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Biochar incorporation increased nitrogen and carbon retention in a waste-derived soil

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Abstract

The synthesis of manufactured soils converts waste materials to value-added products, alleviating pressures on both waste disposal infrastructure and topsoils. For manufactured soils to be effective media for plant growth, they must retain and store plant-available nutrients, including nitrogen. In this study, biochar applications were tested for their ability to retain nitrogen in a soil manufactured from waste materials. A biochar, produced from horticultural green waste, was added to a manufactured soil at 2, 5 and 10 % (by weight), then maintained at 15 ºC and irrigated with water (0.84 mL m^{-2} d^{-1}) over 6 weeks. Total dissolved nitrogen concentrations in soil leachate decreased by 25.2, 30.6 and 44.0 % at biochar concentrations of 2, 5 and 10 %, respectively. Biochar also changed the proportions of each nitrogen-fraction in collected samples. Three mechanisms for biochar-induced nitrogen retention were possible: i) increased cation and anion exchange capacity of the substrate; ii) retention of molecules within the biochar pore spaces; iii) immobilisation of nitrogen through microbial utilisation of labile carbon further supported by increased soil moisture content, surface area, and pH.

Dissolved organic carbon concentrations in leachate were reduced (-34.7 %, -28.9 %, and -16.7 %) in the substrate with 2, 5 and 10 % biochar additions, respectively. Fluorescein diacetate hydrolysis data showed increased microbial metabolic activity with biochar application (14.7 ± 0.5, 25.4 ± 5.3, 27.0 ± 0.1, 46.1 ± 6.1 µg FL g^{-1} h^{-1} for applications at 0, 2, 5, and 10 %, respectively), linking biochar addition to enhanced microbial activity. These data highlight the potential for biochar to suppress the long-term turnover of SOM and promote carbon sequestration, and a long-term sustainable growth substrate provided by the reuse of waste materials diverted from landfill.

Keywords

Waste materials, sustainability, biochar, manufactured soil, nitrogen, carbon

1. Introduction

Within the European Union (EU) the legislative framework on waste management is provided by the EU Waste Framework Directive (Directive 2008/98/EC). This sets the following waste hierarchy to be applied as a priority order in member states: prevention, preparing for reuse, recycling, other recovery and disposal. As such, disposal to landfill is the least favoured option meaning that a large amount of biodegradable waste must be diverted from landfills to other organic waste management practices, where it can be recovered and utilised.

Mineral and organic waste materials, derived from a range of industries and activities, have potential for reuse as components of manufactured soils. Such soils are generally appropriate for urban development and landscape management (green areas), and as high value substrates (Koolen and Rossignol 1998). Their uses include manufacture of topsoils for urban grasslands (Haraldsen et al. 2014), addition of waste sand as a soil amendment (de Koff et al. 2010), and as materials for the horticulture, agriculture, amenity and restoration markets (Jones et al. 2009).

Increased use globally is driving a range of detrimental impacts on topsoils, including decreased agricultural productivity and enhanced release of greenhouse gases (Harter et al. 2014). The effective production, deployment, and management of manufactured topsoils may serve as a means of alleviating pressure on topsoil resources, alongside low-impact waste management (Arbestain et al. 2009, Belyaeva and Haynes 2009, Belyaeva et al. 2012, Braga et al. 2019, Mattei et al. 2017). However, to ensure its effective and sustainable deployment, a detailed understanding of the complex nutrient dynamics and key system influencers is first required. Nutrients are essential for plant growth, so a manufactured soil will require robust nutrient retention and storage capabilities. Nutrient dynamics within all soils are influenced by ecological communities; therefore, for a manufactured soil to be an effective plant growth medium, it must support a diverse ecological community. Under conditions of constant temperature and moisture, microbial diversity within soils is impacted predominantly by soil pH, carbon to nitrogen (C : N) ratio and, to a lesser extent, phosphorus (Dumbrell et al. 2010). A previous study of a manufactured soil linked high C : N ratios to carbon limitation in the soils, leading to mineralisation of soil organic nitrogen (Schofield et al. 2018). This was evident from a sustained increase in dissolved nitrate concentrations in soil leachate as the nitrogen within the organic molecules was quickly converted to this form (Bingham and Cotrufo 2016). As the measured nitrate concentrations approached the European Union threshold of concern for nitrate groundwater and river pollution, this functioning was a potential problem for deployment in areas where soil leachate could impact on ground or surface waters. Additionally, the macronutrient imbalance highlighted the need for a soil management protocol to achieve effective sequestration of both carbon and nitrogen over the lifetime of the substrate.

Biochar is a solid, carbon-rich material derived from biomass by pyrolysis in an oxygen-limited atmosphere; it has been widely acknowledged as an effective tool for environmental management (Lehmann and Joseph 2010). Once incorporated into soil, biochar affects its physicochemical and biological properties, which have importance with regard to agronomic productivity. These include increased pH (Spokas et al. 2009, Zhang et al. 2014), water holding capacity (Lehmann et al. 2011), ion exchange capacity (Godlewska et al. 2017), improved soil nutrient status (Agegnehu et al. 2015, Clough et al. 2013, Li et al. 2018a, Saarnio et al. 2018), microbial activity (Godlewska et al. 2017, Lehmann et al. 2011) and soil structure (Downie et al. 2010). Biochar application to soils may also contribute to climate change mitigation through decreased greenhouse gas emissions (Awasthi et al. 2017, Harter et al. 2014, Oldfield et al. 2018, Spokas et al. 2009), and the promotion of diverse microbial populations (Anderson et al. 2011, Lehmann et al. 2011). When these factors are considered alongside the demonstrated large mean residence time for biochar in soils, the production and application of biochar is considered positive, in terms of a reduction in greenhouse gas emissions and carbon sequestration, when compared to biomass waste management (Keith et al. 2011). A life cycle assessment estimated the energy and climate change impacts and economics of biochar systems (Roberts et al. 2010). Here, analyzed feedstocks were agricultural residues (corn stover), yard waste, and switchgrass energy crops. System net energy was greatest with switchgrass (4899 MJ/ton dry feedstock). Net greenhouse gas emissions for stover and yard waste were negative, at -864 and -885 kg CO_2 equivalent emissions reductions per ton of dry feedstock respired. Of these total reductions, 62-66 % were from C sequestration in biochar. Woolf et al. (2010) estimated the maximum sustainable technical potential of biochar to mitigate climate change and calculated that total net emissions could be reduced by 130 Pg CO_2-C equivalent, over the course of a century without endangering food security, habitat or soil conservation.

