Geochemical Changes, Plant Growth and Ecosystem Recovery in Soils after High Temperature Remediation

Andrew Pape

A Thesis presented for the Degree of Doctor of Philosophy

Department of Civil and Environmental Engineering
University of Strathclyde

Abstract

Thermal and smouldering remediation can effectively remove toxic organic compounds from contaminated sites, opening them up for re-development or environmental enrichment. However, these treatments generate elevated temperatures in the soil (40-1200°C) that may impact its quality, particularly its ability to support plant growth and microbial activity. This thesis demonstrates the effects of such heating on soil properties, the impact on plant and microbial systems, and potential methods for rehabilitating soils to improve plant growth after remediation.

Soils subjected to heating underwent a number of changes including losses of organic carbon (≥250°C), nitrogen (≥500°C), and clay sized particles (≥500°C). These changes resulted in significantly lower plant growth, microbial abundance, and microbial activity in soils heated above 500°C indicating that remediation techniques operating above this temperature will require additional soil improvement steps. In addition, soil toxicity was generated at temperature regimes of 105-250°C and ≥750°C in some soils.

For two soils subject to smouldering remediation, organic soil conditioners (compost and anaerobic digestate) were used to improve the yield of biomass crops. In a calcium-rich topsoil, toxic conditions generated during smouldering (pH>11) were too severe and biomass crop growth could not be established. In a

nutrient-poor soil, higher levels of productivity were established with compost amendment, but high levels of zinc in the anaerobic digestate proved toxic.

A tipping point was identified (500°C), due to losses of nitrogen and labile carbon, above which soil conditioning will be required to facilitate biological activity. Soil conditioners can be used to improve biological activity, but interactions between soil type, remediation temperature, and soil conditioner will be significant in developing soil conditioning programmes. Thermal and smouldering remediation can mitigate toxicity of contaminated sites; however, soil conditions after remediation need to be taken into account to facilitate desired land use, including additional treatment steps when required.

Declaration of Authenticity

This thesis is the result of the original research conducted by Andrew Pape. Where others have contributed this has been fully acknowledged. All external sources used in this study have been correctly referenced. It has been composed by the author and has not been previously submitted for examination that has led to the award of a degree.

Signed

Date

Copyright

The copyright of this thesis belongs to the author under the terms of the United Kingdom Copyright Acts as qualified by University of Strathclyde Regulation 3.50. Due acknowledgement must always be made of the use of any material contained in, or derived from, this thesis.

Acknowledgments

I would like to thank Drs Charles Knapp and Dr. Christine Switzer for the opportunity to study this fascinating area and providing invaluable advice and support. I would also like to thank staff and students at Strathclyde University for support ranging from help with analytical methods to just being a good sounding board for ideas. I would particularly like to thank: Neil McCosh, Dr Stephanie Zihms, Andrew Robson, Aoife Brennan, Mara Knapp and Dr Tatyana Peshkur. In addition, thanks goes to EPSRC for funding the project through a Doctoral Training Account. Finally, I would like to thank my wife Rhona for supporting me through the last three and a half years.

Preface

The main body of this thesis consists of three self contained chapters each with their own introduction, methodology, results and discussion and conclusions. Each of these chapters is intended to be published in a peer-reviewed journal in a similar format to how they appear in this thesis:

Chapter 2

PAPE, A., SWITZER, C. AND KNAPP, C. (2014). Effects of high temperature remediation processes on soil geochemistry. Submitted to *Environmental Science and Pollution Research* in February 2014, currently under review.

Chapter 3

PAPE, A., MCCOSH, N., SWITZER, C. AND KNAPP, C. (2014). Impacts of thermal and smouldering remediation on plant growth and soil ecology. To be submitted to *Geoderma* in May 2014.

Chapter 4

PAPE, A., SWITZER, C. AND KNAPP, C. (2014). - Biomass crop production on formerly contaminated soils subjected to thermal and smouldering remediation. Submitted to *Bioenergy Research* in May 2014, currently under review.

In addition the following conference presentations have resulted from this work:

Oral Presentations:

PAPE, A., SWITZER, C. AND KNAPP, C. (2013). *The Growth of Biomass Crops on Contaminated Sites Post-Remediation*. 6th Scottish Postgraduate Symposium on Environmental Analytical Chemistry, Glasgow, Scotland

PAPE, A., SWITZER, C. AND KNAPP, C. (2013). *The Impact of Thermal Remediation on Soil Rehabilitation*. European Geosciences Union General Assembly 2013, Vienna, Austria

SWITZER, C., ZIHMS, S., PAPE, A., ROBSON, A. AND KNAPP, C. (2013). *Effects of Aggressive Remediation on Soil Properties and Function*. European Geosciences Union General Assembly 2013, Vienna, Austria

PAPE, A., SWITZER, C. AND KNAPP, C. (2012). *The Impact of Remediation on Rehabilitation*. 5th Scottish Postgraduate Symposium on Environmental Analytical Chemistry, Aberdeen, Scotland

Poster Presentations:

PAPE, A., SWITZER, C. AND KNAPP, C. (2012). *The Impacts of Thermal and Smouldering Remediation on Soil Properties Related to Rehabilitation and Plant Growth*. European Geosciences Union General Assembly 2012, Vienna, Austria

PAPE, A., SWITZER, C. AND KNAPP, C. (2011). *Changes to Soil Properties During Aggressive Remediation*. 4th Scottish Postgraduate Symposium on Environmental Analytical Chemistry, Glasgow, Scotland

Contents

Chapter 3 – Impacts of Thermal and Smouldering Remediation on Plant Growth and Soil Ecology

Chapter 4 - Biomass Crop Production on Formerly Contaminated Soils Subjected to Thermal and Smouldering Remediation

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

List of Tables

Chapter 2

Chapter 3

Appendix IV

Chapter 1 – Introduction

1.1 Background

Re-development efforts to restore the economic and environmental value in brownfield sites face many obstacles. In particular, residual contamination with hazardous chemicals remains a major hurdle, contributing to around 300,000 known sites and 2.5 million suspected sites in the EU alone (Van Liedekerke *et al.,* 2014). Non-aqueous phase liquids (NAPLs) consist of hydrocarbons and other organic compounds with highly varied levels of toxicity and environmental mobility. Organic contaminants account for approximately 54% of Europe's contaminated soil inventory (van Liederkerke et al, 2014) and many thousands of contaminated sites worldwide. In the UK the contaminated land regulations (2000), under part IIA of the Environmental Protection Act 1990, requires local authorities to identify and bring about the remediation of contaminated land. This sets out a legal obligation, on top of the economic and environmental considerations, to deal with sites where the potential for harm to the environment or human health exists.

This has resulted in a substantial interest in developing appropriate remediation processes including bioremediation (Bamforth and Singleton, 2005), chemical oxidation (Sirguey *et al*., 2008), thermal (Buettner and Daily, 1995), and smouldering remediation (Switzer *et al.,* 2009). As these methods can remove contaminants from a site without the need to landfill contaminated material they also fit in with the UKs Zero Waste policy, which requires the reduction or elimination of waste streams going to landfill. Thermal (40-1000˚C) and

smouldering (500-1200˚C) can significantly reduce health and environmental impact from pollutants; however, the temperatures generated during these treatments may decrease the suitability of remediated site for specific uses. For land uses that require semi-natural systems where the soil is required to support plant growth and microbial activity, understanding how soil heating alters soil and interacts with subsequent biological systems will be key for successful regeneration after remediation.

1.2 Thermal and Smouldering Remediation

Thermal remediation techniques have advantages over bio-remediation and chemical oxidation. In general they are faster and can deal with higher levels of contamination (Heron *et al*., 2009). In addition no new chemicals need to be introduced to the soil environment like they do for chemical oxidation. Thermal remediation relies on two basic principles; either heating is used to increase the vapour pressure of volatile contaminants so they can be removed by a vacuum system (Heron *et al*., 2005) or; very high temperatures are used to initiate pyrolysis and oxidation reactions in which organic molecules are broken down (Switzer *et al.,* 2009).

As mentioned, for more volatile chemicals, soil heating often forms part of a vapour extraction system. High removal efficiencies for volatile chemicals such as trichloroethene (Buettner and Daily, 1995), toluene (Heron *et al*., 2005), and low

molecular weight poly-aromatic hydrocarbons (Robinson *et al*., 2009) can be achieved at low temperatures (~100°C). A number of methods can generate these temperatures in-situ, including steam injection (Heron *et al*., 2005), electrical resistivity heating (Buettner and Daily, 1995), microwave heating (Robinson *et al*., 2009), and conductive heating (Heron *et al*., 2009).

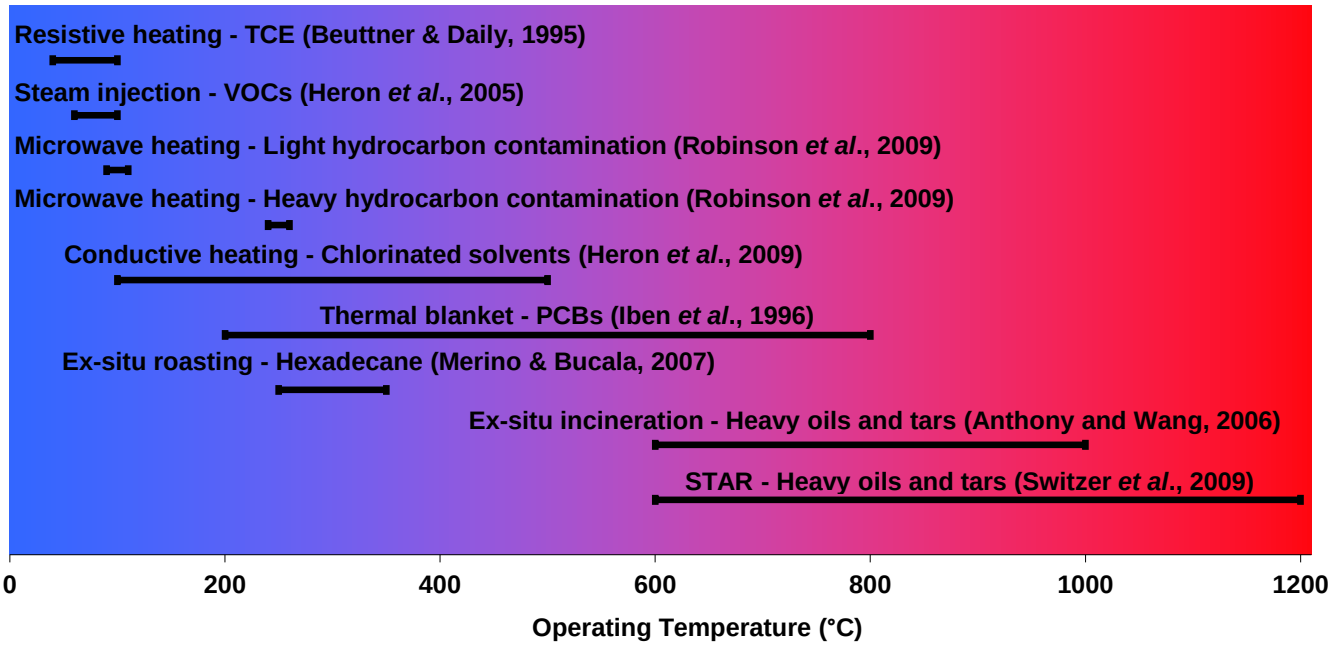


Figure 1.1 – Target compounds and approximate operating temperatures of a variety of thermal remediation techniques.

As the volatility of compounds decreases, higher temperatures may be required to remove them from soils. For instance, polychlorinated biphenyls require temperatures of at least 250°C to be removed (Iben *et al*., 1996) and hexadecane requires temperatures of 300°C or more (Merino and Buccalá, 2007). As volatility decreases further, oxidation of contaminants to carbon dioxide using elevated temperatures becomes more practical. Low volatility chemicals such as dioxins,

furans (Kasai *et al*., 2000), and coal tars (Anthony and Wang, 2006) can be destroyed or altered by ex-situ incineration with operating temperatures of up to 1000°C. Such mixtures can also be removed by both in-situ (Hansen et al., 1998) and ex-situ heating (McGowan et al., 1996), but temperatures of 400-500˚C must be maintained for extended periods (up to several weeks for in-situ treatments) at a high energetic cost.

An important point to note from this is that treatment temperature is not an open choice for remediation engineers. The temperature selected has to be, in some part at least, based on the type of contaminant (particularly its volatility), the levels in the soil and the desired remediation objectives. This then feeds into technology choice as certain methods are only able to create limited temperature ranges in the soil, for instance resistive heating is only able to heat soil up to the boiling point of water as it needs the soil to act as an electrical conductor (Buettner and Daily, 1995). Overall thermal remediation covers a wide range of temperatures and contaminants (Figure 1.1) but suitable remediation techniques for a given situation may limited.

One of the draw backs of thermal remediation techniques, particularly those operating at high temperatures, is the high energetic cost involved in raising soil temperatures. This could have both financial and environmental (carbon emissions from energy productions) implications. Smouldering remediation utilises the inherent chemical energy in organic contaminants to drive the remediation process

thereby reducing the reliance on external heat sources. This system has been shown to remove low volatility hydrocarbon contaminants such as coal tars and crude oils from heavily contaminated soils (80,000-320,000ppm) with a very high removal efficiency 99.5%+ (Pironi *et al*., 2009). Both in-situ and ex-situ treatment systems (Switzer *et al.,* 2009; Pironi *et al.,* 2011; Switzer *et al.*, 2014) have been developed and are in the process of being deployed.

In practical terms this technique involves heating an initial volume of contaminated soil to the ignition threshold of the contaminant in question (often around 300°C), and then introducing an airflow to cause the contaminants to ignite. The contaminants burn in the liquid phase in the soil rather than in the gas phase so a slow, smouldering combustion occurs. This creates a smouldering front which moves through the soil destroying the contaminants through exothermic pyrolysis and oxidation reactions. The heat produced during these reactions is sufficient to heat the next portion of soil to the ignition threshold ensuring a self-sustaining remediation process with energy inputs limited to the initial ignition stage. Smouldering generates peak temperatures of 500-1200˚C (Switzer *et al.*, 2014) in the soil, peak temperatures depending largely on the contaminant and soil condition.

During this thesis a small scale, ex-situ system was used. This was based on the work done by Switzer *et al.* 2009. The equipment was set up according to figure 1.2. A quartz column (3L) was used as the reaction vessel. At the bottom of this a

heating element and air diffuser were emplaced in around 5cm of clean sand. Above this the column was filled with contaminated soil until 10cm of the beaker remained. Above this was another layer of clean sand that prevented contaminants pooling and conventional gas phase combustion from occurring. A central line of thermocouples was used to monitor the progress of the remediation. To start the remediation process the heater was turned on until the first thermocouple reached 300°C. At this point the air was turned on and the heater turned off and the reaction allowed to progress until the production of smoke stopped and temperatures of the top thermocouple start to decline. At this point the air was turned off and the soil allowed to cool before being removed from the column.

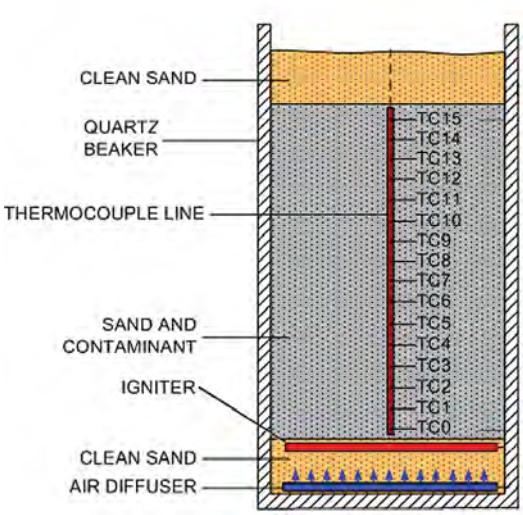


Figure 1.2 – Smouldering remediation ex-situ set up

By targeting treatment systems to contaminant and site conditions, significant reductions in contaminant loads can be achieved. However, generation of elevated temperatures in the soil environment affects its suitability for future use by altering it physically and chemically (Certini, 2005; Galang *et al,.* 2010; Ulery *et al,.* 1996; Glass *et al*., 2008; Gray & Dighton, 2006). If future land uses require the soil to

sustain biological activity, changes to the soil must be overcome. Such situations would include: subsequent bio- or phyto-remediation of residual contaminants; growth of biomass crops for energy production; environmental restoration and enhancement; and creation of green spaces in larger developments. Understanding how soil geochemistry changes with the range of heating temperatures utilised during current remediation methods (40-1200˚C) and how these changes interact with biological systems will be essential to successfully re-developing contaminated sites.

1.3 Aims and Objectives

The aim of this thesis is to assess and enhance the ability of soils subject to thermal or smouldering remediation to support biological activity, particularly plant growth. To achieve this aim, the following objectives will be achieved:

1. Quantify the physical and chemical changes that soils undergo as they are subjected to increased levels of heating, with particular attention paid to parameters that will be important for plant growth.
2. Translate chemical and physical changes upon heating into measurable impacts on biological systems, including plant growth, microbial abundance, and microbial activity in the soil.
3. Demonstrate sustainable methods of soil restoration to enhance plant growth and biological activity.

These objectives feed directly into the structure of the thesis (Fig 1.1) with an experimental chapter given over to each objective. Chapter 2 addressed changes to soil properties on heating. This includes a literature review of the current state of knowledge of the effects of soil heating on soil properties and experimental work covering changes to soil properties over the full range of treatment temperatures currently utilised in remediation. Following this Chapter 3 covers the effect of heating on plant growth and soil microbiology. Again the knowledge base currently available was reviewed and experiments carried out. Heat treated soils were used and the growth of two plant species (*Trifolium pratense* and *Festuca rubra*) was observed and evaluated after a seven-week growth period. The influence of supplementation with microbes on plant growth and microbial population recovery was also investigated. Finally Chapter 4 examined a practical application for contaminated land, the growth of biomass crops, and the influences of soil heating or smouldering on this potential new land use. The ability of organic amendments (compost and anaerobic digestate) to enhance productivity was tested. Together, these Chapters allowed definitive conclusions to be drawn about the role of heating conditions on the ability of remediated soils to be restored as biological media.

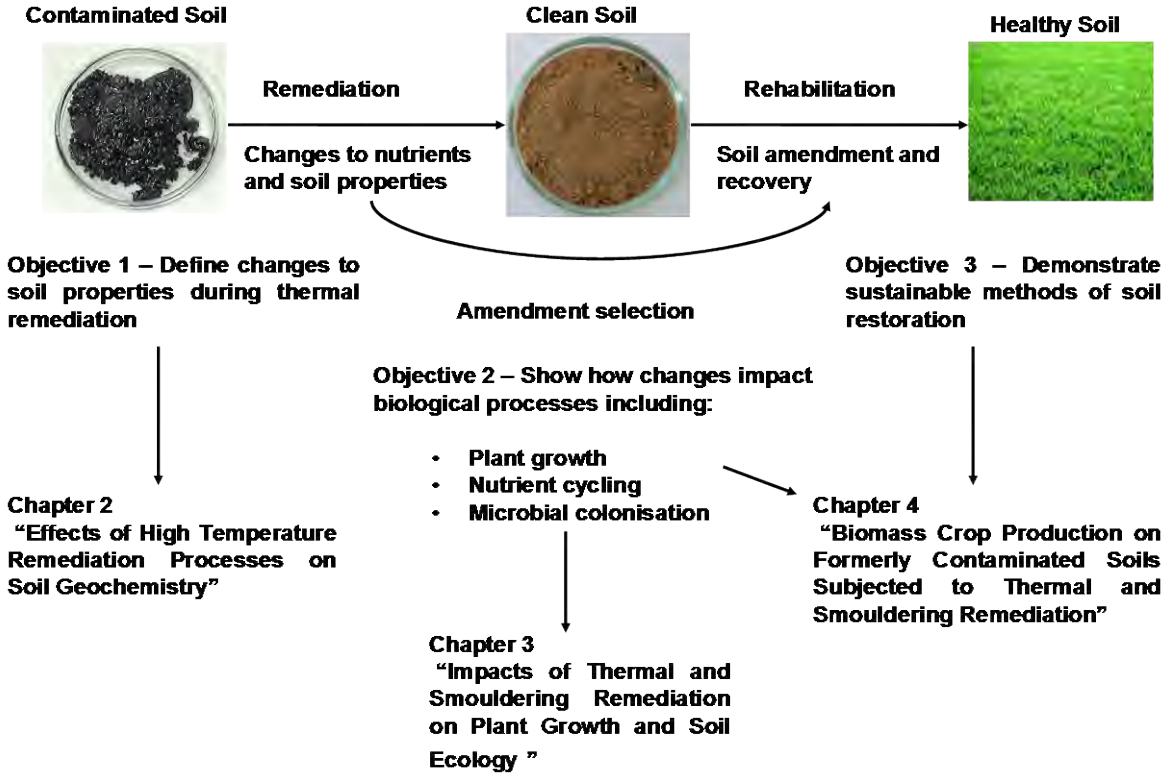


Figure 1.3. Relationship between objectives and structure of the thesis

1.4 Soil Samples and Preparation

A number of soils were used in the preparation of this thesis. Different soils were used in different chapters due to availability and purposes of the work, the use and designation of soils in different chapters is listed in table 1.1. Soils were sampled by removing litter and digging out a large volume of soil from 0-20cm depth form a single square sampling point. This soil was then air dried, sieved and thoroughly homogenised. The aim of this sampling strategy was not to provide a site representative sample, instead it was intended to provide a single large and very homogenous sample of each soil to be used throughout this study. This would

ensure that consistent results could be obtained for each soil over the full 3.5 year duration of the practical work for this thesis. The basic properties of each soil are listed in table 1.2.

Table 1.1 – Soil sample locations, descriptions and designation in different chapters

				Soil Designation		
Soil	Location	Grid Reference	Description	Chapter 2	Chapter 3	Chapter 4
A	Glasgow	NS 54852 66676	Urban garden soil	A		
B	Aberdeenshire	NJ 72528 35982	Rural garden soil	B	1	
C	Aberdeenshire	NJ 74702 33364	Pine forest soil	C		
D	B&Q	n/a	Commercial soil		2	1
E	Rest and Be Thankful	NN 23827 09269	Nutrient poor subsoil			2

Table 1.2 – Basic soil properties

Soil	pH	Organic Content	Texture	Nitrogen Content
A	7.4	11.3%	Sandy loam	1.1g/kg
B	5.6	12.1%	Loam	1.1g/kg
C	4.1	20.0%	Silt loam	1.3g/kg
D	7.4	17.3%	Sandy loam	1.0g/kg
E	4.1	2.8%	Loamy sand	0.3g/kg

1.3 References

Anthony, E.J. & Wang, J. 2006. Piolt plant investigations of thermal remediation of tar-contaminated soil and oil-contaminated gravel. *Fuel*, **85**, 443-450

Bamforth, S.M. and Singleton, I. (2005). Review: Bioremdiation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology*, **80**, 723-736

Buettner, H.M. & Daily, W.D. 1995. Cleaning contaminated soil using electrical heating and air stripping. *Journal of Environmental Engineering*, **121**, 580-589

Certini, G. 2005. Effects of fire on properties of forest soils: A review. *Oecologia*, **143**, 1-10

Hansen, K.S., Conley, M., Vinegar, H.J., Coles, J.M., Menotti, J.L. and Stegemeier, G.L. (1998). In situ thermal desorption of coal tar. *IGT/GRI International Symposium on Environmental Biotechnologies and Site Remediation Technologies* Orlando, Florida (Avaiable:http://www.terratherm.com/pdf/white%20papers/paper20-11-6-09.pdf)

Heron, G., Carroll, S. & Nielsen, S.G. (2005). Full-scale removal of DNAPL constituents using steam-enhanced extraction and electrical resistive heating. *Ground Water Monitoring and Remediation*, **25**, 92-107

Galang, M.A., Markewitz, D. & Morris, L.A. 2010. Soil phosphorus transformations under forest burning and laboratory heat treatments. *Geoderma*, **155**, 401-408

Gray, D.M. & Dighton, J. 2006. Mineralisation of forest litter nutrients by heat and combustion. *Soil Biology and Biochemistry*, **38**, 1469-1477

Glass, D.W., Johnson, D.W., Blank, R.R. & Miller, W.W. 2008. Factors effecting mineral nitrogen transformations by soil heating: A laboratory-simulated fire study. *Soil Science*, **173**, 387-40

Heron, G., Parker. K., Galligan, J. and Holmes, T.C. (2009). Thermal treatment of eight CVOC source zones to near nondetect concentrations. *Ground Water Monitoring and Remediation,* **29**, 56-65

Iben, I.E.T., Edelstein, W.A., Sheldon, R.B., Shapiro, A.P., Uzgiris, E.E., Scatena, C.R., Blaha, S.R., Silverstein, W.B., Brown, G.R., Stegmeier, G.L. & Vinegar, H.J. 1996.

Thermal blanket for in-situ remediation of surficial contamination: A pilot test. *Environmental Science and Technology*, **30**, 3144-3154

Kasai, E., Harjanto, S., Terui, T., Nakamura, T. and Waseda, Y. (2000). Thermal remediation of PCDD/Fscontaminated soil by zone combustion process. *Chemosphere*, **41**, 857-864

McGowan, T.F., Greer, B.A. and Lawless, M. (1996). Thermal and non therma technologies for remediation of manufactured gas plant sites. *Waste Management*, **16**, 691-698

Merino, J. & Bucalá, V. 2007. Effect of temperature on the release of hexadecane from soil by thermal treatment. *Journal of Hazardous Materials*, **143**, 455-461

Pironi, P., Switzer, C., Rein, G., Fuentes, A., Gerhard, J.I. & Torero, J.L. 2009. Small-scale forward smouldering experiments for remediation of coal tar in inert media. *Proceedings of the Combustion Institute*, **32**, 1957-1964

Robinson, J.P., Kingman, S.W., Snape, C.E., Shang, H., Barranco, R. and Saeid, A. (2009). Separation of polyaromatic hydrocarbons from contaminated soils using microwave heating. *Separation and Purification Technology*, **69**, 249-254

Sirguey, C., Silva, P.T.S., Schwartz, C. and Simonnot, M.O. (2008). Impact of chemical oxidation on soil quality. *Chemosphere*, **72**, 282-289

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G. & Torero, J.L. (2009). Self-sustaining smoldering combustion: A novel remediation process for non-aqueous-phase liquids in porous media. *Environmental Science and Technology*, **43**, 5871-5877

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G. and Torero, J.L. (2014). Volumetric scale-up of smouldering remediation of contaminated materials. *Journal of Hazardous Materials*, **268**, 51-60

Ulery, A.L., Graham, R.C. & Bowen, L.H. 1996. Forest fire effects on soil phyllosilicates in California. *Soil Science Society of America Journal*, **60**, 309-315

Van Liedekerke, M., Prokop, G., Rabl-Berger, S., Kibblewhite, M. and Louwagie, G. (2014*). Progress in the management of contaminated sites in Europe*. European Commission Joint Research Centre, Institute for Environment and Sustainability. Ispra Italy

Chapter 2 - Effects of high temperature remediation processes on soil geochemistry

A. PAPE, C. W. KNAPP & C. SWITZER

2.1 Preface

This Chapter was submitted for publication in *Environmental Science and Pollution Research* in February 2014 and is currently under review. This paper investigates the chemical and physical changes that occurred in three soils (A, B and C) subjected to heating and smouldering remediation. A. Pape as the main author was responsible for experimental design, practical work, data analysis, and writing the paper. C.W. Knapp and C. Switzer, as project supervisors, provided advice on experimental design, data analysis, and presentation as well as editorial comments during preparation of the chapter.

2.2 Summary

High-temperature strategies have been used to aggressively remediate contaminated land. While often successful in removing high-levels of contamination, the high temperatures have the potential to change the geochemical properties of soils. Here, the effects of soil heating and smouldering, a novel high temperature treatment, are investigated, and their effects on soil quality are discussed. Soil from three different locations underwent seven different high-temperature treatments including an ambient control; thermally-treated at 105°C, 250°C, 500°C, 750°C and 1000°C; and a smouldering treatment, which resulted in exposure temperatures of 1000-1100°C. The effects on micro and macro nutrients, organic content, and other physical and chemical properties were quantified. Heated samples showed significant losses of carbon (>250˚C), nitrogen and clay sized particles (>500˚C), and phosphorus (1000˚C). Additional changes in available nutrients were observed with a trend towards low availability at high temperatures. Results from the smouldering experiments were similar to those for the equivalent heating temperature; however, secondary factors such as residence time need to be taken into account. Soil heating and smouldering are effective remediation tools but can lower soil quality by nutrient removal and physical changes. To counter these changes, appropriate rehabilitation strategies need to be tailored for specific land uses and treatment regimes.

2.3 Introduction

Chemical contamination is one of the main obstacles to redeveloping and restoring economic value to brownfield sites. This is particularly the case for areas with non-aqueous phase liquid (NAPL) contamination, which consists of hazardous organic substances that can be particularly recalcitrant in soils. These sites frequently require aggressive, high-temperature processes for effective remediation. These processes include steam injection (Heron *et al.,* 2005), electrical heating (Buettner & Daily, 1995), microwave heating (Robinson *et al*., 2009), conductive heating (Heron *et al.*, 2009) and smouldering (Switzer *et al.,* 2009; Pironi *et al.,* 2011). When deployed effectively for site and contaminant conditions, these processes can significantly reduce contaminant levels in the subsurface within short periods of time. While the potential now exists to return heavily contaminated soils to a more natural state, it remains important to understand the effects of these high remediation temperatures on soil quality.

Table 2.1. Examples of high-temperature remediation strategies, associated operating temperatures and pollutant removal efficiencies.

Heating Type	Temperature (°C)	Pollutant	Removal Efficiency	Source
Conductive	90+	Chlorinated solvents	98%+	Heron *et al* ., 2009
Electrical	38-100	TCE	Not recorded	Buettner & Daily, 1995
Steam injection	80-125	VOCs	99.85%+	Heron *et al* ., 2005
Microwave	100	Light hydrocarbons	95%+	Robinson *et al* ., 2009
	250	Heavy hydrocarbons	95%+	Robinson *et al* ., 2009
Thermal Blanket	250-800	PCBs	98%+	Iben *et al* ., 1996
Ex-Situ Bulk Heating	300	Hexadecane	99.9%+	Merino & Bucala, 2007
Ex-Situ Incineration	800	Crude Oil and Tar	100%	Anthony & Wang, 2006
Smouldering	600-1200	Coal Tar/Crude Oil	99.9%+	Switzer *et al* ., 2009
Smouldering	1000-1100	Coal Tar	99.98%+	Soils A, B, C

Elevated temperatures during natural phenomena, such as wildfires, have been associated with changes to nutrient content, destruction of organic matter and changes to the texture and physical properties of soils (Certini, 2005; Galang *et al.,* 2010; Ulery *et al.,* 1996). However, these circumstances only impact the upper soil layers for a relatively short period of time whereas changes during thermal and smouldering remediation may be more severe as they affect greater depths (Certini, 2005). Additionally, some techniques generate soil temperatures higher than the 200-500°C (Certini, 2005) commonly associated with wildfires (Table 2.1).

Using wildfire research, predictions can be made about the effects of different temperatures on specific soil properties; however, knowledge remains limited of the effects when temperatures exceed 500°C. For example, changes in nutrient status are expected from the higher temperatures. While soil nitrogen increases during wildfires due to inputs from combusted surface fuels (Certini, 2005; Covington & Sackett, 1992), high-temperature remediation will volatilise soil nitrogen (both organic and inorganic) (Glass *et al*., 2008; Gray & Dighton, 2006). Additionally, phosphorus is either lost to the atmosphere as P_2O_5 (Galang *et al*., 2010; Raison *et al*., 1985) or becomes less bio-available (Kang & Sajjapongse, 1980). The loss of organic matter and microorganisms further disrupts natural geochemical cycling and exacerbates nutrient loss (Certini, 2005; Glass *et al*., 2005; Smith *et al*., 2008).

Besides the potential of accelerated nutrient loss at elevated temperatures, other physical-chemical properties can be affected. High temperatures have been found to decompose clay minerals (Ulery *et al*. 1996; Terefe *et al*., 2008; Ketterings *et al*., 2000). This subsequently releases exchangeable bases and other ions, resulting in an increase in pH and electrical conductivity (EC) (Granged *et al*., 2011; Ketterings *et al*., 2000). This causes the remaining clay fraction to aggregate into larger particles (Terefe *et al*., 2008), ultimately affecting the texture, hydraulic properties and long term structure of the soil. Most importantly, the cation exchange capacity (CEC) declines, along with the soil's ability to retain key nutrients such as ammonium, calcium, and potassium (Kang & Sajjapongse, 1980).

Limited information exists on the effects of high-temperature remediation on soil quality. This information is important for implementation of follow-up, biological remediation as well as restoration of soil function after remediation.

This research aims to provide an encompassing examination of how physical-chemical properties change over the full range of temperatures utilized during thermal and smoldering remediation processes, testing the hypothesis that such heating will have a significant impact on soil properties. This information will help predict potential impacts associated with specific remediation processes and better inform rehabilitation decisions. To further validate these findings, the properties of soils treated by a novel smoldering technology will also be compared to the effects of heating alone. This work represents an initial contribution towards a holistic

regeneration scheme that takes into account the desired land use and assures the restoration of economic and environmental value.

2.4 Methodology

2.4.1 Soil Samples

After litter removal, soil samples were collected from the surface horizon (0-20cm) at three locations: “sample A” from an urban environment in Glasgow (Scotland); “sample B” from a horticultural setting in north-east Scotland; and “sample C” from similar locale as “B”, but from a long-established Sitka Spruce (*Picea sitchensis*) plantation. These samples were chosen to represent different locations (urban against rural) and land uses (grass and weeds against established forest) to see if any significantly different responses were apparent. The samples were air dried and sieved to <2mm. Six treatments were created by heating soils at different temperatures, each representing a different thermal treatment: control, 105°C, 250°C, 500°C, 750°C and 1000°C. Approximately 200g of each soil were spread on a ceramic tray to a depth of 4cm, oven-dried at 105°C for 24 hours to avoid the inhibitory effect of moisture content during further heat treatments (Glass *et al*., 2008), and subsequently heated at their respective temperatures in a muffle furnace (Nabotherm P330, Lilienthal, Germany) for one hour. For each sample this treatment was repeated three times and the replicates thoroughly mixed to provide enough material for analysis.

Additionally, as a seventh treatment, a sample of each soil was artificially contaminated with coal tar (80g/kg) and treated via smouldering combustion according to Switzer *et al.* (2009) with modifications to allow for soil permeability, namely a reduced air flow and pressure of 25lpm at 1bar. Briefly, a heating element and air diffuser were emplaced in 5cm of clean sand in the bottom of a 3 liter quartz beaker. This was then filled to within 10cm of the top with contaminated soil and capped with 10cm of clean sand. K-type Thermocouples were emplaced starting 1cm from the heating element to monitor temperature during the remediation process. The heater was turned on until the first thermocouple reached 300°C at which point the air diffuser was turned on and the heater turned off. The air supply was kept on until the reaction had terminated and the column left to cool before the remediated soil was removed. To verify efficacy of the treatment procedure, soil samples underwent accelerated solvent extraction (ASE; Dionex350, Thermo Scientific; Waltham, MA, USA) and analysis for total extractable petroleum hydrocarbon (TPH) by Thermo Scientific Focus Gas Chromatography with flame-ionization detection.

