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The mycorrhizal status of plants colonizing a calamine spoil mound in southern Poland

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Abstract The arbuscular mycorrhizal (AM) status of two plant communities on a calamine spoil mound (rich in cadmium, lead and zinc) in southern Poland was surveyed: an undisturbed grassland community and an early succession community that developed after complete removal of the surface layer of the calamine substrate about 10 years earlier. The undisturbed site harbored 40 herbaceous species making up 87% of the absolute cover. AM colonization was recorded in 25 species accounting for 77% of the relative cover. Species with 51–75% AM root colonization such as *Festuca ovina* and *Leontodon hispidus* dominated the undisturbed turf, contributing 45% to the relative cover. *Carex* ssp. were the most abundant nonmycorrhizal plants and accounted for 9% of the relative cover. Spores of *Glomus aggregatum, G. constrictum, G. fasciculatum, G. pansihalos, Glomus* sp. and *Entrophospora* sp. averaged 25 per 100 g dry substrate at the undisturbed site. The disturbed site was colonized by 25 species accounting for 17% of the absolute cover. Among the AM plants, most abundant were the species with up to 20% AM root colonization, such as *Agrostis stolonifera* and *Thymus pulegioides*, wich accounted for 24% of the relative cover. Nonmycorrhizal species, such as *Biscutella laevigata, Cardaminopsis arenosa, Gypsophila fastigiata* and *Silene vulgaris*, dominated the early succession community and contributed 64% to the relative cover. Spores of *G. fasciculatum* and *Entrophospora* sp. averaged 20 per 100 g dry substrate at the disturbed site.

Key words Mycorrhiza · Arbuscular mycorrhizal fungi · Calamine spoil mound · Heavy metals

Introduction

Heavy-metal tolerance in plants results from various biochemical and physiological adaptations (Verkleij and Schat 1990). Mycorrhiza formation may contribute by providing a metal exclusion barrier and improving plant nutritional status (Turnau et al. 1993; Weissenhorn et al. 1995a). The ameliorating function of mycorrhizal symbiosis has been documented in plants with ericoid mycorrhiza (Bradley et al. 1981, 1982) as well as in several species of ectomycorrhizal plants (Denny and Wilkins 1987; Colpaert and Van Assche 1993).

The role of arbuscular mycorrhiza (AM) in metal uptake remains largely unclear (Galli et al. 1994; Haselwandter et al. 1994). There is ample laboratory evidence that AM plants are more efficient than nonmycorrhizal in the acquisition of micronutrients such as Cu and Zn when available at low concentrations (Faber et al. 1990). However, field relationships between AM colonization, shoot Cu or Zn concentrations and plant biomass are not fully understood (Sanders and Fitter 1992b). When these or other metals are present in excess, AM colonization may result in metal toxicity and decrease in plant biomass (Killham and Firestone 1983). Other evidence suggests that AM may be beneficial to the host under metal stress, although, this depends strongly on plant growth conditions, AM fungus and the metal (Weissenhorn et al. 1995a). Several AM fungal strains have been isolated from metal-enriched soils, indicating that AM fungi are able to develop heavy-metal tolerance mechanisms (Gildon and Tinker 1981; Weissenhorn et al. 1993; Griffioen 1994).

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This paper presents results of a survey of the AM status of plants colonizing a calamine spoil mound that originated from lead and zinc ore mining in Boleslaw in southern Poland. The spoil mound contained Cd, Pb and Zn at concentrations greatly exceeding natural soil levels (Godzik 1991; Kabata-Pendias and Pendias 1992). The history of ore mining in this region extends back to medieval times (Dobrzanska 1955). The vascular plant species occurring at the spoil mound constitute an example of a highly specialized calamine flora associated with extensive deposits of zinc ores (Dobrzanska 1955; Ernst 1974). The metal-tolerant vegetation of the calamine site offers an opportunity to investigate the role of AM in plant interactions with heavy metals at a functional community level.

