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Changes in organic molecular marker signatures in soils amended with biochar during a three-year experiment with maize on a Fluvisol

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Abstract: Biochar is widely used as a soil amendment to improve soil properties and as a tool to absorb net carbon from the atmosphere. In this study we determined the signatures of organic molecular markers in soil following the incorporation of 5 and 10 t/ha biochar in a Fluvisol, cultivated with maize at the experimental field of the ISSAPP "N. Poushkarov" institute in Bulgaria. The *n*-alkane distribution in the biochar treated soils was uni- or bimodal maximizing at n-C17 alkane, n-C18 or C18 branched alkanes, i.e. there was an imprint of biomass burning, e.g. from the biochar due to predominance of short chain (< C20) homologues and increased microbial activity (presence of branched alkanes). This is also confirmed by the values for the average chain length (ACL) of n-alkanes which indicated prevalence of homologues of shorter chain (20–21 C atoms) in the variants of longer biochar residence time. There was evidence of trans-13-docosenamide, which originated from biochar. Fatty acids and fatty alcohols distributions also implicate microbial contribution to soil organic matter (SOM), supporting the suggestion that biochar addition can improve soil microbiological status.

Keywords: Biochar; Organic molecular markers; Fluvisol.

INTRODUCTION

Biochar is now unequivocally considered as a promising soil amendment, improving soil fertility as well as providing a long-term and sustainable sink of atmospheric carbon (Lehmann et al., 2006). Biochar influences soil physical, physico-chemical properties, microbial characteristics, pH, electrical conductivity (EC), cation exchange capacity (CEC), nutrient availability and therefore plant yields (Montanarella and Lugato, 2013). Biochar can also contain persistent pollutants such as polycyclic aromatic hydrocarbons (PAHs) (de Resende et al., 2018). The properties of biochar and its effect on soil have therefore been the focus of a large body of studies.

In our previous work (Petkova et al., 2018), biochar amendment on the Alluvial Meadow Soil (Fluvisol) from the experimental field of the "N. Poushkarov" Institute of Soil Science, Agrotechnologies and Plant protection in Bulgaria on which wheat and maize were cultivated in crop rotation, led to stimulation of soil microflora and increased CO_2 emission. In another study on the same experimental field (Atanassova et al., 2020) on a Fluvisol amended with 3 t/ha biochar, alkanes distribution was in the range < nC_{24} with prevailing "even" over "odd" predominance (EOP) of the homologues. The free lipid signature revealed anthropogenic and/or microbial sources of soil organic matter, due to the presence of both short chain compounds, e.g. from thermal fragmentation in biochar, and microbial alkanes. Kuzyakov et al. (2014), studying biochar stability, decomposition and transformation conclude that neutral lipids content was stable and only slightly decreased over 3.5 years following biochar addition.

In our studies of organic geochemical markers composition in anthropogenic and technogenic soils (Atanassova and Mills, 2016; Atanassova et al., 2020), a wide variety of organic markers and metabolites were analyzed, incl. contaminants that are "free", "neutral" and "labile", and not "bound" in the soil matrix, therefore are more prone to release or degradation in the environment.

Fatty acids, and especially phospholipid fatty acids (PLFA) analysis have long been used to study soil microbial community structure and responses to various agricultural management. In studies with anthropogenic soils and or matrices that contain burnt (pyrolyzed) organic matter, however, it has been shown that alkanes distribution brings about detailed and complex view of sources and origin of soil organic matter (SOM), because of their higher hydrophobicity and stability to degradation, and also their use as a *proxy* to determine past vegetation environments (Atanassova et al., 2012; Eckmeier and Wiesenberg, 2009; Thomas et al., 2021).

The aim of this study was to assess the effects of two different doses of biochar application (5 and 10 t/ha) on the soil biomarker signatures after maize harvest at the end of a 3-year experiment with maize cultivated Fluvisol and evaluate sources of organic matter including contaminants.

MATERIALS AND METHODS

The study site involved a three-year field experiment of a plot of 25.2 m^2 sown with maize at the Institute's experimental field (42°10.8' N, 24°32.5' E) in Tsalapitsa, Plovdiv region, in the Eastern Central Bulgaria. The soil characteristics are described in Harizanova et al. (2022), i.e. clay content (15 %), total N 0.107 %, pH 6.00–6.50, CEC 16.6 cmol.kg^{-1}, total organic carbon 0.5% determined by dry combustion according to DIN 19539 and CEC by saturation with K malate (pH 8.2). All soil samples are taken at depth 0–20 cm.

