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Legacy effects of former charcoal kiln sites on the forest vegetation of a Mediterranean area

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Legacy effects of former charcoal kiln sites on the forest vegetation of a Mediterranean area

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Riassunto

Parole chiave: carbone da legna, carbonaia, condizioni edafiche, crescita degli alberi, composizione floristica, diversità della vegetazione, effetti ecologici, paesaggio forestale, rinnovazione arborea.

Obiettivo: Analizzare gli effetti a lungo termine determinati dalla secolare attività di produzione del carbone sull'ecologia ed il paesaggio forestale in area Mediterranea. Lo studio è articolato in modo da perseguire tre obiettivi principali: i) valutare l'impatto delle carbonaie sulla vegetazione arborea ed erbaceo-arbustiva in relazione a fattori abiotici; ii) esaminare gli effetti del suolo sulla crescita e lo sviluppo di specie arboree in giovane età; iii) quantificare e caratterizzare l'"eredità" lasciata da questa attività a livello di paesaggio forestale, attraverso un'analisi della distribuzione e della morfologia dei siti di produzione in zone differenti dell'Italia centrale.

Metodi e risultati: La ricerca è stata effettuata nei tre tipi di bosco storicamente più utilizzati per la produzione del carbone: foreste di sclerofille, querceti termofili decidui e faggete. In un primo studio di tipo *esplorativo* è stata esaminata la rinnovazione arborea e la vegetazione erbaceo-arbustiva su un campione di 61 carbonaie abbandonate da almeno 60 anni, in cui sono state anche analizzate le principali caratteristiche del suolo e la disponibilità di luce. Contemporaneamente si è condotta un'analisi *sperimentale* tramite la realizzazione di un "common garden" in cui sono state seguite la germinazione, la crescita, e la mortalità di tre specie forestali dominanti (*Quercus ilex, Q. cerris, Fagus sylvatica)* allevate su suolo di carbonaia. Infine, tramite censimento a terra e telerilevamento da dati LIDAR, sono state identificate e caratterizzate tutte le carbonaie presenti in aree campione dei tre tipi forestali (approccio *inventariale*). Dai primi due studi è emerso un chiaro effetto negativo delle carbonaie sull'affermazione della rinnovazione arborea, mentre è risultata positivamente influenzata la vegetazione del sottobosco in termini di diversità specifica, composizione e produttività. Tali effetti sono legati a variazioni delle caratteristiche del suolo e di disponibilità di luce. L'esperimento di "common garden" ha dimostrato che le risposte delle tre specie arboree al suolo di carbonaia sono diverse, in parte contrastanti o deboli. Infine, dai rilievi a terra e dalla foto-interpretazione delle immagini telerilevate, è risultata, seppur con alcune differenze tra i tre tipi di bosco, una più alta densità di carbonaie ed una loro differente morfologia rispetto ad altri paesi europei, anche a livello di profilo del suolo, in relazione alla diversa metodologia ed ai diversi scopi per cui venivano realizzate.

Conclusioni: Le carbonaie costituiscono una delle più evidenti eredità lasciate dall'attività umana nei boschi mediterranei ed esercitano ancora effetti significativi sul sistema suolo-vegetazione, in precedenza sconosciuti. Esse rappresentano quindi delle "micro-isole" ecologiche di origina antropica, fino ad oggi non individuate come tali, che aumentano

l’eterogeneità stazionale e la biodiversità dell’ambiente forestale. A livello di paesaggio forestale la magnitudine di tali effetti è significativa a causa dell’elevato numero di tali siti e della superficie complessiva da loro occupata, con piccole differenze fra gli ecosistemi forestali analizzati.

Significato e impatto dello studio: Il valore dell’eredità culturale, paesaggistica ed ecologica lasciata da una delle più antiche forme di uso del bosco merita di essere conservato. La gestione forestale, specialmente nelle aree protette, dovrebbe considerare questo aspetto, attualmente trascurato. Infine, il presente studio suggerisce un contributo significativo di questi siti alla capacità di stoccaggio di carbonio nel suolo forestale, un aspetto che merita ulteriori ricerche.

Articoli inerenti l’argomento di Tesi:

1. Carrari E., Ampoorter E., Verheyen K., Coppi A., Selvi F. Lack of recolonization by woody species in former charcoal kilns in Mediterranean forests. Sottomesso a: *iForest.* (PAPER I; pp. 40 - 59).
2. Carrari E., Ampoorter E., Verheyen K., Coppi A., Selvi F. Former charcoal sites serve as microhabitats for the conservation of understory vegetation diversity and enhanced productivity in Mediterranean forests. Manoscritto accettato: *Applied Vegetation Science*. (PAPER II; pp. 60 - 81).
3. Carrari E., Ampoorter E., Bussotti F., Coppi A., Garcia Nogales A., Pollastrini M., Selvi F. Effects of charcoal site soil on germination, growth and mortality of forest trees: results of a two-year common garden experiment Manoscritto in preparazione. (PAPER III; pp. 82 - 98).
4. Carrari E., Ampoorter E., Bottalico F., Chirici G., Coppi A., Travaglini D., Verheyen K., Selvi F. The old charcoal kiln sites in Mediterranean forest landscapes of Central Italy. Da sottomettere a: *Quaternary International*. (PAPER IV; pp. 99 - 122).

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5. Carrari E., Ampoorter E., Coppi A., Selvi F. (2015). Diversity of secondary woody species in relation to species richness and cover of dominant trees in thermophilous deciduous forests. *Scandinavian Journal of Forest Research.* DOI: 10.1080/02827581.2015.1081981.
6. Ampoorter E., Coppi A., Selvi F., Carrari E., Auge H., Baeten L., Fotelli M., Radoglou K., Verheyen K. Overstorey-understorey relations and their driving mechanisms in mature European forest types. Manoscritto accettato: *Perspectives in Plant Ecology, Evolution and Systematics.*

7. Selvi F., Coppi A., Carrari E. Impact of pine invasion on the taxonomic and phylogenetic diversity of a relict Mediterranean forest ecosystem. Sottomesso a: *Forest Ecology and Management*.
8. Carrari, E., Capretti, P., Santini, A., & Luchi, N. (2015*). Hymenoscyphus fraxineus* mycelial growth on media containing leaf extracts of different Oleaceae*. Forest Pathology*. DOI: 10.1111/efp.12238.

Abstract

Keywords: charcoal hearths, charcoal kilns, forest landscapes, legacy effects, forest recolonization, soil conditions, tree growth, species composition, vegetation diversity, wood charcoal.

Aims: Analysing the long-term effects of the centennial activity of charcoal production on forest ecosystems of the Mediterranean area. The study is structured in order to achieve three main aims: i) evaluating the impact of kiln sites on the forest vegetation (tree, shrubs and herbaceous species) in relation to abiotic factors; ii) examine the effects of the charcoal-enriched kiln site soil on the early life stages of major forest trees; iii) quantifying and characterizing the legacy of such activity at the landscape level, through the analysis of the distribution and morphology of production sites in different environments of central Italy .

Methods and Results: The research was carried out in three forest types traditionally exploited for wood charcoal production, sclerophyllous maquis, mixed oak forest and beech forest. In a first *exploratory* study, we examined tree regeneration and understorey vegetation on a sample of 61 kiln sites, abandoned at least 60 years ago, together with the main soil characteristics and light conditions. At the same time, an *experimental* work was performed by setting up a common garden to compare germination, growth and mortality in three major forest trees (*Quercus ilex*, *Q. cerris*, *Fagus sylvatica*) grown on soil of kiln sites and control sites. Finally, an *inventory* work was carried out in sample quadrats using field surveys and LIDAR data, to determine the density, size, surface and other morphological parameters of kiln sites in the three forest types. In the first two studies we found a negative effect of kiln sites on tree regeneration and forest recolonization, whereas the understorey vegetation was positively influenced in terms of species diversity, compositional variations and biomass production. These effects are related to variations in the characteristics of soil and light, also influenced by the kiln sites. The common garden experiment showed that the responses of forest trees to kiln site soil are different, in some cases contrasting, or weak. The inventory study showed that, compared to other European countries, kiln sites are denser but smaller and with different morphology, also in terms of soil profile, with some differences between forest types. Such differences are probably due to the different methods of preparation and the different purposes for which they were made.

Conclusions: Charcoal kiln sites are one of the most striking legacies left by the millennial human activity in the Mediterranean woodlands. This study shows that such sites have persistent effects on the vegetation via changes in soil and light conditions. Hence, they represent ecological “micro-islands” of anthropic origin that increase the diversity and fine-scale heterogeneity of the forest ecosystem. The relatively high number of these

sites and their total area per unit surface suggest that the magnitude of their effects at the forest level may not be negligible.

Significance and Impact of the Study: The significance of the cultural, landscape and ecological heritage of one of the oldest forms of forest use deserves some form of protection. Hence, forest management, especially in protected areas, should consider this aspect which is currently neglected. Finally, the present study suggests a significant contribution of these sites to the storage capacity of carbon in forest soils, an aspect that should be further investigated**.**

Papers concerning the topics of the Thesis.

1. Carrari E., Ampoorter E., Verheyen K., Coppi A., Selvi F. Lack of recolonization by woody species in former charcoal kilns in Mediterranean forests. Submitted to: *iForest.* (PAPER I; pp. 40 - 59)
2. Carrari E., Ampoorter E., Verheyen K., Coppi A., Selvi F. Former charcoal sites serve as microhabitats for the conservation of understory vegetation diversity and enhanced productivity in Mediterranean forests. Accepted ms: *Applied Vegetation Science* (PAPER II; pp. 60 - 81).
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4. Carrari E., Ampoorter E., Bottalico F., Chirici G., Coppi A., Travaglini D., Verheyen K., Selvi F. The old charcoal kiln sites in Mediterranean forest landscapes of Central Italy. To be submitted to: *Quaternary International* (PAPER IV; pp. 99 - 122).

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1. Introduction: Charcoal kiln sites in the Mediterranean forests

1.1 Legacy effects of past land uses on forests

In the last decades, an increasing number of studies have highlighted the impact of historical human activities on forest ecosystems, especially coppicing, wildfires, controlled burning, livestock husbandry grazing and plant domestication. In central and northern Europe, it is well documented that these traditional land uses have shaped the natural ecosystems since the times of the first civilizations and still affect present-day soil properties (Baeten et al., 2010; Dupouey et al., 2002; Glatzel, 1991; Verheyen et al., 1999). Via their influence on soil characteristics, past land use forms have also strongly affected plant species diversity, composition and productivity of woodlands, especially in Mediterranean region (Arianoutsou, 2001; Bartha et al., 2008; Blondel, 2006; Kopecký et al., 2013; Lloret and Vilà, 2003; Nocentini and Coll, 2013). Here, the succession of civilizations that waxed and waned in the different countries over several millennia determined the greatest impacts on biota and ecosystems (Blondel, 2006). However, similar evidence exists for North America, despite the shorter duration of intensive human utilization of woodlands (Foster, 1992; Fraterrigo et al., 2005).

Hence, when trying to understand the drivers of the present-day structural, compositional and ecological features of woodlands communities it is of crucial importance to consider the long-lasting consequences of the past land uses (e.g. Hermy and Verheyen, 2007; Peterken et al., 2014). Nevertheless, such effects are still incompletely known due to their complex nature and the limited number of studies focusing on this topic.

One of the most important and widespread uses of the forests in the past was the production of wood charcoal, whose effects are the subject of the present investigation.

1.2 Wood charcoal: a long history of use

Charcoal is the first synthetic material produced by man, as shown by the artworks of ca. 38.000 years ago found in some caves of southern France (Antal, 2003) and one of the main source of energy since the Iron age.

During the past millennia charcoal was produced for a wide variety of purposes such as domestic use, industrial processes, and as a heat source for the production of specialized materials for agricultural and other human needs. This wide range of uses is described in a diversity of scientific journals, which explains a certain difficulty in being aware of the complete literature on this topic (Scott and Damblon, 2010).

Wood charcoal production is one of the oldest forms of forest use, that existed since the Neolithic and continued for millennia (Ludemann, 2010; Montanari et al., 2000). This is documented in Egypt and in the Near East from the third millennium BC (Lugli and Pracchia, 1995), and in the whole Mediterranean region since the Iron Age (Blondel, 2006). Archaeological remains show that the Etruscans produced wood charcoal for the smelting of iron and bronze in southern Tuscany (Mariotti Lippi et al., 2000). In the fourth century BC Theophrastus describes the carbonization method and Plinius the Elder mentions the plant species suitable for the different uses of charcoal and the products of charcoal burning (Montanari et al., 2000). Thanks to Plinius, we know that the same method of charcoal production was in use until the middle of the last century (Baroni et al., 2011).

In more recent times, sporadic historical information can be found in a few documents dating back to the late Middle Age. Technical descriptions of charcoal burning can be found since the 16th century (Montanari et al., 2000). Most of the deforestation which was carried out in the medieval time, especially in the Mediterranean countries, has been commonly ascribed to the increasing need of charcoal caused by the spread of metallurgy. A similar situation occurred probably in China during the Han period (206 BC to AD 220; Montanari et al., 2000). In the Modern age, documents on charcoal production are also mainly related to costs and problems of the iron and steel industry (Arrigoni et al., 1985).

In most of the northern and central European countries, this practice was abandoned in the 19th century due to the rapidly increasing and widespread use of coal (Deforce et al., 2012). Contrastingly, in many Mediterranean regions the importance of wood charcoal production increased during the industrial revolution because of the lack of other fuel sources. In most parts of central Italy, as for example in southern Tuscany, the metallurgic activity was continued with the same methodology from 1377 to the end of the 19th century (Arrigoni et al., 1985). Since the Etruscan period, this area has been of utmost importance for the production of metals thanks to the iron mines of the Elba Island, to their proximity to the sea, and the presence of vast forested areas in the Tuscan Maremma. These were an inexhaustible source of all types of wood needed for the metallurgic production by the Etruscans. Different types of wood charcoal were in fact required for this activity: that made from oak wood (*Quercus* sp.), named “legno forte”, was used for the furnaces, while that made from the wood of *Populus* sp., *Salix* sp. and *Alnus* sp., named “legno dolce” was used for the iron foundries (Arrigoni et al., 1985; Giorgerini, 2009).

When in 1709 Abraham Darby 1° used for the first time in England a furnace with 'charked' coal (carbon coque) instead of lignite, in Italy there was a sort of “environmental issue” about the over-exploitation of forests for the production of wood charcoal. Prominent academics such as Giorgio Santi and Giovanni Targioni-Tozzetti expressed their concern about the deforestation of Maremma, pointing out that charcoal production was a limit for the development of agriculture and other types of use of forests (Arrigoni et al., 1985). The first furnace fueled with lignite was built in Italy in 1878 for the industry of Piombino (a major metallurgical centre on the coast of Tuscany; Iacoviello and Cavallini, 2013), and since that year most of the other Italian industries started to use this fossil fuel, then replaced by carbon coque.

However, production of wood charcoal was such a deeply rooted practice in central Italy that it could not quickly disappear. In fact, it remained an important economic activity for many people involved in the trade of this material, which continued to be the major source of energy for heating and cooking, as well as for the production of high quality steel in small blast furnaces until the years 1950 and 1960 (S.I.L.T.E.M., 1946).

1.3 The production of wood charcoal

The charcoaling process can be divided in three phases: drying phase (removal of moisture from the timber charge), pyrolysis phase (thermal decomposition of the wood to form charcoal and various waste products) and cooling phase. All three phases can occur at the same time throughout the charcoaling process, although the first phase happens primarily during the early stages of the process whilst the other two phases occur during the later stages (Powell, 2008). The charcoaling process was realized by different cultures through centuries with different scopes, but with similar methodology based on the building of wood kilns (Photos 1-2; pg. 135). Others methods have been developed but charcoal produced in kilns was always the most appreciated, because it maintains the 95% of fixed carbon (vs the 75% of other techniques), with a calorific power of 6500-7500 Kcal, a very low percentage of ash (less than 3%) and 6-7% of humidity (Giorgerini, 2009).

Kilns were built on small, elliptical or circular terraces with flat surface, previously prepared in the forest (Deforce et al., 2012; Ludemann, 2003; Montanari et al., 2000; Powell, 2008). Different terms are used in the English literature for these sites, such as *charcoal kiln sites*, *kiln platforms. charcoal hearths and charcoal-burning sites;* in this study the two first terms are adopted indifferently.

In central Italy, kiln platforms were usually prepared along footpaths on hill and mountain slopes (Cantiani, 1955). As this was an exhausting and time-consuming practice, charcoal workers mostly used the platforms left from the former cycles of production in a given area; hence the same were used several times, often for centuries (Giorgerini, 2009).

A short description of the traditional process of charcoal kiln building in Tuscany (Giorgerini, 2009), with its Italian terminology, is given below; this method is basically the same of that adopted in other parts of Europe and other continents.

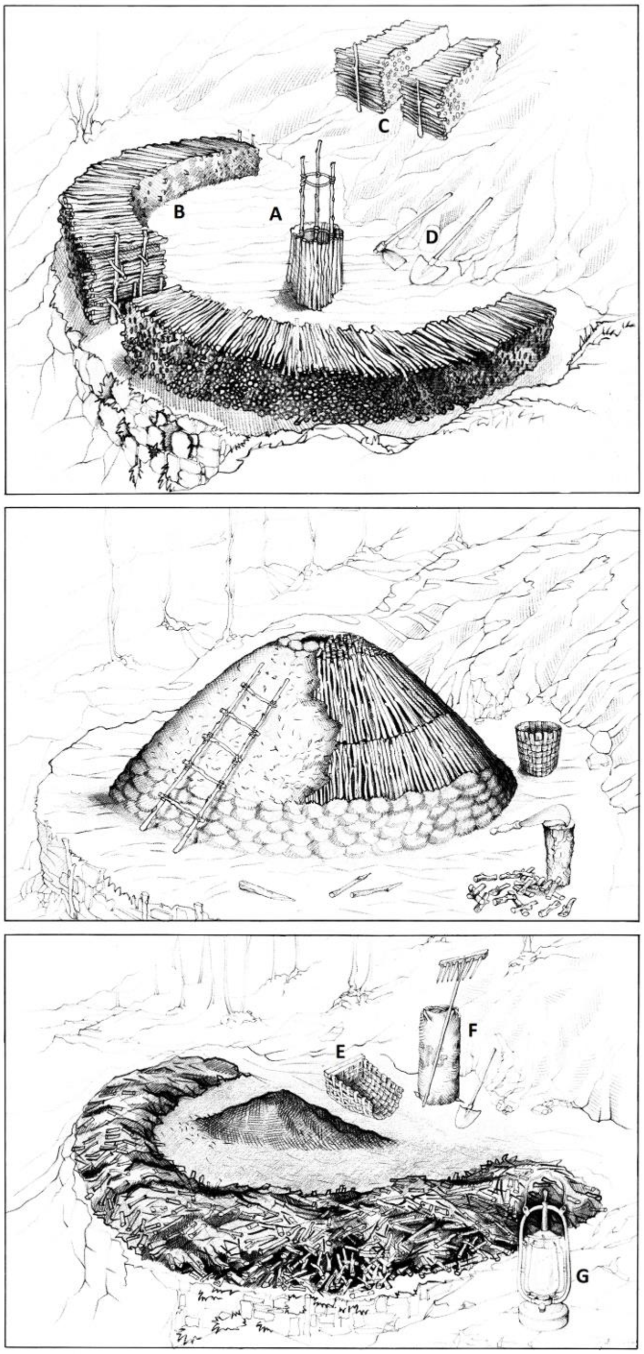

Figure 1. Building process of a wood kiln, showing (from above) the chimney (A), the kiln piled wood (B), the stacked wood (C), the hoe and the shovel (D) the chestnut basket (E), the rake (F) and the ladder (G) (pictures of Sandra Biavati, in Landi et al., 1988).

First, the charcoal worker made a chimney in the middle of the platform ("rocchina" fig. 1a) using small pieces of timber (ca. 10 cm long). The wood was then piled up around this chimney, giving to the whole construction the shape of a cone with a rounded tip and a 10/15 cm-wide hole in the middle. Big clumps collected from the surrounding soil ("pellicce" or "iove") were positioned on the timber, then the pile was covered with a mixture of plant material (leaves, twigs, bark pieces etc.) and organic soil (fig. 1b). The kiln was lighted by entering pieces of ember from the chimney; then some holes were opened first on the top ("fumi") and then in the lower part ("panchini"). On average, the cooking process was conducted at ca. 400 °C (Powell, 2008) and completed in four days. The charred wood was finally collected with special rakes (fig. 1c) (Giorgerini, 2009). Other tools used by charcoal makers were a lantern, two types of baskets, one for collecting ("vaglia") and one for carrying the charcoal, a ladder, a hoe, a shovel, a pruning hook and a lever scale (fig. 1 a,b,c; Landi et al., 1988).

The charcoal was produced in coppice stands and could be obtained from different sizes and pieces of timber: split wood ("carbone da spacco"), large branch pieces ("carbonella"), logs ("carbone da ciocchi") and small branch pieces ("carbone in ramaglia"). According to the old tradition, good quality charcoal should be black but not too dyeing, with a good sound at the touch, not crumbly and producing a small flame. The oak wood charcoal ("carbone forte") weighed 200-250 kg per m^3 while that made from other species with softer wood ("carbone dolce") weighed 130-180 kg (Giorgerini, 2009).

1.4 A glance on charcoal kiln sites in the forests of Europe and North America

Production of wood charcoal was a common practice not only in Europe, but also in the temperate forest ecosystems of North America. Hence, charcoal kiln sites are found in distant parts of the world, where they have been studied from different points of view and for various purposes.

Most of the studies conducted in Europe were focused on the sites of the central and northern countries. In the conifer-dominated woodlands of Norway, kiln sites were investigated using the Airborne Laser Scanning method and were found to occur with an average density of c. 1.2 platforms/ha. Kiln sites were prepared to produce the wood charcoal that was needed for iron production at the ironworks established in the year 1624. These were operational more or less continuously for two centuries, and were closed in the year 1822. Large amounts of charcoal were needed in the blast furnaces in order to achieve sufficiently high temperatures to get iron from ore (Risbøl et al., 2013). An intensive production of wood charcoal for iron metallurgy also existed in Wallonia (Southern Belgium) especially between the years 1750 and 1830 (Hardy and Dufey, 2015a). In a recent study in the woodlands of this region, Hardy and Dufey (2015b) recorded from one to three kiln sites per hectare, with a circular shape and a mean diameter of 10 m. Here, the more or less regular distribution of the platforms and the thickness of the soil layer rich in charcoal remains (ca. 35 cm) showed that the activity was continuous and widespread for a long period. The legacy of charcoal production in the Flemish part of Belgium was different. Results of a study in the Zoersel site showed a less widespread and more clustered distribution of the kiln sites, indicating that the practice was in use only during specific time intervals and not continuous. In fact, the activity was not part of the regular cycle of exploitation of the forest, but was related to exceptional periods of oversupply of wood that originated from the clearing of the coppice stands at the time of its transformation into grassland (Deforce et al., 2012).

In Germany, the production of wood charcoal in kiln sites was deeply rooted both in the northern and southern parts of the country. In the lowlands of Brandenburg, for example, more than 5.000 charcoal kilns were identified in areas formerly covered by *Pinus sylvestris*-dominated woodlands (Raab et al., 2013). Despite such a high number, these authors suggested that charcoal production sites are probably underestimated in the modern, mostly agricultural landscapes of the north German lowlands. In this region, the high density and the large size of the kiln platforms suggested a large-scale charcoal production for the supply of energy to the nearby ironworks. Based on the age of the charcoal remains found here, it is known that the production was active mainly between the 17th and the 19th century, corresponding with the main periods of charcoal burning (Raab et. al., 2013).

In the Baden-Württemberg region, a secular activity of charcoal burning shaped the landscape of the Black Forest. In this area, charcoal kiln sites occur with a considerable density, reaching in some cases more than 150 platforms per square kilometre (Ludemann, 2010). This shows a pronounced dependence of the past economy of south Germany on the wood fuel produced in the forest. A vivid charcoal production based on the local supply of wood continued in late-settled mountainous areas during the Middle Age, which continued until the 18th or the 19th century (Ludemann, 2010).

In southern Europe, the only two studies on this topic were carried out in mountain areas: the Pyrenees and the Alps. In the Vallferrera site (Axial Pyrenees, northeastern Spain) a large number of kiln platforms (942 in an area of 925 ha) were discovered in mixed *Pinus sylvestris*-*Betula pendula* woodlands. This region has been affected by the metal mining and smelting industry for at least 2.000 years, with maximum intensities in the eighteenth and nineteenth centuries, followed by abandonment of the activity. Dendrochronology of the region’s woodlands and historical records indicate that exploitation of wood charcoal for metalworking affected the past vegetation in the area and impeded the development of mature forest (Pèlachs et al., 2009).

Based on the study of Criscuoli et al. (2014), the Val di Pejo site in the Stelvio National Park (eastern Italian Alps) was subject to intensive exploitation for charcoal production since the 16th century. Charcoal was produced from larch stands and then used in the local iron industry. Production ceased only with the destruction of the major iron foundry in the valley (1858). In larch forests, charcoal production was based on extensive clear-cuts, wood chopping and downhill transportation to flat kiln sites with elliptical shape. The relatively large size of the sites suggested that more than one pile of wood was carbonized at a given time (Criscuoli et al., 2014).

In north America, mid-Atlantic forests dominated by oak *(Quercus*), hickory (*Carya*), American chestnut (*Castanea dentata*) and pine (*Pinus* sp.) were broadly exploited for charcoal production at the time of the European settlements (Mikan and Abrams, 1995). Throughout the 17th and 18th centuries, the request of charcoal for the iron furnaces was very high. The production of this material was continued also in later periods, although other fuels became in use especially after the civil war (Mikan and Abrams, 1995). Broad-leaf coppice forests of Canada were cleared at intervals of 20-30 years and kilns were prepared on elliptical platforms of ca. 150 m^2 (Mikan and Abrams, 1995). Kiln sites were considered valuable installations and were used repeatedly until the end of the charcoal iron era (Mikan and Abrams, 1995).

1.5 The hypothesis of kiln sites as ecological “islands”

As a legacy of the widespread and millennial practice described above, an indeterminable number of abandoned kiln sites remain nowadays in many European forests (Ludemann, 2011). According to some authors (Blondel, 2006; Nocentini and Coll, 2013), these sites are particularly numerous in the woodlands of the Mediterranean region, though no studies have attempted to analyse their actual density and/or distribution patterns in this area. Common experience indicates that they are still easily recognizable based on a combination of characteristics such as the flat, regular terrain (Cantiani, 1955; Ludemann et al., 2004; Montanari et al., 2000), the absence of superficial stones and rockiness, the alterations in colour and texture of the topsoil caused by the formation of thick layers (> 20 cm) rich in organic matter and wood charcoal remains (Criscuoli et al., 2014). Common knowledge suggests also that the forest canopy is often sparser compared with the adjacent stands, at least in some forest types of central Italy.

Although the wood charcoal production was ceased at least sixty years ago, the presence in the soil of a distinct “charcoal layer” is not surprising: it is well documented that the condensed aromatic structure of this material allows fragments to persist in soils even over millennial time-scales (Cheng et al., 2008; Wardle et al., 2008; Zimmerman, 2010). Anthropogenic deposits of charcoal dating back to the Neolithic period have been documented in Germany and Italy (Cremaschi et al., 2006; Ludemann, 2010; Schmid et al., 2002) and very old samples (> 8000 years BP) originating from wildfires have been found almost unaltered in forest soils in the Alpine region (Valese et al., 2014), northwestern France (Marguerie and Hunot, 2007), Catalonia (Castellnou and Miralles, 2009) and other areas (Pyne, 1997). Of particular interest is the case of the so-called "Terra Preta de Indio" in Brazil, a relict *Anthrosols* 2500 years old characterized by a high content of charcoal (Lehmann et al., 2004). In this soil type the input of carbonized organic matter was probably due to the production of charcoal in hearths realized by pre-European Amazonians, whereas only low amounts of charcoal were added to soils as a result of forest fires and slash-and-burn techniques (Glaser et al., 2002). It is noteworthy that *Terra Preta* soils have persisted over many centuries despite the prevailing humid tropical conditions and the consequently rapid mineralization rates (Lehmann et al., 2003; Scott and Damblon, 2010).

The long-term stability of charred remains has important implications and applications, such as those in the field of environmental history. Several anthracological studies have been performed to reconstruct the past tree species composition of forest ecosystems through the identification of the woody taxa used to produce charcoal. Such application highlights the usefulness of the old kiln sites in monitoring the vegetation changes through time and the impact of past human activities on present-day ecosystems (Ludemann, 2003; Ludemann et al., 2004; Montanari et al., 2000; Nelle, 2003; Samojlik et al., 2013).

The stability of wood charcoal also causes long term modifications of the soil properties, such as nutrient availability, water holding capacity and other chemical and physical characteristics. Such modifications induced by former human activities have been demonstrated in the case of Terra Preta soils (Glaser et al., 2002), and for the charcoal sites in Canada (Mikan and Abrams, 1995), central Europe (Wittig et al., 1999) and northern Italy (Criscuoli et al., 2014).

In recent times, the short-term effects of charcoal addition to the soil have been widely studied by means of the so-called "biochar" experiments in agricultural systems, especially on crop yields. These studies showed that the practice causes various changes in the structural and functional properties of the soil, which in turn, are likely to have positive effects on seed germination, seedlings establishment and plant growth (DeLuca, 2009; Nelissen et al., 2014). According to Thomas & Gale (2015), biochar is even a promising practice to promote forest restoration thanks to the increased growth and biomass shown by various tree species under experimental conditions. Because of these positive effects, charcoal addition in agricultural systems is increasingly considered an effective way to achieve long-term carbon sequestration in the soil, thus mitigating the impact of climate change (Nelissen et al., 2015; Vaccari et al., 2011).

In natural forest ecosystems, however, mechanisms inhibiting forest recolonization in abandoned kiln sites emerged from field studies conducted in North America (Mikan and Abrams, 1996, 1995; Young et al., 1996). Mikan and Abrams (1996) showed a negative effect of kiln sites on the tree regeneration, probably related to persistent anomalies in nutrient availability still occurring after centuries from their abandonment.

Such contrasting findings show that the response of natural vegetation to accumulation of charcoal in the soil, and more in general to the conditions of the old kiln sites, is still poorly known. Hence, more studies, both observational and experimental, are needed to understand the long-term effects of these altered soils on the establishment and growth of forest trees and, more in general, woodland communities. A special attention should be paid to the lower forest layers and especially to the understorey vegetation, since herbaceous plants are often characterized by a high responsiveness to local site conditions and environmental changes (Gilliam, 2007), and because of the key contribution of this layer to the overall biodiversity of the forest. In most temperate woodlands, the understorey vegetation contains indeed the greatest part of plant diversity (Gilliam, 2007), so its conservation is definitely a priority.

In Europe, only one study was conducted on this topic, which consisted in a phytosociological investigation of former kiln sites in beech stands of Germany (Wittig et al., 1999). In this investigation, the authors demonstrated an effect of kiln sites on the composition of the understorey vegetation, which was significantly different from that in the adjacent forest environment from also an ecological point view.

In the above study, effect on vegetation was most probably associated with changes in soil conditions. Observations in the sites of central Italy suggest that variations in light availability can also contribute to create different environmental conditions at a very local scale. As described above, canopy cover over the old kiln sites can be sparser since trees and shrubs are usually absent within the kiln platform perimeter. Variations in the light regime that can result from this small gap, if present, could, in turn, further influence the understorey (Axmanová et al., 2012; Härdtle et al., 2003; Hofmeister et al., 2009), also through complex interactions with the effects of soil.

For these reasons, the hypothesis can be formulated that the abandoned kiln sites could represent small ecological “islands” *sensu* Stebbins (1976), i.e. areas characterized by a combination of environmental factors abruptly different from the surroundings, and consequently with distinct biotic communities. In the present case, it can be predicted that trees, shrubs and herbaceous vegetation are subject to rapid variations in composition, diversity productivity regeneration dynamics and other ecosystem processes, when compared with the adjacent forest environment.

Such a hypothesis has never been tested with *ad-hoc* observational or experimental investigations, at least for the woodlands in the Mediterranean area. Moreover, unlike for the rest of Europe, information about the number of these potential ecological “islands" per unit surface are not currently available, and no exact data exist about their morphological characteristics and patterns of distribution. Hence, the magnitude of the possible ecological effects on the soil-vegetation system at the forest landscape level cannot be estimated.

1.6 Aims

In the light of what reported above, the present research was developed for the following purposes:

1) Understanding the effects of charcoal kiln sites on forest vegetation in relation to ecological factors (soil and light), specifically:

a. on the diversity of woody species and tree regeneration dynamics;

b. on the diversity, productivity and composition of the understorey vegetation.

2) Assessing the influence of the charcoal-enriched soil of the old kiln sites on the early life stages of major forest trees, especially seed germination, seedling growth and mortality.

3) Describing the legacy effect of charcoal production at the forest landscape level in central Italy, in terms of density, distribution patterns and morphology of the kiln sites.

These topics were investigated in three distinct forest types traditionally used for charcoal production in the Mediterranean area (evergreen sclerophylls, thermophilous deciduous forests with *Quercus* sp., beech forests).

2. Methods and Results

2.1 Research structure

Three approaches were adopted for the purposes described above: **Exploratory**, **Experimental and Inventory.**

The **exploratory** approach was adopted for the first topic, i.e. the characteristics of the vegetation and soil found on charcoal kiln sites compared to control sites. In this part of the study two papers were prepared:

- **Paper I** *Former charcoal kiln sites in Mediterranean forest areas: a hostile microhabitat for the recolonization by woody species*
- **Paper II** *Former charcoal kiln sites as microhabitats affecting understorey vegetation in Mediterranean forests*.

The experimental approach was used in parallel with the previous one to tackle our second task. The results were reported in (provisional title):

- **Paper III:** *Effects of charcoal kiln soil on germination, growth and mortality of forest trees: results of a two-years common garden experiment.*

Finally, the legacy of charcoal production at the forest landscape level was analysed using the inventory approach. This resulted in:

- **Paper IV**: *The old charcoal kiln sites in Mediterranean forest landscapes of Central Italy.*

2.2 Study area

The research was conducted in Tuscany (central Italy; fig. 2). This region is characterized by three major climate types following an altitudinal gradient from sea level to over 1.400 m: 1) meso-Mediterranean along the Tyrrhenian coast, where woodlands are mainly formed by evergreen sclerophylls and especially *Quercus ilex*; 2) supra-Mediterranean on the hill areas in the central part of the region, largely covered by thermophilous mixed forests dominated by various species of deciduous oaks (mainly *Quercus cerris*, *Q. petraea*, *Q. pubescens*); 3) montane-suboceanic on the Apennine range and Mount Amiata covered by beech (*Fagus sylvatica*). Mean annual rainfall and temperature in the area vary from 650 mm and 15 °C respectively along the coast, to 1450 mm and 10.9 °C respectively on the Apennines and Mount Amiata (period 1961-1990, source: Servizio Meteorologico dell’Aeronautica Militare, http://www.meteoam.it/). The study area is characterized by a variety of geolithological formations and soil conditions, but cambisols are the prevalent type according to the Soil Atlas of Europe (European Commission, www.eusoils.jrc.ec.europa.eu). Charcoal production on these areas lasted for centuries and was abandoned at least 60 years ago.