Materials and methods

Site description

The investigated spoil mound is located in Boleslaw (50°17′52″N, 19°28′35″E) west of Kraków in southern Poland. It is composed mainly of the Triassic oolitic limestone and metalliferous dolomite (calamine) from the mined ore (Dobrzanska 1955). The substrate is stony and except for a thin humus layer associated with densely vegetated turfs is not differentiated into horizons.

The spoil mound harbors grassland vegetation with scattered dwarfed pines *Pinus sylvestris* and birches *Betula pendula*. Study of the mycorrhiza in herbaceous plants was carried out in 1989–1990 at two sites of about 100 m^2 each. One site was an undisturbed, densely vegetated turf. Table 1 summarizes some of the chemical properties of the calamine substrate at this site. The second site was a disturbed area where the top layer of substrate had been completely stripped around 1980 and the site left to revegetate naturally. The calamine substrate at the disturbed site contained 0.8% (w/w) organic matter and did not differ significantly in total metal content from the undisturbed site (data not presented).

Herbaceous cover

Absolute cover was defined as a vertical projection of shoot area to the ground surface expressed as a percent of the reference area (Mueller-Dombois and Ellenberg 1974). The absolute cover of herbaceous plant species was estimated in May 1990 by a point intercept method (Mueller-Dombois and Ellenberg 1974). A 10-point frame was placed at random over the investigated sites; the number of sample points was 1000 per site. The relative cover was calculated for each species as its percent contribution to the total plant cover.

Root sampling and preparation

Intact roots of the herbaceous species were sampled in May, June, July and September, as flowering occurred. The stony substrate impeded retrieval of root systems in their entirety. Therefore, after washing, the fine lateral roots were cut off and roots of at least 5 specimens with a total fresh weight of about 1.5 g were pooled, fixed and stored in 50% ethanol. After clearing in 2.5% KOH, roots were stained in 0.05% trypan blue according to Koske and Gemma (1989). Roots were examined with a compound microscope for the presence of structures characteristic of AM such as arbuscules, coils and vesicles. The AM percent root colonization was estimated by a grid intersect method using a dissecting microscope (Giovannetti and Mosse 1980).

Table 1 Chemical properties of the calamine substrate in Boleslaw, southern Poland, assessed in the uppermost 20 cm of the spoil material, which corresponds to the plant rooting zone in the undisturbed turf

pH (H_2O)[a]	6.8
pH (KCl)[b]	6.4
Organic matter (%)[c]	19.5
CEC (cmol kg^{-1})[d]	21.4
Nitrate-N (mg kg^{-1})[e]	13.1
P (mg kg^{-1})[f]	9
K (mg kg^{-1})[g]	141
Ca (mg kg^{-1})[g]	2690
Mg (mg kg^{-1})[g]	434
Total metals (mg kg^{-1})[h]	
Cd	180
Cu	18
Fe	86000
Pb	4560
Zn	49000
DTPA extractable metals (mg kg^{-1})	
Cd	37.4
Cu	1.1
Fe	13.2
Pb	323
Zn	1270

[a] With H_2O (1:2.5 w/v)
[b] With KCl (1:2.5 w/v)
[c] Wet oxidation in $K_2Cr_2O_7$ followed by $FeSO_4(NH_4)_2SO_4$ titration
[d] Ammonium saturation/KCl displacement
[e] Cadmium reduction
[f] Olsen's $NaHCO_3$
[g] Extractable with neutral NH_4OAc
[h] Extractable with hot NHO_3

AM fungi

The AM fungi were assessed in samples of the uppermost 10 cm of the spoil substrate. Three 1-kg samples were collected at random from each site in September 1989. Spores were extracted from the substrate by wet sieving and decanting (Gerdemann and Nicolson 1963). The AM fungi were identified based on their original descriptions, and specimens were deposited in the collection of J. Błaszkowski (Academy of Agriculture, Szczecin, Poland). Two species remain unidentified and are referred to by their reference numbers.

