GLOBAL CHANGE BIOLOGY
BIOENERGY

GCB Bioenergy (2017) **9**, 1618–1626, doi: 10.1111/gcbb.12450

# Hydrochar enhances growth of poplar for bioenergy while marginally contributing to direct soil carbon sequestration

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## Abstract

Hydrothermal carbonization (HTC) has been proposed as an alternative method to pyrolysis for producing C-rich amendments for soil C sequestration. However, the use of hydrochar (HC) as soil amendment is still controversial due to the limited information on the potential benefits and trade-offs that may follow its application into soil. This study investigated the effects of HC starting from maize silage on plant growth in a 2-year controlled experiment on poplar for bioenergy and evaluated HC stability in soil by periodic soil respiration and isotopic ($\delta^{13}C$) measurements. HC application caused a substantial and significant increase in plant biomass after one and two years after planting, and no evident signs of plant diseases were evident. Isotopic analysis on soil and $CO_2$ efflux showed that slightly less than half of the C applied was re-emitted as $CO_2$ within 12 months. On the contrary, considering that the difference in the amount of N fixed in wood biomass in treated and not-treated poplars was 16.6 ± 4.8 g N $m^{-2}$ and that the soil N stocks after one year since application did not significantly change, we estimated that approximately 85% of the N applied with HC could have been potentially lost as leachate or volatilized into the atmosphere as $N_2O$, in response to nitrification/denitrification processes in the soil. Thus, the permanence, additionality and leakage of C sequestration strategy using HC are deeply discussed.

*Keywords:* carbon sequestration, hydrochar, mean residence time, plant yield, poplar bioenergy, soil amendment

*Received 28 February 2017; accepted 5 April 2017*

## Introduction

Minimal fertilizer input and high biomass yield are required to maximize the net benefit of bioenergy crops. Increased yields enhance the profitability for the farmers and the production of renewable energy to offset the use of fossil fuels. As bioenergy crops are expected to expand mainly on marginal and less fertile soils to avoid competition with food production, yield enhancement is not a trivial goal especially considering that intensification must be obtained without adverse environmental impact (Allwright & Taylor, 2016). The most advanced strategies for a sustainable intensification include the selection of traits of interest in cultivated plants for biomass yield and feedstock quality (Van Acker *et al.*, 2014), drought tolerance and pest resistance, but this will hardly overcome the need for sufficient nutrients to sustain plant growth. The cultivation of bioenergy crops should also prevent the loss of organic carbon (C) from soils or eventually maximize C sequestration to enhance their overall impact on $CO_2$ emission mitigation.

Bio-waste, sludge or green household waste is a large source of C and nutrients, which may be exploited to enhance C sequestration and plant nutrition in bioenergy crops. A crude estimate of bio-waste residues, directly accessible and mostly already collected, sums up to about $10 \times 10^9$ tons per year, worldwide (Steinbeiss *et al.*, 2009). Transformations are however required to increase the recalcitrance of organic C-containing

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compounds while enhancing the availability of plant nutrients. The thermochemical conversion of organic material in oxygen-limited conditions is a realistic option to achieve this goal. Carbon-rich and recalcitrant solid residues (charred materials) can become available after the transformation, and those can be subsequently incorporated into soils thus becoming a sustainable negative emissions technology, eventually able to mitigate climate change (Smith, 2016). Several processes have been proposed so far for thermochemical conversion, leading to the production of a wide range of solid residues having different physical and chemical characteristics (Meyer *et al*., 2011). Among those, hydrothermal carbonization (HTC) is of particular interest as it can be used to convert wet biomass, at relatively low temperatures under high pressure and in anoxic conditions (Libra *et al*., 2011). During HTC process, wet biomass undergoes a series of hydrolysis, condensations, decarboxylation and dehydration reactions and this transformation process leads to a two-phase mixture of solid and liquid (slurry) which is conventionally named hydrochar (HC). For the same feedstock, HC has a lower C and higher hydrogen content than the carbonaceous residue of dry pyrolysis or pyro-gasification, which is often called biochar (Libra *et al*., 2011; Kammann *et al*., 2012). The pH of HC obtained from plant residues, such as for instance corn stover, wheat straw, poplar wood and olive residues, is generally lower than biochar (pH <5) (Wiedner *et al*., 2013). The content of nutrients is variable being related to both the processing temperature (Wiedner *et al*., 2013; Schimmelpfennig *et al*., 2015) and on the feedstock composition (Ekpo *et al*., 2016). Although the use of HC as soil amendment has been already proposed (Libra *et al*., 2011), only a very few experiments have been made so far in the field under realistic conditions (George *et al*., 2012; Malghani *et al*., 2013, 2014; Schimmelpfennig *et al*., 2015). Moreover, HC has also been shown to be a promising sorbent of a wide range of pollutants (Sun *et al*., 2011; Eibisch *et al*., 2015; Han *et al*., 2016).

