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ORGANIC MOLECULAR MARKERS IN A FLUVISOL AMENDED WITH ORGANIC AMELIORANTS

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Abstract

Three types of organic ameliorants, sheep manure, sewage sludge from a waste water treatment plant and a plant compost were added to an Alluvial-meadow soil (Fluvisol). There was evidence for well-preserved biomarker signals and higher values of the carbon preference index (CPI) in the sludge and manure-treated soils, while lower values, typical of microbial alkanes, indicating a high degree of degradation were observed in the plant compost amended soil. Sludge and sheep manure originating metabolic products, such as steroids and polycyclic aromatic hydrocarbons (PAHs) have been preserved in the Fluvisol after 104 days of composting.

Key words: organic molecular markers, organic ameiorants, sheep manure, sludge, compost

Introduction. Lipids are major components of soil organic matter due to their hydrophobic nature [1]. They are extremely important for various soil characteristics, such as aggregate stability, water retention capacity and soil fertility in general, as different plant species have a characteristic lipid composition. With good preservation of lipids in soils, the composition of organic molecular markers can be used as an indicator to determine the contribution of the source for a past

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period (paleoenvironment) and related biogeochemical processes. Organic ameliorants to a Fluvisol used in this study have led to increasing soil pH, CEC (cation exchange capacity), C:N ratio, organic matter content, with the domination of humic acids, as well as available macroelements N, P, K [2].

It was found that soil organic matter in a humic Oxisol under various vegetation contains even-numbered *n*-alkanoic acids in the range of C_{16}-C_{32} and long chain alkylic compounds accumulating preferably at depths of 60–100 cm. On the surface, the soil from natural vegetation had a total lipid value higher than the cultivated soils, and showed a predominance of unsaturated alkanoic acids at 60–100 cm. Forest and pasture dominated soils are prone to accumulation of the long-chain ($> C_{20}$) homologues, which may be important with regard to C stabilization and humification processes in subsoil of humic Oxisols [3]. Qualitative and quantitative variations in the molecular characteristics of soil organic matter (SOM) between control and compost-amended soils were found, i.e. increasing amounts and diversified components of fatty acids, *n*-alkanes and various biopolyesters derivatives such as hydroxy-alkanoic and alkandioic acids in the compost-amended soil [4]. A sandy loam composted with anaerobically digested sewage sludge exhibited positive effects of waste amendment, such as increased potentially mineralizable N by a factor of 1.8 and resin-extractable P by a factor of 1.6. However, there were no accumulated effects of waste amendment on the fraction of wet-stable aggregates, or on the microbiological properties. In another experiment by [5], a co-compost amendment from sewage sludge and pruning waste increased the amounts of humic acids (HA) (1.9 times), fulvic acids (FA) (3.3 times), humin (1.5 times), as well as the free organic matter (1.4 times) and free lipids (21.8 times). Incubation of the soils enhanced biological activity mainly in the amended soils, leading to progressive SOC (soil organic carbon) mineralization and humification, mainly humic acids.

The main objective of this study was to define changes in the free lipid biomarkers composition of a low organic matter Alluvial-meadow soil (Fluvisol) following addition of some organic ameliorants, such as plant compost, WWTP (waste water treatment plant) sewage sludge and sheep manure.

Materials and methods. The soil used for composting was Alluvial-meadow soil (Fluvisol) from the Institute's experimental fields in Tsalapitsa, Plovdiv region. Main soil characteristics were, SOC = 0.54 % and CEC = 16.7 cmol.kg^{-1}. Organic soil ameliorants, i.e. compost, sewage sludge and sheep manure were applied. The soil was composted with each of the amendments in a ratio of 1:9. Moisture at 10% was maintained and incubation was continued for 104 days and T 20 °C. The plant compost was produced from an installation for composting separately collected bio-degradable, green waste (branches, tree foliage, plant residues from parks and gardens) and food waste and has the following parameters: organic matter – 66.4%; CEC = 44.7 cmol.kg^{-1}; carbon to nitrogen ratio (C/N) – 11.8; sludge from WWTP, SOC = 14.60 %, CEC = 51.6 cmol.kg^{-1} and car-

bon to nitrogen ratio (C/N) – 4.52; sheep manure SOC = 13.40 %, CEC = 22.5 cmol.kg^{-1} and carbon to nitrogen ratio (C/N) – 8.82.

