Biochar: for better or for worse?

Alessia Freddo

Submitted for the Degree of Doctor of Philosophy

University of East Anglia
School of Environmental Sciences

February 2013

To my Father and my Mother

ABSTRACT

This thesis presents biochar state of the art and investigations into the environmental benefits and potential impacts of biochar application to soil.

Specifically, the opportunity biochar has to increase concentrations of potentially toxic elements (PTE) and polycyclic aromatic hydrocarbons (PAHs) in soil was investigated and contextualised. Results indicated limited environmental impacts in this regard.

The capacity of biochar to interact with organic compounds was studied in two contexts: PAHs absorption and partitioning; and with respect to bioavailability and potential deactivation of herbicides.

Regarding PAH partitioning, sewage sludge biochar (SSBC) was established to be more efficient than sewage sludge (SS) in reducing the bioaccumulation of PAHs in *Lactuca satuva L.* grown in contaminated soil; while increasing significantly ($p < 0.05$) biomass yield, relatively to a soil only control.

Regarding herbicides, biochar amended soil was observed to reduce herbicide extractability (< 2%). This extractability being far lower than that observed in the biochar free control soils (40% and 90%). ^{14}C-radiorespirometry indicated that herbicide sequestration within biochar greatly reduced its bioavailability.

Biochar influence upon weed survival indicated high biochar application rates (5%) to reduce the effectiveness of herbicides, suggesting that biochar incorporation in to soil at these levels could potentially undermine agriculture that relies upon herbicides.

Finally, biochar was tested as microbial carrier. Rhizobacteria survival was established to be higher in biochar produced from redwood than in peat (a common microbial carrier) at high incubation temperatures (25˚C and 35˚C).

In conclusion, biochar addition to soil presents limited direct environmental pollution impact. While biochar absorptivity may be beneficial in mitigating the bioavailability of organic contaminants this trait needs to be considered carefully in agricultural soils where herbicides are relied upon. Given the encouraging results regarding the potential for biochar to act as a microbial inoculant carrier, further research is warranted.

Acknowledgments

I would like to express my sincere gratitude to my supervisor, Brian Reid. His invaluable help in my research and always present encouragement and positivity throughout the course of my PhD have been essential in the completion of this thesis. I could not have asked for a better supervisor.

The assistance and patience of Judith Mayne and Graham Chilvers were very important while undertaking my experiments in the lab, and therefore I am extremely grateful to them.

I would also like to thank Zhu Yong-Guan for the opportunity I had to visit and work in the Institute for Urban Environment (Xiamen, China).

During these years of PhD I had the privilege of meeting many precious people, who helped and supported me. No words can truly express my gratitude to them. Firstly, I would like to acknowledge the Norfolk Baha'i Community, thanks to whom I always felt at home since I first moved to Norwich. Thanks to Natasha for her love and presence in moments of need and to Hutan for our deep conversations and marvellous friendship.

Thanks to my dear friends Karel and Alina, it was a pleasure to live with them. The joy, the sweetness and the utmost inner beauty of my dear Maria, Mi and Maira have uplifted me day by day.

The ever present encouragement, strong support, incredible patience and motivation, which I received from my dearest Robert when I needed it the most, were of crucial importance.

Finally, I want to deeply thank my father, whose unconditional and great love has always been a propellant energy for all my achievements.

Table of contents

INTRODUCTION

1. CLIMATE CHANGE

1.1 Scale of the problem

In the geological past, climate change has been a constant feature. Analysis of marine and lake sediments, ice cores and cave deposits have shown that over the last 100 million years the Earth's climate has fluctuated between warm (interglacial) periods and cold (glacial) periods. These changes have mainly been due to tectonic movements and changes in the Earth's orbit with respect to the Sun. Our present era, the Holocene, started 10,000 years ago, and it is considered a warm period between ice ages. Beyond these natural cycles a sudden and alarming increase in global temperatures has been detected during the last century; these changes being driven by the unprecedented emission of carbon dioxide (CO_2) (Ramanathan, 1988; Oreskes, 2004; Macías and Arbestain, 2010).

Studies on gases trapped in ice cores have revealed that the concentrations of CO_2 before industrialization were approximately 280ppm versus 385ppm at present, indicating a 30% increase of CO_2, equivalent to 160 billion tons. The temperature of the planet is maintained by an established relationship between the concentration of CO_2 and other gases such as methane (CH_4) and nitrous oxide (N_2O) which selectively absorb outgoing radiations re-radiating them both outward and inward (Macías and Arbestain 2010). Higher levels of such gases interfere with this equilibrium, perturbing the climate system.

The reality of anthropogenic climate change has been universally accepted. The IPCC (Intergovernmental Panel on Climate Change) Fourth Assessment Report (2007) lists the evidences of compelling changes in natural and managed systems related to regional climate changes: shrinking ice sheets, droughts and heavy rains and river discharge,

coastal changes, marine and freshwater biological systems changes, terrestrial biological systems changes, effects to agriculture and forestry and, not least, human health threat.

In the last 100 years (1906-2005) the global temperatures increased by 0.74 ± 0.05˚C, but this change has not occurred uniformly (IPCC 2007): in the Arctic the warming has been double the global average in recent decades. Studies have suggested that in the next century global warming could increase temperatures between 1.1 and 6.4˚C with respect to those present prior to the industrial revolution.

1.2 Policies

To keep the temperature rise within a range of 2.0-2.4˚C it would be necessary to stabilise concentrations of CO_2 at 350-400 ppm and CO_2-Ce at 445-490 ppm (the CO_2 equivalent (CO_2-Ce), accounts for the warming effects of both CO_2 non-CO_2 gases). The concentration of CO_2 is already within this range, with the cooling effect of aerosols keeping the CO_2-e within those values. Thus, at present, to stabilize and to even decrease CO_2 emission is an urgent priority and a significant global challenge (Hansen et al. 2008).

Several initiatives planned and on-going offer systematic actions for the mitigation and adaptation to global climate change. These are undertaken by different countries and international organizations such as United Nations Environment Programme (UNEP) and World Meteorological Organization (WMO).

In 2010, leaders of 194 countries signed the treaty of the United Nations Framework Convention on Climate Change (UNFCCC), which states in Article 2: "[…] Ultimate objective [...] is to achieve [...] stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system". The agreement further recognizes that a deep cut of greenhouse gases emissions is required, to hold the global average temperatures rise within 2˚C of pre-industrial levels – the so called 2˚C guardrail (IPCC 2007).

Under the Kyoto Protocol, an international agreement linked to the UNFCCC, the 15 countries that were part of the European Union (EU-15) before 1994 committed to reduce their overall emission of GHG, over a period of five years (2008-2012), to 8% below the levels existing in 1990.

Clean development mechanisms, and use of renewable and non-polluting sources of energy are the most important mitigation measures to reduce carbon emissions. These approaches are being flanked by carbon sequestration. To reach the targets of reducing or limiting emissions of GHG, countries committed to the Kyoto Protocol have primarily national measures. Comparing the efforts of each country is very difficult on account of the incompatible ways to calculate the reductions of emissions, rendering the outcome of implementation strategies complicated to predict and interpret. Never-the-less the emissions monitoring and the projections show that EU-15 are on track to meet this target, as stated in the last update (10 September 2012) of the European Commission report (European and Commission 2012).

The “carbon market” is an additional means introduced by the Kyoto Protocol to stimulate sustainable development through technology transfer and investment, reducing emissions or removing carbon from the atmosphere in a cost-effective way. The “carbon market” is based on three market-based mechanisms: emission trading, clean development mechanism and joint implementation. In this way, the private sectors and developing countries are also encouraged to contribute to the emission reduction efforts

1.3 Renewable energies

Fossil energy resources are not endless. The reserves of oil and gas have been reported to be sufficient for decades and in the case of coal, centuries (IPCC 2007). In 2009 fossil fuels supplied 80.7% of world primary energy demand (The World Bank, 2012), and was responsible for about 85% of the anthropogenic CO_2 emissions produced annually (IEA International Environmental Agency, 2011). As a consequence, to reduce CO_2 emissions and to use energy sources alternative to fossil fuels is of primary importance.

In 2011, the nuclear energy provided 12.3% of the world electricity (The World Bank, 2012). Storm van Leeuwan (2007) reported that, assuming an average operating lifetime of 35 years, with a load factor of 85%, the lifetime of CO_2 emissions from nuclear energy per kilowatt hour (g CO_2/kWh) are between 84 and 122, values that lie between fossil fuel (~750 g CO_2/kWh) and renewable energies (11-62 g CO_2/kWh) emissions (Storm 2007). Moreover, the risks and the environmental impacts associated with the

use of nuclear energy are high; as testified in the recent environmental disaster in Fukushima Daiichi (Japan; March 2011).

Renewable technologies such as hydroelectricity, biomass combustion, and geothermal are the most mature technologies, and, together with water heating, solar photovoltaic, wind, bioethanol and combined heat and power are able to compete in today's energy markets without policy support (IPCC 2007). Currently, a quarter of total global power-generating capacity is covered by renewable technologies which supplies close to 20% of global electricity. Most of this is provided by hydropower.

The capacity of bioenergy (62 GW) at the end of 2010 follows far behind hydropower (1010 GW), wind (198 GW) and solar hot water/heating (185 GW).

The term 'bioenergy' is related to the energy obtained from biomass (Ruane, Sonnino et al. 2010). Biomass sources include biodegradable fraction of products, waste and residues from agriculture, forestry and municipal solid waste. Biomass is used as feedstock to produce several energy carriers in the form of solid fuels (e.g. chips, pellets, briquettes, logs), liquid fuels (methanol, ethanol, butanol, biodiesel), gaseous fuels (synthesis gas, biogas, hydrogen), electricity and heat (IPCC 2007).

Once the feedstock is processed to produce gaseous fuels, heat and power through burning process in a low concentration of oxygen (i.e. pyrolysis process), the by-product released is charcoal. Charcoal itself is a source of energy; it constitutes the primary urban fuel in most developing countries for domestic uses.

Moreover, charcoal (also called *biochar* if applied for environmental purposes) is considered a realistic option to mitigate climate change. Significantly, burning biomass through a pyrolysis process prevents not only the oxidation of micronutrients (e.g. phosphorus, magnesium, manganese), but also carbon volatilization as CO_2. In this way the natural decay of the biomass is avoided and the carbon cycle altered. As a consequence, to produce energy by a feedstock burning process causes an overall negative emission of CO_2. Significantly, due to the high stability of the biochar matrix, biochar represents a storage of carbon that can be recalcitrant for thousands of years (Lehmann, Czimczik et al. 2009) (see Chapter 1).

2. AGRICULTURE CHALLENGES

2.1 Soil organic matter and carbon loss

Soil organic matter (SOM), a complex mixture of carbon and other biologically relevant elements (such as nitrogen, sulphur and phosphorus) provides: carbon and energy sources for soil organisms; contributes to plant nutrition and growth; eases cultivation, and; improves soil structures in terms of aggregates and pores which in turn are important for drainage, soil aeration and containment of erosion (Meredith 1997). This, in turn, enhances water holding capacity at low suctions and earlier warming in spring (Meredith 1997).

SOM finds its origins in the deposition of biota (e.g. plants or animals) cells in soil and their contents and breakdown by microbial population. Animal excretion products, leaf fall and exudates from plant roots are also sources of SOM (Meredith 1997). Organic bound nitrogen, sulphur and phosphorus, carboxylic acid groups, phenolic hydroxyl groups, polysaccharides and gums are some of the chemical structures present in SOM (Meredith 1997). Naturally, the chemical composition of the SOM varies tremendously by ecosystem.

SOM is considered the major pool of C within the biosphere (about 1400 x 10^{15} g C globally), which Post et al (1982) estimated to be roughly twice that in atmospheric CO_2. Techniques such as long-term tillage, withdrawal of grass-leys into rotations and absence of animal or manuring converted native ecosystems into agricultural lands. Today agricultural lands occupy about 40-50% of the Earth's land surface (IPCC 2007). Long term cultivation invariably caused a net C loss from soil (Davidson and Ackerman 1993).

To overcome carbon loss due to agriculture, addition of organic matter to soil (e.g. manures, composts, sewage sludge) has become a common practice in farming systems. Unfortunately the half-lives of these soil carbon amendments are relatively short. For example, Butler and Hooper (2010) reported compost half-lives in soil of up to 10-14 weeks (Butler and Hooper 2010); while the half-lives for the C remaining from sewage sludges ranges from 39 to 330 days (Ajwa and Tabatabai 1994). Due to their low stability in the environment, these organic matter amendment require continued repeated application (this of course being time consuming and at an economic cost).

Studies on Amazon soils have revealed that charcoal was used from ancient civilizations as soil amendment for agricultural purposes (Marris 2006). Although several centuries have past, charcoal is still present in those areas maintaining green and flourishing fields. Further analysis has shown that biochar enhances crop yields and soil properties due to its chemical and physical characteristics (see Chapter 1). Due to such a significant impact to the vegetation, biochar has become of great scientific interest and soil amendment with biochar is evaluated as means to improve soil fertility and crop yields.

Biochar is reported in literature to enhance soil structure, to improve water retention, to increase contents of carbon and nutrients in soil (see Chapter 1). Moreover several studies showed that biochar presents a high sorption capacity for organic compounds, becoming a useful tool in the remediation of polluted land (Yu, Ying et al. 2006; Spokas, Koskinen et al. 2009; Wang, Lin et al. 2009). This aspect, with respect to soil contaminated with polycyclic aromatic hydrocarbons (PAHs) is considered in greater detail in Chapter 3.

References:

Ajwa, H.A. and M.A. Tabatabai, *Decomposition of different organic materials in soils.* Biol Fertil Soils, 1994. 18: p. 175-182.

Brussard, B.T., *Biodiversity and ecosystem functioning in soil.* AMBIO, 1997. 26: p. 563-570.

Buhler, D.D., M. Liebman, and J. Obrycki, *Theoretical and practical challenges to an IPM approach to weed management.* Weed Science, 2000. 48: p. 274-280.

Butler, J. and P. Hooper, *Down to Earth: An illustration of life cycle inventory good practice with reference to the production of soil conditioning compost.* Resources, Conservation and Recycling, 2010. 55: p. 135-147.

Chikowo, R., Faloya, V., Petit S. and Munier-Jolain N.M. *Integrated weed management systems allow reduced reliance on herbicides and long-term weed control.* Agriculture, Ecosystems & Environment, 2009. 132: p. 237-242.

Davidson, E.A. and I.L. Ackerman, *Changes in soil carbon inventories following cultivation of previously untilled soils.* Biogeochemistry, 1993. 20: p. 161-164.

European and Commission, 2012, *http://ec.europa.eu/clima/policies/g-gas/index_en.htm*.

Glaser, B., Haumaier, L., Guggenberger G. and Wolfgang Z. *The Terra Preta phenomenon: a model for sustainable agriculture in the humid tropics.* Naturwissenschaften, 2001(88): p. 301-306.

Hansen, J., Sato, M., Kharecha, P., Beerling, D., Berner, R., Masson-Delmotte, V., Pagani, M., Raymo, M., Royer, D.L. and Zachos, J. C. *Target atmospheric CO_2: where should humanity aim?* Journal of the Atmospheric Sciences, 2008. 2: p. 217-231.

Hayes and W.S. Wilson, Editors. 1997, Humic Substances, Peats and Sludges: Health and Environmental Aspects. The Royal Society of Chemistry: Cambridge

IEA, *CO_2 emissions from fuel combustion Highlights (2011 Edition)*, International Environmental Agency, 2011. p. 9-10.

IPCC, *Intergovernmental Panel on Climate Change: Climate Change* S. Salomon, et al., Editors. 2007, Cambridge Univ. Press: New York.

Lehmann, J., Rillig, M. C., Janice, T., Masiello, C.A., Hockaday W.C. and Crowley, D. *Biochar effects on soil biota - A review.* Soil Biology & Biochemistry, 2011. 43: p. 1812-1836.

Lehmann, J., Czimczik, C., Laird, D. and Sohi, S. *Stability of biochar in the soil*, in *Biochar for environmental management - Science and Technology*, J. Lehmann and S. Joseph, Editors. 2009, Erthscan publisher: London.

Macías, F. and M.C. Arbestain, *Soil carbon sequestration in a changing global environment.* Mitigation and Adaptation Strategies for Global Change, 2010.

Marris, E., *Black is the new green.* Nature, 2006. 442: p. 624-626.

Meredith, J.A., *Soil Organic Matter: Does Physical or Chemical Stabilazation Predominate?*, in *Humic Substances in Soils, Peats and Waters - Health and Environmental Aspects*, M.H.B.

Oreskes, N., *The scientific consensus on climate change.* Science, 2004. 306(5702): p. 1686-1686.

Post, W.M., Emanuel, W.R., Zinke, P. J., Stangenberger, A.G. *Soil carbon pools and world life zones.* Nature, 1982. 289: p. 156-159.

Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C. *Impact of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil.* Chemosphere, 2009. 77: p. 574-581.

Ramanathan, V., *The greenhouse theory of climate change: a test by an inadvertent global experiment.* Science, 1988. 240: p. 293-299.

Richardson, K., W. Steffen, and D. Liverman, *Climate change: global risks, challenges and decisions*, 2011, Cambridge, UK: Cambridge University Press.

Ruane, J., A. Sonnino, and A. Agostini, *Bioenergy and the potential contribution of agricultural biotechnologies in developing countries.* Biomass and Bioenery, 2010. 34: p. 1427-1439.

Storm, v.L., *Nuclear power and global warming*, in *Secure Energy? Civil Nuclear Power, Security and Global Warming*, F. Barnaby and J. Kemp, Editors. 2007, Oxford Research Group: Oxford.

The World Bank, 2012.

http://data.worldbank.org/indicator/EG.USE.COMM.FO.ZS/countries?display=graph.

Wang, H., Lin, K., Hou, Z., Richardson, B., Gan, J. *Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars.* Journal of Soils Sediments, 2009. 10: p. 283-289.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J. and Joseph, S. *Sustainable biochar to mitigate global climate change.* Nature Communications, 2010. 1: p. 1-9.

Yu, X.-Y., G.-G. Ying, and R.S. Kookana, *Sorption and desorption behaviors of diuron in soils amended with charcoal.* Agricultural and Food Chemistry, 2006. 54: p. 8545-8550.

AIMS AND OBJECTIVES

Studies on biochar are relatively recent (Glaser et al. 2001), leaving several aspects unexplored or not fully developed, particularly the potential side effects that biochar could have in the environment. Further research on the impact of biochar in the environment in both the long and the short term is required both to avoid unforeseen consequences and to provide evidences of further potential benefits. It is the aim of the research presented herein to provide answers to some of these questions and to deepen our understanding of soil-biochar interactions.

Towards these ends Chapter 1 aims to account the state of the art, including specifically:

- General definition of biochar
- Physical and chemical description of biochar matrix
- Opportunities represented in biochar to reduce atmospheric CO_2 levels
- The benefits of biochar to soil
- Biochar potential toxicity
- Interaction of biochar with organic compounds (specifically herbicides)

Thereafter, the research reported herein has been designed to present an investigation of various aspects relating to the application of biochar in the environment, assessing:

(i) the negative impacts of biochar in soil in the context of contaminations and agricultural practices, and;
(ii) expanding the present knowledge on the benefits of biochar in food security and microbial carrier properties.

The possible negative impact of biochar once added to soil requires particular attention. Here, the potential for adverse outcomes are considered from two perspectives:

(i) the opportunity to increase levels of toxins, and;
(ii) the implications of herbicide interaction with biochar.

Regarding toxins, biochar is the by-product of a burning process. As such, the formation of polycyclic aromatic hydrocarbons (PAHs) in biochar must be considered and evaluated in order to avoid land contamination and the possibility of PAHs transfer to crops. Depending upon the temperatures applied to the feedstock during the pyrolysis

process there could be different concentration of PAHs in the biochar produced. In addition to PAHs, potential toxic elements (PTE), specifically metal and metalloids might also be present in biochar. PTEs will vary in feedstock and, as a consequence, in biochar produced. There exists the possibility that PAHs and/or PTEs associated with biochar might contaminate the soil and could be bioavailable.

It was the aim of Chapter 2 to:

- Assess levels of PAHs and PTEs in several biochars made of different feedstock and pyrolysis temperatures;
- Contextualise these concentrations with respect to levels in background soil, compost and sewage sludge;
- Comment upon the hazard biochar might represent with respect to these toxicity drivers, following European Union regulations.

Another aspect to be considered was the effects of biochar on agricultural lands and its compatibility with the approaches commonly used in agriculture. The present agricultural system, which started to develop after World War II, is based on the use of herbicides. The use of herbicides is effective to control weed-growth in major crops, to greatly reduce yield losses and to avoid persistent weed infestation (Chikowo, Faloya et al. 2009). Over time the use of herbicides systematically facilitated crops cultivation, promoted the expansion of monocultures and the adoption of reduced tillage systems (Buhler, Liebman et al. 2000). From this prospective the application of biochar to soil to improve soil texture and to enhance crop yields (see Chapter 1), could on one hand be beneficial, while on the other hand, this benefit might be undermined if biochar sorptive capacity for organic compounds reduces herbicide availability and in turn their efficacy. The interaction between biochar and herbicides and herbicide availability after addition of biochar is reported herein.

Specifically Chapter 4 aimed to investigate:

- Herbicide (Isoproturon (IPU)) partitioning in biochar
- Microbial availability of IPU once sequestered in biochar

Extending this research Chapter 5 considers the effects of herbicides on targeted weeds in biochar amended soil. The aims of Chapter 5 were:

- To assess the influence of biochar, at different applications rate (1% and 5%), on the efficacy of three pre-emergence herbicides (mesotrione, pendimenthalin and terbuthylazine) with respect to survival and growth of common broadleaf weeds (*Amaranthus retroflexus* and *Solanum nigrum*).

While biochar capacity to sorb organic compounds could result in adverse outcome in the case of herbicides and their effects on weeds survival, these interactions could be beneficial where soils are contaminated with organic compounds, specifically, in terms of land remediation.

In connection to this, Chapter 3 describes the benefits of biochar (specifically, sewage sludge biochar (SSBC)) applied in PAHs contaminated soils. This chapter compares the outcomes of Sewage sludge (SS) and SSBC amendment to PAH contaminated soil in terms of crop yield and abatement of pollutant soil to plant transfer.

Specifically, the aims of Chapter 3 were:

- To compare the influence of SS and SSBC upon biomass yield into lettuce plants.
- To investigate and compare the bioaccumulation of PAHs following SS and SSBC addition to soil.

Other benefits of biochar in soil could be related to its positive impacts to the soil microbial ecosystem. The literature provides evidence of the opportunity for biochar to influence soil biota, modifying soil biological community composition and abundance (Lehmann, Rilling et al. 2011). The health and diversity of microflora are essential to soil function and to the ecosystem, as it assures soil stability, nutrient cycling, water use efficiency, disease resistance and aeration (Brussard 1997). Currently the relationships between biochar properties, soil biota, and their influence in soil processes have not been systematically described. Therefore, further studies on this subject need to be undertaken.

Chapter 6 explores some of these aspects, particularly in relation to the possibility of using biochar as a microbial inoculant carrier. The addition of certain microorganisms (e.g. *Azotobacter, Bacillus, Clostridium, Frankia, Pseudomonas*, etc) to soil is a

common practice in agriculture and has several applications: promoting plant growth, inhibition of plant pathogens, biodegradation of toxic compound, soil structure improvement and microbial leaching of metals (van Veen et al. 1997; Van Dyke and Prosser 2000).

The specific aims of Chapter 6 were to investigate:

- The potential of dissimilar biochars (maize and redwood feedstock) produced with similar pyrolysis temperature (600˚C) as a carrier alternative to peat for three rhizobia strains (*Rhizobium leguminosarum* bv. *viciae; Rhizobium etli; Rhizobium leguminosarum* bv. *trifolii*) at 4 ˚C, 25 ˚C and 35 ˚C
- Chemical and physical properties of biochars were compared to peat to assess the better suitability as carriers.

References

Van Dyke, M.I. and J.I. Prosser, *Enhanced survival of Pseudomonas fluorescens in soil following establishment of inoculum in a sterile soil carrier.* Soil Biology & Biochemistry, 2000. 32: p. 1377-1382.

Van Veen, J.A., L.S. van Overbeek, and J.D. van Elsas, *Fate and activity of microorganisms introduced into soil.* Microbiology and Molecular Biology Reviews, 1997. 61: p. 121-135.

Structure of the thesis – Alessia Freddo

'Biochar: for better or for worse?'

This thesis is comprised of the following chapters. These have been presented as standalone sections. Some chapters are already published as journal articles, others are under review and some are about to be submitted. Further information in this regard is tabluted below along with a statement of authorship contribution with respect to: the candidate's involvement in experimentation, data processing and manuscript production.

Chapter	Chapter/Paper title and status	Contribution to data and processing	Contribution to writing
1	Application of biochar to soil – A review **In preparation for**: Sustainable science and technology. A. Des Las Heras (Ed.)	Literature review was undertaken primarily by Alessia Freddo, with some material contributed by Brian J Reid.	First author. Lead the preparation of this manuscript with review being provided by co-author Dr. Brian J Reid (Supervisor)
2	Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar **Published in**: Environmental Pollution (2012) 171:18-24	All experimental work and data processing were solely undertaken by Alessia Freddo.	First author. Lead the preparation of this manuscript with review being provided by co-authors Dr. Chao Cai and Dr. Brian J Reid (Supervisor).
3	Reduced bioaccumulation of PAHs by *Lactuca satuva* L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar. **Published in:** Environmental Pollution (2013) 175:64-68	PAH analytical work and data processing were undertaken by Alessia Freddo in collaboration with Dr. Sardar Khan and Ning Wang who undertook the plant component.	The authors (Dr. Sardar Khan, Dr. Brian J Reid, Alessia Freddo and Dr. Chao Cai) contributed equally to the preparation of this manuscript .
4	Influence of biochar on isoproturon partitioning and bioavailability **Under review in**: Environmental Science & Technology	Experimental work was undertaken by Alessia Freddo and Louisa F. Pickering (u/g student). The data processing, as presented, was undertaken by Alessia Freddo. The modeling work was undertaken by Dr. Mick Whelan and Dr. Frederic Coulon (Cranfield).	The preparation of this manuscript was undertaken by Dr. Brian J Reid and Alessia Freddo with modeling work and further review being provided by co-authors Dr. Mick Whelan and Dr. Frederic Coulon.
5	Deactivation of herbicidal activity in biochar amended soil. **Under review in**: Plant and Soil Journal	All experimental work and data processing were solely undertaken and written by Alessia Freddo	First author. Lead the preparation of this manuscript with review being provided by co-author Dr. Brian J Reid(Supervisor)
6	Biochar: a carrier alternative to peat for rhizobia inoculants	All experimental work and data processing were solely undertaken and written by Alessia Freddo	First author. Lead the preparation of this manuscript with review being provided by co-author Dr.Brian J Reid (Supervisor)

Chapter 1

Application of biochar to soil – A review

Application of biochar to soil – A review

Alessia Freddo and Brian J. Reid.
School of Environmental Sciences UEA, Norwich, NR4 7TJ, UK

1. Introduction

Biochar is a product of a biomass burning process in an oxygen limited environment (pyrolysis). This process also produces syngas and bio-oil that can be used in heat and power generation. The yields of each component (syngas and bio-oil and biochar) are dependent upon the temperature of pyrolysis, the residence time of the process and the type of feedstock used.

Biochar holds the potential to reduce atmospheric CO_2 concentrations by sequestering carbon from the atmosphere, into biomass, and 'locking-up' this carbon when this biomass is converted into biochar (Figure 1). Biochar is recalcitrant and physically stable; to the extent that, once applied to soil, it becomes a persistent component within the soil matrix.

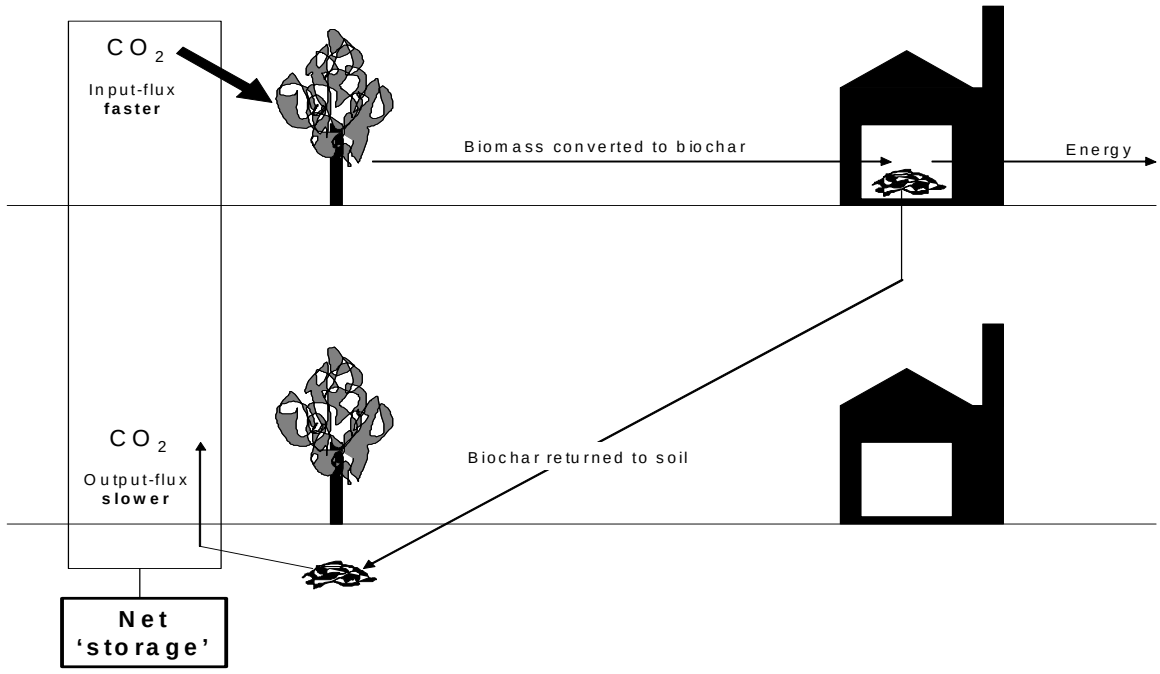


Figure 1: Net carbon gains in a biomass to biochar cycle.

There is mounting evidence that biochar influences a wide range of soil properties in ways which predominantly have the potential to increase agricultural productivity. The nature and extent of such influences varies widely and depend upon: soil type, agro-ecological factors, and the type and quantity of biochar used. The variables affected collectively have a direct bearing on physical, chemical and biological soil characteristics. Yet unlike most other soil amendments, such as fertiliser, manure, compost or lime, the effects of biochar are not yet well understood, either in terms of the precise mechanisms involved or their longevity.

Embracing all of these aspects, the European Commission (Verhaijen et al., 2010) recently defined biochar as:

"charcoal (biomass that has been pyrolyzed in a zero or low oxygen environment) for which, owing to its inherent properties, scientific consensus exists that application to soil at a specific site is expected to sustainably sequester carbon and concurrently improve soil functions (under current and future management), while avoiding short- and long-term detrimental effects to the wider environment as well as human and animal health."

2. Biochar as a climate change mitigation tool

The total carbon (C) present in the planet is, to all intents and purposes, constant (Houghton, 2007). However, the amounts of carbon present in the various environmental compartments, such as the atmosphere, biosphere, pedosphere, hydrosphere and lithosphere can and do change (Maías and Arbestain, 2010). Natural cycles and anthropogenic activities are the main drivers of change. When compared to the amount of C in other compartment, the total amount of carbon present in atmosphere is relatively small (805 Pg C; (Maías and Arbestain, 2010)). In contrast, fossil fuel (5000Pg C; (Archer, Eby et al. 2009)) and soil C reservoirs (3200 Pg C; (Macías and Arbestain 2010)) are much larger. As a consequence the burning of fossil fuels and to a lesser extent changes in land use and soil cultivation practices have resulted in an atmospheric CO_2 increase of 37.5% since the preindustrial era (CO_2 levels have risen from about 280 to 385 ppmv) (IPCC, 2007).

Several studies have shown the necessity to keep the cumulative anthropogenic greenhouse gases emissions below a maximum upper limit (Broecker, 2007; Matthews and Caldeira, 2008; Solomon et al., 2009). Hansen et al (2008) proposed a maximum concentration threshold of atmospheric CO_2 of 350ppm, versus the present 385ppm. Thus, if dangerous changes in the climate are to be avoided, future anthropogenic emissions must approach zero (Hansen et al., 2008). Consequently, global action is necessary to reduce atmospheric CO_2 concentration. Adoption of 'sustainable' or 'low-carbon' or 'carbon-neutral' or indeed 'carbon-negative' approached to global energy provision are key to a strategy to curb CO_2 emission to the atmosphere.

The use of biomass as feedstocks from which to produce energy is not a new concept. However, originality exists where these resources are used to provide energy and *at the same time* the opportunity to sequester carbon from the atmosphere. The pyrolysis of biomass serves to provide energy (via bio-oil and syngas that are subsequently used to run steam turbines) and the purposefully produced material 'biochar'. The conceptual foundations of biochar as an atmospheric CO_2 removal mechanism lie in the photosynthetic processes that produce the biomass for biochar production (Figure 1). As biomass grows, it removes atmospheric CO_2. The production of biochar converts comparatively labile carbon present in the biomass into recalcitrant carbon that resists mineralisation. In this way the rate of return of carbon to the atmosphere is greatly inhibited. It is the difference between the (relatively fast) rate of atmospheric CO_2 sequestration into biomass compared to the subsequent (relatively very slow) rate at which biochar carbon is mineralised that gives rise to net storage of carbon; and by this token the opportunity to produce heat and power by carbon *negative* means.

Several studies have attempted to predict the extent to which biochar can reduce atmospheric CO_2 levels. For example Lehmann et al. (2006) estimate that biochar may be able to sequester 5.5-9.5 Gt C per year, or about 20-35 Gt CO_2 per year by 2100. Lenton and Vaughan (2009) suggest that the capture of CO_2 by plants destined to provide bio-energy and subsequent carbon capture and storage, combine with afforestation and biochar production, may have the potential to remove 100 ppm of CO_2 from the atmosphere. Woolf et al (2010) suggested that biochar can potentially offset a maximum of 12% of current anthropogenic CO_2-C equivalent emissions to the

atmosphere (i.e. 1.8 Pg emissions can be avoided out of the 15.4 Pg of CO_2-C equivalent emitted annually), decreasing significantly the emissions of carbon dioxide by preventing decay of biomass inputs. Moreover, it has been suggested that biochar presence in soil might initiate a positive feedback wherein soil physical and chemical properties are improved and plants yields increased as a result; this feedback further enhancing the amount of CO_2 removed from the atmosphere (Woolf et al., 2010). Additional positive feedbacks might also be realised where biochar suppresses the emissions of other GHGs, such as nitrous oxide and methane (both significant agricultural pollutants and far more harmful in their radiative forcing impact than CO_2). It is stressed, in these regards, that further research is required to substantiate the circumstances under which such positive feedbacks are imitated and sustained.

3. Properties of biochar

3.1 Biochar physical properties

The matrix of biochar has been determined by X-ray diffraction (Lehmann and Joseph, 2009). This work revealed an essential amorphous structure with crystalline areas (Lehmann and Joseph, 2009) consisting of random polycyclic aromatic (graphene) layers rimmed by functional groups (Zhu et al., 2005) and mineral compounds (Lehmann and Joseph, 2009). Associated with the pyrolysis process above 330°C is the formation of polyaromatic sheets which create turbostratic structures (Keiluweit et al., 2010) and increased porosity as temperatures increase. Studies have demonstrated that higher temperatures lead to a decrease in particle size (Downie et al., 2009) and the development of microporosity (< 2nm), which underpin the high surface area of biochar (Downie et al., 2009). Physical properties, of course, vary depending upon the biomass feedstock used and the thermochemical conditions of char formation.

3.2 Biochar chemical properties

Owing to different production conditions and indeed variety in feedstock materials used to produce biochar chemical attributes vary considerably. At an elemental level biochar properties can be ascribed with respect to ratios of C, H, O and N. Particularly, ratios of H/C and O/C are used to determine the degree of biochar aromaticity i.e. the lower is the ratio, the greater is the aromaticity (Kookana et al. 2011). H/C and O/C ratios have

been reported to be higher in biochars produced at low-temperatures, due to incomplete charring of the feedstock; H/C and O/C ratios decrease with increasing temperatures of production (Baldock and Smernik, 2002). Thus, higher temperature chars are inherently more resistant to chemical modifications and therefore are more recalcitrant.

The nutrient content in biochar also varies depending upon feedstock type and pyrolysis conditions used. Higher temperatures and faster heating rates strongly influence the retention nutrients within the biochar formed: nitrogen (N) and sulphur (S) compounds, for example, volatilize at 200˚C and 375˚C respectively; while biochar becomes depleted in potassium (K) when produced above 700˚C and of phosphorous (P) above 800˚C (DeLuca et al., 2009). Minerals such as magnesium (Mg), calcium (Ca) and manganese (Mn) volatilize at temperature above 1000˚C (Neary et al., 1999; DeLuca et al., 2009); pH, electrical conductivity (EC) and extractable NO_3^- tend to be higher with high-temperatures (800˚C), while low temperature (350˚C) result in greater extractable amounts of P, NH_4^+ and phenols. Feedstock type is responsible for different ratio of C/P and C/N; in particular, wood- and nut-based biochars show high ratio of C/P and C/N ratios, while manure- crop- and food-waste biochars have lower ratios (Kookana et al., 2011).

3.4 Influence of physical and chemical properties on biochar stability

The complex structure of biochar affords its great stability in the environment (Schmidt and Noack, 2000): the peculiar cross linking and the steric protection of the refractory macromolecules present in biochar prevent the hyrolytic enzymes to act towards the matrix itself (Derenne and Largeau, 2001; Lehmann et al, 2009a). Nevertheless, some studies show the decay of biochar due to metabolic processes, particularly heterotrophic decomposition (Shneour, 1966; Baldock and Smernik, 2002). Moreover different biochar products result to have different decomposing extents, presenting different physical and chemical structures depending by the feedstock and pyrolysis temperatures used (Lehmann et al, 2009). Biochar found in the Amazon region has suggested millennium scale persistence with radiocarbon dating indicating amazonian dark earths char to be of 500 to 7000 years old (Neves et al., 2003) . Liang et al (2008) found no changes in the aromaticity determined by X-ray techniques from biochar particles coming from the same area. These results providing further evidence of biochar's potential for long-term carbon storage.

4. How can biochar benefit soil?

Although the composition of biochars depends upon the nature of the feedstocks and the operating conditions of pyrolysis, biochars are generally expected to be rich in nutrients. These characteristics can have a direct effect on the plant growth. For example, the addition of 68t C ha^{-1} increased rice biomass by 17 per cent while the presence of 135t C ha^{-1} of biochar enhanced the growth by 43 per cent (Glaser et al., 2002; Lehmann et al., 2003). Improved crop yields have been attributed to improvement in P, K and possibly Cu levels following the addition of biochar (Chan and Xu, 2009).

Biochar has the potential to increase cation exchange, soil water-holding and surface sorption capacity on account of its physical and chemical characteristics of biochar; specifically: its high surface-area, high porosity and variable-charge (Amonette and Joseph, 2009; Yang et al., 2010). Therefore the application of biochar is expected to enhance soil properties in terms of increasing or maintaining the pH of the soils (Rondon et al., 2007), toxin neutralization (Wardle et al., 1998), and reduce soil strength (Chan et al., 2007). Again these properties vary depending upon the properties of the biochar and also on account of the original characteristics of the soil and the plant species of interest. In support of these benefits, Van Zwieten et al (2007) reported a nearly 30-40 per cent increase in wheat height when biochar produced from paper mill sludge was applied at a rate of 10 t ha^{-1} to an acidic soil . Hoshi (2001) suggested that the biomass increase of tea trees (20 per cent in height and 40 per cent in volume) were partly due to the ability of biochar to keep pH constant in soil. Chan et al (2007) found that the dry matter of radish in a pot increased by up to 266 per cent when N fertilizer was applied at 100kg ha^{-1} compared to a control with the same treatment but in absence of biochar.

Another important area where biochar might contribute is to levels of soil carbon. Significantly, modern agricultural practices have resulted in degradation of soil carbon and as a consequence levels of carbon are much lower now than they were several decades ago (Jones et al., 2011). Biochar has recently come to the fore as an additional soil amendment source of carbon. Of greatest significance is the fact that biochar is inherently stable and as a consequence, offers the opportunity to replenish soil carbon reservoirs in a long-lasting way. Measurements of biochar over time were taken; Preston and Schmidt (2006) determined an average of half-life of biochar in coastal temperate rainforest of western Vancouver of 6623 years, while Hammes et al (2008)

calculated a turnover time of biochar from fires in a Russian steppe of only 293 years. There exists uncertainty on the residence of time of biochar as the calculation could be affected by spatial variabilities (Lehmann et al. 2009a) and the decomposition or mineralization of biochar can be affected by several physical conditions. Nevertheless, although biochar is subjected to decomposition processes, its stability remains high over long periods of time.

5. Biochar and soil biota

The peculiar physical and chemical characteristics of biochar have been shown to influence and change soil microsystems (O'Neil et al., 2009; Lehmann et al., 2011). To date the relationships between the biochar phyico-chemical properties and their effects on soil biota and the consequent effects on soil processes are poorly understood (Lehmann et al., 2011). The diversity of soil microbial populations are critical to soil function and ecosystem services, and in turn, it has implications in soil structure and stability, C storage capacity, water use efficiency, nutrient cycling, aeration and pathogens resistance (Lehmann et al., 2011). Therefore, the research related to the application of biochar as a strategy for managing soil biota is a topic of growing interests. Studies on the soil biota present in Terra preta soils demonstrated how the addition of biochar affected the soil biological composition (O'Neil et al., 2009; Kim et al., 2007; Grossman et al., 2010) and increase soil microbial biomass (Liang et al., 2010; O'Neil et al., 2009). However, whether the abundance of microorganisms increases or not it is directly connected to the intrinsic properties of both biochar and soil, and; it may differ for different groups of microorganisms (Warnock et al., 2007; Lehmann et al., 2011). For instance, Makoto et al. (2010) showed that the infection of ectomycorrhizal fungi of larch seedling roots increased by 19-157% after addition of biochar into soil. In turn, decreases in arbuscular mycorrhizal fungi abundance have been observed after biochar addition to soil (Warnock et al., 2010).