This study considered the use of HC as soil amendment in a poplar bioenergy crop. This fast-growing tree species is one of the best candidates for bioenergy production, and the selection of new breeding lines is rapidly developing not only for the high quality of the feedstock product for combustion, but also because it can provide valuable environmental services, in particular soil C sequestration (Ceotto & Di Candilo, 2011; Ceotto *et al*., 2016). This article reports the effects of HC on tree growth, biomass yield and the fate of HC in the soil with implications for its C sequestration potential, the emissions of nitrous oxide and fossil fuel offset.

## Materials and methods

### *HC source and experimental design*

The HC used in this experiment was produced by CarbonSolutions Deutschland GmbH using a CS-HTC90™ reactor and starting from maize silage ($\delta^{13}C_{feedstock} = -12.75‰$). The reactor consisted of two reaction stages with a temperature of 230 °C in stage 1 and a temperature of 180 °C in stage 2 and a mean residence time of 15 and 75 min, respectively. To prevent the water from evaporating, the conversion took place under elevated pressure of 10 to 40 bar. The obtained HC was wet and in the form of a slurry with the lyophilized part (dry material) representing 11% of the total weight (Table 1). The C and N contents of HC were determined using a CHN elemental analyzer (Flash EA 2000 Thermo Fisher Scientific, Bremen, Germany), and $\delta^{13}C$ was determined using a continuous flow isotopic ratio mass spectrometer (CF-IRMS; Delta V Advantage, Thermo Fisher Scientific, Bremen, Germany).

Nutrient content in the lyophilized phase was determined using an inductively coupled plasma optical emission spectrometer (ICP–OES), after mineralization with an Ethos TC microwave laboratory station (Milestone, Bergamo, Italy) (Table 2). Polycyclic aromatic hydrocarbon (PAHs) content in HC was determined by gas chromatography/mass spectrometer (GC/MS) using Soxhlet extraction and 100% toluene as extracting solvent (EPA, 2007). The total PAHs' concentration (i.e., concentration of the 16 priority PAHs for the U.S.

**Table 1** Main characteristics of the applied hydrochar

| | Solid | Liquid phase |
|---|---|---|
| % of total weight | 11% | 89% |
| $\delta^{13}C$ (‰) | −12.4 ± 0.1 | −12.5 ± 0.1 |
| Carbon content (%) | 53.0 | 4.1 |
| Nitrogen content (%) | 2.0 | 0.2 |
| Applied carbon (kg C $m^{-2}$) | 3.14 | |
| Applied nitrogen (kg N $m^{-2}$) | 0.12 | |

**Table 2** Chemical characteristics of the HC (lyophilized phase) used in the experiment

| Element | Concentration |
|---|---|
| Al | 1.2 g $kg^{-1}$ |
| Ca | 8.7 g $kg^{-1}$ |
| Cu | <0.1 mg $kg^{-1}$ |
| Fe | 42.1 g $kg^{-1}$ |
| K | 40.4 g $kg^{-1}$ |
| Mg | 10.1 g $kg^{-1}$ |
| Mn | 84.0 mg $kg^{-1}$ |
| Na | 3.9 mg $kg^{-1}$ |
| P | 8.4 mg $kg^{-1}$ |
| S | 2.7 mg $kg^{-1}$ |
| Zn | 280.8 mg $kg^{-1}$ |
| pH | 4.8 |

Environmental Protection Agency) was 8.8 ± 2.1 mg $kg^{-1}$, lower than the threshold fixed by the International Biochar Initiative (6–300 mg $kg^{-1}$).

The experiment was made in sixteen raised beds (3.0 × 1.0 × 0.50 m) placed in an open field at the Experimental Centre for Tree Nursery in Pistoia (43°55′ N; 10°54′ E; 59 m a.s.l.). These were filled with mixture of 50% peat and 50% pumice substrate (pH = 5.5; C = 48%; N = 0.50%) and fertilized with 2.2 g $m^{-2}$ of N and 5.0 g $m^{-2}$ of $P_2O_5$ before planting. In each raised bed, six whips (0.5 × 0.5 m) of *Populus alba* L. (Villafranca clone) were transplanted in April 2012 and grown for two consecutive seasons. The soil was well watered, as 1.5 l per plant daily was applied during the dry season using drip irrigation. In the first growing season, the following two treatments were applied (number of replicates = 8): control and 100 kg of HC raised $bed^{-1}$ (wet weight) before transplanting (3.1 kg C $m^{-2}$; HC-1). In 2013, one additional treatment was added (number of replicates = 4): 100 kg of HC was added to four of the eight raised beds that were already amended with HC in the first growing season (HC-2).

## Experimental measurements

Poplar trees were cut at the end of each growing season, and stumps were left to re-sprout: Total fresh weight of stems was measured onsite for each individual tree. The wood was then chopped and dried at 70 °C for 40 h to determine dry weight for each treatment. Wood C and N contents at the end of the first year (i.e., control and HC-1) were determined using a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2, Milano, Italy).

