THESE DE DOCTORAT DE
L'UNIVERSITE PIERRE ET MARIE CURIE

Spécialité

Ecole Doctorale des sciences de l'environnement d'Ile de France ED 129

Présentée par

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Pour obtenir le grade de

DOCTEUR de l'UNIVERSITÉ PIERRE ET MARIE CURIE

Sujet de la thèse :

The impact of wildfires on the abundance, composition and recalcitrance of soil organic matter

soutenue le 15 Avril 2013

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DOTTORATO DI RICERCA IN
"*Scienza del suolo e climatologia*"

CICLO XXV

COORDINATORE Prof. Luca Calamai

The impact of wildfires on the abundance, composition and recalcitrance of soil organic matter

Settore Scientifico Disciplinare AGR/14

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Summary

Fire affects more land surfaces than any other natural disturbance. It is a major driving factor in forest ecosystems formation and evolution. It has a strong impact on soil organic matter, primarily by changing its abundance. Also, soil organic matter composition is substantially affected by fire. During the combustion, many reactions take place, leading to the formation of thermally-condensed aromatic compounds characterised by high recalcitrance. However, there are still many uncertainties and contrasting results dealing with fire-induced changes in soil organic matter abundance, composition and recalcitrance.

The aims of this study were: (i) assessing the immediate impact of two wildfires of different severity on soil organic matter composition and abundance; (ii) evaluating the complementary application of thermal and spectroscopic techniques to assess fire-induced changes to soil organic matter; (iii) estimating the long-term effect of a high fire frequency on soil organic matter composition and stability. This investigation provided useful information and contributes to the understanding of the overall impact of fire on soil organic matter.

To carry out this study, we selected some forest sites that have been affected by one or more recent or past wildfires.

Two sites, Orentano (Italy) and Mount Gordon (Australia), were contrasted to highlight the effect of wildfires of different severity on soil organic matter abundance and composition. The results were further analysed to find a possible reliable index of fire severity in soil, eventually useful to define the damage degree of soil quality or charring degree of necromass. Biogeochemical parameters, such as soil sugar and lignin contents and composition, as well as the black carbon spectroscopic properties, did not result useful for this purpose.

At Roccastrada, Tuscany, Italy, the soil of a burnt pine forest was investigated by using a set of thermal and spectroscopic – rapid and cheap – techniques, that efficaciously characterised some important fire-induced changes in soil organic matter composition. They provided indications also on the burning temperatures occurred in soil. Fire produced a substantial reduction in the most labile organic fraction and a relative enrichment in recalcitrant organic matter. Additionally, the fire led to the formation of nitrogen-bearing compounds characterised by a fairly resistant structure.

A topo-climosequence on Mt. Etna was finally analysed for estimating the long-term effect of wildfires on soil organic matter composition and stability. The residence time of the stable soil organic matter fraction – charcoal included – decreased from high to lower elevations, where fire frequency is higher. At lower elevations, labile organic matter, more easily degradable, accumulated in the mineral soil. Macroscopic charcoal fragments were present in significant amounts in the soil profile, showing radiocarbon ages up to 1400-1500 years. High fire frequency resulted to act as a powerful rejuvenating factor to soil organic matter, removing part of the very old, biologically recalcitrant organic matter from the surface horizons.

ii

Sommario

Gli incendi interessano vaste aree di superficie terrestre, più di ogni altro fattore naturale di disturbo. Essi sono un fattore ecologico primario nella formazione e nell'evoluzione degli ecosistemi forestali. Gli incendi hanno un forte impatto sulla sostanza organica del suolo, sulla sua abbondanza ma anche sulla sua composizione, che può subire profonde alterazioni. Durante la combustione hanno luogo reazioni che portano alla neo-formazione di composti aromatici, più o meno condensati, ad elevata recalcitranza. Nonostante gli studi intrapresi da tempo sul tema, ad oggi rimangono molte incertezze sui cambiamenti indotti dagli incendi sulla quantità, composizione e recalcitranza della sostanza organica del suolo.

Gli obbiettivi di questa ricerca sono stati: (i) attestare l'impatto, nel brevissimo periodo, che hanno due fuochi di differente intensità sulla composizione e la quantità della sostanza organica del suolo; (ii) valutare l'applicazione complementare di tecniche spettroscopiche e termiche nello studio dei cambiamenti indotti da un incendio sulla sostanza organica del suolo; (iii) stimare, sul lungo periodo, l'effetto che ha un'elevata frequenza degli incendi sulla composizione e la permanenza della sostanza organica del suolo. Le analisi svolte in questa tesi hanno prodotto diversi dati utili, contribuendo alla comprensione dell'impatto degli incendi sulla sostanza organica del suolo.

Per questo studio sono stati selezionati diversi siti forestali che, recentemente o in passato, sono stati percorsi da uno o più incendi.

I primi due siti studiati sono stati Orentano (Italia) e Mount Gordon (Australia). Questi due siti sono stati paragonati per mettere in risalto l'effetto di incendi di differente intensità sulla quantità e sulla composizione della sostanza organica del suolo. I risultati sono stati analizzati cercando di trovare un indice di facile utilizzo in grado di definire sinteticamente l'intensità dell'incendio percepita al livello del suolo e il grado di deterioramento di quest'ultimo. Lo studio dei principali componenti della lettiera, come gli zuccheri e la lignina, e la composizione della sostanza organica totale rilevata con analisi all'NMR, non hanno fornito alcun parametro utile a risalire al grado di intensità dell'incendio a cui il suolo è stato soggetto.

A Roccastrada, Toscana, è stato analizzato un suolo bruciato utilizzando contemporaneamente tecniche termiche e spettroscopiche contraddistinte dall'essere rapide e relativamente economiche. In questo modo è stato possibile caratterizzare efficacemente alcuni importanti cambiamenti della composizione della sostanza organica del suolo indotti dal passaggio del fuoco. Il passaggio del fuoco ha comportato una significativa riduzione della frazione organica, meno resistente alla degradazione biochimica, ed un relativo arricchimento di quella più recalcitrante. In più, il passaggio del fuoco ha portato alla formazione di composti contenenti azoto re-arrangiati in strutture relativamente resistenti alla degradazione.

Infine è stata analizzata una sequenza climatica/altitudinale di suoli sull'Etna per valutare l'effetto di lungo termine degli incendi sulla composizione e la stabilità della sostanza organica del suolo.

Sull'Etna, alle altitudini meno elevate, dove la frequenza degli incendi è maggiore, il tempo di residenza della frazione stabile della sostanza organica, carbone compreso, si è rivelato minore che alle quote superiori, mentre è stato rilevato un accumulo della frazione meno resistente e più facilmente degradabile. Ciononostante, in alcuni casi il carbone (macroscopico) contribuiva significativamente allo *stock* di carbonio totale nell'intero profilo del suolo, con tempi di permanenza stimati, con la tecnica del radiocarbonio, fino a 1400-1500 anni. Quindi un'elevata frequenza degli incendi sembra avere un "potere ringiovanente" sulla sostanza organica, rimuovendo, dalla superficie dei suoli in maniera non selettiva, parte della frazione con elevati tempi di residenza e biologicamente più recalcitrante.

Résumé

Les feux de forêt affectent une surface plus grande que n'importe quel autre perturbation naturel. Il s'agit d'un facteur majeur dans la formation et évolution des écosystèmes forestiers. Le feu provoque des forts impacts sur les matières organiques des sols, notamment sur leur teneur et composition. Lors de la combustion, plusieurs réactions chimiques ont lieu qui conduisent à la production des nouveaux composés aromatiques condensés thermiquement, caractérisés par leur récalcitrance chimique. Ils existent encore nombreuses incertitudes et résultats contradictoires en ce qui concerne les effets du feu sur l'abondance, composition et récalcitrance des matières organiques du sol.

Le but de cette recherche était : (i) d'attester des impacts immédiats de deux feux de forêt de sévérités différentes sur la composition et l'abondance de la matière organique du sol; (ii) évaluer l'application complémentaire de techniques thermiques et spectroscopiques afin d'attester les changements induits par le feu sur la matière organique du sol; (iii) estimer les effets à long terme d'une fréquence importante de feux sur la composition et la stabilité de la matière organique du sol. La recherche présentée ici fournit des données utiles pour la compréhension des impacts du feu sur les matières organiques du sol.

Nous avons sélectionné ici des sites forestiers affectés par un ou plusieurs incendies, récemment ou dans le passé.

Deux sites, Orentano (Italie) et Mount Gordon (Australie), ont été comparés pour étudier l'effet de la sévérité du feu sur l'abondance et la composition de la matière organique du sol. Les résultats ont été analysés avec l'intention de trouver un paramètre qui puisse représenter à la fois un indice de la sévérité du feu et du degré de la dégradation de la qualité du sol. Pourtant, l'analyse des principaux composants de la matière organique de surface, comme sucres et lignines, ainsi que la teneur des composés aromatiques par spectroscopie RMN de 13C, n'ont pas fourni un paramètre simple capable d'élucider le degré de carbonisation de la necromasse ou la sévérité du feu subie par la surface du sol.

À Roccastrada (Toscane, Italie) le sol d'une pinède brulée a été étudié par des techniques thermiques et spectroscopiques, ce qui nous a permis la caractérisation efficace des modifications majeures provoquées par le feu dans la composition des matières organiques du sol. Ces analyses ont fourni des indications concernant la température du sol pendant le feu, qui a donné lieu à des réductions substantielles des fractions organiques les plus labiles et à des enrichissements relatifs en matières organiques récalcitrantes. En plus, le feu a provoqué la formation de composés azotés caractérisés par leur structure très résistante.

Enfin, une topo-climoséquence au Mt. Etna a été étudiée pour estimer les effets à long terme des incendies sur la composition et stabilité des matières organiques du sol. Dans les endroits où la fréquence des incendies est la plus haute, la teneur de la fraction stable de la matière organique du sol, y compris le charbon, est faible et la matières organiques la plus labile s'accumule dans le

sol minéral. Néanmoins, des quantités significatives de charbon macroscopique y existent avec une âge estimée au ^{14}C de 1400-1500 ans. Les feu fréquences ainsi que les feu d'haute intensité agissent comme un facteur puissant de rajeunissement de la matière organique du sol, en éliminant une partie de la fraction plus ancienne et biologiquement récalcitrante.

Contents

Part I – Thesis text

Part II – Manuscripts

List of Figures – Part I

List of Tables – Part I

Part I – Thesis text

1. INTODUCTION

"A world without fires is like a sphere without roundness —
that is, we cannot imagine it."
(Pausas and Keeley, 2009)

1.1   Wildfire, global change and their interplay

Fire is a widespread phenomenon on the earth surface and it affects more lands than any other natural disturbance. Most probably all of the terrestrial ecosystems experienced at least one fire during their evolution (Bowman et al., 2009). In the forest ecosystems it is often a major driving factor in forest renovation and structure (Certini, 2005; Flannigan et al., 2006; Lavorel et al., 2007). Nowadays, approximately 10-15 x 10^6 ha of boreal and temperate forest, 20-40 x 10^6 ha of tropical forests, and up to 500 x 10^6 ha of tropical and subtropical savannas, woodlands and open forests are burnt every year (Goldammer, 1993).

Fire occurrence is strongly influenced by four factors: weather/climate, fuels, ignition agents and human activities (Johnson, 1996). All these factors are strictly related to each other, whereas not all of them have, actually, the same weight. It is evident, in fact, that human activity has the strongest direct and indirect influence on fire occurrence. Industrial progress led to an unprecedented fire regime change in history, through the increasing in fuel buildup and hazard, resulting in anomalously big, catastrophic wildfires, due to abandonment of farms and to a highly effective fire-suppression policy (Pausas et al., 2008).

Global change, human induced or not, is another indisputable factor that could have a dramatically rapid and profound impact on fire activity, leading to a shift from the natural fire regime to a more intense fire regime that led to the evolution of fire adapted ecosystems and to plant species drift and migration. In the Mediterranean climate regions, where already fire plays a major role in forest ecosystems, climate change is leading to increasing temperature and decreasing precipitation (IPCC Fourth Assessment Report, 2007). This facilitates the spread of fires and their frequency.

Furthermore, the influence of climate change on fire intensity may be a secondary effect as a result of decreased fuel moisture, increased fuel load, increased wind speed, a change to more conducive fuel types or a combination of any of the above (Flannigan et al., 2006). Fire intensity, expressed as the energy released from the combustion, is directly related to fire severity, *i.e.* the loss of above and below organic matter. Both factors will control ecosystem response, such as soil erosion or natural revegetation (Keeley, 2009). Summarising, as a result of global warming, we have to expect an elongation of fire season, an increase in fire frequency and a rise of the occurrence of big fires, with disastrous effects on the forest ecosystems and public health hazard (Flannigan et al., 2006; Keim, 2008).

Fire plays also a major role in carbon dynamics and the occurrence of fires is partly responsible for the greenhouse effect. Large amounts of CO_2, as well as smaller amounts of other greenhouse gases such as methane and nitrogen oxides, are in fact released to the atmosphere via biomass combustion. Also, the decomposition of fire affected vegetation and the increasing of soil surface albedo, due to the darker colour of burning products, produce a higher release of carbon dioxide in the atmosphere (Flannigan et al., 2006). Carbon release from biomass burning is estimated 2.5 Pg C per year (van der Werf et al., 2006) and, although the net C balance has to be considered null, assuming (implausibly) the released CO_2 to be fixed again by re-growing vegetation, there is a negative interplay between climate change and wildfires. In the short term and at regional scale, smoke can have a positive feedback on weather and fire activity reducing local precipitation (Rosenfeld, 1999). Big fires probably have the potential to change even global radiation budgets (Simmonds et al. 2005). Therefore, there is the possibility of a positive feedback, whereby a warmer and drier climate will lead to higher fire frequency that, in turn, will increase carbon emissions from fires, which would feed the global warming (Flannigan et al., 2006).

1.2 Wildfire induced temperatures in soil

Temperatures developed by wildfires are generally in the range of 50° - 1500° and the heat release can vary between 2.1 x 10^3J kg^{-1} and 2.1 x 10^6J kg^{-1} fuel (Neary et al., 1999). Temperatures between 200 and 400 °C define low intensity fires, while temperatures ranging between 700-800 °C are typical of high intensity fires (Kennard and Gholtz, 2001). However, only a small part of the heat generated during a forest fire is radiated to soil. During forest fires, maximum ground temperatures are typically in the range of 200–300 °C, decreasing substantially within a few

centimetres because of the low thermal conductivity of soil, whereas at soil surface temperatures peaks could reach up to 500-700 °C (Neary et al., 1999; Bento-Gonçalves et al., in press).

The degree of soil heating depends on several soil properties. In general, sandy soils are better insulated against heat transfer than fine textured soils, due to a higher content of air-filled coarse pores (Knicker, 2005). In dry soils below-ground temperatures actually rise very slowly because air is a very good insulator (DeBano et al. 1991). Heat, in fact, is transported faster and penetrates deeper in moist soils than in dry soils. In moist soils, water evaporation does not allow the temperatures to go above the water boiling point (Campbell et al. 1995).

All these soil characteristics as well as fire intensity, that in turn depends on topography, wheather (*e.g.* temperature and wind speed) and vegetation characteristics (*e.g.* fuel load, moisture and flammability), concur to define the fire severity that finally is measured as the loss of aboveground and belowground organic matter. Chafer et al. (2004) proposed a visual scale for fire severity based on five levels of vegetal damage:

- Low severity – ground fuel and low shrubs burnt;
- Moderate severity – ground fuel and shrubs to 4 m burnt;
- High severity – ground fuel, shrubs incinerated, canopy scorched;
- Very high severity – ground fuel, shrubs incinerated and canopy completely burnt (all vegetation <5 mm incinerated);
- Extreme severity – all grenn vegetation burnt and stems <10 mm thick incinerated.

1.3   *Fire effect on soil organic matter*

The effect of fire on soil organic matter (SOM) may differ depending on many factors that are correlated with topography, climate, and soil and vegetation features, but also, in particular, on the burning duration and intensity (Certini, 2005). Loss in soil organic carbon starts at temperatures between 100 and 200 °C (Giovannini and Lucchesi, 1997) and at around 460 °C, provided that oxygen is available, almost all of unprotected SOM is combusted (Neary et al., 1999). Hence, fire occurrence implies an immediate net loss of OM from the litter layer and possibly from the mineral topsoil. By a meta-analysis which includes data from thirty studies, Nave et al. (2011) demonstrated that in temperate forest soils, wildfires have relatively consistent effect on soil C and N, principally through the combustion of forest floor, which lost 67% and 69% of its C and N pools, respectively.

Also mineral soil C and N concentration significantly declines (on average 17 and 18% respectively).

The simplest model for biomass burning assumes that the end products are H_2O, CO_2 and salts contained in the ash; complete oxidation of biomass requires a continuous oxygen availability during the combustion process which, in general, does not occur completely in nature (Gonzàlez-Pèrez et al., 2004). Hence, under natural conditions, combustion is often incomplete and a loss of OM is not the only consequence of fire.

Many reactions take place during biomass combustion, *e.g.* dehydration, dehydrogenation, volatilisation of nitrogenous compounds, loss of functional groups (*e.g.* decarboxylation and demethylisation), cyclisation and polycondensation (Hernández et al., 1997; Knicker, 2007). Fire-induecd SOM rearrangement has been partly compared to humification, at least for the increasing of the degree of polymerization of the OM (Gonzàlez-Pèrez et al., 2004). However, the recalcitrance of charred materials is higher compared to naturally humified litter (Knicker, 2011). Additionally thermal treatment removes external O-groups, yielding hydrophobic materials, that Increase soil hydrophobicity (Almendros et al., 1992).

Fire substantially changes SOM composition. SOM is currently described as a complex mixture of biopolymers (Kelleher and Simpson, 2006; Lehmann et al., 2008) with five main biochemical components: carbohydrate, lignin, protein, lipid and fire-derived organic matter (Schmidt et al., 2011; Cécillon et al., 2012). How fire changes the composition and the structure of each of these components is not yet clear and data from the literature are partial and sometimes contrasting.

Polysaccharides are the most abundant litter component and usually are more affected than other SOM components as lignin or lipids. (Neff et al., 2005; Knicker, 2007; Martin et al., 2009). ^{13}C NMR spectroscopy confirms that, under relatively mild heating, the main fire-induced processes are dehydration, dehydrogenation and degradation of O-alkyl C, *i.e.* mainly carbohydrates (Baldock and Smernik 2002; Almendros et al. 2003; González-Pérez et al., 2004), which yield aromatic C and furans (Knicker, 2007). Actually, furans or furan-like structures from cellulose and other carbohydrates are important constituents of the molecular structure of fire-derived organic matter (McGrath et al. 2003). The loss of thermally labile polysaccharides and the proportional increase in aromatic compounds very likely represent a conversion of SOM composition from labile materials to more recalcitrant compounds (Neff et al., 2005).

Lignin, the second major constituent of plant biomass, can be immediately affected by fire in terms of phenols distribution, even at relatively low temperatures, (Kuo et al., 2008; Rumpel et al., 2007; Nocentini et al. 2010). However, lignin is considered somewhat recalcitrant against the heating and

the aromatic skeleton of materials remaining after fire often base on remnants of the lignin backbone (Knicker, 2007). At temperatures <350 °C the predominant fire-induced reaction in lignin structure is dehydration while some decarboxylation occurs at higher temperatures; however, the aromatic rings of lignin structure still remain essentially intact (Sharma et al., 2004).

However, most of the above-mentioned studies were carried out using pure organic matter in laboratory studies. Few investigations have addressed changes in composition of SOM in mineral soil in field studies immediately after fire (e.g. Alexis et al., 2010; Certini et al., 2011) and none of them addressed the effect of different severity fire on SOM composition.

Summarising, fire increases SOM heterogeneity because no original component is totally removed, while new fire-derived aromatic thermally-condensed organic compounds can form in addition to thermal modification of the previously existing C forms, (Kuhlbusch and Crutzen, 1995; Schmidt and Noack, 2000; González-Pérez et al., 2004; Francioso et al., 2011). The new so formed pool is termed pyrogenic organic matter (PyOM).

The combustion residues can play important roles in soil, binding organic pollutants and heavy metals (Glaser et al., 2005), increasing soil carbon sequestration and fertility (Schmidt et al., 2002), potentially offsetting increases in atmospheric CO_2 (Kuhlbusch, 1995; Schmidt & Noack, 2000; Ansley et al., 2006). Furthermore, the analysis (extraction, identification and radiocarbon dating) of macrofossil charred residues buried in soils, *i.e.* charcoal fragments, allow reconstructing fire activities, vegetation and climate of the past (Carcaillet and Brun, 2000; Favilli et al., 2009, 2010).

The formation and the incorporation of charred material into the mineral soil may increase SOM content of this latter (Knicker et al., 2005a). Furthermore, a fraction of the total organic C produced from biomass burning may show high recalcitrance, due to a highly aromatic structure. This latter point received much attention in the last years because part of the charred residues are likely to be sequestered in the stable slow-cycling 'geological' carbon reservoir (Bird et al., 1999; Preston and Schmidt, 2006; Marschner, 2008).

1.4 Pyrogenic organic matter

PyOM has neither defined chemical structure nor unique definition. Actually, there is large confusion, because several terms have been used in the literature for naming the products of incomplete combustions of above and below biomass and the related materials in soil.

Accordingly to Knicker (2011), I decided to use the term 'PyOM' to account for the fact that thermally altered materials is not limited to carbon, but comprises a considerable proportion of heteroaromatic –in particular N-containing- constituents.

The theoretical model of charred materials is a graphene-like structure, *i.e.* stacks of few graphene layers of carbon atoms with a low number of external functional groups (Schmidt and Noack, 2000). For this reason, when it refers limited to C, one of the most used term is pyrogenic carbon (PyC), that is defined as a continuum of fire-altered organic materials, ranging from slightly charred degradable biomass to highly condensed, refractory C compounds (Masiello, 2004) (Fig. 1). At the end of this combustion continuum there is the so called 'black carbon' (BC), the most recalcitrant fraction, whereas the macroscopic pieces left after biomass combustion that can be identified by visual assessment are usually called 'charcoal' (Preston and Schmidt, 2006).

Figure 1. The 'pyrogenic carbon continuum' and variation in the characteristics of pyrolyzed material along the continuum of relevance to isotope studies (from Bird and Ascough, 2012). Environmental alteration potential refers to the degree to which the material is likely to be susceptible to chemical degradation. Transport potential refers to the degree to which the material is likely to be physically dispersed in the environment from its place of formation, controlled largely by particle size. The term 'molecular' acknowledges the fact that a range of distinct molecular and nanometre scale crystalline materials derived from the pyrolysis of biomass also exist, including polycyclic aromatic hydrocarbons and graphene.

	pyrogenic carbon continuum		
	partly charred biomass	charcoal	black carbon (molecular)
pyrogenic carbon content	lower		higher
chemical structure	'disorganized' low aromaticity		'organized' high aromaticity
common particle size	mm and larger	mm-cm	µm and smaller
common formation temperature	<350°C	>350°C	>500°C
material type	solid pyrolysis residue		gas phase condensation products
O/C and H/C	>0.5		<0.5
environmental alteration potential	higher		lower
transport potential	lower (surface)		higher (atmospheric)
porosity	lower		higher

The composition of PyC is highly dependent, among other factors, on the parent material and fire characteristics. In fact, for instance, wood is able to suffer a greater degree of alteration than leaf material, without being entirely consumed (Alexis et al., 2010). Since the thickness and density of wood pieces may induce lower oxygen availability during burning, the resulting wood charcoal could thus have a more condensed structure than leaf charcoal, resulting in a higher stability (Alexis et al., 2010). On the other hand, lower PyC accumulation was observed after intense fires, compared to low to moderate ones, which was attributed to a more complete volatilization during severe heating (Knicker et al., 2006).

PyC is virtually ubiquitous in soil and its contribution to total organic carbon (TOC) could be significant, up to 45% (Schmidt et al., 2002). The estimated annual PyC production from wildfire is 0.05–0.27 Gt C year^{-1} (Kuhlbusch and Crutzen, 1995). This value is only a rough estimation of the PyC release, because none of the methods methods to quantify PyC or BC are able to determine the whole combustion continuum. Hammes et al. (2007), comparing many methods, found highly variable results, which also confirm the complexity of PyC material.

PyOM is considered the most recalcitrant component of SOM, hence representing an important sink of stable C within the global C cycle (Kuhlbusch and Crutzen, 1995; Schmidt and Noack, 2000) and receiving much attention for having the potential to transform labile organic compounds, like polysaccharides and lignin, into highly recalcitrant aromatic structures (Almendros et al., 2003). However, modern studies have demonstrated that PyOM is not as resistant to both biotic and abiotic decomposition as previously assumed (Bird et al., 1999; Cheng et al., 2006; Czimzick et al., 2005; Hamer et al., 2004; Knicker et al., 2006; Singh et al., 2012).

Uncertainties with PyOM identification and quantification may explain why many contradictory turnover times and degradation rates are reported for PyOM or BC (Knicker, 2007). However, some of these uncertainties depend on the biased idea that all PyOM consists in a highly polycondensed aromatic network that resists both thermal and chemical oxidation. On the contrary, PyOM produced during vegetation fires comprises only a minor fraction (if any) of these structures (Knicker, 2011). Knicker et al. (2005b), advanced that most of the aromatic C in charcoal must occur in relatively small clusters with an average size of at most six aromatic rings, hence quite different from a graphite structure.

On such basis, Knicker et al. (2008a) proposed an alternative model of PyOM where it is a heterogeneous mixture of thermally altered bio-macromolecules. The degree of alteration and the recalcitrance of charred material depend upon the nature of the original vegetation and fire conditions. Plant derived PyOM contain discrete proportions of the lignin backbone, but also

furans, anhydrosugars and most likely pyranones from cellulose. Additionally, for plant and litter residues with relatively high nitrogen contents, a significant fraction of PyOM is N-heteroaromatic carbon. Finally, the so defined PyOM can undergo to further alteration that will occur during its aging.

1.5   *Studying the composition of fire-affected SOM*

More works are needed to fully understand the structural fire-induced changes in SOM composition. One of the main limiting factor is the lack of a unique method that allows disentangling the complex nature of SOM and possibly being rapid, cost effective, quantitative, reliable and universal (Cécillon et al., 2012). All available methods for quantifying SOM composition, in fact, share the disadvantage of being unselective, time consuming, expensive and sometimes difficult to compare in terms of results (Kögel-Knabner, 2000; Grandy and Neff, 2008; Cécillon et al., 2012).

In the last years a few techniques were particularly employed for determining most of soil biochemical components simultaneously. Nevertheless, all of them fail to provide a comprehensive insight of the composition of SOM and, hence, of PyOM. One of these technique is analytical pyrolysis, able to provide many molecular features of SOM (Grandy and Neff, 2008; Rumpel et al., 2009). But, such a technique has several limitations and, furthermore, does not allow the quantification of PyOM (Saiz-Jimenez, 1994; Cécillon et al., 2012).

During the last decades, solid state ^{13}C nuclear magnetic resonance spectroscopy (NMR) has become one of the most powerful non-destructive technique able to provide detailed information on the chemistry of organic carbon (Oades et al., 1987; Baldock et al., 1992; Knicker et al., 1996; Skjemstad et al., 1999; Kögel-Knabner, 2000; Mathers et al., 2000; Forte et al., 2006; Knicker et al., 2006). However, it remains inappropriate when designing a routine method, as it is neither rapid nor cost effective. The acquisition of high quality NMR spectra, in fact, requires time (in most cases 24–48 h per sample), expertise and expensive equipment (Cécillon et al., 2012). Furthermore, for improving the quality of the spectra, a treatment of soil samples with strong acids is required to remove paramagnetic minerals (chiefly iron oxides) which strongly reduce the signal-to-noise ratio of the spectra. HF is highly efficient, but typically removes 10–30% organic carbon in mineral soils (Hockaday et al., 2009). Although selective losses of specific groups should be only minor (Rumpel et al., 2006; Knicker et al., 2008b; Knicker, 2011), the possible modification of SOM composition

after HF treatment raises an important question regarding the reliability of NMR analysis (Cécillon et al., 2012).

No single analytical method can entirely account for the chemical complexity of bulk SOM and the continuum of materials that form during fire. As a consequence, a multiple-technique analytical approach, possibly employing rapid, cost effective and reliable methods, appears to be the only viable way to overcome the faults intrinsic in each method.

2. OBJECTIVES

Despite the numerous studies published in the last decades dealing with fire effect on soil organic matter, there are still many uncertainties and contrasting outcomes about this topic. This situation is mainly due to (1) the complexity of SOM and its byproducts of fire, which is a heterogeneous material, (2) to the variety of fire characteristics and (3) to the diversity of the environments in which fire occurs.

The main aim of this thesis is to contribute to the effort sustained so far and to provide useful data to better understand the impact of wildfires on the abundance, composition and recalcitrance of soil organic matter. For this purpose, we selected forest sites, which were characterized by the occurrence of fire with contrasting intensities and focused on three objectives.

I. Assessing the immediate impact of two wildfires of different severity on soil organic matter abundance and composition, with particular attention to the most labile organic fraction *(manuscript I)*

II. Evaluating the meaningfulness of the complementary application of rapid analyses, *e.g.* thermal and spectroscopic techniques, to assess the transformation and the stability of pyrogenic organic matter in a typical Mediterranean burnt forest *(manuscript II)*

III. Estimating the long-term effect of wildfire on soil organic matter composition and stability, under plausible climate change scenario, *i.e.* increased fire frequency *(manuscript III and IV)*

3. SUMMARY OF MATERIALS AND METHODS

We investigated four main study areas affected by wildfires, recently or in the past: at Orentano, Tuscany, Central Italy, at Mount Gordon, Victoria state, South-East Australia, at Roccastrada, again in Tuscany and at Mount Etna, Sicily (Southern Italy) and (Fig. 2).

Figure 2. The study areas; a) in Italy: Orentano and Roccastrada (Tuscany), Mount Etna (Sicily); b) in Australia: Mount Gordon (Victoria state).
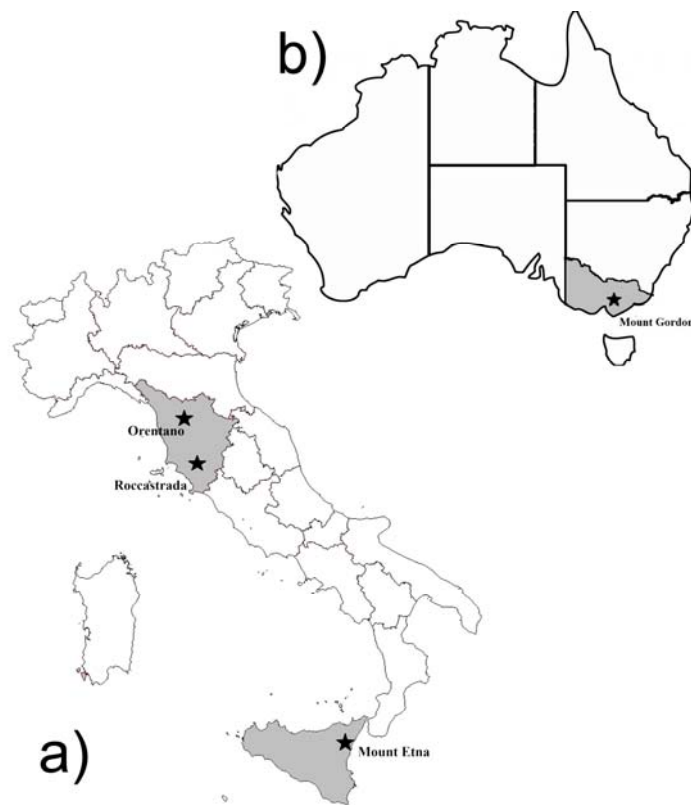
3.1 Comparison of the effects of two wildfires of different severity

This study was carried out at two study sites: Orentano is a mixed Mediterranean forest burnt in July 2011. The burnt area was relatively small, only 3.3 ha, and fire severity, basing on the visual scale of fire severity by Chafer et al. (2004), was classified as moderate to high (Fig. 3).

The study site at Mount Gordon was involved in the sadly famous 'Black Saturday' wildfire, occurring in early February 2009, which burnt 450,000 ha of eucalypt forest in Victoria State and

Figure 3. The Orentano study site as appeared at sampling time, three days after wildfire

Figure 4. A forest affected by 'Black Saturday fire' 10 km far from Mount Gordon (Photo byPeter Campbell from www.wikipedia.org)

caused the tragic 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 substantially higher than any previously reported in Australia (Royal Commission 2009). Resulting fire severity was classified as extreme (Chafer et al., 2004) (Fig. 4).

At each study site, a burnt area and a comparable unburnt area were taken into account. Orentano and Mount Gordon areas are different for many aspects, nevertheless SOM changes at the two sites were contrasted to highlight the effect of different fire severity.

The sampling involved four parallel 20 m transects, laid out 5 m apart, at 5 m intervals. Twenty soil samples were taken each site down to 2.5 cm, after removing the ash, charcoal, or litter layer by a brush. Ten samples from the charcoal layer were collected randomly, from 40 x 40 cm squares.

Table 1. Methods used to assess the immediate impact of two wildfires of different severity on soil organic matter abundance and composition. For details see *manuscript I*

C, N and δ^{13} C concentration	elemental analyser
Lignin phenols	Cupric oxide (CuO) oxidation and analysis by gas chromatography-flame ionisation detector (GC-FID). Quantification of Vanillyl, Syringyl and Cinnamyl phenols
Non-cellulosic neutral sugars	Acid hydrolysis by trifluoroacetic acid (TFA) and analysis by gas chromatography-flame ionisation detector (GC-FID). Quantification of sugars monomers
Bulk SOM composition	^{13}C CP\MAS nuclear magnetic resonance (NMR)

3.2 *Study on the application of thermal and spectroscopic techniques to PyOM characterisation*

This study was carried out at Roccastrada, Southern Tuscany, Central Italy. The investigated site is a typical Mediterranean artificial pine forest of maritime pine (*Pinus pinaster* Ait.) mixed to ancillary trees of *Quercus suber* and *Quercus ilex*. In July 2010 this forest was involved by a wildfire that burned an area of 33 ha, where the forest had been just cut and the felled logs were

lying on the ground. Based on the visual scale of fire severity proposed by Chafer et al. (2004), the wildfire in question was classified as highly severe (Fig. 5)

Figure 5. Roccastrada burnt site as appeared at sampling time two months after wildfire

Soil sampling was random, on six plots from the burnt area and 6 plots from an adjacent unburnt area quite similar to the burnt one prior to the fire. The ash layer, in the burnt area, and the litter layer overlying the mineral soil, in the unburnt area, were sampled separately on a 40 x 10 cm^2 area, using a trowel and a brush. The mineral soil was sampled with a spade down to 10 cm, cutting a 15x15 cm tile of soil.

Table 2. Methods used to evaluate the application of thermal and spectroscopic techniques on PyOM characterisation. For details see *manuscript II*

C and N concentration	elemental analyser
SOM extraction	Soil treatment with a 0.5 M NaOH solution
Thermal analyses	Thermogravimetric (TG) and derivate thermogravimetric analysis (DTG); differential scanning calorimetry (DSC) analysis.
Vibrational spectroscopic analyses	Attenuated total reflectance Fourier Transform infrared spectroscopy (ATR/FT-IR); Raman spectoscopy
Additional spectroscopic characterisation	^{13}C CP\MAS nuclear magnetic resonance (NMR)

3.3 *Study on long-term effects of fire on soil organic matter along a topo-climosequence*

This study was carried out along an elevational gradient on the northeast flank of Mount Etna (Sicily, Italy) ranging from Mediterranean (551 m asl; warm, semi-arid to sub-humid climate) to subalpine (1772 m asl; cold, humid climate) climate zones (Fig. 6). We hypothesised that fire frequency was higher at lower elevated sites and that a comparison between SOM features at six different sites could help to provide predictions on the fate of OM in the soils at higher elevated sites under climate change scenario.

At each site, three soil profiles were opened and sampled at two different depth intervals. Charcoal fragments were separated from soil material by hand-picking or floating and subsequently analysed microscopically for recognising the parent plants.

Figure 6. Location of the investigated sites (topo-climosequence) in the Etna region (Sicily, southern Italy) (from *manuscript III and IV*)
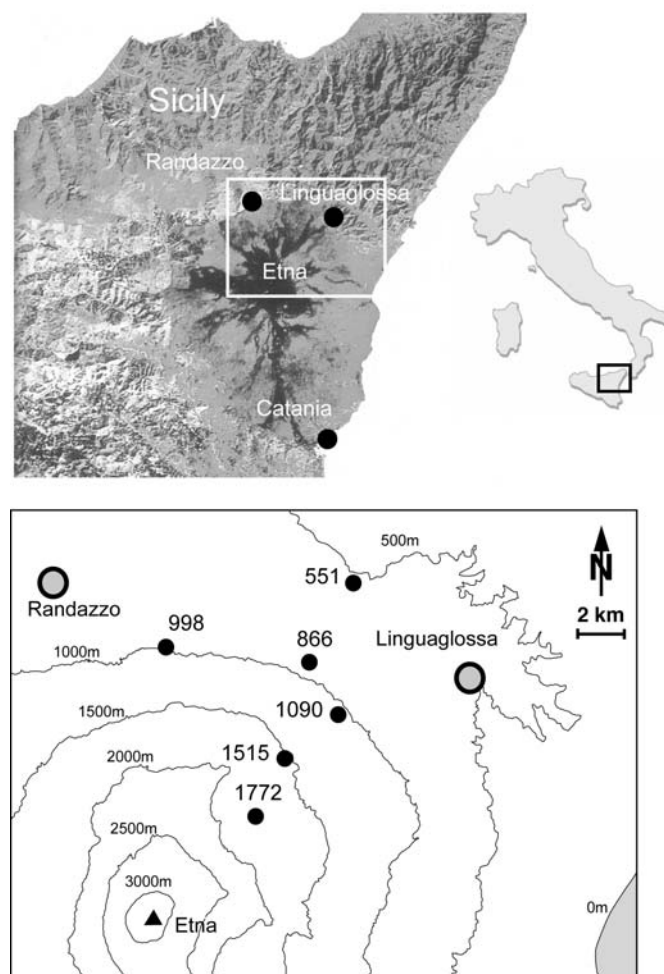
Table 3. Methods used to estimate the long-term effects of fire on soil organic matter along a topo-climo**sequence. For details see** *manuscript III and IV*

C, N	elemental analyser
SOM chemical fractionation	Soil treatment with a 10% H_2O_2 solution
SOM physical fractionation	Na-polytungstate solutions was used to separate four fractions: <1.0 g cm^{-3}, 1.0-1.6 g cm^{-3}, 1.6-2.0 g cm^{-3} and >2.0 g cm^{-3}
Dating of charcoal fragments and SOM stable fraction	Accelerator mass spectrometry (AMS) ^{14}C (Swiss federal institute of technology, Zürich); calibration with OxCal ver.4.1
Black carbon estimation	chemical oxidation resistant carbon (COREC) content determined by oxidation with dichromate solution
SOM composition	^{13}C nuclear magnetic resonance (NMR), diffuse reflectance infrared fourier transform (DRIFT)
Visual observation	Scanning electron microscope (SEM) equipped with an energy-dispersive spectrometer (EDS).

