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

Andrew Pape, Christine Switzer, Neil McCosh, and Charles W. Knapp*

Department of Civil and Environmental Engineering, University of Strathclyde, Scotland, UK

andrew.pape@strath.ac.uk; christine.switzer@strath.ac.uk; neil.mccosh@strath.ac.uk;
charles.knapp@strath.ac.uk

* Correspondence: charles.knapp@strath.ac.uk

Department of Civil and Environmental Engineering,

University of Strathclyde, 5th Floor, James Weir Building

75 Montrose Street, Glasgow, Scotland, UK G1 1XJ

Phone: +44 (0) 141 548 3351

Full version: doi: 10.1016/j.geoderma.2014.12.004
Geoderma (2015) 243/244: 1-9
http://authors.elsevier.com/a/1QD5r3p6n-QLd

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-

44 nutrients such as phosphorus, potassium and calcium by altering geochemistry (Kang and
45 Sajjapongse, 1980; Galang *et al*., 2010). At very high temperatures (e.g., > 1000°C), less volatile
46 nutrients may become lost to the atmosphere, including phosphorus (Galang *et al*., 2010). Compiled
47 literature evidence suggests alterations to chemical conditions depend on remediation temperature.

48 While many previous experiments have focused on relatively lower temperatures and discrete target
49 temperatures, few have examined the full-range of temperatures utilised by remediation
50 technologies. Even less frequently have chemical conditions been compared to multiple biological
51 metrics simultaneously. At low heating temperatures (60-350˚C), complicated relationships exist
52 between heating temperature and plant growth (Cébron, *et al*., 2009; 2011). Combining the results
53 of a number of studies, Johnson (1919) observed that heating in this range could unpredictably have
54 both positive and negative effects on growth depending on soil and plant type. At higher
55 temperatures (>400˚C), results seem more consistent. For instance, Kang and Sajjapongse (1980)
56 observed reduced biomass in rice plants grown in soil heated to 500˚C compared to those heated to
57 200˚C or less. Roh *et al.* (2000), studying the thermal desorption of mercury, found greater plant
58 growth in soil treated at 350˚C compared to 600˚C, despite higher residual toxin levels. Given the
59 range of temperatures now utilised during thermal and smouldering remediation (ambient to over
60 1000˚C), evaluation of the effects of soil heating on plant growth over this full range is important to
61 estimate the effects of specific remediation techniques.

62 In addition to impacts on plants, soil heating can have significant effect on the soils' ability to sustain
63 microbial communities, with consequential impacts on nutrient cycling, organic decomposition, and,
64 in terms of soils with remaining contamination, bio-polishing of residual contaminants (Cébron, *et*
65 *al.*, 2009, 2011; Thion, *et al*., 2012). Successful re-colonisation by microorganisms and plants is
66 essential to sustainable ecosystem recovery. Re-colonisation depends on many factors, but the
67 availability of food (carbon) and nutrients are two key factors. For example, Bárcenas-Moreno and
68 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 *Trifolim 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.

93 Basically, 500g-portions of oven dried soil were spread out in a large crucible, around 4cm deep,
94 heated in a muffle furnace (Nabotherm P330, Lilienthal, Germany) and then held at temperature for
95 one hour; 15-20 portions were bulked together for each soil-temperature treatment. Air-dried soils,
96 without any further heating, represented experimental controls.

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

105 *2.2 Soil Analyses*

106 Further details of the physical and chemical measurements of soils can be found in Pape, *et al*. (in
107 review). Soil pH (BS, 2005) and electrical conductivity (BS, 1995c) were recorded using a Multi 7
108 Mettler-Toledo meter (Columbus, OH, USA) after a two hour extraction in 1:5 soil:water mix. Total
109 organic content was measured by dry ashing at 550˚C for five hours (BS, 2000). Total nitrogen (BS,
110 2001) was measured by quantifying NO_X production during combustion using an Apollo 9000
111 TOC/TN analyser (Teldayne Tekmar, Mason, OH, USA). Inorganic nitrogen species (NH_4^+, NO_3^- and
112 NO_2^-) were measured colorimetrically: an indophenol blue method for NH_4^+; and a sulfanilic acid
113 method for NO_2^- with a hydrazine reduction step for NO_3^- (ADAS, 1985; Bundy and Meisinger, 1994;
114 Shand *et al.*, 2008). In this study, the only form of inorganic nitrogen present in measurable
115 quantities was ammonium. Available phosphate was measured after an Olsen bicarbonate
116 extraction using molybdate/ascorbic acid colorimetry (ADAS, 1985). Cation exchange capacity (CEC)
117 and exchangeable bases (ADAS, 1985) were measured by sequential leaching with ammonium

