

Biochar: carbon sequestration, land remediation and impacts on soil microbiology

Short title: Biochar: carbon, & microbial impacts

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Abstract

Biochar – charcoal used to amend land and sequester carbon – is attracting considerable interest. Its distinctive physical/chemical/biological properties, including high water-holding capacity, large surface area, cation exchange capacity, elemental composition and pore size/volume/distribution, effect its recognised impacts, especially on microbial communities. These are explored in the context of agriculture, composting and land remediation/restoration. Considerable focus is given to mycorrhizal associations, which are central to exploitation in environmental technologies involving biochar. The characteristics of biochar, its availability for nutrient cycling, including the beneficial and potentially negative/inhibitory impacts, and the requisite multidisciplinary analysis (physico-chemical, microbiological and molecular) to study these in detail, are explored.

1. Introduction

Interest in ‘biochar’,¹ the application of charcoal to land, is led by the dual benefits of long term carbon sequestration and potentially positive soil amendment (Dover, 2007; Lal 2009; Tenenbaum, 2009). In particular, the ability of biochar to act as a feasible climate change mitigation technology, implementable at globally significant scale, is recognised (Molina *et al.*, 2009), with the potential to sequester the equivalent of up to 12% of anthropogenic greenhouse gas emissions (Woolf *et al.*, 2010) in ecologically and economically sustainable systems. A key constraint to the commercialisation of the technology is the addition of sufficient value to charcoal to prevent its combustion for traditional applications as a fuel and reductant.

¹ For the purposes of this review ‘biochar’ refers to char (charcoal) that has been, or is intended to be, applied to land.

Proposed benefits in agricultural systems (soil enrichment, reduced input requirement, enhanced yields, land remediation/reclamation) and carbon trading are now being investigated as potential sources of sufficient added value. The pyrolysis process can be tailored in terms of feedstock and conditions to produce chars with desired qualities for specific applications (Dover, 2007). Sustainable systems have been postulated (Mathews, 2008; Preston, 2009; Lee *et al.*, 2010) in which biomass is used to simultaneously produce energy and charcoal, which, upon application to land, removes carbon from the short term photosynthesis-mediated cycle to a long term reservoir. Hence, energy so generated is potentially certifiably carbon negative and can act as a source of revenue from not only its sale but also the generation of tradable carbon credits (Mathews, 2008).

The climate change benefits of biochar depend critically on both the lifetime of the charcoal in soil and the overall life cycle impacts of its production and application. A life cycle analysis of biochar systems made by Roberts *et al.* (2010) highlighted the critical nature of the source material and bioenergy production in realising climate change benefits. Indirect land use change emissions from dedicated crops have the potential to produce net greenhouse gas (GHG) emissions whereas biochar systems with waste or agricultural residues as feedstock provide net carbon sinks, around two thirds of which arise due to carbon sequestration in the biochar. Economic sustainability depends critically on: transport distances; market values for carbon reduction credits; and bioenergy yields. The additional bioenergy available from fast pyrolysis systems makes these more economically viable, therefore, than slow pyrolysis routes to biochar: Brown *et al.* (2011) have demonstrated an internal rate of return as high as 15% by 2015, rising to greater than 25% in 2030, based upon underlying assumptions about the cost of feedstocks and carbon credits. These considerations are especially critical if agricultural waste is the pyrolysis feedstock. Production costs for dedicated energy crops render such biochar uneconomical.

Despite its inherent recalcitrance and limited accessibility through soil particle envelopment (Brodowski *et al.*, 2006) acting as a barrier to enzymes (Ekschmitt *et al.*, 2008), the impacts of biochar on soil biogeochemistry (Liu *et al.*, 2009) and microbial activity are limited by its ultimate mineralization which can vary from thousands of years (Fowles, 2007; Kuzyakov *et al.*, 2009; Grossman *et al.*, 2010) to decades in well aerated tropical soils (Bird *et al.*, 1999). Nguyen and Lehmann (2009) considered, therefore, whether water regimes typical of tropical soils (saturated, unsaturated, and alternating saturated/unsaturated) exerted different effects on the degradation of two biochars (*Zea mays* and *Quercus* spp.) produced at 350 and 600°C. They found that the moisture regime was critical in mineralization (characterized by increased carboxylic and OH functional groups but decreased aliphatic groups) of *Zea mays* stover residue and *Quercus* spp shavings biochar under unsaturated and saturated/unsaturated

conditions, respectively. They attributed this to oxygen availability for abiotic/biotic oxidation and decomposition (Morris *et al.*, 2004). Of the two water regimes, saturated/unsaturated conditions often promote the higher mineralization (Wu and Brookes, 2005) through soil particle disaggregation (Denef *et al.*, 2001), nutrient availability (Binh and Marschner, 2005), microbial activity (Gordon *et al.*, 2008) or microbial biomass turnover (Van Gestel *et al.*, 1991). Since for both plant types, carbon loss correlated strongly with O/C value changes, it was concluded that biochar oxidation was the strongest stability controlling determinant. For the corn stover, the higher charring temperature effected reduced mineralization and oxidation but this was not apparent for the oak.

Together with microbial catabolism and key physico-chemical variables, co-metabolism must also be considered as an important driver of mineralization. To examine this, Kuzyakov *et al.* (2009) added ^{14}C -labelled ryegrass (*Lolium perenne*) biochar produced at 400°C to Haplic Luvisol soil (20% w/w of the C_{org}) and loess (approaching 200% w/w) followed by regular supplementations of glucose ($2.16 \text{ mg g}^{-1} \text{ soil}$) over a period of 1 089 days with biochar decomposition estimated through $^{14}\text{CO}_2$ evolution. In the absence of glucose and even with intensive mixing, decomposition accounted for as little as 0.5% of the biochar per annum. With glucose, however, six-fold increases were recorded for the soil and loess but these decreased after two weeks and three months, respectively, which led the workers to conclude that co-metabolic decomposition was operative. Microbial analysis showed that ^{14}C was incorporated into cell biomass while dissolved organic carbon assay revealed an absence of biochar decomposition product leaching.

Lehmann *et al.* (2009) suggested a double-exponential decay model for biochar carbon loss in soil in which two first order loss processes occur simultaneously: rapid decay (half-life, $\lambda \approx 0.54$ years) of labile carbon; and slow ($\lambda \approx 1,600$ years) mineralisation of fixed biochar carbon. To determine *in situ* longevity, Nguyen *et al.* (2009) studied long-term qualitative/quantitative changes in forest fire recovered cultivated western Kenyan soil dating back 100 years. Seventy per cent losses were recorded in the first 30 years after deposition, which they attributed to decomposition and transport with oxidation (Cheng *et al.*, 2008) the dominant factor. The losses then slowed to reach a final concentration ($3.5 \text{ mg C g}^{-1} \text{ soil}$) of 27.5% of the original, broadly in line with the double exponential decay hypothesis.

Steinbeiss *et al.* (2009) also examined biochar residence in soil. They used greenhouse soil (Eutric Fluvisol and Cambisol) columns (150 g, 25°C day/ 20°C night) with hydrothermally produced ^{13}C glucose and ^{13}C yeast chars (30% initial organic carbon content) and recorded respiratory losses of soil and biochar carbon. Mean residence times of between four (glucose biochar/arable soil) and 29 years (yeast biochar/arable soil) were determined with

the variation accounted for by soil type and biochar quality. As short experiments preferentially probe the kinetics of the rapid decay processes these apparently short mean residence times could have been due to the short timeframe of the experiment. Also, hydrothermal material produced from corn stover (Fuentes *et al.*, 2010) and cherry wood (unpublished results from our laboratory) has a relatively high proportion of aliphatic and carboxy/carbonyl functionality; features expected to increase biological and chemical decomposition rates. Peng *et al.* (2011) reported that as well as increasing aromatic carbon and reducing the O/C ratio, increased pyrolysis temperature also increases estimated lifetime.

In addition to the uncertainties surrounding biochar carbon soil-lifetime there is some debate regarding charcoal's effect on native soil organic carbon stocks. Wardle *et al.* (2008) observed apparent carbon loss from humus in soil/biochar mixtures consistent with charcoal promoting soil microbial growth and, hence, humic decomposition. This loss of soil carbon may offset the GHG sequestration benefits of biochar to some extent possibly due to oxidation of labile biochar components rather than accelerated loss of native soil organic matter (Lehmann and Sohi, 2008). Indeed, Kimetu and Lehmann (2010) have subsequently demonstrated that biochar addition stabilises soil organic carbon better than green manure labile carbon. *Tithonia diversifolia* green manure additions increased CO₂ C loss by 22% while biochar addition reduced soil CO₂ carbon loss by 27% and increased intra-aggregate C per respired C by 6.8 times relative to the green manure. In the longer term, Downie *et al.* (2011), studying soils in ancient Australian Aboriginal oven mounds, showed that historic charcoal additions, between 650 and 1 609 years old, resulted in long-lived, significantly elevated soil carbon stocks, relative to the adjacent soil.

Uncertainties regarding climate notwithstanding, compelling evidence of the impact longevity of biochar in soil was gained by Grossman *et al.* (2010) from molecular analysis (denaturing gradient gel electrophoresis, terminal restriction fragment length polymorphism, cloning and sequencing) of *Terra Preta* Brazilian Anthrosols and adjacent non-Anthrosol soils. For archaea, the divergence was > 90% with higher richness and very similar fingerprints generally observed for bacteria of the four studied Anthrosols. From this they concluded that the strongest determinant of microbial community composition was management by the pre-Colombian Indians rather than soil type or land use.

Agricultural benefits arising from biochar are varied and of presently uncertain and probably complex mechanisms. Reviews by Sohi *et al.* (2010), Atkinson *et al.* (2010) and Joseph *et al.* (2010) have detailed the agricultural implications of biochar and its mechanistic aspects. In this review we briefly discuss the production and characterisation of char and outline the benefits that have been reported before moving on to highlight aspects of mechanisms that are mediated by microbial community responses to biochar amendment. In addition, we consider the potential utility of biochar in composting and microbiological remediation of contaminated land.