Biochar is produced from a range of organic biomass material feedstocks the composition of which, along with the pyrolysis temperature and conditions, influences its physicochemical characteristics and its efficacy as a soil amendment (Li et al. 2018b, Waqas et al. 2018). Increasing the pyrolysis temperature decreases biochar mass yield, and increases pH and total pore volume (Demirbas 2004, Hossain et al. 2011, Li et al. 2018b, Manya 2012, Yuan et al. 2019). Pyrolysis temperatures above 600 ºC increase total concentrations of nitrogen, phosphorus, and potassium; while micronutrients, such as calcium, iron, magnesium, copper, sulfur, and zinc decreased (Hossain et al. 2011, Zhao et al. 2013). This may be a result of increased thermal degradation and aromatisation, which occur at higher pyrolysis temperatures, potentially influencing the bioavailability of nutrient elements by providing a greater number of ion exchange sites (Li et al. 2018b, Zhou et al. 2018). Pyrolysis temperature effects on biochar characteristics and its nitrogen-sorption capacity are feedstock-specific, as the rudimentary porosity and structure are retained (Blackwell et al. 2010, Li et al. 2018a). A range of biochar feedstocks was trialled across a number of studies and can be broadly divided into three categories: wastes, crop residues and purpose-grown feedstock (Hammond 2010). Significant variations between feedstocks have been demonstrated and, whilst some have displayed clear advantages over others, availability and sustainability of the feedstock remain a key factor in their potential as soil amendments (Keith et al. 2011, Mitchell et al. 2015). Pyrolysis is also considered a source of bio-energy and a means of waste disposal, from which biochar is a value-added waste material (Laird 2008). In such circumstances, pyrolysis conditions may represent a compromise between optimal biochar yield and energy production.

For manufactured soils to be effective and sustainable growth media, they must retain and cycle nutrients to support long-term plant growth without the need for significant fertiliser inputs. This study aimed to evaluate the impact of biochar on the efficacy of nitrogen retention, both organic and inorganic, storage and release within a manufactured soil. The test soil, composed of waste materials, has been deployed to support a variety of plants within natural and artificial environments over a 15-year timescale; however, its success as a growth medium has relied on regular fertiliser applications to supply the required nutrients in plant-available form, and significant losses of carbon and nitrogen were apparent in leachate from soil columns measured over a 12-month period (Schofield et al. 2018). The objective of the study was to measure the effect of biochar application to the manufactured soil, at 3 concentrations, on the retention of macronutrients over the experimental period. The results, are discussed and the potential for biochar to improve nutrient retention in this substrate and, by extension, the sustainability of its construction through the reuse of waste materials is evaluated.

2. Materials and Methods

2.1 Biochar production

The pyrolysis conditions under which biochar is produced and the feedstock from which it has been produced have been demonstrated to influence biochar product characteristics. Pyrolysis temperature has been reported to influence certain biochar properties such as yield, pH, recalcitrance (Zhao et al. 2013). High pyrolysis temperatures (>600 ºC) are reported to reduce biochar yields and increase alkalinity (Demirbas 2004, Hossain et al. 2011, Manya 2012). Further, the N concentration for a biochar was found to decrease with increasing pyrolysis temperature (Hossain et al. 2011), whilst other macronutrient concentrations were found to increase (Hossain et al. 2011, Zhao et al. 2013). Other characteristics are reported to be predominantly controlled by feedstock such as biochar C content, CEC, sequestration capacity, mineral content and ash content (Zhao et al. 2013).

Biochar was produced by pyrolysis of a mixed horticultural green-waste feedstock collected from the shredded woody waste feedstock bay at the Eden Project green waste composting facility in Cornwall, SW England (https://www.edenproject.com/). This material consisted of a mix of freshly-shredded palm fronds, bamboo, and mixed temperate hedge trimmings (hawthorn, hazel, beech, holm oak) in approximately equal proportions and was selected to present a readily-available and sustainable (‘cut and come again’) source material. The materials were selected due to their ready availability and their reported efficacy as biochar feedstocks (Sohi et al. 2013, Som et al. 2012, Suthar et al. 2018). The use of mixed feedstocks has been reported to provide a broader range of characteristics to optimise its effectiveness as a soil amendment (Taherymoosavi et al. 2016).

In order to generate a biochar product with improved retention of a range of macronutrients, including N, P, and K a mid-temperature (450 ºC) pyrolysis procedure was devised. The feedstock was oven-dried at 60 ºC for 48 hours, transferred to a glass beaker, wrapped with aluminium foil and placed into a muffle furnace where the temperature was increased from 21 to 450 ºC at a rate of 5 ºC min^{-1}, then held at 450 ºC for 15 min before cooling to room temperature over 12 hours. The average yield was 22.2 ± 1.0 % w/w, calculated as the proportion of solid product to the original feedstock, a lower yield than larger-scale production systems using equivalent conditions, which was 35 % (Bridgwater 2012). Prior to addition to the soil, the biochar particles were ground to pass through a 2 mm sieve.

2.2 Soil composition

The manufactured soil used within this study was prepared using a mixture of available, low-cost waste materials. The freshly-prepared soil comprised both inorganic and organic components to recreate natural soil structure and function. The components were (% by volume) composted bark (32.5 %), composted green waste (32.5 %), china clay sand extract (25 %) and lignite clay (10 %). The soil classification was sandy loam according to ISO 14688-1 (ISO 2002). The composted green waste was produced from the Eden Project's green waste feedstock comprised of a mix of herbaceous and woody plants, predominantly from pruning, thinning and weeding operations. These were mainly shoot materials but included some entire plants plus rootballs; all large and wood material was shredded before addition to the compost windrows. This feedstock was mixed with a small amount of composted food waste (<5 %) 'activator' which was also produced on site by aerobic digestion (Orthodoxou et al. 2015), and composted in weekly-turned windrows for about 3 months or until the core temperature had stabilised to < 20^{o}C.

The pH of freshly-prepared substrate was 6.2–6.8. The air-filled porosity was 25 %, measured through assessments of air-filled porosity of the freshly-prepared substrate following the procedure of Bragg and Chambers (1988). Further details on the soil are given in Schofield et al. (2018).