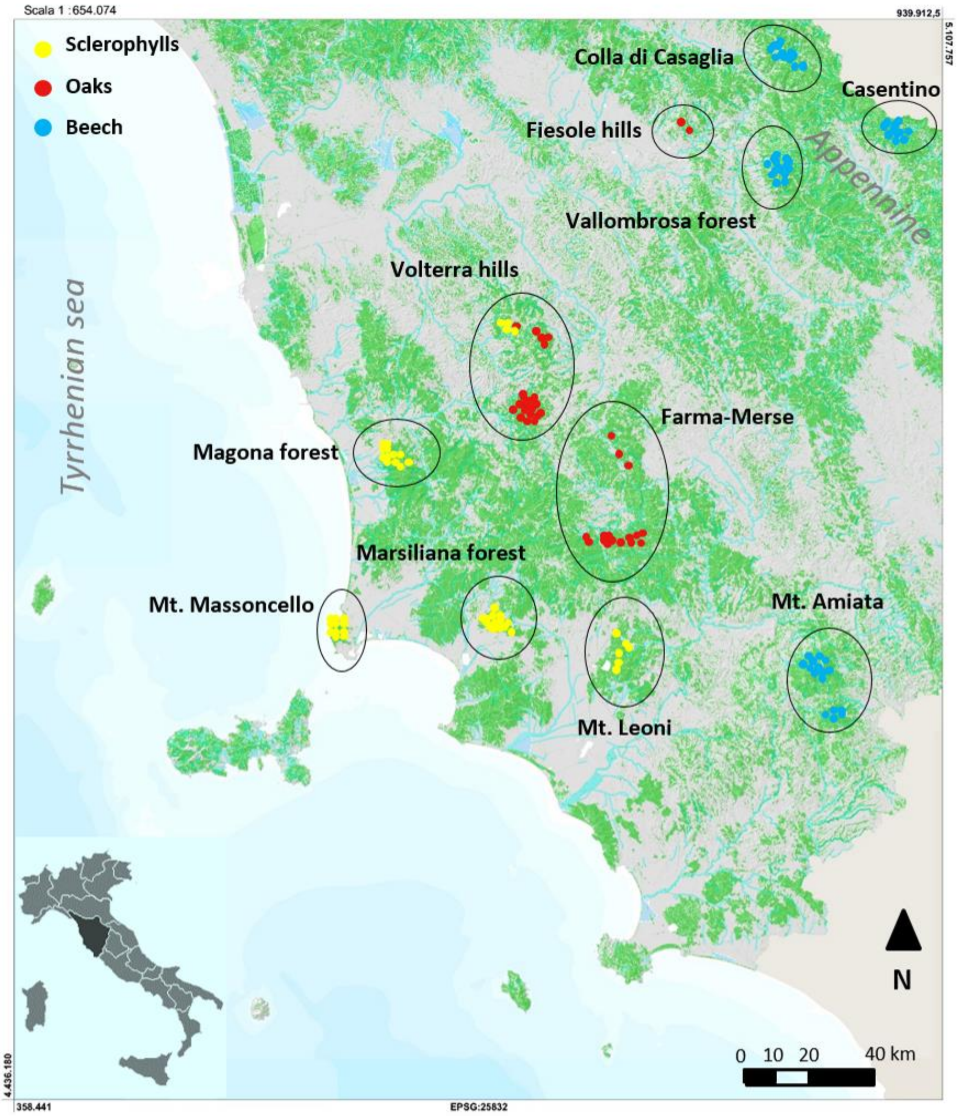

Figure 2 Geographic location of the selected forest areas and the investigated charcoal kiln sites in Tuscany (central Italy).

2.2 Exploratory work (synthesis of Papers I & II):

2.2.1 Data collection

An extensive preliminary search and description of abandoned charcoal kiln sites was conducted in the representative areas of three forest types. From a pool of 154 charcoal kiln sites, 61 were randomly selected among those that were not affected by recent anthropogenic or animal disturbance (e.g. photos 5-12; pp. 138-141). Sampling was conducted in 18 "sclerophyll" kiln sites, 22 "oak" kiln sites and 21 "beech" kiln sites (Appendix S1 – Paper II; pp. 129-130; fig. 2). In the centre of each site, a 3 m x 3 m quadrat hereafter called "kiln plot" (KP; photo 4, pg. 137) was established. In each quadrat, trees were recorded separately for two layers:

i) In the "established tree regeneration" layer (1.3-4 m) we recorded the number of plants for each woody species, either with a single or more stems, number of stems per stool, diameter at breast height of each stem (> 0.5 cm), and mean height of the stems for each stool.

ii) In the "understorey layer" (< 1.3 m) all vascular plant species, both woody and herbaceous, were identified and assessed for ground cover and maximum height. Then the above-ground biomass of all species rooted in a wooden frame of 0.5 m x 0.5 m was clipped, dried and weighed. Total cover and biomass were used as proxies for understorey productivity.

Next, soil core samples were collected and analysed for total C, N and pH; light intensity was also measured (photo 4; pg. 137). For each of the 61 kiln plots, the whole protocol was repeated in a control plot (CP) of the same size, established randomly in the stand adjacent to the kiln platform (photo 10; pg. 140). For a random subsampleof 5 KP per forest type, the density of seedlings of the dominant species of Fagaceae (e.g. all oak species and beech) was measured in the understorey.

2.2.2 Data analyses

i) **Established tree regeneration layer**

The influence of forest type, of the kiln site habitat and of the environmental variables (altitude, parent rock, slope aspect) on the woody species richness and on the structural variables (number of trees/stools, number of stems per plot, their basal area and mean height) was tested using a mixed model approach.

ii) Understorey

To evaluate the effect of kiln site habitat on productivity and diversity of the whole understorey vegetation at different levels (γ or total, α or plot-level and β or among-plots diversity), data were analysed using mixed models. Non-metric multidimensional scaling (NMDS) was used to visualize the understorey compositional differences among plots, testing differences between KP and CP with PERMANOVA. We performed the analysis considering all understorey species and woody species separately. Understorey composition was also analysed using the index of taxonomic distinctness Δ^+ (Clarke and Warwick, 1998) and with an indicator species analysis (Dufrene and Legendre, 1997). Finally, the influence of plot type on light intensity and soil components were tested using a mixed model approach. The comparison between the density of seedlings of dominant tree species in subsamples of KP and CP was conducted with a non-parametric Mann-Whitney U test. All analyses were performed in R 3.1.2 (R core team, 2014).

2.2.3 Results

i) **Established tree regeneration layer**

The number of woody species (eight trees and nine shrubs) was considerably lower in the kiln plots of all three forest types (fig. 3 – PAPER I, pg. 50 and photos 9-10, pg. 140). All species in this layer were taller, denser and with a higher basal area in the control plots, except for beech forests (fig. 3 – PAPER I; pg. 50).

ii) Understorey

Regarding the woody species, significant compositional differences between KP and CP were found only in oak forests (fig. 3a). Species richness was always higher in KP compared to CP for all three forest types (fig. 3 – PAPER I; pg. 50). Concerning the whole understorey community, the effect of charcoal kiln habitat was also positive in terms of species richness and Shannon diversity (Appendix S3 – Paper II; pg. 132). Floristic dissimilarities between kiln plots were larger than between control plots (Appendix S2 – Paper II; pg. 131), and significant compositional differences between the two plot types occurred also in this case (fig. 3b). Graminoid species were more abundant in kiln plots, and 12 indicator species were found in oak forests (e.g. photo 15; pg. 144), while

Anemone nemorosa resulted an indicator for the control plots (photo 16; pg. 144). Cover and total biomass of the understorey were higher on KP, although woody biomass was not significantly different (Appendix S2 – Paper II; pg. 131).

Looking at soil factors, we found consistently higher values of total C, N, C:N ratio, pH in the kiln site habitat. The strongest difference between the two plot types was in total C content, which was ca. two times higher in KP; differences in N content and pH were less pronounced but still significant. Concerning light, PAR values were significantly higher in KP (more than double; Appendix S3 – Paper II; pg. 132).

Seedling density and height in the understorey did not significantly differ between kiln and control plots (Table 2-PAPER I; pg. 52).

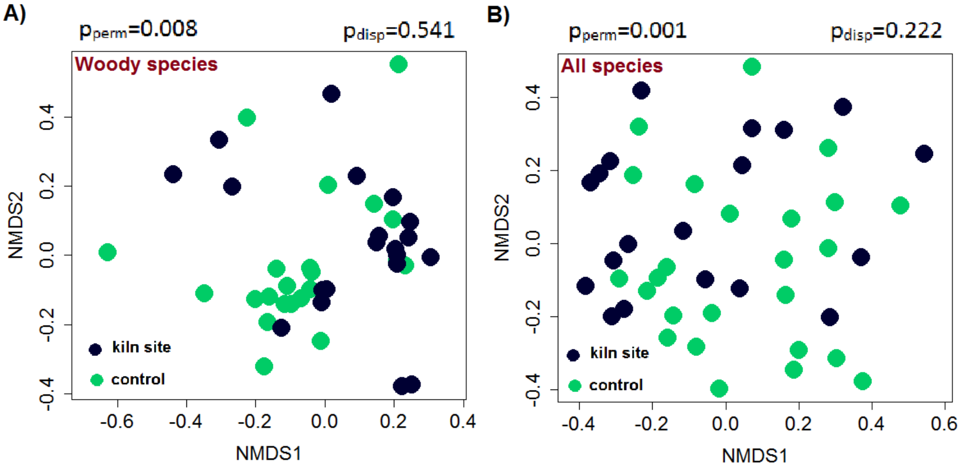

Figure 3 Scatterplot from NMDS based on Bray-Curtis dissimilarity index showing significant compositional differences in the understorey of kiln and control plots of oak forests; considering only woody species (A) and the whole understorey (B). p_{perm} indicates the combined significance of the location and dispersion effect, based on PERMANOVA with 999 permutations; p_{disp} indicates the significance of the dispersion effect.

2.3 Experimental work (synthesis of Paper III)

2.3.1 Sampling design and data collection

We selected the most representative tree species of the three forest types considered in this research (fig. 4): 1) the holm oak (*Quercus ilex*-QI) for evergreen sclerophyll forests; 2) the turkey oak (*Q. cerris*-QC*)* for thermophilous mixed oak forests; 3) the beech (*Fagus sylvatica*-FS) for montane forests. In autumn 2013 we collected the seeds of each species in a single locality, which were then sown in pots filled with the soil collected from a representative charcoal kiln site in the same localities (charcoal soil); the "control" soil was collected from a single spot in the adjacent stands. This allowed the set up of the common garden that was placed in the open spaces of the Faculty of Forestry located in the western outskirts of Firenze (Quaracchi; photos 17-21; pp. 145-149).

Starting from April 2014 we monitored seed germination, growth (height), photosynthetic efficiency and mortality until August 2015, when below-ground and above-ground biomass was also determined. The photosynthetic efficiency, measured in terms of Chlorophyll a fluorescence (ChlF), was determined on subsamples of 20 randomly selected seedlings per species. For biomass measurements, 35 seedlings of each species were randomly collected (photo 22; pg. 149). After extraction from the soil, the roots were washed and cut at the stem junction in order to separate the above-ground biomass. Each part of each seedling was oven-dried at 70°C for 48 hours and then weighed.

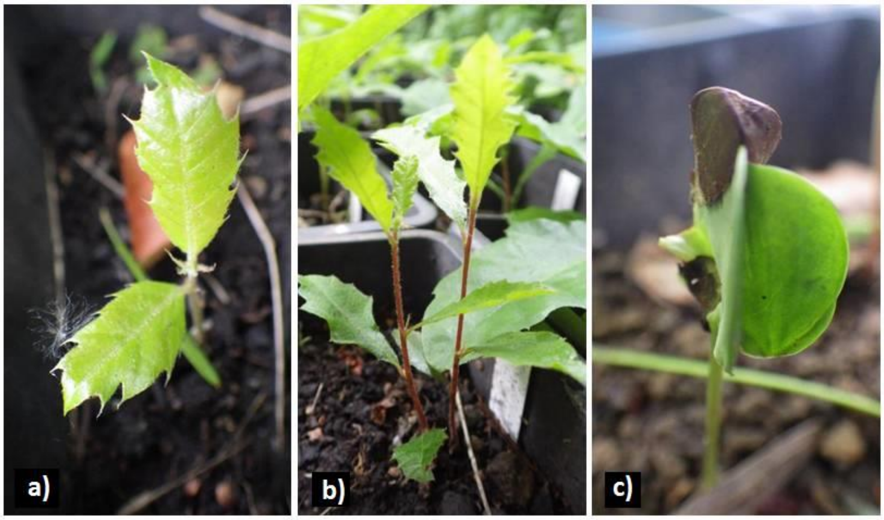

Figure 4 Early growth stages of seedlings of *Quercus ilex* (A), *Q. cerris* (B), *F. sylvatica* (C)

2.3.2 Data analyses

ChlF data were used to calculate the following indices:

- F_v/F_m =[Fm− F0]/Fm = φPo = TR0/ABS = maximum quantum yield of PSII primary photochemistry. F_v/F_m expresses the probability that an absorbed photon will be trapped by the PSII reaction center;
- Ψ_{Eo}, the probability of an electron to reduce the primary quinone acceptor and to move into the electron transport chain beyond PSII;
- Ψ_{Ro} (1-VI), the efficiency of a trapped electron to move into the electron transport chain, from QA^- to the PSI end electron acceptors This is related to the reduction of PSI end-electron acceptors, such as the reduction of NADP;
- PI_{ABS} the performance indices (PIs) measure the potential energy conservation of photons in the intersystem between PSII and PSI;
- $PI_{TOT.}$ the potential energy conservation from photons absorbed by PSII to the reduction flux of PSI end acceptors.

For each species all these indices and height values were averaged for each species for six periods: spring 2014, summer 2014, autumn 2014, winter 2014-2015, spring 2015, summer 2015. Normal distribution and homogeneity of variance for these data and biomass values were tested using the *Lilliefors test* and *Bartlett's test* respectively. All differences between the two soil types were tested by the *t* or the *Mann Withney U test*, depending on normality. All analyses were performed in R 3.1.2 (R core team, 2014).

2.3.3 Results

Seed germination on charcoal soil was higher for QI, while the two other species showed a preference for the control soil, particularly FS (73% on control vs 57% on charcoal; Table 1 – PAPER III; pg. 90).

At the end of summer 2014, mortality of QI and FS seedlings was lower on charcoal, while it was higher in QC, albeit differences were small. In August 2015 survival was always higher on charcoal; the largest difference was recorded for QI followed by QC and FS (Table 1 – PAPER III; pg. 90)

In general, seedlings grew taller on charcoal soil. Differences were generally significant for QC. Instead, no significant differences resulted for FS. Concerning QI, differences were significant only in spring 2014 (Appendix 1–Paper III (pg. 133).

In terms of biomass, no significant differences were recorded, neither considering the total nor the root or the aerial parts separately. However, the root/shoot biomass ratio was consistently higher on charcoal soil (fig. 5 – PAPER III; pg. 93).

The F_v/F_m parameter showed different responses in the three species. In QI it was generally higher on charcoal soil, with the only exception of spring 2015. The response was opposite for QC, which presented a significant better efficiency on control soil, with the exception of spring 2014. The beech presented an intermediate behaviour, with large differences between seasons (fig. 6 – PAPER III; pg. 94). Other parameters measured directly or calculated (provisionally not discussed here) are shown in Appendix 1–Paper III (pg. 133) together with significance levels of the differences.

2.4 Inventory work (synthesis of Paper IV):

2.4.1 Data collection:

The present part of the research was conducted in the forest types used in the exploratoty phase: “sclerophyll”, “oak” and “beech” forests. We selected three main areas for each forest type where charcoal production activity was continued for centuries and abandoned at least 60 years ago, as resulting from local historical documents (e.g. Landi et al. 1988) and common knowledge. These nine areas were distributed along a latitudinal gradient (fig. 1 – PAPER IV; pg. 103). In order to analyse the patterns of distribution and morphology of the kiln sites, two different approaches were adopted:

i) **field inventory** surveys of kiln sites in three 1-ha quadrats randomly selected in the three areas (nine quadrats). For each kiln site the following data were recorded: 1) altitude, 2) slope inclination, 3) slope aspect, 3) tree species composition of the adjacent stands, 4) conservation status, 5) shape, 6) size, and 7) thickness of the charcoal-enriched soil layer.

ii) visual inspection of images generated by high-resolution **Airborne Laser Scanning data (ASL)**, in order to detect kiln sites in the same quadrats used for the first approach. This method was applied in two quadrats oak forests (Tatti and Val di Farma) and one quadrat in beech forest (Vallombrosa).

2.4.2 Data analyses

i) **Field inventory:** the data were then analysed determining firstly the number of kiln platforms for each quadrat and their average density per forest type. Combined with the size data, this allowed to estimate their mean percentage of total surface per hectare in the three forest types. The mean thickness of the soil charcoal layer was also averaged for each forest type. Next, the effects of forest type and slope inclination on density, size and charcoal layer thickness were tested using two model structures with different combination of variables in R 3.1.2 (R core team, 2014). Moreover the frequency of dominant species occurring in the stands next to the kiln sites was determined for each forest type.

ii) **ALS data:** The slope map and the hillshade map were generated from a Digital Elevation Model (DEM). The kilns were detected visually based on the interpretation of the maps on the same 1-ha quadrats where field surveys were carried out. Then, the charcoal kilns identified in the field were used as ground-truth data for evaluating the “overall accuracy” of the ALS-based kiln detection method by direct comparison (Congalton, 1991). The TerraScan software was used for the preparation of the ALS datasets for the three areas (Terrasolid, 2005) and all GIS operations were performed with ArcGIS 10.3.

2.4.3 Results

i) Field inventory

In the total area of 9 ha, we recorded 51 regularly spaced kiln sites, resulting in a mean density of 5.5 sites/ha. Density of kiln platforms was lower in oak-dominated forests (4.7), but here their overall surface proportion was higher (2.3%) due to their larger size. Beech forest included more numerous (6.6) but smaller platforms (fig. 3 – PAPER IV; pg. 108). The dominant species surrounding the kiln sites were the holm oak (*Q. ilex*) for sclerophylls, the turkey oak (*Q. cerris*) in oak forests and the beech (*Fagus sylvatica*) in mountain forests (fig. 4 – PAPER IV; pg. 110). Kiln platforms were invariably elliptical, with the shorter and longer diameter ranging from 3.8 m to 9.3 m, and 4.6 m to 10.8 m, respectively. The charcoal-enriched soil layer was invariably continuous (photo 14; pg. 143), and its thickness ranged from a minimum of ca. 10 cm to a maximum of 46 cm (Table 1 – PAPER IV; pg. 107). Thicker profiles occurred on the steep slopes of mostly mountain beech stands.

ii) ASL data

Most of the kiln sites recorded with field surveys could be detected with hillshade and slope image analysis. On the hillshade maps, the platforms appeared as anomalous spots in the topography, sometimes as small hilly structures some of which showing a depressed area in the centre (fig. 5). On slope images, they appeared as small, dark spots areas with flat surface, mainly located along the altitudinal contour lines. The steeper inclination and the single-layered beech forest cover allowed to detect all sites in the Vallombrosa quadrat, while the lower slope inclination and the multiple-layered oak forest cover with dense shrub layer contributed to the lower accuracy in quadrats of Tatti and Val di Farma.

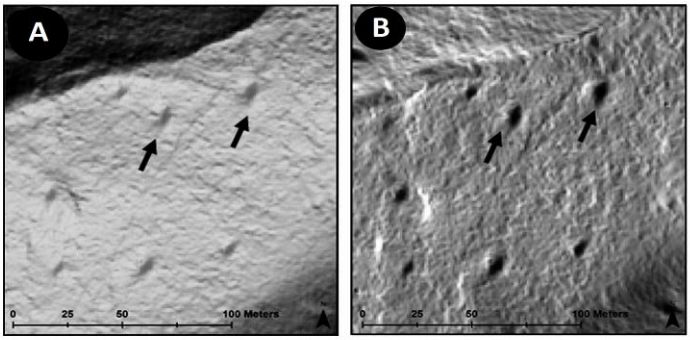

Figure 5 Charcoal kiln sites on the steep slopes of quadrat in Vallombrosa forest. Hillshade image (left) and slope images (right). Arrows indicate two of the platforms that are visible as dark, ovoidal spots

3. Discussion and conclusions

4.1 General discussion

The present research allows a better understanding of the legacy effects of charcoal production activity in the woodlands of a Mediterranean region.

A first striking consequence is the high density of abandoned kiln platforms, confirming that this activity has played a significant role in shaping the present-day aspect of different forest types, as reported by Nocentini and Coll (2013). In the areas examined, the density of kiln sites per unit surface was two- five times higher than in other European forest landscapes (Deforce et al., 2012; Hardy and Dufey, 2015a; Ludemann, 2010; Pèlachs et al., 2009; Raab et al., 2013; Risbøl et al., 2013) probably due to the longer time of intensive forest exploitation for wood charcoal production and the circumstance that this was continued until more recent times mainly due to the lack of other fuels. Practical issues such as the convenience of avoiding the transport of large amounts of firewood out of coppice woodlands often on steep and rough terrains may have also contributed to this intense transformation activity in the forest (Cantiani, 1955; Landi et al., 1988).

Differences were also in terms of size. Kiln platforms in our study region were generally smaller than in the rest of Europe or in North American forests (Deforce et al., 2012; Ludemann, 2010; Mikan and Abrams, 1995; Raab et al., 2015; Risbøl et al., 2013), that may be associated, at least in part, with the different purposes for which these were prepared. In our region, the wood fuel was not only used for iron metallurgy but also for to the production of energy for home heating and cooking, especially in the rural areas, as well for many other minor uses (S.I.L.T.E.M., 1946). Hence, there was a more frequent and continuous request, but usually of smaller amounts.

Some differences in the density and size of the kiln sites were observed among the three forest types, probably associated with the geomorphology of the relative areas and the compositional and structural characters of the stands. In the beech forests, the platforms were significantly smaller and denser than in the oak forests. Most likely, such effects are in part due to slope inclination: the beech is usually found on steep mountain slopes, where it was important to reduce the distance of transport between the places where the stools were cut and the places where these were used to build the kilns. This was achieved by preparing numerous but smaller terraces mainly along the altitude contour lines. Because of their larger size, kiln platforms in the oak forests occupied a higher proportion of surface in spite of their lower density. Coppice stands dominated by oaks or other trees of thermophilous deciduous forests have a higher productivity than either beech or sclerophyll stands (Istituto Sperimentale per l'Assestamento Forestale e per l'Alpicoltura, 1970), which may explain the need for a wider surface to transform larger amounts of wood into charcoal.

On the other hand, our data also showed variations in the size and density of the kiln sites among areas of the same forest type. This is a likely consequence of cultural aspects, since local traditions and uses may have led to the establishment of slightly different uses in similar forest environments and without specific practical reasons. Considering the species which were used for producing charcoal we found no evidence that local people used to make a selection, probably for their similarly high calorific power (Hellrigl, 2006); this is in line with results from anthracological studies in central and southern Europe providing no evidence for the selection for certain taxa (Ludemann, 2003, 2010; Nelle et al., 2010; Pèlachs et al., 2009).

In all sites examined, the “charcoal layer” in the soil profile was single, continuous and rich in charcoal fragments of variable size, in line with findings in the Alpine larch forests of Val di Pejo (Criscuoli et al., 2014). The considerable average thickness of this layer (23 cm) suggests that the same platforms were used repeatedly at given time intervals, in correspondence with the utilizations at the end of each coppice cycle. Without information about the age of the remains, the time of utilization of the same platform cannot be exactly determined. However, local documents and popular knowledge show that production of charcoal was deeply rooted in the local communities and at least centuries old. In south Tuscany, including the Marsiliana forest, it probably started as early as the Etruscan period, which means around the 6th century BC (Arrigoni et al., 1985; Mariotti Lippi et al., 2002).

Soil analysis showed that the total content of Carbon in the first 15 cm of the layer was on average nearly twice than in control soils. Similar evidence was obtained in sites of the Appalachian Mountains (Young et al., 1996), while Criscuoli et al. (2014) measured an even larger difference in a charcoal hearth of the eastern Alps (26.2 kg/m^2 vs. 1 kg/m^2 of total C). In the latter study, as in the present one, this was probably related to the abundance of charcoal fragments still present in the soil despite their abandonment since a time interval of 50-100 years. The condensed aromatic structure of charcoal allows these fragments to persist over millennial time-scales, since the rates of incorporation into the soil matrix are very slow (Cheng et al., 2008; Lehmann and Joseph, 2009). Given such long-term stability, the considerable thickness of charcoal layer, and the proportion of the forest surface occupied by the kilns platforms (up to 2.3%), future estimations of the carbon stock capacity of the woodlands in our region should take into account the potentially significant contribution of these sites. In our study, the higher C/N ratio resulting from the increase in C content was not associated with soil acidification or nutrient shortage, which are both potentially negative for plant growth. As found in previous studies (Criscuoli et al., 2014; Mikan and Abrams, 1996, 1995; Wittig et al., 1999; Young et al., 1996), the soil was in fact generally characterized by a higher pH and N content than in the adjacent stands, despite the wide differences in climate, forest type and parent rock material among the sites investigated.

An important ecological factor also affected by the kiln sites was light availability. Despite differences between the forest types, PAR was in general higher on these sites as a result of the sparser canopy cover. Effect was stronger in the oak forests, in line with findings in deciduous forests of Canada where charcoal platforms were described as “well illuminated” (Mikan and Abrams, 1995). The gap effect was instead less evident in the holm-oak and beech forests, because of the stronger shading power of these trees and their tendency to expand the crown over the kiln site for a better interception of light.

Variations in important factors such as soil and light determined consequent changes on vegetation, both in the understorey and upper forest layers. In all three examined ecosystems, the understorey community on the kiln platforms was considerably different from the adjacent forest environment, in terms of diversity, composition and productivity. Species richness was higher at the whole “kiln site” habitat level (γ-diversity) as well as at the plot-scale level (α-diversity). Decreased soil acidity, increased nutrient content and higher light availability are in fact favourable factors supporting higher diversity in the herb-layer of most European forests (Axmanová et al., 2012; Chytry et al., 2003; Ewald, 2008; Verstraeten et al., 2013). The different environmental conditions also affected the floristic composition of this layer, which included also some transient heliophilic or intermediate shade-tolerant species. These rapidly originate from seed banks or seed rain as in the case of forest gaps of sufficient size (Decocq et al., 2004; Schumann et al., 2003) and can increase the richness and heterogeneity of the understorey. Worth of note is the positive effect of kiln sites on the species richness of woody plants (as many as 45 taxa), including trees and shrubs of different auto-ecology and functional traits. These were mostly present as 1-3 years old seedlings, while older individuals were usually lacking.

Looking at the compositional aspect, it is relevant that differences among the kiln sites (β-diversity) were distinctly larger than those among the control sites. Higher species turn-over is probably also due to various stochastic factors leading to the inclusion of infrequent and non-specialist forest species, similarly to the case of edges in fragmented forest landscapes (Harper et al., 2005). A significant compositional shift also occurred for the woody species in the oak forest, where the gap effect was more pronounced than in the two other forest types. Small gaps have positive effects on tree regeneration (Beckage et al., 2015; Poulson and Platt, 1989; Yamamoto, 2000), and is well documented that these can provide recruitment opportunities for seedlings of various tree species, with positive effects on the overall forest diversity (e.g. Busing and White, 1997; Platt and Strong, 1989). Our findings suggest therefore that kiln sites provide favourable conditions for the germination of seeds and the early establishment of woody species, similarly to the herbaceous plants.

Considering now the taxonomic distinctness of the whole understorey community, this was only slightly negatively decreased compared with control sites, suggesting that charcoal accumulation in the soil does not have deleterious effects on the taxonomic evenness of the floristic assemblage. This appears remarkable since it is well documented that anthropogenic disturbance and different types of pollution can drastically reduce the taxonomic distinctness of various types of biotic communities (Clarke and Warwick, 1998; Stark et al., 2014). The hypothesis of kiln sites as “microhabitat” was also supported by the numerous indicator species that could be identified (12), including herbs, graminoids, shrubs and trees. The widely variable ecological characteristics of these species (e.g. light-demanding or shade-tolerant taxa with high nutrient requirements) suggest that their coexistence is better explained by the interaction of light and soil factors, rather than as a simple “gap” effect. This is in line with evidence from charcoal sites in the beech forests of south Germany (Wittig et al., 1999), where the composition of the understorey community was different mainly due to soil factors, while light was not considered as a driving force for the observed floristic and ecological shifts.

Looking at productivity, it is noteworthy that values of ground cover and total biomass were consistently higher on the kiln sites. Increased light availability is a major driver for this enhanced productivity, which can in turn account for the higher diversity of the herb-layer, as documented in in central European forests (Axmanová et al., 2012; Chytry et al., 2003). At the same time, these results also show that charcoal accumulation or other soil factors do not have detrimental effects on the growth of the understory vegetation, supporting evidence from the several “biochar” studies which indicated an enhanced productivity in crop yields grown on soils amended with charcoal, even in high amounts (Sohi et al., 2010; Vaccari et al., 2011).

On the other hand, other factors such as the zero slope inclination could be considered to explain the higher understorey biomass on kiln platforms. In fact, the flat ground morphology of these terraces is likely to favour the local concentration of water and nutrients especially after rainy events on hill or mountain slopes. While this is a plausible assumption, however, only a few studies have analysed the effect of slope inclination on the herb-layer of temperate forests, and these provided no evidence for such a positive effect on biomass (e.g. Axmanová et al., 2012; Siccama et al., 1970).

Additional evidence on the effects of kiln sites on the forest vegetation comes from the observation of the “established regeneration” layer of woody plants (1.3-4 m). Here, both γ-diversity and plot-level diversity in terms of species richness was significantly lower on the kiln sites in the oak and sclerophyll forests. Such a negative effect also emerged when considering the basic structural variables in the two latter forest types. Here, the number of stools and stems, the basal area and the mean height of the woody plants were consistently lower than in the adjacent stands. Remarkably, a similar detrimental influence on both species richness and structural variables (especially basal area) of the woody component was found in the charcoal platforms of southeastern Pennsylvania (Mikan and Abrams 1995) and the Appalachian mountains (Young et al. 1996). Hence, available data are consistent in showing that the growth and development of the initially numerous trees is then negatively affected by some persistent factors of biotic or abiotic nature that prevent their access and establishment into the higher layers. These factors are ultimately responsible for the substantial lack of forest recolonization in the abandoned kiln sites, but their identification is not straightforward.

Based on results above, neither light nor soil conditions analysed in this study can account for this negative effect. In fact, both types of factors appear generally favourable for plant growth, and doubtless light availability. Most of the soil characteristics are also apparently favourable, such as those reported by Mikan and Abrams (1996) in kiln sites after 110 years from their abandonment. Factors such as higher pH, cation exchange capacity, base saturation and nutrient contents cannot certainly explain the much delayed forest dynamics observed in these sites. Accumulation of charcoal itself is not likely a negative factor also in the light of biochar studies, which demonstrated the positive consequences of charcoal-enriched soils on plant productivity (DeLuca, 2009; Lehmann and Joseph, 2009; Nelissen et al., 2012; Vaccari et al., 2011). Increase of pH, water holding capacity and nutrient content in charcoal-amended soil are known to support plant growth, and this led some authors to even suggest biochar as a promising practice to promote forest restoration (Thomas and Gale, 2015). Hence, some other factor should be searched to explain the impeded forest dynamics of the kiln sites. Among these, Mikan and Abram (1996) suggested the higher electrical conductivity of the charcoal hearth soil, since this is potentially harmful for many plants and especially seedlings. According to these authors, moreover, the higher content of exchangeable Ca, Mg and K can lower the osmotic potential of the soil solution which may cause physiological drought. In the study by Young et al. (1996) in the Appalachian Mountains, it was the lower P content in the soil of charcoal kiln sites which could negatively affect vegetation. Besides the above chemical and physical factors, the biotic components of the soil environment should also be considered. In fact, the repeated combustions and/or charcoal accumulation may have caused deleterious effects on the microbiological communities. For example, Warnock et al. (2007) showed the impact of biochar addition on arbuscular mycorrhizal fungi, while Wallstedt et al. (2002) reported a decrease in both bio-available organic carbon and nitrogen in their ectomycorrhizal system. Also, Gaur and Adholeya (2000) found that the biochar media limited the amount of available P taken up by host plants, indicating that charcoal may in some cases reduce the formation of mycorrhiza by decreasing nutrient availability or creating unfavourable nutrient ratios in soils (Wallstedt et al., 2002).

Soil of charcoal kiln sites also share similarities with forest soils affected by fire, which causes strong heating and sterilization (Mikan and Abrams 1996). Indeed, forest fires negatively affect the diversity and richness of fungi (Longo et al., 2014), and several studies showed a strong impact on ectomycorrhizal formation in conifer forests (e.g. Dahlberg, 2002; Grogan et al., 2000; Torres and Honrubia, 1997). The same effects were found for arbuscular mycorrhizal fungi (Korb et al., 2004) and microbial communities after slash pile burning in forests (Jiménez Esquilín et al., 2007).

Another common “sterilization” effect of fire of is the death of the buried seeds, the incoming seeds and the remaining vegetative structures capable of resprouting. According to Mikan and Abrams (1995) this may be one of the reasons for the lack of formation of a new stand, which can only originate only from newly deposited seeds. Summing up, however, the reasons for the strongly delayed forest recolonization on the old charcoal kiln sites are still poorly understood and require further investigation.

We tried to give a contribution to this issue by means of the common garden experiment, whose results are still under analysis. Twenty months of regular observations highlighted different responses in three species with different ecological and functional traits grown on “natural” kiln site soil, *Quercus ilex* (QI), *Q. cerris* (QC) and *Fagus sylvatica* (FS). Such different responses were in terms of seed germination rate, growth and biomass accumulation and mortality.

