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Impacts of Thermal and Smouldering Remediation on Plant Growth and Soil Ecology

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1. Introduction

Soil contamination remains 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. Typical operating ranges vary from 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 600-1100°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 safe levels where re-use can be considered; however, the effects of treatment conditions on soil quality must be understood in order to support re-development after remediation.

Post-remediation effects are particularly important if the soils are to support plant growth for phytoremediation, biomass crop production, habitat restoration, or urban green space. The effects of heating on soil depend on treatment temperatures and the duration of exposure. Even the lower temperatures (~100°C) utilised in remediation will impact soil biota—killing plant propagules, macro fauna, and microorganisms (Certini, 2005). As treatment temperatures increase, other negative impacts occur, such as charring and subsequent loss of organic matter (Certini, 2005) and atmospheric losses of nitrogen (Glass et al., 2008; Gray & Dighton, 2006). Losses of organic matter and nitrogen will be almost complete for any treatments above 500°C (Glass et al., 2008; Gray & Dighton, 2006), at which point clay minerals breakdown and aggregate, physically altering the soil (Ulery et al., 1996; Terefe et al., 2008; Ketterings et al., 2000). Physical changes to clay minerals and loss of organic matter severely reduce soil’s ability to retain valuable nutrients (Kang and Sajjapongse, 1980). High temperatures also affect the biological availability of many macro-
nutrients such as phosphorus, potassium and calcium by altering geochemistry (Kang and Sajjapongse, 1980; Galang et al., 2010). At very high temperatures (e.g., > 1000°C), less volatile nutrients may become lost to the atmosphere, including phosphorus (Galang et al., 2010). Compiled literature evidence suggests alterations to chemical conditions depend on remediation temperature.

While many previous experiments have focused on relatively lower temperatures and discrete target temperatures, few have examined the full-range of temperatures utilised by remediation technologies. Even less frequently have chemical conditions been compared to multiple biological metrics simultaneously. At low heating temperatures (60-350°C), complicated relationships exist between heating temperature and plant growth (Cébron, et al., 2009; 2011). Combining the results of a number of studies, Johnson (1919) observed that heating in this range could unpredictably 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) observed reduced biomass in rice plants grown in soil heated to 500°C compared to those heated to 200°C or less. 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 residual toxin levels. Given the range of temperatures now utilised during thermal and smouldering remediation (ambient to over 1000°C), evaluation of the effects of soil heating on plant growth over this full range is important to estimate the effects of specific remediation techniques.

In addition to impacts on plants, soil heating can have significant effect on the soils’ ability to sustain microbial communities, with consequential impacts on nutrient cycling, organic decomposition, and, in terms of soils with remaining contamination, bio-polishing of residual contaminants (Cébron, et al., 2009, 2011; Thion, et al., 2012). Successful re-colonisation by microorganisms and plants is essential to sustainable ecosystem recovery. Re-colonisation depends on many factors, but the availability of food (carbon) and nutrients are two key factors. For example, Bárcenas-Moreno and Bååth (2009) observed reduced microbial biomass after 21 days of incubation when carbon levels
had significantly been reduced by heating to 400-500°C. Higher temperatures are even more likely to remove carbon and nutrients, and as a result, microbial re-colonisation of these soils becomes severely inhibited. The extent to which micro-organisms can re-colonise soils, in terms of key-population levels and functional roles, are important in predicting whether long-term viable soil ecosystems are possible without continuous nutritional inputs.

This paper examines how full-range of soil remediation technologies impact *Trifolium pratense* (red clover) and *Festuca rubra* (red fescue), two representative proxies for the effects of thermal and smouldering remediation processes on plants. The red clover was selected for its ability to create rapid vegetative cover, fix atmospheric nitrogen through associations with symbiotic microorganisms, and enhance succession on lithoseric soils (Li and Daniels, 1992; Jefferies *et al.*, 1981). Fescue is a commonly used grass for soil-erosion control and establishing plant growth on bare ground. Changes to soil physical and chemical properties are simultaneously quantified to determine changes to soil characteristics after thermal (105 – 1000 °C) and smoulder treatments. Microbial community recovery and enzyme activity are examined to establish the soils’ capabilities to effectively cycle nutrients. These analyses identify, among the combinations of high temperature treatment and soil types, the ecological impacts to better inform post-treatment interventions to create an effective growing media for the desired land use.

