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## Advanced mine restoration protocols facilitate early recovery of soil microbial biomass, activity and functional diversity

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1 **Advanced mine restoration protocols facilitate early recovery**  
2 **of soil microbial biomass, activity and functional diversity.**

3

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5

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13 **Running title:** Microbes in restored soils

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15 **Abstract**

16

17 Many ecosystem restoration programmes can take over 15 years to achieve  
18 ecosystem functioning comparable to that of an unmodified ecosystem, therefore a  
19 reliable shorter-term method of assessing and monitoring ecosystem recovery is  
20 needed to ensure that recovery is following an appropriate trajectory. Soil microbes  
21 respond to environmental change relatively quickly, and shifts in microbial  
22 communities can reflect the current status of their environment. As well as  
23 potentially acting as 'indicator communities', microbes play an integral role in  
24 restoring ecosystem functions. On an active opencast mine on New Zealand's West  
25 Coast, three main restoration methods are used, differing in cost and restoration  
26 effort. They range from most expensive 1) vegetation direct transfer (VDT), to 2)  
27 biosolids-amended stockpiles that are spread and replanted, and 3) untreated  
28 stockpiles that are spread and replanted. We assessed the impacts of these methods  
29 on soil microbial communities by measuring microbial biomass, dehydrogenase  
30 activity, community level physiological profile (CLPP) and functional diversity as  
31 measured by carbon substrate utilisation, where restored sites were 5 years old or  
32 less. These measures were compared to an unmodified reference ecosystem in the  
33 same location. Microbial activity and biomass were highest in pristine habitats,  
34 followed by VDT and biosolids-amended soils, then untreated stockpile soil. When  
35 compared to all other treatments untreated stockpiled soils had significantly  
36 different CLPPs and significantly reduced microbial biomass and activity; microbial  
37 biomass was an order of magnitude lower than in pristine soils. Functional diversity  
38 and richness did not differ between pristine, VDT and biosolids-amended soils, but

39 were higher than in untreated stockpiled soils. CLPPs did not differ between pristine  
40 habitat soil and VDT soil but biosolids-amended and untreated stockpiled soils were  
41 significantly different to pristine soil. This study has shown that soil microbial  
42 communities are a valuable tool to assess restoration progress, and that ecosystem  
43 restoration can begin in a relatively short time following investment in appropriate  
44 restoration strategy, ultimately benefiting recolonisation by plants and animals.

45

46 Zusammenfassung

47 Viele Ökosystem-Renaturierungsprogramme können mehr als 15 Jahre benötigen,  
48 bevor eine Ökosystemfunktion, die mit der eines nicht modifizierten Ökosystems  
49 vergleichbar ist, erreicht wird. Darum wird eine verlässliche, früher einsetzbare  
50 Methode zur Bewertung und Überwachung der Regeneration des Ökosystems  
51 benötigt, um sicherzustellen, dass die Regeneration einen geeigneten Verlauf nimmt.  
52 Bodenmikroorganismen reagieren relativ schnell auf Umweltänderungen, und  
53 Änderungen der Mikrogen-Gemeinschaften können den aktuellen Status ihrer  
54 Umwelt widerspiegeln. So wie sie potentiell als "Indikator-Gemeinschaften"  
55 fungieren, spielen Mikroben auch eine zentrale Rolle bei der Wiederherstellung von  
56 Ökosystemfunktionen. In einer aktiven Tagebau-Kohlegrube an der  
57 neuseeländischen Westküste werden drei Haupt-Renaturierungsmethoden  
58 angewandt, die sich hinsichtlich der Kosten und des Aufwandes unterscheiden. Sie  
59 reichen von der sehr teuren direkten Übertragung bewachsener Bodenblöcke (VDT)  
60 über die Ausbringung von mit Klärschlamm vermischem Lagerhaldenmaterial, das  
61 ausgebreitet und bepflanzt wird, bis zur Ausbringung von unbehandeltem  
62 Haldenmaterial, das verteilt und bepflanzt wird. Wir erfassten die Auswirkungen

