Using phytostabilisation to conserve threatened endemic species in southeastern Democratic Republic of the Congo

Short title: Conservation and phytostabilisation

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Abstract

Outcrops in the southeastern Democratic Republic of the Congo (DRC) are recognized as some of the largest copper–cobalt orebodies in the world. They support a unique vegetation with nearly 600 metallophytes that include rare and endemic species. Mineral exploitation has increased considerably in the region since the 1900s, affecting both environmental and public health. Phytostabilisation of polluted areas represents an opportunity to decrease the bioavailability of heavy metals in the highly polluted soils that result from ore extraction. Such a technique has been successfully implemented near Lubumbashi with the grass *Microchloa altera*. However, long-term maintenance requires a good understanding of interspecific relationships, such as competition and facilitation. This study tested the establishment success of four herbaceous species from the Katangan Copperbelt by assessing the potential role of *M. altera* as a nurse species. Two annual and two perennial species were sown in an experiment designed to study the influence of soil amendment and vegetation cover on seedling emergence, growth, and survival. These variables were monitored during the vegetation growing season as well as resprouting success for perennials. *Microchloa altera* showed a distinct effect on the emergence and survival of annual and perennial species and negatively affected the growth of individuals belonging to both groups of species.

Introduction

Copper outcrops are widespread around the world (Prasad 1989; Shallari et al. 1998; Gonnelli et al. 2001; Tembo et al. 2006; Ke et al. 2007; Lorestani et al. 2011; Saad et al. 2012; Battogtokh et al. 2013). The southeastern Democratic Republic of the Congo (DRC) hosts some of the largest copper (Cu) and cobalt (Co) orebodies in the world. They are scattered over an area more than 300 km long and 50 km wide named the Katangan Copperbelt (Duvigneaud and Denaeyer-De Smet 1963; Cailteux et al. 2005). The mineralized areas of the Roan Series are part of the Mines Series, which developed during the Quaternary Period (François 1973). Sediments partially covered the Cu-Co outcrops and led to the formation of "copper hills" characterised by soils naturally enriched with Cu and Co. From the top to the bottom of the hills, these soils show an increase in depth and a decrease in heavy metal concentrations (Duvigneaud and Denaeyer-De Smet 1963).

The copper hills support a unique biodiversity with nearly 600 metallophytes occurring in plant communities determined by edaphic factors, including heavy metal concentrations (e.g. Cu and Co) and soil pH (Leteinturier 2002; Saad et al. 2012; Séleck et al. 2013; Malaisse et al. 2016). Two plant communities have been identified according to the heavy metal concentrations in soils: the steppic savannah, found at the base and on the lower slopes of the hills (less than 3,500 mg kg^{-1} and 50 mg kg^{-1} of EDTA-available Cu and Co), and the steppe, found on the upper parts of the slopes (from 3,500 mg kg^{-1} to 10,000 mg kg^{-1} and 50 mg kg^{-1} to 1,000 mg kg^{-1} of EDTA-available Cu and Co) (Séleck et al., 2013). Among all recorded taxa, 56 have been identified as Cu endemics with 44 threatened with extinction (i.e., Vulnerable, VU; Endangered, EN; and Critical, CR) based on IUCN criteria (Faucon et al. 2010). *In situ* and *ex situ* conservation strategies have already been implemented with a mining company, but such methods are focused on small to medium scale conservation areas (Semereab et al. 2009; Le Stradic et al. 2016).

Mining operations contribute to the spread of heavy metals in the environment (Li et al. 2001; Järup 2003; Blacksmith Institute 2007; Narendrula et al. 2012; Sharma et al. 2014; Sherameti and Varma 2015). In southeastern DRC, industrial mining started in the early 1900s near Lubumbashi (then known as “Elisabethville”). Copper and Co were produced in several hydrometallurgical plants and smelters, which emitted zinc, arsenic, and cadmium as by-products (Prasad 1989). These activities increased heavy metal concentrations in the environment, leading to unfavourable physicochemical soil conditions and limited vegetation establishment. In addition, mining activities act as an important source of ground and/or water pollution that puts the health of human populations at risk (Banza et al. 2009; Manda et al. 2010; Shutcha et al. 2010; Cheyns et al. 2014).

Phytostabilisation is a suitable technology to decrease the bioavailability of heavy metals in highly polluted soils (Berti and Cunningham 2000). While this method significantly reduces human and animal exposure to heavy metals (Zhang et al. 2010; Kacprzak et al. 2014; Shutcha et al. 2015), large scale experiments often lead to the establishment of monospecific communities. The criteria used for species selection (high biomass production, dense root systems, or large quantity of propagules) greatly restrict the choice to a limited number of specialists (Rizzi et al. 2004; Mench et al. 2006; O'Dell and Claassen 2006; Mendez and Maier 2008; Shutcha et al. 2010; Parra et al. 2014; Boisson et al. 2016a).

