Abundance and diversity of fungi in a saline soil in central-west New South Wales, Australia

M. Bronicka, A. Raman*, D. Hodgkins & H. Nicol

Soil Research Group, Charles Sturt University, P O Box 883, Orange, NSW 2800, Australia

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Soil salinity is a major threat to agricultural productivity and natural ecosystems in Australia. The effect of rising salinity on soil organisms in general and fungi in particular is almost unknown in Australia. The purpose of the study reported in this paper is to examine the effects of salinity on fungal abundance and diversity in a saline soil in a dryland-agricultural region of central-west NSW. Soil fungal abundance and diversity were compared across four sampling sites in spring 2005 and autumn 2006. Species richness, diversity measurement using Shannon-Wiener index, and evenness measurement using Smith and Wilson index of soil fungi were obtained by sampling soil from 0–10 and 10–20 cm depths, and culturing fungi using Dilution plate and Warcup's plate methods. Soil salinity recorded at the time of sampling did not exceed 2 dS/m, but a negative correlation occurred between soil salinity and soil fungal abundance in spring 2005 and autumn 2006 (ANOVA; $p < 0.05$). No relationship between soil salinity and fungal diversity existed. Penicillium was the dominant species in 30–40 % of the identified fungal samples. Also no correlation existed between low fungal abundance and diversity in salt-affected soils with little or no vegetation cover.

Keywords: salt-tolerant, *Penicillium*, soil ecology, Shannon-Wiener index, Smith and Wilson index.

Soil salinity is a serious environmental problem in Australia. Loss of soil biodiversity, decline in soil condition, and degradation of terrestrial ecosystems are some of the key problems induced by salinity (ANZECC 2001, Salinity Research and Development Coordinating Committee 2002, Goss 2003). In Australia, the area affected by salinity includes c. 4.5 % of arable land, involving an annual loss of Au$ 130 m in agricultural production, Au$ 100 m in infrastructure damage, and at least Au$ 40 m environmental assets (CSIRO Land & Water 2004).

In terrestrial environments, soil salinity inhibits the growth and reproduction as well as kills non-salt tolerant vascular plants;

* e-mail: araman@csu.edu.au

moreover these effects are exacerbated by waterlogging (Lymbery et al. 2003). Salinity in agricultural land usually results in either dwarfed or stunted plants due to a decrease in the availability of readily usable water. Plants not adapted to tolerate drought induced by increasing salinity in the soil generally die. Salts concentrated in soil also exert specific ionic effects on plants: for example, many fruit trees are sensitive to high concentrations of Na^+ and Cl^-, which interfere with their metabolism and nutrient uptake (Wood 1995).

Saline soils contain high levels of dissolved salts of Na^+, Ca^{2+}, Mg^{2+}, usually with a conductivity of saturated extracts (ECe) at 4 dS/m and more (Flowers & Flowers 2005). Rising water tables, consequent to clearing of native vegetation, induce soluble salts to rise to the soil surface (Wood 1989, ANZECC 2001) resulting in salinity. Such soils experience seasonally fluctuating groundwater levels with varying salt concentrations between wet and dry periods of the year. However, little is known about the effect of soil salinity on fungi in arable and agricultural lands in Australia (ANZECC 2001, Rietz & Haynes 2003).

Because soil fungi are sensitive to even subtle disturbances and minor environmental changes (Deacon *et al.* 2006), they are affected by increasing salinity and its consequent effects, such as waterlogging, soil compaction, and greater osmotic pressure. For example, stress due to periodic waterlogging, which induces poor soil aeration and soil moisture beyond optimal limits, restricts growth of fungi (Ghassemi *et al.* 1995, NSW DIPNR 2004). Moreover, when soil is waterlogged, the capacity of fungi to catalyze chemical changes (such as carbon and nitrogen mineralization) is adversely affected (Ghassemi *et al.* 1995, Dix & Webber 1995). When the salt-affected soil environment dries due to high evaporation over the warm, dry summer fungi experience dehydration and desiccation (Varnam & Evans 2000). Under such environment-induced stressful conditions, soil fungi experience elevated osmotic potential, which, in turn, affects growth rate because of decline in the hyphal turgor (Dix & Webber 1995).

Extremely hypersaline soil and aquatic environments usually display low fungal abundance and diversity (Adler 1996); and only the xerotolerant and/or osmotolerant species of *Eurotium, Wallemia,* and *Phaeotheca* exist under such conditions (Butinar *et al.* 2005a, 2005b). Xerotolerant fungi (e.g. species of *Aspergillus* and *Penicillium*) grow on dry material with low matric potentials, whereas osmotolerant species (e.g. *Pichia* sp.) grow at very low (–40 MPa) osmotic potentials (Dix & Webber 1995, Manoharachary *et al.* 2005). In saline environments, fungi lower their internal osmotic potential either through the uptake and accumulation of K^+ or through the accumulation of sugars or sugar derivatives (Wood 1995, Deacon

1997). Even at high saline conditions, some species of soil bacteria and fungi remain active, because mineralization by amidases and deaminases proceeds (Laura 1975, Pathak & Rao 1998), provided salinity had not impaired the overall metabolism of the microbe. Nevertheless, the general consensus that prevails currently is that salinity affects filamentous fungi, resulting in not only reduced abundance and diversity, but also reduced efficiency in their ability to utilize organic carbon (Kis-Papo *et al*. 2003, Rietz & Hayes 2003).