63 dieser Behandlungen auf die Mikrobengemeinschaften im Boden, indem wir die  
64 mikrobielle Biomasse, die Dehydrogenase-Aktivität, das physiologische Profil der  
65 Gemeinschaften und die funktionelle Diversität (gemessen als Nutzung von  
66 verschiedenen Kohlenstoffsubstraten) bestimmten. Die untersuchten  
67 Renaturierungsflächen waren bis zu fünf Jahre alt. Die Messungen wurden mit Daten  
68 aus einem benachbarten naturnahen Referenzökosystem verglichen. Die mikrobielle  
69 Aktivität und Biomasse waren in dem alten Ökosystem am höchsten, gefolgt von den  
70 VDT-Flächen, dem mit Klärschlamm versetzten Material und schließlich dem  
71 unbehandelten Haldenmaterial. Die letztgenannte Behandlung wies verglichen mit  
72 den anderen Varianten ein signifikant abweichendes physiologisches  
73 Profil sowie signifikant reduzierte mikrobielle Biomasse und Aktivität auf. Die  
74 mikrobielle Biomasse war um eine Größenordnung geringer als die von  
75 ursprünglichem Boden. Funktionelle Diversität und funktioneller Reichtum  
76 unterschieden sich nicht zwischen ursprünglichen, mit Klärschlamm gemischten und  
77 VDT-Böden; sie waren aber höher als in unbehandelten Haldenböden. Die  
78 physiologischen Profile der Gemeinschaften unterschieden sich nicht zwischen  
79 ursprünglichem Boden und VDT-Böden, aber sowohl mit Klärschlamm versetzter als  
80 auch unbehandelter Haldenboden unterschieden sich signifikant vom ursprünglichen  
81 Boden. Diese Untersuchung hat gezeigt, dass mikrobielle Gemeinschaften des  
82 Bodens ein wertvolles Mittel sind, um den Rekultivierungsfortschritt abzuschätzen  
83 und dass die Renaturierung relativ kurz nach der Investition in eine angemessene  
84 Renaturierungsstrategie einsetzen kann, wodurch letztendlich die Wiederbesiedlung  
85 durch Pflanzen und Tiere gefördert wird.

86

87 Keywords: Biosolids; Ecological restoration; Microbes; Mining; Vegetation direct

88 transfer; VDT.

89

90

Accepted Manuscript

91 **Introduction**

92

93 To access target resources, opencast mining requires the complete removal of soil,  
94 rock and vegetation, soil and rock mixtures are then regularly stored in large  
95 stockpiles of mixed topsoil and various other rock and soil strata (Boyer, Wratten,  
96 Pizey, & Weber, 2011). In New Zealand, these stockpiles are often used for  
97 restoration of areas where mining is complete. However, the materials selected for  
98 stockpile storage (soil, subsoil and overburden) can have detrimental effects on  
99 stored soil quality, microbial communities and fauna. For example, increased  
100 proportions of rock and a reduction in levels of primary plant nutrients (Nitrogen (N),  
101 Phosphorus (P), Potassium (K) and Carbon (C)) in stockpile soil will decrease soil  
102 quality (summarised in Sheoran et al. 2010). Stockpiles can become stratified such  
103 that at > 1 m depth they can become compacted and anaerobic resulting in loss of  
104 earthworm populations (Boyer et al., 2011), decreased abundance of mycorrhizae,  
105 aerobic bacteria and fungal species, and an increase in soil bacterial to fungal ratio  
106 (Abdul-Kareem & McRae, 1984; Harris, Birch, & Short, 1989).

107

108 Soil quality is important in the restoration of ecosystems because of its role as the  
109 physical, biological and nutrient basis for plant recolonisation and establishment  
110 (Zhang & Chu, 2012). Soil microbial communities are responsible for many  
111 ecosystem functions including decomposition, nutrient cycling, nitrogen fixation and  
112 soil formation (Zhang & Chu, 2011). They also support revegetation processes, which  
113 can further stabilize soils and prevent loss of bulk soil and nutrients through erosion  
114 (Ros, Hernandez, & García, 2003). Restoration following mining should therefore not



115 only aim to achieve plant cover, but also regenerate soil functions that form the  
116 foundations on which ecosystems can persist (Ohsowski, Klironomos, Dunfield, &  
117 Hart, 2012); and microbial assessments can contribute an insight to this process.

118

119 The definition of ecological restoration, as defined by the Society for Ecological  
120 Restoration, is: 'the process of assisting the recovery of an ecosystem... towards a  
121 reference state' (Society for Ecological Restoration, 2013). Following this definition,  
122 studies have shown that after mining, at least 15 years or more may pass before soil  
123 functions are comparable to those of pristine areas (Ruiz-jaen & Aide, 2005; Grant &  
124 Ward, 2007). While restoration of soil functions may take decades, monitoring in its  
125 early stages is essential to assess whether restoration efforts are effective or at least  
126 following a suitable trajectory, and that limited resources (time, finance, machinery)  
127 are not misdirected. Appropriate early monitoring can also be useful to assess the  
128 efficacy of new or previously untested restoration techniques.

129

130 The majority of restoration studies examining ecosystem processes occur within 15  
131 years since restoration was implemented, with most of these in the first 5-10 years  
132 (Wortley, Hero, & Howes, 2013). These studies show that some ecological processes  
133 can have made measurable progress within that time. In particular, soil microbial  
134 communities can serve as early indicators of effective mine restoration, because  
135 they respond relatively quickly to changes in environmental conditions (Sparling,  
136 1992; Emmerling, Liebner, & Haubold-Rosar, 2000; Izquierdo, Caravaca, Alguacil,  
137 Hernández, & Roldán, 2005). They can provide more accurate representations of  
138 short-term recovery, unlike plant species diversity assessments that may prove

139 misleading in the short-term as they could simply reflect the artificial planting  
140 schedules in a particular restoration scheme, rather than genuine re-establishment  
141 (Harris, 2003).

