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Biochar effects on early decomposition of standard litter in a European beech forest (northern Italy)

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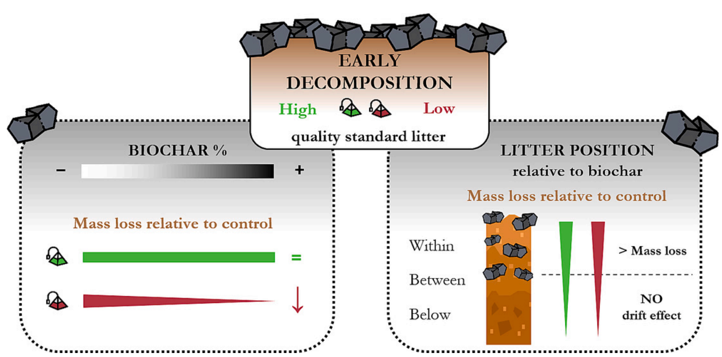
HIGHLIGHTS

- Biochar applications did not increase overall decomposition of standard litter.
- High-quality standard litter decomposition showed negligible responses to biochar applications.
- Low-quality standard litter decomposition decreased as biochar concentration increased.
- Standard litter decomposition was affected by sample position relative to the biochar layer.

GRAPHICAL ABSTRACT



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ABSTRACT

The release of biochar (BC) on forest soil is a strategy aimed at increasing carbon reserves and forest productivity. The effect of BC amendments on the decomposition of different quality litter is, however, poorly understood. With this study we investigate the effects of wood-derived BC applications on early decomposition in a European beech (*Fagus sylvatica* L.) forest through the burial of standard material, i.e. green tea and rooibos tea (high- and low-quality litter surrogates, respectively). Two main questions were addressed: 1) Do BC applications influence the decomposition of high- and low-quality standard litter and, if so, in what way? and 2) Does this effect (if measurable) depend on where the sample is placed with respect to the BC application layer? To test BC amendment effects, four application percentages were employed (0, 10, 20 and 100 %), after which standard litter mass loss was recorded. To investigate the effects of sample position, only three BC application percentages were used (0, 10 and 20 %), with teabags buried at three different depths – within the BC amended layer, between this layer and the unamended soil, and below the latter. Results show that early decomposition of high-quality standard litter was not influenced by BC applications, while a significant reduction in mass loss of low-quality standard litter was observed when the percentage of BC application was higher, specifically of litter

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within the 20 % and 100 % BC amended layers. Decomposition was also affected by sample position relative to the BC layer, exhibiting higher levels of mass loss when samples were placed within the BC amended layer. Overall, BC applications on beech forest soils not only seem to produce negligible effects on the early decomposition rate of high-quality standard litter, but such applications also seem to have the ability to reduce carbon loss following plant material degradation.

1. Introduction

Forests represent the largest carbon (C) sink on land, most of which is stored in soil (Ameray et al., 2021). Timber harvesting operations, however, can significantly affect this important C reservoir in surface soil (up to about 36 cm; James and Harrison, 2016), thereby reducing the overall capacity of forest soils to store C and accumulate nutrients (Achat et al., 2015a; Achat et al., 2015b; Jandl et al., 2007) – a process that is exacerbated when deforestation occurs (Guo and Gifford, 2002). Globally, the negative effect of timber removal on soil C stock is most evident in tropical forests, where logging was found to generate a ca. 8 % reduction in soil C content for the period 1990–2007 (Scharlemann et al., 2014). Regarding Europe, an example of this effect can be seen in the ca. 37 % estimated reduction of C content in Italian forest soils in the 1990s, probably due to the intensification of forest management practices (Fantappiè et al., 2010).

Forest C stocks are also threatened by ongoing climate change and the resulting increase in the frequency of extreme events such as droughts, fires, strong winds and pest outbreaks (Seidl et al., 2017). In the light of this, the reduction of deforestation, the extension of forest harvesting cycles and the promotion of reforestation are key management strategies with which to limit C loss from forest ecosystems (vonHedemann et al., 2020; Ameray et al., 2021). In particular, with reference to the soil compartment, the application of a “soil C sequestration strategy” (Paustian et al., 2019), such as the pyrolysis of wood into biochar (hereafter BC) (ICHAR, 2021) and its subsequent reincorporation into forest soils (Sohi and Kuppens, 2016), appears to be extremely effective in protecting forest soil C stock. Through pyrolysis, which consists of the thermal decomposition of organic matter in the absence of oxygen, wood is de-facto converted into an aromatic, C-rich (>60 %) product. This is characterized by high environmental stability (Conte et al., 2021), lasting even thousands of years (Spokas, 2010), and represents a substrate capable of promoting both C stock within the soils into which it is released (Ohtsuka et al., 2021) and atmospheric CO_2 sequestration (Smith, 2016). In addition, as a result of BC ability to improve both water and nutrient retention of forest soils (Li et al., 2018a), in some cases it can also promote forest growth (Thomas and Gale, 2015; Bruckman and Pumpanen, 2019; Ohtsuka et al., 2021; Vannini et al., 2022), thus increasing a forest’s ability to sequester atmospheric CO_2 and store C through an increase in forest biomass.