4. SYNTHESIS OF THE RESULTS AND DISCUSSION

4.1   Immediate impact of two wildfires of different severity on soil organic matter abundance and composition

At Orentano (OR), where fire severity was moderate/high, the mean concentration of soil carbon slightly increased after the fire occurrence, 100 and 84 g kg^{-1} for burnt and unburnt soils respectively, while the changes of nitrogen concentration and C/N ratio were not statistically relevant. The increase of C concentration was evidently due to the incorporation into the mineral soil of the unburnt woody residues and charred necromass. The significantly lower δ^{13}C value for the burnt soil compared to the unburnt one seems to be related to an incorporation into the top mineral soil of charcoal particles covering the burnt soil. The charcoal particles had in fact a lower mean δ^{13}C (-28.5 ‰) compared to the unburnt soil (-27,4 ‰).

At Mount Gordon (MG), the extremely severe wildfire caused a sharp reduction in soil C and N content. The C content in the bulk mineral soil decreased from 164 to 88 g kg^{-1} while the N content from 5.8 to 4.3 g kg^{-1}, with overall decrease of C/N ratio of soil. We postulates that an accumulation of recalcitrant organic N-forms in the charred material (Almendros et al., 2003; Knicker et al., 2005b; Santin et al., 2008) could have led to a decrease of the C/N in the burnt soil (Fernández et al., 1999; González-Pérez et al., 2004). As at OR, an incorporation of charred material in soil at MG induced a significant change of δ^{13}C value in the burnt soil. However, at MG the burnt soil showed a higher δ^{13}C value compared to the unburnt, -27.5 ‰ versus -27.7 ‰ respectively, because charcoal had a higher δ^{13}C value compared to the unburnt soil.

At OR, the average content of non-cellulosic neutral sugars in the burnt mineral soil slightly decreased compared to the unburnt soil, but this decrease was not statistically significant either expressed on a bulk soil and soil organic carbon (SOC) basis (Fig. 7). On the contrary, in the burnt soil at MG, the net amount of non-cellulosic neutral sugars in the bulk soil dramatically decreased from 21 g kg^{-1} to 9 g kg^{-1} (Fig. 8). However, there is no decrease when data are reported as SOC-normalised content. This means that sugars were not preferentially oxidised by fire, despite they are part of the most thermally labile SOM pool (Manning et al., 2005; De la Rosa et al., 2008; Plante et

al., 2011). The physical protection of sugars by minerals might explain their relative preservation to fire.
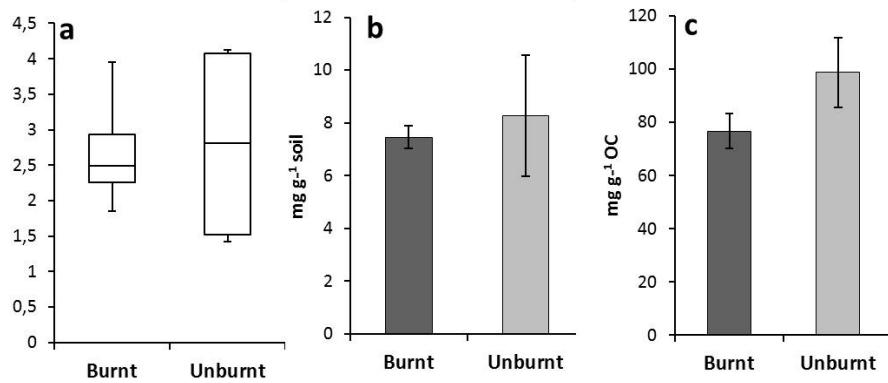
Figure 7. Orentano (Italy): a. Box-plot of GM/AX (galactose + mannose)/(arabinose + xylose) ratio; histogram of non-cellulosic neutral sugars content of burnt and unburnt soil expressed as b. net total amount and c. relative to SOC (bars represent standard deviation; n=4).

The results of sugars content in soil at both OR and MG are in contrast with several studies reporting that fire affects the sugars pool proportionally more than the total SOM (González-Pérez et al., 2004; Kavdir et al., 2005; Knicker et al., 2006; Martin et al., 2009). Martin et al. (2009) even proposed the carbohydrate C to total organic C ratio as a reliable indicator of fire-induced changes on SOM quality, which can also allowing differentiating between low- and high-severity wildfires. Such an indicator, however, does not work in our case, because the sugar content relative to OC did not change because of fire at both OR and MG.
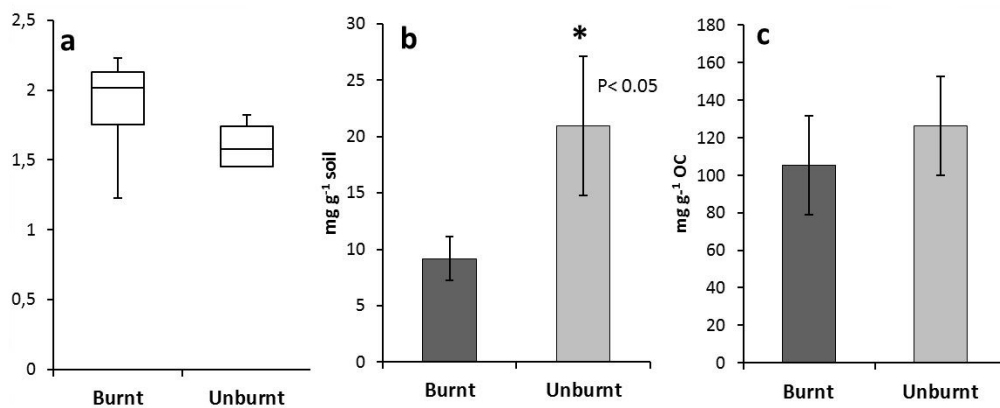
Figure 8. Mount Gordon (Australia): a. Box-plot of GM/AX (galactose + mannose)/(arabinose + xylose) ratio; histogram of non-cellulosic neutral sugars content of burnt and unburnt soil expressed as b. net total amount and c. relative to SOC (bars represent standard deviation; n=4). * indicates significantly different means between burnt and unburnt soil (P<0.05, t-test).

The proportion of microbially-derived sugars in relation to plant sugars was assessed by the mass ratio of hexoses to pentoses sugars, namely (galactose + mannose)/(arabinose + xylose) = (GM/AX). Low (<0.5) or high (>2) GM/AX ratios discriminate between carbohydrates predominantly derived from plants and microbial input, respectively (Oades, 1984; Guggenberger and Zech, 1994).

At both sites, OR and MG, a GM/AX> 2 revealed that in the unburnt soil the carbohydrates were mainly originated by microorganisms (Fig. 7 and 8). This value supports our hypothesis that sugars were present in soil mainly in protected form, by an interaction with soil minerals. The GM/AX ratio was not changed by fire, so indicating that the pentose or hexose sugars were not selectively oxidised by fire.

The sum of vanillyl (V-), syringyl (S-) and cinnamyl (C-) type phenols (VSC), was used to estimate the total amount of lignin in soil. Despite lignin is generally considered to be recalcitrant against the heating, at least more than carbohydrates (Lopez-Capel et al., 2005; Manning et al., 2005; Plante et al., 2009) at OR the amount of VSC phenols in the burnt soil decreased significantly after the fire, both as total amount and the SOC-normalised content (Fig. 9). It is important to remember that lignin is not protected by mineral interactions and could therefore be more prone to loss during fire compared to mineral-protected carbohydrates (Rumpel et al., 2004; Spielvogel et al., 2007).
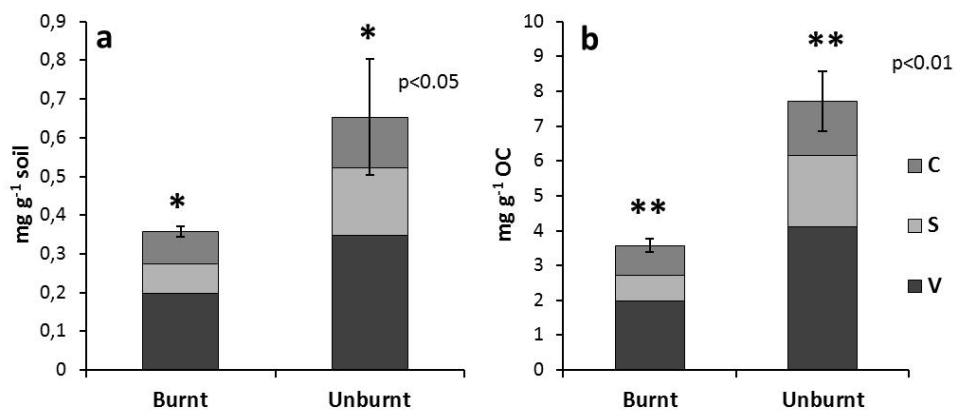
Figure 9. Sum of vanillyl (V), syringyl (S) and cinnamyl (C) units of burnt and unburnt soil at Orentano (Italy) expressed as a. net total amount and b. relative to SOC (bars represent standard deviation; n=4). * and ** indicate significantly different means between burnt and unburnt soil (* P<0.05; ** P<0.01, t-test).

At MG, the total amount of lignin phenols was half in the burnt bulk soil compared to the unburnt one (Fig. 10). VSC content on a SOC basis, instead, was unaffected by fire, as in the case of soil

sugars content. Fire of extreme severiy, hence, apparently oxidised unselectively the bulk SOM, regardless the different resistance to heating of the various SOM constituents.
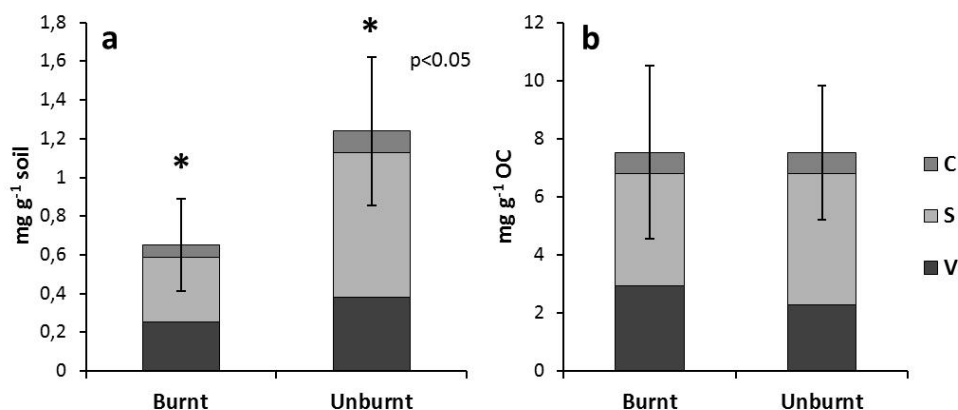
Figure 10. Sum of vanillyl (V), syringyl (S) and cinnamyl (C) units of burnt and unburnt soil at Mount Gordon (Australia) expressed as a. net total amount and b. relative to SOC (bars represent standard deviation; n=4). * indicates significantly different means between burnt and unburnt soil (* P<0.05, t-test).

At both OR and MG, the proportion between the three lignin types phenols (V, S and C) did not change because of fire, indicating that they were oxidised unselectively, in spite of what reported by some authors (*e.g.* Rumpel et al., 2007; Kuo et al., 2008; Certini et al., 2011; Nocentini et al., 2011). These authors also found, in both laboratory and field studies, a higher thermal susceptibility of aldehydes that resulted in an increasing Acid-to-Aldehyde ratio for lignin V and S phenols after a wildfire. Basing on such a result, Certini et al. (2011) suggested a possible index from the Acid-to-Aldehyde ratio for estimating fire severity. Such an index, however, was not applicable to both OR and MG, where lignin units were oxidised without any selectivity.

The ^{13}C CPMAS NMR spectra of burnt and unburnt soils from OR and MG are shown in Fig 11, while Table 4 reports the spectra intensity distribution.

At both OR and MG, fire caused a decrease in the intensity of the alkyl C signal, wich indicates the reduction of the chain length of alkyl compounds, a common occurrence in the burnt soils (González-Perèz et al., 2004). O-alkyl signal was particularly affected only in the burnt soil at MG. Finally, both burnt soils showed an increase of the signal intensity in the aromatic C region, indicatives of PyC accumulation.

As suggested by Knicker et al. (2006), for a more detailed insight into the alteration of the organic matter by fire and its possible correlation with fire severity, was calculated an enrichment factor (EF$_I$) for the alkyl C and the aromatic C shift region according to this equation:

$$EF_i = (I_{csb} X\ C_b)/(\ I_{csu} X\ C_u)$$

where the concentration of the alkyl C and the aromatic C group were estimated by multiplying the relative intensity of the two chemical shift regions, as assessed by NMR analysis (burnt soil, I_{csb}; unburnt soil, I_{csu}), by the C content of the relevant sample (burnt soil, C_b; unburnt soil, C_u).

After calculating the EF for the alkyl C and the aromatic C shift region, the B_{char} index was calculated as follows:

$$B_{char} = EF_{I(aromaric\ C)}/EF_{I(alkyl\ C)}$$

B_{char} is regarded as an index for the degree of charring of the organic C introduced to the soil during and after the fire.

Table 4. Relative distribution (%)[a] of the chemical shift regions 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) is not reported because negligible. EF_I is the enrichment factor for the alkyl C and the aromatic C chemical shift regions. B_{char} is the ratio $EF_{I(aromaric\ C)}/EF_{I(alkyl\ C)}$ and is regarded as an index for the degree of charring of the organic C introduced to the soil during and after the fire.

Site (Code)	Relative distribution (%) of chemical shift region (ppm)						B_{char}
	Alkyl C	O-Alkyl C			Aromatic C	Carboxyl C	
		Methoxyl/N-alkyl	O-alkyl	Di-O-alkyl		carboxyl C/amide/ester	
	0 – 45	45-60	60-90	90-110	110-160	160-185	
Orentano (OR)							
Burnt	30	7	20	6	29	8	
Unburnt	38	6	22	7	20	7	
EF_I	0.93				1.71		1.83
Mount Gordon (MG)							
Burnt	27	8	24	7	24	8	
Unburnt	30	8	31	8	17	6	
EF_I	0.48				0.81		1.69

[a] Maximum error ± 4.

According to the enrichment factor of aromatic C region, at OR there was the largest PyC release to soil, despite the relative low fire severity, while higher C volatilization during the extreme fire

occurred at MG may explain the smaller value of EF_I found there. The degree of charring, as shown by the B_{char} index, was higher at OR, hence suggesting that a higher fire severity does not mean necessarily more advanced charring of the PyC. The extreme fire at MG might have led mostly to complete loss of OM.
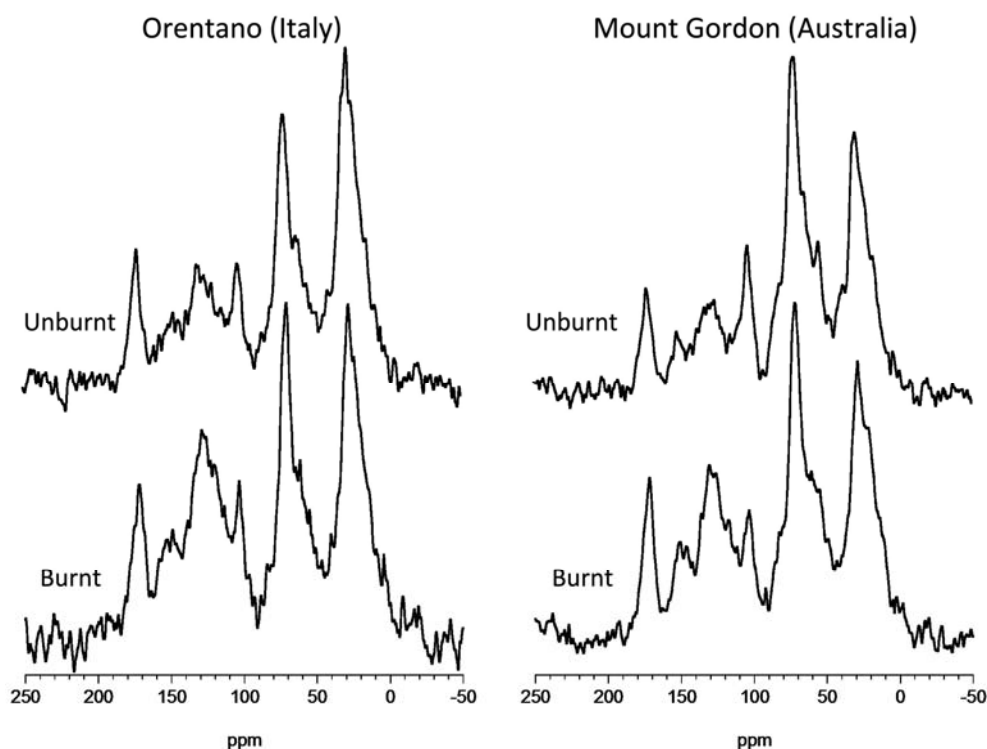
Figure 11. ^{13}C CPMAS NMR spectra of the mineral soil from Orentano and Mount Gordon.

4.2 *Evaluating the complementary application of rapid analyses to assess the transformation of pyrogenic organic matter*

At Roccastrada, the burnt soil displayed a significant enrichment in SOM abundance compared to the unburnt soil, 25% vs 11%. Such a result, although not so marked, has been reported in several studies (*e.g.* Almendros et al., 1988; González-Vila et al., 2002; Certini et al., 2011), and it is due to a substantial incorporation into the top mineral soil of partly charred aboveground biomass and forest floor after a fire.

Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of the bulk burnt soil showed a couple of peaks at 506 and 632 °C, associated with a small mass loss, which are absent in the unburnt soil and (Fig 12). These high temperatures are peculiar for the oxidation of highly refractory organic matter; the presence of these compounds is hence consistent with hermal

reactions occurred in soil during the fire event leading to the formation of polycondensed aromatic carbon compounds, *i.e.* black C (De la Rosa et al., 2008).
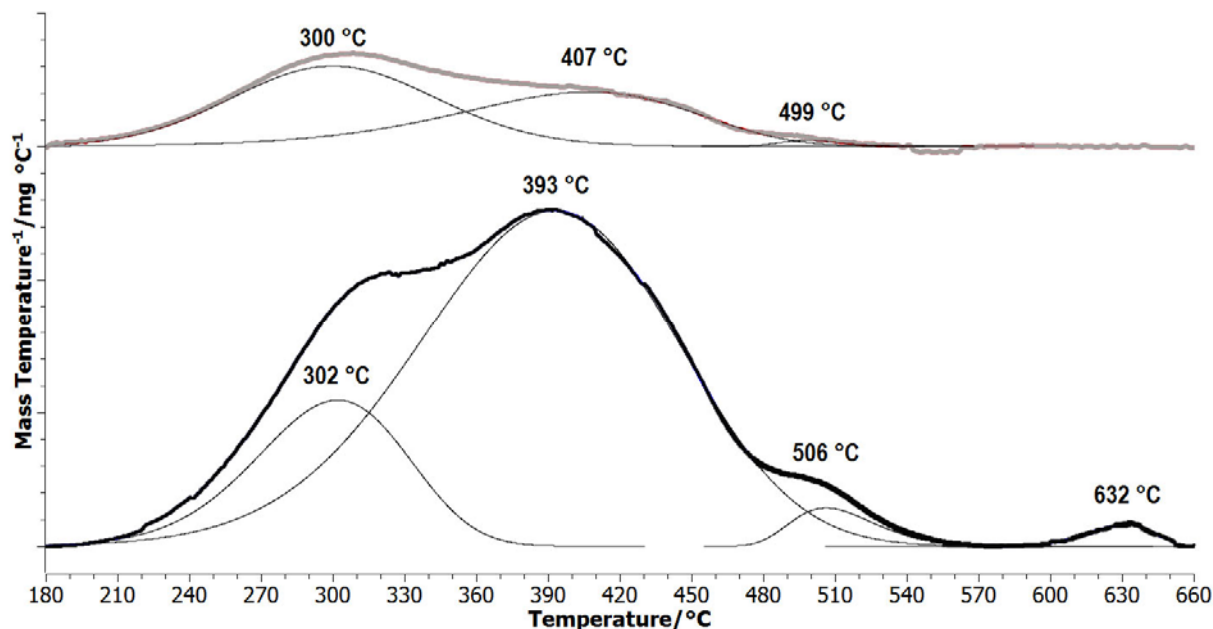
Figure 12. DTG of samples from unburnt (grey) and burnt (black) soils at Roccastrada. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the mass loss. (*from manuscript II*)

The SOM extracted from the burnt soil was characterized by much lower values of enthalpy of combustion (ΔH) than the one extracted from the unburnt soil, particularly for the two first exothermic reactions, as revealed by differential scanning calorimetry (DSC) curves (Fig 13). This indicates that fire caused a substantial reduction of the most labile fraction of SOM and a relative enrichment in more recalcitrant organic compounds.

The DTG thermogram of charcoal was characterized by three peaks: the first one at 407 °C, with a weight loss of 37%, and two refractory peaks at 501 and 658 °C, with relative weight losses of 3.7% and 1.2%, respectively. The DSC thermogram, instead, was composed only by two peaks: one at 376 °C, whose enthalpy of combustion was higher (6068 mJ mg^{-1}) and a second less intense at 437 °C (681 mJ mg^{-1}). It means that the charcoal particles were not completely charred because they still contained a part degradable below 500 °C. Furthermore, these findings confirm that the structure of charcoal formed under natural conditions, is quite different from the almost pure graphitic structures described for the most recalcitrant fraction of PyC (Knicker, 2007). In fact, graphite shows thermal decomposition reactions at around 800 - 900 °C.
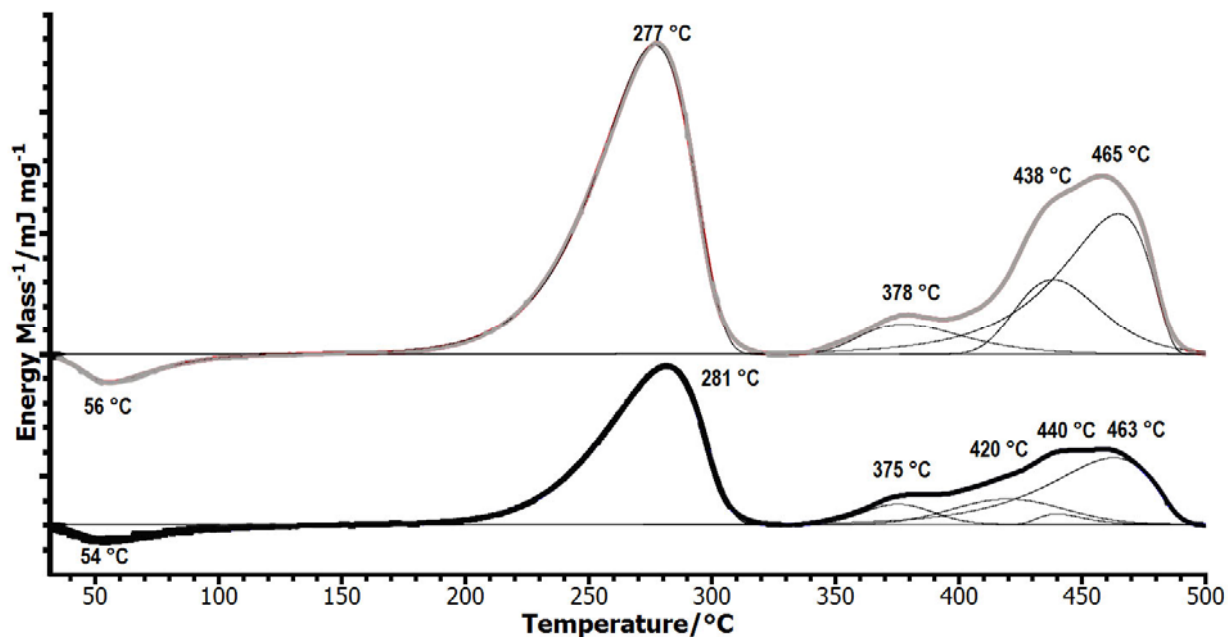
Figure 13. DSC of SOM fraction extracted from unburnt (grey) and burnt (black) soil samples at Roccastrada. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the energy transfers. (*from manuscript II*)

The attenuated total reflectance (ATR) Fourier Transform Infrared Spectroscopy (FT-IR) spectra of extracted SOM fraction from the burnt and the unburnt soils were quite similar (Fig 14). They diverged only in the 2400-2000 cm^{-1} region, where the SOM from the burnt soil revealed the presence of νCN stretching vibration (Francioso et al., 2009). The formation of CN groups probably arises from the dehydration of aromatic amides or from the ring scission and subsequent rearrangement of N heterocyclic polyaromatic molecules. Pyrolytic organic N formation is however debated, even if it has been detected in severely charred biomass at temperature around 350 °C (Knicker et al., 2008a). The appearance of these CN groups, hence, suggests that the soil was most probably heated to less than 400 °C.

The occurrence of CN stretching vibration in burnt SOM was confirmed by a sharp, intense peak at 2329 cm^{-1} in the Raman spectrum (Fig 14). This peak occurred at a noticeably lower frequency than the overtones and/or combination bands of graphite, which fall at about 2450 cm^{-1} (Francioso et al., 2011). In our samples, hence, N was not depleted by the burning, but tended to be incorporated into fairly resistant structures. By contrast, no CN group vibration was detected in SOM extracted from the unburnt soil.

Data obtained from thermal and vibrational spectroscopy techniques were contrasted with the analysis on bulk soil and extracted SOM samples performed via ^{13}C NMR spectroscopy. NMR spectra clearly showed the footprint of fire on both specimens (Fig 15). However, the results were
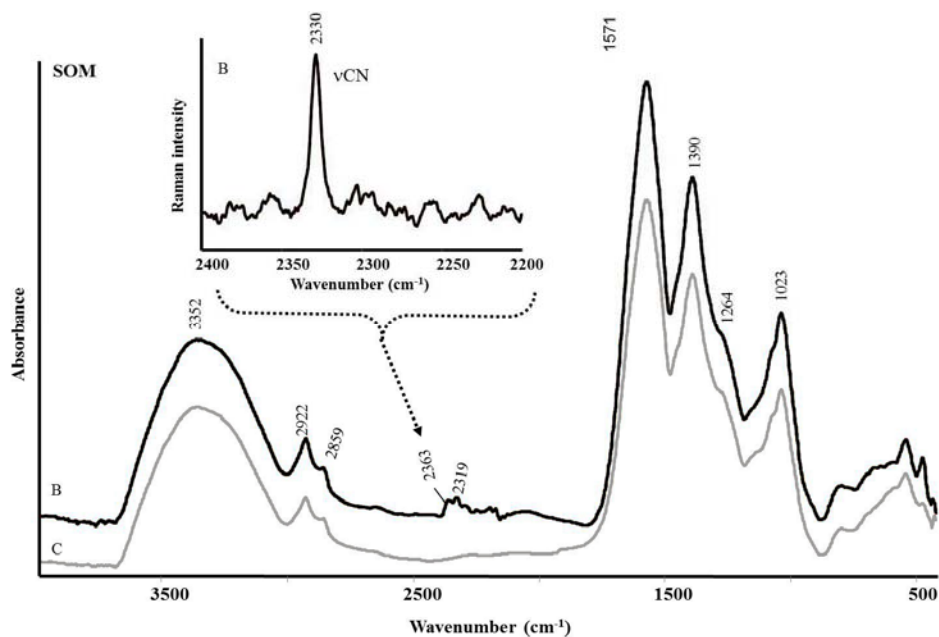
Figure 14. ATR/FTIR spectra of SOM extracted from the burnt (B, black line) and unburnt (C, gray line) soil; top: Raman spectrum of SOM extracted from the burnt soil (B) recorded in the region between 2400-2200 cm^{-1}. (*from manuscript II*)
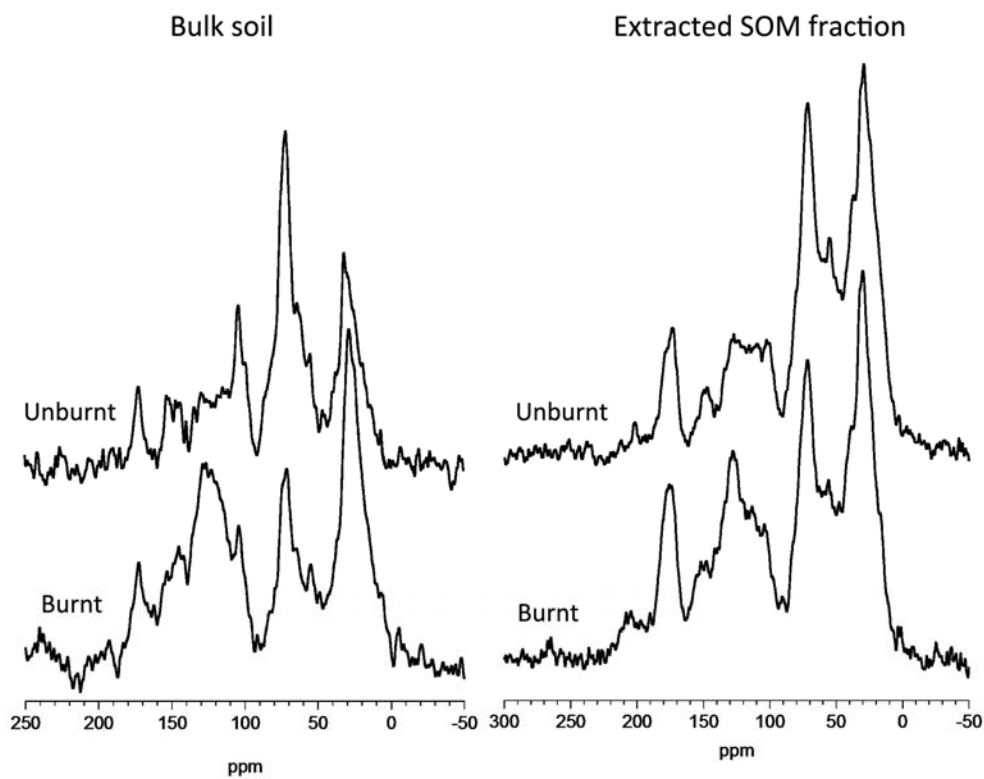
Figure 15. ^{13}C CPMAS NMR spectra of the bulk soil and extracted SOM from the burnt and unburnt area at Roccastrada.

not so different compared to those obtained by the analyses described above. The organic component of soil samples underwent to various fire-induced reactions, the most evident of which were the degradation of O-alkyl C yielding of aromatic C. The signal in the Alkyl C region was more intense in the burnt bulk soil sample compared to the unburnt one, meaning that the lipid fraction resisted better than other SOM components to burning. In the spectra of the extracted SOM fraction, all the differences between the burnt and the unburnt sample were smaller.

Summarising, the combined use of thermal and vibrational spectroscopy techniques provided consistent information concerning the nature of the fire affected SOM, also when contrasted with NMR analysis, which remains a relatively expensive and time consuming technique (De la Rosa et al., 2008; Cécillon et al., 2012)

4.3 *Long-term effect of wildfire on soil organic matter composition and stability under plausible climate change scenario*

The topo-climosequence on Mt. Etna was chosen for evaluating the long-term effect of fire on SOM for three main reasons: (1) here - and the in the Mediterranean basin in general - a higher fire frequency is expected in the near future due to increasing temperature and decreasing precipitation (IPCC Fourth Assessment Report, 2007); (2) on Mt. Etna, wildfires have always been a driving factor in ecosystems composition and landscape modelling. Here, fire occurrence is usual, due to the frequent eruptions of the volcano and the numerous human activities, particularly at lower elevations, *i.e.* the most easily accessible areas. In the whole Mediterranean basin, in fact, human activity considerably contributed - and still contributes - to the incidence of fires (Pausas et al., 2008); (3) since climate change is likely to cause an upslope shift of the ecosystems range boundaries along an elevational gradient, the study of SOM on Mt Etna could help to provide predictions on the fate of OM in the soils at higher elevated sites.

In the investigated soils along the topo-climosequence, the C and N stocks in the whole soil profile showed an increasing trend with decreasing altitude. The fact that such a trend was particularly evident in the subsurface layers (10-40 cm) and more marked for nitrogen than for carbon, seemed a clue of a large contribution of PyOM fraction to SOM. In fact, a higher accumulation of PyOM often takes places in the subsoils (Rumpel et al., 2010; Djukic et al., 2010; Knicker, 2011) and a relatively N-rich biomass (*e.g.* grass vegetation growing in a highly fire-affected area) during charring shows an efficient incorporation of N into the soil (Knicker, 2010).

The hypothesis of a higher abundance of PyOM fraction at lower elevations, where fire frequency is higher, cannot be confirmed. Only a minor part of the SOM accumulated at the low elevations can be ascribed to the stable (H_2O_2 resistant) fraction (Fig 16). A direct evaluation of the black carbon (BC) contribution to total organic carbon (TOC) in soil was not possible: dichromate oxidation treatment was not so straightforward as assumed. In fact, the NMR analysis of the dichromate oxidation resistant fraction showed that this latter consisted mostly of lipids and, hence, only a minor part can be attributed to charred material. Actually the difficulties in detecting BC content in soil were related to the general low content of PyC. In this regard, Knicker et al. (2007) reported that in soil samples with a contribution of aromatic C of less than 20% to total C_{org}, BC is hardly measurable by dichromate oxidation. The NMR analysis actually revealed a low aromatic C content of soil samples, tentatively below 20%. On the contrary, the analysis showed a relative abundance of lipids, in both the bulk samples and all density fractions. A relatively high aromatic contribution (25%) was found only in the $1 - 1.6$ g cm^{-3} soil density fraction at lower elevations.
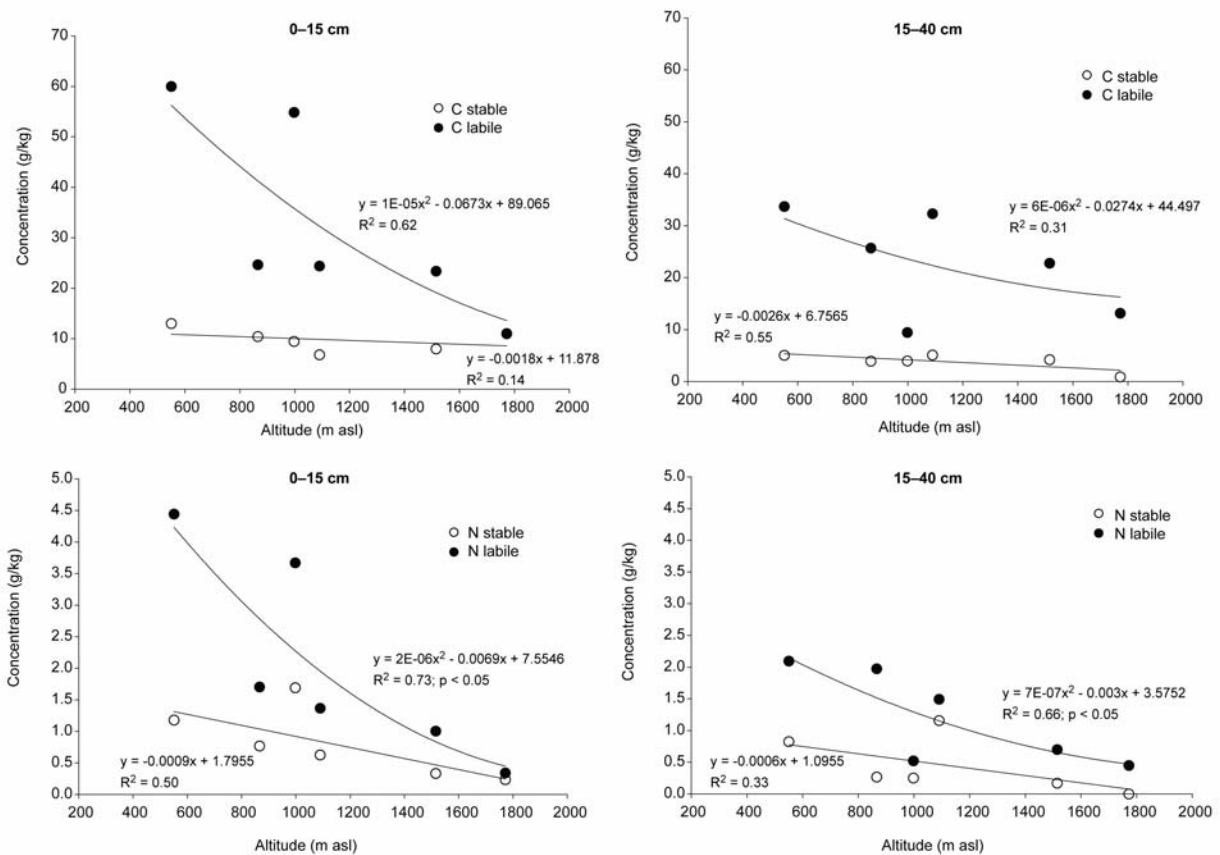
Figure 16. Organic C and total N in the stable and labile SOM fraction along a toposequence on Mt. Etna as a function of soil depth. (from *manuscript III*)

The isolated macroscopic handpicked >2 mm charcoal fraction was present in significant amounts in the investigated soils. In general the C contribution from charcoal to TOC was about 9% on average and up to almost 40% in a couple of soil horizons. There is hence the evidence that (macro)charcoal plays an important role in storing carbon in these soils.

