Biochar and Compost Amendments Enhance Copper Immobilisation and Support Plant Growth in Contaminated Soils.

Jones, Sarah[a]; Bardos, R. Paul[a]; Kidd, Petra S.[b]; Mench Michel[c,d]; de Leij, Frans[e]; Hutchings, Tony[e]; Cundy, Andrew[f]; Joyce, Chris[f]; Soja, Gerhard[g]; Friesl-Hanl, Wolfgang[g]; Herzig, Rolf[h]; Menger, Pierre[i]

[a]r3 environmental technology UK ltd, H9 TOB1, University of Reading Whiteknights Campus, Reading, UK. sarah@r3environmental.co.uk; paul@r3environmental.co.uk.

[b]Instituto de Investigaciones Agrobiológicas de Galicia (IIAG), Consejo Superior de Investigaciones Científicas (CSIC), Santiago de Compostela 15706, Spain. pkidd@iiag.csic.es

[c]UMR BIOGECO INRA 1202, Ecologie des Communautés, Université de Bordeaux, Bât. B2, allée Geoffroy St-Hilaire, CS50023, F-33615 Pessac cedex, France. mench@bordeaux.inra.fr

[d]INRA, UMR BIOGECO INRA 1202, 69 Route d'Arcachon, 33610 Cestas, France.

[e]C-Cure Solutions Ltd. Forest Research, Wrecclesham, Farnham, Surrey, UK. tony@ccuresolutions.com; frans@ccuresolutions.com.

[f] Environment and Technology, University of Brighton, Lewes Road, Brighton, BN2 4GJ, UK. A.cundy@brighton.ac.uk

[g] AIT Austrian Institute of Technology GmbH, Konrad Lorenz-Str. 24, 3430 Tulln, Austria. Wolfgang.friesl@ait.ac.at; Gerhard.soja@ait.ac.at

[h]Phytotech Foundation & AGB, Quartiergasse 12 CH3013 Berne, Switzerland. rolf.herzig.agb@bleuwin.ch

[i]Tecnalia Research & Innovation, Parque Tecnológico de Bizkaia, E-48160 Derio - Bizkaia Spain. pierre.menger@tecnalia.com

Corresponding author: Paul Bardos paul@r3environmental.co.uk Tel: +44(0)118 378 8164; Mob: +44(0)7961 200 118; Fax: +44(0)118 329 0476.

Abstract

Contamination of soil with trace elements, such as Cu, is an important risk management issue. A pot experiment was conducted to determine the effects of three biochars and compost on plant growth and the immobilisation of Cu in a contaminated soil from a site formerly used for wood preservation. To assess Cu mobility, amended soils were analysed using leaching tests pre- and post-incubation, and post-growth. Amended and unamended soils were planted with sunflower, and the resulting plant material was assessed for yield and Cu concentration. All amendments significantly reduced leachable Cu compared to the unamended soil, however, the greatest reductions in leachable Cu were associated with the higher biochar application rate. The greatest improvements in plant yields were obtained with the higher application rate of biochar in combination with compost. The results suggest joint biochar and compost amendment reduces Cu mobility and can support biomass production on Cu-contaminated soils.

Keywords

Organic amendments; sunflower; Leaching tests; Plant trials; Trace elements; In situ stabilisation

Highlights

- Cu-contaminated soil was amended with three biochars singly and with compost
- Leaching tests and plant trials determined Cu mobility and plant growth
- All amendments reduced Cu mobility and facilitated plant growth
- Biochar and compost amendments could facilitate plant growth on Cu-contaminated soil

1 Introduction

Trace element (TE) contamination of soils is a challenging risk management issue. TEs do not degrade over time and are therefore persistent in the environment (Megharaj *et al.,* 2011), and conventional methods for their remediation (e.g. dig and dump) are often costly (Bolan *et al.,* 2014) and non-sustainable. An interesting low input approach to *in situ* remediation of TE contaminated soils may be the use of amendments such as biochar and compost.

Biochar is the carbon-rich end product of biomass pyrolysis. It has diverse potential environmental applications. Amongst other uses, biochar may be used in carbon sequestration, bioenergy production and agricultural waste recycling (Ahmad *et al*., 2014). Biochar may also be an immobilisation agent for TE in contaminated soil (Venegas *et al.,* 2015; Houben *et al.,* 2013; Khan *et al.,* 2013; Beesley & Marmiroli, 2011; Park *et al.,* 2011; Sizmur *et al.,* 2011). Biochar's ability to sorb contaminants has been attributed to an increase in oxygen-containing surface functional groups (carboxyl, hydroxyl and phenolic) with biochar addition to soil (Tong *et al.,* 2011; Uchimiya *et al.,* 2011). Biochar is also highly durable (Gurwick *et al.,* 2013) which could result to a long term immobilisation effect. Whilst biochars have been shown to reduce the bioavailability of TE in soils, further study of this process is required in a range of contaminated soils in order to fully understand both its potential and its limitations.

Compost amendment has also been used in the remediation of contaminated soil. Compost addition can reduce the exchangeable fraction in soil for a number of TE, due to increased cation exchange capacity (CEC) and the strong affinity of metals for organic complexation sites (Bes and Mench, 2008; Fleming *et al.,* 2013). Alvarenga *et al.* (2009) showed that composts derived from green waste and municipal solid waste reduced mobile concentrations of copper (Cu), lead (Pb) and zinc (Zn) as a consequence of altered soil chemical characteristics, including increased pH and organic matter (OM) content.

Biochar and compost incorporation into soil for remediation purposes also provides soil fertility improvements (including nutrient provision, enhanced CEC, improved soil structure and water retention, and pH control). These wider benefits may also be very useful for brownfield sites, which commonly have poor soil quality (Mallik & Karim, 2008; Nixon *et al.,* 2001). Additionally, higher plant yields obtained with organic amendment addition may support increased biomass production on brownfields, and therefore enhanced phytoremediation (Beesley *et al.,* 2011).

In this study, we investigated the effects of three biochars and compost on plant growth and Cu mobility in a contaminated soil from a former wood preservation site. Leaching tests were performed on amended soils to determine Cu mobility, and plant (pot) trials were undertaken to assess impacts on plant growth and plant metal concentrations. Sunflower was selected as the trial plant as it has high adaptability and aesthetic appeal, and is widely used as a biofuel substrate (Zhao *et al*., 2014; Mench *et al*., 2010; Amon *et al*., 2007; Gerçel, 2002). The IBL04 sunflower mother clone was chosen for its relatively high metal tolerance (Herzig *et al.,* 2014;

Nehnevajova *et al.,* 2007, 2009) and has previously been cultivated in the field in amended soils at the Gironde site (Kolbas *et al.*, 2014)

2 Materials and Methods

2.1 Study Site and Soil Sampling

Cu contaminated soil was obtained from a former wood preservation site in the Gironde County Saint Médard d'Eyrans, France (N 44^0 43.353, W 000^0 30.938) (Bes *et al.,* 2010). Soil was sampled randomly and collected in February 2014 with an unpainted stainless steel spade from the P7 sub-site (0-25 cm depth) which has previously been investigated by Bes & Mench (2008), Mench & Bes (2009), and Bes *et al.* (2010). Soil material (5 subsamples totalling 100 kg) was manually homogenised and sieved to 4mm. The P7 soil (WRB classification: Eutric gleysol; pH_{water} 7; LOI 3%) is largely classified as a sandy loam. At the P7 sub-site, wood was dipped in creosote and Cu sulphate as preservative treatments. The P7 soil consequently has high levels of Cu and polycyclic aromatic hydrocarbon (PAH) contamination (see supplementary materials for detailed soil analysis of: PAHs, trace elements, hydrocarbons and BTEX - http://doi.pangaea.de/10.1594/PANGAEA.846932).

2.2 Amendments

Three biochars were tested: BC1, BC2 and BC3. BC1 (pH_{water} 10; LOI 49%) was a specialised biochar agent, *C-Cure Metal*, developed and patented for the remediation of metal contaminated substrates (C-Cure Solutions™ Ltd, Farnham, UK)[1](patent number: WO2009016381A2). BC2 (pH_{water} 10; LOI 42%) and BC3 (pH_{water} 10; LOI 40%) were produced by the AIT (Austrian Institute of Technology GmbH) in co-operation with Sonnenerde GmbH using chopped poplar wood previously harvested from the P7 sub-site. BC2 and BC3 were produced via pyrolysis at 525°C in a Pyreg reactor (Pyreg GmbH, Dörth, Germany) with a residence time of approximately 15-20 minutes. BC2 was used unaltered. BC3 was mixed with 20% Fe_2O_3 purchased from VWR (VWR International GmbH, Darmstadt, Germany). Fe_2O_3 was trialled in an attempt to improve the number of sorption sites. Iron oxides have known sorption capabilities and have been applied as intended "sinks" for certain TE (Komárek *et al.,* 2013; Cundy *et al.,* 2008; Cornell & Schwertmann, 2003). Compost (pH_{water} 8; LOI 18%) made from green waste and sandy soils/sand was purchased in France and was stored at the Gironde site for one year under tarpaulin. Soil and amendments were transported to IIAG-CSIC[2], Spain, where the leaching and plant trials were carried out.

