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Ecotoxicological characterization of biochars: Role of feedstock and pyrolysis temperature

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HIGHLIGHTS

- Soil respiration was explained by the volatile content of the materials applied.
- Collembolan toxicity was generally not observed at typical application rates.
- Toxicity was feedstock dependent and generally unaffected by charring temperature.
- The toxicity observed in some materials was mostly explained by soluble Na.
- Bioassays were shown to be useful in biochar quality evaluation schemes.

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ABSTRACT

Seven contrasting feedstocks were subjected to slow pyrolysis at low (300 or 350 °C) and high temperature (550 or 600 °C), and both biochars and the corresponding feedstocks tested for short-term ecotoxicity using basal soil respiration and collembolan reproduction tests. After a 28-d incubation, soil basal respiration was not inhibited but stimulated by additions of feedstocks and biochars. However, variation in soil respiration was dependent on both feedstock and pyrolysis temperature. In the last case, respiration decreased with pyrolysis temperature ($r = -0.78$; $p < 0.0001$, $n = 21$) and increased with a higher volatile matter content ($r = 0.51$; $p < 0.017$), these two variables being correlated ($r = -0.86$, $p < 0.0001$). Collembolan reproduction was generally unaffected by any of the additions, but when inhibited, it was mostly influenced by feedstock, and generally without any influence of charring itself and pyrolysis temperature. Strong inhibition was only observed in uncharred food waste and resulting biochars. Inhibition effects were probably linked to high soluble Na and NH_4 concentrations when both feedstocks and biochars were considered, but mostly to soluble Na when only biochars were taken into account. The general lack of toxicity of the set of slow pyrolysis biochars in this study at typical field application rates ($\leq$20 Mg ha^{-1}) suggests a low short-term toxicity risk. At higher application rates (20–540 Mg ha^{-1}), some biochars affected collembolan reproduction to some extent, but only strongly in the food waste biochars. Such negative impacts were not anticipated by the criteria set in currently available biochar quality standards, pointing out the need to consider ecotoxicological criteria either explicitly or implicitly in biochar characterization schemes or in management recommendations.

1. Introduction

Biochar use as soil conditioner is currently an important topic of research (Gurwick et al., 2013) and related to potential benefits in the context of agricultural yield, carbon sequestration, waste management and clean energy production (Lehmann and Joseph, 2009; Sohi et al., 2009; Kookana et al., 2011), as well as the more recently claimed role in land reclamation (Beesley et al., 2011; Xie et al., in press). The capacity of biochar technologies to process any carbon-rich waste may allow upcycling of waste surplus or low quality wastes such as sewage or tannery sludges (Muralidhara et al., 1982; Bridle and Pritchard, 2004; Hossain et al., 2010; Méndez et al., 2013). Pyrolysis technologies have been shown to change pollutant burden of the original feedstocks, such as the usual potentially toxic element concentration increases due to mass losses (Koppolu et al., 2003; Méndez et al., 2012; Farrell et al., 2013) and the formation of PAH or dioxins (Schimmelpfennig

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and Glaser, 2012; Hale et al., 2012). More recently, toxic effects of volatile organic compounds (VOCs) resulting from the re-condensation of pyrolysis liquids and gases on biochar have been demonstrated (Buss and Mašek, 2014). The variety of usable feedstocks and pyrolysis procedures leads to a wide range of resulting biochars in terms of pollutant composition and burden, including biochars with unsuitable properties as a soil amendment, though still useful for other environmental benefits, e.g. charcoal use, bioenergy generation and carbon sequestration without soil application.

The soil application of some biochars might unfavorably impact soil quality. Some authors suggest a need to demonstrate both the benefits of biochar to soil health and lack of detrimental effects to the environment (Verheijen et al., 2010). However, research about possible negative impacts of biochars on soil biota is rarely addressed despite the existence of large-scale field trials and sales of biochar products in the market place (Busch et al., 2013). The potential impacts on soil biota might be roughly separated into those mediated by direct negative effects such as pollutant release and excessive salinization or liming (Liesch et al., 2010; McCormack et al., 2013), but also by indirect effects, such as a decreased albedo (Genesio and Miglietta, 2012) if associated with excessive soil heating or drying.

Most products used in agriculture conform to industrial or regulatory standards to ensure that they can be safely used in soil, although for biochar this would require an agreement on the main characteristics to be taken into account (Joseph et al., 2009). Several biochar quality guidelines have been recently proposed such as the IBI Biochar Standard (IBI, 2013), the European Biochar Certificate (EBC, Schmidt et al., 2012) or the UK Biochar Quality Mandate (BQM, Shackley et al., 2013). In these standards, environmental risks are accounted for by the inclusion of limit values for physicochemical properties, including pollutants such as heavy metals, dioxins/furans, PAHs, PCBs or BTEX. However, the use of chemical analyses for this purpose has several limitations such as the fact that total concentrations do not necessarily relate to the bioavailable fraction or the final uptake by organisms (Van Straalen et al., 2005); that non-target toxic substances might also be present and not assessed; and that the combined toxicity of all the chemicals present cannot be assumed to be easily predicted since additive, synergistic and antagonist effects can occur. The use of bioassays for biochar characterization overcomes such limitations, since biochar effects on indicator organisms integrate any of the processes previously described. Although bioassays have also some intrinsic limitations such as a low ecological relevance, because only short-term effects for particular cultured species are assessed, they offer a genuine possibility to assess the actual effects in exposed individuals. Bioassays are increasingly used as a tool for the prospective assessment of environmental risks of substances before its marketing, release, or agricultural use (Brock, 2013), and a necessary complement to the traditional chemical characterization. Bioassay-based approaches may complement physicochemical characterization for the quality assessment of biochars, similar to what has been proposed for the characterization of wastes in the EU (Moser and Römbke, 2009).

Bioassays are not included in all of the currently available biochar quality standards, with the exception of the germination assay which is mandatory in the IBI standard (IBI, 2013). Studies exist utilizing plants (Solaiman et al., 2012; Rogovska et al., 2012; Busch et al., 2013), soil fauna (Liesch et al., 2010; Van Zwieten et al., 2010; Busch et al., 2012; Hale et al., 2013; Marks et al., 2014), as well as aquatic organisms (Hale et al., 2013; Oleszczuk et al., 2013), but the utility of bioassays potentially used in the context of biochar ecotoxicological characterization is still to be rigorously assessed. Furthermore, while ample data exist on the influence of the feedstock and/or the pyrolysis procedure on biochar composition, recalcitrance, or nutrient retention (Novak et al., 2009; Bruun et al., 2011; Hossain et al., 2011; Singh et al., 2012; McBeath et al., 2014; Nelissen et al., 2014), their influence on ecotoxicological effects is not yet well understood.

