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Establishing grassland mixtures on mine wastes – a two-year mesocosm study

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ABSTRACT

Plant growth on mine wastes is restricted by the lack of water, nutrients, phytotoxic responses and the absence of a seedbank. In a mesocosm study, we addressed the establishment of vegetation on metalliferous mine wastes from two seed mixtures. Besides the composition of the vegetation and the increase in plant cover and biomass over time, we studied concentrations of heavy metals in the shoot and analyzed the quantity of throughflow, its pH and EC to follow pollutant discharge. We hypothesized that the types of mine wastes and sown grasslands will affect species composition and the formation of a protective plant cover. Our platform was well-suited to study build-up and succession of a vegetation layer and its potential to stabilize mine wastes. However, the establishing community was less diverse than expected. The dilution of wastes increased species number and biomass, and we found a reduction of material discharge with increasing vegetation cover. Over time, drainage was reduced, while pH of the throughflow did not change. However, it was higher under the addition of greywater. Interestingly, the use of greywater led to a higher biomass in one mixture and slight changes in the chemistry of the throughflow and the plant matter.

STATEMENT OF NOVELTY

Here, we present an integrative method to test the greening potentials of mine wastes. In the mesocosm approach different mine wastes, additives and seed mixtures can be screened and the potential of the establishing vegetation to reduce drainage and runoff may be addressed at the same time. Furthermore, analyses of pollutants in plants, soil substrates and drainage waters serve to study the phytoextraction and phytostabilization potentials of the established vegetation and their ecological services.

KEYWORDS

Drainage; ecological engineering; grey waters; heavy metals; phytostabilization

Introduction

Mine waste tailings overcast vast areas in former mining districts, but the establishment of a dense permanent vegetation cover preventing erosion, run-off and pollutant leaching will be restricted due to the phytotoxicity, unfavorable pH of the substrate as well as the lack of water, nutrients and a seedbank (Tordoff *et al.* 2000; Albrecht *et al.* 2022; Dachroth 2002). In Germany, open pits, quarries, tailings and mining operations amount to 4.3% (1479 km^2) of the settled area (Destatis 2022), but information on the share of rehabilitated (in German: recultivated) sites, types of secondary habitats, and their ecological services are lacking. A comprehensive overview of the mineral processing of hard rock ores, the various types of resulting mine wastes and their associated environmental impacts is given in Lottermoser (2007).

Undoubtedly, the directed establishment of vegetation on abandoned sites using seed mixtures, composts, biochar, liming and irrigation will significantly speed up the natural succession (Kirmer 2019; DIN 2021). While approaches and vegetation technologies are being applied on most of the opencast post-mining sites in *e.g.* densely settled Europe (Dumbeck 2014; Pflug *et al.* 2014; Philipps *et al.* 2017), active restoration and greening of such substrates are rarely foreseen in other regions and climates, and it will take decades until suitable growth conditions will be achieved (Navarro-Ramos *et al.* 2022). Only after centuries, former mining sites in humid regions will be covered by forests as a climax vegetation, but pollution levels, *e.g.* heavy metal concentrations, will still remain high eventually constituting a permanent threat to the associated organisms and food webs. On the long run, land use and climatic changes may lead to the removal and destabilization of the protecting vegetation cover, so that pollutant discharges may consecutively affect the bordering environments. Moreover, higher frequencies of flash floods in a warmer atmosphere may increase the risk of landslides and severe erosion from the mostly loose mine and slag deposits. The 2019 Brumadinho (Minas Gerais, Brazil) disaster may be regarded as an example case for potential dangers of badly managed mine wastes under extreme climatic events. However, in Central Europe, the high standards of environmental stewardship applied in

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post-mining will cause only small environmental pressures even in times of climate change (Rüdiger *et al.* 2020; Lottermoser 2023).

In the EU, registers of potentially contaminated sites are available for the more recent mining and brownfield sites, but knowledge of the exact locations of medieval artisanal and small-scale mining (ASM) sites fell into oblivion elsewhere. Despite the potential environmental hazards due to the mining legacy, the larger ancient tailings and slag dumps can gain interest in re-mining activities, *e.g.* for strategic and renewable energy metals in a circular economy (*e.g.* Kamradt *et al.* 2012, Büttner *et al.* 2020; Mulenshi *et al.* 2021, Suppes & Heuss-Aßbichler 2021). Ironically, some of the extremely polluted metalliferous tailings are nowadays recognized by nature conservation as an important refuge for a highly specialized, rare flora, namely the distinct serpentine and calaminarian grasslands. Latter open formations are protected by the European NATURA2000 legislation (EUNIS habitat type code E1.B2, Annex II habitat type 6130) and some of the characteristic metallophytes, *e.g. Noccaea caerulescens*, are being regarded as model species to investigate the mechanisms of metal detoxification and hyperaccumulation in plants (Baker *et al.* 2010; Kozhevnikova *et al.* 2020). Besides using plant species as bioindicators for heavy metals, van Veen and Lottermoser (2017) discussed bioaccessibility tests for metals and metalloids at mine sites to study the rehabilitation potentials of plants. Furthermore, Tesnerová *et al.* (2017) presented approaches to combine field and laboratory tests for the phytotoxicity and reclamation success at post-mining sites in the Czech Republic.

The overall objective of present study was to develop and test a standardized mesocosm approach for the establishment of vegetation on mine waste and to assess its direct phytostabilization potentials. Repeated vegetation relevés served to identify biodiversity patterns and resilient species and intermediate harvests were performed to study growth conditions. Chemical analyses in the soil mixtures and the plant biomass allowed us to address the metal phytoextraction potentials and continuous collection of throughflow and chemical analyses of the samples enabled us to follow nutrient and pollutant leaching from the mine waste under the vegetation.