Lipids analysis. Extractions were performed by sonication of the soil (4 g) in 16 ml of acetone:hexane (1:1) using Julabo USR 3, 35 kHz, 200 W, Julabo Labor technik GMBH for 20 min. The solvent extracts obtained were dried over Na_2SO_4, evaporated in vacuo (Labconco Centri Vap concentrator at 50 °C) and the solvent was replaced with dichloromethane: hexane (1:1). An internal standard (2-nonadecanone) was added, dried in vacuo, then derivatized with BSTFA + 1% TMS (heated for 1 hour at 70 °C [6]. After completion of the derivatization, the derivatives were cooled, reconstituted to 1 ml with DCM and analyzed by GC/MS.

Gas chromatography–mass spectrometry (GC/MS). Gas chromatograph Agilent 7890A with a 5975 ° C mass-selective detector (splitless injection mode) was used for analysis of the lipid extracts. Separation was performed on HP-5ms capillary column (30 m×0.25 mm I.D., film thickness, 0.25 µm) and He was used as a carrier gas. The GC programme was the following: initial T 60 °C, hold 1 min, linear ramp 10 °C/min to 180 °C, ramped at 4°/min to 300 °C, hold 15 min, MS detection full scan, mass to charge ratio (m/z) 50–1000, cycle time 2.28 scans/s and EI ionization 70 eV. Identification was based on comparison of the mass spectra of chromatographic peaks to NIST-MS library, authentic standards, GC retention times and additional interpretation of mass fragmentation patterns. For quantitative analysis, reference compounds, *n*-alkanes, acids and alcohols were used for calibration. The fragment ions for quantification were: m/z 57 for the *n*-alkanes, m/z 75 for the *n*-alcohols and m/z 117 for the *n*-fatty acids. The peak areas for each component were compared with the peak area of the fragment ion (m/z 58) of the 2-nonadecan one standard by assuming the same response factor for analytes as for the internal standard. Compound ratio (carbon preference index CPI) is calculated: $CPI_1 = \Sigma(C_{17}\text{-}C_{31})/\Sigma(C_{18}\text{-}C_{24})$ for the whole range of detected*n*-alkanes [7] and average chain length ACL for *n*-alkanes: $(\sum C_i \times i)/\sum C_i$, where C_i is the concentration of the *n*-alkane containing *i* carbon atoms [10].

Results and discussion. The main eluted compounds in the control soil (unamended soil) and those ameliorated with organic amendments are presented in Table 1. Some of the major eluted compounds (without the main homologous series of alkanes, fatty acids and alcohols) indicate the presence of plant sterols and palmitic and stearic acid esters, which were previously found to be solubilized in micelle-like microparticles and nanosoluble organic colloids [11]. Distributions corresponding to mixtures of isomers of branched C_{17} – C_{22}alkanes including *iso*- and *anteiso*- isomers are presented in Fig. 1. Compound ratios providing information on various sources of sediment organic matter were calculated according to [9].

A predominance of "odd" vs. "even" homologues of alkanes was found in all the studied variants, except in the compost amended soil (Fig. 1). The alkane dis-

Table 1

Some major compounds in the soils studied

Retention time	Alluvial-meadow soil amended with plant compost
15.004	2-propenoic acid, tridecyl ester
17.013	isopropyl myristate
20.526	7-methyl-Z-tetradecen-1-ol acetate
20.726	palmitelaidic acid, 9-hexadecenoic acid
24.068	E-8-Methyl-9-tetradecen-1-ol acetate (suspected)
27.364	palmitic acid, vinyl ester
27.513	hexanedioic acid, bis(2-ethylhexyl ester
27.667	myristic acid, 2,3-bis(hydroxy)propyl ester
31.272	hexadecanoic acid, 2,3-bis[(trimet hydroxy]propyl ester
34.717	octadecanoic acid, 2,3-bis hydroxy]propyl ester
43.265	β-Sitosterol
43.528	β-Amyrin
Retention time	Alluvial-meadow soil, amended with sludge from WWTP
15.010	2-propenoic acid, pentadecyl ester
15.537	3-dodecene, 1-(benzyloxy)-4-methyl
15.611	9-octadecenoic acid (Z)-, phenylmethyl ester
17.064	2H-pyran, 2-(7-heptadecynyloxy)tetrahydro-
17.316	2-pentadecanone, 6,10,14-trimethyl
18.741	5,6,6-trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one
19.811	methyl 2-hydroxy-tetradecanoate
20.932	hexadecanoic acid
21.213	kaur-16-ene (coal biomarker)
21.550	D-homoandrostane, (5α,13α)
27.278	kauran-19-oic acid, methyl ester
31.272	hexadecanoic acid, 2,3-bishydroxy]propyl ester
34.711	2,3-Bis [hydroxy]propyl stearate
38.939	coprostan-3-ol (in feces)
39.208	5α-cholestane, 3α-(hydroxy)
39.374	5β-cholestan-3α-ol
39.981	cholestan-3-one, (5β)
40.370	cholesterol
40.547	3-[(hydroxy]cholestane
40.999	5α-Ergost-8(14)-ene
41.892	a-homocholest-4a-en-3-one
42.144	24-ethylcoprostanol
42.361	Stigmasterol
43.265	β-Sitosterol
43.460	24-ethylcoprostanol
44.192	β-Amyrin

