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DOC Dynamics in Soil Water, Root Exudates and Plant Leaf Litter Decomposition: A High-Resolution MS Study.

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Abstract

Within the current century, temperatures are predicted to rise by 2–7°C. This will be caused by the increase of atmospheric greenhouse gas concentrations, especially carbon dioxide (CO_2) (Wu et al., 2011). Soil represents a potential source, buffer and sink, depending on the balance of photosynthesis, respiration of decomposer organisms, and stabilization in soil (Dungait et al., 2012) The hypothesis that stability of soil organic matter against microbial decomposition is inherent in the molecular structure, i.e. recalcitrance, has been questioned recently. “Humic substances”, the former operational classification of “stable organic matter”, is recently interpreted as comprising low molecular weight compounds as well as partially decomposed structurally “recalcitrant” biomacromolecules (Sutton et al., 2005). A set of various factors has to be met both in space and time for actual decomposition, leading to complex interactions, which are not fully understood yet. Metals play an essential role in these interactions as co-factors for enzymes or micronutrients for living organisms; however, high concentrations of metals can be toxic for organisms. Plant covers can limit the damage caused by anthropogenic contamination of soils. At present, willow species are in the focus of interest for the bioremediation of cadmium and zinc-contaminated soils.

As a consequence of the above, dissolvable soil organic matter (soil water, root exudates, plant leaf litter) from *Salix* species known to hyper-accumulate cadmium, was analyzed in this present study. With the plant leaf litter a decomposition experiment was conducted in different soil types with magnetite (Fe_3O_4)-amendments. Mass spectra were obtained by high-resolution Fourier-transformed mass spectrometry with an LQT-Orbitrap. Analytes were aligned on the basis of estimated elemental compositions. Atomic ratios between O and C, H and C and molecular mass were visualized in two- and three-dimensional van Krevelen diagrams and interpreted on the basis of empirically defined areas for compound classes. Also multivariate statistical analysis was performed.

The high-resolution-FT-MS-analysis yielded characteristic spectra, allowing differentiation of soil water, root exudate and plant leaf litter samples. Undecomposed plant leaf litter indicated the presence of analytes that can be attributed to proteins, phenols and lipids; decomposed plant leaf litter samples showed analytes that can be interpreted as aliphatic compounds; analytes in root exudates mainly presented in the empirically created carboxyl-rich alicyclic group; and soil water yielded mainly analytes considered as lipids and their condensation products. Differences between the treatments in the plant leaf litter decomposition experiment could be attributed to soil type; no effect of the magnetite treatment was shown.

1. Introduction

After the geological and marine C pools, the terrestrial C pool represents the third largest global carbon store and comprises more than 2500 gigatons (Gt) C (Dungait et al., 2012; Falkowski et al., 2000). The largest terrestrial C reservoir is soil organic matter (SOM), sometimes also called natural organic matter (NOM). Within the first meter of soil, almost 1600 Gt C are stored; three meters into the ground, even up to 2300 Gt C can be found (Jobbágy and Jackson, 2000). Photosynthetic primary producers, plants and cyanobacteria, incorporate 120 Gt of atmospheric carbon dioxide in their biomass per year (Lorenz et al., 2007). Four to six percent of the total assimilated C/year remain in the terrestrial biosphere. For longer periods, this input is balanced by export of dissolved (DOC) and particulate organic carbon to the marine carbon pool (Schimel et al., 2001).

Within the current century, temperatures are predicted to rise by 2–7°C. This will be caused by the increase of atmospheric greenhouse gas concentrations, especially CO_2 (Wu et al., 2011), for which soil represents a potential source, buffer and sink, depending on the balance of photosynthesis, respiration of decomposer organisms, and stabilization in soil (Dungait et al., 2012). Organic carbon is regarded as the most substantial portion of SOM in soils that also contains vital elements for life such as nitrogen (N), phosphorus (P) and sulfur (S). Oxygen, nitrogen and sulfur facilitate complex bond formation with transition metals, many of which represent essential nutrients for living organisms and co-factors for many enzymes (Appenroth, 2010). If present in higher concentrations, though, they turn into toxic heavy metals.

Recalcitrance, the principle that molecular structures are inherently resistant to microbial decomposition, was initially regarded as a substantial factor that contributed to SOM stability (Gleixner et al., 2001; Krull et al., 2003; Sollins et al., 1996; von Lützow et al., 2006). However, the fact that old SOM is resistant to decomposition has been questioned recently (Kögel-Knabner et al., 2008; Marschner et al., 2008). The generally assumed notion is that decomposition requires specific combinations of substrates, microbial extracellular enzymes, oxygen, water and heat, but the distribution of these factors in space and time has to be determined yet. Furthermore, it is still doubtful whether the enzymatic controls have been properly characterized yet (Dungait et al., 2012). In the latter context, a set of poorly understood and often overlooked abiotic decomposition processes may be involved which represent a considerable part of the so-called environmental factors (Swift et al., 1979).

We have to assume that SOM presents a complex mixture of still chemically reactive—in aqueous environments—polymers, whose chemical reactions have not been analytically assessed so far. The most accessible fractions of potential analytes most probably are formed by

low-molecular weight compounds, which still can be extracted by aqueous and organic solvents (DOC) and concomitantly reflect the chemical reaction milieu in a specific soil compartment.

Traditionally, SOM is analyzed by solid-state ^{13}C NMR and pyrolytic gas chromatography, which are linked to a mass spectrometer (Baldock et al., 1991). Assuming that the soluble low molecular compounds, that surround the polymers, reflect the actual chemistry occurring on its surface more than any analysis of the solid material, e.g. solid state ^{13}C NMR, the main objective of the present study was to explore whether high resolution mass spectra of the soluble SOM fraction, specifically the water and alcohol-soluble fraction, provide sufficient information to study its dynamics. Ultrahigh resolution electrospray mass spectroscopy, ESI-FT-ion cyclotron (ICR) MS and FT-LTQ Orbitrap MS, both well known for their high mass accuracy (< 1 and 3 ppm respectively) have been shown to represent promising tools to explore SOM dynamics (Hernandez et al., 2012; Hertkorn et al., 2008; Kim et al., 2003; Ohno et al., 2010).

Soil contamination with heavy metals occurs as a consequence of human activities and phytoremediation with metal-hyperaccumulating plants represents one possibility to limit the caused damage (Zhao and McGrath, 2009). At present, willow species are in the focus of interest in terms of bioremediation of cadmium and zinc-contaminated soils (Rosselli et al., 2003). Consequently, *Salix* clones with such specific properties, *Salix smithiana*, *S. matsudana* x *alba* and *S. dasyclados*, were chosen as model plants for this study. In several experiments, plant leaf litter decomposition, root exudate and soil water samples were analyzed by LTQ-Orbitrap MS. Multivariate statistics and van Krevelen diagrams were performed to determine if

(1) the method provides specific information about chemical reactions in different soil compartments;

(2) differences between plant leaf litter decomposition in different soil types can be monitored;

(3) the addition of iron (magnetite) affects litter decomposition in a detectable fashion;

(4) there are any relationships between root exudation and soil water DOC.

2. Material and Methods

2.1. Plant material

Cuttings of three different species of willow, S*alix smithiana, S. matsudana x alba*, and *S. dasyclados*, were used in this study. The general propagation and culture conditions are described in Vaculik et al. (2012). Willow cuttings were planted in separate pots filled with a Cd-/Zn contaminated cambisol (A-horizon, Arnoldstein, Austria) (Muhammad et al., 2012). Willows and soil were provided by Dr. Markus Puschenreiter (BOKU, University of Natural Resources and Life Sciences, Vienna, Austria). Soil material originated from Arnoldstein, where Zn and Cd from metal smelters were deposited over centuries.

In this study, the plants were cultivated in a greenhouse at 16–33°C with 45% relative humidity, at a 12:12 light–dark cycle and watered with decalcified water. After leaf shedding, the plants were overwintered at 3°C (frost-free) in a temporarily open greenhouse, which was closed only when temperatures fell below 0°C, and brought back to the closed greenhouse at the beginning of spring (Fig. 1).

2.2. Experiments

Unless indicated otherwise, all chemicals were obtained from Sigma Aldrich (Schnelldorf, Germany) and of analytical grade.

Soil water

After overwintering and several weeks of acclimation in the greenhouse until leaves were developed fully (13.5.2011), soil water samples were obtained from six pot-grown *Salix matsudana x alba* and eight potted *Salix smithiana* plants (Figure 1). Macro-rhizons (Rhizosphere Research Products, Wageningen, Netherlands) were introduced into the pots 24 hours before. One hour prior to soil water extraction, plant pots were saturated with decalcified water. Soil water samples were stored at 4°C. After filtration (Whatman 40 cellulose filter, Kent, UK) samples were dried in two steps: first by rotary evaporation at reduced ambient pressure in a 37°C water bath and, second, by freeze-drying to remove water traces.

Root exudates

Ten days after soil water extraction root exudates were recovered (23.5.2011) from same plants as have been utilized for soil water collection. Plant roots (Figure 1) were washed with a sodium acetate buffer (25mM, pH 5.5) for 5 hours. Samples were filtered (Whatman 40 cellulose filter, Kent, UK) and dried in two steps; first by rotary evaporation at reduced ambient pressure in a 37°C water bath and, second, by freeze-drying.

Plant leaf litter

After leaf shedding, plant leaf litter from all *Salix* species, S. *smithiana, S. matsudana x alba, S. dasyclados*, was collected and stored in paper bags at ambient temperature for 2 months. A portion of the plant leaf litter (0.7 g), was pulverized, extracted by 10 ml methanol, to which 1% (v/v) of acetic acid was added and were left to stand in amber glass vials for two days at ambient temperature. After filtration (Whatman 40 cellulose filter, Kent, UK), the extracts were dried as described.

Litter bag experiment

Litter bags were filled with approx. 3.5 g air-dried willow leaves (pooled from all *Salix*) and buried in 1 l plastic boxes filled with approx. 900 ml soil (Figure 1). These microcosms were kept in the greenhouse at 15–18°C, 45 % relative humidity and were maintained humid with decalcified tap water. Commercial potting soil (mixture of white peat, wood fiber and clay minerals, pH_{ad} 7.1; Balkon- und Blumenerde, Kranzinger GmbH, Straßwalchen, Austria) and Cd-/Zn-contaminated clay-humus soil from Arnoldstein, Austria (clay- humus soil/cambisol, pH_{ad} 6.5) were used. Soils were sieved (diameter 2mm). For enhanced transition metal concentration treatment, magnetite (Fe_3O_4) was added at 1‰ weight of the soils dry mass. After six months, the litter bags were recovered (Figure 1) and dried at ambient temperature.

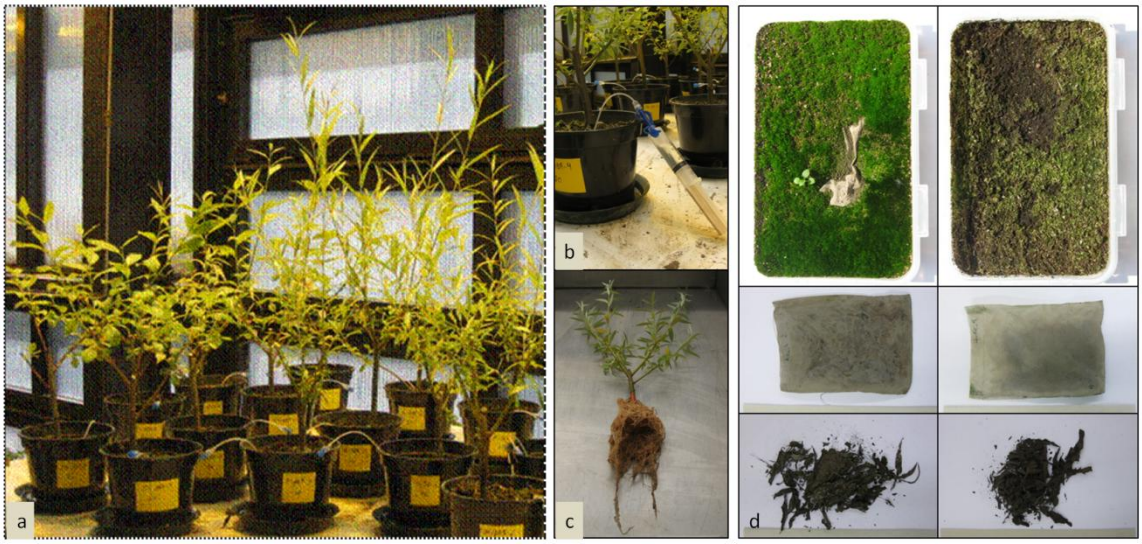

Figure 1: Experimental setup. (a) *Salix smithiana* (left) and *S.matsudana* (right) with rhizons inserted in the pots. (b) Soil water was extracted by rhizons attached to syringes for vacuum. (c) Roots were washed and put into sodium acetate buffer to collect root exudates. (d) Litter bag decomposition experiment: Cd-contaminated cambisol treated with magnetite (left) and potting soil treated with magnetite (right). Microcosms were filled with soil and litter bags (top). A dense moss cover developed on the cadmium-contaminated cambisol whereas the potting soil supported scarcely any cover. After 6 months, litter bags (middle photo series) were recovered and dried. Cambisol litter bags showed iron oxides. Decomposed plant leaf litter (bottom photo series) was used for methanol-extraction.

2.3. Chemical analysis

Elemental analysis

Carbon and Nitrogen weight percent of dried samples were obtained by an Elemental Analyzer (EA1110, CE Instruments, Wigan, UK) coupled to an Isotopic Ratio Mass Spectrometer (DELTAplus Finnigan MAT, Thermo Fisher Scientific, Waltham, MA).

High-resolution mass spectrometry

Dried samples were suspended in methanol with 1% formic acid (v/v) added at a concentration of 1mg*ml^{-1}. Methanol was used as a solvent to stabilize spray formation (Reemtsma, 2009) and formic acid was used to enhance ion formation. Dried samples were diluted and analyzed within 24 hours to omit extensive oxidative decomposition.

Mass spectra were analyzed by a nano-electrospray interface coupled to an LTQ-Orbitrap Hybrid Mass Spectrometer (Thermo Scientific, Waltham, MA). Soft ionization provides intact molecules for molecular mass analysis (Werner et al., 2008). Samples were directly infused at a flow rate of 1µl min^{-1}. After positive ionization, total ions were detected in a linear ion trap. Signals were Fourier transformed (FT-Resolution 100.000). Each sample was scanned for one minute in total, in 3 microscans 5 s^{-1} from m/z 100.00 to 2000.00. A mass accuracy of < 5 ppm with 95% probability was shown for an LQT-Orbitrap MS at a dynamic range of 5000 m/z (Makarov et al., 2006). Between the analyses, solvent blanks were measured. The next sample was introduced when no masses of the previous analysis showed in the spectrum of the pure solvent.

2.4. Data analysis and statistics

Estimation and filtering of elemental composition

Elemental composition was estimated with Xcalibur 2.1 software module (Thermo Scientific, Waltham, MA, USA). All masses to which a charge of 0 was assigned, were excluded from further analysis due to their uncertain status (pers. commun., Thermo tech support). Electrospray ionization may also lead to sodium adduct formation (Sleighter and Hatcher, 2007).

Elemental ranges were set as follows: C_n 1-50, H_n 1-100, O_n 0-30, N_n 0-6, S_n 0-1, Na_n 0-1.

In order to obtain "realistic" elemental composition of the mass fragments the data set was filtered by criteria similar to those that were suggested by other studies (Chen et al., 2011; Stubbins et al., 2010):

(1) C < 50; (2) 2 = H = (2C+4); (3) 0 = O = (C+2); (4) O/C < 1.2; (5) 0.3 ≤ H/C; (6) N/C < 0.5; (7) S/C < 0.2; (8) DBE = 1 + 0.5 * (2C – H + N); (9) DBE (double bond equivalents) ≤ 40, integer number.

By applying these filters, 999 total unique chemical formulas (≥ 5% intensity of the largest mass) were reduced to 368. Thus, 33 masses with elemental composition assignment remained for root exudates, 18 for soil water, 40 for undecomposed plant leaf litter and 283 for decomposed plant leaf litter.

Mass alignment

Alignment of masses with identical elemental composition was performed in R 2.14.1. A script was developed (see Appendix) that created a list of all unique elemental compositions from all spectra. The *m/z* value for one molecule can oscillate within the instrument error between different samples. Conversely, elemental formulas were estimated within the instrument error (± 5 ppm). The *m/z* values were transformed: *m/z* - H^+ * charge. The script explored whether the spectrum contained a signal for a specific elemental formula, formed the total sum of all detected intensities in the spectra and created an average mass value. This approach was stimulated by similarly described procedures (Kujawinski and Behn, 2006)

Sample type mass patterns

For each sample type averaged m/z values and median relative intensities (≥ 5%) were plotted. Within sample types the median relative intensities were determined for each unique elemental composition. Within the filtered unique formula list, median relative intensities of formulas with molecular masses within 100 u mass windows were summed up. The total median relative intensities across the whole mass range (100–2000 u) were referred to as total relative abundance. All these tasks were carried out with MS Excel 2007 (Microsoft Corp., Redmond, CA).

Van Krevelen diagrams

A van Krevelen diagram represents an xy-plot of atomic ratios between O and C and H and C and facilitates assessing the quality status of kerogen and petroleum (van Krevelen, 1950). Atomic ratios of estimated elemental compositions were plotted. The oxidation state of the analytes was interpreted according to the position in the van Krevelen diagram. Carbon dioxide represents the highest oxidation state of carbon with an O/C ratio of 2. The H/C ratio separates compounds according to the degree of saturation, whereas the O/C ratio separates compounds according to oxidation (Wu et al., 2004).

