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Identification and characterization of proteins, lipids, and metabolites in two organic fertilizer products derived from different nutrient sources

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Abstract

The biochemical composition of organic fertilizers largely determines their nutrient supply characteristics following soil application as well as their potential impact on soil microbial communities. Yet, limited information is available regarding the biochemical composition of organic fertilizers derived from different nutrient sources. Here, we qualitatively analyzed the presence and abundance of proteins, lipids, and metabolites in a liquid fish fertilizer (LFF) product and a type of granular organic fertilizer (GOF) commonly used in organic vegetable production, using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Our results suggest that the presence and abundance of proteins, lipids, and metabolites differ greatly between GOF and LFF. The qualitative analysis shows LFF as a rich source of metabolites, while complex proteins and long-chain saturated fatty acids are dominant in GOF. The degree of biochemical composition complexity may help explain the varying impacts of different types of organic fertilizers on nutrient availability, soil health, and environmental quality.

Keywords: Biochemical composition, LC–MS/MS, Liquid chromatography–tandem mass spectrometry, Lipidomics, Metabolomics, Nutrient availability, Organic nutrient source, Proteomics

Introduction

Organic fertilizers derived from various animal and plant-based byproducts have been widely used as nutrient sources for organic vegetable crop production. Compared to synthetic chemical fertilizers, organic fertilizers tend to have lower nutrient content along with variability in composition that depends on ingredients. However, organic fertilizers often contain beneficial microorganisms and are generally rich in organic carbon (C) [1]. Applications of organic fertilizers have been shown to increase soil organic matter content, enhance overall soil enzyme activity [2], and influence microbial community

composition through addition of C and nitrogen (N)-rich organic compounds [3–6]. At the same time, the nutrient release process of organic fertilizers relies substantially on the complex biochemical transformation activities mediated by soil microbes [7] in addition to environmental conditions.

Early studies indicated that the general C/N ratio and nutrient analysis of organic fertilizers might not offer sufficient information for predicting nutrient release patterns, while the biochemical composition of organic fertilizers could be highly associated with mineralization kinetics [8, 9]. Numerous methods have been developed to estimate N availability in soils after organic fertilizer application, such as incubation-based N mineralization studies [10, 11]. Most recently, the extractable soil protein pool was suggested to be a soil health indicator of potentially available organic N [12]. Plant metabolite

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compounds also influence soil nutrient cycling, such as C and N mineralization, through their impact on soil organisms [13]. The input of lipids, especially the long-chain compounds, play an important role in soil health enhancement including the accumulation of soil organic matter content [14]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) with high-throughput capacities has been demonstrated to be a sensitive and powerful analytical tool for detecting proteins, lipids, and other metabolites from biological materials of various sources including soil [15] and plant tissue samples [16–18]. However, limited information is available regarding specific biochemical compounds contained in organic fertilizers that may affect soil microbial communities and nutrient availability as well as environmental quality. LC–MS/MS has rarely been used to compare biochemical compositions of organic fertilizers with different nutrient sources. Here, we chose to examine two commercially available organic fertilizer products, a liquid fish fertilizer (LFF) derived from enzymatically digested fish proteins and a granular organic fertilizer (GOF) containing feather meal and other animal waste materials, which represent commonly used nutrient sources for organic crop production [19].

Therefore, the objective of this pilot study was to employ LC–MS/MS to identify and characterize proteins, lipids, and metabolites in two commonly used commercial organic fertilizer products for qualitative comparisons of their biochemical characteristics.

Materials and methods

The LFF analyzed in this study can be used in organic fertigation systems through drip irrigation, while the GOF may be used for preplant application and/or sidedressing during the crop production season. Both organic fertilizer products have higher levels of N than phosphorus (P) and potassium (K) (Figure 1A; Additional file 1: Table S1). Equal amounts of LFF (100 μ L) and GOF (100 mg) were individually extracted for proteins, lipids, and metabolites. The equivalent mass of 100 μ L LFF was determined as 97.5 ± 1.0 mg. Lipids were extracted via a modified Folch method [20], metabolites were extracted by ice-cold methanol, and proteins by acetone/methanol precipitation. The protein pellet was reconstituted in 0.2% surfactant enhancer and concentrations were measured via a Qubit Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). In-gel protein digestion was performed using trypsin as the enzyme (Promega Corporation, Madison, WI, USA). LC analysis for all was performed on a Dionex UltiMate 3000 RSLCnano system (Thermo Fisher Scientific Inc.). Lipid and metabolites were separately injected (5 μ L) on an Acclaim PepMap RSLC C18 column (Thermo Fisher Scientific Inc.) using

acidified mobile phases of water and acetonitrile for metabolites and water, acetonitrile, and isopropanol for lipids. Proteins (10 μ g) were separated on a PepMAP column using acidified water and acetonitrile.

