Fate of soil organic carbon and polycyclic aromatic hydrocarbons in a vineyard soil treated with biochar

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Abstract

The effect of biochar addition on the levels of black carbon (BC) and polcyclic aromatic hydrocarbons (PAHs) in a vineyard soil in central Italy was investigated within a two year period. Hydropyrolysis (HyPy) was used to determine the contents of BC (BC_{HyPy}) in the amended and control soils while the hydrocarbon composition of the semi-labile (non-BC_{HyPy}) fraction released by HyPy was determined by gas chromatography-mass spectrometry, together with the solvent-extractable PAHs. The concentrations of these three polycyclic aromatic carbon reservoirs, changed and impacted differently on the soil organic carbon over the period of the trial. The addition of biochar (33 ton dry biochar ha^{-1}) gave rise to a sharp increase in soil organic carbon which could be accounted for by an increase of BC_{HyPy}. Over time, the concentration of BC_{HyPy} decreased significantly from 36 to 23 mg g^{-1}, and as a carbon percentage from 79% to 61%. No clear time trends were observed for the non-BC_{HyPy} PAHs varying from 39 to 34 µg g^{-1} in treated soils, not significantly different from control soils. However, the concentrations of extractable PAHs increased markedly in the amended soils, and decreased with time from 153 to 78 ng g^{-1} remaining always higher than those in untreated soil. The extent of the BC_{HyPy} loss was more compatible with physical rather than chemical processes.

Introduction

Biochar is the carbonaceous residue from biomass pyrolysis that has been proposed as an amendment in agricultural practices to increase soil organic carbon (SOC) and to restrain the growth of atmospheric CO_2 through soil carbon sequestration.[1-4] The real or possible benefits and drawbacks of using biochar in agro-environmental management are being debated as experimental data emerge from field studies.[5-7] The knowledge of recalcitrance of carbon that can be sequestered in soil and the potential contamination from polycyclic aromatic hydrocarbons (PAHs) are crucial issues for evaluating the environmental impact of biochar and its role on SOC dynamics. For characterising SOC, the refractory fraction of biochar can be described as black carbon (BC), the pyrogenic carbon produced from the partial combustion of organic materials, including biomass and fossil fuels. In analogy to BC, the biochar matrix comprises complex assemblage of polyaromatic structures along with heteroaromatic components and alkyl moieties from thermally degraded biomacromolecules.[8-10] Although the inherent complexity of biochar makes comprehensive characterization extremely challenging, the degree of aromaticity and ring condensation have been considered to be key structural factors affecting thermal resistance and environmental recalcitrance.[11-12] It is often assumed that environmental recalcitrance is intimately connected with thermal stability associated with these structural factors (aromaticity and ring condensation)[13-15], thus thermo/chemical parameters have been proposed to classify the carbon sequestering potential of different biochars by a number of techniques, including thermogravimetry,[11,16] flash pyrolysis,[17,18] and hydropyrolysis (HyPy).[19-21]

Amongst these techniques, the characterization of biochar by HyPy is of interest because as well as quantifying BC in soils accurately,[22] it defines BC in terms of the smallest polyaromatic structures present. In HyPy, the sample mixed with a suitable catalyst is pyrolysed in the presence of hydrogen at high pressure to promote the reductive removal of thermally labile organic matter leaving a refractory carbonaceous residue. This residue, named as BC_{HyPy} [22] or stable polycyclic aromatic carbon (SPAC),[19] comprises polyaromatic units larger than 7 rings[20] and has an atomic

H/C ratio below 0.5.[22] The organic fraction that is evolved by thermal reductive cleavage, denoted as non-BC$_{\mathrm{HyPy}}$, revealed the presence of PAHs comprising ≤ 7 rings.[21] These non-BC$_{\mathrm{HyPy}}$ PAHs are supposed to be less permanent ("semi-labile") compared to larger PAH structures that constitute the BC$_{\mathrm{HyPy}}$ macromolecular network.[19] It is hypothesized that the semi-labile fraction comprises PAHs occurring in a variety of different interactions with the amorphous organic matter (e.g. biopolymers and humic substances) and the carbonaceous materials (e.g. BC) occurring in soil. The level of individual PAHs in biochar and soil of interest in environmental quality guidelines can be determined by extraction with appropriate solvents and analysis by gas chromatography-mass spectrometry (GC-MS). However, due to the strong interaction of PAHs with the carbonaceous matrix[23],only a minor fraction of extractable PAHs in biochar was found to be bioavailable.[24]

Clearly, the incorporation of biochar into soil systems represents an input of PAHs.[25] Besides of being a source, biochar may also act as sink for PAHs via adsorption.[26] Therefore, biochar could affect the persistence of PAHs in soils probably by reducing their bioavailability.[27]

Several laboratory incubation experiments have demonstrated the degradability of biochar and BC in soils or model systems and provided valuable information on the extent of mineralization in relation to char types[13,14,18,28] and load,[29] environmental conditions[30-32] and soil characteristics.[33-35]

The few studies in the literature reporting on the fate of biochar in field experiments have shed light on its role on SOC dynamics and highlighted the importance of the changes to soil properties that occur after biochar incorporation as well as the chemical and physical changes that biochar undergoes after soil incubation[36-39]. However, more investigations in field and with different techniques are needed to increase our knowledge on the impact of biochar on organic carbon pools in treated agricultural soils.