2.4.2 Analysis of soil parameters

A number of measurements related to soils' ability to provide key nutrients for plants and microorganisms were determined. Each measurement was performed in triplicate to allow for statistical analysis. Total organic carbon (TOC) was determined by carbon dioxide production following combustion after a phosphoric acid sparge

to remove carbonates (BS, 1995a), and total nitrogen (TN) by the Dumas method (BS, 2001a); both were determined using a TOC/TN analyser (Teledyne Tekmark Apollo 9000, Thousand Oaks, CA, USA). Available nitrogen was calculated as the sum of inorganic nitrogen species extracted with potassium chloride (ADAS, 1985); extracts were analysed colorimetrically via the Bertholt reaction for ammonium (Bundy & Meisinger, 1994), and a sulfanillic acid method for nitrite directly and nitrate after a hydrazine reduction step (ADAS, 1985; Shand *et al*., 2008). Phosphate was quantified colorimetrically using an ascorbic acid/ammonium molybdate method (ADAS 1985; BS, 1995b) after an Olsen extraction for plant available P (BS, 1995b) and an *aqua-regia* digest for total P (BS, 2001b). Exchangeable bases were measured after displacement with ammonium acetate (ADAS, 1985) using ion chromatography (Metrohm 850/858; Cheshire, UK) after dry ashing and dissolution in 5mM HCl to remove the extractant (Jackson, 2000; Basta & Tabatabai, 1985). Concurrently, CEC was determined by displacing the soil-held ammonium with acidified potassium chloride (ADAS, 1985) and analysed via the Bertholt reaction (Bundy & Meisinger, 1994). pH and electrical conductivity (Mettlor-Toledo International Inc, Columbus, OH, USA) were recorded after a 1:5 soil/water extraction using an appropriate probe (BS, 2005; BS, 1995c), with this extract also being analysed for soluble bases using ion chromatography. Bio-available copper and zinc were extracted using 0.05M ammonium-EDTA at pH7 as a chelating agent (ADAS, 1985) and analysed using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES, Thermo Scientific Hemel Hempstead, UK).

The soil samples were also analysed for a number of physical factors. Particle-size distribution was determined using wet sieving with the pipette method (BS, 2009). Ash content was quantified following combustion at 550°C for four hours (BS, 2000a), and particle density was determined by volumetric displacement of water (BS, 2000b). Bulk density, pore space and the relative abundance of air and water at a set negative head pressure of -10cm was determined using soil columns and a sand table (BS, 2000b) to give an indication of water balance in the soil.

Results were compared by graphical analysis and analysis of variance (ANOVA) with a Tukey post-hoc test to determine whether any significant changes occurred with increasing temperature. To facilitate comparisons between nutrient changes in the different soils the data sets were corrected to account for mass loss during heating and normalized for initial concentration. This allowed the level of nutrients to be expressed as a percentage of the initial content and changes to be directly comparable between the three soils. Significance was set at $\alpha = 0.05$ for all statistical tests; and 95% confidence intervals were calculated for each mean value.

2.5 Results and Discussion

2.5.1 Background Characteristics of Soil Types

Three soils types were collected, each representing a different environment: urban (soil A), rural (soil B) and forest (soil C). These soils were selected as they were likely to have different properties, this helped identify if any changes occurred related to soil type rather than just heating temperature. The detailed character of the soils is

listed in Tables 2.2 and 2.3 as the control treatments. The soils differed in organic content (C > B ≈ A), pH (A > B > C), phosphorus content (A > B > C), and bioavailable metals (e.g., K, Ca, Mg, Cu and Zn; A > B > C). The variability in soil quality allows us to determine whether soil-dependent effects occur within each temperature treatment.

2.5.2 Changes to Soil Properties upon Heating

The physical properties of the soil visibly changed after exposure to high temperatures, the most apparent of which was colour change. At 250˚C, the soil was darkened by the charring of organic matter, and there was a corresponding loss of mass (Table 2.2). At 500˚C and 750˚C, clear vertical stratification of blackened and reddened layers resulted from progressive oxidation of char and exposure of oxidised iron minerals (Ulery & Graham, 1993). At 1000˚C more advanced oxidation was apparent, and bright-red iron oxides were visible.

Heating resulted in slight increases in the particle density (Table 2.2), towards the value that is typically assumed for the mineral component of soil (2.65g/cm^3) (BS, 2000b). Heating also resulted in an increase in bulk density as heating destroyed organic matter causing aggregates to break down and allowing the remaining soil particles to pack closely together. However at 1000˚C, the fusion of soil aggregates in soils A and B reduced bulk density. Above 500˚C, the soils experienced a loss of the organic component and a reduction in the level of chemically and structurally active clay-sized particles (Table 2.2). These losses resulted in reduced wet-soil cohesion and a change in textural class towards sandier soil types

Table 2.2. Physical properties of control, heated, and smouldered (SM) soils. The ranges of standard deviations, based on triplicate analysis, for each test are listed at the bottom of each column; significantly similar (ANOVA) are grouped and denoted by lettered post-scripts.

	Mass	Ash	Texture				Bulk	Particle			
	Lost	Content	Sand	Silt	Clay	Classification	Density	Density	Porosity	Water	Air
Temp (C)	(%)		(% of Mineral Component)				(g/cm3)		(% Volume)		
Soil A											
Control	-	88.69 a	60.4 a	23.1 a	16.5 a	Sandy loam	1.04 a	2.52 ab	58.82 ab	49.80 ab	9.02 abc
105	-	89.95 b	62.3 a	22.5 a	15.2 ab	Sandy loam	1.00 b	2.46 a	59.43 ab	48.75 bc	10.69 a
250	4.62 a	94.92 c	63.3 a	24.1 a	12.6 b	Sandy loam	1.08 c	2.60 bcd	58.28 bc	51.01 ab	7.27 bc
500	11.39 b	98.77 d	71.8 b	24.2 a	4.1 c	Loamy sand	1.07 c	2.66 c	59.70 ab	55.35 d	4.35 d
750	11.96 b	99.80 e	83.7 c	14.7 b	1.6 cd	Loamy sand	1.02 ab	2.64 cd	61.61 d	54.79 d	6.82 cd
1000	11.65 b	99.99 e	94.2 d	5.4 c	0.4 cd	Sand	0.96 d	2.58 ad	62.77 d	48.99 abc	13.78 e
SM	-	99.92 e	92.1 d	7.0 c	0.9 d	Sand	1.08 c	2.55 a	57.46 c	47.61 c	9.85 ab
Soil B											
Control	-	87.91 a	40.5 ab	45.3 a	14.3 a	Loam	1.00 a	2.48 a	59.68 a	52.99 ab	6.69 a
105	-	89.48 b	37.8 a	48.7 ab	13.4 a	Loam	1.03 ab	2.45 a	57.90 ab	51.53 ab	6.37 ab
250	6.49 a	94.74 c	38.8 ab	48.5 ab	12.7 a	Loam	1.21 c	2.49 a	51.58 c	45.60 c	5.98 ab
500	10.02 b	97.79 d	40.5 ab	54.4 b	5.1 b	Silt loam	1.22 c	2.66 b	54.18 cd	51.49 a	2.69 b
750	11.86 c	99.81 e	45.0 b	52.5 b	2.4 bc	Silt loam	1.08 b	2.63 b	58.81 a	53.32 ab	5.49 ab
1000	12.32 d	99.96 e	70.8 c	28.8 c	0.3 c	Loamy sand	0.98 a	2.62 b	62.72 e	54.23 b	8.49 a
SM	-	99.78 e	61.0 d	38.0 d	1.0 c	Sandy loam	1.18 c	2.66 b	55.58 bd	48.17 d	7.41 a
Soil C											
Control	-	79.94 a	28.7 a	50.6 a	20.7 a	Silt loam	0.78 a	2.23 a	65.22 a	60.68 a	4.54 ab
105	-	82.60 b	29.6 a	51.5 a	18.9 a	Silt loam	0.75 a	2.31 a	67.50 b	59.25 a	8.25 c
250	12.30 a	92.69 c	31.3 a	56.3 a	12.4 b	Silt loam	0.88 b	2.37 a	62.88 c	58.34 a	4.54 ab
500	15.17 b	96.30 d	45.8 b	52.4 a	1.8 c	Sandy loam	0.89 b	2.63 b	66.27 ab	63.73 b	2.54 b
750	18.14 c	98.85 e	60.0 c	38.5 b	1.5 c	Sandy loam	0.87 b	2.65 b	67.09 ab	64.97 b	2.13 b
1000	19.25 d	100.04 f	77.1 d	22.7 c	0.2 c	Loamy sand	0.87 b	2.61 b	66.68 ab	59.59 a	7.09 ac
SM	-	99.86 f	72.4 d	27.1 c	0.5 c	Loamy sand	1.15 c	2.64 b	56.37 d	50.79 c	5.58 ac
St.Dev.	0.07-0.53	0.01-0.63	0.23-6.47	0.53-5.14	0.1-2.38		0.00-0.05	0.01-0.11	0.07-1.85	0.11-1.20	0.00-0.86

Table 2.3. Chemical properties of control, heated, and smouldered (SM) soils. The ranges of standard deviations, based on triplicate anlysis, for each test are listed at the bottom of each column; significantly similar (ANOVA) are grouped and denoted by lettered post-scripts.

	Basic Soil Properties				Total Content (g/kg)		Bio-Available Content (mg/kg)					
Temp (C)	TOC (%)	pH	E.C. (µS/cm)	C.E.C. (cmolc/kg)	N	P	N	P	K	Ca	Mg	Cu	Zn
Soil A													
Control	5.47 a	7.36 a	168 ab	22.6 a	1.14 a	2.39 a	8.6 a	170 a	449 a	3667 a	194 a	23.7 a	45.6 a
105	5.37 b	7.04 a	212 a	22.5 a	1.16 a	2.39 a	9.2 a	144 b	372 b	3670 a	176 b	22.0 ab	36.4 b
250	2.66 c	7.16 a	862 c	14.3 b	1.33 b	2.70 a	48.3 b	230 c	315 c	3603 a	154 c	21.7 b	57.5 c
500	0.14 d	7.99 b	864 c	4.9 c	0.09 c	2.74 a	0.3 c	138 b	174 d	2673 b	134 d	3.9 c	26.9 d
750	0.04 d	8.36 b	753 d	1.8 d	0.00 c	2.89 a	0.3 c	75 d	95 e	1556 c	110 e	1.6 d	6.5 e
1000	0.02 d	8.86 c	426 e	0.0 e	0.00 c	1.60 b	0.6 d	9 e	33 f	692 d	54 f	0.7 d	1.1 f
SM	0.05 d	8.40 b	117 b	0.0 e	0.02 c	0.60 c	0.9 d	5 e	22 f	150 e	15 g	4.0 c	8.8 e
Soil B													
Control	5.48 a	5.56 a	62 a	21.9 a	1.09 a	1.27 ab	8.3 a	110 a	114 a	1287 a	109 a	6.9 a	21.0 a
105	4.80 a	5.18 b	112 b	22.2 a	1.00 a	1.27 ab	9.0 a	107 a	89 b	1354 a	101 b	6.8 a	19.3 a
250	2.65 b	5.61 a	410 c	9.8 b	1.02 a	1.70 a	134.3 b	121 a	88 b	857 b	63 c	4.2 b	16.6 b
500	0.82 c	6.41 cd	426 c	3.1 c	0.39 b	1.51 a	7.1 a	94 b	196 c	569 c	81 d	2.3 c	5.1 c
750	0.21 c	6.73 c	321 d	1.0 cd	0.01 c	1.51 a	0.3 cd	62 c	153 d	341 d	2 e	0.9 d	1.1 d
1000	0.04 c	8.21 e	51 a	0.4 d	0.00 c	0.32 c	0.2 d	3 d	34 e	88 e	0 e	0.1 d	0.3 d
SM	0.12 c	6.06 d	299 d	0.8 cd	0.08 c	0.81 bc	1.1 ac	62 c	112 ab	288 d	3 e	0.6 d	1.4 d
Soil C													
Control	8.20 a	4.11 a	110 a	30.1 a	1.28 a	0.87 a	9.4 a	33 a	66 ab	118 a	49.5 a	1.2 ab	4.9 a
105	7.62 a	3.58 b	302 b	29.9 a	1.12 a	0.87 a	10.6 a	33 a	59 bc	80 bcd	44.2 a	1.3 bc	4.5 ab
250	3.00 b	4.66 c	266 c	11.4 b	1.54 b	1.11 a	108.8 b	51 b	92 a	78 cd	35.3 bc	1.7 c	4.1 b
500	2.24 b	5.07 d	267 c	3.5 c	0.64 c	1.14 a	13.1 a	66 c	189 d	86 bc	96.3 d	1.3 bc	1.5 c
750	0.42 c	5.70 e	306 b	1.9 d	0.08 d	0.89 a	0.9 c	74 c	164 d	85 bc	85.9 d	0.7 ad	0.4 d
1000	0.03 c	8.01 f	23 d	0.0 e	0.00 d	0.09 b	0.1 d	4 d	35 c	70 d	21.3 c	0.1 e	0.1 d
SM	0.09 c	5.73 e	176 e	0.2 e	0.02 d	0.31 b	0.4 d	35 a	59 bc	92 b	36.5 bc	0.3 d	0.9 e
St.Dev.	0.01-0.5	0.01-0.35	0.3-41.6	0.01-2.1	0.01-0.11	0.03-0.5	0.1-13	1-11.9	1.3-20.1	0.6-255	0-13	0-0.83	0.03-1.8

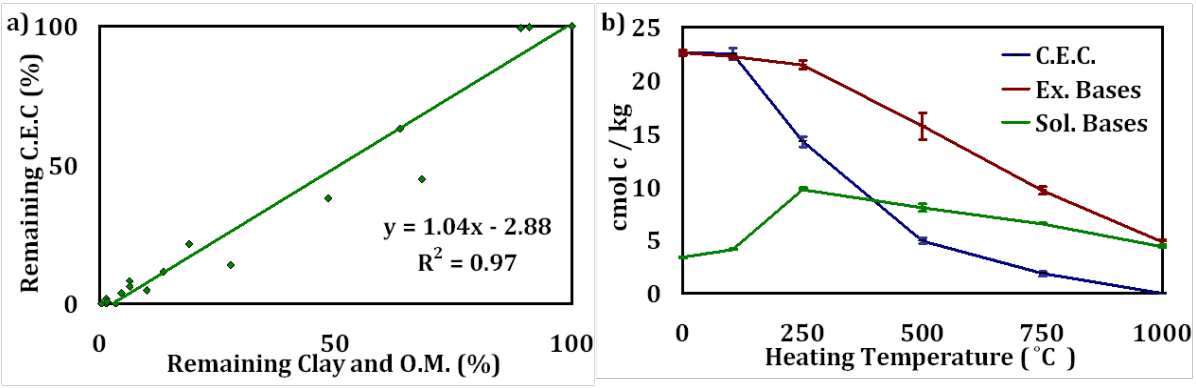


Figure 2.1. a) Correlation of reductions in CEC with reductions in the sum of the organic and clay content of all three soils, b) Relative levels of total soluble and exchangeable bases compared to CE.C for soil A, other soils showed a similar pattern but lower levels of base saturation due to initial soil conditions.

As exposure temperature increased from ambient to 1000°C, TOC decreased, pH increased, and CEC decreased (Table 2.3). Nearly all organic content was removed from the soil after exposure to 750°C. The loss of organic matter and clayed sized particles correlated with the reduction of CEC (Figure 2.1a) until all soil-CEC values approached zero at 1000˚C (Table 2.3). Reduced CEC reflects the soils' inability to retain cations such as calcium, potassium and ammoniacal nitrogen, which was evident in the increased proportion of exchangeable bases which were also water soluble as heating temperature increased (Figure 2.1b). A resulting spike in electrical conductivity (EC) was observed in soils A and B at 250°C (Table 2.3) as leachable bases increased; in soil C, this spike in EC was absent due to the low initial level of base saturation (Table 2.3). By 1000°C, almost all the remaining

exchangeable bases were water soluble due to the very low levels of CEC, this would leave these soils highly vulnerable to leaching.

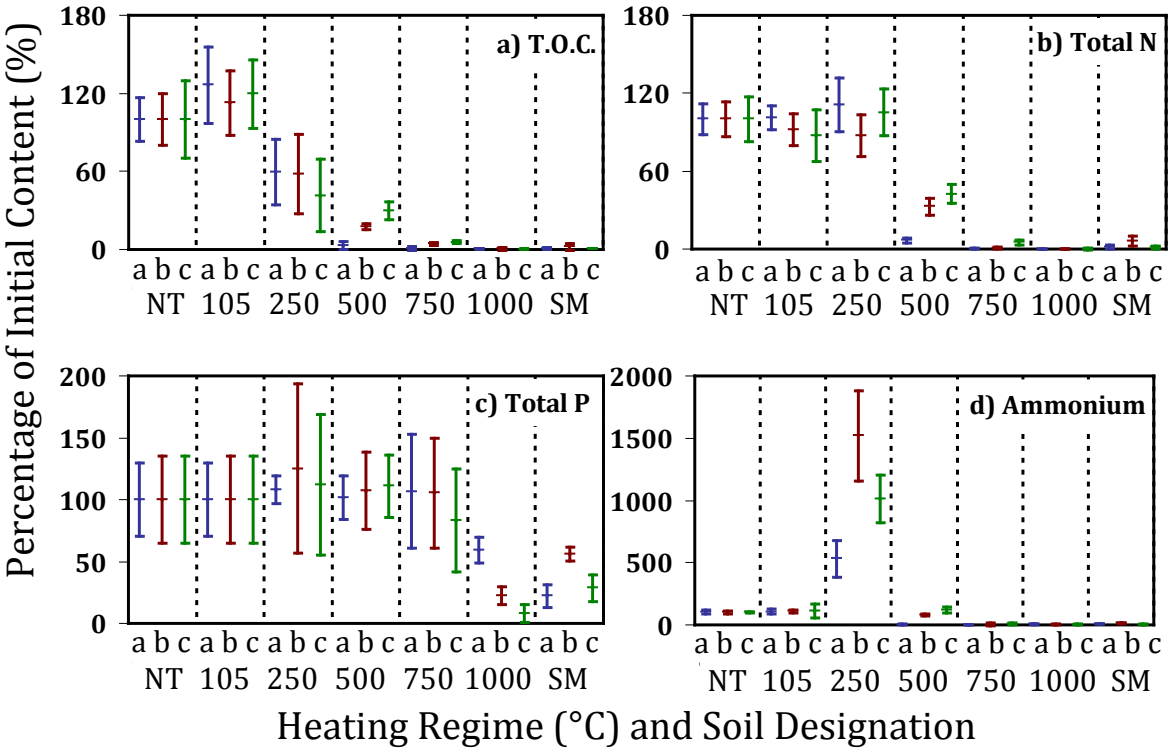


Figure 2.2. Soil properties of Total Organic Carbon (TOC), Total Nitrogen (TN), Total Phosphorus (P) and Ammonium show consistent changes with increasing temperature in all three soils relative to controls that were not treated (NT). Error bars represent the 95% confidence interval and all value are normalised to the initial soil concentrations.

In addition to nutrient holding capacity, nutrient levels were dependent on heating temperature. Loss of nitrogen was apparent at temperatures greater than 500°C, which was consistent with previous studies (Glass *et al*., 2008; Gray & Dighton, 2006). This pattern of loss was due to the volatilisation of nitrogen and consistent

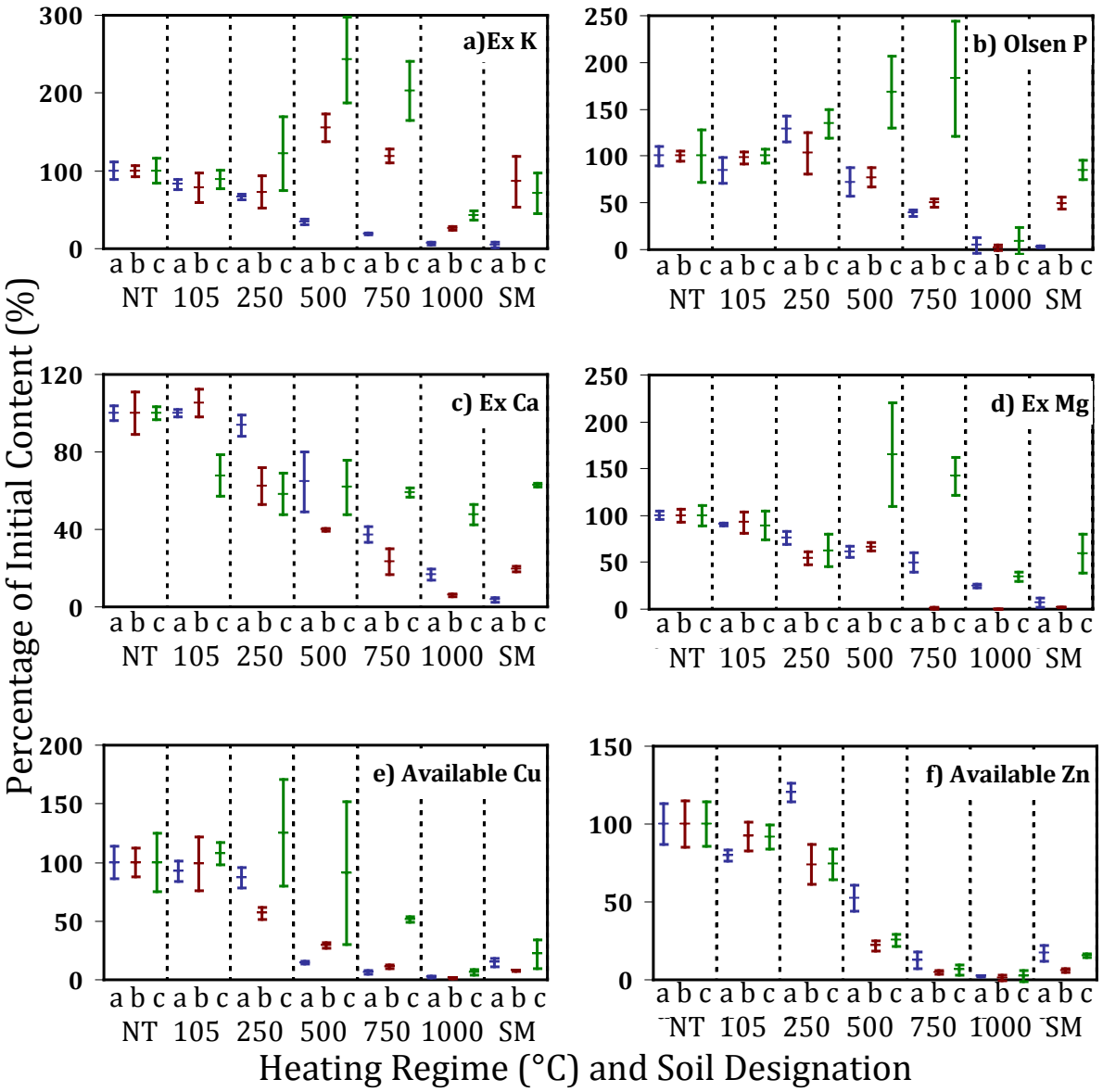


Figure 2.3. Soil properties of exchangeable potassium (Ex K), Olsen phosphorus (P), exchangeable calcium (Ex C), exchangeable magnesium (Ex Mg), available copper (Cu), and available zinc (Zn) show variable responses to heating in different soils relative to controls that were not treated (NT). Error bars represent the 95% confidence interval and all value are normalised to the initial soil concentrations

in the three soils studied, with almost complete loss by 750°C (Figure 2.2). Interestingly, heating briefly affected the bioavailability of ammonium in the soil. In all three soils, ammonium levels increased at 250°C (Figure 2.2) from the pyrolysis of organic matter. At temperatures of 500°C and above, ammonium levels and total nitrogen levels declined significantly. Loss of nitrogen compounds from the rhizosphere may have significant effects on the potential for plant and bacterial regrowth after remediation.

At 1000°C, the loss of phosphorus, another essential nutrient, was observed (Figure 2.2). The cause for its decline remains unknown; it may be due to volatilisation (Galang *et al*., 2010), but partitioning into a more recalcitrant form may have been possible as the *aqua-regia* used is not able to fully digest all siliceous materials. Either way, phosphorus decline will result in phosphate deficiencies for plants and microbes in soils heated at very high temperatures.

So far, the aforementioned nutrients have shown similar patterns of change in all the soils tested, and the effects have shown to be predictable. However, the bioavailability of many nutrients exhibited soil-dependent concentration patterns across the intermediate temperature profiles (Figure 2.3). Certain macro-nutrients (Ca, Mg, K and P) and micro-nutrients (Cu and Zn) displayed this behaviour. At 1000°C in all three soils, mineral availability declined, but remained relatively unpredictable at mid-range temperatures. This general reduction is likely attributed

to the formation of new mineral phases. For example, the formation of insoluble minerals such as copper oxides and calcite have been observed above 300°C (Iglesias *et al*., 1997; Wei *et al*., 2001), as well as the encapsulation of active soil components into the interior of fused aggregates (Ulery & Graham, 1993) or glassy matrices (Ketterings *et al*., 2000). These types of changes can explain the reduction, with some variation, of calcium, zinc and copper levels above 500˚C. However, these processes do not explain the increases in available potassium, magnesium and phosphorus levels seen in some soils between 500˚C and 750˚C (Figure 2.3). One possible explanation for these increases could be the loss of the clay fraction in the soil that occurred in the same temperature range (Table 2.2). Heating has different effects on different clay minerals with some decomposing and others dehydrating, dehydroxylating and aggregating (Ulery *et al*., 1996). If the clay minerals decomposed, then releases of interlayer potassium and magnesium would be expected, as would the release of any adsorbed phosphate causing the spikes seen in soils B and C as the clay fraction disappeared. The presence and type of clay may explain why the patterns are different between the three soils as the mineralogical make up is unlikely to be similar. If the mineralogy is linked to specific changes in the availability of these nutrients, then it may be possible to predict changes based on soil type. Future work classify the initial soil minerology and any changes during heating using methods such as XRD would be valuable to determine if this is the case.

2.5.3 Effects of Smouldering Remediation on Soil

It is important to understand the effects of actual operating conditions over a range of remediation processes. As such, smouldering remediation was carried out to remediate soils contaminated with coal tar (Switzer *et al.*, 2009). Figure 2.4 shows that the three soils achieved different mean peak temperatures, approximately 1100°C for soil A and 1000°C for soils B and C during smouldering. The residence times at peak temperature was a few minutes during smouldering process, compared to one hour in the laboratory heated soils. However, this brief residence time was sufficient to remove over 99.98% of the contaminant from the soil (Table 2.1).

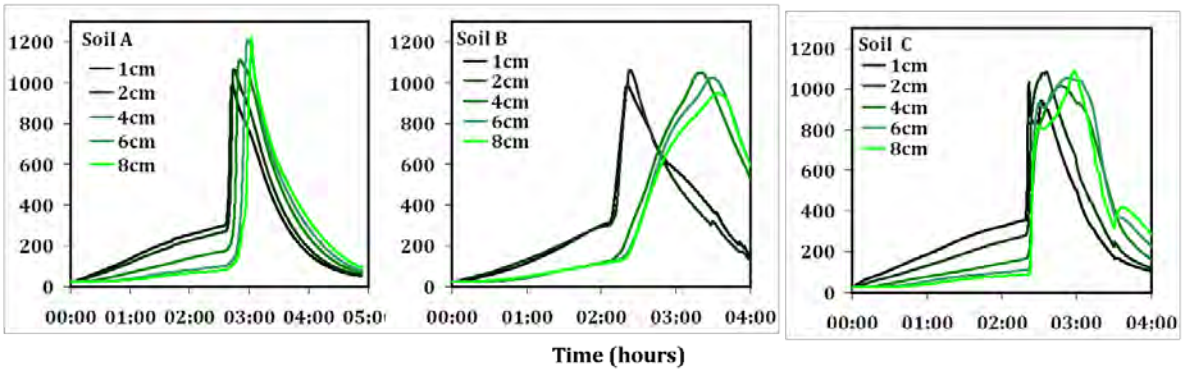


Figure 2.4. Temperature evolution profiles versus time during the smouldering treatments of soils A, B, and C. Distances reported are the distance of the thermocouple above the heating element.

The same chemical and physical tests were carried out on smouldered soils. Contamination with coal tar and subsequent smouldering remediation removed 98-

99% of the TOC, increased the soil pH, and reduced the CEC to near 0 (Table 2.3). Electrical conductivity was more variable, in the range of 100-300 µS/cm, which represented values in the low to moderate range when compared to heat treatments of the same soils. Equivalent reductions were found in nitrogen, organic carbon and clay levels to soils heated at 1000°C (Tables 2.2 and 2.3); in the heated soils these chemical and physical properties stabilised below exposure temperatures of 750°C.

Where soil properties were still in a state of dynamic change at 750°C, smouldering conditions became important in predicting changes. When smouldered, soil A showed more severe nutrient depletion than the 1000°C heated treatment; for example, bio-available calcium was 75% lower, due to the high temperature achieved (1100°C). This temperature also caused soil A to undergo more extensive physical changes with some of the soil minerals melting and fusing into a single solid block as new minerals formed on cooling. Aggregation occurred to a limited extent at 1000°C with the formation of a crumb-like material that easily broke down with hand pressure. Following smouldering, the aggregates were so durable that chiselling was required to break them apart. In a second smouldering run, the same effect was observed in this soil, but with a patchier distribution.

In comparison, soils B and C showed changes to bio-available phosphorus, calcium and potassium that were equivalent to changes observed between the 750°C and 1000°C in the heated treatments. This intermediate change was probably due to

the low residence time at peak temperatures as soil changes during heating have been shown to have a temporal component (Galang *et al*., 2010; Marcos *et al*., 2007). This is significant as it means deficiencies of certain nutrients could depend on the exact temperatures and durations achieved during smouldering. This shows that the data from the heated soils can be used to predict the effects of remediation techniques. However, it may be necessary to consider factors other than temperature when making predictions; in particular residence time at operating temperatures should be considered.

2.5.4 Implications for Remediation Processes

Thermal remediation techniques expose soils to a wide range of temperatures and exposure durations. Initial contamination with NAPLs may provide some protection to soil surfaces but have other effects on soil chemistry. Once the soil itself is exposed directly to high temperatures, based on experimental evidence, it is possible to predict the likely impact on soil quality. For many in-situ thermal techniques designed to remediate volatile molecules, such as conductive, steam injection, electrical and microwave heating, the operational temperatures are around 100°C for periods of weeks to months (Heron *et al*., 2005; Buettner & Daily, 1995; Heron *et al*., 2009). Therefore, these treatments are unlikely to have long-term impacts on soil properties. However, sterilisation may occur at temperatures greater than 100°C (Certini, 2005), which affects the populations available for bio-remediation and nutrient cycling. Nutrient replenishment may not be required, but re-populating the soils may be beneficial. Inoculation could be accomplished by

adding compost, compost teas (Lowenfields & Lewis, 2010) or other liquid inoculants with active microbial populations.

Nutrient deficiencies become an issue with more aggressive methods. For example, microwave heating, thermal blanket and ex-situ bulk heating (all >250°C) are likely to result in changes to the chemical and physical properties of the soil. There is a risk of nitrogen depletion, depending on temperature and residence time; the reduction of organic content and associated physical changes which can enhance leaching potential. Additionally, the increase in ammonium levels, which occurred at 250°C, may be toxic to certain plants (Britto and Kronzucker, 2002). Another effect relevant at this temperature, but not explored in this study, is the development of water repellence (DeBano, 2000). This could potentially cause problems with re-wetting the soil for plant establishment, but also increase run-off and erosion problems before a vegetative cover is established. Plants may be able to stabilise the soil and re-introduce carbon. Organic amendments may help plants overcome potential phytotoxicity by adsorbing toxic constituents, facilitating bio-transformation and improving the physical properties of the soil. In addition, these amendments may help re-establish the microbial community.

The higher temperatures associated with smouldering (>1000°C), incineration (800°C) and conductive and thermal blanket treatments close to the heating source (up to 800°C) will impact site use. The most significant problem with these soils will likely be deficiency of nutrients, particularly nitrogen, which will hinder plant

growth and microbial colonisation in these soils. In addition, nutrient retention and vulnerability to erosion will be affected by textural and physical changes and the loss of organic matter. One possible solution to the nutrient deficiency could be to use chemical fertilizers; however, with the low CEC and changes to the soil minerals, chemical nutrients may become lost quickly by leaching. Organic additions, such as compost and bio-solids, may be more appropriate to use (Alvarenga *et al*., 2008; Hargreaves *et al*., 2008, Bendfelt *et al*., 2001) as the nutrients are slowly released by decomposition so that microbes or plants have a steady source of nutrients to support their establishment in the soil. Hopefully the slow release nutrients will be incorporated into biomass before they have the opportunity to leach from the soil. Organic amendments also benefit by improving soil structure and providing an inoculation of soil microorganisms to help restore natural nutrient cycles (Alvarenga *et al*., 2008; Hargreaves *et al*., 2008, Bendfelt *et al*., 2001).

These strategies have been used in analogous situations to improve soil quality, such as the reclamation of nutritionally poor mine spoils (Alvarenga *et al*., 2008; Hargreaves *et al*., 2008, Bendfelt *et al*., 2001). Additionally, the use of green manures could be beneficial to limit cost and provide more sustainable solutions. Green manures are mixtures of nitrogen fixing plants (e.g., legumes) and organic-matter building plants (e.g., grasses) that are used in agriculture to improve soil fertility and structure. These have been shown to be important in the ecological restoration of mine wastes (Li & Daniels, 1994; Jefferies *et al*., 1981) and could help soils subject to high temperature treatments.

2.6 Conclusion

Very few studies have compared thermal-related effects on soils over the full range of remediation temperatures. The results suggest that major chemical and physical changes occur in the soils, which can impact the success of subsequent remediation strategies. For instance bioremediation requires an optimal C:N ratio to facilitate microbial activity (Bamforth & Singelton, 2005), therefore the loss of nitrogen above 500˚C is likely to hinder the metabolic breakdown of residual contaminants. These changes will also alter soil quality for plant growth, with effects becoming more severe as temperature increases, suggesting that further soil improvement steps are required if agriculture, biomass production or environmental enrichment is the long term goal for a site. Additionally unique to this study is the inclusion of soils undergoing smouldering remediation, which is one of the most aggressive in terms of temperatures generated with peak temperatures easily exceeding 1000 ºC with contaminants such as coal tar (see Fig 3). In some cases, nutrient-poor soil may be beneficial, particularly for industrial redevelopment with below-ground infrastructure. In cases where nutrient-rich soil is desirable, this study outlines specific problems associated with aggressive soil heating and suggests specific treatments after remediation that may facilitate a greater range of land uses and ensure that remediated sites are not just clean but of use to society and the local ecosystem.

2.7 Acknowledgements

The research was supported by the Department of Civil & Environmental Engineering and Glasgow Research Partnership in Engineering (GRPe). AP was supported by EPSRC Doctoral Training Account at University of Strathclyde. The authors thank Stephanie Zihms and Neil McCosh for their help, advice and support. Smouldering combustion of liquids is a patented remediation process (e.g. USA 8132987 with others pending, PCT Application PCT/GB2006/004591, priority date 10th December 2005, and PCT/US2012/035248, priority date 29 April 2011).

