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The role of arbuscular mycorrhizal fungi in plant community establishment at Samphire Hoe, Kent, UK – the reclamation platform created during the building of the Channel tunnel between France and the UK

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Abstract. Samphire Hoe is a newly-created land platform comprising the sub-seabed material excavated during the construction of the Channel tunnel. It represents a unique resource where the arrival and establishment of arbuscular mycorrhizal fungi (AMF) within a sown plant community on a low nutrient substrate can be monitored. Arbuscular mycorrhizal fungi invasion was monitored in a number of ways: by assessing the degree of root colonisation within the roots of plants on the site, by using a successive trap culture technique to determine AMF species richness, and by using sterile substrate bins to determine the extent of wind-borne and rain-dispersed immigration of AMF propagules into the site. Levels of colonisation of indigenous plants by AMF were high in May–June (the pre-flowering phase of growth for many plants) reflecting the important role of the mycorrhizal symbiosis in dry, low nutrient soils. Twelve species of AMF were identified, representing a relatively high diversity for a recently deposited subsoil. An on-site experiment indicated that inoculum of AMF could enter the site within 8 months and that wind dispersal and/or rain were possible vectors. A field experiment compared the outplanting performance of commercially-produced *Elymus pycnanthus* seedlings (in a commercial compost with added nutrients) with seedlings produced in a low nutrient substrate and inoculated with AMF isolated from the site (a mixture of 5 species of *Glomus*) or left uninoculated. After 14 months in the field seedlings, inoculated with the indigenous AMF, had the same tiller production as commercially-produced plants, despite slower initial growth. In contrast, non-mycorrhizal controls grew very poorly with a greater frequency of plant mortality compared with the other treatments. *Elymus* seedlings inoculated with the indigenous AMF ultimately produced approximately seven times the mean number of seed spikes per surviving plant as commercially-produced seedlings and five times greater weight of seed spike. A phyto-microbial approach to the revegetation of nutrient-poor soils is proposed to stimulate plant successional processes as a economically-viable sustainable input for landscaping anthropogenic sites.

Key words: arbuscular mycorrhizal fungi, *Elymus pycnanthus*, increased flowering, landscaping, nurse crops, species richness

Introduction

Samphire Hoe is a 36 ha landscaped reclamation platform site created from chalk marl (5 000 000 m^3) excavated during the creation of the Channel tunnel. The tunnel spoil was deposited behind an 1800 m long sea wall at the base of Shakespeare Cliff, 3 km west of Dover, UK. It is managed by Eurotunnel Developments Limited and the White Cliffs Countryside Project. To minimise the environmental impact of the site after completion of the tunnelling, the contractors, Transmanche-Link (TML), landscaped the site and contracted research into planting regimes and erosion control with the aim of providing an ecologically interesting area which was accessible to the public (Kershaw et al. 1995). Research was undertaken by Wye College, UK, to find the optimal mixture of plant species that would grow in the chalk marl (a highly calcareous clay) and provide stability to the soil. Four seed mixes were formulated for different areas of the site which were applied by hydroseeding along with NPK fertiliser (Kershaw et al. 1995).

In 1992, *Lolium perenne* L. was planted across the site to act as a 'nurse' planting for the seed mixes sown at the same time, since it provides rapid initial cover and stability, behaves as a short-term perennial and is non-persistent on low-fertility soils. These studies showed that low levels of fertilizer application applied to *L. perenne* swards, sown with a wild flower mix, resulted in low productivity and maximum species richness. In contrast elevated N and P levels stimulated the biomass of *L. perenne* at the expense of the wild flower species (Mitchley et al. 1996). As intended, the nurse species had entirely disappeared from the site by 1998 with the exception of a few remnant patches in the north of the site. In addition to seeding the area with the pre-determined plant species, an inoculum of rhizobial bacteria was applied to stimulate the development of the important legume-bacterial symbiosis. However, there was no attempt to introduce inoculum of the second and equally important, plant symbionts, the arbuscular mycorrhizal fungi (AMF).

Arbuscular mycorrhizal fungi form obligate symbioses with the majority of terrestrial plants in most terrestrial ecosystems (Brundrett 1991). The root systems of most plants in natural communities contain an assemblage of species of AMF (Merryweather and Fitter 1998). Van der Heijden et al. (1998a,b) have shown that below ground species richness of AMF is a major factor in the maintenance of plant biodiversity and to ecosystem stability and function. They concluded that increasing the species richness of AMF in grasslands led to the increased spread of highly responsive herb species at the expense of relatively unresponsive grasses. The extraradical mycelium (ERM) of AMF can enter the roots of many plant species in a plant community resulting in long-lived interconnections between plants. This may explain how AMF can influence plant community development.

There have been no studies on Samphire Hoe of the role played by AMF in stimulating the establishment of plant diversity at the site. The chalk marl was initially assumed to be a virgin substrate, with respect to the presence of AMF. Previous

studies have indicated that wind is a major dispersal agent of AMF propagules into virgin ecosystems (Allen 1987; Allen et al. 1989) sometimes aided by the activities of small mammals (Allen 1987; Warner et al. 1987). Therefore, the dynamics of invasion of the chalk marl by AMF on Samphire Hoe under the seed mixes sown, if it occurred at all, required further study to evaluate the species richness of AMF.

The first aim of this investigation was to establish the extent of mycorrhization of plants at Samphire Hoe 6 years after it was established, during a period when root growth was most active. Secondly, the species richness of AMF at Samphire Hoe was investigated by trapping the AMF (Stutz and Morton 1996), present in the roots and rhizosphere of field plants in greenhouse pot-cultures, to enhance sporulation for identification of AMF species. Thirdly, trials were set up to determine the contribution of wind blown inoculum of AMF to the initial colonisation of the original substrate. Finally a field trial was established at the exposed Western end of the site, using AMF isolated from plants on the same site. The aim of this trial was to compare the outplanting performance of seedlings of the salt-tolerant coastal grass, sea couch *Elymus pycnanthus* (Godron) Melderis pre-inoculated with indigenous AMF, with non-mycorrhizal and commercially-produced seedlings.

