Diverse assemblages of ascomycetous fungi occur in woodland, grassland and cropping soils of northern-central New South Wales, Australia

David J. Midgley[1,3], Peter A. McGee[1], Michael I. Stewart[2] and Jennifer A. Saleeba[1,4]

[1]School of Biological Sciences, University of Sydney, NSW 2006, Australia. [2]School of Mathematics and Statistics, University of Sydney, NSW 2006, Australia. [3]Present address: CSIRO Flagship Energy Transformed, Julius Avenue, North Ryde, NSW 2113, Australia. [4]Author for correspondence. Email: jenny.saleeba@sydney.edu.au.

Abstract

Soils of the Brigalow Belt biogeographic region of NSW Australia host endangered, remnant vegetation communities and economically important agriculture, yet we know little of the fungal communities in the area. The ascomycete communities of woodland, grassland and cropping soils of the region were profiled using terminal restriction fragment length polymorphism and amplified ribosomal DNA restriction analysis (ARDRA). Two hundred and thirty one unique terminal restriction fragments (TRFs), putative taxa, were identified. Diversity of TRFs differed significantly between sites. ARDRA-profiling identified 51 unique ARDRA-types, 40% of which could not be identified except as ascomycetes. More specifically, some putative plant pathogens were associated with cultivated soils, where potentially suppressive Trichocomaceae were absent. Fungi cultured from soil indicated Trichocomaceae and potentially pathogenic species of *Fusarium* were present in the cultivated soils. Differences in ascomycetous community composition are discussed from an ecological and agricultural perspective.

Key words: Microbial diversity, ascomycetes, Brigalow woodland, community composition, ARDRA, TRFLP.

Introduction

The Brigalow Belt biogeographic region of northern-central New South Wales (NSW) and southern Queensland hosts a mosaic of intensive agriculture, grassland and endangered remnant woodlands. The clearing of woodlands in the region for intensive agriculture represents one of the most rapid and dramatic changes in vegetation communities ever documented (McAlpine *et al.* 2002; Seabrook *et al.* 2006). Given the economic and environmental importance of the region, it is surprising that the microbial diversity of these soils has attracted little attention. Soil fungi are critically important to many processes including the cycling and translocation of minerals (Warcup 1951; Wainwright 1988; Cairney 1992; Boddy 1999; Cairney 1999; Dighton 2003). Understanding of these communities is therefore crucial for both sustainable agriculture and restoration of ecosystems.

To date, only one study of fungal diversity has been conducted in the Brigalow Belt biogeographic region (Midgley *et al.* 2007a). The diversity of basidiomycete communities in soil at seven field sites with different vegetation communities indicated a higher diversity of potential plant pathogens in cultivated soil. The phylum Ascomycota contains many important groups including most of the fungi that cause disease and species that suppress plant pathogens (Christensen 1989; Aptroot 1997; Berbee 2001; Hawksworth 2001; Weller *et al.* 2002; Cairney & Meharg 2003; Tedersoo *et al.* 2006) leading to the prediction of enrichment of potential pathogens in culivated soils. However, intensification of agriculture has a major and unpredictable impact on soil microbial communities (Matson *et al.* 1997). The current study compared the diversity and composition of ascomycete communities in six sites with different vegetation and land use.

Materials and Methods

Field sites and soil sampling

Soil samples were collected at six 750 m^2 field sites in northern-central New South Wales in April, 2004 (Midgley *et al.* 2007a). Briefly, the six sites represent each of the major uses of the region and include a *Acacia harpophylla* (Brigalow) woodland; a nearby grassland where trees and shrubs are suppressed by grazing, Claremont Nature Reserve (Claremont); a eucalypt woodland within a forest, Jacks Creek State Forest (Jacks Creek) and a herbaceous plain which has also been cleared of trees and shrubs, Warrumbungle National Park (Warrumbungle). Two agricultural plots at the Australian Cotton Research Institute Narrabri (ACRI), a cotton-wheat rotation plot (cotton-wheat) and a 7 year fallow plot (fallow) were included for comparison. Twelve samples of ca 500 g of soil were collected using a cleaned trowel from a soil depth of 5–15 cm from random positions within each 750 m^2 field site. Samples were kept at 4°C in transit to the laboratory prior to soil DNA extraction.