This study reports for the first time the combined application of HyPy and solvent extraction to soil samples deriving from a multiannual time-scale biochar incubation experiment of a cultivated soil[40,41] to track the fate of stable (BC$_{\mathrm{HyPy}}$) and semi-labile (non-BC$_{\mathrm{HyPy}}$ and solvent extractable

PAHs) fractions in the amended and control plots to understand the effect of biochar addition on SOC.

Experimental

Field experiment

The field experiment performed in a vineyard at the “Marchesi Antinori - La Braccesca Estate”, Montepulciano, Tuscany, Italy (43°10′15″ N, 11°57′43″ E) was previously described.[40,41] Overall, the experiment consisted of 15 plots, each 7.5 m in width and 30 m in length, including 4 vineyard rows and 3 inter-rows. Specifically, 10 plots were investigated as depicted in Figure 1. We have analysed two treatments (five replicates plots each): with biochar (amended; two applications, in 2009 and 2010 at the same rate, with a total application corresponding to 33 t ha^{-1} of dry biochar (considering an incorporation depth of 0.3 m and a soil density of 1.2, the application used in the experiment corresponded to 0.91% in weight)) and without biochar as a control. The agricultural soil was classified as sandy-clay-loam[42] textured with 70% sand, 15% silt and 15% clay. The soil characteristics were as follows: pH 5.37, total C 0.77%, total N 0.24%, total H 0.43%, and a cation exchange capacity of 12.1 meq 100 g^{-1}.[40, 41]

Soil was sampled four times from 2011 to 2013 (August 2011, December 2011, May 2012 and May 2013). For the 10 replicates (5 replicates X 2 treatments) soil was sampled in 5 randomly chosen points in the inter-row space of each replicate by means of a soil core sampler at 0-30 cm; from these sub-samples an average sample for each replicate was obtained (5 replicates x 2 treatments x 4 sampling dates = 40 samples). Each sample was air-dried, sieved (mesh size: 2 mm) in order to obtain homogeneous samples free of stones, larger roots and other coarse fragments, and stored at - 20 °C before analysis.

The biochar used in the experiment was a commercial charcoal provided by “Romagna Carbone s.n.c.” (Italy) obtained from dried (10% humidity) orchard pruning biomass (*Pirus communis, Malus domestica*, *Persica vulgaris, Vitis vinifera*) through a slow pyrolysis process with a transportable ring kiln of 2.2 m in diameter and holding around 2 t of feedstock. A peak temperature of 500 ◦C was hold for 2.5 hours, the average heating rate before reaching the peak temperature was 15-18 °C/min.[40]

Analysis

Biochar analyses were performed on a sub-sample obtained by mixing three individual specimens (about 5 g each) withdrawn in different places from the original biochar sample (1 Kg), then thoroughly homogenized by grinding with an agate mortar and pestle, sieved (mesh size: 2 mm), oven dried at 40 °C for 72 h, and stored at - 20 °C prior to analysis.

The carbon, nitrogen, hydrogen and sulfur (C,H,N,S) contents of the biochar and carbon content in soil samples were determined by combustion using a Thermo Scientific FLASH 2000 Series CHNS/O Elemental Analyzer (Thermo Fisher Scientific, Waltham, US). About 2-4 mg of biochar or soil test samples were analysed and compared by calibration with the Reference Material 2,5-bis(5-tert-butyl-2-benzo-oxazol-2-yl)thiophene.

The content of carbonate in soils and the possible contribution of inorganic carbon from biochar ash was negligible (< 0.1%) as confirmed by comparing total organic carbon measured after hydrochloric acid (HCl) treatment and total carbon on a set of treated and amended soil samples. Therefore, the total carbon determined in soil corresponded to the total organic carbon and was termed SOC for uniformity.

The ash content of the biochar was measured by heating samples in a muffle at 550 °C for 6 hours. The oxygen content of biochar was calculated by difference from the mass balance.

