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Assessment of the Potential of Umbrella Thorn [*Vachellia tortilis* (Forssk.) Galasso & Banfi] for the Rehabilitation of Sub-Sahelian Mining Sites at Essakane, North-Eastern Burkina Faso

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Establishment of plant communities at mine sites with significant disturbance depends upon factors such as the presence of efficient mycorrhizal fungi and rhizobia. Field and greenhouse experiments were conducted to assess growth performance of umbrella thorn as a potential tree species for reforestation of mine sites in arid areas. In the first experiment, seedlings were transplanted onto waste rock stockpile (WR) and tailings (TLG) with high arsenic (As) content on sites at the Essakane gold mine. Trees were assessed for growth and survival 3 years after planting. In experiment 2, soil was sampled on four sites across a disturbance gradient from mining waste (WR and TLG) and artisanal gold mining to undisturbed natural soil (NS). Each soil was planted with two separate leguminous trap crops (cowpea and umbrella thorn) in pots to check for microsymbionts. At 3 years, trees grew better on TLG soils with greater arsenic contamination than WRs in the field. Although tree establishment was moderate, with <50% survival, overall results suggest the ability of umbrella thorn to tolerate As contamination levels up to 1,300 ppm and, therefore, its potential for reforestation. Soil pH has shown strong effects on soil nutrient content. In particular, ammonium was the dominant form of mineral nitrogen (N) in the more neutral pH NS soils, while nitrate was present in the more alkaline WRs. Denitrification likely resulted in high N loss where nitrate dominates, reflecting the poor performance of N-deficient trees on WRs compared to TLG soils. Growth trends of umbrella thorn in potted-soils were consistent with those reported on TLG and WR soils in plantations.

Keywords: disturbed soil, arsenic contamination, mycorrhizae, rhizobia, mining sites, reforestation, Sahel

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INTRODUCTION

Mining causes major site disturbance due to the displacement and piling of topsoil, overburden and tailings, especially in surface mining operations. Overburden and tailings have specific properties and affect environmental quality and ecosystem services through various forms of environmental pollution (Zhuang et al., 2009; Lima et al., 2016). Mining wastes originating from deep rock strata are generally unstable and prone to leaching at the Earth's surface, with increased solubility of trace metals (Sheoran et al., 2010; Bruneel et al., 2019). Surface mining produces typical by-products of extraction and processing that are devoid of vegetation cover (Schueler et al., 2011) and which are frequently impoverished in terms of their microbial symbionts and nutrients, making their rehabilitation more difficult (O'Dell et al., 2007; Carrenho et al., 2018; Lacalle et al., 2018; Chileshe et al., 2020).

Previous studies have focused on the use of vegetative technologies as a biotool for mine reclamation (Asensio et al., 2013; Chibuike and Obiora, 2014; Mensah, 2015; Tetteh et al., 2015; Riaz et al., 2020) as an alternative to unsuccessful conventional reforestation. Since these technologies depend upon several factors such as environmental conditions, biological potential, and bioavailability of soil contaminants (Alkorta and Garbisu, 2021), success would depend upon the capacity of plants to deal with the specific properties of these sites and climatic conditions. Therefore, knowledge of the physicochemical properties of mining wastes is a prerequisite, either for matching plant species potentially adapted to rehabilitation operations (Ntloko et al., 2021), or for improving adaptation capacities of candidate species to site-specific soils. In addition, it has been reported that the interaction of plants, soil components, and microorganisms may also be critical for the management of contaminated soils (Pajuelo et al., 2011, 2019; Lacalle et al., 2018).

As root symbioses have revealed an increased capacity for some plant species to perform strongly on dry and nutrient-poor soils (Anderson et al., 2008; Ndiaye et al., 2011; Carrenho et al., 2018; Kumar et al., 2018; Samba-Mbaye et al., 2020), symbiotic N_2-fixing rhizobia and arbuscular mycorrhizal fungi (AMF) are especially important in nitrogen and phosphorus acquisition by plants (Duponnois et al., 1998), which have been previously used for the rehabilitation of mining sites (Bruneel et al., 2019; Festin et al., 2019) and even for promoting plant tolerance on contaminated soils (Kuffner et al., 2010; Lenoir et al., 2017). Yet, the success of rehabilitation activities strongly depends upon the effectiveness of fungi and other microbial populations in the substrate, together with its physicochemical characteristics (Quoreshi, 2008; Nadeau et al., 2018). Soil pH is often reported as a key factor having strong effects on the solubility and speciation of metals both in the bulk soil and in soil solution (McGrath et al., 1995; Giller et al., 1999; Ma et al., 2016). Metal concentrations can almost double for each unit decrease in pH, which in excess are much more toxic to microorganisms such as rhizobia than to other soil biota growing on the same soils (Giller et al., 1999).

Burkina Faso, like other countries in the Sahel region, has experienced expansion of its mining sector for at least a decade. Although environmental protection is imposed upon mining sites, there is little information on the nature and extent of pollutants (Porgo and Gokyay, 2017) and effective interventions that could be implemented for their control. At the Essakane gold mine in northeastern Burkina Faso, the major constraint linked to wastes lies in the high arsenic contents. Shrubby species such as *Senegalia laeta* (R.Br. ex Benth.) Seigler & Ebinger, *Vachellia tortilis* (Forssk.) Galasso & Banfi (Kyalangalilwa et al., 2013) and *Balanites aegyptiaca* (L.) Del. dominate the woody vegetation component. Although phytoremediation techniques were implemented earlier at the site using several tree and herbaceous species, which included *V. tortilis* (Fabaceae, Mimosoideae) among them, the effectiveness of these interventions has not yet been evaluated.

