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Abundance and composition of free and aggregate-occluded carbohydrates and lignin in two forest soils as affected by wildfires of different severity

Giovanni Mastrolonardo[a, b, *]

giovanni.mastrolonardo@gmail.com

Cornelia Rumpel[b]

Claudia Forte[c]

Stefan H. Doerr[d]

Giacomo Certini[a]

[a]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Università degli Studi di Firenze, ~~Piazzale delle Cascine 28, 50144~~ Firenze, Italy

[b]CNRS, Institute of Ecology and Enviroment Paris (IEES, UMR Université Paris VI et XII – CNRS – IRD), Campus AgraParisTech, Thiverval–Grignon, France

[c]Istituto di Chimica dei Composti OrganoMetallici (ICCOM), UOS Pisa, CNR, Pisa, Italy

[d]Geography Department, Swansea University, Singleton Park, Swansea SA28PP, UK

*Corresponding author at: Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente (DISPAA), Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy.

Abstract

Organic matter is the soil component most affected by wildfires, both in terms of abundance and composition. Fire-induced alteration of soil organic matter (SOM) depends on heating intensity and duration, oxygen availability and other factors related to topography, climate, soil and vegetation features. Particularly affected by fire is the litter layer, but SOM from the uppermost mineral soil can also experience some major changes. In this study, we investigated the direct impact of fire on molecular SOM parameters in density fractions isolated from the top 2.5 cm of mineral soil in two forests that recently experienced wildfires of different severity. One, located in Tuscany, Central Italy, is a mixed forest of Downy oak and Maritime pine, developed on Acrisols formed on sandy lacustrine deposits, affected by a moderately severe fire. The other, located in Victoria, South-East Australia, is a mixed-species eucalypt forest, developed on a Cambisol formed on sandy Devonian sediments, affected by an extremely severe fire (the infamous 'Black Saturday' fire). The purpose of this study was the assessment of fire-induced changes on amount and composition of the bulk SOM and SOM associated to soil fractions having different ~~density~~densities. We used 1.8 Mg m^{-3} as density cut-off and distinguished between free and aggregate-occluded SOM. In particular, the analyses focused on abundance and composition of two major SOM components, proposed as molecular indicators of fire severity: the non-cellulosic neutral sugars, digested by trifluoroacetic acid (TFA), and the lignin-derived phenolic monomers, released by cupric oxide (CuO) oxidation. The chemical structure of both bulk SOM and SOM fractions were analysed by solid-state ^{13}C nuclear magnetic resonance spectroscopy.

In contrast to the moderately severe fire affecting the Italian site, the extremely severe fire at the Australian site caused substantial loss of SOM from the top mineral soil. Both fires had major effects on SOM composition. In spite of the evident impact they experienced, neither hydrolysable sugars nor lignin phenols resulted to be reliable indicators of fire severity. Moreover, both fires apparently broke up soil aggregates, hence promoting the release of some occluded organic matter. The fire-induced changes of SOM observed have implications for the C cycle, so highlighting the critical role of wildfire occurrence and severity in climate change.

Keywords: Soil organic matter; Soil aggregates; Non-cellulosic neutral sugars; Lignin; Soil density fractions; ^{13}C CPMAS NMR

1 Introduction

Fire is a major ecological factor, affecting more land surface than any other natural disturbance (Lavorel et al., 2007; Scott et al., 2014). Soils suffer many direct and indirect consequences of fire, which can involve the physical, mineralogical, chemical and biological properties, either temporarily or permanently (Bento-Gonçalves et al., 2012; Certini, 2005; Neary et al., 1999). The organic component of soil is the one most impacted by fire, in terms of both content and composition (González-Pérez et al., 2004; Certini et al., 2011). The assessment of the overall effect of fire on soil organic matter (SOM) is a complex task because burnt soils are generally a patchwork of areas affected by burning to different degrees. Actually, besides the factors correlated with site, soil and vegetation features, differences in heating intensity and duration, oxygen availability and type of combustion (smouldering or flaming), may induce different SOM transformations

(González-Pérez et al., 2004; Rumpel et al., 2007; Shakesby and Doerr, 2006). Most often fire causes a substantial reduction or even complete removal of the litter layer (Bento-Gonçalves et al., 2012; Certini et al., 2011; Nave et al., 2011). However, inconsistent results regarding quantitative changes in the mineral soil are reported in the literature. For instance, meta-analyses by Johnson and Curtis (2001) and Nave et al. (2011) have contrasting outcomes, concluding that, in the short-term, the A horizon does experience, respectively, a significant fire-induced increase or decrease in C content. However, divergent results from different studies can arise from methodological discrepancies, related to sampling strategy, including soil depth interval considered, the time elapsed since fire, as well as local conditions, as for example vegetation/climate type.

In terms of SOM quality, the reactions that occur during combustion, i.e., dehydration, dehydrogenation, volatilisation of nitrogenous compounds, decarboxylation, demethylation, demethoxylation, cyclisation and polycondensation (Hernández et al., 1997; Knicker, 2007), can substantially change the composition of the parent material. Charcoal formation is the most common outcome of wildfires and essentially represents the temperature and oxygen-depletion dependent transformation of the organic compounds into highly recalcitrant aromatic structures (Alexis et al., 2010). Once incorporated into the soil, charcoal may resist decomposition for centuries or even millennia, thereby sequestering carbon (Egli et al., 2012; Kuhlbusch and Crutzen, 1995; Schmidt and Noack, 2000). Carbohydrates are believed to be among the components of SOM most easily affected by fire (Certini, 2005; González-Pérez et al., 2004; Knicker et al., 2006). On this basis, Martín et al. (2009) proposed the ratio of carbohydrates to total SOM as an index of fire impact on SOM quality. In principle, such an index also allows low and high soil burn severity wildfires to be differentiated. Lignin, which, after carbohydrates, is the second most abundant component of plant residues in terrestrial ecosystems, is rather resistant to fire and is totally oxidised only at 400–450 °C (DeBano, 1991; Kuo et al., 2008). Nevertheless, despite the high heat resistance of its backbone (Knicker, 2011; Sharma et al., 2004), lignin is affected by fire at much lower temperatures (200–250 °C), at least in terms of the distribution of phenols (Certini et al., 2011; Nocentini et al., 2010; Rumpel et al., 2007). Therefore, the phenolic composition of lignin has potential as an indicator of fire occurrence and severity. Quantifying post-fire lignin phenols and sugars in soil might be useful to evaluate the wider impact of fire on soil quality.

In the mineral soil, the effects of fire are usually confined to the top few centimeters because of the low thermal conductivity of both minerals and air in pore spaces. Therefore, it is important to avoid sampling at substantial depths, because this is likely to result in a dilution of the fire effects on soil.

In this study, we investigated the top 2.5 cm of mineral soil of two forests, located in Italy and Australia, which have been affected by recent wildfires of moderate and extreme severity, respectively. The purpose of using two contrasting sites in terms of forest type and fire severity was to assess changes to SOM quality resulting directly from the fire and explore its implications. We focused particularly on the non-cellulosic neutral sugars, i.e., those digested by trifluoroacetic acid (TFA), and lignin-derived phenolic monomers, i.e., those released by cupric oxide (CuO) oxidation. The changes SOM experienced at the two sites were compared and contrasted, hypothesising common fire-related alterations in SOM mainly driven by fire severity. Furthermore, we analysed the quantity and composition of SOM allocated to soil fractions having different densitydensities, distinguishing between free and aggregate-occluded SOM.

