Scientific report of MOLTER Exchange Grant 2344 (André Hilscher)

DEGRADATION OF POTENTIAL MARKERS OF PYROGENIC ORGANIC MATTER IN HUMIC SUBSTANCES

General benefits of my stay from January 10th to April 1st in 2010:

The purpose of my stay at the Instituto de Recursos Naturales y Agrobiología de Sevilla was to learn new analytical methods for the pyrogenic organic material (PyOM) characterisation and to use those techniques for studying the alteration of PyOM during controlled humification. The work included the lipid extraction procedure, the GC-MS analyses and the data evaluation and interpretation. The collaboration with this institution was very useful for me because the research group is engaged in investigation of vegetation burnings since a long time. It was an important experience to learn to manage the laboratory work and to discuss the results with scientists in a foreign country. I established new scientific connections in my work field, which will be of great help in the future since I am highly interested in pursuing an academic career. I had the opportunity to give an oral presentation at the institute. I met Dr. José A. GONZÁLEZ-PÉREZ, who is the chairman of the IHSS conference in 2010. Following his invitation, the data will be presented at the conference. The obtained data will be an essential part of my dissertation and are presently summarized for publication in an international well recognized journal. The submission of this paper is planned to occur this summer.

Scientific results:

Prior to the stay in Spain, the PyOM produced at 350°C for 1 (1M) and 4 minutes (4M) from rye grass (*Lolium perenne*, Gr) and pine wood (*Pinus sylvestris*, P) were subjected to aerobic biotic incubation (Incub) for 48 days. In order to obtain more insights into the fire-induced alteration of the plant materials and possible modifications by microbial degradability of PyOM we separated and characterised the lipid fraction. The lipid fraction was soxhlet-extracted from the plant material, the fresh and incubated PyOM samples with CH_2CL_2: CH_3OH (3:1) for 8 h. The lipid extracts were filtrated and the water was removed by adding K_2SO_4. Subsequently the solvent was purged with nitrogen. The weight of the lipid fractions was determined to allow quantification. Then the polar lipid sub-fractions were derivatised by adding 2M trimethylsilyldiazomethane for the methylation of carboxylic acids. *N,O*-bis(trimethylsilyl)trifluoroacetamide was used to react with polar compounds to replace labile hydrogens on a wide range of polar compounds with a $-Si(CH_3)_3$ group (silylation) to prepare

volatile and thermally stable derivatives for gas chromatography and mass spectrometry. The derivatised neutral and acid liquid sub-fractions were analysed using GC-MS. The GS-MS data were qualitatively interpreted and selected compounds were quantified using the software Enhanced Chemstation. We decided to waive pyrolysis-GC-MS measurements because already the GC-MS analysis of the lipid fraction allowed the characterisation of typical biomarkers of biomass burning and the latter method is less critical in terms of producing thermal artefacts.

First results indicate that a consecutive decrease in lipid extract yield with prolonged charring times. The fresh grass and the pine wood showed comparable lipid concentrations of 67 and 62 mg g^{-1}, respectively. The grass-dervived PyOM contains more lipids than that of pines. The lowest lipid contents were found for the more charred pine (P4M) with only 3 mg g^{-1}. The smaller lipid content of the PyOM compared to fresh plant material is caused by cracking and volatilisation losses during the charring process.

Untreated rye grass straw (Gr 0M) revealed a predominance of long chain odd numbered n-alkanes within the aliphatic hydrocarbon (Fig 1 and Table 1). The fresh pine wood (P 0M) showed higher contributions of mid chain n-alkanes maximising at C19 and smaller long chain n-alkanes (C25 to C33; Table 1) contents. This points to a higher enrichment of plant waxes in the grass straw. The molecular ratios ACL (average chain length) and CPI (carbon preference index) decrease with increasing charring time for the grass-derived PyOM. This means that the relative predominance of long chain even n-alkanes is lower vs. straw material and larger amounts of mid chain n-alkanes (C19 to C25) are present caused by thermal induced break down process of long chain odd numbered n-alkanes. For the pine char no change of the ACL and a concomitant decrease of the CPI value were observed. The grass and pine char obtained after 4 minutes of charring revealed comparable n-alkanes distribution patterns (Fig. 1) with maximising at C20 to C22. The total n-alkanes amount is reduced by the factor 6.7 for the P4M in comparison with Gr4M. The n-alkenes are present in very low amounts and it was not possible to quantify them.