2.3 Mesocosms

A range of biochar concentrations has been applied to soil, ranging from 0.02 % in studies from the 1980s and 1990s (Glaser et al. 2001), while more recent work has applied biochar concentrations ranging from 0.4 to 9 % (Asai et al. 2009, Rondon et al. 2007, Steiner et al. 2008, Steiner et al. 2007). In this study, biochar was added to equal mixes of the manufactured soil at concentrations of 0, 2, 5 and 10 % (w/w, oven-dry-mass basis), henceforth BC0, BC2, BC5, and BC10, respectively. To ensure homogeneous mixing, the biochar-soil mix was moistened using high-purity water (HPW; 18.2 MΩ cm; 10 % v/w) and packed into mesocosms in triplicate. The mesocosms were opaque PVC pots (i.d. 110 mm, depth 100 mm) (Figure 1). To aid drainage, the base of each mesocosm was perforated with 5 mm holes, and to minimise fine particulate losses a 100 µm mesh was placed inside.

The mesocosms were maintained unplanted and covered, to minimise evaporative losses, in a controlled temperature room (15 ºC) for 6 weeks. The temperature was that employed during previous experiments on the soil was within the annual range reported for the region Schofield et al. (2018). In that study, irrigation of the soil over 6 weeks reduced NO_3^-, DON and DOC concentrations by 99, 36 and 27 %, respectively. As such, the 6 week experimental period was deemed a suitable period to measure the effect of biochar on the retention of N and C in the soil recipe tested. Each mesocosm was irrigated with 10 mL day^{-1} (0.84 mL m^{-2} d^{-1}) HPW adjusted to pH 7 (Schofield et al. 2018).

2.4 Sample collection and analysis

The prepared mesocosms were placed in the controlled temperature room and allowed to settle for 25 days prior to irrigation. Triplicate mesocosms were used for each treatment from which leachate samples were pooled for each treatment. Composite leachate volume for each treatment was recorded prior to filtration through pre-treated HPLC-grade glass fibre filter paper (75 g m^{-2}, 450 µm). After filtration, aqueous samples were stored at -20 ºC in acid-washed HDPE bottles and analysis was performed within 3 weeks of collection. After 6 weeks, mesocosms were extruded and solid-phase samples collected. To minimise any edge-effects linked to irrigation, solid-phase soil samples were taken from the centre and subsampled in triplicate for each mesocosm. Solid-phase analyses were performed in triplicate on the freshly prepared biochar-soil mixture (T0) and on the extruded samples (T6).

2.4.1 Physicochemistry

Cation exchange capacity (CEC; meq 100 g soil^{-1}) was measured in for each mesocosm using the ammonium acetate method (Schollenberger and Simon 1945). Leachate pH was measured in within 30 minutes of collection, while the pH of solid-phase samples was determined according to Rowell (1994), where 25 mL HPW was added to 10 g of air-dried substrate, which was shaken at 120 rpm for 30 minutes and allowed to stand for 1 hour before pH measurement. Moisture content was measured as the difference in substrate mass after drying at 105 ºC for 48 hours (Rowell 1994).

2.4.2 Microbial activity

Enzyme activity was measured using a fluorescein diacetate (FDA) hydrolysis method (Adam and Duncan 2001), where enzymes within the sample convert FDA to fluorescein (FL), producing a yellow supernatant with intensity proportional to enzyme activity. Enzyme activity is directly proportional to bacterial biomass as total bacterial cell counts per g dried soil ($P < 0.05$, Supplementary Figure 1). Sodium phosphate buffer (60 mM; 15 mL) and FDA solution (1000 µg FDA mL^{-1}; 0.2 mL) were added to 2 g of freshly-sampled soil, the mixture thoroughly mixed and incubated at 30 ºC in a water-bath for 30 minutes, followed by centrifuging at 2000 rpm for 5 minutes. The supernatant was immediately analysed a using a UV-vis spectrometer at 490 nm (Hewlett-Packard 8453) and enzyme activity was reported in µg FL g^{-1} h^{-1} (Adam and Duncan 2001).

2.4.3 Dissolved nutrients

Leachate samples were analysed for a number of dissolved analytes. Total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) were measured using high temperature catalytic combustion (Badr *et al*., 2003) using a Shimadzu TNM-1 nitrogen module coupled to a TOC-V analyzer (Shimadzu, Japan). Ammonium (NH_4^+) was quantified using fluorescence spectrophotometry (Holmes et al. 1999). Combined nitrate (NO_3^-) and nitrite (NO_2^-) , and phosphate (PO_4^{3-}) were measured using a Skalar SAN^{++} nutrient analyser according to Kirkwood (1996). As NO_2^- concentrations were considered to be minimal in the soil, the combined NO_3^- and nitrite NO_2^- measurements are henceforth referred to as NO_3^-. Dissolved organic nitrogen (DON) was calculated indirectly by subtraction of dissolved inorganic nitrogen (DIN; $NO_3^- + NH_4^+$) from TDN. Potassium concentrations (total dissolved K) were determined using ICP-OES (Thermo-Scientific iCAP 7000 series) analysis (K detected at a wavelength of 766.4 nm).

2.4.4 Particulate nutrients

Total particulate nitrogen (TPN) and soil organic carbon (SOC) were analysed using a CHN EA1110 Elemental Analyser (Ryba and Burgess 2002). Samples were pre-digested for analysis of SOC using 0.1 M HCl as described by Jones et al. (2004). The quantification of water-soluble N fractions was determined through cumulative extraction with HPW as an adaption of the Bureau Common Reference extraction method (Little and Lee 2010). A sub-sample (4 g) of each substrate was weighed into a centrifuge tube, and 40 mL HPW added. The tube was placed on an orbital shaker for 2 hours at 120 rpm then centrifuged at 3000 rpm for 5 minutes. The supernatant was removed and filtered through 0.7 µm glass fibre filters and stored at -20 °C prior to analysis. A second 40 mL aliquot of HPW was added and samples were replaced on the rotary shaker; this process was repeated so that five sequential extractions were performed for each soil sample. The filtrate was analysed for total extracted nitrogen (TEN), extracted organic nitrogen (EON), extracted nitrate (ENO_3^-), total extracted potassium (TEK) and total extracted phosphate (TEP); cumulative concentrations were calculated from leachate data. Extracted inorganic nitrogen (EIN) comprised NO_3^-+NO_2^- and NH_4^+.

2.5 Statistical analysis

All analyses were performed in triplicate. Data was determined to follow normal distribution (Anderson-Darling test) and as such the following statistical analyses were conducted. One-way analysis of variance (ANOVA) was used to test for significant differences between control and treated samples, and Dunnett's test was employed to determine whether any treatments were significantly different ($P \leq 0.05$) from the control; Tukey's test was used to confirm which treatments, if any, were significantly different from all other treatments. Results were considered significant where $p \leq 0.05$. A Pearson correlation coefficient was used to indicate linear correlation between microbial metabolic activity and leached-nutrient concentrations. Analyses were conducted using Minitab v17.