Therefore, we investigated the effects of a diverse set of biochars on soil basal respiration and collembolan reproduction in a bioassay. The specific objectives of the study were to assess whether charring changes the ecotoxicity of organic soil amendments; how feedstock and pyrolysis temperature affect ecotoxicity; and which amendment properties relate to negative effects.

2. Materials and methods

2.1. Soil, feedstocks and biochars

The soil used in this study was collected in April 2008 in the Cornell Musgrave Research Farm (Aurora, New York). The soil was continuously cropped to corn for decades under standard, regional agricultural management practices. Soil had a 42% sand, 31% silt and 27% clay, total C content of 16.2 g kg^{-1}, total N of 1.6 g kg^{-1}, and a pH around 7 (see Rajkovich et al., 2012 for a more detailed description). Soil was collected after snowmelt and before any pesticide or fertilization was applied. After collection, soil was air-dried, homogenized, and sieved to 5 mm. Soil was stored for two years and before the beginning of the experiment two freezing–thawing cycles (24 h at −20 °C, 24 h at 20 °C) were carried out, ensuring that no fauna remained.

Bull manure with sawdust, corn stover, oak wood and pine wood were obtained from local suppliers in Wisconsin. Digested dairy manure was supplied by AA Dairy (Candor, NY, USA), obtained after the anaerobic digestion of dairy manure and removal of the liquid fraction by a screw press. Food waste was collected from Cornell University dining halls (Ithaca, NY, USA), and included discards from food preparation, unconsumed food and paper plates and napkins. White paper mill waste was obtained in Mohawk Fine Papers Inc. (Cohoes, NY, USA). The materials were dried at 60 °C until constant weight and processed to pass a 2-mm sieve.

Two biochars were obtained from each feedstock (Table 1), obtained by slow pyrolysis at Best Energies (Cashton, WI, USA), and produced at low (300 or 350 °C) and high temperature (550 or 600 °C). A detailed description of the pyrolysis procedure is provided in Enders et al. (2012). The set of biochars in this study was considered as representative, since slow pyrolysis is the most common technology to produce biochar due to its moderate operating conditions and optimization of biochar yields (Xie et al., in press).

Table 1
Source of feedstocks, and pyrolysis procedure to obtain the corresponding biochars.

Material	Feedstock and source	Treatment
BM		Feedstock
BM350	Bull manure w/sawdust, WI local supplier	Slow pyrolysis, 350 °C
BM550		Slow pyrolysis, 550 °C
CS		Feedstock
CS350	Corn stalks, WI local supplier	Slow pyrolysis, 350 °C
CS550		Slow pyrolysis, 550 °C
DDM		Feedstock
DDM300	Digested Dairy Manure Screw Pressed, AA Dairy, Candor, NY	Slow pyrolysis, 300 °C
DDM600		Slow pyrolysis, 600 °C
FW		Feedstock
FW300	Food waste, Cornell dining hall	Slow pyrolysis, 300 °C
FW600		Slow pyrolysis, 600 °C
OW		Feedstock
OW350	Oak, WI local supplier	Slow pyrolysis, 350 °C
OW550		Slow pyrolysis, 550 °C
PMW		Feedstock
PMW300	Paper Mill Waste, Mohawk Fine Papers Inc., Cohoes, NY	Slow pyrolysis, 300 °C
PMW600		Slow pyrolysis, 600 °C
PW350		Feedstock
PW350	Pine, WI local supplier	Slow pyrolysis, 350 °C
P W550		Slow pyrolysis, 550 °C

2.2. Biochar characterization

Biochar and feedstock compositions are summarized in Table 2. Values for the proximate analysis, total carbon and nitrogen, and elemental composition were obtained from Enders et al. (2012), and when not available, obtained by the same methodology. Analyses were carried out in air-dried samples, ground with a mortar and pestle, and sieved to a particle size of 149–850 μm. Proximate analysis (volatile matter, ash and fixed carbon content) was carried out according to ASTM D1762-84 and with the modifications described in Enders et al. (2012). Total carbon and nitrogen were determined by Dumas combustion (PDZ Europa ANCA-GSL, Sercon Ltd., Cheshire, UK) after ball milling (Retsch MM 301, Retsch GmbH, Haan, Germany). Elemental composition was carried using an ICP trace analyzer emission spectrometer (ICAP 61E, Thermo Electron, Waltham, MA) on dry and ground samples after ashing for 8 h at 500 °C and acid digestion, according to the modified ash-method described in Enders and Lehmann (2012). Inorganic carbon was assessed by the Bernard calcimeter method, consisting of the addition of concentrated hydrochloric acid to dry ground samples and measurement of the CO_2 volume released, after calibration with pure $CaCO_3$. Organic carbon was estimated as the difference between total carbon and inorganic carbon content. pH and electrical conductivity were assessed in a 1:20 (w:v) solution (1.5 g of 2 mm-sieved sample in 30 ml of deionized water), orbitally shaken for 2 h, then centrifuged at 1935 ×*g* for 5 min, and filtered through Whatman #1 filter paper prior to analysis, following the recommendations of IBI (2013).

Total PAH contents available for some of the biochars were obtained from Hale et al. (2012).

2.3. Soil mixture preparation and characterization

Biochars and feedstocks were mixed with soil at a rate of 0, 0.2, 0.5, 2, 7, and 14% (w/w), equivalent to an agricultural application of 0, 7.7, 19.4, 77.4, 270.9 and 541 Mg ha^{-1}, respectively. Such estimation was carried out assuming a 0.3-m arable layer depth and the field bulk density of 1.29 Mg m^3 reported in Güereña et al. (2013) for this soil in field plots.

The day before the beginning of the tests, soil mixtures were moistened to 50% of the water holding capacity (WHC), providing a moist and crumbly substrate required in bioassays. WHC was previously determined for each material and concentration, since water retention capacity increase with increasing feedstock or biochar application rates. Moistening of mixtures was carried out with deionized water containing 5% (v/v) of an inoculant solution to reintroduce the indigenous microorganisms. The inoculant was prepared from freshly collected soil in the same field plots where the soil was collected two years before, and consisted of the supernatant of a soil–water slurry (1:10), stirred for 5 min at 150 rpm, settled for 5 min, decanted, and centrifuged for 5 min at 1935 ×*g*.

Eight replicates were prepared for each material and test concentration (6 for the bioassays, 1 for soil basal respiration, and 1 for soil analysis), each consisting of 30 g of wet soil in a sealed 150-mL polyethylene flask. Samples for the assessment of respiration and analysis were incubated for 28 d under dark conditions and 20 ± 1 °C before being analyzed, in parallel to the collembolan reproduction test.