2. Materials and Methods

2.1 Soil Samples and Treatments

We selected two topsoils for this study: an acidic loam (Soil 1) from northeast Scotland, and a commercially available horticultural soil (Soil 2) with a neutral pH. While organic contaminants could contribute to soil structure (Monserie *et al.*, 2009), pristine soils were selected for quasi-baseline purposes. All soils were air-dried and sieved to below 2mm; soils were then oven dried at 105°C for three days to remove moisture before being heat-treated at 250°C, 500°C, 750°C and 1000°C.
Basically, 500g-portions of oven dried soil were spread out in a large crucible, around 4cm deep, heated in a muffle furnace (Nabotherm P330, Lilienthal, Germany) and then held at temperature for one hour; 15-20 portions were bulked together for each soil-temperature treatment. Air-dried soils, without any further heating, represented experimental controls.

Additional portions of each soil type were artificially contaminated with coal tar (80g/kg) and treated via smouldering remediation (SM) (Pironi et al., 2009) to evaluate its related effects. Based on Switzer et al. (2009), a heating element and air diffuser were emplaced in around 5cm of clean sand at the bottom of a 3-litre quartz column. The column was filled with contaminated soil until 10cm of the beaker remained, and another layer of clean sand. A central line of thermocouples was used to monitor smouldering progression. The smouldering process started by heating soils to 300°C, at which point the airflow commenced and the heater was switched off. Smouldering proceeded until smoke production ceased and temperatures declined, at which time airflow was stopped.

2.2 Soil Analyses

Further details of the physical and chemical measurements of soils can be found in Pape, et al. (in review). Soil pH (BS, 2005) and electrical conductivity (BS, 1995c) were recorded using a Multi 7 Mettler-Toledo meter (Columbus, OH, USA) after a two hour extraction in 1:5 soil:water mix. Total organic content was measured by dry ashing at 550°C for five hours (BS, 2000). Total nitrogen (BS, 2001) was measured by quantifying NOX production during combustion using an Apollo 9000 TOC/TN analyser (Teldayne Tekmar, Mason, OH, USA). Inorganic nitrogen species (NH4+, NO3- and NO2-) were measured colorimetrically: an indophenol blue method for NH4+; and a sulfanilic acid method for NO2- with a hydrazine reduction step for NO3- (ADAS, 1985; Bundy and Meisinger, 1994; Shand et al., 2008). In this study, the only form of inorganic nitrogen present in measurable quantities was ammonium. Available phosphate was measured after an Olsen bicarbonate extraction using molybdate/ascorbic acid colorimetry (ADAS, 1985). Cation exchange capacity (CEC) and exchangeable bases (ADAS, 1985) were measured by sequential leaching with ammonium.
acetate and potassium chloride; flame atomic absorption (Perkin Elmer A Analyst 100, Waltham, MA, USA) determined quantity of bases, and CEC was determined colorimetrically (Bundy and Meisinger, 1994). Levels of bio-available copper and zinc were measured by extraction in ammonium-EDTA (ADAS, 1985) and ICP-OES analysis (Thermo Scientific, Hemel Hempstead, UK). Additionally, proportions of clay, silt and sand were measured using wet sieving and sedimentation (BS, 2009) after dispersion in a sodium carbonate/sodium hexametaphosphate solution.

2.3 Plant Growth Trials

Each soil and temperature treatment was further divided by different microbial amendments. Soils were initially wetted to 25% v/m with either sterile de-ionised water controls or microbially amended (MA) with 0.1% m/m of commercial mycorrhizal inoculant and 25% v/m of aerated compost tea (ACT). The ACT comprised of compost (500 mL) in 15L of sterile, deionised water, and juice of one orange; this mixture was aerated for 24hr to encourage microbial growth. The soils were then incubated at 27°C for seven days before being portioned into 200ml pots. Replicates of 3-4 pots in each treatment were planted with nine red clover (Trifolium pratense) or nine red fescue (Festuca rubra) seeds, with additional samples left as unplanted controls. All pots were watered using a wick system to maintain constant moisture content (BS, 2011) and grown 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, and after a further six weeks, they were harvested and the soils stored refrigerated; 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 also recorded for each plant.
2.4 Microbial Analyses