Results and Discussion

3.1 Nitrogen concentrations

Leached-N concentrations in the biochar-amended samples were significantly lower than in the controls (Table 1, $p < 0.05$) for both inorganic and organic N fractions, with higher biochar content samples achieving the greatest reduction. However, there was no significant difference observed between BC2 and BC5 (Tukey's test, Table 1) supporting previous reports that biochar addition reduced N leaching in soils (Agegnehu et al. 2015, Clough et al. 2013, Saarnio et al. 2018). The total water-extractable nitrogen (TEN) concentration decreased significantly ($p <0.01$) between week 0 and week 6 for all treatments and was most evident in the control (BC0, -64.1 %, Table 2). Whilst biochar incorporation lowered the loss of TEN over the experimental period (Figure 2), there was no apparent trend with regard to biochar content with BC5 showing the lowest proportion of TEN losses over the experimental period (-28.3 % between T0 and T6, Table 2) and with no significant difference ($p >0.05$) between BC5 and BC10 (-44.5 and -47.3 % TEN reduction, respectively) or between BC10 and the control (BC0, -64.1 %).

The proportion of TPN represented by TEN in the solid-phase was reduced following irrigation and was greatest within the control samples (at T0 TEN represented 2.29 % of TPN and 0.88 % at T6 for BC0) and lowest within biochar-amended samples (where TEN represented 2.2, 1.7, and 1.9 % at T0; and 1.2, 1.3, and 1.0 % at T6; for BC2, BC5 and BC10, respectively). This may be attributed, in part, to the increased conversion of TEN to non-water extractable N-fractions through increased microbial activity as a result of biochar amendment, whereby N is incorporated into microbial biomass (Prayogo et al. 2014, Schofield et al. 2018), thereby converting previously water-exchangeable N fractions (TEN) into occluded N.

Reduction of N-leaching in response to biochar incorporation to soil has been reported (Agyarko-Mintah et al. 2017, Awasthi et al. 2017, Clough et al. 2013, Li et al. 2018b, Liu et al. 2017, Sanchez-Monedero et al. 2018, Zhang et al. 2014); however, the mechanisms driving this process, referred to as 'nitrate capture', are poorly understood (Sanchez-Monedero et al. 2018). Mechanisms proposed are as follows:

(1) Adsorption of dissolved inorganic and organic N in anion and cation exchange surface reactions with biochar particles. The extent of this effect is thought to be dependent on the nature of the feedstock with regard to functional groups at the particle surface (Clough et al. 2013, Haider et al. 2016, Sanchez-Monedero et al. 2018). The presence of oxonium functional groups has been attributed to a pH-independent anion exchange capacity (AEC) in biochar, resulting in decreased concentrations of anions, such as NO_3^-, in the leachate of biochar-amended soil (Sanchez-Monedero et al. 2018). However, the AEC of freshly-produced biochar is reportedly rapidly decreased by incorporation with soil due to oxidation (Haider et al. 2016). Some biochars increase the CEC and, therefore, the ability of a soil to retain nutrients. However, our data did not reveal significant increases in CEC within biochar-amended samples (5.76 ± 0.26, 5.72 ± 0.71, 5.47 ± 0.18, 6.03 ± 1.22 meq 100 g $soil^{-1}$ for BC0, BC2, BC5 and BC10, respectively; Tables 2 and 3).

(2) The physical capture of NO_3^- in biochar nano-pores (<10 nm) as observed by Kammann et al. (2015) in surface aged biochar and Li *et al*. (2018b) in freshly-prepared apple wood biochar. The biochar used in this study was freshly-prepared and not subject to long-term surface aging. Therefore, the mixed nature of the green waste feedstock from which the biochar was produced may have served to provide varied physical microstructure and pore-sizes, enabling nutrient retention *via* this mechanism. Biochars produced under higher temperature pyrolysis have been reported to have a larger inner-pore area, which serves to increase NO_3^- retention

(Haider et al. 2016), this may serve to offset the lower N content reported to result from high temperature biochar production (Hossain et al. 2011).

(3) Microbial immobilisation of inorganic-N in the utilisation of labile C resulting in lowered N leaching (Agyarko-Mintah et al. 2017, Clough et al. 2013). The biochar-amended samples had significantly lower DOC leachate concentrations than the control (393 ± 5, 206 ± 4 235 ± 4, 294 ± 5 μg C g^{-1}; for the Control (BCO), BC2, BC5, and BC10, respectively, $P < 0.05$; Table 1), which when considered in combination with reduced leachate concentrations for NO_3^- (-10.2, -17.2, and -28.3 % decrease for BC2, BC5, and BC10 compared to the control (BC0); Table 1) and NH_4^+ (-61.2 % reduction for BC2 and reduction to below the LOD for BC5 and BC10; Table 1) from the biochar-treated soils supports N-immobilisation as a factor contributing to the decrease of leachate inorganic-N concentrations.

3.2 Carbon concentrations

Changes in DOC concentration are an important indicator of microbial activity and rates of organic matter biodegradation within a substrate (Marschner and Kalbitz 2003). Biochar addition promoted organic carbon (OC) retention within the substrate over the experimental period, with a decrease in average leached DOC concentrations measured for the biochar-incorporated substrate compared to the control (-34.7 %, -28.9 %, and -16.7 % in BC2, BC5, and BC10, respectively; Figure 3). This was consistent with solid phase data, where the percentage change in SOC over the experimental period was significantly lower in biochar-amended soils (-28.4, 0.69, and -13.4 % in BC2, BC5, and BC10 compared to -33.2 % BC0; $P < 0.05$; Table 2).

Whilst all biochar treatments had decreased DOC leachate concentrations relative to the control, the BC2 treatment were lowest. This could be linked to a more concentrated leachate, resulting from lower leachate volumes when compared to BC0 (-7.58, -12.5, and -19.7 %, for BC2, BC5, and BC10, respectively; Table 1). The lower leachate volume observed may be, in part, the result of higher porosity of biochar amendments, facilitating greater water-holding capacity (Lehmann et al. 2011). However, the observed effect may also reflect the capacity of the microbial population to utilise available C through mineralisation, with excess labile C being leached.