2.3 Experimental Design

Each of the biochars was trialled as a single amendment at rates of 1% and 3% w/w. Green waste compost (C) was also trialled as a single amendment at application

[1] www.ccuresolutions.com

[2] www.iiag.csic.es

rates of 1% and 2% w/w. Additionally, each of the three biochars was trialled in combination with compost, at the aforementioned application rates. Soils were amended with a total of 20 amendments alongside unamended soil (see Table 1 below).

Table 1: Sample ID showing amendments and rates

Unamended	Compost (1%)	BC3 (3%) + C (1%)
BC1 (1%)	Compost (2%)	BC1 (1%) + C (2%)
BC2 (1%)	BC1 (1%) + C (1%)	BC2 (1%) + C (2%)
BC3 (1%)	BC2 (1%) + C (1%)	BC3 (1%) + C (2%)
BC1 (3%)	BC3 (1%) + C (1%)	BC1 (3%) + C (2%)
BC2 (3%)	BC1 (3%) + C (1%)	BC2 (3%) + C (2%)
BC3 (3%)	BC2 (3%) + C (1%)	BC3 (3%) + C (2%)

Prior to soil amendment, all biochars were air-dried for three days then ground to <2mm. Compost was sieved to <2mm before addition and application rates were amended to allow for moisture content. For incubation trials (see below), 150g aliquots of soil were amended (20 amendments, plus unamended). For plant trials, soil was sieved to 4mm and bulk amended in batches of 3kg (20 amendments, plus unamended). To determine the effect of the soil amendments on Cu mobility and plant growth, leaching tests and plant trials were carried out. pH and Dissolved Organic Carbon (DOC) were measured in parallel to leaching tests.

2.4 Leaching Tests

Leaching tests (adapted from Houba *et al.,* 2000) were carried out to determine the effect of amendment application on Cu mobility in soil. Leaching tests were carried out at three “time points”: “pre-incubation”, “post-incubation” and “post-growth”. “Pre-incubation” leaching tests were carried out 24 hours after amendment by removing aliquots (2.5g) of amended and unamended soil to 50ml centrifuge tubes (4 replicate tubes per amendment) and mixing with 25ml of 0.01M $CaCl_2$. Samples were then placed on a shaker for 24 hours prior to centrifugation at 3752g for ten minutes (J2-MI, Beckman Coulter, Inc., Brea, CA, USA). Samples were extracted for 24 hours as biochar producers C-CURE have found (based on experience over several years) that whilst a 2 hour extraction gives an indication of immediate leachable Cu (i.e. equivalent to exchangeable) it doesn’t give a good indication of releasable metals. A much better assessment of how likely metals are to be released in the longer term is given with a 24 hour extraction. In this sense, the 24h test gives a very conservative assessment of exchangeable/leachable metal levels, but a realistic test of metal stability.

Following shaking, samples were filtered (8-12 µm pore; F2040 - CHMLAB Group, Barcelona, Spain) and analysed for concentration of Cu and other metals using ICP-OES (Varian Vista-Pro, Varian Inc., Palo Alto, CA).

From each 150g amended soil sample (see section 2.3), an 80g sub-sample was removed, moistened with 20ml of water and then stored at 25^{0}C in the dark for two weeks. Throughout the two week incubation, a repeated wet/dry cycle (two days wetting, followed by two days drying) was implemented to replicate conditions that may occur in the environment. Leaching tests as outlined above were repeated following this incubation stage ("post-incubation", 4 replicates). Soils collected after plant growth ("post-growth", 5 replicates) were also tested. Post-growth soils were sieved to and analysed at both <4mm and <2mm.

2.5 pH

To determine pH, four replicate samples (10g) of each amended soil were weighed into 50ml centrifuge tubes. To each tube, 25ml of Milli-Q water was added and pH measured using a Metrohm 632 pH meter (Metrohm AG, Herisau, Switzerland).

2.6 DOC

DOC was determined in the same 1:2.5 (w/v) soil:H_2O extracts after 2 hours of shaking (adapted from Jones and Willett (2006)). Samples were centrifuged at 3752g for ten minutes (J2-MI, Beckman Coulter, Inc., Brea, CA, USA) before paper filtration (8-12 µm; F2040 – CHMLAB Group, Barcelona, Spain). Centrifugation and paper filtering steps were repeated due to the turbidity of samples. Following this, samples were membrane filtered at 0.22µm, then acidified with one drop of reagent grade (70%) nitric acid. After 24 hours, the supernatant of each sample was removed to a clean glass vial before analysis using a Vario TOC Cube (Elementar Analysensysteme GmbH, Hanau, Germany). This method was repeated with soil samples post-incubation (4 replicates) and post-growth (5 replicates).

2.7 Plant Trials

Plant trials were conducted in June-July 2014 using the same series of amendments to determine the effect of biochar and compost addition on biomass yield and plant Cu concentrations. For each amendment type, five replicate pots were prepared with 750g of soil. To each pot, two *Helianthus annuus* L. (sunflower) seeds (IBL04 mother clone, Phytotech, Bern, Switzerland) were added. Plants were watered from below, with water being placed in the saucer underneath pots for uptake. Saucers were kept wet. After germination, seedlings were thinned to one plant per pot. Plants were kept in a greenhouse with natural illumination and a maximum air temperature of 26 ^{o}C. After seven weeks, plants were harvested, washed and separated into roots, stems and leaves. Dry biomass was recorded.

2.8 Analysis of Plant Material

Dried plant material was split into above ground (shoot) and below ground (root) parts and ground manually. Samples of material (0.3-0.8g) were then weighed into glass test tubes. To this, 2ml of nitric acid (analytic reagent grade, 70%) was added and left overnight. Hydrochloric acid (1ml, 37%) was then added to each tube. Samples were digested at 120^{0}C for 9 hours. Samples were transferred to 10ml volumetric flasks and made up to the mark with deionised water. Samples were then analysed via ICP-OES (Varian Vista-Pro, Varian Inc., Palo Alto, CA) for the following elements: Cu, Ca, Fe, K, Mg, Mn, P, and Zn. Blanks and reference material (hay powder No. 129, Community Bureau of References, EU) were also included in each digestion batch to subtract elements from reagents and to check for element recovery. Element recovery was >90% of certified values.

2.9 Statistical Analysis

Statistical analysis was performed using Minitab 17 (Minitab, State College, PA, USA). All datasets were assessed using Anderson-Darling tests. All datasets showed non-normal distributions, which were largely not transformable to represent normal distributions. Therefore, non-parametric statistical analyses were used. Kruskal-Wallis tests were used to determine if there were differences between the soil amendments for the variables measured. Differences between amendments pre- and post-incubation, pre- and post-growth and between 2mm and 4mm post-growth soil were established using Mann-Whitney U tests. Correlations between different variables were established using Rank Spearman correlation. For all tests, a confidence level of 95% was used. Outliers were removed from the post-growth leaching test datasets. For the “2mm” dataset, one outlier was removed from the C (1%) treatment and one from the BC2 (3%) + C (1%) treatment. For the “4mm” dataset one outlier was removed from the BC2 (3%) treatment and one from the BC2 (1%) + C (2%) treatment. Outliers were determined using the Grubbs outlier test.

3 Results

3.1 Leaching Tests

The results of pre-incubation leaching tests showed a significant reduction in leachable Cu across all amendments relative to the unamended soil (see Figure 1). Kruskal-Wallis testing found a significant difference between amendments ($p<0.01$). For biochar only amendments, leachable Cu was reduced in the order: BC1>BC2>BC3 for both the lower and higher rates of application. This trend was repeated in the combined amendments at 1% compost addition. Combined compost and biochar amendments improved the performance of the 1% biochar application rate. The greatest overall reduction in leachable Cu was given by BC1 (3%). This amendment led to a 91% reduction in leachable Cu relative to the unamended samples. Compost alone (1%) proved the least effective amendment; although leachable Cu was still reduced by 47%.