Measurement of the direct effects of salinity on soil fungal abundance and diversity in Australia is limited and studies to date have focussed on microbial biomass and soil carbon. Wong *et al*. (2004) found that soil from the southern tablelands in NSW (34°30’45”S) 149°05’00”E; when subjected to increasing salinity and sodicity suffered significant decline in soil carbon, which was detrimental to biomass accumulation.

Against such a background, our study aimed to determine the effect of salinity on soil fungal abundance and diversity on farmland affected by dryland salinity located at Bray’s Flat in central-west NSW. To achieve the aim, the following questions were addressed: (a) Does increasing salinity affect soil fungal abundance and richness at Bray’s Flat? (b) What dominant fungal species occur in the salt-affected soils at Bray’s Flat? (c) Does the presence or absence of pasture vegetation influence the abundance and richness of fungi in the underlying salt-affected soil at Bray’s Flat?

Materials and Methods

The study area

The study area Bray’s Flat is within a 720 ha property (‘Bellevue’) located in the Gumble Creek subcatchment in central-western slopes of New South Wales (33°04’48”S; 148°38’59”E). Soils in Bray’s Flat are predominantly sodosolic, salic hydrosols (Isbell 1996, NSW DPI 2006). Salinity occurs in 2 % of Bellevue and electrical conductivity (ECe) levels in the topsoil at Bray’s Flat have been recorded between 0 and 35 dS/m (NSW DPI 2005). The Gumble Creek subcatchment includes small remnants of native dry sclerophyll species, e.g., *Eucalyptus tricarpa* (L.A.S. Johnson) L.A.S. Johnson & K.D. Hill (red ironbark; Myrtaceae) and *Callitris glaucophylla* Joy Thomps. & L.A.S. Johnson (white cypress pine, Cupressaceae) amidst a landscape dominated by annual crops and grazing pastures with species such as *Phalaris aquatica* Linn. (harding grass, Poaceae), *Bothriochloa macra* (Steud.) S. T. Blake (red grass, Poaceae), and *Trifolium* sp. (Fabaceae) (Kovac *et al*. 1990, NSW DPI 2006).

Climate is temperate. Average annual rainfall is 700 mm, which is seasonally uniform, except for a slight rise in summer. Minimum daily temperature averages at 13.7 °C in December-February (summer) and 0.1 °C in June-August (winter) (Ellis 1992). Warm temperatures prevailed in January and February 2006, with averages of 24 °C and 22 °C, respectively; cool temperatures in September 2005 and April 2006 with averages of 10 °C and 11 °C, respectively. The study area experiences regular episodes of waterlogging in winters and extended periods of aridity in the summers, occasionally interrupted by brief storms.

Soil sampling procedures

Four sampling sites, H (high salinity: >20 dS/m), M (medium salinity: 10–20 dS/m), L (low salinity: 2–10 dS/m), and Z (zero salinity: 0–2 dS/m) were based on the EMI survey results achieved by the Sustainable Grazing on Saline Lands research team (NSW DPI 2005). One 100 m^2 quadrat was constructed in each sampling site. Each quadrat was divided into twenty-five 2 m^2 plots. Two sampling events, the first in September-November 2005 (hereafter referred as 'spring') and the second in March-May 2006 (hereafter referred as 'autumn') were carried out. For each sampling event, five plots were selected randomly (following Wicklow 1973) from each of the three sampling sites H, L, and Z. To determine the effect of either the presence or the absence of vegetation on fungal abundance and diversity in a salt-affected area, six plots were randomly[1] selected at site M only. In spring and autumn, three 'bare' plots (i.e. less than 50 % of the area of the plot was occupied by either grasses or weeds or both) and three 'vegetated' plots (i.e. more than 50 % of the area of the plot was occupied by either grasses or weeds or both) were sampled from site M.

Three soil samples obtained from every plot from depths of 0–10 cm and 10–20 cm were collected with a sterile spade, placed in plastic bags, and transported to the laboratory in a portable car refrigerator (Engel™: Sawafuji Electronic Co, Model MRFT540DG4, Tokyo, Japan) for analysis. The three soil samples from each plot and depth were bulked and sieved using a clean sieve (2 mm, Endecotts™, London, UK), and stored in plastic containers. Forty-two soil samples were obtained in spring and another set of 42 in autumn.

Soil properties including colour, pedality, and texture were analyzed using techniques described in McDonald *et al.* (1998), and Coyne & Thompson (2006). ECe, pH, and moisture content were determined following Rayment & Higginson (1992). Soil organic

[1] Plots at site M were selected randomly until there were three bare and three vegetated plots.

matter was determined using the 'loss-on-ignition' method (Soil and Plant Analysis Council 2000). The Emerson Aggregate Stability Test (Emerson 1967, Charman & Murphy 1991) was used to measure soil structural stability.