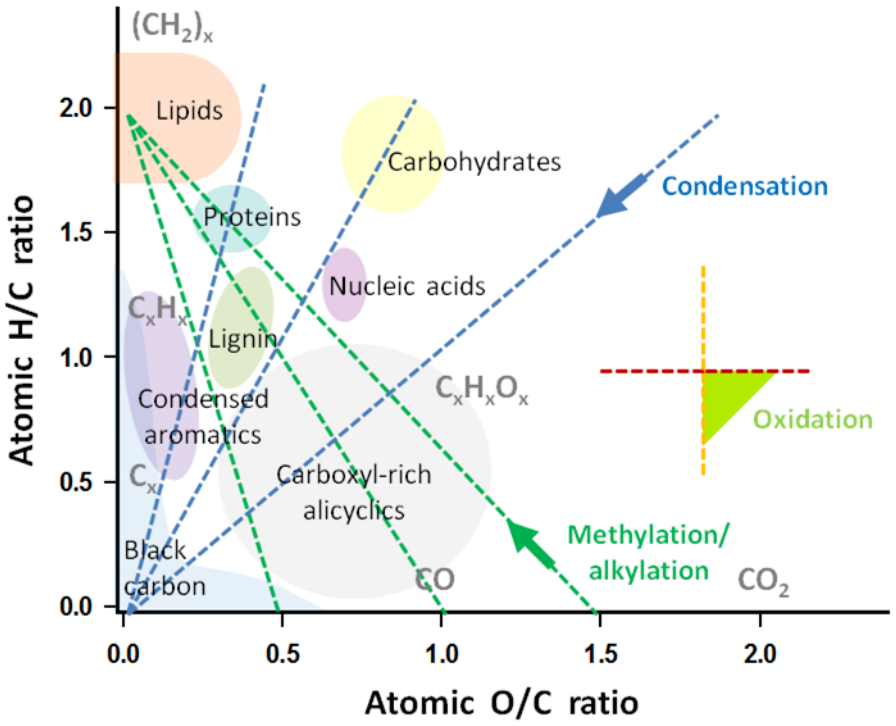

Figure 2: Biochemical groups and processes in the van Krevelen diagram (Hertkorn et al., 2008; Kim et al., 2003; Meija, 2006). Oxidation state of carbon is in grey letters: CO_2 (O/C=2), $(CH_2)x$ (O/C=2) and C_x (O/C=0). According to their biochemical properties, specific compound classes can be located within the diagram. Carboxyl-rich alicylics (grey) are oxidation products from precursor molecules such as lipids (orange), aromats (purple), proteins (turquoise), lignin (green), carbohydrates (yellow), and nucleic acids (pink). Black carbon (blue) is the ultimate condensation product. Intermediates indicate chemical reactions and polymerization of compounds. Chemical changes can be followed. Green lines indicate (de-) methylation or (de-)carboxylation. The arrow is pointed toward alkyl chain elongation, methylation and decarboxylation. Hydration/Condensation is indicated by blue lines. The arrow points in the direction of H_2O loss, i.e. condensation. (De-)hydrogenation (yellow line) and deoxygenation (red line) are indicators for reduction/oxidation. In oxidation the O/C-ratio increases and the H/C-ratio decreases.

The valences of the contributing elements (H = 1, C = 4, O = 2) define the standard scales of the van Krevelen diagram (Hertkorn et al., 2007), within which chemical processes follow straight lines. These lines are defined as H/C=-a (O/C) +b (a=intercept, b=slope): methylation or demethylation b=2; (de)hydrogenation along a vertical line; de(hydration) a=2; oxidation or reduction b=0; decarboxylation a=0, b=2 (Kim et al., 2003). Hertkorn et al. (2007) proposed oxidation as combination of dehydration and increasing O/C ratio (Figure 2, green triangle). Each biochemical group shares characteristic properties according to their elemental ratios (Figure 2). For example, sugar carbohydrates have the typical structure of $(C_6H_{12}O_6)_n$. Their characteristic atomic ratios are O/C (6 O/6 C)=1 and H/C (12 H/6 C)=2. Alkanes follow the structure of C_nH_{2n} so they would plot around O/C=0 and H/C=2. Compounds with long alkyl-chains such as lipids

also show up in this area. Average O/C and H/C ratios were defined (empirically) also for proteins, nucleic acids, lignin and (Chen et al., 2011) condensed aromatics. CRAMS (carboxylic-rich alicylic molecules) are interpreted as oxidation products of precursor molecules. Intermediates indicate the processing of organic compounds, i.e. oxidative and hydrolytic degradation and covalent polymerization of precursors (Hertkorn et al., 2008). Hydrogen-deficient carbon compounds or "black carbon" are regarded as highly condensed remainders. It was pointed out, that even if O/C and H/C ratios of different molecules plot in the same area, it does not necessarily mean that they belong to the same biochemical group (Reemtsma, 2009). Differences in molecular mass between molecules of similar atomic ratios still can be illustrated in a three-dimensional van Krevelen diagram, in which the z-axis represents the mass value.

Statistics

Statistical analysis was performed in R environment (R Development Core Team, 2011), specifically utilizing the R-packages *vegan*, *mass*, *labdsv* and *stats*. Sample sizes were each n = 15 for root exudates, n = 14 for both soil water and plant leaf litter. Litter samples included two undecomposed samples, three samples decomposed in cadmium-contaminated cambisol, three in cadmium contaminated cambisol with added magnetite, three in potting soil and three in potting soil with magnetite added. Nominal factors for statistical analysis were sample type (root exudate, soil water, plant leaf litter), soil type (no soil for undecomposed litter, cambisol, potting soil (+magnetite/– magnetite)) and clone type (*Salix matsudana*, *S. smithiana* and pooled *Salix* plant leaf litter). In the relative intensity data set, relative intensities ≤ 5% were replaced by the value 3. Multivariate statistics were performed only with filtered masses.

The relative abundances of the different analytes in the aligned mass spectra matrix were not distributed normally, so it was appropriate to avoid arithmetic mean-based statistics. As there is no appropriate means of referencing relative intensities of different sample types when different solvents were used (Flerus et al., 2011), the matrix of relative abundances was also analyzed as presence/absence data. Relative intensity data of different sample types can not be compared numerically. Relative intensities fluctuate even between different samples of the same type. Therefore a resemblance matrix was used. To omit two-dimensional analysis (which would result in e.g. Euclidean distances analysis, inappropriate for relative abundances) multidimensional statistics were used. Bray–Curtis index was used as dissimilarity measure for resemblance of relative intensity data, Sørensen and Jaccard-index were used for the categorical data (presence=1, absence=0 data; presence=0 absence=1).

Unconstrained statistical analysis

Firstly, an unconstrained approach was performed in order to explore whether the variance itself separates the data set into groups. Cluster analysis was performed with the average linkage method. A non-metric multidimensional scaling (nMDS) was performed to visualize the resemblance matrices (Anderson, 2001). The correlation (R^2) between the original data set (distance matrix) and the nMDS-configuration was either 1 or close to 1. This resulted in low stress values ($1 – R^2$).

To explore if the total variance in the data set was either due to variance within the groups or due to variance between the groups, the homogeneity of dispersion (i.e. the multidimensional alternative to arithmetic "variance") was tested. In the multivariate space, individual cases within a group share a group centroid (a multidimensional mean). An ANOVA of the multidimensional distances (i.e. dispersion) between individual cases was performed (Anderson, 2006). If the sum of squares for the grouping factor (e.g. sample type) is higher than the sum of squares for the residuals the greater variance is explained by the within-group dispersion than by the dispersion between-groups.

Constrained statistical analysis

Secondly, a constrained approach by Discriminant analysis on principal coordinate axes was performed. The choice of principal coordinates axis numbers for Discriminant analysis was determined by testing the classification success. For each grouping factor (sample type, soil type, clone type, magnetite treatment) and data set, ordinations with increasing numbers of principal coordinate axes were done. The misclassification error was determined for each number of principal coordinate axes by a "leave-one-out"-test. The percentage of variables whose identity was successfully classified in the ordination constituted the classification success. The axes number of successful classification was then used for canonical analysis. To avoid circular references, the axes number was to be less than the number of variables. The vast amount of signal variables (999 masses * 43 spectra) required an extraction of variables that contributed to the ordination and united them in principal coordinate axes. For each grouping factor (e.g. soil type) a different set of variables was crucial for group distinction. Consequently, for each data set and grouping factor a different number of principle coordinate axes was used for canonical discriminant analysis. In the current study, axes numbers ranged from 1–7 (43 variables).

The canonical axes were drawn through the multivariate data point space so that they best separated the groups. The resulting canonical discriminant dimensions explained the variance in the dataset and considered the correlation patterns between the species. The null hypothesis

was verified when the sum of canonical Eigen values equaled the sum of squared canonical correlations (Anderson and Willis, 2003).

The canonical discriminant analysis created dimensions in accordance with the correlation within the principal coordinates axes. Canonical discriminant dimensions themselves correlated with the principle coordinate axes and their supporting variables. Therefore it was possible to retrace the correlation of the canonical discriminant dimensions that separated the cases to the original variables by Spearman rank correlation. Algebraic signs (positive, negative) of correlation coefficients are given (*r*).

3. Results

3.1. Elemental analyses, mass patterns and van Krevelen diagrams

3.1.1. Soil water

Soil water data from 14 samples included 18 analytes after data filtering (see Appendix). The mean nitrogen content (n = 14) was 1.4 ± 0.5% (w/w) and the mean carbon content was 19.4 ± 5.7% (w/w). The median-based soil water spectrum showed its highest abundance (36.2%) between 100–200 u. From 200–300 u a low abundance region followed (1.9%). The region with the second highest abundance was between 300–400 u (30.8%). Mass abundance decreased again between 400–500 u (8.2%) and again rose between 500–600 u (21.2%) and finally faded between 600–700u (1.7%) (Table 1, Figure 3).

Table 1: Analytes in soil water spectra.

	Mass range [u]						
	100-200	200-300	300-400	400-500	500-600	600-700	Total
n	3	1	5	3	5	1	
Rel. abundance [%]	36.2	1.9	30.8	8.2	21.2	1.7	100

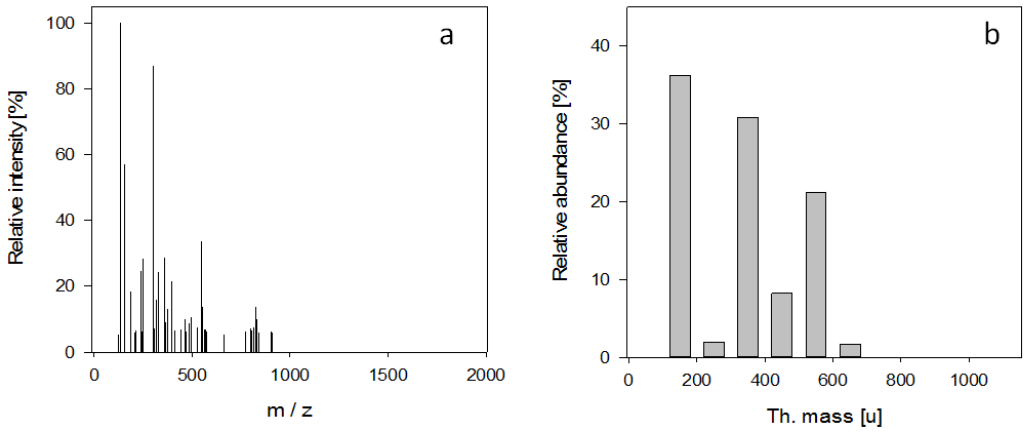

Figure 3: Soil water, 18 analytes from 14 replicates: (a) unfiltered averaged m/z values; (b) relative mass abundance.

Table 2 provides a statistical description of the O/C and H /C ratios of the analytes that were obtained for soil water samples.

Table 2: Soil water: O/C and H/C ratios and theoretical mass, statistical description.

	Mean	±SE	Median	C.I.95%	Maximum	Minimum	Range	100% Rel. abundance
O/C	0.2	0.1	0.2	0.1	0.4	0.0	0.4	0.2
H/C	1.5	0.5	1.8	0.2	2.0	0.4	1.6	1.8
Th.mass [u]	404.31	159.99	420.08	73.91	662.02	136.15	525.87	136.15

The majority of analytes plotted in the aliphate region. Some followed a condensation line to higher condensation, some could be proteins or aromatic (Figure 4a). The median analyte had a mass of 420 u (Figure 4b).

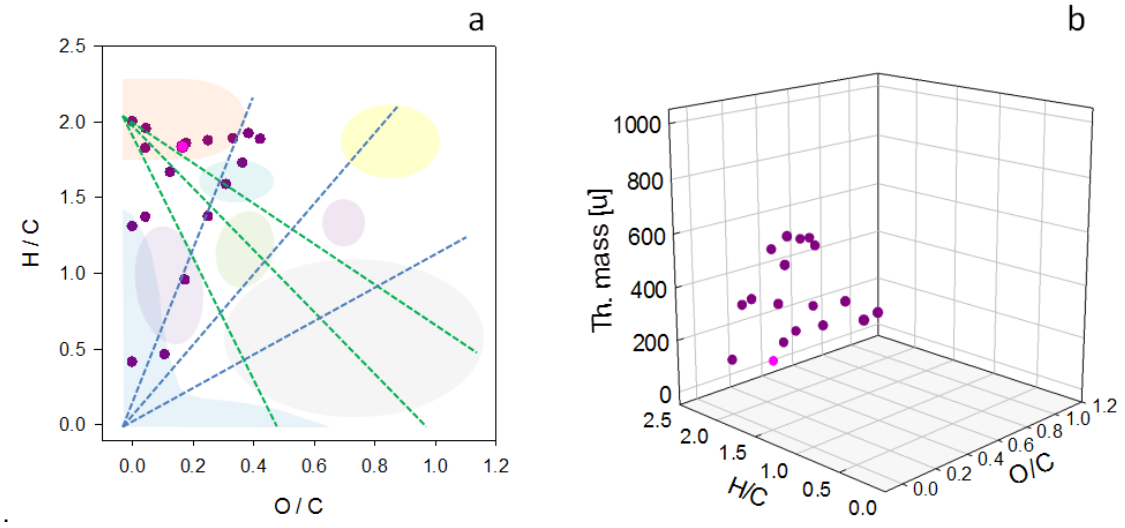

Figure 4: Soil water, 18 analytes from 14 replicates: (a) two-dimensional van Krevelen diagram; (b) three-dimensional van Krevelen diagram; analytes of highest median relative intensity are light pink; the z-axis represents the theoretical mass. Compound class areas: black carbon(blue); aromats(purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

3.1.2. Root exudates

Root exudate data contained 33 analytes from 15 replicates after filtering (see Appendix). No elemental analysis was performed. The root exudate spectra showed no masses between 100–200 u. The second highest mass abundance occurred between 200–300 u and decreased to average abundances between 300–400 u (16.2%) and 400–500 u (12.6%). Peak abundance (43.9%) again showed between 500–600 u tailed to low abundance (1.8%) between 600–700 u. After a 100 u step low abundance between 800-900 u (3.5%) followed. Masses disappeared between 900-1000 u (0.8%) (Figure 5, Table 3).

Table 3: Analytes in root exudate spectra

	Mass range [u]									
xsdudate (15 sample	100-200	200-300	300-400	400-500	500-600	600-700	700-800	800-900	900-1000	Total
n		3	6	8	11	2		2	1	
Rel. abundance [%]		21.2	16.2	12.6	43.9	1.8		3.5	0.8	100

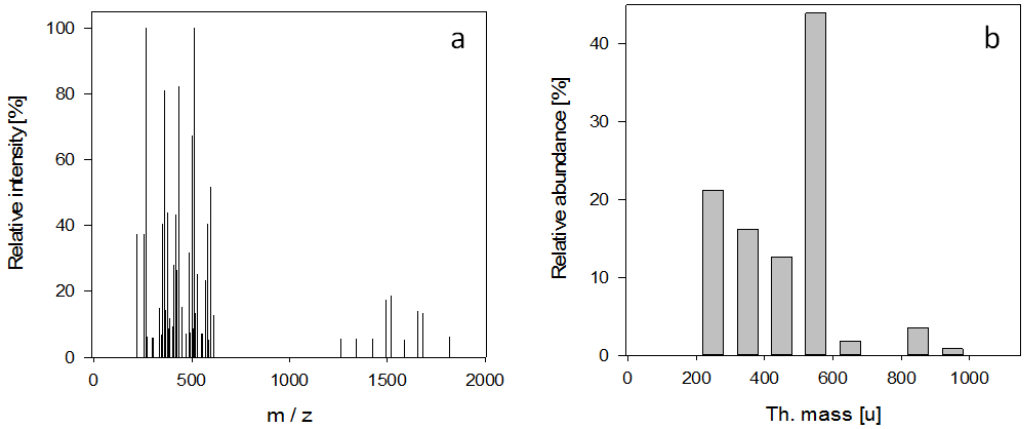

Figure 5: Root exudates, 33 analytes from 15 replicates: (a) unfiltered averaged m/z values; (b) relative mass abundance.

Table 4 provides a statistical description of the O/C and H /C ratios of the analytes that were obtained for root exudate samples.

Table 4: Root exudates: O/C and H/C ratios and theoretical mass, statistical description.

	Mean	±SE	Median	C.I.95%	Maximum	Minimum	Range	100% Rel. abundance	
O/C	0.5	0.3	0.6	0.1	1.1	0.0	1.1	0.5	0.7
H/C	0.7	0.5	0.5	0.2	2.0	0.3	1.7	0.4	0.5
Th.mass [u]	496.14	158.02	471.34	53.91	911.57	219.15	692.42	515.30	515.30

The analyte with 100% median relative intensity as well as the major part of all other present analytes in the root exudates plotted in the CRAM (carboxyl-rich alicyclics) area and were subject to alkylation and reduction. Further analytes showed in the lipid/aliphate and black carbon region and between carbohydrates and nucleic acids (Figure 6a). High mass analytes also were relatively highly oxidized and included the two most abundant analytes. The lowest mass analytes consisted of condensed and alkylated compounds (Figure 6b).

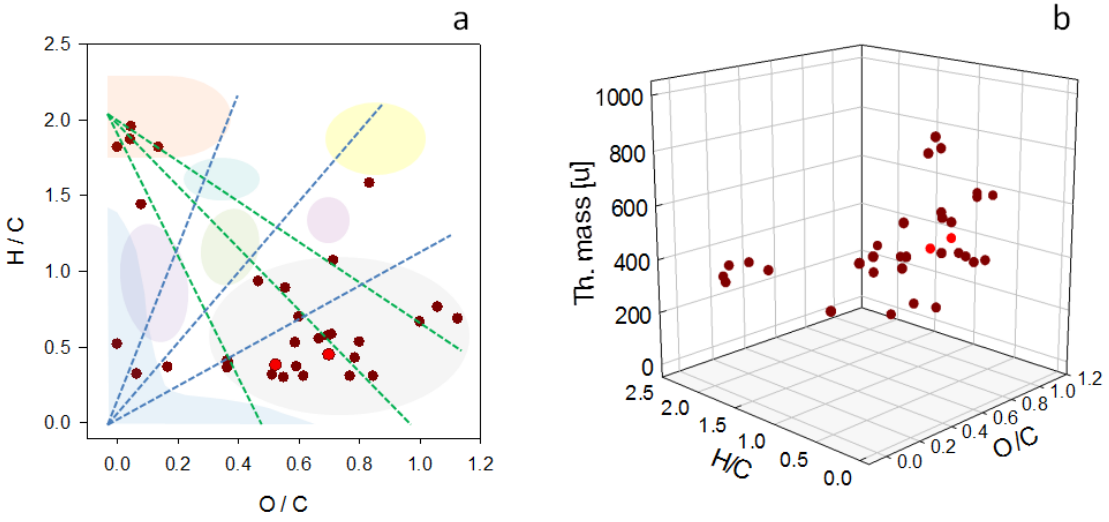

Figure 6: Root exudates, 33 analytes from 15 replicates: (a) two-dimensional van Krevelen diagram; (b) three-dimensional van Krevelen diagram; analytes of highest median relative intensity are light red; the z-axis represents the theoretical mass. Compound class areas: black carbon (blue); aromats (purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

3.1.3. Plant Leaf Litter

Undecomposed plant leaf litter

Undecomposed plant leaf litter samples contained 40 analytes from 2 replicates after filtering (see Appendix). The mean nitrogen content (n = 3) was 0.5 ± 0.2% (w/w) and the mean carbon content was 43.8 ± 5.8% (w/w). Starting at 200-300 u (0.6%), mass abundance rose between 300–400 u (6.5%) to a peak between 400–500 u (36.7%). The second highest abundance (34.6%) showed between 500–600 u, but decreased between 600-700 u (18.0%) and finally disappeared between 700–800 u (3.7%) (Figure 7, Table 5).

