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Production and elimination of water extractable organic matter in a calcareous soil as assessed by UV/Vis absorption and fluorescence spectroscopy of its fractions isolated on XAD-8/4 resins

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ABSTRACT

Water extractable organic matter (WEOM) in soil refers to a broad mixture of water-soluble structures recoverable in laboratory through predetermined procedures. In this work, we investigate the mechanisms controlling the occurrence of WEOM fractions isolated on XAD-8/4 resins in a calcareous soil profile as a function of soil depth and seasonal climatic changes. WEOM was roughly collected on a bi-weekly basis during eight consecutive months from an experimental field in the Mediterranean region, south-east of France. Each fraction was analysed for organic carbon content and further characterised using UV/Vis absorbance and 3D fluorescence spectroscopy.

The concentrations of hydrophobic (HPOA), transphilic (TPIA) and hydrophilic (HPIA) acids fractions isolated from WEOM followed similar decreasing trends with increasing soil depth, but HPOA decreased most sharply. Specific UV absorbance at 254 nm ($SUVA_{254}$) indicated that HPOA's distribution along the soil column is strongly influenced by the selective removal of aromatic structures, which could be controlled by adsorption mechanisms involving organo-mineral interactions and/or by a shift towards less aromatic precursors in deeper soil layers. 3D spectra of fluorescence revealed the presence of four types of fluorophores: two humic-like (A and C) common to all fractions, one lignin-like (H) specific to HPOA particularly in the upper soil layer, and two protein-like (B1 and B2) specific to TPIA. The importance of the humic component in all fractions was verified by the highly significant correlations between their carbon content and the intensity of humic-like peaks. The aging index (IA/IC) and the fluorescence index (FI), two indicators derived from 3D fluorescence spectra, showed an increase in the microbial input and the degree of humification in HPOA as a function of increasing soil depth. By comparison, IA/IC and FI values in HPIA and TPIA indicated that both fractions were composed almost exclusively of microbial-derived structures, regardless of soil depth.

Principal component analysis (PCA) showed the association of temperature (T) to the carbon content in all fractions extracted from the soil layers 0–30 and 30–60 cm, suggesting possible role of microbial activity on their occurrence. The hydraulic regime in soil, controlled by the frequency of rainfall events, affected most significantly HPIA across the entire soil column. PCA, however, failed to identify any consistent trends relating the qualitative properties of WEOM fractions to climatic variables.

1. Introduction

Most of soil organic matter (SOM) derives from inherently recalcitrant plant biopolymers and their degradation by-products (Kögel-Knabner, 2002), stabilised through aggregation, ad/sorption onto soil mineral surfaces and occlusion within soil pores (von Lützow et al., 2006). Water extractable organic matter (WEOM) refers to water-soluble structures either free in the soil matrix, physically trapped within soil's various pores or loosely adsorbed onto soil minerals that are recoverable in laboratory through predetermined procedures (Zsolnay, 2003). Although WEOM represents roughly <1% of total SOM, it is thought to play a key role in most biogeochemical processes in soil (Haynes and Donald, 2005) and to contribute significantly into the global terrestrial carbon budget through the transfer and stabilisation of SOM in deeper soil horizons (Kalbitz and Kaiser, 2008; Kindler et al., 2011).

The occurrence of WEOM in soil under temperate conditions was investigated in a number of recent studies in native (Fröberg et al., 2007; Sanderman et al., 2008) and agro-ecosystems (Akagi and Zsolnay, 2008; Cannavo et al., 2004; Embacher et al., 2007). In either system, the composition of WEOM revealed variable contributions of both labile structures originating from recent organic matter input and refractory humic substances, the main constituents of native SOM. The relative contributions of these contrasting sources, however, was found to

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vary remarkably depending on a multitude of intrinsic and extrinsic variables, including soil depth and seasonal climatic changes. Hence, WEOM found in deeper soil layers reflected predominant contributions of refractory native SOM compared to its counterpart in upper layers where fresh organic matter input contributed more significantly (Fröberg et al., 2007; Sanderman et al., 2008; Toosi et al., 2012). On the other hand, despite the clear seasonality of certain hydrosoluble structures in soil, such as carbohydrates and lignin-derived phenols (Hishi et al., 2004; Kalbitz and Kaiser, 2008), no consistent trends associating WEOM to seasonal climatic changes were identified. This was attributed to the low contribution of seasonally-produced labile structures to the overall composition of WEOM compared to refractory humic substances, known to have a temporal dynamics perceptible in average on decadal basis, and to the rapid elimination of these structures from the soil solution by biotic and abiotic means (Akagi and Zsolnay, 2008; De Troyer et al., 2011; Embacher et al., 2007).