Although in the majority of cases BC application on soils seems to have positive effects on forest productivity, nevertheless there are some “ecological drawbacks” to this practice. In fact, on forest soils, BC applications may modify the activity, abundance and composition of soil microorganisms (Gorovtsov et al., 2020), thus generating alterations in soil organic matter decomposition, i.e. soil respiration (CO_2 emission), with some studies recording positive responses (Zhou et al., 2017; Bruckman et al., 2015; Zhu et al., 2020; Palviainen et al., 2018) whereas negative (Walkiewicz et al., 2020) or even negligible BC effects (Cui et al., 2021; Sarauer et al., 2019) have been found in others. The application of BC on forest soils involves the release of this substrate above the litter layer, which constitutes the third largest C reservoir in European forests (ca. 8.4 %; State of Europe’s Forests, 2020) after soil C (ca. 54 %) and aboveground biomass (ca. 29 %). As a result, changes in litter layer decomposition rates may occur following BC applications which may, in turn, produce either a potential advantage in terms of C sequestration when decomposition rates are reduced, or a potential disadvantage when an acceleration of these rates occurs. At present, studies addressing the effects of wood-derived BC on plant materials of different quality (i.e. different decomposability as a result of different tissues and chemical characteristics) within the same ecosystem are scarce. The studies conducted so far have only investigated the effect of BC amendments on either high-quality litter alone or composite litter consisting of both low- and high-quality material, but they have never focused on the effect of this product on the two types of litter in a single comparative study. Studies have shown, for example, that BC applications (10 t/ha) enhanced the decomposition rate of fresh and old *Quercus serrata* L. leaves which fell above and below the BC application layer, respectively (Minamino et al., 2019), but generated either positive or negligible effects on the decomposition of both shoots and leaves of *Phleum pretense* L. buried in sandy till and medium-fine sandy soils, respectively (−3 cm from the soil surface; Saarnio and Kettunen, 2020). Negligible effects of BC applications (ca. 20 t/ha; different wood-derived origin) were also observed for *Triticum aestivum* L. litter buried in silty-loam soils (−15 cm from the soil surface; Weyers and Spokas, 2014).

The main aim of this study, therefore, is to investigate the effects of different application percentages of wood-derived BC amendments on both high- and low-quality litter by burying standard materials, i.e. green tea and rooibos tea, in a European beech (*Fagus sylvatica* L.) forest. Teabags were employed as an index of litter decomposition (Keuskamp et al., 2013) as this material is widely used to assess the effects of environmental changes on biogeochemical processes (Djukic et al., 2018; Houben et al., 2018; Certini et al., 2023). The current study addresses two main questions: 1) Does standard litter decomposition undergo any changes when placed within the BC amended layer? and 2) Can this effect on decomposition (if measurable) also be transferred beneath the BC amended layer? To answer these questions, we tested four BC applications (0, 10, 20, 100 %) and three burial positions relative to the BC application layer (within the BC amended layer, at the interface between this layer and the unamended soil, and below the latter). Specifically, the first three BC amendments (0, 10 and 20 %) were tested to assess their effectiveness in improving the C stock of the Apennine forest soils, already known to generate no negative effects on tree seedlings (Vannini et al., 2022). The 100 % BC amendment was, on the other hand, implemented to test the most extreme condition. Different burial positions of samples were implemented to detect potential (short-term) changes in decomposition for litter placed below the BC amended layer.

2. Material and methods

2.1. Study site

The study was carried out in a beech forest (44° 23′ 59.7” N, 10° 02′ 08.5″ E) in the Tuscan-Emilian Apennines (Parma, Italy). The study site is characterized by a flat (<5 % slope) and geomorphologically uniform area located ca. 1200 m a.s.l. The site, included in the Tuscan-Emilian Apennines National Park, develops on sandstone belonging to the Macigno Formation. The climate in the area is temperate oceanic (Pesaresi et al., 2017) and is characterized, specifically above 1000 m a.s.l., by a mean annual temperature of ca. 5 °C and mean annual precipitation of 1500 mm, with the warmest mean monthly temperature (ca. 16 °C) being recorded in July (Antolini et al., 2016). The main vegetation types in the montane belt between 1000 and 1700 m are broadleaf deciduous forests dominated by European beech (Ubaldi et al., 1993). Beech forests, such as the one in the present study, have an important role in national forestry, representing almost 12 % of the national forested area (INFC, 2022).

2.2. Experimental design

The study was carried out in 2021 (June–September) and consisted of a manipulation experiment involving the addition of BC to forest soil, produced using wood derived from deciduous broadleaf forests located in the Tuscan-Emilian Apennines (Vannini et al., 2022). Regarding the chemical and physical properties of BC and soil, details are reported in Vannini et al. (2022). Table 1 summarizes the mean pH, surface area and particle size of the BC used. The organic matter content in the BC used, measured after the treatment of the BC samples in the muffle furnace for 24 h at 450 °C, was ca. 96 %.

