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Growth and survival of seedlings of native plants in an impoverished and highly disturbed soil following inoculation with arbuscular mycorrhizal fungi

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Abstract We investigated whether arbuscular mycorrhizas influenced growth and survival of seedlings in an extremely impoverished and highly disturbed soil. Seedlings of four plants species native to the site were either inoculated with native sporocarpic arbuscular mycorrhizal (AM) fungi or fertilised prior to transplanting, and followed over 86 weeks at the site. One treatment was also irrigated with N-rich leachate from the site. In a laboratory experiment, seedlings were fertilised with excess P for 6 weeks, and location of the P store determined. Growth and survival of AM and fertilised seedlings were similar at the site. Inoculated mycorrhizal fungi and roots appeared to extend into the surrounding soil together. P concentration in leaves of all plants was extremely low. Irrigation with leachate increased growth of seedlings. In the laboratory experiment, significantly more P was stored in roots than shoots. We suggest that successful revegetation of extremely disturbed and impoverished sites requires selection of mycorrhizal fungi and plants to suit the edaphic conditions and methods of out-planting.

Keywords Arbuscular mycorrhiza · Soil disturbance · Phosphorus deficiency · Plant growth · Plant survival

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Introduction

Managed re-establishment of plant communities following severe land disturbance, such as strip-mining, is becoming normal practice. Severe disturbance not only removes the vegetation, the remaining soil often lacks a developed structure, organic matter, significant biological activity, and may have low concentrations of available minerals (Bradshaw 1983). Re-establishment of a vegetative cover may require alleviation of these limitations.

Most plants in native vegetation are commonly found with mycorrhiza. Laboratory studies indicate that the presence of mycorrhizal fungi aids establishment and growth of a diversity of plant species in soils with little available P. Mycorrhizal fungi increase plant uptake of minerals especially P, and improve soil structure. Indeed, diversity of fungi and plant species, plant biomass production and removal of P from soil appear to be correlated in experimental microcosms (van der Heijden et al. 1998). Mycorrhizal fungi also enable the establishment of a microbial population in the soil beyond the roots following the spread of hyphae (Jakobsen and Rosendahl 1990). Thus, establishing and increasing the population of mycorrhizal fungi, especially arbuscular mycorrhizal (AM) fungi, appears to be an essential component of the revegetation process at degraded sites (Herrera et al. 1993; Jasper 1995).

Inoculation of plants with AM fungi still only occurs on a small scale in revegetation of disturbed sites. Few studies have clearly demonstrated the benefits of inoculation (Jasper 1994a). The lack of demonstrated benefit may be due to the use of inappropriate strains of fungi, relatively high available P in the soil, inappropriate inoculation of mycorrhizal fungi, inability of introduced AM fungi to establish in the soil, and large variation in rates of plant growth because of heterogeneity of field soils (Fitter 1985; Jasper 1994b) and genetic diversity within plant species. Use of AM fungi and plants adapted to the local soil may be necessary to determine the importance of mycorrhizas to revegetation of disturbed sites.

The lack of benefit to plants of the presence of mycorrhizal fungi may be associated with extremely low concentrations of available P in some soils. Some plant species have relatively low mycorrhizal colonization in roots when growing in soil with low available P (Bolan et al. 1987). In addition, plants from some soils known to have little available P, such as the Hawkesbury sandstone near Sydney, Australia, apparently have limited colonization in their roots (Bellgard 1991). However, many plant species from Australia are adapted to low P soils, have a low critical concentration of P in their tissues, and appear to translocate P within the plant (Beadle 1968; Handreck 1997). Mycorrhizal plants are found widely apparently competing effectively with non-mycorrhizal plants under P-impoverished conditions (Belgard 1991; McGee 1986) indicating that the association benefits the plant. Thus, we predict AM fungi will benefit seedlings of native plants in these severely impoverished soils.

We chose a severely disturbed site on Hawkesbury sandstone that is used for the disposal of waste. A previous attempt to revegetate part of the site using conventional practices resulted in a very limited number of plants and plant species being established. We tested whether survival and growth of plant species native to an impoverished soil improved when colonized by AM fungi from that soil, and whether the effect is related to increased uptake of P. In addition, the water that leached through the waste, called leachate (Pattinson et al. 2000), was irrigated onto the plants to test whether addition of N may aid plant survival and growth.

Materials and methods

The site

Lucas Heights Waste Disposal Centre (LHWDC) is located 30 km south-west of Sydney. The site, on Hawkesbury sandstone, has been excavated and then back-filled with multiple layers of non-putrescible waste. Each excavation is capped to a depth of at least 60 cm with crushed sandstone which is rolled by heavy equipment to create the "soil" (Table 1). The overall goal of revegetation is to return the site to a form indistinguishable from surrounding native vegetation.