118 acetate and potassium chloride; flame atomic absorption (Perkin Elmer AAnalyst 100, Waltham, MA,
119 USA) determined quantity of bases, and CEC was determined colorimetrically (Bundy and Meisinger,
120 1994). Levels of bio-available copper and zinc were measured by extraction in ammonium-EDTA
121 (ADAS, 1985) and ICP-OES analysis (Thermo Scientific, Hemel Hempstead, UK). Additionally,
122 proportions of clay, silt and sand were measured using wet sieving and sedimentation (BS, 2009)
123 after dispersion in a sodium carbonate/sodium hexametaphosphate solution.

124 *2.3 Plant Growth Trials*

125 Each soil and temperature treatment was further divided by different microbial amendments. Soils
126 were initially wetted to 25% v/m with either sterile de-ionised water controls or microbially
127 amended (MA) with 0.1% m/m of commercial mycorrhizal inoculant and 25% v/m of aerated
128 compost tea (ACT). The ACT comprised of compost (500 mL) in 15L of sterile, deionised water, and
129 juice of one orange; this mixture was aerated for 24hr to encourage microbial growth. The soils
130 were then incubated at 27°C for seven days before being portioned into 200ml pots. Replicates of 3-
131 4 pots in each treatment were planted with nine red clover (*Trifolium pratense*) or nine red fescue
132 (*Festuca rubra*) seeds, with additional samples left as unplanted controls. All pots were watered
133 using a wick system to maintain constant moisture content (BS, 2011) and grown in a growth
134 chamber at 27±2°C with 16 hours of light per day. One week after planting, the plants were thinned
135 to two per pot, and after a further six weeks, they were harvested and the soils stored refrigerated
136 (4˚C) for analysis.

137 After harvest, the plants were dried at 70˚C and analysed for shoot and root extension and dry mass
138 of the roots, shoots, and leaves. In addition, frozen sub-samples of the leaves were analysed for
139 chlorophyll content. Chlorophyll was extracted by heating a leaf sample (20-100mg wet weight) in
140 10ml of di-methyl sulfoxide at 70°C for six hours (Hiscox and Israelstam, 1979) and analysed
141 colorimetrically according to the equations of Arnon (1949). For clover, the numbers of symbiotic
142 root nodules were also recorded for each plant.

143 *2.4 Microbial Analyses*

144 To understand the dynamics of the microbial populations, their activities and gene abundances were
145 quantified at the end of the six-week experimental period. To quantify gene abundance in the soil,
146 0.25g samples were extracted using a MoBio (Carlsbad, CA, USA) PowerSoil DNA isolation kit and a
147 cell disruptor (FastPrep24, MP Biomedicals; Solon, OH, USA). Abundance of "total bacteria" was
148 quantified by qPCR using a BioRad (Hercules, CA, USA) sso-Advanced enzyme system analysing
149 portions of the 16S ribosomal RNA gene (Muyzer *et al*., 1993). In addition, bacteria related to
150 nitrogen cycling were quantified using the same system by targeting specific functional genes,
151 including: *nifH* (Poly et al., 2001) for nitrogen fixers, *nirS* (Kandeler *et al*., 2006) and *nirK* (Henry *et al*.,
152 2004) for nitrifiers (nitrite reduction), and *amoA* (Rotthauwe *et al*., 1997) for ammonia oxidising
153 bacteria. These functional genes were normalised to 16S rRNA to represent relative abundances of
154 total bacteria. See Table 1 for list of specific primers and qPCR operating conditions. Quality control
155 included purified plasmid standards (Smith *et al*., 2004; Graham *et al*., 2010), template-free blanks
156 and post-analytical melt curves to verify PCR reaction efficiencies and check for the presence of PCR
157 artefacts.

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

166 *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

191 bioavailability of most nutrients declined in both soils, which would become a concern for plant
192 growth. Additionally, limited levels of copper and zinc could have impacted microbial enzyme
193 systems (discussed later).

