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

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Abstract

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

Implications for practice

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

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

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

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

Introduction

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

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

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

Studies such as Lane et al (2020) underscore the need for active soil management as a basis to facilitate successful (heathland) restoration (see also Holmes. 2001: Benigno et al. 2013; Clemente et al. 2016; Glen et al. 2017). These interventions include the addition of organic matter to increase nutrient levels, retain moisture, and stimulate soil biota (Smith & Read 2010; Muñoz-Rojas et al. 2016; Ngugi et al. 2018). Post-restoration plant establishment and growth is also strongly dependent on symbiotic interactions with fungi (Lunt & Hedger; 2003; Harris 2009; Hart et al 2019). Common European species such as *Calluna vulgaris* and *Erica tetralix* have evolved a close association with ericoid mycorrhizal fungi (ErMF), most notably *Hyaloscypha hepaticicola* (syn. *Rhizocyphus ericae*) and *Oidiodendron maius* (Fehrer et al 2019). ErMF provide the plant with organic N and P, in exchange for photosynthetic carbon (Vohník 2020), and play an important role in the exclusion of toxic metals common in mine waste (Bradley et al., 1982; Read, 1983). Their pivotal role in facilitating ericaceous plant establishment led to the development of commercial ErMF inoculates for commercial cultivation of soft fruits (i.e. *Vaccinium* sp; Koron & Gogala, 2000; Vohník et al 2012), but due to the difficulty associated with large-scale ErMF culture, application to heathland restoration after mining is not widespread (Quoreshi, 2008). Indeed, we are unaware of any previous attempt to assess the viability of commercial ErMF (in combination with soil amelioration and seeding techniques) compatible with industrial-scale heathland restoration projects.

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

Methods

Study System

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

Greenhouse experiment

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

The cuttings were grown on until August 2016 in greenhouse conditions (mean daily temperatures were Min = 14.6 ±0.3°C, Max = 25.1 ±0.5°C) and at that point the surviving 140 plants were transplanted into four treatments, each with 35 replicates. These were: (i) stored topsoil (control), (ii) stored topsoil with commercial ericoid mycorrhizal fungi (ErMF), (iii) stored topsoil with organic matter (OM) and, (iv) stored topsoil with organic matter and commercial ErMF (OM+ErMF). Although the establishment from parent plants was uneven (four parents yielded only one successful cutting each, others up to 23), we assigned cuttings to each treatment group as equally as possible given these constraints.

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

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

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

Field experiment

Study site and experimental treatments

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

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

(ii) ‘Fungi’ – 35 ml ‘Rhodovit’ ErMF inoculant was added to the centre 1 m^2 of each plot. Although subsequent analysis of the vegetation focused on the treated area, we anticipated that successful inoculation would facilitate spread of mycelia beyond.

(iii) ‘Metals’ – Cations were added at the following amounts per plot; sodium 123 g (13.50 mg/kg), calcium – 111 g (12.5 mg/kg), potassium 324 g (35.40 mg/kg), and magnesium 449 g (49.03 mg/kg). These amounts were based on Lane et al., (2020) to increase observed levels in stored overburden to heathland soil concentrations reported by Clarke (1997). Applied in pellet form (Thompson and Morgan, Suffolk, UK), the cations were mixed and broadcast by hand to ensure an even spread.

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

In addition, the following mixed treatment combinations were employed:

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

Soil sampling and analysis

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

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

Vegetation sampling and statistical analysis

In June 2019, plant cover was estimated for each component species in the entire (4×3 m) area of each plot, with the number of individual dwarf ericaceous plants counted in the 1 m^2 centre portion. By the end of the experiment, several squares were lost due to commercial operations leaving only eight replicates per treatment included in the final samples. Analysis of the cover data was performed in three dimensions using metaMDS and ordiellipse to highlight groupings in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2. Once the communities were plotted onto an ordination plot, the physical characteristics of the soil were overlaid as vectors (for variables where $P \leq 0.05$) to facilitate identification of how physical factors varied with, and influenced development of, the various communities. An ANOSIM was performed in the 'vegan' (Oksanen, 2015) package in 'R' v.3.5.2 to examine variation in plant community composition between restoration treatments.