Growth and survival of seedlings at LHWDC

The experiment was set up in a split plot design with five main blocks and three split plots. One of three irrigation treatments: (1) leachate, (2) dam water, or (3) no irrigation (control), was randomly allocated to each split plot. The majority of seedlings irrigated with leachate died between December 1998 and January1999, and the experimental design was altered. Dilute leachate (Pattinson et al. 2000) was irrigated on plots previously irrigated with dam water. The plots irrigated with full-strength leachate were removed from the experiment. Irrigation with dilute leachate commenced in June 1999.

Irrigation pipes were installed parallel to each other, 2 m apart, with 2 m spacing between individual drippers. Seedlings were placed adjacent to each dripper. Seedlings grown in the unirrigated control plots were also planted in a 2 m×2 m grid. The 2-m spacing was selected in an attempt to ensure separation of roots of seedlings and mycorrhizal fungi for the duration of the experiment. Plants were irrigated automatically via drippers for 1 h each day. The flow rate from drippers of 3 l h^{-1} was adequate to produce a leaching fraction large enough to prevent salt build-up (Table 1) in the root zone. Irrigation was interrupted occasionally because of difficulties in filling tanks during periods of heavy rainfall and mechanical breakdowns.

Seed of four plant species, *Dodonaea triquetra* Wendl. (Sapindaceae), *Callitris rhomboidea* R.Br. ex A. & L.C. Rich. (Cupressaceae: both species have large seeds and are exclusively AM, G. S. Pattinson, unpublished data), *Leptospermum polygalifolium* J. Thompson (Myrtaceae), and *Eucalyptus gummifera* (Sol. ex Gaertn.) Hochr. (Myrtaceae: both species have small seeds and were found at the site with both AM and ectomycorrhiza, G. S. Pattinson, unpublished data) was collected from undisturbed vegetation adjacent to LHWDC. After germination on sterile moist sand, seedlings were transplanted into tree tube pots, 5×5×12 cm (width×length×height) filled with autoclaved 30% Lucas Heights soil mix (Pattinson et al. 2000). Half the seedlings were inoculated with a mixed inoculum of the sporocarpic fungi *Glomus pellucidum* McGee & Pattinson and *Glomus atrouva* McGee & Pattinson (McGee and Trappe 2002) by placing approximately 1 g of chopped, colonized fresh root and adherent soil from pot cultures of *Allium porrum* L. adjacent to roots of seedlings at transplantation. The fungi were originally extracted from scats of animals trapped in a similar, undisturbed, habitat (McGee and Baczocha 1994). Seedlings were grown in a glasshouse at 18–22°C for 3 months and hardened outside for 3 weeks before out-planting.

All unfertilised and nonmycorrhizal seedlings died. In an attempt to obtain plants with similar shoots, two or five fertiliser pellets of Osmocote for Australian Natives were added to the pots of mycorrhizal and non-mycorrhizal seedlings, respectively. The shoot growth of fertilised non-mycorrhizal seedlings was slower than mycorrhizal seedlings after 2 months, so the former were fertilised once with a liquid fertiliser, Aquasol, prepared as per the manufacture's directions, applied once at a rate of approximately 50 ml pot^{-1}.

Six mycorrhizal and six uninoculated seedlings each of *D. triquetra*, *C. rhomboidea* , *L. polygalifolium* and *E. gummifera* were sown randomly in each split plot in September 1998, and all were watered once in the first week. Ten litres of organic mulch was placed around the base of each seedling to reduce evaporation. Tree guards were placed around each seedling to reduce damage from pests.

A further five inoculated and uninoculated seedlings of each species from the glasshouse were destructively harvested. The soil was rinsed from the roots and the shoots and the roots weighed. A subsample of roots was cleared and stained (Phillips and Hayman

Table 1 Characteristics of capping material from Lucas Heights Waste Disposal Centre

Soil property	
Particle size analysis, (rocks removed)	8–16% Clay, 15% silt, 23% fine sand, 46% coarse sand
$EC_{1:5}$	0.01–0.1 dS m^{-1} (Department of Natural Resources Queensland 1997)
Cl^- (air-dry soil)	20–100 μg g^{-1}
$pH_{1:5}$ (in water)	4.5–5.5
Total C	<1.0%
Total N	<1.0%
NH_4^+	<5 μg g^{-1}
Available P Bray no. 2	1 μg g^{-1} (0.001%) (Rayment and Higginson 1992)

1970) and the proportional AM colonization determined by the grid intersect method (Giovannetti and Mosse 1980). The shoots were dried at 70°C for 24 h and weighed. P content of the leaves was determined using the ammonium-molybdate method (Allen et al. 1974).

