A BIOGEOCHEMICAL STUDY OF NUTRIENT DYNAMICS IN ARTIFICIAL SOIL

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at Plymouth University has not formed part of any other degree either at Plymouth University or at any other establishment.

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The work in this thesis was primarily the work of the author unless acknowledged otherwise. Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation and knowledge transfer purposes.

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ABSTRACT

Artificial soils have been employed within the Biomes of the Eden Project since its construction in 2000. Produced from sand, bark, composted green waste and lignite clay, these soils were designed to have their nutrient concentrations controlled through careful fertiliser applications. However, following variable environmental conditions, management practices and planting, the soils across the site are performing variably with regard to nutrient retention and storage. Experiments were conducted to assess the performances of an artificial soil in terms of nutrient cycling. This was carried out in three phases:

Firstly, soils from the Humid Tropics and Outdoor biomes were sampled and examined, using a range of analytical techniques, to determine the nutrient characteristics of the established artificial soils from across the Eden Project site. This demonstrated that many of the nutrient concentrations of the artificial soils were consistent with those reported for naturally formed soils within comparable environments. All soil samples were of sandy loam texture (ISO 14688-1), with the sand-sized fraction representing > 50 % of the particle size composition. Statistical analyses suggested that management practices had a greater impact on the nutrient characteristics of artificial soils than environmental conditions.

Secondly, an artificial soil was produced, following the Eden Project protocol, to examine its performance under controlled environmental conditions. This was packed into 4 columns (1 m height by 110 mm diameter), maintained at 15 ^{o}C and subjected to an irrigation regime (delivering 0.14 mL cm^{-2} 18.2 MΩ cm^{-1} water) for 52 weeks. Following 26 weeks of irrigation, 2 of the 4 columns were fertilised. Leachate was analysed for dissolved constituents as were solid samples of the fresh soil and of soil samples collected from the columns following 52 weeks irrigation.

Leachate concentrations for all nutrients, excepting phosphate, were observed to decline over the irrigation period. Leached phosphate concentrations increased from weeks 0 to 2, and then remained relatively constant. Low nitrogen concentrations within the leachate from weeks 2 to 38 were caused by nitrogen immobilisation within the soil, whilst subsequent mineralisation resulted in increased concentrations from Week 38. Analyses of solid phase constituents determined little variation with depth. Fertiliser application demonstrated a significant ($p < 0.05$) increase in leachate concentrations for some dissolved organic nitrogen and nitrate, phosphate, magnesium and calcium and a decrease in pH. Fertiliser application observations showed less prominent differences for the extracted and solid phase constituents.

Thirdly, biochar was applied to the artificial soil at three concentrations (10 %, 5 % and 2 %) plus a control (0 %), to determine whether biochar application may improve nutrient characteristics of artificial soils. The biochar amended soils were packed into mesocosms and maintained at 15 ^{o}C for 6 weeks. In general, leachate analyses demonstrated a decrease in nutrient losses to leaching with increasing biochar concentration, highlighting the potential for improved nutrient retention within the soils.

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PRESENTATIONS

H.K. Schofield, M.F. Fitzsimons, A.D. Tappin, T. Pettitt, G.K. Rollinson, (2014). Analysis of the nutrient content of an artificial soil, Biogeochemistry Research Centre, 6th Annual Conference, Plymouth University, UK (Oral presentation).

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
BC	Biochar content (%) (Chapter 5)
C : N ratio	Carbon to nitrogen ratio
CEC	Cation exchange capacity
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DIP	Dissolved inorganic phosphorus
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
Eh	Redox potential
EOC	Extracted organic carbon
EON	Extracted organic nitrogen
F	Fertilised – in reference to soil columns (Chapter 4)
HPW	High purity water (Millipore system, resistivity 18.2 MΩ cm^{-1}
LOD	Limit of detection
MSDS	Materials safety data sheet
NH_4^+	Ammonium
NO_2^-	Nitrite
NO_3^-	Nitrate
PCC	Pearson correlation coefficient
PO_4^{3-}	Phosphate
POC	Particulate organic carbon
SOC	Soil organic carbon
SOM	Soil organic matter
TDN	Total dissolved nitrogen
TEN	Total extracted nitrogen
TPC	Total particulate carbon
TPN	Total particulate nitrogen
UF	Unfertilised – in reference to the soil columns (Chapter 4)
WHC	Water holding capacity

CHAPTER 1

Introduction

1.1 Overview

The Eden Project is an environmental and educational project located in a disused china clay pit in Cornwall, SW England. It opened in 2001 and has since become one of the UK's leading visitor attractions. It is split into three ecological zones, or Biomes, namely the Humid Tropics, Mediterranean and Outdoor Biomes. The Eden Project designed and constructed artificial soils, from horticultural grit, lignite clay, composted green waste and bark, and used these to support plant growth within the Biomes. The recipe has had a large measure of success as approximately 80,000 tonnes have been deployed onsite. However, inconsistent environmental conditions, plantings and fertiliser additions have led to variable soil performance with respect to nutrient retention.

There are 17 identified essential soil nutrients. These may be classified as either macronutrients or micronutrients, based upon the quantities required by plants. Nitrogen, phosphorus and potassium are often identified as key nutrients for study within soil science because they are required in the largest quantities by plants. As such, they are commonly the limiting nutrients.

Soil conditions, such as temperature, moisture content, pH, cation exchange capacity, texture, water holding capacity and biological community composition, are viewed to have a large impact upon nutrient cycling. As a result they are key factors to consider in the design and execution of nutrient studies.

1.2 Background

1.2.1 The Eden Project

The Eden Project (EP) is an environmental and educational project, built in a previously disused china-clay pit in Cornwall, England. The site is split into three climatic and ecologically defined areas, referred to as biomes; the Humid Tropics (Rainforest), Warm Temperate (Mediterranean) and Outdoor Biomes (Figure 1.1). Each biome houses a diverse ecosystem, containing thousands of plant species and a wide variety of birds, lizards and frogs from across the world. Since its opening in March 2001, the management practices at the Eden Project have changed little and it is therefore necessary to review and assess aspects of the site to ensure best practice. This project focuses upon improving the understanding of the site's soils.

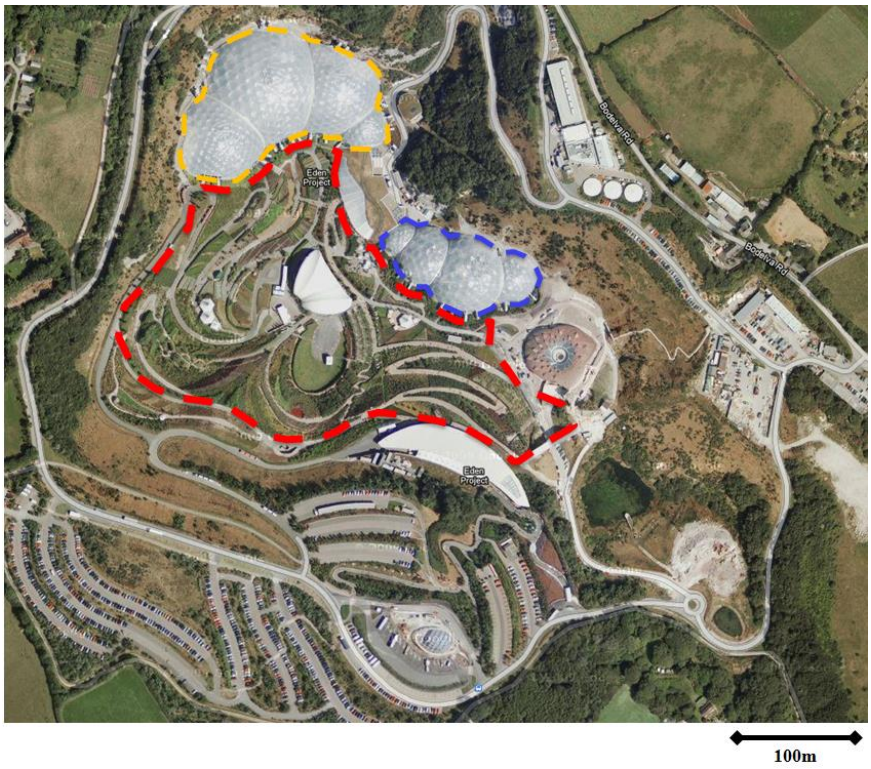

***Figure 1.1**: View of the Eden Project: The Outdoor Biome is highlighted in orange, Humid Tropics Biome in yellow and the Mediterranean Biome in blue (Google Maps, 2012).*

At the time of acquisition the site contained no suitable natural soil. Sourcing and transporting sufficient quantities of natural soils, combined with potential problems such as inconsistency of quality and weed and disease problems, meant that this option was unfeasible. Thus the Eden Project developed its own protocol to prepare approximately 80,000 tonnes of soil for application across the site.

The focus of this work was on soils within the Outdoor and Humid Tropics biomes. The Humid Tropics biome is the larger of the two greenhouse structures, covering an area of 1.5 ha and features tree and plant species from the across world's tropical regions. The temperature within the Humid Tropics biome ranges from 18 to 35 ^{o}C, while a network of misting sprays and a 10 m high waterfall maintain the humidity at approximately 90 % at night, and 60 % during the day. Ground level irrigation pipes provide drip irrigation across the biome.

The Outdoor biome surrounds the two indoor biomes and contains a range of plants from temperate regions of the world, including Europe, the Americas and parts of Asia (Eden-Project, 2009). The Outdoor biome is irrigated predominantly by rainfall, with sprinkler and irrigation systems employed during times of low rainfall.

1.2.2 Artificial soils

Artificial soils are constructed from a mixture of materials with the intention of replicating the properties of a natural soil, whilst bypassing the long timescale associated with natural soil formation. With global soil supplies diminishing, there are a range of negative consequences, including the loss of agricultural productivity and increased release of greenhouse gases. The development of effective artificial soils

offers an alternative to the detrimental removal of natural topsoils, serving to reduce the pressure on this finite resource.

The understanding and knowledge of natural soils has reached a level which allows for the development of artificial soils as a means of alleviating issues associated with the degradation of natural soils. As a result, artificial soils offer the potential for soils to be designed and produced with a wide variety of characteristics to fulfil a range of functions.

Laboratory-based toxicity protocols such as those produced by the Organisation for Economic Co-operation and Development (OECD) and the International Organisation for Standardisation (ISO) use specific artificial soil compositions to minimise the impact of variable soil properties on results and promote precision and comparability between studies. De Silva and van Gestel (2009) reported that certain compositions incorporate components which are not widely available, such as sphagnum peat (which is becoming increasingly scarce) and proposed coco peat as a viable alternative. A significant complication is that, whilst the amount of each compound used for preparation is precisely defined, specific properties of the constituents (kaolin clay and quartz sand) are only briefly defined and may vary between suppliers, leading to variation between soils.

Artificial soils may also be employed in instances where soil has been lost from a site through processes such as degradation or removed due to contamination. In such instances it is common for lost soil to be replaced with translocated natural topsoil, which can be expensive and may result in detrimental environmental consequences associated with land use change. The Eden Project soils represent an important example

of the effective implementation of artificial soil in a large scale land remediation project.

The Eden Project soil was produced from locally-sourced raw materials, the main components were: horticultural grit (a mixture of graded sand 2 to 5 mm with 33 % fine particles, < 2 mm, when oven dried and ungraded sand 2 to 5 mm with 65 % fine particles, < 2 mm when oven dried), lignite clay (a carbonaceous sedimentary rock containing a large fraction of decomposed vegetation), composted bark and composted green waste. Earthworms were also added to the artificial soils around the site to encourage mixing and aeration within the soil (Whitbread-Abrutat, 2004). The resulting Eden Project soils contained a large sand fraction so the focus of this introduction is on soils with sandy texture.

The artificial soil compositions reported for the OECD and ISO protocol for use in toxicology studies, and the Eden Project soils, are shown in Table 1.1. When compared to the OECD and ISO soils, the Eden Project soil has a larger organic matter fraction (10 % and 65 %, respectively) whilst the clay-sized particle fraction is greater within the OECD and ISO soils. It would not have been viable for the Eden Project to employ locally sourced materials were it to replicate the soils outlined by the OECD and ISO protocol. Further to this, the use of sphagnum peat may have led to the introduction of unwanted weed seeds into the soil mix, creating problems within the biomes, It should also be acknowledged that these soils were designed for different purposes, as highlighted by the differences in composition, and would be expected to perform differently.

Table 1.1: *Composition of OECD and ISO artificial soil compared to the Eden Project soil composition.*

Artificial soil	Composition
OECD (1984) and ISO (11268-1, 2012)	10.0 % sphagnum peat 20.0 % kaolin clay ~ 69.0 % quartz sand ~ 1.00 % $CaCO_3$ (adjusting to pH ~ 6.0)
Eden Project topsoil	32.5 % composted green waste 32.5 % bark 25.0 % horticultural grit 10.0 % lignite clay

Whilst artificial soils have been employed in the study of toxicology (De Silva and van Gestel, 2009; Ellis et al., 2007; Saint-Denis et al., 2001; Stabnikova et al., 2005) and waste efficacy studies (Belyaeva and Haynes, 2009; Dayton et al., 2010; Stabnikova et al., 2005), few have characterised an established artificial soil or measured nutrient retention. The work presented in this thesis represents a primary study to directly observe the characteristics of an artificial soil with a view to its performance and potential for improvement.

As an emerging technology, artificial soils have, to date, not been extensively studied; this therefore leaves large scope for their research. In order to select a focus for research the Eden Project was consulted and upon finding that large quantities of fertiliser were required in order to sustain the plant population within the Biomes it was decided that nutrient retention and storage characteristics would serve as the focus for the project.

The Eden Project soils represent an early example of the large scale implementation of artificial soils, as such; their performance is particularly relevant as an indicator of how more recently produced artificial soils may perform over a similar timescale. Since their time of installation, the Eden Project soils have not been the subject of any detailed study. The conclusions and recommendations drawn from this study may be used to

inform management practices for other existing artificial soils and also for the development of future soil compositions.

1.3 Essential soil nutrients

The nutrient supply within a soil governs the efficacy of nutrient acquisition by plants. A soil is subjected to a number of pressures during the process of plant growth. One of the most significant of the pressures is the demand for nutrients. There are 17 essential elements, which are required for healthy plant growth. Carbon (C), hydrogen (H) and oxygen (O) are taken-up from atmospheric carbon dioxide (CO_2) and water (H_2O), whilst the other 13 nutrients are taken-up from the soil and are usually grouped as primary macronutrients (nitrogen, phosphorus and potassium), secondary macronutrients (calcium, magnesium and sulphur) and micronutrients (copper, iron, manganese, nickel, zinc, boron, chlorine and molybdenum) (Brady and Weil, 2008).

Macronutrients are required by plants in relatively large amounts and represent > 0.1 % of dried plant tissue, may be further divided into primary and secondary nutrient groups (Table 1.2). The primary nutrients – nitrogen (N), phosphorus (P) and potassium (K) - are consumed by plants in the largest quantities, whereas the secondary nutrients – calcium (Ca), magnesium (Mg) and sulphur (S) - are required in smaller amounts. Micronutrients – copper (Cu), iron (Fe), Manganese (Mn), Nickel (Ni), Zinc (Zn), Boron (B), Chlorine (Cl) and Molybdenum (Mo) - are required in trace amounts, found to represent < 0.1 % dried plant tissue.

Table 1.2: *Essential nutrients present within soils (Brady and Weil, 2010; White and Greenwood, 2012)*

Macronutrients (> 0.1 % of dry plant tissue)			**Micronutrients (< 0.1 % of dry plant tissue)**
Non-mineral	**Primary**	**Secondary**	
Mostly from air and water	Mostly from soil solids	Mostly from soil solids	From soil solids
Carbon (CO_2) Hydrogen (H_2O) Oxygen (O_2)	***Cations:*** Nitrogen (NH_4^+) Potassium (K^+)	***Cations:*** Calcium (Ca^{2+}) Magnesium (Mg^{2+})	***Cations:*** Copper (Cu^+, Cu^{2+}, Cu chelates) Iron (Fe^{2+}, Fe^{3+} chelates) Manganese (Mn^{2+}, Mn chelates) Nickel (Ni^{2+}, Ni chelates) Zinc (Zn^{2+}, Zn chelates)
	Anions: Nitrogen (NO_3^-) Phosphorous ($H_2PO_4^-$, HPO_4^{2-})	***Anions:*** Sulphur (SO_4^{2-})	***Anions:*** Boron (H_3BO_3, $H_4BO_4^-$) Chlorine (Cl^-) Molybdenum (MoO_4^{2-})

1.3.1 Properties affecting nutrient characteristics

Nutrient availability in soils is characterised by complex interactions between the nutrients and other chemical, physical and biological components of a soil. The balance between the essential nutrients within a soil is important. If more than one nutrient is deficient, the effect of supplying only one of the nutrients will have limited benefit and possibly be harmful (Davies et al., 1993). The possibilities for increasing soil productivity are often constrained by the supply of nutrients, in particular N and P (Spiro and Stigliani, 1996).

The quantity of any given nutrient within a soil is a balance between inputs and outputs. There are a number of ways through which the soil system gains and loses nutrients. Nutrient losses are costly and wasteful, and can be a source of environmental contamination when they reach lakes, rivers and groundwater. Sources of these soluble nutrients in soil, and the ways in which they can be lost, are shown in Table 1.3.

Many important soil chemical properties are controlled by reactions between the soil solution and particle surfaces (Rowell, 1994). Soil solution contains low concentrations of a range of cations, anions and organic molecules (Brady and Weil, 2008). There is a dynamic interface between the soil solution and soil particle surfaces, with the main processes being dissolution and precipitation of salts and minerals and adsorption and desorption on the surfaces of clay and organic matter (Rowell, 1994). Therefore the composition of the soil solution is subject to continuous changes dependent upon temperature, plant uptake of nutrients, wetting, drying and mineralisation of organic matter (Rowell, 1994).

Table 1.3: *Processes contributing to nutrient loss and gain in soils (adapted from Bierman and Rosen (2005).*

Nutrient Gain	Nutrient Loss
Decomposition of plant residues, animal remains and soil micro-organisms.	**Runoff** where dissolved nutrients are lost in water moving across the soil surface.
Organic amendments such as manures, composts, bio-solids, kelp etc.	**Erosion** through wind or water movement removing soil particles from the land surface.
Ground rock products including lime, rock phosphate and greensand.	**Leaching** where dissolved nutrients are lost to groundwater or through field drains.
Inorganic **industrial by-products** such as wood ash or coal ash.	**Gaseous losses** to the atmosphere (primarily losses of different N forms)
N-fixation by legumes.	Crop removal from the soil during **harvesting**.
Weathering of soil minerals.	
Fertiliser applications.	
Atmospheric deposition such as N and S from acid rain or N-fixation by lightning discharges.	
Deposition of nutrient-rich sediment from erosion and flooding	

1.3.1.1 Climatic conditions

Climatic conditions can affect soil nutrient concentrations, with plant-availability and plant tolerance to differing conditions varying significantly. Moisture within an unsaturated soil is influenced by water exchange with both the atmosphere and groundwater, through precipitation, evaporation and transpiration, and is essential in

processes which affect soil ecosystem dynamics and biogeochemical cycles (Chen and Hu, 2004). Low moisture availability within a soil can lead to the plant being unable to absorb water from a soil, leading to wilting.

Microbial populations within the soil are significantly affected by climatic conditions, increasing with increasing temperature and moisture. The rate of soil organic matter decomposition therefore increases with increasing temperature and moisture (Brady and Weil, 2008).

1.3.1.2 Physicochemical properties

1.3.1.2.1 pH

The pH of a soil affects the chemical reactions taking place within, and significantly influences the plant-availability of nutrients (Sims, 1986). As shown in Figure 1.2, nutrient availability varies with pH, and optimal nutrient availability is observed at pH 5.5 to 7.0 (Brady and Weil, 2008). Nutrient uptake may alter soil pH; for example, the form of N used by the plants. N can be adsorbed as either a cation or anion, for each cation that is absorbed by the plant, an H^+ is released into the soil solution, which over time lowers the pH (Mattson et al., 2009). When anions are absorbed by the plant, an OH^- is released into the soil solution, which may promote higher pH values. Over time the H^+ react with the OH^- to form water (H_2O) giving more neutral pH (Lambers et al., 1998; Mattson et al., 2009). The breakdown of soil organic matter also lowers the pH through release of organic acids into the soil solution. Many soils possess a buffering capacity, which minimises the impact of such releases and serves to maintain healthy plant growth conditions (Brady and Weil, 2008).

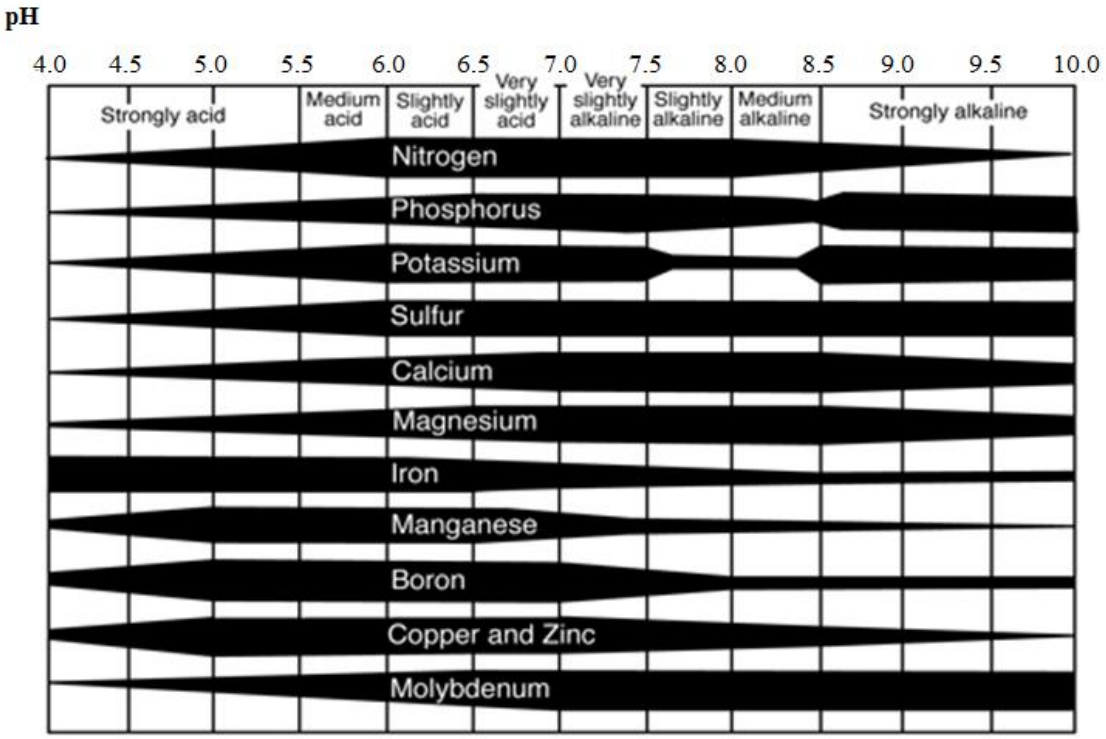

Figure 1.2: *Nutrient availability for plant use as influenced by soil pH for a mineral soil. Bar width represents the proportion which is available to plants. Adapted from Brady and Weil (2008).*

1.3.1.2.2 Surface charges

The surfaces of soil clay minerals and organic matter particles have associated electrical charges (Rowell, 1994). These charges may be either positive, which will retain anions, or negative, which will retain cations. Cations and anions retained electrostatically are easily exchanged with cations and anions in the soil solution. Thus, a soil with a higher cation exchange capacity (CEC) or anion exchange capacity (AEC) has a greater capacity to maintain adequate nutrient quantities (White and Greenwood, 2013).

Sandy mineral soils tend to have a low number of cation exchange sites because they contain fewer clay minerals and organic matter surfaces. This commonly results in a lower nutrient retention than in clay or highly organic soils (Ross and Ketterings, 1995). The electrical charges on some clay mineral and organic matter particles change with

pH, being mainly positive at low pH (increased anion retention) and mainly negative at high pH (increased cation retention) (Rowell, 1994).

The CEC of a soil defines its capacity to adsorb and exchange all types of cations by electrostatic forces (Ross and Ketterings, 1995; White and Greenwood, 2013). These cations are termed exchangeable and those most commonly considered are K, Ca, Mg and NH_4^+. The Na content increases at higher pH and exchangeable Al and H^+ concentrations increase at lower pH (Rowell, 1994). The CEC is an important soil property because of the influence it has over the extent to which soils can meet plant demands (White and Greenwood, 2013). When combined with other measures of soil fertility, CEC is a good indicator of soil quality and productivity (Ross and Ketterings, 1995).

1.3.1.2.3 Texture

Soil texture is determined by particle size distribution and has a significant impact upon soil nutrient retention and storage properties. Soil particles may be comprised of either rock fragments or combinations of inorganic and organic materials cemented together in a porous structure (Barber, 1995). Soil texture determines the surface area of a soil, which has a significant influence on soil properties. Soils composed of mostly clay particles have a larger surface area than soils composed of mostly sand-sized particles (Brady and Weil, 2008). Greater surface area presents a larger number of charged surfaces (cation and anion exchange sites) within the soil, promoting greater nutrient retention and storage (Brady and Weil, 2008).

1.3.1.2.4 Structure

Soil structure regulates a number of functions including water infiltration, percolation and retention, gas exchange, soil organic matter and mineral nutrient dynamics, plant root penetration, soil microbial diversity and activity and susceptibility to soil erosion (Bottinelli et al., 2015). The structure of a soil is influenced by a number of variables: parent material, topography, climate, land use management and biological activity (Bottinelli et al., 2015).

1.3.1.2.5 Water holding capacity

Water holding capacity refers to the amount of water a soil can hold against the force of gravity (Acharya et al., 2014; Milly and Dunne, 1994) and is heavily influenced by the structure, texture, depth and stoniness of a soil (Rowell, 1994). A soil with a low water holding capacity is more likely to lose significant quantities of nutrients to leaching.

1.3.1.3 Biological properties

Plant roots, microbes and animals that makeup the soil biological community produce intracellular and extracellular enzymes, which are responsible for much of the biogeochemical cycling within the soil (Killham, 1994). Many of the main cellular components are composed of essential nutrients, sorbed from the soil as simple inorganic compounds and then converted to organic constituents within the cell (Killham, 1994). The death and decomposition of organisms and their tissues then results in the release of inorganic ions to the soil, where the cycle is repeated.

Within a soil ecosystem stability and resilience are encouraged by the presence of multiple organisms, which carry out each required task within the soil (Brady and Weil, 2008). In general, it is observed that the abundance and diversity of the soil faunal

community is greatest in soils with minimal disturbance, such as those under permanent grassland and natural woodland (Killham, 1994).

Along with the direct impact on nutrient cycling, fauna also have indirect effects on the soil. Macrofauna (soil invertebrates larger than 2 mm), such as earthworms, termites and ants, are considered to play an important role in controlling soil structure dynamics through incorporation of organic material into the soil, which significantly impacts soil porosity and aggregate stability and, therefore, nutrient availability (Bottinelli et al., 2015; Tisdall and Oades, 1982).

The rate of biological productivity in soils is largely affected by water availability and temperature (Austin et al., 2004; Schwinning and Sala, 2004). As such, climate has a significant effect on soil biogeochemical cycles.

1.3.2 Nitrogen

Nitrogen (N) is a constituent of a number of plant components, including all proteins, nucleic acids, and chlorophyll (Brady and Weil, 2010; Russell, 1973). Soil N changes form as a result of the activities of plants and microorganisms (Rowell, 1994). The principal pools and forms of N, and the processes by which they interact within the N cycle, are described below and illustrated in Figure 1.3.

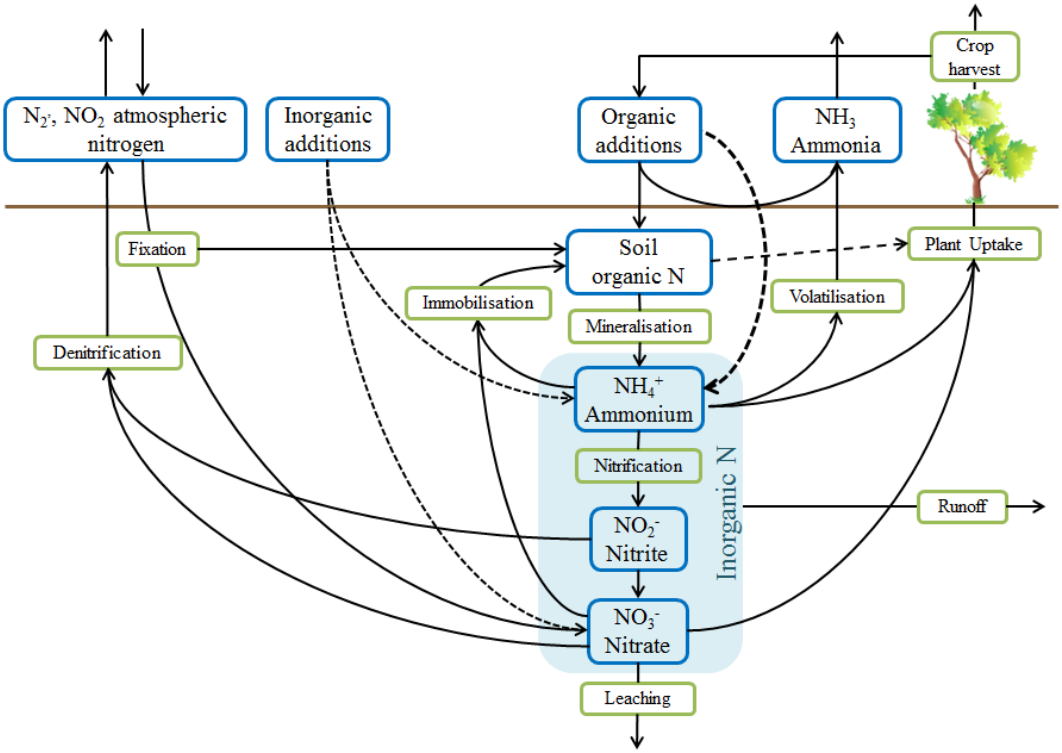

Figure 1.3: *Essential components of the nitrogen cycle (adapted from Cresser et al. (1993) and Rowell, (1994)).*

1.3.2.1 Nitrogen transformations within the soil

The inorganic nitrate (NO_3^-) and ammonium (NH_4^+) ions are the principal forms in which N is taken up from the soil by plant roots (Brady and Weil, 2010; Eshani et al., 1999), though plants may also sorb low molecular weight dissolved organic nitrogen (DON) compounds, such as amino acids and peptides (Burton et al., 2007; Chapin et al., 1993; Farrell et al., 2013; Hill et al., 2011; Jones et al., 2005a). The uptake of NH_4^+ raises the pH of the rhizosphere soil, whilst the uptake of NO_3^- lowers it (Brady and Weil, 2010). These pH changes influence the uptake of other ions such as heavy metals, phosphate (PO_4^{3-}) and micronutrients (Dijkshoorn et al., 1983).

1.3.2.1.1 Fixation

Soil nitrogen is primarily derived from the N_2 present within the atmosphere (Brady and Weil, 2010; Rowell, 1994). The N_2 molecule is very stable (bond energy of 945 kJ

mole^{-1}), which means that N_2 is not generally directly available for plant uptake and must first be converted to another form (Brady and Weil, 2010). Nitrogen fixation requires the destruction of the triple bond and is carried out by specialist soil micro-organisms (both free-living and symbiotically associated with plants) and by lightning (Brady and Weil, 2010; Rowell, 1994).

1.3.2.1.2 Nitrification

The process of nitrification involves the transformation of NH_4^+ to NO_2^- and then NO_3^- by autotrophic nitrifying soil bacteria (Brady and Weil, 2010). Under favourable conditions the nitrification process will occur in two stages: (1) NH_4^+ is converted to NO_2^- by *Nitrosomonas* bacteria and (2) the NO_2^- is then immediately acted upon by a *Nitrobacter* bacteria, producing NO_3^- (Brady and Weil, 2010). The enzyme oxidation process releases energy for the bacteria as shown in equations 1.1a and 1.1b.

$$\underset{Ammonium}{NH_4^+} + 1\tfrac{1}{2}O_2 \xrightarrow{Nitrosomonas\ bacteria} \underset{Nitrite}{NO_2^-} + 2H^+ + H_2O + energy \qquad \textbf{(1.1a)}$$

$$\underset{Nitrite}{NO_2^-} + \tfrac{1}{2}O_2 \xrightarrow{Nitrobacter\ bacteria} \underset{Nitrate}{NO_3^-} + energy \qquad \textbf{(1.1b)}$$

1.3.2.1.3 Immobilisation

Inorganic N ions are converted to organic forms through immobilisation (Equation 1.2). Immobilisation may occur through both biotic and abiotic processes (Brady and Weil, 2010). Biotic immobilisation occurs during the metabolism of organic residues, such as amines ($R–NH_2$), where microorganisms require more N than can be obtained from the decomposing residues, this causes them to scavenge NO_3^- and NH_4^+ from the soil solution (Brady and Weil, 2010), which are then temporarily bound within the soil

microorganisms, to be returned to the soil through mineralisation following the death of the microorganisms (Johnson et al., 2005).

$$R-NH_2 \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} OH^- + R-OH + NH_4^+ \underset{-O_2}{\overset{+O_2}{\rightleftharpoons}} 4H^+ + energy + NO_2^- \underset{-\frac{1}{2}O_2}{\overset{+\frac{1}{2}O_2}{\rightleftharpoons}} energy + NO_3^- \quad \textbf{(1.2)}$$

$$\overset{Mineralisation}{\longrightarrow}$$
$$\overset{Immobilisation}{\longleftarrow}$$

Studies have shown abiotic immobilisation to be most common in forest soils (Colman et al., 2007). Little is known about abiotic immobilisation processes, though it is thought that rapid chemical reactions involving compounds with high C : N ratios are involved (Brady and Weil, 2010). It has been hypothesised, by Colman et al. (2007), that abiotic immobilisation involves the incorporation of NO_3^- and NH_4^+ into soil organic matter (SOM), where it is immobilised as organic nitrogen. Under reduced concentrations of O_2, denitrifying bacteria may also produce some NO and N_2O, which are potent greenhouse gases (Brady and Weil, 2010; Butterbach-Bahl et al., 2002).

The amount and composition of organic N within a soil may be influenced by both biological and abiotic factors, including soil type, quantity and quality of organic matter input, microbial communities, management practices and environmental conditions (Burton et al., 2007). Within many soils, organic N comprises > 90 % of the total N pool (Barbarick, 2006; Brady and Weil, 2008; Fagotti et al., 2012; Haque et al., 2007). Through the process of mineralisation, organic N is converted to the more easily accessed NH_4^+ and NO_3^-.

1.3.2.1.4 Mineralisation

Mineralisation is the reverse of the immobilisation process shown in Equation 1.2 (Brady and Weil, 2008), where organic N contained within the SOM is converted into plant available inorganic forms by the heterotrophic activities of soil microorganisms (Brady and Weil, 2008; Haygarth et al., 2013). Under favourable conditions the second part of the reaction follows the first closely enough to prevent the accumulation of the plant-toxic NO_2^- (Brady and Weil, 2010).

1.3.2.1.5 Denitrification

Where soil oxygen levels are insufficient, denitrification will occur. This is where microorganisms utilise oxygen from NO_3^-, and through this process NO_3^- is rapidly converted to $NO_{(g)}$, $N_2O_{(g)}$ and $N_{2(g)}$ and released into the atmosphere (Equation 1.3) (Barbarick, 2006; Brady and Weil, 2010). Denitrification causes soil N to become unavailable to plants and under poorly aerated conditions this transformation can result in significant loss of NO_3^- from the soil (Barbarick, 2006).

$$\underset{Nitrate}{2NO_3^-} \xrightarrow{-2O} \underset{Nitrite}{2NO_2^-} \xrightarrow{-2O} \underset{Nitric\ oxide}{NO\uparrow} \xrightarrow{-O} \underset{Nitrous\ oxide}{N_2O\uparrow} \xrightarrow{-O} \underset{Dinitrogen}{N_2\uparrow} \quad \textbf{(1.3)}$$

1.3.2.1.6 Ammonia volatilisation

Ammonia (NH_3) volatilisation occurs as a result of the conversion of NH_4^+ to gaseous NH_3, which is released into the atmosphere (Equation 1.4) (Johnson et al., 2005). NH_3 volatilisation is greatest when urea (CH_4N_2O) fertiliser is applied to a soil, but its movement into the soil is restricted (Rawluk et al., 2001). Conditions that affect NH_3 volatilisation include temperature and moisture levels, pH and CEC (Brady and Weil,

2010). The NH_3 gas is in equilibrium with dissolved NH_4^+ according to Equation 1.4 (Brady and Weil, 2010).

$$\underset{Dissolved\ ions}{NH_4^+ + OH^-} \rightleftharpoons \underset{Gas}{H_2O + NH_3 \uparrow} \quad \textbf{(1.4)}$$

1.3.2.2 Nitrogen fertilisation

In crop-based plant systems, N demand frequently exceeds supply. Therefore, in order to promote healthy plant growth and maintain an adequate yield, it is common for fertilisers containing N to be applied to the soils. A range of materials are employed as N fertilisers, with urea (CH_4N_2O) the most widely used in agriculture owing to its high N content and low cost (Taufik et al., 2011). In order to ensure maximum efficiency and yield returns, application of fertilisers must be synchronised with crop uptake (Delin and Engström, 2010).

In circumstances where organic materials with a high C : N ratio, such as wood, are added to a soil, a decrease in the plant available N pool may be observed (Fog, 1988). Within such materials there are large quantities of degradable carbon compounds; however, N is not available for microorganisms to utilise (Jackson and Wright, 2009). This leads the microorganisms to draw on the N within the soil solution pool, diminishing plant-available N supplies (Jackson and Wright, 2009).

Nitrate (NO_3^-) is not efficiently retained by soil particles and is the N-form most vulnerable to leaching (Barbarick, 2006). This has an economic cost and is also a water quality concern (Johnson et al., 2005) as it can have environmental (eutrophication) and human health (blue baby syndrome and stomach cancer) consequences.

1.3.3 Phosphorus

Phosphorus (P) is a major constituent of many plant cell components, playing a role in key processes, such as photosynthesis, respiration, energy storage and transfer, cell division and enlargement (Mullins, 2009; Shen et al., 2011).

Soil P is subject to a range of complex chemical and microbiological reactions (Mullins, 2009), as shown in Figure 1.4. Soil P originates from the weathering of primary minerals and from P additions in the form of fertilisers, animal and plant residues and small quantities from atmospheric deposition (Mullins, 2009). Whilst P is one of the most abundant elements on earth, when compared to other primary plant nutrients its plant availability in soil is relatively low under most soil conditions (Brady and Weil, 2008; Hinsinger, 2001). This is because P ions are highly reactive within soil and water systems and so is readily bound and immobilised (Brady and Weil, 2008; Hinsinger, 2001).

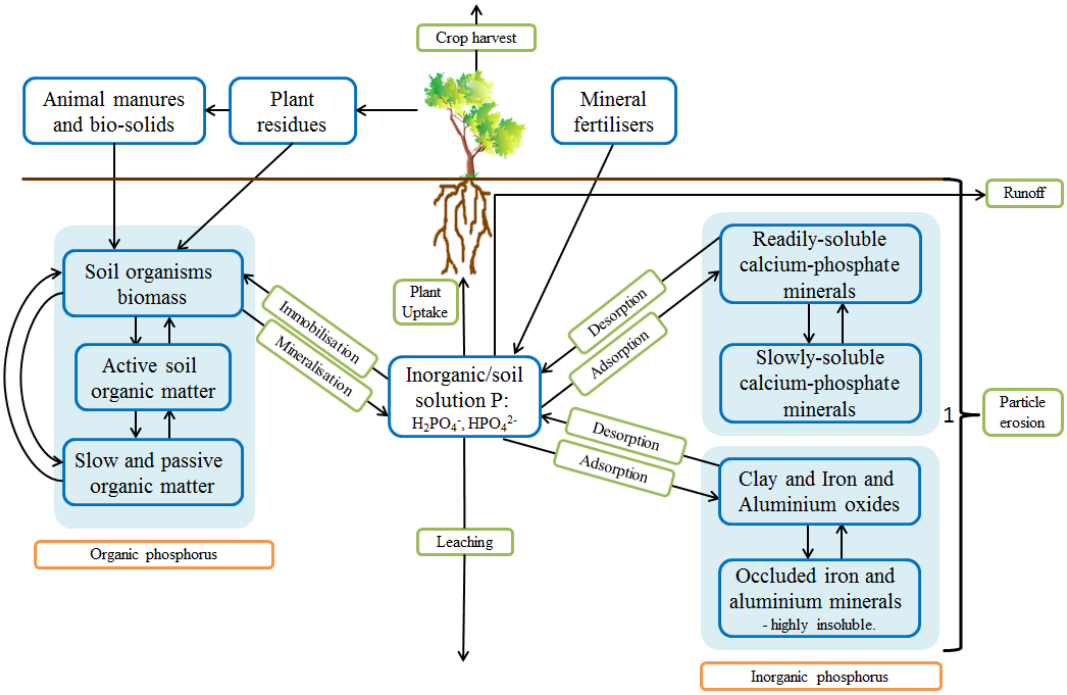

Figure 1.4: *The phosphorus cycle (adapted from Brady and Weil (2008) and Rowell (1994)).*

1.3.3.1 Inorganic phosphorus

Phosphorus (P) in its primary state is found within geological deposits, predominantly as apatite, an insoluble inorganic calcium-phosphate mineral with the formula $Ca_3(PO_4)_2X$, where X may be OH, F or Cl and the Ca may also be substituted for Mn, Sr or Y (Börling, 2003; Gray, 2011). Through the slow process of weathering, P is released into the soil solution (Mullins, 2009), where it is plant available; however, it is also subject to rapid immobilisation and precipitation processes.

Phosphorus (P) is available to plants as dissolved inorganic phosphate (DIP) within the soil solution. Its dominant form is dependent on the localised chemical environment, either $H_2PO_4^-$ in acidic soils, or HPO_4^{2-} in alkaline soils, with a balance in near-neutral soils (Brady and Weil, 2008; Gray, 2011; Rowell, 1994). When the pH is maintained at 6.0 to 7.0, DIP precipitation is at its lowest and therefore plant availability is highest (Brady and Weil, 2010).

Movement of DIP from the soil solution to plant root surfaces is slow. In order to combat this, high root proliferation is found in zones where DIP ions are present and symbiosis occurs between plants and mycorrhizal fungi (Brady and Weil, 2008). The low mobility of inorganic P is due to the reactivity of DIP ions relative to other soil constituents, which also causes high retention (Hinsinger, 2001). As shown in Figure 1.4, DIP may enter the soil solution in a number of ways.

Phosphate ions are easily absorbed to mineral surfaces of which, two groups are important: (1) those containing Ca, (calcite) and (2) those containing Fe and Al (variscite and strengite) (Brady and Weil, 2008). Soil pH has a significant impact on which of these two groups are dominant. At higher pH Ca compounds are stable and

relatively insoluble, so becoming the dominant form. Acidic conditions lead to the dissolution of Ca compounds and their release into the soil solution, leaving Fe and Al compounds as the dominant forms (Brady and Weil, 2008; Gray, 2011; Mullins, 2009). Under both alkaline and acidic conditions, P undergoes reactions, which produce P-containing compounds of decreasing solubility, which are therefore less plant available (Brady and Weil, 2008).

1.3.3.2 Organic phosphorus

Once sorbed by a plant, a fraction of the P becomes part of the plant tissues (Brady and Weil, 2008). As the plants shed leaves and their roots die, or when they are consumed by animals or humans, P is returned to the soil in the form of plant residues, leaf litter and wastes from animals and humans (Brady and Weil, 2008). Organic P typically accounts for 30 to 65 % of total soil P (Turner, 2008).

Organic P, contained within the soil organic matter, is released to the soil solution as DIP, through decomposition and mineralisation, as shown in Equation 1.5 (Hedley et al., 1982; Mullins, 2009). The rate of mineralisation is influenced by soil moisture and temperature, with the highest rates occurring at warmer temperatures, within well drained soils (Brady and Weil, 2008; Hinsinger, 2001). These residues are decomposed by microorganisms, which temporarily immobilise P within their cells, but this is eventually released through mineralisation (Brady and Weil, 2008). Net immobilisation of P is most likely to occur where residues added to the soil have a C : P mass ratio above 300, where P is required by soil microbes for the breakdown of organic matter, while net mineralisation is likely when this ratio is below 200, as the concentration available P is greater than that required by soil microbes (Brady and Weil, 2008).

$$Organic\ P \overset{Microbes}{\rightleftharpoons} \underset{Soluble\ phosphate}{H_2\ PO_4^-} \overset{Fe^{3+}Al^{3+}Ca^{2+}}{\rightleftharpoons} \underset{Insoluble\ fixed\ P}{Fe, Al, Ca\ phosphates} \quad \textbf{(1.5)}$$

$$\xrightarrow{Mineralisation} \quad \xleftarrow{Immobilisation} \qquad \xrightarrow{Adsorption} \quad \xleftarrow{Desorption}$$

1.3.3.3 Phosphorus management

Plant P demand often far exceeds the available supply, making it difficult to achieve high crop yields within agricultural systems (Ohno et al., 2005). In view of this the use of P-containing fertilisers is prevalent. The introduction of soluble P to a soil results in the rapid removal of P from solution (Brady and Weil, 2008), which means that only a small fraction (10 to 15 %) of the P applied in fertilisers and manures is taken up by plants in the year of application (Brady and Weil, 2008). This has led to widespread over-application of P fertilisers, often resulting in the loss of P from the soil to aquatic environments (Pote et al., 1996).

The introduction of P to the aquatic environment can stimulate algal growth, leading to eutrophication and a range of detrimental outcomes. P losses from soils are dependent on the source factors and the transport mechanisms (Börling, 2003; Gburek et al., 2000). This means that fertiliser applications require careful management in order to avoid these detrimental effects.

Phosphorus (P) applied as fertiliser is often sourced through the mining of geological deposits. The minerals are then reacted with acids to bypass the weathering process, with the resultant P applied to the soils as fertiliser. Due to the low solubility and mobility, ore-forming processes are restricted, limiting global P supply and making P a finite resource (Oelkers et al., 2008). This means that P is a finite material, which in

recent years this has led to a significant increase in the cost of P-based fertilisers (Gilbert, 2009).

1.3.4 Carbon and organic matter

Carbon (C) is an essential component for all living organisms. As such, it plays a highly important role within the soil environment. Secondary to the oceans, the terrestrial C reservoir represents a large proportion of global C storage with a capacity over three times larger than the deeper lithosphere and two and a half times larger than the atmosphere (Grotzinger et al., 2006). The interactive processes between the soil, plant and atmosphere are shown in Figure 1.5.

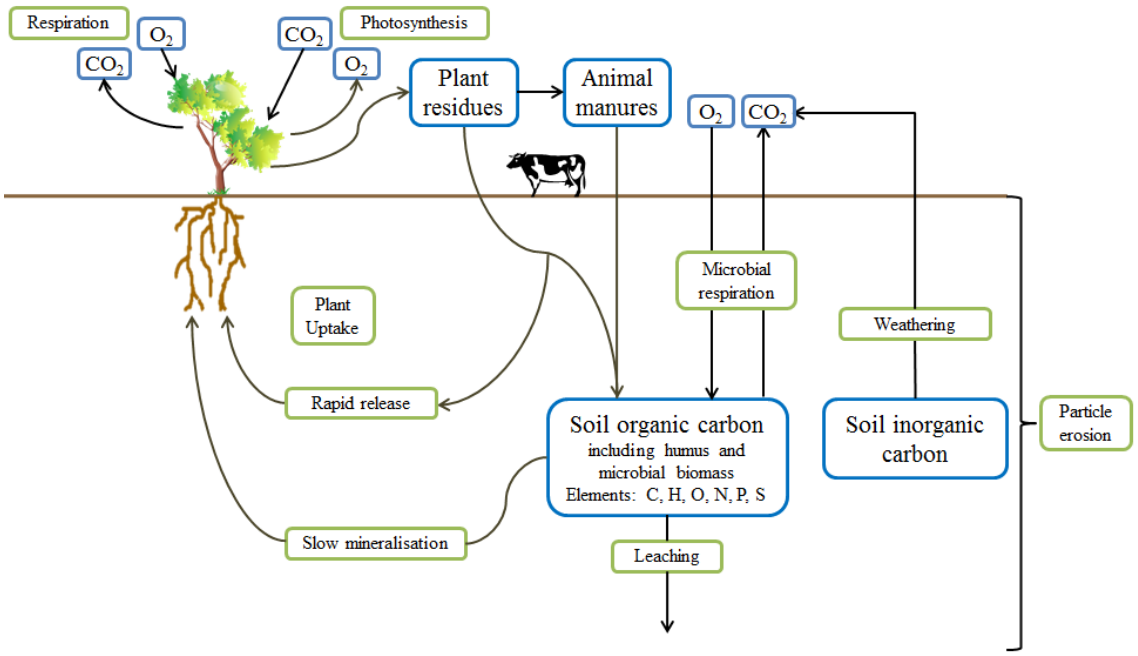

Figure 1.5: *The carbon cycle within the soil environment (adapted from Brady and Weil (2008)).*

There are two types of soil C: inorganic and organic. Soil inorganic C is derived from a reaction between Mg^{2+} and/or Ca^{2+} and carbonic acid in the soil solution, resulting in the formation of carbonates (Brady and Weil, 2008; Sparks, 2003; Wu et al., 2009), such as calcite and dolomite (Lal, 2007), or as calcrete (Walcott et al., 2003). Inorganic

C compounds are an important regulators of atmospheric C dioxide over very long timescales (Walcott et al., 2003), which means that it is relatively stable. Owing to this, soil inorganic C is often overlooked where agricultural production and C sequestration are concerned (Trumbone and Torn, 2003).

Soil organic C (SOC) is a substantial component of SOM, accounting for approximately 50 % of its mass (Brady and Weil, 2008). As such, SOC is commonly used as a measurement of SOM (Walcott et al., 2003). This C fraction of SOM is highly influential with regard to microbial activities within the soil and serves as an important regulator with regard to the mineralisation of other key plant nutrients.

SOM is derived from plant and animal residues at various stages of decomposition, including; cells, tissues of soil organisms and substances synthesised by the soil population (Brady and Weil, 2008). Components of SOM may be subdivided, as shown in Figure 1.6, based on their origin and stage of decay, with their physical, biological and chemical properties influencing soil functions and determining their role as either a sink or source within the global C cycle (Walcott et al., 2003). The living organic matter group is comprised of plants, microbes and animals, which plays an important role in the decomposition of the non-living group and, therefore, nutrient cycling. However, owing to its changeable nature, it is generally disregarded (Walcott et al., 2003).

The major elemental component of SOM is C, though, a range of other nutrients, including N, P, and K, are present within the various constituents. SOM, therefore, acts as a nutrient reservoir within the soil. The rate of SOM decomposition is highly influential with regard to the turnover of plant available nutrients. Within the non-living SOM group, the rate of turnover is highly variable. Historically, non-living organic

materials were sub-divided into fast, slow and passive turnover pools, based on their composition; however, a study by Dungait et al. (2012) suggests that rate of turnover is governed by accessibility.

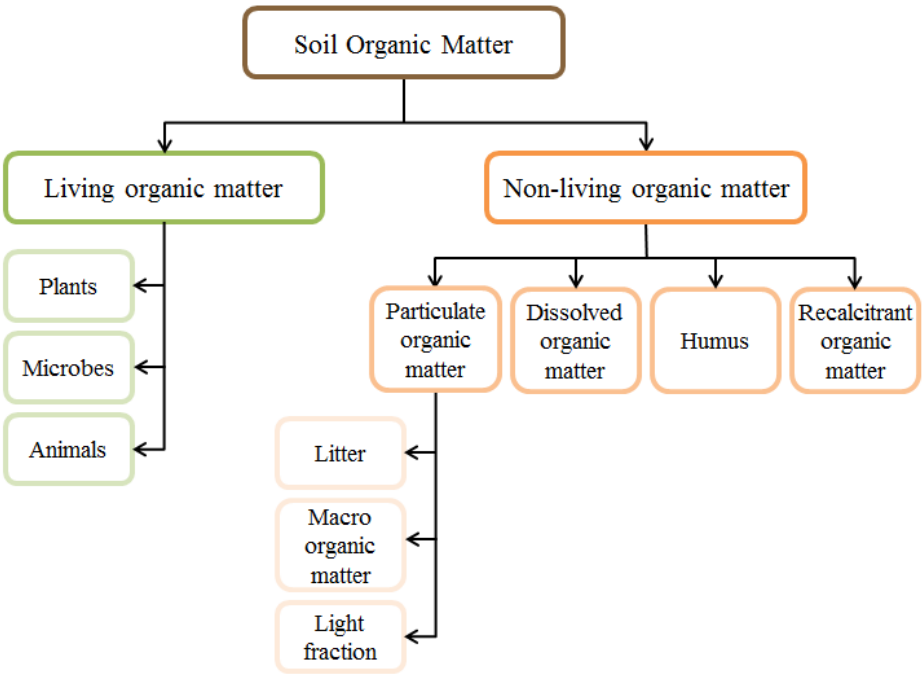

Figure 1.6: *Components of soil organic matter (adapted from Walcott et al. (2003)).*

SOM is a major contributor to the pH buffering capacity of soils (Sparks, 2003) and is also fundamental to the maintenance of soil fertility, serving to affect the mineralisation of key nutrients, the retention of nutrient cations within the soil, structural stability and water holding capacity (Rowell, 1994).

SOM has a net negative surface charge at pH above 3, which increases with increasing pH as a result of dissociation of H^+ from functional groups (Sparks, 2003). This surface charge combined with the large surface area, means that SOM has a greater CEC that clay minerals (Powlson et al., 2013), and may account for up to 80 % of a soil's CEC (Stevenson, 1994). SOM is particularly important within sandy soils, where the clay

content is lower (Sparks, 2003), which serves to reinforce the significance of the role of SOM in the retention of soil nutrients.

1.3.5 Carbon : nitrogen ratio

The breakdown of organic matter by microbes is significantly impacted by the C : N ratio (Hoorman and Islam, 2010). Organic C, is utilised by soil microorganisms, which require C for energy and for building organic compounds (Brady and Weil, 2010). However, the microorganisms also require N for use in the construction of amino-acids, proteins and DNA (Hoorman and Islam, 2010).

The C : N ratio of organic residues within a soil indicates the rate of decomposition and therefore, N availability (Brady and Weil, 2008; Thomas et al., 1998). Typically, the C : N ratio of an organic material applied to a soil will decrease with time (Post et al., 1985). This is because, as decomposition proceeds, easily decomposed material is lost and N is immobilized in microbial biomass and other decay products, leaving behind more recalcitrant material, with slower decomposition rates and lower C : N ratios (Hoorman and Islam, 2010; Post et al., 1985). Factors slowing the decay rate include high lignin content, cool temperatures and poor soil aeration (Post et al., 1985; Waksman, 1936).

For effective breakdown of organic material, soil microbes require 24 g C for every 1 g N (Brady and Weil, 2010). This means that SOM with C : N mass ratios below this are subject to rapid decomposition. The presence of organic matter with a high C : N ratio encourages competition between soil microorganisms (Brady and Weil, 2008), resulting in slower decomposition rates and often causes in the immobilisation of NO_3^- and NH_4^+, depleting the plant available N pool (Brady and Weil, 2010; Russell, 1973).

Where C : N ratio is expressed as a ratio of the content by mass, it is relatively constant for a specific soil, even under a range of management conditions (Russell, 1973). Table 1.4 contains data for soils from different fields at Rothamsted Research, UK. Here, despite a wide range of C contents and land uses the C : N ratio only varies from 8.5 to 12.8.

Table 1.4: *The carbon and nitrogen contents and C:N ratio of some Rothamsted soils (Russell, 1973). The carbon content was been measured using the loss on ignition method and nitrogen by Kjedahl digestion. Data are presented on a dry weight basis.*

Soil	Percent C	Percent N	C:N
Old Woodland 12 – 17 cm	2.38	0.250	9.5
Park Grass, old pasture 0 – 22 cm			
Unfertilised pH approx. 6	3.4	0.28	12.1
Fertilisers and lime pH approx. 7	3.7	0.32	11.6
Fertilisers and no lime pH approx. 5	3.2	0.25	12.8
Subsoil 22 – 45 cm pH approx. 6.5	1.4	0.14	10.0
Broadbalk 0 – 22 cm			
No manure since 1839	0.84	0.099	8.5
Complete fertilisers (annually since 1843)	1.00	0.115	8.7
35 t ha^{-1} farmyard manure (annually since 1843)	2.59	0.251	10.3

1.3.6 Potassium

Potassium (K) is the third most likely nutrient, following N and P, to limit plant productivity (Brady and Weil, 2008). Within plants, K is not incorporated into any cell structures; rather it remains within solution in cells or acts as an activator for a large number of enzymes. As such, it is essential for a wide range of plant processes (Brady and Weil, 2008). The cycling and availability of K (Figure 1.7) within soils is dynamic and readily influenced by management practices.

The K content of soils is predominantly derived from primary minerals, such as micas (biotite and muscovite) and K feldspar (orthoclase and microcline) (Brady and Weil, 2008). The weathering of these minerals releases K into soil solution.

There are four forms of K present in soils: (1) within the crystalline structure of primary minerals, (2) in non-exchangeable positions in secondary minerals, (3) in exchangeable form on soil colloid surfaces and (4) ions within soil solution (Brady and Weil, 2008; White and Greenwood, 2013). All plants are able to access dissolved and exchangeable K within the soil solution and on the colloid surfaces. However, the availability of K from the primary and secondary mineral structures is dependent upon the plant species (Brady and Weil, 2008).

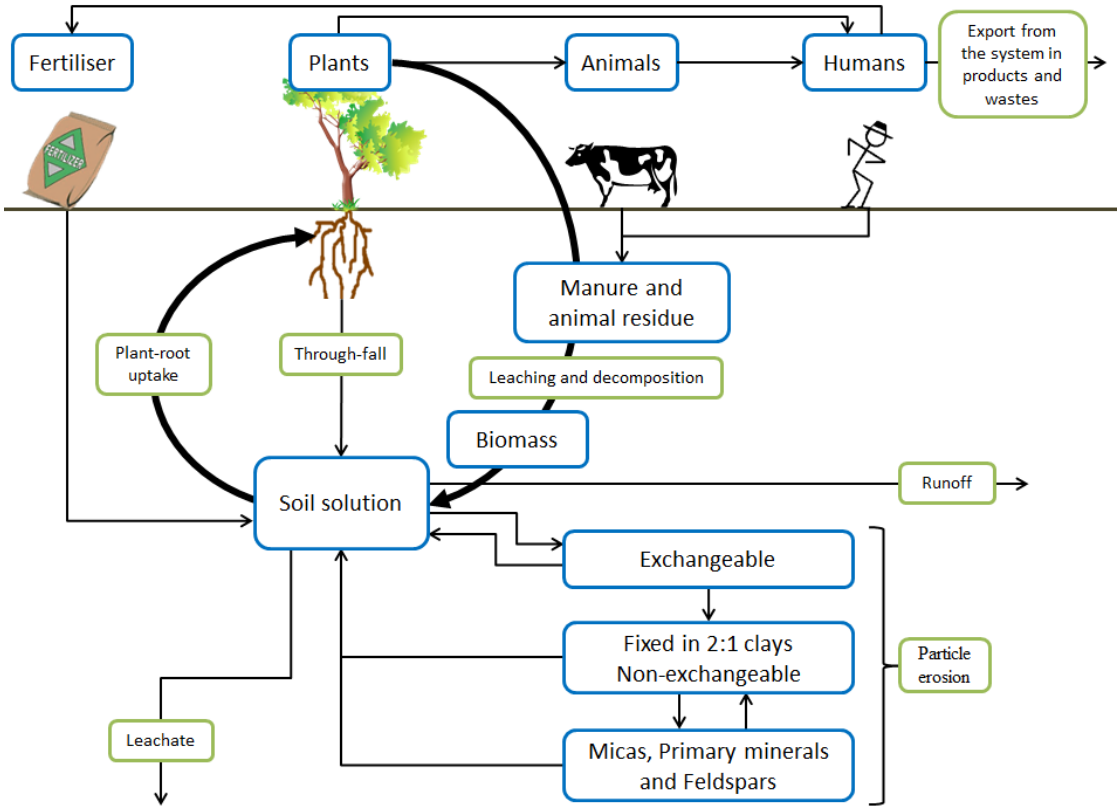

Figure 1.7: *The potassium cycle in soils (adapted from Brady and Weil (2008)).*

Sources of K include plant residues, composts, animal manures and minerals. A small portion of this K may also be leached from plant foliage by rainwater and returned to the soil solution (Brady and Weil, 2008; White and Greenwood, 2013). As K is highly mobile within the soil solution, significant losses are incurred through leaching and

runoff (Alfaro et al., 2004). As high amounts are also required for uptake by plants, it is common for K to be supplemented through fertiliser applications.

Sandy soils have relatively low concentrations of K-bearing minerals and non-exchangeable K (Alfaro et al., 2004; Kayser et al., 2012). This is because sandy soils release little K through weathering and have a low CEC (Kayser et al., 2012), meaning that careful management is required in order to supply sufficient quantities to support plant growth.

1.3.7 Secondary macronutrients

Secondary macronutrients include calcium (Ca), magnesium (Mg) and sulphur (S). Ca is a major component of plant cell walls and also serves in the activation of a number of enzymes (Brady and Weil, 2008). In soils, Ca is principally accessed by plant roots as exchangeable Ca, and from readily weathered minerals, such as carbonates and apatite (Brady and Weil, 2008; Rowell, 1994). These plant-available pools within the soil solution are replenished by three forms: (1) Ca within mineral structures, such as calcite or plagioclase, (2) Ca within SOM (Rowell, 1994) and (3) Ca held to cation exchange sites on clay and humus colloids (Brady and Weil, 2008). Ca solubility decreases with increasing soil pH as shown in Figure 1.2 (Brady and Weil, 2008).

Within plants, Mg is a component of chlorophyll and also serves in the activation of a number of enzymes (Brady and Weil, 2010). Mg is accessed by plant roots from an exchangeable pool on the clay-humus complex (Rowell, 1994). The exchangeable Mg pool is replenished by the weathering of minerals (including dolomite, biotite hornblende or serpentine and certain 2:1 clays), the breakdown of SOM and atmospheric deposition (Brady and Weil, 2010; Rowell, 1994).

Within plants, S is a constituent of amino acids, vitamins, enzymes and aromatic oils (Brady and Weil, 2010). There are three major sources of S for plant uptake: (1) SOM, (2) soil minerals and (3) S gases in the atmosphere (Brady and Weil, 2010). S ions within the soil are positively charged, and are retained within the soil by the cation exchange surfaces on 1:1 type clays (Brady and Weil, 2008). This dependence upon CEC and clay surfaces can lead to S depletion within sandy soils.

1.3.8 Micronutrients

Whilst still considered essential for healthy plant growth, micronutrients are required by plants in relatively small quantities (in dried plant tissue < 0.1 % weight) (Hossner, 2008). Micronutrient elements include boron, manganese, iron, nickel, copper, zinc, molybdenum and chlorine. The occurrence of micronutrients within a soil reflects the nature of its soil forming factors and may also be affected locally, by mining and industrial activities (Harmsen and Vlek, 1985).

The interactions between total and plant-available micronutrient contents differ between elements, with the total values reflecting both the relatively stable fraction and the more dynamic plant-available fraction, which is more readily influenced by soil and climatic conditions (Harmsen and Vlek, 1985).

1.4 Research Objectives and Aims

1.4.1 Rationale

At the time of the production of the artificial soils, plant nutrient retention within the soil was not a prime consideration, as it was initially planned that plant nutrition would be maintained through the use of controlled fertiliser applications (Whitbread-Abrutat, 2004). However, after 10 years of hugely variable environmental conditions, planting, fertilizer practices, incidental introductions of micro-flora and micro- and meso-fauna, as well as general husbandry (mulching and allowing natural organic matter turnover), some Eden Project soils are performing better, in terms of nutrient storage, than was originally anticipated, whilst others remain quite poor. This project seeks to further the understanding of the biogeochemistry of soils manufactured by the Eden Project, relating this to their nutrient retention.

1.4.2 Research aim

To assess the performance of an artificial soil in terms of nutrient cycling.

1.4.3 Research objectives

1. *To characterise the soils established at the Eden Project using appropriate analytical techniques.*
2. *To construct and implement the use of soil column bioreactors to observe the performance of soils with regard to the cycling of key nutrients*
3. *To make controlled changes to the artificial soils determine how this affects the sustainability of the nutrient reservoir.*
4. *To make recommendations for the manufacture of an nutrient-rich soil that can be produced by utilising waste materials available in Cornwall and which has wide application for local projects.*

CHAPTER 2

General analytical and experimental methods

2.1 Introduction

The aims and objectives for this project were achieved through the collection and analysis of a large number of solid and aqueous samples. This chapter describes the materials and methods relating to their collection and analyses.

Analyses were carried out within an ISO-accredited (ISO 9001:2008) laboratory at Plymouth University, with QEMSCAN® analyses conducted at Camborne School of Mines, University of Exeter. Fieldwork was carried out at the Eden Project, Cornwall, UK.

2.2 Cleaning Protocol and Reagents

The high purity water (HPW) referred to in this thesis, was taken from a Millipore system running at a resistivity of 18.2 MΩ cm^{-1}, unless otherwise stated. To minimise contamination, all reagents and standard solutions were prepared under a laminar flow hood. All plastic and glassware was cleaned under a standardised procedure, where it was soaked in 2 % Decon® for 24 hours, rinsed with HPW, soaked in 10 % m/v HCl for a further 48 hours, rinsed again with HPW and dried under a laminar flow hood. Contamination was further avoided, by storing plastic and glassware inside two zip-lock polythene plastic bags. All glassware and filters used for measurement of dissolved organic carbon (DOC) or total dissolved nitrogen (TDN) were combusted at 450 ^{o}C for 6 hours to remove organic residues (Badr et al., 2003).

All reagents were analytical grade, obtained from Alfa Aesar (Lancashire, UK), Fisher Scientific (Loughborough, UK) and Sigma Aldrich (Dorset, UK). All weight measurements $\leq$ 50 g were made using a Salter ER182A balance and recorded to 4

decimal places; weighing of quantities ≥ 50 g was performed using a GF3000 balance and recorded to 2 decimal places.

2.2.1 Certified reference materials

Certified reference materials (CRMs) were used, where appropriate, to support the accuracy of results. There are few commercially available CRMs for soil nutrients and, as such, alternative nutrient CRMs were used. These CRMs were chosen because they contained nutrient concentrations of a similar order of magnitude to those anticipated in samples. Details for any of the CRMs used are recorded in the appropriate sections of this Chapter.

2.3 Fieldwork

Fieldwork was carried out at the Eden Project, and took place on a number of occasions, as detailed in Table 2.1. The majority of fieldwork involved soil collection and preparation; additional detail regarding site visits can be found in the appropriate chapters.

Table 2.1: *Fieldwork dates summary.*

Date	Summary of fieldwork
26/09/11	Preliminary site visit
26/01/12	Sampling across Humid Tropics and Outdoor biomes.
11/02/13	Water sampling and irrigation rate measurement.
25/03/13	Artificial soil production and column packing.
23/01/14	Soil pit digging and sampling.

2.4 Particle-associated constituent preparation and analyses

2.4.1 Soil sampling, preparation and storage

To ensure the collection of enough material for laboratory analyses, approximately 100 g of soil was collected per sample. Sampling was carried out using a plastic trowel, to minimise the potential for metallic contamination. For more extensive sampling, such as in the case of the soil pits, a metal spade was used, with a plastic trowel then employed to clean the sampling-face of the pit. For the initial characterisation analyses, bulked sampling methods were employed at each site, where a number of samples was taken from a set depth (e.g. 0 to 10 cm of topsoil or $\geq$ 20 cm for subsoil) and homogenised to give one representative sample for that soil layer. In all cases, the soil surface was cleared of litter prior to sampling.