The inherent structural heterogeneity of WEOM reflects the diversity of its precursors, the multitude of its production pathways and the factors affecting its transformation in soil. In order to investigate its dynamics and ecological functions in soil, different fractionation procedures had been applied to isolate distinguishable categories based on physical, chemical or biological criteria. Fractionation on weakly polar XAD-8 resins, originally developed in aquatic humic chemistry (e.g. Aiken et al., 1979, 1992; Malcolm and MacCarthy, 1992), is among the most common of these procedures. This approach had been reported to isolate the major part of organic matter in a soil solution, separated into arbitrary hydrophobic and hydrophilic pools (De Troyer et al., 2011; Hunt et al., 2007; Yano et al., 2005). Through integrating hydrophobic XAD-4 resin into the experimental set-up, it was possible to isolate an additional fraction termed by Croué et al. (2003) as "transphilic" *i.e.* of transitional polarity between hydrophobic and hydrophilic fractions. Recently, Hayes et al. (2008) showed that XAD-4 resin isolates structures from WEOM that differ profoundly from operationally-defined fulvic and humic acids in being highly oxidised, significantly enriched in amino acids and carbohydrates, and exhibit structural features suggesting predominantly microbial origins. Esteves et al. (2007), on the other hand, found that structures isolated on XAD-4 resins from different aquatic environments were remarkably enriched in ^{13}C isotopes and hypothesised that such structures are either resulting from selective preservation of recalcitrant biopolymers or from the recondensation of highly degraded simple aliphatic monomers. These structural differences between fractions isolated on XAD-type resins were found closely linked to fundamental functions of WEOM in soil including supporting heterotrophic biological activity (Jandl and Sollins, 1997), interacting with contaminants (Croue et al., 2003) and carbon stabilisation in soil (Guo and Chorover, 2003; Yano et al., 2005).

The advantages of studying WEOM using UV/Vis absorbance and fluorescence spectroscopy are increasingly recognised. Besides their easy operation and rapidity permitting the acquisition of large quantities of data necessary for spatiotemporal investigations, both techniques are low sample demanding and highly sensitive to environmentally relevant low concentrations. UV/Vis absorbance spectra are generally featureless that can serve to extract qualitative data using one or more preselected wavelengths (e.g. Embacher et al., 2007; Jaffrain et al., 2007). Furthermore, changes in the absorbance properties had been successfully used as a tracer of WEOM's functional modifications during adsorption (Guo and Chorover, 2003; Mikutta et al., 2007) and biodegradation studies (De Troyer et al., 2011). Multidimensional fluorescence analysis, on the other hand, is a more comprehensive technique that permits to directly identify the dominant structural classes in a sample, without further signal processing (Chen et al., 2003; Sierra et al., 2005). Moreover, a number of easily determined fluorescence indicators were used to collect precious information on the degree of alteration of WEOM (Corvasce et al., 2006; Zsolnay, 2003) and its potential precursors (Jaffé et al., 2008). Combining data collected from both techniques is therefore considered as a complementary approach that allows their mutual consolidation (He et al., 2009; Jaffé et al., 2008).

In our previous work (Hassouna et al., 2010), we argued that changes in WEOM along the soil column are associated to a shift in its precursors from plant-derived towards microbial-derived and to the influence of organo-mineral interactions dominated by the presence of calcite and the high alkalinity of the soil solution. Our objectives in the present work are (1) to investigate the processes controlling the production and elimination of WEOM in the soil column through the analysis of the optical properties of its subsequent fractions isolated on XAD-type resins, and (2) to determine the effect of seasonal climatic changes on the occurrence of these fractions in each soil layer. Our approach is based on the analysis of organic carbon content and the optical properties of each fraction using UV/Vis absorbance and 3D fluorescence spectroscopy.

2. Materials and methods

Briefly, soil samples were collected on bi-weekly basis during seven consecutive months (October 2002–May 2003) from a field located in the Mediterranean region in the south-east of France. The soil type in the sampling site is fluvic hypercalcaric cambisol (FAO, 2006). Further details about the study site, the main physical and chemical properties of soil, the climatic variables during the sampling period, the sampling strategy, and the procedures applied for WEOM extraction and fractionation on XAD-8/4 resins were presented in Hassouna et al. (2010). We would like to notify that two sets of samples presented in Hassouna et al. (2010) (sampling dates at 28/2 and 24/3) were excluded from the final data processing in the present work due to anomalies in their spectra of fluorescence suggesting possible contamination during the fractionation procedure. As we finally compared the general trends of the whole set of samples of each fraction at each depth ($n = 16$), we assume that the impact of the elimination of these two samples on the overall trends and consequently on our arguments to be negligible.

Prior to chemical and spectroscopic measurements, hydrophobic (HPOA) and transphilic (TPIA) acids fractions were acidified to pH values ~2.0 directly after desorption. All samples were then sparged with CO_2-free N_2 during 10 min in order to eliminate all inorganic carbon traces. All measurements were performed within 24 h after fractionation according to conventional procedures (more details in Hassouna et al., 2010).

UV/Vis absorbance and 3D fluorescence spectra were acquired in 1 cm path-length quartz cells respectively using an Agilent-8453 (Agilent instruments, France) and Perkin-Elmer LS-55 total luminescence spectrometer equipped with an Ozone-free Xenon lamp (150 wt.). All spectroscopic measurements were performed in undiluted samples (DOC$<$17.0 mg l^{-1}).

Specific UV absorbance at 254 nm ($SUVA_{254}$) (l mg^{-1} C m^{-1}) was calculated by normalizing the absorbance at this wavelength by the concentration in organic carbon (mg C l^{-1}) of the corresponding sample. Besides peak identification, 3D fluorescence spectra served to determine (1) the fluorescence index (FI), which corresponds to the ratio of fluorescence intensities at emission wavelengths 450 and 500 nm for an excitation at 370 nm (McKnight et al., 2001) and (2) the aging index, which corresponds to the ratio between the maximum of fluorescence intensities of humic-like peaks A and C (IA/IC) (Fig. 5) (Sierra et al., 2005).

For PCA, we considered seven variables including the concentrations of each fraction, $SUVA_{254}$, IA/IC, FI, rainfall (P), water-filled pore spaces (WFPS) and average daily temperatures (T). The PCs were extracted according to the principal or main axis method where only factors explaining $>$10% of the total variance and Eigenvalues $>$1.0 were considered significant.