Germination responses were not uniform in the three species: while no clear differences were found in QI and QC, FS clearly preferred the control soil, where seed germination was c. 17% higher than in the kiln site soil. This result was in line with evidence from the field, where beech seedlings in the understorey had a significant lower frequency on the kiln platforms than in the adjacent forest. On the contrary, the seedlings of the two oaks were more frequent in the kiln sites, consistently with the lack of a negative effect of charcoal soil on the seed germination of these species. Concerning QI, our results are in line with evidence from a recent experimental study (Reyes et al., 2015), which evidenced the insensitivity of this species to soils added with ash and black carbon, such as those resulting after wildfires. Looking at mortality, the proportion of seedlings died in the second year of the experiment was generally higher than in the first year, probably due to the stronger and longer drought of summer 2015 (data from Lamma Toscana, http://www.lamma.rete.toscana.it). The drought stress of the second year is probably associated with the larger differences of mortality rate on the two soil types, which surprisingly results in a positive effect of charcoal soil. These results seem to support evidence from observations on the Amazonian Anthrosol (Lehmann et al., 2003) and biochar experiments, where it was found that charcoal addition to the soil increases water retention capacity and structural stability (Baronti et al., 2010; Glaser et al., 2002; Yanai et al., 2007). The possibly positive effects of charcoal on the survival of seedlings of forest trees observed in this study should be further tested in different conditions and on a wider a range of species, since it may have important implications for the management of forests and tree plantations under the predicted climate change.

Looking at growth rates, it is noteworthy that charcoal soil had again a mostly positive effect. In fact, plants of QI and largely QC grown on this soil were usually taller than those grown on the control soil. Hence, these data support findings from the various biochar studies mentioned above, where plant growth was enhanced on soils ameliorated with charcoal. On the other hand, the total biomass data did not confirm these growth results. Despite the height differences, weight of the total biomass was not significantly higher on the charcoal soil, probably due to a wide variation between individual plants. Looking closer at these results, however, the root/shoot ratio was consistently higher for the seedlings grown on the charcoal soil, indicating a stronger development of the root systems in these plants. This is surprisingly in line with results of a similar experiment on two North American oak species by Mikan and Abrams (1996), who suggested that such an increased root development may be associated with one or more stress factor such as the physiological drought. However, this is apparently in contrast with the lower mortality observed on charcoal soil indicating that further investigation is needed to unravel the possible multiple effects of charcoal soil on water retention capacity and consequently on the root growth.

Chlorophyll a fluorescence transients also highlighted divergent responses in the three taxa. Based on the index F_v/F_m, QI was characterized by a greater photosynthetic efficiency on the kiln site soil, while QC and FS were mainly negatively influenced. The possible reasons for the reduction in photosynthetic performance in the seedlings of the two latter species grown on charcoal soil are various and should be further investigated. A decrease of the F_v/F_m index has been observed for species grown on substrates with shortage of nutrients (Bussotti et al., 2012), which may appear consistent with the hypothesis of decreased availability of P in soils of North America sites (Young et al., 1996). Unfortunately, little is known about the effects of different levels of single nutrients on photosynthesis, which are likely to vary in different species. In our case, the contrasting response of QI vs. QC and FS is likely associated with the widely diverging morphological, anatomical and ecophysiological traits of these species, as well as to their different sensitivity to soil conditions and nutrient levels. This is not surprising in view of their different edaphic requirements: while QI shows a broad tolerance in terms of pH and nutrients, QC grow on richer but not too alkaline soils and FS generally prefers even deeper substrates with high nutrient contents (Pignatti, 2005).

In the evergreen oak QI, the increased F_v/F_m index on charcoal soil supports findings by Reyes (2015), highlighting the more resilient nature of this drought-tolerant Mediterranean tree in the face of several types of environmental stress. On the other hand, it must be highlighted that this experimental result appears somewhat contradictory with evidence from the field discussed above, which showed the lack of young trees of QI in the "established regeneration" layer. This leads to the assumption that factors other than soil chemistry play a role in the regeneration failure of even this stress-tolerant tree. These may include the impact of wild herbivores, the lack of formation of mycorrhizae or even the altered "natural" structure of the soil in the charcoal sites, all aspects that could not be tested in our 20-months experiment.

Overall, the work conducted in this study, combined with evidence from the existing literature on the topic, shows that charcoal kiln sites have strong and persisting effects on soil and vegetation. Developing methods for the rapid detection of these sites at large spatial scales is therefore important to better understand the magnitude of these effects at the landscape level, as well as to better exploit their potential contribution to more cultural, historical and archaeological issues.

In this perspective, the use of ALS method seems promising. Our first attempt suggests that using only hillshade maps, Digital Elevation Models (DEMs), or Local Relief Models as in previous studies in northern and central Europe (Bollandsås et al., 2012; Deforce et al., 2012; Hesse, 2010; Ludemann, 2011; Risbøl et al., 2013) may not be sufficient to identify the kiln platforms on hilly or mountainous areas. Instead, the combination of hillshade and slope images can give good results in the variable conditions of vegetation and terrain of our region. Accuracy was moderately high for oak forest (c. 75%), and absolute for beech forests (100%), and this possibly for two reasons. First, the better quality of Lidar data in terms of observation point density, confirming the importance of such parameter for the detection of any kind of remains in forested areas (Bollandsås et al., 2012). Second, the fact that beech forests in our region usually occur on steep slopes and have a simple, single-layered structure mostly without shrub layers, unlike the oak forests. These factors make visual identification of the kiln platforms much easier and less prone to errors (Amable et al., 2004; Risbøl et al., 2013). To conclude, however, we support Deforce et al. (2012) and Ludemann (2011) in suggesting that the ALS method cannot completely replace the field traditional work when an absolute precision is needed, especially in the landscapes covered by structurally complex oak-dominated vegetation.

4.2 Conclusions

Overall, the present research demonstrated the existence of important legacy effects of the former charcoal production activity in woodlands, confirming evidence from previous studies in other regions but also contributing new information on aspects that were still largely unknown.

Persistent alterations of soil conditions and light regime are the likely factors for the higher diversity and biomass of the understorey vegetation, but at the same time for the difficulty of woody plants to recolonize these sites. This contrasting effect on plants with different structure and traits results in small, persistent patches often with a well developed herbaceous community, but very sparse, or no shrub and overstorey layers. The blocked or much delayed forest dynamics in the charcoal kiln sites is the major character distinguishing them from "normal" forest gaps, since these usually support a more or less rapid succession of vegetation phases that finally leads to the formation of the stand. While at least some of the causes for the positive effects on the understorey are readily identified, e.g. higher light supply, lower soil acidity and larger amount of N, it remains largely obscure what are the factors drastically reducing the diversity and density of the woody species once they leave the initial "herbaceous phase" and start to grow as shrubs or trees. Although such factors are likely to lie in the soil environment and to act through the root system, results of the common garden experiment on three major forest trees did not show any consistent negative effects that can satisfactorily explain the almost complete lack of established regeneration and overstorey observed in the field. These may have implications for the "forest biochar" issue, and could stimulate more studies aiming at better understanding the long-term effects of charcoal-enriched soil on the development of forest trees. In the charcoal sites, nutrient deficiencies and/or negative influence on the biotic components of the soil (e.g. mycorrhizae) connected with the repeated wood burnings can have an important role, but stochastic factors like destruction or damaging of the woody vegetation by animals and especially wild ungulates can also have a strong, direct impact.

Whatever these factors, circumstantial evidence show that these are a persistent and long-lasting consequence of charcoal production continued for centuries, mostly in the same sites. Hence, combined evidence from the inventory, exploratory and experimental work support the hypothesis that kiln sites form extensive networks of ecological "micro-islands" of anthropic origin, which enhance the fine-scale heterogeneity of the forest landscape and plant diversity in different forest types.

Concerning the magnitude of the ecological effects on soil and vegetation, this is clearly dependent on the density of these sites and, ultimately, on their total surface. Our inventory study showed that density is significantly higher than in other European regions and that total surface is not negligible. However, even better estimations are possible when rapid and effective, semi-automated methods can be used on larger spatial (e.g. landscape) scales, such the ALS technique. From this point of view, the contribution of our study was to show that the combination of hillshade and slope images is probably the best method to identify the kiln sites on the irregular terrains and often complex vegetation structures of our region.

Using this method and, when necessary, the traditional field-based inventory will allow to study the kiln sites and their ecological effects at spatial scales larger than those considered in the present investigation, as well as to explore their potential for more historical-cultural or archaeological purposes.

Inventory work would also be useful in a conservation perspective. Indeed, the old charcoal sites should deserve some forms of protection in the management policy of at least protected areas, since various factors bring a serious threat to their long-term conservation. These include silvicultural practices such as forest track construction, mechanized wood extraction or recreational use by local people or eco-tourists, all of which may cause severe damage by soil erosion, or even destruction. Paying more attention to these neglected sites would contribute to preserve the vivid testimony of one of the oldest forms of forest use throughout the world, and the potentiality for more environmental and historical studies on still little-known topics.

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Paper I

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Former charcoal kiln sites in Mediterranean forest areas: a hostile microhabitat for the recolonization by woody species

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Abstract

Production of wood charcoal is a traditional form of forest use that existed for millennia in the Mediterranean countries and vanished only in the last century. As a result, thousands of abandoned charcoal kiln sites are found in present-day woodlands. These are characterized by peculiar soil conditions caused by the accumulation and long-term persistence of charcoal remains, whose effect on the recolonization capacity of woody species is still unknown. We examined 61 sites, located in evergreen sclerophyllous communities and deciduous broadleaf forests with oaks and beech. At each site, one kiln plot (on charcoal kiln platform) and one control plot were established. On both plots, we examined species richness and composition of trees and shrubs in the understorey (<1.3 m) and in the "established regeneration" layer (> 1.3-4 m). In the latter, main structural parameters such as number of stools, number, dbh and mean height of stems were compared. The density of seedlings of dominant tree species in the understorey was also measured in a subsample of sites per forest type. On the whole, the kiln plot effect was stronger in oak and sclerophyll forests than in beech forests. Significant compositional differences were found only in the former forest type, while species richness was higher in the kiln plots of sclerophyll forests as well. The number of woody species in the established regeneration layer was considerably lower in the kiln plots of all three forest types. All species in this layer were taller, denser and with a higher basal area in control plots, except for beech forests. Seedling density and height in the understorey did not significantly differ between kiln and control plots. We conclude that charcoal kilns provide a favorable microhabitat for only the first regeneration stages of woody species, since their further growth is severely hindered by still unknown abiotic and/or biotic factors. Hence, these sites represent small but persistent ecological "islands" where forest recolonization is substantially lacking despite the long time since their abandonment.

Introduction

Wood charcoal has been the first synthetic material used by man, as shown by artworks of ca. 38.000 years ago in caves of southern France (Antal 2003), and the main source of energy from the Iron Age to the 19th century (Blondel 2006). Its production has continued for millennia, making it one of the oldest forms of anthropogenic forest use in most countries of temperate regions.

Charcoal production, based on the pyrolysis of wood, was carried out in coppice woodlands in special kilns covered by a mixture of soil and plant material. For this purpose, small areas (35-50 m^2) with flat surface and usually semi-circular shape were prepared mainly along footpaths through hill and mountain slopes (Ludemann 2011, WSL 2011). While in most of northern and central Europe this practice was abandoned in the 19th century due to the rapidly increasing and widespread use of coal (Deforce et al. 2012), in most Mediterranean countries the importance of wood charcoal production even increased during the industrial revolution because of the lack of other fuel sources. It generally vanished only in the 1950s, though in some remote mountain areas it is still in use. As a result, remnants of charcoal kiln sites are nowadays widespread in many forest landscapes, with a high density in especially coppice stands (Blondel 2006, Nocentini & Coll 2013). They are characterized by clear alterations of color and texture of the topsoil material due to the charred woody remains resulting from a century-long use (Montanari 2000, WSL 2011). In fact, the condensed aromatic structure of wood charcoal allows fragments and particles to persist in soils and other sedimentary records over millennial time-scales (Cheng et al. 2008, Wardle et al. 2008, Zimmerman 2010), giving an opportunity to reconstruct environmental history and past forest fires (Patterson et al. 1987). Anthropogenic deposits of charcoal dating back to the Neolithic period have been documented in Germany and Italy (Schmid et al. 2002, Cremaschi et al. 2006, Ludemann 2010) and very old samples (> 8000 years BP) originating from wildfires have been found almost unaltered in forest soils, allowing to use them as a proxy of human fire activity during the Holocene in different parts of Europe (e.g. the Alpine region, Valese et al. 2014; northwestern France, Marguerie & Hunot 2007; Catalonia, Castellnou et al. 2009, and other areas, Pyne 1997).

Several anthracological studies were aimed at reconstructing the former forest tree species composition through the identification of the woody taxa used to produce the charcoal, highlighting the usefulness of kiln sites in monitoring the vegetation changes through time (Montanari et al. 2000, Ludemann 2003, Nelle 2003, Ludemann et al. 2004, Samojlik 2013). Concerning the present-day vegetation of these sites, however, only a couple of studies have been carried out to date (Wittig et al. 1999, Carrari et al*., accepted ms*), and none of them focused on the tree regeneration and forest recolonization processes. As a result, information about these aspects and the effects of the long-term persistence of charcoal in the soil on the development of woody species are still unknown. Successful recolonization by these species in charcoal platforms is expected because of the apparently favorable conditions such as the low degree of rockiness, the thick layer of organic matter, and the often higher availability of light (Carrari et al., a, ms. *accepted ms*). In addition, evidence exists that charcoal addition to the soil (the so-called "biochar" practice) can have positive effects on seed germination, seedlings establishment and growth of woody species (DeLuca et al. 2009). According to Thomas & Gale (2015), biochar is even a promising practice to promote forest restoration thanks to the increased growth and biomass shown by various tree species. On the other hand, common knowledge and direct observations indicate that established and adult trees are mostly absent in old charcoal kiln sites, suggesting that tree regeneration is hampered at some stages by biotic or abiotic factors. Considering the relatively long time since their abandonment, such factors are likely to be persistent and may represent a legacy of the past forest use still affecting ecosystem functioning and dynamic processes at the "microhabitat" scale.

Based on an extensive sampling in three major forest types in central Italy (Tuscany), located along an altitudinal gradient from the Mediterranean coast to the mountain belt, this work aims at describing the effects of abandoned charcoal kiln sites on tree regeneration and recolonization of the woody vegetation found in this habitat.

Material and Methods

Study area

This study was performed in the forests of Tuscany (central Italy) in an area located between 42°44'16.08" and 44°3'13.02" N and between 10°29'48.90" and 11°29'0.96"E (Fig. 1). This area (ca. 9.000 km^2) covers an altitudinal gradient from 0 to more than 1.400 m above sea level, therefore including three main climate and forest types, here indicated according to the EEA classification system (European Environment Agency 2007): 1) meso-mediterranean evergreen forest dominated by *Quercus ilex* L. and sclerophyll shrubs, along the Tyrrhenian coast (here named "sclerophyll" forests); 2) supra-Mediterranean thermophilous mixed communities dominated by deciduous oaks (*Quercus cerris* L., *Q. petraea* (Matt.) Liebl., *Q. pubescens* Willd.); these occur on the vast hill areas in the central part of Tuscany ("oak" forests); 3) montane forests with beech (*Fagus sylvatica* L.), on the Apennine range (Casentino and Mugello areas) and volcanic massif of Mt. Amiata ("beech" forests). Mean annual rainfall and temperature in this broad area vary from 650 mm and 15 °C respectively along the coast to 1.450 mm and 10.9 °C respectively on the Apennines (Areonautica Militare, reference period 1961-

1990). The study area is characterized by a variety of geolithological formations and soil conditions, but cambisols are the most prevalent type according to the Soil Atlas of Europe (www.eusoils.jrc.ec.europa.eu).

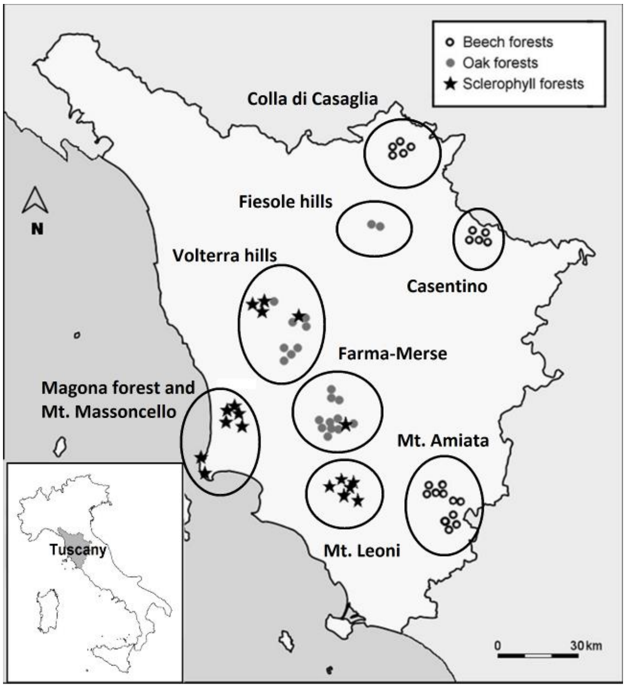

Figure 1. Geographic location of the forest areas and the investigated charcoal kiln sites in Tuscany (central Italy).

Field sampling design and data collection

An extensive search for abandoned charcoal kiln sites was carried out with *ad-hoc* field trips in representative areas of the three main forest types described above. The 154 sites observed, all easily recognizable thanks to the characteristics of the ground surface (Ludemann 2011, 2012), were recorded with a GPS device, numbered, and characterized using simple descriptors such as altitude, slope, aspect, soil type and tree species composition of the adjacent forest stands. From this pool, 61 sites were randomly selected among those that were not affected by recent anthropogenic or animal disturbance (18 “sclerophyll“ sites, 22 “oak” sites, 21 “beech” sites; Fig. 1). The main geographical and environmental variables of these sites are given in Table 1. All of them were located in “ancient forests” sensu Hermy et al. (1999), i.e. with a continuous cover over time and never converted into agricultural land during at least the past three centuries. Furthermore, all but two sites are included in protected areas of the Rete Natura 2000 network, Nature Reserves and National Parks. Charcoal production on these sites lasted for centuries and was abandoned at least ca. 60 years ago, while the coppice-with standards management for the production of firewood was abandoned in later times (ca. three coppice cycles after the last felling) in the oak and beech forests. In the sclerophyll forest areas, this management type was abandoned in more recent times or is even still in use (pers. obs.).

In the centre of the charcoal kiln platform of each selected site, we established a 3 m x 3 m quadrat hereafter called "kiln plot" (KP). The relatively small size and the general shape of the platforms did not allow to use larger quadrats, as this would have resulted in a kiln-forest edge effect with likely consequences on vegetation composition. In each quadrat, we identified all species of tree and shrub seedlings in the understorey (< 1.3 m) and recorded the maximum height and ground cover percentage of each of these species. As "tree" we considered all woody angiosperms or gymnosperms belonging to Raunkiaer's life forms Pscap (scapose phanerophyte) and Pcaesp (caespitose phanerophyte) (Pignatti, 1982). In addition, the density of regeneration of the dominant species of Fagaceae (e.g. all oak species and beech) was measured in a sub-sample of five randomly selected kiln sites in each forest type, by counting the number of individuals of each species < 1.3 m within the quadrat.

Next, we used the 61 kiln plots (3 x 3 m) to record the number of trees/stools, number of stems per stool, diameter at breast height (dbh, cm) of each stem (> 0.5 cm), and mean height of the stems for each stool for all individuals in the "established tree regeneration" layer (1.3-4 m).

For each of the 61 kiln plots, the same protocol was repeated in a control plot (CP) of the same size, established randomly in the stand closely adjacent the kiln platform, e.g. at a distance of 10-20 m from the edge of the KP (depending on local topographic and ground conditions that often required to adjust the exact location of the plot); downhill locations were always excluded to avoid potential charcoal "contamination" by runoff. This allowed to exclude variation between kiln and respective control plot for major environmental variables such as altitude, parent rock material and slope aspect. On the contrary, other factors such as light availability and cover of the herbaceous layer, both potentially influencing tree regeneration, were often different between the two habitats (Carrari et al., *accepted ms*).

Table 1. Main geographical and environmental variables of the studied sites, with number of charcoal kiln (KP) and control plots (CP) for each of the examined forest areas.

Forest	Lat	Long	No. KP	No. CP	Forest Type*	Altitudinal range* (m a.s.l.)	Aspect*	Parent rock material*	Slope	
									kiln	control
Colla di Casaglia	44° 1'57"N	11°29'1"E	5	5	Beech	972-1029	SE/E/NE	marl-sandstone	0	10-50%
Mt. Amiata	42°52'10"N	11°35'3"E	11	11	Beech	846-1268	N/NE/E/SE/S/SW/W/NW	Trachyte	0	20-50%
Casentino	43°48'19"N	11°52'9"E	5	5	Beech	1040-1223	S/SE/E	marl-sandstone	0	5-45%
Volterra hills	43°25'55"N	11° 00'2"E	7	7	oaks/sclerophylls	382-967	E/SE/N/NW/NE	diabase/limestone/sandstone	0	3-40%
Fiesole hills	43°48'15"N	11°20'27"E	2	2	oaks	242-347	W/E	marl-calcareous	0	3-5%
Farma-Merse	43° 5'22"N	11°10'46"E	15	15	oaks/sclerophylls	265-511	N/NE/E/SE/S/SW/W/NW	quarzitic sandstone	0	3-40%
Mt. Leoni	42°56'27"N	11°10'58"E	5	5	sclerophylls	155-437	S/SW/-/W/E	quarzitic sandstone	0	3-35%
Magona Forest/Mt. Massoncello	43°15'50"N	10°37'54"E	7	7	sclerophylls	157-201	W/NW/N/SE	marl-clay/sandstone	0	3-20%

*Variables considered in the starting mixed model as predictors for the response variables.

Data analysis

All analyses were performed in R 3.1.2 (R core team, 2014). First, we calculated the understorey compositional differences in woody species between KP and CP for each forest type, using the cover-weighted Bray-Curtis dissimilarity index. Compositional differences were then visualized by means of non-metric multidimensional scaling (NMDS) (metaMDS function in the vegan package; Oksanen et al. 2013). Differences were tested using a permutational multivariate analysis of variance (PERMANOVA; adonis function in vegan package) with 999 permutations. To verify that such differences were related to the effect of the factor kiln/control (e.g., compositional dissimilarities between kiln and control plots) and not to a dispersion effect (e.g., dissimilarities within each of the two plot types), we tested for multivariate homogeneity of dispersion using betadisper (Vegan package), a multivariate analogue of Levene's test for homogeneity of variances (Anderson 2001, Oksanen et al. 2013).

Density of seedlings of the locally dominant tree species, all belonging to the Fagaceae family, was compared between KP and CP with a non-parametric Mann-Whitney U test (wilcox.test function in the R Stats package, Chambers et al. 1992).

We then looked at the effects of the charcoal kiln platforms on forest recolonization in terms of species richness (SR) at the habitat level (γ-diversity of KP and CP) and plot level (mean SR for KP and CP) found in the understorey and the established regeneration layer, separately for the three forest types.

The influence of forest type (levels: sclerophyll, oak, beech), kiln/control (levels: KP, CP) and environmental variables (altitude, parent rock, slope aspect) on the woody species richness of the understorey and tree regeneration layer was tested using a mixed model approach. First, the effects of all considered variables were fitted, allowing for random variation across "forest areas", in order to remove from the model the variance due to the spatial clustering of the plots [R-syntax: Species richness~ Forest type + Kiln/Control + Altitude + Parent rock + Aspect + (1 | Forest area), using glmer with a Poisson error distribution and loglink, from the lme4 package (Bates et al., 2013)]. Altitude was preliminary log-transformed to fulfil the requirements of normality and homoscedasticity. Starting from this full model, we looked for model parsimony (approach according to Zuur et al. 2009). First, we deleted the random variation across sites while keeping the fixed effect term [R- syntax: Species richness~ Forest type + Kiln/Control + Altitude + Parent rock + Aspect, using glm from the stats package with a Poisson error distribution, log link and parameter estimation via maximum likelihood]. The model yielding the lowest value for Akaike's Information Criterion (AIC; Akaike, 1973) was considered to be most consistent with the data. Once an optimal random structure was found, we searched for the optimal fixed effect structure by comparing the AIC of models with the same random effect structure but a different fixed effect structure (here parameter values were maximum likelihood estimates). In case of over-dispersion, the standard errors were corrected using a quasi-GLM model (Zuur et al., 2009). Accordingly to Zuur et al. (2009) models were validated looking at response residuals (observed minus fitted values, also called ordinary residuals), Pearson residuals, scaled Pearson residuals and the deviance residuals for the optimal quasi-Poisson model.

Using a similar mixed model approach, we tested the effects of forest type and kiln/control factor on seedling density in the understorey and on the structural variables of the established tree regeneration layer (number of trees/stools, number of stems per plot and their basal area and mean height). Number of trees/stools and number of stems were fitted using glmer with a Poisson error distribution, as for the species richness models. Seedling density, basal area and mean height were fitted allowing for random variation across "forest areas" using lmer with a Gaussian error distribution (lme4 package; Bates et al. 2013). In the case of seedling density we did not include in the model environmental factors because they did not differ in such parameters (Appendix 4) and we used just fixed terms; for the other variables we used the R-syntax: y~ Forest type + Kiln/Control + Altitude + Parent rock + Aspect + (1 | Forest area). Furthermore, fixed effect models were tested using gls from the nlme package (Pinheiro et al. 2013) with a Gaussian error distribution, and parameter estimation was calculated with a restricted maximum likelihood. As residual spread changed with the levels kiln/control, we used the varIdent variance structure (nlme package) to weight the models by portion, allowing to achieve homogeneous variances (Zuur et al. 2009).

For the optimal models selected for each variable, we calculated the R-squared (R^2), which refers to the fraction of the total variation in the response variable explained by the model. For models with fixed effects only, the adjusted R^2 of the linear model was reported; for models that (also) contained random effects, a conditional R^2 was calculated according to Nakagawa and Schielzeth (2013) (MuMIn package; Bartoń 2013), indicating the proportion of the variance explained by both the random and fixed effects (not yet applicable for glmer with a Poisson error distribution).

Results

Concerning composition of woody species in the understorey, NMDS analysis yielded different results for the three forest types. Significant differences between KP and CP were found in oak forests (p_{perm}=0.008), where a similar level of compositional variation within these two plot types occurred (p_{disp}= 0.541; Fig. 2); the understorey of beech and sclerophyll showed no compositional differences between the two plot types.

In total, 45 woody species were recorded in the understorey of the 122 plots, of which 26 trees and 19 shrubs. Species richness was always higher in KP compared to CP for all three forest types (22 vs. 14 in sclerophyll forests; 28 vs. 22 in oak forests; 13 vs. 9 in beech forests; Fig. 3). In sclerophyll forests, nine species were unique to KP while only one to CP; in oak forests eight species were unique to KP and two to CP; in beech forests, four species were unique to KP (i.e. *Acer platanoides, Castanea sativa*) and one to CP (*Prunus avium*) (Appendix 1).

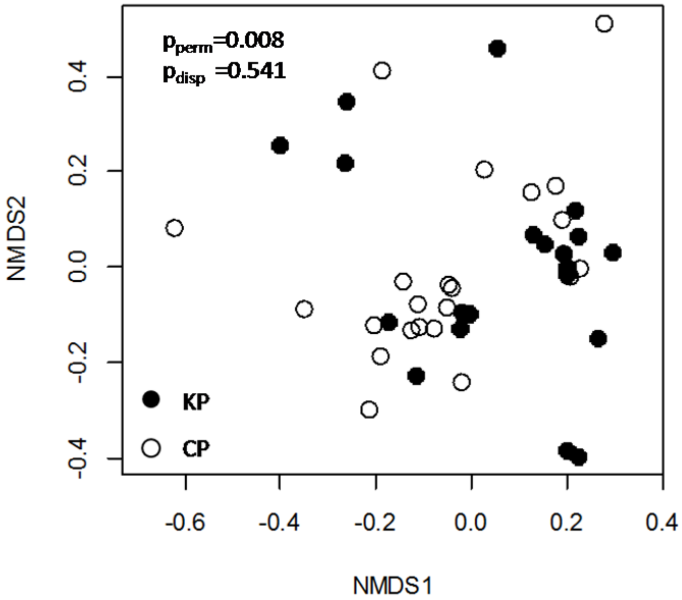

Figure 2 Scatterplot from NMDS based on Bray-Curtis dissimilarity index showing significant compositional differences of woody species in the understory of kiln and control plots in oak forests (p_{perm} indicates the combined significance of the location and dispersion effect, based on PERMANOVA with 999 permutations; p_{disp} indicates the significance of the dispersion effect).

The established tree regeneration layer included a total of eight tree and nine shrub species. The species pool was larger in CP than in KP (Fig. 3). In the of KP sclerophyll forests, this layer contained three woody species (present in 16.7% of the plots), compared to a total of seven species occurring in the total sample of CP (Appendix 1). In oak forests, no species were recorded in KP, while eight woody species were present in this layer in 72.7% of the CP plots (Appendix 2). In this forest type, species richness was much lower in KP than in CP (Tab. 2). No tree species occurred in the established regeneration layer of KP in beech forests, while *Fagus sylvatica* was present in 14.3% of the CP (Appendix 3).

Mixed model results showed that the random variation across the examined forest areas was not relevant for the richness of woody species in the two layers (Tab. 2).

Compared to CP, species richness in the understorey of KP was slightly increased (+0.24), while this was strongly decreased (-2.42) in the established regeneration layer (Tab. 2, Fig. 3). Concerning the other variables, forest and altitude affected species richness of both layers (Tab. 2).

According to the selected model, overall seedling density in the understorey was not affected by the habitat type (Tab. 2), in line with the non-significant differences in the density of each individual species resulting from the Mann-Whitney test; the only exception was *Quercus pubescens* which showed a higher density in the CP of sclerophyll forests (Tab. 4).

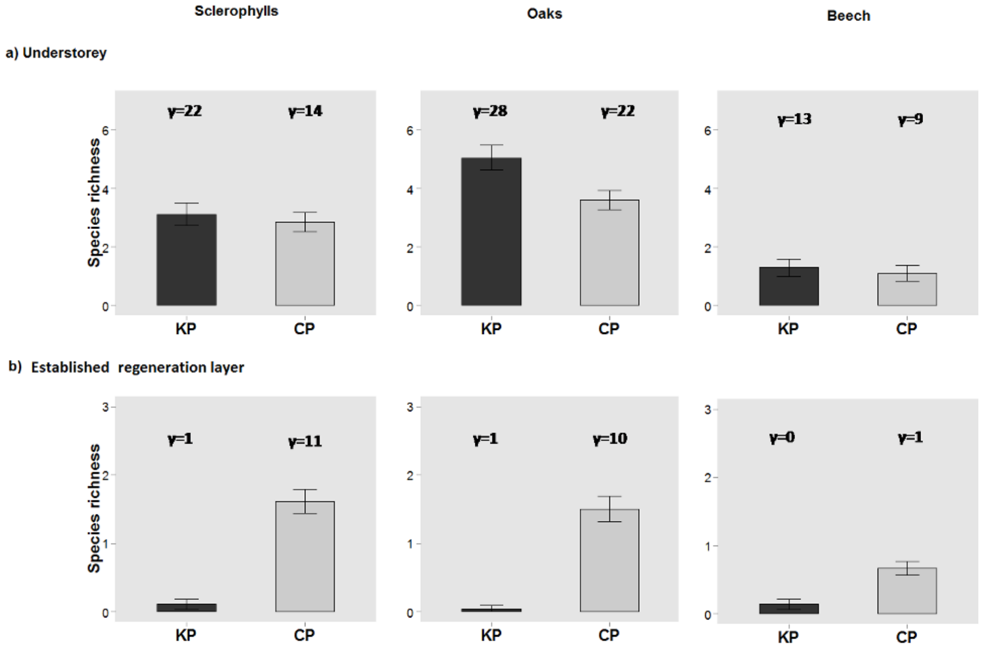

Figure 3 Bar graphs with standard errors showing differences between kiln plots (KP) and control plots (CP) in plot-level species richness for the understorey (a), established tree regeneration layer (b), separately for the three forest types. The total species richness (γ) related to plot type and forest type is also reported above the corresponding bars.

Concerning the structural variables in the established regeneration layer, there were large differences between the three forest types when considering the control plots. As expected, the density of trees and shrubs in the sclerophyll and oak stands was considerably higher than in the beech stands. However, such difference was in general much reduced in the kiln plots, where there was a substantial lack of woody species in all three forest types. Indeed, each single variable resulted affected by the habitat type, though the models did not explain more than 30% of the total variation (Tab. 4). The number of woody stools and stems in KP was always lower than in CP (Fig. 4a-b); in the oak and beech forests, tree species were often nearly completely lacking. Similarly, the mean basal area of KP was always very low compared with CP (Fig. 4c), though the model for this parameter explained only 26% of the total variation. All species in CP were significantly taller than in KP (Fig. 4d).

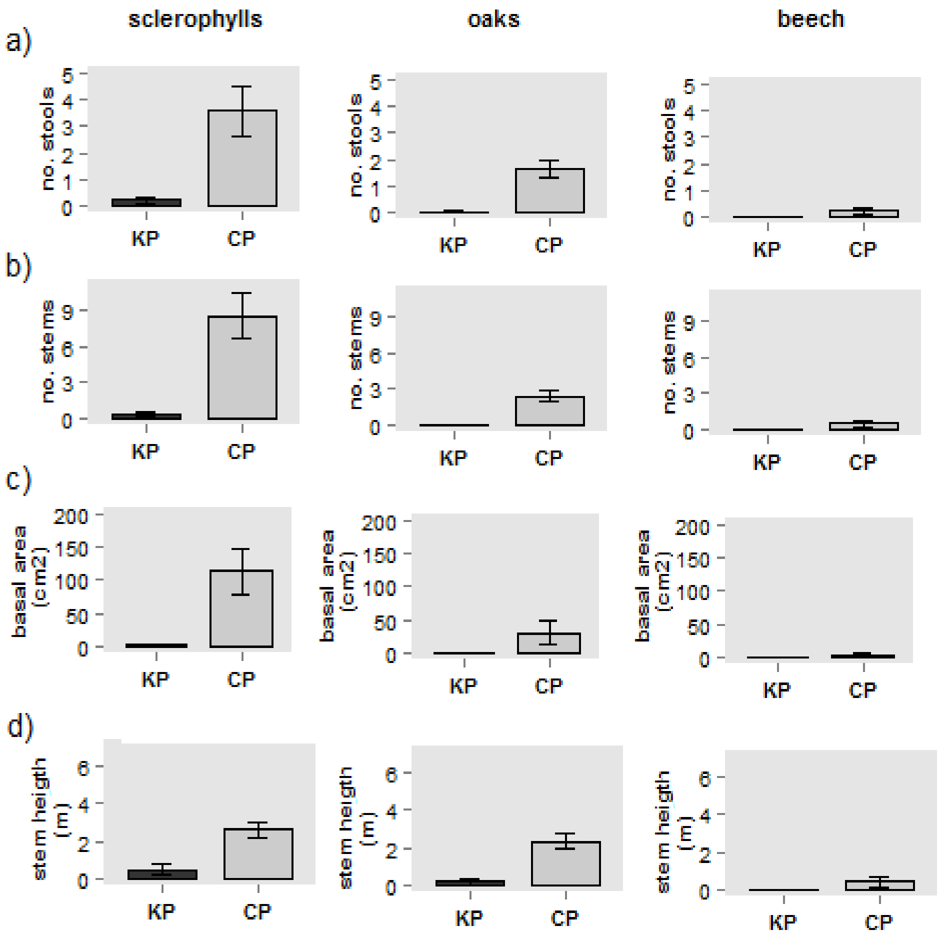

Figure 4 Bar graphs with standard errors showing differences between kiln plots (KP) and control plots (CP) in structural parameters of the established tree regeneration layer (1.30-4 m), separately for the three forest types; a) mean number of stools per plot; b) mean number of stems per plot; c) mean basal area (G, cm^2); d) mean height of tree stems (m).