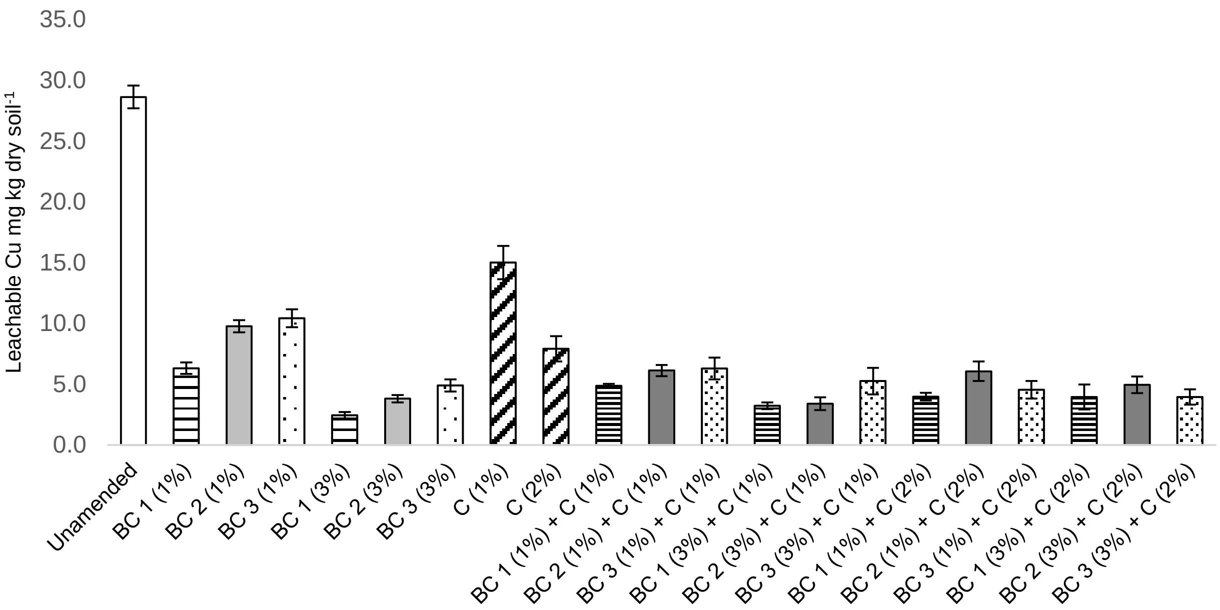


Figure 1: Mean concentration of leachable Cu mg kg^{-1} in pre-incubation soils (± standard error, n=4) (copyright r3 environmental technology uk ltd, 2015; reproduce by permission).

The results for leaching tests following incubation mirrored the pre-incubation leaching tests to some extent, with all amendments reducing mobile Cu relative to the unamended samples. However, the differences between biochars in terms of Cu immobilisation were less discernible post-incubation (see Figure 2). Similar to the pre-incubation leaching tests, significant differences were found between the amendments ($p<0.01$). Additionally, the leachable Cu in the unamended samples decreased by 25% compared to pre-incubation. Mann-Whitney U tests suggested that there were significant differences between pre- and post-incubation datasets (medians: 5.09 mg kg^{-1} and 4.13 mg kg^{-1} respectively, $p=0.01$).

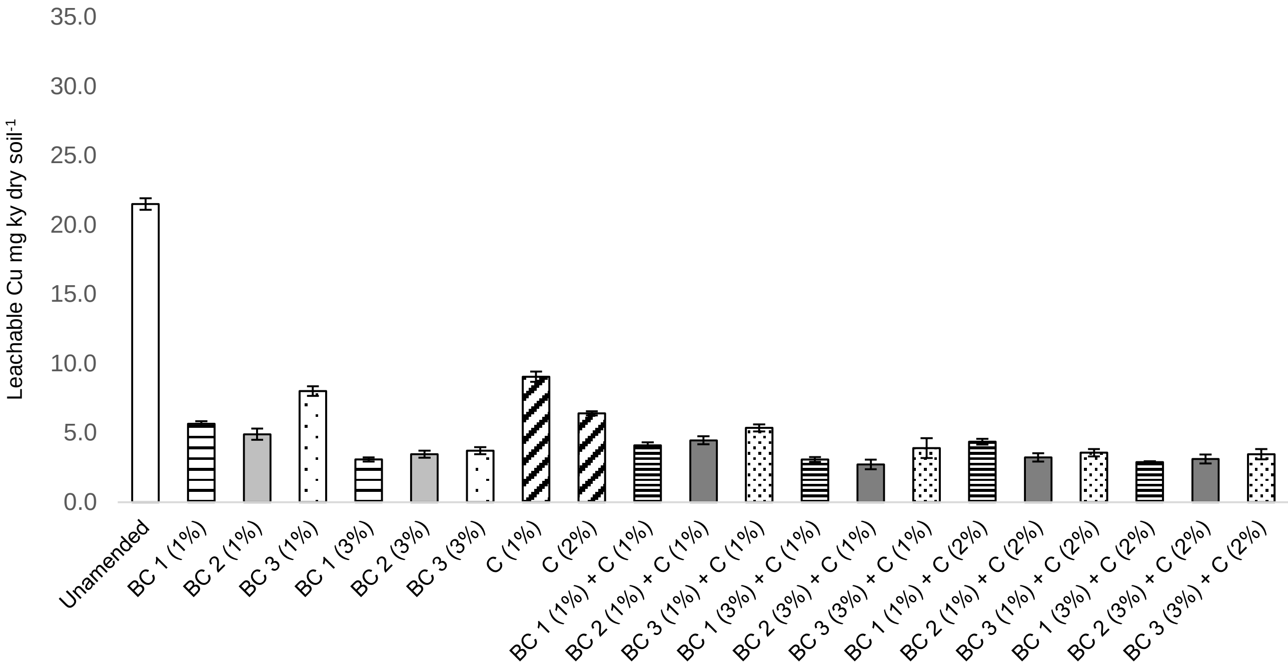


Figure 2: Mean concentration of leachable Cu mg kg^{-1} in post-incubation soils (± standard error, n=4) (copyright r3 environmental technology uk ltd, 2015; reproduce by permission)

The leaching tests performed on soils taken from pots after a seven week (sunflower) growth period again showed a significant reduction in leachable Cu across all amendments compared to the unamended samples (see Figure 3)($p<0.01$). The largest decreases occurred in BC2 (3%) + (1%) and BC1 (3%) + (2%) with 2.76 and 2.92 mg kg^{-1} leachable Cu respectively, compared to 21.8 mg kg^{-1} in the unamended. Mann-Whitney U testing suggested there were no significant differences between leaching tests carried out in soils sieved to 2mm compared to those sieved to 4mm. However, pre-incubation and post-growth (2mm) leaching test datasets were found to be significantly different (medians: 5.09 mg kg^{-1} and 0.91 mg kg^{-1} respectively, $p<0.01$).