Analyses of extractable PAHs in biochar and soils were conducted using the method described in more detail elsewhere.[43] Briefly, 5 g of sample spiked with perdeuterated PAHs (acenapthtene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}) were extracted with 160 ml acetone/cyclohexane (1:1 v/v) mixture for 36 hours. After addition of 1 ml of *n*-nonane, the solvent was evaporated, the mixture cleaned-up by silica gel solid phase extraction and analysed by GC–MS. Occasionally, the procedure was tested analyzing the soil certified reference material ERM–CC013a (manufactured by the Federal Institute for Materials Research and Testing; Berlin, Germany). No significant differences were observed between measured and certified values (relative errors in the -5% +11% range, details in Table S1). Calibrations were performed in the 0.005-2.5 mg/L concentration range for each PAH (serial dilutions of the PAH-Calibration Mix Supelco). Results from calibration and blank analyses (limit of detection and quantitation, R^2) are reported in Table S2. Recoveries of surrogate PAHs (mean values ± s.d. n=40) were 75%±22%, 82%±21 and 83%±23 for acenapthtene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, respectively.

Hydropyrolysis

Hydropyrolysis (HyPy) was performed using the procedure described in detail by Meredith *et al*.[21,22] Briefly, 50-100 mg of biochar sample and 3-4 g of biochar amended soil were loaded with a sulphided molybdenum (Mo) catalyst using an aqueous/methanol 0.2 M solution of ammonium dioxydithiomolybdate [$(NH_4)_2MoO_2S_2$]. Catalyst weight was ~ 5% of the sample weight for soils, ~ 10% for biochar. Analyses were performed under H_2 at 15 MPa from 50 to 250°C at 300°C min^{-1}, then from 250°C to 550°C for 2 min at 8 °C min^{-1}. The evolved products (non-BC_{HyPy}) were quickly removed from the reactor vessel by a hydrogen sweep flow and trapped in a silica gel-filled trap cooled by dry ice; the silica gel was eluted with *n*-hexane and dichloromethane, the organic solutions concentrated, added with 100 µl internal standard solutions (*n*-hexatriacontane and tri-*tert*-butylbenzene, 100 mg l^{-1} each) and analysed by GC-MS (see below).

The weight of the solid residue in the reaction vessel (BC_{HyPy}) was calculated by difference, after weighing the catalyst loaded samples prior to and after each HyPy analysis.

Elemental compositions (C,H,N,S) of the samples before and after HyPy were determined by combustion as described above. The presence of catalyst in the samples (5 %) did not affect significantly the calculation of % $BC_{HyPy.}$ From elemental analysis and weight determinations, the soil concentrations of BC_{HyPy} (mgC g_{soil}^{-1}) and its content relative to SOC (%BC_{HyPy}), on a carbon base, were calculated:

$$\%\mathrm{BCHyPy}\left(\frac{\mathrm{mgC}}{100\mathrm{mgSOC}}\right) = \frac{\text{weight of residue (mg)} * \mathrm{C}\ (\%)}{\text{initial weight of soil (mg)} * \mathrm{SOC}(\%)} * 100$$

$$\mathrm{BCHyPy}\ (\frac{\mathrm{mgC}}{\mathrm{gsoil}}) = \frac{\%\mathrm{BCHyPy} * \mathrm{SOC}}{10}$$

GC–MS analyses of the non-BC_{HyPy} fractions were performed on a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer (Agilent technologies Inc. Santa Clara, CA, USA) operating by electron ionization at 70 eV, and full scan mode in the 35–650 *m/z* range. Analytes were separated with a HP-5MS fused silica capillary column (stationary phase poly[5% diphenyl/95%dimethyl]siloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness), using helium as the carrier gas. Individual *n*-alkanes were quantified from the *m/z* 57 mass chromatograms and by comparison with the added internal standard *n*-hexatriacontane assuming equal response factors for each compound. PAHs were quantified from the mass chromatograms of the molecular ion for each compound and by comparison with added 1,3,5-tri-*tert*-butylbenzene using the relative response factors determined by single calibration point (PAH-Calibration Mix Supelco at 10 mg L^{-1} for each PAH). Procedural blank analyses showed absence of contamination.

Quantitative data are presented as mean values ± standard deviation (s.d., five replicates). An analysis of variance (ANOVA) test was conducted with The “R Foundation for Statistical Computing” R software version 3.1.2 (2014-10-31; http://www.r-project.org) to was to evaluate significant difference between control and biochar amended soils, and between sampling periods. A difference was considered statistically significant at level of $p < 0.05$.

Results

Stable fraction (BC_{HyPy})

The utilised biochar obtained by slow pyrolysis was highly carbonized with 71% C content, and atomic H/C and O/C ratios of 0.26 and 0.11, respectively (Table 1), consistent with a high degree of aromaticity.[12] However, the ash content was rather high (about 20%, Table 1), thus the evaluation of the aromaticity from the O content measured by difference could be inadequate.[12] The impact of this biochar in the amended soils was clearly demonstrated by the higher values of carbon (4.8-3.5

%) in comparison to control soil (0.7-0.9%, Table 2). Considering that the content of inorganic carbon was negligible in these acidic soils, the total content of carbon corresponded to that of SOC (see experimental). The addition of biochar increased significantly the total pool of SOC by over six times (Table 2). An apparent reduction (statistically significant only in the last sampling, Table S3) of SOC in biochar treated soils was measured with time and three years after the biochar application in the vineyard, the SOC in amended soil was 3.8 times higher than that of control soil.