In the literature, the reasons which explain the increase of microbial abundance in presence of biochar are several. For instance, it is reported that in biochar amended soil the nutrient availability (C and micronutrients) increases, either due to biochar-driven improvements in nutrient retention or due to nutrients that are released from biochar (Lehmann et al., 2011). Depending upon the magnitude of nutrient change and the microorganism group, such nutrient availability may be responsible of a microbial

biomass increase (Lehamann et al.; 2011). Moreover, after biochar additions, the pH of soils may increase or decrease, in response to the pH values of biochar, which values can be below 4 or above 12 depending upon the feedstock and the pyrolysis temperatures used to produce the biochar (Chan and Xu, 2009). Thus the living conditions for microorganisms may significantly vary following biochar addition with this, in turn, influencing the total microbial abundance.

Moreover, physical characteristics of biochar may also affect microbial abundance. Cassidy et al. (1996) showed possible attachment of viable microbial cells to surfaces (i.e. flocculation, adsorption to surfaces, covalent bonding to carrier, cross-linking of cells, encapsulation in polymer-gel, entrapment in matrix). The adsorption to biochar may occur via two main processes: hydrophobic attraction or electrostatic forces (Samonin and Elikova, 2004), and; adhesion into pores (Rivera-Utrilla et al., 2001; Samonin and Elikova, 2004). The capacity of bacteria to sorb to biochar surfaces may renders them less leachable in soil (Pietikäinen et al., 2010), and therefore, increase bacterial abundance.

In addition, due to the large surface area and greater water holding capacity (Liang et al., 2006; Downie et al., 2009; Glaser et al., 2002), biochar may retain moist pore spaces that may be available to microorganisms, ensuring higher microbial survival in a drying soil, preventing them stress and thereby, reducing dormancy or mortality.

Given the positive effects of biochar on microorganisms abundance, further investigations in relation to the connections between biochar properties and soil biota, and their implications in soil processes need to be systematically described, in order to develop new strategies to improve soil systems and, at the same time, to avoid inadvertent changes of soil biota.

6. Unintended consequences

5.1 Toxicity

Biochar has been established as a source of considerable benefits with respect of its use as a soil improver (see above and Collison et al., 2009). However, some researchers have reported impaired crop yields where biochar has been implied and it is important to acknowledge this alongside the more numerous reports of benefit. Kishimoto and Sugiura (1985) reported yield reductions of soybean by 37 and 71 per cent when biochar was applied at 5 t ha^{-1} and 15 t ha^{-1}, respectively; they attributed this reduced

yield to micronutrient deficiency under the higher pH conditions following biochar application.

In addition to adverse impacts upon micronutrients, biochar was found to have detrimental impacts upon soils on account of potentially toxic elements contained within its structure. There are two components in particular that are worthy of mention, these being: the presence of Potentially Toxic Elements (PTEs) and organic compounds produced during the pyrolysis process (arguably of greatest significance are polycyclic aromatic hydrocarbons (PAHs)). Both PTEs and PAHs have the potential to interfere with soil quality and may be subject to uptake into/onto products destined for the food chain.

PTEs: Many metal and metalloids are priority substances on account of the adverse effects they have on humans and ecosystems (CEC, 2008). Koppolu et al. (2003) reported the fate of several metals during the pyrolysis of biomass and the results have shown that greater than 98.5% of the metal in the product stream is concentrated in the char formed. The metal concentration was increased 4 to 6 times in the char compared to the feedstock, where the heavy metals were contributing between 0.7 and 15.3% to char mass. In many respects, the positive benefits of biochar parallel those implicit to the disposal of sewage sludge to land. Sewage Sludge Directive 86/278/EEC requires member states to "regulate sewage sludge in agriculture in such a way as to prevent toxic effects on soil, vegetation, animals and man, thereby encouraging the correct use of such sewage sludge". While guidance on sewage sludge application has been implemented (CEC, 1986) no guidance currently exists for biochar application to soil.

Hwang et al (2007) reported metal and metalloid concentrations (mg/kg) in wood-char: zinc 200-12500, copper 70-16000, lead 25-2300 and cadmium 0.05-15. Nevertheless it is important to note that the concentrations of heavy metals are highly dependent on the specific feedstock used during the pyrolysis or depending on sludge type, treatment and wastewater sources (Stevens et al., 2003).

PAHs: Pyrolysis affords the opportunity for the formation of the PAHs, in fact PAH yield has been reported to depend upon high temperature pyrolysis and incomplete combustion reactions (Badger et al., 1960; McGrath et al., 2001). PAH compounds are extremely harmful in humans and animals. These compounds can form adducts with DNA and have been prioritised by USEPA and EU on account of their carcinogenetic, mutagenetic and teratogenic properties (Wassenberg and Giulio, 2004; White and Claxton, 2004). A Working Document on Sludge produced by the European Union

(2000) provides “limit values for concentrations of organic compounds in sludge for use on land” (EU, 2000). The EU proposes a concentration limit of the “sum of the PAHs” equal to 6mg/kg dry matter. The PAHs considered are acenaphthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo-[b+j+k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene. The concentrations of PAHs detected in several studies vary depending by the type sources of sewage sludge. As matter of fact Oleszczuk (2010) reported the PAH sum concentrations in two different sewage sludge equal to 2.72 ± 0.13 mg/kg and 3.54 ± 0.13 mg/kg, while Stevens et al (2003) reported the mean of the sum of PAHs concentrations in sewage sludges equal to 43.47 mg/kg.

Brown et al (2006) analyzed the concentration of PAH in a range of synthetic biochars produced from pine wood pyrolyzed with ramp rates between 30 and 1000 ˚C/h and temperatures between 450 and 1000˚C and it was found that all wood chars contained measurable quantities of PAHs and the concentrations were inversely proportional to the temperatures: 16mg/kg in the 450˚C char material, 7mg/kg in the 525˚C char, and only 3mg./kg for the 1000˚C char).

5.2 Pesticide sequestration

Numerous studies have considered the influence of ‘black carbon’ (of which biochar is a type) upon the partitioning of organic compounds. To date only a handful of studies have specifically considered the potential of biochar to sequester pesticides. However, where research has been undertaken results have been compelling. More generally, numerous studies have shown the capacity of black carbon to sorb organic compounds such as pesticides (Spokas et al., 2009; Wang et al., 2009; Yang et al., 2010; Yu et al., 2009) and drastically reduce their availability in the environment. Research in this field has spanned decades and has provided an in depth understanding of the mechanisms through which organic compounds interact with black carbon matrices (Sander and Pignatello, 2007). The literature accounts research across a broad spectrum of organic compounds. Some of this research relates specifically to pesticides but for the most part research to date has focused upon organic contaminants. Nonetheless, the mechanisms at work with respect to organic contaminants are identical to those that influence pesticide interactions with black carbon matrices. Below we provide a summary of the evidence that supports the fact that organic compounds have a high affinity for black carbon. Some of this evidence relates to biochar specifically while some relates, more

generally, to black carbon. Similarly, the research accounted below considers organic compounds in general and in some instances pesticides specifically.

Studies on sorption and desorption of organic compounds have reported nonlinear isotherms where desorption rates are lower than sorption rates i.e. sorption and desorption display a hysteresis. This hysteresis holds important implications for the transport and bioavailability of sorbents (Braida et al., 2003; Sander and Pignatello, 2005) as it underpins the uptake and subsequent release of sorbents. Lu et al. (2002) and Sander et al. (2006) accounted for hysteresis using a "pore deformation" mechanism. In contrast to surface portion pore deformation entraps sorbent molecules within the physical structure for the matrix. Pore deformation is initiated by the incoming sorbent molecules exerting a pressure on pores smaller to the sorbent molecule causing their expansion (i.e. swelling) thereby allowing the sorbent to enter the pore. Once in the pore the pore contracts around the sorbent molecule physically inhibiting its exchange. In addition, Braida et al (2003) proposed that in response to the penetration of benzene molecules, the polyaromatic walls of black carbon rearrange, thereby opening up new pathways for the sorbent to penetrate. Thus, sectors previously open, close, and in doing so trapping the sorbent molecules inside the matrix. These mechanisms result in irreversible sorption (Sander and Pignatello, 2005). Zhang et al (2009) reported the sorption of phenanthrene on to biochar to exhibit nonlinear sorption that was stronger than that observed for soil and sediments; concluding that biochar presence in soil and sediments would be expected to reduce the bioavailability and influence the ultimate fate of hydrophobic organic compounds in subsurface environments.

At a molecular level, functional groups, mainly O-containing groups present on biochar surfaces can act as both electron donors (i.e. due to the presence of α or π electrons) and as electron acceptors (i.e. due to the unoccupied molecular orbital). This functionality allows both specific and non-specific physiosorption interaction with sorbent molecules (Zhu et al., 2005). Zhu et al (2005) and Sander et al (2005) demonstrated π- π electron donor-acceptor (EDA) interactions between nitroaromatic compounds and the graphene basal plane on a char and on graphite as a model sorbents. In addition to EDA, pH-dependent Coulombic interactions with charged molecules can also occur. Interestingly, Tian et al (2010) reported the interaction of the herbicide isoproturon with biochar to show low hysteresis; concluding that these interactions were predominantly sorption driven rather than pore deformation interactions.

Spokas et al. (2009) showed how the sorption of two common herbicide, atrazine and acetocholor, increased in soil after the addition of biochar Specifically the sorption coefficient Koc values of both the herbicides were greater in unamended soil (atrazine=75$\mu g^{1-1/n}mL^{1/n}g^{-1}$, acetochlor=136$\mu g^{1-1/n}mL^{1/n}g^{-1}$) than in amended soils (atrazine=51$\mu g^{1-1/n}mL^{1/n}g^{-1}$, acetochlor=107$\mu g^{1-1/n}mL^{1/n}g^{-1}$). Yu et al. (2009) considered the influence of biochar produced from *Eucalyptus* spp. at two temperatures (450 and 850˚C). When amended into an Australian red-brown earth (a Xeralf) both of these biochars had a marked effect upon the dissipation, partitioning and phytoavailability of the insecticides carbofuran (aqueous solubility 320 mg L^{-1} (at 20˚C), and a log K_{OW} of 1.52) and chlorpyrifos (aqueous solubility 4 mg L^{-1} (at 25˚C) and log K_{OW} of 4.70). In these experiments leaching was prevented thereby limiting loss mechanisms to degradation and the formation of non-extractable residues. Yu et al (2009) reported that the soil amended with the higher temperature biochar was most effective in reducing the loss of pesticide. This was attributed to its higher surface area, nanoporosity and greater ability to sequester organic compounds (Lua et al., 2004). At the end of their experiment (35d of incubation) a total of 86% of applied chlorpyrifos and 88% of carbofuran residue were lost from the biochar free soil. In contrast, only 44% chlorpyrifos and 51% of carbofuran were lost from the soil amended with biochar (1%).

Regarding phytoavailability the pesticide residues in both above-ground parts as well as below-ground parts of Spring onion (*A. cepa*) for both pesticides were lower in the plants that were grown in soils amended with biochars (Yu et al. (2009)). After 35d of growth the concentration of carbofuran in the under-ground plant parts decreased from 14.4 ± 0.8 in control soil to only 1.8 ± 0.4 mg kg^{-1} in the soil amended with the higher temperature biochar (1%). Similarly, chlorpyrifos uptake into the under-ground plant parts was decreased from 14.1 ± 1.7 to 0.8 ± 0.1 mg kg^{-1} in the presence of the higher temperature biochar (1%).

7. Conclusions

Energy provision through biomass pyrolysis is an approach that may, on a decadal timescale, be relatively simple and cheap to implement at national, regional and global levels. Biochar therefore has the potential to deliver a fast-action climate mitigation strategy and simultaneously boost crop yields when applied to agricultural soils.

Should the application of biochar to soil emerge as significant strategy to atmospheric CO_2 abatement and increase of crop yields, unintended consequences, in particular, soil pollution and sequestration of pesticides, must be given due consideration.

References

Ajwa, H.A., Tabatabai, M.A., 1994. Decomposition of different organic materials in soils. Biol Fertil Soils 18, 175-182.

Amonette, J.E., Joseph, S., 2009. Characteristics of Biochar: microchemical properties, in: Johannes, L., Joseph, S. (Eds.), Biochar for environmental management - Science and Technology. Earthscan publisher, UK and USA.

Archer, D., M. Eby, V. Brovkin, A. Ridgwell, L. Cao, U. Mikolajewicz, K. Caldeira, K. Matsumoto, G. Munhoven, and A. Montenegro. 2009. Atmospheric lifetime of fossil fuel carbon dioxide. Annual Review of Earth and Planetary Sciences 37:117-134.

Badger, G.M., Kimber, R.W.L., Spotswood, T.M., 1960. Mode of formation of 3,4-benzopyrene in human environment. Nature August, 663-665.

Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered *Pinus resinosa*. Organic Geochemistry 33, 1093-1109.

Braida, W.J., Pignatello, J.J., Lu, Y., Ravikovitch, P.I., Neimark, A.V., Xing, B., 2003. Sorption hysteresis of benzene in charcoal particles. Environmental Science and Technology 37, 409-417.

Brown, R.A., Kercher, A.K., Nguyen, T.H., Nagle, D.C., Ball, W.P., 2006. Production and characterization of synthetic wood chars for use as surrogates for natural sorbents. Organic Geochemistry 37, 321-333.

Broecker, W.S., 2007. Climate Change:CO_2 arithmetic. Science 315, 1371.

Butler, J., Hooper, P., 2010. Down to Earth: An illustration of life cycle inventory good practice with reference to the production of soil conditioning compost. Resources, Conservation and Recycling 55, 135-147.

Cassidy, M., Lee, H., Trevors, J., 1996. Environmental applications of immobilized microbial cells: a review. Journal of Industrial Microbiology & Biotechnology 16, 79-101.

CEC, 1986. Commission of the European Communities. Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Official Journal of the European Communities L 181 (86/278/EEC).

CEC, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy.

Chan, K.Y., Xu, Z., 2009. Biochar: nutrient properties and their enhancement, in: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management. Earthscan, London, UK.

Chan, K.Y., Zwieten, L.V., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of green waste biochar as a soil amendment. Australian Journal of Soil Research 45, 629-634.

Collison, M., Collison, L., Sakrabani, R., Tofield, B., Wollage, Z., 2009. Biochar and Carbon Sequestration: A Regional Perspective. A report prepared for East of England Development Agency (EEDA). Project funded under the single programme - DA1 Carbon Reduction Ref. No. 7049.

Davidson, E.A., Ackerman, I.L., 1993. Changes in soil carbon inventories following cultivation of previously untilled soils. Biogeochemistry 20, 161-164.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., 2009. Biochar effects on soil nutrient transformation, in: Lehmann, J., Stephen, J. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan Edition, London.

Derenne, S., Largeau, C., 2001. A review of some important families of refractory macromolecules: composition, origin, and fate in soils and sediments. Soil Science 166, 833-847.

Downie, A., Crosky, A., Munroe, P., 2009. Physical Properties of Biochar, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management - Science and Technology. Earthscan publisher, UK and USA.

EU, 2000. Working Document on sludge, 3rd draft 2000, ENV E 3/LM. European Union.

Freddo, A., Chao, C., Reid, B.J., 2012. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environmental Pollution 171: 18-24.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biology and Fertility of Soils 35, 219-230.

Grossman, J.M., O'Neill, B.E., Tsai, S.M., Liang, B., Neves, E., Lehmann, J., Thies, J.E., 2010. Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. Microbial Ecology 60, 192-205.

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., Cornelissen, G., 2012. Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. *Environmental science & technology*, *46*, 2830-2838.

Hammes, K., Torn, M.S., Lapenas, A.G., Schmidt, M.W.I., 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeosciences Discussion 5, 661-683.

Hansen, J., Sato, M., Kharecha, P., Beerling, D., Masson-Delmotte, V., Pagani, M., Raymo, M., Royer, D.L., Zachos, J.C., 2008. Target atmospheric CO_2: where should humanity aim? Journal of the Atmospheric Sciences 2, 217-231.

Hoshi, T., 2001. Growth promotion of tea trees by putting bamboo charcoal in soil, Proceedings of 2001 International Conference on O-cha (Tea) Culture and Science, pp. 336-345.

Houghton, R.A., 2007. Balacing the global carbon budget. Annual Review of Earth and Planetary Sciences 35, 313-347.

Hwang, I.H., Matsuto, T., Tanaka, N., Sasaki, Y., Tanaami, K., 2007. Characterization of char derived from various types of solid wastes from the standpoint of fuel recovery and pretreatment before landfilling. Waste Manegement 27, 1155-1166.

IPCC, 2007. Intergovernmental Panel on Climate Change: Climate Change in: Salomon, S., Dahe, Q., Manning, M., al., e. (Eds.). Cambridge Univ. Press, New York.

Jones, D.L., Edwards-Jones, G., Murphy, D.V., 2011. Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biology & Biochemistry 43, 804-813.

Keiluweit, M., Nico, P.S., Johnson, M.G., Kleber, M., 2010. Dynamic molecular structure of plant biomass-derived black carbon (biochar). Environmantal Science & Technology 44, 1247-1253.

Kim, J.S., Sparovek, G., Longo, R.M., De Melo, W.J., Crowley, D., 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil Biology and Biochemistry 39, 684-690.

Kishimoto, S., Sugiura, G., 1985. Charcoal as a soil conditioner. Symposium of Forest Products Reasearch, International Achievements for the Future 5, 12-23.

Kookana, R. S., Sarmah, A. K., Van Zwieten, L., Krull, E., & Singh, B. (2011). 3 Biochar Application to Soil: Agronomic and Environmental Benefits and Unintended Consequences. *Advances in Agronomy*, *112*, 103.

Koppolu, L., Agblevor, F.A., Clements, L.D., 2003. Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part II: Lab-scale pyrolysis of synthetic hyperaccumulator biomass. Biomass and Bioenery 25, 651-663.

Lehmann, J., Rilling, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota - A review. Soil Biology & Biochemistry 43, 1812-1836.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmatal management - Science and Technology. Earthshan publisher, UK and USA.

Lehmann, J., Czimczik, C., Laird, D., Sohi, S., 2009a. Stability of biochar in the soil, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management - Science and Technology. Erthscan, London.Lehmann,

Lehmann, J., Pereira de Silva, J., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249, 343-357.

Lehmann, J., Rondon, M., 2006. Biochar soil management in highly weathered soils in the humid tropics in: Uphoff, N. (Ed.), Biological approaches to sustainable soil systems. CRC Press, Boca Raton, FL.

Lenton, T.M., Held, H., Kriegler, E., Hall, J.W., Lucht, W., Rahmstorf, S., Schellnhuber, H.J., 2007. Tipping elements in the Earth's climate system. Proceedings of the National Academy of Sciences of the United States of America 105, 1786-1793.

Lenton, T.M., Vaugham, N.E., 2009. The radiative forcing potential of different climate geoengineering options. Atmospheric Chemistry and Physics Discussion 9, 1-50.

Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J.E., Skjemstad, J.O., Luizão, F.J., Ehgelhard, M.H., Neves, E.G., Wirick, S., 2008. Stability of biomass-derived black carbon in soils. Geochemica et Cosmochimica 72, 6069-6078.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J., Thies, J., Luizao, F., Petersen, J., 2006. Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 70, 1719-1730.

Lu, Y., Pignatello, J.J., 2002. Demonstration of the "conditioning effect" in soil organic matter in support of a pore deformation mechanism for sorption hysteresis. Environmental Science and Technology 36, 4553-4561.

Lua, A.C., Yang, T., Guo, J., 2004. Effects of pyrolysis conditions on the properties of activated carbons prepared from pistachio-nut shells. Journal of Analytical and Applied Pyrolysis, 279-287.

Macías, F., and M.C. Arbestain. 2010. Soil carbon sequestration in a changing global environment. Mitigation and Adaptation Strategies for Global Change:511-519.

Maías, F., Arbestain, M.C., 2010. Soil carbon sequestration in a changing global environment. Mitigation and Adaptation Strategies for Global Change.

Makoto, K., Tamai, Y., Kim, Y., Koike, T., 2010. Buried charcoal layer and ectomycorrhizae cooperatively promote the growth of *Larix gmelinii* seedlings. Plant and Soil 327, 143-152.

Malcolm, J., 1993. The farmer's need for agrochemicals, in: Jones, J.G. (Ed.), Agriculture and the Environment. Ellis Horwood, London, pp. 3-9.

Mallory-Smith, C.A., Retzinger, E.J., 2003. Revised classification of herbicides by site of action for weed resistance management strategies. Weed Technology 17, 605-619.

Matthews, H.D., Caldeira, K., 2008. Stabilizing climate requires near-zero emissions. Geophysical Research Letters 35, L04705.

McGrath, T., Sharma, R., Hajaligol, M., 2001. An experimental investigation into the formation of polycyclic-aromatic hydrocarbons (PAHs) from pyrolysis of biomass material. Fuel 80, 1787-1797.

Mordaunt, A.C., Gevao, B., Jones, K.C., Semple, K.T., 2005. Formation of non-extractable pesticide residues: observations on compound differences, measurement and regulatory issues. Environmental Pollution 133, 25-34.

Neary, D.G., Klopaket, C.C., DeBano, L.F., Folliot, P.F., 1999. Fire effects of below-ground sustainability: A review and synthesis. Forest Ecology and Management 122, 51-71.

Neves, N.G., Petersen, J.B., Bartone, R.N., Silva, C.A.D., 2003. Historical and socio-cultural origins of Amazonian Dark Earths., in: Lehmann, J., Kern, D.C., Glaser, B., Woods, W.I. (Eds.), Amazonian Dark Earths: Origin, Properties, Management. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 29-50.

Olesczuk, P., 2010. Toxicity of light soil fertilized by sewage sludge or compost in relation to PAHs content. Water Air Soil Pollut 210, 347-356.

O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., Thies, J.E., 2009. Bacterial community composition in Brazilian Anthrosol and adjacent soils characterized using culturing and molecular identification. Microb Ecol 58, 23-35.

Pietikäinen, J., Kiikkilä, O., Fritze, H., 2003. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231-242.

Post, W.M., Emanuel, W.R., Zinke, P.J., Strangenberger, A.G., 1982. Soil carbon pools and world life zones. Nature 289, 156-159.

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: A synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397-420

Reid, B.J., Papanikolaou, N.D., Wilcox, R.K., 2005. Intrinsic and induced isoproturon catabolic activity in dissimilar soils and soils under dissimilar land use. Environmental Pollution 133, 447-454.

Reid, B.J., Pickering, F.L., S.Song, Freddo, A., Influence of biochar on the fate, partitioning, transpot and bioavailability of the herbicide isoproturon. Environmental Science & Technology (under review).

Rivera-Utrilla, J., Bautista-Toledo, I., Ferro-García, M.A., Moreno-Castilla, C., 2001. Activated carbon surface modifications by adsorption of bacteria and their effect on aqueous lead adsorption. Journal of Chemical Technology and biotechnology 76, 1209-1215.

Rondon, M., Lehmann, J., Ramirez, J., Hurtado, M., 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. Biology and Fertility of Soils 43, 699-708.

Samonin, V., Elikova, E., 2004. A study of the adsorption of bacterial cells on porous materials. Microbiology 73, 696-701.

Sander, M., Lu, Y., Pignatello, J.J., 2006. Conditioning-annealing studies of natural organic matter solids linking irreversible sorption to irreversible structural expansion. Environmental Science and Technology 40, 170-178.

Sander, M., Pignatello, J.J., 2005. Characterization of charcoal adsorption sites for aromatic compounds: insights drawn from single-solute and bi-solute competitive experiments. Environmental Science and Technology 39, 1606-1615.

Sander, M., Pignatello, J.J., 2007. On the reversability of sorption to black carbon: distinguishing true hysteresis from artificial hysteresis Caused by Dilution of a Competing Adsorbate. Environmental Science & Technology 41, 843-849.

Schlesinger, W.H., 1995. An overview on the carbon cycle, in: Lal, R., Kimble, J., Stewart, B.A. (Eds.), Soils and Global Change. Lewis Publisher, Boca Raton.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: analysis, distribution, implications and current challenges. Global Biogeochemical Cycles, 251-271.

Shneour, E.A., 1966. Oxidation of graphitic carbon in certain soils. Science (New York, NY) 151, 991.

Short, P., Colborn, T., 1999. Pesticide use in the U.S. and policy implications: A focus on herbicides. Toxicology and Industrial Health 15, 240-275.

Solomon, S., Plattner, G., Knutti, R., Friedlingstein, P., 2009. Irreversible climate change due to carbon dioxide emissions. PNAS 106, 1704-1709.

Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C., 2009. Impact of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77, 574-581.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., Jones, K.C., 2003. PAHs, PCBs, PCNs, organochlorine pesticides, Synthetic Musks, and Polychlorinated n-Alkanes in UK. Sewage Sludge: survey results and implications. Environmental Science & Technology 37, 462-467.

Tian, C., Wang, M., Si, Y., 2010. Influences of charcoal amendment on adsorption-desorption of isoproturon in soils. Agricultural Sciences in China 9, 257-265.

Tomlin, C.D.S., 2006. The pesticide manual, 14th edition ed. BCPC Publications, Alton Hampshire UK

UNFCC, 2008. Submission containing ideas and proposals on Paragraph 1 of the Bali Action Plan. , UN Convention to Combat Desertification, Bonn, Germany.

Vencill, W.K., 2002. Herbicide Handbook, 8th ed. Weed Science Society of America, Lawrence, KS.

Verhaijen, F., Jeffery, S., Bastos, A.C., Valde, M.v.d., Diafas, F., 2010. Biochar application to soils. A critical scientific review of effects on soil properties, processes,

and functions. EUR 24099 En Office for the Official Publications of the European Communities, Lugemburg, p.149.

Wang, H., Lin, K., Hou, Z., Richardson, B., Gan, J., 2009. Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars. Journal of Soils Sediments 10, 283-289.

Wardle, D.A., Zackrisson, O., Nilsson, M.C., 1998. The charcoal effect in Boreal forests: Mechanisms and ecological consequences. Oecologia 115, 419-426.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil–concepts and mechanisms. Plant and Soil 300, 9-20.

Warnock, D.D., Mummey, D.L., McBride, B., Major, J., Lehmann, J., Rillig, M.C., 2010. Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. Applied Soil Ecology 46, 450-456.

Wassenberg, D.M., Giulio, R.T.D., 2004. Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A Inhibitors in *Fundulus heteroclitus*. Environmental Health Perspect 112, 1658-1664.

White, P.A., Claxton, L.D., 2004. Benzo[a]pyrene, polynuclear aromatic compounds, Part 1. Mutation Research 567, 227-345.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010. Sustainable biochar to mitigate global climate change. Nature Communications.

Yang, X.-B., Ying, G.-G., Peng, P.-A., Wang, L., Zhao, J.-L., Zhang, L.-J., Yuan, P., He, H.-P., 2010. Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil. Journal of Agricultural and Food Chemistry 58, 7915-7921.

Yu, X.-Y., Ying, G.-G., Kookana, R.S., 2009. Reduced plant uptake with biochar additions to soil. Chemosphere 79, 665-671.

Zhang, J., He, M., Lin, C., Sun, K., Men, B., Zhou, J.L., 2009. Phenanthrene sorption to environmental black carbon in sediments from the Song-Liao watershed (China). Environmental Science & Engineering in China 3, 434-442.

Zhu, D., Kwon, S., Pignatello, J.J., 2005. Adsorption of single-ring organic compounds to wood charcoals prepared under different thermochemical conditions. Environmental Science and Technology 39, 3990-3998.

Zwieten, L.V., Kimber, S., Downie, A., Chan, K.Y., Cowie, A., Wainberg, R., Morris, S., 2007. Papermill char: Benefits to soil health and plant production, Proceedings of the Conference of the Interational Agrichar Initiative, Terrigal, NSW, Australia.

Chapter 2

Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar

Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar

Alessia Freddo[1,2], Chao Cai[2], Brian J. Reid[1*].

[1]School of Environmental Sciences, University of East Anglia, Norwich Research Park, NR4 7TJ, Norwich, UK.

[2] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Science, Xiamen, 361021, China.

Abstract:

Nine dissimilar biochars, produced from varying feedstock at different pyrolysis temperatures, are appraised with respect to concentrations of potentially toxic elements, specifically, metals, metalloids and polycyclic aromatic hydrocarbons (PAHs). Concentrations of the metals and metalloids varied with the following ranges (mg kg^{-1}): 0.02–0.94, Cd; 0.12–6.48, Cr; 0.04–13.2, Cu; 0.1–1.37, Ni; 0.06–3.87, Pb; 0.94–207, Zn and 0.03–0.27, As. Σ_{16}PAH concentrations (16 Environmental Protection Agency PAHs) range between 0.08 mg kg^{-1} to 8.7 mg kg^{-1}. Subsequent comparison with background soil concentrations, concentration applied to the regulation of composted materials (Publicly Available Specification (PAS 100)) and European Union (EU) regulations relating to the application of sewage sludge to agricultural land suggest low risk associated with the concentrations of PTEs observed in biochar. Collectively, results suggest that environmental impacts attributable to metals, metalloids and PAHs associated with biochar following its application to soil are likely to be minimal.

1. Introduction

Biochar is defined as the carbon-rich product obtained when biomass is heated in an oxygen limited environment (Lehmann and Joseph, 2009). Biochar is mainly composed of recalcitrant aromatic C-ring structures, which are reported to have a long half-life in soil (Sombroek et al., 2003). Recently Lenton and Vaugham (2009) contextualised the potential biochar has, with respect to other geo-engineering options, to reduce atmospheric carbon dioxide levels. Scenarios presented by Lenton and Vaughan (2009) suggest decreases in atmospheric carbon dioxide (ΔCO_2 by 2100), as a result of direct air capture and storage, biochar and nitrogen fertilisation of the ocean to be -186 ppm, -37 ppm and -9.3 ppm, respectively. Based on projections regarding the use of renewable fuel by 2100 (180–310 EJ yr^{-1}; (Berndes et al., 2003)) Lehmann et al. (2006) reported carbon sequestration potentials of 5.5–9.5 Pg C yr^{-1} if these renewable fuel targets were met through biomass-to-biochar approaches to energy provision. Collectively, these reports provide a compelling and persuasive argument regarding the potential biochar has to make a considerable difference to atmospheric carbon dioxide levels.

In addition to these carbon sequestration benefits, biochar amendment to soil has also been reported to bring benefits in terms of soil physical, chemical and biological attributes; with a number of authors reporting enhanced plant growth (Glaser et al., 2002; Lehmann et al., 2003; Lehmann and Rondon 2006; Collison et al., 2009). Soil improvements have been linked to three key factors, namely, i) soil fertility (through nutrient provision (Sohi et al., 2009) and influence upon nutrient cycling (Chan and Xu, 2009) on account of changes to cation exchange capacity), ii) changes in soil pH and soil buffering (Liang et al., 2006) and, iii) influences upon soil water dynamics (Major and Lehmann 2006).

Thus, biochar application to soil may offer many benefits and has the potential to contribute to addressing significant challenges that prevail with respect to climate change mitigation, enhanced soil fertility and as a consequence improved crop yield. To date, however, there has been very little attention given to the levels of potentially toxic elements (PTEs) in biochar. The most obvious PTEs for consideration are metal and metalloid elements and polycyclic aromatic hydrocarbon (PAHs).

Regarding metals Koppolu et al. (2003) reported Ni, Zn, Cu, Co and Cr concentrations to increase in char with respect to concentrations in its feedstocks. Results indicated greater than 98.5 % of the metal in the product stream to be concentrated in the char formed and as a result elemental concentration increased by 4 to 6 times (Koppolu et al., 2003). Thus, there is a possibility that PTE levels in biochar may reach inappropriate levels due to enrichment of metal and metalloids within biochar (relative to the concentration in feedstock) during pyrolysis. Subsequently, following the addition of biochar to soil, these metals may adversely affect soil organisms. It is well documented that elevated levels of metals and metalloids can adversely affect abundance and diversity of soil organisms (Creamer et al., 2008). Indeed, elevated metal concentrations have been suggested to have lasting effects on ecosystem functioning (Perry et al., 1989; Creamer et al., 2008); this functioning being dependent, to a large extent, upon soil microflora activity (Donkova and Kaloyanoya, 2008). Fliessbach et al. (1994) reported the potential for metals and metalloids to suppress or even kill sensitive parts of the microbial community lead to shifts in community structure.

Regarding PAHs, these compounds are formed during combustion and pyrolysis processes (Liu et al., 2008) and as a consequnence are likely components within biochar. PAHs form adducts with DNA and have, as a consequence, been prioritized by the USEPA and EU on account of their carcinogenetic, mutagenic and teratogenic properties (Wassenberg and Giulio, 2004; White and Claxton, 2004).

We report here concentrations of metal and metalloid elements and PAHs present in a range of dissimilar biochars produced from contrasting feedstocks under different pyrolysis conditions. These concentrations are subsequently contextualised with respect to concentrations of metals, metalloids and PAHs in other environmental relevant media namely, background soil, sewage sludge, compost and coal.

2. Materials and Methods

2.1 Biochar

The biochars were produced from redwood (<10 cm), rice straw (≤ 20 cm), maize (15-20 cm) and bamboo (≤ 20 cm). Each feedstock was washed and dried before being turned into biochar. For biochar production, the materials were placed in a high performance automatic controlled furnace (GWL-1200, Henan, China), with a

continuous flow of nitrogen through the furnace chamber. A cooling chamber with water was used for passing the off-gas to allow condensation of tars. The biochar was then cooled to room temperature in the presence of nitrogen gas inside the furnace. Each feedstock was converted to biochar using two pyrolysis regimes, specifically, 12 hours at 300˚C and 2.5 hours at 600˚C. Different holding times were previously tested and optimized to reach complete feedstock carbonization for each temperature. In addition to the laboratory produced biochars, biochar was also obtained from a one-quarter scale 500 kW test gasifier (TG) (Refgas UK, Flintshire, UK), fuelled by softwood from a sawmill (herewith this biochar is referred to as 'TG biochar'). The gasification zone of the plant operated under negative pressure (-25 mbar) at around 1000ºC, the pyrolysis section around 500˚C and the "drying zone" at 200˚C. To pass the feedstock from the drying zone to the ash discharge section of the plant took 1 hour.

***2.2 Chemicals*:** Concentrated nitric acid and perchloric acid used in the extraction of metals and metalloids were AR grade and obtained from Sigma, China. The solvents used for PAH extraction (dichloromethane (DCM), acetone and hexane) were HPLC grade and purchased from Fisher Scientific UK. Hydromatrix was provided by Varian (Surrey, UK). Florisil (60-100 mesh) used for in-cell clean up (Hubert et al., 2000) within DCM extractions was provided by Promochem, Germany. Copper (general purpose grade) was purchased from Fisher Scientific. TCL Polynuclear Aromatic Hydrocarbon Mix used to prepare standards for GC-MS analyses was supplied by Supelco, USA. Elemental standards for ICP-MS analysis were obtained from NRCCRM, China.

2.3 Extraction of metal and metalloids

Extraction of metal and metalloids from biochar samples was undertaken using a method adapted from Wong and Li (2004). Biochar samples (0.25g; particle size < 2 mm) were weighed into pre-cleaned Pyrex test tubes and concentrated nitric acid (8mL) and concentrated acid perchloric acid (2mL) added inside the fume hood. The digestion tubes were then progressively heated (inside the fume hood) in an aluminium block at: 50˚C for 3 h, 75˚C for 1 h, 100˚C for 1 h, 125˚C for 1 h, 150˚C for 3 h, 175˚C for 2 h, and 190˚C for 3 h until they were completely dry. Thereafter, the test tubes were cool, 5% nitric acid (10.0 mL) added and the tubes re-heated at 700˚C for 1 h with occasional agitation. Upon cooling, the mixtures were decanted into polyethylene tubes and centrifuged at 1230 x *g* for 10 min.

2.4 Quantification of metal and metalloid concentrations:

Samples were analysed for metal and metalloids using Inductively Coupled Plasma Mass Spectometer (ICP-MS Agilent 7500cx (Agilent Technologies, Inc., USA)). The following elements were determined (m/z shown in parenthesis): Cd (m/z 111), Cr (m/z 53), Cu (m/z 63), Ni (m/z 60), Pb (m/z 208), Zn (m/z 66) and As (m/z 75) with Sc (m/z 45), Ge (m/z 72), Rh (m/z 103) and In (m/z 115) used as internal standards. All data were normalised with the internal standard. Simultaneous mixing of sample and internal standard (100 μg L-1 multielement solution (Agilent)) in the T-piece, prior to flowing to a concentric nebuliser, was achieved using a peristaltic pump.

For quality control, reagent blanks and standard reference materials (NIST SRM 2709 San Joaquin Soil and an internal reference material) were included at a frequency of 10% of the total sample population to evaluate contamination and to assess precision and bias. Samples were randomised prior to analysis. Concentrations were determined using a five-point calibration. The analytical results showed no signs of contamination and that the precision and bias of the analysis were generally within 10%. The recovery rates for most of the heavy metals in the international standard reference material (NIST SRM 2709) were around 80% to 95%. All sample vials were soaked in 10% v/v nitric acid for a minimum of 12 h, washed with ultrapure water, and then oven-dried prior to use.

2.5 Extraction of PAHs

Extraction of PAHs from biochar samples were evaluated by pressurised liquid extraction (PLE) using an Accelerated Solvent Extraction system (ASE 200; Dionex Corp.) following the procedures described by Latawiec and Reid, 2010. ASE extraction cells were packed as follows. On the bottom of each extraction cell, a GF/B filter (Dionex) was placed. Activated copper was placed on top (activation procedure is described elsewhere (Shek et al., 2008)). Extraction cells were then loaded with Florisil (2 g). Each sample (5 g; particle size < 2 mm) was mixed with Florisil (2 g) and Hydromatrix (drying agent: 2 g) and placed in the extraction cell. Remaining head space of the cell was filled with Hydromatrix. Finally, a GF/B filter (Dionex) was placed at the top of the ASE extraction cell and the cells sealed. Samples were extracted in triplicate using dichloromethane (DCM) as the solvent of choice (see supporting

information (Appendix A; Table S1) where the relative performance of a) DCM, b) DCM/acetone (1:1), and, c) acetone/hexane (1:1) is accounted).

Extraction conditions were adopted from EPA Method 3545 and are similar to those employed by others (Mielke et al., 2001; Schantz et al., 1997); 100˚C, 5 min equilibration (heat time), 5 min static (extraction) time, 10.3MPa (103 bar), 60 flush volume, 1 static cycle. The extracted analytes were purged from the sample cell using pressurized nitrogen at 10 bar for 120 s to ensure complete transfer from the cells to the collection vials.

In addition to solvent extraction, water extractions were performed following the method reported in Latawiec and Reid (2010), which presented an extraction technique to assess labile fractions of hydrophobic organic contaminants using ASE 200 (described above) at 200˚C and using a 10 min static time. A flush volume of 20 % was used to prevent possible errors in the system occurring due to high water viscosity at lower temperatures. Samples were mixed with Ottawa quartz sand (20-30 mesh, Fisher Scientfic UK). Extractions were conducted using Milli-Q water (Millipore, USA). After cooling in the collection vial, each extract was transferred into a pear shaped separating funnel (100 mL) and exchanged in to DCM (see Latawiec and Reid (2010)). Extractions were performed in triplicate.

2.5 Quantification of PAH concentrations

Quantification of target PAHs in all extracts was performed using GC-MS fitted with a mass selective detector (Perkin Elmer, Clarus 500). Compound separation was carried out using a fused silica capillary column (Perking Elmer Elite 5MS, 30m) coated with 5 % diphenyl and 95 % dimethyl polysiloxane stationary phase (0.25 mm i.d. x 0.25 mm film thickness). The mass spectrometer was operated at 70 eV in positive ion mode using selective ion response (SIR). The carrier gas was helium (CP grade, BOC UK) at a constant flow of 1 mL min^{-1}. Autosampler injections (1 µL) were performed in the 1:10 split ratio. The oven temperature was programmed as follows: 35˚C (holding time 1.5min) raised to 100˚C at gradient of 25˚C min^{-1}, then at 15˚C min^{-1} to 190˚C (2 min hold) and finally ramped at 10˚C min^{-1} to 270˚C and held for 15 min. Total run time was 35 min. The injector, transfer and ion source temperatures were set at 189˚C, 280˚C and 180˚C, respectively with the detector voltage at 450 V. Identification of PAHs was

made by integrating peak areas at defined retention times and at specific m/z (see Table S2) using Turbomass Software provided with the instrument and by comparison of these peaks with the response of a known concentration of PAHs. Analytical parameters such as detection limit (see Table S2) and quantification of PAHs were determined using standard solutions and appropriate standard calibration curves. Calibration standard with known concentration was inserted every 6 samples to control any possible machine drift within a run. All glassware was acetone rinsed and oven dried prior to use. Total PAH concentrations are reported for the 16 compounds listed by the US Environmental Protection Agency (namely, naphthalene; acenaphthylene; acenaphthene; fluorene; anthracene; phenanthrene; fluoranthene; pyrene; benzo(a)anthracene; chrysene; benzo(b)fluoranthene; benzo(k)fluoranthene; benzo(a)pyrene; indeno(1,2,3-cd)pyrene; dibenzo(a,h)anthracene; benzo[ghi]perylene) and has been denoted in the text as $\sum_{16}$PAH.

2.6 Statistical analysis and calculations

Independent sample t-test and One-Way ANOVA analyses were performed using SPSS 16.0 for Windows. Statistical significance of the influence of extraction temperature on extraction efficiencies of different compounds was determined at 95% confidence interval with the significance level at 0.05 unless stated otherwise. The approach used in the calculation of anticipated PTE concentrations in biochar amended soil is accounted in the supporting information (Appendix A).

3. Results

Metal and metalloid concentrations in dissimilar biochars varied considerably depending upon element and feedstock (Table 1). In ascending concentration (mg kg^{-1}) these were: 0.02 – 0.94 (median 0.03), Cd; 0.12 – 6.48 (median 4.3), Cr; 0.04 – 13.2 (median 5.5), Cu; 0.1– 1.37 (median 0.46), Ni; 0.06 – 3.87 (median: 0.88), Pb; 0.94 – 207 (median 55.6), Zn and 0.03 – 0.27 (median 0.21), As. Considering maximum metal/metalloid concentrations across the biochar types indicated: bamboo biochar to have the greatest concentrations of Ni (1.37 $\pm$ 0.55 mg kg^{-1}), Pb (3.87 $\pm$ 1.08 mg kg^{-1}), Zn (207 $\pm$ 3 mg kg^{-1}) and As (0.29 $\pm$ 0.01mg kg^{-1}); redwood biochar exhibited the

highest concentration of Cd (0.94 $\pm$ 0.01 mg kg^{-1}); while, maize biochar the greatest concentration of Cr (6.48 $\pm$ 1.79 mg kg^{-1}) and Cu (10.6 $\pm$ 0.5 mg kg^{-1}) (Table 1). Comparison of metal/metalloid concentrations following pyrolysis at 300˚C vs. 600˚C indicated: no significant difference ($P > 0.05$) for the majority of pairwise comparisons (12 out of 21 cases); a significant increase ($P < 0.05$) in metal/metalloid concentrations from lower to higher temperature in 6 out of 21 comparisons; and a significant decrease ($P < 0.05$) in concentration in 3 out of 21 cases (Table 1).