All soil samples were placed in labelled, polythene zip-lock bags and transferred to the laboratory within 4 hours. The solid samples were then stored at 4 ^{o}C and liquid samples frozen at -20 ^{o}C. Sample analysis was carried out as rapidly as possible, in most cases within 1 month of sampling.

Soil samples are commonly subject to unintended sorting via different particle densities, shapes and sizes during transport (Schumacher et al., 1990), as such samples were homogenised prior to analysis This sorting can have a major influence on the results of chemical analyses and as such it was necessary to ensure that all samples were homogenised prior to analysis. To encourage homogenisation, the coning and quartering method (Harvey, 2000) was employed to reduce the gross sample size (Figure 2.1).

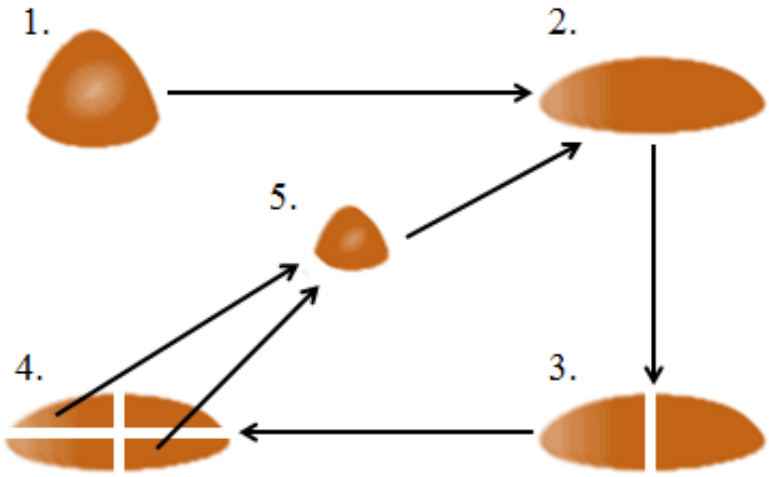

***Figure 2.1**: The coning and quartering process. 1. Sample was formed into a cone shape. 2. The cone was flattened. 3. The flattened cone was then divided in half. 4. Then divided into quarters. 5. The diagonally opposite quarters were taken and either used for laboratory analysis or, if a smaller quantity was required, the process repeated (Harvey, 2000).*

2.4.2 Total Nitrogen, Total Carbon and Organic Carbon

Total nitrogen, carbon and organic carbon were analysed using a CHN EA1110 analyser (CE Instruments), which combusted the samples, converting carbon to carbon dioxide, hydrogen to water and nitrogen to nitrogen dioxide gas (Ryba and Burgess, 2002). The gases were resolved then chromatographically analysed.

Procedure

Approximately 10 g of sample was air dried for 48 hours in a temperature cabinet maintained at 40 ^{o}C. The samples were then ground to pass through an 80 µm sieve.

Each sample was divided into 2 x 10 mL sample tubes one of which was used for the total carbon and nitrogen measurements while the other underwent direct acid digestion, to remove inorganic carbon, allowing for the determination of organic carbon. The acid digestion was performed by adding 1 mL 0.1 M HCl to a sample, which was left for 30 minutes; the supernatant was then removed by vacuum (Figure 2.2). A further 1 mL HPW was added and the sample left for 30 minutes before the supernatant was

removed. This process was repeated until the samples ceased to effervesce (Jones et al., 2004). Once the acid digestion process was complete, samples were dried at 40 ^{o}C for 24 hours.

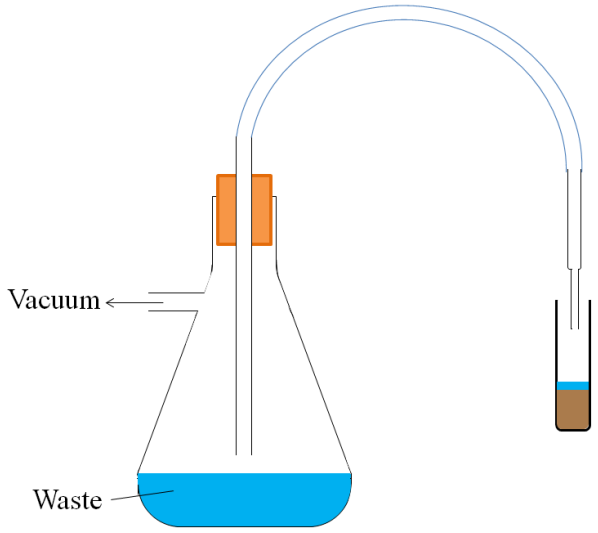

Figure 2.2: *The vacuum system for removing supernatant waste following the acid digestion of a soil sample.*

Following the digestion process, between 6 to 8 mg of each soil sample was accurately weighed into tin cups, using a 5-figure balance (Mettler Toledo AT201), with 3 replicates prepared per sample. The cups were then crushed using tweezers and re-weighed, with the recorded values entered into the software, before being placed onto the auto-sampler. The CRMs used were PACS-1 and Peat Soil Standard (the C and N content of these CRMs is shown in Table 2.2) and empty cups were crushed and used as blanks.

Table 2.2: *The certified carbon and nitrogen values for the certified reference materials employed during the CHN analysis.*

Certified reference material	C (%)	N (%)
PACS-1 (marine sediment)	3.69 ± 0.11	-
Peat soil standard	15.98 ± 0.16	1.27 ± 0.04

Samples were completely combusted in excess oxygen, this reduced C to CO_2, H to H_2O, and N to N_2 and NO_x (Ryba and Burgess, 2002). The combustion products were then passed over high purity copper, heated to 600 ^oC using helium gas as an inert carrier (Ryba and Burgess, 2002).The copper served to remove any oxygen not consumed during the combustion process and converted any NO_x to N_2. The gases were then passed through absorbent traps to leave only the CO_2, H_2O and N_2, which were analysed by gas chromatography with thermal conductivity detection (Ryba and Burgess, 2002).

2.4.3 Mineralogical analyses using QEMSCAN®

The mineralogical characteristics of the soil samples were determined using QEMSCAN® at Camborne School of Mines, Exeter University. QEMSCAN® (quantitative evaluation of minerals by scanning electron microscopy) is a microscopy system which enables quantitative chemical analysis of materials and the generation of high-resolution, mineral maps and images as well as pore structure (Ayling et al., 2012; Gottlieb et al., 2000).

The main benefit offered by QEMSCAN® was the spatially resolved mineralogical data, inferred from chemical spectra, which provided increased information on mineral species (Rollinson et al., 2011). QEMSCAN® only examines solid inorganic material with atomic numbers between 6 (carbon) and 92 (uranium) (Rollinson, 2010), limited by the energy dispersive x-ray spectrometers (EDS) detectors. QEMSCAN® was used to identify and compare any differences in major mineral phases present within a freshly prepared artificial soil and the same soil mix after 1 year of irrigation and management within the soil columns.

Procedure

Approximately 5 g of each sample was dried and sieved (< 2 mm) and then sent to Camborne School of Mines, University of Exeter. There, they were mounted into epoxy resin blocks (30 mm diameter) and polished using water-based lubricants and diamond solutions to give a highly polished surface, as shown in Figure 2.3.

Figure 2.3: *Polished resin sample blocks of freshly prepared artificial soil, analysed using the QEMSCAN® instrument (Photograph courtesy of G.K.Rollinson, December 2013).*

Samples were analysed on the QEMSCAN® 4300, built on a Zeiss Evo 50 SEM (scanning electron microscopy) platform with four light element Bruker Xflsah® silicon drift EDS (Rollinson et al., 2011). An electron beam was rastered across the sample surface, causing the liberation of electrons and x-rays from the sample (Rollinson, 2010). A background threshold was applied via atomic number, allowing particles to be outlined and distinguished from the background (resin mounting). This created a grey-scaled map, based on atomic number (Rollinson, 2010), which guides the x-ray analysis.

X-rays from every point were detected by the energy dispersive x-ray spectrometer detectors and sent to the pulse processors (Rollinson, 2010). The spectra were then compared to a database, allowing for the assignment of each analysis point to a specific

mineralogy and chemistry (Ayling et al., 2012; Rollinson, 2010). Data was collected using iMeasure 4.2 and processed using iDiscover 4.2 & 4.3 to provide an accurate and focussed report, in line with procedures outlined by Rollinson et al. (2011). This involved ensuring the mineral identifications are correct (quality control), developing the database as required and outputting appropriate data.

2.4.4 Moisture content and organic carbon content

Moisture content and organic carbon content were measured concurrently using a two-stage process (Rowell (1994). Moisture content was measured by drying a known weight of soil in an oven at 105 ^{o}C; the difference between the weight of the field moist sample and the oven-dried sample represented the moisture content. Organic carbon content was determined using loss on ignition, by heating the same sample to 450 ^{o}C, at which temperature organic matter is burnt off, the sample was then reweighed (Rowell, 1994).

Procedure

The weight of an empty crucible was recorded using a 4-figure balance, then approximately 10 g of sample was accurately weighed into the crucible before drying at 105 ^{o}C for 16 hours. The crucible was then placed in a desiccator until cool and then reweighed to give the dry soil weight.

For the determination of loss on ignition the crucible was placed in a muffle furnace at 450 ^{o}C for 6 hours, then cooled in a desiccator to cool and reweighed. Moisture content was reported as a percentage relative to the oven-dried sample mass. Loss on ignition was expressed relative to the mass of oven-dried soil in g per 100 g oven-dried soil (Rowell, 1994).

2.4.5 Particle size distribution

Particle size analysis was carried-out on a Malvern Mastersizer 2000, using laser diffraction to determine the particle size distribution of soil samples.

Procedure

Approximately 10 g of each sample was dried at 80 ^{o}C for 16 hours. Any aggregates were then gently broken up using a mortar and pestle and passed through a 1 mm sieve. Each sample was then subdivided into 2 g sub-samples and transferred to sample tubes.

The presence of organic matter (OM) within a sample may cause flocculation of particles, which can lead to erroneous results within samples, so the OM was removed with hydrogen peroxide (H_2O_2) prior to particle size analysis. Each sample was digested in 3 mL 3 % H_2O_2 solution for 16 hours, after which a further 3 mL of 6 % H_2O_2 solution was added and digested for a further 16 hours. Samples were then placed in a water bath at 90 ^{o}C to remove any remaining H_2O_2 solution; the effervescence produced by heating caused any fibrous material to rise to the surface, where it was then removed. This process was repeated 4 times and samples were then dried overnight at 90 ^{o}C.

Samples were moistened with water immediately prior to analysis and mixed to a homogenous paste before being positioned in the auto-sampler. Throughout the analysis sodium hexametaphosphate was added to each suspension to complex cations which may have been bound to clay and silt particles, creating aggregates (particularly Ca^{2+}, Al^{3+} and Fe^{3+}); this substance also acted to suspend any organic matter still present on the surface of the samples.

Particle size was measured by laser diffraction as the suspension passed through a focused laser beam (Malvern Instruments Ltd., 2006). This provided a particle size

distribution range from 0.1 to 2000 µm. The particles scattered the incident light at angles inversely proportional to their size and the angular intensity of the scattered light was then measured using a series of photosensitive detectors (Malvern Instruments Ltd.Malvern, 2006). The particles were then divided into size fractions, based on the results of these analyses. Table 2.3 outlines the particle size classifications used by ISO 14688-1:2002.

Table 2.3: *Soil particle size classifications (ISO 14688-1:2002).*

	Basic soil type	**Sub-type**	**Particle size (µm)**
Fine soil	Clay		< 2.0
	Silt	Fine silt	2.0 – 6.3
		Medium silt	6.3 – 20.0
		Coarse silt	20 – 63
Coarse soil	Sand	Fine sand	63 – 200
		Medium sand	200 – 630
		Coarse sand	630 – 2000
	Gravel	Fine gravel	2000 – 6300
		Medium gravel	6300 - 20000
		Coarse gravel	20000 - 63000
Very coarse soil	Cobbles		63000 - 200000
	Boulders		> 200000

2.5 Dissolved constituents

2.5.1 Sample preparation and storage

Once collected liquid samples were filtered through combusted, HPLC grade, glass fibre filter papers (75 g m^{-2}, 450 μm thickness) using Buchner apparatus. Filters can adsorb nutrients from a sample and, as such, could potentially interfere with the results. As a result all filters were pre-treated with a small amount of sample, prior to filtering. Samples were then subdivided into polypropylene centrifuge tubes (for separate analyses of NH_4^+, NO_3^- + NO_2^-, PO_4^{3-} and metals) and combusted glass vials (for DOC/TDN analysis).

Samples were stored in the dark at -20 ^oC prior to analysis. All bottles had a suitable headspace and were stored and thawed in an upright position (Kirkwood, 1996). This is because the last few millilitres to freeze often have a different composition to the bulk of the sample, and if the bottle volume is completely filled, some of this liquid may be expressed past the closure during the freezing process, leading to unrepresentative results (Kirkwood, 1996). Leaving a sufficient headspace also helped to prevent breakage of storage tubes and vials. Prior to analysis, frozen samples were allowed to thaw to room temperature over a period of 24 hours.

2.5.2 Total dissolved nitrogen and dissolved organic carbon

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using the Shimadzu TOC (total organic carbon) 5000A analyser, coupled with a Sievers NCD (nitrogen chemilluminescence detector) 255 detector as shown in Figure 2.4, following the method outlined by Badr *et al.* (2003).

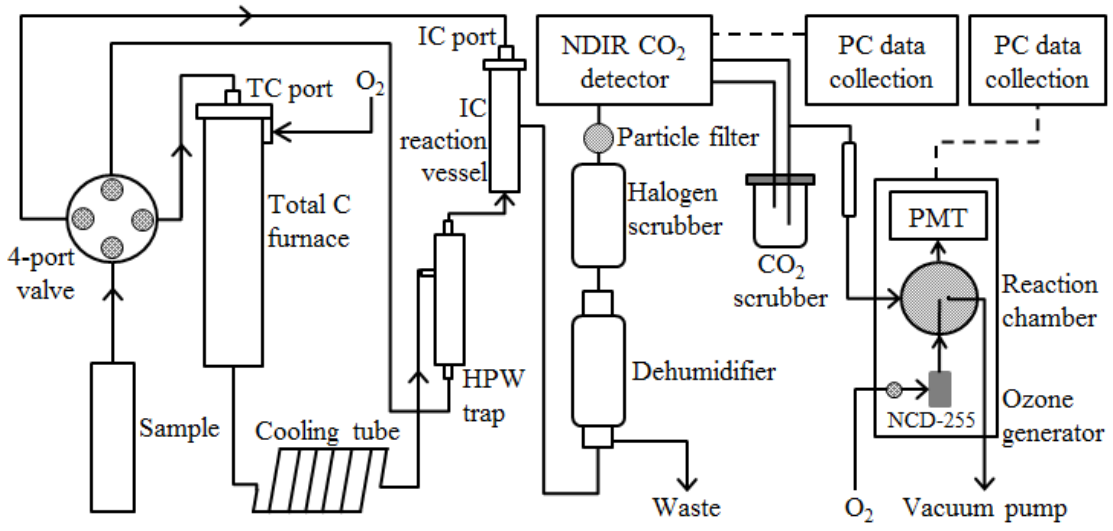

Figure 2.4: *Schematic diagram showing the coupled Shimadzu TOC 5000A HTCO-Sievers NCD 255 nitrogen chemilluminescence detector and the associated hardware (Badr et al., 2003).*

Calibration

Combined DOC/TDN standards were produced using potassium hydrogen phthalate for carbon and glycine for nitrogen. Concentrations ranges were 60 – 600 µM C and 10 to 100 µM N for carbon and nitrogen, respectively. The peak area values for each sample were converted to concentrations using a calibration graph derived from the peak area of the standard solutions. Figure 2.5 shows examples of a typical TDN (a) and DOC (b) calibration graphs.

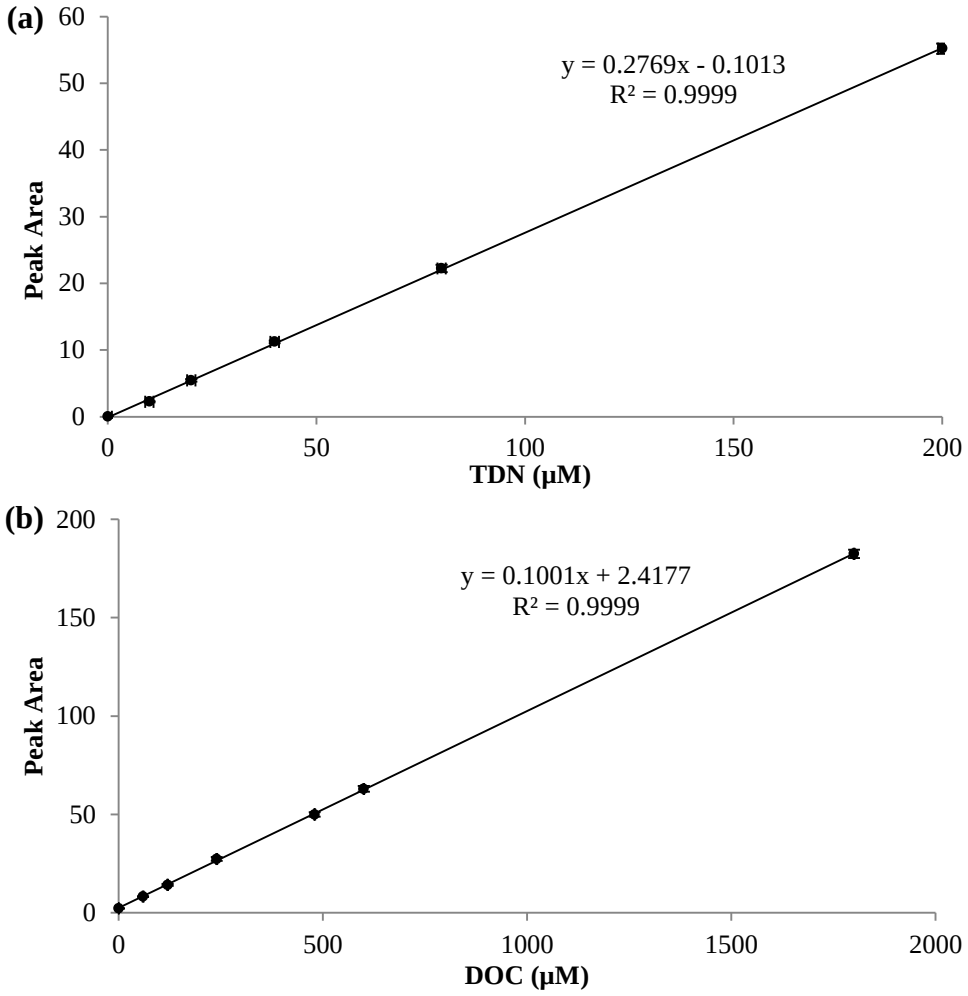

Figure 2.5: a) *Calibration graph as produced and used for total dissolved nitrogen (TDN) analyses (data generated on 15/4/14).* ***b)*** *Calibration graph as produced and used for dissolved organic carbon (DOC) analyses (data generated on 10/3/14).*

Procedure

Samples were acidified to pH 2 using 2 M HCl (100 µL per 100 mL sample) prior to analysis. Samples of approximately 15 mL volume were loaded on to the autosampler in combusted glass vials. Where dilutions were necessary, HPW was used. A deep sea water standard was used as a CRM for each run (the DOC and TDN content of this CRM is shown in Table 2.4).

Table 2.4: *The certified carbon and nitrogen values for the certified reference materials employed during the CHN analysis.*

Certified reference material	C (µM)	N (µM)
Deep sea water	41 – 44	31.50 – 33.75

The first stage of analysis was the removal of dissolved inorganic carbon, by sparging (ca. 8 min^{-1} at 75 mL min^{-1}) using bottled, high-purity (99.999 %) oxygen (Badr et al., 2003). The sample was then injected into the combustion column, where the TDN and DOC in the sample were oxidised to CO_2, NO and H_2O at 680 ^oC, in the presence of a platinum catalyst (Badr et al., 2003).

The combusted gases were dried using an electronic dehumidifier and purified using a halogen scrubber (Badr et al., 2003). The CO_2 was detected by a non-dispersive infra-red detector (NDIRD), the gases exiting the NDIRD were pulled into the nitrogen chemilluminescence detector (NCD) unit using a vacuum pump connected in series, with the chemilluminescence detector (Badr et al., 2003). Any remaining water vapour was removed using a gas dehumidifier. The NO in the combustion gas was then reacted with O_3 to produce NO_2 species (equation 2.1a), which chemiluminesced on decay to its ground state (equation 2.1b). The emitted light (hυ) was collected by a photomultiplier tube and the resulting voltage recorded using a data-acquisition/integration system (Badr et al., 2003). The voltage was stoichiometrically proportional to the total extracted N (Badr et al., 2003). These processes are further illustrated in Figure 2.3.

$$2NO + 2O_2 \rightarrow NO_2^* + 2O_2 \qquad \textbf{(2.1a)}$$

$$NO_2^* \rightarrow NO_2 + h\upsilon \qquad \textbf{(2.1b)}$$

2.5.3 Nitrate + nitrite and phosphate

Dissolved nitrate + nitrite (NO_2^- + NO_3^-) and phosphate (PO_4^{3-}) were analysed simultaneously with a Skalar SAN^{++} analyser, using a continuous flow method on a four-channel system, supplied by an auto-sampler, following the protocol outlined by

Kirkwood (2011). The NO_3^- + NO_2^- value was referred to in the results sections as NO_3^-, as NO_2^- represents an intermediate fraction.

Buffer solution (nitrate ± nitrite)

Ammonium chloride (50 g) was dissolved in approximately 800 mL HPW, which was then adjusted to pH 8.2 with ammonia solution (35 %). Sodium hydroxide (5.5 g) was then added and the solution made up to 1000 mL with HPW. The buffer solution was then de-gassed using helium before 3 mL Brij 35 was added.

Colour reagent (nitrate ± nitrite)

Phosphoric acid (150 mL) was added to approximately 700 mL HPW, 10 g sulphanilamide and 0.5 g N-(1-Naphthyl)ethylene diamine dihydrochloride were then added. This was then made up to 1000 mL with HPW.

Ammonium molybdate (phosphate)

Potassium antimony tartate (0.23 g) was dissolved in approximately 800 mL HPW, 69.4 mL sulphuric acid was added whilst swirling constantly. Once cooled, 6 g ammonium molybdate were dissolved. This was then made up to 1000 mL with HPW and 2 mL FFD6 added.

Ascorbic acid (phosphate)

Ascorbic acid (11 g) was dissolved in approximately 800 mL HPW. Acetone (60 mL) was added, then made up to 1000 mL with HPW and 2 mL of the anionic surfactant FFD6 added.

Standard solutions

Combined nitrate and phosphate standards were made up within a concentration range of 0 to 15 mg L^{-1} for nitrate and 0 to 500 µg L^{-1} phosphate. Sample concentrations were

determined using a calibration curve derived from the peak heights of the standard solutions.

The peak height values for each sample were converted to concentrations using a calibration graph produced through the analysis of standards. Figure 2.6 shows an example calibration graph for, nitrate + nitrite (a) and phosphate (b).

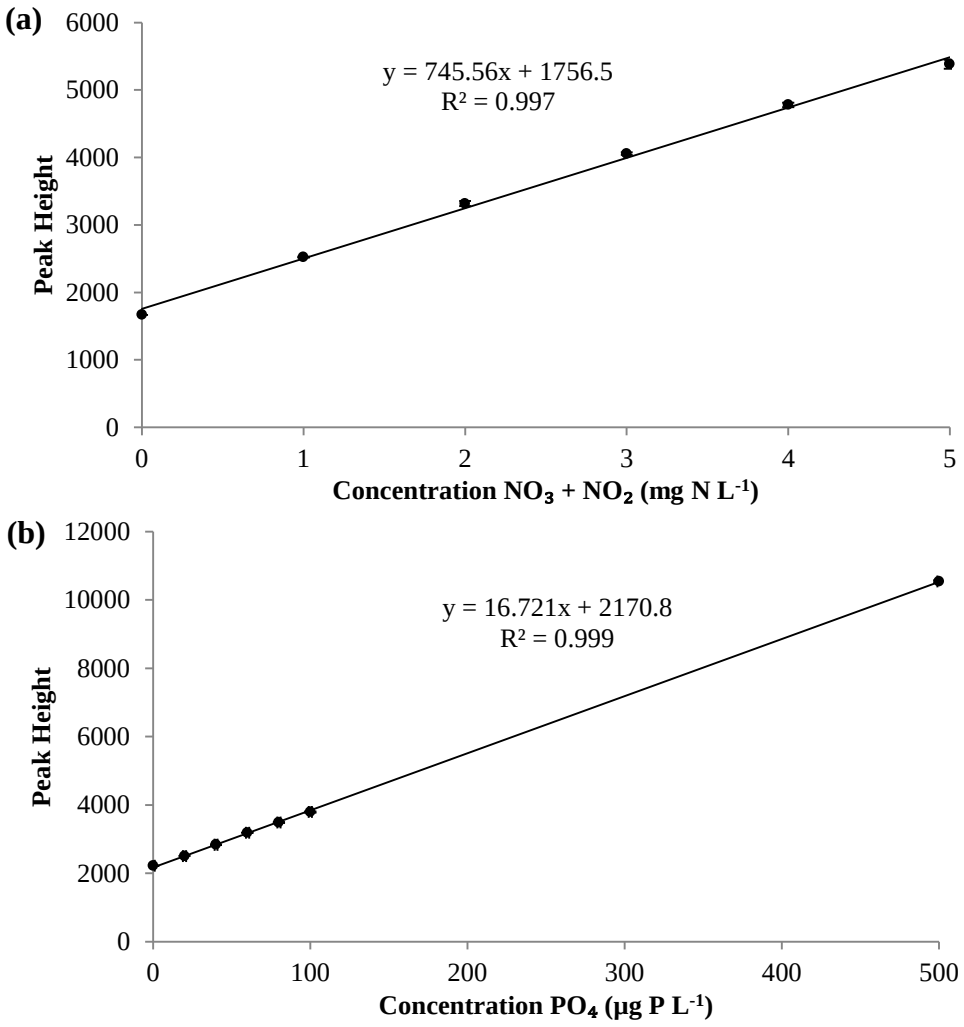

Figure 2.6: a) *Calibration graph as produced and used for nitrate + nitrite analyses (n = 3); data generated on 16/11/13).* ***b)*** *Calibration graph as produced and used for phosphate analyses (n = 3) (data generated on 8/11/13).*

Procedure

Approximately 10 mL sample was transferred to autosampler vials then injected into the system, where it reacted as illustrated in Figure 2.7.

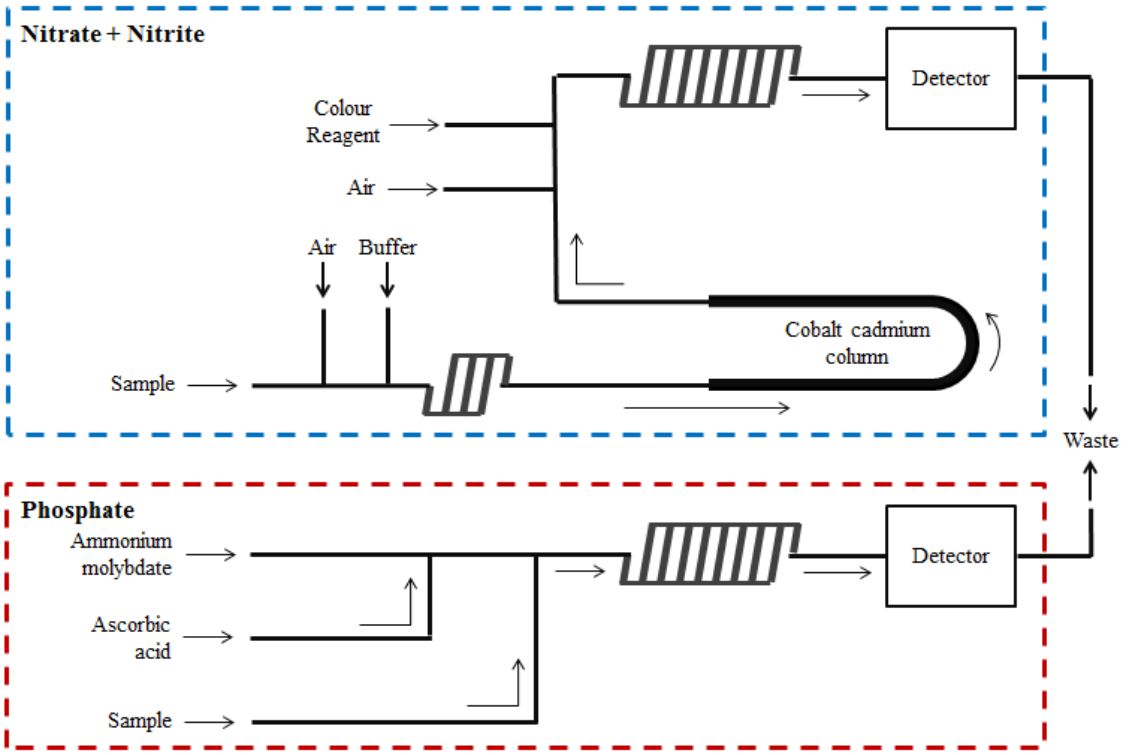

Figure 2.7: *Schematic diagram showing the phosphate and nitrate + nitrite channels of the Skalar San^{++} analyser.*

As direct colorimetric determination of nitrate was not possible, the method proceeded via reduction of nitrate to nitrite (Grabowski et al., 2011) on a column, containing granulated copper-cadmium (Sparks, 2003). The nitrite then reacted with sulphanilamide and coupled with N-(1-naphthyl)ethylenediamine dihydrochloride, to form an intense pink azo dye, which was measured at 540 nm (Kirkwood, 1996; Skalar, 2012a). In order to establish the efficiency of the cadmium column, in converting nitrate to nitrite, two 3 mg L^{-1} standards, one nitrate and one nitrite, were prepared and run at the beginning and end of each set of analyses, standard column efficiency was in the region of 97 ± 0.5 %.

Phosphate reacted with ammonium molybdate and potassium antimony tartate in the ascorbic acidic medium, forming an antimony-phospho-molybdate complex (Kirkwood, 1996). This complex was then reduced by ascorbic acid to produce a blue-coloured

complex, the intensity of which was measured at 880 nm (Kirkwood, 1996; Skalar, 2012b)

The phosphate concentration in some samples exceeded the linearity of the calibration curve and, as such, required dilution. Where dilutions were necessary the nitrate + nitrite and phosphate analyses were carried out separately and samples were diluted in HPW. Figure 2.8 demonstrates the extent of the linearity for the phosphate calibration.

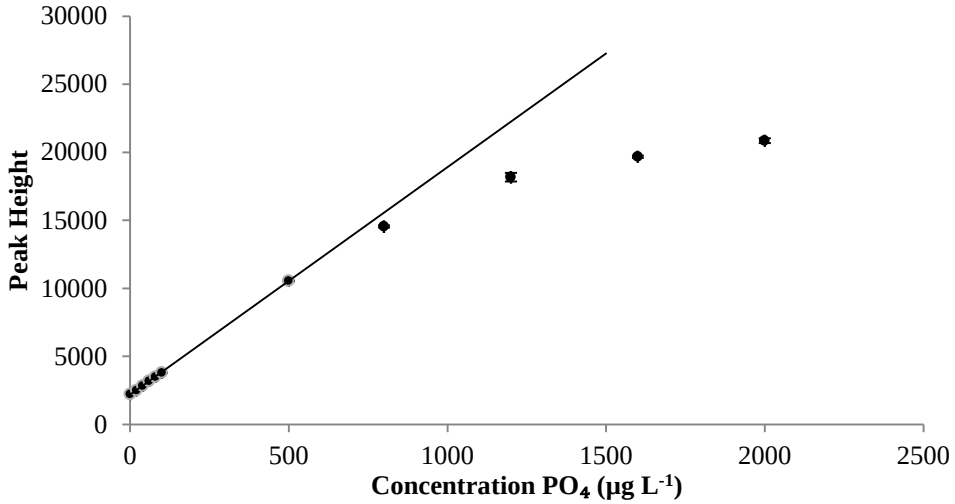

Figure 2.8: *Calibration graph displaying the extent of the calibration linearity for phosphate analyses (n = 3) (data generated on 10/12/13).*

2.5.4 Ammonium

Ammonium concentration was measured on a Hitachi F-4500 fluorescence spectrometer, using the OPA (o-phthalaldehyde) fluorescence spectrophotometry method, as outlined by Holmes *et al.* (1999) and Kérouel & Aminot (2011).

Working reagent

Sodium tetraborate (40 g) was dissolved in 1000 mL HPW, in a glass, amber Winchester bottle. Sodium sulphite (0.4 g) was diluted in 50 mL HPW; 5 mL of which was added to the Winchester bottle, followed by a solution of *o*-phthalaldehyde (OPA)

(5 g dissolved in 50 mL ethanol). The working reagent was prepared a minimum of 48 hours prior to use and was stored in the dark at room temperature.

Standard solutions

Standard solutions were prepared using NH_4Cl to give a concentration range from 0 to 50 µM. The fluorescence values for each sample were converted to a concentration using a calibration graph produced through the analysis of standard solutions. Figure 2.9 shows an example calibration curve for ammonium.

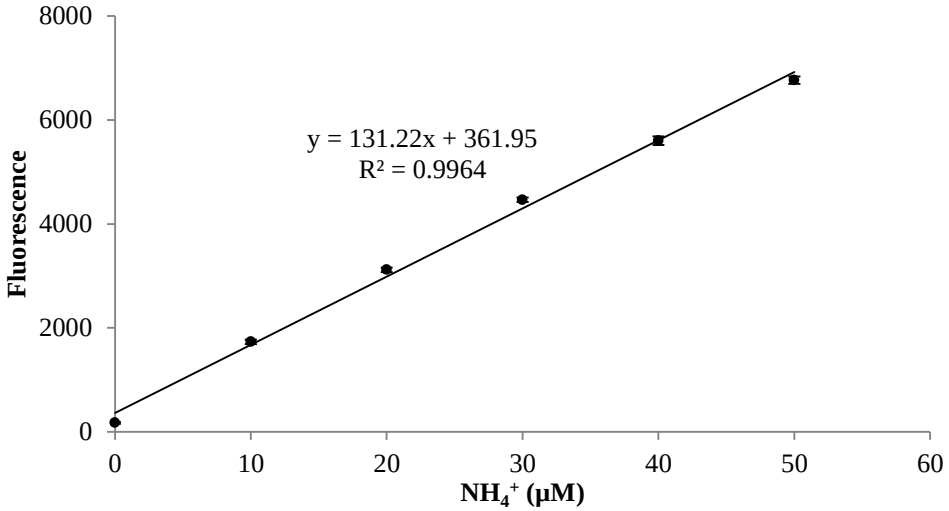

Figure 2.9: *Calibration graph as produced as used for ammonium analyses (n = 3)(data generated on 30/10/13).*

Background fluorescence and matrix effects

Background fluorescence (BF) occurs as a result of the auto-fluorescence of substances within the samples, and must be subtracted from the observed value in order to correctly determine the fluorescence due to ammonium in the sample (Holmes et al., 1999). The BF was measured using sodium tetraborate solution only.

A matrix effect (ME) occurs when substances in the sample, such as organic material, alter the intensity of the fluorescence by reacting with OPA (Holmes et al., 1999). The ME for a sample set was measured using the procedure described in Holmes *et al.*

(1999) in which 4 samples were analysed using the sample matrix and HPW (Table 2.3). Equation 2.2 was used to calculate the ME of the sample, with the letters corresponding to the solutions identified in Table 2.5.

$$ME = \frac{((A-B)-(C-D))}{(A-B)} \times 100\ \% \qquad \textbf{(2.2)}$$

Procedure

A 2.5 mL aliquot of each sample or standard solution was transferred into a 20 mL polypropylene scintillation vial and 10 mL of working reagent added. This was performed in triplicate for all samples and standards, excluding the blank standard, which had a minimum of 5 replicates. For the background fluorescence, 2.5 mL of sample was mixed with 10 mL sodium tetraborate solution; the ME samples were prepared as shown in Table 2.5.

Table 2.5: *Matrix effect sample composition as employed during the ammonium analyses.*

Solution	Working reagent	Sample	High purity water	30 µM Standard
A	10 mL	-	1.25 mL	1.25 mL
B	10 mL	-	2.5 mL	-
C	10 mL	1.25 mL	-	1.25 mL
D	10 mL	2.5 mL	-	-

Once prepared, samples and standards were incubated in the dark for 2 to 3 hours then analysed on the fluorescence spectrophotometer. The excitation and emission wavelength bandwidths used were 340 to 360 and 380 to 600 nm, respectively (Holmes et al., 1999). The slit width used on the detector was 5 mm.

The reaction of OPA with ammonium produced a fluorophore (Holmes et al., 1999). Fluorescence occurred when the fluorophore was excited into a higher energy state, through the absorbance of an incident photon (Lakowicz, 2006); the source in this

instance being a xenon lamp. The fluorophore emits a photon (Lakowicz, 2006), which produced a signal. The energy and relative intensity of the signal could be measured within a wavelength range and the sample ammonium concentration determined using a calibration graph produced from the standard solution data.

2.5.5 Dissolved Organic Nitrogen and Extracted Organic Nitrogen

Dissolved organic nitrogen (DON) and extracted organic nitrogen (EON) were measured. DON represented the soluble organic N fraction present within the soil solution, whilst EON referred to the soil organic N fraction released through extraction with fixed volumes of 2M lithium chloride and HPW. Both DON and EON were measured after filtration through GFF filters (75 g m^{-2}, 450 µm thickness).

DON and EON were determined indirectly as the difference between the TDN and DIN (NO_2^- + NO_3^- and NH_4^+) concentrations as shown in Equation 2.3a. Equation 2.3b shows the standard error calculations used for the separate analyses, allowing the standard error for the calculation to be estimated.

$$DON\ or\ EON = TDN\ or\ TEN - (NO_3^- + NH_4^+) \quad \textbf{(2.3a)}$$

$$\delta DON\ or\ \delta EON = \sqrt{(\delta TDN\ or\ \delta TEN)^2 + (\delta NO3^-)^2 + (\delta NH_4^+)^2} \quad \textbf{(2.3b)}$$

2.5.6 Nutrient analyses using ICP-OES

The column leachate and extractant solutions were analysed for dissolved K, P, Ca and Mg (macronutrients) and Fe (a micronutrient), using an iCAP 7000 series inductively coupled plasma – optical emission spectrometer (ICP-OES).

Standards

Mixed standards were prepared using certified standards in 2 % nitric acid to give a suitable calibration range for the 6 elements (Table 2.6).

Table 2.6: *Concentrations of the mixed standard solutions employed for the ICP-OES calibration.*

Ca. Mg, P (mg L^{-1})	K (mg L^{-1})	Fe (mg L^{-1})
0	0	0.00
20	100	0.02
50	200	0.05
100	500	0.10
200	1000	0.20

Procedure

Nitric acid (2 %) was used as a wash solution between each sample, with the mid-standard used to recalibrate the instrument every 10-15 samples. Between 5 and 10 mL of each sample was required for analyses. The wavelengths used for analyses are listed in Table 2.7.

Table 2.7: *Wavelengths employed for the elemental analyses with ICP-OES.*

Element	Wavelength (nm)
Mg	285.21
Ca	317.93
K	766.49
P	177.50
Fe	259.94

The sample was introduced to the ICP-OES in liquid form, where it was nebulised to an aerosol using argon gas. Larger droplets went to waste, whilst smaller droplets were introduced into the plasma flame, where the sample collided with electrons and charged ions and was itself, broken down into charged ions. The various molecules from the sample broke up into their respective atoms, which then lost electrons and recombined repeatedly in the plasma, giving off light at the characteristic wavelengths. The emitted

light was separated into its component wavelengths and its intensity measured to provide quantification for each sample.

2.5.7 pH and Eh

The pH and Eh of sample solutions were determined within 30 minutes of sample collection and solid sample pH measured within 2 weeks of collection. The pH probe used was a standard glass electrode (VWR) with a Mettler Delta 340 milli-voltmeter, calibrated using buffer solutions of pH 4 and 7, prepared with tablets (Fisher Scientific) dissolved in HPW. The pH of the solid soil samples was measured according to Rowell (1994), as detailed below.

The analysis of solid samples was carried out by drying the sample for 48 hours in an oven at 40 ^{o}C. Sub-samples of 10 g were weighed into 6 centrifuge tubes and 25 mL of HPW added to 3 replicates each per sample (Nelson, 1982; Rowell, 1994). The centrifuge tubes were then placed on a rotary shaker at 120 rpm for 30 minutes, then left to stand for 1 hour before the pH was measured using the calibrated pH meter.

The Eh of soil solution was measured using a double junction potassium chloride ORP electrode (Cole Palmer) with a Metler Delta 340 milli-voltmeter. The voltage measured was then corrected to be consistent with a standard hydrogen reference electrode (+200 mv in this case).

2.5.8 Cation Exchange Capacity

Cation exchange capacity (CEC) was determined through the use of the ammonium acetate method as described by Schollenberger and Simon (1945).

The analysis of solid samples was carried out on air dried soils (40 ^{o}C for 48 hours), 5 g of which were weighed into Erlenmeyer flasks (250 mL) and 25 mL of 1 M ammonium acetate added to 3 replicates per sample and allowed to stand for 16 hours.

The sample was then filtered, with the entire sample transferred to a Buchner funnel, light suction was applied. The soil was then leached with ammonium acetate 5 times using 25 mL each time to ensure maximum cation exchange. Ethanol (95 %) was then used to wash the soil, removing excess ammonium acetate, this was repeated 5 times.

The soil was then leached using 50 mL 1 M potassium chloride to displace the ammonium ions. This leachate collected and analysed for ammonium concentration using the method described in Section 2.5.4. CEC was then calculated using equation 2.4.

$$\text{CEC } (cmol_c\ kg^{-1}) = \frac{(NH_4^+ - N\ in\ leachate - NH_4^+ - N\ in\ ammonium\ acetate)}{RMM\ nitrogen} \quad \textbf{(2.4)}$$

2.5.9 Fluorescein Diacetate Hydrolysis

The fluorescein diacetate (FDA) method reported by Adam and Duncan (2001) was used for measurement of enzymatic activity within solid soil samples. A procedure was developed and utilised for the FDA analysis of liquids, which was applied successfully to the analysis of enzymatic activity within leachate samples. Reagents used for these analyses were adapted according to the sample matrix (i.e. solid or liquid).

Sodium phosphate buffer solution (pH 7.6) 60 mM (for solid)

This was prepared by dissolving 22.74 g sodium phosphate tribasic ($Na_3PO_4 \cdot H_2O$) in 1 L of HPW. Sodium phosphate monobatic dihydrate was then added to achieve pH 7.6.

The solution was stored at 4 ^{o}C and the pH checked on the day of use. For liquid FDA measurements the final concentration of buffer solution was 120 mM.

Fluorescein Diacetate Solution (1000 µg FDA mL^{-1})

The FDA solution was prepared by dissolving 0.1 g fluorescein diacetate (Sigma Aldrich) in 100 mL AR grade acetone and was stored at 4 ^{o}C.

Standard solutions

Standard solutions were prepared using fluorescein sodium salt (Sigma Aldrich) in the appropriate pH 7.6 sodium phosphate buffer solution (60 mM for solid analyses and 120 mM for liquid analyses). The concentrations used for the calibration curve ranged from 0 to 5 mg L^{-1}. Standards were prepared on the day of analyses. The absorbance values were converted to concentrations using a calibration curve, an example of which is shown in Figure 2.10.

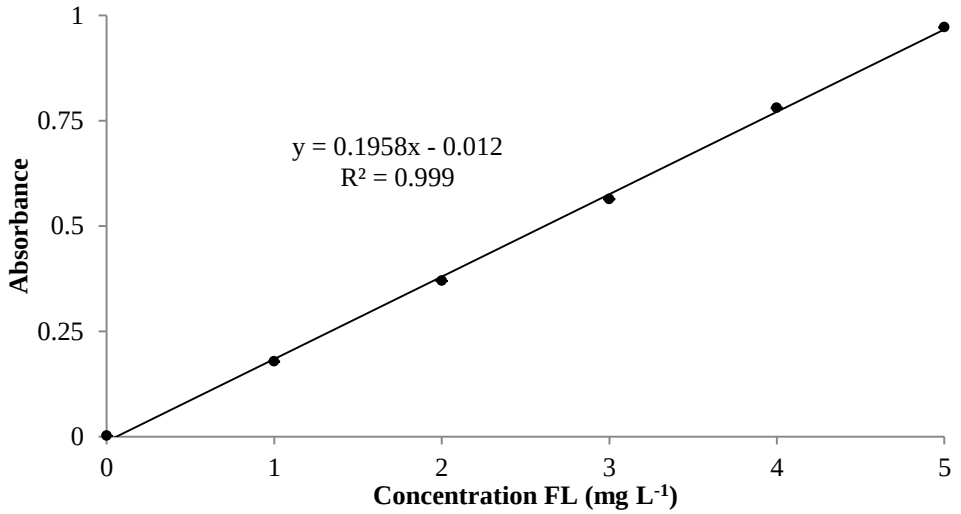

Figure 2.10: *Fluorescein calibration curve used to determine sample fluorescein diacetate concentrations (n = 3) (data generated on 3/2/14).*

Soil analysis procedure

The method of Adam and Duncan (2001) was followed for the analysis of soil samples, with the exclusion of the termination step. Fresh, field-moist soil sample (2 g) was

weighed into a 50 mL centrifuge tube and 15 mL of 60 mM sodium phosphate buffer (pH 7.6) was added. To start the reaction 0.2 mL FDA solution (1000 µg FDA mL^{-1}) was added and the centrifuge tubes shaken to mix. The centrifuge tubes were heated in a water bath at 30 ^{o}C for 30 minutes then centrifuged at 2000 rpm for 5 minutes. The supernatant was immediately analysed at 490 nm on a Hewlett-Packard 8453 UV-vis spectrometer. The fluorescein produced in the reaction gave the supernatant a fluorescent yellow colour; Figure 2.11 demonstrates then colour range within the calibration standards.

A control was used for each soil sample where instead of 0.2 mL FDA solution (1000 µg FDA mL^{-1}), 0.2 mL acetone was added. This allowed for a background measurement of fluorescence to be made.

Figure 2.11: *Fluorescein standards are yellow in colour. Here they increase in concentration from left to right ranging from 0 to 5 mg* L^{-1}.

Liquid sample procedure

Individual parameters of the fluorescein diacetate hydrolysis reaction were studied to optimise the assay for the measurement of leachate samples. These factors included the effect of leachate quantity, amount of substrate and incubation time. Based on reported data and methodology, both temperature (30 ^{o}C) and pH (7.6) were unchanged. It was

found that incubating the soils for 30 minutes at 30 ^{o}C produced the greatest response, as demonstrated in Figure 2.12.

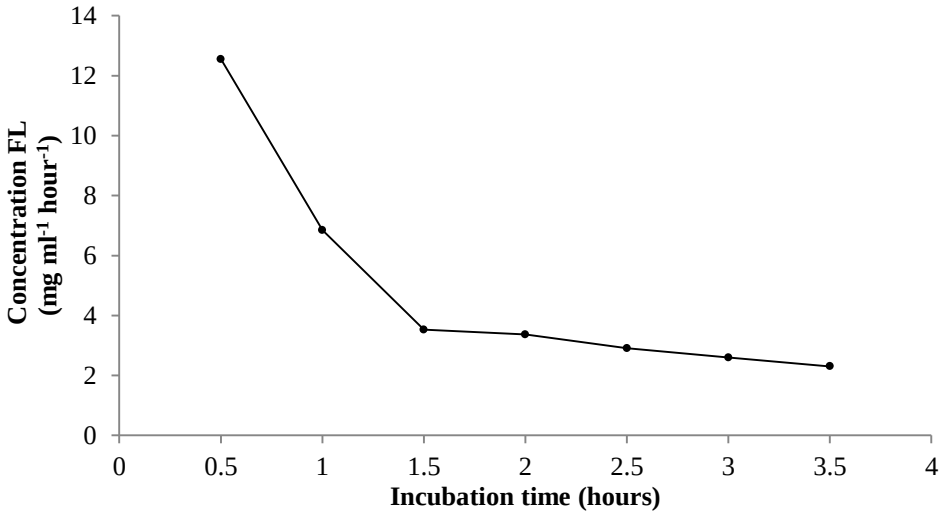

Figure 2.12: *Fluorescein concentration per hour given for a range of incubation times using a leachate sample of unknown concentration (n = 3) (data generated on 4/2/14).*

Leachate was collected from the soil columns and 14.5 mL was immediately pipetted into a 50 mL centrifuge tube, with 15 mL 60 mM sodium phosphate buffer (pH 7.6) then added. To initiate the reaction, 2 mL of FDA solution (1000 µg FDA mL^{-1}) was added and the centrifuge tubes shaken, before being heated in a water bath at 30 ^{o}C for 30 minutes. The supernatant was then analysed at 490 nm.

A chloroform methanol (2:1 v/v) mixture has been widely proposed for the termination of reactions. However, this step was not adopted as some studies reported an interference with the spectrometry. Instead the incubations were instead carried out at staggered time intervals, allowing the immediate measurement of absorbance.

As with the solid analyses, a control was used for each soil sample where the FDA solution was substituted for 2 mL acetone. This allowed for a background measurement to be made.

2.6 Extraction of nutrients from soil samples

2.6.1 Extraction method development

The extractant represents the chemical solution added to the soil sample to dissolve, desorb or exchange a portion of the total amount of a nutrient within the soil sample (Poon and Schmidt, 2010). Typical extractants used for soils vary depending on soil type, pH and climatic conditions some of the most commonly encountered are listed in Table 2.8. When carrying out soil nutrient extractions there are a number of factors impacting the outcome (1) extractant, (2) soil to extractant ratio, (3) soil extractant time and (4) extractant temperature (Li et al., 2012).

Table 2.8: *Literature reported use of soil extractants for different target nutrients.*

Target nutrients	Extracting solution	Reference
NH_4^+, NO_3^-, NO_2^-	Water	(Haney et al., 2006; Moldes et al., 2007)
	KCl	(Haney et al., 2006)
	K_2SO_4	(Atiyeh et al., 2001)
	$CaCl_2$	(Moldes et al., 2007)
P	Mehlich 3	(Azeez et al., 2013; Haney et al., 2006)
	Bray	(Azeez et al., 2013; Haney et al., 2006)
	Olsen P	(Azeez et al., 2013; Haney et al., 2006)
	Water	(Fuhrman et al., 2005; Moldes et al., 2007)
	$CaCl_2$	(Fuhrman et al., 2005)
	H_2SO_4	(Dieter et al., 2010)
	HCO_3^- resin	(Delgado and Torrent, 1997)
K	Water	(Moldes et al., 2007)
	$CaCl_2$	(Moldes et al., 2007)

For the initial site characterisation (Phase 1 - Chapter 3) it was intended to determine the maximum extractable fraction within the soil across the biomes, as such, a strong extraction solution was identified for use. Typically KCl would be employed for this, however, due to the intention to measure K through extraction of soils, it was concluded that KCl would not represent an appropriate extractant and as such LiCl and HPW were

examined as potential alternatives. For the later experimental stages (Phase 2 - Chapter 3, Chapters 4 and 5) the soil extractions were implemented as a means of estimating the concentrations of nutrients susceptible to leaching by rainwater or irrigation. On this basis HPW was employed as the extraction solution.

2.6.1.1 Preliminary extraction study

Preliminary extractions in order to compare LiCl, KCl and HPW were carried out on a loam soil certified reference material (CRM) in order to determine the optimal extractant, extractant concentration and soil to extractant ratio.

Lithium chloride (LiCl), potassium chloride (KCl) and HPW were tested as extractants for N fractions. Three concentrations of LiCl and KCl used were 0.5, 1.0 and 2.0 M; these concentrations were chosen as the most frequently reported concentrations for such investigations. The solid KCl and LiCl were pre-treated by combustion in a muffle furnace at 450 ^{o}C for 6 hours in order to remove any residual nitrogen.

Approximately 0.4 g of CRM was accurately weighed into 50 mL polypropylene centrifuge tubes and 40 mL of extractant added. Three replicates were used for each extractant and three controls (i.e. no CRM was added).

The samples were placed on a rotary shaker for 2 hours at 120 rpm, after which time they were centrifuged at 3000 rpm for 5 minutes. The supernatant was then removed and filtered through Fisher brand HPLC grade GFF papers (75 g m^{-2}, 450 μm thickness) pre-treated with the appropriate extractant. Following the removal of the supernatant, fresh extractant was added to make the volume up to 40 mL and the sample agitated and filtered again; this process was repeated 5 times for each sample. The supernatant was

then stored in acid-washed, combusted glass vials and frozen until 24 hours prior to analysis, at which time the samples were defrosted.

The supernatant was analysed for extractable organic carbon, total extractable nitrogen (Figure 2.13) and extractable ammonium, then an appropriate extractant was selected.

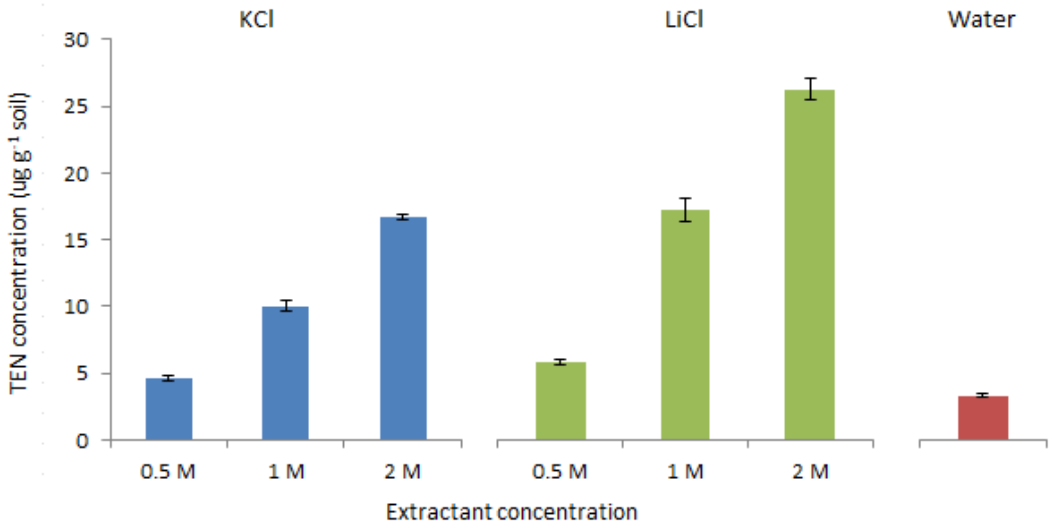

***Figure 2.13**: Total extracted nitrogen (TEN) concentrations (µg g^{-1}) from extractant trials (n = 3) (data generated 12/1/12).*

Based on the results 2 M LiCl was chosen as the most efficient extractant. Following the initial site characterisation, Phase 2 (Chapter 3) extractions were carried out, where a less intensive extractant was required for allow for the comparison of the soil column data gathering during the irrigation of the soil columns (Chapter 4). The intention here was to estimate nutrient concentrations susceptible to leaching, which called for the use of HPW as the extracting solution. The final extraction protocol is described below.

2.6.2 Lithium Chloride extractions

A 0.4 g sample of each soil was weighed into polypropylene centrifuge tubes and 40.mL 2 M LiCl solution added. Each extraction was carried out in triplicate with a 2 M LiCl solution used as a control (n = 3). Samples were placed on a rotary shaker for

2.hours at 120 rpm and then centrifuged at 3000 rpm for 5 minutes. The supernatant was then decanted into a 50 mL syringe and filtered through a 25 mm 450 µm thickness glass microfibre filter membrane, pre-treated with 2 M LiCl. A fresh volume of 2 M LiCl was then added to the centrifuge tube to make it up to 40 mL before being agitated and filtered again. This process was repeated 5 times per sample.

2.6.2 Extractions with high purity water

Extractions with HPW were carried out by accurately weighing approximately 4 g of soil into a centrifuge tube, to which 40 mL HPW was added. The centrifuge tube was then placed on an orbital table for 2 hours at 120 rpm, and then centrifuged at 3000 rpm for 5 minutes. The supernatant was then removed and filtered through pre-treated, Fisher brand HPLC grade glass fibre filter papers (75 g m^{-2}, 450 µM thickness). Following the removal of the supernatant, a fresh aliquot of HPW was added to make the volume up to 40 mL and samples were replaced on the rotary shaker; this process was repeated 3 times per sample. The samples were analysed for DOC/TDN, NH_4^+, and NO_3^- + NO_2^-/PO_4 as described in section 2.5.

2.7 Data Evaluation

Where appropriate, data was converted to mass/volume units following analyses. The limits of detection for each method are shown in Table 2.9 and have been calculated to a 95 % confidence, using the 3 x standard deviation (3σ) of the blank (Miller and Miller, 2000), excepting TDN, Mg, Ca, K, Na and Fe where it was calculated from 3σ lowest standard (Badr et al., 2003).

Table 2.9: *Typical operational limits of detection for the analytical measurements made during soil column leachate and extracted phase analyses.*

Analyses	Limit of Detection	Units
Total Dissolved Nitrogen	0.04	mg N L^{-1}
Nitrate + Nitrite	0.11	mg N L^{-1}
Phosphate	3.82	µg P L^{-1}
Dissolved Organic Carbon	115	µg C L^{-1}
Ammonium	26.8	µg N L^{-1}
Magnesium	0.46	mg L^{-1}
Calcium	0.23	mg L^{-1}
Potassium	2.82	mg L^{-1}
Sodium	1.67	mg L^{-1}
Iron	3.33	µg L^{-1}

Statistical analyses were performed using Minitab® 16, where it was determined whether normal distribution was observed, before the appropriate tests were selected. Further detail of statistical procedures is referred to in the appropriate chapters. Graphical representations of the results were produced using Grapher® (version 7.0) and Excel (Microsoft Office 2010).

CHAPTER 3

The chemical characterisation of established artificial soils from the Eden Project, Cornwall, UK

3.1 Overview

This chapter characterises the established artificial soils within the outdoor and humid tropics biomes at the Eden Project, in order to determine their condition and assess the level of variation across the site. Five topsoil samples, one sub-soil sample and two depth profiles at 5 cm resolution were collected from the Eden Project site and analysed for a range of properties and nutrient concentrations.

The statistical analyses, performed upon the data, suggested that management practices and soil age had a greater impact on the soil properties than differing environmental conditions. It was also found that all topsoil samples were classified within the sandy loam textural class (ISO 14688-1), which has potentially negative connotations for nutrient retention within the soils.

3.2 Introduction

3.2.1 Rationale

In order to address the overall aim for the project and make recommendations for the maintenance of a fertile artificial soil with a large reservoir of slow-release nitrogen, it was first necessary to determine the condition of the soils already established at the Eden Project site.

The Eden Project's artificial soils comprise a topsoil layer of approximately 15 to 30 cm depth, below which is a subsoil layer of a further 40 to 100 cm depth, on top of an impermeable clay layer at the base. It was expected that management practices would be a key influence on nutrient characteristics across the site. It was, however, considered important to explore the nutrient status at various soil sites before any further studies were carried out.

Since the soils were first laid at the Eden Project site, in 2001, they have been subject to a wide range of management practices; as such, there is considerable variability across the site with regard to soil performance. In order to understand the extent of this variation and its impact on nutrient characteristics of the soil, characterisation was carried out on soil samples from a number of locations across the Eden Project site.

Characterisation sampling was split into two Phases. For Phase 1, 6 samples (5 surface samples and 1 sub-surface) were taken from locations in both the Outdoor and Humid Tropics Biomes. Phase 2 focused on the soils within the Humid Tropics Biome, where two soil depth profile studies were carried out.

3.2.2 Research objective

This chapter addresses research objective 1:

- *To characterise the soils established at the Eden Project using appropriate analytical techniques.*

3.3 Experimental design and specific methodology

3.3.1 Sampling strategy

All field sampling took place at the Eden Project site, within the Humid Tropics and Outdoor Biomes. Sampling locations were selected based on discussions with the Eden Project, in which a number of sites were selected to be representative of the range of environments and management practices present at the Eden Project. Phase 1 sampling took place on 26/01/12 at locations marked on Figures 3.1a and 3.1b. Phase 2 sampling was carried out on 23/01/14 in the Humid Tropics Biome; these locations are marked on Figure 3.1a.

Whilst the two sample sets were collected 2 years apart, sampling on both occasions took place in January, as a means of keeping the sampling conditions as constant as possible, to generate comparable data-sets.

The sampling sites for Phase 1 were selected to provide a representative characterisation of soils from the Outdoor and Humid Tropics Biomes across contrasting management histories. There were two sampling locations within the Humid Tropics Biome (Figure 3.1a) and three within the Outdoor Biome (Figure 3.1b). Upon collection, samples were numbered and details of the sampling location, soil composition and management histories were recorded (Table 3.1).

In Phase 2, two soil pits were dug to allow for the sampling of a depth profile. The sites selected were both in the Humid Tropics Biome (Figure 3.1a). Locations provide examples of differing soil age, one original soil (~13 years old) and one relatively recently prepared soil (~2 years old), under similar management practices within the Humid Tropics Biome.

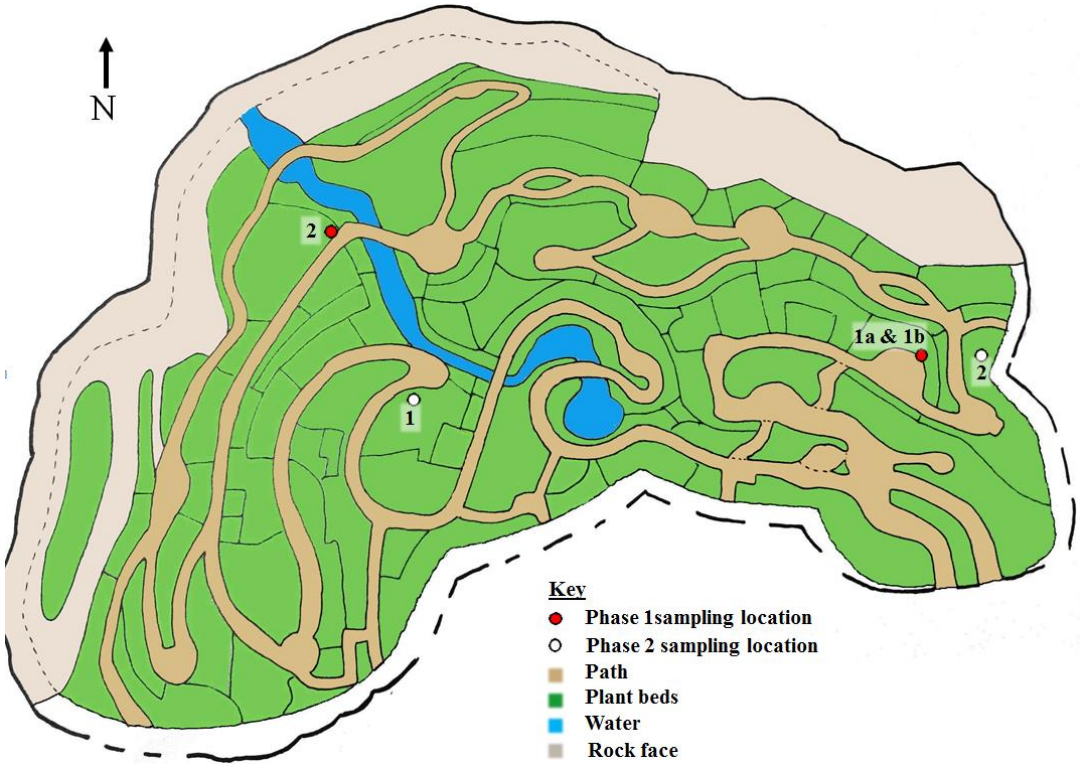

Figure 3.1a: *Map of the Rainforest Biome at the Eden Project including landscape cover and sampling locations.*

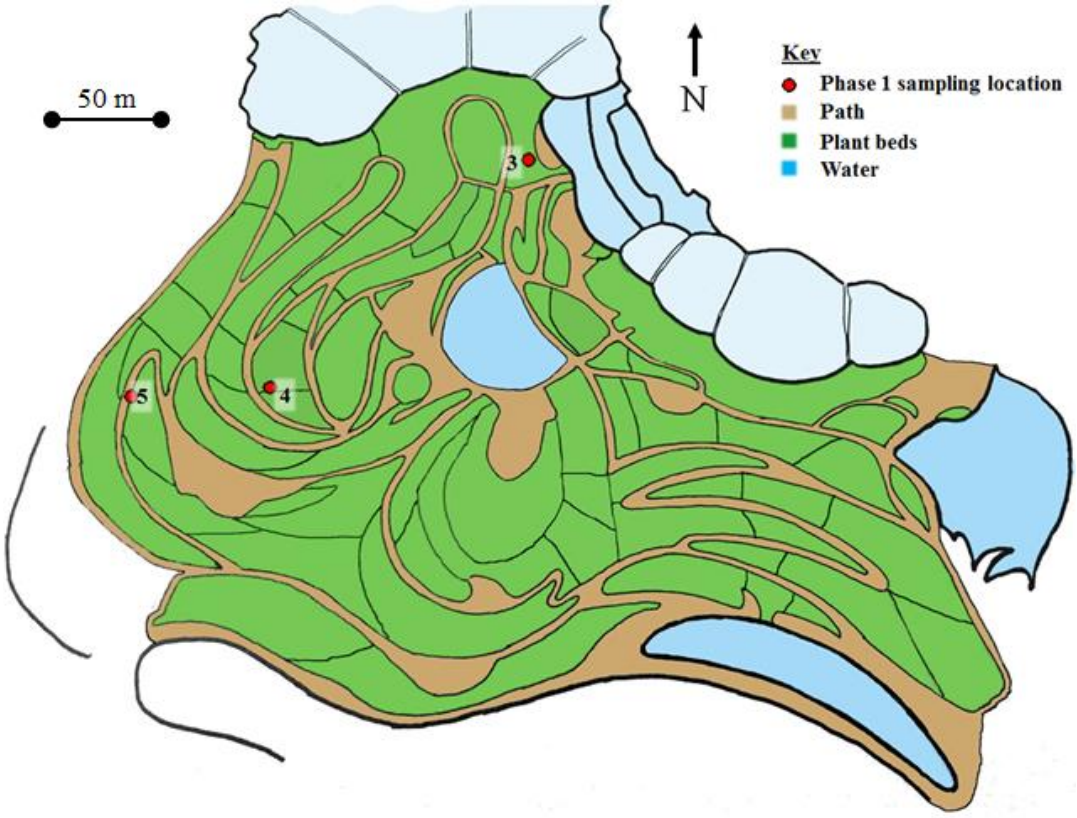

Figure 3.1b: *Map of the Outdoor Biome at the Eden Project including landscape cover and sampling locations.*

3.3.2 Soil sampling

The composition of the soils originally applied to the locations sampled, and the management practices, are detailed in Table 3.1

Phase 1: Initial sampling

Top soil samples were taken from the upper 0 to 10 cm depth, with litter cleared from the surface of the sampling site. Samples 1a, 1b and 2 were taken from the Humid Tropics Biome. Samples 1a and 2 were topsoil samples from locations with contrasting soil management practices, whilst sample 1b was a subsoil sample, taken from 25 to 30 cm depth at the same location as sample 1a. The top-soil depth was subject to variation across the site, typically starting between 10 and 30 cm depth. Samples 3 to 5 were all topsoil samples (0 to 10 cm) from the Outdoor Biome. Sample 3 was taken from a steep

slope, which was subject to biannual fertiliser applications (20 g m^{-2} Vitax® 214). Sample 4 was taken from a flat area which was subjected, annually, to a large number of fertiliser and compost applications. Sample 5 was taken from an area where the base material has been scarified and allowed to mature without fertiliser additions.

Phase 2: Depth profile sampling

The soil pits were dug until the depth of the base material was reached, which was marked by an immediate transition to a very dense clay layer at the base of the former china-clay pit, which could not be penetrated by the spade. Soil samples of ca. 100 g were taken at 5 cm depth intervals throughout the profile. Photographs of the soil profiles are shown in Figure 3.2. Neither of the areas from which Profile 1 and 2 were taken were planted at the time of sampling.

