Lipids source and degradation as revealed by molecular biomarkers in soils after acid-pretreatment: A case of a plantain soil under long-term cultivation

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Abstract. The extractable lipids are important components in soil organic matter (SOM) which were used to trace the sources and degradation of SOM. The protection of lipids by soil mineral have been suggested through organic solvents. But, the extraction efficiency of some lipid compounds was low. This study applied a mild acid treatment to firstly remove most of the reactive mineral particles, and without altering SOM chemical structures in 10% HF/1M HCl (1:4 w: v). Based on the obtained lipid biomarker information, we observed that the lipid extraction efficiency significantly increased by organic solvents on after removal of active minerals. The acid treatment increased the scientific to quantitative the amount of lipids. The minerals showed significant differences in the selective protection to different components of lipids. In this study, the amount range of protected n-alkanoic acids is 73–85%, n-alkanol 41–62% and n-alkanes 26–46%. After the vegetation was replaced, the increased alkenoate and alkane in soil input by the plant tissues of plantain directly, and the alkanols probably input by the hydrolysis of wax esters. Under the interference of man-made tillage activities, the C content in 0-20 cm decreased, suggesting that cultivated activities may enhance SOM degradation and accelerate SOM turnover. Understanding SOM behaviour in this area will provide important information for soil management and to evaluate carbon cycling in human-affected ecological systems.

1. Introduction
Soil organic matter (SOM) is the largest carbon pool in terrestrial ecosystems. It is estimated that the total amount of soil organic carbon storage in the world is about $1.6\times10^{18}$ g, which is higher than the sum of atmospheric carbon pool ($0.75\times10^{18}$ g) and vegetation carbon pool ($0.6\times10^{18}$ g) [1]. Therefore, weak changes in soil organic carbon pool will have a profound impact on soil properties and global climate changes. Xishuangbanna Tropical Rainforest is one of the most diversified areas in China and has been included in the India-Burma Biodiversity Hotspots [2]. It’s natural ecosystems have been transformed into economic agricultural systems on a large scale, and the cultivation of developed economic crops has become an important economic pillar of local society. Vegetation transformation has already pose a serious threat to the biodiversity of the local ecosystem and the property of the soil[3]. Understanding the dynamics of soil organic matter in this region is an important step in assessing the
carbon cycle and will provide important information for soil carbon cycling and fixation mechanisms in the plateau terrestrial system.

Current researchers are focusing on lipid-related information. Lipids are insoluble in water but soluble in organic solvents, including n-alkanoic acids, n-alkanols, n-alkanes, hydroxy alkanoic acids, ketones, steroids, terpenoids, acylglycerols, sugars, and phospholipids and lipopolysaccharides [4]. Lipid biomarkers are not only used to distinguish between plant sources, but also distinguish between microbial and animal sources. Previous studies have systematically summarized lipid biomarkers such as chain-lipids (alkanoic acids, alkanols and alkanes) and cyclic-lipids (steroids, terpenoids)[5]. The carbon preference index(CPI)even-odd advantages of alkanoic acids and alkanols, and the odd-even distribution of alkanes are characteristic of plant-derived dominant performance [6]. These informations will provide important knowledge for studying the SOM turnover and carbon cycle.

Li et al. [7] observed that the content of alkanoic acid increased by 6 times, the increase dominated by long-chain alkanoic acid, and some indication parameters changed significantly before and after mineral removal, such as CPI. Before mineral removal, CPI decreased with increasing soil depth, and CPI increased with soil depth after mineral removal. Zegouagh et al.[8] also observed an increase in polar and long-chain compounds after reactive mineral removal. In the presence of minerals, the extraction efficiency and distribution pattern of lipids are greatly changed. Therefore, this study used the molecular biomarker method and acid treatment to analyze the source and degradation of lipids in the soil after long-term planting of plantain, and evaluated the regional carbon cycle and soil function through a more accuracy approach.