Two mycorrhizal and two uninoculated plants of each species were destructively harvested from each split plot after 20, 66 and 86 weeks. Because of considerable variability in plant growth, mean-sized seedlings were chosen from each treatment, rather than at random. At harvest, as much of the root system as possible was removed. It was possible to extract only a small portion of root system due to the rocky nature of the capping. The soil was rinsed from the roots. A sub-sample of at least 0.5 g fresh weight fine roots was taken, cleared and stained, and the proportion colonized by AM fungi determined. The shoots were dried and weighed. At the first harvest, P content of the leaves of plants grown in control plots was determined. At the second harvest, P content of the leaves, and stems with a diameter <3 mm, of plants grown in the control plots was separately determined. The fine part of the stem is metabolically active and is where differences in P concentration between treatments is possible (Beadle 1968). Because so little of the root system could be recovered, P analyses of roots were not attempted with field plants. Seedling survival was assessed at irregular intervals through the experiment.

The data on mean shoot dry weight, proportion colonisation, proportion of and total P in shoots at out-planting were compared between inoculated and uninoculated seedlings within host treatment by one-way ANOVA. After 20 weeks, the shoot dry weight and proportion colonisation of plants were analysed using split-plot ANOVA, with five main blocks. The main plot treatment was irrigation (nil, leachate, water). Within each plot, the four plant species, either inoculated or uninoculated, were assigned randomly. The analysis examined the effect of irrigation and mycorrhizal inoculation within plant species. Differences among species were not compared. Data were analysed using CoSat (Cohort Software), and differences between means determined using Student-Newman-Keuls (SNK) tests. The data on the proportion colonisation were arcsine transformed prior to analysis (Zar 1996). The data on the concentration of and total P in shoots between inoculated and uninoculated seedlings were compared by one-way ANOVA within host plant treatment.

Spread of AM fungi in soil

Due to the difficulty of recovering roots from the capping material, the establishment of AM fungi under *D. triquetra* only was determined after 22 and 59 weeks by bioassay using seedlings of *Acacia elongata* as trap plants. We used a different plant species because we could recognise the roots of the experimental plant among roots of the trap plant, and *A. elongata* had established in disturbed areas of the site. Two mycorrhizal and two uninoculated *D. triquetra* plants were randomly selected within each split plot. Six-week-old seedlings of *A. elongata* were planted at three predetermined distances (10, 20 and 40 cm, in the first assay: 20, 30 and 40 cm in the second assay) from the base of the *D. triquetra* seedlings, along randomly oriented transects. The seedlings were randomly selected from a given transect, at each distance, and harvested (at 2, 4 and 6 weeks, in the first assay; 4 and 6 weeks, in the second assay). The soil was rinsed from the roots of the trap plant, and the proportion of the roots of the trap plant colonized by AM fungi determined by the grid intercept method (Giovanetti and Mosse 1980).

The mean proportion colonisation of roots of *A. elongata* trap plants grown at three distances from inoculated and uninoculated *D. triquetra* seedlings after 20 weeks in the field, were compared by *t* -tests at each distance, at each harvest. *t* -tests were used instead of ANOVA because of large variation in the colonization data and variable survival of trap plants. The data from the bioassay at 59 weeks were analysed by one-way ANOVA, within the irrigation treatments, at each harvest. Data on proportion colonization were arcsine transformed prior to analysis (Zar 1996).

Soil pH and EC

The EC and pH (1:5 H_2O) (Rayment and Higginson 1992) of the field soil under each treatment were determined from samples of soil collected in each sub plot, adjacent to a randomly selected seedling, at two depths, 0–5 and 15–20 cm, at each harvest. The data on the pH and EC of the soil were analysed by two-way ANOVA, with factors irrigation and depth as factors. Differences between means were determined by Tukey's honestly significant difference (HSD) test.

P allocation in seedlings

As it was impossible to extract a significant proportion of the roots, and minimal P was found in the shoots of field plants, storage of P was examined in a series of laboratory experiments. The sclerophyllous *Acacia longifolia* var. *sophorae* (Labill.) F. Muell, was used because it is also adapted to nutrient-poor sandy soil of the Sydney region and has a wide range of seed size. Seed size may be important if P stored in the seed influences subsequent P nutrition of seedlings. The fully factorial experiment reported here consisted of two seed sizes, small (<0.015 g) and large (>0.022 g), and two mycorrhizal treatments, uninoculated, or inoculated using a plug of approximately 1 g colonised onion root. The fungi were a mixture of *G. pellucidum, G. atroauva*, *Glomus feugianum* (Spegazzini) Trappe and Gerd. and *Glomus macrocarpum* Tul. and Tul. Pregerminated surface sterilised seeds of *A. longifolia* were placed in a small (30×50×80 mm) pot. All pots were then placed in the growth room. A nutrient solution containing 435 μM KH_2PO_4, 3.3 mM KNO_2, 1 mM $Ca(NO_3)_2.4H_2O$, 0.6 mM $MgSO_4.7H_2O$ and 1 ml l^{-1} each of micro-nutrient solution and FeEDTA was applied at a rate of 10 ml pot^{-1} $week^{-1}$. In the data reported here, we were only interested in where P was stored, so each pot received P in excess to requirements for growth of *A. longifolia* (data on P response curve not shown). Five replicates of each treatment were destructively harvested after 6 weeks. Shoot and root dry weight, proportion of root colonised, P content and concentration of shoots and roots, in each size class were determined. Data were analysed using ANOVA and Tukey's HSD tests.

