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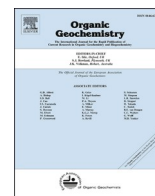
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## Effects of digestate application, winter crop species and development on dissolved organic matter composition along the soil profile

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### ABSTRACT

Applying organic waste products (OWPs) and sowing cover crops are agronomic practices to improve soil health. OWPs can be used in anaerobic digestion. Because microorganisms consume some of the labile molecules, persistent molecules accumulate in digestate. Few studies have investigated the transfer of dissolved organic matter (DOM) in soil that received digestate. Previously, effects of digestate application on dissolved organic carbon (DOC) were compared to those of the original pig slurry under wheat and a mustard catch crop for nine years at a lysimeter experimental site. DOC concentrations after digestate application were higher in the topsoil every year, due to crop development, but did not differ between treatments in the subsoil. The objectives of this study were to determine whether the observed differences in DOC concentrations caused DOM composition to differ, to identify sources (e.g., digestate, root exudation) that may have contributed to the DOM pool and to assess the DOM composition in the subsoil. The DOM composition of lysimeter samples and water extracts from the OWPs applied were analyzed by thermochemolysis coupled with gas chromatography and mass spectrometry, which identified plant- and microbial-derived biomarkers. Under mustard, the DOM pool seemed to contain mainly persistent molecules from digestate that were desorbed due to the increase in pH caused by nitrate uptake. Under wheat, the DOC pool seemed supplied by both digestate and root exudation. After applying digestate, plant-derived molecules decreased, while microbial-derived molecules increased, as depth increased, and molecules may have been sorbed from the topsoil to subsoil.

### 1. Introduction

Dissolved organic matter (DOM) is the most mobile and bioavailable fraction of soil organic matter. It is a source of energy for microorganisms, and its quantity and composition strongly influence soil microbial communities (Eiler et al., 2003; Judd et al., 2006) that are essential for many processes, such as mineralization and nitrification. DOM is composed of various organic elements (e.g. oxygen, nitrogen, phosphorus) and dissolved organic carbon (DOC) (Bolan et al., 2011). It can be transferred and stabilized in the subsoil by interaction with the mineral phase, which can lead to carbon (C) sequestration (Rumpel and Kögel-Knabner, 2011). However, there is also a risk of co-transporting contaminants to groundwater (e.g. heavy metals, pesticides, veterinary antibiotics) by forming complexes with DOM (Weng et al., 2002; Bolan et al., 2011; Gbadegesin et al., 2022), in which the contaminants are

protected from degradation and are spread more easily in the environment, threatening the health of aquatic ecosystems (Dunnivant et al., 1992).

Some farming practices can improve soil health and provide DOM to the soil, such as application of organic waste products. Anaerobic digestion of these products is now common and has many advantages, such as reducing inorganic fertilizer applications, odors, pathogens and greenhouse gas emissions and, most importantly, producing biogas (Holm-Nielsen et al., 2009). Biogas is considered a form of renewable energy, and the European Union encourages its production. Biogas digestate, a by-product of this process that contains DOM, is increasingly produced and recycled on agricultural land (Scarlat et al., 2018). The chemical composition of digestate differs from that of its source material, since microorganisms consume some of its labile molecules during anaerobic digestion, which results in a higher content of chemically

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persistent molecules, such as lignin and non-hydrolysable lipids, and more biological stability than that of the original product (Marcato et al., 2009; Tambone et al., 2009; Häfner et al., 2022). In soil, lignin can be protected from mineralization when bound to mineral surfaces or in aggregates (Angst et al., 2021). Digestate also contains products of degradation, such as carboxylic acids produced during the acidogenesis stage of anaerobic digestion (Liebetrau et al., 2017). Chantigny (2003) highlighted an increase in soil-soluble organic C after application of livestock products, followed by rapid decomposition. However, due to the composition of digestate, the organic matter it provides may persist longer in the soil. Some studies also highlighted auxin-like properties in the DOM or humic-like fraction of some digestates that could bio-stimulate crops (Ertani et al., 2013; Scaglia et al., 2017). Digestates usually have higher concentrations of auxin-like molecules than their original products do (Li et al., 2016), due to microbial transformation of certain aromatic amino acids, such as tryptophan, into auxin during anaerobic digestion (Scaglia et al., 2015). These molecules can be absorbed by plant roots and can promote root development, plant growth or resistance to environmental stresses (Wong et al., 2020; Wu and Dong, 2020). Auxin's effects depend, however, on the distribution of total concentration between endogenous auxin and auxin-like activity from soil and applied products (Pantoja-Guerra et al., 2023). Root development, for example, is promoted at low concentrations of auxin (Li et al., 2016).

The sowing of winter cover crops has many benefits, such as increasing the soil organic matter content, conserving inorganic nitrogen (N) and preventing soil erosion and weed development (Hartwig and Ammon, 2002). Crops can provide DOM via degradation of residues (i.e., roots, leaves, stems) and root exudation (Kalbitz et al., 2000) of low-molecular-weight compounds (Nguyen, 2009). Roots can release carbohydrates (CAR) and organic acids (e.g., lignin-derived, dicarboxylic acids (DA), fatty acids (FA)) by exudation (Vives-Peris et al., 2020). In annual plants, 30–60% of the C fixed by photosynthesis is translocated to the roots, and up to 70% of it can be released into the soil (Neumann and Römheld, 2000). Premrov et al. (2012) measured higher DOC concentration under mustard ( $1.78 \text{ mg L}^{-1}$ ) than under no crop cover ( $1.35 \text{ mg L}^{-1}$ ), Jahangir et al. (2014) highlighted the same trend ( $1.53$  and  $0.90 \text{ mg L}^{-1}$  under mustard and no crop cover, respectively). Lu et al. (2004) and Ge et al. (2015) observed a correlation between root biomass and DOC concentrations. Furthermore, nitrate absorption during crop development tends to basify the rhizosphere (Bloom, 1996; Hinsinger et al., 2003), which can increase the solubility of soil organic matter (Andersson et al., 2000).

Few studies have investigated the transfer of DOM or DOC in the soil after digestate application in the presence of winter crops. In a previous lysimeter study conducted over nine years (Didelot et al., 2025), effects on DOC concentrations and fluxes of digestate application were compared to those of application of the original pig slurry, in association with different winter crops. On a specific date during the drainage season, early in the season for mustard and mid-season for winter wheat, higher topsoil DOC concentrations were observed each year for the digestate treatment, perhaps due to crop root development. The additional DOC measured in the lysimeters 8–9 months after digestate application could have been due to (i) solubilization of the sorbed persistent organic matter from digestate after nutrient uptake by the roots and/or (ii) production of more root exudates through a potential auxin-like effect of the digestate.

In cropland soils, DOM is transferred vertically toward the subsoil. During this transfer, DOC concentrations decrease, for example, from  $16 \text{ mg L}^{-1}$  in the topsoil to  $4.5 \text{ mg L}^{-1}$  in the subsoil (Herbrich et al., 2017). In the previous lysimeter study, similar transfer dynamics were observed (from  $14.4 \pm 6.8 \text{ mg L}^{-1}$  in the topsoil to  $5.0 \pm 7.4 \text{ mg L}^{-1}$  in the subsoil), but DOC concentrations did not differ between digestate and pig slurry treatments in the subsoil, despite differing in the topsoil (Didelot et al., 2025). Most terrestrial DOM is mineralized or retained in the soil, and interaction with particulate amorphous iron and aluminum oxides

was reported as the main stabilization mechanism (Rumpel and Kögel-Knabner, 2011). Kaiser and Kalbitz (2012) developed a conceptual model that explained DOM transfer toward the subsoil, with the topsoil DOM composition having high concentrations of plant-derived molecules (e.g. lignin- and tannin-derived molecules (LT)) and an increase in microbial-derived molecules (e.g. CAR) as depth increased. The lack of difference in DOC concentration between treatments in the subsoil may have been due to mineralization or preferential sorption on the mineral phase between the two depths of the molecules transferred under the digestate treatment.

To test hypotheses about DOM sources, either persistent organic matter from digestate or root exudates, and transfer to the subsoil, undergoing mineralization or sorption on the mineral phase, the present study compared dynamics of the chemical composition of the DOM collected by lysimeters (i) between two treatments (pig slurry and its digestate) in the topsoil at different times during the drainage season, (ii) between two winter crops in the topsoil and (iii) between the topsoil and subsoil. Overall, the objectives of this study were to (i) determine whether the observed differences in DOC concentrations caused DOM composition to differ, (ii) identify sources that may have contributed to the DOM pool and (iii) assess the composition of DOM transferred to the subsoil. To achieve those objectives, this field study is based on the analysis of chemical composition of DOM collected in lysimeters. DOM composition was analyzed using thermochemolysis coupled with gas chromatography and mass spectrometry (THM-GC-MS). This method enables biomarkers to be identified that can provide information about the sources of the DOM pool, including plant-derived molecules (e.g., LT, high-molecular-weight FA), microbial-derived molecules (e.g., low- and medium-molecular-weight FA), as well as CAR and DA, which can come from both sources. The composition of the DOM collected by lysimeters was compared to that of the water-extractable organic matter (WEOM) of the product applied.

## 2. Materials and methods

### 2.1. Study site and soil description

The long-term experimental site, EFELE, forms part of a French observatory for environmental research on organic waste products (SOERE PRO) (described in details by Morvan et al. (2020)). EFELE is a cropland located in western France and is used to compare effects of applying pig slurry or digestate derived from pig slurry on soil and water quality. The digestate was collected from a  $20 \text{ m}^3$  mesophilic ( $38 \text{ }^\circ\text{C}$ ) tank reactor after a retention time of 20 days.

The site's soil is classified as a luvisol-redoxisol and derived from aeolian silt deposited on schist material. The main soil properties from 0–30 cm deep at the beginning of the experiment were 14.2% clay, 71.0% silt, 14.8% sand, 1.1% organic C, a C:N ratio of 9.6 and a slightly acidic pH of 6.1.

### 2.2. Crop rotation and development

The crop rotation consisted of a 2-year succession of maize (*Zea mays*) and winter wheat (*Triticum aestivum*) with a N catch crop of white mustard (*Sinapis alba*). The mustard was sown in September, and two months after (in November, when the drainage season usually begins) the mustard was in the vegetative growth phase (growth of leaves, stems and roots). As a frost-sensitive species, it tended to deteriorate at the end of the drainage season and was chopped up in April. The wheat was sown in November, developed during the drainage season and was harvested in July. The treatments (pig slurry and digestate) were applied in April to the growing wheat or before the sowing of maize (doses are presented in Supplementary Table S1).

