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ABSTRACT

Ultramafic soils are usually marginal in macronutrients (nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca)) for growth of crop plants. Commercial nickel (Ni) agromining is dependent on attaining high yield and high Ni concentration in harvestable biomass of Ni hyperaccumulator species. We previously reported on the biomass responses of two promising tropical 'metal crops' (*Phyllanthus rufuschaneyi* and *Rinorea* cf. *bengalensis*) to rates of N, P, and K fertilisers. Calcium, sulphur (S) and organic matter amendments have varied effects on the biomass production and Ni uptake in temperate Ni hyperaccumulator species used in agromining, but the trends in tropical 'metal crops' are not reported to-date. We investigated the effects of these amendments on the growth performance and the Ni (and other elements) uptake in *P. rufuschaneyi* and *R. bengalensis*. The experiments consisted of a large 12-month randomised growth trial in large pots in Sabah (Malaysia) using ultramafic soils under different treatment levels of soluble Ca and S, and organic matter amendments. We found that Ca and S additions had no significant effects on the growth of *P. rufuschaneyi* and *R. bengalensis*. Organic matter amendments had strong positive effect on the growth of *R. bengalensis* (p <0.05), but we recorded significant negative growth response in *P. rufuschaneyi*. Whereas Ca and S additions improved the Ni uptake in these species, organic matter amendments significantly reduced the shoot Ni concentrations in both species. Our findings indicate that Ca and S additions are important in the agronomy of tropical 'metal crops' to be used in economic Ni agromining, but organic matter amendments may not be useful.

Keywords: *biomass production; hyperaccumulator plants; metal crops; nickel tolerance; nickel yield; organic matter amendments.*

1. INTRODUCTION

Nickel (Ni) agromining involves growing selected Ni hyperaccumulator plants ('metal crops') on sub-economic ore bodies or mineralised (ultramafic) soils, or anthropogenic metal-rich materials (such as contaminated soils, mine wastes, industrial sludges), and processing the biomass after harvesting to recover valuable products such as Ni metal or Ni salts (Barbaroux et al., 2012; Chaney et al., 1998; 2007; van der Ent et al., 2015). Soil and agronomy management practices for agromining based on field and glasshouse studies were recently reviewed (Nkrumah et al., 2016a; 2018a). On the basis of various studies, it is clear that specific agronomic practices in agromining need to be tested with every species and location prior to wider application (Broadhurst and Chaney, 2016; Nkrumah et al., 2018a). Whereas some agronomic practices (*e.g.* NPK or organic matter fertilisation) enhance Ni yield, their usages is dependent on factors that include the nature of substrate under consideration (Nkrumah et al., 2018a). A typical example is the utilization of organic matter amendments in trials involving *Alyssum* species in temperate regions. Broadhurst and Chaney (2016) utilized an aged compost product to investigate the effects of organic matter amendments on the growth and Ni yield of *Alyssum murale*, and found negative responses. In a similar study, increases in biomass production of *A. serpyllifolium* subsp. *lusitanicum*, *A. serpyllifolium* subsp. *malacitanum*, *A. bertolonii* and *Noccaea goesingense* in response to increasing amounts of organic matter amendments were observed, but all treatments significantly reduced both extractable Ni and shoot Ni concentrations in the tested species (Álvarez-López et al., 2016).

Apart from growth responses in 'metal crops', other agronomic measures are targeted at increasing Ni yield by enhancing Ni tolerance (Nkrumah et al., 2016a; 2018a). The effect of Ca addition on Ni uptake by hyperaccumulator plants is species-dependent (Chaney et al., 2008; Robinson et al., 1999). Whereas Ca addition inhibits Ni uptake in the Ni hyperaccumulator *Berkheya coddii* (Robinson et al., 1999), its application to high Mg ultramafic soils with low Ca/Mg quotient ameliorates Ni phytotoxicity and improves annual Ni phytoextraction in *Alyssum* spp. (Brooks et al., 1981; Chaney et al., 2008). Brooks et al. (1981) observed increased Ni tolerance in *Alyssum* species in response to Ca additions to soils with high Ni concentrations (>1000 $\mu g\ g^{-1}$), and consequently these species achieved as high as 4 wt% shoot Ni. Similarly, increases in shoot/root Ni concentration ratio of up to 10-fold in *Alyssum* spp. under varied Ca concentrations in growth media characteristic of ultramafic soils were observed, which is indicative of increased metal translocation (Chaney et al., 2008).