Vachellia tortilis (Forssk.) Galasso & Banfi, which is commonly known as umbrella thorn acacia or Israeli babool, is a multipurpose legume tree that is widely distributed in northern Burkina Faso and useful to communities within the region, and across the Sahel into the Horn of Africa. In the Middle East, this species extends from the Arabian Peninsula into Israel. This study reports on the ability of the umbrella thorn to grow in the gold mining sites of Essakane, which are heavily polluted by mining residues that are rich in arsenic. In the light of the physicochemical and biological properties of soils, the objectives were (1) to assess the success of revegetation 3 years after planting, (2) to assess the physicochemical and symbiotic properties of the plantation soils, and (3) to explore the relationship between soil characteristics and tree performance, which could contribute to a better understanding of the underlying factors influencing the variability of umbrella thorn establishment and growth on site soils.

MATERIALS AND METHODS

Study Site Description

Field experiments and soil sampling were conducted at the IAMGOLD Essakane SA mine sites around Gorom-Gorom, which is a district of Oudalan Province (**Figure 1**). The area is part of the semi-arid transition zone between the Sahara Desert and the Sudan Savanna, which runs from Northern Senegal in the west to Northern Eritrea in the east, and which is home to semi-nomadic pastoralists and subsistence farmers. It covers about 25% of northernmost Burkina Faso and is characterized by dry-savanna shrubs. In addition to a sparse cover of perennial (e.g., *Panicum turgidum* Forssk., *Aristida sieberiana* Trin.) and annual [e.g., *Cenchrus biflorus* Roxb., *Schoenfeldia gracilis* (Kunth.)] grasses, the deep-rooted woody vegetation includes acacias and other legumes. The average annual rainfall in this area is about 400 mm, with mean minimum temperatures ranging from 6 to 20°C in January, which is the coldest month (Nicholson, 2018). Mean maximum temperatures are around 40°C in April-May, just before the rainy season. The Essakane gold mine sites are former artisanal mining sites with decades of disturbance, which cover about 40% of the mine area. In addition to those wastes left by past artisanal mining (AMS), degraded lands are currently expanding mainly because of waste

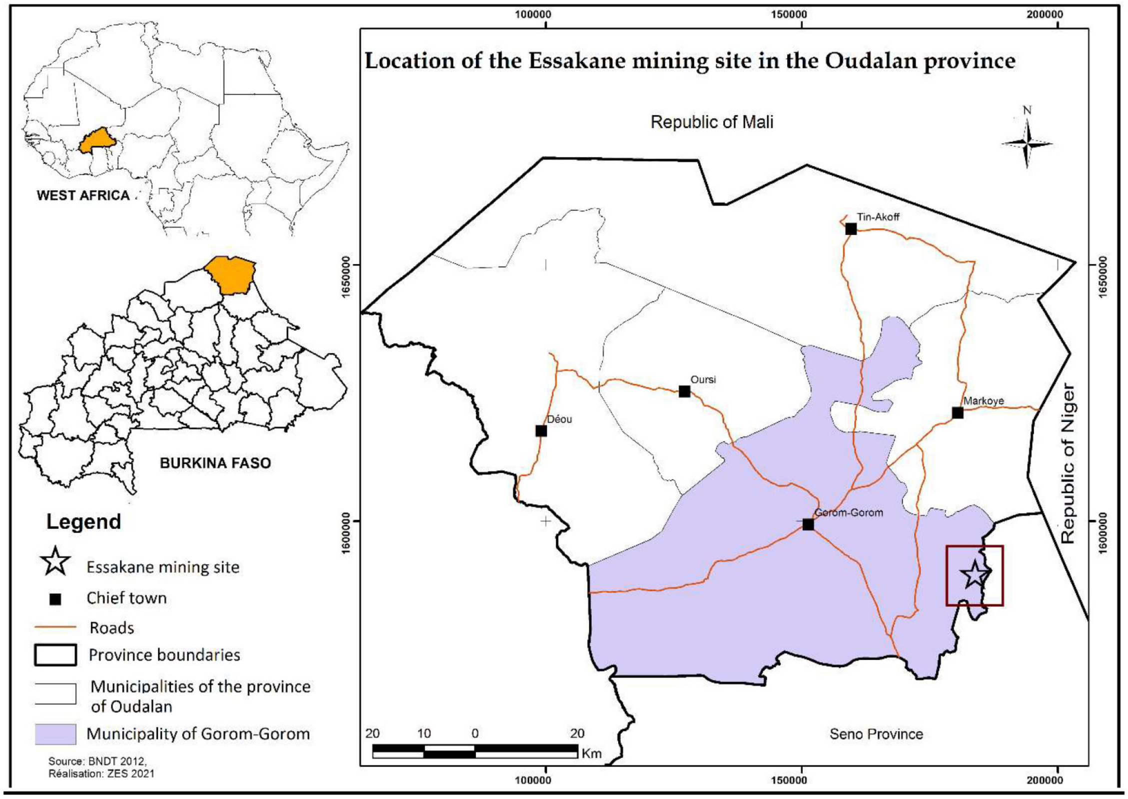

FIGURE 1 | Location of the Essakane mining site in the Province of Oudalan, north-eastern Burkina Faso.

rock stockpile (WR) and tailings (TLG), which are generated by conventional interventions. Stockpiled waste rock is accumulated mounds of unproductive ore (i.e., low gold content), while sludge from the gold extraction process is collected in tailings ponds. AMS is rugged terrain that is located at the border of natural sites (undisturbed soil or NS), which are covered mainly with umbrella thorn and *B. aegyptiaca* (known as desert date or Egyptian balsam).

Field Preparation and Tree Establishment

A revegetation experiment was conducted in the field to assess survivorship and growth of umbrella thorn as a potential tree species for rehabilitating lands that are affected by mining wastes. Prior to planting, the waste rock stockpile was reshaped to obtain a 3:1 slope. Dried tailings were scarified with a Delfino plow, which is commonly employed in restoring degraded arid-land soils following the Vallerani system (Levy, 2016). Seedlings were raised at the Essakane SA nursery using seeds that had been provided by the Centre National des Semences Forestières (CNSF, Ouagadougou, Burkina Faso). Seeds were sown in nursery bags (22 × 7 cm, flat size) containing a mixture of river sand and common nursery compost (50%, v/v). The 3-month-old seedlings were transplanted to the field in August when rains are mostly regular across the Sahel region. The entire field (0.5 ha) was covered by planting 780 seedlings onto the waste rock, while 180 seedlings were planted onto the tailings, at 2 m between-line and 3 m within-line spacing. Arrangements of seedlings were either in staggered rows on the slope when planting on the waste rock pile or on the half-moon ridges on the tailings. After 3 years, the survival, height and crown diameter of the trees were assessed.