142

143 Stockton mine is an active opencast coalmine on the West Coast of New Zealand's  
144 South Island where three primary restoration protocols are employed. Vegetation  
145 direct transfer (VDT) is a relatively recent development in restoration in New Zealand  
146 (Simcock, Toft, Ross, & Flynn, 1999; Ross, Simcock, & Williams, 2000) and is the most  
147 expensive and labour-intensive of the three protocols. It requires lifting patches of  
148 complete ecosystems (all above-ground vegetation and ~30 cm topsoil) and  
149 transferring them to either holding sites, or areas where mining is completed and  
150 requires restoration. VDT sods are laid in a patchwork, where intact sods are placed  
151 alongside each other to achieve ground coverage resembling the donator site.  
152 Typically VDT contains low-medium height scrub vegetation (1-3 m in height) as  
153 larger trees have root networks that become damaged by the process and  
154 experience dieback. The second most labour-intensive restoration strategy is the  
155 mixing of biosolids (dried and sterilised municipal sewage waste) with stockpiled  
156 mine tailings, which are then spread and planted with native vegetation. The third  
157 process is the spread of untreated stockpiled tailings, which are also replanted with  
158 native species. VDT and biosolids soil amendments are relatively new additions to  
159 the Stockton mine restoration protocols that have been implemented on a large  
160 scale only within the last 5 years, and have not yet been thoroughly assessed for  
161 their efficacy in restoring mined areas. To our knowledge few studies have assessed

162 the role of VDT in ecological restoration (Simcock et al., 1999; Ross et al., 2000), and  
163 its impact on soil organisms (Boyer et al., 2011).

164

165 This study assessed soil microbial metrics under the three primary restoration  
166 techniques used on the mine less than five years after their deployment. This  
167 includes measurement of dehydrogenase activity, soil microbial biomass, functional  
168 diversity, and community-level physiological profiles (CLPP). These measurements  
169 were compared to those taken from local unmined native habitat as a benchmark for  
170 restoration success.

171

172

## 173 **Materials and methods**

174

### 175 *Experimental design and site selection*

176

177 Stockton mine has been active since 2008 with an expectancy of 20 years coal  
178 extraction. Prior to mining, the area was pristine native New Zealand bush  
179 vegetation, of typically low to medium height (1-5 m) ranging from native tussock  
180 grassland to beech and podocarp forest. The site undergoing restoration following  
181 coal extraction was Mt Frederick, located on the southwestern area of the Stockton  
182 mine (414248.16 E, 1715045.03 S). Mt Frederick was selected as it was one of the  
183 first areas where mining had been completed. It contains a number of replicated  
184 restoration sites (n=4 for all restoration types) of differing restoration techniques,  
185 including VDT (direct, never stored in holding sites), biosolids soil amendment, and

186 untreated stockpiled soil spreads, and an unmodified pristine native site, all within  
187 0.5 km of one another. The inclusion of areas of unmodified native vegetation was  
188 intended as a benchmark for the restored sites, and was the same state as that prior  
189 to mining. Owing to their location, all sites were a similar size (approximately 200-  
190 300 m<sup>2</sup>), and were subject to similar environmental variables such as slope and  
191 weather conditions at a west to southwest orientation of approximately 1000 m  
192 altitude. Age ranges for restored sites were: VDT 2-5 years, replanted stockpiled soils  
193 2-5 years, and biosolids treated stockpiled soils 3-4 years. Soil stockpiles contain soil,  
194 small fractured rock (generally <5 cm in diameter) and overburden, and can range  
195 from 6 months to 5 years in age before use. Standard practice on Stockton mine is to  
196 1:1 mix 5 years stockpiled soil with 6 months stockpiles prior to spreading and  
197 planting in an attempt to improve soil quality (Thompson, personal communication).  
198 The biosolids spread comprises 1:4 mix of biosolids and the above described  
199 stockpiled soil preparation.

200

201

#### 202 *Soil collection*

203

204 Soils were collected from four separate plots of each restoration type. Fifty 7 cm  
205 deep x 3 cm wide soil cores were taken across each restored and pristine plot  
206 starting in one corner of the plot and following a 'Z' shaped path across the width  
207 and length of the plot to capture the variation in soils within each plot. The 50 cores  
208 were then pooled for each site. Soil was then transported to Lincoln University, New  
209 Zealand and stored at 4 °C until microbial assays were completed.

