Plant Soil (2014) 385:87–105
DOI 10.1007/s11104-014-2198-2

REGULAR ARTICLE

Unintended effects of biochars on short-term plant growth in a calcareous soil

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Received: 1 January 2014 / Accepted: 13 July 2014 / Published online: 6 August 2014

Abstract
Background and aims Biochar has demonstrably improved crop yields in weathered and acidic soils, but studies in calcareous soils are particularly lacking, so biochar effects on plant growth was investigated under these conditions.
Methods Six biochars were obtained from different feedstocks and production technologies. Chemical characterization of fresh biochars included total and extractable nutrients, labile carbon, and Fourier transform infrared spectroscopy. Extractable nutrients were also evaluated in biochar-soil mixtures with a basic (pH >8.2) test soil. Bioassays with lettuce and ryegrass were carried out to relate biochar chemical properties to effects on plant biomass.
Results A sewage sludge slow pyrolysis char was stimulatory to plant growth, as was a slow pyrolysis pine wood char at an intermediate concentration, while gasification and fast-pyrolysis pine and poplar wood chars were strongly inhibitory, with reductions in biomass at realistic application rates of 5–19 t ha^{-1}.
Conclusions Statistical comparison of plant responses with biochar composition led to the assessment that plant responses were most correlated with volatile matter content and total P content, whose availability was likely regulated by pH and Ca content. Potential effects of phytotoxins were considered, but these were seen to be much less probable than effects due to nutrient availability.

Keywords Nutrient availability · Volatile matter · Phosphorus · Effective concentration

Introduction

Biochar is thermally decomposed biomass whose use is destined for application to soils (Granatstein et al. 2009; Sohi et al. 2009; Verheijen et al. 2010). Though very similar to charcoal, a combustible obtained with a traditional technology for storing energy, biochar is defined as pyrolyzed biomass applied to soils to improve crop productivity, enhance soil properties, and increase carbon storage in the soil due to its highly recalcitrant carbon content (Lehmann and Joseph 2009). This practice leads to changes in soil physical (Oguntunde et al. 2008; Asai et al. 2009; Zhang et al. 2012), chemical (DeLuca et al. 2009; Rousk et al. 2013), and biological

Responsible Editor: Simon Jeffery..

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(Lehmann et al. 2011; Domene et al. 2014; Marks et al. 2014) properties. In tropical systems improvements in crop productivity have often been cited (Verheijen et al. 2010), whereas in many cases this is likely due to improvements in soil pH or alleviation of Al toxicity in highly weathered soils (Blackwell et al. 2009). However, not all biochars are created equal as the saying might go, and different materials may cause different crop responses under the same environmental conditions (Gaskin et al. 2010; Deal et al. 2012), thus provoking the question of what biochar properties might lead to observed differences? There is an immense diversity of biochar materials, with important differences in elemental composition (Brewer et al. 2009), ash content (Deal et al. 2012), ion retention and release (Silber et al. 2010), and recalcitrance to biotic decay (Bruun et al. 2008), among others. These differences have important impacts on soil processes, and also given the need for standardization of agricultural products, they require a classification system (Joseph et al. 2009). Recent efforts have included the proposal of appropriate laboratory testing guidelines for biochar characterization (International Biochar Initiative 2013), including a germination test. The germination test proposed by International Biochar Initiative is a rapid method of assessing potential biochar effects on that endpoint, but plant growth bioassays are more complete and of higher significance. Also, whereas much information is already available regarding elemental and chemical transformations associated with distinct pyrolytic production methods, these have not been adequately tied to biological responses.

There are a number of reasons why bioassay screening of biochar is critical if biochar is to be applied on large scale for agricultural purposes or atmospheric CO_2 mitigation as a carbon sink. Firstly, despite many studies promoting biochar application to soil as a soil amendment, mainly centered on agronomical benefits, little attention has been paid to potential unintended effects (Kookana et al. 2011), such as the ecotoxicological risks of its application to soils. Since biochar is produced from biomass, including polluted organic wastes, pollutant content such as heavy metals in biochars could be significant and present potential environmental risks when applied to soils, as has been cautioned for biochars produced from animal manures and sewage sludge (Kookana et al. 2011). Also, organic pollutants may include polycyclic aromatic hydrocarbons (PAHs) and dioxins (Garcia-Perez 2008; Schimmelpfennig and Glaser 2012). The field of soil ecotoxicology has long relied on the use of plant bioassays to assess “safe” levels of potential toxins (Paton et al. 2005), which have been codified in test protocols (e.g. OECD 2006); we would argue that the screening tools should be utilized when contaminants or unintended effects due to biochar application are suspected. Secondly, bioassays with higher plants measure a direct impact on a critical endpoint of this technology, the primary productivity of crops, which may be impacted by biochar’s demonstrated alterations of soil nutrient availability and soil processes (Clough and Condron 2010; Jones et al. 2011; Zimmerman et al. 2011; Bruun et al. 2012). Finally, standardization of biological characterization may allow future screening and materials characterization by biological methods previous to field implementation without the need of specialized equipment, which could be critical for the technology’s adoption in underdeveloped areas of the world.

Biological assessment is not always integrated into the studies undertaking the chemical and physical characterization of biochar, therefore making it difficult to predict outcomes of measured properties on plants or model organisms. Also, recommendations or guidelines for “appropriate” application rates are lacking within the field (Gaskin et al. 2010; Hossain et al. 2010) and bioassays might have a role for this purpose since their elaboration is more cost-effective than field studies. Furthermore, what soil properties may be altered by fresh chars and cause effects on plants in the short term is still under debate.

In this study we addressed the influence of biochar composition on soil-biochar mixture chemical properties and on plant aboveground and belowground biomass. Specifically, the objective was to associate biochar composition and the changes exerted on soil chemical properties with measured responses of above- and below-ground biomass to elucidate possible causes of effects on plant productivity.

Materials and methods

Soil

The test soil corresponded to the 20 cm topsoil of a *Fluventic Haploxerept* (Soil Survey Staff 2010), sandy loam agricultural soil, harvested from the Torre Marimón experimental farm site in Caldes de Montbui

(Catalonia), NE Spain, uncultivated and free of agrochemicals for at least the previous 7 years. Methods used in soil characterization were as follows: texture, Robinson pipette; carbonates, Bernad calcimeter; organic matter, Walkley-Black; nitrogen, Kjeldahl; phosphorus, Olsen extraction with determination by the molybdene ascorbic acid method (Murphy and Riley 1962); exchangeable cations, extraction by 1 M ammonium acetate and determination by ICP-OES; metals, extracted by nitrohydrochloric acid and determined by ICP-OES. Main soil properties are shown in Table 1.

Biochars and biochars characterization

Biochars proceeded from three feedstocks and three pyrolysis methods (Table 2). Biochar aqueous extracts (1:10 g biochar:ml deionized water) were prepared in triplicate by vertical agitation for 24 h at 60 rev min^{-1}, the suspensions were vacuum filtered with Whatman 42 filter paper, and pH and electrical conductivity (EC) were measured immediately. Elemental analyses were carried out on samples dried overnight at 105 °C. Analyses for C and H were carried out using a Flash 2000 C.E. elemental analyzer (Thermo Fisher Scientific), N by a Flash EA 1112 elemental analyzer (Thermo Fisher Scientific), and S by ICO-OES spectrometry using a Varian 725-ES Radial ICP Optical Emission Spectrometer (Varian Inc.). Oxygen content was estimated by difference from ash, C, N, H, and S contents.

Elemental ratios were calculated using the molar concentrations of the elements concerned (Van Krevelen 1961). Metals were analyzed by acid digestion of 0.5 g of sample in Baker Instra-Analyzed HCl - HNO_3 3:1 in a high-pressure teflon reactor heated at 200 °C in an Ethos Plus microwave (Milestone Srl.). The digested sample was filtered and the residue washed with 0.5 M HNO_3 to 100 ml. Determination of Ca, Mg, Na, K, P and Fe were carried out using a Polyscan 61E ICP-MS (Thermo Jarrell-Ash Corp.). Remaining element quantification was carried out using a 7500ce ICP-MS (Agilent Technologies Inc.).