Results

The AM status of the undisturbed turf community

The herbaceous cover in the undisturbed turf was about 87% and comprised 40 species (Tables 2, 3). There were 15 species of nonmycorrhizal plants which constituted 37.5% of species recorded at this site and contributed about 22% to the relative cover. The nonmycorrhizal plants belonged to families Brassicaceae (*Alyssum montanum, Biscutella laevigata, Cardaminopsis arenosa*), Caryophyllaceae (*Cerastium arvense, C. fontanum* subsp. *triviale, Gypsophila fastigiata, Silene vulgaris*), Cyperaceae (*Carex caryophyllea, C. ericetorum*), Polygonaceae (*Rumex acetosella*), Santalaceae (*Thesium alpinum*) and Scrophulariaceae (*Euphrasia

Table 2 Mycorrhizal status of herbaceous plant species, ordered by families, found at the undisturbed site of the calamine spoil mound in Boleslaw, southern Poland. Values listed are means ±SEM (n=3) (*A* arbuscules, *C* coils, *V* vesicles)

Family	Species	Mycorrhizal structures	Percentage of roots colonized
Polygonaceae	*Rumex acetosa* L.	V	4.0 ± 4.0
	Rumex acetosella L.	Absent	
Caryophyllaceae	*Cerastium arvense* L.	V	4.3 ± 2.2
	Cerastium fontanum Baumg. subsp. *triviale* (Link) Jalas	Absent	
	Dianthus carthusianorum L.	VA	14.5 ± 9.2
	Gypsophila fastigiata L.	Absent	
	Silene vulgaris (Moench) Garcke	Absent	
Ranunculaceae	*Ranunculus acris* L.	VA	66.7 ± 8.5
	Ranunculus bulbosus L.	VA	56.5 ± 12.5
Brassicaceae	*Alyssum montanum* L.	Absent	
	Biscutella laevigata L.	Absent	
	Cardaminopsis arenosa (L.) Hayek	V	2.7 ± 2.7
Violaceae	*Viola tricolor* L.	VA	87.3 ± 2.2
Rosaceae	*Potentilla cinerea* Chaix ex Vill.	VA	41.5 ± 17.5
Santalaceae	*Thesium alpinum* L.	Absent	
Fabaceae	*Anthyllis vulneraria* L.	VA	60.0 ± 5.5
	Lotus corniculatus L.	VA	96.0 ± 1.0
	Trifolium pratense L.	VA	84.0 ± 3.0
Linaceae	*Linum catharticum* L.	VA	41.0 ± 14.7
Apiaceae	*Pimpinella saxifraga* L.	VA	54.5 ± 2.5
Plumbaginaceae	*Armeria maritima* (Miller) Willd. subsp. *halleri* (Wallr.) Rothm.	VA	17.3 ± 9.1
Scrophulariaceae	*Euphrasia stricta* Host.	Absent	
	Veronica chamaedrys L.	VA	13.5 ± 1.5
	Rhinanthus angustifolius C. C. Gmelin subsp. *angustifolius*	V	2.7 ± 2.7
Lamiaceae	*Thymus pulegioides* L.	VA	69.5 ± 7.5
	Thymus serpyllum L.	VA	83.0 ± 3.0
Plantaginaceae	*Plantago lanceolata* L.	VA	64.0 ± 31.0
Gentianaceae	*Gentianella germanica* (Willd.) E. F. Warburg	A	18.0 ± 7.0
Rubiaceae	*Galium mollugo* L.	VA	70.0 ± 1.0
Dipsacaceae	*Scabiosa ochroleuca* L.	AC	93.7 ± 0.9
Campanulaceae	*Campanula rotundifolia* L.	VA	83.3 ± 3.8
Asteraceae	*Carlina vulgaris* L.	VA	54.5 ± 2.5
	Hieracium pilosella L.	VA	36.0 ± 4.0
	Leontodon hispidus L. subsp. *danubialis* (Jacq.) Simonkai	VA	54.3 ± 16.2
	Leontodon hispidus L. subsp. *hispidus*	VA	68.0 ± 4.0
Cyperaceae	*Carex caryophyllea* Latourr.	Absent	
	Carex ericetorum Pollich	Absent	
	Carex hirta L.	V	1.5 ± 1.2
Poaceae	*Agrostis stolonifera* L.	VA	50.0 ± 8.5
	Festuca ovina L.	VA	56.3 ± 9.2

stricta). The *Carex* ssp. dominated among the nonmycorrhizal plants, making up about 9% of the relative cover (Table 3). Roots of several species (*Carex hirta, Cardaminopsis arenosa, Cerastium arvense, Rumex acetosa* and *Rhinanthus angustifolius*) had up to 5% of the examined root length occupied by fungal hyphae and vesicles resembling AM vesicles.