2. Material and method

2.1. Sample and preparation
The samples were collected from the plantain plantation of Xishuangbanna, Yunnan Province (101°01'-101°02'E, 22°46'-21°47'N). After removing the top plant litters, soil samples were taken (BA) at different depths, 0-20 cm (S), 20-40 cm (Z), and 40-60 cm (X). The background soil [9] is collected from the uncultivated area outside the plantain planting area, about 100 m away from the edge of the plantain planting area. All samples were freeze dried, ground and passed through 60 mesh. Manually remove the visible roots. In order to remove the reactive minerals, all soil samples were pretreated according to the previous method [10]. Briefly, soil samples were mixed and shaken with acid (10% HF/1M HCl) in a ratio of 1:4 (w:v) for 2 h. The mixture was then centrifuged at 2500 r min⁻¹ for 30 min and remove the supernatant.

2.2. Acid treatment supernatant analysis
All the solutions used and produced during the acid treatment are collected and mixed in a 5L beaker, including 10% HF/1M HCl solution, deionized water (wash soil to pH changesno longer) and 2M NaOH solution (adjust the pH of the mixed solution to neutral), the total volume of each solution is about 4~5L. 40 mL per solution are prepared for TOC determination and then calculate the total organic carbon content. The remaining solution was completely dried in oven at 60 ℃, and the residues was collected and weighed. Then, 15 g of the residues was weighed and subjected to section 2.3 and 2.4, the rest was dried and stored.

2.3. Sequential extraction of free lipid biomarkers
All soil samples and acid treatment residues were extracted as described in the previous study[10]. Briefly, 30 mL of dichloromethane was added to the original soil (20 g), the acid treated soil sample (15 g) and the acid treatment supernatant dried residues (15 g), and sonicated for 15 min. The mixture was then centrifuged at 2500 r min⁻¹ for 20 min. The supernatant was filtered through a glass fiber filter (Whatman GF/A 1.6 μm), and the filtrate was collected. Then, the residue was extracted successively with dichloromethane:methanol (1:1; v/v) and methanol, and the extraction conditions were the same as
above. All filtrates were collected and mixed, concentrated by rotary evaporation, transferred to a 2 mL glass vial and completely dried with N\textsubscript{2} gas. Duplicate for each sample.

2.4. Derivatization and GC-MS detect

The extract will treat with trimethylsilyl compound (TMS). Briefly, the extract was dissolved in 1 mL of dichloromethane: methanol (1:1, v/v). 100 µL of the sample was completely dried by blowing with N\textsubscript{2} gas, and then 90 µL of N, O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) and 10 µL of pyridine were added, and reacted at 70 °C for 3 hours. After cooling, diluted by 400 µL n-hexane. All derivatized samples were detected by GC-MS (Agilent, 7890A GC, equipped with 5975C quadrupole mass spectrometer). TMS derivatives of heptadecanoic acid and ergosterol were used as external standards for extractable lipids quantitative. The GC was equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25µm film thickness). The GC operating conditions were as follows: scan range of 50 to 650 Da, and a solvent delay time of 8 min.

3. Results and discussion

3.1. Soil properties before and after acid treatment

Elemental analysis shown, the carbon content in the soil increased significantly after acid treatment (Table 1), mainly due to the loss of reactive mineral components. TSE/C (TSE: Total solvent extracts; total organic solvent extract based on carbon content) and TSE/S (total organic solvent extract based on soil quality after acid treatment) increase due to organic matter enrichment or concentration after active mineral removal[11] (Table 1), while the increase in TSE/Y (the total organic solvent extract based on soil quality after acid treatment) is due to the improved extraction efficiency of organic solvents after reactive mineral removal.