Recalcitrant black carbon (BC) accounted for the bulk of the additional SOC. This finding can be observed in Figure 2 showing the percentage of the SOC occurring in a stable form as $\%BC_{HyPy.}$ The $\%BC_{HyPy}$ in the untreated soil was on average 6 ± 1% (n=20) of the SOC, a value within the range reported in the literature (BC/SOC 2-13% quartile range),[23] and did not exhibit any trend with time. Hence, the majority of SOC in the control soil occurred in a semi-labile form, probably derived from a combination of lignocellulosic debris, humic acids, microbial biomass including thermally labile charcoal. Amending the soil with biochar created the opposite situation, with the majority of the SOC pool in the form of recalcitrant carbon, with BC_{HyPy} levels of 70 % of SOC on average. Specifically, the soil was amended with biochar two times during two years, then 15 months after the end of the second treatment, the $\%BC_{HyPy}$ was 79% ± 4% (first soil sampling, Figure 2). This value was very similar to that of original biochar (83%, Table 1). Obviously, the effect of biochar addition in soils on the level of BC depended on the level of stable polycyclic aromatic carbon (SPAC) in the original biochar which in turn is governed by the nature of the feedstock and pyrolysis conditions as described by McBeath *et al.*.[19] The high percentage of BC_{HyPy} characterizing the biochar (Table 1) is consistent with the SPAC values reported for aromatized biochars produced at high temperatures,[19] indicative of resistance to degradation in soil. Nevertheless, a gradual decrease of the $\%BC_{HyPy}$ was observed with time down to 61% ± 2% after 36 months following the end of the biochar application (Figure 2), a difference statistically significant (Table S3). The absolute soil concentration of BC_{HyPy} also decreased significantly with time as depicted in figure 3A from 36 ± 5 mgC g_{soil}^{-1} at the first sampling to 23 ± 5 mgC g_{soil}^{-1} after 21 months, representing a loss of 36% (statistical results in Table S3).

The total loss of BC_{HyPy} could be assigned to a variety of factors, including microbial degradation,[30] chemical weathering[13,32] and physical disturbance[37,38] as discussed in the final section.

Semi-labile fraction (non-BC_{HyPy})

Exemplar mass chromatograms of the hydropyrolysates for the non-BC_{HyPy} fraction in control and amended soils are shown in Figures 4B and 4C and these are characterized by the presence of aliphatic hydrocarbons, alkylbenzenes, diphenyls and PAHs. The composition of hydropyrolysates of the treated and untreated soils was similar in terms of tentatively identified compounds, suggesting that the incorporation of biochar did not markedly change the chemical nature of the main hydrocarbons, but rather their relative amount.

Aliphatic hydrocarbons are characterised by *n*-alkanes in the range C_{13} to C_{27} with a distribution centred at C_{16} and C_{18}, and an even carbon number predominance (Tables S4-S5 in supporting information). Iso- and anteiso C_{15} and C_{17} were also identified. Fatty acids are a more probable source of these alkanes as they exhibited an even over odd preference, and typically include a high abundance of C_{16} hexadecanoic (palmitic) acid and C_{18} octadecanoic (stearic) acid.[44] It is known that under HyPy conditions saturated fatty acids are converted into the corresponding alkanes preserving the number of carbon atoms, while cracking into smaller hydrocarbons could occur with unsaturated fatty acids.[45] The skeleton backbone is also preserved so therefore the iso and anteiso hydrocarbons can be assigned to the presence of the corresponding C_{15} and C_{17} acids of bacterial origin.[46] Fatty acids were probably covalently bond as acyl to lipids or macromolecular matrix and make up the aliphatic polymethylene network of the semi-labile BC. These aliphatic compounds were not evident in the non-BC_{HyPy} fraction from the original biochar (Figure 4A).

The non-BC_{HyPy} aromatic fraction of soils comprised monoaromatic rings represented by alkylated benzenes and diphenyls and a PAH pattern dominated by pyrene (Figures 4B and 4C). The ring size distribution was similar to that generated by HyPy from the organic soil component remaining after dichromate oxidation which was dominated by pyrene and minor levels of fluorene, phenanthrene, chrysene, and benzo[*ghi*]perylene among those identified in our study.[21] A similar pattern was observed in the original biochar (Figure 4A) providing evidence of a common polyaromatic backbone featured by specific alternant PAHs (phenanthrene, pyrene, chrysene; benzo[*ghi*]perylene was not detected). Methylated naphthalenes, phenanthrenes and pyrenes were detected as well. It is worth noting that partially hydrogenated PAHs were tentatively identified in the MS-hydropyrolysates suggesting that a degree extent of hydrogenation does occur with signal a peak assigned to dihydropyrene (*m/z* 204) being observed close to that for fluoranthene.

