

Institution: Aberystwyth University		
Unit of Assessment: 6: Agriculture, Food and Veterinary Science		
Title of case study: Use of eDNA analysis in the conservation of grassland fungi		
Period when the underpinning research was undertaken: 2012-2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Dr Gareth Wyn Griffith	Senior Lecturer; Reader	1 October 2008- present
Dr Andrew Detheridge	Research Scientist; Post Doctoral Research Assistant; Post-doctoral Research Assistant - Anaerobic Fungi; Post Doctoral Research Assistant in Soil Science	16 November 2015- 16 January 2016; 19 September 2016- 18 November 2016; 3 January 2017- 31 March 2017; 1 May 2017- 30 September 2019
Period when the claimed impact occurred: August 2013-2020		
Is this case study continued from a case study submitted in 2014? N		
1. Summary of the impact (indicative maximum 100 words) Researchers at Aberystwyth University (AU) developed a novel DNA metabarcoding method using soil eDNA, and have deployed it as a method for rapid assessment of fungal biodiversity in grassland habitats. The method has been used in both commercial and legal contexts, providing evidence and enabling faster decision making for: designation of one site in Birmingham as a Site of Special Scientific Interest (SSSI) - the first ever use of eDNA for this purpose; decisions in four planning applications; and sanctioning of two landowners who contravened Environmental Impact Assessment land-use regulations. These interventions had direct commercial and environmental impacts, and improved public service. Impact on public understanding and participation was achieved through several citizen science projects including collaboration with Royal Botanic Gardens, Kew, in training of citizen scientists in DNA barcoding (using Bentolab), and TV programmes, films and apps raising awareness of fungal conservation.		
2. Underpinning research (indicative maximum 500 words) Several groups of grassland fungi are of conservation concern (some of which have specific legal protection). A novel DNA metabarcoding method was developed by AU researchers for rapid assessment of fungal biodiversity in grassland habitats. The method provides species richness and abundance, and has been validated by comparison with 'standard' fruitbody (mushroom) surveys. In addition, the developed method can be conducted at any time of year and can deliver results within a matter of weeks. Two strands of research have permitted the development of this impact. First, the use of DNA barcoding to define fungal species via DNA sequences (Internal Transcribed Spacer (ITS) and Large Sub-Unit (LSU) barcodes). This has led to revision of many fungal taxa, a process to which we have made a substantial contribution [3.1; 3.2]. Second, the advent of NextGen sequencing (NGS) methods led to the development of DNA metabarcoding, whereby DNA extracted from environmental samples (eDNA) and amplification of the LSU or ITS2 or barcode region using fungal-specific primers, allows sequencing of		

barcode loci from all the fungi present in the eDNA sample. The relative abundance of sequences linked to particular species allows the relative abundance of each species to be assessed. For fungi which are not visible to the naked eye (except when macrofungal species form fruitbodies, for example), this method transforms the assessment of fungal communities. Griffith's research team developed new primers to target the LSU barcode region and undertook NGS on an Ion Torrent platform in a series of projects funded by Welsh Government [3.8; 3.9; 3.10]. A novel bioinformatics pipeline was also developed to analyse the data [3.3; 3.4].

In order to link names to sequences, AU research has contributed to the creation and updating of curated DNA databases, including UNITE [3.5] and RefSeq [3.6]. These databases now include *de novo* DNA barcodes from reference specimens (clearly identified fungal fruitbodies) obtained by the researchers, sometimes via interaction with a diverse range of citizen scientists. The method was validated by comparison with 'traditional' fruitbody surveys to test whether the data from eDNA soil surveys were congruent and optimal storage conditions for transfer preservation of soil samples for eDNA analysis were established [3.7].

