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Potential of various carbonized organic matters as peat substitute in growing media and soil amendment for wheat and sunflower cultivation

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Abstract

Biochar is a stable carbon-rich by-product obtained by pyrolysis of various biomasses. Its use has been recently suggested as peat substitution in potting substrates because of some intrinsic similarities with peat, while the addition to soil as an amendment lacks in open field experiments in the Mediterranean regions. Furthermore, only few studies on biochar impact on crops quality have been published, especially under field conditions in temperate regions.

This work aims to test the potential of selected biochars from different feedstocks, and a composted biochar, to partially or fully substitute peat for tomato and basil growth in a nursery trial. In the case of basil, the assemblage of volatile compounds of basil as well was checked. This work also focused on the effects of biochar as soil amendment on the yield and the content of some nutraceutical compounds of wheat and sunflower.

Overall, I found that high doses of biochar cannot be used in potting substrates for tomato and basil seedlings without negatively affecting their growth. Nonetheless, small doses are not harmful, and on a global scale may represent a precious contribution for preserving the remaining peat resources. The volatile organic compounds of basil were not significantly affected by biochar addition, even with 25% of peat substitution with biochar and up to 50% with composted biochar.. Conversely, high doses of biochar can be added as soil amendment for the cultivation of wheat and sunflower without negatively affecting their growth and nutraceuticals compounds, but this environment-friendly strategy is not feasible from an economic point of view.

Sommario

Per biochar si intende del materiale carbonioso ottenuto dalla pirolisi di biomasse. In anni recenti il suo utilizzo è stato suggerito anche come possibile sostituto della torba per i substrati di crescita nella filiera vivaistica, grazie ad alcune sue caratteristiche che lo rendono simile alla torba, ma sono ancora pochi gli studi in tal senso. L'utilizzo del biochar come ammendante del suolo, invece, è stato molto più studiato, ma sono relativamente pochi gli studi effettuati su prove di pieno campo nelle regioni a clima temperato del bacino del Mediterraneo. In particolare mancano poi studi sul possibile effetto del biochar sulla qualità dei raccolti, specialmente sulle colture tipiche delle nostre regioni.

Gli obbiettivi di questo lavoro sono quelli di testare un biochar compostato e diversi tipi di biochar, ottenuti da biomasse differenti in diverse condizioni di pirolisi, per la sostituzione totale o parziale della torba in una prova di vivaio su piante di pomodoro e basilico. Nel caso del basilico sono stati studiati gli effetti del biochar anche sul profilo dei composti volatili delle foglie. Questo lavoro si è inoltre focalizzato sugli effetti del biochar come ammendante del suolo, in una prova biennale su grano e girasole, sia sulla crescita delle piante che la produzione e la qualità del raccolto in termini di composti nutraceutici.

I risultati ottenuti indicano che non si possono utilizzare alte dosi di biochar nei substrati di crescita senza riscontrare effetti negativi sulla crescita delle piante. Solo dosi basse di biochar possono essere quindi utilizzate per la sostituzione della torba, comunque contribuendo a diminuire lo sfruttamento delle torbiere. Il profilo dei composti volatili del basilico non è stato modificato dall'aggiunta del biochar nel substrato di crescita, alle dosi testate del 25% di biochar in volume e fino al 50% con biochar compostato, ma la biomassa e il colore delle piante sono risultate comunque negativamente impattate. Alte dosi di biochar possono essere aggiunte al suolo, per la coltivazione del grano e del girasole, senza influire negativamente sulla loro crescita e sui composti nutraceutici identificati, sebbene l'utilizzo di biochar in pieno campo ad alte dosi non è una strategia economicamente applicabile.

Resumen

Se entiende por biochar un material rico en carbono obtenido por pirólisis de biomasa. Puesto que dicho material posee propiedades semejantes a la turba, en recientes investigaciones, aun incipientes, se está tratando de evaluar su capacidad para sustituir a este sustrato en cultivos de invernadero. Por otro lado, la aplicación agronómica más frecuente y más ampliamente estudiada es la del uso de biochar como mejorador de suelo. En este aspecto, hay pocos ensayos de campo en las regiones con clima templado de la cuenca mediterránea y en particular, no existen estudios previos sobre su influencia en la calidad de los cultivos típicos de esta región.

El objetivo principal de este trabajo es estudiar, por un lado, la capacidad de un biochar compostado y de varios tipos de biochar obtenidos a partir de diferentes biomasas y bajo diferentes condiciones de pirólisis de actuar como co-sustrato de la turba, sustituyéndola total o parcialmente en cultivos de tomate y albahaca producidos en invernadero. En el caso de la albahaca también se estudia los efectos del biochar sobre el perfil de compuestos volátiles de sus hojas. Por otro lado, se estudia el efecto del biochar como mejorador de suelos agrícolas en un ensayo bienal bajo cultivos de trigo y girasol evaluando su influencia sobre el crecimiento vegetal y sobre el rendimiento de la cosecha, así como la calidad de los cultivos en términos de su riqueza en compuestos nutracéuticos.

Los resultados obtenidos en invernadero indican que en dosis muy elevadas la presencia de biochar afecta negativamente al crecimiento de las plantas, pudiéndose administrar únicamente en dosis bajas. El perfil de compuestos volátiles de la albahaca a las dosis ensayadas (entre el 25% y 50% de biochar) no se vio modificado por la adición de biochar al medio, pero la biomasa y el color de las plantas sufrieron un impacto negativo en su presencia. Del experimento de campo se puede concluir que, en las condiciones ensayadas, los cultivos de trigo y girasol soportan la adición de altas dosis de biochar al suelo sin afectar negativamente ni a su crecimiento ni al contenido de los compuestos nutracéuticos identificados. Sin embargo, el uso de biochar en campo a altas dosis no es una estrategia económicamente viable.

Keywords: recycled waste; charcoal; composted biochar; essential oils; pot experiment; sustainability; growing media; nutraceuticals

1. Introduction

Global agriculture feeds over 7 billion people, but the set of all agricultural practices are also cause of environmental degradation. All the negative impacts of agriculture on environment, such as greenhouse gases emission, occupation of Earth’s land surface, freshwater withdrawals and others, will increase globally over the next years due to population growth. One of the suggested solution for sustainable agriculture and climate change mitigation is biochar, charred organic material that has received increasing attention during last years. Biochar addition to agricultural soils is largely advocated for various reasons related to sustainability. Soil improvement with biochar is often presented as a multiple “winning” strategy, its potential benefits including carbon sequestration, soil fertility enhancement, bioenergy production, heavy metals immobilization and waste disposal (Martos et al., 2020). The environmental benefits of biochar utilization have also been studied during the last decades not only in the field of agronomy but also on global change and pollution mitigation and waste recycling. In particular, biochar production from agricultural and environmental biomass is receiving interest as feasible amendment due to its potential benefits on both agriculture and environment. Biochar and composted-biochar have also been recently suggested as candidates for peat substitution due to some similarities with peat, such as high porosity, low density and high cation-exchange capacity.

1.1 Definition of biochar

Various definitions of biochar had been given during last decades. The “European Biochar Certificate – Guidelines for a Sustainable Production of Biochar” (EBC, 2019) defined biochar as “*a porous, carbonaceous material that is produced by pyrolysis of plant biomasses and is applied in such a way that the contained carbon remains stored as a long-term C sink or replaces fossil carbon in industrial manufacturing. It is not made to be burnt for energy generation*”. Other researchers, Lehmann and Joseph (2009), defined biochar as a carbon-rich product produced by “*so-called thermal decomposition of organic material under limited supply of oxygen (O_2), and at relatively low temperatures (<700°C)*”. As the authors recognized, this process indicates the production of charcoal, but the difference with biochar is that the latter is produced with the intention to add it to the soil. In fact, there is still no fully shared definition for biochar (Guo et al., 2015) and in literature the terms charcoal and biochar are often overlapped creating some confusion. Another term used is pyrogenic carbonaceous materials (PCM), that are defined by Brown *et al*. (2015) as any carbonaceous residues from pyrolysis. PCM is usually the most general term used in scientific literature to describe pyrolysis products from biomass or other materials. The term “char” indicates all PCM originated from natural fires, while charcoal refers to PCM produced from pyrolysis of animal or vegetable matter in kilns for cooking or heating (Brown et al., 2015). As described from Wiedner and Glaser (2015), charcoal is an energy carrier, e.g. for cooking, heating or metallurgy processes, while biochar production is finalized for application to soil, with agronomic or environmental purposes. Many researchers refer to biochar as the carbon-enriched black solids made from pyrolysis or gasification of biomass materials, intended to been used as soil amendment (Guo et al., 2015). Kookana et al. (2011) precised that “*biochar differs from charcoal in regard to its purpose of use, which is not for fuel, but for atmospheric carbon capture and storage, and application to soil”.* The International Biochar Initiative (IBI) defined biochar as a product obtained by thermochemical conversion of biomass under anoxic conditions, which can be applied as an additive to improve soil fertility, mitigate environmental pollution and reduce greenhouse gas emissions. This definition underlines the differences between biochar and other carbon products in their application, and emphasizes the role of biochar in agriculture.

1.2 Historical backgrounds

The term “Biochar” is relatively new, unlike its use that’s historically dates back at least 2000 years (O’Neill et al., 2009). Some regions throughout the world contain charcoal deposit naturally produced by events such as forest and grassland fires (Krull et al., 2008); it is the case for example of the North American Prairie. Large amount of charcoal incorporated into the soil can be found in the Amazon Basin, in this region the use of charcoal is witnessed by the fertile soils known as *Terra Preta* and *Terra Mulata*. The fertility of *Terra Preta* has been attributed to high char content. *Terra Preta de índio*, also known as Amazonian Dark Earths or ADE, are soils that have shown high fertility for thousands of year. Indeed, in these soils high concentrations of nitrogen, phosphorus, potassium and calcium can be found, as well as high amounts of stable soil organic matter (SOM) (Glaser et al., 2001). The presence of charcoal and aromatic humic substances in these soils suggest that residues of incomplete combustion of organic material are persistent in soil. It has not yet clarified if charcoal addition to *Terra Preta* soil was intentional or not; what can be hypothesized is that the indigenous populations started to intensify the agricultural land use after that soil improvement was noted as a consequence of charcoal application (Sombroek et al., 2017). It is estimated that the total area covered by *Terra Preta* is more than 50.000 ha in Central Amazonia (Glaser et al., 2001). Some authors suggested that ADE were intentionally created by the Amerindian population (Kern et al., 2009), with the aim of improving agricultural productivity. There are in fact archaeological evidences that

Amazonian landscapes were transformed by human activities in the proximity of their settlements (Kämpf and Kern, 2005). According to Sombroek et al. (2017) the soils called *Terra Mulata* are the resultant of the intentional application of charred plant materials mixed with products of human and animal activities such as hunting and fishing residues.

The historical utilization of charred material mixed with soil for agricultural purposes is witnessed in many countries (Wiedner and Glaser, 2015), and it is the case for example of the use of charcoal in China from the Shang and Zhou dynasties (Chen et al., 2019). Another example is given for northeast Asian countries like Japan, Korea and also China, where charcoal from rice husk was used as soil amendment (Ogawa and Okimori, 2010). In the same regions wood charcoal was produced using traditional earthen kilns for two thousand years but, because of its expensive price, its utilization was intended only for cooking and house warming and not for agriculture (Wiedner and Glaser, 2015). Some researchers (Cao et al., 2006) analyzed the surface layers of some paddy fields situated in an archeological site in the Yangtze River Delta, near Suzhou in China, that showed high amount of organic matter and rice opals, that have been dated around 4000 B.C. Through solid-state ^{13}C nuclear magnetic resonance it has been possible to reveal the presence of aromatic carbon (C) as the major organic C form present in the fossil surface coat of those prehistoric irrigated rice field. This can be explained by the presence of charred rice residues derived by post-harvest burning. The proposed theory is that those fields were plowed burning rice straw residues at the end of the crop cycle, irrigated to control weeds and rice seeds were directly sown. The use of charcoal in Japanese agriculture can also be found in what can be considered the oldest textbook of sorts entitled "Nogyo Zensho" (Encyclopedia of Agriculture) written by Yasusada Miyazaki in 1697. Miyazaki wrote: "*After charring all waste, concentrated excretions should be mixed with it and stocked for a while. When you apply this manure to the fields, it is efficient for yielding any crop".* Some authors (Glaser et al., 2002; Ogawa and Okimori, 2010) claim that rice husk charcoal has been used since the beginning of rice cultivation in Asia. The traditional cultivation of this crop, supported by organic fertilizers and charcoal, seems to have even an older tradition than that of *Terra Preta* in the Amazon.

Regarding the intentional utilization of charred materials in Europe for agricultural purposes, there are no scientific evidences during the Neolithic period, the first evidence of such use can be traced back to Bronze Age, and it's witnessed by the agricultural use of Plaggen soils. Plaggen soils are widely distributed throughout north-west Europe, and are characterized by a tickened man-made surface layer caused by long-continued manuring (Conry, 1971). As defined by Wiedner and Glaser (2015), the Plaggen horizon consists of "*flaty cut bits and pieces of heath and pasture grass, brought* *into stables, mixed and fertilized e.g. with manure, dung, litter, ash and biochar before applying to the fields".*

1.3 Biochar production

Biochar can be produced with different techniques including slow pyrolysis, fast pyrolysis, gasification, hydrotermal carbonization and combustion, using various feedstocks as starting material: plant tissues, agro-industrial biomass, rice husk, shrimps shell, sewage biosolids, forage plant biomass, paper-mill waste, livestock and human manure (Qambrani et al., 2017). Different types of feedstock and the thermochemical conditions used for the pyrolysis, strongly influence the quality of biochar and consequently its potential use. During pyrolysis cellulose, hemicellulose, lignin and pectin undergo cross-linking, depolymerization and fragmentation at various temperatures. Pyrolysis transforms biomass into biochar, condensable liquid (bio-oil) and non-condensable gasses (syngas) (Qambrani et al., 2017). Different pyrolysis reactors are available, with differences in heating rates, pressures and residence times; these differences affect the proportion of the final products. The yield of biochar, bio-oil and syngas depends in fact also on the type of pyrolysis followed. Table 1 shows reaction conditions and products distribution of various pyrolysis processes.

Table 1. Reaction conditions and product distribution of different pyrolysis as described by Qambrani et al. (2017).

Process	Temperature (°C)	Residence time	Yields %		
			Biochar	Bio-oil	Syngas
Slow pyrolysis	300 – 700	hour – days	35	30	35
Intermediate pyrolysis	~ 500	10 – 20 s	20	50	30
Fast pyrolysis	500 – 1000	< 2 s	12	75	13
Gasification	~ 750 – 1000	10 – 20 s	10	5	85
Hydrothermal carbonization (HTC)	180 – 300	1 – 16 h	50 - 80	5 - 20	2 - 5
Torrefaction	~ 290	~ 10 – 60 min	80	0	20

1.3.1 Slow pyrolysis

Slow pyrolysis consists in a thermal conversion process that is characterized by long residence times and slow heating rates, which implies quite equal distribution of solid, gas and liquid products. This process occurs at atmospheric pressure, heat is supplied from an external energy source, e.g. external heaters, by partial combustion of the biomass feedstock, or by hot-gas recirculation. In slow pyrolysis the biomass is heated in an oxygen limited or oxygen free environment, the heating rates is usually set between 1 and 30 °C min^{-1} (Lua et al., 2004). Slow pyrolysis is considered the most suitable production process to obtain high-quality biochars intended for agricultural use (Song and Guo, 2012). Slow pyrolysis is a simple, robust and inexpensive process ideal for small scale and farm-based biochar production.

1.3.2 Fast pyrolysis

The thermal conversion process of biomass characterized by short residence times, fast heating rates and temperatures between 500 and 1000 °C, is known as fast pyrolysis. In general, lower temperatures and longer vapour residence times lead to higher charcoal production, while high temperatures and longer residence times favour biomass conversion to gasses. Fast pyrolysis produces high yield of bio-oil (75%) from feedstock, non-condensable gasses (13%) and biochar (12%) (Qambrani et al., 2017). The fast pyrolysis is more suitable for bio-oil production, with this process in fact high yields of bio-oil are obtained compared to solid products or gasses. During fast pyrolysis the feedstock decomposes very quickly producing mostly vapours and aerosols, and a little percentage of charcoal and gas. At the end of the process, after cooling and condensation, a dark brown liquid is obtained, which can be used as it is for heating and power application. Theoretically any feedstock biomass can be used for fast pyrolysis, but the majority of work has been carried out on wood.

1.3.3 Gasification

Gasification is a thermochemical process during which a carbon source is mostly converted into syngas (85%), a mixture of hydrogen (H_2), carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4), acetylene (C_2H_2), ethane (C_2H_6) and nitrogen (N_2). Gasification is obtained by reacting the biomass in a controlled oxygen environment, or also using steam without combustion, at high temperatures (700-800 °C) (Cheng et al., 2020; Qambrani et al., 2017). Sohi et al. (2010) defined gasification as a process similar to fast pyrolysis but with a limited supply of oxygen. The solid product of this process is biochar (10%), while the remaining 5%, the liquid product called “tar”, is composed by a mixture of condensable aromatic and oxygenated hydrocarbons compounds (Cheng et al., 2020; Qambrani et al., 2017). Production of tars is a limiting factor for the utilization of biomass-derived syngas, due to their capacity to fouling and blocking downstream equipment and also poisoning liquid-fuel conversion catalysts. Tar also represents an environmental hazard. The removal of tars has represented a technical problem for the commercial implementation of biomass gasification technology (Cheng et al., 2020). There are different methods for tars removal, the complete primary tar removal (in-situ) is difficult to obtain, while secondary tar removal methods (post-gasifiers) like physical tar removal (absorption and adsorption) and chemical tar removal (thermal and cathalytic) are more efficient and economically feasible.

1.3.4 Hydrotermal carbonization

Hydrochar is the product of the process called hydrothermal carbonization (HTC). HTC was discovered by Bergius in 1913 and is now being mentioned as a promising technology to obtain various bio-products from biomasses. This process uses water and catalysts at lower temperatures (180 to 300 °C) under high pressure to convert feedstocks to a different type of products: a solid product called hydrochar, also known as HTC biochar, and a liquid fuel or bio-oil. As it happens during dry pyrolysis, reaction temperature and pressure determines the product distribution (Libra et al., 2011). Hydrochars are acidic, and have low surface areas, less aromatic compounds, and higher CEC compared with those produced by pyrolysis and gasification (Kalderis et al., 2014).

1.4 Physical and chemical properties of biochar

Depending on the temperatures reached during the pyrolysis and the feedstock used as parent material, biochar may vary widely in terms of chemical and physical properties, in particular the most significant changes deal with bulk and surface chemistry (Mukherjee et al., 2011). The environmental potential of biochar depends on its capacity to adsorb and retain reversibly water and nutrients, these properties are controlled by the porosity and the surface chemistry of biochars.

Biochar is mainly constituted by carbon and minerals with different pore sizes (Qambrani et al., 2017), from sub-nanometer micropores to macropores with 10 microns size. Micropores are involved in the high surface area and absorptive capacity of biochar, the liquid-solid adsorption is related to mesopores, while macropores are important for aeration, hydrology, roots development and bulk soil structure. When biochar is applied to soil, its efficiency in terms of sorption ability decreases because bacteria, fungi and nematodes colonize and clog the pores (Qambrani et al., 2017).

Biochar surface is the interface where chemical and biological reactions and interactions take place in soil. McBeath *et al*. (2011) argue that the aromatic ring structure in biochar rise as the pyrolysis temperature increase. The nature of the interactions between biochar and other materials such as soil particles, microorganisms, gasses, dissolved organic matter and water, are determined by the surface charge (Joseph et al., 2010). Therefore, characterizing the functional groups on biochar surface is necessary to understand their chemistry and reactions. As well as aliphatic and aromatic groups, at the surface, biochar can show hydroxyl, epoxy, carboxyl, acyl, carbonyl, ether, ester, amide, sulfonic and azyl groups (Xiao et al., 2018). The typical electronegativity of biochar is due to the presence of some elements like H, N, O, P and S that are associated with the aromatic rings, influencing the cation exchange capacity (CEC) of the charred material. CEC usually increases with biochar age (Mukherjee et al., 2014). Biochar also shows adsorption potential for toxic substances such as aluminum (Al) and manganese (Mn) in acidic soils, and arsenic (As), nickel (Ni), copper (Cu), cadmium (Cd) and lead (Pb) in heavy metals contaminated soils (Uchimiya et al., 2010). Soil aging or hydrogen peroxide treatments can induce, through oxidation, more carboxyl groups in biochars (Singh et al., 2014; Xue et al., 2012). Being some biochar characteristics related to the functional groups, these represents a very important interfacial sites (Xiao et al., 2018).

Biochar also retain some of N, despite during pyrolysis most of the fuel N is lost. Knicker et al. (2008) demonstrated that the N forms are mainly pyrrole-N, amide-N and pyridine-N. The heating process of pyrolysis alters the chemical environment of the carbon (C) in biochar, mostly leading to recalcitrant aromatic structures that are supposed to be resistant to decomposition. For this reason, biochars are considered effective for long-term C sequestration. In fact, it has been proved that biochar macromolecular structure, dominated by aromatic C, makes it more resistant to microbial decomposition compared to uncharred organic material (Verma et al., 2013). As a consequence, biochar has long mean residence time in soil. Warnock *et al*. (2007) reports that biochar is stable up to 10.000 years in soil, with an average of 5.000 years, showing no microbial decomposition. However, a study on biochar stability in soil under field conditions (de la Rosa et al., 2018) revealed that biochar recalcitrance was lower than expected, specifically in the order of decades.

2. The use of biochar in agriculture as soil amendment

The use of biochar as a soil amendment is regulated by the new European regulation on fertilizers, regulation (EU) 2019/1009 of the European Parliament and of the Council (5 June 2019), which establishes rules relating the market of fertilizers in the EU. This regulation changes the regulations (CE) no. 1069/2009 and (CE) no. 1107/2009 and suppresses regulation (CE) no. 2003/2003. The new regulation will be appllied starting from July 16, 2022.

Since the end of Second World War agriculture started to use higher amount of chemical fertilizers instead of the traditional inputs such as manure and compost, often produced locally. With the increase of chemical fertilizers farmers obtained higher crop yields; their immediate action and low cost in fact represented a crucial point in boosting their use in agriculture. However, increasing chemical inputs also causes soil degradation, raises greenhouse gas emissions and can also determine water contamination. Excessive use of agro-chemicals have shown detrimental effects on soil fertility bringing in a decline of organic matter content of many soils, particularly in some Mediterranean regions (Diacono and Montemurro, 2011).

The addition of manure or compost can improve soil fertility but for many farmers these materials are difficult to obtain, and they can also contain pathogens and toxic compounds. Biochar is a sterile material that may raise crop yields and reduce the dependence on fertilizers, by improving soil water and, perhaps, nutrients availability. More confidently, biochar addition stores C in soils and for longer times, as charring process highly increases the stability of C against microbial degradation (Baldock and Smernik, 2002). Biochar could also be a valid solution as soil amendment for raising the performances of degraded agricultural land and improving crop production especially in poor soils. As reported by Batista et al. (2018), biochar has been used as a soil corrector, influencing soil properties and processes, and its addition to soil has been reported to be effective on increasing the availability of nutrients, microbial activity, water retention, reducing at the same time fertilizer requirements, greenhouse gas emissions, nutrient leaching and erosion.

The interest shown by researchers and farmers during the last decades in biochar as a soil amendment derived from the various studies on Amazonian *terra preta* mentioned above. The potential positive effects of biochar as soil amendment are related to its high cation exchange capacity, high surface area, which leads to increase soil pH and water holding capacity (Streubel et al., 2011). The qualities of biochar as soil improver are also related to its potential capacity to provide a good habitat for various organisms, like nitrogen fixing bacteria and mycorrhizal fungi (Ogawa, 1994). The biochar large internal surface area and the high amount of residual pores are responsible of the direct effects, for example retaining water by capillarity. An indirect effect is that it can improve soil aggregation and structure, affecting the water retention capacity of the soil.

The effects of biochar addition on crops yield are debated. As reported by Haider et al. (2017) the responses of crop yields to biochar amendments, in particular under temperate field conditions, are still uncertain. Numerous works reported that biochar as soil amendment generally showed a positive effect on physical, chemical and biological properties related to the soil capacity to support crop growth and productivity (Jindo et al., 2020a). Some meta-analyses (Crane-Droesch et al., 2013; Jeffery et al., 2017, 2011; Liu et al., 2013) reported that with biochar addition to soil the mean crop yield increases of about 10%. These results are confirmed by the observations of Biederman and Harpole (2013) who analyzed 371 independent studies reporting a significant increase in productivity and crop yield after biochar addition, compared with untreated control. Jeffery et al. (2017) observe that the positive effects of biochar as soil amendment have been found mainly in acidic soils, due to the increase of pH, nutrient retention and water holding capacity. However, a review made by Zhang et al. (2016) revealed that of 798 biochar studies, only 26% of them were performed under field conditions. Furthermore, results obtained under field conditions are often differing from laboratory experiments, and showed contrasting findings compared to greenhouse studies (Glaser et al., 2015; Liu et al., 2012). Hammond et al. (2013) reported that the temperate regions particularly lack in fields trials made with biochar addition.

The responses of crop yield and biomass production to biochar addition also vary with crop type. Liu et al. (2013) observed that significantly positive responses were obtained with legumes, vegetables and grasses and Jeffery et al. (2017) reported that rice, wheat, maize and soybean significantly increased their productivity with biochar application. Asahi et al. (2009) hypothesized that crop response to biochar are determined by the nutrient management and pre-existing soil nutrient status. They observed that an higher rice yield was obtained only with low rate biochar addition applied with N fertilizer using a low-yielding crop variety, with a high-yielding variety, in an equivalent treatment, the yield was lower than the control. Other studies on wheat showed no significant differences between the control and low doses of biochar (Blackwell et al., 2007). Some works (Baronti et al., 2010; Vaccari et al., 2011) reported positive effects of biochar addition to soil up to 30% on wheat yield, while others (Tammeorg et al., 2014) reported that the addition of biochar did not significantly affect crop yield or quality of wheat. Olmo et al. (2014) observed that biochar addition did not affect grain quality of wheat if compared to untreated control.

A recent meta-analysis of 105 studies revealed that the addition of high doses of pure biochar, above 10 t ha^{-1}, brings to no yield improvement in temperate environments (Jeffery et al., 2017). Also in a study carried out by Haider et al. (2017) no yield improvements were observed in a four-years experiment with different crops and with different climatic conditions. On the other hand, there are studies even showing negative effects. For instance, Kishimoto and Sugiura (1985) showed a negative effect of biochar (5 t ha^{-1}) on soybean and maize growth, probably related to the alkaline nature of the biochar that increased soil pH with a consequent micronutrient deficiency. The possible negative effects related to biochar addition may be explained also by the release of organic compounds (i.g. benzene, toluene and others) from biochars, such that can suppress germination and reduce plant growth (Spokas et al., 2011). Contrasting results were obtained with biochars produced with temperatures lower than 450 °C, as these chars contain more water repelling organic compounds (Ahmed et al., 2016; Yi et al., 2015), which can negatively influence plant growth (Fang et al., 2014). The hydrophobicity of some biochars can also favour soil erosion due to increased water overflow (Ahmed et al., 2016). One of the critical point highlighted in numerous studies is the potential for biochar to immobilize plant available N (Lehmann et al., 2003; Sohi et al., 2010). The possibility that biochar addition, without a proper nitrogen fertilization, may decrease crop yields was also reported by Asai et al. (2009), just likely linked to N immobilization (Bruun et al., 2012; Novak et al., 2010).

Dai et al. (2020) in their meta-analysis of 153 peer-reviewed works conclude that yield increase is not dependent on the addition of biochar itself but on the consortium of biochar and soil properties. Another meta-analysis of Crane-Droesch et al. (2013) underlines the importance to investigate the effect of biochar addition in soil during an appropriate period of time, as they observed that crop yields increased over time in soils added with biochar, in various works of their database. Therefore, long-term field experiments are needed for a wide understanding of the possible impact of biochar addition on soil properties and crop productivity.

2.1 Biochar as peat substitute in potting substrates

Soil-less culture is, nowadays, the most intensive culture systems for the cultivation of plants, using either inert organic and inorganic materials as substrate, for maximizing yield of crops (Asaduzzaman et al., 2015). Growing media alternative to the soil can be composed of a unique material or a mixture of different materials, and their utilization for horticultural plant production increased considerably during the last decades. There are numerous studies focused on the importance of soilless culture in greenhouses as a valid alternative to traditional open field production for high-value vegetable crops (Asaduzzaman et al., 2015; Cantliffe et al., 2001; Schröder, 1999).

The first organic material that has been standardized as a growing substrates, and that actually represents the most used one, is peat, which is often the unique ingredient for growing substrates commercial formulation (Steiner and Harttung, 2014). It is conventionally recognized that the performance and economics were the most important selection parameters for potting substrates and, in those terms, peat is in many ways the ideal constituent of soilless growing media (Kern et al., 2017). However, due to the rapid climatic changes and increasing attention for the environment by the civil society, there is a great interest towards the utilization of sustainable practices, including agriculture, all around the world. Hence, policymakers, traders and plant growers are asking for the reduction of the negative environmental effects of plant production (Schmilewski, 2014). In this framework peat use present several drawbacks as peat extraction from wetland ecosystems implies their deterioration and re-entry of CO_2 in the atmosphere (Zulfiqar et al., 2019) and is under strict regulations since 20 years at least (Alexander and Bragg, 2014), especially in many European countries, leading to a research for peat substitutes.

As a consequence, during the last decades numerous alternative raw material have been tested to prepare growing substrates for vegetable production: woodchips, coco peat, perlite, vermiculite, zeolite, rockwool, sand, pumice, sepiolite, expanded clay and others. However, none of those seems suitable enough as peat substitute because of their economic and environmental sustainability. For instance, rockwool is too expensive and difficult to dispose due to its un-biodegradability. Perlite and zeolite are less expensive than rockwool and have been used in many countries for the production of many species. However, perlite is produced with mineral undergoing high temperature and this process consumes a lot of energy. Indeed, in choosing possible alternatives for peat substitution, besides costs and performances, the environmental aspect must be carefully taken into account. In last few years, a lot of attention was focused on finding organic derived alternatives including agricultural, municipal and industrial waste materials (Raviv, 2013). The possibility to recycle these waste materials for growing substrates can be a valid solution for the disposal of critic materials that represent an environmental problem.

Biochars have been recently suggested as candidate for peat substitution due to some similarities with peat, such as high porosity, low density and high cation-exchange capacity. There are only a few studies about the performances of biochar as growing substrates for container-grown plant growth. In a recent review (Huang and Gu, 2019) regarding the effects of biochar addition on container-grown plants, it has been reported that 77.3% of the analyzed studies found that certain percentages of biochar addition in growing substrates promoted plant growth, while the 50% of the studies reported a plant growth decrease adding higher percentages of biochar. The effects of biochars on plants grown in containers are dependent on various factors: the type of biochar, the addition rate, plant species and many others. Frenkel et al. (2017) reviewed some studies where biochar was tested at different doses as peat replacement in soilless substrates. In most cases, biochar showed a neutral or positive influence on growth of various plant species compared with peat control, if used at doses lower than 30% (v:v), and in some cases also higher concentrations were found to be not harmful.

In general, large amount of biochar could be used as peat substitute if its physical and chemical properties are similar to those of commercial peat or in the best range for potting-grown plant growth. Among all properties affecting plant species grown in potting substrates, pH could be the most important limiting factor determining the potential use of biochar in growing media (Huang and Gu, 2019). There are also potentially toxic contaminants and compounds in biochar that could have detrimental effects on plant growth. Some chars could contain heavy metals from contaminated feedstocks, like cadmium, copper, lead and zinc.

In conclusion, finding new, renewable and environmentally sustainable organic materials for potting substrates is challenging. In this sense biochar could be a sustainable strategy for the management of some agricultural and industrial wastes, pyrolysis can in fact represent a waste management option which allows the conversion of these biomasses into a material that can be used for potting substrates and as a soil amendment. However, whereas many different biochars have already been tested as amendment of mineral soils for open field crops, although with contrasting results (Spokas et al., 2012), there are a few studies on the utilization of biochar in soilless substrates.

2.2 Biochar as an additive for composting

In recent years, biochar as an additive in composting has received increasing interest. The chemical and physical properties of biochar are able to enhance the composting process, in which biochar itself undergoes oxidation with consequences on its surface chemistry (Sanchez-Monedero et al., 2018). Composting of organic residues and the use of compost represents a solution to reintroduce organic matter to the soil that otherwise would be lost. Compost is every organic material undergone a thermophilic and aerobic decomposition, a process which is precisely called “composting”. Compost is already used in horticulture, and there are several studies about it as growing medium partly or fully replacing peat (Fascella, 2015; Mininni et al., 2015).

The use of carbonized material or ashes to improve composting by accelerating decomposition, stimulating bacterial activity and neutralizing acidity, has been performed for centuries (Ogawa and Okimori, 2010). The composting process can be divided into three phases: mesophilic phase; thermophilic phase; and cooling and maturation phase (Xiao et al., 2017). Some studies demonstrated that the addition of biochar during the various phases of the composting process, can promote the process itself, bringing to a better final product (Chen et al., 2017; Vandecasteele et al., 2016). In particular, the addition of biochar can increase the temperature during the thermophilic phase, so activating the composting process (Chen et al., 2010; Steiner et al., 2010), with a consequent enhancement of the microbial activity (Sanchez-Monedero et al., 2018) that brings many benefits such as a reduction of the composting time and a more rapid stabilization of the composted material (Vandecasteele et al., 2016). Another positive effect of biochar addition into the composting pile is the reduction of N losses during composting (Jindo et al., 2020b); this effect is related to the high adsorption capacity of biochar particles during composting (Dias et al., 2010). However, the recorded effects of co-composted biochar on soil properties and plant growth are contrasting. Some authors (Agegnehu et al., 2016; Schulz et al., 2013) found positive effects on soil fertility and plant growth, but other works reported no synergistic effect of composted biochar on plant growth and that, if a positive effect is observed, the increase in plant productivity is due only to compost addition rather than biochar addition (Seehausen et al., 2017; Wang et al., 2019).

3. Crops quality and the effect of biochar addition on quality parameters of some common Mediterranean crops and herbs

While numerous studies have been published about the effect of biochar addition on soil properties, crop production and plant growth (Agegnehu et al., 2017; Diatta et al., 2020; Jeffery et al., 2011), there is a lack of works on the direct effects of biochar addition, as soil amendment or potting material, on crop quality. Indeed, during the last years, food quality and safety have received growing importance, both in consumers' request and in marketing research.