Materials and methods

Experiment 1 – field survey of the arbuscular mycorrhizal status of plants at Samphire Hoe

Four 50 × 50 m plots were laid out at Samphire Hoe [51°06′39″ N, 01°18′28″ E] (Figures 1 and 3a). Two plots were placed in an area that had been hydro-seeded with native plant seed mixes and two were placed in an area that had received NPK fertiliser and had been planted with the nurse species (*L. perenne*) only (Kershaw et al. 1995; Mitchley and Buckley 1995; Mitchley et al. 1996). The plots (Figure 1) were representative of the vegetation at the East Tip (ET); North-East Mound (NEM); Middle North (MN); and West Tip (WT). A transect was paced out diagonally across each plot from north-west to south-east and one specimen of each of the most commonly occurring plant species was sampled. The chalk marl had a fine particle size, a pH of 7.4–8.3 and was low in organic carbon and N and P levels but relatively high in Ca and Mg (Table 1). The soil had a low water-holding capacity. Soil salinity was variable, with the WT and seaward edges having greater levels of chlorides and Na due to exposure to periodic sea spray and wave intrusion.

Plants were sampled over a 7 week period (2 June–24 July 1998) for the presence of AMF in their root systems. Individual plants, chosen at random, were removed from the soil with a hand trowel. The main root systems, together with attached soil, were sealed in polythene bags and taken to the laboratory. Six samples were taken from ET, 11 from NEM, 13 from MN and 6 samples from WT reflecting the major

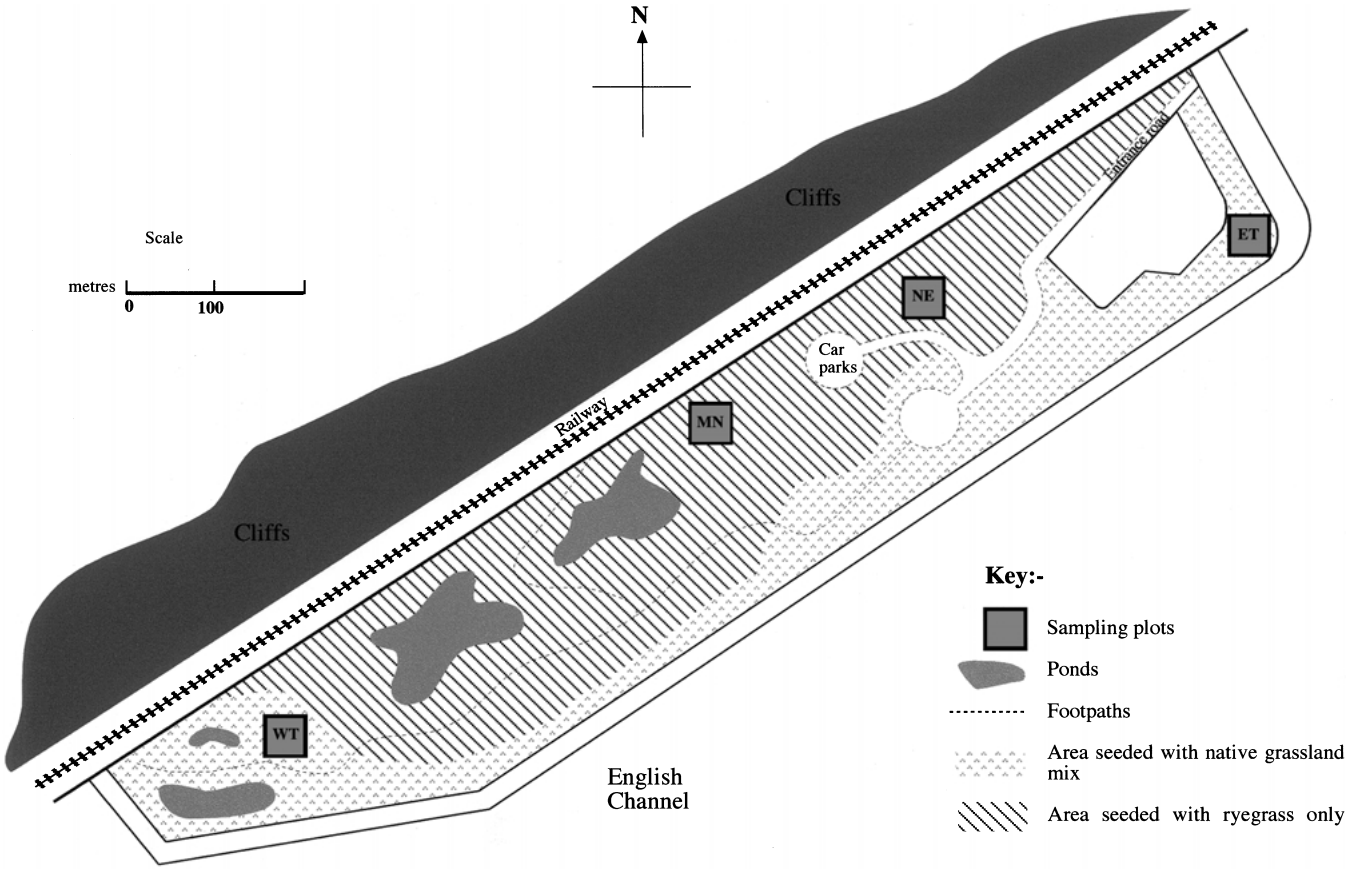

Figure 1. Diagram of Samphire Hoe site showing sampling zones, topography and sowing regimes implemented at the site.

Table 1. Physical and chemical characteristics of the chalk marl spoil in different areas of the Samphire Hoe site, Kent, UK November 1999.

	North-east mound	East tip	Middle north	West tip	Trial site
Composition (%)					
Clay	36.9	32.9	36.1	35.7	38.3
Coarse sand	8.4	12.6	6.5	5.2	8.6
Fine sand	9.9	18.7	12.3	13.7	12.8
Coarse silt	14.6	13.2	15.6	16.7	14.6
Fine Silt	30.3	22.6	29.6	28.6	25.6
pH	7.5	7.4	7.4	7.5	8.3
N (g kg^{-1})	0.48	1.04	0.54	0.53	0.2
P (mg kg^{-1})	74	57	44	63	30
Organic carbon (C)%	0.81	1.57	0.93	0.76	0.41
Organic matter	1.4	2.7	1.6	1.3	0.7
C/N	16.2	15	17	14.4	20.9
Water holding capacity (%)	43.4	45.8	33.4	38.6	51.9
Chlorides (mg kg^{-1})	59.4	93.7	64.8	222	89.4
Exchangeable bases (meq 100 g^{-1})					
K	0.93	1.45	0.84	0.85	1.32
Ca	38.65	37.95	38.4	39.63	33.73
Mg	4.26	5.38	3.85	4.9	6.35
Na	0.41	0.64	0.44	1.03	19.88

plant species which had established in each zone. Only one sample plant per zone was taken for analysis due to restrictions on disturbance at the site as other plant species diversity surveys were ongoing. Approximately 20 cm of young, living, white lateral roots were cut from each root ball and washed thoroughly in tap water. These roots were assessed for percentage root length colonised by AMF using a modified protocol of Phillips and Hayman (1970). The percentage root length colonised was evaluated by mounting a sub-sample of approx. 10 cm of cleared and stained (trypan blue) root on a microscope slide and assessing the presence or absence of typical mycorrhizal structures (arbuscules, vesicles and hyphae) in each field of view at a magnification of $\times 100$ using a compound microscope (Axioskop, Zeiss, Germany). This was used to estimate the degree of mycorrhization and not for direct comparisons of levels of colonisation between plant species.