### 2.3. Climate data

Climate data were obtained using an automatic weather station (CR1000, Campbell Scientific, Utah, USA) at the study site (Supplementary Table S2). The climate is mild oceanic temperate, with a mild winter and warm summer. From 2014 to 2022, the mean annual temperature, rainfall and Penman potential evapotranspiration were 12.8 °C, 681 mm and 731 mm, respectively.

### 2.4. Sampling of drained water

There were two different plots, one was treated with pig slurry and the other one with digestate. On each plot, four fiberglass-wick lysimeters were installed: two at 40 cm depth (topsoil) and two at 90 cm depth (subsoil) (Supplementary Fig. S1). In each lysimeter (area: 0.125 m<sup>2</sup>), the wick is unfolded onto a stainless steel plate, passes through a hole in the center of the plate and is connected to a receiving flask by a Tygon® tube.

### 2.5. Sample collection and analysis

Drainage water was sampled promptly after the end of each drainage event, and ca. 40 mL of each sample were filtered at 0.45 µm and stored at 4 °C until analysis. DOC concentrations were determined using a TOC-V<sub>CSH</sub> analyzer (Shimadzu Corp., Kyoto, Japan) using thermal oxidation. Dynamics of DOC concentrations of seasons 2018–2019 (wheat) and 2020–2021 (mustard) (Fig. 1) were studied by Didelot et al. (2025). There was a specific pattern of DOC concentration that was observed every year (four drainage seasons for mustard and four drainage seasons for wheat), with significantly higher DOC concentrations for digestate from the beginning of the drainage season under mustard and in the middle of the season under wheat. The 2018–2019 and 2020–2021 seasons were representative of the other seasons.

We selected two dates when the DOC concentrations diverged (i.e., significantly higher for the digestate treatment than for the pig slurry treatment) (14 Feb 2019 and 24 Nov 2020) (hereafter, *DOC-div* dates) and two dates when the concentrations converged (i.e., no difference

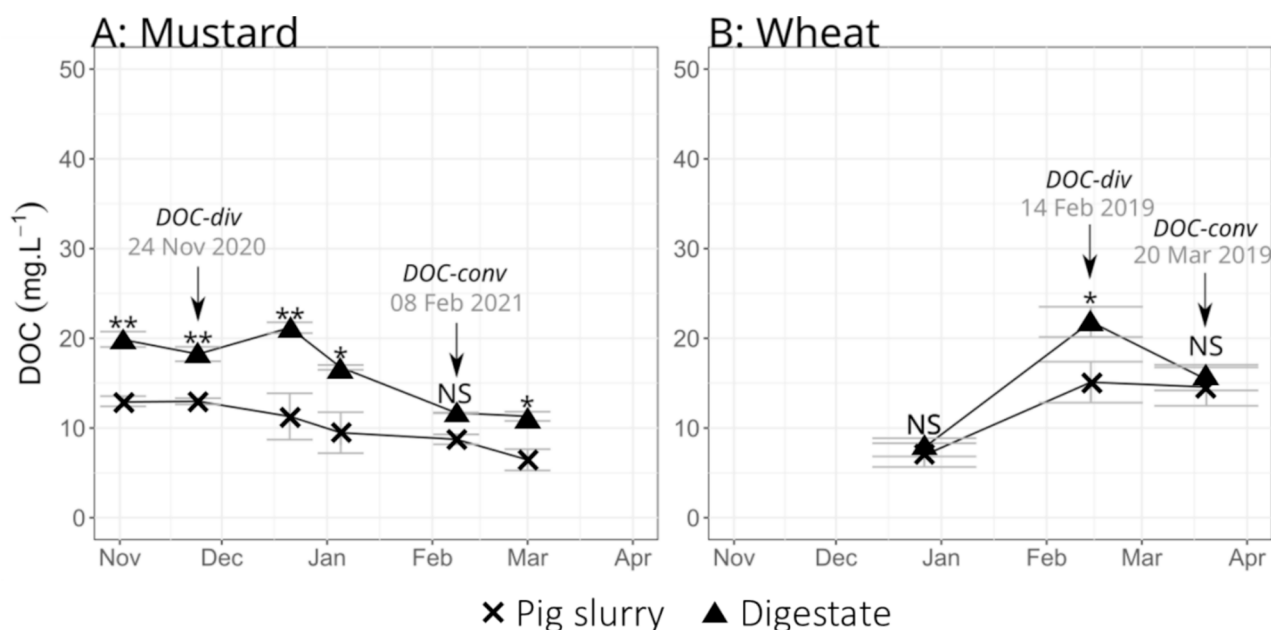
between treatments) (20 Mar 2019 and 08 Feb 2021) (hereafter, *DOC-conv* dates). For the 2020–2021 season, the second drainage event was selected instead of the first event in order to analyze a stabilized DOM composition after the beginning of the drainage season. Samples from the subsoil for the drainage event on 08 Feb 2021 were also analyzed. Approximately 200 mL of each sample collected on these dates were filtered through 0.7 µm fiberglass filters, frozen for 2 days and freeze-dried for 1 week. The lyophilisates were then stored at room temperature in amber-colored flasks until analysis by THM-GC-MS.

### 2.6. Extraction of WEOM from organic waste products

The DOM composition from the lysimeter samples was compared to the WEOM composition of the products applied in 2019, since the applied products were collected from the same farm each year. The organic matter of pig slurry and digestate was extracted using a product: water ratio of 1:5. After agitation at room temperature on an orbital shaker (Stuart SSL1) at 50 rpm for 3 h, the mixture was decanted overnight. The supernatant was then filtered through 0.7 µm fiberglass filters, frozen, freeze-dried and analyzed by THM-GC-MS.

### 2.7. Analysis of DOM composition

For each sample, approximately 2 mg of lyophilisate were introduced into an 80 µL aluminum reactor with an excess of solid tetramethylammonium hydroxide (ca. 3 mg) and 10 µL of a solution of dihydrocinnamic acid d9 (CDN Isotopes, ref. D5666) diluted at 25 µg mL<sup>-1</sup> in methanol as an internal standard. The THM reaction was performed online using a vertical micro-furnace pyrolyser PZ-3030 (Frontier Laboratories, Japan) operating at 400 °C. The products of this reaction were injected into a gas chromatograph GC8860 (Agilent, California USA) equipped with a SLB 5MS capillary column in the split mode (60 m × 0.25 mm ID, 0.25 µm film thickness). The temperature of the transfer line was 321 °C and the temperature of the injection port was 310 °C. The oven was programmed to maintain an initial temperature of 50 °C for 2 min, following an increase to 150 °C at 15 ° min<sup>-1</sup>, a subsequent increase to 310 °C at 3 ° min<sup>-1</sup> where it remained for 14 min. Helium was



**Fig. 1.** Dynamics of dissolved organic carbon (DOC) concentrations (mg L<sup>-1</sup>) (mean of two lysimeter replicates) in topsoil (40 cm) water under a (A) mustard catch crop or (B) winter wheat for two treatments: pig slurry or its digestate. Error bars are average deviations. The treatment effect was tested for each date using ANOVA. Significance: \*\* p < 0.01; \* p < 0.05; NS p > 0.1. *DOC-div* refers to dates when DOC concentrations diverged (higher concentrations for digestate than for pig slurry), *DOC-conv* refers to dates when DOC concentrations converged (no differences between treatments). These dates (arrows) were analyzed by thermochemolysis coupled with gas chromatography and mass spectrometry.

used as the carrier gas, with a flow rate of 1.0 mL min<sup>-1</sup>. Compounds were detected using a mass spectrometer MS5977B (Agilent, California USA) operating in the full scan mode. The temperature of the transfer line was set at 280 °C, the ionization source at 200 °C, and molecules were ionized by electron impact using an energy of 70 eV. The molecules in the samples' DOM and the applied product's WEOM were identified based on their full-scan mass spectra by comparison with the National Institute of Science and Technology (NIST) library and with published data (Nierop and Verstraten, 2004; Nierop et al., 2005; Jeanneau et al., 2014). The analytical uncertainty for this analytical method, expressed as a relative standard deviation, ranged from 10 to 20% depending on the target compounds.

Overall, 46 target molecules were classified into four chemical families: 3 low-molecular-weight DA, 15 FA with 8–22 C atoms (higher-molecular-weight FA were too large to pass through the filter), 23 LT and 5 desoxy aldonic acids as markers of CAR. The molecules analyzed, the *m/z* ratios of their characteristic ions and the mass spectral factor varied (Supplementary Table S3). DA can come from multiple sources, such as plant residues, root exudates and microbial metabolites (Adeleke et al., 2017; Sokolova, 2020). FA can be distinguished, based on the length of their aliphatic chain, into low- and medium-molecular-weight FA (LMWFA and MMWFA, respectively), which are markers of microbial degradation and structure, respectively (Frostegård et al., 1993), except for hexadecanoic and octadecanoic acids, which are ubiquitous, and high-molecular-weight FA (HMWFA), which have a chain of at least 19 C atoms and are plant-derived (Matsuda and Koyama, 1977). LT are plant markers and their proportion might be overestimated in samples due to the conditions caused by tetramethylammonium hydroxide in this method of analysis (Garcette-Lepecq et al., 2001). The use of THM-GC-MS does not enable distinction between lignin- and tannin-derived molecules, since the thermochemolysis with tetramethylammonium hydroxide of lignins and tannins leads to a variety of methylated phenols (Nakagawa-izumi et al., 2004; Kuroda and Nakagawa-izumi, 2006), which can be derived from both lignins and tannins. The source of CAR can be determined by calculating the *deoxyC6:C5* ratio to distinguish plant-derived CAR (ratio < 0.5) and microbial-derived CAR (ratio > 2) (Rumpel and Dignac, 2006). For each molecule, the peak area of the selected *m/z* ratio was integrated and corrected by a mass spectral factor calculated as the reciprocal of the proportion of the fragment used for the integration, related to the entire mass spectrum provided by the NIST library. The relative proportion of each chemical family (i.e., %DA, %LT, %CAR and %FA) was calculated by dividing the area for its molecules by the sum of the peak areas of all molecules analyzed and multiplying it by 100.