Both the lipid and metabolite compounds were analyzed on a Bruker Impact II QqTOF mass spectrometer (Bruker Daltonics, Billerica, MA, USA) using electrospray ionization operated in positive mode. Proteins were analyzed on a Thermo Scientific Q Exactive HF Orbitrap mass spectrometer equipped with an EASY Nanospray source operated in positive mode. All employed data dependent collisionally-activated dissociation. Proteomics data were analyzed in Proteome Discoverer (version 2.4) using the SEQUEST HT searching algorithm. Lipids and metabolites were analyzed in MetaboScape (version 4.0). Full experimental details are provided in Additional file 1.

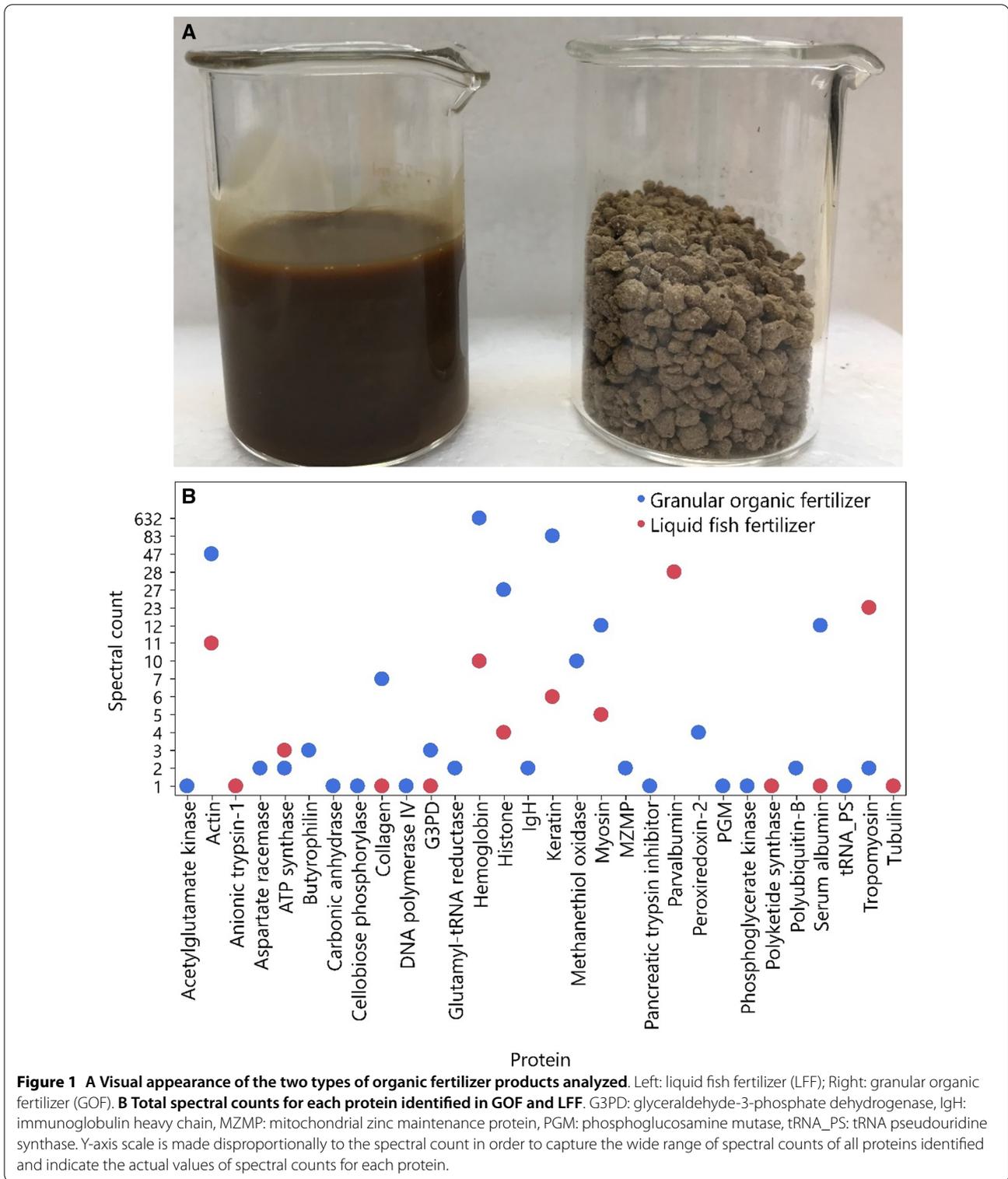
Results and discussion

Proteomics

Figure 1B is a graphical summary of total spectral counts for each protein detected in GOF and LFF products. Hemoglobin (632 spectral counts), keratin (83 spectral counts), and actin (47 spectral counts) were the major proteins detected in GOF (862 spectral counts in total). In contrast, parvalbumin (28 spectral counts), tropomyosin (23 spectral counts), and actin (11 spectral counts) were abundant in LFF (96 spectral counts in total). Degradation of proteins in the soil can be affected by their intrinsic structures including amyloid fibril formation and glycosylation, accessibility to soil microbes or extracellular enzymes, and complex structure formation with other soil organic compounds such as tannins, lignin, and humic substances [21]. For instance, hemoglobin can bind tannins to form protein–polyphenol complex (PPC) that limits N mineralization due to its resistance to decomposition [22]. As a fibrous structural protein, keratin could be highly recalcitrant in response to microbial degradation because of its molecular architecture that involves formation of disulfide bonds, phosphorylation, and glycosylation [23]. The predominance of hemoglobin and keratin found in GOF might imply lower N mineralization rates of GOF relative to LFF.

