed beneath the lysimeter to cut it from the soil beneath. To prevent edge flow liquid petrolatum was injected between the soil and the HDPE pipe. The lysimeters were inverted and 5 cm of fine gravel inserted at the base of the soil and a base plate with drainage outlet was welded to the pipe. The completed lysimeters were installed in a field lysimeter facility under natural rainfall and meteorological conditions. Each soil was sown with perennial ryegrass (*Lolium perennae* L.). In order to replicate typical Irish grazed grassland activities, some of the lysimeter soils were amended with fertiliser and some with both fertiliser and bovine urine, and unamended soils served as controls as described in Stark *et al.* (2007). With the exception of the controls, the lysimeter soils received in 2004 and 2005, 291 kg N ha^{-1} yr^{-1} as fertiliser and 310 kg N ha^{-1} yr^{-1} as urine (Table 2). Treatments were applied in a randomised complete block design with 3 replicates per treatment. Herbage was harvested regularly to correspond with a 28-day rotation of livestock. A series of pipes transported the drainage water (DW) from each lysimeter to storage vessels housed below ground level. Drainage water samples, 200 L from each treatment and control, were collected from the lysimeter facility between June and December, 2005.

2.2 Isolation of hydrophobic acids from drainage waters.

The Ho were isolated from the drainage waters using previously described procedures (Hayes et al., 2008; Malcolm and MacCarthy, 1992). Waters were filtered under pressure (69 kPa) through 0.2 m Sartorius (Goettingen, Germany) cellulose acetate membrane filters. The filtrates were adjusted to pH 2 (HCl) and applied to XAD-8 resin [(poly)methylmethacrylate] (Rohm and Haas, Philadelphia). Two column volumes of 0.01 M HCl were pumped through to ensure that the entire sample had passed through the column. The resin was then desalted with distilled water until effluent conductivities were < 100 pS cm^{-1}. Back elution was carried out using 0.1 M NaOH and the centre cut eluates were H^+ exchanged (Amberlite IR-120, H^+-form; Rohm and Haas, Philadelphia), then freeze dried to give the XAD-8 hydrophobic (Ho) acids.

2.3 Solution State NMR Spectroscopy experimental details

Samples (40 mg) were dissolved in 600 µL of deuterium oxide (D_2O) and titrated to pH 12 using NaOD to ensure complete solubility. Additional samples (40 mg) were dissolved in 600 µL DMSO-d_6.

Samples were analysed using a Bruker Avance 500 MHz NMR spectrometer equipped with a ^1H-^{19}F-^{15}N-^{13}C 5 mm, quadruple resonance inverse probe with actively shielded z-gradient (QXI). 1D solution state ^1H NMR spectra were obtained with 128 scans, a recycle delay of 2 s, 16384 time domain points, and an acquisition time of 0.79 s. Water suppression was achieved using PURGE (Simpson and Brown, 2005). Spectra were apodized through multiplication with an exponential decay corresponding to 1 Hz line broadening, and a zero filling factor of 2. Diffusion-edited (DE) spectra were obtained using a bipolar pulse longitudinal encode-encode sequence. Scans (1600) were collected using a 2.5 ms, 49 gauss/cm, sine-shaped gradient pulse, a diffusion time of 200 ms, 16384 time domain points, 0.82 s acquisition time, and a sample temperature of 298 K.

Heteronuclear multiple quantum coherence (HMQC) spectra were obtained in phase-sensitive mode using echo/anti-echo gradient selection and a ^{1}J ^{1}H-^{13}C value of 145 Hz. Scans (512) were collected for each of the 128 increments in the F1 dimension. A total of 1048 data points were collected in F2, and a relaxation delay of 1 s was employed. The F2 dimension was multiplied by an exponential function corresponding to a 10 Hz line broadening and a zero filling factor of 2. The F1 dimension was processed using a sine-squared function with a ð/2 phase shift and a zero-filling factor of 2.

Total correlation spectroscopy (TOCSY) spectra were acquired in the phase-sensitive mode, using time proportional phase incrimination (TPPI). TOCSY NMR experiments were carried out using 512 scans with 128 time domain points in the F1 dimension and 1048 time domain points in the F2 dimension. A mixing time of 60 ms was used with a relaxation delay of 1 s. Processing of both dimensions used a sine-squared function with a ð/2 phase shift and a zero-filling factor of 2.

Nuclear Overhauser Effect Spectroscopy (NOESY) was obtained with the elimination of zero-quantum interference (Thrippleton and Keeler, 2003). NOESY NMR experiments were carried out using 256 scans with 128 time domain points in the F1 dimension and 1048 time domain points in the F2 dimension. A mixing time of 250 ms was used with a relaxation delay of 1 s. Zero-quantum suppression was achieved through the use of an adiabatic-pulse/gradient pair during the mixing time (Thrippleton and Keeler, 2003). Both dimensions were processed using a sine-squared function with a ð/2 phase shift and a zero-filling factor of 2.

3. Results and discussion

DOM in soil is composed of humic substances and a variety of specific identifiable organic compounds, including carbohydrates and peptides. In this study the hydrophobic acid fraction was isolated using an XAD-8 resin technique (Leenheer, 1981), and is the dominating constituent of bulk dissolved organic matter (DOM) in soil solutions (Asakawa et al., 2006).