Table 5: Analytes in undecomposed plant leaf litter spectra.

	Mass range [u]							
	100-200	200-300	300-400	400-500	500-600	600-700	700-800	Total
n		1	5	20	9	4	1	
Rel. abundance [%]		0.6	6.5	36.7	34.6	18.0	3.7	100

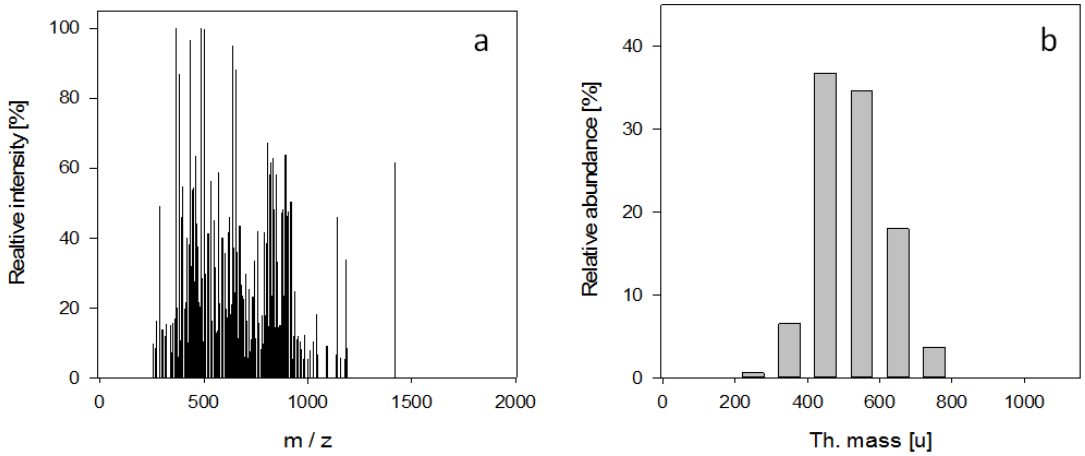

Figure 7: Undecomposed plant leaf litter, 40 analytes from 2 replicates: (a) unfiltered averaged m/z values; (b) relative mass abundance.

Table 4 provides a statistical description of the O/C and H /C ratios of the analytes that were obtained for undecomposed plant leaf litter samples.

Table 6: Undecomposed plant leaf litter: O/C and H/C ratios and theoretical mass, statistical description.

	Mean	±SE	Median	C.I.95%	Maximum	Minimum	Range	100% Rel. abundance
O/C	0.3	0.2	0.3	0.1	0.9	0.0	0.9	0.4
H/C	1.3	0.5	1.3	0.1	2.1	0.5	1.6	0.8
Th.mass [u]	482.02	91.22	477.59	28.27	705.53	221.23	484.30	601.52

The detected analytes plotted in all regions of the van Krevelen diagram, comprising all compound classes except CRAM (Figure 8a). Analyte positions that plotted outside of compound class areas indicated their oxidation. Accordingly, higher masses were characterized by advanced hydrogenation and oxidation (Figure 8b).

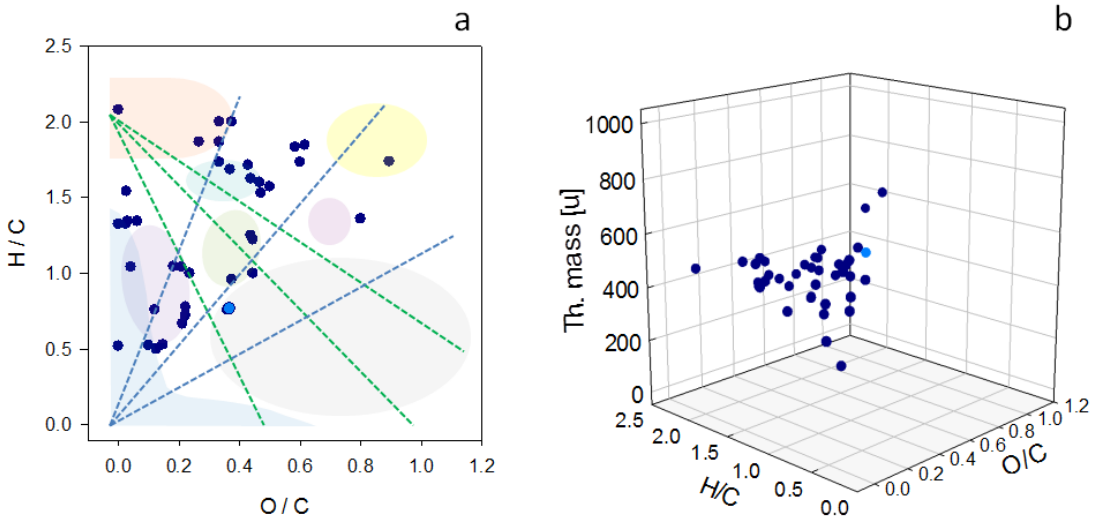

Figure 8: Undecomposed plant leaf litter, 40 analytes from 2 replicates: (a) two-dimensional van Krevelen diagram; (b) three-dimensional van Krevelen diagram; analytes of highest median relative intensity are light blue; the z-axis represents the theoretical mass. Compound class areas: black carbon(blue); aromats(purple); carboxyl-rich alicylics(grey); lignin(green); nucleic acids(pink); proteins(turquoise); carbohydrates(yellow); lipids(orange).

Decomposed plant leaf litter

Decomposed plant leaf litter data contained 283 analytes from 12 samples after filtering (see Appendix). C and N content for the soils and the respective decomposed plant leaf litter are summarized by Table 7.

Table 7: Carbon and Nitrogen weight % (w/w) of decomposed plant leaf litter and soil samples.

	Plant leaf litter		Soil	
	Cambisol+Cd	Potting soil	Cambisol+Cd	Potting soil
Carbon [%]				
Mean	1.6	1.7	0.6	0.89
± SE	0.1	0	0.1	0.07
n	6	6	4	4
Nitrogen [%]				
Mean	41.1	38.1	6.2	25.3
± SE	1.3	1.2	0.1	2.52
n	6	6	4	4

The mass spectra of the decomposed plant leaf litter samples showed no analytes in the low mass range between 100–200 u. Analytes started to appear between 200–300 u (0.7%); they became more numerous between 300–400 u (7.8%). The second highest abundance was observed between 400–500 u (19.4%). They decreased between 500–600 u (7.8%). Middle abundances occurred between 600–700 u (11.5%) and 800–900 u (11.1%). The highest abundance was found between 800–900 u (35.5%). Between 900–1000 u analyte numbers started to decrease (5.7%) and disappeared between 1000–1100 u (0.4%) (Figure 9, Table 8).

Table 8: Analytes in decomposed plant leaf litter spectra.

	Mass range [u]										
	100-200	200-300	300-400	400-500	500-600	600-700	700-800	800-900	900-1000	1000-1100	Total
n		3	15	40	30	49	36	88	18	3	
Rel. abundance [%]		0.7	7.8	19.4	7.8	11.5	11.1	35.5	5.7	0.4	100

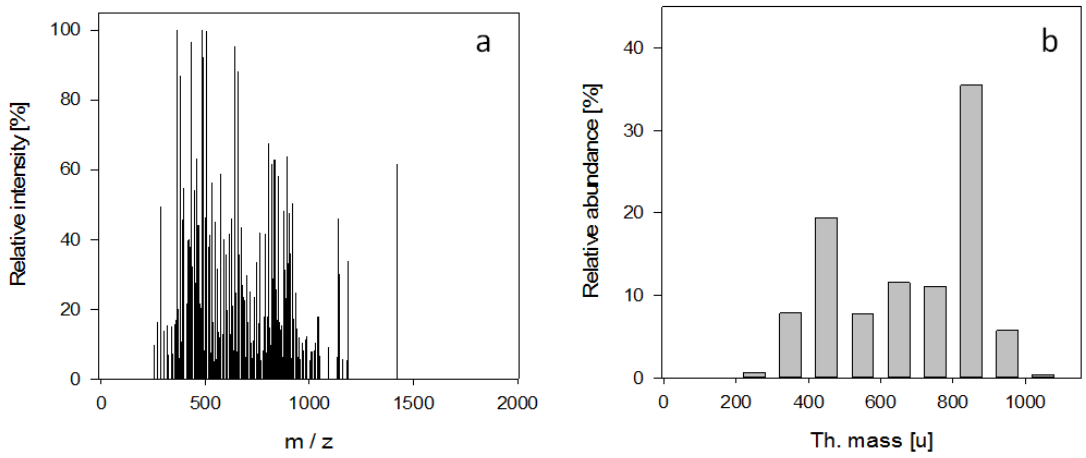

Figure 9: Decomposed plant leaf litter; 283 analytes from 12 samples: (a) unfiltered averaged m/z values; (b) relative mass abundance.

Table 9 provides a statistical description of the O/C and H/C ratios of the analytes that were obtained for decomposed plant leaf litter samples.

Table 9: Decomposed plant leaf litter: O/C and H/C ratios and theoretical mass, statistical description.

	Mean	±SE	Median	C.I.95%	Maximum	Minimum	Range	100% Rel. abundance	
O/C	0.1	0.1	0.1	0.0	1.1	0.0	1.1	0.0	0.1
H/C	1.8	0.3	1.8	0.0	2.1	0.5	1.6	1.3	0.6
Th.mass [u]	688.03	176.63	711.09	20.62	1027.46	255.32	772.13	485.72	365.39

Most analytes plotted in the lipid/aliphate region. The most abundant analytes plotted in the aromatics area and in dehydrogenation direction from the highly alkylated region (Figure 10a). Highly hydrogenated and alkylated analytes varied in their mass (Figure 10b).

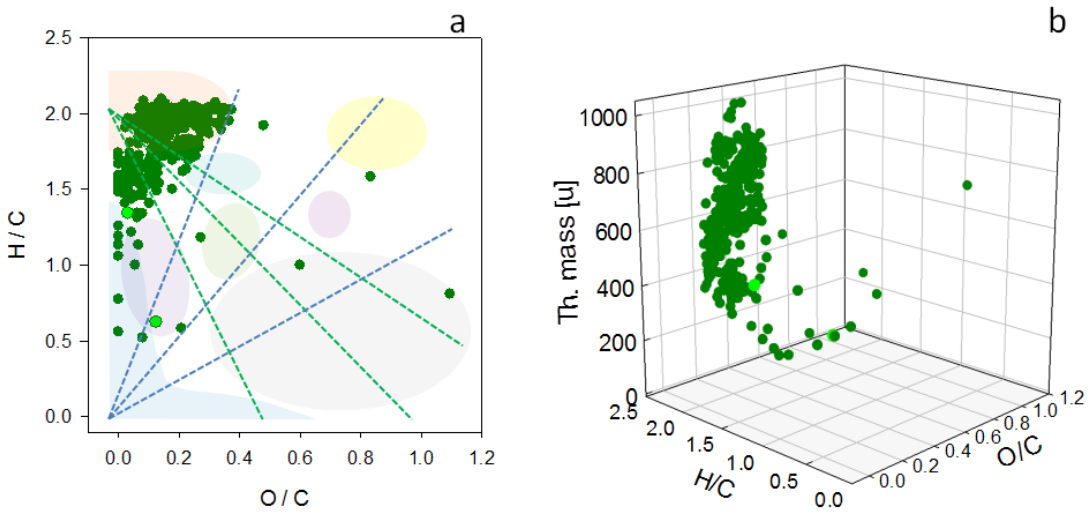

Figure 10: Decomposed plant leaf litter, 283 analytes from 12 samples: (a) two-dimensional van Krevelen diagram; (b) three dimensional van Krevelen diagram; analytes of highest median relative intensity are light green; the z-axis represents the theoretical mass. Compound class areas: black carbon(blue); aromats(purple); carboxyl-rich alicylics(grey); lignin(green); nucleic acids(pink); proteins(turquoise); carbohydrates(yellow); lipids(orange).

3.2. Statistical analysis

3.2.1. All sample types

In order to explore differences between samples, statistics were performed with the whole dataset. Relative intensities were compared to categorical data matrices, presence (1)/absence (0) and absence (1)/presence (0). The resemblance matrices are illustrated by nMDS and by an average linkage cluster analysis (Figure 11). Only the presence (1)/absence (0) categorical matrix provided a separate ordination of the three sample types, soil water, root exudate, and plant leaf litter (Figures 11c and 11d).

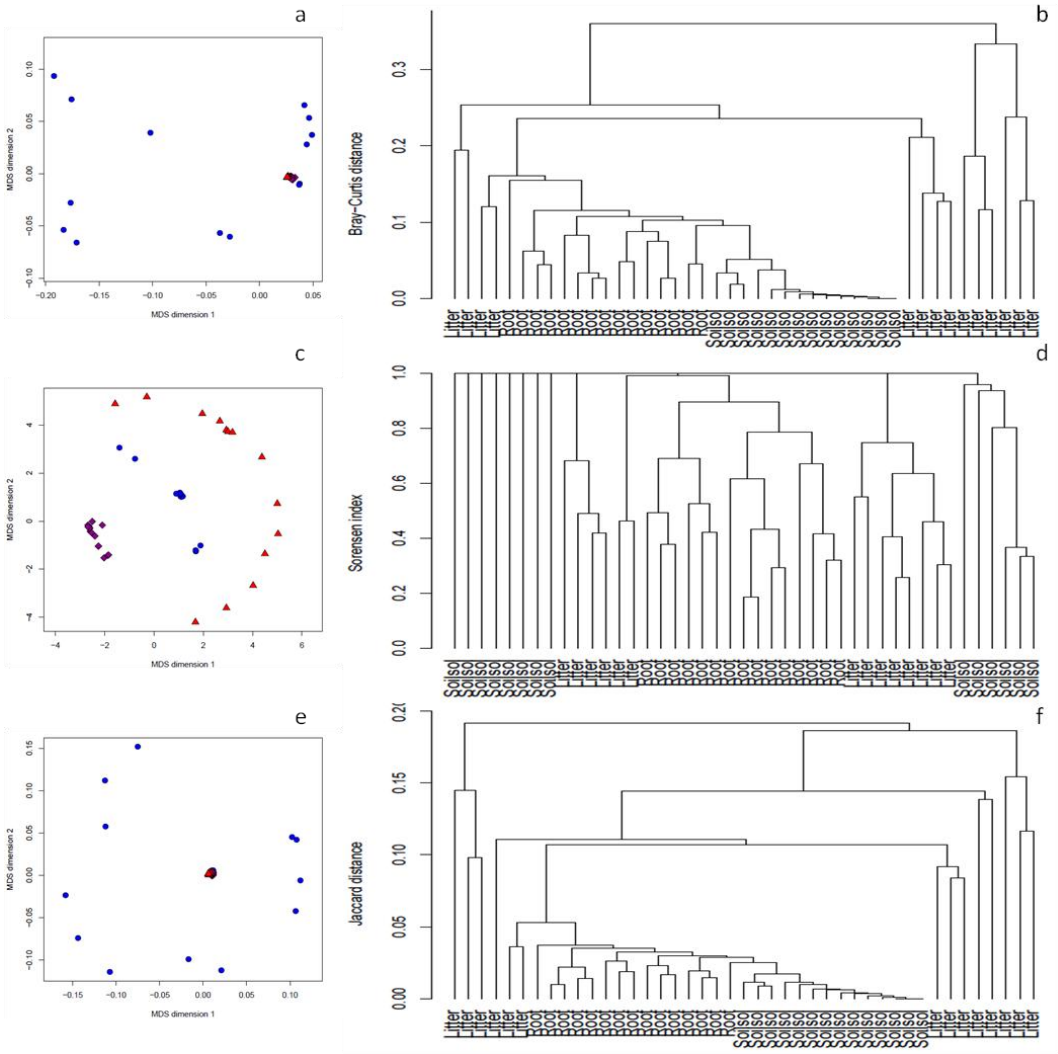

Figure 11: Whole data set, statistical analysis: (a) nMDS plot of Bray–Curtis distances in relative intensity data; (b) average linkage cluster analysis of Bray–Curtis distances in relative intensity data; (c) nMDS plot of Sørensen distances in presence (1)/absence (0) categorical data; (d) average linkage cluster analysis of Sørensen distances in presence (1)/absence (0) categorical data; (e) nMDS plot of Jaccard-distances in absence (1)/presence (0) categorical data; (f) average linkage cluster analysis of Jaccard-distances in absence (1)/presence (0) categorical data. Litter, decomposed and undecomposed plant leaf litter, blue symbols; Soilsol, soil water, red symbols; Root, root exudates, purple symbols.

Canonical discriminant analysis (CDA) of the principal coordinate axes from presence (1)/absence (0) data on factor sample type (100% classification success) yielded two canonical dimensions, CD1 and CD2 (Figure 12). CD1 contributed more to the separation of soil water from root exudates and plant leaf litter (Figure 12b), CD2 contributed more to the separation of root exudates from soil water and plant leaf litter (Figure 12c).

