**Wildfire-induced alterations of topsoil organic matter and
their recovery in Mediterranean eucalypt stands detected with
biogeochemical markers**

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Summary

This study addressed fire-induced changes in topsoil organic matter (SOM) from an eucalypt plantation in Portugal over two years by using three complementary biogeochemical techniques: elemental analysis, analysis of biomarkers from the total extractable lipids (TLE) and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Direct wildfire effects included a marked decrease in soil total organic carbon (TOC) and total nitrogen (TN) content. However, both contents recovered during the two years. Fire also reduced substantially the TLE, producing noticeable changes in its composition. These included the thermal breakdown and cracking of *n*-alkyl compounds. Ratios of short-to-long *n*-alkanes and *n*-fatty acid methyl esters (FAMES) increased and typical carbon number predominance indexes for *n*-alkanes (odd-to-even) and *n*-FAMES (even-to-odd) were altered. Furthermore, the relative abundances of certain markers which are plant-species specific were modified, especially by decreasing terpenoids such as epiglobulol, ledol and globulol which are characteristic of *Eucalyptus globulus*. Other differences observed in the burnt soil were the appearance of levoglucosan, a typical marker for the thermal alteration of polysaccharides, larger relative abundances of lignin-derived compounds (vanillin and methoxyphenols) and the presence of N-heteroaromatic structures, that suggested the accumulation of 'black nitrogen'. The ^{13}C NMR spectra indicated that the wildfire produced a considerable increase in the aromaticity and aromatic condensation of the topsoil SOM. This was reflected in a broadening of the signal of aromatic compounds at the expense of *O*-alkyl and alkyl-C compounds. The continuation of these differences in SOM quality during the two year-study, suggested a slow recovery of soil properties, possibly influenced by a

limited recovery of the vegetation after the fire combined with the fire-enhanced losses of soil.

Introduction

Wildfires drive the behaviour of many biomes across the world, including in the Mediterranean where they are considered to be one of the main causes of soil degradation (Shakesby, 2011). In Portugal, as in the European Mediterranean region as a whole, the frequency and extent of wildfires increased dramatically from the 1960s, because of warmer and drier climate conditions and, in particular, socio-economic changes such as land abandonment and the widespread planting fire-prone tree species (Shakesby, 2011). Continuation of this intensified fire regime are expected to cause important changes in soil organic matter (SOM) quantity and quality and to affect soil carbon sequestration as well as related soil ecosystem services (González-Pérez *et al.*, 2004).

Although many Portuguese ecosystems are fire-prone, the response of their SOM to fire is still poorly known. In general, the effects of fire on SOM content vary greatly, depending strongly on fire intensity and fuel load characteristics. As soil is a good heat insulator (González-Pérez *et al.*, 2004), and effects will decrease markedly with soil depth. Even at 2–3 cm depth, wildfires rarely produce increases of soil temperature that are large enough to result in serious changes in soil and SOM properties, so that recent studies have increasingly focused on the topsoil for assessing heating-induced changes (Badía-Villas *et al.*, 2014).

Fire effects on SOM tend to be very complex. Changes in topsoil SOM content range from total destruction by very intense fires to increases of up to 30% (De la Rosa *et al.*,

2013). Alterations in SOM quality typically consist of an increase in aromatic C through charring at the expense of a decrease in thermo-labile O-alkyl C, and of a conversion of peptide N into heterocyclic structures (Knicker *et al.*, 2006). While such marked rearrangements of C forms by heating can include specific aspects such as, for example, the formation of new water-repellent substances (Atanassova & Doerr, 2011; de Blas *et al.*, 2013), it tends to affect soil health and quality as a whole, including biodiversity and biological activity (González-Pérez *et al.*, 2004). In many cases, however, fire effects on SOM are thought to be reversible, reverting naturally to (almost) pre-fire conditions (González-Pérez *et al.*, 2008).

The recovery of SOM from fire effects continues to be an important research gap. This includes the identification of biogeochemical compounds that are suitable as indicators of the recovery of SOM quality. The soil lipid composition is of special interest, as it is known to be a relatively labile soil OM fraction that is prone to rapid and significant alterations by heating (González-Pérez *et al.*, 2008). The soil free lipid fraction corresponds to a diverse group of hydrophobic substances ranging from simple compounds such as fatty acids, to more complex substances such as sterols, terpenes, polycyclic aromatic hydrocarbons, waxes and resins. These substances play an important role in the incorporation of plant material into SOM, and contain several diagnostic markers for determination of source apportionment and turnover rate (Knicker *et al.*, 2013). González-Pérez *et al.* (2008) also suggested their potential suitability as proxies of the evolution of SOM quality with time-since-fire. Alkyl lipid compounds such as alkanes, fatty acids and alcohols seem to be suitable indicators of high-intensity fires in particular, as high temperatures seem to cause a reduction on their chain length (Tinoco *et al.*, 2006; Knicker *et al.*, 2013). Aliphatic and aromatic hydrocarbons have been

suggested by Wiesenberg *et al.* (2009) as offering specific fingerprint indicators of fire intensity/severity and burnt vegetation type, including those in fire-affected fossil soils.

Several studies have suggested that certain compounds of the soil lipid fraction respond in a specific manner to heating. Examples of such compounds are terpenoids (Almendros *et al.*, 1988), benzenecarboxylic acids (González-Pérez *et al.*, 2004), and various compounds of the alkyl domain (*n*-alkane/alkene pairs and fatty acids) (González-Pérez *et al.*, 2008). In using such lipids as biomarkers, however, due consideration needs to be given to possible alterations when these compounds enter into the soil, either by microbial degradation processes or by re-synthesis of microbial biomass. Furthermore, other sources of such compounds need to be considered as well, in particular as lipids can also derive from decaying root residues and root exudations (Knicker *et al.*, 2013).

Lignin derivatives are widely held to be an important group of biomarkers of fire effects. Lignin is the most abundant polymeric aromatic organic substance in plants and can account for 20–40 % of wood biomass (Sharma *et al.*, 2004). Knicker *et al.* (2008) demonstrated that the lignin backbone can survive charring of plant material. However, it is not yet well understood to which extent the input of such charred lignin residues affects the lignin pool in fire-affected soils. Lignin degradation is a complex process, which includes competitive and/or consecutive reaction steps due to its hindered structure. Lignin decomposes over a broad range of temperatures, because the various oxygen-based functional groups have different thermal stabilities with scissions occurring at different temperatures. While the lignin structure starts to decompose at relatively low temperatures of 150–275° C, the aliphatic side chains start to split off from the aromatic ring at around 300° C and the carbon-carbon bonds between lignin structural units start to cleavage at 370–400° C. At 500–700° C, complete rearrangement of the lignin

backbone takes place, resulting in 30–50 wt% char and the release of volatile products (CO, CO₂, CH₄, H₂) (Sharma *et al.*, 2004).

Our study aims to contribute to a better knowledge of wildfire effects on topsoil SOM and, in particular, on the post-fire recovery of its lipid composition. To the best of our knowledge, this topic has never been addressed in Portugal, in spite of the present-day intensified fire regime and its widespread planting with fire-prone tree species. The study's specific objectives were to (i) assess the direct effects of fire-induced heating on the content and composition of total extractable lipid as well as several ancillary soil properties, by comparing two nearby eucalypts plantations burnt in 2010 with long-term unburnt plantations and (ii) evaluate the recovery of the topsoil from these direct effects by determining the changes in total extractable lipids at both study sites at six-monthly intervals during the first two years after the fire. To this end, we used the three complementary techniques, of elemental analysis, analysis of biomarkers from the total extractable lipids (TLE), and solid state ¹³C nuclear magnetic resonance (NMR) spectroscopy.

Materials and methods

Study area, study sites and soil sampling

The study area was located near the hamlet of Ermida, in the Sever do Vouga municipality, in the Aveiro district of north-central Portugal (40° 08' 46" N; 7° 59' 35" W; 500 m above sea level (Figure 1)). Between the 26th and 28th of July 2010, a wildfire burnt approximately 300 ha of forested lands, predominantly consisting of eucalypt plantations (*Eucalyptus globulus* Labill.). The severity *sensu* Keeley (2009) appeared to

be moderate, as assessed by tree crown scorching, litter consumption and ash colour as indicators and in line with prior studies in the region such as (Prats *et al.*, 2014).