Three random soil samples (1 L each) were collected in each raised bed at two depths (0–15 cm and 15–30 cm) in December 2012 (HC-1) and in December 2013 (control, HC-1, and HC-2). Soil samples were sieved at 2 mm and oven dried at 105 °C for 48 h to determine soil bulk density. Soil organic C and N contents were determined using a CHN Elemental Analyzer (Carlo Erba Instruments, mod 1500 series 2). Prior to C analyses, soil samples were treated with HCl to eliminate carbonates.

Dry subsamples were also acid digested with a microwave oven (CEM, MARSXpress) according to the EPA method 3052. The solutions obtained after the mineralization were filtered (0.45 μm PTFE) and diluted. Total contents of Ca, K, Mg, Na, and P were determined by an ICP optical spectrometer (Varian Inc., Palo Alto, CA, USA Vista MPX) using scandium as internal standard. Soil pH was measured in a soil/water solution at a 1/2.5 ratio.

To measure HC decomposition, soil $\delta^{13}C$ in HC-1 and control plots was measured on soil subsamples using a Finnigan DELTA XP isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). The fraction of HC carbon ($f_{HC}$) present into the soil at the sampling date was calculated using a simplified mass balance equation (Del Galdo *et al*., 2003):

$$f_{HC} = \frac{\delta_{HC\text{-}1} - \delta_{control}}{\delta_{hydrochar} - \delta_{control}}$$

where $\delta_{HC\text{-}1}$ and $\delta_{control}$ are the isotopic signatures of HC-1 and control, respectively, and $\delta_{hydrochar}$ is the isotopic signature of the added HC (−12.43 ± 0.08‰). By multiplying soil carbon stock at sampling date (kg C $m^{-2}$) by $f_{HC}$, it was possible to estimate the amount of HC-C (kg C $m^{-2}$) still present at each soil depth at sampling.

To measure the decomposition of HC with an independent method, periodic soil respiration measurements were performed during the first year of the experiment in control and HC-1 treatments using a portable soil respiration system coupled with an automated chamber (Delle Vedove *et al*., 2007). The $\delta^{13}C$ of the respired $CO_2$ was assessed using the Keeling plot method (Ngao *et al*., 2005; Joos *et al*., 2008) through an online subsampling of the air from the soil respiration chamber using a Picarro G2131-i d13C High-precision Isotopic $CO_2$ Cavity Ring Down Spectrometer (CRDS) (Ventura *et al*., 2015). Instantaneous $CO_2$ concentration and $\delta^{13}CO_2$ were recorded every second by the CRDS in the $CO_2$ concentration range between 500 and 1200 ppm (Fig. 1). Measurement cycles, lasting <10 min, were repeated in three different positions in each container. The fraction of $CO_2$ respiration deriving from HC decomposition ($fr_{HC}$) was calculated using a mass balance approach according to Phillips & Gregg (2001):

$$fr_{HC} = \frac{\delta CO_2 HC\text{-}1 - \delta CO_{2control}}{\delta_{hydrochar} - \delta CO_{2control}}$$

where $\delta CO_2$ HC-1 and $\delta CO_{2\ control}$ are the isotopic signatures of the $CO_2$ emitted from HC-1 and control, respectively, and $\delta_{hydrochar}$ is the isotopic signature of the applied HC (−12.43 ± 0.08‰).

## Statistical analysis

All data in the text and in the tables are reported as mean ± standard deviation (SD), if not differently indicated. Gaussian error propagation technique (GEP) was used in error analysis to analytically determine uncertainty produced by multiple and interacting measurements or variables. For this, the uncertainty associated with each measurement was calculated as deviation of the mean and the classical error propagation theory and equations were used (Lehrter & Cebrian, 2010). Stem biomass, total soil C and N, respired $\delta^{13}CO_2$, and soil respiration fluxes measured on treated and control plots were compared using one-way analysis of variance (ANOVA), followed by the Bonferroni's post hoc test. Data normality and homogeneity of variances were checked before the analysis, and eventually, data were log-transformed to meet the ANOVA 's requirements. When these last were not met, a Kruskal–Wallis one-way analysis of variance on ranks, eventually followed by Tukey's test, was performed. For soil $CO_2$ efflux measurements, significance of differences among treatments and sampling dates was, instead, determined using two-way analysis of variance (testing treatment, sampling date, and treatment x sampling date). The intercepts of the Keeling plots to determine $\delta^{13}CO_2$ were calculated using least squares linear regressions. All statistical analysis and soil respiration data elaborations were performed in SIGMAPLOT 11 (©Systat Software, Inc.) and in STATA 10.1 (© StataCorp, College Station, TX, USA), respectively.