The proximate analysis of biochar products after sample heating at different temperatures was also performed, namely sequential loss on ignition (LOI), volatile matter (VM) and ash content. LOI is a simple method initially developed for estimating the content

Table 1 Test soil main properties

Parameter	Data
Sand (0.05-2 mm)	59.6 %
Coarse silt (0.02-0.05 mm)	12.5 %
Fine silt (0.002-0.02 mm)	10.5 %
Clay (<0.002 mm)	17.0 %
pH (1:5 H_2O)	8.6
Electrical conductivity (25 °C)	210 μS cm^{-1}
Carbonates ($CaCO_3$ equivalent)	6 %
Organic matter	1.60 %
N (total)	0.08 %
P (extractable)	27 mg kg^{-1}
Ca (exchangeable)	5,557 mg kg^{-1}
Mg (exchangeable)	233 mg kg^{-1}
K (exchangeable)	159 mg kg^{-1}
Na (exchangeable)	62 mg kg^{-1}
Cd (acid-extractable)	<0.5 mg kg^{-1}
Cu (acid-extractable)	17 mg kg^{-1}
Ni (acid-extractable)	7 mg kg^{-1}
Pb (acid-extractable)	25 mg kg^{-1}
Zn (acid-extractable)	65 mg kg^{-1}
Hg (acid-extractable)	<40 μg kg^{-1}
Cr (acid-extractable)	10 mg kg^{-1}

Table 2 Biochars studied and identification codes. In the case of the slow pyrolysis chars, heating rate is not applicable as the system was a screw pyrolyser, i.e. the reactor was already functioning at the indicated temperature

Code	Feedstock	Production method	Residence time	Production temperature (°C)
CL	Populus nigra (Poplar) wood chip	Slow	15 m	500 – 550
CR	Populus nigra wood chip	Fast	< 2 s	430 – 510
FL	Thermally dried wastewater sludge	Slow	15 m	500 – 550
PG	Pinus pinaster + P. radiata wood chip	Gasification	10 s	600 – 900
PL	Pinus pinaster + P. radiata wood chip	Slow	15 m	500 – 550
PR	Pinus pinaster + P. radiata wood chip	Fast	< 2 s	440 – 480

of organic matter and carbonate minerals in sediments and sedimentary rocks (Dean 1974). It is a gross measure since some losses via oxidation or dehydratation of other minerals may bias the results (Santisteban et al. 2004), although such biasing will usually be limited in biochars, whose composition is mainly organic. LOI was conducted on ground samples in triplicate in a muffle furnace, first at 375 °C for 18 h, without acid pre-treatment (used for removal of soot and graphitic black carbon) to evaluate biochar organic content except the soot fraction (Gustafsson et al. 1997; Poot et al. 2009); at 550 °C for 5 h to remove soot (Gustafsson et al. 1997), hence representing the complete oxidation of the organic carbon fraction in biochar; and finally at 1100 °C for 5 h, which should mainly remove carbonates (Santisteban et al. 2004). VM was determined in triplicate following ASTM International Guideline (2007) by heating ground samples in a covered (oxygen-limited) crucible at 950 °C for 6 min and determining weight loss, which reflects the uncharred organic content in chars (Deenik et al. 2010). Ash content was also evaluated in triplicate following the same ASTM protocol by heating ground samples in an uncovered crucible at 750 °C for 6 h and determining weight loss, representing the mineral content in chars.

The characterization of the main chemical bonds that are related to functional groups present in the biochars was done by Fourier transform infrared spectroscopy analysis (FTIR) of dry (105 °C), ground biochar samples passed through a 100 μm sieve. Spectra were registered in triplicate at standard infrared resolution (4 cm^{-1}) in the mid-infrared range of 600–4,000 cm^{-1} using a Bruker Tensor 27 spectrophotometer working in attenuated total reflectance (ATR) mode with diamond reflection.

Biochar extractable components

Labile carbon content of the biochars was assessed using a hot water extraction following Rovira and Ramón Vallejo (2007)), hereafter referred as hot water-extractable carbon (C_{hw}). Briefly, oven-dry ground biochar samples of 0.5 g were extracted with 20 ml of deionized water in sealed Pyrex tubes heated in aluminum blocks at 105 °C for 1 h after which the extract was filtered with Whatman 42 filter paper and stored at −20 °C. Later, extracts were evaluated for dissolved organic carbon (DOC) using the acid dichromate method described in Brookes and Joergensen (2006) with the modification that DOC extract and acid volumes were halved.

Water-soluble components of biochar were also assessed in two successive washings. The first was carried out following the biochar extraction methods described above (determination of pH and EC), and extracts were stored afterwards at −20 °C. The filtered residue was dried overnight at 105 °C, weighed, and the process was repeated again. A 5:50 ml dilution of frozen extracts was prepared, from which one homogenized 5 ml sample was taken for ion analysis by liquid chromatography. Ion chromatography analysis of Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+ was carried out with a CS12A Dionex cation column on a Dionex ICS-1,100 Ion Chromatograph (Dionex, Sunnyvale, USA), and for Cl^-, NO_2^- NO_3^-, HPO_4^{2-}, $H_2PO_4^-$, and SO_4^{2-} with a AS4A-SC Dionex anion column on a Dionex DX-100 Ion Chromatograph (Dionex, Sunnyvale, USA). With regards to phosphate measurement, in the methodology used the reported value in practice represents both dihydrogen and monohydrogenated species, and since HPO_4^{2-} is expected to be the most abundant species in the pH range of the present study, phosphate is hereafter considered as HPO_4^{2-}.

Soil-biochar mixtures characterization

The six biochar materials were tested in a wide range of concentrations of 0.4, 0.9, 2.1, 4.9, 11.3 and 26 %, in soil (as dry matter) representing a range of theoretical application rates from 10.6 t ha^{-1} to 676 t ha^{-1} (assuming soil bulk density of 1.3 g cm^{-3}, incorporation to 20 cm depth), with the intention that unrealistically high rates would aid the establishment of recommend maximum application rates and force any effects. Maximum water-holding capacity (WHC) at each biochar concentration was determined by water-saturating 50 g samples for 2 h followed by draining for 24 h at room temperature, and measuring moisture as weight loss after drying at 105 °C overnight. Soil water extracts were obtained at the beginning of the incubation. Briefly, 15 g of soil mixture and 75 ml of deionized water (1:5) were vertically agitated in 150 ml polyethylene cups for 2 h at 60 rev min^{-1}. The extract was subsequently centrifuged and the supernatant was filtered through Whatman 42 filter paper. pH and EC were immediately measured, and the extracts stored at −20 °C. A portion of the extracts for pH and EC determinations above were pooled and a 5:50 ml dilution was prepared, from which a homogenized 5 ml sample was taken for ion chromatography analysis to determine water-soluble concentrations of major cations and anions as described in the previous section.

Plant growth tests

The growth test was conducted following OECD 208 (2006) using *Lactuca sativa* and *Lolium perenne* as test species. Chars were mixed dry with test soil at the concentrations described above before being transferred in equal volumes to plastic 300 ml germination vessels equipped with bottom wicks. Four replicates were prepared per test species and concentration, including control. Ten seeds were placed on the surface of soil-biochar mixtures and lightly covered with soil. After, vessels were placed in a germination chamber equipped with a bottom irrigation system with deionized water. Growth tests were conducted within the germination chamber set at 16:8 light:dark cycle at 300 $\mu E/m^2/s$, and constant 22 °C and 70 % humidity. Seedlings were thinned to the three most vigorous plants after 4 days for *L. sativa* and 7 days for *L. perenne*. Replicates were arbitrarily rearranged within the chamber after the first week. After 14 days the test ended and all plants were carefully removed, their roots washed, rinsed with deionized water, dried at 70 °C for 48 h, and aboveground and belowground parts separated and weighed to obtain dry weight (DW), pooling the three plants in each replicate.

Statistical analysis

All statistical analyses were carried out in R. In the assessment of differences in aboveground and belowground biomass within materials, non-parametric tests were used since homogeneity of variance was violated according to Levene's test. Kruskall-Wallis test was used to test for global differences, and Mann–Whitney post-hoc tests were used to identify significant differences with respect to control at specific biochar concentrations. Dose–response models and effective concentrations (EC_x) were calculated using the *drc* package for R (Ritz and Streibig 2005). EC_x of x =10-20 (i.e., concentrations leading 10-20 % reduction in measured endpoint), equivalent to the no observed effect concentration (NOEC), are commonly established as acceptable limits for potential contaminants (Isnard et al. 2001; Arnold and Cotsifas 2008), which can be also taken as maximum biochar application rates. Therefore, the EC_{10} was chosen as the more conservative baseline for materials comparison. Two model types were considered, Brain-Cousens (BC) models including a hormesis parameter, and log-logistic (LL); that which minimized residual standard error was chosen as the best model. EC_{10} values were thereafter compared with elemental and chemical properties of the fresh biochars using non-parametric Spearman correlations.