For the assessment of chemical properties, soil–water extracts (1:5 w/v) were prepared by adding 20 g of fresh sample to 100 mL of deionized water, orbitally shaking for 30 min at 160 rpm, settling, centrifuging for 5 min at 3600 ×*g*, and filtering through Whatman #1 filter paper. pH and electrical conductivity (EC) were immediately assessed in the extracts. After storage at −20 °C, Cl^-, Br^-, S-SO_4^{2-}, N-NO_2^-, and N-NO_3^- were analyzed in the extracts using an ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA). P-PO_4^{2-} was measured as soluble reactive phosphorus (SRP) in a flow analyzer (FS 3000, OI Analytical, College Station, TX) using the ascorbic acid and molybdate method. N-NH_4^+ was measured by the phenate method as described in APHA-AWWA-WPCF (1985). Elemental content in the extracts (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, S, Se, Si, Sr, Ti, V, Y, and Zn) was assessed by ICP-ES model 61 E trace analyzer (Thermo Jarrell Ash Co, Franklin, MA).

2.4. Bioassays

A minimum test battery consisting of a soil microbial activity test and a collembolan reproduction test were considered to assess the potential impacts on soil biota of the different biochars and uncharred feedstocks in this study.

Microbial activity was assessed as the basal soil respiration (BAS) in soil-material mixtures incubated for 28 d, and measured without disturbance of the soil-mixtures by placing each replicate in 250-mL Mason glass jars for 24 h at 20 ± 1 °C, according to the titration method described in Pell et al. (2006).

Collembolan survival and reproduction were assessed according to the ISO Guideline 11267 (ISO, 1999). Ten individuals, aged 10 to 12 d,

Table 2
Composition of feedstocks and their corresponding biochars; bdl = below detection limit; na = not available; n = 3.

Material	VM %	Ash %	FixedC %	Ctot %	Cinorg %	Corg %	N %	Cd mg kg^{-1}	Cr mg kg^{-1}	Cu mg kg^{-1}	Ni mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}	pH mg kg^{-1}	EC dS m^{-1}	Total PAH μg g^{-1}
BM	84.4	5.3	10.2	43.8	bdl	43.8	0.6	bdl	1.7	13.9	bdl	bdl	65.81	7.58	2.16	na
BM350	58.7	8.3	33	66.3	0.05	66.2	1.3	0.42	2.0	35.6	2.36	3.54	132.96	7.51	2.18	na
BM550	39	10.9	50.1	74.3	0.11	74.2	1.1	0.08	18.6	44.2	14.01	2.47	319.53	10.57	2.77	na
CS	85.2	9.0	5.8	43.4	bdl	43.4	0.5	bdl	1.7	6.4	bdl	bdl	59.42	5.69	1.19	na
CS350	48.9	11.5	39.8	65.2	0.03	65.2	1.2	bdl	2.2	21.5	0.98	1.71	66.03	7.81	1.73	1.61
CS550	37.3	14	48.7	72.2	0.06	72.1	1.00	0.16	2.5	30.5	2.18	4.31	87.81	10.02	1.29	1.76
DDM	74.7	6.92	18.4	45	0.01	50.0	1.7	bdl	1.3	12.6	0.70	0.48	36.45	7.91	1.44	na
DDM300	57.6	39.2	3.2	56.1	0.05	56.0	2.7	bdl	2.3	47.5	5.75	24.27	129.24	8.20	1.87	0.33
DDM600	39.4	18.8	41.7	62.8	0.04	62.8	2.2	bdl	3.1	58.3	3.86	bdl	200.19	10.37	1.65	0.18
FW	51.1	39.2	9.68	42.6	0.51	42.1	2.4	bdl	2.4	4.3	2.83	1.91	20.70	5.40	3.81	na
FW300	45.4	23.3	31.3	65.3	0.12	65.2	5.3	bdl	6.3	41.9	6.40	41.15	49.41	7.50	4.37	0.37
FW600	34.5	52	13.6	32	1.09	30.9	1.2	bdl	8.7	10.9	9.82	bdl	64.17	10.10	4.28	0.09
OW	88.6	2	9.4	47.1	bdl	47.1	0.1	bdl	0.6	106.4	bdl	bdl	47.37	3.98	0.10	na
OW350	60.8	1.1	38.1	74.9	bdl	74.9	0.2	0.55	14.5	120.1	9.10	20.66	109.05	4.49	0.06	na
OW550	38.5	0.6	60.9	87.9	bdl	87.9	0.2	0.11	0.9	25.8	1.23	5.47	15.10	7.42	0.03	na
PMW	60	38.2	1.74	23.5	5.4	18.1	0.1	bdl	3.0	4.2	1.72	2.58	7.07	8.05	0.19	na
PMW300	50.1	50.7	−0.8	21.2	6.23	15.0	0.3	bdl	8.2	17.8	7.09	1.62	25.71	7.58	0.45	0.18
PMW600	41.1	59.1	−0.2	19.2	8.14	11.1	0.1	0.002	11.0	21.2	11.27	13.92	50.52	9.28	0.18	0.27
PW	89.8	1.8	8.3	47	bdl	47.0	0.00	1.40	1.7	131.2	1.37	11.80	45.60	4.53	0.19	na
PW350	56.3	0.6	43.2	70.7	bdl	70.7	0.1	1.40	0.6	13.5	1.25	8.50	20.99	4.72	0.07	na
PW550	40.2	0.8	59	86.8	bdl	86.8	0.1	0.17	4.3	65.3	0.84	36.48	37.57	6.23	0.02	na

were added to each of the already described replicates, thereafter incubated for 28 d under dark conditions at 20 ± 1 °C. At the start of the test and 14 d after, granulated yeast was added to each replicate as a food source to ensure the performance of the individuals. Replicates were aerated twice a week to prevent from anaerobiosis. At the end of the test, soil was poured into a 500-mL Erlenmeyer flask, flooded with water, and stirred in order to float the individuals on the water surface. Then, a picture was taken in order to count the adults and juvenile collembolan by image treatment software.

2.5. Statistical assessment

The statistical analyses were conducted using R software version 2.15 (R Foundation for Statistical Computing). Each bioassay was carried out using the same batch of individuals to ensure that any change in performance was exclusively attributed to the material concentration in soil mixtures and the validity criteria for this test was checked in each case. For comparison purposes, results in each bioassay were expressed as a percentage of the mean performance in the corresponding control, since reproduction varied in the different batches of collembolans used in different tests, which is usual in this parthenogenetic species related to the slight differences in breeding conditions of the different batches (e.g. feeding status) and the interindividual variability observed in this species (Crouau and Cazes, 2003).