Soil Sampling and Analyses

From each of the four study sites, soil samples were collected in mid-January 2011 at a depth of 20 cm. At each site, five core samples were taken every 10 m along three parallel transects using a soil auger, and homogeneously bulked to obtain a composite sample. After bulking, soil samples were air-dried, sieved to pass a 2-mm mesh sieve, and subsampled for physical and chemical analyses. The Bureau National des Sols (BUNASOL, Ouagadougou, Burkina Faso) analyzed the soils for texture, pH, organic matter (OM), total carbon (C), total nitrogen (N), and total phosphorus (P), NH_4–N, NO_3–N, and available P and potassium (K). BVLab (Quebec, QC, Canada) further analyzed soil samples that were collected from the TLG and the waste rock stockpile (WR) for trace metals and exchangeable base cations (Ca^{2+}, Mg^{2+}, and K^+).

Greenhouse Assessment of Soil Symbiotic Properties

An experiment was conducted in the greenhouse to evaluate whole-soil inoculants for legume performance in terms of growth, root nodule, and mycorrhizal root formation using cowpea [*Vigna unguiculata* (L.) Walp.] and umbrella thorn as test crops. Each soil sample was distributed (500 g portions) into plastic pots (7/22 cm, width/height) to grow test crop plants. Study soils consisted of the four field soil types (WR, TLG, AMS, and NS); a sterilized nursery sandy soil was also included as a control. Cowpea seeds that had been provided by Département Productions Végétales (INERA, Kamboinsé, Burkina Faso) were surface-sterilized by soaking for 3 min each in ethanol (96%) and aqueous calcium hypochlorite (3.3% $CaCl_2O_2$ m/v), and then rinsed thoroughly with sterile distilled water. Umbrella thorn seeds were scarified with sulfuric acid (95%) for 30 min, thoroughly rinsed, and soaked in distilled water for 24 h. All pre-treated seeds were aseptically incubated at 30°C to germinate on autoclaved (120°C, for 20 min), moistened cotton wool in Petri dishes. Seedlings of both cowpea and umbrella thorn were transplanted into the potted soils and irrigated daily as needed with tap water. Ten combinations (5 × 2) of soil type and test crop treatments were investigated. The experiment was set up using a complete block design with six replications for a total of 60 individual pot units. Three experimental units were lost at the seedling stage during the trial (two umbrella thorn in the WRs and TLGs; one cowpea in the TLG soil).

Cowpea was harvested 6 weeks after planting, and umbrella thorn after six further weeks. Plant height and nodule number were measured at harvest. Plant shoots and roots (but not nodules, which were retained for further assays) were oven-dried (70°C for 72 h) for biomass determination. Fresh root fragments without nodules were collected at harvest, cleared in 10% KOH, stained with trypan blue, and then assessed for AM root colonization (frequency and intensity), as described by Trouvelot et al. (1986).

Statistical Analyses

Since umbrella tree was only planted in two sites only (either TLG or WR, with no replication options), tree growth data (height, and diameter and circumference of stem) were subjected to non-parametric analyses of the variance (ANOVA) and tests (Wilcoxon, Kruskal-Wallis). Least-squares means and standard errors also were computed. Also, simple regressions were computed to explore relationships between soil pH and other soil properties. Regarding the greenhouse experiment, all data were subjected to analysis (ANOVA) after checking the homogeneity of variance assumption (Levene's Test). Least-squares means and their respective standard errors were calculated, together with simple contrasts for treatment comparisons. All statistical analyses were performed using General Linear Model (GLM) procedures in SAS/STAT software, SAS System Version 9.3 for Windows (SAS Institute, 2011).

RESULTS

Soil Characteristics

The soil textural class is mainly silty, with a high proportion of sand in the NS and AMS (**Table 1**). Much higher silt content and soil alkalinity was found in industrial mine wastes (WR and TLG) than in other soils (AMS and NS). The natural undisturbed site (NS) contained two- to four-fold more sand, yet still had higher soil organic matter and C:N ratios, compared to disturbed sites (WR, TLG, and AMS) (**Table 1**). Total nitrogen content was low with little variation, but extractable NH_4–N and NO_3–N were clearly influenced by soil type. On average, NH_4–N was highest in the natural zone (25.6 mg kg^{-1}), while NO_3–N was highest in the spoil pile (140.2 mg kg^{-1}). Total P concentrations were highest in the disturbed sites, particularly in the WR soils (814.7 mg kg^{-1}) (**Table 1**). Seven trace elements were below instrument detection limits (i.e., <0.02 and <5 mg kg^{-1} for B and Pb) (**Table 2**). Concentrations of 12 trace elements in WRs were 2–6 times higher than those of TLGs, with the exception of arsenic, for which the expected opposite trend was observed (1,300 mg kg^{-1} for TLG versus 345 mg kg^{-1} for WR) (**Table 2**).

TABLE 1 | Average physicochemical properties of soils at mine sites.

Soil characteristic	^{a}Sites			
	WR	TLG	AMS	NS
Clay (%)	24.2 b	17 b	33.2 a	21.6 b
Silt (%)	53.6 a	56.9 a	22.9 b	17.6 b
Sand (%)	22.2 b	26.1 b	41.8 a	60.8 a
Textural class	Silt loam	Silt loam	Clay loam	Sandy clay loam
pH (1:2.5 soil:water)	8.6 b	9 a	8 c	7.1 c
Organic matter (%)	0.2 b	0.2 b	0.4 a	0.7 a
Total C (%)	0.1 b	0.1 b	0.3 a	1.1 a
Total N (%)	0.02 a	0.03 a	0.03 a	0.03 a
C/N ratio	4.8 b	3.6 b	10.2 a	13.6 a
NH_4-N (mg/kg)	7.8 b	9 b	21.4 ab	25.6 a
NO_3-N (mg/kg)	140.2 a	108.2 a	110.7 a	36.9 b
Total P (mg/kg)	814.7 a	131.9 b	126.7 b	93.1 b
Available P (mg/Kg)	1.3 b	2.7 a	1.2 b	1.6 b
Available K (mg/Kg)	6.5 c	30.2 b	82.5 a	115.9 a

Means followed by the same letter within each row are not significantly different at P = 0.05 with the LSMEANS statement for Conover-Iman multiple comparison. Means are based upon three replicated subsamples.
WR, waste rock stockpile; TLG, tailings; AMS, artisanal mine site; NS, natural site; C, carbon; N, nitrogen; P, phosphorus; K, potassium.