2 Materials and methods

2.1 Study sites

The study sites awere Orentano, 30 km east of Pisa, Tuscany, Central Italy, and Mount Gordon, near Marysville, in Victoria State, South-East Australia (Fig. 1; Table 1).



Fig. 1 The study sites as they appeared during the sampling: a) Orentano, two days after the fire, and b) Mount Gordon, two months after the fire.

Table 1 Location and soil pH, bulk density (mean and standard deviation; *n* = 3) and particle size distribution of the burnt and the unburnt soils, sampled at 2.5 cm depth, from Orentano and Mount Gordon study sites.

Site (code)	Sampling area	Location WGS84	Soil pH (H_2O, 5:1)	Bulk density Mg m^{-3}	Sand g kg^{-1}	Silt g kg^{-1}	Clay g kg^{-1}
Orentano (OR) Italy	Burnt	43°47′22.82″N	5.12 (± 0.37)	0.98 (± 0.07)	110	710	170

Italy		10°39′52.30″E					
	Unburnt	43°47′25.94″N	4.22 (± 0.32)	1.11 (± 0.17)	160	660	190
		10°39′50.35″E					
Mount Gordon (MG) Australia	Burnt	37°31′56.30″S	5.90 (± 0.37)	0.81 (± 0.06)[a]	290	520	180
		145°43′17.14″E					
	Unburnt	37°32′54.10″S	4.83 (± 0.03)	0.64 (± 0.14)[a]	330	440	220
		145°37′37.30″E					

[a] *n* = 2 for mean and standard deviation values.

Orentano (hereafter called OR), 20 m a.s.l., has a mean annual precipitation of 893 mm and a mean annual temperature of 14.3 °C. The vegetation cover is a mixed forest of Downy oak (*Quercus pubescentis* Willd) and Maritime pine (*Pinus pinaster* Aiton) with a rich understory of common fern (*Pteridium aquilinum* L.) and *Rubus* spp. Soils formed on sand and stony lacustrine deposits – where quartz is largely dominant and chlorite, illite, kaolinite, and goethite are accessory minerals – and are classified as Haplic Skeletic Acrisols according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). In July 2011, an area of 3.3 ha underwent a wildfire of moderate severity, based on the visual scale of litter and vegetation consumption proposed by Chafer et al. (2004). Most of the tree stems were still standing after the fire and were partly or totally scorched. The soil was entirely covered by charcoal and ash, with no or very little uncharred litter remaining. Soil sampling was carried out three days after the fire on both the burnt area (coordinates WGS84: 43°47′22.82″N, 10°39′52.30″E) and an adjacent (50 ~~meters~~m away) unburnt area having the same characteristics of the burnt one prior to fire occurrence, hence used as control.

At Mount Gordon (hereafter called MG) the sampling area is located 530 m a.s.l., where mean annual precipitation is 670 mm and mean annual temperature is 13 °C. The site was chosen because it represented an end-member in terms of fire severity. The sadly famous 'Black Saturday' fire, which involved also MG, in early February 2009, burned about 450,000 ha of eucalypt forest causing the loss of 173 lives (Royal Commission, 2009). Average fire-line intensity is estimated to have exceeded 70,000–80,000 kW m^{-1}, which is ~~amongst~~ among the highest ever reported in Australia (Royal Commission, 2009). The extreme fire intensity was promoted by particularly extreme weather conditions, such as wind speeds up to 100 km h^{-1} and air temperatures even exceeding 45 °C. Fuel loads were very high, since the forest had not experienced a major fire since 1939 (fuel load, including the litter, amounted to 25–40 Mg ha^{-1}), and fuel moisture was very low (3–4%) because of prolonged drought conditions (McCaw et al., 2009). The sampling site (37°31′56.30″S, 145°43′17.14″E) is a *Eucalyptus* spp. mixed forest 3 km SW of Marysville on the road to Narbethong. The fire removed all ground fuel, green vegetation and woody stems < 10 mm in diameter; accordingly, fire severity was classified as extreme, based on the classification of Chafer et al. (2004). A long unburnt site, approximately 3 km NW of Narbethong (37°32′54.10″S, 145°37′37.30″E) – last burn~~ed~~ by wildfire in 1939 – was selected as control. This site is 8.5 km away from the burnt site and as much as possible similar to the latter in terms of all environmental conditions, soil included. Soils of the area formed on sandy Devonian sediments – where quartz largely prevails and the clay-size fraction comprises vermiculite, illite, and kaolinite – and are classified as Dystric Humic Cambisols according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2014). Sampling was performed in April 2009, two months after the fire and following some light rainfall, but before the more intense winter precipitations had caused significant ash removal via erosion.

2.2 Soil sampling

At both OR and MG study sites the sampling involved four parallel 20 m transects, 5 m apart, at 5 m intervals. Twenty mineral soil samples were taken at each site down to 2.5 cm, after carefully removing the ash, charcoal, or any litter layer by a brush. In both burnt areas, ten samples of charcoal particles were randomly collected in 40 × 40 cm plots.

2.3 Physico-chemical properties

Soil pH was measured potentiometrically using deionised water to soil ratio of 5:1, while particle size analysis was performed according to the hydrometer method. Total C and N contents and stable carbon isotopic composition of the fine earth (the less than 2 mm soil fraction) and charcoal were measured by a Carlo Erba NA1500 elemental analyser coupled to an isotope ratio mass spectrometer (Micromass-Optima). δ^{13} C isotope abundance was reported in per mil (‰) relative to the Pee Dee Belemnite standard (PDB).

2.4 Density fractionation

Density fractionation was carried out on two composite soil samples per site, both obtained combining equal aliquots of ten distinct samples of fine earth. The procedure was based on the method of Golchin et al. (1994), modified according to Sohi et al. (2001) and Cerli et al. (2009). This procedure allows different fractions, related to the spatial arrangement and interactions of organic compounds with minerals, to be separated (Cerli et al., 2012). The method requires a sodium polytungstate (NaPT) solution adjusted at a specific density to isolate a *free light fraction* (f-LF). By addition of the same solution to the precipitated material, followed by ultrasonic dispersion to break down the aggregates, the *occluded light fraction* (o-LF) is separated from the *heavy fraction* (HF), the latter fraction mainly comprising minerals. We performed preliminary trials in order to determine the most suitable density cut off and sonication energy for the fractionation. We first used the density and sonication intensities most commonly found in the

literature, i.e., 1.6 and 1.8 Mg m^{-3} and 100 and 300 J cm^{-3}, respectively (Cerli et al., 2009, 2012; Golchin et al., 1997; Kiem and Kögel-Knabner, 2003), with the ultrasonic energy being calibrated calorimetrically according to Schmidt et al. (1999). Based on the criterion of the highest SOM concentration of the o-LF, i.e., the higher release of the o-LF with the smallest mineral "contamination" (data not shown), we selected 1.8 Mg m^{-3} and 300 J cm^{-3} for soils from both study areas. Hereafter the soil samples were fractionated according to the following procedure: 125 cm^3 of 1.8 Mg m^{-3} dense NaPT solution ~~were~~ was added to 25 g of soil, gently shaken and allowed to stand for ~~one~~ 1 ~~hour~~h. After centrifugation at 6800 *g* for 20 min, the suspended material (f-LF < 1.8 Mg m^{-3}) was separated from the supernatant and filtered on a glass fibre filter (cut-off 0.7 µm) for washing away any residual NaPT. The precipitated material was ultrasonically dispersed in NaPT solution with the same density (soil to solution ratio 1:5) by applying 300 J cm^{-3} and allowed to stand for ~~one~~ 1 ~~hour~~h. After centrifugation at 6800 *g* for 20 min, the o-LF < 1.8 Mg m^{-3} was recovered as described above. All three fractions obtained were repeatedly washed with deionised water until the electrical conductivity of the supernatant was < 50 µS cm^{-1}. They were then oven dried at 50 °C, weighed and analysed for their C and N content. Apart from yielding functionally more homogeneous fractions with a narrower range of properties compared to the bulk soil, this type of density fractionation allows free SOM and SOM associated with minerals and/or physically protected in~~to~~ aggregates to be distinguished.