Results

Growth and survival of seedlings.

In each species, the shoot dry weights of inoculated and uninoculated seedlings were variable at out-planting, though only mycorrhizal *D. triquetra* were statistically significantly (P<0.05) larger than the equivalent uninoculated seedlings. While AM were absent from uninoculated seedlings, patches of ectomycorrhiza were observed in the roots of *E. gummifera* and *L. polygalifolium*. The concentration and total P in shoots were lower in inoculated than in uninoculated seedlings of *E. gummifera*, *C. rhomboidea* and *D. triquetra* (Table 2).

The survival of species differed depending on the irrigation treatment. Between 60 and 100% of seedlings irrigated with full-strength leachate and no more than 10% in the other treatments died (data not shown). Because of the loss of most seedlings irrigated with full strength leachate, the seedlings irrigated with water were irrigated with half-strength leachate, commencing 6 months prior to the second destructive harvest at 66 weeks.

In the first harvest after 20 weeks, the size of the plants varied with mycorrhizal colonisation and irrigation treat-

Table 2 Mean (±SE) shoot dry weight (*SDW*), proportion of arbuscular mycorrhizal (AM) colonisation in roots, and P concentration and total P (μg) in leaves of *Eucalyptus gummifera, Callitris rhomboidea, Dodonaea triquetra*, and *Leptospermum polygalifolium* inoculated (*+AM*) or not (*−AM*) with AM fungi at out-planting

		SDW (mg)	% AM	% P	Total P
E. gummifera	+AM	461±82	90±1*	0.07±0.02	188±10*
	−AM	524±72	0±0	0.16±0.04	547±101
C. rhomboidea	+AM	946±215	89±3*	0.08±0.01*	638±148*
	−AM	1,125±254	0±0	0.19±0.02	1,655±364
D. triquetra	+AM	896±99*	89±1*	0.05±0.01*	330±90*
	−AM	245±45	0±0	0.66±0.04	1,171±194
L. polygalifolium	+AM	1,747±212	73±1*	0.06±0.00	531±84*
	−AM	978±320	0±0	0.05±0.01	276±50

*$P<0.05$ (within host plant treatment)

Table 3 Mean (±SE) SDW, proportion of AM colonisation of roots, and proportion P in leaves of *E. gummifera, C. rhomboidea, D. triquetra*, and *L. polygalifolium* under no irrigation (*Control*), irrigated with dam water, harvested after 20 weeks. Means within irrigation treatments followed by the *same letter* do not differ significantly ($P<0.05$)

		SDW g		% AM		% P Control
		Control	Water	Control	Water	
E. gummifera	+AM	3.98±1.47a	4.65±1.32a	83±5a	69±4b	0.04±0.00
	−AM	5.11±1.64a	9.46±1.32b	0±0c	0±0c	0.05±0.01
C. rhomboidea	+AM	3.60±0.48ab	4.58±0.18a	83±3a	82±2a	0.07±0.01*
	−AM	1.97±0.27b	3.72±0.63ab	0±0c	25±12b	0.05±0.01
D. triquetra	+AM	6.39±2.47a	4.97±2.50a	81±4a	85±2a	0.03±0.01
	−AM	3.83±2.14a	10.08±4.67a	3±3b	3±2b	0.04±0.01
L. polygalifolium	+AM	4.81±1.57a	7.40±1.16a	66±17a	77±7a	0.03±0.00
	−AM	7.26±0.97a	11.41±2.80a	6±6b	8±6b	0.04±0.00

*$P<0.05$ (within inoculation treatment)

Table 4 Mean (±SE) SDW, proportion of AM colonisation of roots, and P concentration in leaves and stems of *E. gummifera, C. rhomboidea, D. triquetra*, and *L polygalifolium* under no irrigation (*Control*) or irrigated with dilute leachate, and harvested after 66 weeks. Means within irrigation treatments followed by the *same letter* do not differ significantly ($P<0.05$)