Facilitation is a positive interaction in assembling ecological communities and is recognized as an important conditional factor for the success of phytoremediation strategies (Frérot et al. 2006; Brooker et al. 2008; Parraga-Aguado et al. 2013; Wang et al. 2014). It occurs when a plant species, referred to as a nurse plant, facilitates the establishment of other species by locally changing biotic and/or abiotic conditions, such as light and nutrient availability (Brooker et al. 2008; Soliveres et al. 2010; Bonanomi et al. 2011; Beltrán et al. 2012). The identification of nurse plants is important to improve germination, growth, and survival of a diversity of plant species in heavy metal-polluted environments (Frérot et al. 2006; Padilla and Pugnaire 2006; Parraga-Aguado et al. 2013; Wang et al. 2014).

While the conservation of threatened endemic or rare species can fail, their integration into projects as key players in phytostabilisation has become a new challenge officially recognized by the Global Strategy for Plant Conservation (Secretariat of the Convention on Biological Diversity 2014). Along with the growing awareness of biodiversity in post-mining restoration practices, threatened metallophytes from the southeastern DRC could be associated with rehabilitation or phytostabilisation strategies (Whiting et al. 2004; International Council on Mining and Minerals (ICMM) 2006; Baker et al. 2010; Faucon et al. 2010; Faucon et al. 2011). Some tolerant grasses have been reported as suitable candidates for phytostabilisation programmes (Shutcha et al. 2010; Boisson et al. 2016a) and *Microchloa altera* has been successfully tested *in situ* near Lubumbashi (Shutcha et al. 2015). This species can provide a basis from which to conduct experiments to create a new dynamic of species colonization and diversification in phytostabilised areas.

The present work aimed to test the potential role of *M. altera* as a nurse plant for the establishment of four species of high conservation value in the Katangan Copperbelt: *Anisopappus davyi*, *Crotalaria cobalticola*, *C. peschiana*, and *Triumfetta welwitschii* var. *rogersii*.

Methods

Plant material and seed collection

The four studied taxa are found in the southeastern DRC in the Katangan Copperbelt (Leteinturier 2002; Faucon et al. 2010). *Anisopappus davyi* S. Moore (Asteraceae) is a pseudo-annual species occurring in natural Cu-rich steppes and in disturbed soils. It is not an endemic species but has been recognized as a hyperaccumulator, and indicator of Cu in soils (Faucon et al. 2010). The flowering period occurs between January and April and the fruiting time ranges from April to August. The species grows slowly and develops a fasciculated root system of up to 10 to 15 cm in depth at the adult stage. *Crotalaria cobalticola* P. A. Duvign. & Plancke (Fabaceae) is an annual species. It is present in Cu-Co outcrop steppes. This strict endemic is listed as Least Concern (LC) in the IUCN classification. Flowers appear in April-

May and fruits mature shortly afterwards, between May and June. It has a shallow root system of 10 cm depth at the adult stage. *Crotalaria peschiana* P. A. Duvign. & Timp. (Fabaceae) is a perennial. It is also a strictly endemic species proposed as Critically Endangered (CR) which is found in the steppic savannah of Cu-Co outcrops (i.e., the lower part of the outcrop) (Duvigneaud et al., 1959, Faucon et al., 2010). The main flowering event occurs at the end of the dry season in August–September, with seeds being produced shortly thereafter. A second, minor, flowering event can occur at the end of the growing season (April–May). Its rooting system includes xylopodia (tuberous roots). *Triumfetta welwitschii* var. *rogersii* (N. E. Br.) Brummitt & Seyani (Malvaceae) is a perennial suffrutex identified as a strictly endemic species of the Katangan Copperbelt. It has been classified as *data deficient* (proposed IUCN status: DD). This taxon is found in both the steppe and the steppic savannah of Cu-Co outcrops. Flowers appear after fires usually at the end of the dry season in September–October. Fruits mature shortly after and co-occur with the flowers. It produces a deep and woody taproot up to 1 m in depth.

The seeds of each taxon were sampled from three seed lots from the University of Lubumbashi (DRC) seedbank, except for *C. peschiana* for which only one population was selected as the species has been reported in too few sites to allow the collection of numerous seeds (Faucon et al. 2010). During seed collection, a minimum of 50 mature and well-developed individuals were targeted to obtain numerous and genetically diverse mature seeds. Because the four species selected for this study are associated with different conservation strategies (i.e., long-term conservation for *A. davyi* and *C. cobalticola* and short term conservation for *C. peschiana* and *T. welwitschii*), their seeds were conserved in different conditions for use in propagation programs (Table 1). *Anisopappus davyi* and *C. cobalticola* were stored in ultra-dry conditions at 25 °C, while *C. peschiana* and *T. welwitschii* were stored at 5 °C. Seedlots for each species were established prior to the experiment by pooling the seeds from different seed samples.