To determine the degree of lignin degradation, the C:V and Al:Ac<sub>V</sub> ratios were calculated, with higher ratios corresponding to less degraded lignin (Ertel and Hedges, 1984; Opsahl and Benner, 1995). The Al:Ac<sub>V</sub> ratio was calculated instead of the more commonly used Ac:Al<sub>V</sub> ratio because it evolves in the same way as the C:V ratio. The C:V ratio was calculated as follows:

$$\frac{\text{area of unit C}}{(\text{area of unit V aldehyde}) + (\text{area of unit V ketone}) + (\text{area of unit V acid})} \quad (1)$$

The details regarding units C and V are indicated in Supplementary Table S3.

The Al:Ac<sub>V</sub> ratio was calculated as follows:

$$\frac{\text{area of unit V aldehyde}}{\text{area of unit V acid}} \quad (2)$$

The percentage of microbial markers (*f<sub>mic</sub>*) equaled the sum of the percentages of LMWFA, MMWFA and microbial-derived CAR (described by Jeanneau et al. (2015)). The percentage of plant-derived markers (*f<sub>plant</sub>*) equaled the sum of the percentages of LT, plant-derived CAR and HMWFA. Hexadecanoic acid, octadecanoic acid and DA were excluded

because they are ubiquitous.

## 2.8. Statistical analysis

Lysimeter data were calculated as the mean ± average deviation of two replicates (for each treatment and depth, Supplementary Fig. S1). Effects of treatment, crop and depth on %DA, %LT, %CAR and %FA were tested by analysis of variance (ANOVA) using R software (R Core Team, 2023). These effects on C:V and Al:Ac<sub>V</sub> ratios, as well as on *f<sub>mic</sub>* and *f<sub>plant</sub>*, were also tested by ANOVA. The assumptions of ANOVA were checked using the Shapiro-Wilk and Bartlett's tests. Differences were considered significant at *p* < 0.05, and means were compared using Tukey's range test. When the parametric conditions could not be met, Kruskal-Wallis (for 1 factor), ANOVA by permutation (for 2 factors) and Dunn's tests were performed instead. To increase the power of the tests, 10 artificial replicates were created using the Excel function RANDBETWEEN (as described by Morelle et al. (2023)) to randomly draw values in a range of ±20% (i.e., the uncertainty in THM-GC-MS measurements) around the value of the sample for each of the molecules considered.

## 3. Results

### 3.1. Chemical composition of WEOM from organic waste products

The 46 molecules identified in the WEOM from pig slurry and digestate by THM-GC-MS were classified as FA, CAR, LT or DA. The WEOM from pig slurry had a high %FA (48.8% of the analyzed compounds), with a predominance of LMWFA and MMWFA (47.6%) compared to HMWFA (1.2%). It also had a high %LT (38.6%) and low %DA and %CAR (9.9% and 2.7%, respectively). The WEOM from digestate was dominated by LT (60.4%) and contained a moderate %DA (17.7%) and low %CAR (1.7%) (Fig. 2). The %FA was 20.1% (i.e., 18.4% LMWFA and MMWFA and 1.7% HMWFA). Overall, *f<sub>plant</sub>* was higher in the WEOM from digestate (89.7%) than in that from pig slurry (59.8%). The *f<sub>plant</sub>* in WEOM from digestate was influenced mainly by the high %LT (60.4%). Overall, *f<sub>mic</sub>* was higher in the WEOM from pig slurry (40.2%) than in that from digestate (10.3%). Whatever the treatment, *f<sub>plant</sub>* predominated.

### 3.2. Dynamics of DOM composition during the drainage season and effects of treatment in the topsoil

Under mustard, on *DOC-div* dates (i.e., higher DOC concentrations for the digestate treatment) (Fig. 1), the DOM composition for the digestate treatment (%CAR: 5.1 ± 1.6% of the analyzed compounds, %FA: 17.9 ± 0.03%, %DA: 22.7 ± 1.6%, %LT: 54.3 ± 0.05%) differed significantly from that for the pig slurry treatment (%CAR: 3.4 ± 1.8%, %FA: 32.5 ± 3.5%, %DA: 16.0 ± 1.6%, %LT: 45.5 ± 3.6%) (Fig. 3A, Table 1). The *f<sub>mic</sub>* was higher for the pig slurry treatment (31.5 ± 2.3%), while *f<sub>plant</sub>* was higher for the digestate treatment (82.6 ± 2.0%) (Table 2). The C:V ratio was higher for the pig slurry treatment (0.06 ± 0.005), and the Al:Ac<sub>V</sub> ratio did not differ between the pig slurry (0.15 ± 0.01) and digestate (0.15 ± 0.001) treatments (Supplementary Table S4). Under mustard, on *DOC-conv* dates (i.e., no difference in DOC concentration between treatments), compared to the *DOC-div* dates, %DA (21.8 ± 5.8%) and %LT (50.9 ± 0.08%) increased for the pig slurry treatment and was constant for the digestate treatment (Fig. 3B, Table 1). The %CAR increased for the pig slurry treatment (5.3 ± 1.6%) and decreased for the digestate treatment (3.0 ± 0.03%). The %FA decreased for the pig slurry treatment (22.0 ± 4.3%) and was constant for the digestate treatment. The *f<sub>plant</sub>* increased for both treatments (mean of 84.2 ± 2.1%) (Table 2). The C:V ratio increased slightly for the digestate treatment (0.08 ± 0.01), and the Al:Ac<sub>V</sub> ratio decreased for the pig slurry (0.09 ± 0.01) and digestate (0.08 ± 0.002) treatments.

Under wheat, on *DOC-div* dates, %DA was significantly higher for the digestate treatment (34.5 ± 7.5% of the analyzed compounds) than for

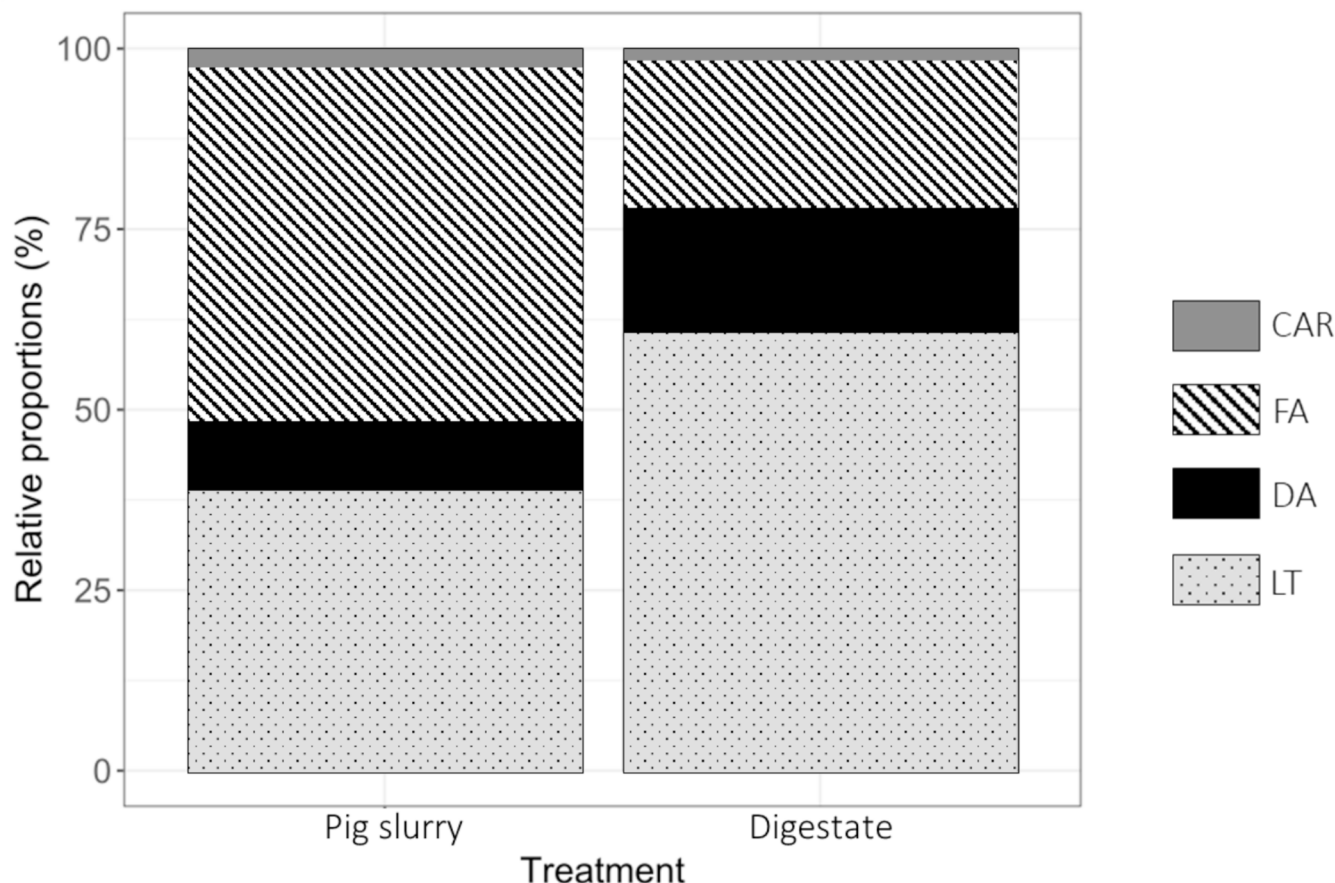


Fig. 2. Relative proportions of four chemical families of molecules in dissolved organic matter of water extracts of pig slurry and its digestate: carbohydrates (CAR), fatty acids (FA), dicarboxylic acids (DA) and lignin-and tannin-derived molecules (LT).

the pig slurry treatment (Fig. 3C, Table 1). The mean %LT ( $40.4 \pm 0.5\%$ ) and %CAR ( $2.1 \pm 0.2\%$ ) did not differ between the two treatments. The pig slurry treatment had a higher %FA ( $28.9 \pm 1.0\%$ ). The  $f_{mic}$  was higher for the pig slurry treatment ( $28.9 \pm 3.2\%$ ), while  $f_{plant}$  was higher for the digestate treatment ( $76.2 \pm 2.7\%$ ) (Table 2). The C:V and Al:Ac<sub>V</sub> ratios were higher for the pig slurry treatment ( $0.23 \pm 0.1$  and  $0.17 \pm 0.02$ , respectively), indicating less degraded lignin. On *DOC-conv* dates, compared to the *DOC-div* dates, %DA increased for the pig slurry treatment (mean of  $35.9 \pm 2.4\%$ ), and was constant for the digestate treatment (Fig. 3D, Table 1). The %LT was constant for the digestate treatment and decreased for the pig slurry treatment ( $32.5 \pm 5.8\%$ ). The %CAR increased for the pig slurry treatment ( $3.7 \pm 1.2\%$ ) and was constant for the digestate treatment, while %FA was constant for both treatments. The  $f_{plant}$  decreased for the pig slurry ( $60.2 \pm 13.2\%$ ) and digestate ( $73.9 \pm 2.5\%$ ) treatments (Table 2). The C:V ratio decreased for both the pig slurry and digestate treatments ( $0.11 \pm 0.06$  and  $0.07 \pm 0.004$ , respectively), and the Al:Ac<sub>V</sub> ratio increased for the pig slurry treatment ( $0.25 \pm 0.03$ ).