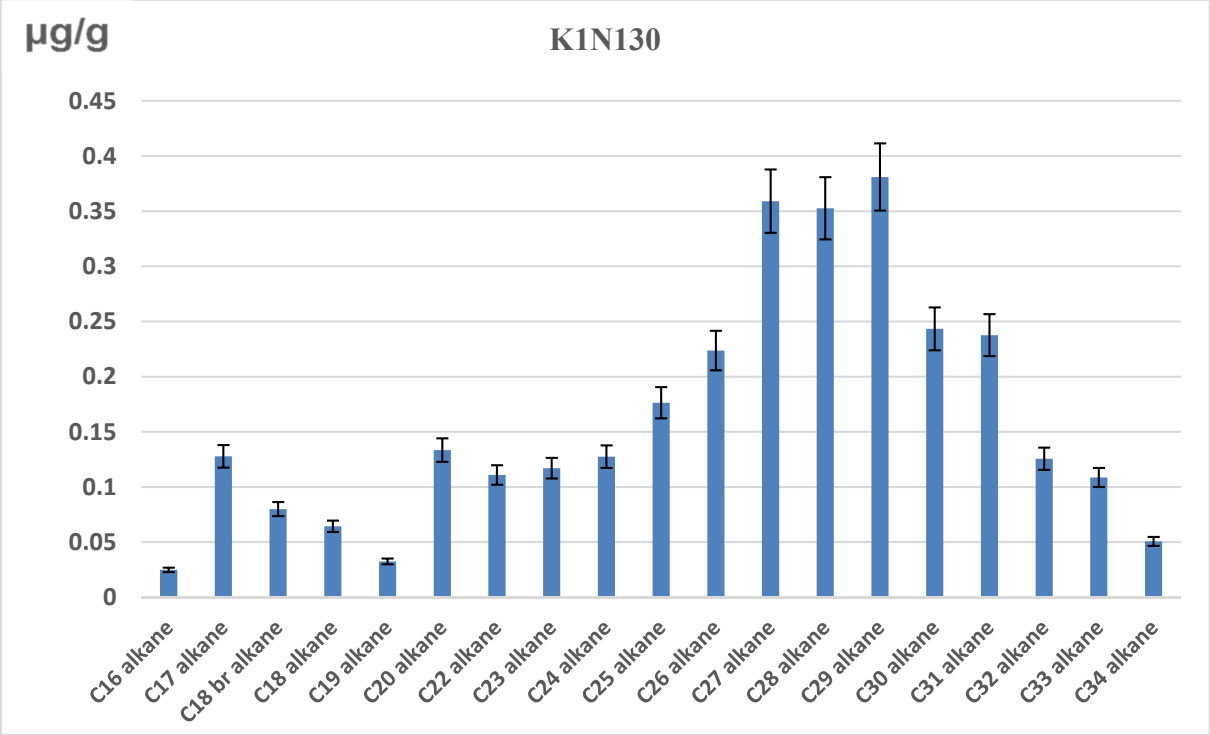

Fig. 1. Chain length distribution of n-alkanes in the control sample without biochar (K_1).

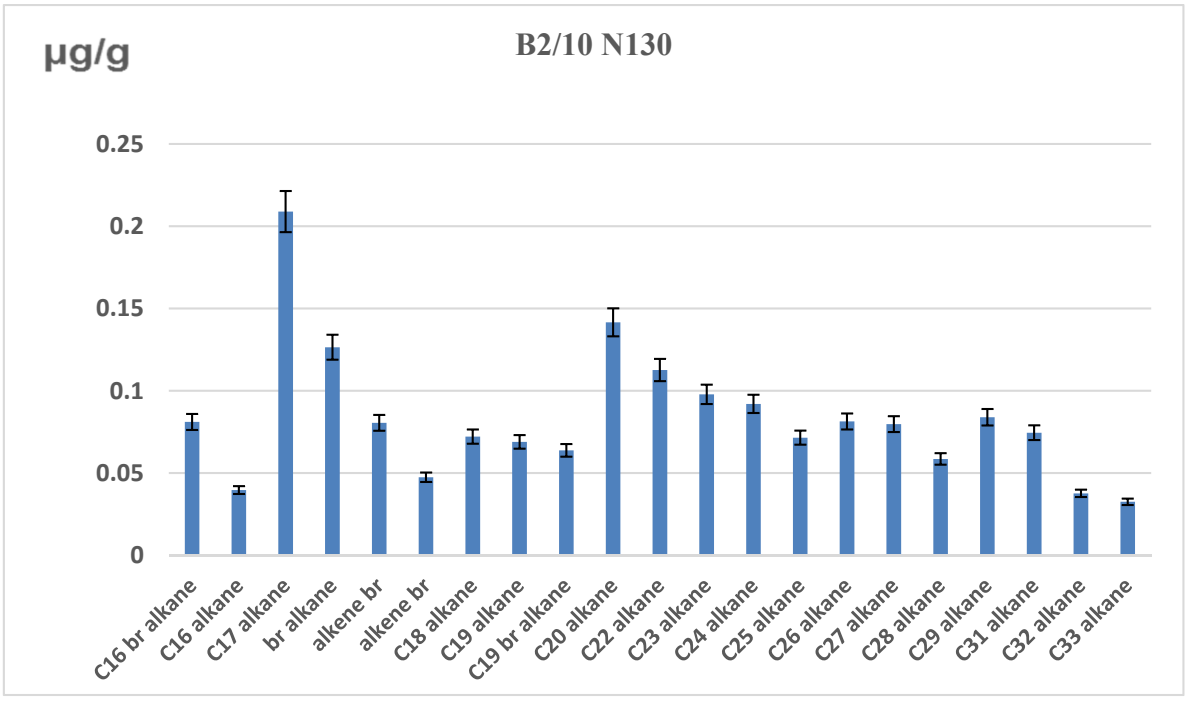

Fig. 2. Chain length distribution of n-alkanes in the sample $B_2$10 N130.

A randomised block design was established with two levels of biochar (5 and 10 t/ha) and two levels of N (ammonium nitrate) fertiliser (130 and 260 N kg/ha), each treatment in four replications. Biochar and 2/3 of the N-fertiliser were applied before maize sowing involving ploughing to a depth of 20 cm. The rest of the N fertilizer 1/3 was applied as additional nutrition at the 10–12th leaf pheno-phase (BBCH scale, Meier, 2001). Phosphorous application was applied in autumn as triple superphosphate and potassium was not applied as soils were rich in mica and illite, and potassium, respectively. The biochar was produced from oak bark at temperature of 400 °C (Nikimol Ltd, Asenovgrad, Bulgaria).

Table 1. Major eluted compounds in the total lipid extracts in the variant $B_2$5N260 (5 t/ha + 260 kg N/ha).