Sulphur addition also plays major role in Ni phytoextraction in Ni hyperaccumulator plants (Broadhurst et al., 2004a, b; 2009). Evidence suggests that S increases Ni tolerance in *Noccaea* Ni hyperaccumulator plants (see Freeman et al., 2004; Na and Salt, 2011). There is a strong correlation between Ni and S localisation at the cell-level in *A. bracteatum and A. murale* in epidermal vacuoles (Asemaneh et al., 2006; Broadhurst et al., 2004a, b; 2009; Küpper et al., 2001). This may be explained by the functioning of SO_4^{2-} as an important counter-ion to Ni^{2+} in the vacuoles of these hyperaccumulator species (Broadhurst et al., 2004a, b; 2009; Küpper et al., 2001). Sulphur addition has also been shown to significantly increase Ni uptake (up to three-fold) in *B. coddii*, mainly because of increase in the concentrations of plant available S (Robinson et al., 1999), although the authors assert reduction in soil pH might also have contributed to the higher shoot Ni accumulation. Therefore, S addition could be a low-cost amendment warranting consideration.

On a global scale, Southeast Asia has some of the largest ultramafic outcrops, with Borneo and Sulawesi together totalling over 23 000 km^2 of ultramafic soils (van der Ent et al., 2013; Galey et al., 2017). Sabah (Malaysia) on the Island of Borneo has extensive ultramafic outcrops, covering an area of 3500 km^2 (Proctor et al., 1988; Repin, 1998). Although unrealised opportunities for Ni agromining exist in Sabah and other tropical regions such as Sulawesi, Halmahera (Indonesia) and Palawan (Philippines) (van der Ent et al., 2013; Nkrumah et al., 2016b; 2018b, c), the effects of Ca and S additions on Ni uptake, tolerance and accumulation of tropical 'metal crops' are not known. Evidence from work in temperate regions suggests that Ca and S additions can have significant effects on the Ni accumulation in *Alyssum* species (Chaney et al., 2008; Broadhurst et al., 2004a, b; 2009). Hence, it is important to study the responses of tropical 'metal crops' to these soil amendments. Furthermore, the growth and shoot Ni responses of tropical 'metal crops' to organic matter amendments have not been reported. We therefore undertook pot trials in Sabah using two local species (*Phyllanthus rufuschaneyi* and *Rinorea* cf. *bengalensis*) to determine whether the trends in temperate regions could be observed in a wet tropical environment. The present study investigates the effects of Ca and S on Ni uptake, tolerance and accumulation in two selected tropical 'metal crops' (*P. rufuschaneyi* and *R. bengalensis*). Furthermore, this study measures the growth and shoot Ni responses of *P. rufuschaneyi* and *R. bengalensis* to organic matter amendments.

2. MATERIALS AND METHODS

2.1 Plant species

The tropical Ni hyperaccumulator species selected for this research project was *Phyllanthus rufuschaneyi* (Phyllanthaceae). This newly-described species is highly light-demanding and is known to be restricted to only two localities in Sabah (*i.e.,* Bukit Hampuan and Lompoyou Hill) (Bouman et al. 2018). The habitat is open secondary scrub that has been impacted by recurring forest fires and excessive logging. Considering that only limited populations exist, and these are highly prone to incessant disturbances, *P. rufuschaneyi* is classified as an endangered species according to IUCN criteria (Bouman et al., 2018). The soil type in the native habitat is young (eroded) hypermagnesian Cambisols on strongly serpentinised bedrock. These soils are characterized by exceptionally high Mg/Ca quotients, *circum*-neutral pH and high available Ni (van der Ent et al., 2016; Echevarria, 2018). *Phyllanthus rufuschaneyi* accumulates exceptional concentrations of Ni in the shoot tissues, with up to 2.5 wt% in the old leaves and 1.23 wt% in the twigs (van der Ent and Mulligan, 2015). The Ni concentrations in the phloem sap and the seeds are high, with mean concentrations of 8830 and 17 570 µg g^{-1} (van der Ent and Mulligan, 2015). Considering the high shoot Ni concentrations, coupled with its multi-stemmed habit, rapid re-growth after coppicing, ease of propagation and pest resistance, *P. rufuschaneyi* is an ideal candidate for use as a Ni 'metal crop' for tropical Ni agromining (Bouman et al., 2018).

Unlike *P. rufuschaneyi*, which is restricted to only two localities in Sabah, *Rinorea* cf. *bengalensis* is widespread in Southeast Asia (Brooks and Wither, 1977; Galey et al., 2017). It occurs on both ultramafic and non-ultramafic soils, with only the populations on ultramafic soils hyperaccumulating Ni, making it a 'facultative hyperaccumulator' (Brooks and Wither, 1977; Brooks et al., 1977; Pollard et al., 2014). In Sabah, *R. bengalensis* is locally common on ultramafic soils, where it can achieve a height of 25 m. In the leaves *R. bengalensis* can accumulate up to 1.70 Wt% Ni, whereas the Ni concentrations in the green phloem semi-solid in the trunk can be up to 79.0 g Ni kg^{-1} DW (van der Ent and Mulligan, 2015). Similar to *P. rufuschaneyi* and other Ni hyperaccumulators in Sabah, *R.* cf. *bengalensis* occurs on *circum*-neutral soils with relatively elevated phytoavailable Mg and Ni concentrations (van der Ent et al., 2016). Considering its previously observed growth-rates in the wild and high Ni accumulation capacity in leaves and bole, *R. bengalensis* clearly has potential to be used as 'metal crop' in tropical Ni agromining.

