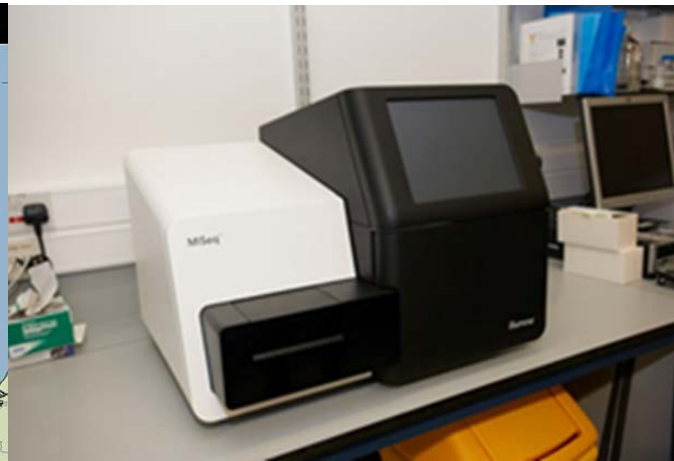
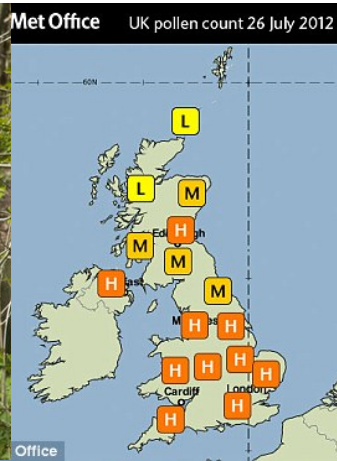
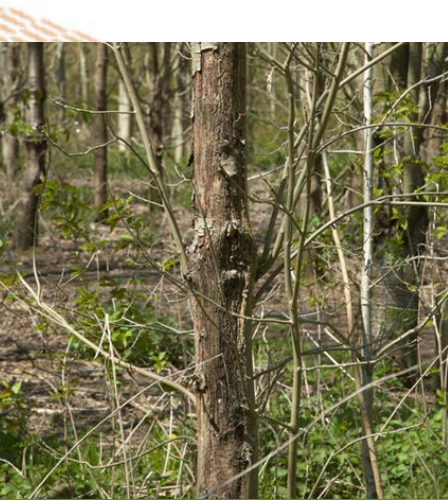




Tree disease surveillance: metabarcoding to identify fungi in spore traps

Ian Adams, Ummei Hany, Rachel Glover, Mel Sapp, Ian Brittain, Judith Turner, Rick Mumford, Neil Boonham



UK Plant Biosecurity Strategy



The screenshot shows the top portion of the Defra website. At the top left is the Royal Coat of Arms and the word 'Department'. To the right is the URL 'www.gov.uk/defra'. Below this is a navigation bar with links for 'free', 'become a member', 'sign in', 'subscribe', and 'search'. A secondary navigation bar features 'the guardian' logo and links for 'jobs', 'dating', 'more', and 'UK edition'. A third bar contains category links: 'UK', 'world', 'politics', 'sport', 'football', 'opinion', 'culture', 'business', 'lifestyle', 'fashion', 'environment', 'tech', 'travel'. A final bar shows 'home', 'environment', 'climate change', 'wildlife', 'energy', and 'pollution'. The main content area has a sub-header 'Ash dieback' and a headline 'Deadly fungus prompts ban on ash tree imports'.

Ash dieback
Deadly fungus prompts ban on ash tree imports

A tree disease has decimated 90% of Denmark's ash population and experts hope to prevent a similar ash dieback in the UK