2.5 Lignin phenols determination

There is at present no method capable of reliably quantifying the total lignin content in soil. Even the cupric oxide (CuO) oxidation, which is perhaps the most widely used method for this purpose, does not succeed in completely depolymerising lignin; hence, it cannot be considered a strictly quantitative method (Kögel, 1986). Nevertheless, CuO oxidation is able to release phenolic monomers and dimers from the end groups of the lignin macromolecules and, therefore, it is a reliable indicator of lignin amount and composition in soil (Otto and Simpson, 2006; Spielvogel et al., 2007; Thevenot et al., 2010). On this basis, we submitted the bulk soil and density fractions to CuO oxidation according to the modified method proposed by Kögel and Bochter (1985) and Kögel-Knabner (1995). Briefly, 50–200 mg of sample (the higher the C concentration, the lower the quantity of sample), 250 mg CuO, 50 mg of glucose, 50 mg of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 15 cm^3 of 2 *M* NaOH were digested in a Teflon pot at 172 °C under N_2 atmosphere for 2 h. Afterwards, ethyl vanillin was added as an internal standard to assess the recovery of lignin products. The solution was adjusted to pH 1.8–2.2 and left overnight for humic acid precipitation. The lignin-derived phenols were then purified by elution through a C_{18} column (International Sorbent Technology) and extracted from the column by adding 5 × 0.5 cm^3 (2.5 cm^3 in total) ethyl acetate. After evaporating ethyl acetate under N_2 flow, the dried residue was dissolved in pyridine containing phenylacetic acid as internal quantification standard, and then derivatised by adding BSTFA [*N*, *O*-bis(trimethylsilyl)trifluoro-acetamide]. The silylated lignin monomers were separated using a HP 6890 gas chromatograph (GC) equipped with a SGE BPX-5 column (65 m × 0.32 mm internal diameter, 0.25 µm film thickness) and a flame ionization detector (FID). The GC oven temperature program was: 100 °C (2 min) to 172 °C at 8 °C min^{-1}, to 184 °C at 4 °C min^{-1}, to 300 °C at 10 °C min^{-1} and isothermal at 310 °C for 5 min. Helium was used as carrier gas and samples were injected in split mode (1:10). CuO oxidation products are composed of vanillyl (V)-units (vanillin, acetovanillone, vanillic acid), syringyl (S)-units (syringaldehyde, acetosyringone, syringic acid), and cinnamyl (C)-units (ferulic and *p*-coumaric acids). The sum of V-, S- and C-type phenols (VSC) was used to estimate the total amount of lignin. Ac/Al_V and Ac/Al_S are the mass ratios of acid-to-aldehyde for vanillyl and syringyl units, respectively. S/V is the mass ratio of syringyl to vanillyl units and C/V is the mass ratio of cinnamyl to vanillyl units. These mass ratios are generally used to assess the state of degradation of lignin, since the Ac/Al ratios increase while S/V and C/V ratios decrease with increasing decomposition. Fire was shown to immediately produce a degraded lignin signature for pure organic matter, increasing the acid-to-aldehyde ratio of V- and S-type units (Nocentini et al., 2010; Rumpel et al., 2007), partly mimicking the effect of microbial degradation.

2.6 Non-cellulosic neutral sugars determination

Sugar monomers were determined according to the method proposed by Amelung et al. (1996), as modified by Rumpel and Dignac (2006). The analysis was performed on both bulk soil and density fractions. Briefly, 200–500 mg of soil, depending on C concentration, ~~were~~ was hydrolysed with 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h. After the samples had cooled down, 0.5 cm^3 of myoinositol (concentration 2 mg L^{-1}) was added as internal standard. Thereafter, the hydrolysed samples were purified by filtration over a glass fibre membrane (cut-off 1.2 µm) and dried using a rotary evaporator (58 °C). Ethylenediaminetetraacetic acid (EDTA) was added, according to Eder et al. (2010), to keep iron in solution in a non-reactive form to avoid possible co-precipitation of dissolved organic carbon. Derivatisation of the samples was carried out in screw top test tubes. Aldoses were reduced to their corresponding alditols after addition of 1 cm^3 $NaBH_4$ dissolved in dimethyl sulfoxide (DMSO). Acetylation was carried out by adding 2 cm^3 acetic anhydride and 2 cm^3 glacial acetic acid, using methylimidazole (2 cm^3) as catalyst. The reaction was stopped after 10 min by 7 cm^3 ice-cold ~~deionized~~ deionised water, which transformed acetic anhydride to acetic acid. The derivatised sugar monomers were extracted by liquid–liquid extraction with 1 cm^3 dichloromethane using a vortex mixer. After the phase separation, the darker organic lower phase was removed with a Pasteur pipette and transferred into a GC vial. The analyses were performed by a HP 6890 gas chromatograph (GC) equipped with a SGE BPX-70 column (60 m × 0.32 mm internal diameter, 0.25 µm film thickness) and a FID. The GC oven temperature program was: 200 °C to 250 °C at 8° C min^{-1} and isothermal at 250 °C for 15 min. Helium was used as carrier gas and samples were injected in split mode (1:10).

The TFA digests the monosaccharides originated from plant-derived hemicelluloses and microbial products, while it is not able to digest crystalline cellulose (Guggenberger et al., 1994). Hence, hereafter, we will use the term sugars to indicate the non-cellulosic neutral polysaccharides. In particular, the sugar monomers detected by this method are: rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose. Fructose is transformed into the same alditol as glucose during the reduction step (Rumpel and Dignac, 2006); however, the fructose content in soil is so low that its contribution can be neglected (Amelung et al., 1996). The concentration of individual sugar monomers was calculated based upon the internal standard myoinositol.

According to Oades (1984), the proportion of microorganism-derived sugars in relation to plant-derived sugars can be roughly estimated from the mass ratio of hexoses/pentoses sugars: (galactose + mannose)/(arabinose + xylose), hereafter called GM/AX. Low (< 0.5) and high (> 2) GM/AX ratios are peculiar of carbohydrates predominantly derived from plants and microorganisms, respectively (Oades, 1984).

2.7 Solid-state ^{13}C NMR spectroscopy

Solid-state ^{13}C cross polarisation with magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectroscopy was used to analyse the bulk soil and the density fractions, with the exception of the heavy one (HF), which was too poor in C to provide reliable information (Mastrolonardo et al., 2013). The specimens subjected to NMR analysis were obtained combining equal aliquots of all independent samples collected from each site. Before ^{13}C NMR analysis, the combined samples were treated with 2% hydrofluoric acid, as described by Skjemstad et al. (1994), to remove paramagnetic minerals, which strongly reduce the signal-to-noise ratio of the spectra. NMR spectra were recorded on a Bruker AMX300-WB spectrometer, working at 75.47 MHz and equipped with a 4 mm CPMAS probehead. The spectra were recorded with a contact time of 2 ms under proton decoupling conditions, and with a spinning rate of 8 kHz. The ^{1}H 90° pulse length was 3.4 μs, the spin-lock field 72 kHz, and the recycle delay 4 s. From 4000 to 40,000 scans were acquired depending on the sample. The chemical shifts were referenced to tetramethylsilane (TMS) using adamantane as external standard.