210

211

212 *Soil microbial biomass*

213

214 Microbial biomass was assessed by the chloroform-fumigation method (Vance,  
215 Brookes, & Jenkinson, 1987). Triplicate 3.5 g soil fresh weight subsamples were  
216 extracted in 15 ml 0.5M  $K_2SO_4$  (at 1:4 soil:extractant ratio) for 30 minutes on a rotary  
217 shaker. Extracts were filtered using Whatman N° 40 filter paper and analysed on a  
218 Total Organic Carbon Analyser TOC-5000A fitted with a ASI-5000A autosampler (TOC;  
219 Shimadzu, Japan). Another set of triplicate 3.5 g subsamples was subjected to  
220 fumigation. These were placed in open vials in a vacuum box, together with 25 ml  
221 ethanol-free chloroform and exposed for 24 h at room temperature in the dark.  
222 Samples were then extracted using the same method for non-fumigated samples.  
223 The difference between the two carbon concentrations in the fumigated and non-  
224 fumigated soils was considered total microbial biomass carbon.

225

226 *Dehydrogenase activity*

227

228 As a proxy for soil microbial activity (Włodarczyk, 2000), dehydrogenase activity  
229 (DHA) was measured by quantifying the rate of reduction of triphenyltetrazolium  
230 chloride (TTC) to triphenyl formazan (TPF) using a modification of the method  
231 described in Alef (1995). 2 g fresh weight soil samples were weighed into sterile glass  
232 vials in triplicate and mixed with 2 ml 0.7% TTC solution made by diluting 0.7 g TTC in  
233 100 ml 100mM Tris (pH 7.5). The samples were shaken on a rotary shaker for 30

234 minutes and incubated in the dark for 24 hours at 30 °C. TPF was extracted by adding  
235 10 ml methanol, and incubating at room temperature for 2 hours with regular  
236 shaking. Samples were filtered through Whatman N°1 filter paper and absorbance at  
237 485 nm measured on a Multiskan GO Microplate Reader (Thermo Scientific, US).

238

239

240 *Community-level physiological profile and functional diversity*

241

242 Biolog EcoPlates™ (Biolog, CA) were used to generate community-level physiological  
243 profiles (CLPPs) based on the microbial communities' ability to utilise different  
244 carbon substrates. EcoPlates can be used to distinguish between communities from  
245 different habitats (Gomez, Ferreras, & Toresani, 2006). They contain 31 carbon  
246 compounds designed to reflect some of those found in root exudates and soils (in  
247 triplicate, plus triplicate controls). A redox-colourant of tetrazolium violet is present  
248 in all wells, which undergoes irreversible colour change when a carbon compound is  
249 metabolised. Functional diversity can be estimated by the range and extent of  
250 carbon compound metabolised.

251

252 2.5 g of soil (dry weight equivalent) was diluted in 22.5 ml of 0.85% NaCl solution and  
253 put on a rotary shaker for 30 minutes. Samples were allowed to settle for 10 minutes  
254 before dilution to  $10^{-2}$ , and 140 µl of this suspension was used to inoculate each well  
255 of the EcoPlates™. Absorbance at 590 nm was measured with a Multiskan GO  
256 Microplate Reader, with initial absorbance taken immediately, and then every 24  
257 hours for 16 days at which point there was no further change in absorbance.

258

259

260 *Statistical analyses*

261

262 Microbial biomass and dehydrogenase activity were compared using analysis of  
263 variance (ANOVA) on GenStat (VSN International), with Fisher's least significant  
264 difference (LSD) for pairwise comparisons. Biomass and DHA data were log  
265 transformed to fit the assumptions of ANOVA. Differences between CLPP patterns of  
266 carbon substrate utilisation were visualized using nonmetric multidimensional  
267 scaling (NMDS) and tested with permutational analysis of variance (PERMANOVA) on  
268 PRIMER software (Clarke & Gorley, 2006). Differences in functional diversity, number  
269 of functions, homogeneity of multivariate dispersion measures of CLPP were also  
270 undertaken in PRIMER. Probabilities for rejecting the null hypothesis below 0.05  
271 were considered significant, and between 0.05 and 0.10 were considered marginally  
272 significant.

273

274

## 275 **Results**

276

277 *Soil microbial biomass*

278

279 Restoration type had a significant impact on soil microbial biomass (ANOVA,  $F =$   
280  $28.97$ ,  $P < 0.001$ ). All treatments were significantly different (LSD,  $P < 0.05$ ; Fig. 1A),  
281 with the exception of stockpiled soil and biosolids-amended soils. Unmodified native

282 soil contained the greatest microbial biomass, followed by VDT, then biosolids-  
283 amended soil and untreated stockpiled soil, the latter being an order of magnitude  
284 lower in microbial biomass carbon than the pristine soil (Fig. 1A).