To understand the dynamics of the microbial populations, their activities and gene abundances were quantified at the end of the six-week experimental 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 disruptor (FastPrep24, MP Biomedical; Solon, OH, USA). Abundance of “total bacteria” was quantified by qPCR using a BioRad (Hercules, CA, USA) sso-Advanced enzyme system analysing portions of the 16S ribosomal RNA gene (Muyzer et al., 1993). In addition, bacteria related to nitrogen cycling were quantified using the same system by targeting specific functional genes, including: *nifH* (Poly et al., 2001) for nitrogen fixers, *nirS* (Kandeler et al., 2006) and *nirK* (Henry et al., 2004) for nitrifiers (nitrite reduction), and *amoA* (Rotthauwe et al., 1997) for ammonia oxidising bacteria. These functional genes were normalised to 16S rRNA to represent relative abundances of total bacteria. See Table 1 for list of specific primers and qPCR operating conditions. Quality control included purified plasmid standards (Smith et al., 2004; Graham et al., 2010), template-free blanks and post-analytical melt curves to verify PCR reaction efficiencies and check for the presence of PCR artefacts.

Several extra-cellular enzyme activities were monitored as indicators of microbial activity in the soil. Monophosphoesterase (acid and alkali) and β-glucosidase were analysed using the nitrophenol conjugate system (Tabatabai, 1994; Eivazi and Tabatabai, 1988; Tabatabai and Bremner, 1969); nitrophenol was produced as enzyme activity removed functional groups (either phosphate or glucose), and quantified colorimetrically at 400nm upon the addition of a strong alkali. Ammonia oxidase activity (Jarvis et al. 2009) was measured by observing the production of nitrite, by sulfanilic acid colorimetry, in the presence of ammonium sulphate and sodium chlorate, a metabolic inhibitor to prevent the oxidation of nitrite to nitrate.

2.5 Data analysis
Statistical analyses were performed using SPSS (v. 20) software. Data distributions were tested using the Kolmogorov-Smirnov test for single populations. Treatment comparisons were performed either by t-tests or one-way ANOVA. Due to statistical similarity among irrigation treatments, the microbial treatments of each soil were combined within each heat treatment and compared by one-way ANOVAs followed by post-hoc t-tests (see supplemental information). Level of significance was pre-determined to be $\alpha = 0.05$.

3. Results and Discussion

3.1 Physical and Chemical Changes to Soil Properties

When subjected to heating, both soil types exhibited similar changes to their particle size distribution. 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 2). At temperatures above 500°C, the proportion of clay-sized particles declined (Table 3) through aggregation and disintegration (Ulery et al. 1996; Terefe et al., 2008; Ketterings et al., 2000). Together, these losses resulted in a significant reduction in the CEC and the soils' ability to retain nutrients and water. Additionally, changes in the structure and cohesion of the soil seemed to occurred, with reduced aggregate formation qualitatively observed in soils heated above 500°C. While not quantified in these experiments, this would be important to monitor in field investigations, as it impacts soil stability and cohesion.

Macro- (N, K, P, Ca, Mg) and micro- (Cu, Zn) nutrient levels in the soils were affected by heating (Table 2). Some organic nitrogen mineralised to ammonium when heated to 250°C in Soil 1, and 105°C - 250°C in Soil 2. Above 500°C, the levels of both inorganic and total nitrogen declined due to volatilisation (Glass et al., 2008). Most other nutrient concentrations showed a negative relationship with heating temperature. Leachable copper, zinc and phosphate declined in both soils; whereas, magnesium and calcium levels were reduced in Soil 1, and potassium in Soil 2. By 1000°C, the
bioavailability of most nutrients declined in both soils, which would become a concern for plant
growth. Additionally, limited levels of copper and zinc could have impacted microbial enzyme
systems (discussed later).

In terms of other chemical properties, the results became more variable and dependent on soil type.
Soil 2 showed variable levels of exchangeable calcium across heating treatments (Table 2). Both
soils increased in pH as observed in other studies (Granged et al., 2011; Ketterings et al., 2000), but
in varying degrees. For Soil 1, pH reached 7.3 at 1000°C, likely due to destruction of organic acids,
which would not be anticipated as a problem for restoration after treatment. In Soil 2, the pH
reached 11.3 in samples heated at ≥750°C, which would severely affect subsequent biological
activity. This high pH was likely 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
Ca$^{2+}$ concentrations at these temperatures.

3.2 Plant Growth Trials

Red clover and red fescue were grown in each of the soil treatments (air-dried controls, 105-1000°C
and smouldered) to quantify the effects of soil changes on plant growth. Based on initial treatment
comparisons (t-tests), treatment temperature was the main driver of treatment variability; as such,
microbial treatments of each soil were combined within each heat treatment and compared by one-
way ANOVA. Plant biometrics showed a negative trend 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 results showed similar patterns. Heat treatments ≥250°C resulted in
statistically lower plant growth (p < 0.01, ANOVA and post-hoc analysis). By 500°C, biomass
production declined by >50% and leaf quality worsened; many samples, particularly clover, showed
lowered chlorophyll content (Table 4). At ≥750°C, negligible plant production was observed.