Preparation of fungal cultures and identification

Dilution plate (Davet & Rouxel 2000, Elmholt & Labouriau 2005) and Warcup's plate (Davet & Rouxel 2000, Deacon *et al.* 2006) methods were used to obtain fungal abundance and diversity because the choice of only one of the two methods would have generated bias towards either sporulating fungi or fungi isolated from mycelia (Varnam & Evans 2000). Czapek-Dox agar medium with the antibiotic streptomycin (30 mg) was used to isolate soil fungi (Davet & Rouxel 2000, Wicklow 1973). After seven days, fungal isolates were counted using 'colony-forming units' (CFUs[2], McInness & Date 2005) and placed into five recognizable taxonomic units (RTUs[3], Standish 2004) based on cultural characteristics observed by Watanabe (2005).

To determine diversity, fungal isolates from the Dilution Plate and Warcup's Plate methods were placed onto Petri dishes with PDA using a sterile inoculating needle (Wicklow 1973, Davet & Rouxel 2000). The plates were incubated in darkness at room temperature ($25\,^{\circ}\mathrm{C} \pm 2\,^{\circ}\mathrm{C}$) and observed for growth. Based on colony morphological features (colour and surface texture) the fungal isolates were classified as single RTUs for identification down to species level. Each RTU represented a 'morphospecies'. RTUs were determined seeking advice and assistance from Michael Priest (Orange Agricultural Institute, NSW DPI, Orange). Chosen RTUs were smeared on to glass slide, stained with aniline blue, and mounted in lactophenol (University of Adelaide 2005). Mounted samples were observed using a compound microscope (Olympus Biological Microscope, Model CHS/CHT, Tokyo, Japan). The results from Dilution plate and Warcup's plate methods were combined to obtain a total mean for fungal abundance and diversity.

Statistical analysis

Fungal abundance and diversity data were analyzed using Excel® for Windows to generate mean values at each sampling site. Data relating fungal abundance to salinity were fitted by linear regression functions using GenStat® (GenStat for Windows 8th Edi-

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[2] CFU – one fungal colony, which corresponds to one colony-forming unit.
[3] RTU – five RTU groups used were based on fungal colony colour, surface texture, colony margin and pattern; molecular technology was not accessible in this study.

tion, VSN International Ltd., Hemel Hempstead, United Kingdom). Analysis of variance (ANOVA) was used to test differences in fungal abundance between plots. Fungal-diversity determination involved calculating richness (number of different RTUs present), diversity using the Shannon-Wiener index (Franklin *et al.* 2001), and evenness using the Smith and Wilson Index (Krebs 1999).

Results

Soil conditions in all the four sampling sites

Spring and autumn sampling revealed soil colour (0–20 cm) ranging from dark brown to grey brown with orange/yellow mottling. Structure varied from massive to platy at 0–20 cm to angular blocky in the subsoil. Texture grade ranged between sandy loam-silt loam (A horizon 0–20 cm) and clay (B horizon 20–80 cm). In spring the mean soil pH (H_2O) was 6.13 at 0-10 cm, 6.02 at 10–20 cm; soil moisture ranged between 0.09 and 0.2 g/g, and the mean soil organic matter was 1.37 % at 0–10 cm and 0.70 % at 10–20 cm. Aggregates showed moderate to strong slaking and dispersion. Surface vegetation cover was on average 80 %. In autumn the mean soil pH was acidic (5.50 at 0–10 cm, 5.32 at 10–20 cm), soil moisture was 0.03 g/g, and the mean soil organic matter was 2.21 % at 0–10 cm and 1.10 % at 10–20 cm. There was minimal to moderate soil slaking and dispersion, and the vegetation cover was again around 80 %.

Overall, soil salinity levels were considerably lower than previous records for Bray's Flat (cf. NSW DPI 2005). Spring salinity at the four sampling sites ranged between 0 and 0.1981 dS/m, with the highest values recorded at site M (0.1981 dS/m at 0–10 cm; 0.0603 dS/m at 10–20 cm). Autumn salinity results at the four sampling sites ranged between 0 and 0.3387 dS/m, with site M again showing the highest salinity values (0.3387 dS/m at 0–10 cm; 0.0864 dS/m at 10–20 cm).

Fungal abundance

In spring, at higher levels of soil salinity, lower mean fungal abundance was detected. The lowest fungal abundance was at the most salt-affected site M, with 7,067 CFUs/g of soil at 0–10 cm and 2,771 CFUs/g of soil at 10–20 cm. There was a negative correlation for the linear regression plot between total fungal abundance and salinity (ANOVA; df=3; F probability <0001). There was also a significant difference between site M and sites H, L, and Z for the two-way analysis of variance (ANOVA; df=3; F probability <0.001; $p<0.05$).

Autumn fungal abundance at site M did not decrease where there was higher soil salinity, with 18,316 CFUs/g of soil at 0–10 cm and 31,911 CFUs/g of soil at 10–20 cm negative correlation for the linear regression plot between total fungal abundance and salinity (ANOVA; df=3; F probability <0001). There was also a significant difference between site M and sites H, L, and Z for the two-way ANOVA (ANOVA; df=1; F probability <0.001; $p<0.05$) between site M and the other three sampling sites (H, L, and Z).

