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1 **Addition of composted green waste and ericoid mycorrhizal fungi fails to facilitate**
2 **establishment of Atlantic heathland species**

3

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14

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18

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20 data and performed laboratory analyses; ML, MH, JE analysed the data, ML, MH, wrote the
21 manuscript with editorial assistance from CB, PL, MK, JE.

22 **Abstract**

23 Post-mining restoration of heathland habitats has met with mixed success. Failures are often
24 ascribed to the complexity of replicating soil conditions: a scarcity of organic matter and
25 microbial symbionts in stored overburden used for restoration is frequently implicated.
26 Nonetheless, systematic investigation of the role of both interventions is lacking. Using a
27 greenhouse trial and a large-scale field experiment within a commercial kaolinite mine site, we
28 explored how the addition of ericoid mycorrhizal fungi (ErMF) and organic matter influenced
29 the establishment of dwarf ericoid species that characterise NW European Atlantic lowland
30 heaths. Neither intervention had any positive effect on ericoid establishment in field or
31 greenhouse conditions. In the greenhouse experiment, organic matter (from commercial refuse)
32 increased heather (*Calluna vulgaris*) cuttings mortality, although surviving plants showed
33 enhanced shoot growth when ErMF were added. All field plots were dominated by
34 combinations of grasses, gorse (*Ulex europaeus*), and bare ground. Establishment of ericaceous
35 plants was remarkably low (< 4%) after three years and *Erica tetralix* and *Calluna vulgaris*
36 abundance in organic matter (which increased pH) or ErMF treatments was reduced compared
37 to untreated control. Although our experiments suggest that research on soil manipulation
38 treatments is required to elucidate the conditions necessary for heathland establishment,
39 corroboration of our greenhouse trial results in field conditions highlights the value of the
40 former in informing the latter. We identify low pH, high lignin (e.g., pine) litter as one
41 potentially worthwhile soil amelioration treatment and suggest how the use of naturally
42 colonised /pre-inoculated 'nursemaid' plants could facilitate heathland restoration.

43

44 **Implications for practice**

45 • Addition of composted green waste in heathland restoration leads to the rapid
46 establishment of mesotrophic grasses in the field.

47

48 • Addition of composted green waste in heathland restoration results in reduced survival
49 of ericaceous plants in laboratory trials.

50

51 • More research is required to investigate the use of ericoid mycorrhizal fungi in large-
52 scale field trials for heathland restoration. These should manipulate the timing of
53 application, species composition and local adaptation of fungal species. Here addition
54 of ErMF did not enhance heathland establishment.

55

56 • No restoration intervention had a positive influence on heathland establishment and we
57 caution that heathland restoration after mineral extraction is not achieved easily within
58 short-timescales.

59

60 **Introduction**

61 Although occupying a relatively small, but increasing, proportion of the global land surface,
62 aggregate mineral extraction causes considerable ecological damage with concomitant
63 biodiversity loss and disruption of ecosystem service provision (Prach & Tolvanen 2016;
64 Salgueiro et al 2020). Post-operation ecological restoration is therefore, desirable, and indeed
65 often essential as a pre-requisite for obtaining a mine concession. All too frequently, however,
66 attempts to reinstate habitats similar to those lost to mineral extraction prove unsuccessful
67 (Hobbs & Harris 2001; Cooke et al 2019; Salgueiro et al 2020). These failures come as no
68 surprise; modern mineral extraction usually necessitates the removal of large areas of
69 vegetation and underlying matter to expose commercially viable mineral deposits in extensive
70 opencast mines. Topsoil, sub soil and overburden layers, later used to recover the area left after
71 extraction are mixed and stockpiled (for as much as 20 years), destroying natural topsoil
72 structure, depleting seed banks and soil microbial communities, and causing major changes in
73 soil biogeochemistry (Rokich et al. 2000; Golos et al. 2016; Merino-Martín et al. 2017; Hart et
74 al 2019).

75 Consequently, a number of factors are likely to combine to limit post-mine restoration success.
76 The large size of many opencast mines is an important barrier to natural recolonization, an
77 effective remediation option only when the target site is small, and surrounded by pre-
78 disturbance vegetation and a supply of plant propagules and mutualist/symbiont species (Holl
79 & Aide 2011; Prach et al. 2014). Nonetheless, chief among the factors preventing restoration
80 are the major changes in local geomorphology and soil biogeochemistry associated with
81 mineral extraction and storage of topsoil forming materials. Indeed, Whisenant (1999)
82 identified abiotic limitations as the first barrier to restoration success and emphasized how
83 physical and/or chemical treatments are necessary before subsequent biological interventions

84 can be implemented. Such interventions may take several years or decades to ameliorate the
85 soil environment before successful restoration of the target plant community is possible.

86 In their study of the natural restoration/recolonization of Atlantic Lowland Heath (ALH) in SW
87 England, Lane et al. (2020) highlighted how even 150 years after kaolinite mining ceased, soil
88 chemistry failed to approach the acidity, organic content, or key soil nutrients characteristic of
89 nearby ALH soils. A likely consequence of this slow pace of natural soil recovery was the
90 failure of ALH species to dominate, or even establish on, former kaolinite quarries and the
91 presence instead of plants more characteristic of acidic or mesotrophic grasslands. Kaolinite
92 deposits frequently occur beneath highly leached, low nutrient, acidic soils, conditions
93 characterised by heathland vegetation making heathland a particularly challenging habitat to
94 restore. Despite the ability of many heathland species to cope with a stressful edaphic
95 environment, the removal and storage of overburden, later used for mine rehabilitation, often
96 reduces nutrient concentrations to levels well below those naturally found in heathland soils
97 and insufficient for plant establishment and growth (Coppin & Bradshaw 1982; Clarke 1993,
98 1997; Lane et al 2020). Overburden storage can also raise pH to levels unsuitable for heathland
99 establishment, and encourage instead recruitment of competitive grass species that limit
100 ericaceous plant establishment (Marrs et al. 1998; Green et al. 2015; Lane et al. 2020).
101 Moreover, reinstatement of a carbon and nutrient limited, highly porous mineral overburden
102 inevitably limits heathland establishment (Diaz et al. 2006; Smith & Read 2010; Machado et
103 al. 2013; Bateman et al. 2018).

104 Studies such as Lane et al (2020) underscore the need for active soil management as a basis to
105 facilitate successful (heathland) restoration (see also Holmes. 2001; Benigno et al. 2013;
106 Clemente et al. 2016; Glen et al. 2017). These interventions include the addition of organic
107 matter to increase nutrient levels, retain moisture, and stimulate soil biota (Smith & Read 2010;
108 Muñoz-Rojas et al. 2016; Ngugi et al. 2018). Post-restoration plant establishment and growth

109 is also strongly dependent on symbiotic interactions with fungi (Lunt & Hedger; 2003; Harris
110 2009; Hart et al 2019). Common European species such as *Calluna vulgaris* and *Erica tetralix*
111 have evolved a close association with ericoid mycorrhizal fungi (ErMF), most notably
112 *Hyaloscypha hepaticicola* (syn. *Rhizocyphus ericae*) and *Oidiodendron maius* (Fehrer et al
113 2019). ErMF provide the plant with organic N and P, in exchange for photosynthetic carbon
114 (Vohník 2020), and play an important role in the exclusion of toxic metals common in mine
115 waste (Bradley et al., 1982; Read, 1983). Their pivotal role in facilitating ericaceous plant
116 establishment led to the development of commercial ErMF inoculates for commercial
117 cultivation of soft fruits (i.e. *Vaccinium* sp; Koron & Gogala, 2000; Vohník et al 2012), but
118 due to the difficulty associated with large-scale ErMF culture, application to heathland
119 restoration after mining is not widespread (Quoreshi, 2008). Indeed, we are unaware of any
120 previous attempt to assess the viability of commercial ErMF (in combination with soil
121 amelioration and seeding techniques) compatible with industrial-scale heathland restoration
122 projects.