2.2 Experimental setup

The experiment was carried out at the substation Monggis of Kinabalu Park (345 m asl; 6°12'1.58"N; 116°45'8.03"E), Sabah (Malaysia). The pot trials were undertaken over a period of 15 months starting from December 2015. The local temperature (average day temperature), humidity and light intensity were 29°C, 75% and 625 µmol m^2 sec^{-1} respectively. We constructed a 25 × 20 m shade house covered with neutral-density shade-cloth (50%) on all sides to permit cultivation of both light-demanding species (*P. rufuschaneyi*) and shade-tolerant species (*R. bengalensis*). Past studies indicate a light level of 20% full sun is conducive for both light-demanding and shade-tolerant species, even though it might not be optimal for either (Denslow et al., 1987; Veenendaal et al., 1996). The roof was made of transparent horticultural polyethylene film, allowing for a relatively uniform light environment and to keep rainfall out so that water supplied to the plants could be controlled through irrigation sprays mounted on the ceiling of the shade house.

Rinorea cf. *bengalensis* (Table 1a) and *P. rufuschaneyi* wildlings (Table 1b) were collected from their native habitats (335 m asl; 6°7'48.82"N; 116°46'21.92"E, and 345 m asl; 6°12'1.58"N; 116°45'8.03"E respectively). Each species was collected from a single location, with *P. rufuschaneyi* seedlings from the same population and *R. bengalensis* from the same mother tree. All seedlings were immediately potted after collection with soil collected from the respective native habitats, and allowed to acclimatise in the shade house for 2 weeks. The wildlings were then transplanted into pots (8 L with 10 kg ultramafic soil). The soil originated from the top 15–20 cm of an unfertilised ultramafic soil (Eutric Hypermagnesic Cambisol) near Pahu village (335 m asl; 6°7'48.82"N; 116°46'21.92"E), Sabah, Malaysia. The soil, totalling 7 tonnes, was sun-dried, homogenized, and sieved through a 1-cm screen. Samples (50 g) of the mixed sieved soil were collected for initial soil chemical analysis reported in Nkrumah et al. (in press), following methods described in Nkrumah et al. (2018b). The wildlings were transplanted and allowed to establish for a period of 3 months before the start of the experiment.

2.3 Treatment application and real-time data collection

In March 2016, plants were blocked by size (Table 1) and then assigned to one of three treatments (Ca, S and organic matter), with five replicates per treatment. For Ca and S treatments: the treatment levels were 'non-treated control', 'treated control', 180 mg E kg^{-1} and 360 mg E kg^{-1}, where E is Ca or S depending on the treatment under consideration (Table 2). For organic matter (OM) amendments, the treatments were 'non-treated control', 'treated control', 5% OM and 10% OM. The 5% OM and 10% OM treatments were added on volume basis, the respective OM were thoroughly mixed with the mineral soil, and same volume of mixed soil put into the pot. All plants received constant adequate levels of N, P, K, Ca and S (including 'treated control' lacking single nutrients for Ca or S comparisons), except in the 'non-treated control' which received no nutrient additions (see Table 2). The OM treatments were applied at the beginning of the experiment, whereas the Ca and S treatments were applied at 3-month intervals, in solution using a syringe inserted through a template over the pot with four holes to ensure uniform application. Each pot received 120 mL of the respective nutrient solution. During treatment application, pots were placed on saucers to avoid treatment losses, and the saucers were removed 1 week after treatment application. The pots were randomly assigned to table space, and to minimize differences in micro-climate and light conditions, the pots were randomised every four months. The plants were watered (with local water tapped from a nearby river) daily to field capacity, except on days when nutrients were dosed. Prior to each Ca and S treatments application, each plant was photographed to obtain information on plant height, leaf count and area. Simultaneously, the stem diameter of each plant was recorded with an electronic calliper to measure stem diameter 10 cm from base at soil level.

2.4 Collection of plant tissue samples for bulk analysis

The plants were harvested after 12 months from the first treatment application (March 2017) and then separated into various fractions: roots, stem and leaves. The plant material samples were then oven dried at 70 °C for five days. Each sample was weighed, ground to fine powder and subsequently packed for transport to Australia and gamma irradiated at Steritech Pty. Ltd. in Brisbane following Australian Quarantine Regulations. The plant tissue samples were digested using 4 mL HNO_3 (70%) and 1 mL H_2O_2 (30%) in a microwave oven (Milestone Start D) for a 45-minute programme and diluted to 40 mL with ultrapure water (Millipore 18.2 MΩ·cm at 25°C) before analysis with ICP-AES (Thermo iCap7400) for Ni, Mg, Ca, and S.