The climate of the study area is classified as humid meso-thermal (Csb in the Köppen classification) having moderately dry but extended summers. The long-term annual temperature was 14.9° C, at the nearest weather station (Castelo Burgães: 40° 51'16"N; 8°22'55"W; 306 m above sea level during 1990–2010) and long-term mean annual rainfall was 1609 mm, at the nearest rainfall station ("Ribeiradio": 40° 44'39"N; 8°18'05"W; 228 m above sea level during, 1990–2010) (SNIRH, 2011). The study area belongs to the Hesperic Massif, one of the region's major physiographic units, and is composed mainly of pre-Ordovician schists and graywackes, although Hercynian granites do occur at several locations (Ferreira de Brum, 1978).

For the present study, two neighbouring eucalypt plantations were selected (Table 1). One was located within the burnt area, designated EB (40°43'30 "N; 8°20'57"W; 150 m above sea level), while the other was located beside the burnt area, designated EU (40°44'15"N; 8°20'46"W; 253 m above sea level). At the base of the two study sites, several soil profiles were sampled and classified. The various profiles corresponded to Umbric Leptosols (IUSS, 2014), comprising shallow A-horizons of 13–20 cm thickness and C- and R-horizons consisting of schists. At both study sites, soil sampling was carried out immediately after the wildfire in August 2010 to study the direct fire effect and then repeated on four occasions at six-monthly intervals until August 2012 to study the post-fire soil recovery.

At each sampling occasion, the topsoil of 0-2 cm depth was collected at five sampling points for each study site. These five sampling points were located at fixed distances along a transect that was laid out from the bottom to the top of the site and which was shifted 1 m across the slope at subsequent sampling occasions. Before sample collection,

litter (EU) and ash (EB) were carefully removed, although a distinct ash layer was no longer recognizable one year after the fire, possibly through incorporation of the charcoal into the topsoil and/or losses by the widespread overland flow in the study area (Prats *et al.*, 2014). Following field collection, the individual soil samples were air-dried at ambient temperature, passed through a sieve with a mesh width of 2 mm, and then stored under dark and cool conditions prior to analysis. The elemental and physical analysis and the extraction of the total lipid fraction were performed with composite samples (one per site and sampling occasion). These composite samples were obtained by mixing subsamples of the five samples from the individual transects (in equal dry-weight proportions).

Elemental and physical analysis

Total carbon (TC) and total nitrogen (TN) contents were determined in triplicate (average values were considered as being representative) on homogenized composite soil samples with an elemental analyser (Perkin-Elmer 2400 series II; Perkin-Elmer, Waltham, MA, USA). Total organic carbon (TOC) was determined with a total organic carbon analyser (Shimadzu-TOC-5050A, Tokyo, Japan). Soil pH (in H₂O) was measured with a glass electrode in the supernatant of a 1:2.5 weight/volume mixture of soil and water. Particle size distribution was determined with a standard method as described by Guitián & Carballas (1976) and texture was classified according to the USDA criteria (SSS, 1999). Maximum water-holding capacity (WHC) of the samples was measured according to Richards & Weaver (1944).

Extraction of the total extractable lipid fraction and biomarker analysis

The free lipid fractions of dry soil samples were Soxhlet-extracted over six hours with an automatic BUCHI B-811 extractor (Büchi Labortechnik AG, Flawil, Switzerland) with a dichloromethane-methanol (3:1 v:v) mixture (suprasolv Merck). Dry and homogenized soils (5–10 grams) were weighed in Whatman cellulose thimbles, which were pre-cleaned for 30 minutes with fresh solvent and subsequently dried. The extract was slowly dried while the solvent was automatically removed (González-Vila *et al.*, 2003). The total extractable lipid (TEL) fractions were obtained by filtering, subsequently concentrated to about 2 ml with a rotary evaporator, dried under pure N stream and stored in desiccators. The TEL yields were determined gravimetrically and related to the total carbon contents of the original samples. An aliquot of the TEL was weighed, re-suspended in dichloromethane (0.5 µl) in a 2 ml vial and dried with sodium sulphate.

Biomarker separation and analysis was conducted with a gas chromatography system Agilent 6890 equipped with a mass selective detector Agilent 5973 (Agilent Technologies, Santa Clara, CA, USA). A fused silica capillary column SE-52 (30 m × 0.32 mm i.d., film thickness 0.25 µm) was used as stationary/separation media and the chromatographic conditions involved increasing oven temperatures from 40 to 100° C at a rate of 30° C minute⁻¹ and from 100 to 300° C at a rate of 6° C minute⁻¹. Helium was used as carrier gas at a flow rate of 1.5 ml minute⁻¹. Mass spectra were acquired at 70 eV ionizing energy. Individual compounds were detected by low resolution mass spectrometry and through comparison with published mass spectra libraries (NIST and Wiley). Traces corresponding to selected homologous series of biomarkers families were obtained by single ion monitoring (SIM) of ions characteristic, such as ion at m/z 57 for *n*-alkanes and ion at m/z 74 for *n*-fatty acid methyl esters (FAMES). The analysis was performed in duplicate. The relative abundance of the compounds was assessed by determining the relative intensities of the traces to the total chromatogram area using the

Agilent ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The corresponding standards deviations were less than 5%, indicating the good reproducibility of the obtained results.

Solid-state ^{13}C nuclear magnetic resonance spectroscopy

Prior to solid-state NMR analysis, soil samples were treated with 10% (v/v) hydrofluoric acid (HF) to remove paramagnetic material that might interfere with the NMR signals as well as to concentrate the OM in the samples (Knicker *et al.*, 2007). It involved shaking 10 g soil in a polyethylene beaker with 50 ml of HF solution for three hour and removing and discarding the supernatant afterwards. This procedure was then repeated five times to obtain a solid residue, containing the concentrated SOM. This residue was then washed three times with distilled water to remove residual HF and, finally, freeze dried.

The solid-state ^{13}C NMR spectra were obtained with a Bruker Avance 600 MHz Wideboard (Bruker Instruments Inc., Germany) operating at a frequency of 150.93 MHz and using zirconium rotors of 4 mm OD with KEL-F-caps. The cross polarization magic angle spinning (CPMAS) technique was applied during magic-angle spinning of the rotor at 15 kHz. A ramped 1H -pulse was applied during the 1 ms contact time to circumvent Hartmann-Hahn mismatches, and a pulse delay of 300 ms was employed. Between 40 000 and 50 000 scans were accumulated, depending on the ^{13}C content of the samples, and line broadenings between 50 and 100 Hz were applied. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). The spectra were quantified by sub-dividing them into the following different chemical shift regions as described in Knicker (2011): alkyl C (0–45 ppm); N-alkyl/methoxyl C (45–60 ppm); O-alkyl C (60–110 ppm); aromatic C and phenol C (110–160 ppm); carboxyl/amide C and carbonyl C (160–245 ppm). The relative ^{13}C intensity distribution

was determined by integrating signal intensity over the above-mentioned chemical shift regions using a modified integration routine as supplied with the instrument's software. At the spinning speed of 15 kHz that we used, the chemical shift anisotropy cannot be completely averaged and causes the appearance of spinning side bands at both sides of the parent material at a distance of the spinning speed (in the present case, between 220 and 275 ppm as well as between 45 and 0 ppm). Therefore, the intensity distribution of spinning side bands were corrected following Knicker *et al.* (2005).

Results and discussion

Total organic carbon, total nitrogen contents and pH

Immediately after the fire, the TOC concentration was reduced by at least 15% at the burnt site (EB) in comparison with the un-burnt site (EU) (Table 2). The wildfire also seemed to have a pronounced direct effect on topsoil nitrogen, increasing the TN concentration by roughly 75%. As a consequence, the TOC:TN ratio at the EB site one month after the wildfire was less than half that at the EU site. These results were in agreement with those reported by other studies fire-affected Mediterranean soils (Almendros *et al.*, 1988; De la Rosa *et al.*, 2008; Badía-Villas *et al.*, 2014). During the subsequent 24 months after fire, TOC content at the EB site remained consistently below that at the EU site. Differences ranged between 15 and 30% but lacked an obvious seasonal pattern. The TOC concentrations were substantially larger immediately after the fire than in the following months at both sites. Specifically, TOC concentrations between months 6 and 24 were 63–71% less at the EU site and 55–69% less at the EB site than during the first month after fire. The large initial TOC concentrations at the EU site could

be related to drier summer conditions in 2008 than in 2009 and 2010, limiting breakdown of SOM. For the EB site, the present results are in agreement with Campo *et al.* (2008), who attributed a reduction of SOM content in the topsoil (0–5 cm) one year after a fire to slow vegetation recovery and post-fire soil losses. Prats *et al.* (2014) reported slow vegetation recovery and marked runoff and erosion in other eucalypt plantations in the burnt Ermida area.