Increases in metal concentrations were only considerable (approximately x 2) for Pb, and Zn in the bamboo biochar (Table 1). On balance, increasing pyrolysis temperature applied to a given feedstock had very little consequential influence upon resultant metal/metalloid concentrations in the resultant biochars.

Table 1: Concentrations (mg kg^{-1}) of metals and metalloids in dissimilar biochars. Like letters indicate no significant difference between elemental concentrations for a given feedstock pyrolised at either 300^0C or 600^0C; while dissimilar letters indicate significant difference.

	Bamboo		Redwood		Maize		TG BC
	300^0C	600^0C	300^0C	600^0C	300^0C	600^0C	500^0C
Cd	0.03 + 0.001a	0.03 + 0.003a	0.94 + 0.01a	0.02 + 0.002b	0.03 + 0.003a	0.03 + 0.01a	0.015 + 0.025
Cr	4.30 + 0.06a	4.39 + 0.21a	4.51 + 0.23a	3.42 + 0.19b	5.09 + 0.27a	6.48 + 1.79a	0.12 + 0.15
Cu	10.0 + 8.1a	6.31 + 0.01a	2.03 + 0.06a	2.06 + 0.07a	10.6 + 0.5a	13.2 + 0.27b	0.04 + 0.01
Ni	1.37 + 0.55a	1.25 + 0.22a	0.42 + 0.03a	0.57 + 0.24a	0.37 + 0.04a	0.59 + 0.09b	0.1 + 0.1
Pb	1.92 + 0.15a	3.87 + 1.08a	0.64 + 0.06a	0.87 + 0.11b	0.06 + 0.11a	1.07 + 0.10b	0.15 + 0.02
Zn	124 + 2a	207 + 3b	38.5 + 3.5a	38.5 + 3.8a	92.0 + 2.3a	53.9 + 3.3b	0.94 + 0.41
As	0.27 + 0.01a	0.29 + 0.01b	0.12 + 0.02a	0.16 + 0.03a	0.25 + 0.03a	0.21 + 0.01a	0.03 + 0.02

3.1 PAH concentrations in dissimilar biochars

$\sum_{16}$PAH concentration varied and depended upon feedstock and the temperature of pyrolysis (Figure 1). The mean $\sum_{16}$PAH concentration ranged between 0.08 mg kg^{-1} and 8.7 mg kg^{-1} (Table 2); the TG biochar had the highest $\sum_{16}$PAH concentration (8.7 ± 1.2 mg kg^{-1}); while the median concentration for the nine biochars was 3.8 mg kg^{-1}. The concentrations of $\sum_{16}$PAH obtained in samples produced at 300˚C were significantly ($P < 0.05$) higher compared to the samples produced at 600˚C (Figure 1). At 300˚C, maize derived biochar indicated the highest concentration of $\sum_{16}$PAH (5.66 ± 1.4 mg kg^{-1}), while concentrations decrease for redwood (4.54 ± 3.73 mg kg^{-1}), bamboo (2.47 ± 0.12 mg kg^{-1}) and rice straw (2.27 ± 0.07 mg kg^{-1}) (Figure 1). This order was not maintained for 600˚C biochar (Figure 1). The concentrations of $\sum_{16}$PAH in the biochars derived at 600˚C were: maize (1.47 ± 0.19 mg kg^{-1}), rice straw (1.15 ± 0.04 mg kg^{-1}), bamboo (1.06 ± 0.13 mg kg^{-1}) and redwood (0.08 mg kg^{-1}) (Figure 1). Of all the biochar matrices assessed, the redwood pyrolised at 600˚C indicated the lowest concentration of $\sum_{16}$PAH (0.08 mg kg^{-1}). This concentration was two orders of magnitude lower than that observed for redwood pyrolised at 300˚C (4.54 ± 3.73 mg kg^{-1}).

Considering individual PAHs, it was observed that, overall, lower molecular weight PAHs were found to be more abundant than the higher molecular weight compounds (Table S1; Figure 2). Naphthalene was the most abundant individual PAH compound in all of the biochar matrices assessed (except for redwood 300˚C and rice straw 300˚C), with concentrations ranging between 0.5 mg kg^{-1} to 5.11 mg kg^{-1} (Table S1; Figure 2); the median concentration across the nine biochars was 1.62 ± 0.04 mg kg^{-1}. Anthracene, fluoranthene and pyrene were the compounds most often found to be present in the biochar matrices (Table S1; Figure 2). Their concentrations ranged from 0.05 mg kg^{-1} to 1.12 mg kg^{-1}. Pyrene was the only compound that showed concentrations higher than the limit of detection for all biochar matrices. Pyrene concentrations were noted for their consistency across biochar types with no significant difference ($p<0.05$) between the biochar types (Table S1; Figure 2). With the exception of fluorene in redwood and rice straw at 300˚C (both 0.12 mg kg^{-1}) and TG biochar (0.3 ± 0.01 mg kg^{-1}); acenaphtene (1.69 ± 0.43 mg kg^{-1}) and acenaphtylene (1.17 ± 0.04 mg kg^{-1}) in TG biochar; benzo(a)anthracene (0.2 ± 0.01 mg kg^{-1}), chrysene (0.36 mg kg^{-1}) and benzo(b)fluoranthene (0.2 mg kg^{-1}) in 300˚C rice straw; the remaining PAHs present

were below the detection limit. The concentrations of naphthalene and fluoranthene were observed with significant differences ($p < 0.05$) depending upon the feedstock and pyrolysis conditions (Table S1; Figure 2).

In light of the TG biochar having the highest $\sum_{16}$PAH concentration (8.7 ± 1.2 mg kg^{-1}) further assessment was made to establish the partition of PAHs in this matrix. Towards these ends a water based non-exhaustive PLE was used. Such an extraction approach has been reported to potentially mimic PAH desorption into aqueous media (Reid et al., 2000) and thereby provide an indication of bioavailability of the organic compound to biological receptors, such as bacteria (Miller and Alexander, 1991; Ogram et al., 1985). Cornelissen et al. (1998) suggested that the bioavailable fraction of a compound to be that which can be rapidly desorbed via the aqueous phase. Results indicated that water based PLE did not liberate PAHs above the limit of detection.

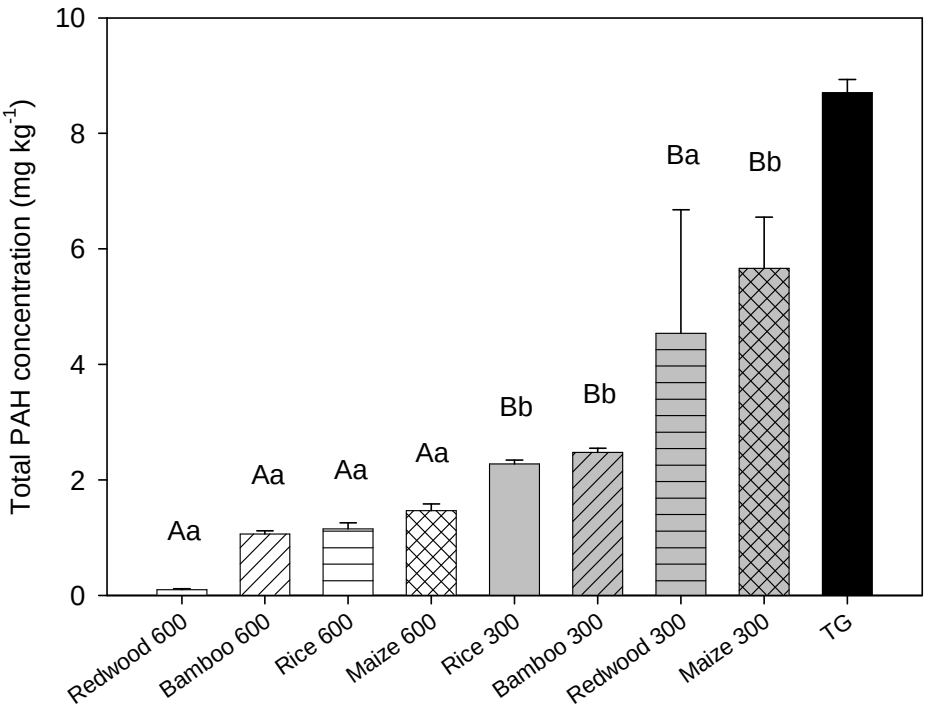


Figure 1: $\sum_{16}$PAH concentrations (mg kg^{-1}) in biochar produced using redwood, bamboo, rice straw and maize (at 600˚ (white) and 300˚C (grey)) and TG biochar (at 500˚C (black)). Error bars represent standard errors (n=3). Upper-case letters indicate significant difference between biochar produced at the same temperature, while lower-case letters indicate significant differences between couplets of biochar made from same feedstock but at different temperatures.

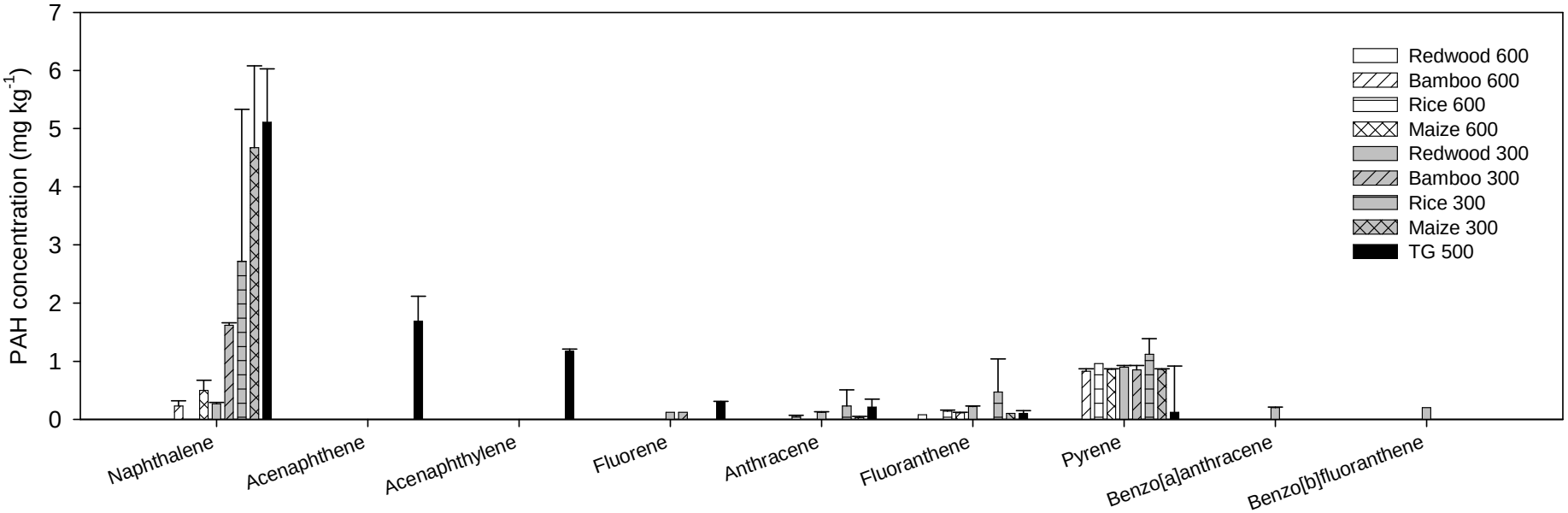


Figure 2: Individual PAH concentrations (mg kg^{-1}) in biochar produced using redwood, bamboo, rice straw and maize (at 600˚ (white) and 300˚C (grey)) and TG biochar (at 500˚C (black)). Error bars represent standard errors (n=3). Missing bars indicate values below detection limit.

Table 2: Maximum, minimum and median concentrations (mg kg^{-1}) metals and metalloids in the nine dissimilar biochar matrices and their comparison with concentrations in background soils, guidance values for sewage sludge, guidance values for compost (PAS, 2011) and concentrations in coal. Where these reference values are approached within one order of magnitude, or exceeded, by the maximum biochar PTE concentrations, this has been highlighted in bold.

	BC Minimum	BC Median	BC Maximum	Background soil (1)	Sewage Sludge (2)	Compost (3)	Coal (4)
Cd	$6.6*10^{-4}$	0.03	0.94	**0.8**	20	1.5	**0.046**
Cr	0.02	4.34	8.27	44	n.a.	100	15.1
Cu	0.03	5.48	18.1	19	1000	200	**30.22**
Ni	0.03	0.46	1.92	25	300	50	**5.27**
Pb	0.12	0.88	4.95	39	750	200	18.98
Zn	0.64	55.63	210.74	**89**	2500	**400**	**17.1**
As	0.01	0.21	0.30	6.2	16	1.0	1.2

1: concentrations in background soil taken from Chen et al. (2001) for As and Zhao et al. (2007) for all other metals

2: guidance values for sewage sludge (E.C. (1986)

3: guidance values for compost (PAS, 2011)

4: concentrations in coal (Wang et al., 2006)

4. Discussion

Given the proposed application of biochar to soil it is important to appreciate the implications of this practice with respect to the associated addition of metals, metalloids and PAHs to soil. Towards these ends, a contextualisation of metal, metalloid and PAH concentrations present in biochar with respect to their concentration in: i) background soils, ii) sewage sludge, iii) compost, and iv) coal is provided.

4.1 Contextualisation of metal and metalloids in biochar

***Background soil concentrations*:** Metals and metalloids are naturally ubiquitous in the environment; their presence in soils resulting naturally from the weathering of the parent materials from which soils are derived. Owing to the use of raw materials these natural levels have been influence by anthropogenic activities; it has been argued, that because of human influence, natural background levels no longer exist on the planet (Reimann and Garrett, 2005). Recently the International Organisation for Standardisation (ISO) defined the term ‘‘ambient background concentration’’ (ABC); this being the resultant trace element concentration in soils that have been subject to moderate diffuse inputs (but not inputs from local point sources that would generally result in concentrations that are greatly elevated) (ISO, 2005). Attempts to define a single background ABC is fraught with difficult because of heterogeneity in regional geology and geochemistry (Reimann and Garrett, 2005). In addition, soil texture also greatly influences trace element concentration in soil (Reimann and Garrett, 2005). Zhao et al. (2007) provided results of a statistical analysis of the soil geochemical data for England and Wales (the National Soil Inventory, NSI), with the aim of estimating local or soil type specific ABCs, for a range of texture classes, for the trace elements: Cd, Co, Cr, Cu, Ni, Pb and Zn (but not As). The median concentration values for the soil texture class ‘fine loamy’ as reported by Zhao et al. (2007) were used to provide context for the metal and metalloid concentrations in biochar (Table 2). Arsenic concentrations vary tremendously and are strongly correlated with soil parent material (O’Neill, 1995). In the UK the soil guidance value (SGV) of 32 mg kg^{-1} is used by the Environment Agency as a safe level of residential use (EA, 2009). Chen et al. (2001), like Zhao et al. (2007), took a statistical approach to assessing background arsenic concentrations in soil (Florida USA). Towards these ends, As concentrations were log-

normally distributed and the baseline background concentration defined as the geometric mean within the 95% range of values (n = 267). The 6.2 mg kg^{-1} background value for undisturbed soils, reported by Chen et al. (2001), was used to provide context for the biochar As concentration. This value was used as it was considerably lower than the UK SGV.

Of the metals and metalloids, only Zn exceeded the background ABC value (of 89 mg kg^{-1}) by a considerable margin. The maximum Zn concentration (bamboo biochar 600˚C) was more than double the ABC value (Table 2) with the lower temperature (300˚C) bamboo biochar and lower temperature (300˚C) maize biochar (Table 1) also exceeding the Zn-ABC value. The maximum concentrations observed for the metals Cd and Cu in biochar were close to the ABC values (Table 2) while the remaining metal and metalloids fell below their respective ABC values by considerable margins: Cr by a factor of 5; Pb by a factor of 8; Ni by a factor of 12; and, As by a factor of 20. It was noted that none of the metal/metalloid median concentrations infringed the soil ABC values (Table 2).

In order to further contextualise the levels of metals and metalloids in biochar calculation of the anticipated resultant concentration of these elements following biochar application (100 t ha^{-1}) to soil was undertaken (see supporting information) (Appendix A; Table S3). Where minimum and median values were used in the calculation the resultant concentrations in the amended soil were *lower* than those in the receiving background soil. Only where maximum concentration values were applied was the resultant concentration of metals observed to increase with respect to the receiving background soil; this increasing being noted for Cd and Zn only. In these cases concentrations were increased by only 3% and 21%, respectively. Thus, it can be concluded that biochar application to soil (up to an application rate of 100 t ha^{-1}) is unlikely to make any real difference to metal and metalloid concentrations in the receiving soil.

***Sewage sludge and composts*:** Sewage sludge and composts commonly applied to agricultural land with a view to improve soil structure and fertility. Across Europe the application of sewage sludge to agricultural land is regulated through the Commission of the European Communities' Council Directive of 12 June 1986 on the protection of the environment, and in particular soil, when sewage sludge is used in agriculture (E.C.,

1986). This document details limiting concentrations for the metals and metalloids: Cd, Cu, Ni, Pb, Zn and As (but not Cr) present in sewage sludge *per se* and in soil to which sewage sludge has been applied.

None of the metal or metalloid concentrations reported here (Table 2) exceeded the sewage sludge guidance values. Of the metals and metalloids, Zn was the only element that came close to approaching the sewage sludge guidance limit; its maximum concentration (bamboo biochar 600˚C) being within a factor of 12. Maximum Cu concentrations (bamboo biochar 300˚C) were within a factor of 50, while maximum Ni (bamboo biochar 300˚C) and Pb (bamboo biochar 600˚C) concentration were with a factor of 150.

While the metal and metalloid concentrations in biochar fall considerably below sewage sludge guidance values it should be acknowledged that the metal and metalloid concentrations set for sewage sludge by the EU Directive are much higher than values set for other media. In more recent years, the Publicly Available Specification (PAS 100) criteria (PAS, 2011) for compost sets concentration thresholds for metals and metalloids at levels that are typically an order of magnitude lower than those for sewage sludge (Table 2). Considering the concentrations of metal and metalloids present in biochar alongside PAS 100 criteria for compost (Table 2) again indicated that none of the metal or metalloid concentrations exceeded the guidance values. Maximum concentration for both Zn and Cd in biochar came closest; fall within a factor of 2 of the PAS 100 criteria; while As fell within a factor of 3; Cr and Cu fell within a factor of about 10, and; Ni and Pb within factors of 25 and 40, respectively.

Coal: Wang et al (2006) reported concentrastion of metals and metalloids in coal. Interestingly, maximum metal and metalloid concentrations observed in biochar were very similar to their levels reported in coal (Table 2). In all cases maximum metal and metalloid concentration in biochar fell within an order of magnitude of the values for coal (Table 2).

4.2 Contextualisation of PAHs in biochar

***Background soil concentrations*:** PAHs released into the atmosphere from pyrolitic processes are returned to the ground surface by atmospheric deposition processes contributing to the PAH burdens of soils (Wild and Jones, 1991). Jones et al. (1989)

reported UK soil $\sum_{14}$PAH concentrations in rural and urban areas to generally fall into the concentration range 0.1- 54.5 mg kg^{-1} with higher concentrations in urban areas, near to point sources, in soils with high organic matter contents and sometimes in soils amended with sewage sludge (Jones et al., 1989). Cousins et al. (1997) analyzed surface soil in either remote, semi-rural and rural regions distributed over the UK where emissions of PAHs could be limited and reported $\sum_{12}$PAH concentrations to range between 0.02 – 7.4 mg kg^{-1} (Cousins et al., 1997). $\sum_{16}$PAH concentrations observed in biochar are comparable to concentrations of $\sum_{14}$PAH and $\sum_{12}$PAH previously reported in background soils (see above and Table 3). Given the similarly in $\sum_{16}$PAH concentrations (0.08 mg kg^{-1} and 8.7 mg kg^{-1} (Table 3)) in biochar to those reported for background soils it is appropriate to conclude that PAHs in biochar are likely to be of minimal concern following biochar application to soil. Furthermore, since PAHs are hydrophobic compounds that associate strongly with organic matter, the physical structure of biochar and its recalcitrance (Sombroek et al., 2003) it is probably that long term bioavailability of biochar associated PAHs is likely to be low. Indeed water based PLE confirmed this in failing to liberate any PAH from biochar at concentrations above limits of detection.

***Sewage sludge and composts*:** It has been reported that the concentrations of PAHs in sewage sludge is variable depending on sludge type, treatment and wastewater source (Stevens et al., 2003). Stevens et al. (2003) provide results on organic pollutants (including PAHs) in digested sewage sludge from 14 UK wastewater treatment plants. Regarding the total PAHs analysed (24 compounds), it was reported that depending on the sewage sludge sample areas (urban/non-urban) the concentrations ranged between 67 to 370 mg kg^{-1} dry weight (dw), (note these values exceed the proposed EU limit (18 to 36 mg kg^{-1} dw)).

Recently, Olesczuk (2010) reported the influence sewage sludges and composts had upon level of PAHs in soils (Olesczuk, 2010). Moreover Oleszczuk (2010) reported ∑PAH concentrations in two sewage sludges and two composts to average ranges between 2.72 ± 0.13 and 3.54 ± 0.13 mg kg^{-1} and between 1.92 ± 0.1 and 3.91 ± 0.24 mg kg^{-1}, respectively. It is noted that the range of concentration (0.08 mg kg^{-1} to 8.7 mg kg^{-1}) reported here for biochar matrices is of the same order of magnitude to those reported by Oleszczuk (2010).

A Working Document on Sludge produced by the European Union (2000) provides "limit values for concentrations of organic compounds in sludge for use on land". The EU proposes a concentration limit of the "sum of the PAHs" equal to 6 mg kg^{-1}dry matter. The PAHs considered are acenaphthene, phenanthrene, fluorene, fluoranthene, pyrene, benzo-[b]fluoranthene, benzo-[j]fluoranthene, benzo-[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene, and indeno[1,2,3-cd]pyrene (denoted $\sum_{11}$PAH). Only at the upper end of the $\sum_{16}$PAH concentration reported here was this 6 mg kg^{-1}dry matter limit exceeded (by a factor of 1.5 times (Table 3)).

Individual median PAH concentrations were in keeping with those reported for compost and in general considerably lower than those reported for sewage sludge (Table 3).

***Coal*:** Laumann et al. (2011) reported that the concentrations $\sum_{52}$PAH in coal from eleven different regions worldwide varied from 6 to 253 mg kg^{-1} depending by the rank and the origin of the coal. Thus, concentrations of $\sum_{16}$PAH in biochar (0.08 mg kg^{-1} to 8.7 mg kg^{-1}) were two orders of magnitude lower than those in coal.

Table 3: Maximum, minimum and median concentrations for individual PAHs (mg/kg), $\sum_{11}$PAH and $\sum_{16}$PAH in the nine dissimilar biochar matrices and PAH concentrations in sewage sludge and compost from the literature.

PAH	Biochar (minimum)	Biochar (median)	Biochar (maximum)	Sewage sludge (mean) (1)	Compost (mean) (2)
Naphthalene	0.23	1.67	5.11	3.7	0.651
Acenaphthylene	< LOD	< LOD	1.69	0.06	<LOD
Acenaphthene	< LOD	< LOD	1.17	4	0.2
Fluorene	0.12	0.12	0.3	5.7	<LOD
Anthracene	0.03	0.11	0.23	0.72	0.06
Phenanthrene	< LOD	< LOD	< LOD	7	0.96
Fluoranthene	0.08	0.21	0.47	4.9	0.4
Pyrene	0.12	0.91	1.12	4.2	1.84
Benzo(a)anthracene	< LOD	0.2	0.2	1.8	<LOD
Chrysene	< LOD	0.36	0.36	2.6	0.67
Benzo(b)fluoranthene	< LOD	0.2	0.2	3	0.35
Benzo(k)fluoranthene	< LOD	< LOD	< LOD	2.2	0.08
Benzo(a)pyrene	< LOD	< LOD	< LOD	2.1	0.12
Indeno(1,2,3-cd)pyrene	< LOD	< LOD	1.3	0.81	< LOD
Dibenzo(a,h)anthracene	< LOD	< LOD	< LOD	0.19	0.3
Benzo[ghi]perylene	< LOD	< LOD	< LOD	1.3	0.88
$\sum_{11}$PAHs (*)	< LOD [$]	1.22[$]	2.11[$]	35.21	4.83
$\sum_{16}$PAHs (+)	0.08[$]	2.28[$]	8.7[$]	44.28	6.51

LOD – level of detection limit; (1) (Stevens et al., 2003) data were obtained through Soxhlet extraction using DCM as extraction solution. This method is equivalent to DCM-ASE extraction method as previously showed in literature (Heemken et al., 1997), therefore the values are comparable with the concentrations of PAHs in biochar presented in this paper.; (2) (Villar et al., 2009) The PAHs were extracted by HPLCD-DAD-FL; (*)The EU sewage sludge proposal considers $\sum_{11}$PAHs (see manuscript); (+)The US EPA considers $\sum_{16}$PAHs (see manuscript); ($) ∑PAH concentrations shown are <u>not</u> the sum of the individual PAH values shown in the rows above; but are: minimum, median and maximum ∑PAH concentrations for actual samples.

5. Conclusions

Collectively, results have indicated that concentrations of metals, metalloids and $\sum_{16}$PAH in biochar to be lower than those reported as acceptable for sewage sludge and either lower, or in keeping with, those acceptable for compost. Significantly, concentrations of metals, metalloids and $\sum_{16}$PAH in biochar were in keeping with those reported for background soils. Subsequent calculation has revealed that biochar application to soil (up to 100 t ha^{-1}) lacks the capacity to elevate metal, metalloid concentrations above background levels. It follows that environmental impacts attributable to metals, metalloids and PAHs associated with biochar following its application to soil are likely to be minimal.

Currently there is no regulation regarding application of biochar to soil. In many respects, the issues relating to the application of biochar as a soil improver parallel those implicit to those relating to sewage sludge and composts. For both of these soil amendments, guidance already exists. In the case of sewage sludge, the Sewage Sludge Directive 86/278/EEC requires member states to 'regulate sewage sludge in agriculture in such a way as to prevent toxic effects on soil, vegetation, animals and man, thereby encouraging the correct use of such sewage sludge', while guidance for compost assumes a quality control approach to limit levels of PTEs.

It is stressed that the feedstocks (bamboo, redwood, rice straw and maize) used in the production of biochar for this research might be described to be of 'low toxicity provenance'; biochar produced from less 'pristine' feedstock, for example, domestic waste may well contain potentially toxic elements in greater abundance. As a consequence, there is a need to develop criteria regarding suitability of potential feedstock with a view to constraining concentrations of potentially toxic elements in the resultant biochars. Particular reservation is attached to the production of biochar from feedstock that could contain chlorinated organic compounds (e.g. polyvinyl chloride (a common plastic) or pentachlorophenol (used in the treatment of timber)) as their pyrolysis *may* result in polychlorinated biphenyl-p-dioxins and furans (PCDD/F) formation.

Acknowledgments

We thank the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-Q02-02) for financial support.

References

Berndes, G., Hoogwijk, M., Broek, R.v.d. (2003). The contribution of biomass in the future global energy supply:a review of 17 studies. *Biomass and Bioenery* **25**, 1-28.

Chan, K.Y., Xu, Z. (2009). Biochar: nutrient properties and their enhancement, in: Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management*. Earthscan, London, UK.

Chen, M., Ma, L.Q., Hoogeweg, C.G., Harris, W.G. (2001) Arsenic background concentrations in Florida, U.S.A. Surface Soils: Determination and Interpretation. *Environmental Forensics* **2**, 117-126

Collison, M., Collison, L., Sakrabani, R., Tofield, B., Wollage, Z. (2009). Biochar and Carbon Sequestration: A Regional Perspective. A report prepared for East of England Development Agency (EEDA). Project funded under the single programme - DA1 Carbon Reduction Ref. No. 7049.

Cornelissen, G., Rigterink, H., Ferdinandy, M.M.A., Noort, P.C.M.V. (1998). Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. *Environmental Science & Technology* **32**, 966-970.

Cousins, I.T., Kreibich, H., Hudson, L.E., Lead, W.A., Jones, K.C. (1997). PAHs in soils: contemporary UK data and evidence for potential contamination problems caused by exposure of samples to laboratory air. *The Science of the Total Environment* **203**, 141-156.

Creamer, R.E., Rimmer, D.L., Black, H. I. J. (2008). Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? *Soil Use and Management*. **24**, 37-46.

Donkova, R., Kaloyanoya, N. (2008). The impact of soil pollutants on soil microbial activity, Soil chemical pollution, risk assessment, remediation and security. Book Series: Nato Science for Peace and Security Series C - Environmental Security, pp. 73-93.

EA (2009): Soil Guideline Values for inorganic arsenic in soil. Science Report SC050021/ arsenic SGV. Sourced http://publications.environment-agency.gov.uk/PDF/SCHO0409BPVY-E-E.pdf

E.C. (1986) Commission of the European Communities' Council Directive of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. Official Journal of the European Communities L 181 (86/278/EEC)

EU, 2000. Working Document on sludge, 3rd draft 2000, ENV E 3/LM. European Union. Fliessbach, A., Martens, A., Reber, H.H. (1994). Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. *Soil Biology & Biochemistry* **26**, 1201-1205.

Glaser, B., Lehmann, J., Zech, W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. *Biology and Fertility of Soils* **35**, 219-230.

Heemken, O.P., Theobald, N., Wenclawiak, B.W., 1997. Comparison of ASE and SFE with Soxhlet, sonication, and methanolic saponification extractions for the determination of organic micropollutants in marine particulate matter. . Analytical Chemistry 69, 2171-2180.

Hubert, A., Wenzel, K.-D., Manz, M., Weissflog, L., Engewald, W., Schuurmann, G. (2000). High extraction efficiency for POPs in real contaminated soil samples using accelerated solvent extraction. *Analytical Chemistry* **72,** 1294-1300.

ISO 2005: International Organisation for Standardisation (2005) Soil quality: Guidance on the determination of background values. ISO 19258: 2005. International Organisation for Standardisation.

Jones, K.C., Stratford, J.A., Waterhouse, K.S., Vogt, N.B. (1989). Organic contaminants in Welsh soils: polynuclear aromatic hydrocarbons. *Environmental Science & Technology* **25**, 540-550.

Koppolu, L., Agblevor, F.A., Clements, L.D. (2003). Pyrolysis as a technique for separating heavy metals from hyperaccumulators. Part II: Lab-scale pyrolysis of synthetic hyperaccumulator biomass. *Biomass and Bioenergy* **25**, 651-663.

Latawiec, A.E., Reid, B.J. (2010). Sequential extraction of polycyclic aromatic hydrocarbons using subcritical water. *Chemosphere* **78**, 1042-1048.

Laumann, S., Miciæ, V., Kruge, M.A., Achten, C., Sachsenhofer, R.F., Schwarzbauer, J., Hofmann, T., 2011. Variations in concentrations and compositions of polycyclic aromatic hydrocarbons (PAHs) in coals related to the coal rank and origin. Environmental Pollution in press, 1-8.

Lehmann, C. J., Rondon, M. (2006), Bio-char soil management on highly-weathered soils in the tropics. *In Biological Approaches to Sustainable Soil Systems* (N.T. Uphoff, ed.), 517-530. CRC Press, Boca Raton.

Lehmann, J., Gaunt, J., Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems - A review. *Mitigation and Adaptation Strategies for Global Change* **11**, 403-427.

Lehmann, J., Joseph, S. (2009). Biochar for environmental management: an introduction, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management - *Science and Technology*. Earthshan publisher, UK and USA.

Lehmann, J., Jr., J.P.d.S., Steiner, C., Nehls, T., Zech, W., Glaser, B. (2003). Nutrient avaiability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil* **249**, 343-357.

Lenton, T.M., Vaughan, N.E. (2009). The radiative forcing potential of different climate geoengineering options. *Atmospheric Chemistry and Physics Discussion* **9**, 1-50.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizao, F.J., Peterson, J., Neves, E.G. (2006). Black carbon increases cation exchange capacity in soils. *Soil Science Society America Journal* **70**, 1719-1730.

Liu, G., Niu, Z., Niekerk, D.V., Xue, J., Zheng, L. (2008). Polycyclic aromatic hydrocarbons (PAHs) from coal combustion: Emissions, analysis and toxicology. *Reviews of Environmental Contamination and Toxicology* **192**, 1-28.

Laumann, S., Miciæ, V., Kruge, M.A., Achten, C., Sachsenhofer, R.F., Schwarzbauer, J., Hofmann, T., (2011). Variations in concentrations and compositions of polycyclic aromatic hydrocarbons (PAHs) in coals related to the coal rank and origin. *Environmental Pollution* in press, 1-8.

Major, M.A.R., Lehmann, J. (2006). Bio-Char applications to a tropical oxisol Increase crop yield and modify water relations, 18th World Congress of Soil Science, Philadelphia, Pennsylvania, USA.

Mielke, H.W., Wang, G., Gonzales, C.R., Le, B., Quanch, V.N., Mielke, P.W. (2001). PAH and metal mixtures in New Orleans soils and sediments. *Science of the Total Environment* **281**, 217-227.

Miller, M.E., Alexander, M. (1991). Kinetics of bacterial-degradation of benzylamine in a montmorillonite suspension. *Environmental Science and Technology* **25**, 240-245.

Ogram, A.V., Jessup, R.E., Ou, L.T., Rao, P.S.C. (1985). Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy) acetic acid in soil. *Applied Environmental Microbiology* **49**, 582-587.

Olesczuk, P. (2010). Toxicity of light soil fertilized by sewage sludge or compost in relation to PAHs content. *Water Air Soil Pollut* **210**, 347-356.

O'Neill, P. (1995). In Heavy Metals in Soils (2nd edn.) (ed. B.J. Alloway). Blackie, London. ISBN: 0751401986.

PAS (2011) PAS 100:2011 Specification for composted materials. ISBN 978 0 580 653070.

http://www.wrap.org.uk/recycling_industry/information_by_material/organics/production.html

Perry, A.D.M., Amaranthus, M.R., Borchers, J.G., Borchers, S.L., Brainerd, R.E. (1989). Bootstrapping in ecosystems. *Bioscience* **39**, 230-237.

Reid, B.J., Jones, K.C., Semple, K.T. (2000). Bioavailability of peristent organic pollutants in soils and sediments - a perspective of mechanisms, consequences and assessment. *Environmental Pollution* **108**, 103-112.

Reimann, C., Garrett, R.G. (2005) Geochemical backgrounddconcept and reality. *Science of the Total Environment.* **350**, 12-27.

Schantz, M.M., Nichols, J.J., Wise, S.A. (1997). Evaluation of pressurized fluid extraction for the extraction of environmental matrix reference materials. *Analytical Chemistry* **69**, 4210-4219.

Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S. (2008). Synthetic polycyclic musks in Hong Kong sewage sludge. *Chemosphere* **71**, 1241-1250.

Sohi, S., Lopez-Capel, E., Krull, E., Bol, R. (2009). Biochar, climate change and soil: A review to guide future research.

Sombroek, W., Ruivo, M.D.L., Fearnside, P.M., Glaser, B., Lehmann, J. (2003). Amazonian Dark Earths as carbon stores and sinks, in: Lehmann, J., Kern, D.C., Glaser, B., Woods, W.I. (Eds.), Amazonian Dark Earths: origin, properties, management. Kluwer Academic Publishers, Netherlands.

Stevens, J.L., Northcott, G.L., Stern, G.A., Tomy, G.T., Jones, K.C. (2003). PAHs, PCBs, PCNs, organochlorine pesticides, synthetic musks, and polychlorinated n-alkanes in UK. Sewage Sludge: survay results and implications. *Environmental Science & Technology* **37**, 462-467.

Villar, P., Villar, M., Callejón, M., Pérez, J.L., Jiménez, J.C., Alonso, E., Guiraúm, A., 2009. Evolution of polycyclic aromatic hydrocarbons (PAHs) and heavy metals in sludge samples from conventional activated sludge wastewater treatment plants. Environmetrics 20, 561-574.

Wassenberg, D.M., Giulio, R.T.D. (2004). Synergistic embryotoxicity of polycyclic aromatic hydrocarbon aryl hydrocarbon receptor agonists with cytochrome P4501A inhibitors in *Fundulus heteroclitus*. *Environmental Health Perspect* **112**, 1658-1664.

White, P.A., Claxton, L.D. (2004). Benzo[a]pyrene, polynuclear aromatic compounds, Part 1. *Mutation Research* **567**, 227-345.

Wild, S.R., Jones, K.C. (1991). Studies on the polynuclear aromatic hydrocarbon content of carrots (*Daucus carota)*. *Chemosphere* **23**, 243-251.

Wang, W., Qin, Y., Wei, C., Li, Z., Guo, Y., Zhu, Y. (2006). Partitioning of elements and materials during preparation of Antaibao coal. *International Journal of Coal Geology*. 68: 223-232

Wong, C.S.C., Li, X.D. (2004) Pb contamination and isotopic composition of urban soils in Hong Kong. *Sci Total Environ*. **319**, 185–95.

Zhao, F.J., McGrath, S.P., Merrington, G. (2007) Estimates of ambient background concentrations of trace metals in soils for risk assessment. *Environmental Pollution*. **148**, 221-229.

Chapter 3

Reduced bioaccumulation of PAHs by Lactuca satuva L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar

Reduced bioaccumulation of PAHs by *Lactuca satuva* L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar

Sardar Khan[a,b], Ning Wang[a], Brian J. Reid[c], Alessia Freddo[a,c], ChaoCai[a*]

[a]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China.

[b]Department of Environmental Science, University of Peshawar, Pakistan.

[c]School of Environmental Sciences, University of East Anglia, Norwich, UK.

Abstract

The influence of sewage sludge (SS) and sewage sludge biochar (SSBC) upon biomass yield and the bioaccumulation of PAHs into lettuce plants grown in contaminated soil ($\sum$16PAH 20.2 ± 0.9 mgkg^{-1}) is presented. All SSBC amendments (2, 5 and 10%) and the 2% SS amendment significantly ($P < 0.01$) increased lettuce biomass. Both SS and SSBC amendments significantly reduced ($P < 0.01$) the bioaccumulation of PAHs at all application levels; with reduction in $\sum$16PAH concentration ranging between 41.8 - 60.3% in SS amended treatments and between 58.0 - 63.2% in SSBC amended treatments, with respect to the control. Benefits in terms of biomass production and PAHs bioaccumulation reduction were greatest where SSBC was used as a soil amendment. At high application rates (10%) SSBC reduced bioaccumulation of PAHs by between 56% and 67%, while SS reduced bioaccumulation of PAHs by less than 44%.

1. Introduction

Industrialization and urbanization have dramatically increased the volume of sewage sludge produced by wastewater treatment plants throughout the world. In China, approximately 30 million tons of sewage sludge was generated in 2010 (Yu, 2011). Agricultural application of sludge has increased dramatically following the passing of the Ocean Dumping Act (1988). In China, 44% of sewage sludge is used in the agriculture sector (this compares with: 71% in UK; 54% in Germany; 54% in Spain; 65% in France; and 60-65% in the USA (Spinosa, 2011; Yu, 2011; Eljarrat et al., 2008)).

Application of sewage sludge to agricultural land delivers well recognized benefits in terms of nutrient addition, increased soil organic matter content (Benckiser and Simarmata, 1994), benefits to soil structure (Richards et al., 2000) and as a consequence benefits for crop yield (El-Motaium and Abo El-Seoud, 2007). However, negative issues relating to sewage sludge application to agricultural land also exist. It is well documented that over application of sewage sludge can adversely affect soil biota (Creamer et al., 2008). In addition, sewage sludge has the potential to introduce pathogens into the soil (Kelley et al., 1984). The transfer of these pathogens on to food and ultimately into the human food chain has also been established (Reilly, 2001).

Biochar is carbon rich material produced through the process of pyrolysis under limited oxygen conditions (Cao et al., 2011). When applied to soil, biochar increases soil cation exchange and water-holding capacities (Glaser et al., 2002; Bélanger et al., 2004; Keech et al., 2005; Liang et al., 2006). In addition, biochar is rich in nutrients (e.g. P, K) and other microelements (e.g. Mg, Ca, Mn) (Neary et al., 1999). Biochar has also been shown to immobilize metals and reduce the bioavailability of hydrophobic organic compounds (HOCs) due to its sorptive capacity (Wang et al., 2011). It is suggested that the application of biochar to soils that have elevated burdens of metals and HOCs may abate problems associated with their toxicity and their transfer onto and into food and ultimately into the human food chain. This scenario is significant in China where rapid urbanization has lead to elevated levels of metals and HOCs (particularly, polycyclic aromatic hydrocarbons (PAHs)) in peri-urban soils that are extensively used for crop

production. This research provides a direct comparison of sewage sludge (SS) and sewage sludge biochar (SSBC) influence upon crop yield and mitigation of PAH bioaccumulation into lettuce plants grown in contaminated soil.

2. Materials and methods

2.1 Chemicals

All solvents were HPLC/spectro grade purchased from Tedia Company Inc, USA. Silica gel, alumina and sodium sulfate were purchased from Sinopharm Chemical Reagent Co Ltd., China. Surrogate standards (PAH-Mix 24 deuterated, LA20950024HE) and reference materials (PAH-Mix 9, XA20950009CY) were purchased from the laboratory of Dr. Ehrenstorfer, Augsburg, Germany.

2.2 Soil sampling

Soil samples (upper horizon, 0-20 cm) contaminated with PAHs were collected from different locations around Sanming Steel Refinery (Fujian Province, China). Soil samples were sieved (2 mm mesh) and homogenized to provide a composite sample. Sub-samples were freeze-dried at -50˚C and 123 ± 2 Pa and stored at -20˚C in paper sacks for future analyses.