The contribution of the main C forms to the total spectral intensity was determined by integration of the corresponding chemical shift regions: 0 to 45 ppm (alkyl C), 45 to 110 ppm (*O*-alkyl C, subdivided in methoxyl/*N*-alkyl C, 45–60 ppm; *O*-alkyl C, 60–90 ppm; di-*O*-alkyl C, 90–110 ppm), 110 to 165 ppm (aryl C, subdivided in aromatic C–H and C–C, 110–140 ppm; aromatic O-substituted C, 140–165 ppm), 165 to 185 ppm (carboxyl C); no signals arising from aldehydes or ketones were observed in the 185 to 220 ppm region.

2.8 Statistics

Data from burnt and unburnt soils were compared by two-tailed paired *t*-test at 95% confidence level (SigmaPlot 12.0). Where data did not show a normal distribution (Shapiro–Wilk test), Spearman rank correlation was used.

3 Results and discussion

3.1 Carbon and nitrogen in the bulk soil

The measured pH values, all below 6, demonstrated that the two soils were carbonate-free, and, hence, that the measured C was entirely in organic forms. At OR, where fire severity was moderate, the concentrations of carbon and nitrogen, and consequently the C/N ratios, did not change significantly between the unburnt and burnt soil (Table 2). Perhaps, the fire-induced loss in SOM, if any, was counterbalanced by the input of organic residues, including charcoal, from aboveground biomass and litter. Doing a meta-analysis, Johnson and Curtis (2001) found a significant increase in soil C in the A horizon of forest soils burnt less than 10 years earlier. They attributed such an increase, at least partly, to accumulation on the ground of unburnt and charred residues. The incorporation of fresh charcoal into the mineral soil at OR was supported by the lower $\delta^{13}C$ value of the burnt soil compared to the unburnt one (Table 2), since charcoal had lower $\delta^{13}C$ than unburnt soil (– 28.5‰ in charcoal versus – 27.4‰ in unburnt soil).

Table 2 Elemental analysis, of charcoal, burnt and unburnt soils (mean values and standard deviation; *n* = 20 for bulk soil samples; *n* = 10 for charcoal samples; *n* = 2 for density fractions).

Site (code)	Sample	Density fraction	% of bulk soil weight	C org g kg^{-1}	N g kg^{-1}	C/N	$\delta^{13}C$ ‰
Orentano (OR) Italy	Burnt soil	Bulk	–	100 (± 34)	5.8 (± 2.0)	17.2 (± 1.7)	– 27.8 (± 0.3)*
		f-LF	15.0 (± 0.6)	216 (± 2)	12.0 (± 0.2)	18.1 (± 0.4)	– 27.9 (± 0.1)
		o-LF	6.5 (± 1.1)	415 (± 20)	21.0 (± 0.6)	19.8 (± 0.4)	– 28.1 (± 0.1)
		HF	78.5 (± 1.7)	31 (± 3)	2.5 (± 0.2)	12.8 (± 0.4)	– 27.3 (± 0.2)
	Unburnt soil	Bulk	–	84 (± 27)	5.2 (± 2.1)	17.0 (± 3.7)	– 27.5 (± 0.3)*
		f-LF	9.1 (± 2.0)	192 (± 14)	10.7 (± 2.5)	18.3 (± 2.9)	– 27.7 (± 0.1)
		o-LF	9.3 (± 2.4)	304 (± 130)	13.9 (± 3.4)	21.3 (± 4.1)	– 28.0 (± 0.1)
		HF	81.5 (± 0.4)	24 (± 1)	2.0 (± 0.4)	12.1 (± 2.0)	– 27.1 (± 0.8)
	Charcoal	–	–	338 (± 62)	16.1 (± 2.6)	20.7 (± 3.6)	– 28.6 (± 0.5)
Mount Gordon (MG) Australia	Burnt soil	Bulk	–	87 (± 13)**	4.2 (± 0.5)**	20.6 (± 2.1)**	– 27.5 (± 0.2)**
		f-LF	9.0 (± 4.0)	171 (± 9)*	7.1 (± 0.4)	24.2 (± 0.2)	– 27.7 (± 0.1)

		o-LF	7.6 (± 0.9)	324 (± 33)	9.6 (± 0.0)	33.7 (± 3.7)	– 27.7 (± 0.1)
		HF	83.4 (± 4.9)	40 (± 3)	2.8 (± 0.1)	14.3 (± 0.6)	– 27.3 (± 0.0)
	Unburnt soil	Bulk		163 (± 55)**	5.8 (± 0.1)**	27.5 (± 3.4)**	– 27.7 (± 0.2)**
		f-LF	20.9 (± 8.0)	252 (± 11)*	8.3 (± 0.1)	30.3 (± 0.8)	– 28.0 (± 0.1)
		o-LF	11.1 (± 1.4)	233 (± 16)	6.6 (± 0.4)	35.5 (± 0.2)	– 28.1 (± 0.3)
		HF	68.0 (± 9.4)	54 (± 19)	3.2 (± 0.5)	16.9 (± 3.2)	– 27.3 (± 0.3)
	Charcoal			196 (± 52)	6.0 (± 1.4)	28.5 (± 9.9)	– 27.4 (± 0.5)

* Significantly different means between burnt and unburnt soil ($P < 0.05$).

** Significantly different means between burnt and unburnt soil ($P < 0.01$).

At MG, where fire severity was extremely high, the burnt soil exhibited substantially lower C and N concentrations compared to the unburnt one: 88 and 164 g C kg^{-1}, and 4.3 and 5.8 g N kg^{-1}, respectively (Table 2). Such fire-induced losses are out of range when compared to data reported by Nave et al. (2011) in their meta-analysis. Evidently, the addition of any charred material from the aboveground biomass at MG was not sufficient to counterbalance the large loss of SOM, although the surface ash layer removed during sampling to expose the mineral soil was considerable (1.7 cm thick on average) and contained substantial amounts of C (62 g kg^{-1}), mainly in the form of charcoal (Santín et al., 2012). The ash layer usually does not remain on hillslopes for a long time because of wind and water erosion (Bodí et al., 2014; Rumpel et al., 2009), but it is conceivable that, in the course of time, at least some of the C it retains will be incorporated into the mineral soil. The C/N ratio experienced a significant fire-induced decrease, which may be due to an accumulation of recalcitrant organic N-forms in the charred material (Almendros et al., 2003; González-Pérez et al., 2004; Mastrolonardo et al., 2014; Santín et al., 2008).

3.2 Carbon and nitrogen in density fractions

The C and N concentrations in soil density fractions are given in Table 2. Light fractions, f- and o-LF, are generally assumed to mainly comprise plant debris, ancillary animal residues, charcoal and microorganisms colonising organic residues (Golchin et al., 1994; Wagai et al., 2009). The main differences between the two fractions consist, generally, in particle size and location within the soil matrix: the f-LF is assumed to feature larger almost undecomposed organic materials, while the o-LF should comprise finer and more altered organic particles (Cerli et al., 2012; Golchin et al., 1994).

The C/N ratio in density fractions of the unburnt soil at both OR and MG supports the expectation that SOM ranges between the less degraded light fractions, having a higher C/N, to the comparably more decomposed heavy fraction showing a lower C/N. These considerations are consistent with the higher $^{13}\delta$ C values measured for HF compared to the light fractions, supporting the hypothesis of a higher decomposition of the former fraction (Roscoe et al., 2001). In spite of the low C and N concentrations, but thanks to its large relative mass (Table 2), HF stores one third of total SOC and almost half of total soil N (Fig. 2).