285

286

287 *Soil dehydrogenase activity*

288

289 Dehydrogenase activity (DHA) significantly differed between treatments (ANOVA,  $F =$   
290  $11.39$ ,  $P < 0.001$ ). Pairwise comparisons (LSD,  $P < 0.05$ ; Fig. 1B) showed that DHA was  
291 significantly lower in untreated stockpiled soil than in all other treatments. Activity in  
292 biosolids-amended soil did not differ from the unmodified site or VDT. However, VDT  
293 had significantly lower rates of DHA than did the unmodified soil. (Fig. 1B). Linear  
294 regression analysis (Fig. 1C) indicates that microbial biomass was not correlated with  
295 DHA treatment means ( $n=4$ ).

296

297

298 *Community level physiological profile and functional diversity*

299

300 Restoration technique significantly affected CLPP of the microbial communities  
301 (PERMANOVA,  $F = 6.526$ ,  $P < 0.001$ ; Fig. 2). Pairwise analyses indicate that VDT did  
302 not differ from unmodified soil or biosolids-amended soil, but biosolids did  
303 marginally differ from unmodified soil (PERMANOVA,  $F = 2.023$   $P = 0.055$ ). CLPP of  
304 stockpiled soil differed significantly from all other treatments (PERMANOVA,  $P <$   
305  $0.05$ ; Fig. 2).



306

307 Richness and Shannon diversity index of CLPPs significantly differed between  
308 treatments (ANOVA,  $F_{richness} = 25.00$ ,  $F_{diversity} = 15.51$ ,  $P < 0.001$ ), where biosolids-  
309 amended soils, VDT and unmodified soils did not differ from one another, but all  
310 were significantly more rich and diverse than untreated stockpiled soil. Dispersion of  
311 CLPP (the distance of points from the centroid within a treatment (Fig. 2), *i.e.*,  
312 variation between samples within a treatment) also differed between treatments  
313 (PERMDISP,  $F = 7.898$ ,  $P = 0.026$ ). Biosolids-amended soil did not differ from  
314 unmodified soil, while VDT had marginally higher dispersion (PERMDISP,  $F = 2.848$ ,  $P$   
315  $= 0.061$ ). VDT and biosolids-amended soils did not differ, but all restoration  
316 techniques and unmodified soils were significantly less dispersed than untreated  
317 stockpiled soil (Fig. 2).

318

319

## 320 Discussion

321

322 Soil microbial communities can be useful early indicators of restoration success  
323 when assessment of plant colonisation or other ecosystem functions may not be  
324 suitable or possible (Sparling, 1992; Harris, 2003; Gomez et al., 2006). Short-term  
325 monitoring in this study indicates that VDT and biosolids addition consistently  
326 restored all measured facets of the soil microbial community towards that of the  
327 reference state. Previous work has found strong differences in soil chemical  
328 properties between soil from different restoration methods on Stockton mine  
329 (Waterhouse, Boyer, Adair, & Wratten, 2014). The differences observed between

330 restoration types in this study, therefore, are likely to be due in part to differences in  
331 soil physical and chemical properties arising from how the soil was treated following  
332 excavation (Abdul-Kareem et al., 1984) and from biosolids use (Sullivan,  
333 Stromberger, Paschke, & Ippolito, 2005). Input of restoration resources beyond that  
334 of spreading untreated stockpiled tailings and replanting them with native  
335 vegetation was beneficial to the aspects measured in this study. VDT was the most  
336 effective protocol for restoring microbial biomass and activity as well as preserving a  
337 community composition somewhat similar to native sites. Biosolids amendments  
338 generally gave intermediate results and stockpile soils were least effective for  
339 restoration. The same order is followed for labour and financial burden. On the  
340 whole, our results suggest that such improvements are proportional to the effort  
341 and cost incurred for each restoration technique, *i.e.*, VDT is the most costly, both in  
342 effort and financially, but restores microbial biomass, CLPP and functional diversity  
343 more effectively than do the other techniques.

344

345 VDT is a process that relocates areas of intact native ecosystem and therefore is an  
346 ecosystem that has experienced, and is recovering from, an extreme disturbance  
347 event. This may explain the reduction in microbial biomass and DHA compared to  
348 the undisturbed habitat (Peacock, Macnaughton, & Cantu, 2001; Lucas-Borja &  
349 Bastida, 2011), and further study would be required to establish if these measures of  
350 microbial communities return to pre-disturbance conditions in the long-term.

351 Nevertheless, the current assessment suggests that VDT is the most effective  
352 restoration method deployed at Mt Frederick. In contrast, restoring areas with  
353 stockpiled soils and stockpiled soils mixed with biosolids are examples of habitats

354 that are being engineered from scratch. For these habitats, measurements in this  
355 study are indicative of whether restoration is accelerating ecosystem development  
356 rather than recovery from disturbance *per se*. Biosolids have been successfully used  
357 to restore microbial communities on copper (Gardner, Broersma, & Naeth, 2010)  
358 and coal (Evanylo, Abaye, Dundas, Zipper, Lemus, et al., 2005) mines, and in metal-  
359 contaminated mine tailings (Brown, Henry, Chaney, & Compton, 2003), and  
360 comparing soils that were amended with biosolids with those that were not shows  
361 that the addition of biosolids leads to an acceleration of restoration in the majority  
362 of microbial measures in this study.