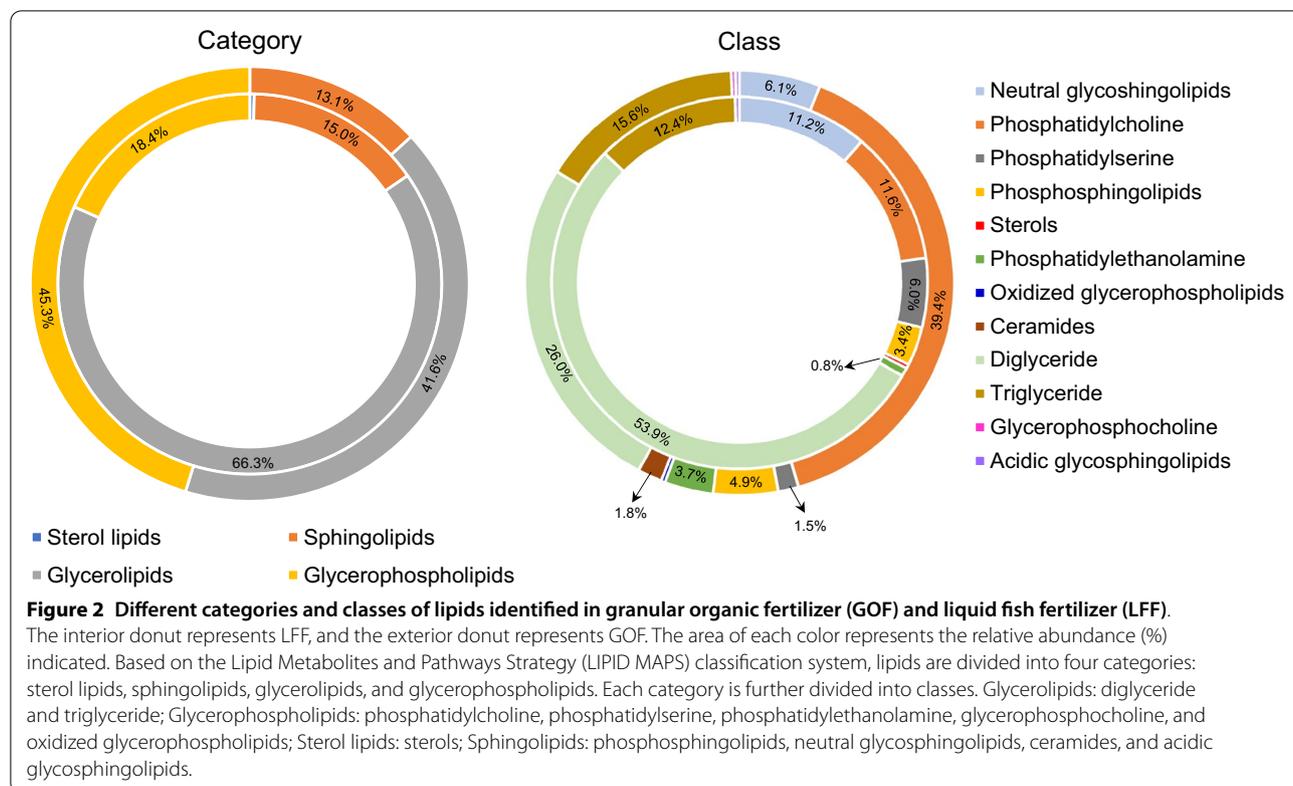
Lipidomics

Phosphatidylcholine (PC) (e.g., C42, C44, C46) and diglyceride (DAG) (e.g., C31, C35, C37, C39, C41, C43, C47) lipids accounted for 39.4% and 26.0% of the lipids in GOF, respectively, whereas DAGs (e.g., C33, C35, C37, C39, C41, C43, C47) showed a higher classification proportion (53.9%) in LFF (Figure 2). Our data also revealed that the majority of DAGs in both LFF and GOF contained highly polyunsaturated fatty acids (e.g., 30:4, 32:3,



34:5, 40:9, 44:12), which could be more susceptible to microbial degradation in the soil than saturated fatty acid molecules [24]. Conversely, PC lipids containing N and P

identified in GOF may degrade and release essential elements rather slowly due to its long-chain structure and the relatively high degree of fatty acid saturation. Overall,



the diverse array of lipids identified in organic fertilizers in our study might indicate the potential impact of organic fertilizer application on improving soil health. For instance, lipids may serve as C and energy sources for various lipid-degrading soil microorganisms, such as *Bacillus*, *Arthrobacter*, and *Pseudomonas* [25]. Long-chain lipids (>C20), in particular, are important in C stabilization and humification processes during the accumulation of soil organic matter [26].

Metabolomics

The identified metabolites (Additional file 1: Table S2) were categorized as amines, amides, polyols, organic acids, steroids, vitamins, isoprenoids, and plasticizers (Figure 3). Comparisons between LFF and GOF suggested that LFF is a richer source of metabolites (Figure 3) and the two organic fertilizers might have different impacts on soil nutrient cycling and soil microbial communities. For example, stearamide (m/z 284.2949; retention time (RT) 36.74 