Table 2. Optimal fixed-effects model structures for the woody species richness at the plot level in the understorey and the established regeneration layer. Models were selected based on AIC criteria (see main text for details). Values for the predictor variables "forest type" (levels: oaks and sclerophylls) and "plot type" (level: kiln plot) and "altitude" are parameter estimates (± standard error) that indicate the relative change of the response variable compared to the first level of the predictor variables "forest type" (level: beech) and "plot type" (level: control plot) or for a unit increment in "altitude" that are incorporated in the intercept. R² refers to the fraction of the variation explained by the optimal model structure; df: degrees of freedom.

Species richness	df	R^2	Overdisp	Intercept	Forest type		Plot type	Altitude[1]
					Oaks	Sclerophylls	Kiln	
Understorey	121	0.435	0.937	1.80±1.18	1.04±0.23	0.52±0.32	0.24±0.11	-0.25±0.17
Tree regeneration	121	0.32	0.918	-2.03±0.57	2.19±0.61	2.46± 0.60	-2.42±0.47	/
Tree density[2]	29	0.30		5.28±0.40	-2.14±0.55	-1.14± 0.57	/	/

[1] square root transformed

[2] VarIdent variance structure has been used

Table 3. Optimal fixed-effects models for the structural variables in established tree regeneration layer, based on AIC selection. The random factor “forest area” was not significant. Values for the predictor variables “forest type” (levels: oaks and sclerophylls),“plot type” (level: control), “Aspect” (levels: E,N, NE, NW, W, S, SE, SW) and “altitude” are parameter estimates (± standard error) that indicate the relative change of the response variable compared to the first level of the predictor variables (beech, control and no exposition, respectively for forest type, plot type and aspect) or for a unit increment in “altitude” that are incorporated in the intercept. R² refers to the fraction of the variation explained by the optimal model structure; df: degrees of freedom; over-dispersion is shown for Poisson distribution.

	df	R²	Ov	Intercept	Forest type		Plot type	Aspect								Altitude[2]
					oaks	sclerophylls	kiln	E	N	NE	NW	W	S	SE	SW	
No. stools	121	0.288	1.546	-0.68± 0.52	1.92±0.49	2.81±0.49	-3.04± 0.46	-0.56±0.31	-0.90±0.28	-16.3±1.10	-0.60±0.38	-1.33±0.35	-1.66±0.37	0.06±0.52	-0.16±0.57	/
No. Stems[1]	121	0.294	2.837	-0.13±0.63	1.62± 0.59	2.87±0.58	-3.30±0.61	-0.24± 0.38	-0.79±0.36	-16.4±1.71	-0.11± 0.41	-1.19± 0.43	-1.05± 0.40	-0.07± 0.77	-0.25±0.83	/
Basal area[2,3]	121	0.261	/	4.65±0.87	/	/	-3.68±0.70	-0.39±0.60	0.17±0.53	0.59±1.07	2.10±0.72	0.27±0.56	0.79±0.55	0.69±0.76	1.88±0.75	-0.05±0.02
Stem height[3]	121	0.306	/	2.70±0.37	/	/	-1.54±0.25	/	/	/	/	/	/	/	/	-0.04±0.01

[1] standard errors corrected by using a quasi-GLM model

[2] square root trasformed variables

[3] VarIdent variance structure was used

Table 4. Density of seedlings of the dominant tree species in the charcoal kiln (KP) and control plots (CP) of the three forests types, with significance of differences (p, Mann-Whitney U test).

Species	Forest type	density/plot (9 m^2)		
		KP *n*=5	CP *n*=5	p
Quercus petraea	Oak	2.50	2.83	n.s.
Quercus ilex	Oak	0.83	2.83	n.s.
Quercus cerris	Oak	4.67	7.33	n.s.
Quercus pubescens	Oak	0.50	0.50	n.s.
Quercus ilex	Sclerophylls	5.00	18.40	n.s.
Quercus cerris	Sclerophylls	0.80	0.80	n.s.
Quercus pubescens	Sclerophylls	3.00	7.00	0.041 *
Quercus suber	Sclerophylls	3.00	1.40	n.s.
Fagus sylvatica	Beech	29.00	28.20	n.s.

Discussion

By extensively sampling in three distinct forest types, this study shows that former charcoal kiln sites represent a peculiar microhabitat for the regeneration of woody species. First, our results indicate that these sites provide favourable conditions for the germination and early establishment of the seeds of trees and shrubs with different ecological requirements and functional traits. Seedlings or young individuals of pioneer shrubs such as *Cytisus scoparius*, *Crataegus monogyna* and *Prunus spinosa* occurred in the understorey together with those of early- successional and late-successional trees such as *Fraxinus ornus* and *Quercus ilex*, respectively, depending on the forest type. In oak forests there was a higher woody species richness and a different species composition on the kiln sites, indicating that charcoal accumulation in the soil does not have detrimental effects on the diversity of trees and shrubs at very early development stages. The flat terrain on the kiln platform is not or only poorly subjected to erosion, the soil is rich in organic matter and nutrients and not strongly acidified, and light availability is generally higher than in the adjacent forest (Carrari et al., *accepted ms*). These factors are likely to promote seed germination and the initial stages of growth, suggesting that kiln sites may act similarly to small gaps with positive effects on regeneration (Poulson and Platt 1989, Yamamoto 2000, Beckage et al. 2008). It is well documented that canopy gaps can provide recruitment opportunities for tree seedlings and thus increase the number of species, explaining why they have figured prominently in empirical and theoretical investigations of mechanisms that promote forest diversity (e.g. Platt & Strong 1989, Busing & White 1997). On the other hand, a sharply reversed situation emerged about diversity in the established regeneration layer including all individuals between 1.3 and 4 m high. Here, both γ-diversity and plot-level diversity in terms of species richness was overall much lower on the kiln sites in oak and sclerophyll forests, clearly indicating less favorable conditions for trees and shrubs at later stages of development.

A similar trend of variation emerged for the structural variables analyzed in this study. When looking at the density of seedlings in the understorey of the three forest types, this was not affected by the plot type, meaning that juvenile trees perform similarly well in the two situations. Above 1.3 m, however, all structural variables were negatively affected by the charcoal kiln habitat, where the number of stools per plot was much lower than in the adjacent stands in the oak and sclerophyll forests. Here, the woody plants were often completely lacking.

Hence, our data show that a strong selection effect occurs in the kiln sites at some later development stages of the woody species, which dramatically reduces the number and abundance of those that are able to leave the understory and reach the upper layers. This effect hinders or at least slows down the recolonization of even the oldest kiln platforms, suggesting that pyrogenic charcoal incorporated in the soil may not always have such a positive influence on tree growth as recently suggested by authors who support the "biochar" practice to promote forest restoration (Thomas & Gale 2015). In our opinion, more experimental investigation is needed to address the long-term effect of this practice on the growth performance of forest trees. Different abiotic and biotic factors are likely involved in the selection effect that we observed, among which water availability, nutritional aspects, accumulation of toxic compounds due to repeated wood pile burning and interactions with biotic communities in the soil. Although pyrogenic charcoal is known to positively affect soil water holding capacity due to its porosity (Karhu et al. 2011, Yu et al. 2013), the actual availability of charcoal-adsorbed water to plants still needs to be assessed (Karhu et al. 2011). A decrease in water availability might occur in the deep soil layers, e.g. those explored by the root system of trees and tall shrubs as they develop towards the adult phase. The presence of drought-tolerant species in the overstorey of the charcoal sites (e.g. *Fraxinus ornus*, *Arbutus unedo*) vs. the lack of some more mesophytic species in the overstorey of the controls (e.g. *Acer campestre*, *Carpinus betulus*) may lend circumstantial support to this hypothesis. On the other hand, this negative effect on water availability may not occur in the topsoil layers supporting the young seedlings, as suggested by their similar density and diversity in the understorey observed in this study. This is also in line with the higher diversity and biomass production of the herbaceous understorey recently found on kiln platforms (Carrari et al., a, ms.

accepted). The nutritional effects of wood charcoal on tree growth are still not completely clear, although it is reported that absorption of phenolic compounds can favour microbial communities and thus nitrification in especially acid forest soils (DeLuca et al. 2009), and that the availability of some macro- and micronutrients is higher in charcoal hearth soils over centennial timescales (Criscuoli et al. 2015). On the other hand, a negative influence could occur indirectly via alterations of the mycorrhizal communities caused by charcoal accumulation and/or fire. For example, Warnock et al. (2007) showed that the negative impact of the biochar addition on arbuscular mycorrhizal fungi was largely due to nutrient effects, while Wallstedt et al. (2002) reported a decrease in both bio-available organic carbon and nitrogen in their ectomycorrhizal system. Also, Gaur and Adholeya (2000) found that the biochar media limited the amount of available P taken up by host plants, indicating that charcoal may in some cases reduce the formation of mycorrhiza by decreasing nutrient availability or creating unfavourable nutrient ratios in soils (Wallstedt et al. 2002). Concerning fire, it is well documented that slash pile burning in forests alters the chemical properties of the soil and has a negative impact on the populations of arbuscular mycorrhizal fungi (Korb et al. 2004) and microbial communities (Jiménez Esquilin et al. 2007). According to Longo et al. (2014) fire occurrence negatively affects the diversity and richness of these fungi, in line with several studies showing a strong impact on ectomycorrhizal formation in conifer forests (e.g. Torres & Hornubia 1997, Grogan et al. 2000, Dahlberg 2002).

Conclusions

The extensive networks of old charcoal kiln sites in Mediterranean forests provide a natural experimental setting to investigate the long-term effects of wood charcoal accumulation in the soil on the growth and development of woody species. This work is the first focusing on the early recolonization processes, and showed a significant effect of these sites. On one hand, they positively influenced the overall richness of woody species at their first stage of regeneration (e.g. in the understorey) in all three forest types, and especially in oak and sclerophyll forests. On the other hand, we found that the further growth and development of trees is negatively affected by some persistent factors of biotic or abiotic nature that prevent their access and establishment into the higher layers, thus causing a substantial lack of forest recolonization. Hence, although abandoned since decades or even centuries ago, charcoal kiln sites are still hostile microhabitats for most woody species. Further experimental investigation is needed to understand the direct of effects of charcoal soil accumulation on the growth and vitality of forest trees, as well as the effects of repeated burning practice on also the mycorrhizal communities involved in the nutrition of trees.

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Paper II

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Former charcoal kiln sites as microhabitats affecting understorey vegetation in Mediterranean forests

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Nomenclature: Pignatti S. (1982)

Abbreviations: KP: charcoal kiln plot; CP: control plot

Abstract

Question Production of wood charcoal is an ancient form of anthropogenic forest use that existed for millennia in the Mediterranean countries and only vanished in the last century. As a result, thousands of abandoned charcoal kiln sites still occur in present-day woodlands. Because of peculiar light and soil properties, the understorey vegetation at these sites may differ from the adjacent stands. Our study investigated for the first time the effects of abandoned kiln sites on understorey vegetation diversity, composition and biomass production in Mediterranean forests.

Location Tuscany, central Italy (42°44'16"N-44°3'13" N; 10°29'49"E-11°29'1"E).

Methods One 3 x 3 m kiln plot on charcoal kiln area and one 3 x 3 m control plot in the adjacent stands were established in 59 representative sites located in three major forest types dominated by evergreen sclerophylls, deciduous oaks and beech. In each plot, diversity and composition of the understorey community were analyzed together with soil factors (content of C, N, C:N ratio, pH) and light conditions (PAR). A 50 cm x 50 cm frame was randomly placed in each plot to measure biomass production.

Results The charcoal kiln habitat positively affected understorey diversity and productivity, as well as the content of C, C:N ratio, pH and light availability. Floristic dissimilarities between kiln plots were larger than between control plots, and significant compositional differences between the two plot types occurred. Graminoids were more abundant on kiln plots, and 12 indicator species were found for this habitat in oak forests. Higher values of cover and biomass showed the lack of detrimental effects of wood charcoal accumulation on understorey productivity.

Conclusions Continued wood charcoal production in Mediterranean woodlands has caused long-lasting effects on the understorey via persistent changes of abiotic factors. Hence, former kiln sites represent anthropogenic microhabitats that increase biodiversity and fine-scale heterogeneity of forest ecosystems. Conservation measures are advocated to preserve them against various external threats.

Introduction

In the last decades, an increasing number of studies has highlighted the impact of historical human activities on forest landscapes. In central and northern Europe, it is well documented that various forms of traditional land use have shaped these ecosystems since the times of the first civilizations and still affect present-day soil characteristics (Glatzel 1990; Dupouey et al. 2002; Plue et al. 2008). Via their influence on soil properties, practices such as coppicing, controlled burning, plant domestication and livestock grazing, have strongly affected the diversity, composition and productivity of the understorey vegetation of European forests, especially in the Mediterranean region (Lloter & Vilà 2003; Blondel 2006; Bartha et al. 2008; Kopecký et al. 2013). Because of the herb layer responsiveness to local site conditions (Gilliam 2007), understanding the present-day structural and compositional features of forest understories is hardly possible without taking into account the long-lasting effects of past land uses (Peterken & Game 1984; Hermy & Verheyen 2007). However, not all the effects of the former human activities on the understory have been investigated, and some of them still remain poorly understood. One of these activities is the production of wood charcoal in forests, which started at least with archaeometallurgy (ca. 4000 B.C.; WSL 2011) and continued for millennia in Europe and the Mediterranean region. Charcoal was the main source of energy since the Iron Age until the 19th century (Blondel 2006). Its production, based on the pyrolysis of wood at ca. 400 °C without oxygen, was realized in coppice stands in special woody kilns covered by a mixture of soil and plant material. For this purpose, small ovoidal terraces (30-45 m^2) known as charcoal kiln sites or charcoal hearths were prepared along footpaths on hill and mountain slopes (Montanari et al. 2000; Ludemann 2003; WSL 2011). In most northern and central European countries this practice was abandoned in the 19th century due to the rapidly increasing and widespread use of coal (Deforce et al. 2012). In the Mediterranean region, however, the importance of wood charcoal raised during the industrial revolution since other fuel sources were largely lacking, and mostly vanished around the year 1950. In some remote mountain areas it even continued until today. As a result, a great number of abandoned charcoal kiln sites are nowadays widespread in many European forests (Ludemann 2011; 2012), and especially in the Mediterranean countries (Blondel 2006; Nocentini & Coll 2013). Their main characteristics are the flat, regular terrain and the alterations in colour and texture of the topsoil caused by the formation of thick layers (> 20 cm) rich in wood charcoal remains (Criscuoli et al. 2014).

Wood charcoal is able to cause significant changes in various structural and functional properties of the soil that are important for plant growth (Nelissen et al. 2014), such as nutrient availability and water holding capacity (DeLuca et al. 2009; Criscuoli et al. 2014). Hence, the presence of charcoal remains has the potential to induce significant variations in the diversity, productivity and composition of the forest understorey. Additional effects can be caused by the often sparse canopy cover of the charcoal sites, which is due to the usual lack of trees and tall shrubs rooted inside the kiln platforms (Carrari et al., b, *ms. submitted*). This is likely to cause variations in light availability which can have, in turn, considerable effects on the understorey (Härdtle et al. 2003; Hofmeister et al. 2009; Axmanová 2011).

For these reasons, abandoned kiln sites may represent small ecological “islands” offering a potential micro-habitat to woodland plant species with functional traits and requirements that differ from those of the species inhabiting the adjacent forest environment. Although such a hypothesis was already supported by a phytosociological investigation in beech stands of Germany (Wittig et al., 1999) no evidence still exists for the woodlands of the Mediterranean area. Compared with those in central Europe, these ecosystems show a higher diversity of compositional and structural types, which may imply different and heterogeneous responses of the understorey species to the charcoal kiln habitat. Accordingly, we used an extensive sampling in three major forest types of central Italy to address the following questions: 1) Do the remnants of former charcoal kiln sites affect the diversity, productivity and composition of the understorey in different forest ecosystems of the Mediterranean area ?; 2) Can this effect be explained by altered soil properties or light conditions?

Materials and Methods

Study area

Field sampling was performed in the forests of Tuscany (central Italy) situated between 42°44'16"N and 44°3'13" latitude N, and 10°29'49"E and 11°29'1" longitude E (Fig. 1). This area is characterized by three climate types following an altitudinal gradient from sea level to over 1.400 m: 1) meso-Mediterranean along the Tyrrhenian coast, where woodlands are mainly formed by evergreen sclerophylls and especially *Quercus ilex*; 2) supra-Mediterranean on the hill areas in the central part of the region, largely covered by thermophilous mixed forests dominated by various species of deciduous oaks (mainly *Quercus cerris*, *Q. petraea*, *Q. pubescens*); 3) montane-suboceanic on the Apennine range and Mount Amiata, a continental “island” of volcanic origin covered by lush forests of beech (*Fagus sylvatica*; Selvi 1997). These communities correspond, respectively, to forest types no. 9, 8 and 7 of the European Environment Agency classification (EEA 2006). Mean annual rainfall and temperature in the area vary from 650 mm and 15 °C respectively along the coast, to 1450 mm and 10.9 °C respectively on the Apennines and Mount Amiata (period 1961-1990, source: Servizio Meteorologico dell’Aeronautica Militare, http://www.meteoam.it/).

The study area is characterized by a variety of geolithological formations and soil conditions, but cambisols are the prevalent type according to the Soil Atlas of Europe (European Commission, www.eusoils.jrc.ec.europa.eu).

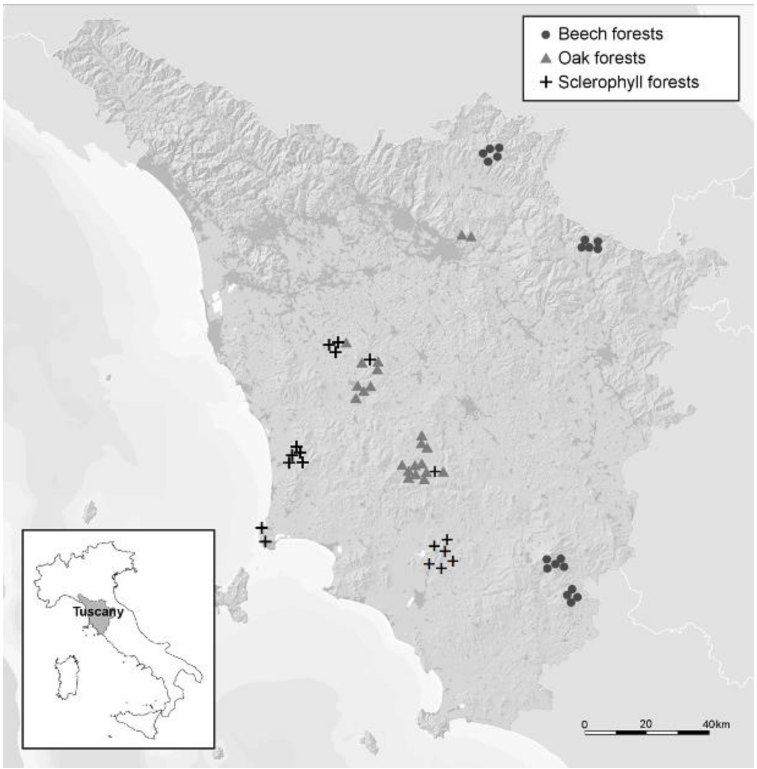

Figure 1 Geographical location of the 59 sites sampled in the study area (Tuscany, central Italy); the three forest types are indicated by different symbols.

An extensive search for abandoned charcoal kiln sites, hereafter referred to as “kilns”, was carried out during *ad-hoc* field trips in representative stands of the three forest types described above (beech, oaks and sclerophylls). The 154 kilns observed were recorded with a GPS device and characterized using simple descriptors such as altitude, slope aspect, soil type, and conservation status of the understorey. From this pool, we selected 59 kilns where the vegetation was not affected by recent anthropogenic or animal disturbance, resulting in 19, 22 and 18 kilns for beech, oak and evergreen sclerophyll forests, respectively (Fig. 1, Appendix S1). Based on local historical documents, these sites are all located in “ancient forests” sensu Hermy et al. (1999), i.e. with a continuous cover since at least the past three centuries. In addition, common knowledge by local rural people indicates that charcoal production on these sites continued for centuries and was abandoned at least ca. 60 years ago.

Data collection

In the centre of each kiln, we established a 3 m x 3 m vegetation plot (*charcoal kiln plot*, KP) inside which all vascular plant species < 1.3 m were assessed for ground cover percentage after their identification following Flora d'Italia (Pignatti 1982). Then, a wooden frame (0.5 m x 0.5 m) was randomly placed inside the KP (*biomass* plot) to clip the above-ground biomass of all species rooted inside. Woody and herbaceous plants (including very young tree seedlings without woody tissues) were kept separately. The clipped biomass was then dried for 72 hours at 40 °C and weighed. Total cover in the vegetation plots and dry weight of the total, herb and woody biomass were then used as proxies for the understorey productivity.

At the four corners and in the centre of the KP, five soil core samples were collected at a depth of 15 cm using steel cylinders. The soil samples were dried and sieved using a 2 mm mesh size, and mixed together to obtain one composite sample per vegetation plot. These samples were analysed for total C, total N (Elementar analyser, type Vario Macro Cube in configuration CNS, with Argon as carrier gas) and pH (H_2O). Light intensity (Photosynthetic Active Radiation, PAR) was measured at 1 m height above the forest floor with a light sensor reader (FieldScout, Spectrum Technologies, Inc., Aurora, Illinois). Measurements were taken at three points along a diagonal of the KP (corners and centre) and averaged for each plot.

For each site, the protocol was repeated in a second 3 m x 3 m vegetation plot (*control plot*, CP), established randomly in the adjacent forest at a distance of 10-20 m from the edge of the KP, excluding downhill locations to avoid potential charcoal "contamination" by runoff. Such a short distance between KP and its relative CP ensured to minimize variation of confounding site factors. Light measurements were performed simultaneously in KP and CP, in order to have identical weather conditions. In the data analyses we refer to the KP-CP pair as a single *site*, in which KP and CP represent different *plot types*.

Data analysis

All analyses were performed in R 3.1.2 (R core team 2014). We started with partitioning the total species pool in six growth forms: trees, shrubs, graminoids (including all grasses of the Poaceae family plus the species of the genera *Carex* and *Luzula*), ferns, vines, and herbs. Understorey diversity was quantified on different spatial scales. The α-diversity was calculated as the total understorey species richness (SR), SR within each growth form and Shannon diversity (H') for all plots (plot-level results). We quantified the understorey compositional dissimilarity (β-diversity) between KP and CP within each site (*intra-site compositional dissimilarity*) using Lennon's distance measure (Lennon et al. 2001; site-level results). Separately for each forest type we also calculated the *inter-site* *compositional dissimilarity* as the mean of the pairwise Lennon dissimilarities of a given plot from all other plots of the same plot type (plot-level results). Gamma diversity (γ) was calculated at the plot type level as the total SR for the pool of plots within a plot type (including therefore all three forest types).

First, in order to avoid pseudoreplications determined by the irregular spatial distribution of the sites, the influence of plot type (KP or CP) on understorey diversity and productivity was tested using mixed models. For understorey SR (not overdispersed) the starting model was fitted with *glmer* function with a Poisson error distribution and *loglink,* for the other variables with *lmer* function with a Gaussian error distribution (*lme4;* Bates et al. 2013). For the intra-site compositional dissimilarity we started from the fixed structure as this response variable is measured between sites. The model selection of each variable followed the protocol of Zuur et al. (2009) where the structure yielding the lowest value for Akaike's Information Criterion (AIC; Akaike 1973) was considered to be most consistent with the data. For models that contained random effects, a conditional R^2 was calculated (Nakagawa and Schielzeth, 2013; *MuMIn* package; Bartoń 2013). Moreover, parameter-specific p-values for each level of the predictors were calculated, using Satterthwaite approximation when models contained the random effect (lmerTest package).

Next, the effect of plot type on the understorey composition was determined as the dissimilarity of each plot against all the other plots, first for all forest types together, then for each forest type, using the Lennon and Bray-Curtis distance measures based on presence/absence and cover data, respectively (*vegdist* function in *vegan* package; Oksanen et al. 2013). Non-metric multidimensional scaling (NMDS) (*metaMDS* function in *vegan* package; Oksanen et al. 2013) was used to visualize the compositional differences between plots. Differences between KP and CP were tested for each couple in the pooled sample and separately for forest types using PERMANOVA; (*adonis* function in *vegan* package; 999 permutations with *strata* ="site"; Anderson 2001; Oksanen et al. 2013). We also tested separately for multivariate homogeneity of dispersion using *betadisper* (*vegan* package) in order to distinguish between the compositional differences determined by the plot type and the dispersion effects within the two plot types (Anderson 2001; Warton et al. 2012).

Third , the index of taxonomic distinctness Δ^+ (Clarke & Warwick 1998) was determined for each plot with the function *taxondive* (*vegan* package; Oksanen 2013), to estimate the degree of taxonomic relatedness of the understorey species in the plot. The index is based on presence/absence data and is defined as the average taxonomic distance between any two species randomly chosen from the plot species pool; this distance is the length of the path connecting these two species traced through a hierarchical tree of classification of the species pool involved. The reference systems of classification used here were those of Smith et al. (2006) for ferns, Christenhusz et al. (2011) for gymnosperms and APG III (Haston et al. 2009) for angiosperms. The randomization test by Clarke & Warwick (1998) was also performed to obtain a confidence funnel graph against which the calculated distinctness values of the plots were checked. This allowed to detect possible effects of KP on the taxonomic distinctness and evenness of the understorey flora.

Fourth, an indicator species analysis (Dufrêne & Legendre 1997) was performed (function *multipatt* in *indicspecies* package; De Cáceres 2013) to identify the species significantly associated with KP or CP for each forest type.

Finally, the influence of plot type on light intensity and soil components were tested using the mixed model approach mentioned above.

Results

Diversity

In total, 240 vascular plant species were recorded across the 118 plots. The γ-diversity at the plot type-level was considerably higher for KP compared to CP, for all forest types together (Appendix S2) as well as for each type (79 vs 64, 141 vs 86, 103 vs 48, in beech, oak and sclerophyll forests, respectively). In beech forests, 15 species were found only in KP vs. 14 in CP, whereas in oak and sclerophyll forests over 50 species were exclusive to KP vs. 11 and 12 to CP.

The optimal models for α-diversity included the random site effect for the total SR, the Shannon diversity, and the SR of graminoids and herbs (Table 1). Kiln plots positively influenced the plot-level SR of all growth forms, except for ferns and vines. The model for the total SR explained 72% of the total variation, while that for the Shannon index accounted for a lower percentage (41%). This index was always higher in KP (Table 1; see Appendix S3 for p-values).

Productivity

Generally, the proxies for understorey productivity showed trends similar to those for diversity described above (Table 1; Appendix S3). Kiln plots had a positive influence on the total cover of the understorey, on the cover of tree seedlings, graminoids and ferns. The total and herbaceous biomass were also positively affected, while no effect on the woody biomass was observed. For all variables (except for shrub cover), the optimal models included the random site variation and explained between 35% and 70% of the total variation (Table 1).

Composition

Permutational analysis of variance revealed significant compositional differences between KP and CP within sites across all plots (p=0.001, Fig. 2), with homogeneity dispersion among plot types (p = 0.259). When considering the three forest types separately, compositional differences between KP and CP were always significant, especially for oak forests ($p_{perm}<0.001$, Fig. 3B); in beech and sclerophyll forests differences were lower but still significant (0.022 and 0.029, respectively; Fig. 3A-C). Dispersion differences between the two plot types were not significant (Fig. 3).

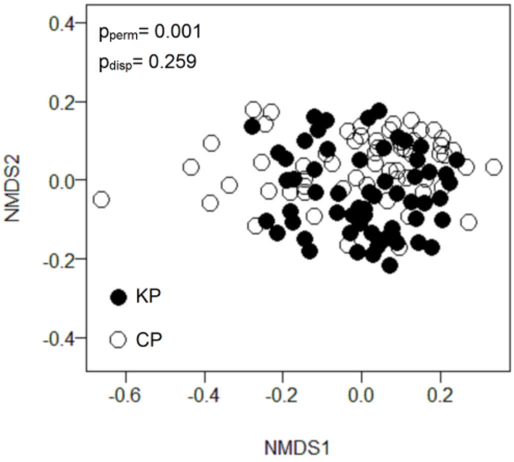

Figure 2 Non-Metric Multidimensional Scaling showing understorey compositional dissimilarity between KP and CP using the cover-weighted Bray-Curtis distance measure; p_{perm} indicates the significance of the combined effect of location and dispersion for each pair of plots (site), based on PERMANOVA; p_{disp} indicates the significance of the dispersion effect.

Regardless of the distance measure used, inter-site compositional variation was highest for KP (Table 1). This means that floristic differences were higher between KP than between CP. In addition, mixed model results showed that the intra-site compositional dissimilarity was not influenced by the forest type (Table 1), indicating compositional differences of the same magnitude between KP and CP in the three forest types (Appendix S3).

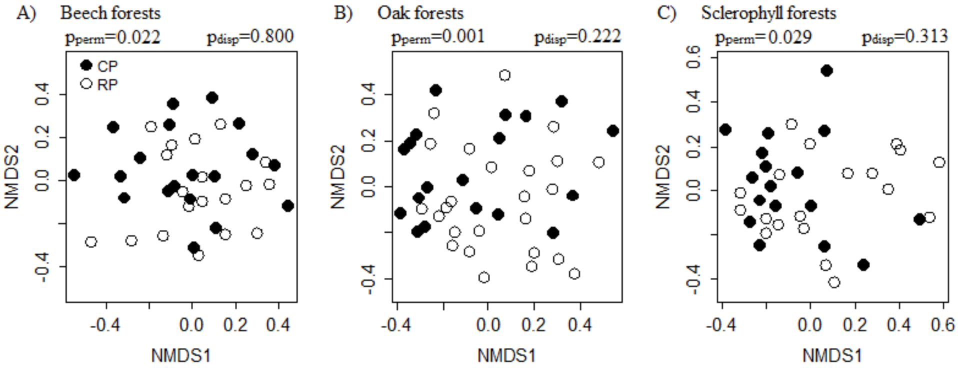

Figure 3 Non-Metric Multidimensional Scaling showing understorey compositional dissimilarity between KP and CP using the cover-weighted Bray-Curtis distance measure separately for beech (A), oak (B) and sclerophyll forests (C); p_{perm} indicates the significance combined of the combined effect of location and dispersion for each pair of plots (site), based on PERMANOVA; p_{disp} indicates the significance of the dispersion effect.

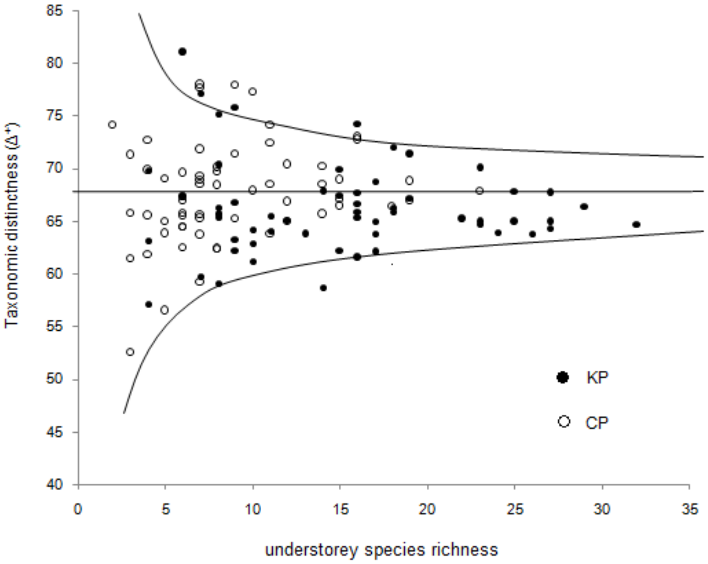

Figure 4 Taxonomic distinctness Δ^+ of the understorey vegetation in kiln (KP) and control (CP) plots in relation to understorey species richness. The horizontal line shows the theoretically expected mean taxonomic distinctness, the 95% confidence from the randomization test is shown (Clarke & Warwick, 1998; see text for further details).

Taxonomic distinctness (Δ^+) was negatively affected by KP, although the model explained only a low percentage of the variation for this response variable (Table 1; Appendix S3). In addition, three KP and four CP in beech forests had Δ^+ values that significantly diverged from the theoretical expected value and above the upper 95% confidence, while only one

KP in sclerophyll forests was below the lower limit (Fig. 4), indicating that the negative effect found by the optimal model was very minor. Indicator species analysis resulted in one indicator herb for CP in beech forests, *Anemone nemorosa*, which occurred in 52% of CP vs.10% of KP. As many as 12 species were instead associated to KP in oak forests, most of which are of early-successional type and have high light requirements, such as *Cardamine hirsuta*, *Cytisus scoparius, Dactylis glomerata, Fraxinus ornus* and *Prunella vulgaris* (Table 2).

Table 2 Indicator species in relation to forest and plot type; frequency indicates the proportion of plots of the associated plot type where the species was observed. p-values from permutation test show the significance of the association. Ancient forest species according to Hermy et al. (1999) are indicated with an asterisk (*). Plant nomenclature follows Pignatti (1982).