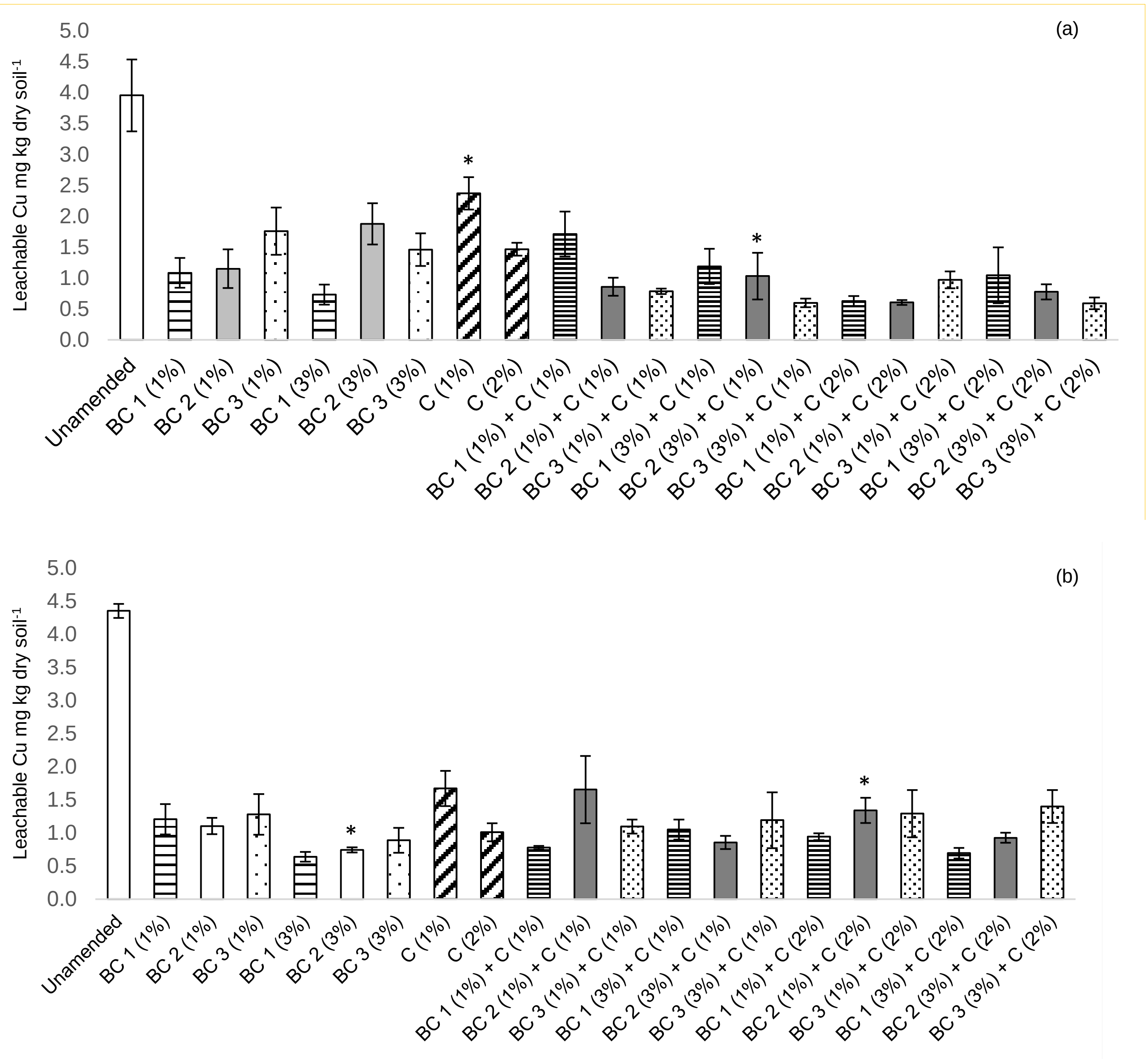


Figure 3: Mean concentration of leachable Cu mg kg^{-1} in post-growth soils, sieved to 2mm (a) and 4mm (b) (± standard error, n=5). *outlier (determined using Grubbs outlier test) has been removed: n=4. Note: change in y-axis scale (copyright r3 environmental technology uk ltd, 2015; reproduce by permission)

A dramatic drop in leachable Cu was determined in the post-growth samples across all amendments and in the unamended samples; a mean reduction of 75% was found compared to pre-incubation leachable Cu. Leachable Cu in the unamended samples decreased by 86% between the two sets of leaching tests, from 28.8 mg kg^{-1} pre-incubation to 3.96 mg kg^{-1} post-growth (4mm).

3.2 pH & DOC

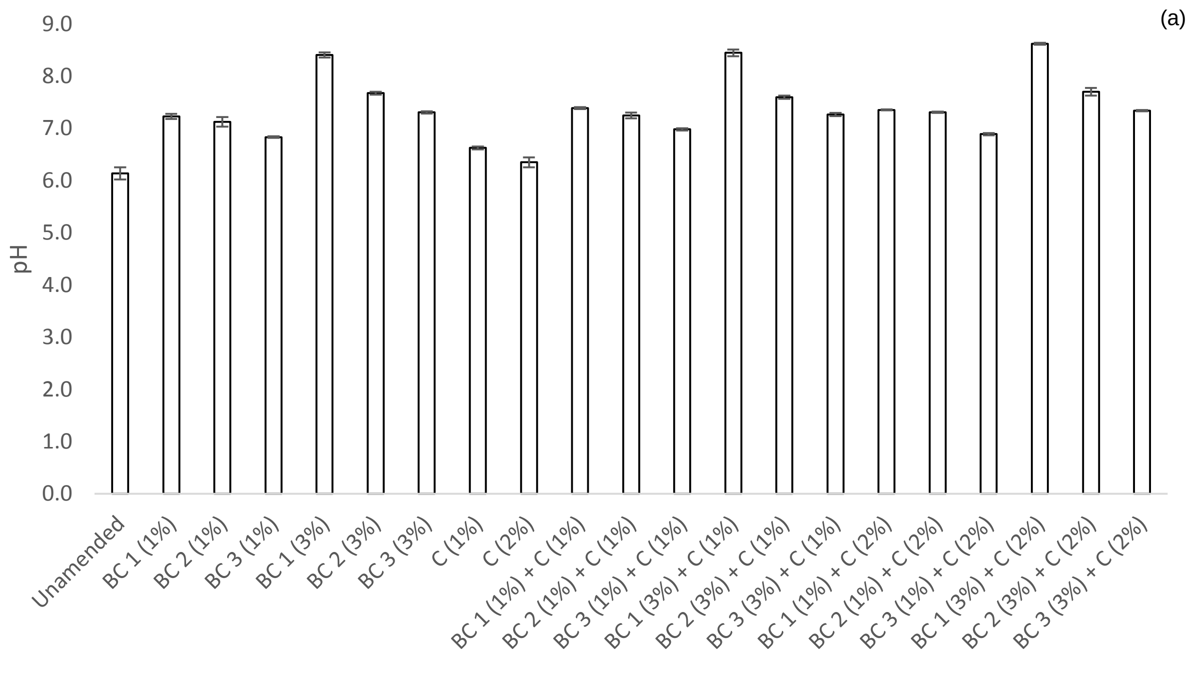
Significant differences were found in soil pH between the amendments at each of the three time points (p<0.01) but pH consistently increased in all amendments compared to the unamended soil (Figure 4Figure 5). However, soil pH varied less between amendments post-growth compared to the pre- and post-incubation tests.

Overall, BC1 (3%) amendments with and without compost were associated with the greatest pH increases. Rank Spearman testing found a negative correlation between pH and leachable Cu pre-incubation. This was found to be significant (r= -0.85, p<0.01). Post-incubation pH also had a significant negative correlation with leachable Cu(r= -0.84, p<0.01). No significant relationship was found between pH and leachable Cu post-growth. Mann-Whitney U testing determined a significant difference between pre- and post-growth pH (Medians: 7.33 and 7.64 respectively, p<0.01) as well as pre- and post-incubation (Medians: 7.33 and 7.41 respectively, p=0.01).

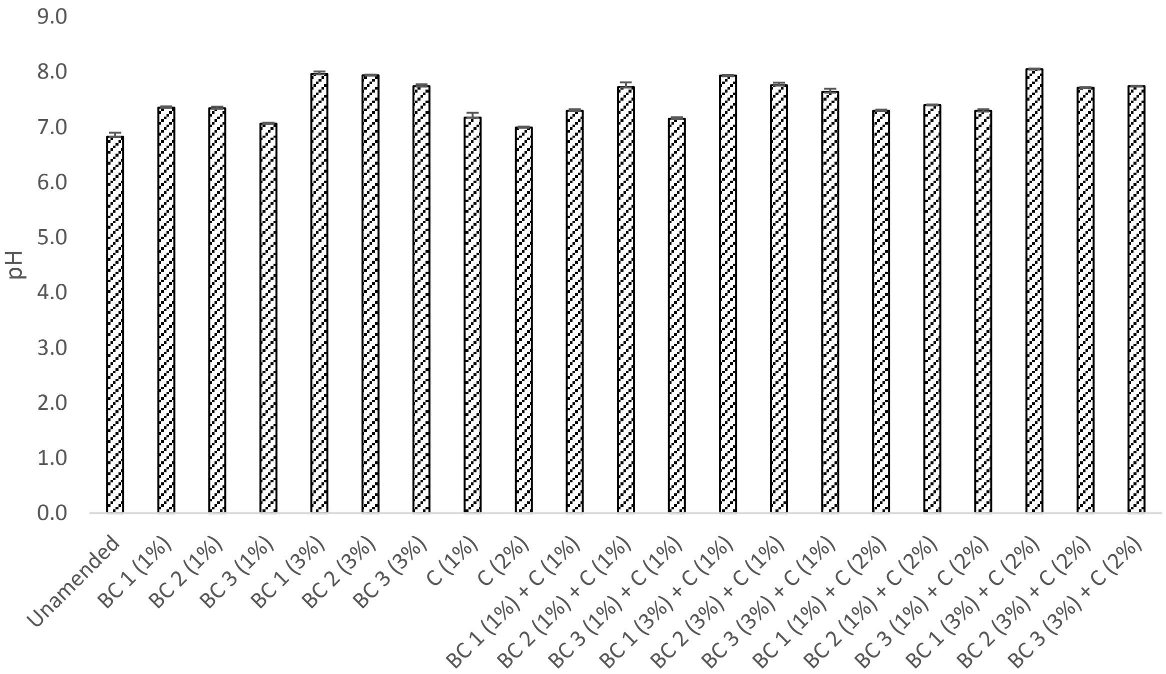
Significant differences were found between amendments for DOC in solution across all time points (Figure 5, p<0.01). Pre-incubation DOC followed a similar trend to soil pH (and showed an inverse relationship with leachable Cu), with BC1 (3%) with and without compost increasing DOC most greatly. A significant correlation was determined between DOC and pH pre-incubation (positive; r= 0.78, p<0.01) and leachable Cu pre-incubation (negative; r= -0.80, p<0.01).

A significant overall increase was found in DOC between pre-incubation and post-incubation (medians: 19.3 mg kg^{-1} and 29.0 mg kg^{-1}, p<0.01). A significant negative correlation was determined between post-incubation DOC and leachable Cu (r= -0.79, p<0.01). No significant relationship could be established between post-growth DOC and leachable Cu. However, post-growth DOC significantly increased compared to pre-growth DOC (medians: 19.3 mg kg^{-1} and 78.5 mg kg^{-1}, p<0.01). This shows a comparable but inverse trend to leachable Cu, which overall significantly decreased post-growth.