363

364 Although microbial biomass significantly differed between VDT and biosolids-treated  
365 stockpile soils, DHA, CLPPs, dispersion and functional diversity did not. The aim of  
366 carbon utilisation profiles in this study was not to compare community species  
367 composition (Bossio & Scow, 1998), but to assess whether the communities have  
368 similar functional diversity and physiological profiles (Garland & Mills, 1991), and  
369 comparing these to an unmodified native habitat can provide a useful measure of  
370 restoration success (Ruiz-jaen et al., 2005). The results in this study indicate firstly  
371 that some input of restoration effort beyond re-spreading and planting stockpiles is  
372 necessary for recovery of microbial CLPPs, dehydrogenase activity and microbial  
373 biomass carbon; and secondly, that biosolids addition and particularly VDT are  
374 especially effective for restoring such functions on the studied site. For example,  
375 both VDT and biosolids reduced the dispersion of CLPPs, resulting in communities  
376 more similar for plots within those treatments (Fig. 2), producing a similar  
377 homogeneity in communities between plots as in the unmodified habitats.

378 Furthermore, richness and functional diversity based on CLPPs was significantly  
379 increased from untreated stockpiled soils to levels similar to unmodified soils. As  
380 VDT is a relatively recently deployed restoration protocol on the Stockton mine, this  
381 is an important finding showing that microbial activity and functional diversity are  
382 not substantially decreased upon relocation of the VDT sods from unmodified areas.  
383 The availability of carbon can have considerable effects on microbial communities  
384 (Bossio & Scow, 1995), which may explain why the addition of biosolids, containing  
385 high levels of carbon, increased CLPP, functional diversity and activity substantially  
386 compared to stockpiled soil alone (Sharma, Rangger, von Lützwow, & Insam, 1998;  
387 Gomez et al., 2006; Zhang et al., 2012).

388

389 Dehydrogenase activity at VDT sites were lower than would be expected for the  
390 given biomass, and *vice versa* for biosolids-treated soil (Fig. 1c). However, similar  
391 functional diversity, richness and CLPP in the two treatments suggests that in  
392 biosolids-treated soil either a larger proportion of the soil microbial biomass in  
393 biosolids was active, or the same proportion was more active and more efficient at  
394 carbon substrate utilization under the conditions used in this study (Haynes & Fraser,  
395 1998).

396

397 Soil microbes mediate key ecosystem functions such as nutrient cycling and organic  
398 matter decomposition, which can in turn influence plant community assembly  
399 (Zhang et al., 2011). In the restoration methods employed at Mt Frederick, biosolids-  
400 amended soils and stockpiled soil spreads are often planted with similar planting  
401 schedules of native plants placed approximately 1 m apart, and when VDT is

402 undertaken, gaps can develop between sods caused by mechanical damage,  
403 vegetation dieback, edge erosion and misalignment. Soil quality will be a key  
404 determinant in the recolonisation of gaps in planting schedules and between sods by  
405 immigrants and subsequent generations. Therefore, organic matter amendment (for  
406 example biosolids), should be considered in order to accelerate ecological succession  
407 in such gaps by ensuring that soil microbial communities have been adequately  
408 restored (Harris, 2009).

409

410 The microbial methods (Biolog EcoPlates, DHA, soil microbial biomass carbon) used  
411 to assess soil activity, functional diversity and CLPP throughout this work are subject  
412 to some limitations. For example, use of Biolog EcoPlates is a coarse method  
413 restricted to carbon substrates and lab-culturability of field microbes (Preston-  
414 Mafham, Boddy, & Randerson, 2002; Campbell, Chapman, Cameron, Davidson, &  
415 Potts, 2003) and DHA is considered a proxy for general soil activity (Włodarczyk,  
416 2000). All of these methods were employed as measures for relative comparison  
417 between sites within the study, and not as absolute measures of soil microbial  
418 community (Preston-Mafham et al., 2002). In spite of the broad nature of rapid CLPP  
419 assessment tools (Lalor, Cookson, & Murphy, 2007) these assessments yielded some  
420 interesting results distinguishing between treatments and agreeing with the benefits  
421 of restoration to microbial communities that has been observed in previous studies  
422 (e.g. Sullivan et al. 2005; Rojas-Oropeza et al. 2010; Zhang & Chu 2012). However,  
423 variations in microbial community composition can cause substantial differences in  
424 function (Strickland, Lauber, Fierer, & Bradford, 2009). Therefore the differences in  
425 community similarity for soil microbes may affect soil processes not measured in this

426 study. Assessment of additional soil functions (e.g. decomposition, nutrient cycling  
427 etc.) should be considered in future work, and over longer time periods to assess  
428 long-term recovery.

