

## Article

# Neutral Sugar Content and Composition as a Sensitive Indicator of Fire Severity in the Andisols of an *Araucaria–Nothofagus* Forest in Southern Chile

Yessica Rivas <sup>1,\*</sup>, Jorge Retamal-Salgado <sup>2</sup>, Heike Knicker <sup>3</sup>, Francisco Matus <sup>4,5</sup> and Diego Rivera <sup>6</sup>

<sup>1</sup> Facultad de Ingeniería y Negocios, Universidad Adventista de Chile, km 12 Camino a TaniIvoro, Chillán 3780000, Chile

<sup>2</sup> Instituto de Investigaciones Agropecuarias, INIA-Quilamapu, Avenida Vicente Méndez 515, Chillán 3800062, Chile; jorge.retamal@inia.cl

<sup>3</sup> Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC), Reina Mercedes Av., 10, 41012 Seville, Spain; knicker@irnase.csic.es

<sup>4</sup> Laboratory of Conservation and Dynamics of Volcanic Soils, Department of Chemical Sciences and Natural Resources, Faculty of Engineering and Sciences, Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco 4780000, Chile; francisco.matus@ufrontera.cl

<sup>5</sup> Network for Extreme Environment Research, Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco 4780000, Chile

<sup>6</sup> Centro de Sustentabilidad y Gestión Estratégica de Recursos (CiSGER), Facultad de Ingeniería, Universidad del Desarrollo, Las Condes 7610658, Chile; diegorivera@udd.cl

\* Correspondence: yessicarivas@unach.cl; Tel.: +56-9-9462-9090



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**Abstract:** Wildfire induces soil alterations that have a long-term impact on soil organic matter (SOM) quality. We postulated that after different fire severities, the neutral sugars in soils can be used as an indicator of soil organic matter quality after fire. The aim of this study was to determine the contribution of neutral sugar, bulk and occluded particulate organic matter (oPOM) affected by wildfire, at different soil depths in an *Araucaria–Nothofagus* Forest, four years post-after fire. The concentration and composition of the neutral sugars in the soils clearly comprised the major fraction in the unburned soil. Medium- and high-severity fires caused a drastic reduction in soil sugars in the bulk soil as well as in the oPOM fractions. The <sup>13</sup>C-CPMAS NMR spectroscopy analysis revealed a high contribution of recalcitrant carbon to the decomposition such as aryl-C and aryl-O derived from charred material, whereas the abundance of O-alkyl C and alkyl C functional groups were decreased. The neutral sugars (Galactose+Mannose/Xilose+Arabinose) revealed a major microbial origin in fire affected areas as the ratio was >2. Therefore, we suggest that the neutral sugar content of soil should be used for monitoring both short- and long-term changes in SOM altered by fires.

**Keywords:** non-cellulosic carbohydrates; <sup>13</sup>C NMR; forest fires; oPOM fractions

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## 1. Introduction

Wildfires are the prevailing disturbance events shaping *Araucaria araucana–Nothofagus* spp. forest landscapes in southern Chile and Argentina [1–3]. Wildfires profoundly impact soil properties [4], mostly by altering the thickness, extent and composition of soil organic matter (SOM) [5–8]. During a fire, the SOM may be affected differently at each mineral soil horizon. For example, fire may deeply influence the particulate organic matter (POM) compared to a more protected SOM in association with the mineral phase and SOM occluded within soil aggregates, i.e., the occluded particulate organic matter (oPOM) [9]. Different forms of organic matter have different effects on soil quality and can respond differently to SOM alteration by fire [10,11]. Studies realized by Golchin et al. [12], Chan et al. [10] and Kölbl and Kögel-Knabner [13], support that the POM is a sensitive indicator of the alteration of soil.

Fire generates large quantities of charred or black carbon (BC), a partially combusted organic material which is assumed to be refractory to microbial decomposition due to relatively high amounts of aromatic compounds [14,15]. If ligno-cellulosic-rich material is burnt, a considerable part of the aromatic C derives from carbohydrates, which are dehydrated and subsequently aromatized [15,16]. It has been shown that the extent of this phenomenon increases with the increasing soil temperatures reached during wildfires [8]. A fire-induced alteration is expected to have a long-term impact on the quality and quantity of different SOM fractions, since above- and belowground fresh C input is the main primary source of carbohydrates in the soil, and it represents a major energy source for microbial growth [17].

The chemical composition of the soil organic matter and the contribution of pyrogenic C along with the net nitrogen (N) mineralization was investigated in soils on permanent plots of Chilean forests of *Araucaria araucana*–*Nothofagus* spp. in the National Park of Tolhuaca, Chile. The site was affected by wildfires of different severities in February 2002 [17]. In previous studies it was shown that the soil N cycling could largely be explained by an increase of aromatic chemical oxidation resistant carbon (CORECarom), a relative measure of charred materials in soil [14]. However, the BC pool played a minor role on the C stabilization due to losses following the transport to deeper soil layers [14]. Alkyl C from charred material seems to enhance soil C sequestration in burned sites.

Martín et al. [18] proposed carbohydrates as an index of fire impact on the SOM quality from soils affected by low and high burn severity wildfires; however, a study from Mastrolonardo et al. [19] questioned whether non-cellulosic neutral sugars and lignin-derived phenolic monomers can be used as molecular indicators of fire severity. Therefore, the effect of fire on neutral sugars as an indicator of severity is unclear and we need further studies to elucidate the complex impact of fire on SOM composition.

In the present study we analyzed the contribution of neutral sugar content in the top and subsurface volcanic (allophanic) soils affected by low and high burn severity wildfires. These soils present a high SOM content (>16%) and the POM have been reported to represent more than 37% of the total C, which represents a fraction mainly preserved by chemical recalcitrance and spatial inaccessibility by microorganisms, showing the importance of this C pool in this ecosystem [20].

We propose that soil sugar composition can be used as a sensitive indicator to evaluate the influence of fire severity on different oPOM fractions, in temperate rain forests. Fire severity would modify the sugar distribution and composition in the topsoil as these are more stable soil fractions, four years after the fire event. The origin and contribution of individual monosaccharides relative to the total carbohydrate content may provide an indicator of fire severity in the POM fraction [21]. For example, arabinose (A) and xylose (X) are mostly of plant origin, whereas mannose (M) and galactose (G) are mainly of microbial origin in soil [22]. For the ratio  $G+M/A+X$  ratio  $< 0.5$ , the contribution is mainly assumed to be from plant origin, whereas for a ratio  $> 2$ , it is assumed to come from a microbial origin [23]. An increase in the ratio is expected in the topsoil after severe fire disturbance in the oPOM fraction of the SOM due to the elimination of vegetation shortly after fire.

The objectives of the present study were to: (i) assess the chemical composition of occluded POM (oPOM) fractions using  $^{13}\text{C}$  NMR spectroscopy in soils affected by different fire severities; (ii) determine the content and composition of neutral sugars; and (iii) evaluate the fate of the sugars in different top (0–5 cm) and subsurface (5–10 cm) soil after wildfires in the Tolhuaca National Park, Araucanía region, Chile.