Pearson correlation was used to link the material's composition with mean BAS and mean collembolan reproduction in each material. Within each feedstock type, the effect of material application rate and pyrolysis temperature on collembolan reproduction was assessed by two-way ANOVA (with biochar rate and pyrolysis temperature as factors), followed by Bonferroni test to assess significant differences in these endpoints with the corresponding controls. This was not possible for BAS, since only one replicate per soil-material concentration was available.

The response variables (BAS and collembolan reproduction) as affected by the exposure to the feedstocks and/or biochars were modeled by Generalized Linear Models (GLMs), including feedstock/biochar concentration and a selection of the explanatory variables measured in each soil-material mixture. Concentrations below the detection limit were assumed to be zero. Prior to model construction, variables with generalized undetectable levels, were excluded as well as those showing high colinearity (Pearson, $r \geq 0.8$). After that, GLMs were constructed assuming a Poisson distribution (*glm* function, stats package) or, when data overdispersion was observed, a negative binomial distribution (*glm.nb* function, MASS package). An initial global model including the last variables was constructed, and then variables were successively removed until the best model was achieved. In Poisson GLM, the *drop1* function (stats package) was used for this purpose, until all the variables selected showed a significant contribution to the model. In the negative binomial GLM, the best model was selected after the removal of more variables using the *vif* function of the HH package, removing sequentially those with highest VIF values until all the variables showed VIF values below 5, and then applying the *dredge* function of the MuMIn package to obtain the best model (lowest AIC), restricted to five explanatory variables at most.

A principal component analysis (PCA) was carried out to ordinate the different materials based on their effects on chemical properties in soil-material mixtures using the *princomp* function of the stats package. The same variables selected for the GLM analysis were used for this purpose, with the exception of the application rate and BAS.

3. Results

3.1. Soil chemical properties in soil-materials mixtures

When the PCA scores of each soil-material mixture in the two main components were plotted and grouped by feedstock or pyrolysis temperature, only unpyrolyzed food waste and its corresponding biochars appeared clearly separated from the other materials along both components, while no clear clustering patterns appeared for pyrolysis temperature (Supplementary Fig. S1). By means of Pearson correlation of the individual scores of each soil-material mixture in each component and the value of each physicochemical property, the main explanatory properties in each component were obtained ($r > 0.75$). Hence, the position of food waste materials in the low values of the first component were indicative of relatively high EC and soluble Al, Ba, Cr, Cl, Fe, K, Na, S, Si, Ti, V, Y and Zn, while the position in the high values of the second component were associated with high levels of soluble Ca and Sr. The first and second principal components explained 33.4 and 14.9% of the observed variability, respectively.

No statistical comparison could be carried out for the chemical properties in soil-material mixtures because only one replicate was available, although some trends are suggested. Soil pH increased slightly with the addition of manure, corn and food waste feedstocks and derived biochars with no apparent effect of pyrolysis (Supplementary Fig. S2). In contrast, pH remained relatively unchanged or decreased after the addition of both charred and uncharred paper mill waste, oak, and pine wood, which in turn were the materials with the lowest pH (Supplementary Fig. S3). The highest salinity was found in food waste materials and animal manures (BM, DDM), while the lowest was observed in wood materials, in accordance with their salt contents (Supplementary Fig. S3). Soil mixture salinity increased linearly with the application rate of feedstocks and biochars with the exception of biochars from paper mill waste, which showed no change, and wood materials which decreased salinity (Supplementary Fig. S4). Unpyrolyzed materials generally showed lower salinity than the corresponding feedstocks, especially in wood materials. Although several ions are contributing to salinity, it was highly and positively correlated with Na (Pearson, $r = 0.95$), Cl^- ($r = 0.88$), and Ba^- ($r = 0.83$) in the set of materials in this study (data not shown).

3.2. Soil respiration

As anticipated, unpyrolyzed feedstocks always showed higher BAS values than the corresponding biochars, with the only exception of digested dairy manure, with similar values. The highest BAS was observed in food waste and paper mill waste, followed by bull manure, digested dairy manure and corn stover, while oak wood and pine wood showed the lowest values (Fig. 1). A trend to lower BAS was observed for the highest temperature pyrolysis biochars produced from digested dairy manure, food waste and paper mill waste, although this was not found for other feedstocks. Accordingly, mean BAS in each material was significantly and inversely correlated with pyrolysis temperature (Pearson, $r = -0.78$), but also with total C, organic C, and fixed carbon ($r = -0.59$, -0.55 and -0.68, respectively), and positively correlated with volatile matter ($r = 0.51$). Pyrolysis temperature was in turn correlated with volatile matter ($r = -0.84$) and fixed carbon ($r = 0.60$), but not with total, organic, and inorganic C contents. When only biochars were considered, mean BAS was negatively correlated with fixed carbon ($r = -0.67$) but not with pyrolysis temperature, and positively related to ash content ($r = 0.62$) and total N ($r = 0.57$).

Modeling of soil respiration response in soil-material mixtures showed a significant negative contribution of pyrolysis temperature and a positive effect of material application rate, pH and soluble Ca (Table 3), in a model explaining 74% of the variance. When only biochars were considered, the model explained 79% of the variance and included a significant negative association with $N\text{-}NO_3^-$ and a positive association with Br^-.

3.3. Collembolan reproduction

Regarding collembolan reproduction, most of the materials assessed did not significantly affect collembolan reproduction at any of the tested