Statistical analyses

Statistical analyses were performed using Statistica version 13.2 (StatSoft Inc., www.statsoft.com) and Microsoft Excel 2013. The shoot biomass (g) of the respective treatment levels are presented as boxplots and in tables. Key to symbols of boxplots: open squares are the ± mean, whiskers are ± standard deviation (SD), circles are outliers and asterisks are extreme outliers. Each value of the growth parameters (height (cm), leaf count and stem thickness (mm)) given in tables represents mean ± SD of five replicate plants. The mean ± standard deviation was determined using descriptive analysis tool, and significant difference was tested using one-way analysis of variance (one-way ANOVA) with confidence level of 95% in the Analysis Toolpak in Microsoft Excel 2013.

3. RESULTS

3.1 Growth responses of two tropical nickel hyperaccumulator plant species to calcium, sulphur and organic matter amendments

The growth responses of *R.* cf. *bengalensis* did not change significantly in the Ca treatments relative to the 'non-treated' control treatment ($p > 0.05$) (Fig. 1; Table 3a). There was 5-fold increase in height in all treatments at the end of the experiment, relative to the start (Table 3a). The highest Ca concentration treatment recorded the highest height growth responses, relative to all other treatments. Considering the leaf count, there was ~3-fold increase at the end of the experiment, relative to the start (Table 3a). There was about ~2-fold increase in the shoot biomass between the Ca treatments and the control treatments (Fig. 1). There were also >2-fold increases in stem thickness for all treatments at the end of the experiment with respect to the start (Table 3a). Similarly, the sulphur treatments did not yield significant increases in all the growth parameters (Fig. 2; Table 3a). However, we observed that the higher the sulphur concentration, the higher the increase in growth response. There was ~4-fold increase in height and leaf count at the end of the experiment, relative to the start (Table 3a). In case of the stem thickness, >2-fold increases were observed in all treatments at the end of the experiment relative to the start (Table 3a). Unlike Ca and S treatments, organic matter additions had strong positive growth effects on *R.* cf. *bengalensis* (Fig. 3). As high as >7-fold increases in height and leaf count were observed at the end of the experiment, relative to the start (Table 3a). We observed that as the organic matter content increased, the higher the growth response (in terms of height, leaf count, stem thickness and leaf area) of *R.* cf. *bengalensis*. The shoot biomass recorded at the end of the experiment was also high for the organic matter treatments. There was a significant difference between the organic matter treatments and the 'non-treated' control treatments ($p < 0.05$).

Similar to the observations in *R.* cf. *bengalensis*, Ca addition had negligible effects on the growth of *P. rufuschaneyi* (Fig. 1; Table 3b). Only slight increases in shoot biomass, height and stem thickness were observed in the Ca treatments, relative to the 'non-treated' control treatments (Table 3b). A similar observation was made in the S treatments (Fig. 2; Table 3b). However, different growth responses were recorded in the organic matter treatments. Unlike in *R.* cf. *bengalensis*, organic matter additions had strong negative effects relative to the control treatments in all the growth parameters recorded in *P. rufuschaneyi* (Fig. 3; Table 3b).

3.2 Elemental concentrations in different plants parts in response to different treatment levels

The Ca concentrations in the leaves of *P. rufuschaneyi* increased upon Ca addition (Table 4a). The mean Ni concentration in the leaves of *P. rufuschaneyi* increased in the order: 'treated control' < 180 mg Ca kg^{-1} < 360 mg Ca kg^{-1} treatments (Table 4a). However, as the added Ca concentration increased, the concentrations of Mg recorded in the leaves of *P. rufuschaneyi* declined (Table 4a). Similar elemental accumulation patterns observed in the leaf fraction of *P. rufuschaneyi* were found in the stem fraction (data not shown). The elemental accumulation in the leaf, fraction of *R. bengalensis* in response to Ca treatments showed increases in concentrations of Ca, S and Ni with increasing Ca treatment levels (Table 4b). However, the concentrations of Mg in these fractions decreased with increasing Ca treatment levels (Table 4b).

In the S treatments, the concentrations of Ni in the leaf fraction of *P. rufuschaneyi* increased significantly with increasing levels of S (Table 4b). Interestingly, the S concentrations in the leaves of *P. rufuschaneyi* in the 180 mg S kg^{-1} were higher than that in the 360 mg S kg^{-1} treatment (Table 4b). In the leaf fraction of *R. bengalensis*, the concentrations of Ca, S and Ni increased significantly with increasing levels of S addition (Table 4b). However, the concentrations of Mg in these fractions decreased with increasing concentrations of added S.

Unlike the Ca and S treatments, the organic matter treatments significantly reduced the concentrations of Ni in the leaf fraction of *P. rufuschaneyi* (Table 4a). However, as the organic matter content increased, the concentrations of Ca in the various fractions of *P. rufuschaneyi* also increased. Likewise, organic matter additions significantly reduced the Ni concentrations in all the fractions of *R. bengalensis* (data not shown). However, Ca concentrations in the various fractions of *R. bengalensis* increased with increasing organic matter content.