Experiment 2 – Species richness of AMF at Samphire Hoe

Trap cultures were set up between 2 June 1998 and 26 July 1998 using the plants collected for Experiment 1. The root ball (4–6 cm diameter with attached soil) of each sample (after removal of some living root for staining) was placed into a 14 cm (diameter) $\times 10$ cm (depth) plastic pot (Figure 2). The aerial parts of the plants were cut back and the remaining volume in the pots was filled with a 2:1 mix of an attapulgite clay (Agsorb 8/16, Oil-Dri Ltd, Wisbech, UK) and a durite sand (a particulate by-product of calcined flint pebbles comprising 97% silica with small percentages of

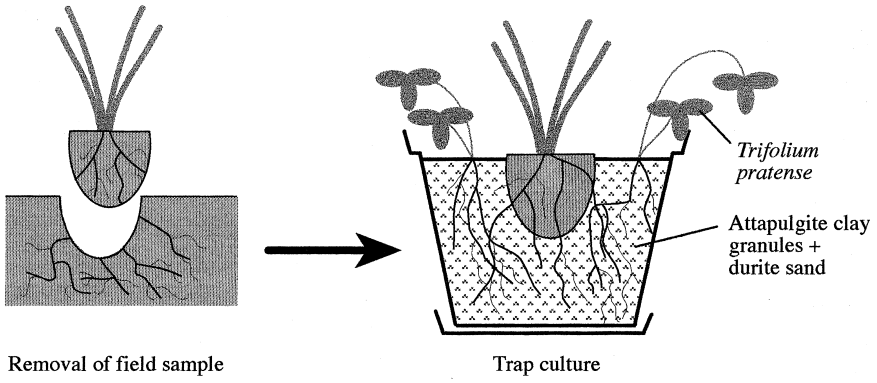

Figure 2. Diagram of trapping procedure used to induce sporulation of arbuscular mycorrhizal fungi from field samples of plants and their root systems over an 18 month period.

iron oxides, calcium oxides and alumina and a mean pH of 8.3 – Bretts Ltd., Canterbury, UK). Seeds of *Trifolium pratense* L. were surface sterilised in 3% (v/v) NaOCl for 5 min and sown around the plant to trap AMF present in the sample (Figure 2). Trap cultures were transferred to a greenhouse (maintained between 8 °C and 34 °C). Plants were watered with de-ionised water as required and supplied with nutrients (N:18 P:0 K:18) once per week using 1.4 g l^{-1} Vitafeed 102 (Vitax Ltd, Leicester, UK) with trace elements.

Arbuscular mycorrhizal fungal spores were extracted from trap cultures by wet-sieving and sucrose density gradient centrifugation [wwwbio.ukc.ac.uk/beg/protocols/extraction.htm]. Two 50 cm^3 cores of the attapulgite clay/durite sand mixture were removed from each trap. The material was flushed through a 500 μm sieve and collected in a 45 μm sieve. The contents of the fine sieve were back-washed into centrifuge tubes and a 60% (w/v) commercial sugar solution was added to the pellet using a syringe. After centrifugation at 3000 rpm for 2 min., the supernatant was washed into a 45 μm sieve and the contents back-washed into a Petri dish. Taxonomic identification was based on spore morphology under a dissecting microscope and on diagnostic slides [wwwbio.ukc.ac.uk/beg/Protocols/slide.htm] examined under a compound microscope at $\times$100–400. Samples of substrate were removed after 3, 8, 13 and 18 months. Four control 'trap cultures' containing the growth medium and *T. pratense* were also incorporated amongst the pots in the greenhouse to check if airborne or splash contamination of pots was occurring. To confirm identification of some AMF species, pure cultures were established from spores using *T. pratense* as a host in a 2:1 attapulgite clay/durite sand medium.

Experiment 3 – the possible agent of dispersal of AMF inoculum to Samphire Hoe

Following the results of Experiment 1 a trial was designed to investigate how inoculum of AMF may have reached the site over the 6 year period of its establishment.

Four plastic bin containers (50 × 30 × 15 cm deep) were filled with 20 l of autoclaved attapulgite clay and placed at four sites across Samphire Hoe (Figure 3b) on 2 September 1998. Each container was placed on a level piece of ground in the centre of a 2 × 2 m heavy gauge polythene sheet that was pegged down and angled away from the container to prevent rain splash from the ground (Figure 3b). The containers were provided with drain holes and were protected with wire netting to prevent animal interference. The containers were collected from the sites and transferred to a greenhouse on 14 June 1999. Each container was seeded with 20 g surface sterilised *L. perenne* and placed into a large, open-ended transparent Sterilin bag and placed on a greenhouse bench (min 18 °C/max 40 °C, relative humidity 60–80%, photoperiod 18 h/6 h light/dark). Watering was carried out using sterilised de-ionised water and no nutrient supplements were applied. On 29 September 1999, eight plants were harvested from the containers at predetermined points to check for percentage root length colonisation by AMF as described above.

