ACCUMULATION AND PRECIPITATION OF MAGNESIUM, CALCIUM, AND SULFUR IN TWO *ACACIA* SPECIES GROWN IN DIFFERENT SUBSTRATES PROPOSED FOR MINE-SITE REHABILITATION[1]

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• *Premise of the study:* Previous studies showed that phyllodes of *Acacia robeorum* accumulate much more magnesium, calcium, and sulfur than those of *A. stipuligera*, and precipitate these elements in phyllodes and branchlets. The substrate in the habitat of *A. robeorum* was mainly siltstone, having significantly higher concentrations of plant-available magnesium and sulfur than the sandy topsoil in the habitat of *A. stipuligera*. It is not known whether the differences in substrates account for the differences in the accumulation and precipitation patterns of magnesium, calcium, and sulfur between these two species.

• *Methods:* Saplings were grown in sandy topsoil or in a topsoil-siltstone mixture proposed for mine-site rehabilitation in a glasshouse. Phyllode magnesium, calcium, and sulfur concentrations of 25-week-old plants were measured. Precipitation of these elements in phyllodes and branchlets was investigated by means of scanning electron microscopy and energy-dispersive X-ray spectroscopy.

• *Key results:* Adding siltstone did not significantly affect phyllode sulfur concentration, but markedly affected magnesium and calcium concentrations in phyllodes of both species. Concentrations of magnesium, calcium, and sulfur in phyllodes of *A. robeorum* were significantly higher than those in *A. stipuligera* phyllodes. For both the topsoil and topsoil-siltstone mixture, mineral precipitates were observed in the two species, with *A. robeorum* having more mineral precipitates containing magnesium, calcium, and sulfur in its phyllodes than *A. stipuligera* did.

• *Conclusions:* The accumulation and precipitation patterns of magnesium, calcium, and sulfur are more species-specific than substrate-affected.

Key words: *Acacia*; alkaline-earth metal; calcium; magnesium; mine-site rehabilitation; mineral precipitation; phyllode; siltstone; sulfur.

Mining is a major activity in the Australian arid zone, and it can severely disturb land surfaces and impact biodiversity in natural ecosystems. In Australia, mine-site rehabilitation is a fundamental part of the mining operation. The rehabilitation objective of most Australian mine-sites is to restore the pre-mining ecosystem, and make it self-sustaining in the long term. Therefore, native plant species, which are adapted to the local conditions, including climate, soil and hydrology, are the first choice for mine-site rehabilitation (Grant et al., 2002). At most mine-sites in highly-weathered areas in Australia, due to lack of sufficient topsoil, revegetation has to be carried out on topsoil diluted with subsoil or overburden, or even directly on mine spoils without capping with topsoil (Mercuri et al., 2006). Mine spoils are often characterized by poor physical, chemical, and biological properties, including poor structure and water-holding capacity, elevated concentrations of heavy metals, low concentrations of macronutrients, little organic matter, acidic pH, low microbial abundance and activities, thus making successful rehabilitation difficult (Gilbert, 2000; Mendez and Maier, 2008).

At Newcrest's Telfer Gold, a region with a sub-tropical semi-arid climate, soil products available for store-and-release cover-system construction include 'outer siltstone' material – a chemically-benign, gap-graded material recovered from deep within the soil profile, and 'topsoil' – oxidised coarse-textured sandy soils harvested from the natural landscape prior to development specifically for the purpose of rehabilitation. For areas not disturbed by mining in the region, vegetation is primarily shrub steppe, dominated by *Triodia* hummock grasses and *Acacia* shrubs; depth of sandy soil is highly variable and underlain by siltstone material of the same origin as the run-of-mine outer siltstone material intended for use as store-and-release cover (observation of our research group). *Acacia* is a large and diverse genus, which is dominant in the vegetation of arid Australia, including the Great Sandy Desert region (Hnatiuk and Maslin, 1988; Grigg et al., 2008; He et al., 2011), and plays an important role in maintaining desert ecosystem stability (Kirschbaum et al., 2008). Many Australian *Acacia* species occur on a range of soil types (Ladiges et al., 2006), and they are important components of rehabilitated ecosystems (Bell et al., 2003).

In a previous study, phyllodes (modified petioles functioning as leaves) of mature plants of *Acacia robeorum* Maslin growing on a rocky sandplain near the Telfer Gold Mine were found to accumulate high concentrations of magnesium (Mg), calcium (Ca), and sulfur (S), and showed the presence of abundant mineral precipitates containing these elements (He et al., 2012a). The S concentration of the phyllodes of *A. robeorum* was up to 42 mg S g^{-1} dry matter (He et al., 2012a), making the plants thiophores, i.e. plants with a S concentration in their leaves ranging from 25 to 82 mg S g^{-1} dry matter (Ernst, 1998). It is noticed that the first 10 cm topsoil at the site of *A. robeorum* had significantly higher concentrations of plant-available Mg, Ca, and S than those at the sites of three other *Acacia* species, and considerably more mineral precipitates containing these elements (He et al., 2012a, b). When looking into the soil profiles, Hoy (2014) found that the site of *A. robeorum* had a much thinner layer of sandy topsoil than sites inhabited by three other *Acacia* species, with the siltstone being very close to or protruding the surface. Chemical analysis of siltstone dug up from a 600 m deep mine pit shows that the siltstone is considerably richer in plant-available Mg and S than the topsoil at the site of *A. robeorum* and those of three other *Acacia* species growing at nearby sites. However, the plant-available Ca concentration of the siltstone is less than that of the topsoil at the *A. robeorum* site and the average value of the topsoil at three other *Acacia* species' sites (Table 1 and Appendix S1, see Supplemental Data with the online version of this article, He et al., 2012a). Therefore, we speculate that *A. robeorum* took up more Mg and S from the siltstone than the other *Acacia* species did at nearby sites, and we hypothesize that if other species were grown in the same Mg- and S-enriched substrate as *A. robeorum*'s substrate, they would also take up significantly more Mg and S, and show the presence of abundant mineral precipitates containing these elements as well.

To test this hypothesis, topsoil and siltstone proposed for mine-site rehabilitation at the Telfer Gold Mine were mixed in different proportions to make substrates with different concentrations of plant-available Mg and S. A glasshouse experiment was designed to investigate the effects of different substrates on the accumulation and precipitation of Mg and S by two *Acacia* species, *A. robeorum* and *A. stipuligera* F. Muell. The reason to select *A. stipuligera* was that it naturally inhabits deep aeolian sand dunes where the plants have the least chance to take up elements such as Mg and S from the siltstone. Accumulation of Ca was studied, together with that of Mg and S, to find out potential relationships between these elements in different *Acacia* species.

MATERIALS AND METHODS

Preparation of substrates —Substrate material for glasshouse experiments was collected from the Telfer gold mine (Newcrest Mining Limited) in the Great Sandy Desert in the Pilbara region, north-western Australia (21°45′S, 122°14′E). Topsoil was collected from areas cleared for mine expansion and stored on-site in stockpiles for less than one year. Siltstone was freshly dug up from a 600 m deep mine pit. Both the topsoil and siltstone were transferred in drums and transported to The University of Western Australia in Perth, and kept in the drums until required for the experiment. To ensure homogeneity of the substrates, both the topsoil and siltstone were sieved through a 2-mm mesh, and air dried. The chemical and physical properties of the air-dried topsoil and siltstone were analysed by ChemCentre (Bentley, Western Australia). The results are presented in Table 1.

Portions of air-dried topsoil and siltstone were transferred into 9-cm diameter, 40-cm high, free-draining PVC tubes to establish two treatments: 1) topsoil only (3.5 kg per pot); and 2) a topsoil-siltstone mixture, for which 1.75 kg topsoil and 1.25 kg siltstone was thoroughly mixed and covered with 0.5 kg topsoil to ensure good germination and seedling establishment.

***Growth of plants*—**The pots were watered to 18% gravimetric soil water content (about 90% water-holding capacity of the substrates) and incubated for one week before sowing the seeds. Seeds of *A. stipuligera* and *A. robeorum* were treated with boiling water for 1 to 2 min and left in cold tap water overnight. Two seeds were sown at 0.5 cm depth in the topsoil in the center of each pot; four weeks after sowing, seedlings were thinned to one plant per pot. There were four replicates for each treatment in a complete randomized design. Plants were grown from July 2010 to January 2011 in a glasshouse at The University of Western Australia (31°59´S, 115°49´E), where the mean day time temperature was 32°C during the experiment, and the light intensity in the glasshouse was about 60% of outdoor light intensity. The pots were watered to 18% gravimetric soil water content every other day, and the plants were harvested 25 weeks after sowing.

***Plant measurements and analyses of phyllode element concentrations*—**When plants were harvested, shoots were severed at the base, roots and nodules were washed thoroughly to remove soil. Shoots, roots, and nodules were dried in an oven at 70°C for 72 h and weighed separately. Oven-dried mature phyllodes (the youngest fully expanded) were ground with a stainless steel coffee grinder for 30 s, about 0.2 g of the ground sample was weighed, digested in hot concentrated HNO_3:$HClO_4$ (3:1) (Zarcinas et al., 1987), and analysed by a Varian Vista-PRO axial ICP-OES (Varian, Inc., Palo Alto, CA, USA) at ChemCentre to determine concentrations of Mg, Ca, and S. Concentration ratios between Mg, Ca, and S were then calculated.