Table 3.1: *Details of the soil samples collected from the Eden Project on 26/01/2012 (samples 1 to 5 and 23/01/14 (Phases 1 and 2), including the original composition. These original soils have subsequently been subject to considerable amendment with mulches since the opening of the EP.*

Sample location	Location description	Soil composition	Notes
Phase 1			
1a	Humid Tropics Biome, under fruit trees, 0 – 10 cm.	25 % graded sand**, 65 % composted bark, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214) and with regular applications of composted green waste.
1b	Humid Tropics Biome, under fruit trees, 25 – 30 cm.	65 % graded sand**, 25 % composted bark, 10 % lignite clay.	Sub-soil
2	Humid Tropics Biome, leaf litter area, 0 – 10 cm depth.	65 % graded sand**, 25 % composted bark, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214) and leaf litter left on the soil surface.
3	Outdoor Biome, adjacent to bridge leading to biomes, 0 – 10 cm depth.	(General mix for outdoors beds) 65 % ungraded* sand (china clay waste product), 25 % composted green waste, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214).
4	Outdoor Biome, *Global Garden* area, 0 – 10 cm depth.	(General mix for outdoors beds) 65 % ungraded* sand (china clay waste product), 25 % composted green waste, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214) and regular applications of composted green waste.
5	Outdoor Biome, scarified clay surface, 0 – 10 cm depth.	Rock base-layer, scarified in two directions at right angles to a depth of approximately 500 mm at 1 m centres.	Base material which has been planted and allowed to colonise.
Phase 2			
1	Humid Tropics Biome Malaysian garden area.	Top 10 cm: 25 % graded sand**, 65 % composted bark, 10 % lignite clay. >10 cm: 65 % graded sand**, 25 % composted bark, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214) and regular applications of composted green waste. Original soil (approx. 13 years old).
2	Humid Tropics Biome, under fruit trees.	Top 10 cm: 25 % graded sand**, 65 % composted bark, 10 % lignite clay. >10 cm: 65 % graded sand**, 25 % composted bark, 10 % lignite clay.	Amended biannually with fertiliser (Vitax® 214) and regular applications of composted green waste. Recently produced soil (approx. 2 years old).

* Sand deemed ungraded if its particle size is 2 – 5 mm with 65 ± 10 % fines (< 2 mm) when oven dried.
** Sand deemed graded if particle size is 2 – 5 mm with 33 ± 5 %) fines when oven dried.

Profile 1 was located within the *Malaysian Garden* area of the Humid Tropics Biome. This site is one of the Biome's shallower soils, with a depth of ca. 60 cm. It has been subject to a biannual fertilisation regime and regular composted green waste application, typical of what has been used throughout the majority of the Humid Tropics Biome.

There were 2 distinct horizons visible within the soil profile, which are highlighted in Figure 3.2.

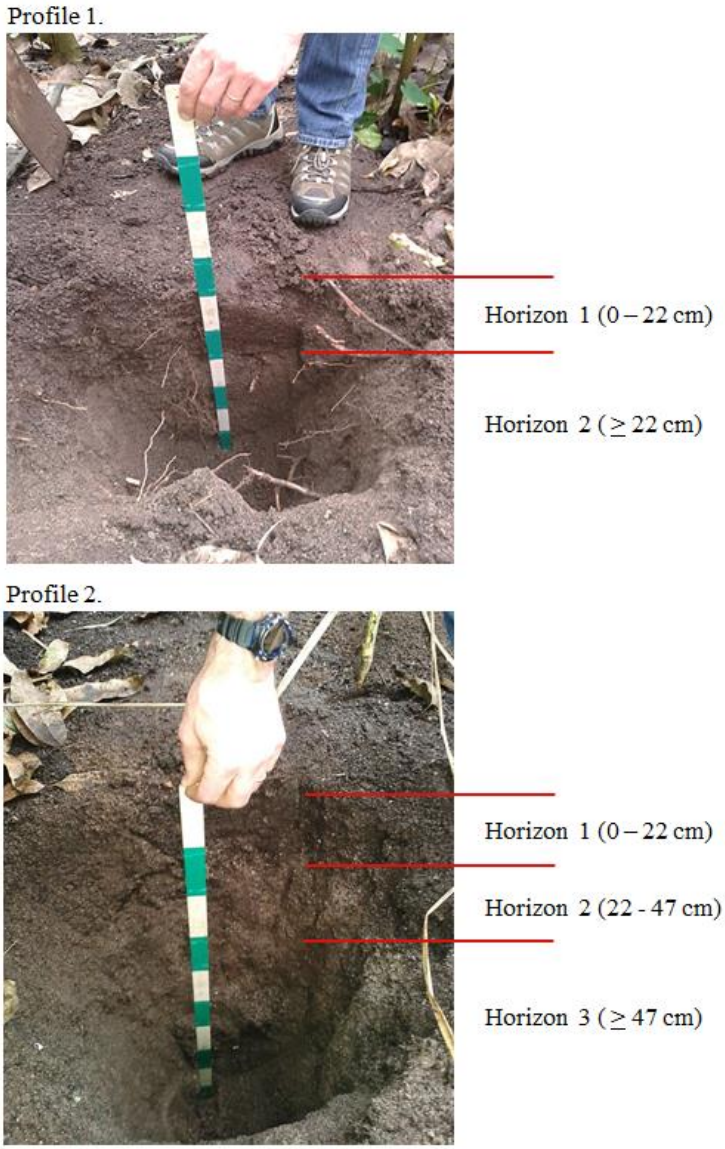

Figure 3.2: *Soil pits, dug at the Eden Project on 23/01/14, with lines indicating visible horizons within the soil profile. Green/white bands show 10 cm increments, though the scale is lost in Profile 2, due to difficult camera angle. Photographs were taken at the time of soil depth profile sampling.*

Profile 2 was located in the fruit tree area of the Humid Tropics Biomes. Until recently (2 years ago) this area had been under a banana plantation, however, due to an outbreak of Panama disease (*Fusarium oysporum f. sp. cubense* Tropical race 4), which required the soil in this area to be removed and replaced with a newly prepared mix. The depth of

this pit was ca. 80 cm and this area had been subjected to biannual fertiliser application and frequent mulch applications, in order to maintain a high organic matter content. Figure 3.2 highlights the position of 3 visible soil horizons within the soil profile, which indicate changes in soil composition.

3.3.3 Analytical Measurements

Following collection, samples were stored at 4 ^{o}C and analysed within 3 months, as recommended in ISO:10381-6 (1993). Both Phase 1 and 2 samples were analysed for pH, moisture content, total particulate carbon, total particulate nitrogen and particle size. For the Phase 1 samples sequential extractions using 2 M LiCl were used to measure exchangeable solid phase analytes, whilst the Phase 2 samples were extracted using high purity water (HPW). The extractants were analysed for nitrate + nitrite (referred to as nitrate), phosphate, ammonium, organic carbon and total nitrogen. Fluorescein diacetate analyses were carried-out on the Phase 2 samples to estimate enzymatic activity levels; these analyses were performed within 24 hours of sample collection.

Between Phase 1 and Phase 2 it was decided that HPW would be employed as an extractant for soil nutrients in place of the 2M LiCl. Details for all the general methods used for the characterisation of the soils collected during Phases 1 and 2 are described Chapter 2 and summarised in Figure 3.3. Results are reported in Sections 3.3 and 3.4.

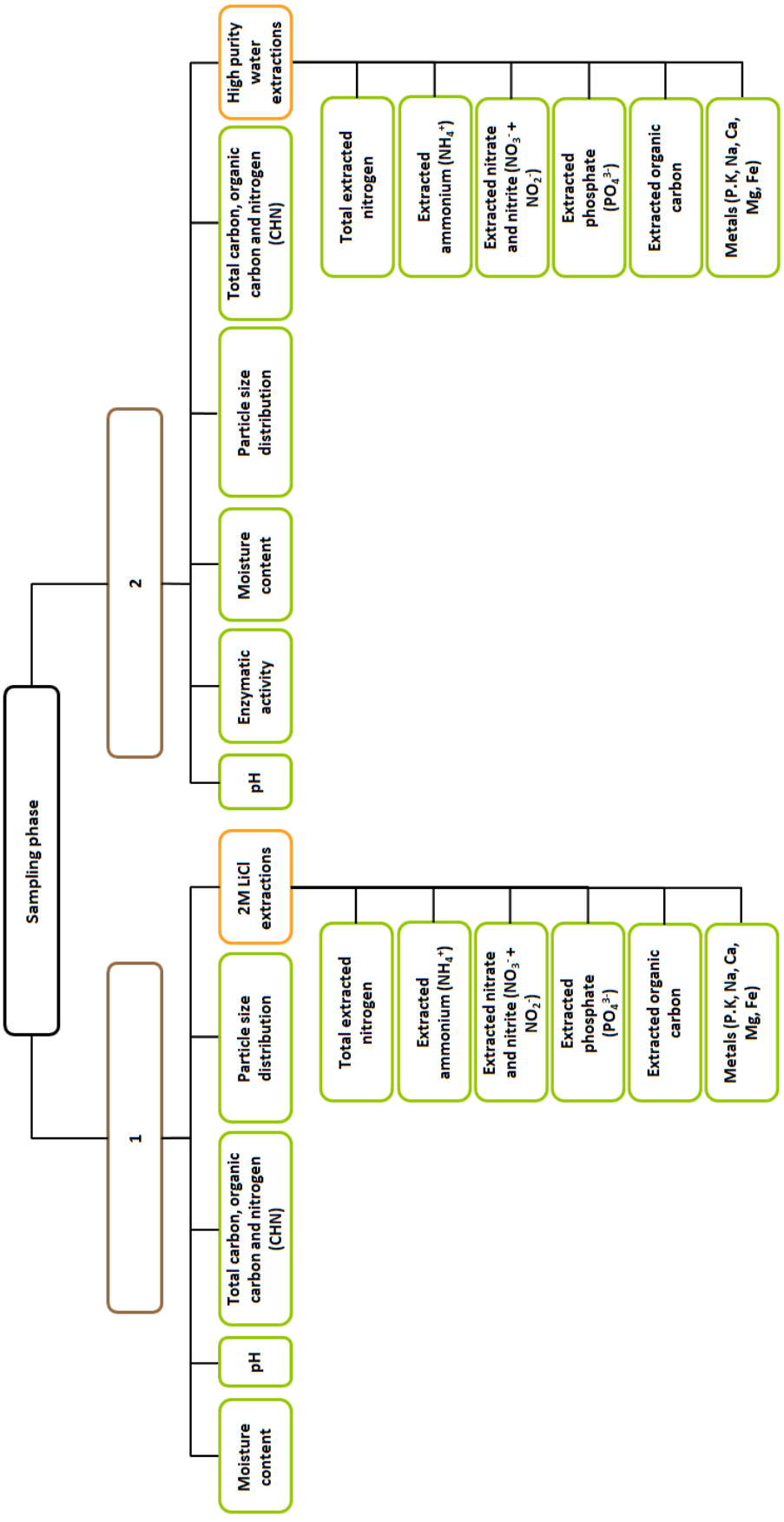

Figure 3.3: *A scheme detailing the analyses performed for the initial characterisation and depth profile of Eden Project.*

3.3.4 Statistical analyses

In order to determine the extent and significance of any differences between two sets of mean values, such as the Outdoor Biome against the Humid Tropics Biome, the fertilised against the unfertilised soils and between the two soil profiles, a one-way analysis of variance (ANOVA) test was employed. This allowed the determination of whether there were any significant differences between the two datasets (Miller and Miller, 2000).

For the Phase 1 and 2 samples a Pearson product-moment correlation coefficient (PCC) was also employed. This test allowed for the determination of whether a linear relationship existed between two datasets, making no assumption as to whether one variable is dependent on the other. The PCC test yields a value between -1 and 1, the closer the value is to 0 the greater the variation between the data points around the line of best fit. A positive value suggests positive correlation and a negative value suggests negative correlation.

The dataset for the Phase 1 soils is small and, as such, any correlations found through statistical analyses of the data suggest a relationship between the soil characteristics rather than offering any definitive confirmation.

3.4 Phase 1: Site characterisation

3.4.1 Results: Initial characterisation

Results for the characterisation of soils across the Eden Project site were comprised of three observations: a comparison of all sampling locations, the average values for soils from the Outdoor and Humid Tropics Biomes, and the average for two management practices:, regular fertiliser addition and no addition.

ANOVA analysis indicated that there was no significant difference ($P > 0.05$) between the Outdoor and Humid Tropics Biomes for any of the characteristics analysed (Table 3.2a). The ANOVA analysis of the management practices (Table 3.2b) suggested that significant differences exist between the TPN, TPC and sand and silt particle size fractions of the analysed soils.

Table 3.2a: *ANOVA comparison of the key soil characteristics at sites within the Outdoor Biome (samples 1a, 1b and 2) vs. the Humid Tropics Biome (samples 3, 4 and 5).*

Soil characteristic	F value	P value
TPN	1.95	0.236
TEN	1.07	0.360
$NO_3^- + NO_2^-$	0.24	0.649
NH_4^+	5.26	0.084
EON	3.24	0.146
$NH_4^+ : NO_3 + NO_2$ ratio	2.06	0.225
PO_4^{3-}	0.06	0.821
TPC	0.37	0.575
POC	2.23	0.157
EOC	5.05	0.088
C : N ratio	5.52	0.079
pH	0.72	0.445
Moisture content	0.86	0.405
Sand	0.17	0.701
Silt	0.18	0.691
Clay	0.75	0.436

Red = significant p values
Blue = insignificant p values

Table 3.2b: *ANOVA comparison of the key soil characteristics at sites subjected to different management practices, under regular fertiliser application (samples 1a, 3, and 4) vs. no regular fertiliser application (samples 2 and 5).*

Soil characteristic	F value	P value
TPN	12.3	0.025
TEN	6.92	0.058
NO_3^- + NO_2^-	1.75	0.256
NH_4^+	< 0.001	0.989
EON	2.25	0.209
NH_4^+ : NO_3 + NO_2 ratio	0.61	0.478
PO_4^{3-}	0.76	0.432
TPC	12.6	0.024
POC	35.2	< 0.001
EOC	1.45	0.295
C-N ratio	5.63	0.077
pH	0.24	0.651
Moisture content	0.88	0.402
Sand	12.8	0.023
Silt	13.1	0.022
Clay	0.57	0.491

Red = significant p values
Blue = insignificant p values

3.4.1.1 Nitrogen

Results from the N analyses are shown in Figure 3.4. Values demonstrate little variation between soils. The comparison of results from each Biome indicated no significant differences between any N fractions. However, when management practices were compared, a significant difference (p = 0.025, Table 3.2b) between the TPN values was observed.

The TPN concentration was highest in soil 4 (11.3 ± 2.5 mg N g^{-1}), with soils 1a (5.98 ± 1.45 mg N g^{-1}) and 3 (6.79 ± 0.25 mg N g^{-1}) also displaying high concentrations. TEN represented the combined extractable inorganic (NO_3^- + NO_2^- and NH_4^+) and EON concentrations in the soils. The TEN concentrations for all soils were significantly lower than the TPN values, representing between 1.27 % and 4.78 % of the TPN. Non-extractable N fractions consistently represented > 95 % of TPN for all soils.

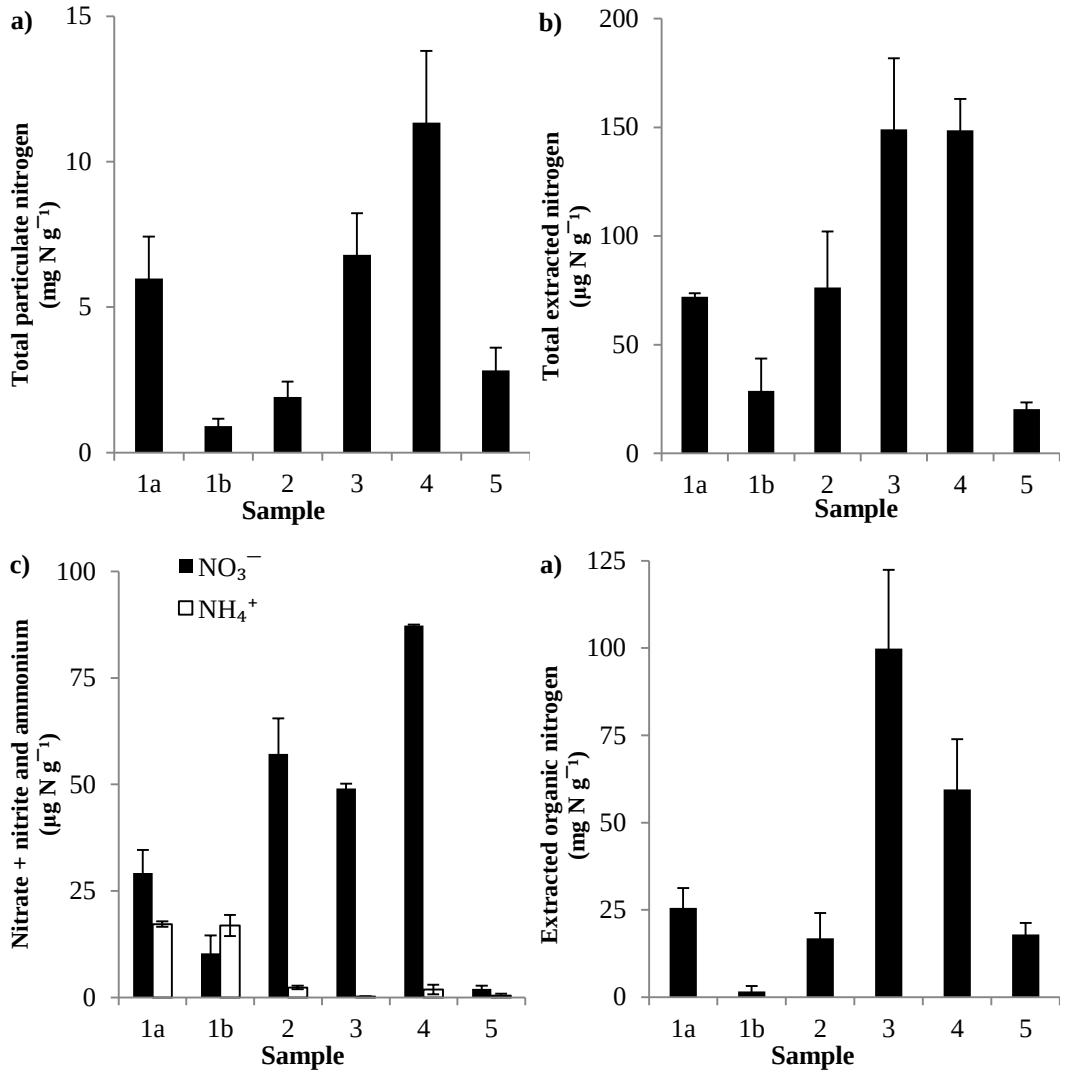

Figure 3.4: *Concentration of N fractions and species for each sampling location within the Eden Project Biomes. Mean and standard deviation were calculated from triplicate soil samples:* ***a)*** *TPN concentration (mg N* g^{-1}*)* ***b)****TEN concentration (µg* g^{-1}*).****c)*** NO_3^- *concentration (µg N* g^{-1}*) and* NH_4^+ *concentration (µg N* g^{-1}*)* ***d)*** *EON concentration (mg N* g^{-1}*).*

The lowest TEN concentrations occurred in soils 1b (28.8 ± 14.8 µg N g^{-1}) and 5 (20.4 ± 3.1 µg N g^{-1}). TEN concentrations were highest in soils 3 (149 ± 33 µg N g^{-1}) and 4 (149 ± 14 µg N g^{-1}), both of which were outdoor soils and subject to regular fertiliser applications, as described in Table 3.1. The representative percentage N fractions from which the TEN was composed for each soil sample are shown Table 3.3.

Table 3.3: *Percentage composition of the total extracted nitrogen fraction extracted from the soils using 2 M LiCl.*

Sample	Percentage composition			
	TEN	**NO_3^-**	**NH_4^+**	**EON**
1a	100	40.6	23.9	35.5
1b	100	35.8	58.5	5.65
2	100	74.9	3.05	22.1
3	100	32.9	0.12	67.0
4	100	58.7	1.27	40.0
5	100	9.69	2.00	88.3

The inorganic N concentrations (NO_3^- and NH_4^+) are shown in Figure 3.4. NO_3^- concentrations were highest for soils 2 (57.1 ± 8.3 µg N g^{-1}), 3 (49.0 ± 1.1 µg N g^{-1}) and 4 (87.3 ± 0.2 µg N g^{-1}), which had comparatively low NH_4^+ concentrations (2.33 + 0.39 µg N g^{-1}, 0.17 + 0.10 µg N g^{-1} and 1.88 + 1.12 µg N g^{-1} respectively). NH_4^+ concentrations were highest in soils 1a (17.2 ± 0.7 µg N g^{-1}) and 1b (16.9 ± 2.5 µg N g^{-1}), with the latter exceeding the NO_3^- concentration. Soil 5 had considerably lower concentrations of NO_3^- (1.98 ± 0.76 µg N g^{-1}) and NH_4^+ (0.41 ± 0.46 µg N g^{-1}). The highest extracted organic nitrogen (EON) concentrations (Figure 3.4) were observed for sample 3 (up to 99.9 µg N g^{-1}), whilst the lowest (1.63 ± 1.56 µg N g^{-1}) were observed within sample 1b.

Mean NH_4^+ : NO_3^- ratios are shown in Figure 3.5. Soil 1b, the sub-soil sample, had the highest NH_4^+ : NO_3^- ratio (1.63), with soils 1a and 5 having intermediate values (0.59 and 0.21 respectively). Ratios for soils 2 and 4 were low (0.04 and 0.02, respectively) and the ratio for soil 3 was 0.00, due to particularly low NH_4^+ concentrations. ANOVA analyses suggested that there was no significant difference between either environmental conditions (p = 0.225, Table 3.2a) or management practices (p = 0.478, Table 3.2b).

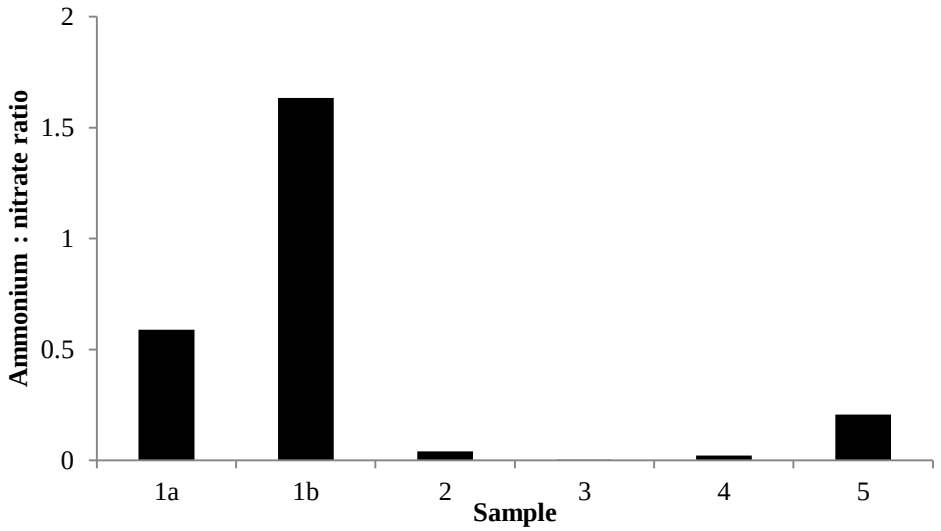

Figure 3.5: *NH_4^+ : NO_3^- ratios for each soil sampling location. Calculated using the mean values from the ammonium and nitrate + nitrite analyses.*

3.4.1.2 Phosphate

PO_4^{3-} concentrations, (Figure 3.6) were highest in soils 2 (5.54 ± 0.10 μg g^{-1}), 3 (5.12 ± 1.46 μg g^{-1}) and 4 (4.59 ± 1.02 μg g^{-1}). PO_4^{3-} concentration was lowest in soil 5 (0.82 ± 0.05 μg g^{-1}). ANOVA analyses determined that there was no significant difference between either of the Biomes (p = 0.821, Table 3.2a) or management practices (p = 0.432, Table 3.2b).

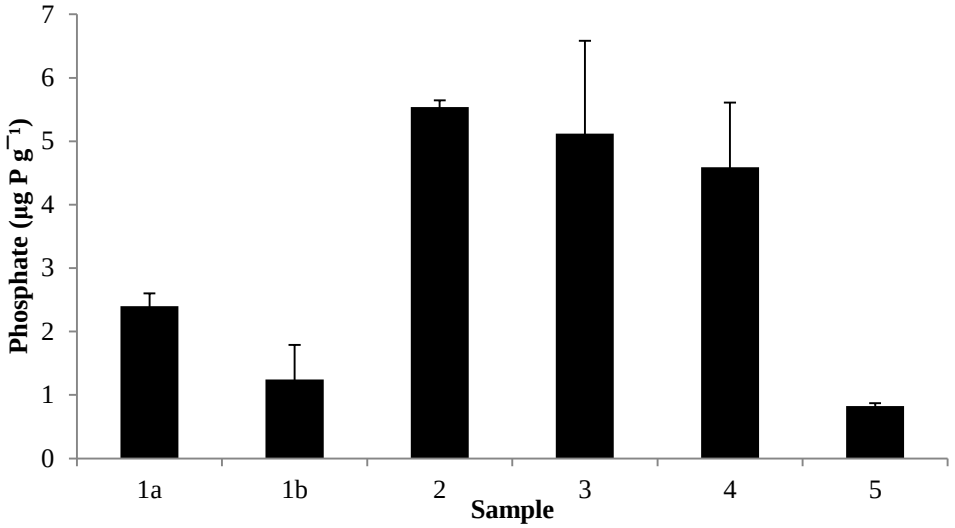

Figure 3.6: *PO_4^{3-} concentrations for each sampling location. Mean and standard deviations were calculated from triplicate samples at each location.*

3.4.1.3 Carbon

TPC and EOC results are shown in Figure 3.7. TPC concentrations were highest in soils 1a (172 ± 11.3 mg g^{-1}) and 4 (136 ± 53 mg g^{-1}), which was consistent with the regular mulch applications to the surface of these soils. Soil 1b had the lowest TPC concentration (53.8 ± 10.8 mg g^{-1}), which was anticipated for a subsoil sample. Soil 2 had the lowest TPC concentration of the top soil samples (83.5 ± 8.2 mg g^{-1}). ANOVA analyses suggest that there is a significant difference in TPC concentration between the different management practices ($p = 0.024$, Table 3.2b).

Soil organic carbon (SOC) concentrations were highest in soils 1a (135 ± 17 mg g^{-1}) and 4 (127 ± 20 mg g^{-1}). The lowest concentration was within sample 1b (sub-soil) (29.6 ± 6.7 mg g^{-1}) and the lowest topsoil concentration in sample 2 (38.9 ± 11.4 mg g^{-1}). ANOVA analyses suggest a significant difference in SOC concentration between the different management practices ($p < 0.001$).

Extracted organic carbon (EOC) concentrations were highest in soils 3 (2.62 ± 0.18 mg g^{-1}) and 4 (3.05 ± 0.09 mg g^{-1}) and lowest in soil 1 with the topsoil sample (1a) having the lowest concentration amongst the topsoils (1.05 ± 0.17 mg g^{-1}). With the exception of soil 1a, EOC values followed a similar pattern to the TPC values. ANOVA analyses revealed no significant differences between either the Biomes ($p = 0.088$, Table 3.2a) or the management practices ($p = 0.295$, Table 3.2b).

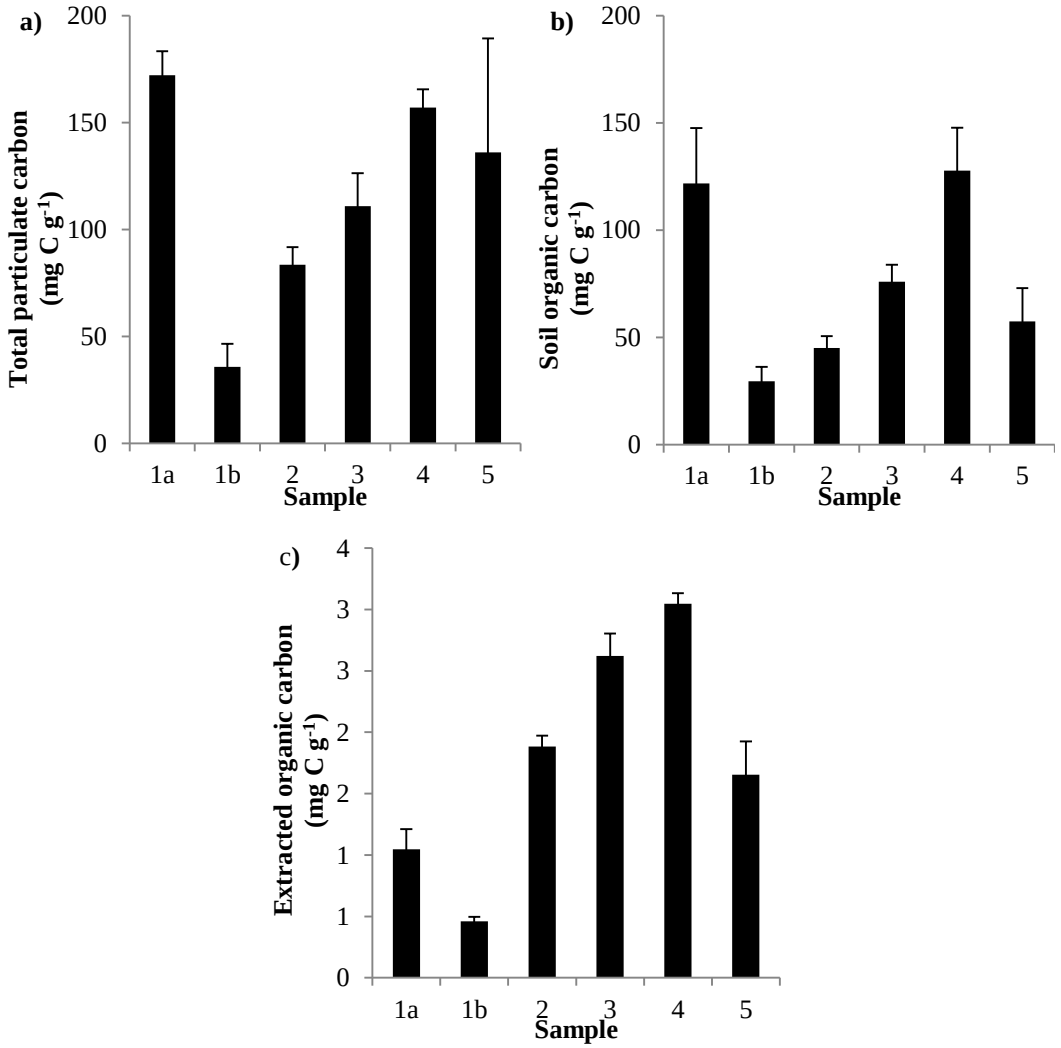

Figure 3.7*: Concentration of C fractions for each sampling location within the Eden Project Biomes. Mean and standard deviation calculated from triplicate samples.* ***a)*** *TPC concentrations (mg* g^{-1}*),* ***b)*** *POC concentrations (mg* g^{-1}*),* ***c)*** *EOC concentration (µg* g^{-1}*).*

3.4.1.4 Carbon : nitrogen ratio

Mean C : N ratios were calculated from the total particulate carbon and total particulate nitrogen data shown in Figure 3.8. The values varied with regard to management practices and Biome across the Eden Project site; however, ANOVA analyses indicate that these differences were not significant ($p = 0.077$ environmental conditions, $p = 0.079$ management practices). Soil 1b (sub-soil) had the highest ratio (32.6) and soils 1a, 2 and 5 ratios were similar (20.4, 23.6 and 20.3, respectively). Soils 3 and 4 showed the lowest values, which were also similar (11.2 and 11.3 respectively).

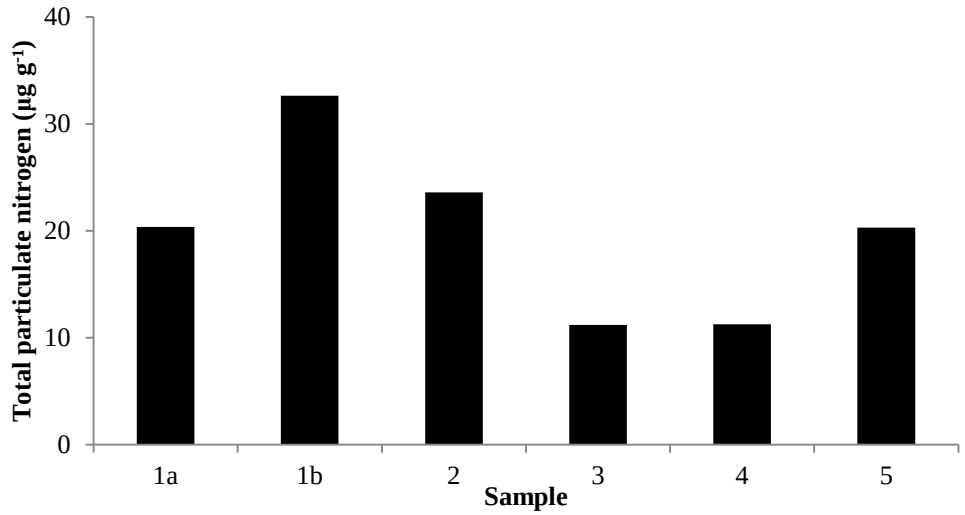

Figure 3.8*: Carbon : nitrogen ratios for each sampling location. Calculated from total particulate carbon and total particulate nitrogen*

3.4.1.5 Physio-chemical characteristics

Figure 3.9 shows the pH values. Soils 1a and 2, the two topsoil samples from the Humid Tropics Biome, were very similar (pH ~ 6.7). The Outdoor Biome soils demonstrated a wider pH range, (6.0 to 7.9). ANOVA analyses confirmed that there was no significant inter-sample variation (p = 0.445, Table 3.2a - environmental conditions, p = 0.651, Table 3.2b - management practices).

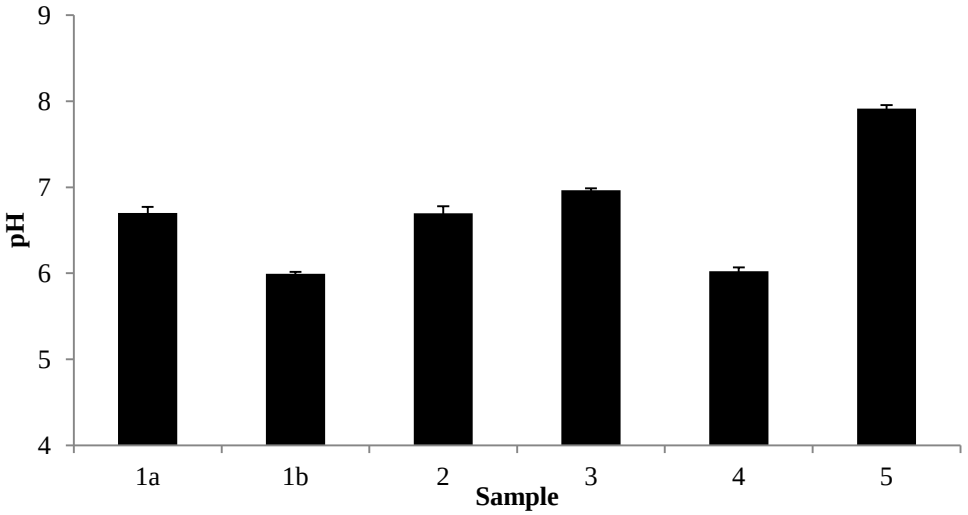

Figure 3.9: *pH values for each sampling location. Mean and standard deviation calculated from triplicate samples.*

Moisture content (Figure 3.10) in top soils ranged from 34.0 ± 1.7 %, (soil 4) to 24.4 ± 0.3 % (soil 3). The sub soil sample (1b) had a moisture content of 14.1 ± 1.1 %. ANOVA analyses indicated no significant variation between the either the Biomes or management practices.

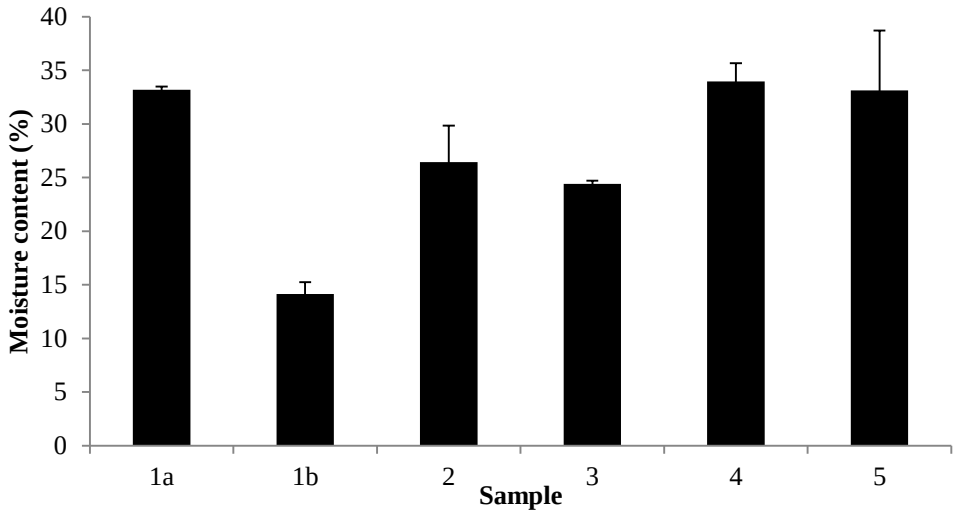

Figure 3.10: *Moisture content values for each sampling location. Mean and standard deviation calculated for triplicate samples from each location.*

Particle size fraction analyses results are shown in Figure 3.11. There was little variation in the clay content between soil samples (p = 0.436, Table 3.2a - environmental conditions, p = 0.491, Table 3.2b - management practices), with all soils having a clay fraction < 1 %. The largest fraction was sand, which accounted for > 50 % in all soils. There was a significant difference between management practices with regard to the proportions of sand (p = 0.023, Table 3.2b) and silt (p = 0.022, Table 3.2b) within the samples (ANOVA). Figure 3.12 indicates that all soils fell within the sandy loam texture category based on the data in Table 3.6.

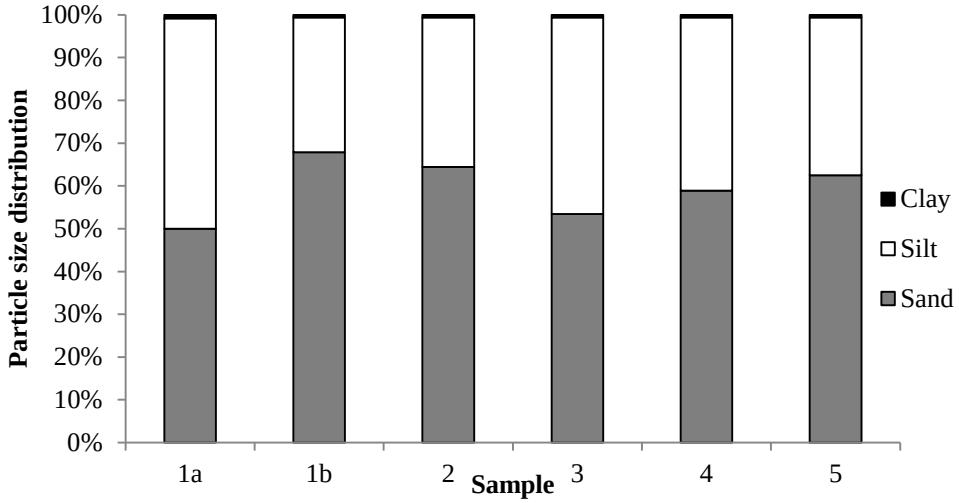

Figure 3.11: *Particle size fractionation for each sampling location. Mean and standard deviations were calculated for triplicate samples from each location.*

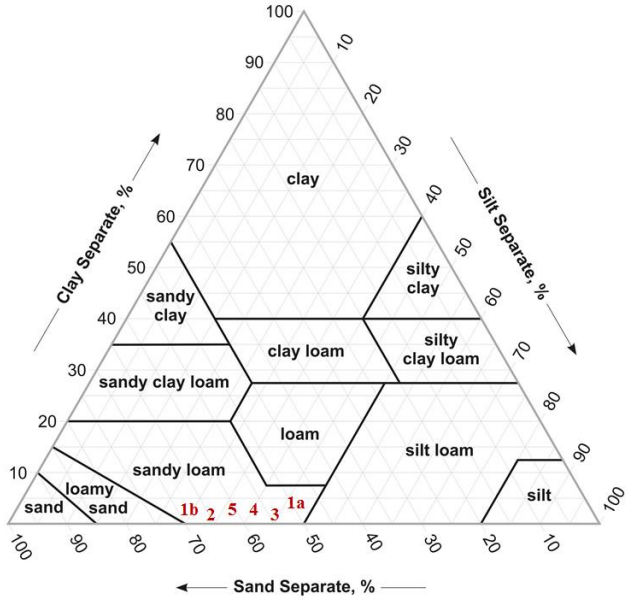

Figure 3.12: *Sampling location texture classes according to ISO14688-1 classification.*

3.4.2 Discussion: Initial characterisation

The results provide a basis from which to determine the nutrient characteristics of a range of soils across the Eden Project site. This discussion focuses on the implications of these characteristics.

The high NH_4^+ : NO_3^- ratios observed within samples 1a (0.59) and 1b (1.63), in comparison to low ratio observed in sample 2 (0.04), also from the Humid Tropics

Biome, may be explained, in part, by the plant types present at this location, as some plants will take up NO_3^- in preference to NH_4^+ (Serna et al., 1992). Before N can be utilised by a plant, it must first be metabolised from its assimilated form. NH_4^+ is metabolised rapidly following uptake, within the roots of a plant, where it reacts with sugars transported from the production site in the leaves, to the roots. NO_3^-, however, is transported to the plant's leaves to be metabolised, where it is first reduced to NH_4^+, before reacting with the sugars. At higher temperatures, a plant's respiration rate is increased (Brady and Weil, 2008), causing the plant to consume sugars at a faster rate. This makes the sugars less available for transport to the roots and involvement in NH_4^+ metabolism (Brady and Weil, 2008).

It could, however, also be argued that the plants present within the Humid Tropics Biome are those which have evolved in warmer regions and, therefore, should be capable of functioning efficiently at higher temperatures. The differences observed may be further accounted for by the differing management practices, with regular composted green waste applications and biannual fertiliser applications being employed at the site of samples 1a and 1b, whereas site 2 was subjected to biannual fertiliser applications, with the leaf litter remaining on the soil surface.

C : N ratios were > 20 for all Humid Tropics Biome soils and one of the Outdoor Biome soils. This was high compared with a number of reported values, such as subtropical forest (11 to 14), arable (9.6 to 15.0) and grass pasture (9.4 to 12.5) (Brady and Weil, 2008; Brookes et al., 1985; Burton et al., 2007; Hood-Nowotny et al., 2010). However, it was consistent with the wide range of values commonly found within the A horizons in tropical regions, where ratio values > 30 are not uncommon (Brady and Weil, 2010).

Sample 1b had the highest C : N ratio (32.63), whilst the TPC concentration within subsoil sample 1b (35.8 ± 10.8 mg C g^{-1}) was lower than that of sample 1a (172 ± 11 mg C g^{-1}); the TPN concentration was lower still, in relative terms(1a = 5.98 ± 1.40 mg N g^{-1} and 1b = 0.91 ± 0.30 mg N g^{-1}). The high TPC concentration within sample 5 (136 ± 53 mg C g^{-1}) was determined to be mostly attributable to inorganic C, through subtraction of the SOC concentration (5.74 mg C g^{-1}), which may suggest that the soil contained a high carbonate concentration, which is a consequence of the soil being composed of the scarified surface of the former china-clay pit floor.

Phosphate is thought to be relatively immobile in most soils because it binds to mineral surfaces (Brady and Weil, 2010; Oelkers et al., 2008), and dissolved PO_4^{3-} tends to be removed from the soil solution, forming compounds which have low solubility (Brady and Weil, 2010). It has been suggested that exchangeable PO_4^{3-} concentration is closely related to soil pH, with maximum solubility found in soils with pH 5 to 7 (Brady and Weil, 2010). The pH range in samples 1a to 4 was 5.99 ± 0.02 to 6.96 ± 0.02, with sample 5 having a pH of 7.90 ± 0.04, therefore, it may be inferred that PO_4^{3-} is at a high solubility within soils 1a to 4, however, in order to further support this a greater number of samples would be required with soils with a range of pH values.

Sand represented a substantial size fraction (> 50 %) within all soil samples, with the clay fraction comprising < 1 %. This implies that the soil surface area is relatively small and, as such, the number of potential exchange sites is reduced. Whilst a larger sand fraction aids the drainage of water through the soil, it is possible that if drainage is too efficient nutrients may be leached from the soil, leading to low concentrations of inorganic N, which is more labile than organic N (Brady and Weil, 2010). One

explanation for the small clay fraction is that over time the smaller fractions have been transported down through the soil profile to be lost in leachate, resulting in the high proportion of larger soil particles. An alternative explanation may be that the clay fraction was lost during the laboratory preparation and analysis stage. However, steps were taken to ensure that any loss was minimised and standard errors between samples are low, which supports the idea that the clay fraction was low for all soils.

Soils within the Humid Tropics Biome (samples 1a, 1b and 2) were watered using drip irrigation systems, whereas soils within the Outdoor Biome (3, 4 and 5) were watered mainly through natural precipitation, with irrigation employed at times of low rainfall. Despite the differing watering mechanisms, moisture content was relatively consistent across the site (ranging from 24.4 ± 0.3 to 33.2 ± 0.32 %), with the exception of sample 1b (14.1 ± 1.1 %), which was anticipated, as it was a subsoil sample, and by design, contained a large sand fraction to aid drainage. The moisture content was likely to have been affected by the particle size composition of the samples and, with similar compositions it follows that they would have similar moisture contents. The use of drip irrigation within the Humid Tropics Biome may also account for the lower nutrient concentrations observed within the samples from the Humid Tropics, particularly 1a, which was subject to the same fertiliser and amendment regime (Table 3.1) as samples 3 and 4, but displayed significantly lower nutrient concentrations.

Considering the minimal variation in the base composition of the soil mix for locations 1 to 4, it is unsurprising that little variation was observed between sampling locations, further to this, the results suggest that soil management practices, such as amendment

additions and plantings had a greater impact on the soil characteristics than the difference in environmental conditions between the two Biomes. Sample 5, which consisted of the scarified and colonised floor of the china-clay pit, differed from the other samples and for a number of characteristics may be considered anomalous.

3.5 Phase 2: Depth profiles of Humid Tropics soils

3.5.1 Results: Depth profiles

The Phase 2 samples were collected to observe the effect of profile depth on soil parameters and chemistry for 2 separate locations of differently aged soils, under similar management practices within the Humid Tropics Biome as described in Table 3.1 (locations shown in Figure 3.1a). Profile 1 was located within the *Malaysian garden* area of the Humid Tropics Biome, which was 60 cm depth and subjected to regular fertiliser (Vitax 214) applications over the 13 years since the Eden Project's opening. Profile 2 was located in the fruit trees area of the Humid Tropics Biome and due to a recent disease break out, the soil in this area was a relatively new mix (2 years old). Neither of the areas from which Profile 1 and Profile 2 were taken were planted at the time of sampling.

Data for both profiles were analysed using the one-way ANOVA statistical test (Table 3.4). The results show that only TEN, NO_3^- + NO_2^-, EON, extracted Ca, extracted Fe and enzymatic activity levels were significantly different.

In general both profiles showed a gradual decline in nutrient concentrations with increasing depth. Pearson correlation coefficient analysis found that all nutrient characteristics (excepting EOC) showed a significant ($p < 0.05$) negative correlation

with depth in both soil profiles (shown in Tables 3.5a and b). In general, there was variation in correlating characteristics between Profiles 1 and 2. Phosphate concentration and moisture content correlated with all nutrient characteristics. This suggests that these two properties were either dependent on or influential over other nutrient properties within both soils.

Table 3.4: *ANOVA values for key soil parameters within profiles 1 and 2 (n =9).*

Soil characteristic	F value	p value
TPN	2.21	0.157
TEN	10.16	0.006
NO_3^- + NO_2^-	11.44	0.004
NH_4^+	0.25	0.623
EON	10.50	0.005
NH_4^+ : NO_3 + NO_2 ratio	4.07	0.061
PO_4^{3-}	1.71	0.209
TPC	1.30	0.272
POC	1.62	0.222
EOC	0.96	0.342
Extracted Mg	2.01	0.177
Extracted Ca	7.36	0.015
Extracted K	0.84	0.372
Extracted Fe	15.49	0.001
C : N ratio	1.51	0.237
pH	1.99	0.178
Moisture content	1.15	0.299
Sand	0.01	0.924
Silt	0.01	0.915
Clay	0.01	0.915
Enzymatic activity	5.82	0.028

Red = significant p values
Blue = insignificant p values

Table 3.5a: *Pearson correlation coefficient values for key soil parameters within depth profile 1.*

Profile 1													
	Depth	**TPN**	**TEN**	**NO_3^- + NO_2^-**	**NH_4^+**	**TPC**	**EOC**	**PO_4^-**	**pH**	**Moisture content**	**Sand**	**Silt**	**Clay**
TPN	-0.903 0.002												
TEN	-0.873 0.005	0.953 <0.001											
NO_3^- + NO_2^-	-0.863 0.006	0.945 <0.001	0.998 <0.001										
NH_4^+	-0.779 0.023	0.678 0.064	0.840 0.009	0.850 0.008									
TPC	-0.882 0.004	0.896 0.003	0.966 <0.001	0.965 0.002	0.905 0.002								
EOC	-0.695 0.056	0.647 0.083	0.803 0.016	0.813 0.014	0.927 0.001	0.879 0.004							
PO_4^-	-0.910 0.002	0.955 <0.001	0.951 <0.001	0.959 <0.001	0.790 0.020	0.932 0.001	0.731 0.040						
pH	-0.243 0.562	0.520 0.187	0.337 0.414	0.305 0.462	-0.194 0.646	0.155 0.713	-0.236 0.574	0.299 0.471					
Moisture content	-0.778 0.023	0.771 0.025	0.918 0.001	0.925 0.001	0.965 <0.001	0.934 0.001	0.947 <0.001	0.834 0.010	-0.028 0.947				
Sand	0.379 0.354	-0.537 0.169	-0.586 0.127	-0.583 0.129	-0.444 0.270	-0.468 0.242	-0.523 0.183	-0.435 0.281	-0.283 0.497	-0.604 0.113			
Silt	-0.335 0.418	0.537 0.169	0.543 0.164	0.541 0.166	0.392 0.337	0.418 0.303	0.476 0.234	0.394 0.334	0.295 0.478	0.557 0.152	-0.998 <0.001		
Clay fraction	-0.671 0.069	0.503 0.204	0.835 0.010	0.830 0.011	0.785 0.021	0.789 0.020	0.817 0.013	0.692 0.057	0.157 0.711	0.886 0.003	-0.892 0.003	0.863 0.006	
Enzymatic activity	-0.889 0.003	0.827 0.011	0.927 0.001	0.922 0.001	0.936 0.001	0.954 <0.001	0.895 0.003	0.864 0.006	0.059 0.891	0.952 <0.001	-0.450 0.263	0.397 0.330	0.793 0.019

Black = PCC value (between 1 and -1).
Red = significant p values
Blue = insignificant p values

Table 3.5b: *Pearson correlation coefficient values for key soil parameters within soil depth profile 2.*

Profile 2													
	Depth	**TPN**	**TEN**	**NO_3^- + NO_2^-**	**NH_4^+**	**TPC**	**EOC**	**PO_4^-**	**pH**	**Moisture content**	**Sand**	**Silt**	**Clay**
TPN	-0.898 <0.001												
TEN	-0.872 0.001	0.874 0.001											
NO_3^- + NO_2^-	-0.778 0.008	0.792 0.006	0.982 <0.001										
NH_4^+	-0.923 <0.001	0.978 <0.001	0.858 0.002	0.777 0.008									
TPC	-0.942 <0.001	0.948 <0.001	0.924 <0.001	0.856 0.002	0.931 <0.001								
EOC	-0.331 0.351	0.193 0.593	0.408 0.242	0.415 0.233	0.170 0.638	0.439 0.205							
PO_4^-	-0.849 0.002	0.986 <0.001	0.869 0.001	0.793 0.006	0.909 <0.001	0.909 <0.001	0.123 0.736						
pH	-0.842 0.002	0.912 <0.001	0.770 0.009	0.689 0.028	0.810 0.004	0.810 0.004	-0.143 0.694	0.921 <0.001					
Moisture content	-0.906 <0.001	0.993 <0.001	0.907 <0.001	0.834 0.003	0.970 <0.001	0.961 <0.001	0.254 0.478	0.981 <0.001	0.892 0.001				
Sand	0.773 0.009	-0.736 0.007	-0.669 0.034	-0.540 0.107	-0.721 0.019	-0.787 0.007	-0.316 0.373	-0.795 0.006	-0.650 0.042	-0.785 0.007			
Silt	-0.754 0.012	0.788 0.007	0.664 0.036	0.535 0.111	0.718 0.019	0.773 0.009	0.279 0.435	0.805 0.005	0.659 0.038	0.785 0.007	-0.998 <0.001		
Clay	-0.730 0.016	0.474 0.166	0.503 0.138	0.408 0.242	0.498 0.143	0.679 0.031	0.672 0.033	0.378 0.281	0.304 0.393	0.503 0.138	-0.661 0.038	0.615 0.058	
Enzymatic activity	-0.482 0.159	0.516 0.127	0.691 0.027	0.722 0.018	0.902 <0.001	0.653 0.041	0.691 0.027	0.495 0.146	0.208 0.565	0.577 0.081	-0.516 0.127	-0.501 0.140	0.519 0.125

Black = PCC value (between 1 and -1).
Red = significant p values
Blue = insignificant p values

3.5.2.1 Nitrogen

Results from the analyses of N components are shown in Figure 3.13. Profile 2 had higher concentrations of TPN within the upper 50 cm of the Profile (shown in Figure 3.13a). The highest TPN concentrations occurred within the upper 5 cm of soil for both Profiles, and concentrations decreased with depth. The lowest concentration for both Profiles occurred below 45 cm, being 1.11 ± 0.10 mg N g^{-1} at 45 to 50 cm in Profile 1 and 1.00 ± 0.04 mg N g^{-1} at 55-60 cm in Profile 2. The smallest N fraction was NH_4^+, concentrations of which ranged from 0.44 ± 0.02 to 3.03 ± 0.45 µg N g^{-1} for Profile 1 and from 0 (< LOD) to 5.37 ± 0.83 µg N g^{-1} for Profile 2.

A N concentration gradient can be seen throughout both profiles, with higher N concentrations observed in the upper layers. In general, the Profile 2 soil had higher N concentrations than profile 1.

TPN concentrations (Figure 3.13a) were consistently higher in Profile 2 than 1 from 0 to 50 cm depth, though this was not statistically significant ($p > 0.05$, Table 3.4). Profiles 1 and 2 showed a distinct decrease in TPN concentration with increasing depth.

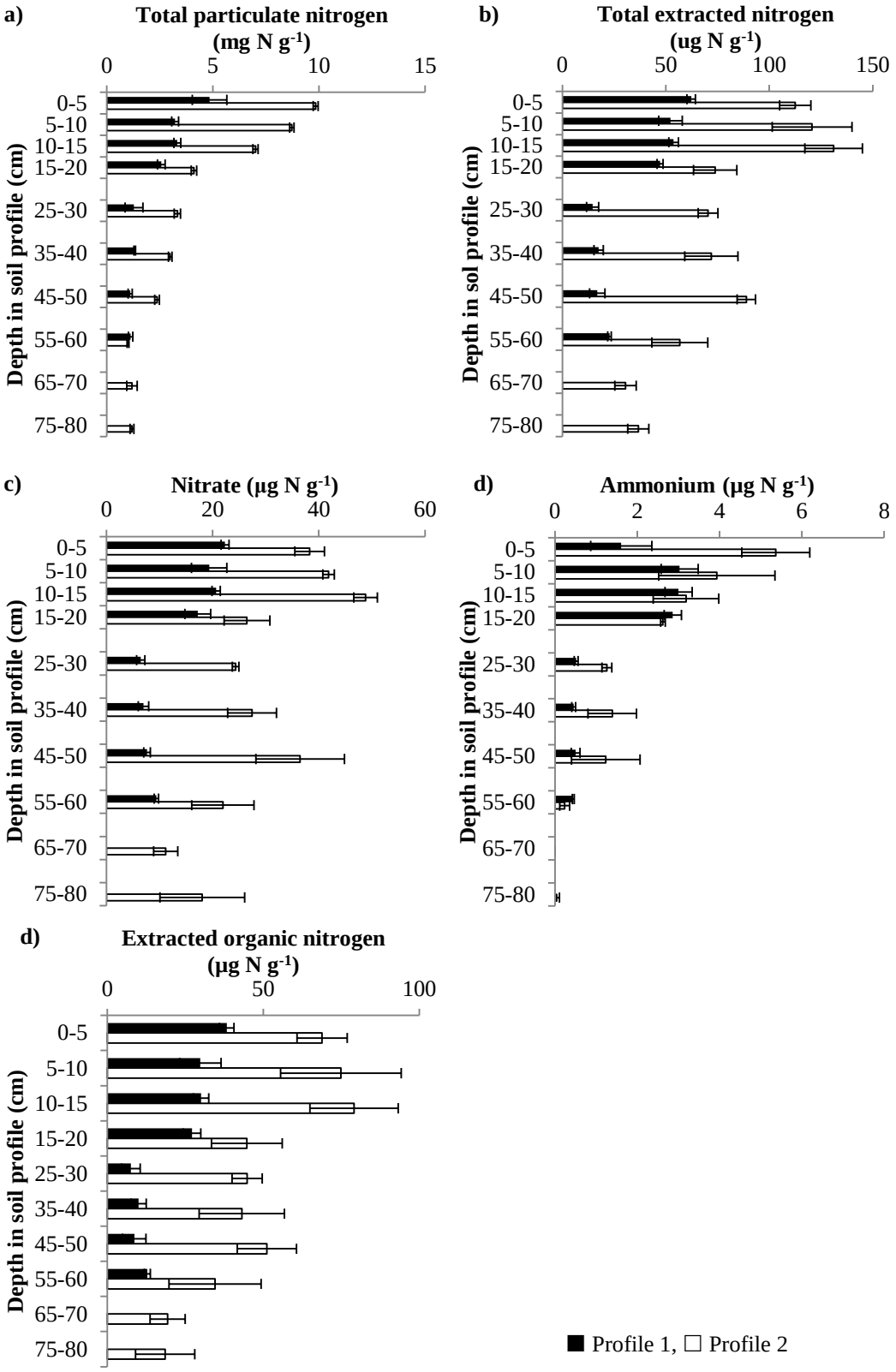

Figure 3.13: *Concentrations of N fractions for each sampling depth within soil profiles 1 and 2. Means and standard deviations were calculated from triplicate samples.* ***a)*** *TPN concentration (mg N g^{-1}).* ***b)*** *TEN concentration (µg g^{-1}).* ***c)*** *NO_3^- concentration (µg N g^{-1}).* ***d)*** *NH_4^+ concentration (µg N g^{-1}).* ***e)*** *EON concentrations ((µg N g^{-1}).*

Non extractable N represents > 97.9 % of the TPN in profile 1 and > 94.3 % of TPN in profile 2, suggesting that the vast majority of N present within the soil was in an unavailable form. Whilst the concentration of TEN decreased with increasing depth, the percentage of TPN composition represented by TEN increased with depth in each case. The percentage of N comprised of the NO_3^- fraction increased with increasing depth in both profiles, being 35.9 to 41.4 % and 34.0 to 49.2 % in profiles 1 and 2, respectively. NH_4^+ and DON fractions decrease in both concentration and proportion of composition with increasing depth. The ANOVA analyses comparing mean TEN values in the two Profiles showed a significant difference ($p = 0.006$, Table 3.4). The representative percentage N fractions from which the TEN was composed for each soil sample are shown in Table 3.6.

Table 3.6: *Percentage composition of the total extracted nitrogen fraction extracted from the soils soil depth profiles using HPW.*

Sample depth	Percentage composition			
	TEN	**NO_3^-**	**NH_4^+**	**EON**
Pit 1				
0 – 5	100	35.87	2.59	61.55
5 – 10	100	37.06	5.79	57.14
10 – 15	100	38.44	5.59	55.98
15 – 20	100	36.40	6.06	57.54
25 – 30	100	44.38	3.52	52.11
35 – 40	100	39.83	2.58	57.59
45 – 50	100	45.40	2.98	51.62
55 – 60	100	41.39	1.95	56.66
Pit 2				
0 – 5	100	34.02	4.77	61.21
5 – 10	100	34.66	3.26	62.08
10 – 15	100	37.22	2.43	60.35
15 – 20	100	35.85	3.55	60.60
25 – 30	100	34.55	1.79	63.66
35 – 40	100	38.12	1.94	59.94
45 – 50	100	41.04	1.39	57.57
55 – 60	100	38.66	0.41	60.93
65 – 70	100	36.54	0.00	63.46
75 – 80	100	49.18	0.11	50.71

The distribution of NO_3^- followed a similar pattern to the TEN distributions for both Profiles (Figure 3.14c). The highest concentration for profile 1 was 22.3 ± 0.8 μg N g^{-1} at 0 to 5 cm and for profile 2 48.8 ± 2.2 μg N g^{-1} at 10 to 15 cm. As with TEN, the NO_3^- concentrations showed an abrupt decrease in concentration from 20 cm depth and results from the ANOVA comparison of the two Profiles revealed a significant difference ($p = 0.004$, Table 3.4).

Extracted organic nitrogen concentrations were significantly higher in Profile 2 than Profile 1 ($p = 0.005$, Table 5.4). The highest concentration in Profile 2 was 79.1 ± 14.1 μg N g^{-1} at 10 to 15 cm depth and within Profile 1 was 38.33 ± 2.32_μg N g^{-1} at 0 to 5 cm depth. Within Profile 2 the lowest concentration (18.6 ± 9.5 μg N g^{-1}) was observed at 75 to 80 cm depth and within Profile 1 was at 25 to 30 cm depth (7.60 3.02 μg g^{-1}).

Figure 3.13d displays the NH_4^+ concentrations for each profile. Profile 1 concentrations were highest at 5 to 10 cm depth (3.03 ± 0.45 μg N g^{-1}) and decreased to 0.44 ± 0.02 μg N g^{-1} at 55 to 60 cm. The NH_4^+ concentrations for Profile 2 also showed a gradual decline, from 5.37 ± 0.83 at 0 to 5 cm to <LOD at 65 to 70 cm. Mean ammonium : nitrate ratios (Figure 3.14) were relatively low at all depths for both soil Profiles (Figure 3.15). This highest values were 0.17 (15 to 20 cm) 0.14 (0 to 5 cm) for Profiles 1 and 2, respectively.

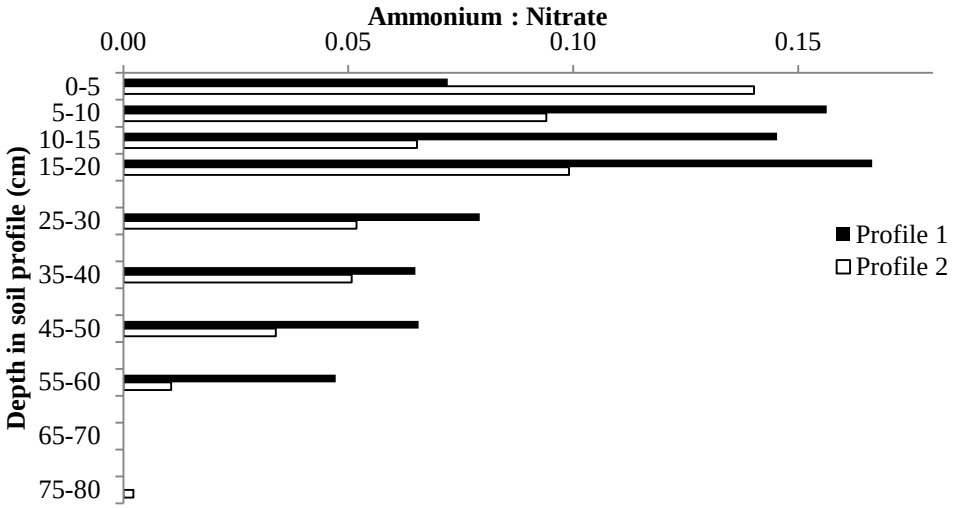

Figure 3.14: *The NH_4^+: NO_3^- ratios for each sampling depth within soil profiles 1 and 2.*

3.5.2.2 Phosphate

PO_4^{3-} concentrations are shown in Figure 3.15; concentrations decreased with depth for both soil Profiles. Soil Profile 2 had the highest overall PO_4^{3-} concentrations, however ANOVA confirmed that there was no significant inter-profile difference ($p = 0.209$, Table 3.4). The highest concentration for soil profile 1 was 20.3 ± 2.5 μg P g^{-1} (10 to 15 cm) and lowest concentration 0.65 ± 0.35 μg P g^{-1} (55 to 60 cm). Soil Profile 2 contained the highest mean PO_4^{3-} concentration of 43.7 ± 2.3 μg P g^{-1} 5 to 10 cm depth though this was not significantly different from the 0 to 5 cm depth (43.1 ± 3.5 μg P g^{-1}), and lowest of 4.26 ± 0.39 μg P g^{-1} at 75 to 80 cm depth.

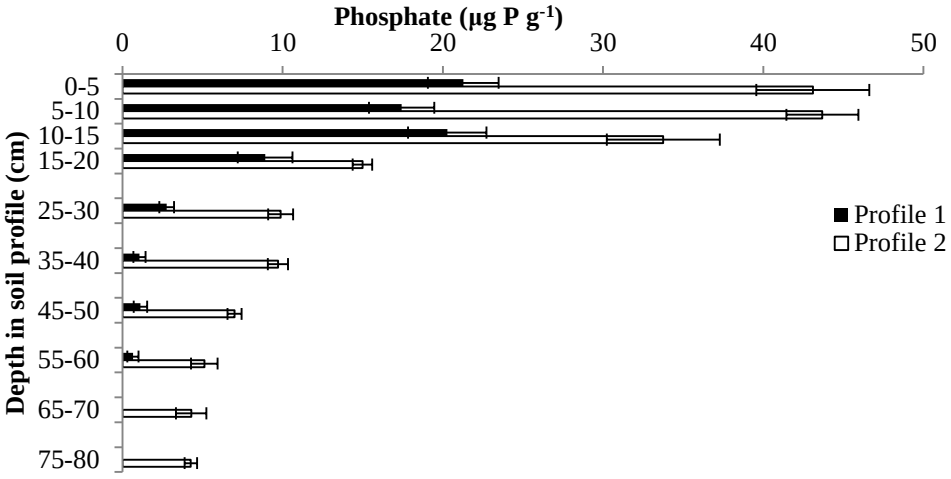

Figure 3.15: *Extracted PO_4^{3-} concentration for each sampling depth within soil profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.*

3.5.2.3 Carbon

TPC results are shown in Figure 3.16a. TPC concentrations for both Profiles varied little from 0 to 15 cm depth and displayed an abrupt decrease between 20 and 25 cm. The highest concentration for profile 1 was 113 ± 1 mg g^{-1} at 10 to 15 cm and the lowest concentration was 40.0 ± 0.4 mg g^{-1} at 25 to 30 cm. The TPC concentration for Profile 2 showed a gradual decrease with depth, from 155 ± 3 mg g^{-1} at 0 to 5 cm to 44.5 ± 0.9 mg g^{-1} at 55 to 60 cm. ANOVA found no significant difference between the two soil Profiles ($p = 0.272$, Table 3.4).