Although leaf biomass production was too limited to determine chlorophyll content, leaves were
visibly chlorotic. These changes related well with the loss of nitrogen and the reduced bioavailability of many macro- and micro-nutrients.

In Soil 2, fescue’s biomass production doubled when the soil was heated to 105°C and 250°C, when compared to the control sample. Heating at 105°C released the nitrogen stored in biomass, whereas heating to 250°C mineralised nitrogen in organic compounds, both of which were evident by enhanced ammonium levels (Table 2). The plants may have benefited from the release of nitrogen that would otherwise be assimilated in microbial biomass (Kaye and Hart, 1997); improved in plant growth have been noted resulting from some fires (Kennard and Gholz, 2001; Madrigal, et al., 2010). Nitrogen deficiency in the control sample was supported by the significantly lower chlorophyll content in the leaves of the fescue compared to the 105°C and 250°C treatments (Table 4). A similar pattern in growth and chlorophyll content was not evident for clover in this soil; the presence of numerous nitrogen-fixing root nodules (Table 5) could have provided additional nitrogen to overcome soil deficiency.

For most plant-soil combinations, the 250°C heat treatment was statistically intermediate between the 105°C and 500°C treatments (Fig. 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 expected given biomass production in other soil/plant combinations and reductions in nutrient levels (Table 2). A possible cause was increased toxicity by a chemical released by the heating. This is more pronounced in clover, as it is known to be more sensitive than grasses (Johnson, 1919). The reason for this sensitivity remains unclear, but large increases in ammonium (+1600%) and exchangeable manganese (+750%) levels (Table 2) were apparent at 250°C in Soil 1, both of which are toxic to plants at high levels (Britto and Krunzucker, 2002; Osborne et al., 1981). Similar but less dramatic increases were observed in Soil 2, perhaps explaining why toxicity was not observed in this soil.

3.3 Microbial Re-colonisation
Soil samples were subject to DNA extraction and gene quantification using qPCR. No consistent patterns in the data were observed between the various plant and microbe treatments within each heat treatment. Airborne and seedborne re-colonisation supplied a range of organisms to the soil. Airborne colonisation of un-inoculated samples was apparent with visible growth occurring within a few days of re-wetting in the 105°C and 250°C treatments. 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 treatment-related differences, results within the plant and microbe treatments were combined to compare temperature-related results with one-way ANOVA.

Levels of 16S-rRNA gene abundance were measured as proxies for overall bacterial abundance. Eight weeks after re-wetting soils, the bacterial populations were similar (≈10^8 genes/g) among the heat treatments <500°C (Fig 2). In these heat treatments, levels of organic matter and nutrients in the soil had not yet significantly declined. The availability of carbon and nutrients, together with the rapid proliferation potential of bacteria, ensured that population levels were fully restored during the period of this experiment.

At ≥500°C, there were fewer bacteria during recovery. The abundance of 16S-rRNA in the soils were two to three order of magnitudes lower in the 500°C, 750°C, 1000°C and smouldered samples, which followed organic matter patterns after heat treatments. Quality of organic matter might be important. While organic matter remained at 3-5% in the soil exposed to 500°C, there were obvious changes in bacterial abundances. Qualitative changes to the organic matter may have played an important role in microbial proliferation. From the biochar literature, chars produced above 300-400°C contained less 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). The recalcitrant organic matter likely reduced the soil’s capacity for heterotrophic bacteria. Interestingly, in both soils, higher abundances were found in the 1000°C heat treatment relative to the 500°C, 750°C and smouldered soils, but lower than the air-dried “control” values.
The abundances of functional genes involved in nitrogen cycling (\textit{nirS}, \textit{nirK}, \textit{amoA} and \textit{nifH}) followed similar patterns as the 16S-rRNA data (Fig. 2). All of the major guilds responsible for nitrogen cycling were present in the same proportions as 16S rRNA in the different heat treatments with some significant differences. This observation is important, as it suggests that nutrient cycling in the soil could be restored when inputs of limited nutrients are added for any of the heat treatments.

The biggest differences among \textit{nirS} and \textit{nifH} abundances were in the smouldered samples. The relative abundances of these genes (as per 16S-rRNA gene values) were quite variable within the smouldered treatments and represent a greater fraction of the microbial community. The reason for the variability remains unclear, but it should be noted that both genes produce enzyme subunits that require non-haeme iron to function. Given the presence of high temperatures and reducing agent (coal tar), changes to iron mineralogy and oxidation state would be likely in smouldered soils.