2.3 Biochar preparation

Sewage sludge obtained from Xiamen Yundang wastewater treatment plant was air dried. SSBC was prepared from SS by pyrolysis at 550˚C for 6 h in a high performance automatic controlled furnace (GWL-1200, Henan, China), under a continuous flow of nitrogen. A cooling chamber, with water, was used for passing the off-gas to allow condensation of heavy tars. The biochar was then cooled inside the furnace to room temperature in the presence of nitrogen gas. Properties of SS and SSBC such as pH, EC, LOI, C, N, S, porosity and surface area are given Table S1.

2.4 Experimental design

PAHs contaminated soil was amended with SS or SSBC (n = 4) at application rates of 2, 5 and 10% (defined as SS2, SS5, SS10 or SSBC2, SSBC5, SSBC10, respectively) (dry weight basis). An un-amended control soil was also prepared (n = 4). Treatments had a total mass of 2 kg. Six uniform seedlings of lettuce (*Lactuca satuva* L.) were transplanted to each treatment pot and then thinned to 4 after one week (Khan et al., 2008). The experiment was conducted in a greenhouse under natural light (12 h) with day temperature of 30 ± 3˚C and night temperature of 24 ± 3˚C. Soils were irrigated with deionized water to maintain the moisture content (60% field capacity). The pots were randomized at regular interval to compensate for light and temperature differences inside the greenhouse. Plants were harvested after 8 weeks following their initial transplanting, and separated into shoots and roots. Shoots were rinsed briefly with deionized water, while roots were first washed with tap water and then with deionized water to remove adhering soil particles. After drying with tissue paper, shoots and roots were freeze-dried at -50˚C and 123 Pa and dry weights recorded.

2.5 PAHs extraction and quantification

Lettuce, SS and SSBC samples (2 g) were extracted with dichloromethane (DCM) and acetone (1:1 ratio) using accelerated solvents extraction (ASE, Dionex-350). The extracts were evaporated to 1 mL using a rotary evaporator and purified using silica chromatography columns prepared with silica gel, Al_2O_3 and capped with Na_2SO_4 (all activated before use; see Khan et al., 2008). Thereafter, the columns were washed with hexane. The concentrated extracts, then were loaded to columns to separate the PAHs from other polar interfering compounds. These columns were eluted with 60 mL mixture of hexane and DCM (7:3), the eluted fractions were again evaporated up to 1 mL using rotary evaporator and transferred to Kuderna-Danish concentrator and rinsed with 10 mL of n-hexane. Afterward, the eluted fraction was again reduced to 1 mL under nitrogen flow and transferred to a vial capped with a Teflon-lined septum for analysis of PAHs. The final concentrated extracts analyzed using gas chromatograph

mass spectrometry (GC-MS, Agilent Technologies 5975C) (see Supporting Information).The GC-MS was equipped with an inert XL MSD with a triple axis detector and used under the selected ion monitoring mode. An HP-5 silica fused capillary column (60 m × 0.25 mm inner diameter × 0.25 µm film thickness) was used with helium as the carrier gas at a constant flow rate of 1 ml min^{-1}. The GC oven temperature was programmed to ramp from 50˚C to 200˚C at 10˚C min^{-1}, then to 300˚C at 10˚C min^{-1} and to then hold for 8 min at this temperature. The injector and detector temperatures were 280˚C and 300˚C, respectively. Mass spectra were acquired at the electron ionization mode, while selected ion monitoring (SIM) mode was carried out using the molecular ions selective for individual PAHs. For quality control and data analysis see the supporting information (Appendix B).

The efficiency of ASE extraction and silica column purification for PAH recovery from soil, sludge, biochar, plant samples and sample blanks was checked with surrogate PAH-deuterated standards (acenaphthene d10, chrysene d12, naphthalene d8, perylene d12 and phenanthrene d10). The results showed satisfactory recovery, with the average recovery ranging from 83.6 ± 8.2% to 96.5 ± 6.4%.

2.6 Data analysis

The data were statistically analyzed using the statistical package SPSS 11.5. The measures were expressed in terms of mean, while the figures presented the mean values and standard deviation of four replicates. Statistical significance was computed using Duncan's multiple range test and Paired-samples t-test, with a significance level of $P < 0.01$.

3. Results and discussion

3.1 PAHs in sludge, biochar and soil

The total PAH concentrations in SS, SSBC and soil were, respectively: 2.95±0.10, 4.35 ± 0.33 and 20.2 ± 0.22 mg kg^{-1} (see Table 1). The PAHs concentrations in sewage

sludge were found to be within permissible limits (Σ9PAHs, 6 mg kg^{-1}) as set by Council of the European Community (CEC, 2000) for sludge application (5 tons of dry weight per ha) to agricultural land. The PAH concentrations in SSBC were below those recently recommended by the International Biochar Initiative (IBI, 2012) (between 6 and 20 mg kg^{-1}) and below those reported by Freddo et al. (2012). Comparison of ∑16PAH concentration in the control soil (20.21 ± 0.22 mg kg^{-1}) with those in the SS10 (18.5 ± 1.0 mg kg^{-1}) and SSBC10 (18.6 ± 1.0 mg kg^{-1}) revealed a reduction in PAH concentration of 10%. This result could be explained by the reduced amount (10%) of contaminated soil present in SS10 and SSBC10. This 'dilution' effect will have contributed to the bioaccumulation reductions discussed below but, it is stressed, that bioaccumulation reductions are of far greater magnitude than that can be attributed to dilution alone.

Table 1: PAH concentrations in sludge, biochar and soil (µg kg^{-1}).

PAH	Sludge	Biochar	Soil
Naphtalene	1596 ± 29	748 ± 18	2357 ± 49
Acenaphthylene	ND	ND	276 ± 12
Acenaphthene	ND	ND	150 ± 15
Fluorene	34 ± 0.9	77 ± 2.1	295 ± 5.6
Phenanthrene	263 ± 31	1139 ± 67	2425 ± 34
Anthracene	33 ± 2.4	70 ± 1.4	425 ± 4.7
Fluoranthene	9.6 ± 0.4	332 ± 20.0	2708 ± 57
Pyrene	221 ± 17	530 ± 22.5	2073 ± 27
Benzo(a)anthracene	120 ± 8.7	174 ± 3.7	1434 ± 3
Chrysene	116 ± 2.4	619 ± 4.9	1220 ± 16
Benzo(b)fluoranthene	163 ± 1.2	226 ± 8.7	2187 ± 19
Benzo(k)fluoranthene	64 ± 0.7	89 ± 3.8	800 ± 10
Benzo(a)pyrene	ND	ND	767 ± 7
Indeno(1,2,3-c,d)pyrene	199 ± 3.0	202 ± 4.8	1960 ± 5
Dibenzo(a,h)anthracene	37 ± 2.0	49 ± 1.0	228 ± 1
Benzo(g,h,i)perylene	91 ± 3.2	92 ± 0.9	901 ± 4
Σ16PAH (mg kg^{-1})	**2.95 ± 0.10**	**4.35 ± 0.33**	**20.21 ± 0.22**

ND - not detected

3.2 Plant biomass

The root biomass production in the SS and SSBC treatments followed a similar pattern of changes as those observed for shoots (Figure 1). In all SSBC treatments, the biomass of shoots was significantly higher ($P \leq 0.01$) than the control soil (Figure 1). These three treatments appreciably improved shoot biomass yields when compared to the control, showing increases of: 71%, 93% and 46% in SSBC2, SSBC5 and SSBC10 treatments, respectively. Shoot biomass in the SS2 treatment was also significantly ($P \leq 0.01$) increased (an increase of 83%) with respect to the control soil (Figure 1). It was noted that no appreciable benefit in terms of shoot biomass was realized following SS application at 5% and 10% (Figure 1). The decrease in plant biomass with increasing SS and SSBC application rates could be related with high C:N ratio which presumably limited N availability, thereby slightly reducing plant yield because fertilizers were not added to these treated soils to normalized the C:N ratios. These findings are consistent with the results in literature (Lehmann et al., 2003; Uzoma et al., 2011). In addition, it is equally plausible that potentially toxic elements (PTEs) associated with SS are exerting a negative effect, at higher SS application rates, on plant growth. Issues relating to PTE toxicity in SS amended soils are very well documented (for example, see: Andrés et al, 2011). In contrast to SS, PTE levels in biochar have recently been contextualized as being consistent with concentration in background soil (Freddo et al, 2012). It follows that biochar addition to soil, regardless of application rate, is unlikely to elevate PTE concentrations. Accordingly ecotoxicity relationg to PTEs in biochar amended soils would not be expected.

The results indicate the greater potential SSBC has over SS with respect to promoting plant growth (particularly at higher application rates). The root biomass production in the SS and SSBC treatments followed a similar pattern of changes as those observed for shoots (Figure 1).

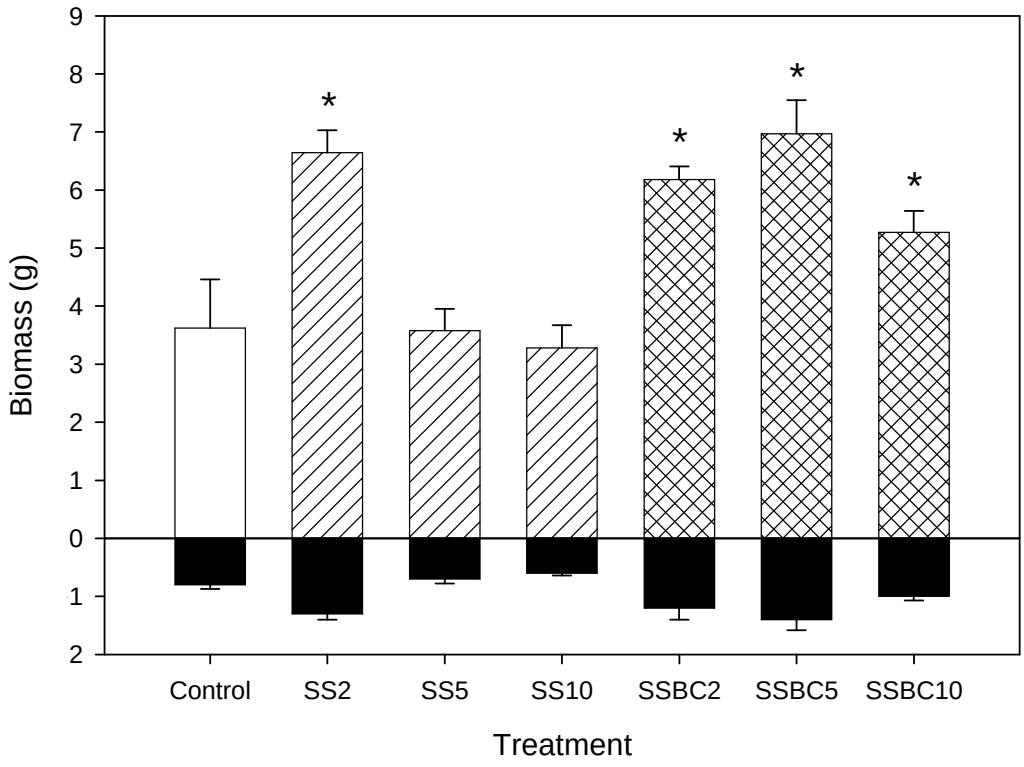


Figure 1: Shoot (white) and root (black) biomass (g dry weight/pot) in the control (un-hatched), sewage sludge amended treatments (SS; hatched) and sewage sludge biochar treatments (SSBC; cross-hatched) at application rates of 2%, 5% and 10%. Error bars indicate $\pm$ 1 standard deviation. Asterisks indicate significantly high whole plant biomass ($P \leq 0.01$) with respect to the control.

3.3 PAHs in plant tissues

All 16 PAHs were detected in lettuce plants grown in control soil and treatments amended with SS and SSBC (Figure 2). The highest single PAH concentration (benzo(a)pyrene: 0.29 mg kg^{-1}) was observed in lettuce grown in the control soil. Fluoranthene was the most prevalent PAH in lettuce grown in soils amended with sewage sludge (from 0.11-0.14 mg kg^{-1}) and biochar (from 0.13-0.15 mg kg^{-1}).

The results indicate that the applications of SS and SSBC significantly ($P \leq 0.001$) decreased the bioaccumulation of both low molecular weight-PAHs (LMW-PAHs) and high molecular weight-PAHs (HMW-PAHs) in lettuce shoots. The concentration of

∑16PAH in lettuce grown in control soil was 1.72 ± 0.08 mg kg^{-1} (Figure 3). The concentrations of total ∑16PAH in lettuce grown in soil amended with SS were all significantly ($P < 0.01$) lower than the control, (mg kg^{-1}): 0.68 ± 0.04 (SS2), 0.86 ± 0.03 (SS5), 1.00 ± 0.04 (SS10). Similarly, the concentrations of total ∑16 PAH in lettuce grown in soil amended with SSBC were all significantly ($P < 0.01$) lower than the control, (mg kg^{-1}): 0.63 ± 0.03 (SSBC2), 0.69 ± 0.04 (SSBC5), 0.72 ± 0.03 (SSBC10). SS application reduced ∑16 PAH bioaccumulation by 41.8-60.3%, while SSBC application reduced total ∑16PAH bioaccumulation by 58.0-63.2% (Figure 3).

At an application rate of 2%, bioaccumulation of individual PAHs in the presence of SS was closely related to values detected in the presence of SSBC (Figure 4A). Thus, SS and SSBC, across all PAHs, had an equivalent influence upon resultant PAH concentrations in lettuce. In most cases SS and SSBC decreased PAH concentrations in lettuce by between 60 and 70% (Figure 4A). Exceptions to this being: phenanthrene (30% decrease), fluoranthene (35% decrease) and pyrene (40% decrease).

At an application rate of 5%, SSBC was observed to be more effective at reducing PAH concentrations in lettuce than SS for the majority of PAHs i.e. data points fell in the upper left portion of the frame (Figure 4B). The only exceptions to this being phenanthrene, fluoranthene and pyrene (Figure 4B). Of these PAHs, phenanthrene and pyrene were noted to be particularly abundant in SS (contributing 8.9% and 7.5% to the ∑PAH concentration, respectively (Table 1)).

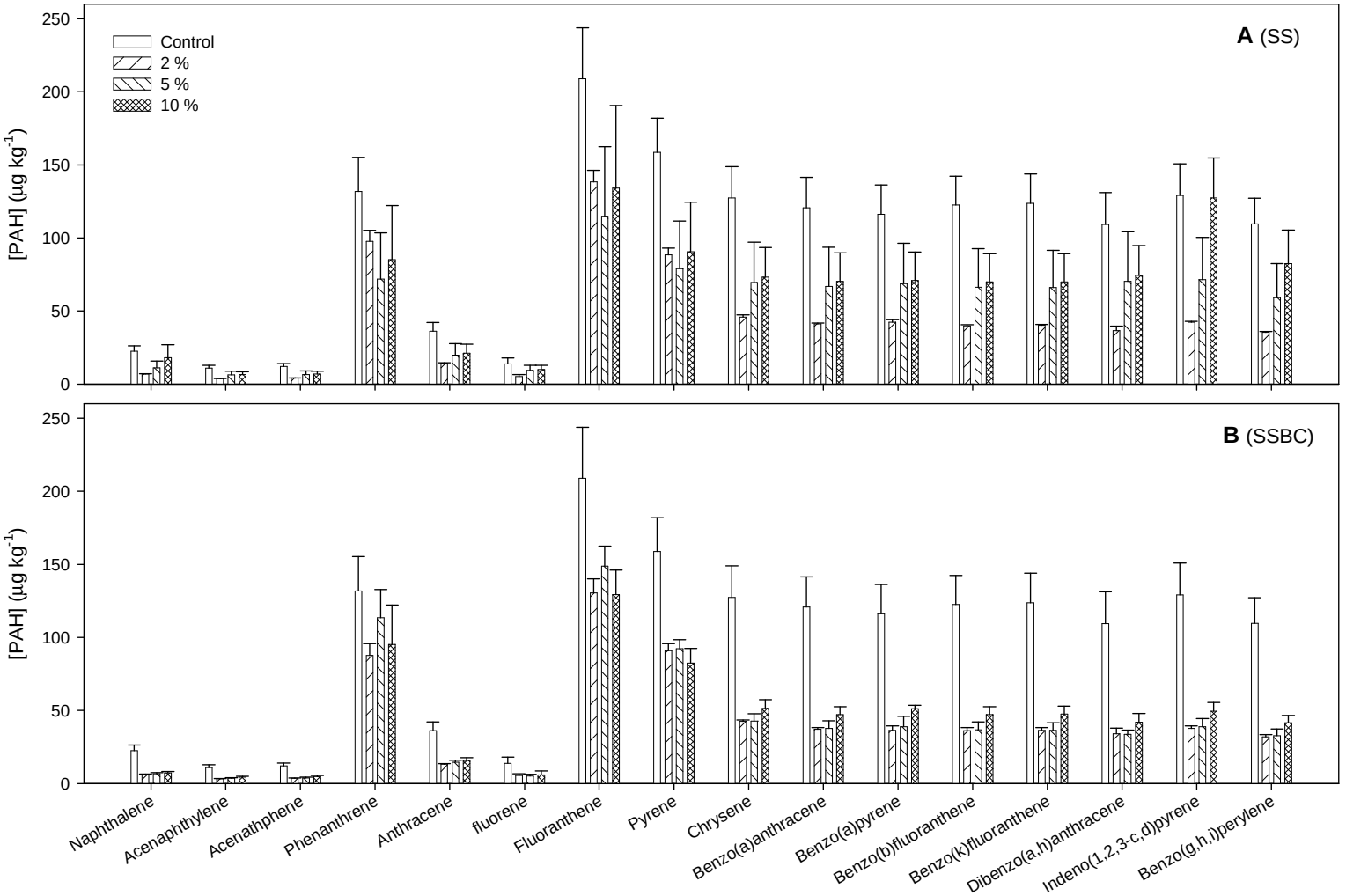


Figure 2: Individual PAH concentration in lettuce shoots grown in control soil and soils amended with SS (A) and with SSBC (B) with amendment applications of 2 %, 5 % and 10 %. Error bars $\pm$ 1 standard deviation (n = 4).

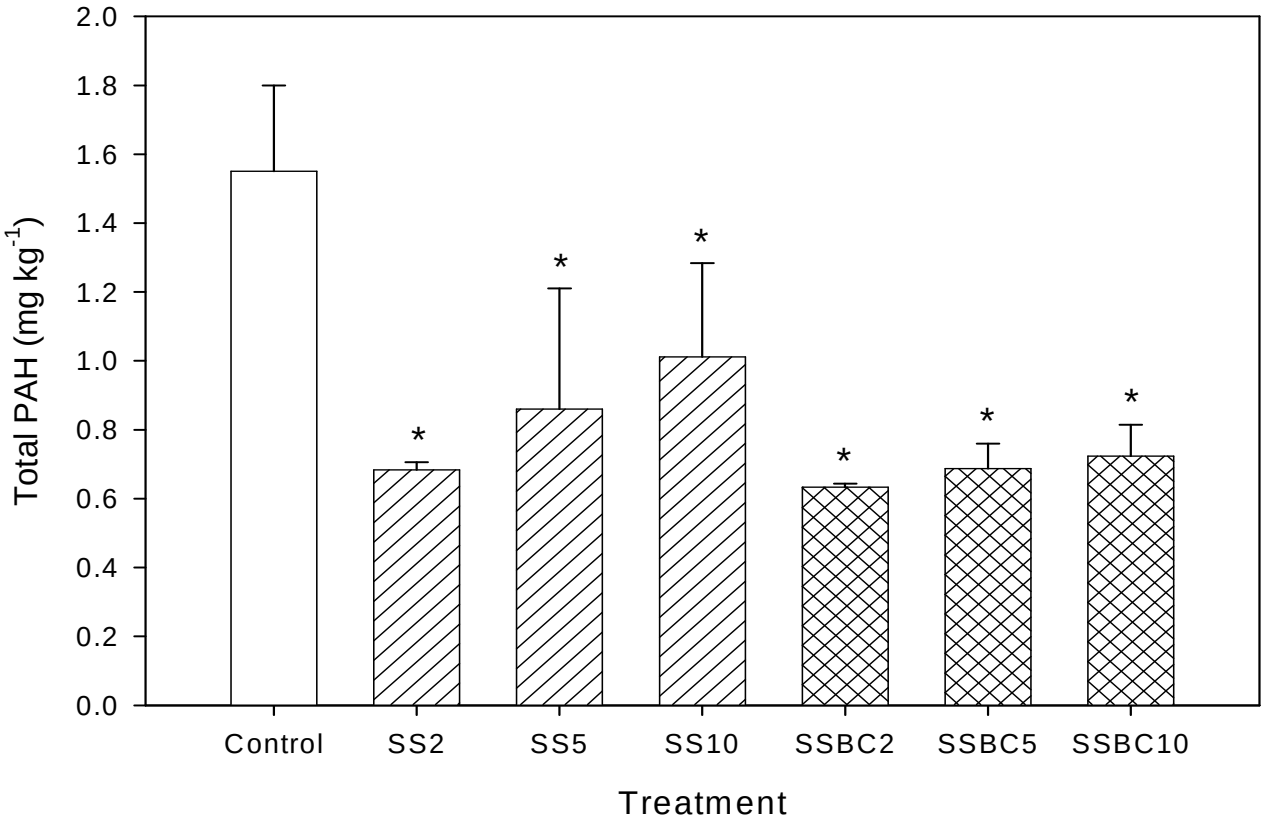


Figure 3: PAH concentrations (mg kg^{-1})in shoot biomass in the control (un-hatched), sewage sludge amended treatments (SS; hatched) and sewage sludge biochar treatments (SSBC; cross-hatched) at application rates of 2%, 5% and 10%. Error bars indicate $\pm$ 1 standard deviation. Asterisks (*) indicate significantlydecreases($P \leq 0.01$) with respect to the control.

These LMW-PAHs would be expected to be of greater availability (Latawiec et al., 2008). It is suggested, therefore, that the higher application rate of 5% may have increased the concentration of *available* LMW-PAHs (phenanthrene and pyrene in particular) and as a consequence bioaccumulation of these PAH increased with respect to the 2% SS application treatments. PAH partitioning in biochar has been reported to be strong; Freddo et al. (2012) reported pressurized subcritical water extraction to be unable to liberate PAHs from softwood biochar (produced at 500˚C) above limits of detection. It is suggested that while SSBC would have provided PAHs to the soil that these were largely non-available and as a consequence were not subject to bioaccumulation.

At an application rate of 10%, SSBC was again observed to have the greatest influence upon PAHs concentrations in lettuce. Only in the case of phenanthrene the reduction of PAHs bioaccumulated in to lettuce relative to extent of bioaccumulation observed in the control soils was more effective in SS (35%) than SSBC (28%) in reducing resultant concentrations in lettuce. Both fluoranthene and pyrene concentration in lettuce were influenced to an equivalent extent by SS and SSBC. In all other case SSBC was more effective at reducing PAHs concentrations in lettuce when compared to SS relative to extent of bioaccumulation observed in the control soils; ranging from 56% to 67% in the SSBC10 treatments and from only 1% to 44% in the SS10 treatments.

Accounting for these difference in bioaccumulation of PAH, biochar has been reported to have a high sorptive capacity which is due to its particular chemical and physical structure (Zhu and Pignatello, 2005; Zhu et al., 2005; Spokas et al., 2009) these properties of biochar are very different to those of other organic matrices, for example, sewage sludge. Furthermore, while biochar may have the potential to deliver additional PAH into the soil, in reality biochar PAHs have very low bioavailability (Freddo et al, 2012).

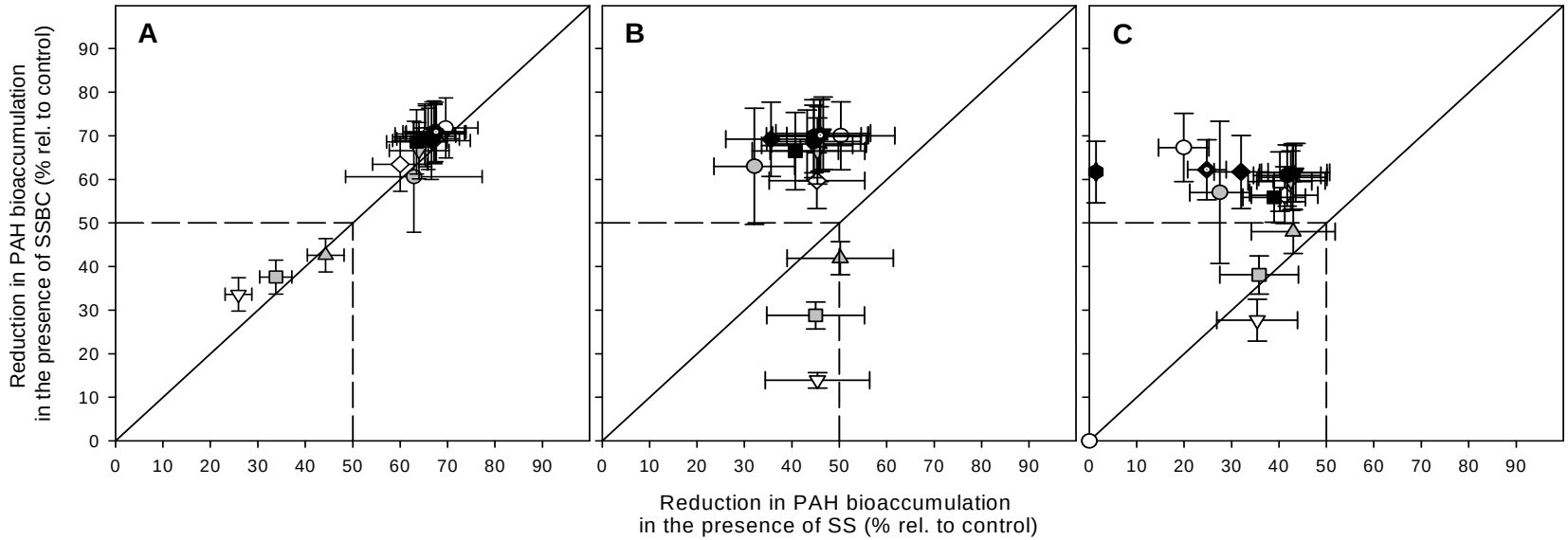


Figure 4: Influence of SS and SSBC amended into contaminated soil at application rates of 2% (A), 5% (B) and 10% (C) upon the % reduction in bioaccumulation of PAHs into *Lactuca satuva* L relative to extent of bioaccumulation observed in the control soils of light (white), medium (grey) and heavy (black) PAHs (see Table S2 symbol key). The solid lines are lines of 1:1 relationship while the dashed lines indicate the 50% reduction in bioaccumulation of PAHs into *Lactuca satuva* L relative to extent of bioaccumulation observed in the control. Errors are root mean square derived combined standard errors for control values (n = 3) and values for respective treatments (n = 4).

Bioconcentration factors (BCFs (Ryan et al, 1988)), were calculated for benzo(a)pyrene (BaP). BaP was chose on account of its greater carcinogenic potential (Kipopoulou et al., 1999; Phillips, 1983; Sims et al., 1974). The BaP BCF for the control ($BFC_{control}$) was 0.15 ± 0.03. This value is in keeping with the mean BaP BCF of 0.16 for lettuce grown in PAHs contaminated industrial soil (Kipopoulou et al., 1999). In the SS amended treatments, the BaP BCFs were lower, with respect to $BCF_{control}$, but were observed to increase appreciably as SS amendment increased: 0.056 ± 0.002 (SS2); 0.094 ± 0.038 (SS5); 0.103 ± 0.028 (SS10); this being attributed to BaP in SS being available for bioaccumulation. The BaP BCFs were also lower, with respect to $BCF_{control}$, in the SSBC amended treatments but, in contrast to SS, BaP BCFs increase much less markedly as SSBC amendment increased: 0.048 ± 0.003 (SSBC 2); 0.053 ± 0.0095 (SSBC 5) and 0.074 ± 0.0034 (SSBC 10); this being attributed to BaP in SSBC being non-available for bioaccumulation due to high sorptive capacity of biochar.

Recently, the application of biochar to soil has been shown to significantly enhance the sorption of PAHs (Tian et al., 2010; Chen and Yuan, 2011). Like other carbonaceous sorbents, biochars have also been found to decrease the bioavailability of PAHs (Hwang and Cutright 2004; Brandli et al., 2008; Beesley et al., 2010). In addition to reducing the opportunity for soil to pore water to plant transfer of PAHs, biochar's influence upon PAHs partition will also reduce the opportunity for PAHs transfer from soil to air to plant (Wild et al., 1991; Kipopoulou et al., 1999; Howsam et al., 2001). Combined, these transfer mechanisms, are likely to underpin the decreased bioaccumulation of PAHs in the SSBC treatments.

4. Conclusions

SSBC and SS applications were effective at significantly reducing the bioaccumulation of PAHs in lettuce. Benefits in terms of biomass production and PAHs bioaccumulation reduction were greatest where SSBC was used as a soil amendment. Given the phytotoxicity of SS at high application rates and risks associated with SS pathogens it is concluded that SSBC represents a promising alternative to SS as a soil amendment.

Acknowledgement

This research was financially supported by the National Natural Science Foundation of China (40801118, 41271324) and Higher Education Commission, Islamabad, Pakistan.

References

Andrés, P., Mateos, E., Tarrasón, D., Cabrera, C., Figuerola, B., 2011. Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna. Applied Soil Ecology 48, 236-242.

Bélanger, N.I., Côté, B., Fyles, J.W., Chourchesne, F., Hendershot, W.H., 2004. Forest regrowth as the controlling factor of soil nutrient availability 75 years after fire in a deciduous forest of southern Quebec. Plant Soil 262, 363-372.

Benckiser G., Simarmata T. 1994. Environmental impact of fertilizing soils by using sewage and animal wastes. Fertilizer Research 37, 1-22.

Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J.L., 2010. Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental Pollution 158, 2282-2287.

Brandli, R.C., Hartnik, T., Henriksen, T., Cornelissen, G., 2008. Sorption of native polyaromatic hydrocarbons (PAH) to black carbon and amended activated carbon in soil. Chemosphere 73, 1805-1810.

Cao, X., Ma, L., Liang, Y., Gao, B., Harris, W., 2011. Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environmental Science & Technology 45, 4884-4889.

CEC, 2000. Council of the European Community, Working Document on Sludge, 3rd Draft, Brussels, 27 April, p. 20.

Chen, B., Yuan, M., 2011. Enhanced sorption of polycyclic aromatic hydrocarbons by soil amended with biochar. Journal of Soils and Sediments 11, 62-71.

Cornelissen, G., Gustafsson, O.R., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environmental Science & Technology 39, 6881- 6895.

Downie, A., Crosky, A., Munroe, P., 2009. Physical properties of biochar. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp.13-32.

Dumontet, S., Scopa, A., Kerje, S., Krovacek, K., 2001. The importance of pathogenic organisms in sewage and sewage sludge. Journal of the Air and Waste Management Association 51,848-860.

Eljarrat, E., Marsh, G., Labandeira, A., Barceló, D., 2008. Effect of sewage sludges contaminated with polybrominated diphenylethers on agricultural soils. Chemosphere 71, 1079-1086.

El-Motaium R. A. And Abo El-Seoud M. A. (2007) Irradiated sewage sludge for production of fennel plants in sandy soil. Nutrient Cycling Agroecosystems 78, 133–142.

Freddo, A., Cai, C., Reid, B.J. (2012) Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environmental Pollution 171, 18-24.

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biology and Fertility of Soils 35, 219-230.

Howsam, M., Jones, K.C., Ineson, P., 2001. PAHs associated with the leaves of three dediduous tree species. II: uptake during a growing season. Chemosphere 44, 55–164.

Hossain, M.K., Strezov, V., Chan, K.Y., Ziolkowski, A., Nelson, P.F., 2010. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (*Lycopersicon esculentum*). Chemosphere 78, 1167-1171.

Hwang, S., Cutright, T.J., 2004. Preliminary evaluation of PAH sorptive changes in soil by Soxhlet extraction. Environment International 30, 151-158.

IBI 2012: The International Biochar Imitative Standardized Product Definition and Product Testing Guidelines for Biochar That Is Used in Soil. Final Version (IBI-STD-01: Revision Date15 May 2012). Downloaded (31 July 2012). http://www.biochar-international.org/sites/default/files/Guidelines_for_Biochar_That_Is_Used_in_Soil_Final.pdf

Keech, O., Carcaillet, C., Nilsson, M.C., 2005. Adsorption of allelopathic compounds by wood-derived charcoal: The role of wood porosity. Plant and Soil 272, 291-300.

Kelley, W. D., Martens, D. C., Reneau, R. B., Simpson, T.W. 1984. Agricultural Use of Sewage Sludge: A Literature Review. Virginia Water Resources Research Center, Virginia Polytechnic Institute and State University Blacksburg, Virginia 24060-3397, pp. 11-13

Khan, S., Aijun, L., Zhang, S., Hu, Q., Zhu, Y-G., 2008. Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. Journal of Hazardous Materials 152, 506-515.

Khan, S., Cao, Q., 2012. Human health risk due to consumption of vegetables contaminated with carcinogenic polycyclic aromatic hydrocarbons. Journal of Soils and Sediments 12, 178-184.

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. , Soil Biology & Biochemistry 43, 385-392.

Kipopoulou, A.M., Manoli, E., Samara, C., 1999. Bioconcentration of PAHs in vegetables grown in an industrial area. Environtal Pollution 106, 369–380.

Latawiec, A.E., Swindell, A.L., Reid, B.J., 2008. Environmentally friendly assessment of organic compound bioaccessibility using sub-critical water. Environmental Pollution. 156, 467-273.

Lehmann, J., da Silva Jr., J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaelogical anthrosol and a ferralsol of the Central Amazon basin: fertilizer, manure, and charcoal amendmends. Plant Soil. 249, 343-357.

Lehmann, J., Joseph, S., 2009. Biochar for environmental management: an introduction. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp.1-12.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Thies, J.O., Luizão, F.J., Petersen, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal 70, 699-709.

Lohmann, R., Macfarlane, J. K., Gschwend, P. M., 2005. Importance of black carbon to sorption of native PAHs, and PCDDs in Boston and New York harbor sediments. Environmental Science and Technology. 39, 141-148.

Matejovic, I., 1997. Determination of carbon and nitrogen in samples of various soils by the dry combustion. Communications in Soil Science and Plant Analysis 18, 1499-1511.

Neary, D.G., Klopaket, C.C., DeBano, L.F., Folliot, P.F., 1999. Fire effects of below-ground sustainability: A review and synthesis. Forest Ecology and Management 122, 51-71.

Northcott, G.L., Jones, K.C., 2001. Partitioning, extractability, and formation of non-extractable PAH residues in soil. 1. Compound differences in aging and sequestration. Environmental Science & Technology 35, 1103-1110.

Peng, J.J., Cai, C., Qiao, M., Li, H., Zhu, Y-G., 2001. Dynamic changes in functional gene copy numbers and microbial communities during degradation of pyrene in soils. Environmental Pollution 158, 2872-2879.

Phillips D. H., 1983. Fifty years of Benzo(a)pyrene. Nature 303, 468-472

Reilly, M., 2001. The case against land application of sewage sludge pathogens. Canadian Journal of Infectious Diseases 12, 205-207.

Rhodes, A.H., Carlin, A., Semple, K.T., 2008. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. Environmental Science& Technology 42, 740-745.

Richards, B.K., Steenhuis, T.S., Peverly, J.H., McBride, M.B. 2000. Effect of sludge processing mode, soil texture and soil pH on metal mobility in undisturbed soil columns under accelerated loading. Environmental Pollution. 109, 327-346.

Ryan, J. A., Bell, R. M., Davidson, J. M., O'Connor, G. A., 1988. Plant uptake of non-ionic organic chemicals from soils. Chemosphere 17, 2299–2323

Selivanovskaya, S.Y., Latypova, V. Z., Gubaeva, L.A., 2006. Microbiological processes in gray forest soil treated with sewage sludge compost. Eurasian Soil Science 39, 443-449.

Sims P., Grover P. L., Swaisland A., Pal K., Hever A., 1974. Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide. Nature 252, 326-328.

Sohi, S., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil. Advances in Agronomy 105, 47-82.

Spinosa, L., 2011. Wastewater sludge: A global overview of the current status and future prospects. IWA publishing ltd. Alliance house 12 Caxton St, London. Pp. 28.

Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C., 2009. Impact of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77, 574-581.

Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biology & Biochemistry 41, 1301-1310.

Tian, C., Wang, M. D., Si, Y. B., 2010. Influences of charcoal amendment on adsorption-desorption of isoproturon in soils. Agricultural Sciences in China 9, 257-265.

Uzoma, K.C., Inoue, M., Andry H., Fujimaki H., Zahoor A., Nishihara E., 2011. Effect of cow manure biochar on maize productivity under sandy soil condition. Soil Use and Management. 27, 205-212.

Wang, F., Bu, Q., Xia, X., Shen, M., 2011. Contrasting effects of black carbon amendments on PAH bioaccumulation by *Chironomus plumosus* larvae in two distinct sediments: Role of water absorption and particle ingestion. Environmental Pollution 159, 1905-1913.

Wang, D., Chen, J., Xu, Z., Qiao, X., Huang, L., 2005. Disappearance of polycyclic aromatic hydrocarbons sorbed on surfaces of pine (*Pinua thunbergii*) needles under irradiation of sunlight: Volatilization and photolysis. Atmospheric Environment 39, 4583-4591.

Wild, S. R., Jones, K. C., 1991. Organic chemicals entering agricultural soils in sweage sludges: screening for their potential to transfer to crop plants and

Chapter 4

Influence of biochar on isoproturon partitioning and bioavailability

Influence of biochar on isoproturon partitioning and bioavailability

Reid, B.J.[1], Pickering, F.L.[1], Freddo, A.[1], Whelan, M.J.[2]., Coulon., F.[2]

[1]School of Environmental Sciences, University of East Anglia,
Norwich, NR4 7TJ, UK.

[2]School of Applied Sciences, Department of Environmental Sciences and Technology
Cranfield University, Cranfield, MK43 0AL, UK.

Abstract

The influence of biochar (5 %) on the temporal loss, partitioning and bioavailability of isoproturon (IPU) in soil was evaluated. Results indicated that biochar had a dramatic effect on IPU partitioning: IPU extractability (0.01M $CaCl_2$) in treatments containing biochar was reduced to < 2 % while, IPU extractability in biochar free treatments ranged between 90 % and 40 % depending on application rate and incubation time. A partitioning box-model was constructed for the soil-biochar matrix and fitted to the experimental data to derive an effective partition coefficient for biochar:water (K_{BW} of 7.82 x 10^4 L kg^{-1}). This was 124 times greater than the apparent K_{foc} value of the biochar free treatments (631 L kg^{-1}). ^{14}C-radiorespirometry assays indicated high competence of microorganisms to mineralise ^{14}C-IPU in the absence of biochar (maximum ^{14}IPU mineralisation was 40.3 $\pm$ 0.9 %). Where biochar was present ^{14}C-IPU mineralisation never exceeded 2 %; indicating that IPU sequestration significantly reduces herbicide bioavailability and the development of catabolic activity. Increasing IPU application from a recommended dose (1 mg kg^{-1}) to x10 this dose was ineffective at redressing IPU sequestration and its consequentially low bioavailability.

1. Introduction

Biochar is defined as the carbon-rich product obtained when biomass is heated in an oxygen limited environment (Blackwell et al., 2009). Biochar is composed primarily of recalcitrant carbon structures (Sombroek et al., 2003). The recalcitrant properties of biochar carbon prevent its mineralisation and as a consequence the addition of biochar to soil results in long-term carbon storage (McGill, 1996; Sohi et al., 2009). In addition to these carbon sequestration benefits, biochar amendment to soil has also been reported to bring benefits in terms of both soil physical and biological attributes, with a number of authors reporting enhancing plant growth following biochar amendment to soil (Glaser et al., 2002; Lehmann et al., 2003; Lehmann and Rondon, 2006; Collison et al., 2009). Soil improvements have been linked to two key factors, namely, i) soil fertility (through nutrient provision and influence on nutrient cycling via changes to cation exchange capacity) (Sohi et al., 2009; Chan and Xu, 2009; Verheijen et al, 2009), and, ii) influences on soil water dynamics (Villarreal et al., 2010).

While these reports highlight the agronomic benefits of biochar and, thereby, support its application to agricultural land, the sorptive capacity of biochar for soil applied herbicides may undermine these benefits if they reduce herbicide efficacy. The extensive sorptive capacity of biochar was recently been reported by Rhodes et al. (2008) and Yu et al. (2009). This capacity has been related to a number of mechanisms, including: i) greater abundance of association sites being present in biochar-amended soil *vs.* soil only, ii) greater affinity between herbicides and the matrix resulting in stronger association and sorption-desorption hysteresis, and, iii) greater opportunity for herbicide entrapment within a more porous biochar-soil matrix. While organic chemical interactions with biochar or other 'black carbon' materials has received considerable attention (Yu et al., 2009; Jonker and Koelmans, 2002; Thorsen et al., 2004; Jonker et al., 2005; Smernick, 2009) there have been relatively few studies on herbicide interaction in soil-biochar mixtures. In addition, existing studies are focused mainly on the influence of biochar abundance rather than on pesticide behavior (Yang and Sheng, 2003; Quilliam et al., 2012).

The research reported herein considers the temporal implications of biochar presence (5 %) on the herbicide isoproturon (IPU) applied to soil at a recommended application rate of 1 mg kg^{-1} d.w. soil and up to ten-times this recommended application rate. This research draws together evidence regarding biochar influence on: i) herbicide

dissipation (loss), ii) herbicide partitioning, and iii) herbicide bioavailability. A simple partitioning model was constructed to describe the interactions between the soil matrix, added biochar and the soil solution. Biochar:water partition coefficient (K_{BW}) have been calculated and compared with conventional soil K_{foc} values.

2. Materials and Methods

2.1 Chemicals

IPU (3-(4-isopropylphenyl)-1,1-dimethyl urea; structure shown in Figure S1) was used as the formulation 'Arelon 500' provided by NUFARM Ltd., UK. Liquid scintillation fluids (Ultima Gold and Ulitma Gold XR) and sample oxidiser cocktails (Carbosorb and Permafluor) were provided by Perkin Elmer, UK. Calcium chloride AR and potassium hydroxide AR were provided by Merck, UK.

2.2 Soil

The upper 10 cm layer of an agricultural silty loam soil, collected from a farm in Edgfield, Norfolk (TG 113 355), was partially dried (residual moisture = 2.6%) and homogenised by screening through a 2-mm sieve. This soil was selected as it had not received any IPU in the preceding three years.