Observations and X-ray microanalyses of mineral precipitates —To prepare and analyse samples, the methods described by He et al. (2012a) were followed. One fresh mature phyllode from each plant was either cut with a double-edged razor blade or fractured by hand, while small sections of fresh branchlets were cut with a double-edged razor blade. All samples were fixed in formalin-acetic acid-alcohol [FAA, formalin : acetic acid : 70% ethanol=1:1:18 (v:v:v)], dehydrated in an ethanol series (70%–95%–100%–dry ethanol), critical point-dried, and then mounted on scanning electron microscopy (SEM) stubs with double-stick carbon tape. The samples were divided into two groups of subsamples. One group was coated with gold (Au) for both SEM imaging and X-ray microanalyses, and the other group was coated with carbon (C) for X-ray microanalyses only. SEM micrographs were captured with a Zeiss 1555 VP-FESEM (Carl Zeiss, Oberkochen, Germany) at 5–10 kV. Qualitative X-ray microanalyses were performed at a 16-mm working distance using a Si (LI) energy-dispersive X-ray spectroscopy system (EDS) (Oxford Instruments, Oxford, England) on the same SEM at 20 kV (He et al., 2012a). A limitation of the EDS technique is that the peaks of some elements (e.g., S) may overlap with Au peaks (from Au-coated samples) to some degree, such that they may not be clearly displayed in the resulting spectra. Carbon-coated samples do not exhibit this limitation; however, they do produce a strong C peak in the spectra which makes it impossible to distinguish coated C from C that exists in the sample itself. Here, X-ray microanalyses were performed on both Au- and C-coated samples in order to provide supplementary information, and hence to resolve the above-mentioned limitations.

Data analysis —The effects of substrate, species, and the effects of interaction between substrate and species on plant growth, phyllode element concentrations and concentration ratios were analysed by performing two-way analysis of variance (ANOVA), using the General linear model, Univariate analysis of variance in the IBM SPSS Statistics 20 software package (IBM Corp., New York, USA). Micrographs were processed using Adobe Photoshop CS5 software (San Jose, CA, USA).

RESULTS

Plant biomass accumulation and phyllode Mg, Ca, and S concentrations**—**Adding siltstone to the topsoil did not significantly affect total plant biomass (including shoot, root, and nodule dry mass) accumulation. Plants of both species produced similar root and nodule dry mass, but *A. stipuligera* produced significantly more shoot dry mass than *A. robeorum* did. There was no significant interaction effect on plant biomass accumulation between substrate and species (Table 2).

The difference between substrates significantly affected phyllode Mg and Ca concentrations, but only affected phyllode S concentration slightly. Phyllodes of *A. robeorum* had significantly higher concentrations of Mg, Ca, and S than those of *A. stipuligera* did. Two-way ANOVA results showed that the effects of interaction between substrate and species on phyllode Mg, Ca, and S concentrations were all significant. The differences between substrates did not have significant effects on phyllode Mg/Ca, Mg/S, and Ca/S. Phyllodes of *A. robeorum* showed a markedly higher Mg/Ca, lower Mg/S and Ca/S than those of *A. stipuligera*. There was no statistically significant interaction between substrate and species on phyllode Mg/Ca, Mg/S, and Ca/S (Table 3).

Locations and elemental compositions of mineral precipitates in plants**—**By means of SEM, mineral precipitates of various morphologies were observed in phyllodes and branchlets of the two *Acacia* species (Figs 1, 2, 3), and EDS revealed that several elements were precipitated in these minerals (Figs 4, 5, Tables 4, 5).

For cells without mineral precipitates, the typical spectrum showed only carbon (C) and oxygen (O) peaks (Figs 4A, 5A). The elemental composition of some mineral precipitates was simple. For example, prismatic crystals (Figs 1C, 2C, 3B, C, I, J) often comprised Ca, C, and O (Fig. 4B, 5B), and sometimes strontium (Sr) (Fig. 5C); these crystals were very likely Ca-oxalates or mixtures of Ca-oxalates and Sr-oxalates. Many mineral precipitates that showed large Ca and S peaks, but only small C peaks, were observed in phyllodes of *A. robeorum*, and these mineral precipitates were presumably mainly composed of Ca-sulfates, while a small proportion of Ca-oxalates was also present (Figs 2G–J, 4M, 5J); in some cases, Mg, potassium (K) and silicon (Si) were detected in these mineral precipitates (Fig. 5K). In phyllodes of *A. robeorum*, there were numerous spherical mineral precipitates (Figs 2D, E) and other types of mineral precipitates (Fig. 2F), in which Mg was sequestered together with Ca, S, C, and O, and sometimes K as well (Figs 4C, D, 5D, E). In branchlets of both species, mineral precipitates with large Ca, K, S peaks and small C peaks, presumably mainly made up of Ca-sulfates and K-sulfates, were present [Figs 3C (unfilled arrow), E, F, K, 4P].

The elemental composition of other mineral precipitates was more complex than that described above. Mineral precipitates similar to those in Fig. 1D [Figs 1E, F, 3B (unfilled arrow), D (unfilled arrow), G], sequestered several alkaline-earth and alkaline metals, including Mg, Ca, Sr, barium (Ba), sodium (Na), and K; the elemental composition of individual mineral precipitates differed from each other to some degree (Figs 4C–H, 5D–G), and these mineral precipitates were presumably complex mixtures of oxalate and sulfate salts of these metals. It was noted that, according to EDS, not all the mineral-like objects in Fig. 1F were mineral precipitates; some of them were starch granules, which had only C and O peaks.

In many cases, aluminum (Al) and transition metals such as manganese (Mn), iron (Fe), copper (Cu), titanium (Ti), and vanadium (V) were also sequestered in mineral precipitates in addition to alkaline-earth and alkaline metals (Figs 1G, H, 2K–N, 4I–L, N, O, 5H, I, Tables 4, 5). Zinc may or may not have been sequestered in some mineral precipitates, but it was not possible to be fully confirmed, because Zn peaks were small and overlapped with Cu peaks (data not shown). Sulfur, C, Si, and O were detected together with these metals (Figs 4 I–L, N, O, 5H, I, Tables 4, 5); these elements were presumably present in the form of oxalate, sulfate, or silicate salts or oxides, for example, silica, oxides of Fe, Ti, and V; there is also a possibility that some metals were present in the form of sulfide.

For both species, the precipitated elements included Mg, Ca, Sr, Ba, Na, K, S, C, and O in both phyllodes and branchlets. However, Mn, Fe, Cu, Al, and Si were only precipitated in phyllodes of the two species; in addition, Ti and V were only detected in some mineral precipitates in phyllodes of *A. robeorum* (Tables 4, 5). In both species, mineral precipitates containing these elements were formed in parenchyma and mesophyll cells, and cells associated with fiber cells in phyllodes, while these elements were precipitated in pith, pith ray cells, xylem fiber cells, phloem parenchyma cells, and cortical parenchyma cells associated with fiber cells in branchlets (Figs 1, 2, 3, and images not shown).

Locations and elemental compositions of mineral precipitates were similar in plants of the same species grown in the topsoil and in the topsoil-siltstone mixture. By estimating the number of mineral precipitates containing certain elements per transverse section of a phyllode, it was noted that *A. robeorum* had more mineral precipitates containing large amounts of Mg, Ca, and S in its phyllodes than *A. stipuligera* did, with phyllodes of *A. robeorum* and *A. stipuligera* having more than 60% and less than 10% cells in a transverse section containing such mineral precipitates, respectively. More mineral precipitates containing Sr and Ba were formed in phyllodes of *A. stipuligera* than in phyllodes of *A. robeorum*; in addition, it appeared that phyllodes of *A. robeorum*, compared with those of *A. stipuligera*, also had more mineral precipitates in which Mn and Cu were sequestered.

DISCUSSION

Our results show that adding siltstone to the topsoil did not significantly affect plant growth and phyllode S concentration of either species, but markedly affected Mg and Ca concentrations in phyllodes of both species. Concentrations of Mg, Ca, and S in phyllodes of *A. robeorum* were significantly greater than those in *A. stipuligera* phyllodes. For both the topsoil and topsoil-siltstone mixture, mineral precipitates were observed in the two species, with *A. robeorum* having more mineral precipitates containing magnesium, calcium, and sulfur in its phyllodes than *A. stipuligera* did. These mineral precipitates were most likely oxalate salts of Mg and Ca, and mixtures of oxalate and sulfate salts of Mg and Ca (He et al., 2012a, b). The accumulation and precipitation patterns of Mg, Ca, and S were more species-specific than substrate-affected. In this study, factors controlling the accumulation patterns of Mg, Ca, and S, and possible causes and functions of sequestering these elements in mineral precipitates in the studied *Acacia* species are discussed. It is very likely that phyllodes of the two *Acacia* species accumulated more Mg, Ca, and S than they required for adequate growth and normal functions (Kirkby, 2011); surplus Mg, Ca, and S were therefore sequestered in mineral precipitates. A series of other metals, both plant-essential (e.g., K, Mn, Fe, Cu) and non-essential (e.g. Na, Al, Sr, Ba, Ti, V), were also precipitated, and in most cases they co-precipitated with Ca. Precipitation of K could be an effective bulk K regulation mechanism in plant tissues and organs, while the precipitation of other metals such as Na, Al, Mn, Fe, Cu, Sr, Ba, Ti, and V might alleviate their potential toxic effects (He et al., 2014). *Acacia robeorum* has potential for the phytoextraction of S at S-enriched sites (Ernst, 1998).