Distinctive differences between the amended and control soils were observed in the mean concentrations of aliphatic and aromatic hydrocarbon constituents (Tables S4-S7 in supporting information). The total concentrations of *n*-alkanes were higher in the control (around 100 µg g^{-1}, Table S4) than in the amended soils (about 40 µg g^{-1}, Table S5). The mean concentrations of non-BC_{HyPy} PAHs were higher in the amended than in the control soil (about 40 µg g^{-1} compared to 20 µg g^{-1}, Tables S6 and S7, see also Figure 3B). However, these differences could not be proved to be statistically significant (with the exception of control vs. treated soils after 9 months from the initial sampling) due to the high dispersion of values in the amended soils caused by the inhomogeneous distribution of biochar particles in the samples withdrawn from the same parcel and possible fluctuations due to seasonal changes in SOC source and decay. Besides, it was shown that PAHs could be heterogeneously distributed in the biochar samples.[47] However, a systematic higher content of non-BC_{HyPy} PAHs in the amended soils was observed across the whole sampling period (figure 3B). Four years after the addition of biochar to soil, semi-labile PAHs apparently were not degraded.

Extractable PAHs

The concentrations of solvent extractable PAHs in amended and control soils are presented in Figure 3C. Almost one year after the last biochar application, the total PAH concentrations in the amended soils (153 ± 38 ng g^{-1}) were significantly higher than those in the control soil (24 ± 3 ng g^{-}

^{1}, n = 5, Figure 3C and Tables S8-S9). The level of PAHs in the amended soils decreased significantly after 35 months down to 78 ± 20 ng g^{-1}, while that of control remained almost constant at 23 ± 3 ng g^{-1} (average in the whole period, n=20). The diminution in concentration of PAHs in the treated soils involved principally the two-four ring PAHs (> 40% loss), and less for the five-six ring PAHs (loss < 40%, Tables S8-S9).

Discussion

The pool of polycyclic aromatic carbon structures comprising the SOC have been operationally differentiated into three fractions: distinctive (GC analyzable) PAH compounds released by solvent extraction[24,43] and HyPy (semi-labile fraction, non-BC_{HyPy}),[19,21] and the undefined large ring polycyclic aromatic carbon matrix resistant to HyPy (stable fraction, BC_{HyPy} or SPAC).[19,21,22] The fate of these fractions in a vineyard soil treated with biochar has been investigated over a time span of about two years and compared to that of a control soil. In both soils, the concentrations of these polyaromatic reservoirs spanned six orders of magnitude: ng g^{-1} (extractable, Figure 3C), µg g^{-1} (semi-labile, Figure 3B) and mgC g^{-1} (stable, Figure 3A). Their relative abundances were also vastly different: on average the extractable PAH fraction accounted for 0.1 % (control)-0.3% (treated) of the total semi-labile PAH pool, which in turn represented 4% (control)-0.1% (treated) of the stable fraction.

The solvent extractable fraction is of importance for the assessment of environmental quality in regulatory procedures. However, exhaustive solvent extraction tends to over-estimate the fraction of bioavailable and bioaccessible PAHs that may pose a threat to living organisms and other methodologies should be used to determine this fraction.[24,48-50] Solvent extractable PAHs increased remarkably (five times on average) after biochar treatment and remained significantly higher than that in the control soil after almost two years (Figure 3C). Similar results were obtained by Quilliam *et al.*[27] who observed a significant increase of PAHs following biochar addition in two different agricultural soils. Although the application of biochar occurred with an inevitable addition of extractable PAHs, the levels in the amended soils remained within the range reported for background soils (<1 to 7,840 ng g^{-1}).[51] Obviously, the level of soil contamination will be determined by the degree of contamination of biochar which is dependent on feedstock materials and process conditions.[24,43,52-54] These data supported the view that when the PAH concentrations in the biochar fulfill the threshold levels proposed by the IBI[55] and EBC,[56] as for the biochar utilised in this study (Table 1, Table S10), the impact to soil is expected to be minimal. However, the large pool of BCH_{yPy} may influence the persistence of mobile PAHs by lowering the microbial activity.[15,27] Alternatively, it may act as a reservoir for PAHs[15] or favoring the sorption of endogenous and environmental PAHs[26] given the relatively high biochar-water partitioning coefficients.[24]