429

430

### 431 **Conclusion**

432

433 Using soil microbial communities as early indicators of restoration success in this  
434 study indicates that on the studied site these communities have benefited from  
435 channeling resources into ecological restoration, including VDT and the addition of  
436 biosolids to stockpiled soils, rather than replanting in untreated stockpile spreads.  
437 Biosolids have received substantial research attention in the context of ecological  
438 restoration, and the results of this study tend to agree with those of others, finding  
439 beneficial effects on the microbial community. VDT, however, has not yet been  
440 implemented globally or on a large scale, and yet shows great promise for ecological  
441 restoration. Ongoing assessment is required to ensure that this recovery is  
442 consistently benefitting soil functions, followed by assessment of other ecosystem  
443 processes (plant and animal recolonisation, biomass production etc.) to quantify  
444 further advanced recovery in the coming years. In the meantime, utilising the  
445 responsiveness of microbial communities can be a valuable tool in monitoring early  
446 recovery of ecosystems following mining.

447

448

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450

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457

458

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577

1 **Figure legends**

2

3 Fig. 1: A) Soil microbial biomass ( $\pm$  SE); (B) soil dehydrogenase activity (DHA) ( $\pm$  SE);

4 (C) DHA ( $\pm$  SE) was not correlated with microbial biomass ( $r = 0.742$ ,  $F = 5.75$ ,  $P =$

5  $0.139$ ). Unmodified native habitat ( $\blacktriangle$ ), VDT ( $\blacksquare$ ), biosolids-amended soils ( $\triangle$ ) and

6 untreated stockpiled soil ( $\circ$ ).

7

8 Fig. 2: Nonmetric multidimensional scaling (NMDS) plot based on Bray-Curtis

9 similarities of carbon substrate utilisation patterns: unmodified native habitat ( $\blacktriangle$ ),

10 vegetation direct transfer (VDT) ( $\blacksquare$ ), biosolids-amended stockpiled soil ( $\nabla$ ),

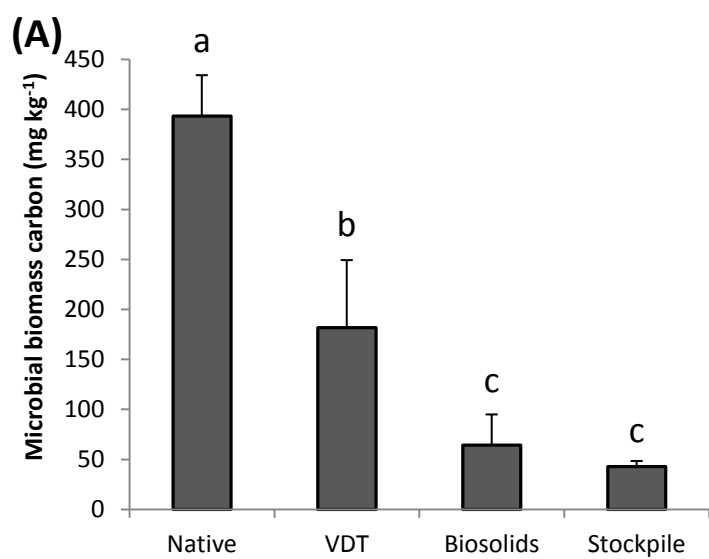
11 untreated stockpiled soil ( $\circ$ ). Unmodified and VDT patterns did not differ, biosolids-

12 amended soil and VDT did not differ, but biosolids did differ marginally from

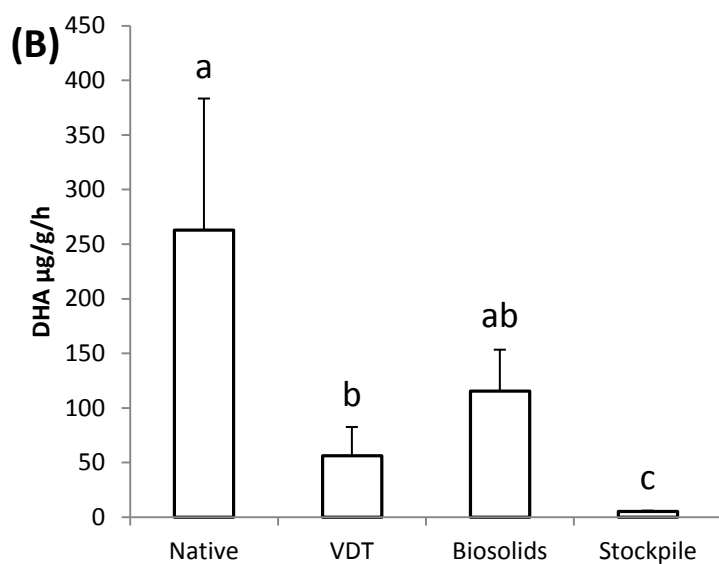
13 unmodified soil. All other pairwise comparisons significantly differed. Stress 0.15.