Adam Vaughan
@adamvaughan_uk

Thursday 4 October 2012 11:58 BST



to save the UK's estimated 80m of the

ready decimated around 90% of

nd in the UK at a Buckinghamshire nursery

the epidemic of Dutch elm disease in the

The screenshot shows an article from 'The Telegraph'. The masthead 'The Telegraph' is at the top. Below it is a navigation bar with links for 'Home', 'Video', 'News', 'World', 'Sport', 'Finance', 'Comment', 'Culture', 'Travel', 'Life', 'Women', 'Fa'. A secondary bar contains 'Politics', 'Investigations', 'Obits', 'Education', 'Science', 'Earth', 'Weather', 'Health', 'Royal', 'Celebrity'. The article's breadcrumb trail is 'HOME > NEWS > EARTH > COUNTRYSIDE'. The headline is 'Ash sites with killer fungus double' and the sub-headline is 'The number of sites found to be infected with the disease threatening 80 million of Britain's ash trees has almost doubled in the past week.' Below the text are social media sharing icons for Facebook, Twitter, Pinterest, LinkedIn, and Email. A large photograph shows a man standing in a forest with many dead, skeletal trees. The bottom of the image is labeled 'Image 1 of 2'.

The screenshot shows a BBC News article. The 'BBC NEWS' logo is at the top left. Below it is a navigation bar with links for 'Home', 'UK', 'World', 'Business', 'Politics', 'Tech', 'Science', 'Health', 'Education', 'Entertainment'. The article's sub-header is 'Science & Environment' and the headline is ''Ash dieback' fungus Chalara fraxinea in UK countryside'. Below the headline is the date '© 25 October 2012' and the category 'Science & Environment'. A large photograph shows a close-up of a tree trunk with a dark, irregular hole or lesion, characteristic of ash dieback.

Task force recommendations

Increase the capacity of the Plant Health Services to undertake surveillance

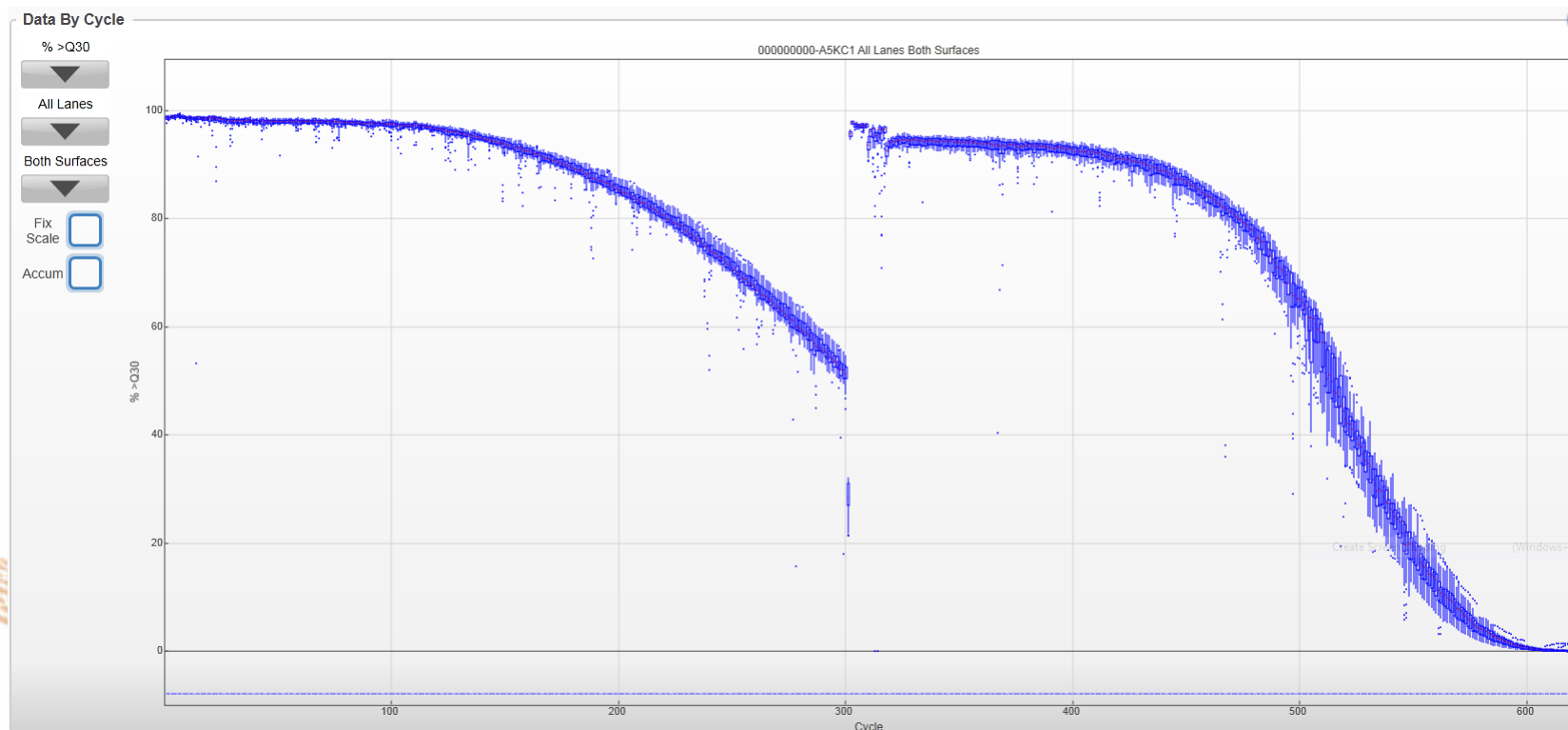
- ***Development of new approaches to; find pests through new detection and identification methods (for example, studying sentinel plants planted at key locations or using molecular techniques for identification of new pest threats in samples from existing networks)***