Study site

The study was performed in Lubumbashi (southeastern DRC). The city of Lubumbashi is located at the extreme south of the Katangan Copperbelt at an altitude of around 1,200 m. This area has a subtropical climate with a rainy season extending from November to the end of March and a dry season from April to October.

This study was based on a phytostabilisation experiment performed by Shutcha et al. (2010). Our work was performed in semi-controlled conditions in the experimental garden of the University of Lubumbashi (11°27’S, 27°28’E). The experiment consists of 24 1 m^2 plots, the soil of which was artificially enriched with copper sulphate (around 1000 mg kg^{-1}) in 2008 (Shutcha et al. 2010). Three different types of soil amendment (lime, organic matter, or unamended) and two types of vegetation cover (with or without vegetation cover) were tested using a full factorial design. In the amended plots, 1 kg of limestone or 22.5 kg of organic matter was mixed into the first 15 cm of soil in 2008. To limit border effects, all plots with the same type of vegetation cover were spatially grouped. The vegetation cover consisted of individuals of *M. altera* (Rendle) Stapf (Poaceae) transplanted in 2009. This species, a caespitose perennial grass forming compact tufts, is frequently found on disturbed sites in the southeastern DRC where it is identified as the first coloniser of mineralized soils impacted by mining activities, and where it can form monospecific stands (Duvigneaud and Denaeyer-De Smet 1963; Shutcha et al. 2010).

Experiment under controlled conditions

Two distinct experiments were performed (Fig. 1). For both annuals (Year 1, Fig. 1) and perennials (Year 2, Fig. 1), 30 seeds of a single target species were sown in a 1 m^2 plot. The seeds were sown at a depth of 1 cm and homogeneously distributed using a grid of six lines and five columns. A border with a minimum width of 10 cm was left on each side of the plots. Soils were slightly ploughed at the sowing location to create favourable germination conditions and avoid seed runoff. Two replications per species were performed.

In the first year (Year 1), the annual species *A. davyi* and *C. cobalticola* were sown in February 2013. Emergence and survival rates were measured once a week until the last week of April 2013. The height of each individual and the number of leaves were measured at the end of the growing season between 15–18 April. In the second year (Year 2), the perennial species *C. peschiana* and *T. welwitschii* were sown in November 2013 and emergence and survival were monitored from February 2014 to March 2014. The height and number of leaves were measured from 15 to 18 April 2014 (Fig. 1). For the perennial species only, we measured the resprouting and the growth (number of leaves and height) at the end of the growing season, in April 2015 (Year 3).

Experiment under in situ *conditions*

The four test species were also sown in a polluted area located near Lubumbashi to simulate a practical case. For 40 years, the area has been contaminated by the deposition of metalliferous fallout coming from the Cu smelter (Gécamines) located 2 km west of our experimental site (Prasad 1989; Shutcha et al. 2015). This site has been populated despite elevated Cu, Co, and Al concentrations in the soil (Narendrula et al. 2012; Faucon et al. 2012; Shutcha et al. 2015). A phytostabilisation test was implemented in 2008 with four types of limestone amendments (control, 0.25, 0.5, or 1 kg m^{-2}) crossed with three types of organic matter addition (control, 4.5, or 22.5 kg m^{-2}) (Shutcha et al. 2015). Each 1 m^2 plot received 16 transplanted individuals of *M. altera* to establish a vegetation cover. Before the start of our experimentation, *M. altera* successfully colonized all the experimental plots, representing a total surface of 72 m^2. In 2014, Cu and Ca concentrations, pH, and organic matter content (C_{org} ‰) did not differ between treatments: 2,826 ± 2,230 mg kg^{-1} Cu (Mean ± SD), 1,18.1 ± 53.7 mg 100 g^{-1} Ca, 13.7 ± 6.1 ‰ of organic C, and a pH KCl of 6.5 ± 0.7. The four species were sown using the same grid method as the one described earlier for the experiment performed under controlled conditions. In total, 720 seeds of each species were sown in 60 plots to assess their establishment. Seedling emergence and survival at the end of the first growing season, as well as resprouting were recorded after one dry season (March 2015).