As reported in Fig. 1, five blocks (replicates), with a surface area of ca. 25 m^2 (5 m × 5 m) each, were selected within the site. Seven 1 m × 1 m plots identified in each block were used to test the effects of seven different treatments on early litter decomposition. This was estimated using purchased standard litter, specifically green and rooibos teabags (Lipton tea, Unilever; green tea EAN 8722700055525, rooibos tea EAN 8711327514348), hereafter named high- and low-quality litter, respectively.

Teabags are, in fact, an economic and ready-for-use plant material widely used in studies investigating litter decomposition dynamics in different ecosystems (Djukic et al., 2018; Petraglia et al., 2019). Not only do teabags avoid the use of local litter but they also avoid bias following the use of plant species with different traits driving decomposition responses (Cornwell et al., 2008). Different lignin (Pengilly et al., 2008; Al Hroob et al., 2019) and nitrogen content (see Keuskamp et al., 2013 for details) in green tea (*Camellia sinensis* L.) and rooibos tea (*Aspalathus linearis* Burm.f.) underlies the quality of litter, i.e. its resistance to microbial degradation. Each teabag employed in our study contained ca. 2 g of tea enclosed in non-woven, 0.2 mm-mesh material.

Treatments were divided into two groups in line with the questions addressed by the study. In order to understand whether BC-amended soils influence the decomposition of litter after its burial within a BC-amended layer, four treatments were carried out for the first group, consisting of 20 cm-deep holes (soil horizon A) filled with the same soil amended as per treatment: with wood-derived BC at 0 % (i.e. only soil), BC at 10 %, then 20 % and 100 % (*v/v*). It is worth noting that the natural soil along the entire excavated profile was dark-coloured, without clear discontinuities in either structure or texture. In each treatment, two teabags for each tea type (green and rooibos) were buried at −8 cm within the modified soil (hereafter, 'within' samples). In order to investigate whether the effect of BC (if measured) could also be transferred to the litter positioned below the BC amended layer (BC drift effect), a second group of treatments was carried out, consisting of 8 cm-deep holes (soil horizon A) dug in each of the remaining three plots, which were then filled with native soil and subsequently covered with a 8 cm-deep soil layer amended with BC at 0 % (only soil), 10 % and 20 % BC (*v/v*). During this procedure, in each treatment two teabags for each tea type were placed in two different positions relative to the amended soil layer: 1) at the bottom of the 8 cm hole before this was filled (hereafter, 'below' samples); and 2) on top of the filled hole but just below the 8 cm amended soil layer (hereafter, 'between' samples). Before digging the holes or placing litter bags on the soil, the entire layer of litter (ca. 2 cm depth), consisting mainly of beech leaves, was removed from the plot, with the original litter layer placed back on the plot after teabag burial. Total rainfall recorded by the meteorological station closest to the study area (Corniglio, Parma, 44°29′14.6"N 10°05′57.2″E) was 99 mm and mean soil temperature was ca. 14 °C (hourly records using HOBO Pendant sensors, one replicate per treatment; Table 2).

Table 1
Mean values of pH, surface area and particle size of the BC used. Results of the chemical analysis area reported in Vannini et al. (2022).

Parameter	Value
pH	8.5
Surface area (m^2/g)	213.3
Particle size (%) <5 mm	90
Particle size (%) <2 mm	70
Particle size (%) <0.5 mm	24

2.3. Mass loss calculation

In June, each teabag was weighed before burial to estimate initial air-dried mass. In September, teabags were retrieved and processed in the lab. BC particles and plant roots were removed from the samples, which were then oven-dried at 65 °C for 48 h and finally weighed to obtain final oven-dried mass.

Decomposition was estimated as mass loss percentage (ML %) by applying the following equation:

$$\mathrm{ML}\ (\%) = \frac{\mathrm{M_0} - \mathrm{M_f}}{\mathrm{M_0}} \times 100$$

where M_0 and M_f represent the initial and final dry mass, respectively. Initial dry mass (M_0) was estimated by multiplying the initial air-dried mass of each teabag by the ratio between air- and oven-dried mass, which was obtained using an independent (unexposed) subset consisting of 15 teabags for each type.

2.4. Data analysis

Data analysis was performed using R software (R Development Core Team, 2022) and packages *nlme* (Pinheiro et al., 2022) and *multcomp* (Hothorn et al., 2008). The effects of the four BC amendments (0 %, 10 %, 20 % and 100 %) on mass loss of "within samples" were assessed by means of linear mixed effects models for green and rooibos tea separately. In these models, mass loss was the response variable, treatment was the fixed effect (4-level predictor) with block and plot considered as random factors. In the case of significant treatment effects on mass loss, differences among treatments were assessed by applying the Tukey's post-hoc test.

To investigate potential BC drift effects, the influence of litter sample position ("within", "between" and "below") and BC amendment (0 %, 10 % and 20 %) on mass loss was evaluated for green and rooibos tea separately. In these models, mass loss was the response variable, whereas position (3-level predictor), treatment (3-level predictor) and their interaction were considered as explanatory variables.