## 2. Materials and Methods

### 2.1. Site Description and Soil Sampling

Soil (Andisol, Soil Survey Staff, 2014) samples were taken from a mixed Chilean forest ecosystem (*Araucaria araucana* (Mol) K. Koch and *Nothofagus* spp.) in the Tolhuaca National Park (TNP) between  $38^{\circ}10'$  to  $38^{\circ}15'$  S and  $71^{\circ}41'$  to  $71^{\circ}50'$  W, at 1100–1300 m a.s.l. in southern Chile. In February 2002, more than half of the park, ca. 6380 ha, was affected by

huge wildfires [24]. The burned area under study was exposed to fires of different severities, ranging from complete tree mortality to slightly damaged vegetation [14]. Fire severity has been operationally defined as the loss of above- and below-ground carbon [25]. In this study the tree mortality (>5 cm diameter breast height (DBH)) was assumed to match these changes [14]. According to the tree mortality observed, the sites were grouped into high severity (HS; >80% of dead trees), medium severity, (MS; 40–80%) and low severity, (LS; 20–40%). An unburned site (U) which was not affected by fire for at least 150 years [2,26] was used as a control.

In February 2006, three plots (1000 m<sup>2</sup>) of HS, MS, LS and U sites were established and considered as independent replicates [14]. All plots presented similar vegetation *Araucaria–Nothofagus* spp. stands with a southern aspect. At each plot, three composed soil samples (0–5 cm and 5–10 cm depth) were taken after removing recognizable plant debris (O<sub>i</sub> + O<sub>e</sub> horizons). A total of 24 soil samples were taken and the visible coarse organic materials were removed by hand picking in the lab. All soil samples were sieved (2 mm), air dried and stored for further analysis. The bulk density was determined by extracting intact steel cylinders (5 cm diameter and 4 cm length) following the methodology of Blake and Hartge [27]. The corrected bulk density was determined by subtracting the volume of the rock fragments >2 mm [28].

## 2.2. Density Fractionation

The soil organic matter was physically fractionated using a sodium polytungstate solution (1.6 g cm<sup>-3</sup>) in order to separate the occluded intra-aggregates organic matter (oPOM) [29,30], which was protected from microbial decomposition and thus not bioavailable for a long period of time. Briefly, about 30 g of dry soil was mixed with 100 mL of sodium polytungstate solution and the soil suspension was gently stirred and allowed to settle overnight. The floating materials were separated by successive stirring and the remaining soil suspension was dispersed ultrasonically at 440 J mL<sup>-1</sup> for 3 min with a sonicator (Bandelin, Sonopuls HD 2200) previously calibrated to avoid excessive disruption of the oPOM [31]. Centrifugation (30 min at 3074×g) was used to separate the occluded POM (oPOM) from the mineral residue. To remove the Na-polytungstate from the POM fractions, the samples were washed several times with deionized water over a sieve of 20 µm mesh size. The obtained oldest and more stabilized organic matter fraction (oPOM < 20 µm) from the washing of the oPOM was washed via pressure filtration until the electric conductivity dropped below 5 µS. All fractions were freeze-dried and stored.

## 2.3. Laboratory Analyses, Total Carbon, and Nitrogen Determination

The soil pH was measured in water (1:2.5, soils: water ratio). The concentration of total C and total N in the whole soils and in each oPOM fraction were determined in duplicates with an elemental Vario analyzer (Vario Macro cube) by dry combustion at 950 °C. Since all soil samples were strongly acidic the measure of total C was assumed to be equivalent to the amount of soil organic carbon (SOC), since it was assumed to be free of carbonate.

## 2.4. Determination of Neutral Sugars

The determination of neutral sugars from the soil samples (~200 mg), according to that described by Spielvogel et al. [32], was completed following three steps: (1) hydrolysis with 4 M trifluoroacetic acid (TFA), according to Amelung et al. [33], (2) acetate alditol derivatization, according to Black and Fox [34] and modified by Rumpel and Dignac [35], and (3) analysis of monomers by gas chromatography (GC). The hydrolysis of non-cellulosic carbohydrates was carried out at 105 °C for 4 h after the addition of 10 mL 4 M TFA, after which the samples were cooled and 1 mL of an internal standard (5 g<sup>-1</sup> mL methylglucose) was added. The soil suspension was separated from the solution by filtration. After removing the TFA by rotary evaporation, the remaining hydrolyzed monosaccharides were dissolved in 2 mL of deionized water containing 1.6 g<sup>-1</sup> myoinositol to calculate the GC response. To increase the pH to 7.0, 0.6 mL ammonia (25%) was added. For the

derivatization, the aldoses were reduced to alditols at 40 °C for 1.5 h after an addition of 1 mL of NaBH<sub>4</sub> (20 g L<sup>-1</sup>) dissolved in dimethylsulfoxide (DMSO). The acetylation was performed by adding 0.2 mL acetic acid and methylimidazole (0.2 mL) as a catalyst to the cooled residue. Meanwhile, 2 mL of glacial acetic acid was added. After 10 min, the reaction was stopped by adding 7 mL ice-cold deionized water; by this process the remaining acetic anhydride was destroyed. We extracted the derivatized sugar monomers by liquid–liquid extraction using a vortex mixer after adding 2 mL dichloromethane. For further details about the gas chromatographic measurements of the sugar monomers, see Spielvogel et al. [32]. The concentrations of the individual sugar monomers (arabinose, fucose, galactose, glucose, mannose, rhamnose, ribose and xylose) were calculated based on the internal standard myoinositol. The recovery of myoinositol during the procedure was such as that obtained by Spielvogel et al. [32], >75%. The mean recovery of the neutral sugars in the physical fractions relative to the bulk soil samples and in the occluded POM fractions were >95%. The sugar contents data (mg g<sup>-1</sup>) were standardized to mg sugar C g<sup>-1</sup> soil.

### 2.5. Solid-State <sup>13</sup>C NMR Spectroscopy

The solid-state <sup>13</sup>C NMR spectra of the bulk soils and their oPOM > 20 µm and oPOM < 20 µm fractions were obtained at the Lehrstuhl für Bodenkunde of the Technische Universität München using a Bruker DSX-200 NMR spectrometer (Biospin, Rheinstetten, Germany) at a resonance frequency of 50.32 MHz. The cross-polarization magic angle spinning technique (CP-MAS) was applied with a spinning speed of 6.8 kHz, using a contact time of 1 ms and a pulse delay of 300 ms. Depending on the material, single scans were accumulated until a reasonable signal-to-noise ratio was achieved. The chemical shift was referenced to the TMS scale and adjusted with glycine (COOH, 176.04 ppm) as an external reference. For quantification, the spectrum was divided into chemical shift regions assigned to the main C functional groups according to Knicker [36] and integrated. The chemical shift regions 0–45, 45–110, 110–140, 140–160 and 160–220 ppm was assigned to alkyl C, O-alkyl C, aryl C, O-substituted aryl C and carboxylic C, respectively. The total aromatic-C contribution was determined by the intensity in the region between 110 and 160 ppm. Owing to insufficient averaging of the chemical shift anisotropy at a spinning speed of 6.8 kHz, spinning side bands of the aromatic C signals occurred at a frequency distance of the spinning speed at both sides of the central signal (300 to 245 ppm and 0 to 50 ppm). Recent NMR studies have confirmed a good reproducibility of the NMR data of soils if a sufficiently low signal-to-noise ratio of the spectra can be achieved [36].