### 3.3. Comparison of DOM composition in the topsoil between wheat and mustard

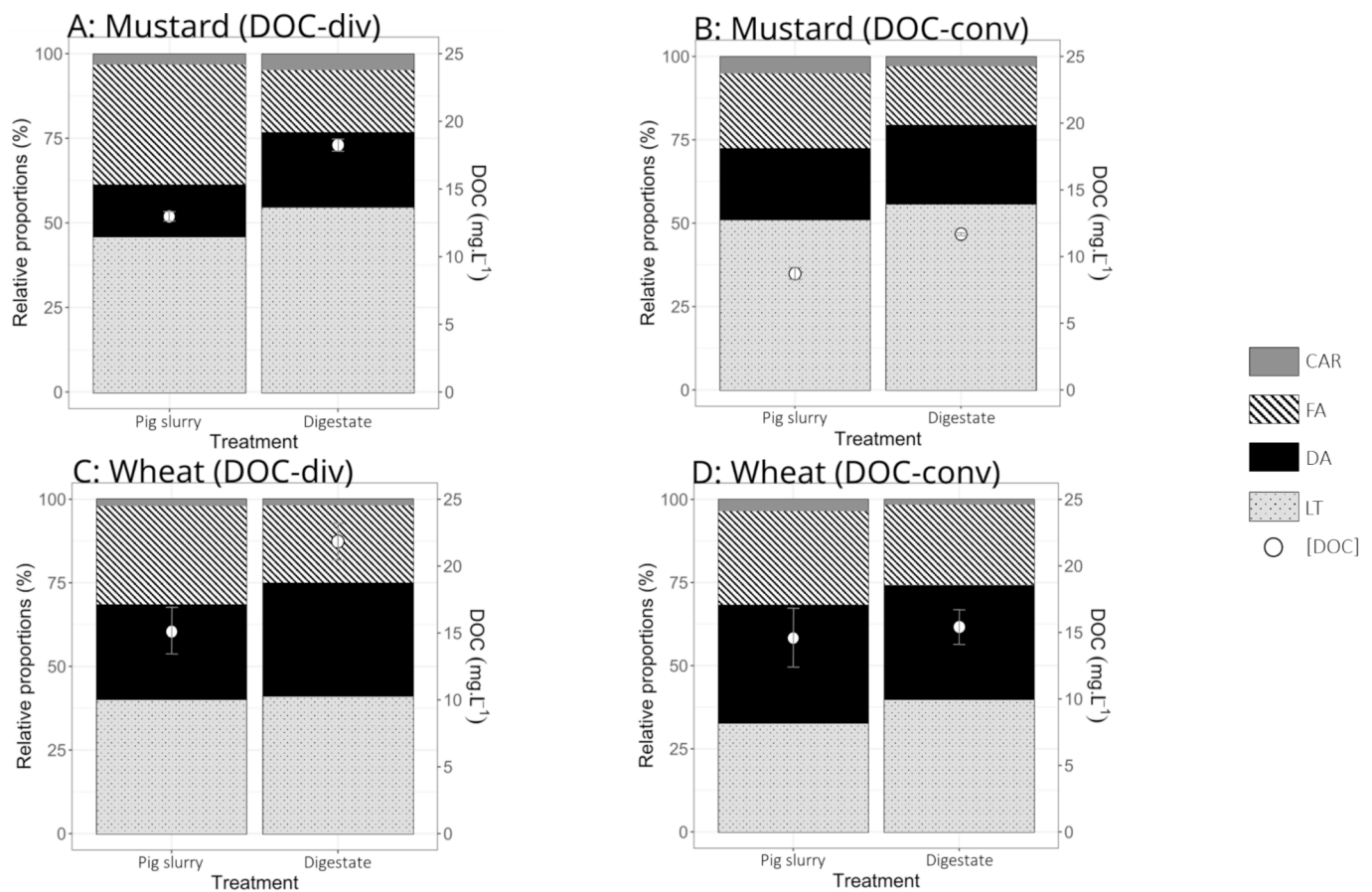
For both treatments (mean of pig slurry and digestate), on *DOC-div* dates, %DA was significantly higher under wheat than under mustard ( $31.7 \pm 2.8\%$  vs.  $19.3 \pm 3.4\%$  of the analyzed compounds, respectively) (Fig. 3, Table 1), p-values are indicated in Supplementary Table S5. Under mustard, %LT was higher ( $49.9 \pm 4.4\%$  vs.  $40.4 \pm 0.5\%$  under wheat), as was %CAR ( $4.2 \pm 0.9\%$  vs.  $2.1 \pm 0.2\%$  under wheat), but %FA did not differ ( $26.6 \pm 8.6\%$  under mustard vs.  $25.8 \pm 3.1\%$  under

wheat). The  $f_{mic}$  did not differ significantly between wheat and mustard ( $26.3 \pm 2.5\%$  vs.  $24.4 \pm 7.1\%$ , respectively) as  $f_{plant}$  ( $73.7 \pm 2.5\%$  vs.  $75.6 \pm 7.1\%$ , respectively) (Table 2). Under wheat, the C:V ratio was significantly higher ( $0.17 \pm 0.06$  vs.  $0.05 \pm 0.009$  under mustard), and the Al:Ac<sub>V</sub> ratio did not differ between the two crops (mean of  $0.15 \pm 0.01$ ) (Supplementary Table S4).

For both treatments (mean of pig slurry and digestate), on *DOC-conv* dates, %DA and %FA were significantly higher under wheat ( $35.4 \pm 0.5\%$  and  $25.9 \pm 2.0\%$ , respectively) than under mustard ( $23.0 \pm 1.3\%$  and  $19.7 \pm 2.3\%$ , respectively). The %LT and %CAR were higher under mustard ( $53.1 \pm 2.2\%$  and  $4.1 \pm 1.2\%$ , respectively) than under wheat ( $36.0 \pm 3.5\%$  and  $2.7 \pm 1.0\%$ , respectively). The  $f_{mic}$  was significantly higher under wheat ( $33.0 \pm 6.7\%$  vs.  $15.8 \pm 2.1\%$  under mustard), and thus  $f_{plant}$  was significantly higher under mustard ( $84.2 \pm 2.1\%$  vs.  $67.0 \pm 6.9\%$  under wheat). The C:V ratio did not differ between the two crops (mean of  $0.08 \pm 0.02$ ), and the Al:Ac<sub>V</sub> ratio was significantly higher under wheat ( $0.20 \pm 0.05$  vs.  $0.09 \pm 0.007$  under mustard).

### 3.4. Differences in DOM composition between the topsoil and subsoil

In the topsoil DOM, higher %LT were measured compared to subsoil DOM (average of pig slurry and digestate under mustard on *DOC-conv* date for each depth:  $53.1 \pm 2.2\%$  vs.  $44.5 \pm 0.6\%$  of the analyzed compounds, respectively) and higher %DA ( $23.0 \pm 1.3\%$  vs.  $5.5 \pm 1.2\%$ , respectively) (Fig. 4), p-values are indicated in Supplementary Table S6. The subsoil DOM had higher %FA than the topsoil DOM ( $42.6 \pm 3.8\%$  vs.  $19.7 \pm 2.3\%$ , respectively) and higher %CAR ( $7.5 \pm 3.3\%$  vs.  $4.1 \pm 1.2\%$ , respectively). The  $f_{mic}$  was higher in the subsoil than in the topsoil ( $38.4 \pm 4.0\%$  vs.  $15.8 \pm 2.1\%$ , respectively), and thus  $f_{plant}$  was higher in



**Fig. 3.** Relative proportions of four chemical families of molecules in dissolved organic matter of lysimeter samples: carbohydrates (CAR), fatty acids (FA), dicarboxylic acids (DA) and lignin- and tannin-derived molecules (LT), and concentrations of dissolved organic carbon (DOC) in mg L<sup>-1</sup> under or mustard (A and B) or winter wheat (C and D) for two treatments: pig slurry or its digestate. The values are the mean of two lysimeter replicates. Error bars are average deviations. *DOC-div* refers to dates when DOC concentrations diverged (higher concentrations for digestate than for pig slurry), *DOC-conv* refers to dates when DOC concentrations converged (no differences between treatments).

**Table 1**

Relative proportions (mean of two lysimeter replicates  $\pm$  1 average deviation) of carbohydrates (CAR) and fatty acids (FA), with details of low-and-medium-molecular-weight FA (LMMWFA) (excluding the ubiquitous hexadecanoic and octadecanoic acids) and high-molecular-weight FA (HMWFA), dicarboxylic acids (DA) and lignin- and tannin-derived molecules (LT) of lysimeter samples under mustard (M) or wheat (W) for two treatments: pig slurry or its digestate. Letters indicate significant differences between treatments among chemical families and based on ANOVA for each drainage date and depth. Significance: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ ; \*  $p < 0.05$ ; NS  $p > 0.1$ . *DOC-div* refers to dates when DOC concentrations diverged (higher concentrations for digestate than for pig slurry), *DOC-conv* refers to dates when DOC concentrations converged (no differences between treatments).