DNA extraction from soil

DNA was extracted from 1.5 g of soil from each sample using the Yan and Vancov method (Midgley *et al.* 2007b).

PCR amplification

The rDNA internal transcribed spacer (ITS) region, including 5.8S rDNA, was amplified in 25 μL reaction volumes using ITS1f/ITS4A primers and standard PCR conditions (Larena *et al.* 2003). The 5′ end of ITS1f was labelled with 6-FAM (Fluorescein, Geneworks) for terminal restriction fragment length polymorphism (TRFLP) analysis.

TRFLP and ARDRA
TRFLP and ARDRA were conducted (Midgley *et al.* 2007a). Twelve amplification products from each site were digested with the restriction endonuclease Taq$^{\alpha}$I (New England Biolabs). Terminal restriction fragments (TRFs) were then identified using the ABI 3730 automated fluorescent DNA sequencer (Applied Biosystems) at Sydney University Prince Alfred Macromolecular Analysis Centre, Sydney, Australia. The number of TRFs detected in each soil sample, for each site was compared using SPSS v.11 (SPSS Inc.). These data were compared using the Kruskal–Wallis test. When significant differences ($P < 0.05$) were observed with the Kruskal–Wallis test, the Mann–Whitney U test procedure was used to identify significantly different pairs ($P < 0.05$). To compare diversity at each site a matrix of presence or absence of individual TRFs was constructed. In this matrix, each peak was scored from 0–12, where 0 denotes the absence of that TRF at a given site, while 1 through 12 indicated the number of samples within each site at which that TRF was recorded. From this matrix an estimate of Simpson's index of biodiversity, 1/D, was computed for each site and all sites were compared (Rogers & Hsu 2001).

For ARDRA, 12 replicate PCR products from each field site were pooled and purified by gel extraction using the QIAquick gel extraction kit (QIAGEN). Approximately 100 ng of purified pooled PCR product was ligated in the pDrive vector (QIAGEN) following the manufacturers directions and subsequently transformed into competent *E. coli* DH5α. Thirty clones for each field site were sorted into Taq$^{\alpha}$I (New England Biolabs) restriction fragment length polymorphism types, designated ARDRA-types, and a representative clone of each RFLP-type was chosen at random for DNA sequencing (Macrogen).

Sequence analysis
Each ITS sequence was divided into ITS1, ITS2 and 5.8S regions and then BLAST searches (Altschul *et al.* 1990) against the GenBank nucleotide database were conducted on each region, using the BLAST program at the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/). For ITS1 and ITS2, matches under 80% identity over less than 80% of the sequence submitted were regarded as below acceptable confidence levels and were not reported. One sequence of each RFLP-type was submitted to the GenBank nucleotide database under the following accession numbers EU520590–EU520640.

Culture of Trichocomaceae
Trichocomaceae were not detected at either of the cultivated sites (cotton-wheat and fallow) using ARDRA, therefore samples from these sites were not used in the culture work. Trichocomaceae were isolated using soil dilution (Markovina *et al.* 2005) from cotton-wheat and Brigalow sites. All colonies were cultured on Czapek Yeast Extract agar (CYA), Malt Extract Agar (MEA) and 25% Glycerol Nitrate Agar (G25N) and morphologically identified (Pitt & Hocking 1997).

Results

TRFLP analyses
In total, 231 unique TRFs were detected. Approximately, 46% of TRFs were unique and occurred only at single field sites: 38 were detected at Jacks Creek, 30 in Brigalow, 24 at Warrumbungle and 11 at Claremont. No unique TRFs were detected in the cotton-wheat or in the fallow samples from ACRI. Twenty four TRFs were detected at all sites (ca 10% of the total TRFs detected). The greatest number of TRF groups was detected at the Jacks Creek, Warrumbungle and Brigalow sites, while the lowest number of TRF groups was detected at the cotton-wheat and fallow field sites (Table 1). Claremont, the grassland site, had an intermediate number of TRFs. This was mirrored by estimates of Simpson's index of biodiversity that was greater at Brigalow, Jacks Creek and Warrumbungle ($P < 0.05$) and lower for cotton-wheat and fallow. The Claremont site had an intermediate index of biodiversity and was significantly lower than Brigalow, Jacks Creek and Warrumbungle, but significantly higher than either ACRI site, cotton-wheat and fallow (Table 1). Overlap in TRF composition was observed between pairs of sites (Table 2). Pairwise identity ranged from 24–69%, with a mean TRF identity between pairs of sites of ca 42%.