4. DISCUSSION

The present study revealed that both Ca and S additions did not have significant effects on the growth of *P. rufuschaneyi* and *R.* cf. *bengalensis*. Whereas organic matter amendments have strong negative effects on the growth of *P. rufuschaneyi*, there is significant positive growth response to these amendments in *R.* cf. *bengalensis* (Fig. 4). This implies that the effect of organic matter amendments on the growth of tropical Ni hyperaccumulator species is species-dependent. Elsewhere, whereas Broadhurst and Chaney (2016) found negative growth responses to organic matter amendments in *Alyssum murale*, Álvarez-López et al. (2016) and Ghasemi et al. (2017) reported positive growth responses to these amendments in *A. bracteata, A. inflata* and *A. serpyllifolia*. These findings confirm that the effect of organic matter on the biomass production of Ni hyperaccumulator plants is different for different species. We must add that the biomass recorded for *A. bracteata, A. inflata* and *A. serpyllifolia*, even under organic matter treatments are limited, and cannot support economic Ni agromining operations. In the tropical setting, *P. rufuschaneyi* is presently the most promising species for economic tropical Ni agromining (Nkrumah et al., 2018a; 2019). Considering the strong negative effects of organic matter amendments on the biomass production of *P. rufuschaneyi,* these amendments will not be useful in practical agromining operations employing these species in the tropics (see Table 5).

Apart from growth responses in 'metal crops', some agronomic measures are targeted at increasing Ni yield by enhancing Ni tolerance (Nkrumah et al., 2016a; 2018a). The present study revealed that Ca addition has significant positive effect on the Ni uptake in both *P. rufuschaneyi* and *R. bengalensis* (Table 4). This finding is in agreement with that of previous studies that reported increases in shoot Ni concentrations in *Alyssum* species in response to Ca addition (Brooks et al., 1981; Chaney et al., 2008). These authors found that Ca additions to high Mg ultramafic soils with low Ca/Mg quotient ameliorates Ni toxicity and improves annual Ni phytoextraction in *Alyssum* species. On the contrary, low Ca increases phytotoxicity of Ni in *Alyssum* species, further confirming the role of Ca addition in enhancing Ni tolerance in *Alyssum* Ni hyperaccumulator species. However, the effects of Ca on Ni uptake by Ni hyperaccumulator plants may be species-dependent; Ca addition inhibits Ni uptake in the Ni hyperaccumulator *B. coddii* (Robinson et al., 1999). Hence, in field agromining operations employing Ca fertilisation, all species need to be tested prior to field application. The present study also found that Ca addition decreases shoot Mg concentrations in both *P. rufuschaneyi* and *R. bengalensis* (Table 4), similar to the finding in *Alyssum* Ni hyperaccumulator species in response to Ca addition (Chaney et al., 2008). Presently, it is not clear how the reduction in shoot Mg concentrations affects Ni uptake in Ni hyperaccumulator plants. The shoot Ca and S concentrations of *P. rufuschaneyi* and *R. bengalensis* increased in response to Ca addition. Even in the absence of Ca addition, *P. rufuschaneyi* and *R.* cf. *bengalensis* have high intrinsic ability to accumulate high concentrations of these elements in the shoots (van der Ent and Mulligan, 2015). In economic Ni agromining, the high shoot concentrations of Ca and S are unwanted 'contaminants' in the eventual 'bio-ore' (Barbaroux et al., 2012; Vaughan et al., 2017). Hence, Ca application needs to be carefully considered in economic agromining operations especially using *R.* cf. *bengalensis*. However, the removal of shoot biomass during agromining may necessarily require Ca application to maintain full yield potential (Bani et al., 2015; Chaney et al., 2008; Nkrumah et al., 2016a; 2018a; van der Ent et al., 2015).

Sulphur addition increases the shoot Ni concentrations of both *P. rufuschaneyi* and *R.* cf. *bengalensis*. Similarly, S addition has been shown to significantly increase Ni uptake in *B. coddii*, mainly due to increase in the concentrations of plant available S (Robinson et al., 1999). In *Noccaea* Ni hyperaccumulator plants, evidence suggests that S increases Ni tolerance (Freeman et al., 2004; Na and Salt, 2011). Several studies report a strong correlation between Ni and S localisation at the cell-level in *A. bracteatum* and *A. murale* in epidermal vacuoles (Asemaneh et al., 2006; Broadhurst et al., 2004a, b; 2009; Küpper et al., 2001). The role of S addition in enhancing Ni tolerance may be explained by the functioning of SO_4^{2-} as an important counter-ion to Ni^{2+} in the vacuoles of these hyperaccumulator species (Broadhurst et al., 2004a, b; 2009; Küpper et al., 2001). Hence, S addition could be a low-cost amendment warranting consideration in tropical economic Ni agromining operations. Applying $CaSO_4$ fertiliser to replace Ca and S removed in harvested biomass would maintain fertility needed for full yield of many species.