(a)



(b)



(c)

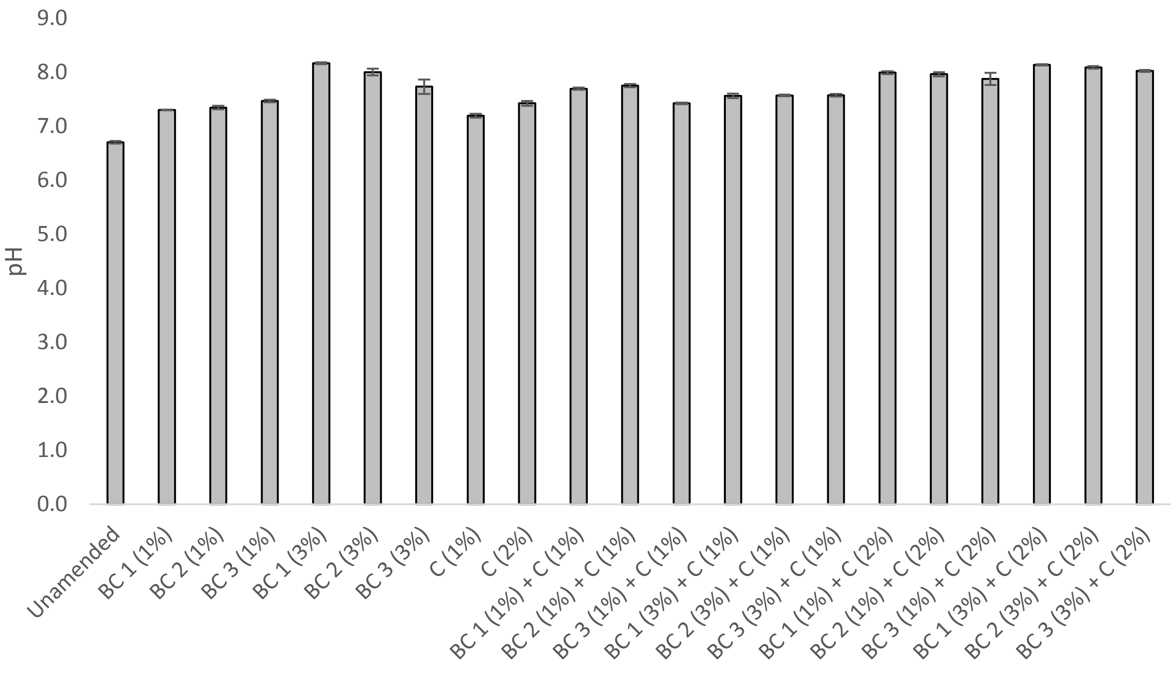
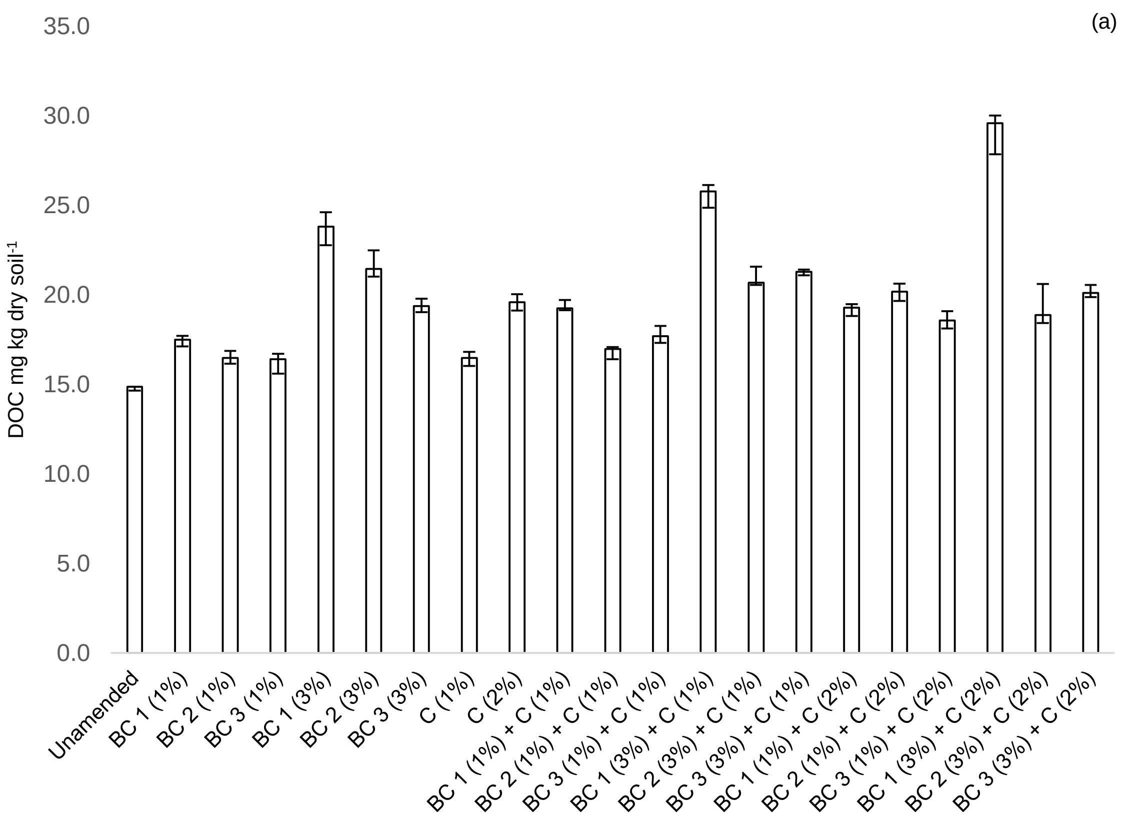


Figure 4: pH ± standard error: pre-incubation (a), post-incubation (b) and post-growth (c), n=3, 3, 5 (copyright r3 environmental technology uk ltd, 2015; reproduce by permission)


(a)
DOC mg kg dry soil-1
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Unamended
BC 1 (1%)
BC 2 (1%)
BC 3 (1%)
BC 1 (3%)
BC 2 (3%)
BC 3 (3%)
C (1%)
C (2%)
BC 1 (1%) + C (1%)
BC 2 (1%) + C (1%)
BC 3 (1%) + C (1%)
BC 1 (3%) + C (1%)
BC 2 (3%) + C (1%)
BC 3 (3%) + C (1%)
BC 1 (1%) + C (2%)
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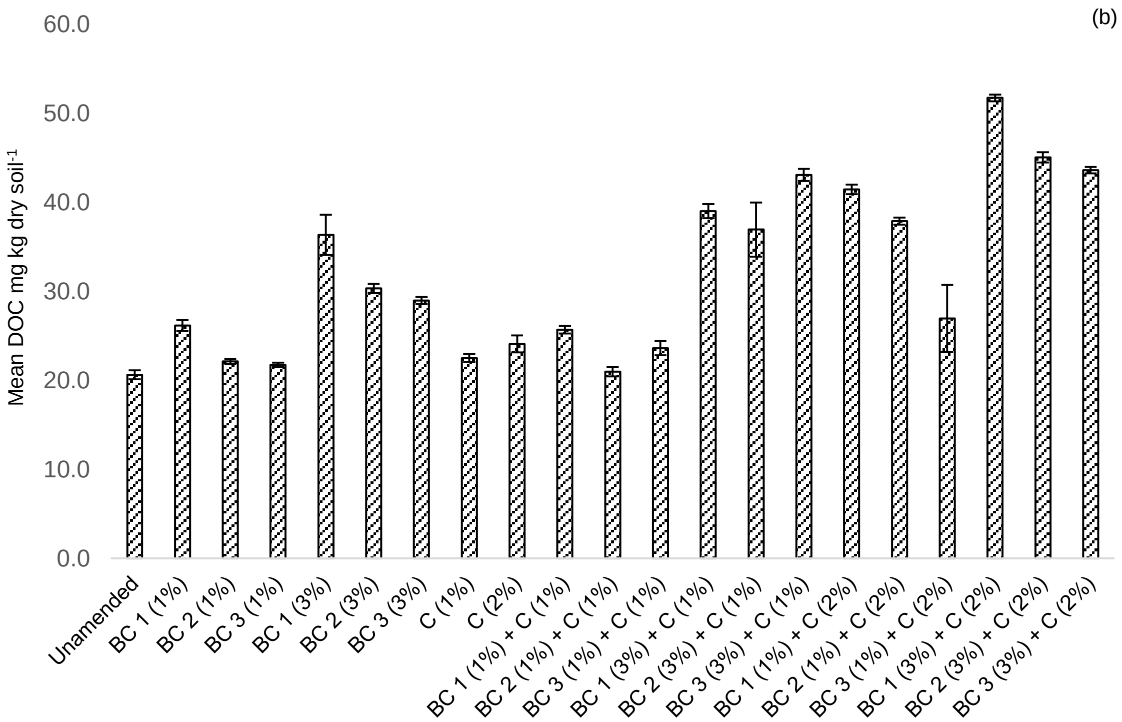
BC 3 (3%) + C (2%)