ARDRA
In total, 51 ARDRA-types were identified from the 180 clones examined (Table 3). Seventeen ARDRA types were found in soil from Claremont, while Jacks Creek and fallow had 4 and 3, respectively (Table 3). Brigalow had 11 ARDRA-types while both cotton-wheat and Warrumbungle had 8.

Obvious chimeric PCR products were excluded. Forty-three percent of all ARDRA-types had less than 80% identity and/or less than 80% query coverage when compared with known ascomycota from the GenBank nucleotide database for any of ITS1, ITS2 or both spacer regions. These fungi could not be reliably identified, except as ascomycetes. Seven taxa were identified with putative affinities to the order Pleosporales (B2, Cn10, Cw1, F2, F3, W1 & W5). Two of these taxa F2 and W5 shared 99.5% ITS sequence identity. Five ARDRA-types with affinities to the Trichocomaceae were identified (B11, Cn7, Cn8, J2 & W4), two of which (Cn8

Table 1 Numbers of terminal restriction fragments (TRFs), and comparisons of Simpson's Index of Biodiversity for each site.

Site	Total TRFs	Mean TRFs sample (±SE)	Estimated Simpson's Index 1/D
Brigalow Nature Park Reserve	119	32.2 (±4.2) bc*	79.4 a*
Claremont Nature reserve	97	25.5 (±2.9) ab	52.9 b
ACRI cotton-wheat rotation	36	15.3 (±1.1) d	23.9 c
ACRI 7 year fallow site	45	26.0 (±1.8) a	31.3 c
Jacks Creek State Forest	135	37.5 (±4.4) c	84.0 a
Warrumbungle National Park	120	37.3 (±3.8) c	87.7 a

*Different letters within a column indicate significant differences.

Table 2 Percentage pairwise identity of TRFs from different field sites.

Sites	Claremont	Cotton-wheat	Fallow	Jacks Creek	Warrumbungle
Brigalow	53	24	31	51	58
Claremont		30	41	48	59
Cotton-wheat			69	32	26
Fallow				30	34
Jacks Creek					54

& W4) had identical ITS sequences. Four ARDRA-types also had affinities to the order Hypocreales (Table 3), three of these (Cw6, Cw8 & F1) were probable *Fusarium* species, while W2 was most likely a *Myrothecium* species. Two ARDRA-types (Cn6 & J3) with affinities to the order Helotiales were detected, one of which (J3), had close affinities to a range of *Leohumicola* species (Table 3). In addition ARDRA-types with affinities to the classes Dothideomycetes (Cn4 & Cn13) and Lecanoromycetes (Cn2), order Chaetothyriales (B5, B8 & J1) and Xylariales (Cw7) were detected.

Isolation of Trichocomaceae

Eighteen species of Trichocomaceae consisting of 9 species of *Aspergillus* and 9 of *Penicillium* were recovered. Brigalow yielded 8 and cotton-wheat yielded 12 Trichocomaceae species. *Aspergillus terreus*, *A.* sp. nov. I & *Penicillium* sp. nov. IV occurred at both sites (Table 3). *Aspergillus candidus*, *A. fumigatus*, *A.* sp. aff. *fumigatus* & *Penicillium lividum* were detected only in Brigalow samples, while *Aspergillus niger* I & II, *A. ustus*, *A.* sp. nov. II, *Penicillium citrinum*, *P. janthinellium*, *P. verruculosum, P.* sp. aff. *citrinum*, *P.* sp. nov. I & *P.* sp. nov. II were detected only in the cotton-wheat samples (Table 3). A number of *Alternaria, Chaetomium, Cladosporium, Coniothyrium, Fusarium, Phoma, Stachybotrys, Trichoderma, Verticillium* species, and a range of non-sporulating fungi, were isolated (Midgley & McGee, unpublished).