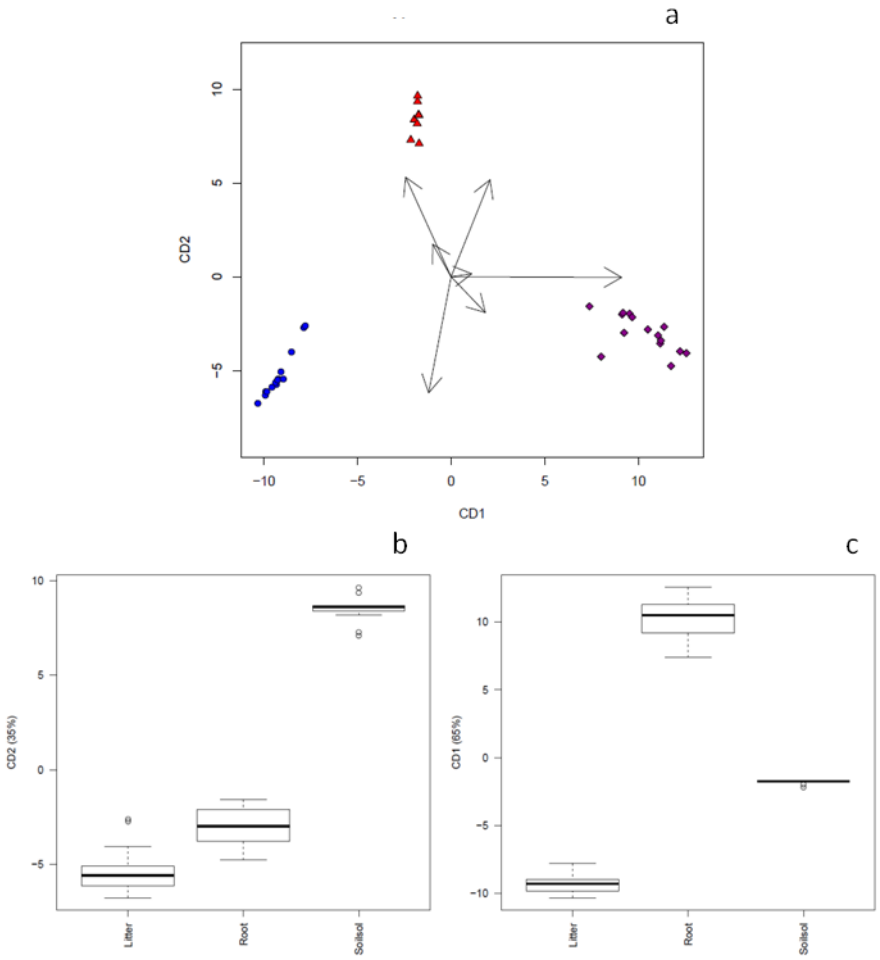

Figure 12: Whole data set, canonical discriminant analysis (CDA) of principle coordinate axes on basis of presence (1)/absence (0) categorical data using the factor sample type: (a) ordination of CDA dimension 1 (64.7% of variance, x-axis) and CDA dimension 2 (35.3% of variance, y-axis); (b) group separation by CDA dimension1; (c) group separation by CDA dimension 2. Arrows indicate the correlation with the seven principal coordinate axes; plant leaf litter, blue symbols; root exudate, purple symbols; soil water, red symbols.

The correlation of canonical discriminant analysis dimensions (CD) with the analytes suggested that CD1 (64.7% var.) positively correlates with a portion of carboxyl-rich aliphatic alicyclics and CD2 (35.3 var.) with a portion of the lipid/aliphate region (Figures 13a and 13b) in both cases over a wide molecular mass range (Figures 13c and 13d).

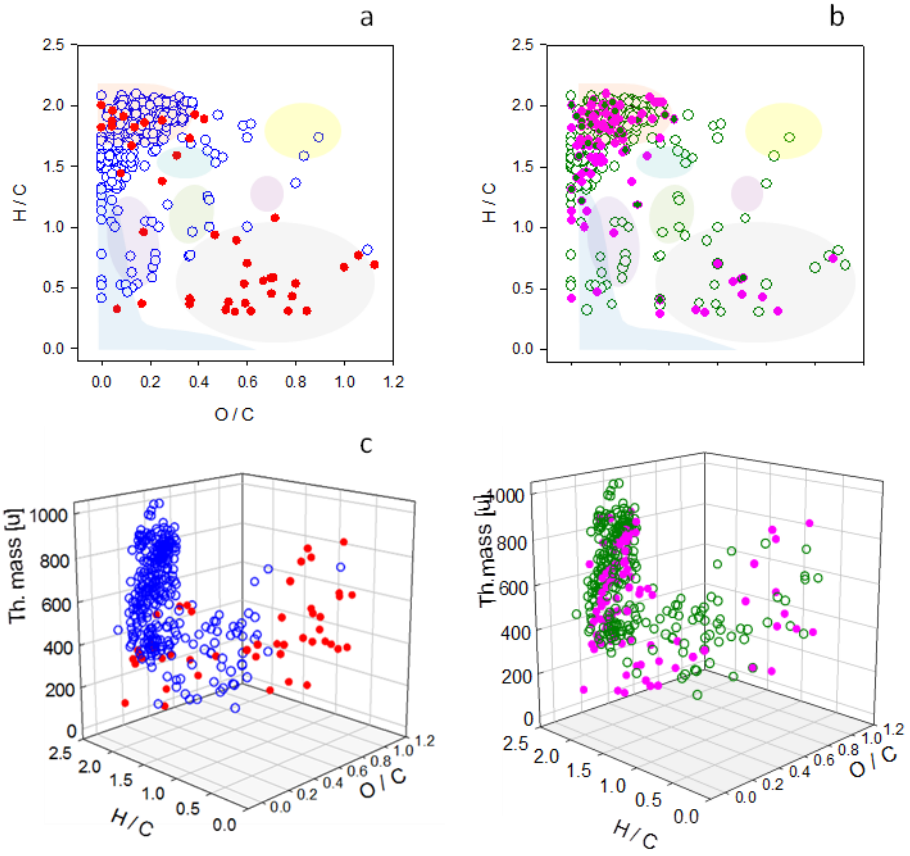

Figure 13: Whole data set, correlation of canonical discriminant analysis dimensions (CD) with analytes in the van Krevelen diagram: (a) CD1, two-dimensional diagram; (b) CD 2, two-dimensional diagram; (c) CD1, three-dimensional diagram ; (d) CD2, three-dimensional diagram. Positive correlations, full circles; negative correlations, hollow circles; the z-axis represents the theoretical mass. Compound class areas: black carbon(blue); aromats (purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

3.2.2. Soil water versus root exudate

The analysis of the whole data set suggested a presence (1)/absence (0) data matrix as the most efficient to ordinate the sample types (see Figure 11). In order to explore the specific differences between soil water and root exudate samples unconstrained and constrained statistical analysis with this data matrix were performed. The multivariate analyses showed a clear separation of the two sample types (Figure 14). The average linkage cluster analysis, however, indicated that the soil water samples did not form a uniform cluster (Figure 14a). The high difference of some of the soil water samples, especially those that can be found in the left region of the cluster diagram, is caused by a strong filtering effect of the applied criteria that reduced the number of analytes to < 3.

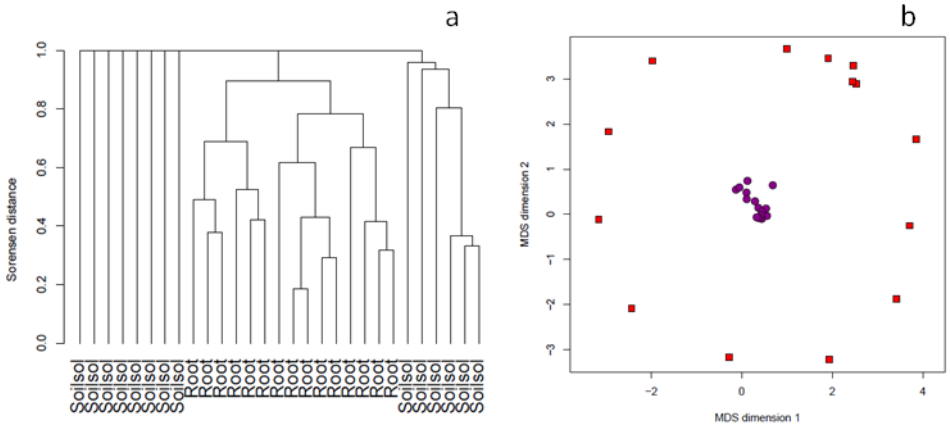

Figure 14: Root exudate and soil water samples, statistical analysis based on presence (1)/ absence (0) categorical data : (a) nMDS plot of Sørensen distances; (b) average linkage cluster analysis of Sørensen distances. Root, root exudates, purple symbols; Soilsol, soil water, red symbols.

Canonical discriminant analysis (CDA) of principle coordinate axes (100% classification success) on factor sample type yielded one dimension, CD1 (100% var.), separating root exudates (negative *r*) from soil water (positive *r*). This was not the case for soil and clone type (data not shown). A correlation of CD1 dimensions with the original data set suggested that analytes in root exudate were higher oxidized than those in the soil water (Figure 15a) but no difference was visible in the molecular mass weight range (Figure 15b).

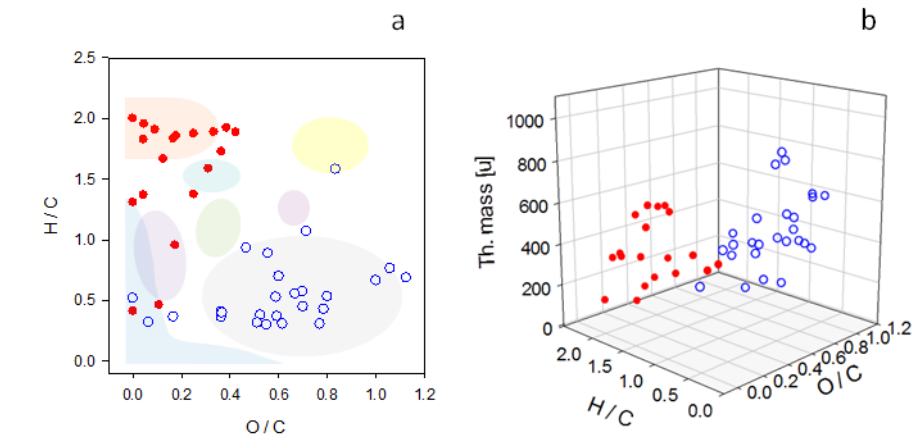

Figure 15: Root exudate and soil water samples, correlation of CDA dimensions (CD) with analytes in the van Krevelen diagram: (a) two-dimensional diagram; (b) three-dimensional diagram, the z-axis represents theoretical masses. Positive correlations, full circles, negative correlations, hollow circles; root exudates, blue symbols; soil water, red symbols. Compound class areas: black carbon (blue); aromats (purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

3.2.3. Plant leaf litter

All plant leaf litter samples

A nMDS of the presence(1)/absence(0) data of plant leaf litter samples yielded an ordination that clearly separated the undecomposed from the decomposed plant leaf litter (Figure 16a). The latter, however, showed no clear ordination but formed two distinct groups of mixed origin. In an average linkage cluster analysis, undecomposed plant leaf litter samples built a distinct cluster but decomposed plant leaf litter from the two soils were divided between two cluster groups (Figure 16b).

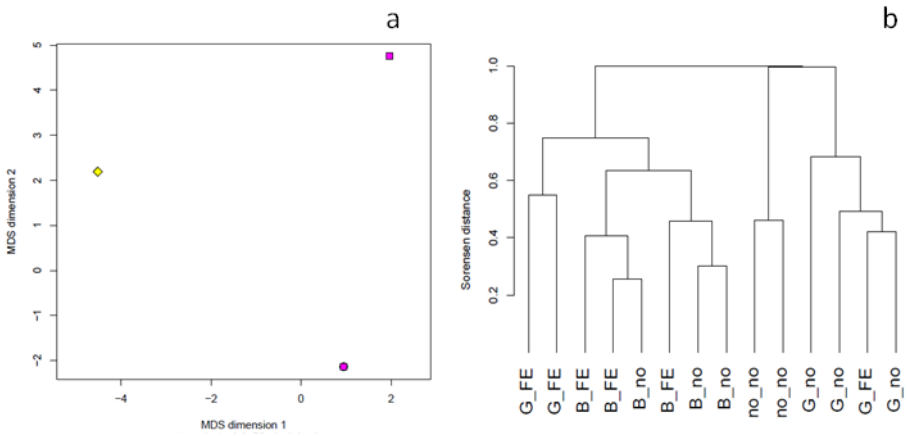

Figure 16: All plant leaf litter samples, statistical analysis based on presence (1)/ absence (0) categorical data : (a) nMDS plot of Sørensen distances; (b) average linkage cluster analysis of Sørensen distances; no_no, undecomposed plant leaf litter, yellow symbol; B_no, decomposed plant leaf litter from Cd-contaminated cambisol (– magnetite); B_FE, decomposed plant leaf litter from Cd-contaminated cambisol (+ magnetite), cyan symbol; G_no, decomposed plant leaf litter from potting soil (– magnetite); G_FE, decomposed plant leaf litter from potting soil (+ magnetite), pink symbol.

Canonical discriminant analysis (CDA) of principle coordinate axes on factor soil type had 100% classification success and yielded 2 dimensions (CD). CD1 (99.4% of variance) separated undecomposed plant leaf litter (negative *r*) from plant leaf litter decomposed in Cd-contaminated cambisol and potting soil (positive *r*) (Figure 17).

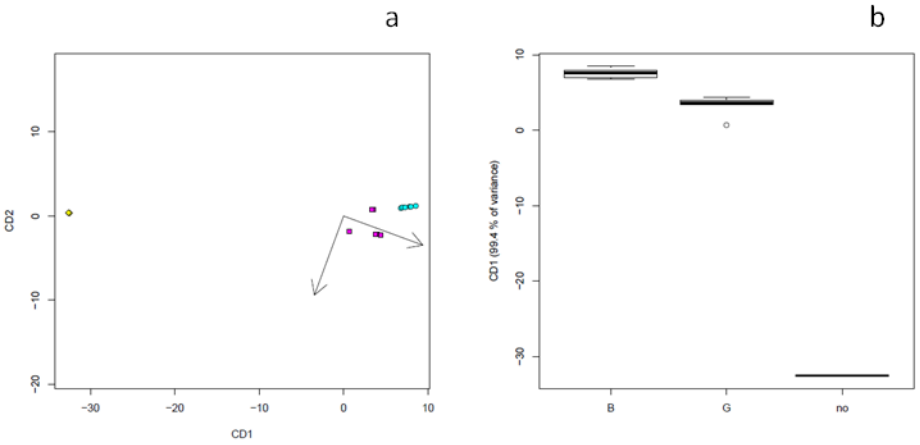

Figure 17: All plant leaf litter samples, canonical discriminant analysis (CDA) of principle coordinate axes based on presence (1)/absence (0) categorical data using the factor soil type: (a) CDA dimension1 (99.4% var., x-axis) and 2 (0.6% var., y-axis); (b) group separation by CDA dimension 1; no, undecomposed plant leaf litter, yellow symbols; B, decomposed plant leaf litter from Cd-contaminated cambisol, cyan symbols; G, decomposed plant leaf litter from potting soil, pink symbol; arrows indicate the correlation with the two principal coordinate axes;

The correlation of CD1 with analytes suggested that undecomposed plant leaf litter contained rather diverse analytes that belonged to various compound classes. By contrast, decomposed plant leaf litter analytes mainly plotted in the lipid/aliphate region (Figure 18a). Sample types did not differ in the molecular mass range (Figure 18b).

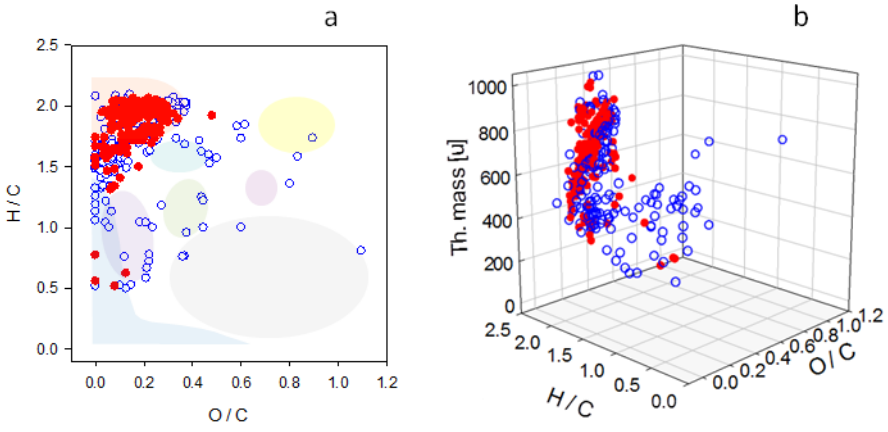

Figure 18: All plant leaf litter samples, correlation of CDA dimension 1 with analytes in the van Krevelen diagram: (a) two-dimensional diagram; (b) three-dimensional diagram, the z-axis represents theoretical masses. Positive correlations, full circles, negative correlations, hollow circles; undecomposed plant leaf litter, blue; decomposed plant leaf litter, red. Compound class areas: black carbon (blue); aromats (purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

Decomposed plant leaf litter samples

A separate multivariate analysis of the decomposed plant leaf litter samples showed a similar picture to that of all plant leaf litter samples (data not shown). Canonical discriminant analysis (CDA) of principle coordinate axes on factor soil type had 100% classification success and yielded one dimension (CD). CD1 (100% of variance explained) separated Cd-contaminated cambisol (negative *r*) from potting soil (positive *r*) (Figure19). CDA of principle coordinate axes on factor magnetite treatment had only 75% classification success and did not separate the factor groups.

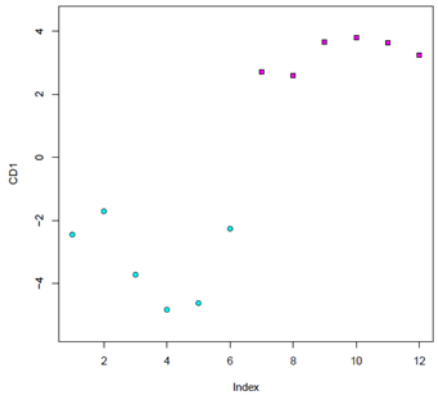

Figure 19: Decomposed plant leaf litter, CDA of principle coordinate axes on factor soil type. CD1, x-axis; sample number, y-axis: Cd-contaminated cambisol, cyan symbols; potting soil, pink symbols.

The correlation with canonical analysis dimension 1 (from canonical discriminant analysis on factor soil type) suggested that the potting soil contained more low-molecular-weight aromatic analytes than the Cd-contaminated cambisol (Figures 20a and 20b).

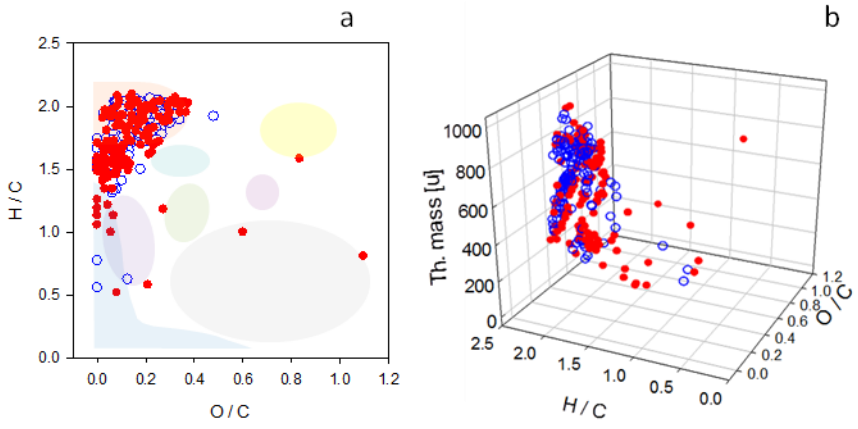

Figure 20: Decomposed plant leaf litter samples, correlation of CD 1 with analytes in the van Krevelen diagram: (s) two-dimensional diagram; (b) three-dimensional diagram, the z-axis represents the theoretical mass. Positive correlations, full circles; negative correlations, hollow circles; Cd-contaminated cambisol, blue; potting soil, red. Compound class areas: black carbon (blue); aromats (purple); carboxyl-rich alicylics (grey); lignin (green); nucleic acids (pink); proteins (turquoise); carbohydrates (yellow); lipids (orange).