Retention time (min)	Compounds sample $B_{2\ (5)}N_{260}$	Retention time (min)	Compounds sample $B_{2\ (5)}N_{260}$
12.035	2,5-Cyclohexadiene-1,4-dione, 2,6- bis(1,1-dimethylethyl)-	27.358	Hexanedioic acid, bis(2-ethylhexyl) ester
12.126	Cyclodecane	27.501	Myristic acid, 2,3-bis, hydroxyl, propyl ester
14.913	2-Propenoic acid, oxybis(methyl-2,1-ethanediyl) ester	28.159	Bicyclo[10.8.0]eicosane(E)
15.445	2-Methyl-Z-4-tetradecene	29.624	Hexadecanoic acid, 2,3-dihydroxypropyl ester
16.189	Trichloroacetic acid, hexadecyl ester	30.557	2-Monopalmitin
16.893	Isopropyl Myristate	30.642	Hexadecanoic acid, 3-[(hydroxy]propyl ester
17.276	Tetradecanoic acid	31.163	Hexadecanoic acid, 2,3-bishydroxy, propyl ester
17.499	1,2-Benzenedicarboxylic acid, butyl octyl ester	32.657	Bis, monostearin
18.289	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	33.400	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
18.895	2-Dodecen-1-yl(-)succinic anhydride	34.001	2-Monostearin
19.393	Metolachlor	34.230	Octadecanoic acid, 2,3-bis[(hydroxy]propyl ester
20.389	cis-9-Hexadecenoic acid	34.436	Trans-13-Docosenamide
20.669	α-Linolenic acid	34.619	Octadecanoic acid, 2,3-bis[(hydroxy]propyl ester
22.403	Octadec-9Z-enol	41.783	Campesterol
22.546	9-Octadecenoic acid	42.184	Stigmasterol
24.034	trans-9-Octadecenoic acid	43.088	β-Sitosterol
25.613	2-Propenoic acid, pentadecyl ester	48.661	Propanoic acid, 3,3'-thiobis-, didodecyl ester

Table 2. Carbon preference indices and average chain length (ACL) for alkanes in selected variants.

	K1 N130	B1/10 N130	B1/10 N260	B2/10 N130	B2/5 N260	B2/10 N260
ACL	26.7	20.3	21.1	24.4	22.1	22.3
CPI	1.05	1.6	1.42	1.13	1.62	1.44

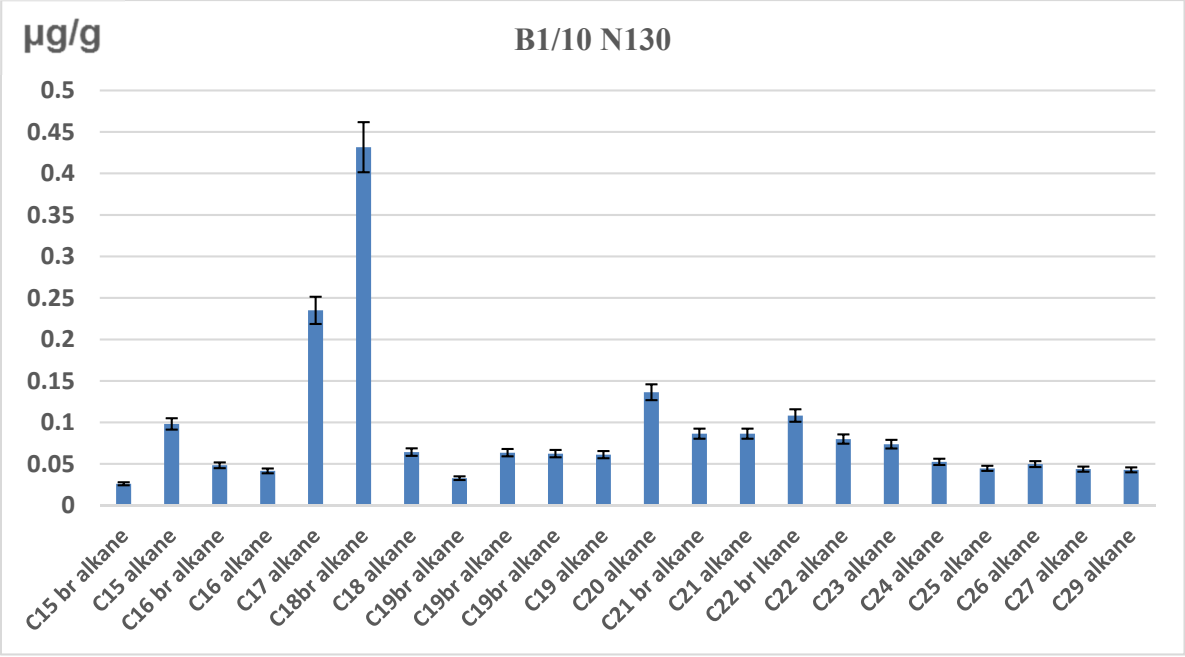

Fig. 3. Chain length distribution of n-alkanes in the sample $B_1$10 N130.

The maize was sown in April 2019, May 2020 and May 2021. The soil samples were taken for analyses in the 3rd year (autumn of 2021). This could allow monitoring of the longer-term effect of biochar addition on the distribution of organic molecular markers both from its introduction in the 1st year of the experiment and the new biochar added in the 2nd year.