**Table 1.** The elemental analysis and extract yields of soils before and after acid treatment

|                | C% | SD  | N% | SD  | SD  | TSE/C | SD  | TSE/Y | SD  | TSE/S | SD  | SL  | CL  |
|----------------|----|-----|----|-----|-----|-------|-----|-------|-----|-------|-----|-----|-----|
| B-S            | 1.75| 0.15| 0.12| 0.01| 17.42| 0.25| 0.12| 0.02| 0.21| 0.02| 0.21| 0.02| 0.66| 0.51|
| B-Z            | 1.29| 0.03| 0.11| 0.01| 13.94| 1.06| 0.11| 0.05| 0.15| 0.07| 0.15| 0.07| 0.65| 0.49|
| B-X            | 0.85| 0.01| 0.09| 0.01| 11.66| 0.85| 0.18| 0.04| 0.15| 0.03| 0.15| 0.03| 0.67| 0.42|
| 30-S           | 1.58| 0.12| 0.14| 0.02| 13.43| 0.77| 0.18| 0.02| 0.28| 0.00| 0.28| 0.00| 0.74| 0.59|
| 30-Z           | 1.58| 0.09| 0.13| 0.01| 14.77| 0.35| 0.12| 0.06| 0.19| 0.08| 0.19| 0.08| 0.73| 0.70|
| 30-X           | 1.29| 0.08| 0.12| 0.00| 12.65| 0.95| 0.12| 0.01| 0.15| 0.01| 0.15| 0.01| 0.73| 0.69|
| AB-S           | 2.53| 0.05| 0.24| 0.00| 12.12| 0.45| 1.21| 0.13| 1.03| 0.14| 3.05| 0.28|
| AB-Z           | 1.92| 0.25| 0.18| 0.00| 12.59| 1.74| 3.26| 1.65| 2.09| 0.84| 6.04| 2.34|
| AB-X           | 1.47| 0.17| 0.17| 0.01| 9.96 | 0.38| 1.07| 0.01| 0.52| 0.05| 1.58| 0.16|
| A30-S          | 2.51| 0.02| 0.20| 0.03| 14.46| 2.10| 1.48| 0.11| 0.96| 0.03| 3.71| 0.25|
| A30-Z          | 1.77| 0.07| 0.15| 0.02| 13.51| 0.91| 1.46| 0.30| 0.70| 0.18| 2.58| 0.43|
| A30-X          | 1.42| 0.05| 0.14| 0.01| 11.58| 0.92| 1.03| 0.27| 0.41| 0.14| 1.48| 0.44|

Note: -S: 0-20cm depth; -Z: 20-40cm depth; -X: 40-60cm depth. 30-: soils planted with plantain for 30 years (BA), B-: original soils [9]; C/N: Atomic ratios; TSE/C: Total solvent extracts on basis of carbon content (mg·g-1 C); TSE/Y: Total solvent extracts on basis of original soil mass (mg·g-1 original soil); TSE/S: Total solvent extracts (mg·g-1 particle mass of the corresponding soil); SL indicates soil particle mass loss after acid treatment; CL represents soil carbon loss (based on the original soil mass) after acid treatment. All data were mean values. SD: Standard Deviation. A: after acid treatment.

After acid treatment, the mass loss [12] of the two soil samples was in the range of 65–67% [9] and 73–74% (BA), respectively. It should be mainly attributed to the loss of mineral quality. The carbon content loss [13] in the range of 42-51% [9] and 59-70% (BA), respectively, which should be mainly attributed to the high hydrophilic organic compounds and fineness particles loss during the removal of reactive minerals. Therefore, we collected and tested the solution produced during the acid treatment.
The results of TOC detection of the solution showed that the TOC content in the solution was in the range of 89.9~106.6 mg·L$^{-1}$ [9] and 114.0~140.0 mg·L$^{-1}$ (BA), respectively, indicating that the organic carbon content in the acid treatment solution was higher. There may be some lipid biomarkers lost with the solution. The results of GC-MS analysis of the acid treatment supernatant dried residual showed that a large amount of sugars and a small amount of alkanes were detected, and no other lipid information was detected. Alkanoic acids and alkanols are more hydrophilic than alkanes, but they are not detected. This phenomenon has not been reasonably explained in the literature. However, the collection and testing of acid treatment solutions will further improve the precise of the quantification of lipid biomarkers in soil.

3.2. The lipid biomarker information interference by reactive mineral

Organic solvent extractable lipids mainly include aliphatic (alkanoic, alkanol and alkane) and steroids [6]. We noticed an increase in the amount of lipids extraction after reactive minerals remove (Figure 1). However, this does not explain the correlation between mineral protection and carbon number, because a large amount of C$_{16}$, C$_{18}$ and its unsaturated alkanoic acid were detected in fresh plantain and bamboo leaf plant tissues, indicating an increase in short alkanoic acid and it may be related to its relatively rich source. The concentration of the alkanol is lower to the alkanoic acid, and the acid treatment also increases the concentration of the alkanol, but not significant compare to alkanoic acid. The amount of alkanol increased by 1.7~1.9 [9] and 2.3~3.7 (BA) times, respectively, indicating that about 41~62% of the alkanol was protected by reactive minerals. The effect of acid treatment on alkanes is the weakest. After acid treatment, the amounts of alkanes increased by 1.4~1.9 times, indicating that 26~46% of alkanes are protected by reactive minerals.