4. Discussion

The application of high-resolution mass spectrometry in studies of soil organic matter dynamics is very recent. This study utilizes a comparatively broad range of sample types, ranging from plant leaf litter to soil water. The therein-identified carbohydrates all represent a portion of soil organic matter (SOM). Their solubility in aqueous and organic solvents presents a barrier that usually excludes molecules that are larger than 1200 u from analysis. The various sample types, decomposed and undecomposed plant leaf litter, root exudates and soil water yielded characteristic spectra that allowed their differentiation, even with the naked eye (see Appendix). Compared to other spectroscopic methods, such as IR and NMR spectroscopy, the advantage of HR-MS lies in the fact that every analyte more or less generates a specific signal in the spectrum, which reveals its molecular mass. In case of both IR and NMR spectra, single analytes create complex spectra that complicate structure elucidation by signal overlap. All chromatography-based methods also suffer from the fact, that the major portion of analytes remains undetected. In general, gas chromatography strongly discriminates against less volatile analytes and even liquid chromatography was shown to fail resolving complex mixtures. A good example represents an analytical study on thearubigins, the most abundant group of phenolics in black tea; LC–MS identified 36 analytes whereas FT-ICR-MS detected more than 5000 (Kuhnert et al., 2010).

In order to assign exact elemental formulas to the masses in the spectrum, filtering rules have to be applied. General recommendations do exist (Kind and Fiehn, 2007), however, here rules specifically recommended for organic matter have been followed (Chen et al., 2011; Stubbins et al., 2010). Moreover, all masses that showed a charge of 0 were excluded from the analysis to avoid including artifacts that could have been generated during the ionization process; they are being ignored in most studies (Stubbins et al., 2010). These prerequisites are important for a realistic assignment of molecular formulas, which is usually performed with a appropriate software, in this present study this has been done with Thermo XCalibur. A complete analyte characterization in the sample will always be hampered by discrimination during ionization. The ESI mode is best suited, since it represents a very soft ionization mode generation H^+ and Na^+ adducts (Sleighter and Hatcher, 2007). Without doubt, the exact procedure of filtering masses to determine elemental formulas for realistic analytes affects the quality of the result, but its standardization is still in progress and not yet completed.

An analysis of the obtained spectra revealed that high-resolution FT-MS with an LTQ-Orbitrap-MS yielded results that allowed discrimination of the sample types. Average van Krevelen diagrams showed that plant leaf litter and decomposed plant leaf litter contained highly diverse analytes. The van Krevelen diagram of the plant leaf litter sample indicated presence of analytes that can be

attributed to the expected compound classes in leaves, proteins, phenols, carbohydrates and some lipids. A comparison of the van Krevelen diagram and the molecular mass range of the detected analytes substantiates the view that during decomposition depolymerization of cellulose and lignin leads to the formation of small molecules that show a tendency to re-polymerize again. This consequently corroborates the hypothes which views such processes as fundamental for the formation of humic acids (Stevenson, 1994). The majority of the detected analytes in the decomposed plant leaf litter, however, are not aromatic but aliphatic compounds, which not only contain oxygen, but also nitrogen and sulfur. The plant leaf litter decomposition experiment was performed in two different soil types, a cadmium-contaminated cambisol and a potting soil. The obtained results reveal that a higher generation of low-molecular weight compounds in the potting soil, which could be interpreted as some sort of a soil-specific effect on the decomposition process. Oxidative depolymerization is catalyzed by transition metals, such as iron and copper (Stevenson, 1994). Addition of magnetite, however, did not affect the process such that an effect could be detected in high-resolution mass spectroscopy.

Other sample types included root exudates and soil water from the same willow species. These samples differed fundamentally from the plant leaf litter samples because the number of detectable analytes was much lower. Root exudates and soil water were chemically different; the former were mainly comprised of carboxyl-rich alicyclic compounds, the latter of lipids and their condensation products. These analytes also often contained nitrogen and to a lesser percentage, sulfur. This concurs with the view that root-exuded plant metabolites somehow trigger different reactions compared to those that can be observed in soil water. It is possible that these differences are caused by exuded trigger solutions of plant roots which exert a stimulating effect on rhizosphere microorganisms (De Nobili et al., 2001).

Despite the preliminary character of this study, it stimulates further exploration of the applicability of high-resolution mass spectroscopy in understanding soil chemical processes. The obtained results support some of the currently pursued hypotheses and thus promise to procure results that facilitate better insights into this complex issue.

5. References

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6. Appendix

6.1. Elemental composition and atomic ratios

Abbreviations: Avg. Mass, averaged mass; Th. Mass, theoretical mass; Decomp. l., decomposed plant leaf litter; B, cambisol ± magnetite; G, potting soil ± magnetite; Fresh l., undecomposed plant leaf litter , Root e., root exudates; Soil w., soil water.

0, absence; 1, presence.

Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
1	135.06582	136.14977	C6H11O1N1Na1	0.2	1.8	0	0	0	1	1
2	**157.07982**	**158.26300**	**C7H14N2S1**	**0.0**	**2.0**	0	0	**0**	0	1
3	184.10812	185.22700	C8H15O2N3	0.3	1.9	0	0	0	0	1
4	**209.10252**	**210.22877**	**C9H17O3N1Na1**	**0.3**	**1.9**	0	0	**0**	2	3
5	218.01942	219.14777	C9H8O5Na1	0.6	0.9	0	0	0	0	0
6	**220.01762**	**221.23400**	**C9H7O2N3S1**	**0.2**	**0.8**	0	0	**1**	0	0
7	253.97592	255.20000	C9H5O6N1S1	0.7	0.6	0	0	0	3	0
8	**254.12962**	**255.32100**	**C15H17O1N3**	**0.1**	**1.1**	0	1	**0**	0	0
9	267.99162	269.22700	C10H7O6N1S1	0.6	0.7	0	0	0	7	0
10	**272.11742**	**273.33077**	**C18H18O1Na1**	**0.1**	**1.0**	0	1	**0**	0	0
11	285.08432	286.24677	C11H13O3N5Na1	0.3	1.2	0	1	0	0	0
12	**300.11882**	**301.29077**	**C12H22O7Na1**	**0.6**	**1.8**	0	0	**2**	0	0
13	300.13402	301.33777	C16H22O4Na1	0.3	1.4	0	0	0	0	1
14	**300.13532**	**301.34877**	**C17H18N4Na1**	**0.0**	**1.1**	0	1	**0**	0	0
15	316.10782	317.36400	C11H19O4N5S1	0.4	1.7	0	0	0	0	1
16	**316.10832**	**317.36677**	**C12H18O1N6Na1S1**	**0.1**	**1.5**	0	1	**0**	0	0
17	318.19692	319.45200	C21H25N3	0.0	1.2	0	1	0	0	0
18	**340.17992**	**341.46577**	**C15H30O3N2Na1S1**	**0.2**	**2.0**	0	1	**0**	0	0
19	342.19552	343.46577	C23H28O1Na1	0.0	1.2	0	1	0	0	0
20	**352.27362**	**353.54577**	**C23H38O1Na1**	**0.0**	**1.7**	3	1	**0**	0	0
21	359.31637	360.58177	C22H43O1N1Na1	0.0	2.0	0	0	0	3	3
22	**359.31712**	**360.59000**	**C22H40N4**	**0.0**	**1.8**	0	0	**0**	0	0
23	364.09896	365.29500	C12H19O10N3	0.8	1.6	0	3	0	0	0
24	**364.09742**	**365.38800**	**C24H15O3N1**	**0.1**	**0.6**	1	0	**0**	0	0
25	364.09760	365.39077	C25H14N2Na1	0.0	0.6	4	0	0	0	0
26	**370.13982**	**371.41177**	**C12H24O4N6Na1S1**	**0.3**	**2.0**	0	0	**2**	0	0
27	375.28987	376.58100	C24H40O3	0.1	1.7	0	0	0	1	2
28	**380.07299**	**381.29700**	**C15H15O9N3**	**0.6**	**1.0**	0	3	**0**	0	0
29	381.07637	382.37100	C24H14O5	0.2	0.6	0	2	0	0	0
30	**381.07647**	**382.37377**	**C25H13O2N1Na1**	**0.1**	**0.5**	0	1	**0**	0	0
31	381.07592	382.38977	C17H17O4N3Na1S1	0.2	1.0	0	0	1	0	0
32	**382.10924**	**383.41400**	**C25H13N5**	**0.0**	**0.5**	0	0	**2**	0	0
33	388.03376	389.33100	C14H15O10N1S1	0.7	1.1	0	0	0	0	0
34	**388.03412**	**389.33377**	**C15H14O7N2Na1S1**	**0.5**	**0.9**	0	0	**0**	0	0
35	393.31222	394.60277	C24H41N3Na1	0.0	1.7	0	4	0	0	0
36	**393.29332**	**394.66200**	**C23H42O1N2S1**	**0.0**	**1.8**	0	0	**0**	0	1
37	394.19722	395.44777	C19H32O7Na1	0.4	1.7	0	0	2	0	0
38	**394.32286**	**395.63500**	**C26H41N3**	**0.0**	**1.6**	1	0	**0**	0	0
39	397.37982	398.67600	C24H50O2N2	0.1	2.1	0	1	0	0	0
40	**407.30202**	**408.58700**	**C22H40O3N4**	**0.1**	**1.8**	0	0	**0**	0	0

Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
41	408.30532	409.67700	C23H43O1N3S1	0.0	1.9	0	0	0	0	0
42	**410.17117**	**411.47677**	**C15H28O4N6Na1S1**	**0.3**	**1.9**	0	0	**2**	0	0
43	412.36975	413.69400	C27H47N3	0.0	1.7	4	0	0	0	0
44	**416.36412**	**417.68200**	**C26H47O1N3**	**0.0**	**1.8**	0	2	**0**	0	0
45	418.98592	420.20600	C13H4O11N6	0.8	0.3	0	0	0	0	0
46	**420.33619**	**421.66477**	**C28H46O1Na1**	**0.0**	**1.6**	3	0	**0**	0	0
47	423.27602	424.58900	C25H36O2N4	0.1	1.4	0	0	0	0	0
48	**424.15047**	**425.45977**	**C15H26O5N6Na1S1**	**0.3**	**1.7**	0	0	**2**	0	0
49	425.15387	426.53377	C24H25O1N3Na1S1	0.0	1.0	0	0	2	0	0
50	**425.98092**	**427.19677**	**C13H4O10N6Na1**	**0.8**	**0.3**	0	0	**0**	0	0
51	426.12977	427.43177	C14H24O6N6Na1S1	0.4	1.7	0	0	2	0	0
52	**426.16607**	**427.47577**	**C15H28O5N6Na1S1**	**0.3**	**1.9**	0	0	**2**	0	0
53	428.36466	429.69300	C27H47O1N3	0.0	1.7	3	0	0	0	0
54	**429.37002**	**430.67677**	**C25H49O1N3Na1**	**0.0**	**2.0**	0	1	**0**	0	0
55	432.33632	433.67577	C29H46O1Na1	0.0	1.6	0	1	0	0	0
56	**432.33810**	**433.68400**	**C29H43N3**	**0.0**	**1.5**	0	4	**0**	0	0
57	433.00152	434.23300	C14H6O11N6	0.8	0.4	0	0	0	1	0
58	**433.33957**	**434.62477**	**C22H45O2N5Na1**	**0.1**	**2.0**	0	2	**0**	0	0
59	433.95592	435.26677	C15H8O12Na1S1	0.8	0.5	0	0	0	0	0
60	**434.35182**	**435.69177**	**C29H48O1Na1**	**0.0**	**1.7**	1	0	**0**	0	0
61	434.35357	435.70000	C29H45N3	0.0	1.6	0	4	0	0	0
62	**438.28742**	**439.60400**	**C25H37O2N5**	**0.1**	**1.5**	1	0	**0**	0	0
63	440.10912	441.41477	C14H22O7N6Na1S1	0.5	1.6	0	0	1	0	0
64	**444.11212**	**445.48877**	**C23H22O4N2Na1S1**	**0.2**	**1.0**	0	0	**0**	0	1
65	446.11952	447.41877	C13H24O8N6Na1S1	0.6	1.8	0	0	2	0	0
66	**446.35182**	**447.70277**	**C30H48O1Na1**	**0.0**	**1.6**	2	0	**0**	0	0
67	446.35370	447.71100	C30H45N3	0.0	1.5	0	4	0	0	0
68	**447.12287**	**448.49277**	**C22H23O4N3Na1S1**	**0.2**	**1.0**	0	0	**1**	0	0
69	447.35510	448.65177	C23H47O2N5Na1	0.1	2.0	5	2	0	0	0
70	**448.33300**	**449.68300**	**C29H43O1N3**	**0.0**	**1.5**	0	4	**0**	0	0
71	448.36742	449.71877	C30H50O1Na1	0.0	1.7	2	1	0	0	0
72	**448.36932**	**449.72700**	**C30H47N3**	**0.0**	**1.6**	0	4	**0**	0	0
73	450.32932	451.61177	C21H44O3N6Na1	0.1	2.1	0	1	0	0	0
74	**450.32572**	**451.70977**	**C24H48O2N2Na1S1**	**0.1**	**2.0**	0	1	**0**	0	0
75	451.32897	452.64277	C25H43O1N5Na1	0.0	1.7	0	2	0	0	0
76	**452.32805**	**453.67100**	**C28H43O2N3**	**0.1**	**1.5**	0	4	**0**	0	0
77	452.30682	453.67400	C31H39N3	0.0	1.3	0	1	0	0	0
78	**452.30492**	**453.68177**	**C23H46O3N2Na1S1**	**0.1**	**2.0**	0	2	**0**	0	0
79	454.12477	455.44177	C15H24O7N6Na1S1	0.5	1.6	0	0	2	0	0
80	**458.19242**	**459.51777**	**C16H32O6N6Na1S1**	**0.4**	**2.0**	0	0	**1**	0	0
81	460.36752	461.72977	C31H50O1Na1	0.0	1.6	1	1	0	0	0
82	**460.36957**	**461.73800**	**C31H47N3**	**0.0**	**1.5**	0	2	**0**	0	0
83	461.37072	462.67877	C24H49O2N5Na1	0.1	2.0	4	2	0	0	0
84	**462.09347**	**463.42077**	**C16H20O7N6Na1S1**	**0.4**	**1.3**	0	0	**2**	0	0
85	462.34877	463.71000	C30H45O1N3	0.0	1.5	0	2	0	0	0
86	**463.09682**	**464.47600**	**C32H16O4**	**0.1**	**0.5**	0	0	**1**	0	0
87	463.09672	464.49477	C25H19O3N3Na1S1	0.1	0.8	0	0	1	0	0
88	**466.09402**	**467.44400**	**C28H13O3N5**	**0.1**	**0.5**	0	0	**0**	2	2
89	466.09422	467.44677	C29H12N6Na1	0.0	0.4	0	0	0	1	1
90	**468.14042**	**469.46877**	**C16H26O7N6Na1S1**	**0.4**	**1.6**	0	0	**1**	0	0
91	468.35917	469.71400	C29H47O2N3	0.1	1.6	0	4	0	0	0
92	**469.36054**	**470.80400**	**C30H50N2S1**	**0.0**	**1.7**	4	0	**0**	0	0
93	470.03717	471.34100	C22H9O8N5	0.4	0.4	0	0	0	0	0
94	**476.39882**	**477.77277**	**C32H54O1Na1**	**0.0**	**1.7**	0	1	**0**	0	0
95	476.40077	477.78100	C32H51N3	0.0	1.6	0	2	0	0	0

Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
96	**478.38012**	**479.75300**	**C31H49O1N3**	**0.0**	**1.6**	0	1	**0**	0	0
97	480.35932	481.72500	C30H47O2N3	0.1	1.6	0	1	0	0	0
98	**482.37502**	**483.74100**	**C30H49O2N3**	**0.1**	**1.6**	0	1	**0**	0	0
99	484.33292	485.71600	C32H43O1N3	0.0	1.3	0	1	1	0	0
100	**484.33522**	**485.76677**	**C29H50O2Na1S1**	**0.1**	**1.7**	0	1	**0**	0	0
101	485.33442	486.65677	C25H45O3N5Na1	0.1	1.8	0	2	0	0	0
102	**488.13017**	**489.45577**	**C15H26O9N6Na1S1**	**0.6**	**1.7**	0	0	**2**	0	0
103	488.40080	489.79200	C33H51N3	0.0	1.5	0	4	0	0	0
104	**489.13352**	**490.52977**	**C24H25O5N3Na1S1**	**0.2**	**1.0**	0	0	**1**	0	0
105	489.40195	490.73277	C26H53O2N5Na1	0.1	2.0	6	2	0	0	0
106	**490.38017**	**491.76400**	**C32H49O1N3**	**0.0**	**1.5**	0	2	**0**	0	0
107	490.37952	491.78277	C25H52N6Na1S1	0.0	2.1	0	0	1	0	0
108	**496.13552**	**497.46000**	**C24H23O9N3**	**0.4**	**1.0**	0	0	**1**	0	0
109	496.13542	497.47877	C17H26O8N6Na1S1	0.5	1.5	0	0	1	0	0
110	**499.98532**	**501.27477**	**C20H6O11N4Na1**	**0.6**	**0.3**	0	0	**0**	1	0
111	500.32787	501.71500	C32H43O2N3	0.1	1.3	0	1	1	0	0
112	**501.32932**	**502.65577**	**C25H45O4N5Na1**	**0.2**	**1.8**	0	2	**0**	0	0
113	504.10422	505.43900	C25H19O9N3	0.4	0.8	0	0	1	0	0
114	**504.10402**	**505.45777**	**C18H22O8N6Na1S1**	**0.4**	**1.2**	0	0	**1**	0	0
115	504.37262	505.80177	C28H54O2N2Na1S1	0.1	1.9	0	1	0	0	0
116	**505.10752**	**506.51300**	**C34H18O5**	**0.1**	**0.5**	0	0	**2**	0	0
117	505.39684	506.73177	C26H53O3N5Na1	0.1	2.0	6	1	0	0	0
118	**505.37592**	**506.73477**	**C29H49O1N5Na1**	**0.0**	**1.7**	0	1	**0**	0	0
119	514.00072	515.29900	C20H9O14N3	0.7	0.5	0	0	0	1	0
120	**514.00092**	**515.30177**	**C21H8O11N4Na1**	**0.5**	**0.4**	0	0	**0**	0	0
121	515.00460	516.40000	C22H8O8N6S1	0.4	0.4	0	0	0	3	0
122	**516.43002**	**517.83777**	**C35H58O1Na1**	**0.0**	**1.7**	1	0	**0**	0	0
123	516.43222	517.84600	C35H55N3	0.0	1.6	0	1	0	0	0
124	**517.43324**	**518.78677**	**C28H57O2N5Na1**	**0.1**	**2.0**	6	0	**0**	0	0
125	520.36742	521.80077	C28H54O3N2Na1S1	0.1	1.9	0	2	0	0	0
126	**521.39174**	**522.73077**	**C26H53O4N5Na1**	**0.2**	**2.0**	6	0	**0**	0	0
127	521.37077	522.73377	C29H49O2N5Na1	0.1	1.7	0	1	0	0	0
128	**524.23767**	**525.69500**	**C40H31N1**	**0.0**	**0.8**	2	0	**0**	0	0
129	533.42817	534.78577	C28H57O3N5Na1	0.1	2.0	6	1	0	0	0
130	**534.44272**	**535.86100**	**C35H57O1N3**	**0.0**	**1.6**	0	1	**0**	0	0
131	536.36242	537.79977	C28H54O4N2Na1S1	0.1	1.9	0	1	0	0	0
132	**538.30332**	**539.67977**	**C30H40O2N6Na1**	**0.1**	**1.3**	1	0	**0**	0	0
133	539.30672	540.64700	C25H48O12	0.5	1.9	1	0	0	0	0
134	**541.31352**	**542.68100**	**C28H42O5N6**	**0.2**	**1.5**	1	0	**0**	0	0
135	548.39882	549.85477	C30H58O3N2Na1S1	0.1	1.9	0	1	0	0	0
136	**549.33837**	**550.69000**	**C26H50O10N2**	**0.4**	**1.9**	0	0	**0**	1	2
137	549.42308	550.78477	C28H57O4N5Na1	0.1	2.0	4	0	0	0	0
138	**550.32252**	**551.67400**	**C26H49O11N1**	**0.4**	**1.9**	0	0	**0**	0	1
139	550.34172	551.78277	C28H52O5N2Na1S1	0.2	1.9	0	0	0	0	1
140	**552.04022**	**553.50800**	**C30H11O5N5S1**	**0.2**	**0.4**	0	0	**0**	0	0
141	552.04052	553.51077	C31H10O2N6Na1S1	0.1	0.3	0	0	0	0	0
142	**557.46450**	**558.85177**	**C31H61O2N5Na1**	**0.1**	**2.0**	6	1	**0**	0	0
143	558.44222	559.88300	C37H57O1N3	0.0	1.5	0	0	1	0	0
144	**565.31232**	**566.69200**	**C29H46O9N2**	**0.3**	**1.6**	0	0	**0**	0	1
145	565.41799	566.78377	C28H57O5N5Na1	0.2	2.0	1	0	0	0	0
146	**567.41952**	**568.90200**	**C32H60O4N2S1**	**0.1**	**1.9**	1	0	**0**	0	0
147	572.42572	573.90900	C42H55N1	0.0	1.3	0	0	0	3	3
148	**573.45941**	**574.85077**	**C31H61O3N5Na1**	**0.1**	**2.0**	6	1	**0**	0	0
149	573.44132	574.91000	C30H62O4N4S1	0.1	2.1	0	2	0	0	0
150	**573.44152**	**574.91277**	**C31H61O1N5Na1S1**	**0.0**	**2.0**	0	1	**0**	0	0
151	574.41622	575.88500	C40H53N3	0.0	1.3	0	0	1	0	0

Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
152	**582.99192**	**584.27077**	**C16H11O18N5Na1**	**1.1**	**0.7**	0	0	**0**	0	0
153	584.15162	585.52200	C27H27O12N3	0.4	1.0	0	0	2	0	0
154	**585.15492**	**586.59600**	**C36H26O8**	**0.2**	**0.7**	0	0	**1**	0	0
155	585.49562	586.90300	C32H66O5N4	0.2	2.1	1	0	0	0	0
156	**585.49581**	**586.90577**	**C33H65O2N5Na1**	**0.1**	**2.0**	2	0	**0**	0	0
157	588.43002	589.91977	C33H62O3N2Na1S1	0.1	1.9	0	1	0	0	0
158	**589.45431**	**590.84977**	**C31H61O4N5Na1**	**0.1**	**2.0**	6	0	**0**	0	0
159	590.41122	591.88400	C40H53O1N3	0.0	1.3	0	0	2	0	0
160	**596.00502**	**597.34877**	**C24H14O17Na1**	**0.7**	**0.6**	0	0	**0**	0	0
161	597.00752	598.29777	C17H13O18N5Na1	1.1	0.8	0	0	0	0	0
162	**597.96322**	**599.32377**	**C26H8O16Na1**	**0.6**	**0.3**	0	0	**0**	0	0
163	597.96260	599.33977	C18H12O18N2Na1S1	1.0	0.7	0	0	0	0	0
164	**600.12542**	**601.52400**	**C30H23O11N3**	**0.4**	**0.8**	0	0	**1**	0	0
165	601.12882	602.51277	C19H33O17N1Na1S1	0.9	1.7	0	0	1	0	0
166	**601.12902**	**602.60077**	**C40H21O4N1Na1**	**0.1**	**0.5**	0	0	**1**	0	0
167	601.49072	602.90477	C33H65O3N5Na1	0.1	2.0	5	1	0	0	0
168	**601.47312**	**602.94800**	**C40H62O2N2**	**0.1**	**1.6**	0	1	**0**	0	0
169	608.13082	609.54977	C33H22O7N4Na1	0.2	0.7	0	0	1	0	0
170	**611.97892**	**613.35077**	**C27H10O16Na1**	**0.6**	**0.4**	0	0	**0**	0	0
171	617.48542	618.90100	C32H66O7N4	0.2	2.1	1	0	0	0	0
172	**617.48566**	**618.90377**	**C33H65O4N5Na1**	**0.1**	**2.0**	5	0	**0**	0	0
173	617.46802	618.96577	C33H65O2N5Na1S1	0.1	2.0	0	1	0	0	0
174	**621.48055**	**622.89177**	**C32H65O5N5Na1**	**0.2**	**2.0**	1	0	**0**	0	0
175	625.52702	626.97077	C36H69O2N5Na1	0.1	1.9	2	1	0	0	0
176	**1259.99610**	**631.47877**	**C30H12O11N2Na1S1**	**0.4**	**0.4**	0	0	**0**	1	0
177	633.48059	634.90277	C33H65O5N5Na1	0.2	2.0	2	1	0	0	0
178	**641.52182**	**642.96700**	**C35H70O6N4**	**0.2**	**2.0**	1	0	**0**	0	0
179	641.52201	642.96977	C36H69O3N5Na1	0.1	1.9	5	2	0	0	0
180	**641.50112**	**642.97277**	**C39H65O1N5Na1**	**0.0**	**1.7**	0	1	**0**	0	0
181	642.52482	644.04100	C44H69O2N1	0.0	1.6	1	0	0	0	0
182	**642.52462**	**644.05977**	**C37H72O1N4Na1S1**	**0.0**	**1.9**	1	0	**0**	0	0
183	647.36902	648.85700	C30H56O9N4S1	0.3	1.9	1	0	0	0	0
184	**649.45442**	**650.90477**	**C36H61O4N5Na1**	**0.1**	**1.7**	0	1	**0**	0	0
185	656.49612	657.93800	C34H67O7N5	0.2	2.0	0	1	0	0	0
186	**656.49262**	**658.03877**	**C38H70O3N2Na1S1**	**0.1**	**1.8**	0	1	**0**	0	0
187	657.51690	658.96877	C36H69O4N5Na1	0.1	1.9	4	1	0	0	0
188	**657.49597**	**658.97177**	**C39H65O2N5Na1**	**0.1**	**1.7**	0	2	**0**	0	0
189	657.52042	659.00900	C40H70O5N2	0.1	1.8	0	1	0	0	0
190	**657.49957**	**659.01200**	**C43H66O3N2**	**0.1**	**1.5**	0	2	**0**	0	0
191	657.49912	659.03077	C36H69O2N5Na1S1	0.1	1.9	0	1	0	0	0
192	**658.52019**	**660.04277**	**C45H68N2Na1**	**0.0**	**1.5**	2	0	**0**	0	0
193	659.53262	660.98477	C36H71O4N5Na1	0.1	2.0	1	0	0	0	0
194	**660.40642**	**661.93800**	**C45H51N5**	**0.0**	**1.1**	0	1	**0**	0	0
195	660.47817	662.01500	C46H63O2N1	0.0	1.4	0	0	0	1	1
196	**661.40972**	**662.92400**	**C33H62O9N2S1**	**0.3**	**1.9**	0	1	**0**	0	0
197	663.49119	664.92877	C34H67O6N5Na1	0.2	2.0	1	0	0	0	0
198	**664.48242**	**666.01477**	**C36H70O5N2Na1S1**	**0.1**	**1.9**	1	0	**0**	0	0
199	672.49117	673.93700	C34H67O8N5	0.2	2.0	0	2	0	0	0
200	**672.49132**	**673.93977**	**C35H66O5N6Na1**	**0.1**	**1.9**	0	1	**0**	0	0
201	672.48747	674.03777	C38H70O4N2Na1S1	0.1	1.8	0	2	0	0	0
202	**673.51192**	**674.96777**	**C36H69O5N5Na1**	**0.1**	**1.9**	3	0	**0**	0	0
203	673.49077	674.97077	C39H65O3N5Na1	0.1	1.7	0	2	0	0	0
204	**673.49432**	**675.01100**	**C43H66O4N2**	**0.1**	**1.5**	0	1	**0**	0	0
205	673.54832	675.01177	C37H73O4N5Na1	0.1	2.0	1	0	0	0	0
206	**673.49472**	**675.01377**	**C44H65O1N3Na1**	**0.0**	**1.5**	0	2	**0**	0	0
207	674.50667	675.95300	C34H69O8N5	0.2	2.0	0	1	0	0	0

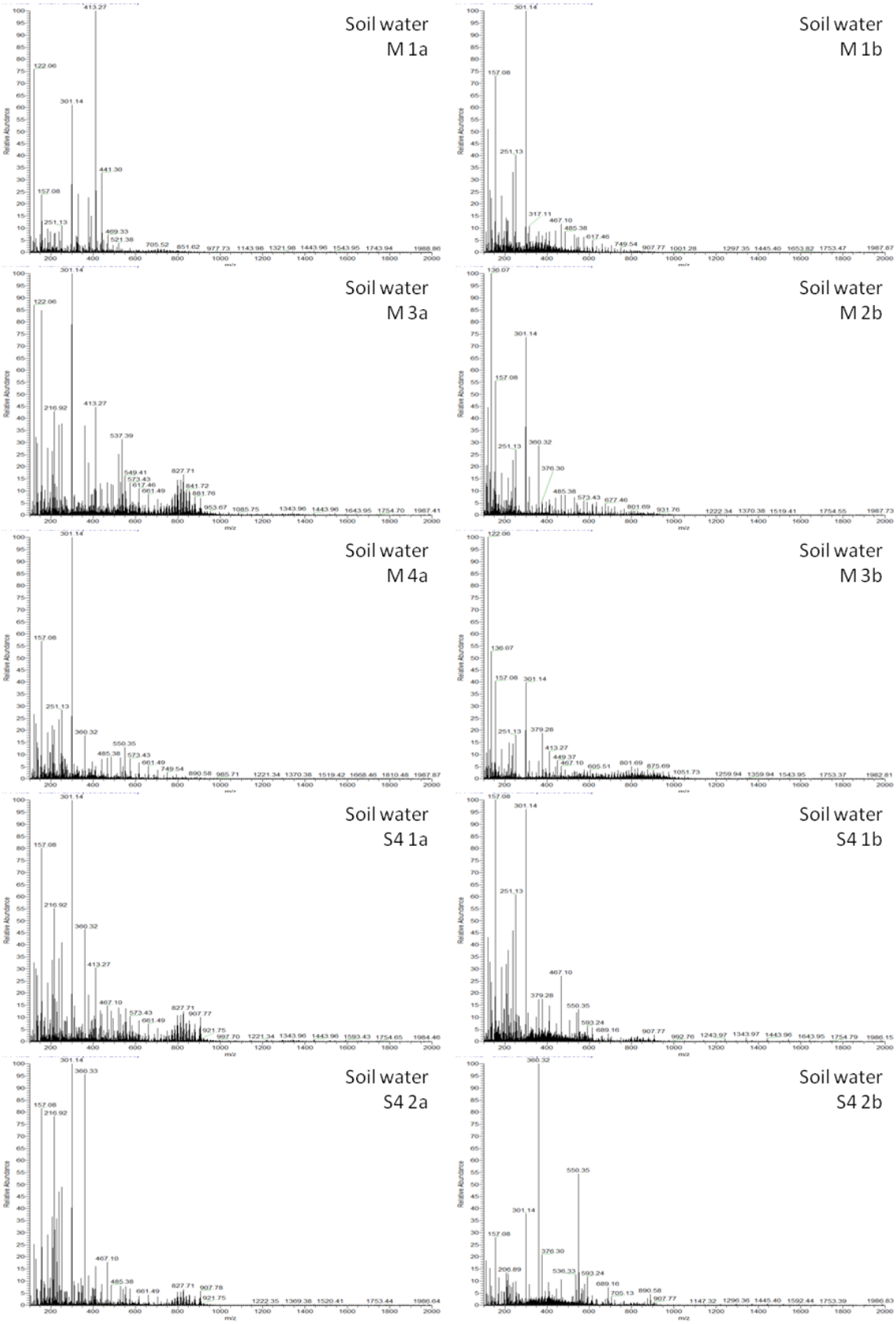
Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
208	**675.52762**	**676.98377**	**C36H71O5N5Na1**	**0.1**	**2.0**	1	0	**0**	0	0
209	676.38052	677.85477	C28H58O9N6Na1S1	0.3	2.1	0	1	0	0	0
210	**677.38392**	**678.92877**	**C37H57O5N3Na1S1**	**0.1**	**1.5**	0	1	**0**	0	0
211	678.46167	679.99777	C36H68O6N2Na1S1	0.2	1.9	0	1	0	0	0
212	**679.39517**	**680.89900**	**C31H60O10N4S1**	**0.3**	**1.9**	2	0	**0**	0	0
213	680.47722	682.01100	C35H71O9N1S1	0.3	2.0	1	0	0	0	0
214	**685.54797**	**687.02000**	**C37H74O7N4**	**0.2**	**2.0**	2	0	**0**	0	0
215	685.54822	687.02277	C38H73O4N5Na1	0.1	1.9	3	0	0	0	0
216	**688.48252**	**690.03677**	**C38H70O5N2Na1S1**	**0.1**	**1.8**	0	1	**0**	0	0
217	689.54312	691.01077	C37H73O5N5Na1	0.1	2.0	1	0	0	0	0
218	**694.45662**	**695.99677**	**C36H68O7N2Na1S1**	**0.2**	**1.9**	0	1	**0**	0	0
219	694.42582	696.00677	C41H60O2N4Na1S1	0.0	1.5	1	0	0	0	0
220	**700.47292**	**702.03600**	**C48H63O3N1**	**0.1**	**1.3**	1	0	**0**	0	0
221	700.47272	702.05477	C41H66O2N4Na1S1	0.0	1.6	2	0	0	0	0
222	**704.15242**	**705.53077**	**C25H34O20N2Na1**	**0.8**	**1.4**	0	0	**1**	0	0
223	705.53812	707.00977	C37H73O6N5Na1	0.2	2.0	1	0	0	0	0
224	**1418.96785**	**711.06700**	**C40H66O3N6S1**	**0.1**	**1.7**	1	0	**0**	0	0
225	1418.97585	711.11000	C45H66O1N4S1	0.0	1.5	0	1	0	0	0
226	**1419.97895**	**711.42500**	**C21H17O23N3S1**	**1.1**	**0.8**	0	2	**0**	0	0
227	1420.97518	712.03700	C36H73O10N1S1	0.3	2.0	3	0	0	0	0
228	**1420.97565**	**712.03977**	**C37H72O7N2Na1S1**	**0.2**	**1.9**	1	0	**0**	0	0
229	713.54692	715.07300	C43H74O6N2	0.1	1.7	0	1	0	0	0
230	**716.44677**	**718.05677**	**C44H62O1N4Na1S1**	**0.0**	**1.4**	0	2	**0**	0	0
231	717.45002	718.88577	C32H65O11N5Na1	0.3	2.0	0	1	0	0	0
232	**720.63722**	**722.15977**	**C43H82O1N6Na1**	**0.0**	**1.9**	0	1	**0**	0	0
233	723.56392	725.07177	C41H75O4N5Na1	0.1	1.8	1	0	0	0	0
234	**728.44092**	**730.01377**	**C39H66O7N2Na1S1**	**0.2**	**1.7**	0	1	**0**	0	0
235	729.57456	731.07577	C40H77O5N5Na1	0.1	1.9	1	0	0	0	0
236	**735.60038**	**737.12677**	**C43H79O3N5Na1**	**0.1**	**1.8**	4	1	**0**	0	0
237	737.61590	739.14277	C43H81O3N5Na1	0.1	1.9	4	1	0	0	0
238	**744.49914**	**746.08900**	**C50H67O4N1**	**0.1**	**1.3**	4	2	**0**	0	0
239	744.49892	746.10777	C43H70O3N4Na1S1	0.1	1.6	2	0	0	0	0
240	**750.50962**	**752.09300**	**C49H69O5N1**	**0.1**	**1.4**	1	0	**0**	0	0
241	758.58642	760.21177	C45H84O5Na1S1	0.1	1.9	1	0	0	0	0
242	**759.59902**	**761.13777**	**C44H83O7N1Na1**	**0.2**	**1.9**	1	0	**0**	0	0
243	759.59992	761.14600	C44H80O6N4	0.1	1.8	1	0	0	0	0
244	**760.47297**	**762.10977**	**C46H66O2N4Na1S1**	**0.0**	**1.4**	0	2	**0**	0	0
245	761.47632	762.93877	C34H69O12N5Na1	0.4	2.0	0	2	0	0	0
246	**761.48122**	**762.99000**	**C39H66O9N6**	**0.2**	**1.7**	0	1	**0**	0	0
247	761.48142	763.13100	C47H70O6S1	0.1	1.5	0	1	0	0	0
248	**761.61602**	**763.16477**	**C45H81O3N5Na1**	**0.1**	**1.8**	1	0	**0**	0	0
249	762.51912	764.11577	C41H76O7N2Na1S1	0.2	1.9	0	1	0	0	0
250	**763.63182**	**765.18077**	**C45H83O3N5Na1**	**0.1**	**1.8**	1	0	**0**	0	0
251	775.55632	777.21277	C48H75N5Na1S1	0.0	1.6	1	0	0	0	0
252	**781.56112**	**783.16000**	**C40H82O10N2S1**	**0.3**	**2.1**	2	0	**0**	0	0
253	781.56142	783.16277	C41H81O7N3Na1S1	0.2	2.0	1	0	0	0	0
254	**782.56502**	**784.23677**	**C50H80O3Na1S1**	**0.1**	**1.6**	1	0	**0**	0	0
255	783.57699	785.17877	C41H83O7N3Na1S1	0.2	2.0	0	2	0	0	0
256	**788.52525**	**790.16077**	**C45H74O4N4Na1S1**	**0.1**	**1.6**	6	1	**0**	0	0
257	802.58662	804.22200	C44H85O9N1S1	0.2	1.9	2	0	0	0	0
258	**802.58682**	**804.22477**	**C45H84O6N2Na1S1**	**0.1**	**1.9**	2	0	**0**	0	0
259	803.58972	805.15500	C45H80O8N4	0.2	1.8	1	0	0	0	0
260	**803.59012**	**805.15777**	**C46H79O5N5Na1**	**0.1**	**1.7**	3	0	**0**	0	0
261	804.50342	806.10177	C40H78O12Na1S1	0.3	2.0	0	1	0	0	0
262	**804.49912**	**806.16277**	**C48H70O3N4Na1S1**	**0.1**	**1.5**	0	1	**0**	0	0
263	804.60229	806.23800	C44H87O9N1S1	0.2	2.0	3	0	0	0	0