### 2.6. Statistical Analysis

An ANOVA test was conducted to determine the significance of the effects of wildfires of different severities on soil characteristics in bulk soil. Significant differences were determined by a Tukey's post-hoc test ( $p < 0.05$ ). A log transformation of the data was applied to achieve normality and homoscedasticity. The statistical procedures were carried out with the software package Statistics for Windows 6.0.

## 3. Results

### 3.1. Soil Characteristics and oPOM Fractions

Four years after fire, the SOC and total N contents of soil tended to decrease in burned sites, according to fire severities, at 0–5 and 5–10 cm ( $p < 0.05$ ) (Table 1). Significant differences were found among the differing fire severities. The C concentration in burned soil varied from 81.2–121.1 g kg<sup>-1</sup> in the topsoil (0–5 cm of depth) to 72.9–107.9 g kg<sup>-1</sup> in the subsurface soil (5–10 cm of depth), whereas the total N contents ranged from 4.1–5.8 g kg<sup>-1</sup> soil (0–5 cm of depth) to 2.6–5.2 g kg<sup>-1</sup> soil (5–10 cm). In the control soil, the C concentration was 156.8 and 162.9 g kg<sup>-1</sup>, for the top and subsurface depth soils, respectively, whereas the total N content was 5.2 and 8.0 g kg<sup>-1</sup>, for the top and subsurface soil depths, respectively. The C to N ratio varied from 14.8 to 31.3 and no significant differences were detected,

except for the highest value. There was no change for the bulk density and soil pH between burned and unburned samples either. Bulk densities were rather low and ranged from 0.41–0.47 Mg m<sup>−3</sup>, while the pH varied from 4.9 to 5.4 and tended to be higher in burned topsoil, except in the HS sites (Table 1).

**Table 1.** Soil characteristics in an Andisol from southern Chile under an *Araucaria–Nothofagus* spp. forest after various fire severities (standard error of the mean is given in brackets,  $n = 3$ ).

| Fire Severity <sup>1</sup> | Soil Depth (cm) | SOC <sup>2</sup> (g kg <sup>−1</sup> ) | Total N (g kg <sup>−1</sup> ) | C/N          |                       | BD <sup>3</sup> (Mg m <sup>−3</sup> ) | pH <sub>water</sub> |
|----------------------------|-----------------|--|-------------------------------|--------------|-----------------------|---------------------------------------|---------------------|
|                            |                 |  |                               |              | (Mg m <sup>−3</sup> ) |                                       |                     |
| U                          | 0–5             | 156.8 (7.6) *                          | 8.0 (0.4) *                   | 19.5 (1.7)   | 0.40 (0.03)           | 4.9 (0.3)                             |                     |
|                            | 5–10            | 162.9 (26.8) *                         | 5.2 (0.6)                     | 31.3 (4.1) * | 0.45 (0.05)           | 5.3 (0.1)                             |                     |
| LS                         | 0–5             | 121.1 (23.9)                           | 5.8 (1.0)                     | 20.9 (3.2)   | 0.46 (0.02)           | 5.4 (0.2)                             |                     |
|                            | 5–10            | 84.1 (12.1)                            | 5.1 (0.8)                     | 16.5 (0.3)   | 0.41 (0.02)           | 5.2 (0.1)                             |                     |
| MS                         | 0–5             | 81.2 (14.9)                            | 5.5 (0.6)                     | 14.8 (0.4)   | 0.44 (0.03)           | 5.3 (0.2)                             |                     |
|                            | 5–10            | 107.9 (17.7)                           | 4.4 (0.4)                     | 24.5 (4.2)   | 0.47 (0.04)           | 5.0 (0.3)                             |                     |
| HS                         | 0–5             | 98.4 (13.9)                            | 4.1 (0.4)                     | 24.0 (4.1)   | 0.47 (0.00)           | 5.1 (0.2)                             |                     |
|                            | 5–10            | 72.9 (13.8)                            | 2.6 (0.2)                     | 28.0 (2.1)   | 0.46 (0.03)           | 5.2 (0.1)                             |                     |

<sup>1</sup> U: unburned site; HS: high severity fire (>80% of dead trees); MS: medium severity (40–80%); LS: low severity (20–40%). <sup>2</sup> SOC: soil organic carbon. <sup>3</sup> BD: bulk density. \* Significant differences ( $p < 0.05$ ) between fire severities and unburned soil.

The burned soil yielded from 8.0 to 16.3 g kg<sup>−1</sup> soil for oPOM  $> 20 \mu\text{m}$  (oPOM<sub>c</sub>) and from 6.6 to 28.3 g kg<sup>−1</sup> soil for oPOM  $< 20 \mu\text{m}$  (oPOM<sub>f</sub>) fraction. In unburned soil, the yielded, varied between 10.5 and 23.0 g kg<sup>−1</sup> soil for the oPOM<sub>c</sub>  $\mu\text{m}$  and between 20.5 and 33.4 g kg<sup>−1</sup> soil for the oPOM<sub>f</sub> (Table 2). The corresponding values for the fractions of the unburned soil were significantly higher (Table 2). The total recovery of the sample material after extraction was approximately 98% of the bulk soil.

**Table 2.** The weight, soil organic carbon (SOC) and total N concentration of occluded particulate organic matter (oPOM) derived from Andisol from southern Chile under *Araucaria–Nothofagus* spp. forest at different fire severities (standard error of the mean is given in brackets,  $n = 3$ ).

| Fire Severity <sup>1</sup> | Soil Depth (cm) | oPOM <sub>c</sub> <sup>2</sup> g kg <sup>−1</sup> soil | oPOM <sub>f</sub> <sup>3</sup> g kg <sup>−1</sup> soil | SOC <sup>4</sup>               |                                | Total N                        |                                |
|----------------------------|-----------------|--|--|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                            |                 |  |  | oPOM <sub>c</sub> <sup>2</sup> | oPOM <sub>f</sub> <sup>3</sup> | g kg <sup>−1</sup> fraction    |                                |
|                            |                 |  |  |                                |                                | oPOM <sub>c</sub> <sup>2</sup> | oPOM <sub>f</sub> <sup>3</sup> |
| U                          | 0–5             | 23.0 (0.9) *   | 33.4 (1.4) *   | 9.7 (0.06) *                   | 15.3 (0.06) *                  | 1.6 (0.06)                     | 1.7 (0.02)                     |
|                            | 5–10            | 10.5 (1.0)   | 20.5 (3.5)   | 3.7 (0.01)                     | 9.8 (0.03)                     | 0.5 (0.04)                     | 0.8 (0.03)                     |
| LS                         | 0–5             | 9.3 (3.5)  | 23.3 (0.3) *   | 4.6 (0.04)                     | 7.7 (0.03)                     | 0.7 (0.03)                     | 1.4 (0.03)                     |
|                            | 5–10            | 13.0 (1.3)   | 28.3 (2.0) *   | 5.9 (0.05)                     | 6.7 (0.04)                     | 0.6 (0.02)                     | 1.0 (0.01)                     |
| MS                         | 0–5             | 8.0 (0.5)  | 3.6 (3.4)  | 3.6 (0.02)                     | 1.2 (0.07)                     | 0.4 (0.03)                     | 0.2 (0.02)                     |
|                            | 5–10            | 10.7 (0.8)   | 12.6 (1.3)   | 3.7 (0.02)                     | 3.7 (0.04)                     | 0.5 (0.02)                     | 0.2 (0.01)                     |
| HS                         | 0–5             | 16.3 (6.3)   | 6.6 (1.7)  | 7.5 (0.05)                     | 2.8 (0.01)                     | 1.6 (0.01)                     | 0.3 (0.22)                     |
|                            | 5–10            | 17.0 (2.7)   | 9.6 (0.6)  | 7.6 (0.03)                     | 2.9 (0.01)                     | 0.9 (0.06)                     | 0.4 (0.03)                     |