The best random effect structure was selected by means of the Akaike information criterion (Zuur et al., 2009) with a random intercept for 'plot' included in all the models. Linear model assumptions were verified by graphical observation of model residuals. Due to heterogeneous variances among sample position, the model investigating BC drift effects for green tea included a variance structure which allowed for different variation in mass loss with respect to sample position.

3. Results

Fig. 2 summarizes mass loss results for high- and low-quality litter buried within the four investigated BC amended soil layers. High-quality litter showed no changes in mass loss when buried within the soil amended with the three different BC application percentages (10 %, 20 % and 100 % BC) when compared to control samples (only mixed soils; C) ($p > 0.05$; $F_{3,16} = 2.5$). On the other hand, low-quality litter showed decreasing mass loss when the BC application percentage was increased ($p < 0.001$; $F_{3,16} = 31.8$). In detail, when compared to mass loss in control (mean 33.1 %), low-quality litter exhibited a decrease in mass

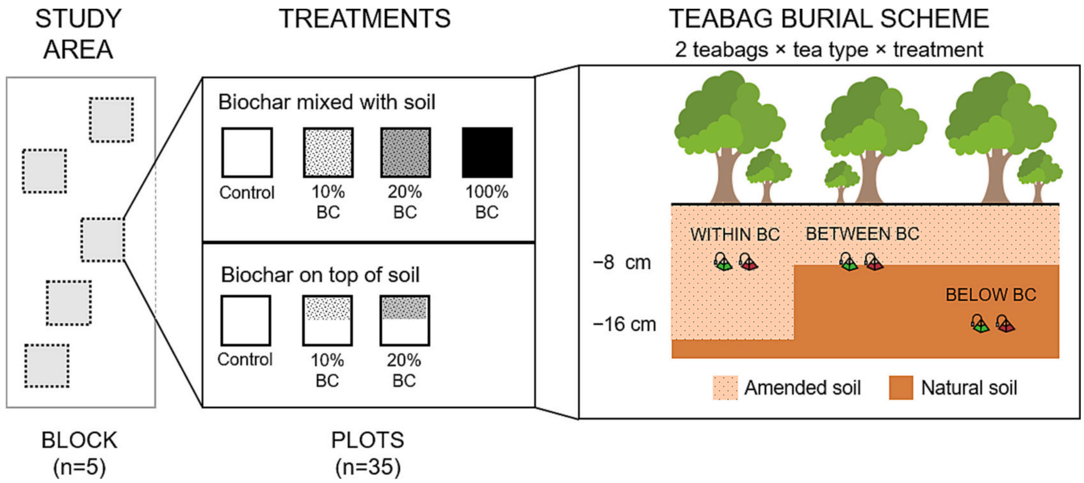

Fig. 1. Scheme illustrating the experimental design.

Table 2
Mean soil temperature and standard error recorded in each treatment during the three-month experimental period: C (BC 0 %, only soil), BC10 (BC 10 %), BC20 (BC 20 %), and BC100 (BC 100 %; only biochar). Teabag position relative to the BC amended layer is specified, with treatments grouped accordingly: "Within BC", teabags incubated in BC; "Between BC", teabags incubated between the BC amended layer and unamended soil; "Below BC", teabags incubated in unamended soil, just below the BC amended layer.

Teabag position	Treatment	Mean (°C)	Standard error
Within BC	C	14.1	0.03
	BC10	13.8	0.02
	BC20	14.3	0.03
	BC100	14.8	0.04
Between BC	C	14.8	0.04
	BC10	14.6	0.03
	BC20	14.9	0.03
Below BC	C	14.1	0.03
	BC10	13.9	0.02
	BC20	14.3	0.03

loss following the application with 20 % BC ($p = 0.039$) and 100 % BC ($p < 0.001$), with significant differences between the treatment with 20 % and 100 % BC also detected ($p < 0.001$).

Fig. 3 shows mass loss responses for high- and low-quality litter buried within, between and below the BC amended layer (0 %, 10 % and 20 %). The effects of BC amendments on mass loss of high-quality litter (Fig. 3a) were not consistent across the sample positions (significant interaction term: $p = 0.033$; $F_{4,60} = 2.8$), with a greater difference between control and BC amendment for the samples located within the amended soil. However, when the three different positions were considered, the treatment effect on high-quality litter was not significant ($p = 0.054$; $F_{2,12} = 3.8$). Regarding low-quality litter (Fig. 3b), treatments did not significantly influence mass loss ($p = 0.112$; $F_{2,12} = 2.6$) and no interactive effect on decomposition was found between treatment and litter position ($p = 0.673$; $F_{4,59} = 0.6$). Litter position alone, nevertheless, significantly affected decomposition of both high- and low-quality litter ($p < 0.001$; $F_{2,60} = 26.6$, and $p < 0.001$; $F_{2,63} = 17.6$, respectively), with higher mass loss recorded for samples incubated in the BC amended layer compared to those located between and below the BC layer. Additionally, but only with regard to low-quality litter, samples located between the BC amended layer exhibited higher mass loss compared to samples located below this layer (Tukey post-hoc test: $p = 0.006$).