Plant growth, and phyllode Mg, Ca, and S concentrations**—**Mine spoils are often chemically, physically, and biologically inferior to normal soil, and hostile to plant growth (Gilbert, 2000; Mendez and Maier, 2008), but there are reports that some plants grown in gypsum mine spoil produce equal or more biomass than those grown in normal soil, with higher concentrations of nutrients such as Ca, P, and K (Rao and Tarafdar, 1998). Plant growth of the present two *Acacia* species was not markedly affected by adding siltstone to the topsoil. For phyllode Mg, Ca, and S concentrations, only the change in Ca might be explained by the differences between the topsoil and siltstone. For both the glasshouse-grown plants and plants grown in the natural habitat, phyllodes of *A. robeorum* always had higher concentrations of Mg, Ca, and S than those of *A. stipuligera* did; the ratios between these elements differed significantly between species (Table 3, Appendix S2, see Supplemental Data with the online version of this article), indicating that these parameters are more species-specific than substrate-affected.

Phyllode Mg/Ca of the same species grown in the topsoil and in the topsoil-siltstone mixture was almost the same. Significant positive correlations between Mg and Ca concentrations across a range of species have also been reported (Thompson et al., 1997; Broadley et al., 2004). It is likely that phyllode Mg and Ca are under strict genetic control, and the Mg^{2+} and Ca^{2+} concentrations may be controlled in part by common regulatory networks at the uptake, transport, and tissue localization levels, due to the chemical similarity of Mg^{2+} and Ca^{2+} ions, although Mg and Ca play different physiological and biochemical roles in plants (Wiesenberger et al., 2007; Broadley et al., 2008). In the current study, phyllodes of *A. robeorum* always showed a markedly higher Mg/Ca than those of *A. stipuligera* did. Investigation of cell wall chemistry and cation-exchange capacity of the roots and shoots (White and Broadley, 2003), and study of various ion or solute transporters (Conn and Gilliham, 2010) may provide valuable information for understanding the species level differences in phyllode Mg and Ca accumulation.

For *A. stipuligera*, the phyllodes in the current study had a lower Mg concentration, but higher Ca and S concentrations than phyllodes of plants grown in the natural habitat in the Great Sandy Desert; the glasshouse plants grown in the topsoil had lower Mg/Ca and Mg/S, but higher Ca/S than plants grown in the natural habitat, while Mg/Ca, Mg/S, and Ca/S of plants grown in the topsoil-siltstone mixture were all lower than those of plants grown in the natural habitat. For *A. robeorum*, Mg, Ca, and S concentrations of the phyllodes in the current study were all considerably lower than those of phyllodes of plants grown in the natural habitat; the glasshouse plants, both grown in the topsoil and in the topsoil-siltstone mixture, had higher Mg/Ca, Mg/S, and Ca/S than plants grown in the natural habitat (Table 3, Appendix S2, see Supplemental Data with the online version of this article).

It has been recently found that two other *Acacia* species in north-western Australia accumulate a significant amount of S on both Ca-rich and low-Ca soils, when surrounding plants had "normal" S concentrations (Hayes et al., 2014), indicating accumulation of S is constitutive in these *Acacia* species. However, plants growing on gypsiferous soils under semi-arid conditions accumulate up to more than 30 mg S g^{-1} dry matter, while plants growing on gypsiferous soils under humid conditions have S concentration not >11 mg S g^{-1} dry matter (Ernst, 1998), indicating soil water availability may impact on the uptake and accumulation of S by plants. We speculate that differences in substrate chemical properties such as soil pH (Lambers et al., 2008), as well as soil water content (García et al., 2008), soil temperature (Lahti et al., 2005), and plant age, may account for differences in the accumulation patterns of Mg, Ca, and S between the glasshouse-grown plants and plants grown in the natural habitat.

***Possible causes and functions of Mg, Ca, and S precipitation in plants*—**According to Kirkby (2011), average concentrations of Mg, Ca, and S in shoot dry matter sufficient for adequate growth are 2, 5, and 1 mg g^{-1}, respectively. In the current study, for plants of *A. stipuligera*, the phyllode Mg concentration was slightly higher than the average sufficient Mg concentration, whereas Ca and S concentrations were both much higher than the average sufficient concentrations. Phyllode Mg, Ca, and S concentrations of *A. robeorum* were all markedly higher than the average sufficient concentrations. Phyllodes of the two *Acacia* species accumulated Mg, Ca, and S more than they required for adequate growth and normal functions, and we assume that surplus Mg, Ca, and S were sequestered in mineral precipitates. It is important to determine the critical deficiency and adequate concentrations of Mg, Ca, and S in phyllodes of the two *Acacia* species, as the critical deficiency and adequate concentrations of nutrients differ between plant species and tissues (Römheld, 2012).

Plants that can survive on gypsum-bearing soils have specific adaptations to cope simultaneously with a surplus of Ca and S (Ernst, 1998). High Ca concentrations in plants may be rendered harmless either by precipitation in cell walls or by accumulation in vacuoles as organic compounds such as Ca-oxalates, but never as Ca-sulfates. For plants growing on gypsiferous soils under semi-arid and humid conditions, about 50% and 60% of the S accumulated is present as sulfate, respectively, but sulfates are often accumulated as flavone sulfates (Ernst, 1998). However, in the current study, the mineral precipitates containing Mg, Ca, and S were most likely oxalates and sulfates of Mg and Ca. Suitable techniques such as X-ray absorption spectroscopy (XAS) could be deployed to confirm the chemical forms of the elements precipitated (Yano and Yachandra, 2009). The mineral precipitates might act as high-capacity sinks of Mg, Ca, and S, and regulate their free cation and anion concentrations in the cytoplasm. As Mg, Ca, and S were sequestered in the mineral precipitates, they were made physiologically and osmotically inactive, and their potential toxic effects could be reduced (Kostman and Franceschi, 2000; Franceschi and Nakata, 2005; He et al., 2014).

For *A. robeorum*, phyllodes of the 25-week-old plants grown in the glasshouse showed much less abundant mineral precipitates containing Mg, Ca, and S than those of mature plants grown in their natural habitat (He et al., 2012a). Precipitation of Mg, Ca, and S might be related to plant/tissue age (He et al., 2012b). Phyllodes of the studied species have long life spans: ten months for *A. stipuligera* and 23 months for *A. robeorum* (Hoy, 2014). In the current study, we tried to compare the formation of mineral precipitates containing these elements in phyllodes of different age, but failed to find pronounced difference, possibly because the duration of the experiment was not long enough. We also speculate that the abundance of mineral precipitates in which Mg, Ca, and S were sequestered was related to the water relations of the plants. As the phyllodes of the plants grown in the natural habitat in the Great Sandy Desert had a lower relative water content than those of the glasshouse-grown plants (E. Hoy, The University of Western Australia, unpublished data; H. He, personal observation), it is very likely that Mg, Ca, and S were more concentrated in the cells, making it much easier to achieve a saturated solution for their precipitation.

***Precipitation of metals other than Mg and Ca*—**As mentioned in the results section, a series of other metals, both plant-essential (K, Mn, Fe, Cu) and non-essential (Na, Al, Sr, Ba, Ti, V), were sequestered in phyllodes and branchlets of the two *Acacia* species, and in most cases they were co-precipitated with Ca. According to the elemental compositions, these mineral precipitates were most likely 1) oxalate salts; 2) mixtures of oxalate and sulfate salts. In some cases, silicate salts, silica, metal oxides, and even sulfides, could also be part of these mineral precipitates.

For K, it is very likely that its uptake was in excess of the plants' requirement, and precipitation of K could be an effective bulk K regulation mechanism in plant tissues and organs (Franceschi and Nakata, 2005; He et al., 2014). Although Na is not essential for plant growth, it is often taken up by plants, and result in K deficiency under limited K supply (Wakeel et al., 2011); sequestering Na in mineral precipitates might alleviate its toxic effects.

Strontium and Ba are not essential for plant growth, but they follow Ca closely during soil-to-plant transfer

(Bowen and Dymond, 1956; Smith, 1971; White, 2001); as plants cannot exclude them selectively, they often cause toxicity (Seregin and Kozhevnikova, 2004; Monteiro et al., 2011). In the current study, both *A. stipuligera* and *A. robeorum* accumulated Sr and Ba in their phyllodes, with the phyllode Sr concentration (130–190 µg g^{-1}) being higher than the Ba concentration (57–96 µg g^{-1}). For both species,the phyllode Ba concentration was significantly greater in plants grown in the topsoil than in plants grown in the topsoil-siltstone mixture, but the difference in phyllode Sr concentration between plants grown in different substrates was not statistically significant. For phyllode Sr/Ca and Ba/Ca of both species, there was no significant difference between plants grown in different substrates; the two species had similar Sr/Ca, but *A. stipulgera* had higher Ba/Ca than *A. robeorum* (Appendices S3, see Supplemental Data with the online version of this article). Co-precipitation of Sr and Ba with Ca and/or Mg was observed in the two *Acacia* species in the current glasshouse experiment, and in some other *Acacia* species grown in their natural haibtat we previously studied (He et al., 2012b), precipitation of Sr and Ba in forms of oxalate and sulfate salts might be a detoxification mechanism (Mazen and El Maghraby, 1998; Mazen, 2004; Franceschi and Nakata, 2005; He et al., 2014).

For micronutrients such as Mn, Fe, and Cu, which may be toxic to plants at high concentrations, precipitation might play an important role in maintaining low concentrations of free cytotoxic cations and detoxifying these elements in plants (Schützendübel and Polle, 2002; Jeong and Guerinot, 2009; Millaleo et al., 2010). Aluminum might be detoxified in forms of oxalate (Mazen, 2004) and silicate salts. Titanium and V are also not nutrients. Titanium is beneficial to plants at low concentrations, but toxic at higher concentrations (Cigler et al., 2010). Currently, effects of V on plants are largely unknown (Kasai et al., 1999; Olness et al., 2005). The mechanisms for Ti and V uptake by plants of *A. robeorum* and their precipitation in the phyllodes are worthy of further study.