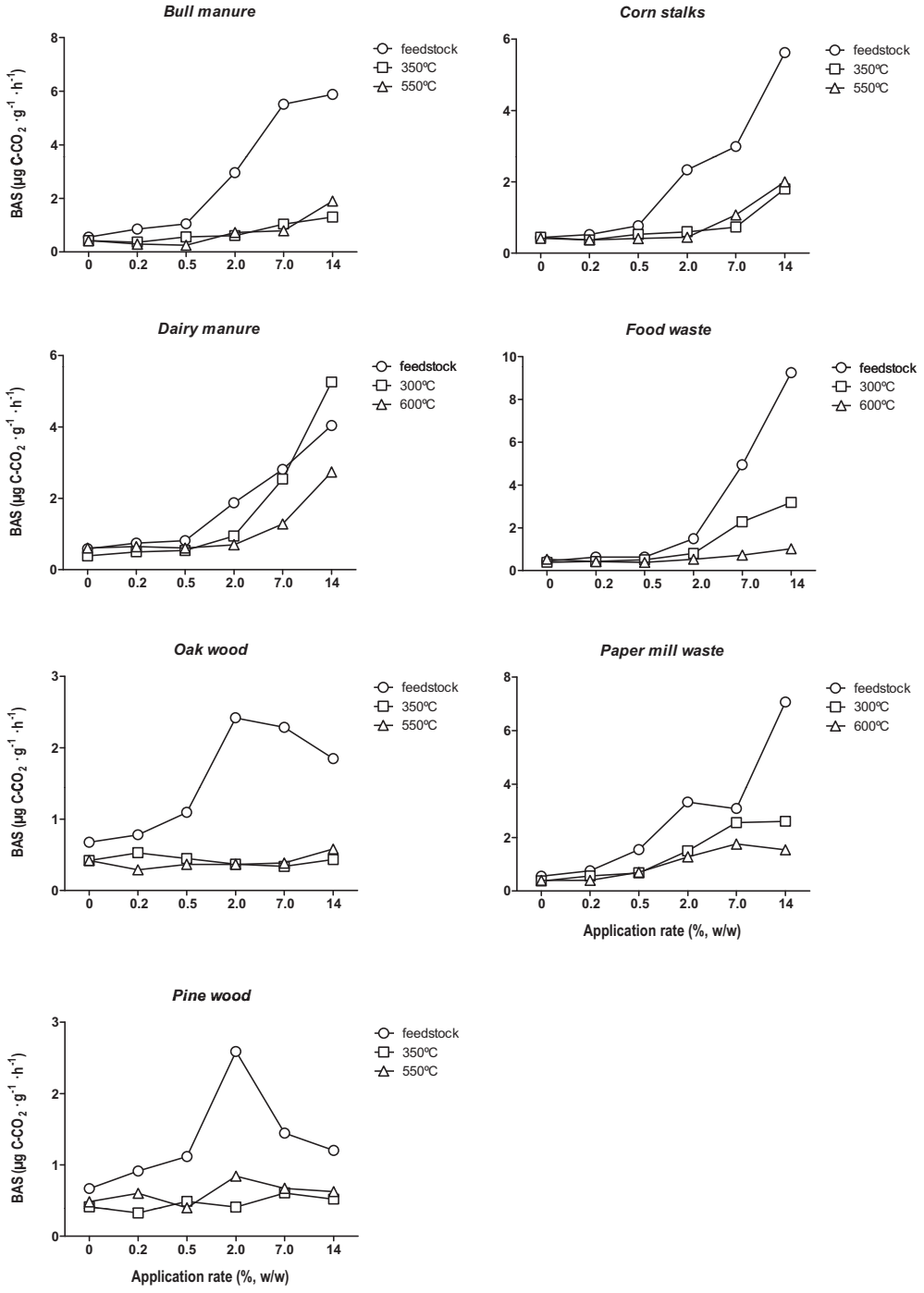

Fig. 1. 24 h-basal soil respiration in each soil-material mixtures at the end of a 28-d incubation at 21 °C; n = 1.

Table 3
GLM models for basal respiration assuming Poisson distribution and combining feedstocks and biochars (left) or only considering biochars (right).

	Estimate	Std. error	z value	Pr(>|z|)		Estimate	Std. error	z value	Pr(>|z|)
Intercept	−4.7626	1.75348	−2.716	0.00661	Intercept	−0.096	0.328715	−0.292	0.7701
Application rate	0.09346	0.0143576	6.5107	<0.0001	Br^-	0.0342	0.007857	4.362	<0.001
Pyrolysis temperature	−0.0021	0.0003511	−6.0621	<0.0001	$N\text{-}NO_3^-$	−0.0108	0.004732	−2.279	0.0227
pH	0.67479	0.250924	2.689	0.00716					
Ca	0.00455	0.0019166	2.372	0.01771					
Null deviance: 143.471 on 125 degrees of freedom					Null deviance: 43.508 on 83 degrees of freedom				
Residual deviance: 36.705 on 121 degrees of freedom					Residual deviance: 9.156 on 81 degrees of freedom				
R^2: 0.74					R^2: 0.79				

concentration (no inhibition in 5 of the 7 unpyrolyzed feedstocks, and in 7 of the 14 biochars). Inhibition was only observed in some of the feedstocks but only at intermediate to high field equivalent application rates (Fig. 2). Namely, slight but significant inhibition was found above an application rate of 0.5% (~19.4 Mg ha^{-1}) with uncharred oak wood, and biochar made from oak wood at 550 °C, and from corn stover at 350 °C, while slight inhibition was observed only above 7% (~77.4 Mg ha^{-1}) with biochar made from bull manure at 550 °C, or paper mill waste at 300 °C. Strong reproduction suppression was observed with uncharred food waste and biochar from food waste made at 300 °C when applied at 7% and above. The degree of inhibition within the same feedstock did not vary whether it was pyrolyzed or not, with the only exception of food waste, with more severe toxicity in the original feedstock than the corresponding biochars. Accordingly, no correlations were found between mean reproduction and pyrolysis temperature and materials composition, with the exception of the negative correlation between reproduction and materials salinity (Pearson, $r = -0.49$, $p < 0.025$, $n = 21$) (data not shown).

The models derived for reproduction in soil-material mixtures showed a significant and negative effect of soluble Na and Fe and a positive effect of S, though the model had low predictability, only accounting for 39% of the variance (Table 4). When only biochars were considered, the negative effect of soluble Na and S was still observed, as well as a negative effect of the application rate and a positive effect of soluble P, with 40% of the variance explained by this model.