TABLE 2 | Trace element concentrations in soils from the two disturbed sites: waste rock stockpile (WR) and tailings (TLG).

Metal	Substrate		Metal	Substrate	
	WR	TLG		WR	TLG
	(mg kg^{-1})			(mg kg^{-1})	
Mercury (Hg)	<0.02	<0.02	Manganese (Mn)	885	370
Silver (Ag)	<0.8	<0.8	Molybdenum (Mo)	<1	<1
Arsenic (As)	345	1,300	Nickel (Ni)	48.25	21.33
Barium (Ba)	36.25	9.67	Lead (Pb)	<5	<5
Cadmium (Cd)	<0.5	<0.5	Zinc (Zn)	62.25	25.67
Cobalt (Co)	25.25	11.33	Boron (B)	<5	<5
Chrome (Cr)	44.75	8	Calcium (Ca)	4,600	3,300
Copper (Cu)	66.25	27	Iron (Fe)	38,250	15,333
Tin (Sn)	<4	<4	Magnesium (Mg)	6,375	996.67

Variation in most soil nutrient contents depended upon soil pH (**Figures 2A–E**). Contents of SOM, total carbon, C:N ratio, assimilable K and NH_4–N (all *P*-values < 0.0001) and, to a lesser extent, total N ($P < 0.05$), decreased with increasing soil pH. In contrast to these trends, NO_3–N increased with increasing pH, while phosphorus showed no consistent variability that was related to the pH (**Figure 2F**).

Establishment and Growth of Field-Grown Umbrella Thorn

Umbrella thorn establishment was relatively poor, with 31% average survival across the mine sites. Tree survival rates ranged from 24% on WR to 38% on TLG soils. Large differences in tree growth characteristics were apparent among the mine waste soils, as determined from assessments that had been conducted

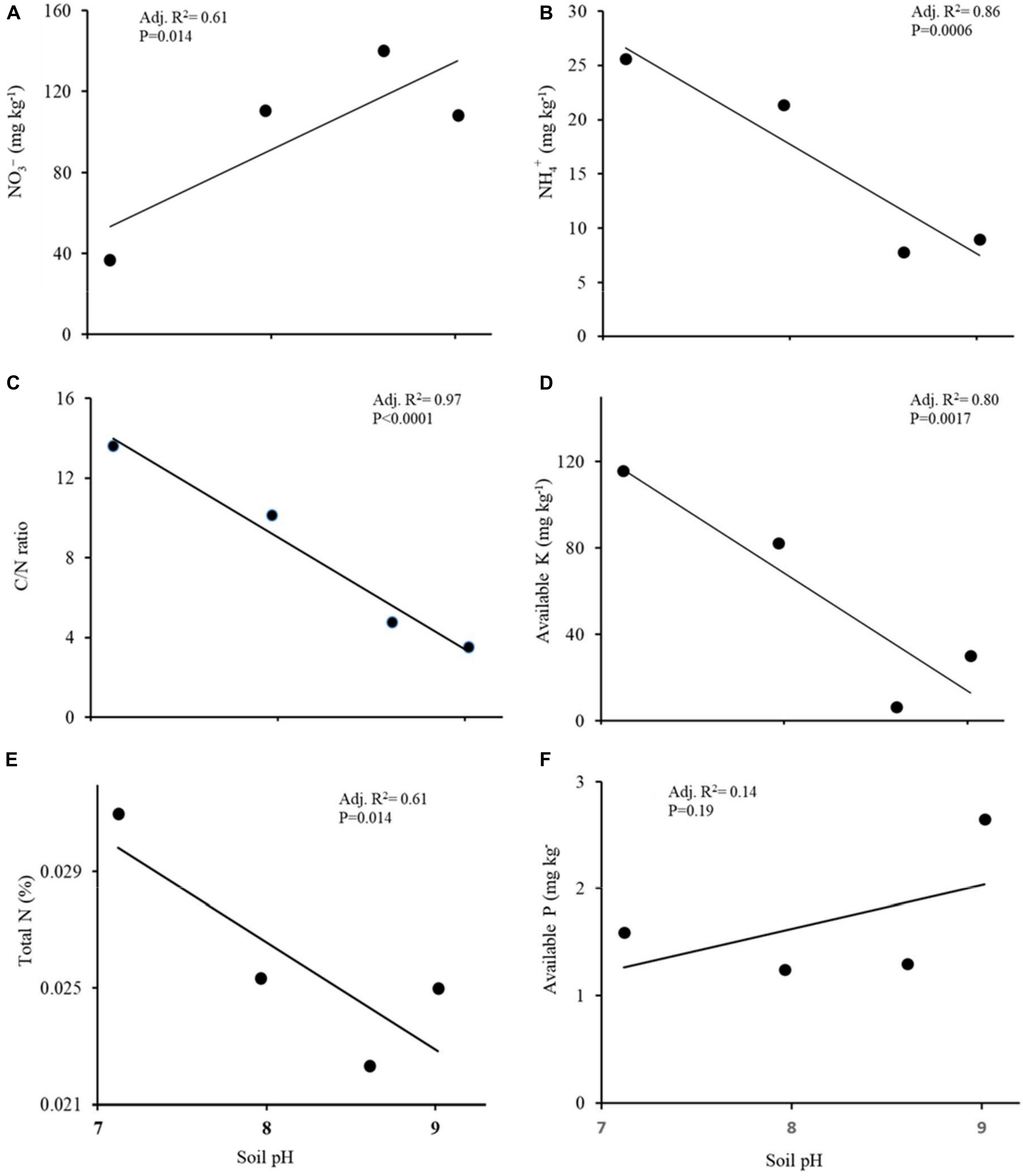

FIGURE 2 | Relationship between selected soil nutrients and soil pH: **(A)** $NO_3{}^-$; **(B)** $NH_4{}^+$; **(C)** C:N ratio; **(D)** available K; **(E)** total N and **(F)** available P. Adjusted coefficient of determination of the regression (Adj. R^2), and its associated probability (P).

