Postprint: Fingerprint Characteristics of Different Soil Organic Matter Fractions in Alpine Grasslands Using Py-GC-MS/MS Technology

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Abstract

Pyrolysis-gas chromatography-tandem mass spectrometry (Py-GC-MS/MS) was employed to investigate the fingerprint differences among five density fractions of soil organic matter in alpine grasslands. Qualitative and quantitative analysis was performed on 150 pyrolysis products, which were categorized based on similar chemical properties into: alkyl compounds, aromatic and polycyclic aromatic hydrocarbons, lignin, phenolic substances, polysaccharides, nitrogen-containing compounds, and chitin. The results revealed that in alpine grassland soils, the F1 fraction (density <1.6 g/cm3) consisted primarily of plant debris. Although this fraction accounted for a small mass proportion of the bulk soil (0.13%), its organic matter content was relatively high (5.7%). This fraction contained abundant lignin and long-chain alkyl compounds (derived mainly from plants), and the content of such compounds gradually decreased with increasing density. The three fractions F2 (density 1.6-1.8 g/cm3), F3 (density 1.8-2.0 g/cm3), and F4 (density 2.0-2.25 g/cm3) exhibited similar chemical properties, with their organic compound contents showing transitional states of increase or decrease across the density fractions. The F5 fraction (density >2.25 g/cm3) constituted the main component of this soil, with a mass content as high as 98%. The microbial fingerprint information in this fraction (polysaccharides and nitrogen-containing compounds of microbial origin) was higher than in the previous four fractions. Simultaneously, recalcitrant substances such as aromatic and polycyclic aromatic hydrocarbons gradually accumulated with increasing density, becoming enriched in the F5 fraction.

Study on the Fingerprints of Soil Organic Components in Alpine Grassland Based on Py-GC-MS/MS Technology

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Abstract

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS/MS) was employed to investigate fingerprint differences among five density fractions of soil organic matter in alpine grassland. In this study, 150 pyrolysis products were qualitatively and quantitatively analyzed and classified according to their chemical properties into alkyl compounds, aromatics and polyaromatics, lignin, phenols, polysaccharides, N-compounds, and chitin. The results revealed that in alpine grassland soils, the F1 fraction (density <1.6 g/cm³) consisted primarily of plant debris. Although this component accounted for only 0.13% of the total soil mass, its organic matter content was relatively high (5.7%). The F1 fraction contained abundant lignin and long-chain alkyl compounds derived mainly from plants, and the content of these compounds gradually decreased with increasing density. The F2 (1.6-1.8 g/cm³), F3 (1.8-2.0 g/cm³), and F4 (2.0-2.25 g/cm³) fractions exhibited similar chemical properties, with their organic compound contents showing transitional patterns of increase or decrease across density fractions. The F5 fraction (density >2.25 g/cm³) represented the major soil component, comprising up to 98% of soil mass. Microbial polysaccharides and N-compounds (primarily from microorganisms) in the F5 fraction were significantly higher than in the first four fractions, indicating that the F5 fraction contained more microbial fingerprint information. Recalcitrant substances such as aromatics and polyaromatics were also found to accumulate in the F5 fraction.

Keywords: soil organic matter; density grouping technique; pyrolysis-gas chromatography-mass spectrometry

Introduction

Soil organic matter (SOM) is a complex mixture composed of plant and animal residues, microbial biomass, and their secretions. Due to its complex structure and composition, SOM research is typically conducted in conjunction with fractionation techniques. The density fractionation technique for SOM originated in the 1980s and operates on the principle of using heavy liquids with densities of 1.6–2.5 g/cm³ to separate SOM into different fractions through sedimentation. Low-density organic matter consists mainly of plant and animal residues and represents an intermediate product between fresh plant material and soil humus, with higher turnover rates than bulk soil. This fraction is composed of cellulose, hemicellulose, and lignin and constitutes only 1.8–3.2% of total soil weight, yet contains 15–32% of total soil organic carbon, serving as an important carbon source for microorganisms. Due to its susceptibility to microbial and enzymatic attack, this fraction has relatively rapid decomposition rates. High-density organic matter is primarily humic substances tightly bound to soil minerals, representing the main component of SOM with extremely slow decomposition and transformation rates. Because different SOM fractions exhibit distinct properties, this technique is frequently applied to evaluate the effects of land use, carbon turnover, nutrient availability, and aggregation processes.

Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS/MS) is an effective method that has emerged in recent years for studying pyrolysis mechanisms. The technique offers significant advantages including minimal sample requirements. The analytical process involves placing samples in a pyrolyzer under controlled heating conditions, causing macromolecular compounds to rapidly decompose into volatile small molecules. These small molecules are then carried by carrier gas into a gas chromatograph-mass spectrometer for separation and identification. By analyzing pyrolysis products qualitatively and quantitatively and correlating them with pyrolysis conditions, the characteristic chromatographic patterns can be used to infer sample composition, structure, and chemical characteristics, thereby establishing intrinsic relationships between pyrolysis products and the original sample’ s composition, structure, and physicochemical properties. This method has been widely applied in polymer science, bioengineering, and geochemistry.

Previous SOM research has primarily focused on total carbon estimation, but the complex structural composition of SOM makes it difficult to understand its sources and degradation processes through carbon quantification alone. Molecular-level SOM research can complement these limitations by identifying specific compound types and their abundances. For example, characteristic biomarkers in soils can distinguish between aboveground and belowground contributions to organic matter and serve as fingerprints for plants, microorganisms, gymnosperms, angiosperms, or microbial sources. The Tibetan Plateau is the world’ s highest and China’ s largest plateau, extensively covered by alpine grassland and alpine meadow soils. As one of the most fragile ecosystems, alpine grassland ecosystems are highly sensitive to global and regional environmental changes. Previous research on alpine ecosystems has concentrated on total soil carbon and nitrogen, with few studies at the molecular level. Conducting molecular-level research on Tibetan Plateau alpine soils will enhance understanding of natural environmental changes and human impacts. This study employs Py-GC-MS/MS to investigate pyrolysis fingerprint differences among five density fractions of alpine grassland soil organic matter.

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1. Study Area Description

The study site is located in the Namco region of the Tibetan Plateau, situated in the plateau' s interior with a typical semi-arid plateau monsoon climate. The area is controlled by the Indian monsoon and westerlies in summer and winter, respectively, with precipitation occurring mainly in summer. The multi-year average precipitation is 410 mm, and the mean annual temperature is -0.6°C. Vegetation consists of alpine grassland dominated by *Stipa purpurea* and *Kobresia macrantha*.

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2. Soil Sample Collection

Soil samples were collected at the Namco Comprehensive Observation Station of the Chinese Academy of Sciences (30°46 44 N, 90°59 31 E) in 2013. In typical alpine grassland areas around Namco, 1 m × 1 m plots were selected. At each sampling point, soils were collected from 0–15 cm depth using an auger in a serpentine pattern. Samples were air-dried after transport to Beijing for subsequent experiments.

2.1 Density Fractionation The heavy liquid used for density fractionation was sodium polytungstate solution prepared at different densities. Following the method described by Gregorich and Ellert, 20 g of mixed sample was added to sodium polytungstate solution at densities of <1.6, 1.6–1.8, 1.8–2.0, 2.0–2.25, and >2.25 g/cm^3. The mixture was shaken at 120 rpm for 20 minutes, then centrifuged. The supernatant was filtered through 0.7 m glass fiber filters (Whatman GF/F), and the material retained on the filter was washed with deionized water until salt concentration was <50 S/cm. All steps were repeated to ensure effective soil separation. The obtained fractions were dried at 60°C, ground to homogenize sample components, and stored for analysis.

2.2 Pyrolysis-Gas Chromatography-Mass Spectrometry (Py-GC-MS/MS) Pyrolysis was performed in a pyrolyzer (PY3030D) directly interfaced with a gas chromatograph-mass spectrometer (Agilent 7890/7000B). Approximately 2 mg of ground soil sample was placed in the pyrolyzer with internal standard C24D50 added for subsequent quantification of pyrolysis products. The pyrolyzer was operated at 600°C with helium as carrier gas.

GC-MS Conditions:
Column: HP-5 (100 m × 0.32 mm × 0.25 m)
Injector temperature: 300°C

Oven program: 40°C (hold 5 min) → 320°C at 3°C/min (hold 25 min)
Ion source temperature: 230°C
Quadrupole temperature: 150°C
Scan range: 50–550 amu
Electron energy: 70 eV

2.3 Statistical Analysis Data compilation and calculation of compound contents and ratios among different fractions were performed in Excel. Principal component analysis (PCA) was conducted using SigmaPlot software.