123 The aim of this study was to determine whether the addition of organic matter and
124 commercially available ErMF to stored overburden influenced establishment of heathland
125 species. To do this we combined (1) a greenhouse experiment focussed on the dominant heather
126 (*Calluna vulgaris*) and (2) a commercial-scale field trial established on kaolinite overburden
127 where the specific goal was to re-establish an ALH community from seed. In so doing, we were
128 able to determine how well a logistically intensive field trial corroborated the results generated
129 by a small greenhouse experiment.

130

131 **Methods**

132 *Study System*

133 Atlantic Lowland Heath is typically dominated by ericaceous plants (*Calluna vulgaris* and
134 *Erica* species – nomenclature follows Stace, 2010), with an associated shrubby Fabaceae (i.e.
135 *Ulex* sp. and other Genisteae species) and distinctive graminoids (e.g. *Molinia caerulea*)
136 community growing on low nutrient, acid soils (Gimingham 1972; Loidi et al. 2010). This
137 vegetation and its often regionally unique component specialist plants supports a host of
138 internationally rare or endangered animal species (Webb et al. 2009). Although present
139 throughout the coastal regions of NW Europe, the habitat is under severe threat from land-use
140 and global change pressures (Fagúndez 2012; Bähring et al. 2017). In SW England, the
141 distribution of ALH regularly coincides with kaolinite deposits, an aluminosilicate mineral
142 produced by in-situ alteration of the plagioclase feldspar component of the granite intrusions
143 throughout the region. Devon is one of the most important global refugia for this habitat with
144 5% of the global total (Devon BAP, 2009). Consequently even though ALH has been
145 designated a conservation priority habitat (JNCC, 2004; Pywell et al. 2011). ALH is under
146 continuing pressure from mineral extraction throughout the region.

147 *Greenhouse experiment*

148 In March 2016, two-hundred *Calluna vulgaris* cuttings were taken from eleven different plants
149 (each displayed new growth and were of a suitable size to withstand tissue loss) located on
150 established ALH at Trendlebere Down, UK (50.3614°N; 03.4432°W). Softwood (new growth)
151 cuttings were taken at the closest growth node (approximately 9 cm from shoot tip), and left in
152 pre-moistened plastic bags to maintain humidity for 24 hours before cultivation in a 50%
153 sand/peat mixture under a mist propagator at 95% humidity to maintain the shoots until root
154 growth took place.

155 The cuttings were grown on until August 2016 in greenhouse conditions (mean daily
156 temperatures were Min = 14.6 ±0.3°C, Max = 25.1 ±0.5°C) and at that point the surviving 140

157 plants were transplanted into four treatments, each with 35 replicates. These were: (i) stored
158 topsoil (control), (ii) stored topsoil with commercial ericoid mycorrhizal fungi (ErMF), (iii)
159 stored topsoil with organic matter (OM) and, (iv) stored topsoil with organic matter and
160 commercial ErMF (OM+ErMF). Although the establishment from parent plants was uneven
161 (four parents yielded only one successful cutting each, others up to 23), we assigned cuttings
162 to each treatment group as equally as possible given these constraints.

163 The organic matter amendment consisted of a 12-week matured green waste compost obtained
164 from Viridor Limited (Taunton, UK), material previously incorporated into artificial soils
165 (Schofield et al., 2018). This material was combined with mineral overburden obtained from
166 Headon China clay works (which had been stored for ~5 years) in an overburden-to-compost
167 ratio of 2:1. Before transplant, rooted *Calluna* cuttings were inoculated with ErMF, by dipping
168 roots into 'Rhodovit' (Symbiom Ltd, Sázava, Czech Republic) a commercially-available
169 mycorrhizal inoculant containing *Oidiodendron maius* and two strains of *Hyaloscypha*
170 *hepaticicola* in nutrient agar broth. The pots were watered to field capacity with rainwater
171 daily.

172 One year later (September 2017) we quantified the number of surviving plants, number of
173 shoots and length of new growth, and number of flowering stalks in each treatment mean (the
174 mean daily temperatures during this period were Max = $19.8 \pm 0.3^\circ\text{C}$, Min = $12.5 \pm 0.2^\circ\text{C}$).
175 After being cleaned of all adhering soil, plant roots were separated from shoots prior to oven
176 drying at 60°C until a constant dry mass was attained and dry weight biomass of roots and
177 shoots established. A sub-sample of the roots were then placed in 3 ml 1M acetic acid for 24
178 hours to clean and rehydrate the roots, before 12 hours immersion in 0.15 ml of Schaffer's
179 black ink to stain any fungi. After washing, we estimated percentage ErMF root inoculation
180 using an Olympus 672110 microscope (400x magnification). Due to low cutting survival in the

181 OM+ErMF treatment group, we assessed the influence of soil treatments on root inoculation
182 in cuttings taken from the same three parent plants.

183 *Field experiment*

184 *Study site and experimental treatments*

185 Located within the Headon China Clay Works (SCR-Sibelco N.V.) near Plymouth, SW
186 England (50.2510°N, 03.5930°W), a 166 m long by 12 m wide SW facing (~30% slope
187 gradient) site situated on a quartz sand waste tip and scheduled for restoration was selected for
188 experimental field trials. Overburden, which had been stored for 5 years on-site, was then
189 spread evenly to a depth of 10 cm. The area was divided into 99 (4 m x 3 m) plots arranged in
190 an 11 x 9 grid pattern, with a 1 m boundary between plots. From these, eleven replicate blocks
191 of nine different treatments were located in a stratified random pattern, such that one replicate
192 treatment was allocated to each row of nine.

193 In October 2016, eight of the nine-treatment groups were seeded with a 173 g commercial seed
194 mixture of heathland species comprised of 34 g each of *Calluna vulgaris*, *Erica cinerea*, *Erica*
195 *tetralix* and *Festuca rubra*, a further 17 g each of *Molinia caerulea* and *Festuca ovina*, and ~3
196 g of *Deschampsia flexuosa* (William Eyre, Bradwell, UK). The plots were broadcast hand-
197 sown to ensure as even a spread of seeds as possible. In addition to the treatment (i) ‘Seeded
198 Control’, which received no further intervention, the following single factor treatments were
199 included:

200 (ii) ‘Fungi’ – 35 ml ‘Rhodovit’ ErMF inoculant was added to the centre 1 m² of each plot.
201 Although subsequent analysis of the vegetation focused on the treated area, we anticipated
202 that successful inoculation would facilitate spread of mycelia beyond.

203 (iii) ‘Metals’ – Cations were added at the following amounts per plot; sodium 123 g (13.50
204 mg/kg), calcium – 111 g (12.5 mg/kg), potassium 324 g (35.40 mg/kg), and magnesium 449

205 g (49.03 mg/kg). These amounts were based on Lane et al., (2020) to increase observed
206 levels in stored overburden to heathland soil concentrations reported by Clarke (1997).
207 Applied in pellet form (Thompson and Morgan, Suffolk, UK), the cations were mixed and
208 broadcast by hand to ensure an even spread.

209 (iv) 'Organic matter' - Having first removed the top 20 mm (~150 kg) of overburden from
210 each plot, 150 kg of Viridor green waste compost was even spread and remixed to a depth
211 of ~100 mm).

212 In addition, the following mixed treatment combinations were employed:

213 (v) 'Fungi' & 'Metals', (vi) 'Organic matter' & 'Fungi', (vii) 'Organic matter' & 'Metals', and
214 (viii) 'Organic matter', 'Fungi' & 'Metals'. The ninth treatment was an unseeded, untreated
215 Control where plots were exposed only to colonisation by windblown or soil-derived
216 propagules.