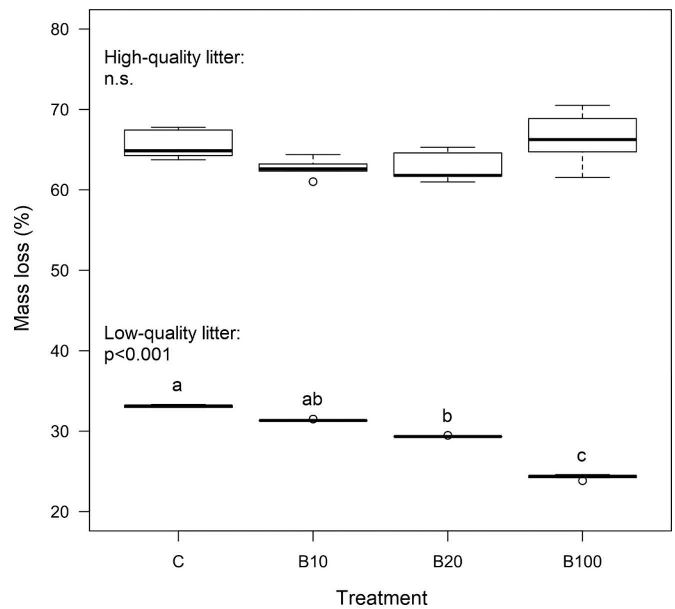

Fig. 2. Mass loss of high- and low-quality litter buried within four different BC amendments: C (control, BC 0 %, i.e. only soil), BC10 (BC 10 %), BC20 (BC 20 %), and BC100 (BC 100 %; only biochar). Different letters indicate statistically significant differences between treatments ($p < 0.05$).

4. Discussion

Decomposition experiments are key to understanding the effects of environmental variables – edaphic factors and/or vegetation – on litter decomposition (Djukic et al., 2018; Petraglia et al., 2019; Kwon et al., 2021). In addition, such experiments are an essential tool for studying the impact of different types of soil amendments on the organic matter content of agricultural soils (Duddigan et al., 2022). In particular, BC applications represent a cost-effective strategy for both improving crop productivity (Palansooriya et al., 2019b) and forest tree biomass (Thomas and Gale, 2015), and also promoting soil C stock (Ohtsuka et al., 2021). Despite this, the effect of BC applications on the decomposition of high- and low-quality forest litter is still poorly understood. The results of this study provide a first indication of the effect of wood-derived BC on the decomposition of forest plant materials of different quality, i.e. high and low quality, by using green and rooibos tea as standardised reference material.