2. Char physico-chemical characterisation

Anaerobic thermal conversion of biomass can be achieved in three different processes: pyrolysis/carbonisation; gasification; and liquefaction. All three give products in three phases, solid, liquid and gas, with the product composition dependent on process conditions. Thus, pyrolysis is characterised by long residence times and moderate temperatures, liquefaction occurs under high heating rates, while gasification is defined by high temperatures, often with additional, though sub-stoichiometric, oxygen.

Pyrolysis typically produces a solid, structured, carbonaceous material which, compared to the feedstock, exhibits a high surface area (Bird *et al.*, 2008), reduced oxygen and hydrogen content (Abdullah and Wu, 2009), and a concentration of nutrients (Aglebor *et al.*, 2010; Gaskin *et al.*, 2008).

Key properties of biochar, such as pH, surface area, volatiles, ash, bulk density and pore-volume, water holding capacity, are sensitive functions of pyrolysis feedstock and process conditions. Surface area is readily discernable *via* classical adsorption techniques, such as N₂ adsorption analysed by the Brunauer-Emmett-Teller (BET) adsorption isotherm (Lua *et al.*, 2004; Brown *et al.*, 2006), while proximate analysis provides quantification of volatile and fixed carbon, as well as residual ash (Antal *et al.*, 2000). Reviews by Atkinson *et al.* (2010) and Joseph *et al.* (2010) summarised the effect of pyrolysis temperature on these properties and the subsequent interactions in soil. The chemical environment of the fixed carbon can be probed by a variety of solid phase ¹³C nuclear magnetic resonance techniques (Sharma *et al.*, 2004; Cheng *et al.*, 2006; Brewer *et al.*, 2009). Char chemistry and surface functionality can be assessed by various techniques including titrimetrically (Boehm, 1994) and spectroscopically by vibrational techniques such as infrared (Sharma *et al.*, 2004; Cheng *et al.*, 2006) and electron energy loss (Cohen-Ofri *et al.*, 2007) spectroscopies. Such investigations demonstrate that biochar properties are a complicated function of feedstock nature (identity, form, *etc.*) and pyrolytic conditions.

Both mass yield and the degree of aromaticity are functions of feedstock and pyrolysis conditions. Temperature, heating rate, sweep-gas flow rate and feedstock particle size are all factors that affect the mass yield of biochar. Özçimen and Ersoy-Meriçboyu (2008) investigated the effect of highest treatment temperature, heating rate and sweep gas flow rate in a 2³ factorial design experiment and found that temperature was the dominant parameter. Similar temperature-induced yield decreases have been reported for pyrolysis of animal residues (Ayllón *et al.*, 2006; Yilmaz *et al.*, 2007), cottonseed cake (Özbay *et al.*, 2001), pinewood bark (Şensöz, 2003), rapeseed (Onay and Koçkar, 2003), sugarcane bagasse (Katyal *et al.*, 2003) and sunflower cake (Gerçel, 2002). Increased sweep gas flow rate, increased heating rate and decreased particle size all lead to decreased char production (Özyurtkan *et al.* (2008), with the former exerting the slightly stronger effect. This indicates that reaction yield is governed by reaction kinetics and thermal and mass transfer considerations.

Temperature (Brewer *et al.*, 2009) and time at temperature (Yip *et al.*, 2011) increase the extent of aromatic structure formation in biochar. Aromaticity is an important determinant of biochar quality in particular contexts. Low aromaticity and small aromatic cluster size implies high surface functionality compared to material characterized by larger aromatic regions, and leads to higher cation exchange capacity in soil (Joseph *et al.*, 2009). This contrasts the increased water retention capacity of elevated temperature material due to higher surface area. On the other hand, a rise in char aromaticity leads to greater recalcitrance in soil with concomitantly protracted sequestration potential. Also, high temperature biochar exhibits high surface area and porosity, both of which can be exploited in adsorption-based remediation technologies.

Just as pyrolysis conditions time alter the properties of the char, so too does the feedstock. The results of studies in Table 1 highlight the range of biochar properties achieved as a function of temperature and feedstock identity. Optimisation of parameters for any given feedstock and application, although a challenge, offers the potential for bespoke biochar tailored for specific resources and applications.

Together with traditional char feedstocks, alternatives have been investigated. Of particular interest, given the thrust of biochar research towards climate change mitigation, is char production from agricultural residues. Carbon rich chars have been produced by pyrolysis of residues of rapeseed (Karaosmanoğlu *et al.*, 2000), rape and sunflower (Sánchez *et al.*, 2009a) under a variety of temperatures and heating rates, while agricultural wastes of relevance in a Turkish context have been investigated widely (Özçimen and Karaosmanoğlu, 2004; Özçimen and Ersoy-Meriçboyu, 2008; Özçimen and Ersoy-Meriçboyu, 2010). Also, pyrolysis of sewage sludge has been shown to generate a good quality char (Sánchez *et al.*, 2009b; Hossain *et al.*, 2011) while casein (Purevsuren *et al.*, 2003)

gives a highly porous product (content of porosity = 20%) with a high nitrogen content (9.02% w/w). Even microalgae have been demonstrated to produce high yields (>1/3 by mass) (Grierson *et al.*, 2009).

In addition to classical pyrolysis processes for char production, alternative routes have been explored. These include hydrothermal carbonisation (Worasuwannarak *et al.*, 2006; Steinbeiss *et al.*, 2009; Fuertes *et al.*, 2010), pressurised pyrolysis (Mahinpey *et al.*, 2009) and microwave pyrolysis (Lei *et al.*, 2009). These latter processes have potential as routes to materials with properties outside the range of normal pyrolysis products and/or higher energy efficiency.

3. Agricultural applications

Research on *Terra Preta* (black earth) – anthropogenically modified Brazilian Oxisols – is well documented (Glaser *et al.*, 2003; Glaser, 2007) with its fertility recognised as long ago as the 16th century (Abend, 2008). Although the efficacy of biochar to promote plant growth (Yamato *et al.*, 2006; Chan *et al.*, 2007a; Steiner *et al.*, 2007a) through, for example, increases in total carbon (van Zwieten *et al.*, 2010), nutrient retention/availability (Asai *et al.*, 2009; Sánchez *et al.*, 2009a), soil moisture holding capacity/permeability (Gathorne-Hardy *et al.*, 2008; Busscher *et al.*, 2010), organic matter and pH, and promoted microbial activity (Chan *et al.*, 2007a), is well recognised, few definitive studies have yet been reported even though, since 2000, global food demand has outstripped supply. Work is, however, in progress globally with different starting materials, production conditions, soil types, loadings, plant species and fertilizer applications (organic/inorganic).

Reviews by Sohi *et al.* (2010) and Atkinson *et al.* (2010) have assessed in some detail the impacts of biochar on agricultural productivity and its potential mechanisms. Briefly, biochar application exerts chemical effects, such as soil pH alteration (Chan *et al.*, 2008; Rodríguez *et al.*, 2009; van Zwieten *et al.*, 2010), changes in cationic properties (Steiner *et al.*, 2007b; Nguyen and Lehmann 2009; Novak *et al.*, 2009), increased availability of N and P (Sánchez *et al.*, 2009a; Hossain *et al.*, 2010), reduced leaching loss of ammonium (Ding *et al.*, 2010), increased organic carbon (Chen *et al.*, 2008, Novak *et al.*, 2009; van Zwieten *et al.*, 2010) and interactions with applied nutrients. Representative results from short-term studies are presented in Table 2. In some cases, significant negative interactions have been observed through, for example, adverse pH alterations (Mikan and Abrams, 1996; van Zwieten *et al.*, 2010) or the action of adsorbed phytotoxic pyrolysis oils (Gell *et al.*, 2011). Biochar addition also alters soil physical properties with water holding capacity (Iswaran *et al.*, 1980; Gathorne-Hardy *et al.*, 2008; Busscher *et al.*, 2010), soil humidity (Steiner *et al.*, 2007b) and top soil saturated hydraulic conductivity (Asai *et*

al., 2009) all increased, while soil strength decreased (Chan *et al.*, 2007a, 2008; Busscher *et al.*, 2010). These effects underpin the short term responses observed in the studies in Table 1.

This complex, and potentially synergic (Steiner *et al.*, 2007a; van Zwieten *et al.*, 2010), combination of physical and chemical changes in the soil environment does not account fully for the observed effects on yield. For example, Kimetu *et al.* (2008), in the Western Kenyan Highlands, investigated whether maize crop declines (through continuous cultivation over 100 years) even in the presence of nitrogen-phosphorus-potassium (120-100-100 kg ha⁻¹) fertilization could be reversed by the addition of biochar. For most degraded sites, maize productivity doubled although this was not accounted fully by enhanced nutrient availability. Similarly, Chan *et al.* (2007a; 2008) attributed plant dry matter yield enhancements in the presence of both biochar and fertilizer to enhanced nitrogen fertilizer use efficiency. Furthermore, the promotion of microbial activity by biochar-induced increases in soil humidity, pH, total nitrogen and the availability of sodium, zinc, copper and manganese was reported by Steiner *et al.* (2007b). They examined the growth of bananas (*Musa* sp.) and guarana (*Paullinia cupana*) on Amazonian upland soil subjected to nitrogen and phosphorus additions through both organic and inorganic fertilizers. Marked increases in basal respiration and microbial efficiency were seen in response to the biochar. Enhanced biological nitrogen fixation rates observed by Rondon *et al.* (2007) were attributed to the increased availability of boron and molybdenum and, to a lesser extent, the improved availability of potassium, calcium and phosphate together with the elevated pH, lower nitrogen availability and aluminium saturation.