194 In terms of other chemical properties, the results became more variable and dependent on soil type.
195 Soil 2 showed variable levels of exchangeable calcium across heating treatments (Table 2). Both
196 soils increased in pH as observed in other studies (Granged *et al*., 2011; Ketterings *et al*., 2000), but
197 in varying degrees. For Soil 1, pH reached 7.3 at 1000˚C, likely due to destruction of organic acids,
198 which would not be anticipated as a problem for restoration after treatment. In Soil 2, the pH
199 reached 11.3 in samples heated at ≥750˚C, which would severely affect subsequent biological
200 activity. This high pH was likely due to the calcination of $CaCO_3$ to highly soluble and alkaline CaO at
201 these temperatures (Giovannini *et al*., 1990), as evidenced by the high electrical conductivity and
202 Ca^{2+} concentrations at these temperatures.

203 *3.2 Plant Growth Trials*

204 Red clover and red fescue were grown in each of the soil treatments (air-dried controls, 105-1000°C
205 and smouldered) to quantify the effects of soil changes on plant growth. Based on initial treatment
206 comparisons (t-tests), treatment temperature was the main driver of treatment variability; as such,
207 microbial treatments of each soil were combined within each heat treatment and compared by one-
208 way *ANOVA.* Plant biometrics showed a negative trend between treatment temperature and plant
209 growth for both species in both soils (Fig 1). Biomass production appeared to be a more sensitive
210 metric than extension, but both results showed similar patterns. Heat treatments ≥250˚C resulted in
211 statistically lower plant growth ($p < 0.01$, *ANOVA* and post-hoc analysis). By 500˚C, biomass
212 production declined by >50% and leaf quality worsened; many samples, particularly clover, showed
213 lowered chlorophyll content (Table 4). At ≥750˚C, negligible plant production was observed.
214 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

239 Soil samples were subject to DNA extraction and gene quantification using qPCR. No consistent
240 patterns in the data were observed between the various plant and microbe treatments within each
241 heat treatment. Airborne and seedborne re-colonisation supplied a range of organisms to the soil.
242 Airborne colonisation of un-inoculated samples was apparent with visible growth occurring within a
243 few days of re-wetting in the 105°C and 250°C treatments. This pattern existed for all of the genes
244 quantified and the same functional guilds were present in both inoculated and un-inoculated
245 samples. Because there were no treatment-related differences, results within the plant and microbe
246 treatments were combined to compare temperature-related results with one-way *ANOVA*.

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

253 At ≥500˚C, there were fewer bacteria during recovery. The abundance of 16S-rRNA in the soils were
254 two to three order of magnitudes lower in the 500°C, 750°C, 1000˚C and smouldered samples, which
255 followed organic matter patterns after heat treatments. Quality of organic matter might be
256 important. While organic matter remained at 3-5% in the soil exposed to 500°C, there were obvious
257 changes in bacterial abundances. Qualitative changes to the organic matter may have played an
258 important role in microbial proliferation. From the biochar literature, chars produced above 300-
259 400°C contained less labile carbon (Rutherford *et al*., 2012; Song and Guo, 2012) due to increasing
260 levels of complex aromatic compounds as oxygen and hydrogen are lost from organic molecules
261 (Kim *et al.*, 2012). The recalcitrant organic matter likely reduced the soil's capacity for heterotrophic
262 bacteria. Interestingly, in both soils, higher abundances were found in the 1000°C heat treatment
263 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 (*nirS*, *nirK*, *amoA* and *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 *nirS* and *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.

288 To determine whether nodulation improved plant growth, samples were grouped by nodule
289 presence; the total biomass production for each plant was compared by t-tests (Fig 3), and
290 statistically higher growth occurred with nodulated plants. Soil 1 heated at 500°C showed the
291 lowest growth improvement; however, it also had the lowest numbers of nodules per plant (Table
292 5). Due to the extreme nitrogen limitation in the 750°C sample (Table 2), these samples shows the
293 most dramatic improvement in growth with biomass production increasing four-fold, and for the Soil
294 2 sample heated at 500°C, chlorophyll content of the leaves tripled. This further supports the
295 hypothesis that nitrogen limitation caused by soil heating affected plant growth. The leaves of the
296 Soil 1 sample at 750°C could not be compared directly to the un-nodulated equivalent due to low
297 biomass production, but chlorophyll content in plants with nodules was triple the value when
298 compared to plants without nodules in the 500°C sample for the same soil. Primary organic nitrogen
299 production is critical for the success of restoration strategies (e.g., Vitousek *et al*. 1989) of thermally
300 remediated sites, particularly if low maintenance and minimal resource input are desired.