![Figure 1. The total amounts of different lipids with soil depth before and after acid treatment.](image-url)
Note: The total amounts of different lipids with soil depth before and after acid treatment. a, c, e: soils planted with plantain for 30 years, b, d, f: original soils; a: 0-20 cm depth; c: 20-40 cm depth; e, f: 40-60 cm depth. Black: before acid treatment; White: after acid treatment. A: alkanoic acid; IA: isoalkanoic acid; C: alkane; O: alkanol; S: steroid.

Before and after the removal of reactive minerals, the distribution characteristics of lipids has changed, which may lead to deviations in the source and degradation information of SOM by biomarkers. A typical example is CPI. CPI is commonly used to indicate the degree of degradation of lipids because the even (alkanoic acid)/odd (alkane) homologs are preferentially synthesized in plant tissues, so the value of CPI is greatest in fresh plant tissues. In order to achieve a parity balance during the degradation process, even (alkanoic acid) / odd (alkane) homologs will preferentially degrade, and CPI will decrease[14, 15]. Our recently reported studies have seen changes in some indication parameters before and after acid treatment[7]. The most obvious change in CPI observed in this study was that in BA, CPI increased with increasing soil depth before acid treatment, but CPI decreased with soil depth after acid treatment.

3.3. The lipid biomarker information by cultivation

After vegetation replacement, all types of lipids were observed the increase for all soil samples (Figure 2). Alkanoic acids increase mainly in short-chain C_{16}, C_{16}, and C_{18}, alkanes increase mainly in long-chain C_{25}, C_{27}, and C_{31}, and alkanols increased generally. The results of GC-MS showed that although the distribution characteristics of lipids in leaves and soil samples were same, the highest alkanoic acid content in leaves was C_{16} and C_{18}, which is the same as the number of alkanoic acids in BA. The increase in the alkane is similar to that of the alkanoic acid, indicating that the increased alkanoic acids and alkanes in the BA may be primarily derived from the input of the plantain plant tissue. A variety of unsaturated C_{18}n-alkanoic acids (C_{18:1}, C_{18:2} and C_{18:3}) were detected in both plant tissues, and C_{18:2} and C_{18:3} alkanoic acids were rarely detected in soil. This is because the double bonds of unsaturated alkanoic acids are easily oxidized [16] and degrade rapidly in the soil. Thus, the direct input of fresh plantain plant tissue observed in BA, but the total organic carbon content decreased in 0-20 cm (cultivation layer), indicating that artificial cultivation activities enhanced lipid degradation in soil.

![Figure 2](image-url)

Figure 2. The distribution of n-alkanoic acids, n-alkanols and n-alkanes in soil planted with plantain and original soil for different depth before and after vegetation changed.
Note: a, d, g: The distribution of n-alkanoic acids in soil planted with plantain and original soil for different depth before and after vegetation changed; b, e, h: The distribution of n-alkanols in soil planted with plantain and original soil for different depth before and after vegetation changed; c, f, i: The distribution of n-alkanes in soil planted with plantain and original soil for different depth before and after vegetation changed; a, b, c: 0-20 cm depth; d, e, f: 20-40 cm depth; g, h, i: 40-60 cm depth.

4. Conclusion
After acid treatment, the extractability of lipids increased significantly. The protection of minerals to alkanoic acid is between 73 and 85%, the protection of alkanoic acid is between 41 and 62%, and the protection of alkanes is between 26 and 46%. The lipid distribution characteristics also changed significantly. Some indication parameters (CPI, ACL, RLS) indicating the source and state of organic matter changed, and even the opposite result appeared; during the acid treatment, some lipids will be lost with the acid treatment solution (the main observations of sugars and alkanes in this study), therefore, reactive minerals should be removed prior to quantitative analysis of lipid biomarkers in soil to ensure accurately analysis of lipid biomarkers, and the acid treatment solution should be collected and tested. Artificial farming activities promote the input of organic matter and enhance the degradation of organic matter, thus, cultivation activities may accelerate the turnover of SOM.

Acknowledgments
This work was financially supported by Yunnan Talents Training Project (KKSY201622013).

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