The contrasting results between the macroscopic and the microscopic pyrogenic carbon mean that either not much of aromatic compounds, besides macro-charcoal, were produced by fire events or they were partially oxidised by a high fire frequency. Furthermore multiple burnings, if temporally too close to each other, do not necessarily lead to an increase in the degree of SOM aromaticity. The new shrub and herb cover developed after the first fire, in fact, provides an available fuel which could burn completely leaving little char. Moreover, the fertilising effect of ash enhances the biomass production and hence the incorporation into the soil of fresh and labile OM, possibly masking PyOC contribution to SOM.

The radiocarbon dating of the stable (H_2O_2 resistant) SOM fraction and of some selected charcoal macroparticles, showed a common elevational trend: an increasing measured age with increasing elevation (Fig 17 and Fig 18). The young ages of charcoal and the stable SOM at low altitudes is due perhaps to mass movements, erosion, or, more likely, to the high frequency of fires, which prevent a longer stabilisation of soil organic matter, charcoal included. On Mt. Etna, at higher elevations, most probably other factors, such as cooler climate, changed vegetation, contributed to the higher age of the stable organic matter fraction. In any event, fire appears to negatively affect the stable SOM turnover rate at lower altitudes where, finally, fires are more frequent.

$y = 197.51x^{0.253}$
$R^2 = 0.84; p < 0.05$
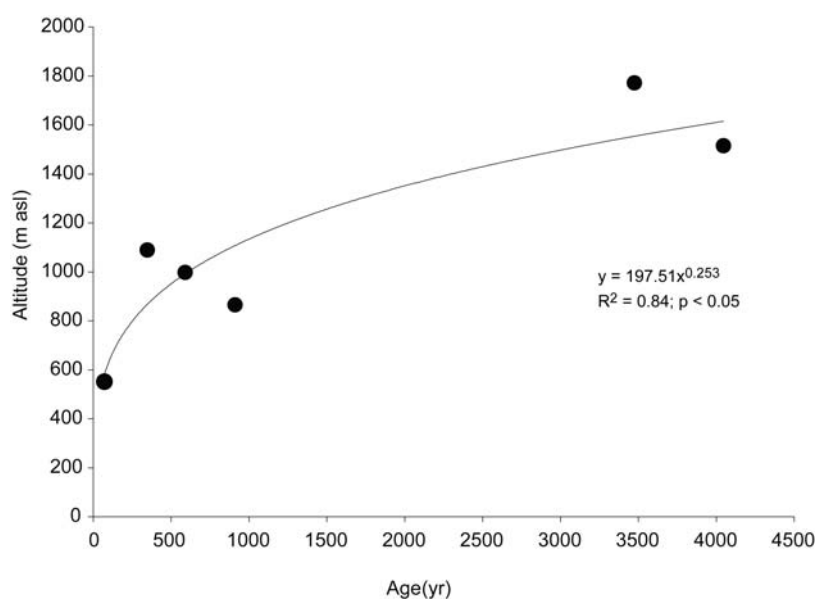
Figure 17. Mean age (calibrated) of the stable fraction of soil organic matter from the 0 – 40 cm depth interval along a toposequence on Mt. Etna as a function of altitude. (from *manuscript III*)

30
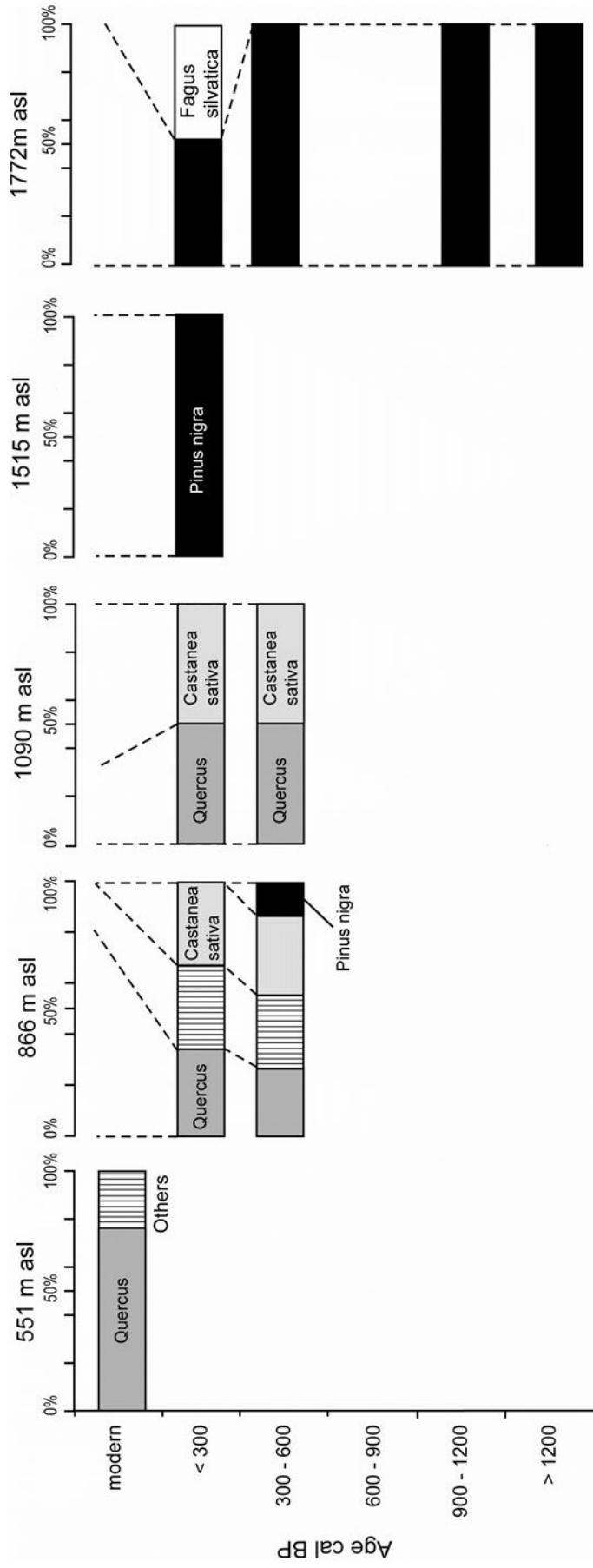
Figure 18. Composition of vegetation along a toposequence on Mt. Etna as a function of time derived from charcoal fragments found in soils. Others = *Lonicera inplexa* (551 m asl), Maloideae (866 m asl). (from *manuscript III*)

5. MAIN CONCLUSIONS, IMPLICATIONS AND PERSPECTIVES

The fire-affected soils at Orentano and Mount Gordon were contrasted to highlight the effect of fires with different severity on soil organic matter composition. At Mount Gordon, a fire of extreme severity resulted in a dramatic loss of total SOM. Nevertheless, at Orentano, a fire of moderate severity significantly affected lignin contents, but not the sugar content, while at Mount Gordon lignin and sugars contents were significantly and similarly affected by fire. Furthermore, at both sites, all lignin units were oxidised without any selectivity.

Overall, in our samples these findings did not provide any simple parameter able to function as a reliable index of fire severity in soil. In fact, the proposed carbohydrate to total organic C ratio (Martin et al., 2009; Rovira et al., 2012), the acid-to-aldehyde ratio for lignin V and S phenols (Certini et al., 2011), and the B_{char} index (Knicker et al., 2006) failed to elucidate the charring degree of necromass or the burnt severity to which the soil was subjected. Such a failure is likely due also to the large variety of soil features and environmental factors affecting fire and its impact on SOM, although it is not yet well defined the relative importance of the various factors. Further data are needed to identify an easy-to-use parameter linked to SOM, useful to define the degree of damage of soil quality that, in turn, could recommend, for instance, if soil reclamation actions are needed.

The study carried out in a burnt forest soil at Roccastrada, based on the application of a set of thermal and spectroscopic techniques to investigate SOM. Although rapid and cheap, such techniques allowed to efficaciously detecting important fire-induced changes in soil organic matter composition. Fire occurrence produced a substantial reduction of the most labile fraction of SOM and a consequent relative enrichment in recalcitrant SOM. Additionally, fire led to the formation of nitrogen-bearing compounds, characterised by a fairly resistant structure. The thermal behaviour of charcoal formed under natural conditions demonstrated the heterogeneity of this material. The results obtained add knowledge on the complexity of charred SOM and encourage using thermal and vibrational spectroscopy analyses as rapid and easy tools for this purpose.

On Mt. Etna, fire frequency appears to play a major role, together with climate, on SOM composition and its stability. At lower elevations, with increasing fire intensity and warmer climate,

the residence time of the stable soil organic matter fraction, charcoal included, decreased and more labile organic matter, easily degradable, was accumulated in the mineral soil. In several of the investigated soil profiles on Mt. Etna macroscopic charcoal was found in significant amounts, contributing up to almost 40% of total organic carbon. Furthermore, the ^{14}C age of some charcoal pieces was even more than 1400-1500 years. Soil charcoal is hence a highly stable pool of carbon in this environment, which also allowed to partly reconstructing the past vegetation. Overall, the study carried out on Mt. Etna suggests that the high fire frequency is a powerful rejuvenating factor for soil organic matter, removing part of the very old, biologically recalcitrant fraction.

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Part II – Manuscripts

Manuscript I

Immediate impact of two forest wildfires of different severity on soil organic matter quantity and composition

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Immediate impact of two forest wildfires of different severity on soil organic matter abundance and composition

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Introduction

Fire is a major disturbance in many ecosystems, affecting more lands that any other natural disturbance (Lavorel et al., 2007). Important socio-economic changes in the last decades, such as abandonment of agriculture and forest management for fire exclusion, contributed to an alteration of fire regimes (Bento-Gonçalves et al., in press; Bormann et al., 2008). In addition, climate change is projected to increase wildfire frequency in many parts of the world (Moriondo et al., 2006; Flannigan et al., 2006). As well as the frequency, the size, seasonality, intensity and severity of wildfires are expected to be influenced by all these changes, affecting, directly and indirectly, all the ecosystem components (Bento-Gonçalves et al., in press).

Soils suffer from many direct and indirect consequences of the occurrence of fires on their physical, mineralogical, chemical and biological properties (Certini, 2005; DeBano, 1991; González-Pérez et

al., 2004). The organic component of soil is the most affected by fire, in terms of both content and composition (González-Pérez et al., 2004; Certini et al., 2011). The assessment of the effect of forest fire on soil organic matter (SOM) is a complex task because different forest fires, *i.e.* smouldering or flaming, may lead to different SOM transformations, which are additionally depending on fire intensity and vegetation type and conditions (Rumpel et al., 2007; Tinoco et al., 2006). Furthermore, wildfires result in an increased heterogeneity in SOM composition (Duguy and Rovira, 2010), making even harder to study a material that was already very complex.

Concerning impact of fire on SOM quantity, in general all the studies agree with a substantial reduction of OM stock in the litter layer (Bento-Gonçalves et al., in press; Bormann et al., 2008; Certini, 2005; Nave et al., 2011). On the contrary, there are contrasting results in the open literature about quantitative changes in the mineral soil. For instance, two meta-analysis, by Johnson and Curtis (2001) and Nave et al. (2011), reported two opposite conclusions: the first work suggested a wildfire-induced increase of mineral soil C, attributed to charcoal input from the litter layer, whereas the second reported a significant declines of C and N soil concentrations due to wildfire occurrence. However, some divergences between different studies can arise from different parameters considered as, for instance, sampling strategy and depth, time elapsed since fire, considering elemental concentration and pool sizes separately, fire intensity and vegetation/climate type, etc.

The reactions that take place during biomass combustion, *e.g.* dehydration, dehydrogenation, volatilisation of nitrogenous compounds, decarboxylation, demethylisation, cyclisation and polycondensation reactions (Hernàndez et al., 1997; Knicker, 2007), can lead to a profound transformation of OM. Perhaps the largest impact of fire on OM is through the formation of pyrogenic carbon (PyC) (Skjemstad and Graetz, 2003). This phenomenon is important also because it represent the transformation of the most labile organic compounds, mainly carbohydrates and lignin, into highly recalcitrant aromatic structures. These latter have the potential, once incorporated

into the soil, to survive to decomposition also for century and millennia and to offset increases in atmospheric CO_2 (Almendros et al., 2003; Ansley et al., 2006; Kuhlbusch, 1995; Schmidt & Noack, 2000).

In addition to complete transformation of biomass into charcoal through high temperatures, SOM is most probably affected by less extreme alterations in the composition of its molecular components. Carbohydrates represent a significant pool in SOM (Martin et al., 2009). They are the main source of energy for soil microorganisms and play an important role in aggregates formation and stabilisation (Kiem and Kögel-Knabner, 2003; Martin et al., 2009; Puget et al., 1998, Rumpel and Dignac, 2006). Several authors reported that the fire effect on carbohydrates is more pronounced than on any other components of SOM (Certini, 2005; González-Pérez et al., 2004; Kavdir et al., 2005; Knicker et al., 2006; Martin et al., 2009). On this basis, Martin et al. (2009) proposed the carbohydrates to total SOM ratio as an index of fire-induced changes on SOM quality; such an index would allow also differentiating between low- and high severity wildfires.

Lignin is the second most abundant component of plant residues in terrestrial ecosystem and it is generally considered to be recalcitrant to degradation, at least compared to other major plant components (*e.g.* polysaccharides and proteins). Lignin has a peculiar behaviour against the fire, because its structure starts to be affected by heating at low temperatures, 200-250 °C, but it is totally oxidised only at relatively high temperatures as 400-450 °C (DeBano, 1991; Kuo et al., 2008; Manning et al., 2005). Hence, lignin is partly preserved against fire, being its backbone highly resistant to heat (Knicker et al., 2008; Sharma et al., 2004), but it is immediately affected by fire in terms of phenols distribution (Kuo et al., 2008; Nocentini et al. 2010; Rumpel et al., 2007; Certini et al., 2011). This might make possible to use the changes in lignin phenols composition as a natural indicator for fire occurrence and fire severity in soil (Certini et al., 2011).

Also isotope composition of burnt materials, as recently reviewed by Bird and Ascough (2012), can vary depending on burning temperatures. For instance, $\delta^{13}C$ of charcoal can vary up to ca.2‰ with

pyrolysis temperature compared to the starting material. $\delta^{13}C$ value of organic materials increase at the beginning of charring reaction and then decrease with increasing charring temperatures (>300 °C) (Czimczik et al., 2002). The analysis of lignin phenols, isotope composition and sugars pools may thus provide a more detailed insight into fire-induced changes to SOM compared to the exclusive quantification of OC (Spielvogel et al., 2007).

The effects of fire are confined to a few cm of top soil because of the low thermal conductivity of earth (Certini, 2005; DeBano, 2000; Knicker, 2007; Rovira et al., 2012; Wan et al., 2001). Nevertheless, often the numerous studies dealing with fire focus on upper soil layers of several cms, resulting in a 'dilution' of fire footprint on soil. The choice of the right sampling depth is indeed of great importance when analysing the immediate impact of a single fire event on soil, due to the detection limits and also to avoid as much as possible cross-contamination with fire affected material from former fires occurred at the same site.

In this study, we investigated the top layer of mineral soil of two forests located in Italy and Australia, which have been affected by wildfires of moderate and extreme severity. The aim of our study was to evaluate the effect of a single wildfire on the quantity and composition of SOM, particularly focusing on the non-cellulosic neutral sugars and lignin phenols content of soil and on the C stable isotopic composition of charcoal and SOM.

Materials and Methods

Study sites and soil sampling

A first study site is Orentano (hereafter called OR), 30 km east of Pisa, Tuscany, Central Italy. Elevation is 20 m asl, mean annual precipitation is 893 mm and mean annual temperature 14.3 °C. The vegetation cover is a mixed forest stand of Downy oak and Maritime pine (*Quercus pubescentis* and *Pinus pinaster)* and an undestorey of common fern (*Pteridium aquilinum)* and

Rubus spp. Soils formed on sand and stony lacustrine deposits and are classified as endoskeletic plinthic Acrisols according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2006).

Wildfire happened in July 2011. Fire severity, based on the visual scale of fire severity by Chafer et al. (2004), was classified as moderate to high. The burnt area was relatively small, only 3.3 ha. Because of the proximity of residential areas, water shooting helicopters were used to stop the fire. Most of the stems, also the shrub ones, were still standing after fire, although partly or totally scorched. Soil sampling was carried out three days after burning, on both the burnt area (coordinates (WGS84) 43°47'22.82"N, 10°39'52.30"E) and an adjacent (50 meters away) unburnt area having the same characteristics than the burnt area prior to the fire.

Table 1. Location, pH, bulk density (mean and standard deviation; n=3) and texture of soil samples.

Site (Code)	Sampling area (Code)	Location WGS84	soil pH (H_2O, 5:1)	bulk density g cm^{-3}	Sand %	Silt %	Clay %
Orentano (OR)	Burnt	43°47'22.82"N	5.12 (±0.37)	0.98 (±0.07)	11	71	17
Italy		10°39'52.30"E					
	Unburnt	43°47'25.94"N	4.22 (±0.32)	1.11 (±0.17)	16	66	19
		10°39'50.35"E					
Mount Gordon (MG)	Burnt	37°31'56.30"S	5.90 (±0.37)	n.d.	29	52	18
Australia		145°43'17.14"E					
	Unburnt	37°32'54.10"S	4.83 (±0.03)	n.d.	33	44	22
		145°37'37.30"E					

The other study site is Mount Gordon (hereafter called MG), near Marysville, in the Victoria State, south-east Australia. The elevation of the sampling area is 530 m asl, mean annual precipitation is

670 mm and mean annual max and min temperatures are 17.7 °C and 8.4 °C respectively. The site was chosen because it represents an end point in terms of fire severity. In fact, in the sadly famous 'Black Saturday' in the early February 2009, some 450,000 ha of eucalypt forest burnt 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 substantially higher than any previously reported in Australia (Royal Commission 2009). This wildfire of extreme fire severity was promoted by particularly favourable wheather conditions, such as wind speeds up to 100 km h^{-1} and air temperatures even exceeding 45 °C. Moreover, fuel loads were very high, since the forest did not experience a major fire since 1939 (fuel load, including the litter, amounted to 25–40 t ha^{-1}), and the fuel moisture was very low (3–4%) because of two weeks of drought (McCaw et al. 2009).

The burnt site chosen for soil sampling (37°31'56.30"S, 145°43'17.14"E) is 3 km SW of Marysville on the Road to Narbethong. It was an *Eucalyptus spp.* mixed forest; tree height was ~30 m on average and the range of DBH (diameter at breast height) was 0.3-1 m. At burnt site, fire removed all ground fuel, green vegetation and woody stems <10 mm in diameter; accordingly fire severity was classified as extreme (Chafer et al., 2004). A long unburnt site - last burn in 1939 - was selected approximately 6 km W-SW of Marysville (37°32'54.10"S, 145°37'37.30"E). This site was 8.5 km far from the burnt area and as similar as possible to the burnt site in terms of time elapsed since the previous fire, vegetation and lithology. Sampling was performed in April 2009, two months after the fire and following some light rainfall, but before the intense precipitation of winter which caused significant ash removal via erosion.

At both study sites, OR and MG, the sampling involved four parallel 20 m transects, laid out 5 m apart, at 5 m intervals. Twenty soil samples were taken each site down to 2.5 cm, after removing the ash, charcoal, or litter layer by a brush. Ten samples of ash/charcoal layer were collected randomly, from 40 x 40 cm areas.

The purpose of this study is to assess what types of changes SOM underwent in the respective sites

because of a fire of moderate and extreme strength respectively. Although an 'orthodox' comparison is not possible, because the two sites are different in terms of environmental and soil properties, SOM changes at the two sites were contrasted to highlight the effect of different fire severity.

Selected basic analyses

Soil pH was measured potentiometrically using a deionised water to soil ratio of 5:1, while the 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 fraction), and charcoal were measured by an elemental analyser (Carlo Erba NA1500) coupled to an isotope ratio mass spectrometer (Micromass-Optima). The measured pH values, all below 6, guarantee that the soil samples are carbonate-free and, hence, that the total C is entirely in organic forms. Results for δ^{13} C isotope abundance were reported in per mil (‰) relative to the Pee Dee Belemnite standard (PDB).

Analysis of lignin phenols

Four soil samples for each site, obtained by combining equal aliquots of all twenty soil samples, were submitted to cupric oxide (CuO) oxidation for the quantification and characterisation of soil lignin according to the modified method of Kögel and Bochter (1985) and Kögel-Knabner (1995). Briefly, the sample (50 mg for organic layers, 150-200 mg for bulk soils), 250 mg CuO, 50 mg of glucose, 50 mg of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and 15 ml 2 M NaOH were digested in a Teflon pot at 172 °C under N_2 for 2 h. Afterward, ethylvanillin 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 over night for humic acid precipitation. Thereafter, the lignin-derived phenols were purified by elution through a C_{18} column (International Sorbent Technology) and extracted from the column by adding 5 x 0.5 ml

(2.5 ml in total) ethyl acetate. After evaporating ethyl acetate under N_2 flow, the dried residue was dissolved in pyridine, containing phenyl acetic 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, to 184 °C at 4 °C/min, to 300 °C at 10° C/min and 310 °C for 5 mins. 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. $Ac/Al_{V,S}$ are the mass ratios of acid to aldehyde units 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. The results are given as mean values obtained from four replicates for bulk soil samples.

Determination of non-cellulosic neutral sugars

On the samples used for the lignin analysis, we determined also the neutral sugar content and composition. Sugar monomers were analysed according to Amelung et al. (1996) as modified by Rumpel and Dignac (2006). Briefly, 500 mg of soil were hydrolysed with 4 M trifluoroacetic acid (TFA) at 105 °C for 4 h. After the samples had cooled down, 0.5 ml Myoinositol (concentration 2 mg l^{-1}) was added as internal standard. Thereafter, the hydrolysed samples were purified by filtration over glass fibre membrane (cut-off 1.2 µm) and dried using a rotary evaporator (58 °C). According to Eder et al. (2010) ethylenediaminetetraacetic acid (EDTA) was added to keep iron in a non-reactive form in solution and to avoid possible co-precipitation of dissolved organic carbon. Derivatisation of the sample was carried out in screw top test tubes. Aldoses were reduced to their

corresponding alditols after addition of 1 ml $NaBH_4$ dissolved in dimethyl sulfoxide (DMSO). Acetylation was carried out by adding 2 ml acetic anhydride and 2 ml glacial acetic acid; Methylimidazole (2 ml) was used as catalyst for the reaction. After 10 min, the reaction was stopped by adding 7 ml ice-cold deionized water to decompose acetic anhydride generating acetic acid. The derivatised sugar monomers were extracted by liquid–liquid extraction with 1 ml 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. GC analysis was performed on a 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 flame ionization detectior (FID). Split injection (1:10) was used. The temperature program started at 200 °C to 250 °C at 8 °C min−1, 15 min isothermal at 250 °C. Carrier gas was Helium.

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, hereinafter, when referring to sugars, we intended non-cellulosic neutral polysaccharides. The sugar monomers detected with 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, fructose concentration in soil is very low and can be neglected (Amelung et al., 1996). The concentration of individual sugar monomers was calculated based upon the internal standard Myoinositol. The results are given as mean values obtained from four replicates for bulk soil samples.

The proportion of microbially-derived sugars in relation to plant sugars was assessed by calculating the mass ratios of hexoses/pentoses sugars: (galactose + mannose)/(arabinose + xylose) = (GM/AX). A low (<0.5) or a high (>2) GM/AX ratio discriminate between carbohydrates predominantly derived from plants and microbial input respectively (Oades, 1984).

Solid-state ^{13}C NMR spectroscopy

Soil samples from the burned and the unburned areas were analysed by solid-state ^{13}C cross polarisation magic angle spinning (CPMAS) NMR. The specimens for this analysis were obtained combining equal aliquots of all of independent samples collected from each site. Before performing the ^{13}C NMR analysis, the samples were submitted to a 2% HF treatment as in Skjemstad et al. (1994), to remove paramagnetic minerals, which strongly reduce the signal-to-noise ratio of the spectra. ^{13}C CP/MAS NMR spectra of HF-treated samples were recorded on a Bruker AMX300-WB spectrometer. A contact time of 2 ms was used and the recycle delay was 4 s. All spectra were recorded at room temperature, spinning at the magic angle at 8.0 kHz.

A semiquantitative determination of the main C forms was done integrating some chemical shift regions of the spectra as in Wilson (1987) and expressing them as percentages of the total area between 0 and 220 ppm: 0 to 45 ppm (alkyl C), 45 to 110 ppm (O-alkyl C, subdivided in Methoxyl/N-alkyl, 45 – 60 ppm; O-alkyl, 60 - 90 ppm; Di-O-alkyl, 90 – 110 ppm), 110 to 165 ppm (aryl C, subdiveded in aromatic C-H and C-C, 112 – 140 ppm; O substituted, 140 – 165 ppm), 165 to 185 ppm (carboxyl C), and 185 to 220 ppm (aldehydes, ketones).

Statistics

Prior to analysis data distributions were checked for normality by Shapiro-Wilks test and data from burnt and unburnt soils were compared by two tailed paired t-test.

Results and Discussions

Carbon and nitrogen in bulk soil

At OR, the mean concentration of carbon slightly increased after fire occurrence (Table 2). Despite N concentration did not change, C/N ratio remained unchanged, highlighting the slight change of C

concentration, even if significant. If litter layer, which, however, is undoubtedly part of soil, is not considered, the moderate fire at OR led to an enrichment of carbon stock of the mineral soil, since bulk density did not increase after the fire (Table 1). Johnson and Curtis (2001), in their meta-analysis carried out on decades of independent results, reported an increase of forest soil C in the A horizon within 10 years after fire. They partly correlated such an increase to an accumulation into the mineral soil of the unburnt residues as charcoal. Furthermore, an incorporation into the mineral soil of charcoal particles covering the burnt site could explain the lower $\delta^{13}C$ value for the burnt soil compared to the unburnt one, since charcoal had a mean $\delta^{13}C$ (-28.5 ‰) lower compared to the unburnt soil (-27,4 ‰).

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

Site (Code)	Sample (Code)	C org g kg^{-1}	N g kg^{-1}	C/N	$\delta^{13}C$ ‰
Orentano (OR)	Burnt soil	100.0 (±34.3)*	5.88 (±2.05)	17.2 (±1.73)	-27.8 (±0.37)*
Itlay	Unburnt soil	84.7 (±27.9)*	5.20 (±2.12)	17.0 (±3.73)	-27.5 (±0.36)*
	Charcoal	338.3 (±62.1)	16.1 (±2.62)	20.7 (±3.63)	-28.6 (±0.52)
Mount Gordon (MG)	Burnt soil	87.8 (±13.2)**	4.27 (±0.52)**	20.6 (±2.17)**	-27.5 (±0.27)*
Australia	Unburnt soil	163.8 (±55.4)**	5.85 (±0.14)**	27.5 (±3.49)**	-27.7 (±0.26)*
	Charcoal	195.9 (±52.1)	6.08 (±1.41)	28.5 (±9.97)	-27.4 (±0.55)

* Significantly different means between burnt and unburnt soil (P<0.05)
** Significantly different means between burnt and unburnt soil (P<0.01)

Extremely severe wildfire at MG caused a sharp reduction in soil C and N concentrations. C in the bulk soil decreased from 164 to 88 g kg^{-1} and N from 5.8 to 4.3 g kg^{-1} (Table 2). Such a high SOM loss is quite unusual if compared to data from other studies, as, for instance, those ones gathered by Nave et al. (2011) in their meta-analysis. However, the extreme and unusual fire severity could partly explain these differences. Moreover, we considered only the top 2.5 cm of soil and thus

avoided a dilution of the fire effect on SOM. Usually, in fact, temperatures at greater depth depths are not high enough to induce noticeably change of SOM features (DeBano, 2000; Knicker, 2007).

Also at MG, fire induced a significant change of $\delta^{13}C$ value in the burnt soil that showed a higher $\delta^{13}C$ value compared to the unburnt soil, -27.5 ‰ and -27.7 ‰ respectively (Table 2). Despite the opposite trend compared to OR study site, also this result could be explained by an incorporation of some charcoal particles from the charcoal and ash layer covering the burnt soil after the fire. At MG site, in fact, charcoal had a $\delta^{13}C$ value higher compared to the unburnt soil.

At MG, C and N content were differently affected by fire, resulting in a significant decrease of C/N ratio of soil. This may be explained by an accumulation of recalcitrant organic N-forms in the charred material (Almendros et al., 2003; Knicker et al., 2005b; Santin et al., 2008), which could have led to a decrease of the C/N in the burnt soil (Fernández et al., 1999; González-Pérez et al., 2004).

Soil non-cellulosic neutral sugars content

At OR, the average content of neutral sugars in the mineral soil of burnt and unburnt soils was 7.4 and 8.2 g kg^{-1} respectively, which corresponded to 76 and 97 g kg^{-1} of TOC (Table 3). These results are in the range of data obtained by other authors in forest soils (e.g. Guggenberger et al., 1994; Rumpel and Dignac, 2006). The burnt soil showed only a slight decrease in neutral sugars, which however was not statistically relevant.

The galactose+mannose to arabinose+xylose ratio (hereafter called GM/AX) before the fire was >2, indicating that sugars were mainly synthetized by microbial population (Guggenberger and Zech, 1994; Oades, 1984). The ratio did not change because of the fire, meaning that none of the plants or microbial derived sugars were preferentially oxidised.

In the burnt soil at MG, the total amount of non-cellulosic neutral sugars in the bulk soil dramatically decreased from 21 g kg^{-1} to 9 g kg^{-1} upon fire of extreme severity (Table 3). However,

if one compares the content of neutral sugar relative to TOC, the difference between burnt and unburnt soil are not significant (105 and 126 g kg^{-1} SOC in burnt and unburnt soil, respectively). This means that apparently the polysaccharides present in mineral soil were not preferentially oxidised by fire, despite sugars are part of the most thermally labile SOM pool, being totally oxidised at relatively low temperatures, ca. 300 °C (Manning et al., 2005; De la Rosa et al., 2008; Plante et al., 2011).

Our results are in contrast with several studies that reported that fire effect on SOM is more pronounced in terms of carbohydrates than in terms of total SOM (Certini, 2005; González-Pérez et al., 2004; Kavdir et al., 2005; Knicker et al., 2006; Martin et al., 2009).

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

Site (Code)	Sample (Code)	Σ Neutral Sugars g kg^{-1} soil	Σ Neutral Sugars g kg^{-1} OC	GM/AX
Orentano (OR)	Burnt soil	7.46 (±0.43)	76.6 (±6.59)	2.79 (±0.90)
Itlay	Unburnt soil	8.27 (±2.30)	98.6 (±13.0)	2.81 (±1.50)
Mount Gordon (MG)	Burnt soil	9.18 (±1.90)*	105.2 (±26.2)	2.00 (±0.44)
Australia	Unburnt soil	20.9 (±6.18)*	126.1 (±26.4)	1.60 (±0.18)

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

Like at OR, the GM/AX ratio at MG did not change following fire, indicating that the pentose or hexose sugars were not selectively oxidised. Pentose and hexose content of the unburnt bulk soil indicated that the carbohydrates were mainly originated by microorganisms. It is reported that sugars of microbial origins participate to the formation and stabilisation of soil aggregates and are, consequently, physically protected within them, as well as stabilised through mineral phase interaction (Kavdir et al., 2005; Kiem and Kögel-Knabner, 2003; Martin et al., 2009; Puget et al.,

1998; Rumpel and Dignac, 2006, Rumpel et al., 2010). Mineral protection might explain the preservation of sugars during fire despite a loss of SOM.

Soil lignin content

Up to now there is no method able to quantify the total content of lignin in soil. CuO oxidation does not completely depolymerize lignin and it is, therefore, not a quantitative method (Kögel, 1986). Nevertheless, CuO oxidation is able to release phenolic monomers and dimers from the outer part of lignin polymer and, therefore, it is widely used as an indicator of lignin amount and composition in soil (Kögel, 1986; Guggenberger et al., 1994, 1995; Kiem and Kögel-Knabner, 2003; Otto and Simpson, 2006; Spielvogel et al., 2007; Thevenot et al., 2010).

At OR, the yield of phenolic CuO oxidation products in the burnt soil significantly decreased after fire occurrence, both in absolute and relative to OC terms (Table 4). Lignin is generally considered to be somewhat recalcitrant, also against the heating (Knicker et al., 2005a). However, lignin is not protected by mineral interactions and could therefore be more prone to loss during fire compared to mineral-protected carbohydrates (Spielvogel et al., 2007; Rumpel et al., 2004).

Table 4. Sum of vanillyl, syringil and cinnamyl units of burnt and unburnt soil (mean values and standard deviation; n=4 for soil samples).

Site (Code)	Sample (Code)	Σ VSC g kg^{-1} soil	Σ VSC g kg^{-1} OC	$(Ac/Al)_v$	$(Ac/Al)_s$	C/V	S/V
Orentano (OR)	Burnt soil	0.35 (±0.01)*	3.57 (±0.18)**	0.74 (±0.37)	0.78 (±0.13)	0.42 (±0.13)	0.38 (±0.22)
Itlay	Unburnt soil	0.65 (±0.14)*	7.71 (±0.86)**	0.58 (±0.22)	0.76 (±0.32)	0.38 (±0.09)	0.50 (±0.11)
Mount Gordon (MG)	Burnt soil	0.65 (±0.23)*	7.53 (±2.97)	0.96 (±0.14)	0.87 (±0.57)	0.24 (±0.10)	1.21 (±0.32)
Australia	Unburnt soil	1.24 (±0.38)*	7.52 (±2.30)	1.05 (±1.21)	0.75 (±0.47)	0.32 (±0.29)	1.96 (±0.59)

* Significantly different means between burnt and unburnt soil (P<0.05)
** Significantly different means between burnt and unburnt soil (P<0.01)

At MG, the yield of phenolic CuO oxidation monomers was half in the burnt bulk soil compared to the unburnt one (Table 4). The VSC content of soil relative to OC, instead, appeared to be unaffected by fire. As in the case of sugars, lignin monomers were not preferentially oxidised by fire compared to other OM constituents. Fire of extreme severity, hence, apparently oxidised unselectively the whole SOM pool, despite lignin is assumed to survive charring to a certain extent if compared to carbohydrates, at least without considering any physical protection of OM in soil.

AT both OR and MG, no one 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 because of fire. Hence, it seems that fire oxidised all the lignin units without distinction and/or that there was not a selective consumption of any vegetal residue. This result is in contrast with data reported by other authors (Certini et al., 2011; Kuo et al., 2008; Nocentini et al., 2011; Rumpel et al., 2007), who found, in both laboratory and field studies, a higher thermal susceptibility of aldehydes that resulted in an increasing Acid-to-Aldehyde ratio for lignin V and S phenols after a wildfire. In the mineral soil, the reaction behaviour of lignins could be changed due to the presence of inorganic catalysts, such as clay minerals. Those were found to profoundly accelerate pyrolysis reactions. Our finding are in contrast to those by Certini et al. (2011), who suggested that a degraded lignin signature of soil may be used as a marker for determining fire occurrence or fire intensity.

NMR analysis

The ^{13}C CPMAS NMR spectra of burnt and unburnt soils from OR and MG are shown in Fig 1, while Table 5 reports the spectra intensity distribution.

The ^{13}C CPMAS NMR spectra of bulk soil from burnt and unburnt soil at OR showed some differences, due to the fire occurrence. The most evident difference was in the aryl C region (110-160 ppm), indicating an enrichment of the aromatic components, clearly correlated to the fire

occurrence, since the majority of the signal at around 130 ppm belongs to charcoal (Skjemstad et al., 1996). Another evident difference was in the alkyl C region (0 – 45 ppm), indicating a loss of aliphatic structures due to fire.

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	30	7	20	6	22	7	8
Unburnt	38	6	22	7	14	6	7
Mount Gordon (MG)							
Burnt	27	8	24	7	18	8	8
Unburnt	30	8	31	8	12	5	6

Table 5. Relative distribution (%)[a] of the chemical shift regions 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) is not reported because negligible.

A slight decrease of the signal intensity was noted in the O-alkyl region of the spectra, most probably assigned to carbohydrate contribution to TOC in the burnt soil.

The ^{13}C CPMAS NMR spectra of burnt and unburnt soil at MG showed not so large differences, despite the extremely high fire intensity. Such result was partly predictable considering the data about the sugars and lignin content that did not show large differences when related to TOC because, apparently, fire did not alter that much relative OM composition.

The burnt soil was still 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 for polysaccharides and amide C of proteins (Knicker and Lüdemann, 1995). In the alkyl C region there was a small difference (2%) between signal intensity in the burnt soil compared to the unburnt. However, looking at the shape of the peak in this region, the little shoulder at 39 ppm of unburnt soil sample,

disappeared in the burnt soil where the shoulder at 20 ppm seemed to be more evident. The signal in the first part of alkyl C region may be attributable to terminal CH_3 groups, while signal in the final part of alkyl C region may be attributable to methylene groups in aliphatic rings and chains (Knicker and Lüdemann, 1995; Tinoco et al., 2006). These results together would indicate the reduction of the chain length of alkyl compounds, that is a common finding in the burnt soils (Gonzàlez-Perèz et al., 2004).