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3. Results

3.1 Fingerprint Characteristics of Different Density Fractions A total of 150 pyrolysis products were identified and quantified (detailed compound information in Table 1). Soil compounds primarily included carbon-containing compounds, nitrogen compounds, phosphorus compounds, and lignin. For analysis, pyrolysis products were grouped by similar chemical properties into alkyl compounds, aromatics and polyaromatics, lignin, phenols, polysaccharides, N-compounds, and chitin.

Total ion chromatograms clearly showed distinct differences among the five fractions in both compound content and composition. The F1 fraction exhibited the strongest peak intensity and highest organic matter content per unit mass. The F2, F3, and F4 fractions showed similar total ion chromatograms. The F5 fraction differed markedly from the first four fractions, with the weakest peak intensity and lowest organic matter content per unit mass.

3.2 Compound Contents in Different Density Fractions Differences in compound contents among fractions are shown in Table 2. Alkyl compounds originate mainly from soil and plant surface wax layers and protective layers of vascular plants. Short-chain n-alkanes and alkenes (C 20) derive primarily from microbial products, either as microbial lipid fragments or from cleavage of long-chain alkanes during pyrolysis. Medium-chain n-alkanes and alkenes originate from plant biopolymers such as cutin and suberin, though plant contributions usually dominate. Short-chain alkyl compounds showed the highest content in the F1 fraction (24.47%) and lowest in F4 (4.07%), with a slight increase in F5 (4.77%). Long-chain alkyl compounds decreased from 42.63% in F1 to 15.16% in F5.

Fatty acids are essential organic components of organisms with high biological specificity, useful for determining organic matter sources in sediments and soils. Different sources exhibit distinct distribution patterns: long-chain saturated fatty acids (C20–C32) originate from higher plant waxes, while short-chain fatty acids (C14–C18) derive mainly from microorganisms. Fatty acid content showed a decreasing trend, with maximum concentration of 3.17% in F1 and minimum of 1.2% in F5.

Aromatics are typically considered protein pyrolysis products, possibly derived from microorganisms or combustion products. Aromatics showed an accumulation trend across fractions, with lowest content in F1 (22.34%) and highest in F5 (40.51%). Polyaromatics may originate from cyclization of aliphatic compounds during pyrolysis or from charred materials. Due to their special chemical structures, these compounds strongly inhibit soil microorganisms. Polyaromatics increased from 11.40% in F1 to 17.60% in F5.

Lignin content was highest in F1 (20.12%) and gradually decreased with increasing density to 3.83% in F5. Lignin is a three-dimensional polymer containing aromatic rings and non-hydrolyzable C-O-C and C-C bonds. Only a few soil microorganisms can degrade lignin, resulting in much slower degradation rates compared to other organic compounds. The recalcitrance of lignin is attributed to both its chemical composition and protection by soil mineral particles. Previous studies show lignin is relatively depleted in small particle-size fractions and enriched in large fractions, with lignin content decreasing as soil mineral particle size decreases.

Although only a few phenolic compounds were identified among pyrolysis products, their relative contribution was substantial. These compounds originate from proteins, lignin, and cellulose, and are abundant in surface soils due to relatively high fresh litter content. Polysaccharides are major soil carbohydrates. Free monosaccharides are produced by microbial metabolism and are readily utilized by microorganisms, so soil carbohydrates exist mainly as polysaccharides. Soil polysaccharides derive from both plants and microorganisms. Xylose and arabinose in soil polysaccharides originate primarily from plant polysaccharides, while microbial polysaccharides are dominated by deoxyhexoses and mannose. Furfural derivatives often come from buried soils and have long residence times because these compounds are recycled during SOM decomposition and humification. Polysaccharides showed a slight accumulation trend with increasing density.

Soil N-compounds include proteins, polypeptides, and amino acids, which can interact with soil mineral surfaces to enhance stability. Adsorption of peptide substances on mineral surfaces, particularly strong adsorption to clay particles, protects proteins. From sand to clay fractions, surface adsorption increases, causing N-compounds to accumulate preferentially in small particle-size fractions. N-compounds increased from 15.55% in F1 to 20.18% in F5.

Chitin-derived pyrolysis products are mainly special N-compound fragments. Chitin typically originates from fungi or arthropods. Chitin content was low across all fractions, possibly derived from arthropod skeletal remains.

3.3 Differences in Degradation Difficulty Among Density Fractions

Although fractions F1–F4 constituted a small proportion of soil mass (0.13%), their pyrolyzable organic matter content was relatively high (5.7%). The F5 fraction is the major soil component. Ratios of alkanes to alkenes, polyaromatics to aromatics, and phenols to lignin all increased across fractions. Since saturated bonds are more degradable than unsaturated bonds, and polycyclic structures are more recalcitrant than single-ring structures, these ratios indicate degradation difficulty. The ratio results showed increasing trends for alkanes/alkenes and polyaromatics/aromatics across fractions, confirming that recalcitrant substances gradually accumulate with increasing soil density.