The linear regression and two-way ANOVA results for both spring and autumn indicate that there was a site interaction between fungal abundance and soil salinity, and that the results for site M were significantly different to the other three sites. Spring and autumn fungal abundance was concentrated at values below 0.1 dS/m (Fig. 1), with autumn showing a greater level of fungal abundance. Fungal abundance was not necessarily greater in the 0–10 cm soil layer and the greatest fungal abundance occurred in autumn (10–20 cm soil layer).

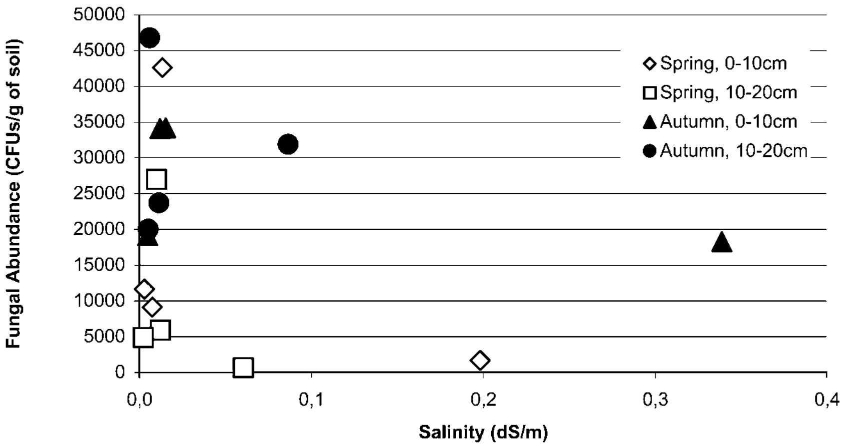

Fig. 1. Scatterplot of mean fungal abundance versus salinity for the two soil layers in spring and autumn.

Fungal diversity

No negative relationship existed between increasing salinity and fungal diversity for spring and autumn. For instance, the mean spring fungal diversity was not the lowest at site M (Tabs. 1 and 3). However, increasing salinity detrimentally affected fungal evenness, with the lowest evenness being at site M. From the 60 identified[4] RTUs isolated in spring (Tab. 2), 21 % were from *Trichoderma* species (Fig. 2). Other soil fungi were species of *Penicillium* (17 %),

[4] From the 3686 pure culture colonies in obtained in spring, 60 of the 101 RTUs were determined to species level.

Fusarium (10 %), *Gliocladium* (6 %), and *Paecilomyces* (3 %). In autumn, 99 RTUs were isolated (Tab. 4), and the most common genus was again *Penicillium* (41 %). Other soil fungi isolated were species of *Gliocladium* (7 %), *Fusarium* (6 %), *Trichoderma* (5 %), *Paecilomyces* (3 %), and *Myrothecium* (3 %) (Fig. 3).

Tab. 1. – Spring fungal abundance, richness, diversity (H') and evenness (R') indices

Site	Abundance	Richness	Shannon (H')	Margalef (R')
H	762	35	0.0495	5.12
M	373	52	0.0205	8.61
L	1042	75	0.0231	10.65
Z	1509	77	0.0356	10.38
Total	3686	101	0.0206	12.18

Tab. 2. – Fungal RTUs isolated from all sampling sites, spring 2005.

RTU	Fungus	No. of colonies	RTU	Fungus	No. of colonies
1	*Paecilomyces lilacinus*	35	**52**	Unknown	6
2	*Fusarium* sp.	63	**53**	*Phoma* sp.	3
3	*Mortierella* sp.	58	**54**	Unknown	5
4	*Penicillium* sp.	14	**55**	*Gliocladium* sp.	2
5	*Trichoderma koningii*	281	**56**	*Penicillium* sp.	11
6	*Gliocladium roseum*	201	**57**	*Penicillium* sp.	20
7	Unknown	12	**58**	Unknown	2
8	*Penicillium* sp.	93	**59**	*Gliocladium viride*	2
9	*Trichoderma* sp.	90	**60**	*Thielavia* sp.	15
10	*Trichoderma hamatum*	346	**61**	Unknown	3
11	Unknown	644	**62**	*Aspergillus* sp.	2
12	Unknown	7	**63**	*Aspergillus* sp.	3
13	*Penicillium* sp.	31	**64**	Unknown	5
14	*Penicillium* sp.	237	**65**	*Penicillium* sp.	6
15	*Penicillium* sp.	2	**66**	Unknown	7
16	*Fusarium oxysporum*	5	**67**	*Aspergillus* sp.	3
17	Unknown	1	**68**	Unknown	1
18	Anamorphic fungus	3	**69**	*Aspergillus* sp.	1
19	*Aspergillus* sp.	11	**70**	*Fusarium* sp.	1
20	Unknown	1	**71**	Unknown	1
21	Unknown	1	**72**	Unknown	1
22	Unknown	14	**73**	*Fusarium* sp.	8
23	Unknown	1	**74**	*Penicillium* sp.	7
24	*Penicillium* sp.	11	**75**	*Penicillium* sp.	29

Tab. 2. – continued.