Biochar amended soil chemistry was evaluated in two steps. First, mixture properties were related to biochar concentration with linear regressions using the *lm* function. Second, the effects of measured mixture chemistry on aboveground biomass and belowground biomass (hereafter AGB and BGB, respectively) and root:shoot of lettuce and ryegrass were assessed using multiple linear regression models (MLM) using the *lm* function in R. Plant responses were standardized with respect to the control for each of the material-concentrations. The following parameters were initially included in the models: pH, EC measured as $\mu S\ cm^{-1}$, and water-extractable nutrients in ionic form as mg kg^{-1} of Ca^{2+}, K^+, Mg^{2+}, Na^+, NH_4^+, Cl^-, NO_3^-, HPO_4^{2-} and SO_4^{2-}. NO_2^- was excluded since it was undetectable in most of the samples in accordance with its low residence time in the soil, as it is quickly converted to NO_3^- under aerobic conditions. Also, concentration was included in the models to account for its association with ion concentration. Following elimination of collinear variables with variance inflation factor >10, the best model was selected as that which minimized the Akaike Information Criteria (AIC) using the *dredge* function in the *MuMln* package. Standardized coefficients, β_i, were calculated for each predictor x_i so as to allow comparison between predictors having different units of measurement; the standardized coefficient is calculated by multiplying the unstandardized predictor coefficient by the ratio of the standard deviations of the predictor and dependent variables. Constant error variance was checked visually using standardized residuals. Outliers were identified using the *outlierTest* function of the *car* package, following Bonferroni p-values for Studentized residuals and removed if corrected p was <0.05, and the Shapiro-Wilk test was used to check normality of model residuals which were taken to be normal if $p>0.05$.

Table 3 Biochar chemistry including pH and EC (SD of three replicates) and elemental content (SD of duplicate samples where applicable; for S and N only mean value is available). Reported O contents are estimated following ASTM International Guideline (2007). C:N and C:P are mass ratios, whereas H:C and O:C are atomic ratios

	CL	CR	FL	PG	PL	PR
pH (H_2O, 1:10)	8.21±0.06	8.14±0.06	8.70±0.11	11.42±0.02	7.29±0.02	8.04±0.04
EC (μS cm^{-1})	784±4	610±3	1624±6	1888±10	565±11	639±2
N (%)	0.48	0.35	2.26	0.12	0.12	0.19
C (%)	81.07±0.11	73.11±0.08	22.34±0.02	71.03±0.16	86.26±0.16	71.76±0.04
H (%)	2.07±0.02	3.27±0.02	1.20±0.02	0.53±0.02	1.97±0.02	3.40±0.04
S (%)	0.04	0.02	1.01	0.08	0.02	0.02
O (%)	12.3	18.8	4.0	8.7	9.1	22.0
C:P	405	1219	4	888	246	1435
C:N	169	209	10	592	719	378
H:C	0.31	0.54	0.45	0.09	0.27	0.57
O:C	0.11	0.19	0.17	0.09	0.08	0.23
P (mg g^{-1})	1.96±0.22	0.57±0.01	51.19±3.27	0.80±0.11	3.51±0.09	0.48±0.07
Ca (mg g^{-1})	9.57±0.23	12.18±0.09	89.11±4.34	92.34±3.65	3.77±0.14	8.27±0.02
Mg (mg g^{-1})	1.31±0.03	1.59±0.04	11.83±0.57	2.59±0.06	0.98±0.04	1.42±0.00
Na (mg g^{-1})	0.96±0.08	1.03±0.02	3.84±0.17	0.78±0.00	0.33±0.05	0.48±0.00
K (mg g^{-1})	6.57±0.23	9.21±0.38	9.09±0.60	8.25±0.29	3.48±0.12	6.40±0.22
Fe (mg g^{-1})	1.97±0.13	1.78±0.05	42.65±2.93	1.53±0.09	1.21±0.02	1.58±0.09
Zn (mg kg^{-1})	130±14	540±7	3074±156	823±121	70±24	181±7
Cr (mg kg^{-1})	213±36	40±1	385±50	26±2	83±21	26±0
Cu (mg kg^{-1})	109±27	29±2	767±87	219±3	27±3	13±0
Ni (mg kg^{-1})	253±22	23±1	249±28	10±2	97±0	25±0
As (mg kg^{-1})	< 2.0	< 2.0	12.4±1.0	< 2.0	< 2.0	< 2.0
Cd (mg kg^{-1})	< 0.5	< 0.5	2.2±0.3	1.2±0.0	< 0.5	< 0.5
Hg (mg kg^{-1})	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Pb (mg kg^{-1})	74.7±14.4	62.1±0.9	277.0±34.4	9.1±0.4	15.7±0.5	10.1±0.1

Results

Biochar and soil mixtures characterization

Biochar elemental composition is shown in Table 3. Slow-pyrolysis wood chars had the highest C content (81-86 %), whereas that of fast-pyrolysis and gasification were similar (71-73 %), and the sewage sludge char was the lowest (22 %) due to its high mineral matter content. The sewage sludge char had the highest N content (2.3 %), followed by the poplar chars (0.35-0.48 %), and pine wood chars the lowest (0.12-0.19 %). P content was highest in the sewage sludge char (5.12 %), followed by the slow-pyrolysis chars (0.20-0.35 %) and lowest in the fast and gasification chars (0.05-0.08 %).

Proximate analyses (LOI, VM, and ash content) and C_{hw} results are shown in Table 4. LOI-375 mainly reflects the organic matter fraction of biochars as well as soot (Gustafsson et al. 1997; Poot et al. 2009), being the main fraction of most wood chars (93-97 %), with the exception of PG (76 %) and FL (12 %). LOI-550, representing the remaining organic fraction (soot) (Gustafsson et al. 1997), was very low in most biochars (0.2-0.7 %) with the exception of PG (2 %) and FL (14 %). Finally, LOI-1100, mainly reflecting carbonate content (Santisteban et al. 2004) was very low in most biochars (0.2-0.9 %) with the exception of FL (4 %) and PG (6 %). As expected, the sewage sludge char had high ash and relatively low VM. Within the wood materials the gasification char had highest ash content (19 %), whereas the other pine wood materials had ash content

Table 4 Proximate analyses of loss on ignition (LOI), volatile matter (VM), and ash contents of fresh biochar samples, expressed as percentage of each fraction with respect to total weight, in addition to hot water-extractable C (C_{hw}), all±SE of three replicates

	Proximate analyses					C_{hw}
Biochar	LOI 375 °C (%)	LOI 375–550 °C (%)	LOI 550–1100 °C (%)	VM (%)	Ash content (%)	µg C g^{-1}
CL	95.10±0.01	0.67±0.05	0.67±0.06	14.75±0.46	4.06±0.09	654±55
CR	93.19±0.34	0.45±0.03	0.90±0.14	27.06±0.31	4.49±0.15	3285±42
FL	12.46±0.36	14.39±0.34	3.97±0.01	14.29±0.34	69.24±0.07	1277±42
PG	76.15±0.16	2.22±0.37	6.1±0.46	15.68±0.31	19.57±0.35	613±42
PL	96.75±0.01	0.18±0.00	0.18±0.01	10.67±0.20	2.57±0.09	933±72
PR	94.96±0.13	0.56±0.00	0.37±0.04	28.07±0.22	2.65±0.09	2684±41

of ~2.5 % and those of poplar ~4.5 %. Fast and slow pyrolysis materials had the greatest VM difference (estimated difference in means 14 %, $p<0.001$), followed by fast and gasification (12 %, $p<0.001$), and least differences in VM were between slow and gasification (2 %, $p=0.040$).

FTIR spectra are shown in Fig. 1. All biochars showed very limited content of aliphatic groups, whose presence is usually strongest at longer wavelengths (C-H stretching 2,800–3,000 cm^{-1}, O-H stretching at 3,300 cm^{-1}) in FTIR spectra (Fig. 1). In the range of 1,030–1,400 cm^{-1}, small peaks of aliphatic methyl and ether/alcohol C-O- and C-O bonds were also evident, though the importance of these was obscured by the strong absorption by aromatic C=C (1570 cm^{-1} and 1415 cm^{-1}) and C =O (1,700 cm^{-1}). Baseline absorption (displacement on the y-axis) is associated with carbonization, aromaticity, and condensation of C (dehydrogenation, rearrangement and polymerization of aromatic rings), which followed in the order of fast pyrolysis < slow pyrolysis < gasification, as also evidenced by the H:C and O:C ratios in Table 3. The FL spectrum noticeably lacked C-bond absorption bands, demonstrating its limited organic content, the most predominant signal being from Si-O (1,015–1,050 cm^{-1}), though some aromatic C=C bonding was evident at 1,410 cm^{-1}.