Materials and methods

Experiments were performed outdoors at the premises of the University of Hohenheim (48°42’51.3"N, 9°12’33.1"E) from 19 April 2021 to 28 June 2022. Daily temperature and rainfall records were obtained from a nearby climate station (Agricultural Technology Center LTZ, https://www.wetter-bw.de). 12 L of the substrates were filled into 40 cm × 60 cm × 6 cm (0.24 m^2) perforated plastic trays, which were put upon taller grey Euro stacking containers (25 L) for the collection of drainage water. A flowchart of the study design and the investigated parameters as well as a map, where the mine waste sites are located are given in the supplementary information (SI-1) to the article. Our experiments were comparable to the *ex situ* trials of Alcantara *et al.* (2015) and Boi *et al.* (2021), but in contrast to their studies on metal mine wastes from Australia and Sardinia, we did not work with planted specimens of a single or pre-cultured species. Instead, we were interested in using different seed mixtures and the establishment of a plant community on mine wastes. Apart from using different grassland mixtures and mine wastes and dilutions, we were also interested in the use of greywaters for the irrigation during the first season.

Substrate mixtures

Two mine wastes were used to prepare the substrate mixtures representing contents of either 12.5 and 6.25% per volume in the treatments. The Davidschacht (D mine dilutions D125 and D06) substrate stemmed from floatation tailings in the north-east of the city of Freiberg (Saxony). The mine was in operation from 1944 to 1964, with an area of the tailings of 6.3 ha (Fritz & Jahns 2017). The wastes derived from the processing of Pb-, Cu- and Zn- ores. The Mansfeld (M mine dilutions M125 and M062) mine waste sample stemmed from a low-grade ore (Kupferschiefer) and waste rock dump near Eisleben (Saxony-Anhalt). The operation of mines in the Mansfeld-Sangerhausen mining district ceased in 1990 and large heaps of mine wastes and environmental burdens are still present to date (Schreck 1998; Matheis *et al.* 1999; Wennrich *et al.* 2004; Borg *et al.* 2012; Kuhn *et al.* 2015).

The D and M mine wastes were first crushed and sieved to 2 mm to yield a similar structure and grain size. They were then mixed with a standard earth LD80 (Fruhstorfer LD80, Gebr. Patzer GmbH & Co. KG, Sinntal Jossa) and washed river sand to obtain dilutions of 12.5 and 6.25% on a volume basis. The standard earth in all treatments corresponded to 50% of the final volume to guarantee an almost uniform physical structure and supply of nutrients. It is composed of peat, volcanic clay and bark humus and is supplied with pelleted slow release fertilizers. According to the supplier, it is free of heavy metals, has a pH of 5.9 and contains 35% organic matter (to convert from OM to organic C, a factor of 0.58 may be applied). Table 1 shows the mixing ratios of the five substrates, as well as the nutrient and metal levels determined in samples of the homogenized material which were taken before filling the trays. Unfortunately, we were not able to determine heavy metal concentrations after the experiments to assess heavy metal extraction by the plants and eventual losses *via* drainage. Before sowing the trays, 12 L of the substrate blends were filled into each of the trays. For each of the four mine waste dilutions six trays were prepared, of which three each were used for the sowing of two grassland mixtures. For the control substrates, we used 18 trays of which six were allocated to the control (CON) and six each for the supply of greywater. CON1 was the treatment with the addition of a half strength, and CON2 was the treatment with a full-strength supply of greywater. An overview of the experimental set-up is given in the supplementary information (SI-1).

Greywater

Greywater was obtained from the Stuttgart Wastewater Treatment Facility (Klärwerke und Kanalbetrieb, Eigenbetrieb

Table 1. Mixing ratios, pH and element composition (nutrients and metals) of the five substrates (CON, and two dilutions of each from the Mansfeld (M) and the Davidschacht (D) mine wastes) that were used in the experiments. In the final columns, heavy metal concentrations in the substrates can be compared to the limits for sandy and clayey agricultural soils that have been set by the German Soil Protection Ordnance (BBSchV 2021).

			Davidschacht		Mansfeld		BBSchV (2021)	
		CON	D125	D06	M125	M06	sandy	clayey
Mixing ratios								
LD80	vol.%	50	50	50	50	50		
Sand	vol.%	50	37.5	43.75	37.5	43.75		
Mine waste	vol.%	0	12.5	6.25	12.5	6.25		
pH ($CaCl_2$)		6.22	6.33	6.32	6.78	6.46		
NO_3^+-N	mg L^{-1}	49.2	42.9	40.5	31.2	46.05		
NH_4-N	mg L^{-1}	1.94	0.76	1.08	1.11	1.1		
N [%]	wt. %	0.067	0.068	0.087	0.119	0.094		
C [%]	wt. %	2.21	2.47	3.14	4.95	3.59		
S [%]	wt. %	0.024	0.952	0.614	0.98	0.359		
K_2O	mg 100 g^{-1}	34	34.5	34.3	38.2	34.5		
P_2O_5	mg 100 g^{-1}	14.6	14.4	13.9	13.8	10.1		
Mg	mg 100 g^{-1}	13.3	20.5	18	20.6	17.7		
Metals and metalloids								
Cu	mg kg^{-1}	5	62	71.8	3772	1904	20	60
Ni	mg kg^{-1}	116	91	127	103	110	15	70
Pb	mg kg^{-1}	6	231	158	3036	1428	40	100
Zn	mg kg^{-1}	12.9	611	292	1586	803	60	200
Cd	mg kg^{-1}	0.1	6.18	3.02	5.82	2.67	0.4	1.5
Li	mg kg^{-1}	2.99	9.88	6.8	15.7	9.02		
Sn	mg kg^{-1}	0.114	6.1	2.81	0.37	0.236		
Fe	mg kg^{-1}	3990	21956	12110	9527	7606		
As	mg kg^{-1}	1.43	2709	1108	35.5	17.3		