In the last decade consumers showed an increasing attention on nutraceuticals use and, thus, there is a strong pressure on the food industry to look for safe substances that can be used in nutrition. Nutraceuticals, the hybrid of "nutrition" and "pharmaceutical", is a wide term that can be defined as "*any substance that may be considered a food or a part of a food, and provides medical or health benefits, including the prevention and treatment of diseases*" (Teoh et al., 2019). Examples of "functional foods" that can provide health benefits are antioxidants, herbals, aromatic plants, cereals and others (Guidi and Landi, 2014; Lee, 2017). Aromatic plants, for example, represent a good resource of bioactive compounds with health benefits. They can offer in fact many nutraceuticals substances such as growth promoters, antimicrobials, antioxidants, flavorings and others, with valuable commercial benefits.

This possible application of aromatic plants and the consequent increasing demand for these species have made them industrial crops. Indeed, the use of aromatic plants is progressively increasing. In this framework, aromatic plants from the Mediterranean basin represent a big source of essential oils and other biologically active compounds (Guidi and Landi, 2014). Growing conditions can strongly affect the content of nutraceuticals substances and the effects of different soils or traditional growing substrates on quality parameters of vegetables and aromatics are well documented (Gruda, 2009; Olle et al., 2012). Studies on this have been published for example on some typical crops and herbs of Mediterranean basin: tomato, sweet potato, basil, wheat, sunflower (Alan et al., 1994; Erekul and Köhn, 2006; Jelacic et al., 2005; Lopez et al., 2004; Najar et al., 2019; Padem and Alan, 1994; Pfister and Saha, 2017). Few studies about the effect of biochar on some quality parameters of horticultural species, aromatic plants and cereals have been published (Massa et al., 2019; Najar et al., 2019; Pandey et al., 2016; Quartacci et al., 2017; Vaccari et al., 2015). Considering that the addition of charred materials as soil amendment or potting substrate represents an opportunity, there is a growing interest in studying their possible effect also on the quality of crops, especially when it comes to largely consumed products, such as horticultural crops, aromatic plants or cereals.

Sweet basil is widely used in the Mediterranean kitchen as a fresh plant. Its extracts are also used in the pharmaceutical products or as pesticides and its essential oil is known to have a high economic value due to the presence of some compounds such as eugenol, linalool and others (Sifola and Barbieri, 2006). Traditionally, this plant has been used in folk medicine for its carminative, stimulant, and antispasmodic properties. Furthermore, basil essential oil has a high economic value due to the presence of some valuable compounds such as monoterpenes, sesquiterpenes and oxygenated compounds. The synthesis of secondary metabolites in plants that produce essential oils is also related to abiotic stresses such as the characteristics of the soil or the growing substrates. For these reasons some studies have been carried out on the effect of different substrates on the essential oil profile of herbs, but there is insufficient and inconclusive work on the effect of biochar addition on the quality of basil oil.

Another widespread crop in the Mediterranean basin is wheat, in particular *Triticum turgidum* ssp *durum* and *Triticum aestivum*, which are respectively used for the production of high-quality semolina for pasta and of flour for bread and biscuits industries. The area interested for wheat cultivation in the Mediterranean countries amounts to 27% of the arable land, and the Mediterranean basin is 60% of the world's growing area for durum wheat (Royo et al., 2017). For thousand years bread made with wheat flour has been one of the principal constituents of human diet, and even today in the Mediterranean countries, according to nutritional guidelines, cereals are placed at the base of food pyramid (Bach-Faig et al., 2011). Wheat is in fact a source of primary nutrients such as carbohydrates and proteins, but also a source of antioxidants. Despite after second world war, local and ancient wheat varieties have been replaced by modern ones, selected for intensive cultivation, the increasing demand of consumers for varieties with greater health potential, nutritional and sensory qualities, renewed the interest in traditional wheat varieties (Dinu et al., 2018; Rocco et al., 2019). Recent studies on the potential health benefits of functional groups from some wheat varieties have renewed the interest in the ancient ones, and in particular on their potential nutraceutical properties (Dinelli et al., 2011; Leoncini et al., 2012). Ancient varieties, defined as those not dwarf and unregistered genotypes that did not undergo modifications during the last century, are in fact receiving interest since some studies suggested that they have healthier and better nutritional profile, more specifically in terms of anti-oxidant and anti-inflammatory properties, than modern varieties (Dinu et al., 2018). Some of the beneficial effects of consuming certain wheat varieties, e.g. ancient ones, are associated with the phytochemicals of wholegrain, which include, for example, phenolics, carotenoids (Heimler et al., 2010) and other antioxidants such as tocopherols, flavonoids and phenolic acids (Vaher et al., 2010). Among these compounds, polyphenols play an important function in contrasting oxidative stress, one of the possible causes of some human diseases. It is known that changes in environmental conditions, particularly in soil quality, can affect the secondary metabolites production such as polyphenols (Chludil et al., 2008). Therefore, it is worth investigating how the addition of soil amendments, such as biochar, can modify the profile or concentration of antioxidants compounds like polyphenols, flavonoids, carotenoids and others.

Sunflower is an important oleaginous plant cultivated for food purposes, and a valid crop to introduce in rotation with cereals. Indeed, wheat-sunflower rotation is a common practice in arable areas of the Mediterranean basin (Ercoli et al., 2014; López-Bellido et al., 2002; Pedraza et al., 2015). In Italy, sunflower cultivation is concentrated in the central regions and in particular in Marche, Toscana and Umbria. During the 1970s sunflower started to be cultivated using traditional varieties. In the next decades other varieties with high oleic acid content, called high oleic sunflower (HOS) varieties, were added, representing a substitute to the monoculture of winter cereals (Spugnoli et al., 2012). Sunflower seeds are also characterized by high antioxidant properties (Karamać et al., 2012; Velioglu et al., 1998). The antioxidant potential of sunflower meal and sunflower seed shells is determined principally by the content of phenolic compounds in the seeds (De Leonardis et al., 2005; Schmidt et al., 2005). Sunflower is highly sensitive to water deficit stress from the early flowering stage to the end of its growth and that that water deficiency can negatively affect oil quality and crop yield in sunflower (Seleiman et al., 2019). The addition of biochar in the soil can have positive effects on water retention and, consequently, producing positive effects on oil quality. Besides the quality as a dietary plant product, sunflower can also represent a good biomass for biofuels production. Due to climate change related issues, increase of energy demand for transport and electricity, there is in fact a growing interest in biofuels obtained from biomasses, in this sense biodiesel from sunflower is one of the most promising solution for bioenergy production in the European countries and particularly in Italy.

4. Aims

This research bases on the following purposes: i) to evaluate the feasibility of some recycled pyrolyzed materials and compost as peat substitutes in potting substrates for seedlings and plan growth; ii) as well as their performances in terms of fresh biomass production and impact on some quality parameters such as leaves color and essential oil volatile compounds; iii) to evaluate the effects of biochar as a soil amendment on crops yield and quality. To reach these goals, three different experiments were conducted:

- **Experiment n°1**. I evaluated the feasibility of recycling by pyrolysis some organic wastes such as chitin from shrimp shells, tomato greens, rice husk, so producing COMs material to be used as peat substitutes in potting substrates for seedlings growth. In order to test if the already used peat can be recycled as growing material, I included pyrolized peat in this study. I hypothesized that, aside the concentration of the charred material in the growing substrate, the different compositions of the feedstocks would result in material with varying nutrient contents and properties, which may have different results on seed germination and plant growth. I tested this hypothesis by preparing peat/biochar mixtures with different substitution rates for a plant nursery trial, the target specie was *Solanum lycopersicon* L., since tomato is one of the most widespread horticultural species in the Mediterranean countries and a big economic resource for some regions.
- **Experiment n°2**. The aim of this experiment was to provide an insight into the possible use of biochar and composted-biochar as peat substitutes for the cultivation of sweet basil, one of the most common aromatic species in our countries. Peat was substituted with doses of 100, 50 and 25 % in volume. The effects were investigated in terms of plant growth, fresh biomass, foliar surface and quality parameters, such as the color of leaves and the essential oil profile composition in terms of volatile organic compounds.
- **Experiment n°3**. I evaluated the effects of biochar addition as soil amendment in a two-years open field trial with wheat and sunflower as target species. An arable soil was amended with doses equivalent to 1, 4 and 20 t ha^{-1}. The aims of this study were to investigate the effects, at field conditions, in terms of soil characteristics, plant growth, fresh and dry biomass and quality parameters such as polyphenols, carotenoids and antiradical activity in wheat and sunflower seeds.

5. Materials and methods

5.1 Experiment n°1: nursery trial on tomato plants (Paper I)

Production of carbonized organic matter (COM) and reference peat substrate: for the present study, rice husks (Ri), chitin from shrimps' shells (Ch), gardening peat (Pe) and dried pellets of green waste of tomato plants (To) derived from a private garden were pyrolyzed at 400 ◦C and 500 ◦C, using a closed custom made stainless steel reactor. The reactor was filled with the feedstocks to about 2/3 of its volume, subsequently flashed with N2 to remove air and put into a preheated muffler for 3 h. Syngas, produced during the pyrolysis was allowed to leave the reactor through a stainless steel tube reaching from the reactor to the outside of the muffler and connected to a gas-trap filled with oil. In this research we decided to summarize our pyrolyzed products as COM in order to avoid misleading and complications with respect to nomenclature and because our pyrolyzed materials were not analyzed for their atomic O/Corg ratios as required for the European Biochar Certificate (EBC, 2019). According to the latter, indeed, a pyrolysed material can be called "biochar" if has got >50% organic carbon (Corg) and atomic H/Corg and O/Corg ratios above 0.7 and 0.4, respectively.

The nomenclature for the COMs includes the abbreviation of the feedstock and the pyrolysis temperature (Ri400, Ri500, Ch400, Ch500, Pe400, Pe500, To400, To500) and is listed in Table 2. As reference material the peat-based gardening substrate was used.

Chemical and physical characterization of the produced biochars: The dried peat and COMs were finely ground before analyses. Their ash contents were measured in aliquots of 0.3 g after heating at 750 ◦C. The pH (H2O) of COMs and peat was obtained from a suspension in distilled water (1:5) with a Crison pH-meter Basic 20, using the method described by Jackson and Beltrán-Martínez (1982) originally set up for soils, and modified by Paneque et al. (2016) for carbonized material.

To determine the water holding capacity (WHC), 6 g of each sample were placed on a Whatman 2 filter placed into a funnel, and saturated with distilled water. For 2 h the water was allowed to percolate through the filter and the funnel, then the weight of the moist samples was measured. The weight difference between dry and moist sample was extrapolated for a duration of the experiment of 12 h according to de la Rosa et al. (2014). Its percentage relatively to the dry weight of the sample resulted in the value for the maximum WHC. The hydrophobicity of pure materials was measured using the methods described by Doerr (1998). In brief, the water drop penetration time (WDPT) and the molarity of an ethanol droplet test (MED) were applied. Five drops of distilled water were placed on the sample surface at a distance sufficient to avoid interferences between the drops. The penetration time of each drop was recorded separately, then the average obtained from the five drops was considered as representative of WDPT. The MED test use drops of known mixtures of water:ethanol placed on the sample surface measuring their infiltration time (Watson et al., 1971). The latter increases with increasing surface tension of the drops that increases with the concentration of ethanol. Therefore, decreasing ethanol concentration until a drop resists to infiltration allows the classification of the samples into surface tension categories limited by two adjacent ethanol concentrations, assigning 1 to very hydrophilic and 7 to extremely hydrophobic. For the present study, we use the following ethanol concentrations: 0, 3, 5, 8.5, 13, 24 and 36 % v:v.

The elemental composition of all the substrates was analyzed at the beginning and at the end of our experiment using an elemental analyzer LECO TruSpec CHNS Micro hosted at CITIUS (Centro de Investigación, Tecnología e Innovación de la Universidad de Sevilla, Spain). The available phosphorus (H2PO4- and HPO42-) was measured following Olsen's method (Murphy and Riley, 1962). Briefly, 50 ml of a 0.5 M solution of NaHCO3 were mixed with 2.5 g of sample for 30 min and extracted after centrifugation and filtration with Whatman 2 filters to determine the concentration of available P in the extracts with a multiparameter analyzer Bran-Luebbe at the Instituto de Recursos Naturales y Agrobiología de Sevilla - Consejo Superior de Investigacion Científicas (IRNAS-CSIC). Available inorganic nitrogen in the samples (NH4+ and NO3-), was quantified after extraction with KCl 1M (Navarro and Navarro, 2013). Data are given as the average of two extraction duplicates. Macro- and micro-nutrients (B, Ca, Cu, Fe, K, Mg, Mn, Na, S, P, As, Ba, Cd, Co, Cr, Sr, Ni, Pb and Zn) were measured in duplicates from the extracts obtained after controlled acidic digestion with ultrapure nitric acid of the samples in a DigiPREP Block Digestion Systems (SCP Science) using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

Pot experiment: For the greenhouse experiment, COMs were mixed with peat-containing gardening soil in the ratios 60/40 and 30/70 and filled into plastic beakers (50 ml volume) which were perforated at the bottom to allow leaching of surplus water. In addition, pots were prepared with pure COMs and pure gardening soil. Each mixture was prepared in triplicate giving a total number of 75 pots (4-treatments × 2-pyrolysys temperature × 3-mixtures × 3-replicates, plus the three potting soil controls). In each pot five certified seeds of *Solanum lycop*ersicum L. var. "Roma" seeds were directly planted onto the substrate. The pots were placed in a greenhouse at 25 °C and 14 h light day^{-1}, utilizing both natural and artificial light during daylight. Pots were spaced 10 cm apart in a completely randomized distribution to avoid variability due to the position in the greenhouse. The pots were initially irrigated with a known amount of distilled water until the substrate reached 50% of its WHC. Every two days, the substrate moisture was restored by replacing evaporated water determined by weight difference. No fertilizers were applied. The germination rate and plant height were measured every two days during the entire experiment. One week after sowing, only the dominant plant was left in each pot. After 40 days, plants were harvested by cutting the above ground biomass and immediately weighted, then dried at 60 °C for 72 h to measure the dry weight biomass.

Statistical design: Data normality was checked with a Shapiro-Wilks test, variables presenting a non-normal distribution were log transformed before further tests. An analysis of variance (ANOVA) was performed to compare the different materials - four feedstocks and two different pyrolysis temperatures, plus the peat control for a total of nine treatments. A unique classification variable was adopted summarizing the three classification factors (type of feedstock, temperature of pyrolysis, and proportions of components in the mixture) used for the calculation of ANOVA for germination and plant development, for a total of 27 treatments. Post hoc pairwise comparisons were carried out using a Duncan’s multiple range test. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

5.2 Experiment n°2: nursery trial on basil (Paper II)

Substrates composition: For this experiment, compost and formally defined biochar were used as growing medium. The biochar used in this experiment was purchased on the market and produced in a syngas plant from woody residues of the pruning of city trees by the manufacturer Econsulenze SAS (Terni, Italy). Its characteristics have been declared by the producer (Table 6 – Chapter 6.2) and according to them this biochar accounts for the I "EBC-Feed" quality class of the European Biochar Certificate (EBC, 2012).

The compost was produced by All Power Labs – SLO Factory (Terni, Italy), using a mixture of organic wastes. Approximately, the compost parent material was: 25% kitchen green waste; 48% sawdust, wood flakes, wood chips; 15% exhausted coffee powder; 5% above-mentioned biochar; 1.5% forest topsoil; 0.5% cane sugar; 5% water. The mixture was prepared using an insulated tumbler rotating within a barrel, designed by the company. The composting lasted one month, checking daily temperature and humidity. Later, the compost was stored for 3 weeks, at room temperature, before being used for our experiment.

For the experiment, seven different substrates were prepared mixing biochar and compost with commercial peat (a mix of Irish and Baltic sphagnum, "Cuore di Terriccio", by Vigorplant Italia SRL) in different volumetric proportions: 100% pure biochar (Char 100); 100% pure compost (Comp. 100); 50% biochar/50% peat (Char 50); 50% compost/50% peat (Comp. 50); 25% biochar/75% peat (Char 25); 25% compost/75% peat (Comp. 25); 100% peat (control) (Table 2).

The pH and electrical conductivity (EC) of pure peat, biochar, compost and their mixtures were measured in a suspension in distilled water (1:2.5) with a XS pH-meter model PC8. The bulk density was measured drying the substrates from the pots at 70 °C until constant weight and then weighting them. Available inorganic nitrogen (NH_4^+ and NO_3^-), and total phosphorous (P) of the substrates were measured using the methods described for experiment n°1.

Experimental design: The experimental design was based on a randomized block scheme and consisted of three replicate blocks per substrate, each comprising 10 pots of 300 ml volume each (210 pots in total). The trial was performed outdoor, with pots placed on a bench equipped with a transparent PE roof. Three seedlings of basil (*Ocimum basilicum*, L. cv. Italiano) were planted in each pot, and then irrigated every two days all trial long, to 100% WHC. Eighty mg of nitrogen, as nitrate (7%), ammonia (5%) and urea (8%), were applied to each pot. Three weeks after the beginning of the experiment, plants were treated with an imidacloprid-based insecticide against cutworms.

Starting from the seventh day, the following variables were measured on the most developed in height plant per pot: height, SPAD values with a leaf chlorophyll meter SPAD-502 Minolta (SPAD - Soil Plant Analysis Development), and color parameters (L*, a*, and b*) of one completely formed leave/plant with a portable colorimeter Minolta Chroma Meter CR-100. The L* parameter accounts for lightness, a* expresses values from green to red, and the b* parameter expresses values from blue to yellow, all together used to determine color differences between samples. Fifty days after transplanting the seedlings, the plants were harvested by cutting all the aboveground biomass. The youngest four completely formed leaves of the dominant plant were collected, weighted, and stored at -80 °C for quantitative and qualitative analysis of the essentials oils. Immediately after harvesting, the total leaf area was measured by scanning all leaves of each dominant plant with a LI-COR LI-3100 Area Meter, and the fresh biomass, leaves plus stems, was weighed and then oven-dried at 105 °C to constant weight. The specific leaf area (SLA, $cm^2\ g^{-1}$) was calculated dividing the leaf area of each plant by the leaf dry weight. The leaf dry matter content (LDMC, (g dry mass g^{-1} fresh mass) was determined as the ratio between the oven-dry mass of leaves and their fresh mass, while the leaf area ratio (LAR, $cm^2\ g^{-1}$) is the ratio between the leaf area and the total dry plant biomass, which accounts for the size of the photosynthetic surface relative to the respiratory mass (Bressan et al., 2020).

VOCs analyses: Volatile organic compounds (VOCs) were extracted from 0.5 g of the last four completely formed leaves that were previously stored at -80 °C. They were combined with 1 ml of heptane as the solvent and tridecane as an internal standard, vortexed for five minutes, sonicated for 15 minutes and then agitated over-night. After centrifugation at 1800 g for 10 minutes, the heptane phase was collected for the gas chromatography mass spectrometry (GC-MS) analysis. The GC-MS analysis was performed with an Agilent 7820 Gas Chromatograph system equipped with a 5977E MSD with EI ionization (Agilent Tech., Palo Alto, CA, USA). One µL of heptane phase was injected in a split/splitless injector operating in splitless mode. A Gerstel MPS2 XL autosampler equipped with liquid option was used. The chromatographic settings were: injector in splitless mode set at 260 °C, J&W innovax column (30 m, 0.25 mm i.d., 0.5 µm df); oven temperature program: initial temperature 40 °C for 1 min, then 5 °C min^{-1} until 200 °C, then 10 °C min^{-1} until 220 °C, then 30 °C min^{-1} until 260 °C, hold time 3 min. The mass spectrometer was operating with an electron ionisation of 70 eV, in scan mode in the m/z range 29-330, at three scans per second. The deconvoluted peak spectra, obtained by Agilent Masshunter software, were matched against NIST 11 spectral library for tentative identification. Kovats' retention indices were calculated for further compound confirmation and compared with those reported in literature for the chromatographic column used. The Kovats retention index of a compound is its retention time normalized to the retention times of adjacently eluting n-alkanes.

To determine the content of each single VOC a calibration curve was built injecting known concentrations of authentic standards (Sigma) into the gas chromatograph-mass spectrometer and expressed as mg g^{-1} dry weight (d.w.). The leaf dry mass weight was determined after drying the residual plant material at 105 °C for 72 h. Relative content (proportions or percentages) of each VOC was expressed as a relative percentage of total VOCs (VOC profile), being calculated on the basis that 100% is equivalent to the sum of all 12 identified compounds.

Statistical analysis: Data underwent one-way analysis of variance (ANOVA) according to a completely randomized block design with three blocks and 30 replicates per treatment. Significant differences among means were determined using Duncan’s post-hoc significance test at $p < 0.05$. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

5.3 Experiment n°3: open field trial on wheat and sunflower (Paper III)

Experimental design: The two-year open field trial started in November 2018 and ended in October 2020, trials were carried out at the experimental farm Azienda Terre Regionali Toscana located in Cesa, Arezzo province. The climate is typically Mediterranean, annual precipitation ranging from 685 to 711 mm and being distributed across 89 rainy days (i.e., with rainfall above 1 mm) (meteorological data recorded at the local weather station). The soils are silty-clay textured (around 10% sand; 45% silt; 45% clay), have a pH around the neutrality, and an organic matter content of about 2%. The principal crops that are cultivated are wheat, as both modern and ancient varieties, sunflower, tobacco, maize, and also some minor crops such as millet, sorghum, quinoa and amaranth. The wheat-sunflower rotation represents one of the typical practice adopted by the farm and since many years the rotation includes ancient wheat varieties.

The experimental design was based on a randomized block scheme and consisted of three replicate blocks each treatment, with plot sizes 6x15 m. We tested three different doses of biochar B1 (1 t/ha), B2 (4 t/ha) and B3 (20 t/ha) plus one control (B0). The biochar used as soil amendment was the same used for experiment n°2.

The target species adopted for the first year of the experiment were four different cultivars of wheat, two “ancient” cvs., Verna (*T. aestivum*) and Senatore Cappelli (*T. turgidum* ssp. *durum*), and two modern cvs., Bologna (*T. aestivum*) and Claudio (*T. turgidum* ssp. *durum*). During the second year the target species was the high oleic hybrid P64HE39 of sunflower. Each wheat genotypes were grown in a sub-plot of 3x7 m, while for sunflower the entire plot was exploited.

The biochar was added once to the plots in November 2018 and was incorporated into the soil by plowing to a depth of 25 cm. Biochar was humidified before addition in the soil to avoid wind dispersion. Wheat seeds, 160 kg ha^{-1} for the ancient cultivars and 200 kg ha^{-1} for the modern cultivars, were sown with a plot seeder in December 2018 and harvested with a plot thresher in July 2019. In March 2020 a NPK fertilizer (12:12:17) was distributed in the field with a dose of 0.4 t ha^{-1}. Sunflower was sown at the beginning of April 2019, pre-emergence herbicide treatment was applied using commercially formulated products, Challenge (1.5 L ha^{-1}), Dual Gold (1 L ha^{-1}) and Most Micro (2 L ha^{-1}). In May, after hoeing, 0.15 t ha^{-1} of urea was distributed. Sunflower was harvested in September 2020.

Physical and chemical characterization of soil samples and biochar: Three soil samples of 0.5 kg each treatment were taken at the end of the growing season of wheat (July 2019) by a coring apparatus fitted with thin-walled stainless steel sample tubes to a depth of 25 cm. Collected soil samples were oven dried at 40 °C and gently grounded and sieved to 2 mm. The so obtained fraction, the fine earth, was further analyzed as following. The Cation exchange capacity (CEC) of each plots was measured of sunflower using the method described by Hendershot and Duquette (1986). Soil pH, EC, and WHC of soil samples were measured using the methods described for experiments n° 1 and n° 2. Total organic C and total N in soil samples was measured by dry combustion (by a Carlo Erba NA 1500 CNS Analyzer, Milan, Italy) after pre-treatment of samples with 6 M HCl at 80 °C to eliminate carbonates (Santi et al., 2006).

Plant growth and production: Just before harvesting, plants’ height was measured for 30 plants each wheat cvs. and, the year after, 36 plants for sunflower. Half square meter of each wheat plot was harvested and the above-ground biomass was weighted. Twelve plants of sunflower each replicate were harvested at the end of the season, weighing the inflorescence heads (capitulas) and the above-ground biomass after oven-drying at 70 °C until constant weight. Seeds were removed from the head inflorescence for laboratory analysis.

Wheat grain and sunflower analyses: Carotenoids of wheat were extracted using 10 g of each sample, middlings and bran, with 100 mL acetone, cold sonicated for 30’. The sample was centrifuged for 5’ at 5000 rpm, the supernatant has been dry evaporated with a Rotovapor and the residue was dissolved in 5 mL acetone. The extracts were subjected to HPLC/DAD analysis. Polyphenols of wheat were extracted using 5 g of middlings and bran with 35 mL of 70:30 EtOH/H2O at pH 3.2 (by HCOOH). For polyphenols of sunflower 1 g of grounded kernel and tegument of each sample was extracted with 25 mL of 70:30 EtOH/H2O at pH 3.2 (by HCOOH). All solvents used were of HPLC grade purity (BDH Laboratory Supplies, Poole, United Kingdom). Both wheat and sunflower samples were shaken for 24 h, centrifuged for 5’ at 14,000 rpm and used for HPLC/DAD analysis.

Quali-quantitative analyses of carotenoids and polyphenols were carried out using an HP 1100 liquid chromatography equipped with a DAD detector and managed by an HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated using a 250 x 4.6 mm i.d., 5 µm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 280, 330, 350 and 450 nm. The samples were analyzed by gradient elution at a flow rate of 0.8 mL/min. For sunflower, compounds were separated using a 250 x4.6 mm i.d., 5 µm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 280, 330 and 350 nm. The samples were analysed by gradient elution at a flow rate of 0.8 mL/min. The mobile phase for carotenoids was a multistep linear solvent gradient system (solvent A: acetone, solvent B: H_2O, pH 3.2 by HCOOH), starting from 80% acetone up to 100% in 30 min, while polyphenols were eluted using the following gradient: from 90% H_2O (adjusted to pH 3.2 by HCOOH) to 100% CH_3CN in 40 min.

Quantification of individual polyphenolic compounds was directly performed by HPL/DAD using a five-point regression curve ($r^2 \geq 0.999$) in the range of 0-30 µg on the basis of authentic standards. The standard used were indolacetic, caffeic, and chlorogenic acids, and Karmpferol 3-glucoside, and Folin-Ciocalteau reagent and were purchased from Sigma-Aldrich (St. Louis, USA). β-carotene standard was purchased from Extrasynthese (Lione, Francia). In particular, flavonols were determined at 350 nm using kaempferol 3-O-glucoside as reference compound while caffeic acid derivatives were determined at 330 nm using chlorogenic acid as reference compound and indolacitic acid derivative at 280 nm using 3-indolacetic acid. Carotenoids were determined at 450 nm using β-carotene as reference compound. For sunflower samples, in particular, caffeic acid derivatives were determined at 330 nm using caffeic acid as reference compound and indolacitic acid derivative at 280 nm using 3-indolacetic acid.

The total phenolic content of wheat samples was determined using the Folin-Ciocalteu method, described by Singleton et al. (1999) and slightly modified according to Dewanto et al. (2002). To 125 µL of the suitably diluted sample extract, 0.5 mL of deionized water and 125 µL of the Folin-Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 mL of a 7% aqueous Na_2CO_3 solution were added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/100 g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 µg/mL ($R^2 = 0.9969$).

Free radical scavenging activity of wheat samples was evaluated with the DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams (1995) and slightly modified. Two mL of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH• (0.0025g/100mL) and the mixture kept at room temperature. After 20 min, the absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin-Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH• solution was checked. The antiradical activity percentage was calculated by the ratio: [DPPH• concentration at t = 20']/[DPPH• concentration at t = 0] .

Statistical analysis: Data underwent one-way analysis of variance (ANOVA) according to a completely randomized block design with three blocks and 3 replicates per treatment. Significant differences among means were determined using Tukey's post-hoc significance test at $p < 0.05$. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

6. Results

6.1 Experiment n°1: nursery trial on tomato plants (Paper I)

In preparing the pyrolysed materials, mass losses increased with pyrolysis temperature for all COMs, whereas ash content increased significantly only for the COMs from chitin (Table 2). With the exception of Ch400, all COMs showed alkaline pH values. Increasing the pyrolysis temperature from 400 to 500 °C had no major impact on the COMs for Pe and To although the latter revealed a considerably high EC at higher temperature.

Table 2. Mass loss of the feedstocks during pyrolysis, ash content, pH (H_2O), and electrical conductivity of COMs (n = 3). Values in the same column followed by the same letter indicate no significant differences at the $P < 0.05$ level.

Sample	Feedstock	Temp. °C	Mass loss %	Ash content (g kg^{-1})	pH	EC (mS cm^{-1})
Ch400	Chitin	400 °C	68.2^{b}	13^{e}	6.7^{e}	0.2
Ch500	Chitin	500 °C	72.3^{a}	18^{d}	7.6^{d}	0.1
Pe400	Peat	400 °C	47.1^{f}	247^{b}	8.8^{c}	1.5
Pe500	Peat	500 °C	53.6^{e}	204^{b}	8.7^{c}	0.6
Ri400	Rice husk	400 °C	57.9^{d}	313^{a}	9.4^{bc}	0.3
Ri500	Rice husk	500 °C	62.9^{c}	363^{a}	10.5^{a}	0.8
To400	Tomato plants	400 °C	57.0^{d}	302^{a}	10.6^{a}	12.5
To500	Tomato plants	500 °C	63.4^{c}	330^{a}	10.0^{ab}	16.6
Peat	Peat (Control)	-	-	159^{c}	6.1^{f}	1.2

In line with the literature (Angin, 2013), higher pyrolysis temperature led to higher C concentrations in all COMs. Chitin COM showed the highest C concentration followed by COMs made from peat, rice husk and tomato. Just To400 revealed C contents below 50%, thus all the others COMs passed the requirement of C concentration posed by EBC for biochar (EBC, 2019). In contrast to the COMs from the vegetal residues, Ch400 and Ch500 were characterized by very high N contents of 9% of the dry weight. Atomic carbon to nitrogen (C/N_{atm}) ratios of the COMs varied between 10 and 140, with chitin and tomato plant chars having the lowest and rice husk chars the highest values (Fig. 1). The control peat showed a C/N_{atm} ratio of 70.

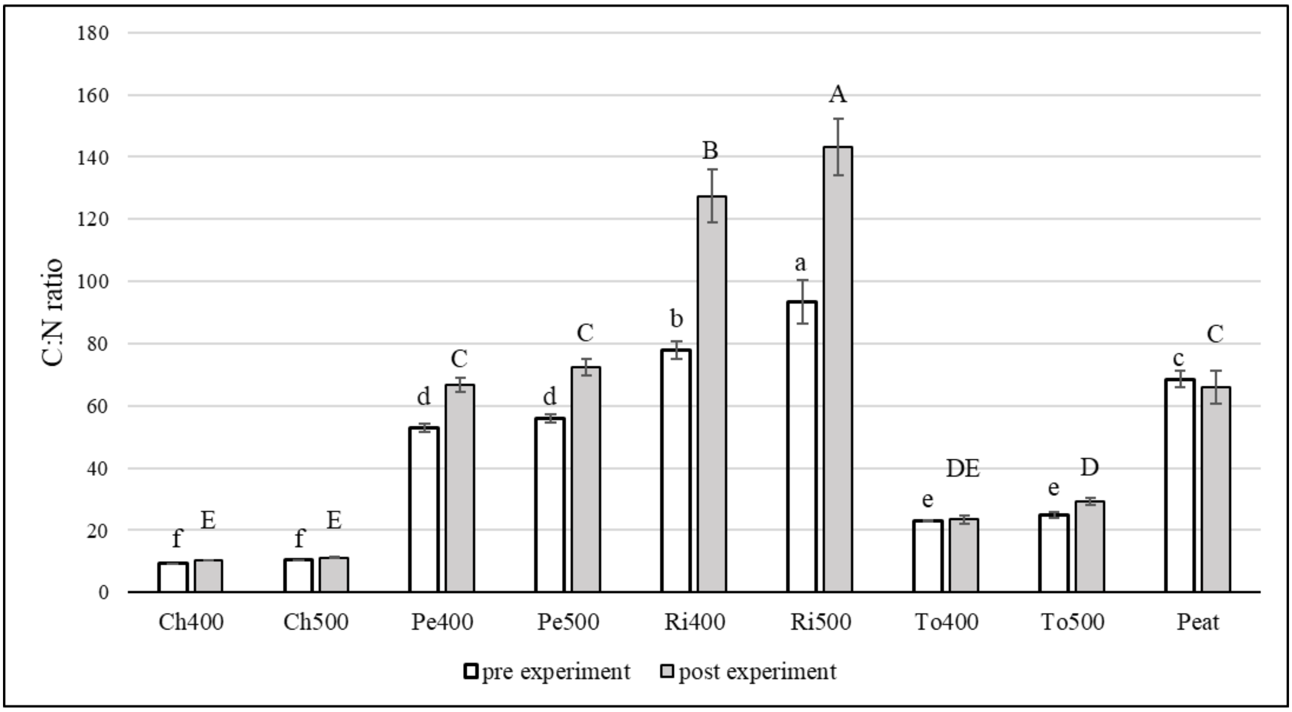


Fig. 1. Atomic carbon:nitrogen ratio of COMs before and after serving as plant growing substrate for 40 days. Values are means of three replicates. Column followed by the same letter indicate no significant differences at the $P < 0.05$ level. Lowercase letters were used for pre-experiment values, capital letters for post experiment values.

The plant- and peat-derived COMs accumulated very low amounts of inorganic N (Ni), less than 9 mg kg^{-1} (Table 3). Only Ch400 had a Ni content (151 mg kg^{-1}) that is above the optimum for growing tomato plants according to Sainju et al. (2003).

Table 3. Pre- and post-nursery experiment values of total nitrogen, carbon and phosphorus for peat and COMs, inorganic nitrogen (Ni) and Olsen-P for peat and COMs. Optimum values for tomato plants are shown as reported by Sainju et al. (2003). Values in the same column followed by the same letter indicate no significant differences at the $P < 0.05$ level.