Discussion

Soils of the Brigalow Belt biogeographic region host genetically diverse assemblages of ascomycetes. The sampling and analyses of TRFLP data reported here indicated that ascomycete biodiversity was significantly greater at the Brigalow, Jacks Creek and Warrumbungle sites than at the Claremont, cotton-wheat or fallow sites. The grassland site, Claremont, had significantly greater biodiversity than either of the agricultural sites (cotton-wheat or fallow), but significantly lower biodiversity than the Brigalow woodland, Jacks Creek or Warrumbungle sites. Interestingly, the Warrumbungle site has a similar site history to the Claremont site having been completely cleared of trees and shrubs for agriculture between 20 and 50 years ago and both remain largely without trees and shrubs, presumably due to grazing pressure. These sites have different fungal communities but both are significantly more diverse than the two agricultural soils. The sites Brigalow and Jacks Creek had different ascomycete composition but the same level of diversity. While all vegetation systems within the Brigalow region have been disturbed to an extent, the abundance of trees and shrubs at Jacks Creeks and the abundance of shrubs at the Brigalow site may indicate greater recovery from disturbance than the other sites. The greater ascomycete diversity at the more stable sites may indicate that the microbial diversity in soil at disturbed sites may increase towards the level in undisturbed sites, over time. The result is consistent with data on basidiomycete communities at the same sites (Midgley *et al.* 2007a).

The lower ascomycete diversity in the agricultural soils (cotton-wheat and fallow), compared to vegetated sites, correlates with land management practices such as monoculture, tillage, fertilisation and irrigation. However the two agricultural sites have other similarities. They have a close geographical proximity, they are subjected to similar environmental conditions and also share similar soil chemistry. Others have reported that changes in land use alter the belowground community of various guilds of microbes (Olsson & Alstrom 2000; Smalla *et al.* 2001; Oehl *et al.* 2003; Midgley *et al.* 2007a, b) and that even relatively mild perturbations in land use, such as change in the crop species, may significantly alter the soil Ascomycota community (Viebahn *et al.* 2005). It is reasonable to conclude that least some of the differences in ascomycete communities at the agricultural and vegetated sites are due to land management practices.

Possible roles of fungi may be inferred if the fungi can be identified. In this study, more than 40% of all ascomycetes detected by ARDRA had low DNA sequence identity with database ITS1, ITS2 or both ITS regions (< 80% identity and/or < 80% query coverage). The lack of identity to the sequences of characterised organisms makes it difficult to infer the possible roles of these fungi in soil. These fungi appear to be poorly characterised and may be undescribed.

Three putative *Fusarium* taxa detected by ARDRA (Cw6, Cw8, F1) are potentially pathogenic species of the crop plants grown in the cultivated soils in this study. Interestingly, previous data from the ACRI cotton-wheat rotation site, suggested that basidiomycetous communities at the site were dominated by *Rhizoctonia*-like *Ceratobasidium* species (Midgley *et al.* 2007a),

Table 3 Putative identities of ascomycete fungi from differing field sites in northern central New South Wales, Australia determined via BLASTN searches between 5.8S, ITS1 and ITS2 regions of cloned sequences and those in the GenBank nucleotide database. B, Brigalow; Cn, Claremont; Cw, Cotton–wheat; F, Fallow; J, Jacks Creek; W, Warrumbungle.