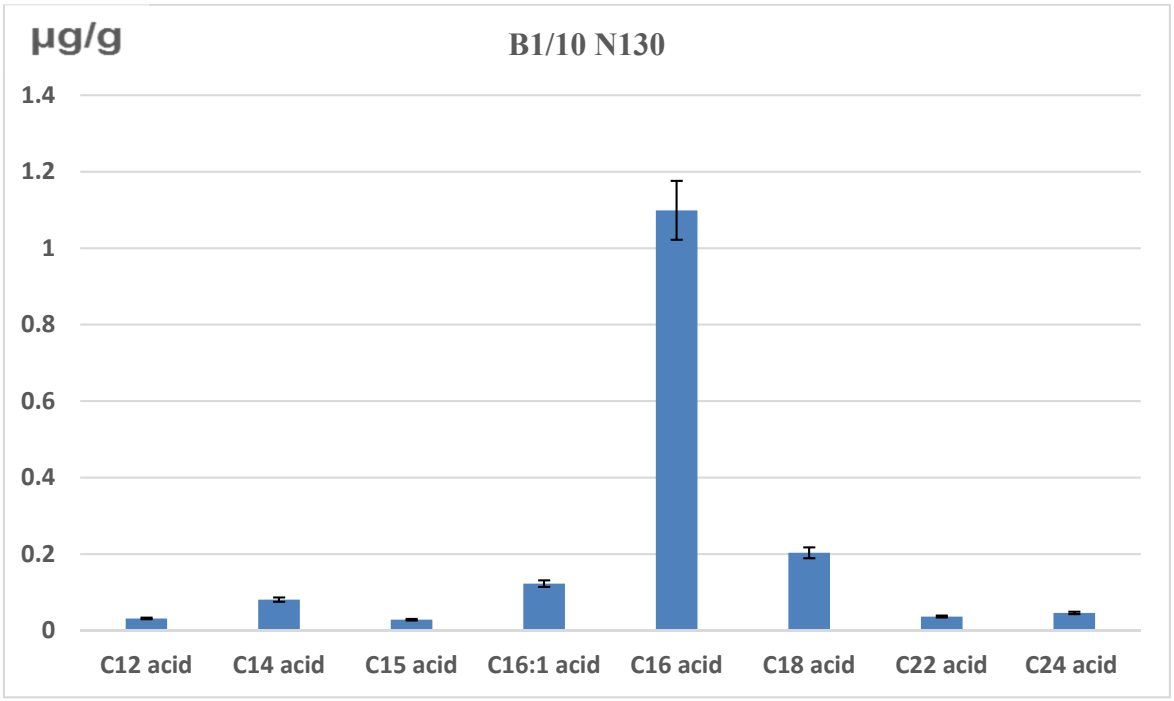

Fig. 4. Chain length distribution of unsaturated and n-fatty acids in the sample $B_1$10 N130.

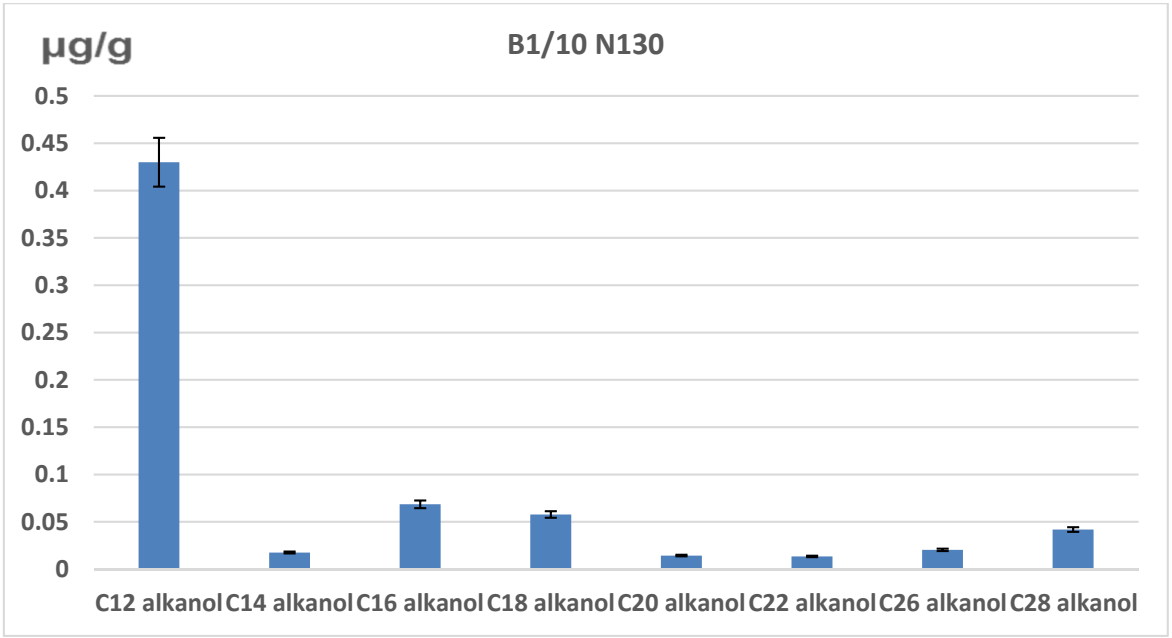

Fig. 5. Chain length distribution of fatty alcohols in the sample $B_1$10 N130.

Soil samples were taken for lipids analysis at depth (0–15 cm) and data from a pooled sample obtained from grid replicates are presented below.

The experimental design and sampling scheme was as follows:

1. Variants from the 1st year of biochar application and monitoring during the 2nd year with no further addition:

K_1 – control – no biochar + 130 kg N/ha

$B_1$5N130 – 5 t/ha + 130 kg N/ha

$B_1$10N130 – 10 t/ha + 130 kg N/ha

$B_1$5N260 – 5 t/ha + 260 kg N/ha

$B_1$10N260 – 10 t/ha + 260 kg N /ha

2. Variants from the 2nd year with new biochar application on new plots:

$B_2$5N130 – 5 t/ha + 130 kg N/ha

$B_2$10N130 – 10 t/ha + 130 kg N/ha

$B_2$5N260 – 5 t/ha + 260 kg N/ha

$B_2$10N260 – 10 t/ha + 260 kg N/ha

3. Sampling in the 3rd year following maize harvest.

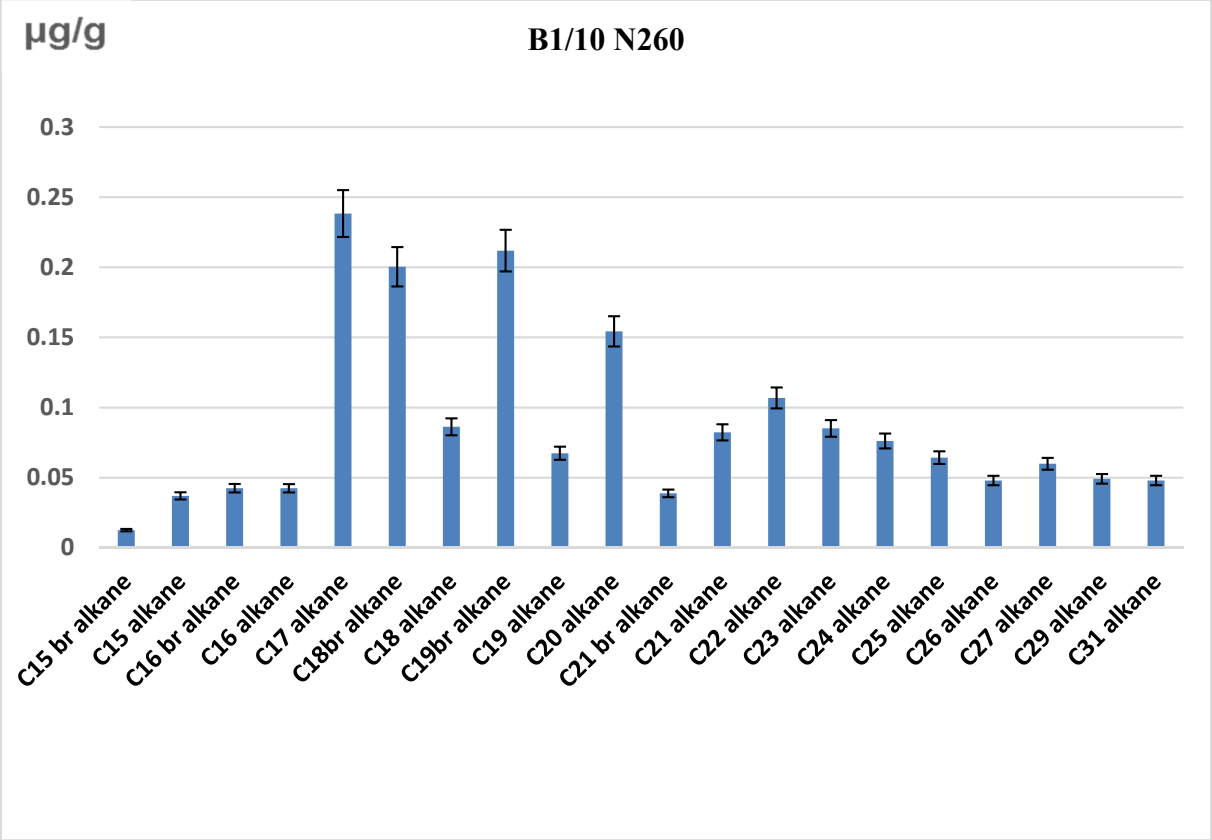

Fig. 6. Chain length distribution of n-alkanes in the sample $B_1$10 N260.

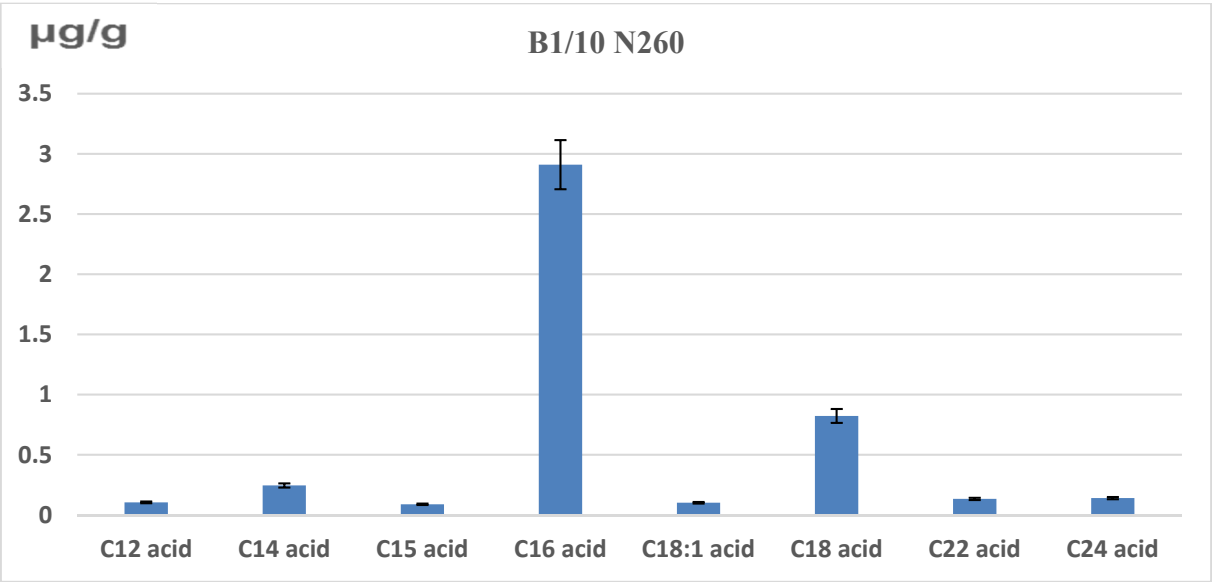

Fig. 7. Chain length distribution of fatty acids in the sample $B_1$10 N260.

Lipids 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, drying over Na_2SO_4, and evaporation in vacuum (Labconco Centri Vap concentrator at 50 °C). The solvent was replaced with dichloromethane:hexane (1:1). Internal standard (2-nonadecanone) was added, solution was dried in vacuum, then derivatized with BSTFA + 1% TMS (heated for 1 hour at 70 °C; modified from Atanassova and Mills (2016)). After completion of the derivatization, the derivatives were cooled, reconstituted to 1 mL with DCM and analyzed by GC/MS (Agilent 7890A with a 5975 °C mass-selective detector, splitless mode).