Stadtentwässerung (SES), Abwasserreinigung HKW). Two variants were prepared, a half-strength solution diluted with tap water 1:1 (v:v) and the undiluted full-strength greywater. Since we could only obtain 300 liters, the waters were only supplied to the unpolluted substrates in present study. For the control (CON) and the four mine waste treatments, tap water was used for the additional irrigation. CON1 and CON2 were the representative treatments, which were hence treated with the diluted and undiluted greywater. Water quality parameters were analyzed by the Core Facility Hohenheim (CFH). The undiluted greywater had a pH of 7.9 and an electric conductivity of 978 µS cm^{-1}. Nitrate, ammonium, phosphorous, calcium, sodium and sulfur levels were 12, 0.2, 0.2, 133, 33 and 38 mg L^{-1}, respectively. Irrigation of the treatments occurred at dry spells and the same water volumes of tap and greywater were supplied to the 42 mesocosms. The volumes were always noted and added to the cumulative precipitation volumes over time. From 19 April until 16 August 2021, mesocosm were irrigated 32 times, *i.e.* 32 L were added to the mesocosms, while 75 L (313 L m^{-2}) stemmed from the precipitation. Mesocoms did not receive extra irrigation after the second harvest and in the following year.

Grassland mixtures, vegetation and plant analyses

Two seed mixtures composing of 50 wild plant species each, were used as experimental vegetation. One corresponds to a typical moist grassland (RIED mixture), while the other represents a rather dry grassland (GRIES mixture). The seeds were purchased from Appels Wilde Samen (Darmstadt, Germany). 8 g of each mixture were blended with 50 g of sand in a plastic beaker and on April 19, 2021, was evenly spread on the surface of the substrate. A 3 mm layer of sand was then sieved a top of each mesocosm, and 1 L of tap water was added to its surface to initiate the germination. Considering the weight percentages and thousand seed masses of the 50 species provided by the supplier, we calculated a seed density of 2894 seeds per mesocosm for the GRIES and 2494 seeds per mesocosm for the RIED mixture. Using the ecological indicator values (EIV) after Ellenberg *et al.* (1991), we derived medians over the 50 species present in the mixtures. In RIED, these were L7, T1, K3, F6, R3 and N4 and in GRIES L8, T6, K3.5, F3, R6.5 and N3. After sowing, mesocosms were arranged at random on four large gardening tables and were rotated clockwise every week to avoid positioning effects. During the establishment phase, we made regular observations of the quantity and identity of germinated species. Identification of the taxa was difficult at the beginning of the experiment. Only after a few weeks, we were well able to differentiate between the species and to perform species-wise vegetation relevés. The development of plants was documented using photographs of all the trays. We also made regular assessments of the total vegetation cover (COV) in 5% intervals. In vegetation analyses, visually determined cover data are to be preferred over ordinal cover classification systems (Damgaard 2014).

On July 19, 2021 (92 days after sowing, DAS), the first harvest (HAR1) was made, cutting the plants at a height of 3 cm above the ground. Dry masses of grasses and herbs were determined apart, so that besides total mass, grass-to-herb ratios could be determined as well. For the plant chemical analyses, grasses and herbs from HAR1 were combined and the heavy metals Cd, Pb, Cu, Fe and Ni as well as the nutrients Ca and K were determined in the shoot dry mass. After drying and milling of the material, chemical analyses were performed at the Core facility Hohenheim (CFH) involving digestion of the ground samples in HNO_3 and

ICP-MS. A second harvest (HAR2) was performed on September 2, 2021 (136 DAS) toward the end of the first experimental season, without separately addressing herbs and grasses. Material was dried at 80 °C for 48 h and weighed but not subjected to chemical analyses. In order to address the re-growth of the vegetation in the second season, a third harvest (HAR3) was performed on June 28, 2022 (435 DAS), in which grasses and herbs were harvested apart to determine the share of the two functional groups. Again, we refrained from chemical analyses of the material.

Drainage water

To determine drainage and loss of material from the mesocosms, throughflow was collected from the stacking boxes underneath the trays. In contrast to Aguilar-Garrido *et al.* (2023), we did not spike our mine wastes with hydrogen peroxide and only allowed rainfall and the applied greywaters to percolate the substrate. Volumes of the water were determined with large plastic cylinders (5 L) after larger rainfall events. Samples of the percolated fluids were taken four times in each of the seasons 2021 and 2022 in labeled scintillation vials (20 mL) for chemical analyses. The rest of the drainage water was discarded. pH and electric conductivity (EC) were determined in all the samples using respective electrodes (WTW Weinheim, Germany) and in the first aliquots, nitrate (NO_3^-) and ammonium (NH_4^-) were measured as well. To convert from EC to total dissolved solids (TDS, mg L^{-1}), a factor of 0.67 (Rusydi 2018) was used.

Data analyses

Means and standard deviations were calculated for each of the original and derived data using tables and graphs visualized using common software. ANOVAs, multiple comparisons and post-hoc tests (Tukey) were performed using the R free software and the relevant packages (R Core Team 2021). In the graphs, we used blue colors for the RIED wet grassland mixtures and orange for the GRIES dry grassland variants.

Twenty-five variables were selected for representative analyses of the data. HAR1 to HAR3 describe the shoot dry masses determined in three consecutive harvests and COV21 and COV22 the maximum vegetation cover in the first and second year of the study as has been derived from visual assessments. GRASS21 and GRASS22 give the share of grasses (Poaceae) in the shoot mass and VOL21 and VOL22 refer to the cumulative volumes of drainage water that had been collected in both years. NO3, NH4, pH1 and EC1 refer to the chemistry of the first flush of drainage water, while pH21, pH22, DRAIN21 and DRAIN22 refer to the seasonal mean pH values and total amounts of solids that had been washed out from the mesocosms in the two years.