The statured fatty acid (FA) homologues of the fresh rye grass and pine wood are different. The rye grass is dominated by the palmitic acid (C16) and stearic acid (C18) (Fig. 2 and Table 2). The palmitic acid accounts with 19.9% of the total ion chromatogram (TIC) of the whole lipid extract (Fig. 3). In contrast, the pine wood contains large amounts of caproic acid (C6). The ACL of 16.4 reflect higher contribution of mid chain FA for grass in comparison with an ACL of 8.9 for the fresh pine wood (Table 2). During the charring process a decrease of the total FA amount by the factor 3.8 and 7.0 for Gr4M and P4M was calculated, respectively,

whereas the fresh rye grass contains 3.8 times more FA than the pine wood. The grass-derived PyOM show no major alterations of the FA distribution pattern. The ACL is did not change and only a slight relative increase of the C6 to C20 homologues was determinate (Table 2). In contrast, a strong depletion of the short chain FA (C6 to C12) was detected with increasing charring time for the pine PyOM (Fig. 2 and Table 2). Especially the caproic acid (C6) content seems to be an indicator for the charring degree of pine wood (Fig. 2).

The fresh grass material contains high amounts of the unsaturated FA 9,15-octadecadienoic acid (C18:2) and 9,12,15-octadecatrienoic acid (C18:3) with 9.6% and 24.5% of the total ion chromatogram (TIC), respectively (Fig. 3 and 3A). After one minute of heating only small residues of 1.7 and 5.5% of the TIC for Gr1M and no contributions for the more charred Gr4M were recovered (Fig. 3A). The grass PyOM contains in contrast to the fresh grass 9-octadecenoic acid (C18:1) and higher amounts of the saturated FA C18, which are more stable against the thermal breakdown processes.

The lipid extract of the fresh pine wood (P0M) contains typical biomarkers for conifers like abietic acid, primeric acid and vanillin derivate which is derived from coniferyl alcohol, the primary aromatic alcohol monomer of gymnosperm lignin (Fig. 4). The P1M char shows a strong accumulation of vanillin (22.7% of TIC, Table 3). This is an agreement with the study of Hilscher et al. (2009), who found an accumulation of methoxyl C and O-aryl C signals in the ^{13}C NMR spectrum of P1M, indicating that some lignin-type structures survived the charring process. No abietic acid and small residues of primeric acid derivates were determinate for the pine chars (Fig. 4 and Table 3). Levoglucosan (1,6-anhydride of glucose), the major tracer from the thermal decomposition of cellulose, is a significant indicator for biomass burning. Levoglucosan is detectable for all PyOM, whereas P1M has with 17.2% of TIC the highest contribution (Table 3). The grass-derived PyOM contains smaller levoglucosan amounts. With prolonging charring time (4 minutes) the amount of levoglucosan is decreased to 13.9% and 2.4% of the respective 1 minute charred PyOM amount for the grass and the pine wood, respectively. This indicates that strongly burnt plant material can be depleted in levoglucosan.

During the 2 months of the aerobic incubation of the char in soil, the cumulative n-alkanes amount of the grass-derived PyOM (Gr1M Incub and Gr4M Incub) was reduced. The recovery is with 61% for Gr1M lower than for Gr4M with 85% (Table 4). The finding is in agreement with the respective NMR data, which point towards the loss of alkyl compounds. The ACL is two carbon homologues shorter than for the fresh grass PyOM because the long chain homologues (>C26) are more degraded than the shorter ones (Table 4). The incubated

pine chars showed no decrease of the n-alkanes. In general, the recovery was 2 to 3 times higher. However, the total amounts of the fresh pine chars are up to 7 times lower. The CPI index for all incubated PyOM was in the same range between 0.9 and 1.1 than for the fresh PyOM (Table 4).

The cumulative recovery of saturated FA shows the same trend as the n-alkanes. The incubated Gr1M and Gr4M lost 39% and 15% of the initial amount (Table 5). The incubated pine chars tend to new formation of FA. The ACL of the FA of all PyOM increased to values around C25, which is comparable to the blank soil incubate (Table 5). The FA homologues larger than C18 showed new formation. This could be explained by partial oxidation of the n-alkanes. The dominate FA homologues is the palmitic acid (C16) whereas for the blank soil palmitic acid and stearic acid have comparable amounts.

The vanillin derivate of the pine char was efficiently decomposed during the short time period of 2 months. Only 8% of the initial amount was recovered for the P1M and the small residues surviving the charring was completely degraded for P4M (Table 6). Lignin seems to be efficiently attacked by the microorganisms. The major levoglucosan content of the P1M was with 77% almost completely decomposed. This indicates that the use of this compound as a tracer for burning may lead to underestimation because under the optimal environmental conditions of laboratory incubation it was rapidly lost.