Soil pH was markedly higher at the EB site than at the EU site, immediately after fire (6.0 and 3.1, respectively). This agrees with the well-recorded ‘liming’ effect caused by the fire, through combustion of organic matter (litter, vegetation) and/or the incorporation of bases (hydroxides and carbonates) with the ash (Ulery *et al.*, 1993). During the following months, the pH at the EB site decreased to values of 4.0–4.3 but remained higher than observed in the EU site (3.2–3.5).

Total extractable lipids: content and overall composition

The amount of total extractable lipids (TEL) normalized to TOC sharply decreased after fire and were more than 75% less at the EB than EU site (Table 2). A decrease in the TEL content was previously observed by Wiesenberg *et al.* (2009) when charring biomass, as well as by Atanassova & Doerr (2011) in soil samples from Australian eucalypt forest heated in a muffle furnace. In contrast, Almendros *et al.* (1988) found a six-fold increase in extractable lipids in recently burnt than in control soils under *Pinus pinea*. The present difference in TEL content during the first month after the fire could be attributed to the drastic decrease in above-ground plant biomass, as is typical after fires of moderate to high severity. In contrast, increases in the TEL contents could occur after fires of low intensity through incorporation of decayed plants and partially charred OM into the soil (Tinoco *et al.*, 2006). In the present study, relative differences in TEL contents between

the two sites appeared to decrease with time after the fire. After two years the TEL concentration at the EB site was 46% less than that at the EU site (108.3 and 199.6 g kg⁻¹, respectively).

The distributions, the compounds detected and their relative contributions to the TEL fraction, for the two study sites on the five different sampling occasions, are shown in Figure 2 and Table 3. In total, 106 compounds were detected and classified into eight main groups according to their structure and origin. These eight main groups comprised terpenes and terpenoids, steroids, *n*-alkanes, *n*-fatty acid methyl esters (FAMES), lignin-derived compounds, aromatic compounds, nitrogenated (N) compounds and other non-specific compounds.

An overview of the relative abundances (calculated as percentages of the total chromatographic area) of these eight main groups of compounds is given in Figure 3. Terpenes, terpenoids and steroids were the groups with the largest relative abundance, and were detected in all the samples. These are major biomarkers components of gums and mucilages from angiosperms and, in particular, grasses, shrubs or herbaceous vegetation. Their relative abundance ranged from 38 to 58% of the TEL in the EB samples and from 76 to 84% in the EU samples (Figure 3). Immediately after the fire, their abundance was 50% smaller at the EB site than at the EU site, and this difference was maintained until 24 months after the fire. Aromatic compounds, lignin-derived compounds and N-compounds were present with a larger relative abundance in the TEL of EB site (11–32%) than in the TEL of the EU site (6–8%). The fire seemed to have produced a strong increase (80%) in the relative abundance of these compounds. Two years later, their relative abundance was still twice as large at the EB site, despite the decrease with elapsed time (post-fire) of their contributions to the TEL fractions. The *n*-alkanes and *n*-FAMES tended to be more relative abundant in the EB samples (19–43%)

than those in EU (9–17%). Among the non-specific compounds, levoglucosan and 1-tricosanol were the most abundant. Their relative abundance was greater at the EB than EU site (3–6 and 0.4–1.3%, respectively, of the TEL). Levoglucosan in particular has been recognized as a marker of vegetation combustion in earlier studies (Kuo *et al.*, 2008).

Terpenes and terpenoids

The terpenes and terpenoids with the greatest relative abundance at both study sites were α -pinene, α - and β - phellandrene, α -and β -gurjunene, isodene, epiglobulol, ledol, globulol, pytone and 3-keto-urs-12-ene. These terpenes or terpenoids were reported for eucalyptus and grassland soils by Otto & Simpson (2005) and Atanassova & Doerr (2010). These compounds had a larger relative abundance at the EU than at the EB site. The EU sample collected immediately after the fire was dominated by the following terpenoids: 1,8- eucalyptol (peak label 5; 1.5 % of the TIC; Table 3); α -and β -gurjunene (peaks label 27 and 28; 2.9 % and 5.6 %, respectively); isodene (peak label 33; 6.0 %); epiglobulol (peak label 36; 4.1 %); ledol (peak label 35; 10.7 %); and globulol (peak label 42; 2.8 %). These are indicators of the presence of *Eucalyptus globulus* (Song *et al.*, 2009; de Blas *et al.*, 2013) and occurred with a smaller relative abundance in the EB samples (Table 3). In addition, some terpenoids such as 1, 8-Eucalyptol, which is a marker of leaves of *Eucalyptus globulus* (Song *et al.*, 2009), were not found in any of the samples collected at the EB site. In contrast, several other terpenoids were only detected in the EB samples: in particular: 4,7-epoxy-5(11)-megastigmen-9-ol (peak label 52; 1.0 %); 1-phenanthrenol, 1,2,3,4,4a,9,10,10a-octahydro-4a-methyl- (peak label 56; 0.9 %); D:A-friedoolean-6-ene (peak label 100; 0.7 %); and α -amyrin (peak label 105; 2.2 %). According to Simoneit (2002), α -amyrin is a marker of angiosperms.

The presence of specific terpene or terpenoid compounds and the relative abundance of others revealed clear differences between the two sites that continued to exist throughout the study period. Overall, the differences suggested that fire produced a drastic and long-lasting decrease in the relative abundance of compounds derived from plant remains, in agreement with the findings of Otto *et al.* (2006), Tinoco *et al.* (2006) and Atanassova &Doerr (2011).

Steroids

The steroids that occurred with the greatest relative abundances in all the samples were cholesterol (peak label 87), cholest-4-ene-3, 6-dione (peak label 89), campesterol (peak label 92) and β -sitosterol (peak label 102). These are common steroids in the waxes of higher plants (phytosterols), which are frequently found in soils and leaf/litter extracts (see Atanassova &Doerr (2011); De la Rosa *et al.* (2013)). Simoneit (2002) also reported that β -sitosterol and cholesterol are molecular tracers for angiosperm, gymnosperm and gramineae whereas campesterol is a molecular tracer for gramineae. In addition, some other steroids compounds (stigmastan-3,5,22-trien (peak label 95); stigmastan-3,5-diene (peak label 97); stigmastan 3β -ol (peak label 104); and stigmastan-3,5-diene-7-one (peak label 106)) were detected with a greater relative abundance in the EB samples than in the EU samples (5.7% and 1.0%, respectively; one month after fire). These could be derived from the natural product β -sitosterol through thermal oxidation (Otto & Simpson, 2005).

Cholesterol was present at both sites. This is a non-specific steroid and can also be produced by soil microorganisms and soil fauna (Otto & Simpson, 2005). The use of the precursor: altered sterol ratio can be suggested as an indicator of the degree of recovery of soil following fire(*ibid*). This ratio is calculated as the sum of the relative abundances of the peak areas of the precursor sterols (β -sitosterol; cholesterol and campesterol) in the

total chromatogram area, divided by the relative abundances of their degradation products (stigmastan 3 β -ol; stigmastan-3, 5-diene-7-one; stigmastan-3, 5, 22-trien; and cholest-4-ene-3,6-dione). A similar ratio has been used to monitor the degree of degradation in three different soils(*ibid*). The values for this ratio were 4–7 times greater in the EU than EB soils. The observed differences in sterol compounds and sterol ratio between the two sites were maintained during the two study years (5 times greater in the EU than EB soils), indicating that the soil at the EB site had not fully recovered from the wildfire in 2010.

Aromatic Structures

Fire caused a pronounced increase in the presence of aromatic compounds (17.4% and 5.5% of the TEL in EB and EU samples, respectively, one month after fire). In general, aromatic structures ranged between 7–17% and 4–6% of the TEL compounds for EB and EU soils, respectively. Similar results have been previously reported by Almendros *et al.* (1988), González-Pérez *et al.* (2008) and Atanassova &Doerr (2011).