3 years after planting. Umbrella thorn grew best on TLG soil in terms of its height, crown diameter, and basal stem circumference ($P < 0.001$, respectively, **Table 3**).

Growth of Test Crops in Mine Soils Under Greenhouse Conditions

Cowpea and umbrella thorn showed large differential responses in height ($P < 0.01$) and shoot biomass ($P < 0.001$) to soil types under greenhouse conditions (**Table 4** and **Figures 3A–F**, **4**). While cowpea height growth was similar on natural and mining soils, umbrella thorn performed very favorably on NS but much less so on mine wastes, resulting in a height difference of 87% ($P < 0.001$, **Table 4** and **Figure 4**). Also, the use of mine waste soils instead of NS always resulted in reduced shoot dry-mass, which was 10% more pronounced in umbrella thorn than in cowpea (**Table 4** and **Figures 3A,B**). Shoot dry-mass was likewise generally higher on artisanal mining soil than on WR and TLG soils, but the difference was especially pronounced in cowpea ($P < 0.01$, **Table 4** and **Figures 3C,D**). In addition, cowpea was more productive on WR soils than TLG soils, while umbrella thorn showed poor growth on both soil types ($P < 0.001$, **Table 4** and **Figures 3E,F**). Cowpea accumulated four times more shoot biomass on WRs than on TLGs (**Figure 3F**).

Quantification of Nodulation Under Greenhouse Conditions

Across all study soils, cowpea formed more nodules (7.8 nodules plant^{-1}) than did umbrella thorn (1.5 nodule plant^{-1}) ($P < 0.001$, **Table 4** and **Figure 5**). For each legume crop, average nodule number varied among soil types, with much higher numbers being found in the natural soil (**Figure 5A**). Also, nodule numbers were higher for artisanal mining site soil (5.8 nodules plant^{-1}) compared with conventional mine waste soils (1.2 nodule plant^{-1}) ($P < 0.05$, **Table 4**). For umbrella thorn, nodule counts were associated with low nodule fresh mass (<0.1 mg plant^{-1}) across soil treatments. Cowpea nodulation assessments, in contrast, resulted in measurable nodule fresh masses (72–209 mg plant^{-1} across soils, except TLGs) (**Table 4** and **Figure 5B**).

Quantification of Arbuscular Mycorrhizal Fungi Root Colonization Under Greenhouse Conditions

Differential responses in mycorrhizal root frequency were evident for both test crops to the mine soils ($P < 0.001$, **Table 4** and **Figure 5C**). Mycorrhiza frequency in natural soil was the highest

TABLE 3 | Summary of analyses of the variance (ANOVA) and means on height, crown diameter, and stem circumference of umbrella thorn 3 years after planting at mine waste sites.

Growth trait	[a]Mine substrate				
	TLG	WR	[b]X^2	[c]DF	[d]$P>$
Height (cm plant^{-1})	202.2 ± 16.4	142.2 ± 16.1	5.7	1	0.017
Crown diameter (cm plant^{-1})	353.9 ± 5.7	137.4 ± 5.6	27.7	1	<0.0001
Stem circumference (cm plant^{-1})	28.6 ± 1.8	13.0 ± 1.0	22.6	1	<0.0001

[a] *WR, waste rock stockpile; TLG, tailings.*
[b] *Kruskal-Wallis Statistic.*
[c] *Degrees-of-freedom.*
[d] *Probability.*

TABLE 4 | Summary of ANOVA of growth, nodulation, and mycorrhization characteristics of umbrella thorn and cowpea plants that were grown in greenhouses on different soils from mining sites.

Source	Shoot					Nodule				Mycorrhization				
	DF	Height (cm)		Dry mass (g plant^{-1})		Number (plant^{-1})		Fresh mass (mg plant^{-1})		DF	Frequency (%)		Intensity (%)	
Species	1	46.3	NS	2616.0	****	378	***	146,289	****	1	270	NS	6	NS
Cowpea vs Umbrella (1)	1	46.3	NS	2616.0	****	378	***	146,289	****	1	270	NS	6	NS
Soil	4	128.3	****	163.2	****	76	*	16,234	***	4	6,147	****	403	*
Nat_S vs mine soils (2)	1	287.0	****	341.3	****	133	*	48,430	***	1	13,613	****	897	*
AMS vs (TLG,WR) (3)	1	159.0	**	95.6	**	153	*	1,367	NS	1	6,400	****	437	NS
TLG vs WR (4)	1	0.2	NS	159.66	***	15	NS	14,466	*	1	133	NS	4	NS
Specie*Sol	4	56.4	**	163.0	****	39	NS	16,233	***	4	1,503	***	108	NS
(1) vs (2)	1	196.4	***	340.6	****	60	NS	48,428	***	1	1,901	**	296	NS
(1) vs (3)	1	3.5	NS	95.4	**	62	NS	1,363	NS	1	4,011	***	133	NS
(1) vs (4)	1	16.4	NS	159.6	***	30	NS	14,467	*	1	33	NS	3	NS
Error	47	14.3		12.4		22		2,913		20	183		134	

**$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$; NS, not significant.*
DF, degrees-of-freedom; WR, waste rock stockpile; TLG, tailings; AMS, artisanal gold mining.