The potential role of S in metal precipitation and S-phytoextraction potential of A. robeorum —For the two *Acacia* species studied, metals were sequestered together with S in most cases, but it is yet not clear whether S played an important role in maintaining low concentrations of free cytotoxic cations in these plants. It has been reported that exposing plants to heavy metals such as Cu, Zn, and Cd increases root sulfate uptake capacity (Nocito et al., 2006). Sulfur ligands such as thiols in glutathione (GSH) and phytochelatin (PC) have a high affinity for heavy metals such as Cd; heavy metal-GSH and heavy metal-PC complexes have been identified as the forms of heavy-metal sequestration in vacuoles and long-distance transport in some plants (Ernst et al., 2008). For the two *Acacia* species in the current study, heavy metals such as Mn, Cu, Sr, and Ba may have formed complexes with S ligands such as thiols during long-distance transport, but were then finally sequestered in mineral precipitates and present as sulfate salts, together with oxalate salts. Confirming the chemical forms of these heavy metals and S using XAS would be helpful for clarifying the roles S plays in heavy-metal sequestration and detoxification, and understanding the underlying mechanisms.

Although phyllode S concentration of *A. robeorum* grown in the glasshouse was less than 10 mg g^{-1} dry matter, it was as high as 42 mg g^{-1} dry matter in the plants grown in the natural habitat (He et al., 2012a), the high water availability might account for the low phyllode S concentration in the glasshouse-grown plants. We propose that *A. robeorum* has potential for phytoextraction of S at S-enriched sites (Ernst, 1998). To assess the phytoextraction potential of S of the plants, plant densities and growth rates in the field should be taken into account. It is necessary to quantify turnover of S and potential toxic metals via litter before designing an appropriate protocol for plant harvest and removal to maximize the phytoextraction potential of the plants, and avoid re-contamination of the soil with S and toxic metals in a cost-effective way.

Conclusions —Adding siltstone to the topsoil did not significantly affect plant growth and phyllode S concentration of either species, but markedly affected Mg and Ca concentrations in phyllodes of both species. Phyllodes of *A. robeorum* showed higher Mg, Ca, and S concentrations, and more mineral precipitates containing large amounts of Mg, Ca, and S than those of *A. stipuligera*. Our results suggest that the accumulation and precipitation patterns of Mg, Ca, and S of the two *Acacia* species are more species-specific than substrate-affected. *Acacia robeorum* shows promise for phytoextraction of S. Factors controlling the accumulation and precipitation of Mg, Ca, and S, and possible roles of mineral precipitation in heavy-metal accumulation and detoxification in the two species warrant further study. Well-designed field trials are required to evaluate the phytoremediation potential of these species on S-enriched and metal-contaminated substrates.

LITERATURE CITED

BELL, J., S. WELLS, D. A. JASPER, AND L. K. ABBOTT. 2003. Field inoculation with arbuscular mycorrhizal fungi in rehabilitation of mine sites with native vegetation, including *Acacia* spp. *Australian Systematic Botany* 16: 131-138.

BOWEN, H. J. M., AND J. A. DYMOND. 1956. The uptake of calcium and strontium by plants from soils and nutrient solutions. *Journal of Experimental Botany* 7: 264-272.

BROADLEY, M. R., H. C. BOWEN, H. L. COTTERILL, J. P. HAMMOND, M. C. MEACHAM, A. MEAD, AND P. J. WHITE. 2004. Phylogenetic variation in the shoot mineral concentration of angiosperms. *Journal of Experimental Botany* 55: 321-336.

BROADLEY, M. R., J. P. HAMMOND, G. J. KING, D. ASTLEY, H. C. BOWEN, M. C. MEACHAM, A. MEAD, ET AL. 2008. Shoot calcium and magnesium concentrations differ between subtaxa, are highly heritable, and associate with potentially pleiotropic loci in *Brassica oleracea*. *Plant Physiology* 146: 1707-1720.

CIGLER, P., J. OLEJNICKOVA, M. HRUBY, L. CSEFALVAY, J. PETERKA, AND S. KUZEL. 2010. Interactions between iron and titanium metabolism in spinach: A chlorophyll fluorescence study in hydropony. *Journal of Plant Physiology* 167: 1592-1597.

CONN, S., AND M. GILLIHAM. 2010. Comparative physiology of elemental distributions in plants. *Annals of Botany* 105: 1081-1102.

ERNST, W. H. O. 1998. Sulfur metabolism in higher plants: potential for phytoremediation. *Biodegradation* 9: 311-318.

ERNST, W. H. O., G. J. KRAUSS, J. A. C. VERKLEIJ, AND D. WESENBERG. 2008. Interaction of heavy metals with the sulphur metabolism in angiosperms from an ecological point of view. *Plant Cell and Environment* 31: 123-143.

FRANCESCHI, V. R., AND P. A. NAKATA. 2005. Calcium oxalate in plants: formation and function. *Annual Review of Plant Biology* 56: 41-71.

GARCÍA, I., R. MENDOZA, AND M. C. POMAR. 2008. Deficit and excess of soil water impact on plant growth of *Lotus tenuis* by affecting nutrient uptake and arbuscular mycorrhizal symbiosis. *Plant and Soil* 304: 117-131.

GILBERT, M. 2000. Minesite rehabilitation. *Tropical Grasslands* 34: 147-153.

GRANT, C. D., C. J. CAMPBELL, AND N. R. CHARNOCK. 2002. Selection of species suitable for derelict mine site rehabilitation in New South Wales, Australia. *Water Air and Soil Pollution* 139: 215-235.

GRIGG, A. M., E. J. VENEKLAAS, AND H. LAMBERS. 2008. Water relations and mineral nutrition of closely related woody plant species on desert dunes and interdunes. *Australian Journal of Botany* 56: 27-43.

HAYES, P., B. L. TURNER, H. LAMBERS, AND E. LALIBERTE. 2014. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *Journal of Ecology* 102: 396-410.

HE, H., T. M. BLEBY, E. J. VENEKLAAS, AND H. LAMBERS. 2011. Dinitrogen-fixing *Acacia* species from phosphorus-impoverished soils resorb leaf phosphorus efficiently. *Plant Cell and Environment* 34: 2060-2070.

HE, H., E. J. VENEKLAAS, J. KUO, AND H. LAMBERS. 2014. Physiological and ecological significance of biomineralization in plants. *Trends in Plant Science* 19: 166-174.

HE, H., T. M. BLEBY, E. J. VENEKLAAS, H. LAMBERS, AND J. KUO. 2012a. Morphologies and elemental compositions of calcium crystals in phyllodes and branchlets of *Acacia robeorum* (Leguminosae: Mimosoideae). *Annals of Botany* 109: 887-896.

HE, H., T. M. BLEBY, E. J. VENEKLAAS, H. LAMBERS, AND J. KUO. 2012b. Precipitation of calcium, magnesium, strontium and barium in tissues of four *Acacia* species (Leguminosae: Mimosoideae). *Plos One* 7: e41563.

HNATIUK, R., AND B. MASLIN. 1988. Phytogeography of *Acacia* in Australia in relation to climate and species-richness. *Australian Journal of Botany* 36: 361-383.

HOY, E. 2014. Plant form and function from an ecohydrological perspective in the Australian arid-zone, with reference to mine-site rehabilitation. PhD thesis, University of Western Australia.

JEONG, J., AND M. L. GUERINOT. 2009. Homing in on iron homeostasis in plants. *Trends in Plant Science* 14: 280-285.

KASAI, M., J. YAMAZAKI, M. KIKUCHI, M. IWAYA, AND S. SAWADA. 1999. Concentration of vanadium in soil water and its effect on growth and metabolism of rye and wheat plants. *Communications in Soil Science and Plant Analysis* 30: 971-982.

KIRKBY, E. 2011. Introduction, definition and classification of nutrients. *In* P. Marschner [ed.], Mineral nutrition of higher plants, 3-5. Academic Press, London.

KIRSCHBAUM, M. U. F., B. HARMS, N. J. MATHERS, AND R. C. DALAL. 2008. Soil carbon and nitrogen changes after clearing mulga (*Acacia aneura*) vegetation in Queensland, Australia: Observations, simulations and scenario analysis. *Soil Biology & Biochemistry* 40: 392-405.

KOSTMAN, T. A., AND V. R. FRANCESCHI. 2000. Cell and calcium oxalate crystal growth is coordinated to achieve high-capacity calcium regulation in plants. *Protoplasma* 214: 166-179.

LADIGES, P. Y., S. R. ARIATI, AND D. J. MURPHY. 2006. Biogeography of the *Acacia victoriae*, *pyrifolia* and *murrayana* species groups in arid Australia. *Journal of Arid Environments* 66: 462-476.

LAHTI, M., P. J. APHALO, L. FINER, A. RYYPPO, T. LEHTO, AND H. MANNERKOSKI. 2005. Effects of soil temperature on shoot and root growth and nutrient uptake of 5-year-old Norway spruce seedlings. *Tree Physiology* 25: 115-122.

LAMBERS, H., L. P. THIJS, AND F. S. CHAPIN. 2008. Plant physiological ecology. 2nd ed. Springer, New York, USA.

MAZEN, A. M. A. 2004. Calcium oxalate deposits in leaves of *Corchorus olitotius* as related to accumulation of toxic metals. *Russian Journal of Plant Physiology* 51: 281-285.

MAZEN, A. M. A., AND O. M. O. EL MAGHRABY. 1998. Accumulation of cadmium, lead and strontium, and a role of calcium oxalate in water hyacinth tolerance. *Biologia Plantarum* 40: 411-417.