Figure 1. ^{13}C CPMAS NMR spectra of samples from Orentano and Mount Gordon: above spectra of mineral soil from unburnt areas, below spectra of mineral soil from burnt areas.
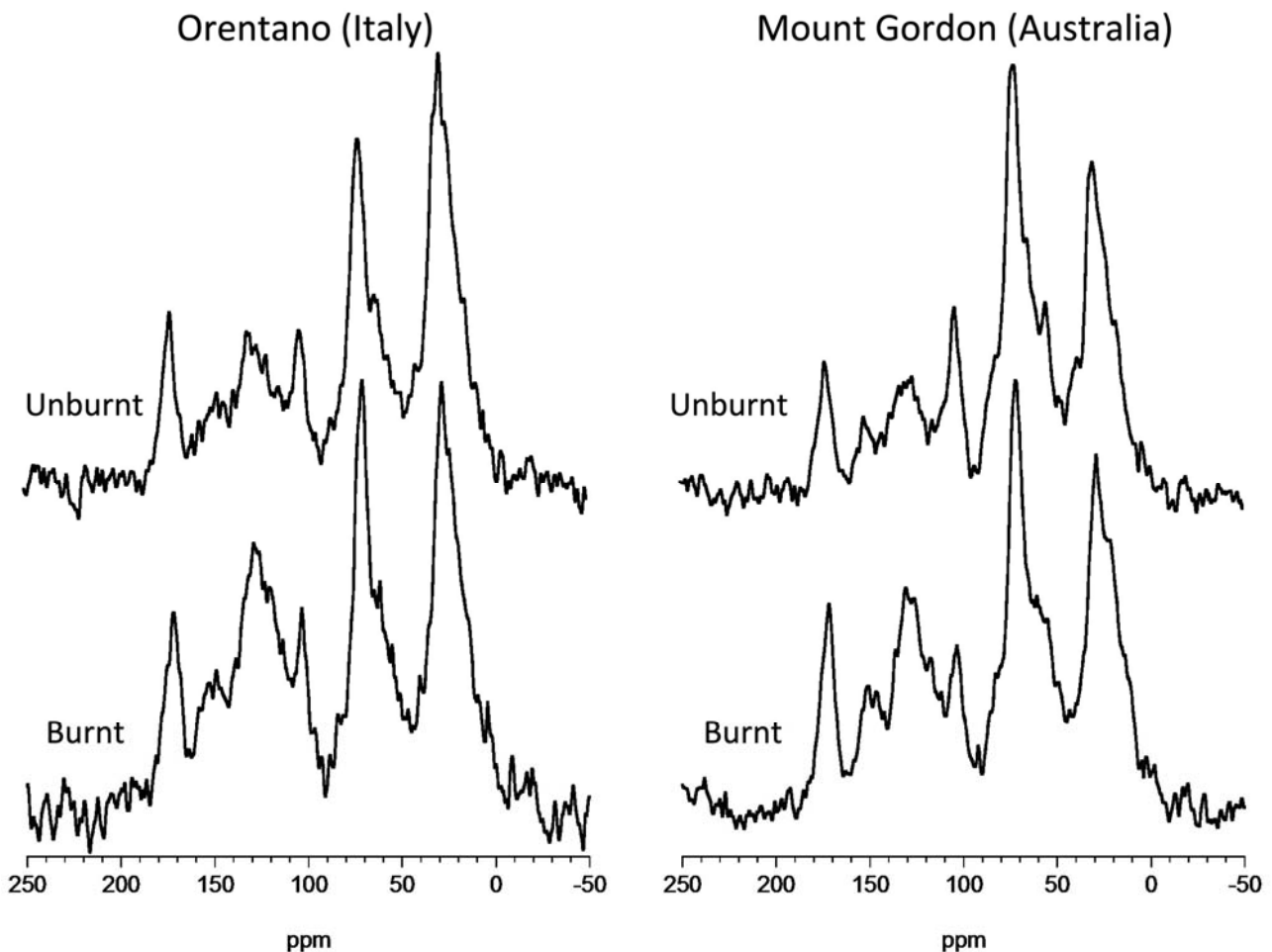
Despite the chemical shift region 45-60 ppm did not show a relative quantitative change in the signal intensity, in the burnt soil the peak at 56 ppm visible in the unburnt soil spectrum became

only a minor shoulder, possibly indicating an oxidation of the methoxil C groups in lignin structure (Hatcher, 1987; De la Rosa et al., 2008).

In the O-alkyl C region the loss of signal intensity was more consistent, 7%, and confined in the range 60-90 ppm, greatly attributable to carbohydrates-derived structures C2, C3, C4 and C5 of hexoses (Knicker and Lüdemann, 1995).

This result would seem to clash with results obtained from sugar quantification. But this data is comprehensive of all sugars in the soil, not only the non-cellulosic ones. Moreover, the results from NMR are relative and not absolute. In fact, in the region 110-160 ppm, representing the unsubstituted and the C- and O-substituted aromatic carbon, burnt soil showed more intense signals due to the formation of new fire-derived aromatic compounds (Skjemstad et al., 1996). Therefore, a relative decrease of the integration value for the O-alkyl C region, was also due to a relative increase of the integration value for aryl C region.

At the aryl C region may be attributable also the phenols belonging to lignin units (Knicker and Lüdemann, 1995). Losses of lignin, hence, could have been counterbalanced by signals from new formed aromatic compounds.

Finally, a slight intensification of the peak at ~165 ppm rather of a decreasing, indicative for carboxyl C, remains hard to explain. In fact, organic matter exposed to severe heating should contain minor amounts of carboxyl C (Knicker et al., 2005a). The oxidation of charred remains and lignin compounds during post-fire humification could partly explain this result (Kögel, 1986; Knicker et al., 2005a), but the short time elapsed between fire occurrence and sampling let us to doubt about this hypothesis.

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Manuscript II

Application of thermal and spectroscopic techniques to assess the changes and the thermal stability of pyrogenic organic matter in a Mediterranean burnt forest

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Abstract

Fire is a major disturbance to vegetation and soil in the Mediterranean basin. The high temperatures may irreversibly alter physical, chemical, and biological soil properties. Soil organic matter (SOM) is the soil component most sensitive to fire, affected both in terms of content and composition. Incomplete combustion yields condensed carbon forms characterized by higher recalcitrance compared to the parent material. In this study, thermogravimetric (TG-DTG), differential scanning calorimetry (DSC), attenuated total reflectance/Fourier transform infrared (ATR/FT-IR) and Raman

spectroscopies were applied to charcoal, mineral soil and extracted SOM fraction to investigate the wildfire effect on the organic matter of a pine forest soil. In order to better understand such an effect, the same investigation was performed on the soil and the litter layer of an adjacent unburnt area. TG-DTG and DSC measurements displayed a substantial reduction of the most labile fraction and a relative enrichment of the recalcitrant and refractory OM pool. The ATR/FT-IR supported the formation in burnt SOM of stable N polyaromatic compounds, that was well highlighted also by the presence of CN groups vibration in Raman spectrum. The study demonstrates that the combined application of thermal and spectroscopic techniques are an efficacious strategy to investigate the effects of fire on soil organic matter, because it is fast and does not require any chemical pre-treatment that could lead to creation of artefacts.

Keywords: Forest fire; Soil organic matter; Charcoal; FT-IR; Normal Raman spectroscopy; Thermal analysis

Highlights: We analyzed the effect of a fire on Soil Organic matter of a Mediterranean forest; Fire caused a loss of labile organic matter in soil; Fire led to the formation of more stable polyaromatic N containing molecules; We collected, rapidly and cheaply, reliable data on SOM changes due to wildfire.

Introduction

In order to completely understand the role of soils as carbon stores it is essential to evaluate the vulnerability of SOM to disturbances or climate change as well as to characterise and identify SOM fractions with short *versus* long residence times (Smith, 2005; Schuur et al., 2008). Fire is a major disturbance and driving factor of ecosystems in the Mediterranean basin, as up to 90% of all forest fires in the EU occur in Mediterranean countries (De la Rosa et al., 2008b), where in the next future,

a higher fire frequency is expected due to Global Change, *i.e.* increasing temperature and decreasing precipitation (IPCC Fourth Assessment Report, 2007), and anthropic activities (Pausas et al., 2008). In the Mediterranean area, soils are dramatically impacted by wildfires (Shakesby, 2011), especially because of post-fire erosion, which upset or even remove the topsoil. Soils suffer many direct consequences because of the occurrence of fires (Certini, 2005). In particular, the organic component of soil is affected in terms of both content and composition (González-Pérez et al., 2004; Knicker, 2007).

In terms of quality, fire increases the heterogeneity of SOM because apparently no original component is totally removed, while new fire-derived aromatic thermally-condensed C compounds can be formed as a consequence of chemical reactions driven by the high temperatures (Schmidt and Noack, 2000; Kuhlbusch and Crutzen, 1995; González-Pérez et al., 2004; Francioso et al., 2011) constituting the so-called pyrogenic organic matter (PyOM). Some constituents of PyOM are reported to have longer residence times in soil compared to the original materials, having a considerable impact on the long-term carbon sequestration potential of soils (Kuhlbusch and Crutzen, 1995; Schmidt and Noack, 2000).

No single analytical method can adequately account for the continuum of materials that form during fire, therefore a multiple-technique analytical approach is required to describe the chemical complexity of PyOM.

Thermal analysis techniques such as differential thermal analysis (DTA), differential scanning calorimetry (DSC), and thermogravimetry (TG), have long been used in the determination of basic soil properties (Siewert, 2004). In particular, these techniques provide a complex pattern of soil physical and chemical properties that can be used, with the accuracy comparable other techniques, to characterize organic matter in bulk soils, isolated soil fractions, humic substances and other organic matters such as forest litter (Dell'Abate et al., 2002; Francioso et al., 2005; Barros et al., 2007; Plante et al., 2009; Tonon et al., 2010; Barros et al., 2011; Fernandez et al., 2011). While the

results obtained on soils are promising, there are a few information on thermal behaviour of SOM affected by fires. Wildfires can result in soil temperatures higher than 300 °C, causing SOM loss and an increase in the relative abundance of aromatic structures (Baldock and Smernik 2002; Knicker et al., 2005). The accumulation of aromatic compounds is due not only to the higher thermal resistance of aromatic structures, but also to formation of new aromatic C forms as a consequence of chemical reactions driven by the high temperatures (Schmidt and Noack, 2000; Francioso et al, 2011). The use of thermal techniques offers the unique opportunity to directly measure changes in thermal stability of SOM as a function of heating effect (Plante et al., 2009) and might be fruitfully used to distinguish PyOM from other SOM fractions (Leifeld, 2007)

However, thermal methods do not provide equivalent information about the chemical composition of samples as other analyses, as spectroscopic ones, do (Plante et al., 2009). Therefore, it is required to use complementary methods to have a more complete information (Barros et al., 2001; Plante et al., 2009)

Infrared spectroscopy (Bornemann et al., 2008; Lammers et al. 2009; Keiluweit et al; 2010; Nocentini et al., 2010), alone or in combination with Raman spectroscopy (Potgieter-Vermaak et al, 2011; Francioso et al., 2011), has been successfully applied to characterize PyOM and charcoal. In fact, aromatic bonds with different substitutions respond directly to infrared spectroscopy, while Raman spectroscopy provides valuable information on both aliphatic and aromatic component. But, spectroscopic analyses have the limit to be only modestly influenced by the physical arrangement of molecules and macro-structures. On the other hand, thermal stability of a material is strongly affected by its physical structures (Plante et al., 2009). Therefore, the parallel use of spectroscopic and thermal techniques is a reliable approach to study PyOM. A great advantage of spectroscopic and thermal methods is that they do not require any chemical pre-treatment of samples, which unavoidably creates some artefacts.

In the present study, we used thermal (TG and DSC) and spectroscopic (FT-IR and Raman) analyses to characterise the effect of a wildfire on the physical and chemical properties of SOM of a typical Mediterranean pine forest. For this purpose, we analysed the bulk soil, extracted SOM fraction and handpicked charcoal laying on the ground of a burnt area, comparing the findings with those carried out in an adjacent unburnt area on the same type of sample with the exception of litter that was analysed instead of charcoal.

Materials and methods

Study site

Figure 2. Location of the study area located in the south of Tuscany region, middle Italy.

The study was carried out in a forest located in the municipality of Roccastrada, Southern Tuscany, central Italy (42° 54' 13.68 "N, 11° 08' 48.24 "E), 25 km away from the coast with an elevation of 360 m above sea level. The site experiences a mean annual temperature of 13.5 °C and a mean

annual precipitation of 897 mm. The forest is a planted pine forest of maritime pine (*Pinus pinaster* Ait.) mixed to ancillary trees of *Quercus suber* and *Quercus ilex.* The understorey is heterogeneous and composed of shrub species typical of Mediterranean vegetation, such as *Arbutus unedo* and *Erica spp.* The soil developed on a metamorphic formation mainly constituted by quartzite and schist sandstone and soils showed an O-A-Bw-C sequence of horizons and were classied as Distri Episkeletic Cambisol according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2006).

The wildfire considered in this study occurred in July 2010 and involved an area of 33 ha, where the forest had been just cut and the felled logs were lying on the ground. Based on the amount of canopy burnt and ground observations using visual scale of fire severity proposed by Chafer et al. (2004), the wildfire in question was classified as very highly severe. This class involved complete consumption of ground fuel and shrubs, canopy completely burnt with all the stems <5mm incinerated.

Sampling

Soil samples were collected two months after the fire. At the time of sampling there was only a scant vegetation regrowth and a charcoal and ash layer covered almost all of the burnt area. The sampling was carried out randomly, on six plots from the burnt area and six plots from an adjacent unburnt area. Fire did not run on the latter because it was stopped by a permanent track. The organic matter above the mineral soil (ash layer in the case of the burnt area, and the litter layer in case of the unburnt area) were sampled separately on a 40 x 10 cm^2 area, using a trowel and a brush. The mineral soil was sampled with a spade down to 10 cm, cutting a 15x15 cm monolite of soil.

All samples were air dried in the laboratory. Afterwards the organic material overlying the mineral soil, burnt or unburnt, was finely ground (saving an intact aliquot) and the mineral soil sieved to 2 mm. Replicates were combined to make single samples for the unburnt (C) and the burnt (B) area.

Elemental analyses

Soil pH was measured potentiometrically using a deionised water to soil ratio of 5:1, and soil texture was determined according to the hydrometer method. Total C and N analysis were performed in duplicates by dry combustion using a CHN analyser (Perkin-Elmer 2400, series 2).

The ash content was determined using Loss On Ignition (LOI) method, firstly oven-drying the samples at 105 °C to constant weight (the relative mass loss from samples was registered as moisture), then keeping them in a muffle furnace at 550 °C for 6 h.

Soil organic matter (SOM) extraction

About 5 g of pulverized mineral soil was treated under N_2 with 50 mL of 0.5 M NaOH and stirred for 24 h. The suspension was centrifuged at 8,000 g for 20 min, and then filtered through a 0.45-µm filter using a Minitan S System (Millipore, Bedford, MA, USA). The solution was dialyzed against Millipore water, using tubing (Cellu SepH1-USA) with a cut-off of 8,000 Da until a neutral pH, then freeze-dried.

Thermogravimetric (TG), derivative thermogravimetric (DTG) and differential scanning calorimetry (DSC) analyses

The TG/DTG curves were obtained by a Mettler TA-STAR, TGA/SDTA 851[e] thermobalance. About 5-10 mg of sample were weighed in alumina crucible and first isothermally heated to 30 °C for 30 min. Afterwards, they were progressively heated at 30 to 800 °C in a dynamic air (80 mL min^{-1}) and nitrogen (80 mL min^{-1}) atmosphere. Heating rate was 10 °C min^{-1}.

Weight loss in each thermograms was expressed as percentage of total dry mass.

The DSC curves were obtained by a Mettler TA-STAR, DSC 821^e calorimeter. Samples, in the 0.5-3.0 mg weight range, were inserted into perforated aluminium pan, within a dynamic air atmosphere (80 mL min^{-1}), and heated at a rate of 10 °C min^{-1} to 500 °C.

Nitrogen atmosphere experiments were not performed on samples because of the too high nitrogen dynamic flux necessary to ensure that no air could enter in the DSC chamber. Samples of indium were used to calibrate the calorimeter.

Enthalpy of combustion, (ΔH), of SOM was directly calculated from the thermograms, as the area between the baseline of the apparatus and the exothermic combustion peak, and was normalised to the dry weight of each sample.

The weight losses and the combustion peaks were estimated by resolving the DTG curves and DSC thermograms with a curve fitting procedure. The position and the initial height and width of the peaks were guessed by the software used for fitting analysis (Wojdyr, 2010). The bands were fitted using logNormal functions derived from the Gaussian function, taking however into account the typical asymmetry of weight losses and combustion peaks.

Spectroscopic analyses

The ATR/FT-IR spectra were recorded using a Nicolet 5700 Thermo-Corporation equipped with a diamond attenuated total reflectance (ATR) accessory (Spectra-Tech, Shelton, CT), by co-addition of 100 scans at a resolution of 4 cm^{-1} in the range 4000-400 cm^{-1}. A background spectrum was recorded prior to collection of each sample spectrum using the diamond crystal only.

The second-derivative spectra of the IR spectrum in the 1850-1250 cm^{-1} region, smoothed with a 5-point smoothing function, were used to define some starting parameters for curve fitting analysis, such as the number and the position of the peak components. The fit was performed by a commercial software (Grams/32 AI 6.00 by Galactic Industries Corporation) on the original spectra

after baseline correction, using the Levenberg-Marquardt algorithm. The IR band components were described as Gaussian functions.

Raman spectra were obtained directly on few mg of extracted SOM, using a Jasco NRS-2000C instrument. Spectra were recorded in backscattering conditions with 4 cm^{-1} spectral resolution, using the 514 nm [Ar+] line. The laser power (Innova Coherent 70) was about 5 mW for the samples, and the total number of scans averaged for each spectrum was 25. The detector was a 160 K frozen digital CCD (Spec-10:100B, Roper Scientific Inc.). The temperature was hold constant at 25 ± 1 °C. The FT-IR and Raman spectra were processed using Grams/386 spectroscopic software (Galactic Industries, Salem, NH, USA).

Statistical analysis

The difference between means of carbon and nitrogen concentrations was tested using the Tukey test at 95% confidence level using STATGRAPHICS software (Statpoint Technologies, Warrenton, VA, USA).

Results and discussion

Elemental analysis and pH

As expected, the pH of burnt soil shifted towards neutrality (Table 1) as a consequence of combustion, favouring the basic cations release from the organic residues (Arocena and Opio, 2003).

The C and N content of litter, charcoal, burnt and unburnt soils and relative extracted SOM are shown in Table 2. Humidity and ash content of burnt soil were significantly higher compared to the unburnt soil. Both these parameters directly depends on the SOM content of samples. A significant enrichment of the SOM fraction in the top mineral soil is reported in several studies (*e.g.*

Almendros et al., 1988; González-Vila et al., 2002; Certini et al., 2011) and is due to a substantial

incorporation of (partly charred) forest necromass after fire occurrence.

Table 6. Location, pH and texture of soil samples

Site	Location	pH	Sand	Silt	Clay
			%	%	%
Unburnt	42°54'15.01" N	4.9	43	40	17
	11° 8'46.18" E				
Burnt	42°54'14.17" N	6.4	41	43	16
	11° 8'48.99" E				

This result is restricted to the top mineral soil and does not mean a net SOM increase for the whole

soil. Usually, in fact, the litter layer is partly or completely incinerated during a wildfire, resulting in

a net loss of SOM.

Table 7. C, N, C/N ratio, moisture and ash content (average ± SD, n= 6 for C and N concentration, n=3 for moisture and ash content) of litter and charcoal, soil and extracted SOM from unburnt and burnt areas

	C (%)	N (%)	C/N	Moisture (%)	Ash (%)
Soil					
Unburnt	7.6 ± 1.8	0.23 ± 0.07*	33	2.0 ± 0.4*	89 ± 1.6*
Burnt	8.3 ± 0.8	0.37 ± 0.05*	22	3.0 ± 0.2*	75 ± 0.5*
SOM					
Unburnt	42.3 ± 0.1	2.2 ± 0.2	19	11 ± 3.2	24 ± 3.7
Burnt	41.4 ± 0.4	2.1 ± 0.1	20	9.2 ± 1.7	24 ± 1.9
Litter	41.6 ± 0.4	1.3 ± 0.1	31	9.4 ± 0.5	17 ± 6.5
Charcoal	33.8 ± 2.0	1.7 ± 0.1	19	3.2 ± 2.7	56 ± 3.6

* significantly different means (P <0.05)

The extracted SOM from both soils did not show substantial difference in C and N, despite a significant difference in the N concentration between the burnt and the unburnt soil ($P < 0.05$). Post-fire N increase in the uppermost layer of soil as observed in our study is not uncommon (Wan et al., 2001; Johnson & Curtis, 2001) and several authors observed an accumulation of organic N-forms, prevalently heterocyclic N, in the charred material (Almendros et al., 2003; Knicker et al., 2005b; Santin et al., 2008). Our results were in agreement with those obtained by Nocentini et al. (2010) and reflect the typical behaviour of soil humic substances in the course of laboratory heating (Almendros et al., 1992; Baldock and Smernick, 2002; Francioso et al., 2009). The persistence of N in soil instead of volatilization, moreover, suggests that the combustion of biomass could be occurred at relatively low temperature (350 °C) and under oxidising conditions (Knicker et al., 2008). The fire-induced increase in N concentration relative to C resulted in a large difference in C/N ratio, much lower in the burnt soil than in the unburnt one.

Thermal analyses

The thermograms (DTG and DSC) of samples under air flow showed different characteristics, dealing with temperature, weight loss (Table 3) and enthalpy of combustion (ΔH) (Table 4). For both burnt and unburnt bulk soil samples we found lowest weight losses (Fig. 2), which are a consequence of a high mineral content that leads to a 'dilution' of the organic component (Schulten et al., 1999; Manning et al., 2005; Lopez-Capel et al., 2005). Nonetheless, a difference is clear: the unburnt soil outcome is characterized by two peaks only, at 300 °C and 407 °C, roughly corresponding to labile and recalcitrant organic matter, respectively (Dell'Abate et al., 2003; Plante et al., 2005; Lopez-Capel et al., 2005). and a little shoulder at 499 °C. (Fig. 2).

In accordance with several studies (Angehern-Bettinazzi et al., 1998, Dell'Abate et al., 2003; Lopez-Capel et al., 2005, 2006; Francioso et al., 2005; Plante et al., 2009), the first peak (300 °C) is produced by the decomposition of more thermolabile compounds, basically aliphatic compounds,

cellulose, holocellulose, proteins, and simple sugars, whereas the second peak (407 °C) is due to decomposition of condensate structures, mainly aromatic components, such as humic acids and lignin (Barros et al., 2011). In the burnt soil a couple of peaks at 506 and 632 °C, absent in the unburnt soil and associated with a small mass loss, were detected; they are peculiar of highly refractory organic matter, viz., black C (De la Rosa et al., 2008a, b). The formation of polycondensed aromatic carbon compounds is actually consistent with the thermal reactions occurred in soil during a fire event.

Figure 3. DTG of soil from unburnt (gray) and burnt (black) samples. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the mass loss.
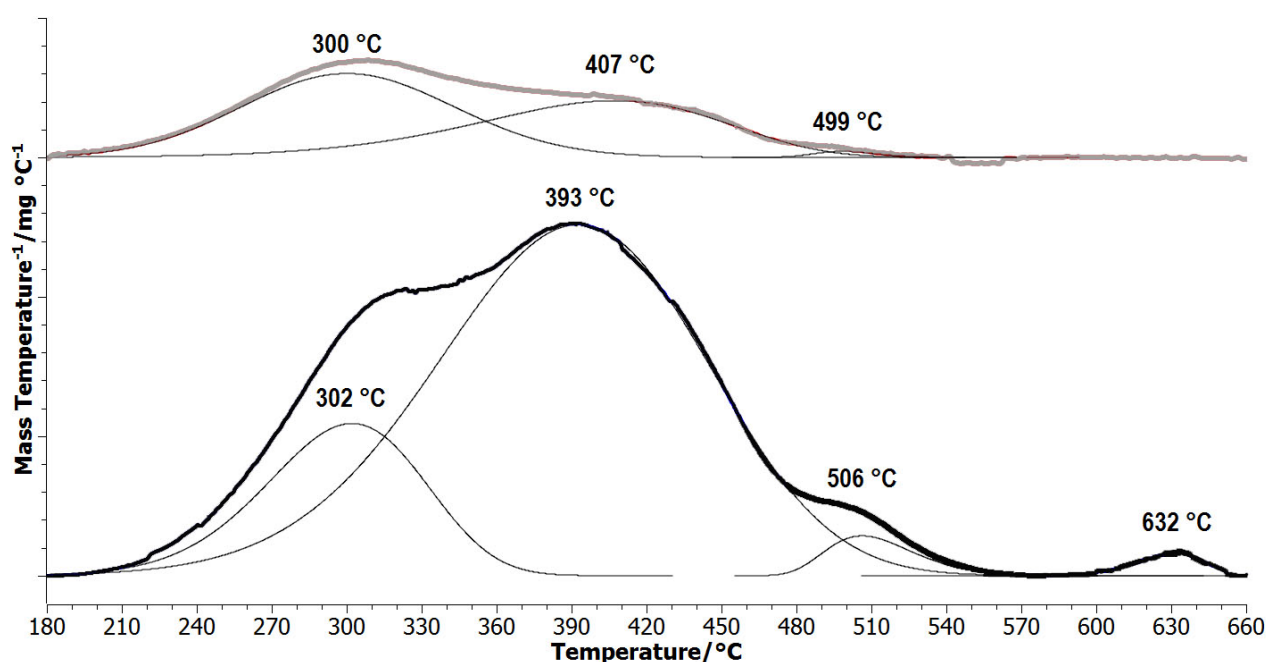
The DSC curves of bulk soil samples showed two main exothermic peaks at about 300 °C (Exo1) and 400 °C (Exo2) (Fig. 3), which correspond to those obtained in the DTG analysis. Both soils showed an endothermic peak at around 55-58 °C relative to water loss. In our experiment, the higher enthalpy of water loss was recorded in the burnt soil (ΔH =-78 mJ/mg), which was not expected at all because soils affected by fire usually are characterized by hydrophobic properties. However, this result is mainly due to the organic matter content of sample, which is the most

hygroscopic component of soil and can hold up to 20 times its weight in water (Ushakov et al., 2011). Also, the great Δ*H* value in the burnt soil might ascribable to the hydration water of salts deposited on the ground after combustion.

DTG	Soil				SOM				Litter		Charcoal	
	Unburnt		Burnt		Unburnt		Burnt					
Peaks	T_{max} °C	Mass Loss (%)	T_{max} °C	Mass Loss (%)	T_{max} °C	Mass Loss (%)	T_{max} °C	Mass Loss (%)	T_{max} °C	Mass Loss (%)	T_{max} °C	Mass Loss (%)
1	300	3.7	302	13.0	277	22.7	278	22.2	309	48.0	407	37.0
2	407	2.9	393	13.5	470	1.5	456	3.1	409	33.0	501	3.7
3	499	0.1	506	0.5	544	7.5	477	1.5			658	1.2
4			632	0.3	598	2.8	534	4.5				
5							589	4.9				

Table 8. Thermogravimetric (TG) analysis results under air flow (dynamic condition) of unburned and burnt soil and their extracted SOM fraction, litter and charcoal. Mass loss is expressed as percentage of total sample mass after subtracting the loss of water (dry mass).

	Soil				SOM				Litter		Charcoal	
	Unburnt		Burnt		Unburnt		Burnt					
Peaks	T_{max} °C	Δ H mJ/mg	T_{max} °C	Δ H mJ/mg	T_{max} °C	Δ H mJ/mg	T_{max} °C	Δ H mJ/mg	T_{max} °C	Δ H mJ/mg	T_{max} °C	ΔH mJ/mg
↓ Endo 1	58	-15	55	-78	56	-452	54	-310	54	-13	62	-93
↑ Exo 1	298	308	310	2284	277	6327	281	3200	325	376	376	6068
↑ Exo 2	383	540	374	3894	378	639	375	298	414	269	437	681
↑ Exo 3	428	29	425	1598	438	896	420	596				
↑ Exo 4			473	220	465	2877	440	109				
↑ Exo 5							463	1560				

Table 9. DSC analysis under air flow (dynamic condition) of unburned and burnt soil and their extracted SOM, litter and charcoal. Exothermic combustion peaks are normalised on the dry weight of each sample

The enthalpy of combustion (ΔH) for Exo1 and Exo2 were considerably higher in burnt soil than in the unburnt one (Table 4). It indicated a higher amount of C content, as also supported by elemental analysis (Table 2), and a more complex organic matter nature (see below IR spectrum of burnt soil). In other studies (*e.g.* Salgado et al., 2009; Leifeld, 2007), the enthalpy of combustion in heated soils in the range 200-350 °C in laboratory experiments was found to be lower than in the unheated soils evidently because the decomposition of a part of the organic matter took place during the heating phase. The differences in the ΔH values between the burnt and unbunrt soils, were attributed to the different OM content between the two sampling areas; nevertheless it was noted that Exo1 reaction took place at a temperature ~12 °C higher in the burnt soil, which. This result might indicate an increase in thermal stability of the labile fraction (De La Rosa et al., 2008b).

Figure 4. DSC of soil from unburnt (gray) and burnt (black) samples. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the energy transfers.
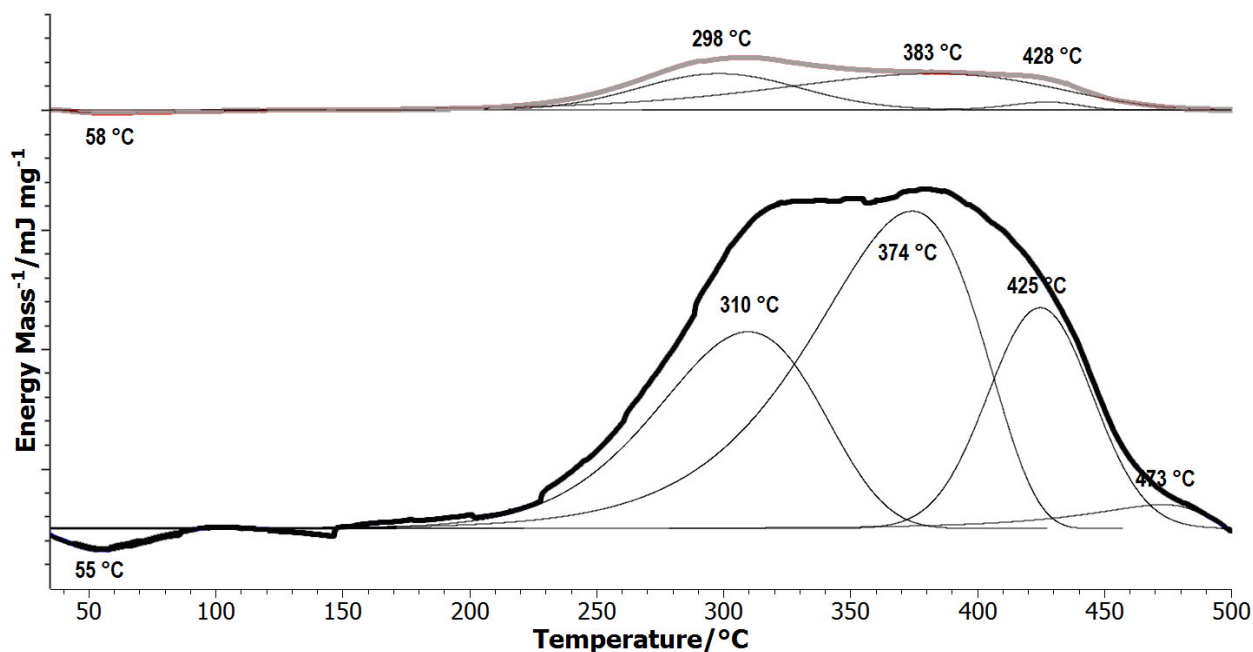
The DTG patterns of the SOM extracted from soil are shown in Fig. 4, while the observed weight losses are displayed in Table 3. Both SOM fractions were characterized by exothermic reactions with similar temperatures and weight losses. These latters occurring in the range 534-598°C were

associated to the highly refractory forms of organic matter such as polycondensed aromatic carbon which accounted for about 10% of total for both the extracted SOMs. Part of these peaks could be actually due to an artifact, *i.e.* char formation from cellulose during sample heating (Kaloustian et al., 2001; Leifeld, 2007; Lopez-Capel et al., 2005; Shapchenkova et al., 2011). Burnt SOM differed from the unburnt one for the presence of one more peak at about 456 °C, likely due to the fire-induced formation of relatively thermostable compounds.

Figure 5. DTG of SOM from unburnt (gray) and burnt (black) samples. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the mass loss.
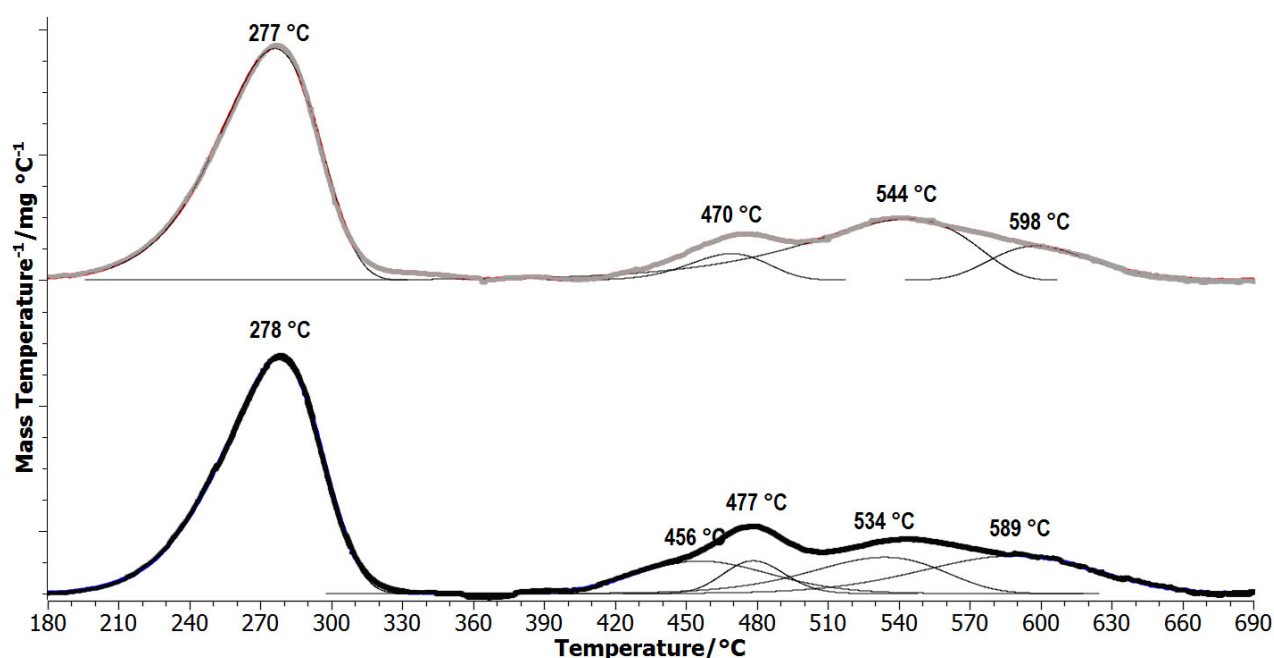
The values of the combustion enthalpy for SOM samples are shown in Table 4. The SOM extracted from the burnt soil showed much lower values of ΔH than the SOM from the unburnt soil, particularly for Exo1 and Exo2. This should indicate that fire occurrence caused a substantial reduction of the most labile fraction.

The DTG and DSC values of litter and charcoal are shown in Table 3 and Table 4. The DTG thermogram of litter showed two prominent peaks at 309 °C and 409 °C. Most of the weight loss

(48%) occurred in correspondence of the first peak and was ascribed to thermal decomposition of hemicellulose, lignin monomers, peptides and fatty acids (Schulten et al., 1999). The peak at 409 °C, which is the result of a 33% of weight loss, could be attributed to the decomposition of aromatic structures (Dell'Abate et al., 2002), although aliphatic macromolecules in the form of long chain hydrocarbons (Dell'Abate et al., 2002; Montecchio et al., 2006) and nitrogen compounds can also contribute to thermal reactions above 400 °C. The DSC thermograms of litter were similar to those obtained by DTG. Taking into account the significant energy produced by Exo1 (ΔH= 376 mJ/mg) seems plausible to attribute this reaction to hemicellulose decomposition, while the low ΔH (269 mJ/mg) in Exo2 might be indicative of some hemicellulose with increased thermal resistance. Large contribution of lignin is improbable since lignin shows a very intense peak over 400 °C requiring great amount of heat liberated (5667 Jg^{-1}) in the reaction.

Figure 6. DSC of SOM from unburnt (gray) and burnt (black) samples. The thin black lines are the results of the peak fitting procedures. The area of each peak was used to determine the energy transfers.
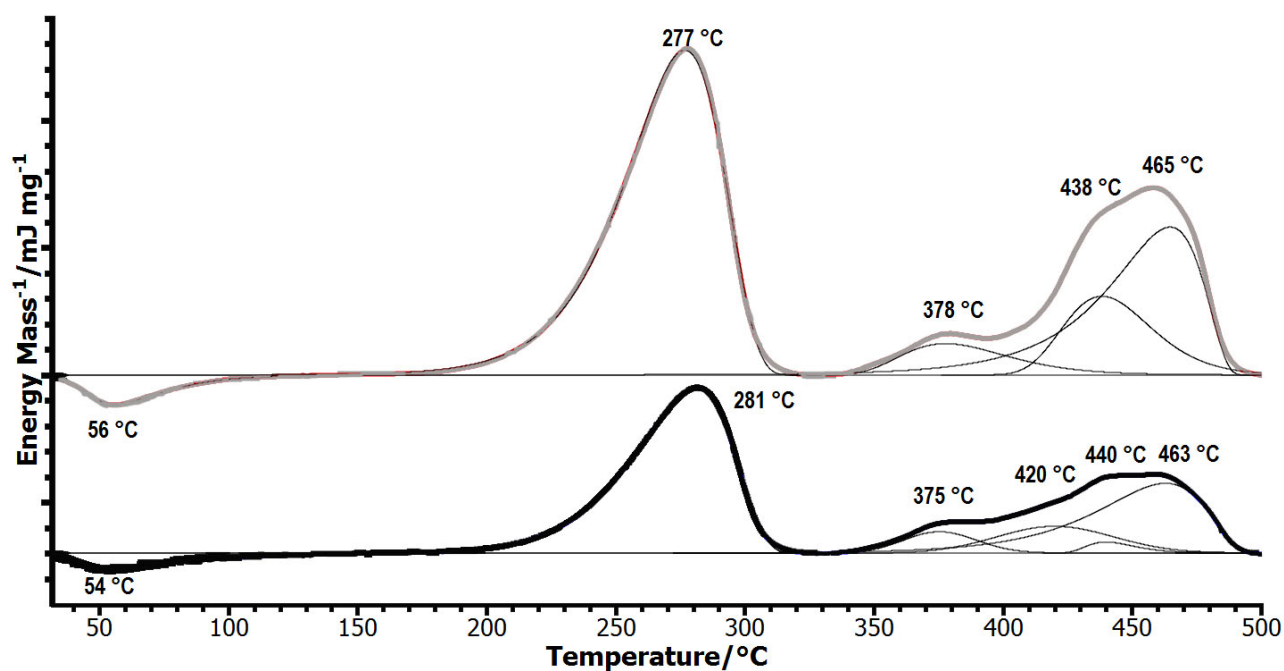
The DTG thermogram of charcoal was characterized by three peaks: the first one at 407 °C, with a weight loss of 37 %, and two extra refractory peaks at 501 and 658 °C, with a relative weight loss of 3.7% and 1.2 %, respectively (Table 3). The high enthalpy of water loss (ΔH =-92 mJ/mg) in charcoal is probably due to the absorption of water molecules inside the highly porous structure of charcoal. The enthalpy of combustion was highest in Exo1 (6068 mJ/mg), while it decreased much for the shoulder at 437 °C (Table 4). These results suggested that the charcoal particles were not completely charred because they still contained a part degradable under 500 °C. These results, furthermore, confirm that the aromatic skeleton of charcoal from wildfires has few graphitic domains (Knicker, 2007). In fact, graphite shows thermal decomposition reactions at around 800 - 900 °C and these were completely absent in our charcoal sample. On the other hand, it is known that charcoal formed under natural fire conditions and the one obtained under laboratory conditions have quite different characteristics.