The increased OC retention in the biochar treated soils is potentially indicative of reduced C mineralisation of the organic material, though the precise mechanism could not be determined from this data. There are six mechanisms to account for biochar-induced reduction of C mineralisation proposed by Jones et al. (2011): 1) the biochar-induced release of soluble humic substances which bind to and inhibit extracellular enzymes involved in soil organic matter (SOM) breakdown; 2) sorption of extracellular enzymes on the biochar surface resulting in the removal of sites of organic matter turnover; 3) release of labile soluble C from the biochar as a preferential C source for the soil biota; 4) a biochar-induced increase in soil pH, stimulating changes to the soil microbial structure; 5) sorption of dissolved organic C into biochar preventing microbial consumption; 6) biochar-induced growth of the microbial community resulting in C storage in microbial tissues, preventing mineralisation.

Whilst it is not possible to attribute the relative influence of any of the specific mechanisms to the observations of this study, microbial metabolic activity was increased by biochar application (Figure 3), which supports conversion of C to biomass and subsequent protection from mineralisation (Jones et al. 2011). However, increased moisture content and sites available for sorption of DOC, consistent with increases in CEC for biochar-amended soils (Table 2) may also have contributed to reduced loss of soil C.

3.3 Microbial activity

All heterotrophic organisms require C as: 1) an energy source, resulting in mineralisation to CO_2; 2) for microbial growth, requiring sufficient supplies of nutrients such as N, P, and K (Marschner and Kalbitz 2003). Thus, microbial activity within soils is closely linked to organic matter and nutrient availability. Biochar incorporation has been hypothesised to modify soil conditions, such that microbial activity is stimulated and SOM biodegradation processes altered (Jones et al. 2011, Mitchell et al. 2015, Prayogo et al. 2014).

The total microbial activity within the solid samples, as determined by FDA hydrolysis, increased with biochar content (72.5, 83.4, and 212 %, for BC2, BC5 and BC10, respectively compared to the control (BC0); Figure 3), whilst leached-N fractions decreased with increasing application rate and leached DOC decreased overall as a general result of biochar application, though BC10 had higher leachate DOC levels than BC2 and BC5 (Figure 3). Biochar application also decreased the leached K^+ and PO_4^{3-} concentrations (Table 1); however, the observed reduction correlated with neither biochar content nor microbial activity (Figure 3).

Biodegradation of organic components are driven by the soil microbial population, and are key to nutrient cycling processes. Early stages of organic matter biodegradation produce organic acids, which lower soil pH and reduce oxyanion surface exchange sites, lowering the soil's CEC (Schofield et al. 2018). The pH was higher in biochar-incorporated samples (at week 6 BC0 = 5.85, biochar-incorporated substrate = 6.04 to 6.35, Table 1), which may be attributed to the increased buffering capacity resulting from biochar incorporation (Sanchez-Monedero et al. 2018, Zhang et al. 2014) and this would, at least, maintain the CEC of the substrate.

The soil C : N ratio is a key soil measurement as, when the availability of N is low, it may limit the biodegradation processes within a soil (Chintala et al. 2014) and the synthesis of new microbial biomass (Marschner and Kalbitz 2003). When the C : N ratio is too high, N immobilisation may occur as it is retained within cell structures of the microbial population (Marschner and Kalbitz 2003). Biochar incorporation lends complexity to the scenario, with studies reporting contradictory outcomes with respect to N-mineralisation, N-immobilisation and labile C availability (Clough et al. 2013).

The high-levels of variability reported are attributed to the variance between biochar feedstocks, production methodologies and soil types. The C : N ratio was calculated using SOC and TPN concentrations, which showed that the C : N ratio decreased with increasing biochar application rate throughout the 6-week study (Table 2). The percentage change in the C : N ratio over the experimental period was greatest in BC0, at -28.7 %; however, a decrease in C : N ratio was also observed for BC2 and BC10, (-26.8 %, and -9.39 %, respectively; Table 2), suggesting that, whilst biochar addition reduced N loss, the continued availability of N was necessary to maintain a healthy nutrient cycle.

The T6 moisture content was higher in biochar-amended samples (BC0 = 15.9%, BC2 = 17.1 %, BC5 = 19.4 %, BC10 = 21.4 %), suggesting that biochar amendment increased moisture content. However, these values were still below the reported optimum moisture content for composting (40- 60 %) and may potentially be limiting microbial activity within the substrate (Haug 1993), although this varies with substrate.

Biochar increased soil enzyme activity (BC0 = 14.7 ± 0.5; BC10 = 46.1 ± 6.1 μg FL g^{-1} h^{-1}). The incorporation of biochar improves physicochemical properties of soil substrates, increasing aeration, surface area, pH, and moisture content, which would be a more favourable environment for nitrifying bacteria, altering structure and diversity of microbial communities and increasing microbial utilisation of DOC, and DN fractions (Agyarko-Mintah et al. 2017, Sanchez-Monedero et al. 2018).

3.4 Effect of biochar application to manufactured soils

Based on the data from this study there was no clear relationship between biochar content and analyte concentrations within the leachate and solid-phase samples. However, it is clear that biochar incorporation to the manufactured soils did reduce loss of N and C to leaching.

The manufactured soils treated with biochar had higher microbial activity values. Whilst microbial activity increased with increasing biochar application rate, the increase was not proportional with biochar content with microbial activity increasing 5.34, 2.46, and 3.13 µg FL g^{-1} h^{-1} per % of biochar added, for BC2, BC5, and BC10, respectively. This suggests that the biochar content may have been in excess of that required for optimal microbial population growth, and that non-biochar dependant conditions became limiting factors for the BC5 and BC10 treatments.

Improved conditions for microbial population growth led to increased utilisation of C and N fractions for incorporation into microbial biomass, thereby, reducing their availability for loss through leaching. Further, increased water holding capacity of the biochar treated soils served to further reduce nutrient losses through lower leachate volumes. Similarly to the microbial activity data, reduction in leached N and C fractions relative to the control (BC0) were not proportionate to the biochar content, suggesting that the 10 % application may be in excess of the quantity required for optimal N and C retention.

The data reported herein provide evidence to suggest that biochar amendment of manufactured soil increases N and C retention, however, it offers limited indication as to the longevity of this effect with Major et al. (2010) reported that following a single biochar application, crop yield was improved for at least 4 years.