The AM plants collected at the undisturbed site belonged to 25 species and contributed about 77% to the relative cover. *Armeria maritima, Dianthus carthusianorum, Gentianella germanica* and *Veronica chamaedrys* had up to 25% of the examined root length colonized by AM fungi and accounted for about 14% of the relative cover. In *Gentianella germanica*, where only arbuscular colonization was observed, massive accumulations of arbuscules were often localized in noticeably swelled root segments. AM root colonization of 26–50% was recorded in *Hieracium pilosella, Linum catharticum* and *Potentilla cinerea*, which contributed about 8% to the relative cover. AM species with 51–75% root colonization dominated the undisturbed site and accounted for about 45% of the relative cover. *Festuca ovina* and *Leontodon hispidus* were most abundant in this group of plants (Table 3). In *Campanula rotundifolia, Lotus corniculatus, Scabiosa ochroleuca, Thymus serpyllum* and *Trifolium pratense*, AM root colonization was over 76% and these species constituted about 5% of the relative cover. The AM colonization in *Scabiosa ochroleuca* was characterized by a high frequency of coiled hyphae.

AM fungi found at this site were *Glomus aggregatum, G. constrictum, G. fasciculatum, G. pansihalos,* unidentified *Glomus* sp. and *Entrophospora* sp. (see Table 5). The mean frequency of AM fungal spores was 25 per 100 g dry substrate.

Table 3 Absolute cover and relative cover of species recorded at the undisturbed and the disturbed sites of the calamine spoil mound in Boleslaw, southern Poland. Species not listed had absolute cover lower than 0.1%. *Asterisks* indicate species with vesicular colonization only

Species	Absolute cover (%)	Relative cover (%)
Undisturbed site		
Nonmycorrhizal		
Carex spp.*	7.5	8.7
Biscutella laevigata	3.3	3.8
*Cardaminopsis arenosa**	2.9	3.3
Gypsophila fastigiata	2.8	3.2
Silene vulgaris	1.5	1.7
Alyssum montanum	0.9	1.0
Rumex acetosella	0.2	0.2
Euphrasia stricta	0.2	0.2
*Rhinanthus angustifolius**	0.1	0.1
Mycorrhizal		
Festuca ovina	23.7	27.3
Leontodon hispidus	8.7	10.0
Dianthus carthusianorum	7.3	8.4
Potentilla cinerea	5.3	6.1
Armeria maritima	4.5	5.2
Anthyllus vulneraria	3.2	3.7
Thymus spp.	2.5	2.9
Lotus corniculatus	2.3	2.7
Ranunculus acris	2.2	2.5
Campanula rotundifolia	2.2	2.5
Pimpinella saxifraga	1.4	1.6
Hieracium pilosella	1.3	1.5
Galium mollugo	1.0	1.2
Plantago lanceolata	0.8	0.9
Ranunculus bulbosus	0.3	0.3
Carlina vulgaris	0.3	0.3
Scabiosa ochroleuca	0.2	0.2
Viola tricolor	0.1	0.1
Total	86.7	100
Disturbed site		
Nonmycorrhizal		
Gypsophila fastigiata	7.3	43.5
Silene vulgaris	2.0	11.9
*Biscutella laevigata**	0.8	4.8
Cardaminopsis arenosa	0.7	4.2
Mycorrhizal		
Thymus pulegioides	2.0	11.9
Agrostis stolonifera	2.0	11.9
Potentilla cinerea	1.3	7.7
Achillea millefolium	0.7	4.2
Total	16.8	100