(b)
Mean DOC mg kg dry soil-1
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Unamended
BC 1 (1%)
BC 2 (1%)
BC 3 (1%)
BC 1 (3%)
BC 2 (3%)
BC 3 (3%)
C (1%)
C (2%)
BC 1 (1%) + C (1%)
BC 2 (1%) + C (1%)
BC 3 (1%) + C (1%)
BC 1 (3%) + C (1%)
BC 2 (3%) + C (1%)
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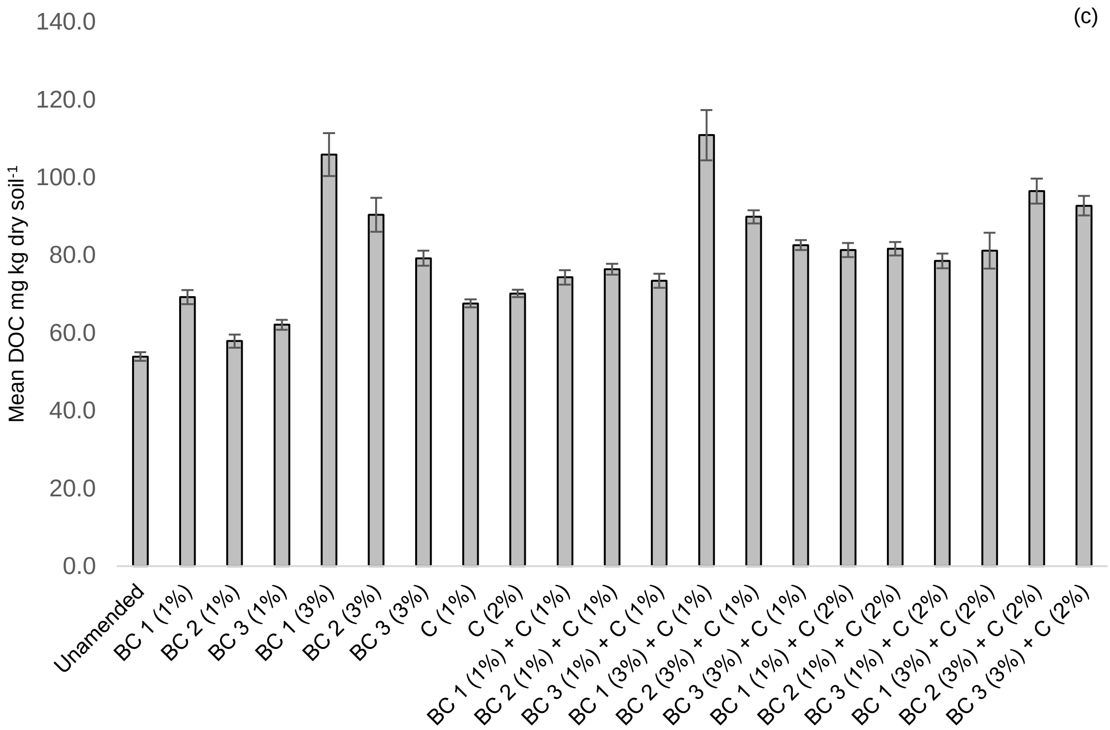

Figure 5: Mean DOC mg kg^{-1} ± standard error: pre-incubation (a), post-incubation (b) and post-growth (c), n=3, 3, 5. Note - change in y-axis scales (copyright r3 environmental technology uk ltd, 2015; reproduce by permission)

3.3 Plant Trials

3.3.1 Plant Height

Significant differences were found between amendments for both plant height and root length ($p<0.01$). Sunflower height over the seven week growth period was greatest in soils amended with BC1 (3%) in combination with compost (Table 2). The most effective amendment (BC1 (3%) + C (1%)) improved plant height by around 36.5cm and root length by around 9cm relative to the unamended samples. At the lower rate of biochar application (1%), compost addition had a greater impact on the plant height of the BC2 and BC3 plants compared to the BC1 samples, with compost addition at (2%) doubling plant height for these two biochars. Plant height and root length were severely reduced in the unamended soils compared with all of the amended samples.

Table 2: Mean values of plant height and root length in the amendments (± standard error, n=5).

Amendment	Mean Plant height (cm) ± standard error		Mean Root length (cm) ± standard error	
Unamended	4.6	±0.9	2.0	±0.3
BC 1 (1%)	23	±1.4	4.1	±0.5
BC 2 (1%)	14	±0.8	7.6	±2.7
BC 3 (1%)	9.9	±0.7	3.2	±0.7
BC 1 (3%)	32	±3.3	11	±2.2
BC 2 (3%)	18	±4.6	6.2	±1.2
BC 3 (3%)	23	±2.3	6.7	±1.5
C (1%)	11	±0.9	2.8	±0.1
C (2%)	25	±2.2	4.7	±1.1
BC 1 (1%) + C (1%)	25	±3.6	4.7	±1.1
BC 2 (1%) + C (1%)	26	±3.6	7.5	±1.5
BC 3 (1%) + C (1%)	13	±1.2	2.9	±0.4
BC 1 (3%) + C (1%)	43	±1.5	11	±1.5
BC 2 (3%) + C (1%)	28	±2.4	12	±2.5
BC 3 (3%) + C (1%)	33	±4.6	5.7	±0.9
BC 1 (1%) + C (2%)	27	±2.0	5.4	±1.3
BC 2 (1%) + C (2%)	30	±1.7	5.6	±1.3
BC 3 (1%) + C (2%)	27	±2.1	7.5	±1.5
BC 1 (3%) + C (2%)	40	±2.3	15	±2.8
BC 2 (3%) + C (2%)	29	±2.1	9.3	±1.1
BC 3 (3%) + C (2%)	34	±3.6	8.4	±1.9

3.3.2 Plant Biomass

Kruskal-Wallis testing found significant differences between amendments for both shoot and root dry biomass (Figure 6) ($p<0.01$). Mirroring plant height data, the most notable increases in shoot biomass were achieved in BC1 (3%), with and without compost. For example, BC1 (3%) + C (1%) yielded on average 0.2g more shoot biomass than BC2 or BC3 with compost at equivalent application rates (68% and 74% increase, respectively). BC3 amended soils generally resulted in the lowest shoot biomass increases relative to the unamended soils, however, BC3 achieved a greater shoot biomass yield than BC2 at 3% both as a single amendment, and with 2% compost.

Even at the lower amendment rate, the compost only amendment increased shoot yields comparably to the lower rate of biochar application. These results are contrary to the leaching test results, where compost only amendments were found to be less effective than biochar only amendments. For the root dry biomass, there was less variation between amended and unamended samples and between the different amendments. Nonetheless, the general trend showed that combined amendments and higher application rates improved root biomass yields.

Rank Spearman testing determined that there was a significant positive correlation between shoot biomass and plant height (r= 0.89, $p<0.01$). No significant relationship was found between shoot biomass and post-growth leachable Cu in soil.

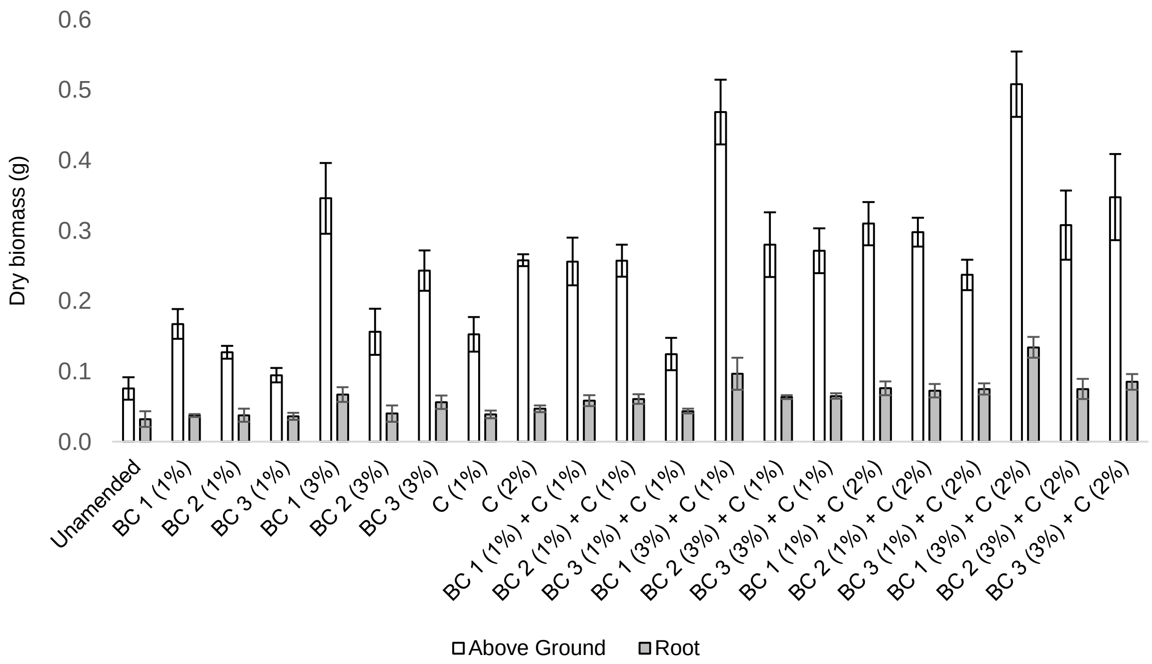

Figure 6: Mean Dry Biomass (g), shoots and roots (± standard error, n=5) (copyright r3 environmental technology uk ltd, 2015; reproduce by permission)

3.3.3 Plant Cu Concentrations

Significant differences, established via Kruskal-Wallis testing, were determined between amendments for Cu concentration in both the shoot biomass ($p<0.01$) and root biomass ($p<0.01$). There was less of a clear trend in the data compared to previous data sets (Figure 7). However, higher application rates and combined amendments overall reduced the Cu concentration in the plant root and shoot biomass. Cu concentration was many times higher in the root samples compared to the shoot plant parts.