As far as high-quality standard litter is concerned, in our study BC

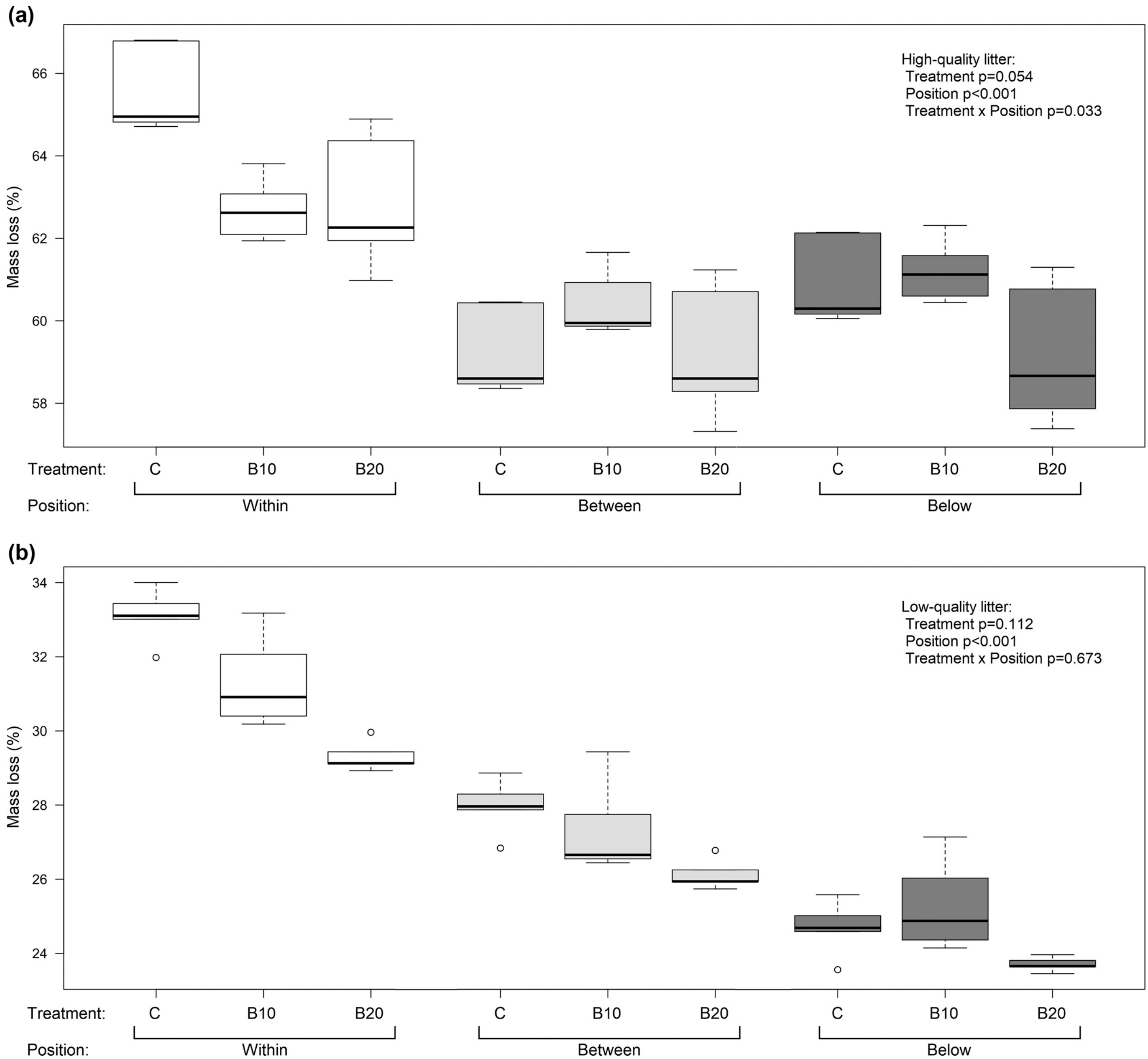

Fig. 3. Mass loss of high- (a) and low-quality litter (b) buried within three different BC amendments, i.e. C (control, BC 0 %, i.e. only soil), BC10 (BC 10 %) and BC20 (BC 20 %), as well as at three different burial positions relative to the BC application layer (within, between and below).

additions from 10 % to 100 % (*v/v*) did not influence mass loss, either when samples were placed within the modified layer or at different burial positions relative to the BC application layer. Considering leaves are the main source of high-quality litter in European beech forests, this result could be seen to indicate that wood-derived BC has only a negligible effect on the early decomposition of European beech leaves. However, it is widely acknowledged that the relationship between the degradation patterns of natural leaf litter and those of green tea is not straightforward (Didion et al., 2016). In fact, although a similarity might be expected, these two matrices not only have different lignin content (Brinkmann et al., 2002; Al Hroob et al., 2019) but also exhibit different C:N ratios (Keuskamp et al., 2013; Didion et al., 2016). Despite this, Didion et al. (2016) found that decomposition dynamics of beech leaf litter in an upland ecosystem (Northern Limestone Alps) were very similar to high-quality standard litter, as were those of *Pinus nigra* leaf litter and low-quality standard litter. Although this study did not specifically analyse the chemical profile of the tea used or that of natural litter, green and rooibos tea could be considered as being similar, to a certain extent, to beech leaves and twigs, respectively.

The absence of any significant effect of wood-derived BC on the early, natural decomposition of high-quality litter in European beech forests obviously refers to the specific parameters of this study, i.e. the soil type tested, the season investigated and the duration of the experiment (three months). However, high-quality litter samples exhibited overall decomposition values consistent with those measured in other European beech forests (Certini et al., 2023), possibly indicating that the decomposition rate of high-quality litter in the northern Apennines is fairly constant. Furthermore, decomposition of beech leaves, as well as the activity of soil microorganisms, does not seem to be related to stand elevation, which affects soil temperature, but rather to soil moisture levels (Gavazov et al., 2014). It is known, in fact, that BC applications increase soil water retention capacity in proportion to the percentage of BC application (Pandian et al., 2016), a pattern which has also been observed in an ongoing experiment in the same northern Apennine beech forest site (data not shown). However, based on the results presented in this study, it seems that soil microorganisms were not affected by such an increase in soil moisture levels.

On the other hand, mass loss of high-quality litter was significantly influenced by sample position relative to the BC amended soil layer, with samples buried within the BC amended layer exhibiting the highest decomposition values; a similar response pattern was also found for low-quality litter even though the decomposition showed a clearer mass-loss negative trend as burial depth increased. The higher mass loss of litter buried within the BC-modified layer may have been caused by its closer proximity to the soil surface, where oxygenation is greater. Assuming, therefore, that similar moisture conditions were present for litter buried within, between, and below the BC layer, the increased litter decomposition for samples buried "inside" the modified layer could be the result of a favourable combination of moisture and oxygenation, as already hypothesized by Fanin et al. (2020). In line with our observations, decomposition of wood stakes of aspen (*Populus tremuloides* Michx.) and pine (*Pinus taeda* L.) was lower for samples located on the soil surface when compared to those buried within forest soils in California and Montana (Page-Dumroese et al., 2019; Jurgensen et al., 2020).