The AM status of the plant community at the disturbed site

The herbaceous cover at the disturbed, naturally revegetated site consisted of 25 species accounting for about 17% of the absolute ground cover (Table 3). Seven species recorded at this site (Table 4) did not occur in the undisturbed turf community (Table 2). Most of them were early succession, nonmycorrhizal plants such as *Reseda lutea* and *Verbascum lychnitis*. The nonmycorrhizal plants constituted 48% of all species recorded at the disturbed site and contributed about 64% to the relative cover (Table 3). Dominant nonmycorrhizal species were *Gypsophila fastigiata* and *Silene vulgaris* from the Caryophyllaceae. Mycorrhizal colonization was not found in a few species, which were also found at the undisturbed site. Most of them, such as *Euphrasia stricta, Gypsophila fastigiata* and *Silene vulgaris,* also lacked AM colonization at the undisturbed site. Others, such as *Dianthus carthusianorum* and *Scabiosa ochroleuca,* were mycorrhizal when collected from the undisturbed turf. *Biscutella laevigata,* which contributed 4.8% to the relative cover, had up to 1% of the examined root length occupied by fungal hyphae and vesicles, the latter resembling those formed by AM fungi.

Functional AM symbiosis indicated by the presence of arbuscules was found in 13 species constituting 52% of all species recorded at the disturbed site. The AM plants accounted for about 36% of the relative herbaceous cover. *Agrostis stolonifera* and *Thymus pulegioides,* which contributed 24% to the relative cover, had up to 20% of examined root length colonized by AM fungi. AM root colonization of 26–50% was recorded in early succession plants such as *Achillea millefolium, Daucus carota, Poa trivialis* and *Tussilago farfara*. None of these species was found at the undisturbed site. Similar colonization levels were recorded in species that were also present at the undisturbed site, such as *Pimpinella saxifraga, Potentilla cinerea* and *Leontodon hispidus* subsp. *hispidus*. Roots of *Galium mollugo*, *Leontodon hispidus* subsp. *danubialis* and *Viola tricolor* had AM colonization levels ranging from 51 to 75%. Roots of *Anthyllis vulneraria* were colonized at over 76%.

Occurrence of spores of *Glomus fasciculatum* and one unidentified *Entrophospora* sp. was recorded at the disturbed site (Table 5). The mean frequency of AM fungal spores was 20 per 100 g dry substrate.

Discussion

The survey of the mycorrhizal status of the calamine vegetation indicated the presence of six AM fungal species with a mean frequency of 20 spores per 100 gram dry substrate. No reports of *Glomus pansihalos* in heavy-metal-enriched soils were found in the available literature. *G. aggregatum* was reported from heavy-metal-polluted soils of Tamil Nadu in India (Sambandan et al. 1992). *G. constrictum* was isolated from a moderately Zn contaminated mine site in Kansas, USA (Shetty et al. 1994). *G. fasciculatum* heavy-metal tolerant strains were found at several sites in the Netherlands (Dueck et al. 1986; Ietswaart et al. 1992). Further surveys of heavy-metal-contaminated sites are needed to assess the distribution of metal tolerance among AM fungal species.

According to Ernst (1990), sites enriched with heavy metals provide a refuge for competitively weak arctic-alpine species able to evolve metal tolerance. The calamine flora of the spoil mound in southern Poland is an assemblage of species of various origins, e.g. *Biscutella*

Table 4 Mycorrhizal status of herbaceous plant species, ordered by families, found at the disturbed site of the calamine spoil mound in Boleslaw, southern Poland. Values listed are means ±SEM (n=3) (*A* arbuscules, *V* vesicles)