ATR/FT-IR and Raman analysis

The ATR/FT-IR spectra of bulk soils were only investigated in the region between 1850 - 1250 cm^{-1} because of strong signal due to the mineral compound vibration. The unburnt soil spectrum (Fig. 6) was dominated by two broad bands at 1630 cm^{-1} and 1440 cm^{-1} manly assigned to OH bending vibrations of the water between phyllosilicates layers (Ellerbrock et al., 2009; Tonon et al., 2010). However, a contribution by vC=O of carboxylate groups (1630 cm^{-1}), vC=C of aromatic rings and C-H deformation and asymmetric stretching of the COO$^-$ group (1460–1390 cm^{-1}) can be not excluded.

The burnt soil spectrum (Fig. 6) was characterized by a set of unresolved peaks. In order to better study the FT-IR spectrum, a curve fitting analysis was performed. The curve fitting procedure actually allowed determining the number and the precise location of the overlapped peaks. The best goodness-of-fit was obtained with a fitting model based on pure Gaussian peak shapes (R^2 = 0.992

± 0.009 SE). The bands at around 1540 and 1389 cm^{-1} can correspond to the G (ca. 1575 cm^{-1}) and

D (ca. 1360 cm^{-1}) graphite as described by Francioso et al. (2011). Usually, the C=C bonds in

graphite rings are not IR-active but the substitution of C with N in the aromatic bonds breaks the

symmetry of rings structure, promoting bond dipoles and making these bands IR-active. The peak at

1289 cm^{-1} probably belongs to C-N and phenolic C-O stretching, whereas the band at 1708 cm^{-1} can

be confidently assigned to C=O stretching vibration in benzene-carboxylic acids (Gonzalez-Perez et

al. 2004). The band at 1708 cm^{-1} can arise from the carbonyl/carboxyl stretching of lignin, which

may still be present at 300 °C (Lammers et al., 2009). The appearance of these components hence

suggests that the soil was heated to less than 400 °C.
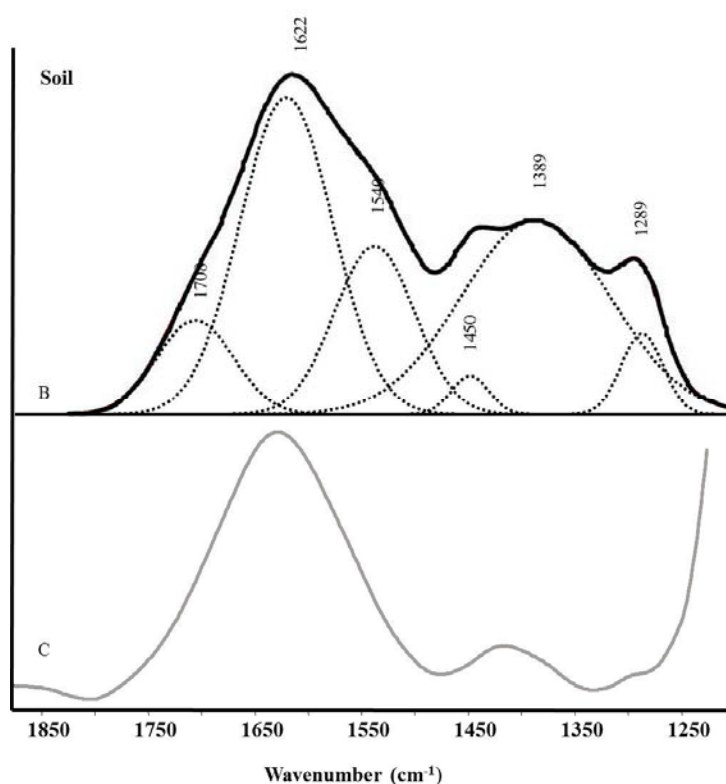
Figure 7. ATR/FTIR spectra of burned (B, black line) and unburned (C, gray line) soil. Curve fitting of B spectrum was obtained by using a Gaussian model.

The SOM extracted from the burnt and unburnt soils showed a similar FT-IR spectroscopic pattern,

at least in the 4000 – 2800 cm^{-1} and 1800 – 400 cm^{-1} (Fig. 7). Major peaks were at 3330 cm^{-1},

assignable to O-H stretching and N-H stretching (Senesi et al., 2003; Francioso et al., 2009), and at

2922 and 2860 cm^{-1}, assignable to aliphatic C-H stretching of CH$_3$ and CH$_2$ groups, respectively. The strong band at 1571 cm^{-1} was due to several group vibrations including aromatic C=C, C=O asymmetric stretching of carboxylate, quinonic C=O and/or C=O of H-bonded conjugated ketones. The band at 1386 cm^{-1} was the result of O-H deformation, C-O stretching of phenolic OH, and CH deformation of CH$_2$ and CH$_3$ groups. The shoulder at 1264 cm^{-1} is generally ascribed to C-O stretching and O-H deformation of carboxyl and C-O stretching of aryl ethers. Finally, the band at 1030 cm^{-1} was produced by the C-O stretching of polysaccharide or polysaccharide-like substances. The spectra diverged in the 2400-2000 cm^{-1} region, where the SOM from the burnt soil revealed the presence of vCN stretching vibration (Francioso et al., 2009). The formation of CN groups probably arises from the dehydration of aromatic amides or from the ring scission and subsequent rearrangement of N heterocyclic polyaromatic molecules. Pyrolytic organic N formation is debated, even if it has been detected in severely charred biomass at temperature around 350 °C (Knicker et al., 2008).

Figure 8. ATR/FTIR spectra of SOM extracted from the burnt (B, black line) and unburned (C, gray line) soil; Raman spectrum of SOM extracted from the burnt soil (B) recoded in the region between 2400-2200 cm^{-1} (top).
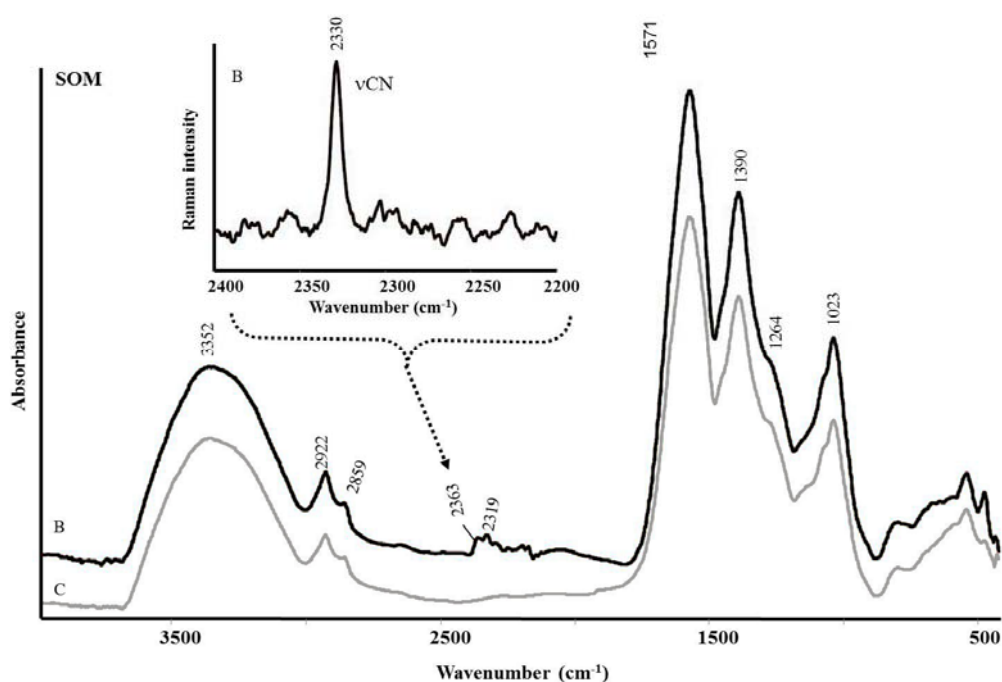
The presence of CN stretching vibration in burnt SOM was confirmed by a sharp, intense peak at 2329 cm^{-1} in the Raman spectrum (Fig. 7). This peak was placed at a noticeably lower frequency than the overtones and/or combination bands of graphite, which fall at about 2450 cm^{-1} (Francioso et al., 2011). It is hence clear that N is not depleted by the burning, but tends to be incorporated into fairly resistant structures. By contrast, no CN group vibration was detected in SOM extracted from the unburnt soil.

Figure 9. ATR/FTIR spectra of litter (top) and charcoal (bottom). The main chemical reactions due to the combustion are indicated by dotted lines.
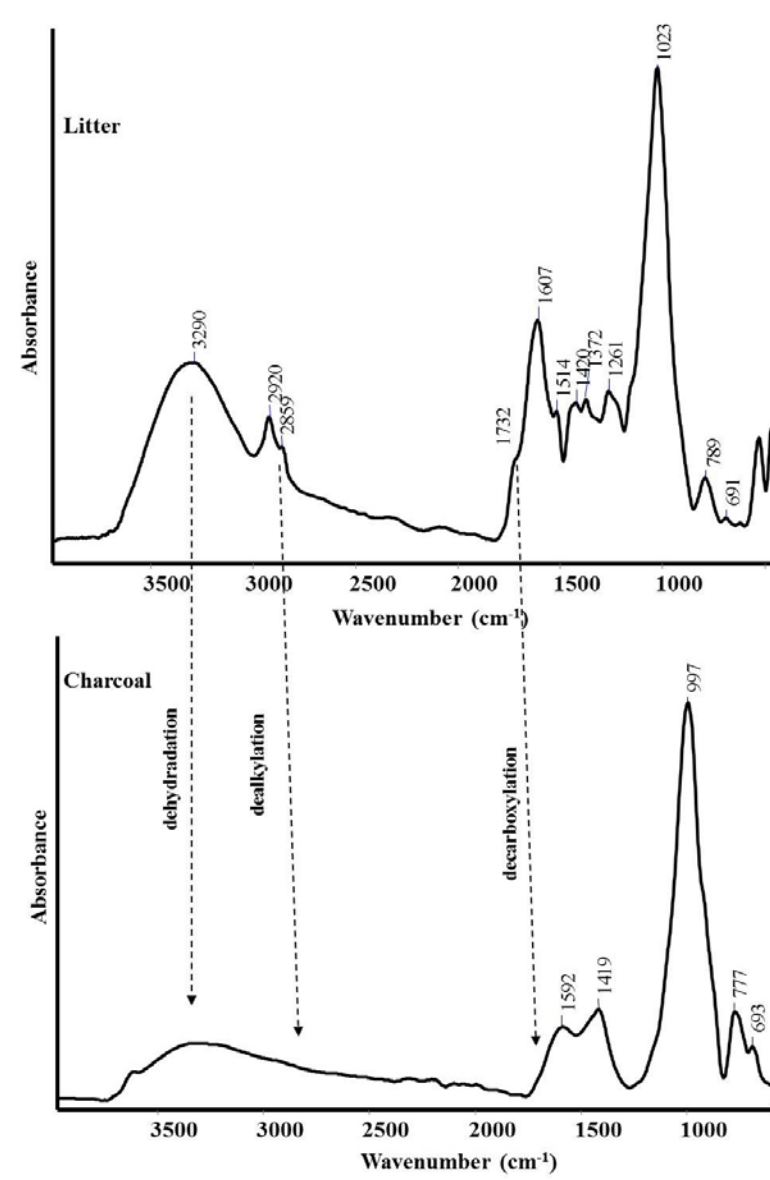
The ATR/FTIR spectra of litter and charcoal are shown in Figure 8. Litter was mainly composed by polymeric components (cellulose, hemicellulose, and lignin). The typical bands of lignin include the band at 1607 cm^{-1} (aromatic C=C stretch), 1514 cm^{-1} (aromatic ring breathing), and 1261 cm^{-1} (C-O stretch). Specifically, the bands at 1514 and 1261 cm^{-1} have been reported to originate from guaiacyl lignin of softwoods (Nuopponen et al., 2006). The bands at 1732, 1372 and 1023 cm^{-1} are from the ester carbonyl groups, such as uronic acid and acetyl groups in hemicellulose (Nuopponen et al., 2006), while the bands at 2920, 2859, and 1420 cm^{-1} have been associated with C-H stretch and bend.

Being the litter the dominant parent material of charcoal, the combustion results to have caused considerable decrease or even disappearance of some functional groups, as shown in Fig. 8. The charcoal spectrum showed a broad absorbance centred at 3300 cm^{-1} assigned to O–H stretching. Two broad peaks at 1582 cm^{-1} and 1417 cm^{-1} were attributed to C=C and C=N stretching of aromatic rings, formed by cyclisation reactions of organic matter at high temperatures components (El-Hendawy, 2006). The band at 1417 cm^{-1} might however partly or totally due carbonates. In fact, it is known that thermal treatment of wood leads hemicellulose to decompose, which at 150-300 °C produces low molecular weight organic acids (such as oxalate and acetate) as a result of acetyl groups cleavage of hemicellulose (Shen et al., 2010), and at around 400 °C carbonates, because of dehydration reaction of these organic acids (Hatakeyama and Liu, 1998). The strong signal at 996 cm^{-1} was finally assignable to silicates (Madejová, 2003).

Conclusions

The results of this study demonstrate that wildfires can substantially change the chemical and physical properties of SOM, even at a relatively considerable depth, considering that fire usually affects only a few cm of soil.

Fire occurrence produced a substantial reduction of the most labile fraction and a relative enrichment of the recalcitrant and refractory OM pool, more resistant against heating and hence, presumably, more stable into soil. In particular, this refractory pool was enriched in N polyaromatic molecules. The appearance of these new components confirms that the soil was affected by temperatures that did not overcome 400 °C. Such a conservation of soil N in recalcitrant form has a great environmental impact because N availability is fundamental to post-fire forest regeneration. Charring, on the other hand, was not complete as part of the burnt material still contained a fraction thermally degradable under 500 °C.

A positive outcome of this work is that the application of a set of thermal and spectroscopic techniques efficaciously characterize some important soil components formed by the burning. Furthermore, the rapidity and the little sampling preparation of these techniques allow to analyse large number of samples, obtaining eventually a robust dataset.

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Manuscript III

Charcoal and stable soil organic matter indicate fire frequency, climate and past vegetation in volcanic soils (Mt. Etna, Sicily)

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Charcoal and stable soil organic matter as indicators of fire frequency, climate and past vegetation in volcanic soils of Mt. Etna, Sicily

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ARTICLE INFO

ABSTRACT

Charcoal fragments in soils are useful to reconstruct past vegetation because the level of preservation is often good enough to determine the tree genus. All forest ecosystems have the potential to burn as a result of naturally occurring or human-induced fires. Forest fires are coupled to climate and are a not-negligible factor of pedogenesis in Mediterranean areas, where they occur frequently. Furthermore, soil organic matter (SOM) is prone to undergo peculiar changes due to forest fires, both in terms of quantity and quality. A soil sequence along an elevational gradient ranging from Mediterranean to subalpine climate zones on slopes of Mt. Etna (Sicily, Italy) was investigated in respect of soil organic C and charcoal. The amount of charcoal and the identification of charred species gave an indication of the fire frequency and vegetation changes that have occurred in the past. The distribution into labile and stable organic fractions provided insight into the stabilisation and turnover mechanisms of SOM. The stable organic matter fraction was measured as the residue of a H_2O_2 treatment. The soils along the altitudinal sequence are variations of Vitric Andosols that developed on a single trachy-basaltic lava flow having an age of 10–15 ky BP. Maquis vegetation dominates at the lower sites of the toposequence, followed by oak- and chestnut-forests at mid elevations, and pine-forest at the highest-elevated sites. Charcoals are older at higher elevations (ages of up to 1.5 ky cal BP). Here, the vegetation type has not changed over the last >1000 years, as all charcoal pieces were identified as *Pinus nigra*. Charred material at the lower sites could be identified as particles of deciduous shrubs, *Quercus*, *Castanea sativa*, *Lonicera implexa* and *Cytisus* spp. with mostly a modern age up to about 300 y cal BP. A similar finding was obtained for the stable (H_2O_2 resistant) SOM. Very high ages for this fraction were found at the highest elevations where it had an age of up to 8.2 ky BP — an age that is close to the start of soil formation. At the lower sites, where frequent bush fires often destroyed a part of the stable fraction, the stable SOM fraction had a maximum age of 1 ky. The studied soils have recorded the signals of the interrelated factors fire frequency, climatic effects and vegetation whose role cannot always be clearly distinguished. With decreasing altitude and with a warmer climate, vegetation changes and fire frequency, org. C and especially nitrogen abundance and the amount of labile SOM increases. At the lower sites, charcoal particles reflect the more recent vegetation probably because the repeated fires here hindered their preservation. Our findings hence suggest that a high fire frequency is a powerful rejuvenating factor for soil organic matter, removing part of the old SOM and promoting plant recolonisation that is a source of young SOM. Fire frequency and intensity on Mt. Etna is, however, moderate enough even at the lowest altitudes for the organic matter pool to be high and not depleted.

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1. Introduction

Earth is an intrinsically flammable planet owing to its cover of carbon-rich vegetation, atmospheric oxygen, seasonally dry climates and widespread lightning and volcanic ignitions (Bowman et al., 2009). Due to global warming, the temperature increase will be pronounced in Central Europe and the Mediterranean areas where, due to

the projected decrease in precipitation, dramatic environmental changes must be expected (Bates et al., 2008; IPCC Fourth Assessment Report, 2007; Moriondo et al., 2006). The Mediterranean climate is characterised by a strong seasonal winter/summer rainfall contrast with the result that the soils and the root zone are dry during the summer, often for several months. The long dry summer period facilitates the occurrence of bush and tree fires, which exert a significant effect on landscape evolution (Naveh, 1990; Shakesby, 2011). Fire has a strong influence on vegetation composition, structure and distribution at both a local and global scale. The general pattern of fire periodicity, seasonality, intensity, duration and size that emerges in any landscape over time comprises the fire regime for that environment and has important consequences for vegetation development. Understanding the fire regime of an area is crucial for fire management purposes and for research concerned with the ecological role of fire (Whelan, 1995). Up to now, the relation between the vegetation type and fire regime has only rarely been investigated in palaeorecords (charcoal, pollen, lake sediments) of the Mediterranean basin (e.g. Sadori et al., 2008). Whether natural or human-induced, fire serves as a regular ecosystem process, influencing vegetation patterns, wildlife distribution, nutrient cycling and many other ecosystem elements (Moody et al., 2006).

Changes in climate and vegetation type are expected to alter the quality and quantity of detritus inputs to soil and also the soil chemical, microbiological and physical processes that regulate the quality of soil organic matter (SOM). The SOM abundance is the product of a dynamic equilibrium between gain and losses. Several investigations have clearly shown that SOM and other soil properties can be influenced by climate (carbon stocks, organic matter chemistry, morphology and functional characters of humus forms) and may quickly react to changing environmental conditions (e.g. Bäumler and Zech, 1994; Bockheim et al., 2000; Laffan et al., 1989; Zanelli et al., 2006). In turn, changes in the quality and quantity of SOM can affect soil chemistry and mineralogy. However, despite advances in our understanding of the specific mechanisms leading to SOM formation and stabilisation, there is a surprisingly poor understanding of how soil quality (including the mineral and organic part) relates to climate and vegetation factors. This knowledge gap compromises our ability to predict the response of SOM storage and several other properties of soils to global change because positive effects of warming on SOM quality could result in a positive feedback on future warming; conversely, warming-related reductions in SOM quality could result in negative feedback.

The effect of fire can be recognised via structural characteristics of soil organic matter (Tinoco et al., 2006) or on the SOM accumulation. On the one hand, fire can cause a substantial loss of SOM in soils (Certini et al., in press; Novara et al., 2010) that may also be made greater due to the subsequent increased erosion effect after a bush fire. On the other hand, a substantial input of charcoal and charred organic matter (black carbon) that are due to fire activities (aboveground biomass) may lead to increased SOM stocks in soils (Eckmeier et al., 2010). Already Schmidt et al. (1999) suggested that burning vegetation produces large amounts of highly refractory organic matter consisting of charcoal and partially charred plant material that can have a major impact on composition, turnover and formation of soil organic matter. Both climate and fire affect the SOM properties and stocks. It is often very difficult to isolate one factor from the other. Furthermore, the climate effect is partially overshadowed because it also influences the state factor vegetation (although this factor is not really fully independent — according to the paradigm of Jenny (1941)).

The Mediterranean area is an interesting region in which to study the effect of fire and climate on SOM fractions. Here, we investigated a soil climosequence along the slopes of Mt. Etna (Sicily), the largest volcano in Europe. A strong effect of the altitude on pedogenesis on the flanks of the volcano was assessed, even when covered by the same vegetation (Fernández Sanjurjo et al., 2003). We hypothesised that fire activity effects pedogenesis because it is higher at the lower sites

(see Egli et al., 2007). This is assumed to affect the SOM characteristics and also the corresponding stocks. It is not yet known what effect the fire frequency has had on the different fractions and nature of SOM in these soils and on the turnover rates of stable and less stable fractions. We therefore performed physical, chemical and isotopic analyses on soils taken from of an altitudinal–vegetational gradient of the volcano. Furthermore, the use of a powerful tool – the analysis (extraction, identification and radiocarbon dating) of macrofossil charcoal fragments buried in soils (Carcaillet and Brun, 2000; Favilli et al., 2010) – allowed us to reconstruct fire activities, vegetation and climate. Through this analysis, a further valuable insight into climate and fire effects on landscape evolution and SOM quantity and quality of Mt. Etna is expected.

2. Investigation area

The Etna volcano was formed at the beginning of the Quaternary in the north-eastern part of Sicily (Branca et al., 2008) and is an isolated mountain region in southern Italy in the centre of the Mediterranean region (35° 50′ N/15° E) with an area of about 1570 km^2 (Poli Marchese, 2004) and a maximum altitude of 3323 m asl. The landscape is characterised by lava-flows of different ages and the most important geomorphological elements are the still active main and secondary craters. According to Poli Marchese (2004) the following vegetation zones can be defined in the Etna region:

a) basal-Mediterranean zone with a thermo-Mediterranean subzone (Oleo-Ceratonion, below 500–600 m asl), a meso-Mediterranean subzone (Quercion ilicis; between 600 and 1100 m asl) and a supra-Mediterranean zone (Quercetalia pubescentis; between 1100 and 1500 m asl)
b) montane-Mediterranean zone between 1500 and 2400 m asl (with Fagetalia sylvaticae, *Astragaletum siculi*, *Pinus nigra* ssp., *Laricio* (Querco-Fagetea), …)
c) Mediterranean high-alpine zone: above 2400 m asl (*Rumici Anthemidetum aetnensis*).

A regional nature park protects a substantial part of the area around the volcano (590 km^2). Human impact is, however, an important environmental factor in the Etna region. Large areas around the Etna volcano have been disturbed over centuries by human activity (agriculture). Undisturbed soils are usually found at high altitudes and on relatively young lava flows. Crops and various fruit orchards can be usually found up to c. 900 m asl (Dazzi, 2007). From 900 m asl, forest vegetation prevails up to about 2200 m asl (the distribution and upper limit depend on the local exposure and morpho-climatic conditions). Also where anthropogenic intervention was restricted to forestation, the choice of the planted species influenced pedogenesis on weatherable substrata (Certini et al., 2001). A wide variation of soil types can be found that is due, among others, to the different ages, climatic features, topography or ash and lapilli input. According to the soil map (1:250000) of Sicily (Fierotti et al., 1988), Dazzi (2007) and Lulli (2007), mature and undisturbed soils of the Etna comprise Eutric or Dystric Cambisols and (Mollic) Andosols along the entire altitudinal sequence. Most of these soils have vitric characteristics (Dazzi, 2007; Lulli, 2007).

3. Material and methods

3.1. Sampling and sites

We investigated six sites along an elevational gradient ranging from Mediterranean (551 m asl; warm, semi-arid to sub-humid climate) to subalpine (1772 m asl; cold, humid climate) climate zones in the Etna region (Table 1, Fig. 1). Soil moisture and temperature regimes vary between xeric to udic and thermic to frigid (Raimondi et al., 1999). Five sites were located on one single lava flow and a sixth one on a separate flow (having the same characteristics). The lava flows were

trachy-basalts (alkali mugearite) that could be attributed to the recent Mongibello chronozone (8 ky) or the ancient Mongibello chronozone (about 15 ky). Dating of lava flows in the Etna region was done by chronostratigraphic studies (Romano, 1979, 1983) and radiometric dating (^{230}Th/^{238}U in Condomines et al., 1982; K–Ar in Gillot et al., 1994). All selected sites (soil profiles) were north-facing and the chemical and mineralogical composition of the parent material was identical (Nater, 2006). Two different types of vegetation can be distinguished along the elevational gradient. At the lower altitudes (4 sites between 551 and 1090 m asl), vegetation is dominated by maquis vegetation and oak/chestnut forests and at the higher altitudes (2 sites at 1515 and 1772 m asl, respectively) by coniferous forests (Corsican pine, *Pinus nigra Arn* ssp. *laricio*).

At each of these 6 sites, the following sampling procedure was applied:

– two soil profiles were sampled by horizons, down to the C or BC horizon (data of one soil profile was already available in Egli et al. (2007)).
– Another 2–3 to profiles (replicates) were sampled at 0–15 and 15–40 cm depth intervals.

With this procedure, a large amount of soil samples per site was collected that enable to cope with the spatial variability of soils (Hitz et al., 2002). From 1 to 3 kg soil material was sampled per soil horizon or soil depth. In fact, to yield reasonable results, unusually large sampling volumes are needed for soils on coarse textures (Hitz et al., 2002), such as those investigated. Soil bulk density was determined by a soil core sampler (100 cm^3, with 2–4 replicates per soil horizon) or by excavated

holes backfilled with quartz sand. Taking advantage of the profile pits, undisturbed soil samples were taken down to the C horizon. The soils did not exhibit any signs of erosion or relocation and therefore they could be considered as natural and as relatively undisturbed.

3.2. Soil chemistry and physics

The soil samples were air-dried, large aggregates were gently crushed by hand and sieved to <2 mm. Total C and N contents of the soil, soil fractions and charcoal pieces were measured using a C/H/N analyser (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) on oven-dried (70 °C, 48 h) and ball-milled material. Soil pH (in 0.01 M CaCl$_2$) was determined using a soil solution ratio of 1:2.5. The total elemental composition of the soil fine earth samples was analysed by X-ray fluorescence spectrometry (XRF). Around 6 g of fine earth material were milled to <60 μm in a Retsch MM400 mill with Zr grinding tools. 4.0 g of the sample powder were mixed with 0.90 g of Licowax® C Micro-Powder PM (Fluxana, Germany), pressed into a 32 mm-pellet and analysed using an energy dispersive X-ray fluorescence spectrometer (SPECTRO XEPOS, SPECTRO Analytical Instruments, Germany).

After a pre-treatment with 3% H$_2$O$_2$, the particle size distribution of the soils was measured by a combined method consisting of sieving the coarser particles (2000–32 μm) and the measurement of the finer particles (<32 μm) by means of an X-ray sedimentometer (SediGraph 5100, Micromeretics, Norcross, GA, USA).

The vitric content of the fine earth fraction was estimated in the field using a hand lens (0.1 mm scale graduated magnifying glass).

Table 1
Characteristics of the study sites in the Etna region.

Elevation (m asl)	Coordinates	Aspect	Slope (°)	MAT[a] (°C)	MAP[b] (mm)	Soil moisture regime	Soil temperature regime	Parent material	Vegetation	Soil type (IUSS Working Group WRB, 2007; Soil Survey Staff, 2010)
551	37.8784° N/15.0900° E	N	0	15.3	1000	Xeric	Thermic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea/Quercion ilicis with *Quercus pubescens, Asphodelus microcarpus, Carlina nebrodensis, Artemisia* spec., *Genista aetnensis, Rubus* spec., *Dryopteris filix-mas*	Vitric Andosol Typic Haploxerand
866	37.8512° N/15.07025° E	N	4	13.1	1100	Udic	Mesic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea/Quercion ilicis with *Quercus ilex, Quercus pubescens Wild*	Vitric Andosol Typic Udivitrand
998	37.8538° N/15.0027° E	N	3	12.5	1100	Udic	Mesic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea/Quercion ilicis with *Quercus ilex, Quercus pubescens Wild, Castanea sativa, Genista aetnensis, Rubus* spec., *Dryopteris filix-mas*	Vitric Andosol Typic Udivitrand
1090	37.8266° N/15.0831° E	N	0	11.9	1150	Udic	Mesic	Ve-Lava (Trachy-basalt)	Supra-mediterranean zone — Macchia Mediterranea / Quercion ilicis with *Quercus ilex, Quercus pubescens Wild, Castanea sativa, Genista aetnensis, Daphne laureola L., Orchis commutata, Muscari* spec., *Rubus* spec., *Dryopteris filix-mas*	Vitri-Mollic Andosol Humic Udivitrand
1515	37.8097° N/15.0640° E	N	4	9.2	1250	Udic	Mesic	Ve-Lava (Trachy-basalt)	Coniferous forest (*Pinus nigra* ssp. *laricio*) with a few deciduous trees (*Fagus sylvatica, Quercus pubescens, Castanea sativa*)	Vitric Andosol Vitric Hapludand
1772	37.7915° N/15.0428° E	N	12	7.5	1400	Udic	Frigid	Ve-Lava (Trachy-basalt)	Coniferous forest (*Pinus nigra* ssp. *laricio, Dryopteris filix-mas, Iuniperus* sp.)	Vitric Andosol Typic Udivitrand

[a] MAT = mean annual temperature (°C).
[b] MAP = mean annual precipitation.
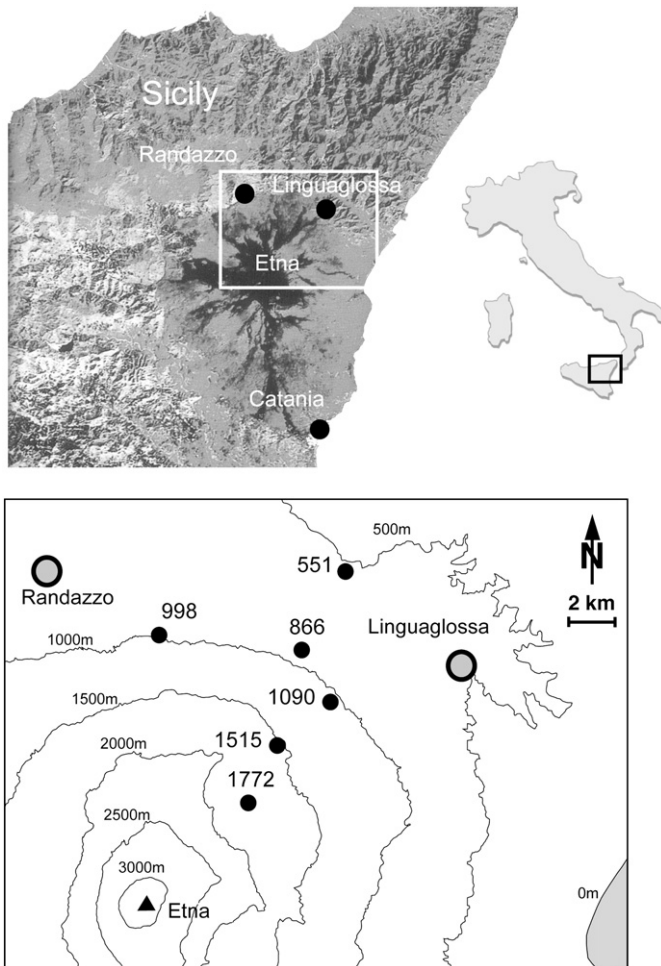
Fig. 1. Location of the investigation sites (toposequence) in the Etna region (Sicily, southern Italy).

The exchangeable base cations and acidity were determined using the BaCl$_2$ method according to MIPAF (1999).

3.3. Soil organic matter fractionation

Assuming that chemical oxidation mimics natural oxidative processes, we treated the soils with 10% H$_2$O$_2$ to eliminate the more labile organic material from the more refractory organic matter (Eusterhues et al., 2005; Favilli et al., 2008; Helfrich et al., 2007; Plante et al., 2004). The fraction left at the end of the treatment usually represents very stable SOM (Favilli et al., 2009). Air-dried fine earth was wetted for 10 min with few ml of distilled water in a 250 ml glass beaker. Afterwards, 90 ml of 10% H$_2$O$_2$ per gramme of soil were added. The procedure was run at a minimum temperature of 50 °C throughout the treatment period. To avoid evaporation of the reagent, the treatment was run in an automated, closed system using steel stirrers. Peroxide treatments were performed for 168 h (7 days). At the end of the treatment the samples were washed three times with 40 ml of deionised water per gramme of soil, freeze-dried, weighted and analysed for total C and N and ^{14}C-dated.

3.4. Charcoal

Charcoal fragments were separated from the soil material by hand-picking or floating and subsequently dried at 40 °C. The individual particles were analysed microscopically and separated into coniferous and broad-leaved tree species (Schoch, 1986) with a stereomicroscope (magnification 6.4–40×, Wild M3Z Leica, Germany). The charcoal fragments from the coniferous trees were further divided at the genus level using a reflected-light microscope (objective 5×, 10×, and 20×, Olympus BX 51, Japan). The observations were compared with a histological wood-anatomical atlas, using an identification key (Schweingruber, 1990). After the identification, some selected charcoal pieces were radiocarbon dated.

3.5. Radiocarbon dating

The CO$_2$ of the combusted samples (soil and charcoal samples) was catalytically reduced over iron powder at 550 °C to elemental carbon (graphite). The obtained mixture was pressed into a target and the ratios ^{14}C:^{12}C (for radiocarbon age) were measured (including the ∂^{13}C) by Accelerator Mass Spectrometry (AMS) using the tandem accelerator of the Institute of Particle Physics at the Swiss Federal Institute of Technology Zürich (ETHZ). The calendar ages were calculated with the OxCal 4.1 calibration programme (Bronk Ramsey, 2001, 2009) based on the IntCal 04 calibration curve (Reimer et al., 2009). Calibrated ages are given in the 2 σ range (minimum and maximum value).

3.6. Statistics

The individual datasets were checked for normal distribution using a Shapiro–Wilk test (SigmaPlot 11.0). This test is robust also with a small number of observations (Jann, 2005). Depending on the data distribution (normal or not normal distribution), the Pearson product-moment or the Spearman rank correlation coefficient was used, respectively. This procedure was checked using a two-tailed test for significance.

4. Results

4.1. General soil properties

The top and subsoils in the study area usually had a sandy loam (Table 2) and in some cases a sandy silt loam or clay loam texture. The soil material contained a significant amount of glass particles (>5%). Soil skeleton was in most cases between 10 and 30% by weight. Grain sizes usually decreased from the parent material to the surface soil horizon where the highest clay and silt contents were found. The general decrease of the grain sizes with depth is a concomitant effect of weathering (the physical breakdown of the coarse glass-particles and chemical–mineralogical transformations) and, presumably, of eolian ashy additions due to the eruptions of Etna.

Criteria for andic soil properties (Soil Survey Staff, 2010) were examined for the whole pedon. Soil density was generally too high to make it typically andic (<0.90 g/cm^3, according to both, the World Reference Base for Soil Resources (IUSS Working Group WRB, 2007) and the Soil Taxonomy (Soil Survey Staff, 2010), although in some horizons rather low values were measured in the topsoil (Table 2). In several soils another important criteria (Al$_o$ + 1/2Fe$_o$>2% within the first 25 cm) did not meet the andic requirements (data not shown; Egli et al., 2007). Nonetheless, the soils can be classified as Andosols as this Reference Soil Group of the WRB classification can have either a vitric or an andic horizon starting less than 25 cm below the soil surface. The requirements for vitric properties were fulfilled in all soils. Furthermore, additional criteria that are used to describe andic properties met the requirements such as an organic C content of less than 250 g/kg in all soil horizons or more than 30% of the fine earth fraction having a particle size >20 μm. In general, the soils were variations of Vitric Andosols (WRB) or Typic Haploxerand to Typic or Humic Hapludand (according to the Soil Taxonomy). The soils usually have a mollic character (with respect to the colour, org. C content and base saturation) at the lower

Table 2
Some physical characteristics of the main soil profiles along the toposequence in the Etna region.