Under constant moisture conditions, aeration and soil temperature are two of the most important factors for soil respiration (considered here as a proxy for soil organic matter loss) (Lloyd and Taylor, 1994; Sierra and Renault, 1996; Vor et al., 2003). However, the limited variation in soil temperature recorded at different burial locations in our study, could indicate a weak contribution of this variable to increased decomposition.

Regarding low-quality litter, BC applications reduced levels of decomposition, but only starting from the 20 % BC amendment, with an additional mass loss decrease of 5 % when litter was incubated in a BC-only matrix (100 % BC). Overall, all BC applications (i.e. from 10 % to 100 % BC) generated an average reduction in decomposition of ca. 5 % when compared to control samples.

According to Fanin et al. (2020) decomposition of low-quality litter is mainly driven by nutrient availability and/or biotic interactions with microbial communities, suggesting that the reason behind the decrease in low-quality litter decomposition as a function of the BC application percentage may be due to the impact of BC in changing i) the nutrient level in the soil and/or ii) the activity of soil microorganisms.

Regarding potential BC effects on soil nutrient levels, a comparison of P and K content in the used BC and in rooibos tea shows that the former has a higher nutritional level than the latter, with the concentration of assimilable P and exchangeable K on the BC surface (Vannini et al., 2022) being higher than that recorded within rooibos tea samples following total chemical digestion (Houben et al., 2018). As far as N is concerned, the amount was not measured within the used BC. Nevertheless, it is generally reported that N concentration in wood-derived BC seems to be lower, i.e. <1 % N (Leng et al., 2020), than that measured within rooibos tea samples, i.e. ca. 1.1 % N (Keuskamp et al., 2013). The greater presence and/or accessibility of nutrients such as P and K on the surface of BC compared to that contained in rooibos tea may, therefore, have "rewritten the interest" of the microbiological soil pool from litter to BC, at least in the early stages of decomposition. In this way, the 'attractiveness' of low-quality litter samples when compared to that of BC may have been reduced. Consequently, increasing the BC application rate may have reduced the interest of soil microorganisms for this type of substrate, with (proportionally) less decomposition of low-quality litter taking place. In line with this, the total fungal population would be more present on substrates with higher C:N ratios, i.e. higher C content, than on those with lower ratios (Rousk and Bååth, 2007). Differences in nutrient composition between BC and the litter employed in this study could also explain the negligible effect of BC applications on the decomposition of high-quality litter. In fact, green tea contains three times more N than rooibos tea (Keuskamp et al., 2013) and ca. five and seven times more K and P, respectively (Houben et al., 2018). As a result, we can presume that green tea samples contain substantially higher amounts of N, K and P even compared with the tested BC. In this case, the bacterial population may have been more 'attracted' by nutrients contained within high-quality litter than those nutrients present on the BC surface. In effect, soil bacterial populations seem to be more present on substrates having a low C:N ratio, as is the case with green tea (Keuskamp et al., 2013), than those with a higher ratio (Rousk and Bååth, 2007), such as the BC used in our treatments.

As for P, which is a fundamental element underlying all forms of life, involved in the formation of molecules crucial for cell metabolism (i.e. the adenosine triphosphate), it is not surprising that increased soil P content simultaneously generates, up to a certain level (Cao et al., 2011), increases in soil respiration (Feng and Zhu, 2019) together with increases in the mineralization of soil organic C (Qualls and Richardson, 2000). In our study, P was probably the main element modifying the nutritional attractiveness for soil microorganisms, since N, although recognised as a limiting factor for litter decomposition (Berg and Matzner, 1997) especially in the early stages (Gill et al., 2021), exhibited a similar (estimated) content in both BC and rooibos tea samples. The amount of K may also have played a role in litter attractiveness because, although it does not appear to be a limiting factor for soil microbial activity (Mori et al., 2019), it has been reported that the amount of K is well correlated with soil microbial activity (Cheng et al., 2013) – added confirmation of the above hypothesis.

Soil microorganism activity may also have been affected by BC applications, explaining the decomposition reduction recorded in low-quality litter. It is well-known that fungi play an important role in lignin-rich litter decomposition (Habtewold et al., 2020), evidenced by the preferential colonization of fungi recently found for rooibos tea as compared to green tea, for which a greater colonization of prokaryotes was detected (Daebeler et al., 2022). If there were a selective effect of BC on microorganisms degrading low-quality litter, fungal activity could have been the driver behind this process. The ability of BC to change soil microorganism abundance, either positively or negatively (Yao et al., 2017; Palansooriya et al., 2019a), is a recognised fact, as are the parameters related to this process, such as variation in available nutrients, soil moisture and pH values. Regarding pH, in particular, the application of the tested alkaline BC (pH 8.5) on acid soils from the Tuscan-Emilian Apennines (pH 4.8; Vannini et al., 2022) may, in all likelihood, have increased overall soil pH (Vannini et al., 2022) and could, in turn, have led to a reduction in growth, development and activity of fungal populations. In line with this hypothesis, Rousk et al. (2009) have pointed out that fungal activity/growth is optimal at pH values between 4.5 and 5, whereas activity is severely limited at pH >5, thus reducing the ability of fungi to decompose litter. Furthermore, significant increases in fungal soil biomass following BC applications have only been found when biochar was applied to soils with a pH ≤ 4.5 (Li et al., 2020), even though exceptions are always possible. Reductions in soil fungal biomass have also been observed following the amendment of pasture soils (7.85 pH) with barley stover BC (7.21 pH), but in this case the occurrence of a negative effect was associated with the presence of toxic elements within the used BC (Yoo and Kang, 2012). Given the limited content of toxic compounds in the BC employed in the current study (reported by Vannini et al., 2022), it could therefore be possible that the increase in pH following the application of BC and its effects on fungal activity/growth is a key factor in limiting the decomposition of low-quality litter. Nevertheless, specific information on the abundance of both bacterial and fungal communities in both litter types and the soil profile is needed to clarify this aspect.