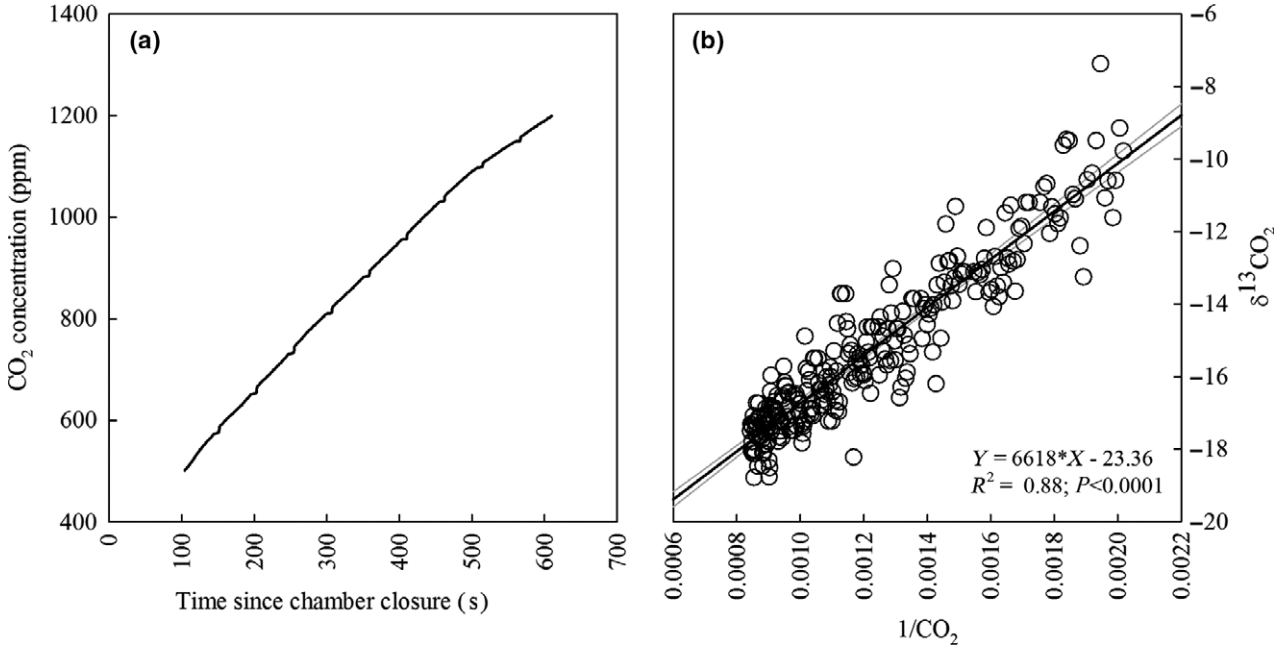


**Fig. 1** Example of measured $CO_2$ increase after chamber closure (a) and related Keeling plot (b) using the automated soil respiration system and the Picarro analyzer. The solid line in panel B represents the regression between $\delta^{13}CO_2$ and $1/CO_2$. In the example (b), the $\delta^{13}CO_2$ of the respired $CO_2$ is equal to the intercept of the regression (−22.36‰). Gray lines represent 95% confidence interval.

## Results

### *Effects on plant growth*

Poplar trees did not show any change in leaf color or other signs of toxicity during all the experimental period. A number of not-identified weed species germinated on treated and not-treated soils and were removed to avoid competition. In the first year (2012), the addition of HC-1 into the soil caused a substantial and significant increase in aboveground biomass compared to the control (Fig. 2).

The cuttings produced the first leaves on May 21st, and over a period of 200 days, they accumulated 41% more dry mass than the controls ($P < 0.05$). HC-treated and control plants also differed in height at the end of the first season with HC-treated plants being 20% taller than the control. After the cut that was made in December 2012, the plants re-sprouted on April 15th, and during a period of 230 days, they accumulated, in both treatments, more biomass than in the previous year (+32%), but again the HC-treated plants grew 37% more in biomass than the control ($P < 0.05$, Fig. 2).

N-content in wood dry biomass was significantly larger ($P < 0.001$) in HC-1 (6.6 ± 1.8 g plant$^{-1}$) than in the control (2.7 ± 0.5 g plant$^{-1}$), but, when those values are compared to the amount of N applied (116 g N m$^{-2}$ with HC + 2.2 g N m$^{-2}$ with fertilization), it appears that HC-1 added much more N to the soil than was actually required to sustain faster growth.

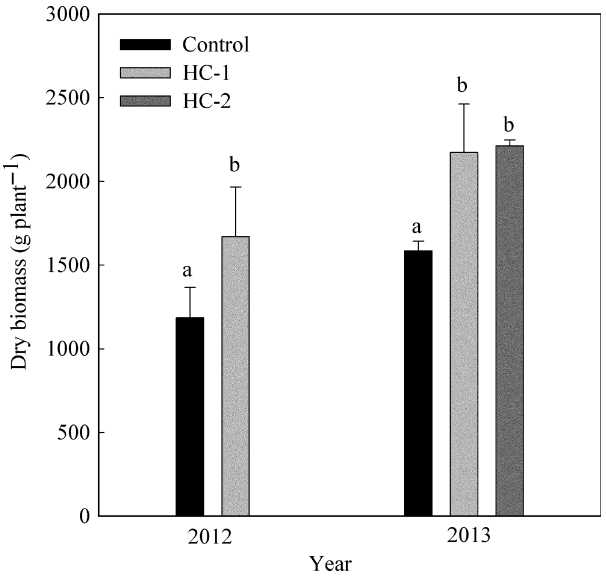


**Fig. 2** Tree biomass at the end of 2012 and 2013 by treatment. Vertical bars indicate standard deviation ($n = 8$ in 2012 and $n = 4$ in 2013, respectively). Different letters indicate a significant difference among treatment within the considered year ($P < 0.05$).