217 *Soil sampling and analysis*

218 In June 2017, a 10 cm soil core (Eijkelkamp Soil & Water, Gisbeek, The Netherlands) sample
219 was taken from the south-west corner of each plot. The sample was subsequently dried in a
220 desiccator at 60 °C, disaggregated, sieved (2 mm mesh) and stored prior to analysis. To
221 measure pH, 10 g of soil in 50 ml deionised water was mixed for 15 minutes with a
222 magnetic stirrer. It was left to settle and quantified using a Hanna 991001 pH and temperature
223 probe (Jones Jr, 2001). Mineral elements were extracted using the Mehlich III method (Jones
224 Jr, 2001), whereby an extraction solution (30 ml) was added to each soil sample (3 g) in
225 centrifuge tubes and mixed on a reciprocating mechanical shaker at 200 rpm for 5 minutes.
226 Samples were subsequently filtered through Whatman 42 filter paper, and the filtrate
227 retained in the dark until analysis. The Na, K, Mg, Ca and P concentrations in the extracted
228 solution was analysed using a Thermo Scientific iCAP7400 ICP-OES instrument; C, H and

229 N were analysed using an elemental microanalysis EA1110 CHN analyser. For the following
230 tests, three sub samples from bulked treatment samples were analysed due to cost. The soil
231 samples (~10 mg) and Peat Standard Soil (~3 mg) were weighed into 6 x 4 mm high purity tin
232 sample pots. These were gently crushed to exclude atmospheric nitrogen. The samples were
233 flash combusted in an oxygen-rich environment and oxidation products measured by a thermal
234 conductivity detector in a column maintained at 65 °C. To measure cation exchange capacity,
235 30 mL 1M sodium acetate was added to 5 g soil samples before mixing for 5 mins at 180 rpm
236 in a reciprocal mixer and centrifuge for 2 mins at 3,500 rpm. The solution was then discarded.
237 This process was repeated once with sodium acetate, then twice again with IMS. Thirty
238 millilitres of 1M ammonium acetate was added to the soil and mixed for 15 mins at 180 rpm
239 in a reciprocal mixer. The sample was centrifuged, and the supernatant diluted and analysed in
240 a flame photometer. Cation exchange capacity (CEC) was calculated using the equation given
241 by Jones (2001).

242 *Vegetation sampling and statistical analysis*

243 In June 2019, plant cover was estimated for each component species in the entire (4×3 m) area
244 of each plot, with the number of individual dwarf ericaceous plants counted in the 1 m² centre
245 portion. By the end of the experiment, several squares were lost due to commercial operations
246 leaving only eight replicates per treatment included in the final samples. Analysis of the cover
247 data was performed in three dimensions using metaMDS and ordiellipse to highlight groupings
248 in the ‘vegan’ (Oksanen, 2015) package in ‘R’ v.3.5.2. Once the communities were plotted
249 onto an ordination plot, the physical characteristics of the soil were overlaid as vectors (for
250 variables where $P \leq 0.05$) to facilitate identification of how physical factors varied with, and
251 influenced development of, the various communities. An ANOSIM was performed in the
252 ‘vegan’ (Oksanen, 2015) package in ‘R’ v.3.5.2 to examine variation in plant community
253 composition between restoration treatments.

254 To examine the effect of treatment on number of ericaceous plants growing in the 1m² centre
255 portion, we applied a two-step approach. First, ANOVA was used to test the effect of the seeded
256 treatments versus the unseeded control was tested across all plots. This was done for *E. tetralix*
257 and *C. vulgaris* separately. Next, ANOVA was performed with minimum adequate models
258 (MAMs). These models were constructed following the iterative procedure outlined by
259 Crawley (2014). This was done including a block factor (i.e. eleven blocks each consisting of
260 all nine treatment plots). In the iterative procedure, first the full factorial model was
261 constructed. The least significant terms were removed from the model, removing insignificant
262 highest order interactions (three-way interaction) first, then second-order terms and so on with
263 the residual standard error examined at each stage of the procedure. Final models were selected
264 when the removal of any factors notably increased the residual standard error. These MAMs
265 tested the effect of treatment (organic matter, fungi, metals, block and all interactions) on the
266 number of *E. tetralix* and *C. vulgaris* as well as concentrations of P, K, Mg, Ca and pH. All
267 analyses were performed in the R studio environment (R core team, 2017).

268 **Results**

269 *Greenhouse experiment*

270 Although long-term heather cutting survival was low (fewer than one third of plants survived
271 to harvest), OM addition had an additional negative impact (Table 1). Moreover, in addition to
272 the fact that compost addition reduced cutting survival to less than a quarter seen in the control,
273 ErMF supplied in isolation or mixture with OM also failed to influence heather survival. For
274 the cuttings that survived, only one intervention (compost addition) had any marked effect on
275 plant growth or flowering, and this was restricted to enhanced shoot biomass in the few
276 surviving plants in single or mixed OM + ErMF treatments. Interestingly, although plants
277 initially exposed to ErMF displayed the expected increase in root inoculation (Table 1),

278 cuttings in the ‘control’ and OM treatments also exhibited substantial root colonisation by
279 fungi. This result might however, highlight a possible limitation in the use of microscopy to
280 distinguish between ErMF and other fungi (Vohník 2020).

281 *Field Experiment*

282 *Plant community composition*

283 Multivariate analysis highlighted a major influence of OM addition on plant community
284 composition (ANOSIM = 0.2531, $p < 0.001$). Specifically, all four OM treatments were
285 clustered (‘top right’ in the nMDS plot - Figure 1) and separate from all other treatment groups,
286 and positively associated with increases in soil pH, CEC and macro- and micro –nutrients.
287 Nonetheless, OM did not promote the establishment of a typical heathland community.
288 Although all four OM treatments were clustered around one common heathland species, the
289 shrub *Ulex europaeus* (Figure 1), acidic or mesotrophic grasses, including *Agrostis stolonifera*
290 and *Festuca* sp., dominated OM plots (Figure 1 and Table 2. It was striking also, that the
291 successful establishment of these grasses together with gorse (*Ulex europaeus*) accounted for
292 the paucity of bare ground in all OM treatment groups (see Table 2), while all other
293 interventions and control plots consistently had at least 25% bare ground. Also remarkable was
294 that despite colonisation by grass and shrubs not included in the original seed mix, recruitment
295 of forb species, even those commonly encountered in acidic heathland soils was exceptionally
296 low.

297 None of the interventions facilitated widespread establishment of the ericaceous species that
298 typify ALH communities; all plant community clusters in the control and ‘OM’, ‘Metal’ and
299 ‘Fungi’ treatments were noticeably disjunct from these target species in the nMDS analysis
300 (Figure 1). At best, target ericaceous species achieved only 1.5% cover in the ‘seeded control’
301 treatment (Table 2). Moreover, the number of *Calluna* and *Erica* sp. individuals recorded in

302 any central 1m² plot 32 months after any intervention was imposed either did not vary from, or
303 in the case of all OM treatments was much reduced in comparison with, the seeded control
304 (Figure 2). These patterns were corroborated by ANOVA and MAM analysis. There was no
305 significant effect of seeded vs unseeded treatments, block, or their interaction on *E. tetralix*
306 (seeded treatment $F_1=0.549$, $p = 0.461$, block $F_{10} = 1.479$, $p = 0.164$, interaction $F_{10} = 0.732$, p
307 $= 0.692$, residual d.f. = 77), or *Calluna vulgaris* (seeded treatment $F_1=0.379$, $p = 0.540$, block
308 $F_{10} = 1.598$, $p = 0.123$, interaction $F_{10} = 0.825$, $p = 0.606$, residual df = 77) abundance.
309 Moreover, addition of OM was associated with a reduction in *E. tetralix* abundance, as
310 illustrated by a model that included the block term and the interaction of OM and block (OM
311 $F_1 = 10.2$, $P = 0.002$, Block $F_{10} = 1.80$, $p = 0.074$, OM \times block $F_{10} = 1.41$, $p = 0.19$, residual
312 df = 76). Similarly, the number of *C. vulgaris* plants was markedly lower in OM plots (OM F_1
313 $= 9.51$, $p = 0.002$, Block $F_{10} = 1.68$, $p = 0.097$, residual df = 87).