Altitude	Soil horizon	Depth	Munsell colour	Bulk density	Skeleton	Sand	Silt	Clay
m asl		(cm)	(dry)	g cm^{-3}	wt.%	g kg^{-1}	g kg^{-1}	g kg^{-1}
551	A1	0–5	5YR 2.5/1	0.45	8.5	476	328	197
	A2	5–15	5YR 2.5/2	1.11	15.3	540	372	88
	Bw	15–65	10YR 3/3	1.40	34.6	699	234	66
	BC	65–85	10YR 4/6	1.37	35.2	827	124	49
866	A	0–15	7.5YR 2/2	1.29	14.0	652	259	89
	Bw	15–40	7.5YR2/2	1.05	15.2	664	261	75
	BC	40–70	7.5YR 3/2	0.78	7.6	462	455	83
998	A1	0–3	7.5 YR 2.5/0	1.05	15.7	625	262	112
	A2	3–20	5YR 2.5/2	1.23	25.5	573	329	98
	Bw	20–70	7.5 YR 5/6	1.64	51.3	769	174	57
	B(C)	70–90	7.5 YR 3/2	1.53	48.1	789	164	47
	C	90–117	7.5 YR 3/2	1.57	52.2	836	117	47
1090	A1	0–6	10YR 3/3	1.01	15.2	673	236	92
	A2	6–25	10YR 3/3	1.09	28.2	656	255	90
	Bw	25–65	5YR 2.5/2	0.75	23.3	429	496	75
	C	65–80		0.60				
1515	A1	0–7	5YR 2.5/1	0.72	14.7	739	148	113
	A2	7–23	10YR 2/2	0.93	15.9	828	106	65
	B1	23–38	5YR 2.5/1	0.84	12.4	812	132	56
	B2	38–98	10YR 3/4	1.00	8.3	732	213	55
	C	98–122		0.71	14.9	723	202	76
1772	A1	0–4	10YR 2/3	0.95	10.0	802	137	61
	A2	4–13	10YR 2/3	1.19	11.0	887	67	46
	AB	13–23	2.5Y 2/4	1.01	8.5	592	342	66
	B	23–100	10YR 6/5	0.78	8.4	791	166	43
	BC	100–140	10YR 6/5	1.04	18.4	666	277	57
	C	140–150	10YR 3/3	1.00	10.0	751	182	67

sites and an umbric one at the highest. The thickness of the topsoil, however, does not fully meet the requirements for a mollic or umbric horizon (except for the soil at 1090 m asl).

The soils were weakly acid and most of them did not show an evident pH gradient within the profile. The pH values of genetic horizons along with total C and N and C/N ratios are reported in Table 3. The total content of the fine earth of one replicate per site is given in Table 4. The chemical composition along and between the soil profiles varies only slightly. Some minor discontinuities along the profiles might be either due to occasional ash input or to weathering (leaching). In general, the soil material can be considered as quite homogeneous and fully fits the expectances for the chemistry of eruptive rocks and ashes of the Mongibello chronozone or the ancient Mongibello chronozone (Pichler, 1984).

Table 3
Organic carbon, nitrogen, C/N ratios and pH-values of the main soil profiles along the toposequence in the Etna region.

Altitude	Horizon	pH	Total C	Total N	C/N
m asl		(CaCl$_2$)	g kg^{-1}	g kg^{-1}	
551	A1	6.30	132.6	10.79	12.3
	A2	5.90	52.6	4.19	12.6
	Bw	5.65	30.7	2.75	11.2
	BC	5.35	10.0	0.85	11.8
866	A	5.95	35.1	2.47	14.2
	Bw	6.35	29.6	2.24	13.2
	B	6.10	49.8	4.60	10.8
998	A1	6.20	90.3	7.68	11.8
	A2	5.65	54.5	4.98	10.9
	Bw	5.50	10.4	0.91	11.4
	BC	5.60	5.7	0.51	11.2
	C	5.70	5.1	0.51	10.0
1090	A1	5.70	45.6	3.54	12.9
	A2	5.45	26.2	2.16	12.1
	Bw	5.15	45.3	4.70	9.6
	C	5.55	8.2	0.71	11.5
1515	A1	5.05	65.2	2.3	28.3
	A2	5.20	22.5	0.79	28.5
	B1	5.50	22.2	0.87	25.6
	B2	5.60	12.7	0.67	19.0
	C	5.55	11.3	0.61	18.5
1772	A1	4.90	24.8	1.02	24.3
	A2	5.00	10.7	0.41	26.0
	AB	5.35	13.2	0.67	19.8
	B	5.70	6.6	0.42	15.8
	BC	5.70	13.8	0.72	19.2
	C	5.80	7.0	0.44	15.9

Table 4
Total content (referred to the organic-free fraction) of major and the most relevant minor components in the fine earth of the soils along the toposequence (one soil profile per site).

Altitude	Soil depth	Na$_2$O	MgO	K$_2$O	CaO	SiO$_2$	Al$_2$O$_3$	Fe$_2$O$_3$	TiO$_2$	P$_2$O$_5$	MnO	V$_2$O$_5$	SrO	ZrO$_2$
m asl	cm	%	%	%	%	%	%	%	%	%	%	%	%	%
551	0–15	1.57	1.69	1.38	5.77	50.25	18.41	9.10	1.92	0.851	0.175	0.031	0.109	0.036
	15–40	1.41	1.47	1.20	4.98	46.76	18.47	8.87	1.87	0.789	0.171	0.033	0.103	0.036
	40–55	2.38	1.94	1.53	6.32	51.52	20.04	9.41	1.96	0.776	0.176	0.039	0.121	0.038
	55–85	3.20	2.07	2.07	7.24	52.63	17.87	9.04	1.93	0.822	0.176	0.021	0.138	0.037
866	0–15	1.89	2.45	1.47	7.54	50.73	16.22	10.28	2.07	0.816	0.182	0.042	0.136	0.033
	15–40	1.86	2.36	1.54	7.21	48.51	15.27	10.19	2.14	0.796	0.178	0.036	0.135	0.034
	40–70	1.46	1.93	1.15	6.41	52.74	18.54	9.41	1.93	0.717	0.179	0.041	0.136	0.033
998	0–15	1.98	2.38	1.39	7.88	54.02	17.82	9.96	2.13	0.811	0.177	0.043	0.156	0.033
	15–40	1.93	2.12	1.24	7.07	49.67	16.79	9.11	1.96	0.693	0.161	0.039	0.145	0.029
	40–90	2.56	2.56	1.74	8.44	49.53	14.44	10.30	2.16	0.926	0.178	0.029	0.156	0.035
1090	0–15	2.12	2.25	1.28	7.28	50.31	16.57	9.40	1.96	0.658	0.169	0.047	0.135	0.032
	15–40	1.43	1.65	1.09	5.65	47.11	15.03	8.85	1.89	0.585	0.163	0.038	0.124	0.033
	40–70	2.74	2.11	1.46	6.33	53.31	18.96	9.18	2.11	0.895	0.187	0.034	0.148	0.040
1515	0–15	2.48	2.42	1.50	8.85	52.17	15.88	9.58	1.92	0.571	0.174	0.045	0.138	0.032
	15–40	2.78	2.49	1.51	9.50	53.71	17.38	9.70	1.99	0.586	0.165	0.047	0.154	0.032
	40–55	1.93	1.83	1.19	5.74	50.18	20.24	9.78	2.13	0.734	0.173	0.044	0.134	0.037
1772	0–15	2.08	2.24	1.56	8.00	49.34	12.88	9.42	1.91	0.496	0.160	0.051	0.129	0.030
	15–40	3.08	2.86	1.56	9.68	53.12	16.66	9.81	1.95	0.538	0.166	0.049	0.144	0.032
	40–55	2.32	2.31	1.46	7.43	52.28	18.85	9.78	2.04	0.721	0.159	0.045	0.131	0.036
	55–70	1.80	2.03	1.18	6.19	52.09	20.44	10.07	2.12	0.718	0.176	0.044	0.134	0.033
	70–80	2.31	3.48	1.30	10.34	50.76	14.08	10.43	1.88	0.463	0.176	0.044	0.127	0.027
	Mean	2.16	2.22	1.42	7.33	50.99	17.18	9.60	2.00	0.713	0.172	0.040	0.135	0.034
	SD	0.52	0.44	0.23	1.43	2.05	2.09	0.48	0.10	0.131	0.008	0.007	0.014	0.003

4.2. Soil organic matter

The mean concentration of organic matter shows a decreasing trend with increasing altitude for both the soil depth intervals 0–15 and 15–40 cm (Table 5). The variability is however relatively high.

With increasing altitude, precipitation increases and temperature decreases (Table 1). According to this observed trend, in such an environment higher temperature leads to an increase of SOC (soil organic carbon) and higher precipitation to a decrease. The C/N ratio changes abruptly between 1090 and 1515 m asl most probably because of to the transition from maquis vegetation, oak and chestnut forests to coniferous stands. The high C/N variability at an altitude of 1515 m asl is predominantly due to an outlier (C/N of 87): without this outlier, the C/N ratio at 1515 and 1772 m asl would be rather similar.

Carbon recovery after oxidation by H$_2$O$_2$ was usually between 13 and 30% (range: 1–50%). The recoveries did not change with increasing soil depth which is rather unusual (Table 6; cf. Egli et al., 2009). A similar behaviour could be measured for nitrogen. Nitrogen recovery was usually between 12 and 33% (range: <1–44%). Also with N, the recoveries did not change with increasing soil depth. Compared to the untreated soils, the H$_2$O$_2$ treatment would be expected to lead to a relative enrichment of nitrogen (lower C/N ratios after the treatment; see Egli et al., 2009; Favilli et al., 2008). This was, if ever, only partially the

case. In topsoil horizons (0–40 cm soil depth) such a trend was detectable, not so in the subsoil. Although only one soil profile per site was investigated regarding the distribution of labile and stable organic matter, which consequently provides a small database, a trend of an increasing proportion of labile SOM with decreasing altitude seems to exist for both considered depth intervals (0–15 and 15–40 cm). These trends have, however, an error probability of >5%. In 0–15 cm soil depth and, less pronounced, in 15–40 cm, the proportion of the labile carbon and nitrogen increased with decreasing altitude (Fig. 2). The altitudinal trends for labile N had an error probability of <5%.

The range of the ∂^{13}C values (Table 7) is typical for C3 plants (Mook and Waterbolk, 1985). A trend (p<0.05) towards lower ∂^{13}C values (and consequently a SOM fraction that is more depleted in ^{13}C) at 0–15 and 15–40 cm soil depth with increasing altitude can be observed (Fig. 3).

4.3. Radiocarbon age of the stable SOM fraction

The ages of the stable organic matter fraction varied considerably: from modern ages up to more than 8 ky old carbon fractions were detectable (Table 7). In some soils an increase in age of the stable SOM with depth was found, while at 1772 m asl the highest age was measured in the surface horizon. At several sites, a modern age of the

Table 5
Some physical properties, C, N and charcoal (as amount of charcoal and C stored in charcoal) of the inventory sites along the toposequence.

| Altitude	Soil depth	Soil skeleton	Density	Ctot	N	C/N	Charcoal	Charcoal C	Charcoal C/Ctot
m asl	cm	wt.%	g/cm^3	g/kg	g/kg		g/kg	g/kg	%
551	0–15	40.3 (±18.3)	0.87 (±0.38)	64.2 (±27.3)	5.05 (±2.38)	13.0 (±0.9)	3.35 (±2.92)	2.40 (±2.09)	3.7
	15–40	47.2 (±31.4)	0.94 (±0.37)	44.1 (±7.6)	3.42 (±0.71)	12.9 (±0.5)	0.00 (±0.00)	0.00 (±0.00)	0.0
866	0–15	14.0 (±3.7)	1.13 (±0.18)	43.4 (±24.2)	2.88 (±1.42)	14.8 (±0.9)	5.11 (±5.40)	3.38 (±3.57)	7.8
	15–40	15.2 (±1.7)	1.08 (±0.03)	40.7 (±15.7)	2.67 (±0.45)	14.9 (±3.3)	20.89 (±17.33)	13.52 (±11.21)	33.2
998	0–15	50.1 (±8.7)	1.02 (±0.13)	64.9 (±7.7)	5.86 (±0.45)	11.1 (±1.3)	0.88 (±1.52)	0.54 (±0.94)	0.8
	15–40	70.6 (±4.3)	1.52 (±0.07)	14.6 (±3.4)	0.90 (±0.37)	16.9 (±2.8)	0.00 (±0.00)	0.00 (±0.00)	0.0
1090	0–15	22.4 (±4.4)	1.01 (±0.05)	33.6 (±5.0)	2.48 (±0.44)	16.9 (±2.5)	2.68 (±2.33)	1.34 (±1.16)	4.0
	15–40	24.3 (±1.6)	0.90 (±0.10)	32.1 (±5.0)	2.40 (±0.31)	12.1 (±1.9)	3.11 (±5.38)	1.53 (±2.65)	4.8
1515	0–15	6.0 (±4.0)	0.89 (±0.13)	27.6 (±9.7)	0.90 (±0.62)	46.8 (±35.3)	4.34 (±3.95)	2.58 (±2.35)	9.3
	15–40	3.7 (±2.4)	1.06 (±0.06)	33.5 (±13.7)	0.93 (±0.23)	35.2 (±5.8)	19.52 (±15.86)	12.16 (±9.88)	36.3
1772	0–15	10.2 (±2.0)	0.97 (±0.15)	37.3 (±11.8)	1.46 (±0.81)	28.6 (±7.4)	1.10 (±2.19)	0.77 (±1.54)	2.1
	15–40	14.0 (±14.1)	1.09 (±0.10)	11.4 (±4.5)	0.32 (±0.16)	37.9 (±5.5)	1.78 (±2.06)	1.11 (±1.28)	9.7

Table 6
Stable and labile C and N fractions in the soils along the toposequence (one soil profile per site).

Altitude	Soil depth	Ctot	Ntot	C stable	C labile	N stable	N labile	C/N tot	C/N stable	C/N labile	C recovery	N recovery
m asl	cm	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg				%	%
551	0–15	73.0	5.62	13.0	60.0	1.18	4.44	13.0	11.1	13.5	17.8	21.0
	15–40	38.7	2.92	5.0	33.7	0.82	2.10	13.3	6.1	16.1	12.9	28.1
	40–55	34.3	2.55	6.5	27.8	0.20	2.35	13.4	32.0	11.8	18.9	7.8
	55–85	6.6	0.6	1.8	4.8	<0.05	0.60	11.0	–	8.1	27.3	–
866	0–15	35.1	2.47	10.4	24.6	0.77	1.70	14.2	13.6	14.5	29.6	31.2
	15–40	29.6	2.24	3.9	25.7	0.27	1.97	13.2	14.6	13.0	13.2	12.1
	40–70	49.8	4.6	3.3	46.5	0.24	4.36	10.8	13.6	10.7	6.6	5.2
998	0–15	64.3	5.36	9.5	54.9	1.69	3.67	12.0	5.6	14.9	14.8	31.5
	15–40	13.4	0.77	4.0	9.4	0.25	0.52	17.4	15.9	18.1	29.9	32.5
	40–90	9.7	0.31	0.1	9.6	<0.05	0.31	31.2	–	30.8	1.0	–
1090	0–15	31.2	1.99	6.8	24.4	0.62	1.37	15.7	10.9	17.9	21.8	31.2
	15–40	37.4	2.65	5.1	32.3	1.16	1.49	14.1	4.4	21.6	13.6	43.8
	40–70	23.9	1.46	3.9	19.9	0.23	1.23	16.4	17.3	16.2	16.3	15.7
1515	0–15	31.4	1.34	8.0	23.4	0.33	1.01	23.4	23.9	23.2	25.5	24.6
	15–40	27.0	0.87	4.2	22.8	0.17	0.70	31.0	24.7	32.6	15.6	19.5
	40–55	16.6	0.19	4.6	23.6	0.08	1.06	87.5	55.9	22.4	27.7	42.1
1772	0–15	22.0	0.58	11.0	11.1	0.24	0.34	38.0	46.4	32.2	50.0	41.4
	15–40	14.0	0.45	0.9	13.2	<0.05	0.45	31.2	–	29.2	6.4	–
	40–55	15.5	0.59	2.3	13.2	0.07	0.52	26.3	34.3	25.2	14.8	11.9
	55–70	31.7	1.32	8.1	23.6	0.23	1.09	24.0	35.6	21.6	25.6	17.4
	70–80	3.2	<0.05	1.7	1.5	<0.05	<0.05	–	–	–	53.1	–

stable SOM fraction was measured in the surface horizon. Either H_2O_2 was unable to oxidise all young OM in these horizons or almost no old carbon exists in these horizons. However, since the H_2O_2 method usually works fine for soils having an organic C content of ≤110 g/kg (Favilli et al., 2008), we assume that in several surface horizons almost all the old OM had been replaced by younger OM.

Rather young ages of the resilient organic fraction were measured in the top- and subsoils at low altitudes.

The measured age in the subsoil of the site 1515 m asl is 8 ky BP and quite close to the age of the soil (also around 8 ky BP). Some of the pristine organic matter evidently still exists in this soil. In the topsoil, the input of fresh organic matter is high and probably replaced all old OM. Also at the site with the highest elevation (1772 m asl), very old ages of stable organic matter were measured (around 6.5 ky BP). At all other sites, the maximum age of the stable organic matter was in the range of 1.1–1.6 ky BP.
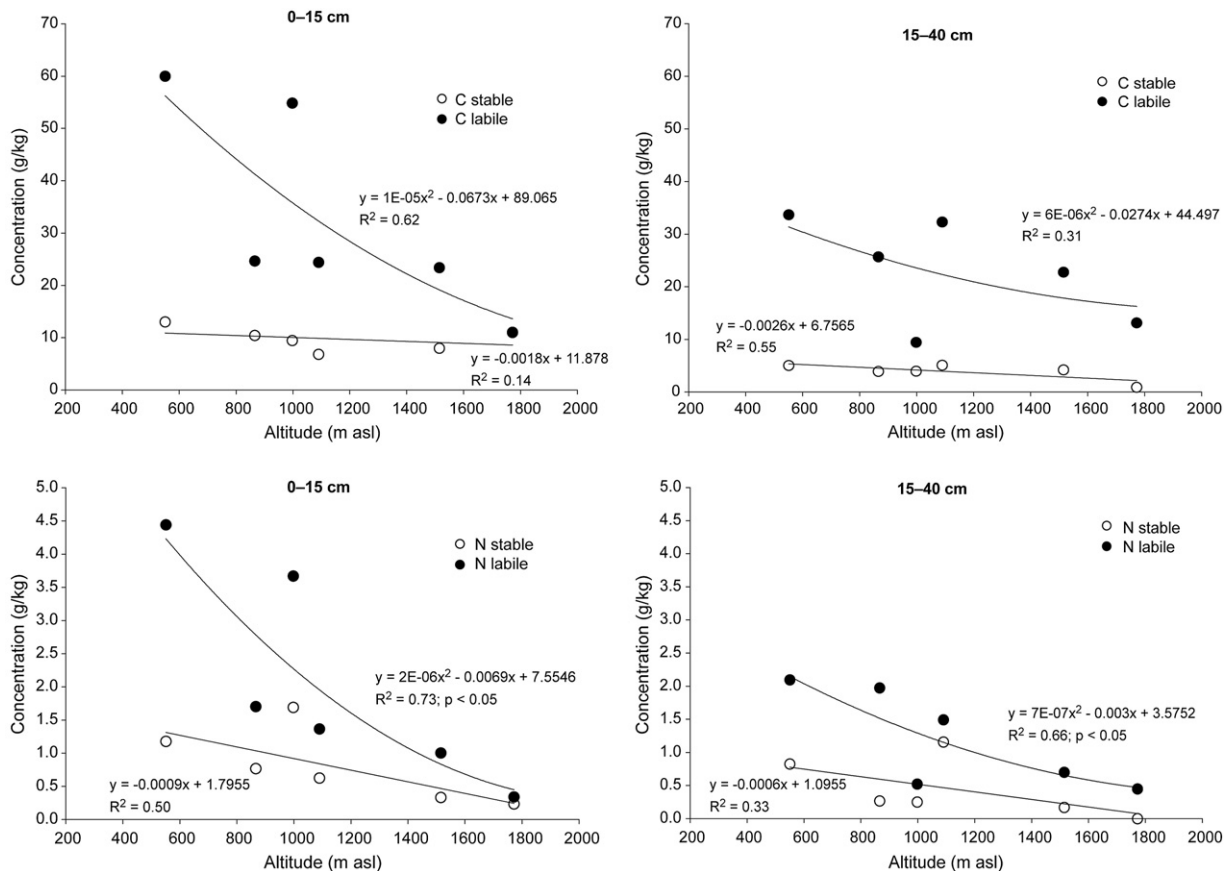
Fig. 2. Organic C and N in the stable and labile fraction along the toposequence (one profile per site) and as a function of soil depth.

Table 7
Age of the stable organic matter fraction in the soils of the toposequence.

| Altitude	Soil depth	^{14}C age	∂^{13}C	Calibrated age[a]
m asl	cm	yr BP ($\pm$SD)	‰	cal yBP
551	0–15	Modern	−19.3 ($\pm$1.1)	Modern
	15–40	50 ($\pm$30)	−19.0 ($\pm$1.1)	31–257
	40–55	1605 ($\pm$35)	−23.9 ($\pm$1.1)	1405–1565
866	0–15	560 ($\pm$30)	−17.4 ($\pm$1.1)	521–644
	15–40	1315 ($\pm$30)	−19.8 ($\pm$1.1)	1179–1296
998	0–15	520 ($\pm$30)	−19.6 ($\pm$1.1)	507–626
	15–40	655 ($\pm$30)	−20.1 ($\pm$1.1)	556–672
1090	0–15	Modern	−21.0 ($\pm$1.1)	Modern
	15–40	740 ($\pm$30)	−18.8 ($\pm$1.1)	660–727
	40–70	1605 {$\pm$35)	−23.9 ($\pm$1.1)	1405–1565
1515	0–15	Modern	−22.0 ($\pm$1.1)	Modern
	15–40	7270 ($\pm$35)	−23.2 ($\pm$1.1)	8011–8170
1772	0–15	5745 ($\pm$35)	−31.4 ($\pm$1.1)	6449–6639
	15–40	350 ($\pm$30)	−32.0 ($\pm$1.1)	315–494
	40–45	1570 ($\pm$30)	−25.2 ($\pm$1.1)	1385–1535

[a] 2-σ range.

These findings support that the elevation – via lower temperatures in the biologically active period, which limit SOM decomposition – and fire activity, which indiscriminately removes all organics from the ground, must have an important impact on the turnover rate of the stable organic matter fraction.

4.4. Charcoal

The age of the charcoal pieces varied from 0 to about 1.6 ky BP (Table 8). At the lowest site, almost all of the charcoal had a modern age. Only one piece at a depth of 55–65 cm was slightly older. The charcoal composition at this site reflects reasonably well the maquis vegetation. Also at 866 m asl, the present-day vegetation (maquis) is predominantly reflected in the charcoal composition. *Castanea sativa* is actually not present but apparently grew here a few hundred years ago. Also here, only rather young ages could be measured. At 1090 m asl, charcoal pieces having an age of several centuries could be found (Table 8).

At the highest site (1772 m asl), increasing ages of charcoal were measured with increasing soil depth. Nonetheless some age inversions could be detected along the profile which is often typical in soils. It usually indicates the downward migration of charcoal pieces, which is plausible in coarse-textured soils like those investigated. Highest ages were recorded at a depth of 55–65 cm with ages in the range of about
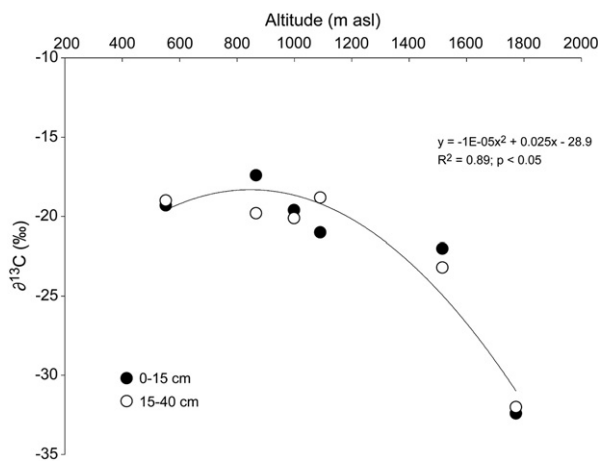
Fig. 3. Relationship between ∂^{13}C of the stable fraction of organic matter from 0 to 15 and 15 to 40 cm soil depth and the altitude. The regression curve is given for the depth 0–15 cm. For both soil depths, a significant correlation (p<0.05) exists between the altitude and ∂^{13}C.

Table 8
Age of some identified charcoal pieces.

Altitude	Soil depth	Plant type	^{14}C age	∂^{13}C	Calibrated age[a]
m asl	cm			‰	cal yBP
551	0–5	*Lonicera*	Modern	−23.8 ($\pm$1.2)	Modern
	5–15	*Quercus*	Modern	−23.1 ($\pm$1.2)	Modern
	5–15	*Fagus silvatica*	Modern	−22.2 ($\pm$1.1)	Modern
	55–65	*Quercus*	Modern	−24.4 ($\pm$1.2)	Modern
	55–65	Deciduous shrubs	175 ($\pm$50)	−23.1 ($\pm$1.2)	0–301
866	15–40	*Fagus silvatica*	175 ($\pm$35)	−23.2 ($\pm$1.1)	0–299
	15–40	*Quercus*	235 ($\pm$35)	−19.0 ($\pm$1.1)	0–426
	15–40	*Castanea sativa*	Modern	−20.4 ($\pm$1.1)	Modern
1090	0–15	*Quercus*	180 ($\pm$35)	−21.0 ($\pm$1.1)	0–301
	15–40	*Castanea sativa*	325 ($\pm$49)	−23.8 ($\pm$1.1)	304–483
1515	15–40	*Pinus nigra*	100 ($\pm$40)	−24.2 ($\pm$1.1)	11–270
1772	0–15	*Pinus nigra*	439 ($\pm$45)	−21.0 ($\pm$1.2)	324–543
	15–25	*Pinus nigra*	310 ($\pm$45)	−21.0 ($\pm$1.2)	290–485
	25–40	*Pinus nigra*	100 ($\pm$40)	−23.2 ($\pm$1.1)	11–270
	55–65	*Pinus nigra*	1600 ($\pm$40)	−22.3 ($\pm$1.1)	1390–1568
	55–65	*Pinus nigra*	1170 ($\pm$50)	−20.6 ($\pm$1.2)	965–1239

[a] 2-σ range.

1–1.6 ky BP. At this site, only charcoal pieces deriving from *P.nigra* could be identified. This shows that the vegetation has not changed over the last 1.6 ky. Some *Fagus sylvatica* charcoal could be related to the age <300 y BP. *F.sylvatica* is not uncommon in these areas. Nowadays, it usually grows at altitudes >1500 m asl. Some individual examples of this species occurred at the highest site.

5. Discussion

Charcoal and charred organic material is present in substantial amounts in the investigated soils. In fact, up to almost 40% of the organic carbon concentration can be charcoal (Table 5). There is evidence that charred organic carbon plays an important role in storing carbon in many soils worldwide such as Chernozems in Russia (e.g. Schmidt et al., 1999), Hapludolls in Argentina (Zech et al., 1997), volcanic ash soils in Japan (Golchin et al., 1997) or in soils of the French Alps (Carcaillet and Talon, 2001). The frequent burning of vegetation produces black carbon that contributes to highly stable organic matter in soils (Schmidt and Noack, 2000). Soil carbon sequestration in the form of charcoal is hence significant in biomes having a dry climate (or periods of dry conditions) and fire-prone vegetation (Carcaillet and Talon, 2001; Rovira et al., 2009).

In general, the stability of depolymerised and microbially transformed detritus is thought to depend on intrinsic soil properties such as surface chemistry (Kleber et al., 2007). Organic fractions having slow turnover rates are mostly found when associated with soil minerals, except for fire-derived organic matter (Marschner et al., 2008). The presence of free particulate charcoal (not bound to the mineral phase) and its significant correlation to org. C is an evidence that not only sorption to mineral surfaces but also molecular recalcitrance is an important factor (Eckmeier et al., 2010).

Although the climate, fire and vegetation interactions are complex in the considered system, fire activity has most probably influenced several important soil processes in the Etna region (Egli et al., 2007). Soil organic carbon (SOC) and especially soil organic nitrogen (SON) seem to accumulate at lower altitudes, because of both specific climatic conditions – a more pronounced change in periods of humidity alternating with periods of droughts – and a higher fire activity. The positive correlations between altitude and SOC or SON content or stock (Figs. 2 and 4) support such a hypothesis.

Furthermore, fire activity (and indirectly also climate) seems to have an impact on the distribution of labile and stable organic matter
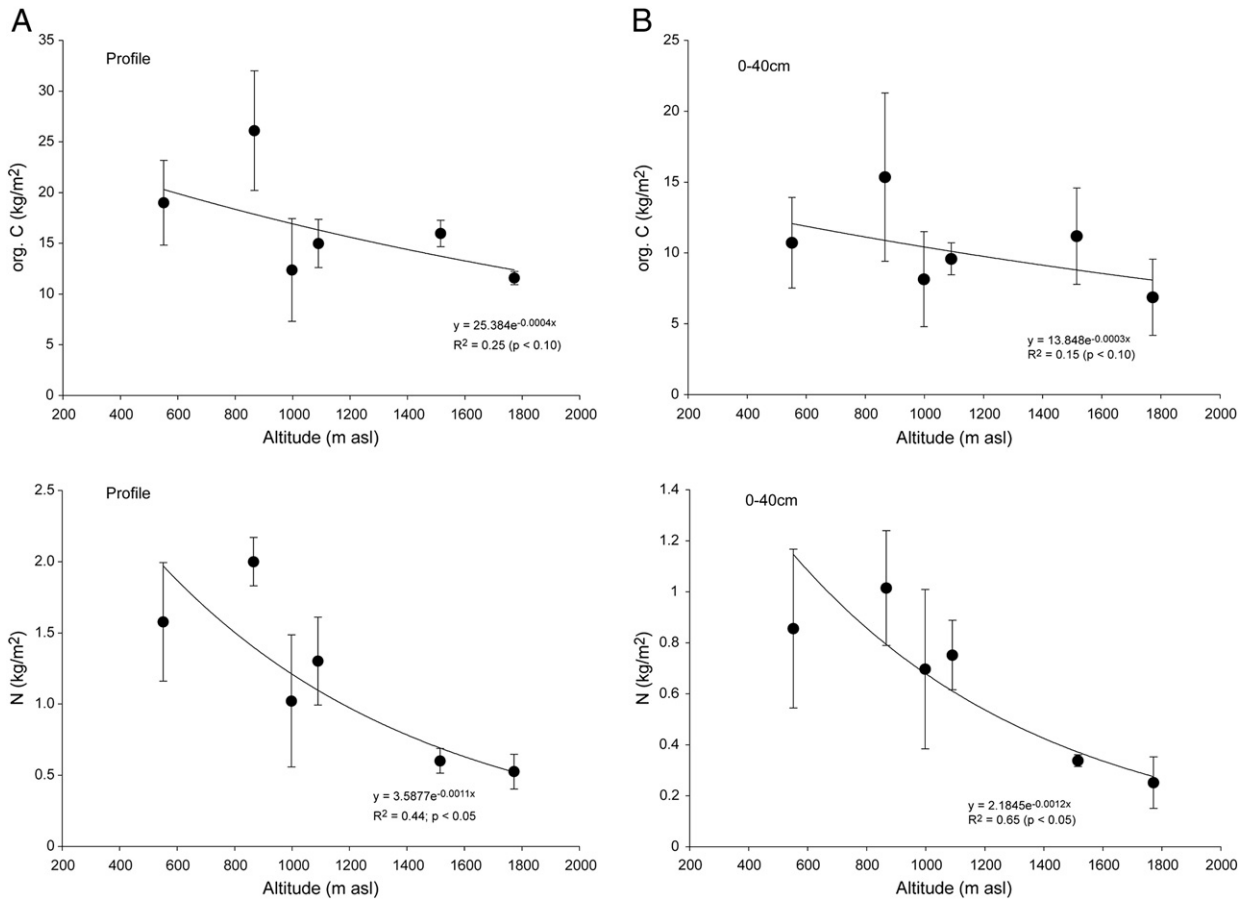
Fig. 4. C and N stocks in the soils along the toposequence. A) shows the values for the whole soil profile (n = 12) and B) the values for the soil depth 0–40 cm (n = 24).

as well as on the age of the stable organic matter fraction (Figs. 2 and 5). With increasing fire intensity (and warmer climate), more labile organic matter is accumulated in the soils and the age of the stable fraction decreases. Consequently, the soils at a lower altitude have a higher amount of labile organic matter. Although no systematic datasets about fire distribution and frequency exist in the investigated region, practical experience of the forestry service supports that in general a higher fire frequency is observed in forests at lower altitudes (especially at altitudes <1000 m asl). According to González-Pérez et al. (2004), the main transformations exerted by fire on soil

humus is the accumulation of new particulate C forms 'highly resistant to oxidation and biological degradation' including the so-called 'black carbon'. Our results do not fully match to this statement. In the investigated soils of the Etna, the fire-affected soil organic matter can be ascribed rather to the labile and, therefore, more easily degradable fraction. The accumulation of this labile fraction at lower altitudes is probably also due to fire damage to the soil biota (and the subsequent lower biodegradation of organic matter, Bárcenas-Moreno and Bååth, 2009) or to the reduced biological activity induced by the aridity of soils during the summer months. According to
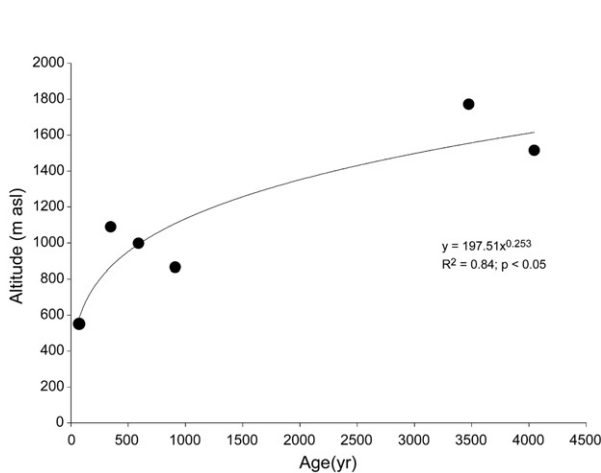
Fig. 5. Mean age (calibrated) of the stable organic matter fraction of the topsoil (0–40 cm) as a function of the altitude.
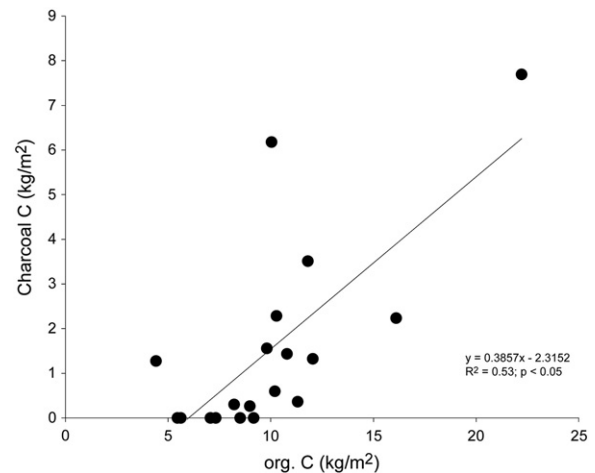
Fig. 6. Correlation between the charcoal and SOC stocks of the individual profiles (one soil profile per site and replicates).

Knicker (2007), char is a heterogeneous mixture of heat-altered bio-polymers having domains of relatively small polyaromatic clusters, but considerable substitution with N, O and S functional groups. Such a concept implies fast oxidation facilitating both microbial attack and dissolution.

In general, a higher org. C (and N) abundance can be measured at lower altitudes of our gradient (Fig. 4) although the trend for C is only significant only at a p-level of around 0.1, for both the whole soil profile and the depth interval 0–40 cm. The correlation between altitude and nitrogen is, however, more stringent. The increase in org. C with decreasing altitude can be at partially attributed to fire activity as there is a significant correlation between the C and charcoal stocks (Fig. 6). A similar tendency is also observed in the dry French Alps where at high elevations, the soil carbon derived from charcoal is negligible whereas at low altitudes (and a consequently warmer climate) the contribution of the charcoal to SOC is considerable (Carcaillet and Talon, 2001). As expected, no significant correlation exists between N stored in the charcoal fraction and the N stocks in the soils. Nitrogen is fixed in a labile fraction that most probably has been chemically altered due to fire effects (black carbon?). The chemical nature of the labile fraction was however not determined.

Increased fire activity at lower altitudes seems to prevent a longer stabilisation of soil organic matter (charcoal included). The age-trend of the stable organic matter with the altitude shows that this fraction is not so very quickly removed (due to burning processes) at high altitudes (Fig. 5), while fire appears to negatively affect the stable SOM turnover rate at lower altitudes where finally fires are more frequent. The young ages could of the stable SOM at low altitudes is due to mass movements (erosion) or, more likely, to fire events that prevent the formation of a very old and stable organic matter fraction. Due to the well-preserved soil profiles and the topography, erosion processes seem to be of minor importance. Major discontinuities in the soil profile are, thus, unlikely. Interaction mechanisms between OM and the mineral matrix also play a role in SOM stabilisation. According to the results of Egli et al. (2007), mineral properties at lower altitudes would be even more favourable to fix stable organic matter (due to the higher amount of kaolinite, imogolite-type material and oxyhydroxides). Consequently, fire activity must have an important impact on the turnover rate of the stable organic matter fraction. Our findings suggest that the high fire frequency is a powerful rejuvenating factor for soil organic matter, removing part of the very old, biologically recalcitrant SOM. The differing vegetation, which is due to the changing climatic and other conditions along the toposequence, also contribute to the observed trend. Our results suggest that the stable SOM reflects a climate signal (with a lower turnover rate at higher elevated sites). As already demonstrated by Tewsbury and Van Miegroet (2007), cooler climatic conditions may lead to a longer residence time of OM in soils. Climate- (and often strongly temperature-) dependent stabilisation of the 'stable' organic matter is, however, controversially discussed in literature (von Lützow and Kögel-Knabner, 2009). Some authors claim that the decomposition rate of the stable OM is not temperature-dependent (e.g. Fang et al., 2005), while according to others (e.g. Melillo et al., 2002), that of labile OM is very temperature-sensitive. Another opinion is that stable OM has even a higher temperature-dependency than labile OM (e.g. Conant et al., 2008; Rey et al., 2008). Furthermore, a fourth group has shown that labile and resistant SOM pools respond similarly to changes in temperature (e.g. Conen et al., 2006). In our studied gradient, most probably several processes (reduced fire activity, cooler climate, changed vegetation) contributed to the higher age of the stable organic matter fraction at higher-elevated sites of Etna. It would seem that none of the contributions made by the individual factors can be fully ruled out.