Applications (0-90 g kg⁻¹ soil) were used to examine nitrogen fixation (isotope dilution method with (¹⁵NH₄)₂SO₄) by common beans (*Phaseolus vulgaris* L.) (Rondon *et al.*, 2007). With biochar (90 g kg⁻¹ soil), the fixed nitrogen proportion increased from 50% (control) to 72%. Increases in the total nitrogen derived from the atmosphere were recorded for 30 g kg⁻¹ soil (49%) and 60 g kg⁻¹ soil (78%) but these decreased to 30% with the highest concentration due to low total biomass production and nitrogen uptake. The workers concluded that the biochar-mediated elevated biological nitrogen fixation was due to the increased availability of boron and molybdenum and, to a lesser extent, the improved availability of potassium, calcium and phosphate together with the raised pH, lower nitrogen availability and aluminium saturation. In contrast to the nitrogen derived from the atmosphere, soil nitrogen uptake decreased by 14 and 17% in the presence of biochar additions of 30 and 60 g kg⁻¹ soil, respectively. Although the results demonstrated clearly that biochar can promote agroecosystem nitrogen input, further studies are required to underpin this practice.

4. Composting

Sustainable physical, chemical and biological properties of soil (Westerman and Bicudo, 2005) rely on continual applications of stabilised compost to reduce erosion, dampen temperature fluctuation, improve water infiltration/retention, control pathogenicity, and provide essential plant nutrients (Hoitink, 1993). Realization of such a product is achieved, in part, by preventing compaction of the compost by the provision of a bulking agent, which supplies carbon and energy (Adhikari *et al.*, 2009) and increases the porosity (Walker, 1993) and, thus, air voids of the pile (Huag, 1993). Since the provision of labile carbon should reduce nitrogen losses, a bulking agent, which supplies this but is dominated by humification-enhancing recalcitrant molecules (Goyal *et al.*, 2005) would be ideal. Although the physical and chemical properties of biochar would commend its use as a bulking agent, this possibility has received little attention (Hua *et al.*, 2009; Dias *et al.*, 2010).

Dias *et al.* (2010) prepared mixtures (1:1 fresh weight) of poultry manure with three different bulking agents, coffee husks, sawdust and *Eucalyptus grandis* biochar, for turned-pile composting (30 weeks) with moisture control. Due to the recalcitrance and high hydrophobicity (Trompowsky *et al.*, 2005) of the carbon, the poultry manure/biochar compost recorded high humification with the humic acids dominating the fulvic acids. Organic matter degradation > 70% (w/w) was, however, recorded and the workers attributed this to microbial catabolism and temperature-promoted abiotic oxidation which, in turn, enhanced further catabolism. The lability of the biochar (Baldock and Smernik, 2002) can be attributed to its method of production (slow, 300-450°C pyrolysis). The alkaline pH of the final product commended its use as an acid-correcting soil conditioner although its post-soil application nutrient cycling and humification still require examination particularly in comparison with biochar-free compost additions.

5. Land restoration/reclamation

5.1 The roles of mycorrhizas

With a research history of 125 years since Frank first termed the higher plant root/fungal interaction 'mycorrhiza', it is unsurprising that an extensive literature exists on these associations. Since the 1970s it has been accepted that most plants are mycorrhizal, commonly arbuscular mycorrhizal or ectomycorrhizal, with the association benefitting the plant by enhancing tolerance to stresses such as drought, salinity, low nutrient (through, for example, promoted phosphorus uptake (Gunderson *et al.*, 2007; Juwarkar and Jambhulkar, 2008)), disease, toxic organic contaminants and heavy metals, and improving soil structure in general (Li *et al.*, 2006), through soil particle formation and aeration promotion, increased legume nitrogen fixation and reduced nutrient leaching

(Mason, 2004). The earlier observations, in turn, stimulated consideration of applications in agriculture (Kendrick and Berch, 1984), forestry (Mexal, 1980) and derelict, degraded and contaminated land restoration/reclamation (Williams and Aldon, 1976; Gardner and Malajczuk, 1988).

Largely driven by economics, rather than pollution amelioration *per se*, land reinstatement continues to attract much research attention. For example, amelioration of heavy metal/metalloid contaminated land (Azcón *et al.*, 2009; Lebeau *et al.*, 2008; Marques *et al.*, 2009) with respect to single chemical species (Cd (Janoušková *et al.*, 2006; Hu *et al.*, 2007; Gonzalez-Chavez *et al.*, 2009); As (Jankong and Visoottiviset, 2008; Smith *et al.*, 2010b)), radioactive contamination (uranium (Dupré de Boulois *et al.*, 2008a); radiocaesium (Dupré de Boulois *et al.*, 2008b)), binary heavy metal systems (Cd/Zn (Krpata *et al.*, 2009; Rashid *et al.*, 2009); Cu/Pb (Huang *et al.*, 2000)) and heavy metal components of generic waste such as fly ash (Cd/Cu/Ni/Pb (Juwarkar and Jambhulkar, 2008; Haynes, 2009)) or tannery effluent (Cr (Khan, 2001)) are active areas of research. Application to the remediation of organics such as petroleum (Lin *et al.*, 2006; Chen *et al.*, 2009), diesel (Gunderson *et al.*, 2007), petrochemical wastes (De Paula *et al.*, 2006) and polycyclic aromatic hydrocarbons (Joner *et al.*, 2001; Joner and Leyval, 2003; Li *et al.*, 2006) such as phenanthrene (Wu *et al.*, 2008a), polychlorinated biphenyls (Chen *et al.*, 2005a), phthalic esters (Wang *et al.*, 2004; Chen *et al.*, 2005b), 3-chlorobenzoic acid (Dittman *et al.*, 2002), and chlorinated phenols, toluene, tetrachloroethylene and 2,4-dichlorophenol (Meharg and Cairney, 2000) continue to be investigated. Insecticides (DDT (Wu *et al.*, 2008b); chlorpyrifos (Korade and Fulekar, 2009)), fungicides (chlorothalonil (Zhang *et al.*, 2007)), herbicides (atrazine (Huang *et al.*, 2007a); triclopyr, imazapyr and sulfometuron methyl (Busse *et al.*, 2004)) and pharmaceuticals such as paracetamol (Khalvati *et al.*, 2010) are also the focus of potential remedial applications of mycorrhizal associations.

Mycorrhizal plants serve important roles through phytoextraction and phytostabilization (Vassilev *et al.*, 2004) and, for heavy metals, the effect exerted depends on contaminant concentration; phytoextraction is operative at low concentrations while reduced bioavailability, through fungal metal binding, is prevalent with high concentrations (Audet and Charest, 2007). The roles of mycorrhiza fungi in combating the challenges of toxic organic molecules can be quite varied. First, the fungus must counter the possibilities of reduced spore germination, root colonization rate and hyphal growth, and the indirect effect of inhibited carbohydrate translocation from the plant (Wang *et al.*, 2006). Once overcome, soil organic pollutant attenuation could result through root accumulation (Huang *et al.*, 2007a). Alternatively, mineralization can occur directly, from fungal catabolism and enzymatic degradation by the constituent enzymes of arbuscular mycorrhizal exudates, or

indirectly, by enhanced root exudation stimulating catabolic rhizospheric microbial activity with this effect diminishing with distance from the root (Joner and Leyval, 2003). Alternatively, the molecule could be taken up by the plant and accumulated (Li *et al.*, 2006). For a phthalic ester such as di(2-ethylhexyl) phthalate, however, it has been shown (Chen *et al.*, 2005b) that the arbuscular mycorrhizal fungus not only catabolises the molecule but also inhibits its uptake and translocation from the roots to the aerial structures (Wang *et al.*, 2004).

5.1.1 Mycorrhizas and biochar

Although the efficacies of mycorrhizas are well documented for phytoextraction and phytostabilization, the literature base is still limited when biochar applications are considered in relation to these amelioration approaches. In laboratory studies, Liiri *et al.* (2007) used acidic coniferous forest soil to examine the impacts of wood ash addition (5 000 kg ha⁻¹) on the growth of ectomycorrhizal Scots pine (*Pinus sylvestris* L.) seedlings in the presence of enchytraeid worms (*Cognettia sphagnetorum*). In contrast to an earlier report by Mahmood *et al.* (2003) of positive impacts on ectomycorrhizal spruce seedlings, the wood ash was found to have a negative effect on seedling biomass which was not overcome by either the mycorrhizal fungi or worms alone but only by the two together thus emphasising the complexity of soil interactions. A critical factor of all additions to soils is the possibility of creating unfavourable nutrient ratios (Wallstedt *et al.*, 2002) by, for example, adding a high C/N ratio biochar with a labile fraction nitrogen sink.

In contrast, and although not recorded in terms of improved seedling biomass, workers have reported both stimulated mycorrhizal fungus spore germination (Rillig *et al.*, 2010) and increased root colonisation in response to biochar (Matsubara *et al.*, 2002; Yamato *et al.*, 2006; Ishii and Kodoya, 2007; Warnock *et al.*, 2007; Rillig *et al.*, 2010) or activated carbon (Herrmann *et al.*, 2004) supplementation probably through enhanced nutrient availability (Ishii and Kadoya, 1994) due to improved soil physico-chemical properties (DeLuca *et al.*, 2006).

A review of mycorrhizal responses to biochar was made by Warnock *et al.* (2007) who considered the possible synergy for promoting soil quality by examining critically four mechanistic hypotheses for its effects on mycorrhizal abundance and/or functioning:

Alteration of soil physico-chemical properties particularly nutrient availability. Although biochar contains limited concentrations of nutrients (Lehmann *et al.*, 2003a; Topoliantz *et al.*, 2005; Gundale and DeLuca, 2006), which could promote fungal proliferation in nutrient poor soils (Treseder and Allen, 2002), both increases (Gundale and DeLuca, 2006) and decreases (Lehmann *et al.*, 2003a) in, for example, available nitrogen, and increases in

phosphorus (Garcia-Montiel *et al.*, 2002) have been recorded following supplementations with the increases facilitated, in part, by the hyphae minimising losses (Allen, 2007). In addition, as discussed by Warnock *et al.* (2007), biochar additions can affect the principal interrelated soil physico-chemical factors of pH (increases/decreases), cation exchange capacity (increases), water holding capacity (increases), bulk density (decreases) and adsorption (increases).