Forest type	Plot type	Indicator species	Growth form	Frequency (%)	p
Beech forests	Control	*Anemone nemorosa**	herb	52	0.003
Oak forests	Kiln	*Brachypodium sylvaticum**	graminoid	41	0.005
		Cardamine hirsuta	herb	27	0.019
		Coronilla emerus	shrub	50	0.042
		Cytisus scoparius	shrub	41	0.046
		Dactylis glomerata	graminoid	27	0.029
		Fraxinus ornus	tree	86	0.008
		Luzula forsteri	graminoid	36	0.017
		Moehringia trinervia	herb	41	0.019
		Poa trivialis	graminoid	27	0.031
		Prunella vulgaris	herb	23	0.047
		Viola alba subsp. *dehnardtii*	herb	45	0.049
		*Viola reichenbachiana**	herb	45	0.037

Soil and light conditions

Mixed model results showed that total soil C and N content, C/N ratio and soil pH were all lower in CP (Table 1; Appendices S2 and S3). The random site effect was included in the optimal models for all the above variables, except C/N ratio. The strongest difference between the two plot types was in total C content, which was ca. two times higher in KP; differences in N content and pH were less pronounced but still significant (Appendix S2). Finally, light availability was higher in KP than in CP (Appendices S2 and S3), especially in oak forests (KP: 55±90, CP: 13±9; $p<0.001$). However, the high variation of PAR values accounted for the low proportion (5%) of total variation explained by the best model ($R^2=0.05$; Table 1).

Table 1 Optimal mixed-effects model structures relating all the response variables to the forest type and plot type [R-syntax: y~ Forest type + Plot type + (1 | Site)]. Values for the predictor variables, forest type (levels: oaks and sclerophylls) and plot type (level: charcoal plot), are parameter estimates (± standard error) that indicate the relative change of the response variable compared to the first level of the predictor variables (beech forest and control plot respectively) that is incorporated in the intercept. R^2 refers to the fraction of the variation explained by the optimal model structure (n: number of observations; '/': predictor variable not present in optimal model structure; 'NA': not applicable; § indicates a random effect for *site*). Response variables: α- diversity, calculated as species richness (SR) (total SR, SR of each growth form) and Shannon index; plot-level understorey total cover, cover of each growth form; total, herb and woody biomass; β-diversity, calculated as inter-site and intra-site compositional dissimilarity using Lennon (L) and Bray Curtis (BC) distance measures; plot-level taxonomic distinctness, soil factors (C, N, C/N, pH), and light (PAR).

Response variable	Unit	n	R^2	Intercept	Forest type		Plot type
					Oak	Sclerophyll	Charcoal kiln
SR total§		118	0.718	1.94 ± 0.10	0.45 ± 0.13	-0.003 ± 0.13	0.51 ± 0.05
SR trees		118	0.095	0.063 ± 0.16	1.08 ± 0.17	0.41 ± 0.19	0.28 ± 0.12
SR shrubs		118	0.156	-0.70 ± 0.23	1.24 ± 0.24	1.37 ± 0.24	0.29 ± 0.15
SR graminoids§		118	0.526	-0.38 ± 0.24	0.28 ± 0.26	-0.73 ± 0.32	0.98 ± 0.17
SR ferns		118	0.109	-1.24 ± 0.30	-0.75 ± 0.51	-1.25 ± 0.65	/
SR vines		118	0.192	-0.80 ± 0.24	1.42 ± 0.27	1.30 ± 0.28	/
SR other herbs§		118	0.578	1.29 ± 0.14	-0.04 ± 0.17	-0.71 ± 0.19	0.72 ± 0.09
Shannon§		118	0.413	1.31 ± 0.11	0.30 ± 0.14	0.10 ± 0.14	0.26 ± 0.08
Cover all§	%	118	0.482	3.43 ± 2.24	7.86 ± 3.08	-0.95 ± 3.23	10.13 ± 1.81
Cover trees§	%	118	0.603	0.077 ± 0.56	1.76 ± 0.73	0.65 ± 0.77	0.90 ± 0.32
Cover shrubs	%	118	0.053	0.082 ± 0.60	2.02 ± 0.82	2.27 ± 0.87	/
Cover graminoids§	%	118	0.346	1.08 ± 0.83	0.54 ± 1.04	-1.76 ± 1.09	2.70 ± 0.67
Cover ferns§	%	118	0.527	0.24 ± 0.18	/	/	0.32 ± 0.18
Cover vines§	%	118	0.696	0.092 ± 0.33	1.13 ± 0.44	0.38 ± 0.47	/
Cover other herbs§	%	118	0.346	1.73 ± 1.53	2.50 ± 1.89	-2.00 ± 1.99	/
Total biomass§	(g/m^2)	118	0.493	3.19 ± 2.84	8.38 ± 3.66	1.10 ± 3.84	5.04 ± 1.90
Herb biomass§	(g/m^2)	118	0.516	0.68 ± 1.21	3.35 ± 1.55	-0.45 ± 1.62	5.38 ± 0.87
Woody biomass§	(g/m^2)	118	0.483	4.70 ± 1.13	/	/	/
Inter-site diss. L §		118	0.382	0.70 ± 0.02	-0.06 ± 0.02	-0.07 ± 0.02	0.05 ± 0.01
Inter-site diss. BC §		118	0.417	0.92± 0.01	-0.05 ± 0.01	-0.04 ± 0.01	0.02 ± 0.01
Intra-site diss. L		59	NA	0.44 ± 0.02	/	/	NA
Intra-site diss. BC		59	NA	0.77± 0.03	/	/	NA
Tax.distinctness Δ+		118	0.088	69.58 ± 0.83	-2.27 ± 0.99	-3.45 ± 0.99	-1.39 ±0.82
C§	%	118	0.688	3.57 ± 0.62	3.43 ± 0.80	2.63 ± 0.84	4.85 ± 0.43
N§	%	118	0.66	0.42 ± 0.02	/	/	0.08 ± 0.02
C/N		118	0.717	10.45 ± 0.50	3.97 ± 0.59	4.44 ± 0.62	7.62 ± 0.49
pH§		118	0.841	5.36 ± 0.17	0.80 ± 0.23	0.63 ± 0.25	0.22 ± 0.06
PAR	µmol m2/s	118	0.05	20.75 ± 6.40	/	/	24.32 ± 9.06

Discussion

In all three ecosystems examined, abiotic factors and understorey vegetation in the charcoal kiln platforms resulted considerably different from the adjacent forest environment, showing that the effects of this traditional activity persist over a time scale of decades or even centuries.

Soil factors and light

A first effect was the increased total C content in the soil, probably caused by the abundance of charcoal fragments to a depth of 15 cm at least. These were still abundant in our samples, indicating slow rates of incorporation and transportation of charcoal C into the soil matrix. This result is supported by evidence from a slash-and-burn experimental study in a temperate forest (Eckmeier et al. 2007) and by the recent finding that total C in centuries old kiln sites in Alpine forests was three times higher than in the adjacent stands (Criscuoli et al. 2014). The condensed aromatic structure of charcoal or black carbon allows fragments and particles to persist in soils and other sedimentary records over millennial time-scales (Cheng et al. 2008; Lehmann & Joseph 2009). Very old charcoal samples (>8000 years BP) originating from wildfires have been found almost unaltered in forest soils (Marguerie & Hunot 2007), and anthropogenic deposits of charcoal dating back to the Neolithic period have been documented in Germany and Italy (Schmid et al. 2002; Cremaschi et al. 2006, Ludemann 2010). Such long-term stability explains why biochar applications in agricultural systems are increasingly considered a promising practice for mitigating the impact of climate change by carbon sequestration (Vaccari et al. 2011; Nelissen et al. 2014).

The higher C content resulted in considerably higher C/N ratio in the charcoal kiln platforms, but the significantly higher pH values and content of total N suggest the lack of detrimental effects for plant growth such as acidification and nutrient shortage. Our results are in line with findings in German beech forests, where the soil of old kiln platforms had higher pH values and supported understorey species with higher nutrient requirements than in the adjacent stands (Wittig et al. 1999). An explanatory hypothesis is that the C fraction deriving from charcoal is mostly biologically inert, due to the refractory structure of the latter and its poor accessibility when physically enveloped by soil particles (Brodowski et al. 2006). Increased nitrogen availability and C/N ratio without significant alteration of soil pH and other soil chemical properties has also recently been found in biochar field experiments (Nelissen et al. 2012, 2014), and nitrogen availability was also improved by biochar addition (DeLuca et al. 2009; Nelissen et al. 2012).

On the whole, light availability was positively affected by the presence of charcoal kilns, mainly due to the lack of adult trees and tall shrubs rooted inside the platforms (Carrari et al., b, *ms. submitted*). Difference was larger in the oak forest due to the relatively high amount of light transmitted by the crowns of the adult adjacent trees, as typical for the deciduous oak species (Bréda 2003). In the two other forest types, differences were smaller due to the stronger shading effect of the beech and the holm oak, whose crowns expand laterally and reduce the canopy discontinuity over the kiln platforms.

Regardless of these differences, increased light availability in especially the oak forest, contributes to an additional edge/gap effect, which is known to affect understorey species richness and composition in most temperate woodlands (Murcia 1995; Gálhidy et al. 2005; Gonzalez et al. 2010).

Diversity and composition

Decreased soil acidity, increased nutrient availability and higher light intensity are assumed to be favourable factors for many European forest herb species (Chytrý et al. 2003; Ewald 2008; Axmanová et al. 2011; Verstraeten et al. 2013), which may explain the higher floristic richness (γ-diversity) in the charcoal kiln habitat. Increased diversity also emerged at the plot-scale, where species richness and Shannon values were higher than in the adjacent stands. Although mean plot-scale alpha-diversity of the forest may have been here underestimated due to the small size of the control plots, this potential problem is reduced by the relatively high number of the plots and the relatively homogeneous distribution of the understory flora in the examined forests.

The peculiar environmental conditions in terms of light and soil also affected the understorey composition on the kiln platforms. Differences between KP and CP are likely due to transient heliophilic or intermediate shade-tolerant species originating from seed banks or seed rain, which can establish also thanks to the reduced canopy cover as in the case of gaps of sufficient size (Schumann et al. 2003; Decocq et al. 2004). Kiln plots induced compositional variations of similar magnitude in the three forest types, implying an equally positive effect on the richness of the total species pools. In addition, floristic differences among KP were distinctly larger than those among CP, showing the significant contribution of these habitats to the understorey species richness at the forest-scale level. Reasons for this floristic variability among kiln platforms are likely associated with various stochastic factors leading to the inclusion of infrequent and non-specialist forest species, similarly to the case of edges in fragmented forest landscapes (Harper et al. 2005). The combined effects of local abiotic factors and competition act as a filter on such rich species pools and results in variable understorey assemblages at the plot-level, as predicted by the resource heterogeneity hypothesis (Ricklefs 1977; Huston 1979). Lower understorey heterogeneity occurs instead under the closed canopy of the stands adjacent to the kilns, mainly due to the smaller species pools upon which this “filter” mechanism can act.

Indicator species of the kiln habitat were only found for the species-rich oak forests, representing four of the six growth forms considered here (all except ferns and vines). The relatively high Ellenberg mean value for light of these species (6.5) is in line with the increased mean PAR values measured in KP (Appendix S2) and the early-successional character of some of them, such as the fast-growing shrub *Cytisus scoparius* and the pioneer tree *Fraxinus ornus.* On the other hand, the occurrence of ancient forest species (*sensu* Hermy et al. 1999) such as *Brachypodium sylvaticum* and *Viola reichenbachiana* shows that the charcoal kiln habitat can provide a suitable niche to also less heliophilic plants with relatively low colonization ability. The geophyte *Anemone nemorosa* resulted positively associated with CP in beech forest, implying a decreased frequency and abundance in the kilns, in line with evidence from charcoal sites in beech forests of Germany (Wittig et al. 1999). Although this ancient forest species is ecologically plastic and its responses to drivers change are difficult to predict (Baeten et al. 2010), the modified topsoil environment may be a reason for its reduced presence on kiln platforms since the superficial rhizome system is sensitive to various forms of chemical and physical alteration of the growth substrate (Shirreffs 1985; Philipp & Petersen 2007).

Kiln understorey was affected also in terms of growth-forms composition. The canopy-gap effect induced by KP can explain the increased total proportion of graminoids, in line with existing evidence that these plants usually benefit from higher light availability (Decocq et al. 2004; Verstraeten et al. 2013). On the other hand, the parallel increase of herbs, among which some shade-tolerant species, and the non-increase of ferns suggest that soil factors specific to the kiln platforms are likely involved in the changes of understorey growth-forms composition. Similar evidence was found in German beech forests, where the shift from the graminoid-dominated understorey of the *Luzulo-Fagetum* to the herb-dominated community of the *Galio odorati-Fagetum* on the kiln platforms was due to soil factors rather than to increased light availability (Wittig et al. 1999).

Enhanced floristic diversity in KP was paralleled by only a very minor negative effect on the taxonomic distinctness of the understorey assemblage. This appears noteworthy, as anthropogenic disturbance and pollution often have a strong negative impact on the taxonomic “spread” of biotic communities in different ecosystems (Stark et al. 1998; Clarke & Warwick 1998). In only one KP the taxonomic distinctness was below the 95% confidence limit of expected values, showing that charcoal accumulation in the soil does not lead to the exclusion or substantial reduction of the number and relative abundance of the taxonomic groups in the understorey community.

Productivity

Understory productivity was enhanced in KP, as consistently shown by ground cover and total and herb biomass values. Hence, the long-term presence of charcoal in the soil does not have detrimental effects on growth and development of the herb-layer, including young seedlings of woody species. Our results from forest ecosystems are the first ones providing circumstantial support to a number of biochar experimental studies in European agricultural systems, where it was demonstrated that even high charcoal application rates can promote crop yields (Baronti et al. 2010; Sohi et al. 2010; Vaccari et al. 2011). Increased water availability and improved structure and aggregate formation in the soil (Lehmann & Joseph 2009), reduced nutrient leaching (Yanai et al. 2007) and nutrient availability (Rondon et al. 2007) are considered key factors accounting for such beneficial effects on plant growth.

Different factors can be involved in the enhanced understory productivity on the kilns, among which light availability is likely an important one. The role of light for biomass production in the herb-layer is well documented, and this in turn, has positive effects on its diversity (Chytrý et al. 2003; Axmanová et al. 2011). Working in various types of central and eastern European deciduous forests, Mölder (2008) and Axmanová et al. (2011) showed that herb-layer species richness monotonically increases with productivity.

Enhanced productivity on KP may also be associated with the flat ground morphology of these terraces, which is likely to favour the concentration of water and nutrients especially after rainy events. Although further studies would be helpful to better understand the influence of slope inclination on the understorey, available evidence does not support any effect on the biomass of the herb-layer in temperate forests (e.g. Axmanová et al. 2011; Siccama et al. 1970).

The lack of differences between KP and CP in the woody biomass is apparently not in line with the higher species richness and cover of tree species on the kiln platforms, but is probably explained by the occurrence of only 1-2 years old seedlings whose above-ground parts were included in the herb biomass.

Conclusions

Based on our findings, abandoned charcoal kiln sites represent stable anthropogenic microhabitats that can increase the diversity, productivity and compositional variability of different types of forest understories, via persistent changes in local soil and light conditions. Because of these long-lasting effects and the multiple potential applications in environmental history and related fields, including soil carbon ecology (Montanari et al. 2000; Ludemann 2004; Ludemann et al. 2003, 2011, 2012; Schoch 2011; Paysen 2011; Criscuoli et al. 2014), specific conservation actions should be considered in the forest management policy of at least protected areas. Together with the impact of wild ungulates, silvicultural practices such as forest track construction, mechanized wood extraction etc. (e.g. Ludemann 2011), or recreational use by local people or eco-tourists are currently the main causes of severe damage or even destruction of these neglected sites. Hence, their identification and inventory could be a first step for conservation programs in protected areas, allowing, for example, to design and/or modify existing tracks or footpaths in a way to leave the platforms unaltered. In addition, increasing the awareness of their historical significance and ecological role may lead to specific rules in regional laws, which could provide another effective tool to conserve the legacy of a vanished form of forest use even outside protected areas.

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Paper III

First draft

Effects of charcoal site soil on germination, growth and mortality of forest trees: results of a two-year common garden experiment

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Keywords: biochar effects, charcoal soil; Chlorophyll a fluorescence; common garden experiment; forest recolonization; kiln hearths; kiln sites; seed germination; seedling biomass; soil effects, tree growth.

Abstract

Charcoal production activity determined a strong legacy effect at the landscape level, particularly in central Italy, with a very high density of kiln sites (i.e. charcoal hearths) in woodlands. In previous studies we demonstrated that that they are similar to micro ecological islands, characterized by a well developed herbaceous community, but without shrub or overstory layer. In this study, we used a common garden experiment in order to assess the influence of the charcoal-enriched soil of the old kiln sites on the early life stages of major forest trees (*Quercus ilex*, *Q. cerris* and *Fagus sylvatica*), especially seed germination, seedling growth and mortality. Moreover in order to monitor their vitality we measured Chlorophyll a fluorescence monthly. At the end of the experiment root and above ground biomass were also measured. On charcoal soil germination was higher only for *Q. ilex*. The other two species preferred control, especially beech. However, at the end of the experiment emerged a clear enhanced survivability of seedlings on charcoal, especially *Q. ilex*. Seasonally, the seedlings resulted taller on charcoal soil, but differences were significant only for *Q. cerris*. The root/shoot ratio was always higher on charcoal soil, but regarding biomass no significant differences were recorded, neither considering the total, or the root or the above ground biomass. The chlorophyll a fluorescence index F_v/F_m showed specie-specific trends, the evergreen species was again positively affected by charcoal, while the two deciduous species negatively. Considering the whole experiment, *Q. ilex* resulted positively affected by charcoal, while the two deciduous species presented some contrasting results. The reasons for the reduction in photosynthetic efficiency in the seedlings on charcoal soil should be further investigated. On the other hand, it must be highlighted that this experimental result appears somewhat contradictory with the lack of forest recolonization on kiln sites in forests, leading to the assumption that factors other than soil chemistry play a role in the regeneration failure of even this stress-tolerant tree. These may include the impact of wild herbivores, the lack of formation of mycorrhizae or even the altered “natural” structure of the soil in the charcoal sites.

Introduction

Charcoal kiln sites are widespread all over Europe (e.g. Deforce et al., 2012; Hardy and Dufey, 2015a; Ludemann et al., 2004; Raab et al., 2013; Risbøl et al., 2013), as well in central Italy (Carrari et al., c, *ms. in prep.*). It is well documented that they are useful spots for archaeological studies, providing charred remains of wood that can be used to reconstruct the past vegetation and the history of forest exploitation (Deforce et al., 2012; Ludemann, 2000; Nelle, 2003). Recently, it was also found that charcoal kiln sites (known also as charcoal hearths) have important legacy effects on vegetation and soil conditions (Carrari et al., c, *ms. in prep*; Criscuoli et al., 2014; Wittig et al., 1999; Mikan and Abrams, 1995). The understorey vegetation of sclerophyll, oak and beech forests of the Mediterranean area resulted positively affected by the centennial charcoal addition in soil (Carrari et al., a, *ms. accepted*). On the abandoned kiln sites, this important component of forest ecosystems was in fact characterized by a higher level of α, β and γ diversity and by a higher production of biomass (Carrari et al., a, *ms. accepted*).

The positive effect of charcoal on this component of the forest ecosystem is supported by numerous studies on the consequences of biochar addition in agricultural systems. Most of these experiments indicated positive effects of wood charcoal on crop yields via improved soil chemical and physical characteristics (Baronti et al., 2010; Sohi et al., 2010; Vaccari et al., 2011). In particular, biochar treatments increased water availability, improved structure and formation of soil aggregates (Lehmann and Joseph, 2009), reduced nutrient leaching (Yanai et al., 2007) and enhanced nitrogen availability (DeLuca, 2009; Nelissen et al., 2012; Rondon et al., 2007). Besides these profitable effects on plant production, the other important factor in favour of biochar is due to the long-term stability of charcoal. In fact, this practice allows sequestration of even large amounts of carbon from the atmosphere, thus mitigating the impact of climate change due to the increase of CO_2 associated with the use of fossil fuels (Nelissen et al., 2015; Vaccari et al., 2011).

On the other hand, a inhibiting mechanism on forest recolonization emerged from the investigation of abandoned kiln sites in North American forests with oaks and beech (Mikan and Abrams, 1996, 1995; Young et al., 1996), as well in sclerophyll, oak and beech forests of central Italy (Carrari et al., b, *ms. submitted*). A strong negative effect on the regeneration and establishment of numerous tree species emerged from these investigations leading to a much delayed or possibly even blocked forest dynamics in these sites (Carrari et al., b, *ms. submitted*). Most of the hypotheses suggested to explain these effects are related with the different soil conditions in kiln sites compared with those in the adjacent forest. These include the shortage of P and Mn (Mikan and Abrams, 1996), a surplus of pH, Ca, K, Na and Mg (Mikan and Abrams, 1996, 1995; Young et al., 1996) or the reduced formation of mycorrhiza (Carrari et al., b, *ms. submitted*).

In addition, other factors have been considered, such as the sterilization of soil caused by the repeated combustions for charcoal production. This may lead to the death of buried seeds, incoming seeds and remaining vegetative structures (Mikan and Abrams, 1995). Finally, the high population density of ungulates, when present, is also a potential negative factor since these animals use the charcoal platforms as preferential sites for grazing (Mikan and Abrams, 1995).

Considering the increasing importance of the biochar practice and its recent consideration for also forest restoration, a first important step is to understand whether the negative effect of charcoal kiln sites on forest regeneration is directly associated with the accumulation of abundant charcoal remains in the soil or it is due to some other external stochastic factor. As stressed by Sohi et al. (2010), in the absence of long-term data (other than those from the Terra Preta in Brasil), development of predictive certainty for the longevity and durability of plant yield and other effects, particularly in relation to specific crop and soil types, is a key issue. Predictability and certainty are required to assign a financial value to the agronomic value of biochar but are also essential to evaluate the environmental sustainability of such practice (Sohi et al., 2010).

Hence, we used an experimental approach to analyse the initial life stages of three major European and Mediterranean forest trees grown on the charcoal-enriched soil of kiln sites abandoned ca. 60 years ago. Parameters analysed were germination rate, growth rate, biomass production and mortality. Moreover we used Chlorophyll a fluorescence transients, to investigate the response of seedlings in terms of photosynthetic efficiency on charcoal soil.

Materials and methods

STUDY SPECIES

We selected the most representative tree species of the three main forest types historically used in Tuscany for charcoal production: 1) the holm oak (*Quercus ilex*-QI) for evergreen sclerophyll forests; 2) the turkey oak (*Q. cerris*-QC*)* for thermophilous mixed oak forests; 3) the beech (*Fagus sylvatica*-FS) for montane forests. These species were dominant and the most frequent in the forest stands adjacent to the kiln sites analysed in a previous study (Carrari et al., c, *ms. in prep.*).

For each species, seeds were collected in autumn 2013 under a few mother trees growing in the close proximity of one or two charcoal sites in the respective forest type.

COMMON GARDEN SET-UP

In the same area of seed sampling, a representative and well preserved charcoal kiln platform was selected in each forest type for the collection of soil. After removal of the superficial litter, this was taken with a shovel at a depth of 1-15 cm (kiln soil). The "control soil" was collected with the same procedure in a single spot adjacent to the sampled kiln site excluding downhill locations to avoid potential charcoal "contamination" by runoff. The two soil types were analyzed for C, N, S, and pH. We then filled 450 pots (15x15x20 cm) with the two types of soil (75 with kiln site and control soil for each species, from the three respective forest types). All the pots were placed in the open in a homogeneous area located at 40 m a.s.l., characterized by a humid temperate climate, with 14.6° C of mean annual temperature and 872.6 mm rainfall (source: Peretola meteorological station). Such conditions represent the mean climate condition for the three forest type. Partial shading was provided to the common garden by the canopy of cultivated ash trees (*Fraxinus angustifolia*).

For each species, 450 seeds in good conditions were sown, placing three seeds per pot in a regular triangle-like design to maximize their distance. The seeds of the beech, which are characterized by an intermediate physiological dormancy (Baskin and Baskin, 2001), were sown on 25/10/2013, after 20 days of stratification at 4 C°, while the two oak species were sown without chilling (Turkey oak on 14/11/2013 and Holm oak on 21/11/2013). Seeds were watered after sowing, and then received only ambient rainfall, except for three emergency waterings on 6th of June, 4th of July 2014 and on 15th of July 2015.

Figure 1 A) common garden experiment with the three species *Quercus ilex*, *Q. cerris* and *F. sylvatica* on the two soil types B) dark-adaptation with leaf-clip of a seedling of *F. sylvatica* for Chlorophyll a fluorescence transients.

DATA COLLECTION

The monitoring of germination, growth rate, photosynthetic efficiency and mortality started in April 2014 and continued until August 2015, when biomass collection was done for a random subsample of plants.

The germination success of the three species was estimated as the percentage of seedlings established at the end of June 2014. The very few seeds germinated after this date were not considered for the other measurements. Similarly, mortality was estimated as the percentage of seedlings that were dead by the end of the first growing season (November 2014, hereafter indicated as M14) and of the whole experiment (August 2015, indicated as M15). The height of each plant was measured monthly from May 2014 until July 2015, together with Chlorophyll a fluorescence (ChlF) transients. The analysis of the fast induction curve of ChlF from PS II has been used to study stress physiology of trees (Pflug and Brüggemann, 2012). Such method is useful to evaluate rapidly the responses to high or low temperature, drought, lack of nutrients, salinity, pollution, ecc. in forest or in controlled experiments (Bussotti et al., 2012). For the two deciduous species (QC and FS) fluorescence was not measured during the winter months (December- 2014-April 2015).

ChlF was measured on a random sample of 20 seedlings per species on the two soil types, using a direct HandyPEA fluorimeter (Plant Efficiency Analyzer, Hansatech Instruments Ltd., Petney, Norfolk, UK) on 2 or 3 leaves (2 before June 2014) after 30 min of sample dark-adaptation with leaf-clips. The measure was repeated each month on the same leaves during the same year. Leaves were changed only when apparently damaged or not healthy for various reasons.

The rising fluorescence was induced by 1 s pulses of red light (650 nm, 3500 µmol m^{-2} s^{-1}) and recorded for that time, starting from 50 µs after the onset of illumination, with 12 bit resolution. The fluorescence induction curve from F0 to Fm is called “fluorescence transient” (OJIP) and its analysis is formalized in the JIP-test (Strasser et al., 2000, 2004). Plotted on a logarithmic time scale, the fluorescence transients show a polyphasic shape. “O” refers to the initial fluorescence level, K (300 µs), J (2 to 3 ms) and I (30 ms) are intermediate levels of the fluorescence emission, and P (500-800 ms - 1s) is the peak level of fluorescence. The latter indicates the highest, or maximal, fluorescence intensity (F_M) when saturating light is applied to the leaf (fig. 2).

The JIP-test defines the maximal (subscript “0”) energy fluxes in the energy cascade for the events Absorption (ABS), Trapping (TR_0), Electron Transport (ET_0), Dissipation (DI_0), and Reduction of End acceptors of PSI (RE_0).

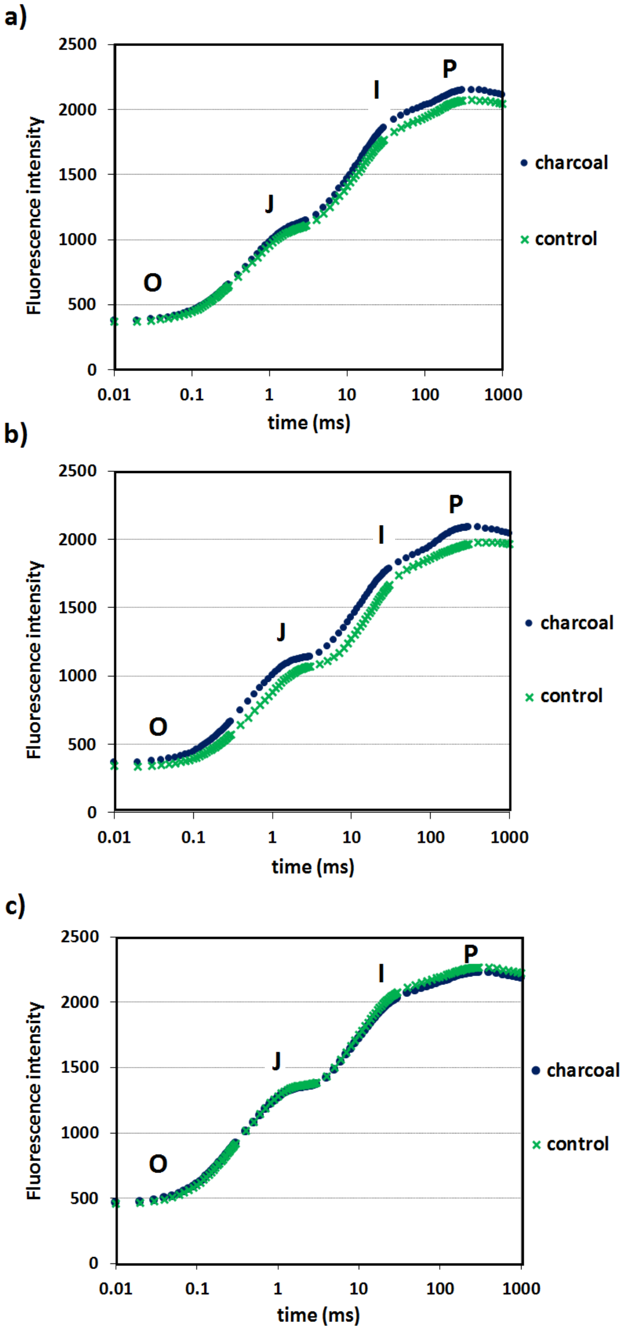

Figure 2 Examples of ChlF “OJIP” transient measurements, comparing leaves of seedlings grown on charcoal soil and control soil of QI (a) QC (b) FS (c) in October 2014. O-J phase refers to the reduction of the primary acceptor of PSII (Q_A); J-I phase refers to the reduction of the pool of plastoquinones; I-P phase refers to the reduction of final electron acceptors (NADP and ferredoxin).

We calculated monthly the following parameters:

- F_V/F_m =[Fm− F0]/Fm = φPo = TR0/ABS = maximum quantum yield of PSII primary photochemistry. F_v/F_m expresses the probability that an absorbed photon will be trapped by the PSII reaction center;
- Ψ_{Eo}, the probability of an electron to reduce the primary quinone acceptor and to move into the electron transport chain beyond PSII;
- Ψ_{Ro} (1-VI), the efficiency of a trapped electron to move into the electron transport chain, from QA^- to the PSI end electron acceptors This is related to the reduction of PSI end-electron acceptors, such as the reduction of NADP;
- PI_{ABS} the performance indices (PIs) measure the potential energy conservation of photons in the intersystem between PSII and PSI;
- $PI_{TOT.}$ the potential energy conservation from photons absorbed by PSII to the reduction flux of PSI end acceptors.

At the end of the experiment (beginning of August 2015), 35 seedlings were randomly collected for biomass measurements. After their complete extraction from the pots, the roots were thorougly washed with water, and then cut at the stem junction in order to separate the abovegroundbiomass. Each part of each seedling was oven-dried at 70°C for 48 hours and then weighed individually.

The germination rate was considered as the percentage of the seedlings born by the end of spring 2014. For each species and soil type, growth and fluorescence parameters were averaged to obtain a single measure for six time intervals:

1. spring 2014: May
2. summer 2014: June- September
3. autumn 2014: October-November
4. winter 2014-2015: December-April
5. spring 2015: May
6. summer 2015: June-July

Height, FV/FM, biomass and fluorescence index were tested for normal distribution using the *Lilliefors test*, and the homogeneity of variance was tested with the *Bartlett's test*.

Differences between soil types were tested by the *t test* or by the *Mann Withney U test*, according to normality test. The other fluorescence parameters are not considered in the present analysis, but mean values are reported in Appendix 1. All analyses were performed in R 3.1.2 (R core team, 2014).

Results

Germination success was lowest for QI on control soil and highest for FS on control soil (tab.1).

Table 1 Percentage of germinated seeds (germination rate), of seedlings that were dead by autumn 2014 (mortality 2014), and at the end of the experiment (mortality 2015) for the three species on the two soil types.

	Q.ilex		*Q. cerris*		*F. sylvatica*	
	charcoal	control	charcoal	control	charcoal	control
Germination rate (%)	56.00	52.89	83.56	85.78	56.89	73.33
Mortality 2014 (%)	0.00	1.33	1.78	0.00	2.22	4.89
Mortality 2015 (%)	1.33	13.78	10.22	17.78	6.22	7.11
Survived (%)	51.56	51.56	80.44	82.22	52.00	67.11

The largest difference was observed for FS, where germination on charcoal soil was 16% lower than in the control soil (tab.1; fig. 3). Seeds of QC also had a lower germination rate on charcoal soil, but here the difference with control was only 2.3% (tab.1; fig. 3). The highest germination rate on charcoal soil was recorded for QI (56%, tab. 1), where the percentage of non- germinated seeds was 3.3% lower than in the control soil (fig. 3).

The lowest total mortality was recorded for QI and the highest for FS on control soil (1.3% and 6.2% respectively; tab. 1). At the end of the summer 2014 this species presented no mortality on charcoal soil vs 1.3% on control (tab.1) the same trend was recorded for FS where mortality was 2.7% lower on charcoal then on control (fig.3). QC presented a different behaviour with no mortality in 2014 on control and 1.78% on charcoal (tab. 1).

At the end of the experiment (2015) the mortality was always lower for seedlings grown on charcoal soil: we recorded differences of 12.5%, 7.6% and 1% respectively for QI, QC and FS (fig.3).

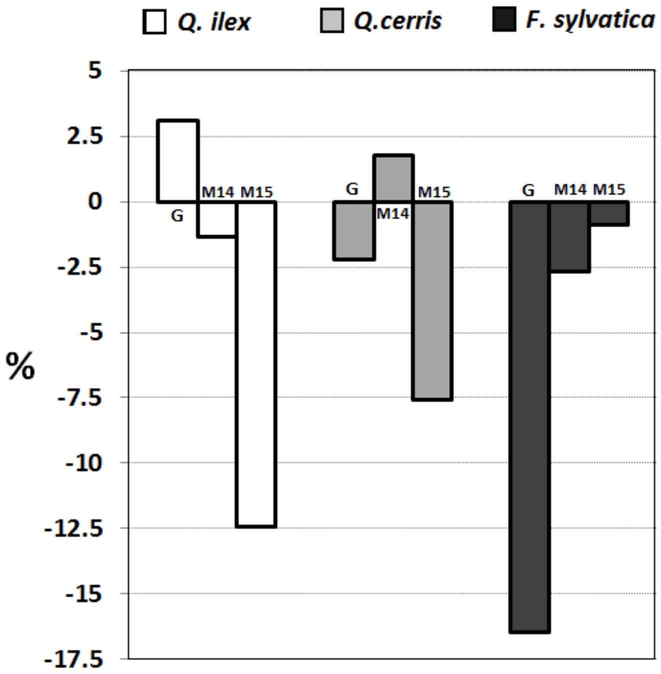

Figure 3 Effect of charcoal soil on seed germination (G) and seedling mortality at the end of the first (M14) and second (M15) summer. Bars indicate the percentage differences with respect to control soil (assumed as zero).