It can be concluded that the composition of aliphatic hydrocarbons in soils offers specific fingerprint indicators for diagnosis of vegetation burning and of burning conditions. Especially, an enrichment of even numbered, short chain n-alkanes were confirmed as reliable indicators for residues of charred biomass in soil. They are applicable for different plant source materials and for varying charring conditions. Even more important, the initial degradation process did not change the n-alkanes distribution. The present study showed further that the commonly used biomarker levoglucosan could lead to underestimation of pyrogenic organic matter because the strongly charred plant remains were depleted in it and it was shown to be efficiently decomposed during the initial microbial degradation of PyOM.

Table 1. n-Alkanes contents of the fresh and brunt plant materials.

chain length	Gr0M µg g^{-1}	Gr4M µg g^{-1}	Gr4M µg g^{-1}	P0M µg g^{-1}	P1M µg g^{-1}	P4M µg g^{-1}
C15		13.9	5.3			0.2
C16		6.0	3.7			0.2
C17		13.5	10.6	2.1	1.2	0.6
C18	1.9	12.1	15.8	4.7	3.3	2.3
C19	3.1	18.3	11.6	14.1	4.3	2.7
C20	2.6	13.3	15.8	3.8	4.8	3.2
C21	3.3	15.9	14.6	3.7	6.2	2.9
C22	1.6	19.2	14.4	3.9	5.4	2.5
C23	3.2	16.0	13.1	2.6	5.0	2.2
C24	2.0	17.7	8.8		3.6	1.7
C25	9.3	9.3	6.2	2.9	2.3	0.5
C26	1.1	8.5	6.3		1.5	0.7
C27	8.3	6.7	3.2	1.0	0.6	0.6
C28	1.1	3.5	2.3		0.4	0.2
C29	9.7	6.5	3.0	0.7	0.4	0.2
C30	0.9	5.1	1.6			0.3
C31	5.3	9.0	3.5	1.3		
C32						
C33	1.7	4.8	1.9			
sum	55.2	199.2	141.7	40.8	38.9	21.0
ACL	25.7	22.2	21.5	20.8	21.6	21.3
CPI	3.7	1.2	1.0	2.3	1.1	0.9

Table 2. Saturated fatty acid contents of the fresh and brunt plant materials.

Fatty Acid **chain length**	Gr0M µg g^{-1}	Gr4M µg g^{-1}	Gr4M µg g^{-1}	P0M µg g^{-1}	P1M µg g^{-1}	P4M µg g^{-1}
C6	21.3			490.1	10.1	
C7	8.0			51.2	4.4	
C8	56.4	53.8	9.1	121.4	12.5	
C9	36.5	70.5	27.2	166.3	28.9	
C10	52.6	61.8	20.4	16.2	6.7	0.5
C11	7.7	33.6	13.4		1.7	0.4
C12	82.0	33.6	29.8		10.0	2.4
C13	11.1	33.1	15.8		3.1	1.5
C14	154.9	151.3	66.4		12.0	6.8
C15	52.5	108.6	44.4		6.1	3.1
C16	3186.3	2487.5	655.7	103.7	52.0	64.4
C17	39.4	38.4	16.1	4.6	2.2	2.4
C18	284.7	279.7	172.3	15.9	8.7	52.7
C19	4.7	11.2	4.1			0.6
C20	64.4	43.8	14.4	14.3	3.4	2.4
C21	11.8	25.6	7.3		1.0	0.5
C22	94.3	82.4	23.2	25.7	7.5	4.1
C23	24.6	52.0	12.0		0.9	0.5
C24	86.1	63.5	19.5	10.1	3.1	2.0
C25	14.3	14.3	4.5			0.3
C26	79.0	19.6	7.1			0.4
C27	5.3					
C28						
total	4378	3664	1163	1019	174	145
ACL	16.4	16.1	16.1	8.9	13.4	16.9
CPI	19.8	8.5	7.0	3.6	2.6	14.9
C6 to C20/ C21 to C28	11.1	13.2	14.8	27.4	13.0	17.5

Table 3. Specific biomarker contents of the fresh and burnt plant materials.