RTU	Fungus	No. of colonies	RTU	Fungus	No. of colonies
25	Unknown	7	**76**	Unknown	3
26	*Penicillium* sp.	15	**77**	Unknown	7
27	Zygomycota	56	**78**	*Penicillium* sp.	1
28	*Mucor* sp.	61	**79**	Unknown	1
29	*Absidia spinosa*	49	**80**	Unknown	1
30	Unknown	24	**81**	*Penicillium* sp.	5
31	Unknown	31	**82**	Zygomycota	1
32	*Penicillium* sp.	20	**83**	*Thielavia* sp.	14
33	*Paecilomyces* sp.	64	**84**	*Penicillium* sp.	20
34	*Fusarium moniliforme*	75	**85**	*Penicillium* sp.	2
35	Unknown	114	**86**	*Acremonium* sp.	10
36	*Fusarium chlamydosporum*	182	**87**	*Penicillium* sp.	1
37	*Penicillium* sp.	13	**88**	*Penicillium* sp.	18
38	*Fusarium* sp.	10	**89**	Unknown	2
39	Anamorphic fungus	5	**90**	*Memnoniella echinata*	1
40	*Trichoderma viride*	57	**91**	Unknown	1
41	*Penicillium* sp.	19	**92**	*Penicillium* sp.	14
42	Unknown	344	**93**	*Penicillium* sp.	7
43	*Penicillium* sp.	3	**94**	*Penicillium* sp.	1
44	*Chaetomium* sp.	7	**95**	Unknown	13
45	*Fusarium* sp.	32	**96**	*Penicillium* sp.	7
46	Unknown	6	**97**	*Penicillium* sp.	7
47	Unknown	6	**98**	Unknown	6
48	Unknown	12	**99**	Unknown	4
49	Unknown	2	**100**	*Penicillium* sp.	8
50	Anamorphic fungus	8	**101**	*Penicillium* sp.	1
51	*Penicillium* sp.	2	**Total**		3686

* Continued – next column

Tab. 3. – Autumn fungal abundance, richness, diversity (H') and evenness (R') indices.

Site	Abundance	Richness	Shannon (H')	Margalef (R')
H	511	53	0.0265	8.34
M	272	44	0.0218	7.67
L	536	58	0.0191	9.07
Z	567	63	0.0306	9.78
Total	1886	99	0.0162	12.99

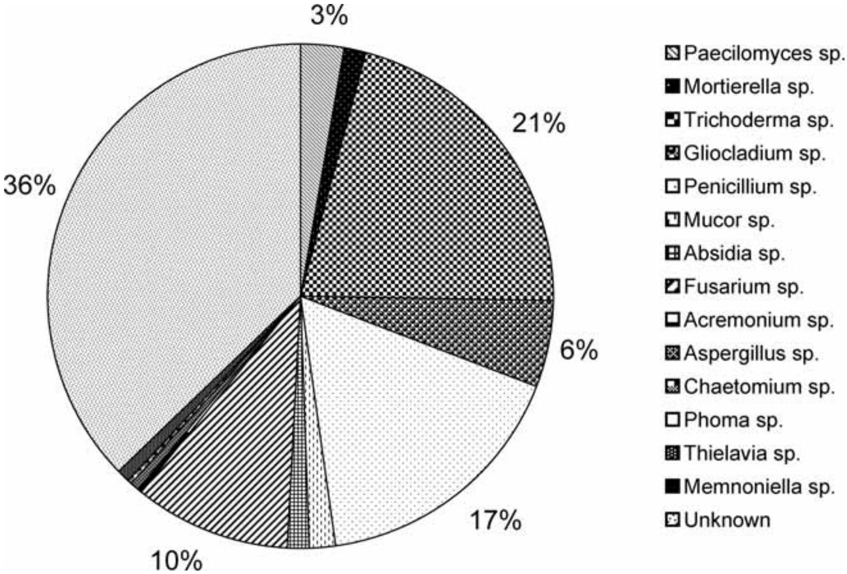

Fig. 2. Fungal genera identified from pure cultures, spring 2005.

Tab. 4. – Fungal RTUs isolated from the sampling sites, autumn 2006.