3.1 Characterisation of the drainage water hydrophobic acids

Two solvent systems were used for the NMR analysis of the Ho; D_2O/NaOD and DMSO- d_6. D_2O or D_2O/NaOD systems are commonly used for studies of DOM (Hertkorn et al., 2006; Kaiser et al., 2003; Kim et al., 2003; Lam et al., 2007; Simpson, 2001; Smejkalova and Piccolo, 2008) and the D_2O/NaOD system in this study enabled comparisons with previous studies. DOM samples in the protonated form (achieved here through exchange with the IR-120 cation exchange resin) are completely soluble in DMSO. DMSO is a dipolar aprotic solvent; hence signals from exchangeable protons, for example, N-H, can be observed. Thus DMSO provides excellent complimentary information for structural studies, especially for protein/peptide components, and in many cases it provides spectra with better defined resonances (Simpson, 2001). Our samples were completely soluble in both solvent systems used. 1D and 2D NMR spectroscopy techniques were used to observe compositional differences in the Ho components in the drainage waters.

Figure 1A shows the ^{1}H NMR spectrum in DMSO-d_6 for the Ho isolated from the poorly drained soil (PDS) treated with fertiliser. Major structural components present include aromatics, lignin (Lig), carbohydrates (Carb), proteins/peptides (P) and aliphatic units. Figure 1B is the diffusion edited (DE) NMR spectrum of the same sample. Signals from larger molecules or rigid molecular associations can be further emphasised by the use of diffusion editing. Diffusion editing “spatially encodes” molecules at the start and then “refocuses” these at the end of the experiment. Species that diffuse and exhibit a high degree of motion during the experiment are not refocused and are essentially gated from the final spectrum (Simpson et al., 2007b). Thus the spectrum produced contains only signals from larger molecules or rigid molecular associations. Because the majority of the signals remain after diffusion editing, it can be considered that the components in the Ho are likely to be larger molecules or very stable aggregates (Simpson, 2002). Main chain methylene signals at ~1.3 ppm are consistent with aliphatic structures from plant-derived waxes/cuticles (Deshmukh et al., 2003) that have previously been identified in humic extracts (Kelleher and Simpson, 2006; Kelleher et al., 2006; Simpson et al., 2003), and to contributions from microbial lipids (Simpson et al., 2007a). In this DE spectrum, the CH_3 signal at ~ 0.8 ppm is likely to be mainly from methylated amino acid side chain residues (Simpson et al., 2007a). This is further dealt with in discussion of Figure 1C.

There is considerable overlap in the 1D NMR resonances. However it has been possible to confirm the suggested assignments by an array of 2D NMR experiments, including HMQC, TOCSY, and NOESY. Applications of 2D NMR for studies of natural organic matter (NOM), and interpretations of the data have been discussed extensively in the literature (Cardoza et al., 2004; Simpson, 2001; Simpson et al., 2001). Briefly, 2D NMR experiments provide increased spectral dispersion as well as additional connectivity information allowing detailed assignments of the chemical functionalities and structural components present (Lam et al., 2007). Figure 2A shows the Heteronuclear Multiple Quantum Coherence (HSQC) spectrum for the Ho isolated from the PDS that was treated with fertiliser. The HMQC experiment detects one bond ^{1}H-^{13}C connectivites in an organic structure (Simpson, 2001). When considered together, the cross-peaks form a specific pattern that can be thought of as the “molecular fingerprint” of a specific structure or class of structure (Kelleher and Simpson, 2006). The HMQC NMR spectrum identifies a range of chemical functionalities present (assignments and references are given in the Figure caption) and suggests that the Ho are a mixture of predominately lignin, protein, carbohydrates, and lipids/cuticlar waxes (Deshmukh et al., 2005; Deshmukh et al., 2003; Kelleher and Simpson, 2006; Lam et al., 2007; Simpson et al., 2007a; Simpson et al., 2007b). This is further supported by the TOCSY (Fig. 2C) and NOESY (Fig. 2D) data. All these components have been assigned previously for NOM (Deshmukh et al., 2003; Hertkorn et al., 2006; Kelleher and Simpson, 2006; Kelleher et al., 2006; Lam et al., 2007; Simpson, 2001; Simpson et al., 2003; Simpson et al., 2007a; Simpson et al., 2007b).

Signals due to *N*-acetyl and/or *O*-acetyl, previously seen in freshwater DOM (Hertkorn et al., 2006; Lam et al., 2007) are evident in region 10 (Fig. 2A, 2B). Acetyl groups (Lam et al., 2007), often found in peptidoglycan from microbial cell walls (Simpson et al., 2007b) and in protein (Simpson et al., 2007a) could indicate microbial inputs. The microbial contributions are most clearly evident from comparisons between spectra for microbial biomass cultured from soil (Simpson et al., 2007a) and those for the Ho in this study. Figures 1B and 1C compare the DE spectrum of the Ho with that obtained for microbes cultured from a Canadian dark grey Chernozem soil. The microbes on which Fig. 1C is based were isolated from a different soil to that from which the Ho for Fig 1B was obtained. The microbes were cultured in a minimal medium with glucose and acetate as carbon sources using a "double spiking approach" (Simpson et al., 2007a). Previous studies have shown that soil microbes give a relatively similar NMR spectrum, irrespective of the soil type from which they are isolated (Simpson et al., 2007a), and the spectrum shown in Figure 1C shows the extent to which the microbial contributions contribute to the Ho.

Comparison of the two spectra indicate that signals from microbial biomass, mainly peaks labelled P, are clearly apparent in the NMR spectrum of the Ho. Characteristic resonances seen for protein/peptide, namely amide (N-H), phenylalanine (Phe), á-protons from amino acid side chains, and methylated side chains are easily distinguishable in both the Ho acid and in the microbial biomass. Furthermore, the region labelled “SC” in Figure 1C represents the side-chain resonances from proteins and peptides. This region can generally be considered as a “fingerprint” region representing the type of peptide/protein present (Simpson et al., 2007a). The side-chain region in the Ho acid matches well with that of the microbes.