Soil analysis

In 2013, soil samples were taken from two composite subsamples collected from the topsoil (15 cm). Prior to physicochemical analyses, soil samples were air-dried and 2 mm-sieved. The pH was measured in 1N KCl with a glass electrode in a 2:5 soil/solution ratio after 2h of equilibration time. Then, the total organic C and N contents were measured in soil samples after dry combustion: C and N were oxidized to CO_2, NO_x and N_2 by heating the soil to at least 900°C in a flow of oxygen-containing gas. The amount of CO_2 released was then measured by Gas Chromatography (GC) and the N content by means of thermal conductivity detection (Margesin and Schinner 2005). Finally, soil extractable concentrations of Cu and manganese (Mn) were measured using 0.5N CH_3COONH_4–EDTA (Lakanen and Erviö 1971). The soil/solution ratio was fixed to 1:5. For the extraction solution, the pH was buffered at 4.65 (Kucak and Blanuša 1998; Faucon et al. 2009; Saad et al. 2012). The supernatant was filtered through an S&S 595 folded filter and analysed using a flame atomic absorption spectrometer (Varian 220), following the norm NF X 31-120.

Data treatment

Kruskal-Wallis rank sum tests followed by Bonferroni tests were performed to compare nutrient contents and metal concentrations in soil samples. The percentage of emergence was calculated as the ratio between the maximum of emergence (annual species) or the maximum number of individuals during February (perennial species) and the total number of seeds sown in the plots. The percentage of survival was the ratio between the number of seedlings occurring at the end of the growing season (April 2013 for annual species and April 2014 for perennial species) and the maximum number of emerging seedlings. These two parameters were calculated for each species and each plot. When normality and homoscedasticity were not met, raw data were transformed using an arcsin of square formula before fitting a two-way ANOVA model (vegetation cover × amendments). The height and number of leaves were compared with a linear mixed effects model (LME) using the 1 m^2 plots as a grouping factor. We determined *probability values* (*P*) with a likelihood test, which consisted of model comparison. When the data did not meet the assumptions of homoscedasticity and normality, they were log or square-root transformed. For the two perennial species, the percentage of resprouting individuals was calculated as the ratio between the number of resprouts and the total number of individuals at the end the growing season (2014). For this analysis, treatments were combined and a LME model was fitted to the data. Means were structured by Tukey's range test (HSD) with an error rate of 5%. Analyses were carried out using the R software version 3.0.1 (R Development Core Team 2010).

Results

Experiment in controlled conditions: edaphic variables

As expected, soil amendments did not induce differences in available Cu concentrations (1,034 ± 248 mg kg^{-1} Cu, $X^2 = 2.66$, $P = 0.26$, Table 2) but led to distinct Ca concentrations in the soil (Table 2). Soils enriched with lime had greater Ca concentrations (34.2 ± 10.4 mg $100g^{-1}$ Ca, $X^2 = 12.7$, $P < 0.01$) than the ones enriched with organic matter (12.6 ± 4.9 mg

100g^{-1} Ca) or unamended soils (17.2 ± 11.4 mg 100g^{-1} Ca). We found that pH was also greater in lime enriched soils (4.6 ± 0.2, $X^2 = 9.06$, $P < 0.05$, Table 2). Finally, the organic matter and the total N content did not vary among soils. According to these edaphic element contents, two soil types can be distinguished: (1) soils amended with lime; and (2) soils amended with organic matter or unamended. However, to conform with the test of Shutcha et al. (2010, 2015), the three categories were kept in the following results.

Experiment in controlled conditions: Emergence, survival and growth of annual species (Year 1)

Mean emergence rates for *A. davyi* and *C. cobalticola* during the first year (Year 1) were 14.2% ± 5.6% and 34.2 ± 18.5 %, respectively. Our results showed that the presence of *M. altera* increased the number of emerging seedlings for both species (Table 3). The mean emergence rate of *A. davyi* in the presence of a vegetation cover was 16.1% ± 4.9%, as opposed to 5.0% ± 2.8% without cover ($F = 19.0$, $P < 0.01$). For *C. cobalticola*, the mean emergence rate was 49.4% ± 19.6% when *M. altera* was used as a nurse plant, which was significantly greater than the percentage without cover (28.8% ± 11.9%, $F = 7.09$, $P < 0.05$, Table 3). For both studied species, we did not find any significant effect of amendment additions on the emergence rate, nor significant interaction between amendment additions and vegetation cover. At the end of the growing season of Year 1, all individuals of *A. davyi* had died whereas *C. cobalticola* showed a greater survival rate in soil amended with lime (53.8%

± 24.9%) in comparison with unamended soils ($F = 4.71$, $P = 0.06$, Table 4). Because of a low percentage of emergence and a high mortality rate at the end of the growing season, the growth of *A. davyi* was not compared with that of other species. For *C. cobalticola*, the vegetation cover had an impact on the height ($F = 4.59$, $P < 0.05$) and the number of leaves ($F = 11.8$, $P < 0.01$). On average, the seedlings were taller with more leaves in plots without cover (2.9 cm ± 1.1 cm; 3.6 ± 1.0 leaves) than in plots with vegetation cover (2.5 cm ± 0.7 cm; 2.4 ± 0.8 leaves).