301 *3.5 Microbial activity*

302 In this study, enzyme assays were used to assess nutrient cycling capability at the end of the growing
303 period to provide an indication of soil recovery. Lower phosphatase activity was observed in Soil 1
304 compared to Soil 2, particularly for alkali phosphatase due to the lower pH of Soil 1 (pH=4.7) (Fig. 4).
305 Few consistent differences were also observed between plant/microbe treatments though; in some
306 instances, the presence of plants in heated samples enhanced phosphatase activity. For example, in
307 Soil 1 the presence of fescue marginally increased activity in the 105°C heat treatments. By far, the
308 most significant effect was phosphatase activity declining with heat treatment, which corroborates
309 previous observations in literature (e.g., Boerner, et al. 2000; Saá, et al., 1993; Serrasolses and
310 Khanna, 1995). This pattern was also evident for the other enzymes tested where exposure at 500°C
311 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.

Acknowledgements

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).

References

Abdelmagid, H.M., Tabatabai, M.A., 1987. Nitrate reductase activity of soils. Soil Biol. Biochem., 19, 421-427

ADAS, 1985. *The Analysis of Agricultural Materials*. Agricultural Development and Advisory Service, London, U.K.

Andreu, V., Imeson, A.C., 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. Pilot plant investigations of thermal remediation of tar-contaminated soil and oil-contaminated gravel. Fuel, 85, 443-450

384 Alvarenga, P., Goncalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-
385 Queda, A.C., 2008. Evaluation of composts and liming materials in the phytostabilisation of a mine
386 soil using perennial ryegrass. Sci. Tot. Environ. 406:43-56

387 Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*.
388 Plant Physiol. 24, 1-15

389 Bárcenas-Moreno, G., Bååth, E., 2009. Bacterial and fungal growth in soil heated at different
390 temperatures to simulate a range of fire intensities. Soil Biol. Biochem. 41, 2517-2526

391 Bendfeldt, E.S., Burger, J.A., Daniels, W.L., 2001. Quality of amended mine soils after sixteen years.
392 Soil Sci. Soc. Am. J. 65:1736-1744

393 Boerner, R.E.J., Decker, K.L.M. Sutherland, E.K. 2000. Prescribed burning effects on soil enzyme
394 activity in a souther Ohio hardwood forest: a landscape scale analysis. Soil Biol. Biochem. 32, 899-
395 908.

396 Bradshaw, A., 1997. Restoration of mined lands – using natural processes. Ecol. Eng. 8, 255-269

397 Britto, D.T., Kronzucker, H.J., 2002. NH4+ toxicity in higher plants: A critical review. J. Plant Physiol.
398 159, 567-584

399 BS - British Standards, 1995. BS 7755 Soil Quality, Section 3.8 Determination of organic and total
400 carbon after dry combustion (elementary analysis). BSI Group, London, UK

401 BS - British Standards, 2001. BS EN 13654 Soil improvers and growing media – Determination of
402 nitrogen, Part 2 Dumas method. BSI Group, London, UK

403 BS - British Standards, 2011. BS EN ISO 22030 Soil quality - Biological methods – Chronic toxicity in
404 higher plants, BSI Group, London, UK

405 Buettner, H.M., Daily, W.D., 1995. Cleaning contaminated soil using electrical heating and air
406 stripping. J. Environ. Eng. 121, 580-589

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

Cébron, A., Beguiristain, T., Faure, P., Norini, M.-P., Masfaraud, J.-F., Leyval, C., 2009. Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. Appl. Environ. Microbiol. 75, 6322-6330.

Cébron, A., Cortet, J., Criquet, S., Biaz, A., Calvert, V., Caupert, C., Pernin, C., Leyval, C., 2011. Biological functioning of PAH-polluted and thermal-desorption treated soils assessed by fauna and microbial bioindicators. Res. Microbiol. 162, 896-907.