2.8 References

ADAS 1985. *The analysis of agricultural materials*. Agricultural Development and Advisory Service, London

Alvarenga, P., Goncalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E. & Cunha-Queda, A.C. 2008. Evaluation of composts and liming materials in the phytostabilisation of a mine soil using perennial ryegrass. *Science of the Total Environment*, **406**, 43-56

Anthony, E.J. & Wang, J. 2006. Piolt plant investigations of thermal remediation of tar-contaminated soil and oil-contaminated gravel. *Fuel*, **85**, 443-450

Bamforth, S.M & Singleton, I.S. 2005. Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology Biotechnology*, **80**, 723-736

Basta, N.T. & Tabatabai, M.A. 1985. Determination of Exchangeable Bases in Soils by Ion Chromatography. *Soil Science Society of America Journal,* **49**, 84-89

Bendfeldt, E.S., Burger, J.A. & Daniels, W.L. 2001. Quality of amended mine soils after sixteen years. *Soil Science Society of America Journal*, **65**, 1736-1744

Britto, D.T. & Kronzucker, H.J. 2002. NH_4^+ toxicity in higher plants: A critical review. *Journal of Plant Physiology*, **159**, 567-584

The British Standards Institute 1995a. *BS 7755 Soil Quality, Section 3.8 Determination of organic and total carbon after dry combustion (elementary analysis)*. The British Standards Institute, London

The British Standards Institute 1995b. *BS 7755 Soil Quality, Section 3.6 Determination of phosphorus – Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution*. The British Standards Institute, London

The British Standards Institute 1995c. *BS 7755 Soil Quality, Section 3.4 Determination of the specific electrical conductivity*. The British Standards Institute, London

The British Standards Institute 2000a. *BS EN 13039 Soil improvers and growing media – Determination of organic matter and ash*. The British Standards Institute, London

The British Standards Institute 2000b. *BS EN 13041 Soil improvers and growing media – Determination of physical properties – Dry bulk density, air volume, water*

volume, shrinkage value and total pore space. The British Standards Institute, London

The British Standards Institute 2001a. *BS EN 13654 Soil improvers and growing media – Determination of nitrogen, Part 2 Dumas method*. The British Standards Institute, London

The British Standards Institute 2001b. *BS EN 13650 Soil improvers and growing media – Extraction of aqua regia soluble elements*. The British Standards Institute, London

The British Standards Institute 2005. *BS ISO 10390 Soil Quality – Determination of pH*. The British Standards Institute, London

The British Standards Institute 2009. *BS ISO 11277 Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation*. The British Standards Institute, London

Buettner, H.M. & Daily, W.D. 1995. Cleaning contaminated soil using electrical heating and air stripping. *Journal of Environmental Engineering*, **121**, 580-589

Bundy, L.G. & Meisinger, J.J. 1994. *Nitrogen Availability Indices.* In : Weaver, R.W, Angle, J.S. & Bottomley, P.S. (eds.) 1994. *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America, Madison USA

Certini, G. 2005. Effects of fire on properties of forest soils: A review. *Oecologia*, **143**, 1-10

Covington, W.W. & Sackett, S.S. 1992. Soil mineral nitrogen changes following prescribed burning in ponderosa pine. *Forest Ecology and Management*, **54**, 175-191

DeBano, L.F. 2000. The role of fire and soil heating on water repellency in wildland environments: a review. *Journal of Hydrology*, **231-232**, 195-206

Galang, M.A., Markewitz, D. & Morris, L.A. 2010. Soil phosphorus transformations under forest burning and laboratory heat treatments. *Geoderma*, **155**, 401-408

Granged, A.J.P, Zavala, L., Jordan, A. & Moreno, G.B. 2011. Post fire evolution of soil properties and vegetation cover in a Mediterranean heathland after experimental burning: A 3-year study. *Geoderma,* **164**, 85-94

Gray, D.M. & Dighton, J. 2006. Mineralisation of forest litter nutrients by heat and combustion. *Soil Biology and Biochemistry*, **38**, 1469-1477

Glass, D.W., Johnson, D.W., Blank, R.R. & Miller, W.W. 2008. Factors effecting mineral nitrogen transformations by soil heating: A laboratory-simulated fire study. *Soil Science*, **173**, 387-40

Hargreaves, J.C., Adl, M.S. & Warman, P.R. 2008. A review of the use of composted municipal solid waste in agriculture. *Agriculture, Ecosystems and Environment*, **123**, 1-14

Heron, G., Carroll, S. & Nielsen, S.G. 2005. Full-scale removal of DNAPL constituents using steam-enhanced extraction and electrical resistive heating. *Ground Water Monitoring and Remediation*, **25**, 92-107

Heron, G., Parker, K., Galligan, J. & Holmes, T.C. 2009. Thermal treatment of eight CVOC source zones to near nondetect concentrations. *Ground Water Monitoring and Remediation*, **29**, 56-65

Iben, I.E.T., Edelstein, W.A., Sheldon, R.B., Shapiro, A.P., Uzgiris, E.E., Scatena, C.R., Blaha, S.R., Silverstein, W.B., Brown, G.R., Stegmeier, G.L. & Vinegar, H.J. 1996. Thermal blanket for in-situ remediation of surficial contamination: A pilot test. *Environmental Science and Technology*, **30**, 3144-3154

Iglesias, T., Cala, V. & Gonzalez, J. 1997. Mineralogical and chemical modifications in soils affected by a forest fire in the Mediterranean area. *The Science of the Total Environment*, **204**, 89-96

Jackson, P.E. 2000. *Ion Chromatography in Environmental Analysis*. In: Meyer, R.A. (ed.) 2000. *Encyclopedia of Analytical Chemistry*. John Wiley & Sons, Chichester

Jefferies, R.A., Wilson, K. & Bradshaw, A.D. 1981. The potential of legumes as a nitrogen source for the reclamation of derelict land. *Plant and Soil*, **59**, 173-177

Kang, B.T. & Sajjapongse, A. 1980. Effect of heating on properties of some soils from southern Nigeria and growth of rice. *Plant and Soil*, **55**, 85-95

Ketterings, Q.M., Bigham, J.M. & Laperche, V. 2000. Changes in soil mineralogy and texture caused by slash and burn fires in Sumatra, Indonesia. *Soil Science Society of America Journal*, **64**, 1108-1117

Li, R.S. & Daniels, W.L. 1994. Nitrogen accumulation and form over time in young mine soils. *Journal of Environmental Quality*, **23**, 166-172

Lowenfels, J. & Lewis, W. 2010. *Teaming with Microbes*. Timber Press, London

Marcos, E., Tárrega, R. & Luis, E. 2007. Changes in a humic cambisol heated (100-500°C) under laboratory conditions: The significance of heating time. *Geoderma*, **138**, 237-243

Merino, J. & Bucalá, V. 2007. Effect of temperature on the release of hexadecane from soil by thermal treatment. *Journal of Hazardous Materials*, **143**, 455-461

Pironi, P., Switzer, C., Rein, G., Fuentes, A., Gerhard, J.I. & Torero, J.L. 2009. Small-scale forward smouldering experiments for remediation of coal tar in inert media. *Proceedings of the Combustion Institute*, **32**, 1957-1964

Raison, R.J., Khanna, P.K. & Woodsa, P.V. 1985. Mechanisms of element transfer to the atmosphere during vegetation fires. *Canadian Journal of Forestry Research*, **15**, 132-139

Robinson, J.P., Kingman, S.W., Snape, C.E., Shang, H., Barranco, R. & Saeid, A. 2009. Seperation of polyaromatic hydrocarbons from contaminated soils using microwave heating. *Separation and Purification Technology*, **69**, 249-254

Shand, C.A., Williams, B.L. & Coutts, G. 2008. Determination of N-species in soil extracts using microplate techniques. *Talanta*, **74**, 648-654

Smith, N.R., Kishchuk, B.E. & Mohn, W.W. 2008. Effects of wildfire and harvest disturbances on forest soil bacterial communities. *Applied and Environmental Microbiology*, **74**, 216-224

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G. & Torero, J.L. 2009. Self-sustaining smoldering combustion: A novel remediation process for non-aqueous-phase liquids in porous media. *Environmental Science and Technology*, **43**, 5871-5877

Terefe, T., Mariscal-Sancho, I., Peregrina, F. & Espejo, R. 2008. Influence of heating on various properties of six Mediterranean soils: A laboratory study. *Geoderma*, **143**, 273-280

Ulery, A.L. & Graham, R.C. 1993. Forest fire effects on soil color and texture. *Soil Science Society of America Journal*, **57**, 135-140

Ulery, A.L., Graham, R.C. & Bowen, L.H. 1996. Forest fire effects on soil phyllosilicates in California. *Soil Science Society of America Journal*, **60**, 309-315

Wei, Y.L., Yang, Y.W. & Cheng, N. 2001. Study of thermally immobilised CU in analogue minerals of contaminated soils. *Environmental Science and Technology*, **35**, 416-421

Chapter 3 - Impacts of Thermal and Smouldering Remediation on Plant Growth and Soil Ecology

Andrew Pape, Neil McCosh, Christine Switzer and Charles Knapp

3.1 Preface

This Chapter will be submitted for publication in *Geoderma* in July 2014. In Chapter 2, significant changes to the physical and chemical nature of soils were found when they were subjected to heating and smouldering remediation. This Chapter investigates the extent to which these changes impact plant growth, microbial colonisation and microbial activity. Two soils were used in this Chapter (Soil 1 and Soil 2) with Soil 1 corresponding to Soil B in Chapter 2.

A. Pape was responsible for the majority of the experimental design, practical work, and data analysis, and was the main author. C.W. Knapp and C. Switzer provided editorial comments in preparing the chapter and advice on experimental design and data analysis and presentation. N. McCosh handled DNA extraction and qPCR of soil samples.

3.2 Summary

Thermal (40-1000°C) and smouldering (600-1100°C) remediation are able to remove significant quantities of organic toxins from contaminated sites, reducing environmental and public health impacts while potentially impacting suitability for subsequent land use. Here, the effects of soil heating (ambient-1000°C) and smouldering remediation (~1000°C) on soil geochemistry, plant growth and microbial abundance and activity were quantified. Both red clover (*Trifolium pratense*) and red fescue (*Festuca rubra*) displayed a largely negative correlation with treatment temperature, exhibiting growth reductions of greater than 50% by 500°C and almost negligible growth at 750°C and above. These trends tied in with geochemical changes in the soil, particularly atmospheric losses of nitrogen and reduced availability of other nutrients (Cu, Zn, P). Microbial abundance and activity showed sharp declines in soils heated above 500°C. Although this transition corresponded to quantitative decline in carbon levels, qualitative changes to carbon compounds were likely to come into play. These changes reduced substrates available to heterotrophic microbes and hindered soil rehabilitation. Thermal remediation techniques operating above 500°C have a major impact on the ability of soils to sustain biological activity. As such, the use of soil conditioners such as compost or organic wastes would be essential in any rehabilitation plan.

3.3 Introduction

Soil contamination is a global problem. Europe faces an estimated 342,000 sites of known contamination and a further 2.5 million potentially contaminated sites (van Liederkerke *et al*, 2014). A great deal of effort has gone into developing remediation processes to remove or reduce the impact of these contaminants in the environment. For organic pollutants such as oils, tars, and polycyclic aromatic hydrocarbons (PAHs), a range of techniques has been developed using heat treatment or combustion processes to volatilise and extract or destroy these contaminants. For thermal treatments, typical operating ranges vary from around 100°C for many vapour extraction methodologies (Heron *et al*., 2005; Buettner and Daily, 1995) to in excess of 1000°C during ex-situ incineration of heavy oils and tars (Anthony and Wang, 2006). Smouldering remediation exposes soils to temperatures of 600 to 1100°C or more (Switzer *et al.,* 2009; Pironi *et al.,* 2011; Switzer *et al.*, 2014). Optimised treatment regimes can significantly reduce the contaminant load of the soil, sometimes to a point where its re-use can be considered; however, the effect of the treatment conditions on soil in relation to subsequent land use needs to be taken into account in order to support redevelopment of sites after remediation.

Remediation effects on soil will be particularly important if the remediated soil is to be used as a growing medium for plants, for phytoremediation, biomass crop production, habitat restoration after remediation, or green space. The effect of

heating on soil will depend on the temperatures reached during treatment and the duration of exposure. Even the lower temperatures (~100°C) utilised during remediation impact soil biota, killing plant propagules, macro fauna, and microorganisms (Certini, 2005). As temperatures increase, charring and subsequent loss of organic matter (Certini, 2005) and atmospheric losses of nitrogen (Glass *et al*., 2008; Gray & Dighton, 2006) begin to occur, both of which will become completely depleted by 500°C. Above this temperature, minerals start to break down and aggregate, physically altering the soil (Ulery *et al*., 1996; Terefe *et al*., 2008; Ketterings *et al*., 2000) and, together with the loss of organic matter (OM), severely reducing the ability of the soil to hold valuable nutrients (Kang and Sajjapongse, 1980). High temperatures also affect the availability of many macro-nutrients such as phosphorus, potassium and calcium (Kang and Sajjapongse, 1980; Galang *et al*., 2010). During very high temperature treatments, less volatile nutrients may be lost to the atmosphere. For instance, at 1000°C, phosphorus in the soil becomes lost (Galang *et al*., 2010). As a result, plant growth may be inhibited in soils where high temperature remediation procedures have been implemented.

At low heating temperatures (60-350˚C), complicated relationships seem to exist between heating temperature and plant growth. Combining the results of a number of studies, Johnson (1919) saw that heating in this range could have both positive and negative effects on growth depending on soil and plant type. At higher temperatures (>400˚C), results seem more consistent. For instance, Kang and

Sajjapongse (1980) saw reduced biomass in rice plants grown in soil heated to 500˚C compared to those heated to 200˚C or less. Similarly Roh *et al.* (2000), studying the thermal desorption of mercury, found greater plant growth in soil treated at 350˚C compared to 600˚C despite higher toxin levels. Given the range of temperatures now utilised during thermal remediation (ambient to over 1000˚C), an overview of the effect of soil heating on plant growth over this full range would be valuable to allow the effects of specific remediation techniques to be estimated.

In addition to impacts on plants, soil heating is also likely to have a significant effect on microbial populations in the soil. These are important for nutrient cycling, organic decomposition and, in terms of contaminated soils, bio-polishing of residual contaminants. Any heating is likely to have a detrimental effect on their population abundance and diversity levels. As such, successful re-colonisation of the soil by microorganisms will be a vital part of sustainable ecosystem recovery. This will depend on many factors, but the availability of food (carbon) and nutrients will be two of the key factors. For instance, Bárcenas-Morena and Bååth (2009) saw reduced microbial biomass after 21 days of incubation when carbon levels had significantly been reduced by heating to 400-500°C. Higher temperatures may remove carbon and nutrients and as a result, microbial re-colonisation of some of these soils may be inhibited. The extent to which micro-organisms can re-colonise soils in terms of population levels and functional roles will be important in predicting if treated soil systems will be viable in the long term without continuous external intervention.

This paper tests the hypothesis that heating soil in the range applicable to thermal and smouldering remediation will affect its' ability to function as a media to support plant growth and microbial activity. Plant growth in heated soils is used as a proxy for the likely effects of thermal and smouldering remediation processes. Changes to soil physical and chemical properties are quantified to determine changes to soil characteristics after treatment. Re-growth of the microbial community and enzymes activity are examined to establish the soils capability to effectively cycle nutrients. These analyses will aid in the identification of combinations of high temperature remediation and soil type require post treatment intervention to create an effective growing media for the desired land use.

3.4 Methodology

3.4.1 Soil Samples and Treatments

Two topsoils were selected for use in this study, an acidic loam (Soil 1) (Soil B in Chapter 2) from the north east of Scotland and a commercially available (B&Q Verve topsoil) horticultural soil (Soil 2) with a neutral pH. In chapter 2 it was clearly seen that different chemical changes can occur in different soils upon heating. Given this it was important to use more than one soil when looking at the effects on biological system, however the samples of soils A and C were not of sufficient volume for further study so a fourth soil sample was introduced. The soils were prepared by air drying and sieving to below 2mm (control). To simulate the effects of thermal remediation, each soil was oven dried at 105°C for 3 days to remove moisture

before being heat-treated at 250°C, 500°C, 750°C and 1000°C in a muffle furnace (Nabotherm P330, Lilienthal, Germany) for one hour (Chapter 2). As an additional treatment, each sieved and air-dried soil was artificially contaminated with coal tar (80g/kg) and treated via smouldering remediation (SM) (Pironi *et al*., 2011) to provide an example of a real remediation technique that utilises high temperatures.

3.4.2 Soil Analysis

Changes to the chemical and physical properties of the two soils were established after each of the heat treatments and the smouldering remediation (Chapter 2). Soil pH (BS, 2005) and electrical conductivity (BS, 1995c) were measured using a Multi 7 Mettlor-Toledo meter (Mettlor-Toledo International Inc, Columbus, OH, USA) after a two hour extraction in 1:5 soil:water mix. Total organic content was measured by loss on ignition (dry ashing at 550˚C for five hours) (BS, 2000). Total nitrogen (BS, 2001) was measured by quantifying NO_X production during combustion using an Apollo 9000 TOC/TN analyser (Teldyne Tekmar Mason, OH, USA). Inorganic nitrogen (NH_4^+, NO_3^- and NO_2^-) was measured colorimetrically using an indophenol blue method for NH_4^+, and a sulfanilic acid method for NO_2^- with a hydrazine reduction step for NO_3^- (ADAS, 1985; Bundy and Meisinger, 1994; Shand *et al.*, 2008). In this study, the only from of inorganic nitrogen present in measurable quantities was ammonium due to the drying and storage of the soil samples. Available phosphate was measured after an Olsen bicarbonate extraction using molybdate/ascorbic acid colorimetry (ADAS, 1985). Cation exchange capacity

and exchangeable bases (ADAS, 1985) were measured by sequential leaching with ammonium acetate to displace the base and then potassium chloride to displace the retained ammonium. The level of bases was determined by flame atomic absorption analysis of the ammonium acetate leachate (Perkin Elmer AAnalyst 100, Waltham, MA, USA). CEC was determined using indo-phenol blue colorimetrically (Bundy and Meisinger, 1994) to quantify the levels of ammonium in the potassium chloride leachate. The levels of bio available copper and zinc were measured by extraction in ammonium EDTA (ADAS, 1985) and ICP-OES analysis (ICP-OES, Thermo Scientific Hemel Hempstead, UK). Additionally, the proportions of clay, silt and sand were measured using wet sieving and sedimentation (BS, 2009) after dispersion in a sodium carbonate/sodium hexametaphosphate solution.

3.4.3 Plant Growth Trials

A sample of each treatment was wetted by adding 25% v/m of sterile de-ionised water with a second sample being microbially amended (MA) by adding 0.1% m/m of commercial mycorhizal inoculant and 25% v/m of aerated compost tea (ACT). The ACT was produced by suspending 500mL of compost in 15L of sterile, deionised water along with the juice of one orange aerating this mixture for 24hr to encourage microbial proliferation (Lowenfels and Lewis, 2010). This mixture was intended to supply a broad ranging microbial community to the soil samples but should provide little or no nutrients. The soils were incubated at 27°C for seven days and mixed thoroughly before being portioned into 200ml pots. This ensured

that each pot within the same treatment contained, as far as possible, the same starting population of microbes. Replicates of each treatment had nine red clover (*Trifolium pratense*) or nine red fescue (*Festuca rubra*) seeds planted, with additional samples left as unplanted controls. All pots were watered using a wick system to maintain constant moisture content (BS, 2011) and allowed to grow in a growth chamber at 27±2°C with 16 hours of light per day. One week after planting, the plants were thinned to two per pot. After a further six weeks the plants were harvested and the soils refrigerated (4˚C) for analysis. Six weeks was considered to be an appropriate period to allow substantial plant growth but prevent root systems from becoming excessively pot bound.

After harvest, the plants were dried at 70˚C and analysed for shoot and root extension and dry mass of the roots, shoots, and leaves. In addition, frozen sub-samples of the leaves were analysed for chlorophyll content. Chlorophyll was extracted by heating a leaf sample (20-100mg wet weight) in 10ml of di-methyl sulfoxide at 70°C for six hours (Hiscox and Israelstam 1979) and analysed colorimetrically according to the equations of Arnon (1949). For clover, the numbers of symbiotic root nodules were recorded for each plant.

3.4.4 Microbial Analysis

To understand the dynamics of the microbial population, its activity and gene abundance were quantified at the end of the growing period. To quantify gene

abundance in the soil, 0.25g samples were extracted using a MoBio (Carlsbad, CA, USA) PowerSoil DNA isolation kit and a cell disrupter. Overall abundance of bacteria was quantified by qPCR using a BioRad (Hercules, CA, USA) Sso Advanced enzyme system analysing portions of the gene encoding the 16S (Muyzer *et al*., 1993) ribosomal subunit. In addition, functional genes relating to nitrogen cycling were quantified using the same system including: *nifH* (Poly et al., 2001) for nitrogen fixation; *nirS* (Kandeler *et al*., 2006) and *nirK* (Henry *et al*., 2004) for nitrate reduction; and *amoA* (Rotthauwe *et al*., 1997) for ammonia oxidation. These functional genes were reported as a fraction of the overall abundance as quantified using the 16S qPCR. See Table 3.1 for list of specific primers used and qPCR operating conditions.

Table 3.1. Primer sets and operation conditions for qPCR

Gene	Primers	Melt	Annealing	Elongation	Source
16s	P1/P2	15s at 95˚C	15s at 55˚C	15s at 60˚C	Muyzer *et al*., 1993
nifH	PolF/PolR	10s at 95˚C	10s at 53˚C	10s at 54˚C	Poly et al., 2001
nirS	nirSCd3aF/nirSR3cd	10s at 95˚C	10s at 55˚C	10s at 60˚C	Kandeler *et al*., 2006
nirK	nirK876/nirK1040	10s at 95˚C	10s at 55˚C	10s at 60˚C	Henry *et al*., 2004
amoA	amoA-1F/amoA-2R	10s at 95˚C	10s at 52˚C	10s at 55˚C	Rotthauwe *et al*., 1997

As an indication of microbial activity in the soil, the activities of several extra-cellular enzymes were quantified. For the phosphorus and carbon cycles, the activities of monophosphatesterase (acid and alkali) and β-glucosidase were analysed using the nitrophenol conjugate system (Tabatabai, 1994; Eivazi and Tabatabai, 1988; Tabatabai and Bremner, 1969). In this system, nitrophenol is produced as functional groups (either phosphate or glucose) are removed by enzyme activity and quantified colorimetrically at 400nm after a strong alkali is added. For the nitrogen cycle, the activity of ammonia oxidase (Jarvis *et al.* 2009)

was measured by observing the production of nitrite by sulfanilic acid colorimetry in the presence of a suitable substrate (ammonium sulphate) and a metabolic inhibitor to prevent the conversion of nitrite to nitrate (sodium chlorate).

3.5 Results and Discussion

3.5.1 Physical and Chemical Changes to Soil Properties

When subjected to heating both soils exhibit similar changes to their physical composition. At temperatures of 250˚C and above, organic matter is lost through pyrolysis and oxidation with only a small fraction of the original content remaining at 750˚C (Table 3.2). At temperatures above 500˚C, clay sized particles start to disappear (Table 3.3) through aggregation and disintegration (Ulery *et al*. 1996; Terefe *et al*., 2008; Ketterings *et al*., 2000). Together, these losses result in a significant reduction in the CEC and affect the ability of the soils to hold nutrients and water. In addition, a qualitative change in the structure and cohesion of the soil was observed, with aggregate formation not apparent in soils heated to temperatures above 500˚C, which will leave such soils vulnerable to erosion in the field (Andreu *et al*., 2001). Collectively, these changes will impact rehabilitation plans in the field; soils heated above 500˚C may benefit from the addition of a bulking agent such as compost.

Table 3.2 - Changes to soil chemistry upon heating. ±S.D.

	Treat. (˚C)	pH	EC (µS/cm)	C.E.C. (cmolc/kg)	O.M. (%)	T. N. (g/kg)	In. N. (mg/kg)	O. P. (mg/kg)
Soil 1	Cont.	4.7 ± 0.0	79 ± 2.9	21.9 ± 2.1	13.6 ± 0.1	1.02 ± 0.08	8.3 ± 0.3	97.5 ± 4.4
	105	4.4 ± 0.1	225 ± 4.6	22.2 ± 0.8	11.7 ± 0.2	1.15 ± 0.08	9.0 ± 0.4	92.7 ± 1.1
	250	5.5 ± 0.1	310 ± 16.1	9.8 ± 0.5	6.9 ± 0.2	1.16 ± 0.06	134 ± 13	85.5 ± 4.2
	500	6.2 ± 0.2	353 ± 7.5	3.1 ± 0.3	2.8 ± 1.0	0.45 ± 0.04	7.1 ± 0.2	39.0 ± 2.7
	750	6.4 ± 0.2	286 ± 14.7	1.0 ± 0.1	0.6 ± 0.1	0.14 ± 0.01	BDL	36.4 ± 2.3
	1000	7.3 ± 0.1	55 ± 2.0	0.4 ± 0.3	0.2 ± 0.1	0.01 ± 0.00	BDL	3.0 ± 0.3
	SM	7.5 ± 0.2	120 ± 13		0.3 ± 0.0	0.08 ± 0.01	BDL	22.1 ± 5.5
Soil 2	Cont.	7.4 ± 0.0	732 ± 16	28.2 ± 4.6	17.3 ± 0.9	1.00 ± 0.10	6.0 ± 0.8	94.0 ± 10.6
	105	7.1 ± 0.0	978 ± 64	16.0 ± 3.2	16.5 ± 1.0	1.15 ± 0.08	11.4 ± 0.4	103.9 ± 4.4
	250	7.3 ± 0.1	1489 ± 81	14.2 ± 3.7	11.6 ± 0.3	0.92 ± 0.07	23.7 ± 1.3	114.0 ± 7.1
	500	9.0 ± 0.0	948 ± 39	6.3 ± 0.9	5.1 ± 0.1	0.62 ± 0.10	6.9 ± 1.5	125.2 ± 9.5
	750	11.3 ± 0.0	1241 ± 8	2.2 ± 0.7	1.9 ± 0.1	0.25 ± 0.04	BDL	51.8 ± 0.9
	1000	11.4 ± 0.1	1410 ± 145	0.6 ± 0.2	0.0 ± 0.0	0.02 ± 0.00	BDL	22.0 ± 0.9
	SM	11.7 ± 0.2	1783 ± 37	0.8 ± 0.3	0.2 ± 0.1	0.10 ± 0.00	BDL	44.9 ± 8.4

	Treat. (˚C)	Ex. Ca (mg/kg)	Ex. Mg (mg/kg)	Ex. K (mg/kg)	Ex. Na (mg/kg)	Ex. Mn (mg/kg)	Av. Cu (mg/kg)	Av. Zn (mg/kg)
Soil 1	Cont.	1287 ± 56	109 ± 3	114 ± 3	127 ± 13	4.3 ± 0.3	6.9 ± 0.3	21.0 ± 1.2
	105	1354 ± 37	101 ± 5	89 ± 9	142 ± 32	10.2 ± 0.9	6.8 ± 0.6	19.3 ± 0.8
	250	857 ± 52	63 ± 3	88 ± 10	138 ± 8	32.4 ± 5.7	4.2 ± 0.2	16.6 ± 1.1
	500	569 ± 5	81 ± 2	196 ± 9	123 ± 8	10.8 ± 2.2	2.3 ± 0.1	5.1 ± 0.3
	750	341 ± 39	2 ± 0	153 ± 5	156 ± 14	5.5 ± 1.2	0.9 ± 0.0	1.1 ± 0.1
	1000	88 ± 4	0 ± 0	34 ± 1	130 ± 4	1.5 ± 0.1	0.1 ± 0.0	0.3 ± 0.2
	SM	254 ± 28	48 ± 10	86 ± 3	124 5	1.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2
Soil 2	Cont.	4056 ± 675	368 ± 22	983 ± 85	241 ± 10	BDL	6.1 ± 0.5	18.6 ± 0.6
	105	2783 ± 893	284 ± 39	806 ± 121	276 ± 12	BDL	6.2 ± 0.8	21.6 ± 3.0
	250	1809 ± 579	144 ± 29	568 ± 49	281 ± 6	13.8 ± 3.9	4.6 ± 0.1	19.4 ± 0.7
	500	2142 ± 296	127 ± 11	400 ± 8	251 ± 30	6.3 ± 1.2	1.8 ± 0.2	5.7 ± 0.3
	750	3678 ± 197	266 ± 9	160 ± 12	249 ± 4	3.5 ± 0.7	1.7 ± 0.1	2.2 ± 0.2
	1000	2154 ± 250	55 ± 4	55 ± 4	268 ± 15	BDL	0.5 ± 0.0	0.8 ± 0.1
	SM	3455 ± 878	106 ± 38	86 ± 7	210 ± 12	BDL	1.2 ± 0.2	1.9 ± 0.3

EC = Electrical conductivity, CEC = Cation exchange capacity, OM = Organic matter content, TN = Total nitrogen, InN = Inorganic nitrogen (ammonium), OP = Olsen phosphate, Ex = Exchangeable, Av = Available

Table 3.3 - Changes to particle size distribution upon heating. ±S.D.

	Soil 1			Soil 2		
Treat. (˚C)	Sand %	Silt %	Clay %	Sand %	Silt %	Clay %
Cont.	37.9 ± 2.5	48.4 ± 2.0	13.7 ± 0.5	70.9 ± 1.1	19.2 ± 1.3	9.9 ± 0.3
105	40.5 ± 2.2	46.1 ± 2.7	13.4 ± 1.1	72.2 ± 0.9	18.1 ± 1.0	9.8 ± 0.2
250	38.4 ± 2.1	49.9 ± 1.4	11.7 ± 0.7	71.1 ± 2.4	20.2 ± 2.2	8.7 ± 0.4
500	43.7 ± 2.6	51.7 ± 2.4	4.7 ± 0.2	86.9 ± 1.3	10.9 ± 1.2	2.2 ± 0.1
750	53.0 ± 0.5	45.5 ± 0.7	1.6 ± 0.2	89.7 ± 0.9	8.3 ± 1.2	1.9 ± 0.3
1000	85.5 ± 2.1	14.5 ± 2.1	0.0 ± 0.1	90.3 ± 0.3	7.2 ± 0.3	2.5 ± 0.1
SM	75.3 ± 1.3	23.6 ± 1.9	1.1 ± 0.6	96.3 ± 0.7	2.2 ± 0.6	1.5 ± 0.3

Macro (N, K, P, Ca, Mg) and micro (Cu, Zn) nutrient levels in the soils were affected by heating (Table 3.2). Some organic nitrogen is mineralised to ammonium when Soil 1 is heated to 250˚C and Soil 2 to 105˚C and 250˚C, which increases its availability; however, above 500˚C, the levels of both inorganic and total nitrogen were reduced due to volatilisation (Glass *et al*., 2008). In any soil treated above 500˚C, nitrogen deficiency will affect biological activity and plant growth. Most other nutrients show a negative relationship with heating temperature. Copper, zinc and phosphate showed reduced bioavailability in both soils; magnesium and calcium in Soil 1 and potassium in Soil 2. By 1000˚C, bioavailability of most of these nutrients was limited in both soils and would be a concern for plant growth. Limited levels of copper and zinc may also impact microbial enzyme systems.

Soil 2 showed variable levels of exchangeable calcium (Table 3.2) due to very high initial levels and chemical changes to calcium compounds as heating progresses. Both soils show increases in pH as seen in previous studies (Granged *et al*., 2011; Ketterings *et al*., 2000). For Soil 1, pH reached 7.3 at 1000˚C, which is not anticipated as a problem for restoration after treatment. In Soil 2, the pH levels in samples heated at ≥750˚C reached 11.3, which may affect subsequent biological activity as this is far higher than what would be found in most natural systems. High pH in soil 2 was due to the calcination of $CaCO_3$ to highly soluble and alkaline CaO at these temperatures (Giovannini *et al*., 1990), as evidenced by the high electrical conductivity and levels of soluble Ca^{2+} concentrations at these temperatures.

In summary, as the soils are heated above 500˚C, reduced nutrient levels are anticipated to affect successful plant growth, as well as the high pH in Soil 2. In terms of microbes, the loss of organic carbon at exposure temperatures above 500°C will be significant as it will reduce the chemical substrates available for heterotrophic respiration.

3.5.2 Plant Growth Trials

To quantify the effects of soil changes on plant growth, red clover and red fescue were grown in each of the soil treatments (control, 105-1000°C and smouldered). T-tests comparing biomass production and extension between equivalent samples inoculated with sterile water or aerated compost tea (see Appendix II for full details) found few statistically significant differences. Instead, treatment temperature was the main driver of variability. Given this, the microbial treatments of each soil were combined for each heat treatment and compared by one-way *ANOVA* followed by multiple t-tests in Microsoft Excel (see Appendix II for full details).

Plant growth data showed a negative interaction between treatment temperature and plant growth for both species in both soils (Fig 1). Biomass production appeared to be a more sensitive metric than extension, but both show similar statistical groupings of results. In both soils, heat treatments of 250˚C and above resulted in statistically lower plant growth. By 500˚C, biomass produced had

declined by 50% or more and leaf quality was reduced, with many samples, particularly clover, showing lowered chlorophyll content (Table 3.4). From 750˚C, negligible plant biomass production was observed. Although leaf biomass production was too limited to determine chlorophyll content, leaves were visibly chlorotic. These changes correlate well with the loss of nitrogen and the reduced bioavailability of many macro and micronutrients as soil heating temperatures increased.

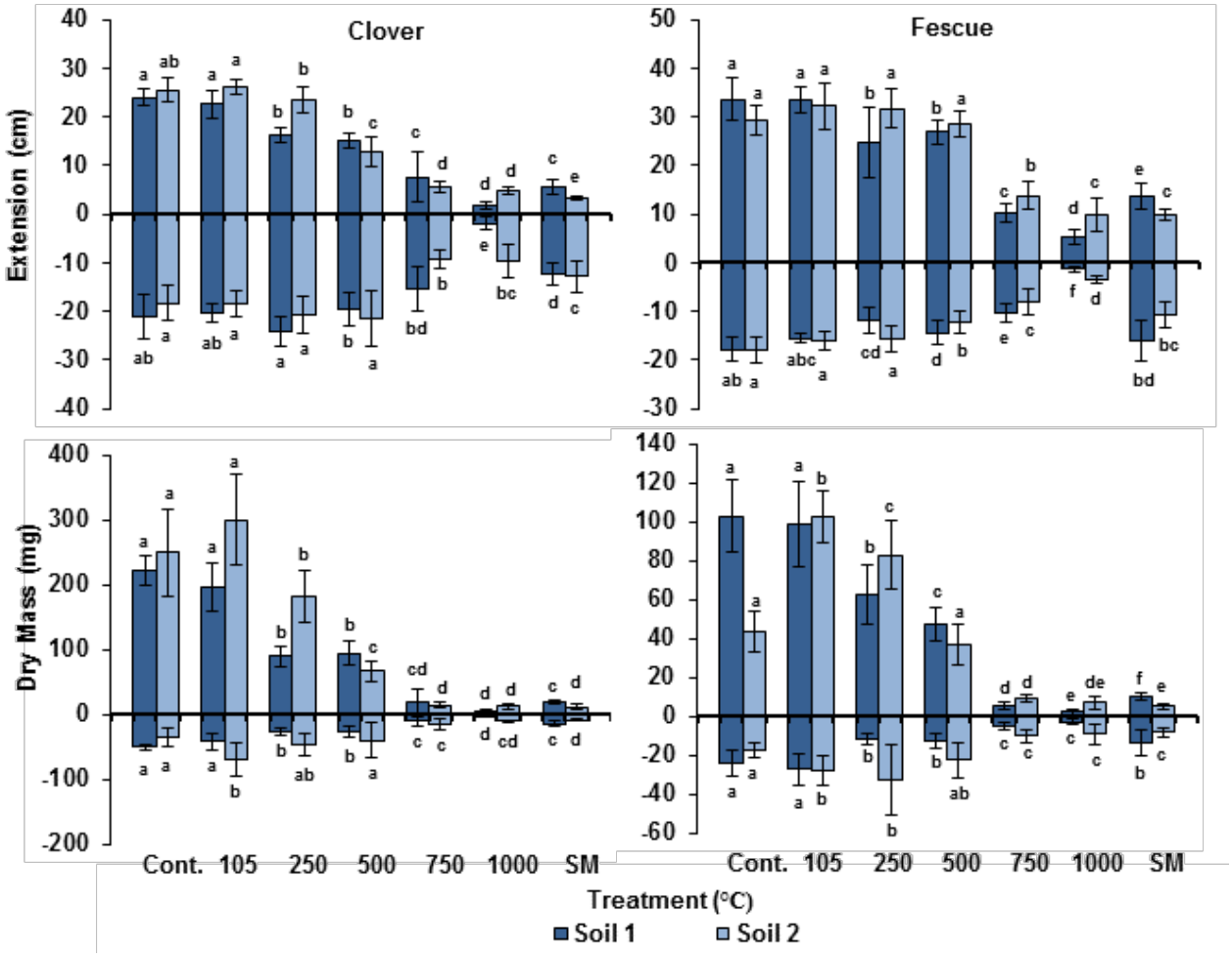


Figure 3.1. Above (positive values) and below (negative values) ground extension and biomass for Red Clover and Red Fescue grown in air dried (Cont.), heated and

smouldered (SM) samples of two soils. Error bars represent one standard deviation and letters denote statistically significant differences in each series.