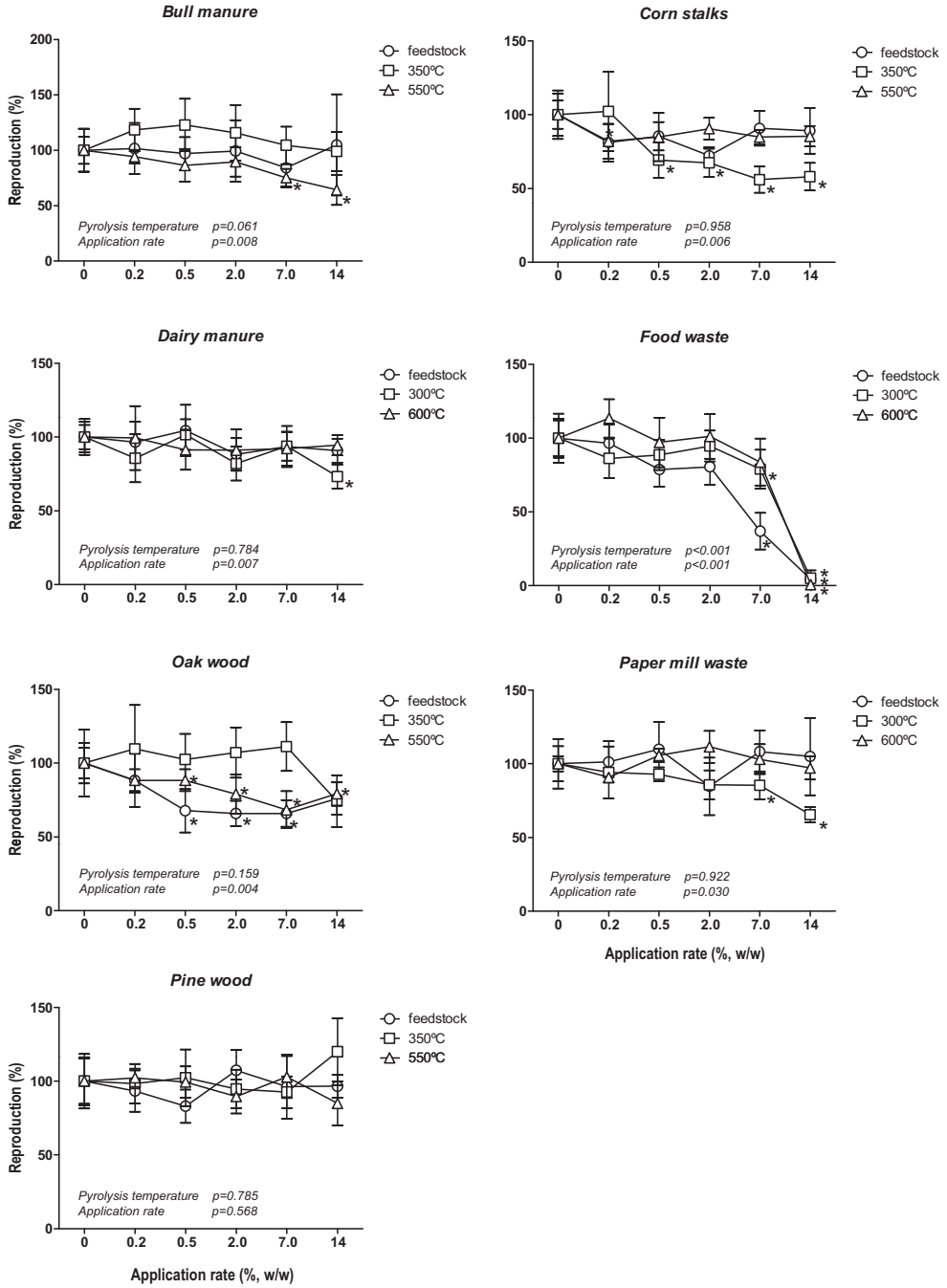

Fig. 2. *Folsomia candida* reproduction in the different soil-material mixtures after 28 d. Mean values are shown and bars correspond to standard deviation. Probability values at the bottom of each graph correspond to the significance of each factor (pyrolysis temperature and biochar application rate) in a 2-way ANOVA. Asterisks within each material for a given application rate indicate significant reduction of reproduction compared to the corresponding control (Bonferroni test, $p < 0.05$); $n = 6$.

When the juvenile number was modeled, a negative effect of soluble Na and N-NH_4^+ was shown, and a positive effect of pyrolysis temperature, explaining 37% of the variance (Table 5). When only biochars were included in the model, again a negative effect of soluble Na was shown, as well as a positive effect of N-NO_2^-, but only accounting 26% of the variance.

4. Discussion

4.1. Potentially noxious compounds in biochars and in mixtures with soil

None of the biochars or feedstocks exceeded the total heavy metal limit values set in the IBI, EBC and BQM guidelines for basic biochar quality (see Supplementary Table S1). However, the limit values for high-grade biochar quality in the BQM guideline were exceeded for Cr (BM550), Cu (BM550, DDM300, DDW600, OW, OW350, PW550), Ni (BM550, PPM600), and Zn (BM550, DDM600) (Supplementary Fig. S5). The soluble metal contents in soil-material mixtures (Supplementary Fig. S6), a fraction proposed to be closely related to metal availability (Peijnenburg et al., 1997), was generally low and in a similar range in different feedstocks, agreeing with the concentrations reported in other studies for plant, manure and biosolid biochars (Farrel et al., 2013, Kloss et al., 2012; Lucchini et al., 2014). Soluble Cu was below the detection limit, soluble Cd generally decreased in biochars compared to the original feedstocks was shown, as well as increases after pyrolysis in soluble Cr, Ni, Pb and Zn for some of the feedstocks. The latter contrasts with studies generally reporting decreased metal leachability after pyrolysis (Hwang et al., 2007; Méndez et al., 2012; Farrell et al., 2013),although explaining this result is out of the scope of the paper. Water-soluble Cd and Zn concentrations in soil-material mixtures fell clearly below those reported to inhibit reproduction of *Folsomia candida* (4 week-reproduction EC50, expressed as soluble Cd, was 0.05–0.8 mg Cd kg^{-1} and 8 mg soluble Zn kg^{-1}) (Van Gestel and Hensbergen, 1997; Van Gestel and Mol, 2003) (Supplementary Fig. S6). Similarly, soluble Pb in soil mixtures fell below the concentrations causing a reproduction inhibition in this species (0.539 mg Pb L^{-1}, equivalent to around 2 mg soluble Pb kg^{-1} according to data and method described in Lock et al. (2006)). This is consistent with the lack of correlation of soluble metals with the collembolan inhibition observed in some soil-material mixtures, and other studies that have reported no or low plant uptake of metals from biochars produced from plant materials or manures (Farrell et al., 2013; Lucchini et al., 2014) and sewage sludge (Hossain et al., 2010). However, Farrell et al. (2013) pointed out in their study that chemical extractions may be unsuitable to predict plant uptake of heavy metals from biochars.

The PAH values available for a subset of the studied biochars were clearly below the limits proposed in the already cited biochar quality standards, with the exception of the ECB limit of 4 μg g^{-1} for premium biochar, exceeded by paper mill waste biochars produced at 300 and 600 °C and pine wood at 350 °C. Lower PAH contents are expected in slow pyrolysis biochars compared to fast pyrolysis and gasification technologies, where recondensation of biooils on biochar's surface may occur (Schimmelpfennig and Glaser, 2012; Hale et al., 2012). The total concentrations of PAH in soil–biochar mixtures with those materials are not expected to cause negative effects on *F. candida* reproduction (Sørensen and Holmstrup, 2005) and that of the closely related species *Folsomia fimetaria* (Sverdrup et al., 2001) after the dilution by mixing with soil and considering the low PAH solubility and bioavailability in soils (Styrishave et al., 2008).