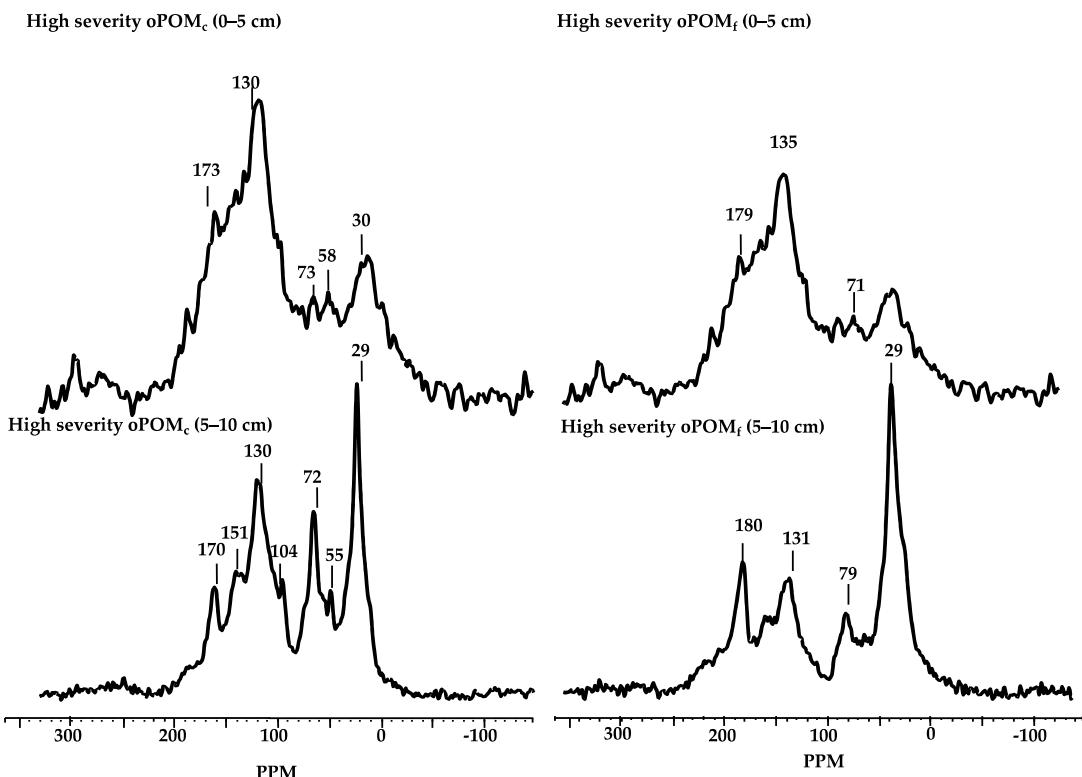
<sup>1</sup> U: unburned site; HS: high severity fire (>80% of dead trees); MS: medium severity (40–80%); LS: low severity (20–40%). <sup>2</sup> Coarse  $> 20 \mu\text{m}$  oPOM (oPOM<sub>c</sub>). <sup>3</sup> Fine  $< 20 \mu\text{m}$  POM (oPOM<sub>f</sub>). <sup>4</sup> SOC: soil organic carbon. \* Significant differences ( $p < 0.05$ ) between fire severities and unburned soil.

The C in burned soil was 3.6 and 7.6 g kg<sup>−1</sup> for the oPOM<sub>c</sub> and was 1.2 and 7.7 g kg<sup>−1</sup> soil for the oPOM<sub>f</sub>, for top and subsurface depth soils, respectively. In the control soil, the C was 3.7 and 9.7 g C kg<sup>−1</sup> soil for the oPOM<sub>c</sub>, for top and subsurface depth soils, respectively, and it showed higher values for the oPOM<sub>f</sub> (9.8 to 15.3 g C kg<sup>−1</sup> soil).

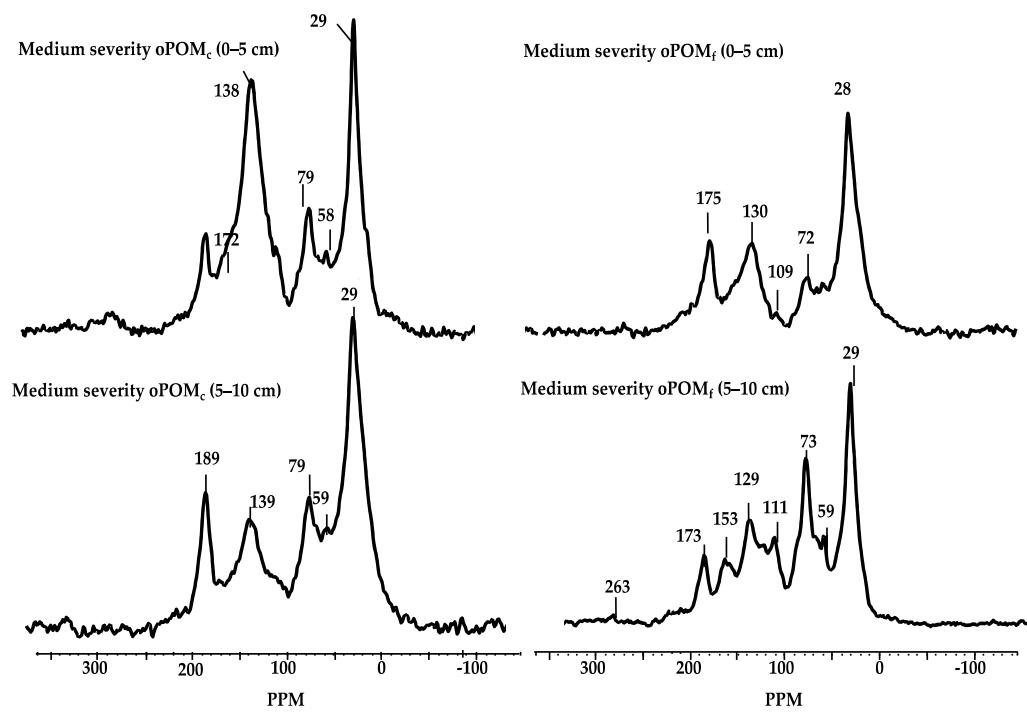
The N in fire-affected soils ranged from 0.4 to 1.6 g N kg<sup>−1</sup> soil for the oPOM<sub>c</sub>, and from 0.2 to 1.4 g N kg<sup>−1</sup> soil for the oPOM<sub>f</sub>. In the unburned soil, N varied from 0.5 to 1.6 g N kg<sup>−1</sup> soil for the oPOM<sub>c</sub> and from 0.8 to 1.7 g N kg<sup>−1</sup> for the oPOM<sub>f</sub>. These results indicate a reduction in the mass and C content in the oPOM fraction post-fire, but there was no major difference with respect to the N pattern.