314 *Soil Properties*

315 The addition of organic matter increased soil pH compared to untreated controls (Table 3),
316 although there were inconsistent differences between OM treatments and all other interventions
317 (see ANOVA and MAM results in Supplementary Data File). Soil concentrations of N, P, K,
318 Mg and Ca were generally elevated in one or more of the OM treatments, but it was noteworthy
319 that this effect was variable amongst the four OM plots. The MAM for potassium concentration
320 included OM, metals, fungi and several interaction terms (Supplementary Data File) with
321 results supporting the trend for increased soil K in plots with added OM and ‘metals’, but
322 reduced in the fungal treatment (Supplementary Data File). For soil P, OM, metals, fungi, the
323 block term and several interaction terms were significant, denoting an increase in the OM and
324 metals treatments, but decline in the fungal treatment (Supplementary Data File). Nonetheless,
325 although macronutrient concentrations tended to increase with OM application (including a
326 three-to-four-fold increase in soil N, P and K between control and at least one ‘OM’ treatment),

327 no comparative increase was apparent for at least one of the ‘OM’/’ErMF’/’Metal’ combination
328 treatments (Table 3). Of the two micro-nutrients considered, the significant OM, metals, fungi,
329 block term and several interaction terms corroborated an increase in mean soil calcium in OM
330 and ‘metals’ treated plots, but reduced in the ‘fungal’ plots (The ANOVA and MAM model
331 also included the near-significant interaction term (‘OM × metals × block’) - see
332 Supplementary Data File). For magnesium, significant metals, fungi, block and several
333 interaction terms highlighted elevated concentrations in OM and metals plots, but reduced
334 concentrations in the fungal treatment (Supplementary Data File).

335

336 **Discussion**

337 We found no evidence in greenhouse or field trials that any of the restoration interventions
338 applied had a positive influence over heathland ericaceous species establishment. This failure
339 was most striking for our large-scale, field trial where our various manipulations of stored
340 overburden had either no, or even negative, effects on the establishment of heathland
341 ericaceous species. Moreover, *Calluna* and *Erica* spp. seedling density was consistently low
342 ($<5 \text{ m}^{-2}$) in all seeded field plots, suggesting that propagule limitation was not the sole factor
343 limiting heathland establishment on newly reinstated overburden. The remarkably high
344 mortality of *Calluna* plants in the greenhouse experiment, coupled with the field experiment
345 where *Calluna* and *Erica tetralix* abundance in all OM plots was much reduced in comparison
346 with all other interventions, strongly suggests that our OM treatment had especially marked
347 negative effects on ericaceous species establishment.

348 There are at least two plausible mechanisms to explain these results. First, and perhaps most
349 pertinent to the greenhouse experiment, OM addition in relatively high humidity might have
350 promoted conditions suitable for the spread of harmful fungi including saprotrophic

351 basidiomycetes that can detrimentally affect mycorrhizal fungi (Shaw et al. 1995; Leake et al.
352 2001). Shaw (2019) also reported how the addition of OM to mine spoil led to the ‘damping-
353 off’ of heathland seedlings as a fungal pathogen killed young plants on waterlogged soils.
354 Consequently, we cannot rule out the possibility that OM addition promoted antagonistic fungi
355 in the field trials. Second, increases in soil macro- and micro-nutrients in field plots, coupled
356 with an increase in soil pH, may have facilitated the rapid establishment of dominant grasses
357 (e.g. *Agrostis* and *Festuca* species) that outcompeted emerging *Calluna* and *Erica* spp.
358 seedlings following OM addition (see Green et al 2015; Tibbett et al 2019; Lane et al 2020).

359 Although some studies (Smith & Read 2010; Wubs et al. 2018; Radujkovi et al. 2020) have
360 suggested that an absence of ErMF can be a limiting factor on ericaceous species recruitment
361 and persistence, we found little evidence that heather establishment or growth benefitted from
362 the addition of one of the few commercially available ErMF sources (‘Rhodovit’). When
363 supplied in isolation or mixture with OM, in the greenhouse trial, ErMF failed to influence
364 *Calluna* survival, growth or flowering. Similarly in the field, emergent plant communities in
365 the ‘Fungi’ or ‘Fungi + Metals’ plots, dominated by bare ground, *Ulex europaeus* and various
366 Graminoids, differed little to those seen in ‘Seeded Controls’. The same was true for *Calluna*
367 and *Erica tetralix* abundance in the central 1 m² portion of each plot (where ErMF was
368 originally applied). Whether our failure stems from the method/ErMF used, the timing of
369 application (Radujkovi et al. (2020) showed that it may take several years to attain levels of
370 ErMF infection equivalent to that of undisturbed soils), and/or stochastic environmental
371 conditions limiting the ericaceous/ErMF interaction is unclear. Nonetheless, our experiments
372 with one of the few commercially available ErMF inoculants do not evidence any consistent
373 benefit to post-mine heathland restoration.

374 It is also apparent from the field experiment that supplementation of some of the various cations
375 thought to limit heathland establishment (Coppin & Bradshaw 1982; Clarke 1993, 1997; Lane

376 et al 2020), had no impact on heath species recruitment or growth. As with the ‘Fungi’ and
377 ‘Fungi + Metals’ treatments, the ‘Metals’ plots were similar to the ‘Seeded Controls’ in being
378 dominated by bare ground, *Ulex europaeus* and Graminoids, while addition of metal cations
379 (alone or in combination with other treatments), had no effect on *Calluna* or *Erica* spp.
380 abundance. The fact that within 9 months of application soil Mg, K and Ca concentrations had
381 declined to levels similar to those in control and other ‘non-metals’ plots, may indicate that
382 winter rain quickly leached these cations from the unvegetated soils (see Duddigan et al. 2020).
383 The only exception was where OM was also added alongside the supplemented cations,
384 suggesting that soil organic content may play a role in nutrient retention as well as provision.

385 Despite our failure to facilitate ericaceous species establishment in greenhouse or field trials,
386 our study offers a number of informative considerations for future research. First, we caution
387 against the supposition that restoration of lost heathland is easily achieved given appropriate
388 management or time. Neither this assumption, nor acceptance that ALH can be replaced with
389 a gorse/mesic grassland sward, should be used to ‘greenwash’ the planning approval process
390 for mine operations (see Firth et al 2020). Second, the fact that ericaceous species establishment
391 was consistently poor in both greenhouse and field trials underscored the value of the former
392 in informing the latter. We strongly recommend therefore, that investigation of putative
393 heathland restoration techniques utilize a comprehensive programme of greenhouse trials
394 before embarking on logistically demanding field experiments. In that vein however, our final
395 recommendation is that despite our results, restoration ecologists and practitioners continue to
396 examine the role of ErMF and OM in post-mine heathland rehabilitation. Specifically, we
397 propose that modification of the OM type used, along with more targeted ErMF inoculation of
398 ‘nursemaid’ plants, including appropriate controls containing killed inoculum and careful
399 design to create conditions suitable for mycorrhizal interactions to develop, might yet offer a
400 way to help facilitate ericaceous species establishment on former mine overburden.

401 When compared with garden waste, municipal compost, of the kind we applied tends to be high
402 in available N and P, but with a neutral pH (see Schofield et al. 2018). The use of OM
403 manipulations dominated by acidic, carbon-based lignin and tannin sources may prove more
404 effective in promoting heathland restoration. Although perhaps not available in the quantities
405 needed for effective wholesale overburden amelioration in large mine restoration projects, a
406 substrate of pine litter, bark and wood chips was shown by Vohník et al (2012) to be effective
407 in facilitating highbush blueberry (*Vaccinium*) growth. Not only does the material offer the low
408 pH demanded by ericaceous species, it can also facilitate the establishment of lignin degrading
409 (and ErMF compatible) basidiomycetes that enhance ericaceous plant growth via the release of
410 nutrients from lignin-rich plant residues (Vohník et al 2012). Highly lignified, low pH litter
411 will likely also degrade slowly enough to limit nutrient release to non-target species (e.g.
412 competitive grasses) to the long-term benefit of ericaceous species establishment. We suggest
413 that future research focus using low pH, lignified litter sources in tandem with ErMF inoculants
414 in greenhouse trials before ‘scaling-up’ to field application. Where quantities of litter sources
415 are limiting, one worthwhile approach may be to plant established target ericaceous species
416 cultivated on low pH lignified litter into mine rehabilitation sites in order to ‘seed’ suitable
417 ErMF and other beneficial soil micro-organisms into surrounding overburden. As the largest
418 field trial of its kind yet performed, our experiment uniquely shows the difficulty associated
419 with heathland reestablishment in post-mining scenarios. Nonetheless, interventions to reduce
420 soil pH and nutrients to limit establishment of competitive non-target species, but facilitate
421 ericaceous species regeneration, may nonetheless be achievable at commercial scales.