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

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soil. Soil Biol. Biochem. 20, 601-606

Fonturbel, M.T., Vega, J.A., Perez-Gorostiaga, P., Fernandez, C. , Alonso, M., Cuinas, P., Jimenez, E. 2011. Effects of soil burn severity on germination and initial establishment of maritime pine seedlings, under greenhouse conditions, in two contrasting experimentally burned soils. Int. J. Wildland Fire. 20: 209-222.

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., Giachetti, M., 1990. Effects of heating on some chemical parameters related to soil fertility and plant growth. Soil Sci. 149, 344-350

Graham, D.W., Trippett, C., Dodds, W.K., O'Brien, J.M., Banner, E.B.K., Head, I.M., Smith, M.S., Yang, R.K., Knapp, C.W., 2010. Correlations between *in situ* denitrification activity and *nir* gene abundances in pristine and impacted prairie streams. Environ. Pollut. 158(10), 3225-3229

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 Biol. Biochem. 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 Sci. **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. Agri. Ecosyst. Environ. 123, 1-14

Henry, S., Baudoin, E., López-Gutiérrez, J.C., Martin-Laurent, F., Brauman, A., Philippot, L., 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. J. Microbiol. Meth. 69(3), 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 Monit. Remediat. 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 Monit. Remediat. 29, 56-65

Hiscox, J.D., Israelstam, G.F., 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57, 1332-1334

Imsande, J. 1985. Inhibition of nodule development in soybean by nitrate and reduced nitrogen. J. Exper. Biol. 37, 348-355.

Jarvis, A., Sundberg, C., Milenkovski, S., Pell, M., Lindgren, P.E., Hallin, S., 2009. Activity and composition of ammonia oxidising bacterial communities and emission dynamics of NH_3 and N_2O in a compost reactor treating organic household waste. J. Appl. Microbiol. 106, 1502-1511

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

456 Johnson, J., 1919. The influence of heated soils on seed germination and plant growth. Soil Sci. 7,
457 1-103

458 Kandeler, E., Deiglmayr, K., Tscherko, D., Bru, D., Philippot. L., 2006. Abundance of *narG*, *nirS*, *nirK*,
459 and *nosZ* genes of denitrifying bacteria during primary successions of a glacier foreland. Appl.
460 Environ. Microbiol. 72, 5957-5962.

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

463 Kaye, J.P., Hart, S.C., 1997. Competition for nitrogen between plants and microorganisms. Trends
464 Evolut. Ecol. 12, 139-143

465 Kennard, D.K., Gholz, H.L. 2001. Effects of high- and low-intensity fires on soil properties and plant
466 growth in a Bolivian dry forest. Plant Soil 234, 119-129.

467 Ketterings, Q.M., Bigham, J.M., Laperche, V., 2000. Changes in soil mineralogy and texture caused
468 by slash and burn fires in Sumatra, Indonesia. Soil Sci. Soc. Amer. J. 64, 1108-1117

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

472 Li, R.S., Daniels, W.L., 1992. Nitrogen accumulation and form over time in young mine soils. J.
473 Environ. Qual. 23, 166-172

474 Madrigal, J., Hernando, C., Guiljarro, M., Vega, J.A., Fonturbel, T., Perez-Gorostiaga, P. 2010.
475 Smouldering fire-induced changes in a Mediterranean soil (SE Spain): effects on germination, survival
476 and morphological traits of 3-year-old *Pinus pinaster* Ait. Plant Ecol. 208, 279-292

477 Monserie, M.-F., Watteau, F., Villemin, G., Ouvrard, S., Morel, J.-L., 2009. Technosol genesis:
478 identification of organo-mineral associations in a young Technosol derived from coking plant waste
479 materials. J. Soils Sed. 9(6), 537-546.

480 Muyzer, G., de Waal, E.C., Uitterlinden, A.G.,1993. Profiling of complex microbial populations by
481 denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes
482 coding for 16S rRNA. Appl. Environ. Microbiol. 59, 695-700.