Experiment in controlled conditions: Emergence, survival, and growth of perennial species (Year 2)

The two perennial species, *C. peschiana* and *T. welwitschii*, had very low emergence rates during the second test (Year 2). The mean percentages were 20.5% ± 5.7% and 7.5% ± 1.8% for *C. peschiana* and *T. welwitschii*, respectively. The presence of vegetation cover marginally affected the percentage of emergence of *C. peschiana* (Table 3), which was slightly lower with cover (11.1% ± 8.3%) than without cover (26.7% ± 10.5%, $F = 4.89$, $P = 0.07$). We did not find any significant effect of amendment additions on the emergence rate of these taxa, nor any significant interaction between amendment additions and vegetation cover. At the end of the growing season of Year 2, *C. peschiana* and *T. welwitschii* showed high survival rates of 82.1% ± 23.2% and 92.7% ± 25.1%, respectively (Table 4). A nearly significant effect of vegetation cover was observed for *C. peschiana* ($F = 3.86$, $P = 0.09$), with a greater percentage of survival in plots with vegetation cover (91.7% ± 20.4%, Table 4).

For *C. peschiana*, the interaction between vegetation cover and amendment addition affected the seedling height ($F = 3.66$, $P < 0.05$). Analyses revealed that the individuals of *C. peschiana* were significantly taller in unamended plots without vegetation cover (4.4 cm ± 2.0 cm) than in the plots amended with lime (2.3 cm ± 1.2 cm) or organic matter (1.7 cm ± 1.1 cm) without vegetation cover. However, the vegetation cover had a significant effect on the number of leaves (*L ratio* = 5.47, $P < 0.05$). A greater number of leaves was observed in plots without vegetation cover (10 ± 7 leaves) than in plots with vegetation cover (7 ± 3 leaves). Finally, the height of *T. welwitschii* individuals was significantly greater in plots without vegetation cover than in plots with vegetation cover ($F = 5.63$, $P < 0.05$).

Experiment in controlled conditions: Resprouting and growth after the dry season (Year 3)

For *C. peschiana*, a greater mean percentage of resprouting (53.3% ± 21.0%) was achieved in plots amended with lime in comparison with plots amended with organic matter (8.3% ± 16.7 %) or unamended plots (12.9% ± 17.7%, $F = 15.5$, $P < 0.01$). The percentage of resprouting for *T. welwitschii* was negatively affected by amendments ($F = 5.79$, $P < 0.05$), with greater values achieved in unamended plots (87.5% ± 25.0%) compared with plots amended with lime (10.0% ± 20.0%) or organic matter (25.0% ± 50.0%). For both species, we did not find any significant effect of vegetation cover on the percentage of resprouting.

The only species to show individual growth variations according to soil and vegetation cover was *C. peschiana*. The height and number of leaves were significantly affected by the interaction between amendment addition and vegetation cover (height: *L ratio* = 9.99, $P < 0.05$, number of leaves: *L ratio* = 19.1, $P < 0.001$). On average, the unamended plots without cover displayed more developed individuals (19.5 cm ± 6.2 cm, 14 ± 7 leaves) than the ones amended with lime but without vegetation cover (7.5 cm ± 5.1 cm, 7 ± 3 leaves).

In situ *experiment*

Under *in situ* conditions in February 2013 (Year 1), the emergence rates of *C. cobalticola* and *A. davyi* were 36.8% and 37.2%, respectively. These values were lower and higher, respectively, than the results found in the experiment performed under controlled conditions. At the end of the growing season of Year 1, the survival rates for *A. davyi* and *C. cobalticola* were 7.5% and 13.6%, respectively. In comparison with the experiment performed under controlled conditions, the survival rate of *A. davyi* was greater, while the survival rate of *C. cobalticola* was more than twice as low. For the perennial species, the emergence rates calculated in February 2014 (Year 2) were 11.7% and 11.5% for *C. peschiana* and *T. welwitschii*, respectively. These values are similar to the ones calculated for the experiment performed under controlled conditions. The survival rates of *C. peschiana* and *T. welwitschii* at the end of the first growing season were 21.4% and 64.3%, respectively. These values were both lower than the ones calculated for the experiment performed under controlled conditions.

In March 2015, the percentage of resprouting was 11.1% (2 individuals) for *C. peschiana*, and 68.5% for *T. welwitschii* (37 individuals).