Results and discussion

As expected, heavy metal concentrations in the treatments with 6.25% of the mine wastes were only half of those than in the substrates that had been blended with 12.5% of the original mine wastes. Levels of Cu, Pb and Zn were highly elevated in the Mansfeld (M) substrates, while Fe, As and Sn reached extreme concentrations in the Davidschacht (D) substrate (Table 1). pH and C contents were slightly higher in the Mansfeld treatments, but S and Cd levels were very similar in both mine wastes. Nevertheless, heavy metal concentrations largely exceeded the limits set by the German Soil Protection Ordnance BBSchV (2021). As expected, heavy metal concentrations were low in the control (CON) substrate, but unexpectedly, those for Ni (116 ppm) were higher than in both mine waste mixtures and even exceeded the BBSchV (2021) limits.

Figure 1. Photographs showing the established vegetation on the mesocosm in the seasons 2021 (left) and 2022 (right). The 42 trays were arranged after blocks for the two grassland mixtures (RIES and GRIES) and after seven rows for the treatments. While the treatments CON, CON 1 and CON2 were based on unpolluted substrates, the other four made use of two mine wastes from either the Davidschacht (D) or the Mansfeld (M).

Development of the vegetation, plant cover and productivity

First seeds started to germinate five days after sowing (April 24, 2021), and we soon noted differences in the number, identity and growth of seedlings between the RIED and GRIES mixture as well as between the different mine wastes. Initial differences in growth and vegetation cover became larger over time and were subsequently responsible for the unequal drainage and loss of solids from the mesocosms. Figure 1 gives a first impression on the vegetation density in the first and second season of the experiment using photographs from each of the trays.

While in the beginning, lowest vegetation cover was recorded on the M125 mixtures irrespectively of the grassland mixture, the GRIES vegetation performed better on the diluted M06 substrate. Although the vegetation cover was generally low in the second year, the M125 still showed the lowest plant cover. Despite the pH and nutrient levels being very similar to the Davidschacht substrates, it may be suggested that the extremely high heavy metal concentrations (Table 1) created phytotoxic effects in the Mansfeld treatments. However, we may not state which of the metals Pb (3036 ppm), Cu (3772 ppm) or Zn (1586 ppm) was most responsible for the inhibition of germination. Interestingly, most of the establishing individuals on the M125 substrates were from the Caryophyllaceae genera *Silene* and *Cerastium*. The vegetation classification, *i.e.* the relevés on copper mine waste sites in the Mansfield area presented by Baumbach *et al.* (2007) and Baumbach (2008) also show that small species from the carnation or pink family (*e.g. Silene*, *Dianthus* and *Minuartia*) may indeed represent typical metallophytes. Likewise, Mengoni *et al.* (2001) were able to confirm the existence of heavy metal tolerant populations of *Silene paradoxa* on serpentine soils. However, lichens instead of higher plants have been addressed as the most conspicuous pioneering organisms on copper slate (Huneck 2006), but we were not able to study lichen or moss growth.

Shoot mass accumulation representing vegetation growth over time, was highest in the beginning of the experiment. Until the first harvest (HAR1), *i.e.* within only 92 days, the controls (CON) produced on average 43 g dry mass per mesocosm, which upscaled to the field represents a remarkable stand biomass of 1.42 t ha^{-1}. In the mine waste treatments, however, a severely reduced growth was observed due to the phytotoxic conditions in the substrates. Figure 2 (left) shows a comparison of how much biomass the two grasslands mixtures (RIED and GRIES) produced on the different substrates.