RTU	Fungus	No. of colonies	RTU	Fungus	No. of colonies
1	*Paecilomyces lilacinus*	48	**113**	*Penicillium* sp.	2
2	*Fusarium* sp.	18	**114**	*Penicillium* sp.	2
3	*Mortierella* sp.	13	**115**	*Penicillium* sp.	1
5	*Trichoderma koningii*	44	**116**	*Penicillium* sp.	3
6	*Gliocladium roseum*	131	**117**	*Penicillium* sp.	1
8	*Penicillium* sp.	3	**118**	*Papulaspora* sp.	4
9	*Trichoderma* sp.	9	**119**	*Penicillium* sp.	45
10	*Trichoderma hamatum*	36	**120**	Unknown	4
11	Unknown	249	**121**	*Penicillium* sp.	6
14	*Penicillium* sp.	83	**122**	*Penicillium* sp.	5
15	*Penicillium* sp.	15	**123**	Unknown	19
18	Anamorphic fungus	5	**124**	Unknown	1
19	*Aspergillus* sp.	2	**125**	Unknown	23
22	Unknown	24	**126**	*Penicillium* sp.	2
24	*Penicillium* sp.	2	**127**	*Acremonium* sp.	1
26	*Penicillium* sp.	88	**128**	*Penicillium* sp.	2
27	Zygomycota	53	**129**	Unknown	4
28	*Mucor* sp.	7	**130**	Unknown	2
29	*Absidia spinosa*	6	**131**	Unknown	8

Tab. 4. – continued.

RTU	Fungus	No. of colonies	RTU	Fungus	No. of colonies
34	*Fusarium moniliforme*	55	**132**	*Penicillium* sp.	3
35	Unknown	77	**133**	*Penicillium* sp.	7
36	*Fusarium clamydosporium*	1	**134**	*Penicillium* sp.	3
37	*Penicillium* sp.	3	**135**	*Aspergillus* sp.	2
38	*Fusarium* sp.	8	**136**	*Penicillium* sp.	6
40	*Trichoderma viride*	2	**137**	*Epicoccum* sp.	2
42	Unknown	1	**138**	*Epicoccum nigrum*	4
45	*Fusarium* sp.	7	**139**	*Myrothecium* sp.	1
46	Unknown	2	**140**	*Penicillium* sp.	53
49	Unknown	1	**141**	*Penicillium* sp.	2
64	Unknown	10	**142**	Unknown	6
70	*Fusarium* sp.	18	**143**	*Cladorrhinum* sp.	2
71	Unknown	2	**144**	*Penicillium* sp.	1
74	*Penicillium* sp.	1	**145**	Unknown	2
79	Unknown	1	**146**	Unknown	1
80	Unknown	12	**147**	*Penicillium* sp.	2
84	*Penicillium* sp.	125	**148**	Unknown	1
91	Unknown	2	**149**	*Penicillium* sp.	1
92	*Penicillium* sp.	29	**150**	Unknown	3
94	*Penicillium* sp.	2	**151**	*Aspergillus* sp.	4
96	*Penicillium* sp.	2	**152**	Unknown	2
99	Unknown	3	**153**	*Penicillium* sp.	4
103	Unknown	4	**154**	*Penicillium* sp.	1
104	*Penicillium* sp.	51	**155**	*Penicillium* sp.	1
105	*Penicillium* sp.	61	**156**	Unknown	6
106	Unknown	32	**157**	*Melanospora* sp.	4
107	*Penicillium* sp.	212	**158**	Unknown	1
108	*Penicillium* sp.	1	**159**	Unknown	1
109	Unknown	1	**160**	Unknown	1
110	Unknown	60	**161**	*Fusarium* sp.	1
111	Unknown	1	**162**	*Penicillium* sp.	1
112	*Penicillium* sp.	3	**Total**		1886

Vegetation cover and soil conditions – site M

The vegetated plots in spring generally had a higher pH (6.0 at 0–10 cm, 5.5 at 10–20 cm), higher soil moisture (0.1 g/g at 0–10 cm and 10–20 cm), and soil organic matter (0.7 % at 10–20 cm) compared with the bare plots (pH – 5.3 at 0–10 cm and 5.5 at 10–20 cm, soil

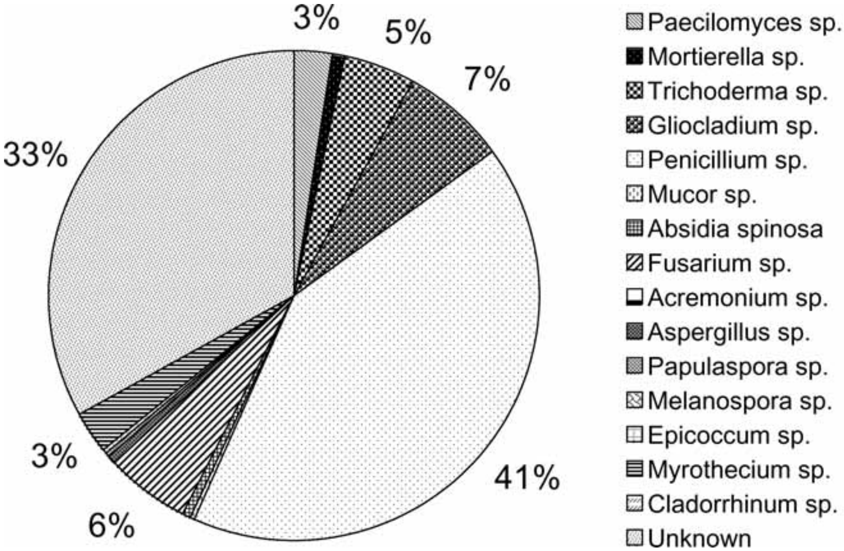

Fig. 3. Fungal genera identified from pure cultures, autumn 2006.