### 3.4 Nodulation and Growth in Clover

Community development in primary succession must include nitrogen-fixing populations to initiate the cycling of organic nitrogen. Based on the DNA concentrations (Fig 2), nitrogen-fixing genes almost uniformly occurred across all heat treatments, within 1-2 orders of magnitude. However, after heat treatment, successful nodulation was limited to samples that received microbial amendments and relatively high-temperature treatments (Soil 1 at 500-750°C, and Soil 2 at 500°C) causing soil to become nitrogen limited (Table 5). Even with the potentially successful microbial inoculants present, nodulation was far from universal, which demonstrates the specificity of conditions and microorganisms required; further, the presence of gene DNA does not guarantee that nodulation will occur. Chemical conditions have an effect as well; at lower heat treatments, the excess nitrogen likely inhibited nodulation (e.g., Imsande 1985), as it comes at an energetic cost to the plants. Further, at higher temperature treatments, soils experienced elevated pH and/or limitation of essential nutrients (e.g., phosphorus) that would limit successful nodulation.
To determine whether nodulation improved plant growth, samples were grouped by nodule presence; the total biomass production for each plant was compared by t-tests (Fig 3), and statistically higher growth occurred with nodulated plants. Soil 1 heated at 500°C showed the lowest growth improvement; however, it also had the lowest numbers of nodules per plant (Table 5). Due to the extreme nitrogen limitation in the 750°C sample (Table 2), these samples shows the most dramatic improvement in growth with biomass production increasing four-fold, and for the Soil 2 sample heated at 500°C, chlorophyll content of the leaves tripled. This further supports the hypothesis that nitrogen limitation caused by soil heating affected plant growth. 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 when compared to plants without nodules in the 500°C sample for the same soil. Primary organic nitrogen production is critical for the success of restoration strategies (e.g., Vitousek et al. 1989) of thermally remediated sites, particularly if low maintenance and minimal resource input are desired.

3.5 Microbial activity

In this study, enzyme assays were used to assess nutrient cycling capability at the end of the growing period to provide an indication of soil recovery. Lower phosphatase activity was observed in Soil 1 compared to Soil 2, particularly for alkali phosphatase due to the lower pH of Soil 1 (pH=4.7) (Fig. 4). Few consistent differences were also observed between plant/microbe treatments though; in some instances, the presence of plants in heated samples enhanced phosphatase activity. For example, in Soil 1 the presence of fescue marginally increased activity in the 105°C heat treatments. By far, the most significant effect was phosphatase activity declining with heat treatment, which corroborates previous observations in literature (e.g., Boerner, et al. 2000; Saá, et al., 1993; Serrasolses and Khanna, 1995). This pattern was also evident for the other enzymes tested where exposure at 500°C represented a critical point, above which spontaneous microbial recovery in soils will unlikely occur.
For other enzymes, differences occurred between the soils and the various plant/microbe treatments. β-glucosidase had the same pattern as the phosphatases in Soil 2. However, in Soil 1, β-glucosidase activity increased in samples heated at 105°C, which (again) likely attributed to nutrient release during and cell lysis of the microbial community. In addition, the combination of clover with microbial amendment resulted in higher activity, attributable to fungal and bacterial symbiosis in the amendment mixture. Despite elevated heat treatments, increased microbial activity can occur if resultant nutrient conditions become favourable (e.g., Stadden, et al. 1998).

Ammonium oxidase showed consistently different patterns of activity between the two soils tested, with almost no activity being observed in Soil 1 (Fig 4) due to the low pH of this soil (pH=4.7). In Soil 2, ammonium oxidation was apparent in all of the plant/microbe treatments at 105°C with the exception of the treatment without any biological amendment, which displayed no activity other than in the un-heated control. In these samples, the gene-abundance data showed equal proportions of amoA genes. As with clover nodulation, a positive DNA assay does not correlate to activity, rather it suggests that the potential is there; metabolic triggers and other biochemical factors, such as gene expression, must first prevail. These bacteria must have been present on seeds as well as in the microbial inoculants. Further amendments are likely required to fully restore nutrient cycling.