Crop	Date	Depth	Treatment	CAR	FA	LMMWFA	HMWFA	DA	LT
M	<i>DOC-div</i>	Topsoil	Pig slurry	3.4 $\pm$ 1.8 <sup>b</sup>	35.2 $\pm$ 3.5 <sup>a</sup>	20.1 $\pm$ 0.8 <sup>a</sup>	0.7 $\pm$ 0.1 <sup>a</sup>	16.0 $\pm$ 1.6 <sup>b</sup>	45.5 $\pm$ 3.6 <sup>b</sup>
			Digestate	5.1 $\pm$ 1.6 <sup>a</sup>	17.9 $\pm$ 0.03 <sup>b</sup>	10.9 $\pm$ 1.1 <sup>b</sup>	0.4 $\pm$ 0.06 <sup>b</sup>	22.7 $\pm$ 1.6 <sup>a</sup>	54.3 $\pm$ 0.05 <sup>a</sup>
	<i>DOC-conv</i>	Topsoil	Pig slurry	5.3 $\pm$ 1.6 <sup>a</sup>	22.0 $\pm$ 4.3 <sup>a</sup>	11.4 $\pm$ 2.4 <sup>a</sup>	0.3 $\pm$ 0.04 <sup>b</sup>	21.8 $\pm$ 5.8 <sup>a</sup>	50.9 $\pm$ 0.08 <sup>b</sup>
			Digestate	3.0 $\pm$ 0.03 <sup>b</sup>	17.4 $\pm$ 2.0 <sup>b</sup>	9.3 $\pm$ 1.9 <sup>b</sup>	0.4 $\pm$ 0.06 <sup>a</sup>	24.3 $\pm$ 2.0 <sup>a</sup>	55.3 $\pm$ 3.9 <sup>a</sup>
W	<i>DOC-div</i>	Topsoil	Pig slurry	2.3 $\pm$ 0.002 <sup>a</sup>	28.9 $\pm$ 1.0 <sup>a</sup>	16.4 $\pm$ 0.07 <sup>a</sup>	1.3 $\pm$ 0.1 <sup>a</sup>	28.8 $\pm$ 7.6 <sup>b</sup>	39.9 $\pm$ 6.6 <sup>a</sup>
			Digestate	1.9 $\pm$ 0.8 <sup>a</sup>	22.8 $\pm$ 2.6 <sup>b</sup>	13.0 $\pm$ 0.3 <sup>b</sup>	1.0 $\pm$ 0.2 <sup>b</sup>	34.5 $\pm$ 7.5 <sup>a</sup>	40.9 $\pm$ 5.7 <sup>a</sup>
	<i>DOC-conv</i>	Topsoil	Pig slurry	3.7 $\pm$ 1.2 <sup>a</sup>	27.9 $\pm$ 7.1 <sup>a</sup>	18.7 $\pm$ 4.1 <sup>a</sup>	1.1 $\pm$ 0.03 <sup>a</sup>	35.9 $\pm$ 2.4 <sup>a</sup>	32.5 $\pm$ 5.8 <sup>b</sup>
			Digestate	1.7 $\pm$ 0.04 <sup>b</sup>	23.9 $\pm$ 6.0 <sup>b</sup>	14.7 $\pm$ 2.0 <sup>b</sup>	1.2 $\pm$ 0.4 <sup>a</sup>	34.9 $\pm$ 4.5 <sup>a</sup>	39.5 $\pm$ 1.4 <sup>a</sup>

**Table 2**

Proportions (mean of two lysimeter replicates  $\pm 1$  average deviation) of microbial-derived molecules  $f_{mic}$  and of plant-derived molecules  $f_{plant}$  of lysimeter samples under mustard or wheat for two treatments: pig slurry or its digestate. Letters indicate significant differences between treatments among chemical families and based on ANOVA for each drainage date and depth. Significance: \*\*\*  $p < 0.001$ ; \*\*  $p < 0.01$ . *DOC-div* refers to dates when DOC concentrations diverged (higher concentrations for digestate than for pig slurry), *DOC-conv* refers to dates when DOC concentrations converged (no differences between treatments).

Crop	Date	Depth	Treatment	$f_{mic}$ (%)		$f_{plant}$ (%)	
Mustard	<i>DOC-div</i>	Topsoil	Pig slurry	31.5 $\pm$ 2.3 <sup>a</sup>	***	68.5 $\pm$ 2.3 <sup>b</sup>	***
			Digestate	17.4 $\pm$ 2.0 <sup>b</sup>		82.6 $\pm$ 2.0 <sup>a</sup>	
	<i>DOC-conv</i>	Topsoil	Pig slurry	17.8 $\pm$ 4.0 <sup>a</sup>	**	82.2 $\pm$ 4.0 <sup>b</sup>	**
			Digestate	13.7 $\pm$ 2.9 <sup>b</sup>		86.3 $\pm$ 2.9 <sup>a</sup>	
		Subsoil	Pig slurry	34.4 $\pm$ 7.9 <sup>b</sup>	**	65.6 $\pm$ 7.9 <sup>a</sup>	**
			Digestate	42.4 $\pm$ 4.3 <sup>a</sup>		57.6 $\pm$ 4.3 <sup>b</sup>	
Wheat	<i>DOC-div</i>	Topsoil	Pig slurry	28.9 $\pm$ 3.2 <sup>a</sup>	***	71.1 $\pm$ 3.2 <sup>b</sup>	***
			Digestate	23.8 $\pm$ 2.7 <sup>b</sup>		76.2 $\pm$ 2.7 <sup>a</sup>	
	<i>DOC-conv</i>	Topsoil	Pig slurry	39.8 $\pm$ 13.2 <sup>a</sup>	**	60.2 $\pm$ 13.2 <sup>b</sup>	**
			Digestate	26.1 $\pm$ 2.5 <sup>b</sup>		73.9 $\pm$ 2.5 <sup>a</sup>	

the topsoil than in the subsoil ( $84.2 \pm 2.1\%$  vs.  $61.6 \pm 4.0\%$ , respectively) (Table 2). The subsoil had a higher C:V ratio than the topsoil ( $0.16 \pm 0.02$  vs.  $0.07 \pm 0.01$ , respectively) and a higher Al:Ac<sub>V</sub> ratio ( $0.2 \pm 0.007$  vs.  $0.09 \pm 0.007$ , respectively) (Supplementary Table S4).

## 4. Discussion

### 4.1. Contribution of organic waste products and crops to the DOM composition

The WEOM composition of digestate and pig slurry observed agreed with those of Tambone et al. (2009), who highlighted degradation of CAR-like molecules and a high concentration of persistent molecules, such as lignin-derived compounds during anaerobic digestion, which could increase biological stability. Monard et al. (2020) analyzed the composition of the  $< 0.7 \mu\text{m}$  fraction from pig slurry and digestate, and estimated %LT of 20% in pig slurry and 53% in digestate, and a low %FA. The higher %DA in the WEOM from digestate (17.7% vs. 9.9% from pig slurry) could be explained by DA production during the acidogenesis stage (Liebetrau et al., 2017).

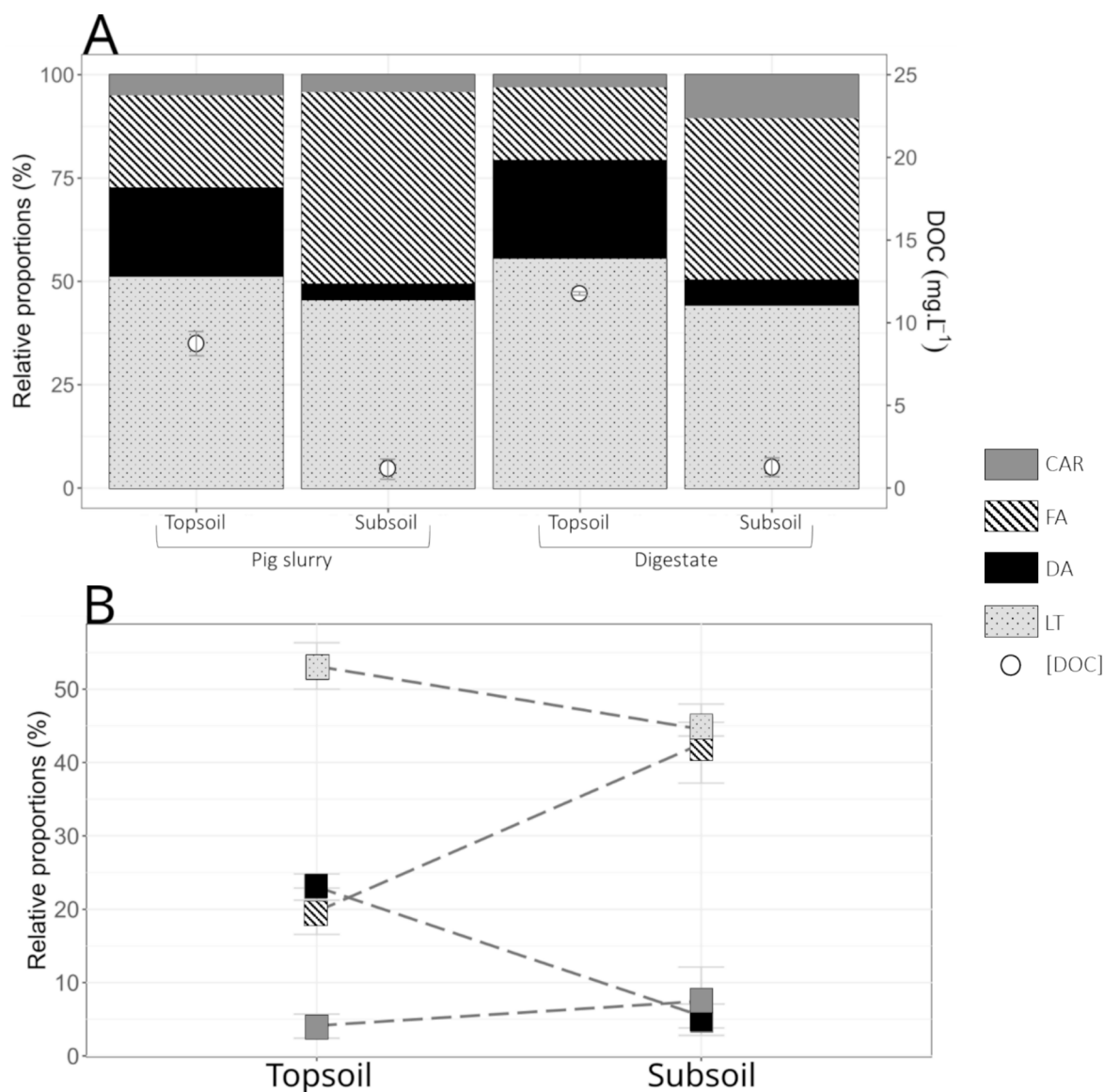
Under mustard, on *DOC-div* dates (i.e., DOC concentrations significantly higher for the digestate treatment), the DOM composition differed between the digestate and pig slurry treatments, with overall high %DA and %LT, and low %FA. These significant differences were detected using ANOVA with 10 synthetically generated replicates, which increased the sample size (from 2 actual replicates to a total of 12 replicates) and thus increased the power of the test. These chemical compositions were similar to those of WEOM from the digestate that was applied to the soil. Digestate was applied ca. 8–9 months before the drainage season and could have been partly mineralized (Chantigny, 2003; Monard et al., 2020), but since the digestate underwent anaerobic digestion and had high biological stability (Tambone et al., 2009), a persistent fraction of the molecules provided by digestate could have been sorbed on the soil. Coward et al. (2018) and Schmidt and Martínez (2019) observed preferential sorption of aromatic or hydrophobic