		SDW g		% AM		% P-Leaves Control	% P-Stems Control
		Control	Leachate	Control	Leachate		
E. gummifera	+AM	19.5±3.5a	33.4±8.0a	52±13a	71±5a	0.02±0.00	0.03±0.01
	−AM	16.2±2.8a	38.3±16.3a	6±3b	5±2b	0.03±0.00	0.03±0.01
C. rhomboidea	+AM	27.1±6.3ab	58.8±17.3a	70±4a	73±4a	0.03±0.00	0.03±0.00
	−AM	13.7±3.9b	14.5±2.8b	35±10b	11±6b	0.03±0.01	0.03±0.00
D. triquetra	+AM	25.3±5.7a	46.6±13.1a	62±12a	74±5a	0.03±0.01	0.03±0.01
	−AM	22.9±11.4a	32.5±12.0a	29±12b	30±8b	0.03±0.00	0.03±0.00
L. polygalifolium	+AM	29.3±9.9a	49.3±15.4a	64±3a	63±9a	0.02±0.00	0.02±0.00
	−AM	35.8±13.8a	49.4±19.2a	14±5b	8±4b	0.03±0.00	0.03±0.01

ments. Seedlings of *E. gummifera* inoculated with AM fungi tended to weigh less than uninoculated seedlings (Table 3; all data on seedlings irrigated with leachate were removed from analysis), and were significantly ($P<0.05$) lighter in the plots irrigated with dam water. Within irrigation treatments, shoot dry weights of inoculated and uninoculated plants of *C. rhomboidea*, *D. triquetra* and *L. polygalifolium* were similar. Seedlings irrigated with water tended to be larger than seedlings grown in the control plots, being statistically significant ($P<0.05$) for *E. gummifera* and *C. rhomboidea* (Table 3).

All inoculated seedlings had AM. Low levels of AM were also observed in some uninoculated seedlings. The concentration and total P in the leaves was statistically higher ($P<0.05$) in the inoculated than uninoculated *C. rhomboidea* seedlings. No significant ($P>0.05$) differences in proportion and total P concentration in leaves were detected for the other plant species (Table 3).

In the second harvest, after 66 weeks, inoculated seedlings tended to be heavier than uninoculated seedlings, though the difference was statistically significant ($P<0.05$) only for *C. rhomboidea*. Irrigated and unirrigated seedlings had similar though variable shoot dry weight. Inoculated seedlings had similar colonisation whether or not they were irrigated. The effect of the factors irrigation and mycorrhizal inoculation interacted within the *C. rhomboidea* treatment only, where differences in means between the inoculated and uninoculated treatments, within the irrigation treatment were compared. The P data on seedlings grown in block one were removed from the analysis, because of intermittent flooding of the block. The concentration of P in leaves and stems, and total P in the leaves were not significantly different ($P>0.05$) between the inoculated and uninoculated seedlings of all plant species (Table 4).

Table 5 Mean (±SE) SDW, proportion of AM colonisation of roots, and P concentration in shoots of *E. gummifera, C. rhomboidea, D. triquetra*, and *L. polygalifolium* under no irrigation (*Control*) or irrigated with dilute leachate, and harvested after 86 weeks. Means with *different letters* are statistically significant ($P<0.05$)

		SDW g		% AM		% P	
		Control	Leachate	Control	Leachate	Control	Leachate
E. gummifera	+AM	73±26a	125±75a	71±4a	49±14ab	0.03±0.01	0.03±0.01
	−AM	79±52a	169±58a	10±10b	6±6b	0.03±0.01	0.02±0.00
C. rhomboidea	+AM	33±11ab	119±40b	59±5a	48±10a	0.04±0.01	0.04±0.01
	−AM	28±11ab	22±11a	10±9b	19±8ab	0.04±0.00	0.03±0.01
D. triquetra	+AM	45±15a	292±144b	61±7ab	74±4a	0.03±0.00	0.03±0.01
	−AM	25±16a	36±9a	29±11b	44±9ab	0.03±0.00	0.03±0.00
L. polygalifolium	+AM	28±7a	95±26b	49±3a	59±1a	0.04±0.00	0.03±0.00
	−AM	24±6a	47±20ab	7±6b	17±10b	0.04±0.00	0.03±0.00

Table 6 Mean (±SE) proportion of AM colonisation of roots of *Acacia elongata* trap plants grown in the field 10, 20 and 40 cm from the base of *D. triquetra* after 20 weeks in the field and harvested at 2, 4 and 6 weeks. *NA* Not analysed