Conclusion

This study demonstrated that the addition of biochar to a soil constructed from waste materials reduced loss of the macronutrients N and C through soil leachate. For N, this is suggested to result predominantly from microbially-induced changes to N-speciation leading to lower N leaching, with some limited further contribution by increased sorption to ion exchange sites - CEC was increased in biochar-amended soils, however this effect was not significant ($p > 0.05$). Carbon retention and storage within the soil was, similarly to N, likely to result from its incorporation into microbial biomass. The increased microbial biomass, in combination with increased soil pH, particulate surface area, and higher moisture content, promoted metabolic activity within the soil, further lowering the concentration of leaching-susceptible DOC and N within the manufactured soil.

Based on these data, biochar-incorporation has the potential to be used as a tool to improve the sustainability of manufactured soils by enhancing conditions suitable to sustain plant growth, by improving moisture content, nutrient retention and carbon storage capacity, whilst lowering dependence on intensive fertiliser applications and reducing both cost and the risk of pollution from excess leaching of major plant nutrients, such as nitrogen (Schofield et al. 2018).

When produced sustainably, biochar is a valuable resource, aligning with the sustainability potential of soils constructed from waste materials, and represents a valuable tool for both waste management and the development of resilient and efficient growth substrates. However, further research is required to develop a full mechanistic understanding of processes such as ‘nitrate capture’ and interactions between the biochar and substrate, and the long-term response of soil microbial populations, which will progress the attainment of optimal deployment conditions and operational procedures.

Soil degradation is a critical and growing global problem while sustainable cities and communities, responsible consumption and production and life on land (Goals 11, 12 and 15, respectively) are core United Nations Sustainable Development Goals. The manufacture of high value soils from waste materials offers international opportunities for food security, carbon sequestration and achieving a circular economy, while alleviating the current acute human and climate pressures on topsoils.

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Tables

Table 1. Cumulative values for leached-N fractions (TDN, NO_3^-, NH_4^+, DON), PO_4^{3-}, K^+, and DOC expressed as µg g^{-1} soil (d.w.); average leachate pH and leachate volume (mL d^{-1}) were determined for leachate samples from each treatment. ANVOA tests, results expressed as *, indicate a significant difference ($p < 0.05$) compared to BC0. Dunnett's test, results expressed as $^{\text{¥}}$, indicate where one treatment was significantly different from the control. Tukey's test results expressed as $^{\text{A,B,C, or D}}$ to indicate whether treatments were significantly different ($p \leq 0.05$) from all other treatments, shared letters indicate no significant difference ($p > 0.05$) between treatments. Nutrient concentrations and leachate volumes were decreased and pH significantly increased for all biochar treatments, compared to BC0. Leachate from BC10 demonstrated the lowest nutrient concentrations, significant for all analytes. BC0 = 0 % biochar treatment (control), BC2 = 2 % biochar treatment, BC5 = 5 % biochar treatment, BC10 = 10 % biochar treatment. DOC = dissolved organic carbon, TDN = total dissolved nitrogen, DON = dissolved organic nitrogen, NO_3^- = nitrate + nitrite, NH_4^+ = ammonium (LOD = limit of detection; 26.8 µg N L^{-1}). PO_4^{3-} = dissolved phosphate. K^+ = dissolved potassium.

		BC0	BC2		BC5		BC10	
		Concentration	**Concentration**	**Δ (%)**	**Concentration**	**Δ (%)**	**Concentration**	**Δ (%)**
DOC	*µg C g^{-1}*	393 ± 5 $^{\text{¥ A}}$	206 ± 4 $^{\text{¥ * B}}$	-34.7	235 ± 4 $^{\text{¥ * C}}$	-28.8	294 ± 5 $^{\text{¥ * D}}$	-16.8
TDN	*µg N g^{-1}*	171 ± 1 $^{\text{¥ A}}$	102 ± 1 $^{\text{* B}}$	-25.2	99.8 ± 1.5 $^{\text{* B}}$	-30.6	85.3 ± 1.9 $^{\text{¥ * C}}$	-44.0
NO_3^-	*µg N g^{-1}*	83.9 ± 2 $^{\text{¥ A}}$	61.1 ± 1.6 $^{\text{¥ * B}}$	-10.2	59.1 ± 1.5 $^{\text{¥ * C}}$	-17.2	55.0 ± 1.2 $^{\text{¥ * D}}$	-28.3
NH_4^+	*µg N g^{-1}*	1.87 ± 0.71 $^{\text{¥ A}}$	0.11 ± 0.00 $^{\text{¥ * B}}$	-61.2	<LOD $^{\text{* C}}$	-	<LOD $^{\text{* C}}$	-
DON	*µg N g^{-1}*	75.0 ± 12.9 $^{\text{A}}$	45.0 ± 3.5 $^{\text{* B}}$	-40.0	41.9 ± 3.5 $^{\text{* B}}$	-44.1	30.2 ± 2.4 $^{\text{¥ * C}}$	-59.7
PO_4^{3-}	*µg P g^{-1}*	33.3 ± 3.1 $^{\text{¥ A}}$	19.2 ± 1.1 $^{\text{* B}}$	-42.5	17.4 ± 4.2 $^{\text{* B}}$	-47.9	21.0 ± 3.6 $^{\text{* B}}$	-36.9
K	*µg K g^{-1}*	400 ± 35 $^{\text{A}}$	343 ± 10 $^{\text{* A}}$	-14.2	350 ± 7 $^{\text{* AB}}$	-12.6	372 ± 8 $^{\text{* A}}$	-7.12
Leachate pH		6.15 ± 0.02 $^{\text{¥ A}}$	6.35 ± 0.02 $^{\text{¥ * B}}$	3.25	6.55 ± 0.02 $^{\text{¥ * C}}$	6.50	6.67 ± 0.04 $^{\text{¥ * D}}$	8.46
Leachate volume	*mL d^{-1}*	9.10 ± 0.28 $^{\text{¥ A}}$	8.41 ± 0.39 $^{\text{* B}}$	-7.58	7.96 ± 0.23 $^{\text{* B}}$	-12.5	7.31 ± 0.34 $^{\text{¥ * C}}$	-19.7

Table 2. Total and extracted N-fractions, total and extracted C and pH for solid-phase samples from each treatment; determined by 5 repeat extractions in high purity water (18.2 MΩ cm). BC0= 0 % biochar treatment (control), BC2 = 2 % biochar treatment, BC5 = 5 % biochar treatment, BC10 = 10 % biochar treatment. T0 = samples collected at the beginning of experiment, T6 = samples collected at the end of the 6-week experiment. SOC = soil organic carbon, TPN = total particulate nitrogen, TEN = total extracted nitrogen, ENO_3^- = extracted nitrate + nitrite, EON = extracted organic nitrogen, TEP = total extracted phosphate. TEK = total extracted potassium. The C : N ratio was calculated from SOC and TPN. The pH was determined for soil in water (1 : 2.5). CEC = cation exchange capacity (CEC; meq 100 g soil^{-1}). Moisture content (w/w, %).