422

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428

429 **References**

- 430 Allison M, Ausden M (2004) Successful use of topsoil removal and soil amelioration to create
431 heathland vegetation. *Biological Conservation* 120: 221–228
- 432 Bähring A, Fichtner A, Ibe K, Schütze G, Temperton VM, von Oheimb G, Härdtle W (2017)
433 Ecosystem functions as indicators for heathland responses to nitrogen fertilization.
434 *Ecological Indicators* 72: 185-193
- 435 Bateman A, Lewandrowski W, Stevens J, Muñoz-Rojas M (2016) Ecophysiological indicators
436 to assess drought responses of arid zone native seedlings in reconstructed soils. *Land*
437 *Degradation & Development* 29:984-993
- 438 Benigno SM, Dixon KW, Stevens J (2013) Increasing soil water retention with native-sourced
439 mulch improves seedling establishment in postmine Mediterranean sandy soils.
440 *Restoration Ecology* 21: 617–626
- 441 Bradley R, Burt AJ, and Read DJ (1982) The biology of mycorrhiza in the ericaceae .8. The
442 role of mycorrhizal infection in heavy-metal resistance. *New Phytologist* 91: 197-209.
- 443 Clarke KR (1993) Non-parametric multivariate analyses of changes in community structure.
444 *Australian Journal of Ecology* 18:117-143
- 445 Clarke CT (1997) Role of soils in determining sites for lowland heathland reconstruction in
446 England. *Restoration Ecology* 5:256-264
- 447 Clemente AS, Moedas AR, Oliveira G, Martins-Loução MA, Correia O (2016) Effect of
448 hydroseeding components on the germination of Mediterranean native plant species.
449 *Journal of Arid Environments* 125: 68-72
- 450 Cooke SJ, Bennet JR, Jones HP (2019) We have a long way to go if we want to realize the
451 promise of the “decade on ecosystem restoration”. *Conservation Science and Practice*
452 2019: e129
- 453 Coppin NJ, Bradshaw AD (1982) The Establishment of Vegetation in Quarries and Open-pit
454 Non-metal Mines. *Mining Journal Books: London*; 112

455 Crawley MJ (2014) *Statistics: An introduction using R*. John Wiley & Sons Ltd. Chichester,
456 UK

457 Devon, CC. (2009) *Devon Biodiversity and Geodiversity Action Plan*. Devon County Council.
458 Devon

459 Diaz A, Green I, Benvenuto M, Tibbett M (2006) Are Ericoid Mycorrhizas a Factor in the
460 Success of *Calluna vulgaris* Heathland Restoration? *Restoration Ecology* 14:187-195

461 Diaz A, Green I, Tibbett M (2008) Re-creation of heathland on improved pasture using top soil
462 removal and sulphur amendments: edaphic drivers and impacts on ericoid mycorrhizas.
463 *Biological Conservation* 141: 1628–1635

464 Fagúndez, J (2012) Heathlands confronting global change: drivers of biodiversity loss from
465 past to future scenarios. *Annals of Botany* 111: 51-172

466 De Graaf MC, Bobbink R, Verbeek PJ, Roelofs JG (1997) Aluminium toxicity and tolerance
467 in three heathland species. *Water Air Soil Pollution* 98: 229–239

468 Duddigan S Gil-Martínez M, Fraser T, Green I, Diaz A, Sizmur T, Pawlett M, Raulund-
469 Rasmussen K, Tibbett M (2020) Evaluating heathland restoration belowground using
470 different quality indices of soil chemical and biological properties. *Agronomy* 10: 1140

471 Fehrer J, Réblová M, Bambasová V, Vohník M (2019) The root symbiotic *Rhizoscyphus ericae*
472 aggregate and *Hyaloscypha* (Leotiomycetes) are congeneric: Phylogenetic and
473 experimental evidence. *Studies in Mycology* 92: 195–225

474 Firth LB, Airoidi L, Bulleri F, Challinor S, Chee S-Y, et al (2020) Greening the grey: making
475 space for nature in the built environment should not be used as a Trojan horse to
476 facilitate coastal development. *Journal of Applied Ecology* 57: 1762-1768

477 Gimingham, CH (1972) *Ecology of heathlands*. Chapman & Hall, London, UK.

478 Glen E, Price EAC, Caporn SJM, et al (2017) Evaluation of topsoil inversion in UK habitat
479 creation and restoration schemes. *Restoration Ecology* 25: 72-81

480 Golos PJ, Dixon KW, Erickson TE (2016) Plant recruitment from the soil seed bank depends
481 on topsoil stockpile age, height, and storage history in an arid environment. *Restoration*
482 *Ecology* 24: 53-61

483 Green I, Evans D, Diaz A (2015) Modifying soil chemistry to enhance heathland recreation: a
484 use for sulphur captured during oil refining. *International Journal of Plant and Soil*
485 *Science* 6: 272-282

486 Grime JP, Hodgson JG, Hunt R. (2007) *Comparative Plant Ecology* 2nd Edn. Dalbeattie,
487 Scotland, Castlepoint Press

488 Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers?
489 Science 325: 573-574

490 Hart M, Gorzelak MA, McAmmond BM, Van Hamme JD, Stevens J, Abbott L, Whiteley AS,
491 Nevill P (2019) Fungal communities resist recovery in sand mine restoration. Frontiers
492 in Forests and Global Change 2: 78

493 Hester AJ, Gimingham CH, Miles J (1991) Succession from heather moorland to birch
494 woodland. III. Seed availability, germination and early growth. Journal of Ecology
495 79: 329-344

496 Hobbs RJ, Harris JA (2001) Restoration ecology: repairing the Earth's ecosystems in the new
497 millennium. Restoration Ecology 9: 239– 246

498 Holl KD, Aide TM (2011) When and where to actively restore ecosystems? Forest Ecology
499 and Management 261: 1558-1563

500 Holmes PM (2001) Shrubland restoration following woody alien invasion and mining: effects
501 of topsoil depth, seed source, and fertilizer addition. Restoration Ecology 9: 71-84.

502 JNCC, (2004) Common Standards Monitoring Guidance for Lowland heath, Version August
503 2004, ISSN 1743-8160.

504 Jones Jr JB. (2001) Laboratory guide for conducting soil tests and plant analysis. CRC press,
505 United States

506 Koron D and Gogala N (2000) The use of mycorrhizal fungi in the growing of blueberry
507 plants (*Vaccinium corymbosum* L.). Acta Horticulturae 525: 101-106

508 Lane M, Hanley ME, Lunt P, Knight ME, Braungardt CB, Ellis JS (2019) Chronosequence of
509 former kaolinite open cast mines suggests active intervention is required for the
510 restoration of Atlantic heathland. Restoration Ecology 28: 661-667

511 Leake JR, Donnelly DP, Saunders EM, Boddy L, Read DJ (2001) Rates and quantities of
512 carbon flux to ectomycorrhizal mycelium following ¹⁴C pulse labelling of *Pinus*
513 *sylvestris* seedlings: effects of litter patches and interaction with a wood-decomposing
514 fungus. Tree Physiology 21: 71–82

515 Loidi J, Biurrun I, Campos JA, García-Mijangos I, Herrera M (2010) A biogeographical
516 analysis of the European Atlantic lowland heathlands. Journal of Vegetation Science
517 21: 832-842

518 Lunt PH and Hedger JN (2003) Effects of organic enrichment of mine spoil on growth and
519 nutrient uptake in oak seedlings inoculated with selected ectomycorrhizal fungi.
520 Restoration Ecology 11: 125-130