moisture – 0.09 % at 0–10 cm and 0.10 % at 10–20 cm, soil organic matter – 1.1 % at 0–10 cm and 0.5 % at 10–20 cm). In autumn the vegetated plots also had a higher pH (6.3 at 0–10 cm, 6.4 at 10–20 cm), and soil organic matter (2.4 % at 0–10 cm, 1.1 % at 10–20 cm) compared with the bare plots (pH – 5.6 at 0–10 cm and 5.0 at 10–20 cm, soil organic matter – 1.5 % at 0–10 cm and 0.9 % at 10–20 cm). The bare plots had higher soil moisture content (0.03 g/g at 0–10 cm, 0.06 g/g at 10–20 cm) than the vegetated plots (0.03 g/g at 0–10 cm, 0.04 g/g at 10–20 cm).

Soil salinity was the highest in the bare plots in spring and autumn (spring, 0.3689 dS/m at 0–10 cm and 0.0857 dS/m at 10–20 cm, autumn, 0.6135 dS/m at 0–10 cm and 0.1545 dS/m at 10–20 cm), with the highest mean soil salinity results being in autumn.

Vegetation cover and fungal abundance

In spring, the bare plots had the highest mean fungal abundance at 0-10 cm with 7,446 CFUs/g of soil and the lowest fungal abundance at 10–20 cm with 886 CFUs/g of soil. In autumn the vegetated and bare plots at 10–20 cm showed the highest fungal abundance with 32,471 CFUs/g of soil and 31,352 CFUs/g of soil respectively. The vegetated plots at 0–10 cm had the lowest fungal abundance with 14,401 CFUs/g of soil, followed by the bare plots at 0–10 cm with 22,231 CFUs/g of soil. Spring fungal abundance was concentrated at values below 0.1 dS/m (Fig. 2), except for the greater abundance at a higher salinity for the bare plots at 0–10 cm. In autumn the bare plots had a fungal abundance above 20,000 CFUs/g of soil over 0.1 dS/m.

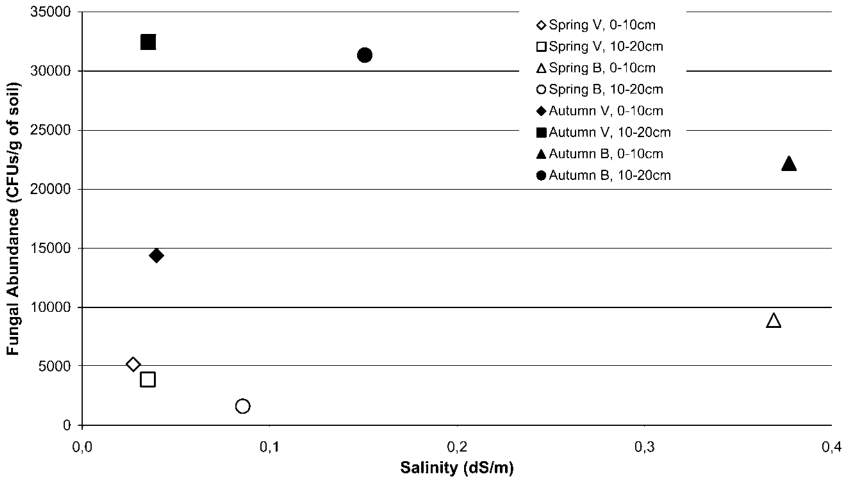

Fig. 4. Scatterplot of mean fungal abundance versus salinity for site M at the bare and vegetated plots in spring and autumn.

Vegetation cover and fungal diversity

Apart from the lower evenness in autumn, the bare plots did not have a reduction in fungal richness, diversity, and evenness in spring and autumn (Tabs. 5 and 6). In spring the vegetated plots at 0–10 cm had the greatest fungal diversity (H' 4.5264) and in autumn the vegetated plots at 0–10 cm had the greatest fungal evenness (E_{var} 4.1489).

In spring 61 % of the identified RTUs isolated in the vegetated plots were from *Trichoderma* species. Other soil fungi were species of *Penicillium* (44 %), *Mucor* (20 %), *Paecilomyces* (9 %), *Fusarium* (8 %), and *Acremonium* (6 %). The identified RTUs isolated in the bare plots were mainly *Penicillium* species (64 %), *Gliocladium* (13 %), *Fusarium* (6 %), *Mortierella* (5 %), *Paecilomyces* (4 %), and *Trichoderma* (4 %).

In autumn the most common genus in the vegetated plots was *Penicillium* (78 %). Other soil fungi isolated were species of *Paecilomyces* (8 %), *Fusarium* (5 %), *Aspergillus* (5 %), *Trichoderma* (5 %), and *Gliocladium* (5 %). The identified RTUs isolated in the bare plots were mainly *Penicillium* species (37 %), *Trichoderma* (36 %), and *Paecilomyces* (9 %).