SOC concentrations (Figure 3.16b) were highest in the upper 20 cm of Profile 1, whilst Profile 2 demonstrated a gradual concentration decrease. The highest SOC concentration for profile 1 was 77.3 ± 0.21 mg g^{-1} at 15 to 20 cm depth and the lowest 21.7 ± 2.3 mg g^{-1} at 55 to 60 cm depth. The highest concentration within Profile 2 was 133 ± 10 mg g^{-1} at 0 to 5 cm and the lowest 25.2 ± 2.1 mg g^{-1}. ANOVA determined that there was no significant difference between the two Profiles ($p = 0.222$, Table 3.4).

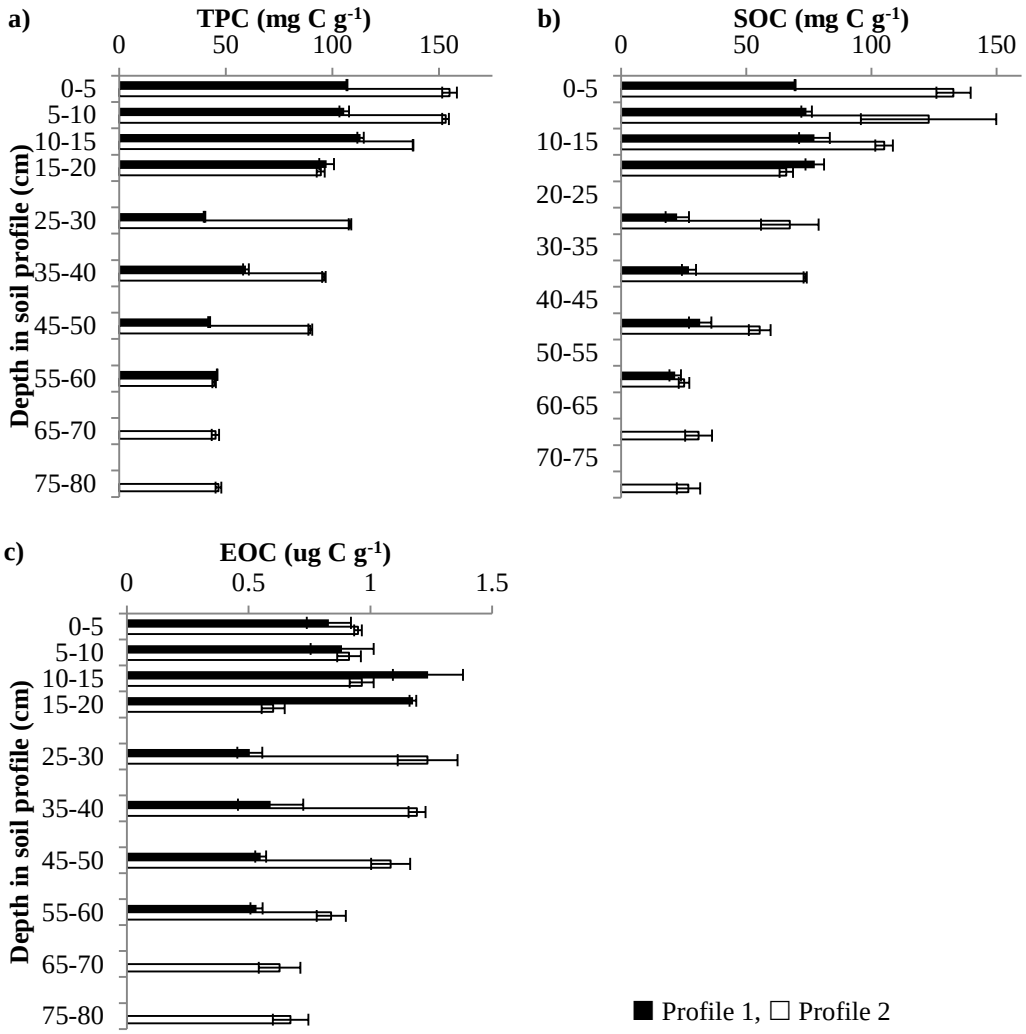

Figure 3.16: *Concentration of carbon fractions for each sampling depth within profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.* ***a)*** *TPC concentration.* ***b)*** *EOC concentration.*

EOC concentrations (Figure 3.16c) varied in both Profiles. The highest EOC concentration for Profile 1 was 1.24 ± 0.14 mg g^{-1} at 10 to 15 cm and the lowest was 0.50 ± 0.05 mg g^{-1} at 25 to 30 cm. The highest EOC concentration for Profile 2 was 1.23 ± 0.12 mg g^{-1} at 25 to 30 cm, and the lowest was 0.60 ± 0.05 mg g^{-1} at 15 to 20 cm. ANOVA determined that there was no significant difference between the two Profiles ($p = 0.342$, Table 3.4).

3.5.2.4 Carbon : Nitrogen ratio

The carbon : nitrogen ratios, shown in Figure 3.17, ranged from 22.0 to 45.0 for Profile 1, and from 15.7 to 44.4 for Profile 2. The lowest values for both Profiles occurred at 0 to 5 cm and both increased with depth.

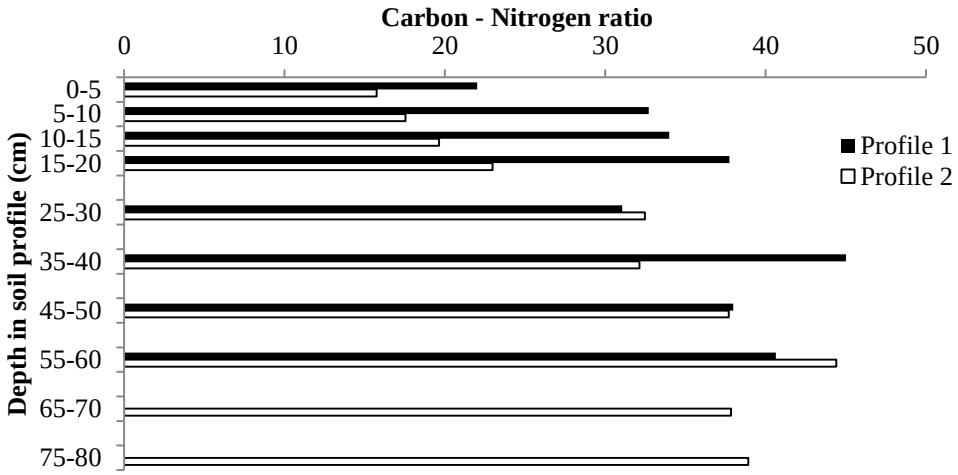

Figure 3.17: *Carbon : nitrogen ratio for each sampling depth within soil profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.*

3.3.2.5 Other extracted ions

Figure 3.18 shows the concentrations (μg g^{-1}) recorded for Mg, Ca, K, Na and Fe. For Profile 1 concentrations of Mg, Ca, K and Fe decreased with depth; Na concentrations were more variable, with the highest concentration occurring at 15 to 20 cm. For Profile 2, concentrations of Ca, K, Na and Fe decreased with increasing depth, Mg concentration varied with depth, the highest concentration occurring at 45 to 50 cm.

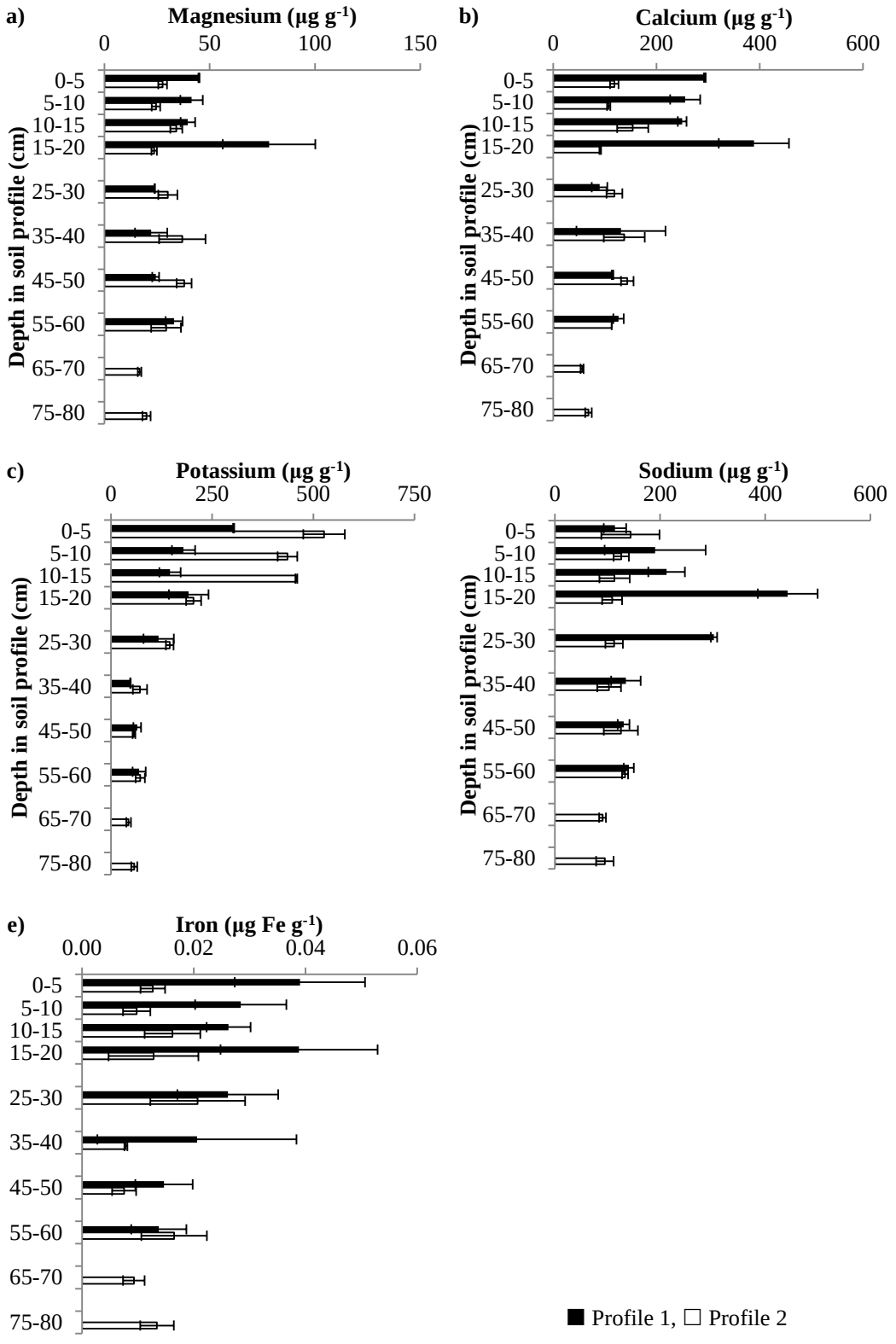

Figure 3.18: *Extracted ions for each sampling depth within profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.* ***a)*** *Mg,* ***b)*** *Ca,* ***c)*** *K,* ***d)*** *Na and* ***e)*** *Fe.*

3.3.2.6 Physio-chemical characteristics

The pH decreased with depth in both Profiles (Figure 3.19). The highest pH value for Profile 1 was 5.53 ± 0.01 (0 to 5 cm) and the lowest was 4.24 ± 0.01 (10 to 15 cm). Below 10 to 15 cm the pH for Profile 1 increased slightly to 4.78 ± 0.06 at 55 to 60 cm. The pH of Profile 2 was higher than Profile 1 from 0 to 25 cm, with a pH of 7.04 ± 0.01 at 0 to 5 cm which declines gradually until it reaches 4.67 ± 0.06 at 25 to 30 cm, after which it remains relatively stable. ANOVA showed there to be no significant difference in pH between the two profiles as a whole ($p = 0.178$, Table 3.4), however a significant difference was observed from 0 to 25 cm depth ($p < 0.001$).

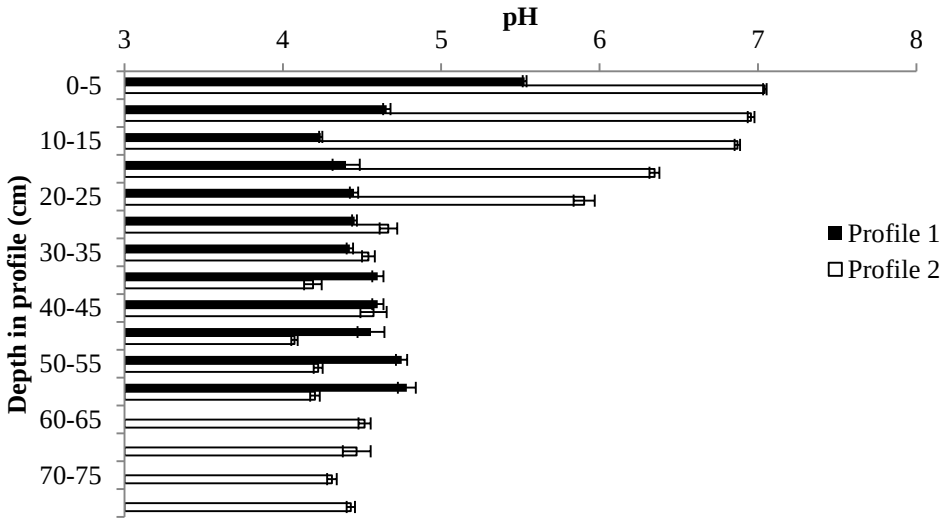

Figure 3.19: *pH throughout soil profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.*

Overall the water content was lower in Profile 1 than profile 2 (Figure 3.20), although, ANOVA determined no significant differences ($p = 0.299$, Table 3.4). The water content for Profile 1 increased with depth from 16.4 ± 0.9 % at 0 to 5 cm to 19.4 ± 1.5 % at 10 to 15 cm, after which lower concentrations between 9.35 ± 0.38 % and 11.7 ± 2.3 % were observed. Water content in Profile 2 decreased in from 31.5 ± 1.0 % (0 to 5 cm) to 9.24 ± 3.52 % (75 to 80 cm).

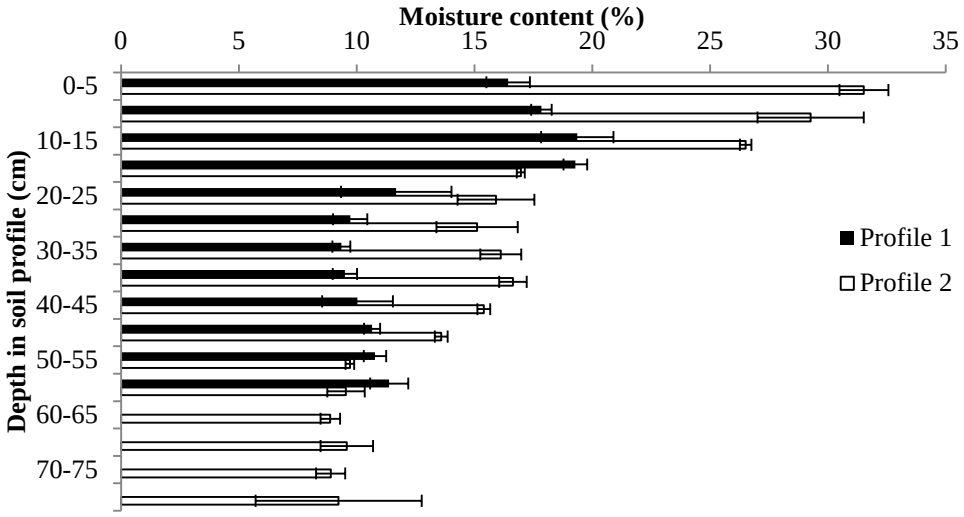

Figure 3.20: *Moisture content throughout soil profiles 1 and 2. Mean and standard deviation calculated from triplicate samples.*

The particle size distribution, shown in Figure 3.21a and b, was consistent for the 2 soil Profiles. Both had a very small clay fraction (≤ 1.01 %) and contained a mixture of sandy loam and loamy sand texture classes (according to the ISO 14688-1 classification). This suggested a relatively small particle surface area within the soil samples.

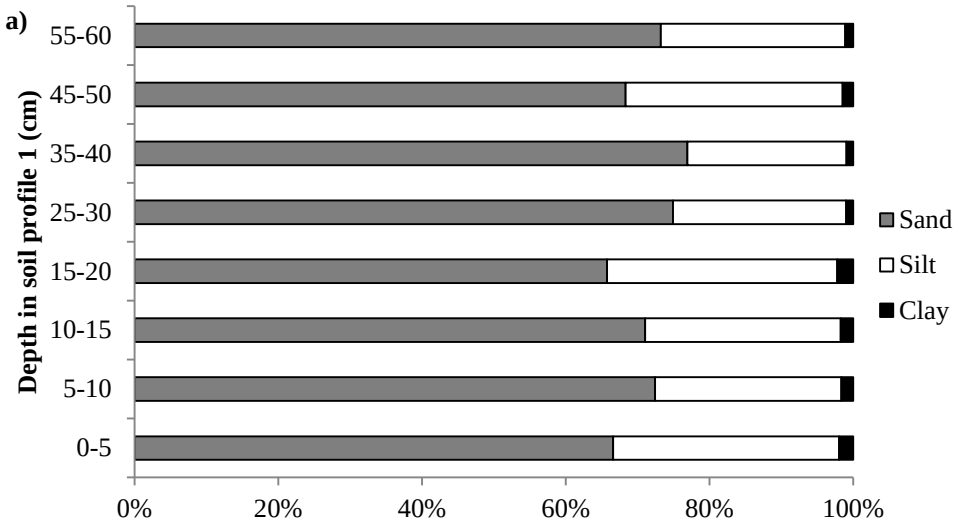

Figure 3.21a: *Particle size composition throughout soil depth profile 1.*

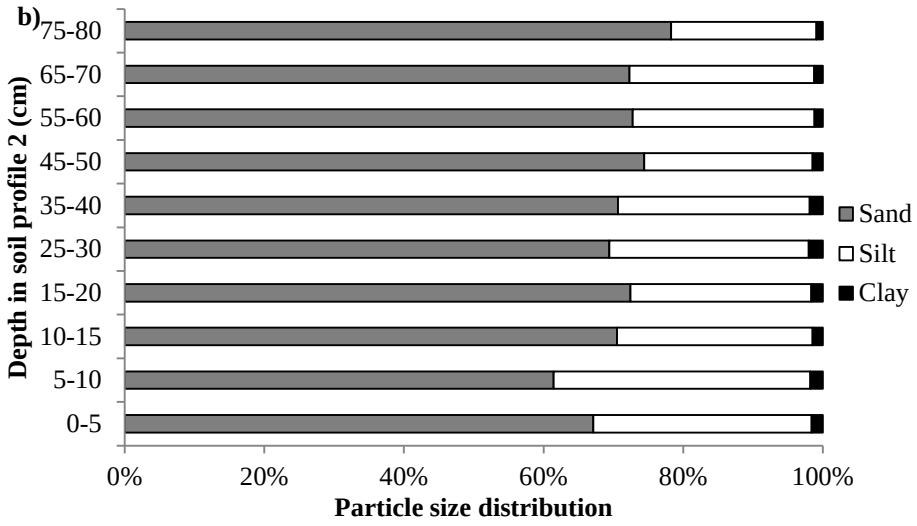

Figure 3.21b: *Particle size composition throughout soil depth profile 2.*

3.3.2.7 Enzymatic activity

Enzymatic activity, expressed as µg Fl g^{-1} hr^{-1}, was highest in the upper 20 cm of Profile 1, ranging from 158 ± 11 to 171 ± 64 mg Fl g^{-1} hr^{-1} (Figure 3.22). Concentrations decreased abruptly from below 20 cm. Profile 2 values varied, ranging from 61.2 ± 8.6 to 124 ± 13 mg Fl g^{-1} hr^{-1}. The difference in enzymatic activity between the two Profiles was significant (p = 0.028, Table 3.4).

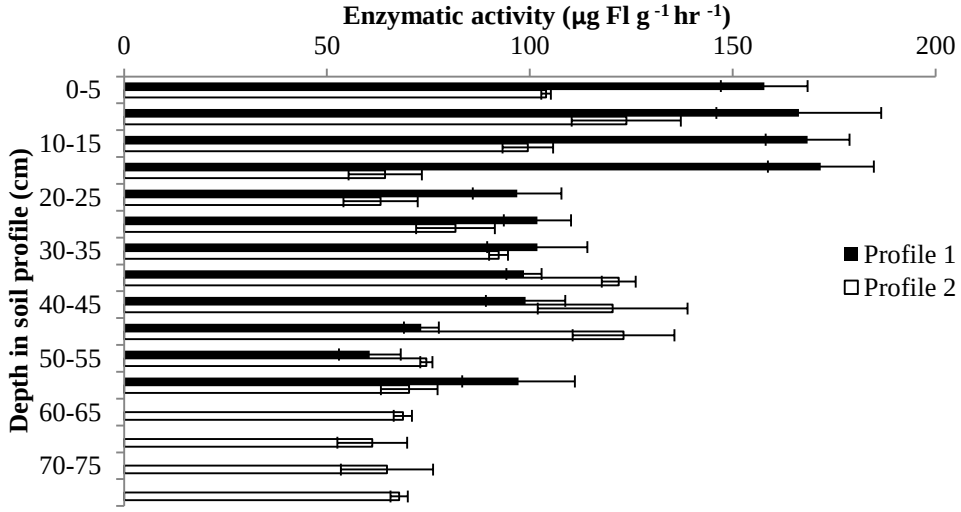

Figure 3.22: *Enzymatic activity, expressed as µg Fl g^{-1} hr^{-1}, in soil profiles 1 and 2. Mean and standard deviations were calculated from triplicate samples.*

3.5.2 Discussion: Depth profile characterisation

The results from Phase 2 provide high resolution observations of nutrient characteristics for 2 soil Profiles, of different ages, subjected to similar management practices within the Humid Tropics Biome at the Eden Project site. This discussion focuses on the effect of the varying management practices at these two locations.

The concentrations for a number of the parameters were significantly higher in the upper part of the Profile (0 to 20 cm) for both soil Profiles. There was a distinct change in nutrient concentrations with increasing depth from 20 cm, which corresponds well with the upper horizon depth (0 to 22 cm depth for Profile 1 and 2) to as measured at the time of sampling (pictured in Figure 3.3). It was observed that the upper horizons of both Profiles had a darker colour, which suggested a higher organic matter content, and this was consistent with the TPC and EOC results. The concentrations within this horizon were larger for Profile 2 than Profile 1, which may be attributed to the lesser age of the soil at Profile 2.

The concentrations within Profile 2 were consistently higher than those in Profile 1. This may be attributed to Profile 2 being a more recently prepared soil mix (2 years old), whilst the management practices at the two locations was similar, with the addition of green waste compost and fertiliser (Vitax® 214) applied to the soil surface at both locations. This suggests that the age of the soil played a significant role in increasing the levels of moisture, enzymatic activity, pH, N (TPN, TEN and NO_3 + NO_2), P (PO_4), TPC and K at the location of Profile 2. The soil composition of the Eden Project soils contains a high proportion (65 %) of organic components (32.5 % composted green waste and 32.5 % bark). The recent preparation of Profile 2 suggests that the organic

matter component of the soil mix may still be under decomposition by soil microbes, which releases various nutrients in to the plant available pool. Whilst, the aged soil in Profile 1 is reliant upon composted green waste and fertiliser applications for nutrients. This may serve to explain the significant differences observed between the two Profiles for many of the nutrient analytes and the C : N ratio. It is interesting to note that EOC concentration demonstrated no significant difference between the two Profiles at the upper depths, but at depths > 25 cm, Profile 2 contained significantly higher concentrations.

Whilst initial observations suggest that the younger age of the soil from Profile 2 increased the nutrient concentration, records do not show at what time the composted green waste additions were made relative to sampling and so it is difficult to determine whether Profile 2 was subject to a more recent applicator than Profile 1 and therefore, this may be a short-term effect in response to a recent application, or a more long-term result of the amendment, or whether it is a more long-term effect of the newer soil mix.

Enzyme activity levels determined throughout the Profiles were within the range of values reported for freshly amended soils, ranging from 90 to 300 μg FL g^{-1} soil hr^{-1} (Sánchez-Monedero et al., 2008). Results for the soil profiles suggest that the higher organic matter component may have caused the increased the enzyme activity, indicating greater microbial abundance within the upper 20 cm of the soil profile. Profile 1 (averaging 116 ± 39 μg Fl g^{-1} hr^{-1}) demonstrated greater enzymatic activity levels than Profile 2 (87.6 ± 24.3 μg Fl g^{-1} hr^{-1}), which was surprising given the higher SOC levels determined within Profile 2 (91.9 ± 51.3 mg g^{-1} in Profile 2 and 65.5 ± 28.1 mg g^{-1} in Profile 1) . However, the more recent production of the soil comprising Profile

2 may serve to explain the difference, with the microbial population of Profile 1 being better established and more extensive than Profile 2.

The gradual decrease in N, PO_4^{3-} and K concentrations with depth for both profiles may be attributable to the fertiliser and composted green waste being added to the surface of the soil and gradually leached through the profile. NO_3^- and K are considered to be particularly prone to leaching and the large sand fraction present throughout the soil profile would have facilitated transport of nutrients down the soil profile. Alternatively, the nutrients were possibly being retained and stored within the upper 20 cm of the profile, with only a small quantity being leached through to the lower profile.

Other possible mechanisms of nutrient loss are: surface runoff, though, the drip irrigation system within the Humid Tropics Biomes will aid nutrient incorporation in to the soil profile in the time following fertiliser additions, minimising loss through this pathway; volatilisation, which is particularly common under warm conditions and in the case of nitrogen; and denitrification, which is more common under anaerobic conditions in saturated soils. The moisture content and large sand fraction observed throughout both soil Profiles, suggests that the conditions were aerobic and, as such, nitrogen loss through denitrification should account for only a small amount of loss.

The difference between the nutrient concentrations for the two soil Profiles supports earlier findings from the Phase 1 characterisation, that there was a significant degree of inter-site variation. This variation, over a relatively small area, is typical of what would be expected with natural soils (Owens et al., 2008; Shen et al., 2011) and demonstrates the effect of varying management practices and plantings, as well as the difference between soils of different ages.

3.6 Synthesis

In combination the results from Phase 1 and 2 support each other in their demonstration of the impact of management practices at the Eden Project site on the nutrient concentrations found within the soils,

ANOVA suggested no significant differences ($p > 0.05$) between the environmental conditions of the Outdoor and Humid Tropics Biomes. However, there were significant differences between management practices expressed as differing TPN and TPC concentrations and the sand and silt particle fractions. TPN concentrations were consistent with values reported for a variety of soils (Table 3.7).

Table 3.7: *Reported TPN concentrations for a variety of soil types.*

Soil Type	Total particulate nitrogen concentration range (mg N g^{-1} soil DW)	Soil characteristics for top soil samples (< 30 cm depth)	Source
This study Phase 1	0.9 – 11.3	Texture: Sandy loam pH: 6.00 – 7.90	
This study Phase 2	1.0 – 9.8	Texture: Sandy loam pH: 4.45 – 7.04	
Arable, Michigan, USA	4.1 – 4.4	Texture: Sandy clay loam pH: 5.7 – 5.8	(Agehara and Warncke, 2005)
Wilderness (Broadbalk), UK	1.3 – 3.7	Texture: Silt loam pH 5.22 – 6.91	(Brookes et al., 1985)
Permanent grass (Park grass), UK	2.1 – 4.0	Texture: Silt loam pH: 5.49 – 6.81	
Subtropical forest	2.1 – 7.5	Texture: Clay pH: 6.0 – 6.6	(Burton et al., 2007)
Grassland, Austria	4.8	*Texture: Sandy loam pH: 5.67	(Hood-Nowotny et al., 2010)
Arable, Austria	1.5 – 3.3	*Texture: Sandy loam pH: 6.21 – 7.15	
Forest, Ivory coast	1.6	*Texture: Sandy clay loam pH: 6.0 – 6.1	(Tie et al., 2010)
Arable, Ivory coast	1.2 - 1.3	* Texture: Sandy clay loam pH: 6.1 – 6.4	

*Texture calculated from particle size distribution data using ISO 14688-1:2002 classification.

Adani et al. (2007) demonstrated that composted green waste addition to a soil over a 4 year period has a significant effect on the composition of the soil organic matter

component. In view of this, that the Eden Project soils are demonstrating variation by management practices is logical, particularly as the original soil compositions demonstrate small variation across the site (Table 3.1).

Limitations

It is important to recognise and acknowledge limitations associated with the work described above. As the sample set for Phase 1 was small (n = 6) any statistical significance should be treated as an association. However, this dataset provides a valuable insight into nutrient concentrations and characteristics across the Eden Project site and the careful consideration given to the selection of sampling locations means that the samples represented the variety of the soils in use.

It is well reported that soil properties are highly spatially variable. Thus a certain amount of uncertainty is associated with depth profiles from a small area. In an ideal situation a minimum of three soil profiles would have been analysed as a means of providing a higher confidence level with regard to the findings from the characterisation of a particular soil. This would have allowed for more robust statistical analyses to be carried out on the dataset. However, this would have resulted in a greater number of samples for analyses and also a greater amount of disruption at the Eden Project site. To counter this, the two sampling sites for the depth profiles were carefully selected based on the performance, management and age of the soils in the two locations.

The use of 2M LiCl as the extraction solution for the initial characterisation allowed for the estimation of the extent of extractable nutrients present within the soils, however, caused problems with the Shimadzu TOC-V analyser. As a result of the combination of a high chloride concentration and the presence of soil colloids within the samples, the

halogen scrubber and catalytic column components had to be replaced. For Phase 2 depth profiles HPW was used as the extracting solution, giving an estimation of the leachable nutrient concentrations within the soils and further, alleviating the problems associated with the Shimadzu TOC-V analyser.

3.7 Conclusions

The reproducibility of the characterisation experiments was good, as demonstrated by the low experimental errors. This is important as it allows for the data to be compared confidently with values reported within the literature and further data from this project.

The upper 20 cm of each profile contained higher nutrient concentrations. The higher organic matter content of the upper horizon may have helped to regulate and retain nutrients. Greater nutrient concentrations were observed within Profile 2, which may be attributed to the greater quantity of organic matter present within this soil through the regular composted green waste additions, which have served as a source of nutrients as well as providing a charged surface for its retention within the soil. With increasing depth the difference between the 2 profiles was lower for most characteristics, suggesting that the effect of soil age diminishes with depth in the soil profile, and that the increased retention of nutrients within Profile 2 only took place in the upper 20 cm of the profile.

There was a limited degree of variation in soil characteristics at the Eden Project site, despite differing environmental factors. This may be answerable to there being little variation between the original soil compositions across the Eden Project site, with the only differences between the soils being introduced through differing management

practices. It may therefore be perceived that the variation in management practices and soil age have had a more significant impact on the soil characteristics than environmental conditions.

CHAPTER 4

Investigation of nutrient retention characteristics of a freshly prepared artificial soil during irrigation and fertilisation amendment

4.1 Overview

A column study was devised and implemented to allow for the detailed observation of the nutrient dynamics of a freshly prepared artificial soil, under controlled environmental conditions and drip irrigation for 52 weeks. The artificial soil was produced from a mix of horticultural grit, lignite, bark and composted green waste, following the Eden Project protocol and packed into 4 columns. Following 26 weeks of irrigation, 2 of the 4 columns were fertilised using Vitax 214 (applied at a N/P/K ratio of 20 g N m^{-2}/ 9 g P m^{-2} / 33 g K m^{-2}) according to the Eden Project protocol, whilst the remaining two columns were unfertilised, serving as controls. Leachate was regularly collected from the base of each column and analysed for dissolved constituents, physicochemical and biological properties. The freshly prepared soil and a 5 cm resolution depth profile of each column, taken following 52 weeks, were analysed for extractable and solid phase constituents, including mineralogical analyses, and physicochemical and biological properties.

Results from the column studies demonstrated significant N immobilisation within the soil, leading to low concentrations of inorganic N within the soil solution. The cause of this is proposed to be the high C : N ratio of the bark component within the artificial soil mix. The soil was generally found to have a high proportion of large sized particles, which was not conducive to nutrient retention.

Following 52 weeks the characteristics for all columns displayed little variation with depth. Fertiliser application gave rise to an increase in leachate concentrations of DON, NO_3^-, PO_4^{3-}, Mg^{2+} and Ca^{2+} and a decrease in pH. Differences between fertilised and unfertilised columns for extracted and solid phase constituents were less prominent.

4.2 Introduction

4.2.1 Research objective

The research outlined in the Chapter addresses research objective 2.

- *To construct and implement the use of soil column bioreactors to observe the performance of the current artificial soil composition with regard to the cycling of key nutrients.*

4.2.2 Rationale

In order to address research objective 2, a long-term soil column study was devised to quantify the nutrient dynamics of the artificial soils typically deployed within the Eden Project Biomes. This served to improve understanding of the nutrient concentrations within the Eden Project soils and to establish the susceptibility of nutrients to loss through leaching. Time series measurements for a number of elements in the soil column leachate, including nitrogen, phosphorus and potassium, allowed for 1) the performance of the artificial soil to be assessed; 2) the nutrient concentrations to be compared to the established soils sampled from within the Eden Project Biomes.

After monitoring the leachate for 52 weeks the contents of the columns were extruded and sampled at a 5 cm depth resolution. In conjunction with data from the fresh soil, this served to identify any changes in soil characteristics, which could be related to nutrient storage and retention within the soils.

4.3 Experimental design and specific methodology

Figure 4.1 *Soil column bioreactors used to assess the nutrient retention characteristics of Eden Project soils.*

Lewis and Sjöstrom (2010) define a soil column as a discrete block of soil, located either outdoors or in a laboratory, which allows for the control or measurement of infiltration. Soil columns are recognised as providing a suitable means of studying soils, allowing for the control of environmental conditions with minimal variability between samples, whilst, also enabling the study of soil properties (Derby et al., 2002).

4.3.1 Column design

Column diameter is a key factor affecting soil dispersivity (Bromly et al., 2007), defined as, the number of flow pathways through the soil. Higher dispersivity is achieved through the use of wider columns (Bromly et al., 2007).

The potential for the formation of preferential flow pathways was a concern, as they can create localised chemical environments, with higher C, N and Fe, and lower pH (Bogner et al., 2012). Packing the top of the columns with glass marbles (10 mm diameter) atop a perforated polypropylene disc on the soil surface (as shown in Figure 4.2) was devised as a means of dispersing the flow across the surface of the soil in order to minimise the occurrence of any preferential flow pathways, through dispersing water, supplied by drip irrigation, across the soil surface.

Each of the 4 columns (Figure 4.2) had an internal diameter of 110 mm, a height of 1000 mm and was manufactured from opaque polyvinylchloride (PVC) to help minimise the potential for algal growth within the columns.

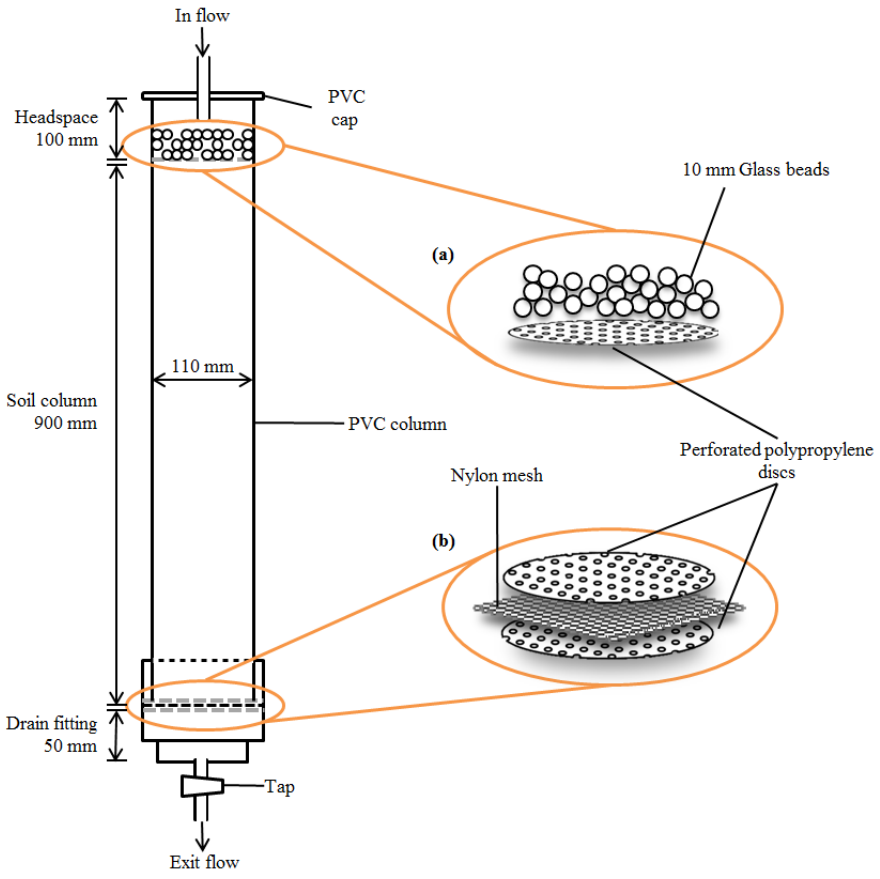

Figure 4.2: *Experimental setup -* ***a)*** *The top of the column contained glass marbles on top of a perforated polypropylene disc.* ***b)*** *The base plate at the bottom of the column contained nylon mesh (100 µm) located between 2 perforated polypropylene discs.*

Table 4.1: *Materials used to prevent drainage problems at the base of the column.*

Author	Material
Cornu et al. (2001)	Nylon mesh, silicon bead layer and a sintered glass disk
Chakrabarti et al. (2005)	Filter paper
Favaretto et al. (2012)	Filter paper and cheese cloth
Hodson and Langan (1999)	Nylon mesh, filter paper, polythene beads and quartz wool
Köhne and Mohanty (2005)	Nylon mesh
Nakamura et al. (2004)	Stainless steel mesh and glass beads
Zhou et al. (2006)	Nylon mesh
Güngör and Ünlü (2005)	Stainless steel mesh

A number of materials have been used to sustain drainage at the base of soil columns (Table 4.1); the most frequently reported materials were filter papers and nylon mesh. For this experimental set-up, a base plate was employed to minimise particulate losses and clogging of the drainage tubing and tap, whilst also being durable. The base plate comprised a layer of nylon mesh (100 µm) located between two perforated polypropylene discs, allowing the water to pass through into the collection vessel, whilst preventing clogging of the tap (Figure 4.2). Nylon, PVC, polypropylene and glass were chosen as materials because of their inert nature, posing a low contamination risk. In order to reduce evaporative losses each column had a cap at the top and base.

A number of leachate collection systems have been described by reported studies, including, vacuum extraction (Derby et al., 2002), tension plates (Cole, 1958), ceramic suction cups (Wagner, 1962) and gravity drainage (Mali et al., 2007). Whilst gravity drainage requires the water content of the soils to exceed field capacity (Derby et al., 2002; Lewis and Sjöstrom, 2010), it was determined that at irrigation rates consistent with those employed at the Eden Project would allow for this method to be effective and thereofre that gravity drainage was the most representative mode of leachate collection. The effective use of gravametric drainage requires a saturated zone at the base of the column before any leachate flow may occur (Lewis and Sjöstrom, 2010), to minimise

the impact of this on the soil a layer of horticultural grit was placed at the base of each column, which served as the saturated zone rather than the soil mix.

It is well known that plant-soil interatctions play an important role with regard to soil biodiversity and ecosystem functioning (Bardgett et al., 2013; Chapin et al., 2009; DeVries et al., 2015). Amoungst the various experimental set-ups reported within the literature, a range of planting conditions were observed. Whilst the inclusion of plants within the experimental set-up allows for nutrient loss by plant uptake to be observered, there are a wide range of negative implications. The choice of plant for such experiments is difficult, particularly given the wide variety of plants found within the Eden Project Biomes, identification of an appropriate and representative species would be highly difficult. Plants may be inconsistent, with certain crops failing, this calls for the use of a greater number of replicate samples. There exist a number of numerical models (SWAP (VanDam et al., 1997) and LEACHM (Hutson and Wagenet, 1987)) which, with the appropriate data inputs, have been used to estimate values for nutrient uptake by a range of plant types from soils, through the assumption that data aquired is derived from studies of unvegetated soils. The ability to employ numerical models in such a manner helps to lower the demands upon the laboratory-based experiments, allowing for focus upon consistency of environmental conditions in which the columns are maintained and further for the focus to remain upon analysis of nutrient concentrations, rather than plant husbandry. Based on these factors it was decided to exclude plants from the experimenta set-up.

4.3.2 Soil composition

The soil composition was designed to represent that employed at the Eden Project at the present time of experimental set-up; consequently, the soil composition employed for this study differs from that examined in Chapter 3 as a result of composition development by the Eden Project over the time since its opening. The mix was designed to have high porosity for effective drainage, whilst it was intended for nutrients to be maintained through regular amendments. The soil mix was developed by the Eden Project under tight time constraints, which have led to some of the nutrient retention issues outlined in Chapter 3. Through reproducing and studying the top soil mix it may be possible to determine methods by which retention of plant available nutrients might be improved.

The soils were manufactured at the Eden Project site on 25/3/13. The upper 70 cm of the soil column comprised topsoil mix. The materials used for the topsoil were composted green waste (composted for 15 weeks), bark, horticultural grit (a locally sourced sandstone based material, particle size range 2 to 6 mm, incorporated to improve drainage) and lignite clay (Table 4.2). The topsoil materials were mixed on a volume basis using a rotary mixer to achieve a homogenous mix of the soil constituents.

Table 4.2: *Composition of the soil mixture used to make the top soil used in the soil columns following current Eden Project protocol.*

Material	Volume (L)	Composition (%)
Composted green waste	16.25	32.5
Bark	16.25	32.5
Horticultural grit	12.50	25.0
Lignite clay	5.00	10.0

The lower 15 cm of each column was packed with 2.5 L (volume) of horticultural grit to simulate a sub-soil, and as a means of maintaining flow at the base of the column.

4.3.3 Column packing

The soil was packed in each column in a manner which encouraged homogeneity and the uniform distribution of particle sizes throughout the profile. Lebron and Robinson (2003) observed that the most effective way to achieve a homogenous mixture was to stir the grains with a small amount of water where the attractive cohesive forces between the water molecules and the particle surfaces hold the soil together.

Sidewall flow may occur as a result of improper packing of the soil or flexing of the column walls (Lewis and Sjöstrom, 2010), where the soil has separated from the walls of the column, creating an airspace (Corwin, 2000). Incidences of sidewall flow have been reported where a soil contains a large-sized soil particle fraction, such as sandy soils (Sentenac et al., 2001). The soils characterised in Chapter 3 displayed a large sand fraction, as such, careful attention was given to column packing to minimise sidewall effects.

The soils were packed into the 4 columns by loading a standard quantity (approximately 100 mm depth) into each, in turn, thereby minimising any difference between the columns and ensuring that a consistent volume of soil mix was added to each column. The soil mixture was moist at the time of loading, which helped to maintain the homogeneity of the mix. Each layer of soil added was gently tapped down to achieve tight packing whilst avoiding air entrapment. The surface of each soil layer added to the column was left uneven before adding the next layer to ensure vertical hydraulic connectivity between layers.

Once the columns were installed and packed with soil, they were allowed to stabilise at 15 ^{o}C, for 2 weeks. This was to allow the soils to settle prior to the commencement of irrigation and leachate collection.

4.3.4 Environmental conditions

It is possible to exercise greater control over both temperature and moisture within laboratory-based column studies. Therefore, there is the potential to implement a wide range of environmental conditions for soil column experiments; this is reflected within the reported studies. Table 4.3 summarises the environmental conditions used in a number of soil column experiments. It is important to tailor the conditions used for column experiments to meet the aims and objectives of the particular investigation.

The average moisture content for the Eden Project soils was 26.5 ± 8.0 and 24.9 ± 11.0 % for the Rainforest and Outdoor Biomes, respectively. The temperatures ranged from 15 to 35 ^{o}C in the Rainforest Biome and 4 to 19 ^{o}C within the Outdoor Biome. Throughout the experiment, the columns were maintained at 15 ^{o}C in a controlled temperature room. This temperature was chosen to be representative of a temperature encountered within the Outdoor, being sufficiently warm to encourage soil processes at such a rate that they may be observed at the chosen sampling resolution and timescale of the study. The irrigation regime was designed to simulate the one employed within the Humid Tropics Biome and Outdoor Biome during times of low rainfall at the Eden Project, outlined in Section 4.3.6.

Table 4.3: *Conditions reported for the implementation and maintenance of soil columns.*

Author	Condition		
	Moisture and Watering	**Temperature**	**System**
Burgos et al. (2006)	Maintained at 70 % WHC*. Simulated rainfall at 415 mm yr^{-1}.	28 ^{o}C	Crop production in sandy texture
Favaretto et al. (2008)	Saturated	19 – 22 ^{o}C Night 24 – 27 ^{o}C Day	Crop production in silt loam texture
Favaretto et al. (2012)	100 % WHC and irrigated with deionised water at 0.5 mL min^{-1}	Not specified	Crop production in silt loam texture
Hilger et al. (2000)	15 % WHC	Not specified	Landfill cover, sandy loam texture
Hubbard et al. (2011)	Saturated	Not specified	Contrasting redox zones
Köhne and Mohanty (2005)	Saturated	22 ^{o}C	Sand at low bulk density
Nakamura et al. (2004)	Saturated	20 ^{o}C	Sandy loam and sand textures
Cornu et al. (2001)	16 mm day^{-1} Rainy season 5 mm day^{-1} Dry season	18 ^{o}C	Subtropical, ferralsol

* WHC = water holding capacity

4.3.5 Irrigation water

Irrigation variables, such as intensity and duration, have a major effect on the infiltration rate of the water. For example, higher intensity and long duration of irrigation result in increased pore-water pressure in the soil, leading to inter-granular pressure; this is potentially damaging to the soil structure (Mitchell, 1962).

Water inflow was controlled using a peristaltic pump, with outflow monitored for volume, flow rate and chemical characteristics. Figure 4.3 illustrates the irrigation system used, with four parallel transparent PVC tubes (internal diameter: 3.175 mm) running through the peristaltic pump to ensure that the same volume of water was delivered to each column.

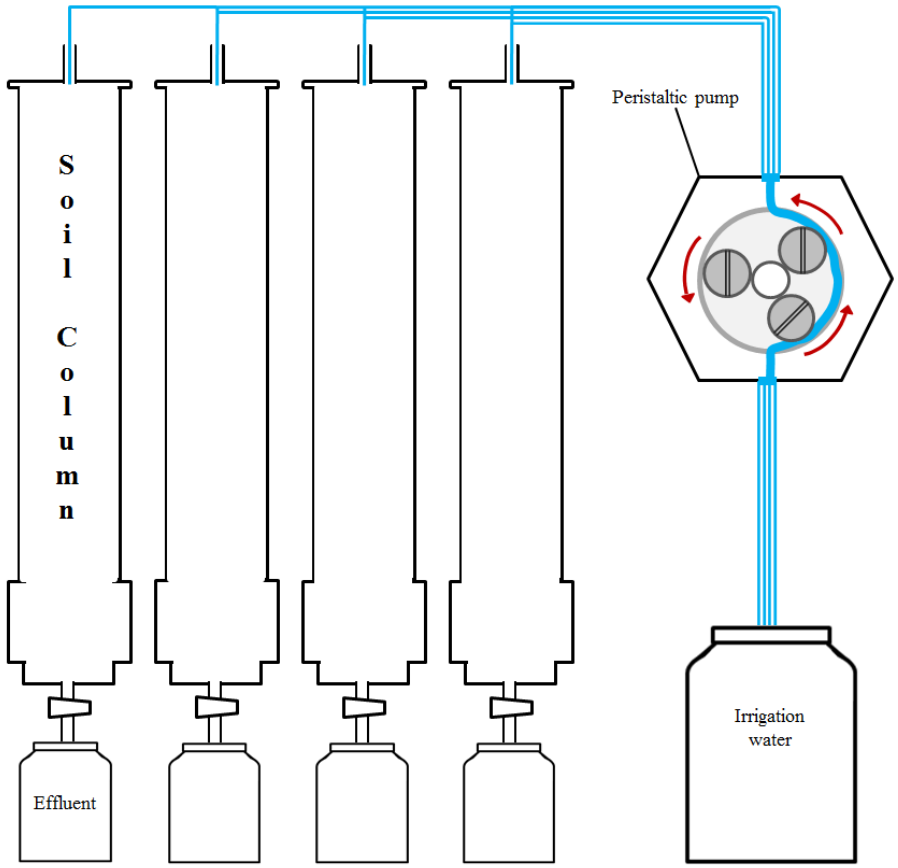

Figure 4.3: *Column irrigation set-up with peristaltic pump. The column watering tubes were made of transparent PVC and had an internal diameter of 3.175 mm. The tubing ran in parallel to ensure that all columns received the same volume of water.*

The quality of water used for irrigation of columns was an important consideration. High purity water was used for the irrigation of laboratory-based soil columns in a number of studies (Burgas et al., 2006; Cornu et al., 2001; Favaretto et al., 2012), whilst simulated rainwater was used in others (Hodson and Langan, 1999; Yang et al., 2006). To determine the most appropriate type of water for use in this experiment a preliminary investigation was carried out, which compared the nutrient content of the Eden Project irrigation water with high purity (18.2 MΩ cm^{-1}) water available at Plymouth University. The Eden Project irrigation water samples were collected from the point of delivery and were immediately analysed for pH. Sub-samples were then analysed for NH_4^+, NO_2^- + NO_3^-, PO_4^{3-}, TDN and DOC. It was found that concentrations of all

analytes measured within the Eden Project water were below the LOD (values reported in Table 2.6). Therefore, due to the lack of significant difference between the two water sources and for reasons of convenience it was decided to employ HPW (18.2 MΩ cm^{-1}) (adjusted to pH 7 with $CaCO_3$) as irrigation water for the duration of the experiment.

During the column experiment, samples were regularly collected from the irrigation water supply and analysed to give baseline concentrations for key measured characteristics. These were consistently below the LOD, with pH approximately neutral throughout.

The Outdoor Biome at the Eden Project is watered naturally (by rainfall) throughout most of the year, with irrigation systems employed to deliver water during times of low rainfall and drought. The Humid Tropics Biome was irrigated using recirculated rainwater at a daily rate of 0.14 mL water cm^{-2} and the irrigation flow rate used for the columns was calculated to accurately replicate this. As a result, each column received 80 to 90 mL of irrigation water daily, delivered over a five minute period six times a day, through drip irrigation regulated by the timer-controlled peristaltic pump. The flow rate of the peristaltic pump, with regard to irrigation water delivery was also checked on a regular basis to ensure that a constant rate was maintained.

Unsaturated soil columns have a negative pressure potential, which means that in order to extract pore-water, suction must be applied to unsaturated soil (Lewis and Sjöstrom, 2010). This would require the use of a vacuum pump and a porous material at the base of the column. Another approach, commonly used, is to allow the free drainage of leachate from the base of the soil column without the application of any suction (Lewis and Sjöstrom, 2010). This approach relies on the presence of a saturated zone at the

base of the column to allow flow to occur (Lewis and Sjöstrom, 2010). With this in mind, a layer of horticultural grit was included at the base of the column to aid drainage at the base and reduce the impact of saturation on the soil profile.

Hydrological properties are often measured in terms of the average amount of time that water remains in its various reservoirs (Pidwirny, 2007). It may take weeks to months for water to move through drainage networks depending upon the complexity of the system (Pidwirny, 2007).

4.3.6 Fertiliser application

The Eden Project employs biannual fertiliser applications across the site in order to maintain the supply of key nutrients (N – P – K) present within the soil; however, little is known regarding the fate of these applications. In order to further examine the fate of applied fertiliser, applications were made to 2 of the 4 columns, using an application rate consistent with that employed in the Eden Project Biomes. Two of the columns remained unfertilised in order to serve as a baseline for soil performance. The columns were fertilised in Weeks 27 and 48 of the trial (10/11/13 and 02/04/14), these sampling dates were chosen to represent the 6 monthly fertiliser application employed across most of the Humid Tropics Biomes at the Eden Project. Vitax® Natural 214 fertiliser was employed, which has an N – P – K ratio of 4.5 – 2.0 – 7.5 and was applied to each of the two columns at 20 g N m^{-2}/ 9 g P m^{-2} / 33 g K m^{-2}. The fertiliser was composed of a range of materials (Table 4.4), many of which were organic, suggesting that the nitrogen component was also organic, encouraging a slow release following application.

4.3.7 Sampling strategy

4.3.7.1 Leachate Sampling

Sample collection was carried out by free drainage of water from the base of the soil column, without the application of any suction. Each column was capped at the top, in order to reduce water loss through evaporation. The columns were unplanted, which meant that there were no water losses due to plant uptake and horizontal translocation of water was ruled out by the non-porous sidewalls. This meant that water, which would otherwise be lost through alternative pathways, travelled through the profile and contributed to the leachate. Further to this, the unplanted nature of the columns may have led to greater nutrient concentrations within the leachate and solid samples, than might be expected within planted columns and as such this must be acknowledged when considered nutrient concentrations.

The leachate collection strategy is outlined in Table 4.4. Leachate samples were collected daily for the first 3 weeks of column irrigation, then every 3 days (Week 4 to 9) and then weekly (from Week 10). From Weeks 1 to 26 all four columns were subject to the same conditions with leachate data averaged for all columns. After fertiliser additions to two of the columns, leachate data was averaged for the duplicate unfertilised (UF) and of fertilised (F) columns.

Immediately following the fertiliser applications the frequency of leachate collection was increased. Leachate was collected in 120 mL, acid washed, polyethylene bottles, filtered through Fisher brand HPLC grade glass fibre filter papers (75 g m^{-1}, 450 μM thickness) and stored at -20 ^{o}C prior to analysis.

Table 4.4: *Leachate sampling strategy employed throughout the column experiment.*

Week	Leachate sampling	
1 - 3	Daily	
4 – 9	Every 3 days	
10 - 26	Weekly	
27 - 30	Daily	Fertiliser application 1 (20 g N m^{-2}/ 9 g P m^{-2} / 33 g K m^{-2})
31 - 33	Every 3 days	
34 - 36	Every 2 weeks	
37 - 47	Weekly	
48 - 50	Daily	Fertiliser application 2 (20 g N m^{-2}/ 9 g P m^{-2} / 33 g K m^{-2})
51 -53	Every 3 days	
		Column extrusion

4.3.7.2 Soil Sampling

Following 52 weeks of irrigation, the soils were extruded from the columns, using a piston mechanism (Figure 4.4) to apply even force across the base of the soil profile, whilst minimising disturbance to the soil. Samples were collected at a depth resolution of 5 cm.

Samples were recovered from the centre of the soil column in order to minimise any edge-effects (e.g. during irrigation or drag against the column edge during the column extrusion). Once sampled, the soils were placed in labelled polythene zip-lock bags and stored at 4 ^{o}C prior to analysis.

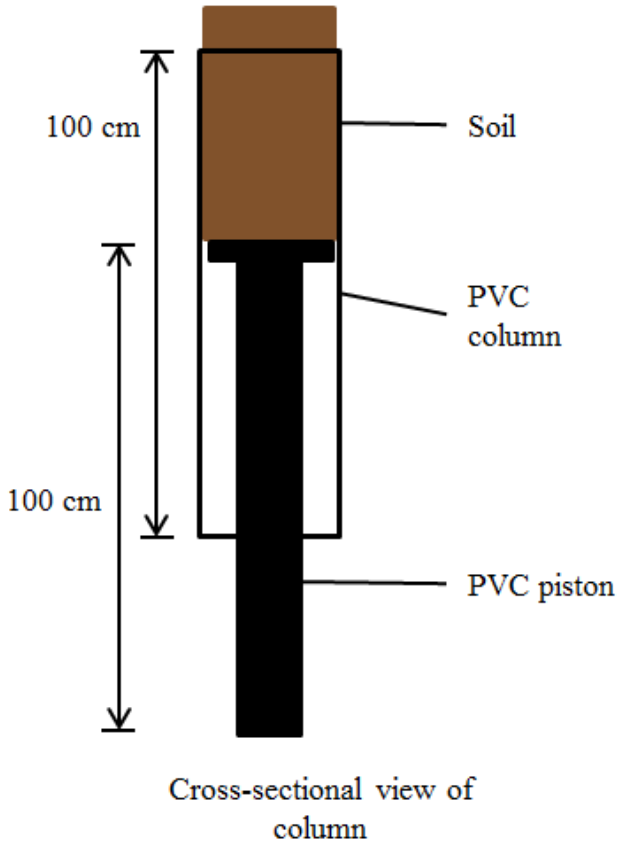

Figure 4.4 *The column extrusion apparatus. The PVC piston was inserted at the base of the column. The column itself was gently and evenly forced down over the piston, extruding the soil from the column for sampling at 5 cm depth intervals.*

4.3.8 Analytical measurements

Leachate and solid samples from the columns were separated and analysed as indicated in Figure 4.5, following methods described in Chapter 2. The leachate samples were analysed for the following *dissolved* analytes: TDN, NH_4^+, NO_3^- + NO_2^-, PO_4^{3-}, K, Fe, Ca, Mg (as ions) and DOC. The pH of the leachate was measured throughout while Eh and enzymatic activity in leachate were monitored during Weeks 48 to 52.

Solid samples of freshly-prepared artificial soil and extruded column sections were analysed for their mineralogical composition, TPN and TPN, pH, CEC, SOM, particle

size distribution, enzymatic activity and the following water-extractable fractions: TEN, NH_4^+, NO_3^-, PO_4^{3-}, K, Fe, Ca, Mg and EOC.

The mineralogical analysis was performed on both freshly prepared soil and extruded samples from the 10 to 15 cm depth interval within the UF and F soil column profiles. This allowed for the observation of any significant changes to the mineral composition over the course of the experiment, whilst also facilitating a comparison between UF and F column samples. The 10 to 15 cm depth was selected as this represents the top soil portion of the soil profile, whilst avoiding the potential disturbance common to the upper layers of a soil profile.

As mineralogical analysis was restricted in terms of the number of samples, freshly prepared soil was analysed in triplicate, with the analyses of samples from the irrigated columns run on single samples. This approach was used as the standard deviation for triplicate analyses of freshly prepared soil was low (RSD = 0.00 to 2.13 %).

NO_3^- + NO_2^- was analysed using the Skalar San^{++} system, however, NO_2^- represents an intermediate form, which is usually present in soil in only trace quantities, thus NO_3^- + NO_2^- will henceforth be referred to as NO_3^-.

4.3.9 Statistical analyses

Leachate data was determined to be of un-normal distribution. As such it was appropriate to use non-parametric tests on this dataset. The Mann-Whitney U test was employed to determine the extent and significance of any differences between leachate from the UF and F columns. A Spearman's rank correlation was used to determine any linear relationships between the characteristics of the UF and F columns.

The solid and extracted phase data was determined to be of normal distribution and as such a one-way analysis of variance (ANOVA) was used to determine the extent and significance of any differences between the UF and F columns. The test was applied to solid and extracted phase analyses, the multiple groups of data generated by the QEMSCAN® analysis and to compare the results from the freshly prepared soil to those subjected to 52 weeks of irrigation.

A Pearson correlation coefficient (PCC) was used to determine any linear relationships between the solid and extracted phase characteristics for the UF and F columns. This test allowed for the determination of whether a linear relationship existed between two datasets, making no assumption as to whether one variable was dependent on the other. Both the PCC and the Spearman's rank tests yield a value between -1 and 1; the closer the value is to 0 the greater the variation between the data points around the line of best fit. A positive value suggests positive correlation and a negative value suggests negative correlation.

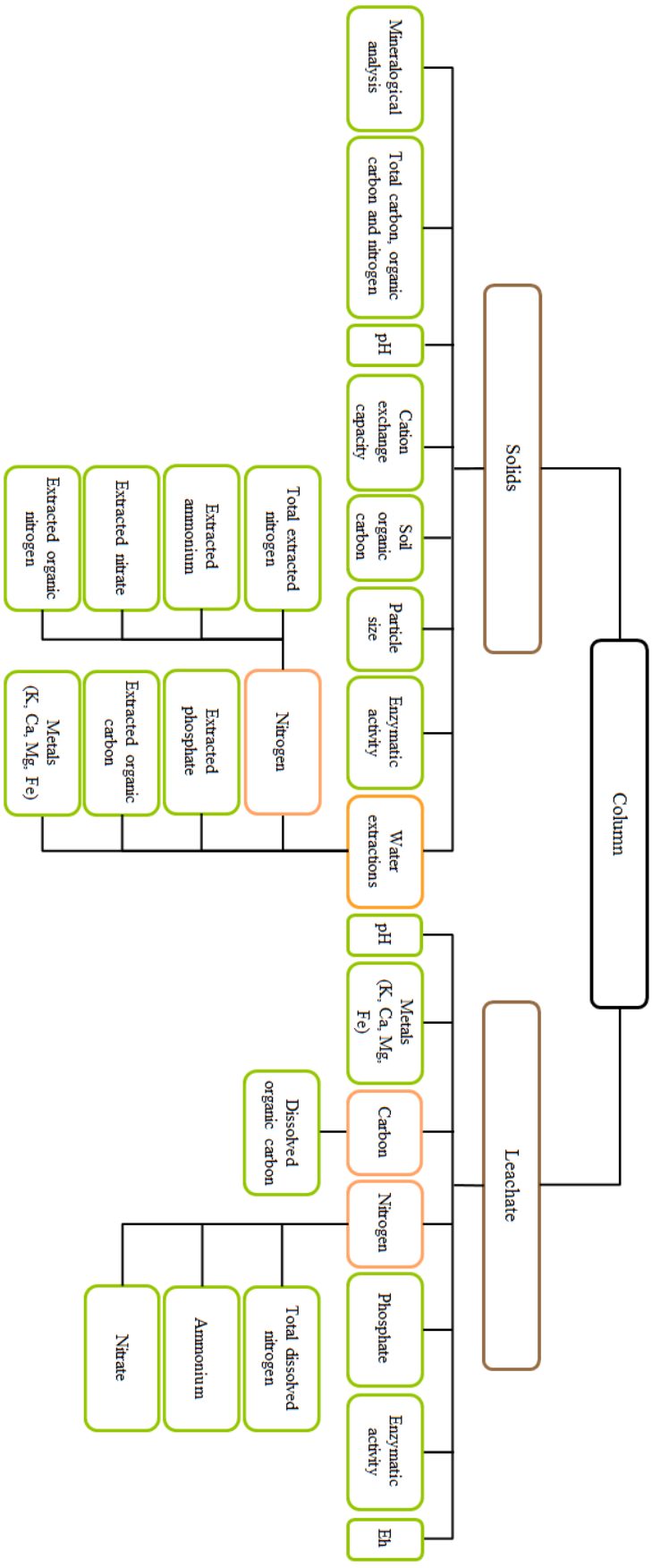

Figure 4.5: *Strategy used for the analysis of leachate and soil samples taken during the column study.*

4.4 Results

The results from this experiment are split into three parts: (1) leachate data; (2) solid soil phase data and (3) water extracted soil constituent data. Data presented for (2) and (3) are on soils extruded from the columns and freshly prepared soil samples.

4.4.1 Results overview

Throughout the 52 week irrigation term the key observations with regard to leachate properties were:

(1) All analysed N fractions, excepting NH_4^+ (which was consistently below the LOD), demonstrated an overall decrease in concentration over the first 27 weeks of irrigation: TDN decreased by 89.3 % (from 14.6 ± 7.6 to 1.51 ± 0.6 mg N L^{-1}) from irrigation Week 6 to 27, DON decreased by 82.6 % (from 14.3 ± 0.5 to 2.25 ± 0.2 mg N L^{-1}) from Week 6 to 27, NO_3^- decreased by 95.2 % (from 6.73 ± 0.92 to 0.25 ± 0.04 mg N L^{-1}) within the first 2 weeks of irrigation. N concentrations within the leachate increased from week 27 for both UF and F columns. Within the UF columns concentrations increased by 95.9 % for NO_3^- (from 0.24 ± 0.01 to 5.87 ± 2.18 mg N L^{-1}) and 75.2 % for DON (from 2.25 ± 0.02 to 9.08 ± 3.94 mg N L^{-1}), between weeks 27 and 52.

(2) PO_4^{3-} concentration increased by an average of 5.55 mg P L^{-1} over the first 6 weeks of irrigation, and from then remained stable until the end of the experiment, with a mean value of 5.31 ± 0.59 mg P L^{-1}.

(3) Mg, Ca, K and Fe each demonstrated an overall decrease in concentration. For Mg and Ca, losses were greatest over the first 6 weeks where leachate concentration declined by 76.8 % and 55.9 %, respectively. K and Fe concentrations showed an initial

increase of 316 mg L^{-1} and 0.17 mg L^{-1}, respectively from Week 1 to 3, following which a gradual and sustained decline was observed throughout the irrigation period where K concentration decreased by 97.1 % and Fe by 66.2 %.

(4) Over the 52 week irrigation term, the pH of the leachate decreased for both UF and F columns, from 6.62 ± 0.51 to 5.96 ± 0.09 and 5.74 ± 0.04, respectively. Eh and enzymatic activity were analysed from Week 48 to 52 and displayed no significant changes ($p > 0.05$) for either the UF or F columns.

(5) Following the 2 fertilisation events, a significant difference ($p < 0.05$) was observed between the UF and F columns for all TDN, NO_3^-, DON, PO_4^{3-}, pH, Mg and Ca. No significant difference was observed between UF and F columns for DOC, K, Fe, Eh and enzymatic activity.

Following analysis of solid samples and extracted constituents of the freshly prepared soils and the UF and F column soils at the end of the 52 week irrigation period, key observations were:

(1) Significant differences in the proportions of some mineral constituents were observed between freshly prepared, UF and F samples following 52 weeks of irrigation. The greatest differences were observed in the proportions of quartz, tourmaline and apatite between the freshly prepared soil and both the UF and F column samples.

(2) Significant differences ($p < 0.05$) between the UF and F columns were observed for moisture content, SOC, enzymatic activity, C : N ratio, extracted Mg, extracted Ca, extracted Fe and particle size distribution.

(3) There were significant differences ($p < 0.05$) between the freshly prepared soil and both the UF and F column soils for TPN, C : N ratio, EOC, TEN, EON, extracted PO_4^{3-}, moisture content and particle size distribution (CEC, NH_4^+ - UF only and extracted Fe - F only).

4.4.2 Leachate analyses

Leachate samples were collected from each column on a regular basis over 52 weeks, as outlined in Table 4.4. Values, reported below, are averages for the columns, n = 12 from Weeks 1 to 26 (after which 2 columns were fertilised), and n = 6 from Week 27.

4.4.2.1 Statistics

Statistical analyses of key characteristics for the leachate data were carried out using a Mann-Whitney U test (Table 4.5). A comparison between the UF and F columns using a Man-Whitney U test (Table 4.5a) revealed significant differences between the UF and F column leachates.

Table 4.5: *Man-Whitney U test results from a comparison key leachate characteristics in UF and F column leachate.*

Characteristic	U value	P value
DOC	0.68	0.109
TDN	4.04	0.040
NO_3^-	6.31	0.003
DON	8.21	0.005
PO_4^{3-}	48.02	< 0.001
Mg	9.77	0.027
Ca	11.18	0.005
K	2.98	0.094
Fe	1.84	0.124
pH	14.21	0.004
Eh	0.01	0.984
Enzymatic activity	2.34	0.460
Leachate volume	54.0	0.474

Red = significant p values
Blue = non-significant p values

Cumulative nutrient losses over the 52 week irrigation period (Table 4.6) were estimated from the leachate data.