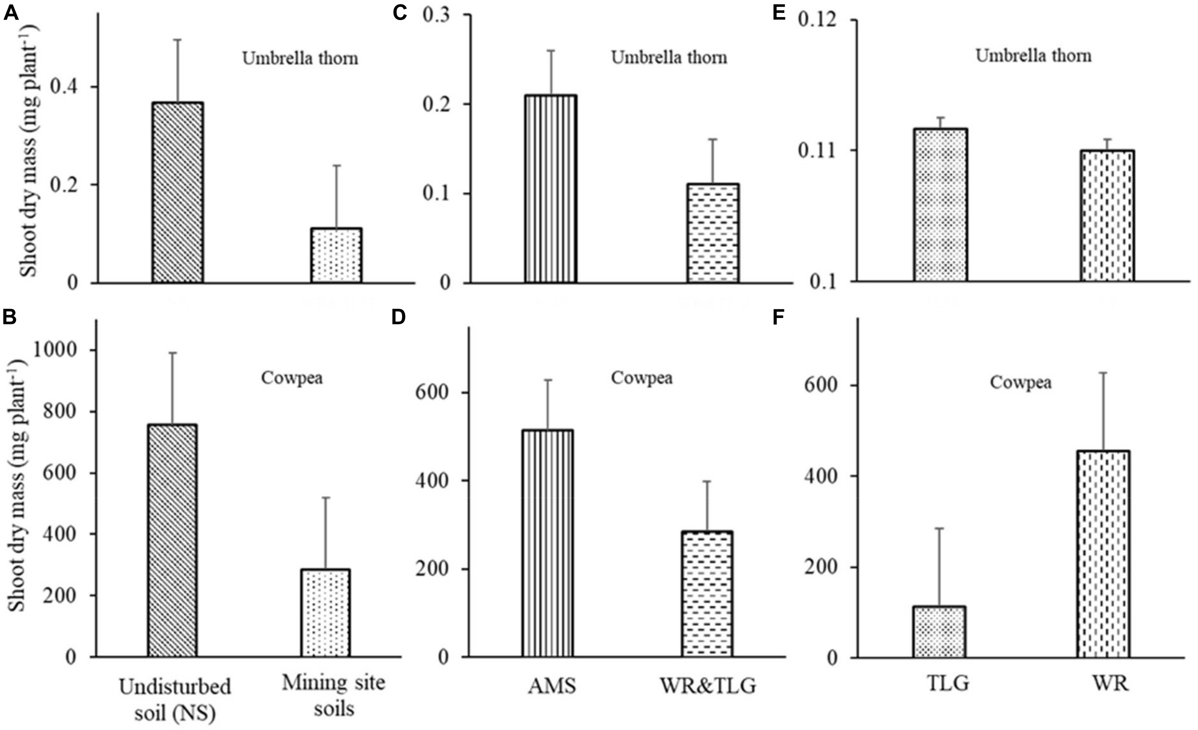

FIGURE 3 | Umbrella thorn **(A,C,E)** and cowpea **(B,D,F)** shoot dry-mass responses to contrasting soil types in the greenhouse: undisturbed-natural soil (NS) vs mine site soils **(A,B)**; soils of the artisanal mining site (AMS) vs soils of the conventional mining site **(C,D)**; and Mine tailings (TLG) vs waste rock stockpile (WR) **(E,F)**. Error bars represent the standard errors of means.

for cowpea, and so for umbrella thorn, when both crops were grown in the mine site soils (**Figure 5C**). While mycorrhizal frequency in the artisanal mining soil was much higher for umbrella thorn than cowpea, both test crops exhibited very low values in the mining TLGs and WRs (**Table 4**). Yet, intensity of mycorrhizal root infection was generally low, with less variability among treatments (**Table 4** and **Figure 5D**). On average, the intensity of mycorrhization was consistently lower for plants that were grown in mining site soils (4%) than in natural soil (18%) (**Figure 5D**).

DISCUSSION

In this study, umbrella thorn had grown better in tailings containing almost four times more arsenic than WR soils. The top performers in terms of growth also had the highest survival rates, indicating that tailings were probably the least detrimental environment for tree establishment, particularly at the initial stage of planting. Overall, these trends in field-grown tree responses to arsenic (As)-enriched mining soil types indicate the ability of umbrella thorn to withstand arsenic contamination levels up to 1,300 ppm and, hence, its suitability for use in revegetation of As-polluted mine sites. However, further testing of umbrella thorn under higher As levels may be required to understand the extent of its resistance/tolerance compared to other species such as huizache or sweet acacia [*Vachellia farnesiana* (L.) Willd.], which was reported as being capable of growing in arid environments containing arsenic concentrations ranging from 4,000 to 32,000 ppm (Armienta et al., 2008). In other studies, harsh growing conditions resulting from high concentrations of various heavy metals in mining wastes, including arsenic, have been reported to reduce the diversity of woody species (30 species) compared to surrounding natural forests (55 species) in Zambia (Festin et al., 2019). With the exception of some legumes that have been identified as being able to establish and nodulate naturally in soils with high concentrations of heavy metals, including As (Carrasco et al., 2005), there is strong evidence that As pollution negatively affects the legume-rhizobium symbiosis (Reichman, 2007; Pajuelo et al., 2008, 2011). The microbial partner is reported to be more sensitive to metal stress than the host plant (Giller et al., 1999; Reichman, 2007), resulting in a decrease in nodulation performance (Pajuelo et al., 2011). Other effects of As on legumes include altered plant growth, root hairs, and photosynthesis, as well as decreased chlorophyll content (Pajuelo et al., 2011, 2019).