Biomarker	Tracer	**Gr0M**		**Gr1M**		**Gr4M**		**P0M**		**P1M**		**P4M**	
		Area %	µg g^{-1}	Area %	µg g^{-1}	Area %	µg g^{-1}	Area %	µg g^{-1}	Area %	µg g^{-1}	Area %	µg g^{-1}
Vanillin (Lignin)	Pine	nn		nn		nn		2	1370	22.7	4077	0.3	11
Primeric acid	Pine	nn		nn		nn		1	927	nn		nn	
Abietic acid	Pine	nn		nn		nn		1	516	nn		nn	
7-Oxodehydroabietic acid	Pine	nn		nn		nn		3	1998	0.4	65	0.8	27
Levoglucosan	burnt	nn		4	1473	2	205	nn	nn	17.2	3080	2	75

Table 4. n-Alkanes recovery of the incubated PyOM.

n-Alkanes	**Gr1M Incub**		**Gr4M Incub**		**P1M Incub**		**P4M Incub**		**soil**
chain length	µg (BV corr)	**RC %**	µg (BV corr)	**RC %**	µg (BV corr)	RC %	µg (BV corr)	RC %	µg
C15	15	**36**	18	**116**	6				
C16	12	**68**	13	**118**	7				1
C17	27	**67**	40	**127**	12	**335**	8	**420**	4
C18	53	**147**	38	**79**	29	**294**	19	**271**	8
C19	40	**73**	41	**117**	20	**157**	9	**110**	5
C20	39	**98**	38	**80**	16	**111**	11	**109**	5
C21	31	**65**	32	**73**	27	**146**	11	**126**	6
C22	42	**73**	37	**85**	34	**211**	15	**209**	6
C23	43	**89**	35	**88**	40	**265**	17	**259**	7
C24	28	**53**	24	**90**	45	**422**	18	**348**	7
C25	16	**56**	16	**85**	37	**545**	15	**937**	5
C26	8	**31**	13	**66**	28	**648**	10	**520**	4
C27	4	**22**	6	**64**	16	**847**	8	**445**	2
C28	3	**25**	4	**56**	10	**792**	4	**628**	1
C29	3	**14**	3	**38**	7	**606**	6	**822**	1
C30			3	**66**	4		2	**237**	0
C31			3	**29**	2		6		0
C32					1				
C33					1		1		
sum	364	**61**	363	**85**	344	**295**	160	**253**	63
ACL	20.6		20.7		22.7		22.9		22.0
CPI	0.9		1.1		0.9		1.0		0.9

Table 5. Saturated fatty acid recovery of the incubated PyOM.

FA chain length	Gr1M Incub µg (BV corr)	RC %	Gr4M Incub µg (BV corr)	RC %	P1M Incub µg (BV corr)	RC %	P4M Incub µg (BV corr)	RC %	soil µg
C6	22		5		**3**	**11**			
C7	15		8		**5**	**34**			0
C8	61	**38**	24	**88**	**37**	**99**	12		0
C9	149	**70**	77	**94**	**76**	**88**	14		0
C10	77	**42**	54	**89**	**30**	**151**	34	**2334**	1
C11	45	**45**	35	**88**	**13**	**253**	7	**650**	1
C12	136	**135**	85	**95**	**68**	**228**	51	**698**	5
C13	74	**75**	47	**99**	**21**	**231**	14	**316**	2
C14	274	**60**	111	**56**	**61**	**170**	62	**307**	21
C15	202	**62**	100	**75**	**41**	**223**	37	**401**	9
C16	4726	**63**	1296	**66**	**403**	**259**	962	**498**	153
C17	98	**85**	36	**74**	**37**	**552**	36	**514**	5
C18	871	**104**	172	**33**	**144**	**548**	535	**338**	160
C19	37	**111**	3	**22**			10	**575**	1
C20	146	**111**	43	**99**	**26**	**258**	41	**574**	7
C21	88	**115**	24	**111**	**7**	**247**	20	**1295**	1
C22	297	**120**	79	**114**	**56**	**247**	93	**757**	5
C23	182	**117**	43	**120**	**9**	**330**	24	**1585**	2
C24	229	**120**	71	**121**	**30**	**324**	35	**591**	7
C25	48	**111**	13	**96**	**3**		11	**1368**	1
C26	65	**110**	20	**95**	**4**		11	**827**	2
C27	6				**1**		6		0
C28	18		8		**2**		27		1
sum	7866	**72**	2352	**67**	**1078**	**206**	2042	**470**	378
ACL	25.5		24.9		24.5		25.8		26.0
CPI	8.4		5.4		4.1		12.0		17.6
C6 to C20/ C21 to C28	7.4		8.1		8.7		8.0		20.0

Table 6. Specific biomarker recovery of the incubated PyOM.