The similarities between the Ho spectrum and that of the microbes, highlights the input of microbial biomass to the Ho isolated from the drainage waters.

Components from plant biomass, in addition to microbial inputs, are also in evidence. There are clear indications for lignin-derived components. While these signals are very clear in the HMQC and NOESY data (Figs 2A, 2D), they are still apparent in the 1D spectra. Figure 1D displays the DE spectrum for a lignin standard (organosolv lignin, Sigma Aldrich). The large resonance centered at ~3.7 ppm is characteristic of the methoxyl of lignin. Comparison of the spectrum for Ho (1B) with that of the Organosolv lignin (1D) clearly indicates that the apex of the central region of the lignin peak (labelled Lig) in the Ho is from the methoxyl of lignin (Simpson et al., 2007b). This is also confirmed by the intensity of the methoxyl signal in the HMQC data (Figure 2A). Additionally, aromatic resonances from lignin at ~6.3-7 ppm (Lig), are evident in the Ho (Figure 1B) and these partially overlap with the signal for aromatic residues in proteins/peptides. Thus it can be concluded that the Ho is likely to be a mixture of soil derived plant and microbial materials that have previously been identified in a range of NOM samples (Hertkorn et al., 2006; Hertkorn et al., 2002; Kelleher and Simpson, 2006; Kelleher et al., 2006; Lam et al., 2007; Simpson, 2001; Simpson, 2002; Simpson et al., 2001; Simpson et al., 2003; Simpson et al., 2004; Simpson et al., 2007a; Simpson et al., 2007b).

3.2 Investigation into the effects of the various treatment regimes

Results have varied with regard to studies of the effects of N on OM decomposition. Concentrations and fluxes of DOC from the forest floor remained unchanged for field additions of N (Currie et al., 1996; McDowell et al., 1998) whereas the DOC release rate was found to have decreased by 20% following N fertilization of a forest soil (Cronan et al., 1992). N addition as urea resulted in the increased release of water-soluble OC from a forest soil (Homann and Grigal, 1992).

Exports of hydrophobic acids in the drainage water from the well-drained and of poorly-drained soils under different treatment applications are shown in Table 3. Both of the control soils had similar exports of Ho acids in their DW. However, the application of fertiliser gave rise to large increases. Exports of Ho were 1.5 times greater from the WDS, and were almost 2.5 times greater from the PDS. This positive correlation between N fertiliser application and total Ho exported in the cases of both soils may have resulted from increased OM matter inputs arising from increase grassland productivity.

This is proportional to N application (McTiernan et al., 2001), and leads to greater returns of OM to the soil via leaf and root decay (Parsons et al., 1991). The additional OM from the increased plant growth would be a potential source of the Ho that would be transported from the plot by rainwater (McTiernan et al., 2001). In addition urea- and ammonium-based fertilisers temporarily solubilise SOM and can, as the result of an increase in soil pH, induce a marked increase in DOC content (Chantigny, 2003; Myers and Thien, 1988). However, this effect has been found to be short-lived (Clay et al., 1995).

The NMR spectra obtained for samples after dissolving in DMSO-d_6, shown in Figure 4, are better resolved but contain the same major structural components seen in D_2O (Figure 3). The contribution of peptides to the Ho is more evident in the DMSO-d_6 spectra, as seen by the double “hump” at ~4-4.4 ppm (á-protons) and by the large amide and methyl resonances (Simpson et al., 2007b). This is most clear in the DE spectra in DMSO (see Figure 5). The DE spectra are dominated by lignin and microbial signatures indicating that these are the largest of the components in the sample.

Regardless of solvent used, the NMR spectra indicate that there is an increase in the lignin contribution to the Ho (Figures 3 and 4: A vs. B, D vs. E) as the result of fertiliser applications. Absolute quantification from such complex 1D spectra is very difficult, as discussed by Simpson et al. (2007b). However, relative quantification of the methoxyl signal is possible from the 2D HMQC spectra. Absolute quantification is not possible because the signal intensity in the HMQC employed in this study is proportional to the one bond coupling constant (^1J ^1H-^{13}C). The intensity of the methoxyl signal with respect to the total intensity of all peaks in the HMQC (with the exclusion of the DMSO peak) provides an estimation of the abundance of lignin in each sample. This, in turn permits the relative increases/decreases in lignin contents in the different samples to be estimated.

Semi-quantitative analysis indicates that, compared to the control, treatment of the soil with fertiliser increased the lignin-derived components in the WD Ho by ca 50%. An increase of 300% was found in the case of the PD Ho. The increases in lignin-derived materials are likely to have resulted from the increased vegetative growth arising from the fertiliser-N amendments.