### 3.2. Solid-State $^{13}\text{C}$ NMR Spectroscopy

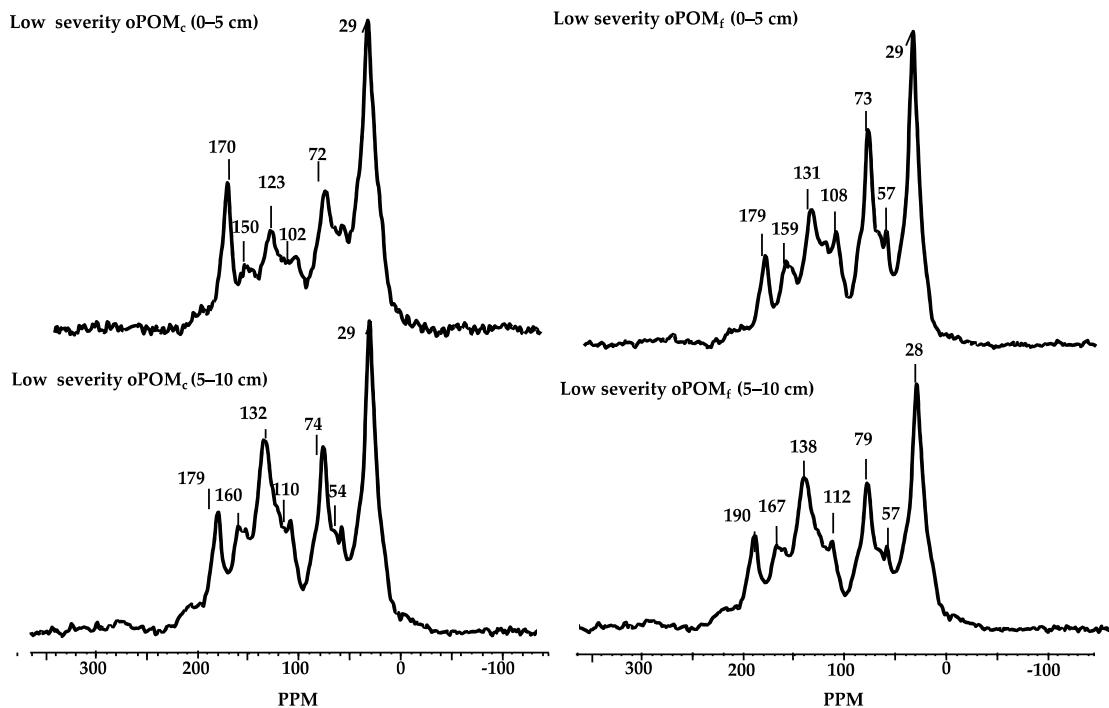
Based on the solid-state  $^{13}\text{C}$  NMR spectra of the fractions of the unburned soil, both oPOM fractions of the fire-affected soils, mainly in the HS and MS, showed a relative decline in intensity in the chemical shift regions of O-alkyl C and alkyl C. This is also true for the oPOM<sub>f</sub> (Figures 1–4, and Table 3). The O-alkyl C region is assigned to the polysaccharides (110 to 60 ppm) and methoxyl C as it occurs in lignin (56 ppm). However, N-alkyl C in peptides and amino sugars can also contribute to this region (60 to 45 ppm) [36]. The alkyl C region contains signals of acetyl C and methyl C in lipids and proteins, hemicellulosic, amino acids, cutin, suberin waxes and other aliphatic bio-macromolecules [37,38]. The signal assigned to aryl C (110–160 ppm), which may originate from lignin, black carbon, and other aromatic compounds, increased from the unburned to burned soils (Figures 1–4, and Table 3). This is particularly true for the spectra of subsamples affected by high severity fire (55–29%). This pattern was most clearly expressed in the spectra of the material from the top 5 cm. The observed alteration implies that the fires not only affected the quantity of oPOM, but also its quality.



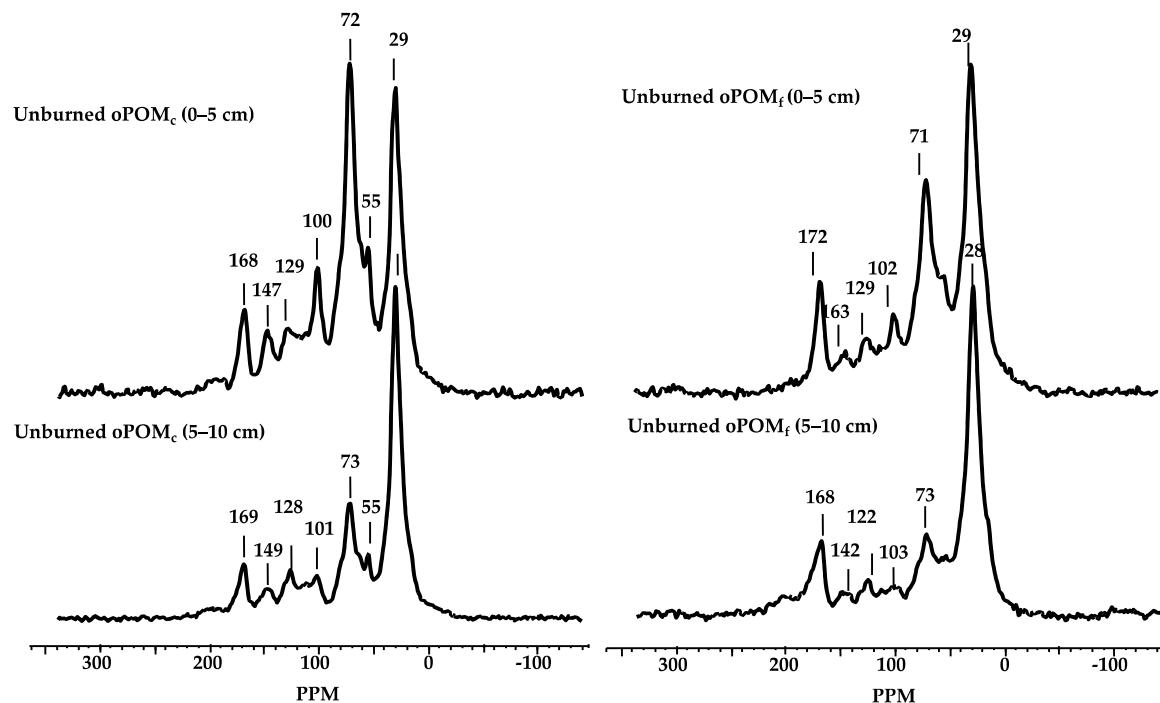
**Figure 1.** Solid-state  $^{13}\text{C}$  NMR spectra of Fine < 20  $\mu\text{m}$  POM (oPOM<sub>f</sub>) and coarse > 20  $\mu\text{m}$  oPOM (oPOM<sub>c</sub>) obtained from depths at 0–5 and 5–10 cm of Andisols under *Araucaria–Nothofagus* spp. forest in south-central Chile, subjected to a high severity wildfire.



**Figure 2.** Solid-state  $^{13}\text{C}$  NMR spectra of Fine < 20  $\mu\text{m}$  POM (oPOM<sub>f</sub>) and coarse > 20  $\mu\text{m}$  oPOM (oPOM<sub>c</sub>) obtained from depths of 0–5 cm and 5–10 cm of Andisols of an *Araucaria–Nothofagus* spp. forest, south-central Chile, subjected to a medium severity wildfire.