Additionally, $\partial^{13}C$ values of the stable organic matter fraction show a nice inverse correlation with altitude (Fig. 3). ^{13}C is a useful tracer for studying the contribution of plant types to the C budget, the decomposition and incorporation of organic material into more stable SOM (Andreux et al., 1989; Desjardins et al., 1994; Garcia-Olivia et al.,

Table 9
Descriptive statistics of the datasets.

	Mean	Median	Skewness	Normal distribution[a]
C stable (g/kg), 0–40 cm	6.81	5.94	0.22	x
C labile (g/kg), 0–40 cm	27.95	24.53	1.06	x
N stable (g/kg), 0–40 cm	0.63	0.48	0.83	x
N labile (g/kg), 0–40 cm	1.65	1.43	1.23	x
C stable (g/kg), 0–15 cm	9.78	9.96	0.10	x
C labile (g/kg), 0–15 cm	33.06	24.53	0.68	x
N stable (g/kg), 0–15 cm	0.81	0.70	0.822	x
N labile (g/kg), 0–15 cm	2.08	1.53	0.72	x
C stable (g/kg), 15–40 cm	3.84	4.08	−1.82	–
C labile (g/kg), 15–40 cm	22.84	24.25	−0.36	x
N stable (g/kg), 15–40 cm	0.44	0.26	0.99	x
N labile (g/kg), 15–40 cm	1.21	1.10	0.22	x
$\delta^{13}C$, 0–40 cm	−21.97	−19.95	−1.63	–
$\delta^{13}C$, 0–15 cm	−21.78	−20.30	−1.91	–
$\delta^{13}C$, 15–40 cm	−22.15	−19.95	−1.98	–
C stock (kg/m²)	15.81	15.05	0.95	x
N stock (kg/m²)	1.10	1.02	0.58	x
Charcoal C (kg/m²)	1.82	1.27	0.62	x
C stock (kg/m²), 0–40 cm	9.95	9.92	1.22	x
N stock (kg/m²), 0–40 cm	0.62	0.57	0.46	x
Charcoal C (kg/m²), 0–40 cm	1.57	0.60	1.94	–
Mean topsoil age (^{14}C)	1573	750	0.91	x

[a] Normality was checked using the Shapiro–Wilk test: x = normal distribution of the dataset; – = not normal distribution of the dataset.

1994; Golchin et al., 1995; Lichter et al., 2008). This trend is primarily due to the changing vegetation and thus the changing litter input into soils but is probably also influenced by the climate (or a combination of both factors). At lower sites, the contribution of C derived from grass (maybe also from some C4 plants) and shrubs is, due to the vegetation type, most probably higher. Also, during decomposition processes some different isotopic fractionation might have occurred at different altitudes, due to the preference accorded by heterotrophic microorganisms to ^{12}C with respect to the heavier ^{13}C (Ågren et al., 1996; Balesdent et al., 1993). Furthermore, Eilmann et al. (2010) showed that maximum $\partial^{13}C$ values were measured after the hottest and driest period of the year in tree rings, and were associated with decreasing growth rates of trees (Scots pine). After biodegradation of the plant residues, we assume that such an influence should also be detectable in the soil organic matter. The $\partial^{13}C$ in early- and late-wood and after biodegradation also in the SOM reflects climatic conditions (Eilmann et al., 2010) and may indicate that moister conditions in soils exist at higher-elevated sites. Furthermore, a higher fire frequency at the lower sites is hypothesised to lead to a depletion of ^{12}C (and to consequently less negative $\partial^{13}C$ values). This would fit observations made by Shrestha (2009) in subalpine soils of Australia: The C and N of soil organic matter were significantly enriched in ^{15}N and ^{13}C isotopes after fire and had not returned to the pre-fire levels five years after the fire. Other authors, however, did not find any significant fire-induced change in $\partial^{13}C$ in burnt areas (e.g. Alexis et al., 2010; Certini et al., in press).

Some detected trends (e.g. $\partial^{13}C$ or the mean age of the stable organic matter) might to be due to an uneven data distribution. This is not very surprising, as some distinct changes in the soil are due to a change in vegetation. Nonetheless, several gradual changes and strict relationships could be found (Figs. 2 and 4) that are either due to climatic conditions or the fire regime. Most of the data had, furthermore, a normal distribution (which would indicate homoscedasticity of the datasets; Table 9). The $\partial^{13}C$ values, however, do not show a normal distribution. The presence of two different datasets is under such circumstances more likely (see above): in this case, the higher sites (having *P.nigra*) can be distinguished clearly from the lower sites.

The factors fire, climate and vegetation are interrelated. Consequently, a clear subdivision is not always possible. The increase of labile C and N with decreasing altitude (Fig. 2), the decreasing age of the
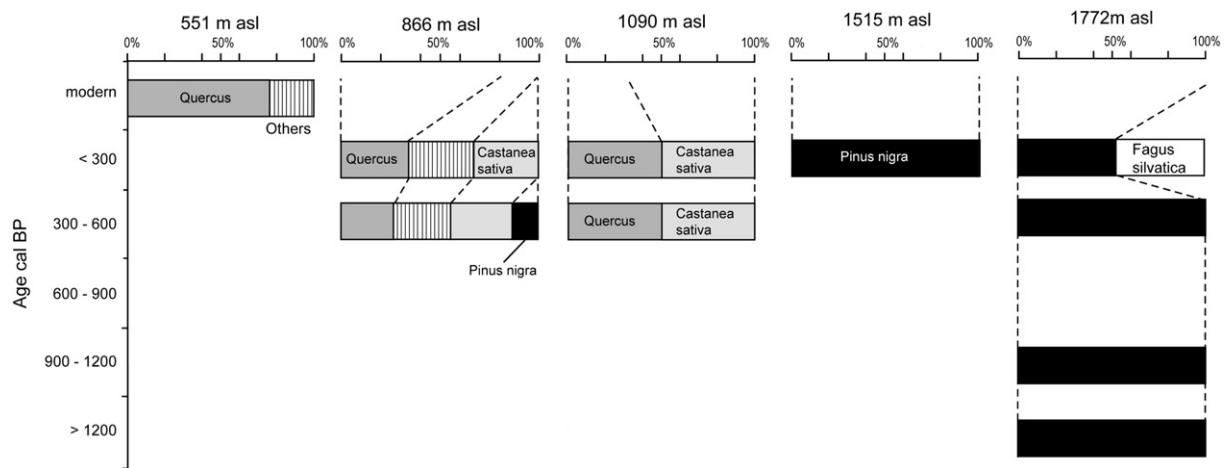
Fig. 7. Composition of the vegetation as a function of time derived from charcoal fragments in the soils. Others = *Lonicera implexa* (551 m asl), Maloideae (866 m asl).

stable OM with decreasing altitude (Fig. 5), the relationship between the charcoal C and org. C abundance (Fig. 6) and the lack of old charcoal fragments (due to continuous removal by fire; Fig. 7; see below) at lower altitudes are explicit signs of an increasing influence of fire on soils with decreasing altitude.

The charcoal composition primarily reflects the three different vegetation systems: maquis at low to mid, chestnut and oak forests (Supra-Mediterranean zone) at mid and pine at high elevations. Preconditions for forest fires are the presence of a substantial amount of flammable trees and litter, a relatively dry climate or periods with drought and thunderstorms (lightning). Another natural factor promoting wildfires in the studied area is the incandescent material erupted by the volcano, as both lava flows and ejecta. Furthermore, human activity also contributes substantially to the incidence of fires in the Mediterranean basin (Novara et al., 2010; Pausas et al., 2008). Similar to findings in the Alps (e.g. Favilli et al., 2010), and as inferred by the identification and dating of charcoal fragments (Fig. 7), vegetation at highly elevated sites on Etna has not changed much during the last centuries to millennia. At low altitudes, the fire frequency seems to be too high to enable preservation of old charcoal particles; here, a reconstruction of the vegetation history was thus hardly possible. Several traced fire events occurred in fact in the Modern Period. At sites >800 m asl, a reconstruction of the vegetation back to the Middle Ages was possible. At the highest altitude, fires that occurred at the end of the Roman period were detectable. This may well reflect a climatic signal of fires, with frequent burning at the lower sites that is accompanied by a continuous removal of older charcoal, but probably also points to the intensity of human impact at lowest altitudes. As shown by Gates and Liess (2001), Hughes (2011) and Henry et al. (2010), human impact has been a determining factor in the evolution of the Mediterranean landscape — in some parts since the Neolithic and Bronze age. Although climate change will alter vegetation composition, future dynamics of mountain forests and soils will be co-determined by anthropogenic fire (Colombaroli et al., 2010).

6. Conclusions

We measured several parameters to investigate the effect of fire on SOM characteristics and to trace back landscape evolution using charcoals. The studied soils have recorded the signals of the interrelated factors fire frequency, climatic effects and vegetation that cannot always be clearly subdivided. Although some of the measured parameters do not have a huge statistical database, they all point into a similar direction. Our results show that fire is a driving factor of landscape

evolution and pedogenesis of the Etna region. In particular, it strongly affects the characteristics of soil organic matter:

- Carbon and especially nitrogen stocks seemed to be higher at lower-elevated than at higher-elevated sites (the trend is however significant only with an error probability of >5%). The accumulation of C correlated with the stocks of charcoal in the soils. The C in charcoal can comprise up to almost 40% of SOC.
- Labile SOM seemed to increase with decreasing altitude (for N this increase was significant whereas for C the error probability was >5%). The increased fire activity with decreasing altitude, the change in vegetation and climate seem to lead to the accumulation of the labile SOM fraction and to the removal of the old and stable one.
- Fire frequency seems to be a powerful rejuvenating factor for soil organic matter. In fact, the age of the stable soil organic matter strongly increased with increasing altitude, so much that in some soils at high altitudes, the stable SOM was close to the age of the soil.
- Natural and human-induced fires have repeatedly affected the landscape evolution. The identification and dating of charcoal pieces reveal that more frequent fires must have occurred at the lowest altitudes. At high altitudes, where a longer charcoal time-sequence was available, the general type of vegetation has not changed greatly over the last centuries to millennia.

The soils have recorded the signal of fire frequency, vegetation and climatic effects. Vegetation is, however, not a fully independent factor as it is influenced by climate and fire. With decreasing altitude and, therefore, with a warmer climate, fire frequency, SOM abundance and the amount of labile SOM seemed to increase. Although fire strongly determines the fate of several soil aspects, fire frequency and intensity on the surveyed area of Mt Etna are, however, still moderate enough even at the lowest altitudes for the SOM pool to be still high and not depleted.

Acknowledgements

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Manuscript IV

Short- and long-term effects of fire on soil organic matter along a toposequence on Mt. Etna, Sicily

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Abstract

Soil organic matter (SOM) can directly be affected by wildfires, both in terms of quantity and quality. The combustion is often incomplete, leading to the formation of pyrogenic organic matter (PyOM), a highly heterogeneous material with prevailing aromatic nature. Focusing our attention on PyOM, we investigated a soil toposequence on Mount Etna, Italy, ranging from 500 m to 1800 m asl, where the fire frequency is assumed to be historically higher at lower elevations due to a warmer and drier climate. We evaluated the long-term effect of fire on the chemical and physical characteristics of SOM along the toposequence and, restricted at two sites having a different recent fire history, also we examined a shorter-term effect. Chemical oxidation of SOM with acid dichromate was used to evaluate the contribution of pyrogenic organic carbon (PyOC) to total SOC. Furthermore, four SOM density fractions were separated and characterised for principal composition, spectroscopic properties (DRIFT and NMR), and particle morphology (SEM). The stocks of C and N as well as PyOC increased with decreasing altitude, hence supporting the hypothesis of higher fire frequency at lower altitudes. The highest C concentrations were found in the density fraction 1.0-1.6 g cm^{-3}. The enrichment of C in this fraction can occur already in the

short-term. At the lower sites, a considerable amount of aromates, and hence a significant part of PyOM, was found in the density fraction 1.0-1.6 g cm^{-3} except for (macro)charcoal that occurs in the lightest fraction (<1.0 g cm^{-3}). The properties of this latter density fraction seemed to be instead rather related to vegetation characteristics.

The dichromate oxidation resulted not to be highly selective for PyOM in the investigated soils, since it isolated a fraction very rich in lipids. The lack of selectivity for PyOM is most likely due to the rather low content of aromates in SOM, although fire frequency was relatively high at the lower elevated sites.

Keywords: wildfires, soil organic matter, density fractionation, pyrogenic carbon, ^{13}C NMR, DRIFT, Mt. Etna.

Introduction

Mediterranean landscape has been shaped by wildfires over millennia, so much that the indigenous vegetation became adapted to face the deleterious effects and acquired the ability to grow and reproduce quickly (Scarascia-Mugnozza et al., 2000). Due to increasing temperature and decreasing precipitation (IPCC Fourth Assessment Report, 2007; Moriondo et al., 2006) in the near future a higher fire frequency is expected in the Mediterranean basin. Soils, which suffer many consequences of the occurrence of fires (Certini, 2005), will probably experience marked C losses because of combustion (Certini et al., 2011) or post-fire erosion (Shakesby, 2011). Of the soil components, soil organic matter (SOM) is in fact the one most directly affected by fire being the fuel in soil. When the fire intensities are not extreme, heating turns SOM to pyrogenic organic matter (PyOM) defined by Masiello (2004) as a continuum of combustion products ranging from slightly charred degradable biomass to highly condensed, refractory soot. Although modern studies have demonstrated that PyOM is not so resistant to decomposition as previously assumed (Bird et al., 1999; Hamer et al., 2004; Knicker et al., 2006), PyOM is still considered a recalcitrant component of SOM (Kuhlbusch and Crutzen, 1995; Schmidt and Noack, 2000). The PyOM structure is highly heterogeneous, mostly depending on the plant materials composition and burning conditions; it appears to be characterised by heteroaromatic forms (González-Pérez et al., 2004; Knicker et al., 2006, 2008a).

Since SOM fractions have a narrower range of properties compared to the bulk, a physical fractionation can be an useful tool to study such a complex mixture of thermally altered biomacromolecules. In particular, density fractionation allows to obtain a light SOM fraction, consisting of slightly decomposed plant and animal residues with a rapid turnover, and a heavy SOM fraction, which includes the SOM involved in organo-mineral complexes that is thought to contain more humified residues with a lower C/N ratio and a lower turnover rate (Glaser et al., 2000). PyOM is usually reported to be mainly concentrated in the light pool (Skjemstad et al., 1990; Golchin et al., 1997, von Lützow et al., 2007).

The aim of this work is to assess the long-term effect of fire on SOM content and composition along a topo-climosequence on Mount Etna (Sicily) where Egli et al. (2012) found evidences that fire activity was higher at lower elevated sites by studying the radiocarbon age of charcoal particles and the stable fraction of soil organic matter. Our hypothesis is that the higher fire frequency at lower elevated sites left a strong fingerprint on SOM, mostly detectable by the accumulation of aromatic compounds. In such an environment, fire is a principal factor of soil formation and a higher fire frequency is expected for the near future. Our study of SOM from a topo-climosequence on Mount Etna shall help to provide predictions on the fate of OM in the soils at higher elevated sites. In this regard, we intended also to look for signals for a short-term effect of fire by comparing two sites of the topo-climosequence having similar properties except the recent fire history.

To accomplish this purpose, soil samples were separated on a density basis into four fractions, which were analysed for principal composition and spectroscopic properties and observed by a scanning electron microscope (SEM). Additionally, a chemical oxidation of SOM using acid dichromate was performed to determine the amount of pyrogenic organic carbon (PyOC) in each fraction.

Materials and Methods

Study sites

The study area is Mount Etna, Sicily (Italy), the largest active volcano of Europe with a maximum elevation 3323 m asl. The Etna landscape is characterised by lava-flows stratigraphically placed according to ages. Poli Marchese (2004) recognised in the Etna region the following vegetation zones:

   a) basal-mediterranean zone with a thermo-mediterranean subzone (*Oleo-Ceratonion*, below 500-600 m asl), a meso-mediterranean subzone (*Quercion ilicis*; between 600 and 1100 m asl) and a supramediterranean zone (*Quercetalia pubescentis*; between 1100 and 1500 m asl)

b) montane-mediterranean zone between 1500 and 2400 m asl (*Fagetalia sylvaticae* with *Astragaletum siculi, Pinus nigra* ssp*., Laricio* (*Querco-Fagetea*)*,* etc.)

c) Mediterranean high-alpine zone: above 2400 m asl (*Rumici Anthemidetum aetnensis*)

Human impact is an important environmental factor in the Etna region. Large areas around the volcano have been disturbed over centuries by human activities, in particular agriculture. Pristine soils are exclusively found at high altitudes and on relatively young lava flows.

We investigated six sites along an elevational gradient on the northeast flank of the volcano ranging from Mediterranean (551 m asl; warm, semi-arid to sub-humid climate) to subalpine (1772 m asl; cold, humid climate) climate zones (Table 1, Fig. 1).
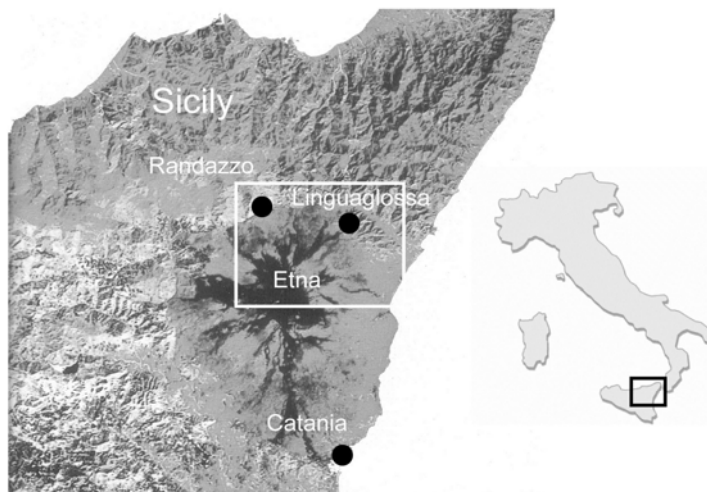
Figure 10. Location of the investigated sites in Mt. Etna region, Sicily, southern Italy.

Soil moisture and temperature regimes vary between xeric to udic and thermic to frigid, respectively (Raimondi et al., 1999). Five sites were located on a lava flow and a sixth one (site 6) on another lava flow having, however, the same composition (Fig. 1). To discriminate the short-term effects of fire on soil organic matter, we compared sites 6 and 4, having both a similar vegetation, altitude, topography and morphological soil properties − but substantially differing in the recent fire history. In fact, during the last 30 years, site 4 burned approximately once per decade while site 6 almost every year (Forest Service, personal communication).

At each site, three soil profiles were opened and sampled at 0-10 and 10-30 cm depth intervals. One to three kg of soil material were taken from either depth interval; such unusually large amounts are actually needed for coarse-textured soils to be representative samples (Hitz et al., 2002). Soil bulk density was determined by a core sampler (100 cm^3), taking 2-4 replicates each depth interval or,

when the stoniness was high, by excavating holes backfilled with quartz sand (irregular hole method, as described in Howard and Singer (1981)). All of the investigated soils did not exhibit any

Table 1. Characteristics of the study sites in the Etna region.

Altitude (m asl)	Site	Coordinates	Aspect	Slope (°)	MAT[1] (°C)	MAP[1] (mm)	Soil moisture regime	Soil temperature regime	Parent material	Vegetation	Soil type (IUSS Working Group WRB. 2006; Soil Survey Staff 2006)
551	5	37.8784° N / 15.0900° E	N	0	15.3	1000	xeric	thermic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea /Quercion ilicis with *Quercus pubescens, Asphodelus microcarpu, Carlina nebrodensis, Artemisia spec., Genista aetnensis, Rubus spec., Dryopteris filix-mas*	Vitri-Mollic Andosol / Humic Haploxerand
866	4	37.8512° N / 15.07025° E	N	4	13.1	1100	udic	mesic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea /Quercion ilicis with *Quercus pubescens Wild, Genista aetnensis, Rubus spec.*	Vitric Andosol / Typic Udivitrand
998	6	37.8538° N / 15.0027° E	N	3	12.5	1100	udic	mesic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea /Quercion ilicis with *Castanea sativa, Quercus pubescens Wild, Genista aetnensis, Rubus spec., Dryopteris filix-mas*	Vitric Andosol / Typic Udivitrand
1090	3	37.8266° N / 15.0831° E	N	0	11.9	1150	udic	mesic	Ve-Lava (Trachy-basalt)	Macchia Mediterranea /Quercion ilicis with *Quercus ilex, Quercus pubescens Wild, Castanea sativa, Genista aetnensis, Daphne laureola L, Orchis commutata, Muscari spec., Rubus spec., Dryopteris filix-mas*	Vitri-umbric Andosol / Humic Udivitrand
1515	2	37.8097° N / 15.0640° E	N	4	9.2	1250	udic	mesic	Ve-Lava (Trachy-basalt)	Coniferous forest (*Pinus nigra ssp. laricio*) with a few deciduous trees (*Fagus silvatica, Castanea sativa*)	Vitric Andosol / Vitric Hapludand
1772	1	37.7915° N / 15.0428° E	N	12	7.5	1400	udic	frigid	Ve-Lava (Trachy-basalt)	Coniferous forest (*Pinus nigra ssp. laricio, Dryopteris filix-mas, Iuniperus sp.*)	Vitric Andosol / Humic Udivitrand

[1]MAT = mean annual temperature (°C). MAP = mean annual precipitation

marked sign of erosion or human disturbance. Additional information about the sites and soils properties are reported in Egli et al. (2012).

OC and N content

The soil samples were air-dried and sieved to 2 mm. Total C and N contents of the fine earth (the less than 2 mm fraction) were measured by a C/H/N Analyser (Elementar Vario EL, Elementar Analysensysteme GmbH, Hanau, Germany) on oven-dried (70 °C for 48 h) and ball-milled material.

Density fractionation

Density fractionation was performed on a composite sample (combining aliquots from the 3 profiles per site) following a procedure adapted from Christensen (1992) which is based on the use of Na-polytungstate solutions with different concentrations. The following fractions were separated: <1.0 g cm^{-3}, 1.0-1.6 g cm^{-3}, 1.6-2.0 g cm^{-3} and >2.0 g cm^{-3}. In brief, 40 g of fine earth was put in 400 mL of deionized water, slightly stirred for 12 h, then centrifuged for 10 min at 5,000 g. The supernatant was skimmed off and evaporated, so obtaining the <1.0 g cm^{-3} fraction. The residual sample was dried at 40 °C and mixed to a sodium polytungstate solution with a density of 1.6 g cm^{-3}. After an ultrasonic treatment of 50 J mL^{-1} for 40 s, we centrifuged the suspension for 30 min at 3000 g. The supernatant was skimmed off and evaporated, so obtaining the 1.0-1.6 g cm^{-3} fraction. The same procedure was finally applied to the residue using a polytungstate solution with a density of 2.0 g cm^{-3}, so as to separate the 1.6-2.0 g cm^{-3} fraction from the >2.0 g cm^{-3} one. All of the fractions were washed three times with deionised water, centrifuging and discarding the supernatants, hence dried at 40 °C.

Hydrofluoric acid treatment

A hydrofluoric acid (HF) treatment according to Gonçalves et al. (2003) was carried out on aliquots of both bulk samples and density fractions before performing the ^{13}C NMR analysis, so as to remove paramagnetic minerals which strongly reduce the signal-to-noise ratio of the spectra. A HF treatment is also required as pretreatment for black carbon quantification by dichromate to allow a complete oxidation of labile SOM, including the physically protected one. In brief, 5 g of soil were shaken at room temperatures with 50 mL of 10% (w/w) HF for 12 h in polyethylene bottles, then centrifuged and the supernatant discarded. The procedure was repeated five times, thereafter the residue was washed five times with 40 mL distilled water and dried at 50 °C.

Although the hydrofluoric acid treatment usually causes some C and N removal, selective losses of specific groups should not occur, as supported by the findings of Knicker at al. (2008b). However, since Rumpel et al. (2006a) showed that NMR spectra of pure organic matter are not influenced by the HF treatment, we expected minor, if any, effects of HF treatment on the results of NMR analysis on the light fractions samples (<2 g cm^3).

Dichromate oxidation

The contribution of PyOC to total SOC was estimated by assuming the chemical oxidation resistant carbon (COREC) to be PyOC as proposed by Knicker et al. (2007).

According to Rumpel et al. (2006b), 250 mg of HF-treated sample was mixed with 40 mL of a potassium dichromate solution (0.1 M $K_2Cr_2O_7$ in 1L of 2M H_2SO_4) in a thermal bath at 60° C for 6 h. The solution was checked during the treatment to detect a possible premature saturation that is revealed by a greenish colour. In this case the oxidation had to be repeated with a larger quantity of sulfochromic solution. To prevent as much as possible such a problem we referred to a C_{org}-to-acid ratio of 2 mg mL^{-1} as suggested by Knicker et al. (2007). Thereafter, the sample was washed at least four times with 50 mL distilled water. The residue was dried at 50 °C and the mass loss was determined. The C and N recovered were calculated by mass balance.

DRIFT measurements

DRIFT measurements were performed on bulk soil and density fractions samples using a Tensor 27, Bruker. Relative peak intensities were used for DRIFT analysis. Spectra were recorded from 4000 to 250 cm^{-1} on pulverised samples dried at 60 °C.

To quantify the relative contribution of single C moieties, we divided the intensity (area) of each peak of the FT–IR spectra by the sum of the intensities of all peaks taken into account (see Table 4) using the software OPUS 6.

NMR spectroscopy

^{13}C CP/MAS NMR spectra of HF-treated samples were recorded on a Bruker AMX300-WB spectrometer at 75.47 MHz, equipped with a 4-mm CP-MAS probe. The 90° pulse was 3.2 μs; 20000 scans were acquired, depending on the sample, with a 4 s repetition time, using a contact pulse of 2 ms and a 60-kHz radiofrequency field. All spectra were recorded at room temperature, spinning at the magic angle at 3.5 or 5.0 kHz.

We applied this technique to the density fractions, combining equal aliquots from the top 10 cm of the soil at the highest altitudes, 1 and 2, and, separately, of the lowest sites, 4 and 6.

A semiquantitative determination of the main C forms was done integrating the chemical shift regions of the spectra and expressing them as percentages of the total area between 0 and 220 ppm: 0 to 45 ppm (alkyl C), 45 to 110 ppm (O-alkyl C, subdivided in Methoxyl/N-alkyl, 45 – 60 ppm; O-alkyl, 60 - 90 ppm; Di-O-alkyl, 90 – 110 ppm), 110 to 165 ppm (aryl C, subdivided in aromatic C-H and C-C, 112 – 140 ppm; O substituted, 140 – 165 ppm), 165 to 185 ppm (carboxyl C), and 185 to 220 ppm (aldehydes, ketones) (Wilson, 1987).

SEM-EDX

Bulk, loose (uncoated) fine earth was observed by a scanning electron microscope (SEM). The principal composition of the focused specimen was assessed by a Dual Beam Quanta 200 3D FEI, equipped with an energy-dispersive spectrometer (EDS) with a Dual BSD detector and a W emitter operating at an accelerating voltage of 20 kV.

Statistics

Datasets were first checked for normal distribution by a Shapiro-Wilk test (SigmaPlot 11.0, Systat Software Inc.) (Jann, 2005), using a two-tailed test for significance. However, since most of them did not show a normal distribution, the Spearman rank correlation coefficient was taken.

Results and Discussions

Carbon and nitrogen in bulk soil and density fractions

The mean concentrations of carbon and nitrogen tended to slightly decrease with altitude in both the 0-10 and 10-30 cm soil layers (Table 2). The C and N stocks showed the same trend, especially for the deep layer (10-30 cm) (Fig. 2). Egli et al. (2007; 2012) found the same elevational trend for soil organic matter in the Etna region. These authors partly correlated the results to the climate and vegetation, inferring that in such an environment higher temperatures should lead to an increase of SOM and higher precipitation to a better mineralisation and consequently a decrease in SOM. The increasing C and N stock trend with decreasing altitude was much more evident in the deeper layer (Fig. 2). An accumulation of PyOM often takes places in the subsoil (Rumpel et al., 2004; Djukic et al., 2010; Knicker, 2011), because the products of the fire combustion from the topsoil are exported in deeper horizons by water percolation, mesofauna activity, and other causes (Schmidt and Noack, 2000; Gonzalez-Perez et al., 2004). Therefore the elevational trend could be also related to the accumulation of fire-derived OM at lower sites, where fire is more frequent.
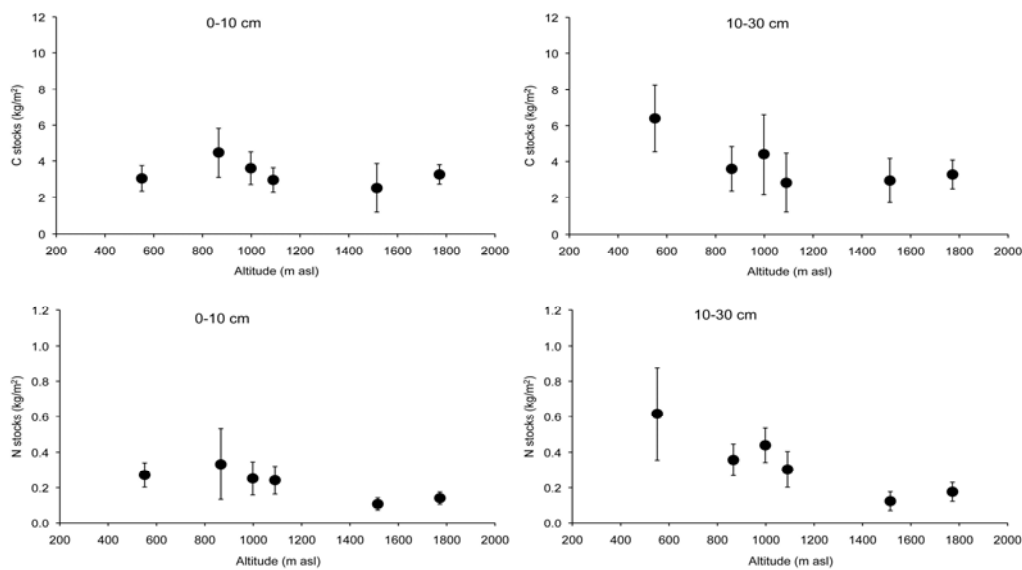
Figure 11 Soil C and N stocks along the toposequence (mean values and standard deviation; n = 4).

The elevational trend, furthermore, is more evident for N than for C in these soils (Fig 2); this could be a further clue of a large contribution of PyOM. In fact, a relatively N-rich biomass (e.g. grass vegetation) during charring shows an efficient incorporation of N into the soil (Knicker, 2010). The lower sites actually have a vegetation cover richer in grass compared to the higher sites dominated by a pine stands with a poor developed understory. Consequently, the more distinct altitudinal trend of N, particularly for the deep layer, also could be due to fire effect. However, fire can affect the presence of nitrogen in opposite ways. Bárcenas-Moreno and Bååth (2009) showed that heating to about 200°C induces a preferential loss of nitrogen over carbon, thus increasing the C/N ratio, whereas heating at higher temperatures, up to 400-500°C, leads to a preferential loss of carbon over nitrogen.

The two sites at the same elevation and with a different recent fire history, site 4 and site 6, did not show any relevant differences in terms of soil C and N concentrations and stocks (Table 2, Fig. 2).

The SOC concentration in the density fractions usually decreased with increasing density in both soil depths, with a single exception for the fraction 1.0-1.6 g cm^{-3} that, on average, had the highest C concentration (Table 3). Site 6, where nearly a fire per year occurred in the last two decades, showed the highest C concentration in the fraction 1.0-1.6 g cm^{-3} in both investigated soil layers. This effect does not seem to be due to climate/vegetation properties, because site 4, having the same altitude and vegetation, had a much lower C concentration in this fraction than site 6. In this regard several authors report that fire-derived carbon is mainly concentrated in the lighter fraction, from 1.0 g cm^{-3} to 2.0 g cm^{-3} (e.g. Skjemstad et al., 1990; Golchin et al., 1997; von Lützow et al., 2007).

Altitude m asl	Soil depth cm	Bulk density g/cm³	Soil skeleton wt-%	Corg g/kg	Norg g/kg	C/N
1772	0 - 10	0.98 (±0.16)	10 (±2)	39.1 (±5.1)	1.69 (±0.38)	23.7 (±3.2)
(Site 1)	10-30	1.13 (±0.12)	8 (±3)	16.9 (±2.9)	0.91 (±0.23)	19.0 (±2.5)
1515	0 - 10	0.87 (±0.12)	8 (±6)	33.7 (±15.1)	1.48 (±0.32)	21.9 (±6.6)
(Site 2)	10-30	1.02 (±0.09)	6 (±6)	16.3 (±6.9)	0.69 (±0.32)	24.4 (±4.4)
1090	0 - 10	1.02 (±0.04)	22 (±4)	39.2 (±5.1)	3.19 (±0.73)	12.5 (±1.2)
(Site 3)	10-30	0.93 (±0.10)	24 (±4)	20.2 (±10.0)	2.23 (±0.64)	9.3 (±3.7)
998	0 - 10	1.06 (±0.13)	43 (±16)	50.6 (±7.0)	3.47 (±0.67)	14.8 (±2.7)
(Site 6)	10-30	1.50 (±0.07)	63 (±17)	25.8 (±12.1)	2.61 (±0.51)	9.9 (±4.0)
866	0 - 10	1.17 (±0.17)	14 (±3)	54.1 (±3.3)	3.92 (±1.51)	15.5 (±6.5)
(Site 4)	10-30	1.02 (±0.12)	14 (±3)	27.0 (±7.1)	2.70 (±0.44)	9.9 (±1.5)
551	0 - 10	0.85 (±0.24)	33 (±21)	57.4 (±6.5)	5.08 (±0.39)	11.3 (±0.8)
(Site 5)	10-30	1.31 (±0.09)	40 (±11)	47.0 (±11.5)	4.47 (±1.63)	10.9 (±1.7)

Table 10. Bulk density and C and N contents of the sampled soils along the toposequence (mean values and standard deviation of n = 4).

In the various fractions nitrogen followed quite similar concentration trends than C. In particular, also for this element the highest concentrations were found in the 1.0-1.6 g cm^{-3} fraction (Table 3). The lighter fractions often showed a higher C/N ratio than the heavier ones, supporting the expectation that the light fractions mainly contained slightly decomposed plant-derived polysaccharides, lignin, cutin, suberin and/or (macro)charcoal (Sollins et al., 2006). Golchin et al. (1997) attributed such a difference in the C/N ratio between density fractions of soil also to preferential adsorption of N-rich organic compounds onto inorganic surfaces in the heavier fractions.

The C and N content in the separated four SOM fractions is given in Fig. 3. Although the lighter fractions had higher C and N concentrations, more than 50% of total SOC and N content were detected in the >2.0 g cm^{-3} fraction due to the higher mass of this fraction. This finding is in contrast to Golchin et al. (1997) who measured, in three Typic Malanudands dominated by Allophane from Japan, an average contribution of 13% to total SOC in the heavy fraction (>2.0 g cm^{-3}).
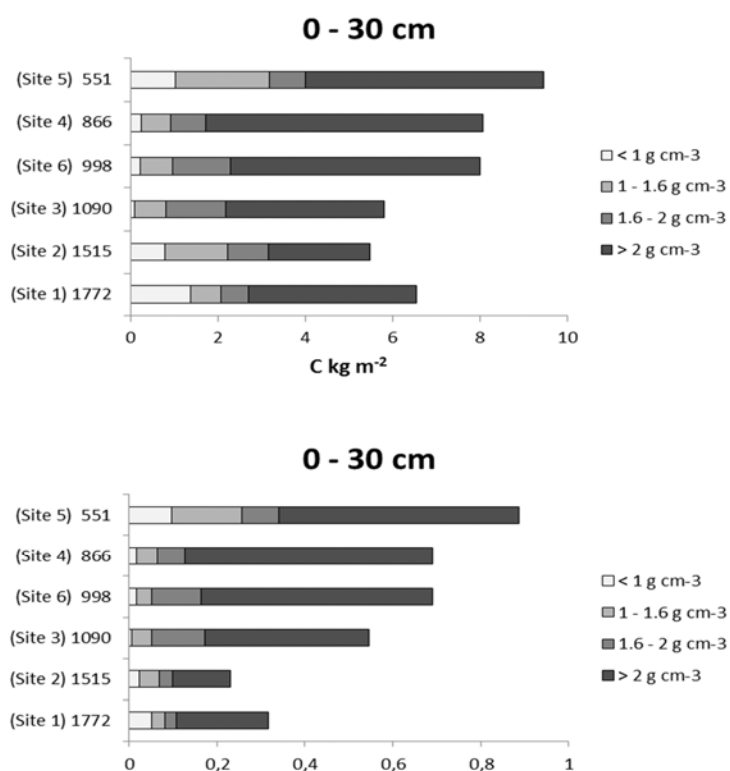
Table 3. C and N concentrations and C/N ratio in density fractions from 0-10 cm and 10-30 cm soil depths along the toposequence.