Grazing can result in the deposition to soils of large quantities of urine-N (400 to 1200 Kg N ha $^{-1}$), and the effects of urine on changes in DOM compositions are not well understood (Rooney et al., 2006). Ho was collected from lysimeter soils amended with both fertiliser-N and urine-N. Applications of fertiliser plus urine (F+U) caused less Ho losses than the treatment with fertiliser alone, (Table 3) but greater than from the control. ^{1}H NMR spectra in both D_2O and DMSO-d_6 solvents show a significant decrease in the lignin-derived signal in the Ho isolated from both F+U treated soils (Figures 3 and 4: B vs. C, E vs. F). This correlates well with the semi-quantitative analysis that suggested a decrease of 70 % (in comparison to the control) in the lignin-derived OM signal for the WDS Ho as the result of treatment of the soil with F + U. A decrease of 3% was found in the case of the PDS Ho as the result of a similar soil treatment. It is probable that this decrease in C export in the drainage water from the F+U treated soils resulted from increased microbial activity in the soil from the addition of urine. Under the aerobic conditions that prevailed in the WDS F+U, the lignin appears to have undergone greater oxidation. Soil respiration was found to be higher from a soil treated with cow urine as the result of an immediate and significant increase in microbial metabolic activity (Lovell and Jarvis, 1996). Urine contains only small concentrations (0.01%) of soluble carbon (Kishan et al., 1989); however, solubilisation of soil organic C has been shown to take place following urine applications (Monaghan and Barraclough, 1993), and that soluble carbon could provide substrate for increased microbial metabolism (Lovell and Jarvis, 1996). Soils treated with varying concentrations of synthetic sheep urine had greater levels of microbial activity than untreated soils (Rooney et al., 2006). Urine deposition has been shown to alter substantially soil microbial communities, in terms of bacterial and fungal counts and respiration rates (Williams et al., 2000). Differences in microbial biomass activity between grassland types are related to differences in substrate availability (Bardgett et al., 1998; Williams et al., 2000). A strong correlation between N immobilization and C mineralization has been found (Barrett and Burke, 2000). Rapid stabilization of N was facilitated by an active microbial community and the availability of a readily minerali sable C substrate. It is likely that increased microbial activity induced by the addition of urine promoted the decomposition of the lignin-derived DOC (observed in the NMR spectra in this study) leading to the decrease in the DOC concentration in the drainage water.

Conversely, cuticular coatings/leaf waxes are known to be highly recalcitrant and to accumulate over time during the degradation of plants (Kelleher et al., 2006). The relative contributions from aliphatic components compared to the lignin components in the DE-NMR spectra for both PDS and WDS increased with applications of F+U (Figure 5, C and F, see arrows). That would correspond to an accumulation of aliphatic components in the Ho. Such may result from a decrease in the more readily degradable fraction (i.e. lignin), resulting in higher concentrations of the ‘less digestible’ cuticular fraction in the soil. Treatment of a grassland soil with sheep urine was found to have led to an increase in the dead or decomposing root mass from 2.2% in the untreated soil (control) to 6.3% in the urine treated soil (Shand et al., 2002). They considered that part of the DOC in the soil solution from beneath the urine patches came from roots damaged by the high concentrations of ammonia (NH_3). That could explain the greater contribution of methylene units, possibly from suberin in the root material, to the spectra of the Ho isolated from the DW of the soils treated with F+U (Figure 5: C and F, see arrows). On the other hand, the signals consistent with protein/microbial contributions are still dominant in the spectra. Such would be expected as both the urea and N should stimulate microbial activity.

There are similarities in the Ho exported from the control soils. The various treatment regimes, however, had greater effects on the PDS. As mentioned, the application of fertiliser caused a greater increase in the exports of Ho from the PDS (Table 3). That could arise, in the case of the poorly drained soil, from the decreased aeration that would impede biological oxidation to carbon dioxide (CO_2) of the increased organic matter (resulting from the application of fertiliser) (McTiernan et al., 2001). On the other hand, rapid decomposition of organic materials may have taken place in the WDS resulting in the removal of less DOM.

In contrast, the F+U application caused a decrease in the Ho from both the PDS and from the WDS, in comparison to the application of fertiliser alone. That could have arisen from increased microbial activity as a result of the urine additions, leading to a greater metabolism of the SOM and leaving less material available to contribute to the DOM.

In summary, the main effects of the varying treatment regimes on the Ho composition from both soils are still not completely resolved. The contribution of lignin components (peak labelled Lig or 6) increased with applications of fertiliser and decreases with fertiliser plus urine addition. The most likely causes of the effects is that the F+U applications lead to an increase in microbial activity causing microbial utilisation of the more degradable lignin components. Irrespective of the causes of these changes it appears that land management practices significantly alter the composition of dissolved organic matter released into drainage water.

3.3 Agricultural/Environmental Significance

Results from the multidimensional solution-state NMR analysis, indicate that the components of Ho in the drainage water of typical Irish grassland soils are complex mixtures of both plant and microbial-derived materials. Strong contributions from lignin and of peptides/proteins of microbial origins were evident in all spectra.

Treatment with fertiliser (F) resulted in an increase in the Ho export from both the WDS and the PDS, and an increase in the lignin contribution to the compositions of the Ho. This is thought to result directly from elevated OM inputs to the soil as the result of increased dry matter production through fertilization. Enhanced microbial activity is brought about by inputs of labile C (Lovell and Jarvis, 1996). Increased microbial activity, stimulated by the addition of urine, could result in a degradation of the increased OM input brought about by fertilization. That is reflected by a lower lignin contribution to the Ho isolated from the fertiliser and urine treatment.

The drainage regime affected the responses of each soil to the treatments. The decreased aeration in the PDS, compared to the WDS, resulted in a lesser decomposition of the increased OM input in the Ho (McTiernan et al., 2001). In contrast, the fertiliser plus urine application gave rise to a decrease in the Ho from the PDS, compared to the treatment with fertiliser alone. A plausible explanation for this might be that the urine may have been transported more slowly through the PDS resulting in a higher level of microbial activity, increased decomposition, and a lower export of Ho.