Results of the fresh biochar washing experiment in the first (W1) and second leachate (W2) are shown in Table 5. Washing generally reduced pH, with the exception of PL. PG and FL showed the largest reductions in pH following washing (0.89-1.69 pH points) due to their high ash contents; reductions in fast pyrolysis material pH were intermediate (0.55-0.74); and slow pyrolysis materials showed little change (0.10-0.12). PG and FL liberated the most salts as expected from their high ash contents. Washing of agronomically important nutrients in respect to their elemental concentrations are shown in Table 6. Extractable N as NO_3^--N and NH_4^+-N was low in all cases. K^+, on the other hand was readily extracted in wood biochars, especially in slow pyrolysis materials, but not in FL. Phosphorous extractability as HPO_4^{2-} was relatively high in the slow pyrolysis materials, whereas much lower amounts were measured for the fast pyrolysis materials, and was below detection limits for PG and FL.

Soluble ion concentration from the soil-biochar mixtures followed the patterns of the fresh chars per material tested, however a few exceptions are noted: increasing biochar concentration generally decreased concentrations of NO_3^-, and increased NH_4^+, as seen in simple regressions in Table 7. Also, Ca^{2+} did not increase in all mixtures. Finally, HPO_4^{2-} was only detected in CL and PL mixtures. Biochar amended soil pH, EC, and WHC are also seen in Table 7. Increases in pH were PG>FL>CR, and decreases were PL>PR>CL. EC increases were highest in PG and FL, though statistically significant increases within realistic application rates were only seen in the latter. As expected, WHC increased in all mixtures.

Plant growth tests

Biochar dosage effects

The coefficient of variation (CV) for *L. sativa* control aboveground (ABG) and belowground biomass (BGB) of was 4 and 5 % respectively, and the CV of *L. perenne* control AGB and BGB was 26 and 31 % respectively. Biochars had diverse effects on AGB and BGB endpoints (Figs. 2 and 3, respectively). For lettuce, Kruskal-Wallis tests detected significant effects of concentration on both AGB and BGB for all biochars (data not shown). For ryegrass all biochars had effects on AGB except PL, and all on BGB except PL and FL. However, Mann–Whitney post-hoc tests for ryegrass revealed significant differences on BGB for FL concentration 11.3 %. Overall correlations between AGB and BGB were very strong and highly significant for both ryegrass (ρ= 0.94, p<0.001) and lettuce (ρ=0.95, p<0.001). Overall, plant growth inhibition, either measured as ABG and BGB were generally observed for the fast-pyrolysis wood biochars and gasification char used here, while stimulation was observed in the sludge biochar and one concentration of a slow pyrolysis char.

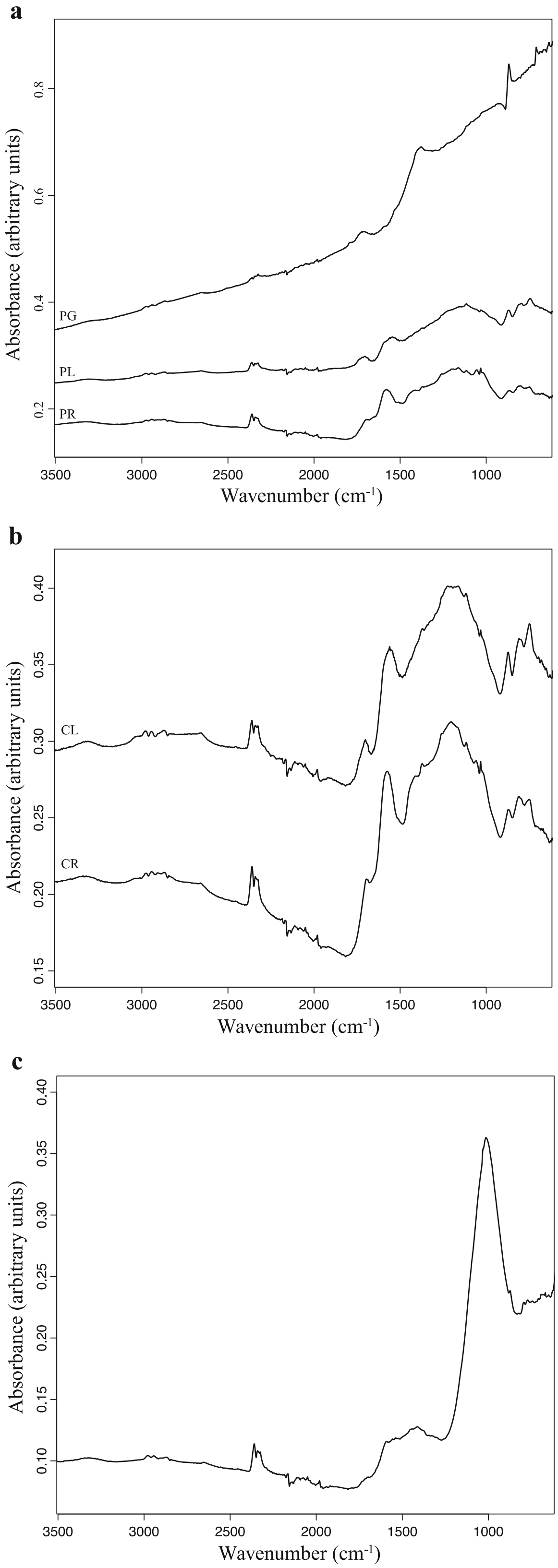

Fig. 1 **a** FTIR spectra of pine feedstock biochars. PG=gasification, PL=slow, PR=fast. **b.** FTIR spectra of poplar feedstock biochars. CL = slow, CR = fast. **c**. FTIR spectrum of slow pyrolysis sewage sludge feedstock biochar ▶

Table 5 Leachate characterization of the first (W1) and second (W2) washing of biochars. Ionic concentrations are expressed as recovered mass in mg kg^{-1} of biochar. 'b.d.l.' indicates concentrations below detection limit. For material codes refer to Table 2

Sample	pH (1:5)	EC (µS cm^{-1} 25 °C)	Na^+ (mg kg^{-1})	NH_4^+ (mg kg^{-1})	K^+ (mg kg^{-1})	Mg^{2+} (mg kg^{-1})	Ca^{2+} (mg kg^{-1})	Cl^- (mg kg^{-1})	NO_2^- (mg kg^{-1})	NO_3^- (mg kg^{-1})	HPO_4^{2-} (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})
CL-W1	8.21	784	368	106	1389	50	137	240	b.d.l.	17	1675	546
CL-W2	8.09	638	251	72	1251	40	97	142	5	19	1391	248
CR-W1	8.14	610	295	32	1154	50	193	175	b.d.l.	15	148	84
CR-W2	7.59	655	280	10	1270	46	161	142	b.d.l.	10	311	78
FL-W1	8.7	1624	374	29	116	137	2094	3009	b.d.l.	b.d.l.	b.d.l.	1282
FL-W2	7.83	967	360	28	107	305	1191	1052	b.d.l.	1	b.d.l.	2333
PG-W1	11.42	1888	306	b.d.l.	2436	b.d.l.	79	834	b.d.l.	28	b.d.l.	798
PG-W2	9.73	570	141	15	982	155	124	243	b.d.l.	18	b.d.l.	287
PL-W1	7.29	565	232	99	952	51	166	210	b.d.l.	21	3,618	263
PL-W2	7.39	253	69	44	480	22	106	119	b.d.l.	17	1,044	74
PR-W1	8.04	639	278	35	1,027	74	270	427	b.d.l.	17	157	131
PR-W2	7.3	360	87	86	465	50	281	202	b.d.l.	8	65	58

Table 6 Recovery of agronomically important nutrients in biochar washing experiment repetitions 1 (W1) and 2 (W2). Expressed as percentage of total elemental concentrations of N, K, and P. Material codes in Table 2

% washing of total elemental concentrations

	NH_4^+-N		NO_3^--N		K^+		HPO_4^{2-}-P	
Material	W1	W2	W1	W2	W1	W2	W1	W2
CL	2.1	1.4	0.1	0.1	21.1	19.0	27.6	23.0
CR	0.9	0.3	0.1	0.1	12.5	13.8	8.4	17.8
FL	0.1	0.1	0.0	0.0	1.3	1.2	0.0	0.0
PG	0.0	1.2	0.5	0.3	29.5	11.9	0.0	0.0
PL	7.7	3.4	0.4	0.3	27.3	13.8	33.3	9.6
PR	1.7	4.2	0.2	0.1	16.0	7.3	10.7	4.4

Dose–response models

EC_{10} values for each of the materials in relation to AGB and BGB are shown in Table 8. BC models were chosen over LL in many cases since they were better able to fit stimulatory effects. EC_{10} show that CR, PG and PR were the most inhibitory materials for all endpoints, whereas EC_{10} for CL, PL and FL corresponded to unrealistically high application rates, causing model non-convergence in some cases, in which case these endpoints EC_{10} were reported as over the maximum concentration tested (>26 %). Considering ryegrass EC_{10}, the order of most-to-least inhibitory did not change between AGB and BGB, though it is seen that roots were more impacted with the exception of PR, in which AGB was slightly more inhibited. For lettuce, AGB inhibition was PR>CR>PG, and BGB inhibition was PG>PR>CR. In contrast to ryegrass, lettuce AGB was overall more inhibited than BGB.