**Figure 3.** Solid-state  $^{13}\text{C}$  NMR spectra of Fine < 20  $\mu\text{m}$  POM (oPOM<sub>f</sub>) and coarse > 20  $\mu\text{m}$  oPOM (oPOM<sub>c</sub>) obtained from depths of 0–5 cm and 5–10 cm of Andisols of an *Araucaria–Nothofagus* spp. forest, south-central Chile, subjected to a low severity wildfire.



**Figure 4.** Solid-state  $^{13}\text{C}$  NMR spectra of coarse  $> 20 \mu\text{m}$  oPOM (oPOM<sub>c</sub>) and Fine  $< 20 \mu\text{m}$  POM (oPOM<sub>f</sub>) obtained from depths of 0–5 cm and 5–10 cm of Andisols of an *Araucaria–Nothofagus* spp. forest, south-central Chile, in unburned soil.

**Table 3.** Solid-state  $^{13}\text{C}$  NMR spectra (%) of occluded particulate organic matter (oPOM) derived from Andisol from southern Chile under *Araucaria–Nothofagus* spp. forest at different fire severities.

| Fire Severity <sup>1</sup> | Soil Depth (cm) | Carboxyl C (160–245 ppm)       |                                | Aryl C (110–160 ppm) |                   | O-alkyl C (45–110 ppm) |                   | Alkyl C (0–45 ppm) |                   |
|----------------------------|-----------------|--------------------------------|--------------------------------|----------------------|-------------------|------------------------|-------------------|--------------------|-------------------|
|                            |                 | oPOM <sub>c</sub> <sup>2</sup> | oPOM <sub>f</sub> <sup>3</sup> | oPOM <sub>c</sub>    | oPOM <sub>f</sub> | oPOM <sub>c</sub>      | oPOM <sub>f</sub> | oPOM <sub>c</sub>  | oPOM <sub>f</sub> |
| U                          | 0–5             | 6.2                            | 8.2                            | 15.4                 | 14.4              | 48.2                   | 37.5              | 30.3               | 45.9              |
|                            | 5–10            | 11.9                           | 6.6                            | 15.4                 | 17.4              | 26.8                   | 29.5              | 45.9               | 46.5              |
| LS                         | 0–5             | 13.0                           | 12.7                           | 21.9                 | 23.7              | 29.1                   | 28.3              | 36.0               | 35.3              |
|                            | 5–10            | 11.7                           | 8.3                            | 21.8                 | 27.0              | 30.2                   | 34.9              | 36.2               | 29.8              |
| MS                         | 0–5             | 13.6                           | 11.9                           | 40.3                 | 28.2              | 21.1                   | 24.2              | 24.9               | 35.8              |
|                            | 5–10            | 13.6                           | 12.6                           | 40.4                 | 23.0              | 20.9                   | 28.0              | 25.1               | 36.4              |
| HS                         | 0–5             | 18.0                           | 22.9                           | 54.4                 | 42.3              | 16.2                   | 22.7              | 11.4               | 12.1              |
|                            | 5–10            | 13.2                           | 14.4                           | 35.7                 | 29.7              | 27.2                   | 25.4              | 23.9               | 31.1              |

<sup>1</sup> U: unburned site; HS: high severity fire ( $> 80\%$  of dead trees); MS: medium severity (40–80%); LS: low severity (20–40%). <sup>2</sup> Coarse  $> 20 \mu\text{m}$  oPOM (oPOM<sub>c</sub>). <sup>3</sup> Fine  $< 20 \mu\text{m}$  POM (oPOM<sub>f</sub>).

### 3.3. Neutral Sugars Content in Bulk Soils and Occluded Particulate Organic Matter in Soil

The contributions of the total neutral sugar in bulk soil are shown in Table 4. Hexoses (mannose, galactose, and glucose) were the most abundant type of sugars found in all areas studied, whereas desoxyhexoses (rhamnose and Fucose) and pentoses (arabinose and xylose) were the scarcest. The highest sugar content in burned soils was found at a depth of 5–10 cm, whereas in unburned soil this was the case within the upper 5 cm depth. The total sugar contents were highest in the U soil ( $223\text{--}357 \text{ mg g}^{-1}$ ), followed by LS ( $127\text{--}220 \text{ mg g}^{-1}$ ), MS ( $154\text{--}186 \text{ mg g}^{-1}$ ) and HS ( $105\text{--}148 \text{ mg g}^{-1}$ ) (Table 4).

**Table 4.** Neutral sugars contents, total sugar contents and sugar ratio of bulk soil, at various fire severities in an *Araucaria–Nothofagus* spp. forest stand, on an Andisol from south-central Chile (standard error of the mean is given in brackets,  $n = 3$ ).

| Fire Severity <sup>1</sup> | Soil Depth (cm) | Rhamnose          | Fucose      | Arabinose   | Xylose      | Mannose     | Galactose   | Glucose     | Total Sugar          | G+M/A+X <sup>2</sup> |
|----------------------------|-----------------|-------------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------------|----------------------|
|                            |                 | Desoxyhexoses     |             | Pentoses    |             | Hexoses     |             |             | mg g C <sup>-1</sup> |                      |
|                            |                 |                   |             |             |             |             |             |             | mg g C <sup>-1</sup> |                      |
|                            |                 |                   |             | mg          |             |             |             |             |                      |                      |
| HS                         | 0–5             | n.d. <sup>3</sup> | n.d.        | n.d.        | n.d.        | 0.87 (0.02) | 1.49 (0.13) | 0.31 (0.21) | 105.3 (12.4)         | n.d.                 |
|                            | 5–10            | 0.07 (0.04)       | 0.03 (0.0)  | n.d.        | 0.03 (0.00) | 0.72 (0.01) | 1.19 (0.03) | 0.75 (0.01) | 147.6 (2.4)          | n.d.                 |
| MS                         | 0–5             | 0.14 (0.01)       | 0.22 (0.00) | 0.19 (0.00) | 0.13 (0.00) | 0.92 (0.01) | 1.87 (0.06) | 2.08 (0.02) | 153.7 (2.3)          | 8.9                  |
|                            | 5–10            | 0.23 (0.00)       | 0.23 (0.02) | 0.17 (0.04) | 0.19 (0.03) | 1.03 (0.04) | 1.81 (0.00) | 1.97 (0.05) | 185.5 (6.1)          | 7.0                  |
| LS                         | 0–5             | 0.02 (0.00)       | 0.02 (0.00) | 0.02 (0.00) | 0.03 (0.00) | 0.91 (0.04) | 1.47 (0.02) | 0.64 (0.00) | 127.5 (4.8)          | 5.3                  |
|                            | 5–10            | 0.23 (0.03)       | 0.26 (0.00) | 0.23 (0.01) | 0.35 (0.00) | 1.02 (0.00) | 1.68 (0.03) | 2.48 (0.07) | 219.5 (3.4)          | 3.3                  |
| U                          | 0–5             | 0.60 (0.01)       | 0.36 (0.01) | 0.72 (0.04) | 0.90 (0.02) | 1.69 (0.06) | 3.11 (0.17) | 6.98 (0.10) | 357.3 (10.9)         | 3.0                  |
|                            | 5–10            | 0.32 (0.02)       | 0.24 (0.01) | 0.34 (0.00) | 0.41 (0.00) | 1.16 (0.01) | 2.12 (0.11) | 3.85 (0.02) | 223.2 (2.9)          | 4.0                  |

<sup>1</sup> U: unburned site; HS: high severity fire (>80% of dead trees); MS: medium severity (40–80%); LS: low severity (20–40%). <sup>2</sup> G = galactose; M = mannose; A = arabinose; X = xylose. <sup>3</sup> n.d.: not determined.