A significant negative correlation was determined between plant Cu concentration and plant biomass for both leaf (r= -0.72, $p<0.01$) and stem data (r= -0.84, $p<0.01$). No relationship was determined between these two variables for the root samples.

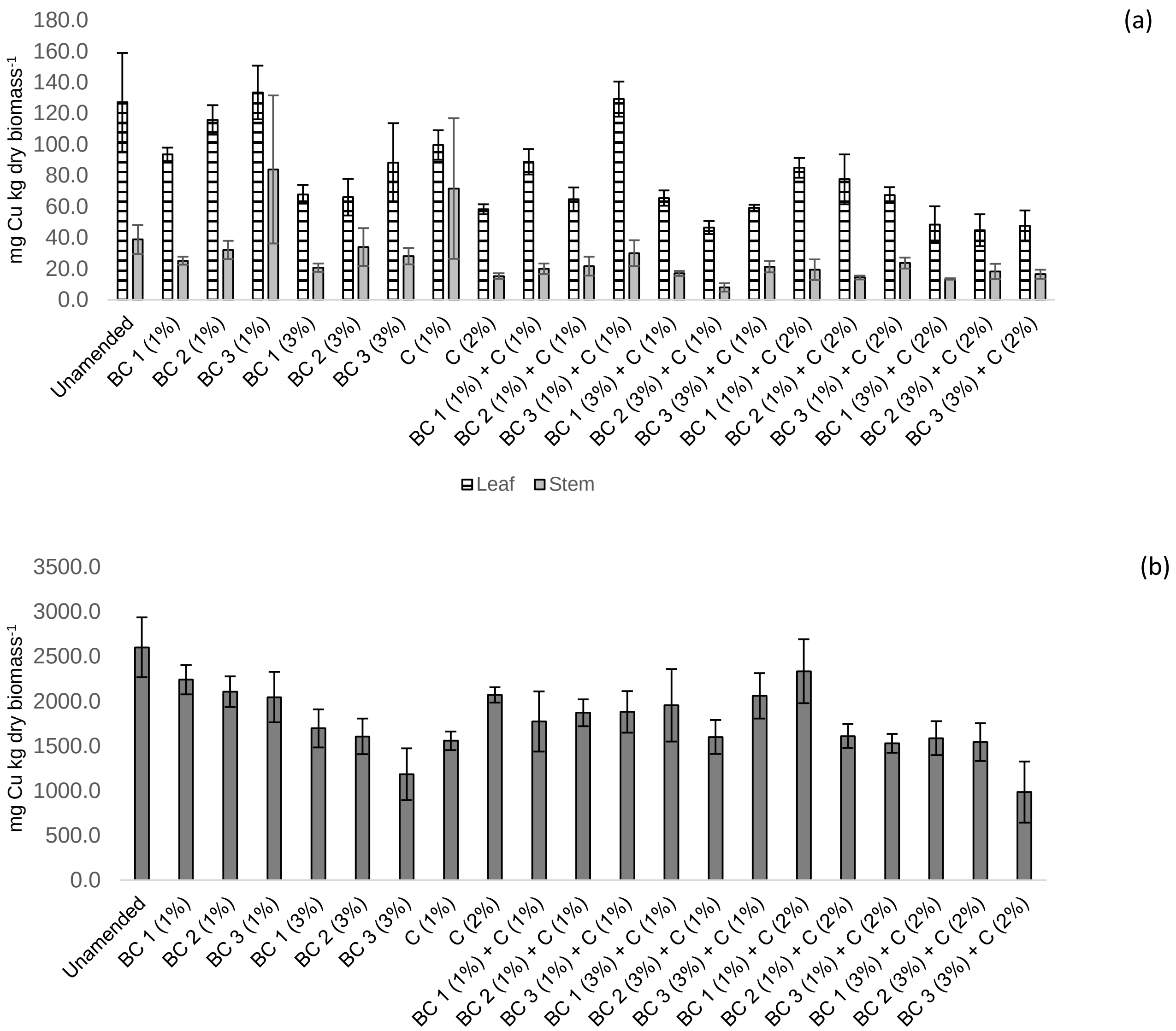

Figure 7: Mean Cu concentrations mg kg^{-1} in plant dry biomass: shoots (a) and roots (b) (± standard error, n=5) (copyright r3 environmental technology uk ltd, 2015; reproduce by permission).

3.3.4 Plant Nutrient Concentrations

Kruskal-Wallis testing determined significant differences between treatments for measured nutrient concentrations in plant shoots, including calcium (Ca) ($p<0.01$), iron (Fe) ($p<0.01$), potassium (K) ($p<0.01$), magnesium (Mg) ($p=0.02$), and phosphorus (P) ($p<0.01$). Nutrient concentrations in the shoot biomass were generally higher in the amended samples than in the unamended soil (Table 3). Overall, higher application rates of biochar and compost increased measured nutrient concentrations. BC1 amended shoots generally had the highest concentration of Mg and K. The three biochars were largely comparable in terms of shoot Ca, Fe and P concentrations. Compost only amendments generally had higher shoot nutrient concentrations than the lower application rate of biochar, however with the higher rate of biochar application, nutrient concentrations were largely comparable to, or better than, compost.

Table 3: Mean nutrient concentrations mg kg^{-1} in shoot dry biomass (± standard error, n=5)

Amendment	Ca (g/kg)		Fe (g/kg)		K (g/kg)		Mg (g/kg)		P (g/kg)	
Unamended	6.41	±1.34	0.25	±0.05	6.32	±1.84	3.74	±0.87	4.18	±1.34
BC1 (1%)	10.8	±1.83	0.12	±0.15	42.8	±5.05	2.69	±0.22	1.92	±0.18
BC2 (1%)	9.32	±1.07	0.16	±0.21	27.4	±2.40	2.97	±0.66	2.32	±0.21
BC3 (1%)	11.8	±2.91	0.44	±0.93	24.6	±4.40	4.28	±0.48	2.87	±0.19
BC1 (3%)	25.8	±1.54	0.10	±0.11	92.6	±7.41	5.00	±0.20	4.80	±0.37
BC2 (3%)	21.3	±4.35	0.26	±0.13	57.7	±7.74	4.03	±0.55	3.03	±0.30
BC3 (3%)	28.5	±11.2	0.22	±0.77	70.1	±20.9	5.36	±1.26	3.20	±0.82
Compost (1%)	12.9	±2.31	0.29	±0.73	9.95	±2.88	3.65	±0.59	2.35	±0.42
Compost (2%)	19.4	±4.28	0.16	±0.16	20.2	±4.00	4.72	±0.53	1.70	±0.28
BC1 (1%) + C (1%)	14.4	±1.31	0.15	±0.21	42.4	±5.82	3.00	±0.40	1.99	±0.22
BC2 (1%) + C (1%)	17.7	±4.33	0.15	±0.14	35.1	±2.54	2.99	±0.43	1.69	±1.00
BC3 (1%) + C (1%)	8.35	±0.47	0.31	±0.42	23.4	±2.30	2.69	±0.07	1.94	±0.13
BC1 (3%) + C (1%)	26.5	±1.34	0.11	±0.19	88.5	±9.86	4.50	±0.20	4.32	±0.55
BC2 (3%) + C (1%)	24.2	±4.68	0.98	±0.14	48.3	±3.42	3.80	±0.31	2.50	±0.22
BC3 (3%) + C (1%	30.0	±3.60	0.12	±0.24	51.1	±5.27	4.09	±0.41	2.75	±0.27
BC1 (1%) + C (2%)	21.1	±3.12	0.13	±0.20	45.5	±8.03	3.38	±0.57	1.79	±0.22
BC2 (1%) + C (2%)	25.1	±4.09	0.17	±0.51	45.9	±4.84	3.90	±0.70	2.29	±0.29
BC3 (1%) + C (2%)	18.3	±2.35	0.16	±0.16	43.9	±8.53	2.99	±0.20	2.00	±0.05
BC1 (3%) + C (2%)	27.4	±7.84	0.06	±0.01	69.3	±7.41	4.14	±0.76	3.46	±0.47
BC2 (3%) + C (2%)	21.9	±6.00	0.10	±0.02	53.1	±7.75	3.78	±0.59	2.78	±0.34
BC3 (3%) + C (2%)	24.4	±5.58	0.11	±0.06	51.3	±5.48	3.89	±0.53	2.52	±0.36

4 Discussion

4.1 Effects of Amendments on Cu Leachability

Our results are in accordance with previous findings (Venegas *et al.,* 2015; Houben *et al.,* 2013; Khan *et al.,* 2013; Park *et al.,* 2011; Sizmur *et al.,* 2011). We found a significant decrease in leachable Cu associated with application of all three biochars relative to the unamended samples. BC1 was generally the most effective biochar in terms of Cu immobilisation, but after incubation and plant growth differences between BC1, BC2 and BC3 were less pronounced. This suggests the effects of time and soil reactions are important factors to consider when measuring the effectiveness of soil amendments on TE concentrations. Fe_2O_3 (haematite) amended BC3 was the least effective biochar for immobilising Cu contrary to initial expectations.