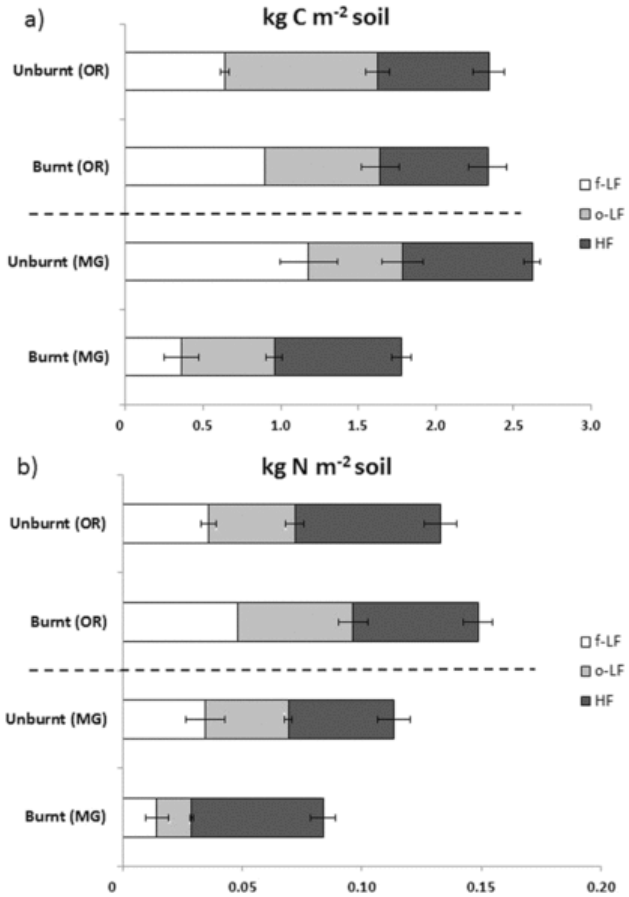

Fig. 2 Soil C and N ~~content~~stocks distribution among density fractions.

Comparing the density fractions from burnt and unburnt soils, we could infer if and how the aggregates were able to protect SOM from heating and if they collapsed as a consequence of fire. As reviewed by Mataix-Solera et al. (2011), the response of soil aggregates to heating by fire is highly variable. Fire can oxidise organic binding agents in aggregates thereby causing their breakdown. Alternatively, a fast vaporisation of the water included within aggregates can lead to their destruction in a similar way as slacking does (Albalasmeh et al., 2013). However, under certain conditions, i.e., for wettable soils with SOM as main binding agent subject to low severity fires, aggregate stability may improve as a consequence of enhanced soil water repellency (Mataix-Solera and Doerr, 2004).

At OR, fire apparently caused an increase in C and N stock and concentration in f-LF (Table 2; Fig. 2). This increase was probably due to the incorporation of some charred residues into the top centimetres of mineral soil and the charring of part of SOM there present. The C stock in the o-LF was slightly lower in the burnt soil compared to the unburnt one, although the C and N concentrations were actually higher (Fig. 2; Table 2). This led us to hypothesise that fire could have caused a partial disruption of aggregates, with a consequent release of mineral particles, with no or little interaction with OM, and OM free particles, which may have become part of the HF and the f-LF, respectively (Fig. 3). The SOM thus released might be more exposed to decomposition because ~~they are~~ it ~~would be~~ is probably more easily accessible by microorganisms and their enzymes. The same phenomenon could have also occurred at MG, here resulting in an overall increase of the HF (Table 2). However, at MG the most affected fraction was the f-LF, which experienced depletion in the C and N content (Table 2; Fig. 2).

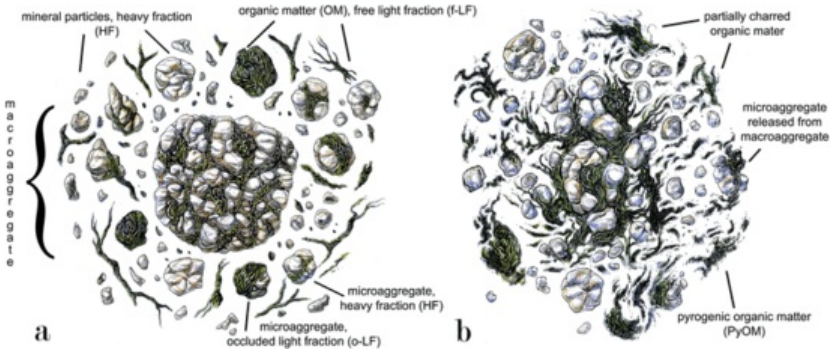

Fig. 3 a) Sketch of a soil macroaggregate and the density fractions of SOM: free light fraction (f-LF), occluded light fraction (o-LF) and the heavy fraction (HF); b) Conceptual model of fire impact on soil organic matter free and occluded in soil aggregates (picture by G. Borgogni).

3.3 Soil ~~polysaccharides~~ polysaccharide content

At OR, neutral sugars in the burnt and unburnt soils amounted to 7.4 and 8.2 g kg^{-1}, respectively, which correspond to 76 and 97 g kg^{-1} of SOC (Table 3), i.e., in the range of data reported by other authors for forest soils (e.g., Guggenberger et al., 1994; Rumpel and Dignac, 2006). The apparent fire-induced decrease in neutral sugars was statistically significant if referred to total SOC, but not in absolute terms. The GM/AX ratio before fire occurrence was > 2, indicating, roughly, that sugars were synthetized mainly by microbial population (Guggenberger and Zech, 1994; Oades, 1984). Fire did not change this ratio indicating that, in principle, neither plant- nor microbial-derived sugars were preferentially affected by fire.

Table 3 Non-cellulosic neutral sugars content and (galactose + mannose)/(arabinose + xylose) ratio (GM/AX) of burnt and unburnt bulk soil and density fractions (mean values and standard deviation; $n = 4$ for bulk soil; $n = 1$ for density fractions).

Site (code)	Sample	Density fraction	Σ Neutral Sugars g kg^{-1} bulk soil[a]	Σ Neutral Sugars g kg^{-1} OC	GM/AX
Orentano (OR) ~~Italy~~Italy	Burnt soil	Bulk	7.46 (± 0.43)	76.6 (± 6.6)*	2.79 (± 0.90)
		f-LF	1.13	111.2	1.28
		o-LF	0.74	86.1	1.24
		HF	4.88	178.9	1.67
	Unburnt soil	Bulk	8.27 (± 2.30)	98.6 (± 13.0)*	2.81 (± 1.50)
		f-LF	1.11	119.7	1.55
		o-LF	0.70	54.6	1.49
		HF	2.50	133.1	2.55
Mount Gordon (MG) Australia	Burnt soil	Bulk	9.18 (± 1.90)*	105.2 (± 26.2)	2.00 (± 0.44)
		f-LF	0.61	88.0	1.42
		o-LF	0.45	52.2	1.30
		HF	3.40	99.1	2.10
	Unburnt soil	Bulk	20.92 (± 6.18)*	126.1 (± 26.4)	1.60 (± 0.18)
		f-LF	5.12	137.6	1.33
		o-LF	0.65	77.9	1.16

	HF	5.01	160.8	2.69	

* Significantly different means between burnt and unburnt soil ($P < 0.05$).

[a] For density fractions, net amount of sugars was calculated by multiplying the sugar content of a given fraction by the mass proportion (% of bulk soil weight) of that fraction.