To examine the effect of treatment on number of ericaceous plants growing in the 1m^2 centre portion, we applied a two-step approach. First, ANOVA was used to test the effect of the seeded treatments versus the unseeded control was tested across all plots. This was done for *E. tetralix* and *C. vulgaris* separately. Next, ANOVA was performed with minimum adequate models (MAMs). These models were constructed following the iterative procedure outlined by Crawley (2014). This was done including a block factor (i.e. eleven blocks each consisting of all nine treatment plots). In the iterative procedure, first the full factorial model was constructed. The least significant terms were removed from the model, removing insignificant highest order interactions (three-way interaction) first, then second-order terms and so on with the residual standard error examined at each stage of the procedure. Final models were selected when the removal of any factors notably increased the residual standard error. These MAMs tested the effect of treatment (organic matter, fungi, metals, block and all interactions) on the number of *E. tetralix* and *C. vulgaris* as well as concentrations of P, K, Mg, Ca and pH. All analyses were performed in the R studio environment (R core team, 2017).

Results

Greenhouse experiment

Although long-term heather cutting survival was low (fewer than one third of plants survived to harvest), OM addition had an additional negative impact (Table 1). Moreover, in addition to the fact that compost addition reduced cutting survival to less than a quarter seen in the control, ErMF supplied in isolation or mixture with OM also failed to influence heather survival. For the cuttings that survived, only one intervention (compost addition) had any marked effect on plant growth or flowering, and this was restricted to enhanced shoot biomass in the few surviving plants in single or mixed OM + ErMF treatments. Interestingly, although plants initially exposed to ErMF displayed the expected increase in root inoculation (Table 1), cuttings in the ‘control’ and OM treatments also exhibited substantial root colonisation by fungi. This result might however, highlight a possible limitation in the use of microscopy to distinguish between ErMF and other fungi (Vohník 2020).

Field Experiment

Plant community composition

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

None of the interventions facilitated widespread establishment of the ericaceous species that typify ALH communities; all plant community clusters in the control and ‘OM’, ‘Metal’ and ‘Fungi’ treatments were noticeably disjunct from these target species in the nMDS analysis (Figure 1). At best, target ericaceous species achieved only 1.5% cover in the ‘seeded control’ treatment (Table 2). Moreover, the number of *Calluna* and *Erica* sp. individuals recorded in any central 1m^2 plot 32 months after any intervention was imposed either did not vary from, or in the case of all OM treatments was much reduced in comparison with, the seeded control (Figure 2). These patterns were corroborated by ANOVA and MAM analysis. There was no significant effect of seeded vs unseeded treatments, block, or their interaction on *E. tetralix* (seeded treatment F_1=0.549, p = 0.461, block F_{10} = 1.479, p = 0.164, interaction F_{10} = 0.732, p = 0.692, residual d.f. = 77), or *Calluna vulgaris* (seeded treatment F_1=0.379, p = 0.540, block F_{10} = 1.598, p = 0.123, interaction F_{10} = 0.825, p = 0.606, residual df = 77) abundance. Moreover, addition of OM was associated with a reduction in *E. tetralix* abundance, as illustrated by a model that included the block term and the interaction of OM and block (OM F_1 = 10.2, P = 0.002, Block F_{10} = 1.80, p = 0.074, OM × block $F10$ = 1.41, p = 0.19, residual df = 76). Similarly, the number of *C. vulgaris* plants was markedly lower in OM plots (OM F_1 = 9.51, p = 0.002, Block F_{10} = 1.68, p = 0.097, residual df = 87).