Experiment 4 – the role of AMF in the establishment of seedlings of Elymus pycnanthus *at Samphire Hoe*

Seeds of sea couch (*Elymus pycnanthus*) were collected on 20 September 1998 from the cliffs adjacent to the Samphire Hoe site to provide plants of local provenance. Some of the seeds were sown on 15 January 1999 at IIB/UKC in a moist, fertilised, soil-less planting medium based on composted bark with slow release fertilizer addition supplied by a commercial company producing *E. pycnanthus* seedlings for large scale outplanting at Samphire Hoe (British Wildflower Plants, Gt. Yarmouth, UK). The seed trays were covered with aluminium foil and put into a heated greenhouse maintained between 10 °C and 25 °C until germination. In parallel, plants were also raised under ambient conditions by British Wildflower Plants. Single seedlings were pricked out into 3.5 × 6 cm plug trays when approximately 2 cm tall. The plants were divided into three treatments: (i) Non-mycorrhizal plants: raised in a sterile medium comprising 5:5:1 attapulgite clay: durite sand:sieved and autoclaved chalk marl soil from Samphire Hoe; (ii) Mycorrhizal plants: raised as for non-mycorrhizal plants except the attapulgite clay/durite sand component contained a mixed inoculum (10% v/v) of AMF (*Glomus claroideum* (Schenck & Smith) emend Walker & Vestberg, *Glomus intraradices* Schenck & Smith, *Glomus geosporum* (Nicol. & Gerd.) Walker, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe and *Glomus* sp. (1) that had been trapped and identified from Samphire Hoe (see Experiment 2); (iii) Commercially-produced plants: This third treatment comprised the commercially-produced seedlings raised by British Wild Flowers which were transferred to IIB/UKC on 18 March 1999. These plants were raised in a similar fashion but pricked out into identical plug trays containing a soilless and fertilised growing compost. All plants from the three treatments were then hardened off in the greenhouse at IIB/UKC for a

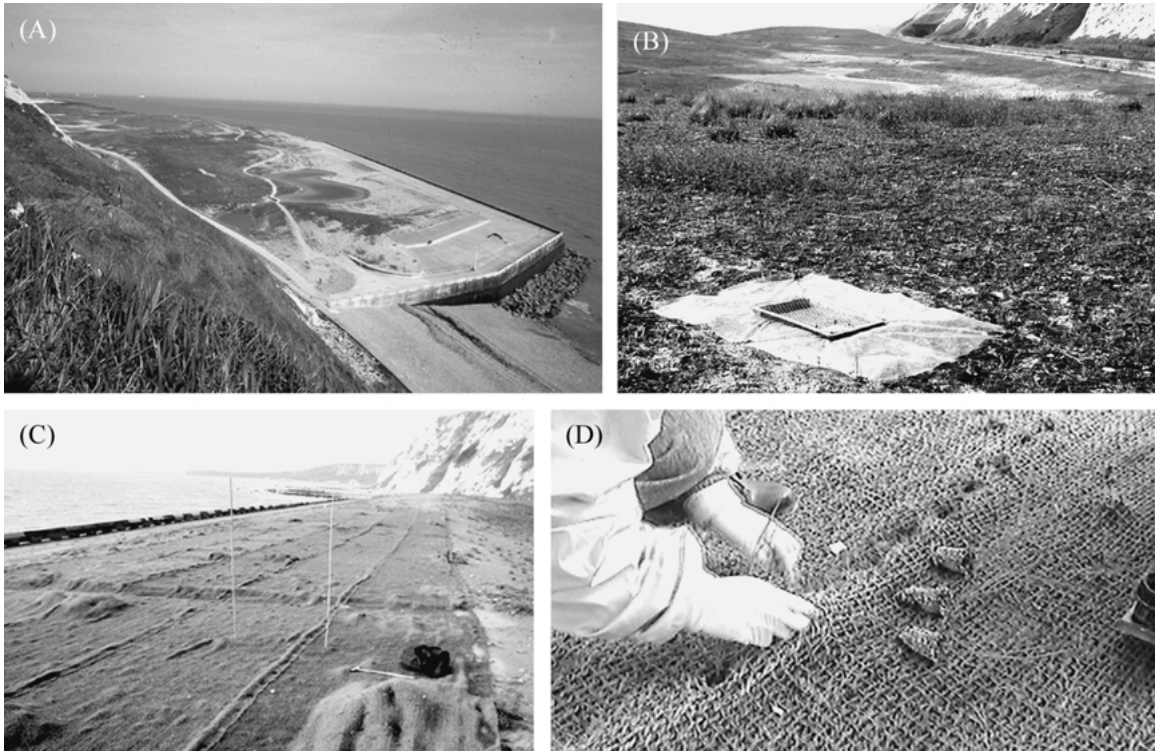

Figure 3. (A) Aerial view of West Tip on Samphire Hoe; (B) Bins placed in the four sampling zones at Samphire Hoe to assess the rate of wind-blown inoculum of AMF onto the site during one year; (C) Coir matting used to aid stabilisation of soil and outplanting of *Elymus pycnanthus* on West Tip; (D) Outplanting of seedling plugs of mycorrhizal, non-mycorrhizal and commercially-produced non-mycorrhizal *E. pycnanthus* on the West Tip coir matting for Experiment 4.

further 4 weeks. Watering was carried out with de-ionised water as required but no additional nutrient supplements were applied. Eight weeks after sowing, the plants were checked for the presence of AMF. Three plants of each treatment were removed from plug trays and the substrate was washed from the root systems with water. A dissecting microscope was used to check for the ERM of AMF before staining the entire root system in trypan blue (see above) and the percentage root length colonised in the mycorrhizal plants was estimated as described above.

On 13 April 1999 the seedlings were planted into coir matting, which provided physical soil protection to aid the establishment of plants, on the western end of Samphire Hoe in a 26×9 m plot on the seaward edge of the platform (Figure 3c). This area is exposed to severe erosion during storms due to prevailing south-westerly winds and was sparsely vegetated with *Beta vulgaris* L., *Limonium binervosum* (G. E. Sm.) C. E. Salmon and *Spergularia* sp. (Figure 3a). The soil was more saline than in the other areas at Samphire Hoe due to frequent incursions of sea spray (Table 1). The experiment was integrated into a pre-determined management plan for re-vegetation of the area and the experimental blocks were set out in a staggered pattern within that area. Nineteen blocks were set out each containing three randomly positioned rows of plants (50 cm gaps) and with individual plants spaced at 10 cm intervals. Each row contained plants from one of the three treatments. Each plug of substrate plus plant was planted through the coir matting in holes drilled with an auger (Figure 3d). The fresh and dry weights of foliage and roots, leaf area, root length and tiller numbers of plants within each treatment at outplanting was measured using five replicates. Tiller numbers were counted on 8 October 1999 and again on 15 June 2000. It was not possible to undertake destructive sampling because of management restrictions at the site so only tiller numbers were recorded. The percentage root length colonised by AMF was again estimated in October 1999 by removing a root sample from the middle plant in each row using a 2 cm soil corer (total of 57 samples).