3. References to the research (indicative maximum of six references)

- 3.1 Lodge, D. J., (... 15 authors....), **Griffith, G.W.**, (... 18 authors....) & Hattori, T. (2014). Molecular phylogeny, morphology, pigment chemistry and ecology in Hygrophoraceae (Agaricales). *Fungal Diversity*, 64(1), 1-99. DOI: [10.1007/s13225-013-0259-0](https://doi.org/10.1007/s13225-013-0259-0)
- 3.2 Adamčík, S., Jančovičová, S., Looney, B. P., Adamčíková, K., **Griffith, G. W.**, Læssøe, T., & Matheny, P. B. (2017). *Hodophilus* (Clavariaceae, Agaricales) species with dark dots on the stipe: more than one species in Europe. *Mycological Progress*, 16(8), 811-821. DOI: [10.1007/s11557-017-1318-9](https://doi.org/10.1007/s11557-017-1318-9)
- 3.3 **Detheridge, A. P.**, Brand, G., Fychan, R., Crotty, F. V., Sanderson, R., **Griffith, G. W.**, & Marley, C. L. (2016). The legacy effect of cover crops on soil fungal populations in a cereal rotation. *Agriculture, Ecosystems & Environment*, 228, 49-61. DOI: [10.1016/j.agee.2016.04.022](https://doi.org/10.1016/j.agee.2016.04.022)
- 3.4 **Detheridge, A.P.**, Comont, D., Callaghan, T. M., Bussell, J., Brand, G., Gwynn-Jones, D., Scullion, J. & **Griffith, G.W.** (2018). Vegetation and edaphic factors influence rapid establishment of distinct fungal communities on former coal-spoil sites. *Fungal Ecology*, 33, 92-103. DOI: [10.1016/j.funeco.2018.02.002](https://doi.org/10.1016/j.funeco.2018.02.002)
- 3.5 Kõljalg, U , Nilsson R.H., [...13 authors...] **Griffith G.W.**, [...24 authors...] Larsson. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, 22, 5271-5277. DOI: [10.1111/mec.12481](https://doi.org/10.1111/mec.12481)
- 3.6 Schoch, C.L., (...29 authors...), **Griffith, G.W.**, Groenewald, J.Z., Groenewald, M., Grube, M., (...51 authors....) & Federhen, S., (2014). Finding needles in haystacks: linking scientific names, reference specimens and molecular data for Fungi. *Database*, 2014, bau061. DOI: [10.1093%2Fdatabase%2Fbau061](https://doi.org/10.1093%2Fdatabase%2Fbau061)
- 3.7 Clasen, L.A., **Detheridge, A.P.**, Scullion, J., **Griffith, G.W.** (2020). Soil stabilisation for DNA metabarcoding of plants and fungi. Implications for sampling at remote locations or via third-parties. *Metabarcoding and Metagenomics*, 4, 135-147. DOI: [10.3897/mbmq.4.58365](https://doi.org/10.3897/mbmq.4.58365)

Research grants

- 3.8 **Griffith, G.W.** The Application of fungal DNA barcoding for the conservation of waxcap grasslands. Welsh Assembly Government. 1 November 2013 - 15 December 2014. GBP45,645.00

3.9 Griffith, G.W. DNA barcoding for the monitoring of grassland fungal populations Welsh Assembly Government-Environmental Resilience and Diversity Fund. 1 September 2012 - 28 March 2013. GBP26,464.00.

3.10 Griffith, G.W. Higher Plant DNA Sequencing in Soil. Welsh Assembly Government (WG contract C343/2017/2018). 1 March 2018 - 31 August 2019. GBP55,999.80

4. Details of the impact (indicative maximum 750 words)

Impact on policy

By localising where rare fungi are present via eDNA, the researchers have been successful in deploying targeted fruitbody surveying to subsequently locate these species [5.1.1; 5.1.2]. Six additional species were located at the Leasowes site in Birmingham, which enhanced the species count at the site by 20%. Thus, the AU 'eDNA guided surveying' has been instrumental in the notification of the Leasowes SSSI [5.1.3], which will be the first instance globally of eDNA being used in the legal protection of a site of conservation concern [5.1.4].

According to the Senior Warden at The Leasowes [5.1.5], the work "...was pivotal in Dudley Council obtaining the designation of SSSI for one of its sites in the West Midland's conurbation..." and that the results "... would target the areas for the field mycologists, saving time and financial cost to the project." This has led to the "... continued the restoration of The Leasowes historic site listed as grade one by Historic England while broadening the appeal of a site that has over 150,000 visitors a year... The SSSI designation also means that further funding ...will be possible so securing funding for a further ten years of management work. All the work described has enabled a very important site to be managed and protected for future generations." [5.1.5].