14

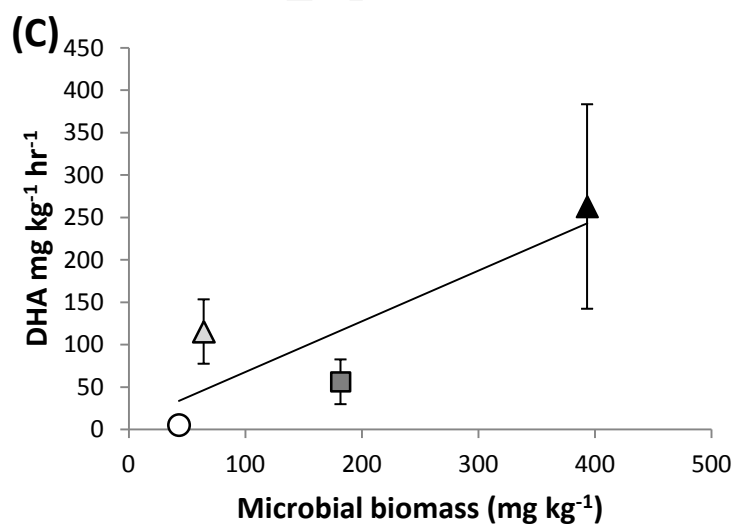
15

16 **Figure 1:**

17



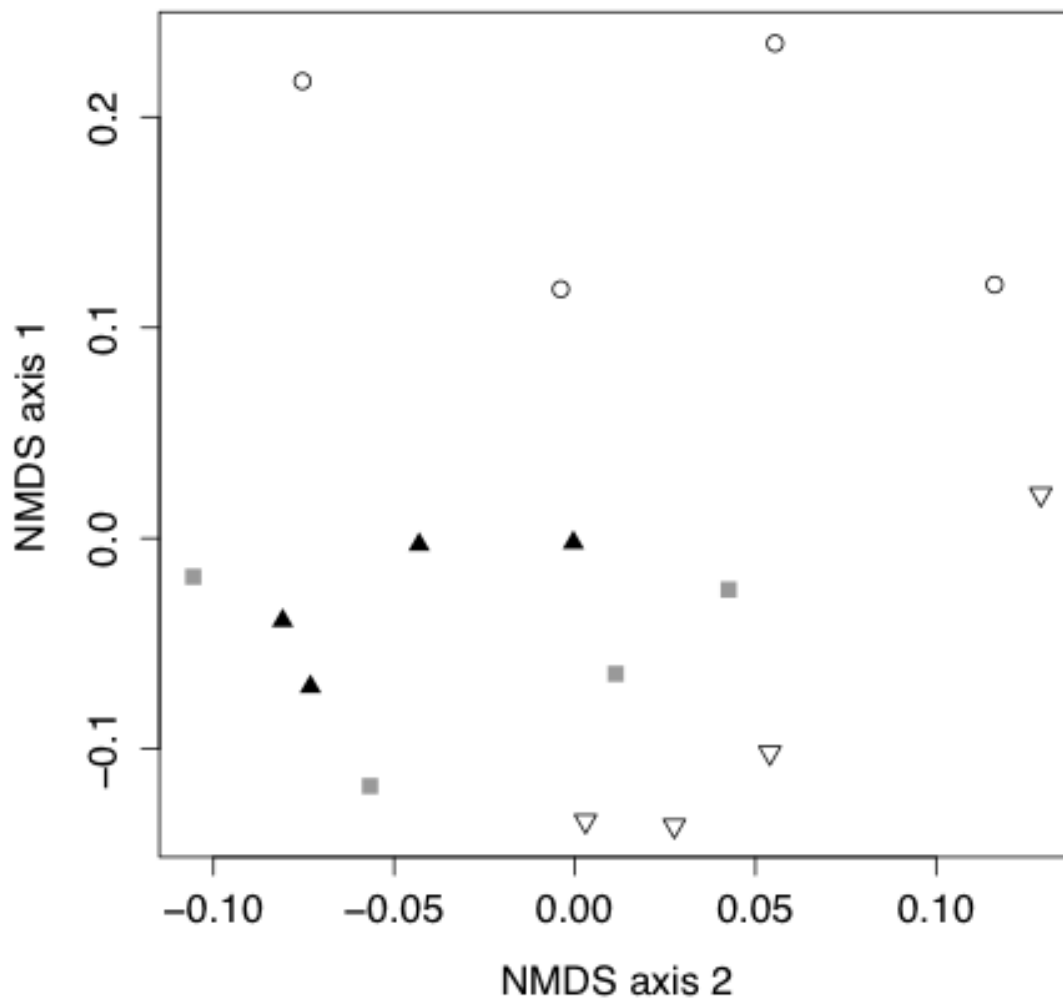
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19

20 **Figure 2:**

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22

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Accepted