MENDEZ, M. O., AND R. M. MAIER. 2008. Phytostabilization of mine tailings in arid and semiarid environments - An emerging remediation technology. *Environmental Health Perspectives* 116: 278-283.

MERCURI, A. M., J. A. DUGGIN, H. DANIEL, P. V. LOCKWOOD, AND C. D. GRANT. 2006. Estimating the unknown components of nutrient mass balances for forestry plantations in mine rehabilitation, Upper Hunter Valley, New South Wales, Australia. *Environmental Management* 37: 496-512.

MILLALEO, R., M. REYES-DIAZ, A. G. IVANOV, M. L. MORA, AND M. ALBERDI. 2010. Manganese as essential and toxic element for plants: Transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition* 10: 476-494.

MONTEIRO, F. A., R. C. NOGUEIROL, L. C. A. MELO, A. G. ARTUR, AND F. DA ROCHA. 2011. Effect of barium on growth and macronutrient nutrition in Tanzania guineagrass grown in nutrient solution. *Communications in Soil Science and Plant Analysis* 42: 1510-1521.

NOCITO, F. F., C. LANCILLI, B. CREMA, P. FOURCROY, J. C. DAVIDIAN, AND G. A. SACCHI. 2006. Heavy metal stress and sulfate uptake in maize roots. *Plant Physiology* 141: 1138-1148.

OLNESS, A., R. GESCH, F. FORCELLA, D. ARCHER, AND J. RINKE. 2005. Importance of vanadium and nutrient ionic ratios on the development of hydroponically grown cuphea. *Industrial Crops and Products* 21: 165-171.

RAO, A. V., AND J. C. TARAFDAR. 1998. Selection of plant species for rehabilitation of gypsum mine spoil in arid zone. *Journal of Arid Environments* 39: 559-567.

RÖMHELD, V. 2012. Diagnosis of Deficiency and Toxicity of Nutrients. *In* P. Marschner [ed.], Marschner's Mineral Nutrition of Higher Plants, 3rd Edition 10.1016/b978-0-12-384905-2.00011-x DOI, 299-312.

SCHÜTZENDÜBEL, A., AND A. POLLE. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. *Journal of Experimental Botany* 53: 1351-1365.

SEREGIN, I. V., AND A. D. KOZHEVNIKOVA. 2004. Strontium transport, distribution, and toxic effects on maize seedling growth. *Russian Journal of Plant Physiology* 51: 215-221.

SMITH, K. A. 1971. The comparative uptake and translocation by plants of calcium, strontium, barium and radium. *Plant and Soil* 34: 369-379.

THOMPSON, K., J. A. PARKINSON, S. R. BAND, AND R. E. SPENCER. 1997. A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytologist* 136: 679-689.

WAKEEL, A., M. FAROOQ, M. QADIR, AND S. SCHUBERT. 2011. Potassium substitution by sodium in plants. *Critical Reviews in Plant Sciences* 30: 401-413.

WHITE, P. J. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* 52: 891-899.

WHITE, P. J., AND M. R. BROADLEY. 2003. Calcium in plants. *Annals of Botany* 92: 487-511.

WIESENBERGER, G., K. STEINLEITNER, R. MALLI, W. F. GRAIER, J. VORMANN, R. J. SCHWEYEN, AND J. A. STADLER. 2007. Mg^{2+} deprivation elicits rapid Ca^{2+} uptake and activates Ca^{2+}/calcineurin signaling in *Saccharomyces cerevisiae*. *Eukaryotic Cell* 6: 592-599.

YANO, J., AND V. K. YACHANDRA. 2009. X-ray absorption spectroscopy. *Photosynthesis Research* 102: 241-254.

ZARCINAS, B. A., B. CARTWRIGHT, AND L. R. SPOUNCER. 1987. Nitric-acid digestion and multielement analysis of plant-material by inductively coupled plasma spectrometry. *Communications in Soil Science and Plant Analysis* 18: 131-146.

TABLE 1. Chemical and physical properties of the topsoil and siltstone. Element concentrations, except those of organic C, total N, NH_4-N, NO_3-N, and total P, are plant-available element concentrations obtained using a Mehlich 3 extraction.

	Topsoil	Siltstone		Topsoil	Siltstone
EC (mS m^{-1})	14	69	Zn (µg g^{-1})	0.2	0.4
pH	6.7	7.7	Mo (µg g^{-1})	< 0.01	< 0.01
Organic C (mg g^{-1})	1.2	< 0.5	Co (µg g^{-1})	0.27	0.06
Total N (mg g^{-1})	0.11	0.18	Na (µg g^{-1})	76	370
NH_4-N (µg g^{-1})	1	< 1	Al (µg g^{-1})	188	75
NO_3-N (µg g^{-1})	6	18	Pb (µg g^{-1})	0.4	0.5
Total P (µg g^{-1})	51	89	Cd (µg g^{-1})	< 0.01	< 0.01
P (µg g^{-1})	1	2	Ni (µg g^{-1})	< 0.1	< 0.1
S (µg g^{-1})	4	110	B (µg g^{-1})	< 0.1	< 0.1
K (µg g^{-1})	28	64	As (µg g^{-1})	< 0.1	0.7
Mg (µg g^{-1})	39	120	Se (µg g^{-1})	< 0.1	< 0.1
Ca (µg g^{-1})	170	150	Sand (%)	94.0	46.0
Mn (µg g^{-1})	9.0	0.5	Silt (%)	0.5	48.0
Fe (µg g^{-1})	18	9	Clay (%)	5.5	6.0
Cu (µg g^{-1})	0.6	2.7			

Notes: All results presented are from measurements of single samples; no replicates were analysed as the substrates had been thoroughly homogenized.

TABLE 2. Two-way ANOVA results of biomass accumulation of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*.

		Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Nodule dry weight (g plant^{-1})
A. stipuligera	T	3.09 ± 1.21	0.55 ± 0.26	0.03 ± 0.02
(means ± SD, $n = 4$)	TS	3.40 ± 0.85	0.39 ± 0.13	0.06 ± 0.04
A. robeorum	T	1.31 ± 0.28	0.51 ± 0.18	0.02 ± 0.02
(means ± SD, $n = 4$)	TS	2.25 ± 0.41	0.50 ± 0.50	0.06 ± 0.05
Substrate effects		$F_{1,12} = 2.651$	$F_{1,12} = 0.280$	$F_{1,12} = 3.811$
		$P = 0.129$	$P = 0.607$	$P = 0.075$
Species effects		$F_{1,12} = 14.223$	$F_{1,12} = 0.061$	$F_{1,12} = 0.002$
		$P = 0.003$	$P = 0.808$	$P = 0.966$
Interactions (substrate * species)		$F_{1,12} = 0.614$	$F_{1,12} = 0.230$	$F_{1,12} = 0.311$
		$P = 0.448$	$P = 0.640$	$P = 0.587$

Abbreviations: T, topsoil; TS, topsoil-siltstone mixture.

TABLE 3. Two-way ANOVA results of concentrations of magnesium (Mg), calcium (Ca), and sulfur (S), and concentration ratios of Mg to Ca (Mg/Ca), Mg to S (Mg/S), and Ca to S (Ca/S) in mature phyllodes of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*.

		Mg (mg g^{-1})	Ca (mg g^{-1})	S (mg g^{-1})	Mg/Ca (g g^{-1})	Mg/S (g g^{-1})	Ca/S (g g^{-1})
A. stipuligera	T	2.3 ± 0.3	15.5 ± 3.2	2.2 ± 1.2	0.15 ± 0.02	1.23 ± 0.53	8.10 ± 3.17
(means ± SD, *n* = 4)	TS	2.4 ± 0.4	15.5 ± 2.4	4.0 ± 2.0	0.16 ± 0.01	0.77 ± 0.48	4.83 ± 2.78
A. robeorum	T	4.8 ± 0.8	22.3 ± 3.4	7.6 ± 0.9	0.22 ± 0.01	0.64 ± 0.09	2.95 ± 0.32
(means ± SD, *n* = 4)	TS	3.4 ± 0.5	15.3 ± 2.3	6.0 ± 0.7	0.22 ± 0.04	0.57 ± 0.11	2.61 ± 0.66
Substrate effects		$F_{1,12} = 5.454$	$F_{1,12} = 5.815$	$F_{1,12} = 0.030$	$F_{1,12} = 0.576$	$F_{1,12} = 2.095$	$F_{1,12} = 2.834$
		$P = 0.038$	$P = 0.033$	$P = 0.865$	$P = 0.462$	$P = 0.173$	$P = 0.118$
Species effects		$F_{1,12} = 41.237$	$F_{1,12} = 5.173$	$F_{1,12} = 31.208$	$F_{1,12} = 30.600$	$F_{1,12} = 4.787$	$F_{1,12} = 11.827$
		$P < 0.001$	$P = 0.042$	$P < 0.001$	$P < 0.001$	$P = 0.049$	$P = 0.005$
Interactions (substrate * species)		$F_{1,12} = 8.417$	$F_{1,12} = 5.833$	$F_{1,12} = 6.532$	$F_{1,12} = 0.012$	$F_{1,12} = 1.189$	$F_{1,12} = 1.872$
		$P = 0.013$	$P = 0.033$	$P = 0.025$	$P = 0.915$	$P = 0.297$	$P = 0.196$

Abbreviations: T, topsoil; TS, topsoil-siltstone mixture.

TABLE 4. A summary of elemental compositions and locations of various mineral precipitates in 25-week-old plants of *Acacia stipuligera*.