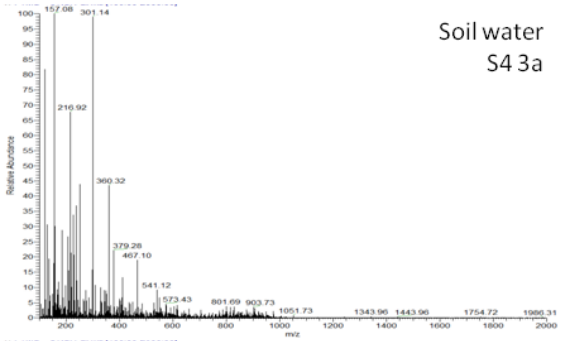
Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
264	**805.50242**	**806.99177**	**C36H73O13N5Na1**	**0.4**	**2.0**	0	1	**0**	0	0
265	805.50722	807.04300	C41H70O10N6	0.2	1.7	0	1	0	0	0
266	**805.55882**	**807.13400**	**C46H74O6N6**	**0.1**	**1.6**	1	0	**0**	0	0
267	805.60582	807.17377	C46H81O5N5Na1	0.1	1.8	2	0	0	0	0
268	**805.50809**	**807.18677**	**C50H73O4N1Na1S1**	**0.1**	**1.5**	0	3	**0**	0	0
269	807.57457	809.15000	C46H76O6N6	0.1	1.7	4	0	0	0	0
270	**808.52432**	**810.13800**	**C41H79O12N1S1**	**0.3**	**1.9**	0	1	**0**	0	0
271	808.63362	810.27277	C45H90O6N2Na1S1	0.1	2.0	1	0	0	0	0
272	**818.58144**	**820.22100**	**C44H85O10N1S1**	**0.2**	**1.9**	4	2	**0**	0	0
273	818.58172	820.22377	C45H84O7N2Na1S1	0.2	1.9	1	0	0	0	0
274	**818.56072**	**820.22677**	**C48H80O5N2Na1S1**	**0.1**	**1.7**	0	1	**0**	0	0
275	818.58602	820.26677	C50H84O5Na1S1	0.1	1.7	0	1	0	0	0
276	**819.58472**	**821.15400**	**C45H80O9N4**	**0.2**	**1.8**	3	2	**0**	0	0
277	819.58506	821.15677	C46H79O6N5Na1	0.1	1.7	3	0	0	0	0
278	**820.59709**	**822.23700**	**C44H87O10N1S1**	**0.2**	**2.0**	5	2	**0**	0	0
279	821.58492	823.12300	C41H82O12N4	0.3	2.0	0	1	0	0	0
280	**821.60032**	**823.17000**	**C45H82O9N4**	**0.2**	**1.8**	2	1	**0**	0	0
281	821.60062	823.17277	C46H81O6N5Na1	0.1	1.8	2	1	0	0	0
282	**821.57962**	**823.17577**	**C49H77O4N5Na1**	**0.1**	**1.6**	0	1	**0**	0	0
283	1655.99825	829.55900	C33H19O23N1S1	0.7	0.6	0	0	0	1	0
284	**1655.99845**	**829.56177**	**C34H18O20N2Na1S1**	**0.6**	**0.5**	0	0	**0**	2	0
285	832.55152	834.21377	C47H78O5N4Na1S1	0.1	1.7	2	0	0	0	0
286	**834.57641**	**836.22000**	**C44H85O11N1S1**	**0.3**	**1.9**	6	1	**0**	0	0
287	834.58192	836.27400	C50H81O5N3S1	0.1	1.6	0	1	0	0	0
288	**835.57967**	**837.15300**	**C45H80O10N4**	**0.2**	**1.8**	3	1	**0**	0	0
289	835.57996	837.15577	C46H79O7N5Na1	0.2	1.7	3	0	0	0	0
290	**835.55882**	**837.15877**	**C49H75O5N5Na1**	**0.1**	**1.5**	0	1	**0**	0	0
291	836.59209	838.23600	C44H87O11N1S1	0.3	2.0	3	0	0	0	0
292	**836.57112**	**838.23900**	**C47H83O9N1S1**	**0.2**	**1.8**	0	1	**0**	0	0
293	837.59526	839.16900	C45H82O10N4	0.2	1.8	3	0	0	0	0
294	**837.59552**	**839.17177**	**C46H81O7N5Na1**	**0.2**	**1.8**	2	0	**0**	0	0
295	837.57452	839.17477	C49H77O5N5Na1	0.1	1.6	0	1	0	0	0
296	**844.70652**	**846.39100**	**C49H99O7N1S1**	**0.1**	**2.0**	1	0	**0**	0	0
297	845.70992	847.32400	C50H94O6N4	0.1	1.9	1	0	0	0	0
298	**846.72222**	**848.40977**	**C50H100O4N2Na1S1**	**0.1**	**2.0**	1	0	**0**	0	0
299	848.53042	850.16300	C42H79O12N3S1	0.3	1.9	0	1	0	0	0
300	**848.53087**	**850.16577**	**C43H78O9N4Na1S1**	**0.2**	**1.8**	0	2	**0**	0	0
301	848.55552	850.20300	C44H83O12N1S1	0.3	1.9	1	0	0	0	0
302	**848.59182**	**850.24700**	**C45H87O11N1S1**	**0.2**	**1.9**	1	0	**0**	0	0
303	849.52872	851.04477	C38H77O14N5Na1	0.4	2.0	0	1	0	0	0
304	**849.53372**	**851.09600**	**C43H74O11N6**	**0.3**	**1.7**	0	1	**0**	0	0
305	849.55882	851.13600	C45H78O11N4	0.2	1.7	1	0	0	0	0
306	**850.53212**	**852.11877**	**C47H76O10N2Na1**	**0.2**	**1.6**	0	2	**0**	0	0
307	850.57127	852.21900	C44H85O12N1S1	0.3	1.9	5	1	0	0	0
308	**850.55027**	**852.22200**	**C47H81O10N1S1**	**0.2**	**1.7**	0	2	**0**	0	0
309	851.55892	853.10500	C41H80O14N4	0.3	2.0	0	1	0	0	0
310	**851.57444**	**853.15200**	**C45H80O11N4**	**0.2**	**1.8**	4	1	**0**	0	0
311	851.57477	853.15477	C46H79O8N5Na1	0.2	1.7	2	0	0	0	0
312	**851.55352**	**853.15777**	**C49H75O6N5Na1**	**0.1**	**1.5**	0	1	**0**	0	0
313	851.61076	853.19600	C46H84O10N4	0.2	1.8	1	0	0	0	0
314	**851.61117**	**853.19877**	**C47H83O7N5Na1**	**0.1**	**1.8**	2	0	**0**	0	0
315	852.58695	854.23500	C44H87O12N1S1	0.3	2.0	4	0	0	0	0
316	**852.56602**	**854.23800**	**C47H83O10N1S1**	**0.2**	**1.8**	0	1	**0**	0	0
317	853.57452	855.12100	C41H82O14N4	0.3	2.0	0	1	0	0	0
318	**853.59016**	**855.16800**	**C45H82O11N4**	**0.2**	**1.8**	3	0	**0**	0	0
319	853.59042	855.17077	C46H81O8N5Na1	0.2	1.8	2	0	0	0	0

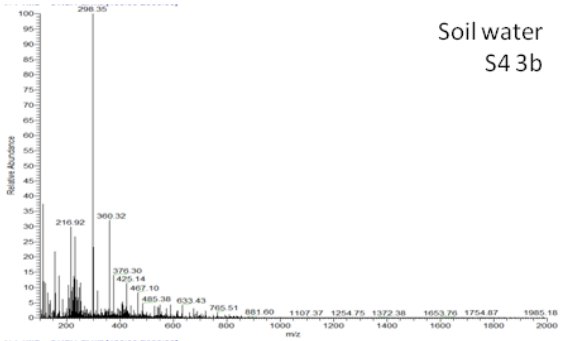
Z	Avg. Mass [u]	Th. Mass [u]	Composition	O/C	H/C	Decomp.l. B	Decomp.l. G	Fresh l.	Root e.	Soil w.
320	**853.56942**	**855.17377**	**C49H77O6N5Na1**	**0.1**	**1.6**	0	1	**0**	0	0
321	856.70632	858.40200	C50H99O7N1S1	0.1	2.0	2	0	0	0	0
322	**858.72192**	**860.41800**	**C50H101O7N1S1**	**0.1**	**2.0**	5	0	**0**	0	0
323	859.72529	861.34000	C50H100O10	0.2	2.0	5	0	0	0	0
324	**860.70111**	**862.39000**	**C49H99O8N1S1**	**0.2**	**2.0**	3	0	**0**	0	0
325	864.55062	866.20200	C44H83O13N1S1	0.3	1.9	1	0	0	0	0
326	**864.58702**	**866.24600**	**C45H87O12N1S1**	**0.3**	**1.9**	1	0	**0**	0	0
327	866.55222	868.15777	C49H80O11Na1	0.2	1.6	0	1	0	0	0
328	**866.56620**	**868.21800**	**C44H85O13N1S1**	**0.3**	**1.9**	5	0	**0**	0	0
329	867.55392	869.10400	C41H80O15N4	0.4	2.0	0	1	0	0	0
330	**867.56932**	**869.15100**	**C45H80O12N4**	**0.3**	**1.8**	3	0	**0**	0	0
331	867.56966	869.15377	C46H79O9N5Na1	0.2	1.7	3	0	0	0	0
332	**868.58182**	**870.23400**	**C44H87O13N1S1**	**0.3**	**2.0**	4	0	**0**	0	0
333	869.58507	871.16700	C45H82O12N4	0.3	1.8	2	0	0	0	0
334	**876.57772**	**878.26677**	**C49H82O6N4Na1S1**	**0.1**	**1.7**	1	0	**0**	0	0
335	878.73957	880.34977	C49H100O7N4Na1	0.1	2.0	0	2	0	0	0
336	**878.73262**	**880.35400**	**C50H97O7N5**	**0.1**	**1.9**	3	0	**0**	0	0
337	880.74834	882.37000	C50H99O7N5	0.1	2.0	5	2	0	0	0
338	**881.73122**	**883.35400**	**C50H98O8N4**	**0.2**	**2.0**	1	0	**0**	0	0
339	884.66475	886.36800	C50H95O9N1S1	0.2	1.9	6	1	0	0	0
340	**892.55662**	**894.21600**	**C44H83O13N3S1**	**0.3**	**1.9**	0	1	**0**	0	0
341	892.55742	894.21877	C45H82O10N4Na1S1	0.2	1.8	0	1	0	0	0
342	**893.55492**	**895.09777**	**C40H81O15N5Na1**	**0.4**	**2.0**	0	1	**0**	0	0
343	894.55812	896.17177	C49H80O11N2Na1	0.2	1.6	0	1	0	0	0
344	**894.70672**	**896.40677**	**C50H100O7N2Na1S1**	**0.1**	**2.0**	0	2	**0**	0	0
345	895.71017	897.33700	C50H96O9N4	0.2	1.9	0	2	0	0	0
346	**897.72572**	**899.35300**	**C50H98O9N4**	**0.2**	**2.0**	0	2	**0**	0	0
347	900.64487	902.32277	C47H94O10N2Na1S1	0.2	2.0	0	2	0	0	0
348	**900.64562**	**902.33100**	**C47H91O9N5S1**	**0.2**	**1.9**	0	1	**0**	0	0
349	900.71732	902.41900	C49H99O7N5S1	0.1	2.0	1	0	0	0	0
350	**901.64732**	**903.25300**	**C47H90O12N4**	**0.3**	**1.9**	0	1	**0**	0	0
351	901.72082	903.34377	C50H97O7N5Na1	0.1	1.9	1	0	0	0	0
352	**903.73611**	**905.35700**	**C49H100O10N4**	**0.2**	**2.0**	4	2	**0**	0	0
353	903.73632	905.35977	C50H99O7N5Na1	0.1	2.0	2	0	0	0	0
354	**905.75199**	**907.37577**	**C50H101O7N5Na1**	**0.1**	**2.0**	3	0	**0**	0	0
355	1820.00490	911.56900	C41H13O21N5	0.5	0.3	0	0	0	1	0
356	**915.64902**	**917.23577**	**C45H91O12N5Na1**	**0.3**	**2.0**	1	0	**0**	0	0
357	916.65446	918.36600	C50H95O11N1S1	0.2	1.9	5	0	0	0	0
358	**917.65762**	**919.28800**	**C50H94O14**	**0.3**	**1.9**	2	0	**0**	0	0
359	920.60342	922.31700	C50H87O10N3S1	0.2	1.7	1	0	0	0	0
360	**922.61006**	**924.22000**	**C47H89O16N1**	**0.3**	**1.9**	1	0	**0**	0	0
361	922.74502	924.36277	C49H100O8N6Na1	0.2	2.0	0	2	0	0	0
362	**936.58372**	**938.27177**	**C47H86O11N4Na1S1**	**0.2**	**1.8**	0	1	**0**	0	0
363	937.58872	939.25277	C44H89O14N3Na1S1	0.3	2.0	0	3	0	0	0
364	**982.72890**	**984.37077**	**C50H100O11N6Na1**	**0.2**	**2.0**	5	0	**0**	0	0
365	984.74521	986.38677	C50H102O11N6Na1	0.2	2.0	3	0	0	0	0
366	**1000.73992**	**1002.38577**	**C50H102O12N6Na1**	**0.2**	**2.0**	1	0	**0**	0	0
367	1025.73069	1027.39300	C50H102O15N6	0.3	2.0	0	3	0	0	0
368	**1025.71929**	**1027.45500**	**C50H102O13N6S1**	**0.3**	**2.0**	0	3	**0**	0	0

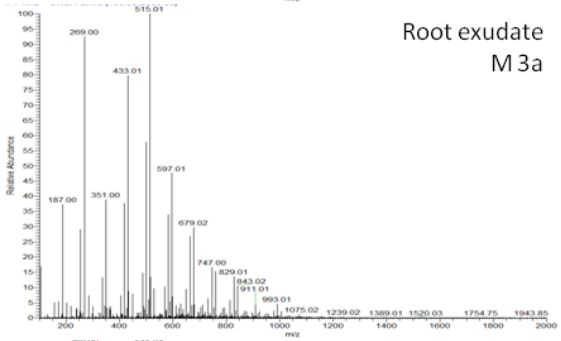
6.2. Mass spectra

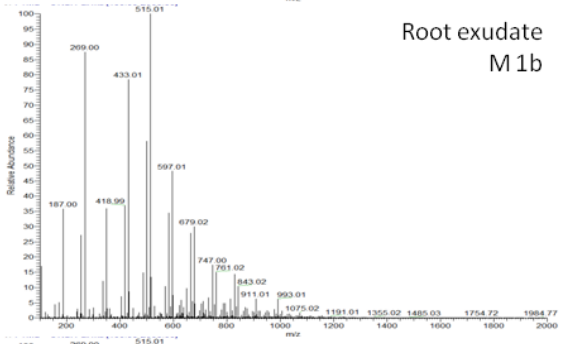
Abbreviations: M, *Salix matsudana x alba;* S, *S. smitihiana*; B, Cd-contaminated cambisol; G, potting soil; FE, + magnetite