Apart from pollutants, excessive NH_4^+, salinization or liming after biochar application have been suggested as potential sources of biological impacts (Liesch et al., 2010). These substances are relatively transient compared to those of metals or persistent organic chemicals, but might cause immediate biological impacts shortly after the application of biochars. Ammonium levels in the materials before addition to soil was not available, but soluble contents in soil-material mixtures showed no clear trends regarding the type of feedstock, pyrolysis temperature or application rate, although a strong increase in NH_4^+ levels after additions of unpyrolyzed food waste and to a lesser extent of unpyrolyzed paper mill waste was observed (data not shown).

On the other hand, the pH increased with pyrolysis and pyrolysis temperature ($r = 0.59$, $p = 0.005$), also when only biochars were considered ($r = 0.59$, $p = 0.025$) (Supplementary Fig. S3), which has been associated with the ash content in a wider dataset by Enders et al. (2012). However, such increase was not associated with drastic pH increases in any of the soil-material mixtures (Supplementary Fig. S2) probably due to the fact that the soil already had a pH around 7. Contradictory results have been published regarding the effect of pH in *F. candida*, which have reported inhibition in reproduction below a pH of 5 (Sørensen and Holmstrup, 2005) or above 7 (Crouau et al., 1999), while others have indicated this species to be relatively insensitive to pH (Domene et al., 2011). Whatever the case, the limited variation in pH values caused by the different materials is unlikely to influence collembolans in our study.

Strong differences in salinity were observed between feedstock types, but not with pyrolysis and pyrolysis temperature (Supplementary Fig. S3), translating into salinity increases in soil mixtures. While no salinity variation was observed in soil-material mixtures with paper mill waste, salinity decreased in mixtures with wood feedstocks and biochars, in turn those with the lowest salt contents (Table 2). On the other hand, a high salinization effect was observed with applications of uncharred food waste and to a lower extent with applications of biochars made from food waste at 300 and 600 °C (Supplementary Fig. S4), with values close to the 2 dS m^{-1}, expected to affect the yield in sensitive crops (Bernstein, 1975). As already indicated, salinity was importantly explained by soluble Na concentrations, which were especially high in soil mixtures with food waste and to a lesser extent in corn stalks and manure materials (Supplementary Fig. S7), known to be highly toxic (Qadir et al., 2005). In a previous study, Rajkovich et al. (2012) tested the phytotoxicity of most of the biochars in our study in pot experiments, and correlated the high Na content in biochars with reduced growth of corn seedlings at the highest application rate tested (7%) in biochars made from dairy manure, paper mill waste, but especially from food waste. Owojori et al. (2009) reported that juvenile production in *F. candida* was significantly inhibited at and above 1.03 dS m^{-1} and reproduction ceased at 1.62 dS m^{-1}, while survival was not affected. The latter value was exceeded with the high application rates of the most saline materials (corn stalks, manures and food waste), and may

Table 4
GLM models for collembolan reproduction (expressed as %) assuming Poisson distribution and combining feedstocks and biochars (left) or only considering biochars (right).

	Estimate	Std. error	z value	Pr(>|z|)		Estimate	Std. error	z value	Pr(>|z|)
Intercept	4.5289	0.0442	102.468	<0.0001	Intercept	4.691	0.04007	117.08	<0.001
Fe	−0.0128	0.0036	−3.526	0.0004	Application rate	−0.0137	0.00527	−2.594	0.0095
Na	−0.0024	0.0003	−8.701	<0.0001	P-$PO4^{2-}$	0.01735	0.00476	3.645	0.00027
S	0.0114	0.0034	3.376	0.0007	Fe	−0.0077	0.00377	−2.053	0.0401
					Na	−0.0019	0.00028	−6.751	<0.001
Null deviance: 285.91 on 125 degrees of freedom					Null deviance: 205.83 on 83 degrees of freedom				
Residual deviance: 173.18 on 122 degrees of freedom					Residual deviance: 121.48 on 79 degrees of freedom				
R^2: 0.39					R^2: 0.409				

Table 5
GLM models for reproduction (juvenile number) assuming negative binomial distribution and combining feedstocks and biochars (left) or only considering biochars (right).

	Estimate	Std. error	z value	Pr(>|z|)		Estimate	Std. error	z value	Pr(>|z|)
Intercept	6.8746	0.0487983	140.878	<0.001	Intercept	6.92744	0.0543107	127.552	<0.001
Temperature	0.00043	0.0001221	3.512	0.00045	$N\text{-}NO_2^-$	0.09709	0.0356535	2.723	0.00647
$N\text{-}NH_4^+$	−0.13	0.0261146	−4.977	<0.001	Na	−0.0022	0.0003644	−5.905	<0.001
Na	−0.0023	0.000329	−6.942	<0.001					
Null deviance: 215.48 on 125 degrees of freedom					Null deviance: 121.603 on 83 degrees of freedom				
Residual deviance: 134.27 on 122 degrees of freedom					Residual deviance: 89.885 on 81 degrees of freedom				
R^2: 0.37					R^2: 0.26				

partly explain some of the observed negative effects on reproduction. Na^+ added as NaCl has been shown to be more toxic than other ions applied at similar concentration (Schrader et al., 1998).

4.2. Bioassays performance

The PCA of the chemical properties in soil-material mixtures only separated the unpyrolyzed food waste and the derived biochars from the other materials and to a lesser extent manure materials and derived biochars (Supplementary Fig. S1). This was associated with high salinity and specifically soluble Na with the addition of food waste.

4.2.1. Effects on BAS

Short-term inhibition of soil respiration has been used for the ecotoxicological assessment of detrimental effects of chemicals (van Beelen and Doelman, 1997; Giller et al., 1998), but also for that of wastes such as alkaline ashes (Pitchel, 1990) or salinized beet vinasse (Tejada et al., 2007). Long-term effects on BAS are less relevant due to the quick selection of resistant microbial taxa able to survive in any new environment (Giller et al., 1998). Although BAS inhibition is not anticipated in most biochars, it is plausible if disruption of microorganism activity occurs. None of the feedstocks and biochars tested showed such negative effects in the short-term, but contrarily BAS was stimulated.