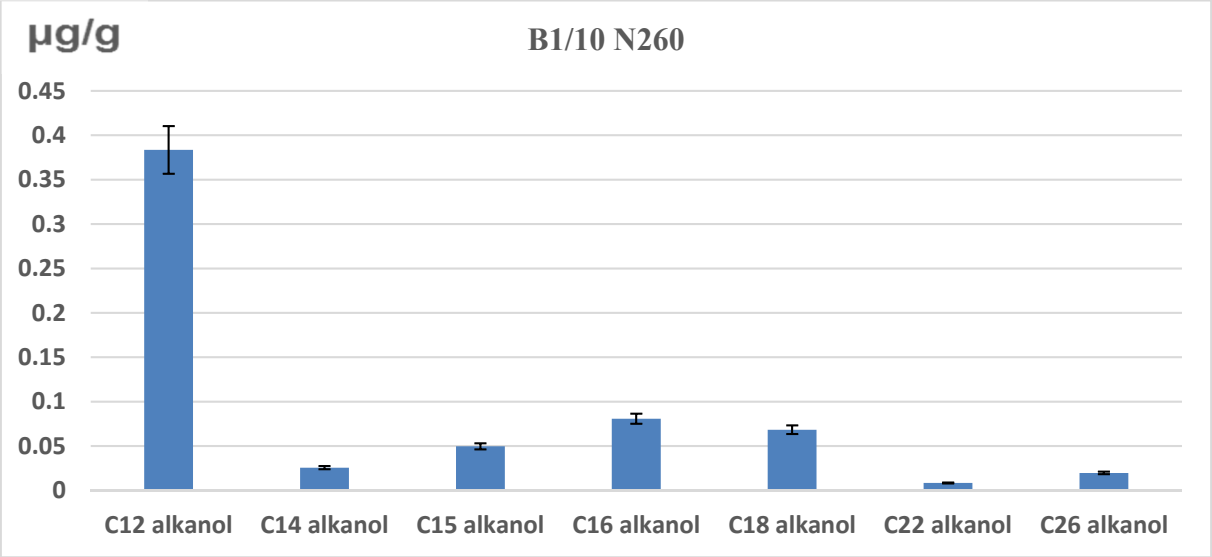

Fig. 8. Chain length distribution of fatty alcohols in the sample $B_1$10 N260.

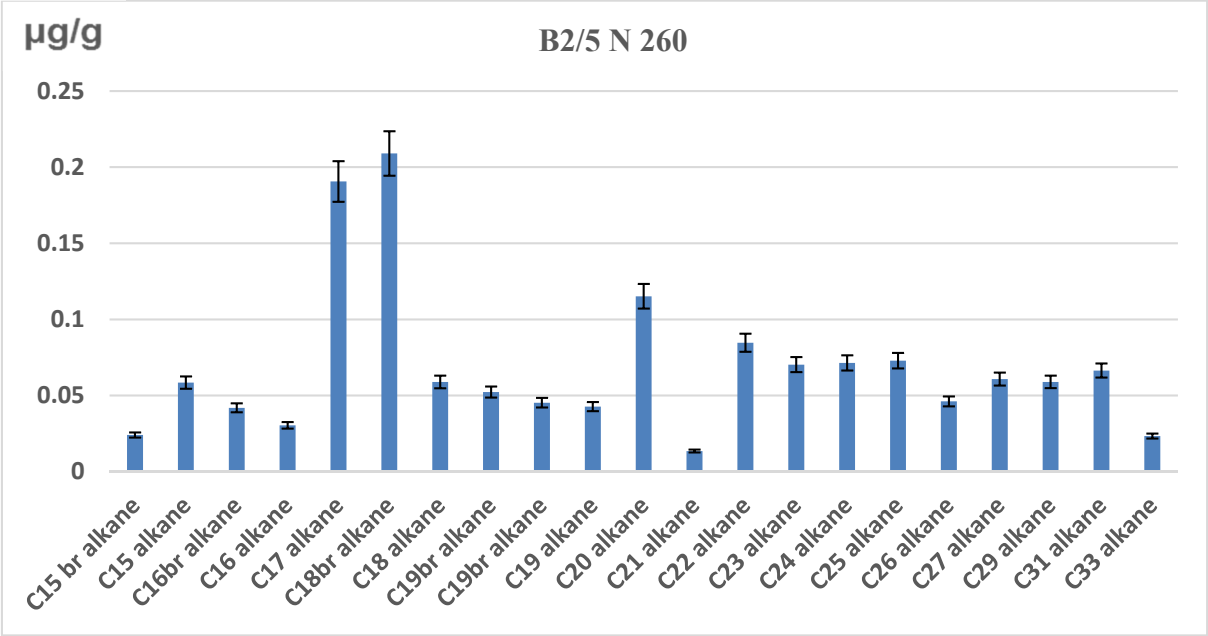

Fig. 9. Chain length distribution of n-alkanes in the sample $B_2$5 N260.

Separation was performed on HP-5ms capillary column (30 m × 0.25 mm I.D., film thickness, 0.25 µm) with He as a carrier gas. The GC programme was, initial temperature of 60 °C, hold 1 min, linear ramp 10 °C /min to 180 °C, ramped at 4 °C/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 08-MS library, authentic standards, GC retention times and interpretation of mass fragmentation.

Compound ratio (carbon preference index for alkanes CPI) was calculated as: CPI1 = Σ (C17–C31)/ Σ(C18–C24) for the whole range of detected n-alkanes (Lichtfouse and Eglinton, 1995) and average chain length ACL for n-alkanes: (∑ Ci × i)/∑ Ci, where Ci is the concentration of the n-alkane containing *i* carbon atoms (Kuhn et al., 2010). Statistical analysis was performed with SPSS 21 for Windows.