Soil Properties

The addition of organic matter increased soil pH compared to untreated controls (Table 3), although there were inconsistent differences between OM treatments and all other interventions (see ANOVA and MAM results in Supplementary Data File). Soil concentrations of N, P, K, Mg and Ca were generally elevated in one or more of the OM treatments, but it was noteworthy that this effect was variable amongst the four OM plots. The MAM for potassium concentration included OM, metals, fungi and several interaction terms (Supplementary Data File) with results supporting the trend for increased soil K in plots with added OM and 'metals', but reduced in the fungal treatment (Supplementary Data File). For soil P, OM, metals, fungi, the block term and several interaction terms were significant, denoting an increase in the OM and metals treatments, but decline in the fungal treatment (Supplementary Data File). Nonetheless, although macronutrient concentrations tended to increase with OM application (including a three-to-four-fold increase in soil N, P and K between control and at least one 'OM' treatment), no comparative increase was apparent for at least one of the ‘OM’/’ErMF’/’Metal’ combination treatments (Table 3). Of the two micro-nutrients considered, the significant OM, metals, fungi, block term and several interaction terms corroborated an increase in mean soil calcium in OM and ‘metals’ treated plots, but reduced in the ‘fungal’ plots (The ANOVA and MAM model also included the near-significant interaction term (‘OM × metals × block’) - see Supplementary Data File). For magnesium, significant metals, fungi, block and several interaction terms highlighted elevated concentrations in OM and metals plots, but reduced concentrations in the fungal treatment (Supplementary Data File).

Discussion

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

There are at least two plausible mechanisms to explain these results. First, and perhaps most pertinent to the greenhouse experiment, OM addition in relatively high humidity might have promoted conditions suitable for the spread of harmful fungi including saprotrophic basidiomycetes that can detrimentally affect mycorrhizal fungi (Shaw et al. 1995; Leake et al. 2001). Shaw (2019) also reported how the addition of OM to mine spoil led to the 'damping-off' of heathland seedlings as a fungal pathogen killed young plants on waterlogged soils. Consequently, we cannot rule out the possibility that OM addition promoted antagonistic fungi in the field trials. Second, increases in soil macro- and micro-nutrients in field plots, coupled with an increase in soil pH, may have facilitated the rapid establishment of dominant grasses (e.g. *Agrostis* and *Festuca* species) that outcompeted emerging *Calluna* and *Erica* spp. seedlings following OM addition (see Green et al 2015; Tibbett et al 2019; Lane et al 2020).

Although some studies (Smith & Read 2010; Wubs et al. 2018; Radujkovi et al. 2020) have suggested that an absence of ErMF can be a limiting factor on ericaceous species recruitment and persistence, we found little evidence that heather establishment or growth benefitted from the addition of one of the few commercially available ErMF sources ('Rhodovit'). When supplied in isolation or mixture with OM, in the greenhouse trial, ErMF failed to influence *Calluna* survival, growth or flowering. Similarly in the field, emergent plant communities in the 'Fungi' or 'Fungi + Metals' plots, dominated by bare ground, *Ulex europaeus* and various Graminoids, differed little to those seen in 'Seeded Controls'. The same was true for *Calluna* and *Erica tetralix* abundance in the central 1 m^2 portion of each plot (where ErMF was originally applied). Whether our failure stems from the method/ErMF used, the timing of application (Radujkovi et al. (2020) showed that it may take several years to attain levels of ErMF infection equivalent to that of undisturbed soils), and/or stochastic environmental conditions limiting the ericaceous/ErMF interaction is unclear. Nonetheless, our experiments with one of the few commercially available ErMF inoculants do not evidence any consistent benefit to post-mine heathland restoration.

It is also apparent from the field experiment that supplementation of some of the various cations thought to limit heathland establishment (Coppin & Bradshaw 1982; Clarke 1993, 1997; Lane et al 2020), had no impact on heath species recruitment or growth. As with the 'Fungi' and 'Fungi + Metals' treatments, the 'Metals' plots were similar to the 'Seeded Controls' in being dominated by bare ground, *Ulex europaeus* and Graminoids, while addition of metal cations (alone or in combination with other treatments), had no effect on *Calluna* or *Erica* spp. abundance. The fact that within 9 months of application soil Mg, K and Ca concentrations had declined to levels similar to those in control and other 'non-metals' plots, may indicate that winter rain quickly leached these cations from the unvegetated soils (see Duddigan et al. 2020). The only exception was where OM was also added alongside the supplemented cations, suggesting that soil organic content may play a role in nutrient retention as well as provision.