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

485 Pape, A., Knapp, C.W., Switzer, C., 2014. Effects of high temperature remediation processes on soil
486 geochemistry. Environ. Sci. Pollut. Res., In review

487 Pironi, P., Switzer, C., Rein, G., Fuentes, A., Gerhard, J.I., Torero, J.L., 2009. Small-scale forward
488 smouldering experiments for remediation of coal tar in inert media. Proc. Combust. Inst. 32, 1957-
489 1964

490 Poly, F., Ranjard, L., Navaret, S., Gourbière, Monrozier, L.J., 2001. Comparison of nifH gene pools in
491 soils and soil microenvironments with contrasting properties. Appl. Environ. Microbiol. 67, 2255-62.

492 Robinson, J.P., Kingman, S.W., Snape, C.E., Shang, H., Barranco, R., Saeid, A., 2009. Separation of
493 polyaromatic hydrocarbons from contaminated soils using microwave heating. Sep. Purif. Technol.
494 69, 249-254

495 Roh, Y., Edwards, N.T., Lee, S.Y., Stiles, C.A., Armes, S., Foss, J.E., 2000. Thermal-treated soil for
496 mercury removal: soil and phytoxicity tests. J. Environ. Qual. 29, 415-424

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

Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene *amoA* as functional biomarker: molecular fine-scale analysis of natural ammonia-oxidising populations. Appl. Environ. Microbiol. 63, 4700-4712.

Saá, A., Trasar-Cepeda, M.C., Gil-Sotres, F., Carballas, T. 1993. Changes in soil phosphorus and acid phosphatase activity immediately following forest fires. Soil Biol. Biochem. 25, 1223-1230.

Séré, G., Ourvrard, S., Schwartz, C., Renat, J.C., Morel, J.L. 2008. Soil construction: a step for ecological reclamation of derelict lands. J. Soil Sed. 8, 130-136. all obstacles to be overcome.

Serrasolses, I., Khanna, P.K. 1995. Changes in heated and autoclaved forest soils of S.E. Australia. II. Phosphorus and phosphatase activity. Biogeochem. 29, 25-41. Shand, C.A., Williams, B.L., Coutts, G., 2008. Determination of N-species in soil extracts using microplate techniques. Talanta 74, 648-654

Smith, M.S., Yang, R.K., Knapp, C.W., Niu, Y., Peak, N., Hanfelt, M.M., Galland, J.C., Graham, D.W., 2004. Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. Appl. Environ. Microbiol. 70, 7372-7377

Song, W., Guo, M., 2012. Quality variations of poultry litter biochar generated at different pyrolysis temperatures. J. Anal. Appl Pyrolysis 94, 138-145

Staddon, W.J., Duchesne, L.C. Trevors, J.T. 1998. Acid phosphatase, alkaline phosphatase and arylsulfatase activities in soils from a jack pine (*Pinus banksiana* Lamb.) ecosystem after clear-cutting, prescribed burning, and scarification. Biol. Fert. Soil 27, 1-4.

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. Environ. Sci. Technol. 43, 5871-5877

Switzer, C., Pironi, P., Gerhard, J.I., Rein, G., Torero, J.L., 2014. Volumetric scale-up of smouldering remediation of contaminated materials. J. Haz. Mater. 268, 51-60

520 Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase
521 activity. Soil Biol. Biochem. 1, 301-307

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

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

527 Thion, C., Cébron, A., Beguiristain, T., Leyval, C., 2012. Long-term in situ dynamics of the fungal
528 communities in a multi-contaminated soil are mainly driven by plants. FEMS Microbiol. Ecol. 82,
529 169-181.

530 Ulery, A.L., Graham, R.C., Bowen, L.H., 1996. Forest fire effects on soil phyllosilicates in California.
531 Soil Sci. Soc. Amer. J. 60, 309-315

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

535 Vitousek, P. M., Matson, P.A., Van Cleve, K. 1989. Nitrogen availability and nitrification during
536 succession: primary, secondary and old-field seres. Plant Soil, 115, 229-239.

Table 1. Primers and reaction conditions for qPCR

Gene	Primers	Melt	Annealing	Elongation	Source
16S-rRNA	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

Table 2 – Changes to soil chemistry upon heating, mean ± S.D.