Discussion

Our results showed that combining the phytostabilisation of polluted areas and the conservation of endemic plant species is complex but feasible in a tropical context as germination is the first, crucial step towards vegetation establishment. The results highlighted that the presence of *M. altera* had a greater positive effect on the emergence of the annual species compared with the emergence of perennial species. The germination rates of *A. davyi* and *C. cobalticola* were approximately twice as high under the vegetation cover. The nurse plant, *M. altera*, facilitated the establishment of annual species (Frérot et al. 2006; Brooker et al. 2008). This cover changed the above-ground microclimatic conditions, most likely reducing water runoff (Levine 2013), buffering extreme temperatures (Callaway and Callaway 2007), and increasing shading (Bader et al. 2007). This facilitation mechanism is the most commonly reported mechanism in tropical ecosystems, followed by an increase in soil fertility and associational refuges (Bonanomi et al. 2011). However, the higher germination rates of these species in Petri dishes (data not shown) suggest that these conditions were still not optimal. *Microchloa altera* can create a deep litter that significantly decreases the amount of light arriving at the soil surface compared with the natural conditions in the copper hills. These modifications were also observed for other grasses and are expected to limit the growth of other species (Callaway and Lawrence 1997; Donath et al. 2007; Ilunga wa Ilunga et al. 2015). The pattern was almost the opposite for the perennial species. Our observations did not support the results of a review of terrestrial ecosystems indicating that assistance was mainly provided to individuals of the same growth form (Bonanomi et al. 2011).

After their establishment, both annual and perennial species showed lower growth under the vegetation cover of *M. alt*era, suggesting that competition was greater than facilitation. Considering that *M. altera* is a cespitose species forming dense tufts 60 cm high, the species could compete for light and resources when the first leaves appear. In natural communities, *A. davyi* and *C. cobalticola* are found mostly in steppe that is characterized by low and open vegetation (Ilunga wa Ilunga et al. 2015). Even if the annual species tend to present a greater growth rate than the perennials (Garnier 1992), the balance between competition and facilitation depends on several factors such as stress and resource gradients (Brooker et al. 2008). The survival of species was either low or non-existent for *A. davyi*. Furthermore, the cover of *M. altera* did not affect the survival rate of the taxa used in this study, except for *C. peschiana* which showed greater survival rates when *M. altera* was present. However, no flowers were observed on the annual species, suggesting that seeds should be established just before the rainy season or that seedlings should be transplanted in the field to complete the life cycle. Translocation is an efficient technique but is more labour-intensive than seed dispersion (Mench et al. 2006) and does not seem to be a viable option for annual species.

During Year 3, some individuals of *C. peschiana* in non-covered plots produced flowers (data not shown) in the field under controlled conditions.

The soil amendment slightly affected the survival of the annual species *C. cobalticola*, the best results being achieved in soils amended with lime. The resprouting and the growth of the perennial species *C. peschiana* after one dry season (Year 3) seemed to be positively affected by Ca concentrations (data not shown). Calcium has a significant effect on plant physiology (Jones and Lunt 1967; Rengel 1992) and on heavy metal availability (Remon et al. 2005; Parra et al. 2014). Córdova et al. (2011) showed that the combination of lime and organic matter increased plant productivity in metalliferous soils. Even if Ca concentrations and pH still presented a difference with the original amendments to those in the study of Shutcha et al. (2010), the organic matter content had been stabilized in all treatments at an average of 14%. However, *T. welwitschii* showed better resprouting ability in unamended soils. This could be explained by the difference in Cu tolerance of both taxa. In comparison with *C. peschiana*, *T. welwitschii* occurs in soils with greater Cu concentrations. The survival percentage and resprouting of C. *peschiana* in the *in situ* experiment (max. 5,000 mg kg^{-1} Cu) was lower than in the experiment performed under controlled conditions (max. 1,300 mg kg^{-1} Cu). *T. welwitschii* had similar mean values between both sites.

While *M. altera* presents advantages for phytostabilisation of polluted soils as it forms a dense vegetation cover (Shutcha et al. 2010; Shutcha et al. 2015), strong evidence exists about the positive effects of species associations with other life forms in polluted soils (Frérot et al.

2006; Padilla and Pugnaire 2006). These interactions should take into consideration that plants have distinct species traits (Ilunga wa Ilunga et al. 2015). Boisson et al. (2017) highlighted that some Cu endemic species, such as *C. cobalticola* or *T. welwitschii,* have distinct shoot trait responses according to the Cu-concentration in cultivated soils. Considering that these trait responses were related to their natural niches along the Cu-gradient on the hills, the selection of suitable candidates should take into account the Cu concentration of the polluted soils.

The plant association should also be chosen with regard to the positive interactions between species, by associating different life forms, phenologies, vegetative heights, Cu-niche optima, or root systems. Grasses living on the copper hills have the capacity to enhance the soil coverage or rhizosphere interactions. Because of low abundance, low seed production, low seed germination in controlled conditions, and a niche optimum at the lowest Cu concentrations, some grasses, such as *Tristachya bequaertii* or *Trachypogon spicatus,* are not suitable in a phytostabilisation context. The grass *Eragrostis racemosa,* however, is a much better candidate (Boisson et al. 2016a).