Plant endpoint models

Each regression model for both plants' BGB and root:shoot endpoints initially met the conditions of model adequacy, whereas for AGB one outlier were identified and removed for each species. Chemical explanatory variables included in the model for each species and endpoint, expressed as standardized coefficients, are shown in Table 9. Biochar concentration was significantly associated with negative effects on AGB and BGB but not for root:shoot endpoints. pH was

Table 7 Linear regressions fit to mixture ion concentration (mg kg-1), water-holding capacity (WHC, w/w), pH, and electrical conductivity (EC) 805 in a series of increasing concentrations of biochar (for codes refer to Table 2) in mixtures of 0, 0.4, 0.9, 2.1, 4.9, 11.3, 26 and 60 % (df=7). 806 Columns are the linear equation (Eq.), the adjusted R2, and the difference between minimum and maximum values (Diff.). Concentrations below 807 detection limit were assigned as 0 concentration, and NA indicates that concentrations were below detection threshold in all or only 1 808 concentration. † indicates that the parameter was below detection threshold in all but two concentrations (CL-HPO42-). NO2- has been omitted 809 due to presence in only three samples (CL-60, 3.9 mg kg-1; PR-0, 3.5 mg kg-1; PR-2.1, 4.9 mg kg-1). Significance codes: <0.05, '*', <0.01, '**', 810<0.001, '***'.

	Ca^{2+}			Cl^-			K^+			Mg^{2+}		
Material	Eq.	R^2	Diff	Eq.	R^2	Diff	Eq.	R^2	Diff	Eq.	R^2	Diff
CL	-0.73x+243*	0.46	75	0.73x+29	0.40	62	7.95x+24***	0.99	474	0.28x+25***	0.89	16
CR	2.88x+216***	0.89	182	1.01x+32***	0.86	71	4.95x+33***	0.99	297	0.44x+20***	0.87	29
FL	21.11x+223***	0.98	1,213	36.11x+74***	0.97	2,088	0.74x+33**	0.77	44	-0.25x+29	0.29	31
PG	-1.91x+263*	0.49	145	9.05x+56***	0.90	509	20.30x+7***	0.98	1,179	-0.31x+26*	0.61	21
PL	2.08x+142**	0.83	138	-0.77x+79*	0.59	51	3.02x+32***	0.94	198	0.14x+29	0.41	13
PR	3.11x+251***	0.94	189	1.97x+30***	0.97	126	2.64x+37***	0.96	164	0.61x+24***	0.98	37
	NH_4^+			NO_3^-			Na^+			HPO_4^{2-}		
Material	Eq.	R2	Diff.	Eq.	R2	Diff.	Eq.	R2	Diff.	Eq.	R2	Diff.
CL	0.17x+25	0.04	22	-0.17x+84	0.08	25	2.06x+35***	0.95	118	3.14x - 3.45**	0.83†	166
CR	0.39x+17**	0.82	25	-0.46x+80**	0.67	30	1.85x+38***	0.97	116	NA	NA	NA
FL	0.28x+24	0.09	36	-0.51x+80*	0.60	31	2.40x+34***	0.94	139	NA	NA	NA
PG	0.70x +14**	0.68	42	-0.28x+23	0.26	30	3.96x+37***	0.94	226	NA	NA	NA
PL	0.60x+14**	0.79	44	-0.60x+86**	0.76	42	-0.19x+64	0.02	32	12.77x - 10***	0.96	805
PR	0.73x+8**	0.70	52	-0.85x+100**	0.66	56	0.76x+32***	0.96	48	NA	NA	NA
	SO_4^{2-}			WHC (% w/w)			pH (1:5 H_2O)			EC (µS cm^{-1})		
Material	Eq.	R2	Diff.	Eq.	R2	Diff.	Eq.	R2	Diff.	Eq.	R2	Diff.
CL	4.02x+50***	0.92	234	4.35x+45***	0.99	262	0.003x+8.59	0.28	0.27	4.99x+132***	0.99	290
CR	0.88x+38	0.19	99	3.81x+55***	0.88	212	0.005x+8.52**	0.71	0.33	3.67x+117***	0.97	228
FL	3.04x+51***	0.93	187	0.32x+39***	0.96	20	0.028x+8.80***	0.88	1.71	12.72x+131***	0.97	739
PG	11.28x+52***	0.92	644	2.79x+48***	0.99	170	0.044x+8.88***	0.92	2.79	9.77x+106***	0.98	572
PL	1.47x+45	0.33	168	2.40x+46***	0.99	147	-0.018x+8.36***	0.86	1.04	2.08x+142**	0.83	138
PR	1.00x+37*	0.42	100	2.39x+47***	0.99	144	-0.006x+8.57**	0.78	0.41	3.62x+126***	0.99	216

significantly associated with increased lettuce AGB and BGB. Ca^{2+}, NH_4^+, NO_3^-, and HPO_4^{2-} were associated with positive effects. For root:shoot, the models selected only Na^+ (neg. for ryegrass, pos. for lettuce), and had very low explanatory power. Specific ionic concentrations and their implications for characterization of biochar materials will be discussed further in the following sections.

Discussion

Feedstock and pyrolysis effects on biochar and biochar-amended soil chemistry

Biochar effects on soil pH and the mechanisms of these are important for understanding real and potential impacts on plant-available nutrients. Biochar pH ranged from slightly to very alkaline. Mechanisms determining pH of water extracts were likely different between the chars owing to their different origins and pyrolysis conditions. In FL, it is likely that a portion of its chloride, sulphate and particularly carbonate salts were easily dissolved in W1, the latter of which contributed to alkalinity and provided large amounts of Ca^{2+} and other basic cations to soil solution (Table 5); in the second washing (W2) pH dropped by one unit, associated with lower concentrations of the same basic cations which had probably been present previously as carbonates and oxides. With regards to PG, it was rich in calcium and potassium ashes (Table 4), and its extracts (W1) had very elevated pH due to hydroxides and carbonates of these elements (Table 5). Washing PG removed a large proportion of K in its ionized form (Tables 5, 6) and strongly decreased pH of this char (Table 5). Oxides, hydroxides, and carbonates of K have a strong neutralizing (or alkalinizing) capacity and are very soluble (Demeyer et al. 2001), so these may have been responsible for high initial basicity. Other wood biochars were less basic owing to their lower ash content and production temperatures (Yuan 2011a; Enders et al. 2012). Applicable to all, hydrolysis of salts which may be present in biochars, especially carbonates such as Na_2CO_3, results in alkalinization (Tan 1993).

Biochars had varying effects on pH of treated soil (Table 7). Biochar-mediated pH changes may be related to their liming potential owing to alkalinity (Yuan et al. 2011b), dissolution or addition of alkaline metals (Kookana et al. 2011; Lehmann et al. 2011), or surface properties such as functional group composition (as mentioned above) which may include carboxyls (strong acids), phenols and carbonyls (weaker acids), and pyrones (basic) (Amonette and Joseph 2009; Joseph et al. 2009). While most often associated with liming effects (Chan and Xu 2009), our results indicate that some biochar materials may cause pH decreases in a calcareous soil (Table 7), whereas the lack of studies documenting such effects has been mentioned elsewhere (DeLuca et al. 2009).

The ionic concentrations reported in Tables 5 and **7** provide plant-available, water-soluble fractions of nutrients. As seen in Table 3, FL had the highest amount of N (2.26 %). In the soil-biochar mixtures, low concentrations of N-species in the FL extracts (in spite of the high N content of the respective feedstock) indicates that the N contained in FL was organically bound or in otherwise insoluble forms (e.g. heterocyclic), as has been found by other authors (Bridle and Pritchard 2004). Despite these considerations, N-species resulted significant in the plant response models (Table 9), although it must be noted that the quantities of soluble N reported here do not represent all plant-available fractions. In alkaline soils, addition of biochars of lower pH may potentially reduce ammonia volatilization and therefore increase plant-available N (Ducey et al. 2013), or conversely, if the biochar pH is higher, decrease it (Ventura et al. 2013).