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

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

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Table 1. Summary of the effects of soil additions of organic matter (OM) and ericoid mycorrihizal fungi (ErMF) on mean (±SE) heather (*Calluna vulgaris*) survival, growth, flowering and proportion of root length colonised by fungi compared to untreated control plants grown in a 50% sand/peat mixture. Significant (p<0.05) differences located by one-way ANOVA or Chi-squared tests are denoted by bold font.

Response	**Control**	**OM**	**ErMF**	**OM & ErMF**	**Test Stat (df)**	***p*-value**
Plant survival (%)	91	23	86	9	$X^2_{(3)} = 37.5$	**<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**

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

Treatment (Mean ± SE)	**Graminoids**						**Shrubs**			**Forbs**	**Bare Ground**
	Agr cur	**Agr sto**	**Fest sp.**	**Jun eff**	**Other**	**Total**	**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

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

Treatment	**pH**	**C (%)**	**N (%)**	**P (µg g^{-1})**	**K (µg g^{-1})**	**C:N**	**Mg (µg g^{-1})**	**Ca (µg 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)

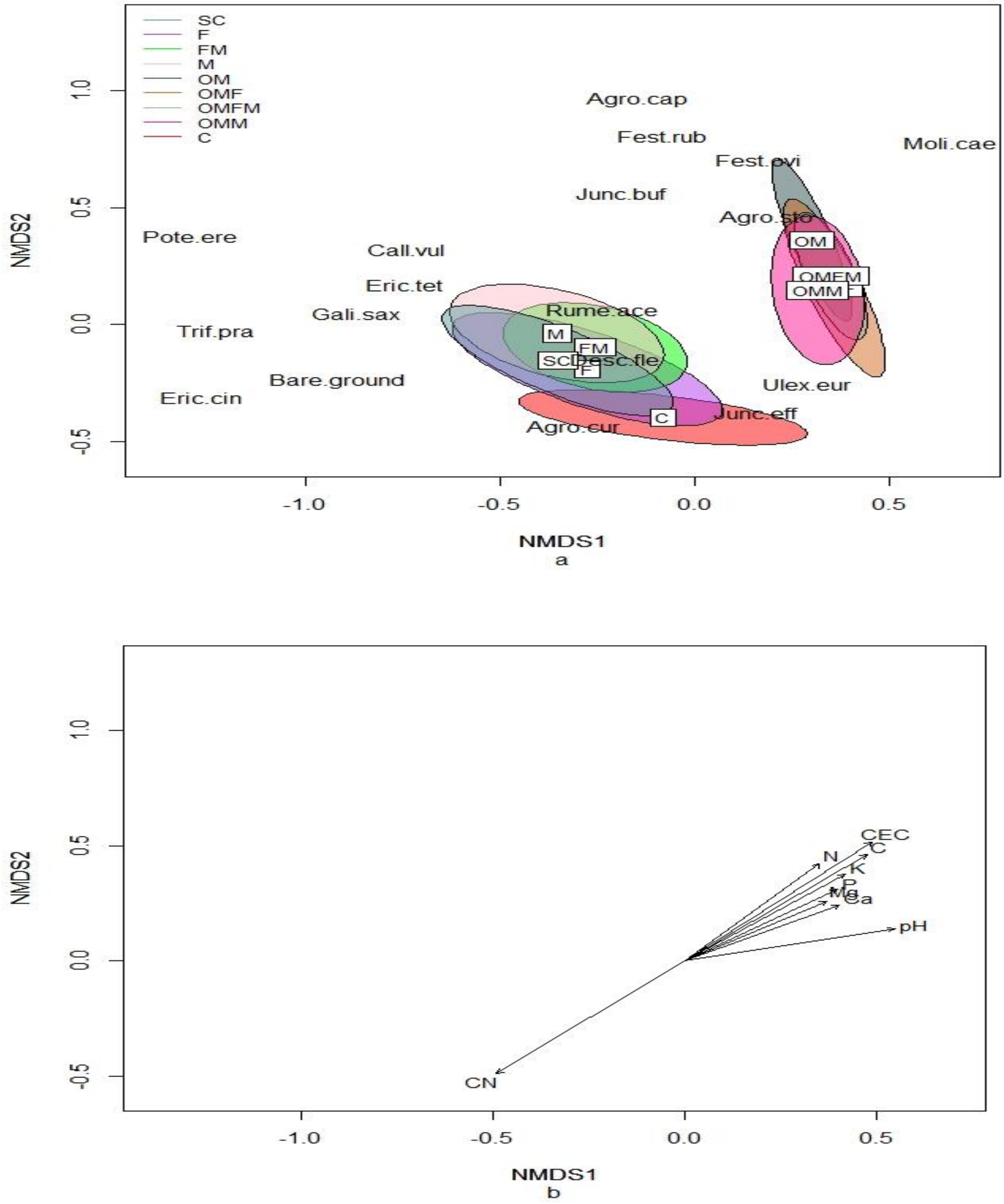
Figure legends

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

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

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

Figure 1


SC
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Eric.tet
Gali.sax
Rume.ace
Trif.pra
Bare.ground
Eric.cin
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Ulex.eur
Junc.eff
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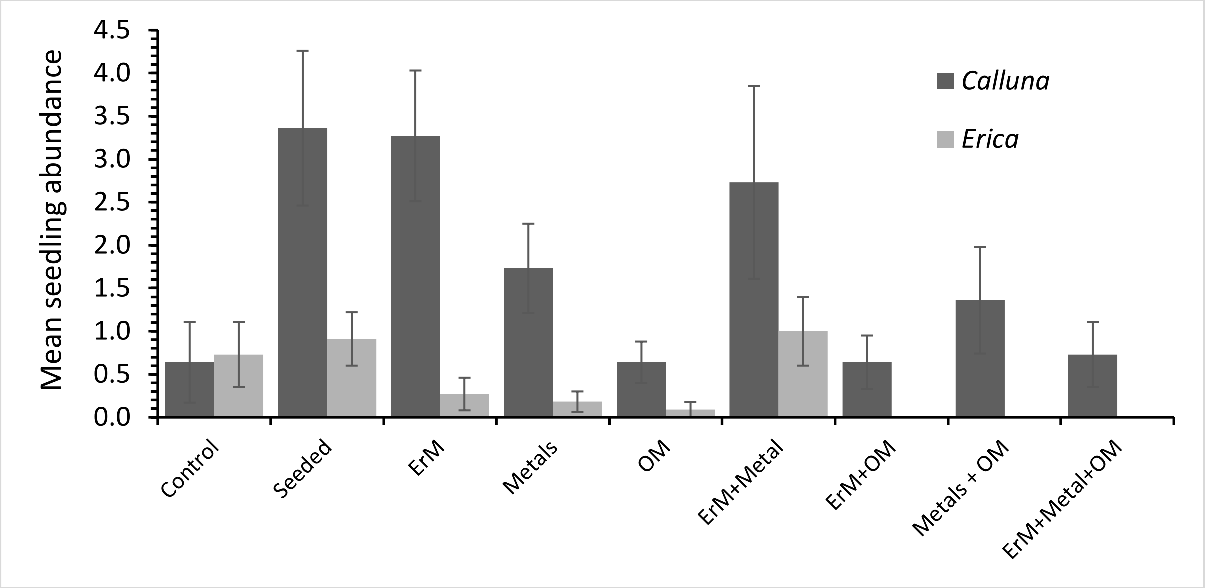
Figure 2


Mean seedling abundance
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Calluna
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Control
Seeded
ErM
Metals
OM
ErM+Metal
ErM+OM
Metals + OM
ErM+Metal+OM