Although changes in pH values are recognised as a key variable for soil fungal activity (Lehmann et al., 2011), soil moisture and/or the immobilization of enzymes critical for lignin decomposition may also have played a role in the reduction of low-quality litter decomposition observed in this study.

The rate of decomposition of low-quality litter such as wood can, in fact, be regulated by soil moisture, with high moisture levels being found to slow down wood decay processes (Piaszczyk et al., 2022). The potential increase in soil moisture levels produced by BC applications (as explained above) may, therefore, have contributed to the limitation of low-quality litter decomposition in our study. It has to be noted, however, that no alteration in decomposition was found for high-quality litter, thus suggesting that soil moisture may have played only a minor role in the degradation of rooibos tea.

On the other hand, immobilization of enzymes critical for lignin decomposition (such as laccase; Brijwani et al., 2010) on the BC surface (Sanchez-Hernandez et al., 2019) may also provide an explanation for the reduction in the decomposition of low-quality litter following BC applications. In fact, Li et al. (2018b) have shown that different types of wood-derived BC are able to increase the immobilization of laccase present in wastewater from ca. 20 % to ca. 60 %. Similarly, Imam et al. (2021) have found that BC derived from rice straw is able to increase the immobilization of laccase by 66 % in aqueous solutions, albeit with pH-dependent immobilization efficiency, i.e. inversely proportional to alkalinity. In the light of these findings, therefore, the activity of laccase might have been reduced by BC in direct proportion to the percentage of BC applied, thereby reducing the decomposition of lignin contained in low-quality litter, leading to lower overall mass loss values in this type of litter.

Finally, no interaction was found between BC treatments and litter location for either high- or low-quality litter, indicating that even at greater depths there was no significant effect of the applications on litter decomposition, at least for the duration of the experiment. It is known that BC can readily percolate into deeper soil layers (Major et al., 2010), even over a relatively long period of time (e.g. one year; Obia et al., 2017). However, in the current study three months may not have been sufficient to transfer fine BC particles ($<$0.5 mm) and thus increase pH, at least in the area between the BC amended and unamended soil layers. Furthermore, even though no effects were detected in litter placed below the 20 % BC amendment in the current study, the possibility of low-quality litter decomposition occurring under an application layer composed of 100 % BC cannot be ruled out.

5. Conclusions

Wood-derived BC applications did not affect early decomposition of high-quality litter, but significantly reduced the decomposition of that of low-quality. This litter exhibited lower decomposition values as BC concentration was increased, although such a response was only detected when litter was buried within the BC amended layer. In fact, both litter types showed no changes in decomposition when litter was placed either between or below the BC amended layer, suggesting that decomposition of litter placed below BC applications was not affected by the BC percentage applied. On the other hand, mass loss was affected by litter position itself relative to BC amendments, with the highest values for samples buried within the BC amended layer. This was, in all likelihood, caused by soil mixing as well as the closer proximity of litter to the soil surface. Interactive effects between BC application percentage and litter position were observed only for high-quality litter. Overall, the application of BC on beech forest soils not only seems to produce negligible effects on the early decomposition rate of high-quality litter, but BC applications of this type also seem to have the ability to reduce C loss following plant material degradation. This means that BC amendments do not generate short-term effects on organic matter turnover in the investigated forest soils but, instead, could increase soil C stock in deciduous broad-leaved forests.

CRediT authorship contribution statement

Andrea Vannini: Formal analysis, Visualisation, Writing – Original draft preparation; **Michele Carbognani:** Formal analysis, Conceptualisation, Methodology, Writing – Original draft preparation; **Giorgio Chiari:** Data curation, Writing – Review and editing; **T'ai Gladys Whittingham Forte:** Data curation, Writing – Review and editing; **Margherita Rodolfi:** Methodology, Writing – Review and editing; **Tommaso Ganino:** Conceptualisation, Funding acquisition, Methodology, Supervision, Validation, Writing – Review and editing; **Alessandro Petraglia:** Conceptualisation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – Review and editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alessandro Petraglia and Tommaso Ganino report financial support was provided by European Agricultural Fund for Rural Development. Andrea Vannini reports financial support was provided by European Union - PON Research and Innovation 2014–2020.

Data availability

Data will be made available on request.

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