Only minor differences between the two soil types were found regarding the growth of the three species, especially QC. During all 2014 seasons, seedlings of this species were significant taller on charcoal soil (p-values in Appendix 1), but this difference disappeared in 2015 (fig. 4b). The QI seedlings grown on charcoal soil were significantly higher compared to those on control soil in spring 2014, and this difference was maintained until the end of the experiment, although without significant differences in 2015 (fig. 4a). In the case of FS plants on control soil were generally smaller, but never significantly (fig. 4c). Mean height values are reported in Appendix 1.

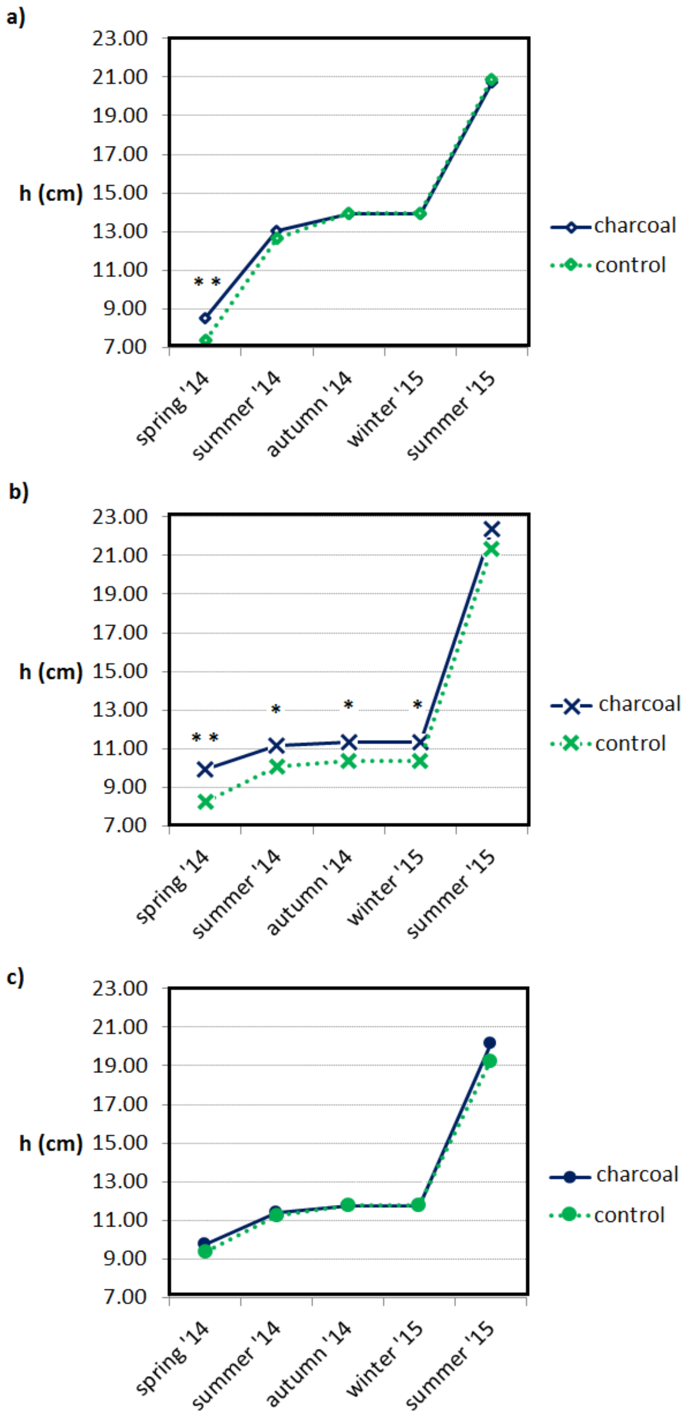

Figure 4 Comparison of the seasonal growth of seedlings of *Quercus ilex* (a) *Q. cerris* (b) and *F. sylvatica* (c) on the two different soil types.

On the contrary, no significant differences could be found in terms of total, root or above-ground biomass. The total biomass of single plants was largely variable, with weight values ranging from 0.02 g to 6.12 g on kiln soil and from 0.13 g to 5.75 g on control soil. Total biomass was generally higher in QI and lower in FS (fig. 5). The larger difference between the two treatments was found in FS, where the above-ground and root biomass produced on kiln soil was 22% and 24% higher than on control, respectively (fig. 5). In QC, despite the lack of significant differences, the root biomass on kiln soil was 13% higher than on control, while the above-ground biomass was more abundant on control soil, resulting in a very minor difference in terms of total biomass (fig. 5). QI showed almost no differences in terms of root biomass, while the above-ground biomass was 13.2% higher on control soil (fig. 5).

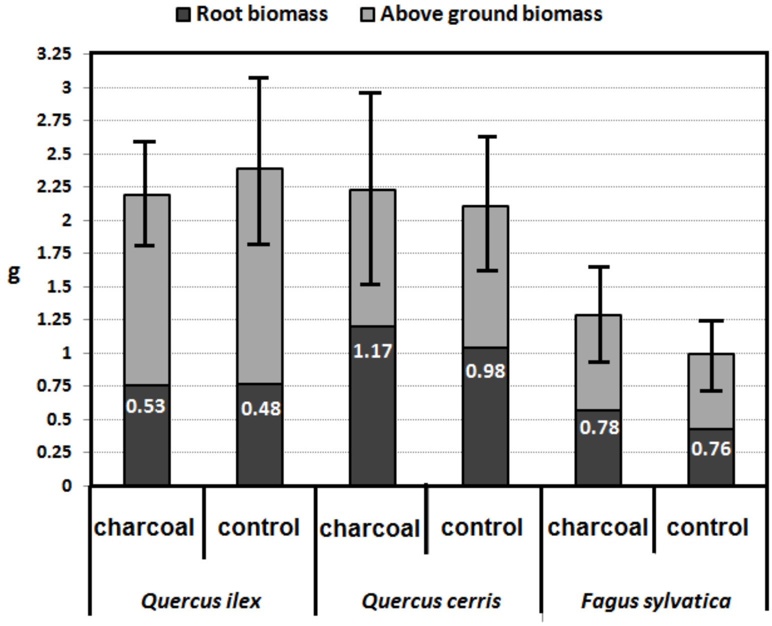

Figure 5 Total biomass values (mean ± sd) per species and soil type. The contribute of above-ground and root system are shown by different colours. The ratio root/above ground biomass is reported on each bar.

The F_v/F_m index presented significant differences for all three species. In QI this was generally higher for seedlings grown on kiln soil, and differences were significant in spring ’14, summer ’14, autumn ’14 and summer ’15; differences were instead not significant in winter ’14 and spring ’15. The response was reversed in QC, which presented a significant higher efficiency in seedlings grown on control soil; only in spring ’14 the index was higher on kiln soil. In spring ’14 and in summer ’15, FS had a higher value of F_v/F_m on control soil, while in summer ’14 photosynthetic efficiency was enhanced on charcoal soil.

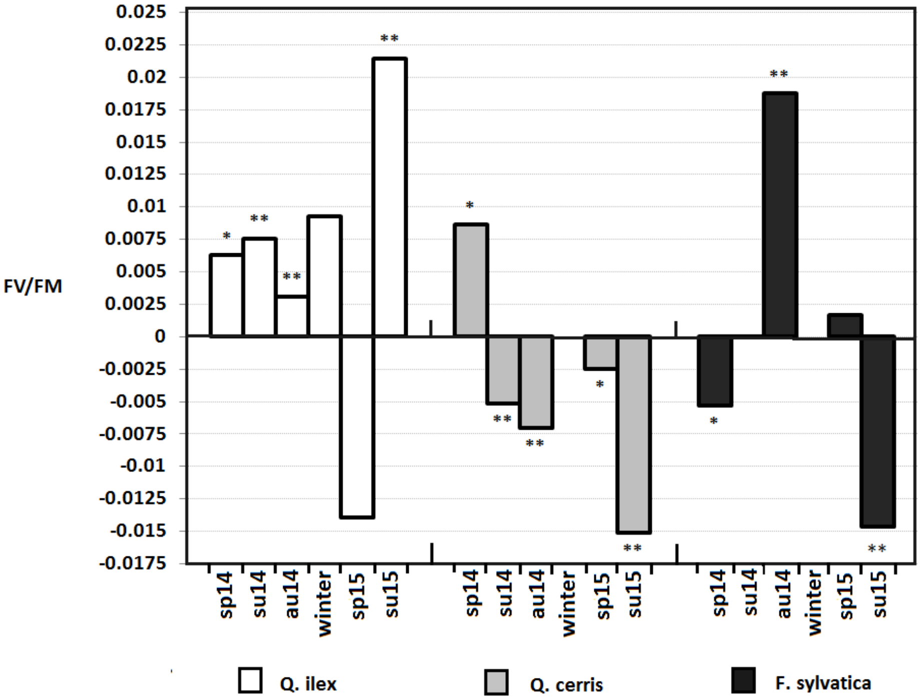

Figure 6 Effect of charcoal soil on the ChlF index Fv/Fm in seedlings of the three species. The index compares the photosynthetic efficiency in spring ’14 (sp14), summer ’14 (su14), autumn ’14 (au14), winter (lacking for the deciduous *Q. cerris* and *F. sylvatica*), spring ’15 (sp15) and summer ’15 (su15). Bars indicate the absolute differences with respect to control soil (assumed as zero). * = $p < 0.05$; ** = $p < 0.001$.

Discussion and conclusions

The three species considered in the present study were variously influenced by charoal soil for the different parameters analysed.

Germination responses were not uniform: while no clear differences were found for QI and QC, FS clearly preferred the control soil, where seed germination was ca. 17% higher than in the charcoal soil. This result is in line with our previous field study (Carrari et al. b, *ms. submitted*), where the beech seedlings had a lower frequency on kiln sites compared with the adjacent forest. On the contrary, the two oaks tended to prefer the kiln sites, which is consistent with the lack of a negative effect of charcoal soil on the seed germination of these species (Carrari et al., b, *ms. submitted*). Concerning QI, our results are in line with evidence from a recent experimental study (Reyes et al., 2015), which evidenced the insensitivity of QI to soils added with ash and black carbon, such as those resulting after wildfires.

Looking at mortality, the proportion of seedlings died in the second year of the experiment was generally higher than in the first year, probably due to the stronger and longer drought of summer 2015 (especially in July; data from Lamma Toscana, http://www.lamma.rete.toscana.it). The drought stress of the second year is probably associated with the larger differences of mortality rate on the two soil types. Again, the effect of charcoal soil on QI was mostly positive: at the end of summer 2015 (second year of the experiment) the seedlings of this species suffered a mortality rate that was 12.5% higher on control soil. The same trend was observed in the two deciduous trees QC and FS, albeit with considerably smaller differences (7.6% and 0.8% respectively). These results seem to support evidence from observations on the Amazonian Anthrosol (Lehmann et al., 2003) and biochar experiments, where it was found that charcoal addition to the soil increases water retention capacity and structural stability (Baronti et al., 2010; Glaser et al., 2002; Yanai et al., 2007). The possibly positive effects of charcoal on the survival of seedlings of forest trees observed in this study should be further tested in different conditions and in wider a range of species, since it may have important implications for the management of forests and tree plantations under the predicted climate change.

Looking at growth rates, it is noteworthy that the effect of charcoal soil was mostly positive. In fact, plants of the three species grown on this soil were usually taller than those grown on control soil; differences were especially marked for the two deciduous species (QC and FS). On the other hand, the total biomass data did not confirm these growth results. Despite the above height differences, weight of the total biomass was not significantly higher on the kiln soil, probably due to a high variation between individual plants. Looking closer at these results, however, it emerges that the root/shoot ratio was consistently higher for the seedlings grown on kiln soil, indicating a stronger development of the root systems in these plants. This is surprisingly in line with results of a similar experiment on two oak species by Mikan and Abrams (1996), suggesting that such an increased root development may be associated with a stress factor, such as physiological drought.

Chlorophyll a fluorescence measurements highlighted species-specific responses in the three taxa. Based on the index Fv/Fm, QI was characterized by a greater photosynthetic efficiency on the kiln site soil, while QC and FS were mainly negatively influenced. This may be due to various reasons. A decrease of the F_v/F_m index has been observed for species grown on soils with shortage of nutrients (Bussotti et al., 2012), which may appear consistent with the hypothesis of decreased availability of P reported above (Young et al., 1996). Unfortunately, little is known about the effects of different levels of single nutrients on photosynthesis, which are likely to differ in different species. In our case, the F_v/F_m parameter suggests a divergent response to kiln soil in QI and the pair QC-

FS, which is likely associated with the contrasting functional traits, ecology and leaf phenology of these species. Indeed, it is well documented that evergreen and deciduous species have widely diverging adaptive traits at morphological, anatomical and physiological level. In addition, they are characterized by a different edaphic ecology: while QI shows a broad tolerance in terms of pH and nutrients, QC avoids too alkaline soils and FS generally prefers substrates with high nutrient contents (Pignatti, 2005). Summing up, overall response to charcoal kiln soil of the two latter deciduous trees was mostly negative, but whether this depends on the presence of the charcoal itself or to some other factors linked to the repeated combustions remains unclear. The reasons for the reduction in photosynthetic efficiency in the seedlings on charcoal soil should be further investigated. In the evergreen oak QI, influence was less pronounced and partly positive, which highlights the more resilient nature of this drought-tolerant Mediterranean tree in the face of several types of environmental stress. On the other hand, it must be highlighted that this experimental result appears somewhat contradictory with evidence from observational studies (Carrari et al., a, *ms. accepted*; b, *ms. submitted*), which showed the lack of young trees of QI in the “established regeneration” layer. This leads to the assumption that factors other than soil chemistry play a role in the regeneration failure of even this stress-tolerant tree. These may include the impact of wild herbivores, the lack of formation of mycorrhizae or even the altered “natural” structure of the soil in the charcoal sites, all aspects that could not be tested in our 20-months experiment.

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Paper IV

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The old charcoal kiln sites in Central Italian forest landscapes

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Abstract

Production of wood charcoal is one of the earliest forms of forest use, existing since millennia in the Mediterranean countries and only vanished in the last century. The legacy of this activity are thousands of abandoned charcoal kiln platforms, in which soil and vegetation are deeply affected. Understanding the consequences of such effects at the forest-level demands a better knowledge of the density, distribution and morphology of these sites, as well as the influence of forest type and local geomorphology on them. We examined these aspects using field surveys and the Airborne Laser Scanning (ALS) approach in 1-ha sample quadrats, spread along an altitudinal gradient and located in three major forest types of Central Italy, namely evergreen sclerophyllous forest, oak-dominated thermophilous deciduous forest and montane beech forest. Density of kiln platforms was lower in oak-dominated forests, but here their overall surface proportion was higher due to their larger size. In beech forests, kiln platforms were more numerous but smaller. Density was intermediate in the sclerophyll forest, where the overall proportion of surface was lowest. The charcoal-enriched soil layer was invariably single and continuous (e.g. not interrupted by mineral layers). Thickness of this layer was not affected by forest type, but increased with slope inclination. Several features of our kiln platforms such as density and shape were distinct from others in Central and Northern Europe, probably reflecting different forest histories and purposes for which they were built. Using ALS, we could detect all kiln platforms in beech forest on steep slopes and approximately 75% of the kilns in oak forests on hilly terrain. Hence, all further ecologically- or archaeologically-oriented study in our region at the landscape level will benefit from the use of hillshade and/or slope ALS images.

Introduction

Based on artworks of ca. 38.000 years ago found in the caves of southern France, it appears that wood charcoal has been the first synthetic material produced by man (Antal, 2003) and one of the main sources of energy from the Iron Age to the 19th century (Blondel, 2006). As such, its production is one of the oldest forms of forest use in the temperate regions, and was continued for millennia to satisfy the needs of the human populations in most European countries. Production of charcoal is based on the pyrolysis of wood at low temperature (ca. 440 °C) without oxygen, and was realized in special wood kilns covered by a mixture of soil and plant material (Ludemann, 2003; Powell, 2008; Deforce et al., 2012). In hill and mountains areas, kilns were usually prepared along footpaths in sites where it was possible to cut the stools in the adjacent stands and concentrate the wood in small, terrace-like platforms prepared for this purpose. In the Mediterranean region, various evergreen and deciduous tree species were used for charcoal production, such as oaks (*Quercus cerris, Q. pubescens* and others, except for *Q. suber*), ash (*Fraxinus ornus*), hop-hornbeam (*Ostrya carpinifolia*) and various secondary woody species (*Sorbus torminalis*) that occur especially in thermophilous deciduous forests, such as the wildservice tree (*Sorbus torminalis*; Carrari et al., 2015). Large sclerophyllous shrubs such as the strawberry tree (*Arbutus unedo*), the heath tree (*Erica arborea*) and the green olive trees (*Phyllirea* sp.) were also used.

While in most northern and central European countries the use of wood charcoal was abandoned in the 19th century due to the rapidly increasing and widespread use of coal (Deforce et al., 2012), the importance of this material in the Mediterranean countries even increased during the industrial revolution, as other fuel sources were largely lacking. Its production and use mostly vanished around the year 1950, though in some remote mountain area it is still in practice today.

The main legacy of this traditional activity are thousands of abandoned charcoal kiln sites disseminated in present-day forests (Ludemann et al., 2004; Blondel, 2006, Nocentini and Coll, 2010). Thanks to the abundant charcoal remains and their persistence in the soil for centuries, these sites provide an opportunity for the reconstruction of former woodland composition and management practices on a stand scale, using anthracological analysis and radiocarbon dating (Ludemann, 2003; Ludemann et al., 2004; Nelle, 2003; Nelle et al., 2010; Pèlachs et al., 2009). However, this also brings along long-lasting ecological effects on the structure, composition and functioning of the soil and vegetation. A first important effect is the strongly increased amount of total carbon in the topsoil layers (Carrari et al., b, *ms accepted*; Criscuoli et al., 2014), suggesting that these sites can contribute significantly to the overall capacity of carbon stock in soils at the forest-level (Criscuoli et al., 2014). Nutrient availability and pH are often increased, which may lead to compositional differences in the understorey vegetation with respect to the adjacent stands (Carrari et al.,b, ms accepted; Wittig et al., 1999). In addition, the process of tree recolonization is altered in abandoned kiln sites which may lead to long-lasting negative effects on forest recovery, as has been documented in Europe and Canada (Mikan & Abrams, 1994, 1996; Young et al., 1996; Carrari et al. a, *ms accepted*).

Evaluating the magnitude of these effects and the contribution of charcoal kiln sites to the long-term carbon stock in the soil at the forest-level demands a better knowledge of their spatial distribution, density and overall surface, and of the thickness of the charcoal-enriched soil layer. Previous inventory studies provided data for Germany and the Alpine area (Hesse, 2010; Ludemann 2011), Belgium (Deforce et al., 2012) and Norway (Raab et al., 2015), but until now only one study has been performed in south Europe (Risbøl et al., 2013). Hence, little evidence exists for the Mediterranean region, where factors like the frequently rough geomorphology of hilly or mountainous areas, the often heterogeneous vegetation landscapes, as well as the diversity of local popular cultures and traditions have probably affected the spatial distribution and the morphology of the kiln sites.

Accordingly, the aims of this work were: 1) to provide a characterization of the charcoal kiln sites in the forest landscapes of central Italy, and 2) to examine the effects of forest type and major geomorphological traits of the local territory (slope inclination, aspect, altitude) on the spatial distribution and morphology of these sites. To this purpose, we used a traditional field-based inventory and the Airborne Laser Scanning (ALS) method. The latter has already been successfully adopted in forest areas of central and northern Europe, but, to our knowledge, still not in areas of southern Europe. By comparing results from the field and the ALS method, it was possible to test the efficacy of the latter for kiln site detection in territories covered by dense oak forests with a multiple-layered structure and a massive shrub layer, or in beech forests occurring on the steep slopes of the Apennine mountain chain.

Material and Methods

Regional setting

The study was performed in the forests of Tuscany (central Italy), located between N42°44'16"N and N44°3'13", and between E10°29'49" and E11°29'1" (Fig. 1). This area is characterized by three major climate and forest types, spread along an altitudinal gradient from sea level to over 1400 m: 1) meso-Mediterranean along the Tyrrhenian coast, where woodlands are mainly formed by evergreen sclerophylls and especially *Q. ilex*; 2) supra-Mediterranean on the hill systems in the central part of the region, largely covered by thermophilous mixed forests dominated by various species of deciduous oaks (mainly *Q. cerris*, *Q. pubescens, Q. petraea*,); 3) montane-suboceanic on the Apennine range and Mount Amiata, where beech (*Fagus sylvatica*) and mixed beech-white fir (*Abies alba*) forests usually occur above 900-1000 m. Mean annual rainfall and temperature in the study area vary from 650 mm and 15 °C respectively along the coast, to 1450 mm and 10.9 °C respectively on the Apennines and Mount Amiata (period 1961-1990, source: Servizio Meteorologico dell'Aeronautica Militare). The study area is characterized by a variety of geolithological formations and soil conditions, but cambisols are the prevalent soil type according to the Soil Atlas of Europe (European Commission 2006).

For each forest type, hereafter indicated as "sclerophyll", "oak" and "beech", we selected three main areas where charcoal production activity was continued for centuries and abandoned about 60 years ago. This information was derived from local historical documents (e.g. Landi et al. 1988) and common knowledge. These nine areas are shown in Fig. 1 and described in Table 1.

In order to analyse the distribution and morphology of the kiln sites, two different approaches were adopted: i) field inventory surveys in all the areas, and ii) visual inspection of images generated by high-resolution ALS data for two oak areas and one beech area (Fig. 1).

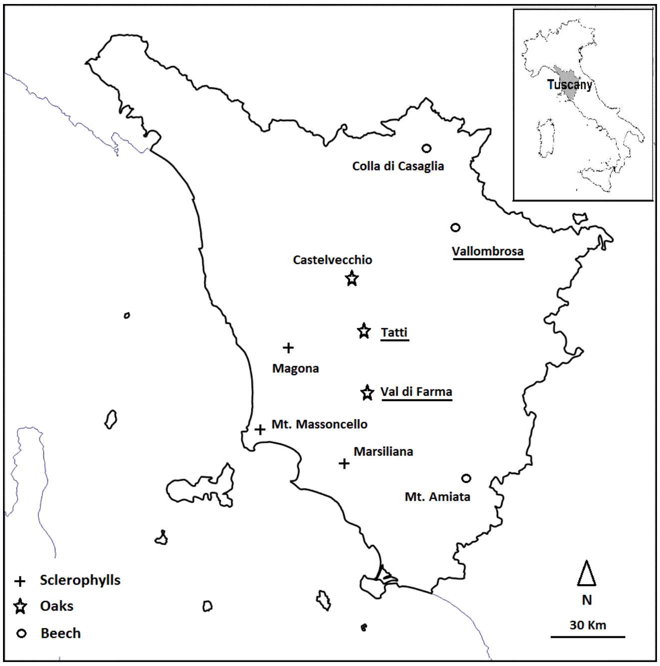

Figure 1. Location of the selected forest areas, with indication of the forest type (different symbols). Areas analysed with the ALS method are underlined.

i) Field inventory

In each forest area, we first identified the zones that were too difficult to access for geomorphological reasons (e.g. slope steepness, rocky outcrops, water courses) using 1:10.000 topographic maps. Next, we randomly selected one point in the remaining accessible zones as the centre of a sample quadrat of one ha. In this way, nine quadrats (Tab. 1) were defined and localized in the field with GPS devices. In each quadrat all the charcoal platforms were then identified and described using the following variables: 1) altitude; 2) slope inclination; 3) slope aspect; 3) tree and shrub species ≥ 4 m high (those potentially used to produce the charcoal) occurring in a circle with 15 m radius external to the perimeter of the platform; 4) conservation status (poor, average, good) based on intensity of soil erosion, impact of human activities and herbivores; 5) shape; 6) size (based on major and minor diameter); and 7) thickness of the charcoal layer, easily distinguishable from the mineral layer for the blackish colour and the abundance of charcoal fragments of various size (Fig. 2C).

For each forest type, we determined the average density of kiln platforms per hectare, their average area proportion (using information on the kiln platform size) and the mean thickness of the charcoal layer.

Next, the effects of forest type and slope inclination on density, size and charcoal layer thickness were tested using two model structures with different combination of variables in R 3.1.2 (R core team, 2014).

First we used a generalized linear model (using glm from the stats package) to test the effect of the forest type, the size and slope inclination of the kiln sites on their density per area, with a Poisson error distribution, log link (lme4; Bates et al., 2014) and parameter estimation via maximum likelihood.

Then we tested the influence of forest type and slope inclination on kiln platform size was tested using mixed models allowing variation between "forest areas" (random factor), in order to remove from the model the variance due to the spatial clustering of the kilns in the nine areas. The starting model was fitted with a linear mixed model (lmer) with a Gaussian error distribution. The model selection followed the protocol of Zuur et al. (2009), where the structure yielding the lowest value for Akaike's Information Criterion (AIC; Akaike, 1973) was considered to be most consistent with the data. The same model selection was used to test the effect of forest type, kiln platform size, slope inclination and random effect of quadrats on the thickness of charcoal layer. For As models contained random effects, a conditional R^2 was calculated (Nakagawa and Schielzeth, 2010; MuMIn package; Bartoń, 2013). The thickness of charcoal layer was also compared between forest types using ANOVA.

ii) ALS

This method was applied in two quadrats within thermophilous deciduous forest (Tatti and Val di Farma) and one quadrat within beech forest (Vallombrosa). In the two former areas, ALS surveys were performed in May 2013 using an ALS50 Leica Geosystems sensor. This instrument recorded four echoes per pulse, with an average laser point density of approximately 4 laser points per m2; the scan-angle was 60°. In the Vallombrosa forest, the ALS dataset was acquired in May 2015 with a RIEGL LMS-Q680i sensor, which recorded the full waveform with an average laser point density of approximately 5 laser points per m2. The scan angle was 30°.

The TerraScan software was used for the preparation of the ALS datasets (Terrasolid, 2005). Standard pre-processing routines were first carried out to remove outlying pulses due to sensor errors. Then the point cloud was classified into ground and non-ground returns on the basis of the adaptive Triangulated Irregular Network (TIN) model algorithm (Axelsson, 2000). Ground returns were interpolated to generate a TIN, which was used to calculate the ground height for each ground return. A Digital Elevation Model (DEM) in grid format with a geometric resolution of 1 m was created. Finally, a slope map (in degree) and a hillshade map were generated from DEM to visualize the micro-topography of the soil surface. All GIS operations were performed with ArcGIS 10.3.

The slope and hillshade maps allowed to identify potential charcoal kiln platforms in the quadrats of the field inventory. They usually appear as small anomalies in the topography on the hillshade model and as flat areas in the slope model. The visual interpretation was performed by an independent interpreter who did not participate to the field inventory work and did not know the position of the kilns. Then, the charcoal kilns identified in the field were used as reference data for evaluating the overall accuracy of the ALS-based kiln detection method (Congalton, 1991).

Results

i) Field inventory

The altitude range of the nine quadrats was 145-230 m, 360-470 m and 1050-1420 m above sea-level for sclerophyll, oak and beech forests, respectively (Tab. 1). Mean slope inclination was higher for the beech forest quadrats (43%) compared to the oak (13%) and sclerophyll forests (11%), with minor differences among quadrats. The sites on the steepest slopes were often provided with robust stone walls built on the downhill side to sustain the platform in a horizontal position (Fig. 2B). The platforms in the sclerophyll areas showed the poorest conservation status due to a significant level of disturbance by human activities. Those in the oak and beech forests were on average well preserved.

Figure 2. Charcoal kiln platforms: A) the elliptical shape and the complete lack of forest recolonization (beech forest, Colla di Casaglia); B) old wall made with volcanic stones to sustain the platform in the beech forests on the steep slopes of Mt. Amiata; C) soil profile through the nearly 30 cm thick charcoal layer in a kiln platform in the oak-forest of Val di Farma.

Table 1. Characteristics of the kiln sites in the nine examined areas (see Fig. 1 for their location); geographical coordinates refer to the central point of the forest area. Each value is the mean of three quadrats per area (n/ha: number of kiln sites per hectare); conservation status of the kiln sites is also averaged for the three quadrats.

Forest area	Latitude	Longitude	Forest type	n/ha	Altitude (m)	Slope inclination (%)	Size (m^2)	Thickness charcoal layer (cm)	Conservation status
Marsiliana	N43.04425°	E10.80938°	Sclerophylls	6	203.0 ± 7.2	5.4 ± 0.9	31.6 ± 4.3	35.3 ± 3.4	Average
Mt. Massoncello	N42.98395°	E10.49700°	Sclerophylls	6	159.0 ± 14.6	32.0 ± 11.0	31.9 ± 7.1	28.0 ± 8.7	Poor
Magona	N43.26512°	E10.63603°	Sclerophylls	4	225.3 ± 2.8	2.0 ± 2.4	24.9 ± 4.3	24.5 ± 5.2	Poor
Tatti	N43.34571°	E10.97231°	Oaks	5	465.5 ± 5.8	6.2 ± 3.2	42.2 ± 6.4	12.8 ± 1.7	Average
Val di Farma	N43.07237°	E11.28179°	Oaks	5	410.4 ± 17.4	14.0 ± 8.2	35.3 ± 5.7	29.0 ± 4.4	Good
Castelvecchio	N43.43400°	E10.99952°	Oaks	5	370.5 ± 6.0	16.3 ± 16.0	55.8 ± 14.8	21.3 ± 2.6	Good
Mt. Amiata	N42.87372°	E11.59816°	Beech	8	1405.0 ± 14.6	14.2 ± 5.8	26.8 ± 4.9	26.0 ± 7.4	Good
Colla di Casaglia	N44.05045°	E11.45977°	Beech	6	1065.3 ± 15.9	45.8 ± 36.1	33.4 ± 8.6	37.2 ± 4.4	Good
Vallombrosa	N43.433950°	E11.342388°	Beech	6	1356.8 ± 15.7	67.7 ± 16.2	22.0 ± 4.9	30.3 ± 0.5	Good

In total, we recorded 51 more or regularly spaced kiln sites, with a minimum of 4 (Magona) and a maximum of 8 (Mt. Amiata) per quadrat (Tab. 1). Despite differences among forest types were not significant, the highest number of platforms for quadrats was found in beech forest, followed by scerophylls and oaks (Fig. 3A). Size and slope inclination had no effect on the number of platforms.

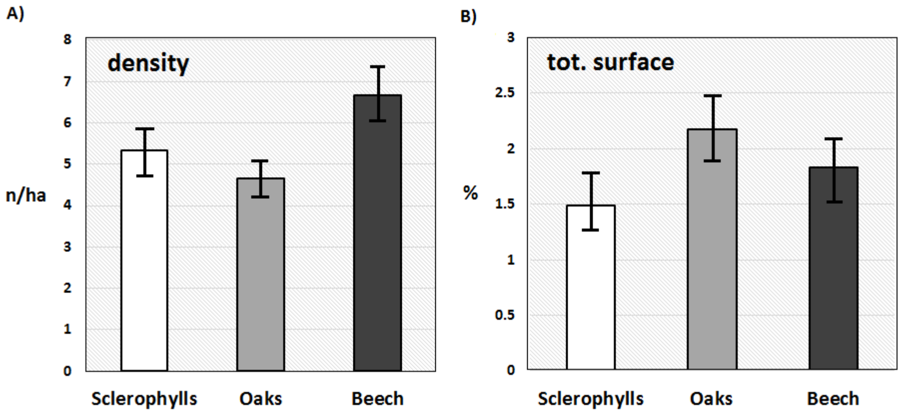

Figure 3. A) Mean number of kiln sites per hectare in the three forest types (± confidence intervals); B) percentage of total surface covered by kiln platforms over the 1 ha quadrat.

These were always elliptical (Fig. 2A), with the shorter and longer diameter ranging from 3.8 m to 9.3 m, and 4.6 m to 10.8 m, respectively. The longer diameter was always oriented along the altitudinal contour lines. The largest platform (ca. 56 m^2), was recorded in an oak quadrat (Castelvecchio), while the smallest (ca. 22 m^2) was in a beech quadrat (Vallombrosa; Tab. 1). Kiln platform size was significantly affected by forest type with higher values in the oak forest (on average 41 m^2) followed by sclerophyll and beech forests (30 m^2 and 27 m^2, respectively) (Tab. 2). Slope inclination had no effect on the size. The best selected model (R^2=0.703) included among-quadrats variation as a random factor and explained ca. 70% of the variation.

The total surface covered by kiln platforms in the 1 ha quadrat ranged from 100 m^2 (Magona) to 253 m^2 (Tatti). Based on mean size and density, the largest proportion of surface was found in the oak forests, where it reached 225.6 m^2, followed by beech (183.2 m^2) and sclerophylls (149.1 m^2); corresponding percentage data are shown in Fig. 3B.

The black charcoal layer containing fragments of woody charcoal was single and continuous in all sites (Fig. 2C), and its thickness ranged from 10 cm (Tatti) to 46 cm (Colla di Casaglia). Kilns platforms in the oak forests showed a thinner charcoal layer (22.4 cm on average) than in the beech forest sites (29.9 cm, *p*-value = 0.0058); an intermediate thickness was found in the sclerophyll sites (27.4 cm). However, the best model structure

($R^2 = 0.412$) revealed slope inclination instead of forest type as a predictor for charcoal layer thickness. The higher the slope inclination, the thicker the charcoal layer (Tab. 2).

Table 2. Optimal random-effects model structures for response variables kiln size and charcoal layer thickness. Models were selected based on AIC criteria (Zuur et al., 2009). Values for the predictor variables "slope inclination" and "forest type" (levels: oaks and sclerophylls) and "plot type" (level: kiln plot) are parameter estimates (± standard error) that indicate the relative change of the response variable for a unit increment in "slope inclination" or compared to the first level of the predictor variables "forest type" (level: sclerophylls) that is incorporated in the intercept. R² refers to the fraction of the variation explained by the optimal model structure; df: degrees of freedom.