Non-targeted method to detect Airborne fungal spores

- Use NGS to determine species present in mixed populations of bacteria, fungi, insects, nematodes, woodchips
- Move from 454 (expensive, time consuming, phased out 2016) to MiSeq (cheap, simple, BUT.....)



MiSeq Error profile



Corrected by combining 2 300bp reads =400bp (PEAR)

Primer design



454

Forward primers

Up to 800bp sequence

Reverse primer



MiSeq (2 stage PCR)

Forward specific primers

Up to 400bp sequence

Reverse primers



Samples tested

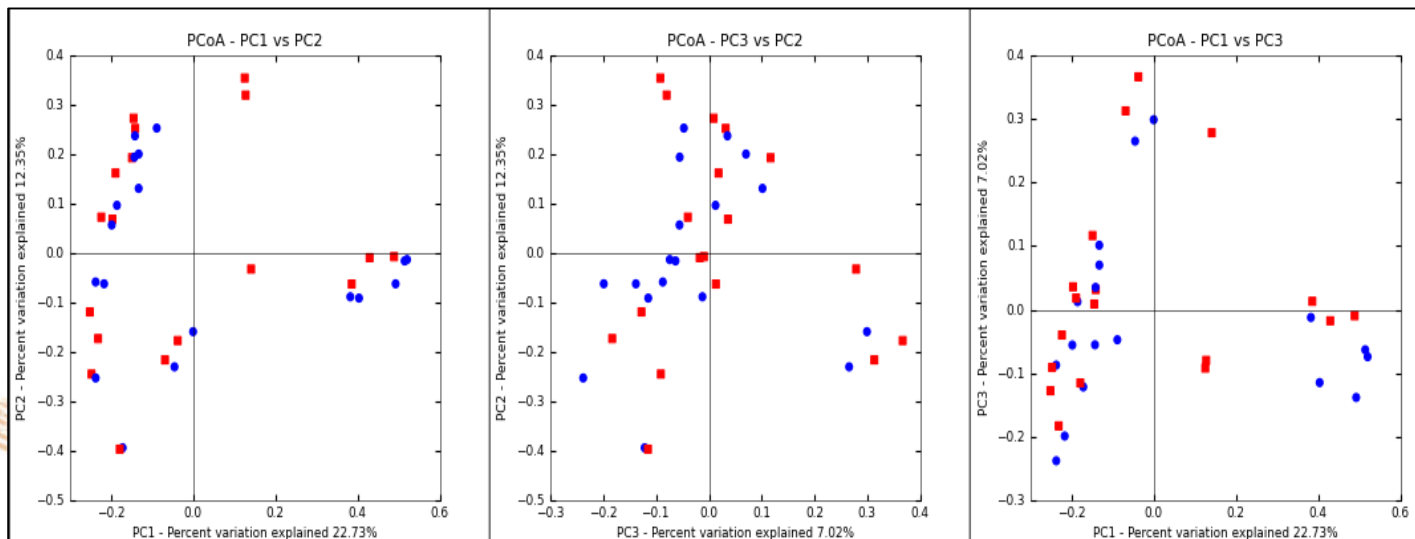
48 sample (sequenced with 454 and MiSeq)

- *Chalara* out break samples
- *Fusarium* spiked samples
- *Chalara* spiked samples
- UK air spore samples

Comparison between platforms

- ITS-1 sequenced range of spore trap samples. (454/MiSeq)

PCA betadiversity (Miseq / 454).