Compost was also shown to significantly reduce leachable Cu compared to the unamended samples, although not as well as biochar. However, there was a general trend that application of biochar and compost combined enhanced Cu immobilisation. Combined application with compost was especially effective at the lower rate of biochar addition. Leachable Cu is likely to have decreased in the presence of compost due to Cu's well-documented strong affinity for OM (Kumpiene *et al.,* 2008).

Notable differences were determined between the three time points in terms of leachable Cu, with a dramatic drop for all amendments post-growth. It is possible for plant growth to decrease Cu solubility in soils (Römkens *et al*., 1999). However, there was also reduction in leachable Cu in the unamended samples, in which plant growth, especially root growth, was very stunted. It is unlikely therefore that the reduction in leachable Cu is attributable solely to plant growth effects. Additionally, the Cu concentrations in the plant shoot and root biomass were an order of magnitude less than the leachable Cu found in soil and therefore not substantial enough in any of the amendments to account for such a significant drop in Cu.

For both pre- and post-incubation leaching tests, soil pH was negatively correlated with Cu leachability. pH is a well-documented driver of metal availability in soil (Kong *et al*., 2014), including Cu availability (Kumpiene *et al*., 2008). Although significant differences were found in pH pre-incubation and post-growth (2mm), there was however a median difference of less than one pH point between the two datasets. It is therefore unlikely that the difference in pH could account for the aforementioned decrease in leachable Cu post-growth.

DOC increases were measured for biochar and compost applications in this study; organic amendments are known to increase DOC in soils (Cao *et al.,* 2003; Antoniadis & Alloway, 2002). Beesley *et al*. (2010) also found increased DOC associated with the application of biochar and green waste compost amendments to soil. Our study showed DOC was significantly increased post-growth compared to pre-growth. Plant growth is known to increase DOC in soils (Römkens *et al.,* 1999), as a result of root exudates. However, in this study, the increase occurred across all amendments and the unamended samples. As plant growth was very limited in the unamended samples, it seems unlikely that plant-growth derived DOC caused the post-growth reduction in leachable Cu. In addition, the significant difference in DOC post-growth combined with a decrease in leachable Cu contrasts with the established nature of DOC-metal interactions. DOC is generally regarded as mobilising soil Cu, as a result of DOC competing with metals for sorption sites or by forming complexes with the metal ions, preventing sorption of metals onto sequestering surfaces (Chirenje *et al.,* 2002; Redman *et al*., 2002; Weng *et al.,* 2002; Giusquiani *et al.,* 1998).

Instead, the observed drop in leachable Cu post-incubation and post-growth could be explained by rewetting processes. Wenzel and Blum (1999) highlight that air-drying soils prior to analysis of mobile metal content can result in the overestimation of metal concentrations, including Cu. Haynes and Swift (1991) demonstrated that air drying soils increased the Cu extractability, but that this effect was reversible; after a two week incubation period following rewetting, Cu extractability had decreased to a level comparable to pre-drying. This trend was attributed to metal-retaining organo-mineral associations being disrupted and then reformed by drying and subsequent rewetting. It is possible then, that the initial leaching test results showed unrealistically high Cu concentrations as a result of this process. The decrease in Cu found in the post-growth leaching tests could accordingly be attributed to an extended period following rewetting allowing the establishment of more stable metal organo-mineral associations.

Nonetheless, even if the unamended soil in the post-growth leaching tests is accepted as giving a “true” representation of leachable Cu in the soil, the effects of biochar and compost are still substantial; a clear reduction in leachable Cu was obtained in the post-growth amended soils compared to the unamended samples.

4.2 Effects of Amendments on Plant Growth and Cu Concentration

All biochar and compost amendments improved sunflower growth relative to the unamended samples, mirroring previous findings (Buss *et al.,* 2012; Beesley *et al.,* 2010). Improved plant growth in Cu contaminated soils with biochar and compost addition may be the result of several factors including decreased Cu bioavailability in the soil (at each time point, all amendments decreased leachable Cu in the soil) and improved soil nutrient and water provision resulting from amendment incorporation into the soil (Bruun *et al.,* 2014; Basso *et al.,* 2013).

The greatest increases in shoot yield were observed in the BC1 amendments at the higher application rate; BC1 amended soil had notably improved growth compared to the other biochars or compost as a single amendment. As the leaching tests suggested that over time differences between biochars became less significant, it is probable that an alternate factor caused the disparity between BC1 and the other two biochars in terms of plant growth. It is possible that differences in nutrient provision between the three biochars was a contributing factor in plant yields. Indeed, Alburquerque *et al.* (2014) found sunflower yields were improved in OM poor, low nutrient, loamy sand by amendment depending on biochar type (in terms of nutrient content) and application rate. In our study, some available nutrients were increased in BC1 compared to the other biochars (see supplementary materials – http://doi.pangaea.de/10.1594/PANGAEA.846932). For example, BC1 generally had the highest concentration of the three biochars of exchangeable cations including Ca, Mg and K. Ca may compete with Cu for plant uptake (Fan *et al.,* 2012). Additionally, plant biomass concentrations of certain nutrients were greater for BC1 amendments. For example, plants grown in soils amended with BC1 (3%), as well as having higher overall biomass, had an average of 92.6 g K kg^{-1} in shoot dry biomass, compared to 70.1 g K kg^{-1} in BC3 (3%) plants, or 57.7 g K kg^{-1} in BC2 (3%).

Compost addition as a single amendment also improved shoot yields comparably to biochar only amendments (in contrast to leaching test results). Additionally, compost further improved the effectiveness of the biochar when applied together. The nutrient provision effects of compost are well established and compost has been shown to improve many soil characteristics, including soil structure and water retention, which can lead to improved yields (Ohsowski *et al.,* 2012; Evanylo *et al.,* 2008; Hargreaves *et al.,* 2008; Aggelides and Londra, 2000). However, the compost used in our research had relatively low OM and plant nutrient levels. Potentially then, an even greater impact might have been seen if a higher-quality compost was used.

Most of the amendments tested significantly reduced Cu concentrations in plant shoots. Where compost and biochar were applied as single amendments, higher application rates decreased concentrations of Cu in plant shoots. Where biochars were applied in combination with compost, combinations mostly improved on the results of single amendments. Kolbas *et al.* (2014) state that Cu availability to plants may be influenced by a range of factors including soil type, DOC, and pH of soil pore waters; and the results of our study (see also supplementary materials – http://doi.pangaea.de/10.1594/PANGAEA.846932) have shown that all of these factors were altered by biochar and compost additions to soil. In numerous studies, biochar addition has been found to decrease contaminant availability to plants (Khan *et al.,* 2013; Cui *et al*., 2011; Park *et al.,* 2011; Namgay *et al.,* 2010). Kloss *et al.* (2014) found that four different biochar types all decreased Cu concentrations in plant tissue. Similar results have been reported for compost application to soil (Ruttens *et al.,* 2006). Karami *et al.* (2011) showed that the addition of both biochar and compost significantly reduced shoot Cu concentrations in ryegrass in comparison to unamended soils.

5 Conclusions

Both biochar and compost as single and combined amendments significantly decreased leachable soil Cu and improved the sunflower shoot yield in a Cu-contaminated soil from a former wood preservation site. Cu concentration in sunflower biomass was also relatively low. It can therefore be suggested that biochar and compost amendments can support sunflower (and potentially other plant) biomass production on Cu-contaminated marginal sites, although site specific testing will be required for each application.

All biochars significantly reduced leachable Cu and facilitated good plant growth. Of the biochars, BC1 was most effective based on application rate. Results suggest that biomass grown on the site could be used to produce biochars for further site improvement by reducing soil contaminant mobility and facilitating plant growth.

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