RESULTS AND DISCUSSION

Biomarker signatures in our study in year 3 following maize harvest indicate some changes, as compared to the lipids distribution at the beginning of the experiment. Table 1 lists the major eluted compounds and the homologous series of alkanes, alkanols and alkanoic acids (see also Fig. 1–12). They indicate the presence of phytosterols (campesterol, stigmasterol, β-sitosterol) and myristic, palmitic and stearic acid esters, which have been attributed to solubilization by micelle-like microparticles and nanosoluble organic colloids (Atanassova et al., 2014). Myristic acid esters were found to accelerate the asymbiotic growth of arbuscular mycorrhizae fungi and serve as a carbon and energy source (Sugiura et al., 2020).

PAHs and PAH derivatives were not detected in the autumn of the 3rd year of the experiment, contrary to the first sampling in year 1 (Atanassova et al., 2022) in which 2,5-furandione and 1H-indene were detected.

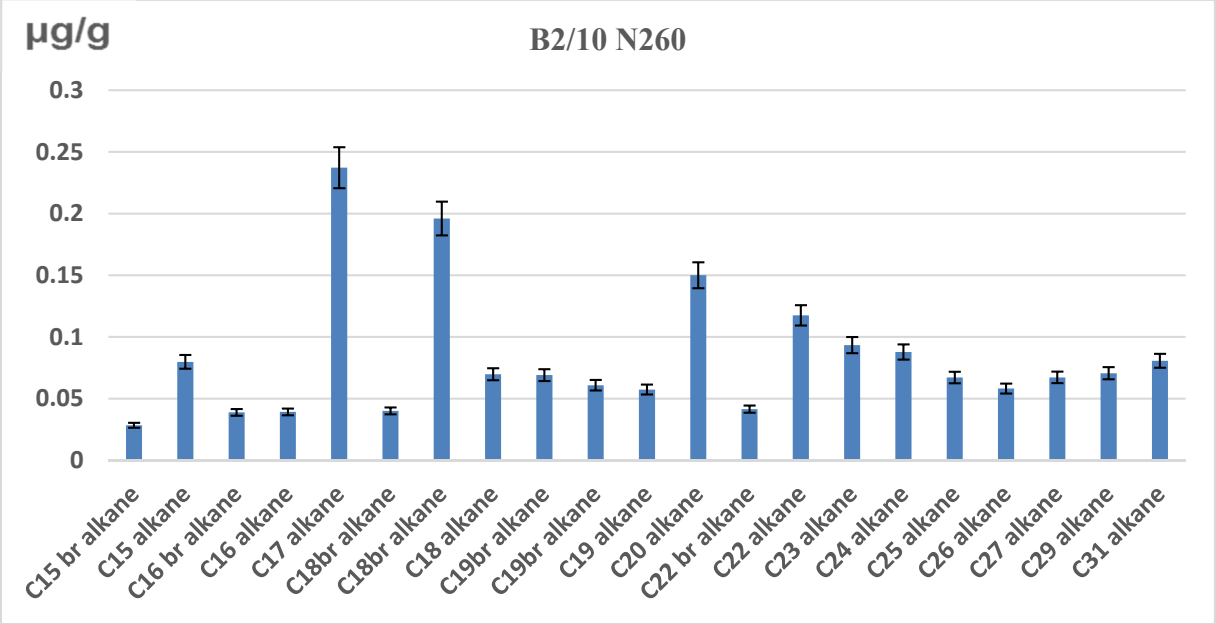

Fig. 10. Chain length distribution of *n*-alkanes in the sample $B_2$10 N260.

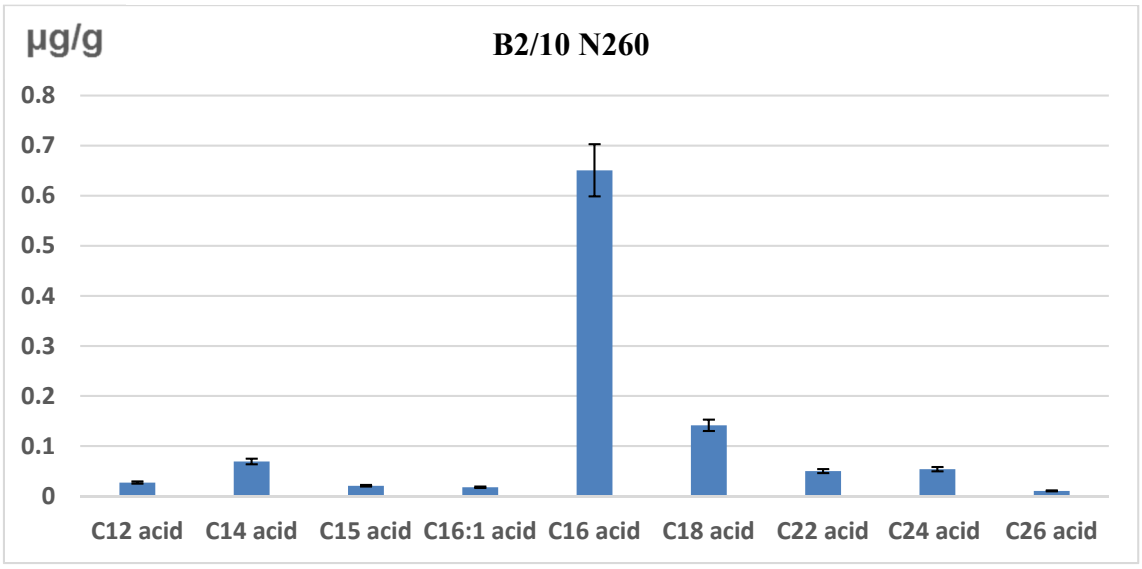

Fig. 11. Chain length distribution of fatty acids in the sample $B_2$10 N260.

Figures 1a–2a present distributions corresponding to mixtures of isomers of branched C15–C31 alkanes, alkenes including *iso- and anteiso-* homologues. It has been found that pyrolysates of biochars were characterized by branched alkanes, short chain n-alkanes/n-alkenes, similarly to what was observed in our previous study and in ancient burnt soils (Eckmeier and Wiesenberg, 2009; Schellekens et al., 2018). A predominance of "odd" vs. "even" homologues of alkanes was found in all the studied variants, except the control soil (Fig. 1). The alkane distribution was uni- or bimodal, maximizing at n-C17 alkane, n-C18 or C18 branched alkanes. In this study, similarly to our previous study (Atanassova et al., 2022), there was an imprint of biomass burning, i.e. most likely from the biochar addition, associated with the predominance of short chain (< C20) homologues and/or increased microbial activity (branched alkanes).