		BC0			**BC2**			**BC5**			**BC10**		
		T0	**T6**	**Δ (%)**	**T0**	**T6**	**Δ (%)**	**T0**	**T6**	**Δ (%)**	**T0**	**T6**	**Δ (%)**
SOC	*mg C g^{-1}*	232 ± 10	155 ± 4	-33.2	211 ± 7	151 ± 2	-28.4	144 ± 32	145 ± 21	0.69	108 ± 0 18	93.5 ± 9.6	-13.4
TPN	*mg N g^{-1}*	10.2 ± 0.1	9.53 ± 0.02	-6.39	9.99 ± 0.04	9.80 ± 0.07	-1.71	10.2 ± 0.0	9.41 ± 0.03	-8	9.17 ± 0.02	8.76 ± 0.05	-4.5
TEN	*µg N g^{-1}*	234 ± 12	83.9 ± 38.5	-64.1	218 ± 4	121 ± 9	-44.5	173 ± 23	124 ± 28	-28.3	173 ± 24	91.2 ± 14.2	-47.3
ENO_3^-	*µg N g^{-1}*	32.4 ± 5.6	9.27 ± 6.76	-71.4	33.1 ± 2.5	14.7 ± 3.8	-55.6	23.6 ± 3.1	16.2 ± 5.0	-31.4	25.0 ± 3.4	11.1 ± 1.7	-55.6
EON	*µg N g^{-1}*	200 ± 13	74.6 ± 39.0	-71.4	185 ± 5	106 ± 9	-42.7	149 ± 23	108 ± 28	-27.5	148 ± 24	80.0 ± 14.3	-45.9
C : N ratio		22.7	16.2	-28.7	21.1	15.4	-26.8	14.2	15.4	8.5	11.8	10.7	-9.39
TEP	*µg P g^{-1}*	123 ± 6	118 ± 5	-3.75	161 ± 36	145 ± 16	-10.3	159 ± 23	157 ± 12	-1.35	199 ± 25	178 ± 16	-10.5
TEK	*µg K g^{-1}*	836 ± 78	266 ± 15	-68.2	1100 ± 256	514 ± 6	-53.3	757 ± 149	400 ± 6	-47.2	1014 ± 180	541 ± 54	-46.7
pH		5.91 ± 0.05	5.85 ± 0.13	-1.02	6.16 ± 0.04	6.04 ± 0.16	-1.95	6.59 ± 0.26	6.35 ± 0.13	-3.64	6.81 ± 0.04	6.35 ± 0.10	-6.75
CEC	*meq.100g soil^{-1}*	5.76 ± 0.28	5.76 ± 0.26	0	4.38 ± 1.70	5.72 ± 0.71	30.6	5.27 ± 0.74	5.47 ± 0.18	3.8	5.54 ± 0.54	6.03 ± 1.22	8.84
Moisture content	*%*	13.0 ± 0.3	15.9 ± 0.4	18.2	19.8 ± 0.4	17.1 ± 0.3	-13.6	11.7 ± 0.8	19.4 ± 0.07	39.7	11.7 ± 0.2	21.4 ± 0.2	45.3

Table 3. Dunnett's test results expressed as x, indicate where one treatment was significantly different from BC0 (control). Tukey's test to determine when a treatment was significantly different ($p \leq 0.05$) from all other treatments, shared letters indicate no significant difference ($p > 0.05$) between treatments.

	Dunnett's test			**Tukey's test**			
	BC2	**BC5**	**BC10**	**BC0**	**BC2**	**BC5**	**BC10**
SOC	x	x	x	A	B	B	C
TPN			x	AB	A	AB	B
TEN	x	x		A	BC	C	AB
ENO_3^-				A	A	A	A
C : N			x	A	A	A	B
TEP	x	x		A	A	AB	B
TEK	x	x	x	A	AB	B	C
pH	x	x		A	B	B	C
CEC				A	A	A	A

Figures

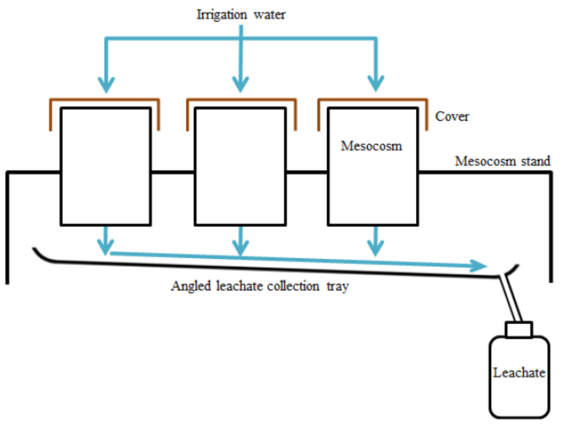


Figure 1. Diagram of the mesocosm set-up used to assess each biochar-amended treatment. Mesocosms (PVC pots, i.d. 110 mm, depth 100 mm) were deployed in triplicate, leachate was sampled cumulatively from each treatment.