	Total carbon (g kg^{-1})		Total nitrogen (g kg^{-1})		Total phosphorus (mg kg^{-1})		N_i (mg kg^{-1})		Olsen-P (mg kg^{-1})	
samples	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.
Optimum							50 - 100		60 - 70	
Ch400	721 ± 3 **b**	713 ± 2 **b**	90 ± 0 **a**	81 ± 0 **a**	1525.2	1436.3	151.2	91.2	0	0
Ch500	759 ± 5 **a**	767 ± 2 **a**	85 ± 1 **b**	79 ± 0 **a**	1703.8	1651.7	7	8.5	0	0
Pe400	651 ± 6 **d**	593 ± 9 **d**	14 ± 0 **d**	10 ± 0 **d**	1278.5	1261.4	1.1	1.3	98.3	93.8
Pe500	684 ± 3 **c**	642 ± 10 **c**	14 ± 0 **d**	10 ± 0 **d**	1437.9	1561.6	1	2.1	76.4	26.6
Ri400	525 ± 4 **f**	506 ± 0 **f**	7 ± 0 **e**	4.7 ± 0 **f**	1481.9	1191.6	1.6	1.2	89.1	263
Ri500	548 ± 4 **e**	536 ± 15 **e**	6 ± 0 **f**	4.4 ± 0 **f**	1053.2	1343.8	0.9	2.4	144	278
To400	486 ± 4 **h**	449 ± 9 **i**	24 ± 0 **c**	22.4 ± 0 **b**	12276.2	12234.0	7.5	1.5	1250	1480
To500	513 ± 10 **g**	479 ± 8 **h**	24 ± 0 **c**	19.0 ± 0 **c**	14258.1	12560.4	2.7	6.6	1510	1550
Peat	437 ± 3 **i**	499 ± 6 **g**	7 ± 0 **ef**	8.8 ± 0 **e**	588.8	593.7	527.7	400.0	167	173

As evident in Table 4, neither the pure COMs, nor the peat showed the optimum concentrations of all the macro and micronutrients for tomato growing. Tomato plant-derived COMs contained the highest concentration of micro- and macro-elements, whereas peat with chitin derived chars exhibited the lowest amount of elements with exceptions for Fe and Mn. None of the tested substrates in the study contained toxic levels of Cu for tomato seedlings, copper toxicity for tomato occurring with a concentration of Cu above 330 mg kg^{-1} with soil pH above 6.5 (Rhoads et al., 1989). Just tomato plant-derived COMs contained toxic level of Zn.

Table 4. Concentration of micro- and macro-elements (mg kg^{-1} and g kg^{-1}) of COMs and peat. Optimum and toxic values for tomato plants are shown as reported by Sainju et al. (2003).

Sample	B	Ca	Cu	Fe	K	Mg	Mn	Na	S	Zn
	mg kg^{-1}	g kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}
Optimum	1.5 - 2.5	1			0.6 - 0.7	0.4 - 0.7	5 - 20			
Toxic	>5						>80			>150
Ch400	0.6	3.7	0.2	0.1	0.1	0.1	2.0	0.3	0.0	6.2
Ch500	0.6	4.0	0.2	0.1	0.1	0.1	3.0	0.3	0.0	7.7
Pe400	11.3	42.8	21.3	1.0	3.2	3.0	179	0.4	2.4	30.2
Pe500	11.0	47.9	25.9	1.0	3.7	3.3	182	0.5	2.5	32.2
Ri400	14.4	1.9	4.2	0.3	5.8	1.4	142	0.5	0.4	34.0
Ri500	12.9	1.8	3.1	0.4	5.7	0.8	98.1	0.6	0.2	22.0
To400	89.2	98.7	28.7	1.0	63.6	11.4	176	4.0	26.4	226
To500	93.2	95.4	25.7	1.0	102.8	12.5	140	4.9	23.1	254
Peat	4.9	21.7	53.9	3.8	1.5	1.5	439	0.2	2.4	16.3

Hydrophobicity of the COMs varied considerably (Table 5). Even the pure peat was extremely hydrophobic, which is also due to the fact that the substrate was dried before the test to ensure equal starting conditions for all materials. A decrease in hydrophobicity was observed when the peat was pyrolyzed. Any increase of the pyrolysis temperatures lowered the hydrophobicity for all the studied materials. The other COMs, those made from chitin, together with tomato plants and rice husk pyrolyzed at 500 ◦C were very hydrophilic, since a complete absorption of water without addition of alcohol was obtained within a few seconds.

Table 5. Assignment of carbonized organic matter (COM) produced at 400 ◦C and 500 ◦C and derived from peat (Pe400, Pe500) rice husks (Ri400, Ri500), tomato plants (To400, To500) and chitin (Ch400, Ch500) to categories of hydrophobicity according to the water drop penetration time (WDPT) and molarity of ethanol drop test (MED).

Class	Descriptive label	Samples
7	Extremely hydrophobic	Peat
6	Very strongly hydrophobic	
5	Strongly hydrophobic	Pe400
4	Moderately hydrophobic	Pe500; Ri400; To400
3	Slightly hydrophobic	
2	Hydrophilic	
1	Very hydrophilic	Ch400; Ch500; Ri500; To500

In line with its extreme hydrophobicity, the pure peat showed a low WHC, whereas all pyrolyzed substrates revealed high WHC except Pe400 (Fig. 2). WHC was two to three folds higher for vegetal derived biochars (rice husk, and tomato plants) than for peat, and up to four times higher for the chitin-derived COMs. Although the differences are not statistically significant due to data dispersion, higher pyrolysis temperature seems to increase WHC for the vegetal-derived biochars, whereas an opposite trend is observed for biochars from chitin. COMs at 30% mixture with peat did not improve significantly the WHC of the growing substrate (Figure 2c). Mixing peat with pyrolyzed rice husk or tomato plants (To400) at low doses even reduced the water retention. Increasing the contribution of COM to 60% of the dry weight did not significantly increase the WHC of the mixtures (Figure 2b).

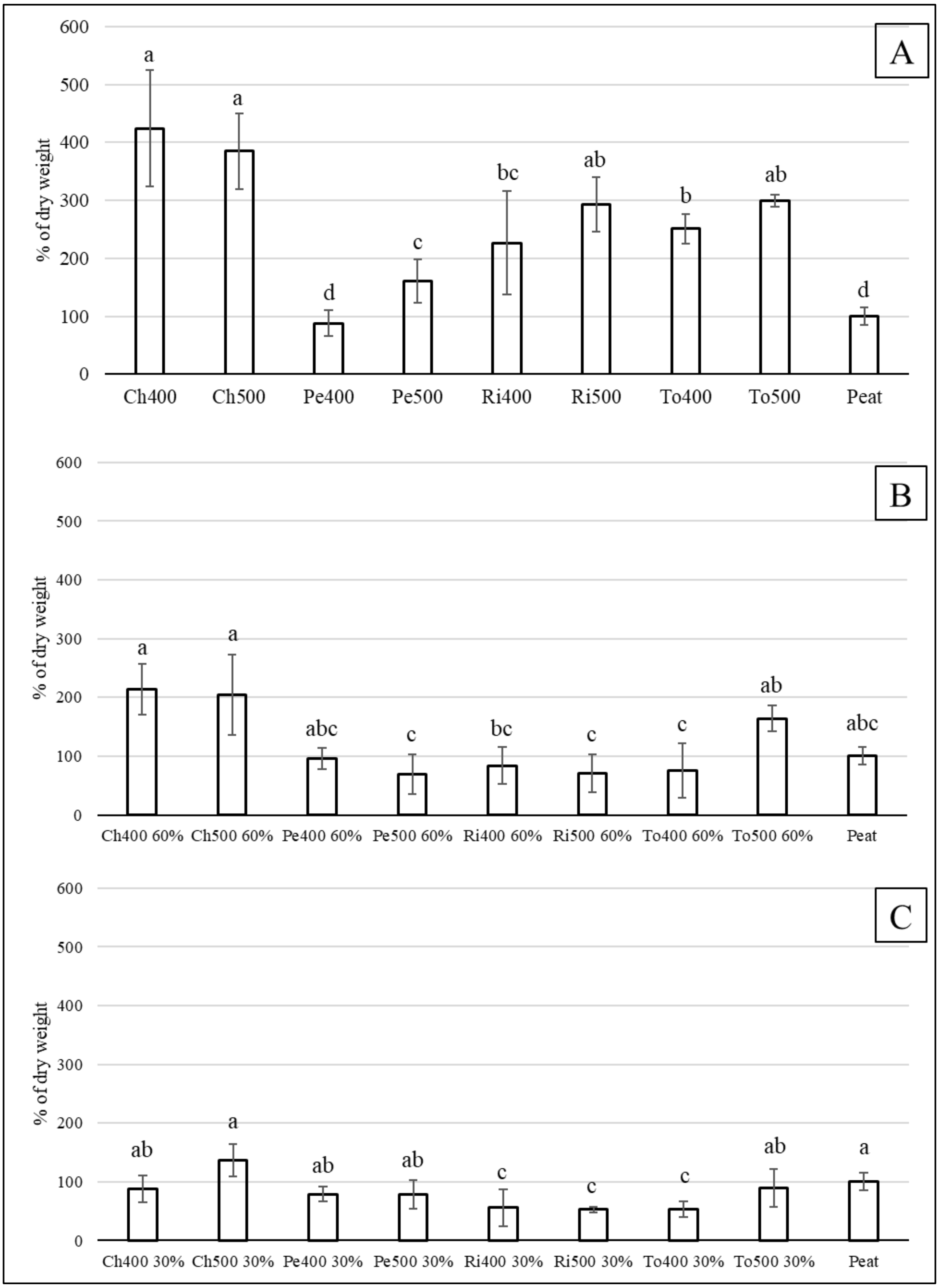
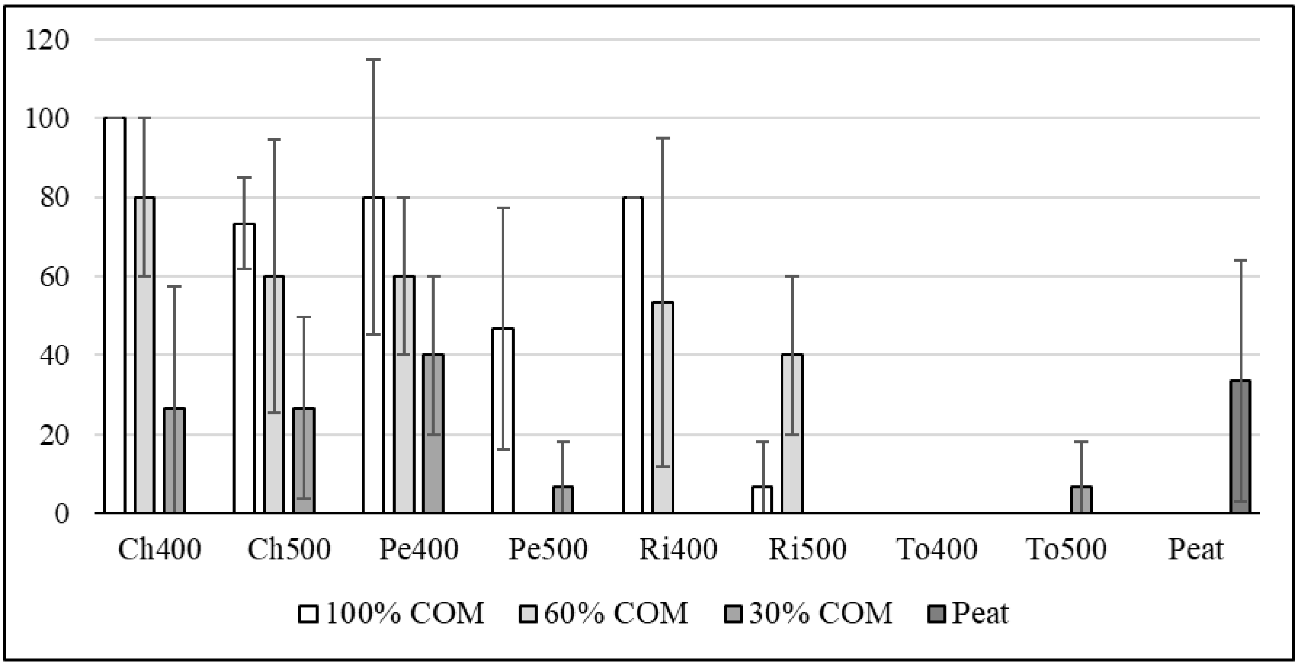


Fig. 2. Water holding capacity (WHC) of A) peat and the products pyrolyzed at 400 ◦C and 500 ◦C of chitin (Ch400, Ch500), peat (Pe400, Pe500), rice husks (Ri400, Ri500) and dried tomato plants (To400, To500), the mixtures with (B) 60% and C) 30% carbonized organic material (COM). Values are means of three replicates; error bars represent the standard deviations. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters.

In testing the different substrates growing tomatoes plants, all treatments, with the exception of pure COMs Ch400 and Ri400, showed high standard deviations in terms of germination rate (Fig. 3). In general, increasing pyrolysis temperature during COMs production and higher peat concentrations tended to have a negative effect on seed germination; even peat control pots experienced very low germination rate (33%) with high data dispersion. First germinated seeds were observed after 5 days for the treatments with 100% char with the exception of tomato chars, Ri400 and control peat. In the substrates with tomato plant COM, no seeds germinated, not even in mixtures with low COM contribution. The highest germination rate was obtained for Ch400 100%, whereas the same COM showed lower germination for the 30% mixture. The germination rate performance for rice husks derived COM was worse than for peat- or chitin-derived COMs.



	Ch400	Ch500	Ri400	Ri500	To400	To500	Pe400	Pe500	Peat control
100%	h	def	fg	-	-	-	gh	fg	cde
60%	ab	a	def	ef	-	-	cde	-	-
30%	c	b	-	-	-	-	cd	-	-

Fig. 3. Germination rates (%) of planted tomato seeds growing on pure peat substrate and on its mixtures with 30% and 60% COM produced at two temperatures (400 and 500 ◦C) of chitin (Ch), peat (Pe), rice husks (Ri) and tomato plant waste (To), control peat (Peat), and the pure COM (100%) 20 days after sowing. Values are means of three replicates; error bars represent the standard deviations calculated. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters in the legend.

After evaluating the germination rate, the impact of COM on plant growth was evaluated focusing on the development of the strongest plant per pot, which was allowed to continue growing for further 40 days after germination, whereas the other plants were removed from the pots. As displayed in Fig. 4, in general best results were obtained with a peat/COM mixture of 60/40. In detail, the substrates with significantly improve performance than the control were Ch500 60%, Ch500 30% and Ch400 60%, while considerably less dry biomass was yielded with all other materials and mixtures (Fig. 4). Increasing the peat concentration of the potting media with the Pe and Ri-derived COMs enhanced the plant height to values comparable to the pure peat experiment but could not compensate the lower dry plant biomass production. The mixed substrates with Ch500 and Ch400 at proportions of 60% and 30% exhibited significantly higher growth compared to the pure peat, both in terms of height of seedlings and biomass.

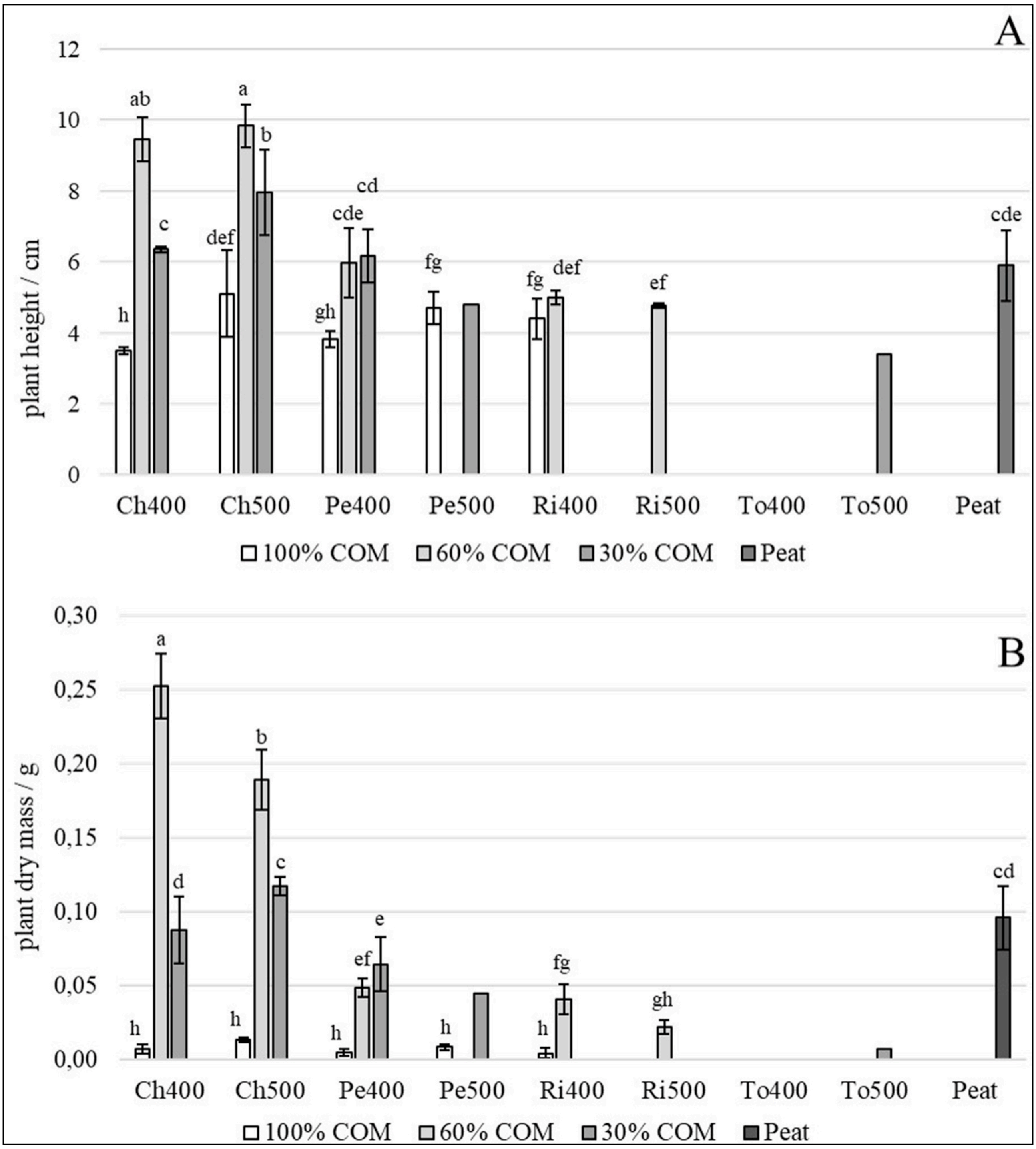


Fig. 4. Height (A) and dry weight (B) of tomato plants growing on pure peat substrate and on its mixtures with 30% and 60% COM produced at two temperatures (400 and 500 ◦C) of chitin (Ch), peat (Pe), rice husks (Ri) and tomato plant waste (To), control peat (Peat), and the pure COM (100%) 40 days after sowing. Values are means of three replicates; error bars represent the standard deviations calculated. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters.

6.2 Experiment n°2: nursery trial on basil (Paper II)

In this experiment both biochar and compost were tested as peat substitute for basil growing at different concentration (Table 7). The biochar properties, according to the producer, are shown in Table 6. Their addition significantly changed the chemical characteristics of the substrate in comparison to the pure commercial peat. In particular, both the pH and EC increased significantly compared to pure peat and differed significantly between the different substrates (Table 7). The EC of Char 100, i.e. 11.9 mS cm^{-1}, was by far higher than those of the other substrates, while pure peat had the lowest value, i.e. 1.6 mS cm^{-1}, the only one in the range 0.5-1.6 mS cm^{-1} reported as optimal for basil seedlings (Solis-Toapanta et al., 2020). All substrate mixtures used had bulk density between 0.23 and 0.32 g cm^{-3}, which is significantly higher than bulk density of the control. The concentration of available inorganic nitrogen (Ni) and total phosphorous of pure materials peat (control), biochar (Char 100) and compost (Comp 100) are shown in Table 8. Pure biochar showed very low values for Ni compared with other material, while the concentrations of total phosphorous were comparable between the three materials.

Table 6. Chemical parameters of the biochar derived from wood provided by the manufacturer. The indicated limit values for heavy metals and H/C refer to the limits given by the European Biochar Certificate (EBC) for a biochar of application class I (EBC-Feed).

Parameter	Unit of measure	Value	Limit values
Humidity at 105 °C	%	74	>20
pH	-	10.7	4-12
salinity	mS/m	68.5	<1000
Organic carbon	% d.m.	87.7	>60
Ashes	% d.m.	11.5	>10
H/C	-	0.01	< 0.7
Total nitrogen	% d.m.	0.7	-
Total phosphorus	% d.m.	1.0	-
Total potassium	% d.m.	0.001	-
Total carbon	% d.m.	88.8	-
Total sodium	% d.m.	0.06	-
Total calcium	% d.m.	2.0	-
Total magnesium	% d.m.	0.4	-
Total lead	mg/Kg d.m.	3.0	10
Total cadmium	mg/Kg d.m.	<0.2	1
Total copper	mg/Kg d.m.	37	100
Total zinc	mg/Kg d.m.	46	400
Total nichel	mg/Kg d.m.	2.0	30
Total mercury	mg/Kg d.m.	<0.2	0.1
Total chromium	mg/Kg d.m.	<0.2	80
polycyclic aromatic hydrocarbons (PAHs)	mg/Kg d.m.	<0.005	6

Table 7. Nomenclature of the potting substrate mixtures used in the experiment, and their pH, electrical conductivity (EC) and bulk density. Values are means ± standard deviation of 3 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test, at the p = 0.05 level

Substrates	Composition	pH	EC	Bulk density
	(in volume)		mS cm^{-1}	g cm^{-3}
Control	100 % peat	6.4±0.0^{f}	1.65±0.04^{g}	0.20±0.00^{e}
Char 100	100 % biochar	10.3±0.2^{a}	11.88±0.02^{a}	0.31±0.01^{ab}
Char 50	50 % biochar/50 % peat	9.4±0.2^{b}	3.48±0.04^{c}	0.27±0.00^{c}
Char 25	25 % biochar/75 % peat	8.4±0.3^{c}	2.09±0.07^{f}	0.23±0.01^{d}
Comp. 100	100 % compost	8.5±0.4^{c}	5.49±0.10^{b}	0.32±0.01^{a}
Comp. 50	50 % compost/50 % peat	7.6±0.2^{d}	3.17±0.01^{d}	0.29±0.01^{b}
Comp. 25	25 % compost/75 % peat	7.1±0.1^{e}	2.35±0.01^{e}	0.24±0.02^{d}

Table 8. Concentration of available inorganic nitrogen (NH_4^+ and NO_3^-) and total phosphorus (P) of control (peat), biochar and compost used for potting substrate.

Substrates	NH_4^+	NO_3^-	P
	mg kg^{-1}	mg kg^{-1}	g kg^{-1}
Control	32.3	778	2.9
Char	0.8	7.1	2.3
Comp.	25.7	55.4	2.2

Only one day after the transplanting, all basil seedlings in the Char 100 showed necrotized areas on leaves and stems of the plants of this treatment. After a further six days, necrosis appeared on top of the seedlings and the youngest leaves in all Comp 100 and Char 50 samples, and on the tenth day, it affected the whole seedlings of those treatments. However, no other plants showed such symptoms or other diseases throughout the trial.

Standard peat control showed better growing variables than the other substrates (Table 9). Negative effects of biochar and compost addition on seedlings' growth and quality were confirmed by SPAD readings and leaf color parameters. Biochar- and compost-including substrates did not show significant differences between each other in terms of total fresh aboveground biomass production; however, plants grown on Char 25 showed higher height and higher leaf dry content than plants grown in compost mixes, while the smallest plants grew on Comp. 50. Leaf color is a crucial quality for marketed fresh basil and we found this aspect to be significantly affected by substrate composition, as revealed by the significant differences in intensity of greenness (-a*), yellowness (+b*) and brightness (L*) between treatments. Seedlings grown in compost and biochar substrates showed higher L* compared to those cultivated on peat. In the green-red axis (a*) seedlings grown on biochar showed the lowest whereas peat the highest value. Plants grown in biochar increased their yellowness (b*) compared to those on the other substrates. Overall, the observed differences of the SPAD values and color coordinates of leaves resulted in a more intense green color of plants grown on peat, with a higher ornamental and commercial value.

Table 9. Final Height (H), total fresh weight (FW), leaf fresh weight (LFW), percentage of fresh leaves weight on total fresh weight (PLW), total leaf area (LA), leaf dry content (LDC), SPAD and color parameters (a^*, b^* and L^*) per plant grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 30 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for $p < 0.05$.

Substrates	H	FW	LFW	PLW	LA	LDC	SPAD	Color parameters		
	cm	g	g	%	cm^2	%	-	a*	b*	L*
Peat	43.9 ± 6.0^{a}	18.4 ± 4.9^{a}	11.3 ± 2.8^{a}	61.9 ± 2.2^{b}	488.1 ± 113.4^{a}	9.0 ± 0.6^{a}	30.8 ± 1.6^{a}	2.5 ± 2.3^{a}	25.0 ± 3.1^{b}	46.0 ± 1.8^{b}
Char 25	35.6 ± 5.0^{b}	13.3 ± 3.9^{b}	8.5 ± 2.3^{b}	64.3 ± 4.1^{ab}	328.6 ± 89.4^{b}	7.7 ± 0.8^{b}	27.7 ± 2.1^{c}	-2.4 ± 4.1^{c}	28.6 ± 3.1^{a}	49.4 ± 2.3^{a}
Comp. 50	26.8 ± 3.3^{d}	10.6 ± 1.4^{b}	7.0 ± 0.9^{b}	66.0 ± 1.7^{a}	270.4 ± 32.2^{b}	6.9 ± 0.3^{c}	28.5 ± 1.6^{bc}	1.8 ± 2.7^{bc}	26.2 ± 1.8^{b}	48.9 ± 2.4^{a}
Comp. 25	29.9 ± 4.0^{c}	11.0 ± 1.2^{b}	7.2 ± 0.8^{b}	65.5 ± 1.6^{a}	292.1 ± 33.9^{b}	7.0 ± 0.5^{c}	29.1 ± 1.8^{b}	-0.6 ± 2.4^{b}	26.1 ± 2.0^{b}	48.6 ± 2.4^{a}

Specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area ratio (LAR) are variables commonly taken into account in agricultural studies, especially with species such as basil where the edible part are the leaves. In particular, SLA is positively correlated with seedlings' growth rate and leaf net photosynthetic rate (Shipley and Vu, 2002). The highest SLA values were clearly associated with the lowest LDMC ones for both compost-containing substrates (Table 10). The SLA of peat and Char 25 did not differ significantly, while the LDMC value of the plants grown in peat was significantly higher than those in Char 25.

Table 10. Specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area ratio (LAR) per plant. Values are means ± standard error of 9 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for p = 0.05.

Substrates	SLA $cm^2 g^{-1}$	LDMC $g g^{-1}$	LAR $cm^2 g^{-1}$
Peat	482.3 ± 36.2^b	0.09 ± 0.01^a	240.8 ± 19.3^b
Char 25	505.7 ± 57.5^b	0.08 ± 0.01^b	256.7 ± 35.6^b
Comp. 50	563.0 ± 15.7^a	0.07 ± 0.00^c	308.4 ± 19.0^a
Comp. 25	587.2 ± 38.2^a	0.07 ± 0.01^{bc}	318.3 ± 26.7^a

Looking at the VOCs content of the basil, I identified ten different monoterpenes, one alcohol (1-octen-3-ol) and one phenylpropanoid (eugenol) (Table 11). Eugenol was the dominant VOC in all the treatments, followed by linalool and cineole. Compounds that reduce the flavor quality of basil such as estragole, camphor, and thymol (Maggio et al., 2016) were absent or present in trace amounts. No methyleugenol, which has been feared as a carcinogen of basil, was found. Data showed significant differences in the concentration of VOCs between treatments only for cineole, terpinen-4-ol, and α-terpineol. The concentration of these three monoterpenes was highest in the plants grown in Char 25, while the lowest amounts were detected in the plants grown in Comp 25. I did not find any significant differences in the total concentration of essential oils compounds between treatments. Results showed that variation in the relative content (%) of monoterpenes was not substrate-dependent, with the only exception of cineole, which was highest in plants grown in Char 25 (Table 12).

Table 11. Concentration of VOCs (mg g^{-1}) on dry weight extracted from basil plants grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 14 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan’s test for p = 0.05.

Substrates	α-pinene	β-pinene	sabinene	myrcene	limonene	cineole	β-cis-ocimene	1-octen-3-ol	linalool	terpinen-4-ol	α-terpineol	eugenol	total conc.
Peat	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.54±0.23^{ab}	0.03±0.02^{a}	0.14±0.04^{a}	2.55±0.84^{a}	0.13±0.08^{ab}	0.10±0.03^{ab}	7.91±2.27^{a}	11.47±3.48^{a}
Char 25	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.73±0.35^{a}	0.03±0.03^{a}	0.14±0.07^{a}	2.73±1.15^{a}	0.17±0.12^{a}	0.12±0.05^{a}	8.91±3.33^{a}	12.92±4.94^{a}
Comp. 50	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.68±0.29^{ab}	0.03±0.03^{a}	0.14±0.06^{a}	2.81±1.12^{a}	0.15±0.12^{ab}	0.11±0.05^{ab}	8.43±3.11^{a}	12.44±4.76^{a}
Comp. 25	0.02±0.01^{a}	0.02±0.02^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.51±0.17^{b}	0.02±0.02^{a}	0.11±0.04^{a}	2.20±0.98^{a}	0.10±0.06^{b}	0.09±0.03^{b}	7.05±2.24^{a}	10.15±3.46^{a}

Table 12. Relative content of VOCs (%), of basil plants grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 14 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan’stest for p = 0.05.

Substrates	α-pinene	β-pinene	sabinene	myrcene	limonene	cineole	β-cis-ocimene	1-octen-3-ol	linalool	4-ol-terpinen	α-terpineol	eugenol
Peat	0.14±0.05^{a}	0.14±0.05^{a}	0.13±0.05^{a}	0.10±0.00^{a}	0.13±0.05^{a}	5.64±1.03^{ab}	0.36±0.05^{a}	1.04±0.17^{a}	22.95±1.91^{a}	2.07±0.19^{ab}	0.84±0.09^{ab}	65.74±2.37^{a}
Char 25	0.20±0.24^{a}	0.14±0.05^{a}	0.14±0.05^{a}	0.11±0.03^{a}	0.11±0.04^{a}	6.75±0.85^{a}	0.34±0.06^{a}	0.89±0.14^{a}	22.10±3.03^{a}	2.23±0.29^{a}	0.89±0.11^{a}	65.49±3.68^{a}
Comp. 50	0.16±0.06^{a}	0.17±0.05^{a}	0.15±0.05^{a}	0.10±0.00^{a}	0.15±0.05^{a}	6.67±0.57^{ab}	0.34±0.06^{a}	0.94±0.14^{a}	23.26±2.51^{a}	2.17±0.28^{ab}	0.87±0.09^{ab}	64.56±2.82^{a}
Comp. 25	0.16±0.06^{a}	0.19±0.10^{a}	0.16±0.05^{a}	0.10±0.00^{a}	0.15±0.05^{a}	6.34±0.77^{b}	0.34±0.05^{a}	0.90±0.28^{a}	21.84±3.15^{a}	2.16±0.16^{b}	0.84±0.09^{b}	66.20±3.50^{a}

6.3 Experiment n°3: open field trial on wheat and sunflower (Paper III)

In the open field experiment, biochar addition did not change significantly the physical and chemical characteristics of the soil even when added at the highest amount, i.e. 20 t ha^{-1} (Table 13). Indeed, the pH, EC, WHC and CEC of soil samples did not differ between treatments. The concentration of total nitrogen and total carbon of soil samples are shown in Table 13. Total soil organic carbon (C) content was affected by biochar application, but significant differences were observed only between the control and B3. Total soil nitrogen was not affected by biochar amendment.

Table 13. Dose of biochar, pH, electrical conductivity (EC), water holding capacity (WHC), cation exchange capacity (CEC), total organic carbon (TOC) content and total nitrogen content (TN) of the different soil treatment. Values are means ± standard deviation of 3 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test, at the $p < 0.05$ level.

Samples	Dose	pH	EC	WHC	CEC	TOC	TN
	t ha^{-1}		µS cm^{-1}	%	meq/100 g	%	%
Control	0	7.6 ± 0.4^a	141 ± 27^a	84.4 ± 4.0^a	42.4 ± 1.4^a	1.12 ± 0.5^b	0.14 ± 0.01^a
B1	1	7.7 ± 0.2^a	180 ± 57^a	85.9 ± 3.1^a	39.7 ± 2.1^a	1.17 ± 0.3^{ab}	0.15 ± 0.00^a
B2	4	7.6 ± 0.2^a	138 ± 27^a	85.3 ± 2.7^a	42.9 ± 0.9^a	1.27 ± 0.8^{ab}	0.15 ± 0.00^a
B3	20	7.7 ± 0.2^a	144 ± 12^a	92.5 ± 5.9^a	41.2 ± 1.5^a	1.35 ± 1.0^a	0.15 ± 0.01^a

As expected, the un-dwarfed ancient cultivarss Senatore Cappelli and Verna were taller and less productive than modern ones (Figure 4). Conversely, the bulk above-ground biomass produced at the end of the growing season, including stems and ears, showed no differences between the cultivars (Figure 7). However, the sowing seed density for modern cvs. was higher. The addition of biochar did not influence the height of each cv. between the various treatments, as well as the biomass and the grain yield, even at the highest dose, i.e. 20 t ha^{-1} (Figure 6, 7 and 8). Total proteins in the ancient cvs. was higher compared with the modern ones (Figure 5). Senatore Cappelli showed the highest protein content while the modern cv. Bologna showed the lowest. However, the protein content of whole grain of each of the four cvs. was not affected by biochar addition.