Clone–RA[a] (Acc. No)	5.8S closest match	ID | OL[b]	ITS1 closest match [c]	ID | OL	ITS2 closest match	ID | OL	Tentative ID
B1 13.3	DQ386141 Mycorrhizal fungal sp. "ML62"	99 | 154	MUC	–	MUC	–	Ascomycota #1
B2 23.3	DQ979726 Fungal endophyte "5095"	99 | 157	MUC	–	AF383952 *Lophiostoma arundinis*	88 | 242	Pleosporales #1
B3 3.3	AF393719 *Cladosporium nigrellum*	98 | 157	MUC	–	MUC	–	Ascomycota #2
B4 3.3	DQ279517 *Hypotrachyna pseudosinuosa*	94 | 156	MUC	–	MUC	–	Ascomycota #3
B5 16.6	DQ885895 *Strelitziana africana*	99 | 158	DQ421073 Uncultured soil fungus "138-16"	96 | 229	AJ507323 *Phaeococcomyces chersonesos*	94 | 264	Chaetothyriales #1
B6 6.6	AJ877106 Uncultured Mycosphaerellaceae	100 | 157	MUC	–	MUC	–	Ascomycota #4
B7 3.3	DQ979738 Fungal endophyte "5279"	99 | 156	MUC	–	MUC	–	Ascomycota #5
B8 13.3	DQ885895 *Strelitziana africana*	100 | 158	DQ421063 Uncultured soil fungus "14-13"	95 | 227	AJ507323 *Phaeococcomyces chersonesos*	95 | 264	Chaetothyriales #2
B9 10.0	EF160075 *Didymella bryoniae*	100 | 157	MUC	–	AF218789 *Phoma* sp.	93 | 258	Ascomycota #6
B10 3.3	AY671913 *Venturia inaequalis*	98 | 158	MUC	–	MUC	–	Ascomycota #7
B11 3.3	DQ336711 *Aspergillus penicillioides*	100 | 157	MUC	–	AF033462 *Eupenicillium rubidurum*	98 | 310	Trichocomaceae #1
Cn1 3.3	AF502889 Leaf litter ascomycete "1000270455"	100 | 155	MUC	–	MUC	–	Ascomycota #8
Cn2 6.6	AF224352 *Dimelaena oreina*	99 | 157	MUC	–	MUC	–	Lecanoromycetes #1
Cn3 3.3	AY916472 *Helicomyces torquatus*	98 | 158	MUC	–	MUC	–	Ascomycota #9
Cn4 10.0	EF160075 *Didymella bryoniae*	100 | 157	EF060611 Uncultured ascomycete "LM283"	97 | 171	DQ981396 *Epicoccum nigrum*	97 | 249	Dothideomycetes #1
Cn5 3.3	EF029186 *Beverwykella pulmonaria*	100 | 158	DQ420831 Uncultured soil fungus "151b32"	98 | 169	DQ420831 Uncultured soil fungus "151b32"	99 | 212	Ascomycota #10

[a] RA = Relative abundance (%), [b] ID = percentage identity, OL = overlap (bp), [c] MUC = Match under cutoff.

Table 3 Continued

Clone–RA[a] (Acc. No)	5.8S closest match	ID | OL[b]	ITS1 closest match [c]	ID | OL	ITS2 closest match	ID | OL	Tentative ID
Cn6 6.6	EF110889 *Sclerotinia sclerotiorum*	100 | 157	DQ420922 Uncultured soil fungus “68a30”	99 | 188	DQ420916 Uncultured soil fungus “174-20”	99 | 203	Helotiales #1
Cn7 3.3	DQ336711 *Aspergillus penicillioides*	100 | 157	MUC	–	AF033462 *Eupenicillium rubidurum*	98 | 251	Trichocomaceae #2
Cn8 3.3	DQ336711 *Aspergillus penicillioides*	100 | 157	MUC	–	AY484896 *Penicillium canescens*	100 | 258	Trichocomaceae #3
Cn9 13.3	DQ420981 Uncultured soil fungus 138-35	100 | 157	DQ420981 Uncultured soil fungus 138-35	92 | 240	DQ420946 Uncultured soil fungus “115-25”	93 | 250	Ascomycota #11
Cn10 6.6	AB245152 *Exserohilum* sp. NK03-1	100 | 157	DQ337381 *Cochliobolus lunatus*	98 | 223	DQ242473 *Bipolaris* sp. 4922	95 | 227	Pleosporales #2
Cn11 3.3	EF104234 *Leptosphaerulina trifolii*	99 | 154	EF060560 Uncultured ascomycete “LM221”	99 | 153	AY293803 *Phoma herbarum*	98 | 267	Ascomycota #12
Cn12 13.3	AF383968 *Vaginatispora aquatica*	100 | 158	MUC	–	MUC	–	Ascomycota #13
Cn13 6.6	EF1600756 *Didymella bryoniae*	100 | 157	DQ979761 Fungal endophyte “5654”	100 | 173	DQ981396 *Epicoccum nigrum*	99 | 249	Dothideomycetes #2
Cn14 6.6	DQ386141 Mycorrhizal fungal sp. “ML62”	99 | 153	MUC	–	MUC	–	Ascomycota #14
Cn15 3.3	AF383968 *Vaginatispora aquatica*	99 | 158	MUC	–	AF383952 *Lophiostoma arundinis*		Pleosporales #2
Cn16 3.3	AF393719 *Cladosporium nigrellum*	96 | 158	MUC	–	MUC	–	Ascomycota #15
Cn17 3.3	AY969640 Uncultured ascomycete dfmo0690_210	98 | 157	MUC	–	MUC	–	Ascomycota #16
Cw1 6.6	DQ865095 *Preussia africana*	99 | 155	AF461664 Uncultured fungus “RFLP103”	79 | 82	AJ279444 Humicola fuscoatra	89 | 208	Pleosporales #3
Cw2 13.3	DQ914435 *Pseudallescheria boydii*	100 | 157	MUC	–	MUC	–	Sordariomycetes #1
Cw3 20.0	DQ914435 *Pseudallescheria boydii*	98 | 157	MUC	–	MUC	–	Ascomycota #17
Cw4 13.3	AB278178 *Phaeoacremonium angustius*	98 | 157	MUC	–	MUC	–	Ascomycota #18
Cw5 3.3	DQ865095 *Preussia africana*	100 | 158	MUC	–	ITS2 not available	–	Sordariomycetes #2