The non-BC_{HyPy} PAH fractions in treated and control soils were not significantly different (Figure 3B) and did not change significantly (statistical results in Table S3 for treated soil) with time suggesting that naturally occurring non-BC_{HyPy} PAHs are dominating this pool turnover. The content of non-BC_{HyPy} PAHs in the original biochar was around 1 mg g^{-1} (Table 1, individual PAHs in Table S10), probably not sufficient to impact markedly the SOC. These PAHs were probably covalently linked[21] or strongly sorbed onto aromatic surfaces, nanopores or occluded sites of the BC_{HyPy} matrix, and therefore not prone to decomposition. On the contrary, biochar amendment increased massively the stable BC_{HyPy} reservoir in comparison to the untreated soil (sixty times, Figure 3A) and ten times on average its proportion to SOC (% BC_{HyPy}, Figure 2). Noteworthy is that its absolute concentration decreased with time with a 36% carbon loss in 21 months (Figure 3A). It is known that biochar can be mineralized by both abiotic (oxidation) and biotic processes in laboratory incubation, however, the reported losses were much lower than those observed in this study (e.g. < 3%,[30,35] < 5%,[18] <12%[28] in days/months, 0.5%-8.9% in five years,[14] 6% after eight years[33]). Small losses (3%) due to respiration were reported in field studies,[38] while significant decomposition of BC (up to 70%) was reported to occur in topsoil in the first 30 years due to physical processes (vertical and lateral export) in addition to chemical mineralisation.[36] Therefore, the decreasing trends of BC_{HyPy} (Figure 3A) could be explained by physical redistribution of biochar particles rather than mineralization, even though the latter can be important in the first period due to priming effects.[57]

The depletion of the relative contribution of BC_{HyPy} (Figure 2) confirmed that the aromatic recalcitrant fraction was lost preferentially in comparison to other SOC components. This finding is in accordance with the studies by Rumpel *et al*. 2009 with rain simulators.[37] These authors found that the lateral and vertical (infiltration) removal of SOC in agricultural soils by water erosion can be significant (up to 55%) and more pronounced for the carbon-rich (less dense) recalcitrant (chemically resistant) BC fraction. The vertical infiltration of pyrogenic carbon was found in soil microcosms.[34] The type of prevailing physical distribution could be governed by the different water regime in tropical and temperate zones, with low-intensity rainfall favoring vertical transport and splash erosion horizontal transport.[37] In our study, erosion could be favored by the gentle slope of the vineyard which may induce a preferential loss of BC as observed for steep slopes with high erosion rates.[58] Based on the results from carbon isotope analysis, Major *et al*.[38] supposed that water runoff was the principal process capable to explain the loss of biochar in a treated soil after two years, being the loss by respiration and vertical transport minimal.

The absolute and relative concentration of BC_{HyPy} did not change significantly in the last two sampling campaigns (April 2012 and May 2013) while the cumulated rainfall increased,[41] indicating that a substantial part of carbon from biochar is resistant to migration processes. In fact, the grape productivity increased (up to 66%) in all the harvests following biochar amendment in the 2010-2014 period, even though the fruit quality remained unaffected. The increased yields were likely to be due to the enhanced soil water content and plant available water in the treated soils in comparison to the control soil.[40,41] Other field studies on vineyards with a slope gradient reported subtle effects on productivity and quality, but the fate of biochar was not investigated.[59] The persistence of BC demonstrated by HyPy analysis supports the role played by biochar in regulating water availability, but potential losses due to SOC dynamics should be carefully evaluated especially in long-term field experiments where abiotic and biotic components drive the carbon stability rather than the inherent biochar recalcitrance.[60] This study demonstrated the usefulness of HyPy to shed light on the characteristics of BC put into the environment when biochar is applied in soil systems.

Associated content

Supporting Information Available

Supporting information contains the results from the analysis of PAHs in CRM soil (Table S1), PAH calibration (Table S2), statistical analysis of treated soils (Table S3), analysis of individual non-BC_{HyPy} alkanes (Tables S4 and S5), analysis of individual non-BC_{HyPy} PAHs (Tables S6 and S7), analysis of individual solvent extractable PAHs (Tables S8 and S9), analysis of individual extractable and non-BC_{HyPy} PAHs in biochar (Table S10). This information is available free of charge via the Internet at http://pubs.acs.org.

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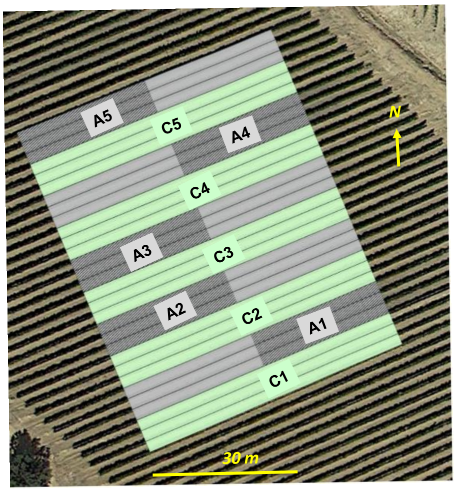

Figure 1. Experimental layout in the vineyard indicating the location of the five plots amended with biochar (A) under the same conditions and the nearby control (C) plots. Photo from GoogleEarth.