Mineral precipitates' type	Spectrum	Elements precipitated	Mineral precipitates' location
Prismatic crystals in Figs. 1C, 3B, C	Figs. 4B, 5B, C	Ca, Sr, C, O	In cells associated with fiber cells in phyllodes; in pith, pith ray cells, xylem fiber cells and cortical parenchyma cells associated with fiber cells in branchlets
Mineral precipitates in Figs. 1D–F, 3B (unfilled arrow), D (unfilled arrow), G	Figs. 4C–H, 5D–G	Mg, Ca, Sr, Ba, Na, K, S, C, O	In parenchyma and mesophyll cells in phyllodes; in pith, pith ray, cortical and phloem parenchyma cells, and xylem in branchlets
Mineral precipitates in Fig. 1G	Figs. 4I, J, 5H, I	Ca, Na, K, Mn, Cu, S, Si, C, O	In parenchyma cells in phyllodes
Mineral precipitates in Fig. 1H	Fig. 4K	Ca, K, Fe, Al, Si, C, O	In mesophyll
Mineral precipitates in Fig. 3C (unfilled arrow), E, F	Fig. 4P	Ca, K, S, C, O	In pith and pith ray cells in branchlets
Mineral precipitates in Fig. 3D (filled arrow)	Figs. 4M, 5J, K	Mg, Ca, K, S, Si, C, O	In xylem in branchlets

TABLE 5. A summary of elemental compositions and locations of various mineral precipitates in 25-week-old plants of *Acacia robeorum.*

Mineral precipitates' type	Spectrum	Elements precipitated	Mineral precipitates' location
Prismatic crystals in Figs. 2C, 3I, J	Figs. 4B, 5B, C	Ca, Sr, C, O	In cells associated with fiber cells in phyllodes; in pith, pith ray cells, xylem fiber cells and cortical parenchyma cells associated with fiber cells in branchlets
Mineral precipitates similar to those in Fig. 1D–F	Figs. 4C–H, 5D–G	Mg, Ca, Sr, Ba, Na, K, S, C, O	In parenchyma cells in phyllodes (images not shown)
Mineral precipitates in Fig. 2D–F	Figs. 4C, D, L, 5D, E	Mg, Ca, K, Fe, S, C, O	In parenchyma and mesophyll cells in phyllodes
Mineral precipitates in Fig. 2L, M	Figs. 4I, J, 5H, I	Ca, Na, K, Mn, Cu, S, Si, C, O	In parenchyma and mesophyll cells in phyllodes
Mineral precipitates in Fig. 2G–J	Figs. 4M, 5J, K	Mg, Ca, K, S, Si, C, O	In parenchyma and mesophyll cells in phyllodes
Mineral precipitates in Fig. 2K	Fig. 4N	Ca, Fe, Al, S, Si, C, O	In mesophyll cells in phyllodes
Mineral precipitates in Fig. 2N	Fig. 4O	Ca, K, Fe, Al, Cu, V, Ti, Si, C, O	In parenchyma cells in phyllodes
Mineral precipitates in Fig. 3K	Fig. 4P	Ca, K, S, C, O	In cortical parenchyma cells in branchlets

ONLINE SUPPLEMENTARY MATERIALS

APPENDIX S1. Chemical properties of the first 10 cm topsoil from the natural habitat of *Acacia stipuligera* and *A. robeorum*.

APPENDIX S2. Concentrations of magnesium (Mg), calcium (Ca), and sulfur (S), and concentration ratios of Mg to Ca (Mg/Ca), Mg to S (Mg/S), and Ca to S (Ca/S) in mature phyllodes of *Acacia stipuligera* and *A. robeorum* grown in their natural habitat.

APPENDIX S3. Two-way ANOVA results of concentrations of strontium (Sr) and barium (Ba), and concentration ratios of Sr to calcium (Ca) (Sr/Ca), and Ba to Ca (Ba/Ca) in mature phyllodes of 25-week-old plants of *Acacia stipuligera* and *A. robeorum.*

FIGURE LEGENDS

Fig. 1. Scanning electron microscopy images of various mineral precipitates (arrow) in phyllodes of 25-week-old plants of *Acacia stipuligera*. (A–B) Transverse-sectional views at different magnifications showing the general structure of the phyllode. (C) Prismatic crystals in cells associated with fiber cells (arrow head); (D) Multiple mineral precipitates in a parenchyma cell. (E) A single mineral precipitate in a parenchyma cell. (F) Multiple mineral precipitates in a mesophyll cell. (G) Mineral precipitates associated with tannin deposit (asterisk) in a parenchyma cell. (H) A single mineral precipitate in the mesophyll. *Abbreviations:* Ep, epidermis; Me, mesophyll; Pa, parenchyma. Scale bars = 200 µm (A), 50 µm (B), 20 µm (C), 2.5 µm (D, G, H), 2 µm (E), 5 µm (F). Panel (H) was adapted from (He et al., 2014).

Fig. 2. Scanning electron microscopy images of various mineral precipitates (arrow) in phyllodes of 25-week-old plants of *Acacia robeorum*. (A–B) Transverse-sectional views at different magnifications showing the general structure of the phyllode. (C) A prismatic crystal in a cell associated with fiber cells (arrow head). (D) Spherical crystals in a parenchyma cell. (E) A spherical crystal in a parenchyma cell. (F–L) Mineral precipitates of varying morphologies in mesophyll cells. (M) Mineral precipitates associated with tannin deposit (asterisk) in a parenchyma cell. (N) Mineral precipitates in a parenchyma cell. *Abbreviations:* Ep, epidermis; Me, mesophyll; Pa, parenchyma. Scale bars = 100 µm (A), 25 µm (B), 5 µm (C, D, N), 2.5 µm (E–K), 1 µm (L), 2 µm (M). Panels (K) and (L) were adapted from (He et al., 2014).

Fig. 3. Scanning electron microscopy images of various mineral precipitates (arrow) in branchlets of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*. (A–G) *A. stipuligera*. (H–K) *A. robeorum*. (A) A whole transverse section of a branchlet of *A. stipuligera*. (B) Prismatic crystals (filled arrow) in cortical parenchyma cells associated with fiber cells (filled arrow head) and mineral precipitates (unfilled arrow) in cortical parenchyma cells (unfilled arrow head). (C–F) Mineral precipitates in pith or pith ray cells. (G) A mineral precipitate in a cortical parenchyma cell. (H) A whole transverse section of a branchlet of *A. robeorum*. (I) A transverse-sectional segment of a branchlet. (J) Prismatic crystals in cortical parenchyma cells associated with fiber cells. (K) Mineral precipitates in a cortical parenchyma cell. *Abbreviations:* Ep, epidermis; Xy, xylem. Scale bars = 200 µm (A, H), 10 µm (B, J, K), 5 µm (C), 2 µm (D–G), 50 µm (I).

Fig. 4. Energy-dispersive X-ray spectra of various mineral precipitates in Au-coated transverse sections of phyllodes and branchlets of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*. (A) Typical spectrum of cells without mineral precipitates. (B–P) Typical spectra of various mineral precipitates. Panels (I), (K) and (N) were adapted from (He et al., 2014).

Fig. 5. Energy-dispersive X-ray spectra of various mineral precipitates in C-coated transverse sections of phyllodes and branchlets of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*. (A) Typical spectrum of cells without mineral precipitates. (B–K) Typical spectra of various mineral precipitates.

Fig. 1.

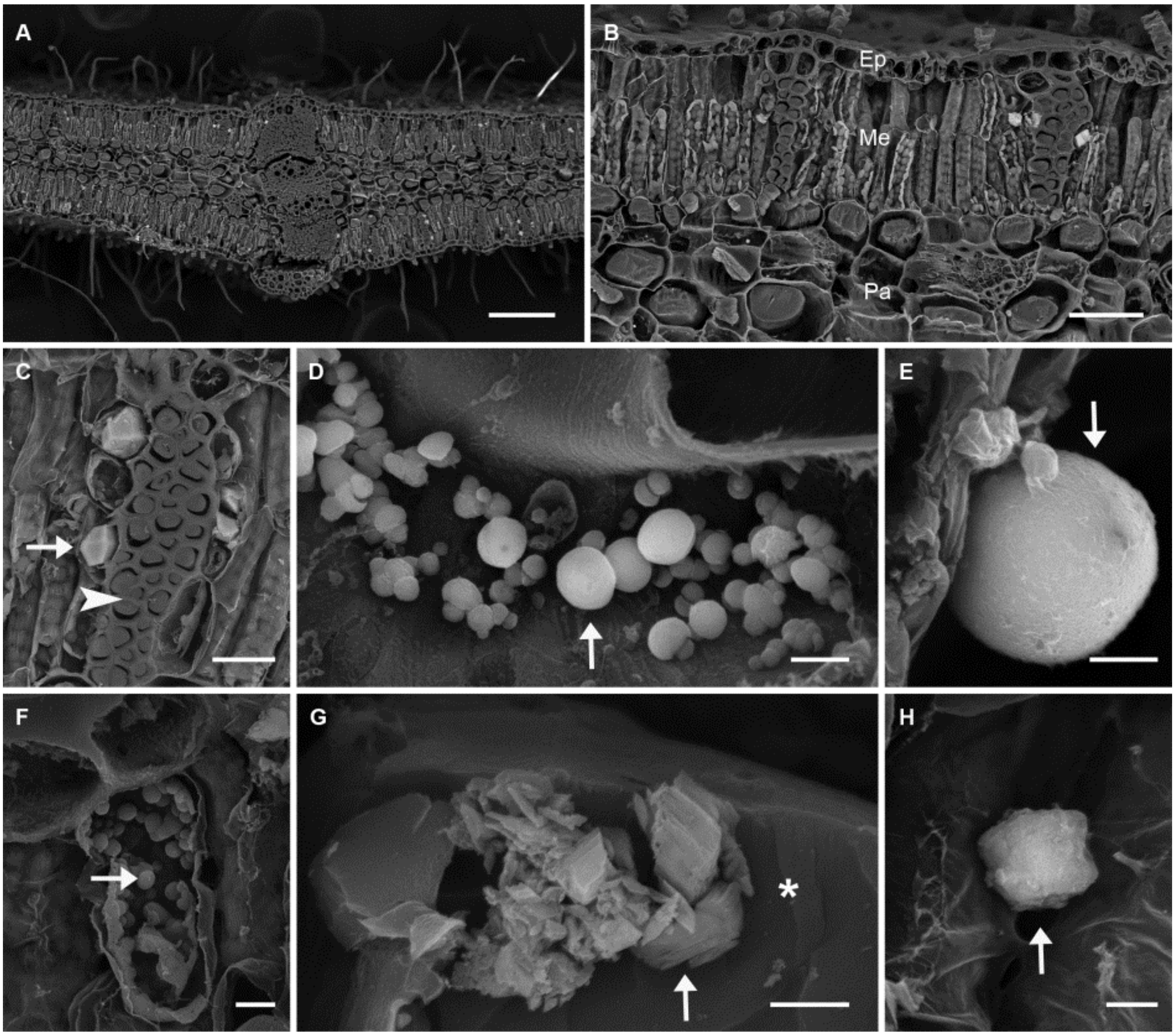


Fig. 2.