Soil depth cm / (site) and altitude m asl	C concentration in fractions (g/kg)				N concentration in fractions (g/kg)				C/N			
	<1 g cm⁻³	1-1.6 g cm⁻³	1.6-2 g cm⁻³	>2 g/cm³	<1 g cm⁻³	1-1.6 g cm⁻³	1.6-2 g cm⁻³	>2 g cm⁻³	<1 g cm⁻³	1-1.6 g cm⁻³	1.6-2 g cm⁻³	>2 g cm⁻³
0 - 10 cm												
(Site 1) 1772	304.3	215.3	234.7	48.7	11.7	9.4	12.5	3.0	25.9	22.9	18.8	16.3
(Site 2) 1515	254.3	275.6	203.5	21.8	7.2	7.6	8.1	2.0	35.6	36.5	25.1	11.1
(Site 3) 1090	209.4	328.8	178.3	31.4	12.2	15.5	13.0	1.3	17.1	21.2	13.7	24.7
(Site 6) 998	215.4	370.5	183.9	76.4	14.9	18.0	14.2	6.8	14.5	20.6	12.9	11.2
(Site 4) 866	244.3	280.1	193.8	91.4	17.2	19.9	17.4	7.5	14.2	14.1	11.1	12.2
(Site 5) 551	244.1	327.7	186.9	43.2	16.6	21.2	16.0	3.7	14.7	15.5	11.7	11.8
mean	245.3	299.7	196.9	52.1	13.3	15.2	13.5	4.0	20.3	21.8	15.6	14.6
sd	33.9	54.3	20.5	26.7	3.7	5.6	3.2	2.6	8.7	8.0	5.4	5.3
10 - 30 cm												
(Site 1) 1772	292.2	249.9	164.0	33.4	10.8	11.3	8.6	2.9	27.1	22.1	19.2	11.6
(Site 2) 1515	235.6	97.2	199.5	11.9	6.4	2.5	7.2	1.0	36.6	38.4	27.6	12.5
(Site 3) 1090	267.8	345.5	103.2	28.7	10.9	14.4	8.4	2.4	24.5	24.0	12.3	11.9
(Site 6) 998	200.1	403.4	162.0	73.3	13.9	18.0	13.1	6.1	14.4	22.5	12.4	12.1
(Site 4) 866	229.6	237.0	156.7	71.2	16.1	17.2	14.3	6.4	14.3	13.8	10.9	11.1
(Site 5) 551	244.1	330.3	162.0	43.9	15.7	20.9	14.0	1.4	15.6	15.8	11.6	31.8
mean	244.9	277.2	157.9	43.8	12.3	14.0	10.9	3.3	22.1	22.8	15.7	15.2
sd	31.9	107.9	31.0	24.4	3.7	6.5	3.2	2.3	9.0	8.7	6.6	8.2

Table 4. Major IR absorption bands and assignments (Piccolo and Mirabella, 1985; Stevenson, 1994; Senesi et al., 2003; Tan, 2003).

Band	Wave number cm⁻¹	Assignment
1	2980 - 2880	Aliphatic C-H stretching (aliphatic methyl and methylene groups)
2	1725 - 1710	C=O stretching of COOH, aldehydes and ketones
3	1660 - 1630	C=O stretching of amide groups. quinone C=O and/or C=O of H- bonded conjugated ketones
4	1620 - 1600	Aromatic C=C. strongly H-bonded C=O of conjugated ketones
5	1535 - 1500	Aromatic rings. amide II vibration
6	1495 - 1470	N-H stretching of proteic amides
7	1470 - 1440	Aliphathic C-H bending
8	1413 - 1333	OH deformation and C-O stretching of phenolic groups
9	1280 - 1200	C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers and phenols
10	1190 - 1127	C-OH stretching of aliphatic, alcoholic O-H
11	1116 - 1050	Secondary alcohols
12	1080 - 1030	C-O stretching of polysaccharides

The amount of C and N in the lightest fraction of SOM often depends more on the vegetation/climate effects than on soil properties, since this fraction has less interaction with minerals. In our toposequence, the highest sites showed the highest amount of C and N in the lightest fraction, probably due to an accumulation of pine litter, which is notoriously a poor quality litter. At the mid site at 1090 m asl the lightest fraction had the lowest amount of C and N, probably due to better environmental conditions for decomposers. Finally, at the lowest elevations, where drought becomes a limiting factor for microorganisms, C and N amount in the lightest fraction increases again.

Figure 12. C and N content distribution among soil density fractions (0 – 30 cm).

DRIFT analysis

As inferred by DRIFT analysis, aliphatic C-H stretching (aliphatic methyl and methylene groups), C-O stretching and OH deformation of COOH, C-O stretching of aryl ethers and phenols and C-OH stretching of aliphatic, alcoholic O-H, appeared to be the dominant groups in the bulk samples from all sites (Table 5). Only small differences seemed to occur with respect to the relative proportion of functional groups.

Also the 'twin' sites 4 and 6 showed only some minor differences: in particular site 4 had less intense signals related to phenolic and aliphatic components and an apparently higher contribution from carboxylic groups than site 6 (Table 5).

The C-OH stretching of aliphatic, alcoholic O-H showed a marked difference in intensity between the more elevated sites, 1 and 2, and the others, especially in the top 10 cm of soil. This difference could be caused by the change in the vegetation type, *i.e.* to the transition from conifer stands to maquis/oak tree vegetation (Table 5).

The relative proportion of methyl and methylene groups in the top soil layer increased with altitude (Fig. 4). Since Simkovic et al. (2008) reported about a decrease in intensity of the FTIR peaks of

Table 5. Distribution (relative proportion) of IR bands with the corresponding absorption bands of bulk soil from either depth (0 – 10 cm and 10 – 30 cm) (see also Table 4).

Soil depth cm / (site) and altitude m asl	1	2	3	4	5	6	7	8	9	10	11	12
0 – 10 cm												
(Site 1) 1772	37.7	0.2	0.8	0.1	1.3	0.2	1.1	2.1	37.1	17.0	1.6	0.7
(Site 2) 1515	34.0	0.0	0.7	0.0	0.7	0.2	0.7	1.8	42.1	17.0	2.2	0.5
(Site 3) 1090	32.8	0.2	1.7	0.2	0.4	0.2	1.7	3.5	23.0	32.8	1.6	1.9
(Site 6) 998	34.0	0.1	1.9	0.4	1.0	0.1	1.7	4.2	17.6	35.0	1.5	2.5
(Site 4) 866	28.3	0.3	0.2	0.4	0.4	0.5	1.4	2.8	34.5	27.8	2.7	0.5
(Site 5) 551	25.5	0.0	0.8	0.1	0.6	0.4	1.3	3.3	40.2	24.2	3.2	0.3
10 – 30 cm												
(Site 1) 1772	25.0	0.1	0.3	0.1	0.4	0.3	0.6	0.7	52.6	17.7	2.0	0.3
(Site 2) 1515	25.3	0.4	2.7	0.2	0.2	0.8	0.6	7.8	33.7	24.0	2.0	2.3
(Site 3) 1090	21.9	0.2	0.6	0.1	0.1	0.1	1.2	2.2	44.1	26.0	2.8	0.7
(Site 6) 998	27.9	0.1	1.7	0.3	1.2	0.4	1.8	4.2	24.9	32.4	3.5	1.6
(Site 4) 866	26.5	0.4	1.1	0.1	0.6	0.3	1.5	3.5	34.0	30.5	0.9	0.5
(Site 5) 551	26.7	0.0	0.1	0.5	0.5	0.3	1.5	4.2	35.1	27.3	3.3	0.5

Table 6. Relative distribution (mean and the standard deviation) of the functional groups of organic matter, with the corresponding absorption bands, for soil fractions from either depth (0 – 10 cm and 10 – 30 cm).

| Soil depth cm / (absorption band)	< 1.0 g cm^{-3}	$1.0 - 1.6$ g cm^{-3}	$1.6 - 2.0$ g cm^{-3}	> 2.0 g cm^{-3}
0 – 10 cm				
Aromatic component (4)	0.4 (±0.2)	0.4 (±0.5)	0.2 (±0.1)	0.4 (±0.3)
Proteins and peptides (6)	0.3 (±0.2)	0.4 (±0.3)	0.4 (±0.1)	0.9 (±0.6)
Aliphatic component (7)	4.8 (±1.4)	6.3 (±1.0)	5.4 (±0.9)	2.9 (±1.4)
Phenolic groups (8)	9.5 (±2.3)	11.6 (±1.0)	11.0 (±2.2)	2.3 (±0.8)
Aliphatic alcoholic (10)	70.8 (±6.1)	65.7 (±4.3)	73.2 (±4.1)	80.7 (±2.5)
Polysaccharides (12)	5.6 (±2.6)	7.1 (±2.0)	5.0 (±1.1)	2.3 (±1.1)
10 – 30 cm				
Aromatic component (4)	0.6 (±0.3)	0.1 (±0.1)	0.2 (±0.2)	0.4 (±0.4)
Proteins and peptides (6)	0.3 (±0.2)	0.7 (±0.2)	0.3 (±0.1)	0.6 (±0.3)
Aliphatic component (7)	3.5 (±0.9)	5.2 (±0.9)	4.6 (±0.7)	2.3 (±1.5)
Phenolic groups (8)	9.2 (±2.8)	11.4 (±1.4)	12.2 (±1.4)	2.5 (±1.3)
Aliphatic alcoholic (10)	70.5 (±5.8)	67.6 (±5.7)	75.2 (±3.7)	79.1 (±2.2)
Polysaccharides (12)	4.2 (±1.6)	6.2 (±3.4)	3.2 (±1.6)	2.2 (±0.6)

122
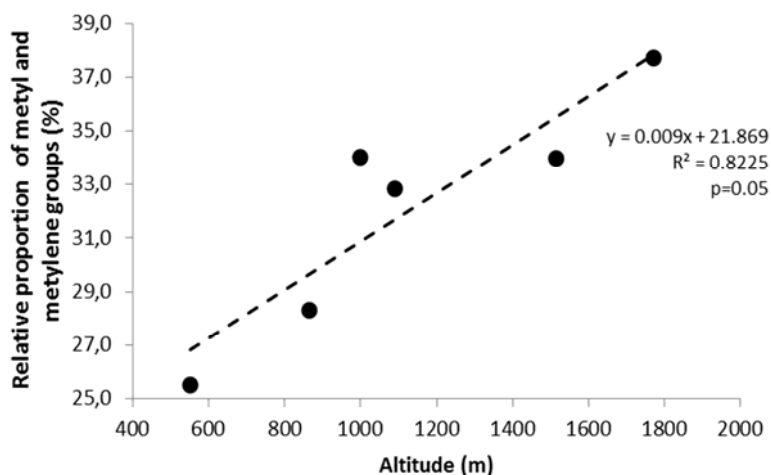
Figure 13. Relationship between the altitude and the relative proportion of methyl and methylene groups. The regression curve is given for the depth 0 – 10 cm.

these functional groups after heating a soil sample, our finding could be an additional evidence that fire frequency at Mt. Etna was historically highest with decreasing elevations.

The hydrophobic character of a soil is essentially due to the methyl, methylene and methine moieties present in aliphatic and aromatic (olephinic) groups (Capriel et al., 1995: Capriel, 1997; McKissock et al., 2003). Ellerbrock et al. (2005) hence proposed a hydrophobicity index, the A/B ratio, where A represents the FTIR band of hydrophobic CHs of methyl and methylene groups (2980 - 2880 cm^{-1}), and B the bands of hydrophilic C=O functional groups, which in our spectra were at 1725 to 1710 cm^{-1} and at 1660 to 1600 cm^{-1} (Table 4). We did not find any trend of the A/B ratio with the altitude (Fig. 5), neither in the topsoil nor in the subsoil.
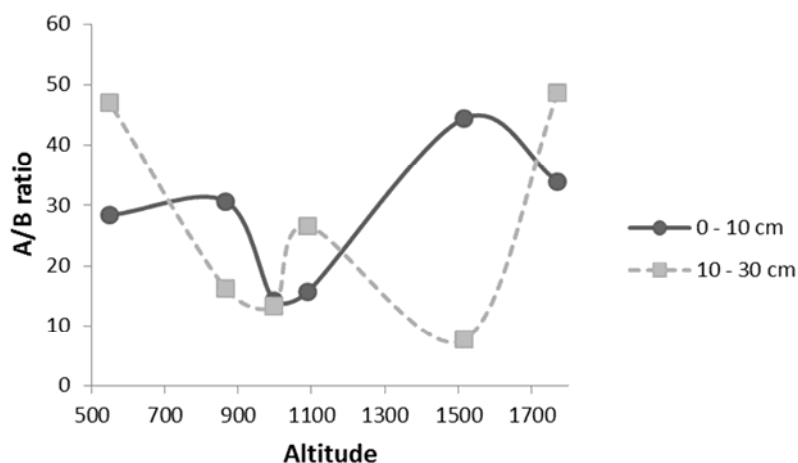
Figure 14. Relation between altitude and the A/B ratio (hydrophobic groups/hydrophilic groups) in the DRIFT spectra of bulk soils for soil depths 0-10 cm and 10-30 cm.

With respect to the soil fractions (Table 6), aliphatic and phenolic compounds, revealed by the peaks at 1470 - 1440 cm^{-1} and 1413 – 1333 cm^{-1} respectively, which are supposed to be mainly related to lignin, they decreased with increasing density, with the exception of the fraction <1.0 g

124

cm^{-3}. Phenolic moieties and polysaccharides, region 1413 – 1333 cm^{-1} and 1080 – 1030 cm^{-1} respectively, showed the largest difference between the light fractions and the heaviest one. Peptides and proteins appeared to increase with increasing density fraction. In the top soil, they were concentrated in the heaviest fraction, probably due to the association with minerals that tends to protect these labile compounds from degradation (Table 6). In the deeper layer, the highest abundance for proteic compounds, however, were found in the 1.0 – 1.6 g cm^{-3} fraction, possibly indicating here a larger concentration of fire derived compounds that integrate in their structures thermally altered N components (Knicker, 2007). The 1.0 – 1.6 g cm^{-3} fraction, in fact, is supposed to segregate fire-derived compounds.

In contrast to expectance, the relative intensity of the DRIFT peaks indicative for aromatic carbon, mostly showed a similar concentration for site 4 and 6, both in the bulk soil and the density fractions. The aromatic components seemed to be even more abundant at site 4 in the 1.0 – 1.6 g cm^{-3} fraction. However, as suggested by Knicker (2007), multiple burning does not necessarily lead to an increase in the degree of SOM aromaticity. The absence of this relationship is due to the fact that the new shrub and herb cover developed after a fire provides an available fuel which could burn completely leaving little char. Moreover, also the previously accumulated charcoal could be oxidised.

^{13}C CP/MAS NMR spectroscopy

Figure 6 shows the NMR spectra and Table 7 shows their intensity distribution for all soil fractions except the >2 g cm^{-3} one, which did not produce any significant signal because of its too low C content, less than 1% in weight after the HF treatment. Only four chemical shift regions were considered in Table 7 since aldehydes and ketones, when present, were negligible.

In general, only a rather small difference in the NMR spectra between the density fractions was detectable, hence indicating a more or less similar SOM composition (Table 7). Baldock et al. (1992) obtained similar results when comparing NMR spectra from different particle size fractions from an Australian Andosol. They explained this behaviour by a rapid decay of fresh organic residues in Andosols that undergo a very slow decomposition once they are protected from microbial attack by the formation of stable organic-aluminium complexes, so minimising differences in SOM chemical structure. A similar process may be hypothesised for the Etna soils.

Clearer differences in chemical composition occur between the soil density fractions for the samples from the lowest sites. There were particularly strong signals in the ranges 60 – 90 and 90 – 110 ppm in the <1.0 g cm^{-3} fraction (Table 7). These are typical of carbohydrate-derived structures and the anomeric carbon of carbohydrates and syringyl units of lignin, respectively (Knicker et al.,

1996). The 1.0 – 1.6 g cm^{-3} fraction showed a less intense signal in the O-alkyl C region and a more intense peak in the aromatic C region compared to the other fractions. This fraction seems to contain more PyOC compared to the other fractions, as shown by the occurrence of major signal at around 130 ppm that belongs to aromatic C (Skjemstad et al., 1996). The 1.6 – 2.0 g cm^{-3} fraction finally showed a pronounced, sharp peak in the alkyl C region, indicating the presence of waxes, cutin, suberin and other lipids (Table 7).

Figure 15. ^{13}C CPMAS spectra of density fractions from composite samples from the topsoil (0–10 cm) of the two highest sites (1+2; below) and the two lowest ones (4+6; above).
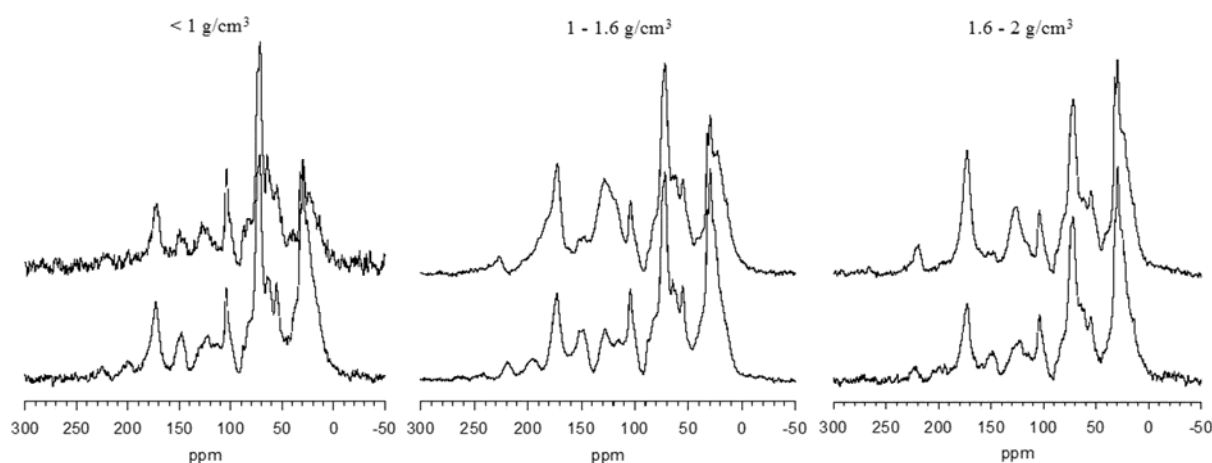
Table 7. Relative distribution (%)[a] of the chemical shift regions of ^{13}C CPMAS NMR spectra of the topsoil (0 – 10 cm). The contribution of aldehydes and ketones (chemical shift region 185-220) is not reported because negligible.

Site/ density fraction	Relative distribution (%) of chemical shift region (ppm)						
	Alkyl C	O-Alkyl C			Aryl C		Carboxyl C carboxyl C/amide/ester
		Methoxyl/N-alkyl	O-alkyl	Di-O-alkyl	C substituted (aromatic C-H and C-C)	O substituted	
	0 – 45	45-60	60-90	90-110	110-140	140-165	165-185
Highest sites (1+2)							
< 1 g cm^{-3}	34	7	28	5	6	9	11
1 – 1.6 g cm^{-3}	30	6	26	4	7	14	13
1.6 – 2 g cm^{-3}	36	5	26	4	6	9	14
Lowest sites (4+6)							
< 1 g cm^{-3}	24	7	35	8	10	6	10
1 – 1.6 g cm^{-3}	25	5	26	4	15	10	15
1.6 – 2 g cm^{-3}	32	7	25	5	9	3	19

[a] Maximum error ± 4.

When composite samples from the two highest sites are compared to those from the lowest sites, (Fig. 6, Table 7), the latter showed much more intense signal in the aromatic region in $1.0 - 1.6$ g cm^{-3} fraction, suggesting an accumulation of charred material. Moreover, the samples from the highest sites have a stronger alkyl C signal in the lightest fraction (<1.0 g cm^{-3}) and a higher contribution of O-substituted aryl C - mainly related to lignin phenols - in all the fractions. These results were probably related more to the different types of vegetation (coniferous stand versus maquis/oak tree) than to fire. However, a reduction of the chain length of alkyl compounds and, hence, a subsequent loss of intensity in the alkyl region is a common occurrence in burned areas (González-Peréz et al., 2004).

SEM observations

Scanning electron microscopy (SEM) showed that the density fractions display different morphologies (Fig. 7).
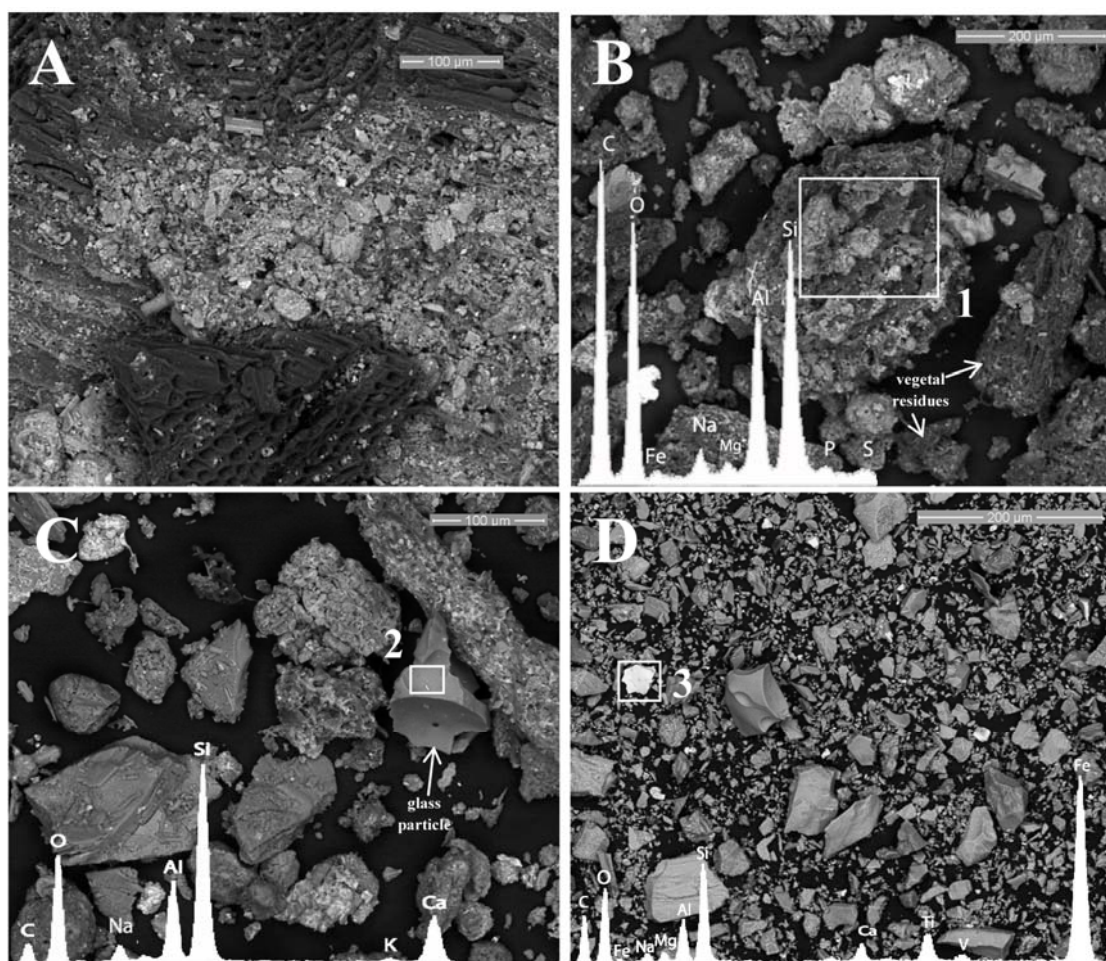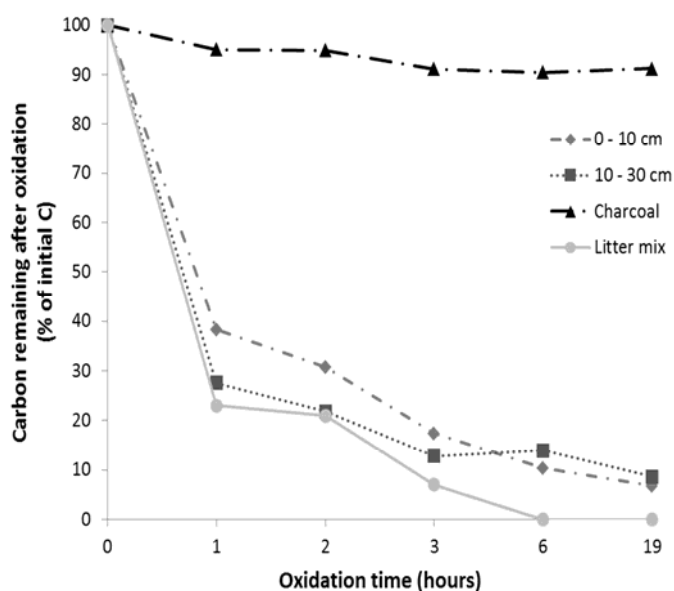

Figure 16. SEM images of the four separated fractions and, in white on the low side, EDX spectrograms of the areas marked off by squares. A) charcoal from the < 1 g cm^{-3} fraction, in which structure cells are clearly visible, B) particles of the 1.0-1.6 g cm^{-3} fraction; insert 1 shows an organo-mineral aggregate , C) the 1.6–2.0 g cm^{-3} fraction; inset 2 shows a glass particle, D) the >2 g cm^{-3} fraction: mineral particles are clearly dominant; inset 3 shows a possible iron (hydr)oxide.

The lightest fraction mostly included undecomposed and slightly decomposed OM whose structure was often still recognisable. Also, a few charcoal fragments were detectable in this fraction. In the fractions $1.0 – 1.6$ g cm^{-3} and $1.6 – 2.0$ g cm^{-3}, many aggregates have an organo-mineral character that undoubtedly helps to protect the organic component from biogenic decay (Christensen, 1992). Finally, the > 2.0 g cm^{-3} fraction was dominated by mineral particles while the organic material was hardly visible.

Pyrogenic organic carbon (PyOC)

Dichromate oxidation as a method to quantify PyOC in soil was originally proposed by Bird and Gröcke (1997). This method exploits the chemically refractory nature of pyrogenic materials, which mostly comprises graphite-like polycondensed aromatic domains (Knicker et al., 2007). However, as Hammes et al. (2007) underlined, this interpretation is based on the erroneous assumption that all of the carbon that survives the treatment is PyOC. On the other hand, part of PyOC is oxidised (Knicker et al., 2007; Rumpel et al., 2006b), while the residual C maintained a marked lipid nature, as demonstrated by Certini et al. (2007) and Knicker et al. (2007, 2008b). In view of these findings, we submitted the organic matter residual from dichromate oxidation, the chemical oxidation resistant carbon (COREC), to NMR analysis for assessing its composition. Another limitation to the use of acid dichromate for PyOC estimation is that the oxidation treatment, with its long ongoing oxidation process, is able to oxidase also part of aromatic carbon (Rumpel et al., 2006b; Knicker et al., 2007). To establish the optimum oxidation time for PyOM isolation, we performed the following experiment. We used a different oxidation time (1, 2, 3, 6, and 19 h) on the following samples: two soil samples (previously treated with HF) were obtained by mixing the same amount of soil from all sites at both depths, $0 – 10$ cm and $10 – 30$ cm. In addition, a mix of ground litter layer from all sites, and ground charcoal particles hand-picked during the sieving procedure (Fig. 8) were used.

Figure 17. Percentage of the initial OC recovered after dichromate chemical oxidation treatment of soil samples from two depths, litter material and ground charcoal particles.

Table 8. Organic C recovered after the HF treatment, content of COREC (chemical oxidation resistant carbon) and C/N of the material left after dichromate oxidation

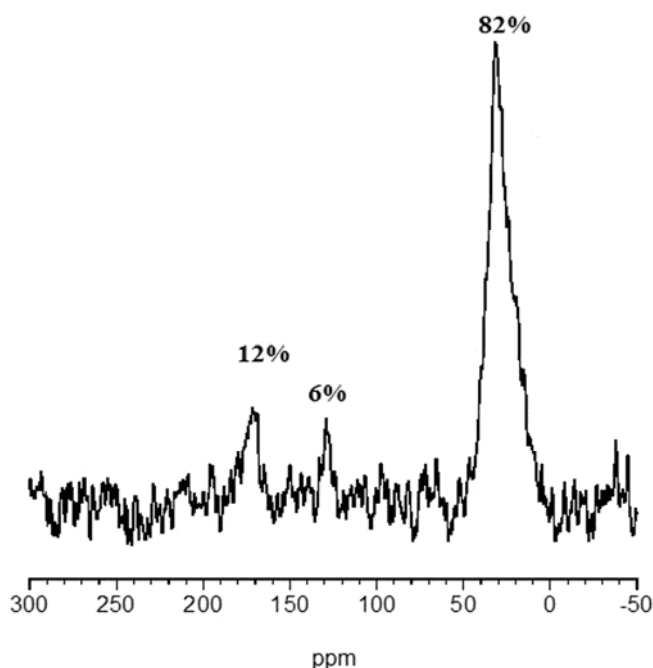| Altitude m asl	Soil depth cm	OC recover after HF % of initial C	COREC % of initial C	C/N
1772	0 - 10	68	9	20
(Site 1)	10 - 30	55	13	37
1515	0 - 10	49	0	n.d.
(Site 2)	10 - 30	86	35	n.d.
1090	0 - 10	49	2	19
(Site 3)	10 - 30	58	17	16
998	0 - 10	57	17	17
(Site 6)	10 - 30	87	4	19
866	0 - 10	62	14	13
(Site 4)	10 - 30	58	27	15
551	0 - 10	63	17	14
(Site 5)	10 - 30	86	14	14

Figure 18. Concentrations of C (A) and N (B) in the organic matter resistant to dichromate oxidation and C and N stocks (C and D) along the toposequence.
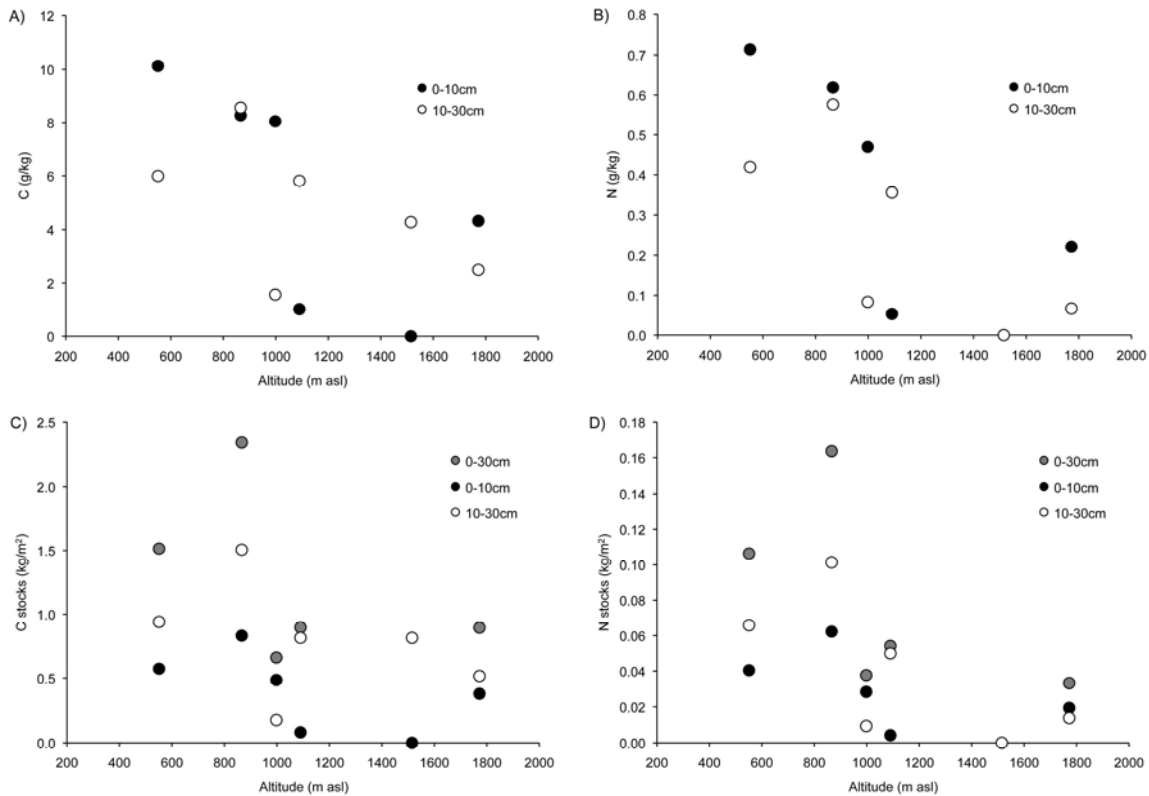
We found that within 3 to 6 h the litter was totally oxidised, while the residual carbon of the soil samples amounted to 10 to 14%. Finally, the carbon of the finely ground charcoal that resisted to the treatment amounted to 91% of the original amount after 6 h and 19 h. Following these results, we chose to apply an oxidation time of 6 h.

Considering both the bulk soil and density fractions, the COREC fraction ranged between 0 and 35% of total OC (Table 8). The total C and N stocks and concentrations of this fraction increased with decreasing altitude (Fig. 9). This would again support the hypothesis of an increasing influence of fire on SOM properties at low elevations. However, the NMR analysis revealed that the COREC had a prevailing lipidic nature, the signal being mostly confined to the $0 - 30$ chemical shift region (Fig. 10); in addition, the C/N ratio of the COREC was similar to the one of the bulk soil (Tables 2 and 8), whereas PyOC should have a high ratio, showing an aromatic nature. Dichromate oxidation resulted not to be much selective for PyOC, hence not really reliable to quantify this latter fraction, at least in our case. In this regard, Knicker et al. (2007) reported that in soil samples with a contribution of aromatic C of less than 20% to total C_{org}, aromatic component of COREC was hardly measurable. Actually, this could be the case for our samples.

The NMR investigation revealed that lipids are resistant to dichromate oxidation, at least at the condition we applied. In these soils, lipids seem to be even more resistant to oxidation than aromatic compounds. Knicker et al. (2007) advanced the idea that lipid structures are protected from the acid treatment due to their hydrophobic nature and that, in general, higher lipid contributions and longer lipid chains enhance the resistance of lipids to oxidation. We share this explanation for our samples. In fact, our soils showed a relatively high abundance of lipids in all the bulk samples and density fractions. Furthermore, the accumulation of the lipid rich COREC fraction at lower altitude, can be explained by the longer lipid chains content of the aliphatic structures present in the bushy xerophilous vegetation typical of Mediterranean areas.

Figure 19. ¹³C CPMAS spectrum of COREC in the top 10 cm of bulk soil of site 5. Numbers represent relative distribution of the three major chemical shift regions (from the left: Carboxylic C; Aromatic C; Alkyl C).

Conclusions

In the investigated toposequence on Mt. Etna, it is not possible to discriminate fire as the only ecological factor that determines SOM properties. However, there are clues that show that it plays an important role. Density fractionation helped to distinguish the influence of different environmental factors, fire included, on SOM characteristics. Climate and vegetation seem to strongly determine the amount and the chemical nature of the very light fraction (<1.0 g cm^{-3}). PyOM, on the other hand, prevails in the fraction $1.0 - 1.6$ g cm^{-3}. The highest C and N concentrations in this fraction were measured at the lowest sites, particularly at the site affected by annual fire.

The stocks of COREC followed an increasing trend with lower altitudes coinciding with a higher fire frequency at these altitudes.

Dichromate oxidation to evaluate the PyOC content in soils proved to be not so straightforward as assumed. In fact the NMR analysis of the dichromate oxidation resistant fraction showed that most of the COREC consisted of alkyl components, namely lipids and only a small part can be attributed to aromatic compounds, in particular to charred material. At the two sites having a similar altitude and vegetation but different recent fire history, the general chemical characteristics of OM were not quite distinct. Nonetheless, the site more affected by fire had the highest carbon concentration in the $1.0 - 1.6$ g cm^{-3} fraction, where PyOM is preferentially confined. A higher spectroscopic signal by aromatic compounds, however, was not detectable compared to the site experiencing less fire events.

Despite of the relatively high fire frequency, the content of aromatic compounds was in general rather modest in the studied toposequence on Mt. Etna. Bulk soil and density fractions were instead rich in lipids. Either not much of aromatic compounds, besides macro-charcoal, were produced by fire events or they partially were oxidised by a high fire frequency that in turns could stimulate the production of fresh labile biomass masking PyOC contribution to SOM. The fertilising effect of ash, in fact, enhances the biomass production and hence the litter incorporation into the soil.

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Acknowledgements

During the three years of my PhD, I met many people that somehow contributed to the achievement of this work. I would like to thank all those people, but particularly those mentioned in this page.

Special thanks are due to Giacomo Certini, for his guidance and his friendship, and to Cornelia Rumpel for his scientific support and for allowing me to develop this joint supervision PhD thesis, spending a very fruitful period at BIOEMCO lab.

A special thank goes to Ornella Francioso for giving me always good advices, for her help and great availability. Thanks also to the "Bologna team", particularly Michele Di Foggia, Sergio Bonora, Paola Nipoti and Antonio Prodi, for all the analysis, fruitful discussions, but particularly for making me feel at home.

Sincere thanks go especially to Markus Egli for the opportunity that gave to me in the "Etna project" and for supervising my stay at Zurich University. The "training period" in soil science that I had there was a milestone in my PhD. Thanks also to Paolo Cherubini for hosting me in his lab at WSL.

I would like to thank Prof. FIorenzo Ugolini for all the suggestions and for revising the English in the manuscripts.

Thanks to Stefano Salvatore for the SEM pictures, Bernardo Maestrini his support, often beyond the research, Remigio Paradelo for all the interesting discussions and for the help with the French, Gregoire Cousin for the help with French translations and bureaucracy, Karen Baumann for the help in the lab.

Thanks to Paola Arfaioli, Bruno Kaegi, Luigi D'Acqui, Tommaso Chiti, Claudia Forte, Andrea Rigaud, Gerard Bardoux, Valerie Puteau, Cyril Girardin, Ottorino Pantani, Daniel Billiou, Stefan Doerr, Ruedi Sailer, Emiliano Sorzoni, Michael Ploetze for supporting me during analysis/sampling.

Thanks to Paola Ricci, Sylvie Faveau, Luca Calamai, Davide Francioli for the help with the bureaucratic stuff.

Thanks also to all the people from the soil science section of the DISPAA of university of Florence, from the BIOEMCO in Grignon and from the Soil Sciences and Biogeography group at Zurich University for the good company, any-kind-of-break, discussions, advices, English improvement and for helping me to make the office a good place where spending time beyond the research.

Last, but not least, a special thanks to my parents, my brothers and sister for being always present and for supporting me, always. And thanks to Giulia for her support to my work and to my life.