Growing concern about climate change has increased interest in the role of DOM in the global carbon cycle (Kalbitz and Kaiser, 2008). This study provides further information on the extent and the composition of the organic C lost from soils through transport in drainage water from Irish grassland. Additions of plants with high lignin content have been proposed as a means of building C stocks (Paustian et al., 1997) in order to sequester C. Aromatic compounds from lignin are considered to be the most stable components of DOM (Kalbitz and Kaiser, 2008). Our study indicates, however, that the stimulation of microbial activity by the addition of urine decreases the recalcitrance of the lignin components in the DOM.

Investigations of the compositions and the extents to which Ho is lost from soils, as influenced by management practises and the processes involved, will help our understanding of the movement of C between the terrestrial and aquatic environments. Such information is important because it provides an insight into an area of the carbon

4. Conclusions

Hydrophobic acids (Ho) were isolated from drainage waters and characterised using solution state NMR. The main conclusions from this study can be summarised as follows:

1, Multidimensional solution-state NMR analysis indicates that the components of the Ho from the drainage water of typical Irish grassland soils are complex mixtures of both plant and microbial-derived materials;

2, Treatment with fertiliser (F) increased the Ho export from both well drained (WDS) and poorly drained (PDS) soils, and increased the lignin contribution to the compositions of the Ho. This possibly resulted from elevated OM inputs to the soil as the result of increased dry matter production through fertilization. Application of a fertiliser plus urine (F+U) mixture resulted in smaller losses of Ho and decreased the lignin-derived signal. This is likely to be attributable to an increase in microbial activity arising from the urine application;

3. The drainage regime affected the responses of each soil to the treatments. Application of fertiliser caused a greater increase in the exports of Ho from the PDS. That reflected the decreased aeration in the PDS, resulting in a lesser decomposition of the increased OM input in the HO. The F+U application gave rise to a decrease in the Ho from the PDS, compared to the treatment with fertiliser alone. The urine may have been transported more slowly through the PDS resulting in a higher level of microbial activity, increased decomposition, a lower export of Ho, and a lower lignin contribution to the Ho.

4. Our study shows that the stimulation of microbial activity by the addition of urine decreases the recalcitrance of the lignin components.

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Table 1: Analyses of the well-drained and of poorly-drained soils.

Soil	Depth (cm)	Total C	Organic C	Total N	C\N ratio	% Sands	% Silts	% Clays
WD	0-10	3.22	3.18	0.3	10.7	45.2	20.4	12.0
	10-20	2.52	2.33	0.24	10.5	44.0	27.9	12.3
	20-30	1.42	1.43	0.14	10.1	48.6	28.0	12.4
	30-40	1.59	1.5	0.13	12.2	41.4	33.1	14.3
	40-50	1.19	1.12	0.08	14.9	40.0	42.5	9.2
	50-60	0.69	0.66	0.05	13.8	42.8	43.2	7.5
	60-70	0.17	0.14	0.02	8.5	46.6	35.7	6.5
	70-80	0.32	0.27	0.03	10.7	46.7	32.4	6.1
	80-90	0.22	0.19	0.02	11.0	50.2	36.4	5.9
	90-100	0.19	0.15	0.02	9.5	42.6	44.7	1.8
PD	0-10	4.36	4.23	0.35	12.5	24.8	35.0	25.2
	10-20	2.72	2.7	0.25	10.9	25.6	35.2	26.3
	20-30	0.83	0.77	0.09	9.2	27.2	34.7	30.0
	30-40	0.34	0.29	0.04	8.5	30.1	17.8	45.6
	40-50	0.25	0.22	0.03	8.3	31.0	34.6	28.7
	50-60	0.22	0.18	0.03	7.3	31.1	34.2	16.1
	60-70	0.14	0.11	0.03	4.7	34.6	34.5	25.3
	70-80	0.14	0.11	0.03	4.7	34.2	35.3	24.0
	80-90	0.12	0.09	0.03	4.0	35.8	34.4	23.9
	90-100	0.11	0.08	0.03	3.7	33.8	36.6	24.3

Table 2: Nutrient application rates to lysimeters.

	Nutrient application rates kg/ha				
	Inorganic Fertiliser		Cow Urine		
Treatment	Urea[1]	CAN[2]	N	P	K
Control	0	0	0	0	0
Fertiliser only	58	233	0	0	0
Fertiliser & urine	58	233	310	0.8	465

1 Urea (46% N) manufactured by Goulding.

2 CAN- Calcium Ammonium Nitrate (27% N) manufactured by Goulding.

2004 At grass sowing all lysimeters received a basal application of NPK of 37, 37 and 74 kg/ha, respectively.

Table 3: Exports of hydrophobic acids (Ho) in the drainage water from the well-drained and of poorly-drained soils under different treatment applications.

	Ho losses mg L$_{1-}$	
Treatment	Well drained soil (WDS)	Poorly drained soil (PDS)
Control	1.62	1.54
Fertiliser	2.42	3.78
Fertiliser & urine	2.25	1.87