The average chain length of alkanes (ACL) was slightly lower for the B1 variants and increased from 20.3 to 24.4 in the B2/10N130 variant (Table 2). The carbon preference index CPI of odd/even n-alkanes O/E did not show significant difference between the B1 and B2 variants. It should be noted that alkane distribution in the control soil differed from those ameliorated with biochar, i.e. it maximized at C29 alkane and was unimodal, and lacking mixtures of isomers of branched short chain alkanes including *iso-* and *anteiso*-homologues, thus indicating an imprint of higher plants. The smaller CPI of odd/even homologues in the control soil might be due to the presence of even chain alkanes which indicates, either degradation of the odd n-alkanes or a shift in plant composition over time, which has been often the case for Tsalapitsa field with crop rotations. Such results have been observed in woodland and grassland soils that have undergone former biomass burning (Kuhn et al., 2010).

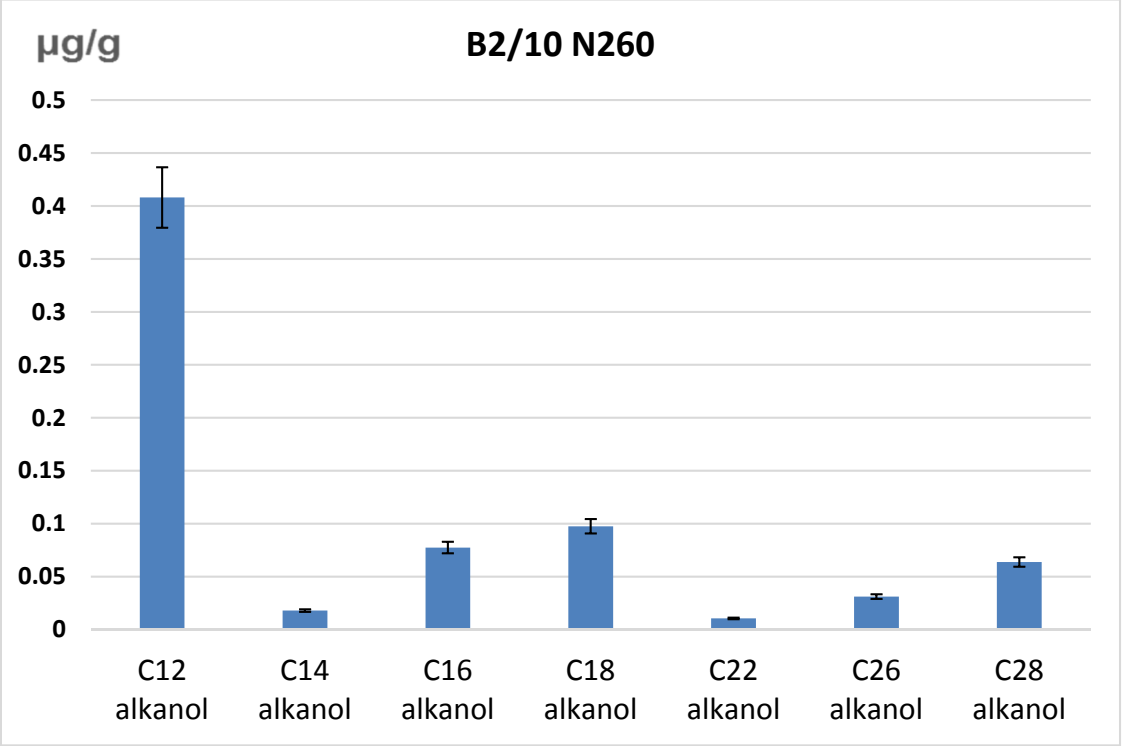

Fig. 12. Chain length distribution of fatty alcohols in the sample B$_2$10 N260.

For fatty alcohols, the predominant ratios of C_{even}/C_{odd} homologues in the C< 26 range indicate the increasing role of microbial source of SOM. A fatty acid distribution in the C4–C26 range, as well as the presence of branched homologues, point at bacterial contribution, while predominance in the C26–C38 range implies origin from higher plants (Naafs et al., 2004; Schnitzer et al., 1986). In our study, fatty acids and fatty alcohols distributions are characterized by predominance of even homologues and a maximum at C16, palmitic acid, and C12 alkanol, in contrast to C18 alkanol in our previous sampling (Atanassova et al., 2022), which implicates predominant microbial sources of SOM.

In the total lipid extracts (Table 1), once again metolachlor was detected, a herbicide, as in the 1st year of the experiment when soils were sampled at 10–12th leaf pheno-phase of maize development, as well as trans-13-docosenamide, a marker of biochar (Atanassova et al., 2012, 2022).

CONCLUSIONS

The post-effect of biochar application on biomarker signatures in a maize cultivated Fluvisol treated with 5 and 10 t/ha biochar was examined in a 3-year experiment. The major homologous classes of organic compounds in the total ion current chromatogram (TIC), revealed that sources of SOM are of microbial origin due to predominance of short chain fatty acids and fatty alcohols (< C20). The alkane distribution (C15–C33) maximized at n-C17, n-C18 or C18 branched alkanes and confirmed a microbial and/or thermal origin of SOM. The organic geochemical marker analysis indicated an imprint of biomass burning, i.e. most likely from the added biochar, which was also supported by the presence of a contaminant, trans-13-docosenamide. The n-fatty acids and n-fatty alcohols distributions also point to predominant microbial sources of SOM, thus confirming our finding that biochar addition enhances microbiological status in the ameliorated soils.

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