Table 1

Continued

Retention time	Alluvial-meadow soil amended with sheep manure
11.943	benzoic acid, 2-(1-oxopropyl)-, methyl ester
12.080	isoxazolo[2,3-b]benz[1,2]oxazine, perhydro-2-methoxycarbonyl-10-methyl-5a-hydroxy-
15.411	1H-indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl-
18.398	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
18.741	hexadecanoic acid, methyl ester
20.240	longifolenealdehyde
20.526	estra-1,3,5(10)-trien-17β-ol
20.726	palmitelaidic acid
27.661	myristic acid, 2,3-bis(hydroxy)propyl ester
31.272	hexadecanoic acid, 2,3-bis hydroxy]propyl ester
34.717	octadecanoic acid, 2,3-bishydroxy]propyl ester
38.939	coprostan-3-ol
40.370	3α-cholest-5-ene
40.616	5-α-ergost-8(14)-ene
41.892	cyclopropa[7,8]cholestan-3-one,3',7-dihydro-, (5α,7β,8α)-
42.247	trimethyl[(3β,5α)-stigmastan-3-yl]oxy]-
43.265	β-Sitosterol
43.454	24-Ethylcoprostanol
43.523	β-Amyrin

tribution was predominantly trimodal maximizing at n-C_{17} alkane, C_{19}/phytane, C_{25}/C_{29} alkane, indicating a mixed origin of OM inputs. In our study, similarly to [10] there was an imprint of higher plants, reflected by the presence of short-chain ($< C_{20}$) alkanes with a preference of *odd/even* homologues. In addition, a number of branched alkanes were detected, thus proving to originate from microbial degradation of long chain n-alkanes. In the compost amended soil there was a reversal in the homologues, indicating a predominant microbial input, CPI = 0.81. The CPI ratio of *odd/even* alkanes for the soils studied, decreased in the order: sheep manure amended, 1.79 > sewage sludge amended, 1.48 > control soil 1.31 > compost amended soil, 0.81. The average chain length ACL decreased in the same order: sheep manure amended (23.2) > sewage sludge amended (21) > control soil (20.3) > compost amended soil (19.8).

Higher values for the CPI and OEP indicate n-alkanes originating from plant waxes and are characteristic of well-preserved biomarker signals, while lower values are typical of n-alkanes originating from microbial sources or indicating a high degree of degradation [10]. It has also been found that the source of short-chain and branched alkanes can be the microbial biofilms found in arid zones [11].

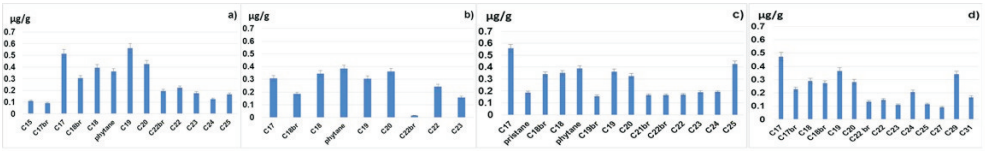

Fig. 1. Chain length distribution of alkanes in the Alluvial-meadow soil (Control), $CPI_{alk} = 1.31$ (a); in the Alluvial-meadow soil, amended with compost, $CPI_{alk} = 0.81$ (b); in the Alluvial-meadow soil, amended with sewage sludge, $CPI_{alk} = 1.48$ (c); in the Alluvial-meadow soil, amended with sheep manure, $CPI_{alk} = 1.79$ (d)