- 521 Machado NDM, Leite MGP, Figueiredo MA, Kozovits AR (2013) Growing *Eremanthus*
522 *erythopappus* in crushed laterite: a promising alternative to topsoil for bauxite-mine
523 revegetation. *Journal of Environmental Management* 129:149-156
- 524 Marrs RH, Snow CSR, Owen KM, Evans CE (1998) Heathlands and acid grassland creation
525 on arable soils at Minsmere: Identification of potential problems and a test of cropping
526 to impoverish soils. *Biological Conservation* 85:69-82
- 527 Merino-Martín L, Commander L, Mao Z, Stevens JC, Miller BP, Golos PJ, Mayence CE,
528 Dixon K (2017) Overcoming topsoil deficits in restoration of semiarid lands: Designing
529 hydrologically favourable soil covers for seedling emergence. *Ecological Engineering*
530 105: 102-117
- 531 Muñoz-Rojas M, Erickson TE, Dixon KW, Merritt DJ (2016) Soil quality indicators to assess
532 functionality of restored soils in degraded semiarid ecosystems. *Restoration Ecology*
533 24: 43–52
- 534 Ngugi MR, Dennis PG, Neldner VJ, Doley D, Fechner N, McElnea A (2018) Open-cut mining
535 impacts on soil abiotic and bacterial community properties as shown by restoration
536 chronosequence. *Restoration Ecology* 26: 839-850
- 537 Oksanen J (2015) Unconstrained Ordination: Tutorial with R and vegan. R package version
538 2.3-4
- 539 Quoreshi AM (2008) The use of mycorrhizal biotechnology in restoration of disturbed
540 ecosystem. In: *Mycorrhizae: Sustainable Agriculture and Forestry*, Z.A. Siddiqui, M.S.
541 Akhtar, K. Futai (Eds.) Springer, Dordrecht. pp. 303-320
- 542 Prach K, Tolvanen A (2016) How can we restore biodiversity and ecosystem services in mining
543 and industrial sites? *Environmental Science and Pollution Research* 23: 13587–13590
- 544 Prach K, Řehouňková K, Lencová K, Jírová A, Konvalinková P, Mudrák O, Študent V,
545 Vaněček Z, Tich L, Petřík P, Šmilauer P, Pyšek P (2014) Vegetation succession in
546 restoration of disturbed sites in Central Europe: the direction of succession and species
547 richness across 19 seres. *Applied Vegetation Science* 17: 193-200
- 548 Pywell RF, Meek WR, Webb NR, Putwain PD, Bullock JM (2011) Long-tErMF heathland
549 restoration on former grassland: The results of a 17-year experiment. *Biological*
550 *Conservation* 144: 1602-1609
- 551 R Core Team (2017). R: A language and environment for statistical computing. R Foundation
552 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- 553 Radujkovic D, van Diggelen R, Bobbink R, Weijters M, Harris J, Pawlett M, Vicca S,
554 Verbruggen E. (2020) Initial soil community drives heathland fungal community

555 trajectory over multiple years through altered plant–soil interactions. *New Phytologist*
556 225: 2140–2151

557 Read DJ (1983) The biology of mycorrhiza in the Ericales. *Canadian Journal of Botany* 61:
558 985–1004

559 Roem WJ, Klees H, Berendse F (2002) Effects of nutrient addition and acidification on plant
560 species diversity and seed germination in heathland. *Journal of Applied Ecology* 39:
561 937–948

562 Rokich DP, Dixon KW, Sivasithamparam K, Meney KA (2000) Topsoil handling and storage
563 effects on woodland restoration in Western Australia. *Restoration Ecology* 8: 196–208

564 Salgueiro PA, Prach K, Branquinho C, Mira A (2020) Enhancing biodiversity and ecosystem
565 services in quarry restoration – challenges, strategies, and practice. *Restoration Ecology*
566 28: 655–660

567 Schofield HK, Pettitt TR, Tappin AD, Rollinson GK, Fitzsimons MF (2018) Does carbon
568 limitation reduce nitrogen retention in soil? *Environmental Chemistry Letters* 16: 623–
569 630

570 Shaw CJ (2019) Damping-off within natural and disturbed kwongan plant communities. PhD
571 thesis, Murdoch University, Australia
572 <https://researchrepository.murdoch.edu.au/id/eprint/56892>

573 Shaw TM, Dighton J, Sanders FE (1995) Interactions between ectomycorrhizal and
574 saprotrophic fungi on agar and in association with seedlings of lodgepole pine (*Pinus*
575 *contora*). *Mycological Research* 99: 159–165

576 Stace C (2010) *New flora of the British Isles*. Cambridge University Press; UK.

577 Tibbett M, Gil-Martínez M, Fraser T, Green ID, Duddigan S, De Oliveira VH, Raulund-
578 Rasmussen K, Sizmur T, Diaz A (2019) Long-term acidification of pH neutral
579 grasslands affects soil biodiversity, fertility and function in a heathland restoration.
580 *Catena* 180: 401–415

581 Vohník M (2020) Ericoid mycorrhizal symbiosis: theoretical background and methods for its
582 comprehensive investigation. *Mycorrhiza* 30: 671–695

583 Vohník M, Sadowsky JJ, Lukešová T, Albrechtová J, Vosátka M (2012) Inoculation with wood
584 decomposing basidiomycete, but not with root symbiotic ascomycetes, positively
585 affects growth of highbush blueberry (Ericaceae) grown in a pine litter substrate. *Plant*
586 *Soil* 355: 341–352

- 587 Walker KJ, Stevens PA, Stevens DP, Mountford JO, Manchester SJ, Pywell RF (2004a) The
588 restoration and re-creation of species-rich lowland grassland on land formerly managed
589 for intensive agriculture in the UK. *Biological Conservation* 119:1–18
- 590 Walker KJ, Pywell RF, Warman EA, Fowbert JA, Bhogal A, Chambers BJ (2004b) The
591 importance of former land use in determining successful re-creation of lowland heath
592 in southern England. *Biological Conservation* 116:289–303
- 593 Walker KJ, Warman EA, Bhogal A, Cross RB, Pywell RF, Meek BR, Chambers BJ, Pakeman
594 R (2007) Recreation of lowland heathland on ex-arable land: assessing the limiting
595 processes on two sites with contrasting soil fertility and pH. *Journal of Applied Ecology*
596 44:573–582
- 597 Webb JR, Drewitt AL, Measures GH (2009) Managing for species: Integrating the needs of
598 England’s priority species into habitat management. Part 1 report. Natural England
599 Research Reports Number 024, Natural England, Peterborough UK
- 600 Whisenant SG (1999) *Repairing damaged wildlands: a process-oriented, landscape-scale*
601 *approach*. Cambridge University Press, Cambridge
- 602 Wubs ERJ, Melchers PD, Bezemer TM (2018) Potential for synergy in soil inoculation for
603 nature restoration by mixing inocula from different successional stages. *Plant and Soil*
604 433: 147-156
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607 **Table 1.** Summary of the effects of soil additions of organic matter (OM) and ericoid
 608 mycorrhizal fungi (ErMF) on mean (\pm SE) heather (*Calluna vulgaris*) survival, growth,
 609 flowering and proportion of root length colonised by fungi compared to untreated control plants
 610 grown in a 50% sand/peat mixture. Significant ($p < 0.05$) differences located by one-way
 611 ANOVA or Chi-squared tests are denoted by bold font.

612

Response	Control	OM	ErMF	OM & ErMF	Test Stat (df)	p-value
Plant survival (%)	91	23	86	9	$\chi^2_{(3)} = 37.5$	<0.001
Shoot length (mm)	6.8 (± 0.5)	7.8 (± 1.3)	6.8 (± 0.5)	4.9 (± 2.0)	$F_{(3,12)} = 1.418$	0.286
Shoot number	32.8 (± 2.8)	22.7 (± 4.4)	33.2 (± 3.0)	38.5 (± 10.5)	$F_{(3,12)} = 2.392$	0.120
Number of flower spikes	3.9 (± 0.7)	4.6 (± 1.9)	2.8 (± 0.4)	3.3 (± 1.7)	$F_{(3,12)} = 0.701$	0.569
Root biomass (g)	1.1 (± 0.1)	1.3 (± 0.1)	1.4 (± 0.4)	1.6 (± 0.6)	$F_{(3,11)} = 0.106$	0.955
Shoot biomass (g)	0.9 (± 0.1)	2.1 (± 0.1)	0.8 (± 0.3)	1.9 (± 0.4)	$F_{(3,12)} = 10.587$	0.0014
ErMF root colonisation (%)	28.9 (± 2.9)	34.2 (± 4.0)	48.0 (± 2.4)	42.1 (± 4.1)	$F_{(3,2)} = 33.561$	0.027