In Soil 2, fescue showed significant growth enhancement when the soil was heated to 105˚C and 250˚C compared to the control sample with biomass production doubling. The organic content (17.3%) of this soil has resulted in a high C:N ratio (≈80:1), creating competition between microbes and plants for nitrogen (Kaye and Hart 1997). Heating to 105˚C released nitrogen stored in microbial biomass and heating to 250˚C mineralised nitrogen in organic compounds, both of which were demonstrated by enhanced ammonium levels (Table 3.2). These processes resulted in increased nitrogen available to plants and enhanced growth, as observed. The case for nitrogen deficiency was supported by the significantly lower chlorophyll content in the leaves of the fescue in the control compared to the 105°C and 250°C heat treatments (Table 3.4). This enhancement is absent for clover, the presence of numerous nitrogen-fixing root nodules (Table 3.5) appear to provide enough nitrogen to overcome the deficiency in the control sample.

For most of the plant/soil combinations, the 250˚C heat treatment is statistically intermediate between the 105˚C and 500˚C treatments (Fig 3.1) in terms of biomass production. In Soil 1, biomass production for clover at 250˚C is equivalent to production at 500˚C and lower than would be expected given the changes to nutrient concentrations (Table 3.2). A possible cause of this was heat-induced toxicity; clover is known to be more sensitive to this toxicity than grasses (Johnson 1919). The reason for this sensitivity is unclear but large increases in ammonium

(+1600%) and exchangeable manganese (+750%) levels (Table 2) are apparent at 250˚C in Soil 1, both of which are toxic to plants at high enough levels (Britto and Krunzucker, 2002; Osborne *et al*., 1981). Similar but less dramatic increases are observed in Soil 2, perhaps explaining why such toxicity was not observed in this soil.

Table 3.4 - Leaf chlorophyll content (mg/g dry mass) for soils inoculated with sterile water (Cont.) and aerated compost tea (M.A.). Values were not determined (n.d.) where biomass was too small for analysis. ±S.D.

	Soil 1				Soil 2			
	Clover		Fescue		Clover		Fescue	
Treatment (°C)	Cont.	M.A.	Cont.	M.A.	Cont.	M.A.	Cont.	M.A.
Cont.	20.9 ± 2.2	20.1 ± 0.9	15.2 ± 2.7	16.1 ± 2.5	27.5 ± 1.8	27.0 ± 1.2	9.7 ± 1.6	11.1 ± 0.3
105	22.0 ± 1.2	23.4 ± 1.2	20.6 ± 1.7	17.9 ± 2.3	19.2 ± 5.3	19.9 ± 5.9	20.9 ± 2.8	19.4 ± 3.0
250	25.1 ± 1.2	21.9 ± 2.9	17.7 ± 1.1	19.2 ± 0.4	19.0 ± 2.9	26.7 ± 3.7	18.7 ± 1.2	18.7 ± 1.6
500	5.8 ± 1.8	7.5 ± 1.7	14.3 ± 1.3	14.2 ± 0.9	4.8 ± 1.2	16.9 ± 4.0	12.2 ± 0.8	15.8 ± 2.6
750	n.d.	18.5 ± 1.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
SM	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

3.5.3 DNA Analysis

A soil sample from each treatment was subject to DNA extraction and gene quantification using qPCR. DNA would have been hydrolysed during the initial heating, with the exception of the control sample, so all DNA observed was from subsequent colonisation. No consistent patterns in the data were observed between the various plant and microbe treatments within each heat treatment. Airborne colonisation of un-inoculated samples was apparent with visible growth

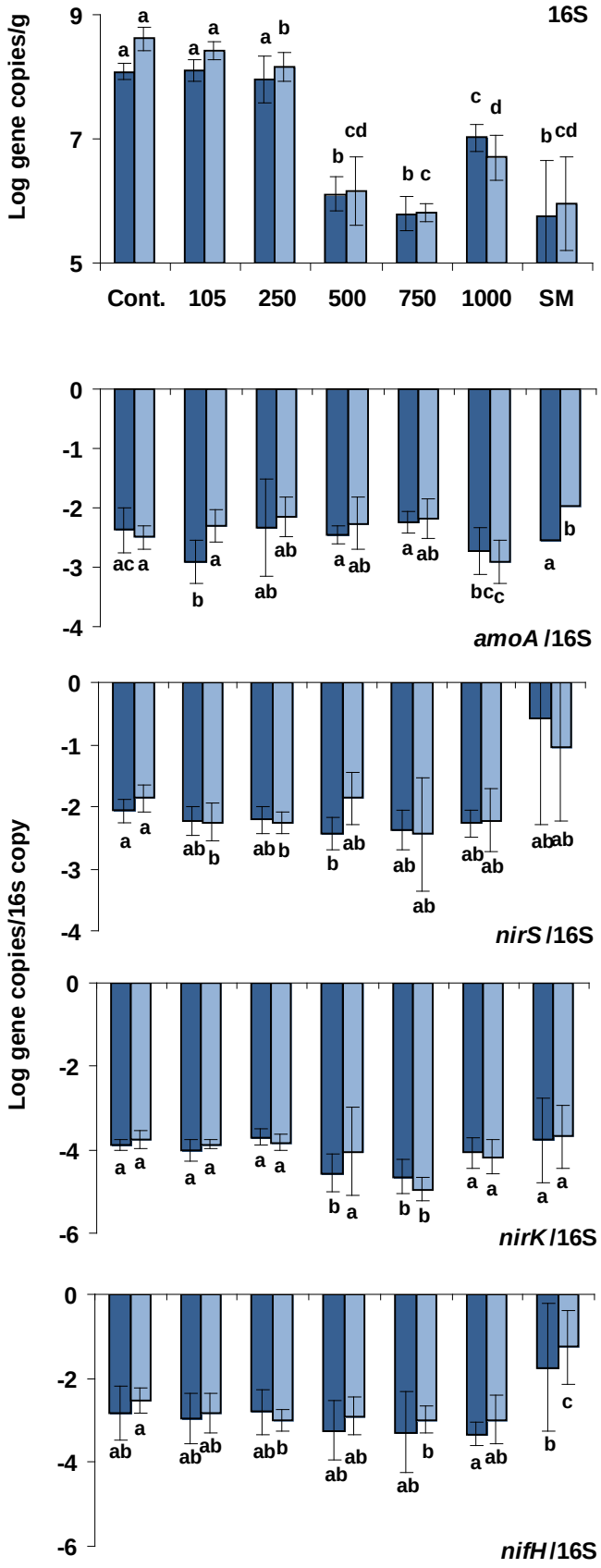


Figure 3.2. DNA quantification in heated soils eight weeks after inoculation. Error bars represent one S.D. and letters denote statistically different groupings

occurring within a few days of re-wetting in the 105°C and 250°C treatments. Airborne and seedborne re-colonisation supplied a range of organisms to the soil. This pattern existed for all of the genes quantified and the same functional guilds were present in both inoculated and un-inoculated samples. Because there were no differences, plant and microbe treatment results were combined for each temperature and analysed using one-way *ANOVAs* followed by multiple t-tests using Microsoft Excel to allow statistical grouping (see Appendix II for full details).

The level of 16S encoding DNA was measured as a proxy for overall bacterial abundance. Eight weeks after re-wetting the

bacterial population was as abundant as the control (≈10^8 gene copies/g) in the 105°C and 250°C heat treatments for Soil 1 and the 105°C treatment for Soil 2 (Fig 3.2). The 250°C treatment is statistically lower than the control and 105°C treatments for Soil 2, but by less than half an order of magnitude. In these heat treatments, the levels of organic matter and nutrients in the soil had yet to significantly decline Instead, increased available levels of nitrogen (Table 3.2) and probably also dissolved carbon (Bárcenas-Moreno and Bååth, 2009) existed due to lysis of microbial cells and heat-induced changes to organic matter. The availability of carbon and nutrients together with the rapid proliferation potential of bacteria has ensured that population levels were fully restored during the period of this experiment.

At 500˚C, there was a step change in bacterial abundance. The number of 16S gene copies in the soils was two to three order of magnitudes lower compared to the control in the 500°C, 750°C and smouldered samples. Lack of abundance correlates with lowered levels of organic matter in these heat treatments, but with 3-5% OM still remaining in the 500°C heat treatments, qualitative changes to the OM also played an important role in limiting microbial proliferation. From the biochar literature, chars produced above 300-400°C are known to contain a significantly lower proportion of labile carbon (Rutherford *et al*., 2012; Song and Guo, 2012) due to increasing levels of complex aromatic compounds as oxygen and hydrogen are lost from organic molecules (Kim *et al.*, 2012). With most soil organisms being heterotrophic, similar phenomena resulting from soil heating may have reduced

bacterial abundance in samples treated above 500°C due to limited energy sources for respiration. For both soils, an increase was seen in gene abundance for the 1000°C heat treatment relative to the other heat treatments, though still far lower than the control values. The reason for this increase is unclear, but enzyme activities in Soil 2 also marginally increased at 1000°C, suggesting enhanced microbial activity.

The abundance of functional genes involved in nitrogen cycling (*nirS, nirK, amoA* and *nifH*) follow the same patterns as the 16S data; when reported as a portion of 16S, few statistical differences are seen (Fig 3.2). All of the major nitrogen cycling guilds were present in the same proportions in the different heat treatments. This observation is significant as it implies that nutrient cycling in the soil will be able to function normally when inputs of carbon and nitrogen are restored for any of the heat treatments. The only exceptions to this consistent observation are *nirS* and *nifH* in the smouldered samples. The proportional results for these genes are quite variable within the smouldered treatments and represent a greater fraction of the microbial population containing these genes. The reason for the variability is unclear but it should be noted that both genes produce enzymes that require non-haem iron to function. Given the presence of high temperatures and a reducing agent (coal tar), changes to iron mineralogy and oxidation state would be likely in smouldered soils, possibly impacting its availability.

3.5.4 Nodulation and Growth in Clover

Through symbiotic relationships with bacteria, certain plant groups, primarily legumes such as the red clover used in this study, can fix atmospheric nitrogen to meet their growth requirements when it is deficient in the soil. Such plants have potential to create rapid vegetative cover and enhance succession on nutrient poor soils (Li and Daniels, 1992; Jefferies *et al*., 1981). However, such activity is dependant on the presence of compatible, symbiotic bacteria (*Rhizobium spp.)* and the availability of other key nutrients. If the conditions are amenable, root nodules will form to house symbiotic bacteria and nitrogen fixation will occur.

Based on the DNA data (Fig 3.2), nitrogen-fixing genes occurred as an almost constant proportion of the population in all treatments. However, after heat treatment at any temperature, successful nodulation was limited to samples that had been supplemented with microbial amendment (Table 3.5). Even when the correct microbes were present, nodulation was far from universal. For lower range heat treatments (105°C in Soil 1 and 250°C in both soils), high availability of nitrogen may have inhibited nodulation (Chambers *et al*. 1980) as it comes at an energetic cost to the plants and symbiosis is only entered into when required. In Soil 2, the higher temperature treatments (750°C, 1000°C and smouldered) did not show nodulation due to the high pH (>11) developed in these samples during heating. In Soil 1, nodulation does not occur at 1000°C and in the smouldered samples, most likely due to the low availability of macro-nutrients nutrients such as

phosphorus (Israel, 1987) and a variety of micro-nutrients (O'Hara *et al*., 1988). Nodulation occurred in three nitrogen-limited, relatively high temperature treatments (Soil 1 treated at 500°C and 750°C and Soil 2 at 500°C).

Table 3.5 - Root nodulation of clover in soils with (MA) and without microbial amendment, ±S.D.

	Soil 1		Soil 2	
Treatment (°C)	Cont.	M.A.	Cont.	M.A.
Cont.	24 ± 9	20 ± 6	32 ± 4	23 ± 8
105	----------	----------	----------	37 ± 17
250	----------	----------	----------	----------
500	----------	8 ± 7	----------	14 ± 7
750	----------	17 ± 19	----------	----------
1000	----------	----------	----------	----------
SM	----------	----------	----------	----------

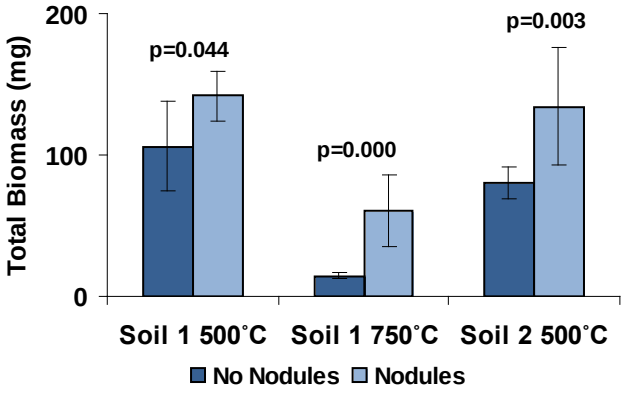


Figure 3.3 – Total biomass for clover separated by nodulation in heated samples were successful nodulation occurred. P-values are reported for t-tests.

To determine if nodulation improved plant growth, samples were grouped by nodulation and the total biomass production for each plant compared by t-tests (Fig 3.3). This approach was slightly different from previous comparisons of microbe

amendment treatment because some plants in amended soils did not nodulate. In all three samples, statistically higher growth was observed in the nodulated plants. Soil 1 heated at 500°C showed the lowest improvement due to the low numbers of nodules per plant (Table 3.5). The other samples had significantly more nodules and showed a greater improvement in growth. Due to the extreme nitrogen limitation in the 750°C sample (Table 3.2), these samples show the most dramatic improvement in growth with biomass production quadrupling. Also for the Soil 2 sample heated at 500°C, nodulation more than tripled the chlorophyll content of the leaves, supporting the hypothesis that nitrogen limitation was reducing chlorophyll content. The leaves of the Soil 1 sample at 750°C could not be compared directly to the un-nodulated equivalent due to low biomass production, but chlorophyll content in plants with nodules was triple the value compared to plants without nodules in the 500°C sample for the same soil. These improvements in growth and photosynthetic potential in leaves could be significant for the restoration of thermally remediated sites, particularly if low financial input is required.

3.5.5 Enzyme Assays

While improvements in microbe levels are important for soil recovery, the microbial population's ability to successfully cycle nutrients is important for the long-term sustainability of post-remediation soils. Enzyme assays give some indication of the rates of nutrient release (acid and alkali phosphatase) and degradation of organic

residues (β-Glucosidase) in soils. Additional assays allow the quantification of specific biochemical transformations (ammonium oxidase) that are essential to biogeochemical cycling. In this study, enzyme assays were used to establish nutrient cycling capability at the end of the growing period to give some indication of soil recovery.

Lower phosphatase activity was observed in Soil 1 compared to Soil 2, particularly for alkali phosphatase due to the low pH of Soil 1 (pH=4.7) (Fig 3.4). Few consistent differences were observed between plant/microbe treatments though in some instances, the presence of plants in heated samples seemed to enhance phosphatase activity. For instance, in Soil 1 the presence of fescue marginally increased activity in the 105°C heat treatments. By far, the most significant effect seen is the relationship between heating temperature and phosphatase activity. Like bacterial abundance, phosphatase activity shows a small decline by 250°C before a clear cut off occurs at 500°C with negligible activity recorded above this temperature, including in the smouldered samples. This pattern was also evident for the other enzymes tested. Exposure at 500°C seems to represent a tipping point above which spontaneous microbial recovery in soils is unlikely to occur.

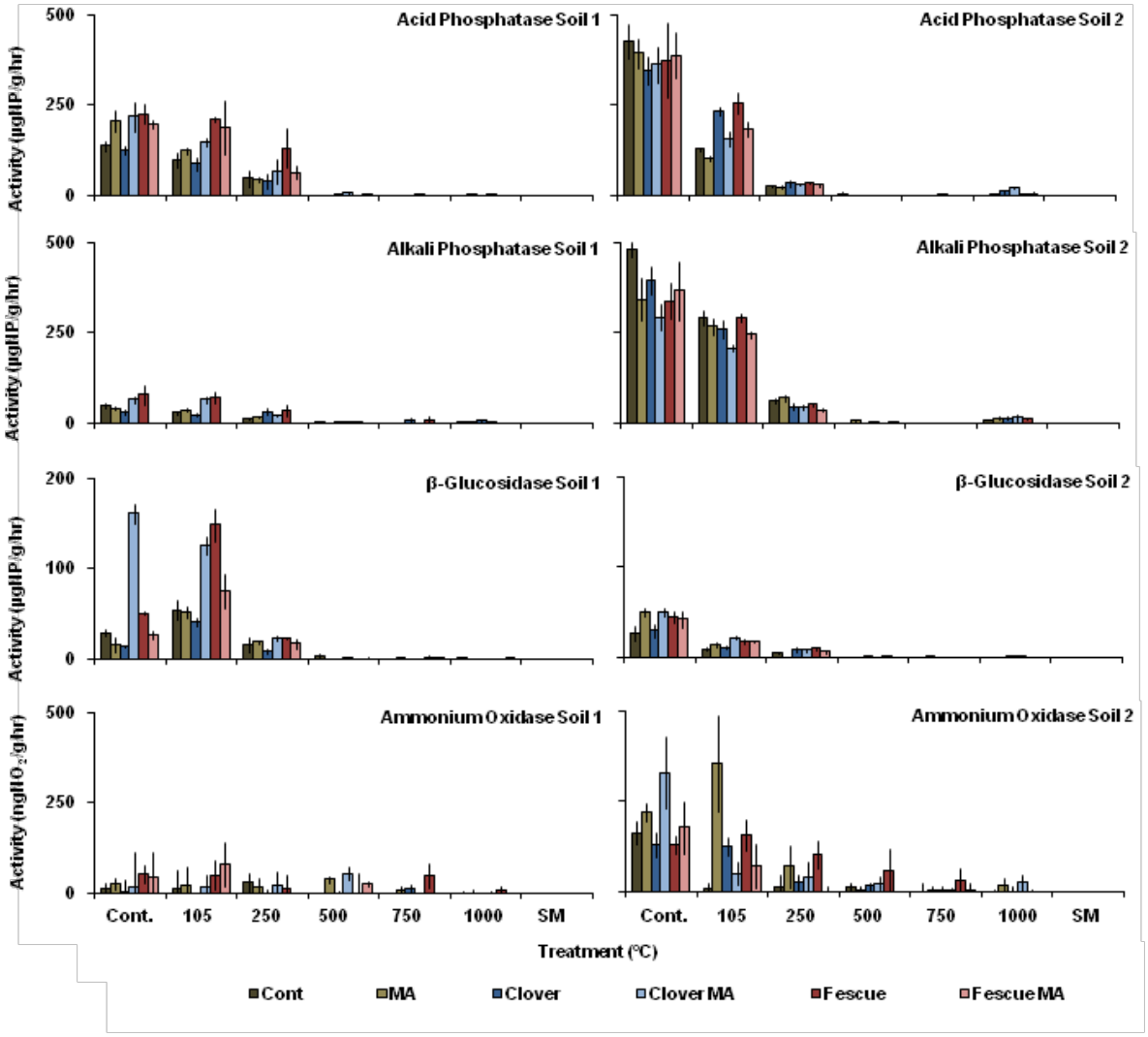


Figure 3.4. Enzyme activity 8 weeks after the inoculation of heated soils with sterile water or aerated compost tea (MA). Error bars represent one standard deviation.

For the other enzymes, differences do occur between the different soils and the various plant/microbe treatments. For the β-Glucosidase, the same pattern is observed as for the phosphatases in Soil 2. However, in Soil 1, the activity of β-Glucosidase was increased in samples heated at 105°C, which is likely due to the release of carbohydrates from microbes killed during the heating and stimulating the production of this enzyme. In addition, the combination of clover with

microbial amendment resulted in large increases in activity that were possibly due to interactions between the clover and symbiotic fungi and bacteria in the amendment mixture.

Ammonium oxidase showed consistently different patterns of activity between the two soils tested, with almost no activity being observed in Soil 1 (Fig 3.4). Absence of activity was due to the low pH of this soil (pH=4.7) making it not conducive to the oxidation of ammonium. Acidic soils tend to have ammonium rather than nitrate as the dominant form of inorganic nitrogen. In Soil 2, ammonium oxidation was apparent in all of the plant /microbe treatments at 105°C with the exception of the treatment with no added plants or microbes, which displayed no activity other than in the un-heated control. In this sample, the DNA data showed equal proportions of amoA genes. As with clover nodulation, it seems that the correct bacteria has to be present before activity will be recorded. These bacteria must be present on seeds as well as in the microbial inoculants. Further amendment may be required to fully restore nutrient cycling.

3.5.6 Implications for Remediation, Rehabilitation and Land Use

A range of thermal remediation techniques optimised for the vapour extraction of high volatility organic pollutants operates at relatively low temperatures (40-250°C), including electrical (Buettner and Daily, 1995), conductive (Heron *et al*., 2009) and microwave (Robinson *et al*., 2009) heating as well as steam injection (Heron *et al*.,

2005). For these remediation techniques, impacts on soil suitability for land uses requiring plant growth will be minimal. Both growth enhancements and mild toxicity are possible at these temperatures. Pilot work will be required to determine if either of these will be a major factor for desired land use. Although spontaneous re-colonisation of sites treated in this way will occur, microbial amendments may be beneficial to ensure that a full range of microbial mediated soil processes can occur. A generic inoculant, such as the aerated compost tea used in this study may be useful, but the opportunity exists to add specific microbes to enhance intended land use. For instance, specific microbes know to be capable of degrading PAHs may be added if the soil is to be treated using a further bio-polishing step.

Treatments that rely on higher temperatures (500°C+) to remove or destroy less volatile compounds, including smouldering (Switzer *et al.,* 2009) and incineration (Anthony And Wang, 2006), will create major challenges for re-establishing plant growth and soil ecosystems. Poor to non-existent plant growth, ineffective microbial re-colonisation and deleterious changes to soil physical properties will all need to be overcome. One method that offers a potential solution to this is the use of organic amendments such as compost that are currently used to create ecosystems on mine spoil heaps where nutrient and organic deprived material has replaced the local topsoil (Alvarenga *et al*., 2008; Bendfelt *et al*., 2001; Hargreaves *et al*. 2008). These will provide nutrients for plants, carbon to encourage microbial

activity, a population of active microbes and will enhance water storage and aggregation.

A more sustainable and economical method to rehabilitate remediated sites could be to use specific combinations of plants and microbes to re-introduce nutrients and organic matter through semi-natural succession (Bradshaw, 1997). In this study, red clover showed significant growth improvements in some nitrogen deficient heat treatments when the correct bacterial symbiont was present to facilitate nitrogen fixation. However, nodulation was not consistent in all high temperature treatments and defining the exact conditions (nutrient limitation and toxicity) when nodulation will be inhibited will be necessary to fully utilise this approach. In addition a range of nitrogen fixing plant species and numerous strains of compatible symbiotic bacteria are available. Further work to develop the most appropriate combinations for local soil and remediation conditions will be necessary on a case-by-case basis. Comprehensive biological restoration would provide a longer-term, sustainable solution to site rehabilitation after remediation and reduce the requirements for external inputs into the system.

3.6 Conclusions

This study aimed to understand the linkages between remediation operating temperatures, changes to soil properties and impacts on biological activity. A turning point was identified at 500°C. Treatments operating below this

temperature will be amenable to biological recovery and support plant growth, though inoculation with an appropriate microbial community will improve the recovery of specific biological processes in the soil. Above 500°C, geochemical changes, most significantly losses of nitrogen and carbon, resulted in poor plant growth and minimal microbial re-colonisation. Such treatments will necessitate more extensive rehabilitation programs that may include the addition of organic amendments or the use of nitrogen fixing assemblages, to re-introduce nutrients, carbon and stabilise soil. By integrating this knowledge with the design of remediation processes, it will be possible to ensure that remediated sites offer environmental and economic benefits in addition to lower environmental hazards.

3.7 Acknowledgments

The research was supported by the Department of Civil & Environmental Engineering and Glasgow Research Research Partnership in Engineering (GRPe). AP was supported by EPSRC Doctoral Training Account at University of Strathclyde. The authors thank Tatayana Peskur for her support with chemical analysis and Andrew Robson for advice about smouldering. Smoldering combustion of liquids is a patented remediation process (e.g. USA 8132987 with others pending, PCT Application PCT/GB2006/004591, priority date 10th December 2005, and PCT/US2012/035248, priority date 29 April 2011).

3.8 References

Abdelmagid, H.M. and Tabatabai, M.A. (1987). Nitrate reductase activity of soils. *Soil Biology and Biochemistry*, **19**, 421-427

ADAS (1985). *The Analysis of Agricultural Materials*. Agricultural Development and Advisory Service, London

Andreu, V., Imeson, A.C. and Rubio, J.L. (2001). Temporal changes in soil aggregates and water erosion after a wildfire in a Mediterranean pine forest. *Catena*, **44**, 69-84

Anthony, E.J. & Wang, J. 2006. Piolt plant investigations of thermal remediation of tar-contaminated soil and oil-contaminated gravel. *Fuel*, **85**, 443-450

Alvarenga P, Goncalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2008) Evaluation of composts and liming materials in the phytostabilisation of a mine soil using perennial ryegrass. Sci Tot Environ 406:43-56

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology,* **24**, 1-15

Bárcenas-Moreno, G. and Bååth, E. (2009). Bacterial and fungal growth in soil heated at different temperatures to simulate a range of fire intensities. *Soil Biology and Biochemistry*, **41**, 2517-2526

Bendfeldt ES, Burger JA, Daniels WL (2001) Quality of amended mine soils after sixteen years. Soil Sci Soc Am J 65:1736-1744

Bradshw, A. (1997). Restoration of mined lands – using natural processes. *Ecological Engineering*, **8**, 255-269

Britto, D.T. and Kronzucker, H.J. (2002). NH4+ toxicity in higher plants: A critical review. Journal of Plant Physiology, **159**, 567-584

The British Standards Institute 1995. *BS 7755 Soil Quality, Section 3.6 Determination of phosphorus – Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution*. The British Standards Institute, London

The British Standards Institute 1995c. *BS 7755 Soil Quality, Section 3.4 Determination of the specific electrical conductivity*. The British Standards Institute, London

The British Standards Institute 2000a. *BS EN 13039 Soil improvers and growing media – Determination of organic matter and ash*. The British Standards Institute, London

The British Standards Institute 2001a. *BS EN 13654 Soil improvers and growing media – Determination of nitrogen, Part 2 Dumas method*. The British Standards Institute, London

The British Standards Institute 2005. *BS ISO 10390 Soil Quality – Determination of pH*. The British Standards Institute, London

The British Standards Institute 2009. *BS ISO 11277 Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation*. The British Standards Institute, London

The British Standards Institute 2011. BS EN ISO 22030 Soil quality - Biological methods – Chronic toxicity in higher plants. The British Standards Institute, London

Buettner, H.M. & Daily, W.D. 1995. Cleaning contaminated soil using electrical heating and air stripping. *Journal of Environmental Engineering*, **121**, 580-589

Bundy, L.G. and Meisinger, J.J. (1994). *Nitrogen Availability Indices.* In : Weaver, R.W, Angle, J.S. and Bottomley, P.S. (eds.) (1994). *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America. Madison USA

Certini, G. 2005. Effects of fire on properties of forest soils: A review. *Oecologia*, **143**, 1-10

Eivazi, F. and Tabatabai, M.A. (1988). Glucosidases and galactosidases in soil. *Soil Biology and Biochemistry*, **20**, 601-606

Chambers, C.A., Smith, S.E. and Smith, F.A. (1980). Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of *Trifolium subterraneum*. *New Phytologist*, **85**, 47-62

Galang, M.A., Markewitz, D. & Morris, L.A. 2010. Soil phosphorus transformations under forest burning and laboratory heat treatments. *Geoderma*, **155**, 401-408

Giovannini, G., Lucchesi, S. and Giachetti, M. (1990). Effects of heating on some chemical parameters related to soil fertility and plant growth. *Soil Science*, **149**, 344-350

Granged, A.J.P, Zavala, L., Jordan, A. & Moreno, G.B. 2011. Post fire evolution of soil properties and vegetation cover in a Mediterranean heathland after experimental burning: A 3-year study. *Geoderma,* **164**, 85-94

Gray, D.M. & Dighton, J. 2006. Mineralisation of forest litter nutrients by heat and combustion. *Soil Biology and Biochemistry*, **38**, 1469-1477

Glass, D.W., Johnson, D.W., Blank, R.R. & Miller, W.W. 2008. Factors effecting mineral nitrogen transformations by soil heating: A laboratory-simulated fire study. *Soil Science*, **173**, 387-40

Hargreaves JC, Adl MS, Warman PR (2008) A review of the use of composted municipal solid waste in agriculture. Agri Ecosyst Environ 123:1-14

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Martin-Laurent, F., Brauman, A. and Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *Journal of Microbiological Methods*, **59**, 327-335

Heron, G., Carroll, S. & Nielsen, S.G. (2005). Full-scale removal of DNAPL constituents using steam-enhanced extraction and electrical resistive heating. *Ground Water Monitoring and Remediation*, **25**, 92-107

Heron, G., Parker. K., Galligan, J. and Holmes, T.C. (2009). Thermal treatment of eight CVOC source zones to near nondetect concentrations. *Ground Water Monitoring and Remediation,* **29**, 56-65

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, **57**, 1332-1334

Israel, D.W. (1987). Investigation of the role of phosphorus in symbiotic dinitrogen fixation. *Plant Physiology*, **84**, 835-840

Jarvis, A., Sundberg, C., Milenkovski, S., Pell, M., Lindgren, P.E. and Hallin, S. (2009). Activity and composition of ammonia oxidising bacterial communities and emission dynamics of NH3 and N2O in a compost reactor treating organic household waste. *Journal of Applied Microbiology*. 106, 1502-1511

Jefferies, R.A., Wilson, K. and Bradshaw, A.D. (1981). The potential of legumes as a nitrogen source for the reclamation of derelict land. *Plant and Soil*, **59**, 173-177

Johnson, J. (1919). The influence of heated soils on seed germination and plant growth. *Soil Science*. 7, 1-103

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D. And Philippot, L. (2006). Abundance of nirS, nirK, narG and nosZ gens of denitrifying bacteria during primary succession of a glacier foreland. *Applied Environmental Microbiology*, **72**, 5957-5973

Kang, B.T. & Sajjapongse, A. 1980. Effect of heating on properties of some soils from southern Nigeria and growth of rice. *Plant and Soil*, **55**, 85-95

Kaye, J.P. and Hart, S.C. (1997). Competition for nitrogen between plants and microorganisms. *Trends in Evolution and Ecology*, **12**, 139-143

Ketterings, Q.M., Bigham, J.M. & Laperche, V. 2000. Changes in soil mineralogy and texture caused by slash and burn fires in Sumatra, Indonesia. *Soil Science Society of America Journal*, **64**, 1108-1117

Kim, K.H., Kim, J.Y., Cho, T.S. and Choi, J.W. (2012). Influence of pyrolysis temperature on physiochemical properties of biochar obtained from the fast pyrolysis of pitch pine (*Pinus rigida*). *Bioresource Technology*, **118**, 158-162

Li, R.S. and Daniels, W.L. (1992). Nitrogen accumulation and form over time in young mine soils. *Journal of Environmental Quality*, **23**, 166-172

Lowenfels, J. and Lewis, W. (2010). *Teeming with microbes*. Timber press, London

Muyzer, G., de Wall, E.C. and Uitterlinden, A.G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16s. *Applied Environmental Microbiology*, **59**, 695-702

O'Hara, G.W., Boonkerd, N. and Dilworth, M.J. (1988). Mineral constraints to nitrogen fixation. *Plant and Soil*, **108**, 93-110

Osborne, G.J., Prately, J.E. and Stewart, W.P. (1981). The tolerance of subterranean clover (*Trifolium subterraneum l.*) to aluminium and manganese. *Field Crop Research*, **3**, 347-358

Pape, A., Knapp, C.W. and Switzer, C. (2014). Effects of high temperature remediation processes on soil geochemistry. *Environmental Science and Pollution Research*, **In review**

Pironi, P., Switzer, C., Rein, G., Fuentes, A., Gerhard, J.I. & Torero, J.L. 2009. Small-scale forward smouldering experiments for remediation of coal tar in inert media. *Proceedings of the Combustion Institute*, **32**, 1957-1964

Poly, F., Monrozier, L.J. and Bally, R. (2001). Improvements in the RFLP procedure for studing the diversity of nifH genes in communities of nitrogen fixers in soil. *Research in Microbiology*, **152**, 95-103

Robinson, J.P., Kingman, S.W., Snape, C.E., Shang, H., Barranco, R. and Saeid, A. (2009). Separation of polyaromatic hydrocarbons from contaminated soils using microwave heating. *Separation and Purification Technology*, **69**, 249-254

Roh, Y., Edwards, N.T., Lee, S.Y., Stiles, C.A., Armes, S. and Foss, J.E. (2000). Thermal-treated soil for mercury removal: Soil and phytoxicity tests. *Journal of Environmental Quality*. 29, 415-424

Rotthauwe, J.H., Witzel, K.P. and Liesack, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine scale analysis of natural amoonia-oxidizing populations. *Applied Environmental Microbiology*, **63**, 4704-4715

Rutherford, D.W., Wershaw, R.L., Rostad, C.E. and Kelly, C.N. (2012). Effect of formation conditions on biochars: Compositional and structural properties of cellulose, lignin and pine biochars. *Biomass and Bioenergy*, **16**, 693-701

Shand, C.A., Williams, B.L. and Coutts, G. (2008). Determination of N-species in soil extracts using microplate techniques. *Talanta*. 74, 648-654

Song, W. And Guo, M. (2012). Quality variations of poultry litter biochar generated at different pyrolysis temperatures. *Journal of Analytical and Applied Pyrloysis*, **94**, 138-145

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G. & Torero, J.L. (2009). Self-sustaining smoldering combustion: A novel remediation process for non-aqueous-phase liquids in porous media. *Environmental Science and Technology*, **43**, 5871-5877

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G. and Torero, J.L. (2014). Volumetric scale-up of smouldering remediation of contaminated materials. *Journal of Hazardous Materials*, **268**, 51-60

Tabatabai, M.A. and Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, **1**, 301-307

Tabatabai, M.A. (1994). *Soil Enzymes*. In: Weaver, R.W, Angle, J.S. and Bottomley, P.S. (eds.) (1994). *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America. Madison USA

Terefe, T., Mariscal-Sancho, I., Peregrina, F. & Espejo, R. 2008. Influence of heating on various properties of six Mediterranean soils: A laboratory study. *Geoderma*, **143**, 273-280

Ulery, A.L., Graham, R.C. & Bowen, L.H. 1996. Forest fire effects on soil phyllosilicates in California. *Soil Science Society of America Journal*, **60**, 309-315

Van Liedekerke, M., Prokop, G., Rabl-Berger, S., Kibblewhite, M. and Louwagie, G. (2014*). Progress in the management of contaminated sites in Europe*. European Commission Joint Research Centre, Institute for Environment and Sustainability. Ispra Italy

Chapter 4 - Biomass Crop Production on Formerly Contaminated Soils Subjected to Thermal and Smouldering Remediation

A. PAPE, C. W. KNAPP & C. SWITZER

4.1 Preface

This Chapter has been submitted for publication in *Bioenergy Research* in May 2014. In previous Chapters it was found that significant changes to soil properties occurred upon heating and smouldering and that these had significant impacts on biological processes. In this Chapter, nutrient amendments were utilised to differentiate between toxicity and deficiency in heated soils. In addition the use of soil conditioners (compost and anaerobic digestate) to improve plant yields on remediated sites was investigated. Two soils were used in this Chapter (Soil 1 and Soil 2) with Soil 1 corresponding to Soil 2 in Chapter 3. The main author, A. Pape, was responsible for the majority of the experimental design, practical work, and data analysis; he also serves as corresponding author. C.W. Knapp and C. Switzer, as project supervisors, provided advice on experimental design, data analysis, and presentation as well as editorial comments during preparation of the manuscript.

