Heathland vegetation ecology relies on positive interactions between fungi and plants

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Background: Heathlands are crucial protected UK habitats supporting endemic flora and fauna. Heathland soils are acidic and mainly comprise sand and peat, fluctuating between extreme dry and wet. In order to access soil nutrients, vascular plant roots and bryophyte rhizoids form mutualistic symbioses with fungi wherein the fungi transfer nutrients to, and receive photosynthates from, their host plants.

In two case studies - one involving a rare lycophyte (*Lycopodiella inundata*) and the other a non-vascular leafy liverwort (*Cephalozia bicuspidata*) − we used a multidisciplinary approach, combining cytology, molecular identification, *in vitro* cultivation, and radio- and stable-isotope tracer techniques, nursery experiments and field recordings to study how the heathland fungal symbiont community can be a critical factor in conservation of rare plants and restoration of heathlands.

Cephalozia bicuspidata

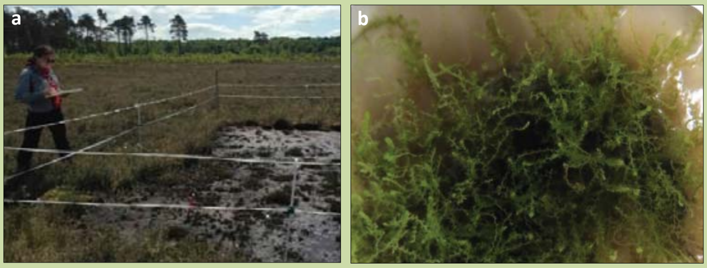

Figure 1. (a) Field measurements of outplanted *Erica tetralix* experimentally inoculated with *Pezoloma ericae* via the liverworts. (b) Culture plate of axenically-grown *Cephalozia bicuspidata* resynthesized with the fungal isolate of *P. ericae.*

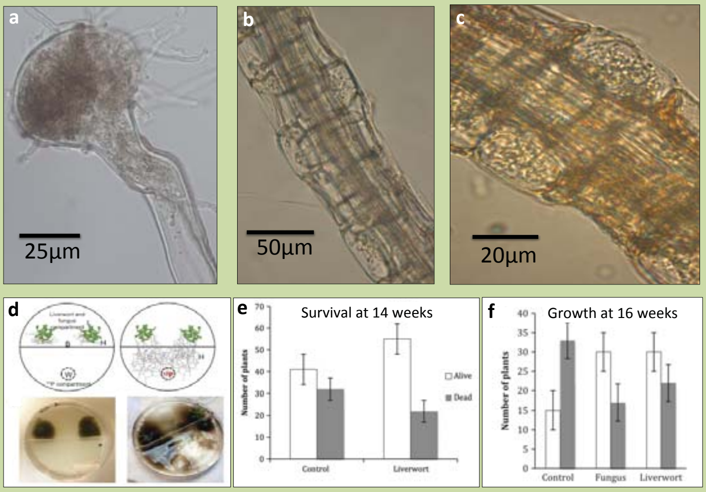

Figure 2. Light microscope images of (a) *Cephalozia bicuspidata* rhizoid, (b) *Calluna vulgaris* and (c) *Erica tetralix* root showing typical colonization patterns of *Pezoloma ericae*. In the liverwort, *P. ericae* only colonises rhizoids while in Ericaceae vascular plant roots, it forms coils in the epidermal cells. (d) Experimental set-up for liverwort:fungus ^{33}P stable isotope experiment demonstrating the fungus transfers phosphorus to the liverwort. Plant-derived ^{14}C was transferred to the fungus and measured using sample oxidation and liquid scintillation counting (Kowal *et al.* 2018). (e,f) Nursery growth experiments testing the efficacy of the colonised liverwort as a fungal inoculum yielded statistically significant support for this technique (*P*-value < 0.001 and 0.01, respectively), using binomial proportion data with a two-sample test for equality of proportions and continuity correction (error bar ±1 SEM).

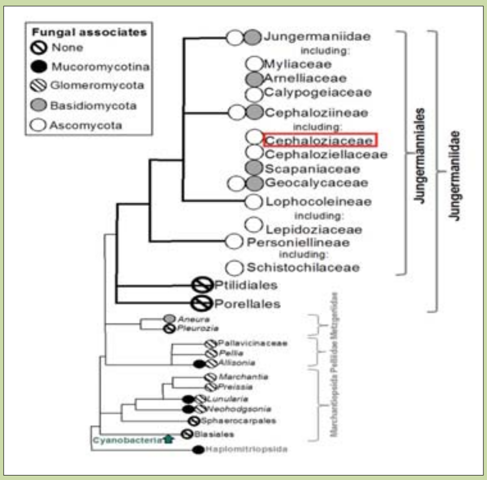

Figure 3. Liverwort phylogram from Field *et. al*. 2015c showing Ascomycota and Basidiomycota fungi associate with many leafy liverworts families. Many of these fungal groups contain mycorrhizal fungi which also associate with a broad range of angiosperms and conifers, thus there is clear potential to explore ecological applications where conventional restoration techniques are insufficient.

Lycopodiella inundata

Figure 4. Field observations showing (a) wet heathland habitat where *Lycopodiella inundata* samples were collected; (b) close-up view of a colony.