Table 4.6: *Estimated cumulative nutrient losses to leaching.*

Characteristic	Unit	Unfertilised	Fertilised
Total leachate volume	L	30.7 ± 4.1	30.1 ± 3.4
TDN	g	70.9 ± 2.4	81.4 ± 1.0
- NO_3^-	g	18.1 ± 0.9	28.0 ± 1.3
- DON	g	53.1 ± 1.5	55.3 ± 1.3
PO_4^{3-}	g	55.0 ± 0.3	61.1 ± 0.4
DOC	g	783 ± 8	759 ± 8
K	g	1775 ± 14	1636 ± 14
Ca	g	463 ± 4	470 ± 4
Mg	g	128 ± 2	131 ± 2
Fe	g	2.02 ± 0.04	1.96 ± 0.03

4.4.2.2 Nitrogen

The leached TDN concentrations for all columns are displayed in Figure 4.6. Concentrations within the first 5 weeks of irrigation ranged from 9.2 ± 1.1 to 14.6.±.1.4.mg N L^{-1}, with a relatively large standard deviation for each sampling, suggesting a large inter-column variation. From Week 6 to 27 the concentrations within all 4 of the unfertilised columns declined steadily, at an average rate of decrease of 0.28.mg N L^{-1} $Week^{-1}$ from 14.6 ± 1.4 to 1.51 ± 0.6 mg N L^{-1}.

The TDN concentrations from the UF columns increased from irrigation Week 28, reaching 10.6 ± 4.43 mg N L^{-1} by Week 52. The leachate concentration from the F columns increased from Week 32. The concentrations from both the UF and F columns were relatively constant from Week 39 to 48, after which another increase was observed in the F columns. The second fertiliser application occurred in Week 48 and whilst both the UF and F TDN concentrations increased, the greater increase occurred in the F columns (up to 24.4 ± 4.3 mg N L^{-1} and 16.4 ± 4.2 mg N L^{-1} for UF and F,

respectively). Mann Whitney-U indicated a significant difference between the values from UF and F columns ($p = 0.040$, Table 4.5a).

The F column leachate displayed a strong positive correlation with time ($p < 0.001$, Table 4.5c), which demonstrated an overall increase in TDN concentration throughout the experimental timescale.

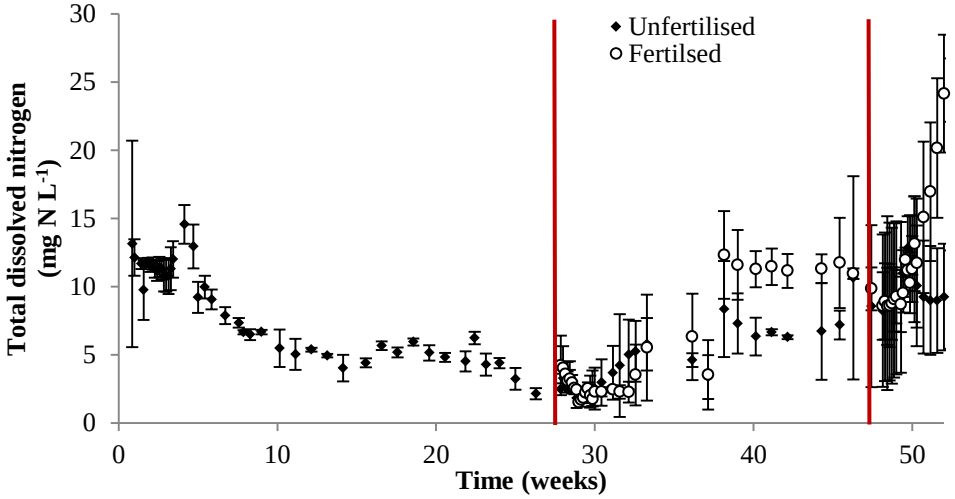

Figure 4.6: *TDN concentrations (mg N L^{-1}) for column leachate. Analyses were carried out in triplicate on samples collected from each column. Leachate was collected from 4 columns (n = 12) from Weeks 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application.*

Dissolved NO_3^- concentrations are displayed in Figure 4.7. An initial decrease in concentration, from 6.73 ± 0.92 to 0.36 ± 0.04 mg N L^{-1}, occurred over the first 2 weeks. From irrigation Week 2 to 27 the concentration in the UF columns remained relatively constant, averaging 0.28 ± 0.17 mg N L^{-1}.

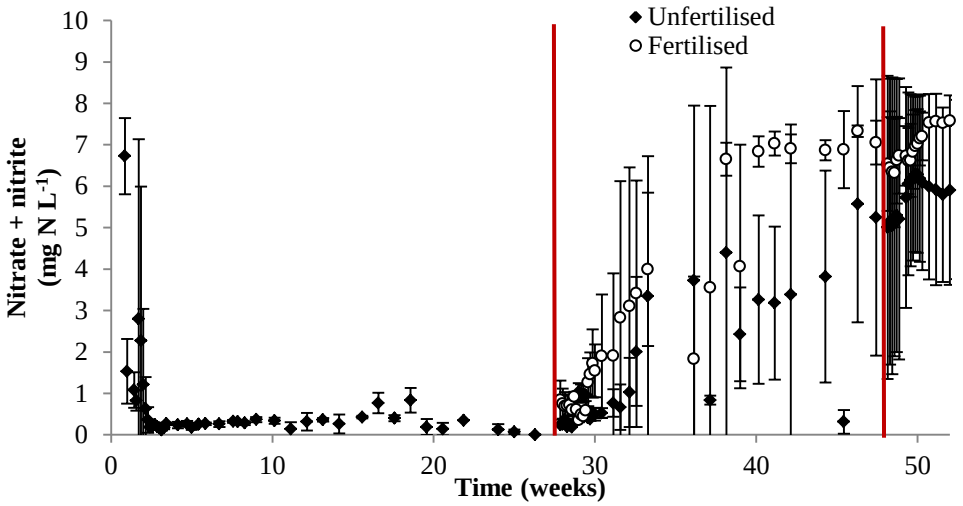

Figure 4.7: *NO_3^- concentrations (mg N L^{-1}) within the column leachate. Analyses were carried out in triplicate on samples collected from each column. Leachate was collected from 4 columns (n = 12) from Week 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

Following fertiliser application in Week 27, an increase in NO_3^- concentration was observed almost immediately within the F columns. The NO_3^- concentration within the UF column also increased, though, initially at a slower rate than the F column (0.41 mg N L^{-1} $week^{-1}$ vs. 0.59 mg N L^{-1} $week^{-1}$ respectively), increasing from 0.24 ± 0.01 to 5.87 ± 2.18 mg N L^{-1} for the UF columns and 0.84 ± 0.47 to 7.54 ± 0.01 mg N L^{-1} for the F columns. It appeared that leaching of NO_3^- (Figure 4.7) was the principal reason for the increase in TDN concentration (Figure 4.6). Over the experimental period a significant increase in concentration was observed in both the UF and F column leachates from Week 27 ($p = 0.003$, Tables 4.5b and c). The difference between the UF and F column treatments was also significant ($p = 0.013$, Table 4.5a).

NH_4^+ concentrations were monitored on a monthly basis and were found to be consistently below the LOD (< 1.12 µg N L^{-1}).

The DON concentrations within the leachate were calculated by subtracting the sum of the DIN fractions (NO_3^- and NH_4^+) from the TDN concentration (Figure 4.8). There was an increase in DON concentration, (2.4 ± 3.4 to 14.3 ± 0.5 mg N L^{-1}) between Weeks 1 and 5. From irrigation Weeks 6 to 9, the concentration declined at an average rate of 1.89 mg N L^{-1} $Week^{-1}$, then at 0.21 mg N L^{-1} $Week^{-1}$ until Week 27.

Fertilisation of the columns appeared to have no immediate effect on the DON fraction, as levels continued to decline. From Week 27 the DON concentration increased in the UF columns and from Week 32 in the F columns, similar to the behaviour of the TDN fraction. The increase in concentration within the UF columns was relatively small (up to 9.08 ± 3.94 mg N L^{-1} on Week 47). The DON concentration from the F columns exceeded this (up to 16.60 ± 2.32 mg N L^{-1} on Week 52). A significant difference ($p = 0.005$, Table 4.5a) in DON concentration between the leachate from the UF and F columns was observed from Week 48 to 52.

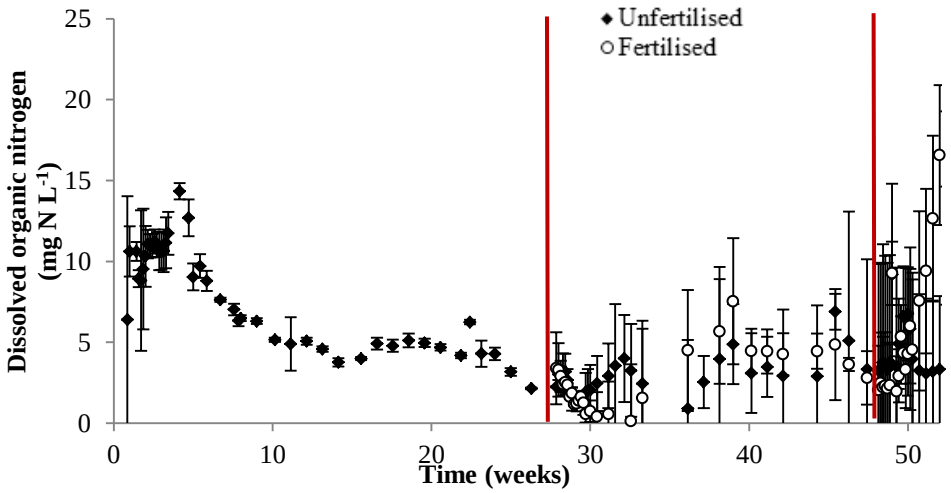

Figure 4.8: a) *DON concentrations (mg N L^{-1}) of column leachate. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Week 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

4.4.2.3 Phosphate

PO_4^{3-} concentrations (Figure 4.9) increased over the first 6 weeks of irrigation, from 0.02 ± 0.01 mg P L^{-1} in Week 1 to 5.57 ± 1.23 mg P L^{-1} in Week 6, an average increase of 1.12 mg L^{-1} $Week^{-1}$. The PO_4^{3-} concentration in the UF column leachate remained relatively stable from Weeks 6 to 39, ranging from 5.80 ± 1.04 to 4.13 ± 0.31 mg P L^{-1}.

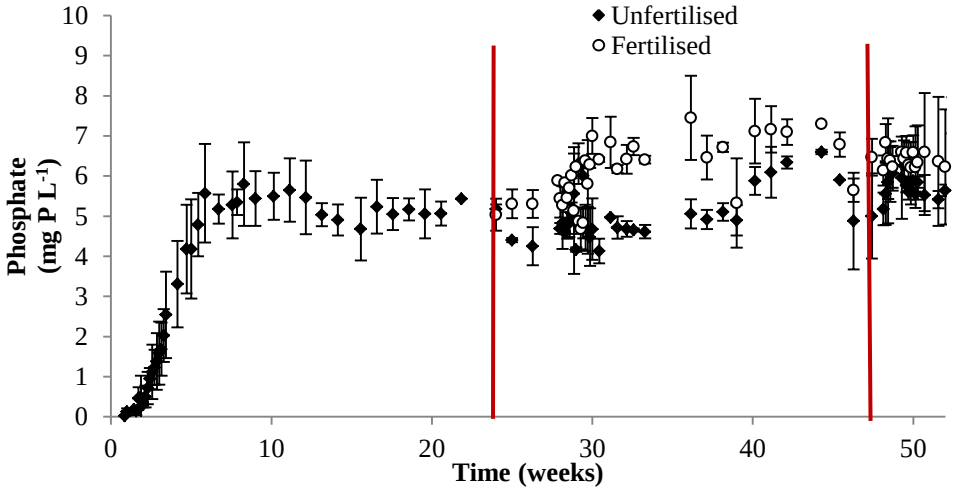

Figure 4.9: *PO_4^{3-} concentrations (mg P L^{-1}) within the column leachate. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Week 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

A significant increase in PO_4^{3-} concentration from irrigation Weeks 28 to 30 of 0.42 mg P L^{-1} $Week^{-1}$ was observed within the F columns, with the concentration of subsequent leachate samples remaining relatively constant. The concentration within the UF columns increased towards Week 52.

Following the first fertilisation, an increase in PO_4^{3-} was observed in all columns, though the greater increase occurred in the F columns. Following the second fertilisation this difference was less pronounced. The difference in PO_4^{3-} concentrations between UF and F columns was significant following each fertiliser applications ($p < 0.001$, Table 4.5a). Both UF and F columns displayed in a significant concentration

increase of dissolved PO_4^{3-} with time ($p < 0.001$ for both UF and F columns, Tables 4.5b and c).

4.4.2.4 Dissolved organic carbon

DOC concentrations of leachate (Figure 4.10) decreased significantly ($P < 0.001$) for both the UF and F columns. The initial concentrations were variable and a general increase was observed from Weeks 1 to 4 (72 ± 30 to 272 ± 29 mg C L^{-1}).

Between Weeks 2 and 4 the mean DOC concentration decrease was lower (6.39 mg C L^{-1} $Week^{-1}$) than from Weeks 4 to 14 (19.6 mg C L^{-1} $Week^{-1}$). The lowest rate of concentration decrease was for Weeks 29 to 52 (0.75 mg C L^{-1} $Week^{-1}$). Fertilisation had no significant effect on the leachate DOC concentrations ($p > 0.05$, Table 4.5a).

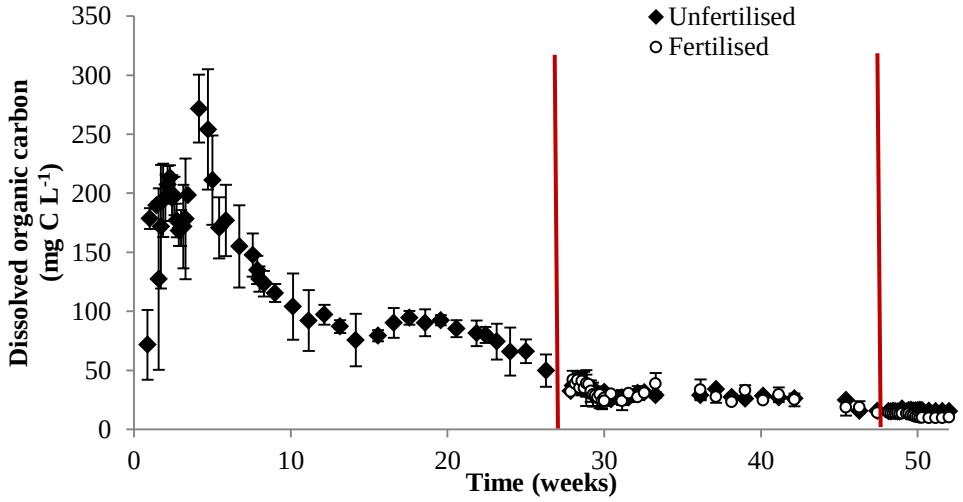

Figure 4.10: *DOC concentrations (mg C L^{-1}) within the column leachate. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Week 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

4.4.2.5 Metals

Mg concentrations in leachate (Figure 4.11a) decreased over the first 5 weeks of irrigation, from 38.6 ± 2.2 to 9.8 ± 1.8 mg Mg L^{-1}. The concentration remained relatively stable thereafter, (9.8 ± 2.0 mg Mg L^{-1} from Weeks 5 to 52). The fertiliser applications appeared to affect the leachate Mg concentrations as there was a significant difference between the UF and F columns ($p = 0.027$, Table 4.5a).

Leached Ca concentration profiles (Figure 4.11b) were similar to those of Mg. After an initial concentration decrease (87.5 ± 3.8 mg Ca L^{-1} to 42.4 ± 8.5 mg Ca L^{-1}) during Weeks 1 to 4, the concentrations varied little (36.4 ± 7.4 mg Ca L^{-1}) in the UF columns. Both fertiliser applications resulted in an increase in dissolved Ca within the leachate, with a larger increase observed following the second fertiliser application. The difference in dissolved Ca concentration between the UF and F columns was significant ($p = 0.005$, Table 4.5a).

Dissolved K concentrations in the column leachate (Figure 4.11c) initially increased from 193 ± 39 mg K L^{-1} at the end of Week 1, to 509 ± 53 mg K L^{-1} after 3 weeks. From Week 3 the concentration then declined at a steady rate of 9.80 mg K L^{-1} $Week^{-1}$ (UF columns) with the concentration reaching 18.0 ± 0.3 mg K L^{-1} at the end of Week 52. The fertiliser additions resulted in no significant difference in dissolved K concentrations between columns ($p = 0.094$, Table 4.5a).

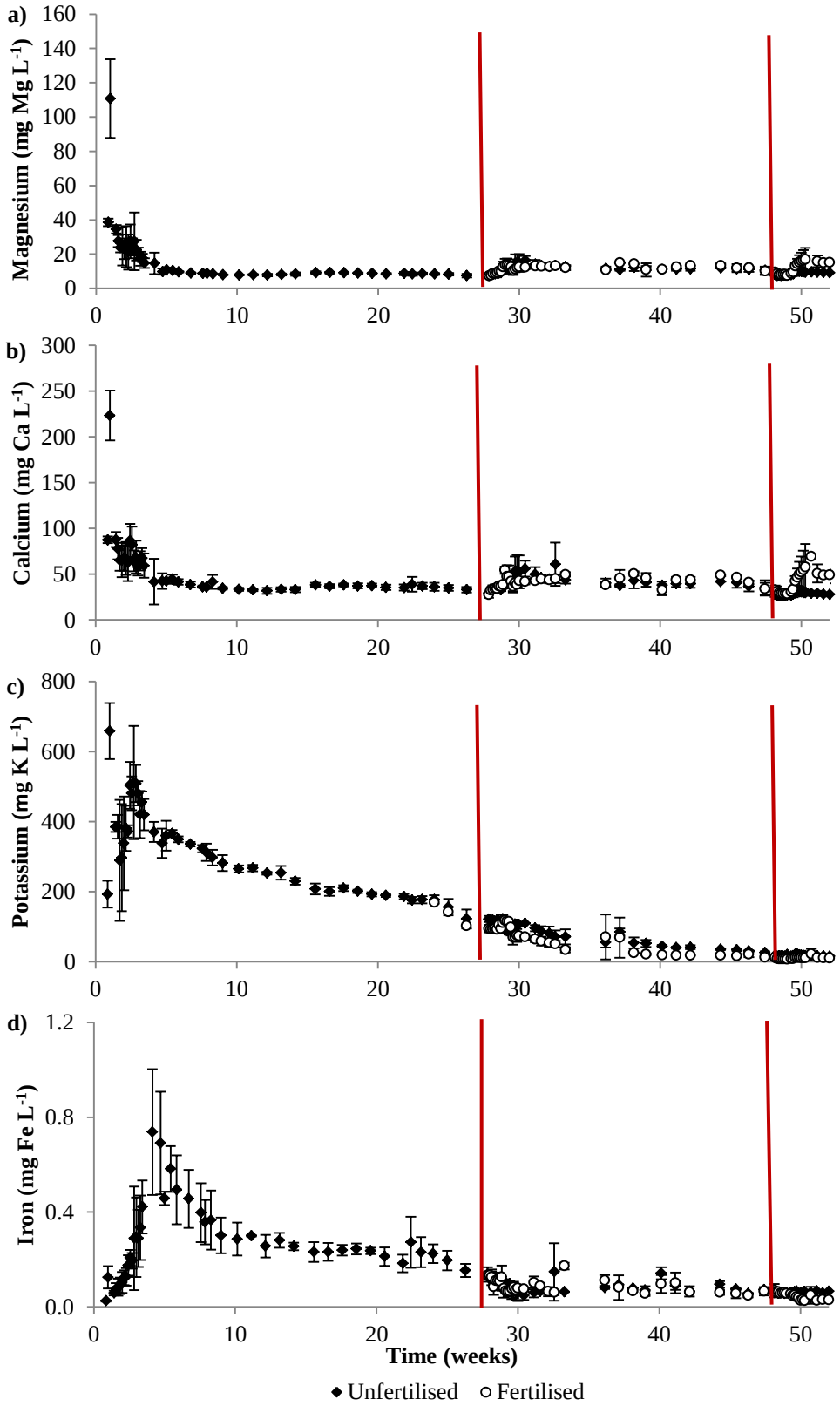

Figure 4.11: *Concentrations of dissolved metals (mg g^{-1})* ***a)*** *Mg,* ***b)*** *Ca,* ***c)*** *K and* ***d)*** *Fe within the column leachate. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Weeks 0 to 26. From Week 27 analyses were carried out on 2 sets of leachate from 2 sets of columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

Dissolved Fe concentrations (Figure 4.11d) increased from < LOD (0.003 mg Fe L^{-1}) to 0.74 ± 0.27 mg Fe L^{-1} between Weeks 1 and 4. The Fe concentration then decreased at a rate of 0.091 mg Fe L^{-1} $Week^{-1}$ from Week 5 to 8, then slowed to 0.007 mg Fe L^{-1} $Week^{-1}$ until Week 26. From Week 31 the dissolved Fe concentration was relatively stable, with the column fertilisation having no significant effect (p = 0.124, Table 4.5a).

4.4.2.6 Physicochemical characteristics

The pH of the leachate samples (Figure 4.12) decreased significantly with time (UF p < 0.001, F p < 0.001, Tables 4.5b and c). The initial mean pH was 6.62 ± 0.51, which decreased to 5.96 ± 0.09 and 5.74 ± 0.04 in the leachate from the UF and F columns, respectively. The F column leachate pH was significantly lower than that of the UF columns (p = 0.004, Table 4.5a). The pH correlated significantly with all characteristics (p < 0.05, Tables 4.5b and c), excluding enzymatic activity for both UF and F columns and dissolved PO_4^{3-} for the UF columns.

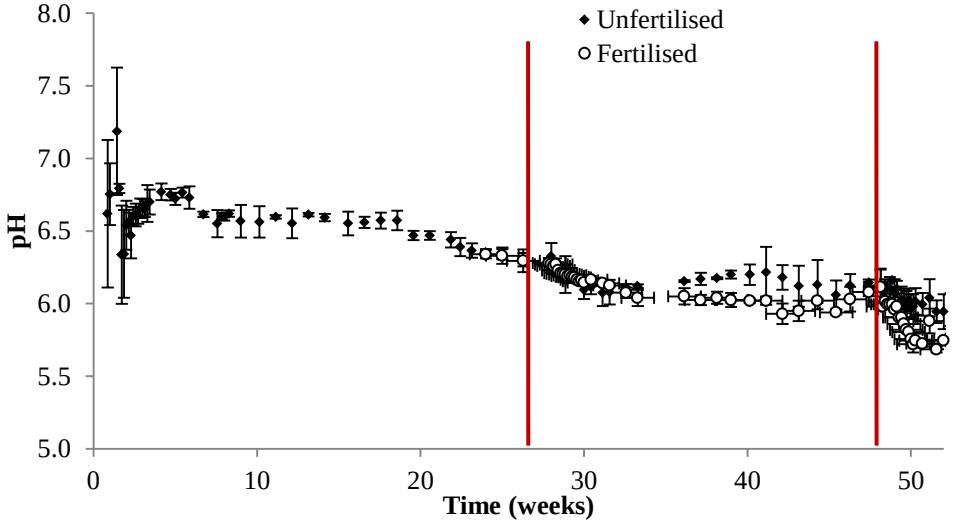

Figure 4.12: *pH for column leachate. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Week 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6). Red lines represent fertiliser application timings.*

The Eh for each leachate sample was measured from Weeks 48 to 52, following the second fertiliser application (Figure 4.13). The columns demonstrated no significant difference between them (p = 0.984, Table 4.5a). The highest Eh values for both treatments were recorded during Week 49 (401 ± 1 and 401 ± 4 mV for UF and F columns, respectively).

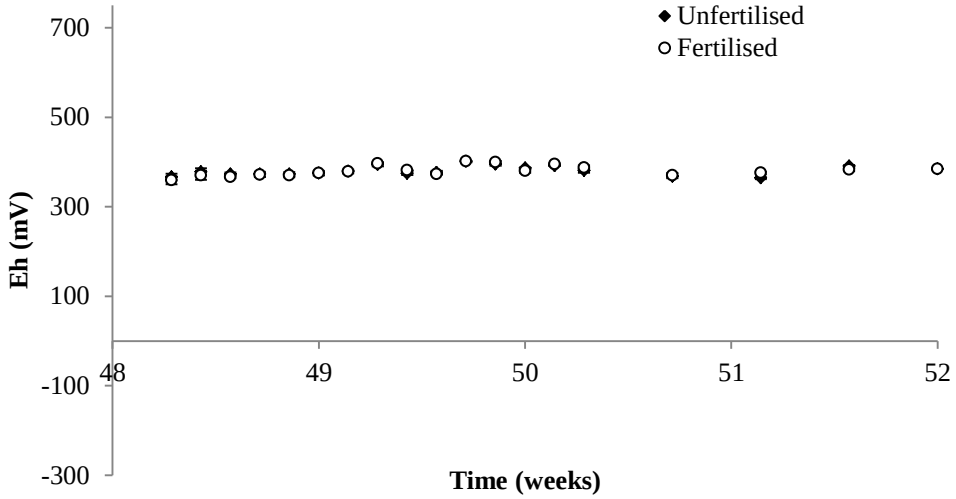

Figure 4.13: *Eh (mV) within the column leachate following the second fertilisation of the soil columns. Analyses were carried out in triplicate on samples from each column. Leachate was collected from 4 columns (n = 12) from Week 48 to 52. Analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6).*

4.4.2.7 Enzymatic activity

Enzymatic activity within the column leachate was monitored following the second fertiliser application, from Weeks 48 to 52 (Figure 4.14). The results indicated no significant differences between the two treatments (p = 0.460, Table 4.5a); however, between Weeks 47 and 50 the average enzymatic activity was higher in the F column samples. The enzymatic activity was then highest in the unfertilised samples. Overall there was no significant correlation between enzymatic activity and any other measured characteristics (p > 0.05, Tables 4.5b and c).

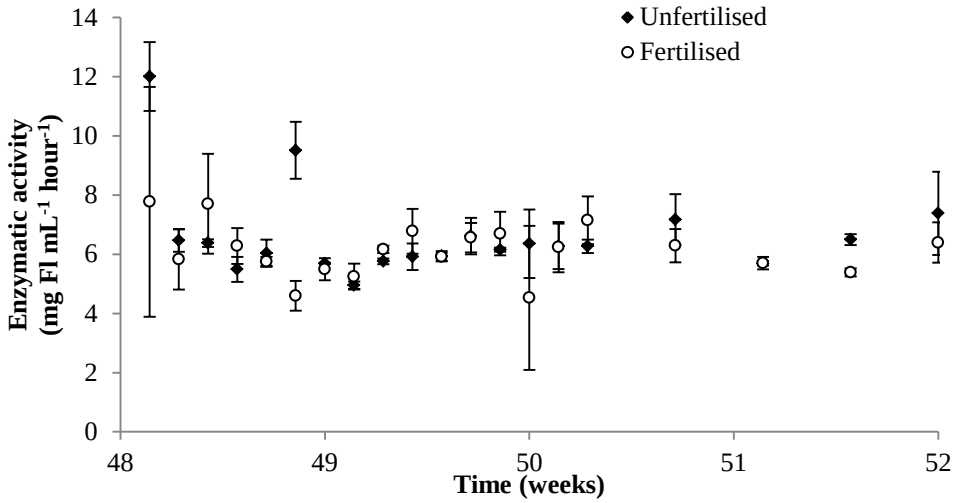

Figure 4.14: *Enzymatic activity (mg Fl mL^{-1} $hour^{-1}$) within the column leachate samples following the second fertilisation of the columns. Analyses were carried out in triplicate on samples collected from each column. Leachate was collected from 4 columns (n = 12) from Week 48 to 52. Analyses were carried out on 2 sets of 2 columns (UF and F) (n = 6).*

4.4.2.8 Leachate volume

The leachate volume from each column was monitored for each sample (Figure 4.15) and varied between 75 to 90 mL day^{-1}. There was observed to be no significant difference between the leachate volume from the UF and F columns ($p > 0.05$, Table 4.5a).

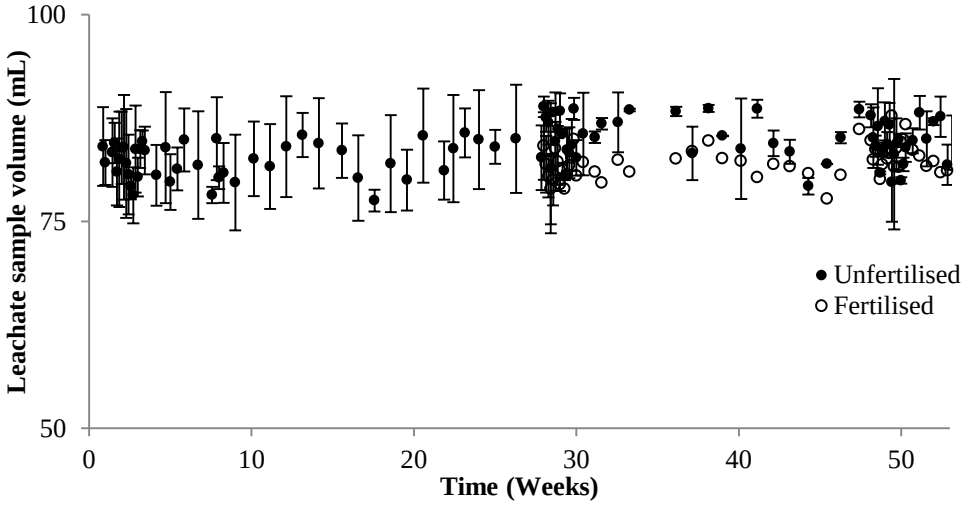

Figure 4.15: *Leachate volume (mL) from each column. Leachate was collected from 4 columns (n = 4) from Weeks 0 to 26. From Week 27 analyses were carried out on 2 sets of 2 columns (UF and F) (n = 2).*

4.4.3 Statistical examination of solid phase and extract analyses

The soils were extruded from the columns after 52 weeks and sampled at a 5 cm depth resolution. For the TPN and TPC, water extractions and particle size analyses, the 0 to 20 cm depth interval was analysed at a 5 cm resolution, with the 20 to 50 cm depth interval analysed at a 10 cm resolution. The pH, moisture content and enzymatic activity measurements were carried out at 5 cm resolution throughout the column. The values reported below are means for each pair of UF and F columns (n = 6). At the time of packing the columns contained 70 cm of soil, but the soil had compacted following 1 year of irrigation to give a soil depth of approximately 50 cm in each column.

The freshly-prepared artificial soils were analysed for various characteristics, in order to identify any significant changes they may have undergone during the column study; the results from the fresh soil analyses are shown in Table 4.7.

Table 4.7: *Characteristics of the freshly prepared artificial soil as packed into the soil columns.*

Characteristic	Units	Value
TPN	mg N g^{-1}	10.2 ± 0.2
TPC	mg C g^{-1}	185 ± 1
SOC	µg C g^{-1}	108 ± 9.6
C : N ratio		22.6 ± 0.4
pH		5.91 ± 0.04
Moisture content	%	13.0 ± 0.3
Sand fraction	%	59.8
Silt fraction	%	39.3
Clay fraction	%	1.0
CEC	$cmol_c$ kg^{-1}	5.76 ± 0.28
TEN	µg N g^{-1}	253 ± 14
Extracted NO_3^-	µg N g^{-1}	166 ± 102
Extracted NH_4^+	µg N g^{-1}	< LOD (26.8)
EON	µg N g^{-1}	246 ± 103
Extracted PO_4^{3-}	µg P g^{-1}	104 ± 4
EOC	µg C g^{-1}	970 ± 80
Extracted Mg	µg Mg g^{-1}	5.20 ± 1.86
Extracted Ca	µg Ca g^{-1}	22.0 ± 4.7
Extracted K	µg K g^{-1}	63.4 ± 3.9
Extracted Fe	µg Fe g^{-1}	9.48 ± 1.13

The mean percentage change to concentrations of each analyte following 52 weeks of irrigation was calculated for the UF and F columns (Table 4.8). Results demonstrated that within the UF and F columns most nutrients, excepting Mg and Ca and NO_3^- to F column only, decreased in concentration during the irrigation period, this is addressed in greater detail within Section 4.5.

Table 4.8: *Mean percentage differences between the UF and F soils at t = 0, and following 52 weeks of irrigation. The positive values indicate and increase in concentration while negative values indicate a concentration decrease).*

Characteristic	Unfertilised	Fertilised
TPN	-7.06 ± 0.3	-9.9 ± 0.3
TPC	-5.95 ± 3.42	-4.32 ± 7.08
EOC	-82.7 ± 38.1	-81.4 ± 22.8
TEN	-80.1 ± 4.7	-72.8 ± 18.5
Extracted NO_3^-	-88.0 ± 26.4	85.1 ± 2.8
EON	-89.8 ± 1.7	-84.0 ± 5.6
Extracted PO_4^{3-}	-48.6 ± 6.4	-45.4 ± 2.6
Extracted K	-90.6 ± 1.3	-26.7 ± 5.7
Extracted Ca	49.6 ± 4.2	-45.5 ± 0.7
Extracted Fe	-14.1 ± 0.2	-56.5 ± 0.1
Extracted Mg	15.8 ± 0.1	55.8 ± 0.0
pH	0.00 ± 0.01	0.85 ± 0.01
CEC	-26.7 ± 6.64	11.5 ± 1.3
C : N ratio	-42.5 ± 0.1	-37.1 ± 0.3
SOC	-4.79 ± 1.09	1.85 ± 0.91
Sand fraction	-20.4 ± 5.0	-11.9 ± 4.2
Silt fraction	27.0 ± 4.8	-88.5 ± 4.7
Clay fraction	149 ± 5	110 ± 7

Statistical analyses were performed using a one-way ANOVA to determine the significance of any difference between the UF and F soil profiles and the freshly prepared soil following extrusion of the columns (Table 4.9). These analyses indicated that many of the measured characteristics had changed significantly during the experiment ($p < 0.05$).

Table 4.9: *Comparison of the characteristics of the freshly prepared artificial soil to the results obtained following 1 year of irrigation, using a one-way ANOVA.*

Characteristic	Freshly prepared v unfertilised soil	Freshly prepared v fertilised soil
	p value	p value
TPN	0.030	0.030
TPC	0.152	0.152
SOC	0.096	0.053
C : N ratio	0.002	0.003
pH	0.836	0.631
Moisture content	< 0.001	< 0.001
Sand fraction	0.003	0.025
Silt fraction	0.006	0.038
Clay fraction	< 0.001	< 0.001
CEC	0.187	0.561
TEN	< 0.001	< 0.001
Extracted NO_3^-	0.400	0.201
Extracted NH_4^+	0.006	0.097
EON	< 0.001	< 0.001
Extracted PO_4^{3-}	0.011	< 0.001
EOC	< 0.001	< 0.001
Extracted Mg	0.705	0.055
Extracted Ca	0.445	0.183
Extracted K	0.807	0.240
Extracted Fe	0.766	0.028

Red = significant p values
Blue = insignificant p values

The one-way ANOVA tests (Table 4.10a) determined that there were significant differences ($p < 0.05$) between the TEN, EON, pH, moisture content, SOC, enzymatic activity, C : N ratio, Mg, Ca, Fe and sand, silt and clay particle size fractions within the UF and F column profiles.

Pearson's correlation coefficient (PCC) analyses between key soil characteristics (Tables 4.10b and c) suggests only a few instances of significant correlation ($p < 0.05$) between soil characteristics, with some results displaying correlation for the unfertilised profiles, but not for the fertilised profiles and vice versa.

Table 4.10a: *One-way ANOVA results for key characteristics between UF and F column profiles.*

Characteristic	ANOVA	p Value
TPN	1.78	0.207
TPC	1.15	0.304
SOC	13.3	0.002
C : N ratio	5.99	0.031
pH	0.79	0.386
Moisture content	2.98	0.087
Sand	17.7	0.001
Silt	15.7	0.002
Clay	29.5	< 0.001
CEC	3.97	0.093
Enzymatic activity	45.0	< 0.001
TEN	5.56	0.036
Extracted NO_3^-	0.52	0.483
Extracted NH_4^+	0.10	0.755
EON	0.31	0.586
Extracted PO_4^{3-}	0.48	0.502
EOC	0.18	0.681
Extracted Mg	18.1	0.001
Extracted Ca	15.6	0.002
Extracted K	1.90	0.193
Extracted Fe	15.7	0.002

Red = significant p values
Blue = insignificant p values

Table 4.10b: *Pearson correlation coefficient values for key characteristics within the unfertilised column profile.*

	Unfertilised											
	Depth	**TPN**	**TEN**	**NO_3^-**	**NH_4^+**	**TPC**	**EOC**	**PO_4^-**	**pH**	**Moisture content**	**Organic matter**	**Clay fraction**
TPN	-0.305 0.472											
TEN	-0.346 0.447	0.862 0.013										
NO_3^-	-0.166 0.722	0.777 0.040	0.897 0.006									
NH_4^+	-0.620 0.138	0.892 0.007	0.758 0.048	0.715 0.071								
TPC	-0.328 0.472	0.590 0.163	0.341 0.454	0.516 0.235	0.745 0.055							
EOC	0.840 0.018	0.072 0.879	-0.131 0.780	0.145 0.756	-0.162 0.728	0.134 0.774						
PO_4^-	0.723 0.067	0.383 0.397	0.222 0.632	0.395 0.381	0.031 0.948	0.139 0.766	0.867 0.012					
pH	0.895 <0.001	-0.123 0.792	0.028 0.953	0.216 0.641	-0.431 0.334	-0.231 0.619	0.721 0.067	0.685 0.089				
Moisture content	0.684 0.029	0.199 0.668	0.224 0.630	0.220 0.635	0.185 0.692	-0.112 0.811	0.613 0.144	0.683 0.020	0.832 0.020			
Organic matter	0.100 0.784	0.509 0.244	0.164 0.725	0.339 0.457	0.402 0.372	0.716 0.071	0.457 0.302	0.614 0.143	0.048 0.918	0.153 0.744		
Clay fraction	-0.671 0.099	0.655 0.110	0.755 0.050	0.734 0.060	0.793 0.033	0.676 0.095	-0.421 0.348	-0.186 0.689	-0.304 0.507	-0.170 0.716	0.274 0.552	
Enzymatic activity	-0.726 0.018	0.061 0.897	-0.050 0.914	-0.240 0.604	0.346 0.447	0.367 0.418	-0.150 0.748	-0.735 0.060	-0.749 0.013	-0.273 0.446	-0.038 0.918	0.394 0.382

Black = PCC value (between 1 and -1).
Red = significant p values
Blue = insignificant p values

Table 4.10c: *Pearson correlation coefficient values for key characteristics within the fertilised column profile.*

	Fertilised											
	Depth	**TPN**	**TEN**	**NO_3^-**	**NH_4^+**	**TPC**	**EOC**	**PO_4^-**	**pH**	**Moisture content**	**Organic matter**	**Clay fraction**
TPN	0.189 0.685											
TEN	-0.305 0.225	0.730 0.063										
NO_3^-	-0.530 0.221	0.244 0.598	0.635 0.125									
NH_4^+	0.888 0.008	0.437 0.327	-0.015 0.975	-0.218 0.638								
TPC	-0.191 0.682	0.405 0.388	0.782 0.038	0.246 0.596	-0.164 0.725							
EOC	0.527 0.225	0.380 0.400	0.188 0.687	-0.498 0.256	0.333 0.465	0.513 0.239						
PO_4^-	0.722 0.067	0.761 0.047	0.403 0.370	-0.131 0.780	0.594 0.159	0.321 0.482	0.843 0.017					
pH	0.973 < 0.001	0.184 0.691	-0.357 0.432	-0.576 0.176	0.827 0.022	-0.225 0.628	0.559 0.192	0.676 0.096				
Moisture content	0.785 0.007	0.189 0.685	-0.182 0.696	-0.595 0.159	0.846 0.016	-0.110 0.814	0.568 0.183	0.703 0.078	0.806 0.029			
Organic matter	0.450 0.192	0.377 0.405	0.632 0.128	0.275 0.551	0.431 0.309	0.644 0.119	0.406 0.366	0.601 0.154	0.142 0.762	0.464 0.176		
Clay fraction	0.861 0.013	0.041 0.931	-0.462 0.297	-0.471 0.286	0.868 0.011	-0.550 0.201	0.183 0.695	0.506 0.247	0.823 0.023	0.860 0.013	0.127 0.787	
Enzymatic activity	0.871 0.001	0.389 0.389	-0.171 0.715	-0.302 0.510	0.923 0.003	-0.255 0.581	0.408 0.364	0.766 0.045	0.847 0.002	0.783 0.007	0.340 0.337	0.833 0.020

Black = PCC value (between 1 and -1).
Red = significant p values
Blue = insignificant p values

Individual components of the freshly prepared soil mixture were analysed for TPN and TPC concentrations, with results displayed in Table 4.11. The TPC concentration was highest in the bark component (416 ± 42 %) and lowest in the composted green waste component (196 ± 45 %). The TPN concentration was highest in composted green waste (15.1 ± 3.7 %) and lowest in the lignite clay component (3.5 ± 0.4 %).

Table 4.11: *TPC (mg C g^{-1}) and TPN (mg N g^{-1}) concentrations and C : N ratios for each component used in the production of the artificial soil.*

Analyses	TPC (mg C g^{-1})	TPN (mg N g^{-1})	C : N ratio
Composted green waste	196 ± 45	15.1 ± 3.7	13.0 ± 3.0
Bark	416 ± 42	12.4 ± 2.2	33.5 ± 4.0
Lignite	234 ± 2	3.5 ± 0.4	67.9 ± 2.1

The C : N ratio was calculated from the TPC and TPN values (Table 4.11). It was found that the ratio was highest in the lignite component (67.9) of the soil mix, with bark also having a mid to high value (33.5). The composted green waste component displayed a much lower value (13.0).

4.4.4 Solid phase analysis

4.4.4.1 Mineralogical analyses

The freshly prepared artificial soil samples were analysed in triplicate, along with single samples from the UF and F columns. The freshly prepared soil results were compared to those of the irrigated soils; where the value for the irrigated soils lay outside the standard deviation of the fresh soils, there was deemed to be a difference.

The results of the mineralogical analyses, by QEMSCAN®, for the freshly prepared soil and the UF and F samples are shown in Table 4.12, with the false colour fieldscan images displayed in Figure 4.16. For the freshly prepared soil n = 3 and for the UF and

F soils n = 1. Comparative study suggested that a small number of mineral proportions were different between the freshly prepared soil and the UF or F soils (Table 4.12). Differences in the proportion of quartz, tourmaline, Mn phases and apatite were observed when comparing the freshly prepared soil with both the UF and F soils. The results suggest that changes in biotite / zinnwaldite / phlogopite, other silicates and ilmenorulite had occurred within the UF soil, and in topaz, and calcite in the F soil.

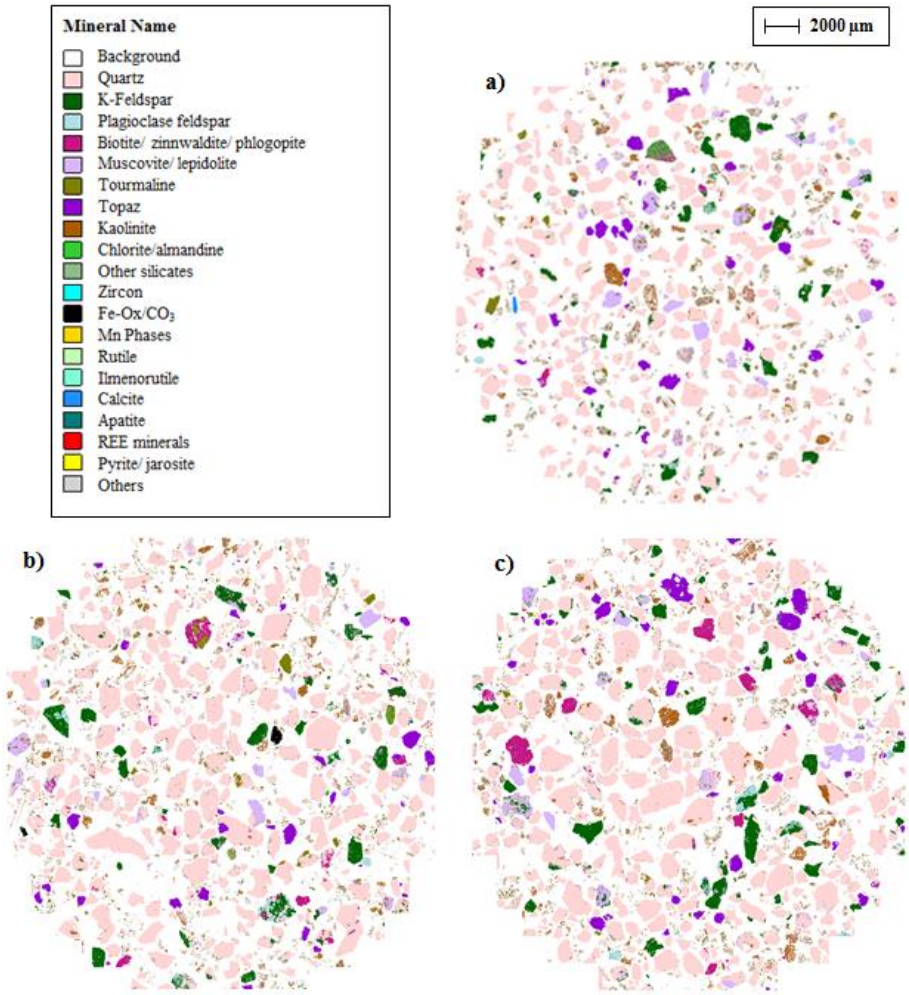

Figure 4.16: *False colour fieldscan images of resin mounted samples.* ***a)*** *An example of the freshly prepared artificial soil.* ***b)*** *Unfertilised column soil from 10 to 15 cm depth following 52 weeks of irrigation.* ***c)*** *Fertilised column soil sampled from 10 to 15 cm depth following 52 weeks of irrigation.*

Table 4.12: Mineral composition for the irrigated fertilised (10 to 15 cm)and unfertilised (10 to 15 cm) soils and of freshly prepared artificial soil. Analysis carried out using QEMSCAN®.

Mineral	Mineral volume (%)		
	Fresh soil	Fertilised	Unfertilised
Quartz	64.1 ± 0.46	66.48	68.04
K-Feldspar	8.70 ± 0.44	8.83	8.13
Plagioclase feldspar	2.87 ± 0.41	2.42	2.75
Biotite/ Zinnwaldite/ Phlogopite	2.23 ± 0.96	3.40	2.33
Muscovite/ Lepidolite	8.40 ± 2.13	6.81	6.40
Tourmaline	1.68 ± 0.37	0.88	2.18
Topaz	4.30 ± 0.58	4.49	3.15
Kaolinite	6.84 ± 0.77	6.32	6.10
Chlorite/Almandine	0.35 ± 0.26	0.12	0.33
Other silicates	0.06 ± 0.01	0.02	0.07
Zircon	< 0.01 ± 0.00	< 0.01	< 0.01
Fe-Ox/CO3	0.03 ± 0.02	0.03	0.30
Mn phases	0.01 ± 0.00	0.02	0.02
Rutile	0.05 ± 0.01	0.04	0.05
Ilmenorutile	0.01 ± 0.00	0.02	0.01
Calcite	0.25 ± 0.13	0.03	0.06
Apatite	0.13 ± 0.01	0.06	0.04
Rare earth element minerals	< 0.01 ± 0.00	< 0.01	< 0.01
Pyrite/Jarosite	0.01 ± 0.00	0.01	0.01
Others	0.01 ± 0.00	0.02	0.02

Red – result different from freshly prepared soil
Blue – no difference from freshly prepared soil

Quartz has a silicate-oxygen tetrahedral structure and represents the major portion of the sand and silt fraction for most soils (Barber, 1995). Quartz weathers slowly, which suggests that the decline in the representative proportion observed over the irrigation period (Table 4.12) was caused by the transport of these molecules, rather than by their breakdown. Feldspars have an alumina-silicate tetrahedral of either potassium (K-feldspar), sodium and calcium (plagioclase feldspar) (Barber, 1995).

Apatite is a group of phosphate minerals; the most common forms have the formula $Ca_5(PO_4)_3(F/Cl/OH)_2$. Apatite minerals are primarily employed in the production of

fertiliser and, as such, are an important source of PO_4^{3-} in soils. Given the high levels of PO_4^{3-} leached during this study, apatite appeared to be particularly important.

4.4.4.2 Nitrogen

TPN concentrations were relatively uniform with depth (Figure 4.17). The average TPN concentrations within the profile of the UF columns were higher than those of the F columns, though no significant difference was determined ($p = 0.207$, Table 4.10a) between either of the treatments and there was no correlation with depth ($p = 0.472$ for UF, $p = 0.675$ for F, Table 4.10b and c). The highest TPN concentration for both treatments occurred at 0 to 5 cm depth (10.4 ± 1.1 mg N g^{-1} and 9.62 ± 1.45 mg N g^{-1} in the UF and F columns, respectively) The lowest concentrations occurred at 5 to 10 cm depth in the UF columns (8.91 ± 0.87 mg N g^{-1}), and at 10 to 15 cm depth in the F columns (8.81 ± 0.78 mg N g^{-1}).

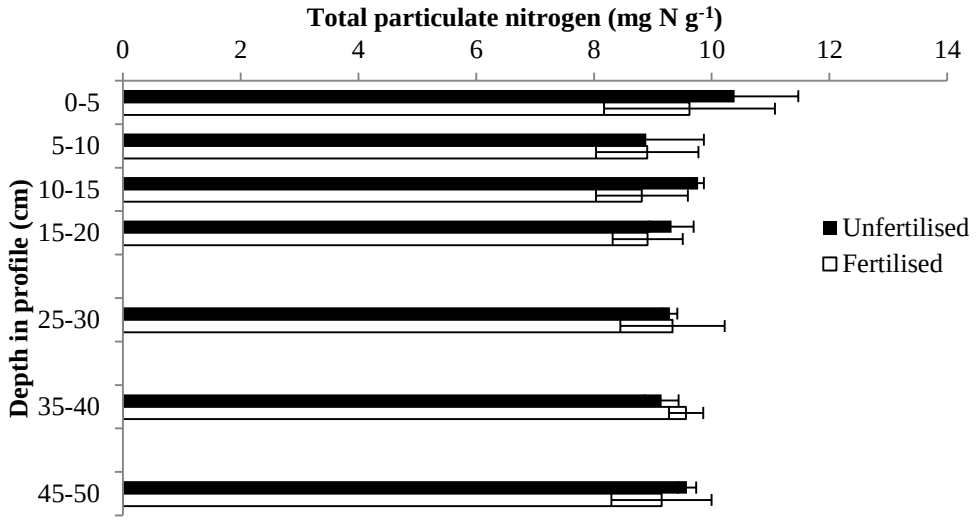

Figure 4.17: *TPN content (mg N g^{-1}) for the UF and F profiles, following 1 year of irrigation. Mean and standard deviations calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

4.4.4.3 Carbon

The TPC results are displayed in Figure 4.18. Concentrations remained uniform throughout the depth profiles for both UF and F columns (173 ± 6 to 183 ± 3 mg C g^{-1}). Statistical analyses using a one-way ANOVA demonstrated no significant difference between treatments (p = 0.304, Table 4.10a).

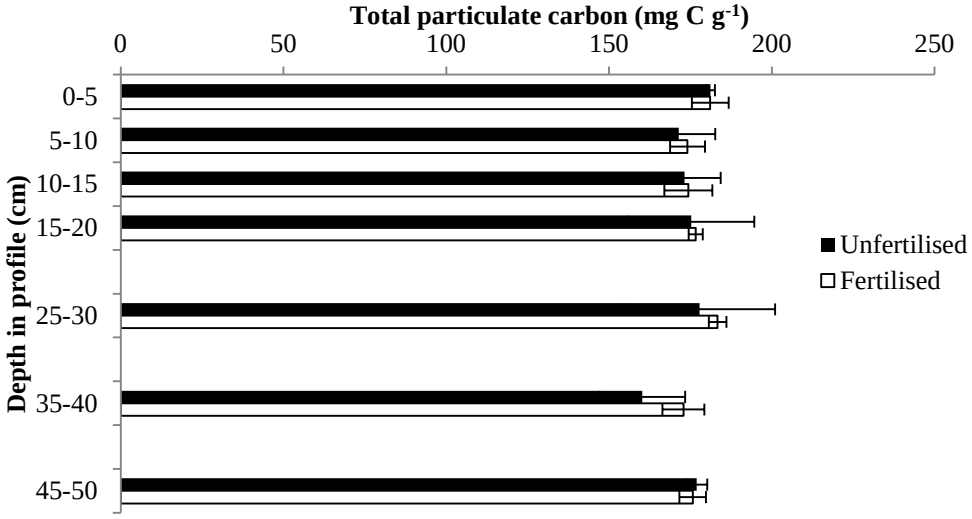

Figure 4.18: *TPC content (mg C g^{-1}) for the UF and F profiles, following 1 year of irrigation. Mean and standard deviation calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

The SOC content (Figure 4.19) within the fertilised profiles ranged from 77.5 ± 98.0 to 104 ± 3 mg C g^{-1} (UF columns) and 88.5 ± 5.8 to 133 ± 35 mg C g^{-1} (F columns). There was no correlation of SOC with depth (UF p = 0.784; F p = 0.192; Table 4.10b and c). In general, the SOC content was significantly higher in the F column profiles, (p = 0.002, Table 4.10a).

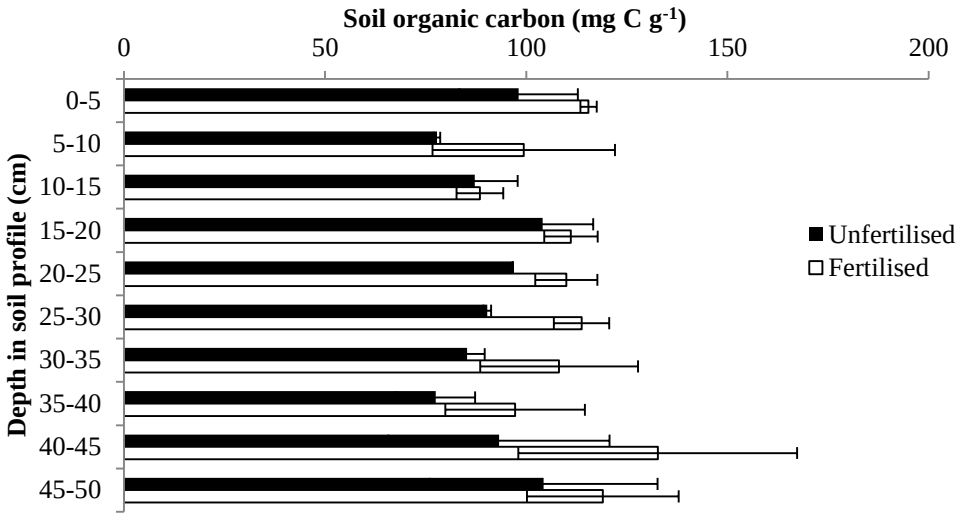

Figure 4.19: *SOC content (mg C g^{-1}) for the UF and F profiles, following 1 year of irrigation, determined through loss on ignition. Mean and standard deviations were calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6)*

4.4.4.4 Stoichiometry

4.4.4.4.1 Carbon : nitrogen ratio

The C : N ratios (Figure 4.20) were calculated using TPC and TPN concentrations. They displayed a relatively narrow range through the profiles, from 16.7 to 19.7 and 17.4 to 19.7 for the UF and F columns, respectively. There was a significant difference between the UF and F columns ($p = 0.031$, Table 4.10a), which suggests that the fertiliser application increased the C : N ratio.

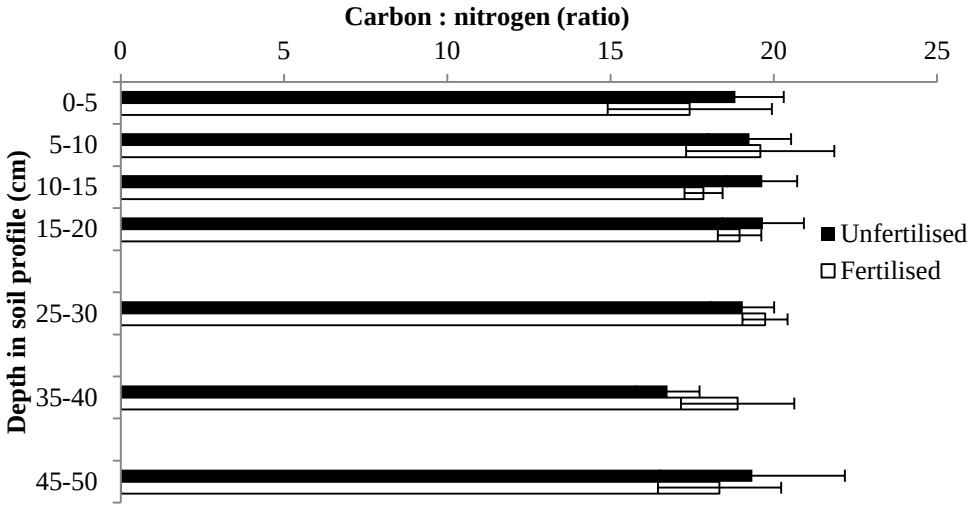

Figure 4.20: *Carbon :nitrogen ratios for the UF and F profiles, following 1 year of irrigation. The data were calculated from TPC and TPN values (n = 6).*

4.4.4.4.2 Carbon : nitrogen : phosphorus ratio

Table 4.13 shows the C : N : P ratios for both UF and F columns and, with the exception of the freshly prepared soil, shows limited variation from the 'Redfield-like' ratio (186 : 13 : 0.1) proposed by Cleveland and Liptzin (2007), who observe that this ratio may vary depending on the microbial community present within the soil, however on average it is well-constrained.

Table 4.13: C : N : P ratios for each depth within the UF and F profiles following 1 year of irrigation. The data were calculated from TPC, TPN and PO_4^{3-} values (n=6).

Depth range (cm)	Unfertilised			Fertilised		
	C	N	P	C	N	P
0-5	181	10.4	0.06	181	9.62	0.06
5-10	171	8.89	0.04	174	8.90	0.05
10-15	173	9.77	0.05	174	8.81	0.05
15-20	175	9.32	0.05	177	8.91	0.05
25-30	178	9.29	0.05	183	9.33	0.06
35-40	160	9.15	0.06	173	9.57	0.06
45-50	177	9.58	0.07	176	9.15	0.06
Freshly prepared soil	150	6.65	0.01			
Redfield ratio	**186**	**13.0**	**0.10**			

4.4.4.5 Physicochemical characteristics

The pH (Figure 4.21) within both the UF and F columns increased with depth, to a degree that was statistically significant (unfertilised p <0.001, fertilised p <0.001, Tables 4.10 and c). There was no significant difference between the UF and F profiles (p = 0.386, Table 4.10a). The minimum and maximum pH for the UF columns were 5.77 ± 0.02 (5 to 10 cm depth) and 6.03 ± 0.04 (40 to 45 cm depth). The minimum and maximum pH measured for the F columns were 5.65 ± 0.13 (0 to 5 cm depth) and 6.03 ± 0.03 (45 to 50 cm depth).

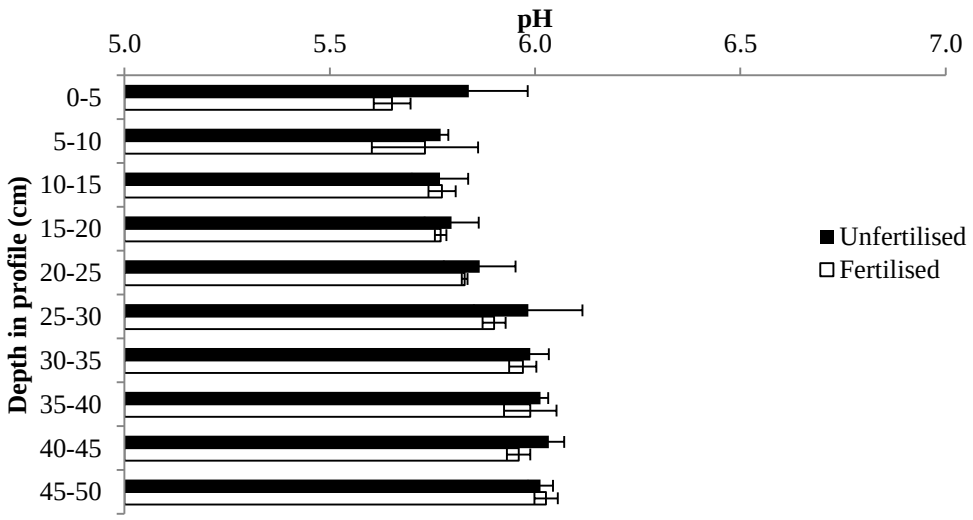

Figure 4.21: *pH for the UF and F column profiles, following 1 year of irrigation. Mean and standard deviation calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

The moisture content (Figure 4.22) of the columns ranged from 32.1 ± 0.6 to 39.7 ± 0.8 % within the UF columns and from 30.3 ± 0.0 to 36.4 ± 1.1 % in the F columns. There was no significant difference between the UF and F columns (p = 0.087, Table 4.10a). There was a significant correlation between depth and moisture content within both treatments (UF p = 0.029, F p = 0.007, Tables 4.10b and c).

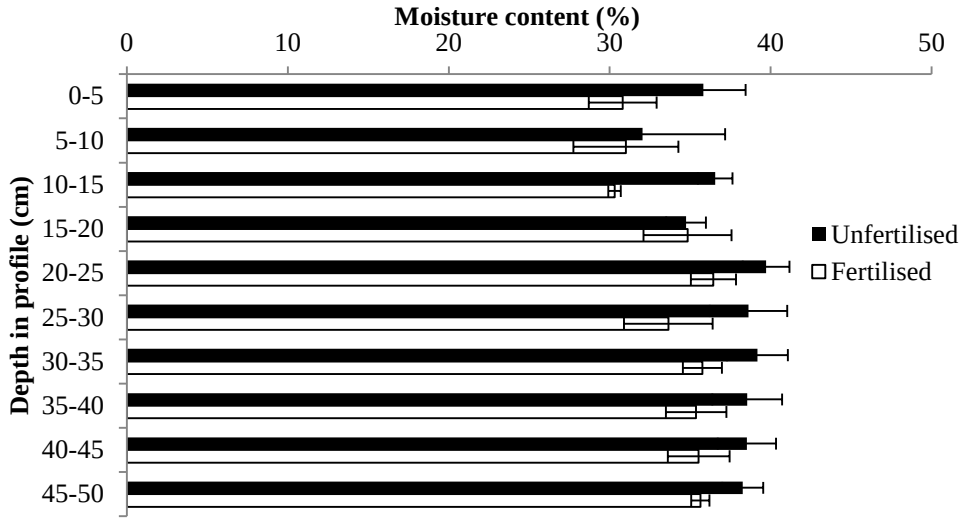

Figure 4.22: *Moisture content for the UF and F profiles, following 1 year of irrigation. Mean and standard deviations were calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

There was little measured particle size variation with depth (Figure 4.23). Sand and silt comprised the largest fractions with depth for both treatments. Changes in particle size distribution were observed (Table 4.7), with the sand fraction decreasing from 59.8 % for the freshly prepared soil to 50.5 and 51.0 % within the UF and F columns, respectively. The clay fraction comprised the smallest proportion of the texture composition (< 2.60 %) in all samples. According to the ISO 14688-1 texture classifications, all samples were classified as either sandy loam or silt loam (ISO14688-1:2002).

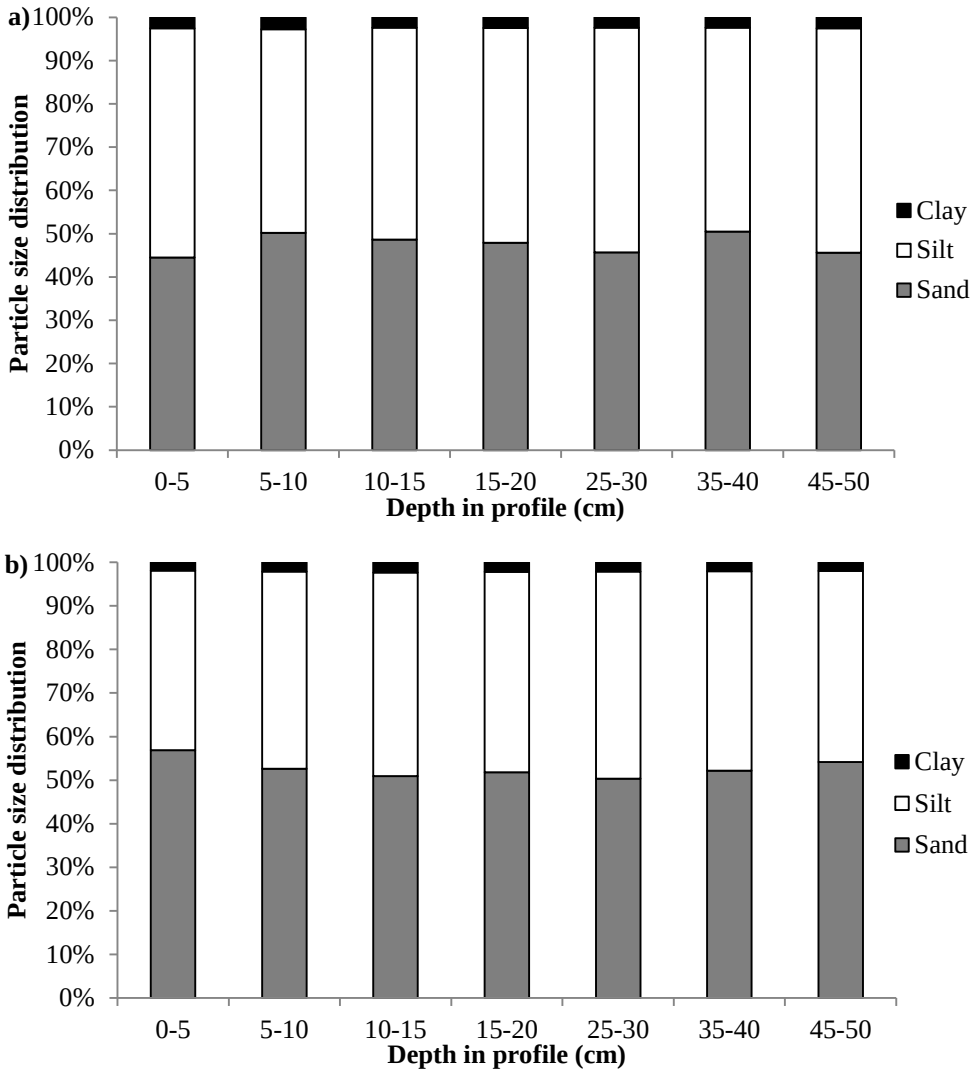

Figure 4.23: *Particle size distribution for the* ***a)*** *UF and* ***b)*** *F profiles. Mean calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

Figure 4.24 shows the CEC values for two depths within the column profiles. The F column samples had the highest CEC values (6.47 ± 1.16 $cmol_c\ kg^{-1}$ at 5 – 10 cm and 6.36 ± 3.50 16 $cmol_c\ kg^{-1}$ at 35 to 40 cm). The UF column sample CEC values, at 5.68 ± 0.28 $cmol_c\ kg^{-1}$, demonstrated no significant difference from those of the freshly prepared soil ($p > 0.05$, Table 4.9). There was no significant difference between the UF and F column CECs ($p = 0.093$, Table 4.10a).