4.2 Summary

Conversion of heavily contaminated sites to biomass crop production would transform a liability into a valuable resource while avoiding negative impacts on land used for food production. Thermal or smouldering remediation can be used to remove or transform a variety of organic and metallic toxins from soils; however, consequences of soil heating such as the destruction of organic matter and loss of nitrogen affect plant productivity and must be overcome. Towards this end, changes to soil chemistry and plant growth of three potential second generation biomass crops (*Phalaris arundinacea, Panicum virgatum* and *Lolium perenne*) were assessed in two soils after heating (105-1000°C) or smouldering remediation (~1000°C). In a fertile horticultural soil, heating above 500°C reduced plant growth due to nutrient deficiency and elevated pH (>11). In a poor-quality acidic soil, heating had minimal impact due to low initial production levels. To examine the effects of nutrient supplementation on productivity, samples of both soils before and after smouldering remediation were conditioned with compost or anaerobic digestate. Plant growth after compost treatment exceeded growth after digestate treatment, though neither amendment was able to overcome the pH shift in the horticultural soil. Biomass crop production on thermally remediated sites remains possible, but success depends on the complex interactions between treatment temperature, soil chemistry and plant productivity. These results illustrate the potential for formerly contaminated sites to be converted into biomass production as well as the necessity of laboratory and pilot studies when designing rehabilitation measures to ensure productive crops.

4.3 Introduction

The use of sustainable forms of energy production, including the use of biomass crops for direct burning or processing into fluid fuels, is being widely promoted to reduce carbon emissions and reliance on fossil fuels [1]. Because the crops are not associated with food production, planting on vacant and Brownfield lands has been suggested to help reduce environmental impact and avoid competition with other land uses [2, 3]. Before effective cropping systems can be established on such sites, potential toxicity issues from chemical contamination need to be examined and mitigated if necessary.

In the case of organic compounds and volatile metals, many high temperature remediation processes have been developed to extract or destroy the contaminants [4-11]. Current technologies vary in operating conditions from ambient temperatures to over 1000˚C, depending on the type of heating involved and remediation objectives. Significant reductions in contaminant concentrations can be achieved in soils [4-11]. Although remediation can remove or reduce toxicity as a barrier to the growth of biomass crops, the effects of exposure to elevated temperatures on soil quality and crop productivity need to be determined to effectively cultivate formerly contaminated soils.

Biomass crops used to produce second generation biofuels include high yielding perennial grasses such as reed canary grass (*Phalaris arundinacea*), switchgrass

(*Panicum virgatum*) and perennial ryegrass (*Lolium perenne*) [12, 13]. With these species, the entire plant is used in biogas [14] or bioethanol [15] production. Although no data exist for the effect of soil heating on the productivity off these species, trials using rice [16], radish, fescue, oats [11] and beans [17] in soils exposed to temperatures above 400-500˚C have resulted in strong negative impacts on plant growth. This inhibition of plant growth corresponds with a reduction in nutrient levels, particularly nitrogen [18, 19]; reductions in the available levels of other macro- [16, 17] and micro- [20] nutrients have been reported in some soils. At lower temperatures, heating has a variable effect on plant productivity with positive effects due to nutrient release or negative effects due to toxicity being reported [21-23]. Significantly low yields following remediation may necessitate amendments to improve soil quality. Determining the temperatures at which deficiency and toxicity limit the growth of the target biomass crops allows for the selection of suitable remediation and rehabilitation methods to ensure optimal biomass production.

Smouldering remediation for hydrocarbon contamination involves a high-temperature front that moves through the treatment zone and destroys contaminants via pyrolysis and oxidation reactions [10, 24]. The heat generated ensures a self-sustaining process so external energy inputs are limited to an initial ignition stage. A successful treatment will remove 99%+ of the contaminants present with temperatures peaking between 600˚C and 1200˚C [10, 24, 25] and an exposure period of hours to days [25]. Although these remediation strategies

effectively remove many contaminants, these soil temperatures far exceed the range associated with reduced plant growth [11, 16, 17].

Soils remediated by high temperature processes may require nutrient supplements; [26] however, the use of chemical fertilisers or liquid wastes such as municipal wastewater [2] may not be suitable for these soils. Soil heating above 500˚C results in the destruction of organic matter [27] and increased sandiness in soils [28, 29]. Both factors significantly reduce nutrient retention in these soils [16]. Instead, the use of solid organic wastes, such as compost or anaerobic digestate fibre, may be preferred as they could also improve soil structure, nutrient retention and water holding capacity. Additionally, this approach aligns with organic re-cycling and zero-waste policies in many countries. These materials have been used to promote re-vegetation in similar situations, such as mine wastes, where nutrient-poor mineral phases have been brought to the surface [30-32].

This study investigated strategies to restore the environmental and economic value of remediated sites. The aims of this study were to quantify the effect of soil heating on the early growth of a selection of second-generation biomass crops and determine the potential of organic amendment of treated soils to enhance biomass crop production. These aims were achieved with series of experiments involving horticultural and nutrient poor soils; heat treatments and smouldering; and nutrient amendment with supplements derived from anaerobic digestate and compost,

4.4 Methodology

4.4.1 Sample Preparation and Analysis

Two contrasting soils were selected for this study to represent a high quality soil with good growth potential and one of lower quality that may be more representative of soil on contaminated sites. The first was a commercial horticultural soil (pH ≈ 7.5) with high organic and nutrient content whereas the second, loamy sand obtained from the Scottish highlands, was low in nutrients and organic matter with an acidic pH (~4.0). Each soil was air dried, sieved < 2mm (control), and dried at 105˚C to remove the inhibitory effects of moisture content on further heating [18]. Portions of each soil (500g) were then heated at 250˚C, 500˚C, 750˚C and 1000˚C in a muffle furnace (Nabotherm P330, Lilienthal, Germany) for one hour. Additional samples of each soil were treated using a smouldering method [10, 24] after artificially being contaminated with 80g/kg of coal tar to support the combustion. This preparation created a total of seven treatment regimes: control, 105˚C, 250˚C, 500˚C, 750˚C, 1000˚C and smouldered (SM).

Chemical and physical properties were quantified for each soil treatment. pH and EC were measured in a 1:5 water extract (Mettler-Toledo International Inc, Columbus, OH, USA) [33, 34]. Particle-size distribution was quantified using wet sieving and sedimentation following dispersion with a sodium hexametaphosphate/sodium carbonate solution [35]. Organic content was

measured by dry ashing at 550˚C for four hours [36]. Total nitrogen was determined on a Teldyne Tekamark Apollo 9000 combustion analyser (Mason, OH, USA) with a chemiluminescent NO_x detector; inorganic nitrogen was measured colorimetrically after a 2M KCl soil extraction using an indophenol blue method for $N\text{-}NH_4^+$, and a sullfanillic acid/NEDD method with a hydrazine reduction step for $N\text{-}NO_3^-$ [37-39]. Cation exchange capacity (CEC) and exchangeable bases (Ca, Mg, K, Mn) were determined after ammonium acetate/potassium chloride leaching via flame atomic absorption for the exchangeable bases (Perkin Elmer AAnalyst 100, Waltham, MA, USA) and indophenol blue colorimetry for the CEC [37, 38]. Available phosphate was measured after an Olsen bicarbonate extraction at pH 8.5 using ascorbic acid/ammonium molybdate colorimetry [37].

4.4.2 Growth Trials

Plant trials were conducted under artificial lighting (Grolux fluorescent tubes) with 16 hours of light per day. For each sample five replicates per grass species (switchgrass (Sunburst, Sheffields.com), reed canary grass and perennial ryegrass (Cotswold seeds)) were performed using 200ml pots with a wick watering system to provide constant moisture conditions [40]. Each pot was planted with ten grass seeds. After one week for reed canary grass and ryegrass and two weeks for switchgrass, the plants were thinned to one per pot. After a further five weeks, the plants were harvested and root and shoot extension was measured. A sample of shoot tissue from each plant was frozen for chlorophyll analysis, which involved

extraction with dimethyl sulfoxide at 70˚C (DMSO) [41] and spectrophotometric quantification at 645nm and 663nm [42]. The remainder of the shoots and all roots were dried at 70˚C to determine dry mass.

4.4.3 Treatment Effect on Biomass Crop Production

For each soil and thermal treatment, each grass species was grown under two levels of nutrient amendment. One set had de-ionised water provided via the wick-watering system [40] throughout the entire growing period. The other set was initially provided with 150ml of Hoaglands [43] nutrient solution then provided with de-ionised water for the rest of the growth period. Additional samples of each grass were grown in washed and sterilised silver sand, with and without nutrient addition, to provide baselines for growth in the conditions provided. Normalised performance was expressed as a ratio of optimal growth based on the values obtained for samples grown in nutrient-amended sand to facilitate comparisons between the grass species. Results over the full temperature treatment range were compared using one-way *ANOVA* followed by multiple t-tests within each soil/grass/nutrient treatment (α=0.05).

4.4.4 Use of Organic Soil Conditioners to Enhance Growth

Smouldered and control samples of each soil were conditioned with either the fibre fraction of food waste anaerobic digestate (sourced from Barkip biogas plant,

Ayrshire Scotland) or commercial compost (B&Q Verve compost) at 5%, 10% and 20% levels by volume. The fibre fraction of anaerobic digestate was selected over the liquid as it would also help improve soil structure and water/nutrient retention. Additionally it was felt that the low CEC of treated soils would lead to unacceptable leaching of nutrients (particularly ammonium) if the liquid fraction was used. Each conditioner treatment was planted with all three grass species and harvested after six weeks. In addition, a sample of each mixture was watered using the same wick system for two weeks to equilibrate before being chemically analysed. pH, EC, available phosphate, $N\text{-}NH_4$ and $N\text{-}NO_3$ were measured as for background information. Exchangeable bases (Na, Ca, Mg, K, Mn in 1:10 1M ammonium acetate extract) [39] and heavy metals (Cu, Zn, Pb, Ni, Cd in 1:5 0.05M ammonium EDTA extract) [39] were quantified by ICP-OES (ICP-OES, Thermo Scientific Hemel Hempstead, UK). Results were analysed using nested *ANOVA* for individual soil/grass treatments and two-way *ANOVA* to compare the impact of the same conditioner on control and smouldered samples of the same grass/soil combination.

4.5 Results and Discussion

4.5.1 Physical and chemical changes after thermal exposure

Comparisons between the untreated samples of the horticultural (Soil 1) and loamy sand (Soil 2) (Table 4.1) revealed clear differences. The loamy sand had approximately 16% of the organic content, 30% of the clay content, and a

correspondingly lower cation exchange capacity than the horticulturakl soil. It also contained less nutrients (Table 4.2), with 75% and 66% lower total and inorganic nitrogen content, respectively; additionally the availability of other macro-nutrients were one to two orders of magnitude lower in the loamy sand (Table 4.2). The loamy sand also had a lower pH than the horticultural soil, which possibly contributed to negligible levels of available phosphate (Table 4.2), as phosphate strongly binds to iron oxides at pH < 4. The horticultural soil (pH = 7.4) was based on a calcium carbonate parent material, as evidenced by the high extractable calcium levels and recognisable fragments of chalk and flint.

Table 4.1 – Effects of soil heating and smouldering on soil properties for horticultural (soil 1) and nutrient poor, acidic (soil 2) soils. Values are reported with ± one standard deviation based on triplicate analysis

	OM (%)	pH	EC (µS/cm)	Sand (%)	Silt (%)	Clay (%)	CEC (cmolc/kg)
				Soil 1			
Control	17.3 ± 0.9	7.4 ± 0.0	732 ± 16	70.9 ± 1.1	19.2 ± 1.3	9.9 ± 0.3	28.2 ± 4.6
105˚C	16.5 ± 1.0	7.1 ± 0.0	978 ± 64	72.2 ± 0.9	18.1 ± 1.0	9.8 ± 0.2	16.0 ± 3.2
250˚C	11.6 ± 0.3	7.3 ± 0.1	1489 ± 81	71.1 ± 2.4	20.2 ± 2.2	8.7 ± 0.4	14.2 ± 3.7
500˚C	5.1 ± 0.1	9.0 ± 0.0	948 ± 39	86.9 ± 1.3	10.9 ± 1.2	2.2 ± 0.1	6.3 ± 0.9
750˚C	1.9 ± 0.1	11.3 ± 0.0	1241 ± 8	89.7 ± 0.9	8.3 ± 1.2	1.9 ± 0.3	2.2 ± 0.7
1000˚C	0.0 ± 0.0	11.4 ± 0.1	1410 ± 145	90.3 ± 0.3	7.2 ± 0.3	2.5 ± 0.1	0.6 ± 0.2
Smouldered	0.2 ± 0.1	11.7 ± 0.1	1783 ± 37	96.3 ± 0.7	2.2 ± 0.7	1.6 ± 0.3	0.8 ± 0.3
				Soil 2			
Control	2.8 ± 0.1	4.1 ± 0.1	31 ± 5	80.8 ± 0.2	16.2 ± 0.2	3.0 ± 0.1	7.0 ± 2.3
105˚C	2.2 ± 0.1	3.7 ± 0.0	39 ± 1	82.4 ± 0.7	15.1 ± 0.4	2.5 ± 0.3	4.5 ± 0.4
250˚C	1.4 ± 0.1	4.3 ± 0.1	106 ± 8	82.5 ± 0.3	14.9 ± 0.2	2.6 ± 0.1	3.8 ± 1.5
500˚C	0.5 ± 0.0	6.0 ± 0.0	149 ± 10	83.2 ± 0.5	14.8 ± 0.3	2.1 ± 0.2	2.0 ± 1.4
750˚C	0.0 ± 0.1	7.0 ± 0.1	119 ± 6	83.3 ± 0.3	14.8 ± 0.4	1.9 ± 0.2	1.0 ± 0.5
1000˚C	0.0 ± 0.0	7.2 ± 0.1	17 ± 1	90.6 ± 1.1	8.8 ± 1.1	0.6 ± 0.1	1.1 ± 1.7
Smouldered	0.4 ± 0.0	8.5 ± 0.1	51 ± 3	86.5 ± 0.5	12.8 ± 0.4	0.8 ± 0.1	2.1 ± 0.7

Despite the evident differences between the two soils, both underwent similar patterns of change when heated. Levels of organic matter decreased at temperatures >250˚C, and the percentage of clay and silt particles decreased at >500˚C - both of which contributed to a lowering of the cation exchange capacity. Heating to 105˚C and 250˚C increased levels of ammonium as organic matter was mineralised, but at >500˚C both ammonium and total nitrogen levels declined due to volatilisation. pH increased in both soils, quite possibly due in part to the loss of organic acids. The extent of the pH increase was greater (i.e., pH > 11) in the horticultural soil as calcination of calcium carbonate to calcium oxide (and hydroxide on wetting) after exposure to temperatures >500˚C [44] was more likely.

Table 4.2 – Effects of soil heating and smouldering on soil nutrient levels. Values are reported with ± one standard deviation based on triplicate analysis

	Total N (g/kg)	N-NH4 (mg/kg)	Olsen P (mg/kg)	Ex. K (mg/kg)	Ex. Ca (mg/kg)	Ex. Mg (mg/kg)	Ex.Mn (mg/kg)
				Soil 1			
Control	1.00 ± 0.10	6.0 ± 0.8	94 ± 10.6	983 ± 85	4056 ± 675	368 ± 22	BDL[b]
105˚C	1.15 ± 0.08	11.4 ± 0.4	104 ± 4.4	806 ± 121	2783 ± 893	284 ± 39	BDL[b]
250˚C	0.92 ± 0.07	23.7 ± 1.3	114 ± 7.1	568 ± 49	1809 ± 579	144 ± 29	13.8 ± 3.9
500˚C	0.62 ± 0.10	6.9 ± 1.5	125 ± 9.5	400 ± 8	2142 ± 296	127 ± 11	6.3 ± 1.2
750˚C	0.25 ± 0.04	BDL[a]	52 ± 0.9	160 ± 12	3678 ± 197	266 ± 9	3.5 ± 0.7
1000˚C	0.02 ± 0.00	BDL[a]	22 ± 0.9	55 ± 4	2154 ± 250	55 ± 4	BDL[b]
Smouldered	0.10 ± 0.00	BDL[a]	45 ± 8.4	86 ± 7	3455 ± 878	106 ± 38	BDL[b]
				Soil 2			
Control	0.24 ± 0.01	2.0 ± 0.8	0.0 ± 0.2	20.5 ± 6.6	14.8 ± 2.2	9.8 ± 2.0	BDL[b]
105˚C	0.25 ± 0.01	2.8 ± 0.9	3.2 ± 0.2	17.5 ± 3.3	14.5 ± 1.1	12.1 ± 1.1	BDL[b]
250˚C	0.25 ± 0.01	47.8 ± 5.5	13.3 ± 1.5	15.1 ± 2.2	14.8 ± 1.1	28.2 ± 2.5	7.8 ± 1.2
500˚C	0.03 ± 0.00	BDL[a]	15.3 ± 0.9	130.0 ± 4.9	17.2 ± 1.6	59.3 ± 6.2	3.5 ± 0.8
750˚C	0.01 ± 0.00	BDL[a]	19.5 ± 0.4	122.3 ± 10.3	23.5 ± 1.1	56.6 ± 7.5	BDL[b]
1000˚C	0.00 ± 0.00	BDL[a]	0.8 ± 0.8	15.5 ± 2.3	14.8 ± 1.0	11.5 ± 0.8	BDL[b]
Smouldered	0.08 ± 0.01	BDL[a]	12.8 ± 2.0	125.1 ± 13.9	50.2 ± 1.4	40.0 ± 5.9	4.8 ± 1.9

[a] Detection limit = 1.5mg/kg

[b]Detection limit = 2.5mg/kg

Dissimilarities between the two soils were observed in the levels of many macro-nutrients. The horticultural soil (Soil 1) experienced a decline in the available levels of most macro-nutrients (K, P, Mg) as heating increased; available calcium levels were highly variable due to calcination. In the loamy sand (Soil 2), macro-nutrient availability (particularly K and Mg) increased between 500˚C and 750˚C, presumably as the clays began to decompose [28].

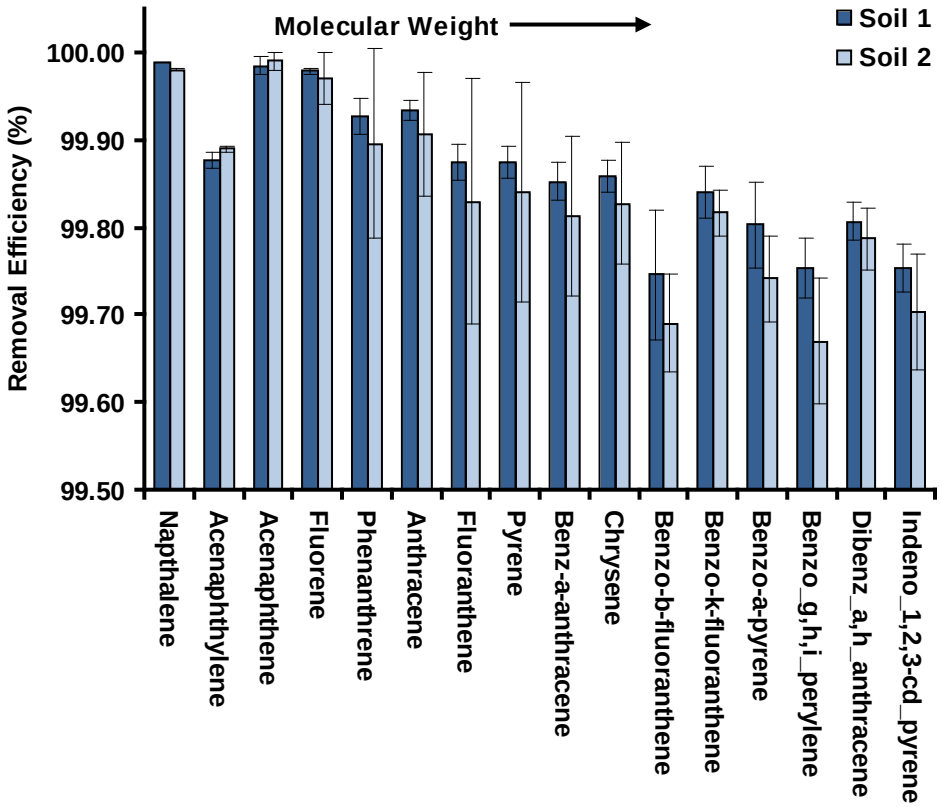


Figure 4.1 – Residual PAH contamination of smouldered soils. Error bars represent one standard deviation based on triplicate analysis

Smouldering resulted in an almost complete removal of the target contaminants, decreasing total PAH levels from ~10,000mg/kg to ~10mg/kg in both soils. Looking at the individual PAHs there is some variation with a negative correlation (r^2 = 0.77 and 0.78) between removal efficiency and molecular mass (Fig 4.1) but in all cases levels are low enough that phytoxicity would not be expected to be an issue. Smouldering remediation changed soil properties (Tables 4.1 and 4.2) most closely resembling the 750˚C heat treatments with a loss of nitrogen, organic matter, clays and, in the horticultural soil, the increase in pH. Although the smouldered soils did consistently reach temperatures of around 1000˚C, they had a low residence time at that temperature and did not exhibit the same changes as soils exposed to 1000˚C for a full hour in the furnace.

4.5.2 Effect of heat treatments on growth

The growth performance of three plants was monitored (reed canary grass, rye grass, and switchgrass) by measuring biomass production, extension and chlorophyll content of six week-old plants. Chemical and physical changes from heating soils impacted biomass crop production, and the trends were not straightforward. For the horticultural soil, exposure at 105˚C and 250˚C caused significantly increased biomass production in all three grass species (one-way *ANOVA*, p=0.05, see S.I. for details) and extension in reed canary grass (Fig. 4.2). Additional ammoniacal nitrogen is presumed to have stimulated plant growth by

supplying the limiting nutrient [45]; mild heating will lyse microbial cells and release nitrogen (Table 4.2) [21]. At higher temperatures, there was a significant decrease in biomass and extension for all three grasses where losses of nitrogen, organic matter, clays and other macronutrients occurred. For the 750˚C, 1000˚C, and smouldered samples biomass production was negligible (Fig 4.1).

The loamy sand (Soil 2) experienced low levels of biomass production at all temperature treatments with few statistically significant differences or consistent trends. This was likely attributed to wide ranging nutrient deficiencies previously discussed (see Table 4.2). While K, P, and Mg availabilities increased at 500˚C and 750˚C, the system had already shifted to extreme nitrogen limitation (>500˚C, see Table 4.2). With low growth under all treatment conditions, this soil would be a candidate for nutrient-supplementation to stimulate biomass crop production.

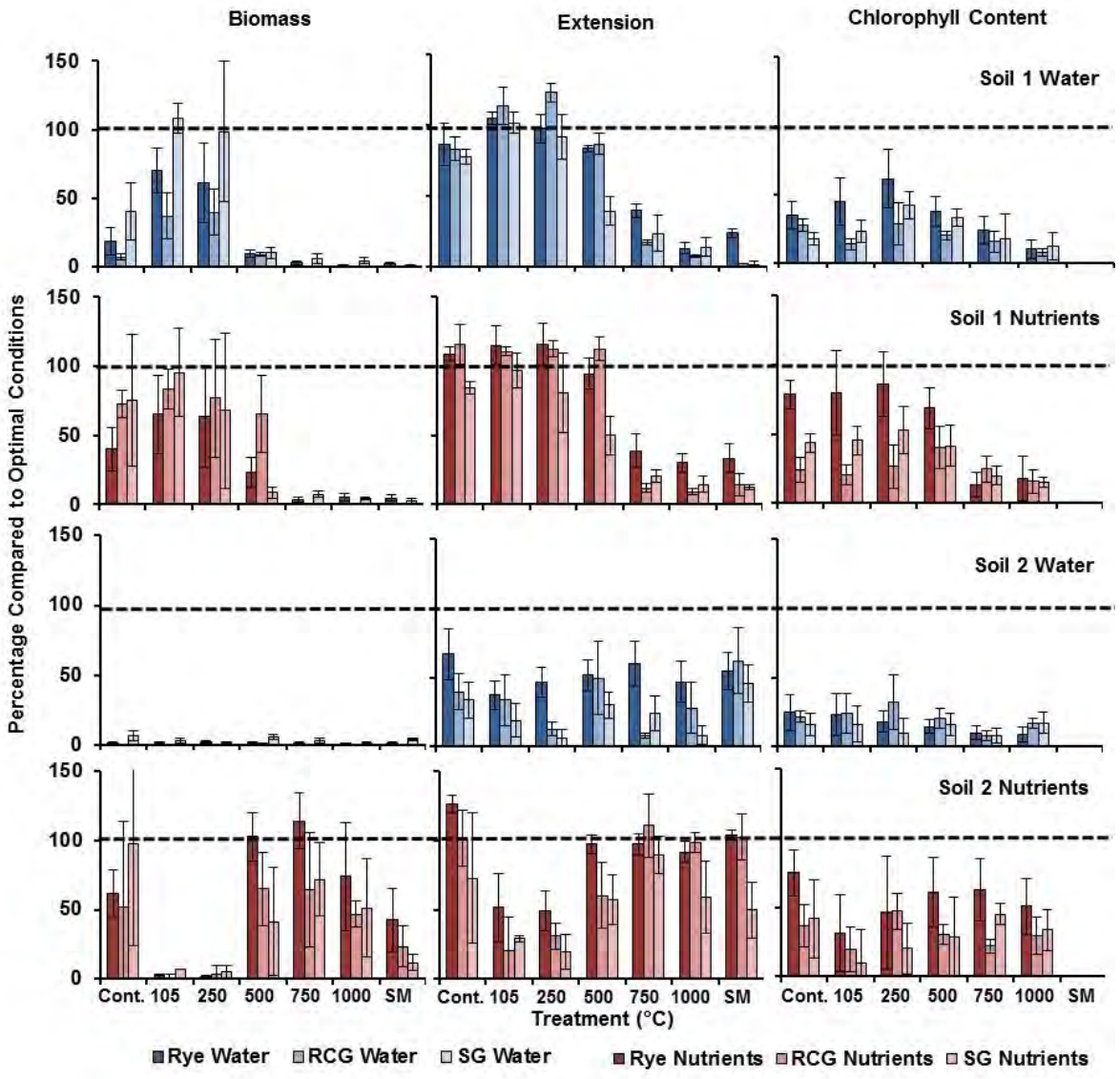


Figure 4.2 – The effect of soil heating and smouldering on the total extension, biomass and chlorophyll content of three grass species after six weeks growth. Optimal growth based on positive controls. Error bars represent 95% confidence interval based on five replicates

4.5.3 Plant growth with nutrient amendment

Half of the plants initially received Hoagland's nutrient supplementation to differentiate whether toxicity or nutrient deficiency affected the plants. For the horticultural soil (Soil 1), increased toxicity appeared to be the main mechanism causing reduced plant growth at higher temperatures; there was limited evidence of improved biomass production with nutrient addition, except at lower temperature treatments: rye grass at "control" temperature only, ≤ 500 ˚C for reed canary grass, and ≤ 250 ˚C for switchgrass (Fig 2). At higher temperatures, the addition of nutrients did not enhance plant growth; heating temperature correlated significantly with increasing pH (Table 4.1), which reached levels inhospitable to plant growth.

For the loamy sand (Soil 2), nutrient amendment increased biomass production, extension and chlorophyll content towards optimal levels (Fig 4.2) in a wider range of temperature treatments. All three grasses improved with nutrient amendment in all thermal treatments except 105 ˚C and 250 ˚C. This soil, especially after elevated temperatures, had minimal organic matter or nutrients, and the nutritional quality of the soil resembled the added amendment.

When the loamy sand was heated at 105˚C and 250˚C, nutrient addition was unable to restore biomass production. Plant toxicity has been observed in other studies at these temperatures [22], but the reason behind it is unclear. PAH production and

hydrophobicity [21-23] were unlikely in this soil due to low initial levels of organic material. Possible explanations at 250˚C included the elevated availabilities of manganese and ammonium (Fig 4.2), both of which could be toxic [46, 47]. Both increased in the horticultural soil at this temperature without any apparent toxicity, although its greater CEC might have reduced their availability.

The reason for toxicity at 105˚C also remains unclear. One possibility could be the low pH (pH=3.7, Table 4.1) generated by this temperature which may be toxic to certain plant species.

4.5.4 Effect of Soil Conditioners on Biomass Crop Growth

To determine whether compost or anaerobic digestate fibre waste could improve crop production, conditioners were added at different levels (0-20% v/v). In the horticultural soil (Soil 1), neither conditioner was able to significantly improve plant growth in the smouldered samples of this soil (Fig. 4.3). Nested *ANOVA* data (Table 4.3) for Soil 1 demonstrates that treatment (smouldered or control) explained most of the variation in the data set. Both conditioners facilitated a reduction in pH and marginally improved root penetration and biomass (Fig 4.3), particularly for ryegrass. The pH remained above 8.7 and biomass production was minimal in smouldered Soil 1 samples when compared to the control sample. In the control samples, both conditions equally improved biomass production for switchgrass and ryegrass, largely as a function of dose. For reed canary grass, biomass production

was lower when conditioned with AD compared to compost, with declining biomass as AD dose increased, suggesting that toxicity may have been an issue.

Table 4.3 – Statistical analysis results for nested *ANOVA* for control and smouldered soils. Lighter areas represent more statistically significant results.

	Soil 1						Soil 2					
f-value (p-value)	Total Mass			Total Extension			Total Mass			Total Extension		
	Rye	RCG	SG	Rye	RCG	SG	Rye	RCG	SG	Rye	RCG	SG
Treatment	37.73 (0.03)	7.94 (0.11)	601.5 (0.00)	149.4 (0.01)	136.9 (0.01)	268.8 (0.00)	0.63 (0.51)	0.28 (0.65)	0.95 (0.43)	2.77 (0.24)	0.51 (0.55)	0.31 (0.63)
Amendment	0.68 (0.59)	2.67 (0.11)	0.07 (0.49)	0.24 (0.79)	0.29 (0.75)	0.37 (0.70)	1.48 (0.27)	3.84 (0.05)	4.13 (0.04)	1.32 (0.31)	2.25 (0.15)	4.63 (0.03)
Dose	18.44 (0.00)	40.38 (0.00)	2.05 (0.03)	18.97 (0.00)	24.18 (0.00)	3.04 (0.00)	45.40 (0.00)	89.83 (0.00)	11.05 (0.00)	10.54 (0.00)	34.32 (0.00)	8.20 (0.00)

For the loamy sand (Soil 2), plant growth data revealed a very different trend. In this soil, both biomass production and extension were similarly enhanced in the control and smouldered soils (Fig 4.3), with the exception of switchgrass when compost was used as the conditioner. AD was less successful, with lower biomass production than compost in most of the samples, except perennial ryegrass in the control soil. In smouldered samples, biomass production was negligible with AD added. Here, amendment type explained a significant portion of the variability for reed canary grass and switchgrass (*ANOVA*; Table 4.3). Significantly more biomass was produced with the AD conditioner in the control when compared to the smouldered samples for Soil 2 (two-way *ANOVA*; p=0.05).

Chemical analysis of the conditioned soils can help explain the differing responses to the AD and compost. The levels of total nitrogen in the two conditioners were

similar (~9 g/kg; *t*-test *p*=0.12,) and both similarly improved ryegrass and switchgrass in control treatments of Soil 1, suggesting that lack of nutrients in the AD was not the issue. Both conditioners increased nutrients in both treatments of Soil 2, including conductivity, phosphate availability and the exchangeable levels of bases (Ca, Na, Mg, K) (Fig 4.4). None increased to levels of obvious concern and remained less than concentrations in the control samples of the horticultural soil, where healthy plant growth was observed.

Of the heavy metals (see Appendix IV), increased availability of zinc was of particular concern (Fig 4.4). AD had a greater impact on the levels bio-available zinc in amended soils compared to compost. In Soil 1, additional zinc was largely irrelevant in smouldered samples due to pH-related toxicity. Control samples of Soil 1 had high levels of organic matter that may have diminished the incremental increases in bio-available zinc, but sufficient zinc remained to impact the sensitive reed canary grass [48]. In contrast, smouldered samples of Soil 2 had almost no available zinc and minimal clay or organic matter to facilitate zinc adsorption (Table 4.1). Addition of AD increased zinc levels and correspondingly reduced biomass production in all three grasses (Fig 4.3). Soil 2, in its original form, had some clay and organic matter (≈3% of each by mass). AD addition resulted in lower available zinc levels compared to smouldered samples, which explains the increased biomass production in control relative to smouldered samples when amended with AD.