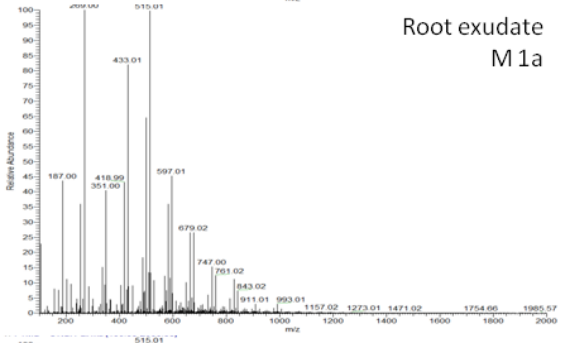


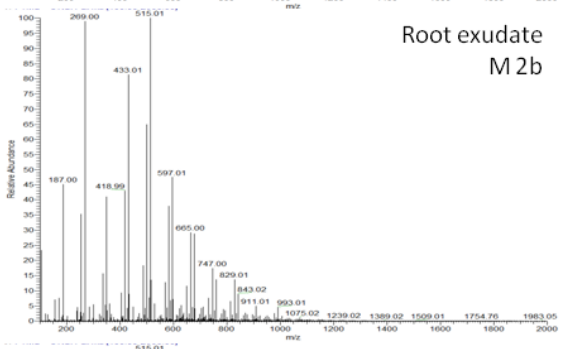

Soil water
S4 3a

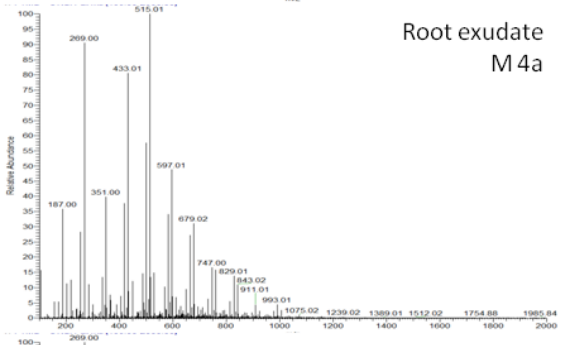

Soil water
S4 3b

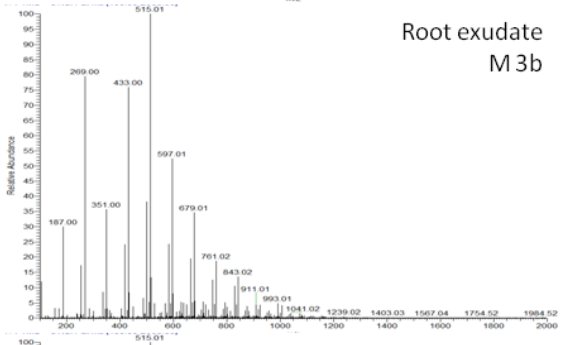

Root exudate
M 3a

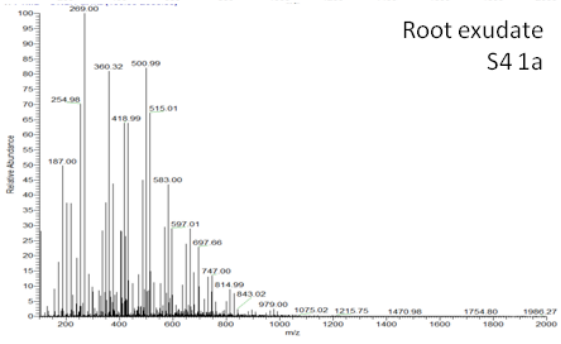

Root exudate
M 1b

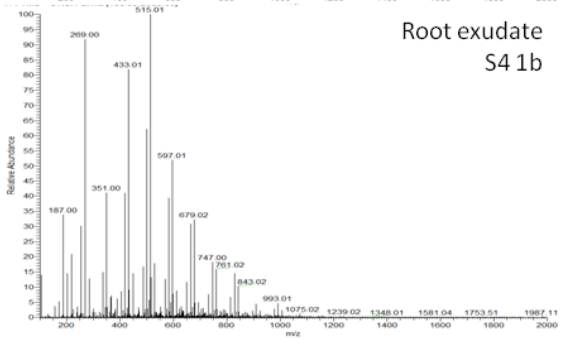

Root exudate
M 1a

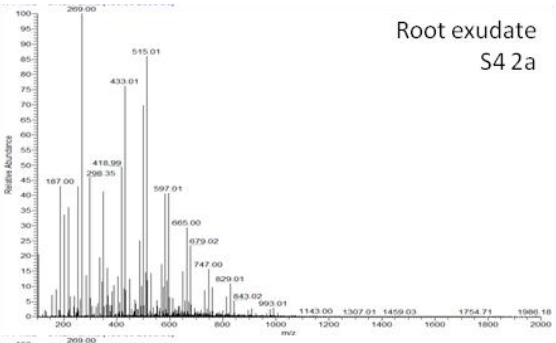

Root exudate
M 2b

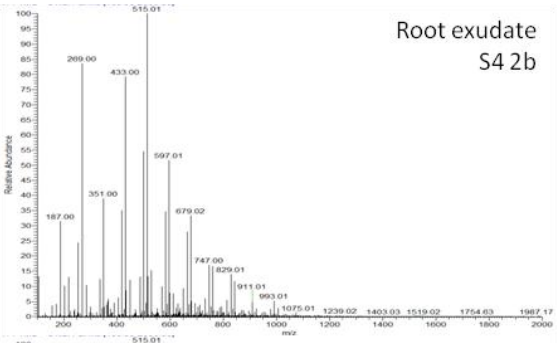

Root exudate
M 4a

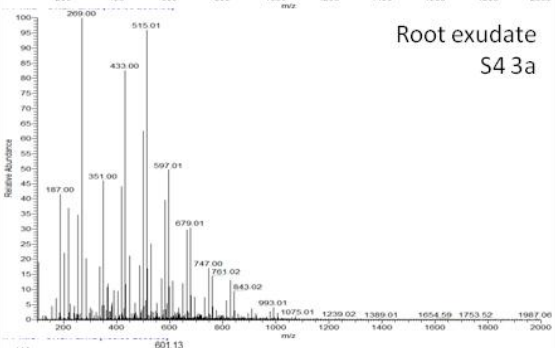

Root exudate
M 3b

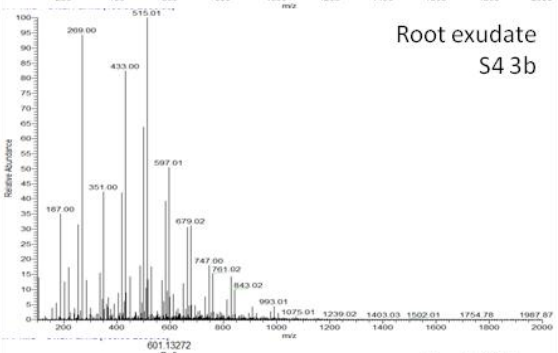

Root exudate
S4 1a

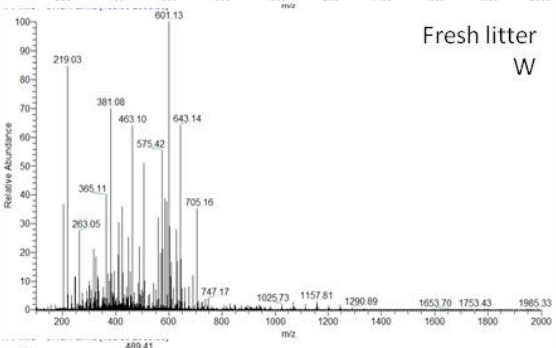

Root exudate
S4 1b

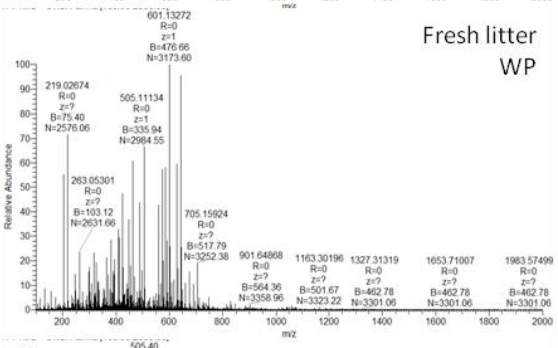

Root exudate
S4 2a

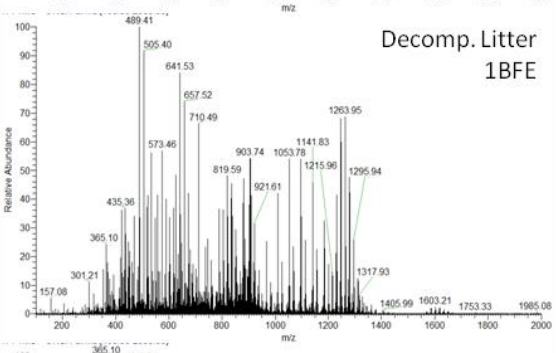

Root exudate
S4 2b

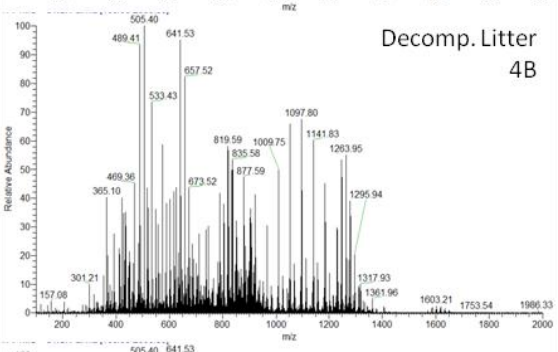

Root exudate
S4 3a

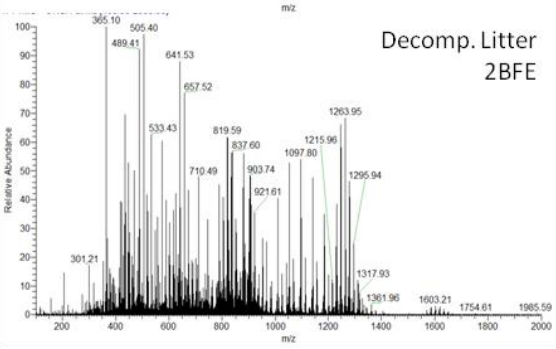

Root exudate
S4 3b

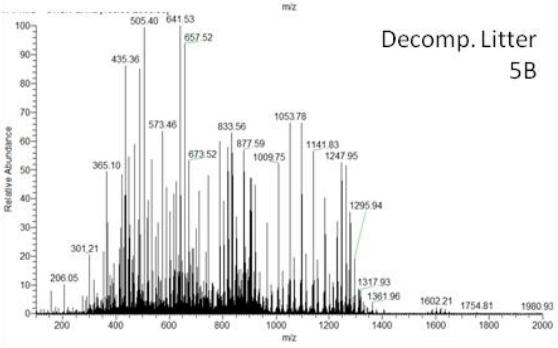

Fresh litter
W

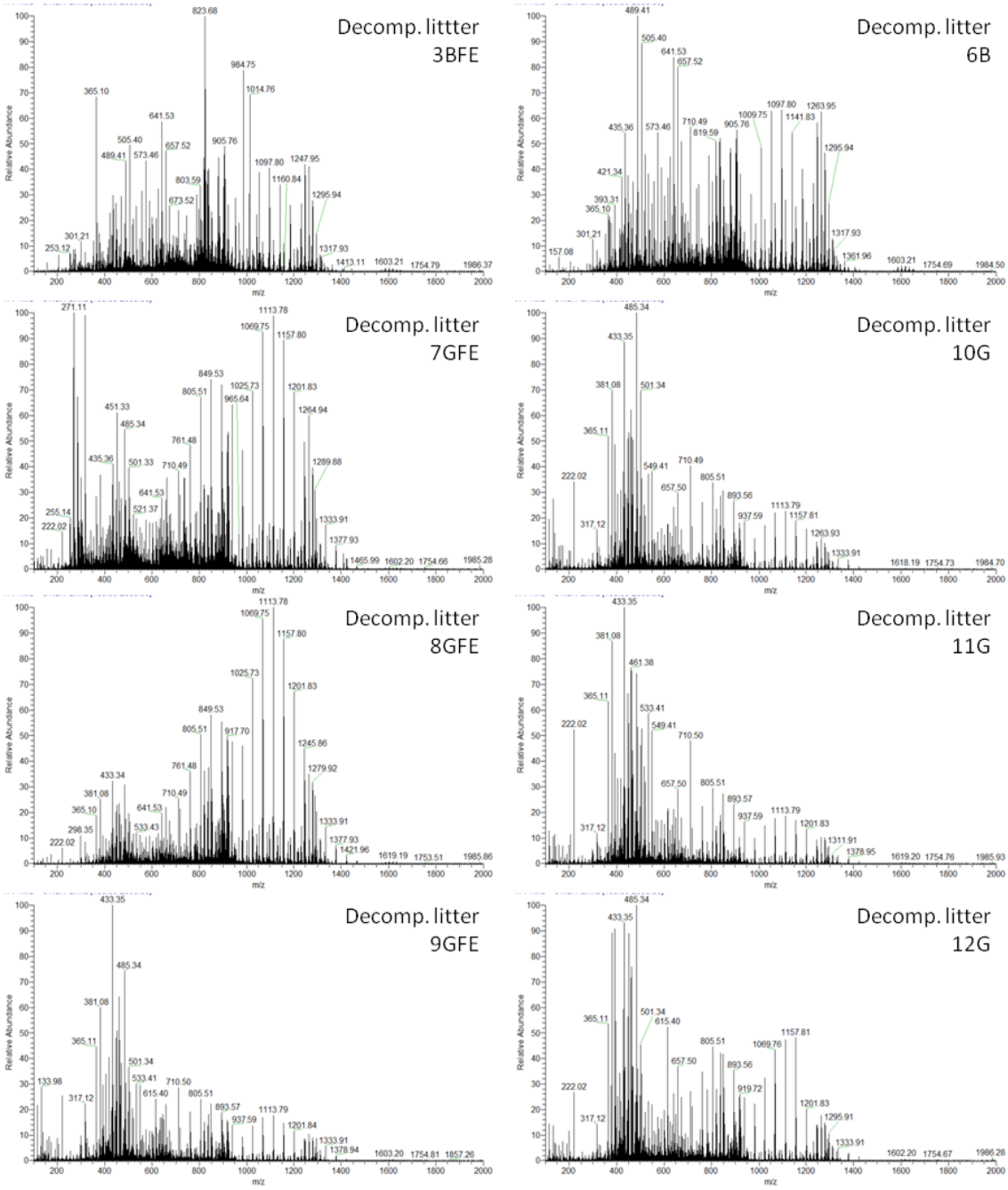

Fresh litter
WP


Decomp. Litter
1BFE


Decomp. Litter
4B


Decomp. Litter
2BFE


Decomp. Litter
5B


Decomp. littter 3BFE
Decomp. litter 6B
Decomp. litter 7GFE
Decomp. litter 10G
Decomp. litter 8GFE
Decomp. litter 11G
Decomp. litter 9GFE
Decomp. litter 12G
Relative Abundance
m/z

6.3. Alignment of mass data: R-script

```
sheets<- c (
        "MEOHMETAC","1BFE","2BFE","3BFE","4B","5B","6B","7GFE","8GFE","9GFE","10G",
        "11G","12G","W","WP","MAB91W","MAB92W","MAB93W","MAB94W","MSK1W",
        "MSK2W","MSK3W","NAACW","S4AB91W","S4AB92W","S4AB93W","S4AB94W",
        "S4SK1W","S4SK2W","S4SK3W","S4SK4W","MAB91B","MAB93B","MAB94B","MSK1B
        "MSK2B","MSK3B","S4AB91B","S4AB92B","S4AB93B","S4AB94B","S4SK1B","S4SK2B
        "S4SK3B","S4SK4B"    )

for (i in 1:length(sheets))
{       data<-read.csv(paste(sheets[i],".csv", sep=""),header=T, sep=","
        dataz<-data[data$Charge!=0,]
        dataz$mzz<- (dataz$m.z-1.007276466212)
        dataz$malz<-(dataz$mzz) * (dataz$Charge)
        data<-dataz[order(dataz$malz),]
        data$file<-sheets[i]
        if (i==1) {return<-data} else {return<-rbind(return, data)} }
data2<- return

data.mat<-      data.frame(matrix(nrow=length(unique(data2$Composition)),
                ncol=length(sheets)+2))
                colnames(data.mat)<-c("Composition", "mz", sheets)
                data.mat$Composition<-unique(data2$Composition)

for (i in 1:nrow(data.mat))       {
        for (j in 1:length(sheets))
        {       print(paste("i =",i,"j =",j))
                data.mat[i, which(colnames(data.mat)==sheets[j])]<-
                sum(data2$Relative[which(data2$Composition==
                data.mat$Composition[i]&data2$file==sheets[j])])        }

                data.mat[i, "mz"]<-
                mean(data2$malz[which(data2$Composition==
                data.mat$Composition[i])])              }
```

Zusammenfassung

Aufgrund steigender Konzentrationen atmosphärischer Treibhausgase (vor allem CO_2), wird ein weltweiter Temperaturanstieg von 2-7°C noch für das laufende Jahrhundert vorhergesagt (Wu et al., 2011). Die organische Substanz in Böden sind CO_2-quelle, -buffer und –speicher. Diese Funktionen sind abhängig vom Zusammenspiel von Photosynthese, der Veratmung durch mikrobielle Zersetzergemeinschaften und der Stabilität der Bodensubstanz (Dungait et al., 2012). Ob die Stabilität der organischen Bodensubstanz gegenüber mikrobiellem Abbau vor allem durch Rekalzitranz, also die strukturelle Zusammensetzung, bedingt ist, wird derzeit diskutiert. Die zunehmende Zahl von Analysemöglichkeiten regt neue Hypothesen zur Zusammensetzung und Entstehung der „rekalzitranten“ Huminstoffe an. Heute wird vermutet, dass in der organischen Bodensubstanz teilweise zersetzte, „rekalzitrante“ Biopolymere (z.B. Lignine, Wachse) mit niedermolekularen Verbindungen (z.b. Zucker, organische Säuren) verbunden sind (Sutton et al., 2005). Für den Abbau organischer Bodensubstanz ist das zeitliche und räumliche Aufeinandertreffen vieler Faktoren erforderlich, die in ihrer Gesamtheit und ihren Wechselwirkungen nach wie vor schwer zu erfassen sind. Schwermetalle übernehmen in diesen komplexen Interaktionen eine wichtige Rolle, unter anderem in Enzymen als Co-substrat und als Mikronährstoff in Pflanzen. Für die meisten Lebewesen sind hohe Konzentrationen jedoch toxisch. Schäden, die durch anthropogene Schwermetallbelastung entstehen, können durch Bioremediation mit Hilfe von spezialisierten Pflanzen gemildert werden. Weiden (*Salix sp.*) werden aktuell zur Sanierung von Cadmium- und Zinn-belasteten Böden eingesetzt.

Für die vorliegende Studie wurde daher organische (Boden-)substanz (Bodenwasser, Wurzelexudate, Laub) von Weidenarten verwendet, deren Fähigkeit zur Hyperakkumulation von Cadmium und Zink bekannt ist. Weidenlaub wurde in einem Experiment in verschiedenen Böden und mit unterschiedlichen Magnetit (Fe_3O_4)-Gaben abgebaut. Die Proben wurden mit hochauflösender Fourier-transformierter Massenspektronomie (FT-MS) analysiert. Die Massensignale wurden anhand geschätzter Elementzusammensetzungen synchronisiert**.** Die Atomverhältnisse von Sauerstoff (O) zu Kohlenstoff (C) und von Wasserstoff (H) zu Kohlenstoff (C) wurden gemeinsam mit der Molekularmasse in van Krevelen Diagrammen dargestellt und auf Basis empirisch definierter Bereiche für Verbindungsklassen interpretiert. Multivariate Statistik wurde durchgeführt. Die hochauflösende FT-MS- Analyse brachte charakteristische Masssenspektren hervor, die es ermöglichten, die Probentypen (Bodenwasser, Wurzelexudate, Laub) voneinader zu unterscheiden und unterschiedliche Verbindungsklassen in den einzelnen Probentypen zu interpretieren.

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Curriculum vitae

Personal information:

Name	Angelika Anna Hofer
Date of birth	20-06-1985
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Education:

October 2010	Start of Diploma thesis at the Department of Terrestrial Ecosystem Research, University of Vienna
November 2008	Intermediate degree in biology, University of Vienna
Since 2005	study in Biology (Ecology), University of Vienna
Since 2004	study in History, University of Vienna and University of Klagenfurt at the Faculty of Interdisciplinary Studies
June 2003	Matura (final exam qualifying for university admission)
1999 – 2003	Secondary School with focus on music, Wiener Neustadt
1995 – 1999	Secondary school with focus on languages, Neunkirchen
1991 – 1995	Primary School

Relevant work experience:

Since October 2010	Project-coworker at the department of Terrestrial Ecosystem Research in FWF-project on *Salix*-species and Metal contamination
Summer 2009/2010	Tutor for the ecological field-course „Kenntnis mitteleuropäischer Lebensgemeinschaften“
Summer 2009	Diploma in ecological design and engineering, Permakultur Austria
Summer 2008/2009	Organization of group-holidays, Vienna Youth and Family Offices
Summer 2007	Work with children of special needs, Dominican Republic
Additional work	Assistance on farms
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