The SOC normalised sugar content slightly decreased due to fire in the f-LF, while in the o-LF and HF it increased (Table 3). A possible explanation for this result could be that carbohydrates interacting with soil minerals and/or incorporated into stable aggregates were relatively preserved compared to the other OM compounds. In fact, it is commonly reported that sugars of microbial origin contribute to the formation and stabilisation of soil aggregates, hence benefiting from physical protection (Kiem and Kögel-Knabner, 2003; Martín et al., 2009; Rumpel et al., 2010). Moreover, they seem to be chemically stabilised by interaction with the mineral phase (Rumpel et al., 2010).

At MG, the absolute concentration of non-cellulosic neutral polysaccharides in the bulk soil was substantially lower in the burnt area than in the unburnt one: 9 and 21 g kg^{-1}, respectively. This apparent decrease, however, was not significant if sugar content was normalised to SOC (Table 3). This suggests that the polysaccharides present in the mineral soil were not preferentially affected by fire, despite being part of the most thermally labile SOM pool (De la Rosa et al., 2008). However, it must be pointed out that the method here used does not detect cellulose C, which may have a different behaviour towards fire compared to non-cellulosic sugars. As observed for OR, the GM/AX ratio at MG indicates approximately that carbohydrates were mainly originated from microorganisms and the ratio did not change after fire occurrence. Most of the sugars were stored in the f-LF and were apparently considerably reduced as a consequence of fire in all fractions, both in absolute terms (normalised to mass proportion of density fractions) and relative to SOC (Table 3).

3.4 Soil lignin content

At OR, the yield of phenolic CuO oxidation products in the burnt soil was significantly lower than in the unburnt one, both in absolute terms and relative to SOC (Table 4). This suggests that lignin was somehow preferentially affected by fire, despite its assumed moderate recalcitrance to heating (Knicker et al., 2005). Fire apparently left the VSC content of SOC associated to HF almost unaltered, while it affected the VSC content of the light fractions, particularly o-LF, both in absolute value and relative to SOC. Therefore, occlusion into aggregates does not seem to guarantee lignin protection against fire. Actually, thermal degradation of lignin polymers in aggregates could be favoured by inorganic catalysts, such as acidic clay minerals (Ohta and Venkatesan, 1992).

Table 4 Sum of vanillyl, syringyl and cinnamyl units (VSC), acid-to-aldehyde ratio of vanillyl and syringyl units, cinnamyl phenols and syringyl phenols-to-vanillyl phenols, in the bulk soil and density fractions (mean values and standard deviation; $n = 4$ for soil samples; $n = 2$ for density fractions).

Site (code)	Sample	Density fraction	Σ VSC g kg^{-1} bulk soil[a]	Σ VSC g kg^{-1} OC	$(Ac/Al)_v$	$(Ac/Al)_s$	C/V	S/V
Orentano (OR) ~~Itlay~~Italy	Burnt soil	Bulk	0.35 (± 0.01)**	3.57 (± 0.18)**	0.74 (± 0.37)	0.78 (± 0.13)	0.42 (± 0.13)	0.38 (± 0.22)
		f-LF	0.13 (± 0.08)	4.1 (± 2.8)	1.57 (± 2.19)	1.43 (± 1.05)	0.56 (± 0.12)	0.76 (± 0.12)
		o-LF	0.08 (± 0.02)	3.0 (± 0.4)	0.57 (± 0.77)	0.65 (± 0.10)	0.59 (± 0.39)	0.97 (± 0.45)
		HF	0.25 (± 0.15)	8.1 (± 1.7)	1.09 (± 0.13)	0.67 (± 0.09)	0.45 (± 0.10)	0.62 (± 0.06)
	Unburnt soil	Bulk	0.65 (± 0.14)**	7.71 (± 0.86)**	0.58 (± 0.22)	0.76 (± 0.32)	0.38 (± 0.09)	0.50 (± 0.11)
		f-LF	0.11 (± 0.01)	6.5 (± 1.8)	0.26 (± 0.14)	1.09 (± 0.78)	0.29 (± 0.10)	0.82 (± 0.08)
		o-LF	0.20 (± 0.09)	8.3 (± 5.2)	0.11 (± 0.12)	0.83 (± 0.52)	0.80 (± 0.04)	0.81 (± 0.13)
		HF	0.17 (± 0.02)	9.0 (± 1.4)	0.92 (± 0.02)	0.60 (± 0.03)	0.33 (± 0.00)	0.57 (± 0.01)
Mount Gordon (MG) Australia	Burnt soil	Bulk	0.65 (± 0.23)*	7.53 (± 2.97)	0.96 (± 0.14)	0.87 (± 0.57)	0.24 (± 0.10)	1.21 (± 0.32)
		f-LF	0.17 (± 0.09)	10.9 (± 1.8)	0.41 (± 0.29)	0.65 (± 0.33)	0.56 (± 0.05)	1.63 (± 0.82)
		o-LF	0.21 (± 0.14)	8.9 (± 5.9)	1.16 (± 0.42)	1.24 (± 0.91)	0.59 (± 0.43)	1.12 (± 0.88)
		HF	0.54 (± 0.25)	16.0 (± 7.1)	0.80 (± 0.28)	0.62 (± 0.17)	0.45 (± 0.13)	1.18 (± 0.14)
	Unburnt soil	Bulk	1.24 (± 0.38)*	7.52 (± 2.30)	1.05 (± 1.21)	0.75 (± 0.47)	0.32 (± 0.29)	1.96 (± 0.59)

		f-LF	0.38 (± 0.10)	11.6 (± 0.4)	1.41 (± 1.43)	7.82 (± 8.69)	0.29 (± 0.09)	1.24 (± 1.02)
		o-LF	0.29 (± 0.14)	21.4 (± 10.3)	0.23 (± 0.27)	6.73 (± 9.00)	0.80 (± 0.05)	1.63 (± 1.30)
		HF	0.46 (± 0.29)	10.1 (± 0.3)	0.80 (± 0.13)	0.52 (± 0.02)	0.33 (± 0.00)	1.44 (± 0.13)

* Significantly different means between burnt and unburnt soil ($P < 0.05$).

** Significantly different means between burnt and unburnt soil ($P < 0.01$).

[a] For density fractions, the net amount of VSC was calculated by multiplying the VSC content of a given fraction by the mass proportion (% of bulk soil weight) of that fraction.

At MG, the absolute yield of phenolic CuO oxidation monomers in the burnt bulk soil was half of that in the unburnt one (Table 4), but such a difference was not significant if values were referred to SOC. As in the case of sugars, lignin monomers did not appear to be preferentially affected by fire compared to other SOM constituents. In the unburnt soil, lignin was almost equally distributed among the different density fractions. Fire mainly affected the f-LF, causing a large decrease in its VSC content. The o-LF showed the highest lignin contribution to SOC and the highest apparent lignin loss as a consequence of fire, while HF even showed a relative accumulation of lignin compounds.

At both study sites, none of the indicators describing the composition and degradation of lignin, i.e., acid-to-aldehyde ratios of V and S-type units, S-to-V and C-to-V ratios, changed significantly in response to fire (Table 4). Hence, it seems that fire unselectively affected all lignin oxidation products, which is in contrast with the higher thermal susceptibility of aldehydes in V and S phenols reported in the literature (Certini et al., 2011; Kuo et al., 2008; Nocentini et al., 2010; Ohta and Venkatesan, 1992; Rumpel et al., 2007). Nevertheless, it must be pointed out that most of the aforementioned studies dealt with plant residues and charcoal in the litter layer, a much more homogenous materials than SOM in the top mineral soil. Consequently, when the analyses are focused on the latter, CuO oxidation products might not be sufficiently sensitive to fire severity. Plotting the sugar vs. lignin contents from burnt soils at OR and MG, we found a fairly good linear correlation that did not occur in the case of the unburnt soils (Fig. 4). This correlation probably depends on fire that, whatever its severity, could affect lignin and sugars leading to an overall decrease of both components. Although based on a relatively small sample size, the positive correlation here found deserves further investigation in future studies to ascertain its wider validity.