2.3 Biochar

Biochar was obtained from a quarter-scale (500 kW) gasifier (Refgas UK, Flintshire, UK), fuelled by waste softwood chips from a sawmill. The gasification zone of the plant operated at around 1000ºC, the pyrolysis section around 500˚C and the "drying zone" at 200˚C. Negative pressure (-25 mbar) was maintained in the reactor. Throughput time from the drying zone to the ash discharge section was 1 hour.

2.4 Microcosms

Microcosms were established in sterile 25 ml glass vials containing either 500 g of air dried soil or a mixture of air dried soil (500 g) and biochar (25 g). Each microcosm was spiked with ^{12}C-IPU at 1, 2, 5 and 10 mg kg^{-1}; with or without ^{14}C-IPU at 40 Bq g^{-1}. A ^{12}C-IPU stock solution (1000 mg L^{-1}) was prepared in ethanol using Arelon 500 and

subsequent dilutions were prepared in MilliQ water such that a given stock solution could be added at a rate of 1 ml per 100 g d.w. soil to achieve the desired IPU dose. Similarly, a ^{14}C-IPU stock solution was prepared such that 200 μL per 100 g d.w. soil achieved the desired radioactivity. Spiking and soil rehydration was carried out as described by Reid et al. (2005). Each microcosm treatment was established in quadruplicate. Microcosms were incubated in the dark between 10°C and 16°C. ^{12}C/^{14}C-IPU treatments were used for assessment of residual IPU and extractable IPU while ^{12}C-IPU treatments were used to assess catabolic competence. The lowest dose of IPU (1 mg kg^{-1}) was selected based upon the regulatory agriculture application rate for IPU of 1.5 kg ha^{-1} with the assumption that IPU would be incorporated to a depth of 10 cm in a soil with a bulk density of 1.5 g cm^{-3}.

2.5 Determination of residual IPU following incubation

Residual IPU remaining in the soil following incubation periods of 1, 13, 34 and 62 days was determined by sample oxidation. Soil samples from the ^{14}C/^{12}C-IPU treatments (1g; n = 4) were placed into cellulose combustion cones and 100 μL of Combustaid™ was added. The samples were then combusted using a Packard 307 Sample Oxidiser over a burn time of 2.5 min. Liberated carbon dioxide was trapped using Carbosorb and eluted using Permafluor. Combustion efficiency was established to be > 97 % with carryover < 0.1 % prior to any samples being processed. ^{14}C-radioactivity in the eluted samples was determined by liquid scintillation counting (Perkin-Elmer Tri-Carb 2900TR liquid scintillation analyser; count time 10 min). The ratio of ^{14}C-radioactivity (per g of soil) and the mass of ^{12}C-IPU (per g of soil) was used to convert activities observed to mass of IPU present.

2.6 Determination of IPU partitioning

An aqueous $CaCl_2$ extraction technique was used to determine easily extractable IPU. While Jonker and Koelman (2002) suggested that aqueous-based extractants (such as $CaCl_2$) may experience difficulties in penetrating black carbon matrices and as a consequence overestimate sorbent absorption, such non-exhaustive extraction techniques have been proposed to better reflect compound ageing phenomena.[21] Mordaunt et al. (2005) justified the use of 0.01 M $CaCl_2$ as an extract to simulate the readily available fraction of pesticides, including IPU. Samples of ^{14}C/^{12}C-IPU spiked

soils (3 g, n=4) were weighed into Teflon centrifuge tubes and 0.01 M $CaCl_2$ (30 mL) added. Tubes were then placed on their sides on a flatbed shaker and shaken for 18 h at 100 r.p.m (IKA Labortechnik KS501). Thereafter, samples were centrifuged (at 2000 r.p.m. for 20 min; Sigma laboratory centrifuge 4K15). A sample of supernatant (10 mL) was then removed and added to a liquid scintillation vial containing Ultima Gold XR (10 mL). Samples were stored in the dark for a minimum of 24 h before ^{14}C-radioactivity was determined by liquid scintillation counting (Perkin-Elmer Tri-Carb 2900TR liquid scintillation analyzer; count time 10 min). Soil samples containing no ^{14}C-IPU were processed in a similar manner and used to blank-correct the activities observed. The fractions of IPU easily extracted into $CaCl_2$ are reported relative to residual activity at time of extraction (not the originally spiked activity).

2.7 Determination of IPU catabolic competence

^{14}C-radiorespirometry was used to determine the catabolic competence of microbes to degrade IPU (Reid et al., 2005; Semple et al., 2007; Mordaunt et al., 2005; Posen et al., 2006). Catabolic competence is defined as the relative ability of the microorganisms in a given treatment type to mineralise ^{14}C-IPU to $^{14}CO_2$ (the level of competence being reported as extent (%) of mineralisation). Samples of ^{12}C-only IPU spiked soil (10 g, n=5) was added to sterile Schott bottles (250 mL) containing sterile distilled water (30 mL) and a spike of ^{14}C-IPU added (250 Bq in 100 µl of ethanol). A vial containing 1M KOH (1 mL) was suspended from the top of the Teflon lined respirometer lid to capture $^{14}CO_2$ produced by microbial mineralization of the freshly added ^{14}C-IPU spike. The flasks were shaken (100 r.p.m.; IKA Labortechnik KS501) and the vials replaced following respirometer assay times of: 12 h, 1 d, 2 d, 4 d, 6 d, 8 d, 10 d, 12 d, 17 d, and 22 d. Once vials were removed Ultima Gold scintillation fluid (5 mL) was added, the samples shaken and stored in the dark for a minimum of 24 h before ^{14}C-radioactivity was determined by liquid scintillation counting (Perkin-Elmer Tri-Carb 2900TR liquid scintillation analyzer; count time 10 min). All results were blank-corrected using CO_2 traps obtained from respirometers that were not spiked with ^{14}C-IPU.

2.8 Estimating a partition coefficient for biochar

A simple portitioning model was constructed in order to estimate IPU partition coefficients between organic carbon and water, and between biochar and water. A four

phase system consisting of soil solids, biochar, air and water was considered. System dimensions and physical and chemical parameters used are presented in the Supporting Information (Appendix C). In the soil solids, the chemical was assumed to sorb only to soil organic carbon and to biochar.

The total mass of IPU, M_T, is assumed to be the sum of the IPU masses in the organic carbon phase (M_C), in water (M_W), in air (M_A) and, if present, in biochar (M_B):

$$M_T = M_C + M_W + M_A + M_B = m_C \cdot C_{OC} + V_W \cdot C_W + V_A \cdot C_A + m_{BC} \cdot C_{BC} \quad (1)$$

where m_C is the mass of organic carbon present in the system (kg), C_{OC} is the IPU concentration in organic carbon (mg kg^{-1}), V_W is the volume of water in the system (L), C_W is the concentration of IPU in water (mg L^{-1}), V_A is the volume of air in the system (L), C_A is the concentration of IPU in air (mg L^{-1}), m_{BC} is the mass of biochar carbon in the system (kg) and C_{BC} is the IPU concentration on biochar carbon (mg kg^{-1}), assuming that the chemical only sorbs to carbon.

The equilibrium partition coefficients for air:water (K_{AW}); organic carbon:water (K_{OC}); and biochar : water (K_{BW}) are defined as follows:.

$$K_{AW} = \frac{C_A}{C_W} \quad (2)$$

$$K_{OC} = \frac{C_{OC}}{C_W} \quad (3)$$

$$K_{BW} = \frac{C_{BC}}{C_W} \quad (4)$$

The partition coefficients were then used to rearrange Eq. 1 to yield:

$$M_T = m_C.K_{OC}.C_W + V_W.C_W + V_A.K_{AW}.C_W + m_{BC}.K_{BW}.C_W \quad (5)$$

As it is widely accepted that IPU sorption in soils is most appropriately described using the Freundlich equation (Singh et al., 2001; Chao et al., 2010) in which the relationship between the sorbed phase (Cs) and the dissolved phase (C_W) is defined as:

$$C_S = K_f.C_W^{1/n} \quad (6)$$

or, in terms of sorption to carbon, as:

$$C_{OC} = K_{foc}.C_W^{1/n} \quad (7)$$

where K_f, $1/n$ and K_{foc} are empirical (fitted) coefficients. Eq. 5 can be rearranged assuming that no biochar is present, by substituting Eq. 7 for the $K_{OC}.C_W$ term, as follows:

$$\frac{M_T - C_W.(V_W + V_A.K_{AW})}{m_C} = K_{foc}.C_W^{1/n} \quad (8)$$

There are two unknowns in this equation K_{foc} and $1/n$. We estimated values for these terms by trial and error optimisation using the Solver feature in Microsoft Excel so as to minimise the combined root mean squared error (RMSE) between the left hand side (which contains only known terms) and the right hand side of Eq.8 (which contains the unknown terms) calculated for all sample times and all IPU doses in the biochar-free treatments. Specifically,

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(X1_i - X2_i)^2}{N}} \quad (9)$$

where i is a single combination of nominal IPU dose and sampling time, $X1_i$ is the C_{OC} calculated from the left hand side of Eq. 8 for combination i and $X2_i$ is the C_{OC} calculated from the right hand side of Eq. 8 for combination i, using a common pair of K_{foc} and$1/n$ values (the optimised values) for all sampling times (1, 13, 34 and 62 days) and nominal IPU doses (1, 2, 5 and 10 mg kg^{-1}) [N = 16].

The shape of the IPU sorption isotherm for biochar is unknown. We can derive the following definition of K_{BW}:

$$K_{BW} = \left(\frac{\frac{M_T - m_C.K_{foc}.C_W^{1/n}}{C_W} - (V_W + V_A.K_{AW})}{m_{BC}} \right) \quad (10)$$

For the purpose of estimating K_{BW}, we assume that C_W is equivalent to the mass of IPU extracted in $CaCl_2$ divided by the volume of water in the soil plus the volume of the $CaCl_2$ extractant used. In all cases, we assume that K_{AW} can be calculated from published values of the Henry's law constant.

***2.9 Statistical Analysis*:** Student t-tests were used to assess significant difference between paired treatments and ANOVA *post hoc* Tukey tests using SPSS 16.

3. Results and discussion

***3.1 Influence of biochar on IPU loss*:** One day after spiking all of the amended treatments contained the intended dose of IPU within a tolerance of approximately 20 % (Figure 1). Levels of IPU achieved in couplets of biochar and biochar-free treatments were not significantly different ($p < 0.05$) at this time (Figure 1). Upon incubation negligible IPU loss occurred from any of the treatments (regardless of biochar presence or absence and IPU dose applied) (Figure 1). Across all treatments, no significant differences ($p < 0.05$) were observed in comparisons between IPU concentration at 1 d and 62 d.

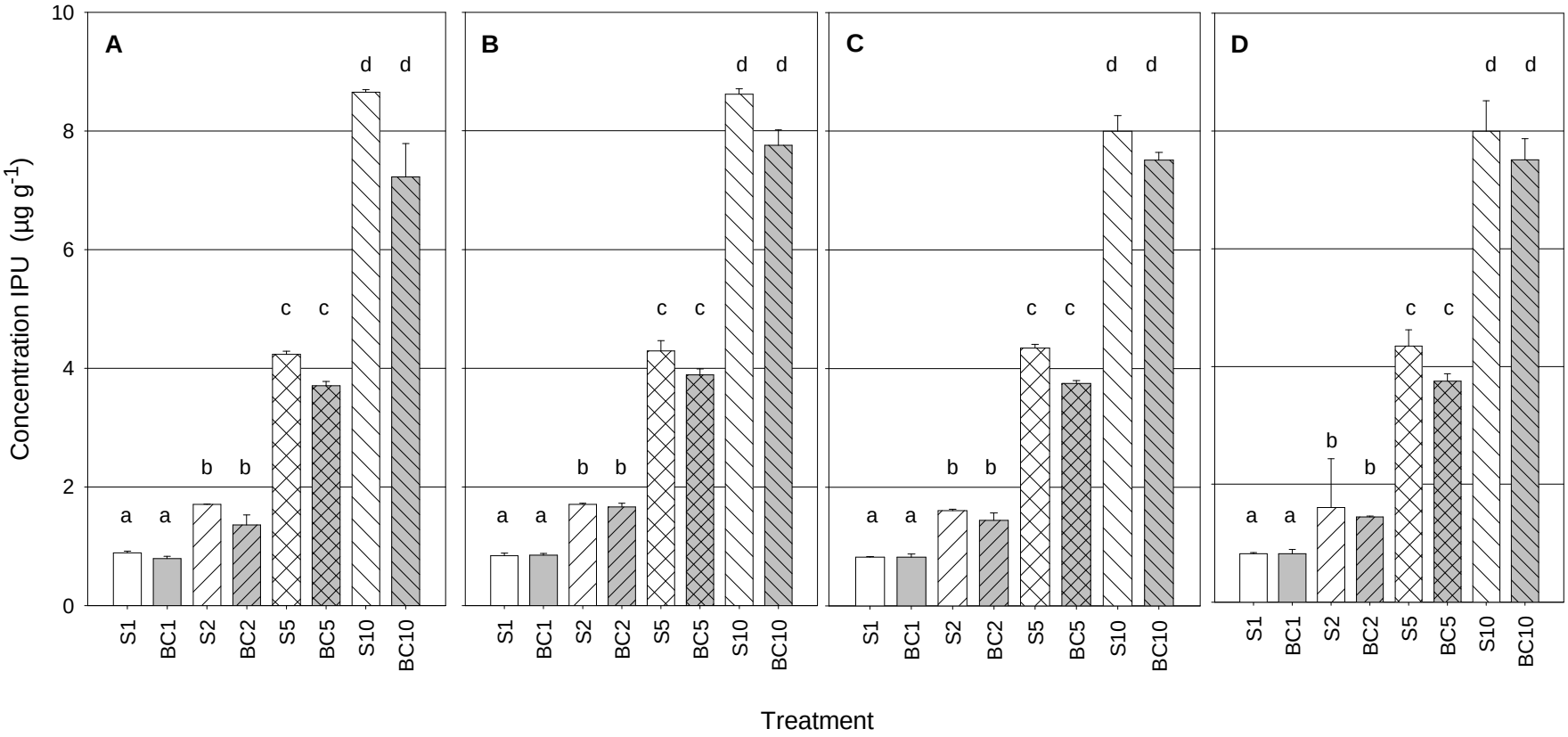


Figure 1: Residual IPU concentrations in soils amended with target doses of 1 mg kg^{-1}, 2 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1} (denoted 1, 2, 5, and 10, respectively) in soils containing biochar (5 %) (grey bars – denoted BC) and biochar free controls (white bars – denoted S) following incubation periods of 1d (A), 13d (B), 34d (C) and 62d (D). Error bars = standard error of the mean (n = 4). For each incubation period, different letters indicate significant difference between biochar and biochar free treatments at a given IPU dose.

3.2 Influence of biochar on IPU partitioning

Extractability of IPU was significantly ($p < 0.01$) lower in treatments containing biochar compared to biochar-free treatments (Figure 2). This was true irrespective of the initial concentration of IPU and incubation time. At all IPU doses and after all incubation times IPU extractability was < 2 % (i.e. IPU sequestration was > 98 %) in all biochar treatments. In contrast, IPU extractability was between 90 % and 40 % (depending upon dose and incubation time) in biochar-free treatments. Temporal decreases in IPU extractability ('ageing') were evident in all of the biochar-free treatments (extractable IPU fractions were significantly different ($p < 0.05$) between samples extracted after 1 d and those extracted after 62 d).

Numerous studies have shown that the increased contact time leads to a decrease in organic compound extractability from soil (see review articles Semple et al., 2007; Reid et al., 2000).

In contrast to the biochar-free treatments, soil containing biochar showed an almost immediate and complete ageing effect (Figure 2). Biochar is known to provide a variety of functional groups at the edges of stacked carbon sheets formed during pyrolysis, including hydroxyl, amino, ketone, ester, nitro aldehyde, and carboxyl groups (Verheijen et al., 2009). This makes the surface of biochar particles highly reactive (Amonette et al., 2009). Davies and Jabeen (2002) suggested that IPU binds to surfaces through interaction between carboxyl and amino acid groups on the molecule. This type of bonding would significantly reduce the extractability of IPU in biochar amended soils. Independent of chemical bond formation, biochar provides ample opportunity for enhanced physical entrapment within its highly porous matrix. Previous work has noted that nanoporosity can enhance pesticide recalcitrance (Rhodes et al., 2010).

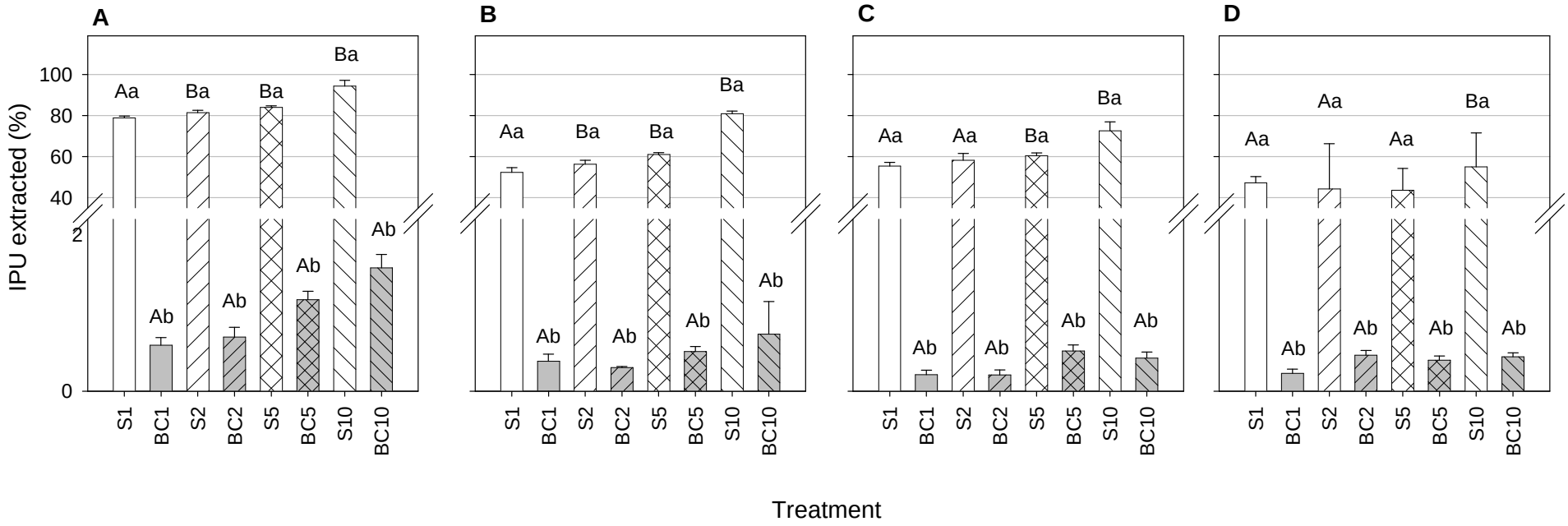


Figure 2: Extractable IPU (% residual concentration at time of extraction) in soils amended with target doses of 1 mg kg^{-1}, 2 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1} (denoted 1, 2, 5, and 10, respectively) in soils containing biochar (5 %) (grey bars – denoted BC) and biochar free controls (white bars – denoted S) following incubation periods of 1d (A), 13d (B), 34d (C) and 62d (D). Error bars = standard error of the mean (n = 4). Upper-case letters indicate significant difference with increasing IPU dose at a given incubation time, while lower-case letters indicate significant differences between couplets of with biochar and biochar free treatments at a given IPU dose.

3.3 Derivation of apparent IPU partition coefficients

The best-fit values of K_{foc} and 1/n were, respectively, 631 L kg^{-1} and 0.75 (RMSE = 42.4 mg kg^{-1} which is 20.2 % of the average C_{OC} value derived using the left hand side of Eq. 7 for all combinations of nominal dose and sampling time). The agreement between the measured and best-fit estimates of C_{OC} (LHS and RHS of Equation 7) are displayed graphically in Figure 3 (the slope of the best fit line was 0.91 and the r^2 value was 0.93). This suggests that the Freundlich isotherm was a reasonable approximation for sorption, as observed elsewhere (Singh et al., 2001; Chao et al., 2010). It also suggests that a single pair of K_{foc} and 1/n values provides a reasonable description of IPU sorption in all biochar-free treatments and sampling times, although there appears to be a slight underestimation of sorption on day 62 for the high dose treatments. This estimate of K_{foc} is higher, but within a factor of five, compared with K_{foc} values for IPU reported for agricultural soils in the literature (112 to 138 L kg^{-1}) (Worrall et al., 1996; Cooke et al., 2004) the derived value for 1/n is similar to the value of 0.8 reported elsewhere (Semple et al., 2007). The IUPAC database reports K_{foc} values for IPU ranging from 36 to 241 L kg^{-1}. The difference between the value of K_{foc} derived here and those reported elsewhere may be due to differences in the test systems employed. We have assumed that the soil solids are in equilibrium with an aqueous volume consisting of the soil solution and a volume of $CaCl_2$ extractant. However, if complete equilibrium did not occur during extraction then the mass in the aqueous phase could have been underestimated resulting in an overestimation of apparent sorption. In any case, it should be emphasised that the purpose of deriving an estimate of the sorption coefficient here is for comparison with sorption to biochar in relative terms.

From Eq. 10, a mean value for K_{BW} of $7.82 \times 10^4 \pm 1.04 \times 10^4$ L kg^{-1} and a mean value for $\log(K_{BC})$ of 4.83 ± 0.06 were obtained. Comparison of K_{BW} with the value of K_{foc} suggests that the sorption of IPU to biochar carbon is more than two orders of magnitude higher than sorption to soil carbon (123 times higher per unit mass than sorption to soil organic carbon). It is well known that organic pollutants sorb more strongly to activated carbon and black carbon than to soil and sediment organic matter (Yang and Sheng, 2003; Yang and Sheng, 2006; Yu et al., 2006; Xu et al., 2008). Recently, Sopeña et al. (2012) reported sorption isotherm parameters for IPU in soils containing different quantities of biochar, although they did not attempt to derive a

separate coefficient for biochar as we have done. They report that K_f (see Eq. 6) increased with increasing biochar content, with K_f for soil containing 2% biochar by mass more than 5 times higher than K_f for biochar-free soil. This is considerably lower than the sorptivity implied by our data, possibly due to the additional apparent sorption afforded by the physical protection associated with a higher biochar mass used in our experiment.

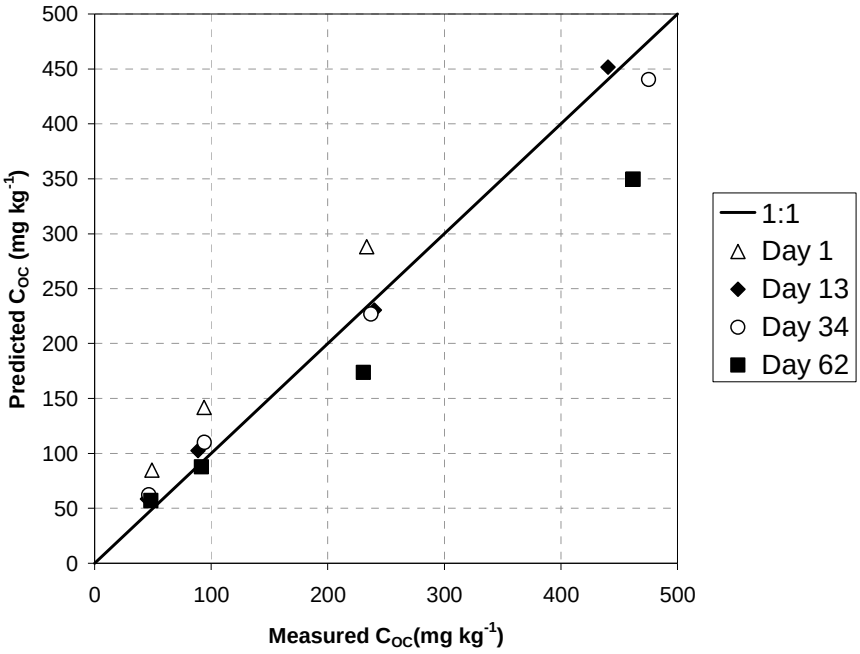


Figure 3: Comparison of the IPU concentration sorbed to soil organic carbon calculated from the left hand side of Equation 9 and that calculated from the Freundlich isotherm (right hand side of Equation 9) for different sampling times and IPU doses. The slope of the best fit line is 0.91 and the r^2 value is 0.93.

3.4 Influence of biochar on IPU-catabolic competence

Levels of catabolic competence (as indicated by the extent of ^{14}C-IPU mineralisation) in the control treatments (to which neither biochar nor IPU had been added) did not vary significantly ($p > 0.05$) temporally with respect to the levels observed at 1d (7 ± 2 %) (Figure 4A and S2). ^{14}C-IPU mineralisation values were consistent with those reported by Reid et al. (2005) for agricultural soil that had not been pre-exposed to IPU (with

IPU mineralisation ranging from 4 % to 6%) and to soils of low catabolic activity reported by Posen et al. (2006) (wherein IPU mineralisation ranging from 12 % to16 %). In the absence of biochar, IPU promoted increased catabolic competence with incubation time up to 34 d (Figure 4A). This increase in catabolic competence was noted in all of the IPU treatments ranging in IPU concentrations from 1 mg kg^{-1} to 10 mg kg^{-1} (Figure 4). At the time of maximum catabolic competence (34 d), the extents of mineralisation in the 1, 2, 5 and 10 mg kg^{-1} treatments were 31 $\pm$ 3 %, 38 $\pm$ 1 %, 33 $\pm$ 2 % and 40 $\pm$ 0.9 %, respectively. These levels of catabolic competence were significantly ($p < 0.05$) greater than in the control treatment and significantly greater with respect to the 1 d values for the respective treatments; implying significant changes in IPU catabolic competence with time and in response to IPU dose. Reid et al. (2005) reported temporal increases in IPU catabolic activity following IPU addition to soils that were previously un-exposed to IPU, while Posen et al. (2006) reported levels of IPU mineralisation ranging between 33% and 44% in soils that were catabolically competent. Subsequently the level of IPU mineralisation was observed to significantly ($p < 0.05$) decrease in all treatments with respect to the 34 d values (Figure 4A).

Levels of IPU catabolic competence in the treatments containing biochar were never > 1.5 % (Figure 4B). Given the marked effect of biochar on IPU extractability (Figure 2) it would appear that biochar effectively sequestered IPU and as a consequence impeded mass transfer of substrate to microorganisms thereby reducing catabolic competence. The modelling work of Bosma et al. (1997) indicates that should substrate mass transfer be supressed, to below a critical threshold, then exploitation of substrates by microorganisms will be prevented. These results highlight the profound influence biochar has upon herbicide bioavailability and are in agreement with other studies where biochar or black carbon has been reported to reduce organic compound bioavailability (Thorsen et al., 2004; Rhodes et al., 2010; Sopeña et al., 2012; Burgess et al., 2004). Specifically, these results complement those recently reported by Jones et al. (2011) that showed 'agronomic application rates' (10 - 100 t ha^{-1}) of biochar lead to suppressed simazine biodegradation. While Jones et al.(2011) attributed this suppression to reduced herbicide availability our results extend the mechanistic understanding of suppression by providing an appreciation of microbial factors; specifically, the lack of catabolic competence where biochar is present.

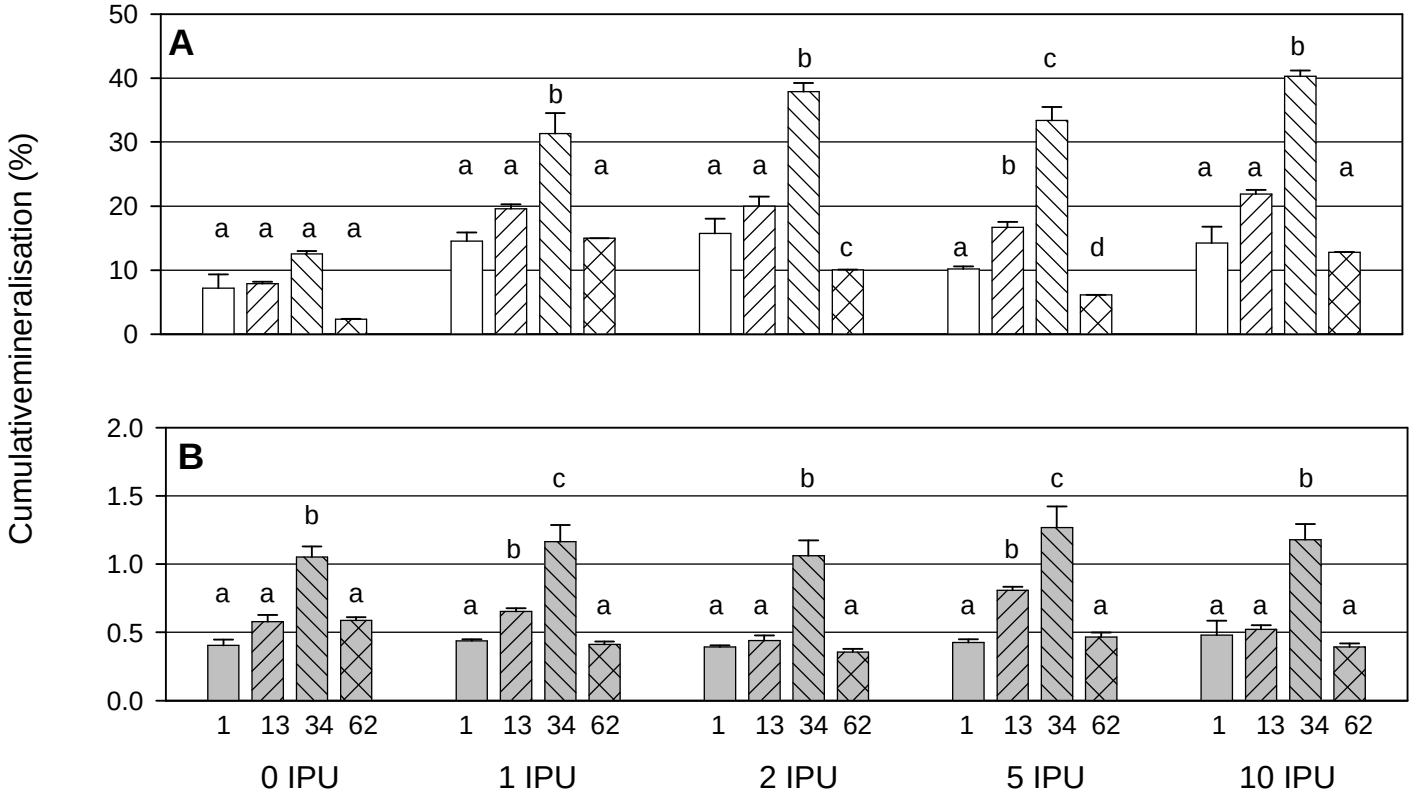


Figure 4: Catabolic activity with respect to IPU (^{14}C-IPU cumulative mineralisation (%) after 22d assay time). Frame A shows biochar free treatments with bars grouped by IPU dose applied (in the order: control, 1 mg kg^{-1}, 2 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1}) to allow temporal response to IPU to be visualised after 1d, 13d, 34d and 62d incubation time. Frame B shows the with biochar (5 %) treatments (bars grouped as for Frame A). Error bars display standard error of the mean (n = 4). Lower case letters indicate significant difference within groups of bars.

3.5 Environmental benefits

A key finding of this research has been to establish the significant influence which biochar can have on IPU partitioning. Our results indicate that efficient (> 98 %) and rapid (within 1 d) IPU sequestration can occur when soil was amended with biochar (5 %), even when IPU application was increased 10-fold (i.e. at a nominal dose of 10 mg kg^{-1} IPU availability after 62 days was very low (Figure 2)). On one hand this could be seen as a beneficial outcome as herbicide sequestration reduces non-target receptors exposure - for example, soil biota, groundwater, surface waters and aquatic organisms therein. Non-target effects of herbicides including IPU on soil biota and aquatic organisms have been extensively reported (see reviews Fleeger et al., 2003; Freemark et al., 1995); as has IPU contamination in surface and groundwaters (Baran et al., 2008). Biochar application, thus, has the potential to provide environmental protection by reducing pollutant transport. With this in mind it may be possible to direct biochar placement with enhanced herbicide sorption; for example, incorporating it within soils along riparian corridors to provide 'buffer strips' to mitigate herbicide transfer to surface waters. Reduced herbicide availability in soils may also reduce the potential for herbicide uptake to food crops and upwards into the food chain. Such reduced soil to plant transfer of pesticides has been reported for insecticides (Pylypiw et al., 1997) and herbicides (Pylypiw et al., 1993). Yu et al. (2009) showed that plant uptake of the insecticides chlorpyrifos and carbofuran was markedly reduced when they were applied to soils containing biochar (1 %).

3.6 Adverse implications relating to reduced herbicide availability

While many authors have suggested the application of biochar to farmland as potentially beneficial in the context of carbon storage (Lehmann et al., 2006; Gaunt et al., 2008; Laird, 2008; McHenry, 2009) the sequestration of herbicide in biochar amended soils would be expected to be impaired the efficacy of herbicides as a weed control agents. In order to contextualise the potential scale of relevance, from a

herbicide perspective, the FAO land use database (2009) was used to establish land areas under ‘arable or permanent crop’ designations and these areas then compared with projections for biochar deployment.

North America, Europe, Japan and Australia have been reported to account for the use of 80% of all herbicides sold for agricultural use (FAO, 2008). These countries account for a total area of 563 $x10^6$ ha under ‘arable or permanent crop’ designations (FAO, 2008); this value being approximately one third of the global area designated under ‘arable or permanent crop’ landuse (FAO, 2008).

Lehmann et al. (2006) reported biochar burial to hold the potential to sequester 224 Pg of carbon. This level of C-sequestration would be sufficient to offset half the increase in atmospheric CO_2 which has been observed from preindustrial levels to the present day. In order to realise this C-storage potential through biochar burial in land designated as being under arable or permanent crop a biochar application rate of 140 t_{carbon} ha^{-1} would be required. The 5 % biochar application rate used in our research (which equates to 4.3 % biochar incorporated to 30 cm in a receiving soil with a bulk density of 1.5 g cm^{-3}; assuming biochar to have a carbon content of 70%) is consistent with this application rate. Thus, while biochar may hold real potential to reduce atmospheric CO_2 concentrations should biochar application be restricted to arable and permanent crop land where herbicides are *not* relied upon the carbon–abatement potential of 224 PgC would be reduced to 149 PgC.

The formation of bound residues (as supported by Figures 2 and 4) is a further concern for two reasons: i) the potential for incremental increases in bound residue levels following repeated herbicide application and ii) the potential for future release of these residues and associated environmental impacts. Given very high fraction IPU sequestered (Figure 2) and the resultant impediment to the development of catabolic activity (through which degradation can take place) (Figure 4) the development of every increasing burdens of bound residues would be anticipated. Further research is warranted to establish the capacity of biochar to enhance bound residues and to

consider i) the bioavailability of these residues to soil biota, and ii) the implications which these residues might have for biologically mediated processes.

It is submitted that the use of agrochemicals is a cornerstone in modern agricultural practice and a reduction in agronomic efficacy could pose a threat to yields and undermining food security. Our data indicate that biochar, when applied at application rates consistent with those used in carbon storage scenarios (Lehmann et al., 2009), has the potential to dramatically reduce herbicide bioavailability (Figures 2 and 3). Given the one third quotient of global arable and permanent crop land use area reliant upon herbicide applications, we urge that due consideration is given to where biochar should be applied to soil and at what levels. In order to protect food security and to mitigate against the accumulation of herbicide bound residues, it is concluded that would not be prudent to apply high levels of biochar to land where herbicides are relied upon.

References

Amonette, J. and Joseph, S. Characteristic of Biochar: mirco-chemical Properties, In: *Biochar for Environmental Management: Science and Technology*, Lehmann, J., Joseph, S. Eds. Earthscan, London, 2009.

Baran, N. ; Lepiller, M. ; Mouvet, C. Agricultural diffuse pollution in a chalk aquifer (Trois Fontaines, France): Influence of pesticide properties and hydrodynamic constraints. J. Hydrol. **2008**, 358, 56-69.

Blackwell, P.; Reithmuller, G.; Collins, M. Biochar Application to Soil. In *Biochar for Environmental Management: Science and Technology*; J. Lehmann, S. Joseph, Eds.; ISBN 9781844076581. Earthscan, London 2009; pp 207-226.

Bosma T.N.P.; Middeldrorp P.J.M.; Schraa G.; Zehnder, A.J.B. Mass transfer limitation of biotransformation: quantifying bioavailability. Environmental Science and Technology. **1997**, 31, 248-252.

Burgess, R.M. and Lohmann, R. Role of black carbon in the partitioning and bioavailability of organic pollutants, Environ. Toxicol. Chem. **2004**, 23, 2531–2533.

Chan, K.Y.; Xu, Z.H. Biochar Nutrient properties and their enhancements, In: *Biochar for Environmental Management: Science and Technology*; Lehmann, J., Joseph, S. Eds.; Earthscan, London 2009; pp 67-84.

Chao T.; Mi-dao W.; You-bin S. Influences of charcoal amendment on adsorption-desorption of isoproturon in soils. *Agric. Sci. China.* **2010**, 9, 257-265

Collison, M., Collison, L., Sakrabani, R., Tofield, B., Wallage, Z. *Biochar and Carbon Sequestration: A Regional Perspective*; report prepared for East of England Development Agency (EEDA) 2009.

Cooke, C.M.; Shaw, G.; Collins, C.D. Determination of solid-liquid partition coefficients (Kd) for the herbicides isoproturon and trifluralin in five UK agricultural soils. *Environ. Pollut*. **2004**, 132, 541-552

Davies, J.E.D. and Jabeen, N. The adsorption of herbicides and pesticides on clay minerals and soils, Part 1. Isoproturon. *J. Inclusion Phenomena and Macrocyclic Chemistry*. **2002**, 43**,** 329–336.

FAO (2009) http://faostat3.fao.org/home/index.html

Fleeger, J.W.; Carman, K.R.; Nisbet, R.M. Indirect effects of contaminants in aquatic ecosystems. *Sci. Total Environ*. **2003,** 317, 207-233.

Freemark, K. and Boutin, C. Impacts of agricultural herbicide use on terrestrial wildlife in temperate landscapes: A review with specific reference to North America. *Agric. Ecosyst. Environ*. **1995**, 52, 67-91.

Gaunt, J. L. and Lehmann, J. Energy balance and emission associated with biochar sequestration and pyrolysis bioenergy production. *Environ. Sci. Technol*. **2008**, 42, 4152–4158.

Glaser, B.; Lehmann, J.; Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. *Biol. Fertil. Soils*. **2002,** 35, 219-230.

Jones, D.L., Edwards-Jones, G., Murphy D.V. Biochar mediated alterations in herbicide breakdown and leaching in soil. Soil Biology and Biochemistry. 2011, 43, 804–813.

Jonker, M. T. O.; Hawthorne, S. B.; Koelmans, A. A. Extremely slowly desorbing polycyclic aromatic hydrocarbons from soot and soot-like materials: evidence by supercritical fluid extraction. *Environ. Sci. and Technol.* **2005**, 39, 7889-7895.

Jonker,M.T. O. and Koelmans, A. A. Extraction of polycyclic aromatic hydrocarbons from soot and sediment: solvent evaluation and implications for sorption mechanism. *Environ. Sci. and Technol*. **2002**, 36, 4107–4113.

Laird, D.A. The Charcoal Vision: a Win-Win-Win Scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.* **2008**, 100, 178-181.

Lehmann, C. J., and Rondon, M. (**2006**) Bio-char soil management on highly-weathered soils in the tropics. In *Biological Approaches to Sustainable Soil Systems* (N.T. Uphoff, Ed.), CRC Press, Boca Raton, pp 517-530.

Lehmann, J.; da Silva, J.P.; Steiner, C.; Nehls, T.; Zech, W.; Glaser, B. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. *Plant Soil*. **2003**, 249, 343–357.

Lehmann, J.; Gaunt, J.; Rondon, M. Bio-char sequestration in terrestrial ecosystems - A review. *Mitig. Adapt. Strat. Global Change*. **2006**, 11, 403-427.

McGill, W.B. Review and classification of ten soil organic matter (SOM) models. In: *Evaluation of soil organic matter models.* Powlson, D.S., Smith, P., Smith, J.U. Eds.; Springer Verlag, Berlin Heidelberg 1996; pp. 111-132.

McHenry, M.P. Agricultural bio-char production, renewable energy generation and farm carbon sequestration in Western Australia: Certainty, uncertainty and risk. *Agric. Ecosyst. Environ.* **2009**, 129, 1-7.

Mordaunt C.J.; Gevao B.; Jones K.C.; Semple K. T. Formation of non-extractable pesticide residues: observations on compound differences, measurement and regulatory issues, *Environ. Pollut.* **2005**, 133, 25-34.

Posen, P.; Lovett, A.A.; Hiscock, K.M.; Evers, S.; Ward, R.; Reid, B.J. Incorporating variations in pesticide catabolic activity into a GIS-based groundwater risk assessment. *Sci Total Environ*. **2006**, 367, 641-652.

Pylypiw, H.M. ; Arsenault, T.L.; Thetford, C.M.; Mattina, M.J.I. Suitability of microwave-assisted extraction for multiresidue pesticide analysis of produce. *J Agric. Food Chem*. **1997**, 45, 3522-3528.

Pylypiw, H.M.; Bugbee, G.J.; Frink, C.R. Uptake of pre-emergent herbicides by corn – distribution in plants and soil. *Bull. Environ. Contamin. Toxicol*. **1993**, 50, 474-478.

Quilliam R.S.; Marsden K.A;, Gertler C.; Rousk J.; DeLuca T.H.; Jones D.L. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agriculture Ecosystems and Environment.* **2012**. 158, 192-199.

Reid, B.J.; Jones, K.C.; Semple, K.T. Bioavailability of persistent organic pollutants in soils and sediments - a perspective on mechanisms, consequences and assessment. *Environ. Pollut.* **2000**, 108, 103-112.

Reid, B.J.; Papanikolaou, N.D.; Wilcox, R.K. Intrinsic and induced isoproturon catabolic activity in dissimilar soils and soils under dissimilar land use. *Environ. Pollut.* **2005**, 133, 447-454.

Rhodes, A.H.; Carlin, A.; Semple, K. T. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. *Environ. Sci. and Technol*. **2008**, 41, 3952-3958.

Rhodes, A.H.; McAlister, L.E.; Rongrong, C.; Semple, K.T. Impact of activated C on the mineralisation of phenanthrene in soils, *Chemosphere*. **2010**, 79, 463-469.