Response variables	df	R²	Intercept	Slope inclination	Forest type		Random effect
					Oaks	Beech	
kiln size	51	0.703	30.53±4.74	**/**	11.80±6.70	-3.64±6.68	Forest area
charcoal layer	51	0.412	24.17±2.18	0.11±0.05	/	/	Forest area

In total, 14 trees and shrubs ≥ 4 m were recorded in the forest around the kiln platforms (Fig. 4). As expected, species composition in this belt was different in the three forest types. *Quercus ilex* was the most frequent tree in the sclerophyll quadrats (27.5% on the total sites; Fig. 4), followed by *Arbutus unedo* and *Erica* sp. (mostly *E. arborea*); *Viburnum tinus*, *Phyllirea* spp. and the deciduous oak *Quercus pubescens* were present with a lower frequency. *Quercus cerris* was always present in the sites of the oak quadrats (29.4%, Fig. 4), while other dominant tree species such as *Castanea sativa, Quercus petraea, Q. ilex, Populus tremula, Ostrya carpinifolia, Arbutus unedo, Erica arborea* and *Fraxinus ornus* occurred with a frequency < 10% (i.e. ca. 1/3 of kiln sites in oak forests). In the beech quadrats, kiln sites were always surrounded by *Fagus sylvatica*, while *Abies alba* was present in half of the sites.

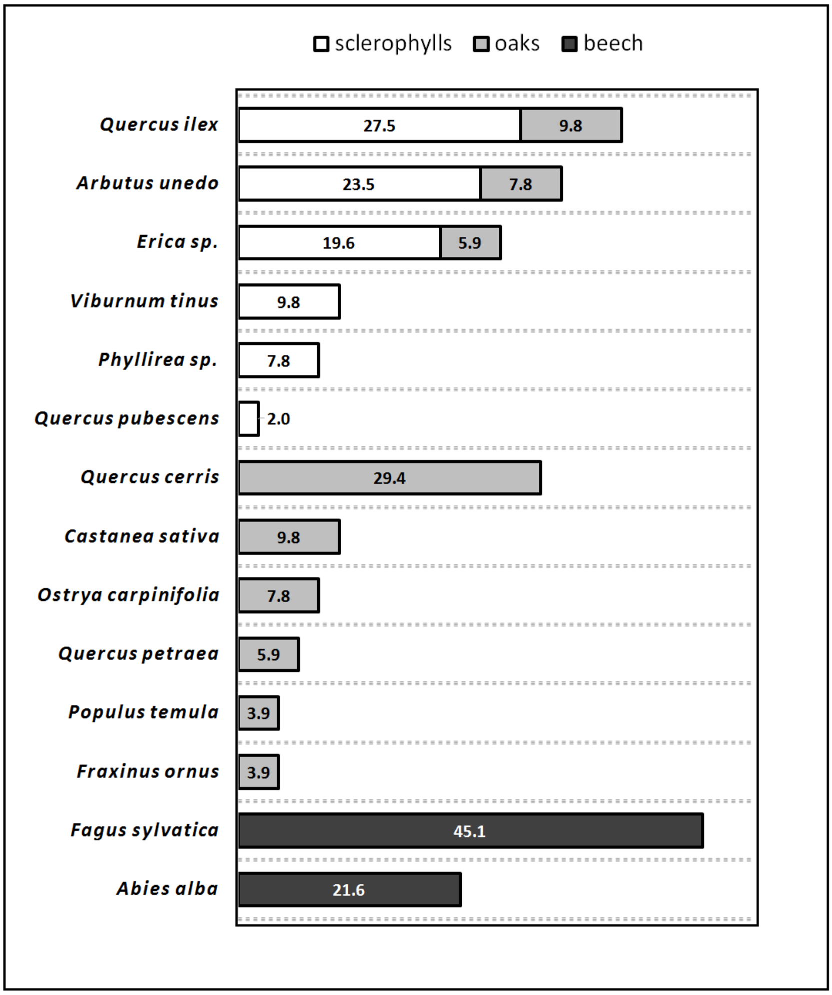

Figure 4. Frequency (%) of the dominant woody species in the stands adjacent to the kiln sites. Percentages are reported on the total number of kiln sites.

ii) Airborne Laser Scanning data

Most of the kiln sites recorded with field surveys in the quadrats in Vallombrosa, Tatti and Val di Farma could also be detected with hillshade and slope image analysis. On the hillshade map (Fig. 5A), the platforms appeared as deviating spots in the topography, e.g. small hilly structures, sometimes with a depressed area in the centre. On slope images (Figs. 5B, 6A,B,C), they appeared as small, dark spots areas with a flat surface, mainly located along the altitudinal contour lines. These could be more easily distinguished on the steep slopes of the mountain area of Vallombrosa than in the hilly areas of Tatti and Val di Farma. The steeper inclination and the single-layered beech cover allowed to detect all six platforms in the Vallombrosa quadrat (overall accuracy = 100%), while the lower slope inclination and the multiple-layered oak forest cover with dense shrub layer contributed to the lower accuracy in Tatti and Val di Farma (overall accuracy = 71% and 80%, respectively). In the former area (Tatti), hillshade and slope images showed 7 sites, of which two were not actually observed in the field (and that did not exist); in the latter area (Val di Farma) the ALS-based method showed 4 sites, failing to identify one site that was clearly observed in the field (Figs. 6A,B).

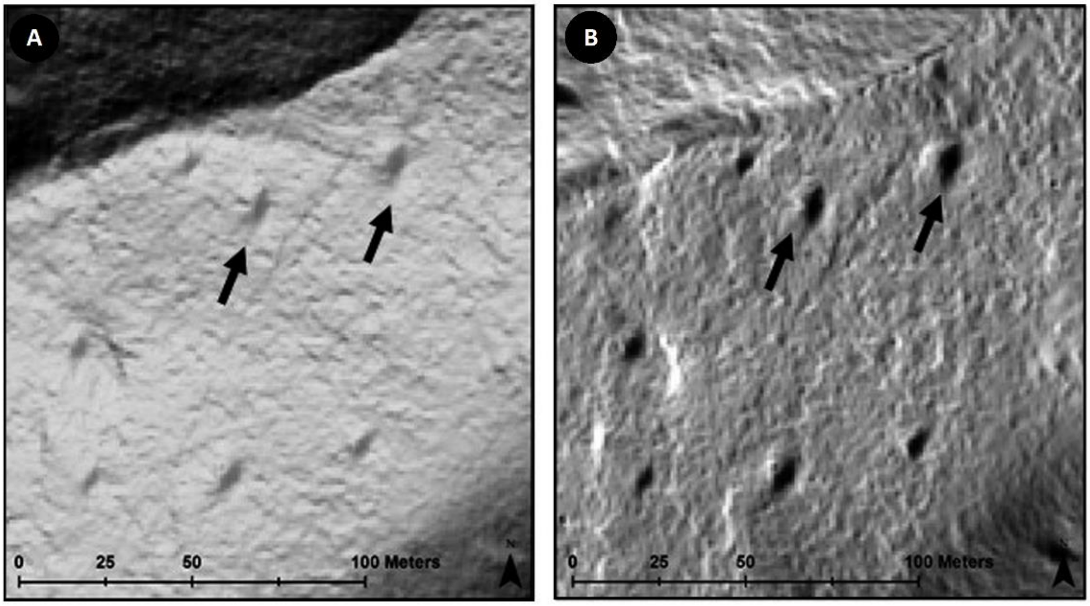

Figure 5. Kiln sites detected on the slope map of the beech quadrat in Vallombrosa. (A) Close-up of hillshade and (B) slope images of the beech quadrat in Vallombrosa. Arrows indicate two of the platforms that are visible as dark, elliptical spots.

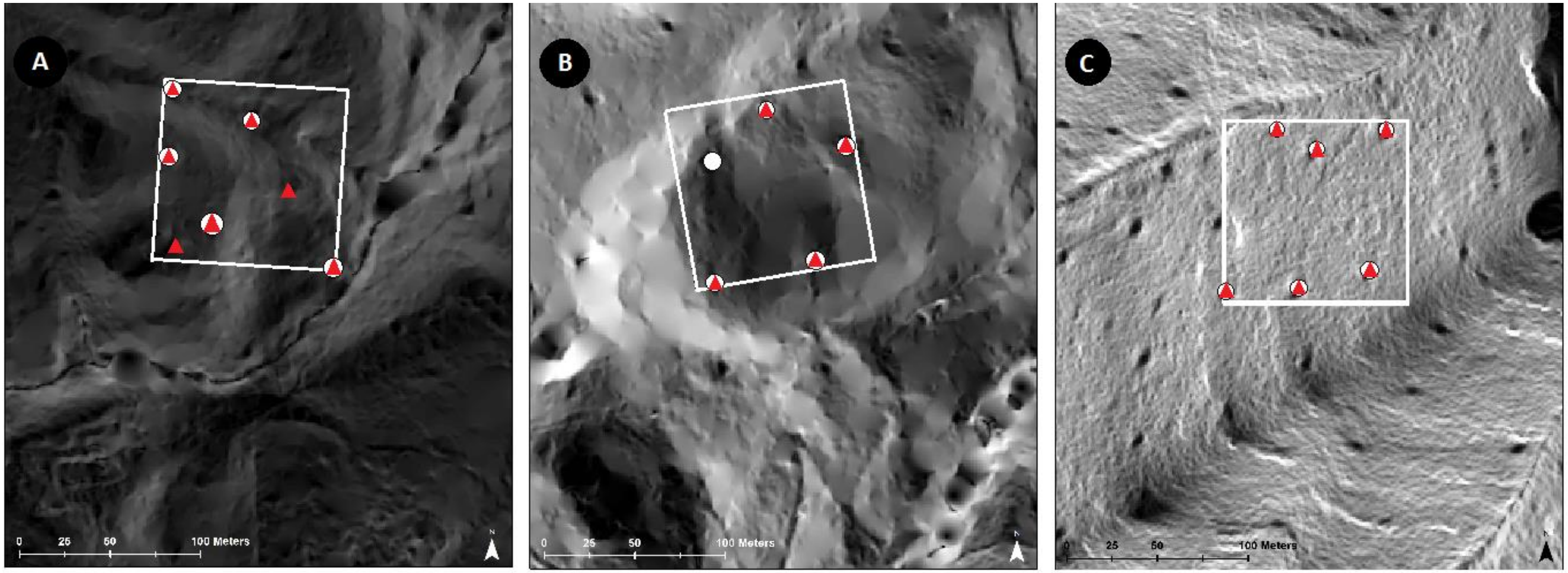

Figure 6. Kiln platforms included in the 1-ha quadrats, shown as white squares, detected on the slope maps of the oak forest areas of A) Tatti and B) Val di Farma C) Vallombrosa. The sites detected from visual interpretation of the maps are indicated by the small, red triangles; the small, white circles show the platforms that were inventoried in the field.

Discussion

Field inventory data

The density of kiln sites in our forest areas was not as high as reported in previous studies from the Mediterranean region, e. g. up to 40 sites per ha (Blondel, 2006). However, it was approximately five times higher than in other European forest landscapes. Around one site was detected in Norway, NW Belgium, Pyrenees, and Northern Germany (Deforce et al., 2012; Pèlachs et al., 2009; Raab et al., 2013; Risbøl et al., 2013), while the mean density in the Black Forest in SW Germany was 1.5/ha (Ludemann, 2010) and similarly from one to three sites were detected in Wallonia (Hardy and Dufey, 2015b). Such a difference is likely associated with the more intensive forest exploitation in the Mediterranean countries, where charcoal provided the energy for the everyday needs of the local populations until more recent times. In addition, the practical problem of carrying away large amounts of firewood from coppice woodlands on rough terrains has been a major reason for its transformation to a much lighter material directly in the forest. People of central Italy, especially in montane areas, preferred to spend days in the forests to produce charcoal than to bring heavy loads of firewood with donkeys and mules along tracks on steep and rocky terrains (Cantiani, 1955; Landi et al., 1988).

While kiln platforms were always elliptical in our study, a prevalently circular shape has been documented in Belgium and Germany (Deforce et al., 2012; Ludemann, 2010; Raab et al., 2013) and a variable shape was observed in Norway, with circular, oval, square or irregular shapes (Risbøl et al., 2013). Based on information that we obtained from the local people, the elliptical shape was adopted to facilitate collection of the charcoal from the two sides of the platform, which always extended along the altitude contour lines of the hill slope. Soil erosion on the downhill side can have accentuated the elliptical shape in some cases. Despite variations between forest types, the kiln sites in our study were generally also smaller than in C and N Europe. On average, the platforms had a mean major diameter of 7.2 m and a minor one of 5.5 m, resulting in a mean surface of ca. 30 m^2, similarly to what was observed in the Pyrenean woodlands (Pèlachs et al., 2009). In more northern countries they ranged from 8-12 m in diameter, as in the Black forest (Ludemann, 2010), up to 18 m in N Germany (Raab et al., 2013), as well as in the larch forests of the Alpine region where they measured, on average, 94 m^2 (Criscuoli et al., 2014).

The larger size of the kiln platforms documented in the studies mentioned above may be associated, at least in part, with the different purpose for which these were prepared. In other regions, charcoal was often produced for metal processing in foundries (Pèlachs et al., 2009; Deforce et al., 2012; Criscuoli et al., 2014), which required large amounts of fuel in specific periods. In the area, the production of such woody fuel was not only addressed to iron metallurgy; charcoal was also the main source of energy for home heating and cooking, as well for many other minor uses (S.I.L.T.E.M., 1946), then possibly the request was more frequent, but of smaller amounts.

Related to this aspect are possibly also the differences in the distribution patterns. In Belgium, for example, charcoal kilns were found to be irregularly distributed in space and time, e.g. spatially clustered and probably linked to specific but infrequent events also corresponding to periods of over-supply of wood due to forest clearings (Deforce et al., 2012). On the contrary, the importance of wood charcoal for the everyday life of local people through the centuries can explain the more even spatial distribution of these sites in our region, and their apparently continuous use through time.

In our study, size were found to be affected also by the forest type. Model results showed that kiln sites were significantly smaller and denser in beech forests than in oak forests, where they were larger but less numerous per unit surface. Most likely, such effects are associated with both the geomorphology of the areas where these woodlands occur and the compositional and structural characters of the forest communities. Slope inclination may be one of the factors for the indirect effects. Beech forests of central Italy occur in the mountain belt, usually higher than 1.000 m a.s.l. and often on steep slopes, as in our sample quadrats (on average 43% inclination). In such a condition, it was important to reduce the distance of transport of the wood material between the places where the stools were cut and the charcoal kilns. This was achieved by preparing numerous but smaller platforms mainly along the altitude contour lines. On the other hand, such a hypothesis was not supported by a significant effect of slope inclination on size and density of the kilns sites, which may indicate the role of other factors not included in this study, such as the length of the coppicing cycle and the consequently variable amount of wood produced by the forest. More direct effects are likely associated with the different tree species composition of the forest types and their consequently different levels of productivity. From local (Tuscan) productivity tables, the average wood volume in twelve-years oak coppices is 45.2 m^3/ha, vs. 40.6 m^3 and 35.5 m^3 in, respectively, beech and sclerophyll stands with the same age and under similar conditions (Istituto Sperimentale per l’Assestamento Forestale e per l'Aplicoltura, 1970). Such differences in the levels of productivity contribute to explain the higher proportion of surface covered by the kiln sites in the more productive oak forests compared with beech and, even more, sclerophyllous forests.

On the other hand, our data also show variations in the size and total surface of the kiln sites among the kilns within the same quadrat. The site-random effect was in fact included in the model to remove among-quadrat variation. This is possibly due again to local differences in forest productivity but also to cultural aspects, since local traditions may have led to the establishment of slightly different practices in similar forest environments.

In all three forest types, the different tree species currently occurring in the stands around the kilns are most likely the same that were used to produce the wood charcoal in former times. We found no evidence that local people used to make a selection among these species, also due to their similarly high calorific power (from 17.78 MJ/kg in *Quercus cerris* to 18.84 MJ/kg in *Castanea sativa*; Hellrigl, 2006). Anthracological studies in central and southern Europe also showed that charcoal remains in the soil reflect the composition of the vegetation in the immediate surroundings, with no indications for the selection of certain taxa (Ludemann, 2003, 2010; Nelle et al., 2010; Pèlachs et al., 2009).

In all sites, the charcoal layer in soil was single, continuous and rich in charcoal fragments of variable size, and about 23 cm thick. This is a bit higher than recent observations from a larch forest of the eastern Italian Alps (Criscuoli et al., 2014), but lower than the average measured in Wallonian forests (Hardy and Dufey, 2015b). The considerable average thickness of this layer suggests that the same platforms were used repeatedly at given time intervals, in correspondence with forest utilizations at the end of the coppice cycles. This supports the hypothesis that charcoal kilns built on small, man-made terraces in hilly terrains were repeatedly used, in contrast with those prepared on flat terrains, that were often used only once, as for example in the Zoerslen forest in Belgium (Deforce et al., 2012). In our sites, the time interval between two consecutive utilizations of the same kiln platform was usually very short (6-12 years), since charcoal production for home energy required the use of young coppice stools of small diameter. Such a short rotation can explain the fact that the charcoal profile was single and continuous, in contrast to what expected for other sites that were repeatedly used at larger time intervals and therefore with a discontinuous charcoal profile.

Repeated use of the sites on hill and mountain slopes is also supported by the positive effect of slope inclination on the thickness the charcoal layer, as shown by the best model selected for this variable. The steeper the slope on which the platform was placed, the harder was the work to build it, which often required to prepare stone walls on the downhill side to sustain the terrace in a horizontal position (Fig. 2B). Such an investment of time and energy was done in the perspective of a repeated use of the same kiln site for long periods, which mostly probably resulted in the formation of a thicker charcoal profile than in the sites on less steep terrains. Indeed, kiln platforms on the steeper slopes of the beech forests showed on average a thicker charcoal profile (31.2 cm vs. 29.3 and 21 cm in sclerophyll and oak forest). Model results did not indicate forest type as a significant predictor for this variable mainly due to the large variation among the three sclerophyll quadrats. The six kilns of the sclerophyll quadrat in Marsiliana had a remarkably thick charcoal layer, despite the only moderately steep slopes of this area. This is possibly due to the long period of intensive human exploitation of the woodlands in this southern part of Tuscany, that started as early as the Etruscan period (6th century BC, Mariotti Lippi et al., 2002). Based on local documents, production of charcoal was deeply rooted in the local communities and most probably already in use in the Iron age.

Based on the few available studies, the amount of total C contained in the soil of abandoned charcoal kiln platforms is usually considerably higher than in the soil of the adjacent forest environment. In sites of the same region investigated here, Carrari et al. (*ms accepted*) found that total C was on average nearly twice than in control soils (10.5% vs. 5.65%), while Criscuoli et al. (2014) measured an even higher difference (26.2 kg/m^2 vs. 1 kg/m^2 of total C in the whole anthropogenic layer) in charcoal kilns in a larch forest of the Italian Alps. Similar evidence was obtained in Canada, where organic matter in charcoal kilns was 13.9% vs. 5.6% in the control soils (Mikan and Abrams, 1995). In the two Italian studies mentioned above, most of the C was in the form of carbon, whose condensed aromatic structure allows the fragments to persist in the soils over millennial time-scales (Cheng et al., 2008). Given the long-term stability of this material and the fact that it is accumulated in thick soil layers over a significant proportion of the forest surface (up to 2.3%), these sites will have to be considered in future estimations of the carbon stock capacity of the woodlands in our region.

Airborne Laser Scanning (ALS) method

The combination of hillshade and slope images derived from ALS data emerged as a promising approach for the detection of kiln sites in the variable conditions of vegetation and terrain of our region. Using only hillshade maps, Digital Elevation Models (DEMs), or Local Relief Models as in previous studies in N and C Europe (Bollandsås et al., 2012; Deforce et al., 2012; Hesse, 2010; Ludemann, 2011; Risbøl et al., 2013) was not sufficient to identify the kiln platforms in the case of hilly or mountainous areas such as those sampled here.

Overall detection accuracy was 100% in the case of the Vallombrosa beech forest, where kiln sites were unambiguously identified thanks to the lack of natural "morphological equivalents", as already observed in Germany (Hesse, 2010). Accuracy was bit lower in the case of the two quadrats with oak-dominated vegetation. In one of these (Val di Farma), one kiln site inventoried in the field was not detected by ALS data, while in the other one (Tatti) two sites that could not be observed in the field were detected by this method. Reasons for the higher precision in the beech forest depend firstly on the higher ALS point density that was available for this area, compared with the two other quadrats. Indeed, Bollandsås et al. (2012) suggested that detection success of cultural and archaeological remains in forests increases with increasing density of points. Also, the steep slopes, the simpler (e.g. single-layered) forest structure and the lack of shrub vegetation contributed to such an elevated accuracy in this quadrat. In the two oak areas, the less inclined slopes and the structural density of the forest stands have probably limited the efficacy of this method, since more complex patterns of shadowing and texture result in more complex vegetation landscapes on irregular terrains (Amable et al., 2004). For example, some features created by the uprooting of large trees in points with low slope inclination may have caused misinterpretations (Hesse, 2010). According to Ludemann (2011), factors such as (1) bad conservation, by e.g. erosion, forest road construction, wood transport etc., (2) heterogeneities of the ground surface, or (3) vegetation with dense herb or shrub layer can reduce the reliability of the ALS method. Hence, this method cannot completely replace the field-based inventories when an absolute precision is needed (Deforce et al., 2012).

Unfortunately we could not test the applicability of the method in a typical Mediterranean forest landscape with very dense and relatively low communities for evergreen, sclerophyllous trees and shrubs (the "maquis"). No ALS data were in fact available for the sclerophyll areas. To our knowledge, no previous studied provided evidence on this aspect, which therefore requires further investigation.

Conclusions

This study allows a better knowledge and understanding of a major legacy of the human activities in the forests of the Mediterranean region, and shows that some characters of the kiln sites in our study area differ from those in other parts of Europe. In the coppice woodlands of central Italy, the repeated events of wood charcoal production in the same sites have left thousands of small platforms more or less regularly spaced in the forest landscapes. As expected, geomorphological factors and forest type affected some of the morphological variables of the kilns platforms, but local traditions and practices have also contributed to this differentiation.

The magnitude of the effects on the soil-vegetation system at the forest level may not be neglected, taking into account the high average density, relative total surface, and amount of charcoal in the soil. The data provided here could be the basis for further studies focusing on the contribution of the kiln sites to the carbon stock capacity of forest soils in our region, an aspect that has never been considered to date.

Further studies focusing on the ecological effects or the more historical, archaeological or anthracological aspects of the kiln sites in our region will benefit from the use of the ALS method. Using both hillshade and slope images is most appropriate in areas comparable to ours. When a slightly lower accuracy can be accepted, the ALS method will allow the inventorying of the sites at large spatial scales with various potential applications for more ecological and historical-archaeological investigations. When a 100% detection accuracy is required, fieldwork will remain necessary, especially in thermophilous deciduous forests on hilly terrains.

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Appendices

Appendix 1- PAPER I: Woody species in sclerophyll forests

Frequency and height (mean ± standard deviation of height, as maximum for trees and shrubs <1.30 m and mean for that one >1.30) of the species recorded in the kiln plots (KP) and charcoal plots (CP) in understorey and in tree regeneration layer in sclerophyll forests (18 plots). No significant differences in height between KP and CP were found (all p-values > 0.05, Mann-Whitney U-test, not shown).

	Frequency (%)		Height (cm)					
	KP	CP	KP			CP		
Understorey (<1.30 m)								
Acer obtusatum	5.6	0.0	5.0	±	0.0	0.0	±	0.0
Arbutus unedo	11.1	5.6	2.0	±	0.0	110.0	±	0.0
Crataegus monogyna	5.6	0.0	12.0	±	0.0	0.0	±	0.0
Erica arborea	5.6	0.0	70.0	±	0.0	0.0	±	0.0
Fraxinus angustifolia	5.6	5.6	5.0	±	0.0	5.0	±	0.0
Fraxinus ornus	83.3	77.8	17.0	±	20.4	14.9	±	26.2
Juniperus communis	5.6	0.0	130.0	±	0.0	0.0	±	0.0
Laburnum anagyroides	5.6	0.0	7.0	±	0.0	0.0	±	0.0
Myrtus communis	0.0	5.6	0.0	±	0.0	130.0	±	0.0
Ostrya carpinifolia	5.6	5.6	5.0	±	0.0	3.0	±	0.0
Phillyrea angustifolia	5.6	16.7	3.0	±	0.0	56.0	±	36.8
Phillyrea latifolia	16.7	38.9	39.3	±	33.9	60.0	±	47.7
Pistacia lentiscus	5.6	0.0	20.0	±	0.0	0.0	±	0.0
Pyrus amygdaloides	5.6	0.0	7.0	±	0.0	0.0	±	0.0
Prunus spinosa	16.7	0.0	10.0	±	4.4	0.0	±	0.0
Quercus cerris	16.7	5.6	5.3	±	0.7	8.0	±	0.0
Quercus ilex	38.9	33.3	12.9	±	13.1	39.8	±	46.9
Quercus petraea	5.6	5.6	6.0	±	0.0	12.0	±	0.0
Quercus pubescens	5.6	27.8	6.0	±	0.0	9.4	±	3.4
Quercus suber	5.6	5.6	5.0	±	0.0	4.0	±	0.0
Sorbus torminalis	5.6	11.1	9.0	±	0.0	12.5	±	13.4
Ulmus minor	5.6	0.0	8.0	±	0.0	0.0	±	0.0
Viburnum tinus	44.4	38.9	12.0	±	10.0	6.9	±	6.4
Tree regeneration (1.30-4 m)								
Arbutus unedo	5.6	27.8	2	±	0	3.3	±	0.4
Cytisus scoparius	5.6	0.0	3	±	0	0.0	±	0.0
Erica arborea	5.6	16.7	1.5	±	0	2.7	±	1.0
Fraxinus ornus	0.0	5.6	0	±	0	2.5	±	1.0
Juniperus communis	0.0	5.6	0	±	0	3.5	±	0.0
Phillyrea latifolia	0.0	11.1	0	±	0	2.3	±	0.8
Quercus ilex	5.6	11.1	4	±	0	4.0	±	0.0
Viburnum tinus	0	11.1	0	±	0	3.1	±	0.4

Appendix 2- PAPER I: Woody species in oak forests

Frequency and height (mean ± standard deviation of height, as maximum for trees and shrubs <1.30 m and mean for that one >1.30) of the species recorded in the kiln plots (KP) and charcoal plots (CP) in understorey and in tree regeneration layer of oak forests (22 plots). No significant differences in height between KP and CP were found (all p-values > 0.05, Mann-Whitney U-test, not shown).

	Frequency (%)		Height (cm)					
	KP	CP	KP			CP		
Understorey (<1.30 m)								
Acer campestre	27.3	13.6	9.7	±	7.0	16.0	±	10.6
Acer monspessulanum	18.2	0.0	9.8	±	7.4	0.0	±	0.0
Acer obtusatum	9.1	4.5	5.0	±	0.0	5.0	±	0.0
Acer pseudoplatanus	13.6	9.1	6.3	±	3.2	6.5	±	2.1
Carpinus betulus	13.6	9.1	3.0	±	1.0	3.0	±	0.0
Cornus mas	22.7	4.5	19.0	±	15.6	18.0	±	0.0
Cornus sanguinea	4.5	4.5	90.0	±	0.0	100.0	±	0.0
Crataegus monogyna	18.2	13.6	34.8	±	24.9	66.7	±	58.4
Erica scoparia	0.0	9.1	0.0	±	0.0	23.5	±	12.0
Euonymus europaeus	9.1	18.2	19.0	±	15.6	50.0	±	38.5
Fagus sylvatica	22.7	0.0	8.6	±	5.7	0.0	±	0.0
Fraxinus ornus	86.4	86.4	25.3	±	30.5	14.1	±	16.2
Ilex aquifolium	0.0	13.6	0.0	±	0.0	15.7	±	10.2
Laburnum anagyroides	4.5	0.0	0.0	±	0.0	0.0	±	0.0
Laurus nobilis	4.5	4.5	38.0	±	0.0	13.0	±	0.0
Ostrya carpinifolia	54.5	54.5	5.3	±	7.8	2.8	±	1.3
Phillyrea latifolia	13.6	13.6	3.3	±	1.5	58.0	±	4.1
Pyrus pyraster	4.5	0.0	4.0	±	0.0	0.0	±	0.0
Prunus avium	9.1	0.0	6.0	±	1.0	0.0	±	0.0
Prunus spinosa	36.4	4.5	16.8	±	24.2	8.0	±	0.0
Quercus cerris	40.9	18.2	8.9	±	4.4	10.5	±	6.4
Quercus ilex	22.7	36.4	27.4	±	28.5	34.6	±	42.3
Quercus petraea	9.1	4.5	10.0	±	7.1	8.0	±	0.0
Quercus pubescens	9.1	9.1	10.0	±	7.1	10.0	±	7.1
Sambucus nigra	4.5	0.0	32.0	±	0.0	0.0	±	0.0
Sorbus domestica	13.6	4.5	24.7	±	12.3	50.0	±	0.0
Sorbus torminalis	36.4	18.2	12.3	±	12.8	3.5	±	1.7
Taxus baccata	4.5	0.0	3.0	±	0.0	0.0	±	0.0
Ulmus glabra	4.5	4.5	14.0	±	0.0	32.0	±	0.0
Viburnum tinus	4.5	0.0	3.0	±	0.0	0.0	±	0.0

Tree regeneration (1.30 - 4 m)				
Carpinus betulus	0.0	4.5	0 ± 0	2.5 ± 0.0
Cornus mas	0.0	13.6	0 ± 0	3.0 ± 1.0
Cornus sanguinea	0.0	0.0	0 ± 0	0.0 ± 0.0
Crataegus monogyna	0.0	4.5	0 ± 0	2.5 ± 2.5
Fraxinus ornus	0.0	22.7	0 ± 0	3.3 ± 0.4
Ostrya carpinifolia	0.0	9.1	0 ± 0	3.2 ± 0.4
Quercus ilex	0.0	2.7	0 ± 0	2.5 ± 0.5
Sorbus domestica	0.0	0.0	0 ± 0	0.0 ± 0.0
Sorbus torminalis	0.0	9.1	0 ± 0	2.9 ± 0.6
Taxus baccata	0.0	4.5	0 ± 0	2.0 ± 2.0

Appendix 3- PAPER I: Woody species in beech forests

Frequency and height (mean ± standard deviation of height, maximum for trees and shrubs <1.30 m and mean for that one >1.30) of the species recorded in the kiln plots (KP) and charcoal plots (CP) in understorey and in tree regeneration layer of beech forests (21 plots). No significant differences in height between KP and CP were found (all p-values > 0.05, Mann-Whitney U-test, not shown).

Species	Frequency (%)		Height (cm)	
	KP	CP	KP	CP
Understorey (<1.30 m)				
Abies alba	28.6	23.8	5.5 ± 2.5	5.2 ± 3.0
Acer campestre	4.8	4.8	6.0 ± 0.0	4.0 ± 0.0
Acer obtusatum	4.8	4.8	7.0 ± 0.0	6.0 ± 0.0
Acer platanoides	4.8	0.0	6.0 ± 0.0	0.0 ± 0.0
Acer pseudoplatanus	19.0	23.8	10.8 ± 6.2	9.8 ± 4.3
Castanea sativa	4.8	0.0	6.0 ± 0.0	0.0 ± 0.0
Crataegus laevigata	4.8	0.0	2.0 ± 0.0	0.0 ± 0.0
Crataegus monogyna	4.8	0.0	1.0 ± 0.0	0.0 ± 0.0
Fagus sylvatica	23.8	28.6	8.6 ± 5.7	32.2 ± 39.6
Fraxinus ornus	14.3	9.5	4.7 ± 2.3	7.5 ± 6.4
Populus tremula	4.8	4.8	18.0 ± 0.0	7.0 ± 0.0
Prunus avium	0.0	4.8	0.0 ± 0.0	7.0 ± 0.0
Prunus spinosa	4.8	0.0	5.0 ± 0.0	0.0 ± 0.0
Quercus cerris	4.8	4.8	8.0 ± 0.0	18.0 ± 0.0
Tree regeneration (1.30 - 4 m)				
Fagus sylvatica	0.0	14.3	0.0 ± 0.0	3.0 ± 1.8

Appendix 4- PAPER I: Environmental variables for the seedling density plots

Geographical coordinates, altitude, slope aspect and parent rock of the subsample of plots where seedling density was determined was with number of charcoal kiln (KP) and control plots (CP) for each examined forest area.

Forest name	Latitude	Longitude	n. KP	n. CP	Forest Type*	Altitudinal range* (m a.s.l.)	Aspect*	parent rock material*
Foreste Casentinesi	43°48'19"N	11°52'9"E	5	5	beech	1040-1223	S/SE/E	marl-sandstone
Volterra hills	43°25'55"N	11° 0'2"E	5	5	oaks	382-967	E/SE/N/NW/NE	diabase/limestone/sandstone
Mt. Leoni	42°56'27"N	11°10'58"E	5	5	sclerophylls	155-437	S/SW/-/W/E	quarzitic sandstone

Appendix S1 - PAPER II:

Geographic coordinates (latitude and longitude according to the reference system WGS84), main geographic area, specific forest name and forest type of the 59 sampled sites. Each site is identified by an ID number (Site ID).