Further application of 33 kg m$^{-2}$ of HC in 2013 (HC-2) had a negligible effect on plant growth, and the

difference in biomass between the treated and the control plants was 40% ($P < 0.05$), while the difference between the plants that received 66 and 33 kg $m^{-2}$ of HC was 2% ($P > 0.05$; Fig. 2).

*Effects on soil C and N*

At the end of the first year (December 2012), soil C and N stocks at 0–15 cm were significantly higher in HC-1 than in control plots ($P < 0.001$; Table 3), while no difference was observed at the deepest soil layer (15–30 cm). At the end of the second year, while the difference in the upper layer became negligible, the deepest layer showed higher C and N stocks than control even if such a difference was not significant.

The isotopic analysis of soil samples, combined with prior knowledge of $\delta^{13}C$ of the applied HC ($-12.43 \pm 0.08‰$) and control ($-26.33 \pm 0.05‰$), revealed that the relative contribution of HC to soil C stock (f) in the upper layer decreased from 16 to 4% after 206 and 575 days since application, respectively (Table 4). Such a fraction increased from 7 to 20% at 15–30 cm soil depth during the same period of time (Table 4 and Fig. 3). Overall, 47% of the C applied with HC-1 was lost within a year after application (Table 4).

Both the total $CO_2$ efflux and its isotopic signature were significantly different between the two treatments ($P < 0.001$ and $P = 0.024$), between sampling dates ($P < 0.001$ and $P = 0.010$), and their interaction ($P < 0.001$ and $P < 0.001$) (Table 5). The relative contribution of HC-1 to the total $CO_2$ efflux decreased with time from 39% to almost zero in the period ranging from 31 to 393 days since application. This confirmed the occurrence of a rapid decomposition of the HC-contained C labile fraction immediately after application.

*Effects on other soil nutrients*

A single application (HC-1) increased concentrations of Ca, K, Na, and P in the upper soil layer at the end of

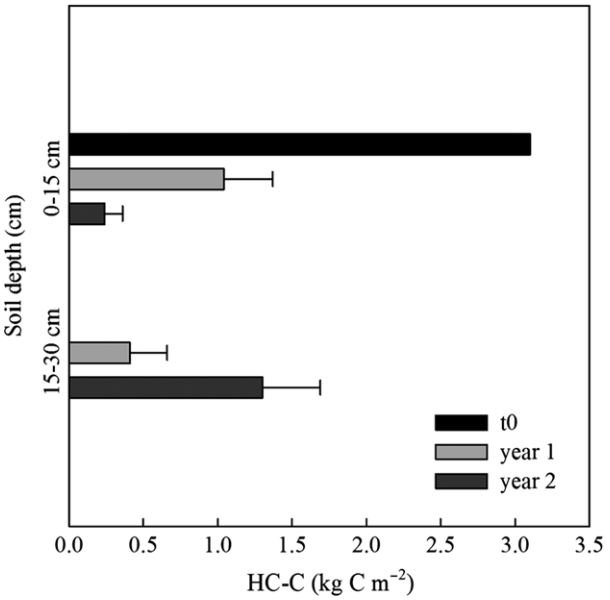


**Fig. 3** Hydrochar-C (HC-C) in HC-1 at 0–15 and 15–30 cm at the beginning of the experiment (t0), after one year and two years. Vertical bars are standard deviation ($n = 4$).

**Table 3** Soil carbon and nitrogen by soil depth in the experimental treatments. For HC-1, data are reported for both 2012 (Year 1) and 2013 (Year 2) sampling. Different letters indicate a significant difference among treatments ($P < 0.05$)

| | kg C $m^{-2}$ | | | kg N $m^{-2}$ | | |
|---|---|---|---|---|---|---|
| Treatment | 0–15 cm | 15–30 cm | Total | 0–15 cm | 15–30 cm | Total |
| Control | 6.0 ± 0.1 b | 5.8 ± 0.4 b | 11.8 ± 0.4 b | 0.16 ± 0.004 c | 0.42 ± 0.03 b | 0.58 ± 0.03 b |
| HC-1 (Year 1) | 6.5 ± 0.1 c | 5.8 ± 0.6 b | 12.3 ± 0.5 b | 0.19 ± 0.01 b | 0.47 ± 0.08 b | 0.66 ± 0.08 b |
| HC-1 (Year 2) | 6.0 ± 0.2 b | 6.5 ± 0.2 ab | 12.5 ± 0.1 b | 0.18 ± 0.01 bc | 0.53 ± 0.05 b | 0.70 ± 0.06 b |
| HC-2 | 6.9 ± 0.1 a | 7.5 ± 0.7 a | 14.4 ± 0.7 a | 0.24 ± 0.02 a | 0.71 ± 0.09 a | 0.95 ± 0.08 a |