Semple, K.T.; Doick, K.J.; Wick, L.Y.; Harms, H. Microbial interactions with organic chemicals in soils: Definition, Processes and measurements. *Environ. Pollut*. **2007**, 150, 166-176.

Semple, K.T.; Morriss, A.W.J.; Paton, G.I. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *Eur. J. Soil Sci*. **2005**, 54, 809-818.

Singh, N.; Kloeppel, H.; Klein, W. Sorption behaviour of metolachlor, isoproturon, and terbuthylazine in soils. *J Environ Sci Health B.* **2001**, 36, 397-407

Smernick, R. J. Biochar and the sorption of organic compounds, In *Biochar for Environmental Management: Science and Technology.* Lehmann, J., Joseph, S. Eds.; Earthscan, London 2009; pp 67-84.

Sohi, S.; Loez-Capel, E.; Krull, E.; Bol, R. *Biochar's roles in soil and climate change: A review of research needs*. CSIRO Land and Water Science Report 05/09; 2009.

Sombroek, W.; Ruive M. de L.; Fearnside P. M.; Glaser B.; Lehmann J. Amazonian Dark Earths as carbon stores and sinks. In: *Amazonian Dark Earths: origin, properties, management.* Lehmann J, Kern DC, Glaser B, and Woods WI, Eds.; Kluwer Academic Publishers. 2003; pp 125-139.

Sopeña F.; Semple K.; Sohi, S.; Bending G. Assessing the chemical and biological accessibility of the herbicide isoproturon in soil amended with biochar. *Chemosphere*. **2012**, 88, 77–83.

Thorsen W.A. and Cope W.A., D. Shea. Bioavailability of PAHs: effects of soot carbon and PAH source. *Environ. Sci. and Technol*. **2004**, **38**, 2029–2037.

Verheijen, F.G.A.; Jeffery, S.; Bastos, A.C.; van der Velde, M.; and Diafas, I. *Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions*. EUR 24099 EN, Office for the Official Publications of the European Communities, Luxembourg; 2009.

Villarreal, L.; Pete Waller; P.; Slack, D.C. *Water conservation in biofuels development: Greenhouse and field crop production with biochar*. Lap Lambert Academic Publishing ISBN: 3838392418, 2010.

Worrall, F.; Parker, A., Rae, J.E.; Johnson A.C. Study of the equilibrium adsorption of isoproturon onto soils, subsoils and clay minerals. Eur. J. Soil Sci. **1996**, 47, 265-272.

Xu C.; Liu W.; Sheng G.D. Burned rice straw reduces the availability of clomazone to barnyardgrass. *Sci. Total Environ*. **2008**, 392, 284-289.

Yang Y.N. and Sheng G.Y. Enhanced pesticide sorption by soils containing particulate matter from crop residue bums. *Environ. Sci. Technol.* **2006**, 37, 3635-639.

Yang, Y. and Sheng, G. Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. *Environ. Sci. and Technol*. **2003**, 37**,** 3635–3639

Yu X.Y.; Ying G.G.; Kookana R.S. Sorption and desorption behaviors of diuron in soils amended with charcoal. *J. Agric. Food Chem*. **2006**, 54, 8545-8550

Yu, X.Y.; Ying, G.G.; Kookana, R.S. Reduced plant uptake of pesticides with biochar additions to soil, *Chemosphere*. **2009**, 76, 665-671.

Chapter 5

Deactivation of herbicidal activity in biochar amended soil

Deactivation of herbicidal activity in biochar amended soil

Alessia Freddo and Brian J. Reid*

School of Environmental Sciences, University of East Anglia, Norwich, UK

Abstract

The influence biochar (0%, 1% and 5%) on the efficacy of three pre-emergence herbicides (mesotrione, pendimenthalin and terbuthylazine) with respect to survival and growth of two common broadleaf weeds (*Amaranthus retroflexus and Solanum nigrum)* is presented.

In these instances the viability of targeted weeds, after 22 d of growth assay, in the herbicide amended soil containing biochar were tested and compared to the number of viable weeds in the control treatments (to which neither biochar nor herbicide had been added).

Results indicated biochar presence (5%) significantly ($p < 0.05$) reduced the effectiveness of two of the herbicides (mesotrione and pendimenthalin) and the weed survival was significantly ($p < 0.05$) different to the control treatments. In contrast, these herbicides were almost 100% effective when applied to biochar free soil.

These results are significant in so much as they provide *direct evidence* that biochar incorporation to soil can undermine the efficacy of soil applied herbicides.

1. Introduction

Biochar has been associated with several priority environmental issues, including: renewable energy, mitigation of climate change, soil improvement and waste management (Lehmann and Joseph, 2009; Roberts et al., 2009). Biochar is a product of a biomass burning in an oxygen limited environment (pyrolysis) (Yaman S., 2004). This process produces: gases (syngas), liquids (bio-oil) and solids (biochar), with the yields of each component being dependent upon the temperature and the residence time of the process (Sohi et al., 2009). Owing to its recalcitrant structure biochar is a stable store of carbon (Lehmann et al., 2006).

Biochar has a high surface-area, high porous and variable-charge. As a consequence, biochar has the potential to increase cation exchange capacity and water-holding capacity of soil (Glaser et al., 2002; Bélanger et al., 2004; Keech et al., 2005; Liang et al., 2006); in addition biochar is rich in nutrients such as potassium, phosphorous and microelements such as magnesium, calcium and manganese (Neary et al., 1999). Reports have shown increasing bioavailability and plant uptake of phosphorus and alkaline metals following biochar application to soils along with fertilisers (Lehmann et al., 2003; Glaser et al., 2002; Steiner et al., 2007). Several studies have reported positive benefits on plant growth following biochar addition to soil (Lehmann et al., 2003; Glaser et al., 2002; Rondon et al., 2007; Steiner et al., 2007).

Other, studies have shown the capacity of biochar to sorb organic compounds such as pesticides (Yang et al., 2010; Wang et al., 2009; Yu et al., 2009; Spokas et al., 2009) and reduce drastically their availability in the environment. Thus, while biochar provides benefits (as accounted above) that might support increased agricultural productivity, the sorptive capacity of biochar, particularly with respect to herbicides, may counteract these benefits.

In order to provide an insight into the sorption mechanism of biochar, several approaches have been used to elucidate the physico-chemical properties of biochar. X-ray diffraction has shown biochar to have an amorphous structure with crystalline areas (Lehmann et al., 2009) consisting of random polycyclic aromatic (graphene) layers (Zhu et al., 2005) rimmed with functional groups (Zhu et al., 2005).

Zhu et al. (2005) have suggested that the functional groups present in biochar are mainly O-containing groups. Naturally, O-functionality can vary depending on

pyrolysis temperature, carbon source and partial pressure of O_2 used during biochar production (Zhu et al., 2005). Significantly, this functionality can undergo specific and non-specific physiosorption interaction with sorbent molecules (Zhu et al., 2005). Zhu et al. (2005a) and Sander et al (2005) have demonstrated π- π electron donor-acceptor interactions between nitroaromatic compounds and the graphene basal plane of char. Li et al. (2009) studied the effect of the pH on pesticide sorption on the organic amendments and showed an increase of sorption with a decrease in pH.

In addition, studies on sorption and desorption of organic compounds have revealed nonlinear isotherms in biochar (with desorption rates being lower than sorption rates (Tian et al., 2010)). This hysteresis has important implications for the transport and bioavailability of sorbents (Sander et al., 2005; Braida et al., 2003) such as pesticides. Lu et al (2002) and Sander et al (2006) explain this hysteresis through a "pore deformation" mechanism according to which the incoming sorbents molecules exert a pressure in pores smaller than the adsorbate molecules causing them to expand. Similarly, Braida et al (2003) proposed that in response to the penetration of benzene molecules, the polyaromatic walls of biochar rearrange and open up new pathways for the adsorbent to penetrate and for sectors, previously opened, to close, trapping the molecules inside the matrix.

While the influence of biochar upon herbicide partitioning has been reported the resultant implications for the effectiveness of herbicides to perform their role (weed control) remains limited (Graber et al., 2012; Kookana et al., 2010; Nag et al. 2011). The objectives of this study were to investigate (i) the influence of biochar upon the efficacy of three herbicides (mesotrione, pendimenthalin and tributilazine) (ii) to compare the effects of different amounts of biochar upon the efficacy of these herbicides, and (iii) to compare the physiologic characteristics of the weeds grown in the presence of biochar/herbicide to those grown in biochar/herbicide free soils.

2. Materials and Methods

2.1 Materials

Biochar was produced from a one-quarter scale 500 kW test gasifier (Refgas UK, Flintshire, UK). The feedstock used was waste softwood. The gasification zone of the plant operated at around 1000^{o}C, the pyrolysis section around 400^{o}C to 500^{o}C and the

"drying zone" at 200^{o}C. Negative pressure (-25 mbar) was operated by the reactor. To pass the feedstock from the drying zone to the biochar discharge section of the plant took 1 hour.

A silty loam soil (Sheringham Series) was collected from an agricultural area of Norfolk, UK (grid ref. TG 11210 35466) at depth of 0-10 cm; it was air-dried and sieved (2 mm) prior to use.

The herbicides: mesotrione, pendimenthalin and terbuthylazine were supplied by Syngenta (Guildford, Surrey, UK). Selected properties of these herbicides are shown in Table 1.

Amaranthus retroflexus and *Solanum nigrum* used in the plant growth experiments were also supplied by Syngenta, UK. These are common annual weeds (Sutton et al., 2002).

Table 1. Properties of the herbicides used [26] and their rates of application

	Mesotrione	**Pendimenthalin**	**Terbuthylazine**
Chemical Group	Triketone	Dinitroaniline	Triazine
Vapour Pressure	5.70x10^{-3} mPa at 25ºC	1.94 mPa at 25ºC	0.15 mPa at 25ºC
Water Solubility	160 mg L^{-1} at 20ºC	0.33 mg L^{-1} at 20ºC	8.5 mg L^{-1} at 20ºC
Log K_{ow}	0.11	5.2	3.21
Composition (purity)	≥92%	≥90%	≥96%
Density	1.2 x 10^{-3} g/ml	0.374 g/ml	1.1 g/ml
Recommended application rate	Pre-emergence (100-225 g ha^{-1}) Post emergence (70-150 g ha^{-1})	Pre- and post emergence (0.6-2.4 g ha^{-1})	Pre- and post emergence (0.6-3 g ha^{-1})
Application rate used in this research	0.021 g m^{-2}	0.17 g m^{-2}	0.3 g m^{-2}

2.2 Experimental approach

The soil was mixed with 1 % and 5 % biochar (2 mm particle size). Soil treatments were subdivided in 8 plastic containers (23 cm length, 17 cm width, 15 cm height (surface area = 0.0391 m^2); a closed system prevented leaching loss of water or herbicides. To each box 1.5 kg of biochar-amended soils was added. In addition 8 boxes were also filled with 1.5 kg of biochar free soil. Half of each set of boxes were used with no herbicide added. The soil in the remaining boxes was dosed (Table 1) with recommended applications of herbicide (calculation of these application rates are shown in the Supporting Information (Appendix D) with doses used provided in Table 1).

A. retroflexus was tested as target of mesotrione and pendimenthalin, and *S. nigrum* was tested as the target for terbuthylazine (Sutton et al., 2002). For each species 500 seeds were sown at a depth of 2 cm in each treatment. Herbicides were then sprayed homogeneously across the surface of the treatment at recommended application rates (Table 1). The weed growth assays were maintained in a growth chambers at 15ºC with a 12 h lighting cycle for 22 days. Treatments were watered every 2 days to maintain soil moisture. Viable numbers of weeds were recorded every 2 days (perished weeds were not included in the count). In order to assess physiological responses individual plants of *A. retroflexus* grown both with and without herbicide (pendimenthalin) were carefully removed from the treatments following the 22 d growth period. The plants were washed and the length of the roots and shoots were recorded. Thereafter, roots and shoots were oven-dried at 60˚C for several days to obtain constant weight. The final dry weight was recorded.

Statistics

Independent sample t-test and One-Way ANOVA were performed using SPSS 16.0 for Windows. Statistical significance of weeds survival was determined at 95% confidence interval with the significance level at 0.05.

3. Results

3.1 Seedling emergence and temporal survival in untreated soil

The emergence and survival of the weed seeds A. *retroflexus* and *S. nigrum* in the untreated soil and soil amended with 1% and 5% of *biochar in absence of herbicide* is shown in Figure 1a and 1b, respectively.

The trend of growth of *A. retroflexus* seeded in the untreated soil began on day 12 and reached a maximum number of plants after 22 days (26.3 ± 4.11). *A. retroflexus* seeds in the 1 % biochar treatment started to grow from day 12. *A. Retroflexus* survival in the 1 % biochar treatments was significantly greater ($p < 0.05$) compared to the weeds in untreated soil throughout the experiment; with 69.2 ± 12.9 plants viable on day 22 in the 1 % biochar treatment. Germination of *A. retroflexus* in 5 % biochar treatments started on day 8. The number of viable plants in the 5 % biochar treatment was significantly higher ($p < 0.05$) when compared with the biochar free soil throughout the experiments; with 71.7 ± 11.7 plants viable on day 22 in the 5 % biochar treatment. Thus, application of biochar at both 1 % and 5 % increased *A. retroflexus* viability. These results are in agreement with previous reports which have shown the benefits of biochar amendment to soil in terms of fertility and productivity, increasing seed germination, plant growth, and crop yields (Lehmann et al., 2006a; Lehmann et al 2003; Glaser et al., 2002).

In contrast to *A. retroflexus* germination success and survival, *S. nigrum* seeds did not show a significant difference ($p > 0.05$) in the presence of neither 1 % or 5 % biochar when compared to the soil only control (figure 1b). These results highlight that different weed species respond differently to the presence of biochar.

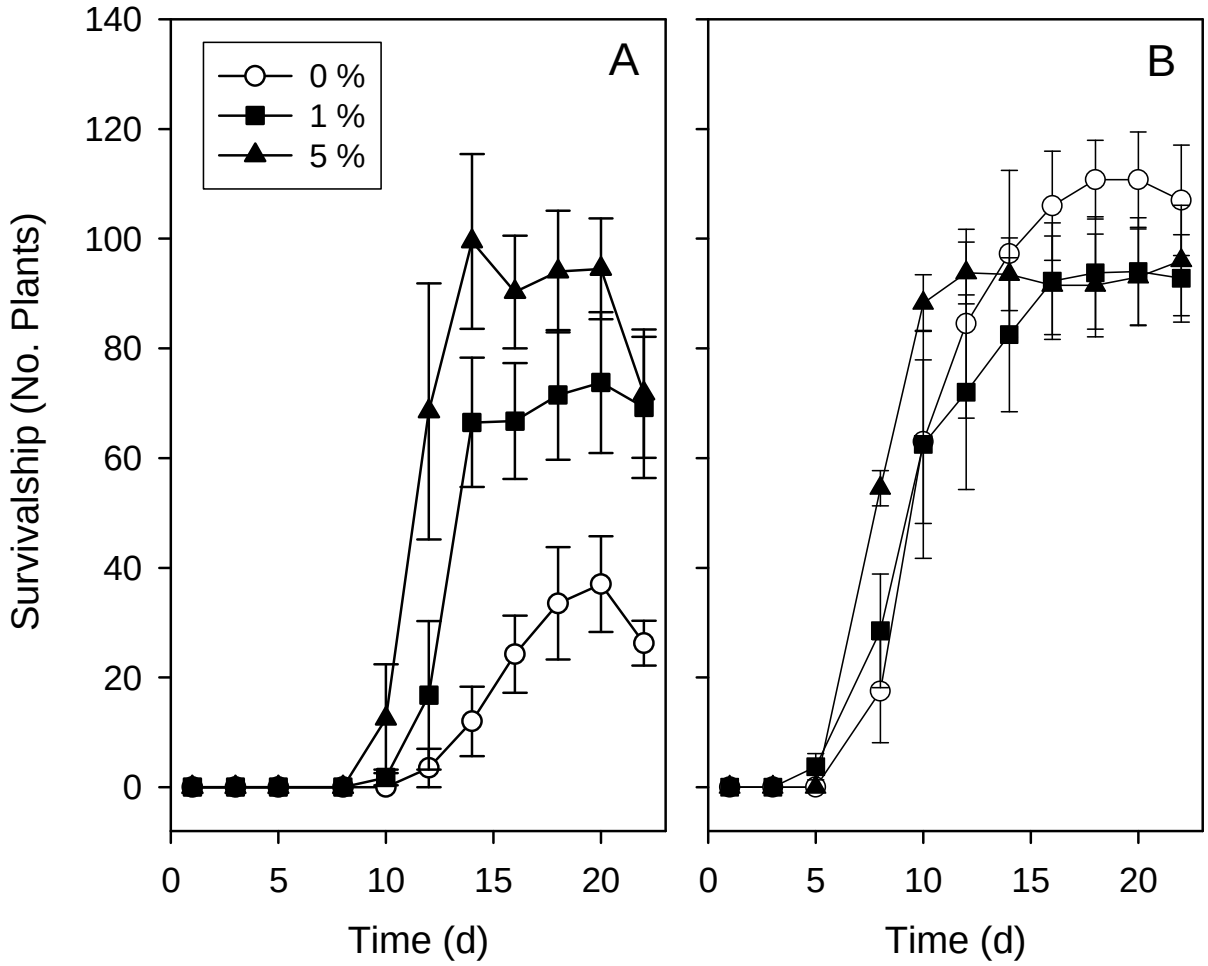


Figure 1 – Weed survival in the absence of herbicide of: *Amaranthus retroflexus* (A) and *Solanum nigrum* (B); in biochar free treatments (○), 1 % biochar treatments (■) and 5 % biochar treatments (▲). Mean values shown ± 1 standard error (n = 4).

3.2 Seedling emergence and temporal survival in herbicide amended treatments

A. retroflexus with mesotrione

A. retroflexus survival in both biochar free soil and the 1 % biochar treatment in the presence of mesotrione followed a similar trend although the growth of *A. retroflexus* observed in soil with 1% biochar was significant different ($p < 0.05$) from day 12 to day 18 (Figure 2a). Both biochar free treatments and the 1% biochar treatment reached maximum survival at day 8 and day 5, respectively (Figure 2a). There the number of *A. retroflexus* plants decreased in both the treatments until reaching a total absence of plants on day 22 (Figure 2a). In the 5 % biochar treatment *A. retroflexus* started to grow from day 5 (Figure 2a). Growth was, however, significantly lower ($p < 0.05$) than that observed in both the biochar free treatments and the 1 % biochar treatments (Figure 2a). The number of *A. retroflexus* plants from day 12 and day 14 in the 5 % biochar treatments dosed with mesotrione was significantly greater ($p < 0.05$) than those observed in the biochar free soil and the 1 % biochar amended treatments (Figure 2a). At the end of the assay (22 d) the number of *A. retroflexus* plants was significantly ($p < 0.05$) higher in the 5 % biochar treatments with respect to the biochar free soil. Thus, while weeds showed their natural response to the herbicide in the biochar free treatments and 1 % biochar treatments the efficacy of mesotrione in the 5 % biochar treatments was compromised and weed plants remained viable.

A. *retroflexus* with pendimenthalin

The presence of pendimenthalin was effective in reducing the survival of *A. retroflexus* in biochar free soil; germination in the biochar free soil was minimal (Figure 2b). Where biochar was present at 1 % germination success of *A. retroflexus* was again low in the presence of pendimenthalin (Figure 2b). In contrast, where biochar was amended to soil at 5 % *A. retroflexus* germination success and survival was much better (Figure 2b). *A. retroflexus* survival peaked at 20 d with the number of viable plants in the 5 % biochar treatments being significantly ($p < 0.05$) higher than those in the biochar free soil (the number of viable plants in the 1 % biochar treatments were also significantly ($p < 0.05$) higher than in the biochar free soil at this time) (Figure 2b). Subsequently, *A. retroflexus* survival in the 5 % biochar treatments decrease but remained much higher and significantly ($p < 0.05$) different to than in the biochar free treatments (Figure 2b).

On account of high levels of survival in all of the treatments weed plants from this pairing of weed and herbicide were considered further with respect to physiological parameters (see section 3.3).

***Solanum nigrum* with terbuthylazine**

Irrespective of biochar presence/absence or its level of amendment *S. nigrum* germinated well in the all of the treatments to which terbuthylazine was added (Figure 2c). Weed survival reached a maximum at 12 days in all treatments. Thereafter, terbuthylazine was effective at killing *S. nigrum* seedings. At the end of the assay (22 days) survival was greatest in the 5 % biochar treatments (45 ± 6.1) followed by the 1 % bichar treatments (31.5 ± 9.4) with the biochar free soil showing the lowers survival (21.7 ± 9) (Figure 2c) but there was no significant difference ($p > 0.05$) between these treatments.

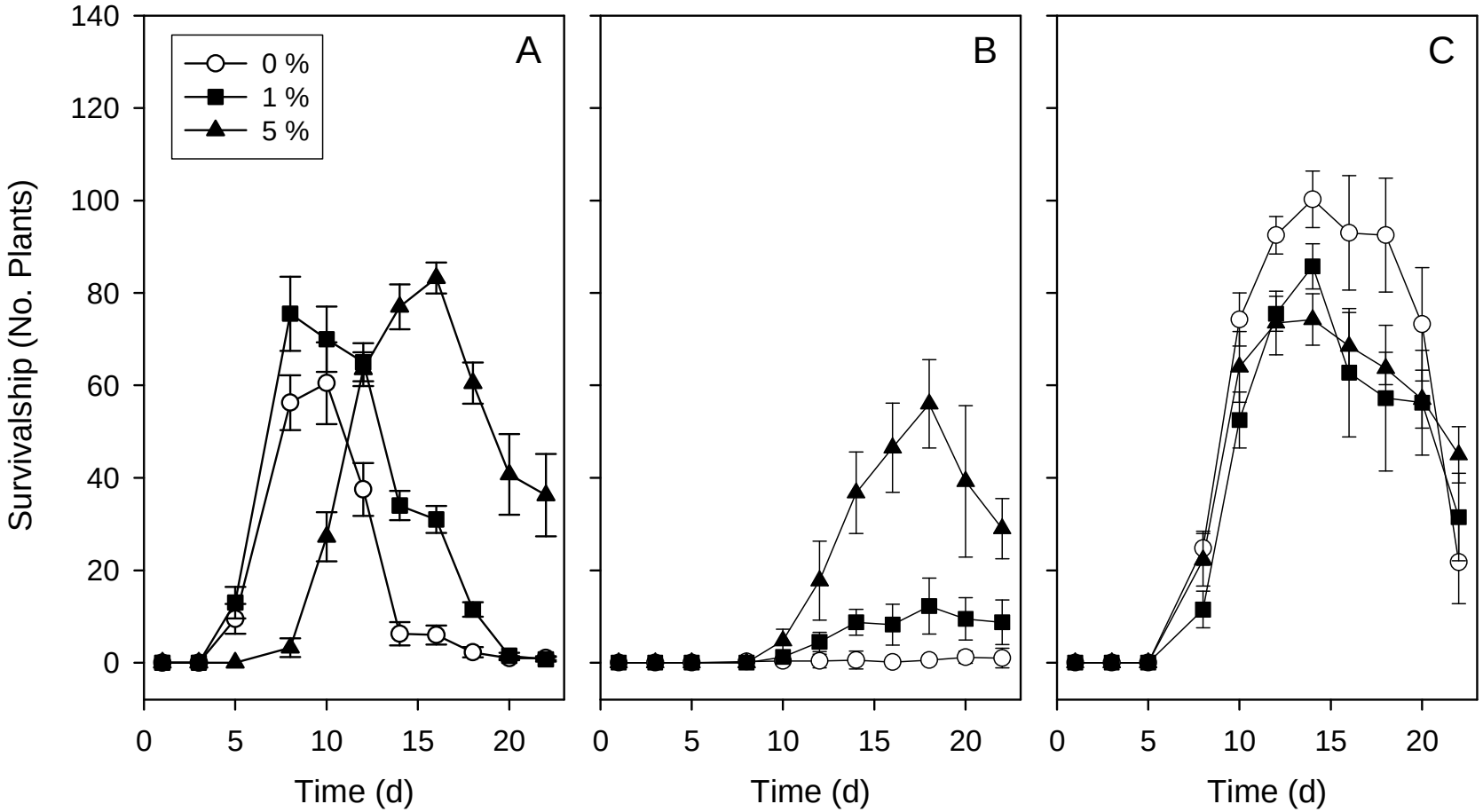


Figure 2 – Weed survival in the presence of herbicide of: *A. retroflexus* in treatments dosed with mesotrione (A), *A. retroflexus* in treatments dosed with pendimenthalin (B) and *Solanum nigrum* in treatments dosed with terbuthylazine (C); in biochar free treatments (○),1 % biochar treatments (■) and 5 % biochar treatments (▲). Values shown ± 1 standard error (n = 4).

3.3 Survival comparison with the biochar free unamended soil treatments

In order to provide a common base line, with which to compare all of the results, survival of the weed plants (at the conclusion of the assay – 22 days) was considered as the difference between weed survival in the treatments with respect to the control (biochar and herbicide free soils) expressed as a percentage relative to survival in the control (Figure 3).

When subjected to exposure to mesotrione *A. retroflexus* survival decreased in the biochar free treatments (-96 % ± 2 %) and the 1 % biochar treatments (-97 % ± 2 %) (Figure 3a). Thus, mesotrione efficacy was equivalent where soil only treatments and the 1 % biochar treatments were compared. It was noted that the efficacy of mesotrione in the 1 % biochar treatments was set against the positive influence biochar had in the absence of herbicide (+164 % ± 49 %). In contrast, the 5 % biochar treatments exposed to mesotrione indicated an increase in weed viability (+ 38 % ± 34 %). Reduced herbicidal efficacy in the 5 % biochar treatments could be attributed to: i) herbicidal activity being deactivated by the sorptive capacity of the biochar (Yang et al., 2010; Wang et al., 2009; Spokas et al., 2009; Yu et al., 2006), ii) the beneficial influence biochar had (when applied at 5 %) upon *A. retroflexus* survival in the absence of herbicide, or; a combination of both factors.

A. retroflexus viability when subject to exposure to pendimenthalin resulted in a decrease in weed viability in the biochar free treatments (-85 % ± 8%) and in the 1 % biochar treatments (-67 % ± 18 %). Where biochar was present at 5 % weed viability increased despite pendimenthalin being present (+10 % ± 25%). It was noted *A. retroflexus* viability was greatly improved by the presence of biochar both at 1 % and 5 % amendment. When 1 % and 5 % biochar was added to soil the number of *A. retroflexus* plants increase significantly ($p < 0.05$) compared with the biochar-free soil.

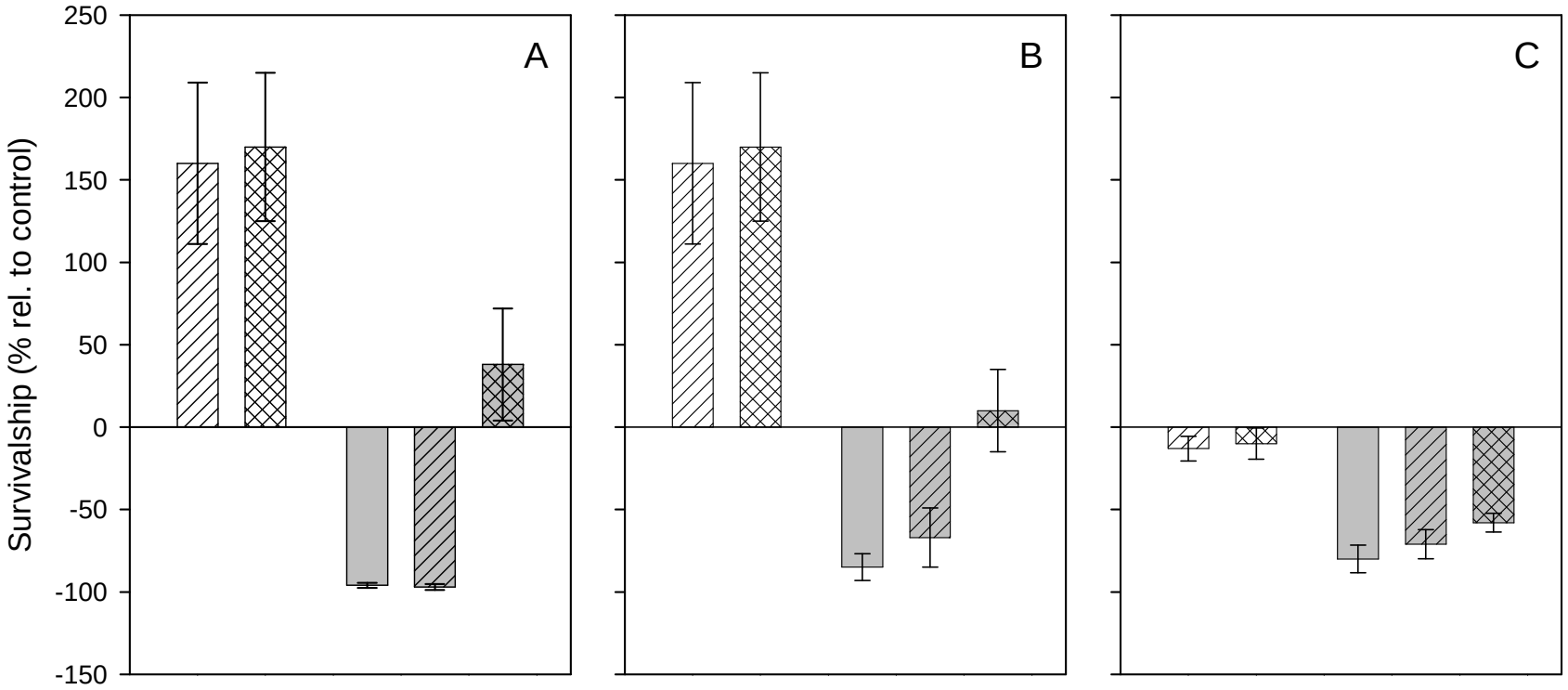


Figure 3 – Weed survival at the end of the assay (22 d) expressed relative to the soil only herbicide free treatments. Hatched bars are treatments with 1 % biochar, and; cross-hatched treatments with 5 % biochar. White bars are herbicide free while grey bars have been dosed with herbicide: *A. retroflexus* in treatments dosed with mesotrione (A), *A. retroflexus* in treatments dosed with pendimenthalin (B) and *S. nigrum* in treatments dosed with terbuthylazine (C). Values shown ± 1 standard error (n = 4).

S. nigrum did not show significant differences between the untreated soil and the soil amended with biochar. When exposed to terbuthylazine weed survival was dramatically reduced in the biochar free soil and the treatments containing biochar at both 1 % and 5 % application rate. These results highlight that the addition of biochar at higher concentrations (5 %) can drastically reduced the efficacy of herbicides but that the deactivation of herbicidal activity is variable in its extent depending upon herbicide used and the weeds it is used target.

3.4 Physical characteristics of the weeds in untreated and treated soil

The median fresh weight of the plants (*A. retroflexus*) grown in the biochar free soil was 3.5 mg. Significant increases in fresh weight ($p < 0.05$) of plants grown in soil amended with 1 % (5.3 mg) and 5 % (5.9 mg) biochar were observed with respect to the biochar free control soil (Figure 4a). When herbicide (pendimenthalin) was added the fresh weight of the plants in the biochar free soil was 3.7 mg. In herbicide applied treatments containing 1 % and 5 % biochar these weights were 3.2 mg and 3.5 mg, respectively. None of the fresh weights were significant ($p < 0.05$) different to those observed in the biochar free control soil.

In the absence of herbicide the length of the plants grown with biochar increases significantly ($p < 0.05$) with increasing amounts of biochar: the median length of the epigeal plant parts in the biochar free soil was 4 mm; in soil amended with 1% biochar 10 mm, and; with 5 % biochar 16 mm (Figure 4b). Thus, following 22 days of assay time, weed lengths were doubled in the 1 % biochar treatments and quadrupled in the 5 % biochar treatments. These results were expected in light of previous research that has reported enhance plant growth in biochar amended soils (Lehmann et al., 2006).

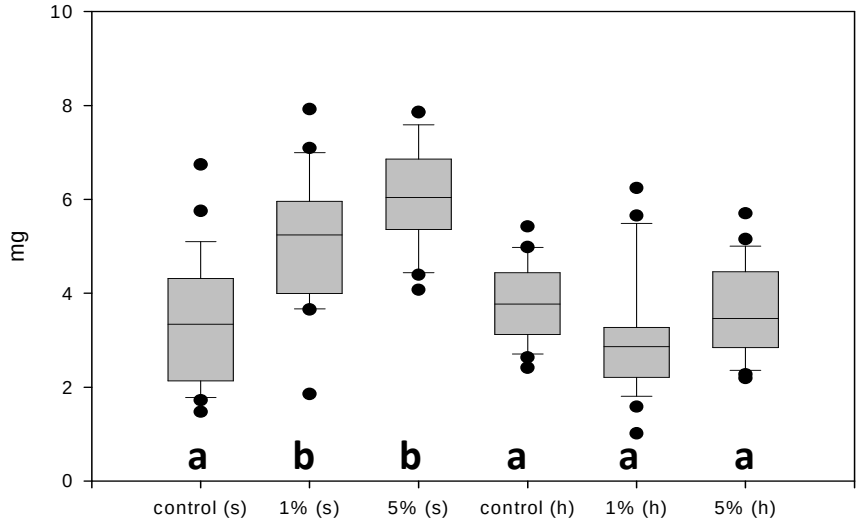
In the presence of herbicide (pendimenthalin) the epigeal part of plants grown in biochar free soil was observed to be significantly shorter ($p > 0.05$) than epigeal length observed in the control soil (Figure 4b). In contrast, the epigeal part of the plants grown in the presence of biochar did not show a significant difference ($p < 0.05$) when compared to the control soil (Figure 4b). These results indicate that under standard

condition pendimenthalin was able to affect the length of the weeds, but that the addition of biochar to soil maintained stem growth at lengths similar to those observed in the biochar-free control soil.

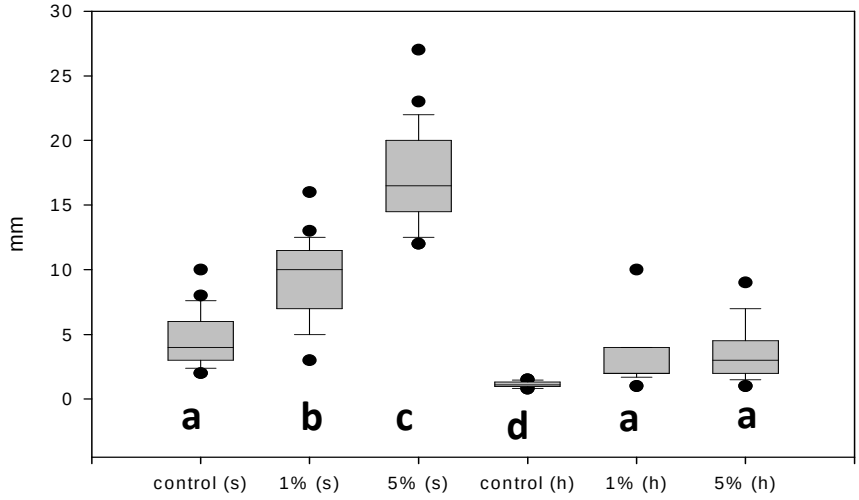
Considering hypogeal plant parts (Figure 4c) in the absence of herbicide median root lengths in the biochar free soil was 22 mm; in the 1 % biochar treatments 35 mm, and; in the 5 % biochar treatments 30 mm (Figure 4c). Significant ($p < 0.05$) increases were observed where length of the roots in the 1 % and 5 % of biochar treatments were compared to root lengths in the biochar free soil.

The addition of herbicide (pendimenthalin) resulted in a significant ($p < 0.05$) decrease in roots lengths observed in the biochar free soil. However, where root lengths in soil containing 1 % and 5 % of biochar were compared to with those in the biochar free control soils no significant ($p > 0.05$) difference were observed (Figure 4c) .

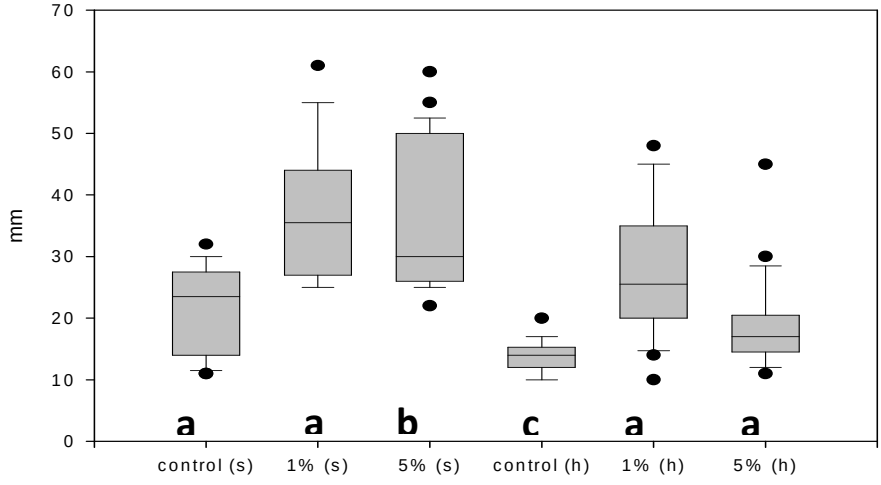
In terms of stem and root length these results indicate that pendimenthalin was effective in undermining weed physical structure in the biochar free soils. However, where biochar was present these results indicated that disruption to weed physical structure did not take place with both epigeal and hypogeal lengths showing no significant ($p > 0.05$) decrease with respect to the biochar free control soil to which no herbicide was added.



A



B



C

Figure 4 – Fresh weight (A), stem length (B) and root length (C) of the plants grown in untreated soil, 1% biochar amended soil, 5% biochar amended soil in absence (s) and presence of pendimenthalin (h) and control. Different letters below the bars indicates significant difference ($p < 0.05$)

4. Discussion

The benefits of herbicide application with respect to improved crop productivity have been well established (e.g. Gianessi and Reigner (2007) reported a loss of rice, soybean, cotton and wheat of 53%, 26%, 20% and 25% respectively without the use of herbicide; Abernathy J.R. (1981) accounted for a loss of 36% of the total U.S. corn crop as a result of using only available cultural and mechanical control practices without the use of herbicides). On balance the agricultural benefits associated with biochar (e.g. improving soil fertility) should be considered alongside potentially detrimental outcomes, specifically, herbicide deactivation.

Of course, the extent to which herbicidal activity might be deactivated in presence of biochar will be dependent upon biochar properties, these in turn being dictated by feedstock type and pyrolysis conditions used to produce the biochar. The physical and chemical characteristics of biochar vary considerably depending by the feedstock and the pyrolysis conditions applied (Chan et al., 2009).

Downie et al (2009) reported abundance of macropores in biochar produced at higher pyrolysis temperatures. The importance of macropores (>50 nm diameter) in influencing the sorption-desorption hysteresis of organic compounds (such as herbicides) has been correlated to the decrease in particle size and thus the increase of the specific surface area (Tian et al., 2010). The biochar applied in this research was produced at a relatively moderate temperature (~ 450^{o}C); given the results of Tian et al (2010) herbicide sorption might be expected to be greater where higher temperature biochars are used.

The results reported here suggest that a high biochar application rate (5 %) was required to result in a reduction in herbicidal activity (Figure 3). Biochar application rates reported in literature varies, ranging between 1.5 t ha^{-1} to 135 t ha^{-1} (Asai et al. (2009), Blackwell et al. (2007), Chan et al. (2007), Chan et al. (2008), Gaskin et al. (2010), Hossain et al (2010), Ishii and Kadoya (1994), Lehmann et al. (2003), Jeffery et al., 2011, Kimetu et al. (2008), Major et al. (2010), van Zwieten et al. (2009), Yamato et al. (2006)). Incorporation of biochar, at an application rate of 100 t ha^{-1}, to

soil to a depth of 10 cm would result in a biochar content of approximately 5 % (see Supporting Information for calculation (Appendix D)). This research has indicated that an application rates of 1 % biochar did not deactivate the herbicides tested with respect to the target weeds used in the experimental assays (Figure 3). Based upon these results limiting biochar application to ensure a resultant biochar loading up to ~1 % would be advisable. This loading, of course, being dependent upon both biochar application rate (t ha^{-1}) and the depth to which biochar is incorporated.

Results reported here relate to three herbicides of contrasting physical and chemical properties (Table 1). It is noted that deactivation of herbicidal activity was observed for both mesotrione and pendimenthalin; these compound representing the maximum and minimum values for aqueous solubility (160 mg L^{-1} and 0.33 mg L^{-1}, respectively) and octanol-water partition coefficients (0.11 and 5.2 (log K_{OW}), respectively) of the herbicides tested. The third herbicide, terbuthylazine, had aqueous solubility and a log K_{OW} value between those of mesotrione and pendimenthalin (Table 1). While the deactivation of more hydrophobic compounds might be expected to be more pronounced than of less hydrophobic compounds (on account of stronger partition to biochar/geosorbents (Nag et al., 2011)), the results supported here do not support this. Further research is required to screen the sorption of contrasting herbicides, the implication of this sorption with respect to the changes in herbicidal efficacy and to evaluate possible desorption mechanisms over the time. A broader dataset of this type will determine relationships between herbicides properties and their vulnerability in the presence of biochar.

5. Conclusions

At high application rates of 5 % biochar was effective at deactivating the herbicidal activity of two of the three herbicides tested. These herbicides represent different groups of herbicide, namely, the triketone and dinitroaniline classes. At more moderate biochar application rates (1 %) deactivation of herbicide was not observed. These results highlight a need for caution when biochar application is made to

agricultural land that is reliant upon the use of soil application herbicides in order to ensure herbicide phytoavailability is maintained. Further work on the partitioning of the three herbicides in biochar amended soil is required to understand the extent of herbicide adsorption and bioavailability at different application rate of biochar. Moreover, this initial study was conducted under controlled laboratory condition. Further research is required to establish the potential for herbicide deactivation in biochar amended soils at the field scale. In addition, further herbicide classes should be considered and evaluation made of their relative resilience to deactivation in biochar amended soils.

Acknowledgements

We are grateful to Syngenta Jealott's Hill International Research Centre for the provision of herbicides and weed seeds used in this research and for the technical advice they provided.