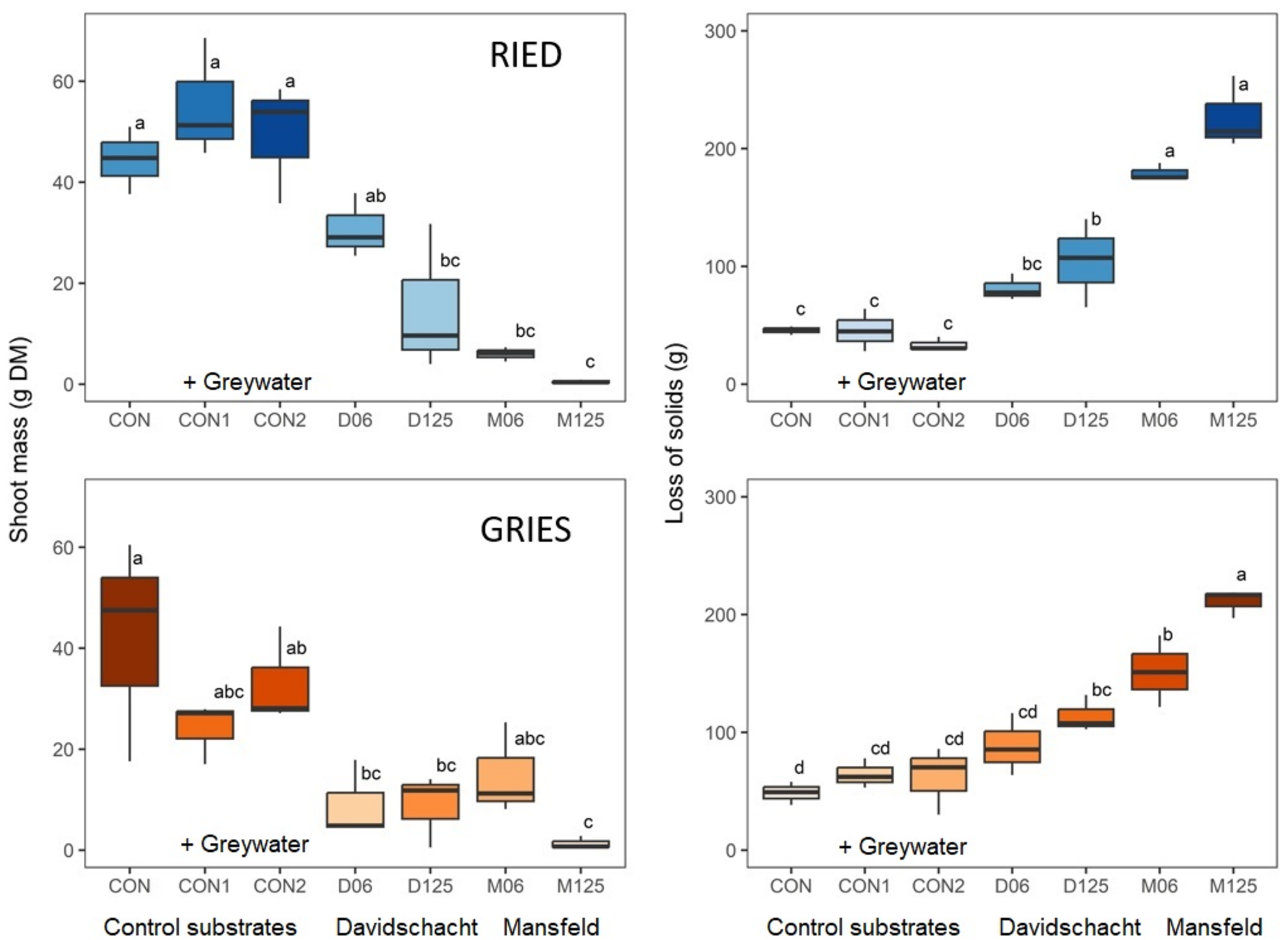

Figure 2. Dry shoot biomass produced in the wet (above, blue) and dry (below, orange) grassland mixtures during the first 92 days of the experiment (left) and loss of solids due to the draining of water in the first season (right). In each of the panels, boxplots are arranged after the control (CON, left) greywater (CON1, CON2) and mine waste treatments (see x-axes). The intensity of color shading is sorted after the median values. Same letters above the boxes indicate non-significant differences between the treatments in post-hoc multiple comparisons (Tukey tests).

The same trend in productivity was observed at the other two harvests (HAR 2 and HAR3, for the data refer to the table in SI-2), which were made in the autumn of the first year and in the early summer of the following year. However, plant growth was largely reduced due to the lack of nutrients. While at HAR2 in the fall of the first season, only 12 g on average were produced in the mesocosms of the CON treatments, productivity averaged to 21 g in the second season, *i.e.* half of the growth in the first harvest. Obviously, the slow-release fertilizer, which was present in the same amounts in all of the trays initially, had been exhausted and the take-off of the biomass at the first harvest meant that nutrients could not be re-mineralized in the mesocosms. Adding to this loss of resources, the leached nutrients were also not available anymore to later sustain the regrowth of the vegetation.

Interestingly, the use of greywater on the control substrates had contrasting effects in the RIED and the GRIES grassland mixtures. While the supply of greywater resulted in slightly more biomass in the wet grassland mixture, the opposite was observed in the dry grassland mixture (Figure 2). However, the biomass of greywater-treated mesocosms was not significantly different from the CON treatment, which only received tap water. Different growth of the grassland mixtures under greywater supply may in principle be explained by the availability of slightly more nutrients. In the more productive wet grassland species of the RIED mixture this could have led to a boost, while the less productive species in the dry grassland mixture were probably not able to respond to the more fertile conditions.

Ecological differences between seed mixes and mine wastes and correlations between parameters

The median Ellenberg indicator values presented in the materials and methods section confirm that the GRIES grassland mixture represents a vegetation ecologically better adapted to habitats with a lower moisture and nutrient level. At the same time, the ecological indicator value for reactivity (R6.5) for the GRIES grassland suggests that it prefers less acidic conditions than the RIED (R3) grassland. However, these evaluations are based upon the species that were present in the seed mix in the first place. Since we could not determine all the species that had established, we were unfortunately not able to address the ecological behavior and diversity indices in the mesocosm vegetation. At the same time, slight differences in pH resulting from the supply of greywaters to the CON substrate could have resulted in a different performance of the grassland mixtures. It has also been reported that surfactants contained in greywaters can alter the hydrophobicity of soils (Maimon *et al.* 2017). This may then result in differences in *e.g.* the wettability of aggregates and may affect the cation or anion exchange capacity. However, we do not have information on the levels of tensides of the applied greywater. Still, we observed slightly higher although not significantly higher electric conductivity in the first flush (EC1) of drainage waters in the CON1 and CON2 as compared to the CON treatments in both grassland mixes and in the RIED vegetation the mean pH values (pH21) were elevated in the first drainage flush from the greywater variants (for data on the pH and EC of drainage, waterrefer to SI-2).

Although both grassland mixtures were unable to establish on the phytotoxic M125 substrate, we observed a different performance of the mixtures on the other substrates. The RIED seed mixture produced more biomass than the GRIES mixture on the D06 and D125 treatments, whereas GRIES performed better than RIED on the probably more toxic M06 substrate. This points to the above proposed better "ecological performance" of slow growing and basiophilous dry grassland species on the slightly more alkaline mine waste. However, the M125 substrate was equally hostile for the GRIES seedlings.

Vegetation cover and biomass production showed clear relationships across the treatments studied in both grassland mixtures. For the RIED vegetation, we found a slightly stronger relationship (R^2 0.88) between cover (COV21) and shoot mass (HAR21) than for the GRIES vegetation (R^2 0.83). Table 2 gives a concise correlation matrix for 25 variables relevant for the outcome of the experiment and the separate interpretation of the results obtained for the GRIES and RIED dry and wet grassland vegetation. Obviously, the different species composition may be regarded as the driver of growth, the quantity and quality of drainage water as well as the accumulation of heavy metals in the plant biomass. It can be observed that relationships among the parameters were generally stronger for the more productive established RIED grassland vegetation. The stronger negative relationship between shoot mass and mass of drained solids suggests a better suitability of the more productive vegetation to prevent leaching, but the overall loss of material was not different between the grassland mixtures in treatment-wise comparisons.