**Table 4** Soil $\delta^{13}C$, fraction of hydrochar-C (f), and remaining hydrochar-C (HC-C) in HC-1 at different depths and sampling dates since application ($n = 4$)

| | $\delta^{13}C$ | | f | | Remaining HC-C (kg C $m^{-2}$) | | |
|---|---|---|---|---|---|---|---|
| | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | 0–15 cm | 15–30 cm | Total |
| Time 0 | - | - | - | - | - | - | 3.1 |
| Year 1 | −24.05 ± 0.70 | −25.32 ± 0.56 | 0.16 ± 0.05 | 0.07 ± 0.04 | 1.04 ± 0.33 | 0.41 ± 0.24 | 1.45 ± 0.41 |
| Year 2 | −25.73 ± 0.29 | −23.49 ± 0.78 | 0.04 ± 0.02 | 0.20 ± 0.06 | 0.24 ± 0.12 | 1.30 ± 0.39 | 1.54 ± 0.41 |

**Table 5** Total soil $CO_2$ efflux in control and HC-1 (HC-$CO_2$ total efflux) and $CO_2$ flux due to hydrochar decomposition (HC-$CO_2$ efflux). Significant differences are reported for comparison between control and HC-1 within each single date ($n = 4$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

| Days since application | $CO_2$ efflux (g C $m^{-2}$ $day^{-1}$) | | Respired $\delta^{13}C$ (‰) | | f | HC- $CO_2$ efflux (g C $m^{-2}$ $day^{-1}$) |
|---|---|---|---|---|---|---|
| | Control | HC | Control | HC | | |
| 31 | 5.21 ± 1.33 | 10.49 ± 0.72*** | −25.44 ± 0.80 | −20.31 ± 1.07*** | 0.39 ± 0.11 | 4.13 ± 1.20 |
| 53 | 5.65 ± 1.29 | 7.52 ± 2.01 | −26.42 ± 0.87 | −23.79 ± 1.50* | 0.19 ± 0.12 | 1.41 ± 0.98 |
| 67 | 5.17 ± 1.34 | 8.30 ± 0.44* | −24.17 ± 0.65 | −25.53 ± 1.61 | −0.12 ± 0.15 | −0.96 ± 1.20 |
| 127 | 5.78 ± 0.66 | 5.85 ± 1.37 | −25.92 ± 1.04 | −24.99 ± 2.86 | 0.07 ± 0.23 | 0.40 ± 1.30 |
| 393 | 3.99 ± 1.51 | 2.16 ± 0.50 | −23.99 ± 1.71 | −25.93 ± 0.81 | −0.17 ± 0.17 | −0.36 ± 0.38 |

the first year (0–15 cm; Table 6), while no differences were detected for Mg (Table 6). No differences were observed for all nutrients at greater depth (Table 6). At the end of the second year, no differences were detected at both depths for the single HC application.

In the case of two consecutive applications (HC-2), nutrients concentrations were (with the exception of Mg) always significantly higher at 0–15 cm depth, while no differences were observed at greater depth (Table 6).

## Discussion

According to the Intergovernmental Panel for Climate Change (IPCC), an effective and sustainable C sequestration activity implies that $CO_2$ which is removed from the atmosphere is stored in terrestrial and marine sinks in a permanent and additional way, without leakage. *Permanence* is the desired timescale in which C is retained in sinks and it is normally assumed to be in the order of centuries. *Additionality* means that the reduction in the emissions or the enhancement of the removals is additional to any that would occur in the absence of the activity. *Leakage* refers to the situation in which a C sequestration measure, directly or indirectly, triggers an activity, which in whole or part, counteracts the C effects of the initial activity.

When C storage is followed by release within a couple of months or years, it cannot be considered permanent. There has been much debate on the possibility to enhance C sequestration of forests and plantations by increasing their growth rates. In a recent paper, Körner (2017) pointed out that *'unless the residence time of C is maintained or enlarged, faster growth does not mean there is more C sequestration'*. Accordingly, the most realistic option to achieve a permanent and effective C sequestration in terrestrial ecosystems is to add exogenous sources of C into soils, bearing in mind that the C, which is added in this way, should also not involve any leakage. Hydrochar (HC) is a potential large exogenous source of C (Table 1), but our results showed that 47% of the C added into the soil was lost through decomposition during the first year since application (Table 4). This matches previous observations of Malghani *et al.* (2013, 2014) made using the same HC of this study, while contradicting the general assumption that chars with O:C ratio <0.4, H:C ratio <0.6 and black carbon >15% are the best suited for sequestering C into soil (Schimmelpfennig & Glaser, 2012) and previous incubation experiments (Naisse *et al.*, 2014). On the other hand, more than half of the C added with HC did not decompose further, as clearly shown by the isotopic signature of the respired $CO_2$ fluxes (Table 5). Soil measurements provided solid evidence for a fast translocation toward deeper soil horizons, where decomposition is generally lower because of a low microbial density and reduced oxygen content (Kuzyakov *et al.*, 2000). However, the assumption that vertical migration favors a permanent C sequestration requires some caution: It has been shown that the addition of fresh C into deeper soil layers can prime microbial activity leading to increased decomposition of ancient buried C which is bound to soil minerals (Fontaine *et al.*, 2007). Similarly, Naisse *et al.* (2015) reported faster SOM mineralization after HC application into soil. Other studies have instead shown that biochar addition may have a SOM protection effect, finally leading to decreased decomposition rates of the original SOM (Keith *et al.*, 2011; Wang *et al.*, 2016; Riaz *et al.*, 2017).