Benzene carboxylic acid (peak label 10); 4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-one (peak label 31); 9-ethyl-anthracene (peak label 34); naphthalene, 1,6-dimethyl-4-(1-methylethyl) (peak label 44); and anthracene,1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl- (peak label 61) were the aromatic compounds with the largest relative abundances in the EB samples. Most of these compounds are usually released during incomplete combustion processes, as occur during wildfires, and indicate the presence of char (Tinoco *et al.*, 2006). The only aromatic compounds detected in the EU samples were 4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-one and naphthalene,1,6-dimethyl-4-(1-methylethyl), both of which could be also derived from vegetation residues.

n-alkanes

The *n*-alkanes series ranged from C₁₇ to C₃₃ and revealed bimodal distribution with a maximum at C₂₉ for EU samples and at C₂₄, C₂₅ and C₂₉ for EB samples. In the TEL, *n*-alkanes represent 11–17% of the EB samples and 2–4% of the EU samples. Figure 4 shows changes in the relative abundance of the *n*-alkanes and Table 4 shows the average chain length (ACL), carbon preference indexes (CPI) and short to long ratio of *n*-alkanes.

The distribution of *n*-alkanes showed that immediately after the fire the ACL was slightly reduced in the EB samples (27.4–27.7 one month after fire); however, this difference increased with time (24.8–26.8; two years after fire). Fire also caused a loss of the odd-over-even predominance (CPI decreased for EB samples) and the ratio of short-to-long *n*-alkanes ($\sum C_{17}-C_{25}:\sum C_{26}-C_{33}$) increased 2–6 times. This indicates that the fire altered the abundance of native *n*-alkanes by thermal cracking of long chain homologous molecules. The loss of odd-over-even predominance and an increase in short chain molecules with even numbers of carbon atoms was previously reported for ancient soils subject to biomass burning (Eckmeier & Wiesenberg, 2009), fire affected forest soils (González-Pérez *et al.*, 2008; De la Rosa *et al.*, 2013) and for charred biomass (Wiesenberg *et al.*, 2009; Knicker *et al.*, 2013). The largest short to long chain ratio ($\sum C_{17}-C_{25}:\sum C_{26}-C_{33}$) was reached two years after fire so the differences between EB and EU soils were amplified over this period. The same occurred for the ACL and CPI indexes between EU and EB soils. These changes indicate that the evolution of the molecular composition of the EB soil OM was different to that of the unaffected soil, probably because of fire induced degradation/erosion processes.

n-fatty acid methyl esters (FAMES)

The series of *n*-fatty acid methyl esters (FAMES) ranged from C₁₄ to C₃₀ with a predominance of even-numbered C- chains, and a maximum at C₁₆, C₂₂ and C₂₄ for all the samples (Figure 4). These compounds represent the 7–26% of the TEL for EB samples and 7–14% for EU samples. Immediately after the fire, the ratio short:long chain increased in the EB sample relative to those of EU soil (2.6 to 1.9; table 4) and these differences were maintained during the two years. The ACL was slightly smaller in EB samples than in the unaffected soils (19.6–19.9 and 20.6–21.2, respectively; Table 4). These results confirm the oxidative scission of long chain homologues in the soils affected by fire as previously described by González-Pérez *et al.* (2008) and Atanassova &Doerr (2011) without any clear indication of the recovery of the OM at a molecular level to the pre-fire conditions.

It is known that some unsaturated acids (C_{17:1}, C_{18:1} and C_{18:2}), may be from a bacterial contribution to soil OM (Wiesenberg *et al.*, 2010). Such molecules were detected in both the EB and EU soils. According to Wiesenberg *et al.* (2010), the ratio of unsaturated to saturated fatty acids [(C_{18:1} + C_{18:2}):C₁₈] may indicate the ongoing degradation of plant biomass towards soil OM. This ratio was calculated as the sum of the relative abundances of the peak areas of the unsaturated acids in the total chromatogram area, divided by the relative abundance of their saturated fatty acids. Figure 5 compares their presence in the EB and EU soils. The decrease in the relative abundance of unsaturated FAMES in the EB soils could correspond to a diminution in the microbiological population affected by heat. The presence of C_{18:2} one year after the fire in EB samples could result from a seasonal increase in the above-ground biomass of perennial plant species (Elgersma *et al.*, 2003), and was in agreement with the increase of content of TOC observed 12 months after the fire (70 to 110 g/kg).

Lignin compounds

Lignin-derived compounds were detected at both sites and its relative abundance ranged from 4 to 11% of the TEL in the EB samples, and was around 2% in the EU samples. These structures are characteristic of plant and wood material (Sharma *et al.*, 2004). The larger relative abundance of lignin compounds in the EB soils could be explained by their greater thermal stability in comparison with other compound series. Vanillin (peak label 25); syringaldehyde (peak label 43); and acetosyringone (peak label 47) were the lignin compounds that occurred with the largest relative abundances in the EB samples. In contrast, methyleugenol (peak label 53) was the compound with the largest relative abundance in the EU samples, whereas vanillin (peak label 25) was not detected. Vanillin is the primary aromatic alcohol monomer of gymnosperm lignin and also is a marker for pine (Knicker *et al.*, 2013) and eucalypt wood (Nunes *et al.*, 2010), whereas acetosyringone is a marker of angiosperm lignin (Simoneit, 2002). Although with a small relative abundance, several other methoxyphenol compounds (peak labels 18, 32 and 49) were detected in the EB samples. The presence of such compounds could indicate that fire caused a thermal degradation of lignin structures and the incorporation of monomeric lignin compounds to the TEL fraction. These compounds were also detected in the Py-GC/MS chromatogram of the charred plant residues from humic substances (Knicker *et al.*, 2005) and in the burnt soil from a Mediterranean forest (De la Rosa *et al.*, 2008; Knicker *et al.*, 2013).

Nitrogenated (N)-compounds

N-compounds were only detected in the EB site, with a relative abundance of 0.2–3 % of the TEL. For all burnt soil samples the most abundant nitrogenated compounds were 1H-isoindolene-1,3 (2H) dione (peak label 29) and N-methyl-N-benzyl-dodecanamine (peak

label 69). These N-compounds showed a larger relative abundance in the EB sample collected immediately after the fire (3.6% and of the TEL), but decreased in the samples collected 12 months later (0.2% of the TEL). The relative abundance of the iso-indolene compound in the EB samples agreed with the increases in heterocyclic N-compounds reported previously after fire in Mediterranean soils (Knicker *et al.*, 2005; De la Rosa *et al.*, 2008) and are related to the aromatic N-structures ('black nitrogen') formed during the heating of soil OM and plant biomass. The greater content of those compounds in the EB than in EU samples, immediately after fire is related to the increase of TN content (13.5 and 7.8 g kg⁻¹ for EB and EU samples, respectively, after one month). The relative abundance of N-compounds and TN content at the EB site was strongly correlated (Spearman rank correlation coefficient of 0.78). In addition, two other N-compounds were detected in the EB samples with a small relative abundance ($\leq 0.6\%$): these were benzenemethanamine, N N-dimethyl-(peak label 6) and N-methyl-N-benzyltetradecanamine (peak label 75) and may be considered as peptide-derived structures.

Other compounds

Levoglucosenone and levoglucosan and (peaks label 12 and 20, respectively) were only detected in the EB samples. These compounds are derived from the thermal decomposition of cellulose and have been proposed as a marker for different types of vegetation combustion, such as softwood, hardwood and grass (Otto *et al.*, 2006; Kuo *et al.*, 2008). The presence of levoglucosan and other anhydrosugars was also detected in the Py-GC/MS chromatogram of burnt soils from a Mediterranean forest (De la Rosa *et al.*, 2013). They represented 1.7% of the TEL immediately after the fire but decreased to 0.6% after six months. These results suggest that the partially-burnt remains of the

vegetation deposited on the soil surface were lost by post-fire erosion processes and/or by chemical degradation in the early aftermath of the fire, which agrees with the findings of Knicker *et al.* (2013). On the other hand, levoglucosan is produced by heating cellulose, a process that involves bond cleavage by transglycosylation, fission and disproportionation reactions (Simoneit, 2002): in addition levoglucosenone can be formed through the dehydration of levoglucosan. Thus, it can be used as a marker of moderate-high thermal alteration of soil OM.

The alcohol 1-tricosanol was also present with a relative abundance between 1–5.7% and 0.4–1.1% of the TEL for EB and EU soils, respectively. The alcohol homologues $>n$ -C₂₀ are characteristic of vascular plant wax whereas $<n$ -C₂₀ may be derived from microbial sources (Simoneit, 2002).