Table 3 Continued

Clone–RA[a] (Acc. No)	5.8S closest match	ID | OL[b]	ITS1 closest match [c]	ID | OL	ITS2 closest match	ID | OL	Tentative ID
Cw6 20.0	DQ315564 *Fusarium solani*	100 | 157	EF060567 Uncultured ascomycete "LM231"	100 | 215	AB255352 *Fusarium* sp. E033	96 | 153	Hypocreales #1 Probable *Fusarium* sp.
Cw7 3.3	EF119336 *Pestalotiopsis clavis-pora*	100 | 158	MUC	–	MUC	–	Xylariales #1
Cw8 20.0	EF155534 *Fusarium oxysporum*	100 | 158	DQ393594 *Acremonium cyanophagus*	93 | 194	AY188919 *Fusarium oxysporum*	97 | 243	Hypocreales #2 Probable *Fusarium* sp.
F1 50.0	EF155534 *Fusarium oxysporum*	100 | 157	EF155534 *Fusarium oxysporum*	100 | 177	EF155534 Fusarium oxysporum	98 | 207	Hypocreales #3 Probable *Fusarium* sp.
F2 6.6	DQ865095 *Preussia africana*	100 | 157	MUC	–	MUC	–	Pleosporales #4
F3 43.3	DQ826430 *Phoma pinodella*	100 | 157	AB266850 *Didymella bryoniae*	100 | 153	DQ993290 *Phoma tracheiphila*	100 | 210	Pleosporales #5
J1 43.3	DQ885895 Strelitziana africana	100 | 158	DQ421069 Uncultured soil fungus "9b29"	96 | 231	AJ507323 *Phaeococcomyces chersonesos*	95 | 265	Chaetothyriales #3
J2 16.6	DQ336711 *Aspergillus penicillioides*	100 | 157	AY213679 *Penicillium turbatum*	92 | 197	AY484896 *Penicillium canescens*	96 | 257	Trichocomaceae #4 Probable *Penicillium* sp.
J3 16.6	AY706328 *Leohumicola lenta*	100 | 157	AY706329 *Leohumicola minima*	94 | 183	DQ497978 *Leohumicola verrucosa*	93 | 221	Helotiales #2 Probable *Leohumicola* sp.
J4 23.3	AY970168 Uncultured ascomycete "dfmo1059_179"	100 | 157	AY970168 Uncultured ascomycete "dfmo1059_179"	87 | 147	MUC	–	Ascomycota #19
W1 6.6	EF104234 *Leptosphaerulina trifolii*	99 | 154	EF060560 Uncultured ascomycete "LM221"	100 | 153	AY293803 *Phoma herbarum*	98 | 267	Pleosporaceae #6
W2 3.3	EF151002 *Myrothecium roridum*	100 | 158	AJ301999 *Myrothecium verrucaria*	98 | 194	AJ302003 *Myrothecium verrucaria*	99 | 251	Hypocreales #4 Probable *Myrothecium* sp.
W3 20.0	DQ897651 Septoria sp. Slp-1	100 | 158	AM262410 *Cyathicula* sp. "SS-1847"	99 | 160	EF029230 *Cordyceps cylindrica*	93 | 195	Possible chimeric ARDRA-type #1
W4 20.0	DQ336711 *Aspergillus penicillioides*	100 | 157	MUC	–	AY484896 *Penicillium canescens*	100 | 258	Trichocomaceae #5 Probable *Penicillium* sp.
W5 40.0	DQ865095 *Preussia africana*	100 | 158	MUC	–	MUC	–	Pleosporales #7
W6 3.3	AY773472.1 *Gamsylella parvicollis*	99 | 157	MUC	–	MUC	–	Ascomycota #20
W7 3.3	DQ885895 *Strelitziana africana*	100 | 158	DQ421065 Uncultured soil fungus "151b36"	99 | 229	DQ421059 Uncultured soil fungus 137-59	100 | 222	Ascomycota #21
W8 3.3	DQ307316 *Dendryphiella vinosa*	100 | 158	MUC	–	MUC	–	Ascomycota #22