3.6 Implications for Remediation, Rehabilitation, and Land Reuse

A range of thermal remediation techniques optimised for the vapour extraction of highly volatile organic pollutants operates at relatively low temperatures (40-250°C; Buettner and Daily, 1995; Heron et al., 2009; Robinson et al. 2009); their impacts on soil suitability for plant growth are comparatively minimal. However, given the possibility that either growth enhancement or mild toxicity are possible at these temperatures, pilot studies would be beneficial to determine whether any detrimental effects would impact desired land use. Although spontaneous re-colonisation of
sites would eventually occur, microbial amendments would be beneficial to enhance community development.

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), 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 are all obstacles to be overcome (Séré, et al., 2008). Reclamation strategies, such as slow-release nutrient additions (such as compost) provide nutrients for plants and microbial activity, and enhanced water storage and aggregation; however, this could incur purchasing, transportation and spreading costs.

The use of specific combinations of plants and microbes to re-introduce nutrients and organic matter through semi-natural succession (Bradshaw, 1997) would be one economical restoration strategy that could provide a long-term sustainable solution. In this study, red clover improved biological activity in nitrogen deficient soils when the correct bacterial symbionts were simultaneously present to facilitate nitrogen fixation. This could complement, and-or follow the short-term amendment strategy. Further work to develop the most appropriate combinations for local conditions 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.

1. Conclusions

This study aimed to understand the linkages between remediation operating temperatures, changes to soil properties and impacts on biological activity. A critical temperature threshold was observed 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 improves the
recovery of specific biological processes in the soil. Above 500°C, geochemical changes, most
significantly losses of nitrogen and carbon, result 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.

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liquids is a patented remediation process (e.g. USA 8132987 with others pending, PCT Application
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Table 1. Primers and reaction conditions for qPCR

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<th>Gene</th>
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<th>Melt</th>
<th>Annealing</th>
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<td>P1/P2</td>
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<td>15s at 55°C</td>
<td>15s at 60°C</td>
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<td>nifH</td>
<td>PolF/PolR</td>
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<td>10s at 55°C</td>
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<td>10s at 95°C</td>
<td>10s at 52°C</td>
<td>10s at 55°C</td>
<td>Rotthauwe et al., 1997</td>
</tr>
</tbody>
</table>
Table 2 – Changes to soil chemistry upon heating, mean ± S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>E.C. (µS/cm)</th>
<th>C.E.C. (cmolc/kg)</th>
<th>Organic Matter (%)</th>
<th>Total N (g/kg)</th>
<th>Inorganic N (mg/kg)</th>
<th>Organic P (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil 1</td>
<td>Air dried</td>
<td>4.7 ± 0.0</td>
<td>79 ± 3</td>
<td>21.9 ± 2.1</td>
<td>13.6 ± 0.1</td>
<td>1.02 ± 0.08</td>
<td>8.3 ± 0.3</td>
</tr>
<tr>
<td>105°C</td>
<td>4.4 ± 0.1</td>
<td>225 ± 5</td>
<td>22.2 ± 0.8</td>
<td>11.7 ± 0.2</td>
<td>1.15 ± 0.08</td>
<td>9.0 ± 0.4</td>
<td>92.7 ± 1.1</td>
</tr>
<tr>
<td>250°C</td>
<td>5.5 ± 0.1</td>
<td>310 ± 16</td>
<td>9.8 ± 0.5</td>
<td>6.9 ± 0.2</td>
<td>1.16 ± 0.06</td>
<td>134 ± 13</td>
<td>85.5 ± 4.2</td>
</tr>
<tr>
<td>500°C</td>
<td>6.2 ± 0.2</td>
<td>353 ± 8</td>
<td>3.1 ± 0.3</td>
<td>2.8 ± 0.1</td>
<td>0.45 ± 0.04</td>
<td>7.1 ± 0.2</td>
<td>39.0 ± 2.7</td>
</tr>
<tr>
<td>750°C</td>
<td>6.4 ± 0.2</td>
<td>286 ± 15</td>
<td>1.0 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.14 ± 0.01</td>
<td>n.d.</td>
<td>36.4 ± 2.3</td>
</tr>
<tr>
<td>1000°C</td>
<td>7.3 ± 0.1</td>
<td>55 ± 2</td>
<td>0.4 ± 0.3</td>
<td>0.2 ± 0.1</td>
<td>0.01 ± 0.00</td>
<td>n.d.</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Smoulder</td>
<td>7.5 ± 0.22</td>
<td>120 ± 1313</td>
<td>0.5 ± 0.22</td>
<td>0.3 ± 0.0</td>
<td>0.08 ± 0.01</td>
<td>n.d.</td>
<td>22.1 ± 5.5</td>
</tr>
</tbody>
</table>