¹³C NMR spectroscopy

The CP-MAS ¹³C NMR spectra of the HF-treated soils collected 1, 12 and 24 months after the fire and, their relative intensity distribution are displayed in Figure 6 and Table 5, respectively. The NMR spectra revealed remarkable differences in the chemical composition of SOM in the fire affected and unaffected soils. All spectra of the top 2 cm of EU soils had the typical intensity distribution expected for spectra obtained from soils under forest (Knicker *et al.*, 2005; Otto *et al.*, 2006; De la Rosa *et al.*, 2008) and characterized by two dominant peaks, the signal attributable to carbohydrates in the *O*-alkyl C region between 60 and 110 ppm (34 to 37% of the total ¹³C-intensity) and the intense signal in the chemical shift region between 0 and 45 ppm, typically assigned to alkyl C (36 to 38 % of the total ¹³C-intensity) in lipids and amino acids. During the experiment, no major changes in the intensity distribution were observed. Indications for the presence of lignin are given by the signals in the chemical shift region of *O*-substituted

aromatic C between 160 and 140 ppm and the signal in the region between 60 and 45 ppm, which is often assigned to methoxyl carbons. However, some response in this region can also be produced by N-alkyl C, associated with peptides and amino sugars. This region contributed 8–9% of the total ^{13}C -intensity in the EU soils. The proportions of aromatic C (160 and 110 ppm) of 13% of the EU samples are typical of soils lacking contributions from charred material (Knicker *et al.*, 2007), and are similar to the 15% reported for fresh plant material by Knicker *et al.* (2008). Peaks for carboxyl/amide contributed about 4% to the region between 160 and 185 ppm and for carbonyl C 2% to that between 245 and 185 ppm.

The fire apparently resulted in a considerable increase as well as broadening of the signal in the sp^2 C region, at the expense of the *O*-alkyl and alkyl C signal (from 13 to 38%). The intensity of the aryl-C region decreased from 38 to 28 and then to 20 % at one, 12 and 24 months after fire, respectively. Concomitantly, the signal intensity for alkyl C increased from 26 to 42%, probably because of a relative increase in plant residues. However, fatty acids, waxes and resins also contribute to this region (Tinoco *et al.*, 2006). The signal attributable to carbohydrates in the *O*-alkyl C region (60–110 ppm) was reduced sharply to 23–26% (it ranged 34–37% for the EU samples). It has been widely reported that heating SOM leads to a loss of *O*-alkyl C structures (González-Pérez *et al.*, 2004; Tinoco *et al.*, 2006). The initial vegetation recovery after a wildfire is typically dominated by herbs and shrubs, and is likely to result in considerable litter production. The present results indicate that post-fire vegetation recovery was rather limited, agreeing with what other post-fire studies in the study region reported ((Prats *et al.*, 2014).

The signal corresponding to alkyl C (0–45 ppm) was also reduced in the EB samples, which is in agreement with the breaking of alkyl structures shown from the analysis of the TEL. However, the signal intensity in the region between 60 and 45 ppm, assigned to

methoxyl carbons of lignin and hemicelluloses, remained constant with a contribution 7%. The signals at 153 and 145 ppm probably corresponded with C₃ and C₅ of syringyl units and to C₃ and C₄ of guaiacyl, respectively, associated with lignin structures (Knicker *et al.*, 2007). The signal at 105 ppm remained in the spectra of all samples collected after the fire. This region in the signal corresponds to cellulosic structures, although non-protonated aromatic carbons in tannins may also contribute (Mengchang *et al.*, 2008).

Conclusions

The present results indicate that a moderate-severity wildfire produced substantial changes in the quantity and quality of SOM in the topsoil of eucalypt plantations. The wildfire appeared to have resulted in marked decreases in TOC and TEL contents as well as in a clear increase of the TN content. These initial differences between the burnt and the long-unburnt eucalypt plantations diminished during the 24 months following the fire but at a pace that suggested a limited recovery from the direct fire effects. The biomarkers in the TEL fractions indicated that the wildfire modified topsoil SOM by (i) thermal breakdown of the alkyl-chains, increasing the ratios of short-to-long *n*-fatty acids and *n*-alkanes; (ii) increasing and altering the characteristic CPIs; (iii) increasing the relative abundance of aromatic compounds, most probably through the input of charred biomass, as was also suggested by the ¹³C NMR spectra; (iv) decreasing the relative abundances of specific biomarkers of vegetation and, in particular, *Eucalyptus globulus* such as the terpenoids epiglobulol, ledol and globulol; (v) introducing compounds that are thermal degradation products from steroids, such as stigmastan-3,5-diene (derived from β -sitosterol); (vi) increasing the relative abundance of lignin compounds, indicating the greater thermal stability of lignin moieties compared with other SOM chemical structures;

(vii) introducing levoglucosan, indicating thermal alteration of biomass; and (viii) introducing heteroaromatic N-compounds, pointing towards thermal transformation of SOM.

The limited post-fire recovery of soil over two years reflected the poor growth of vegetation and was observed in other studies in the area and these reported the losses of soil from runoff and erosion. The slow soil recovery is also reflected in the ^{13}C NMR spectra, revealing a weak enrichment in the relative abundance of carbohydrates in the burnt topsoils during the first two years after fire.

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713 **TABLES**

714 **Table 1** Topsoil (0–2 cm) characteristics at the recently burnt and the long-unburnt
 715 eucalypt plantation.

	716	
	Eucalypt Burnt (EB)	Eucalypt Unburnt (EU)
Slope angle / ° degrees	20	25
Density / g cm ³	1.15	0.88
Water holding capacity / ml g ⁻¹	1.2	0.9
Sand / %	67.3	69.2
Clay / %	23.7	22.0
Silt / %	9.0	8.8
Texture	Sandy clay loam	Sandy clay loam

717 **Table 2** Average total organic carbon (TOC), total nitrogen (TN), ratio TOC:TN, pH,
718 and total lipids extract (TEL) of the topsoil (0-2cm) at the burnt site (EB) and the long-
719 unburnt (EU) eucalypt plantation at 1, 6, 12, 18 and 24 months after a wildfire.

Time after fire (Months)	Code	TOC / g kg ⁻¹	TN / g kg ⁻¹	TOC:TN	pH (in H ₂ O)	TEL / g kg ⁻¹ TOC
1	EB1	154	13.5	11	6.0	70.0
	EU1	184	7.8	24	3.1	307.8
6	EB6	96	3.6	27	4.1	80.8
	EU6	127	2.2	58	3.2	308.7
12	EB12	97	4.6	21	4.2	110.3
	EU12	115	2.4	48	3.4	218.2
18	EB18	107	4.2	26	4.3	80.6
	EU18	130	4.5	29	3.4	243.5
24	EB24	84	2.7	31	4.0	108.3
	EU24	118	3.9	30	3.5	199.6

720 **Table 3** Compounds of the topsoil lipid fraction and their relative abundances at the
721 recently burnt and long-unburnt eucalypt plantation at 1, 6, 12, 18 and 24 months after a
722 wildfire, detected using GC-MS and calculated as percentages of the total
723 chromatographic area, respectively.