Mycorrhizal fungi/soil bacteria interactions. Warnock *et al.* (2007) considered the various interactions between mycorrhizal fungi and soil bacteria and identified the important provision of bacterial metabolites (flavanoids, furans and raffinose), which promoted hyphal growth and subsequent root colonization. The authors also considered mineral provision through, for example, bacterial phosphate solubilisation (Kothamasi *et al.*, 2006) as a key factor. The effects of biochar on these interactions remain to be resolved although Warnock *et al.* (2007) speculated that the supplement may provide nutrients and/or reduced carbon compounds, either directly or indirectly through adsorption. The provision of a high surface area for microbial attachment and growth could also be an important element.

Plant-fungus signalling interference and biochar attenuation of allelochemicals. Signalling between mycorrhizal fungi and the host plant is a feature of the rhizosphere (Harrison, 2005). Key plant root secretion compounds include carbon dioxide, flavanoids, sesquiterpenes and strigolactones (Warnock *et al.*, 2007). Biochar addition could, therefore, exert both direct (adsorption/desorption of signalling compounds or inhibitory allelochemicals/toxic molecules) and indirect (pH change) effects. Although the provision of a sink for fungicidal molecules would promote growth and root colonization, the adsorption of chemical signals would be disadvantageous.

Provision of physical protection against fungal grazers. As discussed above, the physical properties of different biochars can vary with respect to cation sorption capacity (Gundale and DeLuca, 2006) and pore distribution with pore diameters > 16 µm reported (Hockaday *et al.*, 2007). Whilst providing surfaces for attachment and growth (Samonin and Elikova, 2004), pore colonisation can afford protection against grazing species (Pietikäinen *et al.*, 2000; Warnock *et al.*, 2007). Although the aromatic fused ring clusters convey considerable recalcitrance to biochar, with accepted estimates for soil residence times of 5 000 to 10 000 years (Swift, 2001; Krull *et al.*, 2003), both pore sizes and porosity can change through, for example, organic matter adsorption (Kwon and Pignatello, 2005). In well aerated tropical soil, however, residence times of < 100 years have been estimated (Bird *et al.*,

1999) with mineralization attributed to microbial catabolism (Hamer *et al.*, 2004) and abiotic oxidation (Cohen-Ofri *et al.*, 2007).

The efficacy of biochar to promote arbuscular mycorrhizal fungal abundance in roots (percent colonisation) must be questioned following the results of a comprehensive study made by Warnock *et al.* (2010) who examined this together with soil abundance (hyphal length). The host plant was *Plantago lanceolata* while three different soil types and five biochars and ten applications were used. For all supplementations, root fungal abundance remained either unchanged or decreased. The latter was recorded for *Pinus contorta* Douglas ex. Loudon wood, and was accompanied by decreases in soil phosphorus availability, and both peanut shell and mango wood biochars, which were characterised by phosphorus increases. Elevated plant biomass production was recorded for a single treatment only. These results contrasted earlier studies (Matsubara *et al.*, 2002; Yamoto *et al.*, 2006) where increased abundances were recorded. To find an explanation for their results, Warnock *et al.* (2010) considered the key variables of pH, phosphate availability, decreased soil organic matter decomposition through biochar sorption and phenolics/polyphenolics –cidal/-static effects and highlighted that considerable work still needs to be done before biochars can be used with confidence in land restoration/reclamation programmes.

Although this discussion has focussed on mycorrhizal fungi, it must be recognised that biochar can be a determinant of both pathogenic and saprophytic fungal activities. Matsubara *et al.* (2002), for example, demonstrated that tolerance of asparagus seedlings to *Fusarium oxysporum* was enhanced by the presence of biochar although fresh organic matter gave comparable results. Elad *et al.* (2010) reported similar results but with citrus wood charcoal when 1-5% (w/w) supplementations of sandy soil were found to convey systemic resistance of pepper and tomato to *Botrytis cinerea* (grey mould) and *Leveillula taurica* (powdery mildew) and pepper to the mite *Polyphagotarsonemus latus* Banks. The continued efficacy of biochar is, in turn, dependent on saprophytic fungal activity which, through extracellular enzymic activity and hyphal growth/penetration, can violate the integrity of the material.

5.2 Soil/sediment remediation

The ability of biochars to abate soil/sediment pollutants through sorption and sequestration has been recognised increasingly (Bornemann *et al.*, 2007; Nguyen *et al.*, 2007; Chen *et al.*, 2008; Yu *et al.*, 2010; Chen and Yuan, 2011) with high microporosity and surface area and heterogeneous surface physico-chemical properties (James *et al.*, 2005) the key factors (Lohmann *et al.*, 2005; Yu *et al.*, 2006).

Pesticides. An insight of the possible role of biochar in mitigating the genotoxicity of pentachlorophenol in sediments was given by Cui *et al.* (2009). With a concentration of $200 \mu\text{g kg}^{-1}$, the molecule exerted genotoxicity effects on the target species earthworm (*Eisenia fetida*) but these decreased with increased concentrations of crop residue ash black carbon. The workers cautioned, however, that high concentrations of ash black carbon (10%) also exerted genotoxicity as recorded by an increased DNA lesion. Later work by Lou *et al.* (2011) with rice straw biochar showed that pesticide concentrations of 50 mg kg^{-1} sediment reduced markedly wheat seed growth but a biochar supplementation of 2 % (w/w) increased both the germination rate and root elongation, which they equated to an extraction liquid PCP concentration reduction from 4.53 to 0.17 mg l^{-1} thus commending the adsorption remediation approach.

As a commonly used herbicide in New Zealand silviculture, terbuthylazine was the target molecule of Wang *et al.* (2010) in their studies of sorption in two pine plantation pumice soils (low organic matter and organic-rich topsoil) through organic amendments (thermally dried digested/undigested biosolids and biochars, 350/700°C). In contrast to the undigested and digested biosolids topsoil additions, which had near negligible effects on herbicide adsorption, both biochars, particularly the 700°C material, promoted this. Further, desorption was also resisted more strongly by the biochars than the biosolids. In conclusion, the workers suggested that through the intervention of biochar, groundwater could be protected from the hydrophobic herbicide.

The two carcinogenic herbicides atrazine and acetochlor were the focus of studies made by Spokas *et al.* (2009) who used Waukegan silt loam soil amended (2-60% w/w, 24-720 t ha⁻¹) with CQuestTM (500°C) biochar to examine respiration, nitrous oxide production, methane oxidation and herbicide transformation (atrazine, $1.04 \mu\text{g g}^{-1}$ soil; and acetochlor, $1.06 \mu\text{g g}^{-1}$) and retention (atrazine/acetochlor, $0.23\text{-}22.67 \mu\text{g g}^{-1}$ soil). For all the biochar concentrations used, both CO₂ and N₂O productions were reduced as too was CH₄ oxidation thus increasing its inimical environmental impact potential. Carbon dioxide suppression was attributed to reduced mineralization while the methane oxidation decreases were thought to be due to selective use of sorbed organic compounds. No mechanism for the N₂O decreases was tendered. As anticipated by the workers, biochar addition promoted herbicide adsorption although with atrazine it had no effect with the highest initial concentration ($22.67 \mu\text{g g}^{-1}$ soil). The workers compared the biochar with other types of soil organic matter and concluded that its efficacy was lower. Earlier studies by Yang and co-workers (Yang and Sheng, 2003; Sheng *et al.*, 2005), for example, with diuron, bromoxynil and ametryne in the presence of wheat and rice biochars had recorded much higher sorption capacities. Together with sorption, herbicide bioavailability is dependent on mineralization which is

slowed by the presence of biochar. Thus, although both leaching and runoff may be minimised by biochar application, herbicide phytotoxicity may also be reduced thus necessitating increased application.

Cao *et al.* (2009) also examined biochar (dairy manure, 200/350°C) sorption of atrazine and observed that the herbicide was partitioned into its organic phase. This contrasted with their commercial activated carbon control where surface sorption was the operative mechanism.

Studies on the insecticides chlorpyrifos and carbofuran were made by Yu *et al.* (2009) who used *Eucalyptus* spp wood chips biochars (450/850°C) to reduce the bioavailabilities of the insecticides to Spring onion (*Allium cepa*). Pesticide (50 mg kg⁻¹ soil) loss through degradation and/or sequestration in the sandy loam decreased as the biochar concentration (0 – 1% w/w soil) increased although plant uptake decreased to 10% (chlorpyrifos) and 25% (carbofuran) in the presence of 1% biochar (850°C) compared with the control. The workers attributed the phytoavailability reduction of both molecules to the affinity/sequestration potential of the biochar and suggested that a strategy could be developed to reduce plant uptake. In developing management practices it must be recognised, however, that biochar sorption potential can be masked by organo-mineral soil biochar interactions (Singh and Kookana, 2009).

The fungicide pyrimethanil, which inhibits methionine synthesis, was the focus of work by Yu *et al.* (2010) with two (450 and 850°C) *Eucalyptus* spp biochars. In 24-hour soil equilibration tests, sorption coefficient and isotherm non-linearity both increased with biochar supplementation particularly for the higher temperature material and this was attributed to its greater surface area and microporosity.

Other organic molecules. Zhu *et al.* (2005) examined sorption of apolar (cyclohexane, 1,2-dichlorobenzene, 1,4-xylene, 1,2,3,5-tetramethylbenzene and 1,3,5-triethylbenzene) and polar (*o*-cresol, 4-nitrotoluene, 2,4-dinitrotoluene and 2,4,6-trinitrotoluene) molecules by five different maple (*Acer* sp.) wood biochars. Principal among the conclusions were: polar molecules sorbed to a higher degree than apolar compounds; the larger molecules suffered from steric exclusion; and platinum catalyst facilitated hydrogenation to remove O functionality promoted sorption by both molecule types by reducing competitive adsorption by water molecules.

Sorption studies of neutral organic contaminants (benzene and nitrobenzene) on two wheat (*Triticum aestivum* L.) biochars (300/700°C) were reported by Chun *et al.* (2004). The lower temperature material was characterized by partial carbonisation, a high organic carbon content (40-50%), reduced surface area (< 200 m² g⁻¹) and an oxygen content > 20%. In contrast, the 700°C product was well carbonized and had a high surface area (> 300 m² g⁻¹)

with low organic matter (< 3%) and oxygen (< 10%) contents. The two biochars exhibited different adsorption patterns with carbonized surface adsorption the dominant mechanism for the 700°C product and surface adsorption and lower partition into the residual organic matter phase operative for the lower temperature material. Both biochars exhibited higher surface affinity for the polar nitrobenzene than the non-polar benzene which they attributed to the surface acidity/basicity of the biochars.