Mined bare soils are generally low in nutrients and organic matter (Bradshaw and Chadwick 1980). Plants can change the soil properties to improve the performance of conspecifics and enhance the probability of the species to monopolize its local habitat. This process is called positive plant–soil feedback (Van der Putten et al. 2013). Adding species that are able to increase nutrient contents is essential to preserve long-term vegetation in these soils (Whiting et al. 2004; Gan et al. 2013). Primary successional stages are often characterized by the symbiosis between plants and N-fixing bacteria and are considered a positive plant–soil feedback (Van der Putten et al. 2013). The Fabaceae *C. cobalticola* and *C. peschiana* produce nodules and thus have the ability to fix nitrogen (N) from the air, thus increasing N availability for the plants. Even outside its natural habitat, *C. cobalticola* tolerates higher Cu concentrations than *C. peschiana* (Boisson et al. 2016b). While this association would be difficult in soils polluted by cadmium because N-fixation is inhibited (Furini 2012), Cu does not seem to reduce the nodule biomass in a Cu-tolerant Fabaceae (Gan et al. 2013). The creation of an ecological succession in a bare soil would create a heterogeneous environment, having small-scale spatial variability of soil parameters that represents opportunities for the successful recruitment and establishment of non-tolerant plant species (Mench et al. 2010). In the context of Cu-Co rich bare soils in the region of Lubumbashi, the choice of *C. cobalticola*, *C. peschiana*, *T. welwitschii* and several grasses identified by Boisson et al. (2016a) such as *E. racemosa*, *Andropogon schirensis* and *Sporobolus congoensis* could enhance the successional stage, thus promoting the recolonization of bare soils.

Conclusion

Facilitation and competition are key components to consider in the implementation of *ex situ* strategies promoting endemic plant species. This study highlighted that species living in the Cu-Co soils of the southeastern DRC present complex and dynamic interactions according to vegetation stages and the local conditions of the site. Although these results are interpreted for four species only, this practical and theoretical question needs to be developed for other metalliferous ecosystems or vegetation occurring on singular soils.

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Table 1.

Details of the four species used, including their conservation status, collection sites and seed conservation methods. Legend: LC = least concern, CR = critically endangered, DD = data deficient, R.H. = relative humidity in air. Strict endemic refers to species having all populations restricted to metalliferous soils (Faucon et al., 2010).

	Conservation status	IUCN status*	Life form	Root system type	Fruiting time	Collecting sites	Number of individuals collected	Conservation method
Anisopappus davyi	Hyperaccumulator	-	Pseudo-annual	Branched	April-August	Fungurume IV Kabwelunono Kakalalwe 1	70 100 60	R.H. < 5 % Ambient T°C
Crotalaria cobalticola	Strict endemic	LC	Annual	Branched	May-June	Goma 3 Kakalalwe Kavifwafwaulu 1	130 50 75	R.H. < 5 % Ambient T°C
Crotalaria peschiana	Strict endemic	CR*	Perennial	Tuberous	August-September April-May	Kazinyanga	75	Ambient R.H. T° 5°C
Triumfetta welwitschii	Strict endemic	DD*	Perennial	Taproot	September-October	Fungurume VIII Kabwelunono Kavifwafwaulu 4	150 75 75	Ambient R.H. Ambient T°C

* Proposed IUCN classification (Faucon et al., 2010)

Table 2.

Edaphic content in copper (Cu, mg kg^{-1}), pH, calcium (Ca, mg 100g^{-1}), organic matter (C_{org}, %), and total nitrogen (N_{tot}, %) under controlled conditions. Different letters indicate significant difference according to Kruskal-Wallis test. Treatment abbreviations: Lime refers to a plot with limestone amendment, OM refers to a plot with organic matter amendment and UA refers to a plot without amendment (*i.e.*, unamended)

	Lime	OM	UA	X^2	*P*
Cu (mg kg^{-1})	1,040 ± 146^{a}	939 ± 297^{a}	1,122 ± 272^{a}	2.66	**0.26**
pH KCl	4.6 ± 0.2^{a}	4.2 ± 0.1^{b}	4.3 ± 0.2^{b}	9.06	**< 0.05**
Ca (mg 100g^{-1})	34.2 ± 10.4^{a}	12.6 ± 4.9^{b}	17.2 ± 11.4^{b}	12.7	**< 0.01**
C_{org} (‰)	13.8 ± 1.3	13.8 ± 1.4	14.5 ± 2.8	0.18	**0.91**
N_{tot} (%)	0.141 ± 0.007	0.141 ± 0.010	0.146 ± 0.018	0.05	**0.98**

Table 3.

Percentage of emergence (%) of the four species among the amended and the vegetation cover treatments (controlled conditions experiment). Data were analysed using a two-way ANOVA followed by a mean range test. Different letters indicate significant difference according to Tukey's HSD range test with an error 5 %. Treatment abbreviations: Lime refers to a plot with limestone amendment, OM refers to plot with organic matter amendment and UA refers to a plot without amendment (*i.e.*, unamended). Interactions were not significant.