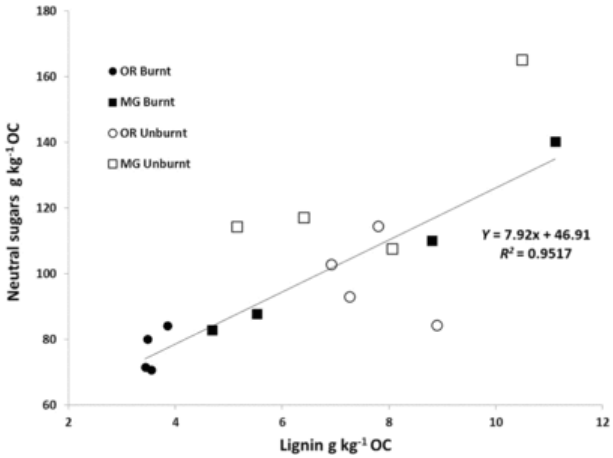

Fig. 4 Relationship between the OC-normalised contents of lignin and neutral sugars, in burnt and unburnt bulk soils from Orentano and Mount Gordon. A regression curve is provided for the burnt bulk soils.

3.5 NMR analysis

The ^{13}C CPMAS NMR spectra of bulk soil from burnt and unburnt areas at OR and MG are displayed in Fig. 5, while Table 5 shows the percent distribution of the total signal among the seven chemical shift regions commonly identified. The most evident difference between the burnt and unburnt soil specimens was the more intense signal of the former in the aromatic C region (110–160 ppm). This was clearly due to input of charred material, whose signal is centred at ~ 130 ppm (Skjemstad et al., 1996). At OR, this enrichment was counterbalanced by a decrease in alkyl-C (0–45 ppm region) and a less substantial decrease in *O*-alkyl C (60–90 ppm). The two sharp peaks at around 72 and 104 ppm in the burnt soil revealed the persistence of substantial amounts of polysaccharides, possibly cellulose. Therefore, contrarily to what is commonly found (e.g., Certini et al., 2011; Knicker et al., 2005, 2006), here *O*-alkyl C does not seem to be the most fire-affected C form. At MG, in spite of the extremely high fire severity, spectra did not show major differences, apart from the evident enrichment in aromatic C in the burnt soil. Both burnt and unburnt soils were characterised by dominant signals in the alkyl C region, generally assigned to lipids and other aliphatic compounds, and in the *O*-alkyl C region, indicative of polysaccharides and amide C of proteins (Knicker and Lüdemann, 1995). The decrease in intensities of these signals, plausibly attributable to fire, was modest.

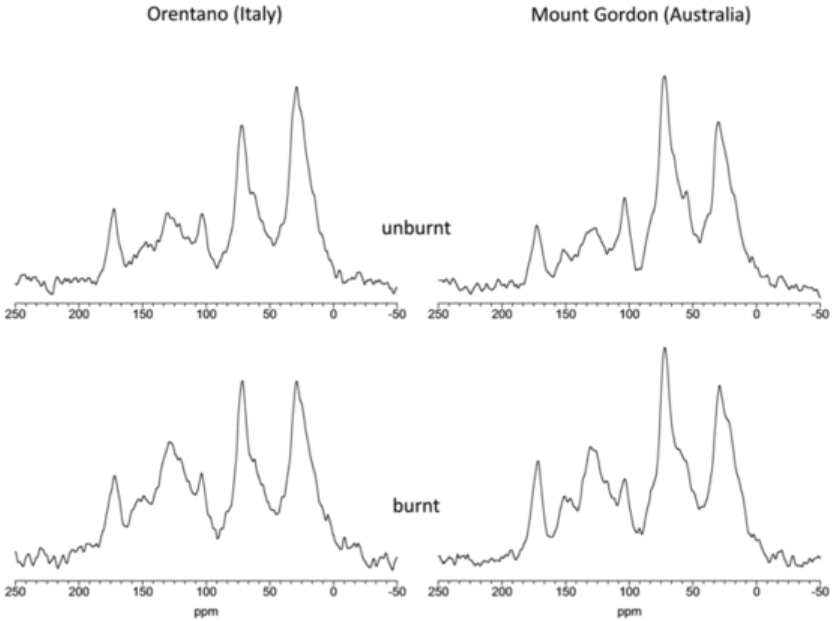

Fig. 5 ^{13}C CPMAS NMR spectra of the burnt and unburnt bulk uppermost mineral soil from Orentano and Mount Gordon.

Table 5 Percentage distribution (%)[a] of the signal intensity between selected chemical shift regions (ppm) of ^{13}C CPMAS NMR spectra of bulk soil at Orentano (OR) and Mount Gordon (MG). The contribution of aldehydes and ketones (chemical shift region 185–220 ppm) is not reported because negligible.

Site (code)	Relative distribution (%) of chemical shift region (ppm)						
	Alkyl C	*O*-Alkyl C			Aryl C		Carboxyl C
	0–45	Methoxyl/*N*-alkyl 45–60	*O*-alkyl 60–90	di-*O*-alkyl 90–110	C–substituted (aromatic C–H and C–C) 110–140	O–substituted 140–160	Carboxyl C/amide/ester 160–185
Orentano (OR)							
Burnt soil							
~~b~~Bulk ~~soil~~	30	7	20	6	22	7	8
f-LF ~~< 1.8 Mg m^{-3}~~	31	8	24	7	22	4	4
o-LF ~~< 1.8 Mg m^{-3}~~	34	7	23	6	16	7	6
Unburnt soil							
~~b~~Bulk ~~soil~~	38	6	22	7	14	6	7
f-LF ~~< 1.8 Mg m^{-3}~~	33	8	29	8	13	3	6
o-LF ~~< 1.8 Mg m^{-3}~~	31	6	24	6	22	5	5
Mount Gordon (MG)							

Burnt soil							
~~b~~Bulk ~~soil~~	27	8	24	7	18	8	8
f-LF ~~< 1.8 Mg m^{-3}~~	30	8	26	8	16	5	7
o-LF ~~< 1.8 Mg m^{-3}~~	31	6	23	8	24	4	4
Unburnt soil							
~~b~~Bulk ~~soil~~	30	8	31	8	12	5	6
f-LF ~~< 1.8 Mg m^{-3}~~	31	11	30	9	10	5	4
o-LF ~~< 1.8 Mg m^{-3}~~	30	7	24	9	16	7	6

[a] Maximum error ± 10%.