Like Chun *et al.* (2004), Chen and Chen (2009) distinguished between the adsorbent carbonized organic matter and partition phase non-carbonized organic matter which, together with the surface and bulk properties, control sorption. The workers chose orange peel (cellulose, hemicelluloses and pectin) for their starter material and produced chars at nine different pyrolysis temperatures between 150 and 700°C. After analysis (elemental, BET-N₂ surface area and Fourier transform infrared spectroscopy), sorption of nonpolar (naphthalene) and polar (1-naphthol) hydrophobic molecules from water was used to determine the relative contributions of adsorption and partition in biochar sorption. For the pyrolysis temperature of 150°C, the sorption isotherms for both molecules were near linear indicating the dominance of partition through the presence of an amorphous aliphatic fraction (Chen *et al.*, 2008). With higher pyrolysis temperatures, however, the isotherm shape moved from linear to Freundlich indicating the increased contribution of adsorption, due to increased sorbent aromaticity (Chen *et al.*, 2008), and the importance of pyrolysis temperature for bespoke biochar production. Further evidence for this isotherm shape change was reported by Chen and Yuan (2011) who examined the efficacy of pine needle biochars (100, 300, 400 and 700°C) to sorb polycyclic aromatic hydrocarbons (PAHs) and reported reduced soil supplementation requirements to achieve total sorption for the higher temperature materials.

Huang and Chen (2010) examined the sorption of polar (*p*-nitrotoluene, *m*-dinitrobenzene and nitrobenzene) and non-polar (naphthalene) molecules to different rice straw ash charcoals, including 800°C biochar, and recorded comparable sorption properties, which commended the more cost-effective charcoal supplement for organic contaminant sequestration.

From this brief discussion it can be seen that a number of studies have recorded reduced mineralization of organic chemicals in soils through effective adsorption. Work by Zhang *et al.* (2005), however, showed that biochar nutrients, particularly phosphorus, stimulated both microbial cell growth and benzonitrile mineralization prior to catabolism slowing through adsorption.

Together with the positive roles of biochars in promoting microbial catabolism and sequestering organic contaminants in soil, it must be recognised that they can, at times, have a negative effect. At the plant level, Yang *et al.* (2006), for example, found that biochar addition reduced the herbicidal effect of diuron to grass.

Metals/Metalloids. Derelict/degraded land is often characterized by a lack of top soil and, therefore, a susceptibility to improvement through, for example, compost addition. Where the land is contaminated with metals the supplementation may also result in immobilisation (Gadepalle *et al.*, 2007) albeit on a temporary basis (van Herwijnen *et al.*, 2007) although this could be extended by the application of biochar (Hartley *et al.*, 2009). An alternative view is that, following carbon-rich additions, the metals/metalloids may be displaced as organic complexes (Cao *et al.* (2003).

Greenhouse pot trials, with three arsenic contaminated ($60\text{-}72\text{ mg kg}^{-1}$) soils subjected to biochar application (20% v/v) and planted with *Miscanthus x giganteus*, were made by Hartley *et al.* (2009). In comparison to green waste compost (30% v/v), which improved crop yield and increased soil pore water dissolved carbon concentrations and, thus, iron and arsenic mobility, the effects of biochar were much less pronounced although increased pore water arsenic was apparent with two of the soils. The workers speculated that a rise in pH might have been responsible since arsenic mobility is reduced in acidic soils due to iron oxide surface adsorption (Madejón and Lepp, 2007). Also, bioavailable nutrients, such as phosphorus, as a consequence of biochar addition (Lehmann *et al.*, 2003a), would have competed with the arsenic for binding sites.

Pot trials (60 days), complemented by *Lolium perenne* L. var Cadix shoot emergence testing, were also used by Beesley *et al.* (2010) to examine the mobility, bioavailability and toxicity of zinc, cadmium, arsenic and copper, together with PAHs, in the presence of added commercial green waste compost and hardwood biochar. For copper and arsenic, both supplementations effected > 30-fold soil pore water increases, which were attributed to dissolved organic carbon and pH increases. In contrast, the zinc and cadmium concentrations both decreased with biochar proving more effective for the latter. Since, as discussed above, biochar may be used as a compost bulking agent, it may be speculated that, compared with green waste compost, this compost type could have improved efficacy to reduce the bioavailability of inorganic and PAH soil contaminants.

Studies with lead were made by Cao *et al.* (2009) who used dairy manure biochar (200/350°C) to examine metal sorption. The biochar, particularly the 200°C material, was found to be six-times more effective than commercial activated carbon. Sorption followed a dual Langmuir-Langmuir model due to lead precipitation ($\beta\text{-Pb}_9(\text{PO}_4)_6$,

200°C; $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$, 350°C) (84-87%) and surface sorption (13-16%). In contrast, activated carbon sorption obeyed a single Langmuir model which the workers attributed to surface sorption. They also considered the dual sorption of lead and atrazine and recorded little competition for biochar sorption in contrast to the strong competition on activated charcoal.

6. Soil microbial response to biochar

The observed actions of biochar on soil microbiological activity result from at least three effects: alteration of physico-chemical interactions, such as increased water and nutrient retention; electron donor provision; and provision of habitat.

Physico-chemical effects. The presence of biochar in soil promotes enhanced water holding capacity (Pietikäinen *et al.*, 2000), nutrient adsorption capabilities (Dünisch *et al.*, 2007, Major *et al.*, 2009), dissolution-precipitation, acid-base reactions, redox reactions (Joseph *et al.*, 2010) and cation retention (Lehmann 2007). Wardle *et al.* (1998) suggested that nutrient adsorption facilitated increased availability and Ortega-Calvo and Saiz-Jimenez (1998) showed that co-adsorption of substrate and microorganisms increased bioavailability.

Organic molecule sorption by biochar is a function of its aromaticity (Chen *et al.*, 2008; Chen *et al.*, 2009) and, hence, pyrolysis temperature (Wang *et al.*, 2010; Yu *et al.*, 2009) since increases lead to higher aromaticity (Brewer *et al.*, 2009), and elevated herbicide (Wang *et al.*, 2010) and pesticide adsorption capacity and phytoavailability (Yu *et al.*, 2009). Although cation retention is enhanced by the presence of biochar relative to soil organic carbon, this is a poorly understood function of biochar age (Lehmann, 2007).

The potential for more subtle physico-chemical modifications of soil microbial ecology is the focus of much work. As discussed above, since flavanoid plant signals have similar adsorption properties to low molecular weight polycyclic aromatic hydrocarbons (Shaw and Hooker, 2008), it has been postulated that there may be negative effects on bioavailability of these molecules in the rhizosphere, potentially affecting interactions such as nitrogen fixation (Shaw, 2010). Furthermore, several researchers (Thies and Grossman, 2006; Thies and Rillig, 2009) observed that microbial extracellular enzymes may interact with biochar in a variety of presently poorly understood ways.

In summary, the surface properties of biochar exert positive effects on soil nutrients and cations. Co-adsorption can lead to increased local nutrient concentrations for microbial community species and enhanced water retention

while organics adsorption leads to reduced run-off loss, although there may be competing negative effects on nutrient phytoavailability and microorganism-plant signalling arising from sorption. Although it has been shown (Durenkamp *et al.*, 2010) that supplementation (3.5/28 mg C g⁻¹ soil) of silty clay loam, loamy sand and Chinese red loam with beech (500⁰C) and maize straw/wood waste (350-400⁰C) biochars did not decrease the fumigation extraction efficiencies of biomass carbon or biomass ninhydrin-nitrogen, sorption phenonema could have important implications for chemical extraction methodologies.

Substrate provision. Biochar, although generally perceived as an inert soil material, undergoes changes on two different timescales. Initially, residual adsorbed pyrolysis products act as substrates for soil biota and, consequently, soil microbial populations are affected by the quality of the applied material. Biochar quality, in turn, depends on feedstock and pyrolysis conditions (Zimmerman, 2010). In particular, flash- and low-temperature pyrolysis give rise to residual bio-oils and re-condensed materials (Steiner *et al.*, 2007c). Low molecular weight oxygenated volatile organic compounds (acids, alcohols and carbonyls) serve as substrates (Steiner *et al.*, 2007c) in low concentrations, but are toxic to microorganisms at higher concentrations (Deenik *et al.*, 2010; Steiner *et al.*, 2007c) as are polycyclic aromatic hydrocarbons, cresols and xylenols (Thies and Rillig, 2009). To probe recalcitrance, Hilscher and Knicker (2011) made 28-month microcosm incubation studies with ¹³C- and ¹⁵N-enriched *Lolium perenne* biochar (350⁰C) in Bw horizon Cambisol and elucidated the humification processes at different times with solid-state ¹³C and ¹⁵N NMR spectroscopy. Following incubation, changes were recorded in the proportion of O-containing functional groups (increased) and aryl C and N-containing heterocyclic structures (both decreased) from which the workers concluded that the attendant physical and chemical property changes both made the biochar more labile and increased its adsorption capacity. Also, the degradation of N-heterocyclic domains suggested important implications for the soil nitrogen cycle.

Increased microbial biomass (Zackrisson *et al.*, 1996) respiration and respiration efficiency (Steiner *et al.*, 2007b) have been reported in response to biochar treatment. Studies of microbial respiration by researchers at Rothamsted, UK, for example, have shown that initial ‘flushes’ of CO₂ evolution following biochar addition, returned to control levels after a few days (Durenkamp and Brookes, 2010). Comparable studies by Smith *et al.* (2010a) with *Panicum virgatum* biochar (500⁰C) supplementations of two silt loam soils also recorded immediate CO₂ evolutions, which continued for six days, from the char in proportion to the addition. The workers concluded that the pyrolysis bio-oil condensates were the likely labile carbon pool. Supporting evidence for this was provided by Luo *et al.* (2010) who reported that lower temperature biochars resulted in enhanced CO₂ evolution

due to higher amounts of water-extractable organic carbon. Biochar mediated CO₂ evolution must also be considered in the context of greenhouse gas emissions with reports of increased methane and decreased nitrous oxide releases from rice paddy supplemented with 10-40 t ha⁻¹ (Zhang *et al.*, 2010).