| Soil 2        | Air dried | 7.4 ± 0.0 | 732 ± 16 | 28.2 ± 4.6 | 17.3 ± 0.3 | 1.00 ± 0.10 | 6.0 ± 0.8 | 94.0 ± 10.6 |
| 105°C         | 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°C         | 7.3 ± 0.1 | 1490 ± 80 | 14.2 ± 3.7 | 11.6 ± 0.3 | 0.92 ± 0.07 | 23.7 ± 1.3 | 114.0 ± 7.1 |
| 500°C         | 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°C         | 11.3 ± 0.0 | 1240 ± 10 | 2.2 ± 0.7 | 1.9 ± 0.1 | 0.25 ± 0.04 | n.d. | 51.8 ± 0.9 |
| 1000°C        | 11.4 ± 0.1 | 1410 ± 150 | 0.6 ± 0.2 | 0.0 ± 0.0 | 0.02 ± 0.00 | n.d. | 22.0 ± 0.9 |
| Smoulder      | 11.7 ± 0.22 | 1783 ± 3737 | 0.8 ± 0.3 | 0.2 ± 0.1 | 0.10 ± 0.00 | n.d. | 44.9 ± 8.4 |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Exch. Ca (mg/kg)</th>
<th>Exch. Mg (mg/kg)</th>
<th>Exch. K (mg/kg)</th>
<th>Exch. Na (mg/kg)</th>
<th>Exch. Mn (mg/kg)</th>
<th>Avail. Cu (mg/kg)</th>
<th>Avail. Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil 1</td>
<td>Air dried</td>
<td>1290 ± 60</td>
<td>109 ± 3</td>
<td>114 ± 3</td>
<td>127 ± 13</td>
<td>4.3 ± 0.3</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>105°C</td>
<td>1350 ± 40</td>
<td>101 ± 5</td>
<td>89 ± 9</td>
<td>142 ± 32</td>
<td>10.2 ± 0.9</td>
<td>6.8 ± 0.6</td>
<td>19.3 ± 0.8</td>
</tr>
<tr>
<td>250°C</td>
<td>857 ± 52</td>
<td>63 ± 3</td>
<td>88 ± 10</td>
<td>138 ± 8</td>
<td>32.4 ± 5.7</td>
<td>4.2 ± 0.2</td>
<td>16.6 ± 1.1</td>
</tr>
<tr>
<td>500°C</td>
<td>569 ± 5</td>
<td>81 ± 2</td>
<td>196 ± 9</td>
<td>123 ± 8</td>
<td>10.8 ± 2.2</td>
<td>2.3 ± 0.1</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td>750°C</td>
<td>341 ± 39</td>
<td>2 ± 0</td>
<td>153 ± 5</td>
<td>156 ± 14</td>
<td>5.5 ± 1.2</td>
<td>0.9 ± 0.0</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>1000°C</td>
<td>88 ± 4</td>
<td>0 ± 0</td>
<td>34 ± 1</td>
<td>130 ± 4</td>
<td>1.5 ± 0.1</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Smoulder</td>
<td>254 ± 28</td>
<td>48 ± 10</td>
<td>86 ± 3</td>
<td>124 ± 55</td>
<td>1.7 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.2</td>
</tr>
</tbody>
</table>