With regards to potassium, its non-selection in the multiple linear models indicates that its concentrations were not limiting for plant growth. Fresh FL had very little soluble K (2.4 % recovered following W1 and W2), though the elemental concentration was similar to that of the other materials (Table 3). Recovered K from wood biochars following W1 and W2 was between 23 % (PR) and 41 % (PG) (Table 5). Overall, washings of the wood biochars show that K was highly available in these materials. During low-temperature pyrolysis (<500 °C) K takes plant-available forms, and is considered particularly available (Amonette and Joseph 2009). Accordingly, in the soil biochar mixtures, extracted K^+ increased with concentration in all biochars (Table 7). Regarding other important alkali metals, Ca^{2+} increased in CR, FL, PL, and PR, and Na^+ increased with concentration in all cases except PL, PG having the

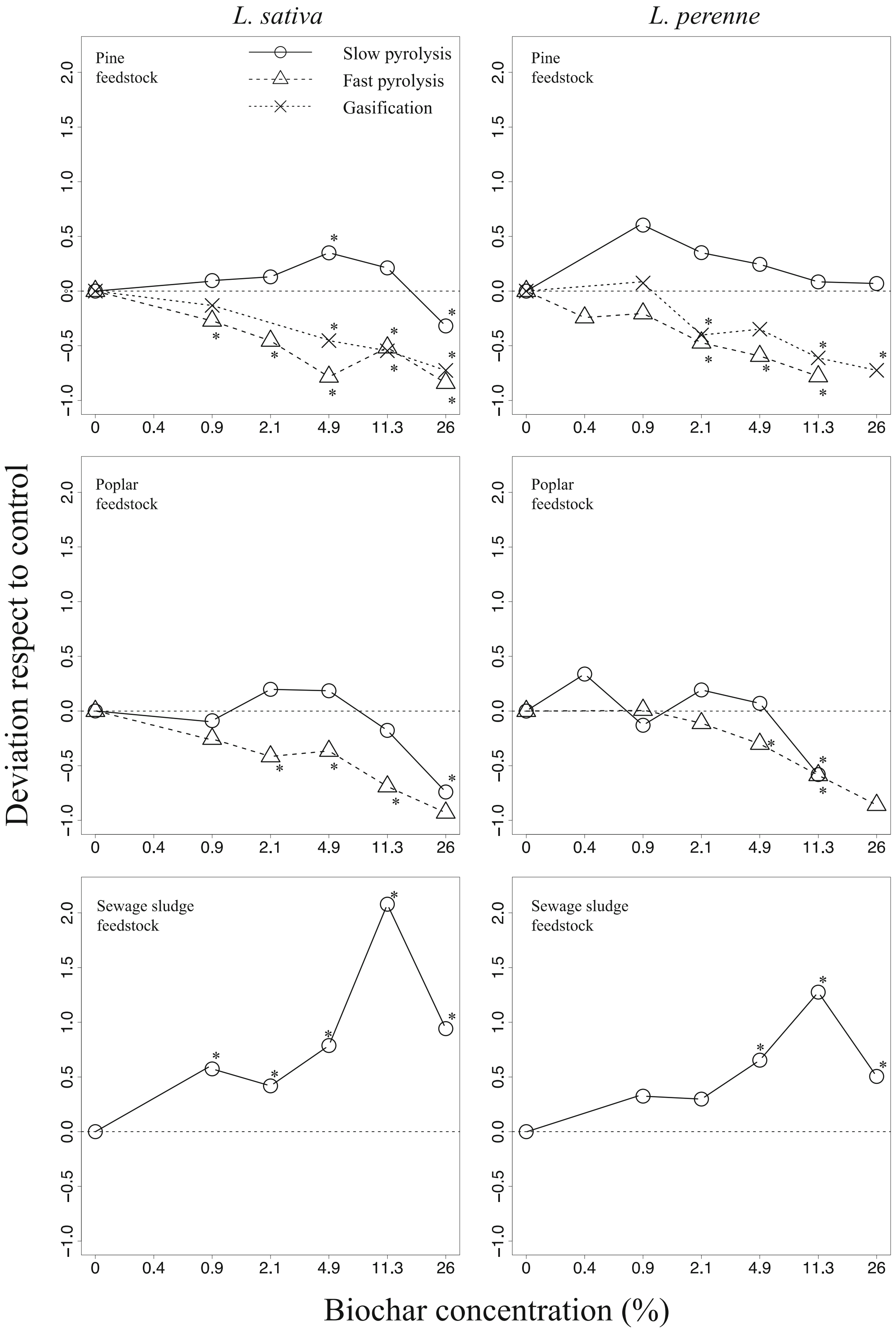
L. sativa
L. perenne
Pine feedstock
Poplar feedstock
Sewage sludge feedstock
Slow pyrolysis
Fast pyrolysis
Gasification
Deviation respect to control
−1.0
−0.5
0.0
0.5
1.0
1.5
2.0
0
0.4
0.9
2.1
4.9
11.3
26
*
Biochar concentration (%)

Fig. 2 Plant responses for aboveground biomass of *Lactuca sativa* (lettuce), and *Lolium perenne* (ryegrass). Y-axis unit is deviation of concentration mean with respect to control e.g. 0 =[equivalent to] control, 1 = control + (1 * control), −1 =control - (1 * control), and x-axis indicates the biochar concentration. Statistical differences with respect to control as evaluated by Mann–Whitney *U* test are indicated with *

highest Na^+ loads, reaching 92 mg kg^{-1} at 26 % concentration.

Soluble P was generally scarce in soil-biochar mixtures, only detectable in CL and PL mixtures. P extractability in the washing experiment was relatively high in the slow pyrolysis materials, and undetectable in PG and FL. Since biochar often strongly affects pH, and the availability of P is largely pH-dependent, the possibility that biochar may modify P availability has been previously recognized (DeLuca et al. 2009). Higher solution pH values and higher Ca:P ratios increase the precipitation of phosphate to less soluble forms (Song et al. 2002). When calcium is abundant (Ca:P of 3.33) it can precipitate with phosphate as quickly as 10 min in a pH 8.00 solution (Song et al. 2002). The alkalinity of the soil of this study (Carreira and Lajtha 1997), as well as the high Ca content and/or the high alkalinization capacity of leachates in FL and PG might explain the low soluble P release from these chars due to phosphate retrogradation. Our results suggest that both biochar liming capacity and Ca release may have been responsible for the low availability of P in PG and FL, and magnified by the fact of being tested in an alkaline soil. PG presented high elemental Ca:P ratio of 117, and though Ca washability was not high, pH was very elevated in leachates (9–11). Regarding FL, it presented a low Ca:P ratio (1.74), but extremely high concentrations of washable Ca. Phosphate precipitation has been described by previous studies with pyrolyzed sewage sludge, though importantly it was also found that P availability increased with incubation time (Bridle and Pritchard 2004; Yao et al. 2010). Yao et al. (2010)) demonstrated that solubility of P in pyrolyzed sewage sludge was increased by humic acids functioning as chelating agents. Precipitation reactions may also explain why soluble P was highest in slow pyrolysis chars (PL, CL), which had lower Ca:P ratios (1.07 - 4.8), low washable Ca content, and similar degree of alkalinization of the leachates. Finally, the fast pyrolysis chars, with intermediate Ca:P ratios (17–21), and intermediate Ca washability, presented intermediate P soluble content. The above reactions are of particular importance in calcareous soils with high abundances of base cations; biochar increases soil solution concentrations of free divalent cations, increasing precipitation of P as Ca or Mg phosphates (Chintala et al. 2014). The variation in soluble P with biochar addition therefore might have an important role on plant performance, since this, together with N, is the main nutrient limiting primary productivity in ecosystems (Elser et al. 2007), and in fact in our study these nutrients were associated with higher plant biomass (Table 9).

Relating biochar properties and their effect on soil chemistry to effects on plants

Fast-pyrolysis materials (CR, PR) along with the gasification material (PG) had the greatest negative effects on biomass (Table 8). The AGB EC_{10} of these materials also fall within realistic potential application rates of 5.5-51.2 t ha^{-1} for ryegrass, and 5.2-8.3 t ha^{-1} for lettuce. The other materials, CL, PL, and FL, were not harmful in any realistic range of application rates, CL having the lowest AGB EC_{10} of these at 173 t ha^{-1} in ryegrass, however it is noted that these conclusions are only valid in the context of the test species and test soil utilized.