min) was detected in LFF but not in GOF. It is interesting that capsaicin (m/z 308.2218; RT 15.05 min) was found in GOF but absent in LFF (Additional file 1: Table S2). Although the source of capsaicin is unclear, the GOF that contains capsaicin might potentially demonstrate a deterrent or repellent effect on certain fungi, insects, and mammals due to the irritant

property of capsaicin [27]. Four fatty acid amides including pipericine (m/z 336.3260; RT 40.04 min), macamide (m/z 346.3100; RT 37.08 min), docosanamide (m/z 340.3572; RT 42.44 min), and erucamide (m/z 338.3416; RT 41.87 min) appeared more abundant in LFF than GOF (Table S2), but statistical analyses with replications were not performed in this exploratory study. These amides may participate in stimulatory activities associated with soil microbial metabolism [28]. Two phenols, including *p*-coumaric acid ethyl ester (m/z 193.0861; RT 35.01 min) and gingerol (m/z 277.1798; RT 19.20 min), were also abundant in LFF (Table S2). As a product of acidic hydrolysis of *p*-coumaric acid ethyl ester, *p*-coumaric acid can increase soil dehydrogenase activity and abundance of soil bacterial and fungal communities [29, 30]. However, gingerol, another compound abundant in LFF, may display antimicrobial activity [31]. Additionally, given the relatively high acidity (pH=3.5) and abundant level of organic acids of LFF (Additional file 1: Tables S1, S2), its application could potentially result in a reduction in soil pH and suppression of certain soilborne pathogens in the longer term [32]. The low pH of LFF may be attributed to the abundance of bile acids, such as 3 β -hydroxy-5-cholenoic acid (m/z 357.2787; RT 22.11 min), 3-oxo-cholic acid (m/z 424.3060; RT 17.59 min), cholic acid (m/z 426.3214; RT 18.98 min), and nutriacholic acid (m/z