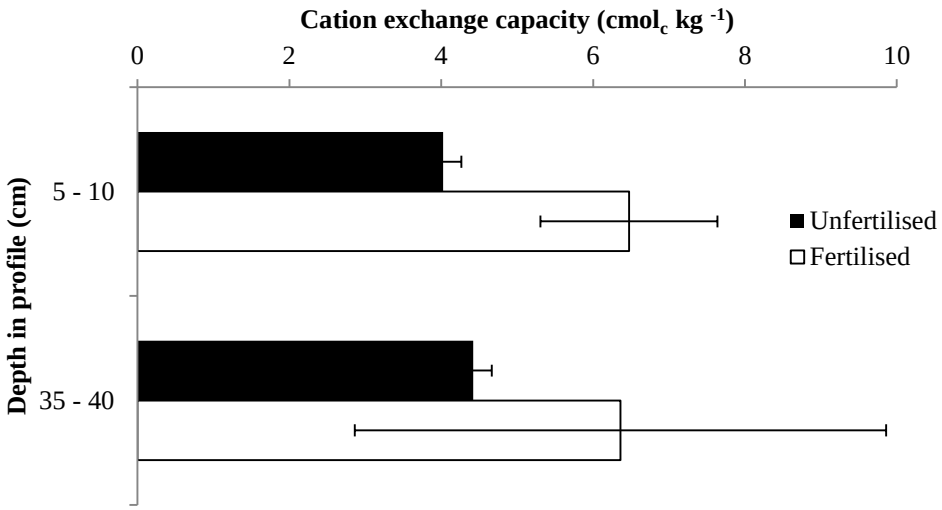

Figure 4.24: *CEC (cmol$_c$ kg^{-1}) for a typical root zone (5 to 10 cm) and at 35 to 40 cm depth. The mean and standard deviation were calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

4.4.4.6 Enzymatic activity

The mean enzymatic activity was highest at all depths within the UF columns (Figure 4.25). The highest activity within the UF column was 293 ± 88 mg Fl g^{-1} hr^{-1} at 10 to 15 cm decreasing to 158 ± 11 mg Fl g^{-1} hr^{-1} at 40 to 45 cm depth. In contrast, the highest concentration for the F column was 160 ± 15.4 mg Fl g^{-1} hr^{-1} at 45 to 50 cm with a minimum occurring at 15 to 20 cm depth (85.1 ± 6.94 mg Fl g^{-1} hr^{-1}).

The enzymatic activity was correlated with depth in both profiles, though positively within the UF column ($p = 0.018$, Table 4.10b) and negatively within the F column ($p = 0.001$, Table 4.10c). The one-way ANOVA revealed no significant difference between the two profiles ($p < 0.001$, Table 4.10a).

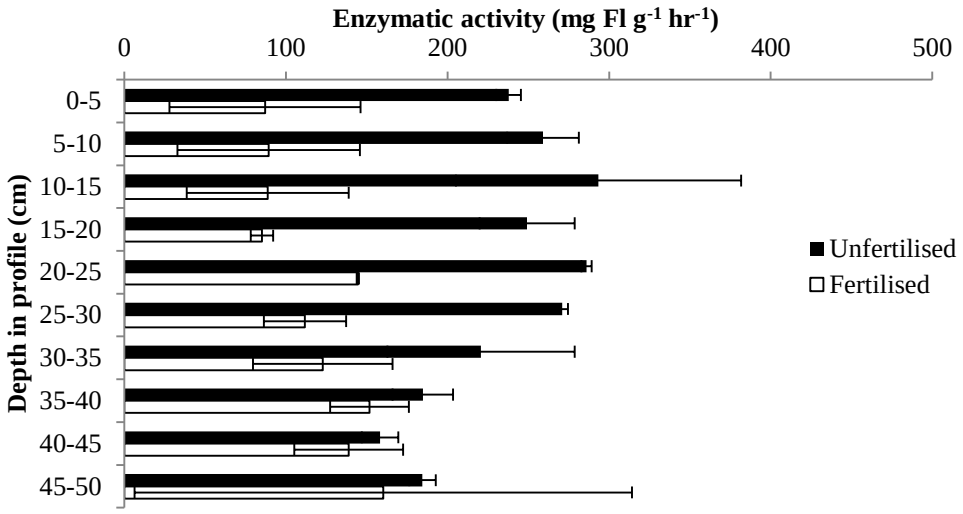

Figure 4.25: *Enzymatic activity (mg Fl g^{-1} hr^{-1}) for the UF and F profiles, following 1 year of irrigation. Mean and standard deviations were calculated from 2 sets of 2 columns (UF and F). Analyses were run in triplicate for each sample (n = 6).*

4.4.5 Extracted constituent analyses

4.4.5.1 Nitrogen

Results from the N fraction analyses are shown in Figure 4.26. Non-extractable N represented > 99.3 % of TPN in the UF columns and > 98.9 % in the F columns, demonstrating the dominance of an unavailable N fraction throughout the profile. The TEN concentrations (Figure 4.24a) decreased with depth for both the UF and F columns, with the highest concentrations occurring at 0 to 5 cm.

The TEN concentrations (Figure 4.26a) were highest in the F columns. The highest concentration for both treatments occurred at 0 to 5 cm depth (68.9 ± 14.8 and 105 ± 49 μg N g^{-1} in UF and F columns, respectively), with a significant difference between mean values (p = 0.036, Table 4.10a).

There was a decrease in TEN concentration with depth for both the UF and F profiles, though this was not significant ($p = 0.447$ for UF, $p = 0.225$ for F, Tables 4.10b and c). The largest decrease occurred in the F column (42.0 μg N g^{-1}).

The extracted NO_3^- concentrations (Figure 4.26b) were highest at 0 to 5 cm depth for both the UF and F soil profiles, though there was no significant difference ($p = 0.483$, Table 4.10a) in concentration (UF = 47.2 ± 6.3 μg N g^{-1} and F = 45.7 ± 8.1 μg N g^{-1}). In the F columns concentrations decreased below 0 to 20 cm; in the UF columns the concentration dropped below 5 cm depth and remained fairly constant from 5 to 20 cm (ranging from 9.6 to 10.9 μg N g^{-1}), before increasing towards the bottom of the profile.

NH_4^+ represented the smallest N fraction at all depths within both the UF and F columns. The F columns displayed the largest variability in NH_4^+ concentration (Figure 4.26c), with the concentration increasing with depth; a minimum occurred at 10 to 15 cm (2.51 ± 0.50 μg N g^{-1}) and a maximum at 45 to 50 cm depth (8.69 ± 2.25 μg N g^{-1}), though this was not a statistically significant correlation with increasing depth in the soil profile ($p = 0.221$, Table 4.10c). The NH_4^+ concentration in the UF columns varied less (3.68 ± 0.87 to7.36 ± 0.86 μg N g^{-1}).

EON concentrations varied with depth (Figure 4.26d). There was no significant difference between the UF and F column concentrations ($p = 0.586$, Table 4.10a).

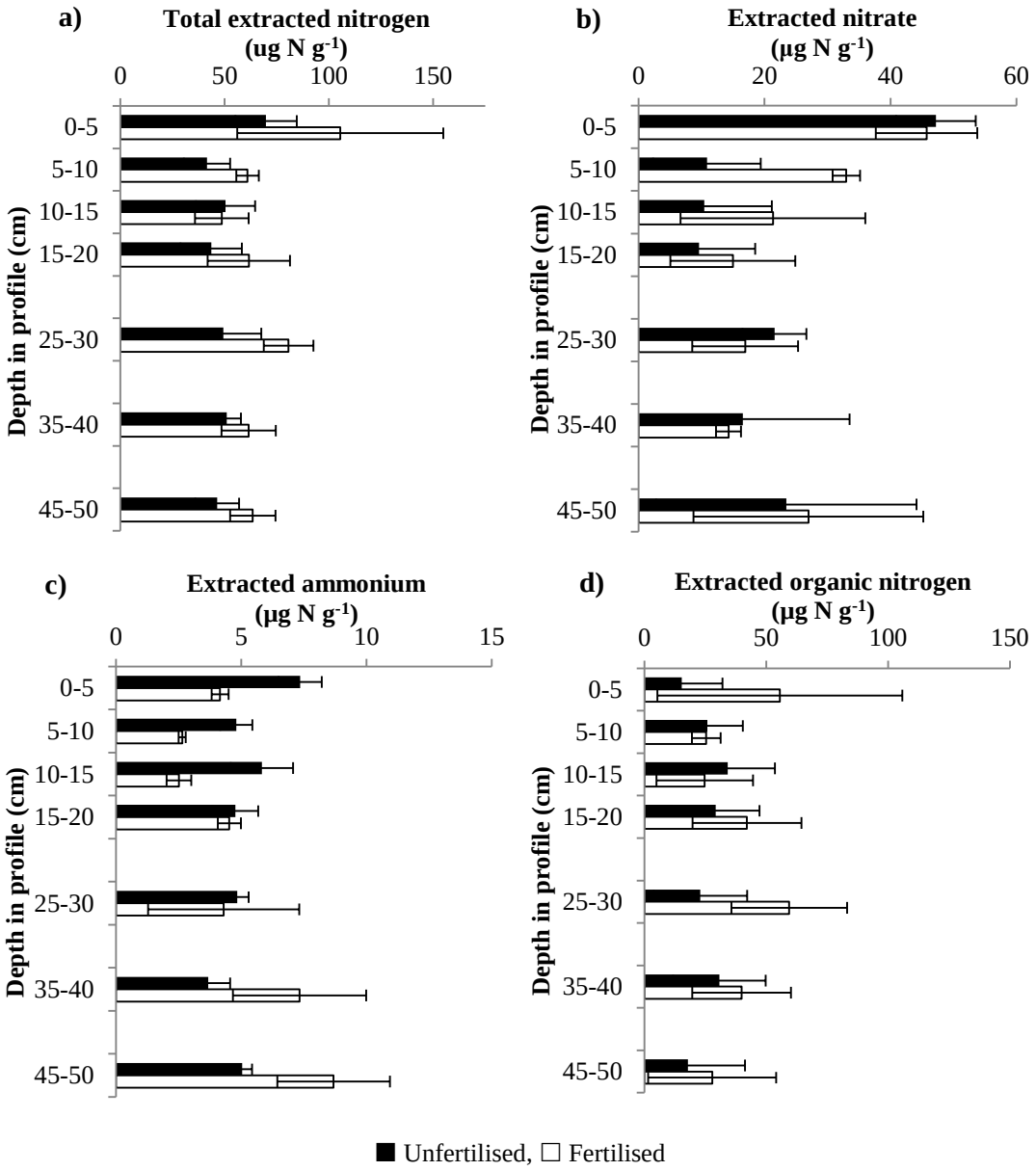

Figure 4.25: *Extracted N fraction concentrations (µg N g^{-1}) for* ***a)*** *TEN,* ***b)*** *NO_3^-,* ***c)*** *NH_4^+ and* ***c)*** *EON for the UF and F profiles, following 1 year of irrigation. Mean and standard deviation calculated from 2 sets of 2 columns (UF and F). Analyses run in triplicate for each sample (n = 6).*

4.4.5.2 Phosphate

The PO_4^{3-} concentrations are shown in Figure 4.27. The UF columns concentrations varied with depth, with a high concentration from 0 to 5 cm depth, which decreased at 5 to 10 cm, and then increased with depth. The F columns displayed a similar pattern, with less variation. The highest average concentration occurred at 45 to 50 cm depth for

both treatments (UF: 74.9 ± 4.7 µg P g^{-1}; F: 6.16 ± 1.11 µg P g^{-1}). Whilst the highest concentration occurred within the UF profiles, the overall average for all depths within the profile was greater in the F column (56.8 ± 4.6 µg P g^{-1} compared with 53.5 ± 11.9 µg P g^{-1}). There was no significant difference in PO_4^{3-} concentration between the two treatments (p = 0.502, Table 4.10a).

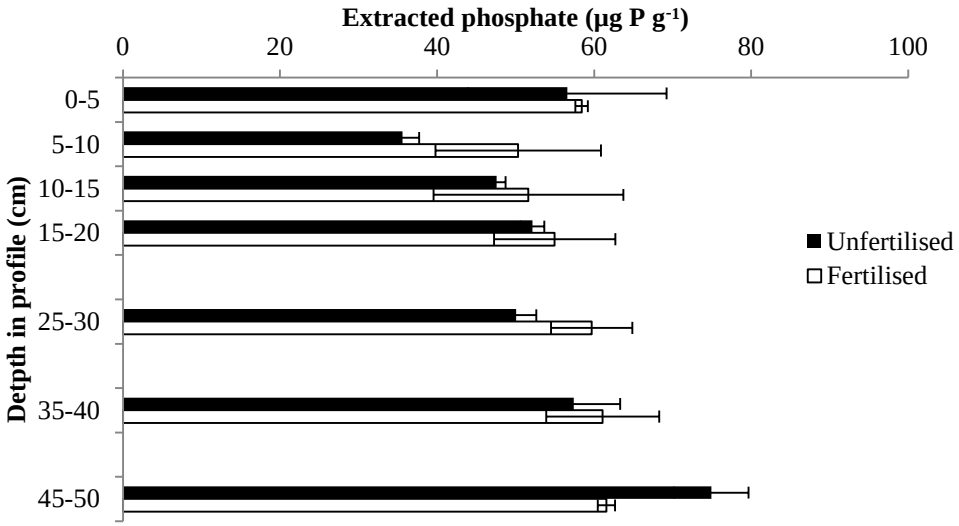

Figure 4.27: *Extracted PO_4^{3-} concentrations (µg P g^{-1}) for the UF and F profiles, following 1 year of irrigation. Means and standard deviations were calculated from 2 sets of 2 columns (UF and F). Analyses were made in triplicate for each sample (n = 6).*

4.4.5.3 Carbon

The EOC concentrations increased with depth in the UF columns, from 136 ± 48 to 306 ± 3 µg C g^{-1} (Figure 4.28). The F columns were more variable, with the highest concentration (259 ± 59 µg C g^{-1}) occurring at 25 to 30 cm depth and the lowest (134 ± 25 µg C g^{-1}) at 10 to 15 cm. There was no significant difference between the two treatments (p = 0.681, Table 4.10a).

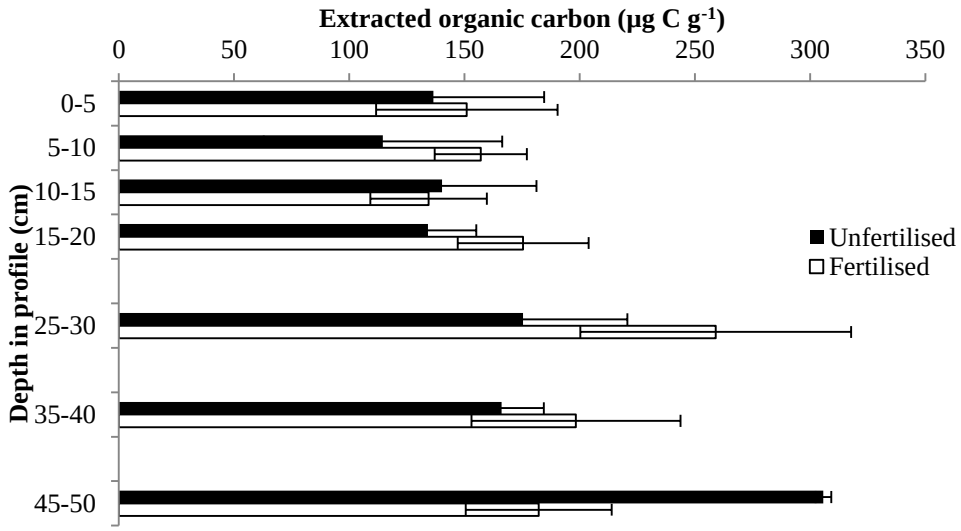

Figure 4.28: *EOC concentrations (µg C g^{-1}) for the UF and F profiles, following 1 year of irrigation. Means and standard deviations were calculated from 2 sets of 2 columns (UF and F), with analyses run in triplicate (n = 6).*

4.4.5.4 Metals

Figure 4.29 shows the concentrations (µg g^{-1}) of Mg, Ca, K and Fe with depth in the columns. The metal concentrations followed a similar profile in the UF columns, with the lowest concentrations occurring at mid-depth profile followed by an increase to the highest concentrations. The F columns contained higher concentrations of Mg, Ca and Fe at depth, with the highest concentrations occurring 30 to 40 cm depth.

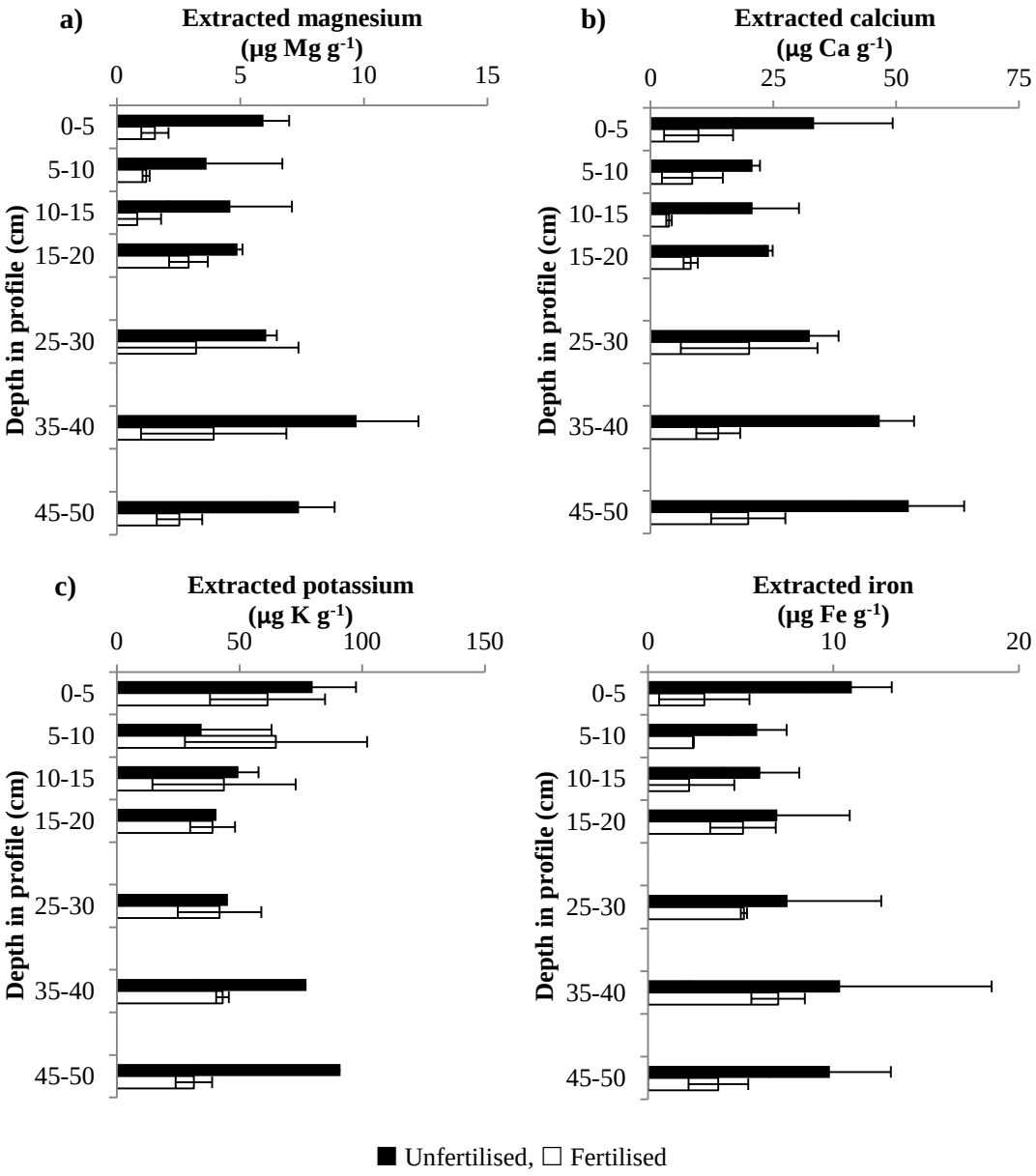

Figure 4.29: *Concentrations of extracted metals (µg g^{-1})* ***a)*** *Mg,* ***b)*** *Ca,* ***c)*** *K and* ***d)*** *Fe for the UF and F profiles, following 1 year of irrigation. Means and standard deviations were calculated from 2 sets of 2 columns (UF and F), with analyses run in triplicate (n = 6).*

4.5 Discussion

This discussion aims to interpret the data from the leachate and soil profile analysis, focusing on the observed changes to the soil and leachate characteristics which occurred during the 52 week experimental term.

4.5.1 Nitrogen

Nitrate concentrations within the leachate (Figure 4.7) decreased by 95 % during the first 2 weeks of irrigation (from 6.73 to 0.36 mg N L^{-1}), with the NO_3^- concentrations in the leachate remaining low (< 0.83 mg N L^{-1}) from Weeks 2 to 27. DON was therefore the principal form of N within the leachate, declining much slower from Weeks 5 to 32 (from 11.1 to 1.6 mg N L^{-1}). Only low molecular weight DON (e.g. peptides, amino acids, urea, polyamines, small polypeptides) is available for plant uptake (DiTomaso et al., 1992; Jones et al., 2005b). It has, however, been suggested that most DON within soil solution is of a high molecular weight (Jones et al., 2005b), and thus, most is unavailable for plant uptake.

After the initial flush of leachable inorganic from the columns brought about by the commencement of the irrigation, the inorganic N concentration within the leachate remained low. This may be attributed to immobilisation of inorganic N, through its utilisation by the soil microorganisms in the breakdown of soil organic matter, of which there were large quantities within incorporated into the soil mix. Blagodatsky et al. (1998) reported rapid N immobilisation, following an application of N-containing fertiliser to a silt loam soil. The C : N ratio of organic materials within the soil is of particular significance with regard to N immobilisation, affecting the ease with which the organic material may be decomposed by soil microorganisms (Nicolardot et al.,

2001). The high C : N ratio of the organic bark component within the soil mix will thus have had a significant impact upon the immobilisation of N within the soils, discussed in further detail below.

From Week 28 a significant increase in the TDN concentration was observed for both the UF and F soil columns (Figure 4.6). This increase in TDN was driven by an increase in NO_3^- and DON concentrations (Figures 4.7 and 4.8) (NH_4^+ concentrations were consistently below the LOD). Whilst the increase in leached N concentration in F columns coincided with the application of fertiliser, it was also observed within the UF columns. It must therefore be assumed that the concentration increase was caused by the mineralisation and subsequent release of previously immobilised N, through the mineralisation process, triggered by a shift in the soil microbial population. The decomposition of organic material within the soil is a major driver of such processes, with the characteristics of an organic material having a substantial effect on its decomposition (Thomas et al., 1998).

During the decomposition of organic material, microorganisms utilise the C component to produce CO_2. In order to do this effectively the microbial population multiplies rapidly, requiring readily-available N for incorporation into their cells (Brady and Weil, 2008). Where sufficient N is not available within the decomposing organic material, the microorganisms exploit the inorganic N within the soil solution (Cheshire et al., 1999). Through this process the inorganic N is converted to organic forms, which are unavailable for direct plant uptake (Cheshire et al., 1999) resulting in low NO_3^- concentrations within the leachate, as seen between Weeks 2 and 28 (< 0.83 mg N mL^{-1},

Table 4.7). A similar immobilisation trend was observed by Blagodatsky et al. (1998) over a 2 week experimental period.

There are a number of factors which influence the rate and impact of the decomposition of organic matter within the soil, including: C : N ratio, particle size, lignin content of the organic matter and environmental conditions (soil pH, moisture, aeration and temperature). Organic material represented 65 % of the composition of the artificial soil mix, of which half was comprised of composted green waste and half bark. The composted green waste, which had a lower C : N ratio (13.0) than the overall soil mix (22.6), would have been rapidly decomposed by soil microorganisms (El-Sharkawi, 2012). However, the bark had a high C : N ratio (33.5), and may, therefore, have promoted N immobilisation within the soil. This also suggests that N was the limiting factor in the decomposition of the bark component of the soil.

Organic matter decomposition is reported to cause N immobilisation within soils, Barney and Colt (1991) and Boyer et al. (2012) reported significant N immobilisation within bark amended soils. The bark component of the soil mix had a relatively large particle size, which meant that a smaller surface area was exposed to physicochemical and microbial attack (Thomas et al., 1998). The composted green waste however, had a considerably smaller particle size, which exposed a larger surface area for microbial decomposition. Whilst the composition of both the bark and the composted green waste has not been explored in great depth by this investigation, it may be hypothesised that bark had a high lignin content, which made it more resistant to microbial decomposition, whilst the composted green waste may have contained a lower lignin content allowing for it to be readily decomposed (Thomas et al., 1998). In view of this

it may be suggested that the more easily-decomposed composted green waste component would have been decomposed in preference to the bark component.

The temperature and irrigation regime, under-which the columns were maintained, remained relatively constant throughout the experiment. Whilst they may have been a limiting factor to the rate of organic material decomposition, the effect remained fixed throughout. The leachate pH values were observed to decline (by 0.66 (UF) and 0.88 (F) within the leachate) throughout the irrigation term, though, no significant pH difference was observed between the freshly prepared and UF and F soils ($p > 0.05$, Tables 4.9 and 4.10a). This suggests that the pH decrease may have been brought about by the release of soluble organic acids through the breakdown of the organic matter (Ritchie and Dolling, 1985), however leachate DOC concentrations declined throughout irrigation (from 213 mg C mL^{-1} at Week 2 to 14.9 mg C mL^{-1} (UF) and 10.6 mg C mL^{-1} (F) by Week 52) and without further DOC fractionation it is difficult to determine the extent to which this may have contributed to the decline in pH.

Studies have demonstrated that fungal activity plays a much greater role in N immobilisation than bacteria (Cheshire et al., 1999; Thomas et al., 1998), with specific species favouring the breakdown of different organic materials (Thomas et al., 1998). It may therefore be hypothesised that different groups of microorganisms were responsible for the decomposition of different organic matter components within the soil. Group 1 was partially responsible for the initial NO_3^- release (Weeks 1 to 2), where the composted green waste was rapidly decomposed, and Group 2 was responsible for the decomposition of the more recalcitrant bark, causing the NO_3^- release seen from Week 28. This is supported by the findings of Henriksen and Breland (1999) who

modelled N immobilisation in soils through a microcosm experiment and determined that the application of organic residues (e.g. straw) to soils served to retard N mineralisation and resulted in greater N immobilisation.

Further, the following may be speculated. Subsequent to the decomposition of the composted green waste, the microbial population size of Group 1 decreased, releasing inorganic N into the soil through cell lysis and subsequent mineralisation. The irrigation then served to flush this from the soil to be collected as leachate. Following this a population transformation occurred, where microorganisms more adept in the decomposition of more recalcitrant materials (Group 2) became prominent. In order to decompose this recalcitrant material - with a high C : N ratio, inorganic N from the soil solution was utilised by the Group 2 microorganisms, lowering the plant availability and leachable concentrations. The population size of the Group 2 microorganisms was eventually constrained by environmental factors, such as nutrient availability, leading to a stationary phase, with the population remaining constant. The exponential growth and stationary phases may be reflected from Weeks 2 to 28 within the NO_3^- data, where concentrations remained low and stable as the Group 2 microorganisms decomposed the more recalcitrant organic matter.

Once the decomposition had progressed to a point where the C : N ratio of the OM had been lowered through the release of CO_2 into the atmosphere, causing C to become limiting, the population underwent logarithmic decline, where NO_3^- was released as a result of the lysis of the microbial cells, demonstrated by increased dissolved NO_3^- concentration from Week 28 onwards. Figure 4.30 demonstrates the theoretical relationship between concentrations of NO_3^- and changes in the microbial population.

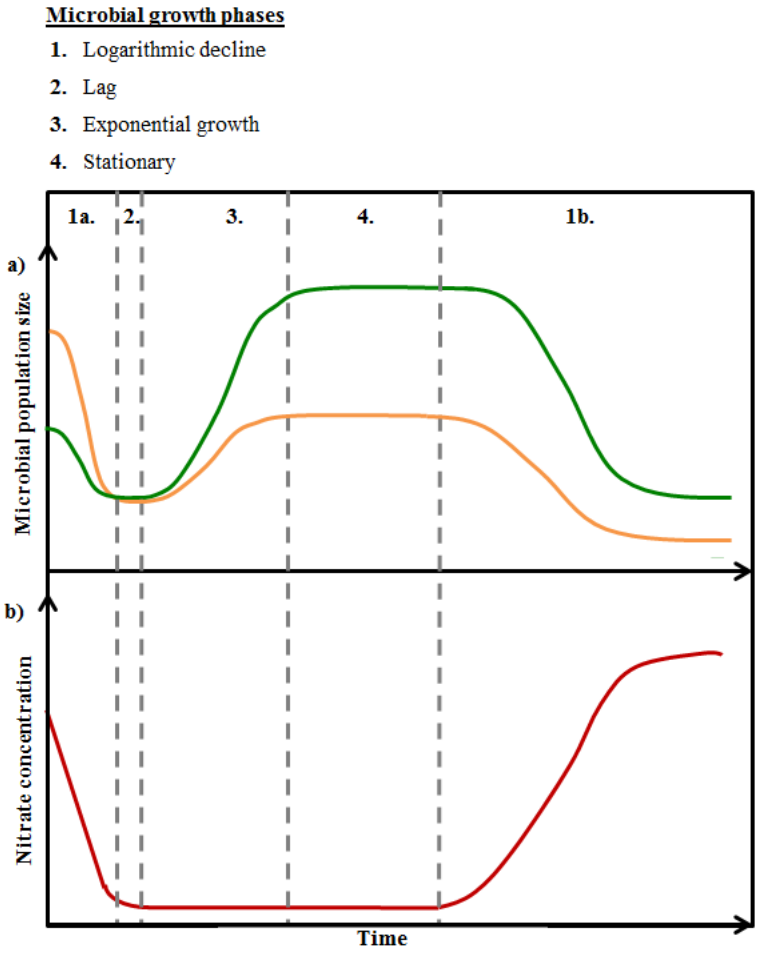

Figure 4.30: *A proposed model for the relationship between NO_3^- concentrations within the leachate and responses of the microbial population during decomposition of the organic matter component of the soil mix.* ***a)*** *The theoretical microbial population dynamic curves* ***b)*** *concentrations of NO_3^- measured within the column leachate. The microbial growth curve adapted from Killham (1994). The orange line represents population of microorganisms which favour the easily-decomposed composted green waste, whilst the green line represents the population facilitating the breakdown of more recalcitrant organic material. The proposed stages of the microbial population dynamics in relation to NO_3^- concentration are:*
1a. *The composted green waste within the soil mix was decomposed rapidly by the microbial population; once the majority of this material had been decomposed there was a decline in population size, leading to the release of inorganic N into the soil through cell lysis; this release is shown in the graph and occurred during the initial flushing effect of irrigation on dissolved NO_3^- concentrations.****2.*** *A short lag phase - where the microbial population adjusted to the alternative organic matter (bark), through changes in the dominant microorganism from those which can utilises easily-decomposed organic matter (orange line), to species which can better utilises more recalcitrant organic matter (green line). During this phase the population size remained stable.* ***3.*** *As the microbial population began to breakdown the bark component of the soil, its numbers increased exponentially.****4.*** *A point was reached where the population size was limited by environmental factors other than food supply, the stationary phase. The exponential growth and stationary phases cause the NO_3^- concentrations to remain low and stable throughout, as a result of N immobilisation within the cells of the increasing microbial population.* ***1b.*** *Once the C : N ratio of the bark component within the soil had been sufficiently lowered, the microbial population declines, with NO_3^- being released as a result of cell lysis demonstrated here by the logarithmic decline phase.*

The timescale over which the Group 1 microorganisms decomposed the more easily-accessed organic matter cannot be determined from the data. However, given that the columns were allowed to stabilise for 2 weeks prior to the start of irrigation, it may be presumed that the majority of green waste decomposition took place during this period.

The microbial immobilisation and subsequent mineralisation, as described above, is supported by the decrease in TPC relative to TPN concentrations within the soil, which was brought about through either the increase in N present within the soil or, more likely, through the breakdown and release of soil carbon as CO_2 by the microbial population (Brady and Weil, 2008). Burgos et al. (2006) reported similar immobilisation and re-mineralisation of N over a 40 week period following the application of a range of materials with high C : N ratios to sandy textured soils.

Nitrate accounted for 21.0 to 67.6 % of the TEN concentration as NH_4^+ in the leachate was below the LOD (<1.12 µg N L^{-1}). This suggests that, either the conditions within the soil favoured rapid conversion of NH_4^+ to NO_3^- via nitrification, or that NH_4^+ was strongly bound to the surface of soil particles and was not easily leached from the soil (Li et al., 2012); however the higher proportion of large size particles suggests a small surface area and an associated low number of binding sites must be acknowledged as probable.

A mass balance was calculated to estimate the overall losses of N and to identify the fractions involved (values shown in Table 4.14). A greater overall N loss occurred within the F than the UF columns (1.01 mg N g $soil^{-1}$ in the F and 0.72 mg N g $soil^{-1}$ within the UF columns). A significant fraction of N was unaccounted for (0.72 mg N g $soil^{-1}$ in F and 0.42 µg N g $soil^{-1}$ in UF columns) suggesting an alternative mode of N

loss from the soil columns. Within a natural system N may be lost from soils through crop removal, denitrification, erosion, runoff, leaching and volatilisation (Gentry et al., 2009). Within the column system denitrification, volatilisation and leaching represent the means through which N loss may occur.

Ammonia (NH_3) may be volatilised when ammonium (NH_4^+) is dissolved in water at near neutral to alkaline pH (Rochette et al., 2013). The pH conditions within the column were consistent with those required for NH_3 volatilisation, solid phase pH ranged from 5.65 to 6.03 and within the leachate 5.69 to 7.19. Denitrification is the production of nitrous oxides through the reduction of nitrate, commonly occurring in environments with limited O_2 (Ussiri and Lal, 2013). Stehfest and Bowman (2006) reported that N_2O emissions increase with increasing SOC concentration due to increased availability of substrate for the microbial community in soils. The SOC content of the columns decreased over the irrigation period suggesting that the availability of SOC was greater during the earlier phase of the experiment, so any denitrification occurring as a result of SOC availability may have occurred during this period.

Table 4.14: *Nitrogen mass balance estimated from the solid and leachate values from this experiment making the assumption that the column masses remained unchanged throughout the experimental period.*

	Unfertilised		**Fertilised**	
	Freshly prepared soil	**Following 52 weeks irrigation**	**Freshly prepared soil**	**Following 52 weeks irrigation**
TPN in column (g)	97.2	90.3	84.3	75.9
TPN in column (mg N g^{-1})	10.2	9.48	10.2	9.19
Column N loss (mg N g^{-1})		0.72		1.01
N loss by leaching(mg N g^{-1})		0.30		0.29
% N loss form column		7.06		9.90
% loss represented by leaching		41.4		28.3
Unaccounted loss (mg N g^{-1})		0.42		0.72

4.5.2 Phosphorus

When compared with other major nutrients, under most soil conditions, P is the least mobile and least plant available (Hinsinger, 2001). In the environment P is most commonly found in the form of dissolved inorganic phosphate (DIP) (Barancíková et al., 2007) and its low mobility is attributed the highly reactive nature of DIP ions relative to other soil nutrients, resulting in the long-term retention of P in unavailable forms (Hinsinger, 2001). For this investigation DIP was measured as PO_4^{3-}.

Following a lag-period of 1 week with little change in concentration, the PO_4^{3-} concentrations in the leachate increased for 5 weeks until Week 6, when concentrations become relatively stable. This was consistent with results reported Li et al. (1997) and Broschat (1995). They measured nutrient concentrations within the leachate of compost amended-soils and also observed a period of minimal concentration increase for dissolved PO_4^{3-}, lasting from between 6 days and 4 weeks, before an increase in concentration. Broschat (1995) suggested that fixation within the soil may have caused the initially low dissolved PO_4^{3-} concentrations, which then increased following saturation of the soil's adsorption capacity.

Phosphorus losses from soils are dependent on source factors and transport mechanisms (Börling, 2003; Gburek et al., 2000) and the PO_4^{3-} concentrations within the leachate may be explained through PO_4^{3-} buffering, where PO_4^{3-} is sorbed to - or desorbed from - soil particles. Within the environment this refers to a two stage process: (1) rapid surface adsorption onto - or desorption from - reactive particle sites, followed by (2) a slow penetration by solid-state diffusion of this PO_4^{3-} into - or out of - sub-surface horizons within the interior of particles (Froelich, 1988). The equilibrium between solid

and solution is governed by the environmental conditions Eh, pH, CEC and organic matter (Froelich, 1988; Reddy et al., 2005).

Apatite is a primary source of mineral P in soil. The weathering process is typically too slow to provide sufficient P to meet crop demand (Shen et al., 2011). However, direct application of PO_4^{3-} rocks has been demonstrated to be relatively effective for crop growth in acidic soils (Shen et al., 2011). Whilst the conditions within the soil column were slightly acidic (pH 5.9) this is unlikely to explain the extent of P concentration within the leachate. Soil fungi are known to increase plant available P concentrations through the promotion of the dissolution of various PO_4^{3-} minerals, including apatite (Rosling et al., 2007). The role of fungi in the breakdown of soil organic matter was supported by the leachate N concentrations.

It should be noted that comparison of apatite values from the freshly prepared soils and those from the UF and F columns suggests a decrease in the relative composition represented by apatite over the irrigation period (-0.06 and -0.04 % for UF and F columns respectively, Table 4.11). Minerals within the apatite group vary in their solubility (ranging from log K ~ -122 to log K ~ -107.5) (Guidry and Mackenzie, 2003), depending on the particle size and the soil pH (Oelkers et al., 2008).

Whilst it is possible that the decrease in apatite may have been caused by transport through the soil profile, the relative stability of most other mineral components occurring within the soil during this study suggest that the decline may have been caused by the breakdown of this mineral, though this cannot be verified. Mass balance calculations (Table 4.13) suggest that cumulative PO_4^{3-} losses within the leachate for the 52 weeks experiment account for an additional 0.14 and 0.21 mg g^{-1} of the water

extracted PO_4^{3-} within the UF and F columns respectively, when compared to the freshly prepared soil. This suggests that PO_4^{3-} was converted from a previously unavailable form and released into the soil solution. The mass balance calculations (Table 4.15) estimate that approximately 0.17 and 0.13 mg g^{-1} of PO_4^{3-} were lost through the breakdown of in apatite from the UF and F columns respectively, which may account for some of the leached PO_4^{3-},with organic matter decomposition providing another source.

Table 4.15: *Phosphate and apatite mass balance estimated from solid and leachate values from this experiment making the assumption that the column messes remained consistent throughout the experimental period.*

	Unfertilised		**Fertilised**	
	Freshly prepared soil	**Following 52 weeks irrigation**	**Freshly prepared soil**	**Following 52 weeks irrigation**
Extracted PO_4^{3-} (g)	0.95	0.48	0.83	0.50
Extracted PO_4^{3-} (mg P g^{-1})	0.10	0.05	0.10	0.06
Column loss of PO_4^{3-} (mg P g^{-1})		0.05		0.04
PO_4^{3-} loss by leaching (mg P g^{-1})		0.19		0.25
% PO_4^{3-} loss from column		50.0		40.0
% PO_4^{3-} loss represented by leaching		191		246
Apatite				
Representative composition (%)	0.13	0.04	0.13	0.06
Total apatite in columns (g)	12.4	3.81	10.7	4.96
Apatite loss (g)		8.58		5.78
Apatite loss (%)		69.2		53.8
Apatite lost (mol)		0.02		0.01
Total PO_4^{3-} in apatite loss (g)		1.60		1.08
PO_4^{3-} in apatite loss (mg P g^{-1})		0.17		0.13

It has been observed that compost may serve as a major source of bioavailable P in soil (Barker, 1997) and may be reasoned that the trends observed for the PO_4^{3-} concentrations within the leachate were caused by P mineralisation, through the decomposition of the soil organic matter component. Initially the PO_4^{3-} released through the decomposition of organic material within the soil mix may have been adsorbed by

minerals, becoming insoluble. With the continued decomposition of the organic matter component and associated PO_4^{3-} release, the available adsorption sites within the soil became saturated, resulting in the loss of PO_4^{3-} from the soil through leaching. The stabilisation of the leachate PO_4^{3-} concentration was likely as a result of an equilibration between the soil and irrigation water. This trend is supported by studies of Garcia-Albacete et al. (2014) and Spohn and Kuzyakov (2013), who identified the significance of organic matter decomposition by the soil microbial population to the mineralisation of P within soils.

Adsorption is of PO_4^{3-} is closely related to soil texture (Sposito, 1989), with smaller particles containing the highest number of charged binding sites. The addition of a small quantity of clay or Al or Fe oxides can greatly reduce PO_4^{3-} transport within soils (Favaretto et al., 2012). Studies by Leinweber et al. (1999) and Liu et al. (2012) reported relatively high levels of PO_4^{3-} leaching in soils with a relatively small clay fraction. The small clay fraction results in fewer binding sites for solubilised PO_4^{3-} ions to sorb to, leading to greater PO_4^{3-} loss through leaching, consistent with this experimental data.

4.5.3 Carbon

The DOC concentrations within the leachate samples decreased at a steady rate throughout the irrigation study, with the concentration remaining stable for the final 6 weeks of irrigation. This final trend suggests that a equilibrium state between the irrigation water and the soil was reached. There was a significant ($p < 0.05$, Table 4.7) decrease in EOC for both the UF and F soils by the end of the 52 weeks of irrigation (82.7 and 81.5 %, respectively). However, there was no significant difference in EOC

concentration throughout the depth profile ($p > 0.05$, Table 4.8a) suggesting that loss of particulate C was relatively even throughout the profile. Over the 52 week irrigation period a larger concentration of EOC was leached from the UF soil (62.0 μg g^{-1}) than the F soil (44.9 μg g^{-1}); however a higher percentage of EOC losses from the F soil were unaccounted for by leached concentrations (76.9 % in F soil, 61.2 % in UF soil), suggesting alternative modes of C loss from the soil.

As discussed above, the C content of the soils has a significant effect on the nutrient cycling processes within the soils. The TPC concentrations demonstrated no significant differences between UF and F profiles ($p > 0.05$, Table 4.8a). All depths, in both UF and F columns, demonstrated a differing levels of decrease in TPC concentration ranging from 0.89 to 13.4 %, though this was not found to be significantly different ($p > 0.05$, Table 4.7). The decline in TPC between the freshly prepared soil and both the UF and F soils following 52 weeks of irrigation is reflected in the decreased C : N ratio (T_0 = 22.6 UF = 16.7 to 19.7 and F = 17.4 to 19.7). The reduction in TPC content within the soil over the irrigation period may be attributed to the decomposition of organic matter within the soil and its subsequent release through respiration and leaching.

4.5.4 Physicochemical and biological properties

Typically, a higher proportion of organic matter is present within the upper layers of a soil, resulting in greater moisture content within this organic horizon. However, this was not observed within the columns, where moisture content was highest at depth. This may be explained in terms of this experiment, where the soil mix was homogenised and packed into the columns, resulting in a relatively uniform soil composition throughout the profiles and further to this, there were no applications of organic material, which

increases water retention, to the surface of the columns. In view of this it may be inferred that the variation in moisture content was more likely to have been caused by gravitational movement rather than variations in water holding capacity throughout the profile.

The pH of the soil influences nutrient availability, affecting the oxidation state and solubility of ions present within the soil (as demonstrated in Figure 1.2) thereby affecting the activity of micro-organisms responsible for breaking down organic matter and chemical transformations in the soil (Lambers et al., 1998). The pH decreased throughout the experimental term, and may have influenced nutrient solubility, as demonstrated by significant positive correlations between pH and nutrient concentrations (DOC, TDN, NO_3, Mg, Ca, K, Fe and Eh (and PO_4-UF only)) (Tables 4.5b and 4.5c).

Silt- and sand-sized particles represented the majority of the particle fraction throughout the column profiles, with the clay sized fraction consistently small (< 2 %). This suggests that the overall surface area of soils throughout the column profiles was small, resulting in fewer nutrient binding sites than in soils with a larger clay fraction. Comparison of the UF and F columns following 52 weeks irrigation with the particle size distribution data for the freshly prepared artificial soils demonstrated little change over the irrigation period.

The CEC of a soil is of particular importance for nutrient retention and storage. The CEC values for the artificial soils were consistent with those reported for sandy loam soils, which range from 2 to 12 $cmol_c\ kg^{-1}$ (Brady and Weil, 2008; Rowell, 1994). Soils with a larger clay fraction report much higher CEC values (up to 60 $cmol_c\ kg^{-1}$) and

therefore better support nutrient retention (Rowell, 1994). Typically, organic matter is reported to have a net negative charge, which suggests that soils with a higher organic matter content may have a greater CEC (Sparks, 2003).

4.5.5 Fertiliser application

There were no significant differences between the TPC concentrations found in the UF, F or freshly prepared soils. However, the C : N ratios throughout the profiles of both treatments were significantly lower than those of the freshly prepared soils. This suggests that the change in C : N ratio was more influenced by changes to the total nitrogen concentrations than carbon concentrations.

A significant difference in the leachate pH was observed between the UF and F columns; with the latter being lower ($p < 0.001$, Table 4.5a). The fertiliser was composed of a range of materials (detailed in Appendix A) including kali vinasse, fish meal and kieserite, all of which contain sulphur and are commonly applied to soils to reduce pH (Modaihsh et al., 1989). However, this effect was not reflected within the solid phase as overall there was no significant difference between the pH of the freshly prepared soil, and the UF or F soils ($p > 0.05$, Table 4.7), which suggests that there may have been a buffering effect taking place within the soil caused by the addition of metals (K) within the fertiliser.

The effect of the fertiliser applications on the leached N concentrations was small in comparison to the overall N increase which occurred in both the UF and F columns, as discussed previously. The second fertiliser application led to a significant ($p = 0.005$, Table 4.7) increase in the N leachate concentration. This may be attributed to the first fertiliser application coinciding with the large N release, with the N concentration

within the leachate already increasing, whilst the second fertiliser application took place once the N concentration within the leachate had stabilised. The DON concentration demonstrated the greatest increase, which may have been attributable to the fertiliser containing a large organic N fraction; relative to inorganic N fractions (components are detailed in Appendix A).

The extractable Mg, Ca and Fe and leachate Mg, Ca and K concentrations were significantly different between the UF and F soil columns and co-varied with pH throughout the profiles, which was confirmed by a close correlation between the values ($p < 0.05$, Tables 4.8b and 4.8c). These changes may have been caused by changes to the CEC of the soil as a result of the fertiliser application. The organic nature of a number of components within the fertiliser would have provided a greater number of exchange sites within the soil, however, no statistically significant difference ($p > 0.05$, Table 4.10a) between the CEC in the UF and F columns were observed.

The leached PO_4^{3-} concentrations in the F columns increased following fertiliser application. However, there was no significant difference ($p > 0.05$, Table 4.8a) measured between the extracted PO_4^{3-} concentrations of the UF and F soils. This further supports that the P leached from the soil was being controlled by equilibrium with the irrigation water.

The enzymatic activity values from the solid samples were significantly lower throughout the depth profile of the F columns. It should be noted that no significant difference was observed between the enzymatic activity values within the leachate of either the UF or F columns. This corresponds with a study by Ramirez et al. (2010), where microbial respiration rates were observed to decrease as a result of N-containing

fertiliser applications, and responses were observed to be similar across a range of ecosystems. Ramirez et al. (2012) suggests that this reduction in microbial activity is caused by the shifting of metabolic capabilities of soil bacterial community in response to the N application, yielding communities less capable of decomposing the more recalcitrant soil carbon pools. The SOC data from the soil columns may support this theory. With less microbial decomposition of organic matter occurring within the F columns as a result suppression from the N fertiliser application the significantly ($p = 0.002$, Table 4.9a) higher SOC content observed within the F columns (Figure 4.19) may be explained. Further support for this theory may have been obtained through monitoring CO_2 fluxes from the columns, it would have been anticipated that with less decomposition of organic matter occurring within the F column, the CO_2 release from F columns would have been lower than that of the UF columns.

4.5.6 Synthesis

Results suggest that irrigation rate may have been one of the main processes influencing nutrient losses from the soil, with equilibrium reached for PO_4^{3-} and DOC. This suggests that the EP could benefit from reduction of their irrigation rate within the Biomes.

Nitrate was quickly leached from the soil, after which low N concentrations persisted, possibly due to the immobilisation of N during the breakdown of more recalcitrant organic material. A mass balance (Table 4.14) of N throughout the experiment showed a decrease of 7.06 % in the total N content within the UF columns and a decrease of 9.90 % in F columns. Significant proportions (58.6 % for UF and 71.7 % for F) are unaccounted for, and may be attributed to NH_3 volatilisation and denitrification.

The pH of the soil decreased throughout the experimental term and influenced nutrient availability by solubilising ions present within the soil. The concept of pH change may impact the activity of micro-organisms responsible for breaking down organic matter and most chemical transformations in the soil (Lambers et al., 1998). As indicated in Figure 1.2, mineral soils with pH ranging from 6.5 to 7.5 have the greatest nutrient availability.

The extractable PO_4^{3-} concentration in the solid samples extruded from the column after 52 weeks were lower than in the freshly prepared soil. This was consistent with high concentrations measured in the leachate throughout the experiment and the calculated PO_4^{3-} release from apatite minerals.

The initial concentrations of nutrients within the leachate collected from the columns were variable; with a large standard deviation (for example during the first 9 weeks of irrigation for TDN and DOC variations of 44.5 and 26.4 % respectively, were measured). This suggests that there was a stabilisation period within the columns, lasting approximately 9 weeks (17 % of experimental period) from the time that irrigation commenced. The variation then decreased to average 14.0 % and 12.9 % (RSD) for TDN and DOC respectively.

In soils the potential for significant hydrologic loss of mobile nutrients is directly related to the size of the exchangeable nutrient pool and the proportion in solution at any given time (Robertson et al., 1999). Field-moist soils were used for the determination of water-extractable nutrient concentrations in this study, meaning that a significant proportion of the concentrations presented above will have been present within the soil solution and extractable phase. However, the data give an indication of

the concentrations present within the soil profiles relative to each other, thereby serving to meet the aim of the investigation. The drying of soils has been reported to significantly affect the K and P concentrations within a soil sample (Attoe, 1947; Dowdy and Hutchenson, 1963; Pote et al., 1999).

Particle size distribution is a key factor with regard to nutrient dynamics in any soil (Silver et al., 2000). In this instance it appears that the high proportion of large sized particles served to limit the nutrient retention and storage capabilities of the soil, through their proportionately small surface area and associated lack of charged sites to which nutrients may bind.

4.6 Conclusion

Column experiments provide an important means of examining the behaviour of soil nutrients within a controlled system. This experiment was designed to control potentially important physicochemical variables such as temperature and moisture content, which would undoubtedly have significant impacts on the experimental outputs. In restricting these variables a valuable insight has been gained into the behaviour of the nutrients within the soils, and a greater understanding of the mechanisms taking place. This study represents the first time the Eden Project soils have been studied in such detail and further, to the best of the author's knowledge, the first time the nutrient characteristics and retention of artificial soils have been observed in such a manner.

The microbial population has been suggested to have had a significant impact on the nutrient concentrations observed within the soil leachate, particularly N and P. The

immobilisation of N within the soil columns was most likely caused by the decomposition of organic material, with bark having the most significant effect as a result of its high C : N ratio. This bark decomposition resulted in later re-mineralisation of N and transfer into soil solution.

PO_4^{3-} was suggested to have been released through the microbial decomposition of organic matter within the soil. The initial lag-phase displayed by the leachate concentrations, suggested P adsorption within the soil, with the subsequent increase in leached PO_4^{3-} resulting from saturation of available adsorption sites, with PO_4^{3-} being leached in response to changes in the equilibrium between the soil and the irrigation water. It would therefore be anticipated that in the long-term these values would begin to decline as the decomposable organic matter stocks within the soils decreased.

The clay-sized particulate component of the soil was small, and consistent with that found within the Humid Tropics Biome soil pits (as described in Chapter 3). Hence, the overall surface area of the soils was small, meaning that there were a lower number of charged binding sites within the soil. A potential consequence of this is that nutrients released through the breakdown of organic residues and fertiliser application, are highly susceptible to leaching from the soil, particularly under the continuous drip irrigation system employed at the Eden Project. This reduces the nutrient residence time in the soil and the timescale over which plant uptake of the nutrients may occur. Thus, a continuous supply of nutrients from an external source is thought to be required in order to meet the plant demands.

The Eden Project soils have been in place since 2001, and have been subjected to inconsistent regimes of mulch and fertiliser application. Whilst the high N and PO_4^{3-}

concentrations observed within the leachate at the termination of the column experiment were not likely to have been sustained long term, regular application of mulch and fertiliser, as practiced at the Eden Project, is likely to lead to recurring nutrient leaching. The NO_3^- immobilisation observed as a result of the bark application would exercise significant impact on the performance of plants and, as such, planting should be avoided during the period of high immobilisation, following application of any high C : N ratio materials.

The readiness with which N and PO_4^{3-} nutrients were leached from the soils within the columns may be extrapolated to reflect the performance of the Eden Project soils. This means that in order to maintain sufficient N and PO_4^{3-} concentrations for the support of healthy plant growth, large quantities of fertiliser application are required. Alongside the negative impacts on the plants, the high levels of nutrients lost from the soils have negative implications for the environment (Spiro and Stigliani, 1996).

In order for the soil nutrient retention and storage capabilities to improve, it will be necessary to amend the soil composition, particularly within the root zone. A reasonable first step would be to increase the soil surface area, through increasing the clay content, thus increasing the number of nutrient binding sites and reducing the potential for losses and increasing the long-term nutrient availability within the soil.

Improvements to nutrient retention within the Eden Project soils, and other similar artificial soils, will have important implications, not only with regard to plant growth and economic savings (lower fertiliser costs), but also through improvements in water quality in the form of lower concentrations of N and P in the water draining from the soils.

CHAPTER 5

The effect of biochar amendments on nutrient retention of Eden Project soils

5.1 Overview

A study was devised to determine the potential for biochar to improve the nutrient retention of artificial soils. Biochar was added to the artificial soil mix at 3 concentrations (10 %, 5 % and 2 %) plus a control (0 %) and packed into mesocosms. These were maintained under controlled environmental conditions with irrigation for 6 weeks. Leachate was collected on 6 occasions and analysed for dissolved constituents, physicochemical and biological properties. Samples were of the freshly amended soil and of the irrigated amended soils were taken and analysed for solid-phase and extractable constituents and physicochemical and biological properties.

Many of the observations detailed within the results section are consistent with those reported for other biochar studies. Results demonstrated that biochar application helped to reduce losses of key nutrients through leaching, however on a mass basis there was no clear distinction between biochar concentrations. Overall nutrient leaching decrease may be attributed to the reduced leachate volume, leading to the inference that biochar application increased the water holding capacity. Enzymatic activity was increased following biochar application, suggesting that biochar encourages the biological community, with this having potentially significant implications for the breakdown of SOM and nutrient cycling within the soils. With appropriate management the application of biochar at the Eden Project site could lead to greater soil resource efficiency.

5.2 Introduction

5.2.1 Research objectives

The research outlined in this chapter addresses research objective 3.

- To make controlled changes to the artificial soils determine how this affects the sustainability of the nutrient reservoir.

5.2.2 Rationale

The experimental plan for the project included the investigation included the exploration of the potential for amendment to the soil composition used by the Eden Project. If justified on the basis of data obtained from the soil column experiments, trials to measure improvements in the nutrient retention and storage capability of the artificial soil resulting from amendments would be made. Observations recorded within Chapters 3 and 4 demonstrate a range of soil characteristics and processes, which have been identified as detrimental to the nutrient retention and storage capabilities of the soil. Key characteristics and behaviours included a very small (<1 %) clay fraction with particle size being consistently large throughout the soils, a low CEC, high levels of N immobilisation by the soil microbial population and the subsequent N release following remineralisation, sustained P leaching from the soil as a result of organic matter decomposition. On the basis of these observations it was decided that through increasing the number of available charged binding sites within the soil, improvements to the nutrient retention and storage capabilities may follow.

Following consultation with the Eden Project, biochar was identified as the amendment for this study due to the Eden Project's intention to install a pyrolysis unit for the

production of biochar from the site's green waste material. This chapter reports on the efficacy of biochar applied to the artificial soil used by the Eden Project. Observations reported in Chapters 3 and 4 indicated that the Eden Project artificial soils had poor nutrient retention and storage capabilities, relating to the high proportion of large sized particles, resulting in low water holding capability and a small surface area on to which nutrients may bind.

5.2.3 Biochar

Biochar is a fine-grained carbonaceous form of charcoal, manufactured with the intention of being applied to a soil in a deliberate manner (Lehmann et al., 2011). It is produced through pyrolysis: the thermo-chemical decomposition of organic materials, in the absence of oxygen (Lehmann and Joseph, 2010). The physical characteristics of biochar depend upon both the feedstock and the pyrolysis conditions under which it is manufactured. An additional factor influencing the choice of studying biochar as an amendment was the intention to install a pyrolysis unit on site at the Eden Project.

Historically, biochar has been connected with soil management practices. One example is the Terra Preta (*black earth*) soils within the Amazon basin, which boast distinctly greater fertility than soils in the immediate surroundings and have been dated as far back as 9000 years B.P. (Sohi et al., 2010). It is thought that the pre-Columbian people created and managed this area through the addition of incompletely combusted remnants from domestic fires, and by carrying out controlled in-field burning. The extent of these deposits suggests that applications were increasingly deliberate, presumably as a management strategy to address low soil fertility (Sohi et al., 2010).

Residually, the Terra Preta soils still display elevated SOC content, and enhanced N, P, K, and Ca status (Sohi et al., 2010).

The application of biochar as a soil amendment in more recent times has been reported to have a significant impact on soil properties, processes and functions. Increased pH and water holding capacity, improved soil structure, increased nutrient retention, decreased N_2O and CH_4 emissions, reductions in leaching of inorganic N, adsorption of anthropogenic chemicals (e.g. steroid hormones) and adsorption of heavy metals have all been reported (Anderson et al., 2011; Downie et al., 2010; Manyà, 2012; Spokas et al., 2009). The potential of biochar to increase the retention of soil moisture and nutrients was of particular interest to this study.

Biochar is reported to offer manifold benefits as, further to its application as a soil amendment, it offers a means of long-term C sequestration, and a source of bio-energy and waste disposal (Laird, 2008). As the Eden Project aims to operate sustainably the production and use of biochar onsite aligns with their ethos.

5.3 Experimental design

5.3.1 Overview of experimental design

As a preliminary step to explore the potential for soil improvement through the application of biochar to the artificial soils, a short-term mesocosm experiment was devised.

Figure 5.1: *Replicate soil mesocosms used to measure the effect of biochar application on nutrient retention in artificial soils at the Eden Project.*

A biochar was produced from waste organic residues (predominantly softwood chippings), sourced onsite at the Eden Project. It was mixed with the artificial soil at 3 concentrations (2, 5 and 10 %), plus a control (0 %). Mesocosms were then established in triplicate using the soil-biochar mixes (Figure 5.1). The mesocosms were maintained under the same conditions as the soil columns (Chapter 4) and were irrigated and incubated for 6 weeks, during which time leachate was sampled. Following the 6 week incubation period, the mesocosm soil was extruded and analysed for physicochemical and biological characteristics.

5.3.2 Mesocosm design

In order for a plant population to benefit from the application of biochar to soil, it needs to be present at depths commonly accessed by roots (Blackwell et al., 2010) and at which biochar can easily be incorporated in the soils already established at the Eden Project. In view of this, a mesocosm of 100 mm depth was selected.

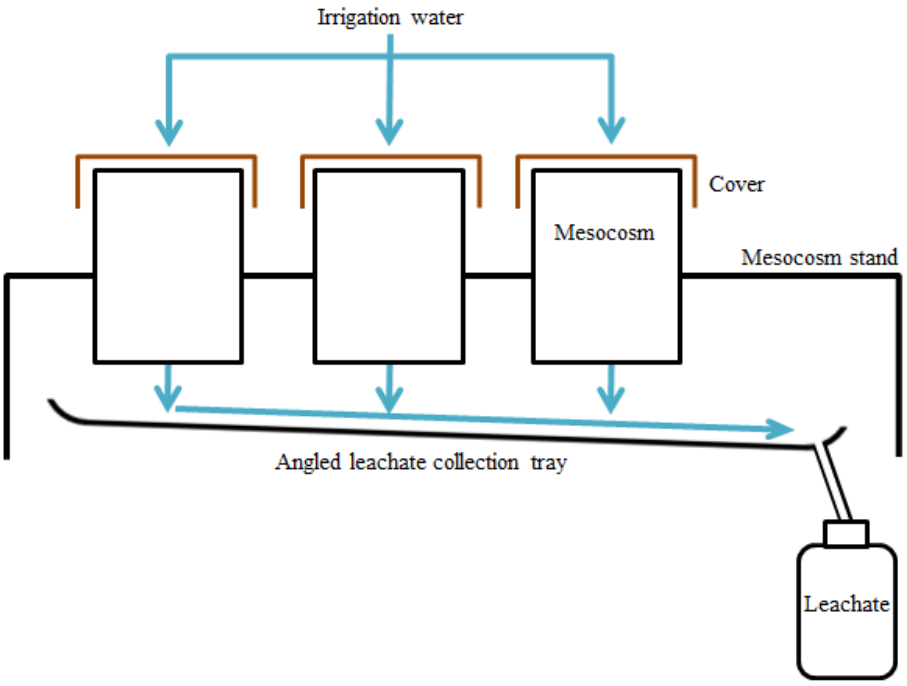

Figure 5.2: *Mesocosm set-up as used for the amendment study Mesocosms were made up in PVC pots which were 110 mm diameter by 100 mm height.*

The mesocosms were opaque PVC containers, with an internal diameter of 110 mm at the top and 90 mm at the base with a depth of 100 mm. As shown in Figure 5.2 the mesocosms were suspended above a collection tray, which directed the leachate to an acid washed 100 mL polyethylene collection bottle. The base of each mesocosm had 10 outlets (5 mm diameter) in order to aid drainage. A mesh (100 µm pore size) was laid at the base of each mesocosm, prior to soil packing, in order to minimise soil loss through the outlets.

5.3.3 Environmental conditions and irrigation

The mesocosms were maintained within a controlled temperature room at 15 ^{o}C for the duration of the study. This temperature was chosen to represent temperatures found within the Eden Project Biomes and was consistent with the temperature to which the soil columns were subjected.

Irrigation of the mesocosms was performed by hand on a daily basis, using pH-adjusted 18.2 MΩ cm^{-1} water. Each mesocosm received 0.84 mL m^{-2} irrigation water day^{-1} (10 mL day^{-1}), consistent with the irrigation rate used at the Eden Project.

5.3.4 Soil composition

The artificial soil base used for these experiments was from the same batch used for the soil column experiments, prepared onsite at the Eden Project on 25/03/13. This soil was used because the soil column study (Chapter 4) provided valuable insight to its behaviour and characteristics under irrigation, allowing for the generation of a dataset comparable to that from Chapter 4.

5.3.5 Biochar production

5.3.5.1 Feedstock

A varied selection of feedstock materials for the production of biochar have been reported, which can be broadly divided into 3 categories: wastes (e.g. municipal solids), crop residues and purpose grown feedstock (Hammond, 2010).

During the biochar production (pyrolysis) process the volatile organic compounds within the feedstock structure are lost, which causes the biochar to retain the rudimentary porosity and structure of the feedstock (Blackwell et al., 2010). Hence the

feedstock used to produce the biochar has a large impact upon its physical and chemical properties and, therefore, on its effectiveness as a soil amendment (Downie et al., 2010; Sun et al., 2014; Zhao et al., 2013).

The Eden Project generates large quantities of plant residue, which would typically be composted on site and either used as a component of the soil mix or applied to the soil as a mulch amendment. As plant residue was readily available at the site, it was chosen as the feedstock.

5.3.5.2 Pyrolysis

Aside from the effect of different feedstock materials, biochar characteristics are further influenced by production variables such as temperature, time and pyrolysis atmosphere (Zhao et al., 2013). Reported pyrolysis methods are listed in Table 4.1.

Table 5.1: *Reported pyrolysis conditions producing biochar.*

Author	Pyrolysis		
	Temperature (^{o}C)	**Heating rate and time**	**Yield (%)**
Cheng et al. (2006)	350	16 hours at constant temperature	33.2
Fang et al. (2015)	450 and 550	5 – 10 ^{o}C min^{-1} held for 40 minutes at peak temperature	/
Herath et al. (2013)	350 and 500	3.1 and 5.1 ^{o}C min^{-1} respectively, until temperature reached and then cooled	27.0 – 35.0
Hossain et al. (2011)	300, 400, 500 and 700	10.0 ^{o}C min^{-1} until temperature reached and then cooled	52.4 – 72.3
Keith et al. (2011)	450 and 550	5 – 10 ^{o}C min^{-1} held for 40 minutes at peak temperature	/
Mukherjee et al. (2011)	400 and 650	26 ^{o}C min^{-1} held for 3 hours at peak temperature	/
Rondon et al. (2007)	350	1 hour at constant temperature	/
Sun et al. (2014)	200, 300, 400 and 600	5 hours at constant temperature	22.7 – 48.4
Wardle et al. (1998)	450	15 minutes at constant temperature	/
Yuan et al. (2011)	300, 500 and 700	20 ^{o}C min^{-1} held for 4 hours at peak temperature	9.24 – 34.9
Zhao et al. (2013)	200, 350, 500 and 650	18 ^{o}C min^{-1} held for 4 hours at peak temperature	26.8 – 45.1

In general, pyrolysis conditions demonstrate a trend with regard to certain soil properties displayed by the resultant biochar. Higher pyrolysis temperatures (> 600 ^{o}C) have been observed to reduce biochar yields and increase alkalinity (Demirbas, 2004; Hossain et al., 2011; Manyà, 2012; Yuan et al., 2011). The concentration of N was found to decrease with increasing temperature, whilst P, K and micronutrient (Ca, Fe, Mg, S, Cu and Zn) concentrations increased (Hossain et al., 2011; Zhao et al., 2013). Higher pyrolysis temperatures tend to produce biochar with a larger surface area (Downie et al., 2010).

In view of these variations, it was important to consider the aim of the biochar application, which in the case of the Eden Project soil, was to determine the potential for biochar amendment to reduce nutrient loss through leaching and, in so doing, enhance plant available nutrient concentrations (particularly N, P and K). To best meet this aim, a mid-range pyrolysis temperature of 450 ^{o}C was chosen.

Raw material was dried at 60 ^{o}C for 48 hours prior to pyrolysis, which was performed using a muffle furnace programmed to increase from room temperature (approximately 21 ^{o}C) to 450 ^{o}C at a rate of 5 ^{o}C min^{-1}. The final temperature was held for 15 minutes, before cooling to room temperature. The average yield obtained during the biochar production was 22.2 ± 1.0 %, which is low compared to larger scale production systems using equivalent conditions (35 % yield) (Bridgwater, 2012).

5.3.6 Preparation for incubation

A study by Lehmann et al. (2003) suggested that biochar with a particle size of approximate 20 mm behaved identically to biochar sieved to below 2 mm, with regard to nutrient uptake and crop yield. This further suggests that biochar particle size does

not play an overriding role in soil fertility enhancement and may, therefore, be chosen as a function of practicality or cost.

Ideal biochar particle sizes have not been determined, however, where biochar particle size is fine there is increased risk of particles and bound nutrients being leached from the soil. The probability of this occurring in the Eden Project soils, which have a coarse texture, would be high. It was therefore decided that the biochar would not be finely ground but passed through a 2 mm sieve, prior to application to the soil.

Content refers to the quantity of biochar applied to the soil (% w/w) and has been demonstrated to be of significance with regard to the magnitude of changes witnessed within the soil. The literature reports a range of biochar contents, summarised in Table 5.2. For this investigation 3 contents were chosen: 10 %, 5 % and 2 % with a control (0 %) w/w (henceforth referred to as BC10, BC5, BC2 and BC0, respectively). These contents were chosen to observe the effect of varying biochar quantities on soil properties, with a view to determining the most suitable treatment for the Eden Project soils.

Table 5.2: *Biochar contents reported in the literature.*

Author	Content (% w/w)
Cheng et al. (2006)	4
Keith et al. (2011)	1, 2 and 4
Hyland et al. (2010)	0.2, 0.5, 2 and 7
Novak et al. (2009)	0.5, 1 and 2
Rondon et al. (2007)	3, 6 and 9
Yuan et al. (2011)	1
Zheng et al. (2013)	1, 2, 5

Once the biochar was added to the soils and mixed, samples were packed in to the mesocosms. The mix was loaded into triplicate mesocosms, in 2 cm layers in turn, to

minimise any composition difference between each, whilst also ensuring a consistent volume of soil mix in all samples. To help maintain homogeneity, the soil-biochar mixes were moist at the time of loading. As with the packing of the soil columns (Chapter 4), each layer of added soil mix was gently tapped down to achieve tight packing and avoid air entrapment, with the surface of each layer remaining uneven as a means of encouraging hydraulic connectivity throughout the mesocosm.