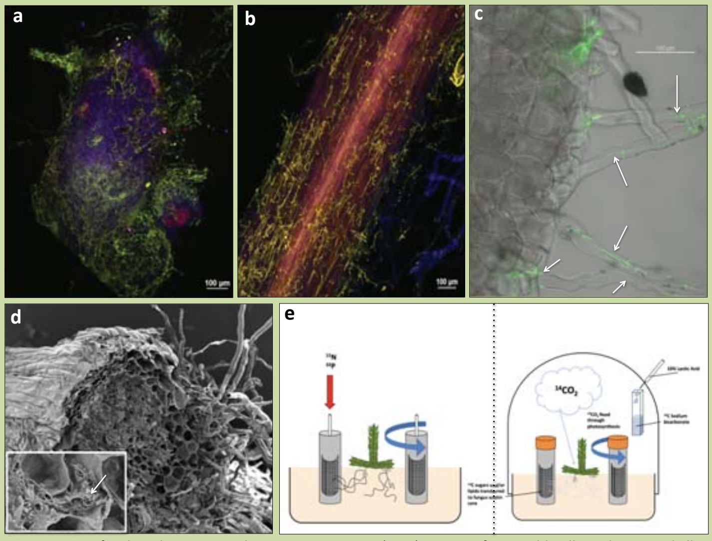

Figure 5. Confocal and scanning electron microscope (SEM) images from wild-collected *Lycopodiella inundata* colonies, Thursley Common, Surrey, UK. (a) Cross-section of protocorm and (b) sporophyte root stained with WGA-FITC, showing extent of fungal colonization by Mucoromycotina; (c) detail of young sporophyte hairs colonized by fungal hyphae (arrowed). (d) SEM image of a cross-section of a protocorm showing fungal proliferation in intercellular spaces; inset: close-up of an intracellular space colonised by Mucoromycotina (arrow). (e) set up of nutrient exchange experiments (plant fixed C transmitted to the fungus for fungal fixed N & P delivered to the plant).

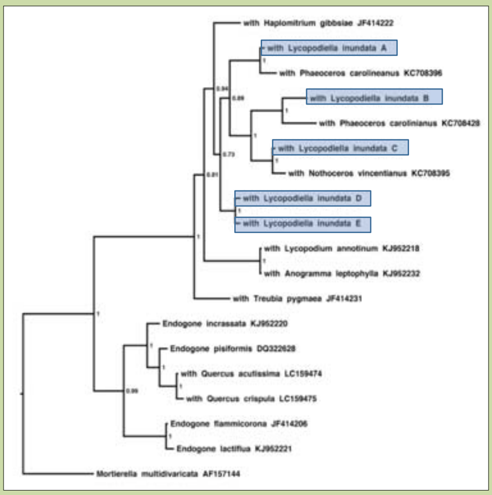

Figure 6. Mucoromycotina fungal DNA sequences from *L. inundata* symbionts showing their relative position to *Endogone* fruit bodies and *Endogone*-like sequences sourced from wild extant non-vascular land plants (Haplomitriopsida liverworts - *Haplomitrium* and *Treubia*, hornworts - *Phaeoceros* and *Nothoceros)* and vascular plants (fern - *Anogramma*; angiosperm - *Quercus*). Bayesian inference using Mr Bayes; Nst=2, Rates=invgamma; 1M generations; sequences of full 18S gene sequenced using molecular cloning; Genbank reference sequences. Further samples collected from other sites are also colonised by Mucoromycotina.

Main findings: Both plants associate exclusively with one mycorrhizal fungus group despite numerous other mycorrhizal fungi occurring in adjacent vegetation. *Lycopodiella inundata* gametophytes and sporophyte roots associate with Endogonales (Mucoromycotina). Cytological analyses supported by molecular results show widespread colonization in the gametophyte and early-sporophyte (with corm) life stages. Once a root cap forms and develops, the presence of colonized cells is greatly reduced. We are currently testing whether there is a nutritional mutualism occurring between *L. inundata* and Mucoromycotina and using stable isotope techniques, obtaining a better understanding of the functionality at the plant-fungus-soil interface underlying heathlands. A key question is whether temporal factors and plant-life stages influence fungal colonization.

Cephalozia bicuspidata associates exclusively with *Pezoloma ericae* (Ascomycota). We also demonstrate, for the first time, nutritional mutualism between a leafy liverwort and fungus. As neighbouring Ericaceous plants also associate with *P. ericae,* we show in glasshouse experiments a potentially practical application of colonized liverworts as an 'ecological fertilizer' to promote re-establishment of ericaceous plants. Working with Natural England, pilot field tests were initiated to inform conservation efforts. Further testing may provide an effective and sustainable means for restoring habitats. Our results demonstrate the efficacy of *C. bicuspidata* and *P. ericae*, but there are many other potential pairings between non-vascular liverworts and vascular plants sharing the same symbiont for use in habitat restoration (Figure 3).

Conclusion: These two examples demonstrate the importance of studying the heathland fungal symbiont community as a critical factor in conservation of both rare plants and restoration of habitats.

References: Hoysted *et al.* 2019. *Plant Physiology* DOI:10.1104/pp.19.00729; Kowal *et al*. 2018. *Annals of Botany* **121**:221-227; Kowal *et al.* 2016. *Journal of Functional Ecology* **30**: 1014-1023; Field *et al.* 2015. *New Phytologist* 205: 743-756; Field *et al. Trends in Ecology & Evolution* **30**: 477-486; Field *et al.* ISME J **10**: 1514-1526; Pressel *et al.* 2010. *Journal of Systematics and Evolution* **54**: 666-678; Rimington *et al.* 2015. *New Phytologist* **205**: 1394-1398.

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