Most studies have reported an initial increase in respiration shortly after the application of biochar to soil (Steinbeiss et al., 2009; Novak et al., 2010), as a result of the initial stimulation of microbial activity caused by the easily mineralizable C fraction present in most biochars (Lehmann et al. 2009, Kolb et al., 2009; Jones et al., 2011). Such increased activity might also be coupled with shifts in microbial communities better able to use this fraction, as shown by Jin (2010), who reported an increase in the number of taxa using simple organic compounds such as sugars or cellulose (Zygomycota), and a decrease in the groups using more complex organic carbon such as lignin (Basidiomycota and Ascomycota). This has also been supported by some studies showing increases in C-cycle soil enzymatic activities short-term after the application of biochar (Bailey et al., 2011). It has been suggested that volatile matter, measured according to ASTM standard methods, initially developed to measure the quality of coals as fuels, might correlate with biochar persistence and stimulation of respiration (Deenik et al., 2010; Zimmerman et al., 2011), which is confirmed in our study, although this relationships has not always confirmed and attributed to priming effects (Zimmerman et al., 2011; Dempster et al., 2012).

The generalized stimulation of BAS in the materials in our study was explained by its positive correlation with volatile matter content and a negative correlation with pyrolysis temperature, and total, organic and fixed carbon. Only fixed carbon remained negatively correlated with BAS when feedstocks were excluded and only biochars were considered, which is consistent with the previous statement since fixed carbon is known to increase with higher pyrolysis temperature and lower volatile matter (Enders et al., 2012). Modeling of BAS in soil-material mixtures confirmed the negative contribution of pyrolysis temperature, but also a positive association with application rate, pH and soluble Ca levels when data from all the materials were pooled. When only biochars were considered, higher BAS was associated with lower NO_3^- levels and Br^- that might be explained by an increased microbial transformation or assimilation of these compounds.

A similar effect of pyrolysis temperature on soil respiration has been reported in other studies. According to Baldock and Smernik (2002) lower mineralization would be expected for those biochars produced at higher temperatures, which in turn presented higher degree of aromatic carbon (aryl groups). In our study the effect of pyrolysis temperature on BAS is clearly mediated by its effect on volatile matter content, since both parameters are correlated in the set of materials in this study (Pearson, $r = -0.86$), and also when only biochars were considered ($r = -0.84$).

4.2.2. Effects on collembolan reproduction

In our study, no strong inhibition of reproduction of the slow pyrolysis biochars and feedstocks was observed, except in the food waste materials. Furthermore, no significant effect of pyrolysis temperature on such inhibition was found, and only salinity, mostly explained by soluble Na, was significantly correlated with the negative effects observed in the food waste feedstock and derived biochars. Only for food waste, pyrolysis was able to significantly decrease toxicity, probably due to volatile matter losses after pyrolysis and the resulting reduction of soluble NH_4^+ which is released by mineralization.

The negative effect of salinity and soluble Na on collembolan reproduction could be at least partly related to the interruption of egg development due to osmotic effects causing dehydration (Schrader et al., 1998). Regarding NH_4^+, it has been linked to negative impacts on soil fauna after the application of nitrogenated fertilizers (Seniczak et al., 1994) or labile organic wastes (Domene et al., 2007), but also to biochars: Liesch et al. (2010) reported that the high earthworm toxicity of a poultry litter biochar was suggested to be related by the high pH and gaseous NH_3 emissions. No correlation was found in our study between toxic effect and the total metal contents in feedstocks or biochars or their soluble content in soil-material mixtures. Our results contrast with those of Marks et al. (2014), whom reported *F. candida* reproduction stimulation of different slow and fast pyrolysis biochars at similar application rates, as well as strong inhibition in a gasification biochar due to its high liming capacity.

4.3. Usefulness of soil bioassays in the context of biochar characterization

Ecological risk assessment is an increasingly used tool by environmental authorities in the United States (USEPA, 1998) and the European Union (EC, 2003), and defined as a process for evaluating the likelihood of adverse ecological effects occurring as a result of exposure to stressors (Gentile et al., 1993). Risk is assessed comparing the exposure concentrations with the concentrations causing biological effects (Brock, 2013). Data from bioassays become the main source of biological data due to the obvious challenges of applying pollution at the field scale. Conversely, data from bioassays can be used to define safe application rates of pollutants or materials. This approach used for pollutants can also be taken for potentially polluted materials, as it has been proposed for the prospective risk assessment of wastes in the EU for the consideration of a waste as ecotoxic (Moser and Römbke, 2009). The same approach could be used for the certification of biochars,

ensuring its safe use in soil while preserving the wide range of environmental benefits.

The results from our study support a generalized lack of toxicity for most biochars at 0.5% (~19.7 Mg ha^{-1}), in the range of the typical one-time biochar applications for quality soil management, mostly below 20 Mg ha^{-1} (Jeffery et al., 2011; Biederman and Harpole, 2013). Similarly, in a previous study that tested all the biochars used in our study with the exception of bull manure biochars (Rajkovich et al., 2012), no phytotoxicity was observed below the 2% application. Caution needs to be exercised when application rates are maximized to sequester C for climate change mitigation, and rates of individual additions be limited for those biochars that show toxicity at high applications. Furthermore, our results point out the suitability of soil ecotoxicological tests for the detection of problematic biochars that would not be excluded from application according to the available quality standard guidelines alone, which mostly rely on physicochemical characterization and do not include recommendations about application rates or site-specific use. Earthworm and enchytraeid avoidance tests, together with plant germination tests, have been proposed as suitable for the ecotoxicological characterization of biochars before its application in biochar trials (Major, 2009). A variety of OECD and ISO standardized ecotoxicological tests exist for soil organisms that could be easily adapted for biochar testing.

5. Conclusions

Heavy metal content and alkalinity were characteristic for each feedstock, but generally increased after pyrolysis and with pyrolysis temperature. Alkalinity was the highest in paper mill waste, manures and corn stalk materials, and the lowest in wood materials, but in most materials increased with pyrolysis and pyrolysis temperature. On the other hand, salinity was strongly influenced by that of the original feedstock, but did not vary with pyrolysis or pyrolysis temperature. The highest salinity values were observed in food waste materials and the lowest in paper mill waste and wood materials.

Basal soil respiration was not impaired but always stimulated by feedstock or biochar application and positively correlated with volatile content of these materials. Regarding collembolans, toxicity was feedstock dependent and generally unaffected by pyrolysis or pyrolysis temperature, with strong inhibition only observed in food waste feedstock and biochars. Soluble Na was identified as the main factor responsible for inhibition in this study.

A generalized lack of toxicity was observed at concentrations in the range of usual field biochar applications rates (<20 t ha^{-1}), indicating low short-term toxicity risk of the slow pyrolysis biochars used in this study. Bioassays were demonstrated useful for detecting potentially ecotoxicological effects of biochars, not captured by the physicochemical limit values set in different biochar quality standards currently available, which do not provide guidance for application rates specific to soil or crop types. This is why ecotoxicological tests are proposed as important criteria to develop management recommendations.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.12.035.

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