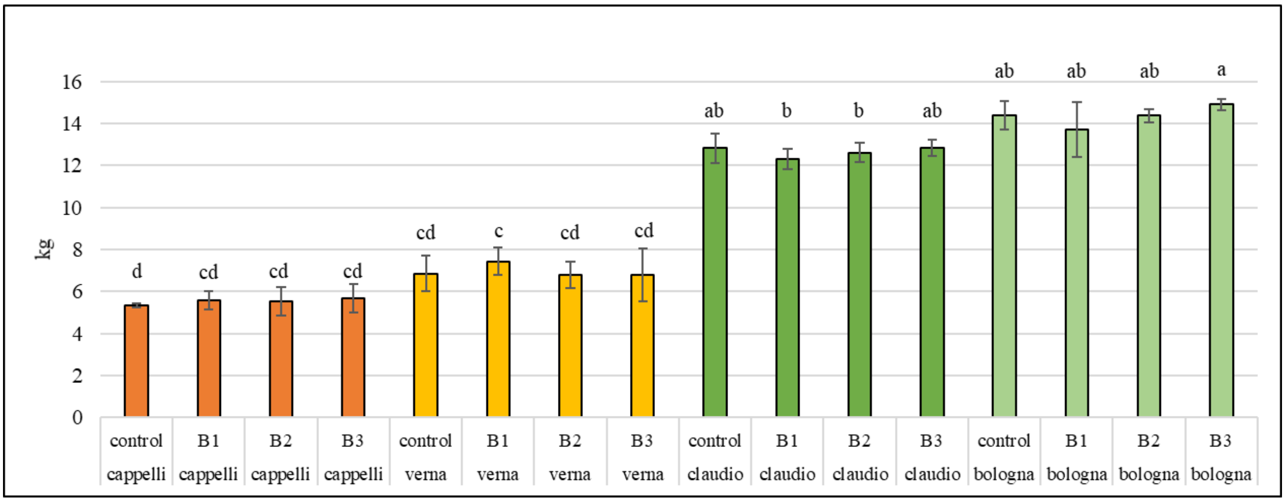


Fig. 4. Grain production/plot (kg) for each wheat cv. under 3 different doses plus the control. Values are means of three replicates. The comparison was performed for different doses within each cv. The significant differences were shown by different letters (Tuckey's test for $p < 0.05$).

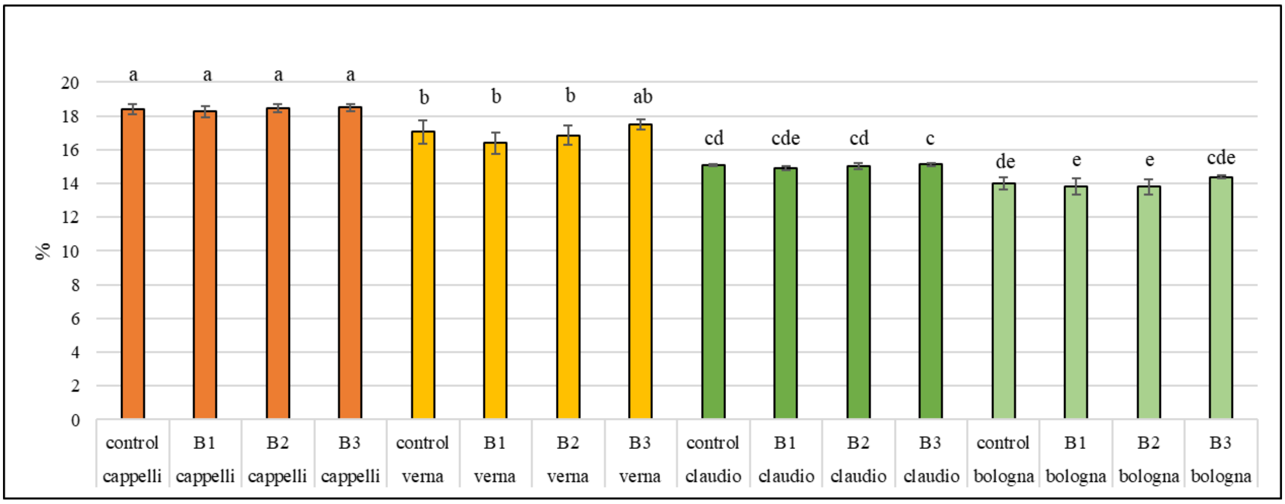


Fig. 5. Total protein content of wheat seeds for each cv. under 3 different doses plus the control. Values are means ± standard deviation of 3 replicates. The comparison was performed for different doses within each cv. The significant differences were shown by different letters (Tuckey's test for $p < 0.05$).

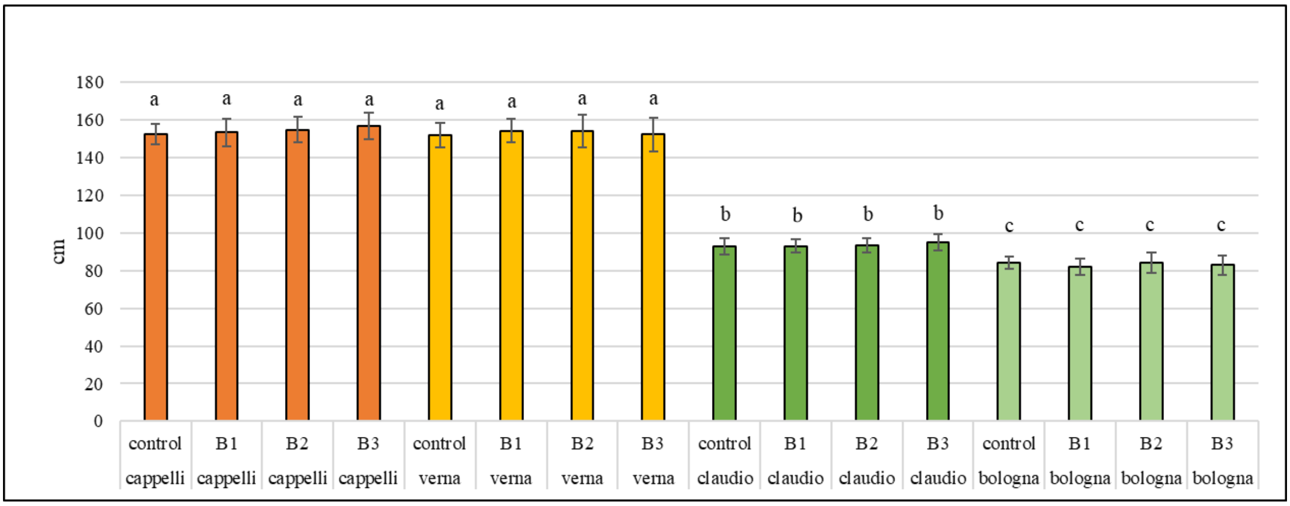


Fig. 6. Plant height (cm) for each wheat cv. under 3 different doses plus the control. Values are means ± standard deviation of 20 replicates. The comparison was performed for different doses within each cv. The significant differences were shown by different letters (Tuckey's test for $p < 0.05$).

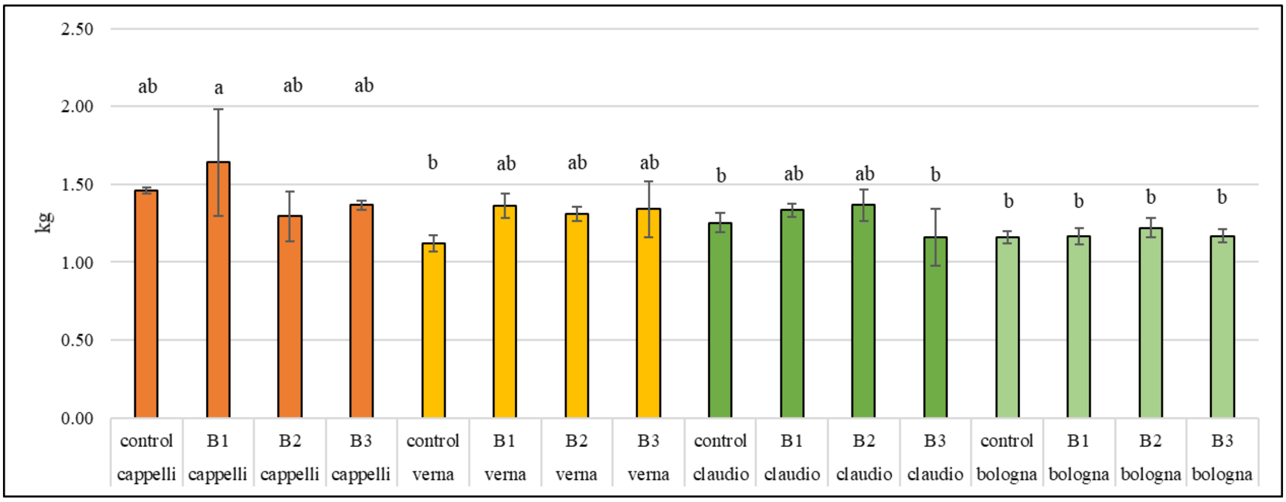


Fig. 7. Plant fresh biomass (kg) for each wheat cv. under 3 different doses plus the control. Values are means ± standard deviation of 3 replicates. The comparison was performed for different doses within each cv. The significant differences were shown by different letters (Tuckey's test for $p < 0.05$).

As Verna is one of the most cultivated ancient cultivars in Tuscany for making bread, we took into account just this cv. for the analysis on the concentration of total polyphenols (also known as total phenolics), carotenoids, flavonoids and caffeic acid in the middlings and bran (Table 14). These compounds are strictly related to the quality of grain and flour and are unevenly distributed in the kernel, for this reason the analysis were performed on middlings and bran separately. We observed significant differences in the concentration of total polyphenols in the middlings according to the dose of biochar added to soil as significantly higher values were obtained by B2 and B3 treatments compared to the control. Differences were also found in the bran for treatments B1 and B2 that showed a significantly higher values of total pholyphenols compared to the other treatments. Significant differences in flavonoids concentration in the bran were also observed, as B3 treatment showed the highest values while the control the lowest values. B3 was also the treatment with most abundant caffeic acid derivatives in the bran, although it did not differ significantly with the control; in this case the concentration of caffeic acid derivatives seems not to follow a trend related with biochar amount. Overall, biochar application seems to led to an increase of the concentration of polyphenols, flavonoids and caffeic acid derivatives. On the other hand, the concentrations of carotenoids in the middlings and in the bran, and the concentration of flavonoids in the middlings, were not affected by biochar addition at tested doses.

Table 14. Concentration of total polyphenols, carotenoids, flavonoids and caffeic acid derivatives of middlings and bran of Verna as affected by different doses of biochar. Values are means ± standard error of 9 replicates. Values in the same column followed by different letters indicate significant differences, based on Tukey's test for p = 0.05

Samples	Polyphenols mg(GAE)/g		Carotenoids mg/g		Flavonoids mg/g		Caffeic a.d. µg /g
	middlings	bran	middlings	bran	middlings	bran	bran
Control	1.08±0.08^{b}	1.77±0.11^{b}	5.78±0.65^{a}	3.87±0.34^{a}	0.43±0.07^{a}	0.04±0.01^{b}	7.7±1.4^{ab}
B1	1.18±0.07^{ab}	1.92±0.07^{a}	5.92±0.28^{a}	4.04±0.69^{a}	0.40±0.02^{a}	0.06±0.02^{ab}	6.3±1.9^{b}
B2	1.29±0.06^{a}	1.92±0.04^{a}	5.51±0.40^{a}	3.52±0.41^{a}	0.44±0.12^{a}	0.05±0.01^{ab}	5.8±0.7^{b}
B3	1.23±0.16^{a}	1.70±0.12^{b}	5.36±0.41^{a}	3.92±0.37^{a}	0.41±0.05^{a}	0.07±0.03^{a}	9.1±2.4^{a}

The radical scavenging activity of antioxidants was calculated on samples of control and B3 only for Verna. The capacity of wheat extract to scavenge the stable DPPH radical is shown in Figure 8. The T-test showed no differences between the two treatments, control and B3, both in middlings and in bran.

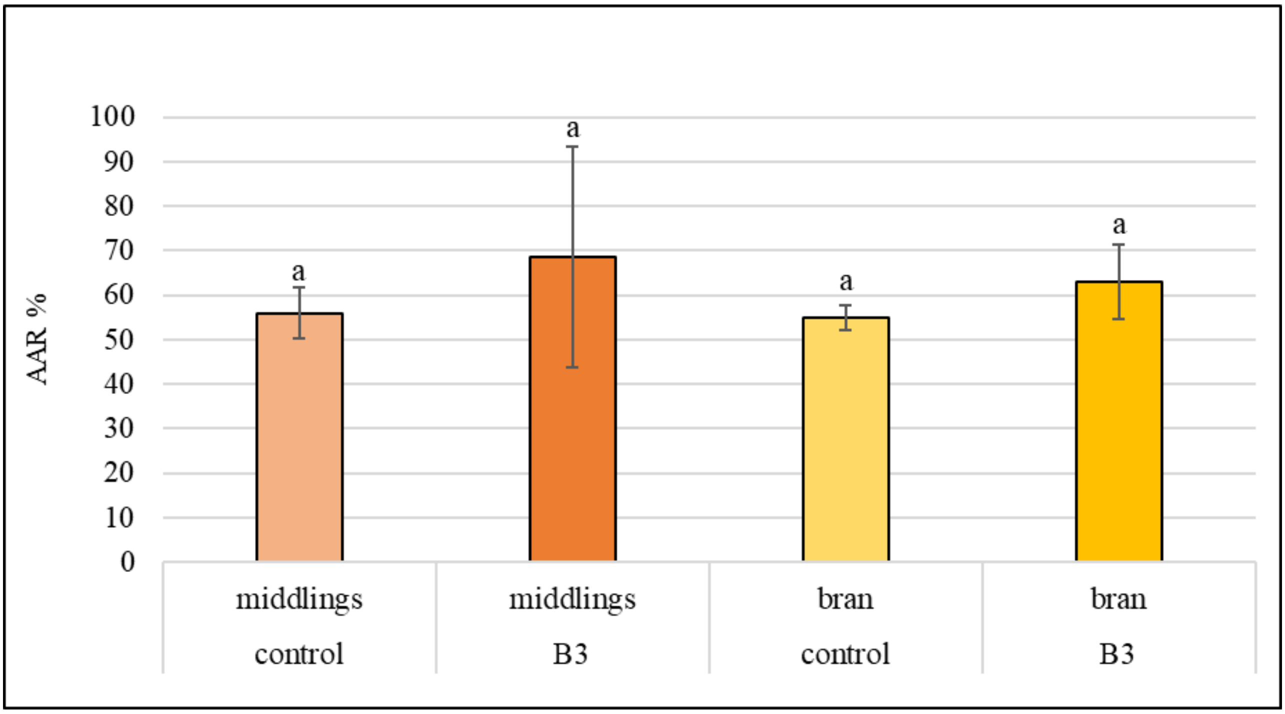


Fig. 8. Antiradical activity percentages of middlings and bran in the control and the B3 treatment. Values are means ± standard deviation of 3 replicates. Letters indicates significant differences according to T-test for $p < 0.05$.

Sunflower hybrid P64HE39 cultivation followed the wheat on the same field. Also the growth parameters of sunflower such as plant height, total fresh weight of above-ground biomass, fresh weight of capitulas, as well as the crop yield calculated as dry seeds weight, were not affected by biochar addition, even at the highest amount (Table 15).

Table 15. Plant height (PH), fresh above-ground biomass weight (FABW), capitulas fresh weight (CW) and dry seeds weight (DSW) as affected by biochar addition. Plant height values are means ± standard error of 36 replicates; FABW, CW and DSW are means ± standard error of 3 replicates. Values in the same column followed by different letters indicate significant differences, based on Tukey's test for $p = 0.05$

Samples	PH	FABW	CW	DSW
	cm	kg	kg	G
Control	192±16^{a}	4.36±0.76^{a}	1.56±0.32^{a}	724.04±59.54^{a}
B1	196±11^{a}	4.13±0.83^{a}	1.42±0.22^{a}	709.16±127.12^{a}
B2	193±11^{a}	4.05±0.40^{a}	1.45±0.22^{a}	653.99±17.59^{a}
B3	199±8^{a}	4.81±0.82^{a}	1.53±0.17^{a}	744.18±115.82^{a}

Total polyphenols concentrations of sunflower dry seeds (kernel and tegument) are shown in Figure 9. The concentration seems to decrease with the increase of biochar application, but the data showed a high standard deviation and the statistical analysis results showed no significant differences between means according to Anova.

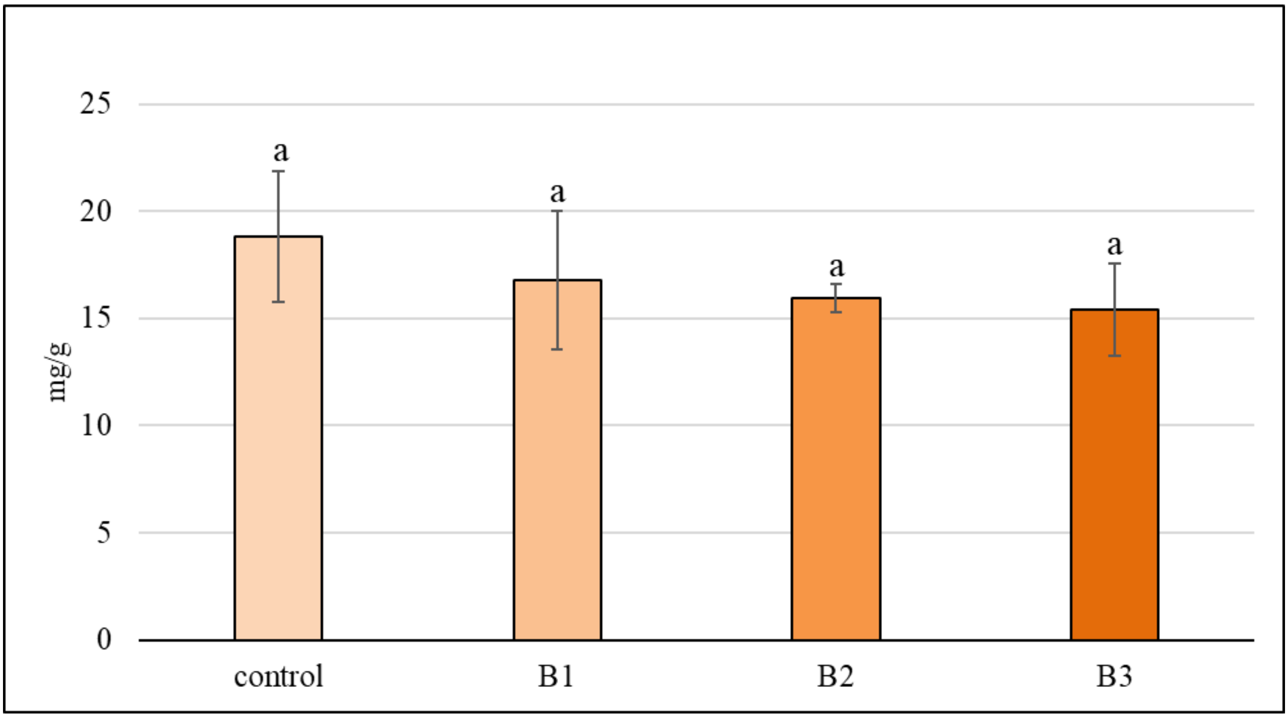


Fig. 9. Concentration of total polyphenols (mg g^{-1}) of sunflower dry seeds (kernel and tegument) as affected by different amounts of biochar added to the soil. Values are means ± standard error of 3 replicates. The letters indicates significant differences according to Tuckey's test for $p = 0.05$.

7. Discussions

In a historical period characterized by scarcity of resources and climatic changes, the production of nursery plants using alternative growing substrates has much to offer as an environmentally friendly industry. Finding new, renewable and environmentally sustainable organic materials for growing media is a challenge but certainly represents also an opportunity. In this context, using renewable resources and recycling waste material can be a win-win choice, not only in the production of nursery plants but also for using these materials as soil amendment.

7.1 Biochar as growing media and compost additive

Taking into account the materials studied, with the exception of biochar from chitin, all the biochars produced from waste material and tested in this study showed too high pH and EC values ($8.7<\text{pH}<10.6$; $300<\text{EC}<16600$ μS cm^{-1}) for being optimal growing media. The optimum pH for most vegetable seedlings is in fact reported to be between 5.8 and 6.8 (Huang et al., 2019b); moreover, most crops grow properly in soils with EC values between 100 and 1000 μS cm^{-1}. For these reasons EC and pH can be limiting factors when biochar is added at high concentrations in potting substrates, without a proper correction. Also, high EC of biochars is common indicator of salinity, which can have a negative effect on plant growth (Prasad et al., 2019). Huang and Gu (2019) suggested that high doses of biochars cannot be used in potting substrates without the addition of acidic compounds. Indeed, high pH and EC of substrates can explain the bad performance of plant growth in the experiments with basil and tomato seedlings. All basil seedlings in the Char 100 died soon, after just one day. After a further six days, necrosis appeared on top of the seedlings and the youngest leaves in all Comp 100 and Char 50 samples, which died soon after. On the other hand, high pH-values might not have been the single responsible for our observations. In the potting experiment with tomato also the presence of some toxic compounds, such as B and Zn, above their toxicity levels can explain the lack of germination of tomato seeds. Furthermore, Alpaslan and Gunes (2001) observed that boron toxicity symptoms on tomatoes appeared at concentrations of 5 mg kg^{-1} and increase with high salinity. Note that this threshold was exceeded by all COMs except those derived from chitin and peat. Unexpectedly, increasing the peat concentrations in the substrate composition tended to have a negative effect on tomato seed germination. This result is related to the initial condition of the peat, which was dried, and the resulting high hydrophobicity, which may have prevented an adequate imbibition of tomato seeds, a very important step for an adequate germination (Liu et al., 1993). The hydrophobicity of the COMs varied considerably. In general, increasing the pyrolysis temperatures lowered the hydrophobicity of the studied materials, although it is known that charring enhances the aromaticity commonly associated with higher hydrophobicity. This, however, did not have an overall positive impact on tomato seed germination as increasing pyrolysis temperature production of COMs tended to have a negative impact on seed germination.

With regard to plant growth, with the exception of chitin-derived COMs, none of the other materials we tested on both tomato and basil had comparable results with standard peat. In my experiments the rate of peat substitution by charred peat-, rice-, tomato-COMs and wood-derived biochar were too high for positively affecting plant height and biomass production. Best results for tomato growth were obtained with a peat/COM mixture of 60/40. The high N content of the chitin-derived COMs may have contributed to the improved performance of the plants with respect to the other COMs. Even having high level of available P in the COMs, as in the case of COMs from tomato plants, can be detrimental as together with a high pH it may reduce the availability of others micronutrients. Another main issue is that the plant- and peat-derived COMs, even those that can be properly defined as biochar as in the case of the one used for basil growth, accumulated very low amounts of inorganic N (Ni), i.e., less than 9 mg kg^{-1}, which may lead to N deficiency if these materials are used as growing substrate without being properly fertilized with regards to N. Therefore, considering the N deficiency of these materials along with the presence of some compounds that can be toxic for plant growth, I can conclude that none of the tested substrates is per se a suitable substrate able to optimally supply all nutrients to the tested plants and many others. However, N fertilization, dilution of these materials with peat and/or washing and desalting may overcome the negative impacts described.

The detrimental effect of biochar and compost at a substitution rate of even 25% v.v., concerned not only on basil height, biomass and foliar surface, but also on some qualitative traits such as SPAD readings and color parameters, which are strictly correlated to the plant nutritional status (Dispenza et al., 2016). The SPAD values can be used as an indicator providing a quick and objective estimation of quality for green-leaved foliage (Wang et al., 2005). My results are partially in line with those of Yu et al. (2019), who overall reported lower growth for basil seedlings grown in substrates with high doses of biochar compared to growth performances obtained with the commercial peat-based propagation mix. Their results indicated that biochar can be mixed safely with peat moss up to 50% for basil seedling production in a greenhouse. With regard to compost addition to peat for growing basil there are contrasting results in the literature. Hewidy et al. (2014) observed that growing media comprising up to 30% compost in volume enhanced some plant variables like basil height, dry mass, and essential oil content, compared to pure peat moss, while DeKalb (2014) reported negative effects on basil height and weight by just 20% compost in the substrate, observing an inhibition of seedlings emergence in substrates with pH higher than 8.3.

Pandey et al. (2016) highlighted the importance of the substrates' nutrients content as they observed that the use of biochar in a pot experiment without fertilizers was not sufficient to boost the growth of the basil plant. Indeed, the lower SPAD values I found for basil grown on substrates added with compost or biochar and the lower N concentration in leaves may be related to a lower availability of N in these substrates. Several studies report that biochar and compost in soilless substrates can lead to N immobilization, consequently reducing the availability of this important element for plants (Blok et al., 2017; Burger et al., 1997; DeKalb et al., 2014; Huang et al., 2019).

Substrate composition significantly affected also leaf color, a crucial quality for marketed fresh basil, as revealed by the significant differences in intensity of greenness (-a*), yellowness (+b*) and brightness (L*) between treatments. Seedlings grown in compost and biochar substrates showed higher L* compared to those cultivated on peat. In the green-red axis (a*) seedlings grown on biochar showed the lowest whereas those grown on pure peat the highest value. Plants grown in biochar increased their yellowness (b*) compared to those on the other substrates. Overall, the observed differences of the SPAD values and color coordinates of leaves resulted in a more intense green color of plants grown on peat, with a higher ornamental and commercial value.

Another important quality parameter of basil is its essential oil profile, not only for fresh consumption but also for pharmaceutical purposes. Factors that can affect essential oils production and composition are mostly based on the individual genetic variability, the phenological stage, the kind of organ of the plant, and environmental factors, such as light, precipitation and soil/substrate characteristics, including pH and water availability (Barra, 2009; Johnson et al., 1999). In particular, Bernstein et al. (2010) observed that the production of essential oils in basil increases because of salinity stress associated with a decrease in plant biomass. Also other works on basil (Ekren et al., 2012) and on sage (Ben Taarit et al., 2009) reported that ecological conditions as salinity stress can influence some essential oil composition and content. However, in my experiment the addition of biochar or compost did not significantly influence the total concentration of essential oils compounds, despite the large differences in terms of EC between the substrates. Also the variation in the relative content (%) of monoterpenes was not significantly affected by the different substrates, with the only exception of cineole, which showed the highest values in plants grown in Char 25. However, this last result was expected as the terpene profiles of plants, i.e., the relative contents of volatile terpenes, are under strong genetic control and usually little affected by abiotic factors (Casano et al., 2011; Michelozzi et al., 2008; Pandey et al., 2016; Squillace, 1976).

7.2 Biochar as soil amendment

The degradation of agricultural soils has become a global problem limiting crop production. Soil erosion, soil acidity, low fertility and salinization of cultivable soils are widespread problems. The utilization of biochar as soil amendment to improve soil quality and productivity, has been suggested since many years. Application of biochar to soils is reported to enhance soil quality and plant growth (Guo et al., 2020), through important changes in soil physiochemical and biological properties, consequently increasing many crop yields. Furthermore, biochar can also represent a valid tool in controlling soil pH and improving the water content. Despite various works reported an increase in crop productivity of various species after biochar application, alone or combined with fertilizers (Jeffery et al., 2011), I did not observe any positive effects in the open field trial with wheat and sunflower, neither on the yield nor on the height of the plant or their above-ground biomass. Even at the maximum dose of 20 t ha^{-1}, that can be considered for itself impracticable for the majorities of farmers because of the high cost of biochar, I did not observe significant changes in the pH, EC, CEC and WHC of amended soil, which was comparable to the control soil, showing a high buffering capacity of the soil. My results are in line with those obtained by Paneque et al. (2016) in a similar study on sunflower in a Calcic Cambisol (pH=8.5), which is a common agricultural soil of the Mediterranean basin, where these authors observed no significant differences in soil pH added with different doses of biochars (pH $\geq$10) from various feedstocks at a maximum dose of 15 t ha^{-1}. Despite the high EC of the biochar used for the field trial of my experiment, the EC of amended plots was lower than the value of 270 μs cm^{-1} that are recommended for most common crops such as sunflower. Regarding the WHC of soil samples, our results are in contrast with the observations made by Verheijen et al. (2019) on sandy and sandy loam soils, who reported that the effect of biochar on WHC is usually positive, especially on sandy soils, and with the work by Paneque et al. (2016), who observed that the application of 15 t ha^{-1} of biochar as soil amendment, increased significantly the WHC of a Calcic Cambisol. However, my experiment was set up in a very different soil in texture terms, being silty-clay, hence much finer of the ones cited above. Actually, the effects are strongly related to the type of soil, the applied biochar dose and the feedstock, with sandy soils showing the higher improvement and wood-derived biochar the most performant feedstock. Otherwise, Cooper et al. (2020) in a study on biochar and composted-biochar as soil amendments, with application rates of 9 and 70 t ha^{-1}, observed that biochar significantly increased soil pH, while compost significantly increased pH and CEC even six years after application. They concluded that high rates of both biochar and compost are beneficial for soil properties affecting pH and CEC. In our experiment no differences between soil samples were observed for the CEC, also at the maximum application rate. CEC is a measure of the quantity of readily exchangeable cations neutralizing negative charge in the soil, in other words the CEC is the total capacity of a soil to hold exchangeable cations. The sources of soil CEC are organic matter and clay minerals. Jeffery et al. (2011), in a statistical meta-analysis on the relation between biochar and crop productivity, either yield and biomass produced, reported that the major positive effects were obtained in acidic and neutral soils, while the soil where I performed the trial is slightly alkaline, with a silty-clay texture. These results suggested that some of the mechanisms involved in yield increase could be the liming effect and the rise in the water holding capacity of the soil, after biochar application, and also an improved nutrients availability. Same authors found that the major positive effects were observed when biochar was added at a rate of 100 t ha^{-1}, anyway they also observed an increase in crop productivity even at application rates of 10, 25, and 50 t ha^{-1}. These application doses are, from an economic point of view, completely unfeasible for farmers.

Total soil organic C is one of the most important indicators of soil quality (Andrews et al., 2004; Laird et al., 2010), in my field experiment the addition of the maximum dose of biochar (20 t ha^{-1}) significantly increased the total C content, measured after 10 months from the application. Even in this case the little increase in total C is not feasible from an economic point of view, as the application costs are not justified for the possible benefits.

Regarding the quality of harvested seeds, I observed higher percentage of proteins in the ancient cvs. Verna and Senatore Cappelli, as expected. In fact, it’s known that ancient wheat cvs. differ from modern ones also in terms of protein content (Giunta et al., 2020). The protein content is an important factor that is strictly related to grain quality and moreover contributes the most to the EU Quality Index for durum wheat (Giunta et al., 2020). However, the wheat protein content was not impacted by biochar addition at any does for any cv. Factors that can affect grain protein percentage are the genotype, environment, management and the interaction of these factors. Between the environmental conditions, drought and temperature contribute the most to determining variations in wheat quality traits (Giunta et al., 2020), properties that would be affected by biochar addition. The color of whole wheat grain is usually considered as a quality indicator, color is mostly related to the presence of carotenoid and their esters (Luthria et al., 2015). No differences on carotenoids concentration, both in middlings and bran, of cv. Verna after biochar addition were observed. Biochar amendment, even at high doses, does not seem to negatively affect the carotenoids’ concentration in wheat.

Wheat bran represents a good source of polyphenols, which strongly contribute to the total antioxidant activity. Polyphenols are secondary metabolites of plants and are usually involved in defense against ultraviolet radiation or attacks by plant pathogens. Verma et al. (2008) reported that the antioxidant activity in wheat bran is highly correlated with its total phenolic content. Phenolics, in fact, have an ability to act as radical scavengers. In a study on modern and ancient varieties of *T. durum* and *T. aestivum*, Heimler et al. (2010) reported that atmospheric conditions are the main factors which causes differences in free polyphenols and antiradical activity of wheat. Polyphenol synthesis and accumulation is usually improved in response to biotic or abiotic stresses, as observed by Ramakhrisna and Ravishankar (2011) who also reported that salinity of soil is one of the factors that can bring to an increase of polyphenols content in plant tissues. The same authors found that flavonoids perform protective functions against drought stress; these compounds are moreover implicated to protect plant growth in soils contaminated with high amount of toxic metals. The most present phenolic compounds in cereals are phenolic acids and flavonoids, which act as antioxidant (He and Giusti, 2010) and are the largest group of naturally occurring polyphenols (Žilić, 2016). In my experiment I observed an increase of total polyphenols of the middlings of cv. Verna in B3 and B2 treatments, with a significant difference with the control and the lowest biochar dose B1. Differently, for bran samples B1 and B2 showed the highest results. Sample B3 also showed the highest values for the flavonoids and the caffeic acid derivatives present in the bran. Factors that can influence total flavonoids are the genotype, environment and genotype-by-environment interaction. Also soil nitrogen is able to affect flavonoids content of vegetables and fruit; furthermore, a higher nitrogen supply may lead to a decrease in the total polyphenols content of grape fruits and no differences for tomato fruits (Heimler et al., 2017). The polyphenols content of seed samples in my experiment cannot be explained by the availability of nitrogen, which was similarly provided in all treatments by a synthetic fertilizer. Shamloo et al. (2017) suggested that some abiotic factors, such as high temperatures, can force wheat plants to produce a higher amount of flavonoids as a defense mechanism against the environmental changes. Khlestkina (2013) underlined that a lot of studies confirmed the relationship between the flavonoids biosynthesis and abiotic stresses. For example, the excessive moisture of soil at some stages of cereals growth can induce some changes in flavonoids concentrations, as they can prevent negative effects of excessive moisture. Biochar is reported to improve the soil moisture as observed by Haider et al. (2017) in a four-year field study on different crops including wheat, but without any improvement in yields.

Taking into account the total polyphenols of sunflower seeds, analyzing kernel and tegument together, the control showed the higher concentration of these compounds, while other samples exhibited lower concentration as the dose of biochar increased. Anyway, such differences were not validated by the statistical analysis of data.

Regarding the antiradical activity of Verna flour, bran samples showed strong DPPH free radical scavenging activities if compared with middlings samples. DPPH assay is one of the most widely employed methods for screening antioxidant activities of plant extracts. The higher the antiradical value, the more efficient the radical scavenging activity of the samples. These results are in line with those of Liyana-Pathiran and Shahidi (2007), who found that the ability to scavenge DPPH radicals in wheat fractions was higher in bran than in other fractions such as feed flour, whole grain and flour. The same authors reported that the concentration of bioactive constituents was higher in the external layers of wheat grain; moreover, they observed that the bran fraction alone demonstrated a higher antioxidant activity than that of other milling fractions. My results showed that total polyphenols concentration is higher in the bran than in middlings, confirming this trend. In my study the addition of biochar did not affect the quality parameters of wheat cvs., not even the nutraceutical compounds of cv. Verna and the polyphenols content of sunflower seeds, also when added at the highest dose.