Organic matter amendments significantly decrease shoot Ni concentrations in both *P. rufuschaneyi* and *R. bengalensis*. Clearly, organic matter amendments are not useful in economic tropical Ni agromining operations employing *P. rufuschaneyi*, but could be considered when employing *R.* cf. *bengalensis* (Table 5). Elsewhere, organic matter amendments have negative effects on the Ni uptake in *Alyssum* hyperaccumulator species (Álvarez-López et al., 2016; Broadhurst and Chaney, 2016). The low shoot Ni concentrations in these species in response to organic matter amendments may be explained by the reduction in soil extractable Ni (Álvarez-López et al., 2016; Broadhurst and Chaney, 2016). High soil extractable Ni is always a desired property for economic Ni agromining operations (Nkrumah et al., 2016a; 2019), further confirming that organic matter amendments may not be beneficial for economic Ni agromining operations. However, the nature of some substrates may necessarily warrant the use of organic matter amendments to support normal plant growth, especially as industrial mine wastes are potential sites.

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TABLE CAPTIONS

Table 1a. Growth parameters of *Rinorea* cf. *bengalensis* at the beginning of the experiment expressed as mean ± standard error.

Table 1b. Growth parameters of *Phyllanthus rufuschaneyi* at the beginning of the experiment expressed as mean ± standard error.

Table 2. Composition of the various treatment levels used in the growth trial. OM represents organic matter, and was mixed with the ultramafic soils on volume basis at the start of the experiment in the organic matter treatments.

Table 3a. Growth parameters of *Rinorea* cf. *bengalensis* as increment over a year expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Table 3b. Growth parameters of *Phyllanthus rufuschaneyi* as increment over a year expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Table 4a. Leaf elemental concentrations of *Phyllanthus rufuschaneyi* under calcium, sulphur and organic matter treatments expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Table 4b. Leaf elemental concentrations of *Rinorea* cf. *bengalensis* under calcium, sulphur and organic matter treatments expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Table 5. Leaf nickel yield per plant (mg) of *Phyllanthus rufuschaneyi* and *Rinorea* cf. *bengalensis* in the 'non-treated control', calcium, sulphur and organic matter treatments under optimum conditions. Leaf Ni yield (expressed as mean) is calculated as a product of mean Ni concentration and the corresponding mean dry biomass of the leaf fraction.

FIGURE CAPTIONS

Fig. 1. Effects of calcium addition on the shoot biomass of *Rinorea* cf. *bengalensis* and *Phyllanthus rufuschaneyi.* Key to symbols of boxplots: open squares are the ± mean, whiskers are ± standard deviation (SD), circles are outliers.

Fig. 2. Effects of sulphur addition on the shoot biomass of *Rinorea* cf. *bengalensis* and *Phyllanthus rufuschaneyi.* Key to symbols of boxplots: open squares are the ± mean, whiskers are ± standard deviation (SD), circles are outliers.

Fig. 3. Effects of organic matter amendments on the shoot biomass of *Rinorea* cf. *bengalensis* and *Phyllanthus rufuschaneyi*. OM represents organic matter. Key to symbols of boxplots: open squares are the ± mean, whiskers are ± standard deviation (SD), circles are outliers.

Fig. 4. Images showing growth responses to organic matter amendments in *Phyllanthus rufuschaneyi* (small leaf blades) and *Rinorea* cf. *bengalensis* (large leaves).

Table 1a. Growth parameters of *Rinorea* cf. *bengalensis* at the beginning of the experiment expressed as mean ± standard error.

Treatments	Height (cm)	Leaf count	Stem thickness (mm)
Untreated control	20 ± 3.5	2.0 ± 1.0	4.0 ± 0.4
Calcium			
0 mg Ca kg^{-1}	14 ± 1.5	4.0 ± 1.0	3.4 ± 0.3
180 mg Ca kg^{-1}	13 ± 1.5	4.0 ± 1.0	3.6 ± 0.3
360 mg Ca kg^{-1}	19 ± 2.3	5.0 ± 1.0	3.9 ± 0.3
Sulphur			
0 mg S kg^{-1}	16 ± 1.5	3.0 ± 1.0	3.6 ± 0.2
180 mg S kg^{-1}	15 ± 1.3	3.0 ± 1.0	3.5 ± 0.3
360 mg S kg^{-1}	11 ± 2.5	4.0 ± 1.0	2.7 ± 0.2
Organic matter			
0% OM	16 ± 4.5	4.0 ± 1.0	3.7 ± 0.7
5% OM	16 ± 2.3	5.0 ± 1.0	3.5 ± 0.3
10% OM	19 ± 1.0	5.0 ± 1.0	4.2 ± 0.2

Table 1b. Growth parameters of *Phyllanthus rufuschaneyi* at the beginning of the experiment expressed as mean ± standard error.