Distance from colonised host (cm)	Week 2		Week 4		Week 6	
	+AM	−AM	+AM	−AM	+AM	−AM
Not irrigated 10	15±5*	0±0	26±3*	0±0	43±5*	2±2
20	12±4*	0±0	29±4*	0±0	43±12*	1±1
40	0±0	0±0	4±2	0±0	16±11*	0±0
Irrigated 10	14±6	1±1	41±4*	2±2	NA	16±12
20	8±6	1±1	28±5*	3±3	56±8*	13±7
40	0±0	0±0	0±0	9±7	4±3	0±0

*$P<0.05$ (between mycorrhizal treatments at the given distance)

After 86 weeks, differences in the shoot dry weights of seedlings of each species in the control treatment were not statistically significant ($P>0.05$). However, for both exclusively AM species *C. rhomboidea* and *D. triquetra* in the irrigated plots, inoculated seedlings were significantly heavier ($P<0.05$) than uninoculated plants. All plants had AM present in roots, though a larger proportion of root of inoculated plants was colonised. Irrigation with dilute leachate after 66 weeks did not significantly influence the level of colonisation. The concentration of P in leaves of all seedlings was similar whether irrigated with dilute leachate, or inoculated with AM fungi (Table 5).

Apart from the effect of leachate at the first harvest, treatment did not influence survival of seedlings. More plants of all species died during summer than any other season (data not shown).

Spread of fungi in field soil

In the control and water-irrigated plots, the fungi had spread 40 cm from the point of planting by 20 weeks. In plots irrigated with full-strength leachate, trap plants remained largely uncolonized (data not shown). In the control plots, no mycorrhizal colonization was detected in the trap plants grown adjacent to the uninoculated *D. triquetra* seedlings until 6 weeks. In plots irrigated with water, trap plants became colonized when adjacent to the uninoculated *D. triquetra* seedlings at all harvests though at comparatively low levels (Table 6). The proportion of colonization of trap plants harvested at 4 and 6 weeks was increased by inoculation (Table 7). The level of colonization was greatest in the control plots ($P<0.05$) but the difference was not significant in plots irrigated with dilute leachate, and at 20 cm at 6 weeks.

Table 7 Mean (±SE) proportion of AM colonisation of roots of *A. elongata* trap plants harvested after 4 and 6 weeks, grown in the field 20, 30 and 40 cm from the base of *D. triquetra* seedlings after 59 weeks

Distance from colonised host (cm)	Week 4		Week 6	
	+AM	−AM	+AM	−AM
Not irrigated 20	25±6*	2±2	20±6	8±8
30	15±7*	0±0	30±6*	4±4
40	17±2*	0±0	37±6*	3±3
Irrigated 20	17±7	9±1	29±4	24±6
30	18±8	4±2	31±10	24±11
40	20±10	1±1	22±10	18±8

* $P<0.05$ (between mycorrhizal treatments at the given distance)

Soil pH and EC

The pH of the soil measured prior to planting was similar at both depths. The EC of the soil was highly variable though tended to be higher at 0–5 cm than 15–20 cm (Table 8). The pH of soil was similar in plots irrigated with dilute leachate and the control after approximately 58 weeks. However, the pH of the soil at 15–20 cm in the irrigated plots was significantly ($P<0.05$) lower than at 0–5 cm. The EC of the soil in different irrigation treatments and depths was similar (Table 8).

Table 8 Mean (±SE) pH and EC (μS cm^{-1}) of soil sampled at 0–5 and 15–20 cm, from the control, and water plots prior to planting and after 58 weeks. Means that are followed by *different letters* differ significantly ($P<0.05$) at time of harvest

Sample date	Treatment	pH		EC	
		0–5	15–20	0–5	15–20
Out-planting	Control	4.7±0.2 [a]	4.4±0.3 [a]	95±42 [ab]	29±4 [b]
	Water	4.2±0.1 [a]	4.3±0.2 [a]	181±93 [a]	51±14 [b]
After 58 weeks	Control	5.8±0.7 [ab]	5.4±0.6 [ab]	53±16 [a]	62±34 [a]
	Dilute leachate	7.3±0.4 [a]	5.1±0.3 [b]	102±18 [a]	60±5 [a]

Table 9 Mean (±SE) shoot dry weight, % P and % colonisation of seedlings of *Acacia longifolia* var. *sophorae* inoculated (*AM*), or not inoculated (*NM*), and harvested after 6 weeks

	Seed Size	SDW	%P Shoot	Root	%AM
NM	Small	50±8*	0.05±0.01	0.7±0.4	0
	Large	75±8	0.04±0.01	0.7±0.3	0
AM	Small	38±3*	0.08±0.01*	0.9±0.3	54±4
	Large	81±7	0.03±0.01	0.5±0.2	51±8

* $P<0.05$ (within inoculation treatment)

P allocation in seedlings

Percentage colonization was similar in inoculated seedlings from both seed sizes. AM did not form in roots of uninoculated seedlings. Shoots were significantly heavier ($P<0.05$) in seedlings grown from large compared to small seeds. P concentration was similar within classes of seed sizes. Most importantly, significantly higher concentrations of P were found in roots than shoots for all treatments ($P<0.05$). In addition, inoculated seedlings grown from small seeds had significantly ($P<0.05$) higher concentrations of P in their shoots, than inoculated seedlings grown from large seeds (Table 9).

