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Pyrolysis-mass spectrometry and gas chromatography-flame ionization detection as complementary tools for soil lipid characterization

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ABSTRACT

Lipid biomarker profiles are a powerful tool for assessing soil microbial community structure, but intensive laboratory work and data analysis are needed to construct profiles from phospholipid fatty acids and other common biomarkers. Pyrolysis mass spectrometry (Py-MS) is a alternative method that provides a rapid and sensitive 'fingerprint' of soil lipids and may be sufficient to characterize lipids from various sites. The objective of this work was to evaluate the capacity of pyrolysis metastable atom bombardment time-of-flight mass spectrometry (Py-MAB-TOF-MS) to provide replicable analysis of soil lipids, compared to a routine gas chromatography-flame ionization detection (GC-FID) method. Soils were collected from six agricultural fields under soybean, corn and asparagus production. Soil lipids extracted with 1:2 chloroform:methanol solvent were analyzed with Py-MAB-TOF-MS or transesterified into fatty acids and then analyzed by GC-FID. The two methods were complementary, but distinct: lipid fingerprints, generated from Py-MAB-TOF-MS spectra, included extractable soil lipids from microbial, animal and plant origins plus non-living organic matter in the samples, whereas fatty acid profiles generally represented lipids from soil bacteria and fungi. We conclude that the soil lipid fingerprints generated from Py-MAB-TOF-MS present more variability than lipid biomarker profiles from the GC-FID method because they include a broader group of extractable soil lipids. Further work is needed to identify the molecular fragment masses in Py-MAB-TOF-MS spectra that could precisely identify soil lipids of microbial origin.

1. Introduction

Characterization of soil lipid biomarkers is a powerful tool for studying the structure of living biotic communities as well as the source, turnover and stabilization of non-living organic matter [1–3]. A wide array of lipid molecules could be used as biomarkers, including fatty acids, sterols, respiratory quinones and alkanes. In living biota, fatty acids are the major building blocks of phospholipids, glycolipids and neutral lipids such as sterol esters, monoacylglycerols, diacylglycerols and triacylglycerols [4]. For example, the lipid biomarker profile obtained from analysis of total lipid fatty acids is an appropriate tool for assessing how agricultural practices and environmental stresses affect the soil microbial community [5,6].

Generating lipid biomarker profiles for assessing soil microbial communities or other studies on the chemical structure and biological origin of soil lipids demands intensive laboratory work (lipid extraction, fractionation, derivatization, chromatography) and data analysis. Simpler 'fingerprinting' techniques might be adequate for the characterization of soil lipids extracted from living organisms and other soil organic matter fractions. Available fingerprinting techniques include: Raman spectroscopy, Fourier transform infrared spectroscopy, direct infusion mass spectrometry and Py-MS [7–10]. Fingerprinting techniques have the potential to be more rapid and have a higher throughput than profiling methods because they do not require chromatographic separation or derivatization of the target compounds, however, they require chemometric interpretation of the complex analytical data generated from simultaneous acquisition of hundreds of metabolites [8].

Among fingerprinting techniques, Py-MS has several advantages, such as speed of analysis, sensitivity and high sample throughput. In most Py-MS systems, the chemical compounds in a sample are desorbed and volatilized during a rapid heating phase, followed by ionization with electron impact, and detection by mass spectrometry [7]. This technique was successfully used, sometimes with a thermally assisted hydrolysis and methylation step, to discriminate and classify bacteria [11], fungi [12], higher

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plants [13] and soil organic matter [14]. The modified Py-MS system in this study (Py-MAB-TOF-MS) has a metastable atom bombardment capacity that permits better control of the ionization energy and reduces chemical fragmentation during ionization, compared to electron impact ionization [15,27]. Recently, Py-MAB-TOF-MS systems have been used to fingerprint lipids of plant, animal and microbial origins [16–18,27]. We are not aware of any studies that have used a Py-MAB-TOF-MS system to characterize soil lipids, but hypothesize that a Py-MAB-TOF-MS system will produce a replicable lipid fingerprint that will complement the fatty acid biomarker profile generated by GC-FID for the same set of soil samples.

The objective of this work was to evaluate the reproducibility and pattern of the lipid fingerprint produced by the Py-MAB-TOF-MS system, compared to the lipid biomarker profile generated by the conventional GC-FID method, for six agricultural soils with diverse cropping histories.

2. Experimental

2.1. Soil collection and handling

The soils used in this study were mixed, frigid Typic Endoaquents collected from the top 15 cm of agricultural fields in southwestern Québec, Canada that were under soybean (S1, S2, S3 and S4), corn (CORN) and asparagus (ASP) production. Each soil sample was a composite of 18–25 cores (15 cm long, 3 cm internal diameter) collected from random locations in each field and mixed together. Immediately after collection, half of each soil sample was frozen at −20 °C to preserve soil lipids. The other half of the sample was air-dried, sieved through a 2 mm mesh sieve and sent for soil physical and chemical analysis. Agricultural practices and selected soil characteristics for each field are reported in Table 1.