Tab. 5. – Spring fungal abundance, richness, diversity (H') and evenness (R') results for site M

Plot	Abundance*	Richness	Shannon (H')	Margalef (R')
Vegetated (0–10 cm)	134	27	0.0198	5.31
Vegetated (10–20 cm)	118	21	0.0496	4.19
Vegetated (Total)	252	44	0.0272	7.97
Bare (0–10 cm)	34	15	0.0373	3.97
Bare (10–20 cm)	69	23	0.0406	5.20
Bare (Total)	103	32	0.0264	6.69

*Fungal abundance calculated from pure cultures.

Tab. 6. – Autumn fungal abundance, richness, diversity (H') and evenness (R') results for site M

Plot	Abundance*	Richness	Shannon (H')	Margalef (R')
Vegetated (0–10 cm)	33	14	0.0279	3.72
Vegetated (10–20 cm)	46	21	0.0415	5.22
Vegetated (Total)	79	23	0.0275	5.03
Bare (0–10 cm)	87	24	0.0233	5.15
Bare (10–20 cm)	107	30	0.0241	6.21
Bare (Total)	194	37	0.0223	6.83

*Fungal abundance calculated from pure cultures.

Discussion

Changes in environmental conditions, such as soil salinity, induce stress on soil microbial communities. Severity and duration of changes in environmental conditions affect fungal survival rates and their ability to adapt and establish in the soil environment (Macdonald 1977, Varnam & Evans 2000). Stress induced by soil salinization can lead to either decline in numbers of fungi or changes in their diversity (Varnam & Evans 2000). Keeping these in view, we evaluated and determined the effect of salinity on the abundance and diversity of fungi in Bray's Flat.

Soil conditions during spring and autumn were non-saline due to regular storm events in spring and summer (2005–2006), which may have flushed salts from the topsoil (0–20 cm), and thus moderated them to the levels recorded in this study. However, these transient levels are consistent with seasonal variation of ECe in topsoil underlain with sodic subsoil in large areas of south-eastern Australia (Rengasamy 2002), where rising groundwater is not *the* key determinant factor at the site all year around.

At a higher salinity level (over 0.1 dS/m) fungal abundance at Bray's Flat was detected to be low, which accords with the findings of Adler (1996) and Butinar *et al.* (2005a, 2005b). Because soil fungi are sensitive to disturbance and environmental change (Deacon *et al.* 2006), future research at Bray's Flat when soil salinity will be more than 4 dS/m would provide an interesting comparison to the detrimental effects on fungal abundance from increasing salinity in non-saline soils. Fungal diversity in this study was not necessarily lower at a higher salinity. The salinity ranges at the present study may not have been high enough to be detrimental for fungal diversity. The results may change once the ECe is over 2 dS/m (i.e. when salinity becomes a problem for most of the vascular plants).

Low evenness results for site M compared with the results from sites H, L, and Z indicate that dominant fungi were present at greater levels of soil salinity. But conflicting evenness results on the presence of dominant fungal species at site M rendered it difficult to determine whether dominant fungi were present only in bare salt-affected areas. The most dominant fungus at Bray's Flat in both spring and autumn was *Penicillium* and *Trichoderma*, other salt-tolerant species, such as *Fusarium, Trichoderma, Gliocladium*, and *Aspergillus* occurred in varying degrees. Dix and Webber (1995) found similar results establishing that species of *Penicillium* were generally dominant in temperate areas, whereas species of *Aspergillus* were dominant in either arid or semiarid areas. Should *Bray's Flat* become hypersaline in the near future, then species of *Aspergillus* are likely to gain dominance over the others (Suryanarayanan & Hawksworth 2005).

This study also found that although the bare plots had greater salinity levels compared with vegetated plots, the absence of vegetation at site M did not have a detrimental effect on fungal abundance and diversity. The levels of soil-organic matter were not lower when vegetation was absent at site M and so the fungi still had a nutrient supply for reproduction and growth (Dix & Webber 1995, Varnam & Evans 2000). It would be worth investigating fungal abundance and diversity at Bray's Flat when the level of soil organic matter was lower in the plots with little vegetation.

Conventional culturing techniques such as the Dilution Plate and Warcup's Plate methods are in currency in microbial ecological studies (Subba Rao 1999, Elmholt & Labouriau 2005, Deacon et al. 2006). However, these methods have a disadvantage of being selective and only those fungi suitable to the 'nutrients' in those media will grow and sporulate (Garrett 1981, Cannon 1996). Molecular methods such as Universally Primed PCR (UP-PCR) (Lubeck &

Lubeck 2005) and gradient gel electrophoresis (DGGE) (Anderson & Cairney 2004), although appropriate, could not be used in the present study due to their inaccessibility.

In conclusion, in the current study we have found that even at low levels salinity in soil at Bray's Flat does detrimentally affect soil fungal abundance, while fungal diversity did not drop with greater levels of salinity. *Penicillium* and *Trichoderma* to be the dominant fungi at Bray's Flat, with 17 % of the identified RTUs in spring and 41 % of the identified RTUs in autumn. We also have found that the absence of vegetation cover at a salt-affected site may not necessarily have a reduced fungal abundance and diversity.

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