The NMR spectra of the light density fractions from OR and MG are shown in Figs. 6 and 7, while the signal distribution among the different chemical shift regions of the spectra is reported in Table 5. The light fractions from the unburnt soils had similar patterns in the two sites, although the o-LF revealed a more advanced stage of alteration than f-LF, as indicated by a lower *O*-alkyl C to alkyl C ratio (Baldock et al., 1992) and a much higher signal in the aryl C region (Golchin et al., 1994). In particular, the higher relative intensity observed in the aromatic region at ~ 150, 130 and 115 ppm for o-LF with respect to f-LF suggests higher lignin content (Golchin et al., 1994; Hatcher, 1987). The NMR analysis indicated that the light fractions in the soil were to some extent affected by fire at both sites (Figs. 6 and 7). At OR, the f-LF from the burnt soil showed a higher intensity for the peak at 130 ppm, and less intense signals in the alkyl (0–45 ppm), *O*-alkyl (60–90 ppm) and carboxyl C (160–185) regions compared to the counterpart from the unburnt soil, overall indicating charring processes. In the o-LF, the peak at 56 ppm (ascribable to lignin methoxyl carbon) and the signal at around 150 ppm (*O*-substituted phenolic carbon) decreased much more in the burnt soil compared to the unburnt one, suggesting lignin decomposition, in accordance with the CuO results (Table 4). At MG, the most evident fire-induced change to the spectra of the light fractions in the soil was the increase of the peak at 130 ppm, while ancillary differences are the decrease of the peaks at about 150 and 53 (lignin), and the intensification of the peak at 174 ppm (carboxylic C). The latter was unexpected, because organic matter exposed to severe heating generally loses carboxyl C (Knicker et al., 2005).

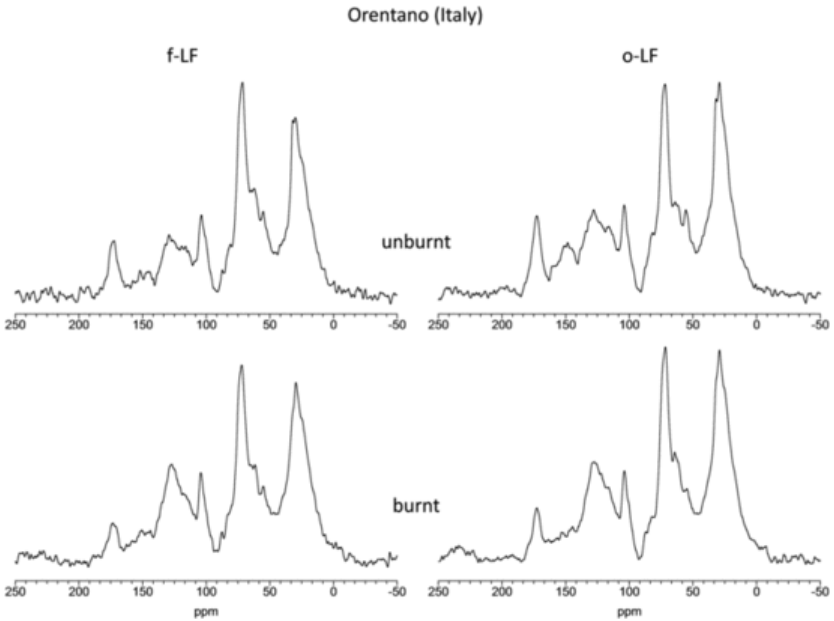

Fig. 6 ^{13}C CPMAS NMR spectra of the light density fractions, f-LF and o-LF, of the burnt and the unburnt soil from Orentano.

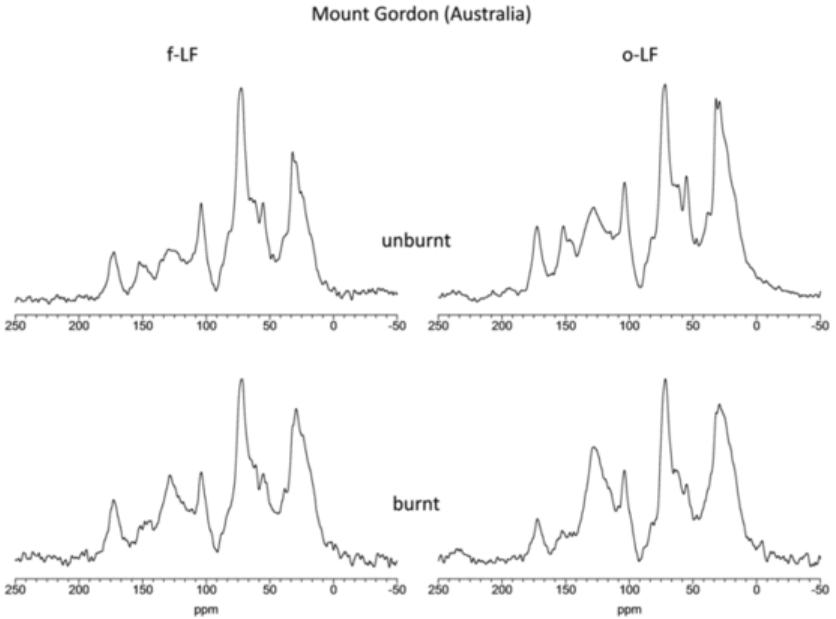

Fig. 7 ^{13}C CPMAS NMR spectra of the light density fractions, f-LF and o-LF, of the burnt and the unburnt soil from Mount Gordon.

4 Conclusions

The parallel investigation at Orentano and Mt. Gordon, two areas recently affected by wildfires of markedly different severity, showed that in both cases fire had a marked impact on composition of SOM from the uppermost mineral soil. This impact was partly independent of fire severity. At Orentano, Italy, where fire severity in the mixed oak-pine forest was moderate, soil did not experience any significant loss of carbon; on the contrary, some charred material from the organic layer and the standing vegetation had joined the mineral soil. At Mt. Gordon, Australia, where an extremely severe fire had burnt the eucalypt forest, ~~SOM~~soil experienced a substantial carbon loss not counterbalanced by the incorporation of charred materials, although it is likely that over the time some of the OM still retained in the ash layer will be partly incorporated into the mineral soil.

Density fractionation allowed three SOM pools with different characteristics and turnover time in soil to be examined, and the fire impact on each of them to be assessed. In spite of the different fire severity at the two study sites, we found similar fire impacts on the SOM assumed to be occluded in aggregates. Apparently, fire partly disrupted aggregates, causing the release of the organic matter contained in them ~~SOM from this fraction~~. Such SOM redistribution could imply substantial changes to the C cycle. It is reasonable, for example, hypothesising a higher availability of the released SOM to microorganisms. This outcome of fire, together with the significant immediate loss of SOM due to combustion and the increase in mean residence time of charred residues, should be taken into account when accounting for the fire impact on C balances.

In terms of SOM composition, at OR lignin was preferentially affected by fire compared to non-cellulosic neutral polysaccharides, particularly in the SOM fraction assumed to be occluded into aggregates. At MG, where lignin and sugars significantly decreased in response to fire occurrence, apparently none of the main C forms, either in the bulk soil or the density fractions, were preferentially affected by the fire. Variables formerly proposed as reliable indicators of fire severity in soil, i.e. the sugar-to-total organic C ratio and phenols ratio in lignin, did not provide encouraging results in this study. Further studies are needed to elucidate the complex impact of fire on SOM composition and to ultimately identify the chemical legacies that are most useful to reconstruct fire history.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.geoderma.2015.01.006. These data include Google map of the most important areas described in this article.

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Appendix A. Supplementary data

The following KMZ file contains the Google map of the most important areas described in this article.

Multimedia Component 1

Map KMZ file containing the Google map of the most important areas described in this article.

Highlights

- We examined the soils of two forests affected by wildfires of different severity.
- Only the extremely severe fire led to a substantial loss of soil organic matter (SOM).
- SOM combusted by fire in both soils was partly compensated by charcoal incorporation.
- Both fires apparently caused partial disruption of aggregates, so freeing some occluded SOM.
- Sugars were better preserved than lignin against fire of moderate severity.

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