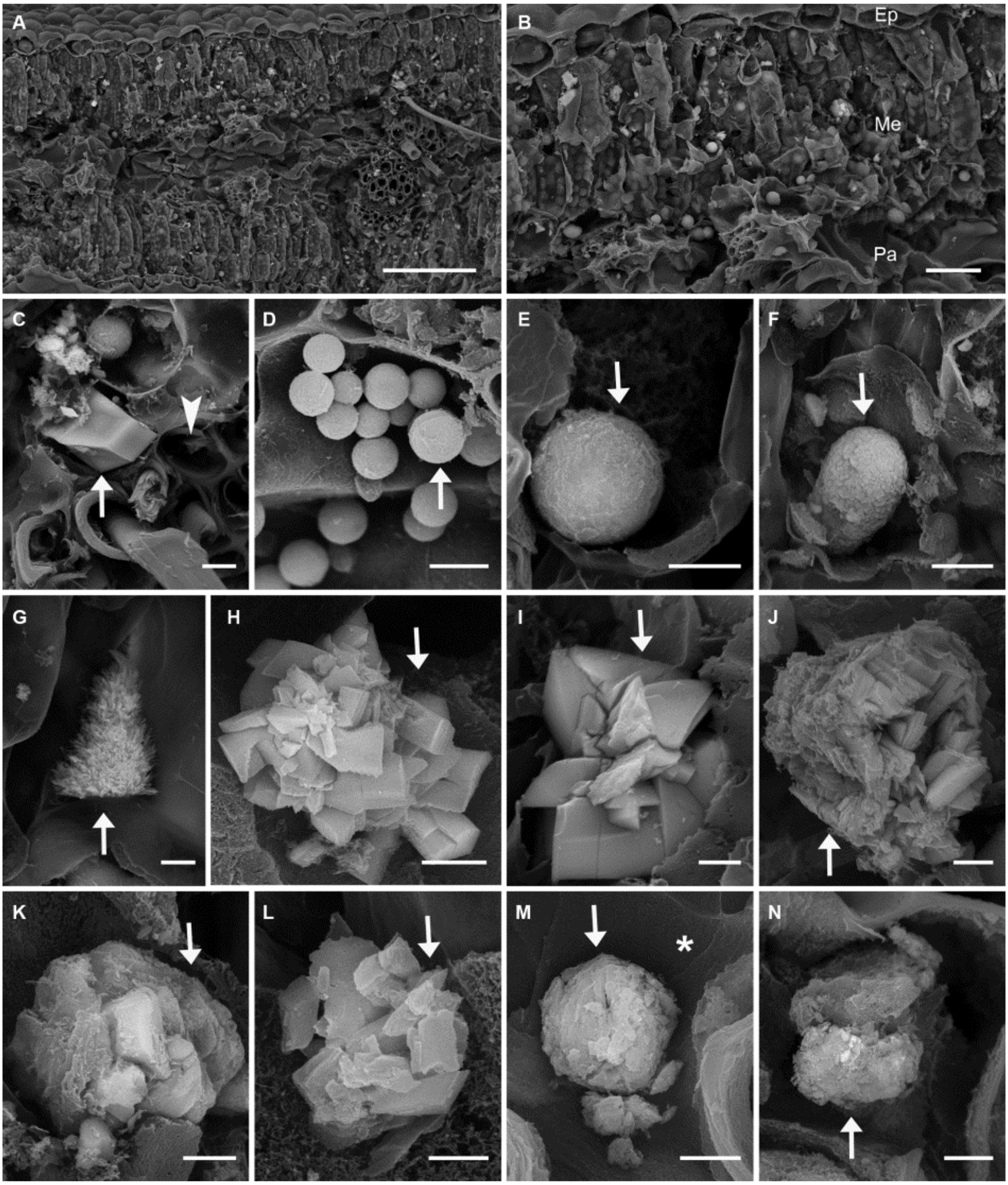


Fig. 3.

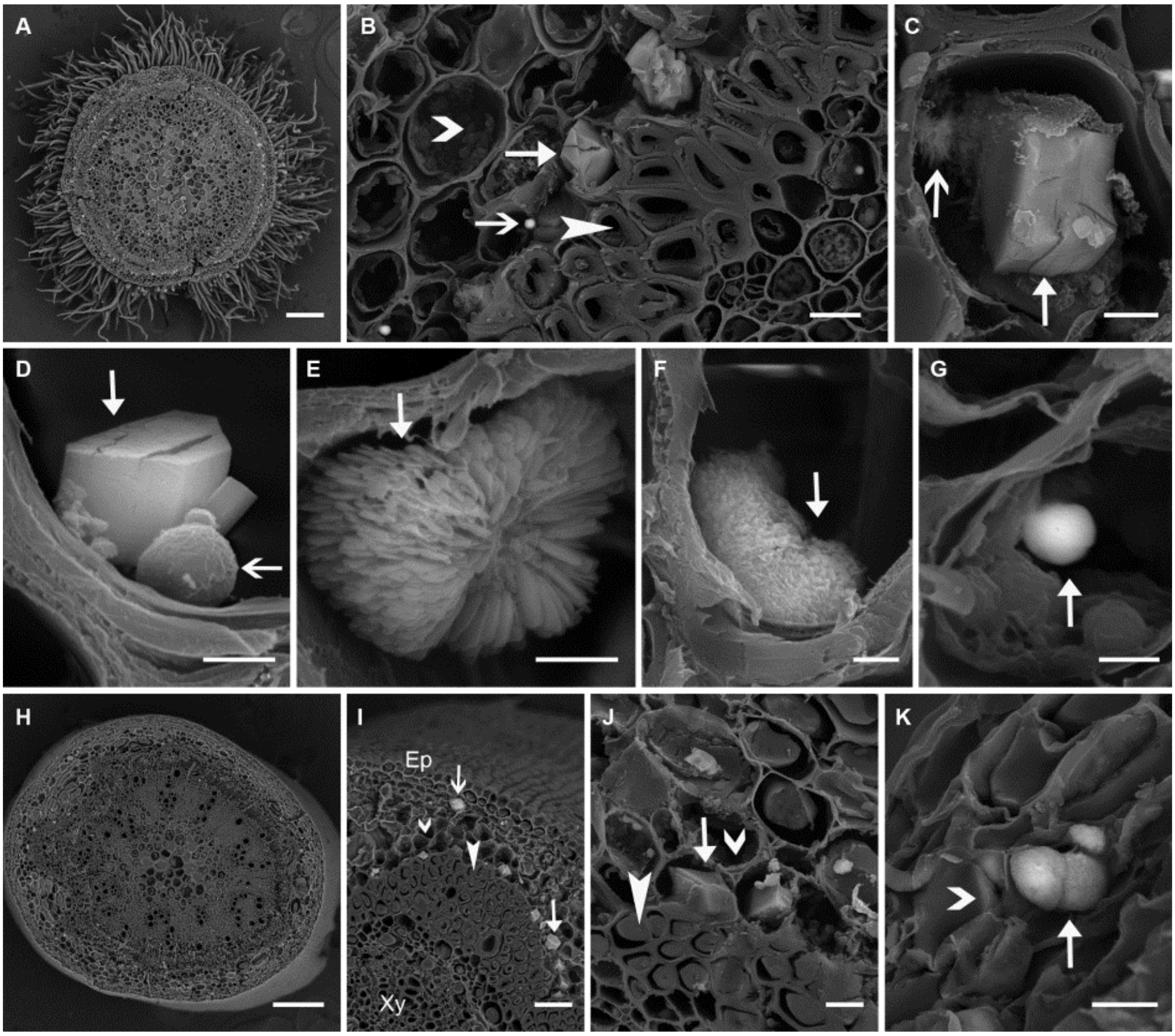


Fig. 4.