The addition of exogenous C into the soil may have neutral, decremental or incremental effects on the net primary production (NPP). Decremental effects may occur in different ways, such as, for instance, through inhibitory effects on seed germination and the initial seedling growth rates (Schimmelpfennig *et al.*, 2014; Fang *et al.*, 2015; Reibe *et al.*, 2015), but not any toxic effect was observed on the poplars in our study. The germination of weeds in the HC-treated soil suggested the absence of any inhibitory effects also on herbaceous species. Our results apparently conflict with previous observations made in laboratory (Busch *et al.*, 2012, 2013), in pot (George *et al.*, 2012), and in field experiments (Malghani *et al.*, 2014), where toxic effects associated with a general decrease in dry biomass yield and/or in plant height were reported for different species,

**Table 6** Soil nutrient concentrations (g $kg^{-1}$) in the experimental treatments at 0–15 and 15–30 cm soil depth. For HC-1, data are reported for both 2012 (Year 1) and 2013 (Year 2) sampling. Different letters indicate a significant difference among treatments ($P < 0.05$)

| | 0–15 cm | | | | | 15–30 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Ca | K | Mg | Na | P | Ca | K | Mg | Na | P |
| Control | 13.55 ± 0.36 b | 8.49 ± 0.23 c | 4.32 ± 0.40 a | 1.29 ± 0.08 b | 0.83 ± 0.06 b | 9.80 ± 0.40 a | 7.92 ± 0.71 a | 2.83 ± 0.18 a | 1.11 ± 0.08 a | 1.34 ± 0.29 a |
| HC-1 (Year 1) | 17.68 ± 1.04 a | 9.51 ± 0.03 ab | 4.19 ± 0.36 a | 1.64 ± 0.16 ac | 1.23 ± 0.12 b | 10.41 ± 0.73 a | 8.23 ± 0.92 a | 2.95 ± 0.17 a | 1.05 ± 0.11 a | 1.47 ± 0.31 a |
| HC-1 (Year 2) | 13.82 ± 0.92 ab | 8.89 ± 0.77 bc | 4.04 ± 0.13 a | 1.42 ± 0.15 bc | 1.12 ± 0.14 b | 10.76 ± 1.79 a | 8.32 ± 0.85 a | 2.87 ± 0.18 a | 1.06 ± 0.07 a | 1.11 ± 0.13 a |
| HC-2 | 18.48 ± 0.09 a | 9.92 ± 0.47 a | 4.24 ± 0.27 a | 1.7 ± 0.04 a | 1.36 ± 0.02 a | 10.64 ± 2.11 a | 9.53 ± 1.11 a | 3.09 ± 0.19 a | 1.19 ± 0.15 a | 1.49 ± 0.25 a |
| *P-value* | 0.009 | 0.04 | 0.63 | <0.001 | 0.008 | 0.79 | 0.12 | 0.27 | 0.32 | 0.18 |

but it is noteworthy to remark, here, that those experiments were made with crop species starting from seeds. The observed toxicity on germination and initial plant growth in those experiments, but not on plant regeneration from cuttings in our study, may explain such a discrepancy.

Incremental effects may occur when plant growth is stimulated in response to an amelioration of the soil physical properties and/or of soil fertility. The addition of charred substances into soil has been repeatedly proven to have incremental effects, thus achieving the double goal of sequestering C and stimulating plant growth (Lehmann *et al.*, 2006; Baronti *et al.*, 2010). The same occurred for our HC application, which largely stimulated biomass growth in poplar mainly through the supply of additional nutrients, in particular N (Table 2). Positive effect of fertilization has been repeatedly reported for poplars (Coleman *et al.*, 2006; Ceotto *et al.*, 2016), and other studies made with HC confirmed that, when the initial toxic effects disappears, the nutrients contained in the amendment have positive effects on growth and crop productivity (Malghani *et al.*, 2014). Those positive effects saturated, however, after the first year since application (HC-1) and two consecutive applications (HC-2) did not further increase the total biomass of the poplars (Fig. 2). Overall, the large growth stimulation effect of HC that we observed in a bioenergy crop such as poplar may have additional indirect consequence on $CO_2$ emission mitigation. Assuming that the energy content of poplar is almost constant when expressed on a dry weight basis (19.8 ± 0.1 MJ $kg^{-1}$; Kauter *et al.*, 2003), the observed HC-driven mean increase of 5.3 ± 2.3 t $ha^{-1}$ $yr^{-1}$ indicates that approximately 106 ± 46 GJ $ha^{-1}$ $yr^{-1}$ of additional bioenergy may potentially become available in HC-treated soils. Assuming an emission factor for natural gas equivalent to 56.1 ± 3.8 t $CO_2$ $TJ^{-1}$, the fossil fuel energy offset would decrease net $CO_2$ emission of about 6.2 ± 2.8 t $CO_2$ $ha^{-1}$ $yr^{-1}$.