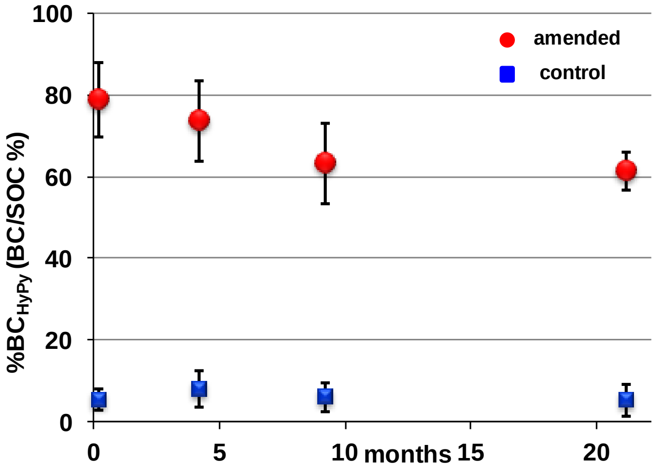

Figure 2. Black carbon from HyPy (%BC_{HyPy}) as percentage of soil organic carbon in the biochar amended (red circles) and control (blue squares) soils vs. sampling time (months from the first sampling). Mean values ± 2*s.d. (n=5).

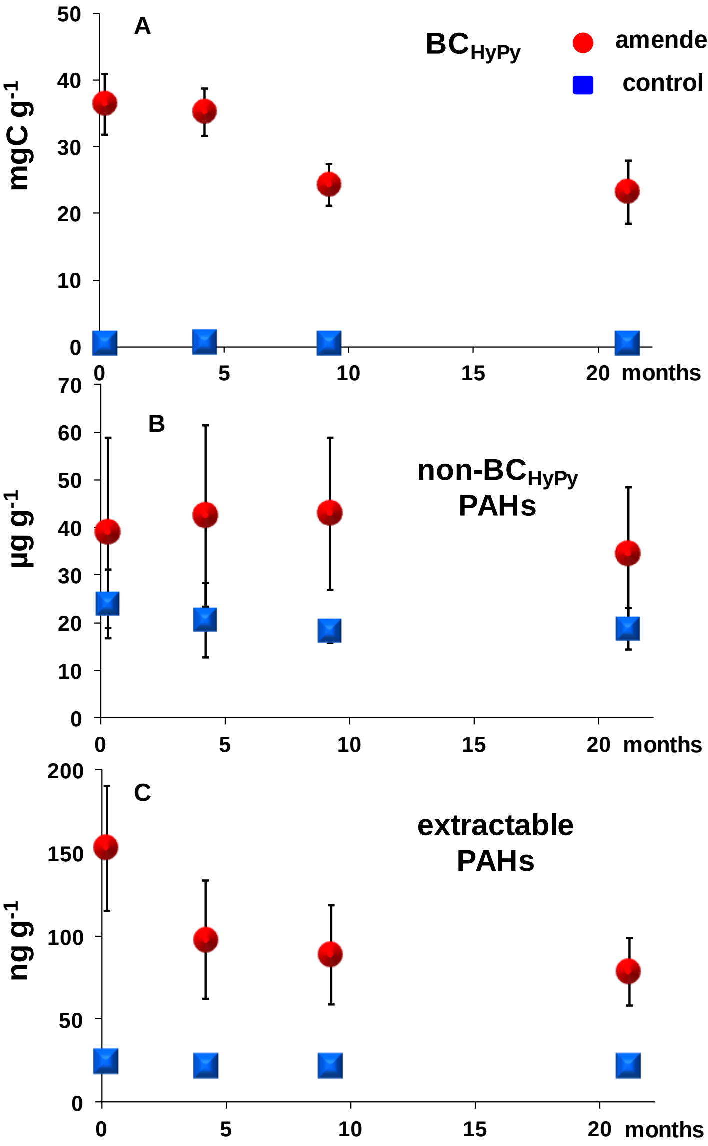

Figure 3. Concentration vs. sampling time (months from the first sampling) of biochar amended (red circles) and control soils (blue squares) of different polycyclic aromatic fractions. (A) stable BC$_{HyPy}$, (B) non-BC$_{HyPy}$ PAHs, and (C) solvent extractable PAHs. Mean values ± s.d. (n=5, error bars not visible when smaller than square size).