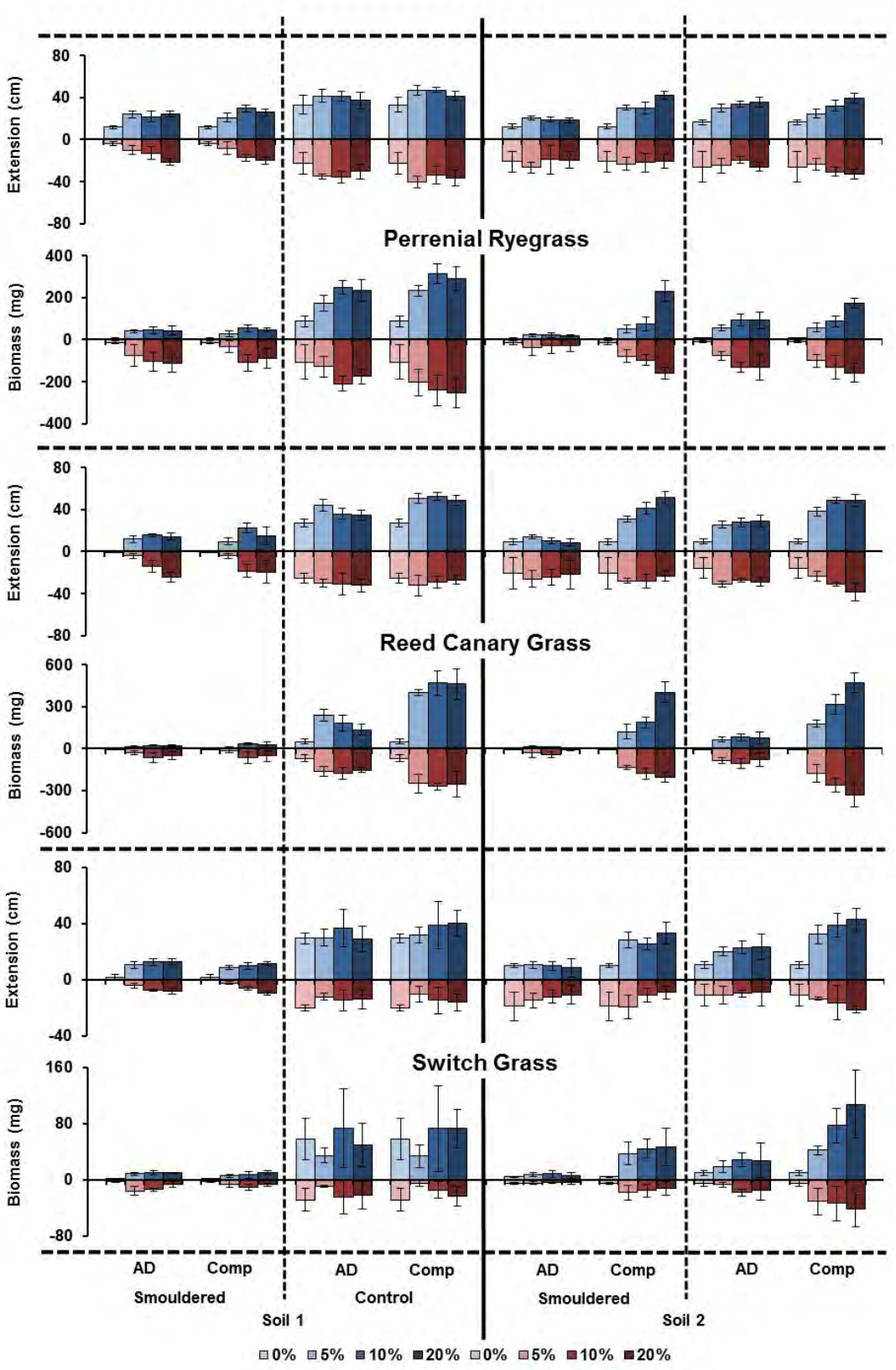


Figure 4.3 – Biomass and extension (roots in red, shoots in blue) for smouldered and control soils conditioned with anaerobic digestate (AD) or compost (comp). Error bars represent 95% confidence interval based on five replicates

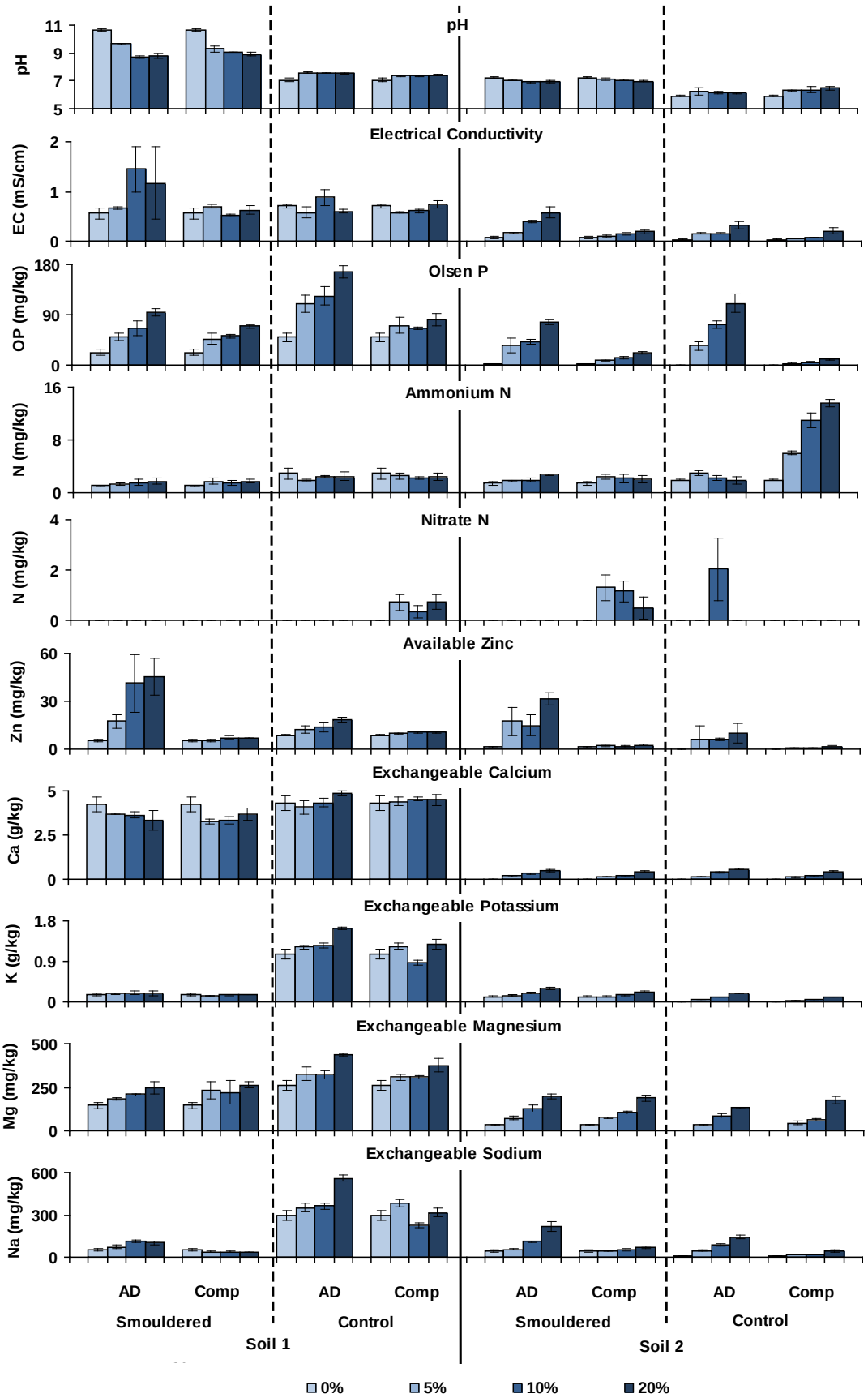


Figure 4.4 – Chemical analysis for smouldered and control soils conditioned with anaerobic digestate (AD) or compost (comp) two weeks after re-introducing moisture. Error bars represent one standard deviation based on triplicate analysis

4.5.5 *Implications for Thermal and Smouldering Remediation*

Numerous thermal remediation techniques operate at relatively lower temperatures (40-250°C), including electrical, conductive and microwave heating and high temperature steam injection [4-7]. The effect of the equivalent temperatures on biomass crops was extremely variable. The highly organic horticultural soil at these temperatures showed improved growth, whereas the acidic sandy loam soil became toxic to plants. Other studies have shown similarly variable results [21-23]. With over 14,000 soil classifications being recognised by the USDA [49] predicting toxicity due to heating in a given soil may be extremely challenging.

Understanding the causative agent of plant toxicity and linking it to soil parameters such as mineralogy, pH, texture and organic content is needed. Until such knowledge is available, small-scale pilot trials are essential to identify potential pitfalls before they occur. Past studies have shown that inoculation with microbes and flooding or leaching with water can counteract toxicity in heated soils [50-51], and this may be a necessary step to ensure good biomass crop production on some soils when thermally remediated at lower temperatures (105-250°C).

At higher temperatures (>500°C), growth of plants was limited by nutrient deficiencies [11, 16-19]. Poor biomass production was observed in both soils at these temperatures. In the horticultural soil, nutrient deficiency was not the main

cause of reduced plant growth. A dramatic increase in the pH of this soil was observed due to calcinations of carbonates, which prevented any substantial growth of biomass crops when full nutrient supplementation and organic soil conditioners were added. Even at the highest application rates (20%), the organic conditioners were unable to reduce pH to a suitable level. Additional acidifying measures will be required to allow the production of biomass crops. These measures may include the use of elemental sulphur [52], pyritic peat [53] or pyritic mine wastes [54], which could help to reduce pH to a level whereby plants could become established.

In the loamy sand, minimal biomass production above 500°C was due to nutrient limitation; improved production levels were achieved when nutrients were supplied. Thermal and smouldering remediation did not decrease the suitability of the soil for biomass crop production. Soil quality on contaminated sites is often highly variable due to limited primary production, artificial parent materials and low pedogenic periods since site abandonment. In practice, the investment to establish a productive biomass cropping system on an aggressively remediated soil (>500°C) is equivalent to the investment required for a similar uncontaminated soil if initial soil quality is low.

Care must be taken when selecting appropriate organic conditioners for remediated soils. Soils subject to thermal remediation above 500˚C have low levels of organic matter and clays, which will reduce the soil's ability to sequester heavy metals and

organic compounds into less bio-available forms. Many organic residues that are being widely promoted for soil conditioning may contain elevated levels of potentially toxic elements [32, 55, 56], particularly heavy metals such as copper, zinc and cadmium, and normal dosages appropriate for normal soil systems may need to be revised for soils subject to aggressive remediation.

4.6 Conclusion

Biomass crop production after thermal or smouldering remediation has been demonstrated as a viable subsequent use for formerly contaminated soils. Appropriate amendments would be required to facilitate this transformation. As with remediation itself, site-specific conditions will affect the processes required to convert post-remediation soil into productive use. Toxicity generated in the soil by heating may require specific amendments or treatment steps. Post-remediation soils may become more susceptible to toxicity from organic soil amendments if soil texture and organic content have been affected. A number of interactions between soil type, treatment temperature, amendment type and plant growth complicate predictions. Thorough laboratory and pilot trials will be essential to the design of post-remediation treatments to ensure optimal crop productivity. Biomass crop production presents a viable way of restoring economic and environmental value to derelict, post-remediation sites.

4.7 Acknowledgements

The research was supported by the Department of Civil & Environmental Engineering and Glasgow Research Partnership in Engineering (GRPe). AP was supported by EPSRC Doctoral Training Account at University of Strathclyde. The authors thank Tatayana Peskur and Aoife Brennan for their support with chemical analysis, Andrew Robson for advice about smouldering and Richard Lord for useful discussions about biomass crops and soil amendments. Smoldering combustion of liquids is a patented remediation process (e.g. USA 8132987 with others pending, PCT Application PCT/GB2006/004591, priority date 10th December 2005, and PCT/US2012/035248, priority date 29 April 2011).

4.8 References

[1] Dhillon RS, von Weuhlisch G (2013) Mitigation of global warming through renewable biomass. Biomass Bioenerg 48:75-89

[2] Gopalakrishnan G, Negri MC, Wang M, Wu M, Snyder SW, Lafreniere L (2009) Biofuels, land and water: A systems approach to sustainability. Environ Sci Technol 43:6094-6100

[3] Smith SL, Thelen KD, MacDonald SJ (2013) Yield and quality analyses of bioenergy crops grown on a regulatory brownfield. Biomass Bioenerg 49:123-130

[4] Buettner HM, Daily WD (1995) Cleaning contaminated soil using electrical heating and air stripping. J Environ Eng 121:580-589

[5] Heron G, Carroll S, Nielsen SG (2005) Full-scale removal of DNAPL constituents using steam-enhanced extraction and electrical resistive heating. Ground Water Monit R 25:92-107

[6] Heron G, Parker K, Galligan J, Holmes TC (2009) Thermal treatment of eight CVOC source zones to near nondetect concentrations. Ground Water Monit R 29:56-65

[7] Robinson JP, Kingman SW, Snape CE, Shang H, Barranco R, Saeid A (2009) Seperation of polyaromatic hydrocarbons from contaminated soils using microwave heating. Sep Purif Technol 69:249-254

[8] Iben IET, Edelstein WA, Sheldon RB, Shapiro AP, Uzgiris EE, Scatena CR, Blaha SR, Silverstein WB, Brown GR, Stegmeier GL, Vinegar HJ (1996) Thermal blanket for in-situ remediation of surficial contamination: A pilot test. Environ Sci Technol 30:3144-3154

[9] Anthony EJ, Wang J (2006) Pilot plant investigations of thermal remediation of tar-contaminated soil and oil-contaminated gravel. Fuel 85:443-450

[10] Switzer C, Pironi P, Gerhard JI, Rein G, Torero JL (2009) Self-sustaining smoldering combustion: A novel remediation process for non-aqueous-phase liquids in porous media. Environ Sci Technol 43:5871-5877

[11] Roh Y, Edwards NT, Lee SY, Stiles CA, Armes S, Foss JE (2000) Thermal-treated soil for mercury removal: Soil and phytoxicity tests. J Environ Qual 29:415-424

[12] Shield IF, Barraclough TJP, Riche AB, Yates NE (2012) The yield response of the energy crops switchgrass and reed canary grass to fertiliser applications when grown on a low productivity sandy soil. Biomass Bioenerg 42:86-96

[13] Lewandowski I, Scurlock JMO, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatus grasses as energy crops in the US and Europe. Biomass Bioenerg 25:335-361

[14] Seppälä M, Paavola T, Lehtomäki A, Rintala J (2009) Biogas production from boreal herbaceous grasses – Specific methane yield and methane yield per hectare. Bioresource Technol 100:2952-2958

[15] Kallioinen A, Uusitalo J, Pahkala K, Kontturi M, Viikari L, von Weymarn N, Siika-aho M (2012) Reed canary grass as a feedstock for 2nd generation bioethanol production. Bioresource Technol 123:669-672

[16] Kang BT, Sajjapongse A (1980) Effect of heating on properties of some soils from southern Nigeria and growth of rice. Plant Soil 55:85-95

[17] Nishita H, Haug RM, Hamilton M, Alexander GV (1970) Influence of soil heating on the growth and elemental composition of bean plants. Soil Sci 110:61-70

[18] Glass DW, Johnson DW, Blank RR, Miller WW (2008) Factors effecting mineral nitrogen transformations by soil heating: A laboratory-simulated fire study. Soil Sci 173:387-40

[19] Gray DM, Dighton J (2006) Mineralisation of forest litter nutrients by heat and combustion. Soil Biol Biochem 38:1469-1477

[20] Nishita H, Haug RM (1974) Water and ammonium acetate-extractable Zn, Mn, Cu, Cr, Co, and Fe in heated soils. Soil Sci 118:421-424

[21] Kitur BK, Frye WW (1983) Effects of heating on soil chemical properties and growth and nutrient composition of corn and millet. Soil Sci Soc Am J 47:91-94

[22] Johnson J (1919) The influence of heated soils on seed germination and plant growth. Soil Sci 7:1-103

[23] Paradelo R, Devesa-Rey R, Cancelo-González J, Basanta R, Pena MT, Díaz-Fierros F, Barral MT (2012) Effect of compost mulch on seed germination and plant growth in a burnt forest soil from NW Spain. Soil Sci Plant Nutr 12:73-86

[24] Pironi P, Switzer C, Rein G, Fuentes A, Gerhard JI, Torero JL (2009) Small-scale forward smouldering experiments for remediation of coal tar in inert media. P Combust Inst 32:1957-1964

[25] Switzer C, Pironi P, Gerhard JI, Rein G, Torero JL (2014) Volumetric scale-up of smouldering remediation of contaminated materials. J Hazard Mater 268;51-60

[26] Pape A, Knapp CW, Switzer C (2014) Effects of high temperature remediation processes on soil geochemistry. Environ Sci Pol Res, In review

[27] Certini G (2005) Effects of fire on properties of forest soils: A review. Oecologia 143:1-10

[28] Ulery AL, Graham RC, Bowen LH (1996) Forest fire effects on soil phyllosilicates in California. Soil Sci Soc Am J 60:309-315

[29] Terefe T, Mariscal-Sancho I, Peregrina F, Espejo R (2008) Influence of heating on various properties of six Mediterranean soils: A laboratory study. Geoderma 143:273-280

[30] Alvarenga P, Goncalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E, Cunha-Queda AC (2008) Evaluation of composts and liming materials in the phytostabilisation of a mine soil using perennial ryegrass. Sci Tot Environ 406:43-56

[31] Bendfeldt ES, Burger JA, Daniels WL (2001) Quality of amended mine soils after sixteen years. Soil Sci Soc Am J 65:1736-1744

[32] Hargreaves JC, Adl MS, Warman PR (2008) A review of the use of composted municipal solid waste in agriculture. Agri Ecosyst Environ 123:1-14

[33] The British Standards Institute (2005) BS ISO 10390 Soil Quality – Determination of pH. The British Standards Institute, London

[34] The British Standards Institute (1995) BS 7755 Soil Quality, Section 3.4 – Determination of the Specific Electrical Conductivity. The British Standards Institute, London

[35] The British Standards Institute (2009) BS ISO 11277 Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation. The British Standards Institute, London

[36] The British Standards Institute (2000) BS EN 13039 Soil improvers and growing media – Determination of organic matter and ash. The British Standards Institute, London

[37] Bundy LG, Meisinger JJ (1994) Nitrogen Availability Indices. In: Weaver RW, Angle JS, Bottomley PS (eds.) (1994) Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties. Soil Science Society of America, Madison, USA

[38] The British Standards Institute (2011) BS EN ISO 22030 Soil quality - Biological methods – Chronic toxicity in higher plants. The British Standards Institute, London

[39] ADAS (1985) The Analysis of Agricultural Materials. Agricultural Development and Advisory Service, Lodon

[40] Shand CA, Williams BL, Coutts G (2008) Determination of N-species in soil extracts using microplate techniques. Talanta 74:648-654

[41] Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332-1334

[42] Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1-15

[43] Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Calif AES Circ 347;1-32

[44] Giovannini G, Lucchesi S, Giachetti M (1990) Effects of heating on some chemical parameters related to soil fertility and plant growth. Soil Sci 149:344-

350

[45] Kaye JP, Hart SC (1997) Competition for nitrogen between plants and microorganisms. Trends Evol Ecol 12:139-143

[46] Fujimoto CK, Sherman GD (1948) Manganese availability as influenced by steam sterilisation of soils. Am Soc Agronomy J 40:527-534

[47] Britto DT, Kronzucker HJ (2002) NH_4^+ toxicity in higher plants: A critical review. J Plant Physiol 159:567-584

[48] Korzeniowska J, Glubiak ES, Igras J (2011) Applicability of energy crops for the phytostabilisation of soils moderately contaminated with copper, nickel and zinc. J Food Agric Environ 9:693-697

[49] USDA (1999) Soil Taxonomy: A basic system of soil classification for making and interpreting soil surveys. United States Department of Agriculture, Washington, USA

[50] Rovira AD, Bowen GD (1966) The effect of micro-organisms upon plant growth II. Detoxification of heat sterilised soils by fungi and bacteria. Plant Soil 25:129-142

[51] Warcup JH (1957) Chemical and biological aspects of soil sterilisation. Soils Fert 20:1-5

[52] Walker KJ, Warman EA, Bhogal A, Cross RB, Pywell RF, Meek BR, Chamber BJ, Pakeman R (2007) Recreation of lowland heathland on ex-arable land: assessing the limiting processes on two sites with contrasting soil fertility and pH. J Appl

Ecol 44:573-582

[53] Dunsford SJ, Free AJ, Davy AJ (1998) Acidifying peat as an aid to the reconstruction of lowland heath on arable soil: a field experiment. J Appl Ecol 35:660-672

[54] Bradshaw A (2000) The use of natural processes in reclamation – advantages and difficulties. Landscape Urban Plan 51:89-100

[55] Alvarenga P, Palma P, Goncalves AP, Fernandes RM, Cunha-Queda AC, Duarte E, Vallini G (2007) Evaluation of chemical and ecotoxicological characteristics of biodegradable organic residues for application to agricultural land. Environ Int 33:505-513

[56] Shi J, Yu X, Zhang M, Lu S, Wu W, Wu J, Xu J (2010) Potential risks of copper, zinc and cadmium pollution due to pig manure application in a soil-rice system under intensive farming: A case study of Nanhu, China. J Environ Qual 40:1695-1704

Chapter 5 – Conclusion

5.1 Key Findings

The aim of this thesis was to understand how thermal and smouldering remediation affected the ability of soils to support plant growth and microbial activity, and demonstrate ways to enhance plant growth if required. To this end, a series of experiments were carried out (Chapters 2-4) that addressed theses aims. A number of key findings have contributed knowledge to help ensure that remediated sites are environmentally and economically useful.

5.1.1 Threshold for Biological System Recovery

Experiments involving plant growth and microbial re-colonisation in heated soils consistently demonstrated a tipping point above which un-assisted biological recovery does not occur. In these experiments, this occurred at 500°C and was a result of major compositional changes in the soil. It has been routinely observed that atmospheric loss of nitrogen was the most significant factor for the recovery of plants; for microbes, the loss, and possibly alteration, of organic carbon was a limiting factor in re-colonisation. Additionally both plants and microbes were affected by significant reductions in the level of other nutrients as heating temperatures increased, particularly phosphorus, copper and zinc. For soils exposed to >500°C (including smouldering remediation) the use of organic conditioners, such as compost, to re-introduce nutrients and organic matter would be advisable. Such conditioners also act as a source of microbes for re-colonisation and improve structure and cohesion in the soil.

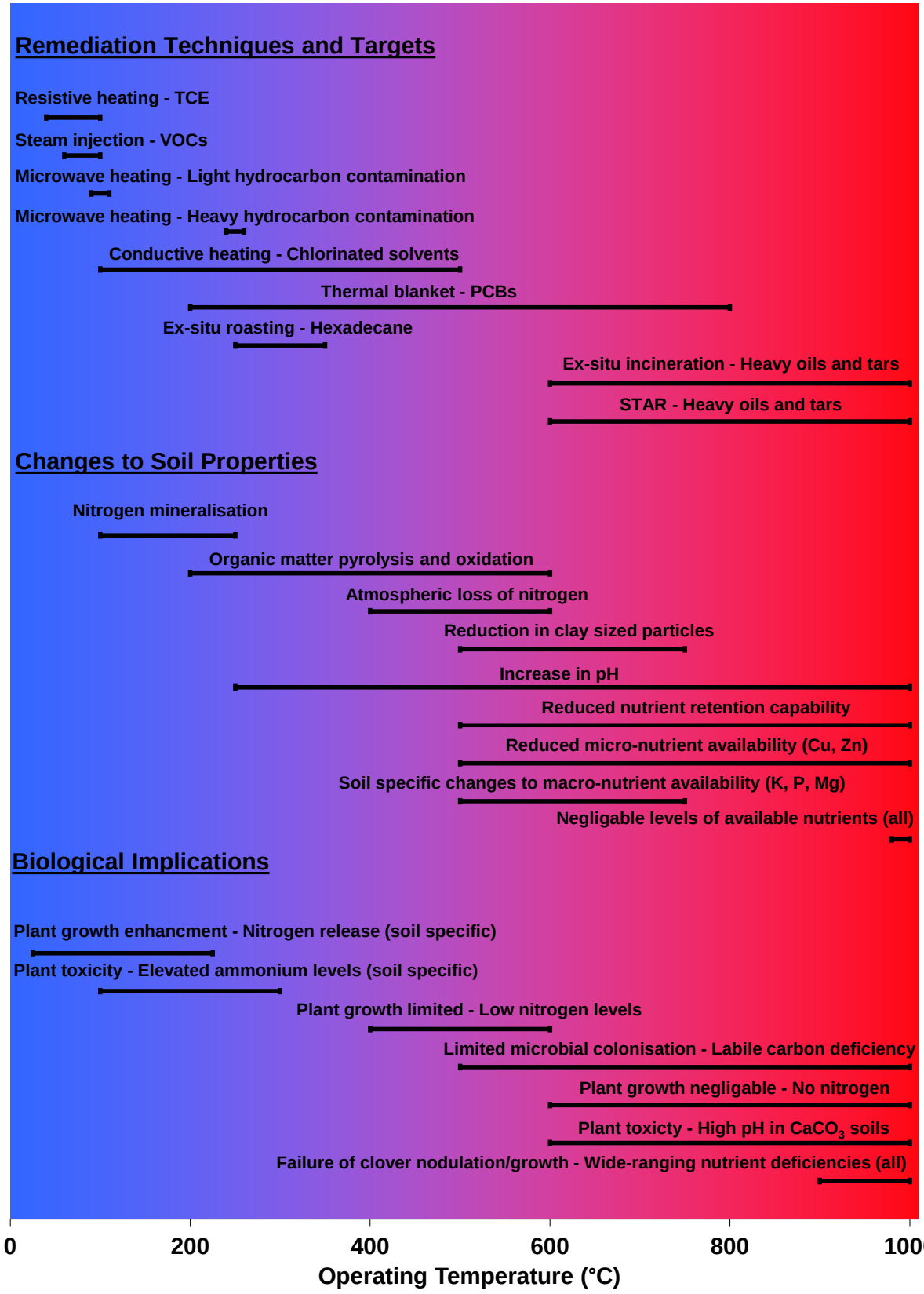


Figure 5.1 – Summary of the range of temperatures utilised during remdiation, changes to soil properties and biological implications.

5.1.2 Nitrogen Mineralisation and Toxicity

One key finding of this study is a consistent increase in the concentration of ammonium in the soil to very high levels at heating temperatures around 250°C. This change was consistently seen in all soils and in the same soil in different chapters. The magnitude of the increase varied between the soils but for some levels exceeded 100ppm. This far exceeds normal levels for ammonium in soils and it seems very likely that it was responsible for observed plant toxicity at this temperature. This could have important implications for restoring soils that have been treated by low temperature thermal remediation.

5.1.3 Soil Diversity – Chemistry and Toxicity

For all soils tested, certain parameters underwent the same changes when subjected to heating; for example, both carbon and nitrogen predictably declined as heating temperature increased. For other important nutrients, their bio-availability changed as a function of soil type and heating temperature. This was clearly seen for the exchangeable bases and phosphate in all chapters, where some soils show increased availability of these nutrients at 500-750°C. It has been theorised that this was due to differing clay mineralogy between soils and their varying decomposition rates and elemental composition. Another factor that showed substantially different changes between soils was pH. In all tested soils, pH increased as a function of heating temperature, usually to around pH 7-8; however in one soil based on a calciferous parent material, pH increases to >11 due to the calcination of abundant calcium carbonates in this soil. Different levels of phytotoxicity were observed in other soils (Chapters 3 and 4) at lower heating

temperatures (105-250°C), due to the increased availability of nutrients that become toxic at higher levels (ammonium).

The different responses of soils to heating chemically and biologically are significant as it becomes difficult to extrapolate results to other sites and soils with any degree of certainty. Until such variances are linked to measurable soil properties (such as organic content, soil classification and mineralogy), conducting thorough lab and pilot scale studies would be advisable before full scale remediation and rehabilitation programs are undertaken. Detailed site evaluation will help ensure that site-specific solutions can be developed to ensure that remediated sites are fit for their intended use and bring them one step closer to being economically or ecological valuable.

5.1.4 Restoring the Value of Remediated Sites

When low plant productivity and microbial activity are observed after remediation, methods need to be developed to restore plant and microbial activity to soil. In this thesis, the use of organic soil conditioners was investigated to see if they could improve the yield of biomass crops grown on smouldered soils. Results were inconsistent. Although crop productivity was restored in one of the soil/conditioner treatments tested, it was seen that the answer to this question was not simple and depended on a variety of factors. Heat induced toxicity and negative interactions between potentially toxic elements (PTEs) and soil conditions in the smouldered soils both prevented successful restoration. Careful consideration needs to be

taken of the suitability of soil conditioners used in terms of PTEs and whether they can solve all of the problems created by remediation. Further study is needed to fully explore nutrient replacement in soils after remediation. Bespoke treatments may be required for certain soil and remediation combinations.

5.2 Future Work

5.2.1 Linking Variable Responses to Soil "Type"

From the work presented here, chemical changes and toxic responses of plants vary across soil conditions and heat treatments. Variable plant growth responses occur particularly at around 250°C. At the moment, lab and pilot studies are required for individual soils, replacing this empirical approach with a deterministic understanding of what is causing such variation would be extremely valuable for practitioners of thermal remediation, wildfire researchers and managers and agronomists considering soil sterilisation. Such knowledge would change on a fundamental level how these practitioners approach the problems of restoring ecological function to degraded ecosystems.

One possible way to achieve this would be to gather a range of soils representing different parent materials, land uses, soil classifications and mineralogy (20+) and subject them to the same heat treatments, chemical analysis and plant toxicity assays used in this thesis (Fig 5.1). By analysing such data sets using ordination techniques (advanced statistics), such as principle component analysis (PCA) and redundancy analysis (RDA), it may be possible to link variation in toxicological and geochemical responses to specific characters of the original soil. This could be used

to create a predictive model to indicate which soils will cause toxicity in which temperature ranges, potentially saving a great deal of time for researchers in the future. Such work could also expand to cover a greater range of toxicological metrics, including responses of microbial communities and arthropod and earthworm toxicity assays.

5.2.2 Effects of Soil Heating on Bioremediation Potential

Where thermal or smouldering remediation is unable to reduce contaminant load to desired target levels, soil bioremediation or phytoremediation will be a valuable polishing step. Soil heating has an impact on microbial abundance and activity. In addition, heating has an impact on the bio-availability of contaminants due to the loss of organic matter that facilitates adsorption at higher temperatures. It is essential to see how heating affects bioremediation simple one-contaminant systems, complex system with mixtures of contaminants and real-life systems after thermal or smouldering remediation if it is to be used as an effective polishing step. Additionally, heat sterilises soil and provides the opportunity to bio-augment microbial community with minimal competition from resident populations. This combination could be a powerful remediation tool. Two-step remediation programs, where low temperature thermal remediation extracts more volatile contaminants and engineered degradation communities degrade the rest, may have a more sustainable impact on soils than relying on more aggressive, high temperature processes alone.

5.2.3 The Use of Nitrogen Fixing Plants in Site Restoration

It was shown in this thesis that the nodulation of clover with nitrogen fixing bacteria could significantly enhance its growth in nutrient-limited, heated soils. This could play an important role in low-input site restoration and soil improvement following thermal remediation. For this to be a real possibility this idea needs explored further. This includes looking at the potential of different legumes and specific strains of symbiotic bacteria to fulfil this role after differing thermal conditions and in different soil types. As part of this, understanding the constraints on legume growth and nodulation in terms of macronutrients, such as phosphorus, and micronutrients such as iron, copper and molybdenum, will be important. Where specific nutrient requirements are understood, these could be added to thermally remediated soils when identified as deficient to kick-start a successional process in the soil.

5.2.4 Rehabilitation for High pH After Smouldering

One of the key limiters for productive plant system on calciferous soils after smouldering remediation was found to be a high pH (>11). Finding ways to effectively reduce this pH to a more normal range will be essential to restoring soil function in these cases. The use of soil conditioners such as elemental sulphur, pyritic peat or pyritic mine wastes should be investigated to see if they can mitigate against this problem.

This thesis demonstrated that effective thermal and smouldering remediation programs can be followed by ecosystem recovery and plant growth on sites. To

facilitate this consideration of the effect of remediation on soil properties and biological activity, and how this interacts with factors such as soil type and the properties of soil conditioners, needs to be considered and investigated. Such knowledge will help ensure remediated sites provide an economic or environmental benefit o the surrounding area.

Appendix I – Detailed Methodologies

Standard Operating Procedure

University of Strathclyde Glasgow

INTRODUCTION

i Operation	
Title	Chemical, Physical and Microbiological Analysis of Soils and Analysis of Plant Material
Department	Civil and Environmental Engineering

ii Person Undertaking Work			
Name:	Andrew Pape	**Position:**	Ph.D. Student
Signature:		**Date:**	
Department	Civil Engineering		

iii Contents

SECTION 1.1 – ANALYSIS OF EXTRACTABLE PHOSPHORUS

1.1.1 Introduction
Only a small portion of the total phosphorus in soil is available for use by plants and microbes. There are several extraction methods used as a measure of P availability including Mechlich-1, Bray-1 and Olsen (Kuo 1996). In the UK the Olsen method is a suggested standard method for P availability (ADAS 1985; BS 1995). This method involves extraction of the soil with a CaCO3 solution and the colorimetric determination of phosphate concentration.

1.1.2 Safety Documents to be Consulted	
COSHH	Extraction of nutrients/metals from soil for analysis Analysis of liquid samples (straight or extract) for phosphate
Risk Assessment	Extraction of nutrients/metals from soil for analysis

1.1.3 Equipment and Consumable Required	
Extraction	Orbital or end over end shaker 15ml centrifuge tubes 100ml volumetric flasks Centrifuge
Analysis	350µl 96 well flat bottomed microplates Orbital shaker Microplate reader 15ml centrifuge tubes Spectrophotometer

1.1.4 Reagents and Standards	
Phosphate stock	Dry and dissolve 1.099g KH_2PO_4 in 250ml water with 1.25ml conce. HCl and 1 drop of toluene for 1000mg l^{-1} stock
Dilute sulphuric acid	Dilute 8ml concentrated acid to 100ml with de-ionised water
Ammonium molybdate	1.2% - Dissolve 12g ammonium molybdate and 0.3g antimony potassium tartartae in 600ml water, add 150ml conce. H_2SO_4 and make up to 1l with water (store cool place up to 3 months): shortly before use dilute 12.5ml to 100ml with water for working solution
Ascorbic acid	Dissolve 1.5g in 100ml water – make on day of use
Olsen extractant	Dissolve 42g $NaHCO_3$, 5ml polyacrylamide solution (0.5g in 1000ml water) in 1l water adjusted to pH 8.5 with 1M NaOH – make on day of extraction

1.1.5 Procedure
Extraction for available phosphorus - Weigh 0.5g of soil into a centrifuge tube and add 10ml of Olsen extractant. Mount on the end over end or orbital shaker (horizontally) and shake for 30 minutes before centrifuging to remove solid matter. Include both blanks and positive controls in each run. Perform all extractions in triplicate Extract analysis – • Dilute stock to 10mg l^{-1} by diluting 1ml stock in 100ml extractant, range for cal 0-7mgl^{-1}

Micro determination:

- Add 50ul standard/sample to two wells
- Add 10ul sulphuric acid and stand for 5 min
- Add 200ul ammonium molybdate
- Add 50ul ascorbic acid and shake for 1 hour
- Analyse at 880nm

1.1.6 Analysis and Reporting of Results

Use the following calculation to determine the available P content in the soil:

$[P\text{-}PO_4]$ (mg/kg) = $((Abs_{Sample}\text{-}c) / m /$ mass of sample (g) / c.f.) x extraction volume (ml) x dilution factor

Where the calibration curve is linear in the form y = mx + c and c.f. is the oven dry content (105°C) of the material expressed as a fraction

1.1.7 References

ADAS (1985). *The analysis of agricultural materials*. Agricultural Development and Advisory Service, London

The British Standards Institute (1995). *BS 7755 Soil Quality, Section 3.6 Determination of phosphorus – Spectrometric determination of phosphorus soluble in sodium hydrogen carbonate solution*. The British Standards Institute, London

Kuo, S. (1996). *Phosphorus*. In: Bigham, J.M., Bartels, J.M., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T. and Sumner, M.E. (eds.) (1996). *Methods of Soil Anlaysis Part 3: Chemical Methods*. Soil Science Society of America, Madison WI

SECTION 1.2 - ANALYSIS OF EXTRACTABLE INORGANIC NITROGEN SPECIES

1.2.1 Introduction
Inorganic nitrogen species (NO_3^-, NO_2^- and NH_4^+) are the main form of plant available nitrogen in soils. To extract these from the soil a strong KCl (ADAS 1985) solution is used as the K^+ ions are able to displace the strongly bound NH_4^+ ions. The extracted ions are then analysed calorimetrically using an indophenol blue (Bundy and Meisinger 1994) method for NH_4^+ and a sulfanillic acid method for NO_2^- (ADAS 1983). NO_3^- cannot be directly analysed and has top be reduced to NO_2^- using hydrazine before analysis (Shand *et al*. 2008).

1.2.2 Safety Documents to be Consulted	
COSHH	Extraction of nutrients/metals from soil for analysis Ammonium analysis Nitrate and nitrite analysis
Risk Assessment	Extraction of nutrients/metals from soil for analysis

1.2.3 Equipment and Consumable Required	
Extraction	Orbital shaker 15ml centrifuge tubes Centrifuge
Analysis	350µl 96 well flat bottomed microplates Orbital shaker/incubator Microplate reader 15ml centrifuge tubes Spectrophotometer

1.2.4 Reagents and Standards	
Ammonium stock	Dry and dissolve 0.3821g NH_4Cl in 1000ml water for 100mg l^{-1} stock
Nitrite stock	Dry and dissolve 0.1232g $NaNO_2$ in 1000ml water for 25mg l^{-1} stock
Nitrate stock	Dry and dissolve 0.7218g KNO_3 in 1000ml water for 100mg l^{-1} stock
Extractant	2M KCl: 149.1g in 1l DI water
Ammonium analysis	• EDTA: 6g Na_2EDTA/100ml DI water pH7 • Phenol-nitroprusside :7g phenol and 34mg sodium nitroprusside in 100ml water, store in dark fridge • Buffered hypochlorite: 1.48g NaOH, 4.98g Na_2HPO_4, 20ml 5% NaOCl to 100ml with water pH11.4-12.2
Nitrite analysis	• NEDD: 0.1g in100ml of DI water • Sulfanilic acid: 1g in 100ml 2M HCl (47.5ml conce HCl in 250ml water)
Nitrate analysis	• Sulfanillic acid and NEDD: As above • Sodium hydroxide: 40g in 1l water • Copper/zinc catalyst: 35.4mg $CuSO_4.5H_2O$ and 900mg $ZnSO_4.7H_2O$ in 1l water • Hydrazine sulphate: 1.71g in 1l water • Catalyst mix: 1 part each of the above three solutions

1.2.5 Procedure

Extraction – Weigh 2g of soil into extraction tube and add 10ml of 2M KCl. Mount horizontally on the orbital shaker and shake for 1 hour before centrifuging to remove solid matter. Include both blanks and positive controls in each run. Perform all extractions in triplicate.