References

Asai, H., Samson, B.K., Stephan, H.M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T., Horie, T., 2009. Biochar amendment techniques for upland rice production in Northern Laos: 1. Soil physical properties, leaf SPAD and grain yield. Field Crops Research. 111, pp. 81-84

Abernathy, J.R., 1981. Estimated crop losses due to weeds with nonchemical management, in: Pimentel, D., (Ed), CRC Handbook of Pest Management in Agriculture (Volume 1). CRC Press, Florida

Bélanger, N.I., Côté, B., Fyles, J.W., Chourchesne, F., Hendershot, W.H., 2004. Forest regrowth as the controlling factor of soil nutrient availability 75 years after fire in a deciduous forest of southern Quebec. Plant Soil. 262, pp.363-372.

Blackwell, P., Shea, S., Storer, P., Soilaman, Z., Kerkmans, M., Stanley, I., 2007. Improving wheat production with deep banded oil mallee charcoal in Western Australia, Proceedings of the International Agrichar Conference. Terrigal, NSW

Braida, W.J., Pignatello, J.J., Lu, Y., Ravikovitch, P.I., Neimark, A.V., Xing, B., 2003. Sorption hysteresis of benzene in charcoal particles. Environmental Science and Technology. 37, pp. 409-417.

Chan, K.Y., Zwieten, L.V., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of green waste biochar as a soil amendment. Australian Journal of Soil Research. 45, pp. 629-634.

Chan, K.Y., van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochars as soil amendments. Australian Journal of Soil Research. 46, pp. 437-444

Chan, K.Y., Xu, Z., 2009. Biochar: nutrient properties and their enhancement, in: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management. Earthscan, London, UK.

Downie, A., Crosky, A., Munroe, P., 2009. Physical Properties of Biochar, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management. Earthscan, London, UK, pp. 13-29.

Gaskin, J.W., Speir, R.A., Harris, K., Das, K.C., Lee, R.D., Morris, L.A., Fisher, D.S., 2010. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. Agronomy Journal. 102, pp. 623-633

Gianessi, L.P., Reigner, N.P., 2007. The value of herbicides in U.S. crop production. Weed Technology. 21, pp. 559-566

Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biology and Fertility of Soils. 35, pp. 219-230.

Graber, E., Tsechansky, L., Gerstl, Z., Lew, B., 2012. High surface area biochar negatively impacts herbicide efficacy. Plant and Soil, 1-12.

Jeffery, S., Verheijen, F.G.A., Van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment. 144, pp. 175-187.

Hossain, M.K., Strezov, V., Yin Chan, K., Nelson, P.F., 2010. Agronomic properties of wastewater sludge biochar and bioavailability of metals in production of cherry tomato (lycopersicon esculentum). Chemosphere. 78, pp. 1167-1171

Ishii, T., Kadoya, K., 1994. Effects of charcoal as a soil conditioner on citrus growth and vesicular-arbuscular mycorrhizal development. Journal of the Japanese Society for Horticultural Science. 63, pp. 529-535

Kimetu, J.M., Lehmann, J., Ngoze, S.O., Mugendi, D.N., Kinyangi, J.M., Riha, S., Verchot, L., Recha, J.W., Pell, A.N., 2008. Reversibility of soil productivity decline with organic matter of differing quality along a degradation gradient. Ecosystems. 11, pp. 726-739.

Keech, O., Carcaillet, C., Nilsson, M.C., 2005. Adsorption of allelopathic compounds by wood-derived charcoal: The role of wood porosity. Plant and Soil. 272, pp. 291-300.

Kookana, R.S., 2010. The role of biochar in modifying the environmental fate, bioavailability, and efficacy of pesticides in soils: a review. Soil Research 48, 627-637.

Lehmann, J., da Silva Jr, J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B. 2003. Nutrient avaiability and leaching in an archaelogical Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil. 249, pp. 343-357.

Lehmann, J., Gaunt, J., and Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems - A review. Mitigation and Adaptation Strategies for Global Change. 11, pp. 403-427.

Lehmann, J. and Rondon, M., 2006a. Biochar soil management in highly weathered soils in the humid tropics in Biological approaches to sustainable soil systems, N. Uphoff, Editor. CRC Press: Boca Raton, FL.

Lehmann, J. and S. Joseph, Biochar for environmental management: an introduction, in Biochar for environmatal management - Science and Technology, J. Lehmann and S. Joseph, Editors. 2009, Earthshan publisher: UK and USA.

Li, R., Wen, B., Zhang, S., Pei, Z., Shan, X., 2009. Influence of organic amendments on the sorption of pentacholorphenol on soils. Journal of Environmental Sciences. 21, pp. 474-480.

Liang, B., Lehmann, J., Solomon, D., Kinyangi, J., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luizao, F.J., Peterson, J., Neves, E.G., 2006. Black carbon increases cation exchange capacity in soils. Soil Science Society America Journal. 70, pp. 1719-1730.

Lu, Y. and Pignatello, J.J., 2002. Demonstration of the "conditioning effect" in soil organic matter in support of a pore deformation mechanism for sorption hysteresis. Environmental Science and Technology. 36, pp. 4553-4561.

Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2010. Maize yield and nutrition during 4 years after biochar application to a Colombian savannah oxisol. Plant and Soil. 333, pp. 117-128.

Nag, S.K., Kookana, R., Smith, L., Krull, E., Macdonald, L.M., Gill, G., 2011. Poor efficacy of herbicides in biochar-amended soils as affected by their chemistry and mode of action. Chemosphere. 84, pp.1572-1577

Neary, D.G., Klopaket, C.C, DeBano, L.F., Folliot, P.F. 1999. Fire effects of below-ground sustainability: A review and synthesis. Forest Ecology and Management. 122, pp. 51-71.

Roberts, K.G., Gloy, B.A., Joseph S., Scott, N.R., Lehmann, J., 2009. Life cycle assessment of Biochar systems: estimating the energetic, economic, and climate change potential. Environmental Science and Technology. 44, pp. 827-833.

Rondon, M.A., Lehmann, J., Ramirez, J., Hurtado, M., 2007. Biological nitrogen fixation by common beans (*Phaseolus vulgaris L.*) increases with bio-char additions. Biology and Fertility of Soils. 43, pp. 99-708.

Sander, M. and Pignatello, J.J., 2005 Characterization of charcoal adsorption sites for aromatic compounds: insights drawn from single-solute and bi-solute competitive experiments. Environmental Science and Technology. 39, pp. 1606-1615.

Sander, M., Lu, Y., Pignatello, J.J., 2006. Conditioning-annealing studies of natural organic matter solids linking irreversible sorption to irreversible structural expansion. Environmental Science and Technology. 40, pp. 170-178.

Sohi, S., Lopez-Capel, E., Krull, E., Bol, R., 2009. Biochar, climate change and soil: A review to guide future research. CSIRO Land and Water Science Report, 05/09.

Spokas, K.A., Koskinen, W.C., Baker, J.M., Reicosky, D.C., 2009. Impact of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere. 77, pp. 574-581.

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., Luis, J., de Macedo, L.V., Blum, W.E.H., Zech, W., 2007. Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered central Amazonian upland soil. Plant and Soil. 291, pp. 275-290.

Sutton, P., Richards, C., Buren, L., Glasgow, L., 2002. et al., Acitvity of mesotrione on resistant weeds in maize. Pest Management Science. 58, pp. 981-984.

Tian, C., Wang, M.-d. and Si, Y.-b., 2010. Influences of charcoal amendment on adsorption-desorption of isoproturon in soils. Agricultural Sciences in China. 9, pp. 257-265.

van Zwieten, L., Kimber, S., Morris, S., Chan, K., Downie, A., Rust, J., Joseph, S. Cowie, A., 2009. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant and Soil. 327, pp. 235-246.

Wang, H., Lin, K., Hou, Z., Richardson, B., Gan, J., 2009. Sorption of the herbicide terbuthylazine in two New Zealand forest soils amended with biosolids and biochars. Journal of Soils Sediments. 10, pp. 283-289.

Yaman, S., 2004. Pyrolysis of biomass to produce fuels and chemical feedstocks. Energy Conversion and Management. 45, pp. 651-671.

Yang, X.-B., Ying, G.-G., Peng, P.-A., Wang, L., Zhao, J.-L., Zhang, L.-J., Yuan, P., He, H.-P. 2010. Influence of biochars on plant uptake and dissipation of two pesticides in an agricultural soil. Journal of Agricultural and Food Chemistry. 58, pp. 7915-7921.

Yamato, M., Okimori, Y., Wibowo, I.F., Anshori, S., Ogawa, M., 2006. Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea, peanut and soil chemical properties in south Sumatra, Indonesia. Soil Science and Plant Nutrition. 52, pp. 489-495.

Yu, X.-Y., Ying, G.-G., Kookana, R.S., 2006. Sorption and desorption behaviors of diuron in soils amended with charcoal. Agricultural and Food Chemistry. 54, pp. 8545-8550.

Yu, X.-Y., Ying, G.-G., Kookana, R.S., 2009. Reduced plant uptake with biochar additions to soil. Chemosphere. 79, pp. 665-671.

Zhu, D., Kwon, S., Pignatello, J.J., 2005. Adsorption of single-ring organic compounds to wood charcoals prepared under different thermochemical conditions. Environmental Science and Technology. 39, pp. 3990-3998.

Zhu, D., Pignatello, J.J., 2005a. Characterization of aromatic compound sorptive interactions with black carbon (charcoal) assisted by graphite as a model. Environmental Science and Technology. 39, pp. 2033-2041.

Chapter 6

Biochar: a carrier alternative to peat for rhizobia inoculants

Biochar: a carrier alternative to peat for rhizobia inoculants

Alessia Freddo and Brian Reid

School of Environmental Sciences UEA, Norwich, NR4 7TJ, UK

Abstract

Biochar has been evaluated as a microbial inoculant carrier as an alternative to peat. Two types of biochar, produced from different feedstock materials (redwood and maize) pyrolysed at 600˚C, were tested for their performance in maintaining survival of three rhizospheric bacteria strains (*Rhizobium leguminosarum bv. viciae; Rhizobium etli; Rhizobium leguminosarum* bv. *trifolii*) under different temperature conditions: 4˚C, 25˚C and 35˚C. Results showed high (between 50% and 75%) and very high (> 75%) survival of the strains at higher temperatures when inoculated in redwood biochar; these being significantly higher ($p < 0.05$) to survival recorded in maize biochar and peat. High and very high microbial survival was observed in peat when stored at lower temperatures. Analysis of the chemical and physical properties of the carriers revealed that the higher specific surface area, higher water holding capacity, higher labile carbon and essential micro-nutrients content of biochar were the key elements for a more favourable habitat for rhizobia (particularly at 25˚C and 35˚C). The increase in the pH in maize biochar precluded an equal level of microbial survival to redwood biochar.

1. Introduction

While in the recent past, the use of fertilizers and pesticides have promoted higher crop yields, the same approaches today are failing to guarantee similar improvements (Archer, Eby et al. 2009). Alternative approaches are needed to improve crop yield. Eco-friendly methods include improved water use and soil management, restricting chemical fertilizer and pesticide use, and application of crop rotation (Kim, Sparovek et al. 2007). In addition to these, enhancing soil microbial attributes is also beneficial.

Soil fertility and physico-chemical properties rely upon soil biodiversity and biological processes. When the micro-ecosystem is improved in soil, plant growth is enhanced and the sustainability of the environment is maintained, obtaining beneficial effects on crop yields (Archer, Eby et al. 2009).

The rhizospheric soils present several microorganisms which positively influence crop productivity (Archer, Eby et al. 2009). The plant growth promoting rhizobacteria (PGPR) are rhizospheric microorganisms have the means to enhance plant growth. The presence of PGPR in the rhizosphere prevents pathogen infections to the plant by secreting antifungal metabolites. In addition, PGPR ensure to the plant an efficient nitrogen fixation, an improvement of mineral solubilisation and uptake, availability of growth promotion hormones and tolerance of environmental stress (Liang, Lehmann et al. 2006; Grossman, O'Neill et al. 2010; Liang, Lehmann et al. 2010).

The quality of the soil highly influences which rhizobia species colonize the rhizosphere. Herridge (2002) reported that soils are rhizobia host specific, and that soil acidity and scarcity of carbon matter are inversely proportional to rhizobial population (Makoto, Tamai et al. 2010; Warnock, Mummey et al. 2010). Thus the inoculation of PGPR to soil has been a long-established and successful practice to enrich the quality of different soils and thereby allow a better growth of legumes and crops in otherwise difficult soil conditions. For several decades PGPR have been introduced into agricultural lands through soil inoculation or bacterial coating on seeds, roots, or tubers (Cassidy, Lee et al. 1996; Bond 2010) or, more recently, using inoculant carriers. Although many studies have reported the beneficial effects of these practices results are quite variable (Cassidy, Lee et al. 1996). The major obstacle of this technique is developing a reliable and effective inoculant technology.

The main limitation when using an inoculant carrier is given by the type of the material used. The essential characteristic of a carrier is: to have a good capacity to deliver a certain threshold of viable cells in good physiological condition to the soil surrounding the root (Trevors 1991; Rivera‐Utrilla, Bautista‐Toledo et al. 2001; Pietikäinen, Kiikkilä et al. 2003; Samonin and Elikova 2004; Kookana 2010; Graber, Tsechansky et al. 2012). In order to ensure this, the carrier used should have properties which meet the needs of the microbial physiology (high water holding capacity, pH buffering capacity, cations and/or anions exchange capacity and to be non-toxic to rhizobia); of the plant (good adhesion to seed), and; of industrial requirements (easy to sterilize, readily and inexpensively available, survival during storage) (Makoto, Tamai et al. 2010). Many different carrier materials have been studied and used, in both slurry and powder forms. Examples of carriers are: mineral soil (silt loam) (Chao and Alexander 1984), soybean/peanut oil (Kremer and Peterson 1983), alginate beads (Bashan 1986), and peat (Thompson 1980).

The carrier must be able to support a high number of microorganisms. Sometimes the chemistry and the physical structure of a carrier are able to carry high numbers of only one specific strain (Yardin, Kennedy et al. 2000). Peat soil is the material repeatedly shown to be suitable for several PGPR: *Rhizobium* (Yardin, Kennedy et al. 2000), *Agrobacterium radiobacter* (Yardin, Kennedy et al. 2000), *Penicillium bilaii* (Rice, Olsen et al. 1995), *Pseudomonas fluorescens* (Vidhyasekaran and Muthamilan 1995), and others (Gagné, Dehbi et al. 1993). Peat has also been reported to meet most of the criteria listed above (Tilak, Pal et al. 2010). Peat has become a widely used carrier for agricultural applications (Thompson 1980). However, peat is often either not readily available (Chao and Alexander 1984; Graham-Weiss, Bennett et al. 1987) or present in preserved wet-lands where its extraction is forbidden (Daza, Santamarıa et al. 2000). Moreover the exposure of inoculants to high temperatures and dry conditions during shipping, storage and planting often results in decreased viable cell numbers and N_2-fixing effectiveness of the rhizobia (Kremer and Peterson 1983). Due to these limitations, more readily available carriers have been studied and investigated (Thompson 1980; Stephens and Rask 2000; Hungria, Loureiro et al. 2005).

Recently biochar has become of great interest in regards to its wide potentials in several environmental issues, most significantly biochar has been viewed as a strategy to mitigate climate change (by reducing the emission of carbon dioxide during heat and

power production) and as a mean to enhance the quality and fertility of soil, due to the intrinsic chemical and physical characteristics of biochar. In addition, studies on the effect of biochar in relation to the microbial biomass have shown microbial population to increases where biochar has been added to soil (Lehmann, Rilling et al. 2011). Though very little is known about the mechanisms which promote higher microbial abundance, properties such as high water holding capacity (Lehmann, Rilling et al. 2011), high organic contents, environmental safety and non-toxicity (Freddo, Cai et al. 2012), together with advantages such as relatively readily and inexpensive availability, and its long stability in soil, make biochar a potentially good alternative to peat as microbial inoculant carrier.

To date the number of studies which have tested the potential of biochar/charcoal as microbial carrier are extremely limited (Newbould 1951; Kremer and Peterson 1983; Ogawa 1989; Saranya, Krishnan et al. 2011).

The properties of biochar produced from different feedstock and pyrolysis temperature can vary dramatically, thus different effects on inoculant organisms can be expected. The research presented here compares the survival of three PGPR strains (*R. leguminosarum* bv. *viciae; R. leguminosarum bv. trifolii; R. etli*) following their inoculation into peat and into biochars produced using redwood and maize feedstock (at 600˚C). Cell viability was established at three incubation/storage temperatures (4 ˚C, 25 ˚C and 35 ˚C) over 60 days of storage. In this way evaluation of which carrier was most suitable was made.

2. Material Methods

2.1 Carriers

The biochars used as carriers were produced from maize (BM) and redwood (BR). Each feedstock was washed and dried and then pyrolised into biochar. To produce the biochar, the materials were placed in a high performance automatic controlled furnace (GWL-1200, Henan, China), with a continuous flow of nitrogen through the furnace chamber. A cooling chamber with water was used for passing the off-gas to allow condensation of tars. Biochar was cooled to room temperature in the presence of nitrogen gas inside the furnace. The pyrolysis regimes took 2.5 hours at 600˚C.
The control carrier was moss peat, provided by PJ McAnulty & Co; Coalisland, Dungannon.

2.2 Chemical and Physical characteristics of the carriers

Carbon (C) and nitrogen (N) content of each carrier was analysed using CHNS-O Elemental Analyzer (EA1108, Carlo Erba Instruments).

Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES, Varcan Vista-Pro Axial) was used to determine the following chemical elements (wavelength light): Al (396 nm); As (189 nm); B (250 nm); Ca (316 nm); Cd (229 nm); Co (239 nm); Cr (268 nm); Cu (325 nm); Fe (260 nm); K (766 nm); Mg (285 nm); Mn (258 nm); Mo (202 nm); Na (590 nm); Ni (232 nm); P (214 nm); Pb (220 nm); S (182 nm); Sr (422 nm); Zn (214 nm). Before the analysis, the samples (0.5g) were mixed with HNO_3 (5 mL) and microwave digested (High Performance Microwave Digestion System, Milestone EthosOne) for 20 minutes to reach 240˚C and then for 25 minutes at steady temperature (~220˚C). Once digested, the sample were diluted with 20 mL of milliQ water and loaded in the ICP-OES. The operating conditions of the ICP-OES were: power, 1.200 kW; plasma flow, 15.0 L min^{-1}; Auxulary flow, 1.50 L min^{-1}; nebulizer (seaspray concentric nebulizer) flow, 0.75 L min^{-1}. The values of the chemical elements are reported in mg kg^{-1} in Table 1.

The pH of peat (pH = 4.1) and maize biochar (pH = 9.3) was adjusted to 7 with $CaCO_3$ and HCl respectively. The pH of redwood biochar was 7.

The water holding capacity (WHC) and moisture content (Mc) of peat, BM and BR over 60 days were calculated.

The physical structure of peat and biochar was evaluated using Scanning Electron Microscopy (JSM4900LV, JEOL Ltd, Japan). Before the analysis, the samples were first mounted on double sided carbon adhesive, fitted on aluminium stubs, and then gold coated by a Polaron SC7640 High Resolution Sputter Coater (operating parameters: 30 sec at 20mA; power 2.1kV; pressure 4 10^{-2}mbar; average distance form gold target, about 50mm).

2.3 Preparation of the carriers

Maize and redwood biochars were pulverised using a ball mill. 5g dry weight of each matrix were disposed in glass jars and closed hermetically using a lid. The jars were autoclaved 3 times at 121˚C for 90 min. The sterility of the carriers was tested in duplicate by plating 100µl of one fold diluted matrix on nutrient agar medium. The

dilution was made by shaking for 30 minutes 1g of soil/biochar that was thereafter diluted in 10 mL distilled water.

2.4 Bacterial strains and inoculum preparation

The rhizobia used were *Rhizobium leguminosarum* bv. *viciae* strain 3841, *Rhizobium etli* strain MP4 DO6 and *Rhizobium leguminosarum* bv. *trifolii*, provided by John Innes Centre, Norwich, UK. Strains were grown at 25˚C in 250 mL Erlenmeyer flasks containing 100 mL of tryptone yeast medium. The cultures were incubated at 25˚C on a rotary shaker operating at 200 rpm for 5 days.

Portions (ca. 10^{11} cells per mL) of each strain were inoculated aseptically from the media of growth to the pre-autoclaved peat and biochar samples. 0.1% (wt/wt) sucrose was added (1 mL) as a nutrient supplement. The final moisture provided at the beginning of the assay was 70% in peat and 40% in biochars. Different moisture contents are in relation to the different water holding capacity of the carriers. The inoculated jars were kept in the dark at 4˚C, 25˚C and 35˚C for 60 days.

2.5 Enumeration

The survival of each strain was tested over time (7 days, 14 days, 30 days and 60 days). 1g of sample was diluted in a polyethylene tube with 10 mL of TY medium. The tube was then shaken for 30 min on a rotary shaker at 150 rpm. Samples were then plated using the drop plate method. Each TY agar plate was divided into four quarters and each quadrant was reserved for one dilution in the series. Each dilution was dispensed in five evenly spaced of 10 µl drops. Petri dishes were inverted and incubated at 25˚C for 5-6 days. Thereafter, colonies were counted with the aid of a 10X magnifier. The number of colonies was considered reliable when ranging between 3 and 30 per drop.

Statistical analysis

Independent sample t-test and One-Way ANOVA were performed using SPSS 16.0 for Windows. Statistical significance of the colonies forming units (CFU) in different temperature conditions and between strains was determined at 95% confidence interval with the significance level at 0.05.

3. Results

3.1 Analysis of the carrier materials

The concentrations of the chemical elements of the three carrier materials are reported in Table 1. Peat presented average values significantly higher ($p<0.05$) than BR and BM in the following elements: Al (670 mg kg^{-1}), Fe (683 mg kg^{-1}), Na (311 mg kg^{-1}), Ni (1.11 mg kg^{-1}), Pb (2.7 mg kg^{-1}) and S (4802 mg kg^{-1}), Sr (24 mg kg^{-1}). In peat, Zn (5.33 mg kg^{-1}) concentrations were significantly lower ($p < 0.05$) than in BR and BM .

BM showed average concentrations of Cu (10 mg kg^{-1}), K (30200 mg kg^{-1}), Mo (1.2 mg kg^{-1}) and P (2190 mg kg^{-1}) significantly higher ($p < 0.05$) to peat and BR. BR had significantly higher average concentrations ($p < 0.05$) than peat and BM of Ca (3250 mg kg^{-1}) and Mn (456 mg kg^{-1}). Co, Mg, Sr and Zn showed values significantly different between the three carriers: Co showed higher concentrations in BR (6.82 mg kg^{-1}) than in BM (4.23 mg kg^{-1}) and peat where no traces of Co were detected. Concentrations of Mg were higher in peat (2850 mg kg^{-1}), BM (1370 mg kg^{-1}) and BR (889 mg kg^{-1}) respectively. Average concentrations of Sr were higher in peat (24 mg kg^{-1}), BR (15 mg kg^{-1}) and BM (4.55 mg kg^{-1}) in sequence. Finally, Zn presented higher concentrations in BM (51 mg kg^{-1}) and lower in BR (31 mg kg^{-1}) and peat (5.33 mg kg^{-1}).

Table 1: Chemical elements concentrations (mg kg^{-1}) (± standard error) in redwood biochar, maize biochar and peat. For each element, different letters indicate significance difference ($p < 0.05$) between the carriers.

	Redwood Biochar (mg kg^{-1})	Maize Biochar (mg kg^{-1})	Peat (mg kg^{-1})
Al	37 ± 0.33a	54 ± 3.1a	671 ± 11b
As	0.21 ± 0.34a	0.02 ± 0.17a	0.12 ± 0.05a
B	5.86 ± 0.1a	4.96 ± 0.22a	5.22 ± 0.09a
Ca	3252 ± 163a	1229 ± 291b	1895 ± 124b
Cd	0.02a	0.07 ± 0.02a	0.06 ± 0.02a
Co	6.82 ± 0.15a	4.23 ± 0.19b	N.D.
Cr	0.27 ± 0.01a	0.7 ± 0.09a	0.98 ± 0.21a
Cu	2.75 ± 0.29a	11 ± 0.89b	2.48 ± 0.29a
Fe	21 ± 0.59a	75 ± 6.83a	683 ± 90b
K	951 ± 15a	30206 ± 111b	115 ± 4.17a
Mg	889 ± 8.37a	1373 ± 111b	2845 ± 23c
Mn	456 ± 14a	16 ± 0.98b	13 ± 0.43b
Mo	0.45 ± 0.1a	1.18 ± 0.16b	0.43 ± 0.01a
Na	73 ± 33a	61 ± 25a	312 ± 19b
Ni	0.74 ± 0.11a	0.57 ± 0.07a	1.11 ± 0.11b
P	74 ± 2.53a	2190 ± 178b	125 ± 2.02a
Pb	0.25 ± 0.09a	0.1 ± 0.03a	2.72 ± 0.22b
S	828 ± 198a	820 ± 171a	4802 ± 127b
Sr	15 ± 2.14a	4.55 ± 0.88b	24 ± 0.8c
Zn	31 ± 0.85a	50 ± 3.15b	5.33 ± 0.13c

N.D. – not detected

The carbon (C) content in BR was found to be the highest (91.2%) compared to BM (80%) and peat (50%). Nitrogen (N) had higher values in peat (0.8%) than in BM (0.7%) and BR (0.3%).

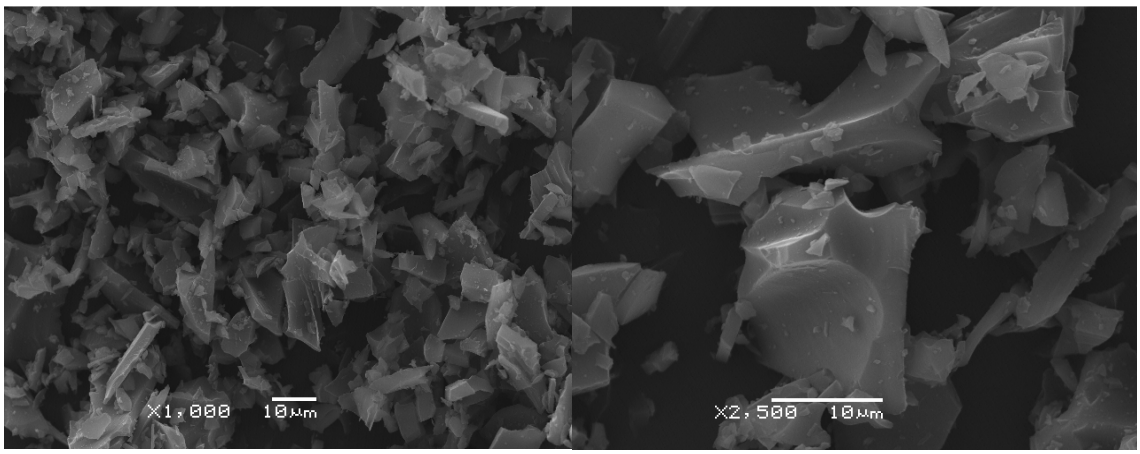
Over the 60 days assay, pH presented constant values in BR (7) and peat (6.7), while increased over the time in BM (from 7.5 in day 0 to 8.5 in day 60).

WHC presented the following values on dry matter: peat, 5.62 mL g^{-1}; BM, 2.46 mL g^{-1}, and; BR 1.95 mL g^{-1}. The moisture content (Mc) in peat and biochars at 4˚C, 25˚C and 35˚C was tested after 60 days. Results indicated (Table 2) that between day 0 to day 60, the Mc loss at 4˚C was 27% in peat, 39% in BM and 51% in BR; at 25˚C the Mc loss in peat was 25%, in BM was 21% and in BC 20%; at 35˚C peat lost 97% of Mc while BM and BR lost 93% and 92% of Mc respectively.

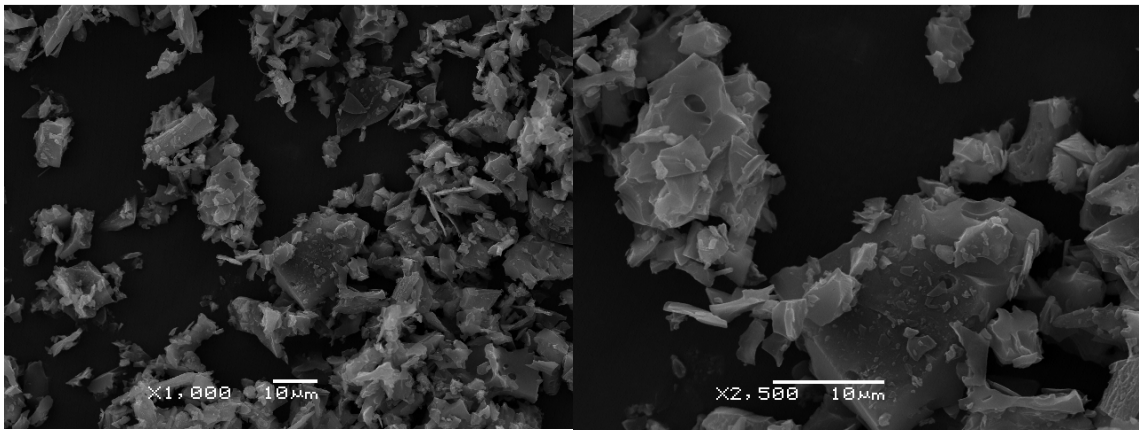
Table 2: Moisture loss (%) from day 0 to day 60 in peat, maize biochar and redwood at 4˚C, 25˚C and 35˚C.

	Peat	Maize Biochar	Redwood Biochar
4˚C	27%	39%	51%
25˚C	25%	21%	20%
35˚C	97%	93%	92%

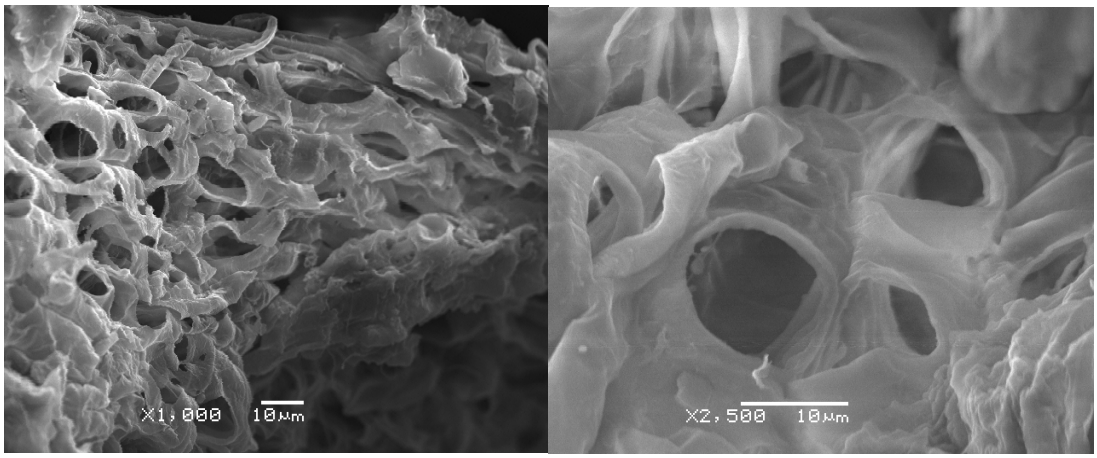
The physical structure of the carriers was also taken into account. The carriers were observed through scanning electron microscope (SEM). These images are presented in Figure 2. The specific surface areas calculated for each material were as follows: 0.01 m^2/m^3 (peat), 0.4 m^2/m^3 (BR) and 0.56 m^2/m^3 (BM).



A a



B b



C c

Figure 2: SEM images of redwood biochar (A/a), maize biochar (B/b), and peat (C/c).

3.2 Survival of *Rhizobia* in peat

The initial cell density of *R. leguminosarum bv. viciae* and *R. etli* was 9 x 10^{10} cells per g of peat, and 1.4 x 10^{10} cells per g of peat of *R. leguminosarum bv. trifolii*. The survival of the three strains inoculated in peat for 60 days and stored at 4˚C, 25˚C and 35˚C is shown in Figure 3.

Counts made at 7, 14, 30 and 60 days show that the survival for all organisms tested was greatest at 4˚C and lowest at 35˚C (Figure 3).

In *R. leguminosarum bv. viciae*, the cell survival at 4˚C was relatively constant over the 8 weeks, with a lower survival on day 7 (this data point also noted for its higher standard deviation). The relative cell loss in *R. leguminosarum bv. viciae* was only 1% at the end of the assay (day 60). On day 7, the relative survival of *R. leguminosarum bv. viciae* stored at 25˚C was 30% which was significantly lower ($p < 0.05$) than the relative survival recorded at 4˚C (82%) on the same date. At 35˚C, *R. leguminosarum bv. viciae* did not survive after 7 days.

R. etli behaved similarly (no significant difference; $p > 0.05$) at 4˚C and 25˚C during the first two weeks of storage, with a respective relative loss of cell survival of 32% and 30% after 7 days. The survival was relatively constant at both temperatures after 14 days, with an additional relative increment of 0.39% at 4˚C and 2% at 25˚C. On day 30, the survival of *R. etli* showed a marked decrease at 4˚C and 25˚C. However the relative survival at 4˚C (55%) was significantly higher ($p<0.05$) than the relative survival recorded at 25˚C (28%). After 60 days, half (50%) of the initial cells inoculated in peat survived at 4˚C; while no cells were detected when stored at 25˚C. The survival of *R. etli* at 35˚C was significantly lower ($p<0.05$) than the survival recorded at 4˚C and 25˚Cover the entire storage period. The relative survival of *R. etli* after the first week was 34% when stored at 35˚C and 28% was observed after 14 days.

R. leguminosarum bv. trifolii did not survived after 7 days at 35˚C. Over the 60 days of the assay, *R. leguminosarum bv. trifolii* relative survival at 4˚C was significantly higher ($p<0.05$) than the relative cell survival at 25˚C. On day 7, *R. leguminosarum bv. trifolii* relative survival at 4˚C was 91% and over the time the survival decreased until reaching a relative survival on day 60 of 64%. At 25˚C, 30% of *R. leguminosarum bv. trifolii* survived after the first week. The number of cells stored at 25˚C decreased during the assay, until no survival was recorded after 30 days.

3.3 Survival of *Rhizobia* in maize biochar

The initial cell density of *R. leguminosarum bv. viciae, R. etli* and *R. leguminosarum bv. trifolii* was 1 x 10^{12} cells per g of BM, 9 x 10^{10} cells per g of BM and 1.4 x 10^{11} cells per g of BM, respectively. The relative survival of *R. leguminosarum bv. viciae, R. etli* and *R. leguminosarum bv. trifolii* inoculated in BM for 60 days and stored at 4˚C, 25˚C and 35˚C is shown in Figure 3.

Counts made in different times over the assay showed that the survival for all the strains followed a similar trend to the strains inoculated in peat, with greatest survival at 4˚C and poorer at 35˚C.

R. leguminosarum bv. viciae and *R. etli* did not survived at higher temperature (35˚C) after 7 days, while *R. leguminosarum bv. trifolii* relative survival was only 23% on day 7 and no survival was recorded after 14 days.

The survival of *R. leguminosarum bv. viciae* showed no significance difference ($p<0.05$) if stored at 4˚C or 25˚C during the first 30 days of assay. The number of cells of *R. leguminosarum bv. viciae*, stored at 4˚C and 25˚C, decreased at the end of the assay mutually significantly differently ($p<0.05$). More than half (53%) of the cell density survived at 4˚C and only 27% survived at 25˚C.

The trends of *R. etli* survival were significantly different ($p<0.05$) at 4˚C and 25˚C. On day 7, 60% and 33% of *R. etli* relatively survived at 4˚C and 25˚C respectively. While no cells of *R. etli* were counted after 14 days when stored at 25˚C, 67% of *R. etli* survived at 4˚C. The survival of *R. etli* at 4˚C decreased with a final number of survived cells equal to 30%.

R. leguminosarum bv. trifolii behaved significantly differently ($p<0.05$) at 4˚C and 25˚C. At 4˚C *R. leguminosarum bv. trifolii* presented a gradual decrease of survival, with a final relative loss of cells of 53% on day 60. At 25˚C, after 30 days no cells of *R. leguminosarum bv. trifolii* were detected.

3.4 Survival of *Rhizobia* in redwood biochar

The initial CFU of *R. leguminosarum bv. viciae* that was inoculated in BR and stored at 25˚C and 35˚C was 5.1 x 10^{8} cells per g of BR, while 4.8 x 10^{9} cells per g of BR was inoculated in BR and stored at 4˚C. *R. etli* and *R. leguminosarum bv. trifolii* were

inoculated in BR with an initial cell density of 2.4 x 10^{10} cells per g of BR and 5.2 x 10^{8} cells per g of BR and incubated at 4˚C, 25˚C and 35˚C.

The results obtained with BR over the survival of the *R. leguminosarum bv. viciae, R. etli* and *R. leguminosarum bv. trifolii* (Figure 3) were very much different to the results observed in peat and BM.

In contrast to the trends observed with peat and BM, only *R. leguminosarum bv. viciae* responded positively to lower temperature (4˚C), with a final relative survival on day 60 equal to 77%. In contrast, *R. etli* and *R. leguminosarum bv. trifolii* showed a constant decrease in cells survival, with a respective relative survival of 29% and 0% on day 60. *R. leguminosarum bv. viciae* did not show a significant difference ($p < 0.05$) in survival at 25˚C and 35˚C during the first 14 days. The relative survival at 25˚C started to decrease on day 30 (80%) but it was significantly higher ($p<0.05$) to the relative survival recorded at 35˚C (52%). At 25˚C, 70% of *R. leguminosarum bv. viciae* survived after 60 days while, no cells survived at 35˚C. After an initial increase in cell numbers in *R. etli* after 7 days at 25˚C (+ 9%) and 35˚C (+ 9%), the survival in *R. etli* decreased. After 60 days the relative survival at 35˚C was to 58%. On day 14 the cells at 25˚C also decreased (-25%) but increased again on day 30 with a relative survival of 93%, and after 60 days the relative survival of *R. etli*.was 100.4%

Also *R. leguminosarum bv. trifolii* showed an increase of cell density. After 14 days the relative survival at 25˚C and 35˚C was 101% and 100% respectively. However both trends showed a decrease of cells survival over the assay. The final survival of *R. leguminosarum bv. trifolii* at 25˚C and 35˚C was significantly different ($p < 0.05$) and equal to 72% and 50% respectively.

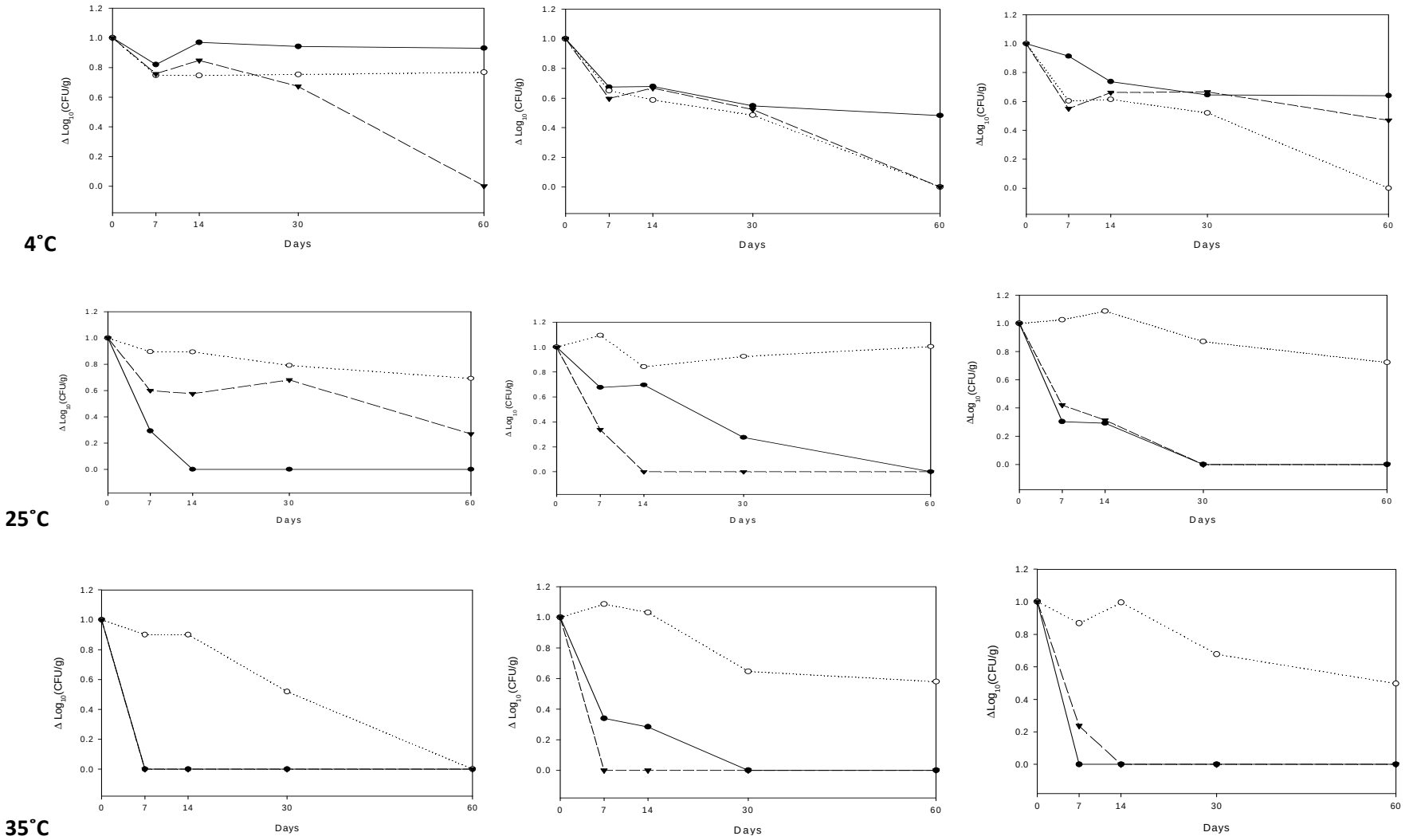


Figure 3: Relative performance of survival (Δ Log_{10}(CFU/g)) over 60 days of *Rhizobium leguminosarum* bv. *viciae* (column A)*; Rhizobium etli* (column B)*; Rhizobium leguminosarum* bv. *trifolii* (column C), in: redwood biochar (white circle), maize biochar (triangle) and peat (black cirlcle) incubated at 4^0C (upper frames), 25^0C (middle frames) and 35^0C (lower frames).