In the long term, biochar is a recalcitrant form of carbon. A 50 nm spatial resolution near-edge X-ray adsorption fine structure spectroscopy (NEXAFS) map of the distribution of carbon environments in carbon particles from Amazonian Dark Earths demonstrated that surface oxidation occurs only over very long timeframes (Liang *et al.*, 2006). Nevertheless, increases in soil microbial biomass, respiration and respiration efficiency have been reported for these (Thies and Rillig, 2009; Thies and Suzuki, 2003) relative to surrounding native soils. Furthermore, differences in soil microbial community composition, relative to similar, adjacent native soils, have been demonstrated by sequencing (O'Neill *et al.*, 2009) and genetic fingerprinting (Grossman *et al.*, 2010). It is apparent, therefore, that biochar addition affects microbial ecology beyond the timescale of adsorbed residuals metabolism. Presumably, adsorption processes lead to alterations in soil substrate concentration and availability over the longer term (Wardle *et al.*, 1998). Hence, the soil microbial population, which establishes following biochar amendment varies according to biochar type (electron donors), amount, frequency of addition and age (longer term effects) (Joseph *et al.*, 2010).

Habitat provision. Pores in biochar can provide support surfaces for microbial colonisation which, together with enhanced water holding capacity, can afford a suitable habitat for microorganisms. Pietikäinen *et al.* (2000) compared microbial communities of biochar and pumice supports and found that the former supported larger numbers with higher respiration rates than either pumice or activated charcoal, with relatively low and relatively high specific surface areas and adsorption capacities, respectively. These results were interpreted as evidence that elevated microbial abundance required a high surface area in combination with high water retention.

Also, the two materials supported distinct population types as demonstrated by phospholipid fatty acid analysis. These differences in microbial ecology remain unresolved although they may result from pore size variations affording protection from fungal grazers (Warnock *et al.*, 2007), or effecting pH and local concentrations of pore gases (Thies and Rillig, 2009). Electron microscopy work by Luo *et al.* (2010) demonstrated that the pores of lower pyrolysis temperature biochars provide secure environments for microorganisms.

Discussions of soil microbial responses to biochars would be incomplete without consideration of enzyme activities. Bailey *et al.* (2011) examined enzyme activity (β -glucosidase, β -N-acetylglucosaminidase, leucine

aminopeptidase and lipase) in the presence of *Panicum virgatum* biochar in three soil types (Palouse silt loam, Quincy sand and Warden sandy loam) but recorded inconsistent results with both increased (enzyme function chemical enhancement) and decreased (substrate sorption) activities apparent. Although not proven in the study, the workers speculated that enzyme activity promotion may have been due to the release of an enzyme-specific allosteric upregulator, such as ethylene, from the biochar.

6.1 Microbiological/molecular analysis

Although the combined and enhanced role of char and soil microbial populations in ecosystem amelioration are recognised (Glaser *et al.*, 2002; Warnock *et al.*, 2007), limited research has been reported of microbial diversity/functional response to the approach (Pietikäinen *et al.*, 2000; Warnock *et al.*, 2007; 2010); Steinbeiss *et al.*, 2009). The distinctive physical/chemical/biological properties of biochar, including high water-holding capacity relative to activated charcoal, large internal surface area, cation exchange capacity, elemental composition and a wide range of pore size/volume/distribution (Pietikäinen *et al.*, 2000), effect its recognised influence on microbial ecology. Thus, it adsorbs soluble organic matter/gases/inorganic nutrients, and so provides a robust habitat for diverse soil microbial associations protected from predation/desiccation (Warnock *et al.*, 2007). Its sorptive properties can, however, lead to biases in total microbial activity assessments and these necessitate comprehensive investigation.

To date, studies of microbial community responses to biochar have relied on assays such as soil respiration (CO₂ evolution), colorimetric and fluorescent measurements, culturing, hyphal abundance, phospholipid fatty acid analysis (PLFA), restriction fragment length polymorphism (RFLP) automated ribosomal intergenic spacer analysis (ARISA) and sequencing (Kolb *et al.*, 2009; O'Neill *et al.*, 2009; Steinbeiss *et al.*, 2009; Smith *et al.*, 2010; Warnock *et al.*, 2010; Bailey *et al.*, 2011; Khodadad *et al.*, 2011). An example is 16S rRNA gene analysis of pepper and tomato rhizosphere communities in the presence of biochar (Graber *et al.*, 2010). Although the plants were grown in soilless media by fertigation – the application of water soluble products through irrigation - the researchers recorded an increased abundance of culturable communities in augmented treatments with 16 of the 20 unique isolates affiliated with plant growth promoting and/or biocontrol agents. Similarly, preliminary investigations in our laboratory with denaturing gradient gel electrophoresis (DGGE) and nutrient agar (10% strength) cultivations revealed increased numbers of colony forming units, predominance of Gram-negative rods but reduced diversity *re* species richness and numerical dominance in a biochar-supplemented domestic garden soil. Other researchers (Khodadad *et al.*, 2011) have used molecular quantitative polymerase chain reaction

(qPCR) and nested PCR-ARISA) and culture-dependent analyses to investigate the effects of two biochar types on forest soils with and without burn histories. Briefly, the high temperature biochar-supplemented soils showed peaks in viable cell counts and 16S rRNA gene copy numbers. However, despite increased rates of respiration, a general decrease in the diversity of total bacteria was observed with direct plating and qPCR showing a numerical decrease in relative abundance especially in unburnt soils augmented with a low temperature Oak250 biochar. Nonetheless, sequencing showed an increase in the relative abundance of Actinobacteria and Gemmatimonatedes. The authors concluded that their study was consistent with previous findings where biochar augmentation resulted in an enrichment of specific taxa, especially the Actinobacteria, in fire-impacted soils. Environmentally ubiquitous, particularly in soils, members of this phylum are central to organic material decomposition and humus formation (Ventura *et al.*, 2007) and, therefore, the carbon cycle including biochar development and soil application.

A review by Nielsen *et al.* (2011) examined critically 26 published microbial ecology studies (reflecting 85 experimental observations) to highlight the importance of recognising the diversity-function relationship within the context of soil biodiversity and carbon cycling. They proposed that magnitudes of positive – linear, redundant/asymptotic or idiosyncratic, neutral and negative effects on the cycle would be dependent on soil species richness, traits and community composition. Although made for the carbon cycle, one of their principal conclusions, that studies on general and specialized ecosystem function in relation to biodiversity in response to anthropogenic activities were essential, is also relevant to investigations on soil biochar application. Therefore, shifts in soil microbial composition, species richness and evenness, as related to key functional/process stability and attendant enzyme activity, must be determined. Consequently, Bailey *et al.* (2011) measured biological activity of β -glucosidase, β -N-acetylglucosaminidase, lipase, and leucine aminopeptidase to establish, generically, soil capacity to metabolise carbohydrates, lipids and proteins in the presence of biochar. They reported highly variable responses of the four enzymes in three different soils to 2% (w/w) augmentation. Therefore, extensive proteomic investigations should provide potentially more conclusive data that complement the colorimetric and fluorescent assays, which, in this instance, showed either increased or decreased activity.

Nevertheless, obvious and considerable knowledge gaps still exist. For example, extensive research over the last two decades has established that culture-based analyses of complex ecosystems such as soils, sediments and surface-/groundwater are still limited largely to $\leq 5\%$ of all microbial species (Amman *et al.*, 1995; Head *et al.*, 1998). Consequently, molecular analysis of mixed and unknown species by techniques such as DNA-/RNA-based conventional and quantitative polymerase chain reaction (PCR) (Lee *et al.*, 1996; Bach *et al.*, 2002; Koenigsberg

et al., 2005), DGGE (Muyzer and Smalla, 1998), PCR-single strand conformation polymorphism (SSCP) (Schwieger and Tebbe, 1998), fluorescent *in situ* hybridization (FISH), t-RFLP (Hartman and Widmer, 2008), and clone libraries has become routine. Therefore, comprehensive approaches must be taken with the analyses of total and functional microbial communities central to determining biochar impacts on specific ecosystems. For example, the expression of key catabolic genes and enzymes from known populations/genera/species can be targeted through qPCR, transcriptomics (McGrath *et al.*, 2008; Shrestha *et al.*, 2009; Vandenkoornhuysen *et al.*, 2010) and (meta)proteomic analysis (Maron *et al.*, 2007; Schneider and Riedel, 2010), while community profiling can be made with DGGE, T-RFLP, 'qfingerprinting' (Ramette, 2009) and ARISA (Danovaro *et al.*, 2006; Kovacs *et al.*, 2010). Increasingly, these methods are complemented by novel software applications (Yu *et al.*, 2005; Schloss *et al.*, 2009; Giongo *et al.*, 2010) that have been developed specifically to process the vast data that often result from molecular microbial ecology studies of complex interacting associations. Also, as already discussed, mycorrhizas continue to be the focus on the impact of biochar on soil populations, probably because they play an important role in rhizospheric interactions. Joseph *et al.* (2010) presented a summary of microscopy, chromatography and spectroscopy based studies to investigate/propose mechanisms of root growth in biochar-supplemented soils. Generally, root hairs can penetrate the water-filled macropores, influence local mineral, electron donor/acceptor and redox gradients and, consequently, affect the distribution of attendant microorganisms, including mycorrhizal fungi. Furthermore, bioturbation via biochar ingestion by soil macrofauna such as worms, termites and larvae will redistribute the material, its surface-attached communities and their inherent functional capacities especially vertically through the soil profile. The macrofauna can also have direct impacts on surface area, aeration, nutrient availability, environmental parameters such as pH (*e.g.* the termite gut is generally alkaline with pH >10 or 12 found in some species (Brune *et al.*, 1995; Brune and Friedrich, 2000)) and, thus, microbial occurrence/activity. Hence, although in a non-biochar study, this bioturbation phenomenon was also observed by Liu *et al.* (2011) where burrows of the earthworm *Aporrectodea caliginosa* showed increased microbial herbicide degradative enzyme activity. Generally, these physical and biochemical mechanisms create unique micro-geographical ecosystems within the soil structure. Therefore, the recent application of (macro)ecology analytical tools/indicators in molecular microecophysiology (Prosser *et al.*, 2007), such as DGGE/T-RFLP data interpretation for taxa-area/biogeography relationships in (arbuscular) mycorrhizal fungal (van der Gast *et al.*, 2010) and bacterial (Bell *et al.*, 2005) populations, should facilitate the measurement of microbial spatial and temporal distribution in the presence of biochar.