Ammonium analysis –

- Add 2.5ml stock to 100ml matrix to give 2.5mgl^{-1} working solution, range for cal 0-2.5mgl^{-1}

Micro determination:

- Add 60ul of standard/sample to two wells
- Add 15ul EDTA stand 5 min
- Add 25ul phenol nitroprusside stand 1 min
- Add 50ul hypochlorite stand 1 min
- Add 160ul DI water an shake at 37˚C for 1 hour
- Analyse at 636nm after 10 minutes of cooling

Nitrite analysis –

- Dilute stock to 0.25mgl^{-1} by making 1ml up to 100 with matrix, range 0-0.25mgl^{-1}

Micro determination:

- Add 250ul standard/sample to two wells
- Add 25ul sulfanillic acid and shake for 20min
- Add 25ul NEDD and Shake for 1 hour
- Analyse at 535nm

Nitrate analysis –

- Dilute stock to 1mgl^{-1} by diluting 1ml in 100ml matrix, range 0-1mgl^{-1}
- In addition nitrite standards of 0-1mgl^{-1} are required, dilute stock by making 4ml up to 100ml with matrix
- Run both nitrate and nitrite standards

Micro determination:

- Add 140ul of standard/sample to two wells
- Add 60ul of catalyst mix and shake for 45min
- Add 75ul sulfanillic acid and shake for 20min
- Add 20ul NEDD and shake for two hours
- Analyse at 535nm

1.2.6 Analysis and Reporting of Results

For each form of nitrogen use the following calculation to determine the soil content:

[N] (mg/kg) = ((Abs_{Sample}-c) / m / mass of sample (g) / c.f.) x extraction volume (ml) x dilution factor

Where the calibration curve is linear in the form y = mx + c and c.f. is the oven dry content (105°C) of the material expressed as a fraction.

The three values can be added together to provide total inorganic nitrogen in the soil.

1.2.7 References

ADAS (1985). *The analysis of agricultural materials*. Agricultural Development and Advisory Service, London

Bundy, L.G. & Meisinger, J.J. (1994). *Nitrogen Availability Indices.* In : Weaver, R.W, Angle, J.S. & Bottomley, P.S. (eds.) 1994. *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America, Madison USA

Shand, C.A., Williams, B.L. & Coutts, G. (2008). Determination of N-species in soil extracts using microplate techniques. *Talanta*. 74, 648-654

SECTION 1.3 – TOTAL ORGANIC CARBON AND TOTAL NITROGEN VIA COMBUSTION

1.3.1 Introduction
Soil carbon and nitrogen can be measured by observing the production of CO2 and NOx during catalysed combustion by non dispersive infra red and chemiluminescnece respectively.

1.3.2 Safety Documents to be Consulted	
COSHH	See PUWER folder for TOC analyser
Risk Assessment	See PUWER folder for TOC analyser

1.3.3 Equipment and Consumable Required	
Sample preparation	Mortar and Pestle
Analysis	TOC analyser Analytical balance Temperature controlled hot plate

1.3.4 Reagents and Standards	
TOC stock	Dry and dissolve 0.5314g of hydrogen potassium phthalate in 100ml water with a few drops of concentrated nitric acid for 2500mgl^{-1} stock
TN stock	Dry and dissolve 0.1353g of 99% L-Asparagine monohydrate in 100ml water for 250mgl^{-1} stock
Phosphoric acid 20%	Dilute 120ml of 85% phosphoric acid to 500ml in water

1.3.5 Procedure
Sample preparation – Dry sample (air or oven as required) and grind a sub-sample into a fine powder with a mortar and pestle. This will increase homogenisation and allow small samples to be weighed.
Analysis • Turn on TOC/TN, boat sampler and air flow (zero grade air and oxygen). Log in to software start run and allow equipment to equilibrate (2-3 hours). • Insert boat and move into furnace to remove carbonates. • In triplicate add 40µl of blank or standard to boat and insert into furnace to create calibration curve (working range 0-250 for TN and 0-2500 for TOC). • Weigh sample into boat (0-10mg, for roughly 5% TOC 1mg would be best) add 10µl phosphoric acid and heat on hotplate at 70˚C for 10 minutes to remove carbonates. • Insert sample into furnace and record area for TOC and TN. Analyse samples in triplicate.

1.3.6 Analysis and Reporting of Results
For carbon and nitrogen use the following calculation to determine the soil content: [C or N] (mg/kg) = ((Reading-c) / m / mass of sample (g) / c.f.)

Where the calibration curve is linear in the form y = mx + c and c.f. is the oven dry content (105°C) of the material expressed as a fraction.

1.3.7 References

The British Standards Institute 1995a. *BS 7755 Soil Quality, Section 3.8 Determination of organic and total carbon after dry combustion (elementary analysis)*. The British Standards Institute, London

The British Standards Institute 2001a. *BS EN 13654 Soil improvers and growing media – Determination of nitrogen, Part 2 Dumas method*. The British Standards Institute, London

SECTION 1.4 – CATION EXCHANGE CAPACITY AND EXCHANGEABLE BASES

1.4.1 Introduction
As well as nutrient such as nitrogen and phosphorus plants also require large amounts of potassium and lesser amounts of other bases such as magnesium and calcium. In soil these are derived from the weathering of rocks and one of the most important factors effecting whether they stay in the soil or are leached out is the cation exchange capacity (CEC). CEC is basically a measure of the number of negatively charged sites in the soil capable of holding these positively charged nutrients. It is due to combination of permanent surface charges on clays, ionised functional groups on organic matter and pH dependant charges on clay edges and iron oxides. To measure it all these sites are filled with ammonium by leaching ammonium acetate through the soil (ADAS 1985). After rinsing this ammonium is removed by leaching with acidified potassium chloride and quantified colorimetrically using the berholt reaction (Bundy and Meisinger 1994). In addition the original ammonium acetate leachate can be used to quantify the level of exchangeable bases in the soil. This is traditionally done by atomic adsorption flame spectroscopy (FAAS), flame photometry or inductively coupled plasma (ICP-OES/MS) but in the absence of these instruments ion chromatography can be used to quantify these bases (Basta and Tabatabai 1985). To facilitate this the ammonium in the extraction solution needs to be removed by dry ashing and the bases need to be re-dissolved in a dilute hydrochloric acid solution.

1.4.2 Safety Documents to be Consulted	
COSHH	Extraction of nutrients/metals from soil for analysis Ammonium analysis
Risk Assessment	Extraction of nutrients/metals from soil for analysis

1.4.3 Equipment and Consumable Required	
Leaching	50ml syringes PP tubing Clamp flow regulators Glass wool 100ml volumetric flasks
C.E.C. NH_4 analysis	350µl 96 well flat bottomed microplates Orbital shaker/incubator Microplate reader 15ml centrifuge tubes Spectrophotometer
Exchangeable base analysis	20ml porcelain crucibles 50ml plastic centrifuge tubes Hot plate IC

1.4.4 Reagents and Standards	
Ammonium acetate	Dissolve 77g of ammonium acetate in one litre of water
Ethanol	Any high strength (80%+) ethanol solution
Potassium chloride	Dissolve 100g of KCl in 1000ml of water. Adjust the pH to 2.5 with 1M HCl (approx. 2.5ml per litre)
Ammonium stock	Dry and dissolve 0.3821g NH_4Cl in 1000ml water for 100mg l^{-1} stock

Ammonium analysis	• EDTA: 6g Na_2EDTA/100ml DI water pH7 • Phenol-nitroprusside :7g phenol and 34mg sodium nitroprusside in 100ml water, store in dark fridge • Buffered hypochlorite: 1.48g NaOH, 4.98g Na_2HPO_4, 20ml 5% NaOCl to 100ml with water pH11.4-12.2
Chromatography standards	1000ppm standards of Na, K, Mg,, Ca, Li and Mn bought from a reputable source

1.4.5 Procedure

Leaching procedure:

- Pack each syringe with a plug of glass wool followed by 2g of soil then another plug of glass wool (in triplicate for each sample). Attach an inch of tubing with a clamp flow regulator to each syringe.
- Attach each syringe to clamp stand and insert the tubing into a 100ml volumetric flask. Close the flow regulator and fill the syringe with ammonium acetate. Adjust the regulator until the flow rate is 2-3 drops per second. Leach small portions of ammonium acetate through the column until the volumetric flask until it is almost full. Remove the flask, make to the mark and retain for analysis.
- Rinse the outside of the column and tubing with de-ionised water. Leach 50-60ml of ethanol through the sample to remove any excess ammonium not bound to the soil. Safely dispose of the leachate.
- Put the tubing in another 100ml volumetric flask and repeat the leaching procedure with potassium chloride. Retain the leachate for analysis.

Ammonium analysis for CEC –

- Add 2.5ml stock to 100ml matrix to give 2.5mgl^{-1} working solution, range for cal 0-2.5mgl^{-1}

Micro determination:

- Add 60ul of standard/sample to each microplate well
- Add 15ul EDTA stand 5 min
- Add 25ul phenol nitroprusside stand 1 min
- Add 50ul hypochlorite stand 1 min
- Add 160ul DI water an shake at 37˚C for 1 hour
- Analyse at 636nm after 10 minutes of cooling

ExBase analysis by ion chromatography:

- Add 15ml of ammonium acetate leachate to a small crucible and heat on a hot plate just below boiling point until all the liquid has evaporated.
- Ash the dry sample at 450˚C for four hours to remove the ammonium acetate.
- Add 15ml of 5mM HCl to the cooled crucible and heat at 70˚C on a hotplate for 20 minutes. Pour the contents into a graduated 50ml centrifuge tube. Repeat twice more then make the volume up to 50ml with 5mM HCl.
- Analyse for Na, K, Mg, Ca, Li and Mn on an ion chromatograph according to the manufacturers instructions.

Alternatively samples may be analysed by FAAS using an appropriate method or handed of to the Scottish Environmental Technology Network (SETN) for ICP-OES analysis.

1.4.6 Analysis and Reporting of Results

To determine the C.E.C the first thing to do is calculate the ammonium concentration in the soil:

$[N\text{-}NH_4]$ (mg/kg) = ((Abs_{Sample}-c) / m / mass of sample (g) / c.f.) x etxractant volume (ml) x dilution factor

Where the calibration curve is linear in the form y = mx + c and c.f. Is the oven dry content (105°C) of the material expressed as a fraction

To express C.E.C. in its accepted units (centimoles of charge per kg) this needs to be converted:

C.E.C. ($cmol_C/kg$) = ([$N\text{-}NH_4$] (mg/kg) x charge of ammonium x 10)/ molar mass of nitrogen (g)

So C.E.C. ($cmol_C/kg$) = (10 x [N-NH4] (mg/kg))/14

For individual bases use the following calculation to determine the soil content:

[Base] (mg/kg) = ((Reading-c) / m / mass of sample (g) / c.f.) x extraction volume (ml) x dilution factor

Where the calibration curve is linear in the form y = mx + c and c.f. is the oven dry content (105°C) of the material expressed as a fraction.

1.4.7 References

ADAS (1985). *The analysis of agricultural materials*. Agricultural Development and Advisory Service, London

Basta, N.T. & Tabatabai, M.A. (1985). Determination of Exchangeable Bases in Soils by Ion Chromatography. *Soil Science Society of America Journal,* 49, 84-89

Bundy, L.G. & Meisinger, J.J. (1994). *Nitrogen Availability Indices.* In : Weaver, R.W, Angle, J.S. & Bottomley, P.S. (eds.) 1994. *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America, Madison USA

SECTION 1.5 – AVAILABLE HEAVY METALS/TRACE NUTRIENTS

1.5.1 Introduction
The availability of heavy metals is important in determining any potential toxicity issues for plants and other soil biota. However, some metals (Cu and Zn) are also essential micronutrients so available levels are also important for identifying any potential deficiencies in soil systems. One of the common methods for determining available metal content for Cu, Zn, Cd, Ni and Pb is to extract the soil with a buffered NH_4EDTA solution (ADAS 1985). After filtration this solution can be analysed by a variety of methods such as ICP and FAAS.

1.5.2 Safety Documents to be Consulted	
COSHH	Extraction of nutrients/metals from soil for analysis
Risk Assessment	Extraction of nutrients/metals from soil for analysis

1.5.3 Equipment and Consumable Required	
Extraction	15ml centrifuge tubes End over end shaker 0.45µm MCE syringe filter (fisher, product code 10460031) 20ml plastic syringes

1.5.4 Reagents and Standards	
Extracting solution (0.05M NH_4EDTA)	Dissolve 14.6g of EDTA in 950ml of water and add 8ml of 35% ammonia solution. Add 1M nitric acid or ammonia to correct the pH to 7. Make up to 1l. Note, if the pH is below 7 the EDTA will not easily dissolve until the pH has been corrected.

1.5.5 Procedure
Weigh out 2g of sample into three centrifuge tubes. Add 10ml of extracting solution, close the tubes and spin on end over end shaker for 1 hour at 20˚C. Filter through the syringe filters and refrigerate the filtrate. Samples were analysed by ICP-OES at the Scottish Environmental Technology Network (SETN) labs. Samples were handed off to their technician and results were received as a liquid concentration. Alternatively samples could be analysed using an appropriate FAAS method. Include blank samples in the analysis to account for any metals in the extracting solution.

1.5.6 Analysis and Reporting of Results
For each metal the following calculation was used to determine the concentration in the soil: [Metal] (mg/kg) = Liquid concentration (mg/l) x etxractant volume (ml) x dilution factor / c.f. / sample mass (g) Where c.f. Is the oven dry content (105°C) of the material expressed as a fraction

1.5.7 References
ADAS (1985). *The analysis of agricultural materials*. Agricultural Development and Advisory Service, London

SECTION 1.6 – AQUA-REGIA SOLUBLE CONTENT

1.6.1 Introduction
Aqua regia digestion of soil provides a pseudo total content of non-volatile elements such as metals and phosphorus in soil (BS 2001). The value can only be described as pseudo total as some soil components, such as silicates, are resistant to digestion. More aggressive methods are available such as hydrofluoric acid digestion and perchloric acid digestion however they are more hazardous, for many applications aqua regia will provide sufficient data while limiting risk. Soil samples are finely ground then digested in by boiling in a 3:1 mix of hydrochloric and nitric acid. Large amounts of organic matter and black carbon will also reduce digestion efficiency – in these cases an additional ashing step can be introduced before wet digestion to improve digestion efficiency.

1.6.2 Safety Documents to be Consulted	
COSHH	Aqua regia digestion of soil samples
Risk Assessment	Aqua regia digestion of soil samples

1.6.3 Equipment and Consumable Required	
Digestion	50ml beakers with watch glasses or 10ml Hach digestion tubes with plastic lid (not lids with rubber septas) Hot plate ore digestion block Anti bumping granules Hardened quantitative filter paper (Fisher QT 280, product code 11732375) Plastic filter funnels 100ml or 50ml volumetric flasks Mortar and pestle 15ml plastic centrifuge tubes

1.6.4 Reagents and Standards
Concentrated nitric acid
Concentrated hydrochloric acid

1.6.5 Procedure
Digestion procedure: • Grind the sample into a fine powder using the mortar and pestle • Add 0.5g of sample to three beakers • Add 9ml of hydrochloric and 3ml of nitric acid and a few anti bumping granules to each beaker • Cover and rest at room temperature for 16 hours (overnight) • Using a hot plate bring the acid to the boil for two hours, making sure non of the samples dry out • After cooling pour the digestate into a 100ml volumetric flask and rinse the beaker three times with water, adding each rinse to the volumetric flask

- Bring the flask to volume with water, mix and allow any residue to settle
- Filter a portion of the sample into a centrifuge tube for storage/analysis

Alternate method for high organic/black carbon samples:

- Add 0.5g of the finely ground sample to three Hach tubes
- Heat in a furnace at 550˚C for four hours (no char should remain, if it does higher temperatures may be required)
- Allow to cool then add 3ml of hydrochloric and 1ml of nitric acid to each tube
- Loosely cap and rest at room temperature for 16 hours (overnight)
- Seal the tubes and digest in a digestion block at 120˚C for two hours
- Cool and prepare samples as above using a 50ml rather than 100ml volumetric

Samples were analysed by ICP-OES at the Scottish Environmental Technology Network (SETN) labs. Samples were handed off to their technician and results were received as a liquid concentration. Alternatively samples could be analysed using an appropriate FAAS method or colorimetrically for P content. Include blank samples in the analysis to account for any metals in the aqua regia.

1.6.6 Analysis and Reporting of Results

For each element the following calculation was used to determine the concentration in the soil:

[Element] (mg/kg) = Liquid concentration (mg/l) x digestate volume (ml) x dilution factor / c.f. / sample mass (g)

Where c.f. Is the oven dry content (105°C) of the material expressed as a fraction

1.6.7 References

The British Standards Institute (2001). *BS EN 13650 Soil improvers and growing media – Extraction of aqua regia soluble elements*. The British Standards Institute, London

SECTION 1.7 – ANALYSIS OF pH AND ELECTRICAL CONDUCTIVITY (EC)

1.7.1 Introduction
pH and EC can be simply measured in a 1:5 soil:water extract using an appropriate meter.

1.7.2 Safety Documents to be Consulted	
COSHH	n/a
Risk Assessment	See PUWER folder for appropriate metre

1.7.3 Equipment and Consumable Required	
Extraction	15ml centrifuge tubes End over end shaker 0.45µm MCE syringe filter (fisher, product code 10460031) 20ml plastic syringes

1.7.4 Reagents and Standards	
pH	Appropriate bought in standards to calibrate meter
EC	Appropriate bought in standards to calibrate meter

1.7.5 Procedure

- Weigh 2 grams of soil into 3 centrifuge tubes and shake for 2 hours
- With sample still in suspension measure and record pH according to manufacturers instructions
- Filter samples using the syringe filters
- Measure and record EC according to manufacturers instructions

1.7.6 Analysis and Reporting of Results

Report mean of the three measurements

1.7.7 References

The British Standards Institute 1995c. *BS 7755 Soil Quality, Section 3.4 Determination of the specific electrical conductivity*. The British Standards Institute, London

The British Standards Institute 2005. *BS ISO 10390 Soil Quality – Determination of pH*. The British Standards Institute, London

SECTION 2.1 – ORGANIC MATTER AND ASH CONTENT BY COMBUSTION

2.1.1 Introduction
Organic content is determined by dry ashing at 550°C for four hours and observing the change in mass.

2.1.2 Safety Documents to be Consulted	
COSHH	n/a
Risk Assessment	See furnace PUWER folder

2.1.3 Equipment and Consumable Required
Muffle furnace Porcelain crucibles 4d.p. analytical balance

2.1.4 Reagents and Standards
n/a

2.1.5 Procedure
• For each sample heat 3 crucibles at 550°C for four hours • After cooling weigh each crucible (Mcru) • Add ~10g of soil to each crucible and dry at 105°C for 48 hours • After cooling weigh each crucible (Mdry) • Heat at 550°C for 4 hours • After cooling weigh each crucible (Mashed)

2.1.6 Analysis and Reporting of Results
Organic content = 100- (((Mashed-Mcru)/(Mdry-Mcru))*100)

2.1.7 References
The British Standards Institute 2000a. *BS EN 13039 Soil improvers and growing media – Determination of organic matter and ash*. The British Standards Institute, London

SECTION 2.2 – ANALYSIS OF PARTICLE SIZE DISTRIBUTION

2.2.1 Introduction
The texture of a soil has important implications for water balance, structure and nutrient retention. To asses texture the relative proportion of sand (2mm to 53µm), silt (53µm to 2µm) and clay (less than 2µm) sized particles needs to be quantified. The first step involved in this process is to remove organic matter from the sample that may cement particles together by digestion with hydrogen peroxide. In addition it may be necessary to remove other cementing materials such as gypsum or iron oxides (see BS). Following this the particles must be fully separated using a dispersant before the relative proportions are determined by wet sieving and sedimentation. By comparing these values to classification tables the textural class can be determined.

2.2.2 Safety Documents to be Consulted	
COSHH	Particle size distribution
Risk Assessment	Particle size distribution

2.2.3 Equipment and Consumable Required	
Organic matter removal	250ml Conical flasks Watch Glasses Hotplate 250ml Plastic bottles Large centrifuge 50ml Syringe with plastic tubing attached
Dispersion	250ml plastic bottle End over end shaker
Particle separation	63µm Test sieve with bottom container 500ml Glass cylinders 10ml glass pipettes Large plastic funnel Parafilm Aluminium foil dishes Spatula

2.2.4 Reagents and Standards	
Organic matter removal	Make up a 30 Volume solution of hydrogen peroxide by diluting bought in solutions appropriately
Dispersing agent	Dissolve 33g of sodium hexametaphosphate and 7g of anhydrous sodium carbonate in 1l of water. Store out of sunlight.

2.2.5 Procedure
Organic matter removal (if present):

- Weigh out 10-15g of soil in a 250ml conical flask
- Thoroughly wet with 30ml of water and then add 30ml of hydrogen peroxide solution
- Allow the mixture to react overnight before gently heating to around 100˚C on a hotplate until reaction stops
- If excess foaming occurs a few drops of ethanol or octan-2-ol may be added as an anti-foaming agent
- Quantitatively transfer the digested soil to a plastic bottle, fill with water and centrifuge
- Remove supernatant containing dark organic residues. Repeat until the supernatant is largely colourless but take care to centrifuge enough so clays are not still in suspension and removed

(If necessary additional steps for the removal of soluble salts, gypsum and iron oxides can be used – see BS for details)

Dispersion:

- Add 25ml dispersing solution to each sample.
- Fill the plastic bottle with water until it contains 150-200ml
- Spin on the en d over end shaker for 18hours to disperse the aggregates (overnight) in a controlled temperature environment (20˚C)

Particle separation (in controlled temperature environment of 20˚C):

- For each sample oven dry (24hours) and weigh (in grams to three d.p.) three foil dishes (Mdish1-3)
- Empty the contents of a sample bottle onto the sieve so the liquid drains into the container, thoroughly rinse the bottle and wash any fines through the sieve using a wash bottle
- Transfer any sample retained on the sieve into dish 1
- Using the funnel transfer the liquid to the 500ml cylinder and make to 500ml with water. Cover with parafilm, thoroughly mix by inverting at least 20 times and then place on a level surface.
- After 56 seconds pipette a 10ml portion of the liquid from 20cm below the surface into dish 2. After a further 7 hours and 44 minutes pipette another 10ml this time from 10cm depth into sample dish 3.
- Oven dry the three dishes to a constant weight and weigh in grams to three d.p. (Mdry1-3)

Dispersant correction (in controlled temperature environment of 20˚C):

- Oven dry (24 hours) 3 sample dishes and weigh in grams to three d.p.
- Add 25ml dispersant to a cylinder, make to 500ml with water, cover with parafilm and mix by inverting at least 20 times.
- After 56 seconds pipette three 10ml samples from 20cm depth in to the three dishes
- Oven dry to constant mass and weigh in grams to three d.p. Use this data to compensate for the dry mass of the dispersant (Dry mass – dish mass = Mdisp)

2.2.6 Analysis and Reporting of Results

Mass of sand = Mdry1 – Mdish1
Mass of clay = (Mdry3 - Mdish3 – Mdisp) x 50
Mass of silt = ((Mdry2 – Mdish2 – Mdisp) x 50) – Mass of clay

% of individual component = (Mass of component/(Mass of sand + mass of silt +mass of clay) x 100

2.2.7 References

The British Standards Institute 2009. *BS ISO 11277 Soil quality – Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation*. The British Standards Institute, London

SECTION 3.1 – DEGRADATION ENZYMES - ACID AND ALKALI PHOSPAHTASE AND B-GLUCOSIDASE

3.1.1 Introduction
These enzymes are involved (though far from exclusively) in the biodegradation and cycling of carbon (β-Glucosidase), and phosphate (Acid Phosphatase) and will give some indication of how well the microbial communities are functioning in terms of nutrient cycling. Both acid phosphatise and β-glucosidase assays are based on the hydrolysis of p-nitrophenol conjugates (p-nitophenyl phosphate (PNP) and p-nitrophenyl-β-glucopyranoside (PNG) respectively) and are quantified by the measuring the absorbance due to the nitrophenol produced which turns yellow under alkaline conditions.

3.1.2 Safety Documents to be Consulted	
COSHH	1. Phosphomonoesterase (Acid and Alkaline) NP based Assays for Soil 2. Glycosidase (α and β glucosidase and galactosidase) NP based enzyme assays for soil
Risk Assessment	1. p-Nitrophenol (NP) based enzyme assays for soil

3.1.3 Equipment and Consumable Required
350µl 96 well microplates 37˚C Water Bath 15ml centrifuge tubes Centrifuge Microplate reader

3.1.4 Reagents and Standards	
Modified Universal Buffer (MUB) Stock Solution	Dissolve 12.1g of THAM, 11.6g of maleic, 14g of citric acid and 6.3g of boric acid in 1l of water
MUB pH 6, 6.5 and 11	Titrate 100ml of MUB stock with 0.5M HCl to desired pH and make up to 500ml with water. For pH 11 titrate with 0.1M NaOH
p-nitrophenyl phosphate (PNP)	Dissolve 0.420g of PNP in 25ml of MUB pH 6.5 (refrigerate)
p-nitrophenyl β-glucopyranoside (PNG)	Dissolve 0.188g of PNG in 25ml of MUB pH 6.0 (refrigerate)
0.5M $CaCl_2$ solution	Dissolve 3.675g of $CaCl_2$ in 50ml of water
NP Stop Solution (THAM pH 12.1)	Dissolve 1.21g of THAM in 100ml of water and adjust pH to 12.1 using 0.5M NaOH
p-Nitrophenol (NP) Standard 1000mg/l	Dissolve 1g of p-Nitrophenol in 1l of water

3.1.5 Procedure

Acid Phosphatase Assay –

- For each sample add 4ml of MUB pH 6.5 to six 15ml centrifuge tubes containing 1g of soil
- To four of the tubes (the samples) add 1ml of PNP and incubate all three tubes at 37°C for one hour after mixing well
- Add 1ml CaCl2 solution to each tube then add 4ml of NP stop solution to each tube
- To the tubes with no PNP (the control) now add 1ml of PNP
- Dilute the NP stock solution to 50mg/l by making 5ml of stock up to 100ml with MUB pH 6.5
- Create standards in the range of 0-50mg/l and add 5ml of each to a tub along with 1ml of CaCl2 and 4ml of NP stop solution
- Centrifuge all the tubes to obtain a clear supernatant and pipette 0.3ml from each eppendorf into a microplate well and read the absorbance at 400nm or use a spectrophotometer with a 1cm path length

Alkaline Phosphatase Assay –

- As for acid phosphatase but using MUB pH 11 instead of MUB pH 6.5

β-Glucosidase Assay –

As for acid phosphatase but using MUB pH 6 instead of MUB pH 6.5 and PNG instead of PNP

3.1.6 Analysis and Reporting of Results

$$\text{Activity} = \text{Mass of End Product Produced} \div \text{Mass of Sample} \div \text{Incubation Time}$$

$$\text{Activity} = \frac{\left(\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}\right) \times \text{Volume}}{\text{Sensitivity} \times \text{Sample Mass} \times \text{Incubation Period} \times c.f.}$$

Where the calibration curve is linear in the form y = mx + c (sensitivity = m) and c.f. is the oven dry content (105°C) of the material expressed as a fraction

3.1.7 References

Eivazi, F. and Tabatabai, M.A. (1988). Glucosidases and galactosidases in soil. *Soil Biology and Biochemistry*, **20**, 601-606

Tabatabai, M.A. and Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, **1**, 301-307

Tabatabai, M.A. (1994). *Soil Enzymes*. In : Weaver, R.W, Angle, J.S. and Bottomley, P.S. (eds.) (1994). *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America. Madison USA

SECTION 3.2 – AMMONIA OXIDASE ACTIVITY

3.2.1 Introduction
Ammonia oxidation is an important soil process. The activity of the enzymes responsible can be measured by observing the production of nitrite in the presence of ammonium and a suitable inhibitor to prevent nitrite being converted to nitrate

3.2.2 Safety Documents to be Consulted	
COSHH	Ammonium oxidase assay
Risk Assessment	Ammonium oxidase assay

3.2.3 Equipment and Consumable Required
350µl 96 well microplates 15ml centrifuge tubes Centrifuge Microplate reader

3.2.4 Reagents and Standards	
Am.Ox. Substrate	1mM ammonium sulphate – Dissolve 0.1321 g in 1litre of water
Am.Ox. Inhibitor	1.5M Sodium chlorate – Dissolve 1597g in 100ml of water
2M Potassium chloride	Dissolve 75g in 500ml water
Ammonium chloride buffer	Dissolve 5g of ammonium chloride in 450ml of water. Adjust pH to 8.5 using an ammonia solution and make solution up to 500ml
Colour reagent	Dissolve 2g of sulphanilamide and 0.1g of NEDD in 150ml of water and slowly add 20ml of conce. phosphoric acid. Make up to 200ml. Solution should be colourless and needs to be made up daily.
Nitrite stock solution	Dissolve 0.4926g of dried sodium nitrite in 100ml of water. Store at 4˚C for max. 3 weeks.
Calibration standards	Dilute stock to 10ppm using water. Dilute to range 0-1ppm in water with 2M KCl added at 20% of total standard volume.

3.2.5 Procedure

- For each sample add 4ml of substrate and 0.02ml of inhibitor to six 15ml centrifuge tubes containing 1g of soil
- Incubate four tubes at 20°C and two at -20°C for five hours
- Add 1ml of potassium chloride solution to each tube (defrost frozen tubes)
- Pipette 125ul of each sample/standard into a microplate well followed by 75ul of buffer and 50ul of colour reagent
- After 15 minutes measure colour produced at 520nm

3.2.6 Analysis and Reporting of Results

$$\text{Activity} = \text{Mass of End Product Produced} \div \text{Mass of Sample} \div \text{Incubation Time}$$

$$\text{Activity} = \frac{\left(\text{Abs}_{\text{Sample}} - \text{Abs}_{\text{Control}}\right) \times \text{Volume}}{\text{Sensitivity} \times \text{Sample Mass} \times \text{Incubation Period} \times c.f.}$$

Where the calibration curve is linear in the form y = mx + c (sensitivity = m) and c.f. is the oven dry content (105°C) of the material expressed as a fraction

3.2.7 References

Berg, P. and Rosswall, T. (1985). Ammonium oxidiser numbers, potential and actual oxidation rates in two Swedish arable soils. *Biology and Fertility of Soils*. 1, 131-140

Kandeler, E., Poll, C., Frankenberger, W.T. and Tabatabai, M.A. (2011). *Nitrogen Cycle Enzymes*. In :Dick, R.P. (ed.) (2011*). Methods of Soil Enzymology*. Soil Science Society of America. Madison USA

Tabatabai, M.A. (1994). Soil Enzymes. In : Weaver, R.W, Angle, J.S. and Bottomley, P.S. (eds.) (1994). *Methods of Soil Analysis: Part 2 – Microbiological and Biochemical Properties*. Soil Science Society of America. Madison USA

SECTION 3.3 - ANALYSIS OF SOIL DNA TARGET GENES

3.3.1 Introduction
The following report presents the standard operating procedure for analysing soil DNA for specific genes. This report covers 16S, nirS, nirK, amoA and nifH.

3.3.2 Safety Documents to be Consulted	
COSHH	Extraction of DNA from soil samples for analysis
Risk Assessment	Extraction of DNA from soil samples for analysis

3.3.3 Equipment and Consumable Required	
Extraction	MoBio soil DNA extraction kit Centrifuge Fridge (5°C) Micropipettes
Analysis	350µl 96 well flat bottomed microplates BioRad iQ5 PCR Cycler Micropipettes

3.3.4 Reagents and Standards		
Sigma Aldrich primers	**nirS**	nirSCd3aF (5'-AACGYSAAGGARACSGG)
		nirSR3cd (5'-GASTTCGGRTGSGTCTTSAYGAA)
	nirK	nirK-876 (5'-ATYGGCGGVAYGGCGA)
		nirK-1040 (5'-GCCTCGATCAGRTTRTGGTT)
	amoA	amoA-1F (5'-GGGGTTTCTACTGGTGGT)
		amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC)
	nifH	nifH: pol1F (5'-TGCGAYCCSAARGCBGACTC)
		nifH: pol2R (5'-ATSGCCATCATYTCRCCGGA)
	16S	5'-CCTACGGGAGGCAGCAG-3'
		5'-ATTACCGCGGCTGCTGG-3'
Enzyme reagent (volumes required per well / sample)	5µl Bio-Rad SsoAdvanced SYBR Green Supermix 3µl nuclease free water 0.5µl primer of target primer (i.e. one of the above)	
DNA Standards	Serial dilutions from a known DNA concentration (e.g. 10^3 - 10^9)	
Water	Nuclease free water	

3.3.5 Procedure

DNA Extraction (taken from MoBio soil DNA extraction kit protocol):

1. Add 0.25 – 0.5g of soil to **PowerBead Tubes** and gently vortex to mix;
2. Add 60 µl of Solution C1 and invert several times or vortex briefly;
3. Secure **PowerBead Tubes** onto MP FastPrep 24 ribolyser and run for 20 secs at 5M/S. Allow to cool and repeat;
4. Transfer the supernatant to a clean **2 ml Collection Tube**. **Note**: Expect between 400 to 500 µl of supernatant. Supernatant may still contain some soil particles;
5. Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 5 minutes;
6. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*;
7. Avoiding the pellet, transfer up to, but no more than, 600 µl of supernatant to a clean **2 ml Collection Tube**;
8. Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes;
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 x *g*;
10. Avoiding the pellet, transfer up to, but no more than, 750 µl of supernatant into a clean **2 ml Collection Tube**;
11. Shake to mix Solution C4 before use. Add 1200 µl of Solution C4 to the supernatant and vortex for 5 seconds;
12. Load approximately 675 µl onto a **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Discard the flow through and add an additional 675 µl of supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. Load the remaining supernatant onto the **Spin Filter** and centrifuge at 10,000 x *g* for 1 minute at room temperature. **Note**: A total of three loads for each sample processed are required;
13. Add 500 µl of **Solution C5** and centrifuge at room temperature for 30 seconds at 10,000 x *g*;
14. Discard the flow through and centrifuge again at room temperature for 1 minute at 10,000 x *g*;
15. Carefully place spin filter in a clean **2 ml Collection Tube**. Avoid splashing any **Solution C5** onto the **Spin Filter**;
16. Add 100 µl of **Solution C6** to the centre of the white filter membrane. Alternatively, nuclease free water may be used for elution from the silica Spin Filter membrane at this step;
17. Centrifuge at room temperature for 30 seconds at 10,000 x *g*;
18. Discard the **Spin Filter**. The DNA in the tube is now ready for any downstream application. No further steps are required.