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616 **Table 2** Effect of different rehabilitation treatments on mean (\pm SE) cover of the most common plants recorded in plots three years after the start of a lowland
617 heath restoration experiment on a kaolinite mine site in Dartmoor, SW England. Key to plant species: *Agrostis curtisii*; Agr sto, *Agrostis stolonifera*; Fest sp.
618 includes *Festuca ovina* & *F. rubra*; Jun eff, *Juncus effusus*: ‘Other’ includes *Agrostis capillaris*, *Deschampsia flexuosa*, *Juncus buffonius*; Cal vul, *Calluna*
619 *vulgaris*; Erica sp, includes *Erica cinerea* & *E. tetralix*; Ule eur, *Ulex europaeus*: ‘Forbs’ included *Rumex acetosella*, *Potentilla erecta*, *Galium saxatile* and
620 *Trifolium pratense*

621

Treatment (Mean \pm SE)	Graminoids					Total	Shrubs			Forbs	Bare Ground
	Agr cur	Agr sto	Fest sp.	Jun eff	Other		Cal vul	Erica sp.	Ule eur		
Control	9.1 (2.2)	1.5 (1.0)	2.4 (1.9)	13.2 (3.9)	0	26.1	0.6 (0.5)	1.1 (0.8)	55.0 (6.7)	0.5	27.7 (7.7)
Seeded control	6.8 (1.5)	6.4 (1.7)	8.2 (3.8)	8.8 (2.9)	1.8 (0.8)	32.4	1.8 (0.9)	2.6 (1.4)	37.7 (6.2)	0.1	30.9 (4.6)
Fungi	4.2 (1.9)	4.5 (1.6)	9.1 (4.0)	12.9 (3.7)	1.4 (0.7)	32.1	0.7 (0.3)	0.4 (0.3)	40.5 (6.9)	0.1	29.5 (5.2)
Fungi + Metals	6.4 (1.5)	10.6 (4.4)	12.8 (4.1)	11.6 (3.2)	0.9 (0.6)	44.5	1.2 (0.6)	1.1 (0.7)	40.8 (6.5)	0	29.5 (4.8)
Metals	4.3 (1.4)	6.4 (1.5)	14.6 (4.6)	12.6 (6)	0.9 (0.6)	38.8	0.9 (0.5)	2.0 (1.0)	33.6 (5.2)	0.8	33.2 (5.6)
Organic Matter	4.1 (2.0)	15.5 (3.5)	37.3 (8.7)	8.4 (2.9)	0.9 (0.6)	67.5	0.2 (0.1)	0.2 (0.2)	44.1 (7.5)	0	0
Organic Matter + Fungi	2.3 (1.2)	19.1 (6.2)	23.1 (6.5)	11.8 (4.7)	1.9 (1.1)	58.7	0.1 (0.1)	0	50.9 (8.5)	0	0
Organic Matter + Metals	2.0 (1.2)	14.5 (3.7)	25.4 (6.0)	15.6 (7.1)	0.0 (0.0)	58.5	0.1 (0.1)	0	47.7 (5.2)	0	0.5 (0.5)
Organic Matter +Fungi + Metals	1.4 (1)	13.6 (2.5)	28.7 (6.2)	6.6 (2.8)	0.9 (0.9)	53.0	0.2 (0.1)	0.2 (0.1)	53.6 (4.9)	0	0

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626 **Table 3:** The effects of soil additions of organic matter ('OM'), ericoid mycorrhizal fungi ('Fungi') and cations ('Metals' - i.e. sodium, calcium,
627 potassium and magnesium) and treatments in combination on various soil properties in a china clay mine site located in SW England. Samples
628 were taken and analysed nine months after initial interventions (October 2016) and the establishment of vegetation following broadcast sowing of
629 typical heathland plant species. CEC – Cation Exchange Capacity. Mean (\pm SE) are reported.
630

Treatment	pH	C (%)	N (%)	P ($\mu\text{g g}^{-1}$)	K ($\mu\text{g g}^{-1}$)	C:N	Mg ($\mu\text{g g}^{-1}$)	Ca ($\mu\text{g g}^{-1}$)	CEC (mEq /100g)
Control	4.8 (0.07)	1.8 (0.06)	0.06 (0.01)	18.4 (3.5)	63.8 (5.3)	31.6 (2.2)	51.2 (6.7)	202.0 (55.1)	8.4 (0.2)
Seeded Control	4.9 (0.07)	2.4 (0.07)	0.09 (0.01)	12.3 (2.2)	38.6 (3.4)	26.1 (1.0)	40.5 (3.6)	116.7 (13.9)	11.2 (0.2)
Fungi	4.8 (0.04)	2.3 (0.08)	0.09 (0.01)	8.6 (1.1)	28.6 (1.9)	26.0 (0.9)	36.7 (4.4)	97.2 (9.5)	9.6 (0.2)
Fungi, Metals	4.8 (0.07)	2.9 (0.4)	0.10 (0.01)	10.2 (1.5)	52.5 (7.0)	29.2 (0.9)	47.4 (4.3)	108.6 (8.8)	10.3 (0.2)
Metals	4.8 (0.1)	2.3 (0.2)	0.08 (0.01)	23.4 (3.4)	94.0 (8.2)	26.7 (0.9)	90.7 (12.9)	181.0 (26.5)	10.6 (0.2)
OM	5.2 (0.1)	3.8 (0.7)	0.17 (0.04)	71.7 (7.3)	163.8 (14.0)	22.4 (0.9)	113.1 (7.5)	644.3 (63.7)	13.0 (0.2)
OM, Fungi	5.4 (0.1)	2.5 (0.07)	0.10 (0.01)	33.3 (7.4)	66.2 (3.8)	23.9 (1.2)	57.1 (5.6)	323.3 (65.5)	11.9 (0.4)
OM, Metals	5.6 (0.15)	2.8 (0.23)	0.14 (0.02)	70.0 (17.91)	90.8 (7.46)	20.5 (1.53)	109.1 (15.74)	675.9 (172.61)	12.5 (0.15)
OM, Fungi, Metals	5.2 (0.07)	2.8 (0.2)	0.12 (0.01)	75.8 (13.6)	193.7 (19.5)	22.9 (1.1)	120.6 (13.9)	647.6 (121.1)	13.6 (0.8)

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632

633 **Figure legends**

634

635 **Figure 1:** nMDS of plant community composition cover three years after eight different post-
636 mine restoration interventions were applied to kaolinite mine spoil in southwest England. Stress
637 =0.09; Ordiellipse illustrates community overlap (a); the vectors illustrate key environmental
638 factors (b) (see Table 2). Soil amelioration treatments were based on the addition of Ericoid
639 mycohorrizal fungi, major plant nutrient cations ('metals') and organic matter singly and in
640 combination (shown in 2 dimensions for ease of visualisation) to plots where seeds of heathland
641 plant species were also added (plus an untreated/unseeded control). Key to treatments: C
642 Control, SC seeded Control, F Fungi, M Metal, OM Organic matter, FM Fungi and metals,
643 OMF Organic matter with fungi, OMM Organic matter with metals, OMMF Organic matter
644 with fungi and metals.

645 Key to plant species: *Agro cap*, *Agrostis capillaris*: *Agro sto*, *Agrostis stolonifera*: *Agro cur*, *Agrostis*
646 *curtisii*: *Desc fle*, *Deschampsia flexuosa*: *Fest ovi*, *Festuca ovina*: *Fest rub*, *Festuca rubra*: *Moli cae*,
647 *Molinia caerulea*: *Call vul*, *Calluna vulgaris*: *Eric tet*, *Erica tetralix*: *Eric cin*, *Erica cinerea*: *Ulex eur*,
648 *Ulex europaeus*: *Rume ace*, *Rumex acetosella*: *Pote ere*, *Potentilla erecta*: *Gali sax*, *Galium saxatile*:
649 *Trif pra*, *Trifolium pratense*: *Junc buf*, *Juncus bufonius*: *Junc eff*, *Juncus effusus*.