molecules on the reactive mineral phase, such as lignin-derived compounds that dominate the chemical composition of digestate. Among these compounds, 37% of the WEOM from digestate is acidic LT, and Kaiser et al. (2004) suggested preferential sorption of acidic compounds. Nitrate uptake tends to basify the rhizosphere (Bloom, 1996; Hinsinger et al., 2003), which can increase the solubility of organic matter (Andersson et al., 2000). As a catch crop, mustard absorbs large quantities of nitrate during development, which may increase soil pH (Zuk-Golaszewska et al., 2019). Thus, the sorbed molecules from digestate may have been desorbed into the soil solution and contributed to the DOM pool, particularly under mustard (as a catch crop) and to some extent under wheat (not a catch crop). The digestate from pig slurry studied by Scaglia et al. (2015a, 2017b) contained molecules with auxin-like properties due to anaerobic digestion of aromatic amino acids. Auxin can stimulate root development (Li et al., 2016), and thus the roots that developed may have released more exudates that contributed to the DOM pool. The auxin effect of digestate could be explored in future studies of the digestate used in the present study. For the pig slurry treatment, the overall %FA and %LT were high. The pig slurry initially applied to the soil also contained high %FA and %LT in its WEOM, but some of it may have been degraded rapidly (Chantigny, 2003) in the weeks after application. The WEOM from pig slurry contained 24.2% of acidic LT; thus, some molecules from the pig slurry applied may have been sorbed on soil minerals, but less than the molecules from the digestate applied. Pig slurry application may have partly contributed to the DOM composition in the lysimeter samples.

On *DOC-conv* dates, the DOM composition for the digestate treatment was similar to that on the previous *DOC-div* dates, under both crops. This lack of change may have been due to a supply of DOM from the same sources on both dates for the digestate treatment, but that decreased toward the end of the drainage season, which explained the decreased DOC concentrations that homogenized with those of the pig slurry treatment. In contrast, the DOM composition for the pig slurry treatment changed: %FA decreased and %DA increased under both crops compared to those on the previous dates, while %LT increased under mustard. This could be due to microbial transformation of FA into low-molecular-weight FA and DA (Sempere et al., 2018) and degradation of aboveground lignin, since mustard is frost-sensitive and tended to deteriorate at the end of the drainage season.

Crops may have contributed to soil DOM through root exudation. A large fraction of the C fixed by photosynthesis is released into the soil by exudation of low molecular weight molecules (e.g., CAR, carboxylic acids and LMWFA) (Neumann and Römheld, 2000; Vives-Peris et al., 2020). Low-molecular-weight carboxylic acids (e.g., DA) appear to be a major component of exudates since they are involved in many processes, including nutrient acquisition and metal detoxification (Jones, 1998; Mimmo et al., 2011). As mentioned, the mustard in the present study was sown in September and was undergoing vegetative growth when the drainage season began in November. Mimmo et al. (2011) observed a peak of exudation of low-molecular-weight carboxylic acids (including DA) in soil for 1–3 weeks (depending on the molecule) after the planting of rapeseed (*Brassica napus*), a species similar to mustard, followed by a decrease. Kim et al. (2010) measured high concentrations of low-molecular-weight carboxylic acids (including DA) in the rhizosphere around brown mustard (*Brassica juncea*) roots 5 weeks after germination ( $14\text{--}140 \text{ mg.L}^{-1}$  of total carboxylic acids). Heuermann et al. (2023) analyzed the exudation of white mustard under conditions similar to those of the present study (i.e., sown in September in western Europe, in a silty loam soil, after winter wheat) after 5 weeks of cultivation. They detected CAR (fructose) and some amino acids in the exudates, but no DA. The exudates produced early by the mustard may have been degraded quickly in autumn before the beginning of the drainage. In the present study, wheat, sown in November and still developing during the drainage season, may have finished tillering and reached the jointing stage around February (the *DOC-div* date). Since tiller number and root development are positively correlated (O'Brien, 1979), the root biomass





**Fig. 4.** A: Relative proportions of four chemical families of molecules in dissolved organic matter of lysimeter samples: carbohydrates (CAR), fatty acids (FA), dicarboxylic acids (DA) and lignin- and tannin-derived molecules (LT), and concentrations ( $\text{mg L}^{-1}$ ) of dissolved organic carbon (DOC) under mustard on 08 Feb 2021 for two treatments – pig slurry or its digestate – in the topsoil and subsoil. The values are the mean of two lysimeter replicates. B: Mean percentages of the fractions of both treatments in the topsoil and subsoil. Error bars are average deviations.

that developed may have enabled large quantities of root exudation of the wheat. Chen et al. (2019) highlighted that wheat releases large quantities of low-molecular-weight carboxylic acids (including DA) at the jointing stage, particularly succinic acid (82–87 % of the total organic acids), to attract rhizobacteria that promote plant growth. The exudation of wheat roots could have provided large quantities of DA to the DOM pool on both *DOC-div* and *DOC-conv* dates, since mineralization likely decreased during low winter temperatures (Karhu et al., 2014). Succinic acid represented  $21.3 \pm 2.5\%$  (for both treatments) of the molecules measured in DOM from the lysimeters under wheat on *DOC-div* dates and  $24.5 \pm 1.5\%$  on *DOC-conv* dates. Zhao et al. (2022) analyzed the composition of WEOM from different Chinese soils under wheat and found higher contents of organic acids and CAR compared to those of the control without wheat. Combined with the potentially higher exudation due to the auxin-like effect, this process could explain the high %DA for the digestate treatment under wheat. The higher C:V and *Al:Ac<sub>v</sub>* ratios under wheat indicated the presence of less degraded lignin (Ertel and Hedges, 1984; Opsahl and Benner, 1995), which is

consistent with its later sowing.

In summary, applying digestate could have two effects on DOM composition: a direct effect, in which the persistent molecules provided by digestate itself and sorbed on the soil minerals are solubilized via basifying of the rhizosphere after nutrient uptake, and an indirect effect, in which the digestate has an auxin-like effect on crops, which increases root growth and thus root exudation. Most of the topsoil DOM pool for the digestate treatment may have been provided directly by the applied digestate under mustard, for which exudation did not seem to contribute significantly to the DOM pool on the dates analyzed, and both directly and indirectly under wheat.

#### 4.2. Transfer of DOM from the topsoil to the subsoil

The DOM composition collected by the lysimeters in the topsoil and subsoil were consistent with the conceptual model developed by Kaiser and Kalbitz (2012), with a decrease in plant-derived compounds such as LT and an increase in microbial-derived compounds such as CAR, as

depth increased. The molecules observed in the topsoil underwent microbial transformation. A decrease in the C:V and Al:Ac<sub>V</sub> ratios indicates aerobic degradation of lignin biopolymers (Ertel and Hedges, 1984; Opsahl and Benner, 1995). In the present study, the increase in the ratios observed as depth increased suggests less degraded lignin in the subsoil. These results disagree with those of Xia et al. (2021), who observed degradation of lignin as DOM was transferred towards the subsoil in arable soils. However, these results agree with those of Kaiser et al. (2004), who observed an increase in these ratios as depth increased, which was explained by preferential sorption of acidic LT on mineral surfaces (e.g., aluminum and iron oxides and hydroxides). To verify this hypothesis, we calculated the percentages of acidic LT, which were lower in the subsoil than in the topsoil, perhaps due to sorption. The decrease from topsoil to subsoil was larger for the digestate treatment (from 32.0 ± 1.7% to 8.8 ± 0.2%, respectively) than for the pig slurry treatment (from 24.4 ± 1.5% to 6.7 ± 2.4%, respectively), which could indicate more intense sorption of molecules for the digestate treatment and thus more C storage in the subsoil depending on the duration of this sorption. Since DOM compositions in the topsoil and subsoil were compared for a single drainage date in the present study, future studies should be performed to confirm these observations.

## 5. Conclusions

Since digestate application is a recent practice, its effects on the dissolved compartment have not yet been fully investigated. This interdisciplinary study between biogeochemistry and organic geochemistry aimed to explore the link between DOC concentrations and DOM composition. It provided new information on the sources contributing to the DOM pool in a soil–plant–digestate system under field conditions and several months after digestate application, as well as on the transfer of DOM to the subsoil in this context.

Early in the drainage season for mustard and mid-season for winter wheat, topsoil DOC concentrations were higher each year for the digestate treatment. Under mustard, the DOM composition appeared to be influenced mainly by the recalcitrant molecules provided by the digestate. This effect may also have occurred to a certain extent under wheat, but exudation from its root system also seemed to contribute greatly to the DOM pool. Thus, the crop development seemed to influence both DOC quantity and DOM composition. This study revealed a synergy between digestate and crops that impacts DOM composition, and thus its fate in the environment, as well as that of the potential contaminants it might co-transport.

Although DOC concentrations in the topsoil differed between the treatments, those in the subsoil did not. Analysis of the DOM composition on one date highlighted a decrease in acidic lignin- and tannin-derived molecules as depth increased, particularly for the digestate treatment, which could imply a greater sorption of molecules for this treatment compared to the original pig slurry. Thus, applying digestate might lead to a greater carbon storage compared to the application of the undigested product, and possibly reduce the pressure on climate change.

## CRedit authorship contribution statement

**Anne-Flore Didelot:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **Anne Jaffrezic:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition. **Thierry Morvan:** Writing – review & editing, Project administration, Methodology, Funding acquisition. **Marine Liotaud:** Resources, Methodology, Data curation. **Florian Gaillard:** Resources, Methodology. **Emilie Jardé:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anne Jaffrezic reports financial support was provided by National Centre for Scientific Research. Anne Jaffrezic reports financial support was provided by French National Institute for Agricultural Research INRAE. Thierry Morvan reports financial support was provided by The French Agency for Ecological Transition. Anne-Flore Didelot reports financial support was provided by Brittany Region. Anne-Flore Didelot reports financial support was provided by l'Institut Agro Rennes-Angers. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.orggeochem.2024.104923>.