Biomarker	Tracer	**Gr1M Incubat**		**Gr4M Incubat**		**P1M Incubat**		**P4M Incubat**		**soil**
		µg g^{-1}	RC %	µg g^{-1}	RC %	µg g^{-1}	RC %	µg g^{-1}	RC %	µg g^{-1}
Vanillin (Lignin)	Pine					33	8	nn	0	nn
Primeric acid	Pine									nn
Abietic acid	Pine									nn
Levoglucosan	burnt	nn	0	nn	0	70	23	6	85	nn

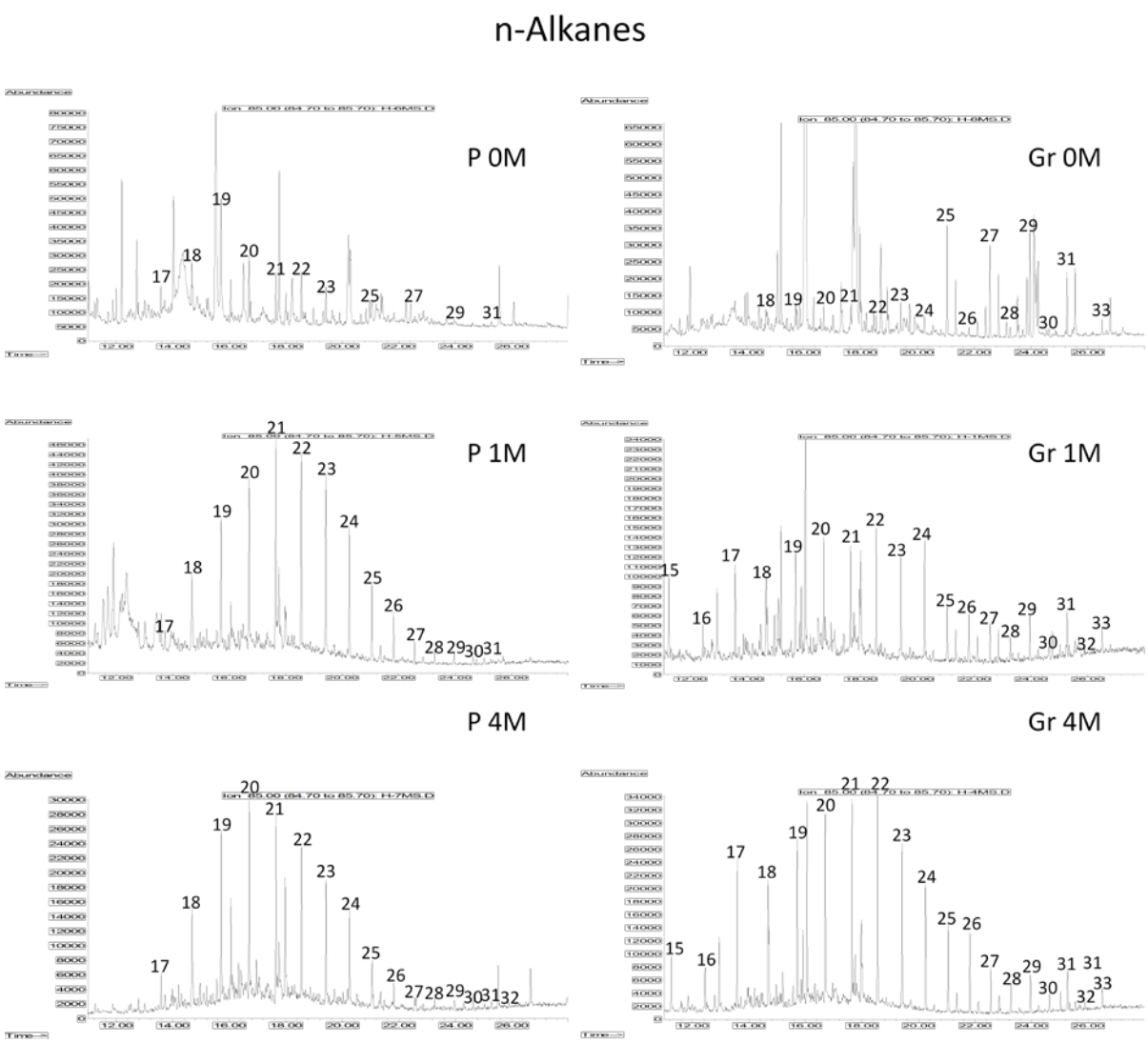

Fig. 1. n-Alkanes distribution of the fresh and charred pant materials (m/z 85).