T-test alpha diversity asking if two platforms sampled from same population $p=0.91$

Detection of known fungi

Wide range of fungi detected Ferry sample 98 (454)-105 (MiSeq) different species

Taxon	Ferry Sample		possible low levels <i>C. fraxinea</i>		Spore tape (Seeded <i>C. fraxinea</i>)		Spore tape (Seeded <i>Fusarium</i>)		<i>C. fraxinea</i> outbreak site	
	454	MiSeq	454	MiSeq	454	MiSeq	454	MiSeq	454	MiSeq
<i>Chalara</i>										
<i>Fraxinea</i>	nd	nd	nd	0.004%	0.233%	0.067%	nd	nd	0.069%	0.010%
<i>Fusarium</i> Spp.	0.327%	0.251%	0.067%	0.098%	0.003%	0.001%	12.64%	19.90%	nd	0.005%

Fungal populations



Taxa	Ferry sample (all species above 1%) MiSeq	comment
<i>Mycosphaerellaceae</i>	0.2%	family including plant pathogens
<i>Periconia spp</i>	0%	wind dispersed fungi
<i>Xenobotryosphaeria calamagrostidis</i>	1%	?
<i>Epicoccum nigrum</i>	5%	plant saprophyte
uncultured <i>Ascochyta</i>	6%	?
<i>Botryotinia fuckeliana</i>	3%	common plant pathogen
<i>Monographella nivalis</i>	2%	grass pathogen
<i>Peniophoraceae spp</i>	1%	wood saprophyte
<i>Cryptococcus albidosimilis</i>	0%	common yeast found in soil
<i>Cladosporium spp</i>	71%	Common airborne fungus



Mock Community fungi

- *Puccinia striiformis* , *Puccinia triticina*, *Fusarium culmorum*, *Alternaria brassicae*, *Phytophthora kernoviae*, *Ceratocystis platani* , *Phytophthora ramorum*, *Cladosporium herbarum* , *Phomopsis* sp., *Microdochium nivale*, *Microdochium Majus*, *Alternaria tenuissima*, *Alternaria mali*, *Ceratocystis parasdoxa*, *Gibberella circinata*, *Pestalotiopsis guepinii*, *Hymenoscyphus pseudoalbidus*, *Mycosphaerella graminicola*, *Glomerella cingulata*, *Fusarium graminearum*.
- Optimum primers for ITS1 ? / Proof reading / non-proof reading Taq?

Task 1: Smaller amplicons

Primer name	Forward/Reverse	Sequence (5-3)	Reference
ITS1	Forward	TCCGTAGGTGAACCTGCGG	White et al, 1990
ITS2	Reverse	GCTGC GTTCTTCATCGATGC	White et al, 1990
ITS3	Forward	GCA TC GATGAAGA ACGCAGC	White et al, 1990
ITS4	Reverse	TCCTCCGCTTATTGATATGC	White et al, 1990
ITS5	Forward	GGAA GTA AAAGTCGTAA CAAGG	White et al, 1990
ITS1-F	Forward	CTTGGTCATTAGAGGAAGTAA	Gardes & Bruns, 1993
ITS4-B	Reverse	CAGGAGACTTGACACGGTCCAG	Gardes & Bruns, 1993
5.8S	Reverse	CGCTGCGTTC TTCA TCG	Vilgalys lab (Google cache pdf of webpage attached below)
5.8SR	Forward	TCGATGAAGAACGCAGCG	Vilgalys lab (Google cache pdf of webpage attached below)
SR6R	Forward	AAGWAAAA GTCGTAACAAGG	Vilgalys lab (Google cache pdf of webpage attached below)
ITS1-F_KYO2f	Forward	TAGAGGAAGTAAAAGTCGTAA	Toju et al, 2012
ITS2_KYO2r	Reverse	TTYRCTRC GTTCTTCATC	Toju et al, 2012
ITS4r	Reverse	CCTCCGCTTATTGATATGC	Toju et al, 2012
Scibetta_wobble	Reverse	GCA RRGACTTTCGTC CCYRC	Scibetta et al, 2012