8. Conclusions

The results of this thesis suggest that:

- Chitin residues can be recycled as a pyrolyzed additive to gardening soil if the lack of nutrients other than N and P can be compensated by other means. All the other biochars or COMs based on wood-poor green wastes exhibited too high salt concentrations. Without prior treatment, it is unlikely that those materials would have the potential to be used to a higher extend as growing substrates for nursery and horticultural crops growing.
- Wood-derived biochar and composted-biochar also showed poor results for basil seedlings growth. Overall, high values of both pH and EC at high concentrations of COMs, biochar or compost resulted to be detrimental to tomato and basil growth, suggesting that much lower doses of biochar and compost for seedlings cultivation can be used foe peat substitution. Furthermore, surviving basil plants, grown with the lowest amounts of biochar (25%) and compost we applied (50% and 25%) were not able to guarantee the standard quality for basil in terms of fresh mass and leaves' color. Nevertheless, the profile of essential oils was not modified by the addition of biochar or compost into the growing substrates, although the total yield of VOCs decreased because of the lower leaves' biomass.
- My results would not support the massive use of alternative media such as charred material or composted wastes as a valid strategy to reduce the use of peat in potting substrates. Anyway, the substitution of peat with charred material even at low doses, can contribute to mitigate the climate change by promoting C sequestration and decreasing greenhouse gases. Thus, it will be essential to find appropriate formulations of biochar/peat/ and added nutrients

if one intends to follow the use of biochar as an efficient strategy for recycling organic waste from agriculture or fishery as soilless substrate with ready-to-use characteristics. The latter is a fundamental need for successful commercializing biochar based growing media.

- Considering the use of biochar as soil amendment, my results suggested that it can be used also at high doses for the cultivation of wheat and sunflower without affecting the soil chemical and physical properties, and the vegetative and qualitative parameters of crops. This application would be a positive strategy for the recycling of some agricultural wastes, previously pyrolyzed, only if they implies disposal costs. Differently, the use of biochar at high doses for soil amelioration is not a viable solution under an economic point of view. In fact, the possible positive performances on crop productivity are not enough to justify the high costs necessary for buying and applying the biochar to the soil.

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9. Manuscripts

9.1 Paper I

Recycling pyrolyzed organic waste from plant nurseries, rise production and shrimp industry as peat substitute in potting substrates

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Research article

Recycling pyrolyzed organic waste from plant nurseries, rice production and shrimp industry as peat substitute in potting substrates

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A B S T R A C T

Organic waste from greens of tomato plants, gardening substrate, rice husks and shrimp-derived chitin were pyrolyzed at 400 °C and 500 °C for 3 h, with the aim to elucidate the feasibility of using such products as replacement of peat in soilless gardening substrates. Characterization of the carbonized organic matter (COM) and the gardening substrate indicated that neither the peat nor the COMs provided the recommended levels of nutrients for the cultivation of tomato plants, although improvements could be obtained using COM/substrate mixtures. The toxicity thresholds for Zn were exceeded significantly by the COMs of the tomato greens and high boron levels were found for all the COMs except for those derived from chitin. In a 40-days pot experiment, germination and development of tomato seeds and plants (*Solanum lycopersicum* L.) were tested on COM/peat mixtures at 30%, 60% and 100% COM substitution rate. The lack of seed germination on the mixtures with COM from tomato greens is best explained with the high salinity of the COM. Best plant growth was obtained with COM from chitin at 60%, most likely because its high N content satisfied best the N-needs of the growing tomato plants without increasing the pH of the growing media. Moreover, an increase of water retention was evidenced for COM/substrate mixtures. Although the use of COM from chitin and rice husks showed promising results, the proposed recycling of organic waste from agriculture or fishery as soilless gardening substrate requires the development of formulations of COM/peat/and added nutrients with ready-to-use characteristics to increase its feasibility.

1. Introduction

The constant increase of organic wastes and the need to reduce the biodegradable fraction allocated in landfills (e.g. EU Directive, 1999/31/EC) still asks for the development of cost-effective and environmentally-friendly solutions (e.g. EU Directive, 2008/98/EC). During the last years, European Commission (EU) adopted an ambitious Circular Economy Package, which includes revised legislative proposals on waste to stimulate Europe's transition towards a circular economy. Accordingly, a common target of the EU is recycling 65% of municipal waste by 2030 (https://ec.europa.eu/environment/waste/target_review.htm). A considerable amount of organic waste derived from agriculture, horticulture or fishing industry is already recycled by composting. Its thermal transformation under pyrolytic conditions (low oxygen or anoxic conditions) into biochar useable as soil amendment has been suggested as an additional approach (Lehmann, 2007; Sohi et al., 2010). However, the cost efficiency of this alternative is still debated (Bach et al., 2016; Lehmann, 2007; Pratt and Moran, 2010).

The high relative value of horticultural crops may provide more economic incentive to apply such materials for the production of soilless substrates used in horticulture and home gardening. Soil-free substrates, composed of a mixture of inorganic and organic components, are the standards for modern greenhouse and nursery manufacturing (Bilderback et al., 2005). Currently, peat represents the most frequently used organic component for growing media formulation, since it guarantees some characteristics such as high porosity, low bulk density and high nutrient retention. However, the use of this material is increasingly questioned since peat drainage and extraction has a large

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impact on wetland ecosystems, one of the most fragile ecosystems of the planet and an unique source of ecosystem services (Clarke and Rieley, 2019). Although covering just 3% of the world's surface, they contain almost a third of all the terrestrial organic carbon (C) which is equivalent to approximately two-third of the entire atmospheric C pool and twice the amount of C found in the entire world's forest biomass (Dunn and Freeman, 2011). Thus, peatlands are recognized as important C sinks, but as soon as peatland is drained its organic matter (OM) decomposes rapidly and turns into a source of greenhouse gases (Barkham, 1993. Therefore, in many European countries, the policy is to decrease the use of peat in potting substrates to protect peat bogs as natural areas (Raeymaekers, 2000). As a consequence, alternative growing media in nurseries are urgently needed.

Biochars have been recently suggested as candidate for peat substitution due to some similarities with peat, such as high porosity, low density and high cation-exchange capacity (Kern et al., 2017; Steiner and Harttung, 2014; Vaughn et al., 2015). Note that according to the European Biochar Certificate (EBC, 2019), carbonized organic matter (COM) has to fulfill certain characteristics to be called biochar. For example, COM containing <50% organic carbon (C_{org}) and with atomic H/C_{org} and O/C_{org} ratios below 0.7 and 0.4, respectively, should not be called biochar. Various works demonstrated that the properties of biochar depend on the used feedstock and the applied production conditions and can vary strongly (de la Rosa et al., 2014; Spokas et al., 2012). Whereas many different biochars have already been tested as amendment for mineral soils, although with contrasting results (Spokas et al., 2012), there are just a few studies on the utilization of biochar in soilless substrates (Bachmann et al., 2018; Banitalebi et al., 2019; Nieto et al., 2016; Steiner and Harttung, 2014; Vaughn et al., 2013). On the other hand, before such materials can be applied in horticulture, their impact on plant growth must be evaluated. Only if their performance as substrate is at least comparable to peat, this strategy is feasible.

Therefore, with the present research, we intended to test the potential of selected COMs from different feedstocks to partially or fully substitute peat for growing tomato plants in a nursery trial. We tested chars derived from chitin, rice husk, greens of tomato plants and peat, which were processed at two different temperatures, 400 °C and 500 °C, for 3 h. The feedstocks were chosen according to certain characteristics related to their chemical composition, and comprised organic waste typically occurring in Mediterranean countries. Chitin is a residue of the crustacean industry representing an important economic sector in particular in Spain, being the most important fishery country of the EU. In 2017, Spain produced approximately 11 × 10^4 t of canned and frozen crustaceans together with 18 × 10^5 t of organic waste only in the fishery sector (USDA Foreign Agriculture Service, 2017). In contrast to woody plant derived feedstocks, it is rich in nitrogen similarly to other feedstocks such as sewage sludge (Paneque et al., 2017), casein and grass residues (De la Rosa and Knicker, 2011; Knicker, 2010) and it is expected to result in a N-rich char. Such products were shown to be an advantage with respect to N fertilization of plants (Leiva-Suárez et al., 2020; Paneque et al., 2020). Thermal treatment of rice husks results in a mineral and silica rich product with the capability to increase levels of exchangeable potassium (K) and magnesium (Mg) (Varela Milla et al., 2013). Rice husks account for approximately 20–25% of the rice's weight and is commonly burned for energy generation and used animal bedding. Worldwide, it was estimated that approximately 782 × 10^6 t of rice were produced in 2018 (FAO, 2019a). For Spain, this number amounted to 808 × 10^3 t (FAO, 2019b).

Greens of tomato plants and used peat substrate accumulate as waste in nurseries. In Europe 23 × 10^6 t of tomatoes were produced in 2018 (FAO, 2019b). Italy and Spain together account for 70% of the tomato production in the European Union. Thus, the establishment of an added-value chain for tomato greens recycling could not only reduce the need of peat but also the space occupied for its storage and composting.

In the present study we evaluated the feasible of recycling those common organic wastes as peat substitute. In order to test if used peat substrate could also be recycled, we included its COM in the study. We hypothesized that aside of the concentration of the COM in the growing substrate, the different compositions of the feedstocks result in COMs with varying nutrient contents and properties, which may affect seed germination and plant growth. We tested this hypothesis by preparing peat/COM mixtures with three different substitution rates (100, 60 and 30%) for a plant nursery trial of 40 days. We used *Solanum lycopersicon* L., since tomato plants represents one of the most cultivated horticultural species in the Mediterranean area and a big economic resource for some regions. We measured height, fresh and dry weight of plants and analyzed the growing substrates for chemical and physical properties.

2. Materials and methods

2.1. Production of carbonized organic matter (COM) and reference peat substrate (Pe)

In order to avoid misleading and complications with respect to nomenclature and the fact that our pyrolyzed products were not analyzed for their atomic O/C_{org} ratios as required for the European Biochar Certificate (EBC, 2019) and the International Biochar Initiative (IBI, 2015), we decided to summarize them as COM. For the present study, rice husks (Ri) (particle size of approx. 0.5 cm) (ARROZÚA S.C.A, Sevilla, Spain), chitin from shrimps' shells (Ch) (particle size: approx. 0.5–1 cm) (Sigma, CAS Number 1398-61-4, practical grade, coarse flakes), gardening peat (Pe) (Floradur, Oldenburg, Germany) and dried pellets (particle size < 1 cm) of green waste of tomato plants (To) derived from a private garden were pyrolyzed at 400 °C and 500 °C, using a closed custom made stainless steel reactor. The reactor was filled with the feedstocks to about 2/3 of its volume, subsequently flashed with N_2 to remove air and put into a preheated muffler (Hobersal, Caldes de Montbui, Spain) for 3 h. Syngas, produced during the pyrolysis was allowed to leave the reactor through a stainless steel tube reaching from the reactor to the outside of the muffler and connected to a gas-trap filled with oil. At the end of the process the carbonized substrates, the muffler and the reactor had to cool down to room temperature, before yielding COM. The nomenclature for the COMs include the abbreviation of the feedstock and the pyrolysis temperature (Ri400, Ri500, Ch400, Ch500, Pe400, Pe500, To400, To500) and is listed in Table 1. As reference material the peat-based gardening soil was used.

2.2. Chemical and physical characterization of the produced biochars

The dried peat and COMs were finely ground before analyses. Their ash contents were measured in aliquots of 0.3 g after heating at 750 °C

Table 1
Mass loss of the feedstocks during pyrolysis, ash content, pH (H_2O), and electrical conductivity of COMs (n = 3). Values in the same column followed by the same letter indicate no significant differences at the $P = 0.05$ level.

Sample	Feedstock	Temperature (°C)	Mass loss (%)	Ash content (g kg^{-1})	pH	EC (mS cm^{-1})
Ch400	Chitin	400	68.2 b	13 e	6.7 e	0.2
Ch500	Chitin	500	72.3 a	18 d	7.6 d	0.1
Pe400	Peat	400	47.1 f	247 b	8.8 c	1.5
Pe500	Peat	500	53.6 e	204 b	8.7 c	0.6
Ri400	Rice husk	400	57.9 d	313 a	9.4 bc	0.3
Ri500	Rice husk	500	62.9 c	363 a	10.5 a	0.8
To400	Tomato plants	400	57.0 d	302 a	10.6 a	12.5
To500	Tomato plants	500	63.4 c	330 a	10.0 ab	16.6
Peat	Peat (Control)	–	–	159 c	6.1 f	1.2

for 5 h. The pH (H_2O) of biochars and peat was obtained from a suspension in distilled water (1:5) with a Crison pH-meter Basic 20, using the method described by Jackson and Beltrán-Martínez (1982) originally set up for soils, and modified by Paneque et al. (2016) for carbonized material. To determine the water holding capacity (WHC), 6 g of each sample were given on a Whatman 2 filter placed into a funnel, and saturated with distilled water. For 2 h the water was allowed to percolate through the filter and the funnel, then the weight of the moist samples was measured. The weight difference between dry and moist sample was extrapolated for a duration of the experiment of 12 h according to de la Rosa et al. (2014). Its percentage relative to the dry weight of the sample resulted in the value for the maximum WHC. The hydrophobicity of pure materials was measured using the methods described by Doerr (1998). In brief, the water drop penetration time (WDPT) and the molarity of an ethanol droplet test (MED) were applied. Five drops of distilled water were placed on the sample surface at a distance sufficient to avoid interferences between the drops. The penetration time of each drop was recorded separately, then the average obtained from the five drops was considered as representative of WDPT. The MED test use drops of known mixtures of water:ethanol placed on the sample surface measuring their infiltration time (Watson et al., 1971). The latter increases with increasing surface tension of the drops that increases with the concentration of ethanol. Therefore, decreasing ethanol concentration until a drop resists to infiltration allows the classification of the samples into surface tension categories limited by two adjacent ethanol concentrations, assigning 1 to very hydrophilic and 7 to extremely hydrophobic. For the present study, we use the following ethanol concentrations: 0, 3, 5, 8.5, 13, 24 and 36% v:v.

The elemental composition of all the substrates was analyzed at the beginning and at the end of our experiment using an elemental analyzer LECO TruSpec CHNS Micro hosted at CITIUS (Centro de Investigación, Tecnología e Innovación de la Universidad de Sevilla, Spain). The available phosphorus ($H_2PO_4^-$ and HPO_4^{2-}) was measured following Olsen's method (Murphy and Riley, 1962). Briefly, 50 ml of a 0.5 M solution of $NaHCO_3$ were mixed with 2.5 g of sample for 30 min and extracted after centrifugation and filtration with Whatman 2 filters to determine the concentration of available P in the extracts with a multiparameter analyzer Bran-Luebbe at the Instituto de Recursos Naturales y Agrobiología de Sevilla - Consejo Superior de Investigacion Científicas (IRNAS-CSIC). Available inorganic nitrogen in the samples (NH_4^+ and NO_3^-), was quantified after extraction with KCl 1M (Navarro and Navarro, 2013) Data are given as the average of two extraction duplicates. Macro- and micro-nutrients (B, Ca, Cu, Fe, K, Mg, Mn, Na, S, P, As, Ba, Cd, Co, Cr, Sr, Ni, Pb and Zn) were measured in duplicates from the extracts obtained after controlled acidic digestion with ultrapure nitric acid of the samples in a DigiPREP Block Digestion Systems (SCP Science) using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

2.3. Pot experiment

For the greenhouse experiment, COMs were mixed with peat-containing gardening soil (Floradur, Oldenburg, Germany) in the ratios 60/40 and 30/70 and filled into plastic beakers (50 ml volume) which were perforated at the bottom to allow leaching of surplus water. In addition, pots were prepared with pure COMs and pure gardening soil. Each mixture was prepared in triplicate giving a total number of 75 pots (4-treatments × 2-pyrolysys temperature × 3-mixtures × 3-replicates, plus the three potting soil controls). In each pot five certified seeds of *Solanum lycopersicum* L. var. "Roma" seeds (HA Huerto y Jardín, Basuri, Spain) were directly planted onto the substrate. The pots were placed in a greenhouse at 25 °C and 14 h light day^{-1}, utilizing both natural and artificial light during daylight. Pots were spaced 10 cm apart in a completely randomized distribution to avoid variability due to the position in the greenhouse.

The pots were initially irrigated with a known amount of distilled water until the substrate reached a moisture of 50% of its WHC. Every two days, the substrate moisture was restored by replacing evaporated water determined by weight difference. No fertilizers were applied. The germination rate and plant height were measured every two days during the entire experiment. One week after sowing, only the dominant plant was left in each pot. After 40 days, plants were harvested by cutting the above ground biomass and immediately weighted, then dried at 60 °C for 72 h to measure the dry weight biomass.

2.4. Statistical design

Data normality was checked with a Shapiro-Wilks test, variables presenting a non-normal distribution were log transformed before further tests. An analysis of variance (ANOVA) was performed to compare the different materials - four feedstocks and two different pyrolysis temperatures, plus the peat control for a total of nine treatments. A unique classification variable was adopted summarizing the three classification factors (feedstocks-pyrolysis temperature-mixture percentage) used for the calculation of ANOVA for germination and plant development, for a total of 27 treatments. Post hoc multiple pairwise comparisons were carried out using a Tukey's HSD (honestly significant difference) test at a $P = 0.05$ significance level. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

3. Results and discussion

3.1. Chemical characteristics of the COMs

Mass losses increased with pyrolysis temperature for all COMs, whereas for the ash contents an increase was only significant for the COMs of chitin (Table 1). These results are comparable with literature reports and are due to the thermal degradation of organic compounds (Agrafioti et al., 2013). With the exception of Ch400, all COMs showed alkaline pH values. Increasing the pyrolysis temperature from 400 to 500 °C had no major impact on the COMs from Pe and To although the latter revealed a considerably high EC at higher temperature. Note that high pH and EC of COMs are common indicators of salinity, which can have a detrimental effect on plant growth (Prasad et al., 2019; Solaiman et al., 2012). Most crops grow properly in soils with EC values between 100 and 1000 $\mu S\ cm^{-1}$. On the other hand, soils with more than 2000 $\mu S\ cm^{-1}$ are considered saline (Soil Survey Manual, 2017). This is the case for COM from To (Table 1).

In line with Angin (2013), higher pyrolysis temperature led to higher C concentrations in all COMs (Table 2). Chitin COM showed the highest C concentration followed by COMs made from peat, rice husk and tomato. Note that only To400 revealed C contents below 50%, thus all the others contain sufficient C to be named biochar according to the requirements of the EBC (EBC, 2019). In contrast to the COMs from the vegetal residues, Ch400 and Ch500 are characterized by very high N contents of 9% of the dry weight. This is related to the fact that chitin is a polymer of *N*-acetylglucosamine in which N accounts for at least 6% of the molar mass, a considerable fraction of which was incorporated into the organic network of the COM. Earlier solid-state ^{15}N nuclear magnetic resonance spectroscopic studies on pyrolyzed N-rich organic matter and proteins identified the converted structures as pyrrole-type units (Knicker, 2010). Such domains are also formed by the heat-induced cyclization of *N*-acetylglucosamine as it was evidenced by solid-state ^{15}N NMR spectroscopy of chitin COM, produced at 400 and 500 °C °C for 4 h (unpublished result) (Fig. S1).

Atomic carbon to nitrogen (C/N_{atm}) ratios of the COMs vary between 10 and 140, with chitin and tomato plant chars having the lowest and rice husk chars the highest values (Fig. 1). The control peat has a ratio of 70.

The plant- and peat-derived COMs accumulated very low amounts of inorganic N (N_i) of less than 9 mg kg^{-1} (Table 2) which may lead to a N deficiency if only COM is used as growing substrate. Only Ch400 had a

Table 2
Pre- and post-nursery experiment values of total nitrogen, carbon and phosphorus for peat and COMs, inorganic nitrogen (N_i) and Olsen-P for peat and COMs. Optimum values for tomato plants are shown as reported by Sainju et al. (2003). Values in the same column followed by the same letter indicate no significant differences at the $P < 0.05$ level.

	Total carbon (g kg^{-1})		Total nitrogen (g kg^{-1})		Total phosphorus (g kg^{-1})		N_i (mg kg^{-1})		Olsen-P (mg kg^{-1})	
Samples	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.	pre exp.	post exp.
Optimum							50–100		60–70	
Ch400	721 ± 3 b	713 ± 2 b	90 ± 0 a	81 ± 0 a	1.5	1.4	151	91.2	0	0
Ch500	759 ± 5 a	767 ± 2 a	85 ± 1 b	79 ± 0 a	1.7	1.7	7	8.5	0	0
Pe400	651 ± 6 d	593 ± 9 d	14 ± 0 d	10 ± 0 d	1.2	1.3	1.1	1.3	98	94
Pe500	684 ± 3 c	642 ± 10 c	14 ± 0 d	10 ± 0 d	1.4	1.6	1	2.1	76	26
Ri400	525 ± 4 f	506 ± 0 f	7 ± 0 e	5 ± 0 e	1.5	1.2	1.6	1.2	89	263
Ri500	548 ± 4 e	536 ± 15 e	6 ± 0 e	4 ± 0 e	1.1	1.3	0.9	2.4	144	278
To400	486 ± 4 g	449 ± 9 g	24 ± 0 c	22 ± 0 b	12.3	12.2	7.5	1.5	1250	1480
To500	513 ± 10 f	479 ± 8 f	24 ± 0 c	19 ± 0 c	14.3	12.7	2.7	6.6	1510	1550
Peat	437 ± 3 h	499 ± 6 f	7 ± 0 e	9 ± 0 d	0.6	0.6	527.7	400.0	167	173

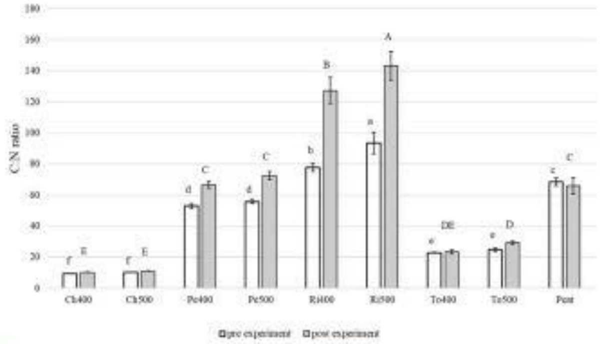

Fig. 1. Atomic carbon:nitrogen ratio of COMs before and after serving as plant growing substrate for 40 days. Values are means of three replicates. Column followed by the same letter indicate no significant differences at the $P < 0.05$ level. Lowercase letters were used for pre-experiment values, capital letters for post experiment values.

N_i content (151 mg kg^{-1}) which is above the optimum for growing tomato plants according to Sainju et al. (2003). Increasing the pyrolysis temperature, on the other hand, considerably reduced the content of plant-available N in Ch500 either by volatilization or by its incorporation into heteroaromatic structures (Torres-Rojas et al., 2020). The high N_i content of the commercial peat used in our experiment is best explained by the use of additives during its production.

Whereas the chitin derived COMs contain no detectable concentrations of Olsen-P, all the other COMs revealed levels which are above those recommended for plant tomato cultivation, i.e. 60–70 mg kg^{-1} according to Sainju et al. (2003). Exceptionally high total P contents and Olsen-P were measured for To400 and To500.

3.2. Contents of heavy metal, macro and micro nutrients of the COMs and the peat

Our analysis showed that the concentrations of As, Cr, Cu, Ni and Pb in all COMs are of no concern since they are below or close to the minimum limit values of potentially toxic elements (PTE_{min}) for composts from source separation in Europe (ENV.A.2./ETU/2001/0024, Annex 2) (Table S1). The concentrations of Cd in the COMs range between PTE_{min} of 0.7 mg kg^{-1} and the maximum limit value (PTE_{max}) of 3 mg kg^{-1}. Zn concentrations of the COMs are below the PTE_{min} (75 mg kg^{-1}), with the exception of the COMs from tomato greens that reached, the concentrations of 227 and 256 mg kg^{-1}, still below PTE_{max} for this element (1500 mg kg^{-1}).

According to Sainju et al. (2003), tomato plants require at least twelve nutrients for normal growth: N, P, K, Ca, Mg, S, B, Fe, Mn, Cu, Zn, and Mo.

As it can be seen in Table 3, neither the pure COMs, nor the peat showed the optimum concentrations of all the macro and micronutrients for tomato growing. Tomato plant-derived COMs contained the highest concentration of micro- and macro-elements, whereas peat with chitin-derived chars exhibited the lowest amount of elements with exceptions for Fe and Mn. As reported by Vijayarengan and Mahalakshmi (2013) up to 100 mg kg^{-1} level of Zn in the soil is beneficial for tomato plants growth, whereas levels >150 mg kg^{-1} are toxic. This threshold is passed by the chars of the tomato greens. Boron is a further element which can be toxic and limit plant growth. Alpaslan and Gunes (2001) observed that on tomatoes, toxicity symptoms appear at concentrations of B of 5 mg kg^{-1} and increase with high salinity. Note that this threshold is passed by all COMs, except those derived from chitin and peat. As reported by Rhoads et al. (1989) tomato plants growth is reduced with soil Cu levels >150 mg kg^{-1}. On the other hand, in soils with pH < 6.5. Cu levels >330 mg kg^{-1} are necessary to observe reduction in plants growth. None of the tested substrates in our study contain toxic levels of Cu for tomato seedlings. Based on the analysis of the nutrients in the COMs, one can conclude that none of them and not even the peat would represent a good substrate which optimally supplies all nutrients. A comparable observation is reported by Banitalebi et al. (2019). Some of the nutrients occur even at levels that may negatively affect plant growth. However, dilution of those by mixing it with peat substrate (Prasad et al., 2019) or washing and desalting may overcome those possible negative impact.

3.3. Physical characteristics of COMs and substrates

Hydrophobicity of the COMs varied considerably (Table 4). The pure peat was extremely hydrophobic, which is – aside others – due to the fact that the substrate was dried before the test to ensure equal starting conditions for all materials. At low moisture, peat turns highly hydrophobic, indeed (Perdana et al., 2018). A decrease of hydrophobicity was observed when the peat was pyrolyzed. Increase of the pyrolysis temperatures lowered the hydrophobicity for all the studied materials, although it is well known that charring enhances the aromaticity commonly associated with higher hydrophobicity. Marshall et al. (2019) suggested that in biochars produced under low pyrolysis temperatures (≤400 °C) tars containing aliphatic and aromatic compounds contribute to augmented hydrophobicity. This effect can be supported by the clogging of the pores by the tars (Das and Sarmah, 2015). However, with the pyrolyzer setup and the pyrolysis temperatures used in our study, tars volatilize and the produced syngas can escape from the reactor. On the other hand, it is assumed that porosity of biochars also increases with increasing pyrolysis temperature and this may have enhanced the water absorption capacity of COM per surface unit (Gray et al., 2014).

As concern the other COMs, those made from chitin, together with tomato plants and rice husk pyrolyzed at 500 °C were very hydrophilic, since a complete absorption of water without addition of alcohol was obtained within a few seconds. These results are in accordance with the

Table 3
Concentration of micro- and macro-elements (mg kg^{-1}) of COMs and peat. Optimum and toxic values for tomato plants are shown as reported by Sainju et al. (2003).

Sample	B (mg kg^{-1})	Ca (g kg^{-1})	Cu (mg kg^{-1})	Fe (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Mn (mg kg^{-1})	Na (g kg^{-1})	S (g kg^{-1})	Zn (mg kg^{-1})
Optimum	1.5–2.5	1			0.6–0.7	0.4–0.7	5–20			
Toxic	>5						>80			>150
Ch400	0.6	3.7	0.2	0.1	0.1	0.1	2	0.3	0.0	6
Ch500	0.6	4.0	0.2	0.1	0.1	0.1	3	0.3	0.0	8
Pe400	11.3	42.8	21.3	1.0	3.2	3.0	179	0.4	2.4	30
Pe500	11.0	47.9	25.9	1.0	3.7	3.3	182	0.5	2.5	32
Ri400	14.4	1.9	4.2	0.3	5.8	1.4	142	0.5	0.40	34
Ri500	12.9	1.8	3.1	0.4	5.7	0.8	98	0.6	0.20	22
To400	89.2	98.70	28.7	1.0	63.6	11.4	176	4.0	26.4	226
To500	93.2	95.4	25.7	1.0	102.8	12.5	140	4.9	23.1	254
Peat	4.9	21.7	53.9	3.8	1.5	1.5	439	0.2	2.4	16

Table 4
Assignment of carbonized organic matter (COM) produced at 400 °C and 500 °C and derived from peat (Pe400, Pe500) rice husks (Ri400, Ri500), tomato plants (To400, To500) and chitin (Ch400, Ch500) to categories of hydrophobicity according to the water drop penetration time (WDPT) and molarity of ethanol drop test (MED).

Class	Descriptive label	Samples
7	Extremely hydrophobic	Peat
6	Very strongly hydrophobic	
5	Strongly hydrophobic	Pe400
4	Moderately hydrophobic	Pe500; Ri400; To400
3	Slightly hydrophobic	
2	Hydrophilic	
1	Very hydrophilic	Ch400; Ch500; Ri500; To500

observations reported by Kinney et al. (2012).

In line with its extreme hydrophobicity, the pure peat showed a low WHC, whereas all pyrolyzed substrates revealed enhanced WHC except Pe400 (Fig. 2). Values of WHC are two to three folds higher for vegetal-derived biochars (rice husk, and tomato plants) than for peat, and up to four times higher for the chitin-derived COMs. Although the differences are not statistically significant due to data dispersion, higher pyrolysis temperature seems to increase WHC for the vegetal-derived biochars, whereas an opposite trend is observed for biochars from chitin. Comparable results were previously reported by Marshall et al. (2019). Whereas pyrolysis at 400 °C had no impact on the WHC of peat, higher pyrolysis temperature went along with higher water retention. In this context, one has also to consider that particle size distribution can have a considerable impact on water retention in the studied substrates. Although not measured in more detail, visual inspection suggested that COM from tomato greens had the biggest particles followed by that of rice husk, peat and chitin. According to these results, it may be concluded that addition of biochar to a soilless substrate could help to avoid increasing hydrophobicity once the peat substrate turns dry. As a consequence, it may allow a faster rewetting of the growing medium and concomitantly improves the water retention of the mixture. Other authors observed that biochar addition increased the air space, the total porosity and the WHC of the substrate (Banitalebi et al., 2019; Méndez et al., 2015; Nemati et al., 2015). However, COMs at 30% mixture with peat did not improve significantly the WHC of the growing substrate (Fig. 2c). Mixing peat with pyrolyzed rice husk or tomato plants (To400) at low doses even reduced the water retention, possibly because the added material offered a preferential flow situation. Most likely, the high hydrophobicity of the peat and preferential flow counteracted against the capacity of the COMs to absorb water. Increasing the contribution of COM to 60% of the dry weight tended to increase the WHC of the mixtures (Fig. 2b), although this increase was not statistically significant.

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3.4. Greenhouse experiment

All treatments, with the exception of pure COMs Ch400 and Ri400, showed very high standard deviations with respect to germination rate (Fig. 3). In the substrates with tomato plant COM, no germination of seeds occurred, not even in mixtures with low COM contribution. First germinated seeds were observed after 5 days for the treatments with 100% char with the exception of tomato chars, Ri400 and control peat. Pairwise comparisons highlighted significant differences between different substrates for germination rate as well as for plant height and biomass dry weight (Figs. 3 and 4). The highest germination rate was obtained for Ch400 100%, whereas the same COM showed lower germination for the 30% mixture. The germination rate performance for rice husks derived COM was worse than for peat- or chitin-derived COMs.

In general, increasing pyrolysis temperature during COMs production and higher peat concentrations tended to have a negative effect on seed germination; even peat control pots yielded in a very low germination rate (33%) with high data dispersion. Most likely, this behavior is related to the dry initial condition of the peat and its hydrophobicity, as confirmed by the positive Spearman correlation coefficients (Table S2) obtained between WHC and germination ($\rho = 0.311$, $P = 0.01$) or total added water and germination ($\rho = 0.608$, $P = 0.01$). Therefore, although the dry peat substrate was irrigated with water after the setup of the experiment, its high hydrophobicity may have prevented an adequate imbibition of tomato seeds, which is a very important step for an adequate germination. For tomato seeds this phase occurs during the first 12 h and it is followed by a second phase characterized by an increment of the water uptake (Liu et al., 1993). Inhomogeneous and insufficient water distribution caused by the peat hydrophobicity are in line with the general tendency of decreasing germination rate with increasing portion of peat in the growing medium.