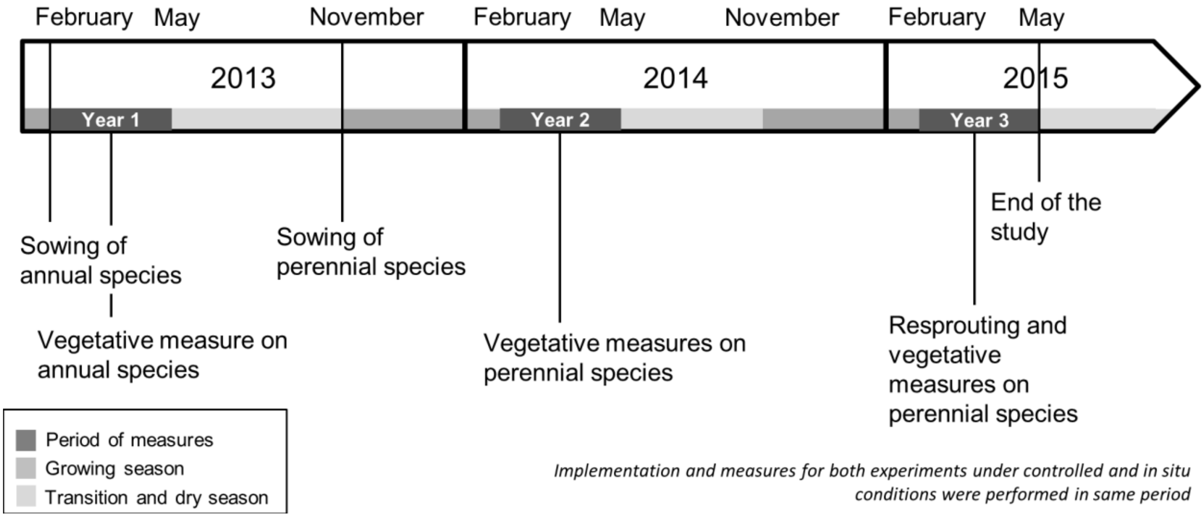
	Lime	OM	UA	*F*	*P*	With vegetation cover	Without cover	*F*	*P*
Anisopappus davyi	10.8 ± 8.8	10.0 ± 8.2	10.8 ± 5.7	0.10	0.90	16.1 ± 4.9^{a}	5.0 ± 2.8^{b}	19.0	**< 0.01**
Crotalaria cobalticola	37.5 ± 18.3	50.0 ± 25.4	30.0 ± 6.7	2.19	0.19	49.4 ± 19.6^{a}	28.8 ± 11.9^{b}	7.09	**< 0.05**
Crotalaria peschiana	16.7 ± 13.1	16.7 ± 11.6	23.3 ± 14.1	0.47	0.65	11.1 ± 8.3^{b}	26.7 ± 10.5^{a}	4.89	**0.07**
Triumfetta welwitschii	11.6 ± 6.9	5.8 ± 3.2	4.1 ± 1.7	2.51	0.16	8.3 ± 3.5	6.1 ± 6.8	1.28	0.30

Table 4.

Percentage of survival (%) of the four species among the amendment and the vegetation cover treatments (controlled conditions experiment). Data were analysed using a two-way ANOVA followed by a mean range test. . Different letters indicate significant difference according to Tukey's HSD range test with an error 5 %. Treatment abbreviations: Lime refers to a plot with limestone amendment, OM refers to a plot with organic matter amendment and UA refers to a plot without amendment (*i.e.*, unamended). Interactions were not significant.

	Lime	OM	UA	*F*	*P*	With vegetation cover	Without cover	*F*	*P*
Anisopappus davyi	0	0	0	-	-	0	0	-	**-**
Crotalaria cobalticola	53.8 ± 24.9^{a}	24.2 ± 9.9^{ab}	14.6 ± 13.8^{b}	4.71	**0.06**	37.8 ± 20.0	23.9 ± 26.4	2.27	0.18
Crotalaria peschiana	87.5 ± 25.0	73.8 ± 37.7	85.9 ± 17.6	0.55	0.60	91.7 ± 20.4	73.1 ± 29.6	3.86	**0.09**
Triumfetta welwitschii	95.8 ± 8.3	100 ± 0	75.0 ± 50.0	0.84	0.48	100 ± 0	80.6 ± 40.0	1.32	0.29

Figures



Implementation and measures for both experiments under controlled and in situ conditions were performed in same period

Fig. 1. Timeline of the study performed between 2013 and 2015 under controlled conditions (University of Lubumbashi, DRC) and in in situ conditions (polluted area of Lubumbashi, DRC).