	Treatment	pH	E.C. (µS/cm)	C.E.C. (cmolc/kg)	Organic Matter (%)	Total N (g/kg)	Inorganic N (mg/kg)	Organic P (mg/kg)
Soil 1	Air dried	4.7 ± 0.0	79 ± 3	21.9 ± 2.1	13.6 ± 0.1	1.02 ± 0.08	8.3 ± 0.3	97.5 ± 4.4
	105°C	4.4 ± 0.1	225 ± 5	22.2 ± 0.8	11.7 ± 0.2	1.15 ± 0.08	9.0 ± 0.4	92.7 ± 1.1
	250°C	5.5 ± 0.1	310 ± 16	9.8 ± 0.5	6.9 ± 0.2	1.16 ± 0.06	134 ± 13	85.5 ± 4.2
	500°C	6.2 ± 0.2	353 ± 8	3.1 ± 0.3	2.8 ± 0.1	0.45 ± 0.04	7.1 ± 0.2	39.0 ± 2.7
	750°C	6.4 ± 0.2	286 ± 15	1.0 ± 0.1	0.6 ± 0.1	0.14 ± 0.01	n.d.	36.4 ± 2.3
	1000°C	7.3 ± 0.1	55 ± 2	0.4 ± 0.3	0.2 ± 0.1	0.01 ± 0.00	n.d.	3.0 ± 0.3
	Smoulder	7.5 ± 0.22	120 ± 1313	0.5 ± 0.22	0.3 ± 0.0	0.08 ± 0.01	n.d.	22.1 ± 5.5
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

	Treatment	Exch. Ca (mg/kg)	Exch. Mg (mg/kg)	Exch. K (mg/kg)	Exch. Na (mg/kg)	Exch. Mn (mg/kg)	Avail. Cu (mg/kg)	Avail. Zn (mg/kg)
Soil 1	Air dried	1290 ± 60	109 ± 3	114 ± 3	127 ± 13	4.3 ± 0.3	6.9 ± 0.3	21.0 ± 1.2
	105°C	1350 ± 40	101 ± 5	89 ± 9	142 ± 32	10.2 ± 0.9	6.8 ± 0.6	19.3 ± 0.8
	250°C	857 ± 52	63 ± 3	88 ± 10	138 ± 8	32.4 ± 5.7	4.2 ± 0.2	16.6 ± 1.1
	500°C	569 ± 5	81 ± 2	196 ± 9	123 ± 8	10.8 ± 2.2	2.3 ± 0.1	5.1 ± 0.3
	750°C	341 ± 39	2 ± 0	153 ± 5	156 ± 14	5.5 ± 1.2	0.9 ± 0.0	1.1 ± 0.1
	1000°C	88 ± 4	0 ± 0	34 ± 1	130 ± 4	1.5 ± 0.1	0.1 ± 0.0	0.3 ± 0.2
	Smoulder	254 ± 28	48 ± 10	86 ± 3	124 ± 55	1.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.2
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

Table 3 - Changes to particle size distribution upon heating, mean ±S.D.

	Soil 1			Soil 2		
Treatment	Sand (%)	Silt (%)	Clay (%)	Sand (%)	Silt (%)	Clay (%)
Air dried	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°C	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°C	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°C	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°C	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°C	85.5 ± 2.1	14.5 ± 2.1	0.0 ± 0.1	90.3 ± 0.3	7.2 ± 0.3	2.5 ± 0.1
Smoulder	75.3 ± 1.3	23.6 ± 1.9	1.1 ± 0.6	96.3 ± 0.7	2.2 ± 0.6	1.5 ± 0.3

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.

	Soil 1					Soil 2			
	Clover		Fescue			Clover		Fescue	
Treatment	Control	M.A.	Control	M.A.		Control	M.A.	Control	M.A.
Air dried	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°C	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°C	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°C	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°C	n.d.	18.5±1.2	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.
1000°C	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.
Smoulder	n.d.	n.d.	n.d.	n.d.		n.d.	n.d.	n.d.	n.d.

Table 5 - Root nodulation of clover in soils with microbial amendment (M.A.) and without (control), ±S.D.

	Soil 1		Soil 2	
Treatment	Control	M.A.	Control	M.A.
Air dried	24 ± 9	20 ± 6	32 ± 4	23 ± 8
105	0 ± 0	0 ± 0	0 ± 0	37 ± 17
250	0 ± 0	0 ± 0	0 ± 0	0 ± 0
500	0 ± 0	8 ± 7	0 ± 0	14 ± 7
750	0 ± 0	17 ± 19	0 ± 0	0 ± 0
1000	0 ± 0	0 ± 0	0 ± 0	0 ± 0

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.