5.3.7 Sampling strategy

5.3.7.1 Leachate sampling

The leachate from the triplicate samples for each content was collected as illustrated in Figure 5.2, without the use of any suction. As with the column study in Chapter 4 the mesocosms were designed with a cap, non-porous sidewalls and remained unplanted, which removed the potential for water loss through evaporation, translocation or plant uptake. There was a 3.5 week delay between commencing irrigation and yielding leachate from all treatments, at which point sample collection took place on a 4 day cycle as outlined in Table 5.3.

***Table 5.3**: Sampling strategy employed throughout the column experiment.*

Date	Action
03/05/14	Biochar applied to soils and commencement of daily irrigation
27/5/14	Leachate collection date
31/5/14	
04/06/14	
08/06/14	
12/06/14	
14/06/14	Final leachate collection and mesocosm extrusion

The weights of filtered particulates were recorded during leachate filtration to measure effects of biochar application on particulate losses.

5.3.7.2 Solid sampling

After 6 weeks of irrigation the mesocosms were extruded. In order to reduce the impact of any edge effects, which may have occurred throughout the irrigation period, soil samples were taken from the centre of each mesocosm. Samples were placed in labelled polythene zip-lock bags and stored at 4 ^{o}C prior to analysis.

5.3.8 Analytical measurements

Leachate and solid samples from the mesocosms were analysed using the strategy outlined in Figure 5.3, according to the analytical methods described in Chapter 2. The leachate samples were analysed for the following dissolved analytes: TDN, NH_4^+, NO_3^-, PO_4^{3-}, K, Fe, Ca, Mg and SOC. The pH was monitored throughout the study.

Solid samples were taken from the freshly prepared soil and the extruded mesocosms; these samples were analysed for TPN and TPC, pH, CEC, SOC, particle size distribution, enzymatic activity and the following water extractable analytes: TDN, NH_4^+, NO_3^- + NO_2^-, PO_4^{3-}, K, Fe, Ca, Mg and DOC (Figure 5.3).

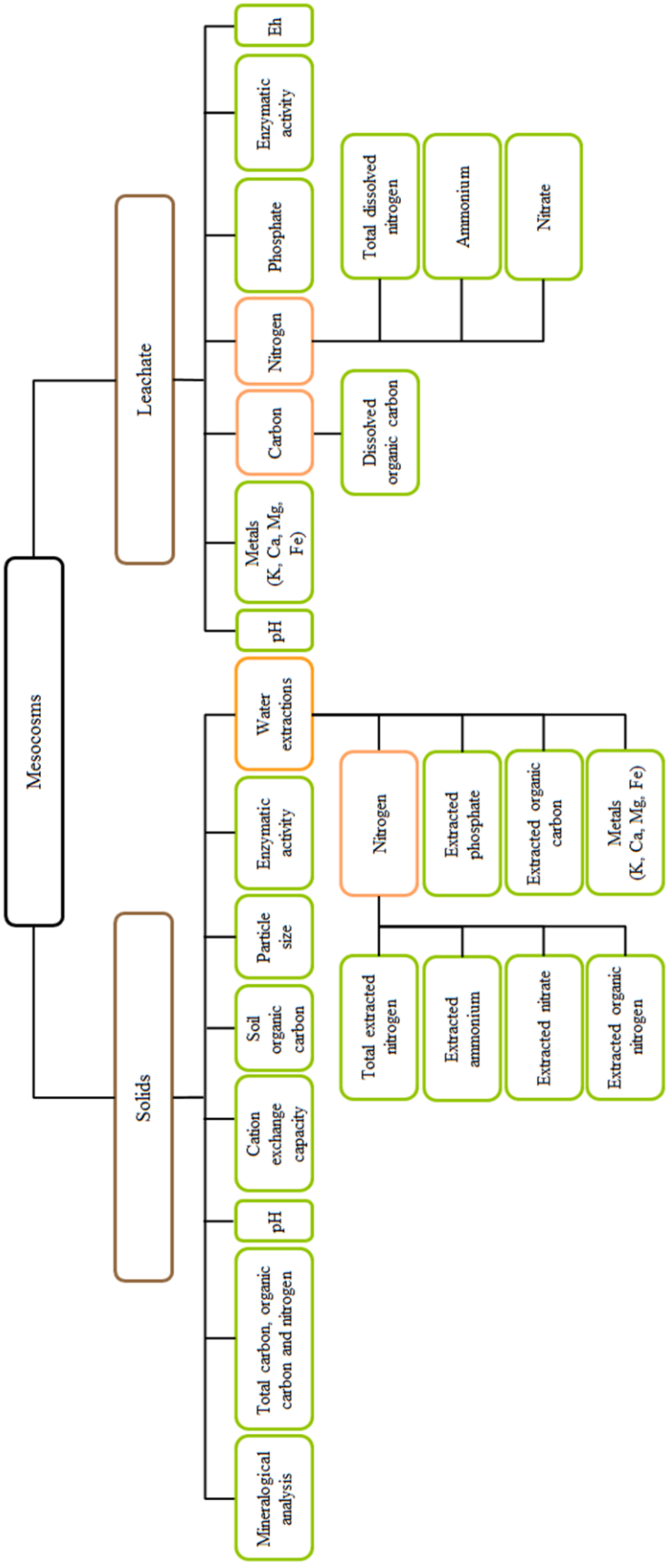

Figure 5.3: *Strategy used for the analysis of leachate and soil samples taken during the column study.*

5.3.9 Statistical analyses

Statistical analyses were carried out to allow for the comparison of leachate and solid data for each biochar application, and to compare the freshly amended samples with those sampled after 6 weeks of irrigation. The data was determined to follow normal distribution and as such parameter statistical analyses were employed. The Dunnett's and Tukey's tests were used for this purpose, alongside a one-way ANOVA. The Dunnett's test is a parametric multiple comparison procedure, which allows the mean for each treatment to be compared to the control in order to determine whether a specific biochar application had a significant difference on the measured characteristics (Cardinal and Aitken, 2006). The Tukey's test varies from the Dunnett's test, in that it allows for the comparison of each treatment to all other treatments, including the control.

A Pearson correlation coefficient (PCC) was used to determine any linear relationships between the characteristics and the biochar content (BC) and irrigation time, both for leachate and solid phase characteristics. This test allowed for the determination of whether a linear relationship existed between the datasets, making no assumption as to whether one variable was dependent on the other.

5.4 Results

The results from this experiment are split into three parts: (1) the analyses of the leachate collected throughout the 6 weeks of irrigation; (2) analyses of the solid and (3) extracted phase components of the freshly prepared soils and of soils following their extrusion from the mesocosms.

5.4.1 Results overview

Throughout the 6 week irrigation term the key observations, with regard to leachate properties, were:

(1) Statistical analyses determined that for all analysed characteristics at least one biochar content yielded significantly different values compared to the control. Leachate volumes decreased with increasing biochar content (BC2 =7.58 %, BC5 = 12.5 %, BC10 = 19.7 % decrease, compared to control) as did particulate loss within the leachate (BC2 = 34.0 %, BC5 = 39.6 %, BC10 = 66.0 % decrease, compared to control).

(2) The TDN, NO_3^-, NH_4^+, Mg and Ca losses through leaching were less in soils with greater biochar content. Whilst biochar application reduced losses of PO_4^{3-}, DOC and K, there was no correlation with biochar content. Increasing biochar content also increased the pH and Eh within the leachate. Losses of DON and Fe increased with increasing biochar application.

Following analysis of solid samples and extracted constituents of freshly amended soils and amended soils following the 6 week irrigation period, key observations were:

(1) In general there was a decrease in nutrient concentration over the 6 week irrigation period, which was significant for TPN, TPC, TEN, NO_3^-, EON, Mg, Ca and K.

Typically the representative size of the concentration decrease was observed to decrease with increasing biochar content.

(2) For BC2 soils it was observed that for all characteristics, except pH, TPC, PO_4^{3-}, sand and silt particle size fractions, there was no significant difference from the control. The BC5 soils were significantly different to the control soils for pH , TPC, SOC, PO_4^{3-}, extracted Mg, extracted Fe, sand and silt particle size reactions. The BC10 soil data were significantly different from the control for the same characteristics as BC5 plus TPN, clay particle size fraction, moisture content and enzymatic activity.

5.4.2 Leachate analyses

Leachate samples were collected from each biochar treatment on a regular basis from Weeks 3 to 6, as outlined in Table 5.3. One-way ANOVA of the leachate demonstrated that for all analysed characteristics, at least one biochar content yielded significantly different values (Table 5.4). The Dunnett's test further served to identify which of the treatments produced values significantly different from the control (Table 5.5); box plot diagrams for each leachate parameter are contained in Appendix C. The results of the Dunnett's test demonstrated that all characteristics, excluding Fe, were significantly different ($p < 0.001$, Table 5.4) from the control values. The Tukey's test revealed that for a number of characteristics there was no significant difference between the BC2 and BC5 soils.

Table 5.4: *Results for one-way ANOVA results with Dunnett's test to confirm whether the average values (n = 18) for any treatments were significantly different from the control. The Tukey's test was used to confirm which treatments were significantly different from one another.*

Characteristic	One-way ANOVA	Dunnett's test			Tukey's Test			
	p	BC2	BC5	BC10	BC0	BC2	BC5	BC10
DOC	< 0.001	✓	✓	✓	D	C	B	A
TDN	< 0.001	✓	✓	✓	C	B	B	A
NO_3^-	< 0.001	✓	✓	✓	D	C	B	A
DON	< 0.001	✓	✓	✓	C	B	B	A
PO_4^{3-}	< 0.001	✓	✓	✓	B	A	A	A
pH	< 0.001	✓	✓	✓	D	C	B	A
Mg	< 0.001	✓	✓	✓	C	B	B	A
Ca	< 0.001	✓	✓	✓	C	B	B	A
K	< 0.001	✓	✓	✓	B	B	AB	A
Fe	< 0.001			✓	B	B	B	A
Eh	< 0.001	✓	✓	✓	D	C	B	A
Leachate volume	< 0.001	✓	✓	✓	C	B	B	A
Leached particulate weight	< 0.001	✓	✓	✓	C	B	B	A

Boxes containing a tick (✓) demonstrate a significant difference from the control.
Boxes which do not share a letter are significantly different.

The leachate collection method meant that there was the potential for leachate losses through evaporation. To minimise the impact of this on the experimental outcomes the collection trays were covered and the data processed in terms of percentage difference alongside concentration values. The average values for replicate samples (n = 3) collected on 6 sampling occasions (n = 18) for each analyte/parameter are shown in Table 5.5. The average percentage change throughout the 6 weeks of irrigation was calculated for each biochar content. The data demonstrate that most analytes/parameters had changed as a result of the biochar applications; predominantly the most significant changes occurred in samples from the highest biochar application.

Table 5.5: *Mean concentration values (n = 18) for leachate samples, with the percentage difference of each treatment from the control concentrations.*

Characteristic	Average concentration				% change from control		
	BC0	**BC2**	**BC5**	**BC10**	**BC2**	**BC5**	**BC10**
DOC (mg C L^{-1})	34.0 ± 1.4	22.2 ± 0.8	24.2 ± 0.6	28.3 ± 1.4	34.7 ± 3.4	28.9 ± 2.4	16.7 ± 5.0
TDN (mg N L^{-1})	147 ± 15	110 ± 4	102 ± 4	82.3 ± 3.3	25.1 ± 11.7	24.0 ± 11.6	39.0 ± 12.2
NO_3^- (mg N L^{-1})	72.7 ± 2.2	65.3 ± 1.3	60.2 ± 1.4	52.1 ± 2.6	10.1 ± 2.0	17.1 ± 2.3	20.2 ± 4.9
DON (mg N L^{-1})	75.0 ± 12.9	45.0 ± 3.5	41.9 ± 3.5	30.2 ± 2.4	39.9 ± 7.8	44.1 ± 8.3	59.8 ± 8.1
PO_4^{3-} (mg P L^{-1})	33.3 ± 3.1	19.2 ± 1.1	17.4 ± 4.2	21.0 ± 3.6	42.5 ± 5.9	48.0 ± 24.2	36.9 ± 17.0
Mg (µg Mg L^{-1})	27.9 ± 4.7	16.3 ± 0.7	13.1 ± 0.7	7.69 ± 0.93	-3.17 ± 0.33	-6.53 ± 0.33	-8.51 ± 0.52
Ca (µg Ca L^{-1})	83.4 15.0	49.2 ± 2.3	39.3 ± 2.0	23.7 ± 1.6	41.1 ± 4.1	52.8 ± 5.0	72.4 ± 12.2
K (µg K L^{-1})	400 ± 35	343 ± 10	350 ± 7	372 ± 8	41.0 ± 4.7	52.9 ± 5.1	71.6 ± 6.5
Fe (µg Fe L^{-1})	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.12 ± 0.02	14.2 ± 3.0	12.6 ± 2.1	7.1 ± 2.2
pH	6.15 ± 0.02	6.35 ± 0.02	6.55 ± 0.02	6.67 ± 0.04	31.1 ± 15.3	-9.49 ± 33.8	-206 ± 18
Eh (mV)	378 ± 2	381 ± 2	390 ± 3	396 ± 1	-0.97 ± 0.46	-3.22 ± 0.73	-4.77 ± 0.31
Leachate volume (mL)	9.10 ± 0.28	8.41 ± 0.39	7.96 ± 0.23	7.31 ± 0.34	/	/	/

A Pearson correlation coefficient (PCC) was calculated to determine whether the biochar content and irrigation time correlated with the characteristics analysed within the leachate (Table 5.6). All characteristics, except DOC and K, correlated with the biochar content. The PCC values correlated negatively, apart from pH, Fe and Eh, suggesting that an increase in biochar content reduced the leachate concentrations of most measured constituents. None of the characteristics correlated significantly with time.

Table 5.6: *Pearson correlation coefficient values for comparison between biochar content and irrigation time against measured leachate characteristics.*

Characteristic	Biochar application		Irrigation time	
	PCC value	p value	PCC value	p value
DOC	-0.192	0.369	0.036	0.869
TDN	-0.872	< 0.001	-0.165	0.442
NO_3^-	-0.957	< 0.001	-0.049	0.820
DON	-0.806	< 0.001	-0.209	0.326
PO_4^{3-}	-0.488	0.016	-0.071	0.740
pH	0.948	< 0.001	-0.072	0.739
Mg	-0.870	< 0.001	-0.163	0.446
Ca	-0.864	< 0.001	-0.179	0.403
K	-0.174	0.417	-0.313	0.136
Fe	0.850	< 0.001	0.158	0.461
Eh	0.948	< 0.001	0.003	0.990

Red = significant p values
Blue = non-significant p values

Cumulative nutrient losses over the 6 week irrigation period (Table 5,7) were estimated from the leachate data.

Table 5.7: *Estimated total leached nutrient concentrations for each biochar content.*

Characteristic	Unit	Biochar content (%)			
		0	2	5	10
Total leachate volume	L	234 ± 12	204 ± 9.3	197 ± 12	178 ± 13
TDN	mg	87.3 ± 0.7	55.3 ± 0.0	50.3 ± 0.1	39.8 ± 2.7
- NO_3^-	mg	42.7 ± 1.1	33.2 ± 0.0	29.75 ± 0.1	25.6 ± 1.7
- DON	mg	16.6 ± 1.3	21.2 ± 0.1	21.3 ± 0.1	37.1 ± 2.5
PO_4^{3-}	mg	202 ± 5	98.0 ± 0.1	87.7 ± 0.1	106 ± 7
DOC	mg	200 ± 3	112 ± 0	118 ± 0	137 ± 9
K	g	2.41 ± 0.07	1.75 ± 0.02	1.73 ± 0.02	1.84 ± 0.12
Ca	mg	552 ± 3	254 ± 0	196 ± 0	117 ± 8
Mg	mg	173 ± 1	84.1 ± 0.0	65.5 ± 0.0	38.0 ± 2.6
Fe	mg	0.23 ± 0.02	0.14 ± 0.00	0.21 ± 0.00	0.58 ± 0.04

5.4.2.1 Leachate volume

The average leachate volumes decreased with increasing biochar content (Figure 5.4). The control (BC0) had the highest average leachate volume (9.10 ± 0.28 mL day^{-1}) and BC10 the lowest (7.31 ± 0.34 mL day^{-1}). Statistical analyses suggested that differences

in leachate volumes were significant ($p < 0.001$, Table 5.4) with all biochar treatments having significantly lower leachate volumes than the control (Table 5.4). There was no significant difference between the BC5 and BC2 soils, as determined by Tukey's test (Table 5.2).

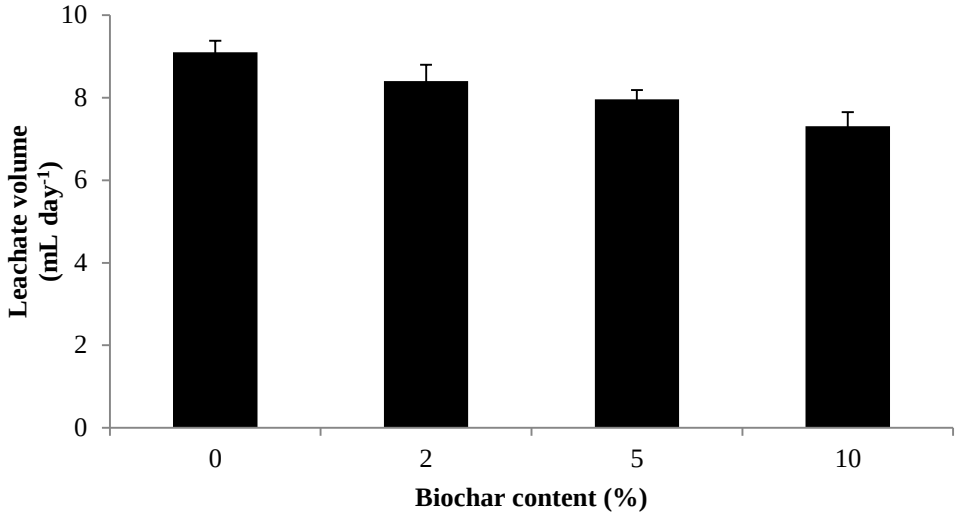

Figure 5.4: *Average leachate volume collected from each biochar treatment over 24 hours collection was carried out on dates shown in Table 5.3 (n = 6).*

The average particulate loss within the leachate for each biochar application was calculated (Figure 5.5a). The biochar content had a significant impact on the particulate loss in leachate ($p < 0.001$, Table 5.4). The control sample had the highest particulate loss, with the lowest observed within the BC10 soil, most likely attributed to the decrease in soil proportion with increasing biochar content. Figure 5.5b further illustrates the difference in particulate loss between biochar contents.

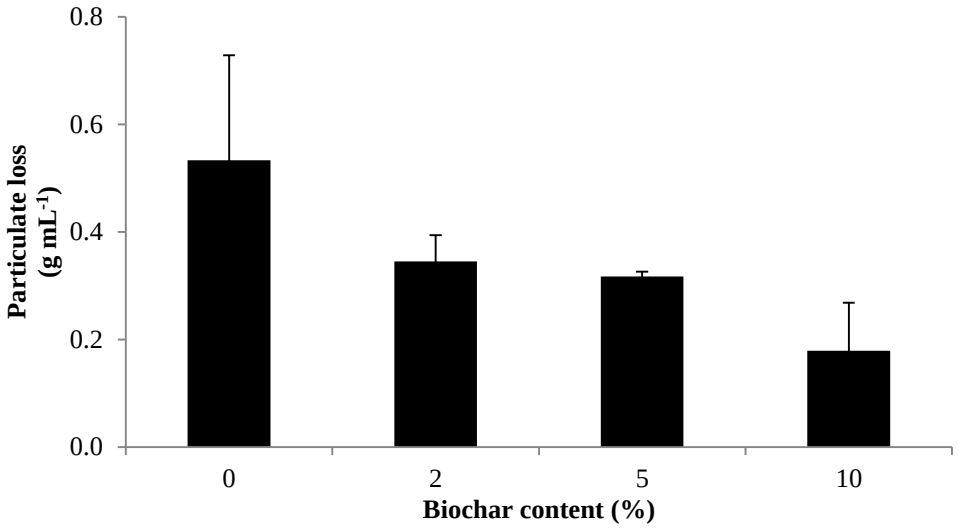

Figure 5.5a: *Average loss of particulate in leachate for each biochar content over 6 week leachate collection period (n = 6).*

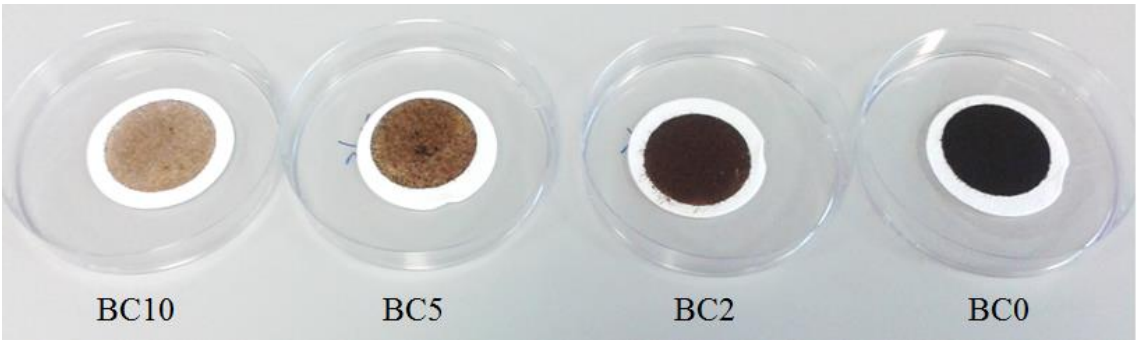

Figure 5.5b: *Filters following filtration of leachate from mesocosms, sampled on 8/6/15.*

5.4.2.2 Nitrogen

The TDN concentrations within the leachate samples are shown in Figure 5.6. All amended soils had significantly lower concentrations within the leachate compared to the control (14.5 ± 1 mg N L^{-1}; $p < 0.001$, Table 5.6), with the BC10 soil having the lowest average concentration (8.11 ± 0.3 mg N L^{-1}). However, no significant difference was observed between the BC5 and BC2 biochar content soils (10.6 ± 0.6 mg N L^{-1} and 10.9 ± 0.4 mg N L^{-1}, respectively). The control concentrations decreased over the irrigation period, whilst the amended samples' TDN concentrations were relatively stable throughout, reflected in smaller standard deviations than the control (Figure

5.6b). The biochar applications reduced TDN losses, through leaching, by 24.0 to 39.0 % (Table 5.5).

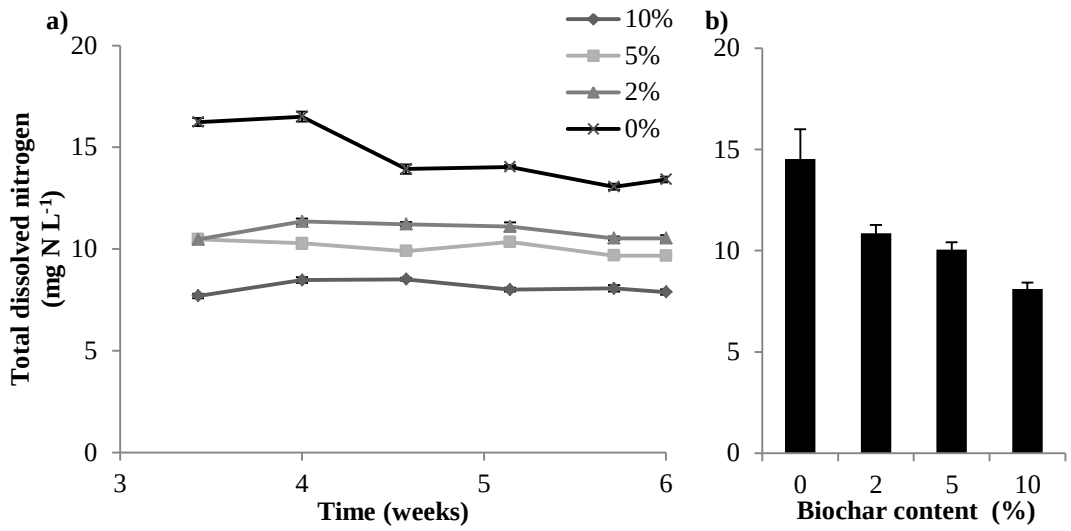

Figure 5.6: *TDN concentrations within leachate samples taken from each biochar treatment.* ***a)*** *Time series for leachate from each biochar treatment.* ***b)*** *Average concentrations within the leachate for each biochar treatment.*

Concentrations of dissolved NO_3^- in leachate are shown in Figure 5.7; they decreased significantly as a consequence of the biochar applications ($p < 0.001$, Table 5.4). All amendment contents were significantly different from the control (average 7.27 ± 0.22 mg N L^{-1}). The leached NO_3^- concentrations decreased with increasing biochar content. The BC10 amended soil had the lowest concentrations (mean 5.2 ± 0.3 mg N L^{-1}), while the BC2 and BC5 soils had concentrations of 6.53 ± 0.13 and 6.02 ± 0.14 mg N L^{-1}, respectively. There was a large difference in NO_3^- concentration within the leachate between the control and BC2 soil, which suggests that even a small quantity of biochar serves to improve the retention of these N species within the soil. On average the biochar applications reduced NO_3^- losses by 10.1 to 20.2 % (Table 5.5).

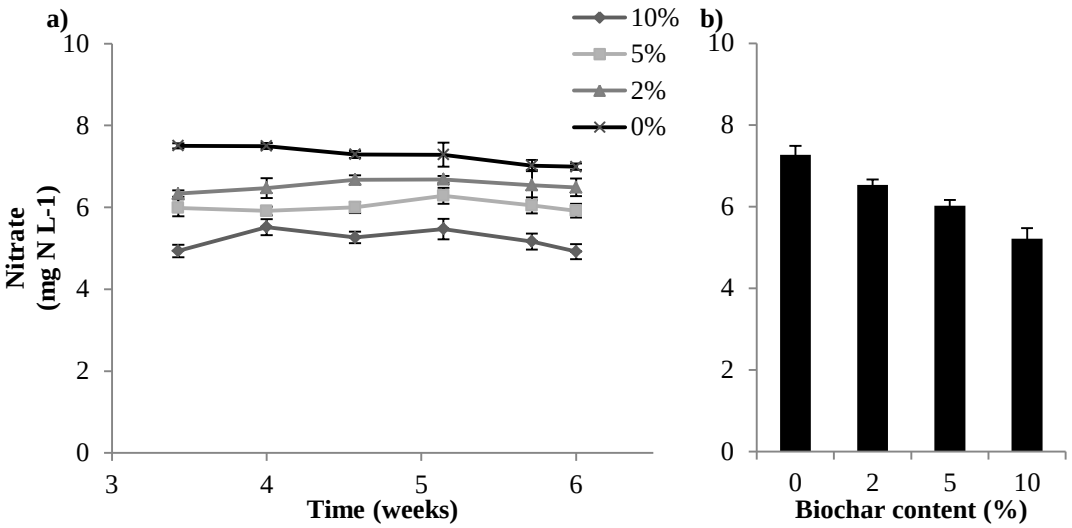

Figure 5.7: *NO_3^- concentrations within leachate samples taken from each biochar treatment.* ***a)*** *Time series for leachate from each biochar treatment.* ***b)*** *Average concentrations within the leachate for each biochar treatment.*

NH_4^+ concentrations (Figure 5.8) within the leachate were consistently below the LOD (1.2 µg N L^{-1}) for all BC10 and most BC5 samples. The control treatment concentrations decreased with time, consistent with the soil column data, with any dissolved or loosely-bound NH_4^+ quickly leached from the soil.

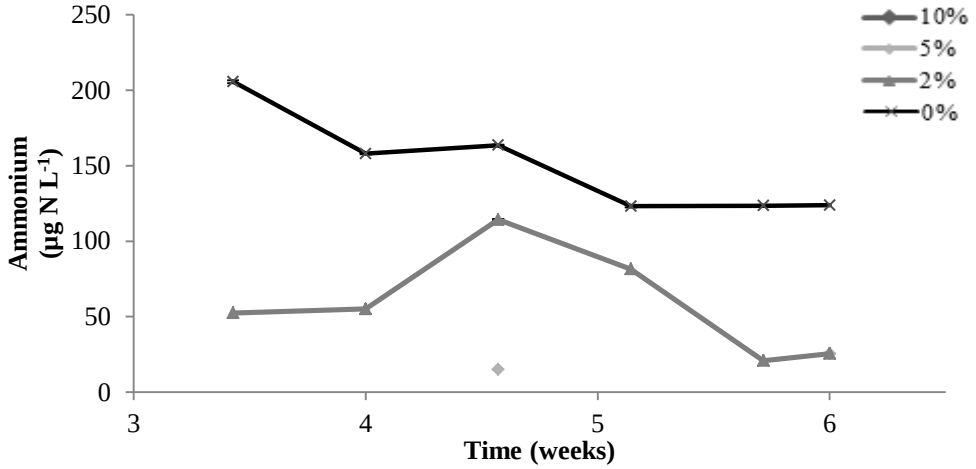

Figure 5.8: *NH_4^+ concentrations within the leachate samples taken from each biochar treatment. Dissolved concentrations were below the limit of detection for all BC10 and all, but 2 BC5 sample.*

DON concentrations within the leachate (Figure 5.9) were significantly higher in the control (7.14 ± 1.22 mg N L^{-1}) content compared with BC2, BC5 and BC10 ($p < 0.001$,

Table 5.4). The BC10 leachate had the lowest DON concentrations (2.89 ± 0.24 mg N L^{-1}) with no significant difference between content BC2 and BC5 concentrations (4.03 ± 0.34 mg N L^{-1} and 4.31 ± 0.34 mg N L^{-1} respectively). On average biochar application reduced DON leachate concentrations by 39.9 to 59.8 % (Table 5.5).

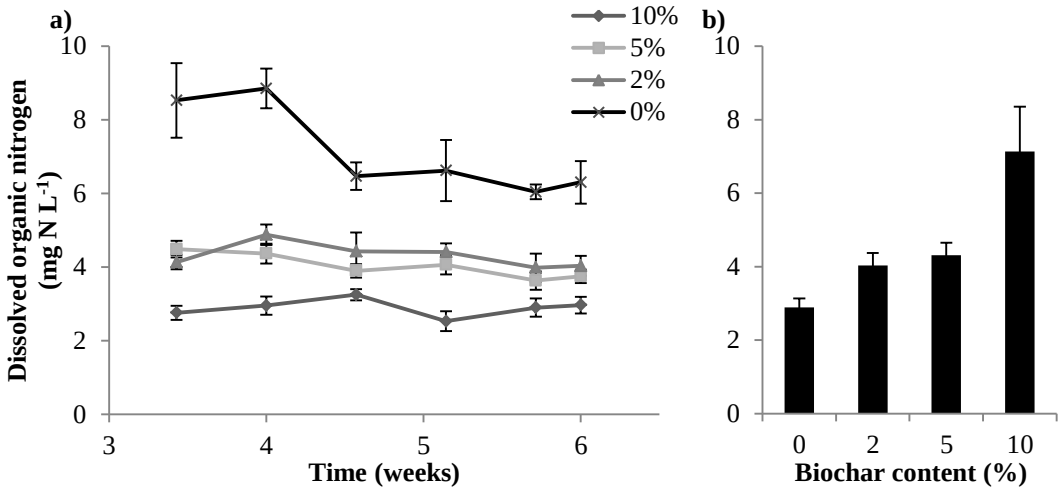

Figure 5.9: *DON concentrations in leachate. The DON concentration was calculated by subtracting the NO_3^- and NH_4^+ (where values >LOD) concentrations from those for total dissolved nitrogen.*

5.4.2.3 Phosphate

Dissolved PO_4^{3-} concentrations (Figure 5.10) were consistently highest in the control sample leachate (33.3 ± 3.1 compared with a range of 17.4 ± 4.2 to 21.0 ± 3.6 mg P L^{-1} for the amended soils). Biochar application had a significant effect on dissolved PO_4^{3-} concentrations within the leachate ($p < 0.001$, Table 5.4), though results from the BC10, BC5 and BC2 contents were variable, with no significant difference observed between them ($p > 0.05$, Table 5.4). On average the biochar applications reduced PO_4^{3-} losses through leaching by 36.9 to 47.9 % (Table 5.5).

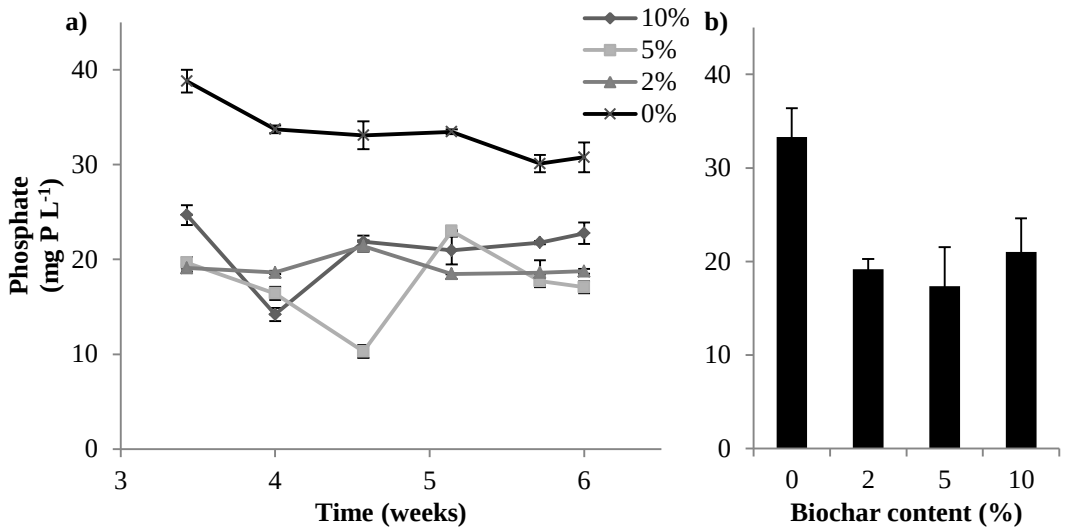

Figure 5.10: *Dissolved PO_4^{3-} concentrations in leachate sampled from each biochar treatment.* ***a)*** *Time series for leachate from each biochar treatment.* ***b)*** *Average concentrations within the leachate for each biochar treatment.*

5.4.1.4 Carbon

DOC concentrations (Figure 5.11) were highest in the control samples (34.0 ± 1.4 mg C L^{-1}) and lowest in BC2 samples content (22.2 ± 0.8 mg C L^{-1}), a reduction of 34.7 %. The DOC concentrations of all biochar applications were found to be significantly different from the control concentrations ($p < 0.001$, Table 5.4).

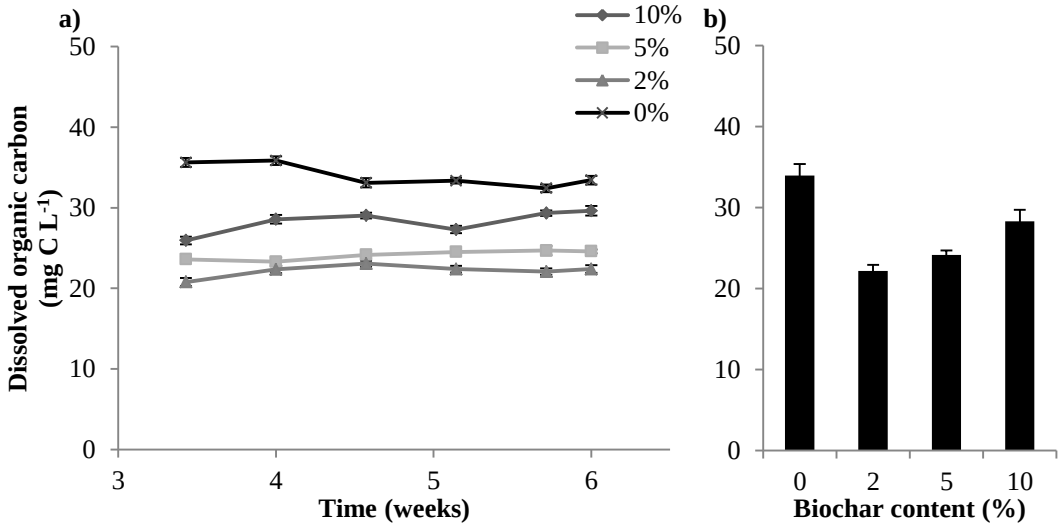

Figure 5.11: *DOC concentrations for the leachate samples of each biochar treatment.* ***a)*** *Time series for leachate from each biochar treatment.* ***b)*** *Average concentrations within the leachate for each biochar treatment.*

5.3.2.5 Metal ions

Dissolved Mg concentrations in the leachate are shown in Figure 5.12a; they decreased with increasing biochar content, with the highest and lowest concentrations measured in the control (27.9 ± 4.7 µg L^{-1}) and BC10 (7.69 ± 0.93 µg L^{-1}), respectively. Leachate samples from all biochar contents were found to be significantly different from the control samples (Table 5.4), with no significant difference measured between BC2 and BC5. The biochar amendments reduced Mg loss through leaching by 41.4 to 72.4 % (Table 5.5). The dissolved Mg concentration in control leachate decreased with time, whilst concentrations from the biochar applied samples remained stable.

The concentrations of Ca within the leachate were similar in trend to Mg (Figure 5.12b), with the control samples containing the highest dissolved concentrations (83.4 ± 15.0 µg L^{-1}) and BC10 having the lowest (23.7 ± 1.6 µg L^{-1}). The control leachate concentration decreased over the experimental period, whilst the biochar amended samples remained relatively stable, as demonstrated by the lower standard deviation values (Figure 5.12b). Concentrations for the biochar amended samples were found to be significantly different from the control, with there being no significant difference between the BC2 and BC5. Biochar applications reduced the dissolved Ca leachate concentrations by 41.0 to 71.6 % (Table 5.5).

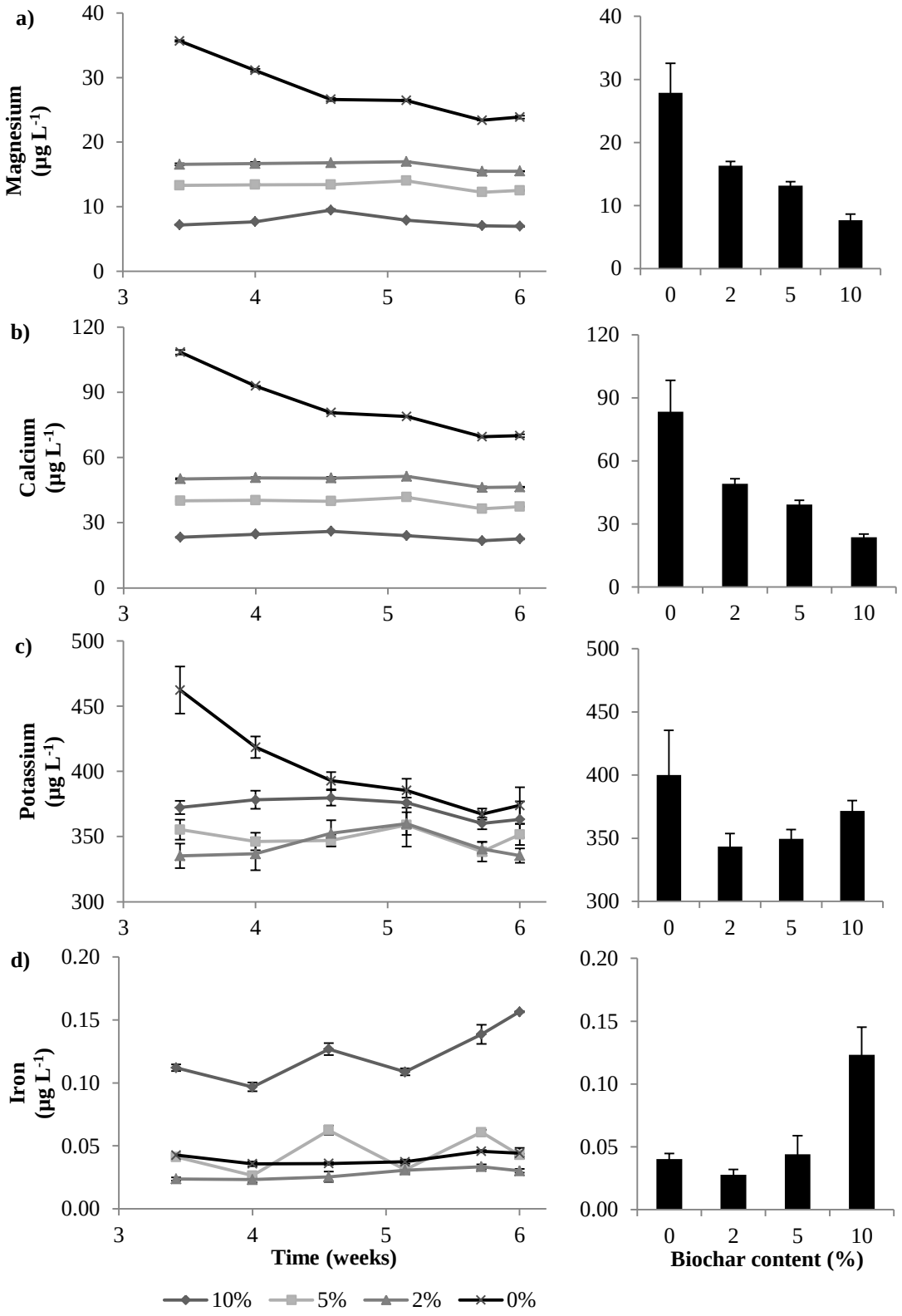

Figure 5.12: *Dissolved concentrations (µg L^{-1}) of selected metals within leachate samples of control soils and biochar amendments. The analyses were carried out in triplicate on samples collected from each treatment.* ***a)*** *Mg,* ***b)*** *Ca,* ***c)*** *K,* ***d)*** *Fe.*

Dissolved K leachate concentrations (Figure 5.12c) were highest in the control samples (400 ± 35 µg L^{-1}) and lowest, on average, in the BC2 samples (343 ± 10 µg L^{-1}). Concentrations within the leachate from the control samples decreased throughout the experimental period to give concentrations consistent with the biochar applied samples between weeks 4 and 5. On average, the values for all biochar applied samples were significantly different from the control values according to Dunnett's test data (Table 5.4). However, the concentrations were variable so that that there was no significant difference between average concentrations for different biochar applications as determined using the Tukey test (Table 5.4).

Dissolved Fe concentrations in the leachate samples are shown in Figure 5.12d. There was no significant difference between the control concentrations and those from BC2 and BC5 according to data from a Dunnett's test (Table 5.4). The BC10 concentrations were significantly higher than all other samples (average 0.12 ± 0.02 µg L^{-1}).

5.4.2.6 Physicochemical characteristics

The pH of the leachate samples remained stable for all treatments throughout the sampling period (Figure 5.13). Biochar application resulted in a significant increase in leachate pH, with all biochar containing samples having a significantly higher pH than the control ($p < 0.001$, Table 5.4). The highest (6.67 ± 0.04) and lowest (6.15 ± 0.02) average pH were measured in leachate from BC10 and control samples, respectively.

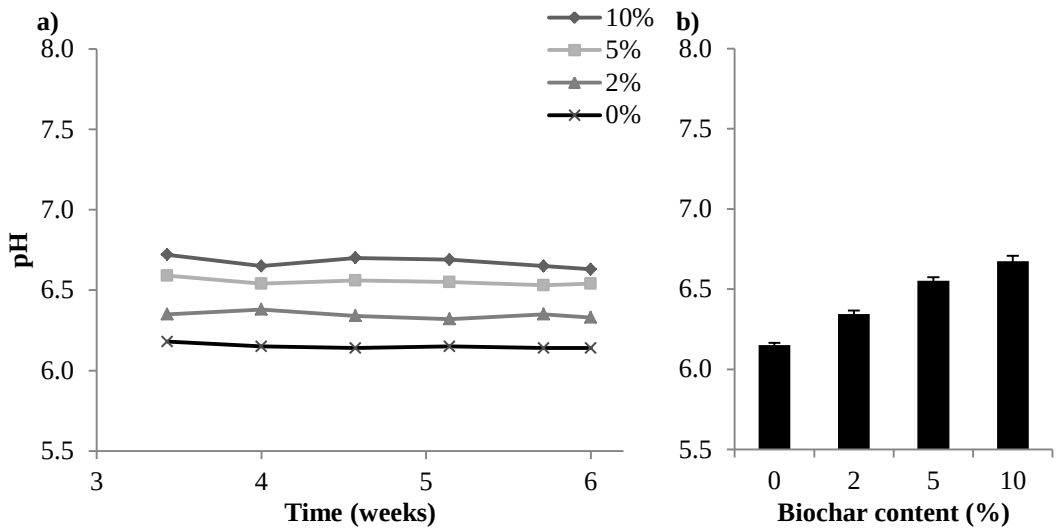

Figure 5.13: *Leachate pH data for biochar-amended and control samples.* ***a)*** *time series data for each biochar treatment,* ***b)*** *average pH for all treatments over the sampling period.*

The Eh values increased with increasing biochar content (Figure 5.14). BC10 samples had the highest average Eh (399 ± 1 mV) with the lowest values measured in the control (377 ± 2 mV). This suggests that the biochar addition increased the water holding capacity of the soil, which is commonly attributed to the porosity of the biochar material (Joseph et al., 2010).

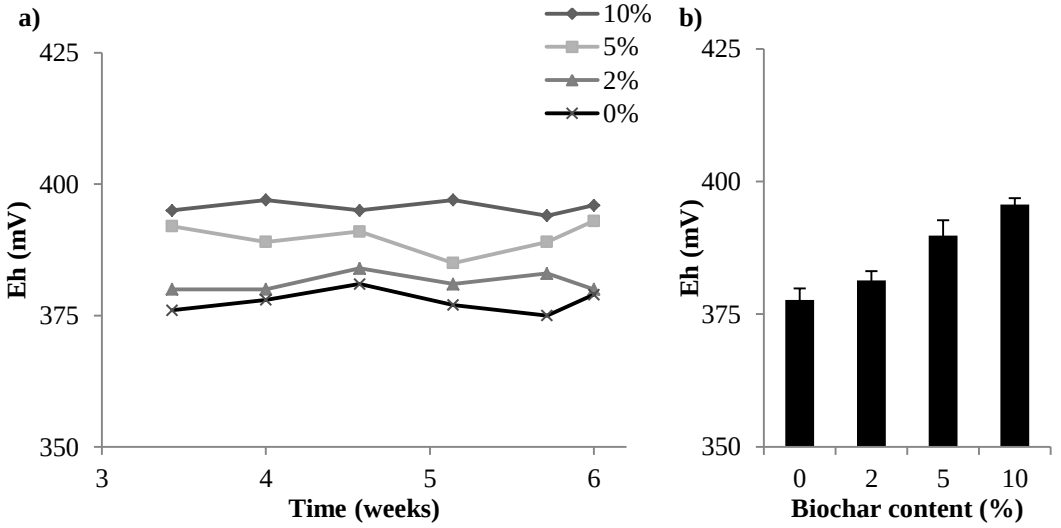

Figure 5.14: *Eh measured in leachate samples of biochar-amended and control soils.* ***a)*** *Time series data for each biochar treatment,* ***b)*** *average Eh for all treatments over the sampling period.*

5.4.3 Statistical analysis of solid phase and extract analyses

The soils were extruded from the mesocosms following 6 weeks of irrigation and analysed for the analytes and parameters shown in Figure 5.3. The data reported below are averages for each biochar application quantity (BC10, BC5, BC2 and BC0). Fresh biochar amended soils were also analysed in order to identify any significant changes.

One-way ANOVA was performed to determine whether the irrigation period had any significant effect on the characteristics of the four different biochar contents, to indicate the resilience of the biochar application in the short-term. Results from the one-way ANOVA are shown in Table 5.8. The TPN, TPC, TEN, NO_3^-, EON, Mg, Ca, and K displayed significantly different results following the 6 week irrigation period ($p < 0.05$, Table 5.8).

Table 5.8: *Results for one-way ANOVA to test for significant differences in analyte concentrations and parameters of biochar-amended soils before and after the 6 weeks of irrigation.*

Characteristic	p value
pH	0.110
TPN	0.003
TPC	0.002
C : N ratio	0.734
EOC	0.083
TEN	< 0.001
Extracted NO_3^-	< 0.001
EON	< 0.001
Extracted PO_4^{3-}	0.190
Extracted Mg	< 0.001
Extracted Ca	< 0.001
Extracted K	< 0.001
Extracted Fe	0.302
Sand fraction	0.637
Silt fraction	0.568
Clay fraction	0.239
SOC	0.768
CEC	0.121

Red = significant p values
Blue = insignificant p values

The one-way ANOVA was applied to compare the values for the 3 treatments and control before and after irrigation (Table 5.8). The test revealed that the different biochar applications had significantly different pH, TPN and TPC, C : N ratios, sand and silt particle size fractions, SOC and enzymatic activity. A number of characteristics did not change significantly in response to biochar content.

A Dunnett's test was performed in addition to ANOVA to determine whether data for each biochar application content were significantly different from the control (Table 5.8). This demonstrated that for all characteristics except pH, TPC, PO_4^{3-}, sand and silt, the BC2 sample did not differ significantly from the control. The content BC5 data were significantly different from the control for pH, TPC, PO_4^{3-}, Mg, Fe, sand and silt particle size fractions, and SOC. The BC10 soil data was significantly different from the control for the same characteristics as BC5 plus TPN, clay particle size fraction, moisture content and enzymatic activity.

Table 5.9: *Results for the one-way ANOVA showing differences between contents for a range of soil characteristics following 6 weeks of irrigation. Dunnett's test was used to confirm which treatments were significantly different from the control (✓indicates a significant difference from the control).*

Characteristic	ANOVA test	Dunnett's Test		
	p value	BC2	BC5	BC10
pH	0.024	✓	✓	✓
TPN	0.030			✓
TPC	0.016	✓	✓	✓
SOC	0.024		✓	✓
C : N ratio	0.016			✓
EOC	0.079			
TEN	0.168			
Extracted NO_3^-	0.204			
EON	0.223			
Extracted PO_4^{3-}	0.027	✓	✓	✓
Extracted Mg	0.080		✓	✓
Extracted Ca	0.935			
Extracted K	0.112			
Extracted Fe	0.069		✓	✓
Sand fraction	0.024	✓	✓	✓
Silt fraction	0.024	✓	✓	✓
Clay fraction	0.082			✓
Moisture content	0.041			✓
CEC	0.622			
Enzymatic activity	0.041			✓

Red = significant p values
Blue = non-significant p values

The average percentage change to each analysed characteristic following 6 weeks of irrigation was calculated for each biochar content sample (Table 5.9). Results demonstrated that most nutrients decreased in concentration during the irrigation period. These losses generally decreased with increasing biochar content.

Table 5.10: *Average percentage differences between the biochar-amended soils at t = 0, and following 6 weeks of irrigation. The positive values indicate and increase in concentration while negative values indicate a concentration decrease).*

Characteristic	Biochar content (%)			
	0	**2**	**5**	**10**
TPN (mg N g^{-1})	-6.87 ± 0.00	1.09 ± 0.00	-7.93 ± 0.00	-4.29 ± 0.00
TPC (mg C g^{-1})	-0.08 ± 0.00	9.13 ± 0.00	-6.36 ± 0.03	-13.7 ± 0.1
EOC (µg C g^{-1})	-35.2 ± 19.9	-20.6 ± 9.2	22.7 ± 3.2	-4.00 ± 0.63
TEN (µg N g^{-1})	-73.9 ± 19.9	-44.5 ± 9.2	-12.7 ± 3.4	-41.8 ± 19.9
Extracted NO_3^- (µg N g^{-1})	-72.0 ± 2.0	-55.1 ± 0.8	-29.0 ± 0.4	-55.2 ± 0.5
EON (µg N g^{-1})	-71.5 ± 3.8	-42.5 ± 6.8	-12.6 ± 2.5	-40.2 ± 14.0
Extracted PO_4^{3-} (µg P g^{-1})	-3.67 ± 0.36	-10.33 ± 5.4	-0.32 ± 0.55	-6.74 ± 6.21
Extracted K (µg K g^{-1})	-69.6 ± 14.0	-43.6 ± 1.35	-46.1 ± 10.6	-40.6 ± 15.5
Extracted Ca (µg Ca g^{-1})	-73.4 ± 14.1	-25.6 ± 2.2	-23.9 ± 1.5	-34.3 ± 5.2
Extracted Fe (µg Fe g^{-1})	-35.2 ± 0.1	-24.2 ± 0.0	42.0 ± 0.4	26.4 ± 0.0
Extracted Mg (µg Mg g^{-1})	-49.1 ± 0.5	-51.2 ± 1.0	-33.4 ± 0.1	-59.7 ± 0.2
pH	-0.95 ± 0.00	-3.25 ± 0.00	-3.73 ± 0.00	0.83 ± 0.00
CEC ($cmol_c$ kg soil $^{-1}$)	0.25 ± 0.00	-31.2 ± 0.0	5.34 ± 0.00	8.42 ± 0.00
C : N ratio	7.56 ± 0.01	8.02 ± 0.01	4.84 ± 0.01	-5.32 ± 0.05
SOC	-3.04 ± 0.00	0.22 ± 0.00	17.8 ± 0.1	-40.0 ± 1.2
Sand fraction	-4.07 ± 0.09	1.58 ± 0.02	5.94 ± 0.04	12.0 ± 0.1
Silt fraction	6.43 ± -0.09	-2.72 ± 0.03	-11.3 ± 0.0	-22.9 ± 0.1
Clay fraction	-9.24 ± 0.00	20.8 ± 0.0	-4.57 ± 0.00	-23.0 ± 0.0

5.4.4 Solid phase analyses

5.4.4.1 Nitrogen

The TPN concentrations are shown in Figure 5.15. There was a small, but significant decrease in concentration following the irrigation period for the control (BC0), BC5 and BC10 contents ($p = 0.003$, Table 5.8). Statistical analyses indicated that biochar content had a significant effect on the TPN concentrations ($p = 0.030$, Table 5.9).

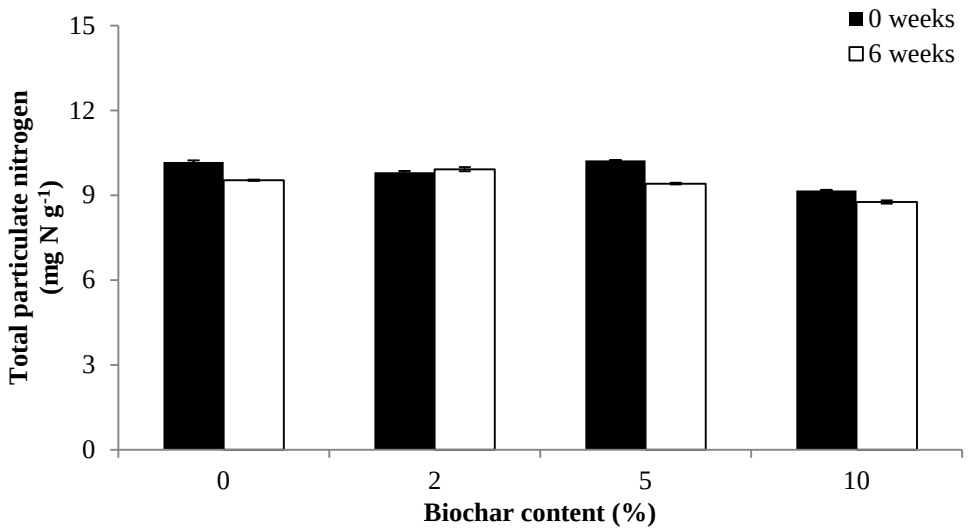

Figure 5.15: *TPN concentrations for each biochar content amendment at t = 0 and following 6 weeks of irrigation, (T_0 samples n = 3, for week 6 samples n = 9).*

5.4.4.2 Carbon

TPC concentration (Figure 5.16) increased with increasing biochar content, from 170 ± 1 mg C g^{-1} within the fresh control (0 %) sample to 401 ± 6 mg C g^{-1} within the freshly amended BC10 biochar samples. Following the 6 week irrigation period the largest reduction in TPC occurred in BC10, decreasing by 13.7 ± 0.1 % (Table 5.10). The BC2 and control (0 %) TPC concentrations increased following irrigation, by 9.13 ± 0.00 % and 0.08 ± 0.00 %, respectively. Statistical analyses indicated that biochar content and irrigation resulted in significant differences in TPC concentrations within the soil ($p < 0.016$ and $p = 0.002$ respectively, Tables 5.8 and 5.9).

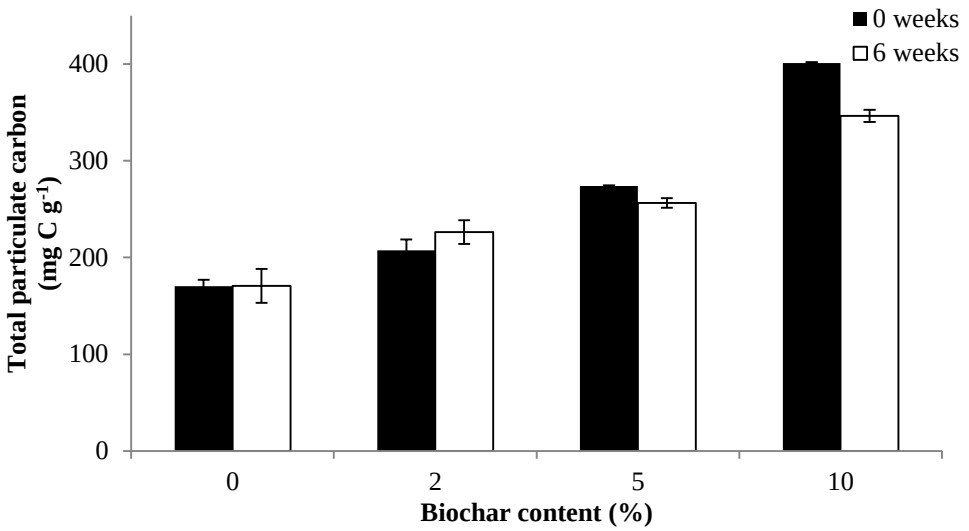

Figure 5.16: *TPC concentrations for each biochar amendment at t = 0 and following 6 weeks of irrigation, (T_0 samples n = 3, for week 6 samples n = 9).*

The SOC concentrations are shown in Figure 5.17. The BC10 content sample had the highest average concentration (232 ± 9.6 %), however, there was no significant difference between the BC10 and BC5 content samples. The biochar amended soils were significantly different from the control (0 %) values (p = 0.024, Table 5.9). Interestingly, there was no significant difference in SOC concentration as a result of the irrigation period (p > 0.05, Table 5.8).

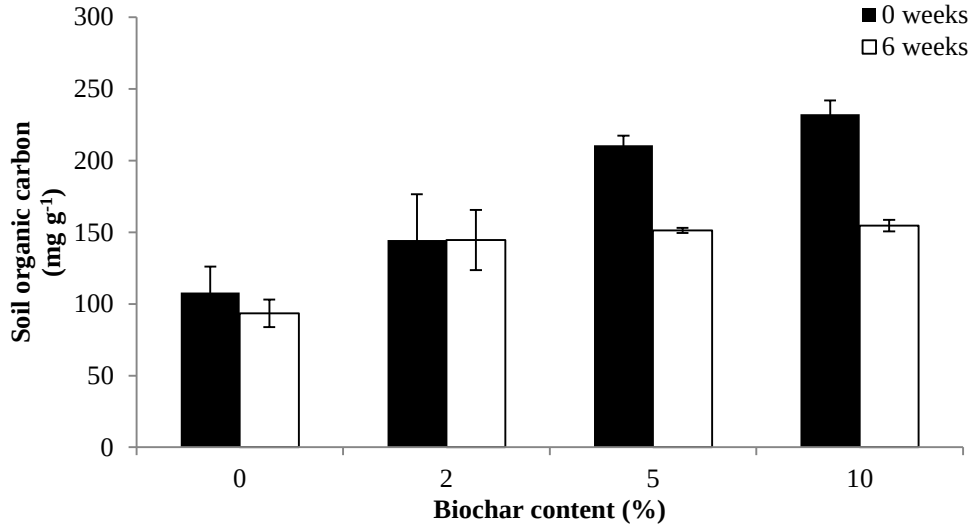

Figure 5.17: *SOC concentrations for each biochar amendment at t = 0 and following 6 weeks of irrigation, (T_0 samples n = 3, for week 6 samples n = 9).*

5.4.4.3 Carbon – nitrogen ratio

The C : N ratio increased with increasing biochar content (Figure 5.18). The highest ratio was 25.3 ± 1.8 in BC10 at T_0 and the lowest occurred in the freshly prepared control sample at T_0. There was no significant difference in C : N ratio after 6 weeks of irrigation ($p > 0.05$, Table 5.8), though a significant difference was observed between samples with different biochar content ($p < 0.016$, Table 5.9).

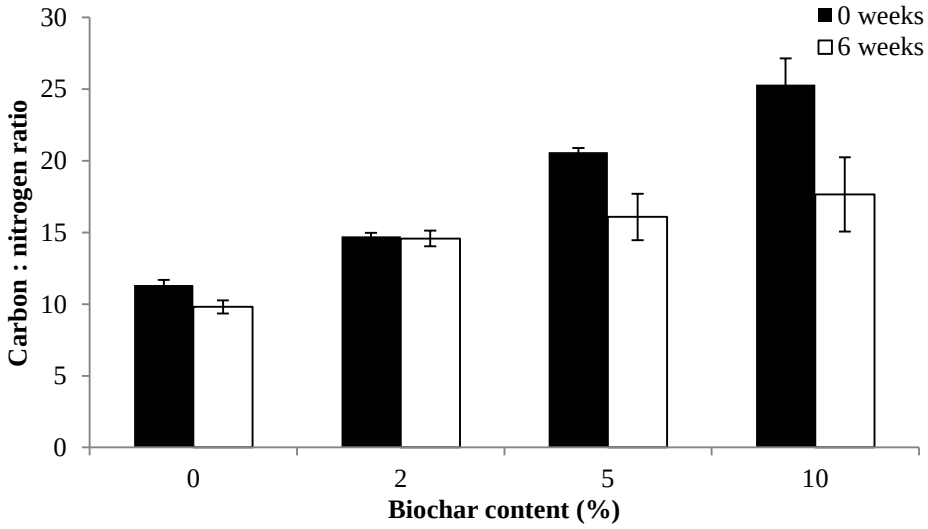

Figure 5.18: *C : N ratios for each biochar application, both for freshly amended samples and after 6 weeks of irrigation. The ration was calculated from soil organic carbon and total particulate nitrogen concentrations within the solid phase.*

5.4.4.4 Physicochemical characteristics

The pH increased with increasing biochar content (Figure 5.19). The highest pH occurred in BC10 samples (6.87 ± 0.13) and the lowest occurred in the control (0 %) sample (5.85 ± 0.13), both following the 6 week irrigation period. ANOVA analyses indicated a significant difference between the different biochar samples ($p < 0.001$, Table 5.9). There was a significant difference between the freshly amended samples and following 6 weeks of irrigation ($p = 0.024$, Table 5.8).

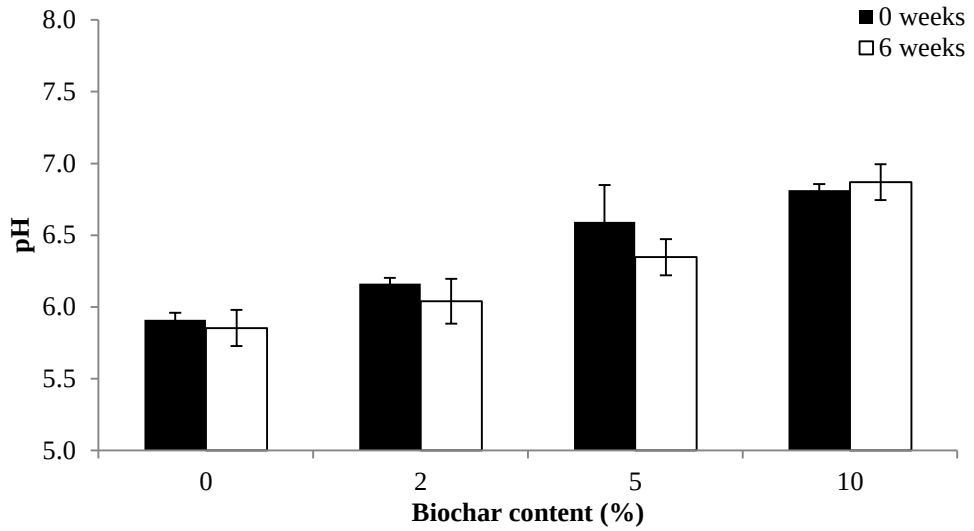

Figure 5.19: *pH values for each biochar application, both as freshly amended samples and following 6 weeks of irrigation. The mean values and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

The moisture content of samples increased with increasing biochar content (Figure 5.20). BC10 content had the highest value (21.4 ± 0.22 %) and the control sample the lowest (13.0 ± 0.3 %). The difference between treatments was statistically significant ($p = 0.041$, Table 5.9).

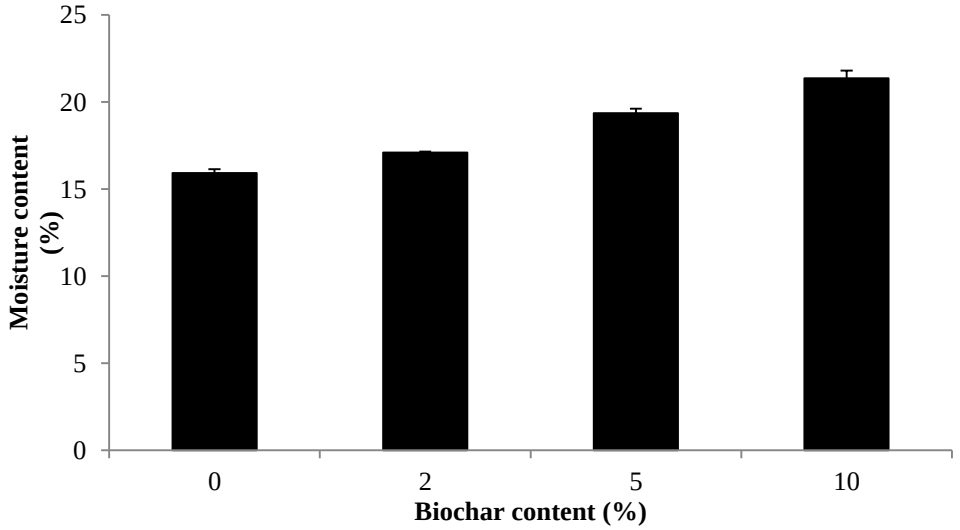

Figure 5.20: *Moisture content values for each biochar application, following 6 weeks of irrigation. The mean values and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

The particle size fraction data for each sample are shown in Figure 5.21. The largest sand sized fraction was observed in the BC10 samples for the freshly amended soil (T_0; 65.6 %) and the lowest occurred within the control sample following 6 weeks of irrigation. Whilst the sand fraction increased with increasing biochar content, the silt fraction concurrently decreased significantly ($p = 0.024$, Table 5.9). This suggests that the biochar particles were within the sand sized fraction (63 to 2000 µm). The average values suggest a decline in the sand size fraction over the irrigation period for all samples, though this was not statistically significant ($p > 0.05$, Table 5.8).