Family	Plant species	Mycorrhizal structures	Percentage of roots colonized
Polygonaceae	*Rumex acetosa* L.	Absent	
Carophyllaceae	*Cerastium fontanum* Baumg. subsp. *triviale* (Link) Jalas	Absent	
	Dianthus carthusianorum L.	Absent	
	Gypsophila fastigiata L.	Absent	
	Silene vulgaris (Moench) Garcke	Absent	
Ranunculaceae	*Ranunculus repens* L.	Absent	
Brassicaceae	*Biscutella laevigata* L.	V	0.7 ± 0.8
	Cardaminopsis arenosa (L.) Hayek	Absent	
Resedaceae	*Reseda lutea* L.	Absent	
Violaceae	*Viola tricolor* L.	VA	60.0 ± 1.0
Rosaceae	*Potentilla cinerea* Chaix ex Vill.	VA	42.6 ± 5.8
Fabaceae	*Anthyllis vulneraria* L.	VA	90.0 ± 10.0
Apiaceae	*Daucus carota* L.	VA	33.0 ± 6.0
	Pimpinella saxifraga L.	VA	26.5 ± 1.5
Scrophulariaceae	*Euphrasia stricta* Host.	Absent	
	Verbascum lynchnitis L.	Absent	
Laminaceae	*Thymus pulegioides* L.	VA	13.5 ± 1.5
Rubiaceae	*Galium mollugo* L.	A	67.0 ± 4.0
Dipsacaceae	*Scabiosa ochroleuca* L.	Absent	
Asteraceae	*Achillea millefolium* L.	VA	46.0 ± 7.0
	Leontodon hispidus L. subsp. *danubialis* (Jacq.) Simonkai	VA	52.5 ± 7.5
	Leontodon hispidus L. subsp. *hispidus*	VA	27.5 ± 2.5
	Tussilago farfara L.	VA	27.5 ± 7.5
Poaceae	*Agrostis stolonifera* L.	VA	18.3 ± 2.3
	Poa trivialis L.	VA	49.5 ± 0.5

Table 5 Arbuscular mycorrhizal (AM) fungal species found on the calamine spoil mound in Boleslaw, southern Poland. Values listed are means ±SEM (n=3). No sigificant difference in spore numbers was detected using a Kruskal-Wallis one way analysis of variance of ranks (*P*=0.9)

AM fungal species	Spore number per 100 g dry substrate
Undisturbed site	
Glomus aggregatum Schenck & Smith emend. Koske	1 ± 1
Glomus constrictum Trappe	33 ± 33
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	7 ± 7
Glomus pansihalos Berch & Koske	100 ± 63
Unidentified *Glomus* sp. (ref no. 79)	4 ± 4
Unidentified *Entrophospora* sp. (ref no. 94)	4 ± 4
Disturbed site	
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	25 ± 25
Unidentified *Entrophospora* sp. (ref. no. 94)	16 ± 15

laevigata and *Thesium alpinum* are mountain species; *Anthyllis vulneraria* and *Scabiosa ochroleuca* are associated with dry calcareous soils; *Armeria maritima, Carex ericetorum, Rumex acetosella* and *Thymus serpyllum* are typical of dry silicaceous soils, whereas *Alyssum montanum, Cardaminopsis arenosa, Dianthus carthusianorum, Potentilla arenaria* and *Silene vulgaris* are found equally often on both types of soils (Dobrzanska 1955).

The contribution of the AM species to the undisturbed calamine community (62.5% of recorded species accounting for 77% of the relative cover) appeared to be lower than in grassland vegetation on soils with similar characteristics such as high metal or calcium carbonate content. A serpentine grassland community on soil with toxic levels of Cr and Ni was reported to be comprised of 93% AM species with 98% relative cover (Hopkins 1987). The AM species contributed 88% to the grassland vegetation on the surface of a 32-year-old dry sedimentation basin of a soda factory and accounted for about 99% of the relative cover (Pawlowska 1991). On the other hand, plant communities from high elevations in the calcareous part of the Tatra mountains exhibited very similar or even lower levels of AM species than in the calamine community (Dominik et al. 1954). In an association *Oxyria digyna-Papaver burseri* developed on scree at 1700–1750 m above sea level, only 46% of the species were mycorrhizal (Dominik et al. 1954). In a turf of *Saxifragetum perdurantis* at a similar height but on fixed scree, AM was found in 64% of the species. In a turf of *Firmetum carpaticum* at 1500 m, AM species constituted about 70%, as found for the association *Carex tatrorum-Carduus glaucus* on fixed scree at 1150 m (Dominik et al. 1954).