<p>| Soil 2        | Air dried | 4060 ± 680 | 368 ± 22 | 983 ± 83 | 241 ± 10 | n.d. | 6.1 ± 0.5 | 18.6 ± 0.6 |
| 105°C         | 2780 ± 890 | 284 ± 39 | 806 ± 121 | 276 ± 12 | n.d. | 6.2 ± 0.8 | 21.6 ± 3.0 |
| 250°C         | 1810 ± 580 | 144 ± 29 | 568 ± 49 | 281 ± 6 | 13.8 ± 3.9 | 4.6 ± 0.1 | 19.4 ± 0.7 |
| 500°C         | 2140 ± 300 | 127 ± 11 | 400 ± 8 | 251 ± 30 | 6.3 ± 1.2 | 1.8 ± 0.2 | 5.7 ± 0.3 |
| 750°C         | 3680 ± 200 | 266 ± 9 | 160 ± 12 | 249 ± 4 | 3.5 ± 0.7 | 1.7 ± 0.1 | 2.2 ± 0.2 |
| 1000°C        | 2150 ± 250 | 55 ± 4 | 55 ± 4 | 268 ± 15 | n.d. | 0.5 ± 0.0 | 0.8 ± 0.1 |
| Smoulder      | 3460 ± 880 | 106 ± 38 | 86 ± 7 | 210 ± 12 | n.d. | 1.2 ± 0.2 | 1.9 ± 0.3 |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil 1 Sand (%)</th>
<th>Soil 1 Silt (%)</th>
<th>Soil 1 Clay (%)</th>
<th>Soil 2 Sand (%)</th>
<th>Soil 2 Silt (%)</th>
<th>Soil 2 Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air dried</td>
<td>37.9 ± 2.5</td>
<td>48.4 ± 2.0</td>
<td>13.7 ± 0.5</td>
<td>70.9 ± 1.1</td>
<td>19.2 ± 1.3</td>
<td>9.9 ± 0.3</td>
</tr>
<tr>
<td>105°C</td>
<td>40.5 ± 2.2</td>
<td>46.1 ± 2.7</td>
<td>13.4 ± 1.1</td>
<td>72.2 ± 0.9</td>
<td>18.1 ± 1.0</td>
<td>9.8 ± 0.2</td>
</tr>
<tr>
<td>250°C</td>
<td>38.4 ± 2.1</td>
<td>49.9 ± 1.4</td>
<td>11.7 ± 0.7</td>
<td>71.1 ± 2.4</td>
<td>20.2 ± 2.2</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>500°C</td>
<td>43.7 ± 2.6</td>
<td>51.7 ± 2.4</td>
<td>4.7 ± 0.2</td>
<td>86.9 ± 1.3</td>
<td>10.9 ± 1.2</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>750°C</td>
<td>53.0 ± 0.5</td>
<td>45.5 ± 0.7</td>
<td>1.6 ± 0.2</td>
<td>89.7 ± 0.9</td>
<td>8.3 ± 1.2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>1000°C</td>
<td>85.5 ± 2.1</td>
<td>14.5 ± 2.1</td>
<td>0.0 ± 0.1</td>
<td>90.3 ± 0.3</td>
<td>7.2 ± 0.3</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Smoulder</td>
<td>75.3 ± 1.3</td>
<td>23.6 ± 1.9</td>
<td>1.1 ± 0.6</td>
<td>96.3 ± 0.7</td>
<td>2.2 ± 0.6</td>
<td>1.5 ± 0.3</td>
</tr>
</tbody>
</table>
Table 4 - Leaf chlorophyll content (mg/g dry mass) for soils inoculated with sterile water (control) and aerated compost tea (M.A.). Values were not determined (n.d.) where biomass was too small for analysis. ±S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil 1</th>
<th>Soil 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clover</td>
<td>Fescue</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>Air dried</td>
<td>20.9±2.2</td>
<td>20.1±0.9</td>
</tr>
<tr>
<td>105°C</td>
<td>22.0±1.2</td>
<td>23.4±1.2</td>
</tr>
<tr>
<td>250°C</td>
<td>25.1±1.2</td>
<td>21.9±2.9</td>
</tr>
<tr>
<td>500°C</td>
<td>5.8±1.8</td>
<td>7.5±1.7</td>
</tr>
</tbody>
</table>
Table 5 - Root nodulation of clover in soils with microbial amendment (M.A.) and without (control), ±S.D.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil 1 Control</th>
<th>Soil 1 M.A.</th>
<th>Soil 2 Control</th>
<th>Soil 2 M.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air dried</td>
<td>24 ± 9</td>
<td>20 ± 6</td>
<td>32 ± 4</td>
<td>23 ± 8</td>
</tr>
<tr>
<td>105</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>37 ± 17</td>
</tr>
<tr>
<td>250</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>500</td>
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<td>8 ± 7</td>
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<td>14 ± 7</td>
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<tr>
<td>750</td>
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<td>17 ± 19</td>
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<td>0 ± 0</td>
</tr>
<tr>
<td>1000</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>
Figure captions:

Figure 1. Above and below ground extension and biomass for Red Clover and Red Fescue grown in air dried, heated and smouldered (SM) samples of two soils. Error bars represent one standard deviation and letters denote statistically significant differences in each series.

Figure 2. DNA quantification in heated soils eight weeks after inoculation. Error bars represent one S.D. and letters denote statistically different groupings.

Figure 3 – Total biomass for clover separated by nodulation in heated samples were successful nodulation occurred. P-values are reported for t-tests.

Figure 4. Enzyme activity eight weeks after the inoculation of heated soils with sterile water or aerated compost tea (MA). Error bars represent one standard deviation.