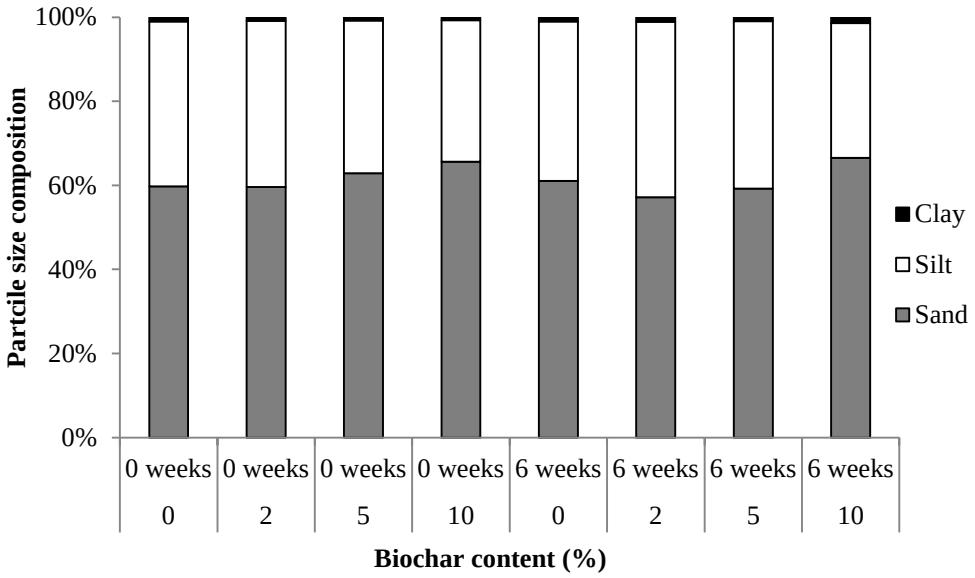

Figure 5.21: *Soil particle size distribution for each biochar application, both as freshly amended samples and following 6 weeks of irrigation. The mean values and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

The CEC for each biochar application is shown in Figure 5.22. The biochar content and 6 week irrigation period had no significant effect on the CEC ($p > 0.05$ for both, Tables 5.8 and 5.9), with the values ranging from 4.38 ± 1.70 to 6.03 ± 1.22 $cmol_c$ kg $soil^{-1}$.

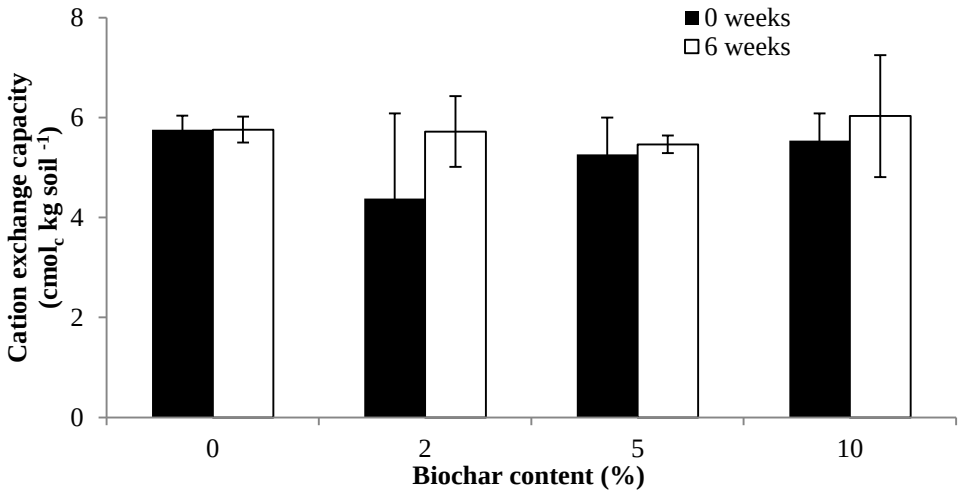

Figure 5.22: *CEC for each biochar application, both as freshly amended samples and following 6 weeks of irrigation. Mean and standard deviation calculated from 3 replicates analysed in triplicate (n = 9).*

5.4.4.5 Enzymatic activity

Enzymatic activity (Figure 5.23) was highest in the 6 week irrigated BC10 samples (46.1 ± 0.49 µg Fl g^{-1} hr^{-1}) and lowest in the control samples (14.7 ± 6.0 µg Fl g^{-1} hr^{-1}). The one-way ANOVA determined that biochar had a significant impact on the enzymatic activity of the samples (p = 0.041, Table 5.9). However, the Dunnett's test (Table 5.9) further indicated that only the BC10 sample had a significantly different enzymatic activity value to that of the control (BC0).

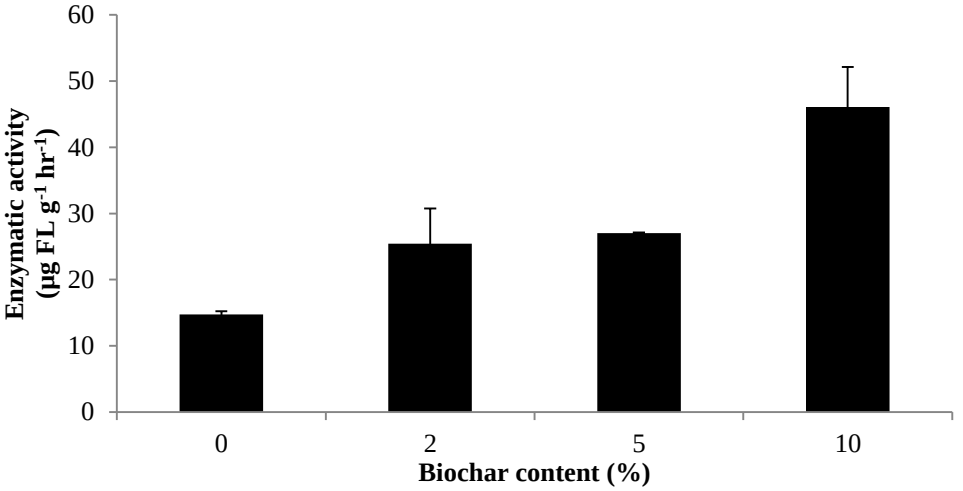

Figure 5.23: *Enzymatic activity values for each biochar application, following 6 weeks of irrigation. The mean and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

5.4.5 Extracted constituent analyses

5.4.5.1 Nitrogen

Results from the N fraction analyses are displayed in Figure 5.24. The non-extractable N fraction represented 98.0 to 99.2 % of the TPN present, alluding to a substantial unavailable N fraction within the samples. The TEN concentrations (Figure 5.24a) were significantly reduced for all treatments over the 6 week irrigation period ($p < 0.001$, Table 5.8). However, there were no significant differences between the different biochar contents ($p > 0.05$, Table 5.9). NH_4^+ concentrations were below the LOD for BC10 and BC5 within the freshly amended soils and 32.3 ± 5.6 and 46.9 ± 5.9 μg N g^{-1} in BC2 and the control, respectively. NH_4^+ concentrations were consistently below the limit of detection for all samples following 6 weeks of irrigation.

The highest TEN concentration was measured in the fresh BC0 sample (233 ± 12 μg N g^{-1}) and the lowest in the control following the 6 week irrigation period (83.8 ± 38 μg N g^{-1}). This suggests that whilst the control had the highest TEN concentration within the T_0 samples, it was subject to the largest decrease in concentration (73.9 ± 19.9 %; Table 5.10). The biochar amended samples had a much lower percentage decrease after the 6 week irrigation period, with the smallest loss occurring in sample BC5 (12.7 ± 3.4 %).

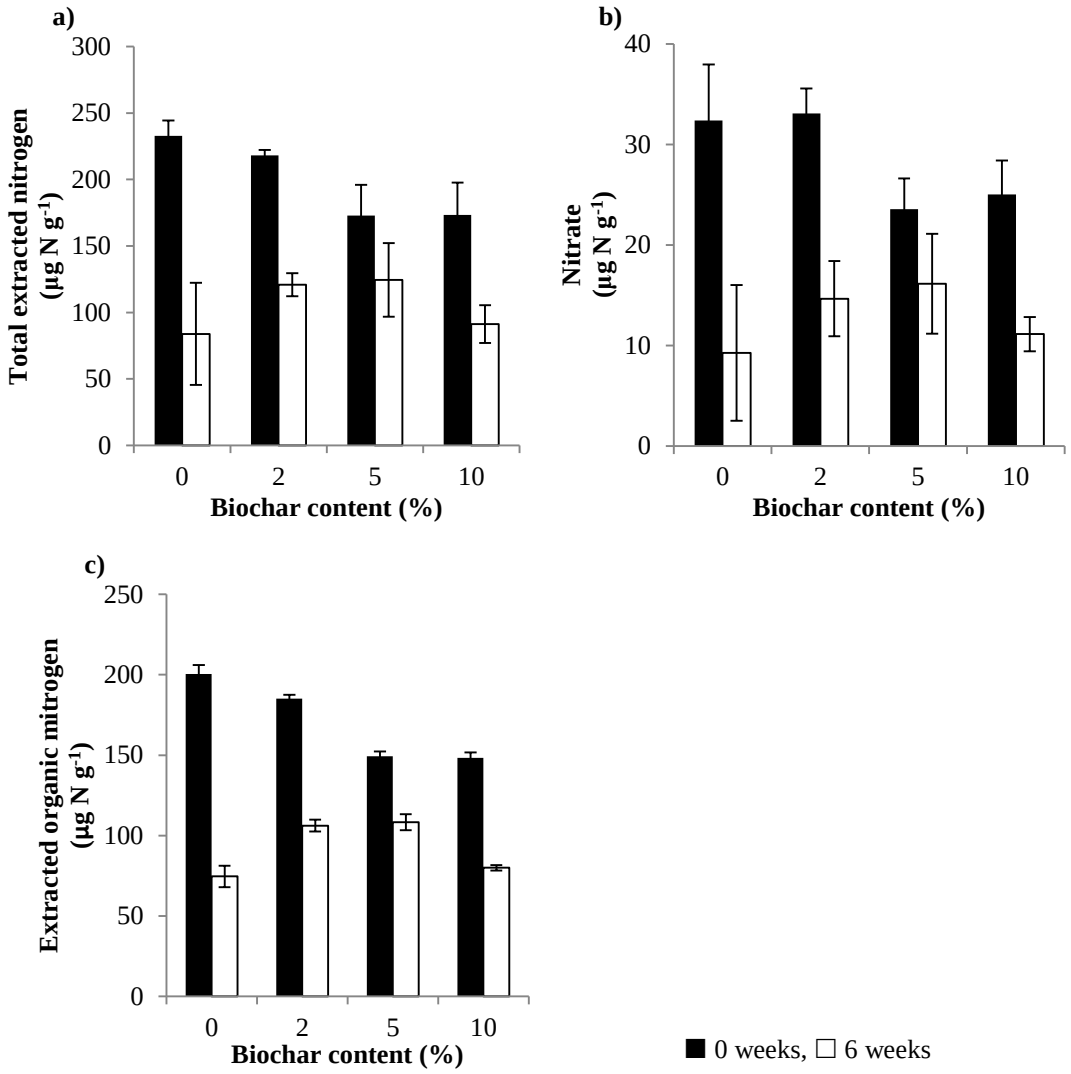

Figure 5.24: *N fraction concentrations for each biochar content. The means and standard deviations were calculated from 3 replicates analysed in triplicate.* ***a)*** *TEN (µg N g^{-1}).* ***b)*** *NO_3^-(µg N g^{-1}).* ***c)*** *EON (µg N g^{-1}).*

The NO_3^- concentrations are shown in Figure 5.24b. The trends were similar to the TEN data, with all concentrations decreasing significantly following irrigation ($p < 0.001$, Table 5.8). Statistical analyses indicated no significant difference between the treatments following the irrigation period ($p > 0.05$, Table 5.9). The highest NO_3^- concentrations were measured in the BC2 samples pre-irrigation (33.1 ± 2.5 µg N g^{-1}, respectively). The lowest concentrations occurred in the post-irrigation control (BC0) samples (9.27 ± 6.8 µg N g^{-1}). As for TEN, the largest percentage decrease in NO_3^- post-

irrigation was measured in the control sample (72.0 ± 2.0 %, Table 5.9), while the smallest decrease occurred in the BC5 samples (29.0 ± 0.4 %, Table 5.10).

The EON concentrations (Figure 5.24c) reflected the same trend as the other extracted N fractions; with the control sample showing the largest percentage decline in concentration (71.5 ± 3.8 %, Table 5.10) and the BC5 samples the smallest (29.0 ± 0.4 %). Irrigation had a significant impact on the EON concentrations within the samples ($p < 0.001$, Table 5.8). There was no significant difference between the biochar samples following the 6 week irrigation period ($p > 0.05$, Table 5.9).

5.4.5.2 Phosphate

The PO_4^{3-} concentration for each biochar content is shown in Figure 5.25. Statistical analyses indicated that the data for all biochar contents was significantly different from the control (BC0) sample ($p = 0.027$, Table 5.9). The highest PO_4^{3-} concentration was observed in the pre-irrigation BC10 sample (199 ± 25 μg P g^{-1}) and the lowest concentration within the control (BC0) sample post-irrigation (118 ± 5 μg P g^{-1}). Irrigation caused a small decline in concentration for all samples, which was not significant ($p > 0.05$, Table 5.8).

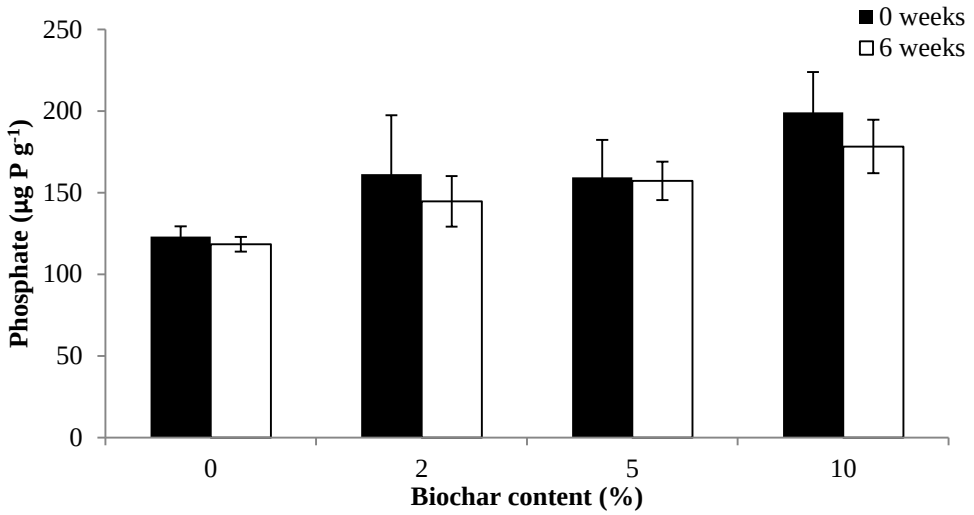

Figure 5.25: *PO_4^{3-} concentrations for each biochar sample. The means and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

5.4.5.3 Carbon

The EOC concentration for each biochar application is shown in Figure 5.26. The highest and lowest EOC concentrations were measured in the pre- and post-irrigation control (BC0) samples (255 ± 21 and 170 ± 7 µg C g^{-1}, respectively). Overall there were no significant differences between each treatment or as a result of irrigation ($p > 0.05$, Table 5.8 and 5.9).

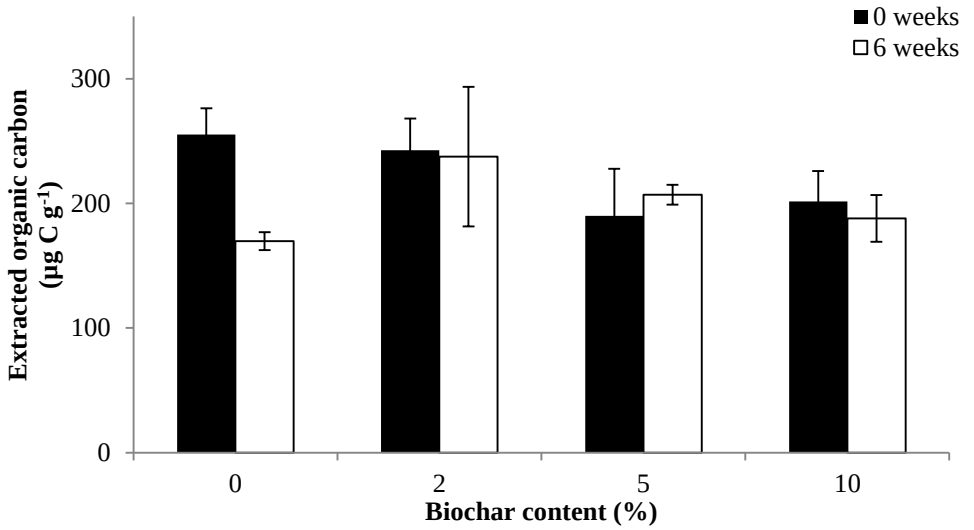

Figure 5.26: *EOC concentrations for each biochar sample. The means and standard deviations were calculated from 3 replicates analysed in triplicate (n = 9).*

5.4.5.4 Metals

Extracted Mg concentrations (Figure 5.27a) were highest in the pre-irrigation control sample (31.0 ± 3.4 µg g^{-1}) and lowest in irrigated BC10 sample (4.18 ± 1.43 µg g^{-1}). Results display an overall decline with increasing biochar content. The irrigation period had a significant impact on the Mg concentrations ($p < 0.001$, Table 5.8) and following the 6 weeks of irrigation there was no longer any significant difference between biochar contents ($p = 0.08$, Table 5.9). The Dunnett's test suggested that the BC10 and BC5 sample concentrations were significantly different to the control.

Extracted Ca concentrations are shown in Figure 2.27b and demonstrate variability between contents in the pre-irrigation samples, with the control concentration being substantially higher (92.4 ± 0.00 µg g^{-1}) than the BC10, BC5 or BC2 samples (58.5 ± 0.2 to 66.8 ± 10.5 µg g^{-1}). Following irrigation there was no significant differences observed between the contents ($p > 0.05$, Table 5.9); however, there was a significant concentration decline in extracted Ca for all treatments ($p < 0.001$, Table 5.8). The largest percentage decline was observed in the control sample (73.4 ± 14.1 %) and the smallest within the 5 % sample (23.9 ± 1.5 %).

The extracted K concentrations, shown in Figure 2.27c, were not significantly different between samples ($p > 0.05$, Table 5.9), though, a significant decline was measured following the irrigation period ($p < 0.001$, Table 5.8). The largest percentage decrease occurred in the control sample (69.6 ± 14 %), with little variation between biochar-containing samples (40.6 ± 15.5 % to 46.1 ± 10.6 %).

The extracted Fe concentration (Figure 5.27d) was highest in the pre-irrigation control samples (8.88 ± 2.39 µg g^{-1}) and lowest in the pre-irrigation BC10 samples

(2.89 ± 0.47 µg g^{-1}). In general, the extracted Fe concentration decreased with increasing biochar content, though, no significant difference between treatments was observed ($p > 0.05$, Table 5.9). The behaviour of Fe was variable; as concentrations increased following irrigation in the BC5 and BC10 and samples and decreased in the BC2 and control (BC0) samples. Irrigation had no significant effect on the results ($p > 0.05$, Table 5.8); however, the largest percentage decline in concentration was observed in the control samples ($35.2 \pm .0.4$ %).

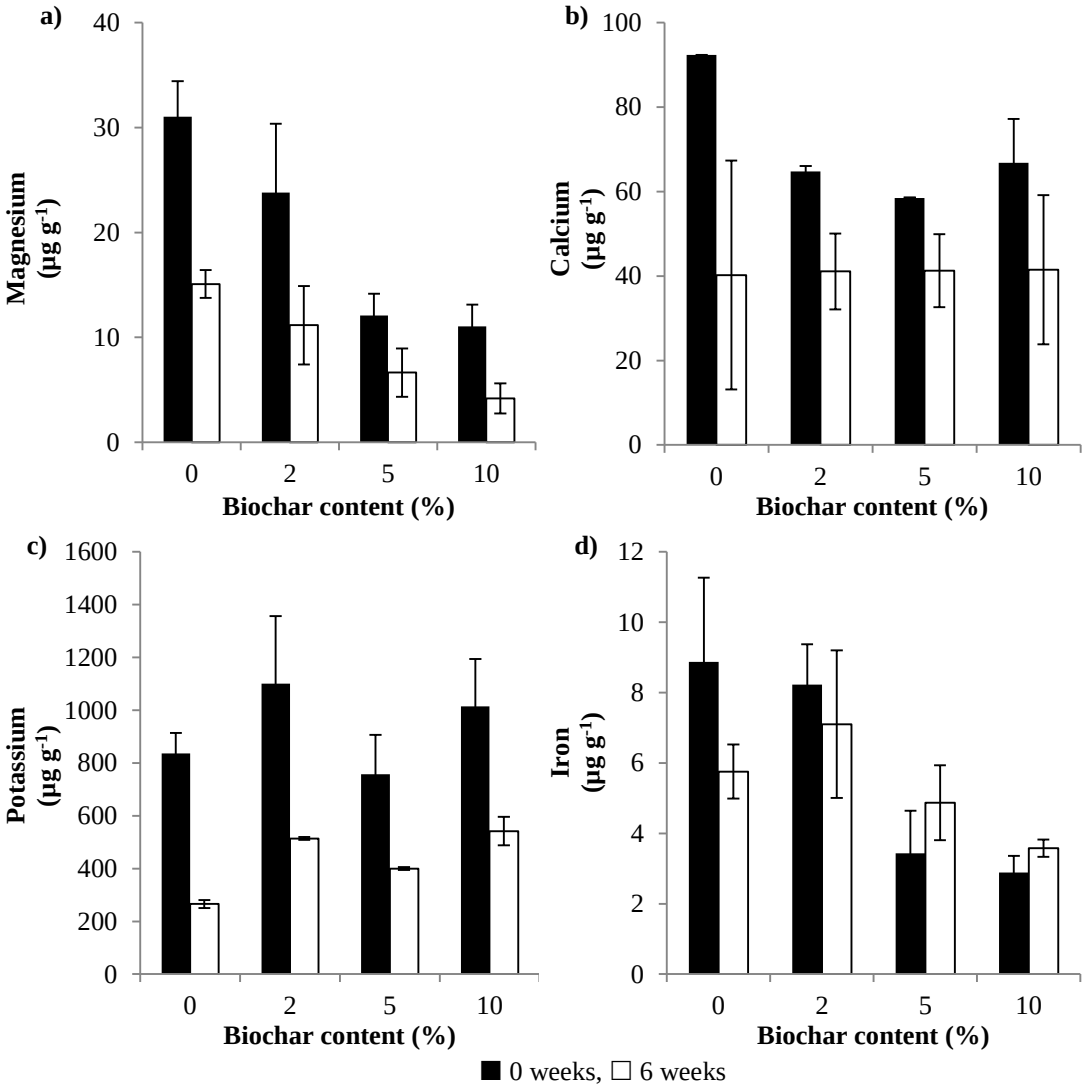

Figure 5.27: *Extracted ions for each biochar application for freshly amended samples and following 6 weeks of irrigation. Mean and standard deviation calculated from 3 replicates analysed in triplicate (n = 9).* ***a)*** *Mg,* ***b)*** *Ca,* ***c)*** *K and* ***d)*** *Fe.*

5.5 Discussion

This discussion will use the data described in Section 5.3 to further examine the potential for biochar application to improve the nutrient retention and storage capabilities of the artificial soils at the Eden Project, and more generally.

Many of the observations encountered within the results section are consistent with those reported for other biochar studies (Anderson et al., 2011; Downie et al., 2010; Manyà, 2012; Spokas et al., 2009). Nutrient concentrations within the leachate collected from the mesocosms were, with the exception of DON and Fe, lower in the biochar amended soils than in the control sample, demonstrating that biochar serves to retain nutrients within the soils, through increasing the surface area of the soil mix and therefore the number of nutrient binding sites, though, no significant difference in CEC was observed between the amended and control samples.

5.5.1 Nitrogen

The mass balance calculations for N within the biochar amended soils (Tables 5.7 and 5.11) demonstrate that, in general, biochar application reduced the total N loss (BC0 0.89 mg N g^{-1}, BC2 0.46 mg N g^{-1}, BC5 0.36 mg N g^{-1} and BC10 0.64 mg N g^{-1}, Table 5.11). The unaccounted for N loss from the control sample over the 6 week irrigation period (0.72 mg g^{-1}) was equal to that of the F columns and greater than that of the UF columns (0.42 mg g^{-1}) over 52 weeks (Table 4.14, Chapter 4). This suggests that there was a greater amount of N lost through means other than leaching within the soil mix for this study than the column study. Biochar treatment significantly decreased the leached N concentration within the soils the extent of which was consistent across all treatments (BC0 0.17 mg N g^{-1} and BC2, BC5 and BC10 0.10 to 0.11 mg N g^{-1}, Table

5.11). The proportion of N loss represented by leaching was variable across the treatments (BC0 19.2 %, BC2 22.7 %, BC5 29.1 % and BC10 16.5 %, Table 5.11), however represented a smaller percentage than found within UF columns (41.4 %, Table 4.14, Chapter 4). This suggests that biochar treatment may have had some impact upon the N concentrations within the leachate, though this is not consistent across all treatments.

Table 5.11: *Nitrogen mass balance estimated from the solid and leachate values from 11this experiment making the assumption that the mesocosm masses remained unchanged throughout the experimental period.*

		Biochar content (%)			
		0	**2**	**5**	**10**
TPN in mesocosm (mg)	Time 0	5238	5257	4894	3555
	6 weeks	4784	5012	4721	3314
Total N lost from mesocosm (mg N g^{-1})		0.89	0.46	0.36	0.64
Total N lost in leachate (mg N g^{-1})		0.17	0.10	0.11	0.11
% N loss from mesocosm		8.68	4.65	3.53	6.77
% N loss represented by leaching		19.2	22.7	29.1	16.5
Unaccounted N loss (mg N g^{-1})		0.72	0.35	0.26	0.53

Whilst inorganic N concentrations within the leachate decreased with increasing biochar content, the DON concentrations increased. On a mass basis, the BC2 application demonstrated the lowest percentage N loss through leaching and the BC5 application the lowest overall N loss. Unaccounted (non-leached) N losses from the mesocosms were observed to be lower in biochar amended soils than the control. However, this effect did not increase with biochar application rate, with the BC2 application demonstrating the lowest non-leached N loss. It has been reported that NO_3^- concentration within the leachate may be reduced as a result of more easily degradable C presence within biochar produced through lower temperature pyrolysis, which leads to greater N immobilisation, thus reducing NO_3^- leaching (Clough et al., 2013),

however, lower NO_3^- concentrations within the leachate may also be attributed to loss of NO_3^- through denitrification stimulated by the additional C (Clough et al., 2013).

Leached NH_4^+ concentrations were below the LOD (26.8 µg N L^{-1}, Table 2.9, Chapter 2) in the BC5 and BC10 samples. This is consistent with reported behaviours within biochar amended soils (Clough et al., 2013; Schomberg et al., 2012), which is inferred to be attributable to increased CEC, however, there were found to be no significant CEC differences between treatments, Saleh et al. (2012) suggest that physical entrapment within the biochar pore structures may be responsible for the reduction in leached NH_4^+. The uncertainty surrounding the understanding of these mechanisms highlights the requirement to advance the understanding of mechanisms responsible for the adsorption of N forms onto biochar surfaces.

5.5.2 Phosphate

The PO_4^{3-} mass balance (Table 5.12) demonstrates that whilst the total leached PO_4^{3-} decreased within increasing biochar content, when considered on a mass basis, biochar content was observed to have a varied impact on upon the leached PO_4^{3-} losses. The effects of biochar application on P availability have been noted to be inconsistent across differing soils, treatments and timescales (Nelson et al., 2011). The inconsistency of the effect of biochar application on P availability is reported by Xu et al. (2014). DeLuca et al. (2010) explore the mechanisms by which biochar may directly and indirectly influence the P cycle, either as (1) a direct source of soluble P salts and exchangeable P. Biochar contains a large amount of P and thus may serve as a direct source of P. (2) Biochar acts as a modifier of soil pH and ameliorator of P complexing metals (Al^{2+}, $Fe^{3+/2+}$ and Ca^{2+}), which has significant implications for the sorption and desorption

reactions in soils. (3) Biochar acts as a promoter of microbial activity which therefore impacts P mineralisation.

Table 5.12: *Phosphate mass balance estimated from the solid and leachate values from 11this experiment making the assumption that the mesocosm masses remained unchanged throughout the experimental period.*

		Biochar content (%)			
		0	**2**	**5**	**10**
Total water extracted PO_4^{3-} (g)	Time 0	62.6	89.5	76.3	70.0
	6 weeks	60.3	84.2	75.2	67.4
PO_4^{3-} lost from mesocosm (mg P g^{-1})		4.61	9.81	2.15	6.91
PO_4^{3-} lost in leachate (mg P g^{-1})		0.40	0.18	0.18	0.28
% P loss from mesocosm		3.75	5.84	1.35	3.73
% P loss represented by leaching		8.59	1.88	8.54	4.06
Unaccounted P loss (mg g^{-1})		4.22	9.63	1.96	6.63

5.5.3 Carbon

As anticipated, biochar application increased the C content of the soil. However, observations demonstrate that over the 6 week irrigation period significant concentrations of SOC were lost from the BC5 and BC10 mesocosms and TPC from the BC0, BC5 and BC10 mesocosms. The source of the SOC loss may be the decomposition of the composted green waste or bark components of the soil mix, or potentially the degradation of the biochar. Results reported within Chapter 4 suggested that the decomposition of organic matter components within the soil mix may have contributed to N immobilisation within the soil over 27 weeks, during which time N concentrations within the leachate were low. In order to explore the potential source of C loss within the soil a C mass balance was calculated for the study (Table 5.13).

Whilst this investigation employed the same soil mix as studied in Chapter 4, the results from this investigation suggest that the C content within the BC0 and BC2 remained relatively unchanged (+ 0.2 g within BC0 and + 11 g within BC2) over the 6 week

experimental period. This suggests that the decomposition of the organic material within the soil mix was had a lesser impact upon the decrease in TPC and SOC within the samples, further suggesting that C may have been leached through biochar degradation.

Table 5.13: *Carbon mass balance estimated from the solid and leachate values from 11this experiment making the assumption that the mesocosm masses remained unchanged throughout the experimental period.*

		Biochar content (%)			
		0	**2**	**5**	**10**
TPC in mesocosm (g)	Time 0	86.7	110	131	152
	6 weeks	86.9	121	123	131
Total C lost from mesocosm (mg C g^{-1})		/	/	17.6	54.7
Total C lost in leachate (g)		0.39	0.21	0.25	0.36
SOC lost from mesocosm (g)		4.03	/	21.2	49.3
% C loss from mesocosm		/	/	6.44	13.6
% of C loss represented by leaching		/	/	1.40	0.66
Unaccounted C loss (mg g^{-1})		/	/	17.4	54.4

Although biochar C is generally viewed as a stable form of C, there is evidence of biochar degradation in soils (Cheng et al., 2008; Nelson et al., 2011). The high C : N ratio of biochar means that biochar degradation would result in N immobilisation and has been cited as an explanation for reduced N uptake and visual N deficiencies in some biochar-amended soils (Cheng et al., 2008; Lehmann et al., 2003).

The impact of biochar upon the metal concentrations was variable. Biochar application was observed to decrease potassium leaching from the soil, though the biochar application quantity had variable effect. Ca and Mg decreased with increasing biochar application quantity and Fe was highly variable with no significant difference between the control and biochar amended samples.

5.5.4 Physicochemical characteristics

Biochar application significantly reduced the leachate volume with increasing biochar content, from which it may be inferred that biochar increased the soil water holding capacity. Increased water holding capacity further explains the reduction in leached nutrient concentrations with increasing biochar content.

The pH of both the leachate and the solid samples increased significantly with increasing biochar content, suggested to have been caused by metal oxides present within the biochar as a result of the pyrolysis process.

A key characteristic of Eden Project soils, revealed during the solid column experiments (Chapter 4), was the significant loss of nutrients via leaching from the soil. This was attributed to the consistently large sand, and small clay fractions within the soil samples, resulting in a smaller soil surface area and so fewer charged particle surface sites to which nutrients may bind. The biochar applications increased the sand sized fraction within the soil, but the large surface area, which is typical of biochar particles (Lehmann and Joseph, 2010; Mukherjee et al., 2011), likely resulted in an increase in the number of available sites to which nutrients could attach, so reducing loss to leaching.

Biochar application to soils is reported to increase the soil CEC (Major et al., 2010), with the biochar particles having a negative surface charge caused by carboxylate groups on the surface of the biochar itself, and exposed carboxylate groups of organic acids sorbed by the biochar (Cheng et al., 2006; Novak et al., 2009). Whilst CEC did not change significantly in the amended samples ($p > 0.05$, Table 5.9), Cheng et al. (2006) observed that CEC was greater in *aged* rather than *fresh* biochar.

The pH of both the leachate and the solid samples increased significantly with increasing biochar content. This may have been caused by metals (primarily K^+, Ca, Si and Mg), present within the feedstock, forming metal oxides during the pyrolysis process (Novak et al., 2009; Steiner et al., 2007). Once present within the soil environment, these oxides trigger the release H^+ and Al^{3+} species into solution, through exchange, altering the soil pH (Novak et al., 2009; Sparks, 2003). The application of biochar to the Eden Project soil resulted in a pH close to 7, which is the optimum pH for availability of many important soil nutrients to plants (Figure 1.2).

5.5.5 Enzymatic activity

Enzymatic activity was increased following biochar application, which suggests that biochar encourages the biological community, with potentially significant implications for the breakdown of SOM and nutrient cycling within the soils.

Biochar has been shown to change the composition and abundance of the biological community within a soil and, as such, may have an indirect impact on plant growth (Lehmann et al., 2011). Changes in the microbial community composition, or increases in activity (Figure 5.23) induced by biochar application, may further affect nutrient cycling, plant growth and the cycling of SOC (Lehmann et al., 2011; Wardle et al., 1998).

It is suggested that the application of biochar with high levels of volatile organic compounds (VOCs) may cause the inhibition of *Nitrosomonas* bacteria, leading to decreased nitrification within the soil (Nelson et al., 2011). This resulted in increased NH_4^+ concentrations within the soil; however, this was not evident within the results from this investigation.

5.5.6 Synthesis

The impact on the analyte concentrations within the leachate, solid and extractable phases were unclear, with some of the analysed characteristics displaying no significant relationship between biochar content and nutrient retention. Causes of this variation may be either that the biochar has an effect on selected nutrients within the soil, retaining some nutrients whilst encouraging the leaching of others or the biochar itself serving as a source of nutrients and thereby directly contributing to the available nutrient pool (Nelson et al., 2011).

Biochar application significantly reduced the leachate volume, which decreased with increasing biochar content, implying that biochar increased the soil water holding capacity. This was consistent with the increasing moisture content measured in biochar-amended samples. The increased water retention in the biochar-amended soils may be related to altered percolation patterns, residence time and flow pathways, brought about by changes in the soil surface area, bulk density, porosity, pore size distribution and aggregation of soil (Manyà, 2012; Thies and Rillig, 2009; Zheng et al., 2013). The reduction in nutrient leaching may be attributed to this increased water holding capacity, leading to a slower percolation velocity, and increased residence time for nutrients at the soil surface. A further advantage of increased water holding capacity is a reduced irrigation volume requirement.

As results from the soil column (Chapter 4) experiments indicated, the leachate initially contained high concentrations of TDN which gradually decreased over the first 20 weeks. Although the time scale for this experiment was considerably shorter, nutrient concentrations in the leachate and solid samples were comparable to those measured in

the soil columns (Chapter 4) and certain soil properties changed significantly over the experimental period.

The data from these amendments provided an important insight into the behaviour of a biochar amended soil. It provided little indication of the long term performance, though, Major et al. (2010) reported that a single biochar application improved crop yield for at least 4 years following application. This suggests that, whilst improvements to soil properties and yield might not be permanent, they provide improvements in soil quality over a longer period than fertiliser or mulch applications have demonstrated (Chapter 4). As such, biochar offers a less labour intensive, more cost effective approach to maintaining the nutrient concentrations within the Eden Project soils.

Prior to any large scale biochar application at the Eden Project site, a number of issues require further investigation in order to fully utilise the benefits available. The biochar used for this study was produced from a mixture of plant residue material of varying size and type, collected on site. To avoid differing and unpredictable biochar properties, this material would require homogenising prior to pyrolysis to ensure effective implementation across the Eden Project site. Alternatively, the biochar could be processed to optimise the particle size. A more detailed study to determine the most effective feedstock composition would be advisable. The pyrolysis method used for this study had a low yield, averaging 22.2 ± 0.1 %. A number of studies have reported higher yields, whilst maintaining the beneficial properties of the biochar (Table 5.1). This suggests that it may be beneficial to develop pyrolysis procedure in order to optimise yield and biochar properties.

Whilst care was taken, variation in the packing of the mesocosm may have led to variations in the flow patterns between samples, however, the use of replicate samples reduced this uncertainty.

This investigation focused upon providing a detailed insight into the effects of biochar application on the properties of artificial soils. Typically biochar would be applied to the organic horizon within the soil, where it would have the greatest impact on nutrient availability to plants through the root zone. Observing the effects of biochar application on plant growth would provide further means of determining the extent to which biochar application may improve nutrient availability.

5.6 Conclusion

In general biochar application appears to have decreased the total N and P loss within the soils, however, the effect of this did not increase with biochar content. The representative proportion of N and P loss to leaching was decreased by biochar application, though results were also found to be variable with application quantity.

Biochar application led to an increase in nutrient retention within the artificial soil. The increased nutrient retention observed within the biochar amended samples, demonstrated by the leached nutrient load values (Table 5.7), could be attributed to the increased water holding capacity, which reduced nutrient leaching within the soil, increasing nutrient residence time and availability to plant roots. The pH was most likely altered by the introduction of metal oxides into the soil mixture, which caused an increase in soil pH. The biochar application served to increase the soil pH to close to 7 (neutral), which would improve availability of nutrients to plant roots within the soil.

With appropriate management the application of biochar at the Eden Project site could lead to greater resource efficiency, which would serve to reduce fertiliser requirement and increase run-off water quality. However, further investigations to determine the optimal combination of feedstock and pyrolysis conditions would be highly advisable, together with small scale application trials within the Biomes to determine whether the beneficial outcomes determined by this laboratory-based investigation may be extrapolated to the Eden Project Biomes under various plantings.

The data suggests that the BC10 biochar sample produced the greatest changes to soil properties in this investigation; however, this was not consistent across all nutrients with variable performance noted for N and P leachate concentrations. The 10 % application quantity also may be uneconomical, with regard to the amount of feedstock required to produce enough biochar for such an application rate. As such, smaller application of biochar may be more realistic across the site, with higher contents used selectively for areas with lowest quality soil performance.

CHAPTER 6

Conclusions and recommendations

6.1 Overview

The aim of the work presented in this thesis was to make recommendations for the production of a fertile artificial soil with a large reservoir of slow-release nutrients that meet the nutrient requirements of the plants at the Eden Project.

This chapter summarises the main findings of this thesis and presents the research conducted in context, making recommendations for the improvement of the Eden Project's artificial soil and suggesting directions for future research. The first 3 research objectives have been met through the experimental studies detailed within the preceding chapters and research objective 4 is addressed within Section 6.3 of this chapter.

6.2 Summary and conclusions

The Eden Project is a rare example of an established artificial soil. The variety of plant species, environmental conditions and management techniques mean that it is an ideal location to study a wide range of artificial soil processes that are relevant to other artificial soils. Whilst artificial soils have been referred to across a number of disciplines, it is rarely studied as the primary subject, commonly used as a substrate with consistent composition within toxicological studies.

This thesis represents one of the first systematic studies in artificial soils research and has taken a direct approach to their study, using a broad range of techniques to thoroughly examine their nutrient dynamics, with a view to improving their nutrient retention. The main findings and conclusions from this study are presented here with reference to the corresponding research objective.

6.2.1 To characterise the soils established at the Eden Project using appropriate analytical techniques.

The observations in Chapter 3 of this thesis demonstrate that the nutrient concentrations for a number of artificial soils sampled across the Humid Tropics and Outdoor Biomes at the Eden Project site were found to be highly variable with regard to soil characteristics, particularly nutrient concentrations. The variation between soils was observed to be influenced more greatly by management practices and age, than environmental conditions.

Within the established artificial soils, the greatest nutrient concentrations were observed within the upper 20 cm of the soil profiles, where it is suggested that the greater organic matter content served to regulate and retain nutrients. Particle size was generally large, with soil samples classified as sandy loam texture, resulting in lower soil surface area and the associated lowered nutrient retention capabilities, particularly lower down the profile where the organic matter content was lower.

The limited information regarding historic management practices across the Eden Project site has restricted the ability of this investigation to determine the full extent to which the variation in management practices has impacted the nutrient retention capabilities of the soil; however, it is clear that disconnected management has had a detrimental effect on the overall soil health across the site.

6.2.2 To construct and implement the use of soil column bioreactors to observe the performance of the currently artificial soil composition with regard to the cycling of key nutrients.

An artificial soil was produced from a mix of horticultural grit, lignite, bark and composted green waste, following the Eden Project protocol and packed into 4 columns. The columns were irrigated over a 52 week period with leachate collected from the base of each and analysed for key nutrients and physicochemical properties. After 28 weeks irrigation 2 of the 4 columns were fertilised with Vitax® 214. Following 52 weeks irrigation the columns were extruded and analysed for solid phase and extractable phase characteristics.

Inorganic N concentrations within the leachate quickly decreased and remained low for a significant period (approximately 26 weeks), following which significant quantities of both inorganic and organic N were released. This behaviour suggests that the N had previously been immobilised, with the increase in leached N a result of mineralisation. It has further been postulated that this immobilisation occurred as a result of the microbial decomposition of recalcitrant organic material within the soil mix.

Phosphate concentration within the leachate displayed an initial increase from which time it remained stable and high. The PO_4^{3-} is suggested to have been released through the decomposition of organic matter within the soil mix, with the stability of the PO_4^{3-} concentrations being cause by the equilibrium between the irrigation water and the soil. This therefore suggests that following the completion of organic matter decomposition within the soil would result in the decrease in PO_4^{3-} concentrations within the soil, leading to the requirement for supply through either fertiliser applications or further

organic matter additions to the soil. Other nutrient concentrations behaved much as anticipated within a closed system, demonstrating a decrease in leachate concentrations throughout the 52 week experimental period.

Solid samples demonstrated relatively uniform distribution of nutrients throughout the soil profiles with regard to depth, which was in contrast to what would be anticipated within natural soils and with the results from Chapter 3: Phase 2. Typically, natural or anthropogenic additions to the soil (e.g. litter, organic matter, and fertiliser) over time lead to the formation of soil horizons, which have distinct physical, chemical and biological characteristics. This was not unexpected within this investigation as there were only fertiliser applications to 2 of the 4 columns.

Fertiliser application resulted in significant differences in a range of leachate characteristics including all N fractions (except NH_4^+), PO_4^{3-}, Mg, Ca and pH. In the solid phase significant differences between the UF and F samples were observed in SOC, C : N ratio, TEN, Mg, Ca, Fe and enzymatic activity. It was observed that the fertiliser application had a greater effect on the concentrations of leached nutrients, than nutrients within the solid and extracted phases, which suggests that the effects of fertiliser application to the soils were short-lived.

6.2.3 To make controlled changes to the artificial soils determine how this affects the sustainability of the nutrient reservoir.

Biochar was employed as a means of determining the potential for improvement to the nutrient retention of the artificial soils. Biochar was identified for study owing to its manifold reported benefits following soil application and also due to the intention for the installation of a pyrolysis unit at the Eden Project site. Biochar was incorporated

into the soil at 3 concentrations (10 %, 5 % and 2 %), plus a control (0 %). The soil-biochar mixtures were packed into mesocosms, and were irrigated under controlled environmental conditions for 6 weeks.

In general, it was found that biochar application reduced the losses of key nutrients through leaching. However, the effect of nutrient leachate reduction was did not increase with increasing biochar content, particularly for N and P, where the 5 % application content demonstrated the lowest losses. Biochar application had no significant effect on the CEC of the soils, which suggests that in this instance the increased nutrient retention may be attributed to the increased water holding capacity rather than sorption of nutrients to biochar surfaces.

6.3 Recommendations

The fourth research objective for this project was *to make recommendations for the manufacture of a nutrient-rich soil that can be produced by utilising waste materials available in Cornwall and which has wide application for local projects.*

Whilst the effect of the following recommendations would require close monitoring, to ensure that plant available nutrient concentrations within the soil are sufficient, they may offer beneficial outcomes for the Eden Project from both an economic and environmental standpoint.

Recommendation 1- Discontinue the use of bark within the soil mix.

Results from Chapter 4 determined that, whilst promoting other beneficial soil properties relating to structure and nutrient retention, the inclusion of a significant bark component within the soil mix resulted in significant levels of nitrogen immobilisation

within the soil columns. Through the reducing the proportion of the high C : N ratio organic matter component, bark, within the soil mix and by instead increasing the proportion of the lower C : N ratio composted green waste, there will be a lower amount of N immobilisation occurring within the soil. This will lead to greater plant N availability within the soil thereby reducing the requirement for fertiliser supplement.

Recommendation 2 - Prepare soils 1 year in advance of use.

The artificial soil mix within the columns demonstrated a stabilisation period, during which time organic material was decomposed by the microbial population and nutrients were subsequently released in response to the microbial behaviour. Following the 52 weeks irrigation, nutrient concentrations (excepting N) within the leachate were determined to have stabilised, therefore, by allowing a 1 year stabilisation period between soil preparation and implementation, allowing the organic matter decomposition to take place and promoting the formation of a more stable humic material.

The combination of Recommendations 1 and 2 may mean that a stabilisation period of less than 52 weeks may be employed; however, further investigative work would confirm this and serve to optimize the process.

Recommendation 3 - Increased proportion of clay

The CEC of the soil was determined to be consistently low throughout the columns. This may be attributed to the low clay content of the soil. Therefore the use of a higher proportion of clay within the soil mix would serve to provide a greater number of charged exchange surfaces to which nutrients may bind, thereby increasing the nutrient retention capabilities of the soil.

One alternative to the use of a greater lignite clay component would be the use of clay balls, which may incorporated into the soil to serve as localised areas of high CEC, whilst their shape and size also serving to limit compaction within the soils.

Recommendation 4 – Lower overall particle size

Results from Chapters 3 and 4 demonstrate a consistently large particle size within the soils across the Eden Project site. Through decreasing the representative proportion of the sand fractions and in doing so, lowering the overall soil particle size would serve to increase the surface area of the soil. Increased surface area would provide a greater number of potential nutrient binding sites, aiding nutrient retention within the soil. This would further offer benefit through increasing the water holding capacity of the soil, thereby increasing the soil solution residence time, prolonging the time nutrients are within the plant root zone.

The use of a consistently sized sand fraction (e.g. 2.5 mm sand particles) in place of horticultural grit may aid porosity and aeration, whilst also resisting compaction as demonstrated in Figure 6.1. The use of such a strategy would also serve to encourage drainage through-out the soil profiles.

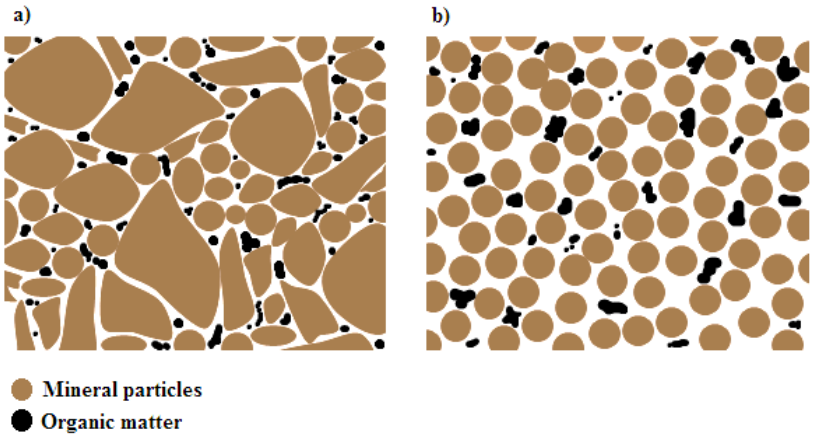

Figure 6.1: *a) Mixed particle sizes – fewer pore spaces, due to filling by organic matter and smaller sized particles. b) Consistently sized particles – more air-filled pore space.*

Recommendation 5 – Reduction of rock phosphate application to organic matter amended soils

The results from the column study outlined in Chapter 4, demonstrated that the freshly prepared artificial soil leached significant quantities of PO_4^{3-} during the column experiment as a result of organic matter decomposition. This observation has led the Eden Project team to reduce its applications of rock-phosphate, which has the potential to reduce the concentrations of PO_4^{3-} through leaching, whilst also serving to lower the cost and quantity of the finite material used.

Recommendation 6 – Reduction of irrigation rate

The nutrient concentrations determined within the leachate collected from the soil columns, demonstrate that is a significant pathway through which nutrients are lost from the Eden Project soils. The transport of nutrients, particularly P and N, leached from soils to water-bodies has potentially detrimental environmental effects (e.g. eutrophication). In this scenario P is more harmful than N, as the ratios required are 16:1 (N : P ratio). Whilst the Eden Project minimises the extent of this through collecting all leachate and runoff from its soils, treating and reusing it as irrigation water the potential for harm may be further circumvented through reconsidering the irrigation volume supplied to the Biomes. Through the design of targeted systems, where water is distributed according to specific plant demand, the quantity of nutrient loss through leaching may be further reduced and potentially detrimental environmental effects avoided.

6.4 Future research

The recommendations outlined in Section 6.3 have been tailored to address the low nutrient retention within the artificial soil. The observations discussed within this thesis have highlighted the need for further research within a number of areas in order to further develop the potential of artificial soils. These suggestions are outlined below and could serve to provide greater insight into the effective use of artificial soils both at the Eden Project site and on an industrial scale.

Variation of environmental conditions

The examination of the effect of different environmental stresses on the experimental outcomes would be particularly interesting with regard to determining the magnitude of their impact on the nutrient dynamics within the soil. This would be relevant with regard to the development of artificial soil production protocol for a range of environmental conditions. In particular, it is suggested that temperature and moisture have a significant effect on the rate of organic matter decomposition (Kirschbaum, 2006), which as determined in Chapter 4 has a significant impact on nutrient availability.

The Eden Project soils are also subject to annual temperature fluctuations the extent of which is highly likely to have an impact on the nutrient dynamics within the soil. In studying these effects it may be possible to tailor the management practices across the Eden Project site to the environmental conditions.

Influence of microbial population

Through closely studying the microbial populations within the soil it may be possible to determine the immobilisation and mineralisation processes within the soil, which may

serve to confirm the nitrogen immobilisation and re-mineralisation hypothesis within Section 4.5.1, or allow for the exploration of an alternative hypothesis.

Impact of plant growth

Assessment of the extent to which plant uptake affects the nutrient dynamics within the soil, and also determining the effect of any of the recommended changes to the soils may have on plant growth, through growth of plants within soil columns (e.g. mycorrhizal associations). The selection of an appropriate plant species would require careful consideration, particularly in the case of the Eden Project, where an extensive range of plants are grown over a relatively small area. The successful propagation of plants may vary between species and as such it would be interesting to compare a plant considered to be easily cultured at the Eden Project against a more difficult to culture species, to determine the suitability of the soil to support a range of species

Further study of biochar

The 10 % biochar application had the greatest impact on leachate nutrient levels, assuming a biochar production yield equalling that reported within large scale production (35 %), application at this rate within only the Humid Tropics Biome would require approximately 104 tonnes of feedstock. Whilst biochar application would be on a less frequent basis than current fertiliser or mulch applications, the production of this quantity of biochar would be a significant undertaking. It is therefore advised that a lower content, between 2 and 5 %, should be applied, along with the use of targeted applications across the site. A long-term biochar study would also determine whether the behaviours observed within the columns study (Chapter 4) are encountered within biochar amended soils.

A more detailed understanding of the composition of the carbon within the biochar, particularly the carboxylate group characteristics would enable further understanding of the potential for increased cation exchange capacity with biochar amended soils (Cheng et al., 2006). This could be achieved through analysis of the biochar by nuclear magnetic resonance spectroscopy (^{13}C).

Variation of irrigation water volume

Assessment of water requirement by plants across the site may allow for the development of a targeted irrigation water regime, where the delivery of excess irrigation water may be avoided. As demonstrated by the data within Chapters 4 and 5, large concentrations of nutrients were lost from the soils within the leachate. By reducing the volume of irrigation water supplied to the soils, the amount of nutrients lost through leaching may be reduced.

Longer time series experiments

An extension of the timescale for the experiments with chapters 4 and 5 would have allowed for the further monitoring of the nutrient dynamics. In particular, further monitoring of N concentrations and fractions within the leachate to determine how long the increased N concentrations were sustained and observe the rate of decline and the timescale over which this would have occurred, to provide greater insight into the extent of the mineralisation process within the soil. Further to this a longer experimental period for a biochar study would give greater insight into the long term benefits offered by biochar application.

By extending the timescale over which the nutrient dynamics within the soil are observed there above recommendations may be tailored for the further improvement of the nutrient retention within the artificial soils.

Impact of differing components

It is important that soils produced using the same protocols perform consistently with regard to nutrient retention. Exploring the variations in the properties of soils produced following the same protocol using materials sourced from different suppliers, would provide insight into the variability between batches and may offer further information on the variability in the performance of the soils across the Eden Project site.

The materials from which artificial soils are produced should be sustainable and readily available. In certain situations materials required by the soil production protocol may be unavailable, exploring the potential for the substitution of materials would further the potential for the widespread use of artificial soils.

Exploration of the potential for designer soils

Many of the above suggestions require decisions to be made as to plants species or environmental conditions, which highlights the requirement for a range of artificial soil protocols for specific purposes. This gives rise to the concept of *designer* soils, wherein a soil may be tailored for function based on the specific requirements, conditions and material availability. It is equally important for artificial soils to be produced from sustainable materials sourced locally to the site of their intended use. This would be an exceptionally large undertaking, with protocol for soil production varying on both a national and international basis.

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Appendix A

The MSDS sheet for the Vitax (111, 214 and high K) fertiliser shows the materials from which the fertiliser was composed.

VITAX SAFETY INFORMATION SHEET

IDENTIFICATION OF PREPARATION

NATURAL PELLETED 111, 214 AND HIGH K

Packaging: 25 kg LDPE sacks

AND COMPANY Vitax Ltd, Owen Street, Coalville, LE67 3DE, Tel: 01530 510060

COMPOSITION Pelleted Compound fertiliser for Organic Farming & Growing Systems

Analyses:			
111	4.5	4.5	4.5
214	4.5	2.0	7.5
High K	0.5	0.5	20

Products contain a mixture of some of the following:

Cocoa shell	Kali Vinasse	Composted Breeder
Fish Meal	Calcified Seaweed	Poultry Litter
Gypsum	Kieserite	Rock Phosphate
Extracted Cocoa Meal	Lignite	Reddslag
Extracted Rape Meal	Sand	Fritted Trace Elements

HAZARDS IDENTIFICATION Not classified as dangerous

FIRST AID MEASURES Accidental over exposure may result in the following symptoms:

Eye Contact – dusty/gritty material expected to cause irritation to eyes.

Skin Contact – repeated and/or prolonged contact may cause irritation.

Ingestion – small quantities are unlikely to be harmful. Large quantities may give rise to gastro intestinal disorders.

Inhalation – high concentration of dust may be irritating to trachea and lungs. Inhalation of decomposition gases (eg in a fire) may cause serious lung effects.

Eye Contact – irrigate eyes with copious amounts of eyewash solution or water for at least 15 minutes. Obtain medical advice if the symptoms persist.

Skin Contact – wash the affected area with soap and water. If the irritation persists obtain medical advice.

Ingestion – do not induce vomiting. Rinse mouth and give water to drink. obtain medical attention if more than small quantities have been swallowed.

Inhalation – remove from source of exposure to dust. Keep warm and at rest. Obtain medical advice if symptoms persist. Persons who have inhaled decomposition gases (eg in a fire) should seek medical advice and be kept under medical supervision for at least 48 hours.

FIRE FIGHTING MEASURES When the fertiliser is not directly involved in the fire use the best means available to control the fire.

When the fertiliser is involved in the fire avoid breathing the fumes and wherever possible wear an approved mask when fighting the fire or when fumes are being emitted. Call the fire brigade. Use plenty of water and open doors and windows to give maximum ventilation. If the water containing the fertiliser enters any drain or water course, inform the appropriate water authorities immediately.

ACCIDENTAL RELEASE MEASURES Clean up spillage promptly. Sweep up spills carefully to minimise dust. Transfer to heavy duty plastic bags or drums suitably labelled and keep safe for disposal. Dispose of by use on farm, by spreading thinly on open ground or to authorised waste facility. Take care to avoid the contamination of water courses and drains. Inform the appropriate water authority in the event of accidental watercourse contamination. Refer to exposure controls/ personal protection and disposal consideration for further details.

HANDLING & STORAGE Store in original containers, tightly closed in a secure, well ventilated, cool but frost-free, dry area away from foodstuffs and herbicides. Do not block stack pallets.

EXPOSURE CONTROLS/ Occupation exposure standards have been established for nuisance dusts.

PERSONAL PROTECTION Normal good hygiene practices should be observed. Do not eat, drink or smoke when handling spillage. Wear gloves, overalls, goggles and dust mask where dust cannot be adequately controlled by engineering measures.

PHYSICAL & CHEMICAL PROPERTIES		
	Appearance	brown pellets
	Odour	faint organic odour
	pH	ca.7
	Boiling point	decomposes under intense heat
	Melting point	decomposes under intense heat
	Flash point	none
	Flammability	not flammable
	Autoflammability	none
	Explosivity	none
	Oxidizing properties	none
	Vapour pressure	N/A
	Bulk density	900-1400 kg/m3
	Solubility	partly soluble in water
	Other data	none

STABILITY AND REACTIVITY Product decomposition under intense heat may release toxic nitrogen and sulphur oxide fumes. Stable under normal conditions. Avoid high temperatures.

TOXICOLOGICAL INFORMATION Based on product components, ingestion of large quantities may cause abdominal pain, nausea, vomiting and diarrhoea.

To the best of our knowledge physical, chemical and toxicological properties have not been fully investigated.

ECOLOGICAL INFORMATION Product contains nutrients essential to plant growth. Do not exceed recommended application rates.

DISPOSAL CONSIDERATIONS Dispose of waste through a reputable waste disposal contractor and in accordance with the Environmental Protection Act 1990.

TRANSPORT INFORMATION Not classified as dangerous for carriage.

REGULATORY INFORMATION Not classified as dangerous for supply.

Occupational Exposure Standards for nuisance dusts in air 10 mg/m3 (8 hr), total inhalable 5 mg/m3 (hr) respirable.

Other Regulations

Health & Safety at Work Act 1974

Environmental Protection Act 1990.

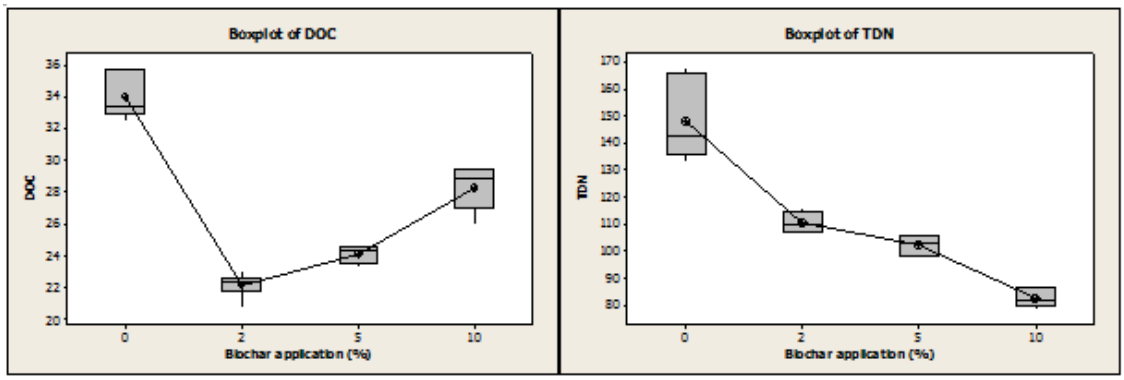
OTHER INFORMATION The product label provides information on the use of the product: do not use otherwise, unless you have assessed any potential hazard involved and the safety measures required.

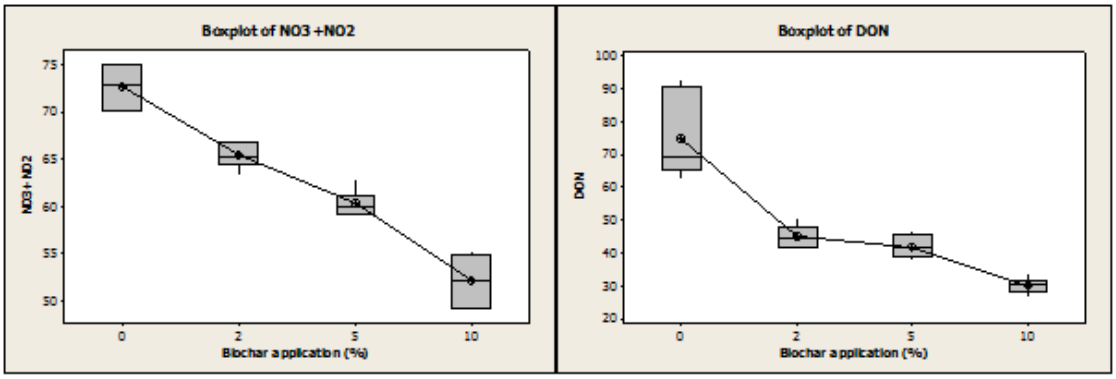
Prepared by VITAX LTD for Health & Safety purposes from the best knowledge available at the time of printing.

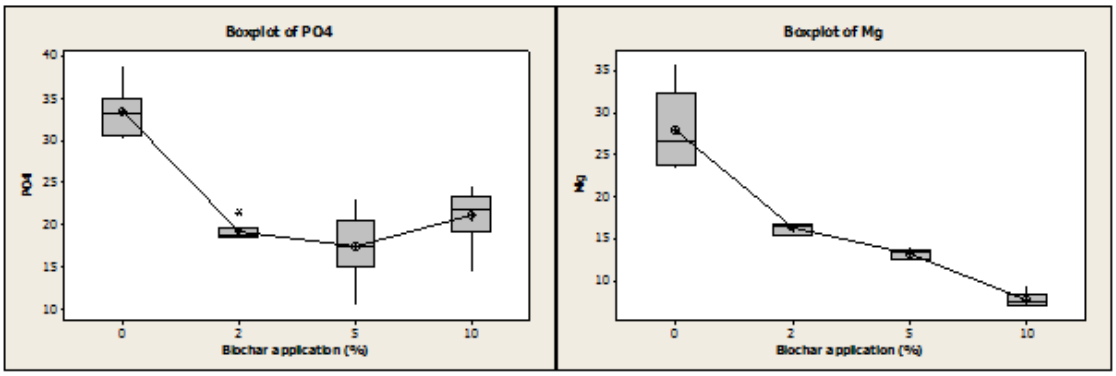
December 2000

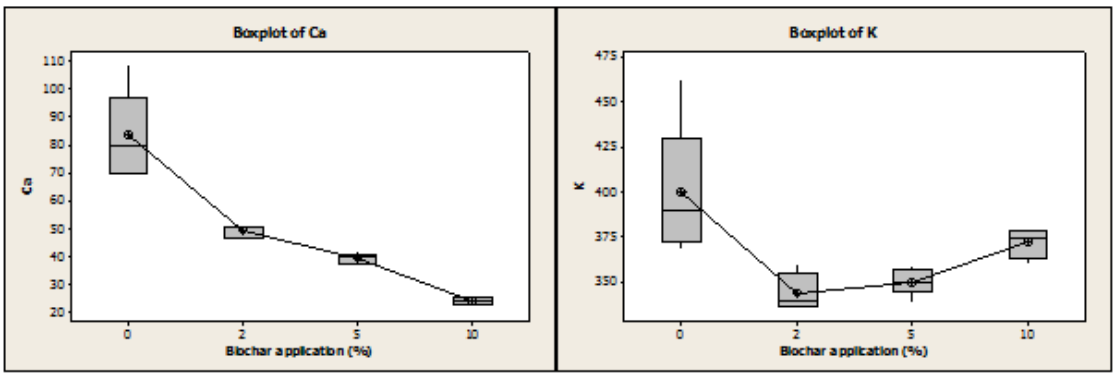
Appendix B

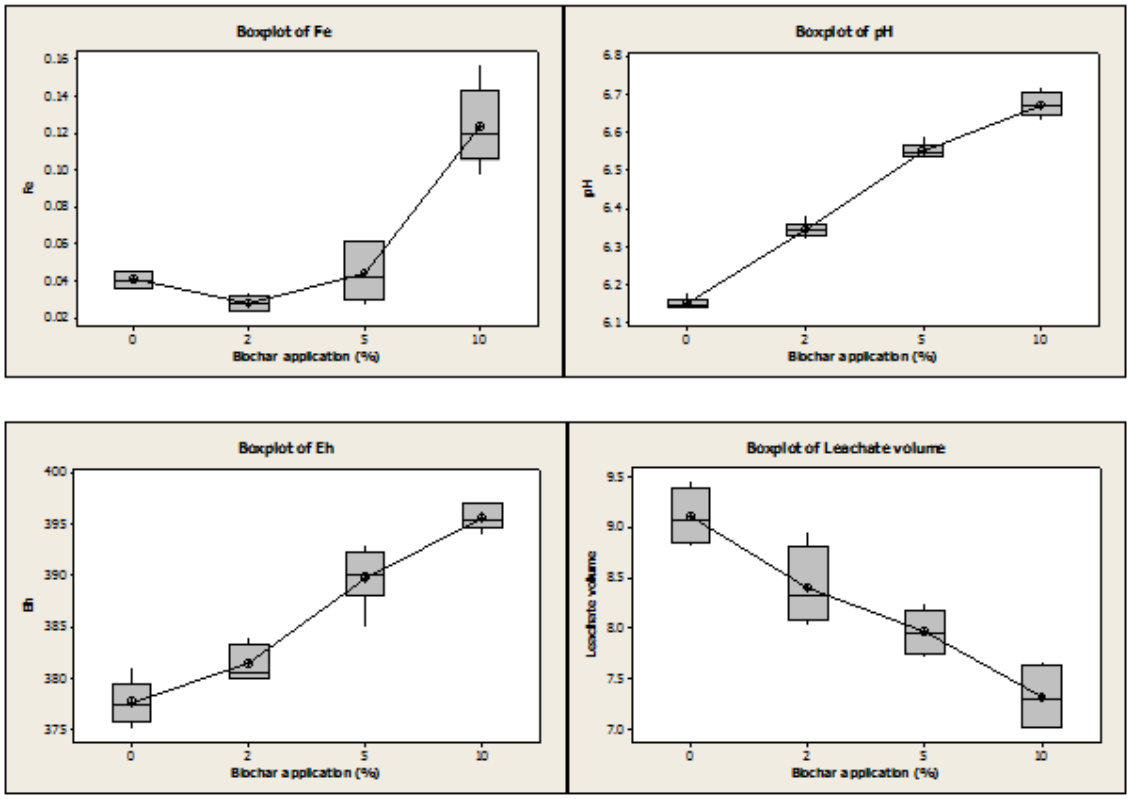
Box plot diagrams for nutrient concentrations (DOC, TDN, NO3- + NO2-, DON PO43-, Mg, Ca, K, Fe) and physicochemical analyses (pH, Eh and volume) of leachate sampled from each biochar concentration in Chapter 5. Biochar application was demonstrated to significantly reduce the concentration of nutrients within the leachate with increasing biochar content.


Boxplot of DOC
DOC
Biochar application (%)
Boxplot of TDN
TDN
Biochar application (%)


Boxplot of NO3+NO2
NO3+NO2
Biochar application (%)
Boxplot of DON
DON
Biochar application (%)


Boxplot of PO4
PO4
Biochar application (%)
Boxplot of Mg
Mg
Biochar application (%)


Boxplot of Ca
Ca
Biochar application (%)
Boxplot of K
K
Biochar application (%)


Boxplot of Fe
Fe
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0
2
5
10
Biochar application (%)
Boxplot of pH
pH
6.8
6.7
6.6
6.5
6.4
6.3
6.2
6.1
0
2
5
10
Biochar application (%)
Boxplot of Eh
Eh
400
395
390
385
380
375
0
2
5
10
Biochar application (%)
Boxplot of Leachate volume
Leachate volume
9.5
9.0
8.5
8.0
7.5
7.0
0
2
5
10
Biochar application (%)