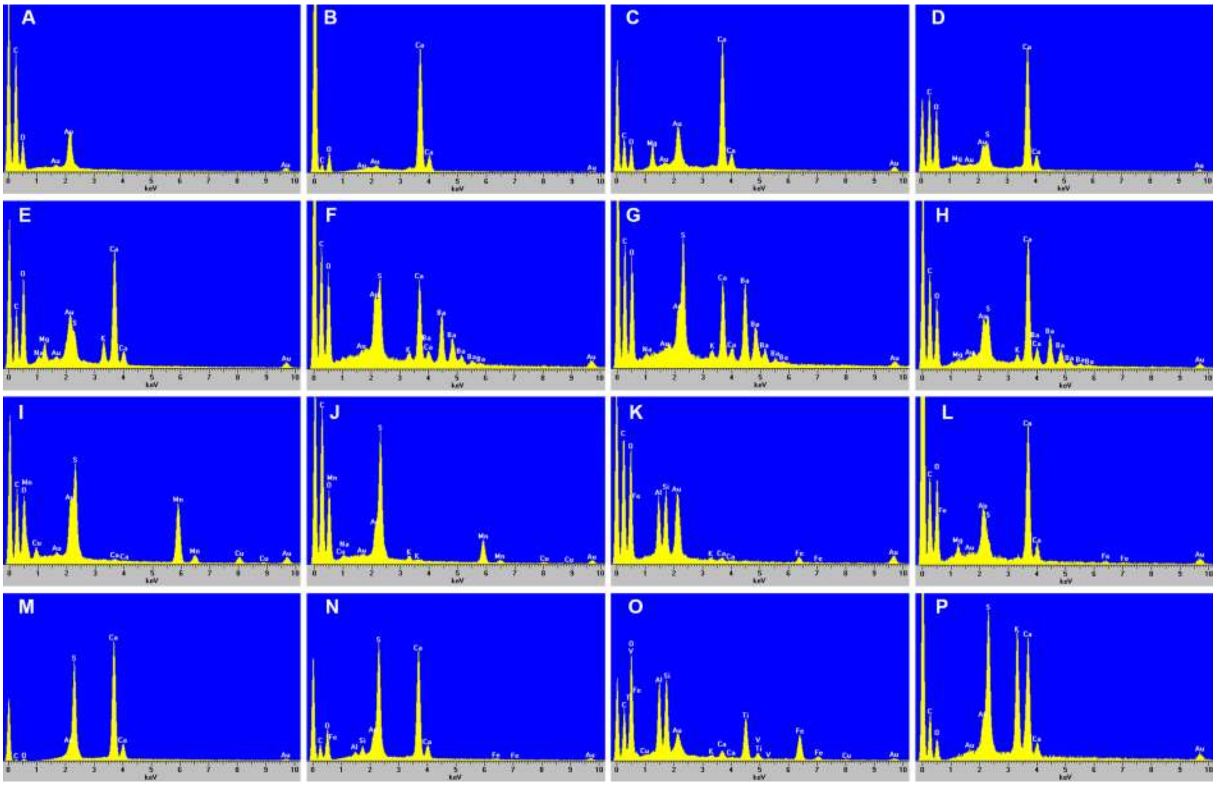
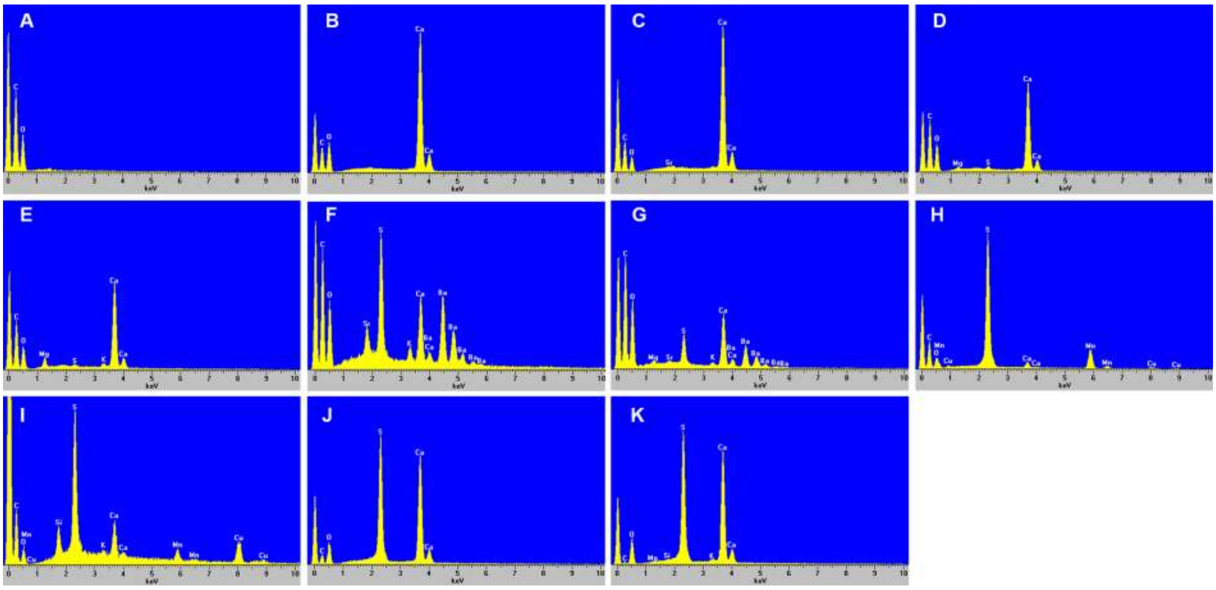


Fig. 5.


A
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APPENDIX S1. Chemical properties of the first 10 cm topsoil from the natural habitat of *Acacia stipuligera* and *A. robeorum*. All values are presented as means ($n = 2$, and each sample was a bulk sample of soil collected under the crowns of three shrubs). Data of *A. robeorum* were modified after He et al. (2012a).

	A. stipuligera	*A. robeorum*		*A. stipuligera*	*A. robeorum*
EC (mS m^{-1})	1	3.5	Zn (μg g^{-1})	0.2	0.2
pH	5.7	6.7	Mo (μg g^{-1})	<0.01	0.02
Organic C (mg g^{-1})	1.2	2.3	Co (μg g^{-1})	0.12	1.85
P (μg g^{-1})	2	5	Na (μg g^{-1})	<1	1
S (μg g^{-1})	1	14	Al (μg g^{-1})	110	280
K (μg g^{-1})	18	71	Pb (μg g^{-1})	0.2	0.6
Mg (μg g^{-1})	22	80	Cd (μg g^{-1})	<0.01	<0.01
Ca (μg g^{-1})	82	385	Ni (μg g^{-1})	<0.1	0.3
Mn (μg g^{-1})	14	170	B (μg g^{-1})	< 0.1	0.5
Fe (μg g^{-1})	9	23	As (μg g^{-1})	<0.1	1.8
Cu (μg g^{-1})	0.1	2.9			

APPENDIX S2. Concentrations of magnesium (Mg), calcium (Ca), and sulfur (S), and concentration ratios of Mg to Ca (Mg/Ca), Mg to S (Mg/S), and Ca to S (Ca/S) in mature phyllodes of *Acacia stipuligera* and *A. robeorum* grown in their natural habitat. All values are presented as means ± SD (single-sample *t*-test, df = 5, $P < 0.001$ for all data). Data of Ca, Mg, and S concentrations of *A. robeorum* were modified after He et al. (2012a).

Species	Mg (mg g^{-1})	Ca (mg g^{-1})	S (mg g^{-1})	Mg/Ca (g g^{-1})	Mg/S (g g^{-1})	Ca/S (g g^{-1})
A. stipuligera (means ± SD)	3.4 ± 0.9	9.4 ± 2.7	1.3 ± 0.1	0.37 ± 0.06	2.61 ± 0.57	7.25 ± 1.81
	(*t* = 9.537)	(*t* = 8.475)	(*t* = 27.323)	(*t* = 14.966)	(*t* = 11.150)	(*t* = 9.821)
A. robeorum (means ± SD)	10.3 ± 0.9	72.0 ± 8.1	42.2 ± 2.9	0.14 ± 0.02	0.25 ± 0.03	1.70 ± 0.10
	(*t* = 27.266)	(*t* = 21.762)	(*t* = 35.945)	(*t* = 15.430)	(*t* = 19.301)	(*t* = 41.283)

APPENDIX S3. Two-way ANOVA results of concentrations of strontium (Sr) and barium (Ba), and concentration ratios of Sr to calcium (Ca) (Sr/Ca), and Ba to Ca (Ba/Ca) in mature phyllodes of 25-week-old plants of *Acacia stipuligera* and *A. robeorum*.

		Sr (μg g^{-1})	Ba (μg g^{-1})	Sr/Ca (mg g^{-1})	Ba/Ca (mg g^{-1})
A. stipuligera	T	130 ± 29	96 ± 14	8.4 ± 0.2	6.5 ± 2.3
(means ± SD, *n* = 4)	TS	131 ± 17	75 ± 12	8.4 ± 0.3	4.9 ± 1.1
A. robeorum	T	190± 36	88 ± 17	8.5 ± 0.4	4.0 ± 0.6
(means ± SD, *n* = 4)	TS	130 ±26	57 ± 18	8.5 ± 0.6	3.7 ± 0.9
Substrate effects		$F_{1,12} = 4.608$	$F_{1,12} = 11.787$	$F_{1,12} = 0$	$F_{1,12} = 1.857$
		$P = 0.053$	$P = 0.005$	$P = 1$	$P = 0.198$
Species effects		$F_{1,12} = 4.598$	$F_{1,12} = 2.750$	$F_{1,12} = 0.181$	$F_{1,12} = 7.426$
		$P = 0.053$	$P = 0.123$	$P = 0.678$	$P = 0.018$
Interactions (substrate * species)		$F_{1,12} = 4.770$	$F_{1,12} = 0.383$	$F_{1,12} = 0.111$	$F_{1,12} = 1.002$
		$P = 0.050$	$P = 0.548$	$P = 0.745$	$P = 0.337$

Abbreviations: T, topsoil; TS, topsoil-siltstone mixture.