The development of seed spikes was monitored in the summer of 2000. Spikes were first observed on 30 June 2000 when an initial count was made. A further four counts were made in consecutive weeks until numbers indicated that no more seed spikes were being produced. On 6 September 2000 the seed spikes from all plants in the experiment were harvested. This date was chosen as seeds had fully matured but were still firmly attached to the seed spike. The seed spikes from each replicate block treatment were bulked together and placed into paper envelopes and air dried for 48 h at 50 °C before weighing.

Statistical analysis

Data were tested for normality and analysed using analysis of variance (ANOVA) on Minitab version 12 for Windows. When a significant F-value was obtained ($P = 0.05$),

treatment means were separated by the Least Significant Difference (LSD). The standard error of the treatment mean is presented in bar charts.

Results

Experiment 1

Thirty plant species were sampled for colonisation by AMF and the greatest plant diversity was observed in the NEM and MN areas (Table 2). This observation is supported by studies undertaken by Wye College, London University, UK, working on this site (J. M. Mitchley, unpublished data). All 30 plant species had very high percentage of root length colonisation by AMF except for *Hippophae rhamnoides* L. (sea buckthorn) which had a very fibrous root system and root clusters which were difficult to obtain and stain. *Cardaria draba* L. (Brassicaceae), however, had low numbers of colonised roots.

Experiment 2

The trapping technique employed proved very successful in inducing sporulation by species of AMF in sufficient numbers to allow identification. Table 3 summarises the occurrence of the twelve species of AMF isolated from trap cultures established and monitored over an 18 month period. Table 3a shows that greatest number of species (9) were isolated from the soil collected at NEM and MN plots and the least (6) were found in traps from plants taken from ET and WT plots. Four species of AMF, *Glomus claroideum*, *Glomus geosporum*, *Glomus intraradices* and *Glomus mosseae* were common to all plots. *Glomus fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske and *Acaulospora morrowiae* Spain & Schenck were only found in NEM and MN plots. Table 3b shows that the numbers of species of AMF identified increased up to final harvest. Again, *G. claroideum*, *G. geosporum*, *G. intraradices* and *G. mosseae* sporulated most frequently and were identified at each harvest. *Acaulospora morrowiae* and *Glomus coronatum* Giovannetti appeared only at one harvest. *Glomus etunicatum* Becker & Gerdemann, *G. fasciculatum*, *G. microaggregatum* Koske, Gemma & Olexia and *Entrophospora infrequens* (Hall) Ames & Schneider appeared only in the latter two harvests. It should be noted that no spores of AMF were isolated from the four control trap pots which were sampled over the same period as plants remained non-mycorrhizal. There was no apparent relationship between plant species trapped and number or taxon of AMF species isolated. It was interesting that four different AMF were isolated from traps of sea buckthorn (*H. rhamniodes*) even though roots were not found with mycorrhizal structures.

Table 2. Percentage root length colonised of individual plants from four sampling zones at Samphire Hoe (samples collected between 2 June 1998 and 25 July 1998).

Sampling zone	Host plant	Root length colonised (%)	Sampling zone	Host plant	Root length colonised (%)
East Tip	*Silene nutans*	63	West Tip	*Ononis repens*	62
	Plantago lanceolata	99		*Lotus corniculatus*	94
	Festuca rubra	54		*Crepis capillaris*	46
	Ononis repens	73		*Silene nutans*	74
	Picris echioides	62		*Hippophae rhamnoides*	0
	Daucus carota	61		*Limonium binervosum*	63
North-East Mound	*Hypochoeris radicata*	65	Middle North	*Parapholis incurva*	12
	Festuca ovina	66		*Koeleria macrantha*	17
	Trifolium repens	78		*Festuca rubra*	75
	Hieracium pilosella	95		*Rumex acetosa*	38
	Holcus lanatus	50		*Picris echioides*	73
	Anthyllis vulneraria	85		*Cirsium arvense*	86
	Spergularia marina	87		*Senecio jacobea*	55
	Plantago coronopus	84		*Centaurium erythraea*	66
	Tussilago farfara	69		*Blackstonia perfoliata*	79
	Plantago media	94		*Cardaria draba*	5
	Ononis repens	100		*Anagallis arvensis*	50
				Lotus corniculatus	38
				Lolium perenne	69

Experiment 3

Levels of root length colonised by AMF of trap plants of *L. perenne* sown in samples of substrate from the bins from the ET, NEM and MN samples were 5% or less in the eight sub-sample cores sampled. The percentage of root length colonised in samples taken from the trap plants sown in the WT bin, however, averaged $23.0 \pm 12.5\%$.

Table 3. The species of AMF isolated from plants sampled from Samphire Hoe and trapped over an 18 month period. (a) Species richness of AMF from four different sampling zones; (b) Species richness of AMF at each harvest.

	Sampling sites			
AMF species identified	East Tip	N-E Mound	Mid. North	West Tip
(a)				
Glomus mosseae	×	×	×	×
G. geosporum	×	×	×	×
G. intraradices	×	×	×	×
G. claroideum	×	×	×	×
G. etunicatum	×	×	×	
G. microaggregatum	×			
G. coronatum			×	
G. occultum		×		
Glomus sp. 1 (*G. fragilistratum*-like)			×	×
G. fasciculatum		×	×	
Acaulospora morrowiae		×	×	
Entrophospora infrequens		×		×
Total	6	9	9	6
	Harvest			
	1	2	3	4
(b)				
Glomus mosseae	×	×	×	×
G. geosporum	×	×	×	×
G. intraradices	×	×	×	×
G. claroideum	×	×	×	×
G. etunicatum			×	×
G. microaggregatum		×	×	×
G. coronatum	×			
G. occultum				×
Glomus sp. 1 (*G. fragilistratum*-like)		×	×	×
G. fasciculatum	×		×	×
Acaulospora morrowiae		×		
Entrophospora infrequens			×	×
Total	6	7	9	10

Experiment 4

After 8 weeks growth in the greenhouse, the plants receiving AMF inoculum had $95 \pm 5\%$ of their root length colonised whilst non-mycorrhizal and commercially produced plants had no colonisation. The growth of seedlings of *E. pycnanthus* at outplanting, 14 weeks after germination, is detailed in Table 4. Plants from both the non-mycorrhizal treatment had significantly greater leaf and root biomass than the mycorrhizal plants for all growth parameters measured. The commercially produced plants also had significantly greater leaf biomass than the mycorrhizal plants. The root parameters measured were also greater than the mycorrhizal plants but this was not quite significant at $P = 0.05$.