Interestingly, in the first year, the RIED mixtures had a greater share of grasses in the total shoot mass in the controls that were supplied with tap and greywater (refer to data in supplementary information SI-2). In those mesocosms, the share of grasses reached 75%, while in the GRIES mesocosms, Poaceae only contributed to 50% of the mass. In the mesocosms that were supplied with D wastes, the opposite was found. Under these stressful edaphic situations, the GRIES vegetation had a higher share of grass mass than the RIED variants, and the grass share was even higher than in the control. In the GRIES mixtures, only the species *Anthoxanthum odoratum* was present, suggesting that it can well cope with the stress exerted from metal mine wastes. Although we were not able to perform detailed analyses on species identities and differences in the plant composition, we noted a decrease in plant diversity in the mine waste substrates from the 6.25 to the 12.5% treatments. We are aware that facilitation and plant competition will be modified along stress gradients and that this will probably be driven by the initial species composition, the availability of resources and time. In the second year, the share of grasses was generally higher in the mine waste treatments than in the first year irrespective of the grassland mixture, but in the RIED mesocosms that were supplied with tap water in the previous year

Table 2. Correlation matrix for 25 variables addressed in the mesocosm study. Positive product moment coefficients are indicated in lighter and darker blue ($r > 0.5$, $r > 0.8$) and negative relationships are indicated in lighter and darker orange ($r < -0.5$, $r < -0.8$). The upper triangle refers to the calculation of data from the GRIES grassland, while the lower represents the data from the RIED mesocosms. For the abbreviations of the parameters refer to the materials and methods section.

	HAR1	HAR2	HAR3	COV21	COV22	VOL21	VOL22	GRAS21	GRAS22	CD	PB	CA	CU	FE	K	NI	ZN	NO3	NH4	pH 1	EC 1	pH21	pH22	DRAIN21	DRAIN22
HAR1		0.55	0.83	0.80	0.62	−0.68	−0.24	−0.17	−0.03	−0.67	−0.15	−0.15	−0.19	−0.02	0.22	0.24	−0.35	−0.03	−0.73	0.49	−0.60	0.34	0.61	−0.66	−0.61
HAR2	0.81		0.78	0.81	0.74	−0.42	−0.40	−0.28	−0.13	−0.56	−0.37	0.12	−0.26	−0.33	0.32	−0.26	−0.24	−0.16	−0.58	0.56	−0.55	0.49	0.49	−0.63	−0.67
HAR3	0.71	0.86		0.85	0.82	−0.57	−0.13	−0.10	0.06	−0.73	−0.47	−0.20	−0.44	−0.33	0.19	−0.08	−0.48	−0.18	−0.82	0.65	−0.76	0.53	0.69	−0.77	−0.66
COV21	0.83	0.89	0.73		0.70	−0.40	−0.27	−0.22	−0.08	−0.77	−0.33	−0.02	−0.29	−0.16	0.34	0.00	−0.41	−0.14	−0.73	0.69	−0.71	0.59	0.74	−0.74	−0.74
COV22	0.75	0.73	0.78	0.62		−0.44	−0.29	−0.20	−0.01	−0.64	−0.43	−0.10	−0.40	−0.39	0.11	−0.31	−0.30	−0.16	−0.60	0.50	−0.60	0.35	0.56	−0.59	−0.57
VOL21	−0.78	−0.72	−0.62	−0.75	−0.61		0.35	0.08	0.02	0.52	0.07	0.11	0.15	−0.12	−0.11	−0.31	0.26	−0.04	0.54	−0.40	0.39	−0.30	−0.40	0.61	0.40
VOL22	−0.65	−0.50	−0.61	−0.37	−0.53	0.53		0.58	0.30	−0.11	−0.02	−0.53	−0.11	−0.05	−0.43	0.17	−0.20	0.14	−0.15	−0.15	0.12	−0.16	0.01	0.26	0.58
GRAS21	0.68	0.49	0.45	0.49	0.67	−0.61	−0.27		0.43	0.04	−0.19	−0.73	−0.39	−0.03	−0.63	−0.02	−0.38	0.29	−0.11	0.03	0.10	0.05	0.08	0.05	0.54
GRAS22	0.17	0.10	0.00	0.17	−0.02	−0.21	0.08	0.31		0.10	−0.45	−0.76	−0.66	0.08	−0.52	0.13	−0.64	−0.58	−0.09	0.25	−0.35	0.38	0.29	−0.27	0.15
CD	−0.75	−0.72	−0.74	−0.73	−0.81	0.60	0.39	−0.67	0.13		0.18	0.33	0.26	0.25	0.14	0.10	0.32	−0.14	0.64	−0.44	0.53	−0.18	−0.71	0.49	0.41
PB	−0.47	−0.41	−0.14	−0.54	−0.35	0.51	0.14	−0.58	−0.64	0.13		0.27	0.90	0.36	−0.18	0.31	0.80	0.34	0.23	−0.68	0.71	−0.68	−0.60	0.67	0.32
CA	−0.80	−0.76	−0.69	−0.86	−0.75	0.76	0.29	−0.80	−0.30	0.86	0.48		0.59	0.06	0.78	−0.02	0.53	0.43	0.28	−0.26	0.23	−0.19	−0.43	0.12	−0.37
CU	−0.54	−0.41	−0.31	−0.60	−0.36	0.56	0.07	−0.52	−0.82	0.20	0.79	0.56		0.16	0.15	0.18	0.91	0.47	0.25	−0.57	0.65	−0.64	−0.63	0.63	0.16
FE	0.06	0.15	0.09	−0.03	−0.13	−0.13	−0.21	0.04	−0.10	−0.01	0.45	0.10	0.36		0.05	0.77	−0.11	−0.10	0.05	−0.38	0.15	0.11	−0.17	−0.07	0.14
K	−0.43	−0.34	−0.17	−0.53	−0.17	0.54	0.07	−0.47	−0.80	0.04	0.79	0.50	0.78	0.02		0.13	0.00	0.38	−0.06	0.21	−0.17	0.31	−0.05	−0.36	−0.48
NI	0.21	0.43	0.32	0.28	0.03	−0.31	−0.22	0.16	−0.05	−0.26	0.38	−0.17	0.24	0.92	−0.07		−0.16	−0.10	−0.32	−0.07	−0.12	0.10	0.04	−0.26	−0.02
ZN	−0.70	−0.65	−0.49	−0.83	−0.45	0.76	0.20	−0.56	−0.70	0.44	0.82	0.76	0.87	0.11	0.84	−0.10		0.42	0.40	−0.57	0.74	−0.75	−0.68	0.78	0.20
NO3	−0.55	−0.37	−0.22	−0.49	−0.36	0.73	0.25	−0.59	−0.28	0.23	0.74	0.48	0.53	−0.13	0.59	−0.15	0.54		0.05	−0.24	0.52	−0.44	−0.23	0.35	0.37
NH4	−0.41	−0.54	−0.48	−0.58	−0.34	0.76	0.02	−0.39	−0.19	0.49	0.16	0.60	0.38	−0.26	0.33	−0.47	0.58	0.50		−0.65	0.70	−0.47	−0.74	0.72	0.51
pH 1	0.69	0.66	0.60	0.72	0.57	−0.79	−0.35	0.48	0.41	−0.47	−0.68	−0.67	−0.80	−0.08	−0.59	0.09	−0.81	−0.67	−0.66		−0.74	0.80	0.80	−0.79	−0.56
EC 1	−0.88	−0.76	−0.68	−0.86	−0.68	0.84	0.48	−0.69	−0.17	0.76	0.51	0.89	0.60	−0.14	0.57	−0.32	0.78	0.74	0.62	−0.79		−0.72	−0.85	0.90	0.77
pH21	0.65	0.65	0.60	0.73	0.59	−0.70	−0.42	0.36	0.35	−0.42	−0.85	−0.64	−0.75	−0.22	−0.61	−0.05	−0.79	−0.62	−0.45	0.90	−0.74		0.60	−0.80	−0.46
pH22	0.84	0.81	0.83	0.82	0.75	−0.73	−0.44	0.57	0.07	−0.84	−0.20	−0.82	−0.45	0.08	−0.28	0.31	−0.63	−0.44	−0.61	0.72	−0.85	0.67		−0.79	−0.60
DRAIN21	−0.86	−0.80	−0.75	−0.88	−0.66	0.90	0.58	−0.57	−0.23	0.68	0.65	0.86	0.70	−0.05	0.67	−0.27	0.89	0.66	0.62	−0.87	0.92	−0.86	−0.83		0.73
DRAIN22	−0.73	−0.57	−0.70	−0.54	−0.53	0.54	0.89	−0.31	0.12	0.60	−0.04	0.46	0.12	−0.26	0.08	−0.33	0.31	0.22	0.19	−0.44	0.65	−0.47	−0.66	0.69	