2.2. Soil lipid extraction

All organic solvents used in this study were HPLC grade. Glassware and laboratory equipment were prepared following the recommendations of White and Ringelberg [19]. Soil lipids were extracted with an ASE 200 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA, USA) according to the operating conditions described by Macnaughton et al. [20], which consisted of one heating cycle at 80 °C and 8280 kPa during 5 min, three static cycles of 15 min each at the same temperature and pressure, rinsing of the transfer lines and sample cell with the solvent and purging with N_2 for 180 s between each sample. Between 6 and 8 g of freeze-dried soil was packed into a 11-mL stainless steel ASE vessel that had been rinsed with 1:2 chloroform:methanol solvent. Soil lipids were extracted with 1:2 chloroform:methanol solvent, dried under N_2 gas and quantitatively transferred to a vial using 1:2 chloroform:methanol, dried under N_2 gas and re-dissolved in 1 mL of 1:2 chloroform–methanol prior to direct analysis with the Py-MAB-TOF-MS. Soil lipid extracts were transformed to fatty acid methyl esters (FAMEs) before analysis by GC-FID (see below).

2.3. Analysis of soil lipid extracts using the Py-MAB-TOF-MS system

Soil lipid extracts were analyzed with a Py-MAB-TOF-MS (Dephy Technologies, Montreal, Canada) as described elsewhere [18,21]. One microliter sample was applied to the pyroprobe (Pyroprobe 2000 pyrolyzer; CDS Analytical, Oxford, PA, USA). Pyrolysis was achieved by ramping the probe temperature by 20 °C ms^{-1} from ambient to 1100–1200 °C, with a final hold time of at least 50 s. The probe was specially modified to enable helium flow (1–2 mL/min) through the quartz capillary, thus enhancing transfer of pyrolysis products into the MAB source. The ionization gas was N_2, which has an ionization energy of 8.67 eV (85%) and 11.88 eV (15%). The mass range was scanned between 40 and 1000 m/z. Each soil lipid extract (one per composite sample) was analyzed five times.

2.4. Preparation of the fatty acid methyl esters and analysis with the GC-FID system

Soil lipid extracts were transformed to FAMEs before analysis by GC-FID. Total lipid fatty acid methyl esters (TL-FAMEs) were prepared by mild alkaline transesterification of the soil lipid extract [19]. Ester-linked fatty acid methyl esters (EL-FAMEs) were also generated by subjecting unmodified soil samples to direct mild alkaline transesterification [5]. The EL-FAMEs were extracted after the transesterification step. In both preparations, the fatty acid methyl esters were not fractionated from the rest of the lipid biomarkers. The TL-FAMEs are most like to contain mainly extractable free lipids. Using an alkaline reagent for the transesterification, only ester-linked fatty acids could be targeted and not the free fatty acids, that are most likely coming from plants [20]. The EL-FAME method by directly transmethylating the fatty acids could also recovered fatty acids that were ester-linked to insoluble (in organic solvents) minerals and organic macromolecules such as cutin and suberin. After drying under N_2, the TL-FAMEs and EL-FAMEs were dissolved with 1-mL of iso-octane containing 25 ng μL^{-1} of methyl-nonadecanoate (C19:0) internal standard. Each TL-FAME mixture (one per composite sample) was analyzed five times, and each EL-FAME mixture (one per composite sample) was also analyzed five times. This involved injecting 5 μL of each analytical replicate in split mode (50:1) with a gas chromatograph (Hewlett Packard 6890) equipped with an Ultra-2 capillary column (cross-linked 5% diphenyl-95% di-methylpolysiloxane; length, 25 m; internal diameter, 0.20 mm; film thickness, 0.33 μm; Agilent J&W 19091B-102). Hydrogen was the carrier gas (68.9 kPa), nitrogen was the "makeup" gas (30 mL min^{-1}), and air was used to support the FID flame. The temperature program ramped from 170 °C to 270 °C at 5 °C min^{-1} and was held at 270 °C for 2 min. Inlet and detector temperatures were 250 °C and 300 °C, respectively. The retention times of the peaks were converted to equivalent chain length (ECL) values [22]. Identification of peaks was based on comparison of retention times (ECLs) to commercial FAMEs standards (Supelco 37 Component FAME Mix cat.#47885-U; Supelco Bacterial Acid Methyl Esters cat.#47080-U; Matreya PUFA-2 cat.#1081; Matreya Bacterial Acid Methyl Esters CP Mix cat.#1114; Matreya cis-11-Hexadecenoic Acid cat.#1208 and Matreya 10-Methyloctadecanoate cat.#1763, used directly or derivatized if needed), and led to the identification of 70 TL-FAMEs and 78 EL-FAMEs. The settings were the same as those used in the MIDI protocol (MIDI, Inc., Newark, Delaware, USA, www.midi-inc.com) [5,22], thus, peak identity could be validated by sending a subset of all samples to a certified laboratory (Laboratoire de Santé Publique du Québec, Sainte-Anne-de-Bellevue, QC) using the MIDI system. The MIDI library was built/designed to allow the identification of fatty acids from microbial origin. However, microbes and plants share some common fatty acids.

2.5. Calculations and statistical analysis

The Py-MAB-TOF-MS analysis of the total lipid extracts gave ion masses ranging from 40 to 1000 m/z (m/z, mass-to-charge ratio). The ion intensity for each mass was normalized to percent total ion counts. From the GC-FID data, the percent area of each FAME was calculated as: (peak area of each identified FAME/total peak area of all identified FAMEs) × 100%.

This work was considered exploratory, since we are not aware of other studies that attempted to characterize soil lipids with Py-MAB-TOF-MS. Analysis of variance was used to distinguish the