Treatments	Height (cm)	Leaf count	Stem thickness (mm)
Untreated control	12 ± 3.0	2.0 ± 1.0	2.8 ± 0.4
Calcium			
0 mg Ca kg^{-1}	8.0 ± 1.5	3.0 ± 1.0	2.3 ± 0.2
180 mg Ca kg^{-1}	9.5 ± 1.5	4.0 ± 1.0	2.8 ±0.2
360 mg Ca kg^{-1}	15 ± 2.0	3.0 ± 1.0	3.0 ± 0.3
Sulphur			
0 mg S kg^{-1}	15 ± 1.0	4.0 ± 1.0	3.6 ± 0.3
180 mg S kg^{-1}	8.0 ± 0.5	3.0 ± 1.0	2.1 ± 0.3
360 mg S kg^{-1}	10 ± 2.0	3.0 ± 1.0	2.7 ±0.2
Organic matter			
0% OM	9.2 ± 1.0	4.0 ± 1.0	2.5 ± 0.2
5% OM	16 ± 3.5	5.0 ± 1.0	3.7 ± 0.4
10% OM	17 ± 4.0	4.0 ± 1.0	3.3 ± 0.2

Table 2. Composition of the various treatment levels used in the growth trial. OM represents organic matter, and was mixed with the ultramafic soils on volume basis in the organic matter treatments.

Treatments	**Treatments Levels**	**mg kg^{-1} after 3 × application**	**mg kg^{-1} after 3 × application**			
		NaH_2PO_4	**NH_4NO_3**	**KCl**	**Na_2SO_4**	**$CaCl_2 \cdot 2H_2O$**
Non-treated Control	–	–	–	–	–	–
Calcium treatments:						
	Treated control	180	180	180	60	–
	180 mg Ca kg^{-1} treatment	180	180	180	60	180
	360 mg Ca kg^{-1} treatment	180	180	180	60	360
Sulphur treatments:						
	Treated control	180	180	180	–	60
	180 mg P kg^{-1} treatment	180	180	180	180	60
	360 mg P kg^{-1} treatment	180	180	180	360	60

Table 2–continued.

Treatments	**Treatments Levels**	**mg kg^{-1} after 3 × application**	**mg kg^{-1} after 3 × application**			
		NaH_2PO_4	**NH_4NO_3**	**KCl**	**Na_2SO_4**	**$CaCl_2.2H_2O$**
Organic matter treatments:						
	Treated control	180	180	180	60	60
	5% OM	180	180	180	60	60
	10% OM	180	180	180	60	60

Table 3a. Growth parameters of *Rinorea* cf. *bengalensis* as increment over a year expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Treatments	Height (cm)	Leaf count	Stem thickness (mm)
Untreated control	60 ± 3.5 d	10 ± 1.0 b	4.5 ± 0.5 b, c
Calcium			
0 mg Ca kg^{-1}	57 ± 10 d	10 ± 2.0 b	4.8 ± 0.5 b, c
180 mg Ca kg^{-1}	85 ± 10 b	11 ± 2.0 a, b	5.0 ± 0.3 b
360 mg Ca kg^{-1}	90 ± 8.0 a	12 ± 1.0 a, b	4.4 ± 0.7 b, c
Sulphur			
0 mg S kg^{-1}	80 ± 8.5 b	13 ± 1.0 a	5.2 ± 0.3 b
180 mg S kg^{-1}	72 ± 5.5 c	10 ± 1.0 b	5.2 ± 0.6 b
360 mg S kg^{-1}	92 ± 9.5 a	10 ± 1.0 b	5.9 ± 0.2 a
Organic matter			
0% OM	80 ± 16 b	10 ± 1.0 b	5.0 ± 0.5 b
5% OM	100 ± 14 a	11 ± 1.0 a, b	5.8 ± 0.6 a
10% OM	110 ± 10 a	12 ± 1.0 a, b	6.3 ± 0.4 a

Table 3b. Growth parameters of *Phyllanthus rufuschaneyi* as increment over a year expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Treatments	Height (cm)	Leaf count	Stem thickness (mm)
Untreated control	47 ± 6.0 c	19 ± 3.0 b	3.5 ± 0.5 c
Calcium			
0 mg Ca kg^{-1}	90 ± 6.5 a	25 ± 5.0 a	7.5 ± 0.7 a
180 mg Ca kg^{-1}	66 ± 5.5 b	13 ± 3.0 b	6.0 ± 0.5 a, b
360 mg Ca kg^{-1}	73 ± 15 b	15 ± 4.0 b	5.0 ± 0.7
Sulphur			
0 mg S kg^{-1}	97 ± 10 a	24 ± 8.0 a	6.2 ± 0.8 a
180 mg S kg^{-1}	79 ± 10 b	20 ± 5.0 a, b	7.0 ± 0.5 a
360 mg S kg^{-1}	71 ± 7.5 b	22 ± 5.0 a, b	6.5 ± 0.5 a
Organic matter			
0% OM	77 ± 15 b	23 ± 4.0 a, b	6.3 ± 0.7 a, b
5% OM	50 ± 8.5 c	15 ± 5.0 b	5.5 ± 0.4 b
10% OM	38 ± 2.5 d	5.0 ± 2.0 c	3.8 ± 0.2 c