Table 4. Growth parameters of mycorrhizal, non-mycorrhizal or commercially produced *Elymus pycnanthus* plug seedlings ($n = 5$) at time of planting on Samphire Hoe in April 1999.

	Leaf				Root		
Treatment	Fresh weight (mg)	Dry weight (mg)	Area (cm^2)	Tiller no.	Fresh weight (mg)	Dry weight (mg)	Length (cm)
AMF-inoculated	358	90	9	2.2	409	58	29
Non-AMF	778	212	18	4.2	742	108	39
Commercial plants	608	132	12	1.8	790	116	39
LSD ($P = 0.05$)	384	101	6	0.7	NS	NS	NS

Colonisation levels of the roots of *E. pycnanthus* by AMF 6 months after outplanting (October 1999) was still greater than 60% in pre-inoculated plants but negligible in both uninoculated and commercially produced seedlings (data not presented). Tiller numbers were counted at the same time, 6 months after outplanting, and the AMF-inoculated and commercially produced plants had significantly greater numbers of tillers per plant than the non-inoculated plants raised in the attapulgite clay/durite chalk marl mixture (Figure 4). However, 14 months after outplanting, there was no significant difference between the numbers of tillers produced by AMF-inoculated

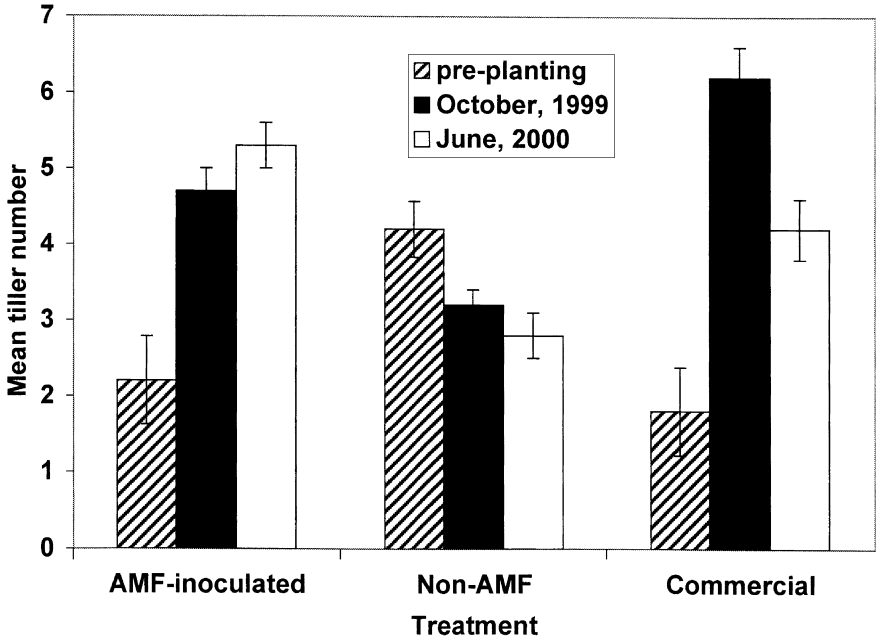

Figure 4. Mean tiller numbers of mycorrhizal, non-mycorrhizal and commercially produced non-mycorrhizal *Elymus pycnanthus* seedlings at outplanting, 6 months and 14 months after planting at Samphire Hoe (West Tip). Error bars are standard errors of the mean for each treatment at each sampling.

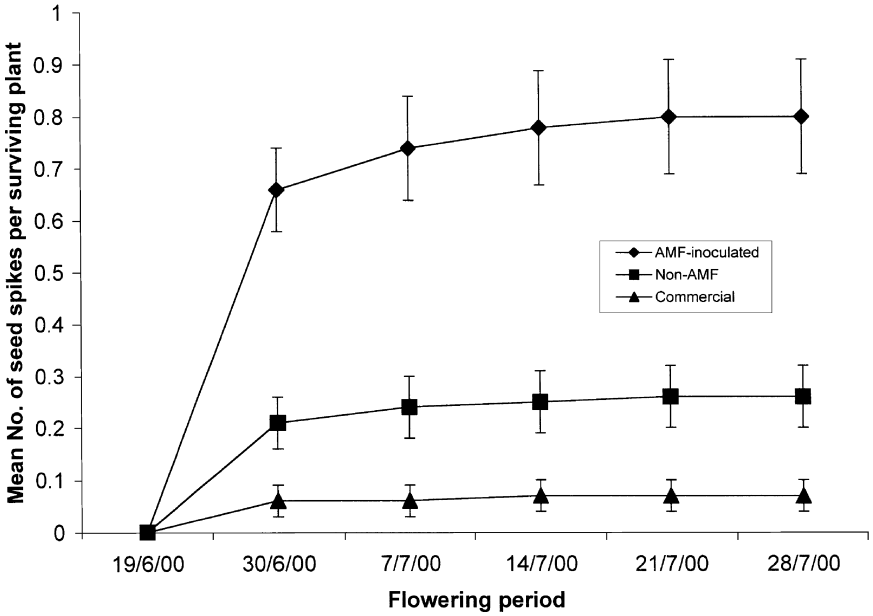

Figure 5. Mean seed spike numbers of surviving *Elymus pycnanthus* plants inoculated with AMF, non-mycorrhizal and commercially produced plants 15 months after outplanting at Samphire Hoe. Error bars are standard errors of the mean for each treatment at each sampling.

or commercially produced plants. The mean number of tillers produced per plant by non-inoculated plants raised in the attapulgite clay/durite chalk marl mixture continued to decline as the mortality rate increased (Figure 4).

Elymus plants inoculated with AMF produced significantly greater numbers of seed spikes than the non-mycorrhizal or the commercially produced plants 15 months after outplanting. The mean number of seed spikes per surviving mycorrhizal plant was around four times greater than that of the non-inoculated plants and more than six times that of the commercially produced plants (Figure 5). The mean yield of seed spikes per treatment block was also more than five times greater in AMF-inoculated plants compared with both non-inoculated and commercially produced plants (Figure 6). Whilst there were significantly fewer seed spikes on surviving commercially produced *Elymus* plants, the mean individual seed dry weight was twice that of mycorrhizal or non-mycorrhizal treatments 17 months after outplanting.