The use of HC and of any other charred substance into soils for C sequestration has the necessary requisites of additionality, as it involves a voluntary action specifically aimed at C sequestration.

$CO_2$ leakage (*sensu* IPCC) may occur in HC application in two ways. In a first instance, agricultural residues, which are thermochemically transformed in the HTC process, subtract a fraction of the organic C and nutrients from crops and soils where they originate. While the C contained in the residues would be mostly returned to the atmosphere, if the residues were incorporated into soils, the nutrients which are removed must be instead replaced by fertilizers which may be produced from fossil sources or involve high emission costs to be transported from the production place to the

application site. It is important to highlight, here, that although the incorporation of residues into soil may bring back a fraction of the nutrients that were originally taken up by the crop, it may have negative effects on yield in particular in monocultures. Recent studies analyzing the occurrence of self-inhibition on growth in different species proposed that an accumulation of extracellular self-DNA into the soil may be at the origin of such negative growth effect, over the long term (Mazzoleni *et al*., 2015), thus suggesting that the removal, rather than the incorporation of residues, may be beneficial as far as monocultures or agricultural short-rotations are concerned. A second potential source of $CO_2$-equivalent leakage may occur if HC application enhances the emissions of other greenhouse gases such as $N_2O$ and $CH_4$. We did not measure $N_2O$ emissions in this study but considering that the difference in the amount of N fixed in wood biomass in treated and not-treated poplars was $16.6 \pm 4.8$ g N $m^{-2}$ and that the soil N stocks after one year since application did not significantly change (Table 4), we can estimate that approximately 85% of the N applied with HC could have been potentially lost as leachate or volatilized into the atmosphere as $N_2O$, in response to nitrification/denitrification processes in the soil. Such leakage estimate cannot be unfortunately better documented on the basis of our experimental data, but this aspect certainly deserves some attention as $N_2O$ global warming potential is 298 times greater than $CO_2$ so that even a small emission may offset a significant fraction of the net emission reduction realized by HC. Even though HC has been shown to be a promising sorbent of a wide range of pollutants (Sun *et al*., 2011; Eibisch *et al*., 2015; Han *et al*., 2016), the possible groundwater contamination through N leaching after HC addition into soil should be better assessed in future studies. In fact, the nutrient retention potential of HC (i.e., nitrate and ammonium) differed strongly with nutrient and the type of carbonized feedstock, as well as amended soil type.

## Conclusions

HC application in short rotation forestry is recommended as it is very effective in stimulating plant growth. Such stimulation is accompanied by a less effective soil C sequestration potential which we estimated to be around 50% of the applied C. This last conclusion has a high degree of uncertainty because of the duration of our experiment (2 years) and the lack of a proper understanding on the priming/protection of charred substances on soil organic matter.

Poplar cuttings are very tolerant to potential toxic effects of HC. This is not the case for all species as, when the same HC was applied to wheat and rapeseed, the seedlings showed a reduced growth in biomass and height (Malghani *et al*., 2014). The causative agent of such toxicity should be urgently identified.

The supply of large quantities of mineral nutrients, which is the major advantage of the HC use, warns that the dose used in our experiment (30 tC $ha^{-1}$) was excessive as most of the applied N was not used by the plants. This calls for the need of a careful examination of dose–response functions to finally develop best practices for HC application in short rotation forestry. This is also important to minimize risks of $N_2O$ emissions. Due the high relative HC mobility into the soil, the choice of the right dose is likely to diminish the risk of contamination of the water table by drainage. A reduction in the dose applied, however, inevitably reduces the C sequestration potential of HC, but not the potential for fossil fuel substitution due to enhanced biomass growth.

## Acknowledgements

This study was financially supported by the European Commission through the EuroChar project (FP7-ENV-478 2010ID-265179) and AgroPyroGas project funded by Regione Toscana, Italy. The authors would like to thank V. Zwing at Carbon Solutions CS Deutschland GmbH; Ce.Spe.Vi. s.r.l., Pistoia for the support in the field experiment; Dr. Luisa Andrenelli and Dr. Adriano Baglio (Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, DISPAA, University of Firenze) for laboratory analysis; Dr. Anita Maienza, Dr. Emanuela Pusceddu, Dr. Sara Di Lonardo, and Mario Lanini (IBIMET-CNR) for sampling support operations. G.A. during data analysis and paper preparation was partially supported by a German Academic Exchange Service (DAAD, Germany) scholarship for a three-month research period at Chair of Silviculture, University of Freiburg, Germany. I.C. was supported by a Ph.D. grant from Fondazione Edmund Mach, Trento. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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