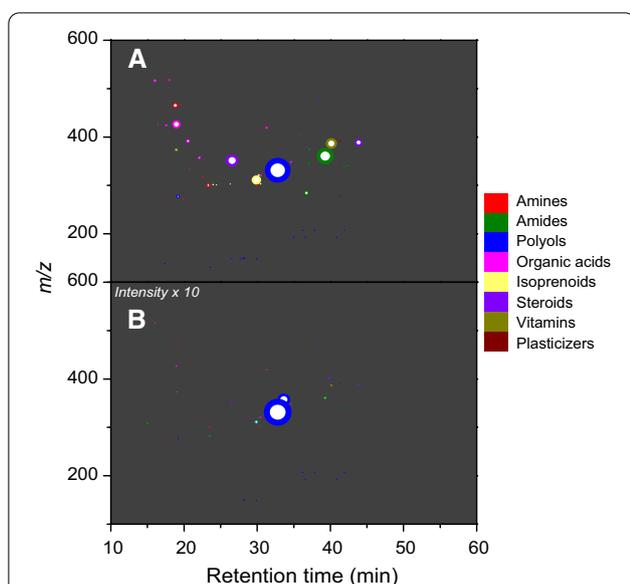


Figure 3 Survey overview of metabolite presence and relative abundance broken down by class and functionality for liquid fish fertilizer (A) and granular organic fertilizer (B). These compounds were annotated and categorized with the help of PubChem based on the general chemical structures of compounds. The compound abundance was determined using the spectral and analyte metabolite matches compiled in MetaboScape. The circle color represents compound category and circle size is proportional to the compound abundance. The abundance values were scaled up by an intensity factor of 10 in plot B in order to make dots visible in plot B.

391.2844; RT 20.55 min) (Table S2). Moreover, bacterial degradation products (e.g., androstadienediones) of bile acids could also pose a potential risk, such as reduced reproduction rates, to invertebrates in agricultural soils [33]. Similarly, as a steroid hormone, progesterone (m/z 315.2319; RT 28.67 min), which was discovered in both LFF and GOF (Additional file 1: Table S2), might accumulate in soil and cause adverse impacts on the environment [34].

Conclusions

Our qualitative analysis is the first study attempting to elucidate the biochemical composition of organic fertilizers, explore its linkage to nutrient availability of organic fertilizers, and envision its impact on soil quality and health. The dominance of complex proteins and long-chain saturated fatty acids contained in GOF suggests that GOF might decompose and release nutrients at a slower rate in the soil relative to LFF. A diverse variety and abundance of metabolites were identified in GOF and LFF, indicating potentially different impacts of these organic fertilizers on soil microbial communities and nutrient availability. Future research can examine more organic fertilizer products

derived from various nutrient sources and include quantitative analysis for in-depth comparisons. Overall, our study demonstrates the complexity in biochemical composition of organic fertilizers and suggests the need to further understand how organic fertilizers with different biochemical profiles influence nutrient cycling, soil health, and environmental quality.

Abbreviations

LFF: Liquid fish fertilizer; GOF: Granular organic fertilizer; GC/MS: Gas chromatography–mass spectrometry; LC–MS/MS: Liquid chromatography–tandem mass spectrometry; PPC: Protein–polyphenol complex; PC: Phosphatidylcholine; DAG: Diglyceride.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13765-021-00625-2>.

Additional file 1: Table S1. Basic characteristics of the two organic fertilizer products used in this study. **Table S2.** List of metabolites identified by LC–MS/MS in liquid fish fertilizer and granular organic fertilizer.

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Authors' contributions

JL and XZ designed the experiment and wrote the manuscript. LSB, MNK, and KBB performed the LC–MS/MS analysis and helped with data analysis and interpretation. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Declarations

Competing interests

There is no conflict of interest.

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