## Data availability

Data will be made available on request.

## References

- Adeleke, R., Nwangburuka, C., Oboirien, B., 2017. Origins, roles and fate of organic acids in soils: a review. *South African Journal of Botany* 108, 393–406. <https://doi.org/10.1016/j.sajb.2016.09.002>.
- Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. *Soil Biology and Biochemistry* 32, 1–10. [https://doi.org/10.1016/S0038-0717\(99\)00103-0](https://doi.org/10.1016/S0038-0717(99)00103-0).
- Angst, G., Mueller, K.E., Nierop, K.G.J., Simpson, M.J., 2021. Plant- or microbial-derived? A review on the molecular composition of stabilized soil organic matter. *Soil Biology and Biochemistry* 156, 108189. <https://doi.org/10.1016/j.soilbio.2021.108189>.
- Bloom, A.J., 1996. Nitrogen dynamics in plant growth systems. *Life Support & Biosphere Science* 3, 35–41. <https://pubmed.ncbi.nlm.nih.gov/11539158/>.
- Bolan, N.S., Adriano, D.C., Kunhikrishnan, A., James, T., McDowell, R., Senesi, N., 2011. Dissolved organic matter: biogeochemistry, dynamics, and environmental significance in soils. *Advances in Agronomy* 110, 1–75. <https://doi.org/10.1016/B978-0-12-385531-2.00001-3>.
- Chantigny, M.H., 2003. Dissolved and water-extractable organic matter in soils: a review on the influence of land use and management practices. *Geoderma, Ecological Aspects of Dissolved Organic Matter in Soils* 113, 357–380. [https://doi.org/10.1016/S0016-7061\(02\)00370-1](https://doi.org/10.1016/S0016-7061(02)00370-1).
- Chen, S., Waghmode, T.R., Sun, R., Kuramae, E.E., Hu, C., Liu, B., 2019. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome* 7, 136. <https://doi.org/10.1186/s40168-019-0750-2>.

- Coward, E.K., Ohno, T., Plante, A.F., 2018. Adsorption and molecular fractionation of dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environmental Science & Technology* 52, 1036–1044. <https://doi.org/10.1021/acs.est.7b04953>.
- Didelot, A.-F., Jardé, E., Morvan, T., Lemoine, C., Gaillard, F., Hamelin, G., Jaffrézic, A., 2025. Disentangling the effects of applying pig slurry or its digestate to winter wheat or a catch crop on dissolved C fluxes. *Agriculture, Ecosystems & Environment* 378, 109285. <https://doi.org/10.1016/j.agee.2024.109285>.
- Dunnivant, F.M., Jardine, P.M., Taylor, D.L., McCarthy, J.F., 1992. Cotransport of cadmium and hexachlorobiphenyl by dissolved organic carbon through columns containing aquifer material. *Environmental Science & Technology* 26, 360–368. <https://doi.org/10.1021/es00026a018>.
- Eiler, A., Langenheder, S., Bertilsson, S., Tranvik, L.J., 2003. Heterotrophic bacterial growth efficiency and community structure at different natural organic carbon concentrations. *Applied and Environmental Microbiology* 69, 3701–3709. <https://doi.org/10.1128/AEM.69.7.3701-3709.2003>.
- Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M., Nardi, S., 2013. Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (*Zea mays* L.) plantlets. *Journal of Geochemical Exploration* 129, 103–111. <https://doi.org/10.1016/j.gexplo.2012.10.001>.
- Ertel, J.R., Hedges, J.I., 1984. The lignin component of humic substances: distribution among soil and sedimentary humic, fulvic, and base-insoluble fractions. *Geochimica et Cosmochimica Acta* 48, 2065–2074. [https://doi.org/10.1016/0016-7037\(84\)90387-9](https://doi.org/10.1016/0016-7037(84)90387-9).
- Frostegård, Å., Tunlid, A., Bååth, E., 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Applied and Environmental Microbiology* 59, 3605–3617. <https://doi.org/10.1128/aem.59.11.3605-3617.1993>.
- Garcette-Lepecq, A., Derenne, S., Largeau, C., Bouloubassi, I., Salot, A., 2001. Thermally assisted hydrolysis and methylation of kerogen-like organic matter in a recent sediment off the Danube delta (northwestern Black Sea). *Journal of Analytical and Applied Pyrolysis* 61, 147–164. [https://doi.org/10.1016/S0165-2370\(01\)00133-4](https://doi.org/10.1016/S0165-2370(01)00133-4).
- Gbadegesin, L.A., Tang, X., Liu, C., Cheng, J., 2022. Transport of veterinary antibiotics in farmland soil: effects of dissolved organic matter. *International Journal of Environmental Research and Public Health* 19, 1702. <https://doi.org/10.3390/ijerph19031702>.
- Ge, T., Liu, C., Yuan, H., Zhao, Z., Wu, X., Zhu, Z., Brookes, P., Wu, J., 2015. Tracking the photosynthesized carbon input into soil organic carbon pools in a rice soil fertilized with nitrogen. *Plant and Soil* 392, 17–25. <https://doi.org/10.1007/s11104-014-2265-8>.
- Häfner, F., Hartung, J., Möller, K., 2022. Digestate composition affecting N fertiliser value and C mineralisation. *Waste Biomass Valor* 13, 3445–3462. <https://doi.org/10.1007/s12649-022-01723-y>.
- Hartwig, N.L., Ammon, H.U., 2002. Cover crops and living mulches. *Weed Science* 50, 688–699. [https://doi.org/10.1614/0043-1745\(2002\)050\[0688:AIACCA\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050[0688:AIACCA]2.0.CO;2).
- Herbrich, M., Gerke, H.H., Bens, O., Sommer, M., 2017. Water balance and leaching of dissolved organic and inorganic carbon of eroded Luvisols using high precision weighing lysimeters. *Soil and Tillage Research* 165, 144–160. <https://doi.org/10.1016/j.still.2016.08.003>.
- Heurmann, D., Döll, S., Schwenker, D., Feuerstein, U., Gentsch, N., von Wirén, N., 2023. Distinct metabolite classes in root exudates are indicative for field- or hydroponically-grown cover crops. *Frontiers in Plant Science* 14. <https://doi.org/10.3389/fpls.2023.1122285>.
- Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. *Plant and Soil* 248, 43–59. <https://doi.org/10.1023/A:1022371130939>.
- Holm-Nielsen, J.B., Al Seadi, T., Oleskowicz-Popiel, P., 2009. The future of anaerobic digestion and biogas utilization. *Bioresource Technology* 100, 5478–5484. <https://doi.org/10.1016/j.biortech.2008.12.046>.
- Jahangir, M.M.R., Minet, E.P., Johnston, P., Premrov, A., Coxon, C.E., Hackett, R., Richards, K.G., 2014. Mustard catch crop enhances denitrification in shallow groundwater beneath a spring barley field. *Chemosphere* 103, 234–239. <https://doi.org/10.1016/j.chemosphere.2013.11.072>.
- Jeanneau, L., Jaffrézic, A., Pierson-Wickmann, A.-C., Gruau, G., Lambert, T., Petitjean, P., 2014. Constraints on the sources and production mechanisms of dissolved organic matter in soils from molecular biomarkers. *Vadose Zone Journal* 13. <https://doi.org/10.2136/vzj2014.02.0015>.
- Jeanneau, L., Denis, M., Pierson-Wickmann, A.-C., Gruau, G., Lambert, T., Petitjean, P., 2015. Sources of dissolved organic matter during storm and inter-storm conditions in a lowland headwater catchment: constraints from high-frequency molecular data. *Biogeochemistry* 12, 4333–4343. <https://doi.org/10.5194/bg-12-4333-2015>.
- Jones, D.L., 1998. Organic acids in the rhizosphere—a critical review. *Plant and Soil* 205, 25–44. <https://doi.org/10.1023/A:1004356007312>.
- Judd, K.E., Crump, B.C., Kling, G.W., 2006. Variation in dissolved organic matter controls bacterial production and community composition. *Ecology* 87, 2068–2079. [https://doi.org/10.1890/0012-9658\(2006\)87\[2068:VIDOMC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[2068:VIDOMC]2.0.CO;2).
- Kaiser, K., Guggenberger, G., Haumaier, L., 2004. Changes in dissolved lignin-derived phenols, neutral sugars, uronic acids, and amino sugars with depth in forested Haplic Arenosols and Rendzic Leptosols. *Biogeochemistry* 70, 135–151. <https://doi.org/10.1023/B:BIOG.0000049340.77963.18>.
- Kaiser, K., Kalbitz, K., 2012. Cycling downwards – dissolved organic matter in soils. *Soil Biology and Biochemistry* 52, 29–32. <https://doi.org/10.1016/j.soilbio.2012.04.002>.
- Kalbitz, K., Solinger, S., Park, J.-H., Michalzik, B., Matzner, E., 2000. Controls on the dynamics of dissolved organic matter in soils: a review. *Soil Science* 165, 277. <https://doi.org/10.1097/00010694-200004000-00001>.
- Karhu, K., Auffret, M.D., Dungait, J.A., Hopkins, D.W., Prosser, J.I., Singh, B.K., Subke, J.-A., Wookey, P.A., Ågren, G.I., Sebastia, M.-T., 2014. Temperature sensitivity of soil respiration rates enhanced by microbial community response. *Nature* 513, 81–84. <https://doi.org/10.1038/nature13604>.
- Kim, K.-R., Owens, G., Kwon, S., 2010. Influence of Indian mustard (*Brassica juncea*) on rhizosphere soil solution chemistry in long-term contaminated soils: a rhizobox study. *Journal of Environmental Sciences* 22, 98–105. [https://doi.org/10.1016/S1001-0742\(09\)60080-2](https://doi.org/10.1016/S1001-0742(09)60080-2).
- Kuroda, K.-I., Nakagawa-izumi, A., 2006. Tetramethylammonium hydroxide (TMAH) thermochemolysis of lignin: improvement of the distribution profile of products derived from β-aryl ether subunits. *Journal of Analytical and Applied Pyrolysis* 75, 104–111. <https://doi.org/10.1016/j.jaap.2005.04.011>.
- Li, X., Guo, J., Pang, C., Dong, R., 2016. Anaerobic digestion and storage influence availability of plant hormones in livestock slurry. *ACS Sustainable Chemistry & Engineering* 4, 719–727. <https://doi.org/10.1021/acscuschemeng.5b00586>.
- Liebetrau, J., Sträuber, H., Kretzschmar, J., Denysenko, V., Nelles, M., 2017. Anaerobic Digestion, in: Wagemann, K., Tippkötter, N. (Eds.), *Biorefineries, Advances in Biochemical Engineering/Biotechnology*. Springer International Publishing, Cham, pp. 281–299. <https://doi.org/10.1007/10.1007/10.2016.67>.
- Lu, Y., Watanabe, A., Kimura, M., 2004. Contribution of plant photosynthates to dissolved organic carbon in a flooded rice soil. *Biogeochemistry* 71, 1–15. <https://doi.org/10.1007/s10533-004-3258-0>.
- Marcato, C.-E., Mohtar, R., Revel, J.-C., Pouech, P., Hafidi, M., Guirese, M., 2009. Impact of anaerobic digestion on organic matter quality in pig slurry. *International Biodeterioration & Biodegradation* 63, 260–266. <https://doi.org/10.1016/j.ibiod.2008.10.001>.
- Matsuda, H., Koyama, T., 1977. Early diagenesis of fatty acids in lacustrine sediments—II. A statistical approach to changes in fatty acid composition from recent sediments and some source materials. *Geochimica et Cosmochimica Acta* 41, 1825–1834. [https://doi.org/10.1016/0016-7037\(77\)90214-9](https://doi.org/10.1016/0016-7037(77)90214-9).
- Mimmo, T., Hann, S., Jaitz, L., Cesco, S., Gessa, C.E., Puschenreiter, M., 2011. Time and substrate dependent exudation of carboxylates by *Lupinus albus* L. and *Brassica napus* L. *Plant Physiology and Biochemistry* 49, 1272–1278. <https://doi.org/10.1016/j.plaphy.2011.08.012>.
- Monard, C., Jeanneau, L., Le Garrec, J.-L., Le Bris, N., Binet, F., 2020. Short-term effect of pig slurry and its digestate application on biochemical properties of soils and emissions of volatile organic compounds. *Applied Soil Ecology* 147, 103376. <https://doi.org/10.1016/j.apsoil.2019.103376>.
- Morelle, J., Jeanneau, L., Laverman, A.M., 2023. Organic carbon quality impacts benthic microbial nitrate reduction. *Wetlands* 43, 99. <https://doi.org/10.1007/s13157-023-01747-7>.
- Morvan, T., Lemoine, C., Gaillard, F., Hamelin, G., Trinkler, B., Carreaux, L., Petitjean, P., Jaffrézic, A., 2020. A comprehensive dataset on nitrate, nitrite and dissolved organic carbon leaching losses from a 4-year lysimeter study. *Data in Brief* 32, 106029. <https://doi.org/10.1016/j.dib.2020.106029>.
- Nakagawa-izumi, A., Kuroda, K., Ozawa, T., 2004. Thermochemolytic behavior of β-β lignin structures in the presence of tetramethylammonium hydroxide (TMAH). *Organic Geochemistry* 35, 763–774. <https://doi.org/10.1016/j.orggeochem.2004.01.020>.
- Neumann, G., Römhild, V., 2000. The release of root exudates as affected by the plant's physiological status. In: Pintor, R., Varanini, Z., Nannipieri, P. (Eds.), *In: The Rhizosphere: Biochemistry and Organic Substances at the Soil-Plant Interface*. CRC Press, Boca Raton, pp. 41–93. <https://doi.org/10.1201/9781420005585.ch2>.
- Nguyen, C., 2009. Rhizodeposition of Organic C by Plant: Mechanisms and Controls. In: Lichtfouse, E., Navarrete, M., Debaeke, P., Véronique, S., Alberola, C. (Eds.), *Sustainable Agriculture*. Springer, Netherlands, Dordrecht, pp. 97–123. [https://doi.org/10.1007/978-90-481-2666-8\\_9](https://doi.org/10.1007/978-90-481-2666-8_9).
- Nierop, K.G.J., Preston, C.M., Kaal, J., 2005. Thermally assisted hydrolysis and methylation of purified tannins from plants. *Analytical Chemistry* 77, 5604–5614. <https://doi.org/10.1021/ac050564r>.
- Nierop, K.G.J., Verstraten, J.M., 2004. Rapid molecular assessment of the bioturbation extent in sandy soil horizons under pine using ester-bound lipids by on-line thermally assisted hydrolysis and methylation-gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 18, 1081–1088. <https://doi.org/10.1002/rcm.1449>.
- O'Brien, L., 1979. Genetic variability of root growth in wheat (*Triticum aestivum* L.). *Australian Journal of Agricultural Research* 30, 587–595. <https://doi.org/10.1071/ar9790587>.
- Opsahl, S., Benner, R., 1995. Early diagenesis of vascular plant tissues: lignin and cutin decomposition and biogeochemical implications. *Geochimica et Cosmochimica Acta* 59, 4889–4904. [https://doi.org/10.1016/0016-7037\(95\)00348-7](https://doi.org/10.1016/0016-7037(95)00348-7).
- Pantoja-Guerra, M., Valero-Valero, N., Ramírez, C.A., 2023. Total auxin level in the soil–plant system as a modulating factor for the effectiveness of PGPR inocula: a review. *Chemical and Biological Technologies in Agriculture* 10, 6. <https://doi.org/10.1186/s40538-022-00370-8>.
- Premrov, A., Coxon, C., Hackett, R., Kirwan, L., Richards, K., 2012. Effects of over-winter green cover on groundwater nitrate and dissolved organic carbon concentrations beneath tillage land. *The Science of the Total Environment* 438, 144–153. <https://doi.org/10.1016/j.scitotenv.2012.08.043>.
- R Core Team, 2023. *R: A Language and Environment for Statistical Computing*. <https://www.r-project.org/>.
- Rumpel, C., Dignac, M.-F., 2006. Gas chromatographic analysis of monosaccharides in a forest soil profile: analysis by gas chromatography after trifluoroacetic acid hydrolysis and reduction–acetylation. *Soil Biology and Biochemistry* 38, 1478–1481. <https://doi.org/10.1016/j.soilbio.2005.09.017>.

- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter—a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158. <https://doi.org/10.1007/s11104-010-0391-5>.
- Scaglia, B., Pognani, M., Adani, F., 2015. Evaluation of hormone-like activity of the dissolved organic matter fraction (DOM) of compost and digestate. *Science of the Total Environment* 514, 314–321. <https://doi.org/10.1016/j.scitotenv.2015.02.009>.
- Scaglia, B., Pognani, M., Adani, F., 2017. The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property. *Science of the Total Environment* 589, 36–45. <https://doi.org/10.1016/j.scitotenv.2017.02.223>.
- Scarlat, N., Dallemand, J.-F., Fahl, F., 2018. Biogas: developments and perspectives in Europe. *Renewable Energy* 129, 457–472. <https://doi.org/10.1016/j.renene.2018.03.006>.
- Schmidt, M.P., Martínez, C.E., 2019. The influence of tillage on dissolved organic matter dynamics in a Mid-Atlantic agroecosystem. *Geoderma* 344, 63–73. <https://doi.org/10.1016/j.geoderma.2019.03.001>.
- Sempere, R., Charrière, B., Castro-Jiménez, J., Kawamura, K., Panagiotopoulos, C., 2018. Occurrence of  $\alpha$ ,  $\omega$ -dicarboxylic acids and  $\omega$ -oxoacids in surface waters of the Rhone River and fluxes into the Mediterranean Sea. *Progress in Oceanography* 163, 136–146. <https://doi.org/10.1016/j.pocean.2017.07.002>.
- Sokolova, T., 2020. Low-molecular-weight organic acids in soils: sources, composition, concentrations, and functions: a review. *Eurasian Soil Science* 53, 580–594. <https://doi.org/10.1134/S1064229320050154>.
- Tambone, F., Genevini, P., D'Imporzano, G., Adani, F., 2009. Assessing amendment properties of digestate by studying the organic matter composition and the degree of biological stability during the anaerobic digestion of the organic fraction of MSW. *Bioresource Technology* 100, 3140–3142. <https://doi.org/10.1016/j.biortech.2009.02.012>.
- Vives-Peris, V., De Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports* 39, 3–17. <https://doi.org/10.1007/s00299-019-02447-5>.
- Weng, L., Temminghoff, E.J., Lofts, S., Tipping, E., Van Riemsdijk, W.H., 2002. Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil. *Environmental Science & Technology* 36, 4804–4810. <https://doi.org/10.1021/es0200084>.
- Wong, W.S., Zhong, H.T., Cross, A.T., Yong, J.W.H., 2020. Plant Biostimulants in Vermicomposts, in: *The Chemical Biology of Plant Biostimulants*. John Wiley & Sons, Ltd, pp. 155–180. <https://doi.org/10.1002/9781119357254.ch6>.
- Wu, S., Dong, R., 2020. Nutrients and Plant Hormones in Anaerobic Digestates. In: Meers, E., Velthof, G., Michels, E., Rietra, R. (Eds.), *Biorefinery of Inorganics*. John Wiley & Sons, Ltd, pp. 231–246. <https://doi.org/10.1002/9781118921487.ch5-3>.
- Xia, S., Song, Z., Wang, Y., Wang, W., Fu, X., Singh, B.P., Kuzyakov, Y., Wang, H., 2021. Soil organic matter turnover depending on land use change: coupling C/N ratios,  $\delta^{13}\text{C}$ , and lignin biomarkers. *Land Degradation & Development* 32, 1591–1605. <https://doi.org/10.1002/ldr.3720>.
- Zhao, C., He, X., Dan, X., He, M., Zhao, J., Meng, H., Cai, Z., Zhang, J., 2022. Soil dissolved organic matters mediate bacterial taxa to enhance nitrification rates under wheat cultivation. *Science of the Total Environment* 828, 154418. <https://doi.org/10.1016/j.scitotenv.2022.154418>.
- Zuk-Golaszewska, K., Wanic, M., Orzech, K., 2019. The role of catch crops in in the field plant production—a review. *Journal of Elementology* 24. <https://doi.org/10.5601/jelem.2018.23.3.1662>.