In order to identify the key factors explaining plant growth inhibition responses, AGB responses at high concentrations (average of biomass at concentrations 4.9 and 11.3 % with respect to control) were correlated with measured biochar parameters described above. P content of biochars resulted strongly significantly and positively correlated with plant biomass (p=0.003, ρ=1.0 for both species) (Fig. 4). Similarly, biochar C:P was strongly negatively correlated with plant biomass (p=0.003, ρ=1.0 for both). These correlations reflect previously discussed results, whereas growth stimulation was observed in sewage sludge biochar with the highest P content, and inhibition was observed in fast pyrolysis biochars with the lowest P content. We reiterate that P content must be distinguished from its availability (soluble P), which we assume was chemically regulated by the alkalinity or calcium richness of the soil and the biochar.

A strong relationship was also found between VM content and plant performance, with significant negative correlations of VM with biomass (p=0.017, ρ=−0.94

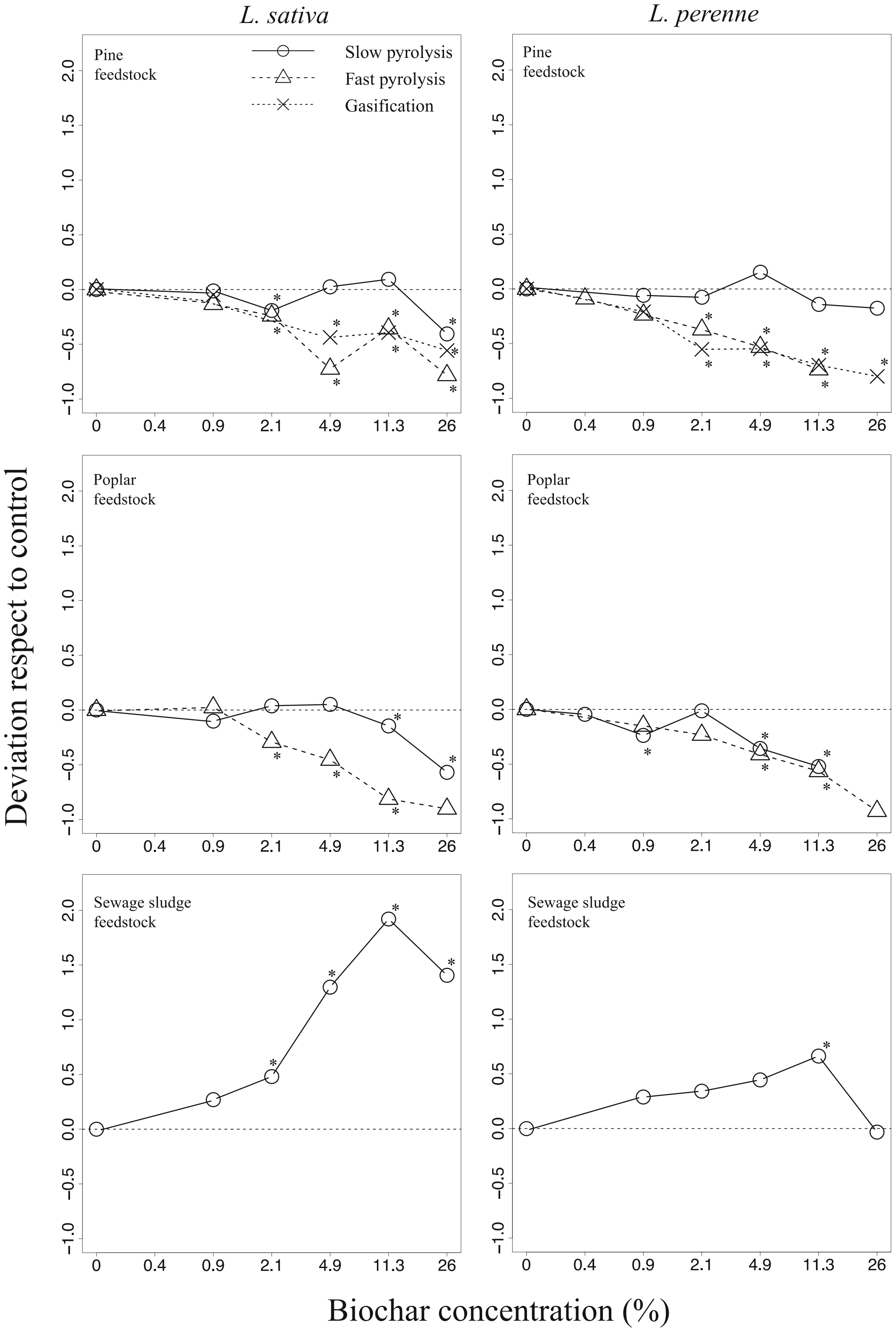

Fig. 3 Plant responses for belowground biomass of *Lactuca sativa* (lettuce), and *Lolium perenne* (ryegrass). Axes are as in Fig. 2. Statistical differences with respect to control as evaluated by Mann–Whitney *U* test are indicated with *

Table 8 Fit dose response models and EC_{10} (CI lower, CI upper) of each species-material combination for aboveground biomass. LL.3 indicates fit of a 3-parameter log-logistic model, and BC.4 indicates fit of a 4-parameter Brain-Cousens model. Using EC_{10} as criteria, within species results are ordered from most to least inhibitory. Finally, EC_{10} concentration values have been transformed to t ha^{-1} assuming application to 20 cm depth and bulk density of 1.3 g cm^{-3}. Material codes in Table 2

Test organism	Biochar	Curve fit	EC_{10} (%)	EC_{10} (t ha^{-1})
L. perenne	PR	LL.3	0.21 (−0.12,0.53)	5.5
Aboveground	PG	BC.4	1.61 (0.03,3.18)	41.9
	CR	LL.3	1.97 (−0.15,4.10)	51.2
	CL	LL.3	6.64 (−5.43,18.73)	172.6
	FL	NA	>26	>676
	PL	NA	>26	>676
L. sativa	PR	LL.3	0.20 (0.01,0.38)	5.2
Aboveground	CR	LL.3	0.32 (−0.41,1.06)	8.3
	PG	LL.3	0.45 (−0.12,1.02)	11.7
	CL	BC.4	10.87 (6.38,15.35)	282.6
	PL	BC.4	19.176 (12.95,25.39)	498.7
	FL	NA	>26	>676
L. perenne	PR	LL.3	0.33 (0.06,0.59)	8.6
Belowground	PG	BC.4	0.45 (−0.13,1.03)	11.7
	CR	LL.3	0.94 (−0.50,2.39)	24.4
	CL	LL.3	2.77 (−1.68,7.22)	72
	FL	BC.4	25.29 (13.70,36.89)	657.5
	PL	NA	>26	>676
L. sativa	PG	LL.3	0.35 (−0.26,0.96)	9.1
Belowground	PR	LL.3	0.91 (0.11,1.72)	23.7
	CR	LL.3	1.06 (0.10,2.02)	27.6
	CL	LL.3	10.35 (3.29,17.41)	269.1
	PL	BC.4	23.96 (−4.61,52.54)	623
	FL	NA	>26	>676

for both) (Fig. 5). VM has been proposed as a critical factor leading to reductions in plant productivity due to its influence on N-immobilization in weathered soils (Deenik et al. 2010; Bruun et al. 2012). Higher VM indicates higher labile carbon content and may stimulate microbial biomass, resulting in significant N immobilization (Bruun et al. 2012). Alternatively, the possibility of phytotoxic compounds in chars with high VM has also been suggested (Villar et al. 1998; Van Zwieten et al. 2009; Deenik et al. 2010). Although IR absorption at some wavelengths was obscured by strong aromatic peaks, thus limiting identification of potential differences, strong effects due to phytotoxic pyrolytic condensates seems unlikely due to the similarity of peaks in slow (stimulating or non-inhibitory) and fast pyrolysis (strongly inhibitory) FTIR spectrograms (Fig. 1), though we lack the data to support this assertion.

Regarding potentially hazardous heavy metals such as Cu, Cr, and Zn, concentrations in soil-char mixtures were compared to the values limiting applications of sewage sludge for alkaline soils (pH≥7) set in the Working Draft for the use of sewage sludge in agriculture (European Commission 2000). FL slightly exceeded the Zn limit (200 mg kg^{-1} dm) at 11.3 %, exceeded the Cr limit (100 mg kg^{-1} dm) at 26 %, and the Cu limit (100 mg kg^{-1} dm) at 26 %. PG exceeded the Zn limit at 26 %. As such, limits were only reached at highly unrealistic application rates. Additionally, bioavailability of heavy metals should be low both due to the alkaline pH of the soil (Uchimiya et al. 2011a) and the high metal sorption capacity of biochars (Uchimiya et al. 2011b).