Despite measurable success of revegetation at 3 years, due in part to the arsenic tolerance of umbrella thorn, tree survival, and growth were highly variable, with establishment rates that were much lower than 50%. This can mainly be attributed to the generally harsh environmental conditions under which revegetation is being implemented. Poor crop establishment and low productivity are common characteristics of production systems in sub-Saharan Africa (SSA). The constraints include

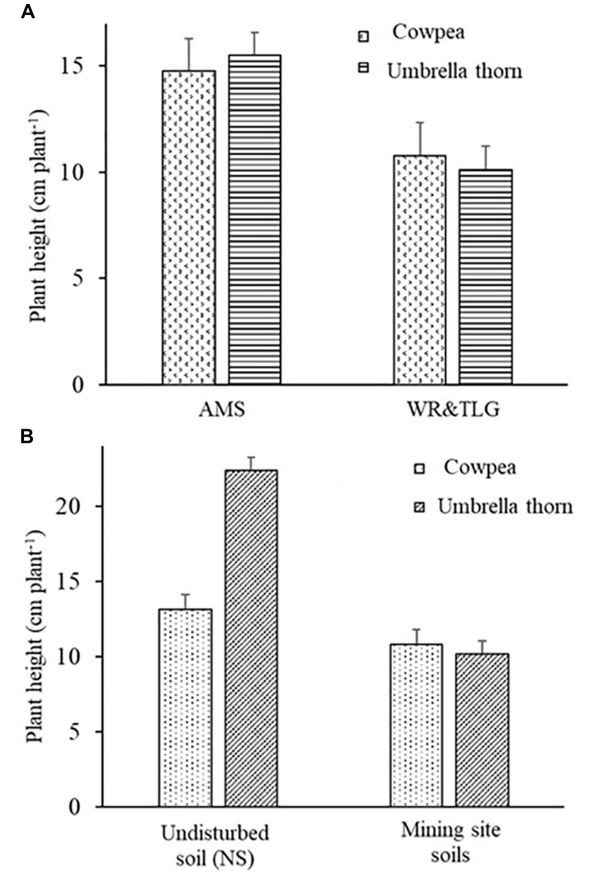

FIGURE 4 | Umbrella thorn and cowpea height growth responses to contrasting soil types in the greenhouse: Soils of the artisanal mining site (AMS) vs soils of the conventional mining site **(A)**; Undisturbed-natural soil (NS) vs mine site soils **(B)**. Error bars represent the standard errors of means.

frequent drought, insufficient rain, low nutrient availability and high temperatures, among others (Zahran, 1999; Pule-Meulenberg and Dakora, 2007; Tully et al., 2015; Ayangbenro and Babalola, 2020).

The soils that were studied showed a pH gradient ranging from relatively neutral in the adjacent undisturbed soil to basic in the case of soils that were disturbed by mining. Although most soil characteristics are strongly correlated (results not shown), variation in soil chemical properties with soil pH showed that ammonium was the dominant form of mineral nitrogen in unpolluted soil. For the same soils, values were highest for total nitrogen, assimilable potassium, carbon and the C:N ratio. In alkaline mining soils, in contrast, nitrate was the main form of mineral nitrogen. Overall, both the C:N ratio and ammonium decreased with increasing nitrate, indicating active nitrogen mineralization in mining soils. Therefore, the difference in umbrella thorn growth may be explained either by its preference for ammonium over nitrate, or by the unavailability of nitrate for uptake, resulting in N-deficiency on mining site soils. Earlier studies using potted soils or aeroponic cultures reported a preference of several N_2-fixing tree species for NH_4^+ when this nutrient was supplied at moderate rates (Goi et al., 1993; Weber et al., 2007). This preference was confirmed in mature eucalyptus forest by Pfautsch et al. (2009), who reported that ammonium was the preferred form of nitrogen for the dominant stringy gum (*Eucalyptus regnans* F. Muell.) and N_2-fixing *Acacia* spp. Preference for nitrate has been suggested from field studies based upon variability of nitrate/ammonium ratios under stands of acacias and eucalypts in the Congo (Tchichelle et al., 2016), but no such preference could be confirmed by subsequent studies in the nursery (Epron et al., 2016).

Relatively low growth of umbrella thorn with nitrate-enriched soils in the present study might reflect effects of factors other than the preference for mineral nitrogen forms. One of these factors could be intrinsic soil conditions, where iron and arsenic were simultaneously present, together with nitrate. Wang et al. (2018) demonstrated that under such conditions, the activities of denitrifying microorganisms, coupled with both iron and arsenic oxidation result in substantial immobilization of As and loss of N. It has been further demonstrated that metal toxicity reduces nitrate reductase activity, affecting assimilation of nitrate due to impaired use of the substrate (Rai et al., 2004). In addition to nitrogen, the combination of many other potential factors, such as the availability of phosphorus, which has not been shown to be related to pH, and the availability of micronutrients, which were not measured, may have contributed to plant response trends. Many metals that are essential to life, such as Na, K, Cu, Zn, Co, Ca, Mg, Mn, and Fe, can be toxic when present above certain threshold concentrations (McGrath et al., 1995; Giller et al., 1999; Pajuelo et al., 2011; Chibuike and Obiora, 2014).

In the pot experiment, the most obvious difference between umbrella thorn and cowpea was latter's greater ability to nodulate in all soils, unlike the umbrella thorn that only formed a few nodules. The presence of nodules in both cases suggests that rhizobia that are compatible with both cowpea and umbrella thorn survived long-term exposure to heavy metals in soils at the mine site (Giller et al., 1999; Pajuelo et al., 2011). With access to N_2-fixation as an additional nitrogen source (in addition to soil nitrogen), cowpea showed equal height growth across all soil types. Yet, biomass was lowest in disturbed soils, suggesting that N_2-fixation was not efficient enough to meet nitrogen requirements of the cowpea plants. Growth of umbrella thorn, which was poor in mining soils and much better in undisturbed soil, suggests that the seedlings were primarily dependent upon mineral nitrogen, while nitrate may not have contributed much to nutrition. It also suggests that umbrella thorn nodulation was probably due only to ineffective rhizobia that have tolerated soil metal stress (Giller et al., 1989). When taken together, these results demonstrate that nitrogen deficiency, in part, may have been a major constraint to the establishment and healthy growth of umbrella thorn in plantations.