The increased application of molecular microbial ecology techniques has also led to greater awareness of both their potentials and limitations. This in turn has facilitated the improvement of existing tools and/or application of novel ones such as microarrays. Subject to successful nucleic acid recovery, these platforms, unlike their counterparts, typically circumvent PCR/amplification biases, are (semi-)quantitative, high throughput and allow simultaneous analyses of community composition, structure and function in increasingly complex ecosystems (Wu *et al.*, 2001; Li and Liu, 2003; Zhou *et al.*, 2010). Also, while less laborious and faster than other ecogenomic protocols, they are generally more sensitive. As a result, greater microbial diversity in urban aerosol, subsurface soil, subsurface water and spacecraft clean rooms has been recorded, for example, with phylogenetic 16S rRNA gene microarray (PhyloChip) analyses than with 'universal' (16S rRNA) and functional gene clone libraries alone (DeSantis *et al.*, 2007; Probst *et al.*, 2010). Similarly, the application of genechips to both pristine and contaminated environments revealed rich, diverse and robust microbial communities (He *et al.*, 2007; Hollister *et al.*, 2010; Xu *et al.*, 2010). Consequently, attempts to address the recognised and new challenges of high throughput microbial ecology platforms, including competitive chemistries/hybridization, difficulties with creating and maintaining conditions that are optimum for all array probes, even detection of different signal intensities, specificity, attendant data analysis, *etc.* (Avarre *et al.*, 2007; He *et al.*, 2010) continue to be made (Liang *et al.*, 2010; Oh *et al.*, 2010) to facilitate wider and more comprehensive applicability. For example, combinations of genechips with other tools such as FISH have been developed and applied to monitor both bacterial and fungal populations (Metfies and Medlin, 2008). Also, although they recognized some limitations for application on high density arrays, Cao *et al.* (2002) and Avarre *et al.* (2007) recommended several improvements including the use of nanoparticles to increase hybridization specificity. However, any co-extraction of biochar particles during nucleic acid recovery, and subsequent application on nanoparticle-enhanced arrays, would possibly present interesting challenges regarding surface charge interactions. For example, any preferential/competitive binding of the biochar to the nanoparticle-probe moieties could potentially result in inhibitory binding of the environmental nucleic acids to the probes on the arrays. . Nevertheless, any improvements to these already powerful tools should, ultimately, benefit the requisite detailed and comprehensive analyses of the full impacts of contemporary biochar applications. These can be realised and exemplified during gene expression studies especially in contaminant degradation in the presence of biochar. Furthermore, differences in soil types, land use histories, application regimes and experimental designs complicate direct comparisons between (microecophysiology) investigations of contemporary soil biochar augmentation (Kolb *et al.*, 2009). The consistent and now well established platforms such as the PhyloChip and GeoChip 2.0/3.0, and

their demonstrable applicabilities in diverse ecosystems, will facilitate comparisons of microbial community structural and functional responses to biochar, including references to specific biogeochemical cycles/processes (C, N, metal resistance, organic contaminant remediation, *etc.*).

As discussed above, the results of some studies infer the need for repeat applications of biochar for sustained efficacy. The implications of this approach on the soil physical structure, mineral composition (Carson *et al.*, 2007), microbial abundance (Warnock *et al.*, 2010), nutrient and contaminant bioavailability, particularly in relation to biotoxicity and soil 'health' (Dawson *et al.*, 2007; Federonkova *et al.*, 2010), must be determined. Therefore, small bespoke microarrays could also be developed to test the biotoxicity of specific chars using single strains (Johnson *et al.*, 2008) and/or model communities and then enriched indigenous catabolic communities.

Notwithstanding their analytical power/capacity, ecogenomic tools do not provide phenotypic or physiological characterization of novel and previously uncultivated microbial strains. This results in a discrepancy that compromises a more complete understanding of microbial diversity in specific ecosystems, particularly soil. As a result, extensive research is underway to address the well recognised limitations (Amman *et al.*, 1995) of culture-based methods so that seemingly important community members (*e.g.* as identified by numerical dominance or relative abundance) or representatives of functionally significant groups can be cultivated for further analyses. Despite the debate for and against cultivation, with or without molecular analyses (Tyson and Banfield, 2005; Ritz, 2007), the approach has been exemplified by many researchers such as Bollmann *et al.* (2010) who used a diffusion chambers to cultivate and study the physiology of strains from acidic, heavy metal-contaminated sites. Naturally, the isolates were capable of growing in low pH conditions in the presence of high concentrations of nitrate and heavy metals. Subsequent 16S rRNA gene analyses showed representation of Actinobacteria, Firmicutes, Bacteroidetes and Alpha/Beta/Gamma Proteobacteria. Stevenson *et al.* (2004) used several strategies to mimic the soil environment including incubation atmospheres of elevated CO₂ (5% v/v) and O₂ (2% v/v) concentrations. Subsequent plate wash PCR and sequencing revealed a predominance of Acidobacteria and Verrucomicrobia in the agricultural soil and guts of wood-feeding termites. Similarly, Joseph *et al.* (2003) resuspended soil samples in VL55 medium (Sait *et al.*, 2002) prior to plating on a 1/100 dilution nutrient broth and recovered 350 isolates of the subdivisions of five key phyla including Acidobacteria, Proteobacteria, Verrucomicrobia, Gemmatimonadetes and Actinobacteria. Due to the widely observed ubiquity of their 16S rRNAs or 16S rRNA genes in soils (Joseph *et al.*, 2003; Stevenson *et al.*, 2004), these phylogenetic groups were also recorded as indicators of soil microbial ecological response to biochar, with Verrucomicrobia (Grossman *et al.*, 2010) and Actinobacteria (Khodadad *et al.*, 2011), for example, showing higher relative abundances due to the

augmentation. Therefore, culture-dependent tools/approaches, including community-level physiological profiling (Chaer *et al.* 2009; Bougnom *et al.*, 2010), ‘habitat’ or ecological media, such as soil extract broth/agar (Liebeke *et al.*, 2009; Bastida *et al.*, 2010; Bučková *et al.*, 2010), simulated natural environments and protracted incubation times (*e.g.* up to 12 weeks; Sait *et al.*, 2002) will be essential for understanding the physiological responses of soil microbial communities to different biochars.

Furthermore, it is well recognised that one of the principal challenges of successful contemporary application and exploitation of biochar is understanding the attendant complex soil-microorganism-biochar interactions. As a result, the application of magnetised biochars (C-Cure Solutions Ltd., www.forestresearch.gov.uk) may be applicable in relatively simple laboratory-scale reactors with slurries and liquid cultures. Thus, the biochar can be extracted to make comparative determinations of the distribution of free-living (planktonic) and surface-attached (biofilm) microbial communities with time. The latter could then entail specific analyses of preferential attachment between the biochar and soil surfaces. This can be achieved, for example, with combinations of culture, molecular, spectroscopy, microscopy and chromatography based techniques as discussed in this and other biochar-related reviews. Also, nanoparticles have been applied increasingly in microbial ecology to exploit the potential of magnetic carrier technology for simultaneous concentration, separation/recovery and purification of biomolecules (Sebastianelli *et al.*, 2008). This tool would, therefore, enhance the recovery of the active/growing and functional community members. These potentially powerful tools within the context of soil microbial community response to biochar could be extended with additional fundamental investigations using the thymidine analogue, bromodeoxyuridine (BrdU) (Borneman, 1999; Hamasaki *et al.*, 2007; McMahon *et al.*, 2010). The defining principle of the BrdU immunocapture approach – enrichment and recovery of populations that grow in response to specific stimuli and incorporate the BrdU label into *de novo* DNA, should facilitate analysis of the physiological response of probably the functionally significant components of the soil communities. Naturally, careful experimental design to maximize microbial community recovery and subsequent analysis must be central to studies that employ combinations of the magnetic biochar, nanoparticle and BrdU based approaches to avoid possibly competitive and/or inhibitory interactions. Nevertheless, these fundamental investigations can then be complemented by potentially more rapid and precise fingerprinting techniques such as denaturing high-performance liquid chromatography coupled with T-RFLP (Penny *et al.*, 2010), to expediently link the physiological/phenotypic and phylogenetic characteristics of the soil populations, especially numerically and/or functionally significant members.