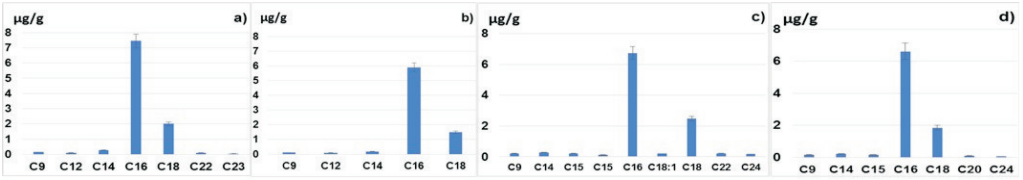

Fig. 2. Chain length distribution of fatty acids in the Alluvial-meadow soil (Control) (a); in the Alluvial-meadow soil, amended with compost (b); in the Alluvial-meadow soil, amended with sewage sludge (c); in the Alluvial-meadow soil, amended with sheep manure (d)

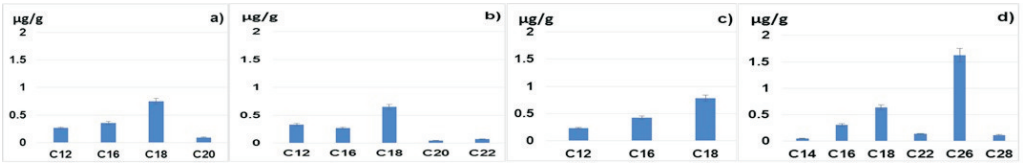

Fig. 3. Chain length distribution of fatty alcohols in the Alluvial-meadow soil (Control) (a); in the Alluvial-meadow soil, amended with compost (b); in the Alluvial-meadow soil, amended with sewage sludge (c); in the Alluvial-meadow soil, amended with sheep manure (d)

The distribution of fatty acids and alcohols (Fig. 2, 3) was maximized at palmitic acid, C_{16}, as well as the C_{18} alcohol. Only in the sheep manure amended soil, was there a shift to longer chain fatty alcohols, C_{26} and C_{28}, and maximum at C_{26}.. In our previous study [12] with the same soil (Fluvisol) on which biochar was deposited, the distribution of alkanes was maximized at the short-chain *n*-alkane (*n*-C_{18}), and for fatty acids and alcohols, similarly at palmitic acid, C_{16}, as well as the C_{18} alkanol. In the TIC chromatogram were absent long chain fatty acids (> C_{20}) and alkanols (> C_{20}), which implicate mainly microbial sources to SOM. Some of the major compounds detected in the extracts (Table 1) are biomarkers, such as 24-ethyl coprostanol in the sheep manure amended soil, which is formed from the biohydrogenation of β-sitosterol in the gastrointestinal tract of higher animals [13]. A number of steroids were also detected in the sewage sludge amended soil, i.e. (5α-cholestane, 3α-(hydroxy); 5β-cholestan-3α-ol; cholestan-3-one, (5β); cholesterol; 3-[(hydroxy]cholestane; 5α-Ergost-8(14)-ene; a-homocholest-4a-en-3-one, kaur-16-ene, a coal biomarker. In the sheep manure amended soil, coprostan-3-ol (fecal contamination); 3α-cholest-

5-ene, 5-α-ergost-8(14)-ene and another PAH derivative 1H-indene, 2,3-dihydro-1,1,3-trimethyl-3-phenyl- were detected. Other compounds were isoxazolo[2,3-b]benz[1,2]oxazine, perhydro-2-methoxycarbonyl-10-methyl-5a-hydroxy- [14,15]; 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, a flavonoid in mushrooms or pharmaceutical materials; longifolene aldehyde, in the high-boiling fraction of pine resins; myristic acid, found naturally in oil and butter fat [16] and present in the compost ameliorated soil (Table 1).

Conclusions. The study involved treatment of an Alluvial-meadow soil with organic ameliorants, such as sheep manure, sewage sludge from a waste water treatment plant and a plant compost. There was evidence for well-preserved biomarker signals and higher values of the CPI in the sludge and manure-amended soils, while lower values typical of microbial alkanes indicating a high degree of degradation in the plant compost amended soil. Sludge and sheep manure originating steroids and PAHs derivatives were preserved in the soil after 104 days of composting. Sources of SOM, as shown by the main homologous series of alkanes, fatty acids and alcohols were dual, plant and microbial.

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