Table 4a. Leaf elemental concentrations of *Phyllanthus rufuschaneyi* under calcium, sulphur and organic matter treatments expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Treatments	Calcium	Magnesium	Sulphur	Nickel
Calcium				
0 mg Ca kg^{-1}	3500 ± 825 c, d	3000 ± 250 d, e	2200 ± 90 c	9800 ± 1000 c
180 mg Ca kg^{-1}	4460 ± 840 c	4930 ± 570 a	2820 ± 230 b	10 000 ± 870 b
360 mg Ca kg^{-1}	4630 ± 730 c	3660 ± 230 b	2670 ± 135 b	11 600 ± 950 a, b
Sulphur				
0 mg S kg^{-1}	4270 ± 855 c	3870 ± 350 a, b	1960 ± 90 d	9770 ±920 c
180 mg S kg^{-1}	4330 ± 830 c	3040 ± 255 d, e	3780 ± 430 a	10 000 ± 1130 b
360 mg S kg^{-1}	4510 ± 825 c	3290 ± 310 d	2910 ± 215 b	12 200 ± 1310 a
Organic matter				
0% OM	4550 ± 890 c	3730 ± 380 b	1970 ± 90 d	12 400 ± 1400 a
5% OM	6580 ± 860 b	2750 ± 220 e	2740 ± 510 b	10 200 ± 825 b
10% OM	6980 ± 1190 a	2760 ± 535 e	2320 ± 275 c	7150 ± 1190 d

Table 4b. Leaf elemental concentrations of *Rinorea* cf. *bengalensis* under calcium, sulphur and organic matter treatments expressed as mean ± standard error. Mean ± standard error followed by the same letter are not significantly different (p <0.05) according to the Duncan-Waller K-ratio t-test.

Treatments	Calcium	Magnesium	Sulphur	Nickel
Calcium				
0 mg Ca kg^{-1}	12 300 ± 1800 d	3310 ± 255 a, b	3050 ± 190 d	3880 ± 610 d
180 mg Ca kg^{-1}	14 400 ± 1330 c	3270 ± 270 b	3370 ± 140 c	3930 ± 410 c, d
360 mg Ca kg^{-1}	16 900 ± 1920 b	2980 ± 260 c	3640 ± 170 b	4200 ± 455 b, c
Sulphur				
0 mg S kg^{-1}	17 000 ± 1500 b	3190 ± 130 b, c	1580 ± 120 e	5150 ± 450 a
180 mg S kg^{-1}	10 200 ± 735 e	3120 ± 215 b, c	3140 ± 155 d	2930 ± 280 e
360 mg S kg^{-1}	13 100 ± 1100 c	2750 ± 175 c, d	3680 ± 155 b	4470 ± 465 b, c
Organic matter				
0% OM	16 000 ± 1440 b	3050 ± 195 b, c	3720 ± 160 a, b	4980 ± 615 a, b
5% OM	19 000 ± 1760 a	2460 ± 130 e	3720 ± 205 a, b	4030 ± 505 c
10% OM	18 000 ± 1380 a, b	3400 ± 285 a	3880 ± 165 a	3150 ± 350 e

Table 5. Leaf nickel yield per plant (mg) of *Phyllanthus rufuschaneyi* and *Rinorea* cf. *bengalensis* in the 'non-treated control', calcium, sulphur and organic matter treatments under optimum conditions. Leaf Ni yield (expressed as mean) is calculated as a product of mean Ni concentration and the corresponding mean dry biomass of the leaf fraction.

Treatments	***Phyllanthus rufuschaneyi***	***Rinorea* cf. *bengalensis***
Non-treated control	180	120
Calcium	325	200
Sulphur	300	150
Organic matter	200	195

Calcium Treatments


Sulphur Treatments
Phyllanthus rufuschaneyi
Rinorea cf. bengalensis
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
p >0.05
a
b
b
b
Shoot biomass (g)
0
10
20
30
40
50
Non-treated control
Treated control
180 mg S kg-1
360 mg S kg-1


Organic Matter Treatments
Phyllanthus rufuschaneyi
Rinorea cf. bengalensis
Median
25%-75%
Non-Outlier Range
Outliers
Extremes
Shoot biomass (g)
0
10
20
30
40
50
60
70
80
90
100
a
b
c
d
Non-treated control
Treated control
5% OM
10% OM


Non-treated control
Treated control
5% organic matter treatment
10% organic matter treatment