Discussion

The field experiment examined the growth and survival of four native plant species inoculated with native sporocarpic AM fungi, or fertilised, in a highly disturbed, extremely mineral-poor soil. Plants required either mycorrhizas or small amounts of fertiliser for their survival and growth. The field soil was successfully inoculated with AM fungi by sowing seedlings colonized prior to out-planting. The fungi established in the soil and spread rapidly to at least 40 cm from the base of the plant within 20 weeks. Uninoculated, unfertilised seedlings did not survive to out-planting, leading us to suggest that mycorrhizas are increasing the uptake of minerals by plants under these conditions. The interpretation is tentative because plants used in the field experiment continued growing at extremely low concentrations of P in shoot tissue and soil, and many became mycorrhizal prior to completion of the experiment. These results differ from those of Bolan et al. (1987), perhaps due to their use of pasture plants adapted to soils with higher available P than the lowest rates used in their experiments.

Establishment and spread of AM fungi have been problems in other field studies. Spread of hyphae observed here is similar to other studies where the rate of spread of AM fungi was in the range of 0.8–3.5 cm $week^{-1}$ (Mosse et al. 1982; Powell 1979). The colonizing potential of AM fungi in the soil also increased over the duration of the experiment, indicating that the fungi may reach densities found in neighbouring undisturbed habitats. Although not determined accurately because of the difficulty of extracting roots, the AM fungi and the roots of the host seemed to spread through the soil at similar rates. These results indicate that the two AM fungi used in the inoculum mix can tolerate the soil conditions at LHWDC. Presumably other sporocarpic AM fungi collected from similar plant communities and soil types will also tolerate these soil conditions and can be used in attempts to revegetate highly disturbed sites.

The establishment of mycelium of AM fungi in disturbed soil is important because the hyphae are likely to increase the structural stability of the soil (Miller and Jastrow 2000; Tisdall 1994; Tisdall and Oades 1979; 1982). Aggregation of soil ensures the formation of free-draining pores within the profile, enabling drainage of excess rainfall and aeration of the root zone, possibly improving root elongation, and plant growth and survival. Poor plant survival and growth in earlier attempts to revegetate the adjacent site were associated with water logging and soil compaction, despite the survival of some plants with ectomycorrhiza. We noted adequate drainage around inoculated plants towards the end of this experiment, though development of soil aggregation was not determined.

The presence of mycelium of AM fungi is also likely to enable the establishment of a diversity of plant species dispersed into the site as seeds. Seeds of potentially mycorrhizal plant species that germinate in soil with an established mycelium of AM fungi may have immediate access to minerals (Francis et al. 1986; Read et al. 1976) and C from established plants (Francis and Read 1984; Read et al. 1985). Improved nutrition may be particularly important for plant establishment in impoverished soils (Read et al. 1976), such as at LHWDC, and for species with small seed reserves (Allsopp and Stock 1995). If recruitment of plant species is enhanced, the density of mycorrhizal fungi may thereby increase (Genny et al. 2001), ultimately leading to a complex biota at the site including mycophagous animals (McGee and Baczocha 1994).

Low levels of AM fungal colonization were found in uninoculated seedlings from 20 weeks and in all plant species after 66 weeks. The mycorrhiza appeared especially common in irrigated treatments. While some propagules may have been present at the site at out-planting, the increasing number of colonized plants indicates that fungal propagules dispersed into the site. Spores of AM fungi can be dispersed by wind (Warner et al. 1987) and in water flowing across the soil surface. Although not determined in all cases, colonization arose from an *Acaulospora* sp. that has been isolated from elsewhere at LHWDC (G. S. Pattinson, unpublished data). The presence of *Acaulospora* leads to the question of whether or not deliberate inoculation is necessary. Colonization associated with *Acaulospora* was relatively limited, and delayed, especially in unirrigated seedlings. The development of mycorrhizas was so delayed that seedlings are unlikely to survive following seed germination. Inoculation of the soil with fungi tolerant of conditions at the site will be essential to ensure reasonable plant survival. In addition, tests of the leachate tolerance of the isolate of *Acaulospora* indicate that it is more sensitive than the inoculated species (MacIntyre and P. A. McGee, unpublished data). In the absence of fertiliser, inoculation with many fungi tolerant of the edaphic and environmental conditions is an important component for revegetating highly disturbed sites (Reddell and Milnes 1992), though use of fertiliser may suffice where dispersal of several fungi is common.