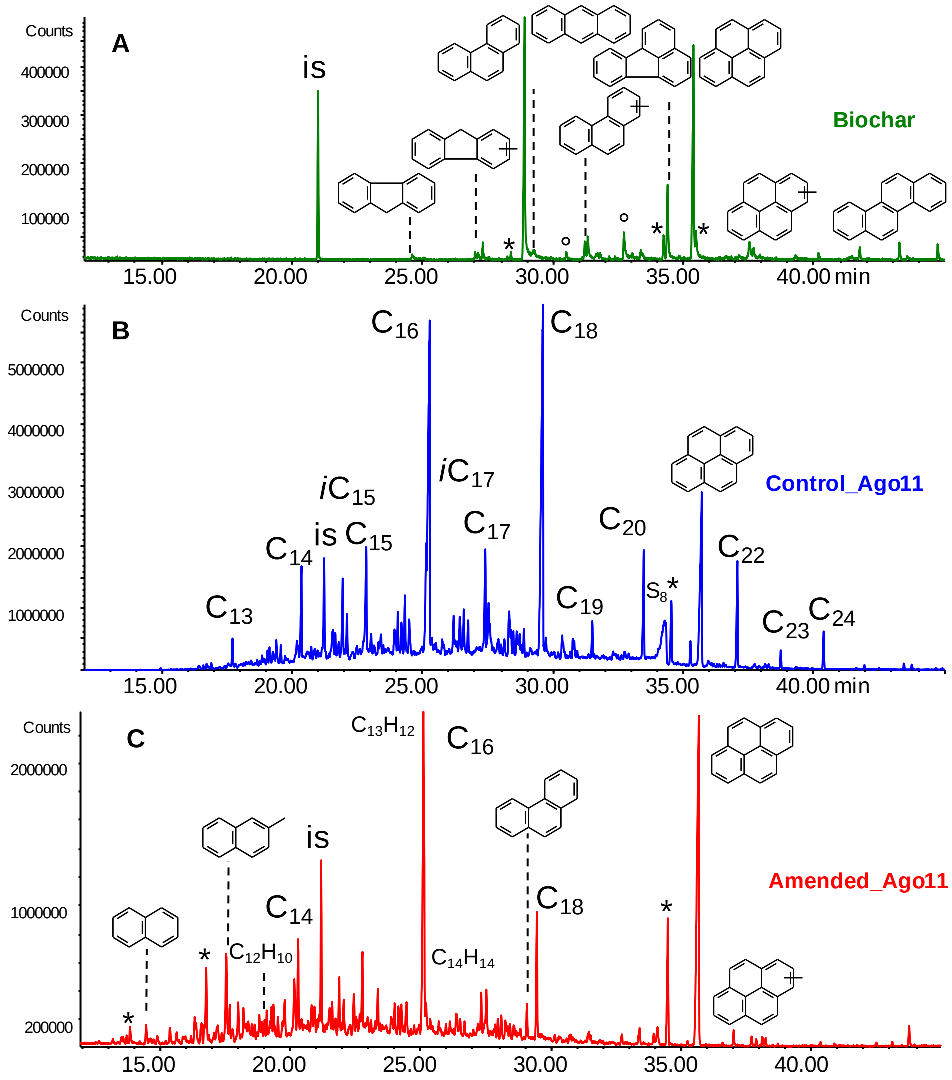

Figure 4. Examples of total ion chromatograms for the hydropyrolysates (non-BC_{HyPy} fraction). From top to bottom: (A) biochar, (B) control soil and (C) soil amended with biochar sampled in August 2011. Cx: *n*-alkanes with x carbon atoms, i: iso/anteiso, CxHy: biphenyls, °: phenylnaphthalenes (tentative), *: probably hydrogenated PAHs.

Table 1. Elemental analysis (oxygen by difference), atomic H/C and O/C ratios, ash content, solvent extractable PAHs, non-BC_{HyPy} PAHs and $\%BC_{HyPy}$ ($\%BC_{HyPy}$/SOC) of the biochar applied in the field experiment (mean values ± standard deviation s.d., n=3 on a dry basis).

parameter	**units**	**mean value ± s.d.**
C	%	71.4 ± 1.2 %
H	%	1.54 ± 0.11
N	%	0.72 ± 0.05
S	%	0.59 ± 0.05
O	%	5.9 ± 0.7
Ash	%	19.9 ± 1.5
H/C	atomic	0.26
O/C	atomic	0.11
extractable PAHs	$\mu g\ g^{-1}$	3.8 ± 0.8
non-BC_{HyPy} PAHs	$mg\ g^{-1}$	1.1 ± 0.2
$\%BC_{HyPy}$	%	83 ± 3

Table 2. Soil organic carbon (SOC) of the soil treated with biochar and untreated soil (control) in different sampling periods (months elapsed after the last biochar application). Values are mean values ± s.d., n=5, % on a dry basis.

	Aug 2011	**Dec 2011**	**May 2012**	**May 2013**
SOC%	**months 0**	**4**	**9**	**21**
Control soil	0.76±0.21	0.76±0.21	0.83±0.21	0.91±0.12
Biochar amended soil	4.79±0.58	4.30±0.83	3.97±0.75	3.49±0.29

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