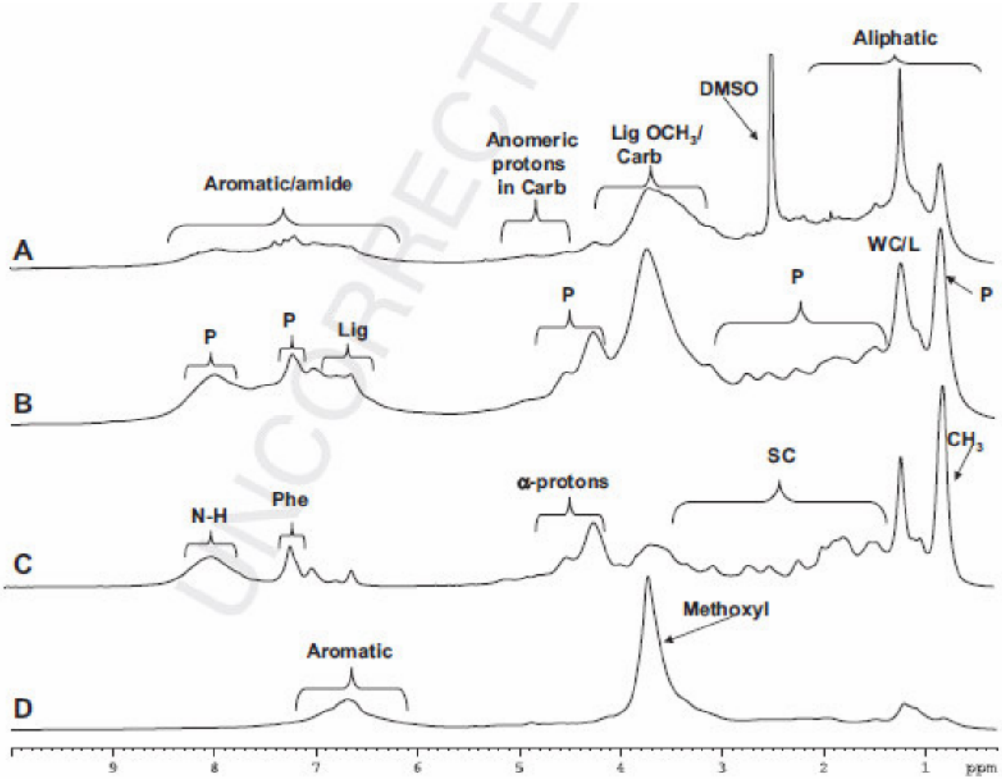

Figure 1. (A), ^{1}H NMR spectrum in DMSO- d_6 for Ho isolated from the PDS treated with Fertiliser. (B), Diffusion edited ^{1}H NMR spectrum in DMSO- d_6 for the Ho. (C), Cultured soil microbes. (D), Organosolv Lignin. Assignments include lignin (Lig),

carbohydrates (Carb), protein/peptides (P), waxes, cuticles and lipids (WC/L), protein/peptide side chains (SC), phenylalanine (Phe) and amide (N-H).

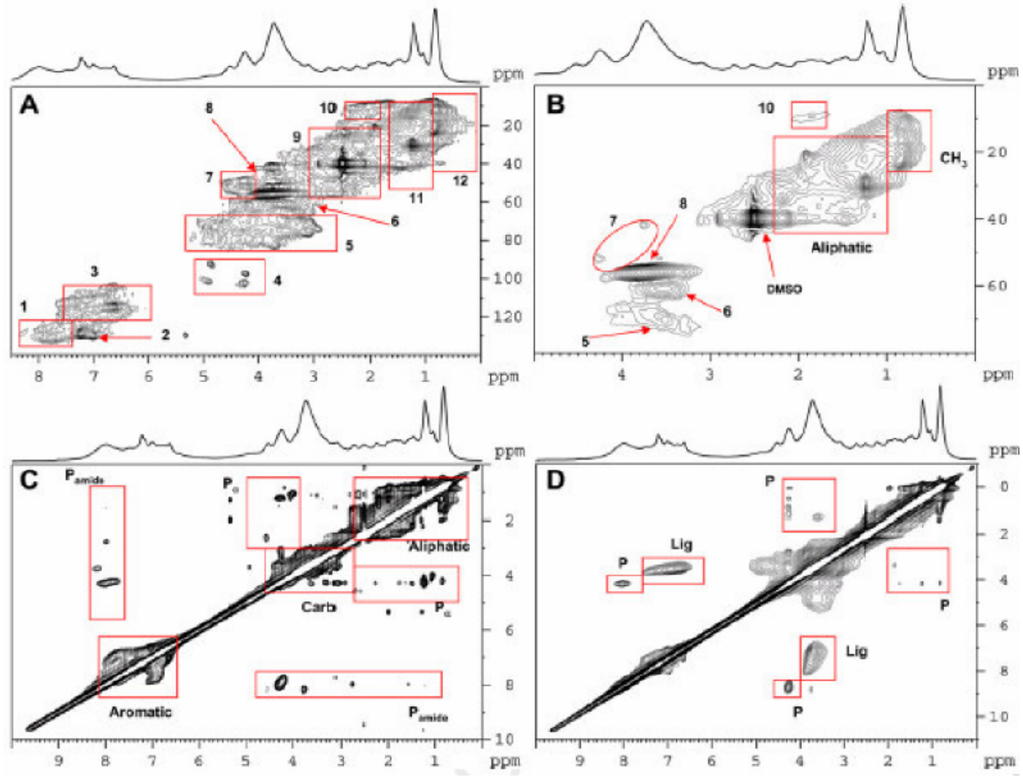

Figure 2. Various 2D NMR spectra of the Ho isolated from the PDS treated with Fertiliser. (A), HMQC Spectrum, main assignments can be summarized as 1, p-hydroxybenzoate aromatics in lignin (Kelleher and Simpson, 2006; Simpson et al., 2004); 2, phenylalanine in peptides (Kelleher and Simpson, 2006; Simpson et al., 2007a) ; 3, aromatic lignin units (Kelleher et al., 2006; Simpson et al., 2004); 4, anomeric protons in carbohydrates (Kelleher and Simpson, 2006; Lam et al., 2007); 5, methine in carbohydrates (Kelleher and Simpson, 2006; Lam et al., 2007); 6, methylene units in carbohydrates (Kelleher and Simpson, 2006; Lam et al., 2007); 7, α-protons in peptides and proteins (Kelleher and Simpson, 2006; Simpson et al., 2007a; Simpson et al., 2007b); 8, methoxyl in lignin (Kelleher and Simpson, 2006; Simpson et al., 2003; Simpson et al., 2004); 9, aliphatic linkages including signals from various lipids and plant cuticles (Deshmukh et al., 2005; Deshmukh et al., 2003; Simpson et al., 2003; Simpson et al., 2007b), and side-chain protons in peptides (Kelleher and Simpson, 2006; Simpson et al., 2007a); 10, *N*-acetyl and/or *O*-acetyl