Conversely, the oPOM fraction followed a trend comparable to that observed in the bulk soils (Table 5). In most cases, the neutral sugar contribution to the total C was higher in the oPOM fractions than in the bulk soil samples (Table 4). The sugar contents of the oPOM ( $\text{oPOM}_f + \text{oPOM}_c$ ) until 10 cm of depth were the highest in the U soil ( $300\text{--}292 \text{ mg g C}^{-1}$ ), followed by LS ( $250\text{--}221 \text{ mg g C}^{-1}$ ), MS ( $170\text{--}156 \text{ mg g C}^{-1}$ ) and U ( $125\text{--}66 \text{ mg g C}^{-1}$ ). In general, in all fire severities the contribution of sugar was higher for the  $\text{oPOM}_f$  than for the  $\text{oPOM}_c$ . However, the U soils showed no clear difference between fractions. Moreover, the highest sugar concentration was found between 5 and 10 cm of depth for HS and MS soil in comparison to LS and U soil where the highest concentration was observed between 0 and 5 cm of depth.

**Table 5.** Neutral sugar contents of the occluded particulate organic matter (oPOM) fraction  $> 1.6 \text{ g cm}^{-3}$  (see above), at various fire severities in an *Araucaria–Nothofagus* spp. forest stand on an Andisol from south-central Chile (standard error of the mean is given in brackets,  $n = 3$ ).

| Fire Severity <sup>1</sup> | Soil Depth (cm) | Fraction (oPOM) ( $\mu\text{m}$ ) | Rhamnose      | Fucose            | Arabinose   | Xylose      | Mannose     | Galactose   | Glucose     | Total Sugar          | G+M/A+X <sup>2</sup> |
|----------------------------|-----------------|-----------------------------------|---------------|-------------------|-------------|-------------|-------------|-------------|-------------|----------------------|----------------------|
|                            |                 |                                   | Desoxyhexoses |                   | Pentoses    |             | Hexoses     |             |             | mg g C <sup>-1</sup> |                      |
|                            |                 |                                   |               |                   |             |             |             |             |             |                      |                      |
|                            |                 |                                   | mg            |                   |             |             |             |             |             |                      |                      |
| HS                         | 0–5             | $\text{oPOM}_c$ <sup>3</sup>      | 0.04 (0.00)   | n.d. <sup>4</sup> | 0.18 (0.01) | 0.27 (0.00) | 1.06 (0.07) | 1.75 (0.01) | 1.59 (0.00) | 27.9 (0.5)           | 6.3                  |
|                            |                 | $\text{oPOM}_f$ <sup>5</sup>      | 0.28 (0.02)   | 0.18 (0.01)       | 0.59 (0.00) | 0.63 (0.00) | 0.51 (0.02) | 2.13 (0.08) | 3.47 (0.02) | 37.8 (3.6)           | 2.2                  |
| MS                         | 0–5             | $\text{oPOM}_c$                   | 0.09 (0.00)   | 0.07 (0.00)       | 0.31 (0.00) | 0.52 (0.00) | 1.04 (0.03) | 2.02 (0.01) | 2.78 (0.05) | 48.7 (0.4)           | 3.7                  |
|                            |                 | $\text{oPOM}_f$                   | 0.28 (0.02)   | 0.19 (0.00)       | 0.14 (0.00) | 0.13 (0.00) | 1.11 (0.03) | 2.07 (0.02) | 2.01 (0.04) | 75.7 (0.2)           | 11.5                 |
| MS                         | 5–10            | $\text{oPOM}_c$                   | 0.22 (0.02)   | 0.18 (0.02)       | 0.52 (0.01) | 0.40 (0.02) | 1.21 (0.00) | 2.44 (0.07) | 2.73 (0.08) | 62.8 (1.8)           | 3.9                  |
|                            |                 | $\text{oPOM}_f$                   | 0.60 (0.00)   | 0.54 (0.02)       | 0.29 (0.01) | 0.24 (0.01) | 1.51 (0.00) | 2.94 (0.03) | 4.14 (0.14) | 94.1 (1.6)           | 8.4                  |
| LS                         | 0–5             | $\text{oPOM}_c$                   | 0.45 (0.01)   | 0.44 (0.01)       | 0.32 (0.02) | 0.20 (0.02) | 1.42 (0.02) | 2.81 (0.04) | 3.39 (0.13) | 80.3 (2.3)           | 7.6                  |
|                            |                 | $\text{oPOM}_f$                   | 0.05 (0.00)   | 0.03 (0.02)       | 0.11 (0.00) | 0.10 (0.01) | 1.02 (0.04) | 1.66 (0.06) | 2.03 (0.07) | 89.7 (3.6)           | 12.9                 |
| LS                         | 5–10            | $\text{oPOM}_c$                   | n.d.          | n.d.              | n.d.        | 0.06 (0.00) | 0.88 (0.01) | 1.26 (0.01) | 1.49 (0.01) | 67.8 (0.2)           | n.d.                 |
|                            |                 | $\text{oPOM}_f$                   | 0.87 (0.00)   | 0.39 (0.01)       | 0.79 (0.01) | 0.95 (0.02) | 1.90 (0.17) | 2.88 (0.09) | 5.73 (0.14) | 181.3 (4.4)          | 2.6                  |
| U                          | 0–5             | $\text{oPOM}_c$                   | 0.51 (0.01)   | 0.33 (0.01)       | 1.23 (0.00) | 1.46 (0.00) | 0.98 (0.16) | 3.45 (0.17) | 5.26 (0.68) | 92.3 (8.5)           | 1.6                  |
|                            |                 | $\text{oPOM}_f$                   | 0.64 (0.05)   | 0.32 (0.05)       | 0.35 (0.09) | 0.49 (0.00) | 0.87 (0.40) | 2.10 (0.05) | 2.61 (0.04) | 128.5 (1.2)          | 3.3                  |
| U                          | 5–10            | $\text{oPOM}_c$                   | 0.90 (0.11)   | 0.50 (0.00)       | 1.30 (0.03) | 1.58 (0.01) | 1.42 (0.09) | 3.96 (0.17) | 8.50 (0.29) | 173.9 (5.8)          | 1.9                  |
|                            |                 | $\text{oPOM}_f$                   | 0.90 (0.03)   | 0.50 (0.03)       | 0.96 (0.03) | 1.19 (0.06) | 1.35 (0.01) | 3.56 (0.18) | 8.00 (0.25) | 126.4 (4.5)          | 2.3                  |

<sup>1</sup> U: unburned site; HS: high severity fire (>80% of dead trees); MS: medium severity (40–80%); LS: low severity (20–40%). <sup>2</sup> G = galactose; M = mannose; A = arabinose; X = xylose. <sup>3</sup> Coarse > 20  $\mu\text{m}$  oPOM ( $\text{oPOM}_c$ ). <sup>4</sup> n.d.: not detected. <sup>5</sup> Fine < 20  $\mu\text{m}$  POM ( $\text{oPOM}_f$ ).