The recovery, particularly from soils, of high quality, high quantity nucleic acids suitable for a variety of downstream analyses and representative of all microbial communities remains a challenge. Also, expediency through fast methods that allow rapid processing of large sample numbers is a key consideration for the development and application of efficient/robust/balanced protocols. Extensive research has been made on this fundamental step as reviewed by Schneegurt *et al.* (2003) and Kirk *et al.* (2004). A few examples of how some typical bottlenecks can be addressed have been provided by: Gabor *et al.* (2003), who used indirect environmental DNA extraction for subsequent gene bank construction; Kabir *et al.* (2003), who investigated three extraction protocols for downstream real-time quantitative PCR; Braid *et al.* (2003), with focus on chemical flocculation for the removal of PCR inhibitors; and Delmont *et al.* (2011), who compared different strategies to circumvent extraction biases while recovering currently inaccessible communities especially for metagenomic studies. For biochar, addressing these limitations was explored by Durenkamp *et al.* (2010), who studied the efficacy of K₂SO₄ fumigation extraction to recover microbial biomass carbon and ninhydrin-N following biochar or activated charcoal supplementation of three soil types. They found that carbon recovery was dependent on soil type, biochar and extractant concentration. Also, Grossman *et al.* (2010) modified the protocol of a commercial soil DNA extraction kit with an additional phenol-chloroform purification step followed by nested PCR. These steps enhanced the detection and subsequent exploration of the bacterial and archaeal diversity in *Terra preta* by DGGE and T-RFLP (Personal communication). Generally, different nucleic acid extraction methods have proved successful as demonstrated by application to high throughput platforms, which must be sensitive and specific, such as phylogenetic and functional microarrays. Since the addition of biochar could introduce further difficulties to the fundamental challenge of microbial surface attachment, stable isotope labelled substrates could be used in studies that combine these with respiratory measurements (Neufeld *et al.*, 2007; Schwartz, 2007). Therefore, ¹³CO₂ evolution, for example, would establish the activity of the complete biome while the molecular-based analysis would reflect only the recoverable sub-communities of the biochar-augmented soil. Notwithstanding the obvious costs, bespoke radio-/stable isotope labeled chars could also be developed for definitive investigations of mass/carbon balance calculations and recalcitrance (Smith *et al.*, 2010) in comparison to their availability as electron donors or susceptibility to re-mineralisation (Zimmerman, 2010).

The successful design and implementation of comprehensive microbial ecology investigations is underpinned increasingly by the use of toolboxes with a suite of relevant and complementary culture-/molecular-based techniques, which address the inherent limitations of each. For example, the key challenges of sample collection, handling, processing and analysis, to ensure accurate representation of the original ecosystem, can be

circumvented with *in situ* methodologies. Thus, despite their well established limitations such as costs, representative/even labelling, and signal detection sensitivity against potentially competitive backgrounds, especially with real environmental samples (Neufeld and Murrell, 2007), further developments in tools that can be used for whole communities *in situ* or with minimal disruption *ex situ*, including substrate-tracking autoradiography (STAR)-FISH (Lee *et al.*, 1999; Ouverney and Fuhrman, 1999), Raman/infrared spectroscopy (Singer *et al.*, 2005; Harz *et al.*, 2009), Raman-FISH (Huang *et al.*, 2007b), and Fourier transform infrared (FT-IR) spectroscopy (Wharfe *et al.*, 2010), will be fundamental to biochar research. Also, rapid clone library management schemes (Grant and Ogilvie, 2004) and extensive metagenomic analysis with techniques such as 454-pyrosequencing (Liu *et al.*, 2009; Rothberg and Leamon, 2008), to identify and quantify (occurrence/distribution/prevalence) species components, in the presence and absence of biochar, will be invaluable. Ultimately, the responses of total and functional microbial communities in pristine and contaminated environments will have to be determined for both different biochars and application regimes, and in relation to specific ecosystems.

Summary

The application of char to soil, as biochar, especially for increased and sustained agricultural output, was practised by ancient civilisations in different parts of the world. This practice is receiving renewed research interest, particularly for carbon sequestration, and, potentially, composting and restoration/amelioration of contaminated environments. This review, therefore, considers findings of biochar application to diverse ecosystems and in the presence of a wide range of organic and inorganic compounds such as heavy metals, pesticides, polycyclic aromatic hydrocarbons, *etc.* Reported and new knowledge gaps to be addressed for successful exploitation in agriculture and several environmental biotechnologies are identified. Thus, examples of specific pathways and their relevance in mycorrhizal interactions to effect increased crop yield, and phytoremediation, via phytoextraction/phytoaccumulation, are explored together with possible underpinning physical, chemical and biological mechanisms. Similarly, the potentially inhibitory effects of biochar, such as decreases in molecule bioavailability during bioremediation programmes, pesticide adsorption with reduced phytotoxicity and nutrient sequestration in agricultural soil, are discussed. The longevity of char in relation to its biodegradability and carbon and trace elements source, and as a co-metabolism substrate are additional essential considerations.

Generally, the recognised historical benefits seem to justify contemporary biochar exploitation although the mechanisms are complex and require further study. Specifically, the efficacies of different chars in different

ecosystems are dependent on the source material, production conditions, application regimes, target molecules and site specific parameters. As a result, there is need for comprehensive and consistent/reliable tool kits to explore the attendant mechanisms from both multi-disciplinary and multi-phasic perspectives. Therefore, established/novel cutting edge tools to elucidate char physico-chemical properties prior to, during and post application to soils are considered. These should be complemented by molecular microecophysiology techniques for definitive studies of microbial community response for directed and sustainable exploitation in the different biotechnologies to then establish robust monitoring programmes. Consequently, some of the literature cited provides examples of tool kits, with protocols used to study historical biochar exploitation in, for example, Amazonian Dark Earths, thus establishing particularly relevant platforms for comparisons with new scenarios.

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1 Tables

2 Table 1: Biochar properties as a function of feedstock

Char	Proximate analysis						Surface area / m ² g ⁻¹	Cation exchange capacity / meq (100 g soil) ⁻¹	Author
	Temp / °C	Carbon content / g kg ⁻¹	Yield / %	Volatiles / %	Fixed carbon / %	Ash / %			
Wood	200	525							Baldock and Smernik (2002)
Cellulosic char	300	440							Shafizadeh and Sekiguchi (1983)
Wood	350	673							Baldock and Smernik (2002)
Corn (<i>Zea mays</i>) residue	350	675							Nguyen and Lehmann (2009)
Cellulosic char	400	765							Shafizadeh and Sekiguchi (1983)
Pitch pine (<i>Pinus rigida</i>) wood	450						< 10		Brown <i>et al.</i> (2006)
Grapeseed (<i>Vitis vinifera</i>)	450		49 - 53						Özçimen & Ersoy-Meriçboyu, (2008)
Chestnut shell (<i>Castanea sativa</i> Mill.)	450		55 - 60						Özçimen & Ersoy-Meriçboyu, (2008)

Cellulosic char	500	804						Shafizadeh and Sekiguchi (1983)
Rapeseed cake (<i>Brassica napus</i> L. ssp. oleifera)	500		18.70	63.70	17.60			Özçimen & Karaosmanoğlu (2004)
Poultry (<i>Gallus domesticus</i>) litter	500						38.3	Gaskin <i>et al.</i> (2008)
Peanut (<i>Arachis hypogaea</i>) hulls	500						4.63	Gaskin <i>et al.</i> (2008)
Pine (<i>Pinus taeda</i>) chips	500						5.03	Gaskin <i>et al.</i> (2008)
Switchgrass	500		7.1	39.5	52.5	50.2		Brewer <i>et al.</i> (2009)
Corn stover	500		11.1	54.7	32.4	20.9		Brewer <i>et al.</i> (2009)
Rape	550		13.6	64.6	21.8			Sánchez <i>et al.</i> , (2009a)
Sunflower	550		13.4	57.7	28.9			
Grapeseed (<i>Vitis vinifera</i>)	550	43 - 45						Özçimen & Ersoy-Meriçboyu, (2008)
Chestnut shell (<i>Castanea sativa</i> Mill.)	550	48 - 50						Özçimen & Ersoy-Meriçboyu, (2008)
Corn (<i>Zea mays</i>) residue	600	790						Nguyen and Lehmann (2009)
Pitch pine (<i>Pinus rigida</i>)	750						400	Brown <i>et al.</i> (2006)

wood							1
Grapeseed (<i>Vitis vinifera</i>)	750	39.45	50.96	9.59		Özçimen & Ersoy-Meriçboyu, (2010)	2
Hardwood	unspecified	19.7	63.8	13.9	19.7	Brewer <i>et al.</i> (2009)	
Switchgrass (<i>Panicum virgatum</i>)	unspecified				7.7 – 7.9	Boateng (2007)	

1 Table 2: Agricultural effects of biochar

Crop	Trial Type	Soil	Region	Biochar	Application Rate	Effect	Authors
Radish (<i>Raphanus sativus</i>)	Pot	Alfisol	Australasia	Greenwaste pyrolysis	10 - 100 t ha ⁻¹	At highest rates with nitrogen (100 kg ha ⁻¹) application, +280% yield, compared to +95% in absence of biochar	Chan <i>et al.</i> (2007a)
Radish	Pot	Alfisol	Australasia	Poultry litter pyrolysed at 450 and 550 °C	0-50 t ha ⁻¹ +/- 100 kg N ha ⁻¹	+42% at 10 t ha ⁻¹ without N +96% at 50 t ha ⁻¹ without N with N, lower temp material more effective	Chan <i>et al.</i> (2008)
Cherry tomato	Pot	Chromosol	Australasia	Wastewater sludge pyrolysed at 550 °C	10 t ha ⁻¹	64%	Hossain <i>et al.</i> (2010)
Maize	Field	Degraded Amazonian	South America			Biochar doubled maize yield	Kimetu <i>et al.</i> (2008)
Maize	Pot	Top soil and subsoil	South America	Sugarcane bagasse	50 g kg ⁻¹ soil +/- biodigester effluent (100 kg N ha ⁻¹)	Biochar increased green biomass growth of maize in top soil absence and presence of effluent. Biochar increased green biomass in presence of effluent in subsoil	Rodríguez <i>et al.</i> (2009)
Wheat Soybean Radish		Ferrosol and Calcerosol	Australasia	Pyrolysed papermill waste	10 t ha ⁻¹	Up to 225% increase in biomass production (soybean only: negative responses for wheat and soybean)	van Zwieten <i>et al.</i> (2010)

Rice (<i>Oryza sativa</i> L., cv Wuyunjing 7)	Field		China		10 and 40 t ha ⁻¹	Increase in rice yield of upto 14% in highest application rate and in the absence of applied N	Zhang et al. (2010)	1 2
Maize	Pot	Ultisol	China	Rice straw pyrolysed at 250 - 400°C for 2 – 8 hours.	1% ± NPK	Increased maize yield of 146% in the presence of NPK and 64% in its absence.	Peng et al. (2011)	3