With respect to the substrates containing tomato plant COMs, the lack of germination may have been caused by the high salinity and high amount of micro-nutrients such as B and Zn above their toxicity levels. Indeed, the very alkaline pH of many COMs is often stated as one of the main limiting factor for the use of COMs in potting substrates since it can inhibit seed germination (Horne et al., 1996; Pierce et al., 1999; Solaiman et al., 2012). Huang and Gu (2019) suggested that without the addition of acidic compounds, high substitution rates of COMs to growing media cannot be feasible. Nonetheless, in our experiments, the pure Pe and Ri COMs allowed germination rates as high as 80% despite alkaline pH-values. Thus, high pH-values might not have been the single responsible for our observations. For instance, although high levels of Olsen-P we found in the COMs from tomato plants are not considered as

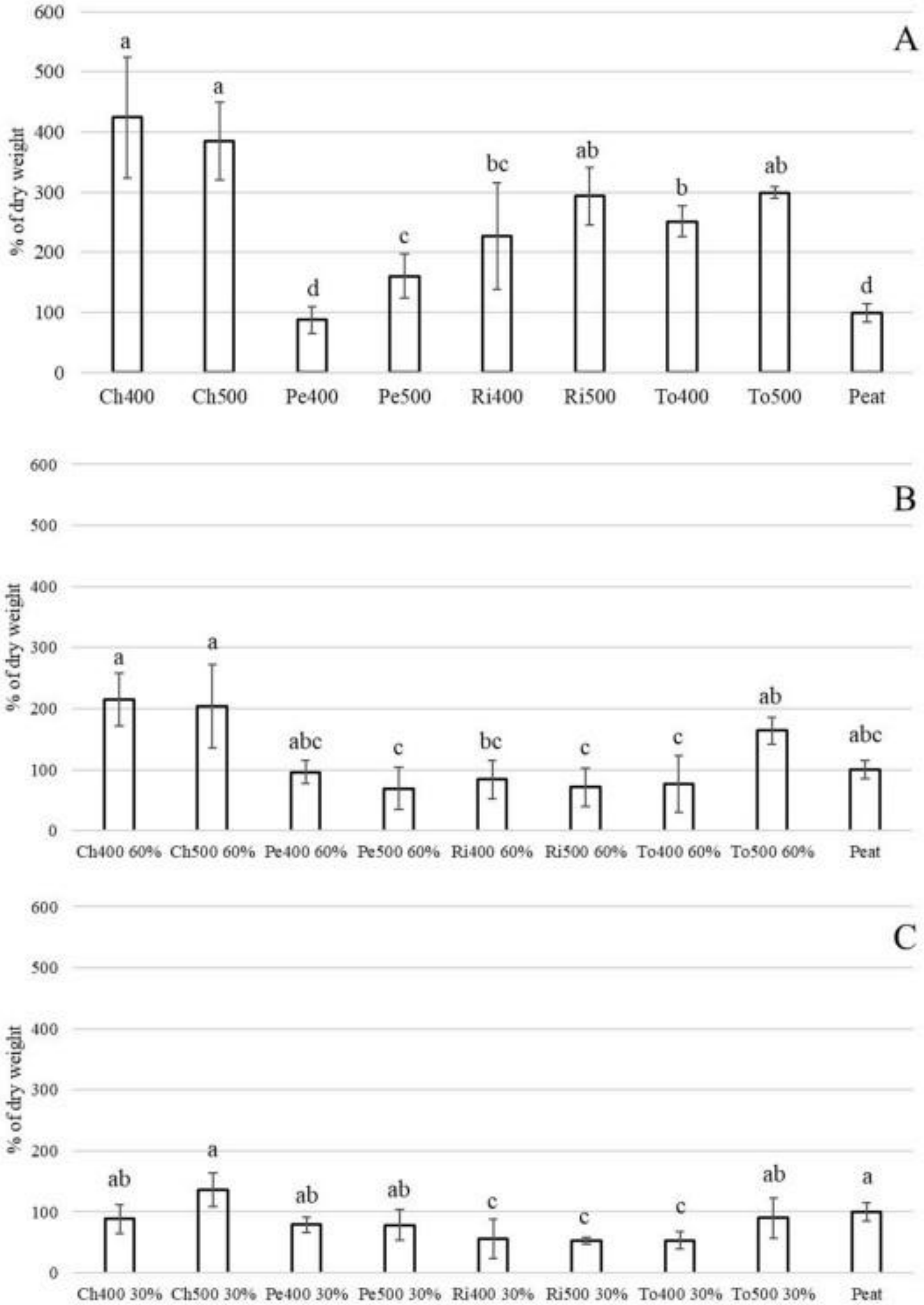

Fig. 2. Water holding capacity (WHC) of A) peat and the products pyrolyzed at 400 °C and 500 °C of chitin (Ch400, Ch500), peat (Pe400, Pe500), rice husks (Ri400, Ri500) and dried tomato plants (To400, To500), the mixtures with (B) 60% and C) 30% carbonized organic material (COM). Values are means of three replicates; error bars represent the standard deviations. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters.

toxic, together with a high pH they may reduce the availability of others micronutrients.

As reported by Gascó et al. (2016) a possible biochar phytotoxicity on seed germination represents a complex issue and depends on the type of biochar but also on the type of seeds. In their work, the same biochars which were proven to be toxic and inhibited germination of some plant species, promoted germination on others, compared to standard peat control. Other studies reported decreasing of both germination and survival rates of sunflowers with addition of pyrochar from sewage sludge at high concentration (Paneque et al., 2016), possibly due to the production of pyrogenic phytotoxic compounds. Graber et al. (2010) suggest that biochars with chemicals that are proven to be phytotoxic at high concentration may stimulate plants growth at low doses.

However, this was not tested in our study. Here, the negative impact on germination is better explained by the high salinity and the presence of high levels of B and Zn in the COMs, since Spearman correlation

	Ch400	Ch500	Ri400	Ri500	To400	To500	Pe400	Pe500	Peat control
100%	h	def	fg	-	-	-	gh	fg	cde
60%	ab	a	def	ef	-	-	cde	-	-
30%	c	b	-	-	-	-	cd	-	-

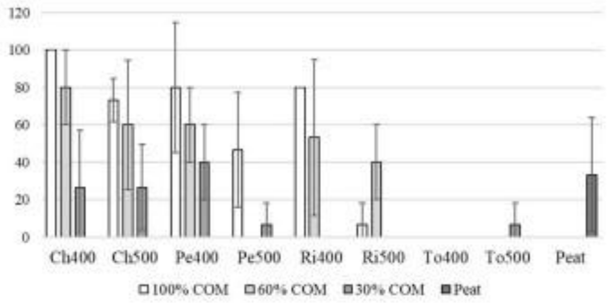

Fig. 3. Germination rates (%) of planted tomato seeds growing on pure peat substrate and on its mixtures with 30% and 60% COM produced at two temperatures (400 and 500 °C) of chitin (Ch), peat (Pe), rice husks (Ri) and tomato plant waste (To), control peat (Peat), and the pure COM (100%) 20 days after sowing. Values are means of three replicates; error bars represent the standard deviations calculated. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters in the table below the figure.

coefficients indicated negative correlation between germination rate and contents of Na ($\rho = -0.667, P = 0.05$), K ($\rho = -0.702, P = 0.05$) and B ($\rho = -0.745$, $P = 0.05$) in the COMs (Table S3). Although biochar salinity does not represent a problem for its use as soil amendment (Gascó et al., 2016; Paneque et al., 2016), the concentration of COMs derived cations is less diluted in a pot experiment than in a large volume of field soil.

The impact of COM on plant growth was followed by focusing on the development of the strongest plant per pot, which was allowed to continue growing for 40 days after germination whereas the other plants were removed from the pots. The substrates with significantly better performance than the control were Ch500 60%, Ch500 30% and Ch400 60% (Fig. 4). In line with this, considerably less dry biomass was yielded with all other materials and mixtures. Increasing the peat concentration of the potting media with the Pe and Ri-derived COMs enhanced the plant height to values comparable to the pure peat experiment but could not compensate the lower dry plant biomass production.

Different authors stated that small addition of COMs in soilless substrates seems to increase plant height and biomass of tomato (Altland et al., 2015; Altland and Locke, 2012; Graber et al., 2010; Massa et al., 2019; Vaughn et al., 2013). Thus, it may be concluded that in our experiments the rate of peat substitution by Pe, Ri and To derived COMs were still too high for positively affecting plant biomass production (Fig. 4B). However, statistical analysis indicated that contents of B, K and Na are negatively and significantly correlated not only with germination but also with plants growing after germination (Table S3). Indeed, with the exception of COM from chitin, all COMs contain B concentrations above the toxicity threshold (Table 3). In particular for the COM derived from tomato greens, the high pHs (Table 1) may also have contributed to our results, since they are significantly associated with low plant growth (Pierce et al., 1999; Prasad et al., 2019).

In contrast, the substrates mixed with Ch500 and Ch400 at proportions of 60% and 30% exhibit a statistically significant higher growth compared to the control peat, both with respect to the height of the seedlings and biomass production. As depicted in Fig. 4, best results were obtained with a peat/COM mixture of 60/40. The high N content of the chitin-derived COMs may have contributed to the improved performance of the plants with respect to the other COMs. Indeed, the considerable lowering of the N_i contents in the Ch400 treatment with prolonged experiment time would support this suggestion (Table 2). Although the C or N content of the other COMs showed marginal changes during plant growth, an increase of their C/N was observed, which may also be related to N uptake by the plants (Fig. 1, Table 2). Such an effect was not evidenced for the Olsen-P. However, no statistically significant correlation between plant performance and N or P-contents of COMs and COM/peat mixtures was obtained (Table S3). Possibly, the positive effect of additional N on plant growth was masked by other COM properties and a more detailed experiment must be designed to test this hypothesis.

On the other hand, high N contents in the substrates with 100% Ch-COMs did not result in improved plant performance compared to the pure peat substrate. This may be explained by their low contents of micro nutrients or potassium. In the COM/mixtures this deficiency may not be as important since they are supplied by the peat.

3.5. *Implications for the use of COM as peat substitute*

Published studies on the effects of COMs on plant growth in soilless substrates show diverging results. According to the review paper by Huang and Gu (2019), the addition of biochars to growing medium without the use of fertilizers can have positive effects on plant growth in the majority of analyzed works (77.3%). On the other hand, 50% of the studies show a suppression of plant growth caused by addition of biochars in potting substrates because of high biochar application rates. Aside from the fact that different plants need different growing conditions and act differently with stress, COMs produced from different feedstocks and under different conditions vary with respect to their characteristics and the composition of their organic and inorganic components. Islam et al. (1980) found that tomato plants have their best growing conditions in substrates/soils with pH values ranging between 5.5 and 6.5. But increasing the pH from 5.5 to 8.5 had neither effect on tomato plants growth, nor in yields. Sainju et al. (2003) recommended pH values for tomato growth to be between 6.5 and 7.5. In our study, we identified the pH of the added COM as a key factor for the performance of tomato plant seedlings. Furthermore, considering that high pH values of the COMs correlated significantly with their B, K and Na contents, our observations may have resulted from the combination of several negative factors. Possibly, removal of cations by washing processes may not only decrease the level of potentially toxic elements but could also decrease the pH and salinity of the COMs. Such a treatment may be needed if biochar from green waste is considered as additive for potting media as our COM from tomato plants did not even allow seed germination. Another possible strategy could be the thermophilic co-composting of COMs with green waste or other composting material. This microbial process has demonstrated to activate the biochar by increasing the presence of functional reactive groups on its surface which helps to reduce the nutrient leaking from composting windrows, thus enhancing biochar agronomical value (Kammann et al., 2015).

As Sainju et al. (2003) reported, nitrogen is the most restricting element for tomato growth because tomato plants remove large amount of this element from the soil. The high need of N is certainly responsible for the better performance of plants growing on substrates mixed with

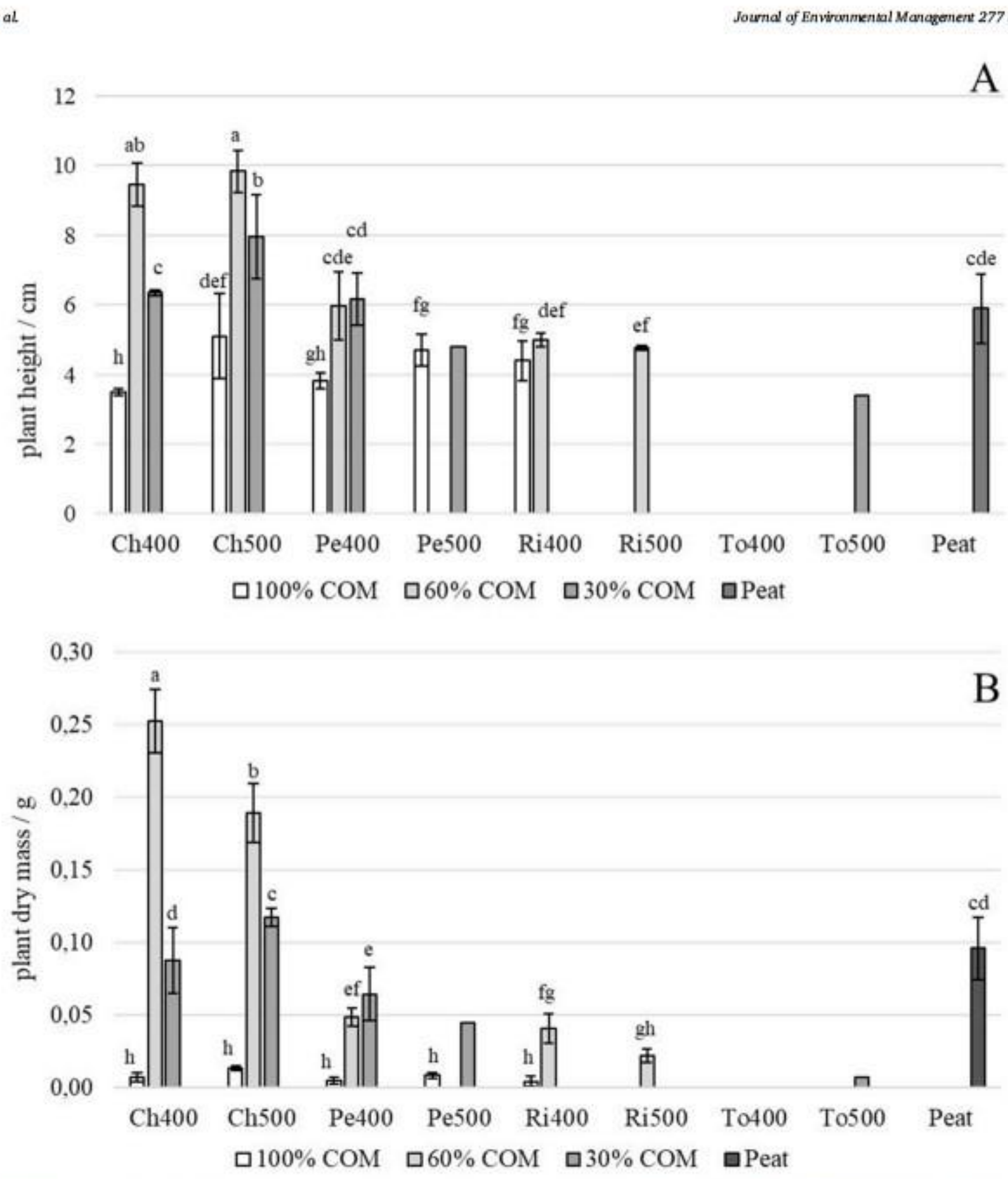

Fig. 4. Height (a) and dry weight (b) of tomato plants growing on pure peat substrate and on its mixtures with 30% and 60% COM produced at two temperatures (400 and 500 °C) of chitin (Ch), peat (Pe), rice husks (Ri) and tomato plant waste (To), control peat (Peat), and the pure COM (100%) 40 days after sowing. Values are means of three replicates; error bars represent the standard deviations calculated. Significant differences between treatments at a $P < 0.05$ level are indicated by different letters.

COM from chitin. In addition, the latter are not increasing the pH of the growing media. On the other hand, as indicated by the low production of plant biomass, using solely COM from chitin is likely to lead to a deficiency of other plant nutrients which has to be compensated either by addition of peat or the respective minerals.

4. Conclusions

Our results suggest that chitin residues can be recycled as a pyrolyzed additive to gardening soil if the lack of nutrients other than N and P can be compensated by other means. Biochars or COMs based on wood-poor green wastes are likely to exhibit comparably high salt concentrations as we observed for tomato plant residues. Without prior treatment, it is unlikely that those materials would have the potential to be used to a higher extend as growing substrates for nursery and horticultural. Thus, it will be essential to find appropriate formulations of COM/peat/and added nutrients if one intends to follow the use of COM as an efficient strategy for recycling organic waste from agriculture or fishery as soilless gardening substrate with ready-to-use characteristics. The latter is a fundamental need for successful commercializing COM-based growing media.

CRediT authorship contribution statement

Marco Nocentini: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - original draft, Visualization. **Marco Panettieri:** Methodology, Validation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing, Supervision.

José María García de Castro Barragán: Methodology, Conceptualization. **Giovanni Mastrolonardo:** Supervision, Validation, Writing - review & editing. **Heike Knicker:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111436.

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9.2 Paper II

Effects of biochar and compost addition in potting substrates on growth and volatile compounds profile of basil (*Ocimum basilicum* L.)

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Effects of biochar and compost addition in potting substrates on growth and volatile compounds profile of basil (*Ocimum basilicum* L.)

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Abstract

Peat-based growing media are the standard for nursery production of seedlings and for the cultivation of some low-size vegetables during the entire growth cycle. Despite the excellent characteristics of peat, alternative more environmental-friendly materials are needed for the near future. Many countries are in fact limiting the mining of peat to preserve as much as possible peatlands, which are one the main reservoir of carbon in terrestrial ecosystems. However, the full or partial substitution of peat must guarantee a qualitative and quantitative commercial standard without detrimental effects on the target species. This nursery research was conducted for assessing the influence of biochar and compost on yield and essential oil profile of basil, which is especially appreciated for making seasoning sauces. In a 50-days pot experiment we evaluated the performances of both pure biochar from pruning of urban trees and composted kitchen scraps, testing the growth and the volatile compounds profile of sweet basil (*Ocimum basilicum* L.) assessed via gas chromatography-mass spectrometric (GC/MS). High substitution rates of biochar (100% and 50%) and compost (100%) resulted in seedlings death after few days from transplantation. Best plant growth and color parameters were obtained with standard peats. The identified basil aroma compounds were ten different monoterpenes, one alcohol and one phenylpropanoid. Biochar and compost application did not affected basil essential oil composition, with the sole exception of cineole that showed higher concentration and relative content in plants grown in substrate substituted with biochar at 25% (v.v.). Conversely, taking into account the lower plant biomass, the total yield of essential oils decreased in biochar/compost substituted substrates.

Keywords: charcoal; composted biochar; essential oils; pot experiment; sustainability; growing media

1. Introduction

Peat-based substrates are the standard growing media in nurseries and greenhouses, and a major component of potting mixtures for commercial plant production, because of their excellent characteristics as low bulk density and high water holding capacity (WHC) and cation exchange capacity (CEC). Despite the excellent characteristics of peat, now environmental issues and economic reasons impose to reduce its use. Indeed, many European countries have agreed to limit peat mining to preserve peatlands (Fascella, 2015), which are fragile ecosystems with very important ecological and social values (Clarke and Rieley, 2019). The reduced availability on the markets resulted in increasing prices for peat (*Zulfiqar et al.*, 2019). Another important aspect that must be considered is that peat can be a vehicle for plant pathogens, for example soil-borne fungi like *Phytium*, *Fusarium* and *Rhizoctonia* (Hoitink and Kuter, 1986), which implies the applications of numerous fungicides over times for the production of seedlings. Thus, alternative potting materials are strongly desirable.

Biochar (Kern et al., 2017; Nocentini et al., 2021) and compost (Raviv, 2013) are considered as two alternative choices due to their high porosity and biochemical resistance. Moreover, the pyrolysis and composting processes, if correctly implemented, guarantee a material free of plant pathogens, thus avoiding having to resort to fungicides. Furthermore, since both materials can derive from waste, they can also be an interesting solution for a sustainable waste management (Jindo et al., 2020b). The research to ascertain the suitability of these materials as growing media has begun a few years ago, and have led to contrasting results (*e.g.* Huang & Gu, 2019; Lazcano et al., 2009; Zaller, 2007). Biochar is the by-product of the pyrolysis process (*i.e.* heating at high temperatures under low-oxygen conditions) of any type of organic matter. Biochar added as amendment in potting substrates has shown various positive characteristics such as high structural stability, porosity and water holding capacity (Jindo et al., 2020b; Kern et al., 2017). Additionally, it is able within certain limits to suppress some soil-borne pathogens (Gao et al., 2019; Jaiswal et al., 2019, 2018). Being the properties of different biochars mostly inherited from the parent feedstock and the pyrolysis process, each biochar shows different characteristics (de la Rosa, et al., 2014). As a consequence, the effects of biochar addition in substrates may vary depending on the material used and the target species, as well as the applied dose (Jindo et al., 2020b; Muter et al., 2014). Such a variability is perhaps the main reason for the contrasting results obtained so far from experiments on the subject (Bachmann et al., 2018; Huang & Gu, 2019; Nieto et al., 2016; Steiner & Harttung, 2014; Vaughn et al., 2013).

Compost is every organic material undergone a thermophilic and aerobic decomposition, a process which is precisely called "composting". Compost is already used in horticulture, and there are several studies about it as growing medium partly or fully replacing peat (Bünemann et al., 2018; Hoitink and Fahy, 1986; Mininni et al., 2015; Raviv et al., 1998). It shows appreciable characteristics such as nutrient availability, high porosity and water retention, and very low cost (Zhang et al., 2013). As for biochar, such characteristics strongly depend on parent materials, as well as on the composting process (Fascella, 2015). The percentage of compost that can be used in potting substrates must be carefully evaluated to avoid negative effects on plant growth as, for instance, those derived from a too high amount of soluble salts (Zhang et al., 2013). Compost can also be added with biochar. The addition of this latter to the composting mixture has proven to have several positive effects, such as increased aeration, reduced ammonia volatilization, and improved humification (Jindo et al., 2020b, 2016; Zhang et al., 2014). It has also been observed that biochar may increase the temperatures of the composting process, and also extend the thermophilic phase of composting, this phase having the strongest effect on the transformation of organic matter into compost (Godlewska et al., 2017).

Sweet basil (*Ocimum basilicum* L.) is an annual aromatic plant, cultivated worldwide as an appreciated culinary herb and widely used in the food processing industry. Its high content of phytochemicals with potential beneficial effects on health has further increased its demand. In fact, the essential oil extracted from basil leaves and flowers is used as a flavoring agent in foods, perfume, medicines, and cosmetic products (Maggio et al., 2016). In spite of its widespread cultivation in nursery, only few studies deal with the possibility to use biochar and/or compost in growing media for basil. The appropriateness of using biochar for this purpose is controversial, because some studies report positive results (Graber et al., 2010; Méndez et al., 2015), others negative (Steiner and Harttung, 2014). Furthermore, the type of substrate has been reported to influence many basil variables besides plant growth, such as the color and chemical composition of its tissues; however, basil has been investigated even less in this regard (Burdina and Priss, 2016; Najar et al., 2019), although its content in total phenolic compounds, carotenoids and especially essential oils, is crucial for its commercialization (Ahmed et al., 2019). Indeed, for the fresh market plants with intense green leaves are demanded, which requires paying close attention to their chlorophyll content (Makri and Kintzios, 2008). As regards the essential oil composition, sweet basil has many different classes of compounds such as mono and sesquiterpene hydrocarbons, oxygenated mono and sesquiterpenes, aliphatic alcohols, aldehydes, esters, ketones, acids, aromatic compounds. The major aroma constituents of basil are linalool, estragole, methyl cinnamate, eugenol, and 1,8-cineole (Baczek et al., 2019; Pushpangadan and George, 2012). In the Italian sweet basil the dominant compounds are eugenol, methyleugenol, eucalyptol (cineole) and linalool (Calín-Sánchez et al., 2012). Linalool and eugenol are responsible for the characteristic taste of basil, and together with cineole, have been studied for a long time due to their wide uses in food stocks and medicine (Chang et al., 2008). The essential oil of sweet basil has also demonstrated antifungal and antibacterial activity and has been proven to be effective against some plant pathogens (Carović-Stanko et al., 2010; Gaio et al., 2015; Oxenham et al., 2005).

This work aims at providing an insight into the possible use of biochar and compost as peat substitutes for the cultivation of sweet basil. For this purpose, we designed an experiment where the effects of the application of different dose of these materials to growing substrates were investigated in terms of plant growth, fresh biomass, extent foliar surface and quality parameters, such as the color of leaves, and the essential oil profile composition in terms of volatile organic compounds (VOCs).

2. Materials and methods

2.1 Substrates composition

The used biochar, which is commercialized for agricultural purposes, was produced in a syngas plant from woody residues of the pruning of city trees by the manufacturer Econsulenze SAS (Terni, Italy). Its characteristics have been declared by the producer (Table S1) and account for a class I “EBC-Feed” quality according to the European Biochar Certificate (EBC, 2012).

The compost was produced by All Power Labs – SLO Factory (Terni, Italy), using a mixture of organic wastes. In detail the compost parent material was: 25% kitchen green waste; 48% sawdust, wood flakes, wood chips; 15% exhausted coffee powder; 5% above-mentioned biochar; 1.5% forest topsoil; 0.5% cane sugar; 5% water. The mixture was prepared using an insulated tumbler rotating within a barrel, designed by the company. The composting lasted one month, checking daily temperature and humidity. Later, the compost was stored for 3 weeks, at room temperature, before being used for our experiment.

For the experiment, seven different substrates were prepared mixing biochar and compost with commercial peat (a mix of Irish and Baltic *sphagnum*, “Cuore di Terriccio”, by Vigorplant Italia SRL) in different volumetric proportions: 100% pure biochar (Char 100); 100% pure compost (Comp. 100);

50% biochar/50% peat (Char 50); 50% compost/50% peat (Comp. 50); 25% biochar/75% peat (Char 25); 25% compost/75% peat (Comp. 25); 100% peat (control) (Table 1).

The pH and electrical conductivity (EC) of pure peat, biochar, compost and their mixtures were measured in a suspension in distilled water (1:2.5) with a XS pH-meter model PC8. The bulk density was measured drying the substrates from the pots at 70 °C until constant weight and then weighting them. The growing material compression was standardized before measuring.

2.2 Experimental design

The experimental design was based on a randomized block scheme and consisted of three replicate blocks per substrate, each comprising 10 pots of 300 ml volume each (210 pots in total). The trial was performed outdoor, with pots placed on a bench equipped with a transparent PE roof. Three seedlings of basil (*Ocimum basilicum*, L. cv. Italiano) were planted in each pot, and then irrigated every two days all trial long, to 100% water holding capacity (WHC). Eighty mg of nitrogen, as nitrate (7%), ammonia (5%) and urea (8%), were applied to each pot. Three weeks after the beginning of the experiment, plants were treated with an imidacloprid-based insecticide against cutworms.

Starting from the seventh day, the following variables were measured on the most developed in height plant per pot: height, SPAD values with a leaf chlorophyll meter SPAD-502 Minolta (SPAD - Soil Plant Analysis Development), and color parameters (L*, a*, and b*) of one completely formed leave/plant with a portable colorimeter Minolta Chroma Meter CR-100. The L* parameter accounts for lightness, a* expresses values from green to red, and the b* parameter expresses values from blue to yellow, all together used to determine color differences between samples.

Fifty days after transplanting the seedlings, the plants were harvested by cutting all the aboveground biomass. The youngest four completely formed leaves of the dominant plant were collected, weighted, and stored at -80 °C for quantitative and qualitative analysis of the essentials oils. Immediately after harvesting, the total leaf area was measured by scanning all leaves of each dominant plant with a LI-COR LI-3100 Area Meter, and the fresh biomass, leaves plus stems, was weighed and then oven-dried at 105 °C to constant weight. The specific leaf area (SLA, cm^2 g^{-1}) was calculated dividing the leaf area of each plant by the leaf dry weight. The leaf dry matter content (LDMC; g dry mass g^{-1} fresh mass) was determined as the ratio between the oven-dry mass of leaves and their fresh mass, while the leaf area ratio (LAR, cm^2 g^{-1}) is the ratio between the area of the leaf lamina and the total dry plant biomass, which accounts for the size of the photosynthetic surface relative to the respiratory mass (Bressan et al., 2020).

2.3 VOCs analyses

Volatile organic compounds (VOCs) were extracted from 0.5 g of the last four completely formed leaves, that were previously stored at -80 °C. They were combined with 1 ml of heptane as the solvent and tridecane as an internal standard, vortexed for five minutes, sonicated for 15 minutes and then agitated over-night. After centrifugation at 1800 *g* for 10 minutes, the heptane phase was collected for the gas chromatography mass spectrometry (GC-MS) analysis.

The GC-MS analysis was performed with an Agilent 7820 Gas Chromatograph system equipped with a 5977E MSD with EI ionization (Agilent Tech., Palo Alto, CA, USA). One µL of heptane phase was injected in a split/splitless injector operating in splitless mode. A Gerstel MPS2 XL autosampler equipped with liquid option was used. The chromatographic settings were: injector in splitless mode set at 260 °C, J&W innovax column (30 m, 0.25 mm i.d., 0.5 µm df); oven temperature program: initial temperature 40 °C for 1 min, then 5 °C min^{-1} until 200 °C, then 10 °C min^{-1} until 220 °C, then 30 °C min^{-1} until 260 °C, hold time 3 min. The mass spectrometer was operating with an electron ionisation of 70 eV, in scan mode in the m/z range 29-330, at three scans per second.

The deconvoluted peak spectra, obtained by Agilent Masshunter software, were matched against NIST 11 spectral library for tentative identification. Kovats' retention indices were calculated for further compound confirmation and compared with those reported in literature for the chromatographic column used. The Kovats retention index of a compound is its retention time normalized to the retention times of adjacently eluting *n*- alkanes. To determine the content of each single VOC a calibration curve was built injecting known concentrations of authentic standards (Sigma) into the gas chromatograph-mass spectrometer and expressed as mg g^{-1} dry weight (d.w.). The leaf dry mass weight was determined after drying the residual plant material at 105 °C for 72 h. Relative content (proportions or percentages) of each VOC was expressed as a relative percentage of total VOCs (VOC profile), being calculated on the basis that 100% is equivalent to the sum of all 12 identified compounds.

2.4 Statistical analysis

Data underwent one-way analysis of variance (ANOVA) according to a completely randomized block design with three blocks and 30 replicates per treatment. Significant differences among means were determined using Duncan's post-hoc significance test at $p < 0.05$. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

3. Results and discussion

3.1 Chemical and physical characteristics of substrates

Biochar and compost addition significantly changed the chemical characteristics of the substrate in comparison to the pure commercial peat. In particular, the pH increased significantly (Table 1). The optimum pH for most vegetable seedlings is reported to be between 5.8 and 6.8 (Huang et al., 2019) and, with the exception of the control, none of the experimental substrates fell within this range. According to Frerichs et al. (2020) the best pH for basil is between 5.5 and 6.0, while pH above 7.0 would inhibit the growth of this species.

Table 1. Nomenclature of the potting substrate mixtures used in the experiment and their pH, electrical conductivity (EC) and bulk density. Values are means ± standard deviation of 3 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test, at the p = 0.05 level

Substrates	Composition (in volume)	pH	EC mS cm^{-1}	Bulk density g cm^{-3}
Control	100 % peat	6.4±0.0^{f}	1.65±0.04^{g}	0.20±0.00^{e}
Char 100	100 % biochar	10.3±0.2^{a}	11.88±0.02^{a}	0.31±0.01^{ab}
Char 50	50 % biochar/50 % peat	9.4±0.2^{b}	3.48±0.04^{c}	0.27±0.00^{c}
Char 25	25 % biochar/75 % peat	8.4±0.3^{c}	2.09±0.07^{f}	0.23±0.01^{d}
Comp. 100	100 % compost	8.5±0.4^{c}	5.49±0.10^{b}	0.32±0.01^{a}
Comp. 50	50 % compost/50 % peat	7.6±0.2^{d}	3.17±0.01^{d}	0.29±0.01^{b}
Comp. 25	25 % compost/75 % peat	7.1±0.1^{e}	2.35±0.01^{e}	0.24±0.02^{d}

The EC differed significantly between substrates (Table 1). High EC reflects high levels of soluble salts that decreasing osmotic potential may reduce the availability of water to plants, causing a reduction in germination and plant growth (Nieto et al., 2016). Other studies (Rajkovich et al., 2012; Revell et al., 2012) reported that the osmotic stress caused by high biochar EC might negatively affected plant growth. The EC of Char 100, i.e. 11.9 mS cm^{-1} was by far higher than those of the other substrates, and the pure peat had the lowest value, i.e. 1.6 mS cm^{-1}, the only one in the range 0.5-1.6 mS cm^{-1} reported as optimal for basil seedlings (Solis-Toapanta et al., 2020), which, however, is considered a moderately salt-tolerant plant (Ding et al., 2020). On the other hand, Morano et al. (2017) argued that the best outcomes in basil yield and leaf quality are obtained with a EC of 2.8 mS cm^{-1}, while Walters and Currey (2018) reported that ECs up to 4.0 mS cm^{-1} did not depress significantly the growth of sweet basil. Overall, an increase of pH and EC in the substrate would have a general detrimental effect on basil grown (Nobile et al., 2020).

The optimal range in bulk density (BD) for potting substrates is between 0.2 and 0.5 g cm^{-3}, as higher BDs have negative effects on roots growth and development (Andika et al., 2014). All substrate mixtures we used had BD between 0.23 and 0.32 g cm^{-3}, which are a significantly higher than BD of the control.

3.2 Growth of the seedlings

Only one day after the transplanting, all seedlings in the Char 100 showed total necrosis of leaves and stems. This symptom was evenly distributed on the totality of the plants of this treatment. After a further six days, necrosis appeared on top of the seedlings and the youngest leaves in all Comp 100 and Char 50 samples, and on the tenth day, it affected the whole seedlings of those treatments. However, no other plants showed such symptoms or other diseases throughout the trial.

Death of seedlings in pure biochar or compost are likely due to the very high pH and EC of these materials. In fact, these materials showed pH above 10 and 8, respectively, values seemingly incompatible with healthy plant growth. Huang (2019), in a similar study, reported that basil seedlings died after some weeks in a potted growing medium made of 90% in volume of hardwood biochar, with a pH around 11, mixed with compost and commercial peat. In addition, in the case of pure biochar, its high salt content, revealed by the high EC, is likely to have contributed to the immediate necrosis of plants. Similar conclusions were reached by Nocentini et al. (2021) in tomato. Biochar could have a negative effect through some toxic compounds reported to form during the pyrolysis, such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and persistent free radicals (PFRs) (Zheng et al., 2018). However, with the exception of PAHs that were below the reference limits (Table S1), we did not make any investigation in this regard and we took for granted the insignificant presence of these compounds being the used biochar a marketed product.

3.3 Basil vegetative variables

Standard peat control showed better growing variables than the other substrates (Table 2). Negative effect of biochar and compost addition on seedlings' growth and quality was confirmed by SPAD readings and leaf color parameters, which are strictly correlated to the plant nutritional status

(Dispenza et al., 2016). The SPAD values can be used as an indicator providing a quick and objective estimation of quality for green-leaved foliage (Wang et al., 2005). Higher SPAD and colorimeter values reveal a higher availability of some macro- and micro-nutrients involved in the biosynthesis of chlorophyll (Netto et al., 2005). Overall, biochar- and compost-including substrates did not show significant differences between each other in terms of total fresh aboveground biomass production; however, plants grown on Char 25 showed higher plants and higher leaf dry content than plants grown in compost mixes, while the smallest plants grew on Comp. 50. Yu et al. (2019) reported that basil seedlings grown in different biochar-amended mixes at higher doses than 50% peat/hardwood biochar mix showed significantly lower growth index and fresh and dry weight than those grown in the commercial propagation mix. On the other hand, using of biochar up to 50% of the substrate mix led to a greater or similar basil growth compared to that obtained with the commercial substrate (Yu et al., 2019). Conversely, Pandey et al. (2016) observed that the use of biochar in a pot experiment without fertilizers was not sufficient to boost the growth of the basil plant.

Table 2. Final Height (H), total fresh weight (FW), leaf fresh weight (LFW), percentage of fresh leaves weight on total fresh weight (PLW), total leaf area (LA), SPAD and color parameters (a^*, b^* and L^*) per plant grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 30 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for p = 0.05.