the stimulating effect had completely vanished. We suspect that the loss of nutrients from the mesocosms at the later stage led to a dominance of grasses rather than their potentially higher tolerance of heavy metals. Compared to dicotyledonous herbs, the roots of grasses optimize the acquisition of limited soil resources and their root system is longer lasting than that of dicots. This will probably make them efficient settlers in mine wastes and create high phytoremediation and rehabilitation potentials in the more productive grass species (Pandey and Singh 2020; Rabêlo *et al.* 2021).

Overall, the longevity, growth of roots, runners and ramets as well as the associated mycorrhiza will probably be more important for the formation of a permanent vegetation on mine waste than the aboveground biomass (Vannoppen *et al.* 2017). Nevertheless, the accumulation of organic carbon in these ecosystems will be necessary for the development of the soils and the associated microbial communities (Thouin *et al.* 2022) needed for the initiation of a plant succession. A high initial biomass production will thus always be preferable since it will act as an ecosystem engineer reducing the erosion and drainage as well as enhancing the C and probably the nutrient content on the long run. Apart from the initial differences in C contents in the different substrates (Table 1), we do not have information on the soil carbon concentrations at the end of the experiment and on how much carbon was taken off by the drainage water.

We also noticed that the biomass of the RIED vegetation had a stronger positive impact on the pH of the drainage water than that of the established GRIES mixture (Table 2). The results may thus indicate that more positive charged cations will be drained from the wet grassland system, whereas H^+ ions will remain in the root zone. This could point to a slight acidification of the mine waste, but we are not sure if this will eventually lead to a stronger mobilization and higher availability of heavy metals.

Differences in the uptake of heavy metals between seed mixtures and treatments

Looking at the phytoextraction of metals from the different substrates, we found positive linear relationships between the soil and shoot concentrations of the heavy metals. As expected, the strength of the relationships decreased in the following order Cd (R^2 0.9) > Zn > Cu > Pb > Fe (R^2 0.3), representing the differences in mobility and plant availability of the metals. Figure 3 shows the concentrations of copper (Cu) and cadmium (Cd) that were measured in the shoot mass from the intermediate harvest 92 days after the onset of