DNA Analysis:

1. Add 1.5µl of sample DNA per microplate well and add 8.5µl of reagent to each well. Cover the microplate with film and place into the qPCR tray. **Note**: Mix and use the enzyme reagent mix on day of analysis as it does not keep. Use one primer per target DNA;
2. Add at least five standards and two blanks to each microplate to ensure PCR efficiency;
3. Update the program protocol with corresponding DNA temperatures. An initialisation step set at 98°C for 3 mins, followed by 40 cycles of set temperatures (see table below). A melt curve is created with 1°C increments from 55 – 95°C for 6 secs;

Table 1 – Annealing temperatures for target DNA

Step	Temperature (°C)			
	nirS	**nirK**	**amoA**	**nifH**
Denaturing	95	95	95	95
Annealing	55	55	52	53
Elongation	60	60	55	54

4. Create a detailed PCR report and Melt Curve report upon run completion.

3.3.6 Analysis and Reporting of Results

The software associated with the PCR machine quantifies the data. Correct values for mass of soil extracted, extract volume and dilution factor

3.3.7 References

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Martin-Laurent, F., Brauman, A. and Philippot, L. (2004). Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. *Journal of Microbiological Methods*, **59**, 327-335

Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D. And Philippot, L. (2006). Abundance of nirS, nirK, narG and nosZ gens of denitrifying bacteria during primary succession of a glacier foreland. *Applied Environmental Microbiology*, **72**, 5957-5973

Muyzer, G., de Wall, E.C. and Uitterlinden, A.G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16s. *Applied Environmental Microbiology*, **59**, 695-702

Poly, F., Monrozier, L.J. and Bally, R. (2001). Improvements in the RFLP procedure for studing the diversity of nifH genes in communities of nitrogen fixers in soil. *Research in Microbiology*, **152**, 95-103

Rotthauwe, J.H., Witzel, K.P. and Liesack, W. (1997). The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine scale analysis of natural ammonia-oxidizing populations. *Applied Environmental Microbiology*, **63**, 4704-4715

SECTION 4.1 – LEAF CHLOROPHYLL CONTENT

4.1.1 Introduction

Heating soil samples is likely to not only effect the quantity of plant matter produced but also its quality. One measure of this would be the chlorophyll content of the leaves which would be dependent on the amount of nitrogen and certain micronutrients (incl. copper) available to the plant but would also indicate how much photosynthesis is going on. To extract the chlorophyll frozen samples of fresh leaf tissue are heated to 65˚C in DMSO (di-methyl sulphoxide) (Hiscox and Isrelstam 1979) and the amount of chlorpophyll determined by colorimetric analysis at 645nm and 663nm according to the equations of Arnon (1949).

4.1.2 Safety Documents to be Consulted

COSHH	Extracting chlorophyll from plant tissue
Risk Assessment	Extracting chlorophyll from plant tissue

4.1.3 Equipment and Consumable Required

1.5ml Eppendorf tubes
15ml polypropylene centrifuge tubes
-20˚C freezer
Water bath at 65˚C
96 well microplates
Microplate reader

4.1.4 Reagents and Standards

Di-methyl sulphoxide (DMSO)

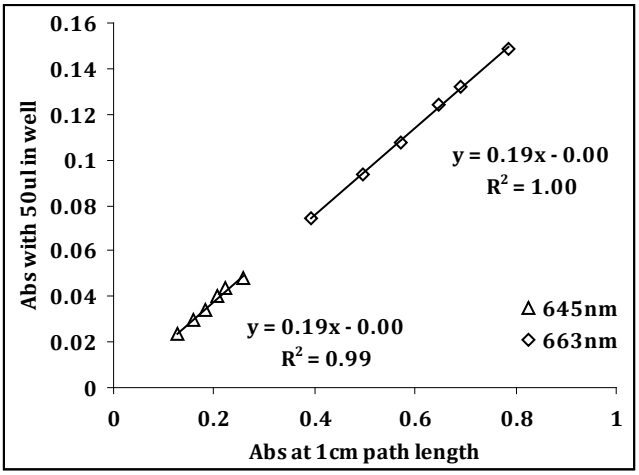
4.1.5 Procedure

- Weigh 0.02-0.1g of fresh leaf tissue into a 1.5ml eppendorf (label only on lid) and record the mass to 4 d.p. Freeze the sample at -20˚C until analysis. Retain a portion of each leaf sample for moisture content determination (see section 1).
- To extract the chlorophyll cut the top off each eppendorf tube and drop into a 15ml centrifuge tube. Add 10ml of DMSO to each tube and stand them in a water bath at 65˚C.
- Every 15 minutes invert the tubes several times to ensure mixing.
- For each leaf type create a time series to determine when extraction is complete. Every 15 minutes remove the same two samples. Pipette 150µl of each into three microplate wells and analyse at 645nm and 663nm. Return the samples from the wells to the tubes and return to the water bath. When the absorbance values ceases increasing extraction is complete. This time series only needs to be performed once for each leaf type.
- When extraction is complete remove the tubes from the water bath and allow to cool. Once cool pipette 50µl of each sample into two microplate wells. Include DMSO blanks on each plate then analyse on the microplate reader at 645nm and 663nm. Record the results as sample absorbance – blank absorbance.

4.1.6 Analysis and Reporting of Results

To convert the absorbance values to a concentration of chlorophyll the equations determined by Arnon (1949). Although some authors have revised the values slightly (Barnes et al. 1992) it was found that the natural variation between plants made the difference between using the two equations statistically insignificant, though Barnes equations generally produced a marginally higher result. Arnons' equation is:

$$\text{Chlorophyll (mg/l)} = 20.2Abs_{645} + 8.02Abs_{663}$$



However the equations of Arnon are based on a 1cm path length so need to be converted for the path length for 50µl in a microplate well. To do this a concentrated chlorophyll extract was dilute to several different concentrations and the absorbance determined using each system. The results were plotted (left) and the trend lines used to alter the equations so:

$$Abs_{1cm} = -(1/0.19)\, Abs_{50\mu l}$$

$$Abs_{1cm} = 5.26\, Abs_{50\mu l}$$

Therefore:

$$\text{Chlorophyll (mg/l)} = 106.25Abs_{645} + 42.19Abs_{663}$$

To convert to content in leaf:

$$\text{Leaf content (mg/g)} = (\text{extract volume} \times \text{chlorophyll (mg/l)}) \div \text{dry leaf mass (g)}$$

For this to be valid the relationship between chlorophyll content and absorbance must be within the linear range. To ensure this take the sample with the highest absorbance, dilute to 0.75, 0.50 and 0.25 of the initial concentration and measure the absorbance of these dilutions and the initial solution at 645nm and 663nm. If these absorbances produce a linear plot then the results are within the acceptable range. If not the linear range needs to be established and samples diluted to be within it.

4.1.7 References

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 24, 1-15

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S. and Davison, A.W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophylls *a* and *b* in lichens and higher plants. *Environmental and Experimental Botany*. 32, 85-100

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*. 57, 1332-1334

Appendix II – Chapter 3 Statistics

Table II.1 – Statistical analysis for plant extension, Chapter 3

		Aboveground Extension									Belowground Extension								
	Treat.	T-test p	Cont.	105	250	500	750	1000	SM	Group	T-test p	Cont.	105	250	500	750	1000	SM	Group
Soil 1 Clover	Cont.	0.91		0.29	0.00	0.00	0.00	0.00	0.00	a	0.85		0.75	0.20	0.55	0.06	0.00	0.00	ab
	105	0.00			0.00	0.00	0.00	0.00	0.00	a	0.66			0.03	0.64	0.05	0.00	0.00	ab
	250	0.56				0.21	0.01	0.00	0.00	b	0.90				0.03	0.00	0.00	0.00	a
	500	0.56					0.01	0.00	0.00	b	0.19					0.10	0.00	0.00	b
	750	0.33						0.04	0.37	c	0.07						0.00	0.18	bd
	1000	0.54							0.00	d	0.13							0.00	e
	SM	0.26	ANOVA p=0.00							c	0.42	ANOVA p=0.00							d
Soil 2 Clover	Cont.	0.05		0.59	0.12	0.00	0.00	0.00	0.00	ab	0.54		0.95	0.22	0.20	0.00	0.00	0.01	a
	105	0.29			0.03	0.00	0.00	0.00	0.00	a	0.66			0.18	0.18	0.00	0.00	0.00	a
	250	0.41				0.00	0.00	0.00	0.00	b	0.09				0.71	0.00	0.00	0.00	a
	500	0.01					0.00	0.00	0.00	c	0.08					0.00	0.00	0.00	a
	750	0.00						0.06	0.00	d	0.06						0.79	0.02	b
	1000	0.37							0.00	d	0.17							0.07	bc
	SM	0.72	ANOVA p=0.00							e	0.01	ANOVA p=0.00							c
Soil 1 Fescue	Cont.	0.54		0.95	0.03	0.01	0.00	0.00	0.00	a	0.94		0.07	0.00	0.04	0.00	0.00	0.43	ab
	105	0.09			0.03	0.00	0.00	0.00	0.00	a	0.64			0.02	0.34	0.00	0.00	0.74	abc
	250	0.96				0.51	0.00	0.00	0.01	b	0.00				0.12	0.29	0.00	0.07	cd
	500	0.29					0.00	0.00	0.00	b	0.41					0.01	0.00	0.42	d
	750	0.00						0.00	0.04	c	0.68						0.00	0.02	e
	1000	0.09							0.00	d	0.72							0.00	f
	SM	0.78	ANOVA p=0.00							e	0.32	ANOVA p=0.00							bd
Soil 2 Fescue	Cont.	0.19		0.16	0.19	0.56	0.00	0.00	0.00	a	0.98		0.15	0.12	0.00	0.00	0.00	0.00	a
	105	0.36			0.86	0.07	0.00	0.00	0.00	a	0.88			0.77	0.00	0.00	0.00	0.00	a
	250	0.23				0.08	0.00	0.00	0.00	a	0.14				0.01	0.00	0.00	0.00	a
	500	0.30					0.00	0.00	0.00	a	0.78					0.01	0.00	0.23	b
	750	0.70						0.03	0.01	b	0.72						0.00	0.08	c
	1000	0.02							0.92	c	0.67							0.00	d
	SM	0.62	ANOVA p=0.00							c	0.69	ANOVA p=0.00							bc

Table II.2 – Statistical analysis for plant biomass production, Chapter 3

		Aboveground Biomass									Belowground Biomass								
	Treat.	T-test p	Cont.	105	250	500	750	1000	SM	Group	T-test p	Cont.	105	250	500	750	1000	SM	Group
Soil 1 Clover	Cont.	0.63		0.19	0.00	0.00	0.00	0.00	0.00	a	0.73		0.17	0.00	0.00	0.00	0.00	0.00	a
	105	0.15			0.00	0.00	0.00	0.00	0.00	a	0.24			0.02	0.04	0.00	0.00	0.00	a
	250	0.30				0.66	0.00	0.00	0.00	b	0.22				0.78	0.00	0.00	0.00	b
	500	0.46					0.00	0.00	0.00	b	0.21					0.01	0.00	0.02	b
	750	0.14						0.13	0.98	cd	0.23						0.05	0.21	c
	1000	0.73							0.00	d	0.52							0.00	d
	SM	0.82	ANOVA p=0.00							c	0.15	ANOVA p=0.00							c
Soil 2 Clover	Cont.	0.51		0.17	0.03	0.00	0.00	0.00	0.00	a	0.31		0.01	0.17	0.67	0.01	0.00	0.00	a
	105	0.52			0.00	0.00	0.00	0.00	0.00	a	0.59			0.07	0.04	0.00	0.00	0.00	b
	250	0.35				0.00	0.00	0.00	0.00	b	0.17				0.54	0.00	0.00	0.00	ab
	500	0.01					0.00	0.00	0.00	c	0.13					0.04	0.02	0.01	a
	750	0.00						0.38	0.17	d	0.05						0.13	0.03	c
	1000	0.00							0.66	d	0.25							0.06	cd
	SM	0.01	ANOVA p=0.00							d	0.15	ANOVA p=0.00							d
Soil 1 Fescue	Cont.	0.27		0.73	0.00	0.00	0.00	0.00	0.00	a	0.17		0.49	0.00	0.01	0.00	0.00	0.03	a
	105	0.01			0.01	0.00	0.00	0.00	0.00	a	0.01			0.00	0.00	0.00	0.00	0.01	a
	250	0.41				0.05	0.00	0.00	0.00	b	0.28				0.55	0.00	0.00	0.52	b
	500	0.34					0.00	0.00	0.00	c	0.46					0.00	0.00	0.77	b
	750	0.00						0.02	0.00	d	0.48						0.05	0.02	c
	1000	0.22							0.00	e	0.06							0.01	c
	SM	0.51	ANOVA p=0.00							f	0.32	ANOVA p=0.00							b
Soil 2 Fescue	Cont.	0.00		0.00	0.00	0.21	0.00	0.00	0.00	a	0.18		0.00	0.04	0.20	0.00	0.00	0.00	a
	105	0.54			0.03	0.00	0.00	0.00	0.00	b	0.44			0.53	0.21	0.00	0.00	0.00	b
	250	0.55				0.00	0.00	0.00	0.00	c	0.02				0.19	0.01	0.01	0.01	b
	500	0.29					0.00	0.00	0.00	a	0.57					0.01	0.00	0.00	ab
	750	0.55						0.28	0.00	d	0.03						0.63	0.18	c
	1000	0.05							0.14	de	0.02							0.62	c
	SM	0.46	ANOVA p=0.00							e	0.86	ANOVA p=0.00							c

Table II.3 Statistical analysis for DNA quantification (qPCR), Chapter 3

		Soil 1								Soil 2							
	Treatment	Cont.	105	250	500	750	1000	SM	Group	Cont.	105	250	500	750	1000	SM	Group
16s	Cont.		0.74	0.45	0.00	0.00	0.00	0.00	a		0.06	0.00	0.00	0.00	0.00	0.00	a
	105			0.38	0.00	0.00	0.00	0.00	a			0.04	0.00	0.00	0.00	0.00	a
	250				0.00	0.00	0.00	0.00	a				0.00	0.00	0.00	0.00	b
	500					0.08	0.00	0.39	b					0.19	0.07	0.62	cd
	750						0.00	0.92	b						0.00	0.67	c
	1000							0.02	c							0.07	d
	SM	ANOVA p=0.00							b	ANOVA p=0.00							cd
amoA	Cont.		0.03	0.92	0.64	0.49	0.14	0.30	ac		0.17	0.06	0.28	0.10	0.04	0.00	a
	105			0.16	0.02	0.00	0.41	0.05	b			0.46	0.90	0.54	0.01	0.04	a
	250				0.73	0.80	0.33	0.55	ab				0.64	0.93	0.00	0.24	ab
	500					0.11	0.16	0.17	a					0.71	0.02	0.17	ab
	750						0.03	0.04	a						0.01	0.26	ab
	1000							0.33	bc							0.00	c
	SM	ANOVA p=0.11							a	ANOVA p=0.00							b
nirS	Cont.		0.19	0.22	0.03	0.39	0.11	0.09	a		0.04	0.00	0.99	0.24	0.16	0.15	a
	105			0.85	0.21	0.68	0.81	0.06	ab			0.89	0.10	0.68	0.92	0.05	b
	250				0.14	0.62	0.65	0.07	ab				0.05	0.70	0.84	0.05	b
	500					0.82	0.28	0.04	b					0.25	0.21	0.15	ab
	750						0.75	0.05	ab						0.66	0.05	ab
	1000							0.06	ab							0.06	ab
	SM	ANOVA p=0.00							ab	ANOVA p=0.01							ab
nirK	Cont.		0.34	0.09	0.01	0.00	0.28	0.77	a		0.31	0.57	0.56	0.00	0.06	0.81	a
	105			0.04	0.02	0.01	0.72	0.58	a			0.69	0.71	0.00	0.13	0.57	a
	250				0.00	0.00	0.06	0.89	a				0.65	0.00	0.10	0.65	a
	500					0.73	0.07	0.12	b					0.09	0.78	0.52	a
	750						0.03	0.09	b						0.00	0.01	b
	1000							0.50	a							0.19	a
	SM	ANOVA p=0.01							a	ANOVA p=0.01							a
nifH	Cont.		0.71	0.96	0.30	0.35	0.12	0.15	ab		0.19	0.01	0.12	0.02	0.12	0.02	a
	105			0.65	0.46	0.49	0.20	0.11	ab			0.47	0.84	0.54	0.64	0.00	ab
	250				0.25	0.31	0.07	0.15	ab				0.62	0.90	0.95	0.00	b
	500					0.93	0.77	0.06	ab					0.70	0.77	0.00	ab
	750						0.91	0.07	ab						0.99	0.00	b
	1000							0.05	a							0.00	ab
	SM	ANOVA p=0.03							b	ANOVA p=0.00							c

Appendix III – Chapter 4 Statistics

Table III.1 – Statistical analysis for plant growth in heated and smouldered soils, Soil 1, Chapter 4

	Soil 1	Total Extension									Total Biomass								
	Treat.	Mean	Cont.	105	250	500	750	1000	SM	Group	Mean	Cont.	105	250	500	750	1000	SM	Group
Rye Water	Cont.	86.8		0.09	0.38	0.75	0.00	0.00	0.00	ab	18.7		0.00	0.05	0.06	0.01	0.01	0.01	a
	105	105.0			0.45	0.00	0.00	0.00	0.00	a	69.9			0.25	0.00	0.00	0.00	0.00	b
	250	97.8				0.16	0.00	0.00	0.00	ab	61.2				0.02	0.03	0.02	0.02	b
	500	83.9					0.00	0.00	0.00	bc	9.8					0.00	0.00	0.00	c
	750	40.1						0.00	0.00	c	3.1						0.03	0.11	d
	1000	13.3							0.00	d	1.3							0.27	e
	SM	24.3	ANOVA p=0.000							e	1.9	ANOVA p=0.000							de
Rye Nutrients	Cont.	108.9		0.40	0.39	0.04	0.00	0.00	0.00	a	39.9		0.07	0.13	0.05	0.00	0.00	0.00	a
	105	115.2			0.96	0.03	0.00	0.00	0.00	a	65.0			0.95	0.01	0.00	0.00	0.00	a
	250	115.6				0.04	0.00	0.00	0.00	a	64.0				0.02	0.01	0.01	0.01	a
	500	94.4					0.00	0.00	0.00	b	23.3					0.01	0.01	0.01	b
	750	38.8						0.21	0.47	c	3.6						0.25	0.34	c
	1000	30.2							0.58	c	5.0							0.76	c
	SM	33.5	ANOVA p=0.000							c	4.6	ANOVA p=0.000							c
RCG Water	Cont.	83.4		0.00	0.00	0.49	0.00	0.00	0.00	a	7.0		0.01	0.00	0.07	0.00	0.00	0.00	a
	105	113.9			0.21	0.00	0.00	0.00	0.00	b	36.9			0.74	0.00	0.00	0.00	0.00	b
	250	123.4				0.00	0.00	0.00	0.00	b	39.8				0.00	0.00	0.00	0.00	b
	500	87.0					0.00	0.00	0.00	a	9.1					0.00	0.00	0.00	a
	750	17.4						0.00	0.00	c	0.5						0.13	0.01	c
	1000	7.7							0.00	d	0.4							0.00	c
	SM	2.4	ANOVA p=0.000							e	0.2	ANOVA p=0.000							d
RCG Nutrients	Cont.	115.8		0.48	0.63	0.67	0.00	0.00	0.00	a	72.6		0.12	0.79	0.54	0.00	0.00	0.00	a
	105	110.7			0.62	0.68	0.00	0.00	0.00	a	83.4			0.71	0.15	0.00	0.00	0.00	a
	250	112.3				0.97	0.00	0.00	0.00	a	76.9				0.55	0.01	0.01	0.01	a
	500	112.5					0.00	0.00	0.00	a	65.6					0.00	0.00	0.00	a
	750	11.9						0.15	0.71	b	0.6						0.18	0.06	bc
	1000	9.1							0.22	b	0.7							0.02	b
	SM	14.2	ANOVA p=0.000							b	0.3	ANOVA p=0.000							c
SG Water	Cont.	49.7		0.00	0.10	0.00	0.00	0.00	0.00	a	40.3		0.00	0.02	0.02	0.01	0.01	0.01	a
	105	64.7			0.26	0.00	0.00	0.00	0.00	b	108.1			0.64	0.00	0.00	0.00	0.00	b
	250	58.5				0.00	0.00	0.00	0.00	ab	98.6				0.00	0.01	0.01	0.01	b
	500	25.3					0.06	0.00	0.00	c	10.3					0.05	0.02	0.00	c
	750	15.2						0.19	0.02	cd	5.7						0.44	0.03	d
	1000	9.1							0.01	d	4.5							0.04	d
	SM	1.0	ANOVA p=0.000							e	0.6	ANOVA p=0.000							e
SG Nutrients	Cont.	53.5		0.10	0.79	0.00	0.00	0.00	0.00	a	75.5		0.37	0.78	0.02	0.02	0.01	0.01	a
	105	61.4			0.30	0.00	0.00	0.00	0.00	a	95.4			0.28	0.00	0.00	0.00	0.00	a
	250	51.3				0.06	0.01	0.01	0.01	ab	67.9				0.02	0.04	0.04	0.03	a
	500	31.8					0.01	0.00	0.00	b	8.7					0.50	0.04	0.02	b
	750	13.1						0.11	0.01	c	7.5						0.02	0.04	b
	1000	9.2							0.41	cd	4.6							0.23	c
	SM	7.8	ANOVA p=0.000							d	2.4	ANOVA p=0.000							c

Table III.2 – Statistical analysis for plant growth in heated and smouldered soils, Soil 2, Chapter 4

	Soil 2	Total Extension									Total Biomass								
	Treat.	Mean	Cont.	105	250	500	750	1000	SM	Group	Mean	Cont.	105	250	500	750	1000	SM	Group
Rye Water	Cont.	67.0		0.02	0.07	0.16	0.53	0.11	0.29	a	1.8		0.57	0.17	0.97	0.78	0.16	0.74	a
	105	37.2			0.19	0.07	0.03	0.32	0.08	b	1.5			0.18	0.57	0.49	0.99	0.48	a
	250	46.8				0.49	0.18	0.99	0.36	ab	2.4				0.22	0.33	0.06	0.43	a
	500	51.8					0.39	0.61	0.72	ab	1.8					0.83	0.29	0.78	a
	750	59.8						0.26	0.64	a	1.9						0.25	0.93	a
	1000	46.9							0.46	ab	1.5							0.32	a
	SM	55.0	ANOVA p=0.054							ab	1.9	ANOVA p=0.502							a
Rye Nutrients	Cont.	126.6		0.00	0.00	0.00	0.00	0.00	0.00	a	61.9		0.00	0.00	0.00	0.00	0.46	0.10	a
	105	51.9			0.84	0.00	0.01	0.02	0.01	b	2.2			0.39	0.00	0.00	0.01	0.01	b
	250	49.3				0.00	0.00	0.00	0.00	b	1.7				0.00	0.00	0.01	0.01	b
	500	97.3					1.00	0.28	0.10	cd	102.8					0.27	0.12	0.00	cd
	750	97.3						0.29	0.12	cd	114.3						0.04	0.00	c
	1000	91.8							0.02	c	74.0							0.10	de
	SM	104.2	ANOVA p=0.000							d	42.8	ANOVA p=0.000							e
RCG Water	Cont.	39.8		0.56	0.00	0.49	0.00	0.10	0.61	a	0.7		0.36	0.01	0.22	0.02	0.02	0.95	a
	105	33.6			0.06	0.30	0.03	0.41	0.36	a	0.6			0.07	0.09	0.05	0.09	0.39	ab
	250	12.7				0.02	0.10	0.09	0.02	b	0.3				0.02	0.78	0.91	0.07	ab
	500	49.6					0.02	0.11	0.80	a	1.2					0.04	0.04	0.24	a
	750	7.9						0.03	0.02	b	0.3						0.68	0.08	b
	1000	27.9							0.10	a	0.3							0.08	b
	SM	62.1	ANOVA p=0.001							a	0.8	ANOVA p=0.002							ab
RCG Nutrients	Cont.	101.7		0.00	0.00	0.02	0.51	0.75	0.94	ab	52.4		0.03	0.02	0.83	0.87	0.52	0.12	ac
	105	20.4			0.40	0.03	0.00	0.00	0.00	c	1.2			0.29	0.00	0.01	0.00	0.01	b
	250	31.0				0.03	0.00	0.00	0.00	c	3.9				0.00	0.01	0.00	0.02	b
	500	60.5					0.01	0.02	0.01	b	65.1					0.98	0.13	0.01	a
	750	111.1						0.30	0.53	a	64.6						0.31	0.05	a
	1000	98.6							0.64	a	47.0							0.01	ac
	SM	102.7	ANOVA p=0.000							a	23.5	ANOVA p=0.000							c
SG Water	Cont.	21.4		0.11	0.00	0.65	0.29	0.01	0.19	a	7.2		0.03	0.01	0.39	0.03	0.01	0.09	a
	105	12.2			0.06	0.16	0.53	0.13	0.01	ab	3.4			0.07	0.02	0.95	0.04	0.18	bc
	250	4.1				0.00	0.02	0.41	0.00	bc	1.3				0.00	0.06	0.79	0.03	bd
	500	19.2					0.44	0.00	0.07	ac	5.9					0.01	0.00	0.05	ab
	750	15.5						0.05	0.03	ac	3.4						0.03	0.16	c
	1000	5.2							0.00	b	1.5							0.00	d
	SM	29.1	ANOVA p=0.000							a	4.4	ANOVA p=0.000							bd
SG Nutrients	Cont.	46.4		0.10	0.03	0.60	0.34	0.67	0.39	abc	97.3		0.03	0.01	0.13	0.50	0.21	0.03	a
	105	18.5			0.10	0.01	0.00	0.06	0.08	ad	6.7			0.37	0.04	0.00	0.02	0.08	bc
	250	12.5				0.00	0.00	0.02	0.02	d	5.4				0.03	0.00	0.02	0.04	b
	500	36.6					0.01	0.92	0.53	b	41.3					0.11	0.61	0.10	ad
	750	57.0						0.06	0.01	c	72.0						0.24	0.00	a
	1000	37.5							0.54	bc	51.5							0.03	a
	SM	31.6	ANOVA p=0.001							b	11.7	ANOVA p=0.000							dc

Nested ANOVAs for Perennial Ryegrass Soil 1

Biomass

Source	DF	SS	MS	F	P
Treatment	1	1.61666E+06	1.61666E+06	37.725	0.025
Amendment	2	85707.2080	42853.6040	0.675	0.528
Dose (%)	12	762147.7771	63512.3148	18.436	0.000
Error	64	220476.6197	3444.9472		
Total	79	2.68499E+06			

Extension

Source	DF	SS	MS	F	P
Treatment	1	30439.5031	30439.5031	149.376	0.007
Amendment	2	407.5562	203.7781	0.237	0.792
Dose (%)	12	10300.0625	858.3385	18.968	0.000
Error	64	2896.1000	45.2516		
Total	79	44043.2219			

Nested ANOVAs for Perennial Ryegrass Soil 2

Biomass

Source	DF	SS	MS	F	P
Treatment	1	60022.4746	60022.4746	0.634	0.509
Amendment	2	189355.9845	94677.9922	1.476	0.267
Dose (%)	12	769577.2033	64131.4336	45.402	0.000
Error	64	90400.7674	1412.5120		
Total	79	1.10936E+06			

Extension

Source	DF	SS	MS	F	P
Treatment	1	2015.0281	2015.0281	2.772	0.238
Amendment	2	1453.9063	726.9531	1.318	0.304
Dose (%)	12	6620.3625	551.6969	10.540	0.000
Error	64	3350.0000	52.3437		
Total	79	13439.2969			

Nested ANOVAs for Reed Canary Grass Soil 1

Biomass

Source	DF	SS	MS	F	P
Treatment	1	2.78190E+06	2.78190E+06	7.935	0.106
Amendment	2	701169.1188	350584.5594	2.669	0.110
Dose (%)	12	1.57654E+06	131378.0507	40.375	0.000
Error	64	208253.8859	3253.9670		
Total	79	5.26786E+06			

Extension

Source	DF	SS	MS	F	P
Treatment	1	44627.6281	44627.6281	136.988	0.007
Amendment	2	651.5563	325.7781	0.299	0.747
Dose (%)	12	13079.5375	1089.9615	24.176	0.000
Error	64	2885.4000	45.0844		
Total	79	61244.1219			

Nested ANOVAs for Reed Canary Grass Soil 2

Biomass

Source	DF	SS	MS	F	P
Treatment	1	246871.9736	246871.9736	0.283	0.648
Amendment	2	1.74707E+06	873533.7634	3.844	0.051
Dose (%)	12	2.72704E+06	227253.6435	89.829	0.000
Error	64	161911.0887	2529.8608		
Total	79	4.88289E+06			

Extension

Source	DF	SS	MS	F	P
Treatment	1	2045.2531	2045.2531	0.505	0.551
Amendment	2	8092.6812	4046.3406	2.247	0.148
Dose (%)	12	21613.0625	1801.0885	34.326	0.000
Error	64	3358.1000	52.4703		
Total	79	35109.0969			

Nested ANOVAs for Switchgrass Soil 1

Biomass

Source	DF	SS	MS	F	P
Treatment	1	76824.1341	76824.1341	601.502	0.002
Amendment	2	255.4409	127.7204	0.073	0.930
Dose (%)	12	20891.7335	1740.9778	2.050	0.034
Error	64	54339.6861	849.0576		
Total	79	152310.9946			

Extension

Source	DF	SS	MS	F	P
Treatment	1	24412.5781	24412.5781	268.815	0.004
Amendment	2	181.6312	90.8156	0.368	0.699
Dose (%)	12	2957.5375	246.4615	3.035	0.002
Error	64	5196.6000	81.1969		
Total	79	32748.3469			

Nested ANOVAs for Switchgrass Soil 2

Biomass

Source	DF	SS	MS	F	P
Treatment	1	20163.7702	20163.7702	0.954	0.432
Amendment	2	42280.9149	21140.4575	4.130	0.043
Dose (%)	12	61421.6268	5118.4689	11.046	0.000
Error	64	29656.8680	463.3886		
Total	79	153523.1799			

Extension

Source	DF	SS	MS	F	P
Treatment	1	816.0031	816.0031	0.314	0.632
Amendment	2	5204.3562	2602.1781	4.632	0.032
Dose (%)	12	6741.2125	561.7677	8.200	0.000
Error	64	4384.3000	68.5047		
Total	79	17145.8719			

Two-Way ANOVAs for Soil 2 Amended with Anaerobic Digestate

Ryegrass

Biomass

Source	DF	SS	MS	F	P
Dose (%)	3	92299	30766	21.66	0.000
Treatment	1	111443	111443	78.46	0.000
Interaction	3	54720	18240	12.84	0.000
Error	32	45454	1420		
Total	39	303916			

S = 37.69 R-Sq = 85.04% R-Sq(adj) = 81.77%

Extension

Source	DF	SS	MS	F	P
Dose (%)	3	1006.42	335.47	5.91	0.003
Treatment	1	1953.01	1953.01	34.40	0.000
Interaction	3	400.07	133.36	2.35	0.091
Error	32	1816.60	56.77		
Total	39	5176.09			

S = 7.535 R-Sq = 64.90% R-Sq(adj) = 57.23%

Reed Canary Grass

Biomass

Source	DF	SS	MS	F	P
Dose (%)	3	2558790	852930	215.92	0.000
Treatment	1	161012	161012	40.76	0.000
Interaction	3	71316	23772	6.02	0.002
Error	32	126409	3950		
Total	39	2917528			

S = 62.85 R-Sq = 95.67% R-Sq(adj) = 94.72%

Extension

Source	DF	SS	MS	F	P
Dose (%)	3	17053.1	5684.37	145.67	0.000
Treatment	1	283.6	283.56	7.27	0.011
Interaction	3	475.1	158.36	4.06	0.015
Error	32	1248.7	39.02		
Total	39	19060.4			

S = 6.247 R-Sq = 93.45% R-Sq(adj) = 92.02%

Switchgrass

Biomass

Source	DF	SS	MS	F	P
Dose (%)	3	1783.6	594.52	3.78	0.020
Treatment	1	4872.6	4872.63	30.96	0.000
Interaction	3	1526.1	508.69	3.23	0.035
Error	32	5036.9	157.40		
Total	39	13219.2			

S = 12.55 R-Sq = 61.90% R-Sq(adj) = 53.56%

Extension

Source	DF	SS	MS	F	P
Dose (%)	3	49.15	16.383	0.26	0.855
Treatment	1	275.63	275.625	4.35	0.045
Interaction	3	605.33	201.775	3.18	0.037
Error	32	2028.30	63.384		
Total	39	2958.40			

S = 7.961 R-Sq = 31.44% R-Sq(adj) = 16.44%

Appendix IV – Chapter 4 Heavy Metal Data

Table IV.1 – Heavy metal data for smouldered and control soils conditioned with anaerobic digestate (AD) or compost (comp) at a variety of levels (%). Values are reported as mg/Kg.

			%	Copper		Zinc		Lead		Nickel		Cadmium	
				Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Soil 1	Smouldered	AD	0	bdl	bdl	5.29	0.66	4.01	0.09	0.53	0.01	0.10	0.01
			5	bdl	bdl	17.40	4.41	3.77	0.14	0.56	0.02	0.08	0.00
			10	bdl	bdl	41.28	18.31	3.55	0.26	0.56	0.07	0.08	0.01
			20	bdl	bdl	45.29	11.70	3.95	1.23	0.73	0.25	0.10	0.04
		Comp	5	bdl	bdl	5.45	0.71	3.01	0.29	0.55	0.11	0.11	0.07
			10	bdl	bdl	7.26	1.12	3.39	0.06	0.57	0.02	0.09	0.01
			20	bdl	bdl	6.84	0.13	3.29	0.04	0.55	0.02	0.07	0.00
	Control	AD	0	2.76	0.55	8.77	0.55	5.81	0.16	0.66	0.12	0.16	0.10
			5	3.35	0.20	12.43	2.15	6.26	0.45	0.68	0.03	0.12	0.00
			10	3.02	0.36	13.91	3.13	6.44	0.49	0.67	0.04	0.12	0.01
			20	4.21	0.98	18.63	1.43	7.33	0.44	0.68	0.05	0.13	0.00
		Comp	5	3.16	0.35	9.64	0.45	6.06	0.42	0.65	0.05	0.11	0.01
			10	3.85	0.78	10.52	0.39	6.74	0.54	0.69	0.02	0.12	0.00
			20	3.16	0.10	10.61	0.26	6.47	0.39	0.63	0.03	0.11	0.01
Soil 2	Smouldered	AD	0	bdl	bdl	1.36	0.22	3.03	0.24	0.07	0.01	0.03	0.01
			5	2.17	0.38	17.45	8.66	3.40	0.09	0.12	0.01	0.04	0.00
			10	1.68	0.24	14.76	6.44	3.39	0.33	0.15	0.02	0.04	0.00
			20	2.53	0.23	31.49	3.75	3.55	0.06	0.24	0.02	0.06	0.01
		Comp	5	1.79	0.33	2.10	0.60	2.86	0.18	0.12	0.02	0.03	0.00
			10	1.96	0.49	1.91	0.32	2.85	0.05	0.12	0.01	0.03	0.00
			20	2.15	0.24	2.66	0.15	3.41	0.04	0.18	0.00	0.03	0.00
	Control	AD	0	bdl	bdl	5.29	0.66	4.01	0.09	0.53	0.01	0.10	0.01
			5	bdl	bdl	17.40	4.41	3.77	0.14	0.56	0.02	0.08	0.00
			10	bdl	bdl	41.28	18.31	3.55	0.26	0.56	0.07	0.08	0.01
			20	bdl	bdl	45.29	11.70	3.95	1.23	0.73	0.25	0.10	0.04
		Comp	5	bdl	bdl	5.45	0.71	3.01	0.29	0.55	0.11	0.11	0.07
			10	bdl	bdl	7.26	1.12	3.39	0.06	0.57	0.02	0.09	0.01
			20	bdl	bdl	6.84	0.13	3.29	0.04	0.55	0.02	0.07	0.00