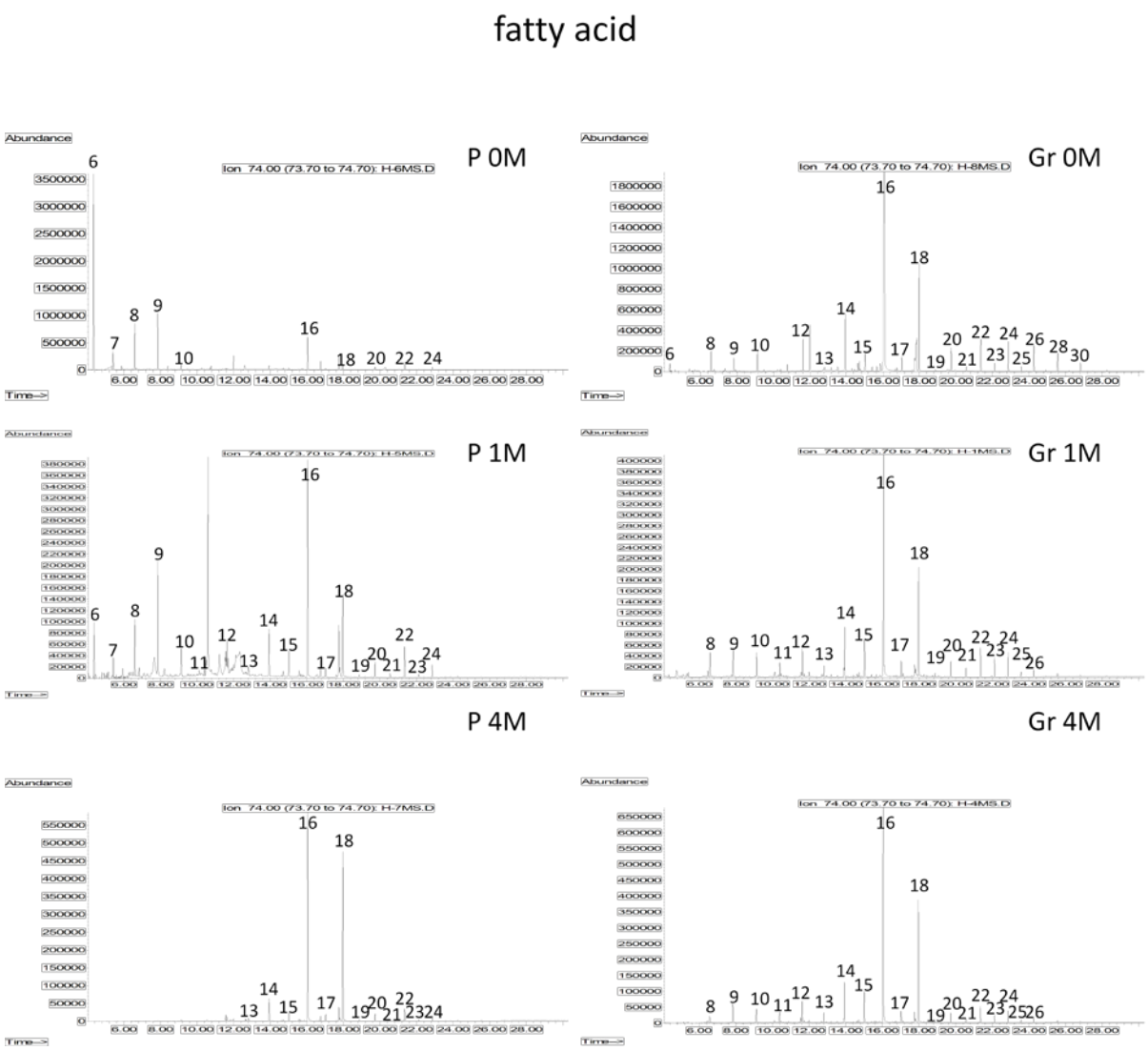

Fig. 2. Saturated fatty acid distribution of the fresh and charred pant materials (m/z 74).