4. Discussion

Table 3 summarises the survival of *R. leguminosarum bv. viciae, R. etli* and *R. leguminosarum bv. trifolii* at the three temperatures using peat, BM and BR after 60 days.

In the literature, rhizobia have been reported to have generally poor growth at temperatures below 10˚C, even if they are tolerant to 4˚C (Graham 1992). Peat was observed to maintain high (between 50% and 75%) or very high (more than 75%) cells survival in all the strains at 4˚C. Similar results are also showed by Chao et al. (1984), who inoculated peat with *R. meliloti* and *R. phaseoli* and stored at 4˚C peat, reporting the best survival in this temperature condition.

In contrast to peat, at 4˚C BM and BR showed medium (between 25% and 50%) and low (less than 25%) survival of *R. etli* and strains C. However, the survival of *R. leguminosarum bv. viciae* was observed to be high (between 50% and 75%) or very high (over 75%) in BM and BR, respectively. This can be explained by the capacity of *R. leguminosarum bv viciae* to grow very well at low temperatures (5˚C) (Drouin, Prévost et al. 1996). Drouin et al (2000) showed cold adaptation mechanisms in R. *leguminosarum bv viciae* similar to psychrotrophic bacteria, which at low temperature induce the synthesis of cold shock and acclimation proteins that may be involved in the maintenance of metabolic functions. Therefore, the results that showed high cell survival of *R. leguminosarum bv. viciae* for all the carriers at 4˚C might be related to the ability of this specific rhizobium to resist and adapt to low temperatures, than the traits of the carriers themselves.

At higher temperatures, only BR was observed to maintain very high (at 25˚C) or high (at 35˚C) survival in all the strains, exception made for *R. leguminosarum bv. viciae* at 35˚C, while peat and BM showed a very low survival in all the strains and temperatures, except for *R. leguminosarum bv. viciae* at 25˚C with about 30% of survival if inoculated to BM. The scarce survival of the strains at 25˚C and 35˚C in BM is probably due to the increase of pH over the time. The pH of BM adjusted to 7.5 was not maintained over the storage period, returning close to the original values (8.5) after 60 days. The optimum pH range for rhizobia is neutral or slightly alkaline (Yadav and Vyas 1973), therefore the conditions created over time in BM were not ideal for the rhizobia survival.

In contrast to BM, the 'natural' pH of BR was optimal for the rhizobia, and will have contributed to ensuring a favourable level of alkalinity during the 60 days of the assay.

The results reported show the efficiency of biochar in enhancing the survival of rhizobia particularly at high temperatures. Moreover, at 25˚C and 35˚C the cell density of *R. etli* and *R. leguminosarum bv. trifolii* calculated after respectively the first or second week of assay, was significantly higher ($p<0.05$) than the initial CFU per g of soil initially inoculated in BR, showing the potential of biochar not only to carry the rhizobia but also to allow their growth. This is a desirable trait for an efficient inoculant carrier (Albareda, Rodríguez-Navarro et al. 2008).

Generally biochar has been described as a matrix with a high porous structure (Downie, Crosky et al. 2009). Such porosity is considered to be an ideal habitat for microorganisms (Lehmann, Rilling et al. 2011), influencing their binding to important nutritive cations and anions (Atkinson, Fitzgerald et al. 2010) and increasing their abundance. It is also reported that pores may facilitate the adhesion of microorganisms to biochar. Samonin and Elikova (2004) present evidences that pore sizes for optimum adhesions may need to be 2-5 times larger than microbial cell-sizes if microorganisms are to enter the pores. If the pores are too large or small may lead to a lower adhesion, either because the curvature is too large to enhance adhesion or because the microorganisms do not fit into the pores respectively (Samonin and Elikova 2004). However, Figure 2 shows that the structure of the biochar used in this study is not particularly porous. The pores observed with the SEM were much smaller than 10µm. Before being inoculated, biochar was been ground mechanically. This operation has possibly destroyed the original structure, creating a high number of small broken organic sheets of biochar, reducing porosity.

This evidence leads to the conclusion that, in this research, the initial proliferation and the final microbial survival have not been facilitated by the porosity of the biochar. However, it is noted that the specific surface area in biochar is much higher than in peat. This has increased notably the surface available to the microorganisms and the possibility for the rhizobia to grow and proliferate, adhering to the surface of biochar through hydrophobic attraction or electrostatic forces, as described by Samonin and Elikova (2004). On the other hand, the electron microscope images (Figure 2) revealed a high level of porosity in peat. As reported for biochar, the pores in peat may have had the role with respect to bacterial protection when stored at low

temperatures, creating a favourable habitat for the rhizobia. This might explain the better survival of rhizobia at 4˚C in peat than in biochar

Other reason for the "good" performance of BR as an inoculant carrier may be found in its moisture content and chemical properties (i.e. nutrient content) which, according to Smith (1992), are two key characteristics of a good carrier (Smith 1992). Although the WHC of BR (1.95 ml g^{-1}) was lower than peat (5.62 ml g^{-1}), possibly due to the different physical structures and to lack of porosity in BR, the % Mc in BR was maintained at a slightly higher level (+5%) than in peat at 25˚C and 35˚C, providing over time a higher moisture availability for growth and survival of the rhizobia (Roughley 1970; Thompson 1980). In contrast, at 4˚C % Mc was lower (-24%) in BR than in peat after 60 days; this being in keeping with the higher survival of rhizobia in peat than in BR at 4˚C.

The high C content is one of the traits that underpins peat as a good inoculant carrier (Albareda, Rodríguez-Navarro et al. 2008). It was noted that the concentration of C in BM was 30% higher than peat and in BR it was 200% higher. Lehmann et al. (2011) divide the composition of biochar into relatively recalcitrant C, labile or leachable C and ash. In contrast to other from other organic material (e.g. peat), biochar is characterized by a larger proportion of aromatic C, specifically fused aromatic C structures (Lehmann et al. 2011), in amorphous or turbostratic forms, depending by the pyrolysis temperature used, lower or higher respectively. These C structures provide in biochar high stability (Nguyen, Lehmann et al. 2010). While the chemical stability may reduce the ability of microorganisms to readily utilize the C as energy source or other nutrients, the fraction readily leachable of biochar may be mineralized as shown in Lehmann et al. (2009) and stimulate microbial activity and abundance (Steiner et al. 2008; Kolb et al. 2009). Deenik et al (2010) and Zimmerman (2010) found, through incubation experiments, a direct relation between the volatile, thus labile, organic matter present biochar and the CO_2 emitted. It is suggested that the content of volatile organic matter of BR and BM could be increased once finely grounded, and therefore allowed a very high access of labile C to the inoculated rhizobia.

The inorganic nutrients present in biochar may also be available to microorganisms (Kolb et al., 2009). BR showed a very high content of several chemical elements, in particular Mn, Co and Zn. Studies on microelement nutrition revealed that the presence of cobalt is essential for the growth of several species of *Rhizobium*, including *R. leguminosarum* and *R. trifolii* (Lowe and Evans 1962). Moreover,

Wilson and Reisenaur (1970) report the importance of Mn and Zn for the growth of several rhizobia, showing a restriction of growth between 0.4-10% and 1-20%, respectively, if the elements were omitted in the medium of growth (Wilson and Reisenauer 1970). These results provide a possible explanation of the high survival of the rhizobia tested over 60 days of assay, showing how the concentration of Co, Mo and Zn, one order of magnitude higher in BR than in peat (Table 1), may have positively influenced growth and survival.

Table 3: Relative level of survival of *R. leguminosarum* bv. *viciae; Rhizobium etli; R. leguminosarum* bv. *trifolii* respectively at 4˚C, 25˚C and 35˚C in peat, maize biochar and redwood biochar on day 60.

		Peat	Maize Biochar	Redwood Biochar
4˚C	*R. leguminosarum bv. viciae*	++++	+++	++++
	R. etli	+++	++	++
	R. leguminosarum bv. *trifolii*	+++	++	+
25˚C	*R. leguminosarum bv. viciae*	+	++	++++
	R. etli	+	+	++++
	R. leguminosarum bv. *trifolii*	+	+	++++
35˚C	*R. leguminosarum bv. viciae*	+	+	+
	R. etli	+	+	+++
	R. leguminosarum bv. *trifolii*	+	+	+++

+, low survival (< 25%); ++, medium survival (between 25%b and 50%); +++, high survival (between 50% and 75%); and very high survival (> 75%)

5. Conclusions

The results presented highlight the potential BR has as a microbial inoculant carrier. The encouraging levels of inoculated cell survival in BR are attributed to the ability of this carrier to: maintain high moisture content, provide liable C and nutrients; these in turn, facilitating very high survival of all *Rhizobium* strains tested at higher temperatures (25˚C and 35˚C). Taken collectively these results suggest RB to support efficient carrier performances that would prove desirable during carrier storing and transporting.

While good results were obtained for peat at lower temperatures this carrier could not match levels of survival observed in BR. The poor efficiency showed using maize biochar are attributed to inapproarite pH conditions in this carrier; this in turn creating an unfavourable environment for rhizobia.

Further studies are needed to establish the ability of biochar to enhance survival of inocula after its introduction into soil, the ability of the inocula to adhere to seed and root surface and be effective in nodulation and plant growth thereafter.

References

Albareda, M., Rodríguez-Navarro, D.N., Camacho, M., Temprano, F.J., 2008. Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biology and Biochemistry 40, 2771-2779.

Atkinson, C., Fitzgerald, J., Hipps, N., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. Plant and Soil 337, 1-18.

Ballard, R., Charman, N., 2000. Nodulation and growth of pasture legumes with naturalised soil rhizobia. 1. Annual Medicago spp. Animal Production Science 40, 939-948.

Bashan, Y., 1986a. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. Applied and Environmental Microbiology 51, 1089-1098.

Bashan, Y., 1986b. Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. Microbiology 132, 3407-3414.

Bashan, Y., 1998. Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnology Advances 16, 729-770.

Bowen, G., Rovira, A., 1999. The rhizosphere and its management to improve plant growth. Advances in agronomy 66, 1-102.

Brown, M.E., 1974. Seed and Root Bacterization. Annual review of phytopathology 12, 181-197.

Chao, W.L., Alexander, M., 1984. Mineral soils as carriers for Rhizobium inoculants. Applied and Environmental Microbiology 47, 94-97.

Cook, R.J., 2000. Advances in plant health management in the twentieth century. Annual Review of Phytopathology 38, 95-116.

Daza, A., Santamarıa, C., Rodrıguez-Navarro, D., Camacho, M., Orive, R., Temprano, F., 2000. Perlite as a carrier for bacterial inoculants. Soil Biology and Biochemistry 32, 567-572.

Downie, A., Crosky, A., Munroe, P., 2009. Physical Properties of Biochar, in: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management - Science and Technology. Earthscan publisher, UK and USA.

Drouin, P., Prévost, D., Antoun, H., 1996. Classification of bacteria nodulating *Lathyrus japonicus* and *Lathyrus pratensis* in northern Quebec as strains of *Rhizobium leguminosarum biovar viciae*. International Journal of Systematic Bacteriology 46, 1016-1024.

Elsas, J.D., Heijnen, C.E., 1990. Methods for the introduction of bacteria into soil: A review. Biology and Fertility of Soils 10, 127-133.

Fages, J., 1990. An optimized process for manufacturing an *Azospirillum* inoculant for crops. Applied microbiology and biotechnology 32, 473-478.

Fages, J., 1992. An industrial view of *Azospirillum* inoculants: Formulation and application technology, 5. International Workshop on *Azospirillum* and Related Microorganisms, Wennigsen(Germany), 6-8 Sep 1991.

Freddo, A., Cai, C., Reid, B.J., 2012. Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar. Environmental Pollution 171, 18-24.

Gagné, S., Dehbi, L., Le Quéré, D., Cayer, F., Morin, J.L., Lemay, R., Fournier, N., 1993. Increase of greenhouse tomato fruit yields by plant growth-promoting rhizobacteria (PGPR) inoculated into the peat-based growing media. Soil Biology and Biochemistry 25, 269-272.

Graham-Weiss, L., Bennett, M.L., Paau, A.S., 1987. Production of bacterial inoculants by direct fermentation on nutrient-supplemented vermiculite. Applied and Environmental Microbiology 53, 2138-2141.

Graham, P.H., 1992. Stress tolerance in *Rhizobium* and *Bradyrhizobium*, and nodulation under adverse soil conditions. Canadian Journal of Microbiology 38, 475-484.

Herridge, D., Gemell, G., Harley, E., 2002. Legume Inoculants and Quality Control, in: Herridge, D. (Ed.), Inoculants and Nitrogen Fixation of Legumes in Vietnam. ACIAR Proceeding 109e. PK Editorial Services, Brisbane, Australia, pp. 105-115.

Hungria, M., Loureiro, M., Mendes, I., Campo, R., Graham, P., 2005. Inoculant preparation, production and application. Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment, 223-253.

Kremer, R.J., Peterson, H.L., 1983. Effects of carrier and temperature on survival of *Rhizobium* spp. in legume inocula: development of an improved type of inoculant. Applied and Environmental Microbiology 46, 1790-1794.

Lehmann, J., Rilling, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota - A review. Soil Biology & Biochemistry 43, 1812-1836.

Lowe, R., Evans, H.J., 1962. Cobalt requirement for the growth of rhizobia. Journal of Bacteriology 83, 210-211.

Nelson, L.M., 2004. Plant growth promoting rhizobacteria (PGPR): Prospects for new inoculants. Online. Crop Management doi 10, 1094.

Newbould, F., 1951. Studies on humus type legume inoculants. I. Growth and survival in storage. Sci. Agr 31, 463-469.

Nguyen, B.T., Lehmann, J., Hockaday, W.C., Joseph, S., Masiello, C.A., 2010. Temperature sensitivity of black carbon decomposition and oxidation. Environmental Science & Technology 44, 3324-3331.

Ogawa, M., 1989. Inoculation methods of VAM fungi: charcoal ball method and rice hull method. Recent Advances in Microbial Ecology. Eds. T Hattori T et al, 247-252.

Piorr, H.P., 2003. Environmental policy, agri evnvironmental indicators and landscape indicators. Agriculture, Ecosystems & Environment 98, 17-33.

Rice, W., Olsen, P., Leggett, M., 1995. Co-culture of *Rhizobium meliloti* and a phosphorus-solubilizing fungus (*Penicillium bilaii*) in sterile peat. Soil Biology and Biochemistry 27, 703-705.

Roughley, R., 1970. The preparation and use of legume seed inoculants. Plant and Soil 32, 675-701.
Samonin, V., Elikova, E., 2004. A study of the adsorption of bacterial cells on porous materials. Microbiology 73, 696-701.
Saranya, K., Krishnan, P.S., Kumutha, K., French, J., 2011. Potential for biochar as an alternate carrier to lignite for the preparation of biofertilizers in India. International Journal of Agriculture, Environment and Biotechnology 4, 167-172.
Singh, J.S., Pandey, V.C., Singh, D.P., 2011. Efficient soil microorganisms: A new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems & Environment 140, 339-353.
Smith, R., 1992a. Legume inoculant formulation and application. Canadian Journal of Microbiology 38, 485-492.
Smith, R.S., 1992b. Legume inoculant formulation and application. Canadian Journal of Microbiology 38, 485-492.
Stephens, J., Rask, H., 2000. Inoculant production and formulation. Field Crops Research 65, 249-258.
Thompson, J.A., 1980. Production and quality control of legume inoculants, in: Bergersen, F.J. (Ed.), Methods for evaluating biological nitrogen fixation. John Wiley, London, pp. 489-533.
Tilak, K., Pal, K., Dey, R., 2010. Microbes for sustainable agriculture. IK International Publishing House Pvt. Limited.
Trevors, J.T., 1991. Respiratory activity of alginate-encapsulated *Pseudomonas fluorescens* cells introduced into soil. Applied Microbiology and Biotechnology 35, 416-419.
Vidhyasekaran, P., Muthamilan, M., 1995. Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. Plant disease 79, 782-786.
Wilson, D., Reisenauer, H., 1970. Effect of manganese and zinc ions on the growth of *Rhizobium*. Journal of Bacteriology 102, 729-732.
Yadav, N.K., Vyas, S.R., 1973. Salt and pH tolerance of Rhizobia. Folia Microbiologica 18, 242-247.
Yardin, M.R., Kennedy, I.R., Thies, J.E., 2000. Development of high quality carrier materials for field delivery of key microorganisms used as bio-fertilisers and bio-pesticides. Radiation Physics and Chemistry 57, 565-568.

Conclusions

The work presented here broadens our understanding of the application of biochar in the environment. The research herein has investigated the potential risks associated with the application of biochar to soil and agricultural lands.

There is currently no regulation regarding the levels of PTEs and PAHs in biochar destined for application to soil. To evaluate the PTE and PAH risks their concentrations in biochar were compared to those in sewage sludge and compost. Results have indicated that concentrations of metal, metalloids and PAHs in biochar are lower than those listed as acceptable by the European Union for sewage sludge and by the Publicly Available Specification (PAS) for compost. Moreover, PTEs and PAHs concentrations in biochar were in keeping with those in background soil. It was concluded that biochar amendment to soil (even at a high application rate of 100 t ha^{-1}) would not elevate metal, metalloids and PAHs concentrations above background levels. Collectively, the results presented imply that the impacts attributable to PTEs and PAHs following biochar application to soil are likely to be minimal. It is suggested that a 'PAS' type criteria system, as applied to compost, could represent a practicable mechanisms through which to regulate biochar application to soil and thereby safeguard against elevations in PTE and PAH concentrations following biochar application to soil.

Regarding the sorption capacity of biochar with respect to organic compounds the research presented herein has evaluated this phenomenon from two different perspectives:

(i) potential benefit in the instance of contaminated lands, wherein biochar might reduce bioaccumulation of organic compounds into crops, and;

(ii) potential threats to the agricultural system where on account of biochar-herbicides interactions deactivating herbicidal activity.

Sewage sludge biochar (SSBC) was compared to sewage sludge (SS) as a soil amendment to PAHs contaminated soil. The presence of both amendments significantly reduced the bioaccumulation of PAHs in *Lactuca satuva*. However, where SSBC was used as soil amendment, the results showed greater benefits. These

findings provide compelling evidence of the positive impacts of biochar, not only in increasing crop yields (as already reported in literature), but also to improve food safety where soil contaminations exist.

In contrast, the sorption of certain organic compounds, specifically herbicides, may be a threat to yields where agriculture relies upon the use of soil-applied herbicides. Results reported herein show the dramatic effect of biochar (5%) had upon the partitioning of the herbicide isoproturon (IPU), and the significant reduction in IPU bioavailability.

Results presented herein have indicated weed survival in presence of herbicides and biochar to be comparable to that in unamended control soils. At high application rates (5%) biochar was effective in deactivating the herbicidal activity of two (mestrione and pendimenthalin) of the three herbicides tested.

Collectively, the results regarding sorption highlight bichar to be effective in mitigating soil to plant transfer of organic compounds (PAHs) from contaminated soil. By the same token herbicide-biochar interactions resulted in reduced availability and bioavailability of herbicides. On one hand this could offer benefits with respect to surface and groundwater protection but on the other hand these results highlight the risk that soil-applied herbicides may become ineffective in the present of biochar.

The use of biochar as soil amendment for agricultural purposes to enhance soil properties and crop yields was extended where biochar was evaluated as an alternative carrier for rhizobia inoculants. The survival of three rhizobia strains (*Rhizobium leguminosarum* bv. *viciae; Rhizobium etli; Rhizobium leguminosarum* bv. *trifolii*) observed in different temperature conditions (25˚C and 35˚C) revealed that biochar (produced from redwood) has a better potential as microbial inoculant carrier than peat. Specific properties of biochar have been attributed to the encouraging levels of cell survival; these including: high water holding capacity, readily liable carbon and high nutrients contents (specifically, manganese, zinc and cobalt). These preliminary results are encouraging in so much as peat is (largely) a non-renewable resource while biochar can be produced on a sustainable basis. The opportunity to potentially replace peat based inoculants with biochar contemporaries could represent appreciable environmental benefits.

Further work

This research has advanced the understanding of different aspects of biochar application to soil. While providing several original insights this research has prompted new research questions, and, with these questions, the opportunity for further research to be developed.

In light of the findings of the research presented herein the following areas of further research are suggested:

1. To establish the potential for herbicide deactivation in biochar amended soil in field plots (rather than laboratory microcosms).
2. To consider the influence of biochar upon several herbicide classes and how herbicide-biochar interactions vary with different biochars (produced from different feedstock and pyrolysis temperatures).
3. To evaluate the capacity of biochar to facilitate rhizobia survival once rhizobia-inoculated-biochar is applied to soil; to establish the ability of the biochar associated inocula to be effective in root nodulation, and; plant growth thereafter.

Appendix A

Environmental contextualisation of potential toxic elements and polycyclic aromatic hydrocarbons in biochar

Alessia Freddo[1,2], Chao Cai[2], Brian J. Reid[1*].

[1]School of Environmental Sciences, University of East Anglia, Norwich Research Park, NR4 7TJ, Norwich, UK.

[2] Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Science, Xiamen, 361021, China

Supporting Methods

Calculation of PTE concentrations in biochar amended soil

In order to calculate resultant metal/metalloid concentrations in a biochar amended soil a biochar application rate is of primary importance. Jeffery *et al.* (2011) reported application rates of 1 t ha^{-1} to 100 t ha^{-1}. In light of this an application rate at the upper end of the range, 100 t ha^{-1}, was used in subsequent calculations to provide a 'potential worst case scenario'.

Resultant concentrations of metals/metalloids following biochar amended to soil were calculated as follows (using the assumptions stated).

Firstly, the incumbent metal/metalloid 'doses' associated with biochar addition (100 t ha^{-1}) were calculated by multiplying this mass of biochar (1 x 10^5 kg) by the concentrations of metal/metalloid therein (Table 3). Minimum, median and maximum values were applied in the calculations. This value is denoted 'A' in Equation 1.

*

Thereafter, the incumbent metal/metalloid masses in 30 cm plough layer of 1 ha of soil were calculated using the ABC background soils justified in the manuscript (reported in Table S3). In this way a given ABC value was applied to a total mass of soil of 5.4×10^5 kg (this being derived as a soil volume contained in 100 m x 100 m x 0.3 m x soil density (assumed to be 1.8 g cm^{-3})). This value is denoted 'B' in Equation 1.

Having obtained the contributing amounts of metal/metalloid from biochar and soil these values were summed and then expressed a proportion of the total mass of material (soil plus biochar (6.4×10^5)) present (Equation 1). In this way the resultant metal/metalloid and PAH concentrations in the amended soil were obtained (Table S3).

$$\text{Resultant concentration} = \frac{A + B}{6.4 \times 10^5} \qquad \text{Equation 1}$$

Supporting Results

Assessment extracting solvent regime rigour

Below (Table S1) is accounted the results of the method appraisal, with respect to their rigour, to extract PAHs using the following solvent extraction regimes: a) DCM, b) DCM/acetone (1:1), and, c) acetone/hexane (1:1).

In terms of total ∑PAH, extraction with DCM was observed to be the most rigorous with the highest ∑PAH concentration (8.7 ± 0.23 mg kg^{-1}) while extraction using DCM/acetone (1:1) and acetone/hexane (1:1) indicated ∑PAH concentration to be 3.96 ± 0.92 mg kg^{-1} and 2.02 ± 0.36 mg kg^{-1}, respectively (Table S1). Moreover DCM extraction, when compared with the other solvents regimes (DCM/acetone (1:1) and acetone/hexane (1:1)), yielded the highest number of PAH compounds (naphtalene, acenaphtylene, acenaphthene, fluorene, antrhracene, fluoranthene, pyrene) (Table S1).

Naphthalene along with acenaphthene were the only compounds observed above the limit of detection for all three solvent regimes. Of the PAHs assessed naphthalene was most abundant in all of the extraction regimes (5.1 ± 0.92 mg kg^{-1} with DCM; 2.4 ± 1.78 mg kg^{-1} with DCM/acetone; 1.0 ± 0.42 mg kg^{-1} with acetone/hexane). Similarly, acenaphthene concentrations decrease in keeping with the same order of solvent regime: (1.17 ± 0.04 mg kg^{-1} with DCM; 0.83 ± 0.07 mg kg^{-1} with DCM/acetone; 0.66 ± 0.09 mg kg^{-1} with acetone/hexane). Although below the limit of detection where DCM/acetone was used as a solvent regime pyrene was extracted with concentrations above the limit of detection where both DCM (0.12 ± 0.08 mg kg^{-1}) and acetone/hexane (0.59 ± 0.58 mg kg^{-1}) were used.

Overall the extraction with DCM gave the greatest opportunity for PAH detection and was therefore used as the method of choice in subsequent extractions.

Table S1. PAH compounds extracted from pilot scale gasification of softwood chips where DCM, DCM/acetone and acetone/hexane were used as extracting solvent regimes. Values are shown ± 1 standard deviation (SD).

		Mean [PAH] mg kg^{-1} (SD)		
PAH (log Kow)	Limit of Detection (mg kg^{-1})	DCM	DCM/acetone (1:1)	acetone/hexane (1:1)
Naphthalene (3.37)	0.5	5.11 (0.92)	2.44 (1.78)	1.04 (0.42)
Acenaphthylene (3.92)	1	1.69 (0.43)	< LOD	< LOD
Acenaphthene (4.00)	0.5	1.17 (0.04)	0.83 (0.07)	0.66 (0.09)
Fluorene (4.18)	0.1	0.3 (0.01)	< LOD	< LOD
Anthracene (4.54)	0.03	0.21 (0.14)	< LOD	< LOD
Phenanthrene (4.57)	0.04	< LOD	< LOD	< LOD
Fluoranthene (5.18)	0.05	0.1 (0.05)	< LOD	< LOD
Pyrene (5.22)	0.1	0.12 (0.08)	< LOD	0.59 (0.58)
Benzo[*a*]anthracene (5.91)	0.05	< LOD	< LOD	< LOD
Chrysene (5.70)	0.05	< LOD	< LOD	< LOD
Benzo[*b*]fluoranthene (5.80)	0.02	< LOD	< LOD	< LOD
Benzo[*k*]fluoranthene (6.00)	0.02	< LOD	< LOD	< LOD
Benzo[*a*]pyrene (6.50)	0.05	< LOD	< LOD	< LOD
Benzo[*ghi*]perylene (6.50)	0.08	< LOD	< LOD	< LOD
Indeno[*123cd*]pyrene (6.65)	0.05	< LOD	< LOD	< LOD
Dibenzo[*ah*]anthracene (6.75)	0.2	< LOD	< LOD	< LOD
∑PAHs		8.7	3.96	2.06

LOD – limit of detection

Table S2. PAH: total residues extracted with DCM. Values are shown ± 1 standard deviation (SD). Retention time (RT) and mass-charge (m/z) applied during GC-MS analyses.

				500˚C (mg kg^{-1}) (SD)	600˚C (mg kg^{-1}) (SD)				300˚C (mg kg^{-1}) (SD)			
PAH (log Kow)	RT (min)	m/z	LOD (mg kg^{-1})	Softwood Pilot	Rice	Bamboo	Redwood	Maize	Rice	Bamboo	Redwood	Maize
Naphthalene (3.37)	7.2	128	0.5	5.11 (0.92)	< LOD	0.23 (0.09)	< LOD	0.5 (0.17)	0.27 (0.02)	1.62 (0.04)*	2.72 (2.61)*	4.67 (1.41)*
Acenaphthene (3.92)	9.8	153	1	1.69 (0.43)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Acenaphthylene (4.00)	9.6	152	0.5	1.17 (0.04)	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Fluorene (4.18)	10.7	166	0.1	0.3 (0.01)	< LOD	< LOD	< LOD	< LOD	0.12	< LOD	0.12	< LOD
Anthracene (4.54)	13	178	0.03	0.21 (0.14)	0.05 (0.02)	< LOD	< LOD	< LOD	0.12 (0.01)	< LOD	0.23 (0.28)	0.03 (0.02)
Phenanthrene (4.57)	13.1	178	0.04	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Fluoranthene (5.22)	16.1	202	0.05	0.1 (0.05)	0.14 (0.02)	< LOD	0.08	0.11 (0.01)	0.22 (0.01)	< LOD	0.47 (0.57)*	0.1
Pyrene (5.18)	16.7	202	0.1	0.12 (0.08)	0.96	0.83 (0.04)	< LOD	0.86 (0.01)	0.9 (0.03)	0.85 (0.08)	1.12 (0.27)	0.86 (0.01)
Benzo[a]anthracene (5.91)	19.8	228	0.05	< LOD	< LOD	< LOD	< LOD	< LOD	0.2 (0.01)	< LOD	< LOD	< LOD
Chrysene (5.70)	19.8	252	0.05	< LOD	< LOD	< LOD	< LOD	< LOD	0.36	< LOD	< LOD	< LOD
Benzo[b]fluoranthene (5.80)	23	228	0.02	< LOD	< LOD	< LOD	< LOD	< LOD	0.2	< LOD	< LOD	< LOD

Benzo[k]fluoranthene (6.00)	23	252	0.02	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Benzo[a]pyrene (6.50)	24.1	252	0.05	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Benzo[ghi]perylene (6.50)	31.7	276	0.08	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Indeno[123cd]pyrene (6.65)	30	276	0.05	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Dibenzo[ah]anthracene (6.75)	30.3	278	0.2	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
∑PAHs				8.7 (1.67)	1.15 (0.04)	1.06 (0.13)	0.08	1.47 (0.19)	2.27 (0.07)	2.47 (0.12)	4.54 (3.73)	5.66 (1.44)

LOD – level of detection limit

* denotes significant difference between like feedstock couplets.

Table S3. Calculated resultant minimum, median and maximum metal/metalloid concentrations (mg kg^{-1}) in soil amended with biochar, at an assumed application rate of 100 t ha^{-1}, and their comparison with background soil concentration (see manuscript for justification). Where

	Background soil	Calculated resultant concentrations (mg kg^{-1})			% change relative to background soil		
	(mg kg^{-1})	Minimum	Median	Maximum	Minimum	Median	Maximum
Cd	0.80	0.7	0.7	**0.82**	-16	-15	**3**
Cr	44	37	38	38	-16	-14	-13
Cu	19	16	17	19	-16	-11	-1
Ni	25	21	21	21	-16	-15	-14
Pb	39	33	33	34	-16	-15	-14
Zn	89	75	84	**108**	-16	-6	**21**
As	6.2	5.2	5.3	5.3	-16	-15	-15

background soil concentrations are exceeded values have been highlighted in bold.

References:

Jeffery, S., Verheijen, F.G.A., Van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment **144**, 175-187.

Appendix B

Supporting information

Reduced bioaccumulation of PAHs by *Lactuca satuva* L. grown in contaminated soil amended with sewage sludge and sewage sludge derived biochar

Sardar Khan[a,b], Ning Wang[a], Brian J. Reid[c], Alessia Freddo[c], Chao Cai [a*]

[a]Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China

[b]Department of Environmental Science, University of Peshawar, Pakistan

[c]School of Environmental Sciences, University of East Anglia, Norwich, UK.

Additional results

Soil and treatment properties: The initial physico-chemical characteristics of the soils were assessed according to standard procedures. The soil pH ($CaCl_2$) was 6.77, electrical conductivity (EC) 0.15 mS cm^{-1}, and loss on ignition (LOI) 16.55 % (Table S1). The contents of N (0.13 %), C (7.05 %) and S (0.31 %) were measured by dry combustion method using macroelementor (VarioMax CNS, Germany) (Matejovic, 1997). Biochar application increased the soil pH by 0.05-0.19 pH units, while sludge decreased the soil pH by 0.02-0.13 pH units.

Table 1S: Characteristics of sludge, biochar and soil

Parameters	Sludge	Biochar	Soil
pH ($CaCl_2$)	5.41±0.21	7.25±0.15	6.77±0.09
EC (mS/cm)	2.58±0.07	1.71±0.10	0.15±0.01
Moisture (%)	2.56±0.07	1.1±0.01	0.76±0.02
LOI (%)	57.93±1.31	48.7±1.42	16.55±0.64
N (%)	3.54±0.11	3.44±0.22	0.13±0.03
C (%)	28.21±1.56	27.08±1.86	7.05±1.17
S (%)	3.38±0.50	4.56±0.73	0.31±0.05
BET Surface Area ($m^2 g^{-1}$)	2.17±0.02	5.45±0.01	ND
Pore Volume ($cm^3 g^{-1}$)	0.0097±0.001	0.0144±0.003	ND
Pore Size (nm)	17.89±1.16	10.54±0.76	ND

ND - not detected

Table S2: Symbol key for Figure 3.

Symbol on Figure 3	Colour on Figure 3 and PAH compound
○	White, naphtalene
□	White, acenaphtylene
△	White, acenaphtene
○	Grey, fluorene
▽	White, phenanthrene
◇	White, anthracene
□	Grey, fluoranthene
△	Grey, pyrene
●	Black, benzo(a)anthracene
▽	Grey, chrysene
▲	Black, benzo(b)fluoranthene
▼	Black, benzo(k)fluoranthene
■	Black, benzo(a)pyrene
⬢	Black, indeno(1,2,3-c,d)pyrene
◆	Black, dibenzo(a,h)anthracene
◈	Black, benzo(g,h,i)perylene

Appendix C

Supporting information

Influence of biochar on isoproturon partitioning and bioavailability

Reid, B.J[1]*., Pickering, F.L[1]., Freddo, A[1]., Whelan, M.J.[2]., Coulon., F[2].

[1]School of Environmental Sciences, University of East Anglia,

Norwich, NR4 7TJ, UK.

[2]School of Applied Sciences, Department of Environmental Sciences and Technology Cranfield

University, Cranfield, MK43 0AL, UK.

Values used to support the fugacity modelling approach

We consider vials with the following properties:

Total internal volume (V_V) = 24.38 cm^3

Mass dry soil added = M_S = 20 g

Assumed soil bulk density 1.4 g cm^{-3}

Assumed density of soil solid phase = ρ_S = 2.6 g cm^{-3}

f_{OC} soil = 0.018 g g^{-1}

Assumed bulk density of biochar = 0.32 g cm^{-3}

f_{OC} biochar = .68 g g^{-1}

% biochar added by weight = 5%

Mass of biochar added = 0.05 * 20 = 1 g

Mass of biochar C added (m_B) = 0.68 * 1 = 0.68 g

Relevant derivations of these properties are as follows:

Volume of bulk dry soil (V_{bulk}) = M_S / ρ_B = 20 / 1.4 = 14.29 cm^3

Porosity of soil (ϕ) = (ρ_S – ρ_B)/ ρ_S = (2.6 – 1.4) / 2.6 = 0.46

Volume of solids (V_S) = (1 – ϕ) . ρ_B = (1 – 0.46) . 1.4 = 7.69 cm^3

Water content (calculated from gravimetric water content of 0.104 g g^{-1}) = 0.4 cm^3 cm^{-3}

Volume water in vial (V_W) = 0.4 . V_{bulk} = 0.4 . 14.29 = 5.71 cm^3

Volume of air in soil = V_{bulk} – V_W - V_S = 14.29 – 5.71 – 7.69 = 0.88 cm^3

Volume of air in vial outside the soil pore space = V_V – V_{bulk} = 23.38 – 14.29 = 10.09 cm^3

Total volume of air in system (V_A) = 10.09 + 0.88 = 10.9 cm^3

Notes regarding isoproturon

The use of IPU (Figure S1) is now prohibited in the UK due to concerns about water pollution and associated risks to aquatic life.[1] IPU has moderate aqueous solubility (65 mg L^{-1}) and a reasonably high log K_{OW} (2.48) which does not immediately suggest a high propensity to leach. However, the risk of water contamination was increased by its widespread use.

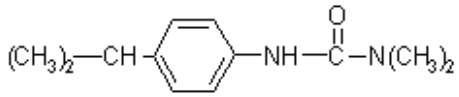


Figure S1: Chemical structure of isoproturon (3-(4-isopropylphenyl)-1,1-dimethyl urea; $C_{12}H_{18}N_2O$)

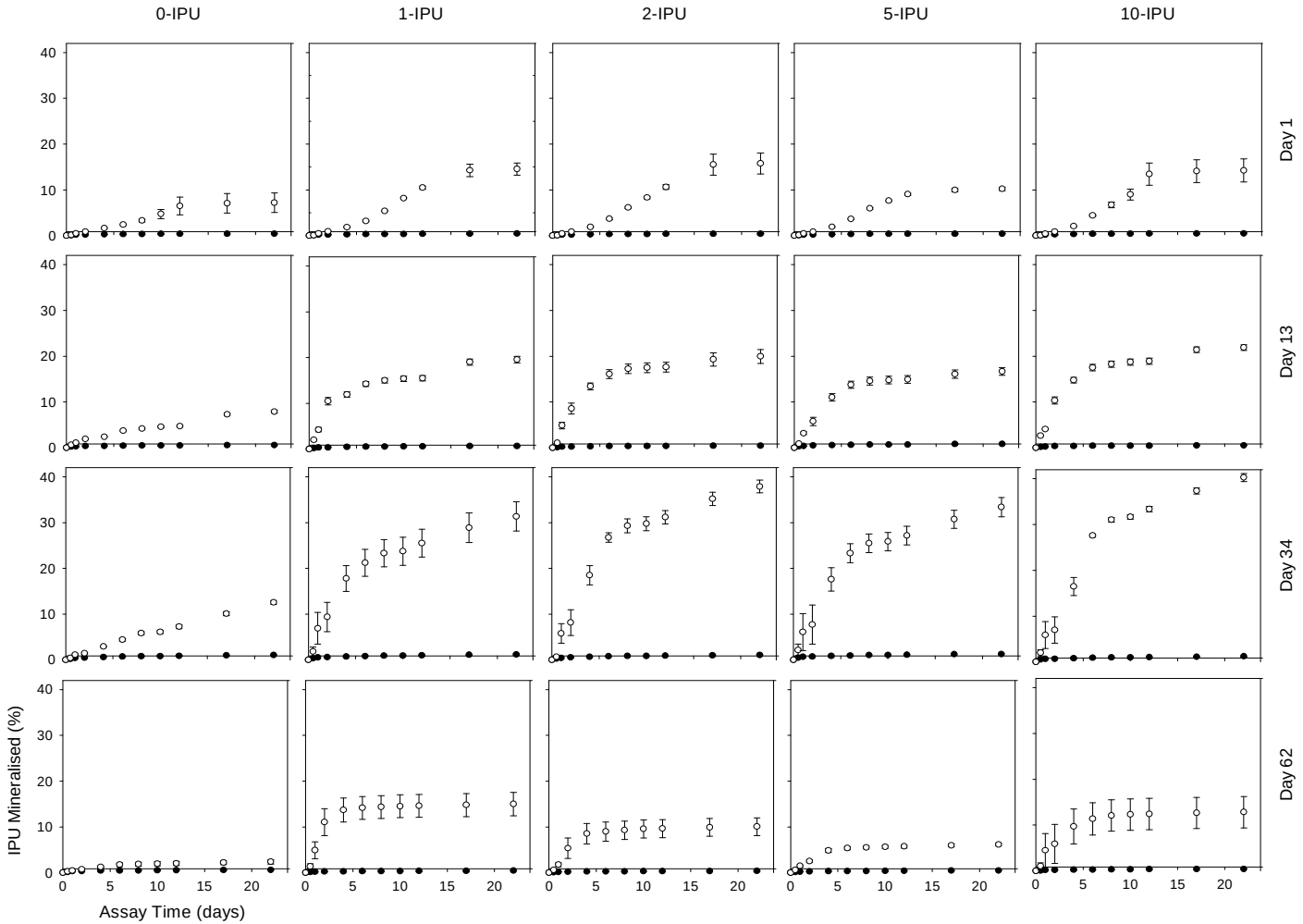


Figure S2: Catabolic activity with respect to IPU (% ^{14}C-IPU mineralisation). Open circles showing biochar free treatments while closed circle show with biochar treatments. IPU dose is organised in the horizontal direction with 1 mg kg^{-1}, 2 mg kg^{-1}, 5 mg kg^{-1}, 10 mg kg^{-1} indicated with column headings of 0 IPU, 1 IPU, 2 IPU, 5 IPU and 10 IPU, receptively. Temporal response is captured vertically with row headings of 1d, 13d, 34d and 62d. Error bars display standard error of the mean (n = 4).

References

(1) Health and Safety Executive. Chemical Regulation Directorate Pesticides, http://www.pesticides.gov.uk/approvals.asp?id=2078 A.

Appendix D

Deactivation of herbicidal activity in biochar amended soil.

Alessia Freddo and Brian J. Reid

School of Environmental Sciences, University of East Anglia, Norwich, UK

Material and Methods

Calculations for herbicide dose rates

The recommended application rate for mesotrione is between 0.1 - 0.225 kg ha^{-1} (Tomlin 2006). The rate used specifically in this study was 0.21 kg ha^{-1}.

The recommended application rate for pendimenthalin is between 0.6-2.4 kg ha^{-1} (Tomlin 2006). The rate used specifically in this study was was 1.7 kg ha^{-1}.

The recommended application rate for terbuthylazine is between 0.6-3 kg ha^{-1} (Tomlin 2006). The rate used specifically in this study was 3 kg ha^{-1}.

To convert these values to a concentration applicable to the limited surface area of the box, the following calculations were undertaken (example for mesotione):

-conversion from kg ha^{-1} to g m^{-2}: 210 g ha^{-1} x 1/10000 ha m^{-2} = 0.021 g m^{-2}

-area of each box: 0.23 m x 0.17 m = 0.0391 m^2

-grams of mesotrione per box: 0.021 g m^{-2} x 0.0391 m^2 = 8.21 x 10^{-3} g

-mesotrione density: 1.2 g mL^1

-volume of mesotrione sprayed per each box: 8.21 x 10^{-3} g / 1.2 g mL^{-1} = 6.84 x 10^{-3} mL

Similar calculations were undertaken for pendimenthalin and terbuthylazine using densities of 0.374 g mL^{-1} and 1.1 g mL^{-1}, respectively.

The calculated amounts of herbicide were sprayed onto the treatments within a total volume of distilled water of 100 mL per box.

Biochar application rate

An application rate of 100 t ha^{-1} would result in a soil loading of approximately 5 % biochar content. This assumes a biochar application to 180 kg of soil. This being derived as a soil volume contained in 100 m x 100 m x 0.1 m x soil density (assumed to be 1.8 g cm^{-3}). Actual resultant biochar loading = 5.5%.