Like N_2-fixing symbioses, mycorrhizae are recognized as playing a crucial role in the revegetation of severely degraded or polluted soils (Pawlowska et al., 1996). In the pot test that was conducted in this study, the frequency and intensity of root colonization were very low and highly variable in mine soils compared to off-site soils. Such erratic colonization of plants may be due either to the low density or diversity

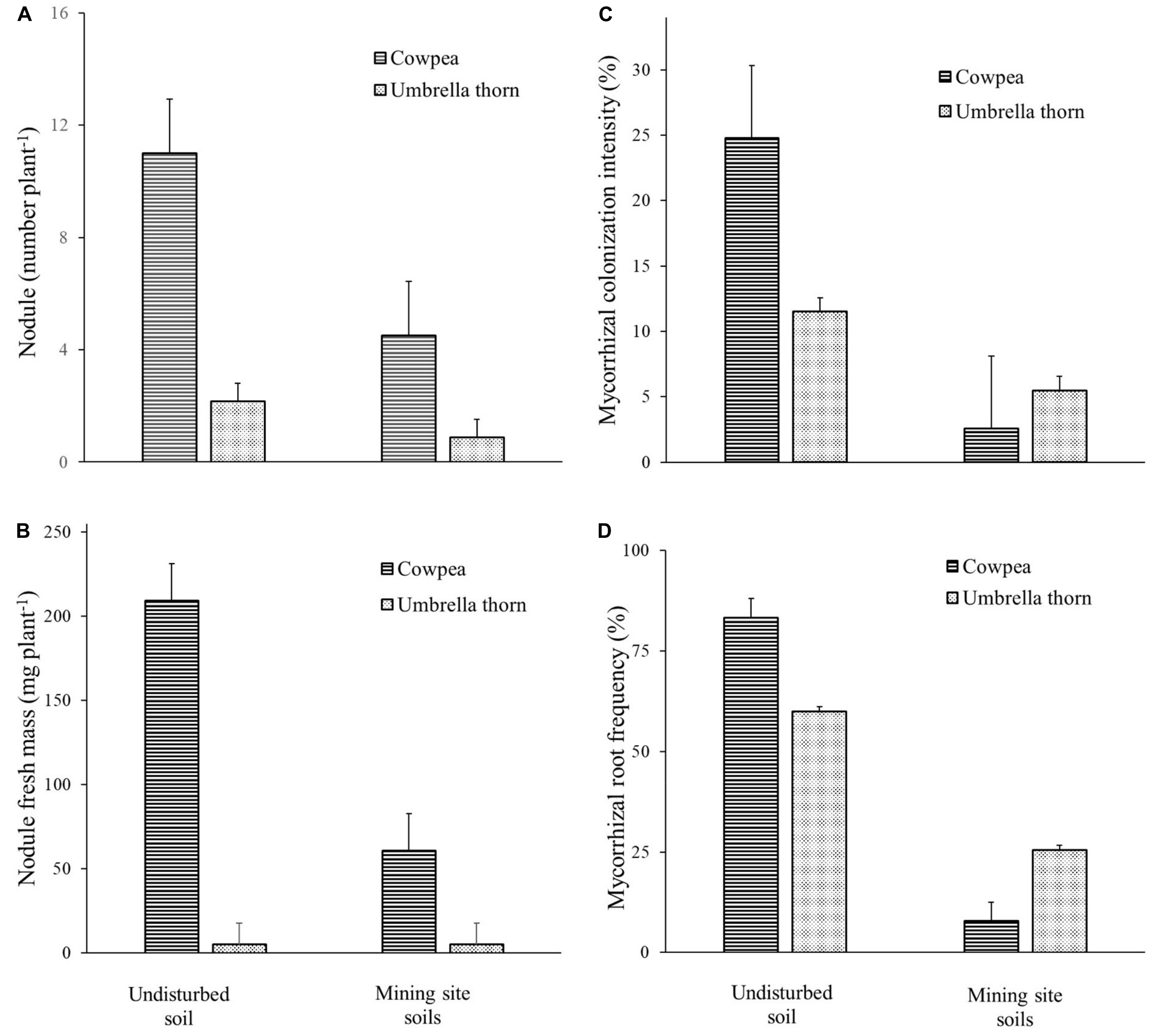

FIGURE 5 | Nodulation **(A,B)** and mycorrhizal colonization of roots **(C,D)** of umbrella thorn and cowpea grown in contrasting greenhouse soil types: **(A)** number of nodules; **(B)** nodule fresh mass; **(C)** intensity and **(D)** frequency of mycorrhization in natural undisturbed soil (NS) compared to soils at the mine site. Error bars represent the standard errors of means.

of fungal propagules in the soils, or to the negative effects of heavy metal contamination on the structure and function of soil microbial communities (Stefanowicz et al., 2008; Khan et al., 2009; Ameen et al., 2021). Therefore, further studies could expand our knowledge regarding the size and diversity of populations of symbiotic microorganisms in mine site environments.

CONCLUSION

In the present study, the umbrella thorn showed a clear ability to tolerate and grow on soils polluted by mine wastes that are rich in heavy metals, especially arsenic, thereby demonstrating its suitability for reforestation of such disturbed sites. Three years after planting, success was greatest on soils that were most heavily contaminated with arsenic, which had the highest pH, as well as nitrate as the dominant form of mineral nitrogen. It is known that in the simultaneous presence of arsenic and iron, denitrification increases the loss of nitrogen, while promoting the transformation of metals in solution. Therefore, nitrogen deficiency could explain the variability in umbrella thorn growth in the field. Differences in field growth were subsequently confirmed in potted soils with umbrella thorn and cowpea as test plants. Under these conditions, root colonization by rhizobia and AMF appeared to be relatively poor and inefficient on soils contaminated with arsenic. Overall, these results suggest that umbrella thorn performance for reforestation of mining sites could greatly benefit from management with efficient and adapted symbiotic technologies.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HY set up the essays, collected the data, and wrote the first draft of the manuscript. HH supported field activities and organized the data. DK, EC, and M-MV mobilized resources for the study. DK and EC contributed to the conception and design of the study. MD performed statistical analysis and discussed sections of the manuscript. MO supervised the field works and edited the "Introduction". All authors contributed to the article and approved the submitted version.

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Conflict of Interest: HY and M-MV were employed by IAMGOLD Essakane SA.

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