Site ID	Latitude	Longitude	Altitude (m a.s.l.)	Geographic area	Forest name	Forest Type
BE1	44°1'56.58"N	11°29'0.96"E	972	N Apennines	Colla di Casaglia	Beech
BE2	44° 2'43.32"N	11°28'4.80"E	1029	N. Apennines	Colla di Casaglia	Beech
BE3	44° 3'5.76"N	11°26'32.46"E	1002	N. Apennines	Colla di Casaglia	Beech
BE4	44° 3'5.46"N	11°26'18.54"E	989	N. Apennines	Colla di Casaglia	Beech
BE5	44° 3'13.02"N	11°25'54.42"E	964	N. Apennines	Colla di Casaglia	Beech
BE6	42°52'10.14"N	11°35'3.12"E	1226	Mt. Amiata	Aia dei Venti	Beech
BE7	42°51'58.50"N	11°35'30.84"E	1211	Mt. Amiata	Aia dei Venti	Beech
BE8	43°48'19.56"N	11°52'8.88"E	1116	N Apennines	Badia Prataglia	Beech
BE9	43°48'30.90"N	11°52'14.28"E	1223	N Apennines	Badia Prataglia	Beech
BE10	43°48'24.36"N	11°52'13.74"E	1183	N Apennines	Badia Prataglia	Beech
BE11	43°48'22.50"N	11°52'10.32"E	1151	N Apennines	Badia Prataglia	Beech
BE12	43°48'11.94"N	11°52'3.06"E	1040	N Apennines	BadiaPrataglia	Beech
BE13	42°52'3.90"N	11°35'29.52"E	1258	Mt. Amiata	Aia dei Venti	Beech
BE14	42°52'8.22"N	11°35'9.00"E	1267	Mt. Amiata	Aia dei Venti	Beech
BE15	42°45'4.68"N	11°39'41.04"E	900	Mt. Amiata	Monte Penna	Beech
BE16	42°44'16.08"N	11°40'14.10"E	891	Mt. Amiata	Monte Penna	Beech
BE17	42°45'3.72"N	11°39'45.00"E	909	Mt. Amiata	Monte Penna	Beech
BE18	42°45'27.30"N	11°39'26.76"E	846	Mt. Amiata	Monte Penna	Beech
BE20	42°52'26.94"N	11°35'46.92"E	1433	Mt. Amiata	AiadeiVenti	Beech
DE1	43°26'3.84"N	10°59'57.60"E	382	Colline Metallifere	Castelvecchio	Oak
DE2	43°25'55.26"N	11° 0'1.80"E	383	Colline Metallifere	Castelvecchio	Oak
DE3	43°26'1.62"N	11° 0'7.08"E	967	Colline Metallifere	Castelvecchio	Oak
DE4	43°25'56.94"N	10°55'2.22"E	438	Colline Metallifere	Montenero	Oaks
DE5	43°21'12.42"N	10°58'24.84"E	405	Colline Metallifere	Berignone-Tatti	Oaks
DE6	43°20'46.44"N	10°58'20.22"E	476	Colline Metallifere	Berignone-Tatti	Oaks
DE7	43°20'59.76"N	10°58'14.82"E	478	Colline Metallifere	Berignone-Tatti	Oaks
DE8	43°20'45.12"N	10°57'30.78"E	506	Colline Metallifere	Berignone-Tatti	Oaks

DE9	43°48'17.28"N	11°20'20.16"E	242	Florentine hills	Valle Sambre	Oaks
DE10	43°48'15.34"N	11°20'27.24"E	347	Florentine hills	Valle Sambre	Oaks
DE11	43° 5'21.54"N	11°10'46.02"E	319	Colline Metallifere	Val di Farma	Oaks
DE12	43° 5'25.26"N	11°11'3.60"E	299	Colline Metallifere	Val di Farma	Oaks
DE13	43° 5'24.03"N	11°10'51.84"E	329	Colline Metallifere	Val di Farma	Oaks
DE14	43° 4'17.94"N	11°13'50.94"E	467	Colline Metallifere	Val di Farma	Oaks
DE15	43° 4'21.60"N	11°16'17.64"E	484	Colline Metallifere	Val di Farma	Oaks
DE16	43° 4'19.97"N	11°16'29.19"E	511	Colline Metallifere	Val di Farma	Oaks
DE17	43° 4'37.68"N	11°12'58.50"E	406	Colline Metallifere	Val di Farma	Oaks
DE18	43° 4'35.94"N	11° 6'47.10"E	430	Colline Metallifere	La Pietra	Oaks
DE19	43° 4'34.86"N	11° 6'49.80"E	445	Colline Metallifere	La Pietra	Oaks
DE20	43° 9'11.94"N	11°13'20.46"E	265	Colline Metallifere	Val di Merse	Oaks
DE21	43°13'35.10"N	11°11'30.18"E	351	Colline Metallifere	Val di Merse	Oaks
DE22	43°10'28.02"N	11°12'20.64"E	438	Colline Metallifere	Val di Merse	Oaks
SC1	43°25'59.52"N	11° 0'9.84"E	368	Colline Metallifere	Castelvecchio	Sclerophyll
SC2	43°25'57.48"N	10°54'41.58"E	437	Colline Metallifere	Montenero	Sclerophyll
SC3	43°26'11.94"N	10°54'37.56"E	321	Colline Metallifere	Montenero	Sclerophyll
SC4	43°25'59.22"N	10°54'45.42"E	436	Colline Metallifere	Montenero	Sclerophylls
SC5	42°56'22.80"N	11°11'15.18"E	176	Maremma	Mt. Leoni	Sclerophylls
SC6	42°56'27.24"N	11°10'58.44"E	155	Maremma	Mt. Leoni	Sclerophylls
SC7	42°57'5.39"N	11° 9'12.70"E	112	Maremma	Mt. Leoni	Sclerophylls
SC8	42°54'44.88"N	11° 8'14.70"E	129	Maremma	Mt. Leoni	Sclerophylls
SC9	42°55'20.58"N	11° 9'27.66"E	345	Maremma	Mt. Leoni	Sclerophylls
SC10	43° 4'21.54"N	11°16'43.20"E	508	Colline Metallifere	Val di Farma	Sclerophylls
SC11	43°16'3.84"N	10°38'22.92"E	201	Maremma	Magona	Sclerophylls
SC12	43°16'2.46"N	10°37'56.88"E	197	Maremma	Magona	Sclerophylls
SC13	42°59'0.90"N	10°29'47.28"E	191	Maremma	Mt. Massoncello	Sclerophylls
SC14	42°59'3.18"N	10°29'48.90"E	171	Maremma	Mt. Massoncello	Sclerophylls
SC15	43°15'58.08"N	10°37'44.58"E	157	Maremma	Magona	Sclerophylls
SC16	43°15'50.18"N	10°37'53.71"E	171	Maremma	Magona	Sclerophylls
SC17	43°16'7.74"N	10°38'3.06"E	172	Maremma	Magona	Sclerophylls
SC18	42°54'50.40"N	11° 9'20.94"E	321	Maremma	Mt. Leoni	Sclerophylls

Appendix S2 - PAPER II:

Mean values ± standard deviation of all measured and calculated understorey-related variables in function of plot type. SR indicates species richness; inter-site diss. and intra-site diss. indicate inter-site and intra-site compositional dissimilarity respectively using Lennon (L) and Bray Curtis (BC) distance measures; Tax. distinctness indicates distinctness (Δ^+); C is the total carbon content, N, the total nitrogen content, C/N the ratio carbon-nitrogen; finally PAR reports values of photosynthetic active radiation. For each variable p-values are given in Appendix S3.

Response variable	Unit	Plot type	
		Control plots	Kiln plots
γ-diversity		132	204
SR total		8.5 ± 4.5	13.9 ± 6.7
SR trees		2.0 ± 1.3	2.6 ± 2.0
SR shrubs		1.4 ± 1.3	1.9 ± 1.5
SR graminoids		0.8 ± 1.0	2.1 ± 1.9
SR ferns		0.2 ± 0.5	0.2 ± 0.4
SR vines		1.3 ± 1.3	1.3 ± 1.1
SR other herbs		3.3 ± 2.8	6.8 ± 4.3
Shannon-Wiener		1.42 ± 0.54	1.65 ± 0.56
Inter-site diss. L		0.66 ± 0.12	0.71 ± 0.08
Inter-site diss. BC		0.90 ± 0.06	0.92 ± 0.04
Intra-site diss L		/	/
Intra-site diss BC		/	/
Tax. distinctness		67.7 ± 4.9	66.3 ± 4.4
Cover all	%	6.1 ± 7.8	16.2 ± 16.0
Cover trees	%	0.9 ± 1.9	1.8 ± 3.3
Cover shrubs	%	1.5 ± 4.5	1.6 ± 3.1
Cover graminoids	%	0.7 ± 0.7	3.4 ± 5.7
Cover ferns	%	0.2 ± 0.8	0.6 ± 1.8
Cover vines	%	0.6 ± 1.3	0.7 ± 1.8
Cover other herbs	%	2.1 ± 2.8	8.1 ± 10.8
Total biomass	(g/m²)	6.6 ± 12.8	11.7 ± 15.3
Herb biomass	(g/m²)	1.79 ± 3.11	7.17 ± 8.11
Woody biomass	(g/m²)	4.86 ± 11.08	4.54 ± 9.06
C	%	5.65 ± 2.98	10.50 ± 3.70
N	%	0.42 ± 0.21	0.50 ± 0.15
C/N		13.3 ± 2.3	20.9 ± 4.1
pH		5.85 ± 0.88	6.08 ± 0.82
PAR	µmol.m2/s	20.7 ± 25.3	45.1 ± 64.8

Appendix S3 - PAPER II:

Effects of forest type and plot type on response variables; p-values referred to models containing random effect (§) were obtained by Satterthwaite approximation, implemented in the *lmerTest* package, those referred for models with fixed effect only are calculated with the function *summary.* See main text for the explanations of response and predictor variables.

Response variable	Unit	Intercept	Forest type		Plot type
			Oak	Sclerophyll	Charcoal kiln
SR total§		<0.001	0.000	0.981	<0.001
SR trees		0.695	<0.001	0.032	0.022
SR shrubs		0.002	<0.001	<0.001	0.044
SR graminoids§		0.115	0.275	0.022	<0.001
SR ferns		<0.001	0.138	0.056	/
SR vines		<0.001	<0.001	<0.001	/
SR other herbs§		<0.001	0.795	<0.001	<0.001
Shannon-Wiener§		<0.001	0.035	0.485	0.002
Cover all§	%	0.163	0.013	0.769	<0.001
Cover trees§	%	0.891	0.020	0.401	0.0071
Cover shrubs	%	0.001	<0.001	<0.001	/
Cover graminoids§	%	0.200	0.606	0.113	<0.001
Cover ferns§	%	0.184	/	/	0.078
Cover vines§	%	0.778	0.014	0.415	/
Cover other herbs§	%	0.001	0.192	0.318	/
Total biomass§	(g/m^2)	0.266	0.026	0.775	0.010
Herb biomass§	(g/m^2)	0.578	0.035	0.783	<0.001
Woody biomass§	(g/m^2)	<0.001	/	/	/
Inter-site diss. L §		<0.001	0.007	0.005	0.001
Inter-site diss. BC §		<0.001	0.000	0.008	0.014
Intra-site diss. L		0	/	/	NA
Intra-site diss. BC		0	/	/	NA
Tax.distinctness ⍰$^{+}$		0.002	<0.001	<0.001	0.044
C§	%	<0.001	<0.001	0.00286	<0.001
N§	%	<0.001	/	/	0.000
C/N		<0.001	<0.001	<0.001	<0.001
pH§		<0.001	0.001	0.014	0.001
PAR	µmol m^2/s	0.002	/	/	0.008

Appendix 1- PAPER III: Mean values ± standard deviation of height and fluorescence indices considered (see pg. 89 for explanation) . P-values from t test or Mann Withney, depending on normality.

			height	p-lev	FV/FM	p-lev	ΨEo	p-lev	ΨRo (1-VI)	p-lev	PIABS	p-lev	PITOT	p-lev
sp14	QI	C	8.53 ± 2.92	<0.001	0.800 ± 0.015	0.045	0.551 ± 0.041	0.027	27.782 ± 6.05	n.s.	0.165 ± 0.027	<0.001	12.378 ± 6.137	n.s.
		N	7.38 ± 2.13		0.793 ± 0.013		0.574 ± 0.049		29.057 ± 10.93		0.197 ± 0.071		17.350 ± 28.496	
	QC	C	9.91 ± 4.31	<0.001	0.798 ± 0.019	0.020	0.558 ± 0.028	0.009	26.539 ± 6.63	n.s.	0.209 ± 0.020	<0.001	15.703 ± 3.343	0.004
		N	8.29 ± 3.36		0.789 ± 0.012		0.577 ± 0.037		27.025 ± 6.25		0.231 ± 0.017		18.013 ± 3.696	
	FS	C	9.77 ± 2.30	<0.001	0.788 ± 0.012	0.049	0.548 ± 0.029	n.s.	20.486 ± 4.10	n.s.	0.149 ± 0.014	n.s.	8.234 ± 2.463	n.s.
		N	9.41 ± 2.24		0.793 ± 0.010		0.545 ± 0.023		21.714 ± 4.07		0.143 ± 0.013		7.659 ± 1.664	
su14	QI	C	13.06 ± 4.40	n.s.	0.810 ± 0.007	<0.001	0.614 ± 0.021	n.s.	38.513 ± 7.48	0.004	0.170 ± 0.021	0.008	16.632 ± 17.502	n.s.
		N	12.67 ± 4.29		0.802 ± 0.010		0.611 ± 0.033		34.841 ± 6.18		0.180 ± 0.015		14.576 ± 3.100	
	QC	C	11.17 ± 4.80	0.030	0.800 ± 0.010	0.004	0.585 ± 0.022	n.s.	29.911 ± 4.73	<0.001	0.192 ± 0.046	0.021	14.287 ± 3.438	<0.001
		N	10.10 ± 4.67		0.805 ± 0.008		0.593 ± 0.023		34.685 ± 5.59		0.197 ± 0.024		16.967 ± 3.626	
	FS	C	11.38 ± 2.29	0.015	0.777 ± 0.012	n.s.	0.572 ± 0.019	n.s.	20.273 ± 3.66	n.s.	0.142 ± 0.018	n.s.	6.781 ± 1.484	n.s.
		N	11.23 ± 2.39		0.777 ± 0.017		0.574 ± 0.024		20.665 ± 3.61		0.147 ± 0.026		7.144 ± 1.611	
au14	QI	C	13.93 ± 4.61	n.s.	0.808 ± 0.009	0.009	0.589 ± 0.024	n.s.	37.788 ± 6.69	n.s.	0.228 ± 0.015	n.s.	26.011 ± 4.885	n.s.
		N	13.97 ± 4.65		0.805 ± 0.007		0.597 ± 0.028		36.660 ± 5.86		0.232 ± 0.019		25.031 ± 5.098	
	QC	C	11.36 ± 4.85	0.035	0.809 ± 0.005	<0.001	0.551 ± 0.032	<0.001	30.745 ± 6.28	<0.001	0.182 ± 0.020	<0.001	15.462 ± 4.012	<0.001
		N	10.41 ± 4.64		0.816 ± 0.015		0.572 ± 0.027		40.902 ± 6.93		0.206 ± 0.020		23.479 ± 5.673	
	FS	C	11.73 ± 2.30	0.035	0.756 ± 0.034	0.002	0.503 ± 0.032	<0.001	17.362 ± 8.98	<0.001	0.188 ± 0.029	<0.001	11.139 ± 4.797	n.s.
		N	11.77 ± 2.23		0.737 ± 0.029		0.476 ± 0.029		11.259 ± 3.26		0.212 ± 0.051		9.856 ± 3.783	
winter	QI	C	/ /	/	0.739 ± 0.021	0.072	0.551 ± 0.037	n.s.	16.911 ± 5.04	n.s.	0.271 ± 0.029	<0.001	15.718 ± 11.102	<0.001
		N	/ /		0.730 ± 0.033		0.555 ± 0.045		14.899 ± 5.26		0.453 ± 0.024		8.725 ± 3.036	
sp15	QI	C	/ /	/	0.783 ± 0.040	0.232	0.436 ± 0.065	n.s.	17.369 ± 11.10	n.s.	0.140 ± 0.028	n.s.	8.255 ± 5.601	n.s.
		N	/ /		0.797 ± 0.020		0.454 ± 0.076		20.292 ± 15.39		0.154 ± 0.040		11.053 ± 11.136	
	QC	C	/ /	/	0.826 ± 0.009	0.032	0.526 ± 0.039	n.s.	31.666 ± 7.46	n.s.	0.174 ± 0.036	n.s.	16.176 ± 6.534	n.s.
		N	/ /		0.828 ± 0.008		0.522 ± 0.031		30.857 ± 6.27		0.171 ± 0.036		15.630 ± 5.910	
	FS	C	/ /	/	0.807 ± 0.013	n.s.	0.573 ± 0.034	<0.001	33.239 ± 6.95	<0.001	0.181 ± 0.026	<0.001	15.779 ± 5.172	<0.001
		N	/ /		0.805 ± 0.010		0.539 ± 0.040		28.082 ± 7.04		0.156 ± 0.030		11.760 ± 4.467	
su15	QI	C	20.71 ± 6.49	n.s.	0.806 ± 0.016	<0.001	0.645 ± 0.039	0.003	47.935 ± 10.32	0.002	0.178 ± 0.026	n.s.	18.586 ± 6.228	n.s.
		N	20.86 ± 7.34		0.785 ± 0.069		0.628 ± 0.041		42.042 ± 13.40		0.184 ± 0.024		17.476 ± 5.783	
	QC	C	22.35 ± 7.68	n.s.	0.795 ± 0.025	<0.001	0.573 ± 0.046	n.s.	27.563 ± 8.02	n.s.	0.143 ± 0.031	n.s.	9.492 ± 4.046	n.s.
		N	21.32 ± 6.16		0.810 ± 0.017		0.567 ± 0.048		30.153 ± 7.94		0.140 ± 0.036		10.290 ± 4.387	
	FS	C	20.16 ± 5.94	n.s.	0.792 ± 0.026	<0.001	0.624 ± 0.038	n.s.	36.311 ± 8.17	<0.001	0.162 ± 0.019	<0.001	12.760 ± 3.695	0.021
		N	19.25 ± 5.44		0.806 ± 0.008		0.629 ± 0.019		0.142 ± 0.02		37.801 ± 5.270		11.330 ± 3.024	

Photo gallery

Photo 1 A burning charcoal kiln on French Pyrenees (Bonhôte, J., 1998. *Forges et forêts dans les Pyrénées ariégeoises: pour une histoire de l'environnement*. PyréGraph, p. 35.)

Photo 2 Reconstruction of a charcoal kiln realized in the Black Forest (Freiburg) in September 2015.

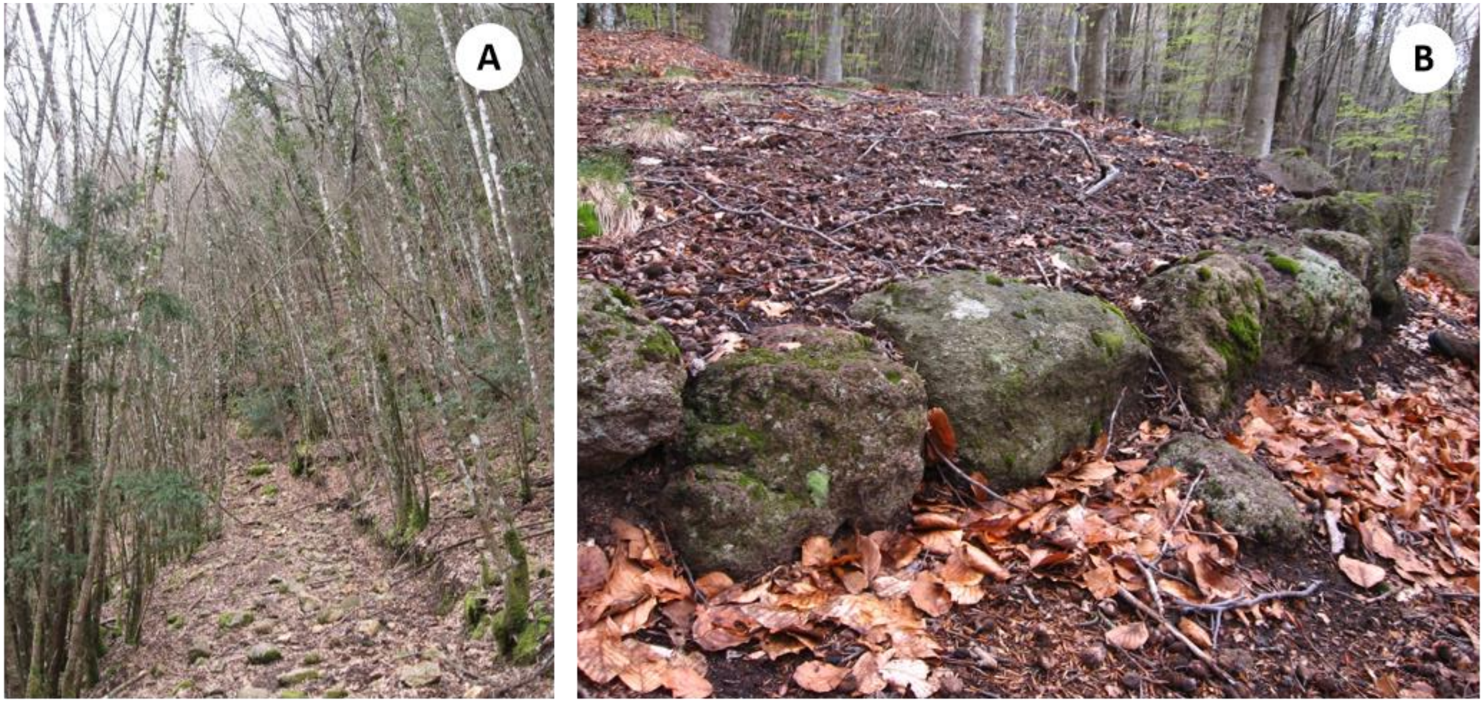

Photo 3 Cultural remains related to charcoal production in the examined forests: A) ancient track for the transport of wood charcoal with mules near the abandoned Middle Age village of Castelvecchio B) typical stone wall on the downhill border of a charcoal kiln site in the beech forest of Monte Amiata.

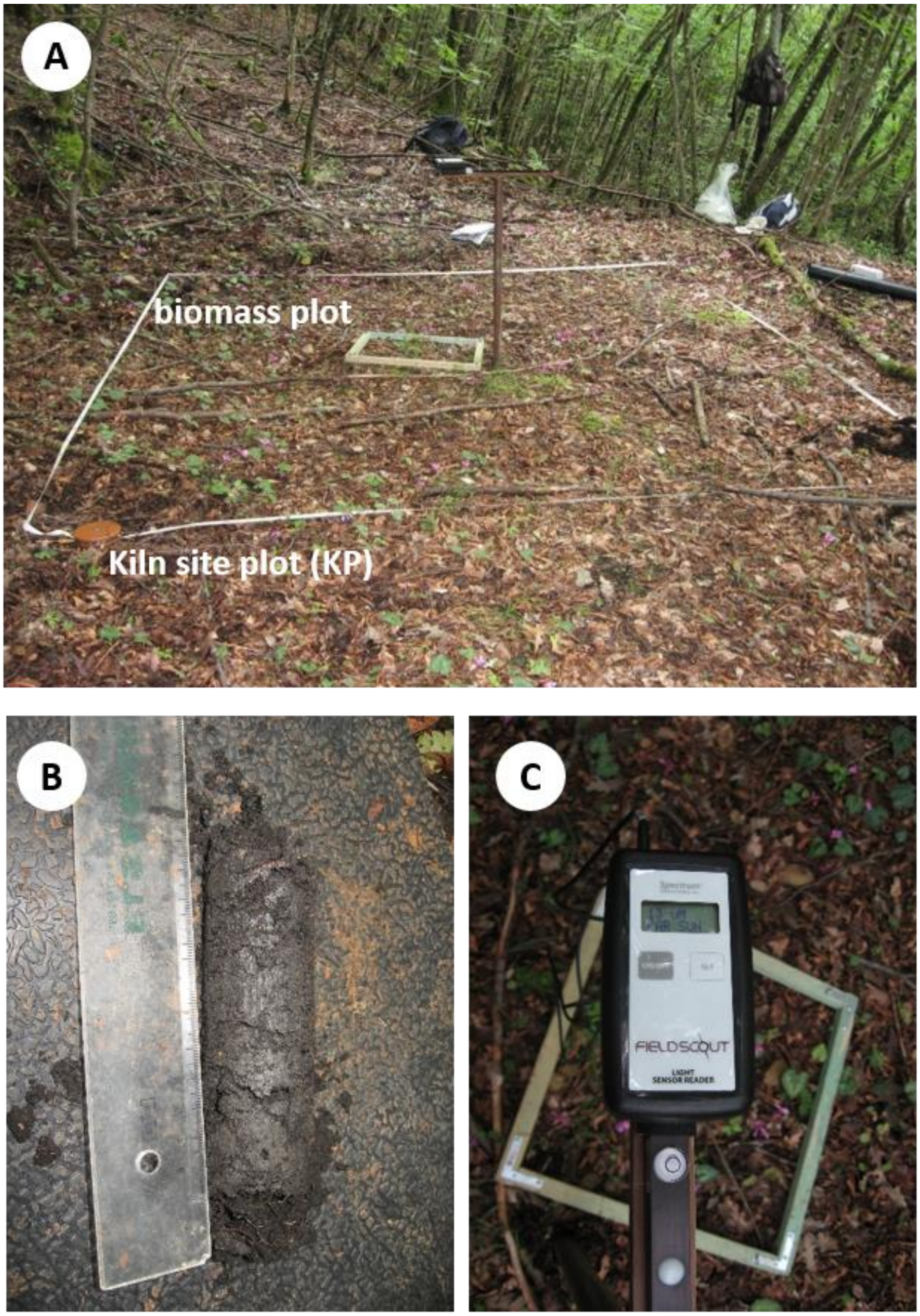

Photo 4 Vegetation sampling in a charcoal kiln site in the oak forest (Castelvecchio), showing the 3x3 m plot and the 0.5x0.5 m biomass plot (A), a 15 cm soil core (B) and the light sensor reader (C).

Photo 5 Charcoal kiln site in the Belagaio forest in winter, showing the flat regular surface and the complete lack of woody vegetation

Photo 6 Charcoal kiln site in the Belagaio forest in winter, showing the regular alignment of oak trees along the uphill border of the platform.

Photo 7 Charcoal kiln site in the Marsiliana forest with *Q. ilex* and *Q. cerris*, showing lack of woody vegetation on its relatively large surface (*Photo by Gianni Mastrolonardo*).

Photo 8 Control area adjacent to the kiln site shown above (photo 4) showing woody vegetation of various species such as *Q. ilex, Q. cerris, F. ornus, Phillyrea latifolia* and others (*Photo by Gianni Mastrolonardo).*

Photo 9 Charcoal kiln site in the oak forest of Tatti (*Q. petraea* and *Q. cerris*), showing abundant herbaceous understorey.

Photo 10 Control area adjacent to the kiln site shown above (photo 6) showing woody vegetation of various species and very sparse herb layer.

Photo 11 Kiln site plot in Castelvecchio oak forest with abundant *Ostrya carpinifolia*. Showing higher cover and biomass of the understorey compared with the adjacent forest.

Photo 12 Dense evergreen maquis in the Marsiliana forest, suddenly interrupted in correspondence of an old charcoal kiln site with mainly shrubby understorey of *Cistus.*

Photo 13 Sampling in a kiln site plot in the beech forest of Casentino.

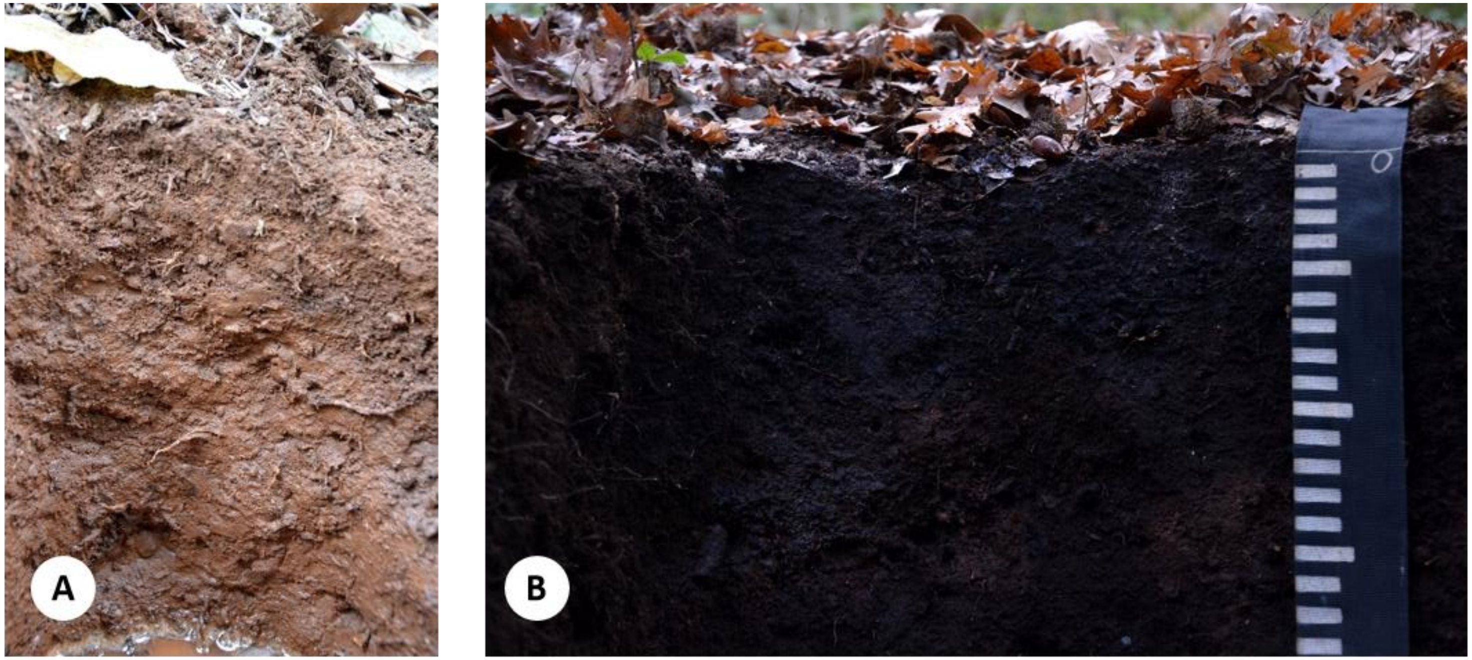

Photo 14 First centimeters of a normal soil profile in Marsilana forest (A) and of a charcoal kiln site (B) in the same area showing the striking difference in colour (*photos by Gianni Mastrolonardo*).

Photo 14 *Luzula forsteri* (Sm.) DC. (Juncaceae), one of the indicator species of the charcoal kiln habitat in oak forests, belonging the funcional group of *graminoids (photo by Federico Selvi).*

Photo 15 *Anemone nemorosa* L., the only species apparently avoiding the charcoal kiln habitat in the beech forests examined in this study *(photo by Federico Selvi)*.

Photo 17 Common garden experiment: pots filled with charcoal soil (darker colour) and control soil (A) and germinating acorn of *Quercus cerris* on charcoal soil

Photo 18 Common garden experiment: *Quercus ilex* seedlings grown in pots with charcoal soil (A) and control soil (B) in August 2014.

Photo 19 Common garden experiment: *Quercus cerris* seedlings grown in pots with charcoal soil (A) and control soil (B) in August 2014; only a few seedlings were dead by the end of the summer.

Photo 20 Common garden experiment: *Fagus sylvatica* seedlings grown in pots with charcoal soil (A) and control soil (B) in August 2014.

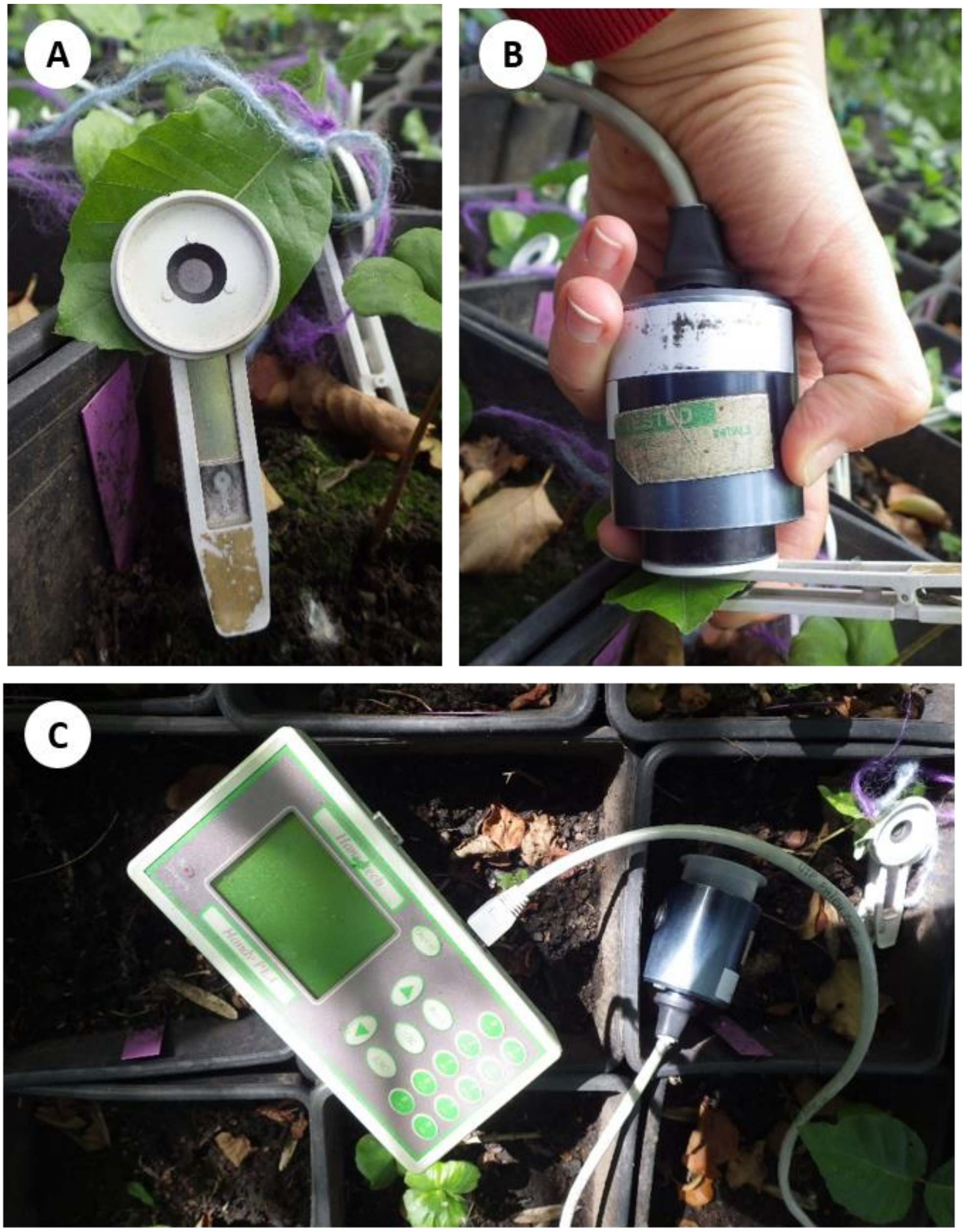

Photo 21 Measurement of ChlA fluorescence with HandyPea: Clips on marked leaves used for dark acclimatation (A) red-light lamp during a measurement (B) and the complete Handy Pea instrument with lamp, monitor and console (C) .