Table 4 Cultured *Aspergillus* and *Penicillium* species from the cotton-wheat and Brigalow field sites.

Soil	Cotton-wheat I	Brigalow I
Aspergillus		
A. candidus	–	1
A. niger I	4	–
A. niger II	1	–
A. fumigatus I	–	4
A. sp. aff. *fumigatus*	–	1
A. terreus	1	2
A. ustus	1	–
A. sp. nov. I	2	1
A. sp. nov. II	1	–
Penicillium		
P. citrinum	1	–
P. janthinellium	1	–
P. lividum	–	1
P. verruculosum	4	–
P. sp. aff. *citrinum*	4	–
P. sp. nov. I	1	–
P. sp. nov. II	1	–
P. sp. nov. III	–	1
P. sp. nov. IV	4	1

a group that includes a number of plant-pathogenic species. Indeed, several pathogens were isolated to culture from the cultivated soil. Cropping may enrich fungal communities for species tolerant of cropping practices (Matson *et al.* 1997; Midgley *et al.* 2007a) including plant pathogens of the crop species grown.

Aspergillus and *Penicillium* species are globally widespread and occur in a range of different soils (Pitt & Hocking 1997). Both genera include species that are implicated in both solubilisation of sparingly soluble forms of phosphorus and in suppression, or antagonism, of plant pathogens (Vassilev *et al.* 2006). This is of particular importance in Australia, where soils are typically nutrient deficient and mobilisation of phosphorus is important for plant productivity. Soil Trichocomaceae were detected by ARDRA at all but the agricultural sites. However, both *Aspergillus* and *Penicillium* species were readily isolated from the cotton-wheat rotation site in our pilot study, with a trend towards greater species richness of Trichocomaceae in soil from cotton-wheat rotation than from Brigalow (13 versus 7 species). The difference between the molecular and culture-based assessment of Trichocomaceae may be due to the differences in the mode of detection of the organisms in the two methods. Trichocomaceae cultures most likely arise from spores, rather than hyphae (Warcup 1951). Data derived from ARDRA reflect the relative abundance of ascomycete ITS templates in soil DNA extractions, and the subsequent PCR amplifications. In this case, spores, sclerotia and hyphae would contribute to the pool of templates. Our data indicate that the abundance of Trichocomaceae ITS templates from the cropping sites is scarce relative to the less disturbed sites, however, the paucity of knowledge of the relative ease of extraction of DNA from spores, sclerotia and hyphae in soil complicates the interpretation of the results. Assuming that the relative abundance of ITS templates is more representative of fungal communities than the relative abundance of spores, our data indicate that diversity of communities of Trichocomaceae appears to be reduced in cultivated soils. The mechanisms for any reduction are unclear (Matson *et al.* 1997). If the communities of Trichocomaceae include species that inhibit plant pathogens (Weller *et al.* 2002), the relative enrichment of pathogens and depletion of suppressive species in cultivated soils has implications for sustainable agriculture.

Molecular and culture-based data from the current study indicate that soils of the Brigalow Belt biogeographic region of NSW host a diverse community of Ascomycota. The composition of the Ascomycota community varies markedly between sites. While some of the differences may be due to land management and soil chemistry, estimates of diversity reported here were equivalent and highest in the sites with growth of trees or shrubs indicating that the ascomycete community may increase in diversity with least disturbance. Potential plant pathogens were readily detected and potentially suppressive fungi were apparently depleted in agricultural soils relative to the other sites. The change in community structure may result in increased plant disease. The relationship between pathogenic and suppressive fungi needs to be tested in further studies. If the relationship is confirmed, the development of processes to enhance the abundance of the fungal taxa that suppress the action of plant pathogens may be one way to reduce the impact of pathogens on crops.

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