Substrates	H	FW	LFW	PLW	LA	SPAD	Color parameters		
	cm	g	g	%	cm^2	-	a*	b*	L*
Peat	43.9 ± 6.0^a	18.4 ± 4.9^a	11.3 ± 2.8^a	61.9 ± 2.2^b	488.1 ± 113.4^a	30.8 ± 1.6^a	2.5 ± 2.3^a	25.0 ± 3.1^b	46.0 ± 1.8^b
Char 25	35.6 ± 5.0^b	13.3 ± 3.9^b	8.5 ± 2.3^b	64.3 ± 4.1^{ab}	328.6 ± 89.4^b	27.7 ± 2.1^c	-2.4 ± 4.1^c	28.6 ± 3.1^a	49.4 ± 2.3^a
Comp. 50	26.8 ± 3.3^d	10.6 ± 1.4^b	7.0 ± 0.9^b	66.0 ± 1.7^a	270.4 ± 32.2^b	28.5 ± 1.6^{bc}	$.1.8\pm2.7^{bc}$	26.2 ± 1.8^b	48.9 ± 2.4^a
Comp. 25	29.9 ± 4.0^c	11.0 ± 1.2^b	7.2 ± 0.8^b	65.5 ± 1.6^a	292.1 ± 33.9^b	29.1 ± 1.8^b	-0.6 ± 2.4^b	26.1 ± 2.0^b	48.6 ± 2.4^a

In the literature there are contrasting results also concerning the effect of compost addition to peat for growing basil. Hewidy et al. (2014) observed that growing media comprising up to 30% compost in volume enhanced some plant variables like basil height, dry mass, and essential oil content, compared to pure peat moss, while DeKalb (2014) reported negative effects on basil height and weight by just 20% compost in the substrate, observing an inhibition of seedlings emergence in substrates with pH higher than 8.3. Manios (2004) measured increased growth of different plant species in peat with 30% compost in volume compared to pure peat, but he found phytotoxic effect with higher doses of compost. In a nursery experiment with an hydroponic solution Bernstein et al. (2010) observed that basil plant height, fresh biomass, and leaf area were reduced under salinity stress.

Mininni et al. (2015) also found that basil seedlings grown on pure peat showed higher fresh weight, dry weight and leaf area but lower SPAD values than those grown on peat-compost mixes. Conversely, in our experiment pure peat showed also the highest SPAD values. Plants lower SPAD values found in the substrates added with biochar or compost can be related to the high pH of biochar and compost, that can reduce Fe availability, with consequent lower leaves' greenness (Huang et al., 2020). Moreover, SPAD is often related to N concentration (Basyouni et al., 2015; Ibrahim and Jaafar, 2013; Ruiz-Espinoza et al., 2010) as chlorophyll incorporates a high amount of adsorbed nitrogen (Hawkins et al., 2009; Ibrahim & Jaafar, 2013; Ruiz-Espinoza et al., 2010). Thus, low SPAD readings usually indicate both low chlorophyll and N concentrations in leaves (Huang et al., 2020). Overall, there is a close relationship between N concentration in leaves and their greenness, as found by several studies (Majkowska-Gadomska et al., 2017; Vrbnicanin et al., 2012; Wu et al., 2007). This has also implications on biomass production, as the yield and biological value of basil are strictly related to the rate of photosynthesis, which is obviously controlled by the chlorophyll (Majkowska-Gadomska et al., 2017). The lower SPAD values we found for basil grown on substrates added with compost or biochar, therefore, may be related to a lower availability of N in these substrates. Several studies report that biochar and compost in soilless substrates could lead to N immobilization, consequently reducing the availability of this elements for plants (Blok et al., 2017; Burger et al., 1997; Burger and Hartz, 1997; DeKalb et al., 2014; Huang et al., 2019). The adsorption of NH_3 or organic nitrogen onto biochar is one of the mechanisms proposed to explain the retention of N in soil amended with charred materials (Jindo et al., 2020a). However, biochars usually show very high C/N ratio and, therefore, high amounts of biochar leads to high C/N values in the substrate, which may imply that the applied N fertilizer is immobilized by microorganisms, so being unavailable to plants (Dumroese et al., 2011).

Leaf color is a crucial quality for marketed fresh basil and we found this aspect to be significantly affected by substrate composition, as revealed by the significant differences in intensity of greenness (-a*), yellowness (+b*) and brightness (L*) between treatments. Seedlings grown in compost and biochar substrates showed higher L* compared to those cultivated on peat. In the green-red axis (a*) seedlings grown on biochar showed the lowest whereas peat the highest value. Plants grown in biochar increased their yellowness (b*) compared to those on the other substrates. Overall, the observed differences of the SPAD values and color coordinates of leaves resulted in a more intense green color of plants grown on peat, with a higher ornamental and commercial value. In general, higher SPAD and colorimeter values reveal a better availability of some macro- and micro-nutrients that are involved in the biosynthesis of chlorophyll in the respective substrate (Netto et al., 2005). Salinity is a factor that can affect pigment content in plants with negative effects on leaves color (Bernstein et al., 2010) and chlorophyll content (Mostafa Heidari, 2011). Our results are consistent with those of other researchers reporting a lower basil biomass and chlorophyll content with increasing levels of salinity (Bekhradi et al., 2015). A negative and significant correlation was found between SPAD and b* values (R=-.383*, p=.05), and between SPAD and L* values (R=-.409*), while a positive and significant correlation was found between SPAD and a* values (R=.495**, p=.01), overall suggesting that the dark green leaves color in the plants is related to a higher chlorophyll content.

3.4 Specific leaf area, leaf dry matter content, and leaf area ratio

Specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area ratio (LAR) are variables commonly taken into account in agricultural studies. SLA and LDMC are important variables in comparative plant ecology because they are associated with important aspects of plant growth (Shipley & Vu, 2002; Zhang, 2005). In particular, SLA is positively correlated with seedlings' growth rate and leaf net photosynthetic rate (Shipley and Vu, 2002). There are also studies on the relationship between SLA and LDMC (Garnier et al., 2001; Wilson et al., 1999), which generally demonstrate that the higher the SLA, the lower the LDMC (Zhang, 2005). We also found that the highest SLA values were associated with the lowest LDMC ones for both compost-containing substrates (Table 3). The SLA of peat and Char 25 did not differ significantly, while the LDMC value of the plants grown in peat was slightly (but significantly) higher than those in Char 25. However, it should also be considered that SLA and its relation with photosynthesis are the result of trade-offs between different functions of the leaf, not only photosynthesis but also competition, storage, damage prevention and support (Dijkstra, 1989). Dijkstra (1989) reported that a higher ratio of leaf dry weight to fresh weight bring to lower SLA, and this was observed also in our experiment. LAR indicates the efficiency with which a plant uses its photosynthetic organs to produce plant material. LAR is the useful leaf area for photosynthesis, which tends to decrease in plants undergone salinity stress as a mechanism to reduce water loss (Bressan et al., 2020). Nevertheless, in our experiment basil plants with higher LAR grew on Comp. 50 and Comp. 25, both substrates having higher EC than Char 25 and Control.

Table 3. Specific leaf area (SLA), leaf dry matter content (LDMC) and leaf area ratio (LAR) per plant. Values are means ± standard error of 9 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for p = 0.05.

Substrates	SLA $cm^2\ g^{-1}$	LDMC $g\ g^{-1}$	LAR $cm^2\ g^{-1}$
Peat	482.3 ± 36.2^b	0.09 ± 0.01^a	240.8 ± 19.3^b
Char 25	505.7 ± 57.5^b	0.08 ± 0.01^b	256.7 ± 35.6^b
Comp. 50	563.0 ± 15.7^a	0.07 ± 0.00^c	308.4 ± 19.0^a
Comp. 25	587.2 ± 38.2^a	0.07 ± 0.01^{bc}	318.3 ± 26.7^a

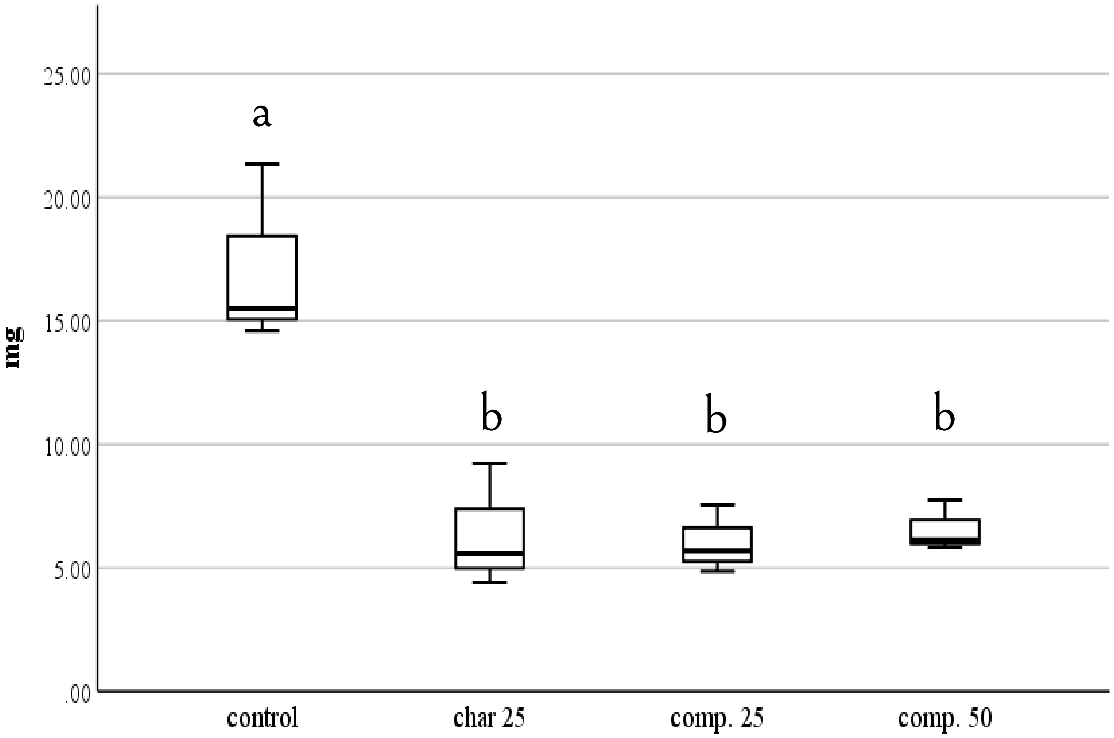
3.5 Volatile organic compounds (VOC)

In our samples we identified ten different monoterpenes, one alcohol (1-octen-3-ol) and one phenylpropanoid (eugenol) (Table 4). Eugenol was the dominant VOC in all the treatments, followed by linalool and cineole. Compounds that reduce the flavor quality of basil such as estragole, camphor and thymol (Maggio et al., 2016) were absent or present in trace amounts. The content of eugenol and methyleugenol is correlated with plant height rather than plant age; methyleugenol is in fact predominant in plants smaller than 10 cm, whereas eugenol is prevalent in taller plants (Chang et al., 2009; Miele et al., 2001). The harvested plants were all taller than 10 cm (Table 2) and no methyleugenol was found, indeed. This latter has been hypothesized to be toxic for humans, although in very high doses (Robison and Barr, 2006). However, the consumption of "pesto" sauce made with small basil plants, may represents an health risk due to the intake of non-negligible doses of methyeugenol (Miele et al., 2001).

Factors that can affect essential oils production and composition are mostly based on the individual genetic variability, the phenological stage, the kind of organ of the plant, and environmental factors, such as light, precipitation and soil/substrate characteristics included pH and water availability (Barra, 2009; Johnson et al., 1999). Nevertheless, in our experiment the addition of biochar or compost did not significantly influence the oil composition of basil plants. Bernstein et al. (2010) observed that the production of essential oils in basil increases because of salinity stress associated with a decrease in plant biomass. Also other works on basil (Ekren et al., 2012) and on sage (Ben Taarit et al., 2009) referred that ecological conditions as salinity stress can influence some essential oil composition and content. In our experiment we did not find significant differences in the total concentration of essential oils compounds between different treatments, despite the large differences in terms of EC between the substrates.

Our data showed significant differences in the content of VOCs between treatments only for cineole, terpinen-4-ol, and α-terpineol. The concentration of these three monoterpenes were highest in the plants grown in Char 25, while their lowest amounts were detected in the plants grown in Comp 25. Conversely, Najafian & Zahedifar (2018) reported that biochar and K addition in potting substrates was able to enhance the relative content of some monoterpenoids and sesquiterpenes in sweet basil oil. Esmaielpour et al. (2017) reported that basil grown on substrates half-substituted with compost showed higher concentration of myrcene, cineole, and sabinene compared to that grown on pure peat. In our study, the highest concentration of sabinene and myrcene were detected in plants grown in Char 25 and Comp. 50, even though those differences were not statistically significant (Table 4). Conversely, the absolute amount of VOCs (per plant), calculated taking into account the dry mass of leaves was significantly higher in peat than all the other substrates, which were comparable each other (Fig. 1).

Figure 1. Boxplot of total concentrations of VOCs (mg) in the leaves (dry mass) between different substrates, basil plants grown on substrate mixtures of peat (control), biochar (25%) and compost (50 and 25%). Values are means ± standard error of 14 replicates. Different letters indicate significant differences between treatments, based on Duncan’s test for $p < 0.05$.



While the absolute amounts of the terpenoids are subjected to environmental factors, the terpene profiles of plants, *i.e.* the relative contents of volatile terpenes are under strong genetic control and usually little affected by abiotic factors (Casano et al., 2011; Michelozzi et al., 2008; Squillace, 1976). As a matter of fact, terpenes profiles are largely used as biochemical markers to characterize plant species, provenance and clones in chemosystematic studies (Casano et al., 2011; Langenheim, 1994). Accordingly, our results showed that variation in the relative content (%) of monoterpenes was not significantly affected by the different substrates, with the only exception of cineole, which showed the highest values for plants grown in Char 25 (Table 5). Our results are partially in agreement with those obtained by Pandey *et al*. (2016) on basil seedlings grown in pot substrate added with a biochar made with wood pruning. They observed that biochar addition, alone or combined with fertilizers, in potting substrates did not affect basil aroma compounds (relative contents), whereas oil yield (ml of oil per 100 g of dried material) was only marginally improved in their two years experiment. However, when biochar was added with chemical fertilizers, crop biomass was enhanced compared to the no-biochar treatment and, hence, while the oil concentration remained comparable, the higher crop biomass obtained resulted in a higher oil production per unit area (Pandey et al., 2016).

Table 4. Concentration of VOCs (mg g^{-1}) on dry weight extracted from basil plants grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 14 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for p = 0.05.

Substrates	α-pinene	β-pinene	sabinene	myrcene	limonene	cineole	β-cis-ocimene	1-octen-3-ol	linalool	terpinen-4-ol	α-terpineol	eugenol	total concentration
Peat	0.02±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.54±0.23^{ab}	0.03±0.02^{a}	0.14±0.04^{a}	2.55±0.84^{a}	0.13±0.08^{ab}	0.10±0.03^{ab}	7.91±2.27^{a}	11.47±3.48^{a}
Char 25	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.73±0.35^{a}	0.03±0.03^{a}	0.14±0.07^{a}	2.73±1.15^{a}	0.17±0.12^{a}	0.12±0.05^{a}	8.91±3.33^{a}	12.92±4.94^{a}
Comp. 50	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.02±0.01^{a}	0.01±0.01^{a}	0.68±0.29^{ab}	0.03±0.03^{a}	0.14±0.06^{a}	2.81±1.12^{a}	0.15±0.12^{ab}	0.11±0.05^{ab}	8.43±3.11^{a}	12.44±4.76^{a}
Comp. 25	0.02±0.01^{a}	0.02±0.02^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.51±0.17^{b}	0.02±0.02^{a}	0.11±0.04^{a}	2.20±0.98^{a}	0.10±0.06^{b}	0.09±0.03^{b}	7.05±2.24^{a}	10.15±3.46^{a}

Table 5. Relative content of VOCs (%), of basil plants grown on substrate mixtures of peat, biochar (25%) and compost (50 and 25%). Values are means ± standard error of 14 replicates. Values in the same column followed by different letters indicate significant differences, based on Duncan's test for p = 0.05.

Substrates	α-pinene	β-pinene	sabinene	myrcene	limonene	cineole	β-cis-ocimene	1-octen-3-ol	linalool	4-ol-terpinen	α-terpineol	eugenol
Peat	0.14±0.05^{a}	0.14±0.05^{a}	0.13±0.05^{a}	0.10±0.00^{a}	0.13±0.05^{a}	5.64±1.03^{ab}	0.36±0.05^{a}	1.04±0.17^{a}	22.95±1.91^{a}	2.07±0.19^{ab}	0.84±0.09^{ab}	65.74±2.37^{a}
Char 25	0.20±0.24^{a}	0.14±0.05^{a}	0.14±0.05^{a}	0.11±0.03^{a}	0.11±0.04^{a}	6.75±0.85^{a}	0.34±0.06^{a}	0.89±0.14^{a}	22.10±3.03^{a}	2.23±0.29^{a}	0.89±0.11^{a}	65.49±3.68^{a}
Comp. 50	0.16±0.06^{a}	0.17±0.05^{a}	0.15±0.05^{a}	0.10±0.00^{a}	0.15±0.05^{a}	6.67±0.57^{ab}	0.34±0.06^{a}	0.94±0.14^{a}	23.26±2.51^{a}	2.17±0.28^{ab}	0.87±0.09^{ab}	64.56±2.82^{a}
Comp. 25	0.16±0.06^{a}	0.19±0.10^{a}	0.16±0.05^{a}	0.10±0.00^{a}	0.15±0.05^{a}	6.34±0.77^{b}	0.34±0.05^{a}	0.90±0.28^{a}	21.84±3.15^{a}	2.16±0.16^{b}	0.84±0.09^{b}	66.20±3.50^{a}

Table S1. Chemical parameters of the biochar provided by the manufacturer. The indicated limit values refer to the limits given by the European Biochar Certificate (EBC) for biochar of class I (EBC-Feed).

Parameter	Unit of measure	Value	Limit values
Humidity at 105 °C	%	74	>20
pH	-	10.7	4-12
salinity	mS/m	68.5	<1000
Organic carbon	% d.m.	87.7	>60
Ashes	% d.m.	11.5	>10
H/C	-	0.01	< 0.7
Total nitrogen	% d.m.	0.7	-
Total phosphorus	% d.m.	1.0	-
Total potassium	% d.m.	0.001	-
Total carbon	% d.m.	88.8	-
Total sodium	% d.m.	0.06	-
Total calcium	% d.m.	2.0	-
Total magnesium	% d.m.	0.4	-
Total lead	mg/Kg d.m.	3.0	<10
Total cadmium	mg/Kg d.m.	<0.2	<1
Total copper	mg/Kg d.m.	37	<100
Total zinc	mg/Kg d.m.	46	<400
Total nichel	mg/Kg d.m.	2.0	<30
Total mercury	mg/Kg d.m.	<0.2	<0.1
Total chromium	mg/Kg d.m.	<0.2	<80
polycyclic aromatic hydrocarbons (PAHs)	mg/Kg d.m.	<0.005	<6

4. Conclusions

Biochar and compost were evaluated as growing potting substrates for sweet basil, alone or mixed with the most usual peat. The results showed that the addition of biochar or compost, also at the lower doses, increased substrate pH and EC. At high concentrations of biochar or compost both pH and EC resulted so high to be most likely detrimental to basil growth. In fact, the substitution of standard peat with 50 % (v/v) or more of biochar and total substitution with compost led seedlings to die just after one or few days from transplanting, suggesting to use much lower doses of biochar and compost for basil cultivation. Furthermore, surviving plants, grown with the lowest amounts of biochar (25%) and compost (50% and 25%) we applied, were not able to guarantee the standard quality for basil in terms of fresh mass and leaves color. Hence, only lower complementary doses, if any, of such alternative growing media could be used to obtain substrates for basil cultivation with ready-to-use characteristics, a fundamental aspect for commercializing biochar-compost based growing media. Nevertheless, essential oil profile of basil was not modified by adding biochar or compost into the growing substrates, although the total amount of VOCs decreased because of the lower leaves biomass. Overall, our results would not support the massive use of alternative media such as charred material or composted wastes as a valid strategy to reduce the use of peat in potting substrates.

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9.3 Paper III

Effects of biochar from wood-pruning as soil amendment on growth and quality of wheat (*Triticum* spp. L) and sunflower (*Helianthus annuus* L.)

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Paper in preparation

Candidate contribution: The candidate conceived the experimental set-up, realized it, carried out measurement, data analysis and writing of the article.

Effects of biochar from wood-pruning as soil amendment on growth and quality of wheat (*Triticum* spp. L.) and sunflower (*Helianthus annuus* L.)

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Abstract

Keywords:

1. Introduction

During the second half of 20th century, agriculture started to use higher amount of chemical fertilizers instead of the traditional inputs such as manure and compost, often produced locally. The higher crop yields obtained by the use of chemical fertilizers, as well as their immediate effect and low cost, represented a crucial point in boosting their use in agriculture. However, excessive use of chemical fertilizers have shown detrimental effects on soil fertility bringing in a decline of organic matter content of many soils, particularly in some Mediterranean regions (Diacono and Montemurro, 2011).

The addition of manure or compost can represent a valid different way to improve soil fertility, but for many farmers these materials are difficult to obtain.

Biochar, a carbon-rich product produced by pyrolyzation of biomasses, could represent a sustainable choice for raising and sustaining crop production, improve soil moisture availability and store C in soils (Baldock and Smernik, 2002). Indeed, biochar affect several soil properties and processes and its addition to soil has been reported to be effective on increasing the availability of nutrients, microbial activity, water retention, reducing at the same time fertilizer requirements, greenhouse gas emissions, nutrient leaching and erosion (Batista et al., 2018). The potential positive effects of biochar as soil amendment are related to its high cation exchange capacity and surface area, which leads to increase the water holding capacity (Streubel et al., 2011).

However, the effects of biochar addition on soil properties and crops yield are contrasting, and the responses of crop yields to biochar amendments, in particular under temperate field conditions, are still unpredictable (Haider et al., 2017). This is due to the numerous variables that can play a role in determining crop yield, e.g. biochar parent material and production conditions, soil and crop type, just mentioning the most important ones. A review made by Zhang et al. (2016) revealed that of 798 biochar studies only 26% of them were performed under field conditions, moreover other authors (Hammond et al., 2013) reported that the temperate regions particularly lack in fields trials made with biochar addition. Furthermore, results obtained under field conditions are often differing from laboratory experiments, and showed contrasting findings compared to greenhouse studies (Glaser et al., 2015; Liu et al., 2012), highlighting the importance of conducting field experiments.

While numerous studies have been published about the effect of biochar addition on soil properties, crop production and plant growth (Agegnehu et al., 2017; Diatta et al., 2020; Jeffery et al., 2011), there is a lack of works on the direct effects of biochar addition on crop quality. Considering that the addition of charred materials as soil amendment is increasingly adopted during last years, studies on the possible effect on the quality of crops should be performed, especially when it comes to largely consumed products, such as horticultural crops or cereals.

During the last years, food quality and safety have received growing importance, both in consumers' request and in marketing research. Consumers also showed a high and increasing attention on nutraceuticals use. Nutraceuticals, the hybrid of "nutrition" and "pharmaceutical", is a wide term that can be defined as "*any substance that may be considered a food or a part of a food, and provides medical or health benefits, including the prevention and treatment of diseases*" (Teoh et al., 2019). Nutraceuticals, also known as functional foods, can be considered foods or substances that can provide health benefits such as antioxidants, vitamins, minerals, herbals, aromatic plants, cereals and others (Guidi and Landi, 2014; Lee, 2017). Nowadays there is a strong pressure on the food industry to look for safe substances that can be used in nutrition. Studies on this have been published for example on some typical crops and herbs of Mediterranean basin: tomato, sweet potato, basil, wheat, sunflower (Alan et al., 1994; Erekul and Köhn, 2006; Jelacic et al., 2005; Lopez et al., 2004; Najar et al., 2019; Padem and Alan, 1994; Pfister and Saha, 2017). But just a few studies about the effect of biochar on some quality parameters of horticultural species and cereals have been published (Massa et al., 2019; Najar et al., 2019; Pandey et al., 2016; Quartacci et al., 2017; Vaccari et al., 2015).

Wheat and in particular both *Triticum turgidum* ssp *durum* and *Triticum aestivum*, for example, are widespread cultivated in the Mediterranean basin, where they are respectively used for the production of high-quality semolina for pasta, and for the production of flour for bread and biscuits industries. The area interested for wheat cultivation in the Mediterranean countries represents 27% of the arable land, and the Mediterranean basin represents 60% of the world's growing area for durum wheat (Royo et al., 2017). For thousand years bread made with wheat flour has been one of the principal constituents of human diet, and even today in the Mediterranean countries, cereals are positioned at the base of food pyramid according to nutritional guidelines (Bach-Faig et al., 2011). Wheat is in fact a source of primary nutrients such as carbohydrates and proteins, but also a source of antioxidants. Despite after second world war, local and ancient wheat varieties have been replaced by modern ones, selected for intensive cultivation, the increasing demand of consumers for varieties with greater health potential, nutritional and sensory qualities, renewed the interest in traditional wheat cultivars (Dinu et al., 2018; Rocco et al., 2019). Recent studies on the potential health benefits of functional groups from some wheat varieties have renewed the interest in ancient cultivars, and in particular on their potential nutraceutical properties (Dinelli et al., 2011; Leoncini et al., 2012). Ancient varieties, defined as those not dwarf and unregistered genotypes, or cultivars that did not undergo modifications during the last century, are in fact receiving interest since some studies suggested that they present a healthier and better nutritional profile, more specifically for improved anti-oxidant and anti-inflammatory properties, compared with modern varieties (Dinu et al., 2018). Some of the beneficial effects of consuming certain wheat cultivars, are associated with the phytochemicals of wholegrain, which include for example phenolics and carotenoids (Heimler et al., 2010), and other antioxidants such as tocopherols, flavonoids and phenolic acids (Vaher et al., 2010). Among these compounds, polyphenols play an important function in contrasting oxidative stress, one of the possible causes of some human deseases.

Wheat-sunflower rotation is a common practice in the arable areas of the Mediterranean basin (Ercoli et al., 2014; López-Bellido et al., 2002; Pedraza et al., 2015). Sunflower is an important oleaginous plant cultivated for food purposes, but it also represents a valid crop to introduce in rotation with cereals. After soybean, rapeseed and safflower, the sunflower represents the fourth most important oilseed crop in the world with high economic value. Furthermore, among the oilseed crops cultivated in the world today, sunflower represents the most important source of high-quality oil for human consumption. Some studies revealed that sunflower seeds contain many phytochemicals that are beneficial for human health (Adeleke and Babalola, 2020). In the case of sunflower, the quality of its oil depends on the ratio of oleic, linoleic and linolenic acids (Ghobadi et al., 2013). Sunflower seeds are also characterized by high antioxidant properties (Karamać et al., 2012; Velioglu et al., 1998); these antioxidant compounds can be found in both sunflower oil and in the oilseed extracted meal. The antioxidant potential of sunflower meal and sunflower seed shells is determined principally by the content of phenolic compounds (De Leonardis et al., 2005; Schmidt et al., n.d.).

The aim of this work was to evaluate the effects of biochar addition as soil amendment in a two-years open field trial with wheat and sunflower as target species, focusing on the effects, at field conditions, in terms of soil characteristics, plant growth, fresh and dry biomass and quality parameters such as polyphenols, carotenoids and antiradical activity in wheat and sunflower seeds. It is known that changes in environmental conditions particularly in soil quality can affect the secondary metabolites production such as polyphenols (Chludil et al., 2008). As a result, investigations are required focusing on antioxidants compounds like polyphenols, flavonoids, carotenoids and others, and in which way the addition of soil amendments such as biochar, can modify their profile or concentration.

2. Materials and methods

2.1 Study area

The study area is the farm Tenuta di Cesa - Terre Regionali Toscana, Italy. The climate is typically Mediterranean, usually the annual precipitation ranges from 685 to 711 mm distributed across 89 rainy days (i.e., with rainfall above 1 mm). The meteorological data were recorded at the local weather station. The principal crops that are cultivated are wheat, both modern and ancient cultivars, sunflower, tobacco, maize, and also some minor crops such as millet, sorghum, quinoa and amaranth. The farm combines traditional productions alongside experimental production and plot trials. The wheat-sunflower rotation represents one of the typical practice adopted by the farm and since many years the rotation includes ancient wheat varieties due to the interest showed by research and consumers.

2.2 Experimental design

The two-year open field trial started in November 2018 and ended in October 2020. The experimental design was based on a randomized block scheme and consisted of three replicate blocks each treatment, plot dimension were 6x15 m. Each wheat genotypes were grown in a sub-plot of 3x7 m, while sunflower was cultivated on the entire plot. We tested three different doses of biochar B1 (1 t ha^{-1}), B2 (4 t ha^{-1}) and B3 (20 t ha^{-1}) plus one control (B0). The biochar was added once in November 2018 and was incorporated into the soil by plowing to a depth of 25 cm. Biochar was humidified before addition in the soil to avoid wind dispersion. Chemicals parameters of the biochar were provided by the manufacturer (Table 1).

Table 1. Chemical parameters of the biochar derived from wood provided by the manufacturer. The indicated limit values for heavy metals and H/C refer to the limits given by the European Biochar Certificate (EBC) for a biochar of application class I (EBC-Feed).

Parameter	Unit of measure	Value	Limit values
Humidity at 105 °C	%	74	>20
pH	-	10.7	4-12
salinity	mS/m	68.5	<1000
Organic carbon	% d.m.	87.7	>60
Ashes	% d.m.	11.5	>10
H/C	-	0.01	< 0.7
Total nitrogen	% d.m.	0.7	-
Total phosphorus	% d.m.	1.0	-
Total potassium	% d.m.	0.001	-
Total carbon	% d.m.	88.8	-
Total sodium	% d.m.	0.06	-
Total calcium	% d.m.	2.0	-
Total magnesium	% d.m.	0.4	-
Total lead	mg/Kg d.m.	3.0	10
Total cadmium	mg/Kg d.m.	<0.2	1
Total copper	mg/Kg d.m.	37	100
Total zinc	mg/Kg d.m.	46	400
Total nichel	mg/Kg d.m.	2.0	30
Total mercury	mg/Kg d.m.	<0.2	0.1
Total chromium	mg/Kg d.m.	<0.2	80
polycyclic aromatic hydrocarbons (PAHs)	mg/Kg d.m.	<0.005	6

The target species adopted for the first year of the experiment were four different cultivars of wheat, two "ancient" cvs., Verna (*T. aestivum*) and Senatore Cappelli (*T. turgidum* ssp. *durum*), and two modern cvs., Bologna (*T. aestivum*) and Claudio (*T. turgidum* ssp. *durum*). Wheat seeds, 160 kg ha^{-1} for the ancient cvs. and 200 kg ha^{-1} for the modern cvs., were sown with a plot seeder in December

2018 and harvested with a plot thresher in July 2019. In March 2020 a NPK fertilizer (12:12:17) was distributed in the field with a dose of 0.4 t ha^{-1}.

During the second year the target species was the high oleic hybrid P64HE39 of sunflower. Sunflower was sown at the beginning of April 2019, pre-emergence herbicide treatment was applied using commercially formulated products, Challenge (1.5 L ha^{-1}), Dual Gold (1 L ha^{-1}) and Most Micro (2 L ha^{-1}). In May, after hoeing, 0.15 t ha^{-1} of urea was distributed. Sunflower was harvested in September 2020.

2.3 Physical and chemical characterization of soil samples and biochar

Soil samples, three sample of 0.5 kg each plot then bulked together so having a composite sample each treatment, were taken at the end of the growing season of wheat (July 2019) by a coring apparatus fitted with thin-walled stainless steel sample tubes to a depth of 25 cm. Collected soil samples were oven dried at 40 °C and gently grounded and sieved to 2 mm. The so obtained fraction, the fine earth, was further analyzed as following. The Cation exchange capacity (CEC) of each plot was measured using the method described by Hendershot and Duquette (1986). The pH and electrical conductivity (EC) of biochar and soil were measured in a suspension in distilled water (1:2.5) with a XS pH-meter model PC8.

To determine the water holding capacity (WHC), 6 g of each sample were placed on a Whatman 2 filter placed into a funnel, and saturated with distilled water. For 2 h the water was allowed to percolate through the filter and the funnel, then the weight of the moist samples was measured. The weight difference between dry and moist sample was extrapolated for a duration of the experiment of 12 h according to de la Rosa et al. (2014). Its percentage relatively to the dry weight of the sample resulted in the value for the maximum WHC. Total organic C and total N in soil samples was measured by dry combustion (by a Carlo Erba NA 1500 CNS Analyzer, Milan, Italy) after pre-treatment of samples with 6 M HCl at 80 °C to eliminate carbonates (Santi et al., 2006).

2.4 Plant harvesting and measurement

Just before harvesting, plants' height was measured for 30 plants each wheat varieties and, next year, 36 plants for sunflower. Half square meter of each wheat plot was harvested and the above-ground biomass was weighted. Twelve plants of sunflower each replicates were harvested at the end of the season, weighing the inflorescence heads (capitulas) and the above-ground biomass after oven-drying at 70 °C until constant weight. Seeds were removed from the head inflorescence for laboratory analysis.

2.5 *Wheat and sunflower seeds quality analysis*

Carotenoids of wheat were extracted using 10 g of each sample, middlings and bran, with 100 mL acetone, cold sonicated for 30'. The sample was centrifuged for 5' at 5000 rpm, the supernatant has been dry evaporated with a Rotovapor and the residue was dissolved in 5 mL acetone. The extracts were subjected to HPLC/DAD analysis. Polyphenols of wheat were extracted using 5 g of middlings and bran with 35 mL of 70:30 EtOH/H2O at pH 3.2 (by HCOOH). For polyphenols of sunflower 1 g of grounded kernel and tegument of each sample was extracted with 25 mL of 70:30 EtOH/H2O at pH 3.2 (by HCOOH). All solvents used were of HPLC grade purity (BDH Laboratory Supplies, Poole, United Kingdom). Both wheat and sunflower samples were shaken for 24 h, centrifuged for 5' at 14000 rpm and used for HPLC/DAD analysis.