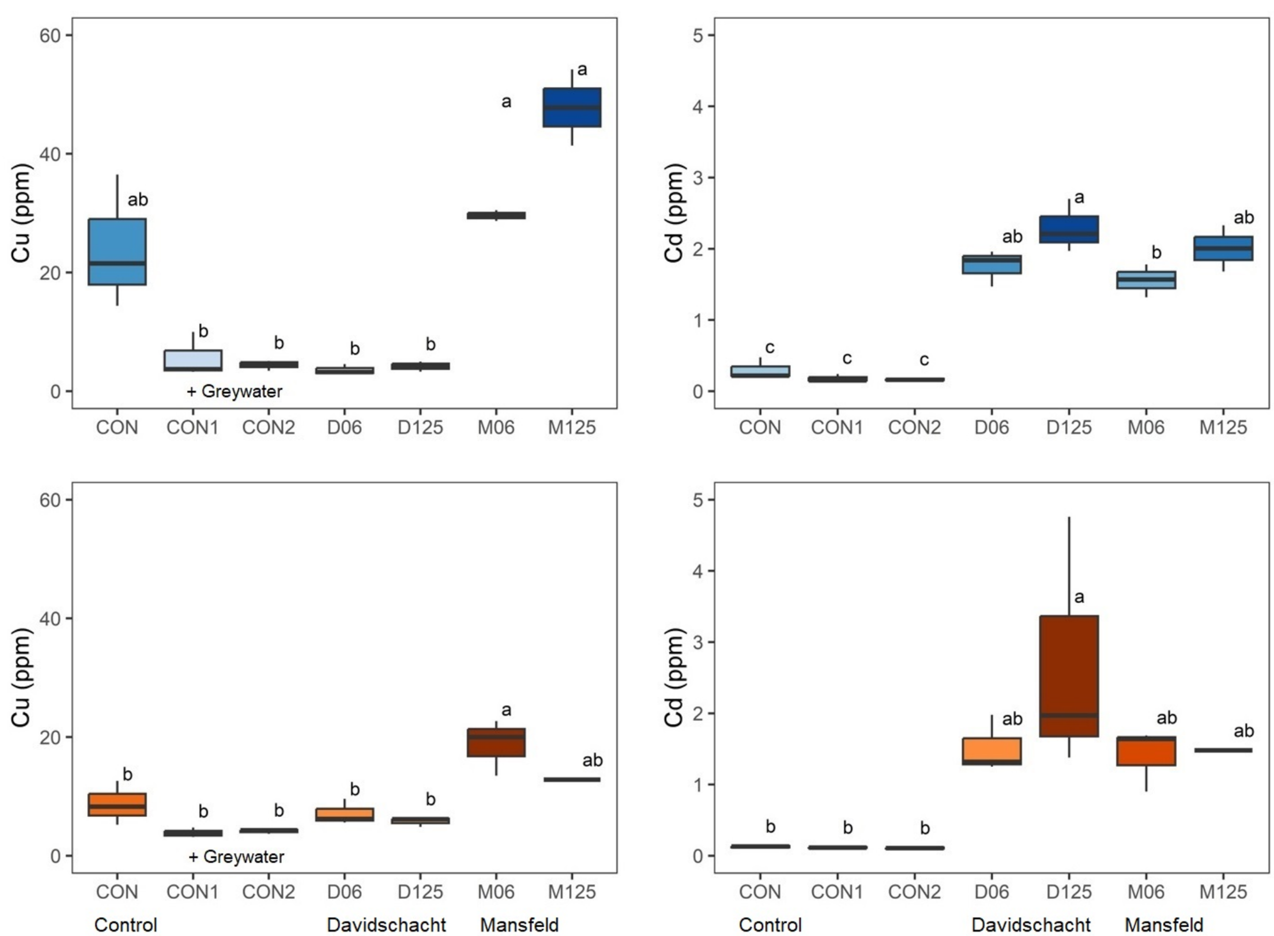

Figure 3. Copper (Cu, ppm) concentrations in the shoot dry biomass produced in the wet (above, blue) and dry (below, orange) grassland mixtures during the first 92 days of the experiment (left) and the respective cadmium (Cd, ppm) concentrations (right). In each of the panels, boxplots are arranged after the control (CON, left) greywater (CON1, CON2) and mine waste treatments (see x-axes). The intensity of color shading is sorted after the median values. Same letters above the boxes indicate non-significant differences between the treatments in post-hoc multiple comparisons (Tukey tests).

the mesocosm study. For the raw data and a graph of the Zn-concentrations, refer to the supplemantary information in SI-2 and SI-3, respectively. No differences in the accumulation of Cd were found between the two grassland mixes. However, in the case of copper, we observed higher concentrations in the more productive RIED than in the GRIES vegetation in the control substrates and those that contained mine wastes from Mansfeld. We assume that the higher share of grasses in the RIED mixture could be involved, but did not analyze heavy metals in different plant groups. Interestingly, the use of greywater reduced the concentrations of copper in both grassland seed mixtures, but this effect was not observed in the other heavy metals. An underlying reason could be that the plant essential ion is stronger bound to the soil colloids or organic matter under the presence of certain chemicals. In contrast, surfactants present in the greywater could also have led to more leaching of copper, but we did not address the concentrations of heavy metals in the drained water.

Conclusion

With the chosen *ex-situ* mesocosm approach we were able to address differences in the establishment of seed mixtures on various types of mine wastes and the ecological service functions of a plant cover to stabilize such substrates, decrease drainage and to initiate a plant succession. We suggest that the research platform is superior to the static drainage tests (White *et al.* 1999) for acid mine or rock drainage (AMD, ARD) and the blending of wastes with additives since it includes phytostabilization as an important ecological service function and is being performed under open air conditions. In present experiment, volumes and chemistry of the drainage waters as well as the mass of total dissolved solids were related to the standing biomass, but we were unable to suggest which plant species or functional types will be most effective in the phytostabilization. In future studies, tested seed mixtures should be less diverse to be able to better follow the performance of single species and functional types. Besides studying the aboveground biomass production and the plant succession, it will be necessary to also address the development of the root system. After the selection of ideal plant combinations, *e.g.* of metallophytes and additives to the substrate in mesocosm experiments, experiments should be extended to field trials on various abandoned mine wastes.

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Data availability statement

Data are available in the supplementary information (SI) to this article.

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