carbohydrates (Hertkorn et al., 2006; Lam et al., 2007); 11, methylene units in aliphatic chains (Kelleher et al., 2006; Simpson et al., 2001; Simpson et al., 2003); 12, methyl groups, a small contribution in this region will be from terminal CH_3 in lipids, though the majority of signals are from peptides (Kelleher et al., 2006; Simpson et al., 2003; Simpson et al., 2007a). (B), is an expanded region of the HMQC. The intense lignin methoxyl signal is clearly evident in region 8. (C), is the TOCSY spectrum which supports assignments made from the 1D and HMQC spectra. Key assignments: aromatic couplings (Kelleher and Simpson, 2006; Simpson et al., 2004); P_{amide} = amide-$_{afry}$ couplings in peptides (Kelleher and Simpson, 2006; Kingery et al., 2000; Simpson et al., 2007a; Simpson et al., 2007b); P_{a}; $_{a}$-protons coupling to amino acid side chains (Kelleher and Simpson, 2006; Kingery et al., 2000; Simpson et al., 2007a; Simpson et al., 2007b); couplings in carbohydrates (Carb) and aliphatic couplings (Deshmukh et al., 2005; Deshmukh et al., 2003; Kelleher et al., 2006; Simpson et al., 2003). (D), is the NOESY spectrum that confirms the strong contribution of P, peptides/proteins with cross-peaks from $_{a}$-protons in amino acid side chains. The most important assignment is the through space interaction between aromatic rings and methoxyl groups indicative of lignin (Lig) (Simpson, 2001).

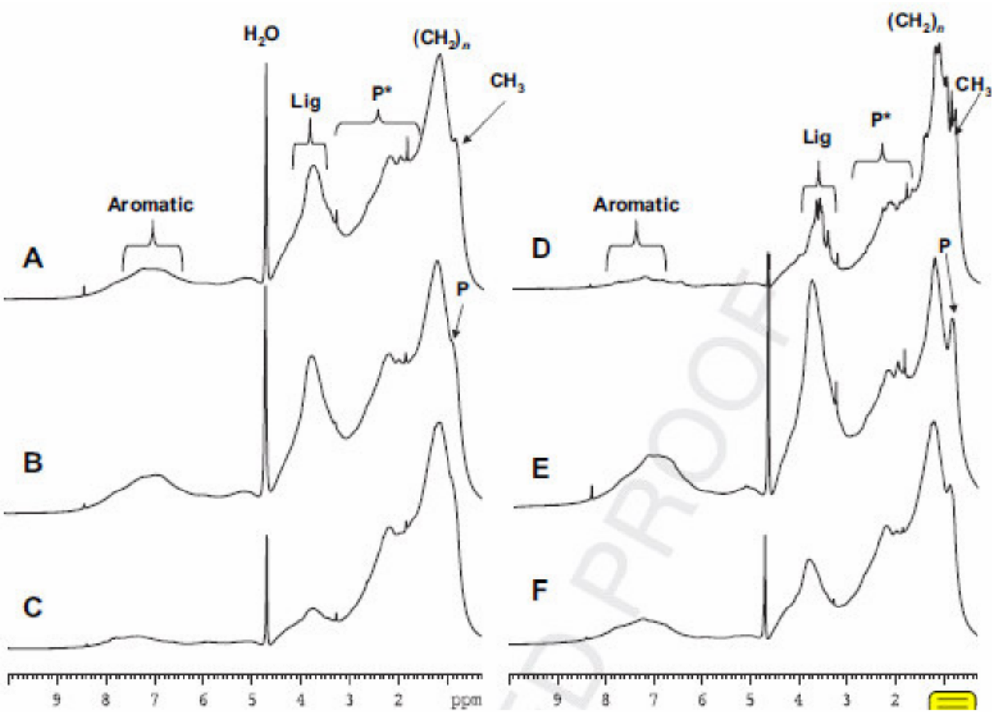

Figure 3. ^{1}H NMR spectra for Ho in D_2O, differing by soil and treatment. (A), WDS Control; (B), WDS Fertiliser; (C), WDS Fertiliser + Urine; (D), PDS Control; (E), PDS Fertiliser; and (F), PDS Fertiliser + Urine. Simple assignments for spectra indicate strong contributions from aromatic functionalities, from P, proteins/peptides; Lig, lignin; Carb, Carbohydrate; $(CH_2)_n$, aliphatic methylene units consistent with aliphatic structures from plant-derived waxes, cuticles and lipids, in addition to contributions from microbial lipids; (CH_3), could be due to methylated amino acid side residues plus contributions from terminal methyl groups from plant-derived residues. P* could contain contributions from other molecules such as Refractory carboxyl-rich alicyclic molecules (CRAM).