Discussion

All of the mycotrophic plants growing on Samphire Hoe had extensive colonisation by AMF at a time when plant growth was maximal and nutrient demand was high prior to flowering. The low levels of available soil nutrients (particularly P) and poor water permeation are likely to have increased the reliance of the plants on the nutrient-

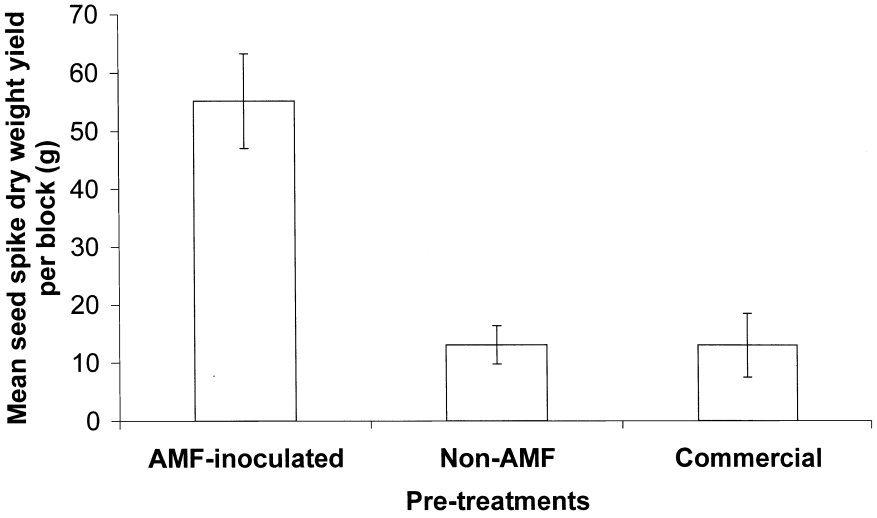

Figure 6. Mean seed spike yield (g dry weight) per treatment block ($n = 19$) of surviving *Elymus pycnanthus* plants inoculated with AMF, non-mycorrhizal and commercially produced plants 15 months after outplanting at Samphire Hoe. Error bars are standard errors of the mean for each treatment.

scavenging ability of the ERM of AMF. The high degree of mycotrophy at the site can be supported by the fact that the reportedly non-mycorrhizal *Cardaria draba* (Brassicaceae) was also mycorrhizal (5% root length colonised). Plant species in the Brassicaceae have been reported to be generally non-mycorrhizal, but with occasional colonisation (DeMars and Boerner 1996) under stressed conditions. However, there are doubts on the benefit of this infrequent relationship to the plant since the symbiosis may be non-functional as no arbuscules have been noted in these studies (DeMars and Boerner 1996).

The highest species richness of AMF was observed in the zones NEM and MN where *L. perenne* had been sown as a nurse cover crop and where the greatest number of different plant species had subsequently established, 6 years after planting (on site survey of plant diversity, unpublished data). *Lolium perenne* is an arbuscular mycorrhizal plant and, as an initial cover vegetation, it was probably an ideal first host for the rapid establishment of incoming AMF. Incoming propagules of AMF would have been able to colonise the dense root network and thus quickly increase soil inoculum levels and produce an extraradical mycelial network between plants. Recent work has provided clear evidence that increasing numbers of species of AMF can influence plant community structure (Van der Heijden et al. 1998a,b) in studies in calcareous grasslands. They also found that different species or groups of species of AMF had unique effects on the growth of several plant species and concluded that natural assemblages of AMF could influence both plant populations and plant community structure. Interestingly, Van der Heijden et al. (1998b) tested some calcareous plant species also found at Samphire Hoe (*Centaurium erythea* Rafn., and

Lotus corniculatus L.) and found them to be dependent on colonisation by AMF for establishment in microcosms. This was also observed for *Blackstonia perfoliata* (L.) Hudson in this study (data not presented).

The results of the bin sampling trial indicates that wind-borne inoculum of AMF (also dispersed by rainfall) is capable of colonising virgin substrates within a year of the exposure to the prevailing elements. Wind dispersal of AMF propagules is recorded from several arid ecosystems (Allen et al. 1989; Warner et al. 1987). The prevailing wind direction at Samphire Hoe is SW. Winds would thus blow across the site from the WT to the ET along the coast south of Samphire Hoe, bringing inoculum from the downwind coastal strip and from the calcareous grasslands on the cliffs above that part of the coastline. Deposition of AMF inocula was greatest at the WT as seen from the significant degree of colonisation by AMF in *L. perenne* grown subsequently in the bins of attapulgite clay positioned at the site. Evidence supporting this mode of entry was found by Warner et al. (1987) in a disturbed arid ecosystem in the USA where wind accounted for the movement of large numbers of spores, particularly *Glomus* spp., from distances up to 2 km. The simultaneous use of the nurse grass, *L. perenne*, at Samphire Hoe with this windblown input of AMF would probably have resulted in a rapid build-up of AMF propagules over the first 2 years. Plant species, which then established in these areas, would have roots growing into a pre-established mycorrhizal hyphal network, thus helping their survival on the site. An alternative source of immigration by AMF propagules could be via the bodies of insects, nematodes and other soil invertebrates. Both Warner et al. (1987) and Allen (1987) showed that animals could also be vectors for the importation and dissemination of AMF propagules. It may be that when the chalk marl was landscaped prior to seeding, inoculum of AMF was also introduced and spread around the site by the earth moving machinery brought in from previous work in areas with established vegetation. Whatever the main reason, the result has been the establishment of a mycorrhizal plant community with high levels of colonisation by AMF during the critical pre-flowering spring–summer season.