In addition, the Conservation Lead Adviser for the West Midlands Area Team at Natural England noted that "*It is due to the advice and evidence gained from Griffith's work that the SSSI was designated at this time, and at the size and scale that it was, thereby providing legal protection on a landscape-scale to a rare and remnant grassland habitat.*" [5.1.6] Furthermore, "... using the results of the DNA analysis we are able to identify the areas of potential interest and change and apply different management practices accordingly.....The eDNA work at The Leasowes has highlighted the potential for the use of this technology in conservation". [5.1.6]

The researchers have also been commissioned to write a number of other eDNA site reports by Natural England [5.1.7] and Natural Resources Wales [5.1.8].

Impact on the environment and conservation

Griffiths' research team have undertaken work for land developers (at the request of local planning officers/ council ecologists) seeking to ascertain whether land proposed for development has conservation value in terms of the fungi present. The primary benefit to the developer is that a decision can be obtained rapidly and at any time of year. In the four cases undertaken to date (Greenacres, Crumlin, Purton Road and Dolygaer [5.2.1 - 5.2.9]), apparently promising grassland sites were subject to eDNA analysis. In three cases, grassland fungal populations of only moderate diversity were identified, and thus the developments were granted permission to continue. However, in the fourth case (Purton Road, Swindon; [5.2.7]), planning was refused, in part due to our discovery of the presence of two waxcap species present on the IUCN's Red List [5.2.6], including *Hygrocybe citrinovirens*.

Where highly diverse fungal populations are found but there are economic pressures for the building development to continue, it is possible to attempt translocation. For fungi this involves the removal and storage of large turves, but this approach is unproven. Griffiths' research team are continuing to work with SES Ltd at the former Severalls Hospital site redevelopment in Essex using eDNA to determine whether waxcap populations can survive such translocations [5.2.10].

The AU research team have also used the method to provide land use assessment [5.3.1; 5.3.2] to the Welsh Government EIA unit in the prosecution of landowners who change use of land, for example through ploughing of permanent pasture without necessary permission, with three separate cases having been undertaken to date.

Impact on public awareness of grassland fungi

Griffiths' research team also interact extensively with citizen scientists (amateur mycologists and other non-professional naturalists). In particular, the AU team can supply specimens of rare fungi which the citizen scientists use as reference specimens to improve their eDNA metabarcoding databases. The team have provided training for groups of citizen scientists, notably Pembrokeshire Fungus Recording Network, to allow them to set up their own DNA barcoding labs [5.4.1], in association with Bentolab and the Royal Botanic Gardens, Kew *The Lost and Found Fungi Project*, whose research lead was trained by Griffith. This collaboration has led to the publication of taxonomic keys supplied free-of-charge to field mycologists [5.4.2].

Other contributions to raising the public profile of grassland fungi include contributions to TV programmes featuring grassland fungi (BBC2, 2017: *The Lake District: A Wild Year*; [5.4.3]), to the recent Plantlife app Waxcap [5.4.4] used at 362 sites throughout the UK and the Isle of Man, and a short film about the eDNA methodology that we have developed [5.4.5].