As ectomycorrhizal (EM) colonization was observed in the roots of *E. gummifera* and *L. polygalifolium* seedlings at the time of out-planting, the influence of AM fungi on plant growth and survival will be discussed for *C. rhomboidea* and *D. triquetra* only. Fertilising seedlings of *C. rhomboidea* prior to out-planting resulted in similar shoot growth in inoculated and uninoculated plants. In *D. triquetra*, mycorrhizal seedlings were heavier than uninoculated seedlings. A lower concentration and quantity of P were found in mycorrhizal seedlings. The presence of AM tended to increase survival and growth of seedlings even though the data may have been confounded by the different nutritional status of the mycorrhizal and non-mycorrhizal seedlings at out-planting. This effect of mycorrhizas could be followed for the duration of the experiment because shoots had a remarkably similar concentration of P, and only low levels of AM colonization were observed in the roots of most uninoculated seedlings, both in the control and irrigated plots.

Inoculation of seedlings with AM fungi was not essential for their establishment and survival when adequate minerals were supplied exogenously. All species used in these experiments never grew beyond the two-leaf stage and died within 3 months in the absence of fertiliser or mycorrhiza (G. S. Pattinson, unpublished data). Uninoculated, fertilised and inoculated seedlings died at similar times and in similar numbers throughout the experiment. AM fungal colonization increased growth rates of *C. rhomboidea* and *D. triquetra*, at least as much as those of fertilised seedlings lacking mycorrhiza, indicating that the primary benefit of AM was due to increased plant uptake of minerals, presumably P.

The role of ectomycorrhiza in the growth and survival of seedlings also requires more careful analysis. The effects of ectomycorrhiza were unclear in this field experiment. However, as ectomycorrhizal species are found in previous attempts to revegetate the site, the fungi may play an important and unrecognised role in similar conditions.

It also appears that the availability of P and N may interact in their effect on plant growth under these conditions. Mycorrhizal seedlings at the last harvest were larger when irrigated with dilute leachate. Leachate is a source of NH_4^+ (Pattinson et al. 2000), indicating a possible interaction between mycorrhizal colonization and N nutrition. The importance of N in mycorrhizal plants growing in P- and N-impoverished soils remains to be explored (Reddell and Milnes 1992; K. A. Hammill, G. S. Pattinson, P. A. McGee and B. G. Sutton, unpublished data).

Differences in growth of plants were difficult to detect. Because root systems were difficult to remove from the crushed rock, we relied on analyses of shoots. Plant species commonly found growing in nutrient-poor soils are often very conservative in their use of nutrients, especially P. The species tend to have low rates of tissue turnover and high degrees of mineral reallocation, and storage in periods when minerals are in excess of requirements. These attributes increase P utilisation efficiency (Beadle 1968; Chapin 1980; Koide 1991). Similar concentrations of P were found in leaves of mycorrhizal plants grown in the field and the P storage experiment (between 0.05 and 0.07% in seedlings at out-planting to the field, and 0.03 and 0.08% P in seedlings fertilised with excess P in laboratory conditions) indicating that leaf P concentrations do not necessarily indicate the P in soil available to seedlings. At least *Acacia longifolia* has the capacity to store P in roots. The other species possibly also have this capacity. Excess P may accumulate in the metabolically active tissues or in a plant part such as the root system (Beadle 1968). These vegetation systems are fire-prone. During severe fire, most of the aboveground plant parts are killed. Many species recover from root-stocks after fire. Storage in roots would reduce P lost in smoke when the shoots are burnt.

P-efficient plants often have inherently slow rates of growth, resulting in low demand for P during periods of growth. Thus additional uptake of P by AM fungi may not result in significantly increased rates of plant growth. These data indicate the importance of examining the entire plant when assessing P uptake.

In conclusion, the field experiment demonstrates that sporocarpic AM fungi collected from undisturbed plant communities in the Sydney region can be introduced into highly disturbed soil in conjunction with native plants. Pre-inoculation of seedlings with AM fungi is not associated with improved uptake of P by plants in the field; rates of plant growth are matched by use of

fertiliser. While the rate of growth of seedlings was probably limited by the efficiency of P utilisation and N availability, at least one native plant species has the capacity to store P in the root system, providing a buffer enabling continued growth. In addition, previous data indicating AM are reduced in roots of plants growing in extremely impoverished soils are not supported by this research. By using plants and fungi adapted to the soil, we demonstrated that plant growth and survival followed a pattern typically found in mineral-rich soils. Presence of AM is probably essential for the return of complex communities formally present at disturbed sites, regardless of the level of available PO_4^{3-} in the soil.

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