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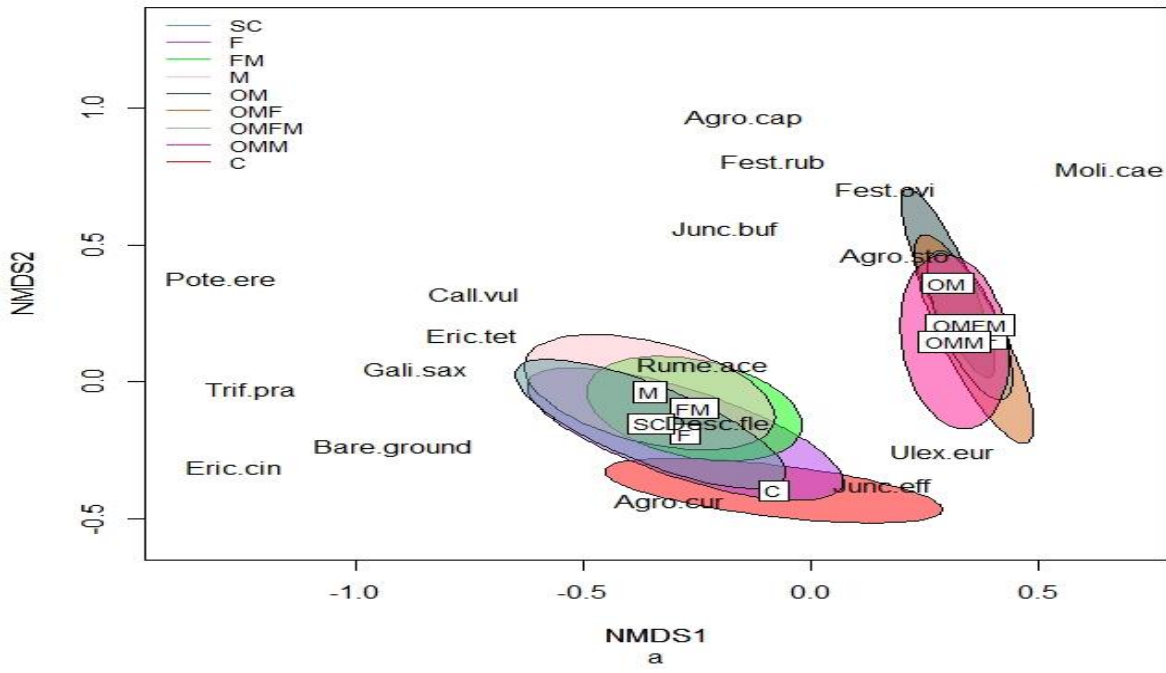
651 **Figure 2:** Influence of soil amelioration treatments on mean (\pm SE) ericoid seedling abundance
652 (*Calluna vulgaris* and *Erica tetralix*) in plots (N = 8) located on former kaolinite mine spoil in
653 SW England. Treatments were based on the addition of Ericoid mycorrhizal fungi (ErMF),
654 major plant nutrient cations (Metal) and organic matter (OM) singly and in combination to
655 plots where seeds of heathland plant species were also added (plus additional
656 untreated/unseeded and untreated/seeded controls). Two *Erica cinerea* seedlings were
657 additional recorded in 'Control' plots.

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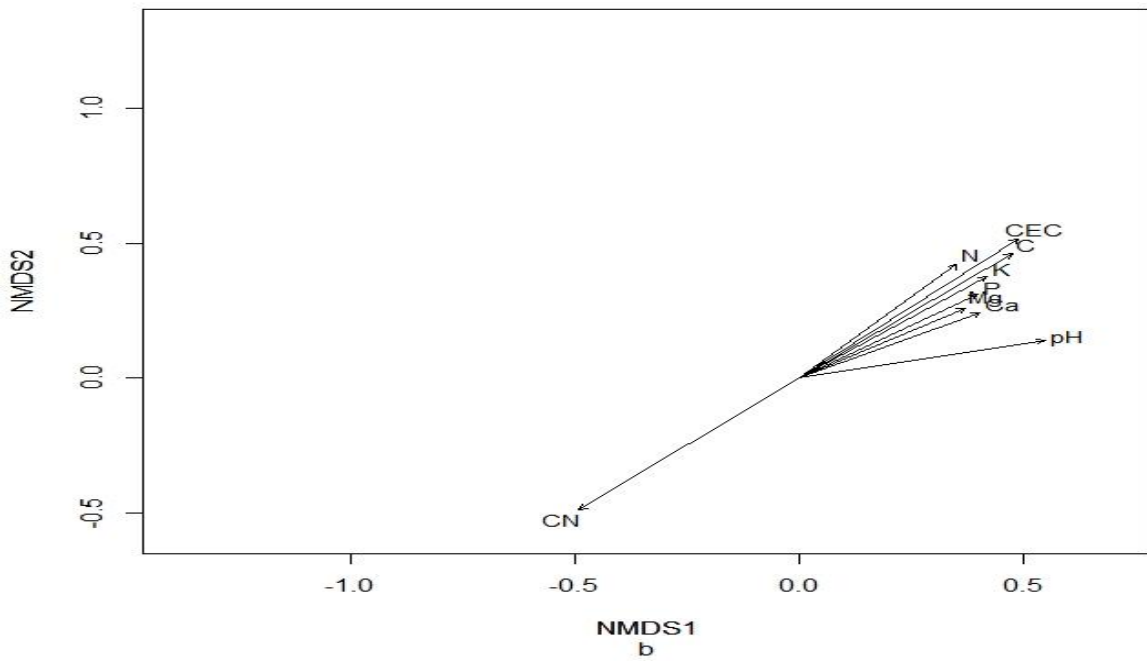
659

660 **Figure 1**

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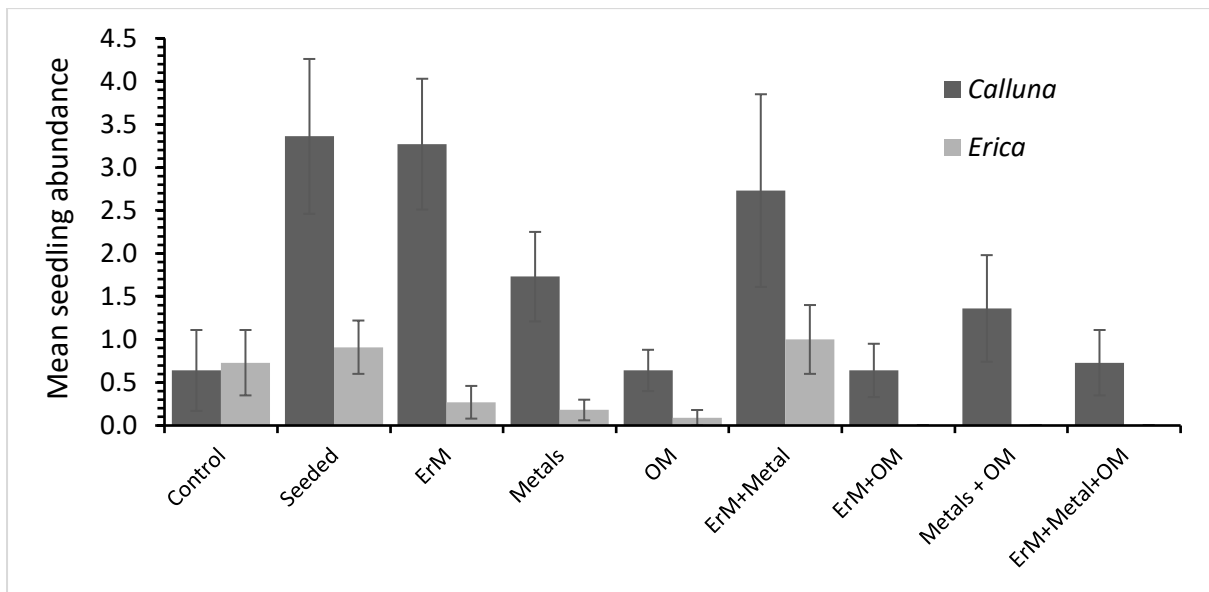
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668 **Figure 2**

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