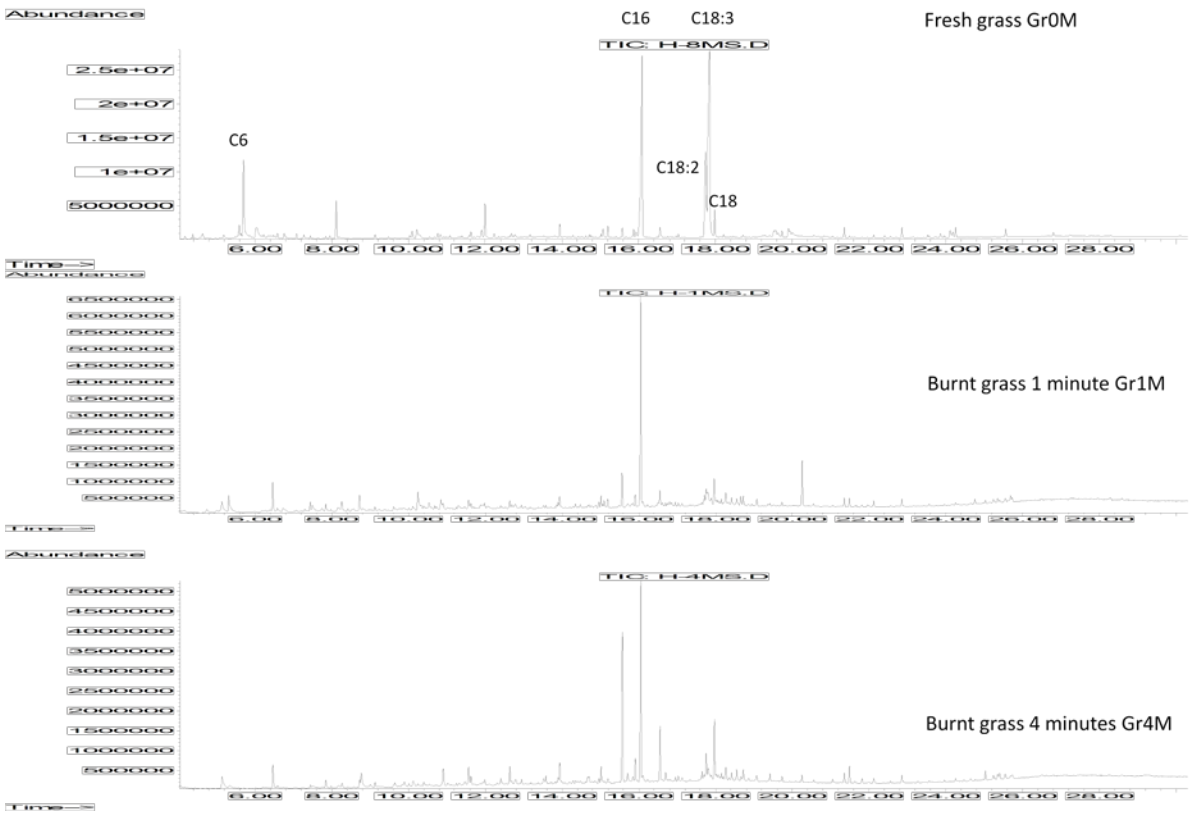

Fig. 3. Total ion chromatogram of the fresh and charred rye grass.