The use of a sequential trapping procedure to induce sporulation and facilitate the identification of AMF from Samphire Hoe was successful. Initial screening of soil samples indicated that the extraction of viable spores was difficult, although some spores of *G. claroideum* were seen in the rhizosphere of *E. pycnanthus* plants at the WT. Many previous studies of AMF richness in disturbed or recolonised mine spoils have involved identification of AMF from spores extracted directly from the field. This approach has been shown to be very unreliable, except in sandy soils (Koske 1987), because of parasitism of spores by other microbes (Lee and Koske 1994; Dodd pers. obs.). The trapping procedure was modified from that used in an arid ecosystem by Stutz and Morton (1996). The difference between the two approaches was that our study did not disturb the trap plants between harvests, whilst Stutz and Morton (1996) initially chopped up roots and soil of their target plant *Prosopis velutina* Woot. and repeated this between trap cycles (3–4 months) using their trap host *Sorghum suda-*

nese (Piper) Staph. Our approach parallels what would be happening on the site and allows AMF which do not like disturbance, e.g. species of *Acaulospora* and *Scutellospora*, to obtain niches in the trap cultures. The numbers of species of AMF identified after 18 months in trap cultures in our study was greatest at final harvest, and may have increased further if trapping had continued. Nevertheless, 40% of the species of AMF which were identified at final harvest were not found after the first harvest. This increase was also observed by Stutz and Morton (1996) in an arid ecosystem, where 75% of the total number of species of AMF identified were not found in the first cycle of trapping.

These observations support the tenet that AMF richness can be grossly underestimated when a limited study is undertaken involving a single 3–4 month trapping regime on a fast growing annual plant. We have been careful to avoid inferring that spore numbers at any stage reflect either abundance or dominance as this is not the case for AMF. Spore densities are often used as a standard measure for abundance, but in AMF they provide more information on carbon allocation by the host to fungal reproduction than niche occupancy, as observed by Stutz and Morton (1996). It is interesting to note that the total number of species identified was 12 in our study and 10 in that of arid systems (Stutz and Morton 1996) and in reclaimed surface mines (Gould and Hendrix 1998). This probably represents an average range for an AMF species assemblage in such water-stressed environments and disturbed landscapes in the early years of revegetation. In all these studies species of *Glomus* predominated, as also noted for similar studies using different isolation methods in dry, tropical regions of Australia (Brundrett et al. 1999). This observation may be related to the unique functional roles played by AMF from different genera in the Glomales in pioneer plant community development (Dodd et al. 2000).

The outplanting of mycorrhizal plants adapted to the prevailing conditions at Samphire Hoe has been shown to aid the establishment and flowering of *E. pycnanthus* seedlings. The fact that seedlings of inoculated plants were initially significantly smaller when produced in greenhouses, due to the resource allocation from the plant to the fungus to establish the mycorrhiza, had no bearing on their subsequent development in the field. The commercially produced seedlings were also non-mycorrhizal at outplanting and remained so up to 6 months later in the field. The better growth and tillering of mycorrhizal and commercially produced non-mycorrhizal plants was therefore the result of two different factors; in the former a reliance on the AMF for nutrient uptake and in the latter the availability of added nutrients and a water retentive planting medium. After 14 months there was no difference between the numbers of tillers per plant in AMF-inoculated or commercially produced seedlings which indicates that the nutrients in the original medium of the latter plants were being depleted. This was supported by data on flowering of *E. pycnanthus* seedlings where the AMF-inoculated plants produced 4 and 6 times more seed spikes on surviving plants than non-inoculated or commercially produced plants, respectively. The mean seed spike yield was also significantly greater in AMF-inoculated plants, although the

mean individual seed weight was significantly greater in the commercially produced plants 15 months after outplanting. These data clearly indicate that early colonisation of *E. pycnanthus* seedlings by adapted AMF can have beneficial effects on the reproductive phase of plant development. There is now an increasing body of evidence (Carey et al. 1982; Koide 2000) highlighting an effect of AMF on reproduction of grasses, growing in natural systems, in P-limiting soils. Natural populations of AMF had no significant effect on P-uptake of field-grown *Vulpia ciliata* (Carey et al. 1982) but did increase seed production. Furthermore, seeds produced by mycorrhizal *Avena fatua* were lighter than those of non-mycorrhizal plants but contained more P (Lu and Koide 1991; Koide and Lu 1992). The seeds from mycorrhizal plants also produced greater offspring vigour than those from non-mycorrhizal plants (Lu and Koide 1991). The effect of early colonisation by AMF may, therefore, be not only to increase seed production but also to improve seed quality in some plant species. Increased numbers of viable seed would greatly increase the chances for survival and spread of this plant species on our site. We have not, however, had time to check the quality of the offspring resulting from the seeds produced in our trial. The interesting point here is that continued monitoring of perennial plants is necessary to establish mycorrhizal benefits following inoculation of young seedlings. Studies of the first season's growth alone may miss the true importance of the symbiosis to plant populations and communities.

The Western end of the Samphire Hoe site had the harshest environmental conditions, e.g. salinity from sea spray and wave intrusion, and there are data to show that hyphal growth of some species of AMF through saline soils is inhibited by NaCl (Koske 1981; McMillen et al. 1998). In more recent work, high salt concentrations, on the Pacific coast of Canada, were found to have a negative effect on the growth of the leguminous coastal foredune plant, *Strophostyles helvola* L. Britton. This negative effect was in part mitigated by the presence of AMF (Tsang and Maun 1999). The data on flowering of mycorrhizal *E. pycnanthus* seedlings compared with non-mycorrhizal plants supports this mitigation role for adapted AMF.

Whilst high input fertilisers and composts or peat substrates are normally used for large scale plant production, a more appropriate strategy for disturbed soils, where revegetation is a prime aim rather than biomass accumulation, would be to establish mycorrhizal seedlings prior to outplanting. This would require less initial inoculum and would be easily incorporated into appropriate growing media (preferably not peat) for seedling production. Our data clearly show this to be a vital ecological input increasing plant fitness, and in the long term, greatly improving the economics of land restoration. The benefits in establishing greater plant fitness and diversity via a rapid establishment of AMF and their mycorrhizal hyphal network in the soil can have spin-off benefits in terms of soil aggregation and erosion control. A combination of management strategies including nurse plantings, slow release fertilisers and mycorrhizal fungi (and other microbiota such as *Rhizobium*) adapted to the edaphic conditions could provide a more sustainable approach to the regeneration of mining

sites (Pfleger et al. 1994) or other anthropogenic sites. Leguminous plants in particular are important contributors to soil fertility and it is also necessary to consider management of populations of the rhizobial symbionts to encourage development of these nitrogen-fixing species. A parallel study of rhizobial diversity on Samphire Hoe (Clapp et al., in preparation) has shown that considerable diversity (based on 16S rRNA gene sequences) already exists within the site despite the relatively short time that it has been in existence. We hope that a phyto-microbial approach can be employed, via direct management or inoculation, in the future in other similar restoration projects where natural plant growth and development is a project aim.

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