Peak label	Compound	Molecular formula	MW	Compound family	Retention time / minutes	Burnt (EB)					Unbunt (EU)				
						1M	6M	12M	18M	24M	1M	6M	12M	18M	24M
1	α -Pinene	C ₁₀ H ₁₆	136	Terpene	5.64	1.6	1.7	1.2	2.1	1.2	1.0	0.9	1.3	0.8	1.3
2	α -Phellandrene	C ₁₀ H ₁₆	136	Terpene	5.98	5.7	7.9	2.9	4.3	1.4	4.3	2.7	3.1	4.2	3.6
3	Benzene, x,x,x,x- tetramethyl	C ₁₀ H ₁₄	134	Aromatic	6.27	0.7	1.6	0.4	0.6	0.2	1.1	0.6	0.5	0.8	0.8
4	β -phellandrene	C ₁₀ H ₁₆	136	Terpenoid	6.36	1.6	2.3	1.3	1.9	1.0	0.9	0.6	0.8	1.4	0.9
5	1,8-Eucalyptol	C ₁₀ H ₁₈ O	154	Terpenoid	6.39	nd	nd	nd	nd	nd	1.5	2.6	1.0	1.9	1.5
6	Benzenemethanamine, N,N-dimethyl-	C ₉ H ₁₃ N	135	N-compound	6.52	0.2	0.6	nd	0.4	nd	nd	nd	nd	nd	nd
7	Benzene, pentamethyl	C ₁₁ H ₁₆	148	Aromatic	7.23	0.7	0.9	0.6	0.9	0.5	0.6	0.6	0.7	0.5	0.7
8	Unknown Terpenoid (m/z 154,121,11,93,81)	C ₁₀ H ₁₈ O	154	Terpenoid	7.72	nd	nd	nd	nd	nd	0.2	0.1	0.2	0.1	0.2
9	Trans-Pinocarveol	C ₁₀ H ₁₆ O	152	Terpenoid	8.19	nd	nd	nd	nd	nd	0.2	0.2	0.2	0.2	0.2
10	Benzene carboxylic acid	C ₇ H ₆ O ₂	122	Aromatic	8.52	2.1	0.5	0.4	0.8	0.5	nd	nd	nd	nd	nd
11	Borneol	C ₁₀ H ₁₈ O	154	Terpenoid	8.67	0.6	0.5	0.5	0.5	0.2	1.3	1.2	1.1	1.0	0.9
12	Levogluconone	C ₆ H ₈ O ₄	144	Others	9.37	0.6	0.03	0.02	nd	nd	nd	nd	nd	nd	nd
13	Nonanoic acid	C ₉ H ₁₈ O ₂	158	FAMES+ FA	10.40	0.5	0.6	0.5	0.4	0.2	0.02	nd	0.1	0.1	0.1
14	Thymol	C ₁₀ H ₁₄ O	150	Terpenoid	11.16	0.2	0.4	0.2	0.2	nd	0.2	0.1	0.1	0.2	0.1
15	2-Methoxy-x-vinylphenol	C ₉ H ₁₀ O ₂	150	Lignin	11.48	0.3	0.3	0.2	0.4	0.2	0.1	0.05	nd	0.1	nd
16	Alkyl benzene	C _x H _y	200	Aromatic	11.82	1.2	1.7	0.7	1.0	0.6	1.1	0.4	1.0	1.0	1.2
17	Longifolene	C ₁₅ H ₂₄	204	Terpenoid	12.01	0.4	0.7	0.4	0.7	0.3	0.8	0.6	0.6	0.9	0.7
18	Phenol, 2,6-dimethoxy (Syringol)	C ₁₀ H ₁₂ O ₄	196	Lignin	12.21	0.5	0.4	0.4	0.4	0.2	nd	nd	nd	nd	nd
19	Dehydroaromadendrene	C ₁₅ H ₂₂	202	Terpenoid	12.24	0.2	nd	nd	nd	nd	0.5	0.5	0.3	0.4	0.2
20	Levogluconan	C ₆ H ₁₀ O ₅	162	Others	12.38	1.1	0.6	nd	nd	nd	nd	nd	nd	nd	nd
21	Decanoic acid	C ₁₀ H ₂₀ O ₂	172	FAMES+FA	12.48	nd	nd	0.3	0.6	nd	0.1	0.1	0.1	0.1	0.1
22	Dehydroaromadendrene	C ₁₅ H ₂₂	202	Terpenoid	12.75	nd	nd	nd	nd	nd	0.4	0.2	0.3	0.3	0.2
23	β -guaiene	C ₁₅ H ₂₄	204	Terpenoid	12.88	0.2	0.3	0.1	0.1	nd	0.7	0.7	0.4	0.3	0.4
24	Unknown Terpenoid (m/z 204, 161, 134, 119,105)	C ₁₅ H ₂₄	204	Terpenoid	13.00	0.5	0.4	0.3	0.4	0.3	1.1	1.0	0.7	0.7	0.7
25	Vanillin	C ₈ H ₈ O ₃	152	Lignin	13.20	2.7	1.6	2.1	1.6	2.0	nd	nd	nd	nd	nd
26	Unknown terpenoid (m/z 204, 161, 119,105,93)	C ₁₅ H ₂₄	204	Terpenoid	13.26	nd	nd	nd	nd	nd	0.9	0.9	1.0	0.6	0.7
27	α -Gurjunene	C ₁₅ H ₂₄	204	Terpenoid	13.62	1.0	1.2	1.3	1.5	1.8	2.9	2.7	1.4	2.0	1.4
28	β -Gurjunene	C ₁₅ H ₂₄	204	Terpenoid	14.12	1.4	1.5	1.0	1.9	0.7	5.6	6.0	3.5	5.5	3.6
29	1H-isoindolene-1,3 (2H) dione	C ₈ H ₅ NO ₂	147	N-compound	14.41	2.1	0.6	0.2	0.5	0.3	nd	nd	nd	nd	nd

30	Aromadendrene	C ₁₅ H ₂₄	204	Terpenoid	14.57	1.0	1.6	2.2	1.6	2.0	1.1	2.1	1.5	1.5	1.5
31	4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-one	C ₁₃ H ₁₆ O ₂	204	Aromatic	14.79	3.6	2.1	1.3	1.6	1.2	2.8	2.7	1.2	2.5	1.4
32	Phenol, 4-methoxy-2,3,6-trimethyl-	C ₁₀ H ₁₄ O ₂	166	Lignin	14.94	1.0	0.6	0.4	0.6	0.4	nd	nd	nd	nd	nd
33	Isoledene	C ₁₅ H ₂₄	204	Terpenoid	15.30	0.8	0.9	0.6	0.9	0.4	6.0	9.8	6.7	6.8	5.5
34	9-ethyl-Anthracene	C ₁₆ H ₁₄	206	Aromatic	15.38	2.4	2.8	1.8	1.3	1.3	nd	nd	nd	nd	nd
35	Dehydroaromadendrene	C ₁₅ H ₂₂	202	Terpenoid	15.97	0.5	0.5	0.6	0.6	0.6	1.4	1.1	1.1	1.0	1.0
36	Epiglobulol	C ₁₅ H ₂₆ O	222	Terpenoid	16.48	0.9	0.9	1.1	1.6	1.0	4.1	3.8	2.7	4.0	2.5
37	Ledol	C ₁₅ H ₂₆ O	222	Terpenoid	16.96	4.0	4.1	3.8	7.5	5.5	10.7	13.8	11.2	15.6	11.5
38	Unknown terpenoid (m/z 204, 189,161, 121,107,93)	C ₁₅ H ₂₄	204	Terpenoid	17.33	nd	nd	nd	nd	nd	4.0	nd	2.6	nd	2.3
39	Valerenal	C ₁₅ H ₂₂ O	218	Terpenoid	17.56	nd	nd	nd	nd	nd	0.5	0.5	0.5	0.5	0.5
40	Spathulenol	C ₁₅ H ₂₄ O	220	Terpenoid	17.88	0.9	0.9	0.6	1.0	0.6	1.9	2.0	1.2	1.4	0.6
41	Xanthotoxin Furanocoumarins	C ₁₂ H ₈ O ₄	216	Terpenoid	17.94	nd	nd	nd	nd	nd	1.2	1.2	0.9	1.3	1.3
42	Globulol	C ₁₅ H ₂₆ O	222	Terpenoid	18.19	1.9	2.1	2.7	4.1	3.1	2.8	4.3	2.8	5.1	5.0
43	Syringaldehyde	C ₉ H ₁₀ O ₄	182	Lignin	18.26	2.9	1.1	nd	nd	nd	nd	nd	nd	nd	nd
44	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	C ₁₅ H ₁₈	198	Aromatic	18.62	2.5	1.8	0.9	1.0	0.2	0.1	0.1	0.01	0.1	0.1
45	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	C ₁₅ H ₁₈	198	Aromatic	18.77	0.8	0.9	0.4	0.7	0.2	nd	nd	nd	nd	nd
46	(-)-Caryophyllenol	C ₁₅ H ₂₄ O	220	Terpenoid	18.98	nd	nd	nd	nd	nd	1.6	1.6	1.5	1.5	1.5
47	Acetosyringone	C ₁₀ H ₁₂ O ₄	196	Lignin	19.16	2.2	2.0	1.2	1.8	0.8	nd	nd	nd	nd	nd
48	Unknown terpenoid (m/z 220,202,187,159,121,107,93)	C ₁₅ H ₂₄ O	220	Terpenoid	19.20	nd	nd	nd	nd	nd	1.3	1.2	1.4	1.4	1.3
49	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	C ₁₀ H ₁₂ O ₄	196	Lignin	19.59	1.3	0.8	0.7	1.2	0.6	nd	nd	nd	nd	nd
50	Unknown terpenoid (m/z 218,178, 139,121, 107,93)	C _x H _y O _z	218	Terpenoid	19.64	0.7	1.1	0.9	1.7	1.0	1.9	1.5	1.8	1.3	1.4
51	Unknown terpenoid (m/z 236, 218,178, 122, 107,95,82)	C _x H _y O _z	236	Terpenoid	19.76	nd	nd	nd	nd	nd	2.2	1.9	3.0	2.3	2.5
52	4,7-Epoxy-5(11)-megastigmen-9-ol	C ₁₃ H ₂₂ O ₂	210	Terpenoid	19.86	1.0	0.9	0.7	1.5	0.7	nd	nd	nd	nd	nd
53	Methyleugenol	C ₁₁ H ₁₄ O ₂	178	Lignin	19.94	nd	nd	nd	nd	nd	2.0	1.5	1.9	1.6	1.7
54	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	FAMES+ FA	19.98	1.6	0.4	0.9	0.6	nd	1.7	0.2	0.6	1.1	0.6
55	Unknown terpenoid (m/z 218,203,175, 136,121,105,91)	C ₁₅ H ₂₂ O ₂	218	Terpenoid	20.34	nd	nd	nd	nd	nd	1.5	0.6	0.6	0.6	0.6
56	1-Phenanthrenol, 1,2,3,4,4a,9,10,10a-octahydro-4a-	C ₁₅ H ₂₀ O	216	Terpenoid	20.46	0.9	1.7	0.9	1.4	0.9	nd	nd	nd	nd	nd
57	Unknown terpenoid (m/z 234,216,198,183,105,91)	C _x H _y O _z	234	Terpenoid	20.51	0.4	nd	nd	nd	nd	1.5	0.7	1.5	1.7	nd
58	3-Hydroxybisabola-1(6),10-dien-2-one	C ₁₅ H ₂₄ O ₂	236	Terpenoid	20.78	nd	nd	nd	nd	nd	1.4	0.5	1.1	0.7	1.1
59	Phytone	C ₁₈ H ₃₆ O	268	Terpenoid	21.44	1.3	1.0	0.8	1.2	0.8	0.9	0.9	1.1	0.9	1.0
60	Unknown terpenoid (m/z 234,216,201,173,135,91)	C ₁₅ H ₂₄ O ₂	236	Terpenoid	21.65	nd	nd	nd	nd	nd	nd	0.3	0.6	nd	0.5