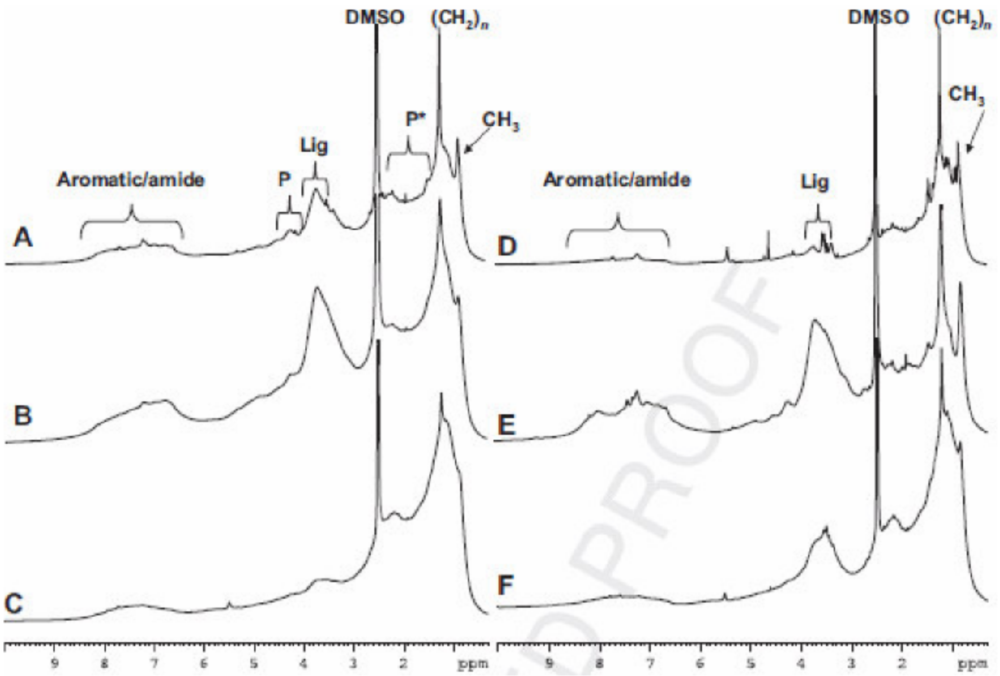

Figure 4. ^{1}H NMR spectra for Ho in DMSO- d_6, differing by soil and treatment. (A), WDS Control; (B), WDS Fertiliser; (C), WDS Fertiliser + Urine; (D), PDS Control; (E), PDS Fertiliser; and (F), PDS Fertiliser + Urine. Assignments are the same as given in Figure 3.

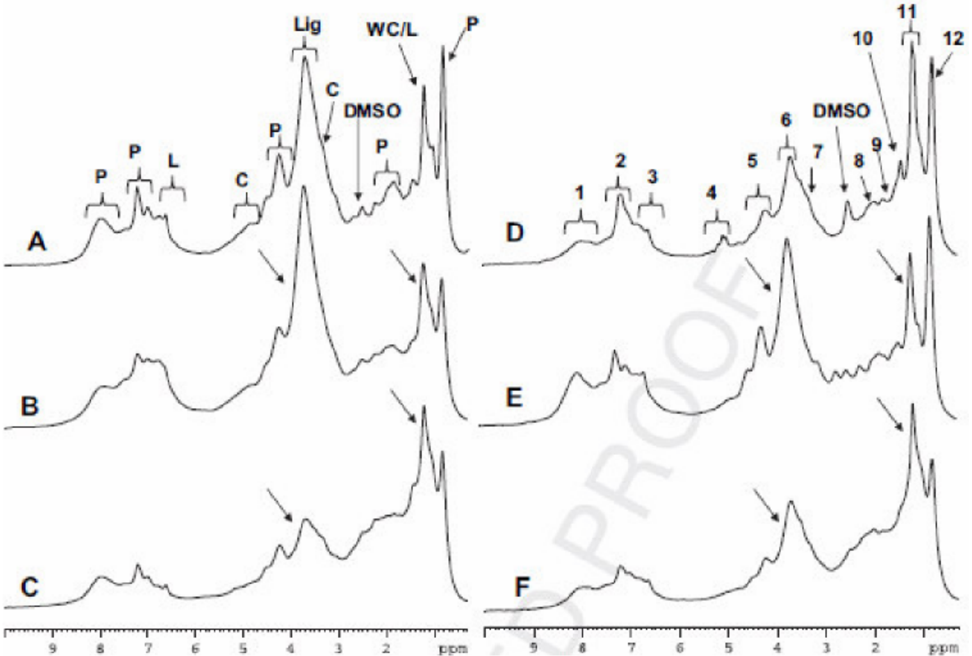

Figure 5. Diffusion edited ^{1}H NMR spectra for Ho in DMSO- d_6, differing by soil

and treatment. (A), WDS Control; (B), WDS Fertiliser; (C), WDS Fertiliser + Urine; (D), PDS Control; (E), PDS Fertiliser; and (F), PDS Fertiliser + Urine. Assignments are the same as shown in Figure 3 in addition to WC/L, which refers to waxes, cutins and/or lipids. More specific assignments shown for spectrum D refer to: 1, amide; 2, phenylalanine; 3, aromatics in lignin; 4, anomeric protons in carbohydrates; 5, á-protons (peptides); 6, methoxyl (lignin); 7, carbohydrate protons; 8, methylene adjacent to a carbonyl; 9, N-acetyl and/or O-acetyl group in peptidoglycan; 10, aliphatic methylene units â to an acid or ester; 11, aliphatic methylene; 12, CH_3. Changes in the relative abundances of Lignin OCH_3 and aliphatic methylene are highlighted by the arrows.

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