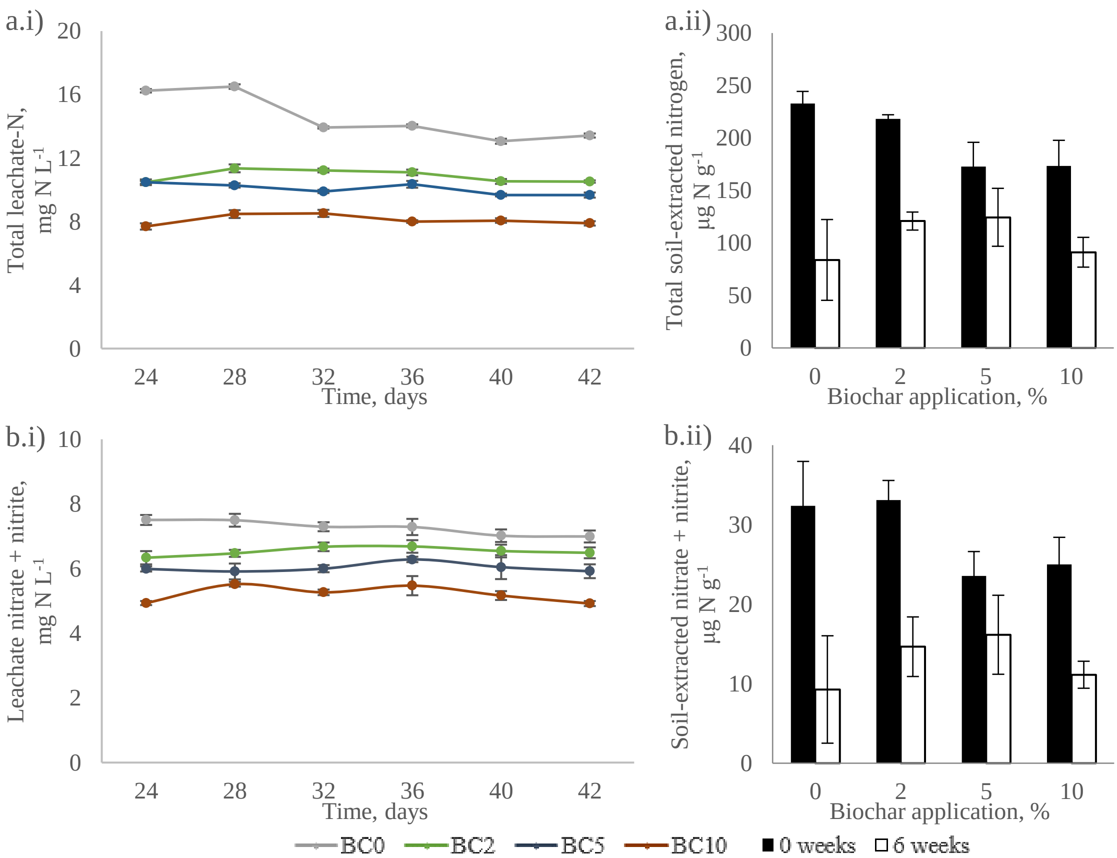


Figure 2. a.i) Time series for total leachate-nitrogen concentrations (mg N L^{-1}) for each biochar treatment. **a.ii)** Total soil-extracted nitrogen (TEN) concentrations at 0 weeks and

following 6 weeks of irrigation. **b.i)** Time series data for leachate-nitrate + nitrite (NO_3^-+NO_2^-) concentrations (µg N g^{-1}). **b.ii)** Total soil-extracted nitrate + nitrite concentrations at 0 weeks and following 6 weeks of irrigation. Analyses were conducted in triplicate.

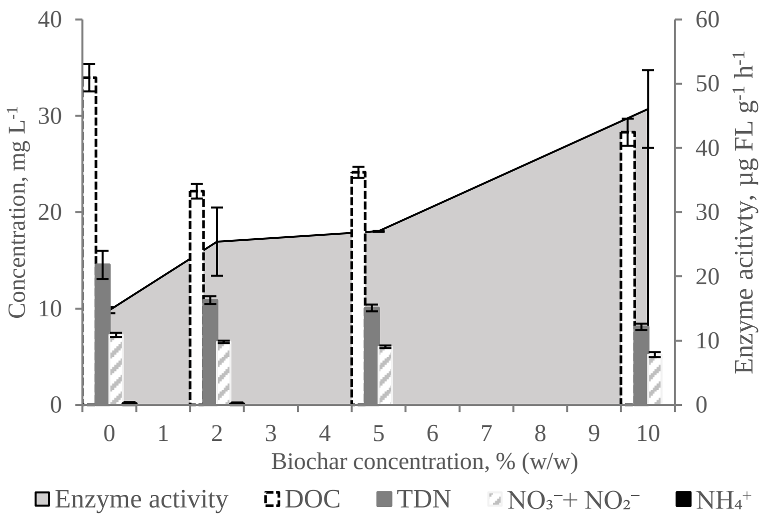
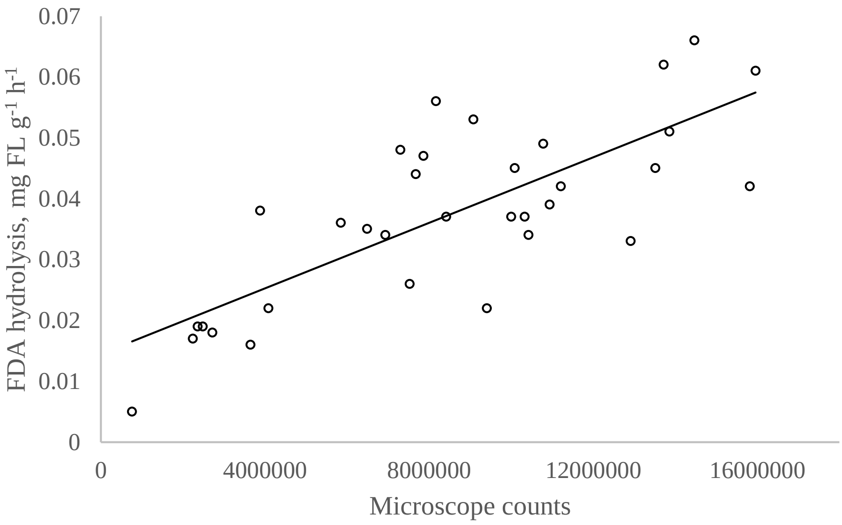


Figure 3. Average leachate concentration for DOC and N-fractions (TDN, NO_3^-+NO_2^-, NH_4^+; mg L^{-1}) and enzyme activity (µg FL g^{-1} h^{-1}) measured within the solid phase following the 6-week irrigation period (n=3). Leachate concentrations for NH_4^+ were <LOD (0.27 µg N g^{-1}) for BC5 and BC10. Pearson correlation demonstrated a significant ($p \leq 0.05$) inverse relationship between enzyme activity (as an indicator for microbial metabolic activity) and TDN (-0.93), NO_3^-+NO_2^- (-0.97), and NH_4^+ (-0.79).

<u>Supplementary material</u>



Supplementary Figure 1. Fluorescein diacetate (FDA) hydrolysis (mg FL g^{-1} d.w. h^{-1}) against microscope bacterial counts for manufactured soil substrate sampled from the Humid Tropics Biome at the Eden Project, Cornwall. The two parameters demonstrate direct proportional linearity (Pearson correlation coefficient $P < 0.05$). On the basis of this, fluorescein diacetate hydrolysis has been used here as an estimation of microbial metabolic activity within the soil and leachate samples.