For the ratio of short-chain (C 20) to long-chain (C>20) alkyl compounds, relationships among fractions were not intuitively comparable, so principal component analysis (PCA) was applied. The first two principal components explained 79.66% and 12.64% of variance, respectively (cumulative 92.3%). Fractions F2 and F3 showed similar properties and could be considered as one group, while other fractions differed significantly.

[Figure 2: see original paper]

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4. Discussion

4.1 Differences Among Five Density Fractions This study employed Py-GC-MS/MS to investigate fingerprint differences among different density fractions of alpine grassland soil organic matter, systematically analyzing the types and distributions of 150 specific compounds. While molecular-level SOM research remains limited in China, the methodology presented here offers insights for studying SOM sources and degradation.

Macroscopic examination of total ion chromatograms revealed differences among fractions, with PCA further demonstrating that F2 and F3 were more similar to each other, while F5 differed substantially from the first four fractions.

Compound content changes across fractions followed clear patterns: (1) With increasing soil density, plant debris decreased, accompanied by reductions in lignin and long-chain fatty acids (primarily plant-derived). Short-chain alkyl compounds, derived from microorganisms or produced by cleavage of long-chain compounds during pyrolysis, showed a decreasing trend from F1 (24.47%) to F4 (9.71%), then increased in F5 (4.77%). This may occur because F1 contains abundant plant material where long-chain alkyl compounds are cleaved during pyrolysis, while F5 contains less plant material, so its higher short-chain alkyl content indicates greater microbial contributions. (2) Aromatics and polyaromatics, being difficult to degrade due to complex molecular structures and scarcity of decomposing microorganisms, accumulated gradually with increasing density. (3) Microbial fingerprint indicators (polysaccharides and N-compounds) increased across fractions.

Saturated bonds are more degradable than unsaturated bonds, and polycyclic structures are more recalcitrant than single-ring structures. Therefore, ratios of alkanes/alkenes and polyaromatics/aromatics were used to assess degradation difficulty. These ratios increased across fractions, confirming that recalcitrant substances accumulate with increasing soil density, making degradation more difficult. The phenol/lignin ratio also increased, suggesting that F1 contains relatively fresh organic matter, while higher-density fractions represent progressively more decomposed and transformed organic matter.

The F1 fraction, composed mainly of plant debris, contains readily degradable substances but has undergone minimal transformation due to recent input. Despite its low soil content, it has high organic matter content. Fractions F2 and F3 represent transitional stages of decomposition and transformation, with similar composition that could be considered as "partially transformed organic matter." The F5 fraction, as the main soil component, has undergone extensive transformation over long residence times, containing more recalcitrant substances and thus representing "deeply transformed organic matter." SOM forms complexes with clay and silt particles, protecting organic matter from microbial access and contributing to stabilization.

4.2 Differences Between Alpine Grassland and Non-Alpine Soils Soils with density <2 g/cm^3 are typically called light fraction organic matter, usually comprising 0.03-2.39% of soil mass while contributing 1-25% of total organic carbon. Light fraction organic carbon accounts for 1.8-3.2% of total organic carbon in arable soils, 0.6-14.7% in grasslands, and 5-48% in forests. The light fraction content of Namco grassland soils (0.13%) falls within these ranges, with light fraction organic carbon comprising 8.2% of total organic carbon—higher than typical grasslands (4-6%) but lower than shrublands (3-63%). This may reflect the region' s unique ecological conditions, with high altitude and cold temperatures resulting in lower organic matter content. With intensifying global warming and human activities, the region's ecosystems have been severely impacted, causing soil degradation and organic matter loss.

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5. Conclusion

This study demonstrated distinct differences among density fractions of alpine grassland soil organic matter. In terms of soil composition, the F5 fraction is the main component, while the F1 fraction, though low in proportion, has relatively high organic matter content per unit mass. Regarding compound composition, lignin content decreased with increasing soil density, being highest in F1 (derived mainly from plant debris) and gradually declining thereafter. Recalcitrant aromatics and polyaromatics accumulated with increasing density. Microbial-derived polysaccharides and N-compounds also increased, indicating that the F5 fraction contains more microbial fingerprint information.

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Note: Figure translations are in progress. See original paper for figures.