61	Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	C ₁₆ H ₂₂	214	Aromatic	22.04	1.1	1.5	1.7	0.7	1.7	nd	nd	nd	nd	nd
62	2-Heptadecanone	C ₁₇ H ₃₄ O	254	Others	22.34	0.5	0.4	0.3	0.5	0.2	nd	nd	nd	nd	0.2
63	FAME C16	C ₁₇ H ₃₄ O ₂	270	FAMEs+ FA	22.78	0.9	1.1	3.9	1.5	4.5	0.7	0.2	1.9	0.3	1.7
64	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	FAMEs+ FA	23.37	1.8	1.7	2.7	2.1	1.8	0.5	0.8	0.9	0.8	0.6
65	Unknown terpenoid (m/z 252,234,224,209,168,107)	C _x H _y O _z	252	Terpenoid	25.01	nd	nd	nd	nd	nd	nd	7.7	6.7	6.8	6.7
66	n-Henicosane (C ₂₁ alkane)	C ₂₁ H ₄₄	296	Alkane	25.51	0.5	0.7	1.4	0.4	1.5	nd	nd	nd	nd	nd
67	FAME C18:1	C ₁₉ H ₃₆ O ₂	296	FAMEs+ FA	25.55	0.2	0.3	3.4	0.9	3.2	1.9	0.6	1.7	0.8	1.6
68	FAME C18:2	C ₁₉ H ₃₄ O ₂	294	FAMEs+ FA	25.57	nd	nd	0.2	nd	0.3	0.5	0.2	0.5	0.1	0.6
69	N-methyl-N-benzyl-dodecanamine	C ₂₀ H ₃₅ N	289	N-compound	25.79	0.8	0.8	nd	0.6	nd	nd	nd	nd	nd	nd
70	FAME C18	C ₁₉ H ₃₈ O ₂	298	FAMEs+ FA	25.93	0.4	0.6	2.2	0.6	2.9	0.6	0.4	0.9	0.4	0.7
71	Oleic acid	C ₁₈ H ₃₄ O ₂	282	FAMEs+ FA	26.11	1.1	0.8	0.9	nd	nd	0.6	0.3	nd	0.4	nd
72	n-Docosane (C ₂₂ alkane)	C ₂₂ H ₄₆	310	Alkane	26.99	0.7	1.0	1.7	0.6	2.1	nd	nd	nd	nd	0.3
73	Phenanthrene, 1-methyl-7-(1-methylethyl)-	C ₁₈ H ₁₈	234	Aromatic	27.42	0.6	0.9	0.5	0.5	0.3	nd	nd	nd	nd	nd
74	n-Tricosane (C ₂₃ alkane)	C ₂₃ H ₄₈	324	Alkane	28.40	0.5	1.0	1.8	0.4	2.6	nd	nd	0.5	nd	nd
75	N-Methyl-N-benzyl-tetradecanamine	C ₂₂ H ₃₉ N	317	N-compound	28.79	0.5	0.4	nd	0.6	nd	nd	nd	nd	nd	nd
76	FAME C20	C ₂₁ H ₄₂ O ₂	326	FAMEs+ FA	28.82	nd	nd	1.1	nd	1.5	0.3	0.1	0.4	nd	0.5
77	n-Tetracosane (C ₂₄ alkane)	C ₂₄ H ₅₀	338	Alkane	29.78	0.8	1.3	1.6	0.9	2.5	nd	nd	0.7	nd	0.2
78	FAME C21	C ₂₂ H ₄₄ O ₂	340	FAMEs+ FA	30.18	0.2	nd	0.8	nd	1.7	nd	nd	0.4	nd	0.5
79	1-Tricosanol	C ₂₃ H ₄₈ O	340	Others	30.99	1.0	1.9	4.1	2.6	5.7	0.4	0.5	0.8	0.6	1.1
80	n-Pentacosane (C ₂₅ alkane)	C ₂₅ H ₅₂	352	Alkane	31.09	0.6	1.6	2.3	0.6	2.2	0.1	0.2	0.5	0.2	0.3
81	FAME C22	C ₂₃ H ₄₆ O ₂	354	FAMEs+ FA	31.49	0.2	0.9	1.4	1.1	1.8	0.5	0.2	1.3	0.3	1.5
82	n-Hexacosane (C ₂₆ alkane)	C ₂₆ H ₅₄	366	Alkane	32.35	0.3	1.5	1.6	0.3	1.1	nd	nd	0.4	nd	nd
83	FAME C23	C ₂₄ H ₄₈ O ₂	368	FAMEs+ FA	32.75	nd	nd	0.9	nd	1.5	nd	nd	0.3	nd	0.6
84	n-Heptacosane (C ₂₇ alkane)	C ₂₇ H ₅₆	380	Alkane	33.57	1.1	2.1	1.6	1.2	1.1	0.3	0.3	0.6	0.4	0.4
85	FAME C24	C ₂₅ H ₅₀ O ₂	382	FAMEs+ FA	33.96	nd	0.5	2.1	0.7	2.5	0.5	0.1	1.5	0.2	1.6
86	n-Octacosane (C ₂₈ alkane)	C ₂₈ H ₅₈	394	Alkane	34.74	0.4	1.4	0.8	0.2	0.4	nd	nd	nd	nd	nd
87	Cholesterol (Cholest-5-en-3b-ol)	C ₂₇ H ₄₆ O	386	Steroid	35.06	1.6	1.2	0.7	1.2	1.0	2.3	1.9	2.4	2.3	2.3
88	FAME C25	C ₂₆ H ₅₂ O ₂	396	FAMEs+ FA	35.14	nd	nd	2.2	1.9	1.3	5.1	4.0	0.2	2.4	1.2
89	Cholest-4-ene-3,6-dione	C ₂₇ H ₄₂ O ₂	398	Steroid	35.55	1.3	1.1	1.3	1.0	0.6	1.4	0.9	1.3	1.0	1.4
90	n-Nonacosane (C ₂₉ alkane)	C ₂₉ H ₆₀	408	Alkane	35.98	1.6	2.3	1.7	2.5	1.2	1.1	1.3	1.6	1.3	1.7
91	FAME C26	C ₂₇ H ₅₄ O ₂	410	FAMEs+ FA	36.27	nd	0.9	1.0	0.9	1.1	0.3	0.2	1.0	0.2	1.1