The calamine plant community at the disturbed site was at an early succession stage. Species with AM colonization represented 52% of recorded species and accounted for about 36% of the relative cover. A low contribution of AM species to early succession communities has been reported in a number of studies and was attributed to a low availability of AM propagules at sites disturbed either naturally or by human activity (Reeves et al. 1979; Pawlowska 1991). Although, there was no significant difference in spore frequencies between the investigated sites, the undisturbed site was characterized by a higher number of AM fungal species and presumably a higher availability of AM propagules in form of a dense mat of colonized roots and fungal hyphae (Friese and Allen 1991). The lack of mycorrhizal structures in roots of *Dianthus carthusianorum* and *Scabiosa ochroleuca* recorded at the undisturbed site concurrent with the presence of the AM colonization in these species in the undisturbed plant community corroborates this explanation.

Metal-tolerant races of nonmycorrhizal species from families such as Brassicaceae (*Alyssum montanum*), Caryophyllaceae (*Silene vulgaris*), Cyperaceae (*Carex caryophyllea*), Polygonaceae (*Rumex acetosella*), Santalaceae (*Thesium alpinum*) recorded at the calamine spoil mound have been commonly found on heavy-metal-enriched soils throughout Europe (Ernst 1974). At many sites, their absolute cover and abundance reached 25% (Ernst 1974). It seems that nonmycorrhizal species make a relatively high contribution to plant communities at metal-enriched sites in comparison to other grassland communities.

The structure and intensity of AM colonization varies within plant populations and depends on factors such as type and spatial availability of inoculum, season, stage of plant development, susceptibility to inoculation, and plant nutritional status (Friese and Allen 1991; Sanders and Fitter 1992a). Flowering was a developmental reference point for the assessment of AM in calamine plants and all plants were collected at this stage. Therefore, it seems that species with a very low colonization intensity and only vesicles present in their roots should not be considered as functionally mycorrhizal, although a role for AM in their development can not be fully excluded. An interesting disparity was observed between the two investigated plant communities in terms of abundance of mycorrhizal species with different root colonization intensities. The undisturbed site was dominated by species with 51–75% root colonization, whereas the disturbed site was dominated by plants with up to 20% root colonization.

The role of AM in plant interactions with excess heavy metals is not fully understood. In *Ehrharta calycina* exposed to simulated acid rain with heavy metal loads, AM colonization resulted in metal toxicity to the plant and decrease in biomass (Killham and Firestone 1983). In *Calamagrostis epigejos* and *Festuca rubra*, AM colonization by metal-tolerant *G. fasciculatum* mitigated negative effect of excess Zn on the root biomass but had no effect on shoot biomass or metal concentration (Dueck et al. 1986). In corn grown in metal contaminated soil under limiting light conditions, AM plants exhibited significantly higher biomass and lower Cd, Cu and Zn concentrations in comparison to nonmycorrhizal controls. In contrast, in plants grown under sufficient light conditions, there was no difference in biomass or Cd concentration between treatments (Weissenhorn et al. 1995a). Field studies on the role of AM in plants under metal stress also appear inconclusive. In AM roots of *Pteridium aquilinum* collected from a Cd-contaminated site, the cytoplasm of the AM fungus contained more Cd than the host cells of the fern (Turnau et al. 1993). Bioavailability of heavy metals did not affect AM colonization intensity in corn plants cultivated in soil polluted by deposition from a Pb-Zn smelter nor did AM colonization prevent plants from metal accumulation (Weissenhorn et al. 1995b). Seasonal changes in the level of AM colonization did not seem to relate to changes in the concentrations of mineral nutrients and toxic metals in populations of *Agrostis capillaris* on soils with heavy metal enrichment (Ietswaart et al. 1992). These findings indicate that, although AM may ameliorate metal stress by improvement of plant nutritional status and formation of a metal exclusion barrier, the interaction is far more intricate and at times may be shifting towards fungal parasitism. Allsopp and Stock (1993) demonstrated in slow-growing sclerophylls from a nutrient-poor environment that AM colonization is a prerequisite for seedling establishment. Heavy-metal-enriched soils are often not only toxic to plants but also nutrient deficient (Shetty et al. 1994). It is conceivable, therefore, that AM plays a similar role in the early development of plants in such environments. Further studies focusing on the early stages of plant establishment at metal-enriched sites are needed.

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