5. Sources to corroborate the impact (indicative maximum of 10 references)

Impact on policy

- 5.1.1** GW Griffith, AP Detheridge and L George (June 2015). *An Assessment of the fungal conservation value of The Leasowes (Halesowen, West Midlands) using NextGen DNA sequencing of soil samples*. Report to Dudley Metropolitan Borough Council in relation to the prospective notification of The Leasowes as SSSI.
- 5.1.2** GW Griffith, AP Detheridge and O. Cavalli & T. Moore (May 2018). Updated eDNA assessment of the fungal conservation value of The Leasowes (Halesowen, West Midlands) including the golf course areas. Report to Natural England in relation to the prospective notification of The Leasowes as SSSI.
- 5.1.3** Natural England (7 February 2019). The Leasowes SSSI Dudley: Notification under Section 28 of the Wildlife and Countryside Act 1981. Including Maps and Supporting Information (<https://consult.defra.gov.uk/natural-england/the-leasowes/>)
- 5.1.4** Press articles relating to the notification of The Leasowes as SSSI (10 - 13 February 2019); The Times; Daily Post; Express & Star; Stourbridge News; DEFRA Press Release; Dudley MBC Press Release; Aberystwyth University Press Release)
- 5.1.5** Testimonial letter from Senior Warden at The Leasowes, Countryside Services, Dudley Metropolitan Borough Council. (13 January 2021)
- 5.1.6** Testimonial letter from Natural England, Conservation Lead Adviser for the West Midlands Area Team (5 October 2020).
- 5.1.7** Griffith, G.W., Cavalli, O., Detheridge, A.P., (2019). An assessment of the fungal conservation value of Hardcastle Crags using NextGen DNA sequencing (NECR258). Natural England Commissioned Report NECR258.
- 5.1.8** Griffith, G.W., Clasen, L.A., Detheridge, A.P., (2019). Use of eDNA analysis of soil samples to evaluate the fungal conservation value of grassland areas in south Wales. Report No 494, commissioned by Natural Resources Wales, 42pp.

Impact on the environment and conservation

Planning application evidence

- 5.2.1** Planning application ([C13/0873/44/LL](#)).
- 5.2.2** eDNA survey of grassland submitted by consultant ecologist to Gwynedd Council (18 October 2013).
- 5.2.3** Gwynedd Council final planning decision 13 March 2014.
- 5.2.4** Extended Phase 1 and Botanical Survey (WYG; Crumlin, Newport). July 2016. (Waxcap eDNA report is Appendix C), linked to planning application: [17/0023/FULL](#).
- 5.2.5** Screenshots of planning application documents, press attention and site maps.

5.2.6 Report by Ecological Solutions Ltd. with fungal eDNA report as Appendix 5 (November 2019).

5.2.7 Final decision of Planning Inspectorate (April 2020).

5.2.8 Preliminary ecological appraisal by Wildwood Ecology Ltd. containing fungal eDNA report (26 September 2019).

5.2.9 Final decision of Brecon Beacons National Park Authority (9 October 2019).

5.2.10 Analysis of soil samples at Severalls Hospital regarding translocation of turves.

Detection of illegal land management activity evidence

5.3.1 GW Griffith, AP Detheridge and J Scullion (January 2016). *Analysis of fungal populations in four unidentified soil sample using Next Generation DNA Sequencing*. Report to Welsh Assembly Government, Environmental Impact Assessment Unit. Location details are redacted.

5.3.2 GW Griffith, AP Detheridge and J Scullion (June 2016). *Analysis of fungal populations in three unidentified soil samples from XXXXX using Next Generation DNA Sequencing*. Report to Welsh Assembly Government, Environmental Impact Assessment Unit (June 2016). Location details are redacted.

Impact on public awareness of grassland fungi

5.4.1 Article from Bentolab website about activities of Pembrokeshire Fungus Recording Network assisted by Griffith and others at Aberystwyth University.

5.4.2 Griffith, G.W. D. Harries and B. Douglas (2019). Earthtongues in the UK: a note on their status with particular reference to recent studies of the genus *Microglossum*.

5.4.3 E-mail thread with Director/Producer of the BBC2 programme "The Lake District: A Wild Year." BBC2 (17 February 2017) (<https://www.bbc.co.uk/programmes/b08flyr2>). Plus credits screenshot. Griffith provided technical advice on waxcaps and fruitbody development.

5.4.4 Plantlife Smartphone App (WaxcApp) launched in October 2021, based on Griffith, G.W., Bratton, J.H. & Easton, G. (2004) Charismatic megafungi; the conservation of waxcap grasslands. *British Wildlife*. October 2004, 31-43.

5.4.5 Film "Waxcaps – film stars of the fungi" <https://www.youtube.com/watch?v=YIUxv98c7TY> (July 2015) about deployment of eDNA technology for surveying of grassland fungi. Subsequently edited with German subtitles and entered into the 'Films about Soil' category at the Innsbruck Nature Film Festival (August 2015).