Primer and polymerase choices

- Higher chao1
 - Higher number of 'rare' OTUs
 - Potentially more amplification and sequencing errors rather than true 'rare' OTUs
- Chosen:
 - Polymerase = phusion
 - Forward primer = Ky02F
 - Reverse primer = its1wobble

Polymerase	observed_species	chao1	shannon
phusion	257851	4026393.443	3.613001552
gold	367985	5908409.965	3.613045931

Primer	observed_species	chao1	shannon
ky02f-its1wobble	270419	4577996.03	3.567338981
its5-its1wobble	67516	1277533.992	3.367191619
ky02f-ky02r	247268	3826687.643	3.569261067
its5-ky02r	40952	560426.5092	3.699479333

QC strategy

- Standard QC strategy:
 - 16.2 million merged reads passed QC
 - Resulted in >600,000 OTUs when clustered at 97%
 - Only 20 species in the mock community...
- Revised QC strategy:
 - 14.2 million merged reads passed QC
 - Resulted in 9,000 OTUs when clustered at 97% - much better!
 - Standard Taxonomic assignment identified the mock community species (although frequently not to Species)

Species-level identification



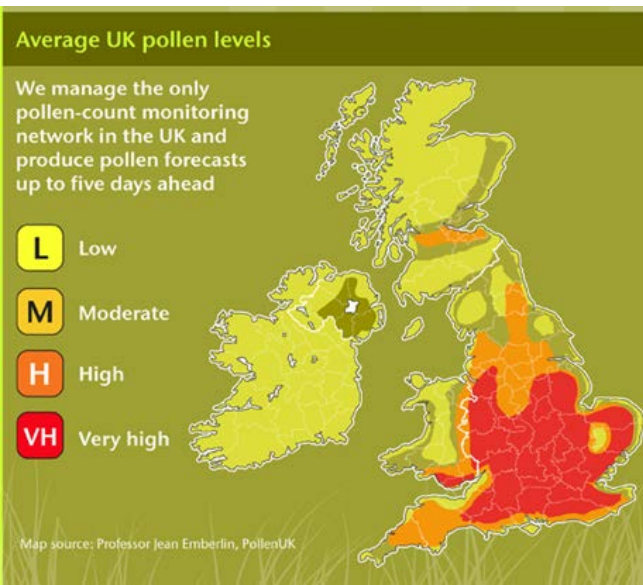
- QIIME standard tool for metabarcoding analysis
- Species-level identification not that important for community comparisons (most are genus level or above)
- To use community profiling techniques effectively for surveillance, species level identifications are required
- Currently developing new methods to provide accurate species level identification

Current work

Plant Biosecurity Strategy

*Development of new approaches to; find pests through new detection and identification methods (for example, studying sentinel plants planted at key locations or using molecular techniques for identification of new pest threats in samples **from existing networks**)*




Potential to use existing networks (pollen network / air quality network / targeted spore traps) to monitor for invasive fungal species



Current work



1. Complete comparison of existing and “new” analysis methods on mock community
2. Compare fungal communities measurable from specialist fungal traps and geographically related spore traps on defined dates.

CropMonitor cyclone traps (2015)	Pollen sticky Traps	Chalara sticky traps (2013+)
 A map of the eastern part of the United Kingdom showing the locations of three cyclone traps. The traps are marked with blue dots and labeled: 'Skeesley' (top), 'Hayward' (middle), and 'Alford' (bottom). Each trap location is enclosed in a red circle.	 A map of the eastern part of the United Kingdom showing the locations of several pollen sticky traps. The traps are marked with green dots. Two of the traps are highlighted with red circles.	 A map of the eastern part of the United Kingdom showing the location of a single Chalara sticky trap. The trap is marked with a black dot and enclosed in a red circle.



Acknowledgments

Funding: Defra Plant Health, BBSRC tree health initiative



Department
for Environment
Food & Rural Affairs