Somewhat surprisingly, C_{hw} had no relationship with either VM or biomass, despite the fact that VM and C_{hw} are proposed as alternative methods for evaluating the most labile fractions of biochars (Joseph et al. 2009). PG, a strongly inhibitory material in the context of this study, had VM and C_{hw} content similar to the non-inhibitory materials CL and PL, indicating that inhibition by this material was probably not due to microbial

Table 9 Multiple linear model results for two plant species endpoints of aboveground (AGB) and belowground (BGB) biomass and root:shoot ratio. Values shown for each chemical parameter are the standardized coefficient β with statistical significance at CI=0.95 indicated as 0.05-0.01 (*), 0.009-0.001 (**), and <0.001 (***). Also provided are the model test statistic, degrees of freedom (df), and significance and adjusted R^2. Material intercepts not shown

	L. sativa			*L. perenne*		
Parameter	AGB	BGB	Root: shoot	AGB	BGB	Root: shoot
Ca^{2+}	0.43 **	0.44 **		0.66 ***	0.43 **	
Mg^{2+}	0.13	0.28		−0.13		
NH_4^+	0.36 *	0.24		0.52 ***	0.47 **	
NO_3^-	0.34	0.36 *			0.25	
Na^+			0.72 **			−0.42 *
HPO_4^{2-}	0.63 ***	0.51 **		0.79 ***	0.73 ***	
pH	0.79 **	1.00 ***	−0.4	0.09	0.27	0.13
Biochar concentration	−1.05 ***	−0.87 ***		−1.16 ***	−1.15 ***	
df	27	28	33	28	28	33
F	7.55	7.83	4.48	10.84	11.18	2.38
p	<0.001	<0.001	0.019	<0.001	<0.001	0.108
R^2	0.57	0.58	0.17	0.63	0.64	0.07

nutrient immobilization. The low P availability in mixtures with this char, as discussed in the previous section, might be an alternative explanation to the high inhibition observed with this material.

Finally, Cr concentration was also positively significantly correlated with plant biomass (data not shown) due to the fact that FL, CL, and PL had the highest Cr concentrations, though this seems to be an artifact since it the opposite of what would be expected and the concentrations are too low to be of real importance or concern.

In the multiple linear models developed for this study, chemical properties explaining AGB and BGB responses were very similar, though somewhat distinguished by magnitude of effect of macronutrients N and P. These nutrients were positively associated with growth, supporting the hypothesis that nutrient limitation caused the plant growth inhibition, whereas increasing biochar concentration had a negative effect, reflecting the general inhibition showed by most chars in this study. It is noted that the models considered here are only representative of the conditions of the study, and the strength and direction of the predictor coefficients are conditioned by the factors included in the model. Their direct extension to other materials sets and species would thus be inappropriate without cross-validation.

The above considerations suggest that reduced nutrient (s) availability, not phytotoxic effects, are the most likely causes of observed growth inhibition in our study. A number of studies of biochar effects in calcareous soils have also documented biochar-induced changes in N and P availability and plant growth. In an apple orchard on a calcareous soil in Northern Italy, Ventura et al. (2013) found reduced soil NO_3^- with biochar treatment, and speculated that strong alkalinity around the biochar might have caused volatilization. From an experiment on a calcareous soil in Idaho, Lentz and

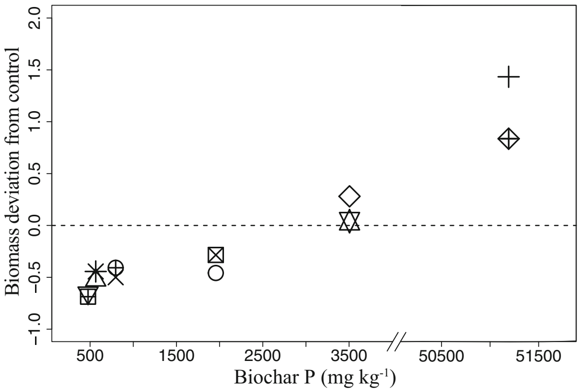

Fig. 4 Fresh biochar P contents (*x-axis*) are plotted against corresponding biomass with respect to the control (*y-axis*, unit as in Fig. 2) at high biochar concentrations (average of performance at 4.9 and 11.3 %) for each test species-biochar pair. Symbols designations for plant-biochar pairs as follows: ○ = Lettuce-CL, △ = Lettuce-CR,+ = Lettuce-FL, × = Lettuce-PG, ◇ = Lettuce-PL, ▽ = Lettuce-PR, ⊠ = Ryegrass-CL, ✳ = Ryegrass-CR,◈ = Ryegrass-FL, ⊕ = Ryegrass-PG, ✡ = Ryegrass-PL, ⊞ = Ryegrass-PR. See Table 2 for material codes

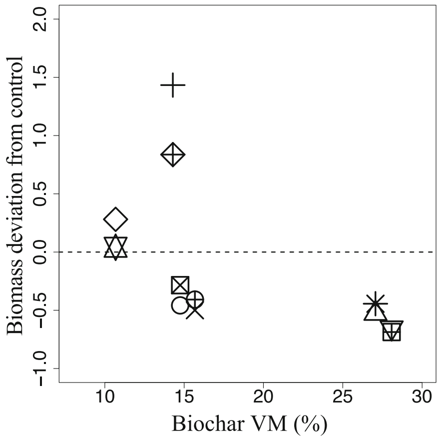

Fig. 5 Fresh biochar volatile matter (VM) contents (*x-axis*) are plotted against corresponding biomass with respect to the control (*y-axis*, unit as in Fig. 2) at high biochar concentrations (average of performance at 4.9 and 11.3 %) for each test species-biochar pair. For symbol designations see Fig. 4

Ippolito (2012) reported lower corn silage production under biochar treatment in one out of 2 years, whereas biochar+mineral fertilizer treated silage had the lowest N concentrations of all treatments considered. Also, Ippolito et al. (2014) and Ducey et al. (2013) found negative, proportional relationships between biochar added and soil NO_3^- concentrations. While the above studies found a negative relationship between biochar addition and soil N availability in calcareous soils, one exception is the study of Zhang et al. (2012) who reported increased maize production in a biochar+mineral fertilizer treatment on a calcareous soil of the Chinese Great Central Plain. This was attributed to an increased N-use efficiency possibly facilitated by increased soil organic carbon added with biochar. All considered, biochar effects on soil N are currently a topic of high interest, and are associated with a multitude of potential mechanisms (Clough et al. 2013). In our study, changes in N availability were more likely due N immobilization rather as discussed above rather than increased N volatilization, especially since the high-VM, growth-inhibiting fast pyrolysis chars did not affect soil pH dramatically or consistently (Table 7), though this does not take into account possible effects due to aeration. Available studies of biochar effects on P availability in calcareous soils have shown contrasting results. Blackwell et al. (2010) found that biochar reduced P fertilizer requirements, whereas this biochar had relatively a high concentration of P (0.96 %). In contrast, Farrell et al. (2013) found that when no fertilizer P was added, biochar appeared to suppress wheat yield, though the biochar in their study had a significantly lower P content (0.03 %). All considered, available information shows that biochar definitely has an impact on both soil P availability and fractionation in calcareous soils (Farrell et al. 2013; Chintala et al. 2014). Based on our and other studies we suspect that biochar characteristics of P content, pH, and free basic cation content are highly important.

Conclusions

The varied biochars considered in this study had diverse effects on soil chemical properties relevant to plant growth. Gasification and fast pyrolysis chars were strongly inhibitory at realistic application rates, while slow pyrolysis chars generally did not affect plant growth. Inhibition was most likely not related to phytotoxic compounds or heavy metals, but rather due to short-term effects on nutrient plant availability, limited by both VM content which increases competition with microorganisms, and P content and/or P availability, the latter of which was in turn likely related to phosphate precipitation to non-available forms due to biochar chemistry and the initial P content. These mechanisms can explain the stimulatory effect of sewage sludge char (low VM and high P content, despite the low water-soluble P in fresh char which likely increased with time), the stimulation or non-effect of slow pyrolysis wood chars (low VM and intermediate P content which was highly available), and the inhibition reported for fast pyrolysis wood chars (with high VM and low P content and availability) and especially for the wood gasification char (low VM but low elemental P and no soluble P).

These results may aid practitioners to avoid any short-term unintended effects of biochars on plant growth that may occur with the application of fresh biochar in an alkaline soil, and also suggest the potential fertilization and acidification value of slow pyrolysis wood biochars under such conditions.

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