92	Campesterol (ergost-5-en-3b-ol))	C ₂₈ H ₄₈ O	400	Steroid	36.78	1.1	0.9	0.7	0.8	0.9	0.7	0.5	0.7	0.6	0.7
93	n-Triacontane (C ₃₀ alkane)	C ₃₀ H ₆₂	422	Alkane	37.00	0.6	0.6	0.4	nd	0.3	nd	nd	nd	nd	nd
94	FAME C27	C ₂₈ H ₅₆ O ₂	424	FAMEs+ FA	37.37	nd	nd	0.8	nd	0.7	nd	nd	nd	nd	nd
95	Stigmastan-3,5,22-trien	C ₂₉ H ₄₆	394	Steroid	38.04	1.6	2.1	0.6	0.6	0.4	0.3	0.5	0.4	0.3	0.3
96	n-Hentriacontane (C ₃₁ alkane)	C ₃₁ H ₆₄	436	Alkane	38.05	3.0	2.1	1.5	2.8	0.4	0.2	0.1	0.1	nd	0.1
97	Stigmastan-3,5-diene	C ₂₉ H ₄₈	396	Steroid	38.24	2.0	1.6	1.4	1.2	1.4	0.2	0.2	nd	0.2	0.3
98	FAME C28	C ₂₉ H ₅₈ O ₂	438	FAMEs+ FA	38.44	nd	nd	1.2	nd	1.1	nd	nd	0.6	nd	0.7
99	D,α,-Tocopherol	C ₂₉ H ₅₀ O ₂	430	Steroid	38.59	0.3	0.3	0.3	0.4	0.3	0.5	0.4	nd	0.3	0.3
100	D:A-Friedoolean-6-ene	C ₃₀ H ₅₀	410	Terpenoid	40.04	0.7	1.3	1.1	1.4	0.7	nd	nd	nd	nd	nd
101	n-Tritriacontane (C ₃₃ alkane)	C ₃₃ H ₆₈	464	Alkane	40.36	1.7	1.2	0.7	1.2	0.9	nd	nd	nd	nd	nd
102	β-Sitosterol (Stigmast-5-en-3b-ol)	C ₂₉ H ₅₀ O	414	Steroid	40.93	1.3	1.0	0.8	1.4	1.2	2.2	2.0	4.2	2.7	4.0
103	3-Keto-urs-12-ene	C ₃₀ H ₄₈ O	424	Terpenoid	41.48	4.3	2.1	2.6	5.9	4.6	0.6	0.8	0.6	0.7	1.0
104	Stigmastan-3b-ol	C ₂₉ H ₅₂ O	416	Steroid	41.67	0.9	1.3	0.9	1.6	1.1	0.1	0.5	0.5	0.5	0.4
105	α-Amyrin	C ₃₀ H ₅₀ O	426	Terpenoid	42.18	2.2	1.1	1.4	3.4	1.6	nd	nd	0.2	0.1	0.2
106	Stigmasta-3,5-dien-7-one	C ₂₉ H ₄₆ O	410	Steroid	42.35	1.2	1.7	1.4	1.3	1.7	0.3	0.2	0.3	0.3	0.3

FAMEs: *n*-fatty acid methyl esters; FA: *n*-fatty acid.

725 **Table 4** Molecular ratios for *n*-alkanes and *n*-fatty acids methyl esters (FAMES) in the
726 soil lipid fraction at the burnt and long-unburnt eucalypt plantations

<i>n</i> -Alkanes																
$\sum C_{17}-C_{25}:\sum C_{26}-C_{33}$ short:long		Diff.	CPI odd:even		Diff.	CPI short		Diff.	CPI long		Diff.	ACL		Diff.		
Time (months)	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	
1	0.5	0.2	0.3	2.9	6.6	-3.7	1.2	3.9	-2.7	5.4	7.7	-2.3	27.4	27.7	-0.3	
6	0.6	0.3	0.3	1.5	5.3	-3.8	1.4	2.0	-0.6	1.5	7.8	-6.3	26.3	27.5	-1.2	
12	1.2	0.7	0.5	1.4	2.0	-0.6	1.3	1.5	-0.2	1.5	2.6	-1.1	25.5	26.2	-0.7	
18	0.6	0.3	0.3	3.4	5.8	-2.4	1.6	2.4	-0.8	6.4	8.4	-2.0	26.6	27.5	-0.9	
24	2.6	0.4	2.2	1.4	3.2	-1.8	1.1	1.6	-0.5	2.4	4.8	-2.4	24.8	26.8	-2.0	
<i>n</i> -FAMES																
$\sum C_{14}-C_{22}:\sum C_{23}-C_{30}$ short:long		Diff.	CPI even:odd		Diff.	CPI short		Diff.	CPI long		Diff.	ACL		Diff.		
Time (months)	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	Burnt		Unburnt	
1	2.6	1.9	0.7	4.6	8.8	-4.2	7.5	14.1	-6.6	2.0	4.8	-2.8	19.6	20.6	-1.0	
6	2.9	1.6	1.3	6.0	7.3	-1.3	9.3	13.9	-4.6	2.6	3.9	-1.3	19.9	20.8	-0.9	
12	2.3	1.6	0.7	6.4	7.8	-1.4	10.1	12.2	-2.1	3.2	4.7	-1.5	19.9	20.9	-1.0	
18	2.9	1.6	1.3	6.4	7.8	-1.4	8.8	11.7	-2.9	3.3	4.9	-1.6	19.6	20.9	-1.3	
24	2.5	1.5	1.0	6.1	7.2	-1.1	10.7	12.0	-1.3	2.6	4.3	-1.7	19.7	21.2	-1.5	

727 Average chain length = $(\sum Z_n \times n) : \sum Z_n$, with Z_n as relative amount of *n*-alkanes with *n* carbons.

728 Carbon preference index of *n*-alkanes = $(\sum C_{17-33\text{odd}} : \sum C_{18-32\text{even}})$

729 Carbon preference index of short chain *n*-alkanes = $(\sum C_{17-25\text{odd}} : \sum C_{18-24\text{even}})$

730 Carbon preference index of long chain *n*-alkanes = $(\sum C_{27-33\text{odd}} : \sum C_{26-32\text{even}})$

731 Diff. = difference between burnt and unburnt site

732 **Table 5** Relative intensity (% of total intensity) in solid state ^{13}C NMR spectra of burnt
733 (EB) and long-unburnt (EU) soil under eucalypt at 1, 12 and 24 months after the
734 wildfire.

^{13}C NMR data								
Sample	ppm						sum	O-Alkyl/Alkyl
	245–185	185–160	160–110	110–60	60–45	45–0		
EB1	3	3	38	23	7	26	100	0.9
EB12	3	4	28	25	7	34	100	0.7
EB24	2	3	20	26	7	42	100	0.6
EU1	2	4	13	37	9	36	100	1.0
EU12	3	4	13	34	8	38	100	0.9
EU24	1	4	13	37	8	36	100	1.0

FIGURE CAPTIONS

Figure 1 Location of the Ermida study area and the two study sites, burnt (EB) and long-unburnt (EU) eucalypt plantations.

Figure 2 GC-MS total ion chromatograms (TIC) of burnt (EB) and long-unburnt (EU) Mediterranean topsoils of eucalypt plantations at 1, 6, 12, 18 and 24 months after the wildfire. ● *n*-alkanes, ☆ *n*-fatty acids+FAMEs. See Table 3 for the identification of the peaks labels.

Figure 3 Cumulative relative abundances calculated as percentages of the total chromatographic area of the major types of compounds identified in soil lipids fraction in burnt (EB) and long-unburnt topsoils (EU) under eucalypt at 1, 6, 12, 18 and 24 months after the wildfire.

Figure 4 Relative abundance of *n*-alkanes and *n*-fatty acid methyl esters (FAME's) in burnt (EB) and long-unburnt (EU) topsoils under eucalypt plantation at 1, 6, 12, 18 and 24 months after the wildfire.

Figure 5 Ratio of unsaturated to *n*-C₁₈ fatty acids in burnt (EB) and long-unburnt (EU) topsoils under eucalypt plantation at 1, 6, 12, 18 and 24 months after the wildfire.

Figure 6 Solid-state ¹³C NMR spectra of burnt (EB) and long-unburnt (EU) topsoils under eucalypt at 1, 12 and 24 months after the wildfire.