Qualy-quantitative analyses of carotenoids and polyphenols were carried out using an HP 1100 liquid chromatography equipped with a DAD detector and managed by an HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated using a 250 x4.6 mm i.d, 5 µm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 280, 330, 350 and 450 nm. The samples were analyzed by gradient elution at a flow rate of 0.8 mL/min. For sunflower, compounds were separated using a 250 x4.6 mm i.d, 5 µm LUNA C18 column (Phenomenex, USA). UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 250, 280, 330 and 350 nm. The samples were analysed by gradient elution at a flow rate of 0.8 mL/min. The mobile phase for carotenoids was a multistep linear solvent gradient system (solvent A: acetone, solvent B: H2O , pH 3.2 by HCOOH), starting from 80% acetone up to 100% in 30 min, while polyphenols were eluted using the following gradient: from 90% H2O (adjusted to pH 3.2 by HCOOH) to 100% CH3CN in 40 min.

Quantification of individual polyphenolic compounds was directly performed by HPL/DAD using a five-point regression curve (r2 ≥0.999) in the range of 0-30 µg on the basis of authentic standards. The standard used were indoleacetic acid, caffeic acid, chlorogenic acid and Karmpferol 3-glucoside, and Folin-Ciocalteau reagent and were purchased from Sigma-Aldrich (St. Louis, USA). β-carotene standard was purchased from Extrasynthese (Lione, Francia). In particular, flavonols were determined at 350 nm using kaempferol 3-O-glucoside as reference compound while caffeic acid derivatives were determined at 330 nm using chlorogenic acid as reference compound and indoleacitic acid derivative at 280 nm using 3- indoleacetic acid. Carotenoids were determined at 450 nm using β-carotene as reference compound. For sunflower samples, in particular, caffeic acid derivatives were determined at 330 nm using caffeic acid as reference compound and indoleacitic acid derivative at 280 nm using 3-indoleacetic acid.

The total phenolic content of wheat samples was determined using the Folin-Ciocalteu method, described by Singleton et al. (1999) and slightly modified according to Dewanto et al. (2002). To 125 µL of the suitably diluted sample extract, 0.5 mL of deionized water and 125 µL of the Folin-Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 mL of a 7% aqueous Na2CO3 solution were added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/100 g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 µg/mL (R2 = 0.9969).

Free radical scavenging activity of wheat samples was evaluated with the DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Brand-Williams (1995) and slightly modified. Two mL of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanol solution of DPPH• (0.0025g/100mL) and the mixture kept at room temperature. After 20 min, the absorption was measured at 517 nm with a Lambda 25 spectrophotometer (Perkin-Elmer) versus ethanol as a blank. Each day, the absorption of the DPPH• solution was checked. The antiradical activity percentage was calculated by the ratio: [DPPH• concentration at t = 20’]/[DPPH• concentration at t = 0] .

2.6 Statistical analysis

Data underwent one-way analysis of variance (ANOVA) according to a completely randomized block design with three blocks and 3 replicates per treatment. Significant differences among means were determined using Tukey’s post-hoc significance test at $p < 0.05$. Spearman's rank correlation coefficients (ρ) were calculated for all the measured variables. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

3. Results

3.1 Chemical and physical characteristics of soil

The soil where the experiment was set up is described by a clay texture (10.7% sand; 43.4% silt; 45.9% clay), a pH of 7.0, and an organic matter content of 1.9%. In this open field trial, the biochar addition to soil did not change significantly the physical and chemical characteristics of the soil even when added at the highest amount, i.e. 20 t ha^{-1} (Table 2). Indeed, the pH, EC, WHC, TN and CEC of soil samples did not differ between treatments. Just total soil organic carbon content was significantly affected by the highest biochar application compared to the control.

Table 2. Dose of biochar, pH, electrical conductivity (EC), water holding capacity (WHC), cation exchange capacity (CEC), total organic carbon (TOC) content and total nitrogen content (TN) of the different soil treatment. Values are means ± standard deviation of 3 replicates. Values in the same column followed by different letters indicate significant differences, based on Tukey's test, at the p < 0.05 level.

Samples	Dose	pH	EC	WHC	CEC	TOC	TN
	t ha^{-1}		µS cm^{-1}	%	meq/100 g	%	%
Control	0	7.6±0.4^{a}	141±27^{a}	84.4±4.0^{a}	42.4±1.4^{a}	1.12±0.5^{b}	0.14±0.01^{a}
B1	1	7.7±0.2^{a}	180±57^{a}	85.9±3.1^{a}	39.7±2.1^{a}	1.17±0.3^{ab}	0.15±0.00^{a}
B2	4	7.6±0.2^{a}	138±27^{a}	85.3±2.7^{a}	42.9±0.9^{a}	1.27±0.8^{ab}	0.15±0.00^{a}
B3	20	7.7±0.2^{a}	144±12^{a}	92.5±5.9^{a}	41.2±1.5^{a}	1.35±1.0^{a}	0.15±0.01^{a}

3.2 Wheat growth, production and quality parameters

As expected, the un-dwarfed ancient cultivars Senatore Cappelli and Verna were taller and less productive than modern ones (Figure 1). Conversely, the bulk above-ground biomass produced at the end of the growing season, including stems and ears, showed no differences between the cultivars (Figure 4). However, the sowing seed density suggested for modern varieties is higher if compared with the ancient ones. Total proteins in the ancient varieties was higher compared with the modern ones (Figure 2). In particular, Senatore Cappelli showed the highest protein content while the modern variety Bologna showed the lowest. The addition of biochar did not influence the height of each variety between the various treatments, as well as the biomass and the grain yield, even at the maximum dose of 20 t ha^{-1} (Figure 1, 3 and 4). Also the protein content of whole grain of each of the four varieties was unaffected by biochar addition.

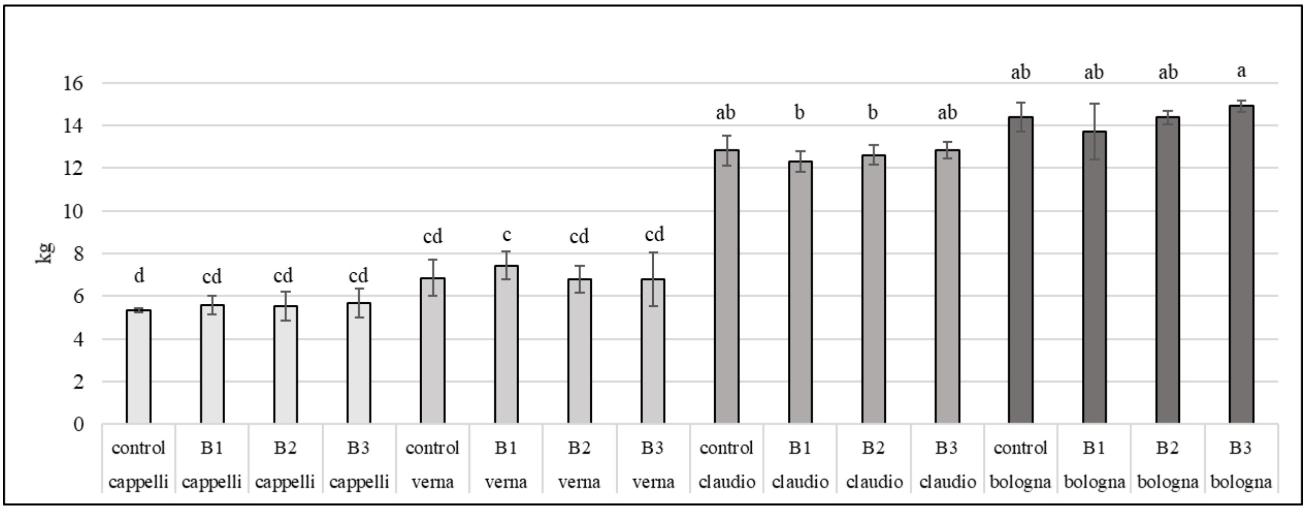

Fig. 1. Grain production/plot (kg) for each wheat variety under 3 different doses plus the control. Values are means of three replicates. The comparison was performed for different doses within each variety. The significant differences were shown by different letters (Tuckey's test for p = 0.05).

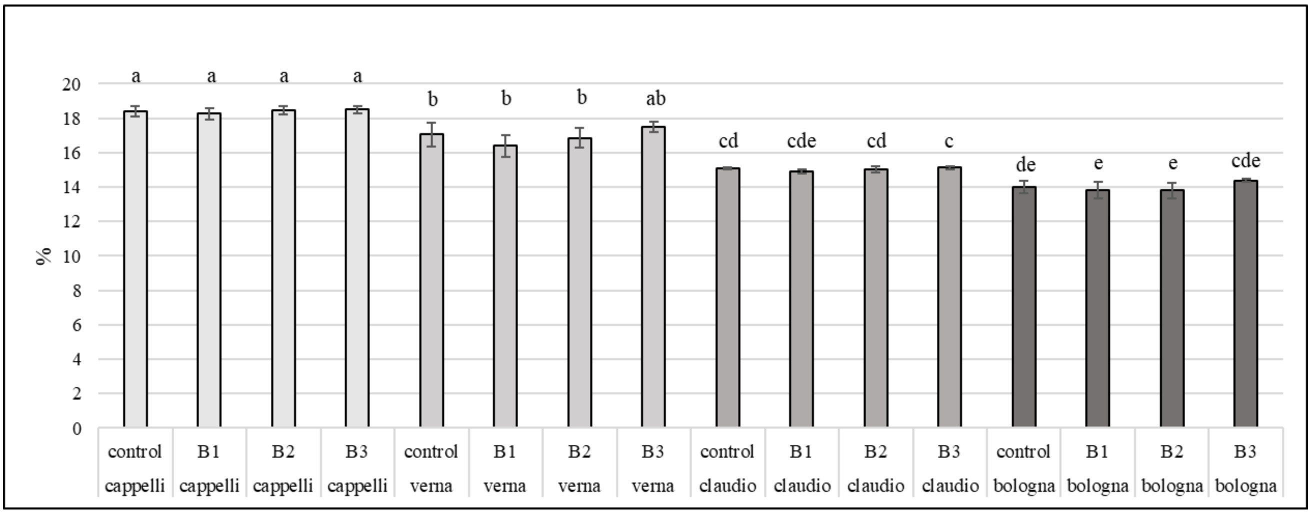

Fig. 2. Total protein content of wheat seeds for each variety under 3 different doses plus the control. Values are means ± standard deviation of 3 replicates. The comparison was performed for different doses within each variety. The significant differences were shown by different letters (Tuckey's test for p = 0.05).

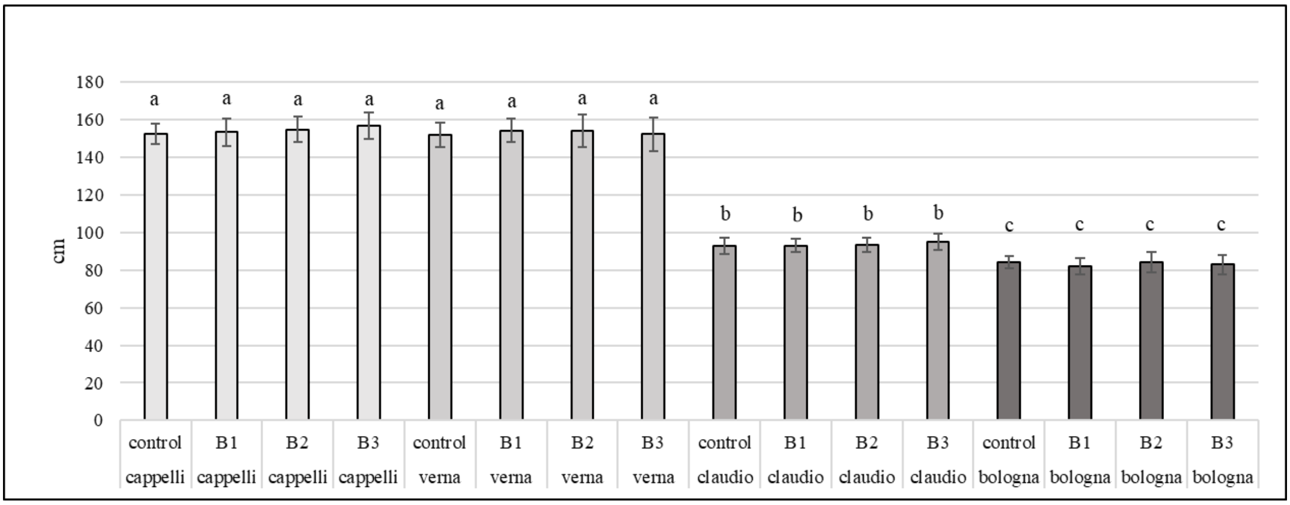

Fig. 3. Plant height (cm) for each wheat variety under 3 different doses plus the control. Values are means ± standard deviation of 20 replicates. The comparison was performed for different doses within each variety. The significant differences were shown by different letters (Tuckey's test for p = 0.05).

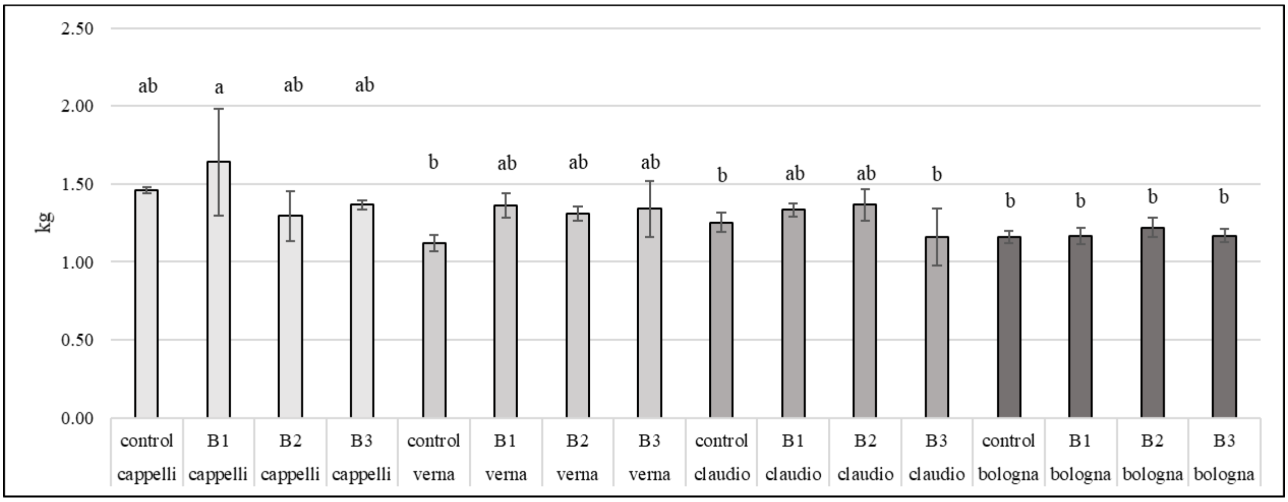

Fig. 4. Plant fresh biomass (kg) for each wheat variety under 3 different doses plus the control. Values are means ± standard deviation of 3 replicates. The comparison was performed for different doses within each variety. The significant differences were shown by different letters (Tuckey's test for p = 0.05).

As Verna is one of the most cultivated ancient variety in Tuscany for bread making, we took into account just this variety for the analysis on the concentration of total polyphenols (also known as total phenolics), carotenoids, flavonoids and caffeic acid in the middlings and bran (Table 3). These compounds are strictly related to the quality of grain and flour and are unevenly distributed in the kernel; for this reason the analysis were performed on middlings and bran separately. We observed significant differences in the concentration of total polyphenols in the middlings. Higher values were obtained by B2 and B3 treatments, while the control showed the lowest value. Differences were also found in the bran, where treatments B1 and B2 showed higher values of total phenolics compared to both B3 and control, which showed similar values. Significant differences in flavonoids concentration in the bran were also observed, B3 treatment showed the highest values while the control the lowest values. B3 was also the treatment with the highest value for caffeic acid derivatives in the bran. Overall, biochar application seems to led to an increase of the concentration of phenolics, flavonoids and caffeic acid derivatives. On the other hand, the concentration of carotenoids, both in the middlings and in the bran, and the concentration of flavonoids in the middlings, were not affected by biochar addition at tested doses.

Table 3. Concentration of total polyphenols, carotenoids, flavonoids and caffeic acid derivatives of middlings and bran of cultivar Verna as affected by different doses of biochar. Values are means ± standard error of 9 replicates. Values in the same column followed by different letters indicate significant differences, based on Tukey's test for p = 0.05

Samples	Polyphenols mg(GAE)/g		Carotenoids mg/g		Flavonoids mg/g		Caffeic a.d. µg /g
	middlings	bran	middlings	bran	middlings	bran	bran
Control	1.08±0.08^{b}	1.77±0.11^{b}	5.78±0.65^{a}	3.87±0.34^{a}	0.43±0.07^{a}	0.04±0.01^{b}	7.7±1.4^{ab}
B1	1.18±0.07^{ab}	1.92±0.07^{a}	5.92±0.28^{a}	4.04±0.69^{a}	0.40±0.02^{a}	0.06±0.02^{ab}	6.3±1.9^{b}
B2	1.29±0.06^{a}	1.92±0.04^{a}	5.51±0.40^{a}	3.52±0.41^{a}	0.44±0.12^{a}	0.05±0.01^{ab}	5.8±0.7^{b}
B3	1.23±0.16^{a}	1.70±0.12^{b}	5.36±0.41^{a}	3.92±0.37^{a}	0.41±0.05^{a}	0.07±0.03^{a}	9.1±2.4^{a}

The radical scavenging activity of antioxidants was calculated on samples of control and B3 only for Verna. The capacity of wheat extract to scavenge the stable DPPH radical is shown in Figure 5. The T-test showed no differences between the two treatments, control and B3, both in middlings and in bran.

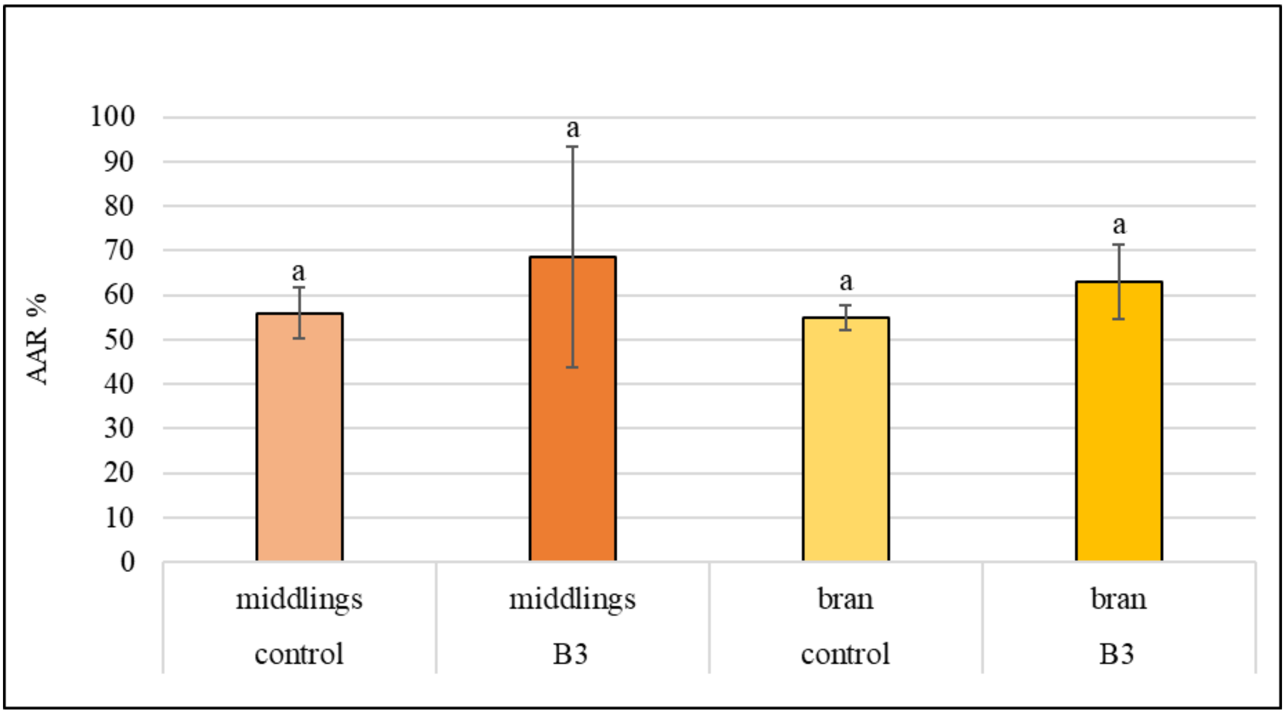

Fig. 5. Antiradical activity percentages of middlings and bran in the control and the B3 treatment. Values are means ± standard deviation of 3 replicates. Letters indicates significant differences according to T-test for $p < 0.05$.

3.3 Sunflower growth, production and quality parameters

The cultivation of sunflower hybrid P64HE39 followed the wheat cultivation on the same plots. The growth parameters of sunflower such as plant height, total fresh weight of above-ground biomass, fresh weight of capitulas, and also the crop yield calculated as dry seeds weight, were not affected by biochar addition, even at the highest amount applied (Table 4).

Table 4. Plant height (PH), fresh above-ground biomass weight (FABW), capitulas fresh weight (CW) and dry seeds weight (DSW) as affected by biochar addition. Plant height values are means ± standard error of 36 replicates; FABW, CW and DSW are means ± standard error of 3 composite samples made of 12 units. Values in the same column followed by different letters indicate significant differences, based on Tukey's test for $p = 0.05$

Samples	PH	FABW	CW	DSW
	cm	kg	kg	g
Control	$192±16^a$	$4.4±0.8^a$	$1.6±0.3^a$	$724.0±59.5^a$
B1	$196±11^a$	$4.1±0.8^a$	$1.4±0.2^a$	$709.2±127.1^a$
B2	$193±11^a$	$4.0±0.4^a$	$1.4±0.2^a$	$654.0±17.6^a$
B3	$199±8^a$	$4.8±0.8^a$	$1.5±0.2^a$	$744.2±115.8^a$

Eight different compounds were isolated from sunflower dry seeds (kernel and tegument): a derivative of indole-3-acetic acid (IAA), 6 caffeoylquinic acids and one feruloylquinic acid. The concentrations of these compounds, the total caffeic derivatives and total polyphenols are shown in Table 5. The concentration of total caffeic derivatives and total polyphenols seems to decrease with the increase of biochar application, but none of the identified compounds were affected in their concentration by biochar addition, even at the highest amount applied.

Table 5. Concentration of isolated compounds (mg g^{-1}) of sunflower dry seeds as affected by different amounts of biochar added to the soil. Values are means ± standard error of 3 replicates. Values in the same string followed by different letters indicate significant differences, based on Tukey's test for p = 0.05.

Compounds	**B0**	**B1**	**B2**	**B3**
	mg g^{-1}	mg g^{-1}	mg g^{-1}	mg g^{-1}
iaa derivative	0.17 ± 0.06^a	0.16 ± 0.03^a	0.15 ± 0.08^a	0.13 ± 0.02^a
3-O-caffeoylquinic acid	0.06 ± 0.02^a	0.06 ± 0.01^a	0.08 ± 0.01^a	0.05 ± 0.01^a
4-O-caffeoylquinic acid	0.14 ± 0.05^a	0.13 ± 0.01^a	0.13 ± 0.04^a	0.10 ± 0.01^a
5-O-caffeoylquinic acid	13.86 ± 2.29^a	12.34 ± 0.45^a	11.66 ± 2.35^a	11.46 ± 0.57^a
5-O-feruloylquinic acid	0.02 ± 0.01^a	0.04 ± 0.02^a	0.04 ± 0.02^a	0.04 ± 0.01^a
3,4-di-O-caffeoylquinic acid	0.11 ± 0.04^a	0.09 ± 0.00^a	0.04 ± 0.00^a	0.07 ± 0.02^a
3,5-di-O-caffeoylquinic acid	1.90 ± 0.41^a	1.86 ± 0.10^a	1.72 ± 0.57^a	1.72 ± 0.13^a
4,5-di-O-caffeoylquinic acid	0.86 ± 0.24^a	0.76 ± 0.05^a	0.78 ± 0.18^a	0.67 ± 0.04^a
total caffeic derivatives	18.66 ± 3.00^a	16.63 ± 0.63^a	15.79 ± 3.42^a	15.33 ± 0.76^a
total polyphenols	18.83 ± 3.05^a	16.79 ± 3.21^a	15.94 ± 0.65^a	15.42 ± 2.15^a

4. Discussions

The potential benefits of adding biochar in the soil as an amendment are still under discussion, numerous works on various crops have been published but with contrasting results. As several meta-analyses (Crane-Droesch et al., 2013; Jeffery et al., 2017, 2011; Liu et al., 2013) underlined, the mean crops yield increased with biochar addition in a range between 10 and 13%. These results are confirmed by the observations of Biederman and Harpole (2013) who analyzed 371 independent studies reporting a significant increase in productivity and crop yield after biochar addition, compared with untreated soil. Anyway, the positive effects of biochar as soil amendment have been found mainly in acidic soils, due to the increase of pH, nutrient retention and water holding capacity (Jeffery et al., 2017, 2011).

The responses of crop yield and biomass production to biochar addition vary with crop type. Important crops such as rice, wheat, maize and soybean significantly increased their productivity with biochar application in some countries (Jeffery et al. 2017). As exposed by some authors (Haider et al., 2020; Jeffery et al., 2017) in a recent meta-analysis of 105 studies revealed that the addition of high doses of pure biochar, above 10 t ha^{-1}, brings to no yield improvement in temperate latitudes. Some studies performed in Tuscany (Baronti et al., 2010; Vaccari et al., 2011) showed positive effects of biochar addition to soil up to 30% on wheat yield, while others (Tammeorg et al., 2014) reported that the addition of biochar, in an open field trial in Finland, did not significantly affect crop yield or quality of wheat. Similar results were obtained by Olmo et al. (2014) who observed that biochar addition did not affect grain quality of wheat if compared to untreated control. Despite various works reported an increase in crop productivity of various species after biochar application, alone or combined with fertilizers (Jeffery et al., 2011), we did not observe any positive effects in the open field trial with wheat and sunflower, neither on the yield nor on the height of the plant or their above-ground biomass.

Even at the maximum dose of 20 t ha^{-1}, that can be considered for itself impracticable for the majorities of farmers, we also did not observ significant changes in the pH, EC, CEC and WHC of amended soil. In fact, at the end of the experiment the pH and EC values of the amended soils were comparable to those of the control, showing a high buffering capacity of the soil. Our results are in line with those obtained by Paneque et al. (2016) in a similar study on sunflower in a Calcic Cambisol (pH=8.5), representing a typical agricultural soil of the Mediterranean basin, where they observed no significant differences in soil pH added with different doses of biochars (pH ≥10) from various feedstocks at a maximum dose of 15 t ha^{-1}. Despite the high EC of the biochar used for the field trial of our experiment, the EC of amended plots, including the maximum dose, was lower than the value of 270 μs cm^{-1} that are recommended for most common crops such as sunflower (Paneque et al., 2016). The excessive salt concentration could be a possible negative factor for plant growth and health, bringing to a reduction of crop yield. However, our results suggest that adding biochar, even at high doses, do not change significantly the EC of amended soil.

Regarding the WHC of soil samples, our results are in contrast with the observations made by Verheijen et al. (2019) who reported that the effect of biochar on WHC is usually positive, and with the work by Paneque et al. (2016), who observed that the application of 15 t ha^{-1} of biochar increased significantly the WHC of the soil. However, the effects are strongly related to the type of soil, the applied biochar dose and the feedstock, with sandy soils showing the higher improvement and wood-derived biochar the most performant feedstock. Otherwise, Cooper et al. (2020) in a study on biochar and composted-biochar as soil amendments, with application rates of 9 and 70 t ha^{-1}, observed that biochar significantly increased soil pH. In our experiment no differences between soil samples were observed for the CEC, also at the maximum application rate. Jeffery et al. (2011), in a statistical meta-analysis on the relation between biochar and crop productivity, either yield and biomass produced, reported that the major positive effects were obtained in acidic and neutral soils. These results suggested that some of the mechanisms involved in yield increase could be the liming effect and the rise in the water holding capacity of the soil, after biochar application, and also an improved nutrients availability. Same authors observed that the major positive effects were observed when biochar was added at a rate of 100 t ha^{-1}, anyway they also observed an increase in crop productivity even at application rates of 10, 25, and 50 t ha^{-1}. These application doses are, from an economic point of view, completely unfeasible for farmers. In fact, as Haider et al. (2020) pointed out, adding biochar at $\geq$ 10 t ha^{-1} is economically challenging since the yield increase, when occurs, does not necessarily cover the investment.

Total soil organic C represents one of the most important indicators of soil quality (Andrews et al., 2004; Laird et al., 2010) and C sequestration in soil represents one of the main environmental benefit coming from biochar application. In our field experiment the addition of the maximum dose of biochar (20 t ha^{-1}) significantly increased the total C content, measured after 10 months from the application. But, given the actual policies, the little increase in total C is not feasible from an economic point of view being the application costs not justified by the possible benefits.

Regarding the quality of harvested wheat seeds, as expected, we observed higher percentage of proteins in the ancient varieties Verna and Senatore Cappelli, it’s known that ancient wheat cultivars differ from modern ones also in terms of protein content (Giunta et al., 2020). The protein content is an important factor that is strictly related to grain quality and moreover contributes the most to the EU Quality Index for durum wheat (Giunta et al., 2020). Factors that can affect grain protein percentage are the genotype, environment, management and the interaction of these factors. Between the environmental conditions, drought and temperature contribute the most to determining variations in wheat quality traits (Giunta et al., 2020). Despite the impacts that biochar can have on soil albedo and water availability to plants, we observed no differences on protein content of the four varieties investigated and no differences on carotenoids concentration, both in middlings and bran, of cv Verna.

Wheat bran represents a good source of polyphenols, which strongly contribute to the total antioxidant activity. Polyphenols are secondary metabolites of plants and are usually involved in defense against ultraviolet radiation or attacks by plant pathogens. The antioxidant activity in wheat bran is highly correlated with its total phenolic content (Verma et al. 2008). Phenolics, in fact, have an ability to act as radical scavengers. In a study on modern and ancient varieties of *T. durum* and *T.*

aestivum, Heimler et al. (2010) observed that atmospheric conditions are the main factors which causes differences in free polyphenols and antiradical activity of wheat. Polyphenol synthesis and accumulation is usually improved in response to biotic or abiotic stresses (Ramakhrisna and Ravishankar, 2011) who also reported that salinity of soil is one of the factors that can bring to an increase of polyphenols content in plant tissues. The most present phenolic compounds in cereals are phenolic acids and flavonoids, which act as antioxidant (He and Giusti, 2010) and are the largest group of naturally occurring polyphenols (Žilić, 2016). In our work we observed an increase of total polyphenols of the middlings of cv Verna in B3 and B2 treatments, with a significant difference with the control and the lowest biochar dose B1. Differently, if we analyze the bran samples, B1 and B2 showed the highest results. Sample B3 also showed the highest value for the flavonoids and the caffeic acid derivatives present in the bran. Factors that can influence total flavonoids are the genotype, the environment and the genotype-by-environment interaction. Also soil nitrogen is able to affect flavonoids content of vegetables and fruit, in particular a higher nitrogen supply would lead to a decrease in the total polyphenols content (Heimler et al., 2017). The biochar used in our field experiment is poor in available inorganic nitrogen, so any differences, if presents, in the polyphenols content of seed samples cannot be explained by biochar nitrogen supply. Shamloo et al. (2017) suggested that some abiotic factors, such as high temperature, can force wheat plants to produce an higher amount of flavonoids as a defense mechanism against the environmental changes. Several are the studies that confirm the relationship between the flavonoids biosynthesis and abiotic stresses (Khlestkina, 2013). For example, the excessive moisture of soil at some stages of cereals growth can induce some changes in flavonoids concentrations, as they can prevent negative effects of excessive moisture. Biochar is commonly reported to improve soil moisture, although we did not find a significant increase of WHC after biochar addition.

Regarding the antiradical activity of Verna flours samples, bran samples showed strong DPPH free radical scavenging activities if compared with middlings samples. These results are in line with those of Liyana-Pathiran and Shahidi (2007), who found that the ability to scavenge DPPH radicals in wheat fractions was higher in bran than in other fractions such as feed flour, whole grain and flour. The same authors reported that the concentration of bioactive constituents was higher in the external layers of wheat grain; moreover, they observed that the bran fraction alone demonstrated a higher antioxidant activity than that of other milling fractions. Our results showed that total polyphenols concentration is higher in the bran than in middlings, confirming this trend. In our study the addition of biochar did not affect the quality parameters of wheat cvs., not even the nutraceutical compounds of cv. Verna and the polyphenols content of sunflower seeds, also when added at the highest dose.

According to numerous papers, the antioxidant activity of sunflower seed is related to phenolic compounds (Adeleke and Babalola, 2020), such as caffeinic acid derivatives. Taking into account the compounds isolated from sunflower seeds, analyzing kernel and tegument together, no differences in the concentrations between the various treatment were observed. Actually, even if not significant, the results showed a decreasing trend in total caffeic acids and polyphenols as the dose of biochar increased.

5. Conclusions

Our results suggested that the use of biochar as soil amendment can be feasible also at high doses for the cultivation of wheat and sunflower without negatively affecting the soil chemical and physical properties, and the vegetative and qualitative parameters of crops. Apart from soil carbon sequestration, we did not find any significant advantage coming from biochar application to soil. Therefore, the latter can represent a positive strategy for the use of some agricultural wastes as by-product of syngas production only if they imply disposal costs. Differently, the use of biochar at high doses for soil amelioration is not a viable solution under an economic point of view. The possible positive performances on crop productivity that can be obtained, are not justified by the high costs necessary for the application of biochar at high doses.

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Appendix

Photo 1. Biochar from wood-pruning as growing medium.

Photo 2. Nursery trial pots with tomato seedlings.

Photo 3. Tomato seedlings grown in (from left to right): pure chitin, 60% of chitin and 30% of chitin

Photo 4. Basil seedlings growing in potting substrates after 15 days from transplanting

Photo 5. Basil plants at the end of the experiment. From left to right: control, 25% biochar, 50% of compost and 25% of compost.

Photo 6. Open field trial site at Tenuta di Cesa (Arezzo) after biochar addition in the plots.

Photo 7. Ancient wheat cultivar Verna grown in untreated control just before harvesting.

Photo 8. Field plots of Sunflower hybrid P64HE39 in August.

Photo 9. Soil plot with the maximum dose of biochar (20 t ha^{-1}) just before the harvesting of sunflower. Biochar particles can be seen on soil surface.

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