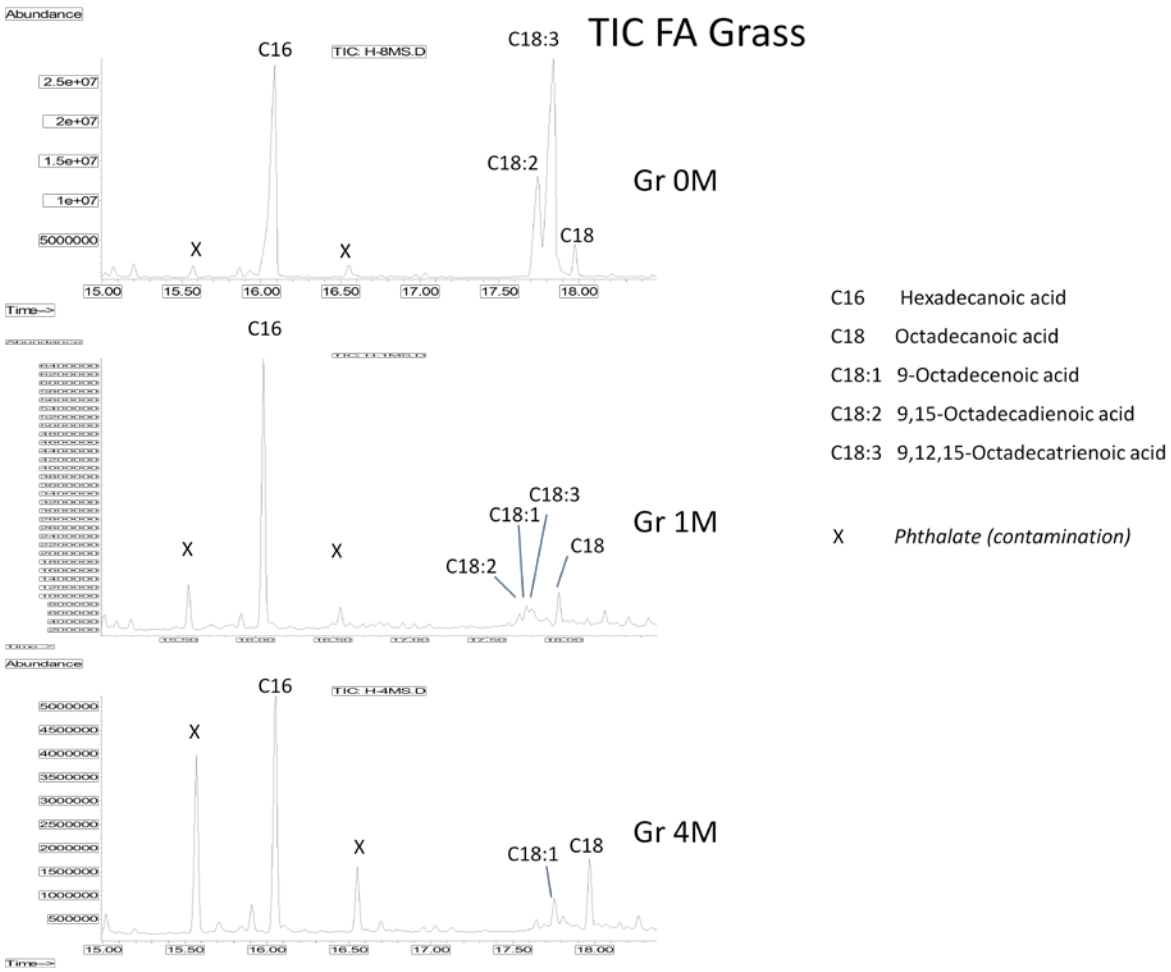

Fig 3A. Total ion chromatogram of selected fatty acids of the fresh and charred rye grass.

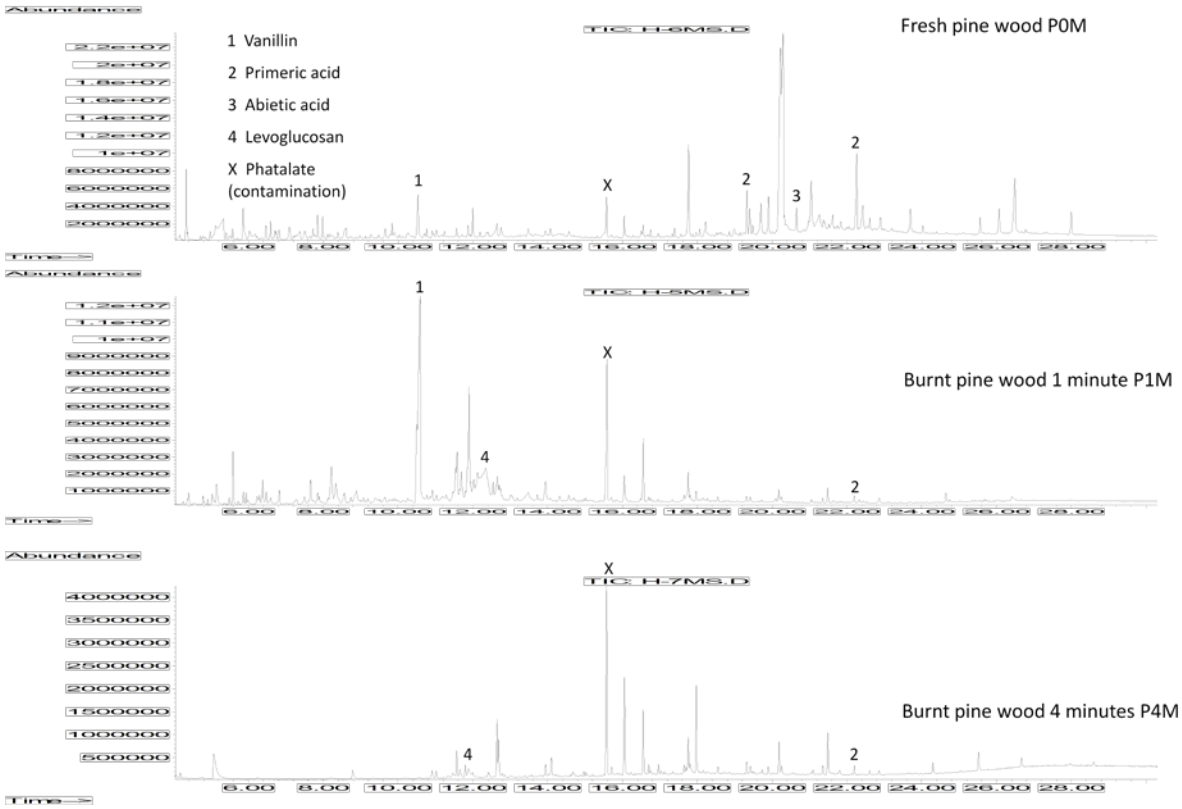

Fig. 4. Total ion chromatogram of the fresh and charred pine wood.