In the bulk soils, the G+M/A+X ratios in the oPOM fractions ranged between 4.5 to 8.9 in burned soils and between 3.0 to 4.0 in unburned soils (Table 4). Due to the loss of forest cover, the ratio of arabinose and xylose was not detected in the HS sites (Table 4). The G+M/A+X ratio in oPOM fraction varied from 1.6 to 12.9 in burned soil and from 1.9 to 3.1 in unburned soil. As a general trend, the burned soils showed the highest ratio.

## 4. Discussion

### 4.1. Effects of Fire on Soil Characteristics, oPOM Fractions and Sugars

Fire induced loss in the SOC and N, particularly in the HS and MS areas, was not counterbalanced by the input of organic residues four years after wildfire. This is mainly related to the oPOM fractions with a fire induced loss in the oPOM<sub>c</sub> and especially in the oPOM<sub>f</sub> where the fire severity was very high (HS). However, this was evident only in the first 5 cm of depth. These fractions were protected from microbial decomposition and are thus not bioavailable. The main difference between the two fractions consists, generally, in the particle size and location within the soil matrix: the larger oPOM consists of relatively fresh, undecomposed organic matter, while the oPOM < 20  $\mu$ m is mostly older and much more decomposed [39].

The decrease in the oPOM fractions can be attributed to organic matter loss and binding agents of the soil aggregates such as neutral sugar (derived from plants and microorganisms) and temporary binding (fungal hyphae, fine roots, bacterial cells) [40–43] attributed to fire. Unlike the oPOM, the stable aromatic compounds were shown to be protected in organo-mineral associations with amorphous clay minerals [44], which are the dominant materials together with the formation of Al-humus complexes [45].

The  $^{13}\text{C}$  NMR spectra of the oPOM fractions (oPOM<sub>f</sub> and oPOM<sub>c</sub>) revealed that fire resulted in a decrease of the relative contribution of alkyl C and O-alkyl C and yielded a relative enrichment of aromatic C, mainly in the 0–5 cm depth (Figures 1–4). Normally only the topsoil of a few cm depths is affected by fire [4,46,47]. However, high aromaticity was still found at 5–10 cm depths, principally in the MS and HS samples for oPOM<sub>c</sub> (Figures 1 and 2). The best explanation for this is that pyrogenic organic matter (PyOM) moved to deeper soils or that oPOM rich in PyOM was translocated to deeper soil [36,48]. On the other hand, the oPOM<sub>c</sub> represented the most decomposed SOM fraction as revealed by the highest alkyl C in unburned soil and low severity of soil, over the other composition of the oPOM fraction and severities. The increase of the alkyl carbon is explained by the selective preservation and in situ synthesis [49,50].

The enrichment of aromatic C (aryl C), in the MS and HS, and principally in the oPOM<sub>c</sub>, could be explained by the new formation of aromatic structures from cellulose [51,52] and other carbohydrates, as well as from peptides, by the dehydration and cyclization reactions [53,54] during the heating of the aggregates.

Alternatively, and more likely, this is due to a preferential input of PyOM from decaying charcoal residues into the soil organic matter fraction, leading to an increase of aryl C (Figures 1 and 2) [48]. The aging of charcoal leads to material which has numerous functional groups to interact with the mineral phase [48]. Subsequently, those interactions are likely to stabilize those partially degraded residues within the oPOM fractions. This explanation is supported by the concomitant increase of aryl C alongside the fire severities [48]. A fast oxidation of charcoal during post-fire humification has also been observed by others [55]. Skjemstad et al. [55] extracted aromatic clusters rich in aryl C groups from oxidized charred residues.

### 4.2. Sources of Sugars in Soil

Plant biomass, including above (leaves, twigs, fruits, flowers) and below ground (roots), is the main primary source of carbohydrates in soil [56]. Soil microorganisms are the secondary source of carbohydrates in soil, i.e., microorganisms synthesize their sugars from plant derived sugar C or other C-containing substances derived from litter or microbial residues [15,57,58]. Microorganisms synthesize mainly hexoses (glucose, mannose, and galactose). Pentoses, particularly arabinose and xylose, are not synthesized by microorganisms in significant amounts [23] and are present mostly in plant residues [59]. Mannose, galactose and glucose were the most common monosaccharides in the burned and unburned soils, both for the bulk material and oPOM fractions, especially the oPOM<sub>f</sub>. The G+M/A+X ratio constituted the often-used indicator to characterize the composition of sugars in soils [15,23,60]. However, Mastrolonardo et al. [19] has questioned whether

non-cellulosic neutral sugars and lignin-derived phenolic monomers can be used as molecular indicators of fire severity. It is true that the mixing of various sugar sources in soil makes it difficult to determine whether their origin is from plants or from microorganisms or a mixing of both; however, regarding our results, fire did induce the decrease in neutral sugar. The GM/AX ratio before wildfire was  $>2$ , indicating that the sugars were synthetized mainly by the microbial community, demonstrating that plant-derived sugars were preferentially affected by fire.

The fire resulted in a shift from pentoses to hexoses, indicating either a consumption or destruction of the plant-derived matter by fire, as well as the new production of microbial compounds. This change may also be explained by a lower input of fire-unaffected plant residues after their combustion during fire. It may also be due to the preservation of a microbial source or accumulation of new carbohydrates by the formation of microbial biomass post-fire. Similar results were discussed by Murayama [61], although the formation of microbial biomass in fire affected soils was suggested based on laboratory incubation experiments. Consequently, four years after fire, the oPOM<sub>c</sub> fraction still retained the most severe effects of the fire regarding the chemical components and changes of the non-cellulosic sugars' composition.

## 5. Conclusions

After four years post-fire, changes were detected in the organic matter quality of oPOM from soils under mixed *Araucaria–Nothofagus* spp. forests. The extent of this change based upon fire severity depends on an increase in aromaticity at the expense of O-alkyl C and alkyl C. However, the oPOM fraction, often neglected in fire affected soils, needs more consideration due to its important role in soil aggregation and as a source of fresh energy for soil microorganisms.

The decrease in carbohydrates, either by a transformation into aromatic compounds or by a reduction of litter and vegetation during fire event, results in a decline in SOM input and consequently in a diminution of both oPOM fractions, especially the oPOM<sub>c</sub>. Thus, fire did not only alter the bulk SOM distribution and composition but also the quality of the binding agent, which has consequences for aggregate stability and post-fire erosion.

The G+M/X+A ratios reveal a predominantly microbial origin in areas affected by medium to high severity of fire, over a plant origin in areas not affected by fire or affected by low severity fire. The pentose ratio from a high severity of fire could not be calculated, due to the loss and reduction of litter and vegetation during the fire event.

Low severity fire did not change the soil chemical and microbial properties and they recovered quickly after fire. The lower ratios of sugar in unaffected areas indicates that plant-derived carbohydrates play a key role rather than microbial carbohydrates.

The origin and contribution of individual monosaccharides relative to the total carbohydrate pool may suggest a good and sensitive indicator of organic matter origin and of ecosystems' recovery after fire.

The results demonstrate that wildfire decreases not only the amount of oPOM, C and F fractions, but that it also has an impact on their quality of SOM.

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